TITLE THE ANALYTICAL CHEMISTRY OF AGRICULTURAL GUANIDINE FUNGICIDES

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THE ANALYTICAL CHEMISTRY OF AGRICULTURAL GUANIDINE FUNGICIDES

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ABSTRACT

The Analytical Chemistry of Agricultural Guanidine Fungicides

Arquimedes Leverenti

A programme of research on agricultural guanidine fungicides, represented by a complex mixture of several components (**guazatine**) and by a single compound (**dodine**), was carried out.

The studies involved the synthesis of several guanidine derivatives and their characterization by spectroscopic methods such as carbon-13 NMR, MS, and FAB/MS. The synthetic routes have been described and different routes for the preparation of guazatine were also established.

Carbon-13 NMR has been shown to be a useful tool in the identification of guanidine structures at the central and terminal positions of the different guanidine molecules, whilst the FAB/MS technique gave complete characteristic fragmentation patterns for the guanidine derivatives.

Acetylacetone and hexafluoroacetylacetone were used as derivatization reagents to convert synthesized guanidine compounds into stable and volatile derivatives to ensure successful gas chromatographic analysis. Novel compounds were also synthesized using both reagents.

GLC and GLC/MS studies have been shown to be powerful techniques in the identification of the important components in the complex mixture of guazatine in the form of their hexafluoroacetylacetone derivatives.

Dodine was also studied in the same way as guazatine and can be analysed by GLC of the hexafluoroacetylacetone derivative.

Mass Spectral data for the various derivatives are presented and possible fragmentation pathways are discussed.

ACKNOWLEDGEMENTS

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I also acknowledge the scholarship provided by the **"Empresa Brasileira de Pesquisa Agropecuária"** (**EMBRAPA**), and would like to thank **Kemiinord AB**, Stockholm, for providing chemical samples, wheat plants and much useful information. Discussions with **Mr. Alf Rautenfeldt** and **Dr. David G. Cameron** (of KenoGard AB) and with **Mr Karl-Erland Stenlö** (of Nobel Central Analytical Services) are gratefully acknowledged.

I would like to thank **Mr Manuel Diamantino** for the kind help provided on technical aspects of chromatographic analysis.

The **Science & Engineering Research Council** (**SERC**) Mass Spectrometry Centre - University College of Swansea is thanked for FAB mass spectrometry services.

DEDICATION

I dedicate this work to my wife, Wilbiria B.C. Laverenti, for her love and happiness which changed my life since I knew her and for the strength and courage that she has given to me to finish this work.

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I - INTRODUCTION

Pesticides control pests on crops and their actions have been recognized for a long time. The three main types of pesticides are insecticides for the control of insects, herbicides for the control of weeds and fungicides for the control of diseases of crops.

Only the larger agrochemical companies can face the expense involved in the discovery and development of pesticides. For example, about 10,000 new compounds have to be tested in order to get only one potential pesticide. This may take some 10 years of intensive studies and around £10 million might be spent until the pesticide can be launched on the market.

Critical steps in the discovery of new pesticides include synthesis, screening against pests, studies of toxicology, field trials, residue analysis, legislation, and marketing, etc.

Every year new pesticides appear on the market because they have a limited period of life, mainly due to the development of pest resistance, the introduction of cheaper competitive pesticides from other companies, and the expiry of patents, etc.

Nowadays, the control of pests is achieved by Integrated Pest Management (IPM) whereby chemical, biological, and mechanical control are utilized in association or individually depending on the pest to be controlled and the availability of resources.

The environmentalists place great emphasis on biological control.

it is not new and has been used by nature from time immemorial. Man observed this behaviour and tried to reproduce it on a large scale to control pests using their natural enemies.

The literature describes many examples of biological control. Some of them have been successful and others failed. The problem with biological control is that we do not have any study over an extended period of time, in order to show whether the natural enemy really controlled the pest, or became a pest itself causing a new problem. Another problem with biological control is that it is also weather dependent.

Genetic engineering is arising as a powerful technique to control pests. New studies in genetic engineering are producing crops resistant to diseases, resistance to attack by insects and selective herbicides controlling weeds without damage to crops.

Biotechnology also has become important as a powerful technique, with the discovery of new biopesticides which are replacing some synthetic pesticides. More studies need to be done because we do not know yet their action and behaviour over a long period of time.

But, apart from all these new methods of pest control, we are still using on a large scale the synthetic pesticides and we will continue to do so for a long time because many cannot be replaced in the near future.

It is important that we should study carefully all the parameters related to the pest, crop and environment before a decision is taken on which method of control to use.

A knowledge of the interrelationship between pests, crops and the environment is needed in order to plan the best combination of techniques that will give suitable control of the pest and produce minimum impact on non-target organisms and the environment.

In the present programme of study a commercial fungicide belonging to

the guanidine group has been chosen for investigation. The product is a mixture of numerous components all with similar biological activity and difficult to separate. The aim is to develop a method for the total analysis of such mixtures and for the determination at residue level of some of the principal components. To do this several studies will be realized including synthesis, structural chemical characterization, and analysis by Gas-Liquid Chromatography, High-Performance Liquid Chromatography, Carbon-13 Nuclear Magnetic Resonance, Mass Spectrometry, Fast Atom Bombardment Mass Spectrometry, and Gas-Liquid Chromatography/Mass Spectrometry, etc.

II- HISTORICAL REVIEW

1- FUNGICIDES

Fungicides are substances or agents that are capable of destroying fungi, thus controlling diseases in plants or animals.

The history of fungicides can be divided into three distinct eras: the first or sulphur era from ancient times to 1882, the second or copper era from 1882 to 1934, and the third or organic fungicide era beginning in 1934, with the introduction of dithiocarbamate fungicides by Tisdale and Williams[1].

Fungicidal action is usually expressed in one or two physically visible ways: the inhibition of spore germination and/or the inhibition of fungus growth. Most fungicides inhibit spore germination or kill the spores immediately following germination. Some of these chemical inhibitors or toxicants also retard or halt fungal growth, when applied after the infectious stage has developed. The newer systemic fungicides have eradicant properties and stop the progress of existing infections by penetrating into the plant and being transported within the cell sap to the untreated parts of the plant.

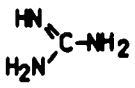
Plant diseases are, like many biological phenomena, difficult to define. We may, however, think of diseased plants as those which have become altered in their physiological and their morphological development to such a degree that signs of such effects are obvious. These

external signs on the plant which are characteristic of a given disease are known as symptoms.

Crops are vulnerable to diseases at every stage of their development, so the defence of our crops must be a continuous effort in order to destroy the initial populations of plant pests (pathogens) or to reduce the rates of increase in the number of pests.

2- GUANIDINE COMPOUNDS

Guanidine (1), is a crystalline, strong, organic base. It was first prepared by Strecker in 1861 by oxidizing guanine with potassium chlorate and hydrochloric acid in the course of his work on constituents of guano, from which the name guanidine is derived[2,3].



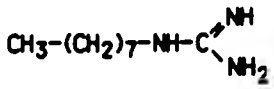
(1)

Guanidine is a deliquescent crystalline solid, readily soluble in alcohol and water; it is volatile and strongly alkaline, absorbs carbon dioxide from the air, and forms crystalline salts; it is a stronger base than any other organic nitrogenous base recorded[4].

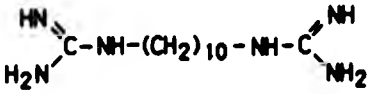
Guanidine is marketed in the form of its salts, such as nitrate, chloride, stearate, silicate, or carbonate (these are more correctly called guanidinium salts). They are used in a variety of areas, thus guanidine hydrochloride is used as an intermediate in the synthesis of pharmaceuticals, guanidinium nitrate in the production of explosives, and guanidine stearate as a release agent for processing of

plastics[5].

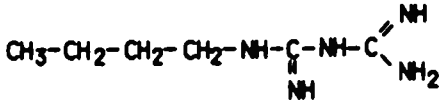
Among the guanidines three groups can be distinguished: monoguanidines, such as *n*-octylguanidine (2), diguanidines such as decamethylenediguanidine (synthalin A) (3), and biguanidines such as *n*-butylbiguanide (buformin) (4).



(2)



(3)



(4)

The short chain guanidine salts are readily soluble in water; the long chain derivatives are more soluble in ethanol/water mixtures. The strong basicity of the guanidines, as well as the tendency for complex formation, may influence their behaviour in biological systems[6].

The biological activity of substituted guanidines was recognized in the mid-thirties when a series of guanidine and biguanidine compounds was reported to have bactericidal and disinfectant properties[7].

3- GUANIDINE FUNGICIDES

The first agricultural guanidine fungicide appeared in 1956 with the development of *n*-dodecylguanidine acetate (5) (known as dodine or Cyprex) by the American Cyanamid Co.[8,9a].

$$CH_3-(CH_2)_{11}-NH-C(=NH_2^+)(NH_2)\ CH_3COO^- \qquad (5)$$

Dodine found wide use because of its ability to destroy established infections of apple scab, a property shown previously only by the organic mercurials[10].

Hudson *et al*[11] presented a comprehensive review on guanidine compounds with antifungal and antibacterial activities.

In 1968 Evans Medical Limited[12] patented a method for the preparation of fungicidal guanidine derivatives having the following general formula:

$$G^1-R^6-\overset{R^1}{\underset{|}{N}}-(R^7-\overset{R^2}{\underset{|}{N}})_n-R^8-G^2$$

where, R^1, R^2 = H or alkyl groups

R^6, R^7, R^8 = may be the same or different, are straight or branched alkylene groups separating adjacent nitrogen atoms by chains of at least two carbon atoms, the total number of carbon and nitrogen atoms in the straight chain between the two groups G^1 and G^2, excluding branching groups, being always greater than 12, n is an integer from 0 - 4,

$$G^1, G^2 = NH-\overset{\overset{N-R^3}{\|}}{C}-N-R^5 \quad (R^4 \text{ on } N)$$

where, R3, R4, R5 = H or alkyl groups having 1 to 4 carbon atoms.

The compounds were intended for pharmaceutical application. They were active against the pathogenic fungus *Candida albicans* and showed antibacterial activity against *Pseudomonas pyocyaneus*, *Staphylococcus aureus* and *Escherichia coli*.

One of the compounds, 9-aza-1,17-diguanidinoheptadecane or bis-(8-guanidino-octyl)amine, was subsequently developed as an important agricultural fungicide under the name guazatine and was produced in the form of the triacetate (10). The compound can be prepared by the reaction of bis-(8-amino-octyl)amine (6) with *S*-methylisothiouronium sulphate (7), followed by conversion to the carbonate and acetate as shown in Figure 1[13].

The first paper showing the fungicidal effects of this guanidine derivative and its salts in the control of seed-borne diseases in cereals appeared in 1968 as the result of joint work between Murphy Chemical Ltd. and Glaxo Research Ltd., both from the UK[14]. The triacetate is particularly useful as a replacement for organomercurial seed-dressings[9b].

It is in this context that KenoGard AB of Stockholm, Sweden, undertook the commercial development of guazatine.

The commercial formulation consists of the acetates of a standardized mixture obtained by 82-84% amidination [using cyanamide (11)] of technical triamine, containing 1,8-diamino-octane (12), bis-(8-amino-octyl)amine (6), and higher oligomers (13)[15,16], and it is to this

mixture that the name guazatine now applies[9c].

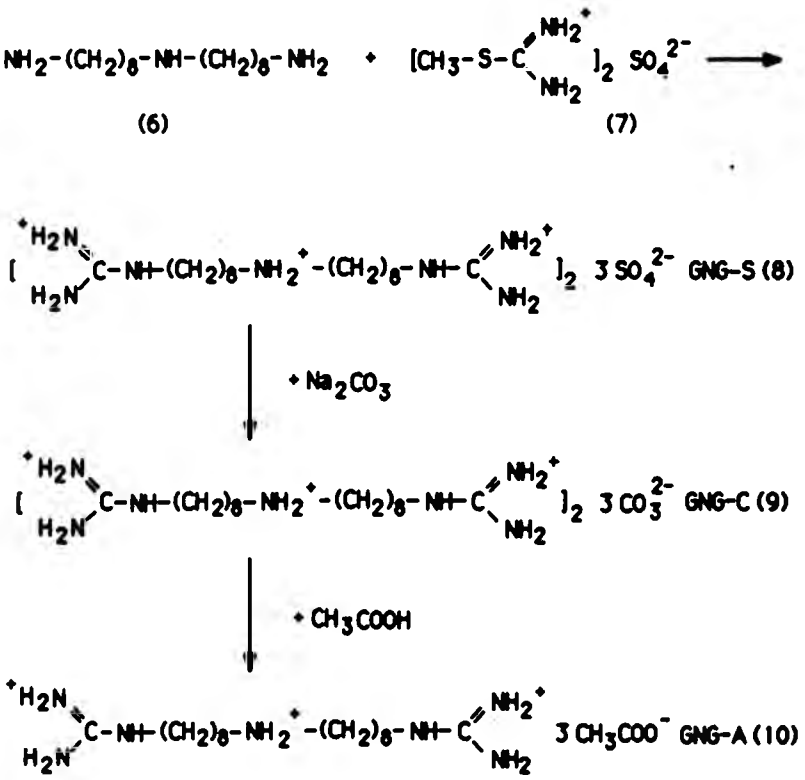


Figure 1. Reaction scheme for the preparation of guazatine (GNG-A) through reaction of triamine with *S*-methylisothiouronium sulphate.

NH_2CN (11)

$NH_2-(CH_2)_8-NH_2$ (12)

$NH_2-(CH_2)_8-[NH-(CH_2)_8]_n-NH_2$ (13)

where, $n \geq 2$

A number of guanidine derivatives are thus present in which one or more of the amino groups (both primary and secondary) have been converted to guanidine.

The principal focus of attention in the present work has been on this guazatine fungicide and some work with dodine has also been carried out.

3.1- SYNTHESIS

The possible reagents for the amidination step in the guazatine synthesis are cyanamide (11)[12,17], *S*-methylisothiouronium sulphate (7)[12,18], or *O*-methylisouronium hydrogen sulphate (14)[19]. *S*-methylisothiouronium sulphate reacts with evolution of methanethiol and so requires careful handling, particularly on the industrial scale. The by-products of the other two reagents are much less toxic and are much more amenable to large-scale handling.

$$CH_3-O-C(=NH_2^+)-NH_2 \; HSO_4^- \quad (14)$$

Kenkyuho described the utilization of *O*-methylisouronium acetate (15) in the production of bis-(8-guanidino-octyl)amine triacetate (10)[20].

$$CH_3-O-C(=NH_2^+)NH_2 \; CH_3COO^- \quad (15)$$

However, the commercial process for guazatine production[21] (Figure 2) does not include the isolation of the triamine resulting from the reforming reaction, and thus a number of polyamines are present.

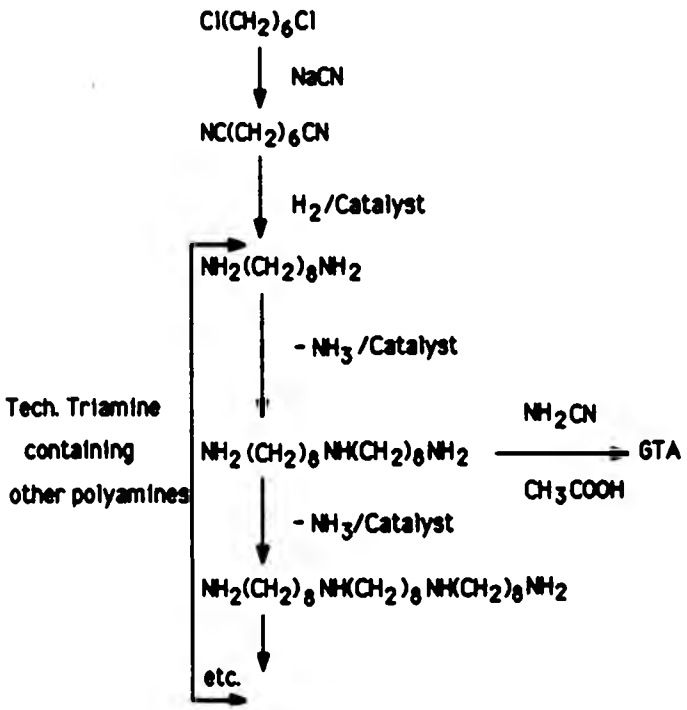


Figure 2. Commercial process for the manufacture of guazatine[21].

The composition of the technical triamine used in this process is gen-

erally as follows: diamine content = 30 ± 5%; triamine content = 40 ± 5%; higher oligomers content = 30%.

Technical triamine and cyanamide in the presence of acetic acid and water give the guanidated amine acetates (GTA).

Table 1. Components of a representative mixture of guanidated amine acetates

Compd.	Mole Frac.	Moles	Molec. Weight as Acet.	Gram of Prod. as Acetates	% w/w of Guanid. Amine Ac.
NN	0.032	0.007	264.36	1.76	0.84
GN	0.295	0.061	306.52	18.81	8.97
GG	0.672	0.140	451.64	48.74	23.23
NNN	0.006	0.001	451.64	0.40	0.19
2/3GNN	0.080	0.012	493.80	5.85	2.79
1/3NGN					
2/3GGN	0.242	0.036	535.96	28.79	13.72
1/3GNG	0.121	0.018	535.96	9.60	4.58
GGG	0.551	0.082	577.43	47.15	22.42
NNNN	0.001		638.92	0.05	0.02
1/2GNNN	0.019	0.001	681.08	0.97	0.46
1/2NGNN					
1/3GGNN	0.131	0.010	723.24	7.11	3.39
1/3GNGN					
1/6NGGN					
1/6GNNG					
1/2GGGN	0.397	0.030	765.40	22.79	10.86
1/2GNGG					
GGGG	0.452	0.034	807.56	27.38	13.05
				209.80	100.00

Theoretically 100 grams of technical triamine amidinated to 82% and neutralized gives 209.80 grams of acetate salts[21].

The average expected isomeric composition of guanidated amine acetates can also be calculated statistically on the basis of the assumption that random guanidation of the various amino groups occurs[21]. Table 1 shows the calculated percentage of each main component of a representative mixture of guanidated amine acetates.

The symbols used stand for: NN = diamine, GN = monoguanidated diamine, GG = diguanidated diamine, NNN = triamine, GNN or NGN = monoguanidated triamine, GGN or GNG = diguanidated triamine, GGG = triguanidated triamine, NNNN = tetra-amine (the main component of the polyamine fraction), GNNN or NGNN = monoguanidated tetra-amine, GGNN or GNGN or NGGN or GNNG = diguanidated tetra-amine, GGGN or GNGG = triguanidated tetra-amine, and GGGG = tetraguanidated tetra-amine. Table 2 shows the chemical structures for these components.

One way in which a study of the various possible components of the product could be made would involve the synthesis of each component and of similar model compounds, and a separate study of these.

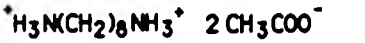
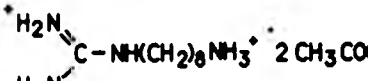
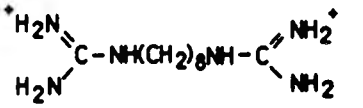
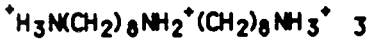
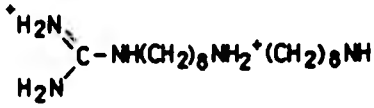
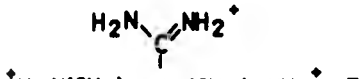
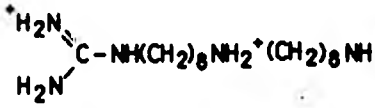
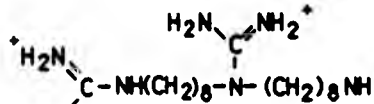
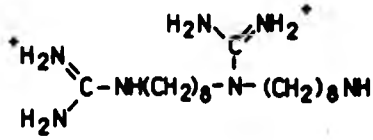
The work involved initially the synthesis of authentic pure samples of bis-(8-guanidino-octyl)amine salts and a series of model compounds under various conditions using at least two different reagents, cyanamide and *S*-methylisothiouronium sulphate.

3.2 - TOXICOLOGICAL AND DEGRADATION ASPECTS

Fungicides, usually, compared with insectidides and herbicides show low mammalian toxicity. Apart from this characteristic they present risk of environmental pollution and residues in foods if not used correctly.

A knowledge of the toxicology and degradation products of guanidine

Table 2. Structures of the components in a representative mixture of guanidated amine acetates.

STRUCTURES	NAME OF THE COMPONENT
	1,8-Diamino-octane diacetate (NN-A)
	1-Amino-8-guanidino-octane diacetate (GN-A)
	1,8-Diguanidino-octane diacetate (GG-A)
	Bis-(8-amino-octyl)amine triacetate (NNN-A)
	N-(8-Amino-octyl)- N-(8-guanidino-octyl)amine triacetate (GNN-A)
	Bis-(8-amino-octyl)guanidine triacetate (NGN-A)
	Bis-(8-guanidino-octyl)amine triacetate (GNG-A)
	1-(8-Amino-octyl)-1-(8-guanidino-octyl)guanidine triacetate (GGN-A)
	1,1-Bis-(8-guanidino-octyl)guanidine triacetate (GGG-A)

fungicides is very important in order to safeguard the farmer and sprayers in the field and the consumers when ingesting food sprayed with fungicides.

Guazatine level causing no toxicological effect:[16]

Rat: 200 ppm, (54.8% active ingredient as acetate in water) equivalent to 5 mg/kg body weight of the active ingredient.

Dog: 200 ppm, (54.8% active ingredient as acetate in water) in the diet, equivalent to 3 mg/kg body weight of the active ingredient.

Estimate of Guazatine Acceptable Daily Intake (ADI) for man:[16]

0 - 0.03 mg active ingredient as acetate/kg body weight.

One of the most important aspects of the understanding of the fate of pesticides in the environment is a knowledge of the degradation mechanisms for the compound in question[22].

By analogy with the catabolism of natural guanidated compounds, a degradative pathway for a guanidated amine as exemplified by guazatine was proposed by Bjork and Siirala-Hansen[23], and is shown in Figure 3.

In a study carried out in Japanese soils, Sato and co-workers showed that when guazatine was added to soil, it was immediatelly and strongly adsorbed to soil particles to become unavailable to micro-organisms and resistant to degradation. The strength of adsorption was comparable to that of bipyridylium herbicides, which are adsorbed by the soil inorganic fraction by a mechanism of ion exchange[24].

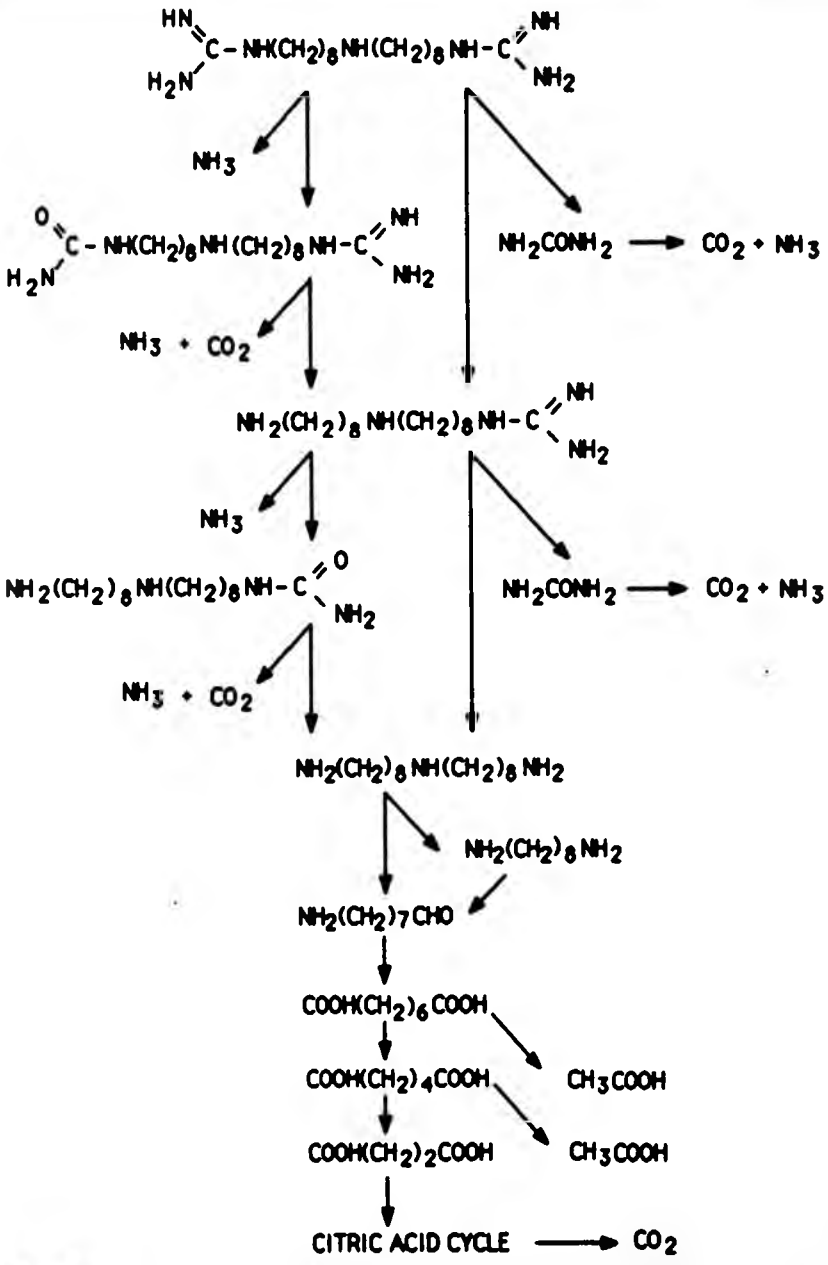


Figure 3. Proposed pathways for the degradation of guazatine[23].

Because of the strong adsorption of guazatine to soil particles, guazatine soil residues were defined as "soil bound residues" unavailable to plants even by rotational crops grown in actual agricultural soils[25]. A soil bound residue is "that unextractable and chemically unidentifiable pesticide residue remaining in fulvic acid, humic acid, and humin fractions after exhaustive sequential extraction with non-polar organic and polar solvents"[26].

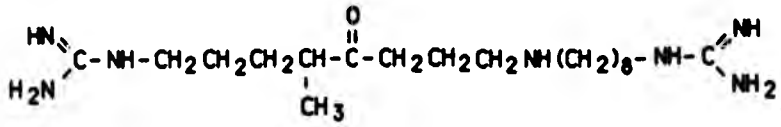
The addition of guanidine hydrochloride releases guazatine from the bound form[27].

Certain plant-components interfere with guazatine and might be the cause of its low recovery[28]. Fructose is a major sugar component of apple and grape and its carbonyl group and hydroxyl group at the C_1-position play an important role in the interaction with the guazatine molecule. A similar result was obtained with sorbose.

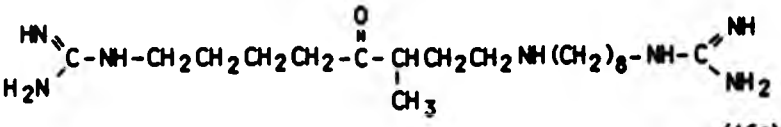
Little is known about the metabolic fate of guazatine in plants. The autoradiographic study of Sato and co-workers with apple fruits over a 12 weeks period following the application of ^{14}C-guazatine triacetate to the fruit-surface showed clearly that: (1) a major part of ^{14}C was retained on the surface, (2) the rate of ^{14}C-uptake was extremely slow, and (3) the order of ^{14}C-concentration in fruit tissue was peel > seeds >flesh > core. They concluded that photolysis was the sole significant transformation pathway of guazatine applied to the plant[29].

Sato *et al*, continuing their study, verified that the first step in the photolysis of guazatine is probably a photosensitized oxidation because guazatine itself absorbs no ultra-violet and visible light. The second step of the reaction (methylation), however, seemed quite extraordinary if phototransformation reactions were involved. It is an unclear feature and further studies are needed to account for the mechanisms of the reaction

and the source of the methyl moiety. They have proposed the major guazatine-photodegradation product (Pm) which was tentatively identified as 4 (or 6)-methyl-5-oxo-9-azaheptadecane-1,17-diguanidine (16 or 16a):



(16) or



(16a)

Pm would be significantly formed under naturally occurring environmental conditions, especially on plant surfaces[30].

Sato *et al*, also verified that the major effect in the dissipation of guazatine from plants was due to mechanical dislodgement by rain because of its low permeability into plant tissues as well as its highly water soluble nature[31].

3.3 - AGRICULTURAL ASPECTS

Some of the diseases partially or totally controlled by guazatine have been described,[14,32,33,34,35] and are as follows: *Alternaria citri, Alternaria herbarum, Alternaria kikuchiana, Botrytis cinerea, Calonectria nivalis, Ceratocystis paradoxa, Cladosporium herbarum, Cochliobolus*

myibeanus, Cochliobolus sativus, Colletotrichum coffeanum, Diplocarpon rosae, Erysiphe cichoracearum, Erysiphe graminis, Fusarium culmorum, Fusarium gramineum, Fusarium nivale, Fusarium oxysporum, Helminthosporium avenae, Helminthosporium gramineum, Leptosphaeria nodorum, Monilia mali, Penicillium digitatum, Penicillium italicum, Phytophthora infestans, Piricularia oryzae, Podosphaera leucotricha, Puccinia recondita, Pyrenophora avenae, Pyrenophora graminea, Pyrenophora teres, Pythium aphanidermatum, Rhizoctonia solani, Rhynchosporium secalis, Sclerotinia borealis, Septoria nodorum, Septoria tritici, Sphaerotheca pannosa, Tilletia caries, Typhula ishikariensis, Uromyces fabae, Ustilago avenae, Ustilago hordei, Ustilago levis, Ustilago nuda, Valsa ceratosperma, Venturia inaequalis.

3.4 - IDENTIFICATION AND ANALYSIS

The lack of suitable analytical methods has delayed attempts to identify guanidine compounds and their derivatives in various substrates.

Research on the guanidinium compounds and their methods of analysis has been stimulated by the discovery that they are involved in many metabolic processes, such as energy transfer, muscle metabolism, and kidney function[36].

Recently, several methods have been reported for the analysis of various guanidino compounds in biological fluids[37], including ion-exchange[36, 38-42], paper[43-46], and thin-layer chromatography[47], coupled with colorimetric determinations by either the Sakaguchi or Voges-Proskauer reactions and gas-liquid chromatography[48-54] after the formation of volatile derivatives.

The greater part of the work that has been done with guanidino compounds is in the medical area. Little is known about guanidino compound analysis in the agricultural area.

A recommended method for the analysis of guazatine at residue level is described by Kobayashi *et al.*[90,55]. Their method is based on the reaction of guazatine with hexafluoroacetylacetone (HFAA) and determination by Flame Thermionic Detector (FTD) Gas Chromatography. They analysed rice grain by this method and found that the lower limit of detection was 1 ng, corresponding to 0.05 ppm in 5 g of sample and the overall average recovery from the rice grain was *ca* 90%.

Lynch presented a method for the determination of guazatine residues in crops and soil. His method was based on the analysis of the parent triamine by Gas-Liquid Chromatography after hydrolysis of the guazatine in an autoclave[56].

Several methods have also been reported for the analysis of various guanidino compounds in biological fluids, including liquid chromatography with phenanthraquinone as a post-column fluorescent derivatization reagent[57-60], benzoin as a pre-column derivatization reagent[61] and ninhydrin as a post-column fluorescent derivatization reagent[62,63].

Mori *et al.* developed a high performance liquid chromatographic (HPLC) method for the fluorimetric determination of guazatine residues in various crops. Their method was based on guazatine reaction with ninhydrin as a post-column derivatization reagent and fluorescent measurement. The lower limit of detection was 10 ng, corresponding to 0.02 ppm in 25 g of crop. The mean recoveries of guazatine from the crops at 0.2-10 ppm level ranged between 79.2 and 99.3%[64].

The screening for pesticide residues in foods should unquestionably be carried out in the first instance using relatively inexpensive chromato-

graphic techniques. However, the difficulties of quantification of individual members of complex pesticide mixtures present at low levels in often intractable food matrices should not be understimated, and if positive residue data are to be put to meaningful use then rigorous confirmation of results is essential[65].

As stated before (See p. 23), guazatine is a mixture of numerous components which are difficult to separate. If we wish to identify its components at residue level new techniques must be used in order to get satisfactory results.

One technique that has recently been increasing in importance for the analysis of polar compounds without conversion to volatile derivatives, necessary for electron impact mass spectrometry, is Fast Atom Bombardment (FAB)[66-69], which allows the direct analysis of such compounds and offers the prospect of quantitative analysis without sample pretreatment[70]. Certain guanidino compounds have already been analysed by Fast Atom Bombardment Mass Spectrometry[71-74].

Nuclear Magnetic Resonance (NMR) spectroscopy has also been employed in studies with pesticides although it is not as suitable for identification at low levels of concentration as is mass spectrometry[75].

In our work with guazatine and its numerous components some of these techniques have been employed in order to elucidate the components present in the mixture and to develop a suitable method for their analysis.

III- EXPERIMENTAL

1- TECHNICAL TRIAMINE

Technical triamine was received from KemoGard AB, Stockholm, and was analytically evaluated and distilled in order to obtain pure diamine and triamine, which were used in preparation of guanidine compounds.

1.1- Evaluation of technical triamine:

A Pye Unicam GCD chromatograph equipped with a flame ionization detector (FID) and fitted with a 180 cm x 4 mm (ID) glass column packed with 5% Apiezon L/6% sodium salt of dimethylaminosuccinamic acid/1% sodium hydroxide on 40-60 mesh Chromosorb W AW was used to analyse technical triamine, diamine and triamine fractions. The temperature of injector, detector and column were 260^{0}C, 300^{0}C and 140^{0}C (for diamine)/230^{0}C (for triamine), respectively. Nitrogen was used as a carrier gas with an inlet pressure of 14 p.s.i..

A Varian 5000 Liquid Chromatograph equipped with an ultraviolet detector at 254 nm and fitted with a partisil 10 µm PAC (Pye Unicam) column was also used to analyse technical triamine, diamine and triamine fractions. The procedure involved the use of dansyl chloride as pre-column derivatization agent[76]. A mixture of hexane and ethanol (9:1) was used as

mobile phase at a flow rate of 1.0 ml/min..

Results from GLC and HPLC showed that the contents of diamine and triamine in technical triamine were in accord with the expected composition, i.e. 30 ± 5% and 40 ± 5%, respectively.

1.2- Distillation of technical triamine:

The distillation of technical triamine was done under reduced pressure. into a 500 ml round bottom flask about 300 grams of technical triamine was added. Another 250 ml round flask was connected to the first through an air-cooled distillation system in order to collect the distilled fractions. A Liebig condenser and vacuum pump were connected to the collector flask, which was placed in an ice/water-bath to cool the fractions. When the first fraction (diamine), b.p. 60-80^{0}C at 0.1 mm Hg was collected, the flask was changed to collect the second fraction (triamine), b.p. 150-170^{0}C at 0.1 mm Hg. The residual fraction (polyamine) was left in the first flask.

Results from gas-liquid chromatography showed that the distilled diamine and triamine fractions were quite pure.

Both fractions were evaluated: diamine, m.p. 43-4^{0}C (*cf.* commercial diamine, m.p. 44-45^{0}C, lit.[77] 52^{0}C), ^{13}C NMR (D_2O): δ, 28.9, 31.4, 34.5, 43.5; FAB/MS: m/z (%), 145 (MH^+,100.0), 128 (16.3), 93 (34.2), 69 (43.2), 58 (15.8), 43 (30.5), 41 (34.7), 30 (35.8). Triamine, m.p. 48-9^{0}C (Found: C, 70.9; H, 13.0; N, 14.5. Calc. for $C_{16}H_{37}N_3$: C, 70.9; H, 13.7; N, 15.5%), ^{13}C NMR (CD_3OD): δ, 27.8, 28.2, 30.4, 33.8, 42.4, 50.6; FAB/MS: m/z (%), 272 (MH^+, 100.0), 255 (7.8), 241 (4.4), 227 (3.2), 213 (3.5), 199 (2.4), 171 (14.8), 157 (20.7), 143 (2.4), 126 (13.8), 112 (7.5), 98 (8.4), 84 (11.7), 70

(12.5), 56 (14.4).

Diamine and triamine sulphates were prepared by adding an equivalent of dilute sulphuric acid to the respective pure amines, followed by rotary evaporation at 50°C and recrystallization with water to obtain the salts.

2- PREPARATION OF THE SALTS OF 1,8-BISGUANIDINO-OCTANE

2.1- Preparation of 1,8-diguanidino-octane diacetate (66-II)

1,8-Diamino-octane (5.0 g, 34.7 mmol; 69.4 meqv) in water (6.8 ml) was neutralized to *ca* 75% using glacial acetic acid (3.1 ml, 51.7 mmol) and the mixture was heated to 75°C. A 50% aqueous solution of cyanamide (2.92 g, 69.4 mmol; 69.4 meqv; in 2.9 ml water) was added dropwise during 3 hours. After an additional reaction time of 1 hour, acetic acid (*ca* 2.1 ml) was added to give a solution of the guanidated amine acetate.

The aqueous solution was evaporated by rotary evaporator and oil vacuum pump at 100°C. The resultant product was recrystallized from a mixture of methanol and acetone (2:1) (*ca* 60 ml), filtered off and dried in a vacuum oven at 65°C to yield 1,8-diguanidino-octane diacetate (2.45 g, 20.3%) as a fine white crystalline solid, m.p. 190-2°C (lit.[78] 202-205°C). (Found: C,48.1; H, 9.2; N, 23.9. Calc. for $C_{14}H_{30}N_6O_4$: C, 48.3; H, 9.2; N, 24.1%); ^{13}C NMR (D_2O/H_2SO_4): δ, 23.5, 28.6, 30.6, 30.9, 44.5, 159.3 [$NH_2\underline{C}(:NH_2^+)NH$], 182.8($CH_3\underline{C}O_2^-$); FAB/MS: m/z (%), 229 (MH^+,100.0), 212 (9.0), 187 (14.1), 172 (7.2), 170 (18.8), 156 (15.1), 142 (9.5), 128 (8.8), 114 (7.3), 100 (8.2), 86 (12.6), 73 (18.6), 59 (12.7).

2.2- Preparation of 1,8-diguanidino-octane sulphate (88-S)

This method followed the same procedure as that described by Brown *et al*[78].

1,8-Diamino-octane (12.3 g, 85.5 mmol) and *S*-methylisothiouronium sulphate (23.8 g, 85.5 mmol) were dissolved in water (50 ml) and heated under reflux for 90 minutes, during which time the methanethiol evolved was collected in potassium permanganate traps. The mixture was then cooled in an ice-bath and washed with 50% aqueous ethanol (*ca.* 30 ml).

The crude product was recrystallized from 50% aqueous ethanol (*ca.* 100 ml) and was dried in a vacuum oven at 60°C to yield 1,8-diguanidino-octane sulphate (17.3 g, 62.2%) as a fine white crystalline solid, m.p. 291-3°C, (Found: C, 36.0; H, 7.9; N, 25.5. Calc. for $C_{10}H_{26}N_6O_4S$: C, 36.8; H, 8.0; N, 25.8%); single peak on HPLC equipped with a differential refractometer detector and a LKB TSK, CM-2SW column; ^{13}C NMR (D_2O): &, 28.5, 30.7, 30.9, 44.1, 159.9 [$NH_2C(:NH_2^+)NH$]; FAB/MS: m/z(%), 327 (51.5), 229 (MH^+,100.0), 212 (10.3), 187 (11.9), 172 (8.5), 170 (20.7), 156 (17.4), 142 (9.2), 128 (8.9), 114 (7.5), 100 (7.2), 86 (9.8), 73 (13.9), 59 (9.3).

2.3- Conversion of 88-S to 1,8-diguanidino-octane carbonate (88-C)

1,8-Diguanidino-octane sulphate (3.3 g, 10 mmol) and sodium carbonate decahydrate (5.7 g, 20 mmol) were dissolved in water (40 ml) and gently heated (55-60°C) under reflux for 30 minutes. The mixture was then cooled in an ice-bath and the colourless solid formed was filtered off, washed with water and dried in a vacuum oven at 60°C. The product was recrystallized from water to give the product in the form of

colourless needles (0.63 g, 21.5%) m.p. 165-70°C (lit.[78] m.p. 172-75°C).

2.4- Conversion of 88-C to 1,8-diguanidino-octane diacetate (88-A)

1,8-Diguanidino-octane carbonate (3.3 g, 12 mmol) and 5% acetic acid (28 ml) were mixed and heated for 15 minutes. The mixture was then cooled and evaporated to dryness in the rotary evaporator at *ca* 60°C and washed with the minimum amount of water (5 ml) and finally with anhydrous diethyl ether (5 ml).

The pure product was dried in a vacuum oven at 60°C to yield 1,8-diguanidino-octane diacetate (3.6 g, 85.7%) as a fine white crystalline solid, m.p. 190-6°C.

3- PREPARATION OF MONOACETYL DERIVATIVES

3.1- Preparation of mono-*N*-acetyl-1,8-diamino-octane

1,8-Diamino-octane (57.6 g, 0.4 mol), ethyl acetate (19.6 ml, 0.2 mol), and ethyl alcohol (20 ml) were heated under reflux for 16 hours at 105-110°C. Solvent (18 ml) was removed on the steam bath and the remainder was fractionated under reduced pressure with a nitrogen bleed (1 mm pressure).

The fraction b.p. 160-176°C/1 mm Hg was collected and refractionated. The monoacetyl diamine boiled at 160-165°C/1 mm Hg (10.9 g, 14.7%) and collected as a white sticky and hygroscopic compound, m.p. 93-99°C (Found: C, 62.0; H, 12.0; N,13.5. Calc. for $C_{10}H_{22}N_2O$: C, 64.5; H, 11.9; N,

15.0%), single peak on GLC; ^{13}C NMR (D_2O): δ, 24.6, 31.2, 32.4, 33.7, 42.3, 43.3, 176.5 ($CH_3\underline{C}ONH$); EI/MS: m/z (%), 187.18048 (MH^+, 35.3). Calc. for $C_{10}H_{23}N_2O$, 187.18104, 186.17162 (M, 2.3), 170.15480 (3.2), 157.14731 (19.9), 142.12308 (8.7), 128.10655 (2.4), 114.09158 (21.6), 100.07623 (16.9), 86.06093 (38.2), 73.05313 (100.0), 55.0576 (24.4).

3.2- Preparation of 1-acetamido-8-guanidino-octane sulphate

Mono-*N*-acetyl-1,8-diamino-octane (1.31 g, 7.04 mmol) and *S*-methylisothiouronium sulphate (0.98 g, 3.52 mmol) were dissolved in water (2.9 ml) and heated under reflux for 90 minutes, during which time the methanethiol evolved was collected in potassium permanganate traps. The mixture was then cooled in an ice bath and the total solution showed the presence of the amidinated compound by ^{13}C NMR analysis (D_2O): δ, 24.7, 28.5, 31.0, 33.1, 42.3, 44.1, 159.7 [$NH_2\underline{C}(:NH_2^+)NH$], 176.6 ($CH_3\underline{C}ONH$).

3.3- Preparation of 1-acetamido-8-guanidino-octane acetate

Mono-*N*-acetyl-1,8-diamino-octane (2.0 g, 10.7 mmol) in water (2.1 ml) was neutralized to *ca* 75% using glacial acetic acid (0.96 ml, 16 mmol) and the mixture was heated to 75°C. A 50% aqueous solution of cyanamide (0 448 g, 10.7 mmol; 10.7 meqv; in 0.47 ml water) was added dropwise during 3 hours. After an additional reaction time of 1 hour, acetic acid (*ca* 0.64 ml) was added to give a solution which showed the presence of the amidinated compound by ^{13}C NMR analysis (CD_3OD): δ, 22.6, 27.7, 28.4, 29.9, 30.1, 40.4, 42.3, 158.7 [$NH_2\underline{C}(:NH_2^+)NH$], 173.3 ($CH_3\underline{C}ONH$), 179.3

($CH_3\underline{C}O_2^-$).

3.4- Attempted conversion of 1-acetamido-8-guanidino-octane acetate to 1-amino-8-guanidino-octane sulphate (GN-8)

1-Acetamido-8-guanidino-octane acetate from the previous preparation (3.3) was heated with 65% sulphuric acid (10 ml) under reflux for two hours at 100°C. The resultant product was evaporated under reduced pressure to give a more concentrated solution; ^{13}C NMR (D_2O/H_2SO_4): δ, 27.8, 28.1, 29.1, 29.5, 30.3, 42.6, 44.4, 159.1 [$NH_2\underline{C}(:NH_2^+)NH$], 164.3 ($NH_2\underline{C}ONH$), 177.9 ($CH_3\underline{C}O_2H$).

4- PREPARATION OF GUANIDATED TRIAMINE ACETATES AND GORGETTINE

The relative proportion of each reagent described in the procedures below is indicated in brackets, where the first figure represents the molecular proportion of the amino compound and the second figure the molecular proportion of the guanidating reagent.

4.1- The technical triamine by the cyanamide method (1:3)

Technical triamine (15.0 g, 55 mmol, calculated as pure triamine) in water (10 ml) was neutralized to *ca* 75% using glacial acetic acid (*ca* 7.0 ml) and the mixture was heated to 75°C. A 50% aqueous solution of cyanamide (7.0 g, 167 mmol; 167 meqv; in 7.0 ml water) was added drop

wise during 3 hours. After an additional reaction time of 1 hour, acetic acid (*ca* 1.9 ml) was added to give a solution of the guanidated triamine acetates (GTA) as the reaction product, ^{13}C NMR (D_2O): δ, 26.0, 28.3, 29.2, 29.4, 30.4, 30.6, 30.7, 42.2, 43.7, 50.0, 51.3, 122.6 [NHC(NH$_2$)NHCN], 158.4 [NH$_2$C(:NH$_2^+$)N], 159.5 [NH$_2$C(NH$_2^+$)NH], 165.8 [NHC(NH$_2$)NHCN], 183.6 ($CH_3CO_2^-$).

Table 1 (See p. 32) presents some possible compounds obtained through this reaction.

4.2- Bis pure triamine

Pure triamine was used as starting material instead of technical triamine and was amidinated to produce guanidated triamine compounds and guazatine, as follows.

4.2.1- Cyanamide method (2:1)

Bis-(8-amino-octyl)amine (0.5 g, 1.85 mmol; 5.5 meqv) in water (0.375 ml) was neutralized to *ca* 75% using glacial acetic acid (0.25 ml; 4.2 mmol) and the mixture was heated to 75°C. A 50% aqueous solution of cyanamide (0.039 g, 0.93 mmol; 0.93 meqv; in 0.04 ml water) was added dropwise during 3 hours. After an additional reaction time of 1 hour, acetic acid (*ca* 0.025 ml) was added to give a solution of the guanidated triamine acetates as the reaction product; ^{13}C NMR (D_2O): δ, 26.3, 28.3, 29.7, 30.7, 31.1, 31.3, 31.6, 42.3, 43.9, 50.2, 51.6, 158.6 [NH$_2$C(:NH$_2^+$)N], 159.8 [NH$_2$C(:NH$_2^+$)NH], 183.7 ($CH_3CO_2^-$).

4.2.2- Cyanamide method (1:1)

Bis-(8-amino-octyl)amine (2.0 g, 7.4 mmol; 22 meqv) in water (1.5 ml) was neutralized to *ca* 75% using glacial acetic acid (1.0 ml; 16.7 mmol) and the mixture was heated to 75°C. A 50% aqueous solution of cyanamide (0.313 g, 7.4 mmol; 7.4 meqv; in 0.35 ml water) was added dropwise during 3 hours. After an additional reaction time of 1 hour, acetic acid (*ca* 0.10 ml) was added to give a solution of the guanidated triamine acetates as the reaction product; ^{13}C NMR (D_2O): δ, 26.1, 28.3, 28.7, 29.3, 29.9, 30.7, 31.0, 42.5, 43.9, 50.3, 51.5, 158.5 [$NH_2\underline{C}(:NH_2^+)N$], 159.7 [$NH_2\underline{C}(:NH_2^+)NH$], 184.0 ($CH_3\underline{C}O_2^-$).

4.2.3- Cyanamide method (1:2)

Bis-(8-amino-octyl)amine (2.0 g, 7.4 mmol; 22 meqv) in water (1.5 ml) was neutralized to *ca* 75% using glacial acetic acid (1.0 ml; 16.7 mmol) and the mixture was heated to 75°C. A 50% aqueous solution of cyanamide (0.626 g, 14.8 mmol; 14.8 meqv; in 0.70 ml water) was added dropwise during 3 hours. After an additional reaction time of 1 hour, acetic acid (*ca* 0.10 ml) was added to give a solution of the guanidated triamine acetates as the reaction product; ^{13}C NMR (D_2O): δ, 26.1, 28.4, 29.3, 30.0, 30.6, 30.9, 31.0, 42.5, 44.0, 50.3, 51.5, 158.6 [$NH_2\underline{C}(:NH_2^+)N$], 159.7 [$NH_2\underline{C}(:NH_2^+)NH$], 184.0 ($CH_3\underline{C}O_2^-$).

4.2.4- Cyanamide method (1:3)

Bis-(8-amino-octyl)amine (2.0 g, 7.4 mmol; 22 meqv) in water (1.5 ml) was neutralized to *ca* 75% using glacial acetic acid (1.0 ml; 16.7 mmol) and the mixture was heated to 75^{0}C. A 50% aqueous solution of cyanamide (0.94 g, 22.4 mmol; 22.4 meqv; in 1.0 ml water) was added dropwise during 3 hours. After an additional reaction time of 1 hour, acetic acid (*ca* 0.25 ml) was added to give a solution of the guanidated triamine acetates as the reaction product; ^{13}C NMR (D_2O): δ, 26.0, 28.3, 29.3, 30.5, 30.9, 42.1, 43.7, 50.0, 51.3, 158.4 [$NH_2\underline{C}(:NH_2^+)N$], 159.5 [$NH_2\underline{C}(:NH_2^+)NH$], 183.4 ($CH_3\underline{C}O_2^-$).

4.2.5- Cyanamide method (1:6)

Bis-(8-amino-octyl)amine (2.0 g, 7.4 mmol; 22 meqv) in water (1.5 ml) was neutralized to *ca* 75% using glacial acetic acid (1.0 ml; 16.7 mmol) and the mixture was heated to 75^{0}C. A 50% aqueous solution of cyanamide (1.89 g, 44.8 mmol; 44.8 meqv; in 1.9 ml water) was added dropwise during 3 hours. After an additional reaction time of 1 hour, acetic acid (*ca* 0.25 ml) was added to give a solution of the guanidated triamine acetates as the reaction product; ^{13}C NMR (D_2O): δ, 26.0, 28.4, 29.3, 30.5, 30.9, 42.0, 43.7, 51.3, 122.4 [$NH\underline{C}(NH_2)NHCN$], 158.3 [$NH_2\underline{C}(:NH_2^+)N$], 159.4 [$NH_2\underline{C}(:NH_2^+)NH$], 165.6 [$NHC(NH_2)NH\underline{C}N$], 183.2 ($CH_3\underline{C}O_2^-$); FAB/MS (after rotary evaporation to remove water): m/z (%) 398 ([$NH_2C(:NH_2^+)NH(CH_2)_8NC(:NH)NH_2(CH_2)_8NHC(:NH)NH_2$], 45) or ([$NH_2C(:NH)NH(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_8NHC(:NH)NH_2$], 45), 381 (4), 356 ([$NH_2C(:NH_2^+)NH(CH_2)_8NH(CH_2)_8NHC(:NH)NH_2$], 79) or

([$NH_2C(:NH_2^+)NH(CH_2)_8NC(:NH)NH_2(CH_2)_8NH_2$], 79] or [[$NH_2C(:NH)NH(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_8NH_2$], 79], 341 (12), 339 (21), 327 (16), 325 (13), 324 (8), 322 (7), 314 [[$NH_2C(:NH_2^+)NH(CH_2)_8NH(CH_2)_8NH_2$], 37] or [[$NH_2(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_8NH_2$], 37], 311 (17), 310 (28), 308 (9), 299 (14), 297 (30), 294 (6), 285 (20), 284 (23), 282 (12), 280 (6), 269 (17), 268 (13), 266 (8), 255 (13), 254 (10), 252 (8), 241 (10), 240 (7), 238 (8), 227 (8), 226 (10), 224 (8), 213 (16), 212 (13), 210 (10), 199 (15), 198 (12), 196 (10), 185 (34), 182 (12), 170 (70), 168 (24), 156 (47), 154 (14), 142 (38), 140 (14), 128 (48), 126 (16), 114 (36), 112 (14), 100 (35), 86 (57), 73 (88), 55 (100).

4.2.6- Cyanamide method (1:12)

Bis-(8-amino-octyl)amine (0.5 g, 1.85 mmol; 5.5 meqv) in water (0.375 ml) was neutralized to *ca* 75% using glacial acetic acid (0.25 ml; 4.2 mmol) and the mixture was heated to 75°C. A 50% aqueous solution of cyanamide (0.939 g, 22.35 mmol; 22.35 meqv; in 1.0 ml water) was added dropwise during 3 hours. After an additional reaction time of 1 hour, acetic acid (*ca* 0.025 ml) was added to give a solution of the guanidated triamine acetates as the reaction product; ^{13}C NMR (D_2O): δ, 26.1, 28.9, 29.7, 31.0, 31.3, 44.2, 51.8, 118.7 (NH_2CN), 123.0 [$NHC(NH_2)NHCN$], 158.7 [$NH_2C(:NH_2^+)N$], 159.8 [$NH_2C(:NH_2^+)NH$], 166.1 [$NHC(NH_2)NHCN$], 183.6 ($CH_3CO_2^-$).

4.2.7- *S*-Methylisothiouronium sulphate method

4.2.7.1- Preparation of bis-(8-guanidino-octyl)amine sesquisulphate (GNG-S) (1:1)

This method followed the same procedure as that described by Hudson *et al*.[13].

Bis-(8-amino-octyl)amine (20.4 g, 75.3 mmol), *S*-methylisothiouronium sulphate (22.7 g, 81.7 mmol), and water (43 ml) were heated under reflux for 1 hour whilst methanethiol which was evolved was collected in potassium permanganate traps. Sulphuric acid (25 ml, 3 N) was then added to the cooled mixture to give a first crop of the sesquisulphate which was washed with 50% aqueous ethanol before drying. Concentration of the mother liquor yielded a second crop. The two crops were combined (11.8 g, 15.6%), m.p. 232-8^{0}C (Found: C, 39.5; H, 8.9; N, 18.1. Calc. for the pentahydrate [$(GNG)_2$, $3H_2SO_4$, $5H_2O$], $C_{36}H_{98}N_{14}O_{17}S_3$: C, 39.5; H, 9.0; N, 17.9%); ^{13}C NMR (D_2O): δ, 28.3, 28.5, 30.7, 30.9, 44.0, 50.4, 159.8 [$NH_2C(NH_2^+)NH$]; FAB/MS: m/z (%), 454 (23), 398 (2), 368 (2), 356 [$[NH_2C(:NH_2^+)NH(CH_2)_8NH(CH_2)_8NHC(:NH)NH_2]$,100.0], 339 (7), 314 (14), 299, (19), 297 (15), 283 (10), 269 (7), 255 (7), 241 (5), 227 (4), 213 (5), 199 (13), 185 (19), 170 (31), 156 (21), 142 (13), 128 (17), 114 (14), 100 (15), 86 (18).

4.2.7.2- Conversion of GNG-S to bis-(8-guanidino-octyl)amine sesquicarbonate (GNG-C)

The sesquisulphate of guazatine (5.9 g, 5.7 mmol) was dissolved in hot

water (26 ml) and treated with a hot solution of sodium carbonate decahydrate (8.9 g, 31.1 mmol) in water (26 ml) to yield a precipitate which was washed with water and then dried in a desiccator to yield the sesquicarbonate (4.5 g, 85.4%), m.p. 131-4^{0}C (Found: C, 51.3; H, 10.8; N, 22.2. Calc. for $C_{39}H_{88}N_{14}O_9$: C, 52.2; H, 9.9; N, 21.9%); FAB/MS: m/z (%) 454 (6.1), 398 (10.0), 380 (3.2), 368 (57.2), 356 ([$NH_2C(:NH_2^+)NH(CH_2)_8NH(CH_2)_8NHC(:NH)NH_2$], 100.0), 339 (11.5), 314 (42.4), 299 (10.8), 297 (21.8), 283 (13.8), 269 (8.6), 255 (10.0), 241 (7.1), 227 (7.8), 213 (12.7), 199 (37.6), 170 (34.3), 156 (32.7), 142 (21.6), 128 (31.9), 114 (28.2), 100 (34.2), 86 (50.7).

4.2.7.3- Conversion of GNB-C to bis-(8-guanidino-octyl)amine triacetate (GNB-8)

The sesquicarbonate of guazatine (0.70 g, 0.78 mmol) was dissolved in aqueous acetic acid (14 ml, 4.7 mmol; 2% w/v) to give a 6% w/v solution of bis-(8-guanidino-octyl)amine triacetate which was analysed by ^{13}C NMR (D_2O): δ, 26.4, 28.3, 28.6, 31.0, 32.0, 44.1, 50.4, 159.9 [$NH_2\underline{C}(NH_2^+)NH$], 184.0 ($CH_3\underline{C}O_2^-$).

This conversion was repeated twice more in order to obtain the pure solid triacetate through two different procedures of evaporation to dryness.

In the first procedure, the sesquicarbonate of guazatine (1.0 g, 1.11 mmol) was dissolved in aqueous acetic acid (20 ml, 6.7 mmol; 2% w/v) to give a 6% w/v solution of bis-(8-guanidino-octyl)amine triacetate which was left standing for about 4 months, after which it was evaporated under vacuum at 100^{0}C to obtain the pure solid compound (0.97 g, 81.0%), m.p.

140-5^{0}C (Found: C, 54.2; H, 10.2; N, 18.5. Calc. for $C_{24}H_{53}N_7O_6$: C, 53.8; H, 9.9; N, 18.3%); ^{13}C NMR (D_2O): δ, 26.3, 28.4, 28.6, 30.7, 31.0, 44.2, 50.5, 159.9 [$NH_2\underline{C}(NH_2^+)NH$], 184.3 ($CH_3\underline{C}O_2^-$); FAB/MS: m/z (%), 454 (2.5), 398 (7.6), 368 (100.0), 356 [$[NH_2C(:NH_2^+)NH(CH_2)_8NH(CH_2)_8NHC(:NH)NH_2]$, 66.6), 339 (7.5), 314 (5.4), 299 (2.4), 297 (7.9), 283 (6.9), 269 (4.2), 255 (4.2), 241 (4.2), 227 (3.9), 213 (7.0), 199 (25.7), 170 (22.1), 156 (17.2), 142 (10.8), 128 (17.3), 114 (13.0), 100 (15.8), 86 (23.4).

In the second procedure, the sesquicarbonate of guazatine (0.30 g, 0.33 mmol) was dissolved in aqueous acetic acid (6 ml, 2.0 mmol; 2% w/v) to give a 6% w/v solution of bis-(8-guanidino-octyl)amine triacetate which was filtered off and then evaporated to dryness under vacuum (oil pump) on a mechanical shaker but without heating. The product was further dried in a vacuum desiccator to leave a viscous residue which was recrystallized from methanol and sodium-dried diethyl ether to give the pure solid compound (0.173 g, 48.3%), m.p. 139-141^{0}C (Found: C, 51.4; H,9.5; N, 17.6. Calc. for $C_{24}H_{53}N_7O_6$. 1.3 H_2O: C, 51.6; H, 10.0; N, 17.6%); ^{13}C NMR (D_2O): δ, 26.1, 28.2, 28.5, 30.6, 30.8, 44.0, 50.3, 159.7 [$NH_2\underline{C}(NH_2^+)NH$], 184.2 ($CH_3\underline{C}O_2^-$); FAB/MS: m/z (%), 454 (3.8), 398 (5.9), 368 (45.0), 356 [$[NH_2C(:NH_2^+)NH(CH_2)_8NH(CH_2)_8NHC(:NH)NH_2]$, 100.0), 339 (7.2), 314 (8.6), 299 (4.0), 297 (10.4), 283 (6.4), 269 (4.6), 255 (5.4), 241 (4.2), 227 (3.8), 213 (6.9), 199 (19.3), 185 (18.1), 170 (24.3), 156 (18.5), 142 (11.7), 128 (16.3), 114 (14.6), 100 (18.1), 86 (27.6).

4.2.7.4- Conversion of BNS-8 to bis-(8-guanidino-octyl)amine triacetate (BNS-A) through the barium acetate method

The sesquisulphate of guazatine (1.0 g, 0.96 mmol) was dissolved in

water (8 ml) and treated with a solution of barium acetate (0.763 g, 3.0 mmol) in 2 ml water at room temperature for 24 hours. The mixture was filtered off and washed with water (*ca* 8 ml). The filtrate was then evaporated to dryness under vacuum (oil pump) on a mechanical shaker but without heating. The product was recrystallized from methanol and sodium-dried diethyl ether to give a solid (0.354 g, 33.2%), m.p. 137-9^{0}C (Found: C, 48.7; H, 9.5; N, 18.2%); ^{13}C NMR (D_2O): δ, 26.3, 28.4, 28.6, 30.7, 30.9, 44.1, 50.4, 159.9 [$NH_2\underline{C}(NH_2^+)NH$], 184.3 ($CH_3\underline{C}O_2^-$); FAB/MS: m/z (%), 454 (27.5), 398 (7.1), 368 (33.9), 356 ([$[NH_2C(:NH_2^+)NH(CH_2)_8NH(CH_2)_8NHC(:NH)NH_2]$, 100.0], 339 (10.1), 314 (13.1), 299 (6.6), 297 (17.7), 283 (11.2), 269 (8.9), 255 (8.1), 241 (7.3), 227 (6.6), 213 (11.1), 199 (27.5), 185 (25.7), 170 (40.4), 156 (26.2), 142 (19.5), 128 (26.6), 114 (22.5), 100 (29.5), 86 (41.7). The FAB/MS results indicate that conversion of sulphate to acetate was not complete: Calc. for acetate (76%) plus sulphate (24%) both hydrated as above: C, 48.7; H, 9.8; N, 17.7%.

4.2.7.5 - Preparation of bis-(8-guanidino-octyl)amine sesquisulphate (BNS-8) (1:2)

Bis-(8-amino-octyl)amine (9.5 g, 35 mmol), *S*-methylisothiouronium sulphate (19.5 g, 70 mmol), and water (20 ml) were heated under reflux for 1 hour whilst methanethiol which was evolved was collected in potassium permanganate traps. Sulphuric acid (11.6 ml, 3 N) was then added to the cooled mixture to give a first crop of the sesquisulphate which was washed with 50% aqueous ethanol before drying. Concentration of the mother liquor yielded a second crop. The two crops were combined

(18.0 g, 47.5%), m.p. 105-30°C (Found: C, 41.6; H, 8.6. Calc. for the dihydrate $C_{36}H_{92}N_{14}O_{14}S_3$: C, 41.5; H, 8.8%); ^{13}C NMR (D_2O): δ, 15.7, 28.5, 30.8, 44.0, 50.2, 159.8 [$NH_2\underline{C}(NH_2^+)NH$].

4.2.8 - Preparation of bis-(8-guanidino-octyl)amine triacetate (GNG-8) through the *S*-methylisothiouronium acetate method

S-Methylisothiouronium acetate was initially prepared by reacting *S*-methylisothiouronium sulphate with barium acetate as follows:

S-Methylisothiouronium sulphate (28.4 g, 10.2 mmol) in water (155 ml) and barium acetate (26.1 g, 10.2 mmol) in water (125 ml) were mixed and stirred at room temperature for 24 hours. The barium sulphate produced was filtered off and was washed with water (50 ml) and dried in a vacuum oven at *ca* 100°C until constant weight (24.3 g, 99.3%).

The filtrate was evaporated to dryness under vacuum (oil pump) on a mechanical shaker but without heating to obtain the solid compound (24.3 g, 81.5%), m.p. 131-3°C. An quantity of this compound (*ca* 5 g) was recrystallized from methanol to give the pure compound (*ca* 2.64 g), m.p. 132°C (Found: C, 32.0; H, 6.5; N, 18.7. Calc. for $C_4H_{10}N_2O_2S$: C, 32.0; H, 6.7; N, 18.7%); ^{13}C NMR (D_2O): δ, 15.8 [$\underline{C}H_3SC(:NH_2^+)NH_2$], 26.2 ($\underline{C}H_3CO_2^-$), 175.8 [$CH_3S\underline{C}(:NH_2^+)NH_2$], 184.3 ($CH_3\underline{C}O_2^-$); FAB/MS: m/z (%), 183 (MH^+ + glycerol, 13.1), 181 ($2M+H^+$, 12.00, 91 (MH^+, 100.0), which was used in the preparation of GNG-A as follows:

Bis-(8-amino-octyl)amine (1.0 g, 3.7 mmol), *S*-methylisothiouronium acetate (1.1 g, 7.4 mmol), and water (2 ml) were heated under reflux for 1 hour at 100°C, whilst methanethiol which was evolved was collected in potassium permanganate traps. Acetic acid (11 ml, 3.7 mmol; 2% w/v) was

then added to the cooled mixture to give the triacetate compound in solution which was evaporated to dryness under vacuum (oil pump) on a mechanical shaker but without heating to give the solid compound (1.4 g, 71.1%) m.p.123-28^{0}C; ^{13}C NMR (D_2O): δ, 26.1, 28.4, 28.5, 29.6, 30.9, 44.1, 50.4, 159.8 [$NH_2\underline{C}(NH_2^+)NH$], 184.1 ($CH_3\underline{C}O_2^-$); FAB/MS: m/z (%), 454 (2.3), 398 (11.1), 368 (68.2), 356 ([$NH_2C(:NH_2^+)NH(CH_2)_8NH(CH_2)_8NHC(:NH)NH_2$], 100.0), 339 (7.5), 314 (18.2), 299 (4.6), 297 (10.4), 283 (6.5), 269 (5.2), 255 (5.7), 241 (4.5), 227 (5.3), 213 (8.2), 199 (24.1), 170 (25.9), 156 (20.6), 142 (13.4), 128 (19.4), 114 (16.5), 100 (19.7), 86 (28.8).

4.2.9- Attempted preparation of 1,1-bis-(8-guanidino-octyl) guanidine triacetate (666-A)

Bis-(8-guanidino-octyl)amine sesquicarbonate (0.5 g, 0.56 mmol), prepared previously (See procedure 4.2.7.2, p. 53), in water (0.2 ml) was neutralized to *ca* 75% using glacial acetic acid (0.25 ml, 4.2 mmol) and the mixture was heated to 75^{0}C. A 50% aqueous solution of cyanamide (0.05 g, 1.2 mmol; in 0.05 ml water) was added dropwise during 3 hours. After an additional reaction time of 1 hour, acetic acid (*ca* 0.04 ml) was added to give a solution of the triguanidated triamine acetates as the reaction product which was analysed by ^{13}C NMR (D_2O): δ, 25.4, 28.6, 29.5, 30.8, 31.1, 31.3, 44.2, 50.5, 51.8, 158.8 [$NH_2\underline{C}(NH_2^+)N$], 159.9 [$NH_2\underline{C}(NH_2^+)NH$], 182.9 ($CH_3\underline{C}O_2^-$). The product could not be crystallized.

5 - PREPARATION OF DI-*N*-OCTYLGUANIDINIUM DERIVATIVES

5.1 - Preparation of di-*n*-octylguanidinium acetate by the cyanamide method

Di-*n*-octylamine (2.41 g, 10 mmol), in water (2.0 ml) was neutralized to *ca* 75% using glacial acetic acid (0.45 ml, 7.5 mmol) and the mixture was heated to 75°C. A 50% aqueous solution of cyanamide (0.43 g, 10 mmol; in 0.5 ml water) was added dropwise during 3 hours. After an additional reaction time of 1 hour, acetic acid (*ca* 0.30 ml) was added to give the acetate which was analysed by ^{13}C NMR (CD_3OD): δ, 14.3, 23.4, 27.0, 27.3, 28.3, 29.9, 32.7, 48.9, 52.1, 157.6, 178.9.

5.2 - Attempted preparation of di-*n*-octylguanidinium sulphate by the *S*-methylisothiouronium sulphate method

Di-*n*-octylamine (7.24 g, 30 mmol) and *S*-methylisothiouronium sulphate (4.45 g, 16 mmol) were dissolved in water (12.5 ml) and heated under reflux for 90 minutes at 100°C, during which time the methanethiol evolved was collected in potassium permanganate traps. The mixture was then cooled in an ice-bath to yield a precipitate which was washed with 50% aqueous ethanol to give di-*n*-octylammonium sulphate (3.09 g, 29.1%), which was analysed by ^{13}C NMR (CD_3OD): δ, 16.7, 25.3, 28.8, 31.6, 34.4, 52.1. No signal at 158-159 ppm was present. The product could not be crystallized.

6 - ACETYLACETONE (AA) DERIVATIZATION

Acetylacetone derivatives were obtained using the method of Palaitis and Curran[79]. Their procedure was repeated with guanidine hydrochloride (7.5 g, 7.9 mmol) being dissolved in sodium hydroxide solution (30 ml, 5N), and water (50 ml), methanol (100 ml), and acetylacetone (50 ml, 50 mmol) were added. Additional methanol was added to bring the volume of the resulting solution to approximately 250 ml. The solution was refluxed on a steam bath for *ca.* 4 hours and, after being allowed to cool to room temperature and being acidified with 1 N aqueous HCl solution, was extracted three times with 100 ml portions of chloroform. The combined extracts were evaporated to dryness and the residue, 2-amino-4,6-dimethylpyrimidine (32), was recrystallized from acetone and isolated as fine white crystals (3.06 g, 31.6%), m.p. 142^{0}C (Found: C, 58.4; H, 7.5; N, 34.1. Calc. for $C_6H_9N_3$: C, 58.5; H, 7.3; N, 34.2%); ^{13}C NMR ($CDCl_3$): δ, 23.7 ($\underline{C}H_3$), 110.5 ($\underline{C}H$), 163.2 ($\underline{C}NH_2$), and 167.9 ($\underline{C}CH_3$); EI/MS: m/z (%), 123 (M^+, 100), 108 (6.4), 96 (29.8), 95 (14.1), 83 (8.8), 82 (13.7), 81 (6.8),67 (11.6), and 66 (8.1).

6.1 - Iodine/acetylacetone derivative

An attempt was made to obtain a iodine/acetylacetone derivative and two trials were carried out differing from each other only in the time of reaction (4 hours and 20 hours). Both trials started with iodine (1.5 g, 5.2 mmol) with other reagents in the appropriate relative proportions according to the method of Palaitis and Curran[79].

After 4 hours of reaction was obtained the probable derivative (0.55 g,

36.2%), m.p. 52°C (Found: C, 59.5; H, 11.3; N, 16.4. Calc. for $C_{18}H_{33}N_3$: C, 74.2; H, 11.3; N, 14.4%). And after 20 hours of reaction the other probable derivative was also obtained (0.86 g, 56.6%), m.p. 50°C (Found: C,59.7; H, 11.1; N, 16.2. Calc. for $C_{18}H_{33}N_3$: C, 74.2; H, 11.3; N, 14.4%). The reason for the low figure obtained for carbon is unknown. Mass spectrometry, however, confirmed that the expected derivative was present in both product: m/z 291 (M^+, 12.8 and 12.5%, respectively), 207 (19), 192 (15.8), 178 (12.8), 150 (18.3), 149 (74.6), 137 (64.1), 136 (100.0), 123 (59.9), 108 (9.6), 83 (13.0), 69 (13.5), and 56 (38.5).

6.2 - 1,8-Diguanidino-octane sulphate/acetylacetone derivative

1,8-Diguanidino-octane sulphate (1.5 g, 4.6 mmol in a 250-ml round-bottom flask equipped with a water-cooled condenser), prepared previously (See procedure 2.2, p. 45), was dissolved in 30 ml of 1N aqueous sodium hydroxide solution, and 20 ml each of water and methanol, and 7 ml of acetylacetone (70 mmol), were added. Additional methanol was added to bring the volume of the resulting solution to approximately 100 ml. The solution was refluxed on a steam bath for *ca* 4 hours and after being acidified with 1N aqueous HCl solution, was extracted three times with 50 ml portions of chloroform. The combined extracts were evaporated to dryness and the residue was recrystallized from acetone to give the product (0.22 g, 13.4%), m.p. 105-110°C (Found: C, 67.6; H, 8.8; N, 23.1. Calc. for $C_{20}H_{32}N_6$: C, 67.4; H, 9.0; N, 23.6%), ^{13}C NMR (CD_3OD): δ, 23.8, 26.9, 29.3, 29.7, 41.4, 109.4 (CH), 162.2 [NHC(:N)N], 167.4 (CCH_3), EI/MS: m/z (%), 356 (M^+,26.5), 234 (14.6), 233 (7.5), 221 (23.1), 220 (43.2), 207 (11.0), 206 (17.0), 192 (23.4), 178 (18.2), 165 (5.6), 164

(12.2), 151 (16.1), 150 (58.8), 137 (56.4), 136 (100.0), 124 (24.2), 123 (66.6), 108 (13.1), 107 (17.8), 67 (18.5), and 55 (13.6).

7 - HEXAFLUOROACETYLACETONE (HFAA) DERIVATIZATION

Hexafluoroacetylacetone derivatives were obtained using the method of Kobayashi *et al*[55].

General Procedure:

Bis-(8-guanidino-octyl)amine triacetate (300 mg, 0.56 mmol) in a saturated sodium bicarbonate solution (5 ml) is heated with hexafluoroacetylacetone (3 ml, 21.2 mmol) in 60 ml of toluene at 100°C for 3 hours, followed by the addition of 20 ml of 5% sodium bicarbonate to hydrolyze the excess hexafluoroacetylacetone.

The organic layer is separated, washed with water (20 ml), dried over anhydrous sodium sulphate, and the solvent is evaporated under reduced pressure. The residue is recrystallized from n-hexane to afford the pure substituted pyrimidine derivative.

7.1 - 1,8-Diguanidino-octane sulphate/HFAA derivative (GS-S/HFAA)

1,8-Diguanidino-octane sulphate (300 mg, 0.92 mmol), prepared previously (See procedure 2.2, p. 45), in a saturated sodium bicarbonate solution (5 ml) was heated with hexafluoroacetylacetone (2.8 ml, 19.8 mmol) in 60 ml of toluene at 100°C for 3 hours, followed by the addition

of 20 ml of 5% sodium bicarbonate to hydrolyze the excess hexafluoroacetylacetone.

The organic layer was separated, washed with water (20 ml), dried over anhydrous sodium sulphate, and the solvent was evaporated under reduced pressure. The residue was recrystallized from n-hexane to afford the pure *N,N'-bis[4,6-bis(trifluoromethyl)pyrimidin-2-yl]-1,8-diaminooctane* (44) substituted pyrimidine derivative (195.6 mg, 37.2%), m.p. 89-90°C (Found: C, 42.2; H, 3.9; N, 15.8. Calc for $C_{20}H_{20}F_{12}N_6$: C, 42.0; H, 3.5; N, 14.7%). EI/MS: m/z (%), 572.15497 (M, 7.6). Calc. for $C_{20}H_{20}F_{12}N_6$: 572,15688, 552.14893 (28.3), $[M-HF]^+$, 532.14088 (35.6), $[M-2HF]^+$, 503.32562 (11.6), 340.12357 (8.8), 336.21640 (9.0), 270.04660 (7.0), 258.04611 (8.0), 245.03506 (15.2), 244.03053 (100.0), $[M-328]^+$, 224.02286 (5.5), 68.99132 (62.9).

7.2 - 1,8-Diguanidino-octane acetate/HFBB derivative (DG-8/HFBB)

1,8-Diguanidino-octane acetate (500 mg, 1.44 mmol), prepared previously (See procedure 2.1, p. 44), in a saturated sodium bicarbonate solution (5 ml) was heated with hexafluoroacetylacetone (5.1 ml, 36.0 mmol) in 100 ml of toluene at 100°C for 3 hours, followed by the addition of 30 ml of 5% sodium bicarbonate to hydrolyze the excess hexafluoroacetylacetone.

The organic layer was separated, washed with water (30 ml), dried over anhydrous sodium sulphate, and the solvent was evaporated under reduced pressure. The residue was recrystallized from n-hexane to afford the pure substituted pyrimidine derivative (45) (275.5 mg, 31.3%), m.p. 84-85°C; ^{13}C NMR (CD_3OD): δ, 27.9, 30.0, 30.3, 42.4, 100.5 ($\underline{C}F_3$), 100.7 ($\underline{C}H$),

159.4 [NHC(:N)N], 164.4 (CCF$_3$); EI/MS: m/z (%), 572 (M, 2.0), 552 (10.3), [M-HF]$^+$, 532 (15.6), [M-2HF]$^+$, 340 (4.1), 270 (4.3), 258 (7.3), 244 (100.0), [M-328]$^+$, 224 (7.8), 69 (11.4).

7.3 - Bis-(8-guanidino-octyl)amine triacetate/HFBB derivative (BNG-A/HFBB)

Bis-(8-guanidino-octyl)amine triacetate (500 mg, 0.93 mmol) prepared previously (See procedure 4.2.7.3: first procedure, p. 54), in a saturated sodium bicarbonate solution (5 ml) was heated with hexafluoroacetylacetone (3.3 ml, 23.3 mmol) in 100 ml of toluene at 100^{0}C for 3 hours, followed by the addition of 30 ml of 5% sodium bicarbonate to hydrolyze the excess hexafluoroacetylacetone.

The organic layer was separated, washed with water (30 ml), dried over anhydrous sodium sulphate, and the solvent was evaporated under reduced pressure. The residue was recrystallized from n-hexane to afford the pure *9-aza-1,17-bis[4,6-bis(tryfluoromethyl)pyrimidin-2-ylamino]-heptadecane* (46) substituted pyrimidine derivative (616.6 mg, 94.4%), m.p. 98-99^{0}C (lit[55] m.p. 105-6^{0}C); ^{13}C NMR (CD_3OD): &, 27.3, 27.6, 27.8, 28.6, 30.0, 31.1, 42.4, 50.2, 100.7 (CF$_3$), 128.5 (CH), 159.4 [NHC(:N)N], 164.4 (CCF$_3$); EI/MS: m/z (%), 699.29129 (2.2), [M$^+$, Cal. for $C_{28}H_{37}F_2N_7$: 699.29298] 659.27925 (3.5), [M-2HF]$^+$, 455.26233 (2.2), [M-244]$^+$, 372.17065 (19.1), [M-327]$^+$,371.16815 (100.0), [M-328]$^+$, 244.02769 (21.2), [M-455]$^+$.

7.4 - Bis-(8-guanidino-octyl)amine sesquicarbonate/HFAA derivative (GNG-C/HFAA)

Bis-(8-guanidino-octyl)amine sesquicarbonate, prepared previously (See procedure 4.2.7.2, p. 53), in a saturated sodium bicarbonate solution (5 ml) was heated with hexafluoroacetylacetone (3.0 ml, 21.2 mmol) in 60 ml of toluene at 100°C for 3 hours, followed by the addition of 20 ml of 5% sodium bicarbonate to hydrolyze the excess hexafluoroacetylacetone.

The organic layer was separated, washed with water (20 ml), dried over anhydrous sodium sulphate, and the solvent was evaporated under reduced pressure. The residue was recrystallized from n-hexane to afford the pure substituted pyrimidine derivative (47) (609 mg, 65.1%), (Found: C, 48.3; H, 5.5; N, 14.2. Calc for $C_{28}H_{37}F_{12}N_7$: C, 48.1; H, 5.3; N, 14.0%); ^{13}C NMR (CD_3OD): δ, 27.8, 28.0, 28.9, 30.0, 30.2, 30.3, 42.4, 50.2, 100.7 ($\underline{C}F_3$), 128.5 ($\underline{C}H$), 159.4 [NH$\underline{C}$(:N)N], 164.5 ($\underline{C}CF_3$); EI/MS: m/z (%), 698 (4.5), $[M-H]^+$, 659 (3.9), $[M-2HF]^+$, 371 (100.0), $[M-328]^+$, 244 (49.5), $[M-455]^+$.

7.5 - Guanidated triamine acetate/HFAA derivatives

Several derivatives were made using the products obtained from pure triamine amidinated with cyanamide in various molar ratios as previously described in procedures 4.2.1 to 4.2.6 (See pp. 49 to 52).

7.5.1 - Cyanamide product (2:1)/HFAA derivative

The resultant product from procedure 4.2.1 (300 mg) in a saturated

sodium bicarbonate solution (5 ml) was heated with HFAA (3.0 ml, 21.2 mmol) in 60 ml of toluene at 100°C for 3 hours, followed by the addition of 20 ml of 5% sodium bicarbonate to hydrolyze the excess HFAA.

The organic layer was separated, washed with water (20 ml), dried over anhydrous sodium sulphate and injected into the GLC (See Figure 46, p. 167).

7.5.2 - Cyanamide product (1:1)/HFBB derivative

The resultant product from procedure 4.2.2 (300 mg) in a saturated sodium bicarbonate solution (5 ml) was heated with HFAA (3.0 ml, 21.2 mmol) in 60 ml of toluene at 100°C for 3 hours, followed by the addition of 20 ml of 5% sodium bicarbonate to hydrolyze the excess HFAA.

The organic layer was separated, washed with water (20 ml), dried over anhydrous sodium sulphate and injected into the GLC (See Figure 47, p. 168).

An aliquot of this solution was taken, evaporated to dryness and diluted with dichloromethane to give a 10% solution and analysed by GC-MS in the Chemical Ionization mode (See Figure 52, p. 174).

7.5.3 - Cyanamide product (1:2)/HFBB derivative

The resultant product from procedure 4.2.3 (300 mg) in a saturated sodium bicarbonate solution (5 ml) was heated with HFAA (3.0 ml, 21.2 mmol) in 60 ml of toluene at 100°C for 3 hours, followed by the addition of 20 ml of 5% sodium bicarbonate to hydrolyze the excess HFAA.

The organic layer was separated, washed with water (20 ml), dried over anhydrous sodium sulphate and injected into the GLC (See Figure 48, p. 169).

7.5.4 - Cyanamide product (1:3)/HFAA derivative

The resultant product from procedure 4.2.4 (300 mg) in a saturated sodium bicarbonate solution (5 ml) was heated with HFAA (3.0 ml, 21.2 mmol) in 60 ml of toluene at 100^{0}C for 3 hours, followed by the addition of 20 ml of 5% sodium bicarbonate to hydrolyze the excess HFAA.

The organic layer was separated, washed with water (20 ml), dried over anhydrous sodium sulphate and injected into the GLC (See Figure 49, p. 170).

7.5.5 - Cyanamide product (1:6)/HFAA derivative

The resultant product from procedure 4.2.5 (300 mg) in a saturated sodium bicarbonate solution (5 ml) was heated with HFAA (3.0 ml, 21.2 mmol) in 60 ml of toluene at 100^{0}C for 3 hours, followed by the addition of 20 ml of 5% sodium bicarbonate to hydrolyze the excess HFAA.

The organic layer was separated, washed with water (20 ml), dried over anhydrous sodium sulphate and injected into the GLC (See Figure 50, p. 171).

7.5.6 - Cyanamide product (1:12)/HFBA derivative

The resultant product from procedure 4.2.6 (300 mg) in a saturated sodium bicarbonate solution (5 ml) was heated with HFAA (3.0 ml, 21.2 mmol) in 60 ml of toluene at 100°C for 3 hours, followed by the addition of 20 ml of 5% sodium bicarbonate to hydrolyze the excess HFAA.

The organic layer was separated, washed with water (20 ml), dried over anhydrous sodium sulphate and injected into the GLC (See Figure 51, p. 172).

A second preparation of this derivative was made with 500 mg of the same product from procedure 4.2.6, in order to isolate and obtain the pure substituted pyrimidine derivative (257.8 mg, 32.6%) m.p. 48-50°C; EI/MS: m/z (%), 585 (42.3), $[M-328]^+$, 371 (4.5), $[M-542]^+$, 258 (100.0), $[M-655]^+$, 244 (57.3), $[M-669]^+$.

7.6 - 1,1-Bis-(8-guanidino-octyl)guanidine triacetate/HFBA derivative (666-A/HFBA)

The resultant product from procedure 4.2.9, (See p. 58), (400 mg) in a saturated sodium bicarbonate solution (5 ml) was heated with HFAA (2.0 ml, 14 mmol) in 60 ml of toluene at 100°C for 3 hours, followed by the addition of 20 ml of 5% sodium bicarbonate to hydrolyze the excess HFAA.

The organic layer was separated, washed with water (20 ml), dried over anhydrous sodium sulphate and injected into the GLC (See Figure 53, p. 176). An aliquot of this solution was also analysed by GC-MS in the Chemical Ionization and Electron Impact modes (See Figure 54, p. 177).

7.7 - 1,8-Diamino-octane/HFAA derivative (NN/HFAA)

1,8-Diamino-octane (300 mg, 2.08 mmol) in a saturated sodium bicarbonate solution (5 ml) was heated with HFAA (6 ml, 42.4 mmol) in 60 ml of toluene at 100°C for 3 hours, followed by the addition of 20 ml of 5% sodium bicarbonate to hydrolyze the excess HFAA.

The organic layer was separated, washed with water (20 ml), dried over anhydrous sodium sulphate and injected into the GLC (See Figure 55, p. 178).

7.8 - Bis-(8-amino-octyl)amine/HFAA derivative (NNN-HFAA)

Bis-(8-amino-octyl)amine (300 mg, 1.11 mmol) in a saturated sodium bicarbonate solution (5 ml) was heated with HFAA (3.0 ml, 21.2 mmol) in 60 ml of toluene at 100°C for 3 hours, followed by the addition of 20 ml of 5% sodium bicarbonate to hydrolyze the excess HFAA.

The organic layer was separated, washed with water (20 ml), dried over anhydrous sodium sulphate and injected into the GLC (See Figure 56, p. 179).

7.9 - Commercial guazatine/HFAA derivative (GTA/HFAA)

Commercial guazatine 70% (1.0 ml, 700 mg active ingredient, 1.31 mmol based on GNG-A component) in a saturated sodium bicarbonate solution (10 ml) was heated with HFAA (3.7 ml, 26.15 mmol) in 120 ml of toluene at 100°C for 3 hours, followed by the addition of 40 ml of 5%

sodium bicarbonate to hydrolyze the excess HFAA.

The organic layer was separated, washed with water (40 ml), dried over anhydrous sodium sulphate and injected into the GLC (See Table 35, p.155 and Figure 40, p. 156). An aliquot of this solution was also analysed by GC-MS in the Electron Impact mode (See Table 36, p. 158).

A second preparation of this commercial guazatine 70% derivative was made but with water being previously removed under reduced pressure at 100°C. The dried product (2.0 g, 3.74 mmol based on GNG-A component) in a saturated sodium bicarbonate solution (15 ml) was heated with HFAA (8.0 ml, 56.5 mmol) in 100 ml of toluene at 100°C for 3 hours, followed by the addition of 40 ml of 5% sodium bicarbonate to hydrolyze the excess HFAA.

The organic layer was separated, washed with water (40 ml), dried over anhydrous sodium sulphate and injected into the GLC (See Table 35, p. 155 and Figure 41, p.157). Part of this solution was evaporated under reduced pressure at 100°C, and dissolved in dichloromethane to give a 20% solution of G70/HFAA derivative which was also analysed by GC-MS in the Electron Impact mode (See Table 36, p. 158 and Figure 42, p. 159).

7.10 -Development sample of guazatine/HFAA derivative (G40/HFAA)

Development guazatine 40% (1.0 ml, 400 mg active ingredient, 0.75 mmol based on GNG-A component) in a saturated sodium bicarbonate solution (5 ml) was heated with HFAA (3 ml, 21.2 mmol) in 60 ml of toluene at 100°C for 3 hours, followed by the addition of 20 ml of 5% sodium bicarbonate to hydrolyze the excess HFAA.

The organic layer was separated, washed with water (20 ml), dried

over anhydrous sodium sulphate, and injected into the GLC (See Figure 45, p. 165).

7.11 - Cyanoguanidine/HFAA derivative

Cyanoguanidine (150 mg, 1.79 mmol) in a saturated sodium bicarbonate solution (2.5 ml) was heated with HFAA (6.3 ml, 9.26 mg; 44.52 mmol) in 30 ml of toluene at 100°C for 3 hours, followed by the addition of 10 ml of 5% sodium bicarbonate to hydrolyse the excess HFAA.

The organic layer was separated, washed with water (10 ml), dried over anhydrous sodium sulphate, and the solution was injected into the GLC.

7.12 - Dodine/HFAA derivative

N-Dodecylguanidine acetate (dodine) (500 mg, 1.74 mmol), in a saturated sodium bicarbonate solution (5 ml) was heated with HFAA (4.9 ml, 34.6 mmol) in 100 ml of toluene at 100°C for 3 hours, followed by the addition of 30 ml of 5% sodium bicarbonate to hydrolyze the excess HFAA.

The organic layer was separated, washed with water (30 ml), dried over anhydrous sodium sulphate and the solvent was evaporated under reduced pressure at 100°C. The residue was recrystallized from n-hexane to afford the substituted pyrimidine derivative (365.1 mg, 52.5%), m.p. 29-30°C (lit.[80] m.p. 34-35°C). (Found: C, 54.2; H, 6.8; N, 10.4. Calc. for $C_{18}H_{27}F_6N_3$: C, 54.1; H, 6.8; N, 10.5%); EI/MS: m/z (%), 399.20935 (M^+, 37.5), 273.06979 (14.4), $[M-(CH_2)_9]^+$, 258.04686 (8.2), $[M-CH_3(CH_2)_9]^+$,

244.03055 (100.0), $[M-CH_3(CH_2)_{10}]^+$. The compound gave one peak on GLC (5% OV-1 at 195-290^{0}C) (See Figure 57, p.180).

8- ATTEMPTED EXTRACTION OF GUANIDATED AMINE ACETATE DERIVATIVES FROM WHEAT PLANTS

The method of Kobayashi *et al.*[55] for the extraction of guazatine from rice grain was adapted for the extraction of guanidated amine acetate derivatives from wheat plants. Two samples of wheat plants, one control and the other treated (*ca* 1.40 ppm of guanidated triamine acetates) were received from Kenogard AB, Stockholm.

General procedure of extraction:

The blended wheat plant (5 g) was shaken with 0.5N NaOH/MeOH (150 ml) at 50^{0}C for 1 hour and filtered through filter paper on a Buchner funnel under vacuum. The filtrate was made up with water to 250 ml, extracted with chloroform (150 ml), and the extract was evaporated under vacuum and dried under a stream of nitrogem. To this residue hexafluoroacetylacetone (0.2 ml) in toluene (7 ml) was added and heated at 100^{0}C for 3 hours. The organic layer was then separated, washed with 5% sodium bicarbonate (1 ml) and water (5 ml), blown to dryness with a stream of nitrogen, and an aliquot of the solution of this material in MeOH (2 ml) was then injected into the GLC.

Using this procedure of extraction several different experiments were attempted:

- Extraction of control wheat plant (5 g)
- Extraction of treated wheat plant (5 g)

- Extraction of GG-A (100 mg, 0.287 mmol) and GNG-A (50 mg, 0.094 mmol) compounds in the absence of wheat plant.

A blended sample of control wheat plant (5 g) was thoroughly spiked with commercial guazatine 70% (1 ml) in water (10 ml). This mixture was well homogenized and the general procedure of extraction described above was carried out. The derivatization step was made with hexafluoroacetylacetone in excess (3 ml) in toluene (10 ml). The organic layer was then separated, washed with 5% sodium bicarbonate (5 ml) and water (10 ml), blown to dryness with a stream of nitrogen, and an aliquot of the solution of this material in methanol (3 ml) was then injected into the GLC.

A clean-up of this final solution by column chromatography was examined. Columns of 1 cm i.d. were filled with three different adsorbents: (a) alumina in the neutral form (aluminium oxide 90, active), (b) alumina in the alkaline form (aluminium oxide "CAMAG" M.F.C., *ca* 100 to 200 mesh), and (c) silica gel 60 (230 to 400 mesh). Columns were eluted with two different solvents: methanol (more polar) and toluene (less polar).

Initially the solution was added to the top of each column (1 ml), eluted with methanol, and fractions of 10 ml were collected. Each fraction was injected into the GLC and the fraction that exhibited all the peaks in the chromatogram (comparable to the results before the clean-up procedure) was evaporated under a stream of nitrogen and transferred to another column with the same adsorbent to be eluted with toluene.

9 - GAS-LIQUID CHROMATOGRAPHIC ANALYSIS

The method of Kobayashi *et al*[55] was adapted and the analysis of some hexafluoroacetylacetone derivatives were carried out on a Varian 3300 chromatograph equipped with a flame ionization detector (FID) and fitted with a 2 m x 4 mm (ID) glass column packed with 5% OV-1 on 100-120 mesh Chromosorb W (HP). The temperature of the detector and injector were both 300^{0}C and the column temperature was programmed as follows: 195^{0}C (2 min), increased at rate of 1.5^{0}C/min to 210^{0}C (4 min), increased at rate of 17.5^{0}C/min to 280^{0}C (5 min), and increased again at rate of 10^{0}C/min to 290^{0}C (9 min). Nitrogen was used as the carrier gas with an inlet pressure of 18 p.s.i. Values between brackets correspond to the time such temperature was kept until it was changed again.

A glass column packed with 4% SE-30 on Chromosorb W-HP (2 m x 4 mm) and an OV-101 (WCOT) 25 m x 0.2 mm capillary column were also used. Different temperature programmes and carrier gas flow rates were also tried.

10 - CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

All carbon-13 and proton NMR spectra were obtained on a Bruker WP 80, which is a high resolution pulse Fourier transform nuclear magnetic resonance spectrometer.

Tetramethylsilane (TMS) or sodium trimethylsilylpropionate (TSP) were used as the reference when samples were dissolved in deuterated organic and aqueous solvents, respectively.

11 - FAST ATOM BOMBARDMENT MASS SPECTROMETRY ANALYSIS

All FAB/MS spectra were obtained at facilities of the Science & Engineering Research Council (SERC) Mass Spectrometry Centre - University College of Swansea, on a VG Analytical ZAB E Spectrometer, with exception for diamine and its sulphate which were obtained at facilities of the School of Chemical Sciences - University of East Anglia, on a VG Analytical ZAB-1F Spectrometer.

IV - RESULTS AND DISCUSSION

1- DIAMINE AND TRIAMINE

Pure samples of 1,8-diamino-octane ("diamine") and of bis-(8-amino-octyl)amine ("triamine") were obtained from the distillation of technical triamine, and were characterized mainly by carbon-13 NMR. Their sulphates were also characterized by carbon-13 NMR. The diamine, its sulphate, and triamine were also studied by fast atom bombardment mass spectrometry.

In order to assign the chemical shifts the carbon atoms were numbered as follows:

H_2N-C-C-C-C-C-C-C-C-NH_2 NN
4 3 2 1 1 2 3 4

^+H_3N-C-C-C-C-C-C-C-C-NH_3^+ SO_4^{2-} N^+N^+
4 3 2 1 1 2 3 4

H_2N-C-C-C-C-C-C-C-C-NH-C-C-C-C-C-C-C-C-NH_2 NNN
5 3 2 1 1 2 4 6 6 4 2 1 1 2 3 5

$[^+H_3N$-C-C-C-C-C-C-C-C-NH_2^+-C-C-C-C-C-C-C-C-$NH_3^+]_2$ $3\ SO_4^{2-}$ $N^+N^+N^+$
5 3 2 1 1 2 4 6 6 4 2 1 1 2 3 5

Table 3 shows the results obtained for diamine, triamine and their sulphates by carbon-13 NMR.

Table 3. Carbon-13 NMR chemical shifts (δ) for diamine, triamine and their sulphates

Compound	δ (ppm)	Intensity	Assignment (C No.)
NN	28.9	18687	1
	31.4	19051	2
	34.5	16393	3
	43.5	17830	4
N^+N^+	28.0	13259	1
	29.5	11723	2
	30.5	12835	3
	43.1	8501	4
NNN	27.8	3397	1
	28.2	3244	2
	30.4	7670	3
	33.8	3008	4
	42.4	3171	5
	50.6	3102	6
$N^+N^+N^+$	28.6	34082	1
	29.7	16059	2
	31.1	27604	3
	32.3	1601	4
	43.1	13181	5
	50.4	18559	6

Spectra for the diamine and its sulphate were run in D_2O while spectra for the triamine and its sulphate were run in CD_3OD.

Only small variations in the chemical shifts were observed when the free bases (NN and NNN) were compared with their respective sulphates (N^+N^+ and $N^+N^+N^+$).

The effects of protonation on the carbon-13 chemical shifts of amino

compounds are complex and may correspond either to shielding or deshielding according to circumstances[81]. For polymethylene diamines, protonation has generally been reported to cause a slight upfield shift for the α-carbon atoms[82].

Fast atom bombardment (FAB) mass spectra were obtained for the diamine and its sulphate in the positive ion mode, using glycerol as matrix, and their results are shown in Table 4.

Table 4 Positive ion FAB/MS data for the diamine and its sulphate

Peak (m/z)	Rel. Int. (%) MN	N⁺N⁺	Assignment
243	—	64.7	$[M+H+H_2SO_4]^+$
214	—	74.7	$[M+H+H_2SO_4-CH_2NH]^+$
197	—	59.5	$[M+H+H_2SO_4-NH_3CH_2NH]^+$
145	100.0	100.0	$[M+H]^+$
128	16.3	20.0	$[M+H-NH_3]^+$
93	34.2	26.3	$[Glycerol+H]^+$
69	43.2	13.2	$[M+H-NH_3-NH_2(CH_2)_2CH_3]^+$
58	15.8	26.8	$[M+H-NH_2(CH_2)_4CH_3]^+$
57	30.5	16.8	$[M+H-NH_3-NH_2(CH_2)_2CH=CH_2]^+$
43	30.5	14.7	$[M+H-NH_3-NH_2(CH_2)_3CH=CH_2]^+$
41	34.7	12.6	$[M+H-NH_3-NH_2(CH_2)_4CH_3]^+$
30	35.8	13.2	$[M+H-NH_2(CH_2)_6CH_3]^+$

Possible fragmentation patterns are shown in Figures 4 and 5, for the diamine and its sulphate, respectively; they are specific for the compounds and serve to characterize them.

Figure 4 (for the diamine) shows the expected m/z 145 ion, $[M+H]^+$, as the base peak. The fragmentation starts with loss of ammonia from the base peak producing a peak at m/z 128, $[NH_2(CH_2)_6]^+$. Three different

routes of fragmentation then appear to be possible from this peak at m/z 128: (a) loss of $[NH_2(CH_2)_2CH_3]$ producing a peak at m/z 69, $[CH_2{=}CH(CH_2)_3]^+$, which by subsequently losing $[(CH_2)_2]$ with cleavage of a C-C bond produces a peak at m/z 41, $[CH_2{=}CHCH_2]^+$; (b) loss of $[NH_2(CH_2)_2CH{=}CH_2]$, by which a peak at m/z 57, $[CH_3(CH_2)_3]^+$ is produced ; (c) loss of $[NH_2(CH_2)_3CH{=}CH_2]$ and production of a peak at m/z 43, $[CH_3(CH_2)_2]^+$. From the base peak, a loss of $[CH_3(CH_2)_4NH_2]$ may also occur with production of a peak at m/z 58, $[NH_2(CH_2)_3]^+$, which by subsequently losing $[(CH_2)_2]$ with cleavage of a C-C bond produces a peak at m/z 30, $[NH_2{=}CH_2]^+$.

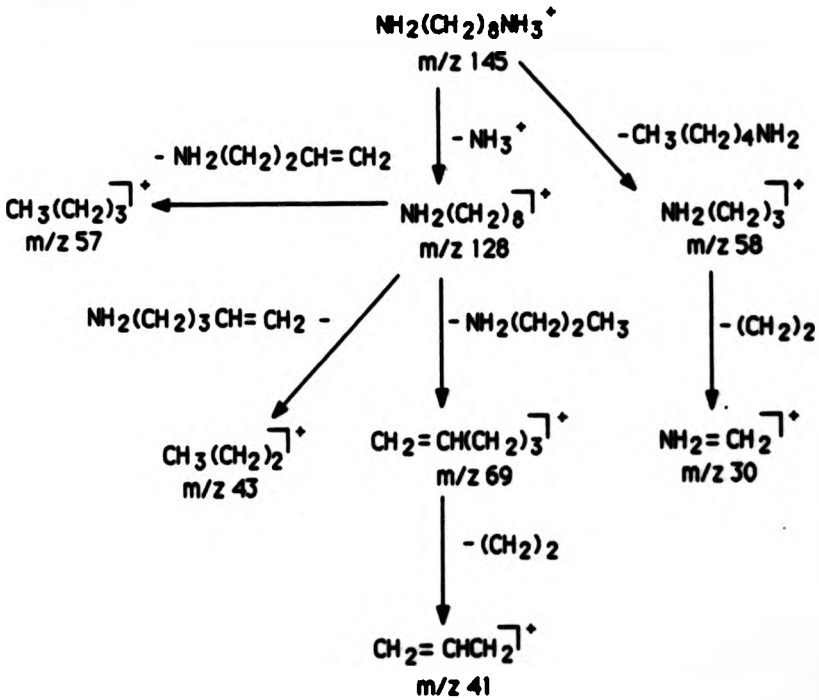


Figure 4. The positive ion FAB/MS fragmentation of diamine.

Figure 5 (for the diamine sulphate) shows an ion at m/z 243, $[M+H+H_2SO_4]^+$, with a relative intensity of 64.7% corresponding to the $[M+H]^+$ ion in association with a molecule of sulphuric acid. Structurally this ion may be the diprotonated diamine plus a hydrogen sulphate ion as shown in the scheme. Two routes of fragmentation that are possible for

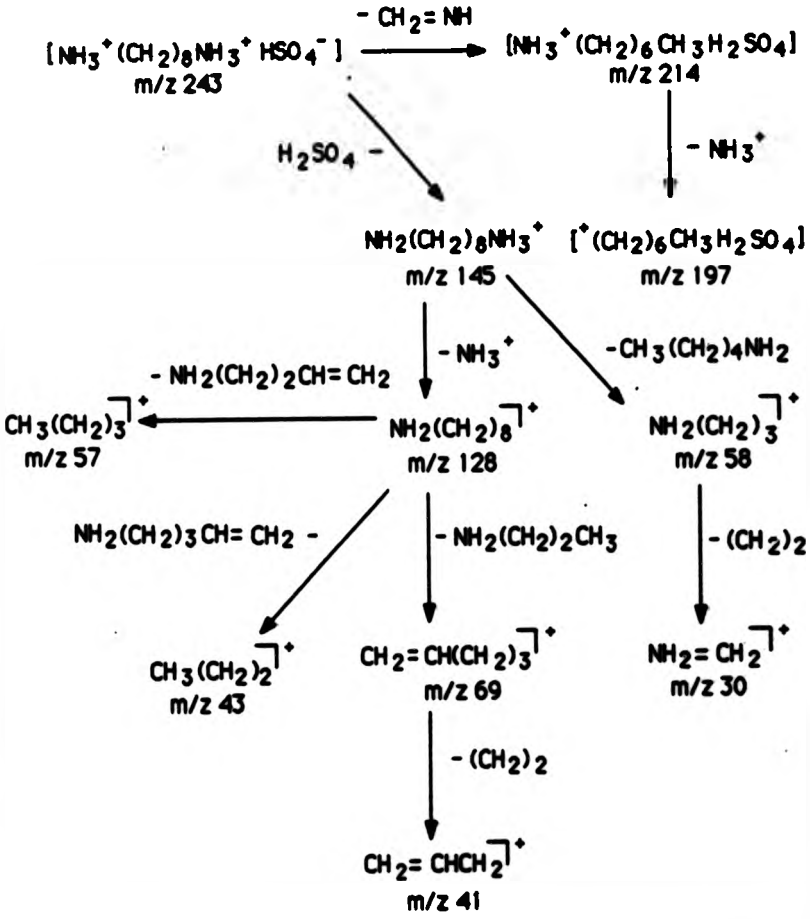


Figure 5. The positive ion FAB/MS fragmentation of diamine sulphate.

this peak ion are: (a) loss of sulphuric acid with consequent production of the base peak at m/z 145, $[NH_2-(CH_2)_8-NH_3^+]$. From this base peak the fragmentation pattern follows that observed in Figure 4; (b) loss of $[CH_2=NH]$ to give a peak at m/z 214, which is assumed to be due to the heptylammonium ion in association with sulphuric acid, $[NH_3^+(CH_2)_6CH_3\ H_2SO_4]$ followed by loss of ammonia to give the peak at m/z 197, $[^+(CH_2)_6CH_3\ H_2SO_4]$. The latter peak is tentatively assigned as the heptyl carbonium ion in association with sulphuric acid.

Table 5. Positive ion FAB/MS data for the triamine using different matrices(a)

Peak (m/z)	Relative intensity (%) GLY	THDE	DGLY	THGLY	3-NOBA	Assignment
284	90.9	35.7	49.5	3.8	2.9	$[M+H+12]^+$
272	100.0	100.0	100.0	100.0	100.0	$[M+H]^+$
255	7.8	7.6	7.6	6.5	5.7	$[M+H-NH_3]^+$
241	4.4	3.7	—	3.5	3.3	$[M+H-NH_3(CH_2)]^+$
227	3.2	—	1.9	2.2	1.4	$[M+H-NH_3(CH_2)_2]^+$
213	3.5	2.9	3.6	1.9	1.9	$[M+H-NH_3(CH_2)_3]^+$
199	2.4	—	4.3	1.7	1.4	$[M+H-NH_3(CH_2)_4]^+$
185	—	—	1.7	1.3	1.7	$[M+H-NH_3(CH_2)_5]^+$
171	14.8	7.6	1.4	1.6	1.4	$[M+H-NH_3(CH_2)_6]^+$
157	20.7	21.0	19.0	10.0	16.7	$[M+H-NH_3(CH_2)_7]^+$
143	2.4	2.4	1.9	1.4	1.9	$[M+H-NH_3(CH_2)_8]^+$
126	13.8	12.9	16.7	3.6	7.6	$[M+H-NH_3(CH_2)_8NH_3]^+$
112	7.5	4.8	7.6	1.1	3.3	$[M+H-NH_3(CH_2)_8NH_3(CH2)]^+$
98	8.4	6.7	8.6	1.6	3.8	$[M+H-NH_3(CH_2)_8NH_3(CH2)_2]^+$
84	11.7	9.5	11.9	2.1	6.0	$[M+H-NH_3(CH_2)_8NH_3(CH2)_3]^+$
70	12.5	12.9	21.4	3.3	8.6	$[M+H-NH_3(CH_2)_8NH_3(CH2)_4]^+$
56	14.4	16.7	27.6	5.0	10.0	$[M+H-NH_3(CH_2)_8NH_3(CH2)_5]^+$

a) Matrices: GLY = Glycerol, THDE = Thiodiethanol (Thiodiglycol), DGLY = Diglycerol, THGLY = Thioglycerol, 3-NOBA = 3-Nitrobenzyl alcohol.

Fast atom bombardment (FAB) mass spectra for the triamine in the positive ion mode were obtained in five CH_2OH-containing matrices (glycerol, thiodiethanol, diglycerol, thioglycerol, and 3-nitrobenzyl alcohol). The results are shown in Table 5.

Possible fragmentation patterns for the triamine are shown in Figure 6.

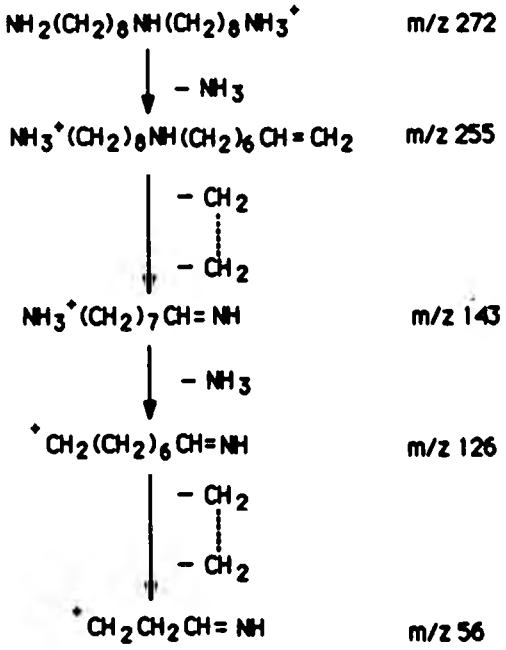


Figure 6. The positive ion FAB/MS fragmentation of triamine.

Figure 6 (for the triamine) shows the expected m/z 272 ion, $[M+H]^+$, as the base peak. The fragmentation starts with loss of ammonia from the base peak (with charge localization possibly changed to the nitrogen at the

other extremity) producing a peak at m/z 255, $[NH_3^+(CH_2)_8NH(CH_2)_6CH{=}CH_2]$ which by subsequently losing $[CH_2]$ fragments with cleavage of a C-C bond to give a series of prominent ions separated by 14 mass units (from m/z 255 down to m/z 143). The fragmentation continues again with loss of ammonia producing a peak at m/z 126, $[CH_2(CH_2)_6CH{=}NH]^+$ which by subsequently losing $[CH_2]$ fragments with cleavage of a C-C bond to give a series of prominent ions separated by 14 mass units (from m/z 126 down to m/z 56).

Triamine has shown strong $[MH+12]^+$ peaks (m/z = 284) in three of the matrices (glycerol, thiodiethanol and diglycerol). Using thioglycerol and 3-nitrobenzyl alcohol matrices the m/z 284 peak was less than about 4% of $[M+H]^+$. The $[MH+12]^+$ peaks are well known in the FAB spectra of amines in glycerol and Lehmann *et al*.[83] have also reported the existence of $[MH+12]^+$ ions in the positive ion FAB spectra of oligopeptides, gradually increasing with time and accompanying the abundant $[M+H]^+$ ions. The same phenomenon has been noticed by Pang *et al*.[84], who have stated that this might be due to the reaction of the amine-containing sample molecule with glycerol or thioglycerol.

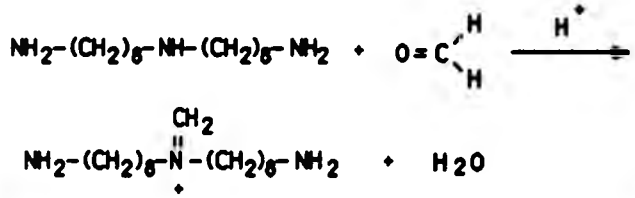


Figure 7. The reaction scheme for the formation of an iminium ion through reaction of formaldehyde with the secondary amino group of the triamine.

Probably the presence of this $[MH+12]^+$ peak in the spectrum of the triamine sample was due to the reaction of formaldehyde produced in the matrix with an amino group of the triamine, e.g. as displayed in Figure 7.

It is also possible that the terminal amine groups of the triamine can undergo condensation with formaldehyde without protonation resulting in Schiff base formation, e.g. as displayed in Figure 8. Significant $[MH+24]^+$ peaks were observed using glycerol and diglycerol matrices but $[MH+36]^+$ peaks were not significantly above the noise level.

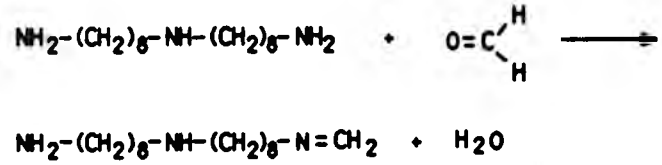


Figure 8. The reaction scheme for the formation of a Schiff base through reaction of formaldehyde with an amino group of the triamine

2- PREPARATION OF THE SALTS OF 1,8-DIGUANIDINO-OCTANE

1,8-Diguanidino-octane (GG) is present in commercial guazatine in the form of the acetate (GG-A). It was therefore necessary to synthesize and characterize this compound.

Diamine was amidinated with cyanamide (Exp. 2.1, p. 44) and 1,8-diguanidino-octane diacetate (GG-A) (17) was obtained directly, as shown in Figure 9.

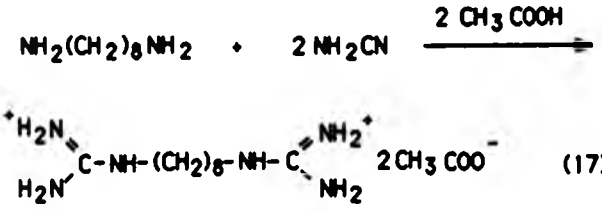


Figure 9. Reaction scheme for the preparation of 1,8-diguanidino-octane diacetate (GG-A).

Diamine was also amidinated with *S*-methylisothiouronium sulphate (Exp. 2.2, p. 45) and 1,8-diguanidino-octane sulphate (GG-S) (18) was obtained, as shown in Figure 10.

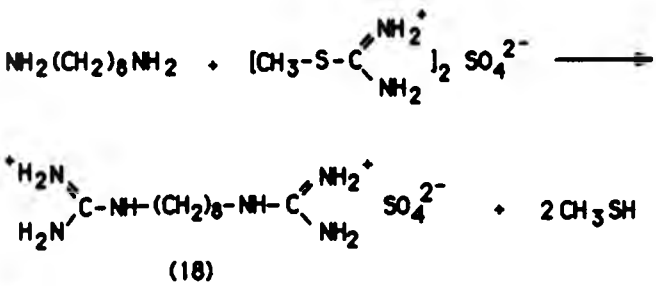
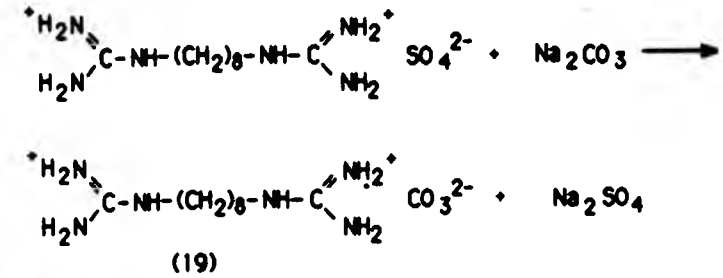


Figure 10. Reaction scheme for the preparation of 1,8-diguanidino-octane sulphate (GG-S).

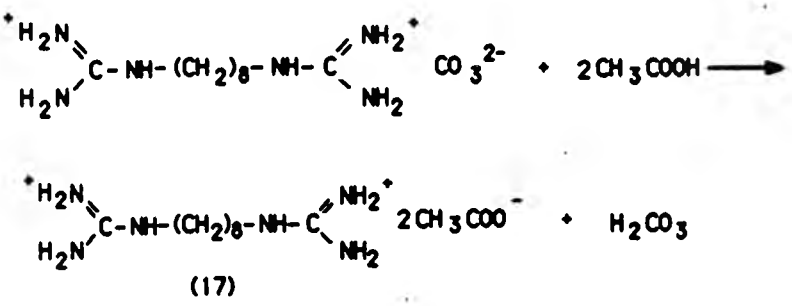
It was first converted to carbonate [GG-C (19); Exp. 2.3, p. 45] as shown in Figure 11,



(19)

Figure 11. Reaction scheme for the conversion of 1,8-diguanidino-octane sulphate to its carbonate (GG-C).

and then to the acetate [GG-A (17); Exp. 2.4, p. 46] as shown in Figure 12.

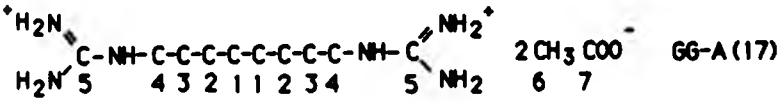


(17)

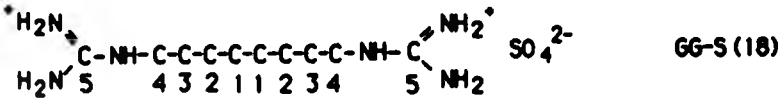
Figure 12. Reaction scheme for the conversion of 1,8-diguanidino-octane carbonate to its acetate (GG-A).

The reason for this indirect route in the preparation of the acetate was because sulphuric acid is a stronger acid than acetic acid so the direct preparation from the sulphate salt is not possible.

The diguanidated diamine salts, GG-S and GG-A, were characterized mainly by carbon-13 NMR. Their carbon-atoms were numbered as follows in order to assign the chemical shifts:



GG-A (17)



GG-S (18)

Table 6 shows the results obtained for GG-S and GG-A which were run in D_2O and D_2O/H_2SO_4, respectively.

Table 6. Carbon-13 NMR chemical Shifts (δ) of 1,8-diguanidino-octane sulphate (GG-S) and diacetate (GG-A)

GG-S[a]		GG-A[a]		GG-A[b]		Assignment
δ (ppm)	Intensity	δ (ppm)	Intensity	δ (ppm)	Intensity	(C No.)
—	—	23.5	6644	23.5	4914	6
28.5	46346	28.5	27121	28.6	6416	1
30.7	46673	30.6	28592	30.6	6802	2
30.9	44767	30.9	28539	30.9	6961	3
44.1	39518	44.2	25351	44.5	5968	4
159.9	12012	159.7	16300	159.3	4499	5
—	—	180.0	4055	182.8	2961	7

(a) *S*-methylisothiouronium sulphate method (Exp. 2.2, GG-S; 2.4, GG-A)
(b) Cyanamide method (Exp. 2.1, GG-A).

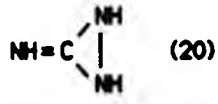
The presence of a guanidine structure at the extremity of GG-S and GG-A compounds was evidenced by the chemical shift around 159.8 ppm, which can be used as a fingerprint of its presence in guanidine compounds.

The carbon-13 chemical shifts for the carbon atoms of the octamethylene chain of GG-S and GG-A are almost identical to those for the protonated diamine, although the values for the α-carbon adjacent to nitrogen (carbon-4) are further downfield by about 1 ppm compared to those for the diamine (See Table 3, p. 77).

There were no differences in the carbon-13 chemical shifts of GG-A prepared by the *S*-methylisothiouronium sulphate and cyanamide methods, with the exception of carbon 7 ($\underline{C}OO^-$) which can vary in chemical shift according to the acidity of the solution used in the nmr measurement.

Table 7 shows the Fast Atom Bombardment (FAB) Mass Spectra results obtained for 1,8-diguanidino-octane sulphate (GG-S) and diacetate (GG-A) (Exp. 2.1, p. 44). The fragmentation pattern for both compounds is shown in Figure 13.

From Figure 13, the fragmentation of GG-S starts with loss of sulphuric acid and production of the base peak at m/z 229 which corresponds to the monoprotonated base. From this peak (where the fragmentation of GG-A also starts), several different routes of fragmentation might occur, involving the initial loss of ammonia (a), cyanamide (b), or guanidine (d) as shown in the Table 7. In addition a peak at m/z 172 results from the loss of CH_3N_3 (c), which has been observed for other guanidine derivatives[73,74] and is thought to be 3-imino-1,2-diazacyclopropane (20).



(20)

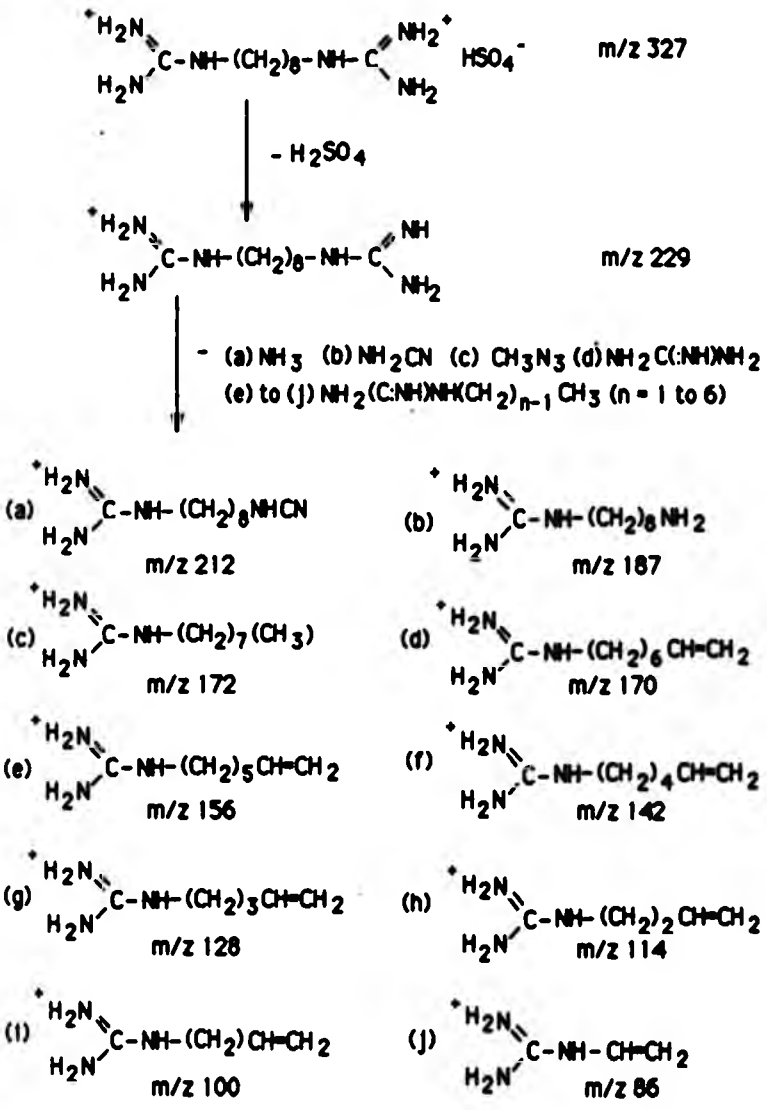


Figure 13. The positive ion FAB/MS fragmentation of 1,8-diguanidino-octane sulphate (GG-S) and diacetate (GG-A).

Table 7. The positive ion FAB/MS data of 1,8-diguanidino-octane sulphate (GG-S) and diacetate (GG-A)(a)

Peak (m/z)	Relative Intensity (%) GG-S	GG-A	Assignment(b)
327	51.5	—	$[M+2H^+ + HSO_4^-]^+$
229	100.0	100.0	$[M+H]^+$
212	10.3	9.0	$[M+H-NH_3]^+$
187	11.9	14.1	$[M+H-NH_2CN]^+$
172	8.5	7.2	$[M+H-CH_3N_3]^+$
170	20.7	18.8	$[M+H-NH_2C(:NH)NH_2]^+$
156	17.4	15.1	$[M+H-NH_2C(:NH)NH_2(CH_2)]^+$
142	9.2	9.5	$[M+H-NH_2C(:NH)NH_2(CH_2)_2]^+$
128	8.9	8.8	$[M+H-NH_2C(:NH)NH_2(CH_2)_3]^+$
114	7.5	7.3	$[M+H-NH_2C(:NH)NH_2(CH_2)_4]^+$
100	7.2	8.2	$[M+H-NH_2C(:NH)NH_2(CH_2)_5]^+$
86	9.8	12.6	$[M+H-NH_2C(:NH)NH_2(CH_2)_6]^+$
73	13.9	18.6	$[NH_2C(:NH)NH_2(CH_2)]$
59	9.3	12.7	[NH2C(:NH)NH2]

(a) GG-A obtained through cyanamide method (Exp. 2.1, p. 44)
(b) M = free base, 1,8-diguanidino-octane

Other peaks arising from C-C cleavages in the octamethylene chain are indicated schematically in Figure 13.

The results presented in Table 7 and Figure 13, clearly show that the fragmentation patterns do not depend on the anion species present in diguanidated diamine compounds, and FAB/MS analysis was a powerful technique for the characterization of these compounds.

3 - PREPARATION OF MONOACETYL DERIVATIVES

The aim was to prepare the monoacetyl derivative of 1,8-diamino-octane, mono-*N*-acetyl-1,8-diamino-octane (21), as shown in Figure 14, and to use it in the preparation of the monoguanidated diamine compound (GN) which is also thought to be present in the commercial guazatine.

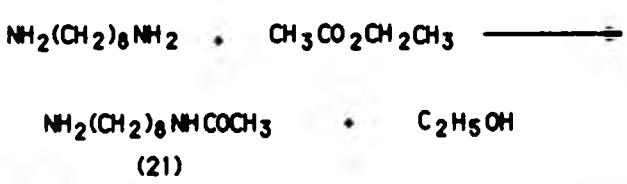


Figure 14. Reaction scheme for the preparation of mono-*N*-acetyl-1,8-diamino-octane.

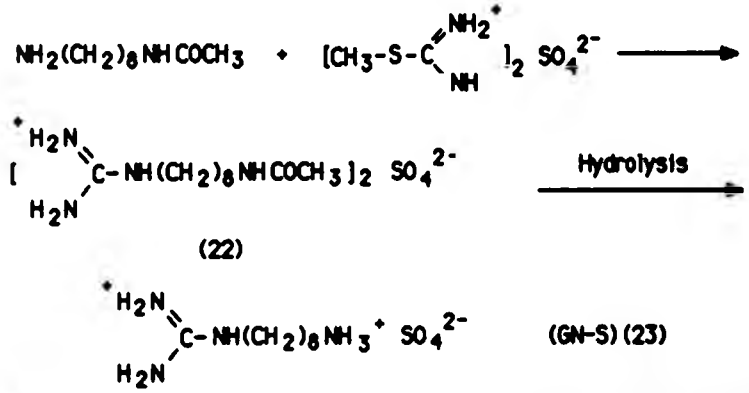


Figure 15. Reaction scheme for the preparation of 1-amino-8-guanidino-octane sulphate (GN-S) through *S*-methylisothiouronium sulphate.

Two different routes of preparation were attempted in order to obtain the monoguanidated diamine compound (GN) through the mono-*N*-acetyl-1,8-diamino-octane derivative (21), one using *S*-methylisothiouronium sulphate, as shown in Figure 15, and the other using cyanamide, as shown in Figure 16.

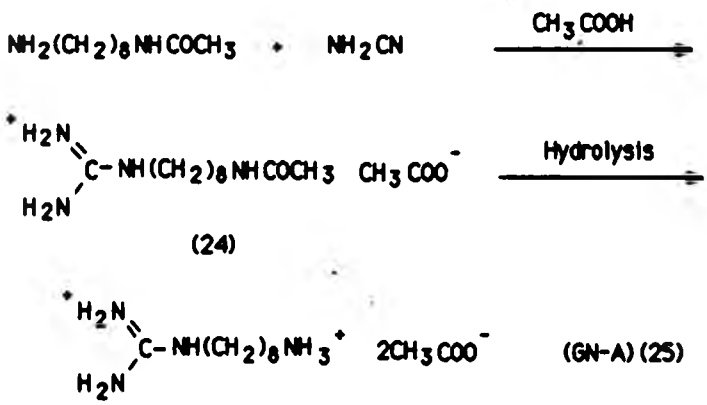


Figure 16. Reaction scheme for the preparation of 1-amino-8-guanidino-octane acetate (GN-A) through cyanamide.

In the synthesis of mono-*N*-acetyl-1,8-diamino-octane (21) (Figure 14), the diamine was protected by a convenient method with ethyl acetate, but further reaction can also occur with production of the diacetyl derivative (26):

$$CH_3\text{-}CO\text{-}NH\text{-}(CH_2)_8\text{-}NH\text{-}CO\text{-}CH_3 \qquad (26)$$

The monoacetyl derivative can however be separated by distillation

and the product obtained in the present work was shown to be pure by gas chromatography.

Table 8 shows the results obtained for the product (21) by carbon-13 NMR which was run in D_2O.

In order to assign the chemical shifts the carbon-atoms were numbered as follows:

$$\underset{2}{NH_2}\text{-C-C-C-C-C-C-C-}\underset{3}{C}\text{-}\underset{4}{NH}\text{-}\underset{1}{CO}\text{-}CH_3 \qquad (21)$$

Table 8. Carbon-13 NMR chemical shifts (δ) of mono-N-acetyl-1,8-diamino-octane

δ (ppm)	Intensity	Assignment (C No.)
24.6	66031	1
28.8	145242	Central carbon
31.0	16281	chain
31.2	131546	"
32.4	11269	"
33.2	7622	"
33.7	33442	"
42.3	81520	2
43.3	51883	3
176.5	26692	4

Table 9 shows mass spectral data with the elemental composition for mono-*N*-acetyl-1,8-diamino-octane and various fragment ions using the electron-impact method. The peak at m/z 187 corresponds to $[M+H]^+$ which is an usual feature in electron-impact spectra.

This is probably due to protonation in the ion source, possibly by transfer of H^+ from another fragment ion, acting in the manner of an ionized chemical ionization reagent gas.

The effect has been noticed recently for a number of carbonyl compounds and amides and is pressure dependent[85].

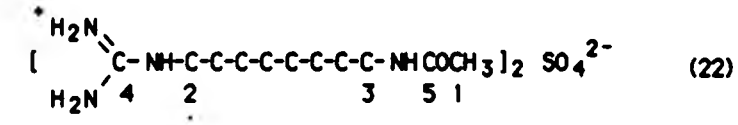
The phenomenon had been noticed by McLafferty who stated that aliphatic amines have a strong tendency to undergo protonation at moderately high sample pressures to yield the characteristic $[M+H]^+$ peak[86].

Table 9. Mass spectral data and elemental composition of ions from mono-*N*-acetyl-1,8-diamino-octane

Exact Mass Observed	Elemental Form. C	H	N	O	Exact Mass Calculated	Error (mm)	R.I. (%)	Remarks
187.18048	10	23	2	1	187.18104	0.6	35.3	$[M+H]^+$
186.17162	10	22	2	1	186.17321	1.6	2.3	$[M]^+$
170.15480	10	20	1	1	170.15449	-0.3	3.2	$[M-NH_2]^+$
157.14731	9	19	1	1	157.14666	-0.6	19.9	$[M-NH(CH)_2]^+$
142.12308	8	16	1	1	142.12319	0.1	8.7	$[M-NH_2(CH_2)_2]^+$
128.10655	7	14	1	1	128.10754	1.0	2.4	$[M-NH_2(CH_2)_3]^+$
114.09158	6	12	1	1	114.09189	0.3	21.6	$[M-NH_2(CH_2)_4]^+$
100.07623	5	10	1	1	100.07624	0.0	16.9	$[M-NH_2(CH_2)_5]^+$
86.06093	4	8	1	1	86.06059	-0.3	38.2	$[M-NH_2(CH_2)_6]^+$
73.05313	3	7	1	1	73.05276	-0.4	100.0	$[M-NH_2(CH_2)_7]^+$
55.05676	4	7	0	0	55.05478	-2.0	24.4	$[M-NH_3(CH_2)_4NHCOCH_3]^+$

Amidination of the monoacetyl derivative was carried out with *S*-methylisothiouronium sulphate (Figure 15, p. 91) and the intermediary

reaction product, 1-acetamido-8-guanidino-octane sulphate (22), was checked by carbon-13 NMR run in D_2O. The carbon atoms of this compound were numbered as follows in order to assign the chemical shifts shown in Table 10:



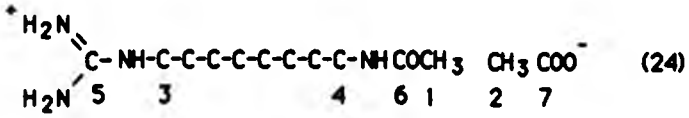
(22)

The chemical shift at 159.7 ppm confirmed that the guanidine structure had been introduced into the monoacetyl derivative but it proved impossible to obtain the product in a crystalline form . The reaction from Figure 15 (See p. 91) was stopped at this stage.

Table 10. Carbon-13 NMR chemical shifts (δ) of 1-acetamido-8-guanidino-octane sulphate.

δ(ppm)	Intensity	Assignment (C No.)
24.7	20024	1
28.5	36085	Central carbon
28.7	33892	chain .
29.5	11554	"
30.7	41691	"
31.0	80238	"
33.1	14584	"
42.3	26175	2
44.1	24961	3
159.7	7267	4
176.6	8363	5

Amidination of the monoacetyl derivative was also carried out with cyanamide in acetic acid solution (Figure 16, p. 92) and the intermediary reaction product, 1-acetamido-8-guanidino-octane acetate (24), was checked by carbon-13 NMR in CD_3OD. Similar results were obtained to those above. The carbon-atoms were numbered as follows in order to assign the chemical shifts shown in Table 11:



(24)

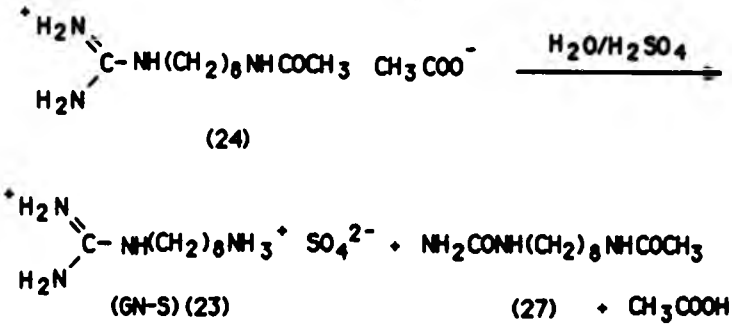
Table 11. Carbon-13 NMR chemical shifts (δ) of 1-acetamido-8-guanidino-octane acetate

δ (ppm)	Intensity	Assignment (C No.)
22.6	29949	1
27.2	15979	2
27.5	16628	Central carbon
27.7	31516	chain
28.4	12355	"
29.9	37237	"
30.1	64842	"
40.4	32087	3
42.3	9208	4
158.7	2905	5
173.3	11548	6
179.3	3792	7

The chemical shift at 158.7 ppm again confirmed that the guanidine derivative of the monoacetyl compound had been formed

although the value was up field by about 1 ppm compared to that for 1-acetamido-8-guanidino-octane sulphate (Table 10, p. 95) and for the GG-S and GG-A compounds (Table 6, p. 87). This variation may be due to the solvent used, *i.e* CD_3OD (D_2O was used with the previous compounds).

1-Acetamido-8-guanidino-octane acetate (24) was heated with 65% sulphuric acid in an attempt to obtain 1-amino-8-guanidino-octane sulphate (GN-S) (23) as shown in Figure 17, but unfortunately the desired product could not be obtained. Although the acetyl group was partially removed, about 50% conversion of guanidine to the ureido group was also observed through carbon-13 NMR results which are shown in Table 12. The spectra were run in D_2O/H_2SO_4.



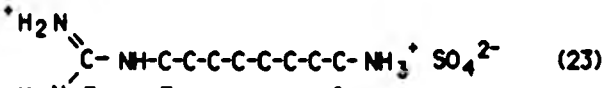
(24)

(GN-S) (23) (27) + CH_3COOH

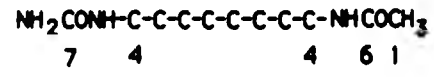
Figure 17. Reaction scheme for the hydrolysis of 1-acetamido-8-guanidino-octane acetate

Because of this undesirable production of the urea derivative (27) instead of 1-amino-8-guanidino-octane sulphate (GN-S), the reaction was stopped at this stage and no further attempts were made.

In order to assign the chemical shifts of the resultant product from the reaction the carbon atoms were numbered as follows:



(23)



(27)

Table 12. Carbon-13 NMR chemical shifts (δ) of the hydrolysis product from 1-acetamido-8-guanidino-octane acetate

δ (ppm)	Intensity	δ (ppm)[a]	Intensity	Assignment (C No.)
21.7	9729	21.7	3217	1
—	—	23.2	62867	(b)
—	—	24.8	3023	Central carbon
27.8	13686	28.4	3768	chain
28.1	17651	28.8	3515	"
29.1	12346	29.7	3730	"
29.5	15438	30.2	2845	"
30.3	20850	31.1	4838	"
42.6	7270	43.0	2494	2
43.8	6354	44.3	1056	3
44.4	11465	44.7	1689	4
159.1	2861	159.6	460	5
164.3	2603	164.9	497	6
177.9	8820	178.0	2064	7
—	—	179.6	38869	(c)

a) In the presence of CH_3COOH in excess
b) Due to $\underline{C}H_3$ of CH_3COOH
c) Due to $CH_3\underline{C}OOH$

4 - PREPARATION OF GUANIDATED TRIAMINE ACETATES AND GUAZATINE

The commercial process for the manufacture of guazatine is shown in Figure 2 (p. 31) and uses cyanamide to amidinate the technical triamine. Some modifications in this procedure were made and some different routes of preparation were attempted in this programme.

Both cyanamide and *S*-methylisothiouronium sulphate were used for the amidination of technical triamine and pure triamine in the production of guanidated triamine acetates and guazatine.

It was noticeable that the reactions using cyanamide tended to give oils, or glass-like, and greasy products much more than the reactions using *S*-methylisothiouronium sulphate and that the products from cyanamide resisted all attempts to induce crystallization.

When cyanamide was allowed to react with technical or pure triamine (in different molar ratios) in the presence of acetic acid, different guanidated triamine acetates were possible as products, as shown in Figure 18.

It was observed that cyanamide tended to react also with the central nitrogen of the triamine molecule producing a triguanidated compound (GGG-A) (31), a reaction not observed with *S*-methylisothiouronium sulphate which produced a diguanidated compound [GNG-S (8); Figure 1, p. 29].

The compounds produced by all these reactions using cyanamide, *S*-methylisothiouronium sulphate, or other reagents. were characterized by carbon-13 NMR and the results are shown in Tables 13 to 17. The spectra were run in D_2O.

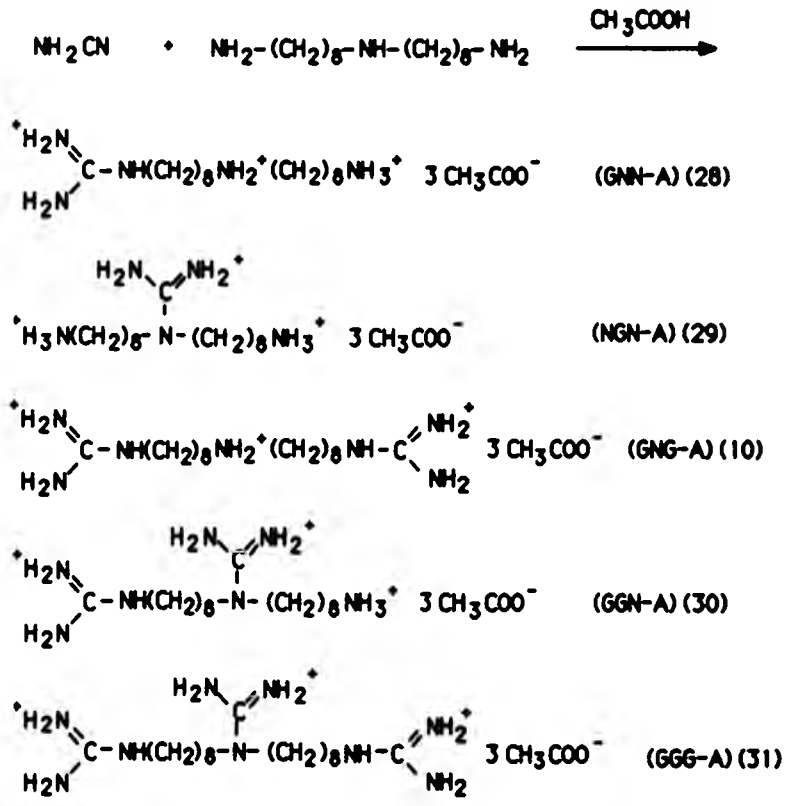
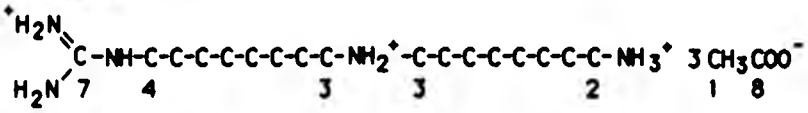


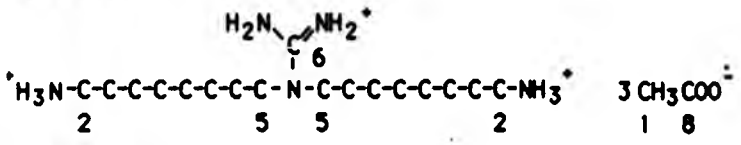
Figure 18. Reaction scheme for the preparation of different guanidated triamine acetates through reaction of cyanamide with pure or technical triamine.

In order to assign the chemical shifts of all possible compounds that might be produced in the preparation of guanidated triamine acetates their carbon atoms were numbered as follows:

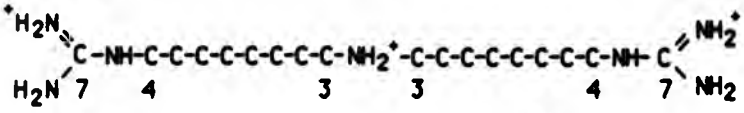
GNN-R:



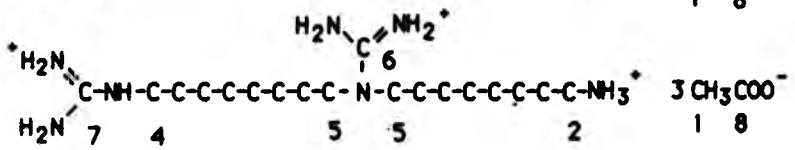
NGN-R:



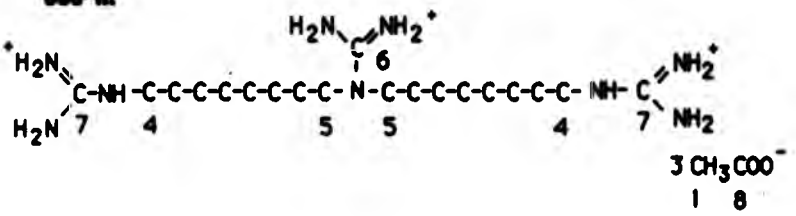
GNG-R:



GGN-R:



GGG-R:



In increasing order of chemical shift signals the carbon atoms can also be numbered as follows:

(1) CH_3 ; (2) $C\text{-}NH_3^+$; (3) $C\text{-}NH_2^+$; (4) C-NH ; (5) C-N-C ; (6) $NC(:NH_2^+)NH_2$ (7) $NHC(:NH_2^+)NH_2$; (8) COO^-

Table 13 shows the results obtained when cyanamide was caused to react with technical triamine in a molar ratio of 3:1, respectively.

Table 13. Carbon-13 NMR chemical shifts (δ) of some guanidated triamine acetates prepared using technical triamine and cyanamide (1:3) as reagents(a)

δ (ppm)	Intensity	Assignment (C No.)
26.0	19919	1
28.3	34620	Central carbon
29.2	11837	chain
29.4	10856	"
30.4	28783	"
30.6	25709	"
30.7	27685	"
42.2	7208	2
43.7	23347	3
50.0	1921	4
51.3	9357	5
122.6	1124	9
158.4	6282	6
159.5	14826	7
165.8	2676	10
183.6	12737	8

(a) Exp. 4.1, p. 48

The assignment of carbon atoms 9 and 10 from Table 13 corresponds to cyanoguanidine (32) which was produced by dimerization of cyanamide that was used in excess.

$$2\,NH_2CN \longrightarrow \underset{10}{HN=\overset{NH_2}{\overset{|}{C}}-NH-}\underset{9}{CN} \qquad (32)$$

From the results of Table 13 it is also possible to conclude that the product obtained is a mixture of several amidinated triamine compounds such as : GNN-A; NGN-A; GNG-A; GGN-A and GGG-A which are present in different relative proportions since the reaction using cyanamide is random and the amidination of triamine can occur at any nitrogen in the molecule[21].

Table 14 shows the results obtained when cyanamide was caused to react with pure triamine in various molar ratios.

The results from Table 14 give an indication that when the concentration of cyanamide that was used was increased from 0.5 to 12 times the molar concentration of triamine, several guanidated compounds were obtained (monoguanidated: GNN-A or NGN-A; diguanidated: GNG-A or GGN-A; and triguanidated: GGG-A), all with different relative concentrations according to the resultant product obtained. Even at a low concentration of cyanamide (2:1), there was a possibility of obtaining the triguanidated compound (GGG-A) which again has shown the preference of cyanamide for the central nitrogen of triamine instead the one at the extremity. When a large concentration of cyanamide was used (1:12) the probability of obtaining the triguanidated compound (GGG-A) as the sole resultant product was greatly increased. Such results will be compared later with those obtained by gas-liquid chromatography.

The assignment of carbon atoms 9 and 10 from Table 14 corresponds to cyanoguanidine (See p. 102), and the carbon atom 11 corresponds to cyanamide (See p. 111).

Table 14. Carbon-13 NMR chemical shifts (δ) of some guanidated amine acetates prepared using pure triamine and cyanamide (in various molar ratios) as reagents(a)

(2:1)		(1:1)		(1:2)		Assignment
δ (ppm)	Intens.	δ (ppm)	Intens.	δ (ppm)	Intens.	(C No.)
26.3	20664	26.1	11436	26.1	6379	1
28.3	40730	28.3	27089	28.4	15581	Central
29.7	21508	28.7	4711	29.3	7262	carbon
30.7	33261	29.3	9698	30.0	6031	chain
31.1	8752	29.9	13780	30.6	7478	"
31.3	3374	30.7	20910	30.9	10307	"
31.6	2150	31.0	10222	31.0	8084	"
42.3	19094	42.5	12392	42.5	4244	2
43.9	2835	43.9	4250	44.0	4681	3
50.2	15282	50.3	8823	50.3	2331	4
51.6	3901	51.5	6900	51.5	5559	5
158.6	1854	158.5	2842	158.6	2654	6
159.8	1082	159.7	2176	159.7	1966	7
183.7	10192	184.0	5764	184.0	3076	8

(1:3)		(1:6)		(1:12)		Assignment
δ (ppm)	Intens.	δ (ppm)	Intens.	δ (ppm)	Intens.	(C No.)
26.0	4235	26.0	29156	26.1	3609	1
28.3	5974	28.4	32044	28.9	6587	Central
29.3	3975	29.3	20113	29.7	3787	carbon
30.5	4327	30.5	25390	31.0	4692	chain
30.9	4795	30.9	28892	31.3	5686	"
31.4	730	—	—	31.5	5602	"
42.1	1637	42.0	4795	—	—	2
43.7	2869	43.7	18061	44.2	3501	3
50.0	593	—	—	—	—	4
51.3	2515	51.3	12649	51.8	2781	5
—	—	—	—	118.7	314	11
—	—	122.4	3790	123.0	1165	9
158.4	1786	158.3	11918	158.7	1432	6
159.5	2184	159.4	19709	159.8	1799	7
—	—	165.6	9831	166.1	1421	10
183.4	2505	183.2	19816	183.6	1915	8

(a) Experimental 4.2.1 to 4.2.6., pp. 49 to 52

Table 15 shows the results obtained when *S*-methylisothiouronium sulphate was caused to react with pure triamine in a molar ratio of 1:1 and also the results from the conversion to acetate through different procedures of evaporation to dryness.

Table 15. Carbon-13 NMR chemical shifts (δ) of guazatine sulphate and acetate prepared using pure triamine and *S*-methylisothiouronium sulphate (1:1) as reagents.

GNG-S(a)		GNG-A(b)		GNG-A(c)		GNG-A(d)		Assignment
δ (ppm)	Intens.	δ (ppm)	Intens.	δ (ppm)	Intens.	δ (ppm)	Intens.	(C No.)
—	—	26.4	3132	26.3	204677	26.1	9462	1
28.3	33021	28.3	5007	28.4	337486	28.2	18219	Central
28.5	45741	28.6	6564	28.6	432919	28.5	22397	carbon
30.7	37197	31.0	7583	30.7	336884	30.6	17910	chain
30.9	52052	32.0	1382	31.0	491549	30.8	26098	"
44.0	19015	44.1	3417	44.2	254169	44.0	12767	3
50.4	17776	50.4	3466	50.5	223056	50.3	11660	4
159.8	5378	159.9	1325	159.9	98542	159.7	4600	7
—	—	184.0	1593	184.3	99975	184.2	3592	8

(a) Exp. 4.2.7.1, p. 53
(b) Compound as 6% w/v solution (Exp. 4.2.7.3, p. 54)
(c) Solid compound obtained by heating (1st procedure of 4.2.7.3, p. 54)
(d) Solid compound obtained without heating(2nd proc. of 4.2.7.3, p. 54)

The guazatine acetate (GNG-A) results from Table 15 clearly show that there were not any significant differences in the chemical shifts following the different procedures used to dry the compound and neither if the compound was finally obtained in solution or in the solid state.

The guazatine sulphate (GNG-S) results were similar to those for the acetates.

Table 16 shows the results obtained for guazatine acetate using two different routes of preparation: barium acetate (Figure 19) and *S*-methylisothiouronium acetate (Figure 21) methods, but for the latter it was necessary to prepare *S*-methylisothiouronium acetate (33) to use as starting material, as shown in Figure 20.

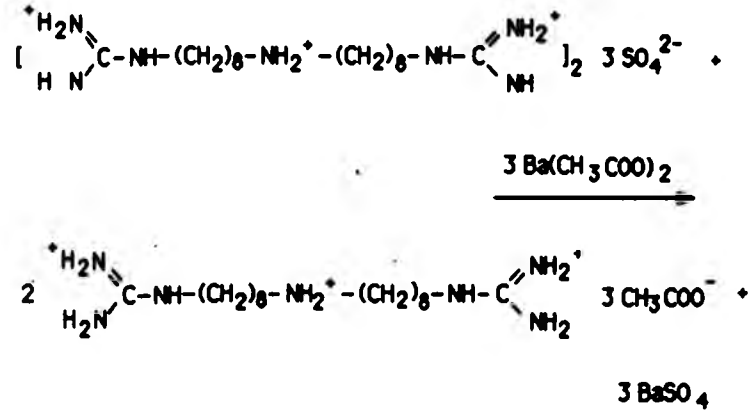
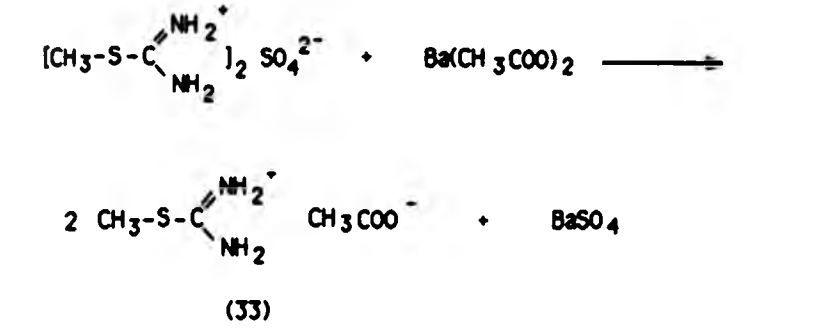


Figure 19. Reaction scheme for the preparation of guazatine acetate through barium acetate.

The results of both methods of preparation of guazatine acetate did not differ from each other and they also agreed with those shown in Table 15. Such results provide alternative methods of preparation for guazatine acetate. Although the use of *O*-methylisouronium acetate (15) has been referred to[20], *S*-methylisothiouronium acetate (33) and its use have not been mentioned in the literature until now. The reagent was prepared from the sulphate by double decomposition with barium acetate solution (See Figure 20). This new method of preparation has an advantage over the use



(33)

Figure 20. Reaction scheme for the preparation of *S*-methylisothiouronium acetate

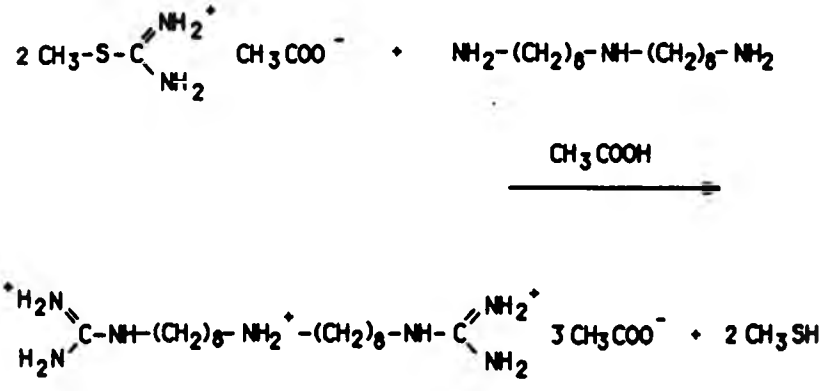


Figure 21. Reaction scheme for the preparation of guazatine acetate through *S*-methylisothiouronium acetate.

of cyanamide and *S*-methylisothiouronium sulphate because there is no risk of production of triguanidated compounds, as is the case with cyanamide, and neither the disadvantage of the need for conversion from

sulphate to carbonate and then to acetate when *S*-methylisothiouronium sulphate is utilized.

Table 16. Carbon-13 NMR chemical shifts (δ) of guazatine acetate (GNG-A) using two different routes of preparation

GNG-A[a]		GNG-A[b]		Assignment
δ (ppm)	Intens.	δ (ppm)	Intens.	(C No.)
26.3	24919	26.1	33609	1
28.4	55063	28.4	60256	Central
28.6	77404	28.5	72961	carbon
30.7	57485	29.6	19320	chain
30.9	85892	30.9	78138	-
44.1	41958	44.1	32821	3
50.4	38956	50.4	35849	4
159.9	13735	159.8	11360	7
184.3	7831	184.1	12956	8

(a) Barium acetate method (Exp. 4.2.7.4, p. 55)
(b) *S*-methylisothiouronium acetate method (Exp. 4.2.7.5, p. 56)

Table 17 shows the results obtained when *S*-methylisothiouronium sulphate was caused to react with pure triamine in a molar ratio of 2:1, respectively.

The product obtained was not very pure, which explains the large range for the melting point, low level of nitrogen, and poor results of carbon-13 NMR which also showed the presence of some of the excess of *S*-methylisothiouronium reagent (δ 15.7). The results have shown, however, that even using double the required concentration of *S*-methylisothiouronium sulphate the amidination at the central nitrogen did not occur to a detectable extent and this confirmed the results

obtained by Hudson *et al.*[13]

Table 17. Carbon-13 NMR chemical shifts (δ) of guazatine sulphate prepared using pure triamine and *S*-methylisothiouronium sulphate (1:2) as reagent(a)

δ (ppm)	Intensity	Assignment (C No.)
15.7	5343	(b)
28.5	27184	Central carbon
30.8	29249	chain
44.0	11935	3
50.2	9792	4
159.8	3113	7

(a) Exp. 4.2.7.5, p. 56
(b) Chemical shift due to *S*-methyl (weak signal).

A development sample of guazatine produced by Murphy Chemical Ltd. (40%, G40) in 1970 from distilled triamine through the *S*-methylisothiouronium sulphate route (Figure 1, p. 29) and the KenoGard AB commercial product (70%, G70), were also characterized by carbon-13 NMR and the results are shown in Table 18.

The results for G40 agreed perfectly with those for the compound synthesized in the present studies through the *S*-methylisothiouronium sulphate and acetate methods for which the results are shown in Tables 15 and 16 (See pp. 105 and 108), respectively, showing that the Murphy product was essentially the single compound, GNG-A.

The results for G70 were also similar to those for the various products synthesized through the cyanamide method (using various molar ratios) which are shown in Tables 13 (for tech. triamine) and 14 (for distilled

triamine) (See pp. 102 and104, respectively).

Table 18. Carbon-13 NMR chemical shifts (δ) of development sample (G40) and commercial guazatine (G70)

G40		G70		Assignment
δ (ppm)	Intensity	δ (ppm)	Intensity	(C No.)
25.9	3661	26.3	112014	1
28.1	10594	28.6	190236	Central
28.4	13234	29.5	68113	carbon
—	—	29.7	62892	chain
30.6	12089	30.7	158078	"
30.8	14326	31.0	162353	"
—	—	42.3	39780	2
43.9	4840	44.0	135176	3
50.1	6530	48.3	5330	4
—	—	51.6	48259	5
—	—	158.6	23939	6
159.6	2784	159.7	48031	7
182.8	3036	184.0	62149	8

From a general view of the results obtained, the presence of the guanidine structure at the central position of the molecule (carbon 6) was evidenced by the chemical shift of about 158.4 ppm and this was observed when cyanamide was used as the reagent for the amidination of pure or technical triamine (Tables 13 and 14). The same signal was not observed when *S*-methylisothiouronium sulphate (Table 15 and 17) or acetate (Table 16) were used.

Carbon-13 chemical shifts for the terminal guanidine groups (carbon 7)

occurred at a slightly lower field of about 159.5 ppm.

Carbon atoms from the central carbon chain (Tables 13 to 18) did not present great variations in chemical shifts. The variations were more pronounced for carbon atoms 2 to 5 where the interaction of the adjacent nitrogen atoms became stronger.

The chemical shifts for these products were distinctly different from those of the reagents used in the reactions. Thus cyanamide shows a single peak at 120.7 ppm, *S*-methylisothiouronium sulphate shows signals at 15.8 and 175.7 ppm for the methyl and isothiouronium carbon atoms, respectively, whilst *S*-methylisothiouronium acetate shows signals at 15.8, 26.2, 175.8 and 184.3 ppm for the *S*-methyl, acetate methyl, isothiouronium and carboxyl carbon atoms, respectively.

Fast Atom Bombardment (FAB) Mass Spectra were obtained for GNG-S, GNG-C, GNG-A, and for a mixture of guanidated triamine acetates (obtained using triamine) in the positive ion mode with glycerol as matrix (Tables 19,20,21).

The fragmentation patterns for GNG-S, GNG-C, GNG-A, and the mixture of guanidated triamine acetates are shown in Figures 23 and 25 (for the mixture) and are complemented by the Tables mentioned above.

Figure 23 and Table 19 [for the bis-(8-guanidino-octyl)amine sesquisulphate and sesquicarbonate] show the expected m/z 356 ion, $[M+H]^+$ as the base peak. In the case of the sulphate salt an additional peak at m/z 454 (relative intensity 23%) appeared which was assigned to the triprotonated base in association with a sulphate anion. In the case of the carbonate salt the same additional peak at 454 (relative intensity 6%) indicated that the conversion of sulphate to carbonate was not a hundred percent and that some sulphate was left. Several different routes of fragmentation might occur from the base peak, involving the initial loss

Table 19. The positive ion FAB/MS data for bis-(8-guanidino-octyl) amine sesquisulphate (GNG-S)[a] and sesquicarbonate (GNG-C)[b]

Peak (m/z)	Rel.Int.(%) GNG-S	GNG-C	Assignment[c]
454	23	6.1	$[M+3H+SO_4^{2-}]^+$
398	2	10.0	$[M+H+C+CH_2O]^+$
380	—	3.2	$[M+H+2C]^+$
368	2	57.2	$[M+H+C]^+$
356	100	100.0	$[M+H]^+$
339	7	11.5	$[M+H-NH_3]^+$
314	14	42.4	$[M+H-NH_2CN]^+$
299	19	10.8	$[M+H-CH_3N_3]^+$
297	15	21.8	$[M+H-NH_2C(:NH)NH_2]^+$
283	10	13.8	$[M+H-NH_2C(:NH)NH_2(CH_2)]^+$
269	7	8.6	$[M+H-NH_2C(:NH)NH_2(CH_2)_2]^+$
255	7	10.0	$[M+H-NH_2C(:NH)NH_2(CH_2)_3]^+$
241	5	7.1	$[M+H-NH_2C(:NH)NH_2(CH_2)_4]^+$
227	4	7.8	$[M+H-NH_2C(:NH)NH_2(CH_2)_5]^+$
213	5	12.7	$[M+H-NH_2C(:NH)NH_2(CH_2)_6]^+$
199	13	37.6	$[M+H-NH_2C(:NH)NH_2(CH_2)_7]^+$
185	19	—	$[M+H-NH_2C(:NH)NH_2(CH_2)_8]^+$
170	31	34.3	$[M+H-NH_2C(:NH)NH_2(CH_2)_8NH]^+$
156	21	32.7	$[M+H-NH_2C(:NH)NH_2(CH_2)_8NH(CH_2)]^+$
142	13	21.6	$[M+H-NH_2C(:NH)NH_2(CH_2)_8NH(CH_2)_2]^+$
128	17	31.9	$[M+H-NH_2C(:NH)NH_2(CH_2)_8NH(CH_2)_3]^+$
114	14	28.2	$[M+H-NH_2C(:NH)NH_2(CH_2)_8NH(CH_2)_4]^+$
100	15	34.2	$[M+H-NH_2C(:NH)NH_2(CH_2)_8NH(CH_2)_5]^+$
86	18	50.7	$[M+H-NH_2C(:NH)NH_2(CH_2)_8NH(CH_2)_6]^+$

(a) Exp. 4.2.7.1, p. 53

(b) Exp. 4.2.7.2, p. 53

(c) M = $NH_2C(:NH)NH(CH_2)_8NH(CH_2)_8NHC(:NH)NH_2$

of ammonia (1), cyanamide (2), or guanidine (4), to give ions at m/z 339, 314, and 297, respectively, as shown in the Table 19 and Figure 23. In ad-

dition a peak at m/z 299 results from the loss of CH_3N_3, as observed for other guanidine derivatives[73,74] (See p. 88). Other peaks arise by cleavage of the various carbon-carbon or carbon-nitrogen bonds along the octamethylene chain (with hydrogen transfer) to give a series of prominent ions as shown in Table 19.

An additional peak at m/z 368 appeared in both compounds, GNG-S (2%) and GNG-C (57%) probably resulting from the formation of an iminium ion by reaction between the free base [M] and formaldehyde produced in the glycerol matrix as shown in Figure 22, and discussed earlier in the case of the triamine (See p. 83).

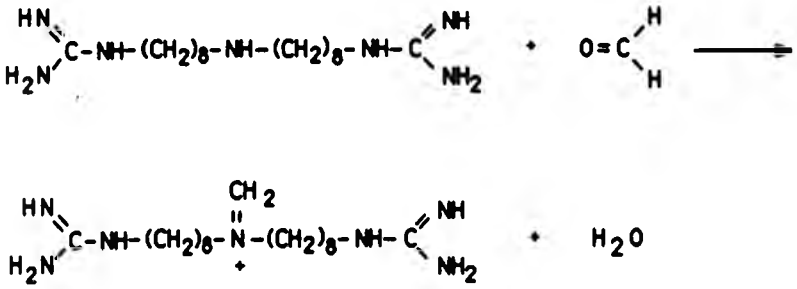


Figure 22. The reaction scheme for the formation of an iminium ion through reaction of formaldehyde with the secondary amino group of the free base of guazatine.

The main reason to use two different procedures to obtain guazatine acetate through conversion of the same carbonate to acetate, via *S*-methylisothiouronium sulphate method, *i.e.* heating and not heating the

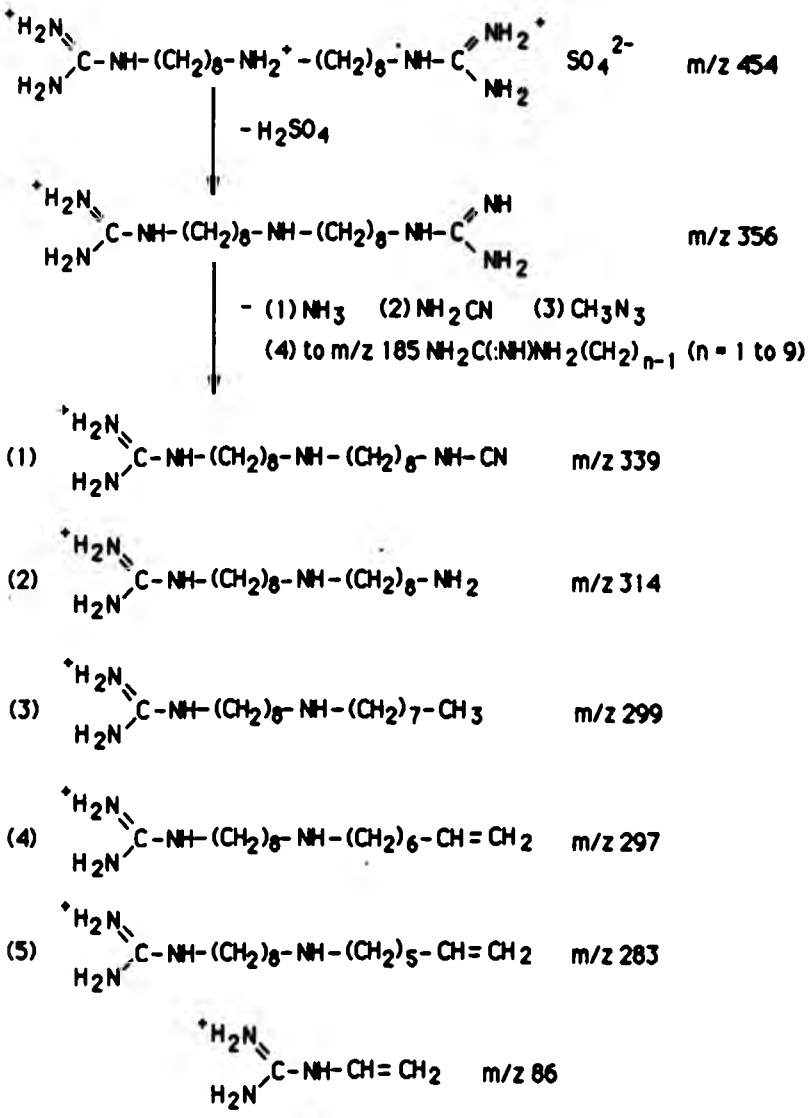


Figure 23. The positive ion FAB/MS fragmentation of bis-(8-guanidino-octyl)amine sesquisulphate (GNG-S), sesquicarbonate (GNG-C), and triacetate (GNG-A).

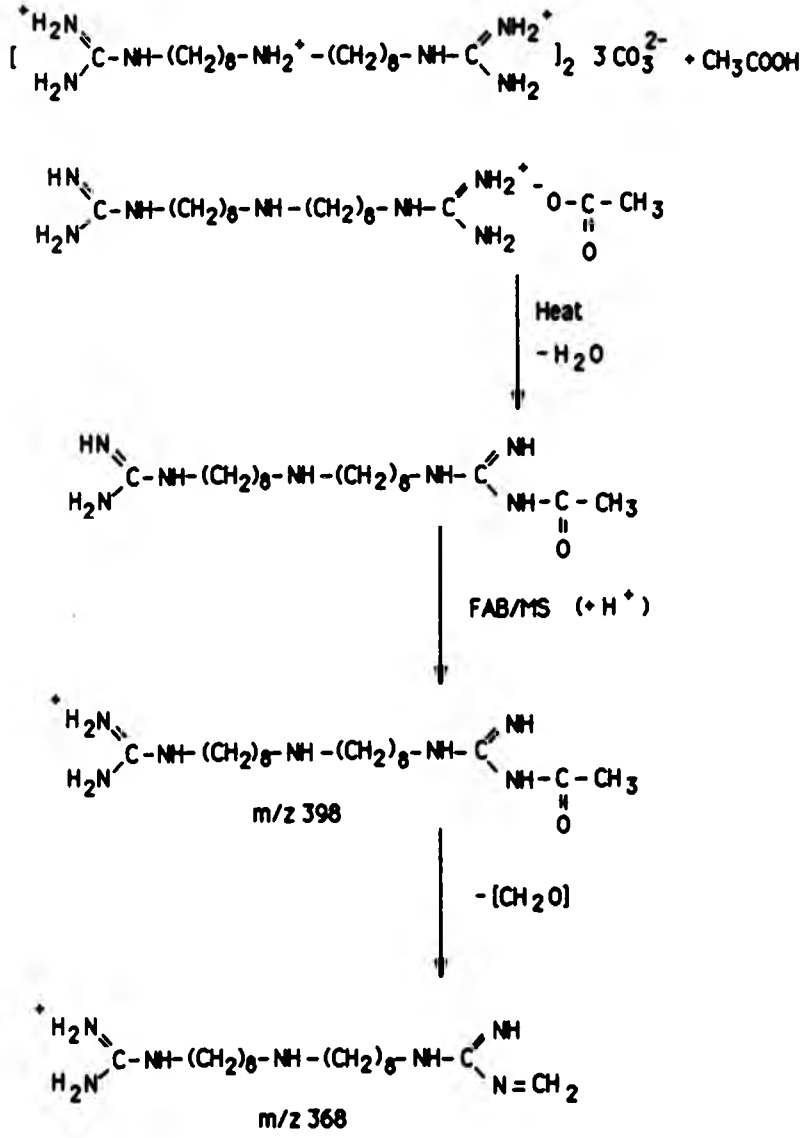


Figure 24. A possible reaction scheme for the acetylation of guazatine.

solution to obtain the pure solid compound (See exp. 4.2.7.3., p. 54), was due the presence of the peak at m/z 368 by FAB/MS. The initial thought was that the peak had originated by acetylation of the guanidino group and subsequent loss of [CH_2O], as shown in Figure 24.

The same 368 peak was however obtained even without heating the solution (Table 20). Different procedures for the preparation of guazatine acetate were therefore attempted in an attempt to explain the presence of this peak at m/z 368, such as the barium acetate and *S*-methylisothiouronium acetate methods (Exp. 4.2.7.4, p. 55 and 4.2.8, p. 57, respectively) in which free acetic acid was not present at any stage. The products also showed the presence of the 368 peak (Table 20).

Figure 23 and Table 20, for the bis-(8-guanidino-octyl)amine triacetate prepared by different procedures, show the expected m/z 356 ion, $[M+H]^+$ as the base peak. The only exception was for the procedure (a) (conversion of GNG-S to GNG-C and after to GNG-A and heating to dry the compound) where the base peak was the ion at m/z 368, $[M+H+12]^+$. In the case of the procedure (c) (conversion of GNG-S to GNG-A through barium acetate method) an additional peak at m/z 454 (relative intensity 27.5%) appeared which was an indication that such conversion was not a hundred percent and some sulphate not converted was left and was identified by FAB/MS. This result also explains the difference in the microanalysis results for C and H when compared with those expected. The presence of this peak at m/z 454 in products of other procedures mentioned above was negligible. The same phenomenon described above, *i.e.* the formation of the peak at m/z 368, also occurred in all cases of GNG-A compounds whatever procedure of preparation was used. Samples from procedures (c) (barium acetate method) and (d) (*S*-methylisothiouronium acetate method) were re-run under FAB with a different matrix (3-nitro-benzyl alcohol), and the peak at m/z 368 was found to be absent. When formaldehyde was added to

Table 28. The positive ion FAB/MS data for bis-(8-guanidino-octyl) amine triacetate (GNG-A) under different procedures of preparation.

Peak (m/z)	Relative Intensity. (%) (a)	(b)	(c)	(d)	Assignment(e)
454	2.5	3.8	27.5	2.3	$[M+3H+SO_4^{2-}]^+$
398	7.6	5.9	7.1	11.1	$[M+H+C+CH_2O]^+$
368	100.0	45.0	33.9	68.2	$[M+H+C]^+$
356	66.6	100.0	100.0	100.0	$[M+H]^+$
339	7.5	7.2	10.1	7.5	$[M+H-NH_3]^+$
314	5.4	8.6	13.1	18.2	$[M+H-NH_2CN]^+$
299	2.4	4.0	6.6	4.6	$[M+H-CH_3N_3]^+$
297	7.9	10.4	17.7	10.4	$[M+H-NH_2C(:NH)NH_2]^+$
283	6.9	6.4	11.2	6.5	$[M+H-NH_2C(:NH)NH_2(CH_2)]^+$
269	4.2	4.6	8.9	5.2	$[M+H-NH_2C(:NH)NH_2(CH_2)_2]^+$
255	4.2	5.4	8.1	5.7	$[M+H-NH_2C(:NH)NH_2(CH_2)_3]^+$
241	4.2	4.2	7.3	4.5	$[M+H-NH_2C(:NH)NH_2(CH_2)_4]^+$
227	3.9	3.8	6.6	5.3	$[M+H-NH_2C(:NH)NH_2(CH_2)_5]^+$
213	7.0	6.9	11.1	8.2	$[M+H-NH_2C(:NH)NH_2(CH_2)_6]^+$
199	25.7	19.3	27.5	24.1	$[M+H-NH_2C(:NH)NH_2(CH_2)_7]^+$
185	—	18.1	25.7	—	$[M+H-NH_2C(:NH)NH_2(CH_2)_8]^+$
170	22.1	24.3	40.4	25.9	$[M+H-NH_2C(:NH)NH_2(CH_2)_8NH]^+$
156	17.2	18.5	26.2	20.6	$[M+H-NH_2C(:NH)NH_2(CH_2)_8NH(CH_2)]^+$
142	10.8	11.7	19.5	13.4	$[M+H-NH_2C(:NH)NH_2(CH_2)_8NH(CH_2)_2]^+$
128	17.3	16.3	26.6	19.4	$[M+H-NH_2C(:NH)NH_2(CH_2)_8NH(CH_2)_3]^+$
114	13.0	14.6	22.5	16.5	$[M+H-NH_2C(:NH)NH_2(CH_2)_8NH(CH_2)_4]^+$
100	15.8	18.1	29.5	19.7	$[M+H-NH_2C(:NH)NH_2(CH_2)_8NH(CH_2)_5]^+$
86	23.4	27.6	41.7	28.8	$[M+H-NH_2C(:NH)NH_2(CH_2)_8NH(CH_2)_6]^+$

(a) Exp. 4.2.7.3, p. 54 (first procedure)

(b) Exp. 4.2.7.3, p. 54 (second procedure)

(c) Exp. 4.2.7.4, p. 55 (barium acetate method)

(d) Exp. 4.2.8, p. 57 (*S*-methylisothiouronium acetate method); m/z 454 (2.3%) might possibly result from sulphate contamination

(e) $M = NH_2C(:NH)NH(CH_2)_8NH(CH_2)_8NHC(:NH)NH_2$

the matrix a dramatic regeneration of the 368 peak was observed and this seems to confirm that under the previous FAB conditions, molecular or ionic species of m/z 355 or 356 undergo condensation with (presumably) H_2CO originating from the glycerol matrix. The FAB spectra of both samples showed $[M+2H]^{2+}$ peaks at m/z 178.5, but no triply-charged species were observed. In addition $[2M+H]^+$ at m/z 711 was observed. In the presence of H_2CO a doubly charged condensation product $[M+12+2H]^{2+}$ at m/z 184.5 was also present.

Several different routes of fragmentation might occur from the base peak which follows the same pattern as that for the sesquisulphate and sesquicarbonate described previously (See p. 111).

Figure 25 and Table 21, for the mixture of guanidated triamine acetates, obtained from pure triamine and cyanamide (Exp. 4.2.4, p. 51), show $[M+H]^+$ ions at m/z 398 (45%), 356 (79%) and 314 (37%) corresponding to the compounds GGG-A, GNG-A or GGN-A, and GNN-A or NGN-A respectively. Such ions arise from a number of different possible structures depending on the site of protonation of the bases and also in the case of the GNG-A or GGN-A and GNN-A or NGN-A components, on the location of the guanidine structure in the molecule. Several routes of fragmentation might occur which are very complex and difficult to show schematically and it is also difficult to identify all the prominent peaks.

Table 21 shows tentatively some of the probable assignments for the prominent peaks that arise during the fragmentation which starts with initial loss of ammonia (1), cyanamide (2), CH_3N_3 (3), or guanidine (4), or by cleavage of the various carbon-carbon or carbon-nitrogen bonds along the octamethylene chain (with hydrogen transfer). The peak at 368 (10%) which is also present for this mixture has the same explanation as stated before and has not been included in the Table.

The presence of the peak at m/z 398 (relative intensity 45%) in the mixture of guanidated triamine acetates, is assigned to the protonated triguanidated triamine compound (GGG). This compound was also confirmed through carbon-13 NMR results (Table 14, p. 104; 1:6) which have shown the presence of a chemical shift at 158.3 ppm due to the carbon that had been introduced by amidination of the central nitrogen of the molecule.

The GNG-S compound also exhibited a weak peak at m/z 398 (relative intensity 2%) (Table 19, p. 112) which might also be due to the triguanidated compound (GGG). This result indicates that the previous statement (See page 108) that *S*-methylisothiouronium sulphate does not produce such compounds (GGG) may not be completely correct. The results of carbon-13 NMR for GNG-S imply that amidination at the central nitrogen of the molecule does not occur under these conditions (Table 15, p. 105). It is clearly important to use both techniques: carbon-13 NMR and FAB/MS, when the identifications of such compound are to be made.

Fast Atom Bombardment (FAB) Mass Spectra for the commercial guazatine (70%; G70) were also obtained. Table 22 shows the prominent peaks from m/z 356 and higher that arise during the fragmentation, with probable assignments which follow the same features as stated before. Some representative peaks which are thought to be due to derivatives of the tetramine appeared in the sample G70 with very small intensities at m/z 567 (1.6%), 525 (2.1%), and 483 (1.8%) corresponding to GGGG, GGGN or GGNG, and GGNN or GNGN or NGGN or GNNG, respectively. Several different routes of fragmentation might occur from these ions but they are complex to show schematically and to identify all the prominent peaks in these spectra would be difficult. The main peaks can be deduced from the previous Tables (19, 20, and, mainly 21).

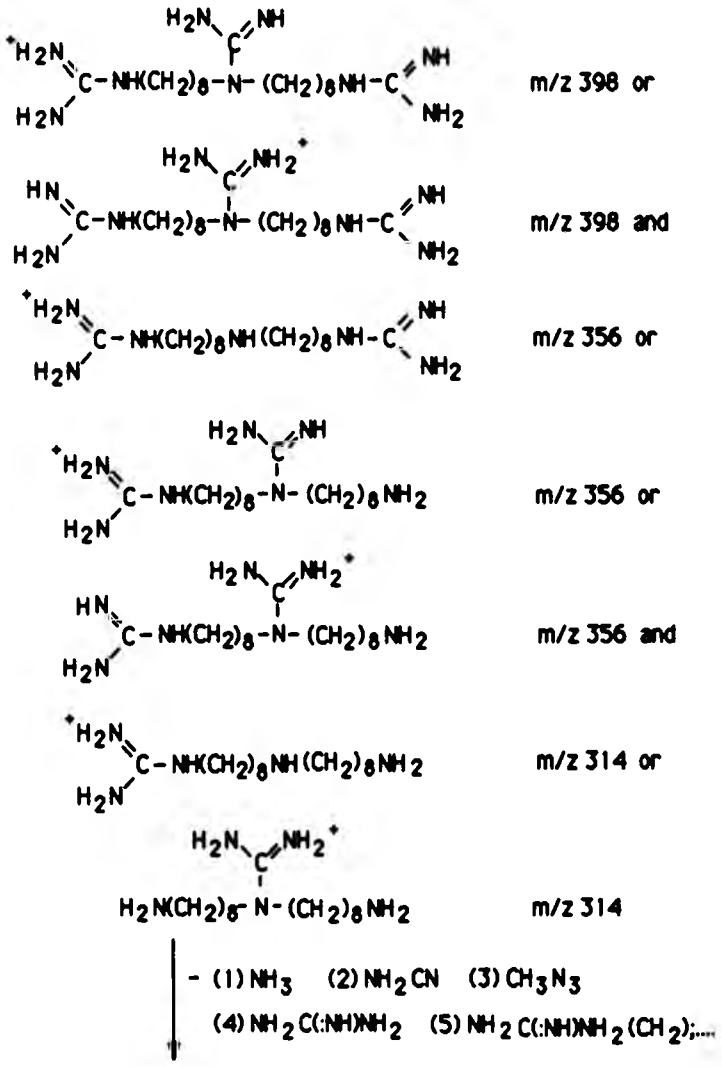


Figure 25. The positive ion FAB/MS fragmentation of a mixture of guanidated amine acetate represented mainly by GGG-A, GNG-A or GGN-A and GNN-A or NGN-A compounds.

Table 21. The positive ion FAB/MS data for a mixture of guanidated triamine acetate compounds

Peak (m/z)	R.I. (%)	Possible Assignment	
454	—		
398	45	$HG^+(CH_2)_8NC(:NH)NH_2(CH_2)_8G$	or
		$G(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_8G$	
381	4	$HG^+(CH_2)_8NC(:NH)NH_2(CH_2)_8NHCN$	or
		$HG^+(CH_2)_8N(CN)(CH_2)_8G$	or
		$G(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_8NHCN$	
356	79	$HG^+(CH_2)_8NC(:NH)NH_2(CH_2)_8NH_2$	or
		$HG^+(CH_2)_8NH(CH_2)_8G$	or
		$G(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_8NH_2$	
341	12	$HG^+(CH_2)_8NC(:NH)NH_2(CH_2)_7CH_3$	or
		$G(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_7CH_3$	
339	21	$HG^+(CH_2)_8NH(CH_2)_8NHCN$	or
		$HG^+(CH_2)_8NCN(CH_2)_8NH_2$	or
		$NH_2(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_8NHCN$	or
		$HG^+(CH_2)_8NC(:NH)NH_2(CH_2)_6CH=CH_2$	or
		$G(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_6CH=CH_2$	
327	16	$HG^+(CH_2)_8NC(:NH)NH_2(CH_2)_6CH_3$	or
		$G(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_6CH_3$	
325	13	$HG^+(CH_2)_8NC(:NH)NH_2(CH_2)_5CH=CH_2$	or
		$G(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_5CH=CH_2$	
324	8	$HG^+(CH_2)_8NCN(CH_2)_7CH_3$	or
		$CH_3(CH_2)_7NC(:NH_2^+)NH_2(CH_2)_8NHCN$	
322	7	$HG^+(CH_2)_8NCN(CH_2)_6CH=CH_2$	or
		$CH_2=CH(CH_2)_6NC(:NH_2^+)NH_2(CH_2)_8NHCN$	
314	37	$HG^+(CH_2)_8NH(CH_2)_8NH_2$	or
		$NH_2(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_8NH_2$	
311	17	$HG^+(CH_2)_8NC(:NH)NH_2(CH_2)_4CH=CH_2$	or
		$G(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_4CH=CH_2$	
310	28	$HG^+(CH_2)_8NCN(CH_2)_6CH_3$	or
		$NCNH(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_6CH_3$	or
308	9	$HG^+(CH_2)_8NCN(CH_2)_5CH=CH_2$	or
		$CH_2=CH(CH_2)_5NC(:NH_2^+)NH_2(CH_2)_8NHCN$	
299	14	$HG^+(CH_2)_8NH(CH_2)_7CH_3$	or

Table 21. cont.

		$CH_3(CH_2)_7NC(:NH_2^+)NH_2(CH_2)_8NH_2$	
297	30	$HG^+(CH_2)_8NH(CH_2)_6CH=CH_2$	or
		$CH_2=CH(CH_2)_6NC(:NH_2^+)NH_2(CH_2)_8NH_2$	or
		$HG^+(CH_2)_8NC(:NH)NH_2(CH_2)_3CH=CH_2$	or
		$G(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_3CH=CH_2$	
294	6	$HG^+(CH_2)_8NCN(CH_2)_4CH=CH_2$	or
		$CH_2=CH(CH_2)_4NC(:NH_2^+)NH_2(CH_2)_8NHCN$	
285	20	$HG^+(CH_2)_8NH(CH_2)_6CH_3$	or
		$CH_3(CH_2)_6NC(:NH_2^+)NH_2(CH_2)_8NH_2$	
284	23	$CH_3(CH_2)_7NC(:NH_2^+)NH_2(CH_2)_7CH_3$	
283	18	$HG^+(CH_2)_8NH(CH_2)_5CH=CH_2$	or
		$CH_2=CH(CH_2)_5NC(:NH_2^+)NH_2(CH_2)_8NH_2$	or
		$HG^+(CH_2)_8NC(:NH)NH_2(CH_2)_2CH=CH_2$	or
		$G(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_2CH=CH_2$	
282	12	$CH_2=CH(CH_2)_6NC(:NH_2^+)NH_2(CH_2)_7CH_3$	
280	6	$HG^+(CH_2)_8NCN(CH_2)_3CH=CH_2$	or
		$CH_2=CH(CH_2)_3NC(:NH_2^+)NH_2(CH_2)_8NHCN$	or
		$CH_2=CH(CH_2)_6NC(:NH_2^+)NH_2(CH_2)_6CH=CH_2$	
269	17	$HG^+(CH_2)_8NH(CH_2)_4CH=CH_2$	or
		$HG^+(CH_2)_8NC(:NH)NH_2CH_2CH=CH_2$	or
		$CH_2=CH(CH_2)_4NC(:NH_2^+)NH_2(CH_2)_8NH_2$	or
		$G(CH_2)_8NC(:NH_2^+)NH_2CH_2CH=CH_2$	
268	13	$CH_2=CH(CH_2)_5NC(:NH_2^+)NH_2(CH_2)_7CH_3$	
266	8	$HG^+(CH_2)_8NCN(CH_2)_2CH=CH_2$	or
		$CH_2=CH(CH_2)_2NC(:NH_2^+)NH_2(CH_2)_8NHCN$	or
		$CH_2=CH(CH_2)_5NC(:NH_2^+)NH_2(CH_2)_6CH=CH_2$	
255	13	$HG^+(CH_2)_8NH(CH_2)_3CH=CH_2$	or
		$HG^+(CH_2)_8NC(:NH)NH_2CH=CH_2$	or
		$CH_2=CH(CH_2)_3NC(:NH_2^+)NH_2(CH_2)_8NH_2$	or
		$G(CH_2)_8NC(:NH_2^+)NH_2CH=CH_2$	
254	10	$CH_2=CH(CH_2)_4NC(:NH_2^+)NH_2(CH_2)_7CH_3$	
252	8	$HG^+(CH_2)_8NCN(CH_2)CH=CH_2$	or
		$CH_2=CH(CH_2)NC(:NH_2^+)NH_2(CH_2)_8NHCN$	or
		$CH_2=CH(CH_2)_4NC(:NH_2^+)NH_2(CH_2)_6CH=CH_2$	
241	10	$HG^+(CH_2)_8NH(CH_2)_2CH=CH_2$	or
		$CH_2=CH(CH_2)_2NC(:NH_2^+)NH_2(CH_2)_8NH_2$	

Table 21. cont.

240	7	$CH_2=CH(CH_2)_3NC(:NH_2^+)NH_2(CH_2)_7CH_3$	
238	8	$HG^+(CH_2)_8NCNCH=CH_2$	or
		$CH_2=CHNC(:NH_2^+)NH_2(CH_2)_8NHCN$	or
		$CH_2=CH(CH_2)_3NC(:NH_2^+)NH_2(CH_2)_6CH=CH_2$	
227	8	$HG^+(CH_2)_8NH(CH_2)CH=CH_2$	or
		$CH_2=CH(CH_2)NC(:NH_2^+)NH_2(CH_2)_8NH_2$	
226	10	$CH_2=CH(CH_2)_2NC(:NH_2^+)NH_2(CH_2)_7CH_3$	
224	8	$CH_2=CH(CH_2)_2NC(:NH_2^+)NH_2(CH_2)_6CH=CH_2$	
213	16	$HG^+(CH_2)_8NHCH=CH_2$	or
		$CH_2=CHNC(:NH_2^+)NH_2(CH_2)_8NH_2$	
212	13	$CH_2=CH(CH_2)NC(:NH_2^+)NH_2(CH_2)_7CH_3$	
210	10	$CH_2=CH(CH_2)NC(:NH_2^+)NH_2(CH_2)_6CH=CH_2$	
199	15	$HG^+(CH_2)_8N=CH_2$	
198	12	$CH_2=CHNC(:NH_2^+)NH_2(CH_2)_7CH$	
196	10	$CH_2=CHNC(:NH_2^+)NH_2(CH_2)_6CH=CH_2$	
185	34	$HG^+(CH_2)_7CH=NH$	
182	12	$CH_2=CHNC(:NH_2^+)NH_2(CH_2)_5CH=CH_2$	
170	70	$HG^+(CH_2)_6CH=CH_2$	
168	24	$CH_2=CHNC(:NH_2^+)NH_2(CH_2)_4CH=CH_2$	
156	47	$HG^+(CH_2)_5CH=CH_2$	
154	14	$CH_2=CHNC(:NH_2^+)NH_2(CH_2)_3CH=CH_2$	
142	38	$HG^+(CH_2)_4CH=CH_2$	
140	14	$CH_2=CHNC(:NH_2^+)NH_2(CH_2)_2CH=CH_2$	
128	48	$HG^+(CH_2)_3CH=CH_2$	
126	16	$CH_2=CHNC(:NH_2^+)NH_2(CH_2)CH=CH_2$	
114	36	$HG^+(CH_2)_2CH=CH_2$	
112	14	$CH_2=CHNC(:NH_2^+)NH_2CH=CH_2$	
100	35	$HG^+(CH_2)CH=CH_2$	
86	57	$HG^+CH=CH_2$	

(a) $G = NH_2C(:NH)NH-$.

Table 22. The positive ion FAB/MS data for commercial guazatine (70%)

Peak (m/z)	Rel. Intens. (%)	Assignment[a]	
567	1.6	$[GH^+(CH_2)_8NC(:NH)NH_2(CH_2)_8NC(:NH)NH_2(CH_2)_8NHG]$	or
		$[G(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_8NC(:NH)NH_2(CH_2)_8NHG]$	
525	2.1	$[GH^+(CH_2)_8NC(:NH)NH_2(CH_2)_8NC(:NH)NH_2(CH_2)_8NH_2]$	or
		$[G(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_8NC(:NH)NH_2(CH_2)_8NH_2]$	or
		$[G(CH_2)_8NC(:NH)NH_2(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_8NH_2]$	or
		$[GH^+(CH_2)_8NC(:NH)NH_2(CH_2)_8NH(CH_2)_8G]$	or
		$[G(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_8NH(CH_2)_8G]$	or
		$[G(CH_2)_8NC(:NH)NH_2(CH_2)_8NH(CH_2)_8GH^+]$	
483	1.8	$[GH^+(CH_2)_8NC(:NH)NH_2(CH_2)_8NH(CH_2)_8NH_2]$	or
		$[G(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_8NH(CH_2)_8NH_2]$	or
		$[GH^+(CH_2)_8NH(CH_2)_8NC(:NH)NH_2(CH_2)_8NH_2]$	or
		$[G(CH_2)_8NH(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_8NH_2]$	or
		$[GH^+(CH_2)_8NH(CH_2)_8NH(CH_2)_8G]$	or
		$[NH_2(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_8NC(:NH)NH_2(CH_2)_8NH_2]$	
398	24.9	$[GH^+(CH_2)_8NC(:NH)NH_2(CH_2)_8G]$	or
		$[G(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_8G]$	
368	6.6	[356+12]	
356	20.0	$[GH^+(CH_2)_8NH(CH_2)_8G]$	or
		$[GH^+(CH_2)_8NC(:NH)NH_2(CH_2)_8NH_2]$	or
		$[G(CH_2)_8NC(:NH_2^+)NH_2(CH_2)_8NH_2]$	or

a) G = $NH(:NH)NH_2$; GH^+ = $NH(:NH_2^+)NH_2$

The Fast Atom Bombardment (FAB) Mass Spectrum was also obtained for the fungicide dodine and the results are shown in Table 23 and Figure 26.

The expected m/z 228 ion, $[M+H]^+$ was obtained as the base peak. Fragmentation of the carbon chain occurs with the loss of hydrocarbon fragments $CH_3(CH_2)_nCH_3$ (n = 0 to 8) to give a series of weak peaks separated by 14 mass units from m/z 198 down to 86.

Table 23. The positive ion FAB/MS data of the fungicide dodine.

Peak (m/z)	Rel. Int. (%)	Assignment(a)
228	100.0	$[M+H]^+$
198	1.3	$[M+H-C_2H_6]^+$
184	1.6	$[M+H-C_3H_8]^+$
170	1.5	$[M+H-C_4H_{10}]^+$
156	1.4	$[M+H-C_4H_{12}]^+$
142	1.8	$[M+H-C_6H_{14}]^+$
128	1.8	$[M+H-C_7H_{16}]^+$
114	1.6	$[M+H-C_8H_{18}]^+$
100	1.6	$[M+H-C_9H_{20}]^+$
86	3.2	$[M+H-C_{10}H_{22}]^+$

a) M = $CH_3(CH_2)_{11}NHC(:NH_2^+)NH_2$.

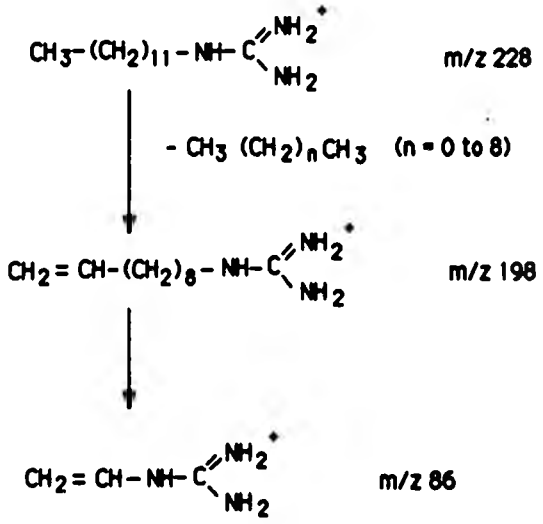


Figure 26. The positive ion FAB/MS fragmentation of the fungicide dodine.

An important feature of the fragmentation process of guanidine compounds was that the positive charge remained on the nitrogen-containing fragment.

Reacting bis-(8-guanidino-octyl)amine sesquicarbonate (obtained through experiment 4.2.7.3, p. 54), with cyanamide in a molar ratio of (1:2), respectively, an attempt to obtain the triguanidated compound (GGG-A) was made (See exp. 4.2.9, p. 58). The resultant product was characterized by carbon-13 NMR and its results are shown in Table 24.

The chemical shift at 158.8 ppm from Table 24 clearly shows that the amidination of the central nitrogen of the molecule of GNG-C was obtained and consequently the triguanidated product (GGG-A) was produced. The same results also show that the resultant product is a mixture of others components besides the GGG-A compound.

Table 24. Carbon-13 NMR chemical shifts (δ) of a probable GGG-A compound.

δ (ppm)	Intensity	Assignment (C No.)
25.4	25272	1
28.6	70219	2
29.5	20647	3
30.8	53058	4
31.1	49819	5
31.3	40131	6
44.2	34929	8
50.5	13083	9
51.8	15038	10
158.8	5546	12
159.9	13464	13
182.9	17554	14

5 - PREPARATION OF DI-*n*-OCTYLGUANIDINIUM DERIVATIVES

In order to compare the reactions of cyanamide and of *S*-methylisothiouronium sulphate with a secondary amino group (such as the central nitrogen of the triamine), di-*n*-octylamine was separately treated with both reagents as shown in Figures 27 and 28, respectively.

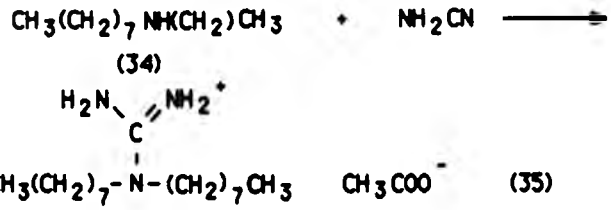


Figure 27. Reaction scheme for the preparation of di-*n*-octylguanidinium acetate, via cyanamide.

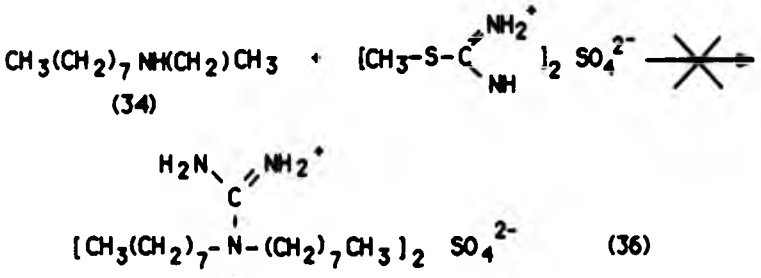
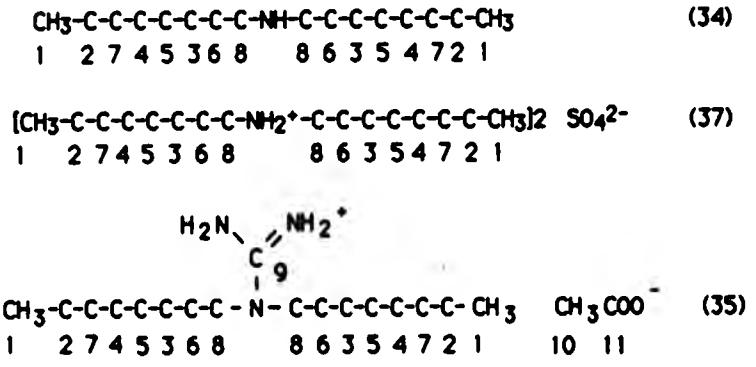


Figure 28. Reaction scheme for the attempted preparation of di-*n*-octylguanidinium sulphate, via *S*-methylisothiouronium sulphate.

Di-*n*-octylamine, its sulphate and the resulting product from Figure 27 (di-*n*-octylguanidinium acetate) were analysed by carbon-13 NMR and the

results are shown in Tables 25 and 26.

To facilitate the assignment of chemical shifts in the amidinated product the carbon atoms for di-*n*-octylamine, its sulphate and di-*n*-octylguanidinium acetate were numbered as follows:



The assignments from Table 25 for di-*n*-octylamine were according to Eggert *et al.*[87], and the same assignments were also used for the sulphate (Table 25) and di-*n*-octylguanidinium acetate (Table 26).

The chemical shift for CH_2 adjacent to nitrogen is at slightly higher field for the di-*n*-octylamine than for the corresponding ammonium and guanidinium salts in this case. The solvent system is however different and the overall molecular structure may also have an effect.

The reaction with *S*-methylisothiouronium sulphate (Figure 28) did not give the desired product. Carbon-13 NMR showed no evidence for the amidinated compound with chemical shift around 158.0 ppm.

Table 25. Carbon-13 NMR chemical shifts (δ) of di-*n*-octylamine and its sulphate.

Amine[a]		Amine[b]		Sulphate[c]		Assignment
δ(ppm)	Intens.	δ(ppm)	Intens.	δ(ppm)	Intens.	(C No.)
14.2	—	14.2	839	16.7	10052	1
23.0	—	22.9	3174	25.3	10588	2
27.8	—	27.7	4172	28.8	11475	3
29.8	—	29.6	5296	—	—	4
29.9	—	29.9	4841	—	—	5
30.1	—	30.6	4411	31.6	14692	6
32.3	—	32.2	4269	34.4	9604	7
50.4	—	50.4	3929	52.1	4495	8

a) From literature (in C_6D_6)
b) Amine (in $CDCl_3$)
c) Sulphate (in D_2SO_4/H_2SO_4)

Table 26. Carbon-13 NMR chemical shifts (δ) of di-*n*-octylguanidinium acetate[a].

δ(ppm)	Intensity	Assignment (C No.)
14.3	12485	1
23.4	14712	2
27.0	7452	10
27.3	10358	3
28.3	7992	4
29.9	15820	5
32.7	11261	6
48.9	9897	7
52.1	1727	8
157.6	1998	9
178.9	2457	11

a) In CD_3OD.

Fast Atom Bombardment(FAB) Mass Spectra for di-*n*-octylamine and its acetate were also obtained and the results are shown in Table 27 and Figure 29.

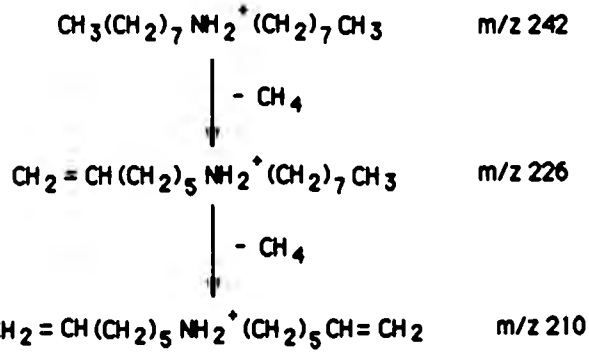


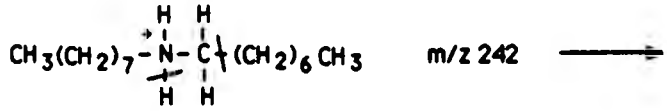
Figure 29. The positive ion FAB/MS fragmentation of di-*n*-octylamine and its acetate.

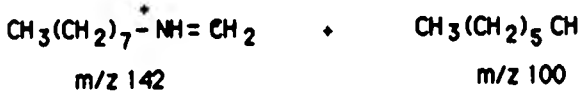
The expected m/z 242 ion, $[M+H]^+$, for di-*n*-octylamine and its acetate was obtained in each case. The fragmentation scheme (Figure 29) is not completely clear but appears to involve loss of two molecules of methane initially. Other fragmentation involving losses of additional CH_2 units give ions such as the following:

$$CH_2=CH-(CH_2)_m-NH_2^+-(CH_2)_n-CH=CH_2$$

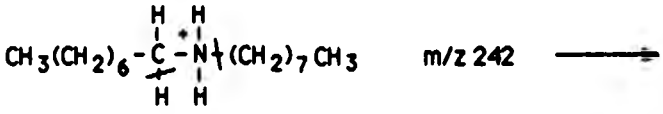
from m/z 210 (m=5, n=5) down to m/z 70 (m=n=0)

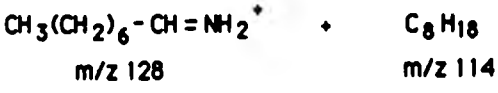
The ion at m/z 142 may be formed by cleavage β to the N atom with H transfer as follows:





And the ion at m/z 128 by cleavage α to the N atom with H transfer as follows:





An additional peak at m/z 254 appeared in the spectra for both di-*n*-octylamine and its acetate (relative intensity variable depending on the matrix used) and might be due to the formation of an iminium ion as described previously (See Figure 7, p. 83), when the sample reacts with formaldehyde originated from the matrix (glycerol). When formaldehyde was also added the peak at m/z 254, $[M+H+12]^+$, was intensified and in the case of di-*n*-octylamine became the base peak (100.0%). Therefore these results confirmed the results obtained previously for the peak $[M+H+12]^+$ in the case of guazatine (See p. 116),and are similar to those obtained by Lehmann *et al*.[83] and Pang *et al*.[84], in the case of primary amino compounds.

Table 27. The positive ion FAB/MS data of di-*n*-octylamine and its acetate

Peak (m/z)	Relative intensity(%) Am.(b)	Ac.(b)	Am.(c)	Ac.(c)	Ac.(d)	Assignment(a)
354	10.5	0.1	0.8	0.1	1.2	
352	25.2	0.2	2.8	0.3	0.6	
254	30.6	2.3	100.0	49.8	100.0	$[M+H+12]^+$
242	100.0	100.0	—	100.0	33.1	$[M+H]^+$
240	43.4	9.6	2.4	8.0	12.3	
226	3.8	—	3.7	3.1	10.2	$[M+H-CH4]^+$
210	2.3	—	—	—	2.6	$[M+H-(CH_4)_2]^+$
196	2.7	—	1.2	1.2	2.3	$[M+H-(CH_4)_2CH_2]^+$
182	3.2	—	1.8	1.1	2.5	$[M+H-(CH_4)_2(CH_2)_2]^+$
168	6.9	—	1.4	1.1	3.0	$[M+H-(CH_4)_2(CH_2)_3]^+$
154	7.4	—	16.5	6.4	13.5	$[M+H-(CH_4)_2(CH_2)_4]^+$
142	17.3	11.1	1.4	10.4	25.1	$[M+H-CH_3(CH_2)_5CH_3]^+$
140	7.4	—	2.0	2.4	4.6	$[M+H-(CH_4)_2(CH_2)_5]^+$
128	2.5	2.0	—	1.5	3.8	$[M+H-CH_3(CH_2)_6CH_3]^+$
126	2.3	—	1.8	1.2	2.6	$[M+H-(CH_4)_2(CH_2)_6]^+$
112	2.3	—	2.0	2.4	3.4	$[M+H-(CH_4)_2(CH_2)_7]^+$
98	3.8	—	2.4	1.2	4.2	$[M+H-(CH_4)_2(CH_2)_8]^+$
84	5.3	—	2.8	1.8	5.8	$[M+H-(CH_4)_2(CH_2)_9]^+$
70	7.6	1.4	2.0	2.4	6.8	$[M+H-(CH_4)_2(CH_2)_{10}]^+$
69	13.0	2.7	2.7	4.2	9.4	

a) $[M+H]^+ = CH_3(CH_2)_7NH_2^+(CH_2)_7CH_3$

b) With glycerol (Am.= Amine, Ac.= Acetate)

c) With glycerol/formaldehyde

d) With glycerol/formaldehyde and increased time.

6 - ACETYLACETONE (AA) DERIVATIZATION

Acetylacetone (38) was used as a derivatization reagent to convert the synthesized guanidine compounds into stable and volatile derivatives to ensure successful gas chromatographic analysis.

Guanidine hydrochloride (39) was first condensed with acetylacetone following the method of Palaitis and Curran[79], as shown in Figure 30.

The acetylacetone derivative of guanidine hydrochloride, 2-amino-4,6-dimethylpyrimidine, (40) was characterized by microanalysis, carbon-13 NMR and low resolution mass spectrometry.

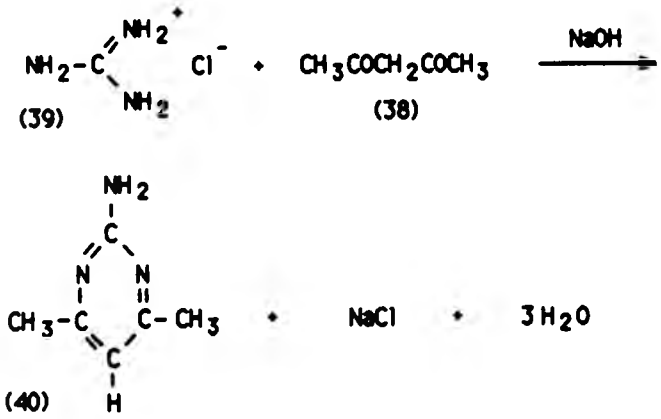
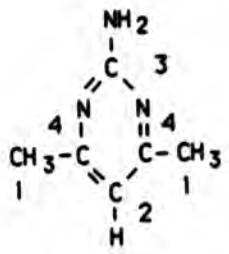


Figure 30. Reaction scheme for the preparation of 2-amino-4,6-dimethylpyrimidine.

In order to assign the chemical shifts the carbon atoms were numbered as follows:



(40)

Table 28 shows the results obtained for the acetylacetone derivative of guanidine hydrochloride run in $CDCl_3$ and the results obtained by Palaitis and Curran[79].

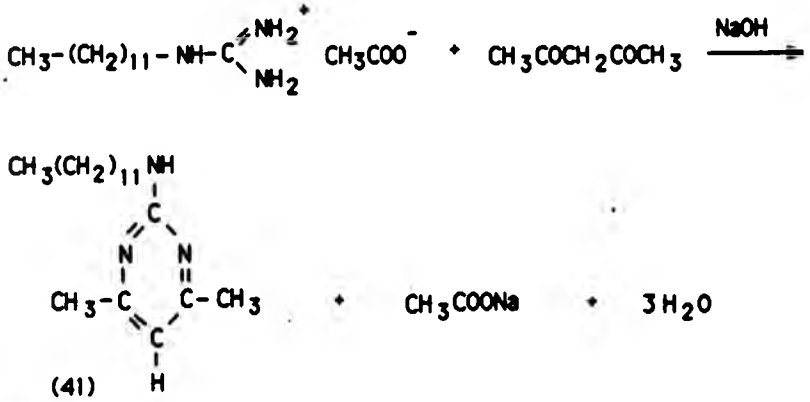
Comparing the results in Table 28, it is clear that the derivative was obtained. The result was also confirmed by electron impact mass spectrometry which showed the presence of the molecular ion at m/z 123 (100%) and a fragment ion at m/z 108 (6.4%), due to loss of methyl.

Table 28. Carbon-13 NMR chemical shifts (δ) of acetylacetone derivative of guanidine hydrochloride, 2-amino-4,6-dimethyl-pyrimidine

Derivative Obtained δ(ppm)	Derivative Literature δ(ppm)	Assignment (C No.)
23.7	23.6	1
110.5	110.3	2
163.2	163.0	3
167.9	167.7	4

6.1- Dodine/acetylacetone derivative

It was expected that the acetylacetone derivative of dodine would be obtained by the reaction shown in Figure 31.



(41)

Figure 31. Reaction scheme for the preparation of dodine/acetylacetone derivative.

The derivative (41) could not be obtained in a pure form but was characterized by low resolution mass spectrometric analysis which showed the presence of the molecular ion at m/z 291, [M] (12.8%) and the fragments at m/z 207 (19.0%), 192 (15.8%), 178 (12.8%), 150 (18.3%), 149 (74.6%), 137 (64.1%), 136 (100.0%), 123 (59.9%), 108 (9.6%), 83 (13.0%), 69 (13.5%), and 56 (38.5%), which are the prominent features of the mass spectrum of the derivative. (Results for 4 and 20 hours of reaction did not differ).

6.2- 1,8-Diguanidino-octane/acetylacetone derivative

The acetylacetone derivative (42) of 1,8-diguanidino-octane was prepared from the sulphate by the reaction shown in Figure 32, and was characterized by microanalysis, carbon-13 NMR and low resolution mass spectrometric analysis. In this case a pure product was obtained.

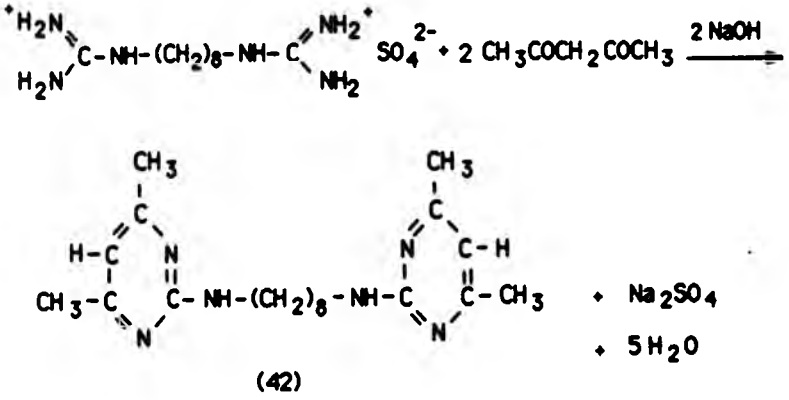
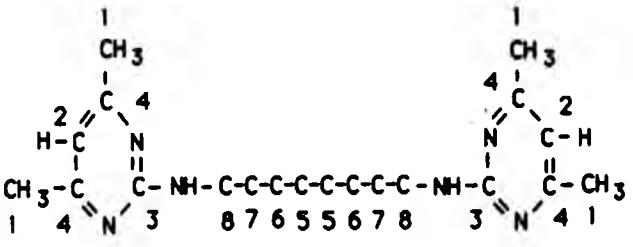


Figure 32. Reaction scheme for the preparation of 1,8-diguanidino-octane/ acetylacetone derivative, from the sulphate (GG-S/AA).

Table 29 shows the carbon-13 data for a GG-S/AA derivative run in $CDCl_3$.

In order to assign the chemical shifts the carbon atoms were numbered as follows:



(42)

Table 29. Carbon-13 NMR chemical shifts (δ) for the acetylacetone derivative of 1,8-diguanidino-octane sulphate.

δ (ppm)	Intensity	Assignment (C No.)
23.8	90950	1
26.9	77699	5
29.3	89837	6
29.7	78615	7
41.4	81915	8
109.4	90546	2
162.2	23002	3
167.4	64545	4

The chemical shifts for carbon atoms 1,2,3, and 4 were comparable to those obtained for the acetylacetone derivative of guanidine hydrochloride (Table 28), whilst the chemical shifts for carbon atoms 5,6,7, and 8 exhibited nearly the same range of values that were found for the central chain of 1,8-diguanidino-octane sulphate itself (Table 6, p. 87). This new compound was further identified by microanalysis and by the mass spectrometric results which clearly showed the presence of the molecular ion at m/z 356, $[M]^+$, (26.5%) and fragment ions at m/z 234 (14.6%), 220 (43.2%), 206 (17.0%), 192 (23.4%), 178 (18.2%), 164 (12.2%), 150 (58.8%),

136 (100.0%), 123 (66.6%), and 108 (13.1%), as shown in Figure 33.

Fragmentation occurs with the loss of one pyrimidine ring attached to $NH(CH_2)_n$ (n=0 to 7) to give a series of peaks separated by 14 units from m/z 234 down to 136.

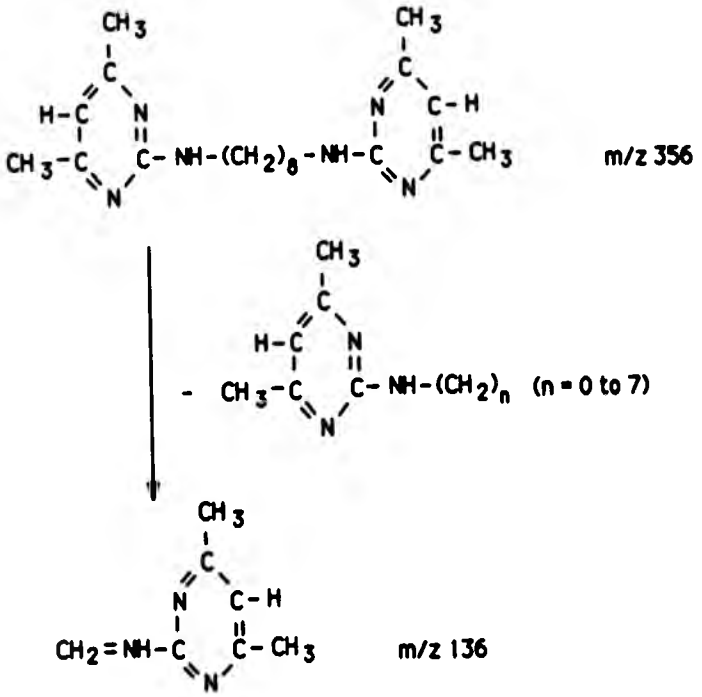


Figure 33. The electron impact mass spectrometry fragmentation of 1,8-diguanidino-octane/acetylacetone derivative.

7 - HEXAFLUOROACETYLACETONE (HFAA) DERIVATIZATION

Kobayashi *et al.*[55] have described the preparation of the hexafluoroacetylacetone derivative of bis-(8-guanidino-octyl)amine for the determination of residue levels of this guanidine in rice grain.

Following the method of Kobayashi *et al.*[55], hexafluoroacetylacetone (43) was also used in the present work as a derivatization reagent to convert the various synthesized guanidine compounds into stable and volatile derivatives to enable gas chromatographic analysis to be carried out.

7.1- 1,8-Diguanidino-octane/HFAA derivative

7.1.1- Preparation from the sulphate

The hexafluoroacetylacetone derivative of 1,8-diguanidino-octane was obtained from the sulphate through the reaction shown in Figure 34.

The identity of this new derivative (44) was confirmed by high resolution mass spectrometry for which the results are shown in Table 30. The molecular ion confirmed the elemental composition of the molecule and other prominent peaks appeared at m/z 552 (7.6%), $[M-HF]^+$, 532 (28.3%), $[M-2HF]^+$, 244 (100.0%), $[M-328]^+$, and 224 (5.5%), $[M-348]^+$, revealing that the desired derivative was obtained.

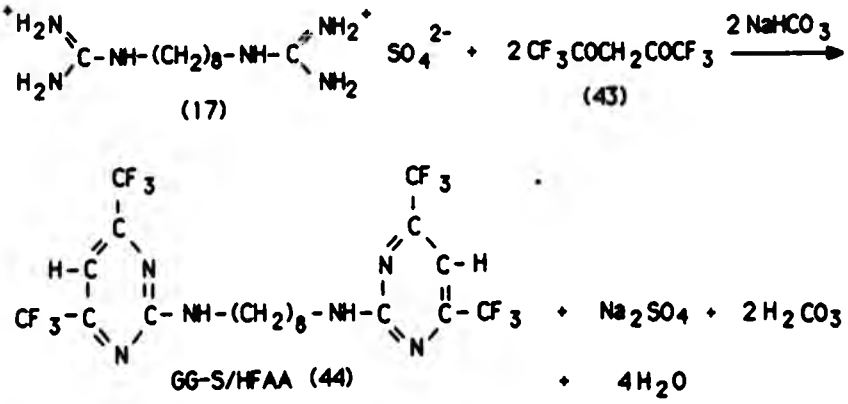
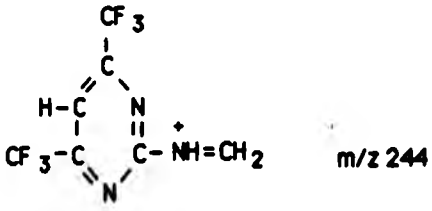


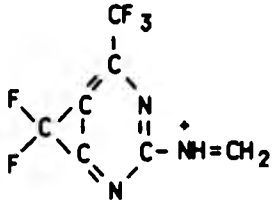
Figure 34. Reaction scheme for the preparation of 1,8-diguanidino-octane/hexafluoroacetylacetone derivative, from the sulphate.

The strong peak at m/z 244 indicates the presence of the pyrimidine ring and adjacent $NHCH_2$ group.



The fragment ion peaks at m/z 552 and 532 were considered to be produced by loss of HF and 2HF, respectively.

The HFAA derivative also exhibited a peak at m/z 224 which may have the structure shown below.



m/z 224

Table 38. Mass spectral data and elemental composition of ions for the hexafluoroacetylacetone derivative of 1,8-diguanidino-octane sulphate (GG-S/HFAA)

Exact Mass Observed	Elemental Formula C	H	N	F	Exact Mass Calculated	Error (mmu)	R.I. (%)	Remarks
572.15497	20	20	6	12	572.15688	0.8	7.6	$[M]^+$
552.14893	20	19	6	11	552.15056	0.6	28.3	$[M-HF]^+$
532.14088	20	18	6	10	532.14424	2.4	35.6	$[M-2HF]^+$
244.03053	7	4	3	6	244.03149	0.4	100.0	$[M-328]^+$
224.02286	7	3	3	5	224.02517	1.9	5.5	$[M-348]^+$

7.1.2- Preparation from the acetate

The hexafluoroacetylacetone derivative of 1,8-diguanidino-octane was also obtained from the acetate by an analogous reaction as shown in Figure 35.

The identity of this derivative (45), which is the same as that (44) derived from the sulphate, was confirmed by low resolution mass spectrometry which showed the molecular ion at m/z 572 (2.0%), $[M]^+$, and other prominent peaks at m/z 552 (10.3%), $[M-HF]^+$, 532 (15.6%), $[M-2HF]^+$, 244 (100.0%), $[M-328]^+$, and 224 (7.8%).

The GG-A/HFAA derivative was also characterized by carbon-13 NMR and the results are shown in Table 31. The spectra were run in CD_3OD.

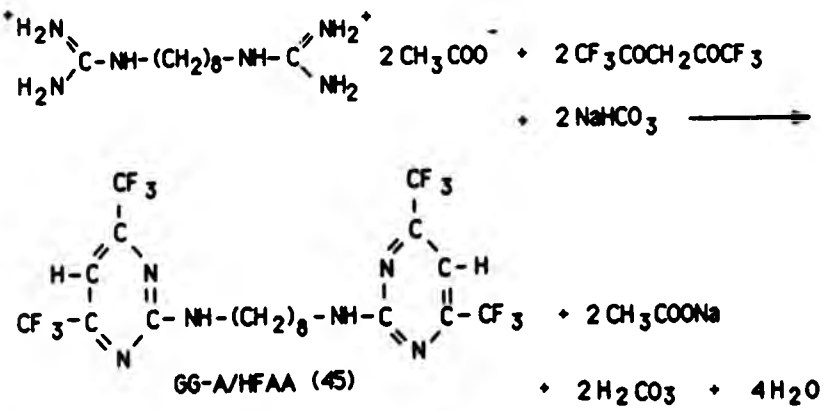
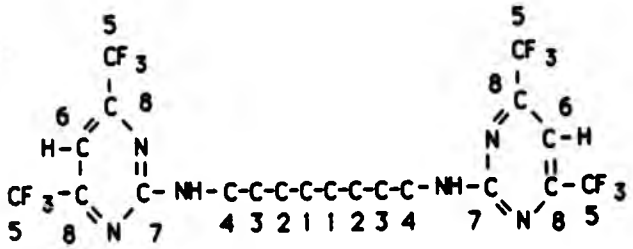


Figure 35. Reaction scheme for the preparation of 1,8-diguanidino-octane/hexafluoroacetylacetone derivative, from the acetate.

In order to assign the chemical shifts the carbon atoms were numbered as follows:



(45)

Table 31. Carbon-13 NMR chemical shifts (δ) for the 1,8-diguanidino-octane acetate/hexafluoroacetylacetone derivative.

δ (ppm)	Intensity	Assignment (C No.)
27.9	11362	1
30.0	16012	2
30.3	12918	3
42.4	10613	4
100.5	3639	5
100.7	4503	6
159.4	1857	7
164.4	1207	8

The assignments were made by analogy with the results for the GG-S/AA derivative which are in Table 29.

The chemical shifts for carbon atoms 1,2,3, and 4 exhibited nearly the same range of values that was found for the central chain of 1,8-diguanidino-octane acetate itself (Table 6, p. 87).

The ^1H NMR spectrum of the GG-A/HFAA derivative had a signal for the pyrimidine ring proton at 7.1 ppm, and integration of this signal indicated the presence of two protons. Thus it was confirmed that the two guanidine groups of 1,8-diguanidino-octane acetate had reacted with HFAA to form two pyrimidine rings as shown.

7.2- Bis-(8-guanidino-octyl)amine/HFAA derivative

The hexafluoroacetylacetone derivative of bis-(8-guanidino-octyl) amine was obtained from the triacetate through the reaction shown in Figure 36, and from the sesquicarbonate by the reaction shown in Figure 37.

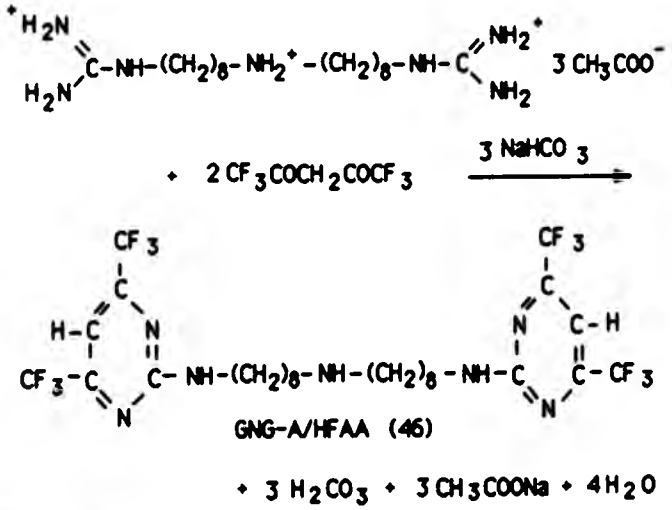


Figure 36. Reaction scheme for the preparation of bis-(8-guanidino-octyl) amine/hexafluoroacetylacetone derivative, from the triacetate.

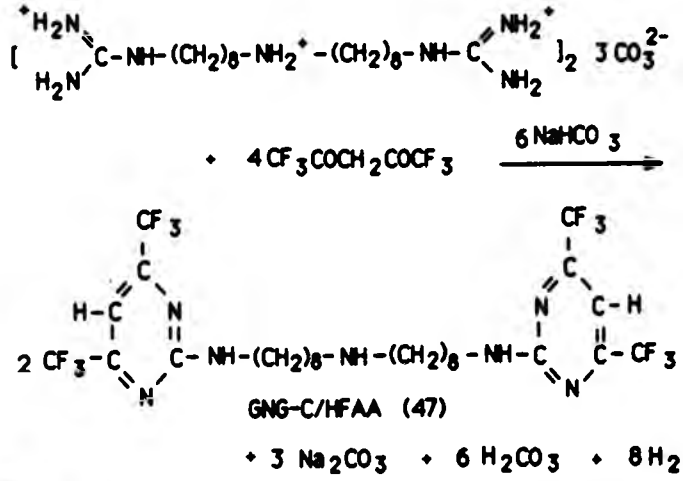
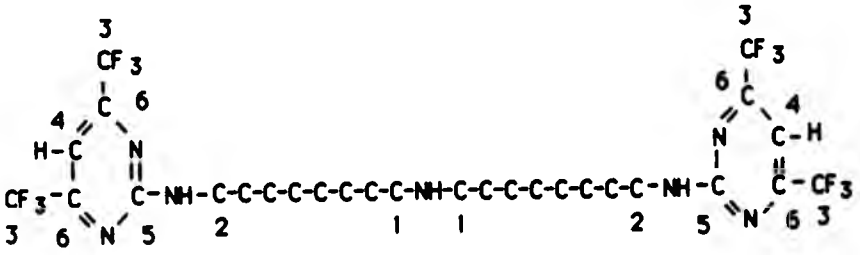


Figure 37. Reaction scheme for the preparation of bis-(8-guanidino-octyl) amine/hexafluoroacetylacetone derivative, from the sesquicarbonate

The derivative in both cases was characterized by carbon-13 NMR and the results are shown in Table 32. The spectra were run in CD_3OD.

In order to assign the chemical shifts the carbon atoms of the derivative were numbered as follows:



(46 or 47)

Table 32. Carbon-13 NMR chemical shifts (δ) of hexafluoroacetylacetone derivative of bis-(8-guanidino-octyl)amine

GNG-A/HFAA(46) δ(ppm)	Intensity	GNG-C/HFAA (47) δ(ppm)	Intensity	Assignment (C No.)
27.3	18547	27.8	54477	Central
27.6	20948	28.0	45214	carbon
27.8	18416	28.9	35722	chain
28.6	3820	30.0	57675	"
30.0	30893	30.2	58552	"
30.1	35141	30.3	53728	"
42.4	15886	42.4	37069	1
50.2	45086	50.2	88425	2
100.7	7821	100.7	15336	3
128.5	3030	128.5	7198	4
159.4	3585	159.4	908	5
164.4	1896	164.5	6654	6

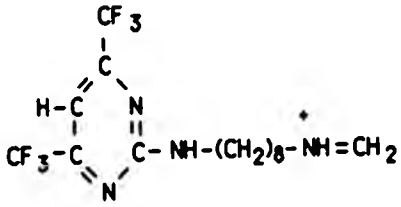
The derivative was also characterized by low resolution mass spectrometry which showed in each case (GNG-A/HFAA and GNG-C/HFAA) an ion at m/z 698 (3.1 or 4.5%), $[M-H]^+$, and other prominent peaks at m/z 679 (3.1 or 0%), $[M-HF]^+$, 659 (7.6 or 3.9%), $[M-2HF]^+$, 371 (100.0 or 100.0%), $[M-328]^+$, and 244 (28.1 or 49.5%), $[M-455]^+$.

In the case of the acetate, high resolution mass spectrometry detected the molecular ion at m/z 699, confirming the elemental composition of the derivative. Confirmation of the composition of some of the major fragment ions was also obtained as shown in Table 33.

Table 33. Mass spectral data and elemental composition of ions for the hexafluoroacetylacetone derivative of bis-(8-guanidino-octyl) amine triacetate (GNG-A/HFAA)

Exact Mass Observed	C	H	N	F	Exact Mass Calculated	Error (mmu)	R.I. (%)	Remarks
699.29129	28	37	7	12	699.29298	0.6	2.2	$[M]^+$
659.27925	28	35	7	10	659.28034	0.2	3.5	$[M-2HF]^+$
455.26233	21	33	4	6	455.26149	-1.4	2.2	$[M-244]^+$
371.16815	15	21	4	6	371.16759	-1.1	100.0	$[M-328]^+$
244.02769	7	4	3	6	244.03149	3.2	21.2	$[M-455]^+$

The strong peak at m/z 371 was considered to be produced by C-C bond cleavage between methylene groups, adjacent to the central nitrogen atom.



m/z 371

The results agreed with those obtained by Sato *et al*[29] and Kobayashi *et al*[55], for the derivative obtained from guazatine triacetate.

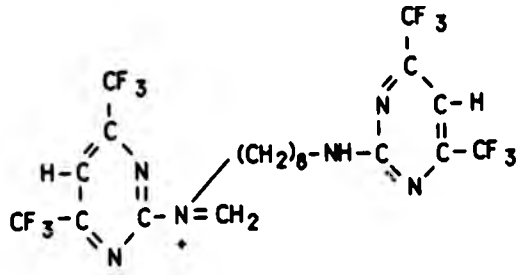
The ^1H NMR spectrum of the GNG-A/HFAA derivative had a signal for the pyrimidine ring proton at 7.1 ppm, and integration of this signal indicated the presence of two protons. Thus it was confirmed that the two guanidine groups of bis-(8-guanidino-octyl)amine triacetate had reacted with hexafluoroacetylacetone to form two pyrimidine rings as shown.

7.3- Cyanamide product (1:12)/HFAA derivative

The hexafluoroacetylacetone derivative of bis-(8-guanidino-octyl) guanidine triacetate was obtained through the reaction shown in Figure 38.

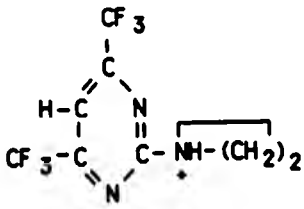
The identity of this derivative (48) was confirmed by low resolution mass spectrometry which showed the fragment ions at m/z 585 (42.3%), $[M-328]^+$, 371 (4.5%), $[M-542]^+$, 258 (100.0%), $[M-655]^+$, and 244 (57.3%), $[M-669]^+$, which are the prominent features of the mass spectrum of the derivative.

The fragment ion at m/z 585 indicates the presence of two pyrimidine rings attached to nitrogen atoms separated by a C_8 chain, thus confirming that the original compound had guanidine groups at both the terminal and central positions in the molecule.



m/z 585

The hexafluoroacetylacetone derivative also exhibited a base peak at m/z 258 which was considered to be produced by C-C bond cleavage between methylene groups and may have the structure shown below.



m/z 258

The ions at m/z 244 and 371 are assumed to be the same as those shown earlier (pp. 140 and 147) in the case of 1,8-diguanidino-octane and the bis-(8-guanidino-octyl)amine derivatives, respectively.

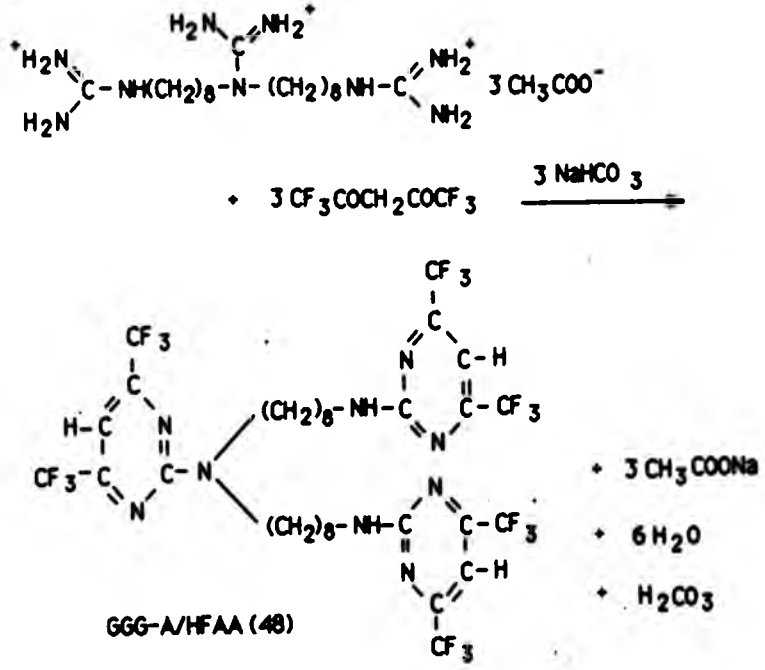


Figure 38. Reaction scheme for the preparation of 1,1-bis-(8-guanidino-octyl)guanidine/hexafluoroacetylacetone derivative.

7.4- Dodine/HFAA derivative

The hexafluoroacetylacetone derivative of n-dodecylguanidine acetate (dodine) was obtained through the reaction shown in Figure 39.

The identity of this derivative (49) was confirmed by microanalysis and by high resolution mass spectrometry which exhibited the molecular ion and its main fragments, as shown in Table 34, which are the prominent features of the mass spectrum of the derivative. Such results also agree with the results obtained by Newsome[80] using another method of

preparation.

The fragment ions at m/z 258 and m/z 244 (base peak) confirm the presence of the pyrimidine ring as shown above.

Several other HFAA derivatives were made and they will be described later in the gas-liquid chromatography section.

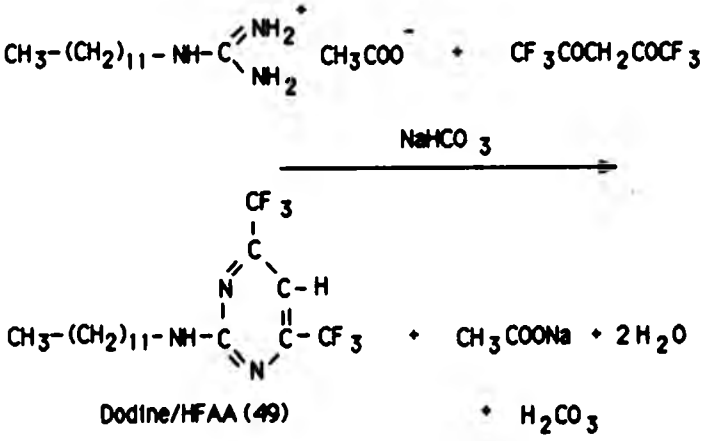


Figure 39. Reaction scheme for the preparation of dodine/hexafluoroacetylacetone derivative.

Table 34. Mass spectral data and elemental composition of ions from dodine/HFAA derivative

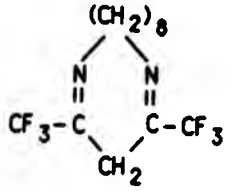
Exact Mass Observed	Elemental Form. C	H	N	F	Exact Mass Calculated	Error (mmu)	R.I. (%)	Remarks
399.20935	18	27	3	6	399.21147	1.6	37.5	$[M]^+$
273.06979	9	9	3	6	273.07062	0.3	14.4	$[M-(CH_2)_9]^+$
258.04686	8	6	3	6	258.04714	-0.3	8.1	$[M-CH_3(CH_2)_9]^+$
244.03055	7	4	3	6	244.03132	0.4	100.0	$[M-CH_3(CH_2)_{10}]^+$

8 - GAS-LIQUID CHROMATOGRAPHY AND GAS CHROMATOGRAPHY/MASS SPECTROMETRY

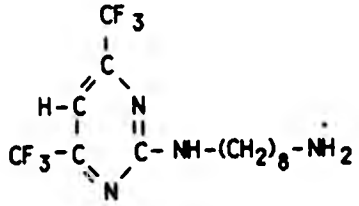
8.1- GLC and GC/MS analysis of commercial guazatine (70%)

In order to obtain all probable hexafluoroacetylacetone derivatives that could be formed from the various isomers present in commercial guazatine, a sample of the 70% aqueous solution was treated with hexafluoroacetylacetone reagent. No attempt was made to isolate the mixed derivatives in this case but the total solution as obtained was used directly for gas-chromatography.

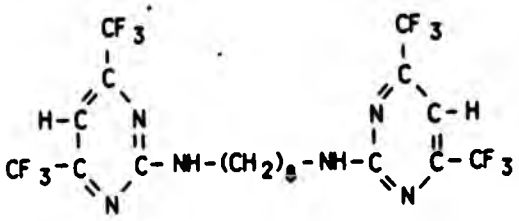
Commercial guazatine (70%) is thought to have the following composition[21]: NN-A (0.56%), GN-A (5.95%), GG-A (15.40%), NNN-A (0.14%), NNG-A (1.26%), NGN-A (0.63%), NGG-A (6.02%), GNG-A (3.01%), GGG-A (14.91%), derivatives of higher ologomeric amine (18.13%), cyanoguanidine (3.99%), and water (30.00%). Therefore the following derivatives might or might not be formed with hexafluoroacetylacetone:



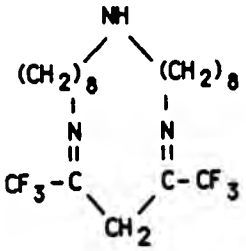
NN-A/HFAA (50)



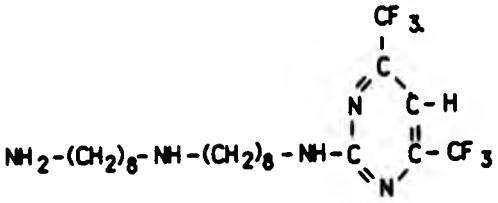
GN-A/HFAA (51)



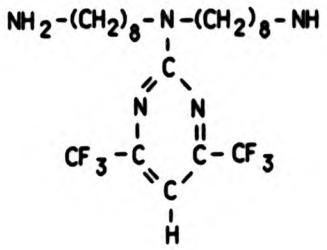
GG-A/HFAA (45)



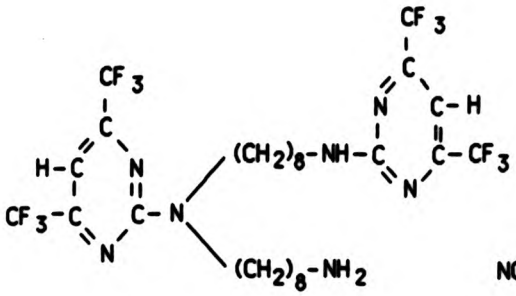
NNN-A/HFAA (52)



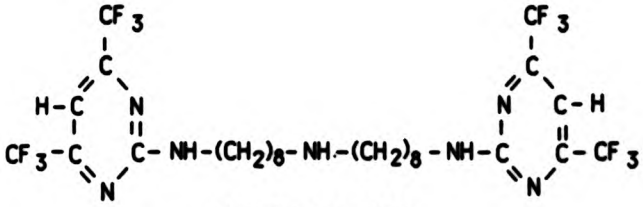
NNG-A/HFAA (53)



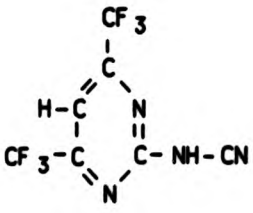
NGN-A/HFAA (54)



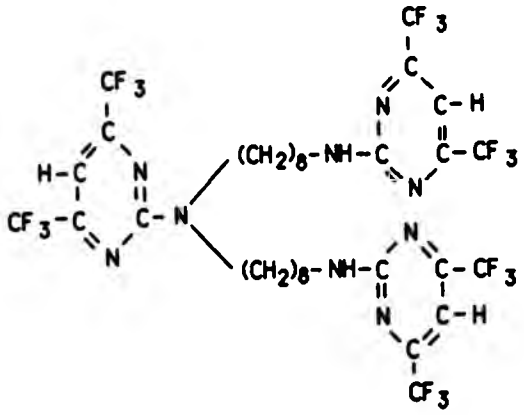
NGG-A/HFAA (55)



GNG-A/HFAA (46)



Cyanoguanidine/HFAA (56)



GGG-A/HFAA (48)

Diluted and concentrated samples of the solution (in toluene) obtained from the reaction of hexafluoroacetylacetone with commercial guazatine 70% (Exp. 7.9, p. 69) were injected into the gas-liquid chromatograph in order to obtain a reference chromatogram showing a series of standard peaks for these derivatives to be used as guide in the identification of some components of guazatine. The results are shown in Table 35 and the chromatograms in Figure 40 and 41, which will be described later with the results for single derivatives.

Both samples were also analysed by GC/MS in the electron impact mode and the results for a typical gas-chromatography/mass spectrum are shown in Table 36 and Figure 42.

Table 35. Results of hexafluoroacetylacetone derivative of commercial quazatine 70% by GLC[a]

Peak No.	Retention Time (min.)		Peak Height (cm)	
	(1)	(2)	(1)	(2)
1	4.1	3.9	2.6	4.6
2	5.3	—	2.8	—
3	7.2	7.0	0.4	0.7
4	8.7	8.5	2.8	2.9
5	11.3	—	0.2	—
6	14.5	14.6	14.2	14.2
7	19.4	—	0.6	—
8	23.2	—	0.2	—
9	25.7	25.4	0.7	0.3
10	26.4	26.1	1.1	0.2
11	27.8	27.6	1.6	2.2
12	28.4	28.2	1.0	3.3
13	29.9	29.7	0.2	0.2
14	32.3	32.2	5.7	10.1

a) Results from four replicates
1) Diluted sample
2) Concentrated sample.

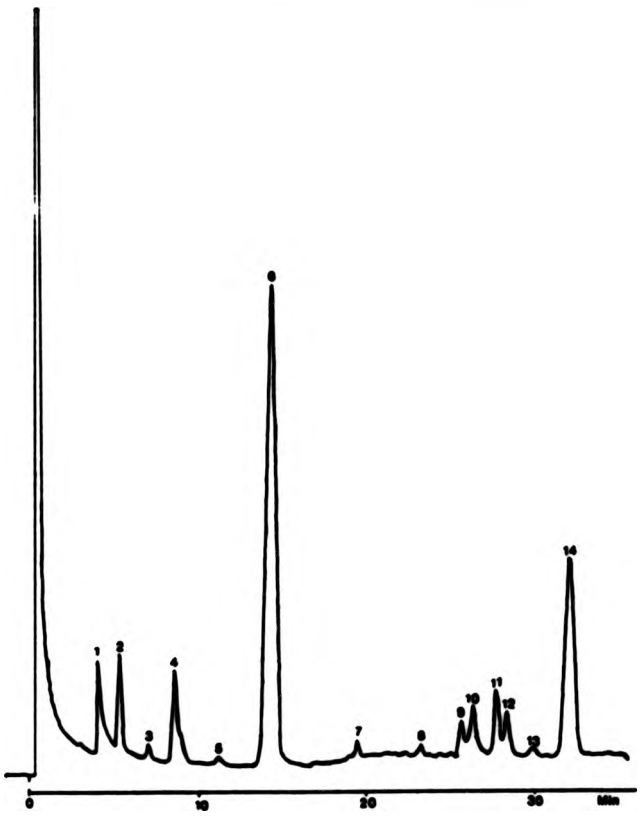


Figure 48. Gas-liquid chromatogram of hexafluoroacetylacetone derivative of commercial guazatine 70% (diluted sample).

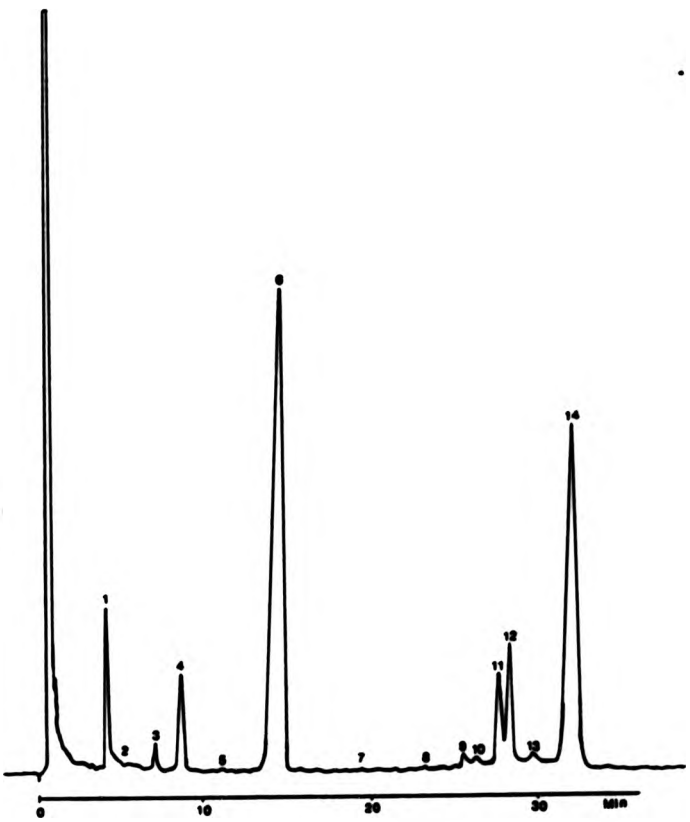


Figure 41. Gas-liquid chromatogram of hexafluoroacetylacetone derivative of commercial guazatine 70% (conc. sample).

Table 36. Results for hexafluoroacetylacetone derivative of commercial guazatine (70%) by GC/MS

Chromatogram peak identifier no.		Possible	m/z (R.I.) Dil./Conc.
Diluted Sol.	Concentrated Sol.	Assignments	
303	683	NG/HFAA	244 (47.0/ 39.8)
			224 (12.4/ 4.9)
337	773	GG/HFAA	552 (4.6/ 1.2)
			532 (13.7/ 2.9)
			244 (100.0/100.0)
			224 (13.1/ 14.0)
491	1130	NGG/HFAA	585 (16.4/ 0.0)
			355 (20.0/ 0.0)
			327 (16.4/ 0.0)
			313 (16.4/ 0.0)
			258 (100.0/ 6.4)
			244 (41.1/ 2.9)
			207 (98.6/ 0.0)
—	1143	GNG/HFAA	258 (0.0/ 34.4)
			244 (0.0/ 14.1)
547	1217	GGG/HFAA	585 (12.9/ 3.6)
			258 (100.0/ 84.3)
			244 (71.4/ 46.3)

The chromatogram peak (identifier number 303 or 683) showed prominent peaks in the mass spectra at m/z 244 (47.0 or 39.8%) and 224 (12.4 or 4.9%) which shows it to be a hexafluoroacetylacetone derivative. The shorter retention time suggests that it is probably from the NG/HFAA derivative formed from 1-amino-8-guanidino-octane.

The chromatogram peak (identifier number 337 or 773) showed prominent peaks in the mass spectra which might have originated from the GG/HFAA derivative characterized previously (See pp. 139 and 141),

and showed the same ions with the base peak at m/z 244.

The chromatogram peak (identifier number 491 or 1130) showed prominent ions which indicate that the compound may be the NGG/HFAA derivative. The presence of two guanidine groups separated by an octamethylene chain is indicated by the fragment ion at m/z 585 (See p. 148) but the retention time is too short for this derivative to have been

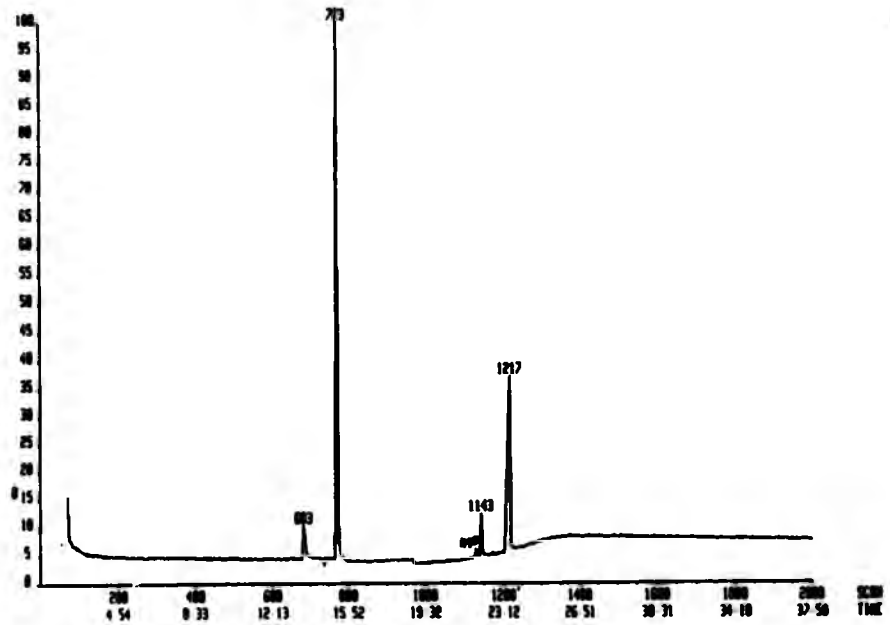


Figure 42. Gas chromatography/mass spectra results for commercial guazatine 70% (concentrated sample).

formed from GGG. Peak (identifier number 1143) showed prominent ions in the mass spectrum as shown in Table 36, and the close retention time to the NGG/HFAA derivative refered to above suggests that it is probably from the GNG/HFAA derivative. The expected ion at m/z 371 (See p. 147) was not however seen under these conditions.

The last chromatogram peak (identifier number 547 or 1217) showed prominent peaks in the mass spectra as shown in Table 36, which are similar to those for the GGG/HFAA derivative characterized previously (See p. 147).

In order to obtain confirmation of the identification of the various peaks in the above chromatograms, comparison was made with hexafluoroacetylacetone derivatives that have been prepared separately from each of the guanidines that were available.

8.2- GLC analysis of GG/HFAA derivative

A solution of GG/HFAA derivative that had been prepared (a) from the sulphate or (b) from the acetate (See pp. 62 and 63) was made in toluene and was injected into the GLC. A typical result is shown in Figure 43, which exhibits a peak with a retention time of 14.6 minutes and corresponds to the peak number 6 from Table 35 and Figures 40 and 41.

A small quantity of the GG/HFAA derivative was also added to a guazatine 70%/HFAA solution and injected into the GLC; the resultant chromatogram exhibited a larger peak height for the peak number 6 and therefore its identity was confirmed.

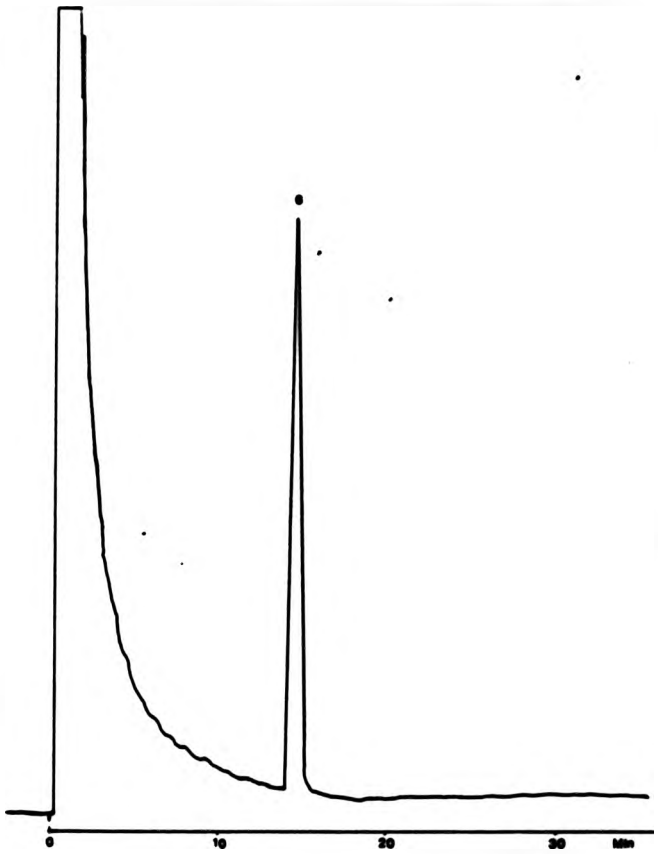


Figure 43. Gas-liquid chromatogram of hexafluoroacetylacetone derivative of 1,8-diguanidino-octane (GG/HFAA) (2.5 mg/ml).

8.3- GLC analysis of GNG/HFAA derivative

A solution of GNG/HFAA derivative that had been prepared (a) from the acetate or (b) from the carbonate (See pp. 64 and 65) was made in toluene and ethanol, respectively, and was injected into the GLC. A typical result is shown in Figure 44, which exhibits a peak with a retention time of 28.2 minutes and corresponds to the peak number 12 from Table 35 and Figures 40 and 41.

A small quantity of the GNG/HFAA derivative was also added to a guazatine 70%/HFAA solution and injected into the GLC; the resultant chromatogram exhibited a larger peak height for the peak number 12 and therefore its identity was confirmed.

A development sample of 40% guazatine triacetate supplied by Murphy Chemical Ltd., and prepared by the *S*-methylisothiouronium sulphate method from distilled triamine (1970), was converted to the hexafluoroacetylacetone derivative (G40/HFAA), in toluene, through procedure 7.10 (See p. 70). GLC analysis gave the result shown in Figure 45 which exhibits a major peak (*ca* 85.5%, based on peak area) with a retention time of 28.4 minutes corresponding to the peak number 12 from Table 35 and Figures 40 and 41, and due to the GNG component as previously identified and shown in Figure 44.

A small peak (*ca* 12.2%, based on peak area) also appeared in the chromatogram with a retention time of 27.4 minutes corresponding to the peak number 11 from Table 35 above which might be due to the GGN component and will be described later for cyanamide product/HFAA derivatives. This result gives an indication that with the *S*-methylisothiouronium sulphate method it may also be possible to obtain guanidation to a small extent at the central nitrogen of the triamine

molecule. It was initially thought that such a reaction occurs only with the cyanamide method. There was, however, no detectable amount of GGG component present.

A very small peak (*ca* 2.3%, based on peak area) with a retention time of 14.5 minutes (corresponding to the peak number 6 from Table 35) was also present in the chromatogram and was due to the GG component as previously identified by Figure 43. This result clearly shows that the triamine used in the manufacture of guazatine 40% (G40) by Murphy Chemical Ltd. contained a small residual impurity of diamine in its composition. When a derivatization of some precipitated material from the bottle of guazatine (G40) was made and injected into the GLC the new chromatogram exhibited a more pronounced peak due to the GG/HFAA derivative (peak number 6) indicating the lower solubility of GG acetate compared to the GNG component.

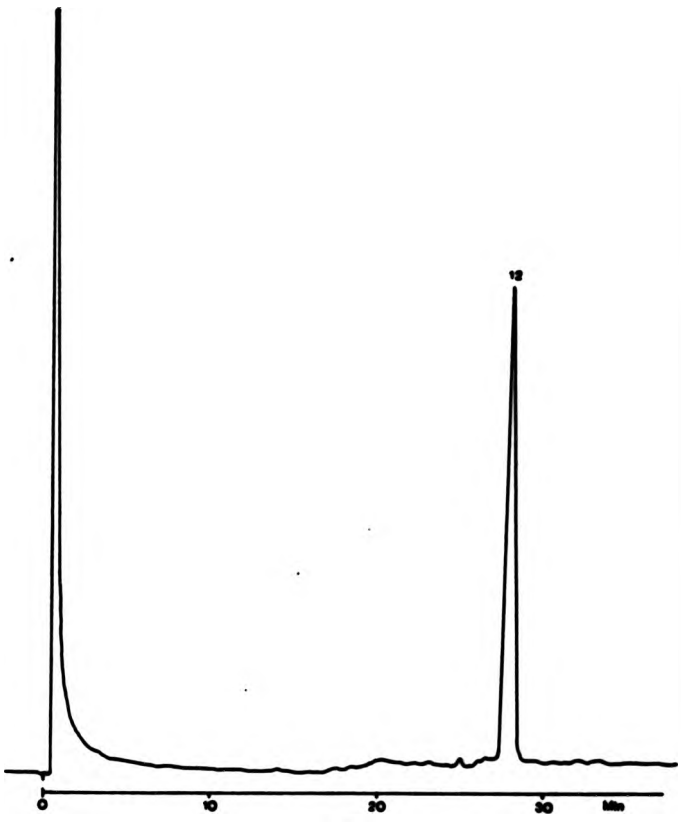


Figure 44. Gas-liquid chromatogram of hexafluoroacetylacetone derivative of bis-(8-guanidino-octyl)amine (GNG/HFAA).

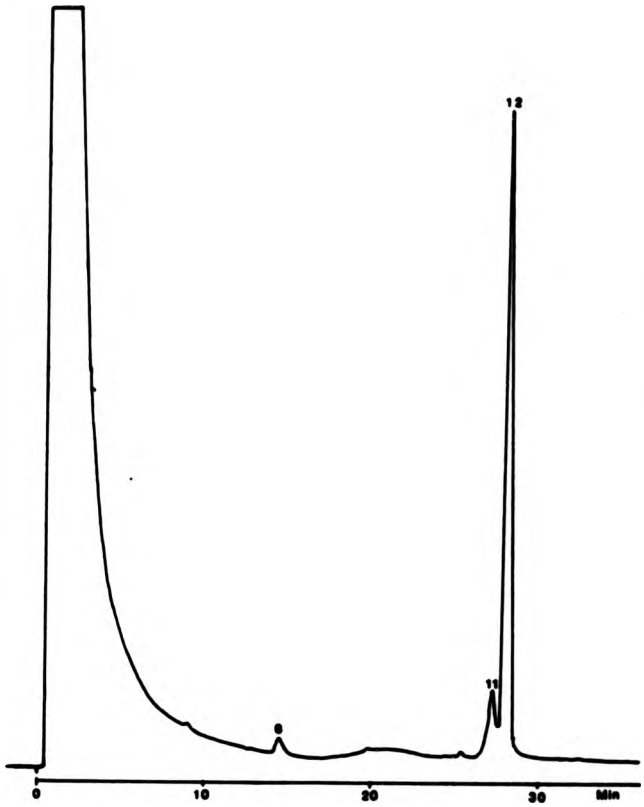


Figure 45. Gas-liquid chromatogram of hexafluoroacetylacetone derivative of development sample of guazatine 40% (G40/HFAA)

In order to obtain information or possible assignments for other peaks in the chromatogram of the hexafluoroacetylacetone derivative of commercial guazatine (Figures 40 or 41), a number of preparations were carried out from the pure triamine, with various molar ratios of cyanamide (2:1, 1:1, 1:2, 1:3, 1:6, and 1:12). These would be expected to give various mixtures of the possible guanidation products containing one, two, or three guanidine groups per molecule.

In each case the total product in solution was converted to the hexafluoroacetylacetone derivatives by the usual procedure and analysed by GLC.

8.4- GLC analysis of cyanamide product/HFAA derivatives

Samples of the solution obtained from the reaction of hexafluoroacetylacetone with cyanamide products (2:1 to 1:12) (Exp. 7.5.1 to 7.5.6, pp. 65 to 68) were injected into the GLC and the results are shown in Figures 46 to 51, respectively, which show the possible presence of GNN, NGN (peaks 9 and 10), GGN (peak 11), GNG (peak 12), and GGG (peak 14) hexafluoroacetylacetone derivatives. From earlier evidence (See p. 98) that guanidation may be first at the central nitrogen atom, peak 9 is tentatively assigned to GNN, and peak 10 to NGN.

As the cyanamide/triamine ratio was increased, the HFAA derivatives showed the same main peaks but with change in their intensities as shown in Figures 46 to 51. The relative proportion of mono-, di-, and triguanidated compounds based on peak area measurement also varied from 62.5 to 0.0%, 36.0 to 3.0%, and 1.5 to 97.0%, respectively, when the ratio was increased from 2:1 to 1:12.

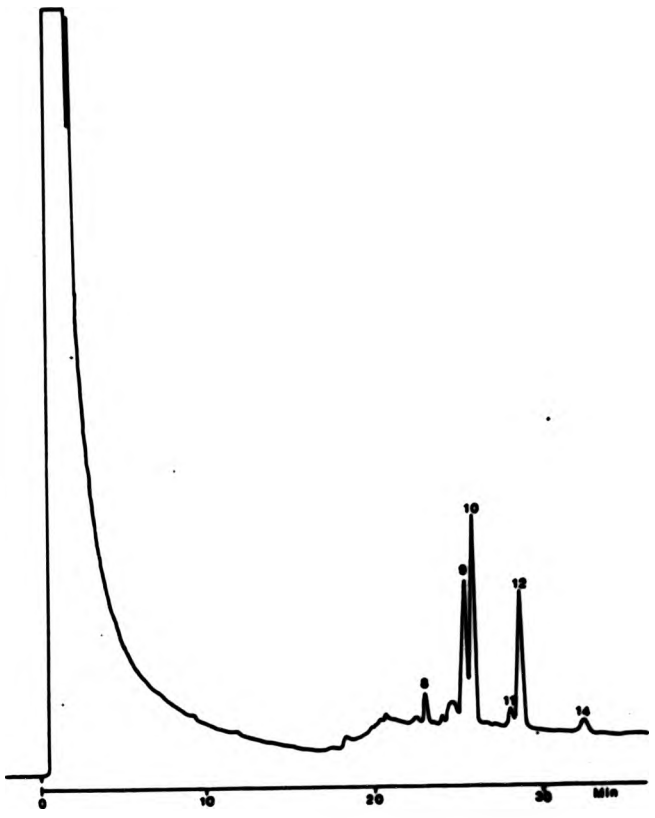


Figure 46. Gas-liquid chromatogram of hexafluoroacetylacetone derivative of cyanamide product (2:1).

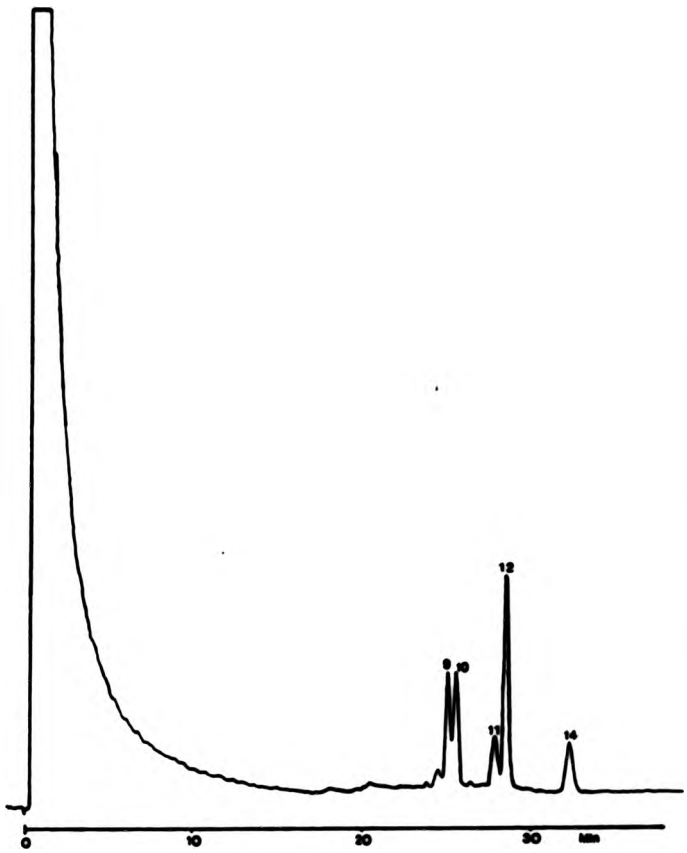


Figure 47. Gas-liquid chromatogram of hexafluoroacetylacetone derivative of cyanamide product (1:1).

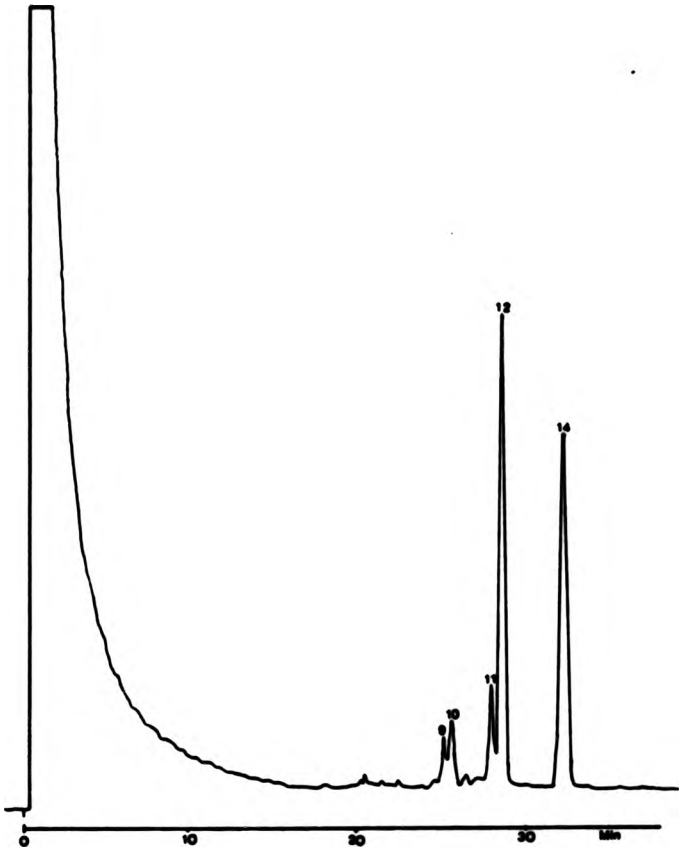


Figure 48. Gas-liquid chromatogram of hexafluoroacetylacetone derivative of cyanamide product (1:2).

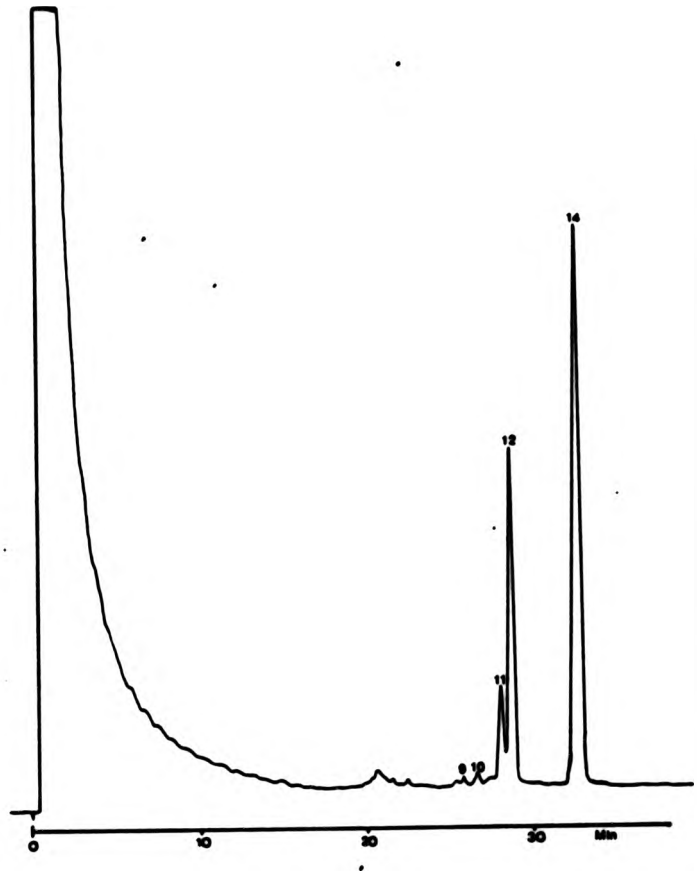


Figure 49. Gas-liquid chromatogram of hexafluoroacetylacetone derivative of cyanamide product (1:3).

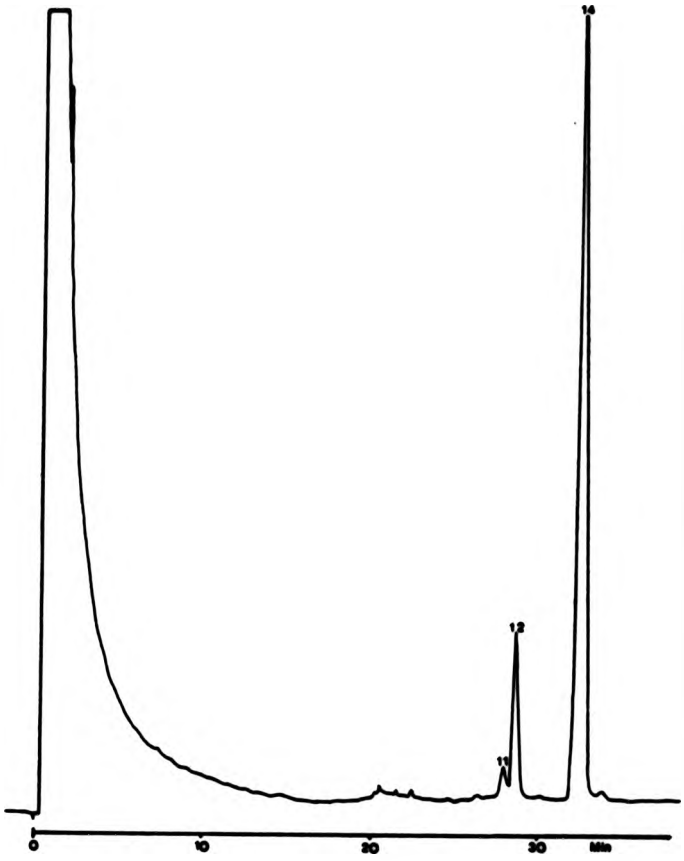


Figure 58. Gas-liquid chromatogram of hexafluoroacetylacetone derivative of cyanamide product (1:6).

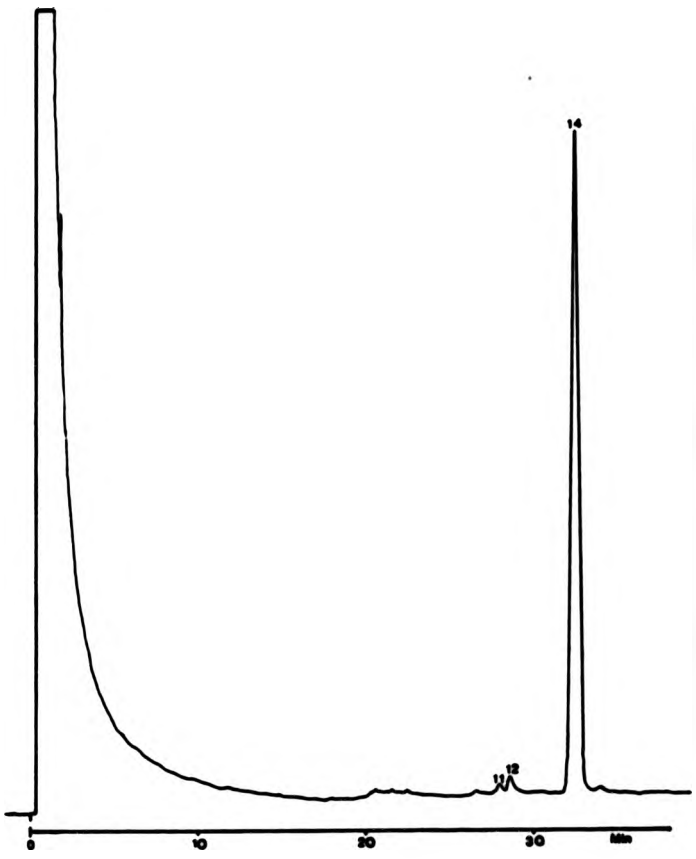


Figure 51. Gas-liquid chromatogram of hexafluoroacetylacetone derivative of cyanamide product (1:12).

The max proportion of GNG (*ca* 46.0%) was obtained with a 1:2 ratio (Figure 48). Under these conditions a small amount of monoguanidated derivatives (*ca* 10.0%) were seem to be present and a significant amount of GGG (*ca* 35.0%) had also been formed. GGG became almost the exclusive product (*ca* 97.0%) when the ratio was increased to 1:12 (Figure 51).

At the smallest concentration of cyanamide, *i.e.* a 2:1 molar ratio of triamine to cyanamide (Figure 46), there was still non-reacted triamine present in the mixture which was also derivatizated by HFAA and gave the peak number 8 shown in the chromatogram of Figure 46.

8.5- GC/MS analysis of cyanamide product/HFAA derivative

The sample of hexafluoroacetylacetone derivative from the cyanamide product (1:1) reaction was also analysed by GC/MS in the chemical ionization mode after it had been evaporated to dryness and diluted with dichloromethane to give a 10% solution.

The chromatogram shown in Figures 40 or 41 was not duplicated under chemical ionization conditions although a 25 m x 0.2 mm OV 101 WCOT column was also used.

Under ammonia chemical ionization conditions with the injector at 250^{0}C there seems to be very little ionization taking place.

A splitless injection of 0.5 µl with a programme of 220^{0}C for 17 minutes, then 16^{0}C/min to 300^{0}C and hold for 25 minutes was used with no success of ionization. Even with a more concentrated sample very little ionization was obtained.

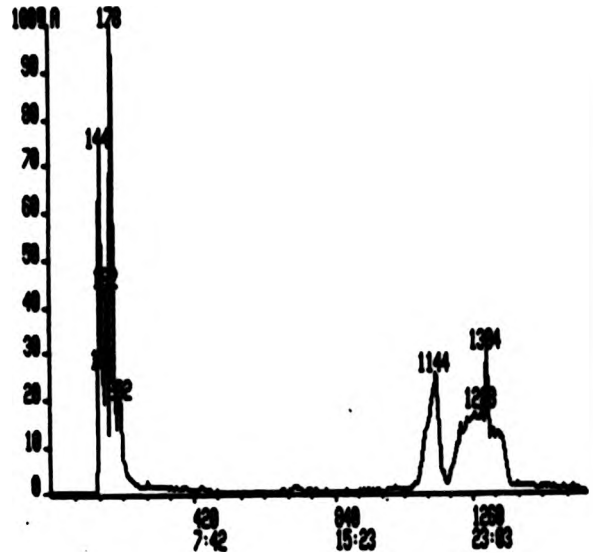


Figure 52. Gas chromatography/mass spectra results for cyanamide product (1:1).

At an injector temperature of 300°C more ionization occurred but, there seemed to be also more decomposition (pyrolysis), giving a blast of early peaks in which some ions were in the region of interest, as well as some later broad peaks as shown in Figure 52.

The only record of interest from these later peaks (Scan 1304) was the ion at m/z 914 (*ca* 25%) which corresponds to the protonated GGG/HFAA derivative.

In addition, evidence for the formation of GGG compound was obtained in the reaction of GNG with cyanamide (Exp. 4.2.9, p. 58). The product yielded a hexafluoroacetylacetone derivative which was shown by GLC to contain GNG, GGG, and an unidentified compound of longer retention time (Figure 53). GC/MS with ammonia chemical ionization confirmed both GNG and GGG by peaks at 700 and 914 (MH^+ peaks), respectively as shown in Figure 54.

The unidentified peaks seem to be of related compounds because they showed prominent peaks at m/z 371 (16.8%) and 244 (30.6%) by GC/MS in the electron impact mode, and m/z 355 (7.5%) by GC/MS in the chemical ionization mode.

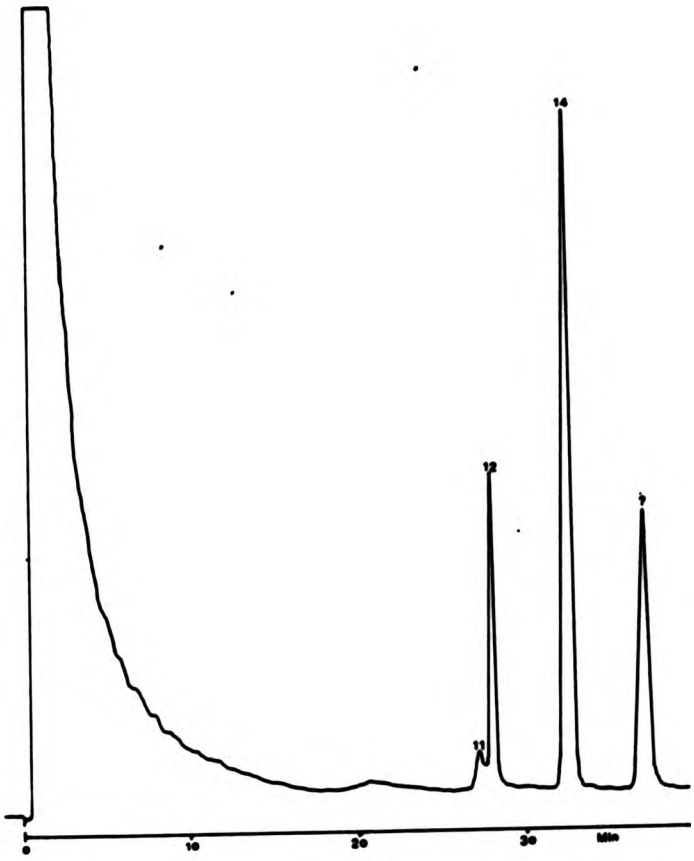


Figure 53. Gas-liquid chromatogram of hexafluoroacetylacetone derivative of a mixture of GNG and GGG compounds.

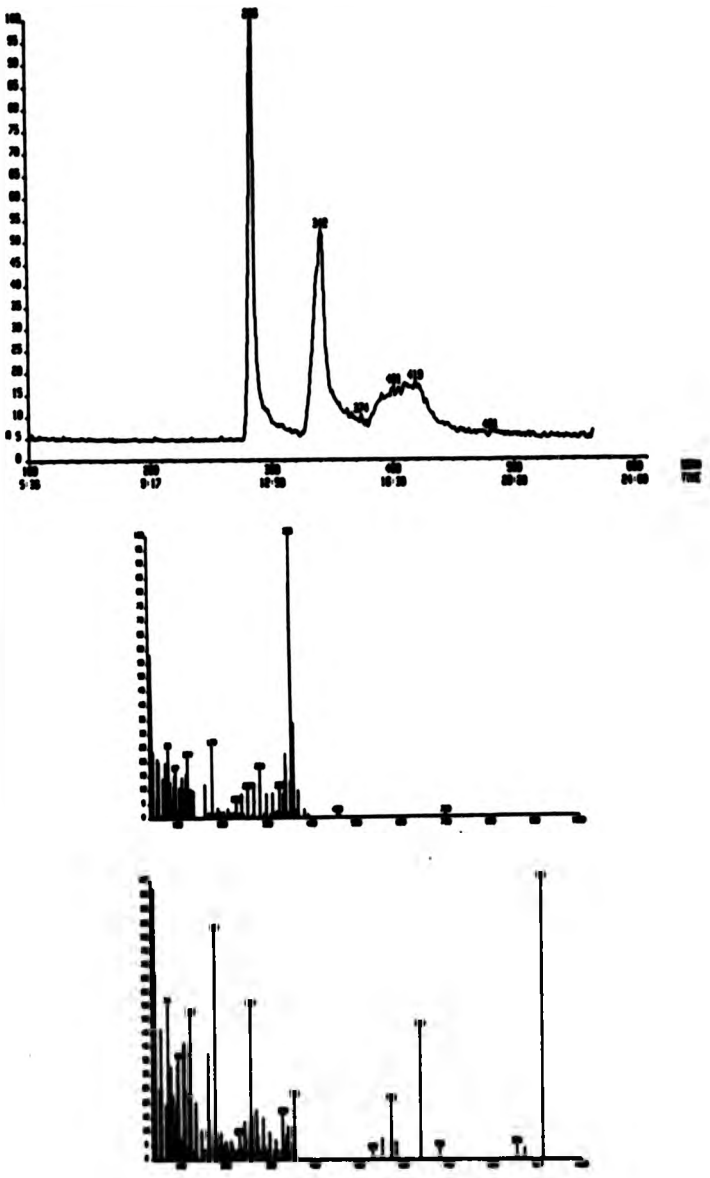


Figure 54. Gas chromatography/mass spectra results of HFAA derivative of a mixture of GNG and GGG compounds.

Some unidentified peaks from Figure 40 or 41 still remain. Some evidence has been obtained that hexafluoroacetylacetone derivatives of diamine and triamine may be formed, as shown in Figures 55 and 56, respectively, although the identity of such derivatives is unknown.

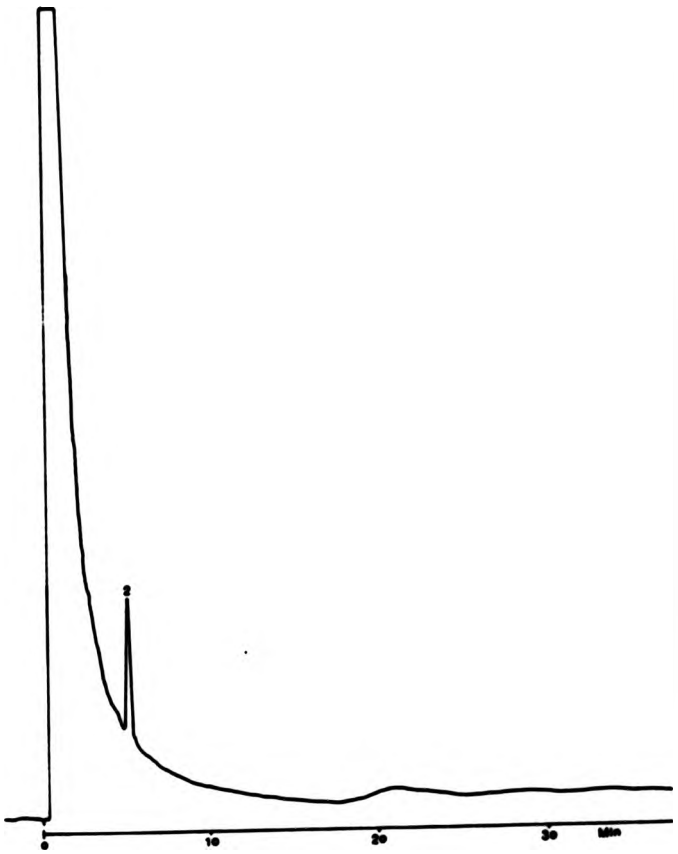


Figure 55. Gas-liquid chromatogram of hexafluoroacetylacetone derivative of diamine.

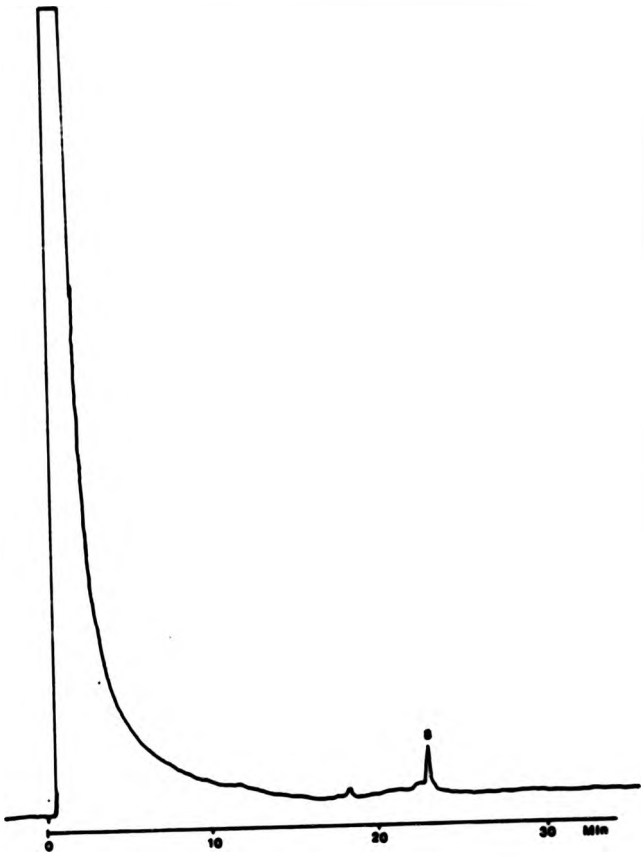


Figure 56. Gas-liquid chromatogram of hexafluoroacetylacetone derivative of triamine.

The experimental trial to make an HFAA derivative of cyanoguanidine (See Exp. 7.11, p. 71) showed no signal in the chromatogram. Lack of reactivity might be due to the strong electronegativity of nitrogen in the cyano group that reduces the nucleophilicity of the guanidine and therefore, the cyanoguanidine/HFAA derivative (56) (See p. 153) is not

obtained under these reaction conditions.

An experimental trial with the hexafluoroacetylacetone derivative of dodine by GLC was made following the procedures of Kobayashi *et al.*[55] and its results are shown in Figure 57. Similar results have been reported elsewhere[60].

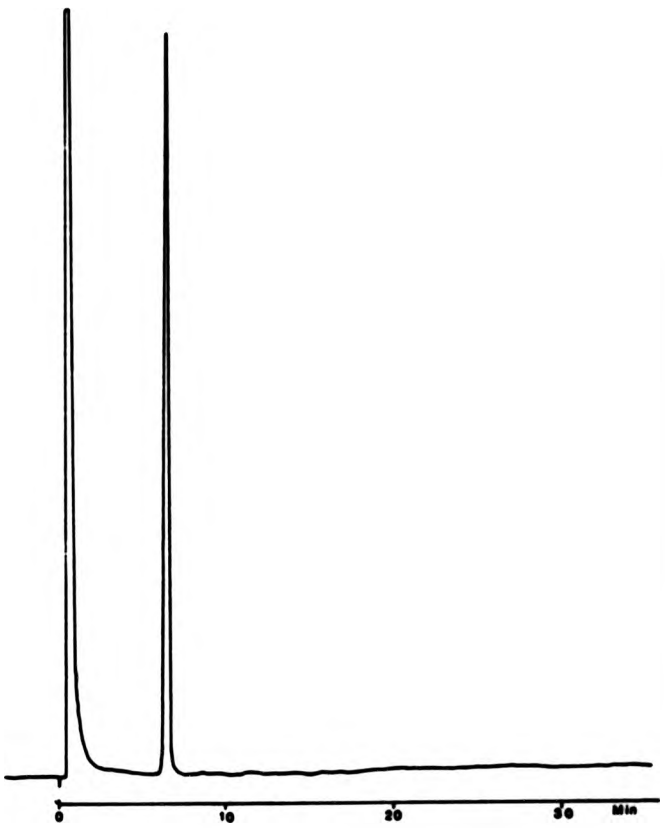


Figure 57. Gas-liquid chromatogram of hexafluoroacetylacetone derivative of dodine.

9 - RESULTS FOR THE ATTEMPTED EXTRACTION OF GUANIDATED AMINE ACETATE DERIVATIVES FROM WHEAT PLANTS

The main aim to use the method of extraction prepared by Kobayashi *et al*.[55] in the present work was to obtain some suitable conditions for the establishment of a residue method of analysis of wheat plants for guanidated amine acetate compounds in the form of hexafluoroacetylacetone derivatives.

Although a different type of sample from their work was used (they used rice grain), an attempt in this programme of research was made.

Under the extraction procedure and chromatographic conditions described earlier (See Exp. 8 and 9, respectively, pp. 72 and 74), the control and treated wheat plants were analysed. Their chromatograms were almost identical and a typical example is shown in Figure 58.

Figure 58 shows several peaks that might be from chemical components of the wheat plant itself or may be due to the same or different components of the wheat plant in the form of hexafluoracetylacetone derivatives which were detected by the Flame Ionization Detector.

In order to know if the co-extractives are detected in their original form or as hexafluoroacetylacetone derivatives an aliquot of treated wheat plant, after it had been extracted with chloroform and before the derivatization step, was injected into the GLC. No peaks on the chromatogram were observed and therefore, this result gives an indication that the co-extractives might be detected in a form of hexafluoroacetylacetone derivatives. Such results also indicate that clean-up procedures are more conveniently executed before the

derivatization takes place, and will be confirmed later when the clean-up procedures are described.

Guanidated amine acetate compounds, represented by GG-A (100 mg) and GNG-A (50 mg) were also studied by this procedure of extraction in the absence of wheat plant. Chromatograms for both compounds showed the presence of their peaks and in the case of the GG-A compound, two other unknown peaks appeared which may be due to contamination. Such results showed that the derivatives are formed and can be analysed by this procedure of extraction although further studies need to be done.

In any case, these results showed the presence of several co-extractives which must be eliminated by a suitable clean-up procedure before the identification and estimation of the quantity of such residues by GLC takes place.

There are several published papers dealing with the clean-up of various sample types and depending on the extent and nature of the co-extractives and the pesticide residue, solvent partition, liquid chromatography (column adsorption or gel, or TLC), and sweep co-distillation are most often used, alone or in combination, for clean-up.[88,89]

From the clean-up procedures described above only column chromatography was tried in this programme of research and the nature of the co-extractives were unknown.

A large concentration of commercial guazatine (G70) was intentionally used to spike the control wheat plant in order to guarantee the presence of the most important guanidine derivatives together with the co-extractives.

Figure 59 shows the GLC results for a spiked control wheat plant with several peaks due to the co-extractives. The presence of GG-A and GGG-A

components as HFAA derivatives were also observed without any interference in their retention time caused by co-extractives. This preliminary result shows that the presence of these two components in wheat plants can be monitored.

GLC results after attempted clean-up on all three adsorbents (neutral alumina, alkaline alumina, and silica gel), eluted with methanol, exhibited several peaks due to the co-extractives and also due to the GG-A and GGG-A components. Such results show that guanidine derivatives were not separated from the co-extractives in this experiment and this probably occurred because the co-extractives and guanidines were all as hexafluoroacetylacetone derivatives and their behaviour on the columns was similar. A different approach to clean-up needs to be studied and it is suggested that the procedure may better be made before derivatization takes place.

Similar results were obtained for alumina (neutral) eluted with toluene. For the other two columns, alumina (alkaline) and silica gel, both co-extractives and guanidine derivatives (GG-A and GGG-A) were retained on the column.

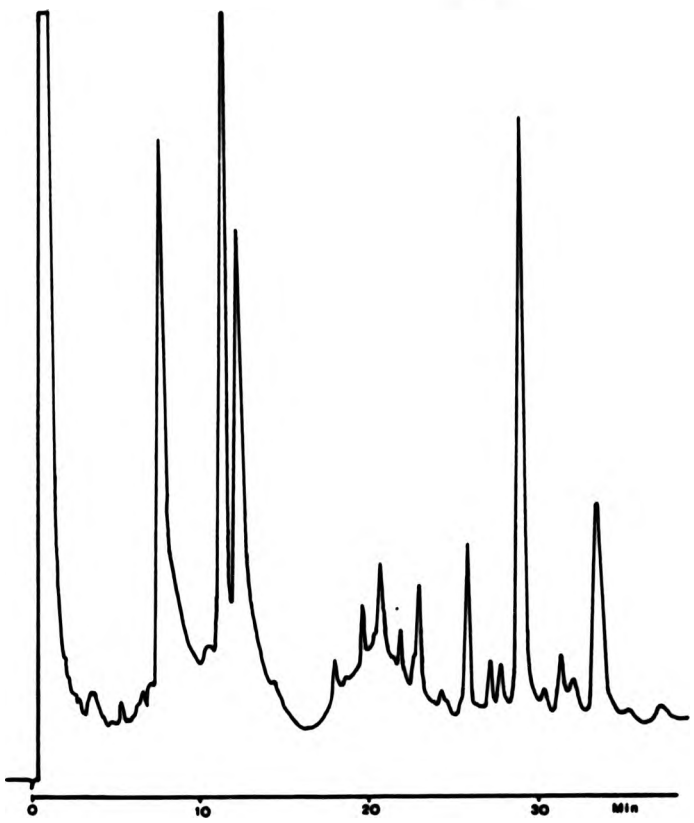


Figure 58. Gas-liquid chromatogram of control wheat plant under extraction procedure.

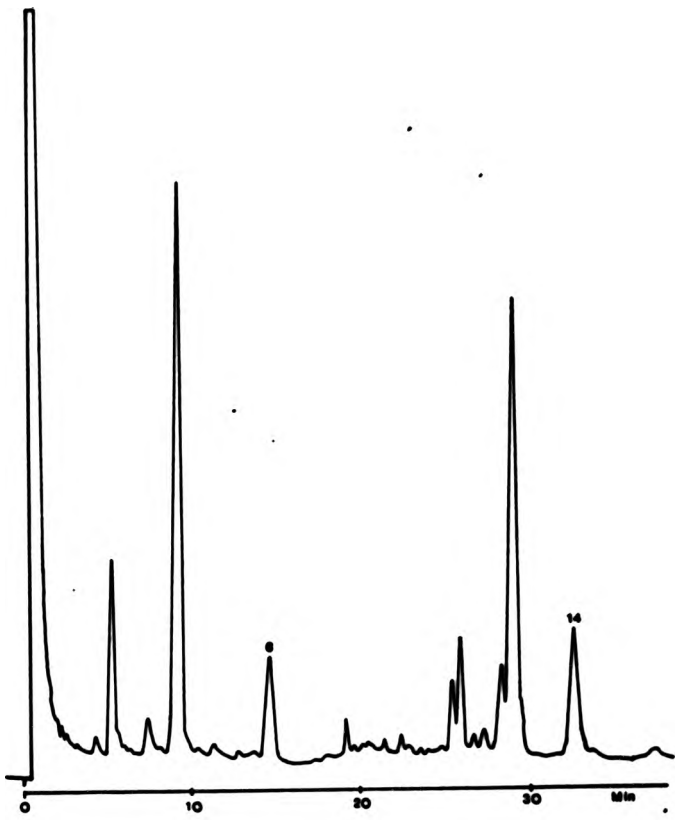


Figure 59. Gas-liquid chromatogram of control wheat plant spiked with commercial guazatine (G70) under extraction procedure.

V - CONCLUSIONS

With the results obtained in this programme of research the following conclusions can be made:

1- Methods for the identification of the principal components of the fungicide guazatine have been established based on the combined use of Carbon-13 NMR, FAB Mass Spectrometry, and GC/MS of the hexafluoroacetylacetone derivatives.

2- The presence of the guanidine structure at the central position of the polyalkylene chain was evidenced by a chemical shift of about 158.4 ppm, whilst for the terminal structure the signal occurred at a slightly lower field of about 159.5 ppm. Both signals can be used as a fingerprint in their identification.

3- The relatively new technique of FAB Mass Spectrometry has also been shown to be a useful tool in the identification of guanidine compounds. Molecular weights are confirmed by intense MH^+ ions in the positive ion spectrum, with the positive charge remaining on the nitrogen-containing fragment during the fragmentation process. $[MH + 12]^+$ ions of various intensity may also arise by reaction with formaldehyde derived from the glycerol matrix.

4- The use of hexafluoroacetylacetone and acetylacetone as derivatization reagents played an important role in gas-liquid chromatography and in GC/MS for the identification of guanidine derivatives. The method based on hexafluoroacetylacetone is suitable for the analysis of the main components of guazatine and for the fungicide

dodine. Residue analysis of guazatine and of dodine in crops, foods, or environmental samples was shown to be feasible. Further work in this area is required after the presence of co-extractives in samples have been removed by adequate procedures of clean-up.

5- New routes to the synthesis of 1,17-diguanidino-9-azaheptadecane triacetate through the use of *S*-methylisothiouronium acetate or by double decomposition of the sulphate with barium acetate have been established.

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