THE MASS SPECTRA OF SOME NATURAL PRODUCTS

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by

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INTRODUCTION

In mass spectroscopy a beam of ions is produced from a substance and separated into a spectrum according to their mass to charge ratios. According to the means adopted to record the spectrum the instrument is known as a mass spectrometer or a mass spectrograph. A mass spectrometer determines the abundance of each species by collecting it individually and measuring after amplification, the current received. All the ions are recorded simultaneously in a mass spectrograph on a photographic plate set in the focal plane of the instrument.

Wien (1) and Thomson produced the first mass spectra by separating a beam of positively charged ions into its component parts. Later Thomson (2) introduced the parabola spectrograph in which a collimated beam of ions was deflected by parallel electric and magnetic fields set perpendicular to the direction of motion of the beam. The positions of the ions emerging were recorded on a photographic plate as a series of parabolas. Each parabola corresponded to ions with a particular mass to charge ratio. With this instrument Thomson (3) was

able to obtain the mass spectrum of a mixture of rare gases. In addition to lines corresponding to helium (mass 4), neon (mass 20) and argon (mass 40) there was a line due to an ion with a mass to charge ratio of twenty-two.

It was already suspected that atoms of different mass occurred in the same element, since many pairs of radioactive materials could not be separated chemically. Soddy (4) had suggested the name "isotope" to denote atoms of the same element which had different atomic weights but occupied the same place in the periodic table. The unidentified ion (m/e = 22) observed by Thomson was, therefore, thought to be an isotope of neon. Aston (5) was later able to demonstrate conclusively the existence of two isotopic forms of neon.

The use of mass spectrometry as an aid to organic analysis was developed by Washburn, Wiley and Rock (6), a development which was stimulated by the demands of the petroleum industry for a rapid and accurate means of analysis of complex hydrocarbon mixtures. The introduction (7) of a heated inlet system allowed the determination of the mass spectra of organic compounds of lower volatility which were stable to higher temperatures provided all metal parts were excluded from the sample

inlet system. The compound to be examined is volatilized and expanded into a heated reservoir which admits the sample into the ionization chamber of the mass spectrometer through a leak. Even when all the metal components are excluded from the sample system some compounds still decompose more or less completely before entry. To obviate this difficulty the direct introduction of samples into the ionization chamber of the mass spectrometer by sublimation from a probe has been pioneered by Reed (8).

As a result of the application of mass spectrometry to organic chemical analysis mass spectrometers of moderate resolving power ($\sim$ 600) were produced commercially. With these instruments it is possible to determine the mass number of the monoisotopic molecular ion. In addition the presence of ions one and two mass numbers above this enables the formula of the molecular ion to be determined. The abundances of the $(P+1)^+$ and $(P+2)^+$ ions can be readily calculated from the natural abundances of C^{13}, H^2 and O^{18} etc. Beynon and Williams (9) have listed the abundances of the $(P+1)^+$ and $(P+2)^+$ ions as a percentage of the parent ion abundance up to a molecular weight of 500 for all possible combinations of carbon, hydrogen, oxygen and nitrogen

which contain six or less oxygen or nitrogen atoms.

Apart from the determination of the molecular formula the mass spectrum of a compound also contains information related to the presence of specific groupings. This information has been obtained from the mass spectra of a group of related compounds and such correlations, as they are called, are often valuable in the determination of an unknown structure. As well as simple fragmentations of the molecular ion by cleavage of one bond many fragment ions have been observed whose constitution has shown that rearrangement of the molecular ion occurred in break-down. In the mass spectra of many paraffins these rearrangements are not specific and have been designated as random by McLafferty (10). With the introduction of a centre of unsaturation or a hetero atom into the molecule highly specific rearrangements with the transfer of a particular hydrogen have been confirmed by deuterium labelling. McLafferty (11) and Biemann (12) have proposed mechanisms for many of these rearrangements which are energetically favoured. Such rearrangements often lead to the formation of a secondary molecular ion and Reed (13) has introduced the term "parent molecular ion" to denote the ion obtained by ionization of the molecule admitted to the ion source.

The use of correlation studies has, however, often been limited by the fact that in compounds other than hydrocarbons, a fragment ion may have more than one constitution. One of the most obvious examples is the $(P-43)^+$ ion which in an unknown may be due to the loss of C_3H_7, C_2H_3O, C_2H_5N or CHNO. Beynon (14) pointed out that a distinction could be made between these possibilities by determining the exact mass of the fragment and the parent molecular ion. A technique for making such a determination has been developed by Nier (15). Nier had also shown the importance of having sufficient resolving power to be able to distinguish between the two possible constituents of a doublet. Such measurements can only be performed on molecular ions in single focusing instruments since a fragment ion formed with an excess of kinetic energy will be deflected along a different path from an ion of the same mass without excess translational energy by the magnetic field. It will, therefore, be recorded as having a mass somewhat larger than the true value.

The pioneering work of Beynon (16) on the use of a double focusing instrument of high resolving power has led to more detailed information on the fragmentation of heterocyclic systems. Under conditions of high

resolving power isobaric pairs or doublets, i.e. CH_4, O and NH_2 can be distinguished and the contribution of each of the constituents to the total abundance of any ion determined. Beynon's demonstrations of the importance of the use of high resolution mass spectrometry and precise mass measurement have forced the commercial development of high resolution mass spectrometers for organic analysis.

CHAPTER I

ALKALOIDS

The use of mass spectrometry in the analysis of the structure of alkaloids has only recently developed, but a considerable literature has already appeared on their fragmentation patterns. Most of this has now been summarized in a book (17) devoted entirely to the mass spectra of the alkaloids. The method is of particular value in correlation studies on various alkaloids from the same species or genus. Frequently these contain the same carbon skeleton and vary only in the type of substitution. The shift of characteristic ions by the presence of the substituent often enables a decision to be made as to where in a molecule it is located. Thus in a molecule A-B-C which fragments to give A^+, substitution in B or C will not affect the mass of A^+ whereas substitution on A will cause a shift to a higher mass number. Such an approach is limited as many substituents drastically change the fragmentation pattern of the whole molecule and the characteristic peaks of the unsubstituted system disappear. This may occur if the

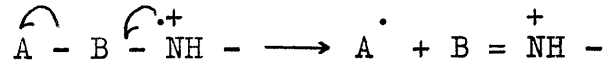
substituent contains a hetero atom which localizes the positive charge and dominates fragmentation by facilitating the cleavage of the bonds α- or β- to it.

The use of infra-red spectroscopy reveals the presence of certain functional groups. The presence and nature of an aromatic system may commonly be so recognized by a characteristic ultra-violet absorption. Mass spectrometry can indirectly deduce these facts but its greatest use is to determine the alicyclic part of the molecule. It is here that the substitution technique has its greatest application. One example of its use was Biemann's elucidation of the carbon skeleton of sarpagine (18). The mass spectrum of O-methyldeoxydihydrosarpagine was very similar to that of a degradation product of ajmaline of known structure, but all the major ions were sixteen mass units higher. The additional sixteen mass units, known to be an oxygen atom, were therefore located in the indole part of the molecule. Once the basic skeleton of the molecule was established the position of the substituents could then be determined.

Another approach used by Biemann (19) consists of degrading an alkaloid by zinc dust distillation. The parent base or mixture of bases so obtained is then separated by gas liquid chromatography and their mass

spectra obtained. The bases are usually derivatives of pyrrole, pyridine, indole, and α- and β-carboline. Valuable information can then be obtained as to the substitution pattern of the alkaloid. A peak or group of peaks corresponding to the parent base is often found in the mass spectrum of the alkaloid and represents a convenient base on which to build the structure of the alkaloid.

In many alkaloids the presence of one or more nitrogen atoms determines the fragmentation pattern, owing to its ability to stabilize the positive charge resulting from fission of a bond β- to it as shown.



Akuammidine alkaloids

Akuammidine has the structure (1), Fig. 1, with the stereochemistry at $C_{(16)}$ as shown. Voachalotine has the reversed configuration at $C_{(16)}$ and is methylated on the $N_{(a)}$ nitrogen atom. Djerassi (20,21) has also investigated this group of alkaloids and has obtained the mass spectrum of polyneuridine which is the $C_{(16)}$ epimer of akuammidine. Using a heated inlet system he obtained a large $(P-18)^+$ ion in the spectrum of

polyneuridine and an abundant $(P-60)^+$ ion in the spectrum of O-acetoxypolyneuridine acetate. These ions were only of low abundance in the spectra of akuammidine and its O-acetate. This striking difference in the spectra of the two compounds, differing only in the stereochemistry at $C_{(16)}$ led Djerassi to suppose that the spectrum of any similar compound might readily be used to determine its stereochemistry.

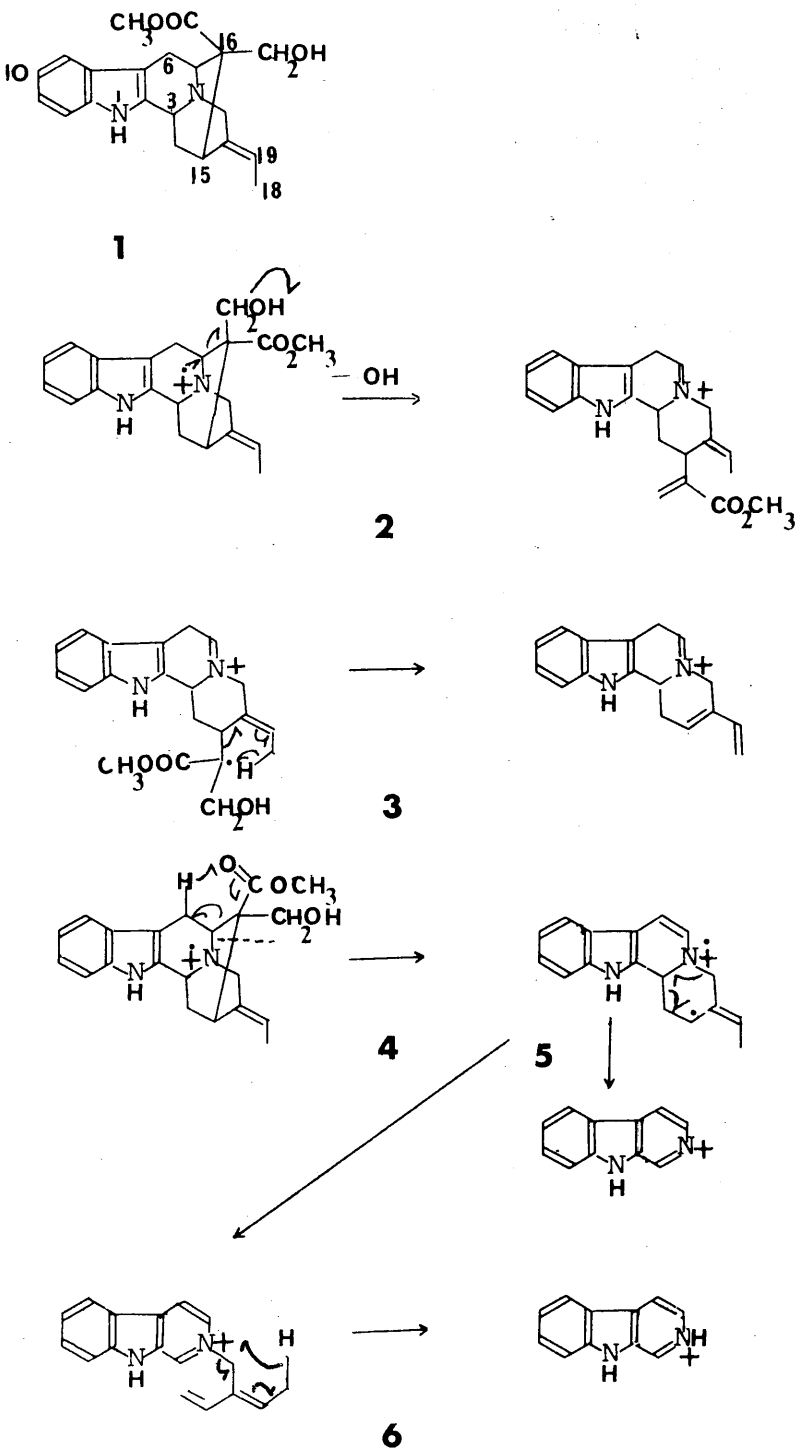
To test the validity of this assumption the mass spectrum of vincamajine alcohol acetate was obtained. Only a $(P-59)^+$ ion, known to be due to the loss of the carbomethoxy group on $C_{(16)}$ was found. There was no $(P-60)^+$ ion, suggesting that the stereochemistry at $C_{(16)}$ was the same as in akuammidine. It was, however, known to be the same as in polyneuridine. This inconsistency could only be explained if the presence of a methyl on the indole nitrogen was altering the mass spectrum. The mass spectra of the acetates of the $C_{(16)}$ epimeric deoxyajmalols (1, Fig. 1, i.e. Hydroxyl at $C_{(9)}$ and methyl on the indole nitrogen atom) were found to be nearly identical. The loss of the carbomethoxy group from $C_{(16)}$ was again prominent but only a small loss of acetic acid was observed. Finally, proof that the hydrogen on the $N_{(a)}$ nitrogen was involved in the elimination of

water or acetic acid was obtained from the mass spectrum of $N_{(a)}$-deuteropolyneuridine acetate. This revealed a facile loss of sixty-one mass units (CH_3CO_2D).

This result was remarkable since methylation of a nitrogen atom is normally assumed not to change the basic fragmentation pattern of a molecule. The mass spectrometric shift technique used by Biemann (22) depends on the fact that methylation of a nitrogen results in specific shifts and not in a modified fragmentation pattern. Since this was so contrary to the weight of accumulated evidence Djerassi redetermined (21) the spectra of akuammidine and polyneuridine using a direct inlet system (23) known to cause the least possible thermal decomposition. No elimination of water from polyneuridine or acetic acid from polyneuridine-O-acetate now occurred showing that previously, thermal decomposition had led to the loss of water from polyneuridine.

There were, however, differences in the abundances of some of the major ions. The parent ion is more abundant in the spectrum of akuammidine (80%) than in polyneuridine (40%), and the ion m/e = 169 which is the base peak in the spectrum of polyneuridine is less abundant (80%) in the spectrum of akuammidine. The difference in conformation at $C_{(16)}$ does therefore alter

FIG I


CH3OOC
16
CH2OH
10
6
1
N
H
3
N
19
15
18
1
CH2OH
CO2CH3
OH
N+
2
CO2CH3
3
CH3OOC
CH2OH
4
OCOCH3
CH2OH
5
6
NH

the related yohimbine alkaloids (20) where deuterium labelling established that over 50% of the hydrogen elided came from $C_{(3)}$. Stabilization of the positive charge by participation of the electron pair of the $N_{(b)}$-nitrogen is unlikely since the additional ring terminating at $C_{(5)}$ would make the formation of a 3,4 double bond difficult. It is likely that the elimination of a hydrogen from $C_{(6)}$ also occurs as this can lead to a resonance stabilized species by participation of the electron pair of the indole nitrogen (26).

Akuammidine (19%), voachalotine (11%) and dihydrovoachalotine (9%) all show the loss of a hydroxyl radical from the parent molecular ion. As such an elimination apparently creates a carbonium ion at the bridgehead carbon $C_{(17)}$ it should not be favoured. Stabilization of the carbonium ion could occur if fission of the 5-17 bond β- to the $N_{(b)}$-nitrogen atom took place. It may well be that the elimination of the hydroxyl radical is initiated by the lone pair of electrons on the $N_{(b)}$-nitrogen atom through a concerted mechanism relieving the strain in the ring system. The mechanism is shown in (2), Fig. 1.

The loss of the bridgehead carbon and its substituents with a hydrogen gives an abundant ion in

dihydrovoachalotine (m/e = 265, 44%), voachalotine (m/e = 263, 68.7%) and akuammidine (m/e = 249, 64.7%). Djerassi has postulated two mechanisms to explain this elimination. The first implies initial rupture of the 5-16 bond to give species (3), Fig. 1, which can then lose $C_{(16)}$ and its substituents with a hydrogen from $C_{(18)}$ through a six-membered transition state. Alternatively fission of the 15-16 bond may be followed by transfer of a hydrogen from $C_{(6)}$ to the carbonyl oxygen as in(4), Fig. 1. This ion was almost absent from the spectrum of normacusine-B ((1), Fig. 1, H for CO_2CH_3), indicating that participation of the carbonyl oxygen is required (20). The spectrum of deshydroxy-methylvoachalotine contains an abundant (15%) ion at m/e = 263 also. Since the spectrum of dihydrovoachalotine also shows this ion, shifted to m/e = 265, the first mechanism cannot operate as it would then lead to the loss of $C_{(19)}$ and $C_{(18)}$ in addition to $C_{(16)}$ and its substituents.

Homolysis of the 15-16 bond therefore appears to be the first step, permitting the close approach of the carbonyl oxygen to the hydrogen on $C_{(6)}$. The removal of this hydrogen is also favoured since it leads to a double bond in conjugation with the indole system.

Further fragmentation of this ion as shown in (4) Fig. 1, yields the abundant β-carboline ions (5) Fig. 1, m/e = 168 (42%) in akuammidine and m/e = 182 (81%) in voachalotine. The stability of the fully conjugated β-carboline system provides the driving force for the re-arrangement. The protonated β-carboline ions m/e = 169 (59%) in akuammidine and m/e = 183 (100%) in voachalotine probably also originate from the ion formed by the loss of $C_{(16)}$. Fission of the 3-14 bond gives the carbolinium ion (6) Fig. 1, this is followed by the transfer of a hydrogen atom from $C_{(18)}$ to the $N_{(b)}$-nitrogen through a six-membered transition state. The same mechanism can apply in the case of dihydrovoachalotine.

There is a group of ions in the spectra of voachalotine and akuammidine fourteen mass units below those of the β-carboline. In voachalotine they are m/e = 167 (16%), 168 (39%), 169 (20%), 170 (29%) and 171 (13%). The ion m/e = 170 in the spectrum of voachalotine and the corresponding ion in that of akuammidine can be explained by rupture of the 5-6, and 3-4 bonds; the result of a retro Diels-Alder reaction. Cleavage of the allylic 14-15 bond gives a stabilized ion as shown in(1), Fig. 2. A similar rearrangement has also been observed in yohimbine and ajmalacine.

The origin of the ion two mass units below m/e = 170 in voachalotine is obscure. Since it must contain two hydrogens less it may well be formed by ring closure of the ion m/e = 170. Beynon (28) has observed the loss of hydrogen after the elimination of carbon monoxide from benzanthrone to give an ion which can only be represented by a highly condensed structure.

In addition to the β-carboline ions there is a group of ions of lower abundance fourteen mass units higher, at m/e = 195 (12%), 196 (18%) and 197 (10%) in the spectrum of voachalotine. The latter two may be assigned unambiguously to methylated analogues of the ions m/e = 182 and 183. The additional carbon atom could be attached either to the $N_{(b)}$-nitrogen atom or to $C_{(3)}$. The fragment ion at m/e = 195 is probably derived from the methylated β-carboline ion (m/e = 196) by the loss of one hydrogen followed by ring expansion. Similar processes have been proposed to account for the loss of hydrogen from other methylated aromatic compounds. Toluene gives a very abundant ion at m/e = 91 formed by the loss of one hydrogen from the molecular ion to yield a tropylium ion (29).

There are abundant ions thirty one mass units below the parent in the spectra of voachalotine (m/e = 355, 45%),

dihydrovoachalotine (m/e = 337, 13.5%) and akuammidine (m/e = 321, 45%) which are apparently due to the loss of the $-CH_2OH$ group and not the methoxyl of the carbomethoxy group since the corresponding ion in deshydroxymethyl-voachalotine is of low abundance (2.7%). The $(P-31)^+$ ion is also abundant (40%) in the mass spectrum of nor-macusine-B. The abundance of this ion is surprising as it requires the formation of a radical or a carbonium ion on the bridgehead carbon atom and there is no obvious manner in which it may be stabilized.

The ions at m/e = 336 (20%) in voachalotine and m/e = 322 (17%) in akuammidine are clearly formed by the loss of formaldehyde. Chemically (30) these compounds are known to undergo a retro-aldol reaction when treated with potassium t-butylate in benzene with the elimination of formaldehyde. The formation of the $(P-30)^+$ ion from the parent molecular ion probably proceeds as shown below, yielding the molecular ion of deshydroxymethylvoachalotine.

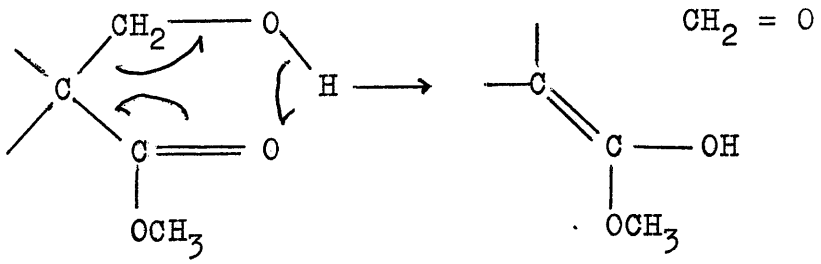
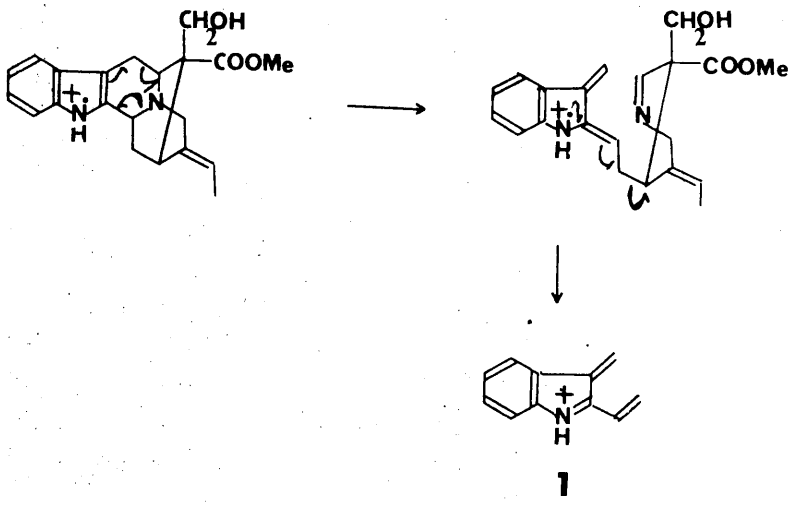


FIG 2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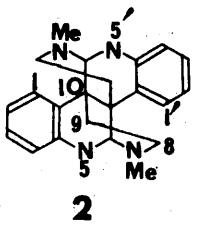
COOMe
N
H
1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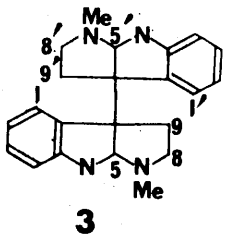
N
5'
N
10
1'
9
N
N
8
5
Me
2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8'
9'
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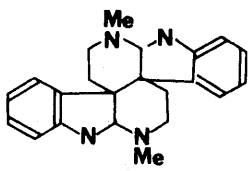
1'
9
N
5
N
8
Me
3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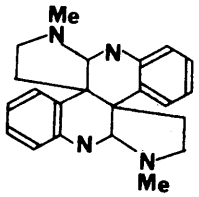
N
N
N
N
Me
4


Me
N
N
N
N
Me
5

The absence of a quaternary centre in deshydroxymethylvoachalotine itself changes the spectrum in a predictable manner. Fission of the 5-16 and 15-16 bonds is no longer so favoured since it gives a secondary rather than a tertiary radical. The parent molecular ion is now the base peak of the spectrum and there are abundant ions corresponding to the β-carboline skeleton at m/e = 182 (80%) and m/e = 183 (71%). As in voachalotine there is an ion (m/e = 263) corresponding to the loss of $C_{(16)}$ and its substituents with a hydrogen atom, it is now considerably less abundant (15%).

Calycanthus alkaloids

Robinson and Teuber (31) have proposed a biosynthetic route for alkaloids of this group by the ββ'-coupling of N-methyltryptamine. Several structures are therefore possible as shown in Fig. 2. Calycanthine has structure (2) Fig. 2. Chimonanthine has the structure (3) Fig. 3 as determined by X-ray analysis of the dibromide; its mass spectrum is consistent with such a structure.

Chimonanthine, Calycanthidine and Folicanthine

The fragmentation patterns of these three alkaloids greatly resemble each other and a detailed comparison indicates that calycanthidine and folicanthine are

N-methylchimonanthine and bis-N-methylchimonanthine respectively.

For chimonanthine the parent molecular ion (m/e = 346) is of low abundance (3.7%) and there is an abundant ion m/e = 173 (71.1%) which cannot be the doubly charged parent ion since there is no doubly charged (P+1) ion at m/e = 173.5. Of all the structures suggested by Robinson and Teuber only (3) Fig. 2 could give such a facile halving of the molecular ion since it requires rupture of only one bond. All other structures would require rearrangement of at least two bonds before halving of the parent molecular ion could occur.

Cleavage is favoured as this bond joins two quaternary centres. The products will be a tertiary carbonium ion and a tertiary radical. Moreover, the bond (10-10') is benzylic and is unusually weak.

The base peak of the spectrum (m/e = 172) represents a more stable ion which may well be formed by the loss of a hydrogen atom from the half molecular ion (m/e = 173), but there is an abundant fragment ion, m/e = 174 (27.9%) indicating that cleavage of the 10-10' bond may also proceed with a hydrogen migration. Elimination of either of the hydrogens on $C_{(9)}$ or $C_{(6)}$ would stabilize the ion. The loss from $C_{(6)}$ is more favoured as it would form an

ionized indole. It is possible to draw six-membered transition states for the transfer of a hydrogen from either $C_{(9)}$ or $C_{(6)}$ to the $N'_{(b)}$-nitrogen atom but in neither case can the hydrogen atom approach within bonding distance of the nitrogen. Fission of the 6'-7' bond would allow the nitrogen to approach more closely but the transfer of a hydrogen through a six-membered intermediate is no longer possible. The hydrogen may, therefore, be transferred from $C_{(6)}$ to $C_{(10')}$ as the 10-10' bond is ruptured, giving the ions m/e = 172 and 174; the charge being preferentially stabilized by the indole system.

The abundant ion at m/e = 171 (23%) correlates well with the known behaviour of 3-substituted indoles. Beynon (26) has found that the base peak of the spectrum of 3-methylindole (m/e = 130) is formed by the loss of a hydrogen atom from the parent molecular ion. He has postulated that the resulting carbonium is stabilized by the lone pair electrons of the nitrogen as in (1) Fig. 3 or by ring expansion to the quinolinium ion (2) Fig. 3. The loss of a hydrogen from the molecular ion of dihydro-pyrrolo[2,3-b]-indole (m/e = 172) is not, therefore, unexpected.

The ion m/e = 130 reported by Beynon in the mass

spectra of 2-methyl- and 3-methylindole is also abundant (49%). It can clearly be formed from the molecular ion by the concerted electron shifts shown (3) Fig. 4, leading to the elimination of the fragment $CH_3N{=}CH_2$ and a stable radical. Rearrangement of the half molecular ion (m/e = 173) by the same mechanism would give this ion. Beynon has observed the elimination of a molecule of hydrogen cyanide from the quinolinium ion (m/e = 130) and this accounts for the fairly abundant ion at m/e = 103 (8.1%).

These ions which could also be formed from alkaloids containing a quinoline or isoquinoline system are of little use in the diagnosis of an indole unit in the molecule. More important in this respect are the ions grouped around the indole molecular ion at m/e = 117 (8.6%). There are ions corresponding to dihydroindole at m/e = 119 (2.9%) and dehydroindole at m/e = 115 (4.1%). Djerassi (32) has observed that these are characteristic of an indole alkaloid.

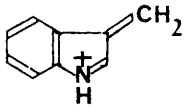
The easy rupture of the 10-10' bond precludes the existence of any abundant ions between the parent molecular ion and the half molecular ion. Elimination of forty-four mass units ($CH_3NH{-}CH_2^{\bullet}$) and one hundred and one mass units ($CH_3{-}NH{-}CH_2{-}CH_2^{\bullet}$ + $CH_3{-}N{=}CH_2$) gives the

ions at m/e = 302 and 245 respectively which, although of low abundance (1% and 3% respectively) are characteristic. Calycanthidine and folicanthine have very similar mass spectra to chimonanthine, their spectra again show the easy halving of the molecular ion. The parent molecular ions at m/e = 360 and 374 suggest that they are methyl-chimonanthine ($C_{23}H_{28}N_4$) and dimethylchimonanthine ($C_{24}H_{30}N_4$) and the great similarity of the spectra shows that the gross molecular structure is the same. Rupture of the 10-10' bond in calycanthidine gives two dissimilar "half units" at m/e = 173 and 187, but in folicanthine only the one half unit (m/e = 187) is formed. As the ion m/e = 187 elides a methyl radical to only a small extent it cannot explain the abundant ions at m/e = 173 and 172 in the spectrum of calycanthidine. The methyl groups in folicanthine must therefore be symmetrically disposed in the molecule. The almost equal split of the abundance of the quinolinium ion between m/e = 130 and 144 in the spectrum of calycanthidine shows the additional methyl group cannot be attached to $C_{(8)}$ as this is elided in the formation of the quinolinium ion. The appearance of this ion at m/e = 144 in the spectrum of folicanthine excludes either of the additional methyl groups from $C_{(8)}$. Substitution of the methyl group on $C_{(6)}$ is unlikely

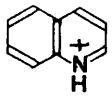
since its loss from the half molecular ion would be facile. The ion m/e = 245 in the spectrum of chimonanthine is shifted by twenty-eight mass units in the spectrum of folicanthine to m/e = 273 and by fourteen mass units in calycanthidine to m/e = 259. It would therefore appear that calycanthidine is $N_{(a)}$-methylchimonanthine and folicanthine is bis-N-methylchimonanthine. Although the mass spectrum cannot eliminate the possibility that the methyl groups are substituted in the benzene rings. When the relationship of calycanthidine and folicanthine to chimonanthine had been established by their mass spectra the absence of an N-H stretching band in the infra-red absorption spectrum determines the methylation pattern of the molecule.

The parent molecular ion of chimonanthine is only of low abundance (3.7%), but this increases with increased methylation. A comparison of the parent ion abundances is not, however, valid since the fragmentation that gives the base peak in chimonanthine and folicanthine gives two ions in the spectrum of calycanthidine. The abundances of the parent molecular ions with respect to the sum of the abundances of the fragment ions in each spectrum are 1.14% for chimonanthine, 5.4% for calycanthidine, and 9.55% for folicanthine. This correlates with the

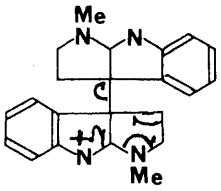
FIG 3

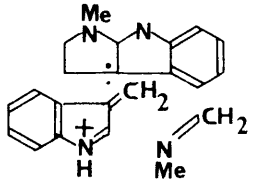


1



2





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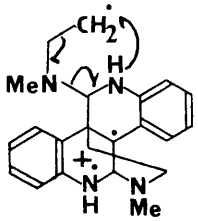
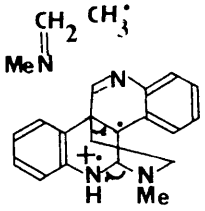
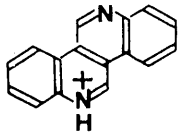


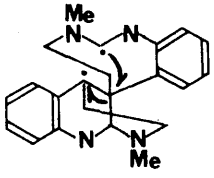


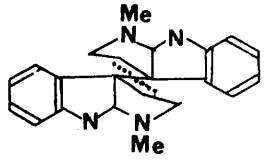
288

4



231





5

decreasing ability of a tertiary nitrogen atom to form a tetravalent ammonium ion. Rupture of the bond joining the $N_{(b)}$-nitrogen to the $C_{(6)}$ is therefore less likely because of the decreased stabilization of the positive charge.

Calycanthine

Chemical degradation of calycanthine gives a variety of bases including β-carboline, skatole, 3-ethylindole, N-methyltryptamine, and calycanine. The mass spectrum shows only the formation of two fragments corresponding to 5,8-dihydrocalycanine (m/e = 232) and a protonated calycanine molecule (m/e = 231).

The parent molecular ion (m/e = 346) is also the base peak of the spectrum. Fragment ions are generally of low abundance indicating that the alkaloid has a more closely knit structure than its isomer chimonanthine. As with the other alkaloids of this group rupture of the bonds at the quaternary centres and the C_6-N_b bonds is favoured. Cleavage of the 10-10' bond in the calycanthine molecular ion cannot lead to the formation of any simple fragment ions as cleavage of at least two other bonds is required before a fragment may be elided. A similar consideration prevents the next most favoured fission

that between a quaternary (C_{10}-$C_{10'}$) and a tertiary centre ($C_{6'}$-C_6) from giving rise to fragment ions. (The stability of the molecular ion and the low abundance of fragment ions is thus accounted for).

Fission of the 9-10 bond now becomes important even though it leads to a less favoured primary radical. The genesis of the major fragment ions (m/e = 288 and 231) can be easily explained on this model. The radical at $C_{(9)}$ is free to approach closely to the hydrogen atom on the $N_{(a)}$-nitrogen. Abstraction of the hydrogen through a six-membered transition leads to the elimination of N-methylmethyleneimine ($CH_3N{=}CH_2$) and a methyl radical and the formation of the ion at m/e = 288 (18.5%). Cleavage of the remaining 9-10 and 6-7 bonds gives the protonated calycanine molecule m/e = 231 (34.8%). The fairly abundant dihydrocalycanine ion at m/e = 232 (20.1%) may be formed simply by fission of both of the 9-10 and 6-7 bonds as these are the most favoured sites for fragmentation to occur.

There are fragments of low abundance, corresponding to fission of the 6-7 bond, at m/e = 316 ($P\text{-}\overset{+}{C}H_4N$), 302 ($P\text{-}\overset{+}{C}_2H_6N$), 259 ($P\text{-}CH_4N\text{-}C_3H_7N$) and 245 ($P\text{-}C_2H_6N\text{-}C_3H_7N$). After fission of this bond the charge will be stabilized by the formation of an "immonium ion" by the $N_{(a)}$-nitrogen.

The other significant ions m/e = 173 (8.1%), 172 (12.1%) and 130 (7.7%) have been correlated previously in the spectrum of chimonanthine with the $N_{(b)}$-methyltetrahydropyrrolo[2,3-b]indole system. Their formation here requires the rearrangement of the parent molecular ion to that of chimonanthine. If the structure of calycanthine is drawn in the most favoured conformation (33) it can be seen that carbon atoms 6, 10, 6' and 10' are close enough to permit such a ring contraction to occur. After initial cleavage of the 6'-10 bond $C_{(6')}$ can now approach $C_{(10')}$ and initiate the ring contraction as in (5) Fig. 3.

The mass spectrum of calycanthine would only eliminate the structure now assigned to chimonanthine from those suggested by Robinson and Teuber. The other two structures could not be easily rejected. The absence of any indole ions would make structure (4) Fig. 2 unlikely whereas structure (3) Fig. 2 would be expected to give these ions.

Calycanine

The parent molecular ion (m/e = 230) is also the base peak and the $(P-1)^+$ ion is the only significant fragment ion (31.8%). The hydrogen is, probably,

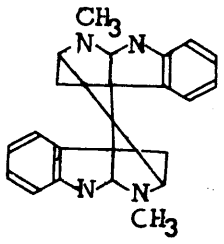
eliminated from one of the carbons adjacent to a nitrogen atom. Elimination of hydrogen cyanide and acetylene from the molecular ion give the fragment ions m/e = 204 (6.4%) and 203 (13.8%) and the further losses of hydrogen are observed at m/e = 202 (15.2%) and 201 (14.0%).

Hodgkinsine

Hodgkinsine is believed to be an alkaloid of the calycanthus group (34). Its structure has not yet been determined but the mass spectrum indicates the formula 1 Fig. 4a The molecular weight determined by conventional means is in the range 340-350 and it was therefore thought to be isomeric with calycanthine and chimonanthine ($C_{22}H_{26}N_4$). The parent molecular ion, however, is at m/e = 344 indicating that it contains two hydrogens less than chimonanthine. The molecular ion is very abundant (93%), consistent with a more closely bonded structure than that of chimonanthine. Abundant fragment ions are present at m/e = 315 (P^+-29), 314 (P^+-30), 302 (P^+-42), 301 (P^+-43), 287 (P^+-57). The molecule is apparently symmetrical for there are fragments corresponding to the further loss of 29, 30, 42, 43 and 57 mass units from the ions listed above.

The base peak of the spectrum, m/e = 172, is similar

FIG 4a



to that of chimonanthine and the fragmentation patterns of the two compounds resemble each other closely at lower masses. The existence of the group of ions at m/e = 115 to 119 also shows that the $N_{(a)}$-nitrogens are contained in an indole system. These were absent in the spectrum of calycanthine an alkaloid which does not contain an indole system. It is, therefore, probable that hodgkinsine also contains the tetrahydropyrrolo[2,3-b]-indole system. The $N_{(a)}$-nitrogen atoms cannot be methylated since this would give an abundant quinolinium ion at m/e = 144. By comparison with chimonanthine, hodgkinsine since it too seems symmetrically arranged, must contain an extra ring. A double bond joining the two halves of the molecule would not be consistent with the relatively easy halving of the molecular ion. The same consideration also excludes the presence of a bond joining the two aromatic rings. A double bond in any other part of the molecule would lead to the formation of two dissimilar ions at m/e = 171 and 173. The additional bond may therefore only connect $C_{(6)}$ and $C_{(6')}$, $C_{(9)}$ and $C_{(9')}$ or $C_{(8)}$ and $C_{(8')}$; the first two of these imply the formation of a four-membered ring.

The first major elimination from the parent molecular ion is of twenty-nine mass units and there is also a loss

of thirty mass units. The $N_{(b)}$-nitrogen atoms must therefore be present as either a CH_3N- or a -NH-CH_2. Since the infra-red absorption spectrum shows the presence of two CH_3N groups the latter possibility may be discarded. A more favoured fragmentation is, however, the loss of forty-two mass units to give the ion m/e = 302 which is accompanied by an abundant ion m/e = 301 formed by the loss of forty-three mass units. These fragmentations in other members of the calycanthus alkaloids lead to the elimination of forty-three and forty-four mass units respectively. The additional ring in hodgkinsine therefore connects $C_{(8)}$ and $C_{(8')}$ and the structure is as shown in (1) Fig. 4*a* A Dreiding model shows the additional six-membered ring, comprising carbon atoms 8,8', 9,9' and 10,10' may exist in either the boat or chair form.

Physostigmine and some eseroline derivatives

In the calycanthus alkaloids two tetrahydropyrrolo-[2,3-b]-indole systems were linked together by a single bond joining two quaternary centres. The spectra were largely made up of fragments derived from fission of this bond. Physostigmine and its derivatives also contain the tetrahydropyrrolo[2,3-b]-indole system but there is now a

methyl group substituted at $C_{(3)}$ of the indole. The $C_{(2)}$-$N_{(b)}$ and the 3-4' bonds (the numbering is shown in (1) Fig. 4) are now the likely bonds to be cleaved. By comparison with chimonanthine there will no longer be an abundant ion resulting from the loss of the substituent at $C_{(3)}$, for this would no longer give a stabilized radical, although the positive ion would be the same as before.

The parent molecular ions of desoxynoreseroline (1, Fig. 4. $R_1 = CH_3$, $R_2 = H$) and desoxybisnoreseroline (1, Fig. 4. $R_1 = R_2 = H$) are also the base peaks of their spectra. Their abundances are 14.92% and 14.58% of the total ion abundances of their spectra respectively. The replacement of the $N_{(b)}$-nitrogen atom by an oxygen atom decreases the stability of the molecular ion which is no longer the base peak although its relative abundance (85.15%) is still high being 11.4% of the total ion abundance. With the introduction of another quaternary centre into the molecule, by the substitution of a methyl at $C_{(2)}$ there is a further decrease in the abundance (35.4%) of the parent molecular ion to only 5.3% of the total ion abundances. This agrees with the view that the presence of a quaternary centre in a molecule generally causes a decrease in the parent ion abundance. In the

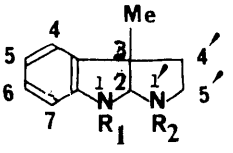
mass spectra of the paraffins the relative height of the parent peak is greatest for the straight chain compound and decreases as the degree of branching increases (35).

The parent molecular ion of $N_{(b)}$-acetyldesoxynoreserpoline is still very abundant (90%) but replacement of the acetyl group by a benzoyl gives a much less abundant parent ion (42%). This results from the greater stability of the benzoyl positive ion compared to the acetylinium ion. Acetylation of both the nitrogens leads, as expected, to an even less abundant molecular ion (20%).

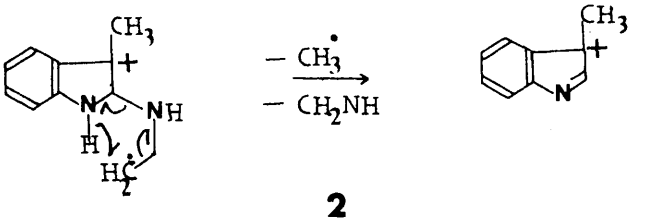
Desoxynoreseroline and Desoxybisnoreseroline

Comparison of the cracking patterns of these two compounds shows that, allowing for the difference in molecular weight, the two closely resemble each other. The loss of a methyl radical occurs which almost certainly represents the loss of the quaternary methyl. This is clearly followed by the elimination of two and three hydrogen atoms with the resulting aromatization of the pyrrole ring. Both compounds elide CH_2NH_2 and $CH_2CH_2NH_2$ to give abundant fragment ions, probably with rearrangement to become substituted quinolinium ions. In each case the fragment that is elided has removed an additional hydrogen

FIG 4


Me
4
5
6
7
1
2
3
1′
4′
5′
N
R1
R2

1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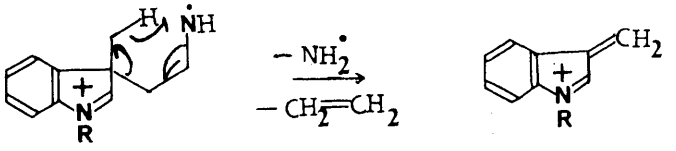
NH
H
H2C
− CH3
− CH2NH
CH3
N

2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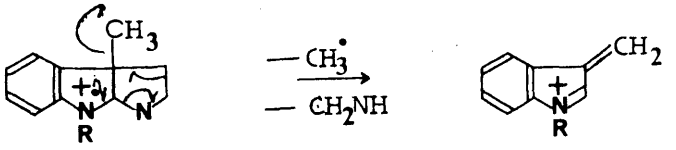
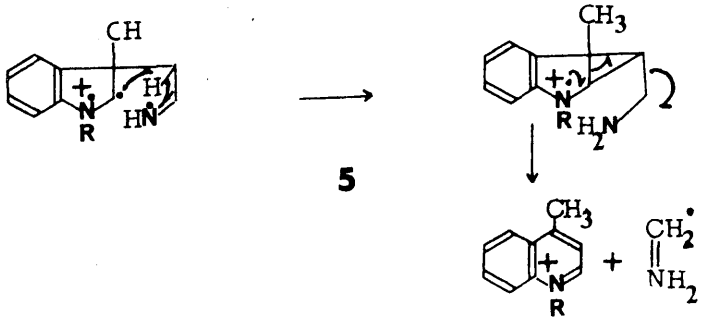
NH
N
R
− NH2
− CH2=CH2
CH2
N
R

3


CH3
N
N
R
− CH3
− CH2NH
CH2
N
R

4


CH
H
HN
N
R
CH3
N
R
H2N
CH3
N
R
+
CH2
NH2

5

atom. There is a slight difference in the behaviour of the two compounds in the elimination of $CH_2CH_2NH_2$. In desoxybisnoreseroline the ion at m/e = 130 (72%) is accompanied by a less abundant ion m/e = 131 (46%) formed by the loss of ring C only. The corresponding ions in desoxynoreseroline m/e = 144 and m/e = 145 are almost equally abundant (87% and 72% respectively).

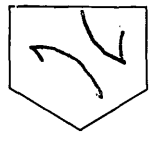
There are three mechanisms by which the elimination of C_2H_6N can take place, only one of these requires the removal of a hydrogen atom from the $N_{(a)}$-nitrogen. Since the elimination of C_2H_5N without a hydrogen takes place to a greater extent when the $N_{(a)}$-nitrogen is methylated it suggests that all three mechanisms may operate. Initial cleavage at the quaternary carbon atom $C_{(3)}$ will enable $C_{(4')}$ to abstract a hydrogen from the $N_{(a)}$-nitrogen in desoxybisnoreseroline through a six-membered transition state. This mechanism is blocked by the substitution of a methyl on the $N_{(a)}$-nitrogen atom. Alternatively a hydrogen may be abstracted from the angular methyl, after the $C_{(2)}$-$N_{(b)}$ bond has been broken, through a similar transition state. A mechanism for the elimination of C_2H_6N can also be envisaged which does not require a hydrogen transfer, but involves the elimination of the angular methyl and a molecule of methyleneimine

(CH_2=NH). These mechanisms are shown in (2, 3 & 4) Fig. 4.

There are abundant fragment ions in the spectrum of desoxynoreseroline and desoxybisnoreseroline formed by the elimination of ethylene at m/e = 160 (12.0%) and 146 (26.7%) and methylene imine at m/e = 159 (30.0%) and 145 (44.4%). This latter ion is probably also formed by the loss of an ethyl radical. The most abundant ion of this group m/e = 144 (72%) in desoxybisnoreseroline or m/e = 158 (64.1%) in desoxynoreseroline is formed by the elision of CH_4N. The resulting ion cannot possess a structure like (1) Fig. 3 since it has an additional methyl on $C_{(3)}$ and must therefore have the quinolinium structure; a probable mechanism for its formation can be seen in (5) Fig. 4. It has been shown in the similar case of physovenine (later this chapter) that this elision is in fact a one step process by the presence of the appropriate metastable ion.

Such eliminations as well as that leading to the loss of $CH_2CH_2NH_2$, occur commonly in cyclic compounds and are often accompanied by the further loss of a hydrogen atom. In the mass spectra of the steroids (Chapter 3) there are ions corresponding to the similar fragmentation of ring D. The spectrum of trans-2-thiabicyclo[3,3,0]-

octane (36) is particularly interesting since it shows typical fragments corresponding to the breakdown of a five-membered ring with hydrogen migration. Abundant fragment ions are formed by the elimination of $CH_2{=}CH_2$, $CH_2{=}S$, $CH_2CH_2CH_2$ and CH_2CH_2S. The mechanism of the formation of these ions can best be represented by the mechanism shown below for cyclopentane.



Either fragment may take the charge. An equivalent process has been postulated by Biemann (37) to explain the fragmentation of six-membered rings in the alkaloid field. Each of these ions is accompanied by a more abundant ion containing one hydrogen less, formed by the loss of $C_2H_5^{\bullet}$, $CH_3S^{\bullet}$, $C_3H_7^{\bullet}$ and $C_2H_5S^{\bullet}$. The formation of these ions is in agreement with the general principle that in carbon, hydrogen, oxygen and sulphur compounds even electron fragments are more stable than odd electron fragments (38) which is apparently the main driving force for the elimination of an additional hydrogen atom.

The most abundant singly charged ions containing a single nitrogen are likely to be those of even mass, since

nitrogen has an even atomic weight and an odd valency. For the same reason the stable doubly charged ions are likely to be those of odd mass. They will, therefore, appear at half integral mass numbers and will always be recognizable. In the spectra of the indoles the abundances of the doubly charged ions are high, 10% of the base peak being not uncommon (26). They are sometimes more abundant than the singly charged species. In 1,7-dimethylindole the ion at m/e = 58.5 is 4.4% of the base peak while the singly charged ion at m/e = 117 is only 3.5%. In common with these and other nitrogen containing compounds the eserolines show a large number of doubly charged ions in their spectra. Nowhere are they as abundant as those in the mass spectra of the indoles; the most abundant in each case corresponds to the removal of a second electron from the $(P-15)^+$ ion.

Tetrahydrofurano(2,3-b)-indoles

These compounds may conveniently be compared with the eserolines. As has already been mentioned they are less stable than the corresponding eseroline giving a much less abundant parent molecular ion. The fragmentations closely resemble those of the eserolines and the elisions of CH_2O, CH_2OH, CH_2CH_2O and CH_2CH_2OH give

abundant fragment ions.

In the spectrum of 2,3-dimethyltetrahydrofurano(2,3-b)-indole the base peak (m/e = 144) is formed by the elimination of CH_2CH_2OH. The mechanisms which lead to the elimination of $CH_2CH_2NH_2$ from desoxybisnoreseroline will operate here and the positive ion may have either the methylene indolinium structure or the quinolinion structure (1) and (2), Fig. 3. Elimination of CH_3O gives rise to an abundant fragment ion at m/e = 158 (63.9%). 3-Methyltetrahydrofurano(2,3-b)-indole shows fragment ions corresponding to both these eliminations in its mass spectrum. However, the base peak is now formed by the loss of CH_3O and the loss of CH_2CH_2OH gives rise to a less abundant fragment ion at m/e = 130 (87.2%). Since the $(P-\overset{+}{C}H_3O)$ has the quinolinion structure the only explanation for its reduced abundance in 2,3-dimethyltetrahydrofurano(2,3-b)-indole would appear to be that the methyl group on $C_{(2)}$ hinders the approach of $C_{(4')}$ to $C_{(2)}$. Expansion of the indole ring to a quinoline might then be more difficult.

In the spectra of the eserolines no distinction could be made between the loss of CH_2NH (29 mass units) or C_2H_5 (29 mass units), the same fragmentation processes in the tetrahydrofuranoindoles lead to the losses of

CH_2O (30 mass units) and C_2H_5 (29 mass units). There are fragment ions at m/e = 145 (17%), 146 (32%) and 147 (20%) corresponding to the losses of CH_2O, C_2H_5 and C_2H_4 from the parent molecular ion of 3-methyltetrahydrofurano-(2,3-b)-indole. Again there are a large number of doubly charged ions in the spectrum; the most abundant of these is the ion $(P-28)^{++}$ at m/e = 73.5 (3.16%) and 80.5 (7.94%) in the spectra of 2-methyl- and 2,3-dimethyltetrahydro-furano(2,3-b)-indole.

3-Methyltetrahydropyrano(2,3-b)-indole

The mass spectrum of this compound illustrates the general conditions that lead to the formation of a stable ion. Elimination of thirty-one mass units (CH_3O) can no longer give an ion which is stabilized by rearrange-ment to a substituted quinolinium ion; the loss of thirty-one mass units does not occur.

Rearrangement to a substituted quinolinium ion could take place after the loss of forty-five mass units (CH_2CH_2OH). The elimination would, however, either require the transfer of a hydrogen through a five-membered ring or cleavage of the pyran ring γ- to the oxygen between two methylene groups, e.g. $-CH_2 \vdots CH_2-CH_2-O-$. The elimination of forty-five mass units occurs to a much

smaller extent (21%) than in the tetrahydrofurano(2,3-b)-indoles.

Loss of ring C gives the base peak of the spectrum at m/e = 131. Since the most abundant ions in the spectrum of a compound containing a single nitrogen atom are generally at even mass numbers, the formation of the molecular ion of 3-methylindole is of interest. The spectrum of 3-methylindole itself has the base peak at m/e = 130 and therefore the stability of the positive ion (m/e = 131) does not seem to be the determining factor in its formation. There is, however, a mechanism (1) Fig. 5 which leads to the elimination of fifty-eight mass units with the elision of formaldehyde and ethylene as stable neutral molecules.

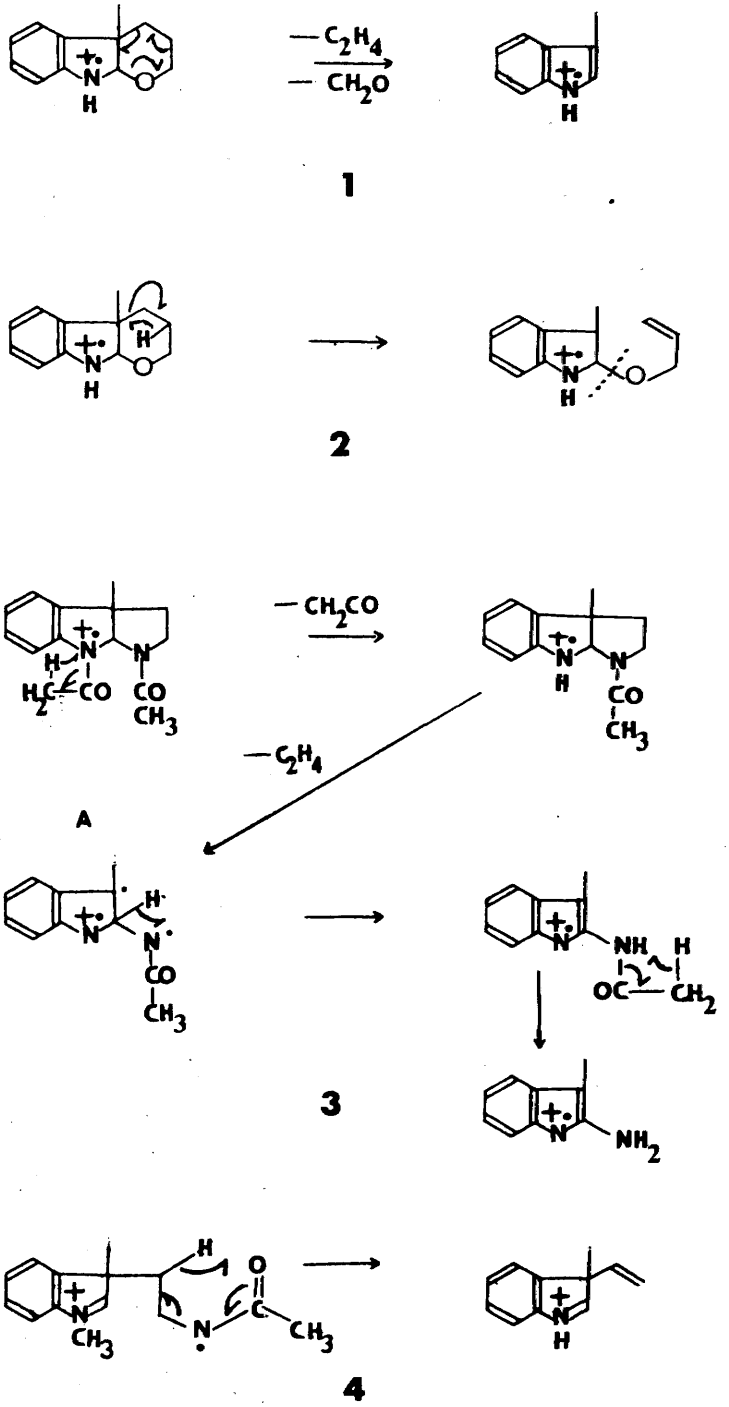
Loss of a hydrogen from the molecular ion of 3-methyl-indole gives an ion at m/e = 130 (48%). Cleavage at the quaternary centre $C_{(3)}$ is followed by the loss of fifty-seven mass units. The capture of a hydrogen by $C_{(3)}$ probably proceeds as in (2) Fig. 5, and is followed by fission of the $C_{(2)}$-O bond. There are two other abundant ions in the mass spectrum at m/e = 147 (14%) and 146 (11%) formed by the loss of C_3H_6 and C_3H_7.

$N_{(b)}$-Acetyldesoxynoreseroline

The presence of the $N_{(b)}$-acetyl group modifies the spectrum considerably. Gilpin (39) found that the mass spectra of a series of aliphatic amides showed two characteristic features. When the acyl substituent of the amide contained a hydrogen atom δ - to the carbonyl group, fragmentation β- to the carbonyl group with the transfer of this hydrogen occurred. Fragmentation between the carbonyl group and the nitrogen atom was followed by cleavage β- to the nitrogen to give an abundant fragment ion. Djerassi (40) has observed that fragmentation β- to the nitrogen atom gave an abundant series of ions in the mass spectra of N-acetylcyclohexylamine and N-acetylcyclopentylamine.

In the spectrum of $N_{(b)}$-acetyldesoxynoreseroline the loss of twenty-eight mass units is marked (45%). Almost certainly this represents the loss of ethylene which was noticeable in the spectrum of the free base. The most favourable structure that can be written for this ion is that of the molecular ion of 2-acetamido-1,3-dimethylindole. Its formation implies the transfer of the hydrogen from $C_{(2)}$ to the $N_{(b)}$-nitrogen after the loss of ethylene. There is an abundant ion at m/e = 160 (72%) formed by the loss of seventy mass units from the

FIG 5



parent molecular ion. Although there is no metastable ion to support the suggestion it would appear that the most favourable method of generating this ion is by the loss of ketene (CH_2=C=O) from the $(P-28)^+$ ion. The elimination of ketene from N-acetates on electron impact is well established (41). N-Acetylcyclohexylamine has as the base peak of its spectrum (40) the ion at m/e = 56 formed by the loss of ketene and C_3H_7 from the parent molecular ion.

The formation of the ion at m/e = 160 by the successive losses of ethylene and ketene from the parent molecular ion is confirmed by the mass spectrum of $N_{(b)}$-benzoyldesoxynoreseroline. Since there are no α-hydrogen atoms in the benzoyl group it cannot form a ketene. The spectrum shows an abundant $(P-28)^+$ ion (52%) and there are ions at m/e = 158 (23.3%) and 159 (24.7%) formed by the loss of the benzoyl group + NCH_2 or NCH_3. There is, however, no abundant ion at m/e = 160.

Only an ion of low abundance (9%) is observed corresponding to the loss of forty-three mass units from the parent ion of $N_{(b)}$-acetyldesoxynoreseroline, the charge preferentially remaining on the acetyl moiety m/e = 43 (32%).

There is a moderately abundant ion at m/e = 172 (20%)

which corresponds to the elimination of $CH_3CONH^{\bullet}$. After cleavage of the $C_{(2)}$-$N_{(b)}$ bond the acetyl group may approach $C_{(4')}$ close enough to abstract a hydrogen from it through a six-membered transition state.

The rearrangement which eliminated CH_4N from the parent molecular ion of desoxynoreseroline leads to the loss of seventy-two mass units ($CH_3CONH-CH_2^{\bullet}$) from N-acetyldesoxynoreseroline to give the ion at m/e = 158 (42.7%). There is a fairly abundant ion at m/e = 159 (28.1%) corresponding to the normal β-cleavage of amides with the elision of $CH_3CON=CH_2$ after fission of the $C_{(2)}$-$N_{(b)}$ bond. The base peak of the spectrum at m/e = 144 is formed by the same processes as in the unacetylated compound.

Benzoyldesoxynoreseroline shows the same general fragmentation pattern with the one important difference already noted. The base peak at m/e = 105 is attributable to the benzoyl ion ($Ph{\cdot}C\overset{+}{O}$).

N,N-Diacetyldesoxybisnoreseroline

The elimination of ketene from the parent molecular ion gives the base peak at m/e = 216. It can be established that this must be exclusively from the $N_{(a)}$-nitrogen. Thus there are no ions at m/e = 186 or

172 corresponding to the elimination of thirty (CH_4N) or forty-four (C_2H_6N) mass units from the base peak as there would be if the $N_{(b)}$-acetyl group had been eliminated. Furthermore the spectrum below the base peak closely resembles that of $N_{(b)}$-acetyldesoxynoreseroline if allowance is made for the additional methyl in the latter. The ions m/e = 230 (90%), 202 (45%), 187 (8.7%), 172 (20%), 160 (71%), 159 (28%), 158 (43%), 157 (10%) and 144 (100%) are all shifted by fourteen mass units to m/e = 216 (100%), 188 (91%), 173 (10%), 158 (12%), 146 (89%), 145 (24%), 144 (35.7%), 143 (12%) and 130 (40%) in the spectrum of N,N-diacetyldesoxybisnoreseroline. The fragmentation of the molecular ion is shown in (3) Fig. 5.

Physostigmine

The parent molecular ion m/e = 275 is abundant (62%) and the base peak of the spectrum at m/e = 218 is formed by the loss of fifty-seven mass units. There are no abundant ions between the parent ion and the base peak. The spectrum below the latter is consistent with the established modes of fission of the eseroline system. Therefore, the loss of fifty-seven mass units is from the side chain. It seems most likely that the hydrogen

gained by the eseroline system is derived from the nitrogen of the side chain with the expulsion of a molecule of methylisocyanate. The rearrangement is illustrated in (1) Fig. 6. The other abundant ions m/e = 160 (40%), 161 (37%), 174 (45%) and 175 (15%) are formed by the established fragmentation modes of the eseroline system.

Physovenine

The structure of this alkaloid was determined by other spectroscopic techniques (42); the mass spectrum alone allows a choice of two structures for the alkaloid. The spectrum obtained by using the heated reservoir system showed signs that decomposition had occurred. The fragmentation pattern resembled those of the tetrahydrofurano(2,3-b)-indoles but the base peak at m/e = 57, shown to be C_2H_3NO by precise mass measurement could not be reconciled with such a structure. The apparent parent molecular ion and the other major fragment ions were shown to be $C_{12}H_{15}NO_2$ (m/e = 205), $C_{11}H_{12}NO$ (m/e = 174) and $C_{10}H_{10}NO$ (m/e = 160) by precise mass measurement. Except for the base peak m/e = 57 the spectrum was therefore consistent with a methoxyl substituted in the benzene ring of 3-methyltetrahydro-

furano(2,3-b)-indole.

When the spectrum was obtained using a probe an abundant parent molecular ion was found at m/e = 262 (19%). Precise mass measurement showed it to be $C_{14}H_{18}N_2O_2$. The ion at m/e = 57 was now much less abundant (12%) and the base peak occurred at m/e = 205.

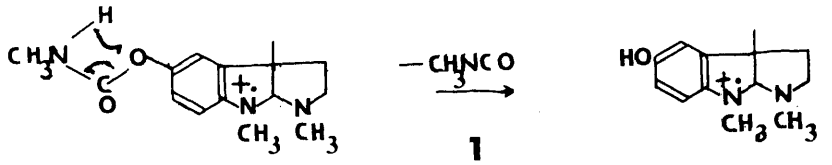
This spectrum closely resembles that of physostigmine with the difference that the parent ion and the base peak are shifted down by thirteen mass units. The other fragments m/e = 174, 161 and 160 are the same however. The difference in the molecular weights of physostigmine and physovenine is known to be that of an $N\text{-}CH_3$ and an O from the precise mass measurement of the parent molecular ion. Therefore the structure of physovenine is (2) Fig. 6.

It was possible to detect three metastable ions in the spectra. The first of these confirmed that the loss of methylisocyanate from the parent molecular ion occurred in one step.

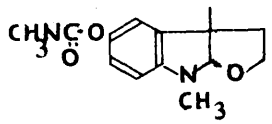
$$262^+ \longrightarrow 205^+ + 57 \quad m/e = 160.5.$$

The losses of CH_3O, to give the ion at m/e = 174 (43.17%), and C_2H_5O to give the ion m/e = 160 (19%) were shown to occur by one step processes from the base peak

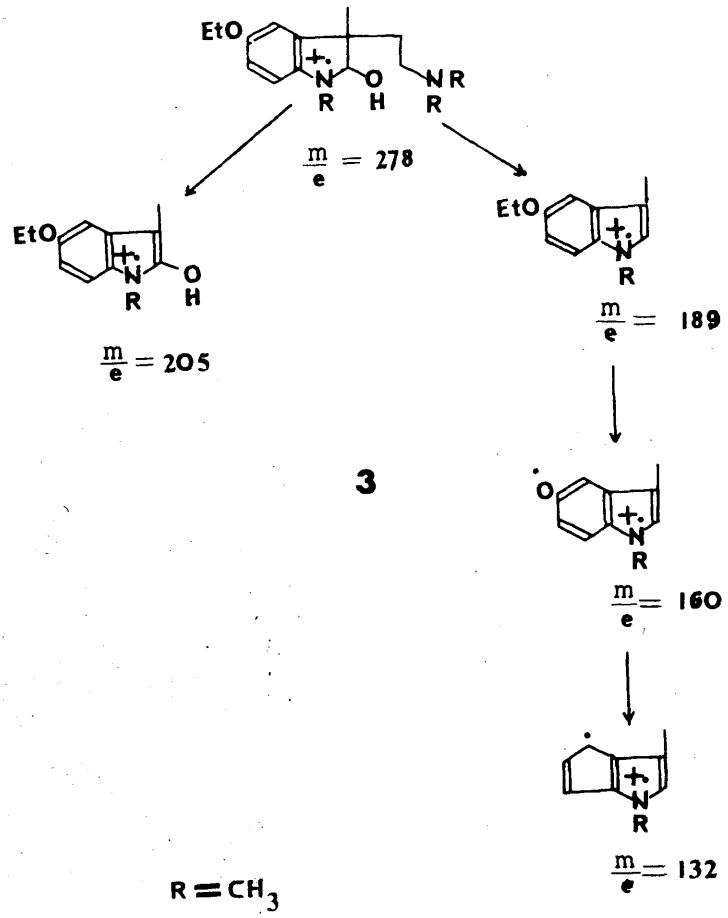
FIG 6



1



2



3

R = CH_3

at m/e = 205.

$$205^+ \longrightarrow 174^+ + 31 \quad m/e = 147.7$$
$$205^+ \longrightarrow 160^+ + 45 \quad m/e = 124.9.$$

Eserethole methine

Although ring C has been opened in eserethole methine, the parent molecular ion is moderately abundant (28%). The base peak of the spectrum at m/e = 58 is consistent with the expected scission β- to the side chain nitrogen atom. Elimination of water from the parent molecular ion gives a prominent ion m/e = 260 (21%) which has the quinolinium structure. It is most likely that this is a 1,3-elimination of water with the additional hydrogen coming from the methyl on $C_{(3)}$ since there is an abundant ion which corresponds to the further loss of fifty-eight mass units ($(CH_3)_2N\text{-}\dot{C}H_2$) from it. If the hydrogen had come from the methylene group on $C_{(3)}$ the resulting quinolinium ion could only lose fifty-eight mass units by cleavage α- to the ring. The remaining fragmentation is shown in (3) Fig.6.

Samples of voachalotine and akuammidine were provided by Professor A. R. Battersby (Liverpool University) and Professor J. Martin (Brussels University). Dr. G. F. Smith (Manchester University) and Dr. E. J. Saxton (Leeds University) provided the samples of the calycanthus alkaloids. Physovenine was provided by Dr. B. Robinson (Nottingham University) and the remainder of the physostigmine group by Dr. G. F. Smith. The spectra were all, except that of physovenine, obtained on a M.S.2 mass spectrometer using a probe insertion system.

[illegible]	[illegible]	140	[illegible]	[illegible]	[illegible]	[illegible]
						[illegible]
102	[illegible]	141	[illegible]	[illegible]	[illegible]	[illegible]
		142	[illegible]	[illegible]	[illegible]	
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

VOACHALOTINE

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
77	9.4	114	1.8	153	5.5	191	1.5
78	2.4	115	7.3	154	15.0	192	3.1
79	12.5	116	3.1	155	9.2	193	3.7
80	1.7	117	3.9			194	7.9
81	8.0	118	2.4	156	4.8	195	12.3
82	2.6	119	1.9	157	20.5	196	17.8
83	2.6	120	1.8	158	8.8	197	10.1
84	0.7			159	2.0	198	4.2
85	2.0	127	6.0			199	0.5
		128	8.8	165	1.1		
91	12.7	129	6.6	166	3.7	204	1.7
92	2.8	130	5.1	167	15.6	205	3.0
93	6.5	131	3.3	168	39.2	206	4.2
94	1.8	132	2.9	169	19.8	207	11.2
95	4.0	133	2.6	170	28.8	208	9.3
96	2.2			171	12.7	209	5.3
97	2.4	140	3.7	172	2.8	210	2.7
						211	1.3
102	1.8	141	3.3	178	2.6	212	0.9
		142	3.7	179	4.0		
103	3.5	143	4.2	180	9.3	218	2.6
104	1.7	144	15.2	181	27.7	219	2.6
105	7.9	145	2.2	182	81.0	220	4.9
106	3.2	146	0.9	183	100	221	5.0
107	10.1			184	25.6	222	3.5
108	2.9	151	2.0	185	3.8	223	2.6
109	2.6	152	4.2			224	1.5

VOACHALOTINE (Contd.)

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
225	1.5	252	2.2	279	0.7	323	-
226	0.7	253	3.5				
		254	0.9	284	1.7	333	5.7
231	2.0			285	1.3	334	9.5
232	2.4	258	0.9			335	44.7
233	3.5	259	2.4	289	1.3	336	20.0
234	3.8	260	2.2	290	0.9	337	4.9
235	10.9	261	5.5			338	1.1
236	4.8	262	8.8	303	1.8		
237	4.0	263	68.7	304	1.8	347	1.7
238	1.3	264	23.6	305	3.7	348	3.7
239	1.8	265	6.4	306	4.0	349	9.7
240	6.8	266	1.8	307	9.5	350	6.6
241	1.8	267	7.0	308	7.9	351	16.3
242	0.9	268	1.8	309	2.2	352	5.1
				310	1.3		
245	1.5	273	1.1	311	1.8	363	5.0
246	2.9	274	0.7	312	1.1	364	37.4
247	4.8	275	3.5			365	59.7
248	4.8	276	2.0	320	1.3	366	84.3
249	12.1	277	2.0	321	1.1	367	28.7
250	18.8	278	0.7	322	1.7	368	6.2

DESHYDROXYMETHYLVOACHALOTINE

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
77	7.4	120	1.3	157	5.4	195	14.4
78	1.6	121	4.2	158	2.7	196	12.1
79	7.2	122	1.3			197	5.1
80	3.2	123	2.8	160	5.4	198	2.4
81	5.4	124	1.3	161	4.4		
82	0.6	125	2.3	162	1.3	203	2.4
83	1.1	126	1.3			204	2.4
		127	4.0	165	2.0	205	2.7
91	4.3	128	6.7	166	3.4	206	3.1
92	1.1	129	2.7	167	11.2	207	2.8
93	10.3			168	34.8	208	3.6
94	2.7	137	2.7	169	14.9	209	2.7
95	3.5	138	3.4	170	11.3		
				171	4.0	217	3.4
103	0.8	140	5.4			218	10.8
		141	4.6	178	1.8	219	6.1
105	0.7	142	4.0	179	3.0	220	5.3
		143	3.8	180	6.5	221	6.7
107	2.2	144	2.4	181	20.0	222	6.7
		145	0.9	182	79.5	223	5.4
113	2.8			183	71.5	224	1.3
114	1.3	151	2.0	184	16.9		
115	5.4	152	3.0	185	3.0	228	2.0
116	2.7	153	4.0			229	2.7
117	1.6	154	9.4	192	2.3	230	2.2
118	2.4	155	5.9	193	4.0	231	2.7
119	2.2	156	2.7	194	5.3	232	2.7

DESHYDROXYMETHYLVOACHALOTINE (Contd.)

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
233	4.0	250	6.5	276	3.4	307	2.0
234	4.0	251	1.6	277	32.4		
235	6.5			278	12.1	321	23.6
236	3.4	259	3.2	279	2.0	322	10.1
		260	1.6			323	2.0
244	1.3	261	5.4	289	1.6		
245	1.3	262	3.4	290	2.2	335	63.3
246	2.0	263	14.8			336	100
247	4.0	264	4.9	304	6.7	337	41.7
248	2.7			305	2.7	338	7.4
249	7.4	275	6.7	306	2.7		

AKUAMMIDINE

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
60	2.9	87	0.9	114	1.8	141	3.4
61	1.7	88	0.5	115	8.6	142	4.2
62	0.9	89	1.7	116	2.9	143	8.4
63	1.6	90	1.4	117	3.4	144	3.9
64	1.8	91	11.4	118	1.6	145	2.2
65	6.0	92	3.4	119	4.4	146	0.7
66	3.0	93	5.6	120	1.9	147	0.7
67	9.2	94	5.4	121	3.3	148	0.7
68	4.7	95	9.9	122	1.4	149	2.1
69	19.6	96	4.1	123	5.1	150	1.1
70	6.4	97	7.9	124	2.2	151	3.0
71	9.3	98	2.7	125	3.3	152	3.0
72	1.3	99	1.3	126	1.5	153	3.2
73	2.8	100	0.5	127	3.7	154	8.4
74	1.3	101	1.4	128	1.9	155	8.1
75	0.9	102	1.7	129	10.6	156	13.4
76	0.9	103	2.4	130	6.4	157	8.9
77	7.9	104	3.2	131	2.2	158	1.9
78	3.4	105	8.2	132	1.4	159	0.9
79	8.4	106	4.5	133	3.1	160	0.9
80	2.6	107	8.5	134	1.6	161	0.9
81	13.9	108	3.3	135	2.4	162	0.6
82	5.4	109	6.9	136	2.2	163	1.1
83	6.4	110	3.5	137	4.5	164	1.5
84	3.8	111	5.2	138	1.9	165	3.6
85	5.4	112	1.9	139	2.7	166	5.2
86	0.8	113	2.0	140	2.9	167	12.0

AKUAMMIDINE (Contd.)

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
168	42.3	195	4.0	222	4.9	249	64.7
169	58.9	196	2.4	223	3.2	250	2.5
170	15.3	197	1.9	224	2.7	251	5.9
171	3.2	198	7.1	225	2.2	252	2.0
172	0.9	199	1.9	226	2.5	253	5.9
173	0.5			227	0.9	254	2.1
174	0.5	201	0.2	228	0.9	255	1.2
175	0.6	202	0.5	229	0.9	256	1.2
176	1.4	203	0.6	230	1.9	257	1.5
177	1.4	204	1.9	231	2.5	258	1.6
178	2.0	205	2.2	232	4.5	259	2.5
179	3.2	206	4.9	233	5.9	260	2.2
180	6.4	207	4.6	234	5.4	261	5.4
181	9.3	208	3.4	235	1.1	262	3.5
182	16.1	209	3.1	236	1.8	263	5.0
183	11.0	210	1.4	237	6.1	264	2.6
184	4.7	211	1.2	238	2.5	265	1.5
185	1.9	212	0.7	239	2.0	266	1.0
186	1.1	213	0.5	240	1.7	267	0.8
187	0.6	214	0.5	241	1.2	268	0.5
188	0.2	215	0.6	242	0.9	269	0.5
189	0.7	216	0.9	243	1.7	270	0.5
190	0.8	217	2.5	244	2.0	271	1.0
191	2.0	218	3.1	245	3.7	272	0.8
192	2.9	219	4.3	246	4.0	273	1.2
193	7.4	220	4.3	247	8.9	274	0.8
194	6.0	221	8.9	248	9.3	275	3.0

AKUAMMIDINE (Contd.)

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
276	1.2	296	1.0	314	0.2	332	2.0
277	0.6	297	1.0	315	0.6	333	5.4
278	0.5	298	0.6	316	0.7	334	6.4
279	0.5	299	0.4	317	1.1	335	11.1
		300	0.2	318	2.7	336	8.4
283	0.5	301	0.6	319	6.0	337	16.5
284	0.5	302	0.6	320	8.2	338	9.4
285	0.6	303	1.0	321	-45.1	339	1.1
286	0.6	304	0.9	322	16.6	340	0.5
287	1.0	305	1.5	323	5.4	341	0.2
288	1.0	306	1.2	324	2.3		
289	3.5	307	1.1	325	1.1	348	0.7
290	4.2	308	1.5	326	0.5	349	2.5
291	4.5	309	0.9	327	0.5	350	16.2
292	4.5	310	0.6	328	0.5	351	73.3
293	18.8	311	0.5	329	0.8	352	100
294	7.9	312	0.2	330	0.6	353	39.4
295	2.7	313	0.2	331	1.5		

DIHYDROVOACHALOTINE

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
77	3.1	109	5.7	142	2.5	169	9.1
78	0.9	110	2.9	143	1.8	170	10.7
79	4.0	111	4.0	144	4.8	171	6.6
80	1.6	112	2.4	145	2.2	172	2.3
81	10.3	113	0.9	146	0.7		
82	5.7					178	2.3
83	8.8	115	2.1	148	0.7	179	3.6
84	2.7	116	0.5	149	1.5	180	7.4
85	4.8	117	1.4	150	1.0	181	15.5
86	0.7	118	0.8	151	3.5	182	74
		119	2.3	152	2.8	183	100
91	3.5	120	0.8	153	3.9	184	26.4
92	0.8	121	0.7	154	5.9	185	5.6
93	3.2	122	0.9	155	5.2	186	2.1
94	1.4	123	2.9	156	4.1		
95	8.8	124	1.5	157	7.6	191	1.4
96	2.7	125	2.4	158	5.1	192	1.8
97	6.0	126	1.3	159	3.1	193	2.7
98	2.0	127	1.7	160	1.8	194	7.1
99	0.9	128	1.8	161	1.6	195	5.9
100	0.7	129	1.0	162	1.6	196	17.6
		130	1.3	163	2.3	197	10.6
104	1.5	131	2.2	164	0.6	198	8.2
105	3.1			165	3.3	199	1.8
106	1.0	139	1.5	166	3.3		
107	2.1	140		167	7.1	205	1.8
108	1.1	141	2.5	168	23.3	206	3.5

DIHYDROVOACHALOTINE (Contd.)

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
207	4.1	238	24.9	279	1.2	323	1.8
208	7.1	239	11.2	280	0.6	324	1.2
209	6.5	240	9.9	281	1.8		
210	4.1	241	4.1	282	0.6	335	7.0
211	3.5					336	4.7
212	2.1	247	1.2	285	2.1	337	13.5
		248	0.6			338	7.8
218	2.3	249	2.3	291	2.9	339	6.1
219	3.3	250	0.4	292	0.6	340	1.4
220	3.8	251	0.6	293	0.6		
221	6.6	252	1.2			349	1.4
222	4.7	253	0.8	305	1.8	350	1.8
223	6.6	254	0.6	306	1.8	351	9.4
224	3.5			307	2.6	352	4.7
225	2.3	263	2.3	308	0.6	353	4.7
226	1.8	264	3.5	309	3.5	354	1.8
		265	44.4	310	1.2		
232	1.8	266	20.6	311	0.6	365	1.2
233	2.3	267	5.6	312	1.8	366	3.5
234	1.2	268	1.4	313	0.6	367	20.9
235	3.5					368	51.5
236	2.6	277	0.6	321	1.2	369	20.0
237	21.7	278	0.6	322	0.6	370	2.6

CALYCANTHINE

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
77	0.8	111	8.1	140	0.8	166	1.0
78	0.4	112	1.6	141	0.8	167	1.6
79	1.6	113	1.6	142	0.8	168	1.8
80	1.2			143	2.7	169	1.6
81	9.0	117	1.5	144	3.4	170	1.2
82	4.0	118	1.0	144.5	1.0	171	3.7
83	12.0	119	2.3	145	2.6	172	12.1
84	3.1	120	1.0	146	0.8	173	8.1
85	5.6	121	1.6	147	1.0	174	3.6
86	0.5	122	0.8	148		175	1.3
		123	4.3	149	2.6	176	0.4
91	1.6	124	1.8	150	0.8	177	1.3
92	0.5	125	5.4	151	2.7	178	0.8
93	1.6	126	0.8	152	1.0	179	1.6
94	1.5	127	1.0	153	1.6		
95	8.1	128	1.6	154	0.8	181	1.6
96	3.6	129	1.6	155	1.2	182	0.8
97	10.6	130	7.7	156	0.8	183	4.0
98	1.8	131	4.0	157	1.8	184	1.6
99	2.4	132	1.6	158	1.3	185	4.4
		133	1.6	159	2.9	186	1.6
105	1.6	134	0.8			187	0.8
106	1.0	135	1.8	161	0.8		
107	1.6	136	0.6	162	0.4	193	0.8
108	1.0	137	2.8	163	1.5	194	0.6
109	6.5	138	0.8	164	0.8	195	1.6
110	2.7	139	2.3	165	2.0	196	1.8

CALYCANTHINE (Contd.)

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
197	2.4	233	9.7	269	1.3	302	15.3
198	1.0	234	2.4	270	1.6	303	8.9
199	1.5	235	0.8	271	5.2	304	2.4
				272	6.8	305	0.6
207	1.0	243	2.0	273	4.0		
208	0.8	244	3.5	274	2.3	314	3.2
209	1.9	245	15.3	275	1.5	315	4.0
210	1.0	246	8.1	276	1.1	316	1.8
211	1.8	247	3.2	277	0.8	317	0.8
212	0.4	248	1.0				
		249	0.8	283	1.0	331	1.3
217	1.6			284	1.0	332	0.6
218	1.6	255	1.6	285	2.4		
219	3.1	256	2.0	286	3.2	344	1.2
220	0.8	257	4.8	287	4.0	345	6.9
221	0.8	258	3.9	288	18.5	346	100
		259	8.1	289	7.7	347	28.1
228	0.8	260	5.2	290	3.5	348	1.6
229	1.9	261	2.4	291	1.0		
230	5.1	262	0.8				
231	34.8	263	0.8	300	2.2		
232	20.1			301	1.8		

CHIMONANTHINE

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
76	1.4			143	7.3	175	5.0
77	7.4	113	0.6	144	6.9	176	1.0
78	1.6	114	0.7	145	4.0	177	0.6
		115	4.1	146	1.4		
80	0.7	116	2.6	147	0.5	203	0.6
81	2.0	117	8.6			204	0.8
82	2.7	118	2.9	151	0.7	205	0.4
		119	2.9	152	0.7		
89	2.1	120	0.5	153	0.5	215	0.4
90	1.6	121	1.9	154	1.2	216	0.8
91	2.5			155	1.7	217	1.2
92	0.7	124	0.6	156	2.2	218	1.0
93	1.0	125	0.6	157	5.8	219	1.0
94	0.7	126	0.6	158	4.7		
95	1.3	127	1.6	159	2.0	230	0.8
96	0.7	128	5.4			231	1.2
97	0.9	129	5.9	165	0.8	232	1.0
98	0.6	130	49	166	0.8	233	0.6
		131	14.8	167	1.0		
101	2.2	132	6.0	168	1.4	242	0.8
102	3.7	133	1.3	169	2.4	243	1.3
103	8.1			170	6.8	244	1.2
104	2.2	139	0.6	171	23.4	245	3.0
105	1.3	140	1.1	172	100	246	1.3
106	1.2	141	1.3	173	71.1	247	0.6
107	0.6	142	3.0	174	27.9		

CHIMONANTHINE (Contd.)

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
255	0.6	270	0.4	282	0.3	314	0.3
256	0.8	271	0.9	283	0.3	315	0.2
257	1.2	272	0.7				
258	0.9	273	0.4	300	0.3	345	1.5
259	1.8	274	0.5	301	0.2	346	3.7
260	1.1	275	0.3	302	1.0	347	1.3
				303	0.5		
269	0.5	281	0.6				

CALYCANTHIDINE

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
77	4.9			159	1.5	257	0.6
78	0.8	115	4.3			258	0.4
79	0.8	116	2.2	165	0.8	259	1.3
80	0.5	117	6.4	166	0.8	260	0.8
81	1.9	118	3.0	167	0.8	261	0.3
82	1.4	119	1.6	168	1.1		
83	2.2	120	0.8	169	3.0	271	0.4
84	0.8			170	5.0	272	0.4
85	1.2	127	0.9	171	15.0	273	0.7
		128	3.2	172	100	274	0.4
91	2.2	129	3.8	173	32		
92	0.8	130	21.1	174	9.2	300	0.4
93	0.9	131	12.0	175	1.5	301	0.4
94	3.5	132	2.7			302	0.9
95	2.0	133	0.8	181	0.8	303	0.6
96	0.9			182	0.8	304	0.2
97	1.5	140	0.8	183	1.5		
		141	0.8	184	4.1	315	0.4
101	0.9	142	1.6	185	12.8	316	1.5
102	3.0	143	5.5	186	74.3	317	0.9
103	6.8	144	26.4	187	31.5	318	0.2
104	1.4	145	15.8	188	15.0		
105	1.4	146	3.0	189	2.6	328	0.4
106	1.1	147	0.8	190	0.5	329	0.4
107	0.8						
		154	0.8	244	0.3	358	1.0
109	1.5	155	1.2	245	0.4	359	2.6
110	0.8	156	1.5	246	0.3	360	21.7
111	1.5	157	4.1			361	8.2
112	0.6	158	3.4	256	0.6	362	1.2

FOLICANTHINE

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
77	1.2	155	1.0	216	0.8	274	2.3
		156	2.9	217	1.3		
91	0.6	157	4.9	218	0.9	285	0.9
92	0.2	158	4.3	219	0.6	286	1.0
		159	2.6	220	0.05	287	1.3
101	0.3	160	0.5				
102	0.8			229	0.7	299	0.5
103	2.6	168	0.5	230	1.1	300	0.7
104	0.4	169	1.8	231	1.3	301	0.4
		170	2.3	232	1.2	302	0.6
115	1.8	171	8.6	233	0.8	303	0.3
116	0.8	172	10.9				
117	1.9	173	2.6	242	0.9	314	0.3
118	0.3	174	0.4	243	1.4	315	0.4
				244	1.6	316	0.9
127	0.4	183	0.7	245	1.8	317	0.7
128	1.8	184	5.2	246	1.0		
129	2.3	185	14.4	247	1.0	330	1.6
130	5.2	186	100			331	0.6
131	3.2	187	52.4	256	1.2		
132	0.6	188	14.0	257	2.6	348	0.5
		189	1.8	258	2.4		
142	1.8	190	1.1	259	2.3	360	1.1
143	6.7			260	1.3		
144	42.7	206	0.1	261	0.8	373	0.5
145	18.5	207	2.6			374	32.0
146	3.7	208	0.6	271	2.0	375	11.7
				272	1.4	376	2.3
154	0.5	215	0.4	273	4.2		

CALYCANINE

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
159	1.2	175	7.5				
160	2.4	176	4.8	199	1.8	227	2.2
161	1.8	177	0.6	200	2.8	228	5.1
				201	14.0	229	31.8
171	0.6	187	1.7	202	15.2	230	100
172	2.7	188	2.2	203	13.8	231	23.2
173	4.2	189	3.0	204	6.4	232	3.6
174	8.5	190	1.0	205	1.0		

HODGKINSINE

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
77	13.2	109	3.6	155	2.9	208	0.6
78	21.9	110	1.5	156	2.9	209	1.5
79	5.1	111	2.2	157	5.8		
80	1.5			158	4.4	214	1.8
81	5.8	115	5.8	159	2.9	215	2.6
82	3.4	116	3.6			216	2.2
83	5.8	117	7.3	164	1.5	217	5.8
84	2.2	118	2.9	165	2.2	218	5.1
85	3.6	119	4.4	166	1.5	219	4.4
				167	2.5	220	0.9
91	5.8	126	1.5	168	4.4		
92	1.5	127	2.2	169	3.5	227	1.8
93	4.1	128	8.0	170	7.3	228	3.6
94	1.8	129	11.7	171	19.0	229	5.1
95	7.7	130	43.7	172	100	230	7.6
96	3.6	131	18.2	173	45.5	231	6.4
97	5.1	132	6.6	174	14.2	232	4.4
98	1.5	133	1.5	175	4.4	233	2.2
				176	2.2		
101	1.5	140	1.5	177	1.5	239	0.7
102	6.6	141	2.9			240	1.9
103	8.0	142	6.6	202	1.9	241	5.1
104	2.9	143	7.9	203	2.2	242	9.5
105	3.6	144	8.2	204	2.5	243	19.0
106	1.9	145	5.1	205	1.8	244	19.7
107	1.5			206	1.5	245	25.7
		154	1.7	207	1.6	246	15.3

HODGKINSINE (Contd.)

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
247	4.4	269	15.3	287	29.2	311	2.9
248	1.5	270	16.8	288	18.2	312	6.7
		271	33.3	289	8.0	313	12.7
253	1.9	272	23.8	290	1.5	314	30.2
254	2.9	273	13.9			315	11.0
255	11.1	274	5.3	296	3.6	316	5.1
256	22.2	275	3.6	297	5.1	317	1.5
257	27.9	276	1.9	298	13.1		
258	19.0	277	0.7	299	15.0	340	0.7
259	38.2	278	0.7	300	29.5	341	0.7
260	33.4	279	1.0	301	63.0	342	4.7
261	11.0	280	1.5	302	44.5	343	7.3
262	2.2	281	3.8	303	40.3	344	93.0
263	0.7	282	5.0	304	12.4	345	44.9
		283	7.3	305	2.2	346	30.6
266	2.2	284	11.5			347	10.2
267	4.4	285	14.6	309	1.0		
268	6.7	286	15.3	310	1.5		

I = Desoxybisnoreseroline

II = Desoxynoreseroline

III = $N_{(b)}$-Acetyldesoxynoreseroline

IV = $N_{(b)}$-Benzoyldesoxynoreseroline

V = Physostigmine

VI = N,N-Diacetyldesoxy-bisnoreseroline

VII = 3-Methyltetrahydrofurano (2,3-b)-indole

VIII = 2,3-Dimethyltetrahydro-furano(2,3-b)-indole

IX = 3-Methyltetrahydropyrano (2,3-b)-indole

X = Eserethole Methine

M/e	I	II	III	IV	V	VI	VII	VIII	IX	X
30	1.4									
31	0.7									
37				0.35						
38				0.87						
39	1.0	2.3			0.32	7.78	14.62	3.24		
40	0.3	1.6			0.19	2.9	16.56	0.62		
41	1.1	3.1			0.61	21.48	3.22	1.75	2.85	
42	1.0	3.3	6.68	0.87	2.64	12.27	2.42	2.11	0.83	12.64
43	0.7	1.4	32.25	0.41	0.48	72.5	2.71	9.53	0.98	1.03
44	2.8	0.8			1.19	25.05	7.22	0.75		12.54
45	0.6						0.77	0.39		23.85
46										1.91
50	1.4	0.7	0.69	2.44	0.26	6.73	4.32	1.26	1.37	
51	4.4	1.4	2.69	1.57	0.39	0.26	11.78	4.12	4.57	
52	2.2	0.7		0.41	0.16	1.19	5.28	1.36	2.02	
53	1.8	0.3			0.51		2.32	1.65	1.25	
54	1.0	0.2			0.32		1.29	0.65	0.65	1.33
55	1.8	0.7			0.48		0.77	1.00		2.54
56	1.3	0.9			1.45	2.11	0.10	0.65		6.72
57	1.3	0.7		1.8	4.21	0.79	0.13	1.20		2.92
57.5								0.32		
58	0.6				5.73	0.53	0.10	0.32		100
58.5	0.4		0.38				0.90	1.17	0.81	
59	0.6	1.4	0.69		0.32	1.32	0.19	0.16	0.21	8.03
59.5									0.15	
60						3.03	0.19			
61				0.46		1.85	0.39			
62	0.6						1.93	0.65	0.65	0.38
63	2.6	1.1	1.23	0.17	0.32	0.79	7.99	3.14	3.29	1.44
64	1.0	0.5		0.46		1.19	1.87	1.62	1.48	

M/e	I	II	III	IV	V	VI	VII	VIII	IX	X
64.5							0.97	1.13	1.28	
65	4.7	1.8	2.07	0.58	0.64	1.32	11.92	6.19	5.67	1.74
65.5		0.1					1.93	1.39	1.98	
66	2.3	0.5	0.69		0.29		2.00	1.36	1.19	1.06
66.5							0.32		0.24	
67	1.8	0.7		4.53	0.35	2.50		0.49	3.26	1.59
68	1.2	0.6		2.26	0.39	1.98		0.32		1.13
69	1.0	1.1		23.52	0.16	30.2		0.65		1.69
70	0.7	0.8		4.64	0.26	6.7	0.32	0.52		4.28
70.5	0.1						0.39	0.97	0.24	
71	0.6	0.2		2.38	0.16	9.75	0.13	1.94		8.06
71.5	0.4	0.2					1.93	5.15	0.74	
72	0.4			0.70	0.16	2.11	1.03	6.42	0.42	14.03
72.5	0.4	0.2	0.38				0.32	1.81	0.24	
73	2.1	1.1	0.69	0.64	1.22	3.96	0.52	0.68	0.15	5.57
73.5							3.16	1.62	3.59	
74	0.4			0.93		4.22	2.97	1.00	1.16	0.96
75	1.5	0.9	0.78	1.04		2.37	4.57	2.27	1.16	0.20
76	2.2	1.4	0.69	5.45		12.93	5.41	2.98	1.54	0.30
77	14.6	7.6	10.60	27.74	1.13	8.57	26.16	16.98	7.98	6.47
78	4.1	2.3	0.54	2.72	0.39	3.43	6.83	5.41	2.55	1.26
78.5		0.5						1.98		
79	3.1	2.7		2.38	0.39	2.37	1.29	2.59	0.62	2.52
79.5	1.2	0.5						0.75		
80	2.2	3.8		0.52	0.42	1.71	0.32	1.30	0.15	1.84
80.5								7.94	0.03	
81	1.2	0.6		7.07	0.71	9.1		2.17		3.42
82	2.5	3.0		2.96	0.58	6.85		0.19		1.76
83	1.8	0.9		6.85		10.04		3.89		2.97
83.5									0.12	
84	0.2			0.70	0.19	4.08		0.23		3.10
84.5									0.09	
85				0.29	0.13	6.33		0.32		0.63
85.5		0.2							0.06	
86		1.3		0.35		1.32		0.23		
86.5		1.3						0.45	0.03	
87		0.2					0.84	2.92	0.33	0.48
87.5					0.13			1.07		
88	0.6						0.97	0.91	0.39	0.45
89	3.7	1.8	2.53	0.64	0.58	1.58	7.21	4.67	2.94	2.04
90	2.1	2.2	2.46	0.29	0.55	1.19	5.99	4.54	2.97	1.54
91	6.1	6.9	3.15	4.18	1.35	7.25	14.05	11.63	5.79	5.01

M/e	I	II	III	IV	V	VI	VII	VIII	IX	X
92	1.3	1.3		0.41	0.32	1.85	2.77	1.88	1.04	0.45
93	2.5	1.2		2.09	0.29	3.03	3.22	1.62	0.80	1.56
93.5		0.5								
94	1.0	1.5		1.04	1.16	1.58	0.64	1.10	0.30	1.84
94.5								1.07		
95	0.7			9.69	0.55	8.05		0.55		3.70
96	0.7			3.65	3.42	3.82		0.36		1.11
97	0.7			7.19	0.71	6.46		0.71		2.59
98				2.61	0.13	3.46		0.29		1.13
99				1.62		2.24		0.26		1.51
100				0.75		1.32	0.19	0.23	0.09	0.20
100.5					0.26					
101	1.8	0.9	1.84	0.52	0.64	0.92	2.51	2.04	0.98	0.48
101.5					1.90					
102	5.1	3.4	3.92	0.93	0.97	0.66	7.92	4.86	2.79	0.75
103	9.7	5.4	5.53	3.02	1.06	2.77	12.82	6.81	5.87	2.39
104	5.9	2.5		9.22	0.74	11.74	6.70	3.14	2.55	1.49
105	2.1	1.0		100	0.68	3.56	3.09	5.41	1.16	2.37
106	2.1	1.6		10.73	0.64	0.40	2.13	2.92	0.89	1.56
107	0.6	0.6		2.73	0.90	1.71		0.65		2.37
108	0.1	0.4		0.93	0.64	0.53		0.26		2.64
108.5										1.18
109	0.5			6.44	1.09	4.08		0.55		4.00
110	0.5			2.15	0.19	2.11		0.32		0.68
111	0.4			3.54		2.64		0.39		2.74
112	0.1			1.74		1.32		0.39		1.59
113	0.4			1.28		0.79		0.81	0.36	0.23
114	1.0	0.8	1.00	0.45		4.97	0.32	2.07	0.47	
115	10.6	9.3	11.59	4.82	1.26	7.60	9.35	13.51	4.39	2.19
116	4.7	4.8	4.84	1.68	1.13	2.36	3.93	6.51	2.05	2.52
117	10.8	9.0	6.68	3.95	2.55	6.68	15.46	11.47	9.46	5.11
118	7.6	6.9	3.61	1.74	1.62	4.98	13.59	7.97	5.58	3.07
119	3.2	0.9	0.23	7.60	1.09	9.83	21.25	2.17	4.99	2.01
120		1.0		0.93	0.84	0.92	3.16	1.56	1.69	1.91
121				1.28	0.51	1.19		0.32		1.44
122				0.58	0.68	0.26				1.49
123		0.7		3.48	0.45	3.28				1.32
124				1.16		1.06				1.66
125	0.3			0.29		2.11		0.32		1.89
126	0.4			0.58		1.45		0.55	0.39	0.18
127	2.2	1.5		0.45	0.26	2.38	1.67	2.59	1.39	0.91
128	7.5	5.9	4.76	2.94	0.58	5.94	8.05	6.81	5.93	1.66

M/e	I	II	III	IV	V	VI	VII	VIII	IX	X
129	10.1	8.6	4.76	2.32	0.64	7.13	7.21	5.77	7.33	2.27
130	72	19.6	11.59	4.98	2.06	40.00	87.2	17.44	48.20	5.72
131	46	9.3	5.91	7.36	4.05	25.47	33.18	13.55	100	5.26
132	18.9	6.1	4.76	3.48	6.60	5.54	25.99	13.84	43.77	15.74
133	9.7	1.8	0.54	2.96	2.55	2.64	2.96	3.34	6.97	5.64
134	1.5	0.4	0.3	0.75	1.32	0.79	0.13	7.13	0.71	3.83
135				2.67	0.39	0.92		2.92		2.90
136				0.46	0.29					0.91
137				1.39	0.16	1.32				2.01
138				0.58		0.66				
139				1.22		3.82		0.32		2.32
140	1.0	0.7		0.35	0.16	2.77		0.91	0.36	
141	1.6	1.4	0.77	2.61	0.32	6.99		1.56	0.56	1.54
142	6.2	5.0	4.07	2.90	0.97	5.93	3.16	5.31	1.25	1.76
143	14.3	17.3	15.59	6.09	1.39	12.13	10.17	17.82	4.45	2.54
144	72	87.0	100	51.5	2.74	35.75	100	100	20.79	5.01
145	44.4	72.2	35.93	18.73	4.15	24.00	16.75	41.25	5.94	5.29
146	26.7	17.3	6.83	5.20	7.56	88.90	31.70	31.43	11.25	6.40
147	5.1	5.8	0.69	1.91	3.67	14.78	19.64	5.67	14.02	5.97
148	0.4			1.74	1.62	3.82	2.77	1.00	2.88	7.03
149				2.09	0.93	2.11		0.29		4.96
150				0.35	0.23	1.19				3.70
151				2.44		2.37				2.69
152				0.64		1.19				1.64
153	0.1			1.10		0.92				0.91
154	1.0	1.3	1.38	0.93	0.26	3.96		0.62	0.65	0.76
155	1.5	1.4	1.15	1.80	0.51	5.80		0.84	0.30	0.78
156	7.3	5.3	7.45	4.18	0.84	9.10	2.32	2.20	1.01	1.74
157	6.4	8.6	9.90	5.37	1.42	8.05	2.25	8.75	0.39	2.97
158	10.3	64.1	42.76	23.34	3.06	12.40	1.67	63.93	1.34	2.30
159	22.7	30.0	28.10	24.75	6.60	6.2	0.45	13.51	1.16	5.34
160	5.0	12.0	71.48	5.45	40.70	2.11	2.51	4.86	3.30	40.52
161	0.9	2.2	12.59	9.10	36.65	1.19	0.19	2.59	2.61	15.19
162	0.4		1.07	2.44	10.24			0.65	0.50	8.03
163			0.54	1.10	1.71					4.28
164				0.46	0.32					1.99
165				2.32		1.45				1.99
166				1.28		1.06				0.83
167			0.69	0.81		1.06				0.83
168		0.5	0.84	0.70	1.61	0.53		0.26	0.12	0.78
169	0.4	1.3	1.54	4.06	0.48	5.41		0.26	0.09	0.93
170	0.6	3.8	3.69	1.74	1.10	1.71		0.71	0.98	0.96

M/e	I	II	III	IV	V	VI	VII	VIII	IX	X
171	1.8	5.2	6.07	2.32	1.10	2.77		0.65	0.47	1.26
172	2.6	5.2	19.73	2.96	2.03	2.64	0.32	2.95	0.27	2.32
173	19.8	10.0	7.06	0.75	5.60	9.90	0.39	1.65	0.21	3.80
174	100	2.9	1.46	0.58	44.95	2.70	5.67	8.10	2.52	8.01
175	18.8	0.2		0.29	14.66		85.15	2.01	0.30	4.05
176	1.1			0.46	3.67		14.17	0.32		13.04
177			0.84	0.58	1.29		2.84			6.87
177.4			Metastable							
178			0.69	1.04	0.26	1.06				7.66
179			1.00	1.33		1.06				6.04
180				1.62		1.32				1.61
181				3.66		5.40				0.68
182				1.16		1.06				0.53
183				0.17	0.16					0.35
184				0.29	0.39					0.76
185		1.1		1.10	0.32	1.32				3.53
186		2.3	1.54	0.87	4.50	1.32		0.13	0.18	3.50
187		14.5	8.75	2.44	4.67	3.56		0.75	0.47	2.87
188		100	2.23	1.68	7.5	90.9		6.48	4.10	10.07
189		15.2	0.84	0.35	2.84	20.04		35.94	58.15	33.77
190		2.1		0.87	2.06	3.30		8.49	18.91	17.07
191				0.93	0.48	0.66		1.30	2.97	5.31
192				0.81		0.53				2.52
193				1.39		1.19				1.26
194				1.10		0.79				0.23
195				0.29		0.53				0.23
196				0.87						
197				0.12						
198					0.32					
199					0.61					1.30
200			0.78		0.64					1.26
201			1.54		2.95	2.37				3.02
202			44.76		2.77	1.45				12.52
203			9.60		8.98	0.53				4.81
204			1.23		2.71	0.13				8.21
205			0.78		0.39	1.06				23.57
206										8.61
207										6.30
208										1.44
209										0.28
212										0.28

M/e	I	II	III	IV	V	VI	VII	VIII	IX	X
213						1.06				0.40
214						1.32				0.53
215			5.14	0.29	0.64	6.59				2.42
216			1.38	1.51	1.29	100				7.00
217				0.75	16.91	26.37				4.78
218				0.58	100	4.2				2.29
219				2.67	23.07	3.69				1.28
220				0.58	3.22	0.53				2.80
221				0.29						0.93
225										Metastable
229			2.07		0.58					0.93
230			90.14		0.58	2.90				3.22
231			23.72	2.20	0.58	3.82				3.75
232			3.45	0.41	0.19	0.79				3.05
233										1.79
234										0.78
235										0.28
236				0.70						0.20
238.9				Metastable						
242					0.23					0.35
243				1.39	0.19	2.11				0.71
244					0.16	0.40				1.79
245					0.32					35.93
246					0.16					11.68
247										1.79
248										0.78
249										0.98
250										7.98
251										2.39
252										0.47
253										0.15
255						0.79				
256						1.06				
257						0.66				0.25
258						20.83				0.78
259						6.46				2.80

M/e	I	II	III	IV	V	VI	VII	VIII	IX	X
260						1.19				20.85
261										6.93
262				0.58						2.29
263				2.03						1.71
264				52.45						0.73
265				18.16						0.18
266				3.48						
267				0.81						
268				0.12						
269				1.22						
274					6.28					
275					61.85					0.23
276					17.60					1.33
277										5.41
278										27.93
279										7.43
280										0.18
290				0.70						
291				1.91						
292				42.40						
293				17.29						
294				3.48						
295				0.58						

PHYSOVENINE

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
50	1.41	70	0.63	87	0.44	109	0.37
51	2.92	70.5	0.09	87.5	0.14	110	0.19
51.5	0.08	71	0.67	88	0.26	111	0.26
52	1.83	71.5	0.43	88.5	0.36	112	0.15
53	1.22	72	0.22	89	1.14	113	0.11
54	0.56	72.5	0.22	90	0.70	114	0.11
55	1.63	73	0.15	91	3.10	115	1.22
56	4.80	73.5	0.29	92	1.47	116	0.93
57	12.28	74	0.15	93	0.64	117	1.48
57.5	0.10	75	0.63	93.5	2.9	118	1.22
58	4.80	76	0.74	94	0.51	119	1.08
58.5	0.24	77	3.50	94.5	0.07	120	0.56
59	0.34	77.5	0.08	95	0.52	121	0.34
60	0.34	78	0.19	96	0.29	122	0.26
61	0.22	78.5	0.41	97	0.39	123	0.26
62	0.48	79	1.51	98	0.21	124	0.14
63	1.81	79.5	0.15	99	0.11	125	0.19
63.5	0.11	80	0.98	100	0.10	126	0.52
64	1.50	81.5	0.32	101	0.37	127	1.05
64.5	0.22	82	0.37	102	0.71	128	2.01
65	2.14	83	0.53	102.5	0.31	129	1.14
65.5	0.22	84	0.41	103	1.03	130	1.35
66	1.14	84.5	0.06	104	0.70	130.5	0.11
66.5	0.10	85	0.40	105	0.93	131	2.30
67	1.15	85.5	0.06	106	0.48	132	2.32
68	0.56	86	0.18	107	0.74	133	1.71
69	0.87	86.5	0.11	108	0.47	134	1.41

PHYSOVENINE (Contd.)

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
135	0.33	156	0.20	176	4.56	206	13.92
136	0.18	157	0.63	177	1.56	207	1.41
137	0.13	158	0.86	178	0.22	208	0.11
138	0.11	159	1.74				
139	0.07	160	18.95	185	0.08	215	0.07
140	0.08	161	8.07	186	0.15	216	0.26
141	0.14	162	4.68	187	0.14	217	0.26
142	0.30	163	0.70	188	1.37	218	0.30
143	0.56	164	0.29	189	1.01	219	0.19
144	1.11	165	0.11	190	0.48	220	0.07
145	1.81	166	0.10	191	0.52		
146	2.43	167	0.10	192	0.11	230	0.07
147	1.78	168	0.07	193	0.07	231	0.33
148	1.44	169	0.07			232	0.11
149	0.44	170	0.09	200	0.10	233	0.07
150	0.15	171	0.11	201	0.10		
151	0.12	172	0.25	202	0.13	260	0.07
152	0.09	173	0.67	203	0.37	261	0.70
153	0.07	174	43.17	204	13.81	262	18.84
154	0.10	175	6.79	205	100.0	263	3.05
						264	0.37

CHAPTER II

XANTHONES

Xanthone

The mass spectrum of xanthone itself has its parent molecular ion (m/e = 196) as the base peak. There are only two abundant fragment ions at m/e = 168 (49.8%), and m/e = 139 (35.8%). The loss of twenty-eight mass units is most likely that of carbon monoxide from the carbonyl function originally present since this elimination requires less rearrangement of the molecular ion. It is most likely that the resulting ion is the molecular ion of dibenzfuran. The elimination of carbon monoxide followed by a hydrogen atom from this system is well known (43). Three metastable ions confirm the stepwise mechanism for the formation of the ion at m/e = 139 in the spectrum of xanthone.

$$196^+ \longrightarrow 168^+ + 28 \qquad m/e = 144$$

$$168^+ \longrightarrow 140^+ + 28 \qquad m/e = 116.8$$

$$140^+ \longrightarrow 139^+ + 1 \qquad m/e = 138.$$

The mass spectrum of the isomeric benzcoumarin (44)

shows the same processes that occur in xanthone giving besides the parent molecular ion as the base peak, abundant fragment ions corresponding to the successive losses of two molecules of carbon monoxide and a hydrogen atom.

Beynon (28) has obtained the mass spectra of anthraquinone and several hydroxy anthraquinones. The parent ion is, in all cases, the base peak of the spectrum and the successive elimination of two molecules of carbon monoxide from the carbonyl groups can be seen. In anthraquinone this fragmentation probably leads to the molecular ion of diphenylene. Both 1-hydroxy- and 2-hydroxyanthraquinones elide a further molecule of carbon monoxide to give a significant peak at mass 140. However, the ion at m/e = 139 is the most predominant ion in this region of the spectrum. No metastable ions were observed to account for the formation of this ion but it seems likely that it has the same structure as the ion formed by the fragmentation of benzcoumarin, xanthone and dibenzfuran. Beynon (28) has suggested the structure (1), Fig. 7, for the ion at m/e = 139.

1,3,8-Trihydroxyxanthone

In 1,4,5,8-tetrahydroxyanthraquinone the successive

loss of molecules of carbon monoxide was found not to be a likely process. The parent molecular ion was the base peak and the second most abundant ion was the doubly charged parent ion. The abundances of the fragments formed by the losses of carbon monoxide from the mono- and dihydroxyanthraquinones were also lower than in anthraquinone itself. 1,3,8-Trihydroxyxanthone shows the same effect for the parent molecular ion (m/e 244) is the base peak of the spectrum and fragment ions are of low abundance. Loss of carbon monoxide from the parent molecular ion gives an ion of low abundance m/e = 216 (5.94%) accompanied by an ion corresponding to the elimination of a formyl radical at m/e = 215 (4.83%). Two metastable ions show that this latter ion is, in fact, formed by the successive elimination of carbon monoxide and a hydrogen atom.

$$244^+ \longrightarrow 216^+ + 28 \qquad m/e = 191$$

$$216^+ \longrightarrow 215^+ + 1 \qquad m/e = 214$$

The most abundant fragment ion in the spectrum is at m/e = 108 (9.78%); it is accompanied by ions at m/e = 107.5 and 109.5 and it is likely that it also is a doubly charged ion. As such it corresponds to the removal of a second electron from the ion at m/e = 216,

a metastable ion does, however, show that it is formed by the elimination of carbon monoxide from the doubly charged parent molecular ion, m/e = 122 (2.32%).

$$244^{++} \longrightarrow 216^{++} + 28 \qquad m/e = 95.5$$

In his examination of the anthraquinones Beynon was able to show by analogy with the monoaminoanthraquinones that the ketonic groups are the first to be eliminated in the monosubstituted series. The loss of carbon monoxide from the phenolic portion is changed to the loss of hydrogen cyanide from the anilinic portion. The monoaminoanthraquinones lose successively two molecules of carbon monoxide and a molecule of hydrogen cyanide. The $(P-28)^+$ ion in the spectrum of 1,3,8-trihydroxyxanthone probably arises from the loss of the ketone group.

There is a fragment ion at m/e = 228 (3.17%) which can only correspond to the loss of an oxygen atom. This is surprising since although the 1-, and 8-hydroxyl groups are both strongly hydrogen bonded to the carbonyl group the 3-hydroxyl is not so bonded. 1,8-Dihydroxy-anthraquinone gives only a $(P-17)^+$ ion on electron impact but the isomeric 1,5-dihydroxyanthraquinone gives only a $(P-16)^+$ ion and no $(P-17)^+$ ion. Beynon (45) reported that the anthraquinones in which the hydroxy groups are substituted in the 2,3,6, or 7-positions around the rings

tend to lose -OH on fragmentation but those substituted 1, 4, 5 or 8-positions giving an internal hydrogen bond to the keto-group show an increased tendency to the loss of an oxygen atom only.

The fragment ion at m/e = 187 is shown to be formed by the successive losses of CO, CO and H from the parent molecular ion by the presence of two metastable transitions.

$$216^{+} \longrightarrow 288^{+} + 28 \qquad m/e = 161.8$$

$$188^{+} \longrightarrow 187^{+} + 1 \qquad m/e = 186 \text{ found } 186.3.$$

3,8-Dihydroxy-1-methoxyxanthone

The parent ion is again the base peak of the spectrum, but fragment ions are more abundant than in 1,3,8-trihydroxyxanthone. There is an abundant $(P-1)^{+}$ ion (19.87%) and since the elimination of one hydrogen from 1,3,8-trihydroxyxanthone gives an ion of low abundance (1%) the hydrogen must be eliminated from the methoxyl group. In 1-methoxy- and 2-methoxyanthraquinone the elimination of a hydrogen was equally favoured so it is unlikely that the proximity of the carbonyl group makes any significant change to this process.

There is an abundant ion (32%) at m/e = 244 corresponding to the elimination of fourteen mass units from

the parent molecular ion. Although this loss does not occur from 1-methoxyanthraquinone it is observed (see later) in the mass spectra of sterigmatocystin, iso-sterigmatocystin, and 1,3,5,6-tetramethoxyxanthone which all contain the 1-methoxyxanthone system. The mass spectrum of 1-hydroxy-3,5,6-trimethoxyxanthone only shows an abundant ion corresponding to the loss of a methyl radical. The driving force for this unusual elimination must be caused by the stability of the $(P-14)^+$ ion. There are three possible tautomeric structures for this ion (3), Fig. 7, one of which is the molecular ion of 1,3,8-trihydroxyxanthone. The structure is in fact the vinylogue of a carboxylic acid and may possess special stability.

The elimination of water from the parent molecular ion gives an abundant ion at m/e = 240 (25.80%), whereas the trihydroxyphenol itself did not lose water at all. A similar situation obtains in the anthraquinones, the elimination of water or hydroxyl from 1-hydroxyanthraquinone gives only fragments of low abundance, but these are each 9% of the base peak of the spectrum in 1-methoxyanthraquinone. It seems most likely that these eliminations lead to the loss of the oxygen from the carbonyl function since the $(P-17)^+$ ion and the $(P-18)^+$ ion

are undetectable in the spectrum of 2-methoxyantraquinone. A metastable ion confirms that the $(P-18)^+$ ion is formed in one step from the parent molecular ion of 3,8-dihydroxy-1-methoxyanthone.

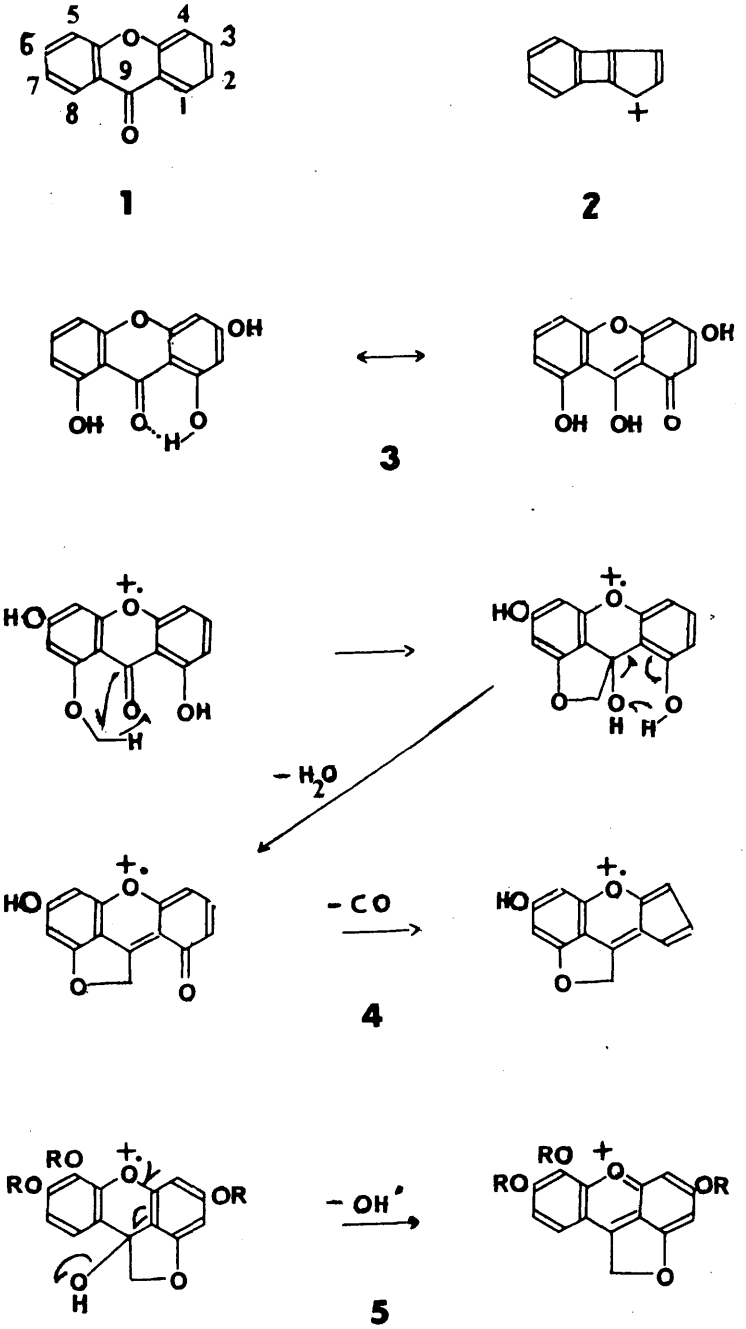
$$258^+ \longrightarrow 240^+ + 18 \quad m/e = 223.5$$

It is significant that although sterigmatocystin and isosterigmatocystin both eliminate a molecule of water 1,3,5,6-tetramethoxyxanthone eliminates a hydroxyl radical and not water. It therefore seems likely that the first step in the elimination of hydroxyl or water is the abstraction of a hydrogen from the methoxyl by the carbonyl oxygen with the formation of a five membered ring as in (4), Fig. 7. Elimination of water through a six membered transition state gives a fully conjugated system. The further elimination of carbon monoxide from what was the 8-hydroxyl group gives the fulvene ion m/e = 212 (36.4%). A metastable ion confirms its formation from the $(P-18)^+$ ion.

$$240^+ \longrightarrow 212^+ + 28 \quad m/e = 187.3.$$

The elimination of carbon monoxide from the parent molecular ion gives an ion at m/e = 230 whose abundance (5.2%) is only slightly greater than the isotope contribution (5.06%) from the $(P-29)^+$ ion (35.27%). There is,

FIG 7



however, an abundant ion at m/e = 115 (8.9%) which probably represents the $(P-28)^{++}$ ion. A metastable ion shows it is formed by the elimination of carbon monoxide from the doubly charged parent ion.

$$258^{++} \longrightarrow 230^{++} + 28 \quad m/e = 102.5.$$

The abundant $(P-29)^{+}$ (35%) is formed by the loss of a formyl radical from the parent molecular ion. Beynon has suggested that the elimination of twenty-nine mass units from 1-methoxyanthraquinone represents the loss of a ketone group with a hydrogen from the methoxyl. This proposal is supported here by the spectrum of 1,3,8-trihydroxyxanthone in which the elimination of twenty-nine mass units occurred in two steps. The elimination of twenty-nine mass units from the parent ion of 3,8-dihydroxy-1-methoxyxanthone occurs in one step as is shown by a metastable ion.

$$258^{+} \longrightarrow 229^{+} + 29 \quad m/e = 203.5.$$

The origin of the abundant $(P-30)^{+}$ ion (26.3%) is uncertain as there are no metastable ions to confirm the mechanism of its formation. Barnes and Occolowitz (45) have recently shown that the $(P-31)^{+}$ ions in the mass spectra of simple phenylmethylethers are formed by the successive losses of formaldehyde and a hydrogen. The

loss of thirty mass units from the parent molecular ion therefore seems likely to be from the methoxyl group, to give the molecular ion of 3,8-dihydroxyxanthone. The further elimination of carbon monoxide gives the moderately abundant $(P-58)^+$ ion at m/e = 200 (7.59%).

$$228^+ \longrightarrow 200^+ + 28 \qquad m/e = 175.5$$

1-Hydroxy-3,5,6-trimethoxyxanthone

Fragment ions are of low abundance in the spectrum of 1-hydroxy-3,5,6-trimethoxyxanthone and doubly charged ions are as prominent as singly charged ions. The loss of a methyl radical gives a fragment ion of low abundance m/e = 287 (5.23%) which then eliminates carbon monoxide to give the most abundant fragment ion m/e = 259 (16%). Two metastable ions confirm that the $(P-43)^+$ ion is formed by this path.

$$302^+ \longrightarrow 287^+ + 15 \qquad m/e = 272.5$$

$$287^+ \longrightarrow 259^+ + 28 \qquad m/e = 233.5.$$

This fragmentation of aromatic methyl ethers has been noticed previously (47) and the driving force for the elimination of carbon monoxide has been attributed to the unstabilized position of the positive charge on the oxygen atom after the loss of the methyl radical. The further loss of the two other methoxyls by the same

fragmentation can be observed. Thus there are metastable ions to show that the $(P-43)^+$ ion loses a methyl radical to give the ion at m/e = 244 (6.35%) which elides carbon monoxide to give the fragment ion at m/e = 216 (9.24%).

$$259^+ \longrightarrow 244^+ + 15 \qquad m/e = 229.9$$

$$244^+ \longrightarrow 216^+ + 28 \qquad m/e = 191.0.$$

There are two fragment ions of low abundance at m/e = 201 (3.35%) and 173 (1.96%) which correspond to the loss of the third methoxyl group.

The loss of a formyl radical in one step gives the fragment ion m/e = 273 (6.55%) but there is no loss of thirty mass units. Elimination of carbon monoxide from the doubly charged parent molecular ion at m/e = 151 (2.96%) gives the moderately abundant fragment ion m/e = 137 (6.41%).

$$302^{++} \longrightarrow 274^{++} + 28 \qquad m/e = 124.3.$$

1,3,5,6-Tetramethoxyxanthone

Methylation of the 1-hydroxyl group again gives rise to a spectrum containing much more abundant fragment ions. There is a fragment ion at m/e = 299 (11.4%) seventeen mass units below the parent. Since 1-hydroxy-3,5,6-trimethoxyxanthone does not give a $(P-17)^+$ ion it could indicate that the methoxyl group on $C_{(1)}$ takes part in

the elimination. As an unusual elimination of water has been correlated previously with the 8-hydroxy-1-methoxy-xanthone structure this confirms the suggestion that rearrangement of the 1-methoxyxanthone takes place on ionization. The mechanism would then be as shown in (5), Fig. 7. After rearrangement the loss of the hydroxyl now attached to $C_{(9)}$ can take place easily by an electron shift from the oxygen of the xanthone ring. Elimination of carbon monoxide from this ion gives an abundant fragment m/e = 271 (15.8%) which loses a hydrogen to give m/e = 270 (20%).

There is no significant ion corresponding to the elimination of carbon monoxide from the parent molecular ion but a prominent ion at m/e = 144 (6.87%) corresponds to the loss of carbon monoxide from the doubly charged parent. A metastable ion shows that a formyl radical is eliminated from the parent molecular ion and it appears likely that it represents the loss of the ketone group together with a hydrogen from the methoxyl on $C_{(1)}$.

$$316^{+} \longrightarrow 287^{+} + 29 \qquad m/e = 260.5.$$

Sterigmatocystin

This compound contains the 8-hydroxy-1-methoxy-xanthone system examined previously and shows the same

loss of fourteen and eighteen mass units. The $(P-18)^+$ ion is abundant (28.9%) but the $(P-14)^+$ ion although more abundant (3.32%) than the $(P-15)^+$ ion (1.43%) is no longer as abundant as in 3,8-dihydroxy-1-methoxyxanthone. A group of prominent ions m/e = 277 (4.86%), 278 (8.6%), 279 (2.6%), 280 (1.3%) and 281 (4.06%) correspond to the elimination of carbon monoxide or a formyl radical from the ions $(P-18)^+$ and $(P-14)^+$. A metastable ion confirms the transitions.

$$324^+ \longrightarrow 306^+ + 18 \quad m/e = 289$$

$$306^+ \longrightarrow 278^+ + 28 \quad m/e = 252.$$

A similar array of ions, m/e = 249 (3.58%), 250 (2.75%), 251 (1.08%), 252 (4.82%) and 253 (2.21%) is formed by a second loss of carbon monoxide from the above series.

The elimination of a formyl radical gives a very abundant fragment ion, m/e = 295 (76%). This loss has previously only given rise to a moderately abundant fragment ion and the loss of CHO from the furan ring system is implied. This is confirmed by the elimination of thirty mass units (CH_2O) from the $(P-29)^+$ ion, a process which gave an abundant $(P-30)^+$ ion in the mass spectrum of 3,8-dihydroxy-1-methoxyxanthone. The $(P-29)^+$ ion may

have the pyrylium structure shown in (2), Fig. 8. This explanation of the origin of the $(P-29)^+$ ion is supported by the spectrum of isosterigmatocystin, (3), Fig. 8, which eliminates twenty-nine mass units to a much less extent (34%).

$$324^+ \longrightarrow 295^+ + 29 \qquad m/e = 268.7.$$

Isosterigmatocystin

The mass spectrum of isosterigmatocystin, (3), Fig. 8, only differs from that of sterigmatocystin in the relative abundances of the major fragment ions. Loss of water from the parent molecular ion occurs to a greater extent and the additional loss of water compared to sterigmatocystin may represent the removal of the 3-hydroxyl group with an α-hydrogen from the furan ring. All the major fragment ions with the exception of the $(P-29)^+$ ion previously noted are more abundant in the mass spectrum of isosterigmatocystin. There are no fragment ions corresponding to the loss of the furan ring.

Jacareubin

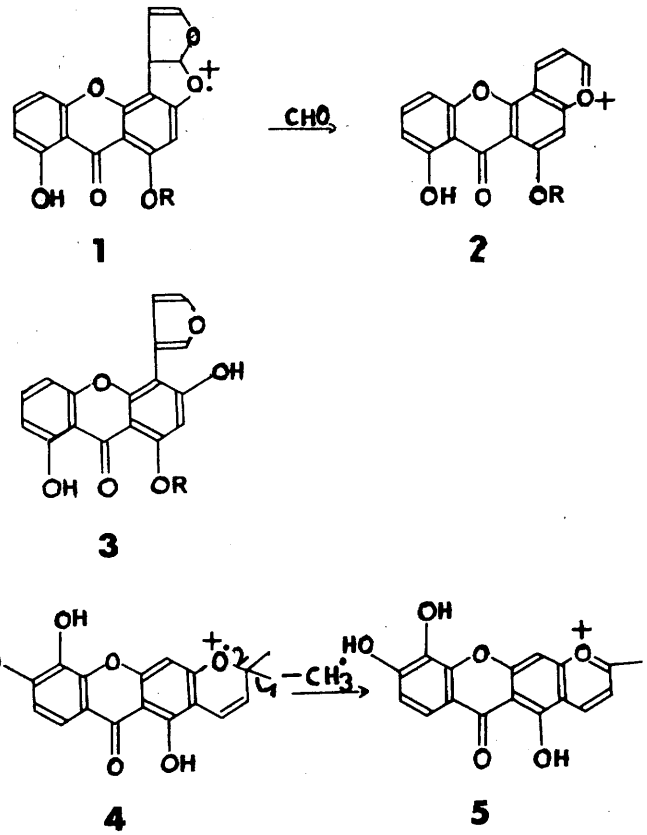
The parent ion of jacareubin, m/e = 326 (20%) is the only one in the spectra of the xanthones which is not the base peak of its spectrum. Loss of a methyl radical from

the parent molecular ion gives the base peak at m/e = 311. Jacareubin, (4), Fig. 8, contains a pyran ring with a gem dimethyl group adjacent to the oxygen. The facile loss of a methyl is therefore not surprising since the resulting ion has the pyrylium structure, (5), Fig. 8. All the hydroxyl groups are strongly hydrogen bonded and it is not unexpected that the $(P-15)^+$ loses an oxygen rather than a hydroxyl group.

$$311^+ \longrightarrow 295^+ + 16 \qquad m/e = 280.$$

Dr. J. G. Underwood (Nottingham University) provided the samples of the xanthones.

FIG 8



R= CH_3

1,3,8-TRIHYDROXYXANTHONE

% Abundance

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
50	2.25	77	2.45	99.5	0.02	121.5	0.08
51	4.14	78	1.05	100	0.41	122	2.32
52	1.81	79	1.43	100.5	0.04	122.5	0.31
53	0.25	80	0.74	101	0.53	123	0.71
54	0.42	81	0.67	101.5	0.08	124	0.50
55	1.46	82	0.11	102	0.73	125	0.17
56	0.19	83	0.05	103	1.16	126	0.10
57	0.71	84	0.10	104	0.38	127	0.05
58	0.28	84.5	0.13	105	0.42	128	0.30
59	0.15	85	0.46	106	0.18	129	0.32
60	0.08	85.5	0.10	107	0.87	130	0.15
61	0.70	86	0.58	107.5	0.15	131	1.48
62	2.15	87	0.99	108	9.78	132	0.46
63	3.57	88	0.59	108.5	0.93	133	0.08
64	1.17	89	0.93	109	0.40	134	0.43
65	1.40	90	0.56	110	0.15	135	0.15
66	1.05	91	1.28	111	0.36	136	1.62
67	0.82	92	1.25	112	0.10	137	2.64
68	0.27	93	0.54	113	0.91	138	0.23
69	6.57	93.5	1.00	114	1.24		
70	0.32	94	0.35	115	0.82	140	0.04
71	0.96	95	0.74	116	0.31	141	0.36
72	0.08	96	0.44	117	0.32	142	0.90
73	0.21	97	0.11	118	0.54	143	0.20
74	0.08	98	0.27	119	0.59	144	0.15
75	0.21	98.5	0.08	120	1.00	145	0.31
76	1.10	99	0.52	121	0.70	146	0.29

1,3,8-TRIHYDROXYXANTHONE (Contd.)

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
147	0.63	169	0.31			218	0.12
148	0.08	170	0.75	197	0.12		
		171	0.52	198	0.35	226	0.04
152	0.22	172	0.08	199	0.23	227	0.28
153	0.54	173	0.10	200	0.30	228	3.17
154	0.06	174	0.18	201	0.12	229	0.54
		175	0.08	202	0.13	230	0.11
157	0.08			203	0.59		
158	0.08	186	0.12	204	0.08	242	0.12
159	0.26	187	3.89			243	1.09
160	0.93	188	0.96	214	0.09	244	100
161	0.31	189	0.15	215	4.87	245	14.24
162	1.11	190		216	5.94	246	2.00
163	0.15	191	0.17	217	0.80	247	0.20
		192					

3,8-DIHYDROXY-1-METHOXYXANTHONE

% Abundance

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
50	5.23	77	7.05	101	1.52	121.5	0.14
51	8.94	78	2.39	102	3.32	122	1.38
52	4.30	79	4.22	103	2.67	122.5	0.14
53	5.65	80	1.71	104	0.76	123	0.87
54	0.79	81	1.24	105	1.26	124	0.70
55	2.75	82	0.16	106	0.84	125	0.45
56	0.11	83	0.16	107	4.58	126	0.82
57	0.65	84	0.06	107.5	0.28	127	1.38
58	1.07	85	0.48	108	8.52	128	2.95
59	0.84	86	0.98	108.5	0.28	128.5	0.14
60	0.42	87	2.25	109	0.76	129	7.05
61	1.40	88	1.26	110	0.42	129.5	0.90
62	6.30	89	3.18	111	0.56	130	1.15
63	11.80	90	1.83	112	0.28	131	2.05
64	3.79	91	4.27	113	1.40	132	0.50
65	3.2	92	5.09	113.5	0.28	133	0.22
66	1.97	93	1.60	114	1.66	134	0.39
67	1.12	93.5	1.01	114.5	0.73	135	0.28
68	0.37	94	0.76	115	8.91	136	3.15
69	15.42	95	2.25	115.5	0.36	137	4.72
70	0.59	96	0.39	116	1.88	138	0.53
71	0.93	97	0.17	117	1.35	139	0.28
72	0.70	98	0.65	118	1.71	140	0.17
73	0.51	99	1.07	119	1.46	141	0.59
74	2.64	99.5	0.14	120	3.60	142	1.06
75	3.74	100	5.11	120.5	0.28	143	0.84
76	2.28	100.5	0.73	121	1.20	144	2.59

3,8-DIHYDROXY-1-METHOXYXANTHONE (Contd.)

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
145	0.98	171	5.31	201	3.32	229	35.27
146	0.73	172	1.26	202	0.56	230	5.20
147	0.45	173	0.39	203	0.51	231	0.65
		174	0.28				
152	0.28			210	0.14	239	0.56
153	0.82	183	0.25	211	2.53	240	25.80
154	0.36	184	0.79	212	36.40	241	11.47
155	2.28	185	3.43	213	6.75	242	6.01
156	0.48	186	0.93	214	3.46	243	5.40
157	0.28	187	3.32	215	3.65	244	31.98
158	1.24	188	5.54	216	2.16	245	4.47
159	0.59	189	0.36	217	0.31	246	0.62
160	0.56						
161	0.28	195	0.17	223	0.42	256	0.67
162	0.45	196	0.28			257	19.87
		197	0.82	225	0.65	258	100.0
168	0.14	198	0.45	226	3.96	259	15.87
169	0.28	199	2.81	227	1.94	260	2.13
170	0.70	200	7.59	228	26.33		

1-HYDROXY-3,5,6-TRIMETHOXYXANTHONE

% Abundance

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
50	2.50	83	0.08	110	0.21	136	0.54
51	3.10			111	0.19	137	6.41
52	0.50	85	0.08	112	0.14	137.5	0.87
53	2.19	86	0.17	113	0.58	138	0.50
		87	0.72	114	0.93		
61	0.37	88	0.58	115	0.83	142	0.41
62	1.65	89	1.78	116	0.64	143	0.37
63	2.65	90	0.68	117	0.81	144	0.62
64	0.83	91	0.27	118	0.21	145	1.20
65	1.01	92	0.58	119	0.52		
66	0.99	93	0.78	120	0.58	150	0.41
67	0.54	94	0.83	121	0.62	151	2.96
68	0.14	95	0.97	122	1.86	151.5	0.45
69	3.84	96	0.14	123	0.77	152	0.29
70	0.04			124	0.17		
71	0.06	98	0.27	125	0.19	158	0.62
72	0.10	99	0.45	126	0.31	159	0.83
73	0.12	100	0.56	127	0.34	160	1.28
74	0.95	101	0.95	128	0.19	161	0.45
75	1.94	102	1.49	129	0.54		
76	0.85	103	0.66	129.5	0.41	170	0.74
77	0.68	104	0.41	130	0.93	171	0.66
78	2.39	105	0.35	131	0.64	172	0.27
79	2.39	106	0.95	132	0.52	173	1.94
80	0.31	107	1.16	133	0.14	174	0.29
81	0.35	108	0.43	134	0.52	175	0.25
82	0.06	109	0.66	135	0.66		

1-HYDROXY-3,5,6-TRIMETHOXYXANTHONE (Contd.)

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
184	0.21	213	1.28	242	0.60	272	2.11
185	1.03	214	1.14	243	1.36	273	6.55
186	1.65	215	1.98	244	6.35	274	1.18
187	1.16	216	9.24	245	1.74		
188	0.68	217	1.18	246	0.25	285	0.91
						286	1.98
197	0.19	226	0.29	255	0.48	287	5.23
198	0.68	227	0.83	256	0.74	288	1.51
199	0.79	228	0.81	257	2.54	289	0.21
200	0.93	229	2.67	258	1.63		
201	3.35	230	1.22	259	16.07	299	0.21
202	0.56	231	1.72	260	2.48	300	0.77
203	0.53	232	0.21	261	0.37	301	3.70
						302	100.00
211	0.25	240	0.21	270	0.48	303	17.82
212	0.35	241	2.11	271	0.97	304	2.48
						305	0.25

1,3,5,6-TETRAMETHOXYXANTHONE

% Abundance

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
50	2.86	91	1.64	121	1.15	142	0.98
51	1.84	92	0.76	121.5	0.46	142.5	0.56
52	0.36	93	1.35	122	0.82	143	1.48
53	2.43	94	1.22	123	0.39	143.5	0.16
		95	0.46			144	6.87
62	2.86			125	0.33	144.5	0.66
63	4.60	98	0.23	126	1.22	145	0.72
64	0.95	99	0.89	127	1.15		
65	1.45	100	1.61	127.5	0.39	149	0.56
66	1.77	101	1.74	128	1.22	150	0.65
		102	0.66	128.5	0.33	150.5	0.59
69	3.39	103	0.92	129	3.58	151	0.92
		104	0.33	129.5	0.33	152	0.62
74	1.48	105	0.59	130	0.53		
75	2.89	106	3.39	131	0.79	154	0.46
76	1.02	107	0.85	132	0.20	155	0.59
77	2.70	108	0.26	133	0.16	156	0.72
78	2.37	109	1.18	134	0.95	157	1.77
79	1.08			135	1.71	158	4.96
		113	1.45	135.5	1.18	158.5	0.89
81	0.46	114	1.64	136	1.18	159	1.28
		115	1.74	136.5	0.66		
86	0.20	116	1.28	137	2.70	165	0.66
87	1.12	117	0.30	138	0.39		
88	0.88	118	0.30	139	0.39	168	0.33
89	0.59	119	0.85	140	0.26	169	1.12
90	1.64	120	1.12	141	0.66	170	1.08

1,3,5,6-TETRAMETHOXYXANTHONE (Contd.)

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
171	1.08	212	1.61	244	1.81	283	1.51
172	1.12	213	2.30	245	0.85	284	0.66
173	0.73	214	1.51			285	13.05
		215	3.91	253	0.66	286	6.84
181	0.56	216	0.85	254	1.64	287	31.22
182	0.66			255	4.27	288	3.85
183	0.66	224	0.49	256	1.25	289	0.69
184	1.31	225	1.05	257	3.02		
185	3.29	226	2.70	258	1.35	297	0.16
186	0.85	227	1.74	259	0.66	298	1.87
187	2.14	228	1.71			299	11.40
188	0.33	229	2.37	267	0.16	300	4.04
		230	2.20	268	0.39	301	3.25
197	1.64	231	0.33	269	2.37	302	3.55
198	0.99			270	20.38	303	0.62
199	1.15	237	0.56	271	15.81		
200	8.51	238	0.66	272	4.77	313	0.23
201	1.97	239	0.66	273	2.63	314	1.54
202	0.52	240	1.31	274	0.32	315	41.57
		241	2.37			316	100.0
210	0.33	242	1.71	281	0.16	317	18.37
211	0.49	243	8.87	282	0.16	318	2.76
						319	0.33

STERIGMATOCYSTIN

% Abundance

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
50	2.22	78	0.45	105	1.08	128	0.83
51	4.71	79	1.56	106	0.28	129	1.17
52	2.67	80	0.98	107	1.50	130	0.45
53	3.12	81	0.83	108	2.87	131	0.61
54	0.35	82	0.43	109	0.24	131.5	0.13
55	1.47	83	0.32	110	0.46	132	1.85
		84	0.19	111	0.50	132.5	7.88
58	0.07	85	0.22	112	1.22	133	3.49
59	0.70	86	0.74	113	1.08	133.5	0.70
60	0.07	87	2.65	114	1.06	134	0.46
61	0.70	88	2.08	115	2.04	135	0.17
62	3.34	89	4.41	116	1.48	136	0.89
63	8.35	90	1.48	117	1.30	137	2.60
64	2.21	91	2.10	118	1.11	138	0.52
65	1.72	92	2.15	118.5	5.01	138.5	0.70
66	0.87	93	0.65	119	2.43	139	4.27
67	0.37	94	0.04	119.5	0.30	140	1.17
68	0.30	95	0.78	120	0.83	141	0.84
69	4.21	96	0.67	121	0.61	142	0.61
70	0.52	97	0.06	122	0.19	143	0.33
71	0.28	98	0.91	123	0.15	144	0.32
72	0.07	99	1.22	124	0.65	145	1.61
73	0.26	100	0.82	124.5	0.93	146	0.54
74	2.63	101	1.87	125	0.74	146.5	0.28
75	4.79	102	1.28	126	1.40	147	2.69
76	3.84	103	1.65	126.5	0.52	147.5	5.53
77	4.51	104	2.23	127	1.52	148	1.71

STERIGMATOCYSTIN (Contd.)

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
148.5	0.24	173	0.85	200	0.28	238	0.19
149	0.24	174	0.26			239	1.15
150	0.72	175	0.24	203	0.33	240	0.59
151	1.19	176	0.15	204	0.09	241	0.43
152	2.45	177	0.09	205	0.24		
153	1.09	178	0.09	206	0.07	248	0.19
154	0.52	179	0.72	207	0.19	249	3.58
155	0.89	180	0.30	208	0.70	250	2.75
156	0.22	181	1.21	209	0.93	251	1.08
157	0.26	182	0.43	210	0.72	252	4.82
158	0.22	183	0.32	211	0.63	253	2.21
159	0.19	184	0.37	212	0.98	254	0.54
160	0.46	185	0.28	213	0.63		
161	0.37	186	0.11			263	0.19
161.5	0.13	187	0.13	220	0.37	264	0.78
162	2.78	188	0.15	221	4.25	265	15.10
162.5	0.50	189	0.35	222	1.17	266	6.23
163	0.85	190	0.48	223	0.98	267	3.51
164	0.26	191	0.36	224	1.46	268	0.89
165	1.47	192	0.22	225	0.82	269	0.43
166	0.37	193	0.48	226	0.41	270	0.19
167	0.32	194	0.32	227	0.19		
168	1.41	195	1.24	228	0.26	276	0.07
169	0.56	196	1.22			277	4.86
170	0.43	197	1.17	235	0.19	278	8.61
171	0.46	198	0.33	236	1.34	279	2.60
172	0.09	199	0.28	237	4.92	280	1.30

STERIGMATOCYSTIN (Contd.)

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
281	4.06	293	1.43	307	10.90	321	0.37
282	1.30	294	3.62	308	2.21	322	0.19
283	0.30	295	76.10	309	1.43	323	7.77
		296	14.69	310	3.32	324	100.0
289	0.43	297	2.34	311	0.59	325	20.00
290	0.19	298	0.30	312	0.13	326	3.67
291	0.28					327	0.48
292	0.35	306	28.86	320	0.37		

ISOSTERIGMATOCYSTIN

% Abundance

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
50	3.20	79	1.38	107	3.28	132	1.73
51	4.41	80	1.12	108	4.06	132.5	9.42
52	2.25	81	0.95	109	0.43	133	3.89
53	3.11	82	0.34	110	0.78	133.5	0.78
54	0.26	83	0.34	111	0.86	134	0.52
55	0.78			112	1.99	135	0.17
56	0.52	86	0.61	113	1.64	136	1.56
57	0.52	87	5.27	114	1.30	137	3.28
		88	3.48	115	3.98	138	2.07
61	1.21	89	4.93	116	3.80	138.5	0.61
62	3.80	90	2.07	117	2.42	139	5.53
63	10.72	91	1.90	118	1.73	139.5	0.35
64	2.68	92	1.99	118.5	5.27	140	1.73
65	2.16	93	0.69	119	2.77	141	1.30
66	1.21	94	0.09	120	0.86	142	0.52
67	0.61	95	0.78	121	0.43	143	0.35
68	0.09	96	0.61	122	0.43	144	0.52
69	3.46	97	0.17	123	0.35	145	1.99
		98	1.21	124	0.69	146	0.78
71	0.09	99	1.98	125	1.30	147	2.07
72	0.17	100	0.86	126	1.64	147.5	5.53
73	0.43	101	2.07	126.5	1.04	148	2.77
74	3.72	102	1.56	127	1.73	148.5	0.24
75	5.10	103	1.47	128	2.51	149	0.52
76	5.45	104	2.76	129	1.12	150	1.47
77	4.32	105	1.73	130	0.43	151	2.16
78	0.26			131	0.69	152	4.41

ISOSTERIGMATOCYSTIN (Contd.)

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
153	1.47	195	1.73	238	2.59	280	1.30
154	0.69	196	1.90	239	1.47	281	7.26
155	1.12	197	1.47			282	3.28
				244	1.56	283	0.69
160	1.12	208	1.04	245	0.43		
161	0.69	209	1.12			289	1.04
161.5	0.13	210	0.61	248	0.34		
162	3.37	211	0.69	249	5.53	292	1.12
162.5	0.19	212	0.78	250	5.96	293	0.86
163	1.56	213	0.61	251	1.90	294	2.85
164	0.52			252	3.37	295	33.96
165	2.42	220	0.78	253	3.37	296	9.42
166	0.43	221	4.32	254	0.95	297	1.90
167	0.43	222	1.38				
168	1.56	223	2.16	263	0.52	306	49.18
169	1.04	224	2.59	264	0.52	307	12.79
		225	1.56	265	24.37	308	2.85
178	1.04	226	0.52	266	8.04	309	4.58
179	0.52	227	0.17	267	7.09	310	7.43
180	2.33	228	0.61	268	1.64	311	1.30
181	0.69	229	0.43				
				276	0.17	322	0.43
192	0.52	235	0.35	277	6.74	323	7.69
193	0.61	236	1.90	278	17.98	324	100.0
194	0.43	237	7.61	279	5.01	325	19.62
						326	3.98

JACAREUBIN

% Abundance

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
50	2.64	77	3.70	106	0.85	133	0.53
51	3.59			107	2.54	134	0.53
52	1.05	79	1.69	108	0.74	135	0.53
53	2.43			109	0.53	136	0.42
54	0.74	81	1.27	110	0.21	137	0.85
55	3.91	82	0.53	111	0.21	138	0.31
56	4.76	83	1.05	112	0.31	139	0.63
57	3.28			113	0.53	140	0.38
58	1.05	87	0.63	114	0.53	141	1.48
59	0.20			115	1.37	142	0.57
60	1.06	89	0.63	116	0.42	143	0.54
61	0.31			117	0.42	144	0.33
62	0.63	91	2.43	118	0.42	145	0.77
63	2.33	92	0.74	119	1.05	146	0.36
64	0.42	93	1.37	120	1.05	147	0.81
65	3.17	94	6.77	121	0.63	147.5	2.07
66	1.90	95	1.16	122	0.63	148	0.71
67	1.16	96	0.31	123	0.63	148.5	0.19
68	0.74	97	0.63	124	0.53	149	0.67
69	1.59			125	0.53	150	0.35
70	0.32	99	0.31	126	0.20	151	0.36
71	1.59	100	0.21	127	1.00	152	1.53
72	0.31	101	0.42	128	1.00	153	2.36
73	1.06	102	0.53	129	0.74	154	0.50
74	0.85	103	0.95	130	0.42	155	1.38
75	1.27	104	0.53	131	0.42	156	2.20
76	1.27	105	3.17	132	0.31	156.5	0.27

JACAREUBIN (Contd.)

M/e	Abundance	M/e	Abundance	M/e	Abundance	M/e	Abundance
157	0.44	238	0.42	271	0.13	298	0.33
158	0.38			272	0.54		
158.5	9.94	244	0.34	273	1.75	307	0.25
159	1.34	245	0.44	274	0.42	308	0.44
160	0.50					309	1.50
161	0.63	253	0.44	280	0.10	310	11.28
162	0.29	254	0.15	281	0.96	311	100.0
162.5	0.19	255	0.38	282	0.61	312	19.31
163	1.86	256	0.46	283	0.46	313	3.22
163.5	0.36	257	0.54	284	0.35	314	0.46
164	0.25			285	1.48		
		265	0.38			323	0.15
202	0.19	266	0.27	293	0.33	324	0.46
203	0.75	267	0.19	294	1.02	325	4.03
204	0.19	268	0.25	295	20.85	326	20.14
205	0.31	269	0.46	296	4.24	327	3.99
		270	0.13	297	1.32	328	1.59
						329	0.40

CHAPTER III

STEROIDS

NOMENCLATURE

The basic carbon system of the steroids and their numbering is illustrated by the example of cholestanol ((1), Fig. 9). If the orientation of a substituent corresponds to the angular methyl groups C_{18} and C_{19}, i.e. above the ring, it is said to be a β-substituent and the bond joining it to the nucleus is represented by a heavy line. Groups lying on the other side of the ring are α-substituents and the bonds are drawn as dotted lines. The example is therefore 5α-cholestan-3β-ol. The side chain at $C_{(17)}$ is normally β-orientated.

The application of mass spectrometry to steroids originates with Reed (48, 49) who examined several steroidal hydrocarbons under low electron voltage conditions and pointed out the advantages to be derived from this, as opposed to other analytical techniques. It was possible to determine the molecular weight and the mass of the $C_{(17)}$ side chain. A 9-11 double bond in

lanostene gave rise to fragments at m/e = 208 and 204, probably formed by a retro Diels-Alder reaction.

Friedland and his co-workers (50) obtained the spectra of a group of 3-sterols and reported that even in the spectra of oxygenated steroids there occurred abundant ions comprising the loss of the $C_{(17)}$ side chain plus forty-two mass units (C_3H_6). The loss of water from these compounds was represented as a 1,2-elimination to give a Δ^3-sterene but recent work has shown that the elimination of water from cyclohexanols is a mixture of 1,3- and 1,4-eliminations (51). When the A-B ring junction was trans (5α) and the 3-hydroxyl α- to the ring, the loss of ring A as 72 mass units was particularly favoured. All the steroids containing a 5-6 double bond showed a large $(P-111)^+$ ion corresponding to the entire loss of ring A with cleavage of the 9,10 and the 5,6-double bond.

Fitches (52) obtained the spectra of a mixed group of about twenty steroids and although criticism has been directed at some of the fragmentations proposed, characteristic features were noted. It was found impossible to obtain a true parent molecular ion from any of the sterol acetates even using a direct inlet system, but in most instances the parent molecular ion

was either very prominent or the base peak.

All the alcohols gave a $(P-18)^+$ ion which also occurred to a less extent in the mass spectra of the ketosteroids. It was suggested as a general rule if the abundance of the $(P-18)^+$ ion was greater than 10% of the parent ion then there is very strong evidence for supposing a hydroxyl group is present. The mass spectra generally confirmed that the mass of the side chain could be inferred from the mass to charge ratio of the most abundant ion in the range m/e = 205-245. This had previously been regarded as a characteristic elimination of the $C_{(17)}$ side chain together with $C_{(13)}$, $C_{(17)}$ and $C_{(18)}$, a suggestion that was criticised by Biemann and Meyerson. They pointed out that such a process required the rupture of three bonds without creating any particularly stable fragments. Biemann proposed the loss of $C_{(15)}$, $C_{(16)}$ and $C_{(17)}$, which involves breaking only two bonds with a hydrogen transfer, perhaps from $C_{(14)}$, might account for this fragmentation. Bergstrom (53) has also observed this fragmentation in his studies on the bile acids.

Djerassi (54) has initiated a comprehensive investigation into the mass spectra of steroidal ketones. Characteristic features were noted, which in most instance

enable one to locate a carbonyl group in a steroid nucleus An extensive investigation (55) was carried out on deuterated androstan-11-ones to establish whether or not the transfer of a hydrogen from $C_{(1)}$ which should proceed through a sterically favourable six membered transition state represented the crucial step; the subsequent fragmentation being directed by the two double bonds thus formed ((2), Fig. 9). The mass spectrum of $C_{(1)}$ deuterated 5α-androstan-11-one showed the virtual absence of any hydrogen transfer from $C_{(1)}$ in the formation of the base peak at m/e = 164, but this occurred to the extent of about 30% in the formation of the satellite peaks at m/e = 151 and m/e = 177. Using the same comprehensive approach Djerassi has elucidated the mechanism of the fragmentations of 16-ketosteroids (56).

The mass spectra of several deuterated α- and β-decalones have been obtained (57, 58) in order to examine the possibilities of using such simple compounds as models to predict the main mass spectral features of larger molecules containing these structures. Virtually all of the main fragmentations were found to be accompanied by hydrogen migrations which were, in some instances, highly specific. Again it was found that the formation of those ions, which required cleavage of a ring, was favoured if

the ring junction was cis although this was more apparent in the α-decalones than in the β-decalones.

The mass spectra of several acyclic ketones (59) show certain preferred fragmentations. The loss of the heavier alkyl group from a ketone $R \cdot CO \cdot R_1$ yields the ion $R \cdot CO^+$ as the base peak of the spectrum. Loss of the smaller alkyl group also gave a prominent ion. If either alkyl group contained a hydrogen - to the carbonyl group β-fragmentation with the migration of this hydrogen led to the molecular ion of a lower ketone. Thus hexan-2-one has its base peak at m/e = 43 and the abundant fragment at m/e = 58 corresponds to the molecular ion of acetone.

Beynon, Saunders and Williams (60) examined the high resolution mass spectra of cyclopentanone, cyclohexanone and cycloheptanone. The parent molecular ions, not unexpectedly, are more abundant than in the acyclic ketones. The base peak of each is the ion at m/e = 55 which was found to be mainly C_3H_3O but the contribution from C_4H_7 increases with ring size.

5α-Pregnan-20-one

The mass spectrum of 5α-pregnan-20-one has its base peak at m/e = 43. High resolution showed that this was

largely C_2H_3O. In his observations on the mass spectra of acyclic methyl ketones Sharkey (59) found that the base peak was invariably at m/e = 43.

The parent molecular ion, m/e = 302, is 57% of the base peak and there is a very abundant $(P-15)^+$ ion. The loss of water from the parent molecular ion gives an unusually abundant $(P-18)^+$ ion and on the basis of the generalization proposed previously by Fitches the presence of an alcohol group would be suspected if the nature of the compound were unknown. Androstan-3,17-dione gives the most abundant $(P-18)^+$ ion previously observed in the spectra of the steroidal ketones; it is about 20% of the base peak which is also the parent molecular ion (52). In 5α-pregnan-20-one the $(P-18)^+$ ion is 25% of the abundance of the parent molecular ion and it seems likely that some specific rearrangement of the molecular ion occurs. It is known that pregnan-20-ones on photolysis readily rearrange to give a mixture of the 20α- and β-18,20 cyclopregnanols (61, 62). McLafferty (63) has drawn attention to the striking similarity of the molecular ion dissociations shown in the mass spectrum to other types of unimolecular decompositions such as those caused by pyrolysis, photolysis, electrolysis or high energy radiation. Therefore, it seems possible that

on electron impact pregnan-20-one may rearrange to the corresponding cyclopregnanols as shown in (3), Fig. 9. The elimination of water from the parent molecular ion of cyclobutanol does not occur to any great extent, the spectrum being dominated by the loss of ethylene to give the base peak at m/e = 44 (64). This elimination here would lead to the loss of fifty-eight mass units which is discussed later, but the presence of a methyl group on the same carbon atom may result in the loss of water with the formation of an exocyclic methylene. A metastable ion confirms the one step elimination of water.

$$302^+ \longrightarrow 284^+ + 18 \qquad m/e = 267.$$

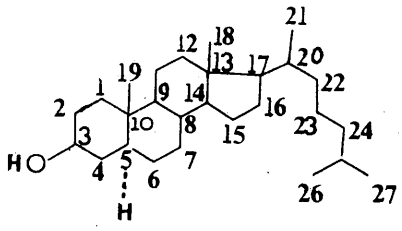
There is a moderately abundant fragment (7.8%) corresponding to the loss of a methyl radical from the $(P-18)^+$ ion since both the methyls $C_{(18)}$ and $C_{(21)}$ are required for the elimination of water from the molecular ion of 5α-pregnan-20-one the methyl group elided is almost certainly $C_{(19)}$. Two metastable ions show that this $(P-33)^+$ can be formed by the following routes:

$$287^+ \longrightarrow 269^+ + 18 \qquad m/e = 252.4$$

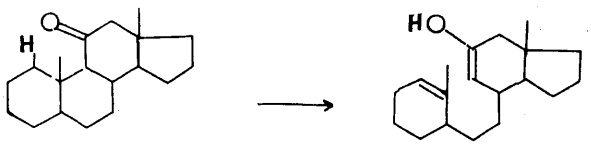
$$284^+ \longrightarrow 269^+ + 15 \qquad m/e = 254.6.$$

Loss of the acetyl groups at $C_{(17)}$ gives the ion at m/e = 259 (9.00%) which is accompanied by two ions,

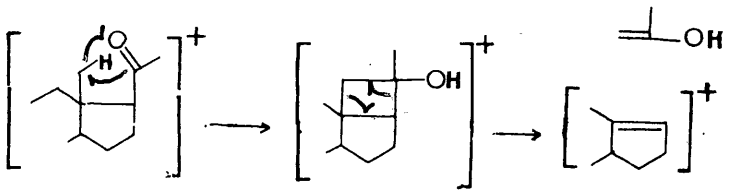
FIG 9


21
12
18
13
17
20
19
22
1
9
14
16
2
10
8
23
24
3
15
HO
5
7
4
6
26
27
H

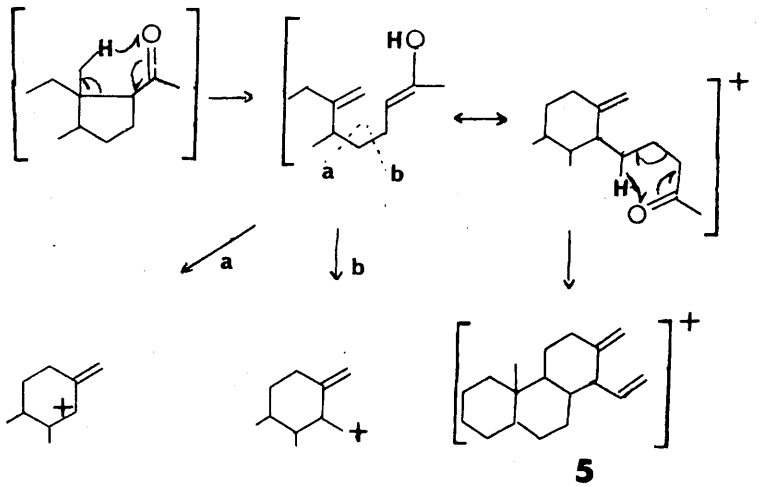
1


H
HO

2


H
OH
OH

3 4


H
HO
a
b
H
a
b

5

6

The probable mechanism of formation of this ion is given in some detail as it explains the formation of the other ions in the high mass region. Abstraction of a hydrogen from $C_{(18)}$ by the carbonyl oxygen proceeds as in (6), Fig. 9, giving an exocyclic methylene at $C_{(13)}$ with concomitant fission of the 13-17 bond. The Dreiding model of the system shows the carbonyl oxygen and the hydrogen to be within bonding distance. The enol that is formed by this rearrangement can revert to a ketone and abstract a further hydrogen from $C_{(15)}$. A metastable ion requires that the elimination of acetone occurs by a one step process and not by the separate loss of a methyl and an acetyl radical. The one step elimination of fifty-eight mass units may occur from the parent ion in another way which cannot be discounted. Thus after rearrangement of the molecular ion of pregnan-20-one to the 18,20-cyclopregnan-20-ol, the known fragmentation of cyclobutanol which led to the loss of ethylene will lead to the loss of the enol form of acetone as shown in (4), Fig. 9.

$$302^{+} \longrightarrow 244^{+} + 58 \qquad m/e = 197.3.$$

Under high resolving power conditions it could be seen that the group of ions at m/e = 229, 231 and 232 were singlets and precise mass measurement showed them to be

$C_{17}H_{25}$, $C_{17}H_{27}$, and $C_{17}H_{28}$ respectively. The ion at m/e = 231 (5.91%) is probably formed by the cleavage (6b), Fig. 9 of the allylic 15-16 bond, and there is also an abundant fragment at m/e = 71 (30.4%) (C_4H_7O) corresponding to the charge remaining with the ketone fragment. The ion at m/e = 232 (6.27%) is formed by the loss of methyl vinyl ketone from the parent molecular ion. The usual fragmentation of five-membered rings leads to the rupture of the 13, 17 and 15,16 bonds.

Loss of seventy-three mass units directly from the parent molecular ion to give the ion at m/e = 229 (3.85%) is unlikely but two other processes could lead to its formation. Loss of a methyl radical from the $(P-58)^+$ ion can occur, and if the loss of methyl does not comprise either $C_{(18)}$ or $C_{(21)}$ the $(P-15)^+$ can eliminate acetone. In fact both of these fragmentations are confirmed by metastable ions.

$$287^+ \longrightarrow 229^+ + 58 \qquad m/e = 214.9$$

$$244^+ \longrightarrow 229^+ + 15 \qquad m/e = 182.7.$$

The one other possible fragmentation namely the loss of C_4H_7 from the $(P-18)^+$ ion is considered to be unlikely as there is no ion at m/e = 247 corresponding to the loss of C_4H_7. Moreover, when $C_{(1)}$, $C_{(2)}$, $C_{(3)}$ and $C_{(4)}$ are

elided from androstane (54) a hydrogen migrates from the ring with them, i.e. there is a loss of fifty-seven mass units.

The most abundant ion in this region of the spectrum at m/e = 217 (71.1%) was shown to be $C_{16}H_{25}$ by precise mass measurements. Its formation could arise by cleavage of the allylic 14-15 bond as shown in (6b), Fig. 9. The resulting carbonium ion is allylically stabilized. The presence of a metastable ion confirms that the loss of eighty-five mass units is a one step process.

$$302^{+} \longrightarrow 217^{+} + 85 \qquad m/e = 156.$$

This ion represents the very characteristic loss of the $C_{(17)}$ side chain plus forty-two mass units from the parent molecular ions of the steroids. Various suggestions have been made as to the origin of the forty-two mass units lost along with the side chain. Loss of $C_{(18)}$, $C_{(13)}$ and $C_{(17)}$ (49) of $C_{(16)}$, $C_{(17)}$ and $C_{(18)}$ (50,52) and of $C_{(15)}$, $C_{(16)}$ and $C_{(17)}$ (66) have all been proposed. In the case of pregnan-20-one it is possible to draw a very favourable mechanism for the formation of this ion, which may account for its high relative abundance.

The ion at m/e = 218 (17.83%) is too abundant to be

merely an isotopic ion (12.58%) corresponding to m/e = 217. There is a more abundant fragment ion at m/e = 84 (53.9%) showing that the charge remains largely with the ketone fragment in this rearrangement. The usual break down of five membered rings leads to the cleavage of the 13-17 and 14-15 bonds. Loss of a methyl radical from the ion at m/e = 218 gives the fragment at m/e = 203 (6.50%).

$$218^{+} \longrightarrow 203^{+} + 15 \qquad m/e = 189.3$$

The abundant fragment ion at m/e = 192 (11.57%) was shown to be $C_{13}H_{20}O$ by precise mass measurement. A metastable ion requires its formation directly from the parent molecular ion by the loss of C_8H_{14}.

$$302^{+} \longrightarrow 192^{+} + 110 \qquad m/e = 122.1.$$

This involves the entire loss of ring A as well as $C_{(6)}$ and $C_{(19)}$. The same process occurs in androstane and in androstan-11-one giving rise to the base peak of the spectrum (54). Extensive deuterium labelling showed that this fragmentation in 11-keto-steroids was not initiated by a hydrogen transfer from $C_{(1)}$ or $C_{(19)}$ through a six membered transition state. Since it has been shown by Djerassi (55) that the hydrogen from $C_{(8)}$ is transferred from the charged fragment during its formation and replaced randomly by a hydrogen from carbons

2, 3, 4, 5 and 6 it would seem most likely that the structure of the ion at m/e = 192 is as shown in (1), Fig. 10.

The abundance of the ion at m/e = 193 is 35% of that at m/e = 192 and as such is too large to be merely an isotopic contribution from it (14.4%). A metastable ion shows that this ion, m/e = 193, eliminates water to give the prominent fragment at m/e = 175 (13.5%). Precise mass measurement confirmed that the ion m/e = 175 was in fact $C_{13}H_{19}$.

$$193^+ \longrightarrow 175^+ + 18 \qquad m/e = 158.7.$$

The fragment ion, m/e = 174, is formed analogously by the loss of water from the ion at m/e = 192. There is no loss of a methyl radical from m/e = 192 nor m/e = 193 and this confirms that the large loss of methyl from the parent molecular ion is from the angular methyl $C_{(19)}$.

Fragment ions at m/e = 159 (5.99%), 161 (11.09%), 162 (10.32%) and 163 (10.29%) were found to be $C_{12}H_{15}$, $C_{12}H_{17}$, $C_{12}H_{18}$ and $C_{12}H_{19}$ respectively but their origin is obscure. Their formation is most likely from the ions at m/e = 217 and 218 by the loss of $C_{(1)}$, $C_{(2)}$, $C_{(3)}$ and $C_{(4)}$ from ring A, a fragmentation which has been observed in androstane and cholestane (54). At high

gain it was possible to detect a metastable ion confirming the formation of the ion, m/e = 161, by this process.

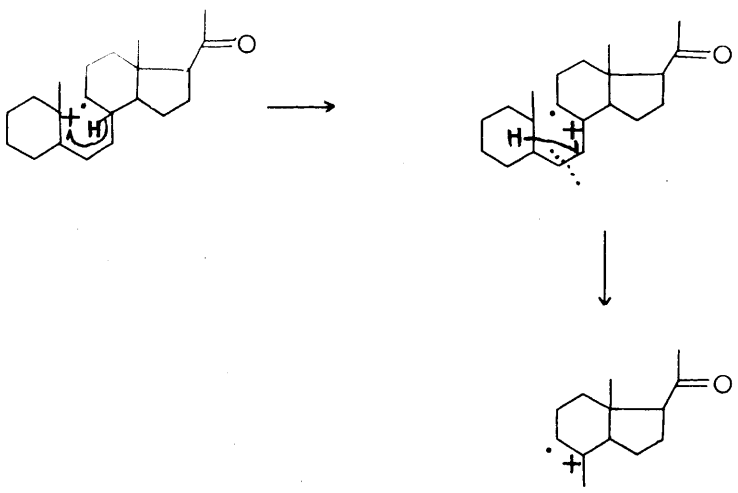
$$217^{+} \longrightarrow 161^{+} + 56 \qquad m/e = 119.5.$$

The largest ion in the range m/e = 143-150 is at m/e = 149 (25.1%); it has been previously attributed (50) to cleavage of the 9-11 and 8-14 bonds with a loss of a hydrogen from rings A and B. For a steroid which does not contain a double bond in rings A and B it will occur at m/e = 149 and will be lowered by two mass units for every double bond in these rings. If an alcohol or an acetate is present in A or B loss of water or acetic acid will leave a double bond in the ring.

5β-Pregnan-3α-ol-20-one

Djerassi (54) obtained the spectra of the steroids with a ketone group in all conceivable nuclear positions to establish general fragmentation patterns which might prove fruitful in structural investigations in the natural product field. The spectra of some derivatives of pregnan-20-one are, therefore, of interest to see if they show the same fragmentations as pregnan-20-one. The introduction of an alcohol group at $C_{(3)}$ should cause some differences in the spectrum noticeably in the abundance of the parent molecular ion.

FIG 10



1

2

the spectrum is again m/e = 43 and is largely CH_3CO^+. The parent molecular ion is now considerably less abundant (8%) and the ion formed by the elimination of water, m/e = 300, is much larger (39%). A metastable ion shows that electron impact induced elimination of water does take place to some extent.

$$318^+ \longrightarrow 300^+ + 18 \qquad m/e = 283.$$

The rearrangements which occurred in pregnan-20-one also occur here, but give rise to ions of much lower abundance. Two ions of low intensity, m/e = 275 (0.56%) and m/e = 274 (1.2%) correspond to the loss of an acetyl radical and a molecule of acetaldehyde observed previously and further fragment ions of low abundance, m/e = 260 (2.18%) and 233 (2.36%) show the elimination of fifty-eight and eighty-five mass units.

The $(P-18)^+$ ion gives rise to an abundant set of secondary ions by the rearrangements of the ketone part of the molecule previously detailed. Thus there are abundant fragment ions at m/e = 257 (7.92%), 242 (8.66%), 230 (10.64%), 229 (7.75%) and 215 (30.70%). Loss of a further molecule of water from the ion at m/e = 300 gives a peak of low abundance at m/e = 282 (2.62%).

Metastable ions exist which confirm the following

transitions:

$$300^{+} \longrightarrow 282^{+} + 18 \qquad m/e = 265$$

$$300^{+} \longrightarrow 242^{+} + 58 \qquad m/e = 195.3$$

$$300^{+} \longrightarrow 215^{+} + 85 \qquad m/e = 154.$$

The rearrangement ion at m/e = 246 is apparently formed by the loss of the unit comprising $C_{(1)}$, $C_{(2)}$, $C_{(3)}$ and $C_{(4)}$ from the parent molecular ion. A metastable ion does, however, show that it is formed by the loss of fifty-four mass units from the $(P-18)^{+}$ ion.

$$300^{+} \longrightarrow 246^{+} + 54 \qquad m/e = 201.7.$$

Friedland and his co-workers also found such a $(P-72)^{+}$ ion in the mass spectra of the sterols; no evidence was adduced to support their suggestion that this was a one step elimination. If the elimination of water was purely thermal then the formation of the $(P-72)^{+}$ ion could be readily explained. After the formation of a 2-3 double bond by thermal dehydration a retro Diels-Alder reaction can remove carbon atoms 1, 2, 3 and 4. However even when the spectrum was obtained using a direct insertion probe, known to cause the minimum of thermal decomposition, the ion at m/e = 246 was still found to be formed by the consecutive losses of water and butadiene. The electron impact induced elimination of water from

cyclohexanols is known to be a mixture of 1,3- and 1,4-eliminations. 1,4-Elimination of water cannot occur from the molecular ion of 5β-pregnan-3α-ol-20-one since the 4-position is occupied by a methyl group. A plausible mechanism for this elimination is shown in (2), Fig. 10; it recalls to mind the decomposition of saturated six-membered rings observed in the alkaloid field. The strain involved in forming the three-membered ring may account for the loss of butadiene. This is virtually the equivalent of a retro Diels-Alder reaction initiated by a three-membered ring. The mass spectra of lanost-9,11-ene acetate and cycloartenol acetate which contains a cyclopropane ring across $C_{(9)}$ and $C_{(11)}$ are almost identical (67); however, in α-amyrin acetate and phyllanthyl acetate where the same situation obtains, considerable differences in the mass spectra occur.

The prominent ion at m/e = 192 in the mass spectrum of 5α-pregnan-20-one is completely absent from those of 5β-pregnan-3α-ol-20-one, its acetate, and 5β-pregnan-3,20-dione. Since the formation of this ion does not require cleavage at $C_{(5)}$ it is unlikely to arise from the difference in the stereochemistry at $C_{(5)}$ and its absence probably only represents the fact that other fragmentations in ring A are now possible.

The largest ion in the group, m/e = 143-149 formed by rupture of the 9,11- and 8,14- bonds is now at m/e = 147 (14.5%) as a consequence of the extra unsaturation introduced by the elimination of water from ring A.

3α-Acetoxy-5β-pregnan-20-one

The parent molecular ion although only of low abundance (0.6%) was observed; Fitches (52) has been unable to obtain the molecular ions of sterol acetates even using a direct inlet system. Cholesteryl acetate has also been reported to give only an ion sixty mass units less than the parent (68). Loss of a methyl radical from the parent molecular ion is negligible but two of the typical rearrangements of the pregnan-20-one system can be observed. The $(P-18)^+$ ion is considerably more abundant than the parent molecular ion and there is an ion of low abundance at m/e = 290 (0.83%) formed by the loss of $CH_3COCH=CH_2$. The elimination of acetic acid from this latter ion proves that this is the correct assignment of the ion m/e = 290. A metastable ion is present for the transition.

$$290^+ \longrightarrow 230^+ + 60 \qquad m/e = 182.5.$$

Loss of acetic acid from the parent molecular ion gives the abundant ion at m/e = 300 which leads to a series

of secondary ions. Shannon (51) has shown that the elimination of acetic acid is a 1,2- process giving rise to a double bond. The formation of a double bond across the 2,3 positions leads to an ion which can then lose carbon atoms 1, 2, 3 and 4 through a retro Diels-Alder reaction. There are prominent fragment ions at m/e = 282 (5.11%), 257 (8.75%), 242 (9.46%), 230 (18.50%), 215 (25.89%) and 201 (6.04%) formed by the losses of H_2O, $CH_3CO^{\bullet}$, CH_3COCH_3, $CH_3COCH{=}CH_2$, $CH_3COCH_2CH_2CH_2^{\bullet}$ and $CH_3COCH_2{-}CH{=}CH_2 + CH_3^{\bullet}$, from the ketone part of the molecule. The ketone rearrangement peaks at m/e = 71 (C_4H_7O) and 84 (C_5H_8O) are still abundant being 16.83% and 13.53% of the base peak of the spectrum respectively. There is a peak of low abundance at m/e = 60 (4.35%) corresponding to the molecular ion of acetic acid, although relative sensitivities were not measured this would seem to indicate that thermal decomposition had not occurred before the sample was admitted to the ionization chamber. A second spectrum showed no changes in the abundances of the major ions and this also indicates that thermal decomposition has not taken place.

5β-Pregnan-3,20-dione

The introduction of a ketone at $C_{(2)}$ or $C_{(3)}$ does

not greatly change the mass spectra of the steroids (54). In 5α-androstan-3-one the principal cleavages involve loss of carbon atoms 1, 2, 3 and 4 with or without one hydrogen, the charge remaining with the tricyclic ring system. This fragmentation is, however, entirely subordinated to the typical steroid fragmentations in cholestan-3-one. Since cleavage at $C_{(5)}$ is required, this fragmentation is dependent on the stereochemistry and Djerassi observed that, as would be expected, this was more prominent in the 5β-enantiomers.

The high mass region of the spectrum of 5β-pregnan-3,20-dione is considerably more abundant than in the 3-alcohol or its acetate. The parent molecular ion is some 20% of the abundance of the base peak, m/e = 43. Loss of water occurs readily giving an ion of greater abundance (m/e = 298, 25.63%) than the parent ion. A peak at m/e = 265 (2.15%) represents the loss of two molecules of water and a methyl radical from the parent ion. Metastable ions confirm the sequence.

$$316^+ \longrightarrow 298^+ + 18 \quad m/e = 281$$
$$298^+ \longrightarrow 283^+ + 15 \quad m/e = 268.8$$
$$283^+ \longrightarrow 265^+ + 18 \quad m/e = 248.$$

The loss of forty-three mass units to give the ion

at m/e = 273 (3.38%) almost certainly represents the loss of the acetyl group at $C_{(17)}$ although elimination can occur from the 3-ketone group. A molecule of acetaldehyde is again lost. The origin of the additional hydrogen is unknown but $C_{(16)}$ is a likely point as this leads to an ionized 16-17 double bond.

$$316^{+} \longrightarrow 272^{+} + 44 \qquad m/e = 234.2.$$

Elimination of acetone again occurs to give a noticeable ion at m/e = 258 (6.10%) and there is an abundant fragment ion corresponding to the loss of eighty-five mass units at m/e = 231 (10.71%). Loss of seventy mass units is, however, much more prominent than it was previously indicating that the elimination of carbon atoms 1, 2, 3 and 4 has led to this peak. The correctness of this interpretation is confirmed by the fact that it is not accompanied by the usual $(P-71)^{+}$ peak and its further breakdown pattern is that of 20-ketopregnane. Thus the elimination of water, acetone and methylvinylketone give the ions at m/e = 228 (8.87%), 188 (4.05%) and 176 (14.35%). These eliminations are all confirmed by the appropriate metastable ions. There are other fragments at m/e = 203 (3.9%), 175 (8.87%) and 161(10.92%) corresponding to the losses of $CH_3CO^{\bullet}$, $CH_3COCH_2CH_2^{\bullet}$ and

$CH_3COCH_2CH_2CH_2^{\bullet}$ from the $(P-70)^+$ ion which are not confirmed by metastable ions.

$$246^+ \longrightarrow 228^+ + 18 \qquad m/e = 211.4$$
$$246^+ \longrightarrow 188^+ + 58 \qquad m/e = 143.7$$
$$246^+ \longrightarrow 176^+ + 70 \qquad m/e = 125.9.$$

The ions at m/e = 71 (19.89%) and 84 (30.35%) are again prominent. If the side chain rule were applied to the fragment ion at m/e = 213 (the biggest peak in the range 205-245) it would show that the side chain was forty-three mass units and that there was an alcohol group and a double bond in the nucleus. The enhanced loss of seventy mass units would indicate that these were present in ring A.

In spite of the increased complexity of the spectra when ring A contained a carbonyl group, an alcohol, or an acetate it was always possible to recognize the basic cracking pattern of the pregnan-20-one system and it is likely that the rearrangements discussed will facilitate recognition of this system in an unknown steroid.

In the light of some of Djerassi's most recent work (69) these results are very informative. He has recently concluded that the presence of a ketone group in the

steroid nucleus does not alter the dominant fragmentation pattern of the steroid except in certain favourable circumstances. He had expected that the fragmentations of many of these would be determined by the initial transfer of a γ-hydrogen to the carbonyl oxygen with concomitant β-fission which had been demonstrated in aliphatic carbonyl compounds (70, 71). Labelling experiments showed that although this mechanism operated in 16-keto steroids it was absent from the mass spectra of 11-keto and 15-keto-steroids. In simple aliphatic carbonyl compounds there is generally no restriction on the formation of a cyclic transition state. This is not the case with ring ketones and Djerassi (69) has proposed that for this mechanism to operate the carbonyl oxygen and the hydrogen must be able to approach to within at least 1.8 Å. When this distance is exceeded Djerassi has been able to rationalize much of the mass spectum by a return to the approach of Beynon (72) involving homolytic fission of the most substituted bonds in the parent molecular ion.

In 16-ketosteroids the minimum distance between the hydrogen on $C_{(22)}$ and the carbonyl oxygen is 1.5 Å and the fragmentation pattern shows an abundant ion corresponding to transfer of this hydrogen with loss of the $C_{(17)}$ side chain and the angular methyl $C_{(18)}$. The

Dreiding model of a 20-keto-steroid shows that the carbonyl oxygen may approach the hydrogen on $C_{(18)}$ just as closely as a carbonyl group at $C_{(16)}$ can approach the hydrogen on $C_{(22)}$. This clearly shows how a carbonyl group on $C_{(20)}$ is able to determine the fragmentation pattern so completely.

The loss of eighty-five mass units which can occur in pregnan-20-ones by a very favourable mechanism is only an example of the usual loss of the $C_{(17)}$ side chain plus forty-two mass units. It may well be that the hydrogen transfer from $C_{(18)}$ is perfectly general. Djerassi has shown that transfer of a hydrogen from $C_{(8)}$, $C_{(14)}$ or $C_{(12)}$ does not occur in this fragmentation (P-42-side chain) and has initiated the synthesis of a $C_{(18)}$ trideutero steroid to confirm that the hydrogen on $C_{(18)}$ is removed in this fragmentation.

5β-Ergost-22,23-ene-3-one

In the $C_{(5)}$ epimeric ketones, coprostan-3-one and cholestan-3-one, it was noticeable (54) that the loss of ring A was only prominent in the 5β-epimer and was entirely subordinated to fragmentation of ring D in cholestan-3-one. The spectrum of 5β-ergost-22,23-ene-3-one was obtained using a direct insertion probe as

there was only a limited amount of sample available; even so the parent molecular ion (62.4%) was no longer the base peak of the spectrum. Fragmentation is dominated by the hydrocarbon part of the molecule, although the stereochemistry at $C_{(5)}$ is the same as in coprostan-3-one. There are fragments of low abundance corresponding to loss of a methyl radical and an isopropyl radical at m/e = 283 (3.5%) and 355 (7.8%). Elimination of water from these ions gives two peaks at m/e = 265 (2.5%) and m/e = 237 (6.5%). Djerassi has postulated a mechanism for the elimination of water from cyclohexanone (74). The genesis of the ion at m/e = 314 is uncertain. That it clearly contains the carbonyl group is shown by the fact that it is shifted to m/e = 316 in the corresponding alcohol and to m/e = 312 in the Δ^4-ketone. A rearrangement of the parent molecular ion is necessary for its formation apparently involves fission of the 22-23 double bond. No metastable ions exist to indicate from which ion it is formed. The loss of ninety-eight mass units gives a much more abundant fragment, however, at m/e = 300 and its formation may well be associated with the stability of the fragment ion. In the corresponding alcohol this ion is found at m/e = 302 and at m/e = 344 in its acetate. The loss of ninety-eight mass units is

therefore from the side chain. Either $C_{(17)}$ or $C_{(21)}$ appears to be the most likely source for the hydrogen removed by fission of the 21-22 bond since this leads to an ionized double bond. Loss of a methyl, probably $C_{(18)}$, from this ion gives the abundant fragment m/e = 285 (12.6%). Delocalization of the positive charge over $C_{(13)}$, $C_{(17)}$ and $C_{(20)}$ probably accounts for the increased loss of a methyl radical from the ion m/e = 300.

There is a group of abundant ions at m/e = 271 (41.4%), 272 (27.5%) and 273 (14.5%), the latter of these can be formed directly from the parent molecular ion by homolytic fission of the 17-20 bond. The ion at m/e = 272 can also be formed directly from the molecular ion by the transfer of a hydrogen from $C_{(16)}$ to $C_{(23)}$ by the usual six membered transition state to form an ionized 16-17 double bond. Loss of another hydrogen gives the fragment ion at m/e = 271 the stability of which can be attributed to delocalization of the charge over $C_{(15)}$, $C_{(16)}$ and $C_{(17)}$. Metastable ions confirm that the loss of the side chain with one or two hydrogens occurs in one step from the parent molecular ion.

$$398^+ \longrightarrow 272^+ + 126 \qquad m/e = 185.9$$

$$398^+ \longrightarrow 271^+ + 127 \qquad m/e = 184.5.$$

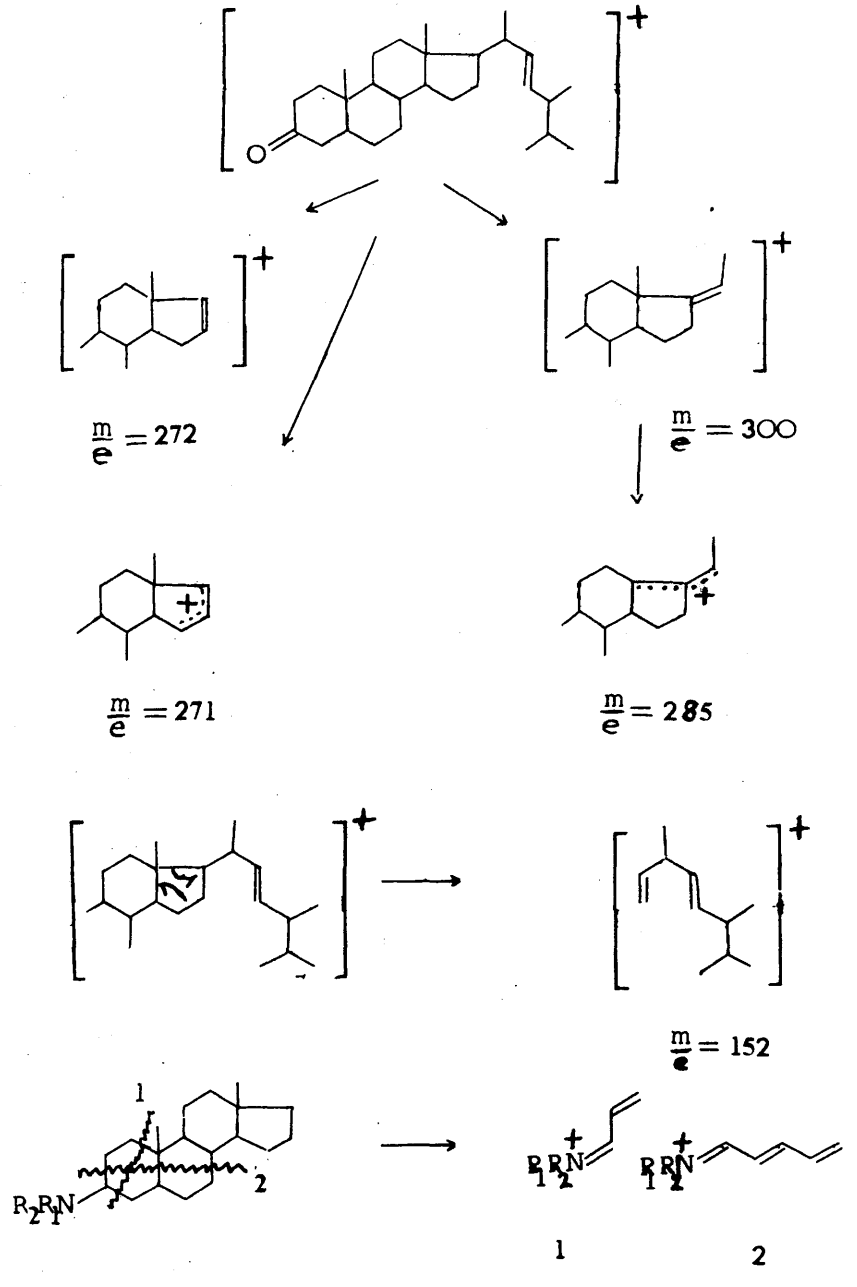
The loss of water from the $(P-125)^+$ ion gives the abundant fragment ion at m/e = 255 (33.2%) and loss of the $C_{(18)}$ methyl gives the prominent fragment at m/e = 258 (14.5%). A metastable ion proves the formation of the ion at m/e = 255.

$$273^+ \longrightarrow 255^+ + 18 \qquad m/e = 238.2.$$

Loss of the $C_{(17)}$ side chain is favoured by the presence of the 22-23 double bond and the contrast between the steroids with a saturated side chain which do not fragment in this way is thus explained.

Rearrangement of ring D, which led to the formation of the $(P-70)^+$ ion and the ion at m/e = 84, in the mass spectrum of pregnan-20-one again takes place. The presence of a double bond in the side chain stabilizes the charge, and the tricyclic ring system with $C_{(15)}$ is eliminated as the neutral moiety, forming an abundant ion m/e = 152. A peak of low abundance at m/e = 166 (3.7%) corresponds to the loss of a tricyclic ring system, the charge remaining on the side chain + $C_{(15)}$, $C_{(16)}$ and $C_{(17)}$. Loss of the side chain and carbon atoms 16 and 17 gave a prominent ion in the spectra of coprostan-3-one and cholestan-3-one, the charge remaining with the tricyclic ring system. Such a fragmentation has been observed previously in the spectra of the alkaloids of the

FIG 11



physostigmine group. It can also be seen in the model compound 1-n-hexadecylhexahydroindan (75) which has the base peak of its spectrum at m/e = 96 formed by the elision of the side chain plus $C_{(1)}$ and $C_{(2)}$. Another prominent fragment ion in its spectrum $C_6H_9^+$ m/e = 81 (51%) may be formed by the same fragmentation as leads to the loss of the steroid side chain plus forty-two mass units.

In 5β-ergost-22,23-ene-3-one, loss of the side chain plus forty-two mass units does not give a particularly abundant fragment ion at m/e = 231 (8.5%). The structures and formation of the major ions in the spectrum are shown in Fig. 11.

Ergost-4,5-22,23-diene-3-one

The presence of an abundant fragment ion at m/e = 124 (21.77%) has been reported (76,77) to be characteristic of Δ^4-3-keto steroids. Its formation involves fission of the 9-10, and 6-7 bonds with the rearrangement of the two hydrogens to ring A. Djerassi (77) has demonstrated by deuterium labelling that the hydrogens originate from $C_{(11)}$ and $C_{(8)}$ and has proposed mechanisms for the formation of this ion. There is also in the spectrum of 5β-ergost-4,5-22,23-diene-3-one an abundant ion m/e = 272 (12.16%) corresponding to the loss of this

fragment from the parent molecular ion. Fragmentation of the side chain proceeds as in ergost-22,23-ene-3-one to give the ions $(P-43)^+$ (18.17%), $(P-84)^+$ (7.44%), $(P-98)^+$ (43.17%), $(P-125)^+$ (48.5%), $(P-126)^+$ (26.9%), $(P-127)^+$ (40.07%). Loss of a methyl radical or a molecule of water from the $(P-125)^+$ ion gives the ions at m/e = 256 (11.91%) and 253 (24.75%).

The relative abundance of the ion formed by the loss of the side chain plus $C_{(17)}$ and $C_{(16)}$ is considerably less (5.46%) than in ergost-22,23-ene-3-one and there is a prominent fragment ion $(P-151)^+$ (29.90%) corresponding to this fragmentation with the gain of a hydrogen by the tricyclic ring system.

5β-Ergost-22,23-ene-3α-ol and 5α-Ergost-22,23-ene-3β-ol

The presence of an alcohol group at $C_{(3)}$ gives rise to a series of abundant fragment ions eighteen mass units below those formed by fragmentation of the side chain and ring D. The mass spectrum of cyclohexanol (64) has its base peak at m/e = 57 (C_3H_5O). There is no ion corresponding to the elimination of this from the parent molecular ions of these two sterols and high resolution mass spectromerty showed that the abundant ion m/e = 57 (30%) was mainly (90%) hydrocarbon. 3-Aminosteroids (78)

give rise to abundant fragment ions at m/e = 54 + R_1 + R_2 and m/e = 80 + R_1 + R_2, the result of fissions (1) and (2), Fig. 11 by the same fragmentations as are observed in N-ethylcyclohexylamine (40). The differences in the mass spectra of the 3-sterols and the corresponding amines can be understood in terms of the greater electron releasing capacity of the amino group compared to the alcohol group. This manifests itself in the greater abundance of $CH_2{=}\overset{+}{N}H_2$ (100%) than $CH_2{=}\overset{+}{O}H$ (8%) in the mass spectrum of 2-aminoethanol (79).

The spectra are very similar to that of the ketone. Thus the spectrum of 5β-ergost-22,23-ene-3α-ol contains the following characteristic ions $(P\text{-}\overset{+}{9}8)$ (21.42%), $(P\text{-}\overset{+}{1}13)$ (8.79%), $(P\text{-}9\overset{+}{8}\text{-}18)$ (13.88%), $(P\text{-}\overset{+}{1}26)$ (16.79%), $(P\text{-}\overset{+}{1}27)$ (33.93%), $(P\text{-}12\overset{+}{5}\text{-}18)$ (58.98%). Loss of water from the $(P\text{-}\overset{+}{1}27)$ ion gives the fragment at m/e = 255 (19.6%) which elides carbon atoms 1, 2, 3 and 4 to give the abundant ion m/e = 201 (12.7%). A mechanism (2) Fig. 10 has been proposed for this previously. Two metastable ions prove the two step formation of this ion from the ion at m/e = 273.

$$273^+ \longrightarrow 255^+ + 18 \qquad m/e = 238.2.$$

$$255^+ \longrightarrow 201^+ + 54 \qquad m/e = 158.3.$$

The characteristic ions m/e = 152 (10.41%) and 166 (4.68%) are again present, and the usual fragmentation of ring D involving the loss of the side chain plus forty-two mass units gives, after elimination of water, the abundant ion at m/e = 215 (16.70%).

3α-Acetoxy-5β-ergost-22,23-ene

A true parent molecular ion was obtained without difficulty using the heated inlet system at 200°C. The shift of some of the peaks by forty-two mass units (CH_2CO) gives a less complicated looking spectrum. The fragment at m/e = 287 in the spectrum of the alcohol is now at m/e = 329 showing that it must be formed by the loss of methyl from the $(P-98)^+$ ion. Loss of the $C_{(17)}$ side chain with one or two hydrogens gives the ions m/e = 316 (6.34%) and 315 (18.7%), and the peaks at m/e = 256 (21.38%) and 255 (30.74%) correspond to the elimination of acetic acid from them. There is a particularly abundant fragment at m/e = 257 (76.92%) which represents the loss of the side chain and a molecule of acetic acid from the parent molecular ion but no ion exists at m/e = 317 to show the loss of the side chain itself.

Stereochemical Differences

Biemann and Siebl (24) have shown that in general

the less crowded of two epimers gives the more abundant molecular ion. In secondary cyclic alcohols the epimer with the equatorial hydroxyl has a more intense molecular ion and fragments are of lower abundance. Djerassi (54) noticed that in a pair of steroidal ketones epimeric at $C_{(5)}$ the 5β-epimer invariably showed a more pronounced loss of ring A.

In the ergost-22,23-ene-3-ols and their acetates examined here the two effects are in opposition; a 3-axially (β) substituted compound being α- at $C_{(5)}$. The parent molecular ion of 5β-ergost-22,23-ene-3α-ol, using a direct insertion probe is more abundant (72.98%) than that of the 5α,3β compound (60.57%). The abundances of the fragment ions are however greater in the 5β compound, the ion at m/e = 201 formed by the loss of ring A and the $C_{(17)}$ side chain plus two hydrogens being 12.7% of the base peak as opposed to 5.7% in 5α-ergost-22,23-ene-3β-ol. The abundances of some of the major fragment ions, e.g. m/e = 201 (15.25%), 257 (76.92%) and 284 (36.63%) were greater in 3α-acetoxy-5β-ergost-22,23-ene than in 3β-acetoxy-5α-ergost-22,23-ene, m/e = 201 (9.53%), 257 (57.38%) and 284 (25.79%), and the stereochemistry at $C_{(5)}$ appears to be the determining factor.

3β-Acetoxy-5α-ergost-7,8-22,23-diene

With the presence of a 7,8-double bond in the nucleus the ions at m/e = 152 and 166, should become insignificant since ionization of this bond to give a tertiary carbonium ion and a secondary radical will be preferred to the formation of a secondary ion and radical by ionization of the 22,23-double bond. It would also be expected that fission of the 5-6- and 9-10- bonds through a retro Diels-Alder mechanism would give abundant fragment ions. Furthermore the loss of the side chain plus forty-two mass units should be assisted since it involves fission of the allylic 14-15 bond.

The mass spectrum supports the prediction that the ions at m/e = 152 and 166 should become less abundant; they are 2.51% and 0.39% of the base peak respectively. But the ion at m/e = 213 (20.93%), formed by the loss of the side chain plus forty-two mass units after elimination of acetic acid, is no longer the most abundant ion in the range m/e = 205-245. The ion at m/e = 229 is 29.35% of the base peak. Another noticeable feature is that the $(P-127-60)^+$ ion (31.31%) was twice as great as the $(P-127)^+$ ion (15.70%) in the 7,8-saturated compound whereas here the $(P-127)^+$ ion (69.13%) is much more prominent than the $(P-127-60)^+$ ion (19.4%). No abundant fragment ion

exists at m/e = 284 corresponding to the expected retro Diels-Alder reaction with the loss of the whole of ring A.

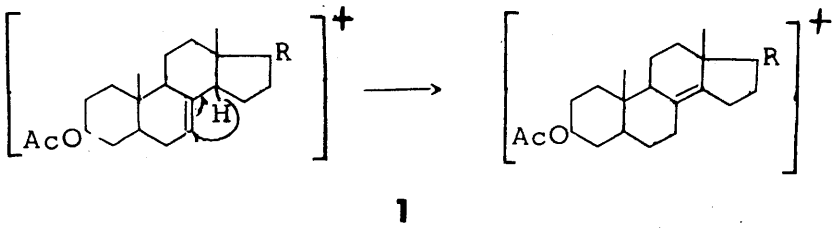
These facts could be best explained by postulating that the hydrogen on $C_{(14)}$ migrates to $C_{(7)}$ forming an 8,14-double bond as in (1) Fig. 12. The stability of the $(P-127)^+$ ion would then be accounted for since the positive charge can be delocalized over $C_{(8)}$, $C_{(14)}$, $C_{(15)}$, $C_{(16)}$ and $C_{(17)}$ as shown in (2), Fig. 12. A favourable structure such as (3) or (4), Fig. 12, can also be postulated for the $(P-151-60)^+$ ion at m/e = 229. The absence of an ion at m/e = 284 formed by a retro Diels-Alder reaction is also accounted for.

Chemically it is known that the 7,8-double bond in a steroid rearranges readily to the 8,14-double bond (80).

5α-Ergost-14,15-22,23-diene-3β-ol

Fragmentation of ring D would not be expected to occur easily and the most likely process should be the loss of the $C_{(17)}$ side chain with one hydrogen. The loss of a methyl radical gives an ion 50% of the abundance of the parent molecular ion at m/e = 383. The most likely methyl to be removed is that on $C_{(18)}$ since the resulting carbonium ion is allylically stabilized. The rearrangement which previously led to the loss of ninety-eight

FIG 12



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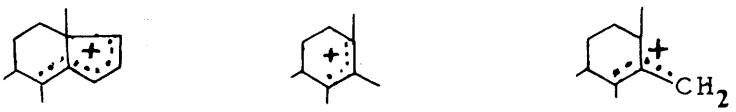
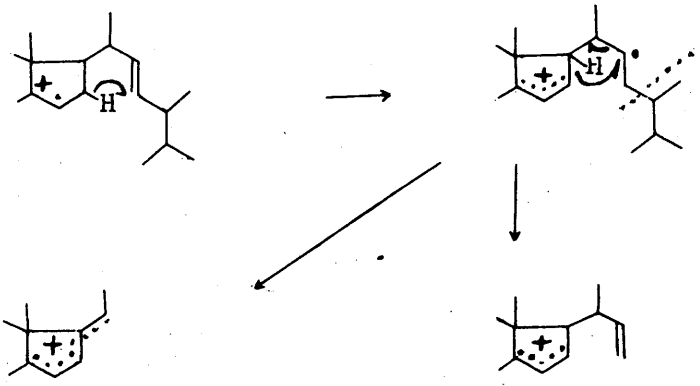


2 3 4



5 6

mass units now gives an abundant $(P-99)^+$ ion (13.33%). A metastable ion shows this to be a one step process.

$$398^+ \longrightarrow 299^+ + 99 \quad m/e = 224.6.$$

The loss of the additional hydrogen from $C_{(16)}$ will lead to the delocalization of the positive charge over carbon atoms 14, 15, 16, 17 and 20 as in (5), Fig. 12.

An abundant ion at m/e = 327 (15.05%) corresponds to cleavage of the 24-25 bond. That the loss of seventy-one mass units is from the side chain and not from ring A is shown by the shift of the ion to m/e = 369 in the spectrum of the acetate. A possible mechanism for its formation is shown in (6), Fig. 12. Transfer of the allylic hydrogen on $C_{(16)}$ to $C_{(23)}$ delocalizes the positive charge over $C_{(14)}$, $C_{(15)}$ and $C_{(16)}$. Homolytic fission of the 24-25 bond eliminates an amyl radical and reforms the 22,23 double bond. As expected the loss of the side chain at $C_{(17)}$ with one hydrogen gives the most abundant ion in the group m/e = 271 (21.64%), 272 (64.77%) and 273 (33.10%). Loss of the methyl on $C_{(18)}$ occurs to give the ion at m/e = 255.

$$272^+ \longrightarrow 255^+ + 15 \quad m/e = 242.9.$$

The presence of fragments at m/e = 247 (5.18%), 246 (12.89%) and 229 (22.07%) can only be explained if

rearrangement of the molecular ion to give an 8,14-double bond occurs. Loss of the $C_{(17)}$ side chain with $C_{(17)}$ and $C_{(16)}$ then gives the ion m/e = 246. A metastable ion proves the formation of m/e = 229 from the ion m/e = 247.

$$247^{+} \longrightarrow 229^{+} + 18 \quad m/e = 212.4.$$

The spectrum of the acetate shows the expected shift by forty-two mass units, of those ions which contained the $C_{(3)}$ hydroxyl group, to higher mass numbers.

Direct Sample Introduction

Reed (8) found that a very convenient method to introduce a sample into the ionization chamber of a mass spectrometer was by sublimation off a probe. Kelly (81) designed a vacuum locked probe system in which the vacuum of the mass spectrometer was not broken. The probe supplied with the M.S.9 is not vacuum locked but an arrangement of valves enables the probe to be inserted by only breaking the source vacuum.

There are two main advantages in the direct inlet system compared to the normal heated reservoir. The first of these is that the sample can be conveniently introduced with the minimum of thermal decomposition. If

the temperature of a heated reservoir system is lowered to reduce thermal decomposition then the pump out time increases. Secondly only a small quantity of sample (100 μgm.) gives an intense and persistent spectrum.

Criticism of direct inlet systems has developed along two lines. The first is that without a leak and an adequate reservoir system the pressure of the sample fluctuates and gives an incorrect mass spectrum. The second is concerned with the possibility that at the high sample pressures associated with a direct inlet technique ion-molecule reactions may occur.

The advantages of the probe are the compelling need for using this system when (i) only a small amount of sample is available, (ii) a compound may decompose thermally. It was impossible to determine the molecular weight of physovenine (Chapter I, this thesis) by mass spectrometry without the direct insertion probe. The mass spectra of a series of aromatic nitro compounds which decomposed vigorously in the heated inlet system have been obtained easily with no signs of decomposition on the probe (82).

The mass spectra of the steroids which had previously been obtained using the heated reservoir were redetermined using the probe to investigate what

differences existed. The spectra were in all cases obtained by scanning the mass range 460-28 in about one minute. All the compounds evaporated in the same way. After it had been observed that the compound had begun to give a spectrum the total ion current recorded by the monitor amplifier was noted every fifteen seconds. When this had remained sensibly constant for about two minutes the spectrum was recorded. After remaining constant for some time the total ion current rose rapidly to a maximum and immediately began to fall again.

During the recording of the spectrum variations of up to ± 5% in the total ion current were commonly encountered. A variation of up to +2% was usually observed when the mass range 460-28 was scanned even using the heated inlet system; when the magnet current was returned to its original position the monitor was observed to do the same. The variations in the total ion current caused by uneven evaporation were relatively slow (± 5% over the whole of the scan) and this difficulty may be obviated by using a faster scan and adjusting the amplifier response time. Counting of a spectrum obtained at such a scan speed is made difficult by the superimposed 50 c/s ripple but this difficulty can be overcome by running another spectrum at a slower rate.

The pressure of the sample on the M.S.9 is measured by an ionization gauge situated on the lead from the ion source to the diffusion pump. It is not possible to measure the pressure inside the ionization chamber. The pressure was usually 10-20 times greater when using the probe (2×10^{-6} - 6×10^{-6} Torr) than when using the heated inlet system. Beynon (83,84) using an M.S.8 with a similar pressure measuring system observed ion molecule reactions in ester mixtures at pressures (4×10^{-5} Torr) some 10 times greater than these. Ion-molecule reactions commonly lead to the addition of a hydrogen atom to the parent molecular ion in a number of organic compounds such as esters, ethers, ketones, aldehydes and nitriles (85). In the case of esters ($P+RCO^+$) ions and in nitriles ($P+RCN^+$) ions have also been reported (83).

The abundances of the ($P+1^+$) ion calculated as a percentage of the parent ion abundances using a probe are shown in the following table, together with those obtained using a heated inlet system and the calculated figures (9). The electron energy was 70 eV.

Formula	Observed (Heated inlet)	Observed (Probe)	Calculated
$C_{21}H_{32}O_2$	23.35	22.98	23.28
$C_{21}H_{34}O_2$	23.68	23.39	23.32
$C_{23}H_{36}O_3$	26.05	27.24	25.55
$C_{28}H_{48}O$	30.90	31.62	31.06
$C_{27}H_{48}O$	30.63	31.08	31.06
$C_{30}H_{48}O_2$	33.46	33.98	33.26
$C_{30}H_{50}O_2$	33.71	33.70	33.30
$C_{30}H_{50}O_2$	32.92	33.69	33.30

In no case was there observed any ion corresponding to the addition of a hydrocarbon group or acetyl group to the molecular ion. All the compounds but 3α-acetoxy-5β-pregnan-20-one showed similar results with variations of ± 0.5% from the calculated values for the $(P+1)^+ : P^+$ ratios. Even though the pressures generated by using the probe were ten to twenty times greater than those used to obtain a spectrum on the heated inlet system no evidence of ion-molecule reactions is apparent. In the normal use of the probe it is true to say that ion-molecule interactions do not give rise to any detectable differences in the mass spectrum. The difference found between the observed and calculated abundances for

3α-acetoxy-5β-pregnan-20-one ($C_{23}H_{36}O_3$) is not of the same order of magnitude as those observed by Beynon (83).

The spectra obtained using a probe do, however, show very marked differences from those obtained using a heated inlet system. The most likely cause of the differences would appear to be the temperature at which the sample is introduced. Using the heated inlet system the compounds were all heated to about 220°C prior to admission into the ion chamber. With the probe the highest temperature the compound can be heated to, if it sublimes without the application of external heating, is about 150°C, the temperature of the ionization chamber.

Cassuto (86) and Osberghaus (87) have investigated the variations in the abundances of parent molecular ions and fragment ions with the temperature of the ion chamber. Cassuto concluded that the probability of formation of the parent molecular ion can only decrease as the temperature increases since an increase in temperature corresponds to an increase in the rate constants of its decomposition reactions. Fragment ions are formed by competition between reactions leading to their formation and those leading to their decomposition except for the final products of decomposition which can only increase with temperature. Therefore, their probability of

formation may pass through a maximum with temperature.

The abundances of some of the major ions in the spectra of the steroids using a probe and a heated inlet system are summarized in the following tables.

Ion	5β-Ergost-22,23-ene-3α-ol		5α-Ergost-22,23-ene-3β-ol	
	Abundance		Abundance	
	Probe	Heated inlet	Probe	Heated inlet
P^+	60.57	14.71	72.98	35.21
$(P-18)^+$	2.61	8.70	6.20	7.91
$(P-18-43)^+$	16.00	5.90	16.70	15.70
$(P-98)^+$	53.73	13.74	47.70	21.42
$(P-98-18)^+$	2.56	8.22	9.22	13.88
$(P-127)^+$	51.70	24.54	49.29	33.93
$(P-125-18)^+$	52.33	30.42	62.81	58.98
$(P-127-18)^+$	5.32	17.64	10.34	19.60

Ion	3α-Acetoxy-5β-ergost-22,23-ene		3β-Acetoxy-5α-ergost-22,23-e	
	Abundance		Abundance	
	Probe	Heated inlet	Probe	Heated inlet
P^+	50.30	16.39	52.78	17.23
$(P-60)^+$	9.96	26.07	23.66	27.08
$(P-98)^+$	43.82	17.31	29.85	10.00
$(P-60-43)^+$	14.94	12.62	22.21	20.39
$(P-127)^+$	60.26	13.25	36.04	18.71
$(P-98-60)^+$	9.46	25.79	25.48	36.63
$(P-125-60)^+$	47.31	57.38	75.71	76.92
$(P-127-60)^+$	14.44	31.31	22.57	30.74

Ion	3α-Acetoxy-5β-pregnan-20-one		5β-Pregnan-3α-ol-20-one	
	Abundance		Abundance	
	Probe	Heated inlet	Probe	Heated inlet
P^+	4.51	0.63	40.18	8.34
$(P-HX)^+$	100.0	51.38	84.56	39.20
$(P-HX-18)^+$	7.46	5.11	5.2	2.62
$(P-HX-43)^+$	11.64	8.75	15.78	7.92
$(P-HX-54)^+$	8.28	7.96	11.95	7.47
$(P-HX-58)^+$	13.04	9.46	14.84	8.66
$(P-HX-70)^+$	22.22	18.50	16.00	10.64
$(P-HX-85)^+$	28.54	25.89	43.44	30.90
m/e = 43	57.32	100	100	100

Ion	5β-Pregnan-3,20-dione	
	Abundance	
	Probe	Heated inlet
P^+	52.94	21.02
$(P-18)^+$	32.26	25.63
$(P-44)^+$	9.32	6.51
$(P-18-43)^+$	7.15	5.74
$(P-70)^+$	35.33	33.32
$(P-85)^+$	10.26	10.71
$(P-70-18)^+$	8.50	8.87
$(P-85-18)^+$	12.43	14.97

Each one of the steroids shows an increased abundance of the parent molecular ion when using a probe. The abundances of the fragment ions do not, however, show any

consistent changes. In the ergost-22,23-enes the $(P-HX)^+$ ion (where X is the substituent at $C_{(3)}$) is always less abundant using a probe but in the 20-ketopregnanes all the fragment ions at the high mass end of the spectrum are more abundant. Some of the fragment ions which have lost the substituent at $C_{(3)}$ are in fact more abundant using the direct inlet system. The ions at m/e = 339 (P-43-HX) in the ergost-22,23-ene-3-ols and their acetates, and the $(P-18-\text{side chain})^+$ ion, m/e = 257, in the spectra of the alcohols show this effect.

The results are therefore what could be expected on the basis of Cassuto's conclusions, namely that the abundance of the parent molecular ion must always decrease as the temperature increases but that the probability of formation of a fragment ion may pass through a maximum.

Samples of the steroids were provided by F. Johnson formerly of the Royal College of Science and Technology, Glasgow.

5α-PREGNAN-20-ONE

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
40	3.69	73	0.44	100	0.13	127	0.32
41	47.4	74	0.38	101	0.32	128	1.31
42	0.95	75	0.25	102	0.32	129	1.86
43	100.0	76	0.38	103	1.80	130	1.11
44	2.81	77	15.2	104	1.64	131	4.5
		78	4.48	105	17.6	132	4.24
50	2.02	79	37.1	106	6.81	133	11.3
51	2.66	80	11.1	107	25.9	134	16.5
52	1.82	81	52.0	108	14.4	135	31.1
53	13.7	82	6.99	109	41.8	136	8.1
54	3.42	83	15.1	110	6.39	137	5.69
55	52.8	84	53.9	111	3.5	138	1.84
56	3.70	85	12.9	112	0.25	139	0.51
57	2.89	86	1.75	113	0.44		
58	1.69	87	0.25	114	0.13	141	0.44
59	2.04	88	0.25	115	1.96	142	0.76
		89	0.50	116	0.82	143	1.25
63	0.76	90	0.32	117	3.98	144	0.38
64	0.32	91	24.7	118	2.34	145	5.5
65	6.12	92	6.31	119	12.58	146	2.5
66	3.74	93	33.0	120	6.70	147	14.5
67	54.2	94	12.4	121	29.3	148	10.6
68	12.7	95	47.2	122	19.2	149	25.1
69	16.0	96	6.59	123	15.7	150	5.61
70	1.41	97	7.50	124	2.72	151	3.55
71	30.4	98	2.26	125	0.82	152	0.19
72	1.71	99	1.60	126	0.13	153	0.13

5α-PREGNAN-20-ONE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
154	0.25	186	0.19	217	71.1	257	6.72
155	0.32	187	3.25	218	17.83	258	5.67
156	0.25	188	2.5	219	3.95	259	9.00
157	0.82	189	5.32	220	0.57	260	2.05
158	0.57	190	1.65			261	0.19
159	5.99	191	1.50	227	1.7		
160	2.32	192	11.57	228	0.57	269	7.84
161	11.09	193	4.0	229	3.85	270	2.00
162	10.32	194	0.38	230	1.95	271	0.32
163	10.29			231	5.91	272	0.19
164	2.06	199	2.46	232	6.27	273	0.82
165	1.21	200	0.44	233	1.61	274	0.19
		201	3.25				
171	1.46	202	2.05	241	0.57	283	0.19
172	0.19	203	6.50	242	2.35	284	14.40
173	3.9	204	1.69	243	2.30	285	4.10
174	6.67	205	1.22	244	15.2	286	0.82
175	13.5	206	1.18	245	5.9	287	34.5
176	3.95	207	0.19	246	0.95	288	8.03
177	3.4			247	0.82	289	0.51
178	0.89	213	1.35			290	-
179	0.51	214	1.19	255	2.99	302	57.0
		215	7.3	256	2.45	303	12.10
185	0.63	216	4.12			304	1.64

5β -PREGNAN-3α -OL-20-ONE

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
40	1.88	70	2.09	97	3.69	124	1.93
41	37.62	71	23.03	98	1.00	125	1.14
42	5.36	72	1.24	99	0.66	126	0.12
43	100.0	73	0.28	100	0.31	127	0.21
44	2.42	74	0.23	101	0.12	128	0.91
45	2.80	75	0.31	102	0.33	129	1.29
		76	0.02	103	1.02	130	0.76
50	0.61	77	11.84	104	1.07	131	5.84
51	1.20	78	3.63	105	22.46	132	5.18
52	0.54	79	28.04	106	8.90	133	12.15
53	10.75	80	6.83	107	26.90	134	7.87
54	2.59	81	33.62	108	13.75	135	11.99
55	33.62	82	4.60	109	14.00	136	3.33
56	2.89	83	6.68	110	1.93	137	2.54
57	5.29	84	33.90	111	3.00	138	0.86
58	2.14	85	10.06	112	0.41	139	0.45
59	0.76	86	1.24	113	0.30	140	0.13
60	0.12	87	0.18	114	0.07	141	0.49
61	0.20			115	1.34	142	0.48
62	0.13	89	0.46	116	0.73	143	1.60
63	0.38	90	0.10	117	3.51	144	0.99
64	0.23	91	22.32	118	2.80	145	9.58
65	3.79	92	4.78	119	13.11	146	3.58
66	2.51	93	27.32	120	9.61	147	14.51
67	28.18	94	14.38	121	19.17	148	5.18
68	8.67	95	32.33	122	7.77	149	8.05
69	11.46	96	3.38	123	6.00	150	1.50

5β-PREGNAN-3α-OL-20-ONE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
151	0.94	178	0.66	205	1.88	236	0.08
152	0.25	179	0.38	206	0.61	237	0.02
153	0.16	180	0.10	207	0.20	238	0.05
154	0.16	181	0.08	208	0.08	239	0.40
155	0.38	182	0.10			240	0.64
156	0.31	183	0.30	211	0.86	241	1.04
157	2.14	184	0.13	212	0.40	242	8.66
158	1.10	185	1.80	213	3.73	243	2.79
159	8.44	186	1.19	214	1.83	244	1.58
160	4.12	187	4.75	215	30.90	245	2.57
161	11.18	188	2.84	216	9.17	246	7.47
162	4.57	189	3.08	217	5.26	247	1.60
163	3.59	190	2.77	218	1.48	248	1.17
164	0.91	191	2.72	219	0.61	249	0.18
165	1.07	192	1.32	220	0.28	250	0.16
166	0.30	193	0.51				
167	0.16	194	0.10	225	0.78	253	1.29
168	0.08	195	0.13	226	0.46	254	0.51
169	0.46	196	0.06	227	3.35	255	2.82
170	0.25	197	0.56	228	1.98	256	1.60
171	1.93	198	0.16	229	7.75	257	7.92
172	1.37	199	1.78	230	10.64	258	2.90
173	6.28	200	1.15	231	3.08	259	1.43
174	3.79	201	5.72	232	0.94	260	2.18
175	7.21	202	1.98	233	2.36	261	0.97
176	3.59	203	2.34	234	1.58	262	0.13
177	1.93	204	3.50	235	0.30	263	0.07

5β-PREGNAN-3α-OL-20-ONE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
264	0.03	274	1.20	287	0.56	304	0.35
265	0.33	275	0.56	288	0.10	305	0.03
266	0.13	276	0.15	289	0.07		
267	2.64					314	0.05
268	0.68	281	0.15	298	0.76	315	0.03
269	0.38	282	2.62	299	0.40	316	0.79
270	0.13	283	1.22	300	39.20	317	0.25
271	0.79	284	0.63	301	9.28	318	8.34
272	1.19	285	10.09	302	1.58	319	2.00
273	0.99	286	2.14	303	1.39	320	0.23

3α -ACETOXY-5β -PREGNAN-20-ONE

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
40	0.91	69	7.39	96	2.48	123	4.27
41	19.72	70	0.69	97	3.05	124	0.85
42	3.11	71	16.83	98	0.57	125	0.53
43	100.0	72	0.98	99	0.49	126	0.02
44	2.22	73	0.14	100	0.33	127	0.24
45	6.37	74	0.24	101	0.04	128	0.49
46	0.08	75	0.14	102	0.20	129	1.24
		76	0.18	103	0.77	130	0.73
50	0.75	77	9.26	104	1.02	131	6.70
51	0.88	78	2.95	105	20.53	132	5.56
52	0.63	79	24.38	106	8.47	133	12.09
53	6.08	80	5.05	107	22.51	134	6.78
54	1.61	81	27.27	108	16.89	135	10.21
55	20.88	82	2.58	109	13.19	136	2.67
56	2.52	83	4.78	110	1.49	137	2.28
57	2.60	84	13.53	111	1.49	138	0.59
58	1.22	85	9.46	112	0.18	139	0.24
59	0.24	86	1.28	113	0.20	140	0.06
60	4.35	87	0.20	114	0.08	141	0.45
61	0.63	88	0.02	115	0.90	142	0.35
62	0.06	89	0.18	116	0.61	143	2.04
63	0.30	90	0.16	117	3.70	144	1.02
64	0.14	91	19.33	118	2.44	145	9.08
65	2.63	92	4.50	119	11.95	146	3.76
66	1.42	93	26.09	120	8.83	147	14.47
67	21.71	94	11.00	121	18.33	148	4.48
68	4.42	95	24.62	122	6.13	149	6.49

3α-ACETOXY-5β-PREGNAN-20-ONE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
150	1.49	177	1.63	204	2.63	231	4.62
151	0.79	178	0.55	205	1.04	232	0.81
152	0.16	179	0.31	206	0.39	233	0.24
153	0.16	180	0.06	207	0.12	234	0.04
154	0.18	181	0.04	208	0.04	235	0.02
155	0.45	182	0.12	209	0.04	236	0.02
156	0.26	183	0.37	210	0.04	237	0.04
157	2.38	184	0.14	211	0.98	238	0.02
158	1.18	185	2.12	212	0.51	239	0.49
159	7.97	186	1.42	213	3.46	240	0.71
160	4.07	187	4.19	214	1.83	241	1.28
161	10.62	188	2.99	215	25.89	242	9.46
162	4.48	189	2.58	216	7.06	243	3.24
163	3.68	190	3.30	217	5.51	244	1.79
164	0.79	191	2.82	218	1.71	245	2.38
165	0.41	192	1.20	219	0.35	246	7.96
166	0.08	193	0.37	220	0.16	247	1.44
167	0.08	194	0.04	221	0.02	248	0.16
168	0.04	195	0.10	222	0.02	249	0.02
169	0.53	196	0.08	223	0.04	250	0.16
170	0.20	197	0.73	224	0.04	251	0.06
171	2.10	198	0.20	225	0.96	252	0.04
172	1.85	199	2.16	226	0.57	253	1.42
173	6.45	200	1.32	227	3.26	254	0.73
174	3.30	201	6.04	228	2.06	255	5.23
175	6.23	202	2.01	229	6.29	256	4.88
176	2.77	203	3.72	230	18.50	257	8.75

3α-ACETOXY-5β-PREGNAN-20-ONE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
258	3.89	275	0.20			325	0.04
259	1.77	276	0.08	298	0.16	326	0.02
260	0.37	277	0.04	299	0.26	327	0.12
261	0.12			300	51.38	328	0.04
262	0.08	280	0.02	301	12.45	329	0.02
263	0.02	281	0.14	302	2.01		
264	0.02	282	5.11	303	0.26	342	3.17
265	0.26	283	1.40	304	0.06	343	0.83
266	0.09	284	0.41			344	0.16
267	3.46	285	12.56	313	0.06	345	0.22
268	0.73	286	2.79	314	0.24	346	0.08
269	0.28	287	0.37	315	0.10		
270	0.08	288	0.12	316	0.20	358	0.06
271	0.77	289	0.08	317	0.12	359	0.04
272	0.55	290	0.83	318	0.06	360	0.63
273	0.12	291	0.18	319	0.02	361	0.16
274	0.07	292	0.04			362	0.04

5β -PREGNAN-3,20-DIONE

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
40	2.67	67	26.91	94	8.36	121	19.33
41	42.34	68	10.92	95	26.91	122	15.74
42	8.71	69	13.28	96	3.43	123	11.64
43	100.0	70	2.00	97	4.72	124	6.15
44	6.30	71	19.89	98	1.28	125	1.79
45	1.74	72	1.38	99	1.18	126	0.41
		73	0.31	100	0.21	127	0.31
47	0.15	74	0.36	101	0.26	128	2.00
48	0.10	75	0.31	102	0.26	129	1.79
49	0.51	76	0.36	103	1.18	130	0.82
50	1.74	77	11.94	104	1.03	131	6.66
51	2.26	78	6.92	105	17.68	132	5.54
52	2.20	79	5.17	106	5.79	133	10.25
53	11.79	80	6.97	107	22.14	134	6.20
54	2.77	81	30.86	108	10.35	135	10.56
55	33.47	82	4.31	109	14.66	136	2.72
56	13.79	83	5.13	110	4.61	137	4.20
57	16.56	84	30.35	111	4.87	138	1.03
58	6.51	85	10.87	112	0.62	139	0.36
59	0.51	86	1.64	113	0.51	140	0.21
60	0.15	87	0.26	114	0.15	141	1.03
61	0.10	88	0.21	115	1.33	142	0.51
62	0.21	89	0.36	116	0.92	143	3.08
63	0.56	90	0.15	117	4.05	144	2.00
64	0.41	91	18.76	118	3.64	145	8.15
65	4.20	92	4.20	119	12.46	146	3.74
66	2.36	93	22.61	120	11.94	147	10.0

5β -PREGNAN-3,20-DIONE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
148	3.23	175	8.87	202	1.54	230	1.13
149	5.07	176	14.35	203	3.90	231	10.71
150	1.33	177	3.02	204	1.28	232	2.87
151	1.18	178	0.56	205	0.51	233	1.90
152	0.21	179	0.67	206	0.15	234	0.36
153	0.15	180	0.15	207	0.10	235	0.05
154	0.15	181	0.21	208	0.05	236	0.05
155	0.62	182	0.21	209	0.46	237	0.10
156	0.41	183	0.87	210	0.15	238	0.21
157	3.74	184	0.41	211	1.18	239	0.62
158	2.41	185	2.67	212	0.36	240	1.13
159	10.87	186	2.36	213	14.97	241	2.20
160	2.92	187	4.51	214	2.92	242	0.82
161	10.92	188	4.05	215	5.48	243	2.41
162	3.59	189	5.48	216	1.49	244	2.36
163	3.69	190	1.64	217	2.56	245	3.49
164	1.28	191	0.97	218	1.49	246	33.32
165	1.13	192	0.41	219	0.31	247	6.05
166	0.21	193	0.41	220	0.05	248	0.67
167	0.15	194	0.10			249	0.10
168	0.15	195	0.46	223	0.21	250	0.05
169	1.03	196	0.21	224	0.15	251	1.64
170	0.51	197	0.82	225	1.59	252	0.41
171	3.59	198	0.46	226	1.69	253	0.41
172	2.41	199	3.49	227	3.59	254	0.31
173	6.56	200	2.00	228	8.87	255	5.74
174	5.07	201	3.28	229	3.13	256	2.10

5β-PREGNAN-3,20-DIONE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
257	1.44	269	2.92	283	6.77	300	0.87
258	6.10	270	1.85	284	1.64	301	5.59
259	2.26	271	1.59	285	0.26	302	1.18
260	0.46	272	6.51	286	0.05	303	0.21
261	0.41	273	3.38	287	0.21		
262	0.05	274	0.62	288	0.10	314	0.26
		275	0.10			315	0.21
265	2.15			297	0.21	316	21.02
266	0.62	280	0.41	298	25.63	317	4.62
267	0.10	281	0.77	299	6.05	318	0.56
268	0.21	282	0.21				

5β -ERGOST-22,23-ENE-3-ONE

M/e	%Z Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
50	0.92	77	10.05	104	0.78	131	5.05
51	1.01	78	2.38	105	16.98	132	1.74
52	0.69	79	31.24	106	4.45	133	9.96
53	11.01	80	6.88	107	30.19	134	8.12
54	3.35	81	58.27	108	11.01	135	11.33
55	100.0	82	26.15	109	43.35	136	2.57
56	5.92	83	36.24	110	7.52	137	8.07
57	17.11	84	4.54	111	4.73	138	1.38
58	1.01	85	1.28	112	0.55	139	0.32
59	0.32	86	0.32	113	0.28	140	0.24
60	0.28	87	0.36	114	0.28	141	0.60
61	0.18	88	0.36	115	0.92	142	0.69
62	0.18	89	0.32	116	0.64	143	1.70
63	0.55	90	0.28	117	3.35	144	0.92
64	0.32	91	18.95	118	1.93	145	7.85
65	3.49	92	3.99	119	11.29	146	2.25
66	1.97	93	35.42	120	4.50	147	11.01
67	46.80	94	16.61	121	16.52	148	3.30
68	14.18	95	46.48	122	9.50	149	10.46
69	78.08	96	17.11	123	19.04	150	2.16
70	11.06	97	10.28	124	7.71	151	3.30
71	7.80	98	1.38	125	6.97	152	26.20
72	0.64	99	0.78	126	1.33	153	3.30
73	0.37	100	0.36	127	0.41	154	0.32
74	0.32	101	0.60	128	2.06	155	0.36
75	0.32	102	0.36	129	1.74	156	0.36
76	0.18	103	0.83	130	0.78	157	1.70

5β -ERGOST-22,23-ENE-3-ONE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundanc
158	0.92	185	1.74	212	0.36	239	1.06
159	7.48	186	0.87	213	3.26	240	0.46
160	2.34	187	4.04	214	1.28	241	1.19
161	10.00	188	1.42	215	4.91	242	0.78
162	3.07	189	3.07	216	1.47	243	2.75
163	9.41	190	0.92	217	4.45	244	2.29
164	1.84	191	1.84	218	1.06	245	3.81
165	1.42	192	0.36	219	1.06	246	1.84
166	3.89	193	0.32	220	0.64	247	1.28
167	0.69	194	0.09	221	0.18	248	0.32
168	0.18	195	0.09	222	0.09	249	0.05
169	0.46	196	0.09	223	0.09	250	0.05
170	0.36	197	0.64	224	0.14	251	0.14
171	1.70	198	0.32	225	0.60	252	0.18
172	0.92	199	3.12	226	0.37	253	1.51
173	4.22	200	1.33	227	1.47	254	0.87
174	1.74	201	3.76	228	1.47	255	34.09
175	4.91	202	2.25	229	4.91	256	7.48
176	2.52	203	10.87	230	5.92	257	3.95
177	5.05	204	1.93	231	8.76	258	14.77
178	0.92	205	1.38	232	2.66	259	4.31
179	0.78	206	0.28	233	1.56	260	0.73
180	0.18	207	0.14	234	0.36	261	0.28
181	0.09	208	0.05	235	0.09		
182	0.09	209	0.05	236	0.36	265	0.32
183	0.45	210	0.05	237	0.28	266	0.32
184	0.28	211	0.64	238	0.83	267	1.84

5β-ERGOST-22,23-ENE-3-ONE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
268	0.60			327	0.78	368	0.18
269	1.24	295	0.18	328	0.91	369	0.18
270	10.64	296	0.14	329	0.60	370	0.32
271	41.75	297	0.60	330	0.18	371	0.09
272	28.22	298	0.78				
273	19.64	299	7.71	337	6.57	379	0.18
274	4.27	300	52.67	338	1.84	380	0.28
275	0.64	301	16.10	339	0.55	381	0.36
276	0.18	302	2.75	340	0.28	382	0.40
277	0.18	303	0.46	341	0.36	383	4.18
278	0.90			342	0.32	384	1.38
279	0.90	309	0.28	343	0.24	385	0.36
280	0.46	310	0.09			386	0.14
281	0.05	311	0.36	353	0.36		
282	1.01	312	0.40	354	0.40	394	0.24
283	4.86	313	0.60	355	7.80	395	0.09
284	1.10	314	6.97	356	2.25	396	1.84
285	13.40	315	1.84	357	0.46	397	0.92
286	3.67	316	0.73	358	0.09	398	62.86
287	1.10	317	0.28			399	19.82
288	0.32			365	2.06	400	4.59
289	0.14	325	0.46	366	0.78	401	0.78
290	0.05	326	0.60	367	0.36	402	0.28

ERGOST-4,5-22,23-DIENE-3-ONE

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
50	0.87	77	14.64	104	1.36	131	10.17
51	0.81	78	3.66	105	23.94	132	3.60
52	1.05	79	33.31	106	5.40	133	18.67
53	11.17	80	5.58	107	26.86	134	7.20
54	1.74	81	44.48	108	8.31	135	17.12
55	100.00	82	18.48	109	39.20	136	5.71
56	5.52	83	45.22	110	5.83	137	10.48
57	19.29	84	4.71	111	4.65	138	2.67
58	1.61	85	1.18	112	0.50	139	0.87
59	0.25	86	0.19	113	0.43	140	0.19
60	0.19	87	0.12	114	0.25	141	1.43
61	0.06	88	3.78	115	2.30	142	1.18
62	0.12	89	0.12	116	1.49	143	4.40
63	0.37	90	0.31	117	6.51	144	2.05
64	0.25	91	28.78	118	2.61	145	13.27
65	10.42	92	5.09	119	16.00	146	4.47
66	2.17	93	32.01	120	5.09	147	29.90
67	42.61	94	8.99	121	21.59	148	9.99
68	8.81	95	41.99	122	10.23	149	24.01
69	87.59	96	13.52	123	26.61	150	3.91
70	11.48	97	15.63	124	21.77	151	2.23
71	8.44	98	1.86	125	9.43	152	5.46
72	0.74	99	0.68	126	1.18	153	0.81
73	0.25	100	0.25	127	0.93	154	0.43
74	0.56	101	0.12	128	3.10	155	1.98
75	0.25	102	0.19	129	3.54	156	1.05
76	0.06	103	1.92	130	1.67	157	5.27

ERGOST-4,5-22,23-DIENE-3-ONE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
158	1.98	185	4.09	212	1.05	239	0.93
159	9.99	186	1.55	213	4.59	240	0.31
160	3.04	187	5.83	214	1.55	241	1.98
161	16.25	188	1.92	215	6.08	242	1.55
162	3.66	189	4.53	216	1.43	243	8.25
163	8.87	190	0.81	217	3.04	244	3.91
164	1.05	191	1.18	218	0.50	245	29.90
165	1.05	192	0.50	219	0.37	246	5.71
166	1.24	193	0.19	220	0.43	247	0.68
167	0.43	194	0.12	221	0.19	248	0.19
168	0.25	195	0.43	222	0.12	249	0.12
169	1.49	196	0.25	223	0.56	250	0.12
170	0.93	197	2.23	224	0.37	251	0.74
171	4.59	198	0.87	225	2.23	252	1.49
172	2.42	199	2.48	226	1.12	253	24.75
173	8.99	200	0.93	227	8.13	254	4.65
174	6.02	201	5.27	228	3.23	255	4.28
175	15.07	202	1.61	229	1.58	256	11.91
176	4.28	203	5.46	230	4.34	257	8.68
177	5.64	204	0.87	231	8.06	258	2.05
178	0.93	205	0.43	232	1.49	259	0.93
179	0.37	206	0.12	233	0.37	260	0.31
180	0.19	207	0.25	234	0.12	261	0.31
181	0.37	208	0.12	235	0.12	262	0.06
182	0.25	209	0.74	236	0.56	263	0.06
183	1.61	210	0.43	237	0.56	264	0.25
184	0.74	211	2.67	238	0.50	265	0.74

ERGOST-4,5-22,23-DIENE-3-ONE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
266	0.31	295	0.43	324	0.31	364	0.12
267	0.74	296	0.37	325	1.05	365	0.06
268	8.31	297	12.72	326	0.43	366	0.06
269	40.07	298	43.17	327	0.99	367	0.19
270	26.92	299	17.80	328	0.43	368	0.25
271	48.51	300	4.22	329	0.12	369	0.12
272	12.16	301	0.87				
273	3.41	302	0.12	335	3.04	378	0.31
274	0.62			336	0.87	379	0.37
275	0.25	307	0.25	337	0.31	380	0.25
		308	0.19	338	0.19	381	4.34
279	0.37	309	0.31	339	0.31	382	1.24
280	1.92	310	0.37	340	0.12	383	0.25
281	4.22	311	1.24			384	0.19
282	0.87	312	7.44	351	0.25		
283	13.77	313	4.28	352	0.74	394	0.87
284	3.60	314	1.12	353	18.17	395	0.50
285	3.66	315	0.25	354	5.52	396	50.00
286	0.81	316	0.12	355	1.36	397	12.84
287	0.25	317	0.06	356	0.19	398	4.09
		318	0.12	357	0.06	399	0.56
293	0.25					400	0.12
294	0.19	323	0.25	363	0.19		

5β-ERGOST-22,23-ENE-3α-OL

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
50	3.63	78	8.62	106	7.85	134	6.28
51	4.68	79	10.53	107	49.72	135	27.22
52	3.11	80	7.29	108	15.79	136	4.77
53	9.68	81	77.49	109	59.34	137	10.67
54	3.88	82	27.77	110	8.64	138	1.89
55	100.0	83	47.55	111	5.93	139	0.72
56	8.34	84	5.99	112	1.16	140	0.38
57	27.50	85	2.68	113	0.68	141	0.97
58	3.37	86	2.45	114	0.41	142	0.84
59	1.03	87	0.65	115	1.70	143	1.73
60	1.20	88	0.34	116	1.03	144	0.92
61	0.52	89	0.61	117	4.00	145	9.27
62	0.70	90	0.31	118	2.69	146	3.16
63	1.52	91	26.86	119	15.51	147	26.04
64	1.79	92	7.21	120	6.88	148	6.34
65	3.22	93	46.37	121	27.68	149	25.41
66	2.20	94	20.51	122	13.97	150	3.97
67	51.63	95	65.88	123	22.50	151	3.93
68	14.07	96	24.05	124	6.78	152	10.41
69	96.55	97	13.88	125	7.81	153	1.71
70	14.07	98	2.56	126	1.45	154	0.91
71	11.34	99	0.65	127	2.57	155	0.49
72	0.96	100	0.39	128	8.80	156	0.44
73	0.84	101	0.80	129	2.35	157	1.32
74	1.76	102	1.25	130	0.72	158	0.81
75	1.39	103	1.26	131	6.16	159	7.51
76	1.73	104	1.26	132	2.44	160	3.95
77	11.43	105	22.87	133	16.97	161	26.95

5β -ERGOST-22,23-ENE-3α -OL (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundanc
162	6.30	190	2.15	218	1.46	246	1.36
163	16.42	191	2.55	219	1.23	247	0.81
164	2.47	192	0.54	220	0.92	248	0.26
165	2.77	193	0.45	221	0.33	249	0.14
166	4.68	194	0.18	222	0.09	250	0.05
167	0.88	195	0.18	223	0.14	251	0.05
168	0.30	196	0.17	224	0.08	252	0.23
169	0.32	197	0.31	225	0.26	253	0.61
170	0.21	198	0.21	226	0.18	254	1.13
171	1.32	199	2.33	227	3.15	255	19.60
172	0.64	200	1.11	228	1.78	256	8.36
173	5.86	201	12.70	229	10.97	257	58.98
174	2.94	203	4.18	230	5.63	258	14.33
175	18.51	203	7.22	231	4.89	259	2.81
176	4.94	204	1.46	232	1.94	260	5.55
177	5.98	205	1.09	233	2.42	261	1.02
178	1.23	206	0.28	234	0.92	262	0.25
179	1.25	207	0.31	235	0.13	263	0.16
180	0.34	208	0.13	236	0.14	264	0.29
181	0.31	209	0.14	237	0.16	265	0.10
182	0.23	210	0.06	238	0.44	266	0.09
183	0.39	211	0.43	239	0.49	267	0.90
184	0.32	212	0.17	240	0.31	268	0.34
185	1.62	213	3.97	241	2.82	269	3.75
186	0.72	214	3.17	242	4.88	270	2.26
187	5.96	215	16.70	243	2.75	271	6.16
188	2.32	216	5.71	244	1.38	272	8.11
189	7.55	217	7.05	245	2.50	273	33.93

5β-ERGOST-22,23-ENE-3α-OL (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundanc
274	16.79	302	21.42	332	0.02	365	0.41
275	3.88	303	4.95	333	0.02	366	0.24
276	0.58	304	0.59	334	0.30	367	3.57
277	0.12	305	0.06	335	0.08	368	1.06
278	0.05			336	0.02	369	0.33
279	0.04	309	0.13	337	1.42	370	0.15
280	0.04	310	0.05	338	0.43	371	0.15
281	0.28	311	0.76	339	15.70	372	0.10
282	0.27	312	0.28	340	4.40		
283	3.51	313	0.39	341	1.07	379	0.06
284	13.88	314	1.02	342	0.17	380	0.31
285	12.70	315	0.40	343	0.20	381	0.40
286	3.14	316	3.63	344	0.10	382	7.91
287	8.79	317	0.92	345	0.04	383	2.73
288	2.70	318	0.16			384	0.66
289	0.35	319	0.07	350	0.10	385	3.66
290	0.21	320	0.07	351	0.04	386	1.25
291	0.07	321	0.04	352	0.06	387	0.21
292	0.07	322	0.06	353	0.12	388	0.06
293	0.07	323	0.16	354	0.10		
294	0.13	324	0.10	355	1.37	396	0.16
295	0.20	325	0.27	356	0.49	397	0.07
296	0.09	326	0.35	357	1.40	398	4.55
297	0.72	327	0.71	358	0.27	399	1.40
298	1.18	328	1.43	359	0.08	400	35.21
299	1.47	329	0.55	360	0.04	401	10.51
300	5.03	330	0.27			402	1.59
301	4.97	331	0.08	364	0.06	403	0.10

5α-ERGOST-22,23-ENE-3β-OL

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundanc
50	3.70	77	12.77	104	1.48	131	7.06
51	12.10	78	7.05	105	19.33	132	2.57
52	8.24	79	25.71	106	7.73	133	14.46
53	14.24	80	7.82	107	46.22	134	7.16
54	6.99	81	68.74	108	15.66	135	21.34
55	100.0	82	28.24	109	64.03	136	5.82
56	14.96	83	43.36	110	8.94	137	14.65
57	36.13	84	0.19	111	7.12	138	2.84
58	13.38	85	5.57	112	1.48	139	1.10
59	3.20	86	0.84	113	1.69	140	0.93
60	2.15	87	1.88	114	0.47	141	1.45
61	1.31	88	0.99	115	4.13	142	1.33
62	1.05	89	0.72	116	1.85	143	1.69
63	4.87	90	1.56	117	6.70	144	1.06
64	15.49	91	38.15	118	2.61	145	7.80
65	5.75	92	12.77	119	14.62	146	2.36
66	4.70	93	39.32	120	5.73	147	20.23
67	52.10	94	18.18	121	25.54	148	7.23
68	14.50	95	59.16	122	13.38	149	22.86
69	89.24	96	25.88	123	32.27	150	5.50
70	10.08	97	16.48	124	6.60	151	9.00
71	11.93	98	3.25	125	9.02	152	11.36
72	2.21	99	1.35	126	1.33	153	2.44
73	1.39	100	1.14	127	1.47	154	0.95
74	8.26	101	2.37	128	2.18	155	0.88
75	3.73	102	5.41	129	3.48	156	0.69
76	5.68	103	1.71	130	1.79	157	1.87

5α -ERGOST-22,23-ENE-3β -OL (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
158	0.95	185	1.22	212	0.15	239	0.49
159	6.60	186	0.57	213	2.47	240	0.27
160	2.85	187	4.38	214	1.33	241	1.79
161	21.85	188	1.18	215	8.93	242	2.06
162	4.76	189	7.08	216	3.01	243	2.32
163	24.37	190	1.71	217	6.28	244	1.47
164	4.89	191	4.05	218	1.64	245	4.45
165	3.94	192	1.07	219	2.04	246	2.09
166	6.41	193	0.95	220	0.82	247	1.50
167	1.08	194	0.72	221	0.42	248	0.42
168	0.61	195	0.36	222	0.15	249	0.15
169	0.57	196	0.27	223	0.15	250	0.15
170	0.49	197	0.42	224	0.32	251	0.15
171	1.31	198	0.21	225	0.19	252	0.15
172	0.72	199	1.81	226	0.27	253	0.25
173	4.42	200	0.80	227	1.66	254	1.54
174	1.85	201	5.71	228	0.93	255	17.64
175	8.70	202	2.34	229	5.12	256	5.98
176	4.49	203	6.83	230	3.48	257	30.42
177	7.02	204	2.63	231	5.61	258	11.19
178	1.71	205	2.13	232	3.03	259	4.49
179	2.04	206	0.89	233	3.03	260	3.18
180	0.70	207	0.57	234	1.22	261	1.03
181	0.34	208	0.23	235	0.19	262	0.23
182	0.29	209	0.17	236	0.19	263	0.02
183	0.19	210	0.17	237	0.17	264	0.17
184	0.32	211	0.34	238	0.55	265	0.49

5α-ERGOST-22,23-ENE-3β-OL (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
266	0.15	296	0.46	329	0.34	367	1.58
267	0.32	297	0.36	330	0.15	368	0.46
268	0.17	298	1.71			369	0.34
269	4.87	299	4.07	334	1.90	370	0.25
270	3.44	300	14.96	335	0.13		
271	17.28	301	7.48	336	0.02	380	0.51
272	12.39	302	13.74	337	1.20	381	0.23
273	24.54	303	3.43	338	0.44	382	8.70
274	10.83	304	0.49	339	5.90	383	4.13
275	3.73			340	1.75	384	0.86
276	0.48	309	0.17	341	0.44	385	1.56
		310	0.11	342	0.15	386	0.42
281	0.21	311	0.36	343	0.15	387	0.27
282	0.17	312	0.32				
283	3.50	313	0.32	350	0.13	396	0.55
284	8.22	314	2.99	351	0.10	397	0.21
285	10.85	315	0.89	352	0.11	398	12.41
286	2.97	316	2.23	353	0.27	399	3.58
287	6.26	317	0.42	354	0.11	400	14.71
288	1.88			355	4.43	401	4.53
289	0.32	325	0.42	356	1.12	402	0.51
290	0.11	326	0.34	357	1.94	403	0.10
		327	0.34	358	0.82	404	0.10
295	0.15	328	0.48				

3α -ACETOXY-5β -ERGOST-22,23-ENE

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
50	1.29	77	11.09	104	1.09	131	7.28
51	1.34	78	7.13	105	26.14	132	2.28
52	0.64	79	38.71	106	10.30	133	16.78
53	7.92	80	7.08	107	60.58	134	7.23
54	2.67	81	81.73	108	19.11	135	26.48
55	90.87	82	22.97	109	59.35	136	3.96
56	4.50	83	44.55	110	8.07	137	9.80
57	21.63	84	4.60	111	3.96	138	1.44
58	0.89	85	1.29	112	0.05	139	0.30
59	0.10	86	0.54	113	0.10	140	0.05
60	14.26	87	0.15	114	0.40	141	0.50
61	1.29	88	0.05	115	1.34	142	0.74
62	0.10	89	0.20	116	0.69	143	1.68
63	0.35	90	0.10	117	4.41	144	0.99
64	0.05	91	27.13	118	2.77	145	9.41
65	2.77	92	6.04	119	16.14	146	4.70
66	1.73	93	55.44	120	7.62	147	31.83
67	52.02	94	20.34	121	31.33	148	6.83
68	11.39	95	64.42	122	10.14	149	25.94
69	100.0	96	22.08	123	18.96	150	3.47
70	11.29	97	6.24	124	5.69	151	3.07
71	9.21	98	0.99	125	10.20	152	9.55
72	0.50	99	0.20	126	1.19	153	1.34
73	0.10	100	0.40	127	0.20	154	0.15
74	0.30	101	0.30	128	0.35	155	0.35
75	0.10	102	0.10	129	1.09	156	0.20
76	0.10	103	0.84	130	0.84	157	1.24

3α-ACETOXY-5β-ERGOST-22,23-ENE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
158	0.94	186	0.84	217	7.18	252	0.10
159	10.79	187	6.63	218	1.39	253	0.40
160	5.10	188	2.77	219	0.50	254	2.52
161	28.81	189	8.17	220	0.69	255	30.74
162	6.68	190	1.83	221	0.10	256	21.38
163	16.68	191	2.18			257	76.92
164	2.97	192	0.40	226	0.05	258	15.54
165	1.53	193	0.30	227	3.42	259	1.98
166	3.56			228	2.72	260	0.25
167	0.59	197	0.20	229	13.17	261	0.25
168	0.15	198	0.10	230	7.92	262	0.05
169	0.15	199	2.48	231	4.90		
170	0.15	200	1.44	232	0.89	264	0.45
171	1.34	201	15.25	233	0.35	265	0.15
172	0.64	202	5.69	234	0.20		
173	6.09	203	7.33	235	0.30	268	0.15
174	4.41	204	1.44			269	10.10
175	21.04	205	0.64	239	0.25	270	2.82
176	4.85	206	0.25	240	0.15	271	1.34
177	5.64	207	0.30	241	4.36	272	0.84
178	0.94			242	10.25	273	0.69
179	0.94	211	0.20	243	5.40	274	0.64
180	0.35	212	0.10	244	1.44	275	0.69
		213	4.21	245	1.93	276	0.25
183	0.30	214	3.96	246	0.30		
184	0.15	215	19.65	247	0.20	281	0.15
185	1.88	216	7.82			282	0.10

3α-ACETOXY-5β-ERGOST-22,23-ENE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
283	5.40	312	0.84	343	1.58	380	0.69
284	36.63	313	0.59	344	10.00	381	0.64
285	17.67	314	2.77	345	2.67	382	27.08
286	3.42	315	18.71	346	0.30	383	7.87
287	0.64	316	6.44			384	1.49
288	0.99	317	1.34	353	0.20	385	0.25
289	0.35	318	0.15	354	0.20		
290	0.30			355	0.10	398	0.10
		325	0.40	356	0.05	399	0.35
296	0.10	326	0.54	357	0.05	400	0.20
297	0.89	327	0.59	358	1.83		
298	3.22	328	3.07	359	0.45	427	1.39
299	0.99	329	5.15	360	0.10	428	0.59
300	0.35	330	1.49			429	0.10
301	0.25	331	0.35	365	0.35		
302	2.82			366	0.15	440	0.15
303	0.74	337	0.10	367	7.62	441	0.15
304	0.20	338	0.15	368	2.33	442	17.23
		339	20.39	369	0.40	443	5.89
309	0.20	340	6.19	370	0.05	444	1.14
310	0.10	341	0.99			445	0.20
311	1.14	342	0.15				

3β -ACETOXY-5α-ERGOST-22,23-ENE

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
50	1.02	77	14.71	104	1.53	131	6.45
51	0.87	78	3.08	105	27.72	132	2.36
52	0.86	79	41.24	106	10.18	133	17.38
53	8.45	80	7.71	107	68.69	134	8.18
54	2.66	81	91.31	108	20.27	135	23.03
55	93.10	82	24.14	109	64.00	136	3.66
56	5.31	83	48.97	110	8.50	137	12.42
57	23.72	84	4.90	111	6.39	138	1.73
58	1.03	85	1.47	112	0.53	139	0.42
59	0.14	86	0.22	113	0.20	140	0.09
60	19.03	87	0.22	114	0.44	141	0.47
61	0.91	88	0.03	115	1.22	142	0.78
62	0.30	89	0.17	116	0.70	143	1.78
63	0.30	90	0.12	117	4.92	144	0.95
64	0.09	91	32.97	118	2.34	145	8.67
65	3.49	92	7.35	119	15.44	146	3.40
66	2.08	93	57.24	120	6.22	147	22.07
67	52.55	94	18.76	121	27.59	148	7.31
68	11.61	95	64.28	122	15.54	149	21.79
69	100.0	96	22.90	123	25.93	150	3.36
70	11.90	97	14.20	124	5.81	151	5.03
71	9.54	98	1.50	125	9.64	152	8.50
72	0.58	99	0.36	126	0.42	153	1.19
73	0.16	100	0.33	127	0.42	154	0.19
74	0.36	101	0.27	128	0.27	155	0.39
75	0.25	102	0.17	129	1.73	156	0.27
76	0.20	103	1.00	130	0.87	157	1.27

3β-ACETOXY-5α-ERGOST-22,23-ENE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
158	0.73	185	1.17	214	3.08	248	0.12
159	7.00	186	0.58	215	13.25		
160	4.30	187	5.09	216	4.62	251	0.16
161	25.79	188	1.83	217	4.89	252	0.17
162	5.51	189	6.82	218	0.89	253	0.36
163	23.59	190	1.66	219	0.86	254	3.36
164	3.58	191	4.19	220	1.08	255	31.31
165	2.55	192	0.70	221	0.28	256	16.12
166	5.15	193	0.34			257	57.38
167	0.77	194	0.11	227	1.87	258	11.93
168	0.14	195	0.09	228	1.70	259	1.69
169	0.31	196	0.06	229	6.87	260	0.41
170	0.20	197	0.20	230	4.73	261	0.81
171	1.08	198	0.12	231	3.46	262	0.25
172	0.52	199	1.69	232	0.73		
173	4.67	200	0.98	233	0.59	269	12.28
174	1.98	201	9.53	234	0.20	270	3.10
175	11.17	202	3.80			271	1.59
176	5.20	203	5.28	239	0.16	272	0.75
177	6.51	204	1.09	240	0.14	273	1.09
178	1.03	205	0.91	241	4.14	274	2.33
179	2.78	206	0.36	242	6.86	275	1.37
180	0.50	207	0.62	243	4.20	276	0.67
181	0.12			244	1.09		
182	0.11	211	0.20	245	3.67	281	0.16
183	0.22	212	0.12	246	0.70	282	0.14
184	0.11	213	3.21	247	0.44	283	4.65

3β -ACETOXY-5α -ERGOST-22,23-ENE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
284	25.79	314	2.70	345	4.45	380	0.28
285	12.29	315	15.70	346	0.66	381	0.30
286	2.53	316	9.26			382	26.07
287	1.23	317	2.19	353	0.12	383	7.93
288	0.92	318	0.23	354	0.12	384	1.43
289	0.39			355	0.12	385	0.28
290	0.22	325	0.12	356	0.12		
		326	0.16	357	0.14	398	0.36
297	0.77	327	0.16	358	2.66	399	0.75
298	3.25	328	0.87	359	0.67	400	0.52
299	1.00	329	4.61	360	0.12	401	0.12
300	0.52	330	1.45				
301	0.73	331	0.20	367	4.51	427	1.13
302	4.00			368	1.53	428	0.41
303	0.89	339	12.62	369	0.36	429	0.09
304	0.17	340	3.57	370	0.11		
		341	0.69			442	16.39
311	0.69	342	0.19	378	0.16	443	5.42
312	0.52	343	1.77	379	0.08	444	0.12
313	0.45	344	17.31				

3β-ACETOXY-5α-ERGOST-7,8-22,23-DIENE

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
50	1.84	78	4.98	106	10.86	134	8.77
51	1.02	79	38.54	107	57.68	135	19.01
52	0.60	80	6.30	108	13.49	136	2.90
53	8.78	81	100.0	109	35.60	137	6.78
54	1.32	82	20.03	110	4.00	138	0.99
55	87.02	83	29.48	111	2.99	139	0.24
56	4.74	84	2.63	112	0.51	140	0.24
57	23.61	85	1.53	113	0.24	141	2.26
58	0.90	86	0.14	114	0.09	142	2.24
59	2.84	87	0.09	115	3.52	143	10.26
60	7.23	88	0.06	116	2.15	144	4.02
61	0.78	89	0.31	117	14.11	145	28.97
62	0.29	90	0.15	118	6.02	146	8.71
63	0.62	91	43.13	119	30.62	147	35.86
64	0.38	92	8.84	120	9.73	148	6.30
65	3.44	93	47.59	121	26.91	149	10.81
66	1.53	94	19.78	122	6.56	150	4.50
67	45.55	95	50.15	123	14.80	151	4.53
68	6.75	96	7.37	124	3.85	152	2.51
69	99.40	97	6.61	125	16.46	153	0.71
70	7.75	98	1.41	126	0.86	154	0.69
71	10.20	99	0.47	127	1.23	155	2.39
72	0.62	100	0.08	128	7.27	156	1.58
73	0.06	101	0.15	129	7.54	157	8.84
74	0.36	102	0.30	130	3.49	158	3.47
75	0.20	103	1.96	131	20.80	159	22.09
76	0.24	104	2.30	132	7.01	160	5.28
77	12.63	105	56.40	133	30.75	161	25.14

3β -ACETOXY-5α -ERGOST-7,8-22,23-DIENE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
162	4.09	190	0.84	218	0.56	246	0.45
163	3.64	191	0.48	219	0.44	247	0.33
164	3.68	192	0.18	220	0.24	248	0.11
165	1.20	193	0.23	221	0.18	249	0.02
166	0.39	194	0.15	222	1.05	250	0.03
167	0.80	195	0.44	223	0.66	251	0.36
168	0.53	196	0.27	224	0.35	252	0.78
169	1.94	197	2.80	225	1.85	253	19.14
170	1.08	198	1.25	226	1.46	254	5.64
171	6.17	199	8.24	227	7.94	255	60.99
172	2.84	200	2.75	228	13.91	256	13.30
173	11.66	201	8.96	229	29.35	257	2.80
174	2.74	202	2.24	230	5.34	258	0.65
175	8.83	203	2.05	231	1.40	259	1.01
176	1.68	204	1.53	232	0.35	260	0.93
177	1.23	205	0.80	233	0.25	261	1.23
178	0.57	206	0.30	234	0.11	262	0.27
179	0.80	207	0.71	235	0.08	263	0.03
180	0.39	208	0.18	236	0.03	264	0.06
181	0.71	209	0.39	237	0.45	265	0.20
182	0.60	210	0.27	238	0.36	266	0.15
183	1.99	211	3.49	239	4.86	267	2.11
184	1.01	212	1.62	240	2.59	268	0.87
185	6.35	213	20.93	241	10.66	269	1.19
186	2.56	214	4.59	242	2.30	270	0.65
187	8.72	215	6.15	243	2.05	271	2.33
188	2.23	216	1.13	244	0.42	272	1.29
189	5.04	217	0.80	245	0.51	273	7.50

3β-ACETOXY-5α-ERGOST-7,8-22,23-DIENE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance		M/e	% Abundance
274	2.80	309	1.56	344	0.78		384	0.12
275	0.93	310	0.47	345	0.08			
276	0.15	311	0.44				395	0.11
277	0.06	312	1.08	351	0.24		396	0.30
278	0.05	313	69.13	352	0.14		397	5.90
279	0.12	314	19.90	353	0.12		398	2.20
280	0.12	315	20.28	354	0.08		399	0.47
281	2.47	316	2.74	355	0.60		400	0.07
282	2.96	317	0.62	356	0.87			
283	3.41			357	0.20		408	0.06
284	0.90	322	0.03	358	0.06	m.s.	409	0.15
285	0.90	323	0.35				410	0.56
286	0.93	324	0.12	363	0.18		411	0.36
287	1.61	325	0.30	364	0.15		412	0.11
288	18.11	326	0.68	365	8.30			
289	3.90	327	3.26	366	2.50		423	0.18
290	0.72	328	1.97	367	0.48		424	0.14
		329	0.41	368	0.12		425	9.16
295	0.89	330	0.06	369	1.10		426	2.68
296	0.48			370	0.48		427	0.54
297	0.36	336	0.06	371	0.08		428	0.11
298	0.39	337	5.59					
299	6.59	338	1.68	378	0.33		438	0.48
300	3.81	339	0.51	379	0.45		439	0.50
301	2.39	340	0.20	380	12.83		440	34.31
302	0.81	341	2.93	381	2.71		441	11.39
303	0.18	342	14.35	382	0.93		442	2.24
		343	4.36	383	0.45		443	0.30
							444	0.05

5α-ERGOST-14,15-22,23-DIENE-3β -OL

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
50	2.19	77	9.17	104	2.68	131	19.06
51	2.94	78	4.30	105	44.85	132	6.33
52	2.24	79	31.38	106	12.18	133	24.36
53	8.83	80	6.80	107	52.45	134	8.66
54	2.25	81	77.95	108	11.41	135	18.92
55	94.15	82	23.21	109	25.22	136	3.05
56	8.17	83	25.22	110	3.38	137	4.05
57	30.09	84	3.75	111	4.69	138	1.05
58	2.61	85	2.50	112	0.80	139	0.52
59	1.45	86	4.71	113	0.58	140	0.44
60	0.31	87	0.94	114	0.31	141	2.55
61	0.63	88	1.31	115	4.61	142	2.05
62	0.69	89	1.57	116	2.91	143	7.15
63	3.04	90	0.47	117	13.79	144	3.59
64	3.26	91	46.86	118	4.97	145	19.63
65	4.88	92	12.43	119	28.37	146	14.18
66	2.17	93	52.30	120	10.91	147	52.16
67	31.96	94	19.35	121	30.38	148	9.68
68	4.38	95	39.98	122	5.81	149	13.68
69	100.0	96	6.06	123	10.77	150	4.36
70	9.30	97	7.48	124	11.84	151	10.62
71	13.23	98	1.51	125	21.20	152	2.71
72	1.10	99	1.10	126	0.61	153	1.15
73	0.65	100	0.42	127	0.30	154	0.72
74	1.75	101	1.45	128	4.44	155	1.90
75	2.17	102	1.81	129	6.86	156	1.35
76	2.61	103	3.24	130	3.92	157	5.15

5α-ERGOST-14,15-22,23-DIENE-3β-OL (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
158	2.52	185	4.80	212	1.10	239	4.03
159	16.75	186	1.89	213	6.89	240	1.61
160	7.49	187	5.95	214	2.16	241	4.12
161	40.70	188	1.68	215	7.24	242	1.29
162	5.57	189	6.20	216	1.83	243	1.10
163	6.01	190	1.23	217	4.63	244	3.13
164	5.48	191	1.31	218	3.12	245	2.85
165	2.27	192	0.63	219	1.42	246	12.89
166	0.66	193	0.27	220	0.47	247	5.18
167	1.00	194	0.24	221	0.19	248	0.82
168	1.26	195	0.58	222	0.25	249	0.68
169	1.68	196	0.28	223	0.54	250	0.16
170	0.94	197	1.81	224	0.31	251	0.50
171	4.52	198	0.74	225	0.91	252	0.63
172	2.30	199	5.22	226	0.69	253	5.75
173	9.73	200	2.08	227	3.68	254	18.77
174	3.07	201	5.13	228	5.92	255	26.80
175	6.69	202	1.43	229	22.07	256	5.67
176	1.27	203	2.83	230	4.53	257	9.85
177	2.01	204	2.55	231	5.04	258	3.05
178	1.23	205	1.46	232	2.47	259	3.15
179	1.09	206	1.29	233	5.78	260	0.88
180	0.31	207	0.42	234	1.07	261	0.19
181	0.72	208	0.36	235	0.31		
182	0.47	209	0.46	236	0.28	267	0.68
183	1.64	210	0.22	237	0.79	268	0.69
184	0.79	211	2.47	238	0.65	269	3.59

5α-ERGOST-14,15-22,23-DIENE-3β-OL (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
270	15.02	299	13.33	330	0.19	368	0.36 m.s.
271	21.64	300	4.41			369	0.52
272	64.77	301	0.77	337	1.57	370	0.30
273	33.10	302	0.27	338	0.42		
274	5.50			339	0.41	376	0.14
275	0.57	307	0.19	340	0.20	377	0.13
		308	0.15	341	0.71	378	0.35
279	0.16	309	5.09	342	0.19	379	0.46
280	0.06	310	1.62			380	8.12
281	3.67	311	0.69	351	0.25	381	4.66
282	1.12	312	0.35	352	0.20	382	1.40
283	0.98	313	1.13	353	1.21	383	10.72
284	0.36	314	0.47	354	0.36	384	3.37
285	1.37	315	0.25	355	2.80	385	0.57
286	1.31	316	0.16	356	0.69		
287	0.63			357	0.14	394	0.41
288	0.09	323	0.35			395	0.22
		324	0.13	363	0.38	396	5.53
294	0.06	325	4.36	364	0.20	397	1.98
295	0.63	326	1.92	365	4.67	398	23.93
296	0.35	327	15.05	366	1.43	399	7.38
297	1.86	328	4.09	367	0.57	400	1.29
298	1.24	329	0.54				

3β-ACETOXY-5α-ERGOST-14,15-22,23-DIENE

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
50	3.27	77	9.88	104	1.67	131	9.84
51	5.25	78	5.30	105	21.35	132	3.71
52	1.85	79	18.42	106	8.02	133	13.36
53	6.23	80	5.21	107	25.22	134	5.32
54	2.58	81	32.40	108	7.57	135	8.02
55	42.52	82	17.33	109	14.43	136	2.27
56	17.36	83	14.16	110	3.55	137	3.12
57	16.75	84	5.82	111	3.50	138	1.76
58	5.81	85	4.22	112	1.41	139	0.84
59	1.46	86	0.95	113	1.14	140	0.87
60	4.64	87	1.25	114	0.61	141	1.75
61	1.62	88	0.56	115	2.73	142	1.60
62	1.16	89	0.87	116	1.61	143	3.91
63	3.04	90	0.57	117	6.82	144	2.56
64	3.13	91	21.33	118	2.94	145	11.44
65	3.28	92	6.99	119	15.11	146	7.68
66	1.76	93	31.14	120	7.10	147	23.97
67	20.61	94	16.46	121	17.23	148	6.01
68	19.53	95	22.39	122	5.87	149	5.74
69	38.59	96	4.96	123	7.04	150	2.52
70	7.71	97	6.21	124	16.53	151	6.23
71	9.39	98	1.92	125	6.05	152	1.54
72	1.23	99	1.41	126	3.32	153	0.73
73	1.44	100	0.79	127	5.97	154	0.87
74	2.85	101	1.70	128	0.43	155	1.38
75	2.22	102	3.21	129	8.32	156	0.97
76	1.77	103	1.99	130	2.17	157	3.63

3β-ACETOXY-5α-ERGOST-14,15-22,23-DIENE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
158	2.13	185	2.50	212	0.72	239	2.21
159	10.46	186	1.18	213	4.37	240	1.03
160	5.07	187	4.02	214	1.47	241	2.72
161	15.58	188	1.56	215	6.17	242	1.13
162	4.02	189	2.77	216	1.84	243	1.32
163	4.08	190	1.03	217	6.36	244	1.13
164	4.82	191	0.97	218	4.39	245	1.36
165	1.98	192	0.70	219	1.61	246	6.41
166	0.92	193	0.64	220	0.76	247	1.98
167	0.68	194	0.49	221	0.41	248	0.56
168	0.74	195	0.62	222	0.46	249	0.33
169	1.30	196	0.66	223	0.48	250	0.46
170	0.81	197	1.27	224	0.44	251	0.69
171	2.88	198	0.85	225	0.97	252	0.63
172	1.46	199	2.97	226	0.77	253	1.84
173	5.20	200	1.34	227	1.96	254	3.82
174	2.14	201	2.96	228	2.37	255	17.63
175	4.42	203	1.39	229	3.35	256	5.72
176	2.95	203	2.75	230	1.34	257	2.83
177	1.77	204	1.77	231	3.36	258	2.17
178	0.96	205	1.26	232	1.73	259	1.18
179	1.07	206	0.86	233	1.12	260	0.81
180	0.71	207	0.61	234	0.55	261	0.66
181	0.70	208	0.53	235	0.34	262	0.45
182	0.70	209	0.48	236	0.44	263	0.29
183	1.29	210	0.54	237	0.49	264	0.55
184	0.72	211	1.17	238	0.51	265	0.82

3β-ACETOXY-5α-ERGOST-14,15-22,23-DIENE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
266	0.53	293	0.33	320	0.27	358	0.48
267	0.83	294	0.33	321	0.26		
268	0.57	295	0.54	322	0.26	360	0.38
269	1.19	296	0.52	323	0.36		
270	1.74	297	0.68	324	0.28	363	0.33
271	2.06	298	6.14	325	0.51	364	0.32
272	5.10	299	3.16	326	0.48	365	2.33
273	2.63	300	3.06	327	1.21	366	0.84
274	3.76	301	2.47	328	0.83	367	0.65
275	8.35	302	1.23	329	0.79	368	0.64
276	1.94	303	0.52	330	0.51	369	21.62
277	0.57	304	0.37			370	7.52
278	0.40	305	0.23	337	10.01	371	1.43
279	0.42	306	0.25	338	0.49	372	0.42
280	0.43	307	0.36	339	0.54		
281	1.36	308	0.33	340	0.39	378	0.47
282	0.77	309	2.77	341	12.80	379	0.69
283	1.96	310	1.04	342	4.95	380	1.24
284	0.78	311	1.74	343	1.16	381	0.84
285	1.09	312	1.32	344	1.31	382	0.82
286	1.11	313	3.38	345	0.46	383	0.51
287	0.67	314	100.00			384	0.37
288	2.04	315	31.65	353	0.34	385	0.26
289	0.66	316	14.92	354	0.36	386	0.46
290	0.45	317	3.22	355	0.94	387	0.25
291	0.30	318	0.77	356	0.63	388	0.23
292	0.36	319	0.31	357	0.47		

3β-ACETOXY-5α-ERGOST-14,15-22,23-DIENE (Contd.)

M/e	% Abundance	M/e	% Abundance	M/e	% Abundance	M/e	% Abundance
394	0.27	410	0.29	425	3.77	438	1.34
395	0.42	411	0.32	426	1.34	439	0.92
396	0.67	412	0.38	427	0.53	440	29.33
397	3.43	413	0.25	428	0.26	441	10.69
398	1.92	414	0.27			442	2.94
399	0.57			436	0.26	443	0.71
400	0.32	423	0.47	437	0.25	444	0.27
		424	0.35				

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