The Constitution of Primycin. Part II.[1,2] The Mass Spectra of the Secoprimycins

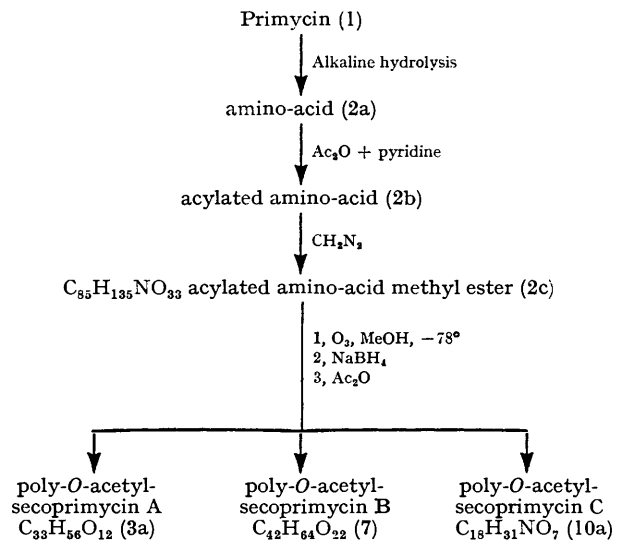
By D. E. Fergus Gracey, Lubomir Baczynskyj, Trevor I. Martin, and David B. MacLean,* Department of Chemistry, McMaster University, Hamilton, Ontario, Canada L8S 4M1

The structures of secoprimycins A and C have been established by application of high resolution mass spectrometry to the secoprimycins themselves, to their simple derivatives, and to products of their chemical degradation. Similarly the carbon skeleton and the oxygenation pattern of secoprimycin B have been established, but the position of the arabinose unit associated with this fragment has been only tentatively assigned. The structure of a minor component formed along with the secoprimycins in some experiments has also been resolved by mass spectrometry. A structure is proposed for the amino-ester that yields the secoprimycins on ozonolysis and reduction.

In previous papers [1,2] the properties and functionality of primycin were discussed and the derivation of secoprimycins A, B, and C was described. The structures of the secoprimycins were deduced by application of chemical degradation, n.m.r. spectroscopy, and mass spectrometry. Here a detailed account of the mass spectrometry of the secoprimycins and their degradation products is given.

In the past decade mass spectrometry has been used extensively in the structural investigation of antibiotics of the macrolide and polyene groups. The exact molecular weight of many antibiotics has been established only in this way. For mass spectrometric study these normally involatile compounds were first converted into their volatile trimethyl silyl ethers or into acetate esters. Methods have also been developed for removal of hydroxy-groups from the molecules in order that they might be converted into hydrocarbons or into saturated fatty acids. Mass spectrometric examination of such derivatives has led to establishment of the carbon framework. Valuable structural information has often been obtained from a study of the fragmentation upon electron impact of these molecules in the form of volatile derivatives or of volatile degradation products. In most cases however, the structural solution depended upon extensive chemical degradation and n.m.r. studies. A review of the foregoing methods has recently appeared.[3]

The secoprimycins † are derived from primycin by the procedure summarized in Scheme 1 and discussed thoroughly before.[1] All the compounds examined in this study are polyhydroxy-compounds, like the secoprimycins, or polyacetates. Examination of the polyhydroxy-compounds was hampered by their low volatility, and the absence of molecular ions because of the loss of water either thermally or upon electron impact. Frequently, primary fragment ions ‡ were absent although ions corresponding to loss of one or more molecules of water from the primary fragment were usually present. In the case of the acetates the molecular ions were usually of low intensity and the spectra were always characterized by the presence of ions resulting from the successive losses of acetic acid from the molecular ion. Ions resulting from successive losses of acetic acid from the primary fragment ions were also invariably observed and were often more intense than the primary fragment. Isobaric ions of the same nominal mass were often present so that high resolution mass spectrometry was essential in the analysis of the mass spectra.



SCHEME 1 The derivation of the secoprimycins

In the preceding paper the chemical degradation of secoprimycin A was described. These reactions are summarized in Scheme 2.§ In the development of the structure (3a) of poly-*O*-acetylsecoprimycin A from the mass spectrometric data the discussion begins with the

† The formula numbers assigned to the secoprimycins, their derivatives, and their degradation products are identical with those used in Part I.[1]

‡ Primary fragment ions in the present context are those formed by cleavage α to an oxygen function or at a branch in the carbon chain.

§ The number and nature of the functional groups is specified for each compound. These data come from the chemical and spectroscopic studies reported in Part I.

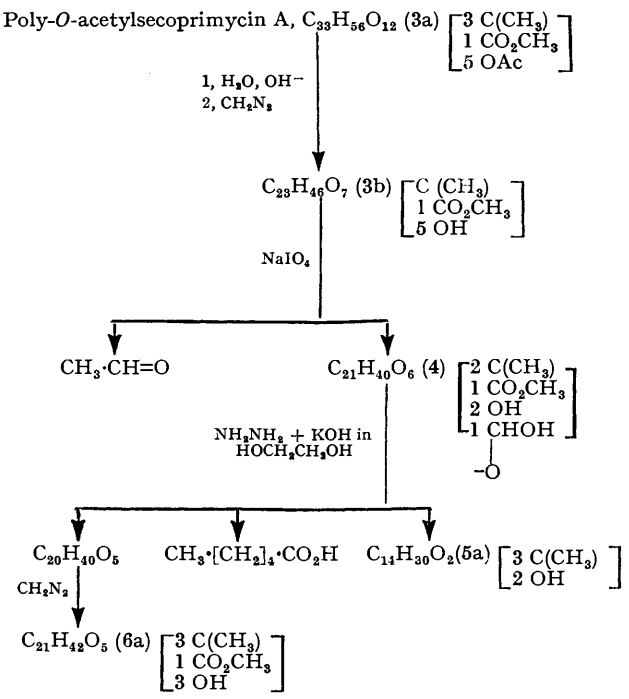
[1] Part I, J. Aberhart, R. C. Jain, T. Fehr, P. de Mayo, and I. Szilagyi, preceding paper.

[2] Part of the contents of this paper was published in preliminary form: J. Aberhart, T. Fehr, P. de Mayo, O. Motl, L. Baczynskyj, D. E. F. Gracey, D. B. MacLean, and I. Szilagyi, *J. Amer. Chem. Soc.*, 1970, **92**, 5816.

[3] K. L. Rinehart and G. E. Van Lear, in 'Biochemical Applications of Mass Spectrometry,' ed. G. R. Waller, Wiley-Interscience, New York, 1972, p. 449.

diol (5a), the least complex member of this group of compounds.

When the ester aldehyde (4) was subjected to Huang-Minlon reduction the reduction product (6a) was obtained only in minor amount. The major product, the crystalline diol (5a), was accompanied by

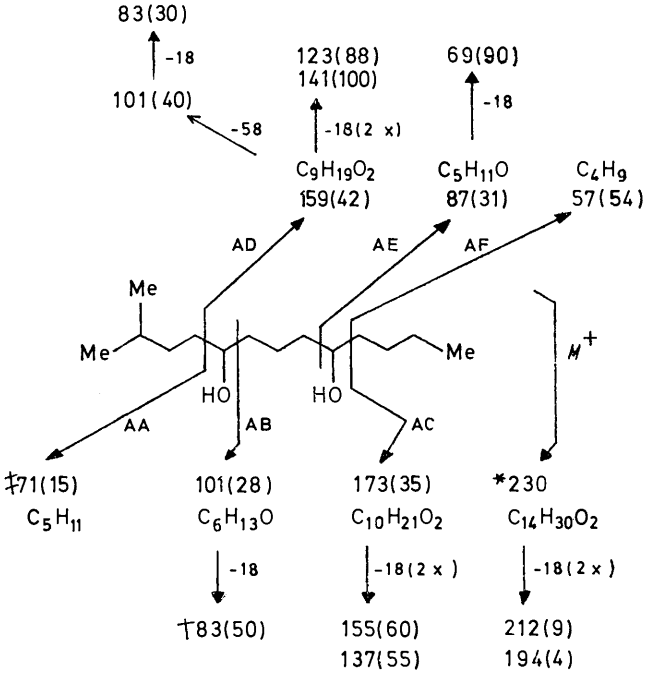


SCHEME 2 Degradation of secoprimycin A

hexanoic acid in equimolar amount, and is apparently formed in a retro-aldol condensation reaction. The mass spectrum of (5a) shows only a very weak molecular ion but has an ion of low intensity at $M - 1$ of composition $C_{14}H_{29}O_2$ and ions at *m/e* 212 and 194 corresponding, respectively, to loss of one and two molecules of water from the molecular ion. The fragmentation of (5a) is outlined in Scheme 3. These and subsequent Schemes do not and are not intended to account for all the fragment ions in the spectrum, but include those ions that define the structure of the compound. Ions AB and AD share a common CHOH group but otherwise constitute different portions of the molecule. The same applies to ions AC and AE. Ions AA and AD together comprise the complete molecule as do ions AF and AC. Thus the mass spectrum of (5a) defines the positions of the hydroxy-groups in the carbon chain and the mass of the hydrocarbon fragments attached to the $-CH(OH)-$ groups at either end of the chain as well as the mass of the hydrocarbon fragment between the sites of the OH groups. The n.m.r. spectrum shows three *C*-methyl groups: two are secondary and in an isopropyl group, and the third is primary.[1] Thus the carbon chain carries a single methyl branch at the penultimate carbon atom. It is not known from these data, however, whether the two methyl groups are associated with the C_4 or the C_5 alkyl fragment.

The second product of the treatment of (4) under Huang-Minlon conditions was an acid, isolated and examined as its methyl ester (6a). Compound (6a) has the same carbon content as methyl hexanoate and (5a) combined. A molecular ion was not present in the spectrum of (6a) but from an analysis of the fragment ions it was possible to deduce the molecular structure.

The fragmentation of (6a) is outlined in Scheme 4. The ions of *m/e* 87 and 159 are identical in composition with the ions AE and AD in the spectrum of (5a) indicating that the same part structure is present in both molecules, but ions corresponding to AB and AC of (5a) are weak or absent in the spectrum of (6a). Thus there was no ambiguity in elaborating structure (6a) from methyl hexanoate and (5a). Only the structure shown is compatible with the chemical data and with the fragmentation of the molecule discussed thus far. It is also compatible with the n.m.r. data, that show the



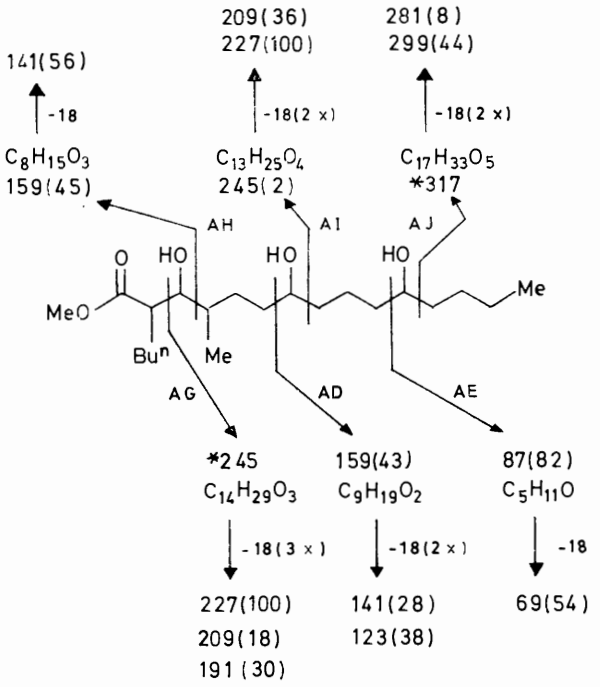
Base and most intense peak *m/e* 141.

SCHEME 3 Fragmentation of the diol (5a)

* In this and in subsequent schemes an asterisk indicates that the ion in question was absent or its intensity was so low that its composition could not be determined by high resolution mass spectrometry. Therefore its composition is inferred. † The compositions of all ions derived from the primary fragment ions were determined by high resolution mass measurements and the losses shown refer to the following compositions: 1, H; 18, H_2O; 42, C_2H_2O; 58, C_4H_{10}; 59, $C_2H_3O_2$; 60, $C_2H_4O_2$. ‡ The first number refers to the *m/e* ratio of the ion in question; the number in parentheses refers to its relative intensity. This notation is used throughout.

molecule has one secondary and two primary *C*-methyl groups but no isopropyl group. The other ions shown in Scheme 4 corroborate the foregoing conclusions.

Although an ion corresponding to the primary fragment AG of Scheme 4 is not present, the spectrum shows ions



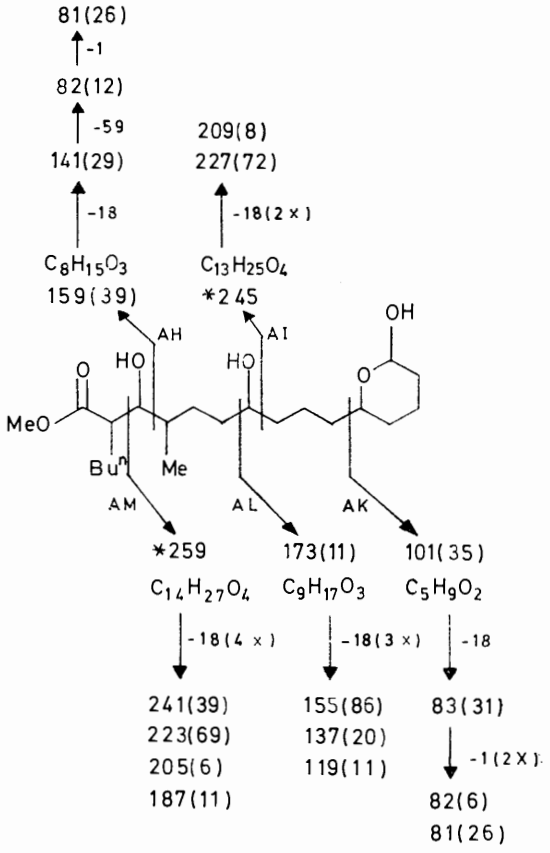
Base and most intense peak *m/e* 227.

SCHEME 4 Fragmentation of the ester (6a)

corresponding in composition to AG — H_2O, AG — $2H_2O$, and AG — $3H_2O$. In Scheme 4 ions AH and AI are present and although AJ is absent AJ — H_2O and AJ — $2H_2O$ have been observed. The presence of these ions serves to locate the sites of the hydroxy-groups in the carbon chain since fragments AG and AH, AD and AI, and AE and AJ share with one another a common $-CH(OH)-$ group but otherwise constitute different portions of the molecule.

The precursor of (6a) is the ester aldehyde (4), which exists in the form of a hemiacetal [1] and is so represented in the formula of Scheme 5. The n.m.r. spectrum of (4) indicates the presence of one primary and one secondary *C*-methyl group. Thus the potential aldehyde function must occupy the same site as one of the two primary *C*-methyl groups of (6a). The mass spectrum shows that ions of the same composition as AH and AI of Scheme 4 are present in the spectrum of (4) (Scheme 5) and therefore the aldehyde function must be situated at the terminus of the main chain and not in the butyl side chain. An ion corresponding to AJ of Scheme 4 is absent in the spectrum of (4) suggesting that the molecule undergoes fragmentation in the hemiacetal form and not in the open-chain tautomeric form. Fragmentation of the molecule from the hemiacetal end of the chain is shown in Scheme 5. Ions AK and AL are both observed in the spectrum and although AM is not present, ions corresponding in composition to loss of H_2O from AM are present as shown. Fragments AI and AL share a common $-CH(OH)-$ group as do AH and AM and in the case of each pair account for the complete molecule and define the sites of the hydroxy-groups.

Compound (4) and acetaldehyde were the products obtained by oxidation of (3b) with periodate, from which it follows that the structure of (3b) must be that shown in Scheme 6. Because of its five hydroxy-groups, this compound has low volatility and a mass spectrum was difficult to obtain. The first recognizable ion appeared at *m/e* 398 ($C_{23}H_{42}O_5$) corresponding to $M - 2H_2O$. In the region below *m/e* 300 there were, however, sufficient ions to characterize the structure. Ions expected by cleavage on either side of the hydroxy-groups are shown in Scheme 6. Ions AH and AI — H_2O are present, as they are in the spectrum of (4), but other ions resulting from cleavage adjacent to the hydroxy-groups in proceeding from the methoxycarbonyl group along the chain were not observed. There is presented in Scheme 6, however, a more revealing picture from the structural viewpoint. The presence of ions AN and AO confirms



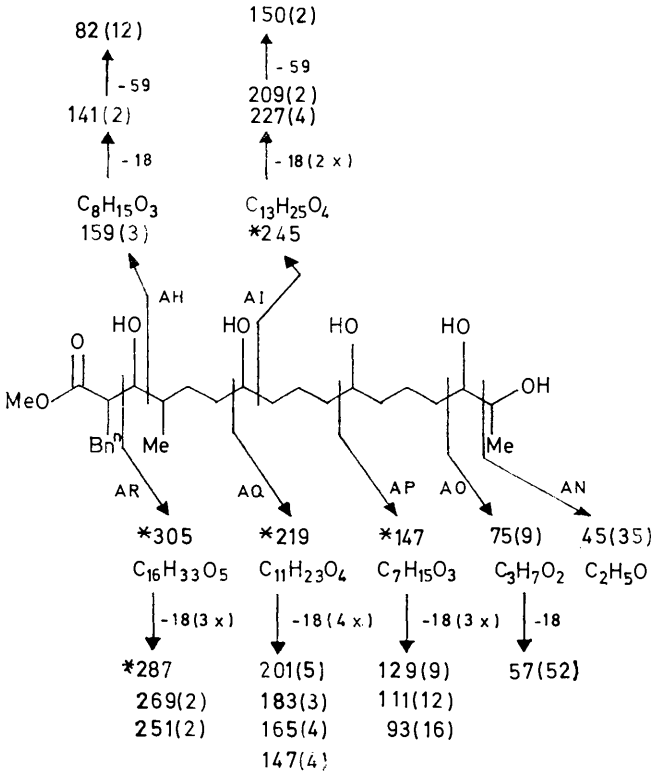
Base and most intense peak *m/e* 55.

SCHEME 5 Fragmentation of the ester aldehyde (4)

the structural features established in the periodate oxidation. The presence of ions resulting from loss of one molecule of water from fragments AP and AQ and

of two molecules of water from AR corroborate the previous findings concerning the structure of the molecule.

The final compound to be examined in this series is poly-*O*-acetyl secoprimycin A (3a). In its spectrum this compound has a weak molecular ion, but it was possible to determine its exact mass by high resolution measurement and thereby establish its composition as $C_{33}H_{56}O_{12}$. The spectrum of (3a) was, however, very complex and was characterized by the presence of ions resulting from five successive losses of the elements of acetic acid from the molecular ion. Similarly the primary fragment ions resulting through cleavages



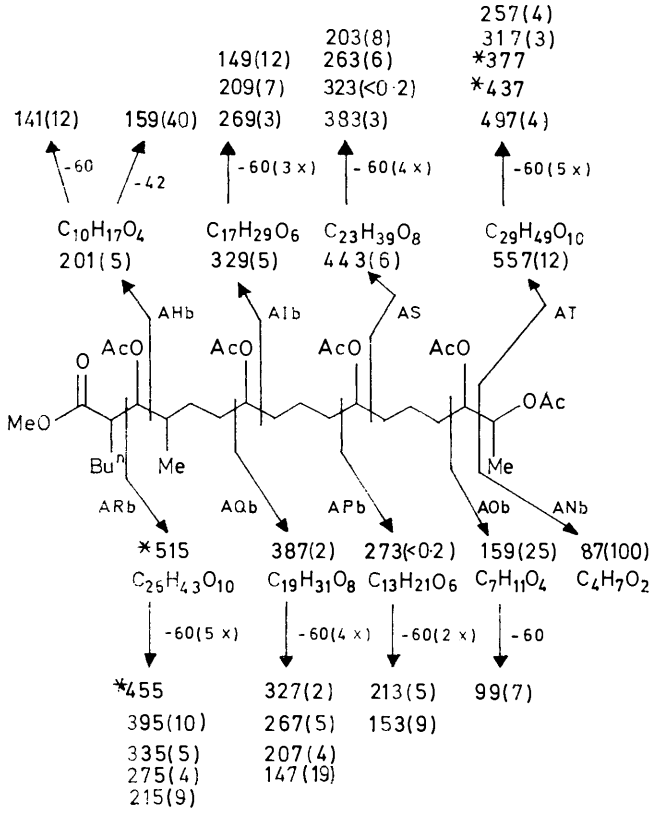
Base and most intense peak *m/e* 74.

SCHEME 6 Fragmentation of the hydroxy-ester (3b)

adjacent to the acetoxy-groups also lost acetic acid readily. Where the primary ions were not observed in the spectrum, ions resulting from the loss of one or more molecules of acetic acid from the primary fragments were always present and served to fix the sites of the acetoxy-groups in the chain. These fragmentations are summarized in Scheme 7.* A comparison of the spectra of (3a) and (3b) shows that the primary ions of high molecular weight derived by cleavages adjacent to the oxygen functions are more likely to be observed in the spectrum of the polyacetate than in that of the polyol.

* An acetylated form of a previously discussed ion is denoted by the same letter but given the suffix b to denote the common origin of the two types from the carbon chain.

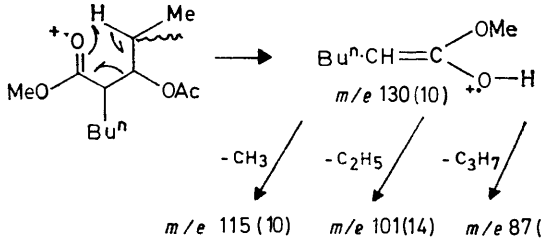
The fragmentation depicted in Scheme 7 substantiates the structural conclusions already made. The Scheme is self-explanatory.



Base peak *m/e* 87.
Most intense peak *m/e* 43 (300%).

SCHEME 7 Fragmention of poly-*O*-acetylsecoprimycin A (3a)

There are two fragmentation processes of (3a) involving the methoxycarbonyl group, both proceeding by the McLafferty rearrangement.[4] The formation of the ion at *m/e* 130 and its further transformations may be rationalized as shown in Schemes 8 and 9. The ion of

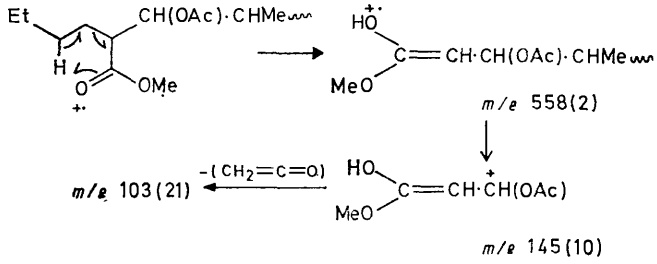


SCHEME 8 McLafferty rearrangement of poly-*O*-acetylsecoprimycin A (3a)

m/e 130 was shifted to *m/e* 133 when OCH_3 was replaced by OCD_3 and thus, there seems little ambiguity about

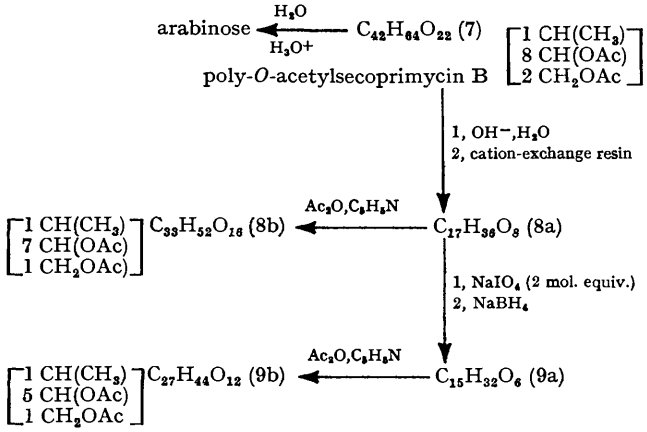
[4] H. Budzikiewicz, C. Djerassi, and D. H. Williams, 'Mass Spectrometry of Organic Compounds,' Holden-Day, San Francisco, 1967, p. 155.

the derivation of this fragment. Elimination of C_4H_8 in another McLafferty rearrangement leads to an ion of *m/e* 588 which may fragment further as shown (Scheme 9) to *m/e* 145 (shifted to *m/e* 148 in the trideuteriomethyl ester) and 103. Initially the observation of an ion at *m/e* 145 was misleading because it suggested that the structural unit, $-CH(OAc)\cdot CH_2\cdot OAc$ was present in



SCHEME 9

(3a). However, the periodate oxidation of (3b) which led to the recovery of acetaldehyde dispelled this notion.



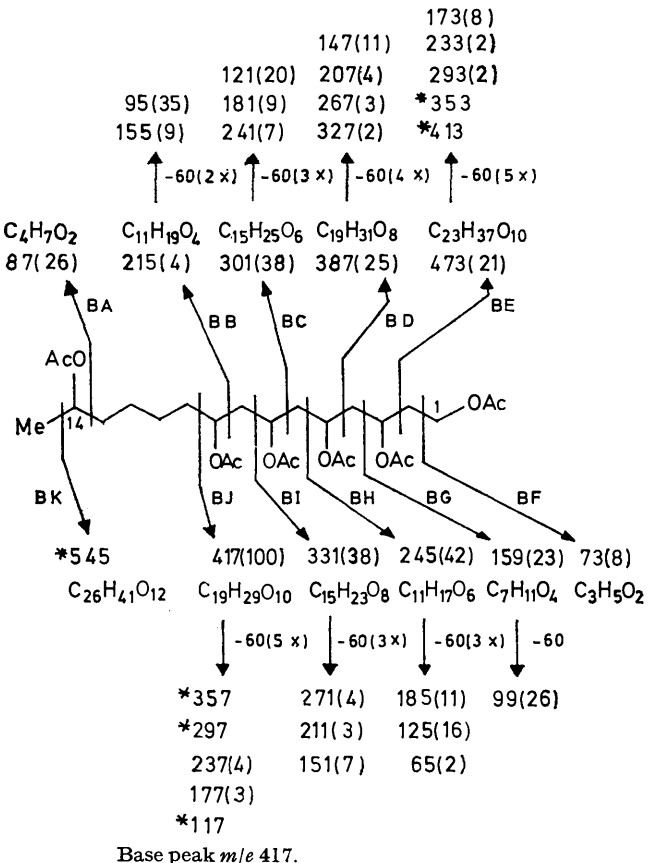
SCHEME 10 Degradation of secoprimycin B

The ion of *m/e* 130 and its further fragmentation products were also present in the spectra of (3b), (4), and (6a).

The fragment formed in the ozonolysis of the amino-ester (2c) originating from the centre of that molecule is the polyacetyl derivative of secoprimycin B (7).[1] It is the fragment of largest mass derived from the ozonolysis and that which carries the arabinose unit. The chemical transformations of secoprimycin B are described in Part I and are summarized here in Scheme 10. In the conversion of (7) into (8a) the ester functions and the arabinose were removed by hydrolysis. The polyol (8a) was acetylated to give the ester (8b) and also subjected to periodate oxidation and subsequent reduction yielding a new polyol (9a), which in turn was acetylated to give (9b).

Only the acetates of this series are reported here because the polyols gave poor spectra in most cases.

Compound (9b) is the simplest member of this series of acetates. Its n.m.r. spectrum showed that there were one primary acetate, five secondary acetates, and one secondary *C*-methyl group. In the absence of other functional groups it follows that (9b) must be a linear molecule with a $-CH_2\cdot OAc$ group at one terminus and a $CH_3\cdot CH(OAc)-$ group at the other. The fragmentation of (9b) and the other acetates of this series occurs characteristically at those carbons carrying the acetoxy-functions and may proceed from either end of the chain as demonstrated in the secoprimycin A series. In Scheme 11 the fragmentation of (9b) is outlined. The ion BA of *m/e* 87 was expected because of the n.m.r. data, and was found in the spectrum. Although its site

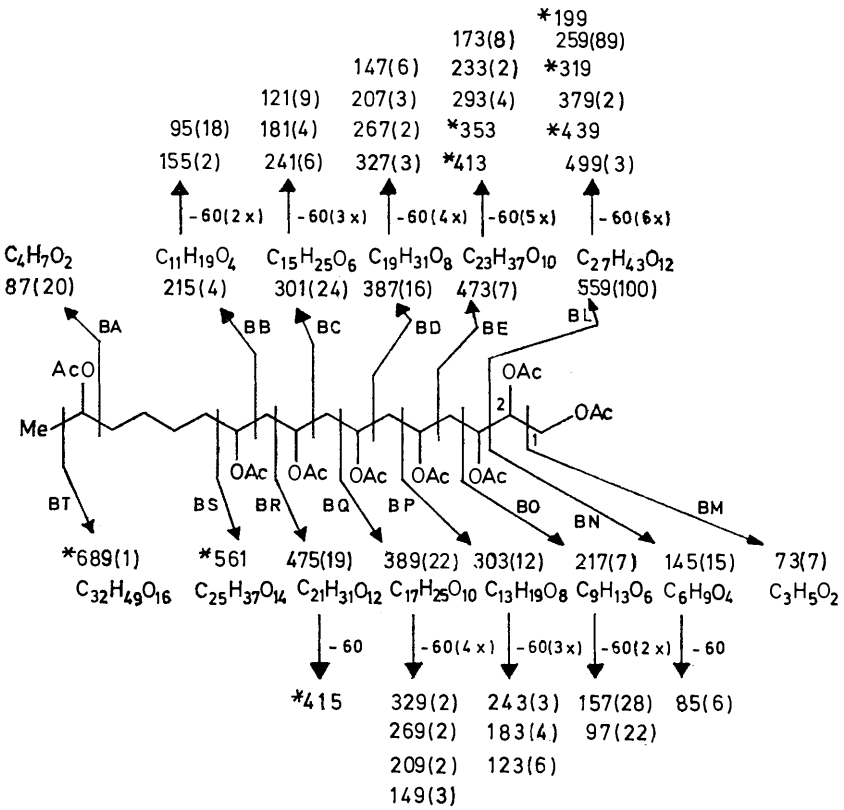


Base peak *m/e* 417.
Most intense peak *m/e* 43 (*ca.* 1100%)

SCHEME 11 Fragmentation of the polyacetate (9b)

of origin is probably as indicated it cannot be said that it is uniquely derived from this site, for the possibility of

its formation in a rearrangement is not ruled out. The presence of ion BB of *m/e* 215 shows that the first $-CH(OAc)-$ is separated from the second by a chain of four methylene groups. The recognition of ions BA and BB defines the structure of this molecule. The derivation of (9a) by periodate oxidation shows that vicinal glycol units are not present and therefore the only tenable structure of (9b) must be that written in Scheme 11. This structure is corroborated by the presence of ions BC, BD, and BE and by the molecular ion. Verification comes from a further examination of spectrum of (9b). The presence of these ions justifies the assignment of structure (8b) as shown, since in the alternative structure discussed above ions BA—BE would not be expected as primary fragment ions. The ion BL was also observed as well as a molecular ion but an ion resulting from cleavage between C-1 and C-2 was not observed. Verification of the structure of (8b) comes from further examination of Scheme 12. The primary ions BM—BR are all observed, as are BT and a molecular ion, but an ion corresponding to BS was not present. The sequence BM, BN, BO demonstrates



Base peak *m/e* 559.
Most intense peak *m/e* 43 (*ca.* 300%).

SCHEME 12 Fragmentation of the polyacetate (8b)

Scheme 11, which also shows fragmentation at the sites of the acetoxy-groups proceeding from the opposite end of the chain. Ions BF—BJ are of mass and composition consistent with the structure proposed.

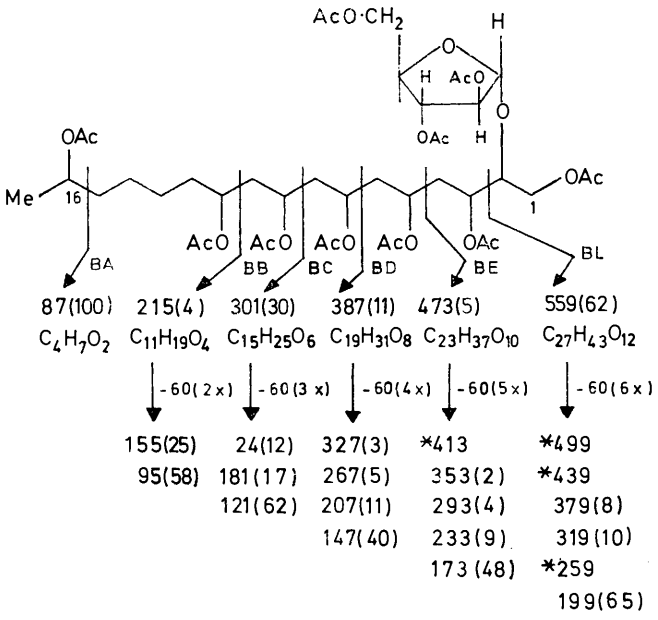
Compound (8a), from which (9a) was derived by periodate oxidation, has the carbon framework shown for (8b) in Scheme 12. The alternative structure in which the $-CH(OH)\cdot CH_2\cdot OH$ unit cleaved in the periodate reaction is joined to (9a) at C-14 rather than at C-1 is precluded on the basis of the n.m.r. data, which showed the presence of a single primary acetoxy-group in (8b), and on the mass spectral data cited below.

Scheme 12 shows the presence of ions corresponding in composition to BA—BE that were found in the conclusively the existence of a terminal three-carbon unit in which each of the carbon atoms bears an acetoxy-group. Thus from the mass spectrum it is possible to deduce the sites of the oxygen functions in the carbon chain.

Poly-*O*-acetylsecoprimycin B (7) is the last compound of this series to be examined. It has an acetylated arabinose unit in place of one of the acetoxy-groups of (8b). The presence of the arabinose unit has a marked effect on the fragmentation of this compound.

In Scheme 13 the fragmentation of the molecule at the acetoxy-functions proceeding from the methyl terminus of the chain is outlined. Ions corresponding in composition to BA—BE and to BL are observed.

Their presence demonstrates that the arabinose unit must be attached at C-1 or C-2. Because of the pronounced tendency of this molecule to lose the arabinose



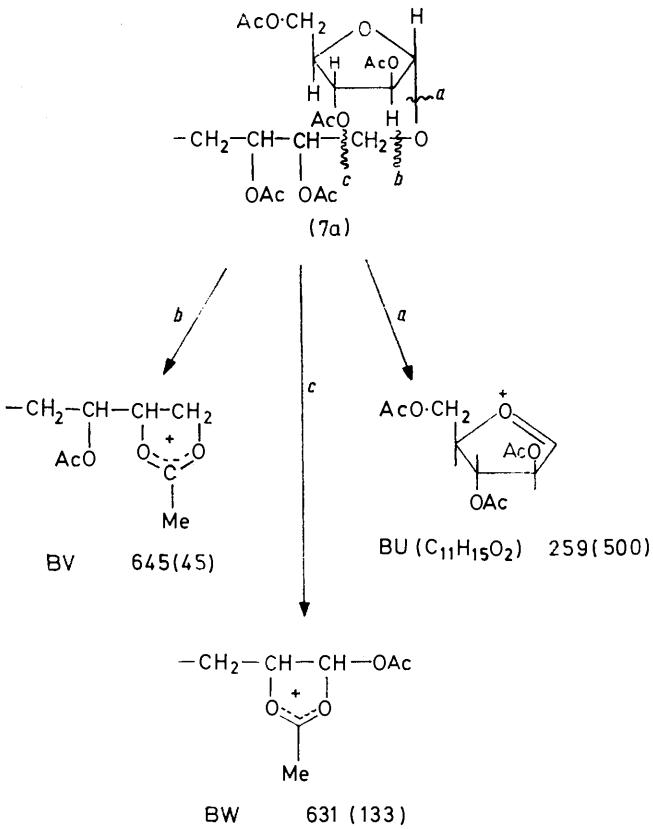
Base peak *m/e* 87.
Most intense peak *m/e* 259 ($C_{11}H_{15}O_7$) (*ca.* 500%)

SCHEME 13 Fragmentation of poly-*O*-acetylsecoprimycin B (7)

unit upon electron impact there is not present a complete series of ions resulting from cleavage at the acetoxy-groups when proceeding along the chain in the opposite direction from that of Scheme 13. An ion at *m/e* 73 ($C_3H_5O_2$) may have the same origin as BM (Scheme 12) or may arise from the CH_2·OAc group of the arabinose unit. An ion at *m/e* 361 ($C_{15}H_{21}O_{10}$) of low intensity corresponds in composition to a fragment containing two carbon atoms of the chain with an associated acetoxy-group and an acetylated arabinose unit. Unfortunately these data do not allow a unique assignment of the arabinose to C-1 or C-2. The spectrum of (7) has a very intense fragment ion (BU) at *m/e* 259 which is characteristic of an acetylated glycoside of a pentose. In the previous paper it was shown that the pentose was arabinose and in the following paper it will be shown that it is a furanoside. Ions typical of a tri-*O*-acetyl-arabinoside are observed at *m/e* 217, 199, 170, 157, 139, 120, 115, and 97.[5]

Two ions, BV and BW, of major interest are found in the high mass region of (7) at *m/e* 645 and 631, respectively. These correspond in composition to $C_{31}H_{49}O_{14}$ and $C_{30}H_{47}O_{14}$, resulting from the loss of $C_{11}H_{15}O_8$ and $C_{12}H_{17}O_8$, respectively, from the molecular ion. In the case of ion BV this loss corresponds in mass to the arabinose unit and its glycosidic oxygen atom and in the case of BW to this same unit plus a methylene group. The simplest explanation of this behaviour is illustrated in Scheme 14, in which only a partial structure is shown with the arabinose unit placed on the terminal carbon atom [structure (7a)]. Fission at *a* leads to BU, at *b* to BV, and *c* to BW. Chemical and n.m.r. evidence presented before, and in the following paper, favours attachment of the arabinose at C-2. The formation of BU and BV in the alternative structure in which the arabinose is attached to C-2 is unexceptional but the formation of an ion at *m/e* 631 requires a more complex fragmentation. Model studies are in progress in an attempt to clarify this apparent anomaly.

Poly-*O*-acetylsecoprimycin C ($C_{18}H_{31}NO_7$) (10a) is the smallest of the three fragments derived from the ozonolysis and subsequent reduction and acetylation of (2c), and the presence of nitrogen in this molecule identifies it as one of the two terminal fragments. Its spectrum, like those of (3a) and (7), has many ions of little structural significance resulting from successive losses of acetic acid and keten from the molecular ion as outlined in Scheme 15. The ions of structural significance are those derived by fission of the carbon chain at branching sites or, more importantly, at sites adjacent to the



SCHEME 14 Postulated fragmentation of structure (7a)

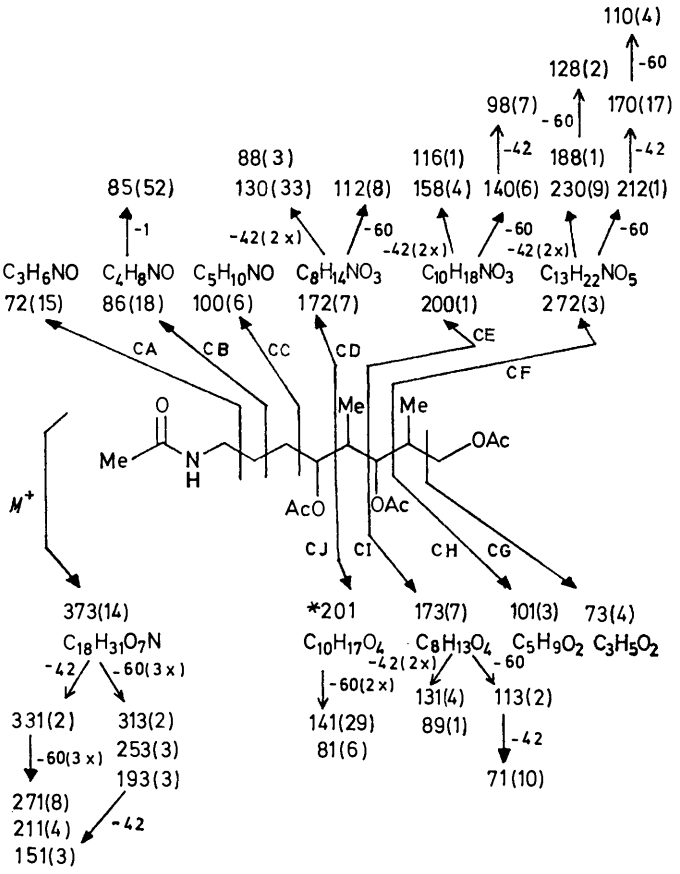
acetoxy-groups. These fragmentations are also outlined in Scheme 15. The pattern is familiar and needs

[5] D. C. De Jongh and K. Biemann, *J. Amer. Chem. Soc.*, 1963, **85**, 2289.

little explanation. The ions resulting from fission from the nitrogen end of the chain are more abundant than those from the acetoxy-end. However, the two fragmentation series overlap and in this way define unambiguously the sites of the acetoxy-functions.

The presence of ions CA—CD suggests strongly that there is an unbranched three-carbon chain emanating from nitrogen and that this unit separates the nitrogen atom from the first carbon atom carrying an acetoxy-group. The fact that the intensity of ion CB is more than twice that of CC militates against branching in the chain between nitrogen and the first acetoxy-group. The second carbon atom carrying an acetoxy-group is separated from the first by a unit of mass 28 and since there are two secondary *C*-methyl groups, as the n.m.r. data indicate, one of them must be located at this site and the unit of mass 28 must be a $-CH(CH_3)-$ group. The presence of ion CF locates the next carbon atom carrying an acetoxy-group. The arrangement of the three remaining carbon atoms, two of which must be in a $-CH(CH_3)-$ group, and the single acetoxy-group is apparent on further examination of Scheme 15. The presence of ions corresponding in composition to CG and CH is indicative of the structural unit $-CH(CH_3)\cdot CH_2\cdot OAc$ at the end of the chain remote from the nitrogen atom, and the presence of CI shows that this unit is joined to a $-CH(OAc)-$ group. Although an ion corresponding to CJ is not present, ions corresponding in composition to CJ — AcOH and CJ — 2AcOH are observed. Ions CF and CH together and ions CI and CE together make up the complete molecule; ions CF and CI share a common $-CH(OAc)-$ but otherwise constitute different portions of the molecule.

The most intense ion in the spectrum of this compound appears at *m/e* 43, as expected of a compound with four acetyl groups; the next most intense is that at *m/e* 85, C_4H_7NO. The origin of the latter is unclear although it may form simply by loss of H from *m/e* 86.



Base peak *m/e* 43.

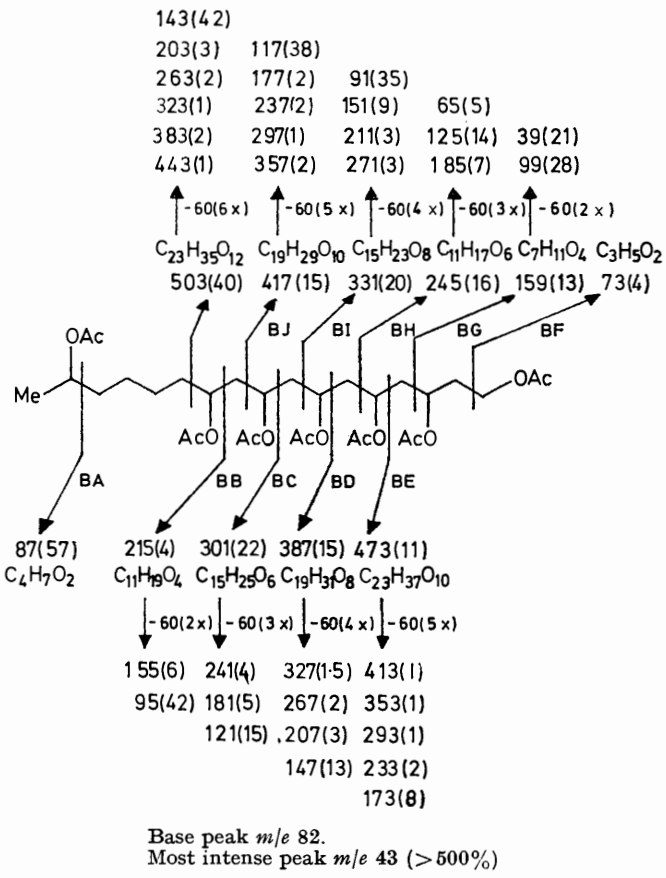
SCHEME 15 Fragmentation of poly-*O*-acetylsecoprimycin C(10a)

Other structures for secoprimycin C were considered but none fitted the mass spectral data as well as that discussed.

The minor product (12a) ($C_{31}H_{50}O_{14}$) was isolated in early ozonolysis experiments along with the secoprimycins. The composition of (12a) was established through high resolution mass measurement and its structure was deduced from an analysis of its spectrum.

There are ions in the spectrum * of the expected mass and composition resulting from the successive losses of seven molecules of acetic acid from the molecular ion.

The spectrum of (12a) is very similar to that of (9b), from which it differs in containing an additional $-CH(OAc)\cdot CH_2-$ group. Its fragmentation is outlined in Scheme 16. Ions corresponding in mass and composition to those found in the spectrum of (9b) are given the same designation (*cf.* Scheme 11). The presence of the additional $-CH(OAc)\cdot CH_2$ group is evident from the mass of the molecular ion and the presence of an ion at *m/e* 503. The fragmentations of (9b) and (12a) are so



Base peak *m/e* 82.
Most intense peak *m/e* 43 (>500%)

SCHEME 16 Fragmentation of the minor product (12a)

much alike that further discussion of (12a) is unnecessary. Formally, (12a) is derived from the acetate of (7) by replacing the arabinose unit, including its glycosidic oxygen atom, with a hydrogen atom, but the manner in which (12b) is formed in the reactions of Scheme 1 is not understood. It may indeed be derived from an impurity.

The amino-acid (2a) from which the secoprimycins were derived was too involatile for mass spectrometric examination but a derivative (2e) was suitable.[1] In (2e) the hydroxy-groups of (2a) have been methylated, the carboxy-group esterified, and the primary amino-group methylated and acetylated. The double bonds and the arabinose unit of primycin are still present but the lactone ring has been opened. It was pointed out before [1] that the spectrum of this compound suggested the presence of a molecular ion at *m/e* 1319. On the instrument used routinely in this investigation, however, a molecular ion was not observed but the spectrum was countable up to *m/e* 1100. Examination of the fragment ions gave useful structural information, as outlined in Scheme 17.

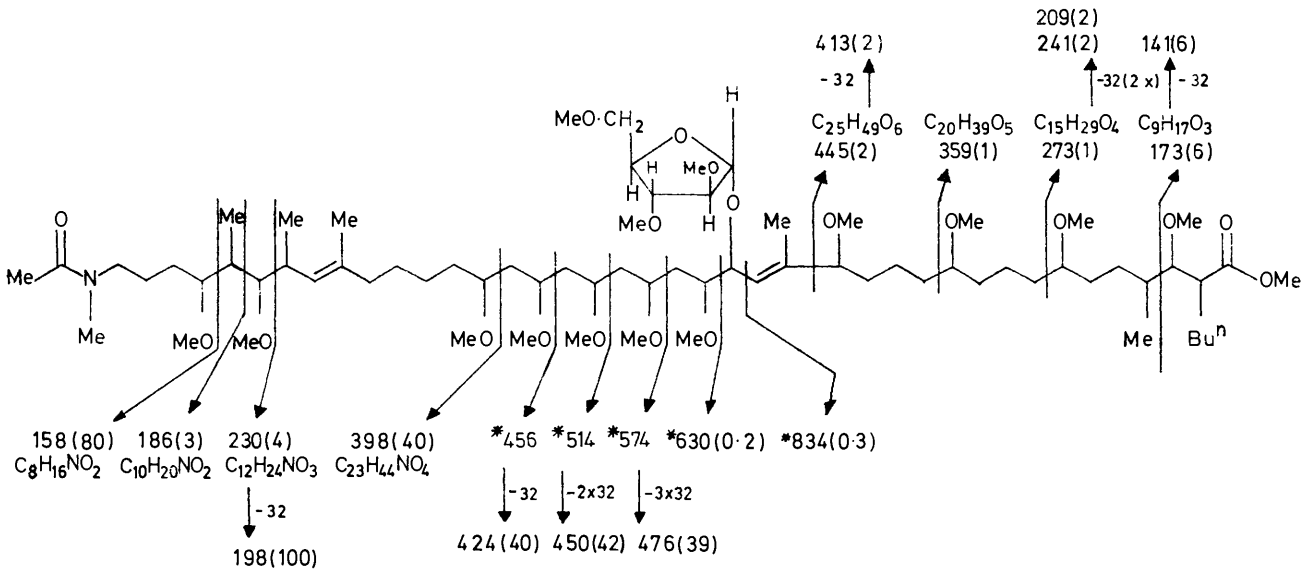
The ions at *m/e* 158 and 230 are analogues of the ions CD and CF of Scheme 15. The presence of these two ions shows that it is the hydroxy-group at C-8 of secoprimycin C that is generated in the ozonolysis and reduction of (2a). In (2e) therefore a double bond should be present at the eighth carbon atom from nitrogen. The next ion of structural significance is that at *m/e* 398. Since it is known [1] that secoprimycin B is derived from the centre of (2a) and therefore of (2e), the ion of *m/e* 398 must comprise all the carbon atoms of secoprimycin C together with some of those from secoprimycin B. The composition of this ion clearly shows that the orientation in the carbon chain of (2a) and (2e) of the fragment that yields secoprimycin B must be that shown in Scheme 17. The primary ions expected at *m/e* 456, 514, and 572 are absent, but ions corresponding to *m/e* 456 — CH_3OH, 514 — $2CH_3OH$, and 572 — $3CH_3OH$ are present and have the compositions expected. Ions that have not been mass-matched are present at *m/e* 630 and 834, as are ions corresponding to the loss of 1, 2, 3, and 4 molecules of methanol from each. The presence of these ions provides evidence for the location of the arabinose unit in the carbon chain of (2e) and by inference in secoprimycin B. Also one can infer that the hydroxy-groups at C-1 and C-16 of secoprimycin B were generated in the ozonolysis–reduction carried out on (2c).

Ions resulting from cleavage at the sites of the methoxy-groups proceeding from the end of the chain carrying the carboxy-group are observed at *m/e* 173, 273, 359, and 445. The observation of these ions verifies the locations assigned to the hydroxy-groups in secoprimycin A and implies that the oxygen function of secoprimycin A generated in the ozonolysis–reduction reactions of (2c) must be that at C-16.

The analysis of the spectrum of (2e) has shown the orientation in (2e) of the fragment that yields secoprimycin B, and has by inference identified those oxygen atoms of secoprimycins A, B, and C that arise in the course of ozonolysis and reduction of (2a). The sites of the double bonds of (2e) may be deduced from a knowledge of the structure of the seco-primycins and a tentative assignment of the location of the arabinose unit in (2e) and (7) may be made.

Further experiments, reported in the following paper, verify these conclusions and provide the information necessary to assign a structure to primycin itself.

* The masses of these ions and their intensities (%) are: 586(4), 526(25), 466(11), 406(15), 346(20), 286(5), and 226(7).



Base peak *m/e* 198.

SCHEME 17 Fragmentation of the polymethoxy-ester amide (2e)

EXPERIMENTAL

Mass spectra were determined on a C.E.C. 21–110B double-focusing spectrometer. Samples were introduced through a direct inlet system. Accurate mass measurements [6] were made by reference to appropriate peaks in the spectrum of perfluorokerosene,[7] either by manual matching of individual peaks or by recording the high resolution mass spectra on Ilford Q-2 photographic plates and subsequent transference of the spectra to magnetic tape using a Gaertner comparator-densitometer linked to a Datex system. The spectra were then processed on a CDC-6400 computer using a modified version of the HIRES program of Tunnicliff and Wadsworth.[8] Intensity values were determined from low resolution spectra and are reported in terms of relative abundance with respect to the designated base peak. The ratios of intensities of isobaric ions were estimated from the high resolution data and used in conjunction with the low resolution data to calculate the relative intensity of each isobaric species. Wherever compositions are reported the measured values of the masses of the ions agreed with the calculated values within ±0·005 atomic mass units.

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[3/1324 *Received*, 22nd *June*, 1973]

[6] K. S. Quisenberry, T. T. Scotman, and A. O. Nier, *Phys. Rev.*, 1956, **102**, 1071.

[7] F. W. McLafferty, *Analyt. Chem.*, 1956, **28**, 306.

[8] D. D. Tunnicliff and P. A. Wadsworth, *Analyt. Chem.*, 1968, **40**, 1826.