The Constitution of Primycin. Part III.[1,2] Degradation of Methylated Primycin, and the Structure of Primycin

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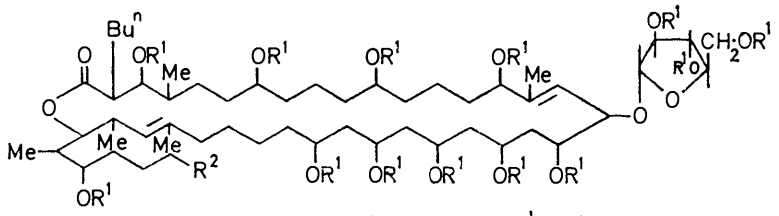
Methylation of primycin with methyl iodide–silver oxide in dimethylformamide gives a trimethylated urea derivative and a trimethylated guanidine derivative. The cleavage of these products by ozonolysis is described. From a study of the products derived from these reactions a structure is proposed for primycin.

In Part I [3] it was shown that alkaline hydrolysis of primycin gave an amino-acid (2a). When a derivative (2b) of this amino-acid was cleaved by ozonolysis and the product reduced with borohydride and reacetylated the poly-*O*-acetylsecoprimycins A (3a), B (4a), and C (5) were obtained. Structures for the secoprimycins have been

[1] Part II, D. E. F. Gracey, L. Baczynskyj, T. I. Martin, and D. B. MacLean, preceding paper.
[2] J. Aberhart, T. Fehr, R. C. Jain, 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] Part I, J. Aberhart, R. C. Jain, T. Fehr, P. de Mayo, and I. Szilagyi, *J.C.S. Perkin I*, 1974, 816.

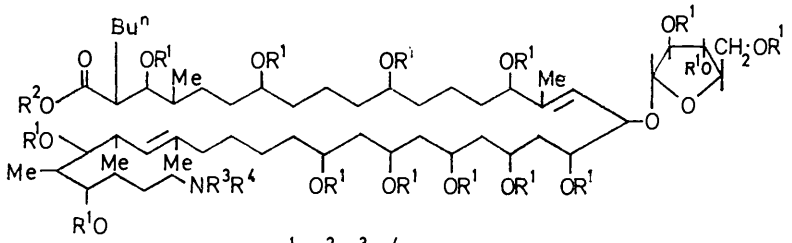
deduced by chemical and by spectroscopic methods and a structure has been tentatively advanced for the amino-acid (2a) based upon a study of its fragmentation upon



(1) a; R^1 = H, R^2 = NH·C(NH_2)=$\overset{+}{N}H_2$, $^1/_2$ SO_4^{2-}

b; R^1 = Me, R^2 = NMe·CO·NMe_2

c; R^1 = Me, R^2 = NMe·C(NMe_2)=$\overset{+}{N}H_2$, Cl^-



(2) a; R^1 = R^2 = R^3 = R^4 = H

b; R^1 = R^3 = Ac, R^2 = Me, R^4 = H

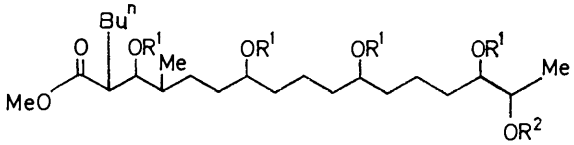
c; R^1 = R^2 = R^3 = Me, R^4 = Ac

electron impact and upon the structures deduced for the secoprimycins.[1] In this paper further degradation studies are reported that confirm the structure (2a) and allow the assignment of structure (1a) to primycin.

Since the derivation of (2a) from primycin involved the use of strong alkali, it was possible that more extensive changes had occurred than the simple hydrolysis of a guanidine and of a lactone. Accordingly, a methylated derivative of primycin was prepared and submitted to the same cleavage procedure in order to check the previous findings: the conditions of the methylation reaction are only mildly basic and degradation or rearrangement of the system would not be expected.

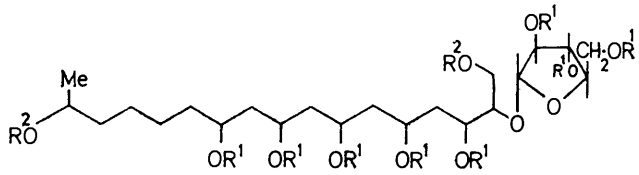
Methylation with methyl iodide and silver oxide in dimethylformamide [4] at room temperature gave a trimethylated urea derivative (1b) and a trimethylated guanidine derivative (1c), together with a minor unidentified product.

Compound (1b), $C_{71}H_{134}N_2O_{18}$, showed ester (1720 cm^{-1}) and amide (1645 cm^{-1}) bands in its i.r. spectrum. Its exact formula follows only from the final constitution of primycin since it was too involatile for examination by mass spectrometry, but its n.m.r. spectrum was informative. Two vinylic methyl groups gave rise to a broad singlet (τ 8·38), and the remaining *C*-methyl groups (*ca.* 4) gave a multiplet between τ 8·97 and 9·22. On the basis of a structure containing four *C*-methyl groups there appeared to be three *N*-methyl groups (τ 7·21). The methoxy-signals appeared between τ 6·51 and 6·76 and it was possible to recognise signals for both the anomeric proton of the arabinoside and the vinyl protons in the region τ 4·75—5·00.



(3) a; R^1=R^2=Ac

b; R^1=Me, R^2=H

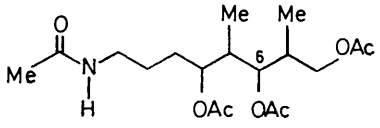


(4) a; R^1=R^2=Ac

b; R^1=Me, R^2=H

c; R^1=Me, R^2=Ac

d; R^1=Me, R^2=CD_3



(5)

[4] R. Kuhn, H. Trischmann, and J. Löw, *Angew. Chem.*, 1955, **67**, 32.

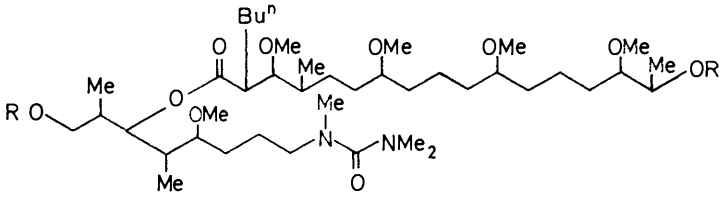
Partial ozonolysis of compound (1b) followed by borohydride work-up gave two main products, the diol (4b) and the hydroxy-ester (6a). The diol (4b), $C_{30}H_{60}O_{12}$, obtained as an oil, had i.r. absorption at 3440 cm^{-1} (OH) and showed n.m.r. signals attributable to a single secondary *C*-methyl group, eight *O*-methyl groups, and the β-anomeric proton of the arabinoside at τ 4·71 (broad singlet). The number and the nature of the hydroxy-groups were inferred from n.m.r. studies of the corresponding diacetate (4c). The n.m.r. spectrum of (4c) showed two acetoxy signals at τ 8·00 and 7·97 and a one-proton multiplet at τ 5·29—4·94 (secondary acetate). The CH_2OAc system gave a multiplet at τ 6·07—5·58, which also contained signals due to CHOR (R ≠ Me). The mass spectrum of the diol (4b) showed, besides the molecular ion at *m/e* 612, intense peaks at *m/e* 581 (M — CH_3O) and 175 ($C_8H_{15}O_4$). The latter represents the methylated arabinose less its glycosidic oxygen atom. These findings corroborated the expected relationship between the diol (4b) and the acetate (4a) and conclusively established that this fragment represented the middle section of primcyin.

Information concerning the site of attachment of the sugar residue came from a study of the diol (4b). Mild acidic hydrolysis of (4b) in methanol gave methyl 2,3,5-tri-*O*-methyl-D-arabinofuranoside (7) and the triol (8). The structure of (7) followed from its mass spectrum, which was identical with that of an authentic specimen,[5] and from data presented in Part I.[3]

The triol (8) showed a molecular composition ($C_{22}H_{46}O_8$) consistent with the loss of a methylated arabinose unit from (4b) through a simple hydrolysis of the glycosidic linkage. Oxidative cleavage of (8) with sodium periodate gave the hydroxy-aldehyde (9), $C_{21}H_{42}O_7$, showing aldehyde i.r. absorption at 1725 and 2710 cm^{-1}. The disposition of the oxygen functions was deduced from an analysis of the mass spectrum.

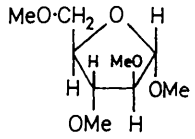
The fragmentation of the methyl ether (9) and of other methylated compounds discussed later in this paper proceeds in much the same way as that of the acetoxy- and hydroxy-analogues discussed in Part II. The major cleavages of the chain occur at the carbon atoms bearing the oxygen functions. The primary fragment ions resulting from these cleavages are invariably accompanied by ions resulting from the loss of one or more molecules of methanol, depending upon the number of methoxy-groups present.

In Scheme 1 the fragmentation of (9) is outlined. The presence of ion BA demonstrates that the aldehyde function is attached to a $-CH(OMe)-$ unit, and the presence of ions BB—BE shows the manner in which the remaining methoxy-groups are disposed. The presence of ion BF identifies the site of the hydroxy-group and

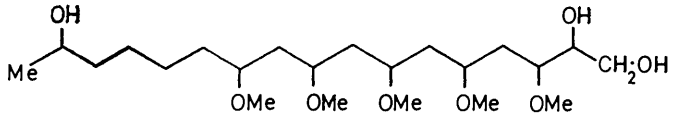


(6) a; R=H

b; R=Ac



(7)



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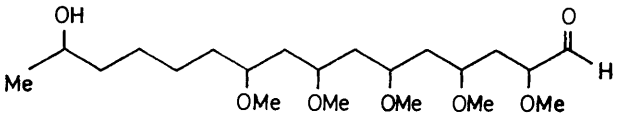
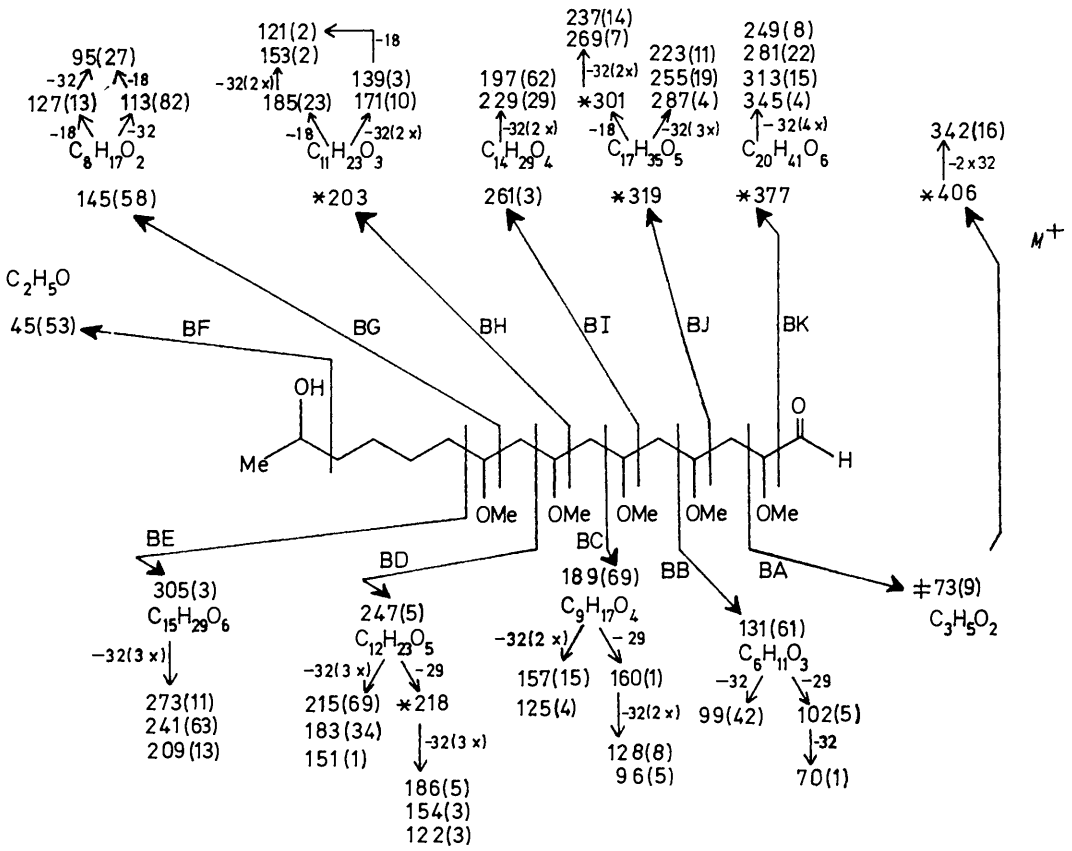


(9)

[5] K. Heynes and H. Scharmann, *Tetrahedron*, 1965, **21**, 507.

that of ion BG shows that the next oxygen function is separated from the first by a unit of mass 56. The other sites of oxygenation are established by the presence of ions BH–CH_3OH, BI, BJ–CH_3OH, and BK–CH_3OH, and by the presence of ions derived from the further fragmentation of all these. The spectrum of (9) is very complex because of the parallel series of fragmentations undergone by each fragment ion, as outlined in Scheme 1, and similar parallel series observed but not discussed for the molecular ion. The structure deduced from the already reported [3] indicated that C-1 represented one of the two sites in secoprimycin B at which a hydroxy-group was introduced in the ozonolysis–reduction of (2a). It thus appears that the arabinose resides at C-2. In Part II evidence, based upon mass spectrometric studies on (2c), was advanced that also pointed to C-2 as the site of the arabinose unit, but, on the other hand, the fragmentation of (4a), the polyacetate of secoprimycin B, cast doubt on this assignment.[1]

In order to clarify this point the free hydroxy-groups



Base and most intense peak *m/e* 71.

SCHEME 1 Fragmentation of the hydroxy-aldehyde (9) †

* Ions denoted with an asterisk in this and subsequent Schemes were either absent or were of such low intensity that the molecular compositions were not determined.

† 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: 18, H_2O; 29, CHO; 32, CH_4O; 59, $C_2H_3O_2$; 60, $C_2H_4O_2$.

‡ The first number refers to the *m/e* ratio of the ion; the number in parentheses refers to its relative intensity.

spectrum of (9) shows clearly that it is a methylated degradation product of secoprimycin B.

The structure of (8) follows from that of (9) since the latter was derived from the former in a periodate oxidation with the loss of a single carbon atom. The triol (8) and 2,3,5-tri-*O*-methylarabinofuranose were derived from (4b) by acidic hydrolysis; thus the arabinose unit of (4b) must reside at C-1 or C-2, a conclusion reached in Part II. The n.m.r. data presented here and in Part I indicate that the arabinose is joined to the alcohol function in an α-linkage. Deuterium labelling experiments of (4b) were trideuteriomethylated, yielding compound (4d), and this was examined by mass spectrometry.

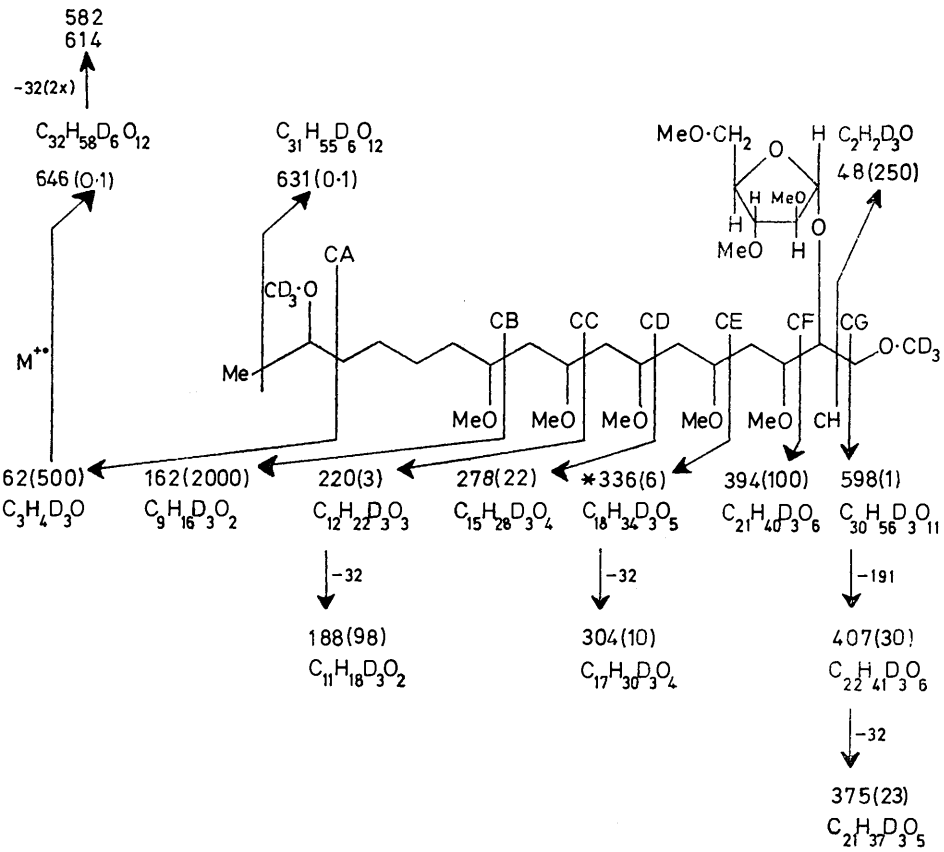
The fragmentation of (4d) is outlined in Scheme 2. A molecular ion of the expected mass and composition was observed but, more important, the ions CG and CH were present. This demonstrates that the arabinose unit is joined to C-2 and not to C-1 and the presence of ions CA—CF confirms the oxygenation pattern already established in the examination of the mass spectra of (9) and the acetate (4a).

The hydroxy-ester (6a), the second compound from

the ozonolysis of (1b), appeared homogeneous on chromatography but the product obtained by its acetylation exhibited two spots on t.l.c. One of these corresponded to the acetate (6b) and the other to a compound $C_{75}H_{142}N_2O_{22}$. The latter, whose characteristics are described in the Experimental section, was apparently derived from the cleavage of only one ethylenic linkage in the entire molecule and was not examined further.

The diacetate (6b), $C_{45}H_{86}N_2O_{12}$ (M^+ 846), showed i.r. absorption corresponding to acetate and tertiary amide functions. The n.m.r. spectrum revealed the presence of five *C*-methyl, two *O*-acetate, three *N*-methyl, and five *O*-methyl groups. The presence of two broad doublets (*J ca.* 7 Hz) at τ 6·09 and 5·95, equivalent to two protons, showed that one acetoxy-group was joined to a methylene that had an adjacent asymmetric centre. A two-proton multiplet centred at τ 5·00 showed that, besides a methine proton on a carbon atom bearing an acetoxy-group, there was a second methine proton, probably on a carbon atom bearing a different ester function. The carbon atoms bearing the acetoxy-groups in (6b) represent the sites of attachment of the middle fragment (4) in the entire molecule, as shown in (1b). Compound (6b) must therefore represent that portion of the primycin molecule from which secoprimycins A and C are derived.

The fragmentation of (6b) is outlined in Scheme 3. The earlier studies showed that secoprimycin C contained nitrogen, and the nitrogen serves as a label in following the fragmentation. The ions DA—DC do not provide new structural information but the presence of ion DD demonstrates that the oxygen function at C-4 is methylated. This site was therefore not involved in the lactone ring or in either of the double bonds of primycin itself. In earlier deuteriation studies it was shown that the primary acetoxy-group of secoprimycin C was generated in the ozonolysis–reduction reactions. We conclude, therefore, that the oxygen at C-6 of secoprimycin C must represent the alkoxy-terminus of the lactone function. This deduction is supported by the presence of ions DE and DF in the spectrum of (6b), since these ions cannot be derived from the secoprimycin A component of (6b). The ions DG—DJ do not provide new structural information but DI and DJ corroborate the findings of Part II regarding the structure of secoprimycin A. In following the fragmentation from the other end of the chain the ions DK—DO, of the expected mass and composition, are all present. The ion DK locates the secondary acetoxy-group in the carbon chain. Thus this site is one of the two in (6b) generated in the ozonolysis–reduction reaction and must correspond to

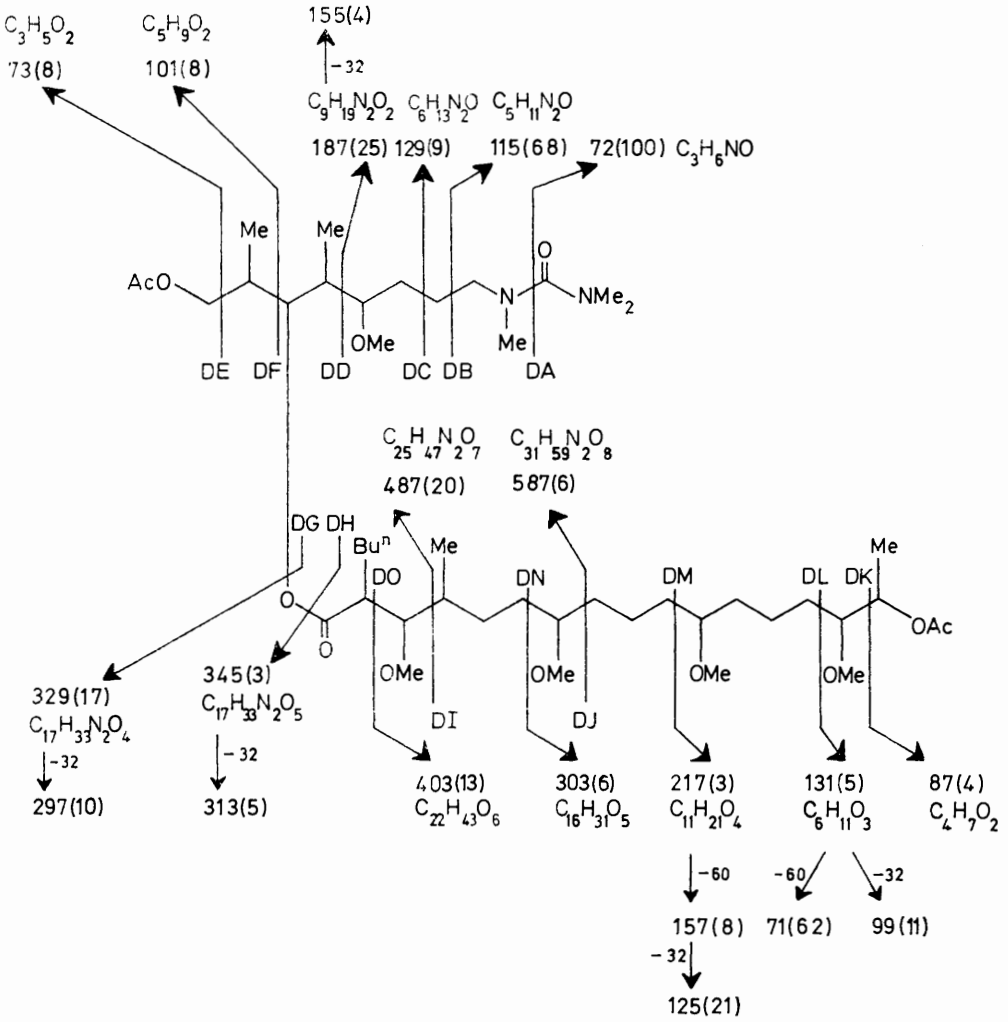


Base peak *m/e* 394. Most intense peak *m/e* 162 (2000%)

SCHEME 2 Fragmentation of the bistrideuteriomethyl ether (4d)

the site of a double bond in primycin. This finding supports the conclusions drawn from the deuteriation studies of Part I. The other ions (DL—DO) locate the oxygenated carbon atoms in the chain, confirming the assignments previously made. The analysis of this spectrum provided the first evidence for the alkoxy-terminus of the lactone function.

OH, ester, and guanidine functions. In the complex n.m.r. spectrum signals associated with five *C*-methyl and three *N*-methyl groups were evident. The guanidine group interfered with other studies and its removal by treatment of (10) with boiling aqueous methanolic potassium hydroxide was undertaken. Three products resulted, the methyl ester (3b) (obtained after diazo-



Base and most intense peak *m/e* 72

SCHEME 3 Fragmentation of the diacetate (6b)

The major component from the methylation of primycin was compound (1c), for which the formula $C_{72}H_{135}N_3O_{17}$,HCl was assigned on the basis of its subsequent degradation. The presence of a guanidine group was indicated by its pK_a value (11·15) and by the observation of i.r. bands at 1600 and 1580 cm^{-1}. Partial ozonolysis of (1c), followed by reduction, gave a neutral compound identified as (4b) by comparison with the sample examined before, and two basic products, (10) and (11), isolated as hydrochlorides.

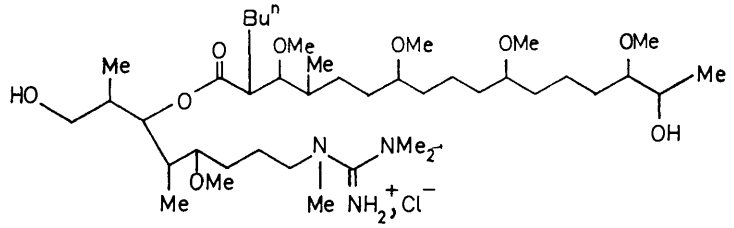
The i.r. spectrum of (10) had absorption attributed to

methane treatment), the trimethylated urea (12a), and compound (6a), identified as its diacetate (6b).

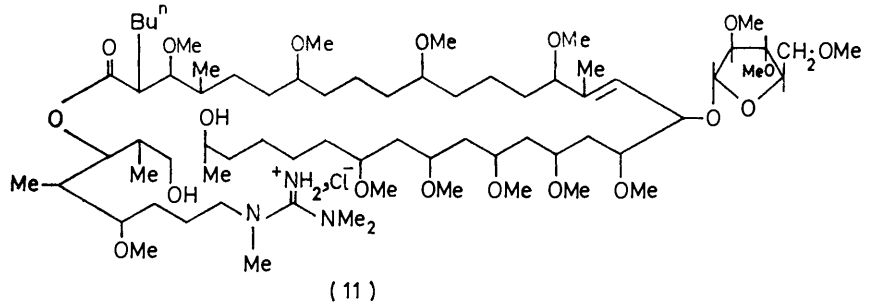
The i.r. spectrum of (12a) showed absorption indicative of hydroxy and urea functions. Acetylation gave the diacetate (12b), $C_{19}H_{36}N_2O_6$, which was used for n.m.r. and mass spectral studies. It was evident from these studies and from its composition that (12b) was related to secoprimycin C.

In the n.m.r. spectrum of (12b) the presence of a one-proton doublet of doublets at τ 4·89 (J 4 and 7 Hz) indicated the presence of an acetoxy-group at C-6.

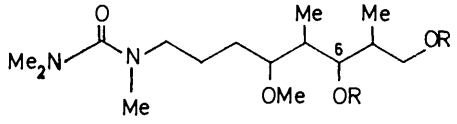
The presence of a two-proton multiplet at τ 6·19—5·99, apparently the AB part of an ABX system, requires an acetoxy-group at C-8 and a methine at C-7. Thus the methoxy-group must be at C-4. Structure (12b) for the diacetate follows also from its mass spectrum. The fragmentation outlined in Scheme 4 is straightforward and discussion is not warranted. The results verify the assignments of the oxygen atoms in the chain already made in the study of secoprimycin C and in this case establish the sites of the methoxy- and the two acetoxy-groups. Using the previously cited evidence that the hydroxy-group at C-8 was generated in the ozonolysis–reduction reaction, it may be concluded that C-6 represents the alkoxy-terminus of the lactone. These findings therefore support those drawn earlier from the mass spectrum of (6b).



(10)



(11)



(12) a ; R=H
b ; R=Ac

The third product (3b) obtained on alkaline hydrolysis of (10) exhibited three almost overlapping spots on t.l.c. that were not separated. The i.r. spectrum of the mixture had absorption attributed to hydroxy and ester functions. The mass spectrum of this compound (Scheme 5) showed that it was a derivative of secoprimycin A. The fragmentation corroborated the previous findings concerning the sites of oxygenation and further discussion is unnecessary. It is of interest, however, that the spectrum showed ions of low intensity at *m/e* 504 and 518 ($M + 14$ and $M + 28$), probably associated with homologues of compound (3b).

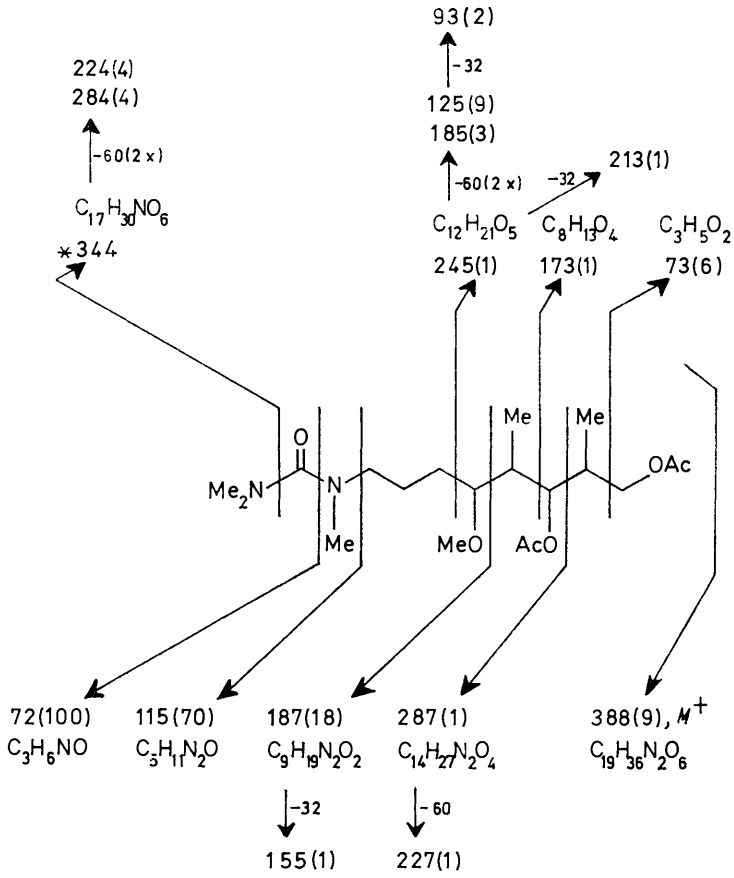
The third product of ozonolysis and reduction of (1c), compound (11), had n.m.r. and an i.r. spectra very similar to those of the starting material. The n.m.r. spectrum of (11), however, showed a new signal at τ 8·82 attributed to a secondary *C*-methyl group evidently formed in the ozonolysis–reduction of (1c). It appears therefore that (11) is formed from (1c) by cleavage of a single double bond and without loss of carbon. A chemical study of (11) provided further insight into the orientation in the original molecule of the fragment yielding secoprimycin B.

When (11) was hydrolysed in hot alkali, and the product was acetylated and treated with diazomethane, compound (13a) was obtained as the major product. The n.m.r. spectrum showed the presence of four *C*-methyl groups [doublets at τ 8·82 and τ 8·99 (J 6·5 Hz), a triplet at τ 9·10 (J 6·5 Hz), and a broad singlet (vinylic) at τ 8·37] an acetoxy-group (singlet at τ 8·00), a methyl ester (singlet at 6·84) and a vinylic proton [broad doublet at τ 4·64 (J 10 Hz)]. From these data and the i.r. data (see Experimental section) it was evident that (13a) did not contain nitrogen and that it corresponded to that

portion of primycin from which secoprimycins A and B were derived. This supposition was substantiated in the following way.

Mild alkaline hydrolysis of the acetoxy-ester (13a) followed by treatment with diazomethane gave the corresponding hydroxy-ester ($\nu_{max.}$ 3440 and 1725 cm^{-1}) (13b). Two-phase chromic acid oxidation of the alcohol gave the methyl ketone (13c). The presence of the sis and borohydride reduction of a compound related to (13c), but having a double bond αβ to the methoxycarbonyl group, and present in admixture with (13c) or (11). The double bond might easily arise by a simple β-elimination of the methoxy-group during the alkaline treatment of (11) or (13b).

In the mass spectrum of compound (14) no homologous ion peaks were observed. Thus homologation in the



Base and most intense peak *m/e* 72

SCHEME 4 Fragmentation of the diacetate (12b)

part structure CH_3–CO–CH_2–CH_2 in (13c) was demonstrated by the presence of a three-proton n.m.r. singlet at τ 7·90 and a two-proton triplet at τ 7·59 (*J ca.* 7 Hz). These findings provide convincing support for the orientation of the fragment yielding secoprimycin B in the molecules (1a—c) and (2a—c).

Cleavage of the double bond of (13c) by ozone followed by borohydride work-up gave the expected methyl ester (3b) and the middle fragment (4b). Besides these two products a very small amount of compound (14) was isolated. The structure of this product was deduced entirely by mass spectrometry (Scheme 6). It is obvious from the fragmentation pattern that this compound is an *O*-methylated degradation product related to secoprimycin A; *cf.* for example the ions common to (14) and (3b) (Scheme 5). Compound (14) may be formed by ozonolysecoprimycin A series of compounds possibly arises in the n-butyl side chain but a rigorous proof is at present lacking.

The discussion so far permits the proposal of structures (1b) and (1c) as the major products of the methylation of primycin and (2b) as the structure of the polyacetoxy-amino-ester. The trimethylated urea derivative (1b) is obviously formed by a partial hydrolysis of the guanidine unit by the mildly basic silver oxide during the course of methylation. Barring the loss of small fragments during methylation the major constituent of primycin should have the structure shown in (1a). The equivalent weight of primycin represented by the structure (1a) is 1127 and this is in good agreement with the experimental value found for primycin.[3]

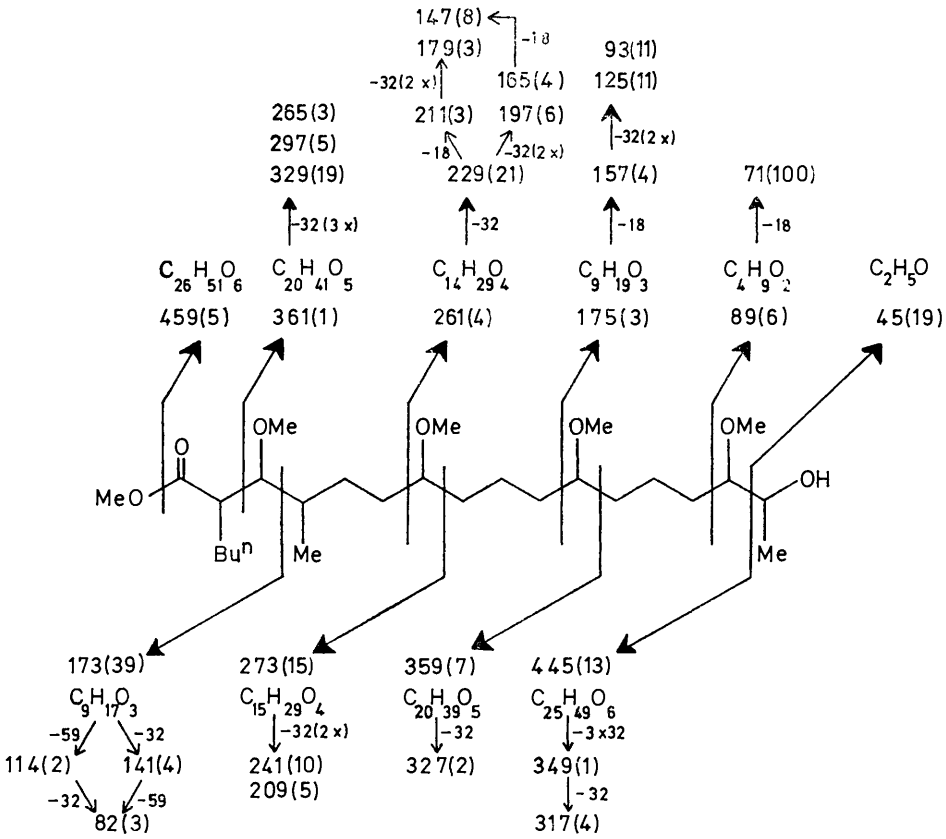
The conditions used for the methylation are mildly

basic and the hydrolysis of esters in the course of this reaction cannot be precluded. However, in the alkaline hydrolysis of primycin reported earlier hexanoic acid was the only volatile acid detected and it was apparently formed in a retro-aldol reaction. Thus the hydroxy-groups of primycin do not appear to be esterified and (**1a**) is the most acceptable structure of the major component of primycin.

biotics. The presence of guanidine and arabinose units also makes it unique amongst the known macrolides whose structures have been established to date.

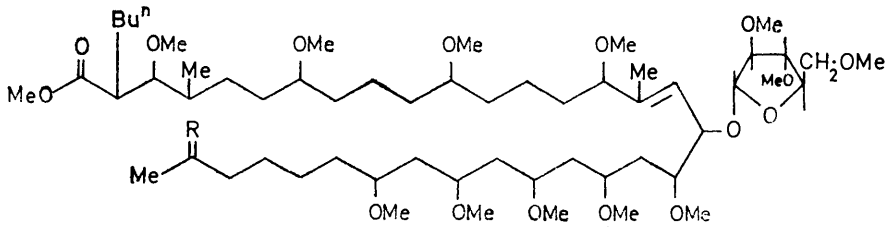
EXPERIMENTAL

All the compounds were colourless oils whose molecular weights were established by mass spectrometry (unless otherwise indicated). The spectra were determined on a



Base and most intense peak *m/e* 71

SCHEME 5 Fragmentation of the hydroxy-ester (3b)



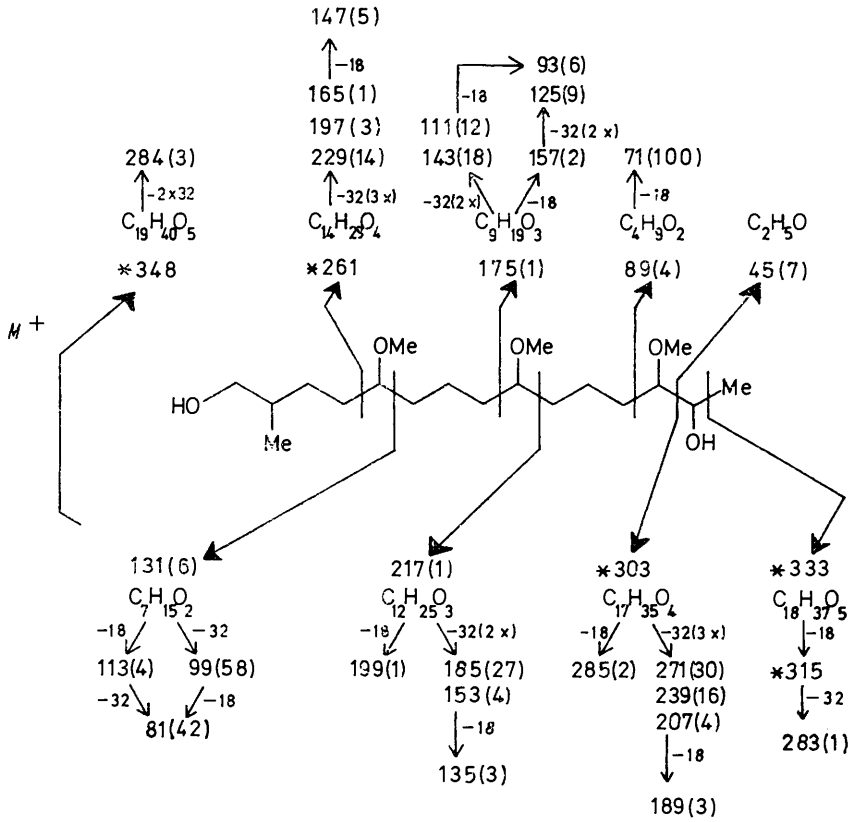
(13) a; R = AcH
b; R = HOH
c; R = O

Primycin is therefore a non-polyene macrolide that has the largest ring size reported so far for this class of anti-

C.E.C. 21-110B double-focusing mass 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 record-

[6] K. S. Quinsenberry, T. T. Scotman, and A. O. Nier, *Phys. Rev.*, 1956, **102**, 1071.
[7] F. W. McLafferty, *Analyt. Chem.*, 1956, **28**, 306.

ing 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 were 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 cyanide (10 g). The solution was extracted with chloroform (4 × 75 ml) and the extract was washed with water, dried, and evaporated *in vacuo* to give an oily residue (4·26 g). The methylation being incomplete (strong OH band in the i.r.), the whole process was repeated to give 4·3 g of an oily residue. This residue in methanol (20 ml) was percolated through a 30 × 1 cm column of Dowex 1-X2 resin (OH^- form; 50—100 mesh). The column was washed with methanol (100 ml) and the alkaline effluent was carefully neutralized with dilute hydrochloric acid. Evaporation at room temperature *in vacuo* gave a viscous oil (4·2 g). Separation by chromatography [$CHCl_3$–MeOH (95 : 5) followed by $CHCl_3$–MeOH (4 : 1) containing 1% v/v of



Base and most intense peak *m/e* 71

SCHEME 6 Fragmentation of the diol (14)

masses of the ions agreed with the calculated values within ±0·005 atomic mass units. The i.r. spectra were recorded on a Beckman IR 10 spectrometer for solutions in chloroform. The n.m.r. spectra were recorded on a Varian HA-100 spectrometer for solutions in deuteriochloroform containing tetramethylsilane as internal standard. T.l.c. was carried out on Merck silica gel GF-254; the same material was used for preparative chromatographic separations. Sodium sulphate was used as drying agent.

Methylation of Primycin.—A mixture of primycin (3 g), dimethylformamide (100 ml), methyl iodide (50 ml), and silver oxide (50 g; freshly prepared) was stirred at room temperature for 2 days to give a thick slurry. The precipitate was centrifuged and washed several times with chloroform. The centrifugate, combined with the washings, was diluted with water (150 ml) and shaken with sodium 2M-HCl] afforded three compounds: the *trimethylated urea derivative* (1b) (770 mg), homogeneous on t.l.c., $\nu_{max.}$ (film) 2920, 1720, 1645, 1490, 1455, 1375, 1090, 1000, and 940 cm^{-1} (Found: C, 65·0; H, 10·3; N, 2·1. $C_{71}H_{134}N_2O_{18}$ requires C, 65·4; H, 10·35; N, 2·15%); the *trimethylated guanidine derivative* (1c) (2·097 g), $\nu_{max.}$ (film) 3360, 2920, 2820, 1725, 1660, 1580, 1455, 1405, 1370, 1250, 1090, 1000, and 950 cm^{-1} (Found: C, 63·6; H, 10·6; Cl, 2·65; N, 3·05. $C_{71}H_{135}N_3O_{17}$,HCl requires C, 63·65; H, 10·25; Cl, 2·65; N, 3·15%); and a third product (542 mg), equiv. wt. *ca.* 1467, pK_a 11·35 (methanol solvent).

Ozonolysis of Compound (1b).—Compound (1b) (482 mg) in methanol (30 ml) was cooled in solid carbon dioxide–acetone and a slow stream of ozone was passed through the

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

solution for 40 min. Sodium borohydride (500 mg) in methanol (10 ml) was added in portions and the mixture was left at room temperature for 15 min. The solution was acidified with hydrochloric acid (2M) and extracted with chloroform to give an oily residue (529 mg). This residue was chromatographed ($CHCl_3$–MeOH) to give the *diol* (4b) (163 mg), which was purified by evaporative distillation (140°; 0·001 mmHg); $\nu_{max.}$ (film) 3440, 2930, 2820, 1460, 1375, 1190, 1090, and 1000 cm^{-1}, τ 8·82 (3H, d, J 6·5 Hz, CH_3), 8·70—8·20 (16H, m, 8 CH_2), 7·60—7·10 (*ca.* 3H, m, partially disappeared on adding D_2O, partly due to OH), 6·65, 6·59, and 6·55 (24H, three apparent singlets, 8 OMe), 6·50—6·05, (9—10H, m), 6·00—5·70 (1H, m), and 4·71br (1H, s, anomeric proton) (Found: C, 58·2; H, 9·75. $C_{30}H_{60}O_{12}$ requires C, 58·8; H, 9·85%), M^+ 612.

Continued elution gave a second fraction (248 mg), homogeneous on t.l.c., that was acetylated with acetic anhydride–pyridine at room temperature and then chromatographed (chloroform containing 2% methanol) to give the *diacetate* (6b), homogeneous by t.l.c., $\nu_{max.}$ (film) 2930, 2870, 2820, 1735, 1645, 1495, 1460, 1370, 1240, 1160, 1090, 1035, and 920 cm^{-1}, τ 9·15—8·91 (12H, m, 4 CH_3), 8·80 (3H, d, J 6·5 Hz, CH_3), 8·75—8·00 (*ca.* 29H, m, CH_2 and CH), 7·96 and 7·95 (6H, two singlets, 2 OAc), 7·22, 7·20, and 7·19 (9H, three singlets, 3 NMe), 7·10—6·72 (7H, m, 5 C*H*OR and NCH_2), 6·69, 6·67, 6·58, and 6·57 (15H, four singlets, 5 OMe), 6·09 and 5·95 (2H, two broad doublets CH_2–OAc), and 5·00br (2H, m, 2 CH·O·COR) (Found: C, 63·6; H, 10·2; N, 3·2. $C_{45}H_{86}N_2O_{12}$ requires C, 63·8; H, 10·25; N, 3·3%).

Further elution gave another compound (80 mg), $\nu_{max.}$ (film) 2930, 2820, 1730, 1645, 1490, 1460, 1370, 1240, and 1090 cm^{-1}, τ 9·15—8·90 (12H, m, 4 CH_3), 8·80 (3H, d, J 6·5 Hz, CH_3), 8·75—8·08 (45—46 H, multiplet having a broad singlet at 8·37 due to vinylic methyls), 7·99 and 7·97 (6H, two singlets, 2 OAc), 7·19 (9H, apparent s, 3 NMe), 5·00br (1H, s, anomeric H), and 4·64 (2H, d, J 10 Hz, vinylic) (Found: C, 62·85; H, 10·25; N, 2·05. Calc. for $C_{75}H_{142}N_2O_{22}$: C, 63·25; H, 10·05; N, 1·95%).

Acetylation of the Diol (4b).—Acetylation of (4b) with acetic anhydride–pyridine at room temperature gave the *diacetate* (4c), homogeneous by t.l.c. The mass spectrum did not give a molecular ion. The peak of highest mass was at *m/e* 632 (M − 2MeOH); $\nu_{max.}$ (film) 2930, 2820, 1735, 1455, 1370, 1240, 1190, 1090, and 1040 cm^{-1}; τ 8·81 (3H, d, J 6·5 Hz, CH_3), 8·74—8·14 (16H, m, 8 CH_2), 8·00 and 7·97 (6H, two singlets, 2 OAc), 7·22 (*ca.* 2H, m, CH_2·OCH_3), 6·70, 6·68, 6·66, 6·62, and 6·58 (24H, all singlets, 8 OMe), 6·54—6·19 (7H, m, C*H*OR), 6·07—5·58 (4H, m, CH_2·OAc and 2 C*H*OR other than methyl ethers), 5·29—4·94 (1H, m, C*H*·OAc), and 4·81br (1H, s, anomeric proton) (Found: C, 58·8; H, 9·25. $C_{34}H_{64}O_{14}$ requires C, 58·6; H, 9·25%).

Conversion of the Diol (4b) *into the Bistrideuteriomethyl Ether* (4d).—A solution of (4b) (5·0 mg) in *NN*-dimethylformamide (0·5 ml) was added to a stirred suspension of sodium hydride (10·0 mg; 57% dispersion) in *NN*-dimethylformamide (1·0 ml) and the resulting mixture was stirred at room temperature for 10 min. Trideuteriomethyl iodide (1·0 ml) was then added and stirring continued for a further 3 h. Water (5 ml) was added and the solution was extracted with ether (4 × 5 ml). The extract was washed with water and evaporated *in vacuo* to yield the ether (4d) (3·8 mg) as an homogeneous oil [R_F 0·8 (alumina–chloroform)], M^+ 646 ($C_{32}H_{58}D_6O_{12}$ by high resolution measurement).

Acidic Hydrolysis of the Diol (4b).—A mixture of compound (4b) (75·4 mg), methanol (5 ml), and hydrochloric acid (5 ml; 0·2M) was refluxed for 20 h. The solution was cooled, carefully neutralized with sodium hydroxide (0·2M), and then extracted with chloroform to give an oily residue (70·7 mg). Chromatography [$CHCl_3$–MeOH (98 : 2)] gave, in order of elution, methyl 2,3,5-tri-*O*-methyl-D-arabinofuranoside (7) (7·8 mg), starting material (7·2 mg) (identified by t.l.c. and i.r.), an unidentified fraction (5·6 mg), and the triol (8) (49·1 mg), $C_{22}H_{46}O_8$ (high resolution mass spectroscopy), $\nu_{max.}$ 3435, 2935, 2825, 1455, 1380, 1235, 1180, and 1075 cm^{-1}, τ 8·83 (3H, d, J 6·5 Hz, CH_3), 8·69—8·14 (16H, m, 8 CH_2), 7·01—6·79 (3H, m, eliminated on adding D_2O, 3 OH), 6·68 and 6·64 (15H, two singlets, 5 OMe), and 6·61—6·17 (9H, m, C*H*·O).

Periodate Oxidation of the Triol (8).—To a solution of the triol (8) (39 mg) in dioxan (2 ml), sodium periodate (27·3 mg) and water (0·5 ml) were added. The solution was kept at room temperature in the dark for 1 h, then extracted with chloroform to give an oily residue (34·4 mg). On t.l.c. it exhibited essentially one spot, but attempts at further purification by chromatography led to several unidentified products and only 6·5 mg of the hydroxy-aldehyde (9) was isolated; $\nu_{max.}$ 3440, 2990, 2930, 2820, 2710, 1725, 1460, 1380, 1240, 1185, and 1090 cm^{-1}.

Potentiometric Titration of Compound (1c).—A solution of compound (1c) (231 mg) in methanol (15 ml) was percolated through a 20 × 1 cm column of Dowex 1-X2 (OH^- form; 50—100 mesh). The column was washed with methanol until the effluent was neutral (pH *ca.* 7). The combined effluent was titrated with hydrochloric acid (0·1N) at the potentiometer; net titre 1·67 ml indicating an equivalent weight of 1383; pK_a 11·15.

Ozonolysis of Compound (1c).—Compound (1c) (944 mg) was subjected to ozonolysis as described for the urea (1b). The reaction mixture in methanol was passed through a 30 × 2 cm column of Dowex 50W-X2 and the column was washed with methanol to give the diol (4b) (187 mg) The column was washed further, first with hydrochloric acid (2M) and then with methanol. These effluents were concentrated *in vacuo*, diluted with water, made just alkaline with sodium hydroxide (2M), and then extracted with chloroform to give an oily residue which was immediately dissolved in methanol (15 ml) and acidified (pH *ca.* 6) with hydrochloric acid (0·01M). The resulting solution was evaporated at room temperature *in vacuo* leaving an oily residue. This residue was chromatographed [$CHCl_3$–MeOH (4 : 1)] to give, in order of elution, the hydrochlorides (10) and (11). Hydrochloride (10) (170 mg) showed $\nu_{max.}$ (film) 3300, 2940, 2820, 1725, 1600, 1580, 1460, 1410, 1255, 1220w, 1160w, 1090, and 900 cm^{-1}; τ 9·18—8·95 (12H, m, 4 CH_3), 8·85 (3H, d, J 6·5 Hz, CH_3), 8·75—7·75 (m, CH_2 and CH), 7·60 and 7·30 (hump, diminished in size on adding D_2O, partly due to OH), 6·90br (s, *N*-methyls), 6·75—6·45 (multiplet with broad singlets at 6·67 and 6·57 due to *O*-methyls), 6·45—5·80 (m, CH·O), and 5·15—4·70 (1H, m, CH·CO_2R). Hydrochloride (11) (4·17 mg) showed $\nu_{max.}$ (film) 3300, 2930, 2820, 1720, 1645vw, 1600, 1575, 1460, 1405, 1375, 1250, 1080, 950, and 900 cm^{-1}. The n.m.r. spectrum was very similar to that of compound (1c) except that it contained, in addition, a three-proton doublet (J 6·5 Hz) at τ 8·82 (secondary Me) and a broad hump at 7·50—7·20 (eliminated on adding D_2O, OH).

Alkaline Hydrolysis of Compound (10).—Compound (10) (94 mg) in potassium hydroxide solution [15 ml; 0·5M in methanol–water (1 : 1)] was refluxed for 10 h. The solution was cooled and then extracted with chloroform. The

mixture obtained after evaporating the extract was treated with ethereal diazomethane and then chromatographed (chloroform containing 2% methanol) to give three products: the diol (6a) (5·2 mg), $\nu_{max.}$ 3440, 2990, 2920, 2870, 1720sh, 1695, 1620, 1500, 1460, 1380, 1205, 1160, and 1090 cm^{-1}; the methyl ester (3b) (43 mg), showing three almost overlapping spots on t.l.c., $\nu_{max.}$ 3560, 3440, 2990, 2930, 2860, 1720, 1460, 1370, 1225, 1165, and 1085 cm^{-1}; and the diol (12a) (23·2 mg), $\nu_{max.}$ 3420, 2990, 2930, 2870, 1620br,s, 1495, 1460, 1385, 1225, 1140, 1080, 975, and 920 cm^{-1}.

Acetylation of the Diol (12a).—The diacetate (12b), $C_{19}H_{36}N_2O_6$ (high resolution mass spectroscopy), obtained after acetylation of (12a) with acetic anhydride–pyridine at room temperature, showed $\nu_{max.}$ (film) 1725 and 1620 cm^{-1} but no absorption in the hydroxy-region; τ 9·12 and 9·09 (6H, two doublets, J 7 Hz, 2 CH_3), 8·69—8·29 (4H, m, 2 CH_2), 8·19—7·60 (2H, excluding the singlet at 7·97, m, 2 CH), 7·97br (6H, s, 2 OAc), 7·22 (6H, s, 2 NMe), 7·20 (3H, s, NMe), 7·04—6·73 (3H, m, CH_2·N, C*H*·OMe), 6·68 (3H, s, OMe), 6·19—5·99 (2H, unresolved AB part of an ABX system, C*H*$_2$·OAc), and 4·89 (1H, dd, J 4 and 7 Hz, C*H*·OAc).

Alkaline Hydrolysis of Compound (11).—A solution of compound (11) (202 mg) in methanol (20 ml) and potassium hydroxide (20 ml; 10M) was refluxed for 4 h. After the solution had cooled it was extracted with chloroform and the extract was evaporated to give an oily residue (180 mg). The aqueous solution was made acidic with dilute hydrochloric acid and then extracted with chloroform to give an oil (14 mg). These extracts, which exhibited the same t.l.c. behaviour, were combined and acetylated with acetic anhydride–pyridine to give, after isolation, a viscous oil (200 mg). Chromatography (chloroform containing 2% methanol) gave the acetate (13a) (99 mg), $\nu_{max.}$ (film) 2930, 2820, 1735, 1730, 1645vw, 1455, 1375, 1245, 1185, 1090, 1000, and 950 cm^{-1}; τ 9·10 (3H, t, J 6 Hz, CH_3), 8·99 (3H, d, J 6·5 Hz, CH_3), 8·77—8·18 (*ca.* 42H, multiplet with a broad singlet at 8·37, vinylic Me, CH_2, and CH), 8·00 (3H, s, OAc), 7·73br (1H, t), 7·58—7·32 (1H, m), 6·84 (3H, s, CO_2Me), 6·76—6·49 (*ca.* 36H, m, 12 OMe), 6·49—6·23 (8—9H, m, C*H*·OR), 6·01—5·78 (1H, m), 5·40 (1H, dd, J 5·5 and 9·5 Hz), 5·12 (1H, dd, J 5·5 and 12 Hz), 5·04br (1H, s, anomeric proton), and 4·64br (1H, d, J *ca.* 10 Hz, vinylic proton); a further fraction (32 mg), shown by t.l.c. to consist of two compounds, was also obtained but was not examined further.

Alkaline Hydrolysis and Chromic Acid Oxidation of Compound (13a).—A solution of (13a) (72 mg) in methanol (5 ml) and potassium hydroxide (5 ml; 0·5M) was kept at room temperature for 12 h, then was carefully acidified with hydrochloric acid, and extracted with chloroform to give the corresponding hydroxy-acid (61·8 mg).

The acid was esterified with ethereal diazomethane to give the hydroxy-ester (13b), $\nu_{max.}$ 3440 and 1725 cm^{-1}; the n.m.r. spectrum lacked the acetoxy-signal but otherwise appeared similar to that of the acetate (13a)

A stirred solution of the hydroxy-ester (13b) (60 mg) in ether (5 ml) was treated dropwise with chromic acid [9] at room temperature. After 5 min, the mixture was diluted with water and then extracted with ether. The extract was washed with 10% sodium hydrogen carbonate solution, then water, dried, and evaporated *in vacuo* to give the keto-ester (13c) (57·4 mg), homogeneous on t.l.c. This residue was dissolved in ether and filtered through a column of silica gel to give (13b) (56·3 mg) as an oil, $\nu_{max.}$ 2930, 2830, 1710br, 1455, 1090, and 1010 cm^{-1}; n.m.r. spectrum very similar to that of the hydroxy-ester (13b) except that the methyl doublet at τ 8·82 was replaced by a three-proton singlet at τ 7·90. There was also a two-proton triplet (J 7 Hz) at τ 7·59 in the spectrum of (13c) (CH_2·CO).

Ozonolysis of the Keto-ester (13c).—The keto-ester (13c) (35·3 mg) was ozonised and the reaction mixture was subjected to borohydride work-up as described for compound (1b). The mixture (35·1 mg) was chromatographed on silica gel [$CHCl_3$–MeOH (99 : 1)] to give the diol (4b) (17·3 mg), the methyl ester (3b) (1·8 mg), and the diol (14) (1·3 mg). The low resolution mass spectrum of (14) showed a molecular ion at *m/e* 348 but it was of low intensity and was not mass matched. The composition of (14) ($C_{19}H_{40}O_5$) follows from an analysis of its fragmentation (Schemes 8 and 9).

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