The Constitution of Primycin. Part I.[1] Characterisation, Functional Groups, and Degradation to the Secoprimycins

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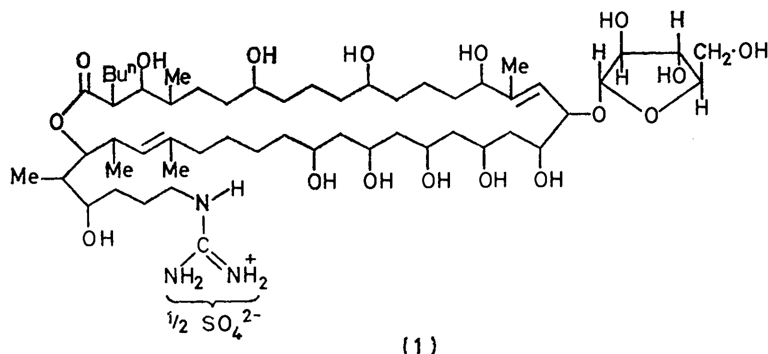
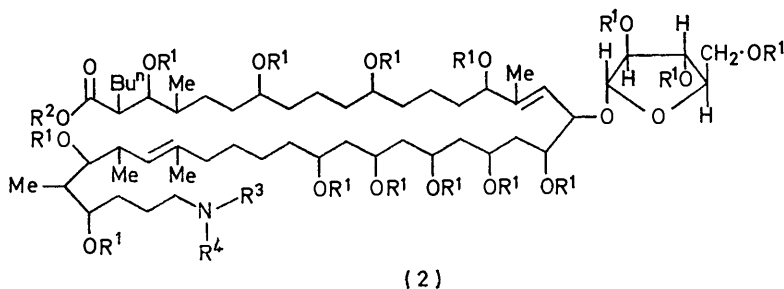
Primycin, the antibiotic agent produced by *S. primycini*, has been found to be the sulphate of a monoalkylated guanidine. The molecule contains six *C*-methyl groups, two ethylenic linkages, and probably between 12 and 15 hydroxy-groups. The presence of a (−)-D-arabinose unit has been demonstrated.

Hydrolysis of primycin with concentrated alkali gives, together with hexanoic acid and other unidentified substances, a long-chain polyhydroxylated amino-acid (2a). This has been acetylated, esterified, and then cleaved by ozonolysis to give three fragments. These were isolated as their borohydride reduction products, the secoprimycins. Evidence is provided which establishes secoprimycin A as 2-butyl-3,7,11,15,16-pentahydroxy-4-methylheptadecanoic acid, B as an arabinosyl derivative of heptadecane-1,2,3,5,7,9,11,16-octaol, the position of the arabinosyl group being undetermined, and C as *N*-(4,6,8-trihydroxy-5,7-dimethyloctyl)acetamide. By using borodeuteride as the reducing agent, the positions of the original ethylenic linkages in the secoprimycins were established.

IN a recent communication [1] we reported, together with the group of Professor D. MacLean at McMaster University, our investigation of the structure of the antibiotic primycin which culminated in the proposal of structure (1) for this substance. Primycin was first isolated in 1954 from growths of an actinomycetes strain, *S. primycini* originating from *Galeria melonella*.[2] It was obtained as a white microcrystalline powder, and appeared to be homogeneous on the basis of paper chromatography, partition column chromatography, counter-current distribution, and fractional crystallisation.[3] Primycin shows an *in vitro* bacteriostatic effect on Gram-positive organisms (both pathogenic and nonpathogenic) as well as on mycobacteria.[4] It forms complexes with polydeoxyribonucleotides and polyribonucleotides, a property also exhibited by streptomycin.[5] However, in animal experiments primycin appeared to be toxic and has, up to the present time, only been used for superficial application.



(1)



(2)

	R^1	R^2	R^3	R^4
a;	H	H	H	H
b;	Ac	H	Ac	H
c;	Ac	Me	Ac	H
d;	H	Me	Ac	H
e;	Me	Me	Ac	Me

In preliminary tests primycin did not reduce Fehling's solution, nor did it react with reagents which normally form derivatives with aldehydes. It exhibited, how-

[1] Part of the contents of this and the following papers have been published in preliminary form: 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.

[2] T. Valyi-Nagy, J. Uri, and I. Szilagyi, *Nature*, 1954, **174**, 1105.

[3] I. Szilagyi, T. Valyi-Nagy, and T. Keresztes, *Nature*, 1965, **205**, 1225.

[4] T. Valyi-Nagy, J. Uri, and I. Szilagyi, *Pharmazie*, 1956, **11**, 304; T. Valyi-Nagy and B. Kelentei, *Arch. Int. Pharmacodyn.*, 1960, **124**, 466.

[5] J. J. Blum, *Arch. Biochem. Biophys.*, 1965, **111**, 635.

ever, a positive Sakaguchi reaction.[6] This observation, taken together with the basicity of primycin (pK_a, 11·2 in MeOH) strongly suggested that the antibiotic was a monoalkylated guanidine derivative. This conclusion was supported by the isolation, from the permanganate oxidation products of primycin,[7] of guanidine itself, albeit in trace amounts.

On fusion with sodium, primycin gave positive indications of the presence of nitrogen and sulphur; there was none for the presence of halogen. Previous studies on primycin had not revealed the presence of sulphur,[8,9] the analytical discrepancy being interpreted, by difference, as additional oxygen.

All the sulphur was found to be present as sulphate ion. This was identified qualitatively as barium sulphate, which was characterised by its i.r. spectrum. The anion was estimated quantitatively by titration with barium perchlorate,[10] the procedure being checked by use of appropriate controls.† The equivalent weight so found was 1143. Titration of the free base, obtained by passing primycin in methanolic solution through a column of Dowex 1-X2 in its basic form, gave an equivalent weight of 1260. The discrepancy is probably due to retention on the columns.

Primycin gave a microcrystalline picrate, m.p. 166—168°, a microcrystalline perchlorate, and a solid, but non-crystalline hydrobromide. Elemental analysis of all three substances showed the complete absence of sulphur, but neither from the analytical figures of these substances, nor from those of primycin (sulphate) itself could an exact empirical formula for the antibiotic be deduced; this situation is not unusual in macrolide chemistry and is compounded in the present instance by problems of solvation. These derivatives are, therefore, not described further.

Little information could be gleaned from the i.r. spectrum of primycin[11] beyond the presence of intense absorption corresponding to strongly bonded hydroxy-functions. Absorption at 1675 cm^{-1} could be attributed to the guanidine function,[12] but the presence of carbonyl absorption in the same region could neither be excluded nor deduced at this stage, hydrogen bonding apparently seriously modifying the lactone absorption.

Only end absorption was observed in the u.v. spectrum, and this was originally attributed entirely to the guanidine function.[13] Comparison with suitable models, however, suggested that, with a molecular weight of the order indicated by the titrations, absorption due to some other function or functions was present. Later studies showed this absorption to be due to isolated ethylenic linkages. Lack of solubility precluded the possibility of hydrogenation studies on primycin itself, and the same restrictions applied to the proper determination of the i.r. spectrum.

At this point it appeared that if primycin contained only three nitrogen atoms, that is, those attributed to the guanidine group; then it must have an empirical formula of the type $C_{50-55}H_{ca.\,100}N_3O_{15-18},0{\cdot}5SO_4$. Indeed, the exact formula ($C_{55}H_{104}N_3O_{17},0{\cdot}5SO_4$) was not obtained until the structure was completely elucidated. The low degree of unsaturation and multiplicity of what were evidently hydroxy-functions led us to suspect that primycin was related to the macrolide group, but that it contained the polyene system common to this family saturated by the addition of the elements of water.

Szilagyi found, in 1963,[8] that under acidic conditions primycin gave a biologically inactive, but Sakaguchi-positive aglycone, together with a sugar. The latter was identified as arabinose by conversion into its *p*-nitrophenylhydrazone. This has been confirmed by repetition, and the sugar itself isolated by using ion-exchange resins. The product was identical (m.p. and mixed m.p.) with (—)-D-arabinose but depressed the m.p. of (+)-L-arabinose. The assignment was supported by the optical rotation, and by conversion, as before, into the *p*-nitrophenylhydrazone.

The agylcone was isolated as an amorphous hydrochloride; it is uncertain whether the material is homogeneous. Nonetheless some further information was obtained from this material which had the advantage over primycin of greater solubility.

This property, for instance, permitted quantitative hydrogenation studies. Hydrogenation over platinum oxide in methanol led to the fairly rapid uptake of 2 mol. equiv. of hydrogen. The aglycone itself showed u.v. end absorption closely similar to that of primycin; after hydrogenation the absorption was compatible with that expected for an alkylguanidine.‡ If, therefore, the acidic treatment had achieved no more than glycosidic hydrolysis these results suggested the presence of two ethylenic linkages in primycin.

As commonly occurs, Kuhn–Roth determination for *C*-methyl groups in the aglycone gave a low figure. However, calibration of the analysis by using erythromycin as a standard suggested that the aglycone contained six *C*-methyl groups for a molecular weight of 1000, a determination eventually found to be correct.

Of the 15—18 oxygen atoms in the molecule, five had been shown to be contained in an arabinose unit,

† Sodium sulphate decahydrate and guanidine sulphate.

‡ The material had ε_{205} 2400, ε_{200} 5000; cyclohexylguanidine hydrochloride had ε_{205} 2600, ε_{200} 5000.

[6] S. Sagaguchi, *J. Biochem.*, 1925, **5**, 25; C. J. Weber, *J. Biol. Chem.*, 1930, **86**, 217; D. Watson, *Experientia*, 1966, **22**, 76.

[7] For analogies see streptomycin: R. L. Peck, C. E. Hoffine, E. W. Peel, R. P. Graber, F. W. Holly, R. Mozingo, and K. Folkers, *J. Amer. Chem. Soc.*, 1946, **68**, 776; tetrodotoxin: R. B. Woodward, *Pure Appl. Chem.*, 1964, **9**, 49; R. B. Conn and R. B. Davis, *Nature*, 1959, **183**, 1053.

[8] I. Szilagyi, Dissertation, Debrecen, 1963.

[9] This indicates the necessity for total elemental analysis at the outset of the study of a structural problem. See, for example, penicillin: H. W. Florey, 'Antibiotics,' Oxford University Press, London, 1949; and senegenin: J. J. Dugan, P. de Mayo, and A. N. Starratt, *Canad. J. Chem.*, 1964, **42**, 491.

[10] J. S. Fritz and S. S. Yamamura, *Analyt. Chem.*, 1955, **27**, 1461.

[11] I. Szilagyi, T. Valyi-Nagy, I. Szabo, and T. Keresztes, *Microchim. Acta*, 1962, **4**, 671.

[12] T. Goto, K. Nakanishi, and M. Ohashi, *Bull. Chem. Soc. Japan*, 1957, **30**, 723.

[13] I. Szilagyi, T. Valyi-Nagy, and T. Keresztes, *Nature*, 1962, **196**, 376.

presumably, since the attachment was cleaved by mild acidic treatment, linked *via* an acetal system. On the assumption that the compound was a member of the macrolide class, and hence contained a lactone group, it could be tentatively concluded that primycin contained 12—15 hydroxy-groups.

The difficulties of analysis and low solubility precluded the possibility of obtaining more meaningful information. A major difficulty was that of handling a salt, and several studies were initiated in an attempt to remove this function. It was finally discovered that strong alkaline treatment (5N-potassium hydroxide at the b.p.) achieved the desired objective. Together with several minor (and unidentified) products the hydrolysis gave one major substance (2a). This could be isolated, as an amorphous material, in 42% yield by chromatography over silica gel.

The molecular weight of this substance was 1100 (osmometry), and there were several indications that it was not a guanidine. First, the Sakaguchi reaction was negative; secondly, the i.r. spectrum lacked the intense absorption at 1675 cm^{-1} attributable to the guanidine; and finally the material appeared to have no separate counter-ion. The product appeared to be, in fact, an amino-acid zwitterion ($\nu_{max.}$ 1560 cm^{-1}).

Hydrogenation of this substance over platinum oxide in acetic acid resulted in the uptake of 2 mol. equiv. in 1 day, but absorption continued thereafter. The presence of unsaturation already suspected was thus further confirmed, but it seemed probable that hydrogenation was being accompanied by hydrogenolysis.

Acetylation of (2a) gave the acetate (2b) ($C_{84}H_{133}NO_{33}$ †) as a sticky glass. Osmometry indicated a molecular weight of 1700 (*M* 1683), and quantitative study of the n.m.r. spectrum suggested (for a molecular weight of 1700) the presence of fifteen acetyl functions. This figure was corroborated by the saponification equivalent. It seemed probable that one of these functions was part of an amide group since acetylation of the amino-group was expected, and the i.r. absorption (1660 cm^{-1}) was appropriate for such a function.

The presence of a free carboxy-group in (2b) was confirmed by direct titration, giving an equivalent weight of *ca.* 1605, and by esterification with diazomethane to give the methyl ester (2c), $C_{85}H_{135}NO_{33}$ (*M* 1697; 1700 by osmometry). Here again the amide function was recognisable from the i.r. spectrum ($\nu_{max.}$ 3450, 1665, and 1520 cm^{-1}) and the introduction of the methoxy-group was revealed by a new n.m.r. signal at τ 6·32.

Hydrolysis of (2c) at room temperature gave the corresponding polyhydroxy-*N*-acetyl methyl ester (2d), the *N*-acetyl and ester methyl signals still being present in the n.m.r. spectrum. Hydrolysis of (2d) with methanolic hydrochloric acid gave (—)-D-arabinose, again [1] identified as the *p*-nitrophenylhydrazone. The continuing presence of the arabinose unit in (2a) was thus assured. Further information with regard to the arabinose function was obtained by oxidation.

Titration of (2d) with periodic acid resulted in uptake of 0·45 mol. equiv. in 70 h. With lead tetra-acetate the same uptake was observed in 10 h, and *ca.* 0·9 mol. equiv. in nearly 100 h. The slow uptake indicated that the arabinose was present in the furanose (in which the vicinal hydroxy-groups are *trans*) rather than in the pyranose form. The total uptake strongly suggested that no other vicinal glycol system was present in (2d) despite the evident massive hydroxylation of the molecule.

The immediate objective, then, was to determine the exact molecular weight of a derivative of (2). As with other primycin derivatives, (2c), the best characterised derivative, was too involatile. Methylation of (2d) with methyl iodide–silver oxide in dimethylformamide [14] gave the ester ether (2e), together with other substances, in low yield. This product, $C_{72}H_{137}NO_{19}$ (*M* 1319) was sufficiently volatile for the molecular weight to be determined mass spectrometrically.‡

Besides the molecular ion at *m/e* 1319 of low intensity a more intense peak indicating the loss of methanol (*m/e* 1287) was observed. Disconcertingly, there were also, relatively (to the molecular ion) weak peaks at 1333 and *ca.* 1345, and even as high as 1364—1366, but the exact masses were only determinable up to 1320. This gave warning that (2), and hence possibly primycin itself, might not be homogeneous, but might contain related homologous substances. Further evidence that this was so emerged from degradation studies.

The n.m.r. spectrum of (2e) provided further structural information. Aside from the absorption expected from the functional groups already characterised, absorption at τ 8·39 suggested the possibility of olefinic methyl groups. In addition to C*H*OMe absorption (4·82—5·20) there was also absorption at τ 4·65 (2H) indicative of possible vinylic protons. There was a likelihood, therefore, that (2) contained two trisubstituted ethylenic linkages, the degree of unsaturation being compatible with results of previous hydrogenation studies.

It seemed probable, on biogenetic grounds, that many of the hydroxy-groups of (2a) would be β to each other, and so any attempt at inter-relating functional groups by chemical means must involve sensitive and unstable intermediates. For this reason the direct trisection of the molecule was attempted. Ozonolysis of (2c) at —78° in methanolic solution, followed by reduction of the ozonide with sodium borohydride [15] *in situ* and subsequent acetylation, gave three products. These substances, which were trivially designated 'secoprimycins' were separated by chromatography.

Secoprimycin A.—The ester acetate (3a) of this substrate had the empirical formula $C_{33}H_{56}O_{12}$ (*M* 644

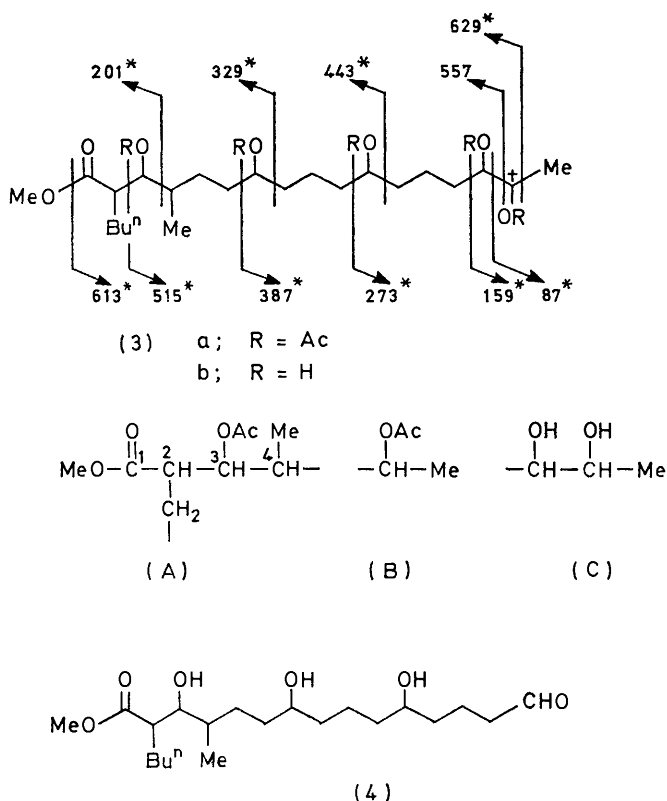
† The exact empirical formula was only known at the termination of the studies, but is given correctly here for simplicity.

‡ We thank Dr. B. C. Das (Gif-sur-Yvette) for this important determination.

[14] R. Kuhn, H. Frischman, and I. Low, *Angew. Chem.*, 1955, **67**, 32.

[15] J. A. Sousa, and A. L. Bluhm, *J. Org. Chem.*, 1960, **35**, 108; D. G. M. Diaper, and D. L. Mitchell, *Canad. J. Chem.*, 1960, **38**, 1976.

from mass spectrometry †) and showed a sharp three-proton n.m.r. singlet at τ 6·54 (C_6D_6) or 6·37 ($CDCl_3$) for the ester methoxy-group. The partial structures (A) and (B) could be recognised by decoupling experiments in deuteriobenzene solution, as follows.



Irradiation at τ 7·26 (the C-2 methine proton absorptions) caused the C-3 methine signal (4·67) to collapse to a doublet (*J* 6·5 Hz). This required, therefore, the presence of a proton at C-4. Irradiation at 4·67 caused collapse of the 2-H signal to an unresolved ‘triplet,’ establishing the contiguity of these protons. Irradiation at 8·3, *i.e.* where the C-4 methine proton signal was expected, caused collapse of the C-3 proton signal to a doublet (*J* 6·5 Hz) and simultaneously the methyl doublet at 9·17 became a singlet. Irradiation at 8·67 (CH_2 region) caused collapse of the C-2 proton multiplet to a broad ‘doublet.’ These results appear to require the part structure (A) for (3a).

In a similar way irradiation at τ 4·95 caused collapse of the methyl doublet at 8·93 and irradiation at 8·93 simplified the multiplet for C*H*OAc at 5·2—4·8. The part structure (B) was thus also required. In sum, from n.m.r. data it could be deduced that (3a) was a methyl penta-acetoxy-carboxylate containing one primary and two secondary methyl groups, and five secondary acetates, and that the systems (A) and (B) were included in this array.

The presence of an acetoxy-group β to the methoxy-carbonyl group in (3a) was demonstrated chemically: treatment of this substance with sodium acetate in acetic anhydride at reflux temperature generated (by β-elimination) an αβ-unsaturated ester. This was readily recognised by its u.v. absorption spectrum ($\lambda_{max.}$ 217 nm; ε 9000).

Hydrolysis of (3a) under mild alkaline conditions, followed by esterification with diazomethane, gave the crystalline hydroxy-ester (3b), m.p. 101—103°. Titration of this pentaol with periodate resulted in the uptake of 0·73 mol. equiv. in 15 h and the liberation of acetaldehyde (characterised as the dimedone derivative). The other product was the aldehyde (4), $C_{21}H_{40}O_6$ (M^+ 388). This apparently existed as a mixture of anomeric hemiacetals since no aldehydic proton signal was visible in the n.m.r. spectrum, and in agreement there appeared to be signals for the anomeric methine protons at *ca.* 6·5 and *ca.* 5·7 of about equal intensity. The presence of the partial structure (C) in (3b) is thus also required.

Modified Huang-Minlon reduction [16] of (4) was then investigated as the first step towards its conversion into a saturated acid and the identification of the carbon skeleton. The products of this reaction were unexpected, consisting of hexanoic acid and a crystalline diol (5a), m.p. 106—107°, $C_{14}H_{30}O_2$ (M^+ 230). The n.m.r. spectrum of the latter showed the presence of three *C*-methyl groups, one primary and two secondary, which, together with that contained in hexanoic acid, made one more than the expected total of three methyl groups from the reduction of (4). A small amount of a crystalline ester (6a), m.p. 95—98°, evidently the expected product, was also obtained from the reduction, and this had the two primary and one secondary methyl groups expected. The cleavage of (4) under the reductive conditions had evidently led to generation of an additional methyl group.

Acetylation of (5a) gave the diacetate (5b), the n.m.r. spectrum of which revealed only two C*H*OAc systems: both alcohol functions in (5a) were, therefore, secondary. Since (4) contained three hydroxy-groups, and since a new methyl group was generated during the reduction it was clear that a reverse aldol-type reaction had occurred in that region of the molecule represented by part structure (A), as represented in sequence (D) ⟶ (E) ⟶ (F). With this information the part structure (G) could be written for (4), leaving the two remaining hydroxy-groups [those present in (5a)] still to be placed. These were located by high resolution mass spectrometry, the main fragmentation being shown on formula (5b).‡ Confirmation came from the fragmentation of (6a) as shown. Combination of (4) with the

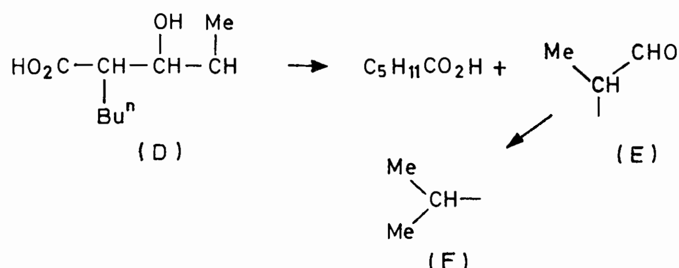
† In this paper reference will be made briefly to certain mass spectrometric data. All these were determined by Professor D. B. MacLean and his colleagues (McMaster University) and are described in much more detail, and together with much further information, elsewhere in this series. His data enable an independent deduction of the representation.

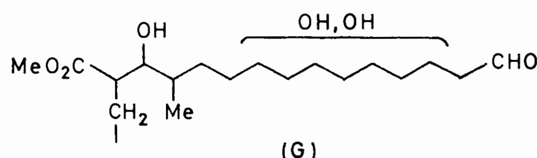
‡ The compositions of peaks marked with an asterik in this and later formulae were established by high resolution measurements.

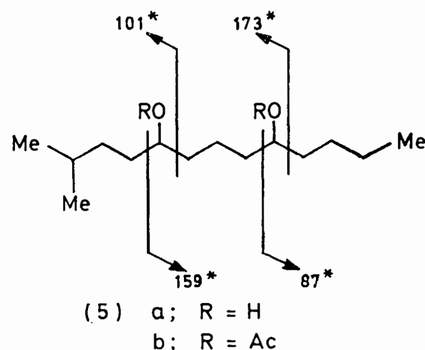
[16] S. Sarel and Y. Yanuka, *J. Org. Chem.*, 1959, **24**, 2018.

part structure (C) established the structure of secoprimycin A methyl ester as (3b). Here again the fragmentation pattern supported the interpretation.

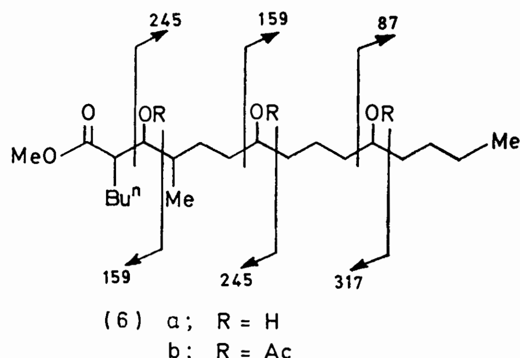
It has already been mentioned that the mass spectrum of the ether ester (2e) indicated the possible presence of homologous or related substances as contaminants.







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



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

The mass spectrum of (5b) showed the expected molecular ion peak at *m/e* 314. A weak peak at *m/e* 342 was also present, possibly due to a homologue containing two more carbon atoms, corresponding approximately to one of the weak higher mass peaks of (2e).

Secoprimycin B.—The polyacetate of this substance (7) had the empirical formula $C_{42}H_{64}O_{22}$ (M^+ 920). The n.m.r. spectrum indicated the presence of only one methyl group (secondary) and, in view of the empirical formula, ten acetoxy-functions (a value supported by the saponification equivalent). Of the latter, two appeared to be primary acetates with the methylene proton signals in the region τ 5·46—6·16 together with the two C*H*OR signals. The signal for the anomeric proton at τ 4·78 appeared as a broad singlet, an indication that the sugar is attached as the α-anomer.[17]

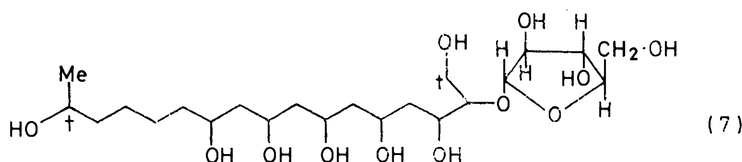
On mild alkaline hydrolysis and passage through a strongly acidic cation-exchange resin the alcohol (8a) was obtained, with concomitant hydrolysis of the glycosidic linkage. This alcohol was crystalline (m.p. 161—163°) and was accompanied by a further substance, m.p. 151—153°, in smaller amount which was not further investigated. Acetylation of (8a) gave (8b), $C_{33}H_{52}O_{16}$ (M^+ 704) as an oil. Titration of (8a) with periodate resulted in uptake of 2 mol. equiv. of oxidant and generation of formaldehyde (characterised as the dimedone derivative). At the same time an aldehyde was produced, which, because of its instability, was reduced directly with sodium borohydride to the crystalline alcohol (9a), m.p. 128—130° (M^+ 308). This overall change corresponded to the cleavage of a vicinal triol and required the system (H) in (8a). The partial structures (B) and (I) were shown to be present in (9b) by decoupling experiments as follows. Irradiation at τ 5·09 caused collapse of the methyl doublet at τ 8·82 to a singlet; irradiation at 8·8, in turn, simplified the *ca.* 5·0 region. Irradiation at 8·25 (methylene) caused collapse of a triplet at 5·93 to a singlet, and irradiation at 5·93 simplified the methylene region.

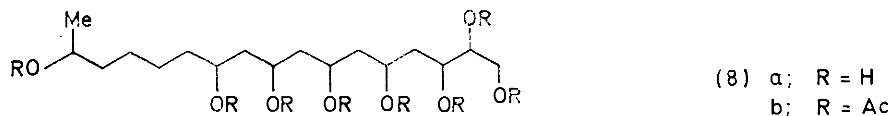
The mass spectrum of (9b) clearly showed the presence of four contiguous methylene groups next to the acetate contained in (B). Since, from periodate experiments, no further contiguous hydroxy-functions remained in (9a), the β location of the remaining acetates in (9b) was required and was confirmed by the main fragmentations indicated. With structure (9) established, structure (8) followed. The placing of the arabinose to give structure (7) is described in the following paper.

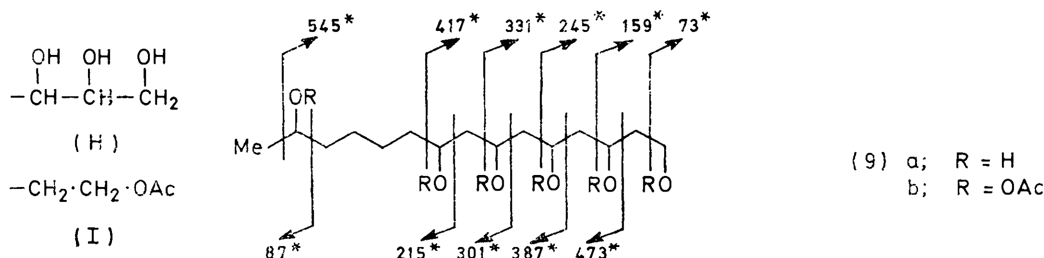
Mild alkaline hydrolysis of the acetate of (7) followed by an isolation procedure not involving exposure to strong acid gave (7) itself, which could not, however, be obtained pure. Acidic hydrolysis gave arabinose, identified as the *p*-nitrophenylhydrazone and as the tetra-acetate. The alcohol (8a), identified as the acetate (8b), was formed at the same time. Evidently in the original experiments the strongly acidic resin was able to hydrolyse the glycosidic linkage and (since none was isolated) to destroy the released arabinose.

Secoprimycin C.—This substance contained the nitrogen function. The acetate (10a) had the empirical formula $C_{18}H_{31}NO_7$ (M^+ 373) and the complete structure could be deduced from the n.m.r. spectrum and from associated decoupling experiments. The chemical shifts are shown on formula (11), more detail being presented

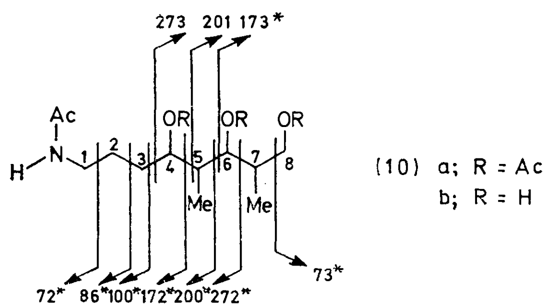
[17] J. D. Stevens and H. G. Fletcher, *J. Org. Chem.*, 1968, **33**, 1799; L. D. Hall and P. R. Steiner, *Canad. J. Chem.*, 1970, **48**, 1155; K. L. Reinhart, W. S. Chilton, M. Hichens, and W. V. Phillipsborn, *J. Amer. Chem. Soc.*, 1962, **84**, 3216.

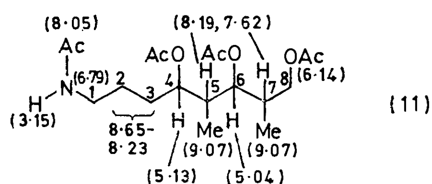


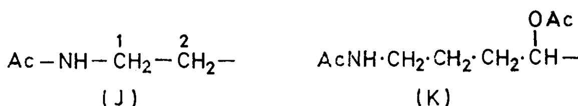


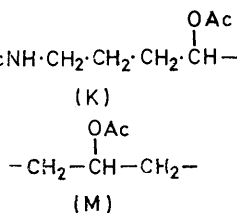


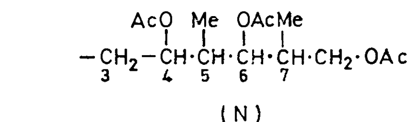
in the Experimental section. Irradiation near τ 3·15 caused collapse of the signal at 6·79 to a triplet (J 6 Hz); irradiation at 6·77 caused collapse of the broad NH signal to a singlet and simplified the 8·23—8·65 region. There was no change in the C-4, C-5, and C-8 proton signals. This requires part structure (J). Irradiation near 8·43 (C-2 and C-3 protons) caused the C-1 methylene signal at 6·79 to a collapse to a doublet (J 6 Hz) and also simplified the C-4 proton multiplet at 5·23, permitting the extension of structure (J) to (K). Since, also, the irradiation had no effect on the signals due to the C-6 and C-8 protons, the part structures (L) and (M) could be excluded.











Simultaneous irradiation at the frequencies corresponding to the C-5 and C-7 protons caused collapse of the τ 9·07 doublet, and the multiplet at 5·13 became an apparent triplet. The C-6 methine doublet of doublets (5·04) became a singlet and the doublet at 6·14 became a singlet. This requires the part structure (N). This interpretation was supported by the fact that irradiation at 6·14 (C-8 protons) simplified the 7·62—8·19 (C-5 and C-7 protons) region without affecting the signals at 8·23—8·65 (C-2 and C-3 protons). Further, irradiation at 5·07 (C-4 and C-6 protons) simplified the multiplet at 8·19—7·62 (C-5 and C-7 protons) and also simplified the C-2 and C-3 proton region at 8·65—8·23 without affecting the doublet at 6·14.

With the requirements implied by (K) and (N) the structure (10a) is deduced. Independent support for aspects of this was obtained from the n.m.r. spectrum of the corresponding hydroxy-amide (10b), obtained by mild alkaline hydrolysis of (10a). This had the empirical formula $C_{12}H_{25}NO_4$ (M 247), and was clearly also a secondary amide ($\nu_{max.}$ 3300, 1635, and 1550 cm^{-1}).

The n.m.r. spectrum ([$^2H_5$]pyridine) showed the methylene protons (C-8) as the apparent AB part of an ABX pattern (τ_A 6·06; τ_B 6·15; J_{AX} 5; J_{BX} 5·5; and J_{AB} 10·5 Hz). The C-6 methine again appeared as a doublet of doublets (τ 5·82; J 4·5 and 7 Hz).

Finally, a complete and independent proof of structure (10a) was obtained from the mass spectral fragmentation pattern. The main fragmentations are shown on formula (10a) and the analysis is discussed in detail in the following paper.

Deuterium Labelling.—When (2c) was ozonised and reduced with sodium borodeuteride instead of borohydride the ester (3a) contained one deuterium atom (*M* 645) at the carbon atom marked with a dagger. The terminal methyl signal in the n.m.r. spectrum of the product appeared as a singlet (τ 8·82) and the 5·4—4·8 region had lost absorption corresponding to one proton. The presence of the deuterium atom indicates the particular alcoholic function introduced during the ozonolysis, and hence marks the site of the original ethylenic linkage cleaved.

The deca-acetate of (7) incorporated two deuterium atoms, one at each of the carbon atoms marked with a dagger. The terminal methyl signal now appeared as a singlet at 8·82, and the regions 6·1—5·5 (CH_2·OAc) and 5·3—4·7 (CH·OAc) had each lost absorption corresponding to one proton. Secoprimycin B thus formed the centre section of the chain constituting the primycin structure; again the daggers mark the sites of the original ethylenic linkages.

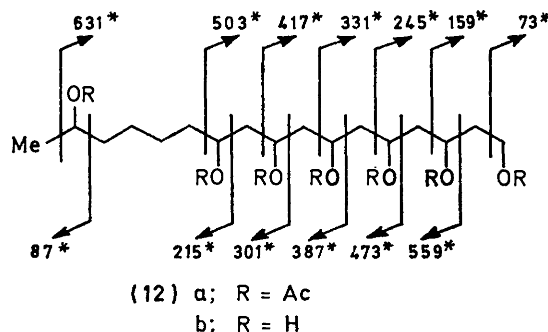
The triacetoxy-amide (10a) incorporated one deuterium atom only and the terminal methylene (CH_2·OAc) signal then integrated for only one proton and appeared as a pair of doublets centred at 6·15 and 6·11, possibly owing to the presence of stereoisomers epimeric at the new chiral centre.

For the completion of the structure of (2) it remained to ascertain its relative orientation in the chain of secoprimycin B, and the location of the arabinose function. For the further elaboration of primycin itself the site of lactonisation must also be ascertained. These aspects are discussed in Part III.

Homogeneity of the Ester (2c).—In earlier experiments, ozonlysis of (2c) which was apparently homogeneous † produced besides the secoprimycins a minor amount of a mixture of two acetates. One of these was the octa-acetate (8b) and the other a hepta-acetate (12a). In addition there was a small amount of a substance greatly resembling (10a) spectroscopically, but differing in t.l.c. behaviour. It may represent an isomer differing in configuration at one of the asymmetric centres, the most probable being C-7.

The hepta-acetate, $C_{31}H_{50}O_{14}$ (M^+ 646), had the partial structures (B) and (L), determined from n.m.r. decoupling experiments as previously described for the acetate (9b). The corresponding alcohol (12b), $C_{17}H_{56}O_7$ (M^+ 352), differed from (8a) only by one hydroxy-group. On attempted periodate oxidation, however, no oxidant was consumed, suggesting the tentative structure (12b). Proof of structure was forthcoming from mass spectrometric studies by Professor MacLean and his collaborators reported in the following paper. The main fragmentation cleavages are indicated on the formula.

The presence of (8b) in the mixture requires no special explanation since dilute acid was used during isolation.



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

The relationship, if any, of (12) to (7) is not understood, and it is clear that (12) is not an integral part of (2). The most probable explanation is that (12) is derived from a contaminant of (2).

Depending on the severity of the reaction conditions in the formation of (2a) a number of minor unidentified products were formed, together with hexanoic acid. No detectable amounts of other volatile acids were produced, and the variation of yield of hexanoic acid with base concentration made it unlikely that it was formed by simple ester hydrolysis. A reversed aldol-type process of the type observed for (4) seems most acceptable.

EXPERIMENTAL

M.p.s were obtained on a Reichert–Kofler hot-stage apparatus. I.r. and u.v. spectra were recorded by means of Beckman IR 10 and Cary 14M spectrometers, respectively. N.m.r. spectra were recorded on a Varian A-60 or HA-100 spectrometer (solvent [^{2}H]chloroform unless otherwise indicated), with tetramethylsilane as internal standard. Unless indicated otherwise, solvents for i.r. and u.v. spectroscopy were chloroform and methanol, respectively. The mass spectra were recorded on a CEC 21-110B spectrometer at McMaster University, Hamilton. T.l.c. was carried out on Camag Kieselgel DF-5 and Merck Silica Gel GF-254; the same materials were used for chromatographic separations. G.l.c. analysis were made with an F & M 700 instrument equipped with 6 ft × 1/4 in columns packed with 10% FFAP on 60—80 mesh Chromosorb P. Samples for analysis were dried at room temperature *in vacuo* for 2—5 days. Molecular weights measured by osmometry were determined on a Hewlett–Packard 301A vapour pressure osmometer.

Primycin.—As received from Medimpex (Hungary) this had m.p. 192—195° (decomp.) (Found: C, 56·7; H, 9·65; N, 3·8; O, 27·15; S, 2·65; Ash, 0; OCH_3 <0·05%), light absorption (EtOH) ε_{205} 14,000, ε_{200} 22,000 (*M* 1500), $\nu_{max.}$

† The purity of (2c) appeared adequate as judged by spectroscopic methods and t.l.c. However when 'pure' (2c) was converted into (2d) which was then purified again and reacetylated, the recovered (2c), although apparently identical with the original sample, gave only the secoprimycins on ozonolysis and reduction (see the borodeuteride experiments in the Experimental section for details).

(KBr) 3400s, 2950, 2880, 2710sh, 1675, 1640sh, 1465, 1385, and 1117 cm^{-1}, τ [$(CD_3)_2SO$] 8·78, 8·37, 8·08, 5·83, 5·32, 4·81, 2·05, and 1·38 (spectrum inadequate for meaningful integration). Primycin gave a positive Sakaguchi reaction, but gave negative results in tests with iron(III) chloride and with copper (Beilstein). It gave a positive Dragendorff test. Fusion with sodium in tests for elements showed the presence of nitrogen and sulphur and the absence of halogens.

Primycin (17·12 mg) in methanol (25 ml) was passed through a column (5 × 1 cm) of Dowex 1-X2 (50—100 mesh) resin in its OH^- form, and eluted with 75 ml of solvent. To the alkaline effluent was added sulphuric acid (0·02N; 2 ml) and the solution was evaporated under reduced pressure at room temperature to *ca.* 6 ml. Methanol (5 ml) was then added and the solution titrated potentiometrically (E.I.L. 23A pH meter) (titre 0·68 ml, giving an equivalent weight of 1260, and pK_a *ca.* 11·2).

Sulphate Titration.—Primycin (28·76 mg) dissolved in methanol was passed through a column (5 × 1 cm) of Dowex 50W-X2 (200—400 mesh) in its H^+ form and the column washed with solvent until the effluent was neutral. The combined washings were adjusted to pH 3 with perchloric acid and titrated with 0·005M-barium perchlorate in methanol–water (4 : 1; pH 3·5 adjusted with perchloric acid); indicator Thorin [2-(2-hydroxy-3,6-disulpho-1-naphthylazo)benzenearsonic acid]; titre 2·153 ml. An average of three such titrations gave an equivalent weight of 1143. The precipitate from the titrations was collected, washed with water, and dried. The i.r. spectrum was identical with that of barium sulphate.[18]

Oxidation of Primycin.—A mixture of primycin (100 mg), saturated aqueous potassium permanganate (100 ml), and potassium hydroxide (500 mg) was heated at 60° for 1 h. The excess of permanganate was destroyed with sodium hydrogen sulphite and the manganese dioxide was filtered off. After acidification (hydrochloric acid) the solution was evaporated under reduced pressure and the residue triturated with ethanol (95%; 5 ml). The presence of guanidine hydrochloride could be shown by t.l.c. [Bu^nOH–HOAc–H_2O (4 : 1 : 5); alkaline ninhydrin [19] spray].

Acidic Hydrolysis of Primycin.—Primycin (1·719 g) in methanol (250 ml) was heated under reflux with hydrochloric acid (0·2N; 250 ml) for 12 h. The cooled solution was passed through a column (2·5 × 30 cm) of Dowex 3 (20—50 mesh) in the free base form. The effluent was then passed through a column (3 × 25 cm) of Dowex 50W-X2 (50—100 mesh) in its H^+ form. The neutral products (166 mg) were obtained by evaporating the effluent to dryness under reduced pressure. T.l.c. on cellulose powder showed a single spot (aniline phthalate: red-brown) in three different solvent systems with the same R_F as arabinose. The sugar crystallised from 95% ethanol in prisms, m.p. 158—159°, mixed m.p. with (−)-D-arabinose not depressed, mixed m.p. with (+)-L-arabinose 133—148°, $[\alpha]_D$ −113° (*c* 0·30 in H_2O), i.r. spectrum identical with that of (−)-D-arabinose; *p*-nitrophenylhydrazone, m.p. 186—188° (from methanol), not depressed on admixture with authentic (−)-D-arabinose *p*-nitrophenylhydrazone, depressed to 163—171° on admixture with the (+)-L-derivative.

Hydrolysis of Primycin.—A mixture of primycin (5 g), aqueous potassium hydroxide (150 ml; 5N), and butanol (a few drops to avoid frothing) was refluxed (nitrogen) for 4 h, cooled, made just acidic with hydrochloric acid (conc.), and evaporated to dryness under reduced pressure. The residue was extracted with chloroform–methanol (4 : 1), the extract was evaporated to dryness, and the residue was chromatographed on silica gel (GF-254; eluant chloroform–methanol–conc. ammonia, 12 : 8 : 1) to give the product (2a) (2·137 g, 42·7 wt.%) as a colourless froth, $\nu_{max.}$ (Nujol) 3300, 1650w, 1560, 1075, and 975 cm^{-1}, *M* (osmometer; methanol solvent) 1100.

In a similar experiment the hydrolysate was passed through a column of Dowex 1-X2 (50—100 mesh) in its basic form. The effluent, after acidification with hydrochloric acid (pH 4) was evaporated; the product showed ε_{200} 22,000, ε_{205} 13,000 (for *M* 1300) together with weak absorption at longer wavelengths. The crude aglycone † (121 mg) in methanol (5 ml) was hydrogenated over platinum oxide (33 mg). After 3 h the uptake was *ca.* 2 mol. equiv. and the product had ε_{200} 5000, ε_{205} 2600.

Hydrogenation of the Amino-acid (2a).—Adams catalyst (30 mg) was added to a solution of the amino-acid (2a) (86 mg) in acetic acid (5 ml) and the mixture was shaken under hydrogen for 26 h. The net uptake was 4·44 ml at S.T.P. [Calc. for 2 mol. (*M* 1010) 4·44 ml].

Acetylation of the Amino-acid (2a).—Acetylation (room temperature; acetic anhydride–pyridine) and then chromatography on silica gel (chloroform–methanol, 9 : 1) gave the *polyacetylated acid* (2b) as a sticky glass, $\nu_{max.}$ 3400, 2940, 2850, 1730, 1660, 1440, 1320, 1225, and 1020 cm^{-1} (Found: C, 59·8; H, 8·1; N, 0·9%. $C_{84}H_{133}NO_{33}$ requires C, 59·85; H, 7·95; N, 0·85%).

Hydrolysis of the Polyacetate (2b).—The polyacetate (2b) (54·8 mg) was treated with 0·2N-sodium hydroxide (5 ml) at room temperature for 16 h and the excess of base was back titrated potentiometrically with 0·1N-hydrochloric acid: net titre 4·6 ml, *i.e.* 14·3 equiv. of alkali per mole of the polyacetate (2b). Two other determinations gave 14·4 and 15·97 equiv.

Potentiometric Titration of the Polyacetate (2b).—A solution of the polyacetate (2b) (57·9 mg) in methanol (a few ml) was percolated through a 10 × 1 cm column of Dowex 50W-X2 (50—100 mesh; H^+ form) and the column was washed with more methanol (100 ml). The combined effluent solution was titrated potentiometrically with 0·1N-sodium hydroxide; a net titre 0·90 ml, equiv. wt. 1605. A second determination gave a value of 1602.

The Polyacetyl Methyl Ester (2c).—Treatment of the polyacetate (2b) with ethereal diazomethane gave the polyacetyl methyl ester (2c) as a syrup, $\nu_{max.}$ 3450, 3030, 2950, 2870, 1730, 1665, 1520, 1430, 1375, 1250, 1110, 1020, and 970 cm^{-1}, τ 9·12br (12H, doublet, *J ca.* 6·5 Hz, 4 Me), 8·85—8·15 (m, CH_2, CH), 8·02, 8·0, and 7·93 (45H, three singlets, 14 OAc, NAc), 6·32 (s, CO_2Me), 5·70 (2—3H, m), 5·45—4·80 (*ca.* 16H, m), and 3·70br (1H, t, *J ca.* 6 Hz, *N*H·CH_2). The saponification equivalents of (2c) as determined for (2b) were found to be 15·2 and 15·0, respectively, in two different estimations for *M* 1700.

The N-*Acetyl Methyl Ester* (2d).—The solutions (after the estimation of the number of *O*-acetate groups) from the alkaline hydrolysis of the polyacetyl methyl ester (2c) were combined and made just acidic (2N-HCl). The product was

† The arabinose decomposed during this process and was retained by the column. This observation was checked by a control experiment.

[18] J. M. Hunt, M. P. Wisherd, and L. C. Bonham, *Analyt. Chem.*, 1950, **22**, 1478.
[19] R. B. Conn and R. B. Davis, *Nature*, 1959, **183**, 1053.

isolated with butanol to give a froth which was treated with diazomethane and chromatographed (chloroform–methanol, 2 : 1) to give the *N*-acetyl methyl ester (2d) as a sticky froth.

Periodate Titrations of the N-*Acetyl Methyl Ester* (2d).—To a solution of the *N*-acetyl methyl ester (2d) (25·95 mg) in methanol (15 mg), aqueous 0·05M-sodium periodate (5 ml) was added, and the volume was made up to 100 ml with water. The solution was kept at room temperature in the dark, and at intervals 10 ml samples were taken, and titrated in the usual way; net titre 122 ml 0·01N-iodine after 24 and 70·5 h, equivalent to 0·45 moles of periodate per mole of (2d) (for $M = 1100$).

Titrations of (2d) with lead tetra-acetate in acetic acid showed uptakes of 0·45 mol. equiv. in 10 h, 0·75 in 48·5 h, and 0·88 in 97 h.

Hydrolysis of the N-*Acetyl Methyl Ester* (2d) *with Hydrochloric Acid*.—A mixture of the *N*-acetyl methyl ester (2d) (19·8 mg), methanol (1 ml), and 0·2N-hydrochloric acid (1 ml) was heated in a closed flask on a steam-bath for 5 h (N_2). After neutralisation (0·2N-NaOH) the solvents were evaporated off. T.l.c. of the residue on cellulose powder (aniline phthalate spray) showed a red-brown spot identical in R_F value with that of authentic (−)-D-arabinose. To the residue in methanol (2 ml), *p*-nitrophenylhydrazine (5 mg) was added and the mixture was heated. The (−)-D-arabinose *p*-nitrophenylhydrazone (0·1 mg), yellow needles, m.p. 183—185° (from methanol), mixed m.p. not depressed on admixture with an authentic sample, mixed m.p. with (+)-L-arabinose *p*-nitrophenylhydrazone 162—166°, was separated by preparative t.l.c. (chloroform–methanol, 4 : 1).

Methylation of the N-*Acetyl Methyl Ester* (2d).—A mixture of (2d) (233 mg), dimethylformamide (10 ml), methyl iodide (2 g), and silver oxide (2 g) was stirred at room temperature for 4 h. More methyl iodide (2 g) was then added and stirring was continued for an additional 16 h. The precipitate formed was centrifuged and washed repeatedly with dimethylformamide. The centrifugate was combined with the washings and then diluted with water (50 ml). Sodium cyanide (1 g) was added and the products were extracted with chloroform. The extract was washed with water, dried, and evaporated; the oily residue (274 mg) showed a strong OH band in the i.r. The procedure was repeated first for 1 day and then again for 3 days. The oily residue (230 mg) obtained after the third treatment was chromatographed (chloroform–methanol–petroleum, 3 : 1 : 6) to give the N-*acetyl methyl ester polymethyl ether* (2e) (76 mg) as a syrup, $\nu_{max.}$ (film) 2930, 2870, 1740, 1650, 1460, 1375, 1270w, 1235w, 1190, and 1090 cm^{-1} (Found: C, 65·45; H, 10·5; N, 1·2. $C_{72}H_{137}NO_{19}$ requires C, 65·5; H, 10·4; N, 1·05%).

Ozonolysis of the Polyacetyl Methyl Ester (2c).—A solution of (2c) (813 mg) in methanol (20 ml) was cooled in solid carbon dioxide–acetone, and a slow stream of ozone was passed through until a slight blue colour persisted in the solution. Sodium borohydride (2·5 g) in methanol (20 ml) was added in portions and the mixture was kept at room temperature for 20 min. The solution was acidified (2N-HCl) and the product isolated with chloroform. Evaporation gave a viscous oil (776 mg) which was acetylated (acetic anhydride–pyridine; room temperature). Chromatography (chloroform–methanol, 9 : 1) gave *methyl* 3,7,11,15,16-*penta-acetoxy*-4-*methylheptadecanoate* (3a) (258 mg) as a syrup, $\nu_{max.}$ 3000, 2960, 2870, 1725, 1460, 1440, 1375, 1230, and 1025 cm^{-1}, τ ($CDCl_3$) 9·14 (t, *J* 6·5 Hz, Me), 9·12 (d, *J* 6·5 Hz, Me), 8·85 (d, *J* 6·5 Hz, Me), 8·9—8·2 (m, CH, CH_2), 7·99, 7·98, 7·97, 7·96, and 7·92 (15H, five singlets, 5 OAc), 7·5—7·2 (1H, m, C*H*·CO_2Me), 6·37 (s, CO_2Me), and 5·34—4·85 (5H, m, 5 CH·OAc), τ (C_6D_6) 9·18 (t, *J* 6·5 Hz, Me), 9·17 (d, *J* 6·5 Hz, Me), 8·93 (d, *J* 6·5 Hz, Me), 8·85—8·30 (m, CH_2 and CH), 8·26, 8·24, 8·22, and 8·19 (5 OAc), 7·4—7·15 (m, C*H*·CO_2Me), 6·54 (s, CO_2Me), 5·2—4·8 (4H, m, C*H*·OAc), and 4·67 (1H, t, *J* 6·5 Hz, C*H*·OAc) (Found: C, 62·0; H, 8·9. $C_{33}H_{56}O_{12}$ requires C, 61·45; H, 8·75%); the deca-acetate (7) (218 mg) as a syrup, $\nu_{max.}$ 3000, 2915, 1735, 1430, 1370, 1235, 1040, 1025, and 980 cm^{-1}, τ 8·82 (d, *J* 6·5 Hz, Me), 8·74—8·11 (16H, m, 8 CH_2), 8·00, 7·95, 7·91, and 7·90 (30H, 10 OAc), 6·16—5·46 (6H, m, 2 CH_2·OAc, 2 CH·O·C), 5·26—4·93 (6H, m, 6 C*H*·OAc), 4·90 (2H, d, *J* 1·5 Hz, C*H*·OAc of furanose), and 4·78br (1H, s, anomeric proton) (Found: C, 54·7; H, 7·1. $C_{42}H_{64}O_{22}$ requires C, 54·75; H, 7·0%); and N-(4,6,8-*acetoxy*-5,7-*dimethyloctyl*)-*acetamide* (10a) (142 mg) as a syrup, $[\alpha]_D^{25}$ +11·2 (*c* 3·72 in MeOH), $\nu_{max.}$ 3440, 2990, 1880, 1730, 1665, 1515, 1455, 1370, 1200—1250, 1020, 965, and 900 cm^{-1}, τ 9·07 (6H, d, *J* 7 Hz, 2 Me), 8·65—8·23 (m, 2 CH_2), 8·19—7·62 (m, 2 C*H*Me), 8·05 (s, NAc), 7·97 and 7·94 (9H, 3 O*Ac*), 6·79 (2H, apparent q, *J* 6 Hz, NH·CH_2·CH_2), 6·14 (2H, d, *J* 6 Hz, CH·CH_2·OAc), 5·13 [1H, m, CH_2·C*H*(OAc)CH], 5·04 [1H, dd, *J* 4·5 and 7 Hz, CHC*H*(OAc)CH], and 3·15br (1H, t, *J* 6 Hz, AcN*H*·CH_2) (Found: C, 57·5; H, 8·35; N, 3·3. $C_{18}H_{31}NO_7$ requires C, 57·9; H, 8·35; N, 3·75%); together with a mixture (61 mg) (single spot on t.l.c.) which on alkaline hydrolysis gave *heptadecane*-1,2,3,5,7,9,11,16-*octaol* (8a), microplates, m.p. 161—163° (from methanol–di-isopropyl ether), $\nu_{max.}$ (KBr) 3300, 2930, 2860, 1630br,w, 1465, 1420, 1380, 1345, 1080, and 920 cm^{-1}; *m/e* 332 ($M^+ - 2H_2O$), 314 ($M^+ - 3H_2O$), 296 ($M^+ - 4H_2O$), 278 ($M^+ - 5H_2O$), and 260 ($M^+ - 6H_2O$); and the heptaol (12b), m.p. 159—161° (from methanol–di-isopropyl ether), characterised as its acetate (see below), $\nu_{max.}$ (KBr) 3300, 2930, 1630br,w, 1465, 1425, 1380, 1345, 1145, 1085, 1055, and 920 cm^{-1}, *m/e* 352, 319 ($M^+ - H_2O - CH_3$), 298 ($M^+ - 3H_2O$), 280·204 (Calc. for $C_{17}H_{28}O_3 \equiv M^+ - 4H_2O$: 280·204), and 262 ($M^+ - 5H_2O$).

Alkaline Hydrolysis of the Penta-acetoxy-methyl Ester (3a).—The number of *O*-acetate groups in compound (3a), determined by a method similar to that described for the polyacetate (2c), was 5·15 (*M* 644). The alkaline solution obtained after the estimation of the number of *O*-acetates was acidified (2N-HCl) and extracted with chloroform–butanol (9 : 1). Evaporation of the extract gave a solid residue which was treated with ethereal diazomethane to give *methyl* 3,7,11,15,16-*pentahydroxy*-4-*methylheptadecanoate* (3b), white microcrystals, m.p. 101—103° [from chloroform–petroleum (b.p. 60—80°)], $\nu_{max.}$ 3380, 2930, 2860, 1725, 1460, 1440, 1380, 1170, and 900 cm^{-1}, *m/e* 398 ($M^+ - 2H_2O$) (Found: C, 63·9; H, 10·5. $C_{23}H_{46}O_7$ requires C, 63·55; H, 10·65%).

Periodate Titration of the Ester (3b).—On titration as for (2d) the substance had consumed 0·65 mol. equiv. of periodate after 1 h, 0·67 after 5 h, and 0·73 after 7 h. There was no further change after 15 h.

Acetaldehyde from the Periodate Reaction of the Ester (3b).—The pentahydroxy-methyl ester (3b) (22·3 mg) was dissolved in dioxan (0·5 ml). Nitrogen was bubbled through very slowly for 20 min and then 0·3M-sodium periodate (0·3 ml), followed by water (0·5 ml), was introduced through the nitrogen inlet tube. A slow stream of nitrogen was passed through the mixture for 3 h and the

acetaldehyde formed was trapped in a hot solution of dimedone (30·2 mg) in dioxan (1 ml) and water (10 ml). The white crystals formed were filtered off, washed with water, and dried at room temperature *in vacuo* (yield 4 mg); m.p. 136—138°. Recrystallisation from aqueous ethanol gave white microplates, m.p. 138—140°, identical (mixed m.p.) with the authentic dimedone derivative of acetaldehyde. The filtrate was concentrated *in vacuo* to give 2 mg more of the derivative.

Methyl 2-*Butyl*-3,7,11-*trihydroxy*-4-*methyl*-15-*oxopentadecanoate* (4).—To a solution of the ester (3b) (830 mg) in methanol (5 ml) a solution of sodium periodate (450 mg) in water (5 ml) was added, and the resulting solution was kept for 30 min in the dark. The excess of periodate was destroyed (mannitol; 150 mg) and the product, isolated with chloroform, was obtained as an oily residue. Chromatography (chloroform–methanol, 98 : 2) yielded the aldehyde (4) (644 mg), as a viscous oil, $\nu_{max.}$ (film) 3400, 2940, 2860, 1735, 1715, 1460, 1360, 1275, 1200, 1110, 1070, 1035, and 980 cm^{-1}, τ ([$^2H_5$]pyridine) 9·15 (t, *J* 6 Hz, Me), 8·85 (d, *J* 6 Hz, Me), 8·8—7·5 (m, CH_2, CH), 7·3—6·9 (1H, m, C*H*CO_2Me), 6·7—6·4 (0·5H, m, anomeric methine proton), 6·26—5·90 (2H, m, 2 C*H*·OH), 6·31 (s, CO_2Me), 5·90—5·55 (0·5H, m, anomeric methine proton), 5·15—4·95 (0·5H, anomeric OH), 4·42br (0·5H, s, anomeric OH), and 4·3—3·9 (hump, OH), *m/e* 388. The presence of half-proton signals suggests that this product is a mixture of approximately equal parts of the α- and β-anomers of either a five-membered or a six-membered hemiacetal.

Huang-Minlon Reduction of the Aldehyde (4).—A mixture of (4) (109·8 mg), ethylene glycol (4 ml), and 85% hydrazine hydrate (4 ml) was heated at 100° for 1 h. After cooling, solid potassium hydroxide (660 mg) was added in portions, the condenser was removed, and the temperature was slowly raised to *ca.* 200°. The mixture was refluxed at this temperature for 2·5 h, then cooled, and the product was isolated with chloroform. A solid residue (32·3 mg) was obtained which, when sublimed (80°; 0·01 mmHg) gave 2-*methyltridecane*-5,9-*diol* (5a) (26 mg), needles, m.p. 106—107° [from chloroform–petroleum (b.p. 60—80°)], $\nu_{max.}$ (KBr) 3340, 3230, 2900, 1450, 1385, 1360, 1140, 1110, 1070, and 935 cm^{-1}, *m/e* 230 and 229·219 (Calc. for $C_{14}H_{29}O_2 \equiv M - 1$: 229·216), τ ([$^2H_5$]pyridine) 9·12 (6H, d, *J* 6 Hz, 2 Me), 9·12 (apparent t, *J ca.* 6 Hz, Me), 8·93—7·93 (17H, m, CH_2 and CH), 6·41—6·08 (2H, m, C*H*·OH), and 5·26—4·93 (OH). The aqueous solution was acidified with 2N-hydrochloric acid and the product isolated with chloroform. The chloroform was removed on a steam-bath by using a small fractionating column, until *ca.* 3 ml of solution was left. This was treated with ethereal diazomethane and then analysed by g.l.c. Only one major peak, identical in retention time (ascertained by mixed injection) with an authentic sample of methyl hexanoate, was detected. By using methyl octanoate as internal standard, 9·72 mg of hexanoic acid, equivalent to 75 mol % of (5a), was estimated to be in the mixture. The solution remaining after the g.l.c. analysis was concentrated on a steam-bath and then cooled in ice–water to give methyl 2-butyl- 3,7,11-trihydroxy-4-methylpentadecanoate (6a) (27·3 mg), microplates, m.p. 95—98° [from chloroform–petroleum (b.p. 60—80°)], $\nu_{max.}$ (KBr) 3340, 3230, 2900, 1450, 1385, 1360, 1140, 1110, 1070, and 935 cm^{-1}, M^+ 374, τ ([$^2H_5$]pyridine) 9·18 (t, *J* 6 Hz, Me), 9·13 (t, *J* 6 Hz, Me), 8·84 (d, *J* 6·5 Hz, Me), 8·75—7·93 (m, CH_2, CH), 7·41—7·13 (1H, m, C*H*CO_2Me), 6·29 (CO_2Me), 6·45—6·05 (3H, m, 3 C*H*·OH), and 4·91 (OH).

Acetylation of the Diol (5a).—Acetylation (acetic anhydride–pyridine; room temperature) gave the acetate (5b), liquid, $\nu_{max.}$ (film) 2960, 1880, 1740, 1470, 1375 (doublet), 1250, 1130, 1030, and 950 cm^{-1}, τ 9·12 (6H, d, *J* 6 Hz, 2 Me), 9·14 (Me), 8·94—8·28 (17H, m, CH_2 and CH), 7·98 (6H, s, 2 OAc), and 5·32—5·02 (2H, m, C*H*OAc).

The Triacetate (6b).—Acetylation of (6a) with acetic anhydride–pyridine gave the triacetate (6b), liquid, τ 9·12 (6H, apparent t, *J ca.* 6 Hz, 2 Me), 9·12 (apparent d, *J ca.* 6·5 Hz, Me), 8·85—8·25 (m, CH_2 and CH), 7·97 and 7·96 (3 OAc), 7·5—7·25 (m, C*H*·CO_2Me), 6·34 (CO_2Me), 5·3—5·06 (2H, m, 2 C*H*·OAc), and 4·97 [1H, t, 6·5 Hz, CH·C*H*(OAc)CH].

Treatment of the Ester (3b) *with Acetic Anhydride and Sodium Acetate*.—A mixture of (3b) (2·3 mg), fused sodium acetate (51·3 mg), and acetic anhydride (1 ml) was refluxed for 24 h, then evaporated, and the residue was treated with potassium hydroxide [1 ml; 0·5N in methanol-water (1 : 1)] at room temperature for 24 h. After acidification and isolation with chloroform–butanol (1 : 1) a solid residue (2·1 mg) was obtained which was treated with ethereal diazomethane. Separation [p.l.c.; chloroform–methanol (4 : 1)] gave 1·5 mg of starting material and 0·3 mg of a product having $\lambda_{max.}$ 217 nm (ε *ca.* 9000). When the starting material was recycled, similar results were obtained.

Alkaline Hydrolysis of the Deca-acetate of (7).—The deca-acetate (1·818 g) in 0·5N-potassium hydroxide (60 ml) was kept at room temperature (nitrogen) for 48 h. The mixture was percolated through a 50 × 2 cm column of Dowex 50W-X2 resin (H^+ form; 50—100 mesh) and the column was washed with more methanol (300 ml) followed by methanol–water (1 : 1). The effluent combined with the washings was evaporated to give a solid residue (1·08 g). Chromatography [chloroform–methanol–conc. ammonia (16 : 4 : 1)] gave the alcohol (8a) (545·5 mg), white microcrystals, m.p. 161—163° (from methanol–di-isopropyl ether), $\nu_{max.}$ (KBr) 3300, 2910, 1620br,w, 1450, 1400, 1370, 1335, 1140, 1070, 1005, and 910 cm^{-1}. Periodate titration showed an uptake of 2 mol. equiv. in 1·5 h, unchanged after 24 h (*M* 343); acetylation with acetic anhydride–pyridine at room temperature or with heating gave a mixture of two products which were not investigated further. The saponification equivalent of the deca-acetate, determined exactly as for (3a), was 9·5.

Acetylation of the Octaol (8a).—Acetylation of (8a) with acetic anhydride–pyridine gave the *octa-acetate* (8b), a syrup, $\nu_{max.}$ (film) 2920, 2850, 1730, 1425, 1365, 1230, 1120, 1020, and 940 cm^{-1}, *m/e* 704, τ 8·82 (d, *J* 6·5 Hz, Me), 8·74—8·11 (16H, m, 8 CH_2), 8·0, 7·98, 7·95, and 7·91 (24H, 8 OAc), 6·14—5·61 (m, C*H*$_2$OAc), 5·3—4·95 (5H, m, 5 C*H*·OAc), and 4·95—4·75 (2H, m, 2 C*H*·OAc) (Found: C, 56·05; H, 7·75. $C_{33}H_{52}O_{16}$ requires C, 56·2;. H, 7·45%).

Periodate Titration of the Octaol (8a).—The titrations were carried out in aqueous solution by the previous procedure. On the assumption of *M* 368 for (8a), after 1·5 h there was an uptake of 1·98 mol. equiv., after 10·25 h, 2·0 mol. equiv., and no further change after 24 h.

Formaldehyde from the Periodate Reaction of the Octaol (8a).[20]—To a solution of (8a) (9·2 mg) in water (2 ml), 0·3M-sodium periodate (0·25 ml) was added, and the solution was kept at room temperature in the dark for 30 min. N-Hydrochloric acid (0·2 ml) was then added, followed by 0·6M-sodium arsenite (0·4 ml, containing an

[20] The procedure used here is a slight modification of that described by R. E. Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1476.

equal volume of 0·6M-sodium hydroxide). The alkaline solution was carefully acidified with N-hydrochloric acid; the solution then assumed a dark yellow colour. More sodium arsenite solution was then added dropwise until the yellow colour just disappeared (at this point the solution was neutral to pH paper). After this, aqueous N-sodium acetate (2 ml) was added, followed by dimedone (80 mg) in 95% ethanol (1 ml; distilled from dimedone), and the mixture was heated on a water-bath (*ca.* 60°) for 1 h, and then kept at room temperature overnight. The formaldehyde dimedone derivative [6·7 mg, 91·8% of theoretical for 1 mole of formaldehyde per mole of (8a)], white crystals, was filtered off, washed with water, and then dried in hot air, m.p. and mixed m.p. 190—191°.

Pentadecane-1,3,5,7,9,14-*hexaol* (9a).—A solution of (8a) (87·6 mg) in water (5 ml) and 0·05M-sodium-periodate (12·5 ml) was kept at room temperature in the dark for 3 h. Sodium borohydride (600 mg) was then added in portions and the mixture was kept at room temperature for 2 h. The alkaline solution was carefully neutralised with 2N-hydrochloric acid and the product isolated with butanol. Evaporation gave a dark brown residue (80·4 mg). Chromatography (chloroform : methanol, 4 : 1) gave the hexaol (9a) (64·8 mg), white microcrystals (45 mg), m.p. 128—130° (from methanol–di-isopropyl ether), $\nu_{max.}$ (KBr) 3300, 2900, 1620vw, 1450, 1410, 1370, 1330, 1125, 1065, and 920 cm^{-1}.

Acetylation of the Hexaol (9a).—Acetylation of (9a) with acetic anhydride–pyridine at room temperature gave the hexa-acetate (9b), a syrup, $\nu_{max.}$ (film) 2910, 2850, 1725, 1425, 1365, 1240, 1120, 1020, and 940 cm^{-1}, τ 8·82 (d, *J* 6·5 Hz, Me), 8·75—8·07 (16H, m, 8 CH_2), 8·01, 8·0, and 7·98 (18H, 6 OAc), 5·93 (t, *J* 6·5 Hz, $CH_2{\cdot}CH_2{\cdot}$OAc), and 5·28—4·59 (5H, m, 5 *CH*·OAc), *m/e* 560 and 559·274 (Calc. for $C_{27}H_{43}O_{12} \equiv M - 1$: 559·275).

Alkaline Hydrolysis of the Deca-acetate (7) *Avoiding Acid Treatment.*—A solution of the deca-acetate (7) (86·9 mg) in methanol (5 ml) and potassium hydroxide [5 ml; 0·5N in methanol–water (1 : 1)] was kept at room temperature (nitrogen) for 28 h, then diluted with water (20 ml), neutralised carefully with acetic acid, and evaporated *in vacuo* at room temperature. The solid residue was extracted with dry pyridine and the extract afforded a solid residue (55 mg) contaminated with inorganic salts. Chromatography [chloroform–methanol–conc. ammonia (14 : 16 : 1)] gave a non-crystallisable product (29·5 mg). Treatment of this product (6·4 mg) with hydrochloric acid exactly as described for the compound (2d), and analysis of the mixture by t.l.c. on cellulose (aniline phthalate spray) showed the presence of arabinose. A major portion of the mixture was converted into the *p*-nitrophenylhydrazone. T.l.c. showed a spot identical in R_F value with that of an authentic sample of (−)-D-arabinose *p*-nitrophenylhydrazone. The minor part of the mixture was acetylated. Two spots, one identical in R_F value with that of an authentic sample of (−)-D-arabinose tetra-acetate and the other identical with that of the octa-acetate (8b) were observed on t.l.c.

Alkaline Hydrolysis of the Triacetoxy-amide (10a).—To a solution of the amide (10a) (88·1 mg) in methanol (10 ml), 0·2N-sodium hydroxide (10 ml) was added. The mixture was kept at room temperature overnight, acidified (N-hydrochloric acid), and evaporated *in vacuo* at room temperature. The residue was chromatographed (chloroform–methanol, 4 : 1) to give N-(4,6,8-*trihydroxy*-5,7-*dimethyloctyl)acetamide* (10b) (46 mg), a syrup, $[\alpha]_D^{28}$ +9·05° (±0·10°) (*c* 4·00 in MeOH), $\nu_{max.}$ (film) 3300, 2930, 1635br,s, 1550, 1440, 1370, 1300, 1190, 1030, and 970 cm^{-1}, *m/e* 247 (Found: M^+, 247·177. $C_{12}H_{25}NO_4$ requires *M*, 247·177).

Acetylation of the Heptaol (12b).—Acetylation of (12b) with acetic anhydride–pyridine gave 1,3,5,7,9,11,16-*hepta-acetoxyheptadecane* (12a), a syrup, $\nu_{max.}$ (film) 2920, 2850, 1725, 1425, 1360, 1230, 1120, 1020, and 940 cm^{-1}, *m/e* 646 (Found: C, 56·95; H, 8·15%; M^+, 646·310. $C_{31}H_{50}O_{14}$ requires C, 57·55; H, 8·15%; *M*, 646·320).

Ozonolysis of the Polyacetoxy-methyl Ester (2c) *with Borodeuteride Reduction.*—A solution of the methyl ester (2c) [128·4 mg; prepared by acetylation of (2d)] in methanol (5 ml) was cooled in solid carbon dioxide–acetone treated with ozone until a light blue colour persisted. Sodium borodeuteride (200 mg) was added in portions and the mixture was left at room temperature for 30 min. The product was isolated with chloroform and gave a thick syrup (120·3 mg), which was acetylated (acetic anhydride–pyridine; room temperature) and then chromatographed to give (t.l.c. comparison) a monodeuteriated compound (45·6 mg) analogous to (3a), as a syrup, τ (A-60 spectrum) 910 (m, 2 Me), 8·82 (s, Me), 8·8—8·07 (*ca.* 23H, m, CH_2 and CH), 7·95br (15H, s, 5 OAc), 7·6—7·3 (1H, m, *CH*·CO_2Me), 6·33 (s, CO_2Me), and 5·4—4·82 (4H, m, 4 *CH*·OAc); a dideuteriated compound (66·6 mg) analogous to the deca-acetate of (7), as a syrup, τ (A-60 spectrum) 8·82 (s, Me), 8·75—8·18 (16H, m, 8 CH_2), 8·0, 7·96, and 7·92 (30H, three singlets, 10 OAc), 6·1—5·5 (5H, m, 2 CH·O·C, CH_2·OAc), and 5·3—4·70 (8H, m, 8 *CH*·OAc); and a monodeuteriated product (24·2 mg) analogous to the triacetoxy-amide (10a), as a syrup, τ 9·07 (d, *J* 6·5 Hz, 2 Me), 8·65—8·22 (4H, m, 2 CH_2), 8·03 (s, NAc), 7·97 and 7·95 (two singlets, 3 OAc), 8·12—7·68 (2H, m, excluding NAc and OAc, 2 CH), 6·77 (2H, apparent q, *J* 6 Hz, (1H, two epimers at CHD·OAc), 5·14 (1H, m, *CH*·OAc), 5·01 [1H, dd, *J* 5 and 6·5 Hz, CH–*CH*(OAc)–CH], and 3·86 (1H, broad hump, AcNH).

Estimation of Hexanoic Acid from the Alkaline Hydrolysis of Primycin (1).—A mixture of primycin (635·5 mg) and 5N-potassium hydroxide (20 ml) was refluxed (nitrogen) for 4 h, cooled to room temperature, and then acidified (pH *ca.* 2; 2N-sulphuric acid). The products were steam-distilled until about 200 ml of distillate had collected. This was made alkaline (phenolphthalein) and then evaporated to dryness. The solid residue was acidified to pH *ca.* 4 with hydrochloric acid in absolute methanol and then esterified with ethereal diazomethane to give a slightly yellow solution (total volume *ca.* 2·5 ml). G.l.c. of the mixture showed the presence of methyl hexanoate (amide,* m.p. and mixed m.p. 99°) as the only volatile ester. By using methyl octanoate as internal standard, 10·82 mg (16·1 mole %) of hexanoic acid was estimated to be present in the mixture.†

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† In a control steam distillation experiment, 14·5 mg (95%) of hexanoic acid was accounted for from an initial amount of 15·2 mg.