Tetrahedron Vol. 35, pp. 105–127

0040-4020/79/0101-0105/$02.00/0

THE ORTHOSOMYCIN FAMILY OF ANTIBIOTICS—I

THE CONSTITUTION OF FLAMBAMYCIN

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(*Received in UK* 18 *May* 1978)

Abstract—The antibiotic, flambamycin, is shown to have the novel oligosaccharide structure (1) associated with two orthoester linkages. It is proposed that flambamycin (1), the everninomicins (28), curamycin, avilamycin, destomycin A (29a), destomycin C (29b), destomycin B (30), hygromycin B (29c), the antibiotics A-396-I (29d) and SS-56C (29e), belong to a new family of antibiotics called the orthosomycins.

Flambamycin[1]‡ is an antibiotic produced by *Streptomyces hygroscopicus* DS 23230. It exhibits a very low toxicity and shows an interesting activity against Gram-positive and Gram-negative cocci and some Gram-positive bacilli.[1] It is practically inactive against Gram-negative bacilli, yeasts and filamentous fungi. This selectivity coupled with, for example, excellent therapeutic *in vivo* activity[1] in mice infected experimentally with *Staphylococcus aureus*, *Streptococcus pyogenes haemolyticus*, or *Neisseria meningitidis* encouraged our structural investigation of flambamycin (1). The constitution previously proposed by us for flambamycin[2–6] requires modification. Additional evidence (Section 4) demands the relocation of the glycosidic linkage between the D-evalose residue (1, D) and the 4 - O - methyl - D - fucose residue (1, E). This intermonosaccharide linkage is now established as involving position −31 rather than position −30.[6]

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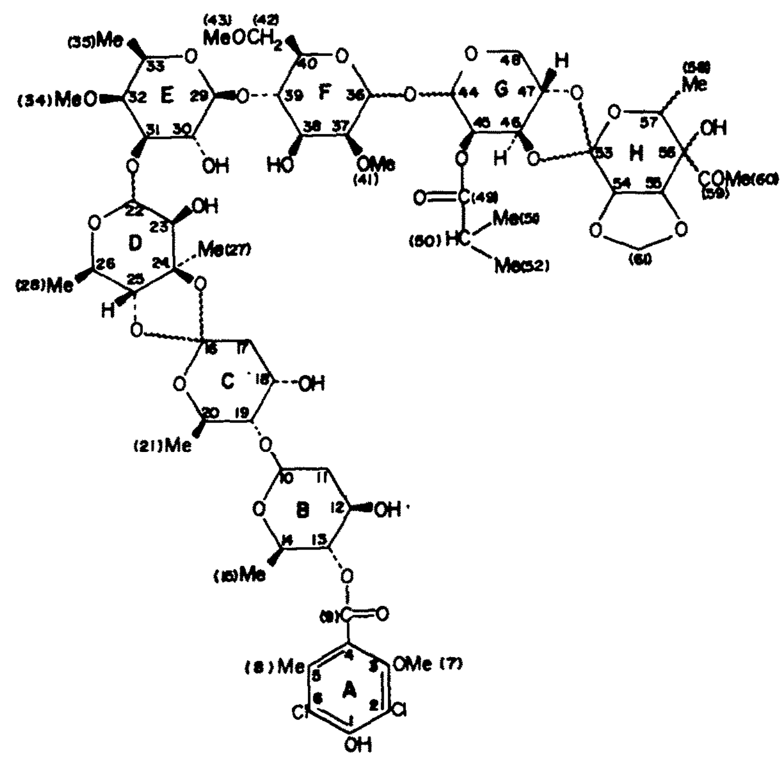
‡This name was first proposed by our colleague, Dr. G. Jolles, Directeur des Rescherches des Division Sante, Rhône-Poulenc, during a discussion of the structural investigation at a dinner held in the restaurant, La Crêpe Flambée, Paris.



1 Flambamycin

Flambamycin, $C_{61}H_{88}Cl_2O_{33}\cdot H_2O$ is a member of a new class of antibiotics which includes curamycin,[7] avilamycin,[8] everninomicin-B,[9] everninomicin-C,[9,10] everninomicin-D[9a,9b,11] and everninomicin-2.[11h] Some progress towards the elucidation of the structures of curamycin[7] and avilamycin[8] has been reported and recently complete constitutions have been announced in preliminary communications for the everninomicins-B,[9d] -C, [9c], -D[10g] and -2.[10h] All these antibiotics are esters derived from dichloroisoeverninic acid (1, residue A). At this point it is also desirable to refer to another common structural feature namely that this family of antibiotics contain orthoester groupings. This unusual feature is discussed in more detail later (Section 11). In addition, the natural occurrence of other antibiotics containing orthoester groupings which are not esters of dichloroisoeverninic acid include the destomycins,[11a,b,c] hygromycin B,[11d] the antibiotic A-396-I[11e] and the antibiotic SS-56C.[11f]

Our structural investigation of flambamycin and its degradation products has involved extensive application of ^{1}H and ^{13}C NMR spectroscopy in association with low and high resolution mass spectrometry. Where appropriate such results are briefly mentioned in this paper: the mass spectral results now reported (Part I) are almost entirely limited to the characterisation of compounds by their parent peaks. Detailed correlation and assignments for the ^{13}C NMR spectra of flambamycin and its degradation products are given in Part II,[12] and their mass spectral fragmentation patterns are discussed in Part III.[13]

(1) *Preliminary characterisation of flambamycin*

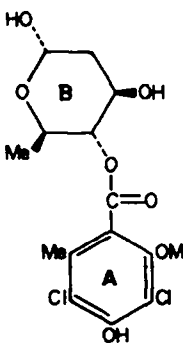
The antibiotic was an optically active colourless compound, m.p. 202–203°, which showed CO absorption (ν_{max} 1735 and 1715 cm^{-1}) in its IR spectrum. Its UV spectrum [λ_{max} 288 nm (ϵ 1725)] indicated the presence of an aromatic chromophore.

Its ^{1}H NMR spectrum (100 MHz) did not demonstrate the presence of aromatic protons but assignments were possible for four OMe groups (singlets, δ 3.95, 3.65, 3.59 and 3.29), one aromatic Me and one Me ketone function (singlets, δ 2.26 and 2.24) one tertiary C-Me (singlet, δ 1.51) and seven secondary C-Me groups (doublets, δ 1.46–1.06). The presence of two Cl atoms was indicated by the mass spectrum which showed fragment ions (m/e 233 and 235) associated with a fully substituted benzoyl cation, $C_6(OH)(Me)(OMe)Cl_2{-}C{\equiv}\overset{+}{O}$.

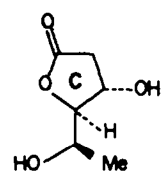
Acetylation with acetic anhydride-pyridine at room temperature gave flambamycin hexa-acetate (ν_{max} 3500, 1785 and 1750 cm^{-1}) indicating the presence of six OH groups plus one or more additional OH groups (ν_{max} 3500 cm^{-1}) which were not acetylated under these mild conditions.

(2) *Identification of acidic hydrolysis products of flambamycin*

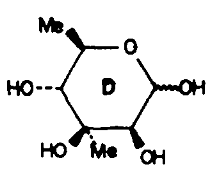
A short period of heating (78°, 30 min) of flambamycin with dilute aqueous hydrochloric acid (0.5% w/v) and ether extraction yielded curacin (2).[7,8] The aqueous hydrolysate was subjected to further heating (100°, 3 hr) with dilute aqueous hydrochloric acid (0.4% w/v) and neutralisation followed by chromatographic fractionation



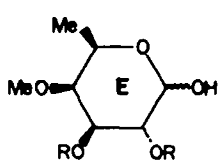
2: Curacin



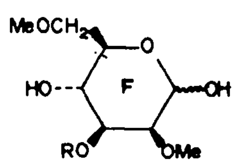
3: 3,5-Dihydroxy-γ-caprolactone



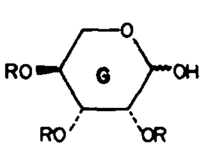
4: D-Evalose



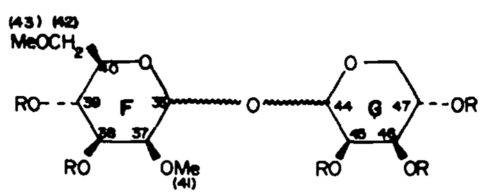
5a: 4-O-Methyl-D-fucose, R = H
5b: R = Me



6a: 2,6-Di-O-methyl-D-mannose, R = H
6b: R = Me



7a: L-Lyxose, R = H
7b: R = Me



8a: Flambabiose, R = H
8b: R = Ac

yielded 3,5 - dihydroxy - γ - caprolactone (3), D-evalose (4), 4 - O - methyl - D - fucose (5a), 2,6 - di - O - methyl - D - mannose (6a), L-lyxose (7a), and a new disaccharide (8a) named flambabiose.

Curacin (2) had been previously obtained as an acidic hydrolysis product of curamycin[7] and avilamycin.[8] The identity of the flambamycin hydrolysis product (2) (characterised as its O-methylglycoside, phenolic O-Me derivative and tri-O-acetate) was firmly established by comparison with the published spectral data.[7,8] on curacin and its derivatives. In our hands, crystallisation of curacin from chloroform gave the α-anomer (2) whose glycosidic configuration followed from its ^{1}H NMR spectrum (glycosidic H, δ 5.29, $J_{1,2}$ 1 and 3 Hz).

3,5-Dihydroxy-γ-caprolactone was previously obtained by the acidic hydrolysis of avilamycin.[8] However, this dihydroxylactone was not isolated as such, but as a crystalline di-O-acetate, m.p. 102°.[8] The dihydroxy-γ-lactone (3) was isolated directly from the flambamycin hydrolysate: it gave a crystalline di-O-acetate, m.p. 113°. Comparison of the IR and ^{1}H NMR spectra of the di-O-acetate,[8] m.p. 102° and the di-O-acetate, m.p. 113° (Experimental) indicates their identity.

From the acidic hydrolysis of flambamycin, we isolated a sugar, $C_7H_{14}O_5$, which at the time of its isolation had not been described previously. In view of the frequent association of antibiotics with unusual carbohydrate residues,[15] a detailed investigation of this sugar, $C_7H_{14}O_5$ was undertaken and the structure 4 was established. Subsequently this structure 4 was allocated to D-evalose, a hydrolysis product of evertetrose-B.[9c] However, our derivation of the configuration of D-evalose (4) is quite different from the method already described,[9c] so it is briefly reported. Our determination of the structure of D-evalose exemplifies a possible approach to a difficult problem which is still encountered in carbohydrate chemistry,[15] namely the determination of configuration at tertiary alcoholic centres of chirality. We believed that this problem could be examined in the case of D-evalose by evaluating the downfield ^{1}H shifts induced by lanthanide shift reagents.

D-Evalose, $C_7H_{14}O_5$ ($M^{+\cdot}$ $-H_2O$, *m/e* 160) was obtained as a mixture of pyranose (4)-, furanose- and aldehydo-forms, which therefore gave a complicated NMR spectrum. D-Evalose (4) yielded a crystalline methyl glycoside, m.p. 132° [δ(C_5D_5N) 4.96 (H-1) and 3.91 (H-2) (AB systems, J_{AB} 1.5 Hz), 3.29 (singlet, OCH_3), 1.67 (singlet, tertiary C–CH_3), 1.48 (doublet, secondary C–CH_3, J 6 Hz)]. D-Evalose (4) also gave a crystalline tetra-acetate, m.p. 132°, [δ(C_5D_5N) 6.29 (H-2), and 5.62 (H-1) (AB system, J_{AB} 1 Hz); 5.56 (H-4), 3.87 (H-5), 1.33 (5-CH_3) (ABX_3 system J_{AB} 9, J_{AX} 0, J_{BX} 6 Hz); 1.62 (singlet, tertiary C–CH_3); 1.98, 1.98, 1.98, 1.94 $(OCOCH_3)_4$]. These assignments for these two derivatives settle the constitution (4) for D-evalose. The configuration at C-4 could be inferred by comparison of the relative downfield shifts obtained when methyl 2-O-methyl D-evalopyranoside (9) was treated with the europium shift reagent, Eu $(fod)_3$; [$(CDCl_3)$ (H-1 δ 4.8$\rightarrow$6.0), (H-2 δ 3.05$\rightarrow$5.7), (H-4 δ 3.7$\rightarrow$12.6), (H-5 δ 3.7$\rightarrow$6.9)]. The dramatic comparative downfield shift of H-4 indicates its *cis*-relation to the tertiary 3-OH group, thus leading to the relative configuration (4) for D-evalose. The absolute configuration (4) for D-evalose has been independently and firmly established by Ganguly and Saksena.[9c] This D-evalose residue is common to everninomicin-B[9d] and flambamycin.

4-O-Methyl-D-fucose (5a), 2,6 - di - O - methyl - D - mannose (6a) and L-lyxose (7a) were initially recognised on the basis of comparison of their ^{1}H NMR spectra, and specific rotations with reported data. The monosaccharides (5a and 6a) had been previously isolated as acidic hydrolysis products of curamycin,[7] avilamycin[8] and everninomicins-B,[9] -C[9e] and -D.[10] L-Lyxose (7a) was also obtained from curamycin and avilamycin whereas the corresponding monosaccharide obtained from everninomicin-B, -C and -D was 2-O-methyl-L-lyxose. 4-O-Methyl-D-fucose (5a; curacose[7a]) was characterised as its triacetate, m.p. 113°. 2,6-Di-O-methyl-D-mannose (6a; curamicose[7b]) was characterised as its triacetate,[8] m.p. 76° and L-lyxose (7a) as methyl - 2,3,4 - tri - O - acetyl - L - lyxopyranoside, m.p. 84°.

The new disaccharide, flambabiose (8a), $C_{13}H_{24}O_{10}$, m.p. 191°, was non-reducing and was clearly associated with 1-1 union of residues derived from 2,6 - di - O - methyl - D - mannose (6a) and L-lyxose (7a). It was characterised as flambabiose penta-acetate, (8b) m.p. 150°. The structure 8a for flambabiose was supported by its ^{1}H NMR spectrum, its ^{13}C NMR spectrum,[12] and the mass spectral fragmentation pattern of its pentaacetate (8b).[13]

(3) *Acidic hydrolysis and identification of formaldehyde*

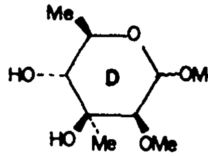
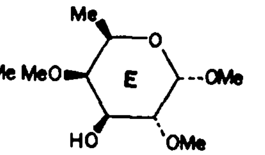
An important product was obtained by the acidic hydrolysis (5N-HCl, 70°, 18 hr) of flambamycin. The product was isolated as formaldehyde 2,4-dinitrophenylhydrazone (65% yield). This high yield was certainly significant and indicated, for example, the presence of a methylenedioxy group. However, it was not possible at this stage to speculate profitably upon the possibility that a methylenedioxy group was associated either with one of the hydrolysis products which had been isolated or with some unidentified portion of the flambamycin molecule.

(4) *Identification of intermonosaccharide linkages*

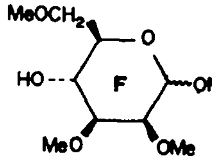
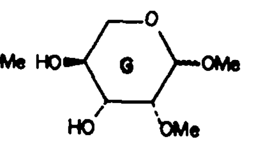
This was carried out using the classical method of permethylation followed by acidic methanolysis yielding four partially methylated mono-saccharides (9–12) in which the positions of secondary OH groups were elucidated by determination of the downfield shift of associated protons. CH–OH, in the derived acetates, CH–OAc.

Flambamycin, methyl iodide and sodium hydride in dimethyl sulphoxide (room temp., 1 hr) yielded "flambamycin permethyl ether", m.p. 147–149°. Direct treatment with boiling methanolic hydrogen chloride (4% w/v, 1 hr) gave a mixture of five Me glycosides (9–13) which were separated by chromatography. The Me glycosides (9–12) were characterised as their O-acetates.

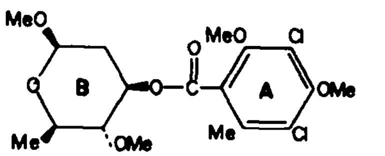
The compound 13 named isocuracin trimethyl ether is clearly related to the curacin (2) residue of flambamycin (1). The migration of the dichloro-isoeverninoyl grouping from position-4 to position-3 of the 2-deoxy-D-rhamnose residue B must have occurred under the basic equilibration conditions associated with the permethylation of flambamycin. The 3-position of the dichloro-isoeverninoyl residue in isocuracin trimethyl ether (13) as compared with its 4-position in curacin (2) was deduced by comparison of the H-3- and H-4- chemical shifts (C_5D_5N) in curacin (2) (δ_3 ~ 4.78, δ_4 5.44), curacin triacetate (δ_3 5.47, δ_4 2.95) and isocuracin trimethyl ether (13) (δ_3 5.47, δ_4 2.95). These results establish that the curacin (2) residue in flambamycin (1) is terminal and is

9 10

11 12



13

linked through the glycosidic oxygen of the 2-deoxy-D-rhamnose residue B.

The isolation of methyl 2 - O - methyl - D - evalopyranoside (9) proved that the 2 - OH group of the D - evalose (4) was free in flambamycin (1). The 2-position of the additional O-Me group in the derivative 9 was established by comparison of the H-2-chemical shifts ($CDCl_3$) of methyl D - evalopyranoside - 2,3,4 - triacetate (*cf.* 4; δ_2 5.67) and methyl 2 - O - methyl - D - evalopyranoside - 3,4 - diacetate (*cf.* 9; δ_2 4.06). Thus the 2-OH group of the D-evalose residue D must be free in flambamycin (1) whereas its O atoms in positions 1 and 4 and possibly 3 are used in bonding in flambamycin.

The determination of the constitution of the methyl glycoside (10) essentially involved the location of the additional O-Me group in either position-2 or position-3 of the 4 - O - methyl - D - fucose residue. The methyl glycoside (10) was first examined by treatment with acetic anhydride - *p* - toluenesulphonic acid. This acid-catalysed transformation yielded a di-O-acetate which was initially formulated[6] as 3,4 - di - O - methyl - D - fucopyranoside - 1,2 - di - O - acetate. This proposal was subsequently recognised as being incorrect. This became clear when it was recognised that the ^1H NMR spectrum of the di-O-acetate could in fact be assigned, in the absence of information regarding the configuration of the anomeric centre, to two possible constitutions; either 3,4 - di - O - methyl - D - fucopyranoside - 1,2 - di - O - acetate or 2,4-di-O-methyl-D-fucopyranoside-1,3-di-O-acetate. It was clarly important to settle this matter unequivocally because this evidence was used to determine the location of the D-E intermonosaccharide linkage in flambamycin (1). This was initially proposed[2-4] as linking C-22 and C-30, however, on the basis of the following additional evidence it has now been established that the D-E intermonosaccharide linkage unites C-22 and C-31.

Base catalysed acetylation of the methyl glycoside (10) yielded methyl 3 - O - acetyl - 2,4 - di - O - methyl - D - fucopyranoside whose constitution was firmly established by its ^1H NMR spectrum (Experimental) (H-1 δ 4.92, doublet, J 4 Hz; H-2, δ 3.82, multiplet; H-3, δ 5.40, double doublet, J 3 and 10 Hz; H-4, δ 3.82, multiplet). These assignments were clearly supported by two spin-decoupling experiments. Irradiation at δ 3.82 causes the collapse of two signals (δ 5.40 double doublet $\rightarrow$ singlet and δ 4.92 doublet $\rightarrow$ singlet). Irradiation at δ 5.40 did not transform the doublet (δ 4.92).

Independent evidence for the existence of the C-22 to C-31 glycosidic linkage in flambamycin (1) and in appropriate degradation products containing residues D and E was provided by the discovery that acetylation of the hydroxyl group at C-30 was associated with a significant upfield shift ($\sim$105 $\rightarrow$ $\sim$100 ppm) in the ^{13}C resonance of the anomeric carbon at C-29. This is discussed in detail in Part II.[12] Finally much reassurance regarding the constitution of flambamycin (1) was provided by the 220 MHz ^1H NMR spectrum of flambeurekanose pentaacetate (24b). This spectrum was obtained after our experimental investigation of flambamycin was completed. However, a detailed interpretation of the 220 MHz ^1H NMR spectrum of flambeurekanose pentaacetate (24b) (Section 8) clearly indicated the presence of an acetoxyl group at position-2 of the 4 - O - methyl - D - fucose residue. Thus the D-evalose residue D must be glycosidically linked to position-31 in flambamycin (1).

The structure of the methyl glycoside (11) follows from the chemical shift (C_5D_5N, δ 5.61) of H-4 in its monoacetate. Thus the 2,6 - di - O - methyl - D - mannose (6a) residue F in flambamycin (1) must be linked through its two O atoms in positions-1 and -4: its association through position-1 with the L-lyxose (7a) residue G has already been established by the formula (8) for flambabiose (Section 2).

The L-lyxose derivative (12) isolated by methanolysis of "flambamycin permethyl ether" was shown to be the 2-O-Me derivative because it yielded methyl 2 - O - methyl - L - lyxopyranoside - 3,4 - diacetate, ($CDCl_3$, δ_3 5.56, δ_4 5.56). Comparison of the structure of flambabiose (8) with that of the 2 - O - methyl - L - lyxose derivative (12) posed an interesting question. Why are the 3- and 4-OH groups of the L-lyxose (7a) residue G *not* O-methylated in "flambamycin permethyl ether"? The answer to this question is provided later (Section 8).

(5) *Isolation and structural elucidation of flambatriose* (14a) *and flambatetrose* (15a)

Mild hydrolysis of flambamycin (1) with dilute aqueous hydrochloric acid (0.5% w/v) initially at 78° (30 min) then 31° (17 hr) gave, after neutralisation and evaporation, a mixture which was fractionated chromatographically yielding flambatriose (14a), flambatetrose (15a) and flambatetrose isobutyrate (17a).† Flambatriose isobutyrate (16a)† was isolated from the acidic methanolysis of flambamycin (Section 6).

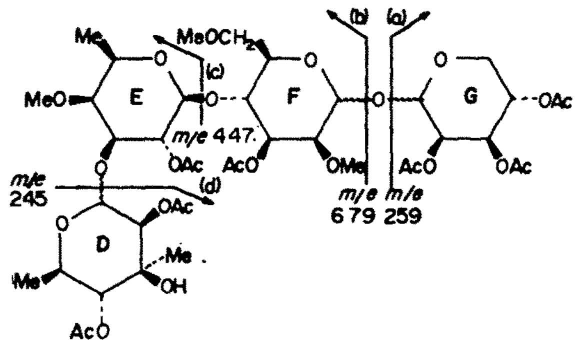
Flambatriose, $C_{20}H_{36}O_{4}$, m.p. 125°, was characterised as its hexa-acetate, m.p. 119°, and hexamethyl ether, m.p. 68–69°. Acidic hydrolysis of flambatriose with aqueous hydrochloric acid (1.8% w/v; 100°; 2.5 hr) gave 4 - O -

†Flambatriose isobutyrate and flambatetrose isobutyrate have been described[2] as single compounds (16a and 17a), but in view of the "doubling" of many of the resonances in their ^1H (Experimental) and ^{13}C NMR spectra,[12] we now believe that these two compounds are in fact mixtures of flambatriose isobutyrate (16a) and its isomer and flambatetrose isobutyrate (17a) and its isomer. In these two pairs of isomers, the isobutyrate group has partially migrated from position-2 to position-3 of the L-lyxose residue G.

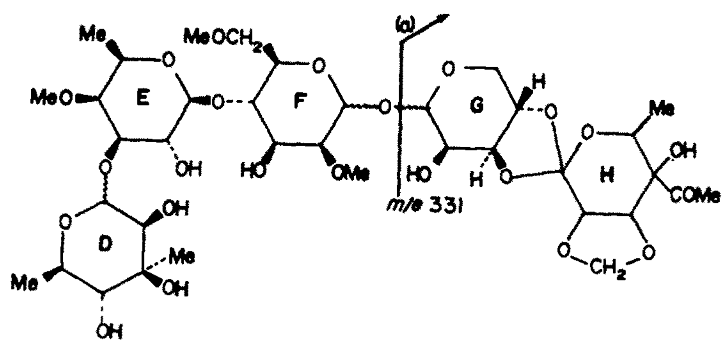
methyl - D - fucose (5a), 2,6 - di - O - methyl - D - mannose (6a) and L-lyxose (7a). Similarly, flambatriose hexamethyl ether on hydrolysis with aqueous sulphuric acid (10% w/v; 100°; 4 hr) yielded 2,3,4 - tri - O - methyl - D - fucose (5b), 2,3,6 - tri - O - methyl - D - mannose (6b), and 2,3,4 - tri - O - methyl - L - lyxose (7b). The location of the OH groups on the three transformation products (5b, 6b and 7b) was established by comparison of their ^{1}H NMR spectra with those of the three derived acetates. These results on the structural investigation of flambatriose when considered in relation to its non-reducing character and the structure of flambabiose (8a) showed that flambatriose had the constitution 14a corresponding with its hexa-acetate (14b) and hexamethyl ether (14c).

Flambatetrose, $C_{27}H_{48}O_{18}$, m.p. 143°, was recognised as a non-reducing tetrasaccharide and was characterised as its hepta-acetate, m.p. 119°, octa-acetate, m.p. 115°, its heptamethyl ether, m.p. 119° and its octamethyl ether, m.p. 96°. A similar acidic hydrolysis (see above) of flambatetrose gave D-evalose (4), 4 - O - methyl - D - fucose (5a), 2,6 - di - O - methyl - D - mannose (6a) and L-lyxose (7a). The relation between flambatriose (14a) and flambatetrose, $C_{27}H_{48}O_{18}$, is clear and the non-reducing character of flambatetrose demanded that the D-evalose residue was glycosidically linked to one of the six available oxygens in flambatriose. This was reduced to two possibilities by consideration of the following mass spectral evidence.[13]

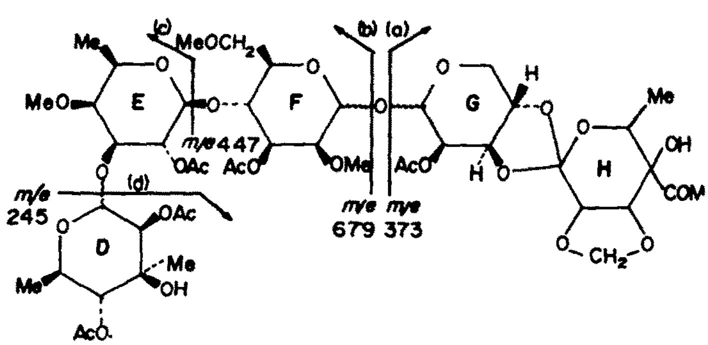
Three derivatives of flambatetrose: its heptamethyl



15b: Flambatetrose hepta-acetate

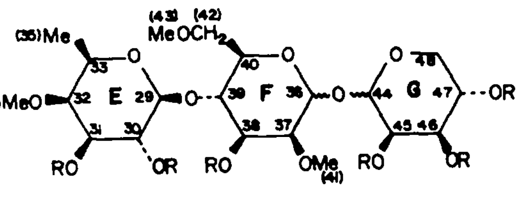


24a: Flambeurekanose

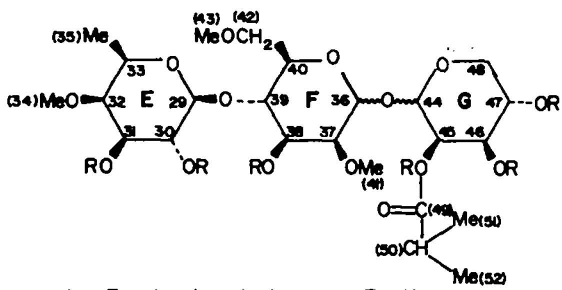


24b: Flambeurekanose penta-acetate

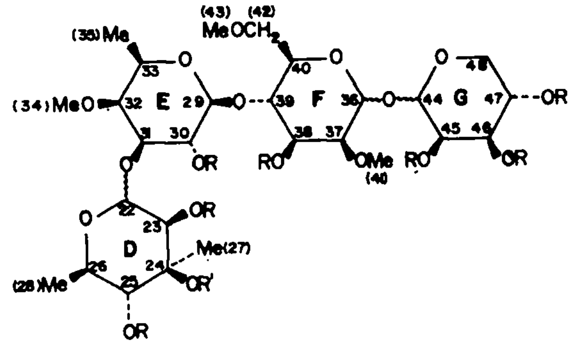
Scheme 1. Mass spectral fragmentation of flambatetrose hepta-acetate (15b), flambeurekanose (24a) and flambeurekanose penta-acetate (24b).



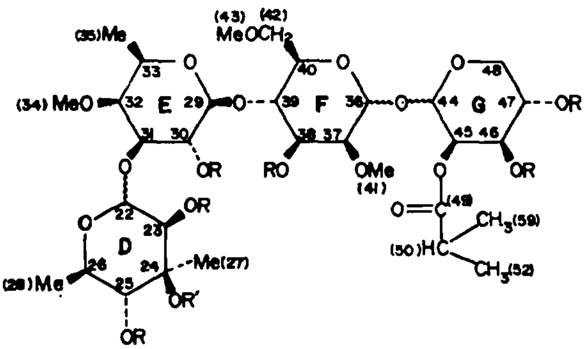
14a: Flambatriose, R = H
14b: R = Ac
14c: R = Me



16a: Flambatriose isobutyrate, R = H
16b: R = Ac



15a: Flambatetrose, R = R' = H
15b: R' = H; R = Ac
15c: R = R' = Ac
15d: R' = H; R = Me
15e: R = R' = Me



17a: Flambatetrose isobutyrate, R = R' = H
17b: R' = H; R = Ac
17c: R' = R = Ac

ether [*m/e* 363, $C_{13}H_{19}O_4(OMe)_4$],† its octamethyl ether [*m/e* 377, $C_{13}H_{18}O_3(OMe)_5$]† and its hepta-acetate [*m/e* 447, $C_{14}H_{22}O_5(OAc)_3$]† gave the indicated fragment ions. The origin of these ions associated with the common cleavage (c) (see **15b** and **24b**, Scheme 1) established that in flambatetrose the D-evalose (**4**) residue was linked glycosidically to either position-2 or to position-3 of the 4 - O - methyl - D - fucose residue. The decision between these two possibilities was made possible by the isolation of methyl 2,4 - di - O - methyl - D - fucopyranoside (**10**) from the acidic methanolysate of "flambamycin permethyl ether" (Section 4). In fact, the isolation of the methyl glycosides (**9**, **10**, **11** and **12**) obviously established the constitution **15a** for flambatetrose and its derivatives (**15b–15e**).

Independently of these chemical degradations and transformations of flambatriose (**14a**) and flambatetrose (**15a**), detailed correlations of their ^{1}H NMR spectra (Experimental) and their mass spectra (Part III[13]) were also possible. The mass spectral fragmentation patterns of flambatriose, flambatetrose and their derivatives (**14b**, **14c** and **15b–15e**) can be interpreted in detail[13] and provide satisfying support for all aspects of the proposed structures. Mass spectral information also provides an excellent basis for proposing structures for the two transformation products: flambatriose isobutyrate (**16a**)† and flambatetrose isobutyrate (**17a**).†

Flambatriose isobutyrate (**16a**), m.p. 115–117°, was characterised as its penta-acetate (**16b**), m.p. 86°. Flambatetrose isobutyrate (**17a**), m.p. 145°, similarly yielded a hexa-acetate (**17b**), m.p. 115°. The ^{1}H NMR spectra of the two pairs, (i) flambatriose (**14a**) and flambatriose isobutyrate (**16a**), and (ii) flambatetrose (**15a**) and flambatetrose isobutyrate (**17a**), showed an entirely acceptable correlation. The presence of an isobutyrate grouping in flambatriose isobutyrate (**16a**) was also clearly supported by its ^{13}C NMR spectrum.[12] Corresponding signals for an isobutyrate grouping were also observed in the ^{13}C NMR spectrum[12] of flambamycin (**1**).

We were now in a position to consider the location of the isobutyrolyloxy group in flambamycin. Comparison of the mass spectral fragmentation patterns[13] of the two pairs (i) flambatriose hexaacetate (**14b**) and flambatriose isobutyrate penta-acetate (**16b**) and (ii) flambatetrose hepta-acetate (**15b**) and flambatetrose isobutyrate hexa-acetate (**17b**) established[16] the location of the isobutyroyloxy group on the L-lyxose residue G. The question posed in the last paragraph of section 4 could now be answered. Clearly the isobutyroyloxy group present in flambamycin (**1**) was cleaved and the resulting OH group was methylated during the generation of "flambamycin permethyl ether". Therefore the isolation of 2 - O - methyl - L - lyxose (**14**) by hydrolysis of "flambamycin permethyl ether" was not incompatible with the location of the isobutyroyloxy group on C-2 of the L-lyxose residue G in flambamycin (**1**).

(6) *Acidic methanolysis of flambamycin*

It was now possible to consider progress towards the determination of the constitution of flambamycin, $C_{61}H_{88}Cl_2O_{33}$,‡ in terms of its relation to four significant degradation products: curacin (**2**), $C_{15}H_{18}Cl_2O_7$; 3,5 - dihydroxy - γ - caprolactone (**3**), $C_6H_{10}O_4$; flambatetrose isobutyrate (**17a**), $C_{31}H_{54}O_{19}$; and formaldehyde, CH_2O. These products had been mainly derived by aqueous acidic hydrolysis, so in order to try to shed some light on the possible nature of the eight unidentified C atoms as

†Fragment ions identified by high resolution mass spectrometry.

‡This molecular formula was not, of course, firmly established until the conclusion of the structural investigation.

well as on the structural origin of formaldehyde, degradation of flambamycin by methanolysis was explored.

Mild treatment of flambamycin with methanolic hydrogen chloride (0.5% w/v, room temp. 90 min) yielded a mixture of curacin methyl glycoside (*cf.* **2**), methyl D-evalopyranoside (*cf.* **4**), flambatriose (**14a**) flambatetrose (**15a**), flambatriose isobutyrate (**16a**) and flambatetrose isobutyrate (**17a**). In addition three new compounds were isolated: flambalactone (**18**), methyl flambate (**19b**) and methyl eurekanate (**20a**; Section 7).

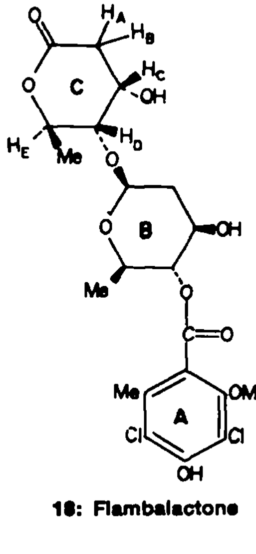
Flambalactone, $C_{21}H_{26}Cl_2O_{10}$, m.p. 217°, has been shown[3] to have the structure (**18**) mainly on the basis of spectroscopic evidence in association with its empirical relation to curacin (**2**), $C_{15}H_{18}Cl_2O_7$, and 3,5 - dihydroxy - γ - caprolactone (**3**), $C_6H_{10}O_4$. Flambalactone (**18**) has been characterised as its mono-O-methyl derivative, m.p. 201°, prepared by methylation of its phenolic OH group with diazomethane. Flambalactone (**18**) yields a tri-O-acetate, m.p. 159°, and a tris-trichloroacetyl carbamate.

The γ-lactone (**3**) shows a CO band at the expected position (ν_{CO} 1780 cm^{-1}) whereas flambalactone (**18**) and its derivatives show (Table 1) a CO band (ν_{CO} 1740 cm^{-1}) indicating that this residue is present in flambalactone as its δ-lactone equivalent.

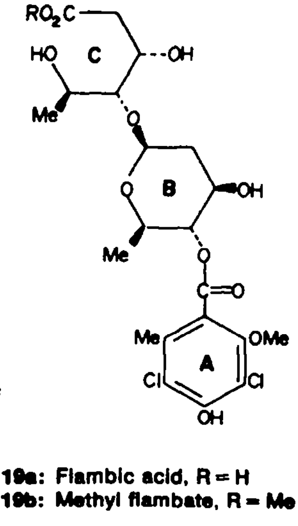
Two possible structures may be considered for flambalactone in which the curacin residue A–B is linked glycosidically to either C-3 or C-4 of a 3,4 - dihydroxy - δ - caprolactone residue C. Clearly, the latter constitution (**18**) is demanded by the marked downfield shift of $\underline{H}_C$ in flambalactone triacetate (δ 5.43) and flambalactone tris-trichloroacetyl carbamate (δ 5.57) (Table 1). The ^{13}C NMR spectrum[12] and the mass spectral fragmentation pattern[13] of flambalactone are in full accord with the formulation (**18**): the indicated relative stereochemistry associated with the δ-lactone is derived from the coupling constants (Table 1): J_{CD} 3; J_{DE} 8–8.5 Hz.

Methyl flambate, $C_{22}H_{30}Cl_2O_{11}$, m.p. 90–92°, was shown to have the structure **19b** by its partial synthesis from flambalactone (**18**) and methanolic hydrogen chloride (0.15% w/v; room temp.). Methyl flambate (**19b**) showed the expected spectroscopic properties (Experimental) and mass spectral fragmentation pattern:[13] it was characterised as a tetra-acetate, m.p. 61–63°.

The isolation of flambalactone (**18**), methyl flambate (**19b**) and flambatetrose isobutyrate (**17a**) by the



18: Flambalactone



19a: Flambic acid, R = H
19b: Methyl flambate, R = Me

Table 1. Chemical shifts and coupling constants of the indicated protons [see (**18**)] and the carbonyl bands (ν_{CO}, $CHCl_3$) associated with the δ-lactone residue of flambalactone and its derivatives

Compound	Chemical shifts, δ$(CD_3)_2CO$						Coupling constants, J/Hz						ν_{CO}/cm^{-1}
	$\underline{H}_A$	$\underline{H}_B$	$\underline{H}_C$	$\underline{H}_D$	$\underline{H}_E$	Me	J_{AB}	J_{AC}	J_{BC}	J_{CD}	J_{DE}	$J_{E,Me}$	
Flambalactone (**18**)	2.98	2.4	3.5–5.0[a]	3.53	4.26	1.39	17	6	6	3	8	6	1740
Flambalactone methyl ether[c]	3.00	2.5	3.7–4.0[a]	3.4–3.39	4.23	1.38	17	6	6	3	8	6	1740
Flambalactone triacetate	3.13	2.5	5.43	3.3–3.89	4.35	1.34	16.5	5	3	3	8.5	6	1740, 1782[b]
Flambalactone tris-trichloroacetylcarbamate	3.32	2.62	5.57	3.8	4.40	1.37	17	5	5	3	8	6	—

[a] Owing to overlap with other signals the chemical shift cannot be determined directly.
[b] Carbonyl band of phenolic O-acetate.
[c] Solvent $CDCl_3$–$(CD_3)_2CO$.

methanolysis of flambamycin under extemely mild conditions was an encouraging result. However, the most exciting development was the isolation of methyl eurekanate (20a), $C_{10}H_{16}O_7$, in significant yield (28%). The elucidation of the constitution of methyl eurekanate[4] is discussed in detail in Section 7 but even a casual inspection of its ^1H NMR spectrum showed the presence of a methylenedioxy group. Acidic hydrolysis of methyl eurekanate gave formaldehyde! Our search for a further structural feature of flambamycin—eurekanic acid, $C_9H_{14}O_7$, or its equivalent—had at last been rewarded and our feelings are, we hope, reflected in our choice of its name.

A particularly significant result was obtained when flambamycin (1) was treated first with methanolic hydrogen chloride and then the mixture of methanolysis products was acetylated directly. Chromatographic fractionation gave reassuringly good yields of flambatetrose isobutyrate hexa-acetate (17b; 18%), flambatetrose isobutyrate hepta-acetate (17c; 45%), methyl flambate tetra-acetate (*cf.* 17b; 65%) and methyl eurekanate monoacetate (20d; 72%).

(7) The constitution of methyl eurekanate (20a)[4]

On the basis of its ^1H NMR and IR spectral properties, in association with appropriate characterisation and degradative studies, the following functional groups were shown to be present in methyl eurekanate (20a), $C_{10}H_{16}O_7$, ($M^{+\cdot}$, *m/e* 248):

(a) MeO_2C–(δ_{Me} 3.78, ν_{CO} 1750 cm^{-1}). The presence of a methoxycarbonyl group was clearly supported by the transformation of methyl eurekanate (20a) into ethyl eurekanate (20b) by ethanolic hydrogen chloride (0.5% w/v, room temp., 18 hr). Furthermore, mild acidic hydrolysis (5N-HCl, room temp., 18 hr) yielded eurekanic acid, which was characterised (acetic anhydride - toluene - *p* - sulphonic acid, room temp., 18 hr) as eurekanic acid diacetate (20f).

(b) Me–CO– (δ_{Me} 2.28, ν_{CO} 1720 cm^{-1}). The presence of a Me ketone function was clearly indicated by the ^{13}C NMR spectrum (Table 2) of methyl eurekanate (δ_{Me} 26.1, δ_{CO} 207.2 ppm) and its mass spectrum showed the loss of a MeCO group and hydrogen transfer (*m/e* 248→204).

(c) –O–CH_AH_B–O– (δ_A 5.10, δ_B 4.89, J_{AB} 0 Hz). These ^1H NMR chemical shifts and coupling constant are highly characteristic[17] of a methylene group located in a 1,3-dioxolan ring. Dimethyl 2,3:4,5 - di - O - methylene-galactarate (21)[18] is an excellent model: its homotopic methylenedioxy groups contain diastereotopic protons (δ_A 5.24, δ_B 5.06, J_{AB} < 1.0 Hz)[18] which show a satisfying correlation with those of methyl eurekanate. The methylenedioxy group in methyl eurekanate (20a) was confirmed by its acidic hydrolysis (5 N HCl, 100°, 6 hr) which gave formaldehyde isolated from the hydrolysate as formaldehyde 2,4-dinitrophenylhydrazone (55% yield).

(d) Me–CH(OH)–C(OH)–. The presence of this secondary-tertiary α-glycol system was supported by the ^1H NMR spectrum of methyl eurekanate which showed two signals (δ 4.15 and δ 2.58) removed by addition of deuterium oxide. Methyl eurekanate yielded a monoacetate (20d), m.p. 87°, with acetic anhydride pyridine (room temp., 18 hr) and a diacetate (20e) with acetic anhydride - toluene - *p* - sulphonic acid (room temp., 24 hr). The ^1H NMR spectrum of methyl eurekanate showed the presence of an A_3X system (δ_A 1.03, δ_X 4.18, J_{AX} 6.5 Hz) characteristic of a secondary-tertiary α-glycol system $C(H_A)_3$–$CH_X(OH)$–C(OH); the proton-H_X signal (δ_X 4.18) showed the expected downfield shift in methyl eurekanate monoacetate (20d) (δ_X 5.39), in its diacetate (20e) (δ_X 5.57) and in its bis-trichloroacetylcarbamate (20g) (δ_X 5.36). The secondary-tertiary α-glycol system of methyl eurekanate was confirmed by its periodate cleavage (sodium metaperiodate, room temp., 35 min), which yielded acetaldehyde isolated (54%) as its 2,4-dinitrophenylhydrazone.

The evidence described above in terms of the sections (a–d) can be summarised by the partial structure shown in Fig. 1. This leads to the four possible constitutional formulae [Fig. 1, (i), (ii), (iii) and (iv)] for methyl eurekanate.

The decision between the four possible constitutional formulae [Fig. 1, (i), (ii), (iii) and (iv)] rests on (a) comparison of the ^1H and ^{13}C NMR spectra of methyl eurekanate and dimethyl 2,3:4,5 - di - O - methylene-galactarate (Table 2) and (b) comparison of the mass spectral fragmentation patterns of methyl eurekanate and trideuteriomethyl eurekanate (Scheme 2).

In addition to the information on methyl eurekanate (Table 1, Scheme 2) which determines the selection of formula (i) (20a) from Fig. 1 for methyl eurekanate, there is additional supporting evidence for this constitution which follows from the general description of our ^{13}C NMR spectroscopic investigation of flambamycin (Part II)[12] and the discussion of the mass spectra of flambamycin and its derivatives (Part III).[13]

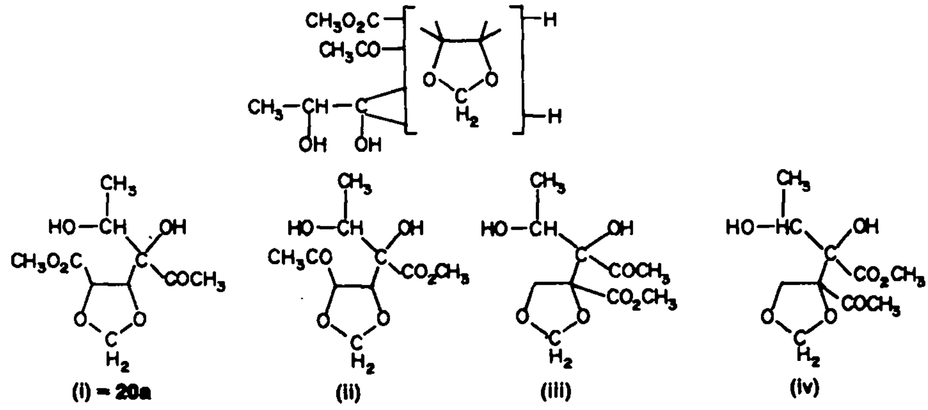
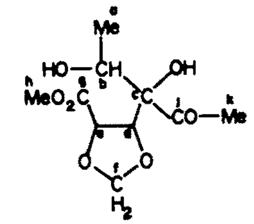
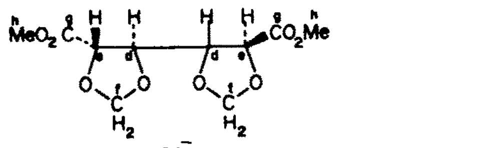


Fig. 1. The partial structure and the derived four constitutional formulae (i–iv) for methyl eurekanate.

Table 2. Comparison of the ¹H and ¹³C chemical shifts (ppm downfield from tetramethylsilane) for corresponding atoms in methyl eurekanate (20a) and dimethyl 2,3:4,5-di-O-methylene galactarate[18] (21). The position of the atoms are indicated by the letters in the formula (20a) and (21)



20a: Methyl eurekanate



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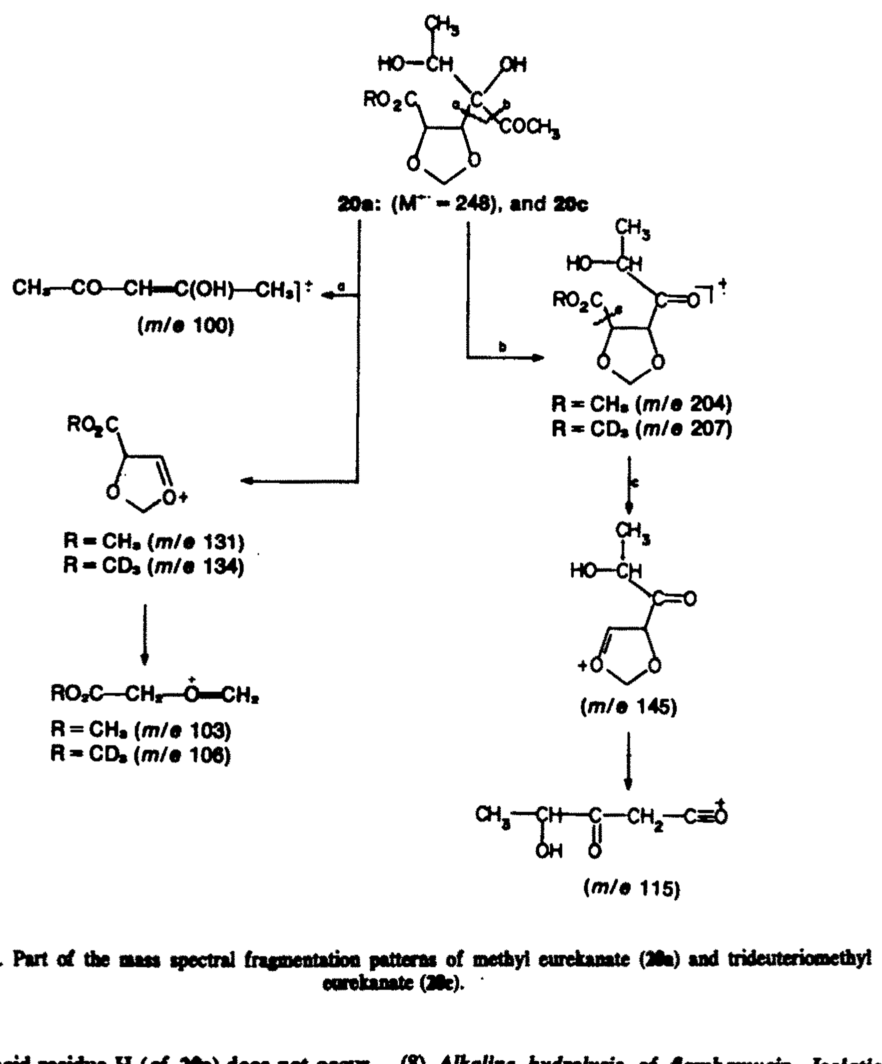
NMR spectra	Me_a	C_bH	C_c	C_dH	C_eH	C_fH_2	C_g	Me_h	C_j	Me_k
(20a) (¹H)	1.03^v	4.18^v	—	4.66^w	4.68^w	5.10,^{y} 4.89^y	—	3.78	—	2.28
(21) (¹H)	—	—	—	4.27^x	4.61^x	5.24,^{z} 5.06^z	—	3.78	—	—
(20a) (¹³C)	17.4	68.4	84.2	74.6	81.5	95.9	171.7	52.8	207.2	26.1
(21) (¹³C)	—	—	—	75.1	78.9	96.8	170.6	52.6	—	—

^{v}A_3X system, J_{AX} 6.5 Hz; ^{w}AB system, J_{AB} 6 Hz; ^{x}AA′BB′ system, $J_{AA'}$ 5.5, $J_{BB'}$ 0, $J_{AB} = J_{A'B'} = 4.0$ Hz[18]; ^{y}AB system, J_{AB} 0 Hz; ^{z}AB system, $J_{AB} < 1.0$ Hz[18].

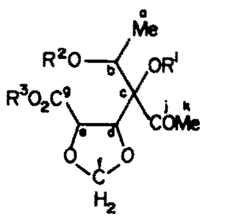


Scheme 2. Part of the mass spectral fragmentation patterns of methyl eurekanate (20a) and trideuteriomethyl eurekanate (20c).

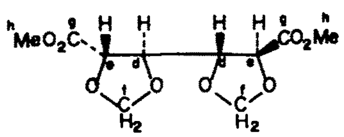
The eurekanic acid residue H (*cf.* 20a) does not occur as such in flambamycin (1) but further investigations (Sections 8 and 9) established its incorporation as an orthoester.

(8) *Alkaline hydrolysis of flambamycin. Isolation and structural elucidation of bamflalactone (23a) and flambeurekanose (24a)*[5]

The degradative evidence discussed so far had been



20a: Methyl eurekanate, R¹ = R² = H, R³ = Me
20b: R¹ = R² = H; R³ = Et
20c: R¹ = R² = H; R³ = CD_3
20d: R¹ = H; R² = Ac; R = Me
20e: R¹ = R² = Ac; R³ = Me
20f: R¹ = R² = Ac; R³ = H
20g: R¹ = R² = $Cl_3CCONHCO$, R³ = Me



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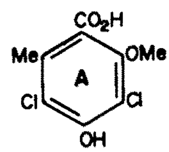
provided by either acidic hydrolysis or acidic methanolysis. It was therefore necessary to explore the possibility of obtaining additional structural evidence by examining the degradation of flambamycin by basic reagents: this approach provided important complementary structural information. At this point, it is important to emphasise that although products were obtained from the alkaline hydrolysis of flambamycin by aqueous sodium hydroxide (10% w/v) at room temperature for various periods, it was not initially appreciated that the genesis of these alkaline hydrolysis products also involved the acid hydrolysis of acid-sensitive intermediates (Section 9). These circumstances initially provided some interesting puzzles in mechanistic interpretation. However, these difficulties were ultimately removed when it was recognised that conditions of mild *acidic* hydrolysis were also involved in subsequent transformations of the so-called *alkaline* hydrolysis reactions of flambamycin. Our understanding of this aspect of these degradative transformations also provided excellent and satisfying supporting evidence for several of the novel structural features of flambamycin (1) which were quite unusual for a natural product. To clarify these matters, it is sufficient to state finally that the mild acidic hydrolysis of various acid-labile intermediates derived from flambamycin occurred during the work up of alkaline hydrolysis products when neutralisation by the addition of aqueous hydrochloric acid was followed by evaporation under diminished pressure. It was gratifying to discover that these acid-labile intermediates could indeed be isolated (Section 9) when the alkaline hydrolysates were neutralised by saturation with carbon dioxide and then worked up under carefully controlled conditions.

The flambamycin constitutional formula (1) can be conveniently divided into eight residues labelled A, B, C, D, E, F, G and H. Evidence for the sequence, D–E–F–G has been provided by the structural elucidation of flambatetrose isobutyrate (**17a**) described in Section 5. The evidence for the sequence A–B–C is based upon the isolation of flambalactone (**18**) and methyl flambate (**19b**) discussed in Section 6. We now present the argument for further structural aspects of flambamycin based upon its alkaline hydrolysis followed by mild acidic hydrolysis.

Alkaline hydrolysis (3 days) of flambamycin followed by acidification yielded dichloro-isoeverninic acid (**22**: 60% yield).



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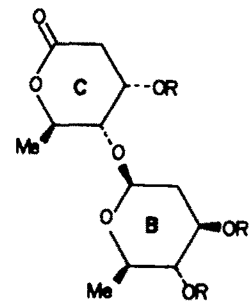


23a: Bamflalactone R = H
23b: R = Ac

Alkaline hydrolysis (5 days) of flambamycin, followed by neutralisation with aqueous hydrochloric acid, careful evaporation and treatment of the residue with acetic anhydride-pyridine (18 hr, room temp.) yielded a mixture of acetates. Chromatographic separation yielded bamflalactone triacetate (**23b**; 36% yield) and flambeurekanose penta-acetate (**24b**, 33% yield).

During our structural investigations, bamflalactone (**23a**) itself has not been isolated but equivalent structural interpretations are possible on the basis of the isolation of its triacetate (**23b**). Bamflalactone triacetate (**23b**), m.p. 131°, had a molecular formula, $C_{12}H_{17}O_4(OAc)_3$, which indicated its relation to residues B and C in flambamycin derived from 2-deoxy-D-rhamnose and a lactone of 3,4,5-trihydroxyhexanoic acid. Bamflalactone triacetate (**23b**) and flambalactone triacetate (Table 1) showed the expected correspondence of spectral properties.

Alkaline hydrolysis (18 hr) of flambamycin followed by neutralisation with dilute hydrochloric acid, careful evaporation and chromatographic fractionation, gave flambeurekanose, $C_{36}H_{58}O_{23}$, m.p. 191–192°, in remarkably high yield (85%). Flambeurekanose was characterised as a penta-acetate, $C_{36}H_{53}O_{18}$ $(OAc)_5$, m.p. 196°. The isolation of flambeurekanose was obviously a highly significant advance and its molecular formula, $C_{36}H_{58}O_{23}$, showed an encouraging relation to the molecular formulae of flambatetrose, $C_{27}H_{48}O_{18}$, and eurekanic acid, $C_9H_{14}O_7$. This possible empirical correlation is summarised by the equation:

$$\underset{\text{Flambeurekanose}}{C_{36}H_{58}O_{23}} + 2H_2O \longrightarrow \underset{\text{Flambatetrose}}{C_{27}H_{48}O_{18}} + \underset{\text{Eurekanic acid}}{C_9H_{14}O_7}$$

This possible correlation was fully confirmed by the mild acidic methanolysis (MeOH–HCl, 0.5% w/v, 90 min, room temp.) of flambeurekanose which yielded flambatetrose (**15a**; 31%) and methyl eurekanate (**20a**; 51%). The problem of proposing a constitutional formula for flambeurekanose could therefore by considered on the basis of dehydrative condensation involving the removal of two molecules of water from one molecule of flambatetrose and eurekanic acid. This approach was also limited by the following facts. (i) The 2-OH group of the L-lyxose residue G is acylated with an isobutyroyloxy group in flambatetrose isobutyrate (**17a**) (see last paragraph of Section 5). (ii) The 3- and 4-OH groups in

Table 3. Comparison of the ^{13}C chemical shifts (ppm downfield from Me_4Si) for corresponding atoms in methyl eurekanate (20a) and flambeurekanose (24a). The positions of the atoms are indicated by the letters in the formulae (20a) and (24a)

	C_a	C_b	C_c	C_d	C_e	C_f	C_g	C_j	C_k
Methyl eurekanate (20a)	17.4	68.4	84.2	74.6	81.5	95.9	171.7	207.2	26.1
Flambeurekanose† (24a)	14.2	83.4	82.1	70.0	80.5	96.7	119.8	210.8	27.6

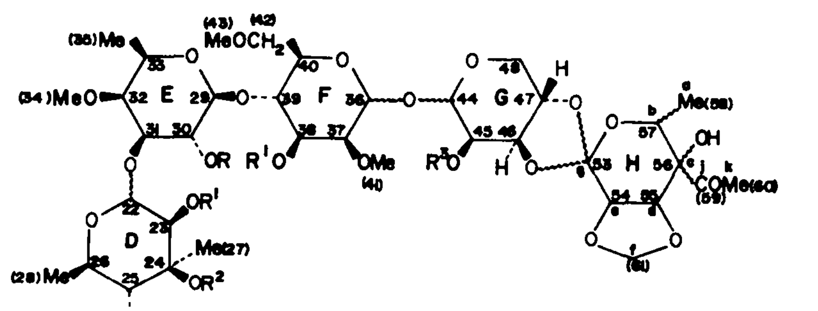
†The ^{13}C assignments for the eurekanic acid residue (H) in flambeurekanose are based upon exclusion by comparison of the ^{13}C spectra of flambatetrose (15a) and flambeurekanose (24a). These assignments are discussed in detail in Part II.[12]

the L-lyxose residue G are not methylated in "flambamycin permethyl ether" (see last paragraph of Section 4). (iii) Flambatetetrose (15a) forms a fully characterised hepta-acetate (15b) whereas under identical conditions (acetic anhydride-pyridine; 18 hr, room temp.) flambeurekanose forms a penta-acetate. (iv) Comparison (Table 3) of the ^{13}C NMR spectra of methyl eurekanate (20a) and flambeurekanose clearly indicates the presence of the Me ketone function, $-\overset{j}{C}O-\overset{k}{M}e$, in both molecules. (v) A similar comparison (Table 3) shows that the ester carbon (C_g; δ 171.7 ppm) in methyl eurekanate (20a) is not present as an ester linkage in flambeurekanose (24a) because C_g shows a dramatic upleld chemical shift (C_g; δ 119.8 ppm) in flambeurekanose. These facts (i–v) show that the union between the flambatetrose and eurekanic acid residues requires the removal of two molecules of water involving the carboxyl group of the eurekanic acid residue and three secondary OH groups (C_2–OH and C_3–OH of the L-lyxose residue plus the secondary-OH of the eurekanic acid residue). These considerations lead to a single constitutional proposal for flambeurekanose (24a) in which the L-lyxose residue G is linked to eurekanic acid residue H via an orthoester grouping. This is compatible with the observed ^{13}C NMR chemical shift (C_g; δ 119.8 ppm), the *stability* of flambeurekanose (24a) towards aqueous alkali and its *instability* towards methanolic hydrogen chloride.

In addition to the chemically based derivation of the constitution (24a) for flambeurekanose, a satisfying alternative proof of structure was provided by a comparison (Scheme 1) of the mass spectral fragmentation patterns of flambatetrose hepta-acetate (15b), flambeurekanose (24a) and flambeurekanose penta-acetate (24b). These results when considered in addition to those reported in Part II[12] leave no doubt about the presence of an orthoester grouping which links the residues G and H.

The fragment (m/e 331) from flambeurekanose corresponds (Scheme 1) with the fragment (m/e 373) from flambeurekanose penta-acetate: these fragments both contain the orthoester grouping and establish the adjacence of the L-lyxose residue G and the eurekanic acid residue H. Furthermore, there is a striking correlation (Scheme 1) between the fragmentation patterns of flambatetrose hepta-acetate and flambeurekanose penta-acetate which show common fragment ions at m/e 245, 447 and 679. The relative stability of this orthoester grouping between the residues G and H under conditions of electron impact is noteworthy.

Reassuring assistance in the determination of the constitution of flambamycin (1) had been provided by ^1H NMR (60 and 100 MHz, Experimental), ^{13}C NMR (Part II)[12] and low and high resolution mass spectra (Part III).[13] However, it was only when a 220 MHz spectrum on flambeurekanose penta-acetate (24b) was obtained after the experimental investigation was completed that we appreciated (i) that at higher resolution many of the ^1H NMR signals of flambeurekanose penta-acetate (24b) could be confidently assigned, (ii) that comparison of the ^1H NMR spectra (100 MHz) of flambabiose penta-acetate (8b), flambatriose isobutyrate penta-acetate (16b), flambatetrose octa-acetate (15c) and flambatetrose isobutyrate hepta-acetate (17c) with the ^1H NMR spectrum (230 MHz) of flambeurakanose pentacetate (24b) was extremely informative and (iii) that the approaches (i) and (ii) led to an independent complementary proof that the constitution first proposed[6] for flambamycin was incorrect and that relocation of the intermonosaccharide D–E linkage to C(22)–O–C(31) now shown in the constitution (1) was required.



24a: Flambeurekanose, $R^1 = R^2 = R^3 = H$

24b: $R^1 = R^2 = Ac$; $R^3 = H$

24c: $R^1 = R^2 = H$; $R^3 = -\overset{O}{\overset{\|}{C}}-CHMe_2$

In the comparison† of the NMR spectra of **8b**, **16b**, **15c**, **17c** and **24b** particular attention was paid to resonances in the range δ 4.3–5.2 which could be attributed to secondary acetates, CH (OAc), glycosidic (anomeric) centres, O–CH–O, and methylenedioxy groups, O–CH_2–O. Although unique assignments to particular signals were not possible, within the range δ 4.3–5.2 informative correspondences (in terms of chemical shift, multiplicities and coupling constants) could be recognised. Alternatively, for pairs of compounds selected from **8b**, **16b**, **15c**, **17c** and **24b**, "subtraction" of common signals meant that additional signals could be assigned to protons which were present in one compound but which were absent from the other compound.

This approach is exemplified by dealing with the compounds studied which exhibited in the range δ 4.3–5.2 signals in accord with the indicated number of protons (**8b**, 7H), (**16b**, 9H), (**15c**, 11H), (**17c**, 11H), (**24b**, 11H). These signals are not individually assignable but they do correspond numerically with the CH(OAc), O–CH–O and O–CH_2–O groupings present in the constitutional formulae **8b**, **16b**, **15c**, **17c** and **24b**. Thus comparison of the ^{1}H NMR spectra of the derivatives **8b** and **16b** shows that the spectrum of **16b** has an additional signal whose chemical shift (δ 4.24) and multiplicity (doublet, $J_{1,2}$ 8 Hz) demands its assignment to the glycosidic C (29)–H of residue E. The coupling constant, $J_{1,2}$ 8 Hz is appropriate for a *trans* diaxial relation of the C (29)–H and the C (30)–H of residue E. In summary, doublet signals ($J_{1,2}$ 8 Hz) are the C (29)–H of flambatetrose octa-acetate (**15c**, δ 4.36, doublet, $J_{1,2}$ = 8 Hz) and flambatetrose isobutyrate hepta-acetate (**17c**; δ 4.39, doublet, $J_{1,2}$ 8 Hz).

It was now possible to compare the 100 MHz, ^{1}H NMR spectrum of flambatetrose derivatives (**15c** and **17c**) with the 220 MHz ^{1}H NMR spectrum of flambeurekanose penta-acetate (**24b**). In flambeurekanose penta-acetate (**24b**) signals due to 11-H appeared in the range δ 4.3–5.2. These included seven singlet signals and four multiplets. The seven singlet signals (δ 4.76, 4.76, 4.97, 5.09, 5.09, 5.23, 5.51) could not be separately assigned because although located such that geminal or vicinal coupling was possible, the coupling constants were obviously close to zero. In contrast the four multiplets in the ^{1}H NMR spectrum of flambeurekanose penta-acetate (**24b**) can only be assigned as follows:

C (25–H) (δ 4.83, doublet, $J_{1,2}$ 10 Hz);

C (29)–H (δ 4.38, doublet, $J_{1,2}$ 8 Hz);

C (30)–H (δ 5.06, double doublet, $J_{1,2}$ 8 and $J_{2,3}$ 10 Hz);

C (38)–H (δ 4.94, double doublet, $J_{2,3}$ 4 and $J_{3,4}$ 10 Hz).

The vicinal *trans*-diaxial relation ($J_{1,2}$ 8 Hz) between C (29)–H and C (30)–H is clear. This necessarily shows that C (30)–H has a chemical shift (δ 5.06) which demands that C (30) bears an acetoxyl group. Therefore the D–E intermonosaccharide linkage must be to C (31).

On the basis of the ^{1}H NMR spectral characteristics of the anomeric protons observed for flambatriose isobutyrate penta-acetate (**16b**; δ 4.24); flambatetrose octa-acetate (**15c**; δ 4.36); flambatetrose isobutyrate hepta-acetate (**17c**; δ 4.39); and flambeurekanose penta-acetate (**24b**; δ 4.38), the intermonosaccharide linkage between residue E and F in these compounds possesses the β-configuration at C (29).

Regarding the methylenedioxy group which is present in flambeurekanose penta-acetate there are several singlet signals (e.g. δ 4.76, 4.97 and 5.09) which are available for assignment to the diastereotopic protons of H_A–C (61)–H_B. The corresponding protons in methyl eurekanate (**20a**) are given in Table 2 (δ 4.89 and 5.10; *J* 0 Hz).

(9) *The constitution of flambamycin* (1)

The derivation of a complete consitution for flambamycin, $C_{61}H_{88}Cl_2O_{33}\cdot H_2O$, could now be considered on the basis of its possible empirical relation to flambic acid (**19a**) and flambeurekanose isobutyrate (**24c**). Flambeurekanose isobutyrate and flambic acid have not been isolated as degradation products of flambamycin, but their hypothetical use in structural proposals is nevertheless fully acceptable, because it is already unequivocally established (Section 5) that the isobutyroyloxy group present in flambamycin is located on C-2 of the L-lyxose residue. The empirical relation between flambic acid, flambeurekanose isobutyrate and flambamycin is summarised by the following equation:

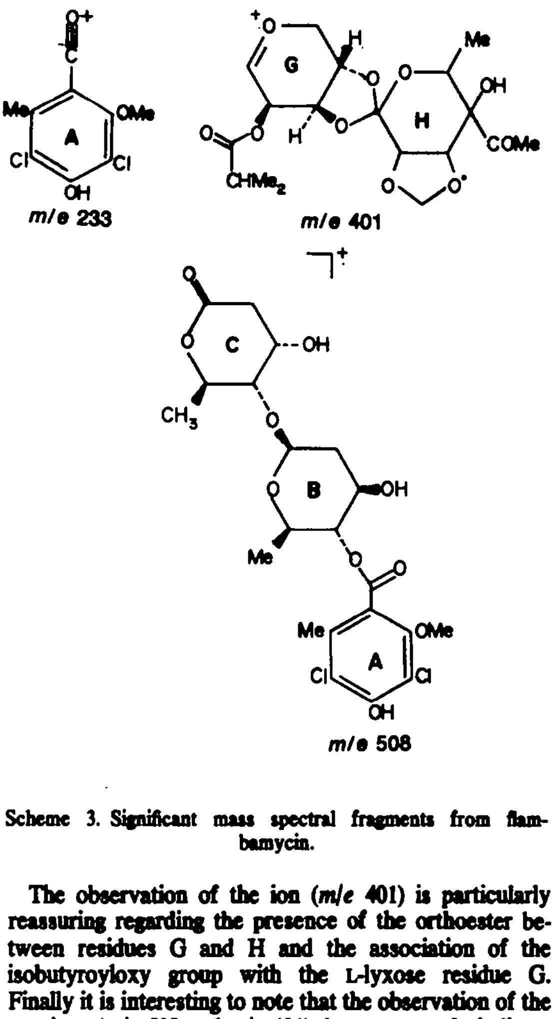
$$\underset{\substack{\text{Flambic acid}\\(\mathbf{19a})}}{C_{21}H_{28}Cl_2O_{11}} + \underset{\substack{\text{Flambeurekanose}\\\text{isobutyrate }(\mathbf{24c})}}{C_{40}H_{64}O_{24}} - 2H_2O \longrightarrow \underset{\substack{\text{Flambamycin}\\(1)}}{C_{61}H_{88}Cl_2O_{33}}$$

Concern about the possibility that this equation could be misleading because flambamycin is normally obtained as a monohydrate, $C_{61}H_{88}Cl_2O_{33}\cdot H_2O$ is not well based because flambamycin has been characterised as a hexa-acetate which is not hydrated. Furthermore, the transformations of flambamycin detailed in Section 10 place, beyond doubt, the view that the derivation of a constitutional formula for flambamycin demands the dehydrative removal of two molecules of water from flambic acid and flambeurekanose isobutyrate. This *bis*-dehydration must involve the generation of a second orthoester grouping between the carboxyl group of flambic acid, one OH group of flambic acid (**19a**) and two appositely placed OH groups of flambeurekanose isobutyrate (**24c**).

Of the six OH groups present in flambeurekanose isobutyrate (**24c**) only the three OH groups located in positions -2, -3, and -4 of the terminal D-evalose grouping (**24c**, residue D) are sterically suitable for possible involvement in an orthoester grouping. This leads to two possible structures for flambamycin of which the constitution (1) was established on the following evidence. (i) Isolation of methyl 2 - O - methyl - D - evalopyranoside (9) from the methanolysis of flambamycin permethyl ether proved that the C_2–OH of the D-evalose residue was free in flambamycin and therefore the C_3–OH and C_4–OH were involved in the orthoester grouping (Section 4). (ii) The ^{13}C NMR spectrum of flambamycin showed two signals (C_5D_5N, δ 119.8 and δ 120.9 ppm) which can be assigned to two orthoester groupings. These signals, which are certainly in accord with orthoester groupings[10g,h,11b,c,d] can, in fact, be respectively assigned to the C–D orthoester grouping (1, C_{14}, δ 120.9 ppm) and the G–H orthoester grouping (1, C_{33}, δ 119.8 ppm). Detailed supporting arguments for these respective assignments are given in Part II.[12] (iii) The hydrolytic transformations of flambamycin (1) discussed in Section

†In order to facilitate the comparison of ^{1}H NMR spectral characteristics of corresponding protons in the five derivatives **8b**, **16b**, **15c**, **17c** and **24b**, the C atoms in the formulae 8, 14, 15, 16, 17 and 24 have been numbered to correspond with the arbitrary numbering of the sixty one C atoms of flambamycin (1).

10 establish the presence of two orthoester groupings located between the C–D and the G–H residues. (iv) Extensive high resolution mass spectral studies of flambamycin and its derivatives are discussed in detail in Part III.[13] However, it is useful at this point to refer particularly to three fragment ions obtained from flambamycin (Scheme 3).



Scheme 3. Significant mass spectral fragments from flambamycin.

The observation of the ion (*m/e* 401) is particularly reassuring regarding the presence of the orthoester between residues G and H and the association of the isobutyroyloxy group with the L-lyxose residue G. Finally it is interesting to note that the observation of the two ions (*m/e* 508 and *m/e* 401) does apparently indicate a difference in ease of cleavage by electron impact of the two orthoester groupings in flambamycin (**1**).

(10) *Constitution of des-isobutyroyl flambamycin* (**25a**), *flambeurekanose flambate* (**26a**), *flambeurekanose flambate isobutyrate* (**26d**) *and des-dichloroisoeverninoyl-des-isobutyroyl flambamycin* (**27**)

Reference has already been made in Section 9 to the experimental investigation of the alkaline hydrolysis of flambamycin. In these initial studies, it was discovered that neutralisation with 2N HCl during work-up resulting in subsequent transformation of acid-labile intermediates. This was circumvented by neutralisation of the alkaline hydrolysates by the passage of carbon dioxide through the reaction products. Cautious work-up then yielded transformation products of flambamycin in accord with our expectation that the ester grouping between the residue A–B and the isobutyroyloxy ester group would both be cleaved by alkaline hydrolysis, whereas in contrast, the two orthoester groupings would be base-stable but cleavable under acidic reaction conditions.

When flambamycin was heated under reflux (40 min) with potassium carbonate in methanol, this gave a potassium salt (99%), which with carbon dioxide in aqueous solution gave des-isobutyroyl flambamycin (**25a**; 81%, m.p. 202–203°). This result provides an informative contrast with the transformation of flambamycin into flambeurekanose (Section 8). Des-isobutyroyl flambamycin (**25a**) with acetic anhydride-pyridine (18 hr, room temp.) gave the expected hepta-acetate (**25b**), m.p. 198–199°.

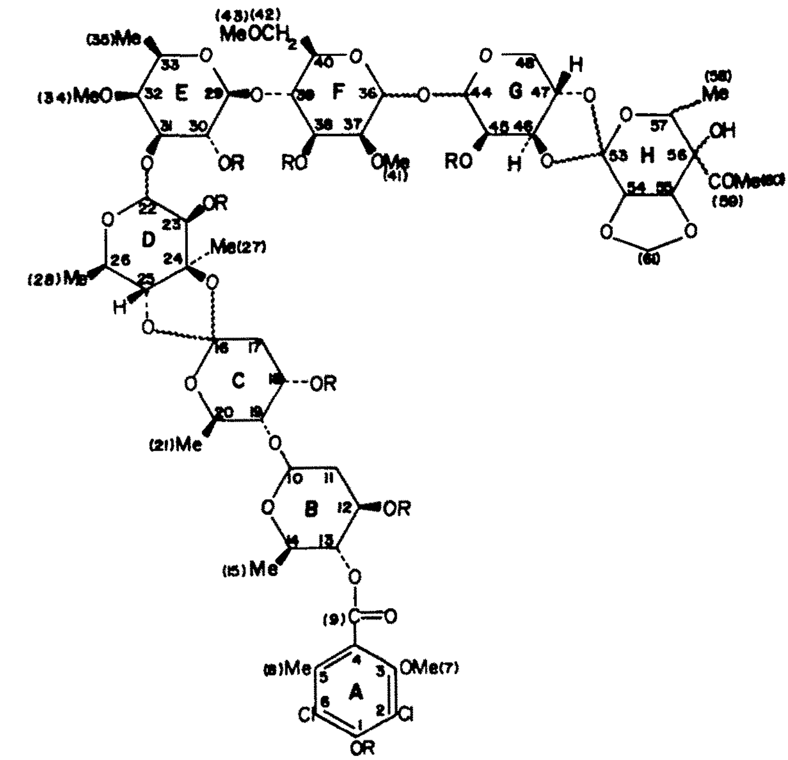
It is noteworthy that a highly selective cleavage of one of the two orthoester groupings in flambamycin is possible. Flambamycin (**1**) with an Amberlyst acidic resin in moist ethyl acetate at room temperature (30 min) is transformed in remarkably high yield (80%) into flambeurekanose flambate isobutyrate (**26d**), m.p. 160–163° characterised as its hepta-acetate (**26e**), m.p. 135–138° and its octa-acetate (**26f**), m.p. 150–153°. The factors which are associated with the mild acidic hydrolysis of the C–D orthoester grouping and the survival of the G–H orthoester grouping, provide a stimulating basis for speculation. Their relative stability is an interesting general aspect of the chemistry of orthoesters which is not, at present, understood.

An entirely analogous transformation occurred when des-isobutyroyl flambamycin (**25a**) was transformed into flambeurekanose flambate (**26a**, 31%), m.p. 174–176°, characterised as its octa-acetate (**26b**), m.p. 187° and its non-acetate (**26c**), m.p. 143–145°. The transformation (**25a**)→(**26a**) also unequivocally established the presence of the orthoester grouping between residues C–D and the direction of cleavage of this orthoester grouping follows from the fact that flambeurekanose flambate (**26a**) forms an octa-acetate (**26b**) under experimental conditions which are known not to result in the acetylation of the tertiary OH group of the D-evalose residue D.

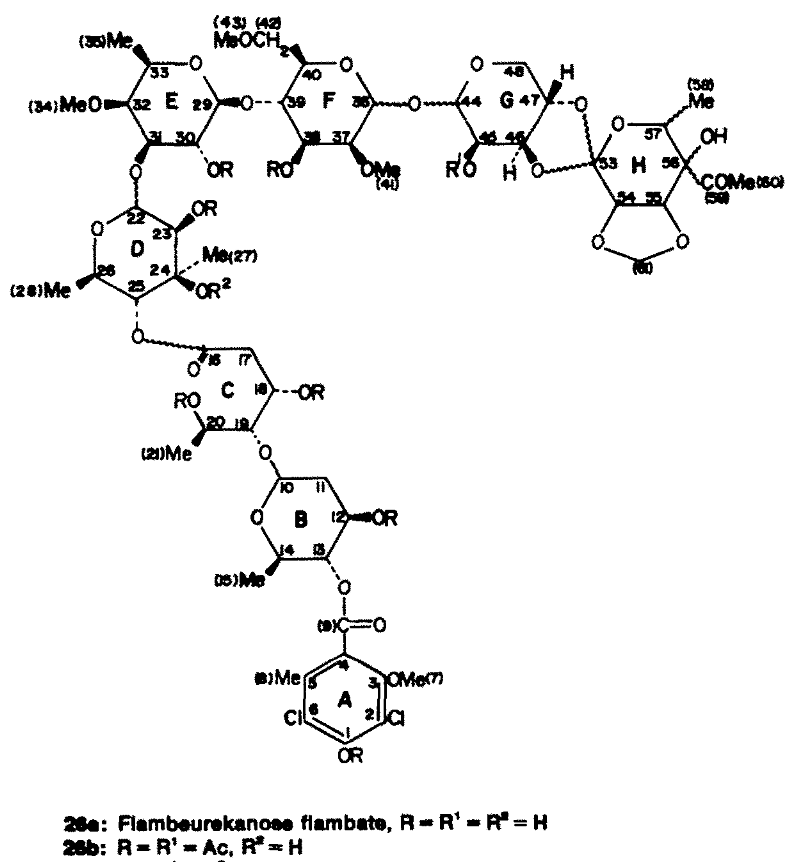
The assignment of structure (**25a**) to des-isobutyroyl flambamycin, structure (**26a**) to flambeurekanose flambate and structure (**26d**) to flambeurekanose flambate isobutyrate was made possible from a comparison of their ^{13}C NMR spectra with that of flambamycin (**1**), where the presence of the C–D orthoester grouping in **1** and **25a**, and the C–D ester grouping in **26a** and **26d** was established from the chemical shift values[12] associated with C-16 in **1** (δ 120.9), **25a** (δ 120.9), **26a** (δ 172.5) and **26d** (δ 172.6). The presence and location of the isobutyroyloxy grouping on C-45 of the lyxose residue G in flambeurekanose flambate isobutyrate (**26d**) was indicated by a comparison[12] of the chemical shift values of C-44 in flambamycin (**1**; δ 95.3) and flambeurekanose flambate isobutyrate (**26d**; δ 95.2) with those of C-44 in des-isobutyroyl flambamycin (**25a**; δ 98.7) and flambeurekanose flambate (**26a**; δ 98.7). This upfield shift (δ 98.7→δ 95.2 ppm) in the ^{13}C resonance of the anomeric carbon at C-44 is associated with acylation of the OH group at C-45, and is discussed in more detail in Part II.[12]

In the transformations (**1**→**26d**) and (**25a**→**26a**) it must be recognised that the hydrolysis of the C–D orthoester grouping is regio-specific in that the derived esters (**26d** and **26a**) contain ester groups located in the secondary position C-25 of the D-evalose residue D.

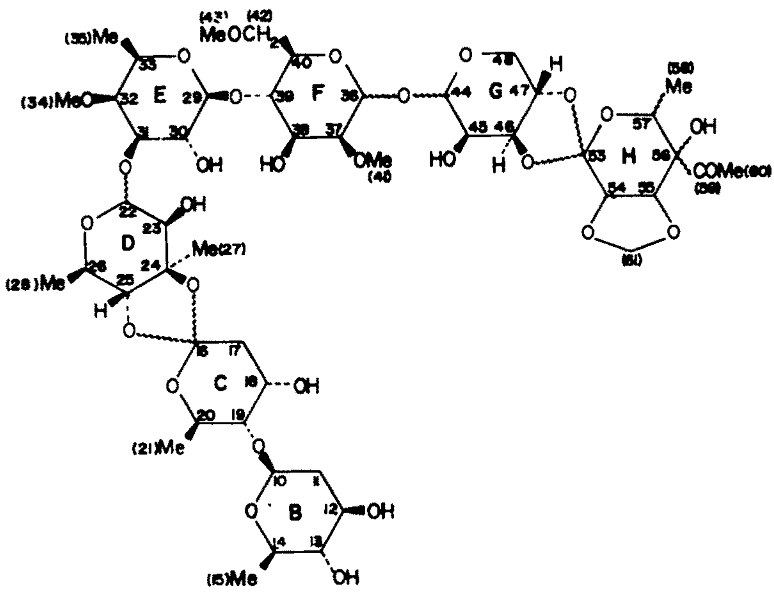
Finally, in accord with expectation based upon the experiences reported in Section 8, flambamycin and aqueous sodium hydroxide (10% w/v) at room tempera-



25a: Des-isobutyroyl flambamycin, R = H
25b: R = Ac



26a: Flambeurekanose flambate, $R = R^1 = R^2 = H$
26b: $R = R^1 = Ac$, $R^2 = H$
26c: $R = R^1 = R^2 = Ac$
26d: Flambeurekanose flambate isobutyrate, $R = R^2 = H$, $R^1 = COCHMe_2$
26e: $R = Ac$, $R^1 = COCHMe_2$, $R^2 = H$
26f: $R = R^2 = Ac$, $R^1 = COCHMe_2$



27: Des-dichloroisoeverninoyl-des-isobutyroyl flambamycin.

ture (24 hr) followed by the passage of carbon dioxide yielded (44%) des-dichloroisoeverninoyl-des-isobutyroyl flambamycin (27, m.p. 212°). In this alkaline hydrolysis (1→27) of flambamycin both ester groupings have been cleaved and both orthoester groupings have been retained.

These results confirm in all respects the more subtle features of the constitution 1 proposed for the antibiotic, flambamycin. The ^{13}C NMR spectra of the compounds 25a, 26a, 26c and 27, provide excellent support for the proposed constitutions and are discussed in detail in Part II.[12]

(11) *The orthosomycins, a new family of antibiotics*

Flambamycin (1) belongs to a new class of antibiotics which includes curamycin,[7] avilamycin[8] and the everninomicins.[9,10] The constitutions of curamycin[7] and avilamycin,[8] have not yet been fully elucidated but considerable degradative evidence has been reported. Recently, complete constitutions have been announced for everninomicin-B (28a),[9d] everninomicin-C (28b),[9c] everninomicin-D (28c),[10e] and everninomicin-2 (28d).[10h]

This new class of antibiotics is apparently characterised by the presence of a number of common structural features: (i) a terminal ester residue A derived from 3,5-dichloroisoeverninic acid (22),[2-10] (ii) a residue C derived from 3,4,5-trihydroxyhexanoic acid,[2-6,8-10] (iii) oligosaccharide sequences associated with various monosaccharide residues derived from 2-deoxy-D-rhamnose, 4-O-methyl-D-fucose (5a), 2,6-di-O-methyl-D-mannose (6a) and L-lyxose (7a), (iv) two orthoester groupings. The constitutional relation between the residues B and C derived from 2-deoxy-D-rhamnose and 3,4,5-trihydroxy-hexanoic acid is noteworthy and could be of biosynthetic significance.[15]

The natural occurrence of orthoesters[19] might be expected to be unusual but the persuasion that they might be encountered was first generated by the elucidation of the remarkable constitution of tetrodotoxin.[20] Subsequently six structurally related antibiotics (29a–e) and (30) were isolated.[11] They were shown to have three residues associated with a diamino-cyclitol (ring C), D-talose or D-mannose (ring B), and a polyhydroxyamino acid (ring A). These antibiotics were unusual in that they contained one orthoester linkage. The structural elucidation of hygromycin B (29e) by ^{13}C NMR spectroscopy was a classic investigation[11d] and particular mention should be made of the recognition that orthoesters were associated with a characteristic chemical shift (δ 120.6; corrected from carbon disulphide to tetramethylsilane as chemical shift reference). Similar chemical shifts have been recorded[11b,c] for the orthoester C atoms in the destomycins (29a,b and 30) (δ 121.2, destomycin A; δ 121.7, destomycin B; δ 121.2, destomycin C).

The presence of two orthoester groupings in flambamycin (1) and the everninomicins (28a–d) is a novel structural feature of these antibiotics. It is now proposed[21] that this new family of antibiotics should be called the orthosomycins in recognition of the presence in their structures of orthoester groupings in association with carbohydrate residues.

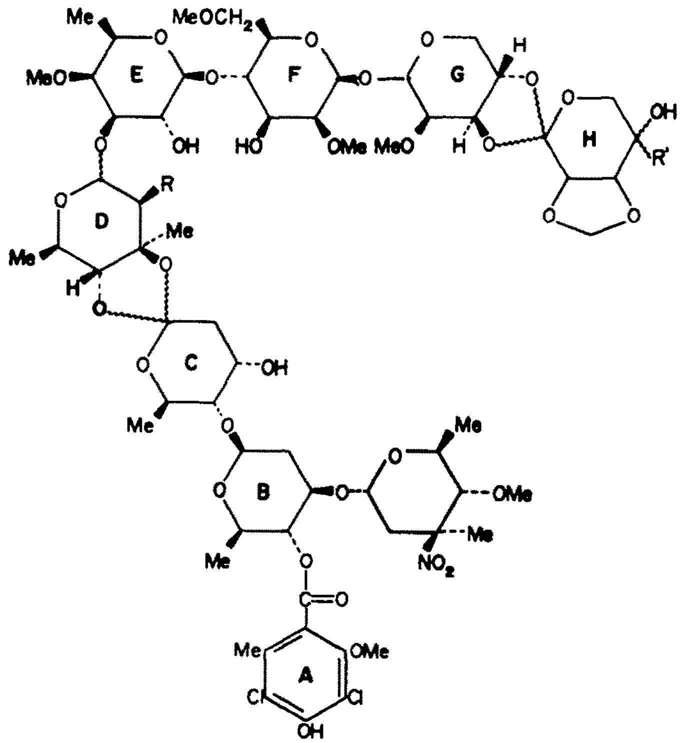
In spite of the structural similarities between the orthosomycins, there are, nevertheless, remarkable structural differences between the complete constitutions of flambamycin (1) and the everninomicins (28). Residues A, B, C, E and F are common.

Residue D is derived from D-evalose (4) in flambamycin (1) and everninomicin-B (28a)[9b] but the corresponding residue in everninomicin-C (28b)[9c], everninomicin-D (28c)[10e] and everninomicin-2 (28d),[10h] is derived from a new sugar D-evermicose.[11b] D-Evermicose is the 2-deoxy-D-evalose.

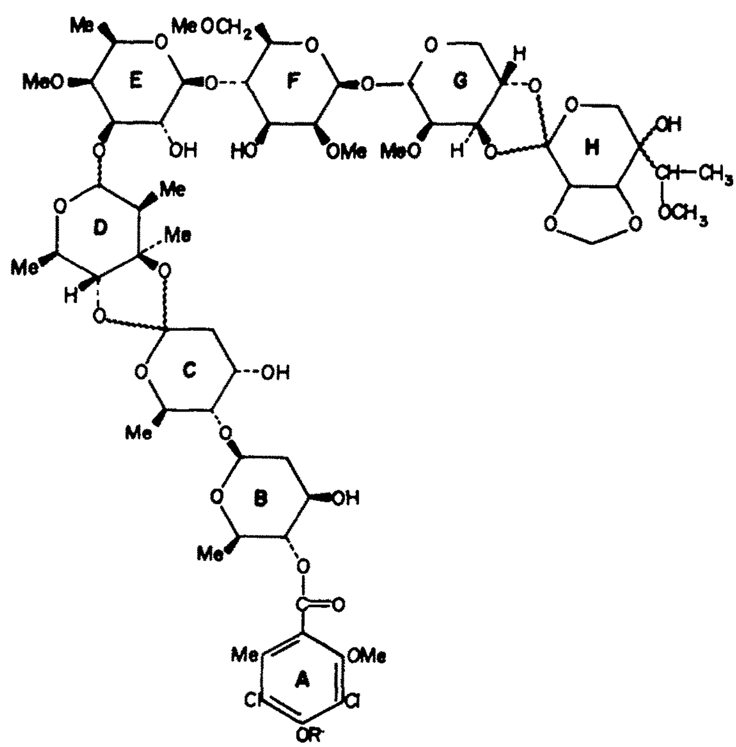
Residue G is derived from L-lyxose (7a). In the case of the everninomicins (28a–d), the L-lyxose residue occurs as its 2-O-methyl ether whereas in flambamycin (1) the residue G is derived from 2-isobutyroyl-L-lyxose.

The degradation of the everninomicins (28a–d) to give products analogous to methyl eurekanate (20a) has been reported.[9d,9a,10g,10h] The residue H in flambamycin (1) does bear an interesting relation to the H-residues which have been identified in the everninomicins (28a–d).

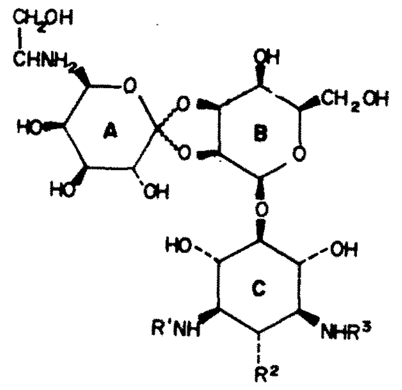
The most striking structural differences between flambamycin (1) and the everninomicins (28a–d) is that the everninomicins contain a nitro-sugar residue. This is

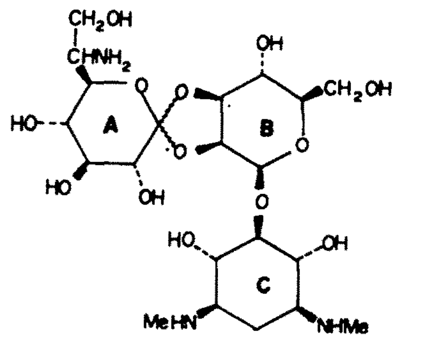


28a: Everninomicin-B, R = OH; R' = CH(OMe)Me
28b: Everninomicin-C, R = R' = H
28c: Everninomicin-D, R = H; R' = CH(OMe)Me



28d: Everninomicin-2





29a: Destomycin A, $R^1 = Me$, $R^2 = R^3 = H$ **30**: Destomycin B
29b: Destomycin C, $R^1 = R^3 = Me$, $R^2 = H$
29c: Hygromycin B, $R^1 = R^2 = H$, $R^3 = Me$
29d: A-396-I (=SS − 56D) $R^1 = R^2 = R^3 = H$
29e: SS − 56C, $R^1 = R^3 = H$, $R^2 = OH$

evernitrose[10a] which is linked glycosidically to residue B. It is interesting to note that the evernitrose residue has been chemically removed from the everninomicins and the products still show antibacterial properties.[10A]

The structural relationships between the orthosomycins may well eventually provide useful information concerning structure-activity correlations among the members of this new family of antibiotics and their transformation products. The mechanism of the biological action of avilamycin has been investigated.[22] The stereochemistry of the reactions of orthoesters is now a subject for detailed exploration.[22] The chemistry and biological activity of the orthosomycins could well be determined by certain aspects of stereoelectronic control characteristic of orthoesters.[23,24]

EXPERIMENTAL

Unless otherwise stated IR spectra were measured in $CHCl_3$ and 60 and 100 MHz ^1H NMR spectra in $CDCl_3$ (chemical shifts on δ scale with respect to TMS as internal reference). Only significant bands from IR and NMR spectra are quoted. High resolution mass spectra were determined with an AEI MS9 spectrometer and low resolution mass spectra with AEI MS12 and V.G. Micromass spectrometers. M.ps were determined with a Kofler hot-stage aparatus. Evaporation refers to evaporation under diminished pressure. Light petroleum refers to the fraction with b.p. 60–80° unless otherwise stated.

Separations of mixtures by chromatography were carried out by the following procedures:

(a) *Method A.* Thick-layer chromatography on silica gel using (i) $CHCl_3$–MeOH (4:1); (ii) $CHCl_3$–MeOH (9:1); (iii) benzene-acetone (2:3); (iv) $CHCl_3$–acetone (9:1); (v) EtOAc; (vi) EtOAc-light petroleum (3:7); (vii) $CHCl_3$–acetone (4:1) as solvents:

(b) *Method B.* Thin-layer chromatography on silica gel using (i) $CHCl_3$; (ii) $CHCl_3$–acetone (9:1); (iii) EtOAc–light petroleum (3:1); (iv) EtOAc–light petroleum (4:1); (v) benzene–EtOH (25:3); (vi) $CHCl_3$–MeOH (4:1); (vii) $CHCl_3$–MeOH (20:1); (viii) $CHCl_3$–MeOH (40:1); (ix) EtOAc; (x) toluene–acetone (3:2); (xi) EtOAc–light petroleum (3:7); (xii) $CHCl_3$–acetone (4:1) as solvents:

(c) *Method C.* Column chromatography on silica using (i) EtOAc–light petroleum (1:1); (ii) EtOAc; (iii) benzene–EtOAc (100:1); (iv) benzene–acetone (3:1) as solvents. Chromatograms (methods A and B) were developed by (marginal) spraying with dil. H_2SO_4 10%, w/v) and heating at 100° for 10 min: all bands were eluted either with acetone or MeOH. During isolation processes the appropriate combination of fractions was determined by examination of their IR spectra and tlc behaviour.

Methods D, E and F. Unless otherwise stated, acetylations were carried out by reaction with either Ac_2O-pyridine for 18 hr (method D) or with Ac_2O - toluene - *p* - sulphonic acid for 3 hr (method E) at room temp. Methylation refers to reaction with sodium hydride - dimethylsulphoxide - methyl iodide for 18 hr at room temp. (method F).

When substances are stated to be identical, their identity has been established by (a) comparison of m.p. and mixed m.p. determination and, where appropriate, (b) comparison of their IR, NMR and mass spectra and their behaviour on tlc.

The numbered headings in the Experimental refer to the corresponding sections in the Introduction.

(1) *Preliminary characterisation of flambamycin*

Flambamycin (1). After isolation, 1 was obtained as colourless micro-needles, m.p. 202–203° (lit.[1] 226–228°), from acetonitrile [Found: C, 50.7; H, 6.1; Cl, 4.8; OMe, 8.6; CMe, 10.5. $C_{47}H_{46}Cl_2O_{29}$ $(Me)_{10}$ $(OMe)_4 \cdot H_2O$ requires: C, 50.9; H, 6.3; Cl, 4.9; OMe, 8.6; CMe, 10.4%]; $[\alpha]_D^{25} -9.3°$ (EtOH); ν_{max} (KBr) 3400, 1735 (ϵ 720), 1717 (ϵ 500) cm^{-1}; λ_{max} (EtOH) 288 nm (ϵ 1725); δ (C_5D_5N) 3.95, 3.65, 3.59, 3.29 (s, 4 OCH_3), 2.26, 2.24 (s, $ArCH_3$, $COCH_3$), 1.51 (s, CCH_3), 1.46–1.06 (d, 7 $CHCH_3$).

Flambamycin hexa-acetate was prepared (Method D; 71%) as colourless crystals, m.p. 177–179°, from EtOAc-*n*-hexane [Found: C, 51.8; H, 5.9; Cl, 4.1. $C_{61}H_{82}Cl_2O_{27}$ $(OAc)_6$ requires: C, 52.0; H, 6.0; Cl, 4.2%]; ν_{max} (KBr) 3500, 1785, 1750 cm^{-1}.

(2) *Identification of acidic hydrolysis products of flambamycin* (1)

Isolation of (i) *curacin* (2), (ii) 3,5 - *dihydroxy* - γ - *caprolactone* (3), (iii) D-*evalose* (4), (iv) 4 - O - *methyl* - D - *fucose* (5), (v) 2,6 - *di* - O - *methyl* - D - *mannose* (6), (vi) L-*lyxose* (7) and (vii) *flambabiose* (8).

A mixture of flambamycin (2.0 g) and dil. HCl (150 ml, 0.5% w/v) was heated at 78° for 30 min and then kept at 31° for 17 hr. The soln was then concentrated to 60 ml, extracted with ether (2 × 100) ml) and the combined ethereal extracts were evaporated. The residue was purified [method A, solvent (ii)] when curacin (0.36 g; 68%) (R_f 0.29–0.38) was obtained.

The aqueous soln after ether extraction was neutralised with Amberlite ion-exchange resin IR-4B (HO^--form) and evaporated. The residue (1.4 g) was heated with dil. HCl (280 ml, 0.4%) at 100° for 3 hr, neutralised as previously, and evaporated. The residual mixture was separated by column chromatography on cellulose (Whatman CF 11 grade) using n-BuOH-water (20:3) as the eluting solvent. Where appropriate, fractions (8 ml) were combined and purified [method B, solvent (vi)] yielding 3,5 - dihydroxy - γ - caprolactone (56 mg, 28%) (R_f 0.64–0.70), 2,6 - di - O - methyl - D - mannose (155 mg, 54%) (R_f 0.40–0.55), 4 - O - methyl - D - fucose (163 mg, 66%) (R_f 0.35–0.44), D-evalose

(195 mg, 70%) (R_f 0.30–0.36), flambabiose (41 mg, 9%) (R_f 0.21–0.29) and L-lyxose (25 mg, 12%) (R_f 0.00–0.10).

(i) *Curacin* (2). This fraction crystallised from $CHCl_3$ as the α-anomer, m.p. 143–145° (lit.[7a] 145°). (Found: C, 46.3; H, 5.0; Cl, 17.9; $M^{+\cdot}$, *m/e* 380. Calc. for $C_{15}H_{18}Cl_2O_7 \cdot 0.5H_2O$: C, 46.3; H, 4.9; Cl, 18.2%; M, 380); $[\alpha]_D^{26}$ +56.7° → 27.5° (EtOH, 24 hr); ν_{max} 3600, 3400, and 1730 cm^{-1}; δ $[(CD_3)_2CO]$ 5.29 (dd, rhamnose residue H-1, $J_{1,2e}$ 1, $J_{1,2a}$ 3 Hz), 4.78 (t, rhamnose residue H-4, $J_{3,4} = J_{4,5}$ = 10 Hz), 4.09 (m, rhamnose residue H-5), 3.86 (s, OCH_3), 2.35 (s, $ArCH_3$), 2.17 (H-2a), 1.74 (H-2e), 4.09 (H-3) (ABX part of an ABXY system, rhamnose residue $-CH_2-$ and H-3), 1.29 (d, rhamnose residue $CHCH_3$, *J* 6 Hz). δ (C_5D_5N) 5.71 (broad doublet, rhamnose residue H-1, $J_{1,2a}$ 3 Hz), 5.44 (t, rhamnose residue H-4, $J_{3,4} = J_{4,5}$ = 10 Hz), 4.78 (m, rhamnose H-3 and H-4), 4.00 (s, OCH_3), 2.54 (s, $ArCH_3$), 1.60 (d, $CHCH_3$, *J* 6 Hz).

Methylation of curacin, using diazomethane, gave curacin methyl ether[7a] which was obtained (92%) as colourless needles (from benzene), m.p. 118° (lit.[7a] 118–119°) [Found: $M^{+\cdot}$, *m/e* 394. Calc. for $C_{14}H_{14}Cl_2O_5$ $(OMe)_2$: M, 394]; ν_{max} 3600, 3450, 1740 cm^{-1}; δ 5.37 (dd, rhamnose residue H-1, $J_{1,2e}$ 1, $J_{1,2a}$ 3 Hz), 4.82 (t, rhamnose residue H-4, $J_{3,4} = J_{4,5}$ = 10 Hz), 3.89, 3.87 (s, 2 OCH_3), 3.70 (m, rhamnose residue H-5), 2.69 (H-2a), 2.20 (H-2e) and 4.15 (H-3) (ABX part of an ABXY system, rhamnose residue–CH_2– and H-3), 2.35 (s, $ArCH_3$), 1.29 (d, $CHCH_3$, *J* 6 Hz).

Curacin methyl glycoside[7b] was obtained (58%) as colourless crystals (from aqueous acetone), m.p. 148–150° (lit.[7b] 148–150°) [Found: $M^{+\cdot}$, *m/e* 394.0586. Calc. for $C_{14}H_{14}Cl_2O_6$ $(OMe)_2$; M, 394.0586]; ν_{max} (KBr) 3500, 3300, 1720 cm^{-1}; δ $[(CD_3)_2CO]$ 4.76 (dd, rhamnose residue H-1, $J_{1,2e}$ 1, $J_{1,2a}$ 3 Hz), 4.79 (t, rhamnose residue H-4, $J_{3,4} = J_{4,5}$ = 10 Hz), 3.87 and 3.28 (s, 2 OCH_3), 3.77 (m, rhamnose residue H-5), 2.35 (s, $ArCH_3$), 2.17 (H-2a), 1.77 (H-2e), 4.07 (H-3) (ABX part of an ABXY system, rhamnose residue $-CH_2-$ and H-3), 1.28 (d, $CHCH_3$, *J* 6 Hz).

Curacin triacetate[7a] was obtained (Method D, 92%) as colourless crystals (from EtOH), m.p. 195° (lit.[7a] 193–194°) [Found: C, 49.7; H, 4.8; Cl, 14.0; $M^{+\cdot}$, *m/e* 506. Calc. for $C_{15}H_{15}Cl_2O_4$ (OAc_3): C, 49.7; H, 4.8; Cl, 13.9%; M, 506]; ν_{max} 1780, 1750 cm^{-1}; δ (C_5D_5N) 6.05 (dd, rhamnose residue H-1, $J_{1,2e}$ 2, $J_{1,2a}$ 10 Hz), 5.34 (m, rhamnose residue H-3 and H-4), 3.89 (m, rhamnose residue H-5), 3.82 (s, OCH_3), 2.37, 2.21, 2.04 and 1.99 (s, $ArCH_3$, 3 $OCOCH_3$), 1.43 (d, $CHCH_3$, *J* 6 Hz).

Curacin tris-trichloroacetylcarbamate was prepared, in hexadeuterioacetone, from curacin and trichloroacetylisocyanate, δ $[(CD_3)_2CO]$ 6.32 (br, rhamnose residue H-1), 5.12 (d, rhamnose residue H-4, $J_{3,4} = J_{4,5}$ = 10 Hz), 4.15 (m, rhamnose residue H-5), 3.92 (s, OCH_3), 2.58 (H-2a), 2.30 (H-2e) and 5.32 (H-3) (ABX part of an ABXY system, rhamnose residue $-CH_2-$ and H-3), 2.34 (s, $ArCH_3$), 1.37 (d, $CHCH_3$, *J* 6 Hz).

(ii) *3,5-Dihydroxy-γ-caprolactone* (3) was purified by short-path distillation at 144° and 0.15 mm Hg, and was obtained as a colourless liquid, $[\alpha]_D^{25}$ +50° (EtOH); ν_{max} 1780 cm^{-1}; δ (C_5D_5N) 4.65 (dq, H-5, $J_{4,5}$ 8, J_{5,CH_3} 6 Hz), 4.35 (dd, H-4, $J_{3,4}$ 6, $J_{4,5}$ 8 Hz), 3.03 (H-2), 2.75 (H-2), 4.97 (H-3) (ABX system with X additionally coupled, $-CH_2-$ and H-3, J_{AB} 18, J_{AX} 4.5 J_{BX} 1.5, $J_{3,4}$ 6 Hz), 1.54 (d, $CHCH_3$, *J* 6 Hz). Acetylation (method D) gave the 3,5-diacetate[8] which was obtained (70%) as colourless crystals, m.p. 113° (lit.[8] 102°), from benzene [Found: C, 52.2; H, 6.2. Calc. for $C_6H_8O_2$ $(OAc)_2$: C, 52.2; H, 6.1%]; ν_{max} (KBr) 1775, 1703 cm^{-1}; δ 5.65 (dq, H-5, $J_{4,5}$ 9, J_{5,CH_3} 6 Hz), 4.43 (dd, H-4, $J_{3,4}$ 4, $J_{4,5}$ 9 Hz), 2.89 (H-2), 2.55 (H-2), 5.65 (H-3) (ABX system with X additionally coupled, $-CH_2-$ and H-3, J_{AB} 18, J_{AX} 5.5, J_{BX} 1, $J_{3,4}$ 4 Hz), 2.05 and 2.00 (s, 2 $OCOCH_3$), 1.40 (d, $CHCH_3$, *J* 6 Hz).

(iii) D-*Evalose* (4) was obtained as a colourless oil $[\alpha]_D^{25}$ −4.9° (EtOH) (lit.[9c] $[\alpha]_D$ 4.7 → 5.2° H_2O). Acetylation (method D) gave the 1,2,3,4 - *tetra - acetate* which was obtained (48%) as colourless crystals, m.p. 132°, from ether-n-hexane [Found: C, 51.7; H, 6.6; $C_7H_{10}O_5$ $(OAc)_4$ requires: C, 52.0; H, 6.4%]; ν_{max} 1760, 1750 cm^{-1}; δ (C_5D_5N) 6.29 (H-1), 5.62 (H-2) (AB system, J_{AB} 1 Hz), 5.56 (H-4), 3.87 (H-5), 1.33 (5-CH_3) (ABX_3 system, J_{AB} 9, J_{AX} 0, J_{BX} 6 Hz), 1.98, 1.98, 1.98, 1.94 (s, 4 $OCOCH_3$). 1.62 (s, CCH_3).

(iv) *4-O-Methyl-*D*-fucose* (5a) was obtained as a colourless oil $[\alpha]_D^{25}$ +81° (EtOH) (lit.[7a] $[\alpha]_D$ +52° H_2O), δ (C_5D_5N) 5.70 and 4.94 (d, H-1, $J_{1,2}$ (α-anomer) 3, $J_{1,2}$ (β-anomer) 7 Hz], 4.22 (dd, H-2, $J_{1,2}$ 7, $J_{2,3}$ 10 Hz), 3.70, 3.68 [s, OCH_3 (α and β-anomers)], 1.37, 1.35 [d, $CHCH_3$ (α and β-anomers), *J* 6 Hz]. Acetylation (method D) yielded (94%) the 1,2,3-*triacetate* as a mixture of α- and β-anomers from which the β-anomer was obtained (30%) as colourless crystals m.p. 113°, from n-hexane [Found: C, 51.6; H, 7.0. $C_7H_{11}O_2$ $(OAc)_3$ requires: C, 51.3; H, 6.6%]; ν_{max} (KBr) 1750 cm^{-1}; δ (C_5D_5N) 6.07 (d, H-1, $J_{1,2}$ 8 Hz), 5.79 (dd, H-2, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz), 5.42 (dd, H-3, $J_{2,3}$ 10, $J_{3,4}$ 3 Hz), 3.92 (dq, H-5, $J_{4,5}$ 1, J_{5,CH_3} 6 Hz), 3.46 (s OCH_3), 2.07, 2.02, 1.94 (s, 3 $OCOCH_3$), 1.28 (d, $CHCH_3$, *J* 6 Hz).

(v) *2,6-Di-O-methyl-*D*-mannose* (6a) was obtained as a colourless oil $[\alpha]_D^{25}$ +6.3° (EtOH) (lit.[7b] $[\alpha]_D^{20}$ + 10.3° (H_2O); δ (C_5D_5N) 5.71 (d, H-1, $J_{1,2}$ 1.5 Hz), 3.51, 3.33 (s, 2 OCH_3). Acetylation (method D) gave the 1,3,4-*triacetate* which was obtained (56%) as colourless needles, m.p. 76° (lit.[8] 80–82°), from ether-n-hexane [Found: C, 49.9; H, 6.7. Calc. for $C_8H_{13}O_3$ $(OAc)_3$: C, 50.3; H, 6.6%]; ν_{max} (KBr) 1730 cm^{-1}; δ (C_5D_5N) 6.43 (d, H-1, $J_{1,2}$ 2 Hz), 5.78 (t, H-4, $J_{3,4} = J_{4,5}$ = 10 Hz), 5.55 (dd, H-3, $J_{2,3} = J_{3,4}$ = 10 Hz), 4.16 (dt, H-5, $J_{4,5}$ 10, J_{5,CH_2} 4 Hz), 3.87 (dd, H-2, $J_{1,2}$ 2, $J_{2,3}$ 3 Hz), 3.56 (centre of AB of ABX system with X additionally coupled, $-CH_2-O$), 3.39, 3.21 (s, 2 OCH_3), 2.06, 2.03, 2.00 (s, 3 $OCOCH_3$).

(vi) L-*Lyxose* (7a) was obtained as a colourless hygroscopic solid, $[\alpha]_D^{25}$ + 13° (H_2O). On boiling with methanolic HCl soln (4% w/v, 3 hr) it was converted to methyl L-lyxopyranoside which, after purification [method B, solvent (ii)] was obtained (60%) as a colourless oil, δ 5.05 (d, H-1, $J_{1,2}$ 2 Hz), 3.37 (s, OCH_3). Acetylation (method D) of methyl L-lyxopyranoside gave the 2,3,4-*triacetate*, which was obtained (52%) as colourless crystals, m.p. 84°, from light petroleum [Found: C, 50.2; H, 6.5. C_5H_6O $(OAc)_3$ requires: C, 49.7; H, 6.3%]; ν_{max} (KBr) 1750 cm^{-1}; δ (C_5D_5N) 5.57 (m, H-2, H-3 and H-4), 4.78 (d, H-1, $J_{1,2}$ 2 Hz), 4.00, 3.64 (ABX system, $-CH_2O$, J_{AB} 11, J_{AX} 5, J_{BX} 9 Hz), 3.28 (s, OCH_3) 2.01, 1.98, 1.98 (s, 3 $OCOCH_3$).

(vii) *Flambabiose* (8a) was obtained as colourless crystals, m.p. 191°, from MeOH–ether, $[\alpha]_D^{26}$ −69.7° (EtOH); δ (C_5D_5N) 5.87 (d, H-1, $J_{1,2}$ 2 Hz), 5.30 (s, H-1), 3.60, 3.25 (s, 2 OCH_3). Flambabiose was hygroscopic so it was characterised by acetylation (method D) which gave *flambabiose penta-acetate* (8b) (55%) as colourless crystals, m.p. 150°, from n-hexane [Found: C, 50.1; H, 6.2. $C_{13}H_{19}O_5$ $(OAc)_5$ requires: C, 50.2; H, 6.2%]; δ 4.85 (d, H-1, $J_{1,2}$ 1.5 Hz), 3.60, 3.33 (s, 2 OCH_3), 2.12, 2.10, 2.05, 2.02, 2.00 (s, 5 $OCOCH_3$).

(3) *Acidic hydrolysis of flambamycin* (1). *Isolation of formaldehyde 2,4-dinitrophenylhydrazone*

A mixture of flambamycin (145 mg) and 5N HCl (10 ml) was heated at 70° for 18 hr whilst a slow stream of N_2 was passed through the mixture and then through a saturated soln of 2,4-dinitrophenylhydrazine in 2N HCl. The formaldehyde 2,4-dinitrophenylhydrazone which separated was obtained (14 mg, 65%) as yellow needles, m.p. 164°, from aqueous ethanol, and was identical with an authentic sample.

(4) *Identification of intermonosaccharide linkages. Permethylation of flambamycin and acidic methanolysis of flambamycin permethyl ether. Isolation of* (i) *isocuracin tri-O-methyl ether* (13) (ii) *methyl 2 - O - methyl -* D *- evalopyranoside* (9), (iii) *methyl 2,4 - di - O - methyl -* D *- fucopyranoside* (10); (iv) *methyl 2,3,6 - tri - O - methyl -* D *- mannopyranoside* (11) *and* (v) *methyl 2 - O - methyl -* L *- lyxopyranoside* (12)

Sodium hydride (2.5 g) was slowly added to a stirred soln of flambamycin (5 g) in dimethylsulphoxide (20 ml) and, after 5 min, MeI (15 ml) was cautiously added (exothermic reaction). The mixture was stirred at room temp. for 1 hr and then was added to water (25 ml). The mixture was extracted with $CHCl_3$ (3 × 75 ml) and the combined $CHCl_3$ extracts were washed with water (4 × 5 ml), dried and evaporated. The residue was dissolved in ether, and the ethereal soln was diluted with n-hexane when flambamycin permethyl ether separated as a colourless solid (3.9 g), m.p. 147–149°.

Flambamycin permethyl ether (2.79 g) was treated with boiling methanolic HCl soln (60 ml, 4% w/v, 1 hr) and the soln was neutralised with sat. $NaHCO_3$ aq, filtered and evaporated. The residue was extracted with ether (2×20 ml) and the combined ethereal extracts subjected to chromatography [method C, solvent (i), then solvent (ii)]. Evaporation of each eluate gave two fractions, (a) (1.25 g) and (b) (1.7 g) respectively.

(i) *Isocuracin tri-O-methyl ether* (13) was isolated by further purification [method C, solvent (iii)] of fraction (a), and was obtained as colourless crystals (0.93 g), m.p. 79–81°, from aqueous EtOH [Found: C, 51.3; H, 6.0; Cl, 16.8. $C_{14}H_{12}Cl_2O_4$ $(OMe)_4$ requires: C, 51.1; H, 5.7; Cl, 16.5%]; ν_{max} (KBr) 1730 cm^{-1}; δ (C_5D_5N) 5.72 (m, rhamnose residue H-3), 4.81 (dd, rhamnose residue H-1, $J_{1,2e}$ 1, $J_{1,2a}$ 3 Hz), 3.93, 3.84, 3.50, 3.29 (s, 4 OCH_3), 3.12 (t, rhamnose residue H-4, $J_{3,4}=J_{4,5}=9$ Hz), 2.35 (s, $ArCH_3$), 1.36 (d, $CHCH_3$, J 6 Hz).

Fraction (b) was further purified [method C, solvent (iv) followed where necessary, by glc, (using either 10% silicone oil on Embacel or QFI on Embacel as supports)] and yielded (ii) *methyl* 2 - O - *methyl* - D - *evalopyranoside* (9) (182 mg), (iii) *methyl* 3,4 - *di* - O - *methyl* - D - *fucopyranoside* (10) (52 mg), (iv) *methyl* 2,3,6 - *tri* - O - *methyl* - D - *mannopyranoside* (11) (88 mg) and (v) methyl 2 - O - *methyl* - L - *lyxopyranoside* (12) (180 mg).

(ii) *Methyl 2-O-methyl-D-evalopyranoside* (9) was obtained as a colourless oil; δ (C_5D_5N) 5.10 (d, H-1, $J_{1,2}$ 3 Hz), 4.54 (dq, H-5, $J_{4,5}$ 7.5, J_{5,CH_3} 6.5 Hz), 3.90 (d, H-4, $J_{4,5}$ 7 Hz), 3.62 (d, H-2, $J_{1,2}$ 3 Hz), 3.50, 3.42 (s, 2 OCH_3), 1.72 (s, CCH_3), 1.53 (d, $CHCH_3$, J 6.5 Hz).

Acetylation (method E) and purification [method B, solvent (xi)] of the product (9) gave (30%) the 3,4-*diacetate* (R_f 0.61–0.71) as colourless needles, m.p. 114°, from light petroleum [Found: C, 53.8; H, 7.9. $C_9H_{16}O_5$ $(OAc)_2$ requires: C, 53.8; H, 7.6%], ν_{max} 1740 cm^{-1}; δ 5.04 (d, H-4, $J_{4,5}$ 10 Hz), 4.64 (d, H-1, $J_{1,2}$ 1.5 Hz), 4.06 (d, H-2, $J_{1,2}$ 1.5 Hz), 3.68 (dq, H-5, $J_{4,5}$ 10, J_{5,CH_3} 6 Hz), 3.34, 3.34 (s, 2 OCH_3), 2.08, 1.96 (s, 2 $OCOCH_3$), 1.62 (s, CCH_3), 1.08 (d, $CHCH_3$, J 6 Hz).

(iii) *Methyl 2,4 - di - O - methyl - D - fucopyranoside* (10) was obtained, as colourless crystals (from ether-light petroleum), m.p. 99–101°, as the α-anomer, δ (C_5D_5N) 4.32 (d, H-1, $J_{1,2}$ 8 Hz), 3.67, 3.60, 3.50 (s, 3 OCH_3), 1.35 (d, $CHCH_3$, J 7 Hz). Acetylation (method E) and purification [method B, solvent (xi)] of the product gave (65%) the 1,3-*diacetate* (β-anomer) as a colourless oil, ν_{max} 1750 cm^{-1}; δ 6.4 (d, H-1, $J_{1,2}$ 3.5 Hz), 5.2 (d, H-2, $J_{1,2}$ 3.5, $J_{2,3}$ 10 Hz), 3.52, 3.42 (s, 2 OCH_3), 2.14, 2.13 (s, 2 $OCOCH_3$), 1.23 (d, $CHCH_3$, J 6 Hz). Acetylation (method D) and purification [method B using EtOAc: light petroleum (1:1) as solvent] of the product gave (61%) the 3-*monoacetate* (β-anomer) as a colourless oil, [Found: C, 52.8; H, 8.2. $C_9H_{17}O_4$ (OAc) requires: C, 53.2; H, 8.1%]. δ (C_5D_5N) 5.40 (dd, H-3, $J_{2,3}$ 3, $J_{3,4}$ 10 Hz), 4.92 (d, H-1, $J_{1,2}$ 4 Hz), 3.82 (m, H-2 and H-4), 3.47, 3.36, 3.30 (s, 3 OCH_3), 2.07 (s, $OCOCH_3$), 1.22 (d, $CHCH_3$, J 7 Hz).

(iv) *Methyl 2,3,6-tri-O-methyl-D-mannopyranoside* (11) was obtained as a colourless oil, δ (C_5D_5N) 4.90 (d, H-1, $J_{1,2}$ 2 Hz), 3.51, 3.46, 3.42, 3.36 (s, 4 OCH_3). Acetylation (method E) gave (88%) the 4-*monoacetate* as colourless needles, m.p. 117°, from light petroleum [Found: C, 51.9, H, 8.0. $C_{10}H_{19}O_5$ (OAc) requires: C, 51.8; H, 8.0%]; ν_{max} 1750 cm^{-1}; δ (C_5D_5N) 5.59 (t, H-4, $J_{3,4}=J_{4,5}$ 10 Hz), 4.49 (s, H-1), 3.60, 3.50, 3.40, 3.31 (s, 4 OCH_3), 2.05 (s, $OCOCH_3$).

(v) *Methyl 2-O-methyl-L-lyxopyranoside* (12) was obtained as a colourless oil, δ (C_5D_5N) 4.98 (d, H-1, $J_{1,2}$ 3 Hz), 3.52, 3.39 (s, 2 OCH_3). Acetylation (method D) gave (39%) after short-path distillation at 150° and 1 mm Hg, the 3,4-*diacetate* as a colourless oil, [Found: C, 50.4; H, 7.2. $C_7H_{12}O_3$ $(OAc)_2$ requires: C, 50.4; H, 6.9%], ν_{max} 1750 cm^{-1}; δ 5.56 (m, H-3 and H-4), 4.48 (d, H-1, $J_{1,2}$ 2.5 Hz), 3.81 (H-2 and CH_2–O), 3.40, 3.32 (s, 2 OCH_3), 2.02, 1.98 (s, 2 $OCOCH_3$).

(5) *Isolation and structural elucidation of flambatriose* (14a) *and flambatetrose* (15a). *Mild acidic hydrolysis of flambamycin* (1). *Isolation of* (i) *flambatriose* (14a), (ii) *flambatetrose* (15a) *and* (iii) *flambatetrose isobutyrate* (17a)

A mixture of flambamycin (5 g) and dil. HCl (380 ml, 0.5% w/v) was stirred at 78° for 30 min and at 31° for 17 hr. The soln was concentrated to 190 ml and extracted first with ether (2×100 ml) and then with $CHCl_3$ (2×100 ml). The acid soln was neutralised with Amberlite ion-exchange resin IR-4B (HO^--form) and filtered. The filtrate was evaporated and the residual mixture (3.7 g) was separated [method A, solvent (i)] yielding *flambatetrose isobutyrate* (0.31 g, 12%) (R_f 0.30–0.35), *flambatriose* (1.05 g, 60%) (R_f 0.25–0.32) and *flambatetrose* (0.22 g, 10%) (R_f 0.05–0.15).

(i) *Flambatriose* (14a) was obtained as a colourless solid, m.p. 125°, from EtOAc-n-hexane (Found: C, 45.1; H, 7.3. $C_{20}H_{36}O_{14}\cdot2H_2O$ requires: C, 44.8; H, 7.5%); $[\alpha]_D^{25}-57.1°$ (EtOH); δ (C_5D_5N) 5.74 (d, H-1, $J_{1,2}$ 2 Hz), 5.18 (s, H-1), 3.64, 3.61, 3.30 (s, 3 OCH_3), 1.28 (d, fucose residue $CHCH_3$ J 6 Hz). Acetylation (method E) of flambatriose and purification [method B, solvent (iv)] of the product gave *flambatriose hexa-acetate* (14b) which was obtained (85%) as colourless crystals, m.p. 119°, from n-hexane [Found: C, 50.6; H, 6.5; $M^{+\cdot}$, m/e 752. $C_{20}H_{30}O_8$ $(OAc)_6$ requires: C, 51.1; H, 6.4%; M, 752]; ν_{max} 1750 cm^{-1}. Methylation (method F) of flambatriose and purification [method B, solvent (v)] of the product gave *flambatriose hexamethyl ether* (14c) which was obtained (85%) as a colourless crystalline solid, m.p. 68–69°, from n-hexane [Found: C, 53.7; H, 8.4; $M^{+\cdot}$, m/e 584. $C_{17}H_{21}O_3$ $(OMe)_9$ requires: C, 53.4; H, 8.3%; M, 584]; δ (C_5D_5N) 5.49 (d, $J_{1,2}$ 2 Hz), 5.01 (s, mannose and lyxose residues H-1), 4.51 (d, fucose residue H-1, $J_{1,2}$ 7 Hz), 4.28 (t, mannose residue H-4, $J_{3,4}=J_{4,5}=9$ Hz), 3.57, 3.57, 3.54, 3.50, 3.44, 3.40, 3.38, 3.35, 3.31 (s, 9 OCH_3), 1.26 (d, fucose residue ($CHCH_3$, J 6 Hz).

Acidic hydrolysis of flambatriose (14a). *Formation of* 4 - O - *methyl* - D - *fucose* (5), 2,6 - *di* - O - *methyl* - D - *mannose* (6), *and* L-lyxose (7). A mixture of flambatriose (95 mg) and dil. HCl (20 ml, 1.8% w/v) was heated at 100° for 2.5 hr, neutralised with Amberlite ion-exchange resin (HO^--form) and filtered. The filtrate was evaporated and the residual mixture separated [method B, solvent (iv)] giving 5 (9 mg, 27%) (R_f 0.35–0.44), 6 (8 mg, 20%) (R_f 0.40–0.55), and 7 (4 mg, 14%) (R_f 0.00–0.10).

Acidic hydrolysis of flambatriose hexa-methyl ether (14c). *Formation of* (a) 2,3,4 - *tri* - O - *methyl* - D - *fucose* (5b), (b) 2,3,6 - *tri* - O - *methyl* - D - *mannose* (6b) and (c) 2,3,4 - *tri* - O - *methyl* - L - *lyxose* (7b). A soln of flambatriose hexa-methyl ether (100 mg) and 2N H_2SO_4 (4 ml) was heated at 100° for 4 hr, neutralised with Amberlite ion-exchange resin (HO^--form) and filtered. The filtrate was evaporated and the residual mixture separated [method B, solvent (v)] giving (i) 5b (12 mg, 34%; R_f 0.60–0.70), (ii) 6b (23 mg, 61%; R_f 0.50–0.60) and (iii) 7b (8 mg, 26%; R_f 0.70–0.75).

(a) *2,3,4-Tri-O-methyl-D-fucose* (5b) was obtained as a colourless oil (Found: C, 51.9; H, 8.6. $M^{+\cdot}$–OH, m/e 189. $C_6H_9O_2$ $(OMe)_3$ requires: C, 52.4; H, 8.8%; M, 206), δ 5.36, 4.50 (d, H-1 (α-anomers), $J_{1,2}$ 3, (β-anomer) $J_{1,2}$ 7 Hz], 4.12 (dq, H-5, $J_{4,5}$ 1, J_{5,CH_3} 6 Hz), 3.61, 3.58, 3.50 (s, unequal intensities, 3 OCH_3 α- and β-anomers), 1.26, 1.25 [d, $CHCH_3$ (α- and β-anomers), J 6 Hz]. Acetylation (method D) and purification [method B, solvent (xi)] yielded (29%) the *monoacetate* (β-anomer) as colourless crystals (from n-hexane), m.p. 73–75° δ 5.40 (d, H-1, $J_{1,2}$ 8 Hz), 3.59, 3.50, 3.50 (s, 3 OCH_3), 1.99 (s, $OCOCH_3$), 1.28 (d, $CHCH_3$, J 6 Hz).

(b) *2,3,6-Tri-O-methyl-D-mannose* (6b) was obtained as a colourless oil [Found: $M^{+\cdot}$ –OH, m/e 205. $C_6H_9O_3$ $(OMe)_3$ requires: M, 222]; δ (C_5D_5N) 5.67 (d, H-1, $J_{1,2}$ 1 Hz), 4.27 (t, H-4, $J_{3,4}=J_{4,5}=9.5$ Hz), 3.49, 3.45, 3.30 (s, 3 OCH_3). Acetylation (method D) yielded (42%), after short-path distillation at 120° and 1 mm Hg, the 1,4-*diacetate* as a colourless oil, δ 6.35 (d, H-1, $J_{1,2}$ 2 Hz), 5.36 (t, H-4, $J_{3,4}=J_{4,5}=9.5$ Hz), 3.64, 3.54, 3.45 (s, 3 OCH_3), 2.23, 2.19 (s, 2 $OCOCH_3$).

(c) *2,3,4-Tri-O-methyl-L-lyxose* (7b) was obtained as a colourless oil, δ (C_5D_5N) 5.24 (d, H-1, $J_{1,2}$ 1.5 Hz), 3.42, 3.39, 3.36 (s, 3 OCH_3). Acetylation (method D) and purification [method B, solvent (xi)] yielded (79%) the *monoacetate* as a colourless oil, δ 6.07 (d, H-1, $J_{1,2}$ 1.5 Hz) 3.57, 3.43, 3.28 (s, 3 OCH_3), 2.01 (s, $OCOCH_3$).

(ii) *Flambatetrose* (15a) was obtained as a colourless solid, m.p. 143°, from EtOAc-n-hexane (Found: C, 49.0; H, 7.3. $C_{27}H_{46}O_{18}$ requires: C, 49.1; H, 7.3%); δ (C_5D_5N) 5.76 (d, $J_{1,2}$ 2 Hz), 5.42 (s), 5.19 (s) (three signals, evalose, mannose and lyxose residues H-1), 4.68 (d, fucose residue H-1, $J_{1,2}$ 6 Hz), 3.65, 3.61, 3.29 (s, 3 OCH_3), 1.26, 1.57 (d, fucose and evalose residues $CHCH_3$, J 6 Hz), 1.53 (s, evalose residue CCH_3). Acetylation (method D) and purification (method B, solvent (vii)] gave *flambatetrose hepta-acetate* (**15b**) which was obtained (33%) as colourless crystals, m.p. 119°, from ether–n-hexane; ν_{max} 1750 cm^{-1}; δ (C_5D_5N) 3.64, 3.52, 3.32 (s, 3 $OC\underline{H}_3$), 2.21, 2.21, 2.00, 2.00, 1.97, 1.97, 1.84, 1.62 (s, 7 $OCOC\underline{H}_3$ and evalose residue $CC\underline{H}_3$), 1.35, 1.27 (d, fucose and evalose residues, $CHC\underline{H}_3$, J 6 Hz). *Flambatetrose octa-acetate* (**15c**) was prepared by acetylation (method E) of flambatetrose and purification [method B, solvent (viii)] and was obtained (36%) as colourless crystals, m.p. 115°, from ether–n-hexane, [Found: C, 51.4; H, 6.94. $C_{27}H_{40}O_{19}$ $(OAc)_8$ requires: C, 51.8; H, 6.5%]; ν_{max} 1750 cm^{-1}; δ 4.36 (d, fucose residue $\underline{H}$-1, $J_{1,2}$ 8 Hz), 3.57, 3.53, 3.37 (s, 3 $OC\underline{H}_3$), 2.11, 2.10, 2.07, 2.07, 2.07, 2.02, 1.97, 1.87, 1.71 (s, 8 $OCOC\underline{H}_3$ and evalose residue $CC\underline{H}_3$), 1.28, 1.21 (d, fucose and evalose residues $CHC\underline{H}_3$, J 6 Hz). Methylation (method F) of flambatetrose and subsequent purification [method B, solvent (v)] of the product yielded *flambatetrose heptamethyl ether* (**15d**; R_f 0.50–0.55) and *flambatetrose octamethyl ether* (**15e**; R_f 0.55–0.60). *Flambatetrose heptamethyl ether* (**15d**) was obtained (23%) as colourless crystals, m.p. 119°, from n-hexane [Found: C, 53.3; H, 8.5. $C_{24}H_{32}O_8$ $(OMe)_{10}$ requires: C, 53.8; H, 8.2%]; δ (C_5D_5N) 5.52 (d, $J_{1,2}$ 2 Hz), 5.09, 5.04 (s), (three signals, evalose, mannose and lyxose residues $\underline{H}$-1), 3.72, 3.68, 3.62, 3.58, 3.58, 3.58, 3.40, 3.37, 3.33, 3.30 (s, 10 $OC\underline{H}_3$), 1.38 (s, evalose residue $CC\underline{H}_3$), 1.30, 1.24 (d, fucose and evalose residues $CHC\underline{H}_3$, J 6 Hz). *Flambatetrose octamethyl ether* (**15e**) was obtained (31%) as colourless crystals, m.p. 96°, from n-hexane [Found: C, 53.5; H, 8.3, M$^{+\cdot}$, *m/e* 772. $C_{24}H_{31}O_7$ $(OMe)_{11}\cdot H_2O$ requires: C, 53.2; H, 8.4%. $\underline{M}$, 772]; δ 5.27 (d, $J_{1,2}$ 2 Hz), 4.76, 4.70 (s, three signals, evalose, mannose and lyxose residues $\underline{H}$-1), 4.35 (d, fucose residue $\underline{H}$-1, $J_{1,2}$ 7.5 Hz), 3.67, 3.60, 3.60, 3.52, 3.48, 3.47, 3.47, 3.46, 3.45, 3.35, 3.27 (s, 11 $OC\underline{H}_3$), 1.29, 1.27 (d, fucose and evalose residues $CHC\underline{H}_3$, J 6 Hz), 1.20 (s, evalose residue $CC\underline{H}_3$).

Acidic hydrolysis of flambatetrose (**15a**). *Formation of* D-*evalose* (**4**), *4-O-methyl-*D*-fucose* (**5**), *2,6-di-O-methyl-*D*-mannose* (**6**) *and* L-*lyxose* (**7**). A mixture of flambatetrose (95 mg) and dil. HCl (20 ml, 1.8% w/v) was heated at 100° for 3 hr, neutralised with Amberlite ion-exchange resin (HO^--form) and filtered. The filtrate was evaporated and the residual mixture separated [method B, solvent (vi)] giving, 2,6-di-O-methyl-D-mannose (19.5 mg, 65%; R_f 0.40–0.55), 4-O-methyl-D-fucose (16 mg, 63%; R_f 0.35–0.44), D-evalose (15 mg, 59%; R_f 0.30–0.36) and L-lyxose (11 mg, 51%; R_f 0.00–0.10), which were identical with those previously isolated (Section 2).

(iii) *Flambatetrose isobutyrate* (**17a**) was obtained as a colourless solid, m.p. 145°, from EtOH–n-hexane (Found: C, 50.6; H, 7.5. $C_{31}H_{54}O_{19}\cdot 2H_2O$ requires: C, 50.9; H, 7.5%); ν_{max} (KBr) 3650, 1740 cm^{-1}; δ (C_5D_5N) 3.73, 3.67, 3.33 (s, 3 $OC\underline{H}_3$), 2.49 [m, $OCOC\underline{H}(CH_3)_2$] 1.58, 1.27 (d, evalose and fucose residues $CHC\underline{H}_3$, J 6 Hz), 1.54 (s, evalose residue $CC\underline{H}_3$), 1.13, 1.07 [d, unequal intensities $OCOCH(C\underline{H}_3)_2$, J 7 Hz]. Acetylation (method D) of flambatetrose isobutyrate and purification [method B, solvent (vii)] of the product gave (51%) *flambatetrose isobutyrate hexa-acetate* (**17b**; R_f 0.37–0.40) as colourless crystals, m.p. 115°, from benzene–light petroleum [Found: C, 51.1; H, 6.7. $C_{31}H_{48}O_{13}$ $(OAc)_6\cdot 1.5H_2O$ requires: C, 51.1; H, 6.9%]; ν_{max} 3600, 1650 cm^{-1}; δ (C_5D_5N) 3.65, 3.60, 3.34 (s, 3 $OC\underline{H}_3$), 2.52 [m, $OCOC\underline{H}(CH_3)_2$] 2.22, 2.21, 2.01, 2.10, 2.01, 1.85, 1.64 (s, 6 $OCOC\underline{H}_3$ and evalose residue $CC\underline{H}_3$), 1.36, 1.29 (d, evalose and fucose residues $CHC\underline{H}_3$, J 6 Hz), 1.14, 1.10 [d, unequal intensities $OCOCH(C\underline{H}_3)_2$, J 7 Hz]. Similar acetylation (method D, 24 hr) of flambatetrose isobutyrate and purification [method B, solvent (iii)] yielded (70%) *flambatetrose isobutyrate hepta-acetate* (**17c**; R_f 0.40–0.60) as colourless crystals, m.p. 124–126°, from EtOAc–light petroleum [Found: C, 51.8; H, 6.9. $C_{31}H_{47}O_{12}$ $(OAc)_7\cdot H_2O$ requires: C, 51.8; H, 6.8%]; ν_{max} (KBr) 1750 cm^{-1}; δ (C_5D_5N) 4.39 (d, fucose residue $\underline{H}$-1, $J_{1,2}$ 8 Hz), 3.63, 3.54, 3.31 (s, 3 $OC\underline{H}_3$), 2.53 [m, $OCOC\underline{H}(CH_3)_2$], 2.19, 2.19, 1.99, 1.99, 1.99, 1.99, 1.82 (s, 7 $OCOC\underline{H}_3$), 1.59 (s, evalose residue $CC\underline{H}_3$), 1.34, 1.27 (d, evalose and fucose residues $CHC\underline{H}_3$, J 6 Hz), 1.14, 1.09 [d, unequal intensities, $OCOCH(C\underline{H}_3)_2$, J 7 Hz].

(6) *Acidic methanolysis of flambamycin* (**1**). *Isolation of* (i) *curacin methyl glycoside* (**2b**) (ii) *methyl* D*-evalopyranoside* (**4b**), (iii) *flambatriose* (**14a**), (iv) *flambatetrose* (**15a**), (v) *flambatriose isobutyrate* (**16a**), (vi) *flambatetrose isobutyrate* (**17a**), (vii) *flambalactone* (**18**), (viii) *methyl flambate* (**19b**) *and* (ix) *methyl eurekanate* (**20a**)

A mixture of flambamycin (5 g) and methanolic HCl soln 375 ml, 0.5% w/v) was kept at room temp. for 90 min. The soln was then neutralised by the addition of $CaCO_3$ and filtered. The filtrate was concentrated to 70 ml, diluted with water (100 ml) and extracted with ether (2 × 100 ml). Evaporation of the combined ethereal extracts gave a colourless oil (1.97 g) which was purified [method A, solvent (ii)] and yielded curacin methyl glycoside (462 mg, 33%; R_f 0.80–0.86), *methyl eurekanate* (102 mg, 12% R_f 0.76–0.80) (see Section 7), *flambalactone* (450 mg, 25%; R_f 0.25–0.35) and *methyl flambate* (101 mg, 5%; R_f 0.10–0.18).

The aqueous soln after ether extraction was extracted with $CHCl_3$ (2 × 100 ml) and the combined $CHCl_3$ extracts evaporated. The residue gave, on purification [method B, solvent (ii)] a further quantity (145 mg, 16%), of *methyl eurekanate* (see Section 7).

The aqueous soln was evaporated and the solid residue (3.5 g) was triturated with anhyd EtOH and filtered. Evaporation of the filtrate gave a colourless oil (2.7 g) which was separated [method A, solvent (i), then solvent (iii)] giving *methyl* D*-evalopyranoside* (750 mg, 67%) [R_f (i) 0.44–0.55; (iii) 0.46–0.54], *flambatriose isobutyrate* (442 mg, 22%) [R_f (i) 0.40–0.48; (iii) 0.13–0.18], flambatetrose isobutyrate [151 mg, 6%] [R_f (i) 0.25–0.40; (iii) 0.05–0.11], flambatriose (219 mg, 13%) [R_f (i) 0.30–0.35; (iii) 0.0–0.05] and flambatetrose (121 mg, 5%) [R_f (i) 0.05–0.15].

(i) Curacin methyl glycoside (**2b**) was identical with that obtained previously (Section 2).

(ii) *Methyl* D*-evalopyranoside* (**4b**) was obtained as colourless crystals, m.p. 132°, from ether–n-hexane (Found: C, 50.1; H, 8.4. $C_8H_{16}O_5$ requires: C, 50.0; H, 8.4%); ν_{max} (KBr) 3450, 3350 cm^{-1}; δ (C_5D_5N) 4.96 (d, $\underline{H}$-1, $J_{1,2}$ 1.5 Hz), 3.95 (m, $\underline{H}$-4 and $\underline{H}$-5), 3.91 (d, $\underline{H}$-2, $J_{1,2}$ 1.5 Hz), 3.29 (s, $OC\underline{H}_3$), 1.67 (s, $CC\underline{H}_3$), 1.48 (d, $CHC\underline{H}_3$, J 6 Hz). Acetylation (method D) and short-path distillation at 130° and 0.3 mm Hg of the product gave the *2,4-diacetate* which was obtained (62%) as a colourless gum, ν_{max} 1750 cm^{-1}; δ 5.17 (d, $\underline{H}$-4, $J_{4,5}$ 9.5 Hz), 5.07 (d $\underline{H}$-2, $J_{1,2}$ 1.5 Hz), 4.91 (d, $\underline{H}$-1, $J_{1,2}$ 1.5 Hz), 4.07 (dq, $\underline{H}$-5, $J_{4,5}$ 9.5 J_{5,CH_3} 6 Hz), 3.63 (s, $OC\underline{H}_3$), 2.89 (br s, $O\underline{H}$), 2.44, 2.40 (s, 2 $OCOC\underline{H}_3$), 1.68 (s, $CC\underline{H}_3$), 1.49 (d, $CHC\underline{H}_3$, J 6 Hz). The corresponding *2,3,4-tri-acetate* was prepared (method E for 18 hr) and obtained (52%) as colourless crystals, m.p. 157°, from n-hexane [Found: C, 53.0; H, 6.7 $C_8H_{13}O_2$ $(OAc)_3$ requires: C, 52.8; H, 7.0%]; ν_{max} (KBr) 1750 cm^{-1}; δ 5.67 (d, $\underline{H}$-2, $J_{1,2}$ 1.5 Hz), 5.20 (d, $\underline{H}$-4, $J_{4,5}$ 10 Hz), 4.68 (d, $\underline{H}$-1, $J_{1,2}$ 1.5 Hz), 3.86 (dq, $\underline{H}$-5, $J_{4,5}$ 10, J_{5,CH_3} 6 Hz), 3.39 (s, $OC\underline{H}_3$), 2.14, 2.10, 1.96, 1.75 (s, $CC\underline{H}_3$ and 3 $OCOC\underline{H}_3$), 1.23 (d, $CHC\underline{H}_3$, J 6 Hz).

(iii) Flambatriose (**14a**), (iv) flambatetrose (**15a**) and (vi) flambatetrose isobutyrate (**17a**) were identical with those obtained previously (Section 6).

(v) *Flambatriose isobutyrate* (**16a**) EtOAC was obtained as a colourless solid, m.p. 115–117°, from EtOH–light petroleum (Found: C, 49.5; H, 7.3 $C_{24}H_{42}O_{15}$ $0.5H_2O$ requires: C, 49.7; H, 7.5%); ν_{max} (KBr) 1730 cm^{-1}; δ (C_5D_5N), 3.71, 3.55 (s, unequal intensities $OC\underline{H}_3$), 3.63 (s, $OC\underline{H}_3$), 3.31, 3.28 (s, unequal intensities, $OC\underline{H}_3$), 2.53 [m, $OCOCH(C\underline{H}_3)_2$], 1.27 (d, $CHC\underline{H}_3$, J 6 Hz), 1.10, 1.04 [d, unequal intensities, $OCOCH(C\underline{H}_3)_2$, J 7 Hz]. Acetylation (method D) of flambatriose isobutyrate and purification [method B, solvent (iv)] of the product gave the *flambatriose isobutyrate penta-acetate* (**16b**) (R_f 0.60–0.66) which was obtained (56%) as colourless microcrystals, m.p. 86°, from EtOAC–light petroleum (Found: C, 51.4; H, 6.7%. $C_{24}H_{37}O_{10}$ $(OAc)_5\cdot H_2O$ requies: C, 51.8; H, 6.9%]; ν_{max} 1750 cm^{-1}; δ 4.24 (d, fucose residue $\underline{H}$-1, $J_{1,2}$ 8 Hz), 3.53, 3.48, 3.35 (s, 3 $OC\underline{H}_3$) 2.31 [m, $OCOC\underline{H}(CH_3)_2$] 2.11, 2.08, 2.04, 2.00, 2.00 (s, $OCOC\underline{H}_3$), 1.28 (d, $CHC\underline{H}_3$, J 6 Hz), 1.19, 1.08 [d, unequal intensities, $OCOC\underline{H}(CH_3)_2$, J 7 Hz].

(vii) *Flambalactone* (**18**) was obtained as colourless crystals, m.p. 217°, from $CHCl_3$ (Found: C, 49.4; H, 5.4; Cl, 14.1; M$^{+\cdot}$, *m/e* 508.0896. $C_{21}H_{26}Cl_2O_{10}$ requires: C, 49.6; H, 5.2; Cl, 14.0%. $\underline{M}$, 508.0903); $[\alpha]_D^{26}$ + 15.7° (EtOH); ν_{max} 1740 cm^{-1}; δ [$(CD_3)_2CO$] 4.87 (m, rhamnose residue $\underline{H}$-1), 4.80 (t, rhamnose residue $\underline{H}$-4, $J_{3,4} = J_{4,5} = 10$ Hz), 4.26 (dq, lactone $\underline{H}$-5, $J_{4,5}$ 8, J_{5,CH_3} 6 Hz), 3.86 (s, $OC\underline{H}_3$), 3.69 (m, rhamnose residue $\underline{H}$-5, lactone $\underline{H}$-3 and $\underline{H}$-4), 2.98 (lactone H-2), 2.40 (lactone H-2) and 6.25 (lactone H-3) (ABX system with X additionally coupled, J_{AB} 17, $J_{AX} = J_{BX} =$ 6 Hz), 2.35 (s ArCH_3), 2.35 (rhamnose residue H-2), 1.76 (rhamnose residue H-2), 4.22 (rhamnose residue H-3) (ABX system with A, B, and X additionally coupled), 1.39, 1.36 (d, lactone and rhamnose residues CHCH_3, J 6 Hz).

Methylation of flambalactone, using diazomethane, gave *flambalactone methyl ether* which was obtained (76%) as colourless crystals, m.p. 201°, from $CHCl_3$–ether [Found: C, 50.5; H, 5.5; Cl, 13.9; $M^{+\cdot}$, *m/e* 522.1057. $C_{20}H_{22}Cl_2O_8$ $(OMe)_2$ requires: C, 50.5; H, 5.4; Cl, 13.6%, M, 522.1059]; ν_{max} 1740 cm^{-1}; δ [$CDCl_3$–$(CD_3)_2CO$] 4.85 (t, rhamnose residue H-4, $J_{3,4} = J_{4,5}$ = 10 Hz), 4.77 (dd, rhamnose residue H-1, $J_{1,2a}$ 10, $J_{1,2e}$ 2 Hz), 3.89, 3.87 (s, 2 OCH_3), 3.00 (lactone H-2) 2.50 (lactone H-2), 6.15 (lactone H-3) (ABX system with X additionally coupled, J_{AB} 16.5, J_{AX} 5, J_{BX} 3 Hz), 2.36 (s, ArCH_3) 1.42, 1.38 (d, lactone and rhamnose residues CHCH_3, J 6 Hz). Acetylation (method D) of flambalactone gave *flambalactone triacetate* which was obtained (82%) as colourless crystals, m.p. 159°, from ether [Found: $M^{+\cdot}$, *m/e* 634.1230. $C_{21}H_{23}Cl_2O_7(OAc)_3$ requires: M, 634.1220]; ν_{max} 1782, 1740 cm^{-1}. δ 5.08 (t, rhamnose residue H-4, $J_{3,4} = J_{4,5}$ = 9 Hz), 4.74 (dd, rhamnose residue H-1, $J_{1,2a}$ 10, $J_{1,2e}$ 2 Hz), 4.23 (dq, lactone H-5, $J_{4,5}$ 8, J_{5,CH_3} 6 Hz), 3.86 (s, OCH_3), 3.59 (m, rhamnose residue H-5 and lactone H-4), 2.93 (lactone H-2), 2.66 (lactone H-2) and 5.51 (lactone H-3) (ABX system with X additionally coupled, J_{AB} 16.5, $J_{AX} = J_{BX}$ = 4 Hz), 2.39, 2.29, 2.06, 2.06 (s, ArCH_3 and 3 $OCOCH_3$), 2.47, 1.71 (ABX system, rhamnose residue CH_2), 1.45, 1.36 (d, 2 CHCH_3, J 6 Hz).

A soln of *flambalactone tris-trichloroacetylcarbamate* was prepared by addition of trichloroacetylisocyanate (2 drops) to a soln of flambalactone (50 mg) in hexadeuterioacetone (0.4 ml); δ [$(CD_3)_2CO$] 5.10 (m, rhamnose residue H-1, H-3 and H-4), 4.40 (m, lactone H-5), 3.89 (s, OCH_3), 3.78 (m, rhamnose residue H-5, and lactone H-4), 3.23 (lactone CH_2), 2.62 (lactone CH_2) and 5.57 (lactone H-3) (ABX system with X additionally coupled, J_{AB} 17, $J_{AX} = J_{BX}$ = 5 Hz), 2.33 (s, ArCH_3), 1.45, 1.37 (d, 2 CHCH_3, J 6 Hz).

(viii) *Methyl flambate* (**19b**) was obtained as a colourless solid m.p. 90–92°, from EtOAc–light petroleum (Found: C, 48.8; H, 5.5. $C_{22}H_{30}Cl_2O_{11}$ requires: C, 48.8; H, 5.6%); ν_{max} (KBr) 1735 cm^{-1}, δ [$(CD_3)_2CO$] 4.76 (t, rhamnose residue H-4, $J_{3,4} = J_{4,5}$ = 10 Hz), 4.75 (dd, rhamnose residue H-1, $J_{1,2a}$ 10, $J_{1,2e}$ 2 Hz), 3.84, 3.62 (s, CO_2CH_3 and OCH_3), 2.71, 2.49 (caproic ester residue CH_2) (AB of ABX system J_{AB} 17, $J_{AX} = J_{BX}$ = 5 Hz), 2.33 (s, ArCH_3), 1.30, 1.21 (d, 2 CHCH_3, J 6 Hz).

Methyl flambate (**19b**) was also obtained from flambalactone when a mixture of flambalactone (50 mg) and methanolic HCl soln (5 ml, 0.15% w/v) was kept at room temp. for 1 hr and the soln was then neutralised by Amberlite ion-exchange resin IR-4B (HO^--form). Filtration and evaporation of the filtrate gave a solid which was dissolved in EtOAc. Addition of light petroleum to the soln gave methyl flambate as a colourless solid (42 mg, 79%), m.p. 90–92°.

(ix) *Methyl eurekanate* (see Section 7)—*Acidic methanolysis of flambamycin* (**1**) *followed by direct acetylation. Isolation of* (i) *flambatetrose isobutyrate hexa-acetate* (**17b**), (ii) *flambatetrose isobutyrate hepta-acetate* (17c), (iii) *methyl flambate tetra-acetate* (*cf.* **19b**) and (iv) *methyl eurekanate monoacetate* (**20d**). A mixture of flambamycin (0.5 g) and methanolic HCl soln (50 ml, 0.15% w/v) was kept at room temp. for 1 hr and the soln was neutralised by addition of Amberlite ion-exchange resin IR-4B (HO^--form). The solid, obtained, after filtration and evaporation of the filtrate, was acetylated (method D) and the mixture of products separated [method B, solvent (iii)]. This procedure yielded flambatetrose isobutyrate hexa-acetate (Section 5) (60 mg, 18%; R_f 0.17–0.29), flambatetrose isobutyrate hepta-acetate (Section 5) (160 mg, 45%; R_f 0.29–0.52), *methyl eurekanate monoacetate* (see Section 7) (73 mg, 72%; R_f 0.83–0.91) and *methyl flambate tetra-acetate* (161 mg, 65%; R_f 0.91–0.99) which was obtained as colourless crystals, m.p. 61–63°, from n-hexane [Found: $M^{+\cdot}$, *m/e* 708.1581. $C_{22}H_{26}Cl_2O_7$ $(OAc)_4$ requires: M, 708.1587]; δ 5.00 (m, rhamnose residue H-3 and H-4, caproic ester residue H-5), 4.53 (dd, rhamnose residue H-1, $J_{1,2a}$ 10, $J_{1,2e}$ 2 Hz), 3.96 (dd, caproic ester residue H-4, $J_{3,4}$ 3, $J_{4,5}$ 7 Hz), 3.85, 3.67 (s, CO_2CH_3 and OCH_3), 3.47 (m, rhamnose residue H-5), 2.97, 2.60 (caproic ester residue CH_2), 5.45 (caproic ester residue H-3) (ABX system with X additionally coupled, J_{AB} 17, J_{AX} 7, J_{BX} 6 Hz), 2.39, 2.28, 2.05, 2.03, 2.01 (s, ArCH_3 and 4 $OCOCH_3$), 1.32, 1.26 (d, 2 CHCH_3, J 6.5 Hz).

(7) *The constitution of methyl eurekanate* (**20a**)

Methyl eurekanate (**20a**) was obtained (Section 6), after short-path distillation at 145° and 0.6 mm Hg, as a colourless oil (Found: C, 48.5; H, 6.5; O, 45.3; $M^{+\cdot}$, *m/e* 248. $C_{10}H_{16}O_7$ requires: C, 48.4; H, 6.5; O, 45.1%. M, 248); $[\alpha]_D^{25}$ −55.2° (EtOH); ν_{max} 3600, 3460, 1750 (ϵ 268), 1720 (ϵ 268) cm^{-1}; δ 5.10 (H_A) and 4.89 (H_B) (AB system, O–CH_2–O, J_{AB} 0 Hz), 4.68 (H_A), 4.66 (H_B) (AB system, O–CH–CH–O, J_{AB} 6 Hz), 1.03 (H_A), 4.18 (H_X) [A_3X system $C(H_A)_3$–CH_X(OH)–, J_{AX} 6.5 Hz], 4.15 (br, OH), 3.78 (s, CO_2CH_3), 2.58 (br, OH) 2.28 (s, $COCH_3$).

Acetylation (method D) of methyl eurekanate (70 mg) and purification [method B, solvent (i)] of the product (R_f 0.50–0.60) gave *methyl eurekanate monoacetate* (**20d**; 36 mg, 44%) as colourless needles, m.p. 87°, from light petroleum [Found: C, 50.0; H, 6.4. $C_{10}H_{15}O_6$ (OAc) requires: C, 49.6; H, 6.3%]; ν_{max} 3450, 1750 (ϵ 569), 1730 (ϵ 285) cm^{-1}; δ 5.39 (H_X), 1.07 (H_A) [A_3X system, $C(H_A)_3$–CH_X(OAc)–, J_{AX} 6.5 Hz], 5.13 (H_A), 4.93 (H_B) (AB system, –O–CH_2–O–, J_{AB} 0 Hz), 4.83 (H_A), 4.58 (H_B) (AB system, O–CH–CH–O, J_{AB} 4 Hz), 4.18 (br, OH), 3.79 (s, CO_2CH_3), 2.36, 2.08 (s, $OCOCH_3$ and $COCH_3$).

Acetylation (method E, 24 hr) of methyl eurekanate (114 mg) and purification [method B, solvent (iv)] of the product (R_f 0.85–0.95) gave the *diacetate* (**20e**) as a colourless syrup (101 mg, 77%); ν_{max} 1750 cm^{-1}; δ 5.57 (H_X), 1.29 (H_A) [A_3X system, $C(H_A)_3$–CH_X(OAc)–, J_{AX} 6.5 Hz), 5.16 (H_A), 4.94 (H_B) (AB system, O–CH_2–O, J_{AB} 0 Hz), 4.87 (H_A), 4.87 (H_B), (s, AB system O–CH_A–CH_B–O, J_{AB} 0 Hz), 3.79 (s, CO_2CH_3), 2.15, 2.13, 2.05 (s, CH_3CO and 2 $OCOCH_3$).

Methyl eurekanate bis-trichloroacetylcarbamate (**20g**) was prepared from methyl eurekanate and trichloroacetylisocyanate in hexadeuterioacetone δ [$(CD_3)_2CO$] 5.36 (H_X), 1.14 (H_A) [A_3X system, $C(H_A)_3$–CH_X($OCONHCOCCl_3$) J_{AX} 6.5 Hz], 5.15 (H_A), 4.79 (H_B) (AB system, O–CH_2–O, J_{AB} 0 Hz), 4.98 (H_A), 4.64 (H_B) (AB system, O–CH_A–CH_B–O, J_{AB} 4 Hz), 3.68 (s, CO_2CH_3) 2.37 (s, $COCH_3$).

Mild acidic hydrolysis of methyl eurekanate. Isolation of eurekanic acid diacetate (**20f**). A mixture of methyl eurekanate (60 mg) and 5N HCl (5 ml) was kept at room temp. for 18 hr, and neutralised by addition of solid $NaHCO_3$. Evaporation and acetylation (method E, 18 hr) of the residual solid gave *eurekanic acid diacetate* as a colourless oil (17 mg, 23%) [Found: $M^{+\cdot}$, *m/e* 318. $C_9H_{12}O_5$ $(OAc)_2$ requires: M, 318]; ν_{max} 1730 cm^{-1}; δ 5.56 (q, CH_3CH, J 6.5 Hz), 5.01 (H_A), 4.85 (H_B) (AB system, O–CH_2–O, J_{AB} 0 Hz), 5.21 (H_A), 4.95 (H_B) (AB system O–CH_A–CH_B–O, J_{AB} 4 Hz), 2.17, 2.07 (s, $OCOCH_3$), 1.32 (d, CHCH_3, J 6.5 Hz).

Acid hydrolysis of methyl eurekanate. Isolation of formaldehyde 2,4-dinitrophenylhydrazone. A mixture of methyl eurekanate (60 mg) and 5N HCl (10 ml) was heated at 100° for 6 hr whilst a slow stream of N_2 was passed through the mixture and into a saturated soln of 2,4-dinitrophenylhydrazine in 2N HCl. The formaldehyde 2,4-dinitrophenylhydrazone which separated was obtained (28 mg, 55%) as yellow needles, m.p. 164°, from aqueous EtOH and was identical with an authentic sample.

Acidic ethanolysis of methyl eurekanate. Isolation of ethyl eurekanate (**20b**). A mixture of methyl eurekanate (20 mg) and ethanolic HCl soln (10 ml, 0.5% w/v) was kept at room temp. for 18 hr. Evaporation and short-path distillation, at 144° and 0.15 mm Hg, of the residue gave *ethyl eurekanate* as a colourless oil; δ 5.09 (H_A), 4.88 (H_B) (AB system O–CH_2–O, J_{AB} 0 Hz), 4.66 (H_A), 4.65 (H_B) (AB system, O–CH_A–CH_B–O, J_{AB} 4 Hz), 4.16 (H_X), 1.02 (H_A) [A_3X system $C(H_A)_3$–CH_X(OH)–, J_{AX} 6.5 Hz], 2.28 (s, CH_3–CO), 1.29 (t, CH_3CH_2–, J 7 Hz).

Periodate oxidation of methyl eurekanate. Isolation of acetaldehyde 2,4-dinitrophenylhydrazone. A soln of methyl eurekanate (74 mg) in water (5 ml) was mixed with a soln of sodium metaperiodate (107 mg) in water (5 ml) and kept at room temp. for 35 min whilst a slow stream of N_2 was passed through the mixture and then through a saturated solution of

2,4-dinitrophenylhydrazine in 2N HCl. The acetaldehyde 2,4-dinitro-phenylhydrazone (34 mg, 54%) which separated was obtained as yellow crystals, m.p. 168°, from aqueous EtOH, and was identical with an authentic sample.

Trideuteriomethyl eurekanate (**20c**). A soln of methyl eurekanate (50 mg) in tetradeuteriomethanolic HCl (0.4 ml, 0.4% w/v) was kept (in an NMR tube) at room temp. until exchange of methoxycarbonyl protons (δ 3.40) was complete (45 min). Evaporation of the soln gave trideuteriomethyl eurekanate as a colourless syrup (50 mg, 99%).

(8) *Alkaline hydrolysis of flambamycin. Isolation and structural elucidation of bamflalactone* (**23a**) *and flambeurekanose* (**24a**)

(i) *Isolation of dichloroisoeverninic acid* (**22**). A mixture of flambamycin (2 g) and NaOH aq (10% w/v, 20 ml) was kept at room temp. for 3 days, acidified with 10N HCl and extracted with EtOAc (3×25 ml). The combined EtOAc extracts were evaporated and the residue was crystallised from ether–light petroleum giving dichlorisoeverninic acid (0.21 g, 60%) as colourless needles, m.p. 132° (lit.[7a] 129–130°) (Found: C, 43.2; H, 3.2; Cl, 28.2. Calc. for $C_9H_8Cl_2O_4$: C, 43.1; H, 3.2; Cl, 28.2%).

(ii) *Isolation of bamflalactone triacetate* (**23b**). A mixture of flambamycin (0.5 g) and NaOH aq (10% w/v, 5 ml) was kept at room temp. for 5 days and neutralised to approximately pH 7 with 2N HCl. The soln was evaporated and the residue extracted with anhyd EtOH. The extract was evaporated and acetylation (method D) of the residue and purification [method C, solvent (v) followed by method B, solvent (iii)] of the product gave *flambeurekanose penta-acetate* (121 mg, 33%; R_f 0.1–0.2) (as described below) and *bamflalactone triacetate* (50 mg, 36%; R_f 0.8–0.9) which was obtained as colourless needles, m.p. 131°, from light petroleum [Found: $M^{+\cdot}$, *m/e* 402. $C_{12}H_{17}O_4$ $(OAc)_3$ requires M, 402], δ 2.03, 2.01, 1.99 (s, 3 $OCOCH_3$), 1.44, 1.18 (d, 2 $CHCH_3$, *J* 6 Hz).

(iii) *Isolation of flambeurekanose* (**24a**). A mixture of flambamycin (2 g) and NaOH aq (10% w/v, 20 ml) was kept at room temp. for 18 hr and neutralised to approximately pH 7 with 2N HCl. The soln was evaporated and the residue was triturated with cold anhyd EtOH. Evaporation of the ethanolic filtrate and fractionation [method A, solvent (i)] yielded *flambeurekanose* (1.05 g, 85%; R_f 0.10–0.20) which was obtained as colourless crystals, m.p. 191–192°, from EtOAc–light petroleum [Found: C, 48.0; H, 6.8; OMe 10.5. $C_{33}H_{49}O_{28}$ $(OMe)_3 \cdot 2H_2O$ requires: C, 48.3; H, 7.0; OMe, 10.4%]; ν_{max} (KBr) 1710 cm^{-1} (ϵ 266); δ 5.35, 5.12, 5.08, 4.99 (br s, 4 anomeric protons); δ (C_5D_5N) 3.64, 3.61, 3.27 (s, 3 OCH_3), 2.40 (s, $COCH_3$), 1.53, 1.25, 1.21 (d, 3 $CHCH_3$, *J* 6 Hz), 1.47 (s, CCH_3).

Acetylation (method D) of flambeurekanose and purification [method B, solvent (ix)] of the product gave *flambeurekanose penta-acetate* (43%; R_f 0.50–0.65), as colourless crystals, m.p. 196°, from EtOAc–light petroleum [Found: C, 51.3; H, 6.5; $M^{+\cdot}$, *m/e* 1068. $C_{36}H_{55}O_{18}$ $(OAc)_5$ requires: C, 51.7; H, 6.4%; M, 1068]; ν_{max} (KBr) 3470, 1755 cm^{-1}; δ (C_5D_5N) 3.62, 3.40, 3.30 (s, 3 OCH_3), 2.44, 2.20, 2.16, 2.00, 1.98, 1.88, 1.61 (s, 1 CH_3CO, 5 $OCOCH_3$ and 1 CCH_3); δ (220 MHz, $CDCl_3$) 5.51, 5.23, 5.09, 5.09, 4.97, 4.76, 4.76 (s, evalose, mannose and lyxose residues H-1, evalose and lyxose H-2, and eurekanic acid residue $-O-CH_2-O-$), 5.06 (dd, fucose residue H-2, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz), 4.94 (dd, mannose residue H-3, $J_{2,3}$ 4, $J_{3,4}$ 10 Hz), 4.83 (d, evalose residue H-4, $J_{4,5}$ 10 Hz), 4.38 (d, fucose residue H-1, $J_{1,2}$ 8 Hz), 3.54, 3.47, 3.36 (s, 3 OCH_3), 2.30, 2.11, 2.10, 2.09, 2.05 (s, 5 $OCOCH_3$), 1.29 (s, evalose residue CCH_3), 1.27, 1.21, 1.04 (d, evalose, fucose and eurekanic acid residues $CHCH_3$, *J* 6 Hz).

Acidic methanolysis of flambeurekanose. Isolation of flambatetrose (**15a**) *and methyl eurekanate* (**20a**). A mixture of flambeurekanose (1.2 g) and methanolic HCl soln (75 ml, 0.5% w/v) was kept at room temp. for 1 hr. The soln was evaporated and the residual mixture separated [method A, solvent (i) followed by method B, solvent (x)] yielding flambatetrose (206 mg, 31%) and methyl eurekanate (152 mg, 51%) which were identical with the substances obtained previously [Section (5) and Section (7)].

(10) *Transformations of flambamycin yielding des-isobutyroyl flambamycin* (**25a**), *flambeurekanose flambate* (**26a**), *flambeurekanose flambate isobutyrate* (**26c**), *and des-dichloroisoeverninoyl-des-isobutyroyl flambamycin* (**27**).

(i) *Des-isobutyroyl flambamycin* (**25a**). A mixture of flambamycin (2.9 g), MeOH (100 ml) and anhyd K_2CO_3 (0.14 g) was boiled for 40 min and the soln was evaporated. The residue was crystallised from EtOH–EtOAc giving the K-salt as colourless crystals (2.68 g, 99%), m.p. 235–240°. The K-salt was dissolved in water (50 ml) and the soln was saturated with CO_2 when *des-isobutyroyl flambamycin* separated as a colourless solid (0.8 g, 81%), m.p. 202–203° (Found: C, 49.1; H, 6.3; Cl, 5.0. $C_{57}H_{82}Cl_2O_{32} \cdot 2H_2O$ requires: C, 49.4; H, 6.3; Cl, 5.1%). ν_{max} (KBr) 3460, 1715 cm^{-1}.

Acetylation (method D) of des-isobutyroyl flambamycin gave *des-isobutyroyl flambamycin hepta-acetate* (**25b**; 61%) as colourless crystals, m.p. 198–199°, from EtOAc–light petroleum [Found: C, 51.8; H, 6.1. $C_{57}H_{75}Cl_2O_{25}$ $(OAc)_7$ requires: C, 51.9; H, 5.9]; ν_{max} (KBr) 3460, 1786, 1752 cm^{-1}.

(ii) *Flambeurekanose flambate* (**26a**). A mixture of *des*-isobutyroyl flambamycin (0.5 g), EtOAc (100 ml), water (0.5 ml) and Amberlyst 15 resin (0.5 g) was stirred at room temp. for 10 min and filtered. Evaporation of the filtrate and purification [method A, solvent (ii)] of the residue gave *flambeurekanose flambate*, which was obtained as a colourless solid (0.15 g, 31%), m.p. 174–176°, from EtOAc–light petroleum (Found: C, 49.3; H, 6.3. $C_{57}H_{84}Cl_2O_{33} \cdot H_2O$ requires: C, 49.4; H, 6.4%); ν_{max} (KBr) 3440, 1725 cm^{-1}.

Acetylation (method D) of flambeurekanose flambate and purification [method A solvent (ii)] gave *flambeurekanose flambate octa-acetate* (**26b**; 26%) as a colourless solid, m.p. 187° from EtOAc–light petroleum [Found: C, 51.8; H, 6.4. $C_{57}H_{76}Cl_2O_{25}$ $(OAc)_8$ requires: C, 51.4; H, 5.9%]; ν_{max} (KBr) 3450, 1785, 1745 cm^{-1}.

Acetylation (method D, RT, 18 hr then 90°, 1 hr) of flambeurekanose flambate and purification [method A, solvent (ii)] gave *flambeurekanose flambate nona-acetate* (**26c**; 36%) as colourless crystals, m.p. 143–145°, from EtOAc–light petroleum [Found: C, 51.1; H, 6.1; Cl, 3.9. $C_{57}H_{75}Cl_2O_{24}$ $(OAc)_9$ requires: C, 51.6; H, 5.9; Cl, 4.1%]; ν_{max} (KBr) 3450, 1785, 1745 cm^{-1}.

(iii) *Flambeurekanose flambate isobutyrate* (**26d**). A mixture of flambamycin (2.0 g), EtOAc (50 ml) and Amberlyst 15 resin (0.5 g) was stirred at room temp. for 30 min and filtered. Evaporation of the filtrate and purification [method A, solvent (iii)] of the residue gave *flambeurekanose flambate isobutyrate*, which was obtained as a colourless solid (1.62 g, 80%), m.p. 160–163°, from EtOAc–light petroleum (Found: C, 50.3; H, 6.34. $C_{61}H_{90}Cl_2O_{34} \cdot H_2O$ requires: C, 50.3; H, 6.4); ν_{max} (KBr) 3450, 1750 cm^{-1}; δ (C_5D_5N) 3.86, 3.50, 3.48, 3.20 (s, 4 OCH_3), 2.37, 2.34 (s, $ArCH_3$ and $COCH_3$), 1.44–1.00 (m, 7 $CHCH_3$ and evalose CCH_3).

Acetylation (method D) of flambeurekanose flambate isobutyrate gave *flambeurekanose flambate isobutyrate hepta-acetate* (**26e**; 48%) as a colourless solid, m.p. 135–138°, from EtOAc–light petroleum [Found: C, 50.9; H, 6.0; Cl, 4.0. $C_{61}H_{83}Cl_2O_{27}$ $(OAc)_7 \cdot H_2O$ requires: C, 51.4; H, 6.1; Cl, 4.0%]; ν_{max} (KBr) 3450, 1782, 1745 cm^{-1}.

Acetylation (method D, RT, 18 hr then 90°, 1 hr) of flambeurekanose flambate isobutyrate and purification [method B, solvent (ii)] gave *flambeurekanose flambate isobutyrate octa-acetate* (**26f**; 35%) as colourless microcrystals, m.p. 150–153°, from EtOAc–light petroleum. δ (C_5D_5N) 3.81, 3.61, 3.38, 3.29 (s, 4 OCH_3), 2.45, 2.38 (s, $ArCH_3$ and $COCH_3$), 2.21, 2.18, 2.16, 2.04, 1.98, 1.98, 1.81, 1.66 (s, 8 $OCOCH_3$ and evalose CCH_3), 1.48, 1.40, 1.36, 1.26, 1.25, 1.18, 1.11 (d, $CHCH_3$, *J* 6 Hz).

(v) *Des - dichloroisoeverninoyl - des - isobutyroyl flambamycin*[12] (**27**). A mixture of flambamycin (0.5 g) and NaOH aq (5 ml, 10%) was stirred at room temp. for 24 hr and the soln saturated with CO_2. Evaporation, trituration of the residue with abs. EtOH (100 ml) and purification [method A, solvent (ii)] gave *des - dichloroisoeverninoyl - des - isobutyroyl flambamycin*,[12] which was obtained as a colourless solid (0.1 g, 44%), m.p. 212°, from EtOAc–light petroleum, ν_{max} (KBr) 3430, 1710 cm^{-1}.

Acknowledgements—We acknowledge a grant from the S.R.C. for the purchase of the AEI MS9 mass spectrometer. We thank Dr. I. McClenaghan and Dr. J. F. Stoddart for the supply of reference compounds, Dr. T. J. Threlfall, Dr. B. J. Peart and Mr. P. Tyson for spectroscopic measurements, Mr. P. J. Warren and Miss D. J. Liddard for technical assistance and Dr. G. Jolles and L. Ninet (Rhône-Poulenc) for their interest and generous supply of flambamycin.

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