Hydrolysis of Flambamycin. The Constitution of Flambeurekanose

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Summary Flambeurekanose, a hydrolysis product of the antibiotic flambamycin, has been shown to be the ortho-ester (**4**).

MILD alkaline hydrolysis (aqueous NaOH, 10% w/v, 24 h., room temp.) of flambamycin followed by mild acidic hydrolysis during work-up, yields (80%) flambeurekanose, $C_{36}H_{58}O_{23}$, m.p. 191—192 °C.† The isolation of flambeure-

† All products have been fully characterised by spectroscopic methods (i.r., n.m.r., and high-resolution mass spectra) and the formation and characterisation of suitable derivatives. Where appropriate, direct comparison has also been made with authentic samples.

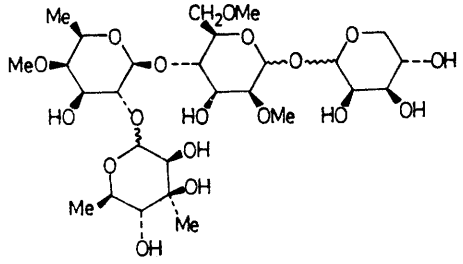
TABLE

Comparison of the ^{13}C chemical shifts (p.p.m. downfield from Me_4Si) for corresponding atoms in methyl eurekanate (**3**) and flambeurekanose (**4**). The positions of the atoms are indicated by the letters in the formulae (**3**) and (**4**).

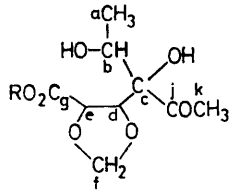
	C_a	C_b	C_c	C_d	C_e	C_f	C_g	C_j	C_k
Methyl eurekanate (**3**)	17·4	68·4	84·2	74·6	81·5	95·9	171·7	207·2	26·1
Flambeurekanose* (**4**)	14·2	83·4	82·1	70·0	80·5	96·7	119·8	210·8	27·6

* ^{13}C Assignments for eurekanic acid residue in flambeurekanose are tentative and are based upon exclusion by comparison of the ^{13}C spectra of flambatetrose (**1**) and flambeurekanose (**4**).

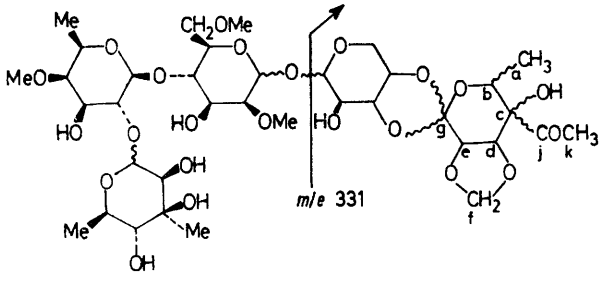
kanose was clearly important as the molecular formula of flambamycin,[1] $C_{61}H_{88}Cl_2O_{33}.H_2O$, is appropriately related to four degradation products: (a) isobutyric acid, $C_4H_8O_2$,



(1) Flambatetrose



(2) R = H, Eurekanic acid
(3) R = Me, Methyl eurekanate

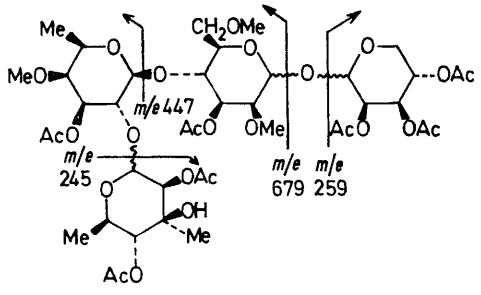


(4) Flambeurekanose

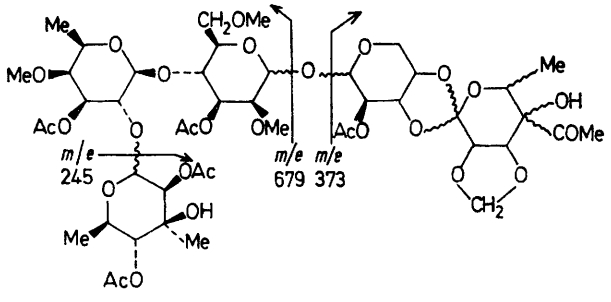
(actually isolated as flambatriose isobutyrate[2] and flambatetrose isobutyrate[2]); (b) flambic acid, $C_{21}H_{28}Cl_2O_{11}$, (actually isolated as methyl flambate[2] and flambalactone[2]); (c) flambatetrose[3] (**1**), $C_{27}H_{48}O_{18}$, and (d) eurekanic acid (**2**), $C_9H_{14}O_7$, (actually isolated as methyl eurekanate[4]).

Flambeurekanose is obviously 'hydrolytically' related to flambatetrose (**1**) and eurekanic acid (**2**) by the equation: $C_{36}H_{58}O_{23} + 2H_2O \rightarrow C_{27}H_{48}O_{18} + C_9H_{14}O_7$. This was confirmed by its mild acidic methanolysis (MeOH–HCl, 0·5% w/v; 90 min., room temp.) which yielded flambatetrose (**1**) and methyl eurekanate (**3**). Flambatetrose is a non-reducing tetrasaccharide derived from the sequence D-evalose, 4-*O*-methyl-D-fucose, 2,6-di-*O*-methyl-D-mannose, and L-lyxose.[3] A novel orthoester linkage between this terminal L-lyxose residue and the eurekanic acid residue has now been demonstrated, thus leading to the constitution (**4**) for flambeurekanose.

The position of the isobutyroyloxy-group in position 2 of the L-lyxose residue of flambamycin has been established.[2] Clearly this 2-isobutyroyloxy-ester is hydrolysed and then the 2-hydroxy-group is methylated under permethylation



(5) Flambatetrose hepta-acetate



(6) Flambeurekanose penta-acetate

SCHEME. Mass spectral fragmentation of flambatetrose hepta-acetate (**5**) and flambeurekanose penta-acetate (**6**).

conditions because one of the products so obtained from flambamycin by permethylation followed by methanolysis is methyl 2-*O*-methyl-L-lyxoside. This glycoside is also produced from flambeurekanose by an identical sequence of reactions. Thus permethylation of flambeurekanose, (NaH–Me_2SO–MeI; 4 h, room temp.) followed by acidic methanolysis (MeOH–HCl, 1% w/v; 1 h, boiling under reflux) and chromatography, yielded *inter alia* methyl 2-*O*-methyl-L-lyxoside.

The possibility that the eurekanic acid residue was associated with an orthoester grouping involving two hydroxy-groups in positions 3 and 4 of the terminal L-lyxose residue was confirmed by the following evidence. Flambatetrose (**1**) forms a fully characterised hepta-acetate,[3] whereas under identical conditions (acetic an-

hydride–pyridine; 18 h, room temp.) flambeurekanose (**4**) forms a penta-acetate, m.p. 195—196 °C (^{1}H n.m.r. spectrum shows signals characteristic of 3 OMe, 3 secondary CHMe, 1 tertiary CMe, 1 CH_3CO, and 5 acetate groupings). The ^{1}H n.m.r. spectrum of flambeurekanose also shows the retention of the acetyl group (δ_{Me} 2·31) of the eurekanic acid residue. The fact that the secondary hydroxy-group of the eurekanic acid (**2**) residue is not acetylatable in the formation of flambeurekanose penta-acetate proves that it is this hydroxy-group which provides the third oxygen of the orthoester grouping in flambeurekanose. There is an acceptable correlation (Table) between the ^{13}C shifts of corresponding carbon atoms in methyl eurekanate (**3**) and the eurekanic acid-derived residue in flambeurekanose (**4**).

The dramatic upfield shift for the ^{13}C chemical shifts (Table) for C_g for the methyl ester carbonyl group in methyl eurekanate (δ 171·7 p.p.m.) to the corresponding signal (δ 119·8 p.p.m.) in flambeurekanose is excellent supporting evidence for an orthoester. Furthermore there is an excellent correlation between this chemical shift (δ 119·8 p.p.m.) and the chemical shifts recorded for the two orthoester groups in everninomycin-D[5] (δ 119·6 and 120·0 p.p.m.) and in olgose[5] (δ 119·8 p.p.m.), a degradation product analogous to flambeurekanose (δ 119·8 p.p.m.). The difference in ^{13}C chemical shifts for C_b in (**3**) and (**4**) is acceptable.

Comparison (Scheme) of the mass spectral fragmentation patterns of flambatetrose hepta-acetate (**5**) and flambeurekanose penta-acetate (**6**) provides independent structural support. The mass spectral fragmentation pattern of flambeurekanose is less informative but there is the conclusive distinction between a fragment (*m/e* 373) from flambeurekanose penta-acetate (Scheme) which is replaced by a fragment (see **4**, *m/e* 331) obviously generated from the orthoester residue derived from eurekanic acid.

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