The Constitution of the Antibiotic Flambamycin

By W. DAVID OLLIS,* CHRISTOPHER SMITH, and IAN O. SUTHERLAND
(*Department of Chemistry, The University, Sheffield* S3 7HF)
and DEREK E. WRIGHT
(*Research Laboratories, May & Baker Ltd., Dagenham, Essex* RM10 7XS)

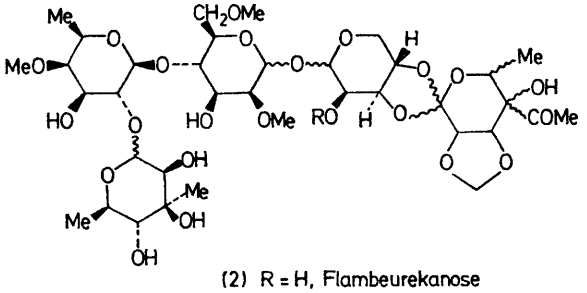
Summary The antibiotic, flambamycin, is shown to have the novel oligosaccharide structure (**4**) associated with two orthoester linkages.

THE antibiotic, flambamycin, produced[1] by *Streptomyces hygroscopicus* DS 23230 shows high *in vitro* activity against gram-positive bacteria and *Neisseria*. We now report the elucidation of the structure of flambamcyin.

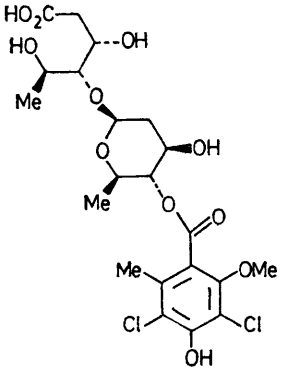
Flambamycin, $C_{61}H_{88}Cl_2O_{33}.H_2O$, m.p. 202—203 °C (lit.,[1] m.p. 226—228 °C) may be formally regarded as a trianhydro-derivative of isobutyric acid, $C_4H_8O_2$, flambic acid (**1**), $C_{21}H_{28}Cl_2O_{11}$, and flambeurekanose[2] (**2**), $C_{36}H_{58}O_{23}$. Flambamycin forms [acetyltrimethylhydantoin (1·1 mol. equiv.)–methyl cyanide, 12 h, boiling under reflux] a monoacetate, $C_{61}H_{87}Cl_2O_{32}(OAc)$, m.p. 170—172 °C.

The location of the isobutyrate residue as an ester involving position 2 of the L-lyxose residue has been firmly established[2,3] in flambatriose, flambatetrose, and flambeurekanose isobutyrates. The derivation of the complete structure of flambamycin therefore involves the dehydrative removal of two molecules of water between flambeurekanose monoisobutyrate (**3**) and flambic acid (**1**). This, in principle, could be most easily achieved by the occurrence in flambamycin of a second orthoester group: the presence of one orthoester group in flambeurekanose (**2**) has been firmly established.[2]

A detailed investigation of the ^{13}C n.m.r. spectra of curacin,[4] flambalactone,[3] flambabiose,[5] flambatriose,[5] flambatriose isobutyrate,[3] flambatetrose,[5] and flambeurekanose[2] (**2**) is highly informative. ^{13}C Chemical shift correlations are

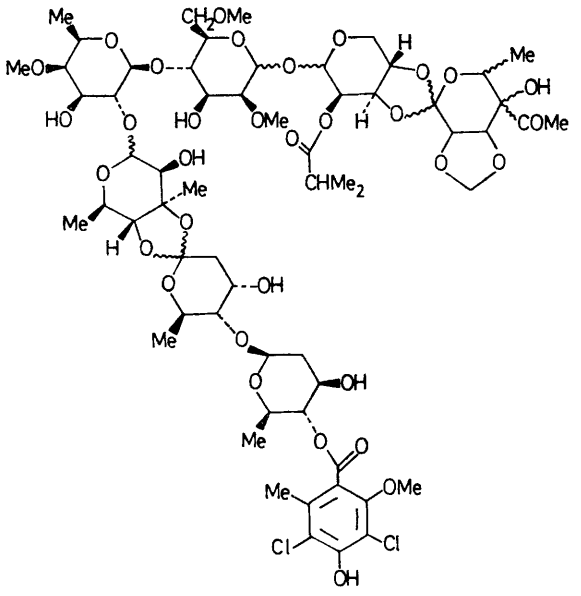


(2) R = H, Flambeurekanose
(3) R = $COCHMe_2$, Flambeurekanose isobutyrate



(1) Flambic acid

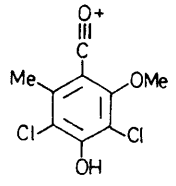
possible between those seven structurally related compounds. In particular, the presence of one orthoester



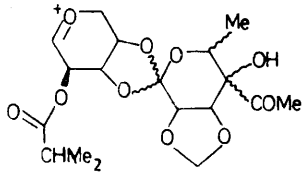
(4) Flambamycin

carbon atom (C_5D_5N, δ 119·8 p.p.m.) in flambeurekanose (**2**) is clear, whereas in flambamycin, *two* ^{13}C orthoester signals (C_5D_5N, δ 119·8 and 120·9 p.p.m.) can be assigned. It has been established that the higher relative intensity of the signal (δ 119·8 p.p.m.) in the ^{13}C n.m.r. spectrum of flambamycin is in fact associated with two carbon atoms: one orthoester carbon and one corresponding with an aromatic quaternary carbon atom present either in curacin [$(CD_3)_2CO$, δ 118·9 p.p.m.] or in flambalactone[3] (C_5D_5N, δ 119·8 p.p.m.). The second orthoester (δ 120·9 p.p.m.) in flambamycin must be associated with the union between the carboxy-group of flambic acid (**1**) and flambeurekanose isobutyrate (**3**). Of the six hydroxy-groups present in flambeurekanose isobutyrate (**3**), only the three hydroxy-groups associated with the terminal D-evalose residue are sterically suitable for possible involvement in an orthoester linkage. This leads to three possible structures for flambamycin of which the constitution (**4**) was established as follows.

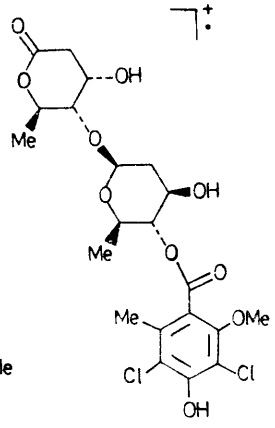
Permethylation (MeI–NaH–Me_2SO, room temp., 18 h) followed by acidic methanolysis (MeOH–HCl, 3·8% w/v, 1 h, boiling under reflux) then separation by t.l.c. yielded five new methyl glycosides which were fully characterised by spectroscopic methods (u.v., i.r., n.m.r.), high-resolution mass spectral fragmentation patterns, specific rotations, and the formation and full characterisation of peroxyacetates. The degradation products were: (i) curacin *O'*-methyl ether 3-*O*-methyl ether methyl glycoside, m.p. 80 °C; (ii) 2-*O*-methyl-D-evalose methyl glycoside (3,4-di-*O*-acetate); (iii) 3,4-di-*O*-methyl-D-fucose methyl glycoside, m.p. 99—101 °C (1,2-di-*O*-acetate); (iv) 2,3,6-tri-*O*-methyl-D-mannose methyl glycoside (4-*O*-acetate); (v) 2-*O*-methyl-L-lyxose methyl glycoside (3,4-di-*O*-acetate).



(5) *m/e* 233



(7) *m/e* 401



(6) *m/e* 508

Acidic hydrolysis (5N-HCl, 70 °C, 18 h) of flambamycin (**4**) gave formaldehyde, isolated from the hydrolysate as its 2,4-dinitrophenylhydrazone (m.p. 164 °C, yield 65%), thus confirming the presence of the methylenedioxy-group. The ^1H n.m.r. spectrum of flambamycin was complicated but clearly indicated the presence of four methoxy-groups, one aromatic methyl group, one methyl ketone, one tertiary methyl, and seven secondary methyl groups.

Extensive high-resolution mass spectral studies[6] were carried out on flambamycin (**4**) and its derivatives. Major fragment ions (**5**), (**6**), and (**7**) confirmed its structure in important respects, particularly the location of the eurekanate and isobutyrate residues.

Flambamycin (**4**) is thus structurally related to the oligosaccharide antibiotics everninomycin-B,[7] everninomycin-C,[8] everninomycin-D,[9] curamycin,[4] and avilamycin.[10]

(*Received*, 20*th February* 1976; *Com.* 181.)

[1] L. Ninet, F. Benazet, Y. Charpentie, M. Dubost, J. Florent, J. Lunel, D. Mancy, and J. Preud'homme, *Experientia*, 1974, **30**, 1270.
[2] W. D. Ollis, C. Smith, and D. E. Wright, preceding communication.
[3] W. D. Ollis, C. Smith, and D. E. Wright, *J.C.S. Chem. Comm.*, 1974, 882.
[4] O. L. Galmarini and V. Deulofeu, *Tetrahedron*, 1961, **15**, 76; E. G. Gros, V. Deulofeu, O. L. Galmarini, and B. Frydman, *Experientia*, 1968, **24**, 323; V. Deulofeu and E. G. Gros, *Anales de Quimica*, 1972, **68**, 789.
[5] W. D. Ollis, C. Smith and D. E. Wright, *J.C.S. Chem. Comm.*, 1974, 881.
[6] S. Jones, W. D. Ollis, and C. Smith, unpublished work.
[7] A. K. Ganguly and A. K. Saksena, *J. Antibiotics*, 1975, **28**, 707.
[8] A. K. Ganguly and S. Szmulewicz, *J. Antibiotics*, 1975, **28**, 710.
[9] A. K. Ganguly, O. Z. Sarre, D. Greeves, and J. Morton, *J. Amer. Chem. Soc.*, 1975, **97**, 1982.
[10] F. Buzzetti, F. Eisenberg, H. N. Grant, W. Keller-Schierlein, W. Voser, and H. Zahner, *Experientia*, 1968, **24**, 320.