Methanolysis of Flambamycin. The Constitution of Methyl Eurekanate

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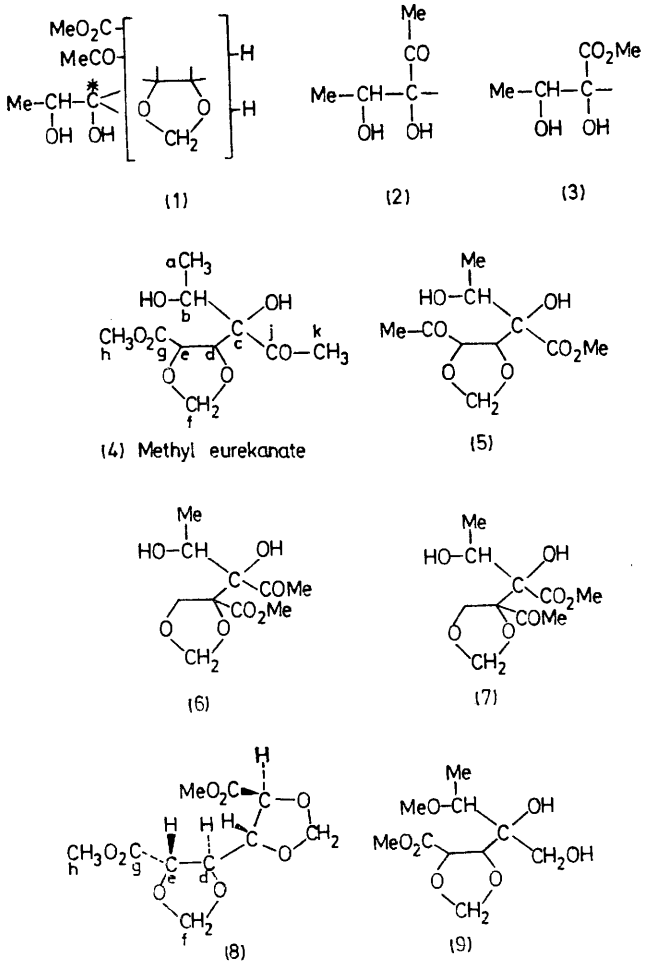
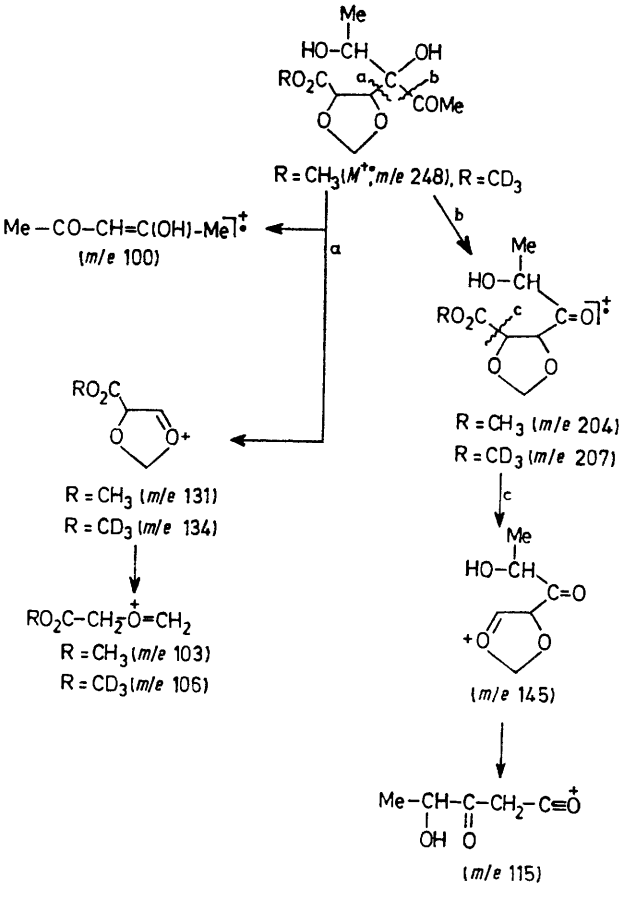
Summary Methyl eurekanate, one of the products from the mild acidic methanolysis of the antibiotic flambamycin, has been shown to have the constitution (**4**).

MILD acidic methanolysis[1] of the antibiotic flambamycin[2] yields a complex mixture of at least nine products of which eight have been fully identified.[1] We now report the constitution (**4**) for the ninth methanolysis product, methyl eurekanate, whose structure is crucial to the complete structure of the antibiotic and relevant to the presence of an orthoester grouping in the antibiotic.

The following functional assignments were initially considered for five of the seven oxygen atoms of methyl eurekanate, $C_{10}H_{16}O_7$ ($M^{+\cdot}$, *m/e* 248) mainly on the basis of the following spectroscopic information [^{1}H n.m.r.† and i.r. ($CHCl_3$)]: (a) CO_2Me (δ_{Me} 3·78, ν_{CO} 1720 cm^{-1}); (b) MeCO (δ_{Me} 2·28, ν_{CO} 1750 cm^{-1}); (c) OCH_AH_BO (δ_A 5·10, δ_B 4·89, J_{AB} 0 Hz). The remaining two oxygen atoms were shown to be associated with two hydroxy-groups (δ 4·15 and 2·58, removed by D_2O addition) located in a secondary–tertiary glycol system. Supplementary evidence reported below led eventually to the partial structure (**1**) for methyl eurekanate.





SCHEME. Part of the mass spectral fragmentation patterns of methyl eurekanate (**4**) and trideuteriomethyl eurekanate.

The presence of a methoxycarbonyl group in methyl eurekanate (**1**) was suspected by its transformation into ethyl eurekanate, $C_{11}H_{18}O_7$ ($M^{+\cdot}$ — CH_3CO, *m/e* 219) by ethanolic hydrogen chloride (1·5% w/v, room temp., 3 h). Its mild acid hydrolysis (5N HCl, room temp., 18 h) yielded eurekanic acid characterised (acetic anhydride, toluene-*p*-sulphonic acid, room temp., 18h) as eurekanic acid diacetate, $C_9H_{12}O_5(OAc)_2$ ($M^{+\cdot}$, *m/e* 318).

The methylenedioxy group in methyl eurekanate was confirmed by acid hydrolysis (5N HCl, 100 °C, 6 h) giving

† All compounds have been fully characterised spectroscopically. Unless otherwise indicated n.m.r. data refer to $CDCl_3$ solutions.

TABLE

Comparison of the ^1H and ^{13}C chemical shifts (p.p.m. downfield from Me_4Si) for corresponding atoms in methyl eurekanate (**4**) and dimethyl 2,3 : 4,5-di-*O*-methylene-galactarate[4] (**8**). The positions of the atoms are indicated by the letters in the formulae (**4**) and (**8**).

	Chemical shifts δ ($CDCl_3$)									
	C_aH_3	C_bH	C_c	C_dH	C_eH	C_fH_2	C_g	C_hH_3	C_j	C_kH_3
(**4**) (^1H)	1·03[v]	4·18[v]	—	4·66[w]	4·68[w]	5·10,[y] 4·89[y]	—	3·78	—	2·28
(**8**) (^1H)	—	—	—	4·27[x]	4·61[x]	5·24,[z] 5·06[z]	—	3·78	—	—
(**4**) (^{13}C)	17·4	68·4	84·2	74·6	81·5	95·9	171·7	52·8	207·2	26·1
(**8**) (^{13}C)	—	—	—	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;[4] [y]AB system, J_{AB} 0 Hz; [z]AB system, $J_{AB} < 1·0$ Hz.[4]

formaldehyde, isolated from the hydorlysate as its 2,4-dinitrophenylhydrazone (m.p. 164 °C, 55% yield). The ^1H n.m.r. spectral characteristics of the methylenedioxy-group (δ_A 5·10, δ_B 4·89; J_{AB} 0 Hz) demonstrated its location in a 1,3-dioxolan ring.[3]

The presence of two hydroxy-groups in the function CH_3–CH(OH)–C*(OH) was supported by the characterisation of methyl eurekanate as a mono-acetate (acetic anhydride–pyridine, room temp., 18 h) $C_{10}H_{15}O_6(OAc)$, m.p. 87 °C, ($M^{+\cdot} - CH_3CO$, *m/e* 247) and a diacetate (acetic anhydride–toluene-*p*-sulphonic acid, room temp., 24 h), $C_{10}H_{14}O_5(OAc)_2$ ($M^{+\cdot} - 1$, *m/e* 331). Methyl eurekanate and trichloracetyl isocyanate gave a bis-trichloracetylcarbamate. The ^1H n.m.r. spectrum of methyl eurekanate clearly showed the presence of an A_3X system (δ_A 1·03, δ_X 4·18, J_{AX} 6·5 Hz) characteristic of the secondary–tertiary glycol grouping, CH_3–CH(OH)–C*(OH). This was confirmed by periodate cleavage (aqueous sodium metaperiodate, room temp., 35 min) yielding acetaldehyde, isolated as its 2,4-dinitrophenylhydrazone (m.p. 168 °C, 54% yield). The expected downfield shift of H_X was observed in the ^1H n.m.r. spectra of methyl eurekanate monoacetate (δ_X 5·39), diacetate (δ_X 5·57), and bis-trichloracetylcarbamate [δ_X 5·36, in $(CD_3)_2CO$]. These results proved that neither of the two unlocated hydrogen atoms in the partial structure (**1**) could be located on C*. Thus the presence of either the grouping (**2**) or the grouping (**3**) could now be considered in relation to the partial structure (**1**). This leads to four possible constitutional formulae (**4**), (**5**), (**6**), or (**7**) for methyl eurekanate.

Comparison of the ^1H and ^{13}C n.m.r. spectra (Table) of methyl eurekanate with that of the model, dimethyl 2,3 : 4,5-di-*O*-methylene-galactarate[4] (**8**) clearly demonstrated that the constitutions (**6**) and (**7**) could be excluded. However, it was still not possible on the basis of the foregoing evidence to determine the relative positions of the methoxycarbonyl and the acetyl groups associated with the remaining two constitutions (**4**) and (**5**). The elucidation of the constitution (**4**) for methyl eurekanate was finally achieved by a detailed examination of the high-resolution mass spectra of methyl eurekanate, trideuteriomethyl eurekanate, and methyl eurekanate diacetate. Only a small part of this extensive mass spectral study is recorded in the Scheme and the information given has been restricted to that which establishes the location of the methoxycarbonyl group.

It may be noted that a eurekanate residue does not occur as such in the antibiotic flambamycin. Flambamycin belongs to a family of structurally related antibiotics which includes the everninomycins,[5] avilamycin,[6] and curamycin[7], and there is an interesting structural correspondence between methyl eurekanate (**4**) and the methyl ester[5a,b] (**9**) obtained by methanolysis of everninomycin-B and everninomycin-D.

(*Received, 20th February* 1976; *Com.* 179.)

[1] W. D. Ollis, C. Smith, and D. E. Wright, *J.C.S. Chem. Comm.*, 1974, 882.
[2] L. Ninet, F. Benazet, Y. Charpentie, M. Dubost, J. Florent, J. Lunel, D. Mancy, and J. Preud'homme, *Experientia*, 1974, **30**, 1270.
[3] R. C. Cookson and T. A. Crabb, *Tetrahedron Letters*, 1964, 679; R. C. Cookson, T. A. Crabb, J. J. Frankel, and J. Hudec, *Tetrahedron Suppl.* 7, 1966, **355**; R. C. Cookson and T. A. Crabb, *Tetrahedron*, 1968, **24**, 2385; S. Sternhell *Quart. Rev.*, 1969, **23**, 236.
[4] I. J. Burden and J. F. Stoddart, *J.C.S. Perkin I*, 1975, 675.
[5] (a) A. K. Ganguly, O. Z. Sarre, D. Greeves, and J. Morton, *J. Amer. Chem. Soc.*, 1975, **97**, 1982; (b) A. K. Ganguly and A. K Saksena, *J. Antibiotics*, 1975, **28**, 707; (c) A. K. Ganguly and S. Szmulewicz, *ibid.*, p. 710.
[6] F. Buzzetti, F. Eisenberg, H. N. Grant, W. Keller-Schierlein, W. Voser, and H. Zähner, *Experientia*, 1968, **24**, 320.
[7] 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.