Methanolysis of Flambamycin. Formation and the Constitutions of Flambalactone, Methyl Flambate, Flambatriose Isobutyrate and Flambatetrose Isobutyrate

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Summary Mild acidic methanolysis of the antibiotic, flambamycin, yields curacin methyl glycoside (**2**), flambalactone (**35**), methyl flambate (**39**), methyl eurekanate, methyl D-evalopyranoside (**10**), flambatriose (**23**), flambatriose isobutyrate (**26**), flambatetrose (**27**), and flambatetrose isobutyrate (**32**).†

ACIDIC methanolysis (MeOH—HCl, 0·5% w/v; 90 min/room temp.) of flambamycin,[1] followed by addition of calcium carbonate, filtration, concentration, addition of water, and extraction with ether gave two fractions, an ether-extractable fraction (A) and an aqueous methanolic soluble fraction (B). Fraction (A) was evaporated and separated [t.l.c., silica; $CHCl_3$–MeOH (9:1)] yielding four

† The structures are given in this and the preceding Communication and are numbered consecutively.

TABLE

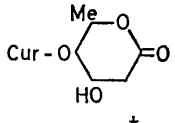
Chemical shifts and coupling constants of the indicated protons [see (35)] and the carbonyl bands (ν_{CO}, $CHCl_3$) associated with the δ-lactone residue of flambalactone and its derivatives.

Compound	Chemical shifts, $\tau(CD_3)_2CO$						Coupling constants, J/Hz						ν_{CO}/cm^{-1} ($CHCl_3$)
	H_A	H_B	H_C	H_D	H_E	Me	J_{AB}	J_{AC}	J_{BC}	J_{CD}	J_{DE}	$J_{E,Me}$	
Flambalactone (35)	7·02	7·6	6·0—6·5[a]	6·47	5·74	8·61	17	6	6	3	8	6	1740
Flambalactone methyl ether (36)[c]	7·00	7·50	6·0—6·3[a]	6·3—6·6[a]	5·77	8·62	17	6	6	3	8	6	1740
Flambalactone triacetate (37)	6·87	7·50	4·57	6·2—6·7[a]	5·65	8·66	16·5	5	3	3	8·5	6	1740, 1782[b]
Flambalactone tris-trichloromethylcarbamate (38)	6·77	7·38	4·43	6·20	5·60	8·63	17	5	5	3	8	6	—

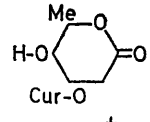
[a] Owing to overlap with other signals the chemical shift cannot be determined directly. [b] Carbonyl band of phenolic *O*-acetate. [c] Solvent $CDCl_3$–$(CD_3)_2CO$.

major compounds: curacin methyl glycoside (**2**),[2] m.p. 148—150°, flambalactone, methyl flambate, and methyl eurekanate.[3] Fraction (B) was evaporated and the residue separated [t.l.c., silica; $CHCl_3$–MeOH (4:1)] yielding methyl D-evalopyranoside (**10**), flambatriose (**23**),[4] flambatetrose (**27**),[4] flambatriose isobutyrate, and flambatetrose isobutyrate. We now report upon our structural investigations of flambalactone, methyl flambate, flambatriose isobutyrate, and flambatetrose isobutyrate.

Flambalactone, $C_{21}H_{26}Cl_2O_{10}$, m.p. 217°, was characterised as its mono-*O*-methyl derivative (diazomethane), $C_{20}H_{22}Cl_2O_8(OMe)_2$, m.p. 201°, and its triacetate (acetic anhydride–pyridine), $C_{21}H_{23}Cl_2O_7(OAc)_3$, m.p. 159°. Flambalactone, its mono-*O*-methyl derivative, and its triacetate all show carbonyl bands (ν_{CO} 1740 cm^{-1}, see Table) indicating that a γ-lactone residue is absent: the lactone (**20**)[4] shows ν_{CO} ($CHCl_3$) 1780 cm^{-1}. On empirical grounds, flambalactone, $C_{21}H_{26}Cl_2O_{10}$, is clearly related to curacin (**1**), $C_{15}H_{18}Cl_2O_7$, and the γ-lactone (**20**),[4] presumably present as its δ-lactone equivalent. This leads to two possible constitutions (**33**) or (**34**) for flambalactone. The decision between these two structural possibilities for flambalactone is clearly possible (see Table) on the basis of the n.m.r. characteristics of the indicated protons (H_A, H_B, H_C, H_D, and H_E) in formula (**35**). The downfield shift of H_C in flambalactone triacetate (**37**) and the tris-carbamate (**38**) derived from flambalactone and trichloroacetyl isocyanate clearly places the curacin residue (Cur)‡ as in (**33**). This leads to the constitution (**35**) for flambalactone and its derivatives (**36**), (**37**), and (**38**). The coupling constants (Table) of the protons associated with the δ-lactone residue define the relative stereochemistry of flambalactone (**35**).

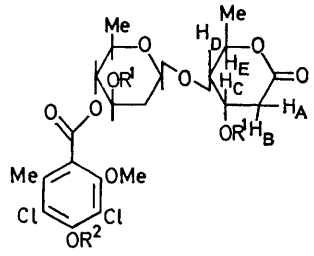


(33)†

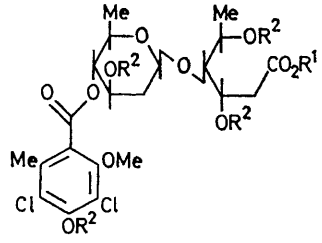


(34)†

The constitution of the methanolysis product, methyl flambate (**39**), $C_{22}H_{30}Cl_2O_{11}$, follows from its alternative synthesis from flambalactone and methanolic hydrogen chloride (0·15% w/v; room temp). Methyl flambate shows the appropriate spectroscopic properties, n.m.r. and ν_{CO}($CHCl_3$) 1735 cm^{-1}. It was characterised as its tetra-acetate (**40**), $C_{22}H_{26}Cl_2O_7(OAc)_4$, which showed a confirmatory mass spectral fragmentation pattern. Ethyl flambate (**41**), $C_{23}H_{32}Cl_2O_{11}$, is analogously formed by the acidic ethanolysis of flambamycin. Compounds structurally analogous to flambalactone (**35**) and methyl flambate (**39**) have been isolated by the aqueous acidic hydrolysis followed by methylation (diazomethane) of everheptose derived from everninomycin-D.[5]



(35) Flambalactone, $R^1 = R^2 = H$
(36) $R^1 = H$; $R^2 = Me$
(37) $R^1 = R^2 = Ac$
(38) $R^1 = R^2 = CONHCOCCl_3$



(39) Methyl flambate, $R^1 = Me$; $R^2 = H$
(40) $R^1 = Me$; $R^2 = Ac$
(41) $R^1 = Et$; $R^2 = H$

Flambatriose isobutyrate, $C_{24}H_{42}O_{15}$, m.p. 115—117° [n.m.r. shows 3 OMe, 1 sec. *C*Me, and 1 isobutyroyl group ($\tau_{Me(A)}$ 8·90, $\tau_{Me(B)}$ 8·96, τ_H 7·47, J_{Me-H} 7 Hz)], on acidic methanolysis gave the methylglycosides of 4-*O*-methyl-D-fucose (**7**), 2,6-di-*O*-methyl-D-mannose (**5**), and L-lyxose (**3**). Acetylation (acetic anhydride–pyridine; room temp.) of flambatriose isobutyrate gave a penta-acetate, $C_{24}H_{37}O_{10}(OAc)_5$, m.p. 86°. Comparison of the mass spectral fragmentation pattern of flambatriose hexa-acetate (**25**) with flambatriose isobutyrate penta-acetate established[6] the

‡ The abbreviation Cur in these formulae is clear in relation to the representation, Cur-OH, for curacin (**1**).

presence of the isobutyroyloxy-group on C-2 of the L-lyxose residue, thus leading to the structure (**26**) for flambatriose isobutyrate.

Flambatetrose isobutyrate, $C_{31}H_{54}O_{19}$, m.p. 145° (n.m.r. shows 3 OMe, 2 sec. *C*Me, 1 tert. *C*Me, and 1 isobutyroyl group) was similarly fully characterised (see above) by acidic hydrolysis and acidic methanolysis. Acetylation yielded a hexa-acetate, $C_{31}H_{48}O_{13}(OAc)_6$, m.p. 114—115°, and a hepta-acetate, $C_{31}H_{47}O_{12}(OAc)_7$, whose mass spectral fragmentation pattern also established[6] the location of the isobutyroyloxy-group on C-2 of the L-lyxose residue in flambatetrose isobutyrate (**32**).

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