Acidic Hydrolysis Products of Flambamycin

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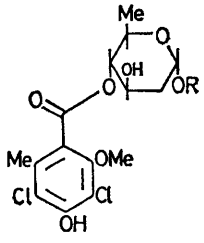
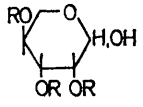
Summary Acidic hydrolysis of flambamycin yields curacin (**1**), L-lyxose (**3**), 2,6-di-*O*-methyl-D-mannose (**5**), 4-*O*-methyl-D-fucose (**7**), D-evalose (**9**), 3,5-dihydroxy-γ-caprolactone (**20**), flambabiose (**21**), flambatriose (**23**), flambatetrose (**27**), and flambatetrose isobutyrate (**32**).

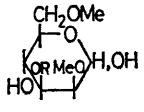
FLAMBAMYCIN is a new antibiotic produced by *Streptomyces hygroscopicus* DS 23, 230.[1] Its possible molecular formula, $C_{61}H_{90}Cl_2O_{34}$, and the nature of a number of its degradation products demonstrate that flambamycin belongs to a family of structurally related antibiotics which includes curamycin,[2] avilamycin,[3] everninomycin-B,[4] and everninomycin-D,[5] whose structures have not been completely elucidated. These antibiotics are oligosaccharides esterified by one dichloroisoeverninic acid residue. We now report structural information on flambamycin as revealed by the study of degradation products† produced by its aqueous acidic hydrolysis.



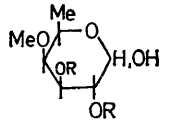
(1) Curacin, R = H
(2) R = Me



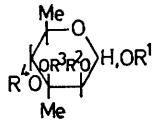
(3) L-Lyxose, R = H
(4) R = Me



(5) 2,6-Di-*O*-methyl-D-mannose, R = H
(6) R = Me

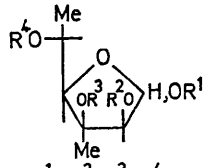


(7) 4-*O*-Methyl-D-fucose, R = H
(8) R = Me

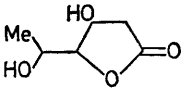


(9) D-Evalose, $R^1=R^2=R^3=R^4=H$
(10) R^1 = Me; $R^2=R^3=R^4=H$
(11) $R^1=R^2=R^3=R^4$ = Ac
(12) R^1 = Me; $R^2=R^4$ = Ac, $R^3=H$
(13) R^1 = Me; $R^2=R^3=R^4$ = Ac
(14) $R^1=R^2$ = Me; $R^3=R^4=H$
(15) $R^1=R^2$ = Me; $R^3=R^4$ = Ac

Mild acidic hydrolysis (HCl, 0·5% w/v; 78°/30 min then 31°/17 h) of flambamycin followed by ether extraction yielded one product identified† as curacin (**1**), m.p. 143—145°.[2] Curacin (**1**) was characterised as its monomethyl ether, m.p. 118—120°, and its triacetate, m.p. 192—194°. After ether extraction and evaporation of the aqueous hydrolysate, further hydrolysis (HCl, 0·4% w/v; 100°/3 h) of the residue followed by neutralisation (Amberlite IR-4B-OH form) and cellulose column chromatography (BuOH–H_2O, 20:3) yielded L-lyxose (**3**),[2,3] 2,6-di-*O*-methyl-D-mannose (**5**), [2,3,5] 4-*O*-methyl-D-fucose (**7**),[2–5] flambabiose, and a novel sugar, $C_7H_{14}O_5$, which at that time had not been described previously. However, during our investigation, D-evalose (**9**) was reported[4] as a hydrolysis product of evertetrose-B, derived from everninomycin-B. The structure proposed[4] for D-evalose (**9**) is identical with that deduced by us, using methods which differ from those previously reported.[4] We obtained the sugar, $C_7H_{14}O_5$, as a mixture of pyranose- (**9**), furanose- (**16**), and aldehydo-



(16) $R^1=R^2=R^3=R^4=H$
(17) R^1 = Me; $R^2=R^3=R^4=H$
(18) R^1 = Me; $R^2=R^3=R^4$ = Ac
(19) $R^1=R^2$ = Me; $R^3=R^4=H$



(20)

forms. The complete characterisation of D-evalose in the pyranose- (**9**) and furanose- (**16**) forms was made on the basis of an extensive study† of the derivatives (**10**)—(**15**) and (**17**)—(**19**), particularly the compounds: (**11**), m.p. 131—132°; (**12**) (M^+· $-H_2O$ 258); (**13**), m.p. 157° (M^+· —OMe 287), and (**17**) (M^+· —OMe 161).

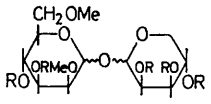
Repetition of the first mild acid hydrolysis of flambamycin followed by ether extraction, neutralisation of the aqueous solution, evaporation, then preparative t.l.c. (silica: $CHCl_3$–MeOH, 4:1) gave four isolable degradation products: a lactone, flambatriose, flambatetrose, and flambatetrose monoisobutyrate. The lactone was identified† as 3,5-dihydroxy-γ-caprolactone (**20**)[3] and was characterised as its diacetate, m.p. 113°.[3]

Flambatriose, $C_{20}H_{36}O_{14}$, m.p. 124—125° (n.m.r. shows 3 OMe and one sec. CMe), gave L-lyxose (**3**), 2,6-di-*O*-methyl-D-mannose (**5**), and 4-*O*-methyl-D-fucose (**7**) on acid hydrolysis (hydrochloric acid, 0·4% w/v; 100°/3 h). Flambatriose was characterised as its hexa-acetate, m.p. 86°, $C_{20}H_{30}O_8(OAc)_6$ (M^+· 752) and its hexa-*O*-methyl derivative, $C_{17}H_{21}O_5(OMe)_9$ (M^+· 584). Acid hydrolysis (H_2SO_4, 10% w/v; 100°/4 h) of flambatriose hexamethyl ether yielded 2,3,4-tri-*O*-methyl-L-lyxose (**4**),[5] 2,3,6-tri-*O*-methyl-D-mannose (**6**),[5] and 2,3,4-tri-*O*-methyl-D-fucose (**8**).[5] These results establish the constitutions of flambatriose (**23**), its hexamethyl derivative (**24**), and its hexa-acetate (**25**).

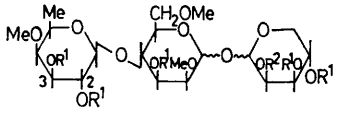
Flambabiose, $C_{13}H_{24}O_{10}$, is a non-reducing disaccharide

† All degradation products have been fully characterised by spectroscopic methods (u.v., i.r., n.m.r., and high resolution mass spectra), mass spectral fragmentation patterns, specific rotations, and the formation and characterisation of appropriate derivatives (peroxyacetates and peroxymethyl ethers). Where appropriate, direct comparison has also been made with authentic samples.

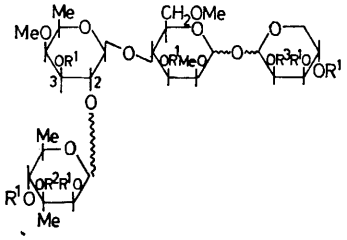
and its n.m.r. spectrum shows signals assignable to two methoxy-groups and two anomeric protons. Flambabiose was characterised as a penta-acetate, m.p. 149—150°, $C_{13}H_{19}O_5(OAc)_5$. The mass spectral fragmentation pattern of this penta-acetate and the spectroscopic properties† of flambabiose (**21**) and its penta-acetate (**22**) established the assigned constitutions and their structural relation to flambatriose (**23**) and flambatriose hexa-acetate (**25**) respectively.



(21) Flambabiose, R = H
(22) R = Ac



(23) Flambatriose, $R^1 = R^2 = H$
(24) $R^1 = R^2 = Me$
(25) $R^1 = R^2 = Ac$
(26) $R^1 = H$; $R^2 = COCHMe_2$



(27) Flambatetrose, $R^1 = R^2 = R^3 = H$
(28) $R^1 = R^3 = Me$; $R^2 = H$
(29) $R^1 = R^2 = R^3 = Me$
(30) $R^1 = R^3 = Ac$, $R^2 = H$
(31) $R^1 = R^2 = R^3 = Ac$
(32) $R^1 = R^2 = H$; $R^3 = COCHMe_2$

Flambatetrose, $C_{27}H_{48}O_{18}$, is a non-reducing tetrasaccharide (n.m.r. shows four anomeric protons, 3 OMe, 2 sec. CMe groups, and 1 tert. CMe) which on acid hydrolysis HCl, 0·4% w/v; 100°/3 h) yielded L-lyxose (**3**), 2,6-di-*O*-methyl-D-mannose (**5**), 4-*O*-methyl-D-fucose (**7**), and D-evalose (**9**). Flambatetrose was characterised as a hepta-*O*-methyl derivative (**28**), $C_{24}H_{32}O_8(OMe)_{10}$ ($M^{+\cdot}$ —MeOH 726), an octa-*O*-methyl derivative (**29**), $C_{24}H_{31}O_7(OMe)_{11}$, m.p. 122° ($M^{+\cdot}$ 772), a hepta-acetate (**30**), $C_{27}H_{41}O_{11}(OAc)_7$ ($M^{+\cdot}$ $-C_2H_2O$ 912), and an octa-acetate (**31**), $C_{27}H_{40}O_{10}(OAc)_8$, m.p. 102°. These results established that flambatetrose was a tetrasaccharide derived from flambatriose (**23**) and D-evalose (**9**). Union of the D-evalose residue [see (**9**)] with the 4-*O*-methyl-D-fucose residue [see (**7**)] was clearly indicated by the mass spectral fragmentation patterns of (i) flambatetrose heptamethyl ether (**28**) (fragment ion, $C_{17}H_{31}O_8$, *m/e* 363), (ii) flambatetrose octamethyl ether (**29**) (fragment ion, $C_{18}H_{33}O_8$, *m/e* 377·2177), and (iii) flambatetrose hepta-acetate (**30**) (fragment ion, $C_{20}H_{31}O_{11}$, *m/e* 447·1882). A decision between the two structural possibilities (glycosidic union of the D-evalose residue either to position 2 or to position 3 of the 4-*O*-methyl-D-fucose residue) for flambatetrose was made possible by the following evidence. Acidic methanolysis of the peroxymethyl ether obtained[7] by direct methylation of flambamycin yields *inter alia* 3,4-di-*O*-methyl-D-fucose methyl glycoside [*cf.* (**7**)]. This establishes the constitution (**27**) for flambatetrose. It may be noted that in flambatetrose (**27**) the D-evalose residue is linked to position 2 of the 4-*O*-methyl-D-fucose residue, whereas in evertetrose-B,[4] the D-evalose residue is linked to position 3 of the 4-*O*-methyl-D-fucose residue.

The structural characterisation of the fifth degradation product, flambatetrose isobutyrate, is discussed in the following paper.[6]

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