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THE ORTHOSOMYCIN FAMILY OF ANTIBIOTICS—III

MASS SPECTRAL STUDIES OF FLAMBAMYCIN AND ITS DEGRADATION PRODUCTS

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Abstract—Mass spectral evidence for the constitution (54) of the antibiotic, flambamycin, is summarised in Schemes 1–10. An analysis of all the evidence given in Parts I, II and III for the location of the two ortho-ester groups flambamycin and its derivatives is presented.

The determination of the constitution (54) of the antibiotic flambamycin has been described in Part I.[1] Brief reference has already been made in Part I (Sections 5, 7 and 9; Schemes 1, 2 and 3) and in five preliminary communications,[2] to our use of low and high resolution mass spectrometry, not only for the determination of molecular formulae, but also for the elucidation of mass spectral fragmentation patterns. The mass spectra of methyl eurekanate (24)[2c] and derivatives of flambatetrose (7)[2b,2d] and flambeurekanose (42)[2d] have been briefly discussed. Our full investigation (Part III) is essentially complementary to (i) the characterisations and degradations reported in Part I,[1] and (ii) the independent reassuring support obtained from the determination of the ^{13}C NMR spectra of flambamycin and other orthosomycins[3] presented in Part II.[4]

We now report on the electron impact mass spectral behaviour (Schemes 1–10) of flambamycin and its degradation products. The same labelling of the residues A, B, C, D, E, F, G and H, and the same numbering of the 61 C atoms of flambamycin (54) used in Parts I[1] and II[4] have been adopted. Common cleavages (with or without hydrogen transfer) are indicated in Schemes 1–10 by the letters a–l. The presence of the residues of the disaccharide (bamflabiose, B–C), disaccharide (flambabiose, F–G), trisaccharide (flambatriose, E–F–G), and tetrasaccharide (flambatetrose, D–E–F–G) in flambamycin (44; Scheme 9) has resulted in easy recognisable cleavage patterns which correspond with those described[5] for other carbohydrate derivatives. Carbohydrates themselves are usually too involatile to provide useful electron impact mass spectra. However, derivatives which have been extensively investigated include peracetates,[6] permethyl ethers,[7] and pertrimethylsilyl ethers.[6d,8]

The cleavage patterns characteristic of esters[9] are assignable to the terminal dichloro-iso-everninoyl residue A and the isobutyroyloxy group located at C-45 of the L-lyxose residue G. There is an interesting difference between the stability on electron impact of the two ortho-ester groups. The ortho-ester group uniting the residues C and D at positions C-16, C-24 and C-25 is clearly more easily fragmented by electron impact then the second ortho-ester group uniting residues G and H at positions C-46, C-47 and C-53. This opinion of the relative ease of cleavage of the two ortho-ester groups present in flambamycin (44) is based upon a comparison of the relative intensities of peaks in various mass spectra (Schemes 1–10) which are assignable either to cleavage-d or to cleavage-l. Furthermore, the observation of peaks corresponding to fragmentation with the retention of the G-H ortho-ester grouping has already been noted in Part I (Schemes 1 and 3).[1]

Our results (Schemes 1–10) on the mass spectra of flambamycin (44) and its derivatives show a satisfying correspondence not only with the mass spectra of carbohydrate derivatives[5–8] but also with mass spectral fragmentations and their interpretations reported for other orthosomycins.[3] These include the mass spectra of Destomycins-A,[10] -B[11] and -C,[12] Antibiotic A-396-1,[13] Hygromycin-B,[14] Antibiotic SS-56-C,[15] the Everninomicins-C,[16] -D[17] and -2[18] and the Avilamycins-A and -C.[19]

In the structural investigation of flambamycin and its degradation products, high resolution mass spectra were determined with an AEI MS9 spectrometer and low resolution spectra were determined with either an AEI MS12 spectrometer or a VG Micromass spectrometer. The compositions of all significant fragment ions associated with the cleavages a–l indicated in the Schemes 1–10 have been established by high resolution measurements. Furthermore, the pathways of fragmentation were revealed by the examination of associated metastable transitions which were obtained by defocussed mode metastable scanning of the first field-free region.[20–23] In order to avoid confusing complexity in the presentation of all this experimental information, we have chosen, with the exceptions of Scheme 4 and Scheme 7, not to

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indicate either (i) the formulae of fragment ions, (ii) the detection of metastable transitions, (iii) fragmentation pathways, or (iv) the structures of fragment cations or cation radicals. This more detailed information is derivable from Schemes 1–10. The observation of hydrogen transfer processes is indicated in Schemes 1–10 by asterisks.

These investigations have provided definite identification of common fragment ions by high resolution measurements. Common fragmentation pathways which could be associated with metastable transitions were also certainly identified.[20–23]

These results are summarised in Schemes 1–10 and are most profitably considered in terms of common cleavages (a–l).

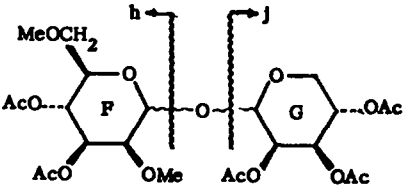
Spectrum of flambabiose penta-acetate (1). This spectrum shows two modes of cleavage h and j characteristic of a non-reducing disaccharide derived from the 2,6-di-O-methyl-D-mannose residue F and the L-lyxose residue G. The two cleavages h and j generate the corresponding stabilised tetrahydropyrylium cations.

Spectra of flambatriose (2) *and its derivatives* (3–6). Cleavage g involving the glycosidic C atom of the 4-O-methyl-D-fucose residue E is observed. This leads to the corresponding tetrahydropyrylium cations (*m/e* 161, 189 or 245) whose composition clearly locates and identifies the substituents associated with C-30 and C-31 of the 4-O-methyl-D-fucose residue E.

The same cleavages h and j identified in Scheme 1 are observable for flambatriose hexa-acetate (3) and the mixture of isomeric flambatriose isobutyrate penta-acetates (6). Only cleavage h is observable in the mass spectrum of flambatriose (2) and only cleavage j in the mass spectra of flambatriose hexamethyl ether (4) and the isomeric flambatriose isobutyrates (5).

As expected, cleavage m of the L-lyxose residue G with the loss of one H atom is also shown by flambatriose hexa-acetate (3) and flambatriose isobutyrate penta-acetate (6). In addition, flambatriose hexa-acetate (3) and flambatriose isobutyrate penta-acetate (6) both show an M-86 peak due to loss of a [CH_2CHOAc] residue associated with C-47 and C-48.

Spectra of flambatetrose (7) *and its derivatives* (8–14). In Scheme 3, in addition to the cleavages g, h and j discussed in Schemes 1 and 2, cleavages e and k are also observed. Cleavage e gives fragment ions (*m/e* 161, 189, 203, 245 or 287) which permit the identification of the substituents (H, Me or Ac) associated with R^1 and R^2 of the terminal D-evalose residue D. Cleavage k (*m/e* 43 or 71) establishes the association of the terminal L-lyxose residue G with either an acetoxy group or an isobutyroyloxy group. The location of these acyloxy groups at C-45 was ultimately established on the basis of (i) permethylation of flambamycin and hydrolysis (Part I, Section 4), (ii) the elucidation of the constitution of flambeurekanose (Part I, Section 8), (iii) ^1H NMR studies (Part I) and (iv) ^{13}C NMR studies (Part II). However, excellent independent support for the location of either methoxy, acetoxy, or isobutyroyloxy groups at C-45 was provided by the observation of appropriate fragment cations (15, 16, 17 and 18) and corresponding metastable transitions. The pyrylium cation (17) contains the substituent (R^2O) originally located at C-45 in the derivatives listed in Scheme 4.



	h	j
	m/e	m/e
(1) Flambabiose penta-acetate	275	259

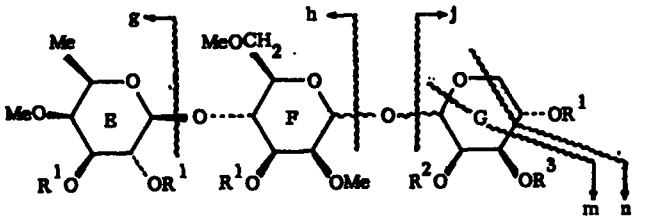
Scheme 1. Mass spectral fragmentation of flambabiose penta-acetate (1).

In Scheme 4, consideration is given to additional modes of fragmentation involving the terminal L-lyxose residue G which yield fragment ions of the types 15, 16, 17 and 18. The informative correlations between corresponding fragment cations (G) derived from derivatives of flambabiose (F–G), flambatriose (E–F–G) and flambatetrose (D–E–F–G) is clearly summarised in Scheme 4.

Spectra of curacin, flambalactone (19), *methyl flambate* (22) *and their derivatives.* Although the constitution of curamycin has not yet been determined,[24] the elucidation of the constitution of its degradation product, curacin,[24a,24c] was of considerable assistance in our initial investigation[2a] of flambamycin (Part I, Section 2).[1] Curacin contains the residues A and B present in flambalactone (19, A–B–C) and methyl flambate (22, A–B–C). The mass spectra of curacin and its derivatives showed the indicated fragment ions (^{35}Cl) assignable to cleavage a: curacin (*m/e* 233), curacin methyl glycoside (*m/e* 233), curacin O-methyl ether (*m/e* 247), curacin tristrimethylsilyl ether (*m/e* 305) and curacin triacetate (*m/e* 275). These peaks are easily identified by their multiplicity and relative intensities which are determined by the relative natural abundances of the ^{35}Cl and ^{37}Cl isotopes. Cleavage a is characteristic of aromatic esters[9] and is to be ascribed to the stability of the acylium cation. Exactly analogous behaviour is shown by the mass spectra summarised in Schemes 5 and 6 and in addition the anticipated cleavages b and c are assignable.

Spectra of methyl eurekanate (24) *and its derivatives* (25–29). The isolation and the structural elucidation of methyl eurekanate[2c] provided a major step forward in the structural investigation of flambamycin (Part I, Section 7).[1] This mass spectral information is summarised in Scheme 7. All the fragment ions (30–41) have been characterised by high resolution measurements and the metastable transitions associated with the indicated fragmentation pathways have been identified.[20–23] The mass spectra of methyl eurekanate (24) and trideuteriomethyl eurekanate (25) have already been compared in Part I (Scheme 2).[1]

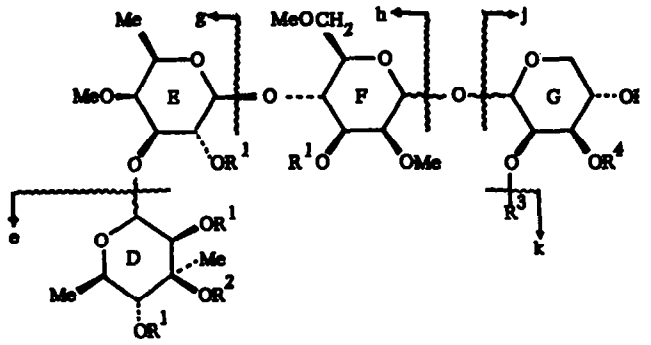
Spectra of flambeurekanose (42) *and its penta-acetate* (43). The relation of flambatetrose (7, D–E–F–G) and methyl eurekanate (24, H) to the constitution of flambeurekanose (42) was fully supported by the mass spectral behaviour summarised in Scheme 8. The observation of a parent peak (*m/e* 1068) in 43 was particularly rewarding and clearly demanded the presence of one ortho-ester grouping. The sequence of residues D–E–F–G–H was revealed by the observation of the cleavages e, f, g, h and j. The resulting fragment ions located the ortho-ester group between residues G and H. The evi-



		R¹=	R²=	R³=	g m/e	h m/e	j m/e	m* m/e	n m/e	M+·
(2)	Flambatriose	H	H	H	161	351				500
(3)	Flambatriose hexa-acetate	Ac	Ac	Ac	245	477	259	649	666	752
(4)	Flambatriose hexamethyl ether	Me	Me	Me	189		175			584
(5)	Flambatriose isobutyrate†	H	$COCHMe_2$	H	161		203			
		H	H	$COCHMe_2$						
(6)	Flambatriose isobutyrate penta-acetate†	Ac	$COCHMe_2$	Ac	245	477	287	677	694	
		Ac	Ac	$COCHMe_2$						

† Cleavage m* = Cleavage m with loss of one hydrogen atom
The origin of these isomers is discussed in Part I (Section 5)[1] and in Part II.[4]

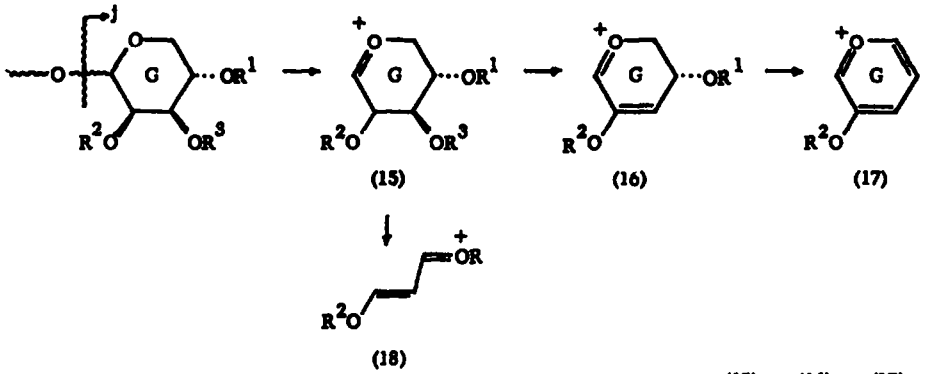
Scheme 2. Mass spectral fragmentation of flambatriose (2) and its derivatives (3–6).



		R¹=	R²=	R³=	R⁴=	e m/e	g m/e	h m/e	j m/e	k m/e	M⁺·
(7)	Flambatetrose	H	H	H	H	161		511	133		
(8)	Flambatetrose hepta-acetate	Ac	H	Ac	Ac	245	447	679	259		
(9)	Flambatetrose octa-acetate	Ac	Ac	Ac	Ac	287			259		
(10)	Flambatetrose heptamethyl ether	Me	H	Me	Me	189	363		175		758
(11)	Flambatetrose octamethyl ether	Me	Me	Me	Me	203	377		175		772
(12)	Flambatetrose isobutyrate†	H	H	H	$COCHMe_2$	161			203	71	
		H	H	$COCHMe_2$	H						
(13)	Flambatetrose isobutyrate hexa-acetate†	Ac	H	$COCHMe_2$	Ac	245	447	679	287	71	
		Ac	H	Ac	$COCHMe_2$					43	
(14)	Flambatetrose isobutyrate hepta-acetate†	Ac	Ac	$COCHMe_2$	Ac	287	447		287	71	
		Ac	Ac	Ac	$COCHMe_2$					43	

† The origin of these isomers is discussed in Part I (Section 5)[1] and in Part II.[4]

Scheme 3. Mass spectral fragmentation of flambatetrose (7) and its derivatives (8–14).



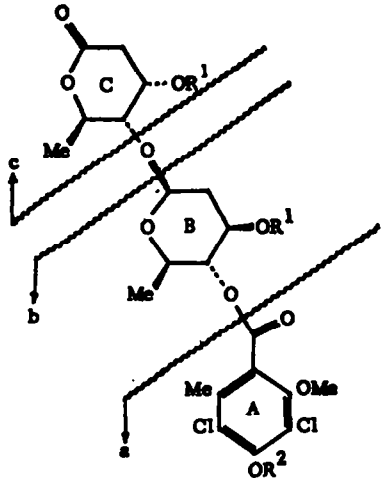
(15) (16) (17)

(18)

		R^1=	R^2=	R^3=	(15) m/e	(16) m/e	(17) m/e	(18) m/e
(1)	Flambabiose penta-acetate	Ac	Ac	Ac	259	199	139	157
(3)	Flambatriose hexa-acetate	Ac	Ac	Ac	259	199	139	157
(6)	Flambatriose isobutyrate penta-acetate †	Ac	Ac	$COCHMe_2$	287	227	167	185
		Ac	Ac	$COCHMe_2$	259	199	139	157
(8)	Flambatetrose hepta-acetate	Ac	Ac	Ac	259	199	139	157
(9)	Flambatetrose octa-acetate	Ac	Ac	Ac	259	199	139	157
(10)	Flambatetrose heptamethyl ether	Me	Me	Me	175	143	111	
(11)	Flambatetrose octamethyl ether	Me	Me	Me	175	143	111	
(13)	Flambatetrose isobutyrate hexa-acetate †	Ac	Ac	$COCHMe_2$	287	227	167	185
		Ac	Ac	$COCHMe_2$	259	199	139	157
(14)	Flambatetrose isobutyrate hepta-acetate †	Ac	Ac	$COCHMe_2$	287	227	167	185
		Ac	Ac	$COCHMe_2$	259	199	139	157

† The origin of these isomers is discussed in Part I (Section 5)[1] and in Part II.[4]

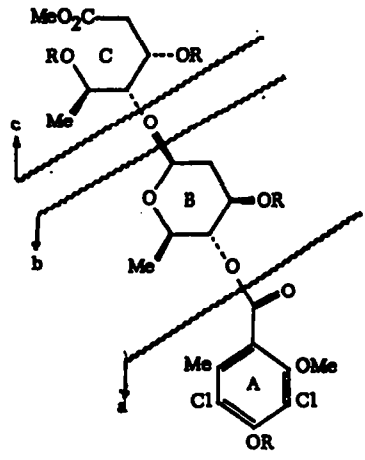
Scheme 4. Complementary mass spectral fragmentation of flambabiose penta-acetate (1), flambatriose acetates (3 and 6) and flambatetrose derivatives (8–14).



		R^1=	R^2=	a m/e	b m/e	b* m/e	c m/e	$M^{+\cdot}$	$M^{+\cdot}-H_2O$
(19)	Flambalactone	H	H	233	363	362	129	508	490
(20)	Flambalactone methyl ether	H	Me	247	377	376	129	522	504
(21)	Flambalactone triacetate	Ac	Ac	275	447	446	171	634	

Cleavage b* = Cleavage b with loss of one hydrogen atom

Scheme 5. Mass spectra fragmentation of flambalactone (19) and its derivatives (20 and 21).



	R =	a m/e	b m/e	b* m/e	c m/e	$M^{+\cdot}$	$M^{+\cdot} - H_2O$
(22) Methyl flambate	H	233	363	362		540	522
(23) Methyl flambate tetra-acetate	Ac	275	447	446	245		

Cleavage b* = Cleavage b with loss of one hydrogen

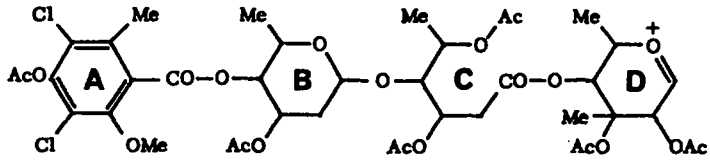
Scheme 6. Mass spectral fragmentation of methyl flambate (22) and its tetra-acetate (23).

dence already discussed in relation to Scheme 3 located this ortho-ester grouping between C-46, C-47 and C-53. An additional argument for the location of this ortho-ester group was also given in a preliminary communication[2d] and in Part I (Section 8 and Scheme 1).[1]

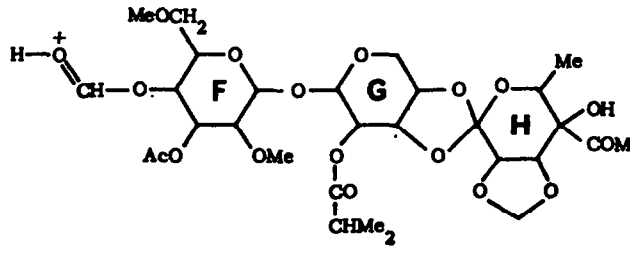
Spectra of flambamycin (44), *flambeurekanose flambate* (48) *and their derivatives* (45–47, 49–51). The mass spectra of these compounds (Schemes 9 and 10) when initially obtained were not very encouraging. Parent peaks were not observed for flambamycin (M = 1418). Significant fragment ions of high mass were observed and characterised by high resolution measurements for flambamycin (*m/e* 637) and its hexa-acetate (*m/e* 679). In the flambeurekanose flambate series, the peaks of highest mass which were observed were flambeurekanose flambate (*m/e* 567), flambeurekanose flambate isobutyrate (*m/e* 637) and flambeurekanose flambate isobutyrate hepta-acetate (*m/e* 679). However, in spite of these initially discouraging results, we persevered and eventually we were fortunate to observe two major fragment ions (*m/e* 921 and *m/e* 679) in the mass spectrum of flambeurekanose flambate isobutyrate octa-acetate (51). The satisfying correlations summarised in Schemes 9 and 10 finally emerged. Comparison of Schemes 9 and 10 with the fragmentation patterns of Schemes 1, 2, 3, 5, 6 and 8 clearly demonstrated that flambamycin and its derivatives (Scheme 9) produced mass spectra associated with cleavages a, b, c, d, f, j and k. Fragment ions associated with the dual cleavages [c + d] and [d + e] were also assignable.

Corresponding cleavages a, b, d, e, f, j and k as well as [f + l] were also discernible in the mass spectra of flambeurekanose flambate and its derivatives (Scheme 10). The most important result was provided by the mass spectrum of flambeurekanose flambate isobutyrate octa-acetate (51; M = 1774). Cleavage e gives the cation (52; *m/e* 921) associated with the sequence A–B–C–D. Cleavage f gives the cation (53; *m/e* 679) associated with the sequence F–G–H.

These two fragment ions (52 and 53) account for all the



(52)
m/e 921



(53)
m/e 679

component C, O and Cl atoms of flambamycin ($C_{61}H_{86}O_{33}Cl_2$) and flambeurekanose flambate isobutyrate ($C_{61}H_{86}O_{34}Cl_2$) with the exception of the residue, C_6O_3, associated with C-30, C-31, C-32, C-33, C-34 and C-35 of the 4-O-methyl-D-fucose residue E. Fortunately residue E is adequately included in the mass spectra summarised in Schemes 2, 3 and 8.

In conclusion, it may be noted that there is an overlap of information in the Schemes 1–10 which defines the sequence A–B–C–D–E–F–G–H in flambamycin (54) as well as the location of all its component C atoms with their substituents.

Epilogue. Flambamycin (54) contains the following oxygen-containing functions which are potentially reactive towards basic or acidic reagents. These include one aromatic ester, one aliphatic ester, two ortho-esters, one methylene acetal and acetals associated with the hexose residues, α-hydroxy methyl ketone, one phenolic OH group and five secondary OH groups.

It was therefore appreciated during the base catalysed and acid catalysed transformations described in Part I that opportunities for various transformations existed. These possible transformations included, for example, (a) hydrolytic cleavage of an ortho-ester and reformation of a different ortho-ester, (b) O-O-acyl migration, (c) interconversion between acetals. These circumstances provided the possibilities that there were not direct structural correlations between precursors and products in some of the following transformations:

(i) Flambamycin → Flambeurekanose

(ii) Flambamycin → Methyl flambate

(iii) Flambamycin → Flambeurekanose flambate isobutyrate

(iv) Flambamycin → Des-isobutyroyl flambamycin

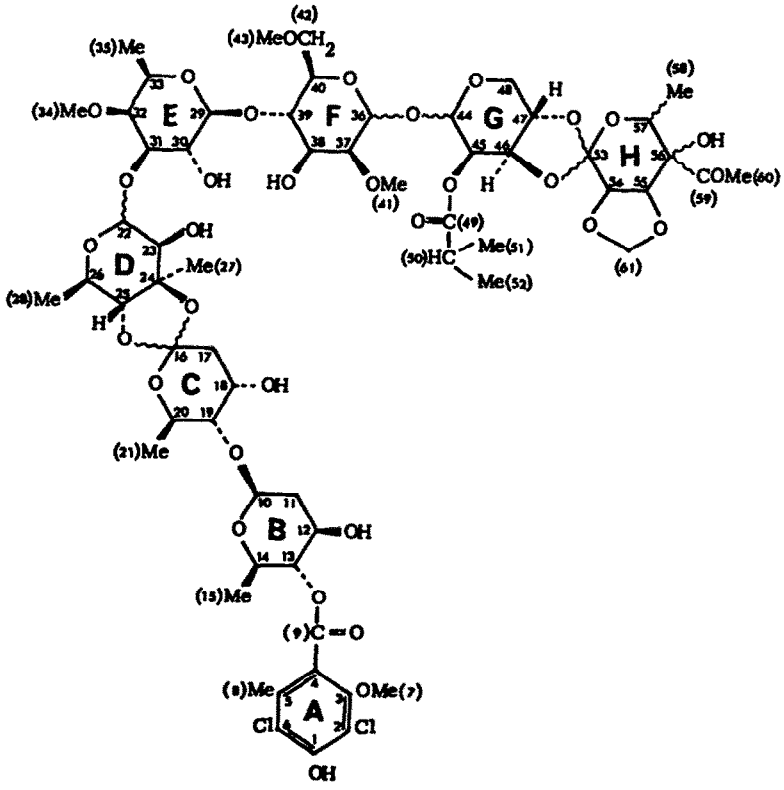
(v) Base catalysed O-methylation of the above transformation products

(vi) Base catalysed or acid catalysed acetylation of the above transformation products.

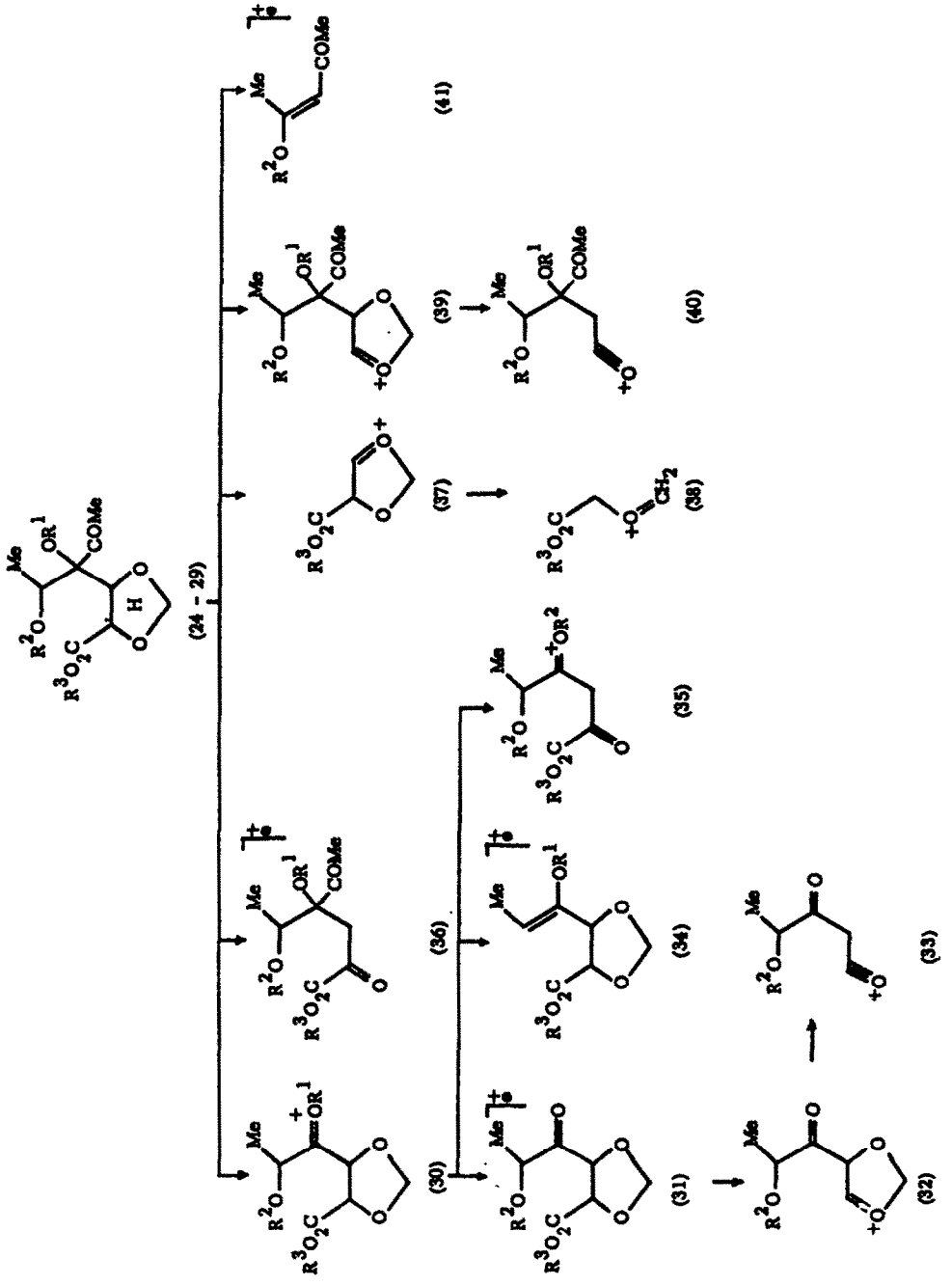
Firm structural evidence has been provided in Parts I, II and III for the constitutions of flambic acid (A–B–C), flambatetrose isobutyrate (D–E–F–G), eurekanic acid (H). Their empirical relation to flambamycin is summarised by Scheme 11.

It has been firmly established that (i) the phenolic OH group at C-1 and the α-hydroxy CO group at C-56 exist as such in flambamycin and its transformation products and (ii) the isobutyroyloxy group is located at C-45 in flambamycin. Thus, in the derivation of all possible structures for flambamycin as depicted in Scheme 11, one is concerned with the creation of *two* ortho-ester groupings by the elimination of $4H_2O$ from the *two* carb :yl groups (C-16 and C-53) of flambic acid and eurekanic acid respectively and the *eleven* alcoholic OH groups located at C-12, C-18, C-20, C-23, C-24, C-25, C-30, C-38, C-46, C-47 and C-57. There are *twenty* different structures containing *two* ortho-esters groupings which can, in principle, be generated by the elimination of $4H_2O$ between *two* different carboxyl groups and *six* different OH groups. The initial decision which therefore has to be made is which *six* of the *eleven* positions bearing O atoms are involved in the *two* ortho-ester groups containing C-16 and C-53. Evidence for the following triads is as follows:

(i) H–C_{12}–OH. This is established by the constitution of curacin (Part I, Section 2), the assigned ^{13}C chemical shifts to C-11 in flambamycin and its derivatives (Part II,



(54) Flambamycin


- 23 -
(24 - 29)
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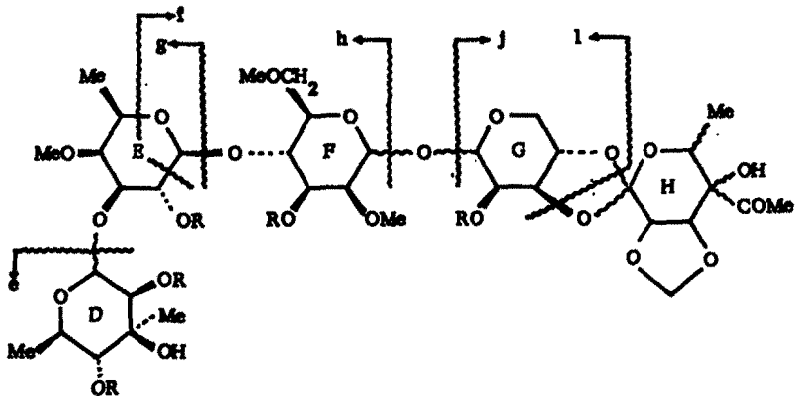
(41)

	R^1=	R^2=	R^3=	$M^{+\cdot}$ m/e	(30) m/e	(31) m/e	(32) m/e	(33) m/e	(34) m/e	(35) m/e	(36) m/e	(37) m/e	(38) m/e	(39) m/e	(40) m/e	(41) m/e
(24) Methyl eurekanate	H	H	Me	248	205	204	145	115	187	175	218	131	103		159	100
(25) Trideuteriomethyl eurekanate	H	H	CD_3		208	207	145	115	190	178		134	106		159	100
(26) Ethyl eurekanate	H	H	Et		219	218	145	115	201	189		145	117	189	159	
(27) Methyl eurekanate monoacetate	H	Ac	Me	332	247		187	157	187			131	103	231	201	
(28) Methyl eurekanate diacetate	Ac	Ac	Me		289		187	157	229			131	103	273		
(29) Eurekanic acid diacetate	Ac	Ac	H	318			187		215					273		

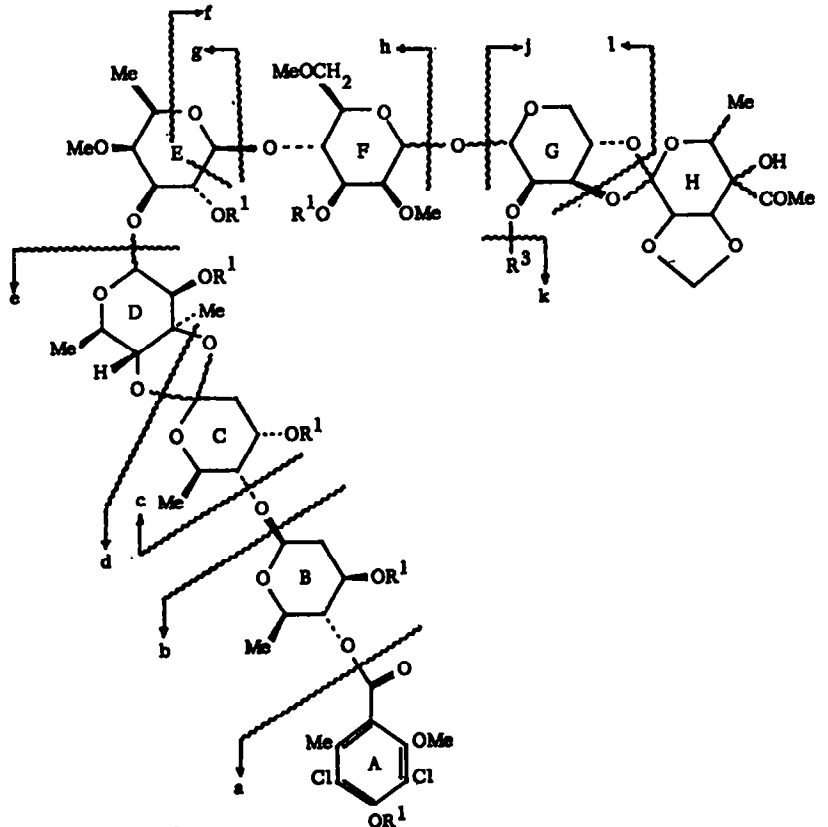
Scheme 7. Mass spectral fragmentation of methyl eurekanate (24) and its derivatives (25–29).



	R =	e m/e	f* m/e	g m/e	h m/e	j m/e	[j+l] m/e	$M^{+\cdot}$ m/e
(42) Flambeurekanose	H	161	567			331	115	
(43) Flambeurekanose penta-acetate	Ac	245		447	679	373	157	1068

Cleavage f* = Cleavage f with gain of one hydrogen atom

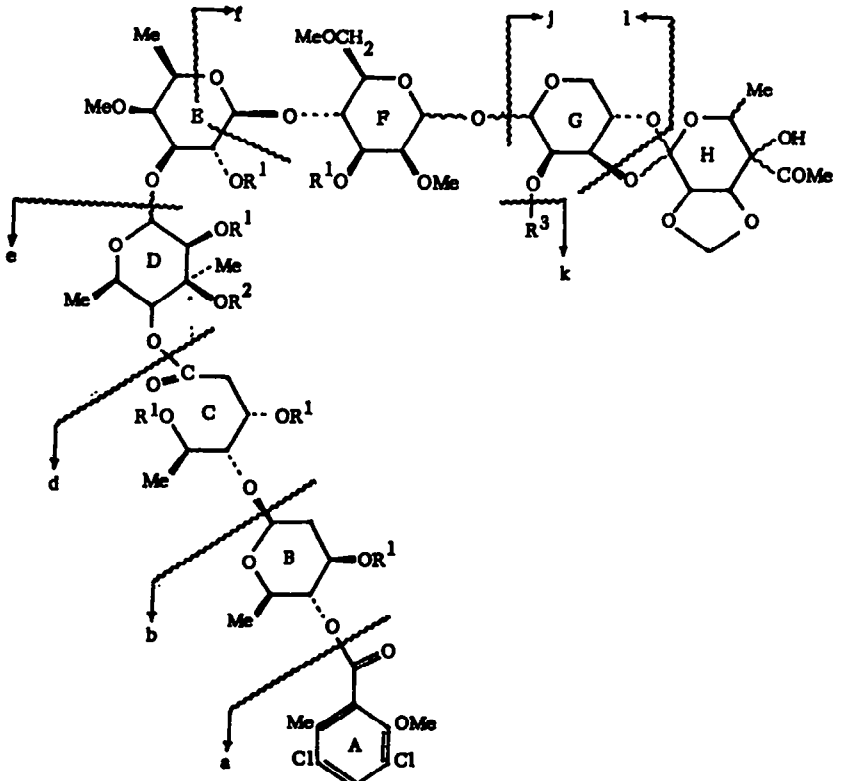
Scheme 8. Mass spectral fragmentation of flambeurekanose (42) and its penta-acetate (43).



	R^1=	R^2=	a m/e	b m/e	b* m/e	[c+d] m/e	d m/e	[d+e] m/e	f* m/e	j m/e	k m/e	[j+l] m/e
(44) Flambamycin	H	$COCHMe_2$	233	363	362	129	508	143	637*	401	71	185
(45) Flambamycin hexa-acetate	Ac	$COCHMe_2$	275	447		171	634	185	679*	401	71	185
(46) Des-isobutyroyl flambamycin	H	H	233	363	362	129		143	567*	331	43	115
(47) Des-isobutyroyl flambamycin hepta-acetate	Ac	Ac	275	447		171		135		373	43	157

Cleavage b* = Cleavage b with loss of one hydrogen atom
Cleavage f* = Cleavage f with gain of one hydrogen atom

Scheme 9. Mass spectra fragmentation of flambamycin (44) and its derivatives (45–47).

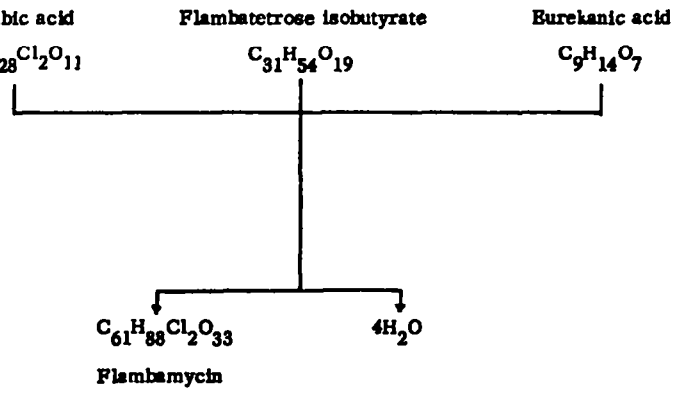


	R¹=	R²	R³=	a m/e	b m/e	b* m/e	d m/e	e m/e	f* m/e	j m/e	k m/e	[f+1]* m/e
(48) Flambeurekanose flambate	H	H	H	233	363	362	508		567*	331		351
(49) Flambeurekanose flambate isobutyrate	H	H	$COCHMe_2$	233	363	362	508		637*	401	71	421
(50) Flambeurekanose flambate isobutyrate hepta-acetate	Ac	H	$COCHMe_2$	275	447				679*	401	71	463
(51) Flameurekanose flambate isobutyrate octa-acetate	Ac	Ac	$COCHMe_2$	275	447			921	679*	401	71	463

Cleavage b* = Cleavage b with loss of one hydrogen atom

Cleavage f* = Cleavage f with gain of one hydrogen atom Cleavage [f+1]* = Cleavage [f+1] with gain of one hydrogen atom

Scheme 10. Mass spectral fragmentation of flambeurekanose flambate (48) and its derivatives (49–51).



Scheme 11.

Table), and the mass spectral cleavage b (Schemes 9 and 10).

(ii) H–C_{18}–OH. This is established by the constitution of flambalactone (19) and methyl flambate (22), the assigned ^{13}C chemical shifts to C-17 in flambamycin and its derivatives (Part II, Table), and by the mass spectral cleavages d and [c + d] (Scheme 9).

(iii) H–C_{23}–OH. This is established by the isolation of methyl 2-O-methyl-D-evalopyranoside (Part I, Section 4), the ^{13}C chemical shift of C-22 in flambamycin and its derivatives (Part II, Table), and by the mass spectral cleavages d and [d + e] (Scheme 9).

(iv) H–C_{30}–OH. This is established by the isolation of methyl 2,4-di-O-methyl-D-fucopyranoside (Part I, Section 4) and the ^{13}C chemical shift of C_{29} in flambamycin hexa-acetate (Part II, Table).

(v) H–C_{38}–OH. This is established by the isolation of methyl 2,3,6-tri-O-methyl-D-mannopyranoside (Part I, Section 4) and the mass spectral cleavage f (Scheme 9).

This evidence (i–v) excludes the involvement of C-12, C-18, C-23, C-30 and C-38 in the ortho-ester groups. Therefore the ortho-ester C atoms (C-16 and C-53) must be linked via O atoms to the remaining *six* positions involving C-20, C-24, C-25, C-46, C-47 and C-57. In flambeurekanose flambate (48) and its derivatives (49–51) it is firmly established that C-16 is linked to C-25 by an ester group. Furthermore, the second ortho-ester must involve ethereal linkage of C-53 to C-46, C-47 and C-57. This is fully confirmed by the methylation studies (Part I, Section 4) and mass spectral behaviour (Schemes 9 and 10; cleavage j).

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