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# THE ORTHOSOMYCIN FAMILY OF ANTIBIOTICS—II

## THE $^{13}C$ NMR SPECTRA OF FLAMBAMYCIN AND ITS DERIVATIVES

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**Abstract**—The important features of the $^{13}C$ NMR spectra of flambamycin (1a) and its degradation products (2–12) are discussed, and assignments are made. The general use of $^{13}C$ NMR spectroscopy for structural investigations of the orthosomycin family of antibiotics is summarised.

The antibiotic flambamycin[1,2] (1a) belongs to a new class of natural product called the orthosomycins.[3] The orthosomycins are characterised by the presence of one or more of the following common structural features: (i) one or two ortho-ester groupings, (ii) one ester group derived from dichloro-iso-everninic acid, and (iii) an oligosaccharide residue derived from a variety of unusual hexoses or diamino-hexitols. Sixteen orthosomycins are known,[3] of which the constitutions of thirteen have been elucidated: knowledge of the absolute configuration at a number of chiral centres is still incomplete.

The structurally characterised orthosomycins include the Destomycins-A,[4,5] -B[5] and -C,[5] Antibiotic A-396-1,[6] Hygromycin-B,[7,8] Antibiotic SS-56-C,[9] the Everninomicins-B,[10,11] -C,[10,12] -D[10,13] and -2,[14] and the Avilamycins-A and -C.[15] The chemical evidence which has been published on curamycin[16] and the sporocuracins-A and -B[17] indicates that these three antibiotics also belong to the orthosomycin family. However, as yet the constitutions of these three compounds have not been fully elucidated.

The importance of the use of $^{13}C$ NMR spectroscopy in the structural elucidation of complex natural products is now fully recognised. However, the investigation in 1970 of Hygromycin-B by this technique must be regarded as a pioneering achievement.[8] Subsequently other orthosomycins have provided interesting applications of the $^{13}C$ NMR spectral properties of carbohydrates[18] and ortho-esters. In particular, the diagnostic value of the $^{13}C$ chemical shift ($\delta \sim 120$ ppm with respect to tetramethylsilane) of ortho-esters is well exemplified by Hygromycin-B ($\delta$ 120.6 ppm),[8] Destomycin-A ($\delta$ 121.2 ppm),[5] Destomycin-B ($\delta$ 121.7 ppm),[5] Destomycin-C ($\delta$ 121.2 ppm),[5] Everninomicin-D ($\delta$ 120.0 and 119.7 ppm),[13] Everninomicin-2 ($\delta$ 120.0 and 119.6 ppm),[14] Avilamycin-A ($\delta$ 120.2 and 118.9 ppm),[15] and Avilamycin-C ($\delta$ 120.2 and 118.8 ppm).[15]

We now discuss the $^{13}C$ NMR spectra of flambamycin (1a), its hexaacetate (1b), and the degradation products (2–12) whose origins have already been described in Part I.[2a] Some $^{13}C$ NMR data have already been briefly reported in Part I. In this paper much additional information is presented in a form (Table 1) which emphasises how the assignment of individual signals to particular C atoms is possible.

The 61 C atoms present in flambamycin (1a) have been arbitrarily designated by numbers, and the labels A, B, C, D, E, F, G and H refer to the eight major residues. Corresponding locants (numbers) and residues (letters) are used in the formulae of curacin (2a, A-B), flambalactone (3, A-B-C), flambabiose (4, F-G), flambatriose (5a, E-F-G), methyl-D-evalopyranoside (6a, D), flambatetrose (7a, D-E-F-G), methyl eurekanate (8, H), flambeurekanose (9a, D-E-F-G-H), des-isobutyroyl flambamycin (10a, A-B-C-D-E-F-G-H), des-dichloro-iso-everninoyl des-isobutyroyl flambamycin (10c, B-C-D-E-F-G-H), flambeurekanose flambate (11a, A-B-C-D-E-F-G-H), and methyl dichloro-iso-everninate (12, A).

The $^{13}C$ NMR spectra of flambamycin (1a) and its degradation products (2–12) are correlated in Table 1. Assignments are listed in relation to the types of C atoms present in the groupings (i)–(x).

(i) *C-Methyl groups.* The spectrum of flambamycin (1a) shows signals attributable to ten C-methyl groups (C-58, $\delta$ 14.3; C-35, $\delta$ 16.3; C-27, $\delta$ 17.8; C-8, $\delta$ 17.8; C-28, 18.( , C-15, $\delta$ 18.6; C-21, $\delta$ 18.8; C-51, $\delta$ 19.2; C-52, $\delta$ 19.3; and C-60, $\delta$ 27.5). Corresponding signals are shown (Table 1) when these C-methyl groups are present in the various degradation products (2, 3, and 5–11).

(ii) *Methine group.* The methine carbon, C-50, of the isobutyroyloxy group is readily assignable in flambamycin (1a, $\delta$ 34.1) and in the degradation products (5b, $\delta$ 34.3) and (11b, $\delta$ 34.1).

(iii) *Methylene groups.* The $CH_2$ groups of the rhamnose residue B (C-11) and the 3,4,5-trihydroxyhexanoic acid residue C (C-17) are readily recognised on the basis of their $^{13}C$ resonances at $\delta$ 41.4 (C-11) and $\delta$ 41.1 (C-17) in the NMR spectrum of flambamycin (1a). In flambamycin hexa-acetate (1b), both these signals are shifted upfield (C-11, $\delta$ 38.8; C-17, $\delta$ 37.3) when the OH groups on adjacent C atoms (C-12 and C-18) are acetylated. Analogous upfield shifts are observed (Table 1) on comparing the $^{13}C$ NMR spectra of des-isobutyroyl flambamycin (10a) with its corresponding hepta-acetate (10b) and flambeurekanose flambate (11a) with its corresponding nona-acetate (11c). Signals assignable to the C-11 resonance are present (Table 1) in the spectra of all

Table 1. The assignment of $^{13}C$ resonances for flambamycin

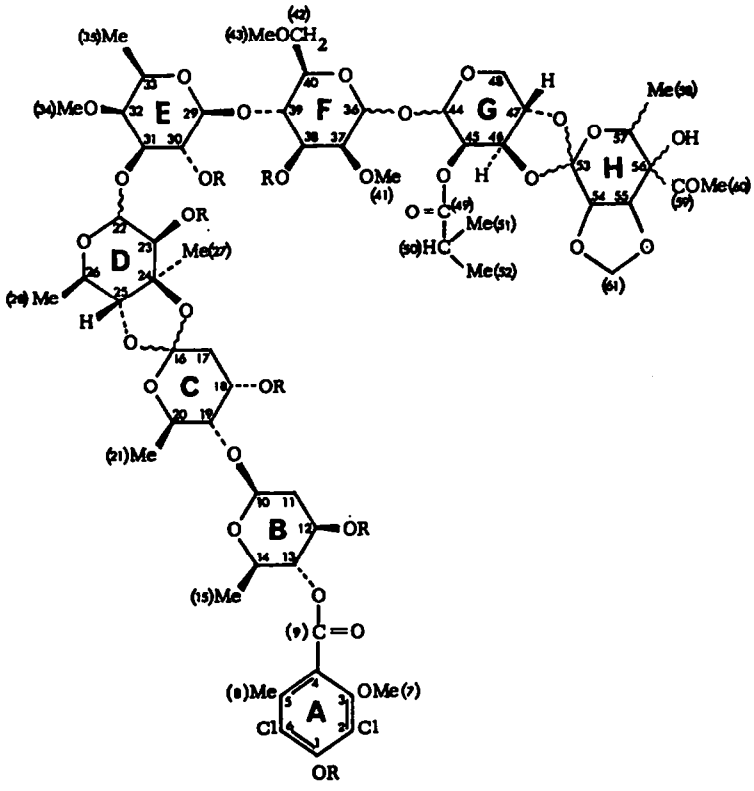
| Functionality | Assignment | 1a | 1b | 2a | 2b | 2c | 3 | 4 | 5a | 5b | 5c |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (i) C-Methyl groups $CH_3-C$ | C-58 | 14.3 | 14.3 | | | | | | | | |
| | C-35 | 16.3 | 16.7 | | | | | | 16.6 | 16.6 | 16.6 |
| | C-27 | 17.8 | 17.3 | | | | | | | | |
| | C-8 | 17.8 | 18.0 | 18.0 | 17.9 | 17.3 | 17.9 | | | | |
| | C-28 | 18.0 | 18.6 | | | | | | | | |
| | C-15 | 18.6 | 18.6 | 18.6 | 18.2 | 17.9 | 18.0 | | | | |
| | C-21 | 18.8 | 19.2 | | | | 19.3 | | | | |
| | C-51 | 19.2 | 20.0 | | | | | | | 19.1 | |
| | C-52 | 19.3 | 20.5 | | | | | | | 19.1 | |
| | C-60 | 27.5 | 27.7 | | | | | | | | |
| (ii) Methine group $-CH$ | C-50 | 34.1 | 34.2 | | | | | | | 34.3 | |
| (iii) Methylene groups $-CH_2-$ | C-11 | 41.4 | 38.8 | 41.1 | 39.8 | 35.7 | 41.3 | | | | |
| | C-17 | 41.1 | 37.3 | | | | 37.2 | | | | |
| (iv) Methoxy groups $CH_3O-$ | C-43 | 59.0 | 59.2 | | | | | 59.0 | 58.9 | 58.9 | 60.9 |
| | C-41 | 61.8 | 60.9 | | | | | 61.5 | 61.6 | 61.6 | 61.1 |
| | C-34 | 61.8 | 61.4 | | | | | | 61.8 | 62.0 | 61.7 |
| | C-7 | 62.1 | 62.5 | 62.2 | 62.2 | 62.5 | 62.1 | | | | |
| (v) Methinoxy groups CH –O | C-12 | | | 66.6 | 66.5 | 69.8 | 68.9 | | | | |
| | C-13 | | | 81.2 | 80.5 | 76.3 | 83.8 | | | | |
| | C-14 | | | 66.1 | 66.5 | 66.0 | 68.7 | | | | |
| | C-18 | | | | | | 76.3 | | | | |
| | C-19 | | | | | | 70.8 | | | | |
| | C-20 | | | | | | 79.6 | | | | |
| | C-23 | | | | | | | | | | |
| | C-24 | | | | | | | | | | |
| | C-25 | | | | | | | | | | |
| | C-26 | | | | | | | | | | |
| | C-30 | | | | | | | | | | |
| | C-31 | | | | | | | | | | |
| | C-32 | | | | | | | | | | |
| | C-33 | | | | | | | | | | |
| | C-37 | | | | | | | | | | |
| | C-38 | | | | | | | | | | |
| | C-39 | | | | | | | | | | |
| | C-40 | | | | | | | | | | |
| | C-42 | | | | | | | | | | |
| | C-45 | | | | | | | | | | |
| | C-46 | | | | | | | | | | |
| | C-47 | | | | | | | | | | |
| | C-48 | | | | | | | 64.6 | 65.1 | 65.0 | |
| (vi) Methylenedioxy group $O-CH_2-O$ | C-61 | 96.9 | 97.0 | | | | | | | | |
| (vii) Glycosidic carbon atoms | C-44 | 95.3 | 95.0 | | | | | 98.2 | 98.9 | {98.4 / 95.7} | 94.7 |
| O–CH–O | C-36 | 96.6 | 95.6 | | | | | 97.1 | 96.9 | 96.6 | 95.6 |
| | C-10 | 101.2 | 100.1 | 92.0 | 98.1 | 98.9 | 101.2 | | | | |
| | C-22 | 102.7 | 100.1 | | | | | | | | |
| | C-29 | 105.2 | 101.7 | | | | | | 105.5 | 105.3 | 101.7 |
| (viii) Ortho-ester carbon atoms | C-53 | 119.8 | 119.9 | | | | | | | | |
| $-C(O)(O)O$ | C-16 | 120.9 | 120.4 | | | | 170.3* | | | | |
| (ix) Aromatic carbon atoms | C-2 | 115.0 | 125.8 | 115.1 | 115.1 | 125.4 | 115.1 | | | | |
| | C-4 | 119.8 | 121.2 | 119.8 | 119.9 | 119.8 | 119.8 | | | | |
| | C-6 | a | 129.2 | 123.2 | a | 129.3 | 122.9 | | | | |
| | C-5 | 133.4 | 133.5 | 133.5 | 133.6 | 133.6 | 133.5 | | | | |
| | C-3 | 152.8 | 152.4 | 152.8 | 152.9 | 152.6 | 152.8 | | | | |
| | C-1 | 153.1 | 146.2 | 152.9 | 153.1 | 146.0 | 153.1 | | | | |
| (x) Carbonyl carbon atoms C=O | C-9 | 166.7 | 167.3 | 166.9 | 166.9 | 167.3 | 166.8 | | | | |
| | C-49 | 175.1 | 175.2 | | | | | | | {176.2 / 176.8} | |
| | C-59 | 210.3 | 210.5 | | | | | | | | |

[a = signal]

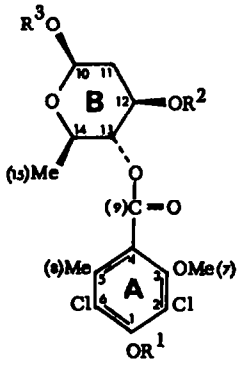
*These signals refer to normal ester carbonyl groups produced by ortho-ester hydrolysis.

(1a) and its degradation products (2–12)

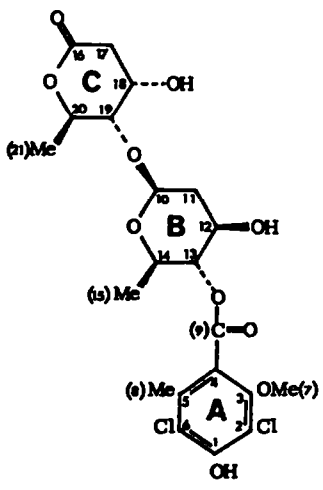
| 6a | 6b | 7a | 7b | 8 | 9a | 9b | 10a | 10b | 10c | 11a | 11b | 11c | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 14.2 | 14.3 | 14.3 | 14.3 | 14.3 | 14.3 | 14.3 | 14.3 | |
| | | 16.4 | 16.7 | | 16.3 | 16.7 | 16.4 | 16.7 | 16.3 | 16.3 | 16.3 | 16.2 | |
| 18.9 | 18.1 | 18.9 | 16.8 | 17.4 | 18.7 | 16.7 | 17.8 | 17.3 | 18.0 | 18.0 | 18.0 | 16.6 | |
| | | | | | | | 17.8 | 18.0 | | 18.0 | 18.0 | 17.8 | 17.7 |
| 20.0 | 20.8 | 19.1 | 18.1 | | 19.1 | 19.4 | 18.0 | 18.0 | 18.2 | 18.4 | 18.6 | 18.4 | |
| | | | | | | | 18.8 | 18.7 | 18.9 | 19.1 | 18.6 | 18.6 | |
| | | | | | | | 19.4 | 18.7 | 19.4 | 20.5 | 20.6 | 17.2 | |
| | | | | | | | | | | | 19.2 | | |
| | | | | | | | | | | | 18.8 | | |
| | | | | 26.1 | 27.6 | 27.6 | 27.7 | 27.6 | 27.7 | 27.7 | 27.7 | 27.6 | |
| | | | | | | | | | | | 34.1 | | |
| | | | | | | | 41.5 | 38.7 | 41.5 | 41.4 | 41.4 | 37.2 | |
| | | | | | | | 41.1 | 37.3 | 40.6 | 40.5 | 40.5 | 37.1 | |
| | | 59.0 | 59.2 | | 58.9 | 59.2 | 59.0 | 59.1 | 59.0 | 59.0 | 59.0 | 59.1 | |
| | | 61.5 | 60.8 | | 61.5 | 61.0 | 61.6 | 60.9 | 61.6 | 61.5 | 61.5 | 60.8 | |
| | | 61.8 | 61.4 | | 61.8 | 61.4 | 61.8 | 61.4 | 61.8 | 61.8 | 61.8 | 61.4 | |
| | | | | | | | 62.1 | 62.5 | | 62.1 | 62.2 | 62.5 | 62.1 |
| | | | | 95.9 | 96.9 | 97.0 | 96.6 | 96.9 | 96.9 | 96.6 | 97.0 | 97.0 | |
| | | 98.9 | 94.7 | | 98.7 | 95.6 | 98.7 | 95.0 | 98.7 | 98.7 | 95.2 | 95.4 | |
| | | 96.8 | 95.6 | | 96.7 | 95.9 | 96.6 | 95.6 | 96.6 | 96.6 | 96.6 | 95.5 | |
| | | | | | | | 101.3 | 100.0 | 101.6 | 101.0 | 101.0 | 100.1 | |
| 103.0 | 100.1 | 102.1 | 99.3 | | 102.0 | 100.0 | 102.8 | 100.0 | 102.8 | 102.0 | 102.1 | 100.1 | |
| | | 105.3 | 101.9 | | 105.1 | 101.9 | 105.2 | 101.7 | 105.2 | 105.2 | 105.3 | 101.9 | |
| | | | | 71.7* | 119.8 | 120.0 | 119.8 | 119.8 | 119.8 | 119.8 | 119.8 | 119.9 | |
| | | | | | | | 120.9 | 120.4 | 120.9 | 172.5* | 172.6* | 172.5* | |
| obscured by solvent ($C_5D_5N$)] | | | | | | | 115.0 | 125.4 | | 115.1 | 115.1 | 125.4 | 115.2 |
| | | | | | | | 119.8 | 121.1 | | 119.8 | 119.8 | 122.9 | 119.2 |
| | | | | | | | a | 123.5 | | a | 123.0 | 124.4 | a |
| | | | | | | | 133.5 | 133.5 | | 133.5 | 133.6 | 133.5 | 133.2 |
| | | | | | | | 152.8 | 152.4 | | 153.0 | 152.8 | 152.0 | 153.1 |
| | | | | | | | 153.2 | 146.2 | | 153.0 | 153.1 | | 153.2 |
| | | | | | | | 166.8 | 167.3 | | 166.8 | 166.8 | 167.4 | 167.4 |
| | | | | | | | | | | | 175.1 | | |
| | | | | 207.2 | 210.8 | 210.5 | 210.8 | 210.8 | 210.8 | 210.8 | 210.8 | 210.8 | |



| | R |
|---|---|
| (1a) Flambamycin | H |
| (1b) Flambamycin hexa-acetate | Ac |



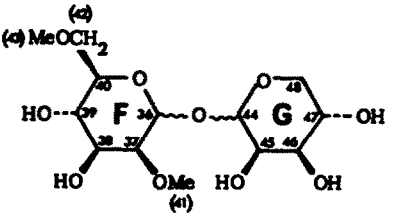
| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| (2a) Curacin | H | H | H |
| (2b) Curacin methyl glycoside | H | H | Me |
| (2c) Curacin methyl glycoside diacetate | Ac | Ac | Me |



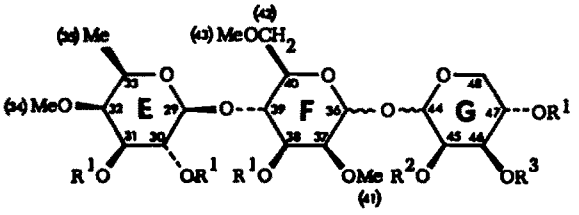
(3) Flambalactone

degradation products containing residue B. Upfield shifts for the signal assignable to the C-17 resonance are detectable in the spectrum of flambalactone (1, $\delta$ 37.2) in which residue C is accommodated in a $\delta$-lactone ring. The C-17 resonance is not significantly shifted in the spectra of flambeurekanose flambate (11a) (C-17, $\delta$ 40.5) and flambeurekanose flambate isobutyrate (11b) (C-17, $\delta$ 40.5) in which residue C is present as the acyclic 3,4,5-trihydroxyhexanoate ester grouping.

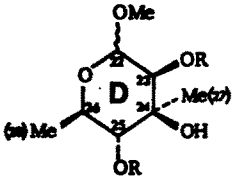
(iv) *Methoxy groups.* The three aliphatic methoxy C atoms C-43, C-41 and C-34 of flambamycin (1a) associated with residues E and F give signals at the high field end of the C–O region (C-43, $\delta$ 59.0; C-41, $\delta$ 61.8; and C-34, $\delta$ 61.8). These signals can be appropriately cor-



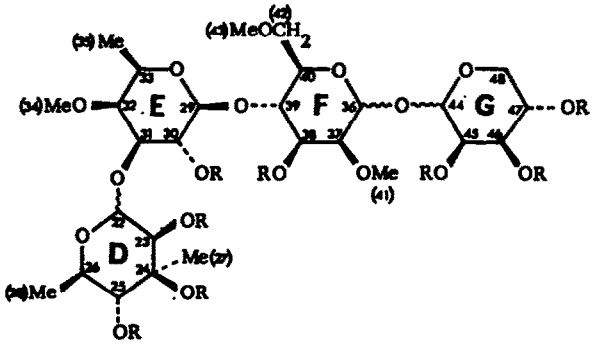
(4) Flambabiose



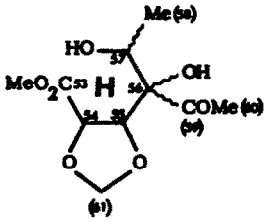
| | | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|
| (5a) | Flambatriose | H | H | H |
| (5b) | Flambatriose isobutyrate | H | $COCHMe_2$ | H |
| | | H | H | $COCHMe_2$ |
| (5c) | Flambatriose hexa-acetate | Ac | Ac | Ac |



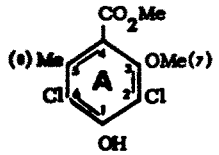
| | | R |
|---|---|---|
| (6a) | Methyl-D-evalopyranoside | H |
| (6b) | Methyl-2,4-di-O-acetyl-D-evalopyranoside | Ac |



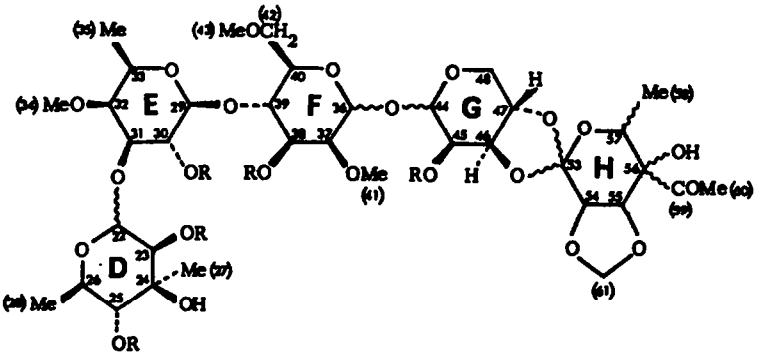
| | | R |
|---|---|---|
| (7a) | Flambatetrose | H |
| (7b) | Flambatetrose octa-acetate | Ac |



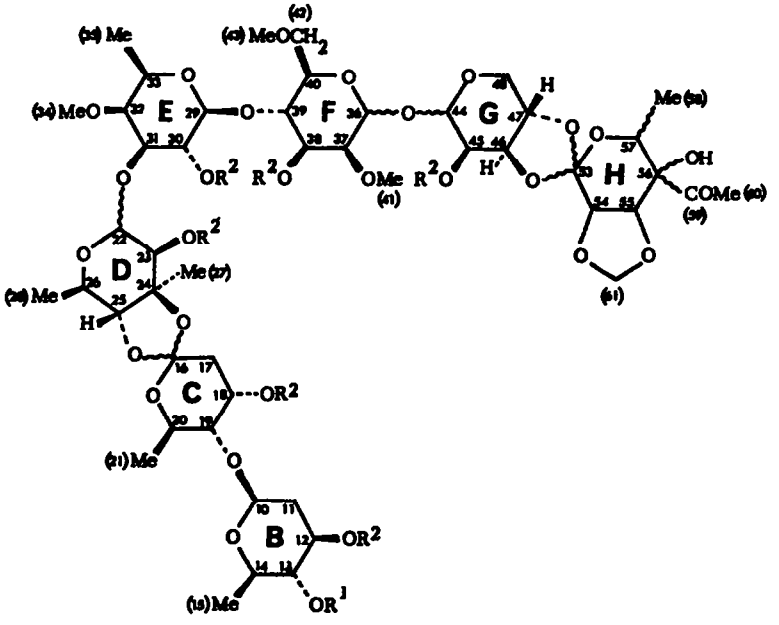
(8) Methyl eurekanate

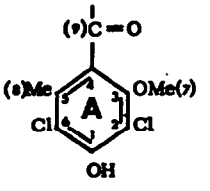
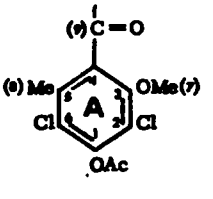


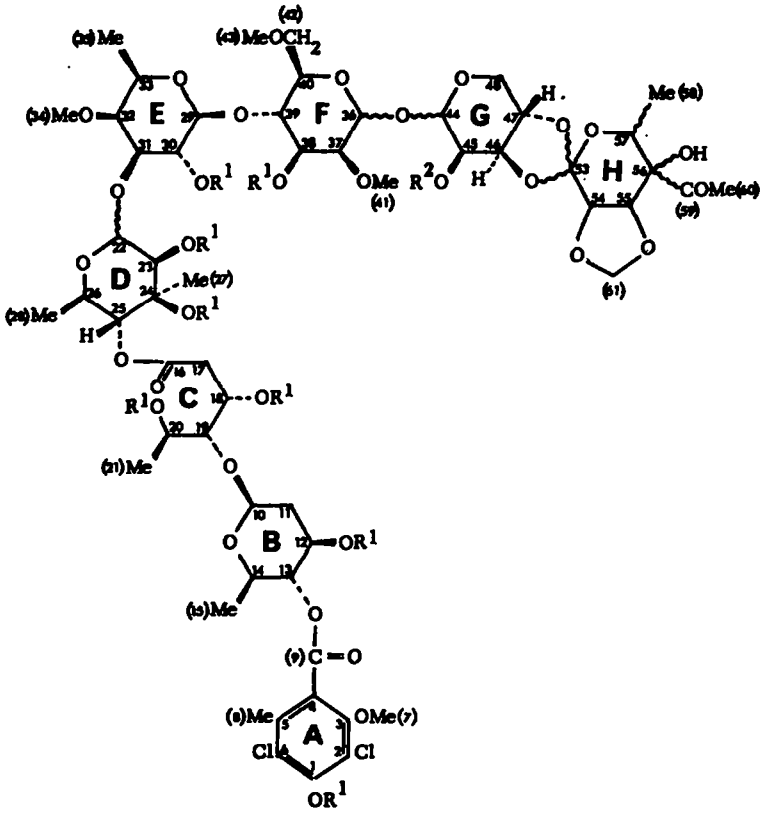
(12) Methyl dichloro-iso-everninate



| | | R |
|---|---|---|
| (9a) | Flambeurekanose | H |
| (9b) | Flambeurekanose penta-acetate | Ac |



| | | $R^1$ | $R^2$ |
|---|---|---|---|
| (10a) | Des-isobutyroyl flambamycin |  | H |
| (10b) | Des-isobutyroyl flambamycin hepta-acetate |  | Ac |
| (10c) | Des-dichloro-iso-everninoyl-des-isobutyroyl flambamycin | H | H |



| | | $R^1$ | $R^2$ |
|---|---|---|---|
| (11a) | Flambeurekanose flambate | H | H |
| (11b) | Flambeurekanose flambate isobutyrate | H | $COCHMe_2$ |
| (11c) | Flambeurekanose flambate nona-acetate | Ac | Ac |

related (Table 1) with corresponding signals in the $^{13}C$ NMR spectra of the degradation products (4), (5a–c), (7a–b), (9a–c), (10a–c) and (11a–c). The aromatic methoxy C atom (C-7, $\delta$ 62.1) of the dichloro-iso-everninoyl residue A of flambamycin (1a) is assignable (Table 1) in the $^{13}C$ NMR spectra of the degradation products (2a–b), (3), (10a–b) and (11a–c).

(v) *Methinoxy groups.* The C–O region of the $^{13}C$ NMR spectrum ($\delta$ 68–88) of flambamycin is very complex. It is therefore reassuring that twenty-three individual C signals are resolved in the spectrum of flambamycin (1a) which can be correlated with analogous signals in the degradation products (2)–(11). However, definite assignments to these twenty-three signals cannot be made.

(vi) *Methylenedioxy group.* The methylenedioxy C atom of flambamycin (1a) (C-61, $\delta$ 96.9) is readily identified in all compounds (8–11) containing the eurekanate residue H.

(vii) *Glycosidic carbon atoms.* The chemical shift of the $^{13}C$ resonance of the glycosidic C atom located at C-44 (residue G) of flambamycin (1a) is affected by the presence or absence of the isobutyroyloxy group at C-45. When the adjacent C atom, C-45, carries a free OH function as in the flambamycin degradation products (4, 5a, 7a, 9a, 10a, 10c and 11a), then the C-44 resonance lies in the range $\delta$ 98.2–98.9. However, when C-45 bears an isobutyroyloxy group (1a, 1b, 5b and 11b) or an acetoxy group (5c, 7b, 9b, 10b and 11c) then the C-44 resonance is shifted to lower frequency ($\delta$ 94.7–95.7). The remaining four glycosidic C atoms, C-10, C-22, C-29 and C-36 associated with residues B, D, E and F of flambamycin (1a) give signals in the expected region of the spectrum and are readily assigned (Table 1) by comparison of the spectrum of flambamycin (1a) with those of its degradation products (2b, 3, 4, 5a, 6a, 7a, 9a, 10a, 10c, 11a and 11b). Comparison of the $^{13}C$ NMR chemical shifts of the glycosidic C atoms C-22, C-29 and C-44 (Table 1) in flambamycin (1a) and its degradation products (5a, 6a, 7a, 9a, 10a and 11a) with those in the corresponding acetylated derivatives (1b, 5c, 6b, 7b, 9b, 10b and 11c) showed upfield shifts (C-22, $\delta \sim 102 \rightarrow 100$; C-29, $\delta \sim 105 \rightarrow 102$; C-44, $\delta \sim 98 \rightarrow 95$ ppm), which played an important role in establishing that ortho-ester migration was not taking place during either the degradative reactions or during the formation of derivatives. This upfield shift of the signals associated with glycosidic C atoms in carbohydrates when an OH group in position 2 is acylated is well documented.[28]

(viii) *Ortho-ester carbon atoms.* The ortho-ester groupings characteristic of the orthosomycin antibiotics,[3] have not been directly detected by any method other than $^{13}C$ NMR spectroscopy. The presence of these groupings, which are essential for the biological activity of these antibiotics,[3] can be readily recognised on the basis of the $^{13}C$ signals assignable to the ortho-ester C atoms C-16 and C-53 of flambamycin (1a) and its various degradation products (9a–b, 10a–c and 11a–c). The

resonance (δ 120.9) in the spectrum of flambamycin (1a) and its derivatives (10a and 10c) is assigned to the C-16 ortho-ester function associated with residue C. The absence of this signal (δ 120.9) in the spectra of flambeurekanose flambate (11a) and flambeurekanose flambate isobutyrate (11b) and its replacement by low field CO signals (11a, δ 172.5; 11b, δ 172.6) indicated very clearly that cleavage of one ortho-ester group involving C-16 to give a normal ester linkage had occurred in the formation of flambeurekanose flambate (11a) and flambeurekanose flambate isobutyrate (11b). Flambalactone (3) has a lactone CO resonance at δ 170.3 associated with C-16. The second ortho-ester group of flambamycin linking the eurekanate residue (H) and lyxose residue (G) also gives rise to a readily assignable $^{13}C$ signal, δ 119.8, associated with C-53. This signal coincides with a signal assignable to the aromatic C atom C-4 (δ 119.8–119.9) in the spectra of flambamycin (1a) and the degradation products (10a, 11a and 11b). Furthermore, this signal (δ 119.8) was clearly visible in the spectra of the degradation products (9a and 10c) which lacked the dichloro-iso-everninoyl residue A. Methyl eurekanate (8) has an ester CO residue (δ 171.7) associated with C-53.

(ix) *Aromatic carbon atoms.* Assignments for the six aromatic C atoms (Table 1) are based upon comparison of the $^{13}C$ NMR spectrum of flambamycin (1a) with the spectrum of the model compound, methyl dichloro-iso-everminate (12),[1] and upon the known effects of substituents on aromatic $^{13}C$ chemical shifts.

(x) *Carbonyl groups*—(a) *Ester carbonyl groups.* The two ester CO resonances (C-9, δ 166.7; C-49, δ 175.1) are found in the $^{13}C$ NMR spectra of compounds containing the dichloro-iso-everninoyl group (residue A) and the isobutyroyl group respectively (Table 1). The CO resonance of the isobutyroyl residue of flambatriose isobutyrate (5b) is observed as two signals of approximately equal intensity (δ 176.8 and 176.2). This observation, in association with the doubling of many other signals in the spectrum of 5b, is believed to be due to the presence of a mixture of isomers (see footnote, Part I, section 5[2a]).

(b) *Methyl ketone group.* The methyl ketone CO resonance of flambamycin (1a) associated with C-59 of the eurekanate residue (H) is readily recognised on the basis of a characteristic $^{13}C$ resonance (δ 210.3). All degradation products (8–11) containing the eurekanate residue (H) also show a corresponding signal associated with C-59 (Table 1).

Our access to a $^{13}C$ NMR spectrometer occurred only after most of the degradative work described in Part I[1,2a] had been completed. It was very reassuring to have this entirely independent confirmation of many structural features which had already been proposed on the basis of chemical degradation, $^1H$ NMR spectroscopy, and high and low resolution mass spectrometry (Part III[2b]). However, access to a $^{13}C$ NMR spectrometer convinced us that the structure which we originally proposed[1] for flambamycin was incorrect and it has now been replaced by the constitution (1a) put forward in Part I.[2a] The evidence for the relocation of the intermonosaccharide linkage between C-22 of the D-evalose residue (D) and C-31 of the 4-O-methyl-D-fucose residue (E) is presented in Part I (Section 4).[2a] Reference is there made to the way in which it was possible to use the characteristic upfield $^{13}C$ shift (δ ~ 105 → 100 ppm) of glycosidic C atoms when the OH group in position 2 of the glycoside residue is acetylated. This well-established phenomenon[18] is of diagnostic value in the structural investigation of mono-, di-, tri- and oligo-saccharides and it has also been extremely useful in the structural elucidation of flambamycin (1a).

The sixteen compounds (2a–c, 3, 4, 5a, 5b, 6a, 7a, 8, 9a, 9b, 10c, 11b, 11c and 12) gave resolved $^{13}C$ resonances (Experimental) which equalled the number of C atoms in their molecular formulae. This was very satisfying. For the other eight compounds, (1a, 1b, 5c, 6b, 7b, 10a, 10b and 11a), some coincidences of signals occurred. This is to be expected and did not detract in any way from the excellent overall concordance between the $^{13}C$ spectra of twenty-four compounds (Table 1).

## EXPERIMENTAL

$^{13}C$ NMR spectra were recorded using a JEOL PFT-100 spectrometer at 25.15 MHz using a sweep width of 6.25 KHz, 8 k data point in the time domain and a pulse angle of *ca.* 30°. Chemical shifts are reported on the δ scale from TMS (increasing ppm corresponding to increasing frequency) and are accurate to ±0.1 ppm. Unless otherwise stated, samples were measured in $d_5$-pyridine containing 5% TMS as internal reference. The $^{13}C$ resonances of flambamycin and its derivatives are listed.

*Flambamycin* (1a). 210.3, 175.1, 166.7, 153.1, 152.8, 133.4, 120.9, 119.8, 119.8, 115.0, 105.2, 102.7, 101.2, 96.9, 96.6, 95.3, 87.9, 83.5, 83.1, 81.8, 81.4, 80.5, 80.5, 79.3, 79.3, 79.3, 79.3, 76.0, 75.1, 73.7, 73.3, 73.1, 71.4, 71.2, 70.8, 70.8, 70.5, 70.5, 70.0, 68.9, 68.7, 68.7, 63.5, 62.1, 61.8, 61.8, 59.0, 41.4, 41.1, 34.1, 27.5, 19.3, 19.2, 18.8, 18.6, 18.0, 17.8, 17.8, 16.3, 14.3.

*Flambamycin hexa-acetate* (1b). 210.5, 175.2, 170.1, 170.1, 170.1, 169.9, 169.2, 167.3, 165.2, 152.4, 146.2, 133.5, 129.2, 125.3, 121.2, 120.4, 119.9, 101.7, 100.1, 100.1, 97.0, 95.6, 95.0, 83.2, 81.8, 81.2, 81.2, 80.6, 80.0, 79.2, 78.3, 75.8, 75.2, 74.1, 73.2, 73.0, 73.0, 71.2, 71.2, 70.5, 70.1, 70.1, 68.7, 63.4, 62.5, 61.4, 60.9, 59.2, 38.8, 37.3, 34.2, 27.7, 21.2, 20.9, 20.5, 20.0, 19.2, 18.6, 18.6, 18.0, 17.3, 16.7, 14.3.

*Curacin* (2a). 166.9, 152.9, 152.8, 133.5, 123.2, 119.8, 115.1, 92.0, 81.2, 66.6, 66.1, 62.2, 41.1, 18.6, 18.0.

*Curacin methyl glycoside* (2b). 166.9, 153.1, 152.9, 133.6, 119.9, 115.1, 98.1, 80.5, 66.5, 66.5, 62.2, 54.6, 39.8, 18.2, 17.9.

*Curacin methyl glycoside diacetate* (2c). 170.1, 167.3, 165.3, 152.6, 146.0, 133.6, 129.3, 125.4, 119.8, 98.9, 76.3, 69.8, 66.0, 62.5, 54.7, 35.7, 21.0, 20.0, 17.9, 17.3.

*Flambalactone* (3). 170.3, 166.8, 153.1, 152.8, 133.5, 122.9, 119.8, 115.1, 101.2, 83.8, 79.6, 76.3, 70.8, 68.9, 68.7, 62.1, 41.3, 37.2, 19.3, 18.0, 17.9.

*Flambabiose* (4). 98.2, 97.1, 81.9, 77.4, 74.9, 72.9, 72.9, 71.5, 68.6, 67.8, 64.6, 61.5, 59.0.

*Flambatriose* (5a). 105.5, 98.9, 96.9, 82.3, 80.9, 79.0, 75.9, 75.4, 73.4, 73.0, 72.0, 71.6, 71.5, 68.2, 65.1, 62.1, 61.8, 61.6, 58.9, 16.6.

*Flambatriose isobutyrate* (5b).† (176.8, 176.2), 105.3 (98.4, 95.7), 96.6, 82.5 (80.8, 80.6), 78.9 (76.0, 75.8), 75.4, 75.4, 73.2 (72.8, 70.6), 71.9, 71.3, 71.3 (69.2, 68.0), 65.0, 62.0 (61.6, 61.4), 58.9, 34.3, 19.1, 19.1, 16.6.

*Flambatriose hexa-acetate* (5c). 170.2, 170.1, 101.7, 95.6, 94.7, 79.8, 78.2, 75.8, 74.7, 74.4, 74.3, 72.8, 71.4, 71.3, 71.1, 70.8, 70.6, 69.8, 69.2, 67.0, 61.7, 61.1, 60.9, 21.2, 20.7, 20.6, 16.6.

*Methyl-D-evalopyranoside* (6a). 103.0, 76.1, 76.1, 73.2, 68.5, 54.8, 20.0, 18.9.

*Methyl-D-evalopyranoside-2,4-diacetate* (6b). 100.1, 76.9, 76.8, 70.7, 66.5, 55.1, 20.8, 20.8, 20.8, 18.1.

*Flambatetrose* (7a). 105.3, 102.1, 98.9, 96.8, 83.0, 82.0, 80.9, 79.0, 76.5, 76.4, 76.0, 73.8, 73.4, 72.9, 72.2, 71.6, 71.6, 71.2, 71.0, 68.1, 65.1, 61.8, 61.5, 59.0, 19.1, 18.9, 16.4.

*Flambatetrose octa-acetate* (7b). 170.1, 170.1, 169.3, 168.9, 101.9, 99.3, 95.6, 94.7, 82.8, 81.2, 80.7, 78.1, 75.9, 74.3, 73.3, 72.9, 72.7, 71.9, 71.7, 71.0, 71.0, 70.6, 70.0, 69.8, 69.2, 68.8, 67.0, 61.4, 60.8, 59.2, 21.6, 21.2, 21.1, 20.6, 20.6, 20.6, 18.1, 16.8, 16.7.

†The signals listed in parentheses are doubled due to the presence of two isomers having the isobutyroyl grouping at C(45)–O and C(46)–O respectively.

*Methyl eurekanate* (8). 207.2, 171.7, 95.9, 84.2, 81.5, 74.6, 68.4, 52.8, 26.1, 17.4.

*Flambeurekanose* (9a). 210.8, 119.8, 105.1, 102.0, 98.7, 96.9, 96.7, 83.4, 82.9, 82.1, 82.0, 80.8, 80.5, 78.9, 76.3, 76.3, 75.8, 75.0, 73.7, 73.7, 73.2, 72.0, 71.3, 71.2, 71.0, 70.0, 69.5, 63.8, 61.8, 61.5, 58.9, 27.6, 19.1, 18.7, 16.3, 14.2.

*Flambeurekanose penta-acetate* (9b). 210.5, 170.4, 170.1, 170.1, 169.4, 169.2, 120.0, 101.9, 100.0, 97.0, 95.9, 95.6, 83.2, 82.3, 81.8, 80.9, 80.0, 79.9, 76.5, 76.5, 76.0, 75.2, 74.1, 73.4, 73.0, 73.0, 71.4, 70.9, 70.9, 70.5, 70.5, 70.0, 63.5, 61.4, 61.0, 59.2, 27.6, 21.1, 21.1, 21.0, 21.0, 20.5, 20.0, 19.4, 16.7, 14.3.

*Des-isobutyroyl flambamycin* (10a). 210.8, 166.8, 153.2, 152.8, 133.5, 120.9, 119.8, 119.8, 115.0, 105.2, 102.8, 101.3, 98.7, 96.6, 96.6, 87.9, 83.4, 83.4, 82.2, 81.8, 81.4, 80.8, 80.5, 79.4, 79.4, 78.9, 75.8, 75.1, 73.8, 73.8, 73.2, 71.1, 70.8, 70.8, 70.8, 70.8, 70.1, 69.4, 68.7, 68.7, 68.7, 68.8, 63.9, 62.1, 61.8, 61.6, 59.0, 41.5, 41.1, 27.7, 19.4, 18.8, 18.0, 17.8, 17.8, 16.4, 14.3.

*Des-isobutyroyl flambamycin hepta-acetate* (10b). 210.8, 170.1, 170.1, 170.1, 170.1, 169.4, 169.4, 167.3, 165.3, 152.4, 146.2, 133.5, 125.4, 123.5, 121.1, 120.4, 119.8, 101.7, 100.0, 100.0, 96.9, 95.6, 95.0, 83.2, 81.8, 81.1, 80.6, 80.0, 79.2, 78.7, 78.3, 75.8, 75.8, 75.2, 74.1, 73.2, 73.0, 73.0, 71.2, 70.4, 70.4, 70.4, 68.7, 63.5, 62.5, 61.4, 60.9, 59.1, 38.7, 37.3, 27.6, 21.2, 21.0, 21.0, 20.5, 20.5, 20.0, 18.7, 18.7, 18.0, 18.0, 17.3, 16.7, 14.3.

*Des-dichloro-iso-everninoyl des-isobutyroyl flambamycin* (10c). 210.8, 120.9, 119.8, 105.2, 102.8, 101.6, 98.7, 96.9, 96.6, 88.0, 83.5, 83.5, 82.2, 81.9, 81.4, 80.9, 80.8, 80.6, 79.3, 79.0, 77.8, 75.9, 75.1, 73.8, 73.8, 73.4, 73.4, 71.6, 71.2, 70.9, 70.7, 70.1, 69.5, 68.7, 68.6, 63.9, 61.8, 61.6, 59.0, 41.5, 40.6, 27.7, 19.4, 18.9, 18.2, 18.0, 16.4, 14.3.

*Flambeurekanose flambate* (11a). 210.8, 172.5, 166.8, 153.0, 153.0, 133.5, 119.8, 119.8, 115.1, 105.2, 102.0, 101.0, 98.7, 96.6, 96.6, 85.7, 83.4, 83.1, 82.1, 82.1, 80.8, 80.5, 79.9, 78.9, 77.2, 76.3, 75.8, 75.0, 73.7, 73.3, 72.7, 72.2, 71.2, 71.2, 71.2, 70.6, 70.0, 69.4, 69.1, 69.1, 67.6, 63.8, 62.1, 61.8, 61.5, 59.0, 41.4, 40.5, 27.7, 20.5, 19.1, 18.4, 18.0, 18.0, 16.3, 14.3.

*Flambeurekanose flambate isobutyrate* (11b). 210.8, 175.1, 172.6, 166.8, 153.1, 152.8, 133.6, 123.0, 119.8, 119.8, 115.1, 105.3, 102.1, 101.0, 97.0, 96.6, 95.2, 85.8, 83.2, 83.2, 81.9, 80.6, 80.2, 79.9, 79.1, 78.9, 77.2, 76.3, 76.1, 76.0, 75.1, 73.7, 73.2, 73.2, 72.7, 72.2, 71.2, 71.2, 70.6, 70.0, 69.1, 69.1, 67.6, 63.5, 62.2, 61.8, 61.5, 59.0, 41.4, 40.5, 34.1, 27.7, 20.6, 19.2, 18.8, 18.6, 18.6, 18.0, 18.0, 16.3, 14.3.

*Flambeurekanose flambate nona-acetate* (11c). 210.8, 172.5, 170.9, 170.9, 170.7, 170.5, 170.1, 170.1, 170.1, 170.1, 169.3, 167.4, 154.0, 152.0, 133.5, 125.4, 124.4, 124.1, 122.9, 119.9, 101.9, 100.1, 100.1, 97.0, 95.5, 95.4, 83.2, 82.4, 82.3, 81.6, 81.3, 80.7, 79.2, 78.9, 78.4, 76.9, 76.8, 76.4, 76.2, 75.9, 75.3, 74.0, 73.2, 72.8, 71.5, 71.1, 70.9, 70.6, 70.2, 70.0, 69.4, 63.5, 62.5, 61.4, 60.8, 59.1, 37.2, 37.1, 27.6, 21.0, 21.0, 21.0, 20.5, 19.9, 19.8, 19.2, 18.6, 18.4, 18.3, 17.8, 17.2, 16.7, 16.6, 16.2, 14.3.

*Methyl dichloro-iso-everninate* (12). 167.4, 153.2, 153.1, 133.2, 119.7, 115.2, 62.1, 52.4, 17.7.

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