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The Orthosomycins — A New Family of Antibiotics (**)

Flambamycin (1), $C_{61}H_{84}Cl_2O_{20}$, contains 32 stereogenic centres. Although the absolute configurations of 23 of these were identified by our earlier studies [1-9] the determination of the absolute configurations of the remaining 9 stereogenic centres has continued to provide us with an interesting challenge.

We now report upon (a) the completion of our structural elucidation of flambamycin and (b) the relation between the stereoformula (1) for flambamycin and the structures of the other orthosomycin antibiotics (Figure 1). In order to assist in the presentation of the structural argument, the 61 carbon atoms of the flambamycin molecule are arbitrarily identified by the sequence of numbers given in the formula (1).

Flambamycin [1-9] belongs to a family of structurally related antibiotics which have been named the orthosomycins [10]. The chemistry and the biological activity of flambamycin is determined by the presence in its structure of two ortho-ester groups at positions C-16 and C-53. The identification of these two ortho-ester groups in flambamycin was essentially revealed by its behaviour in carefully designed experiments towards acidic [2, 3, 4, 7] and basic reagents [5, 7]. In addition, the study of ^{13}C-NMR spectra [8] as well as the elucidation of mass spectral fragmentation patterns [9] provided important complementary evidence concerning the location of these two ortho-ester groups. The elucidation of the absolute configurations of the two ortho-ester groups as depicted in the stereoformula (1) has now been achieved.

Four pharmaceutical companies have been associated with the isolation and biological evaluation of the orthosomycins. These are listed in Figure 1. References to the publications which describe their isolation, antibacterial profile, and the elucidation of their constitutional formulae are given.

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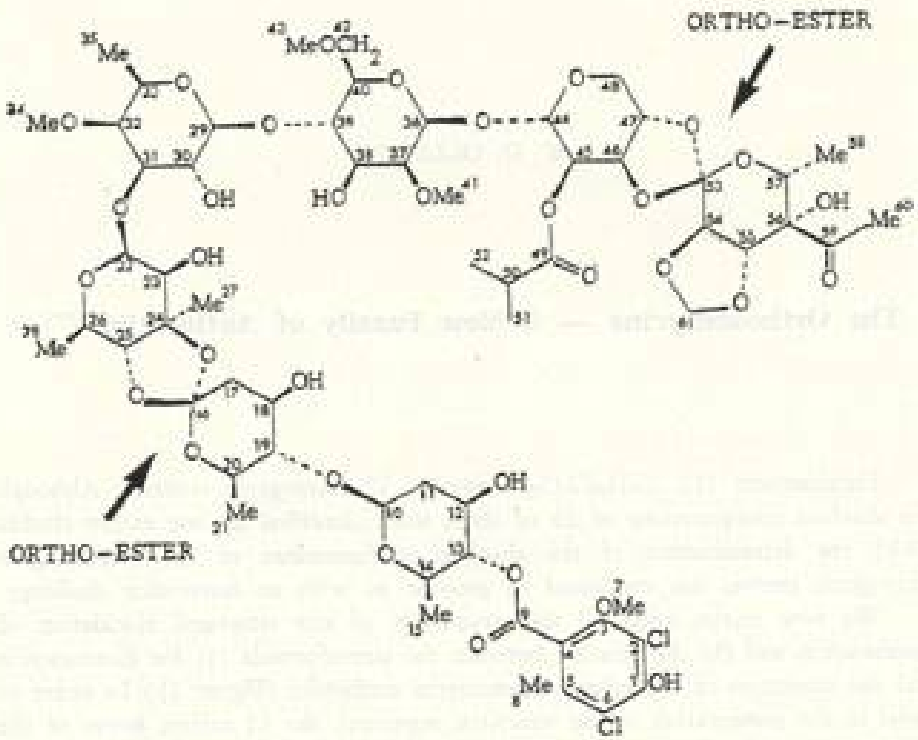
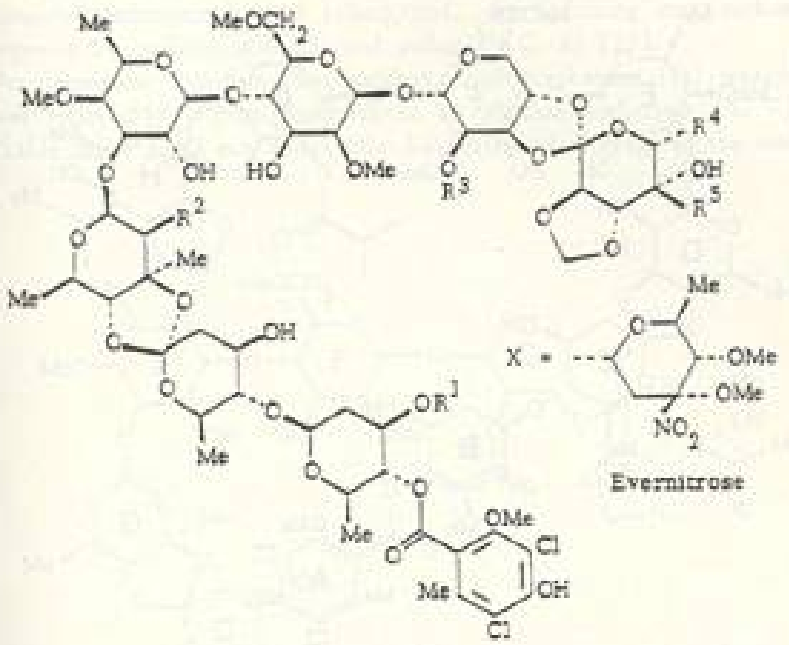


(1) FLAMBAMYCIN

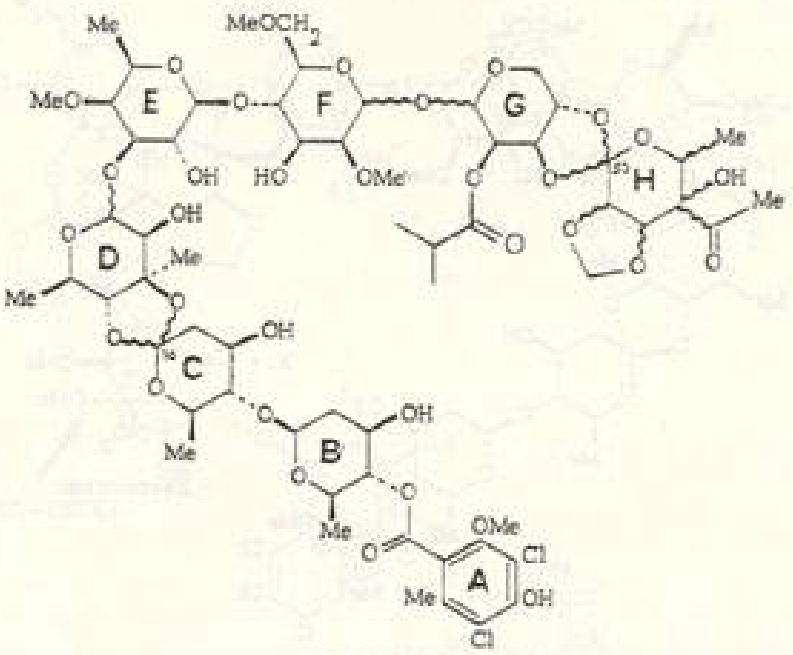
The determination of the constitution of flambamycin involved the identification and location of *one* aromatic ester group, *one* aliphatic ester group, *four* glycosidic (intermonosaccharide) linkages, and *two* ortho-ester groups. The identification of these oxygen-containing functions depended upon the appreciation of differences in reaction pathways produced either by acidic or by basic reagents. Eight residues (A, B, C, D, E, F, G, and H) in flambamycin (2) were identified. The letters A, B, C, D, E, F, G, and H were assigned to each residue in order to identify the relative positions which these eight residues were ultimately shown to occupy in flambamycin. All degradation products and transformation products of flambamycin were characterised as crystalline derivatives (peracetates and permethyl ethers) which were firmly identified by analysis, low- and high-resolution mass spectra, and full spectroscopic (IR, UV, ^{1}H-NMR, ^{13}C-NMR) information. The results of our wide-ranging investigations have been reported [1-9]. These studies led to the proposal of the constitution (2) for flambamycin [7] in which the configurational uncertainties at positions 16, 22, 36, 44, 53, 54, 55, 56, and 57 are indicated.

The remarkable structural coincidences which were detected [12] between flambamycin ($C_{61}H_{88}Cl_2O_{23}$), avilamycin-A ($C_{60}H_{86}Cl_2O_{23}$), and avilamycin-C $C_{61}H_{88}Cl_2O_{22}$) were extremely informative and very reassuring. The essential difference between flambamycin and the avilamycins involved the D residues.



ANTIBIOTIC (Company)	R^1	R^2	R^3	R^4	R^5	References
FLAMBAMYCIN (Rhone-Poulenc)	H	OH	Me_2CHCO-	Me	-CO-Me	1-9
AVILAMYCIN-A (Clba-Geigy)	H	H	Me_2CHCO-	Me	-CO-Me	11, 13
AVILAMYCIN-C (Clba-Geigy)	H	H	Me_2CHCO-	Me		11, 12, 13
CURAMYCIN-A (Squibb)	H	H	MeCO-	Me	-CO-Me	14, 15, 16, 17
EVERNINOMYCIN-B (Schering)	X	OH	Me-	H		17, 18
EVERNINOMYCIN-C (Schering)	X	H	Me-	H	H	17, 19
EVERNINOMYCIN-D (Schering)	X	H	Me-	H		17, 20
EVERNINOMYCIN-2 (Schering)	H	H	Me-	H		17, 21

Fig. 1 - The orthosomycin antibiotics.



(2) FLAMBAMYCIN

In flambamycin, residue D was D-evalose [2, 7], whereas in the avilamycins, residue D was 2-deoxy-D-evalose (D-evermicose) [13]. The structural correspondence between flambamycin, avilamycin-A, and avilamycin-C was dramatically supported by striking similarities which were observed between the ^{1}H- and ^{13}C-NMR spectra of the antibiotics, their transformation products and their derivatives. The ^{1}H- and ^{13}C-NMR spectra were so close that they obviously supported the opinion that corresponding centres of chirality present in the residues D, E, F, G, and H of the antibiotics had corresponding absolute configurations [13].

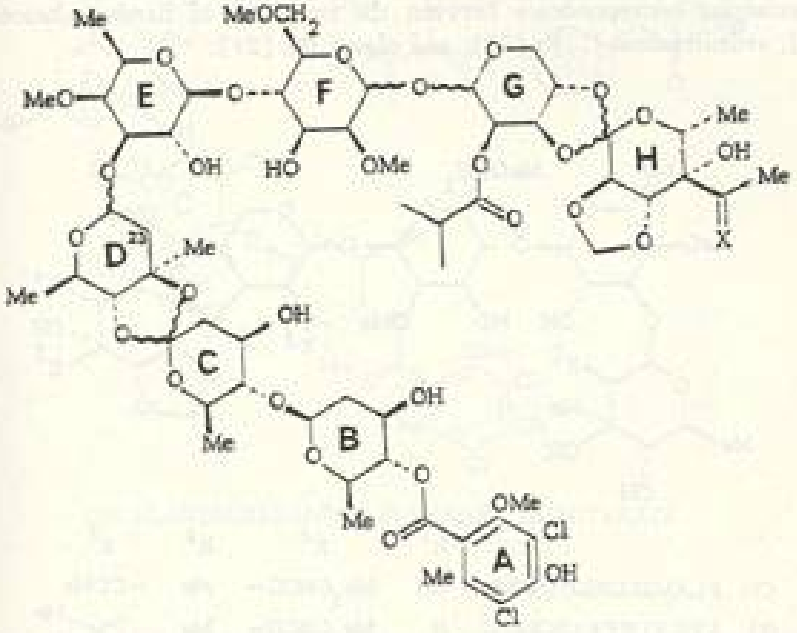
Acid-catalysed methanolysis of flambamycin yields a number of degradation products including methyl eurekanate for which the constitution (3) was proposed [4, 7]. Avilamycin-A similarly yields methyl eurekanate, which was shown by its transformation into L-threonolactone and by an X-ray crystal structure of its mono-acetate to have the absolute configuration (4) [22].

(3) (4)

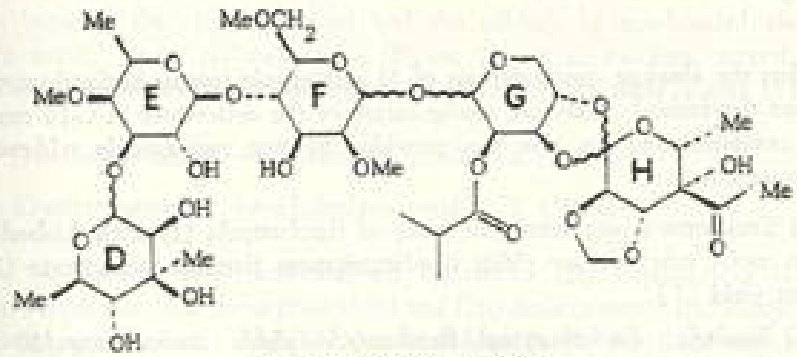
METHYL EUREKANATE

On the basis of the structural correlations which were established between flambamycin, avilamycin-A, and avilamycin-C, the following constitutions could be proposed for avilamycin-A (5) and avilamycin-C (6) [13].

An important transformation product, flambeurekanose (7a) was obtained in good yield (85%) from flambamycin by alkaline hydrolysis [5, 7]. This remarkable result could not involve the base-catalysed cleavage of the ortho-ester



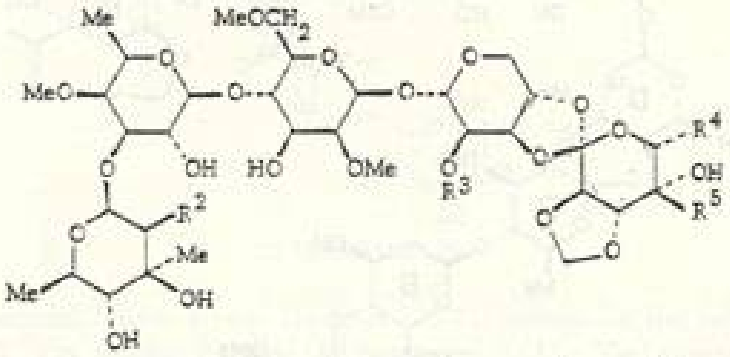
(5) AVILAMYCIN-A (X=O)
(6) AVILAMYCIN-C (X=H, OH)



(7a) FLAMBEUREKANOSE

at C-16, so clearly an acid-catalysed hydrolysis must have occurred as well during work-up.

The complete stereochemistry of flambeurekanose (7b) is based upon the following results. Alkaline hydrolysis of avilamycin-C yielded avileurekanose-C (8) whose stereochemistry was elucidated by X-ray crystal structure examination of its penta-acetate [23]. Mild acid-catalysed hydrolysis of everninomycin-D yields everninomycin-D_1 which undergoes a remarkable cleavage by treatment with diazomethane. One of the products is olgose (9) whose structure was firmly established by X-ray single crystal structure determination of its monohydrate [24]. There is a satisfying correspondence between the structures of flambeurekanose (7b) [5, 7], avileurekanose-C (8) [23], and olgose (9) [24].



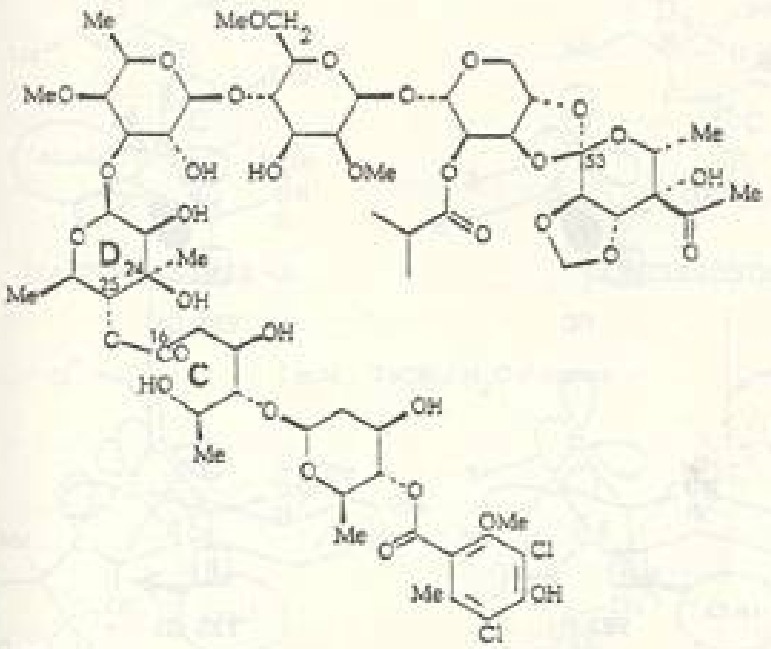
		R^2	R^3	R^4	R^5
(7b)	FLAMBEUREKANOSE	OH	Me_2CHCO-	Me	$-COMe$
(8)	AVILEUREKANOSE-C	H	Me_2CHCO-	Me	
(9)	OLOOSE	H	Me–	H	

Thus the absolute configurations of 31 stereogenic centres in flambamycin (1) had been determined. Only the configuration of the ortho-ester at C-16 remained to be unmasked and the clue was provided by two regiospecific acid-catalysed transformations:

(i) Treatment (room temp./30 min.) of flambamycin (1) with Amberlite 15 resin in moist ethyl acetate yields flambeurekanose flambate isobutyrate (10) in excellent yield [7].

(ii) Similarly, des-isobutyroyl flambamycin yields (room temp./10 min.) flambeurekanose flambate [7].

These two transformations (i) and (ii) are associated with regiospecific retention of the ester group between C-16 and C-25.



(10) FLAMBEUREKANOSE FLAMBATE ISOBUTYRATE

Stereo-electronic control during the acid-catalysed hydration of ortho-esters is now recognised [25-30] as being favoured when there is a stabilising n-σ^* interaction between the cleaved σ-bond and non-bonding electron pairs (Figure 2).

Six particular conformations of ortho-esters are considered (Figure 2) and the presence in each of these six conformations of 2, 1, or 0, antiperiplanar relations between the cleaved σ-bond and the orbitals of non-bonded electron pairs are noted. These rationalisations (Figure 2) are in excellent accord with the regiospecific acid-catalysed transformations of model compounds (Figure 3) [31].

(a) Diastereoisomer A → Hydrolysis product X (100%);

(b) Diastereoisomer B → Hydrolysis product Y (100%).

The regiospecific mild acid-catalysed hydrations [(i) flambamycin (1) → flambeurekanose flambate isobutyrate (10)] and [(ii) desisobutyroyl flambamycin → flambeurekanose flambate] establish the stereoformula (1) for flambamycin and corresponding structures for the other orthosomycins (Figure 1).

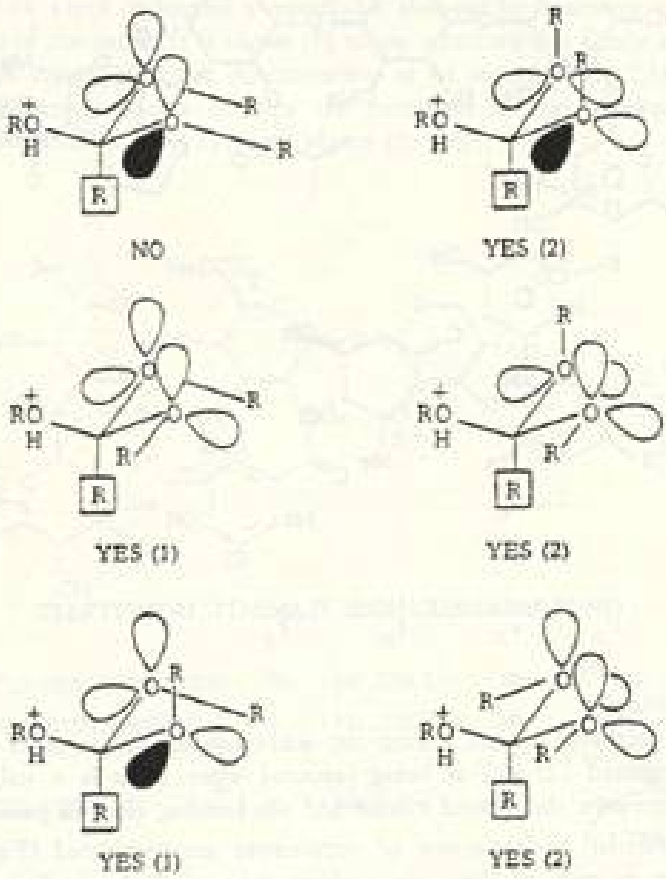


Fig. 2 - Stereoelectronic control during acid-catalysed hydrolysis of ortho-esters.

(1) σ^* is the antibonding orbital of the $-\overset{|}{C}-\overset{|}{O}-$ which is being cleaved.

(2) Stabilising $n-\sigma^*$ interaction requires antiperiplanar relation between the cleaved σ-bond and non-bonded electron pairs.

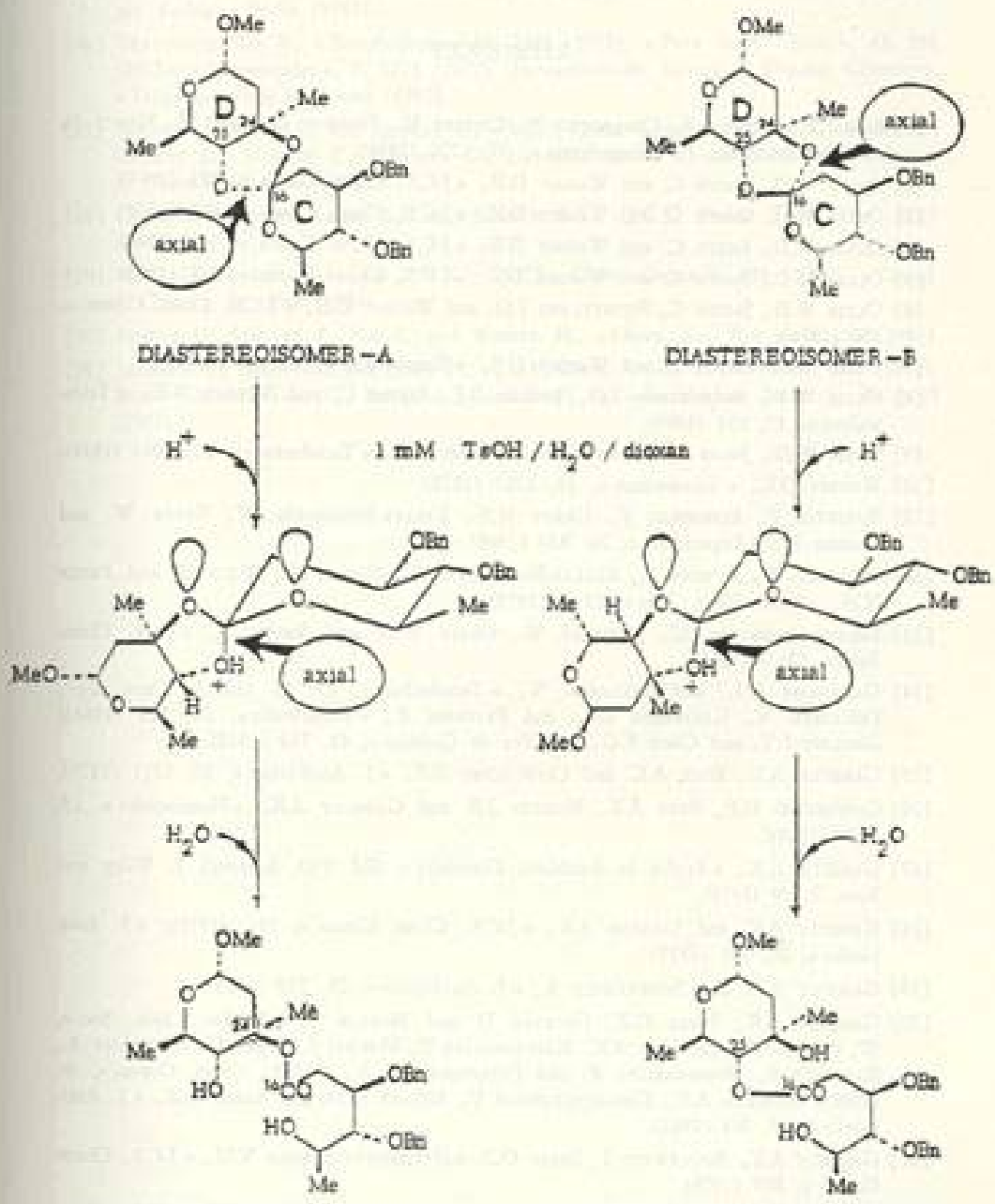


Fig. 3 - Regiospecific hydration of diastereoisomeric ortho-esters. Models for the hydration of flambamycin.

REFERENCES

[1] Ninet L., Benazet F., Charpentie Y., Dubost M., Florent J., Lunel J., Mancy D. and Preud'homme J., « Experientia », *30*, 1270 (1974).

[2] Ollis W.D., Smith C. and Wright D.E., « J.C.S., Chem. Comm. », 881 (1974).

[3] Ollis W.D., Smith C. and Wright D.E., « J.C.S., Chem. Comm. », 882 (1974).

[4] Ollis W.D., Smith C. and Wright D.E., « J.C.S., Chem. Comm. », 347 (1976).

[5] Ollis W.D., Smith C. and Wright D.E., « J.C.S., Chem. Comm. », 348 (1976).

[6] Ollis W.D., Smith C., Sutherland I.O. and Wright D.E., « J.C.S., Chem. Comm. », 350 (1976).

[7] Ollis W.D. Smith C. and Wright D.E., « Tetrahedron », *35*, 105 (1979).

[8] Ollis W.D., Sutherland I.O., Taylor B.F., Smith C. and Wright D.E., « Tetrahedron », *35*, 993 (1979).

[9] Ollis W.D., Jones S., Smith C. and Wright D.E., « Tetrahedron », *35*, 1003 (1979).

[10] Wright D.E., « Tetrahedron », *35*, 1207 (1979).

[11] Buzzetti F., Eisenberg F., Grant H.N., Keller-Schierlein W., Voser W. and Zähner H., « Experientia », *24*, 320 (1968).

[12] Heilman W., Kupfer E., Keller-Schierlein W., Zähner H., Wolf H. and Peter H.H., « Helv. Chim. Acta », *62*, 1 (1979).

[13] Keller-Schierlein W., Heilman W., Ollis W.D. and Smith C., « Helv. Chim. Acta », *62*, 7 (1979).

[14] Galmarini O.L. and Deulofeu V., « Tetrahedron », *15*, 76 (1961); Gros E.G., Deulofeu V., Galmarini O.L. and Frydman B., « Experientia », *24*, 323 (1968); Deulofeu V. and Gros E.G., « Anales de Quimica », *68*, 789 (1972).

[15] Ganguly A.K., Bose A.K. and Cappuccino N.F., « J. Antibiotics », *32*, 1213 (1979).

[16] Cappuccino N.F., Bose A.K., Morton J.B. and Ganguly A.K., « Heterocycles », *15*, 1621 (1981).

[17] Ganguly A.K., « Topics in Antibiotic Chemistry » (Ed. P.G. Sammes), J. Wiley and Sons, *2*, 39 (1978).

[18] Ganguly A.K. and Saksena A.K., « J.C.S., Chem. Comm. », 531, (1973); « J. Antibiotics », *28*, 707 (1975).

[19] Ganguly A.K. and Szmulewicz S., « J. Antibiotics », *28*, 710 (1975).

[20] Ganguly A.K., Sarre O.Z., Greeves D. and Morton J., « J. Am. Chem. Soc. », *97*, 1982 (1975); Ganguly A.K., Karasakalian P., Morton J., Sarre O., Westcott A., Kalleney S., Mangiaracina P. and Papaphilippou A., « J.C.S., Chem. Comm. », 56 (1980); Ganguly A.K., Girijavallabhan V., Miller G.H. and Sarre O.Z., « J. Antibiotics », *35*, 561 (1982).

[21] Ganguly A.K., Smulewicz S., Sarre O.Z. and Girijavallabhan V.M., « J.C.S., Chem. Comm. », 609 (1976).

[22] Kupfer E., Neupert-Laves K., Dobler M. and Keller-Schierlein W., « Helv. Chim. Acta », *63*, 1141 (1980).

[23] Kupfer E., Neupert-Laves K., Dobler M. and Keller-Schierlein W., « Helv. Chim. Acta », *65*, 3 (1982).

[24] Ganguly A.K., Sarre O.Z., McPhail A.T. and Miller R.W., « J.C.S., Chem. Comm. », 22 (1979).

[25] Kirby A.J., *The Anomeric Effect and Related Stereoelectronic Effects at Oxygen*, « Springer Verlag », Berlin (1983).

[26] Deslongchamps P., « Tetrahedron », *31*, 2463 (1975); « Pure Appl. Chem. », *43*, 351 (1975); « Heterocycles », *7*, 1271 (1977); *Stereoelectronic Effects in Organic Chemistry*, « Pergamon Press », Oxford (1983).

[27] Bouab O., Lamaty G. and Moreau C., « J.C.S., Chem. Comm. », 678 (1978); Bouab O., Lamaty G., Moreau C., Pomares O., Deslongchamps P. and Rurst L., « Canad. J. Chem. », *58*, 367 (1980).

[28] Chandrasekhar S., Kirby A.J. and Martin R.J., « J.C.S., Perkin Trans. II », 1619 (1983).

[29] McClelland R.A. and Lam P.W.K., « J.C.S., Chem. Comm. », 883 (1980); « Canad. J. Chem. », 62, 1068 (1984).

[30] Pindur U., Müller J., Flo C. and Witzel H., « Chem. Soc. Rev. », *16*, 75 (1987).

[31] Jaurand G., Beau J.-M. and Sinaÿ P., « J.C.S., Chem. Comm. », 572 (1981); 701 (1982); Beau J.-M., Jaurand G., Esnault J. and Sinaÿ P., « Tetrahedron Letters », *28*, 1103 (1987).