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SYNTHESIS OF LABILOSE A COMPONENT OF THE ANTIBIOTIC LABILOMYCIN AND STRUCTURAL REVISION OF FLAMBAMYCIN BY HIGH RESOLUTION HIGH FIELD ^{13}C N.M.R. SPECTROSCOPY.

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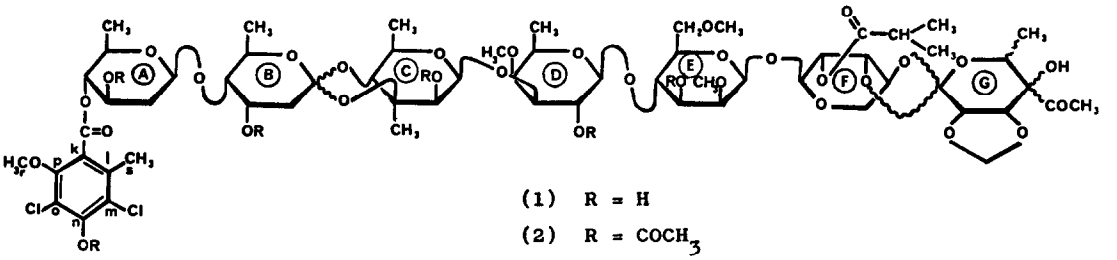
A structure for the antibiotic flambamycin (1) has been previously suggested [1]. However, the configuration at the anomeric centre of evalose (subunit C) and at the two anomeric carbons of the trehalose related moiety (subunits E and F) was not established.We wish to demonstrate here with the help of high resolution high field ^{13}C nmr spectroscopy that (a) the anomeric centre of evalose of (1) has the β-configuration;(b) the anomeric centres of subunits E and F of (1) have the β and α-configuration as shown;(c) the glycosidic linkage between subunits C and D of (1) is of 1⟶3 type in agreement with the related everninomycin antibiotics [2] and in contrast to the previously proposed 1⟶2 linkage in (1) [3, 10].

The 67.89 MHz ^{13}C nmr spectrum of flambamycin (1) and of its hexaacetate (2)(oil $[\alpha]_D$ - 15.1°) were recorded in DMSO-d_6 solution with broad band and without hydrogen decoupling (high resolution).The 94 - 106 ppm region in the spectrum of both (1) and (2) exhibits six signals corresponding to the five oxymethine type anomeric carbons and to the - O-CH_2-O - carbon of subunit G.Inspection of the spectral data given in Table 1 reveals the presence of four equatorially and one axially substituted anomeric centres [4].The coupling constants, chemical shifts and their only possible assignments indicate that the respective anomeric carbons of flambamycin (1) and of everninomycin-B [2a] have the same configuration.The chemical shift contrast between the spectrum of (1) and (2) reflects the presence of two subunits (C and D) having each a free hydroxy group attached to C_2.On the basis of the previously proposed structure for (1) [1] only one anomeric carbon would be expected to be shielded in the spectrum of (2).As a consequence, a structural revision of flambamycin became necessary.In order to confirm this result, the synthesis and spectral examination of four appropriate model compounds (3), (4), (5) and (6) were undertaken.

The known methyl 3,4-O-isopropylidene-β-D-galactopyranoside (7)[5] was selectively tosylated giving (8)(mp. 160-162°,$[\alpha]_D$ +1°) in 84% yield.Treatment of (8) with $AlLiH_4$ afforded as the major product the starting alcohol (7).However, methyl 6-deoxy-3,4-O-isopropylidene-β-D-galactopyranoside (10)(mp. 56-58°,$[\alpha]_D$+16°) was obtained in quantitative yield by transforming (8) with propanthiol into (9)(mp. 129°,$[\alpha]_D$+3°) followed by Raney-Ni desulfurization.The 6-deoxy sugar (10) was then transformed to the 2-O-benzyl (11)(oil,$[\alpha]_D$+82°) and to the 2-O-methyl (12)(mp. 97-99°,$[\alpha]_D$+10°) ethers.Acid hydrolysis of the 3,4-O-isopropylidene group of (11) and (12) afforded respectively

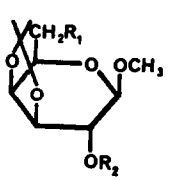
(13) (oil,$[\alpha]_D$+213°) and (14)(mp. 93-94°,$[\alpha]_D$-26°) with quantitative yields.Methylation of (13) furnished (15)(oil,$[\alpha]_D$0°) which was debenzylated in 90% yield giving methyl 6-deoxy-3,4-di-O-methyl-β-D-galactopyranoside (3)(mp. 42-44°,$[\alpha]_D$+6°), 2-O-acetate (4)(mp. 109°,$[\alpha]_D$-20°).Reaction of (14) with di-n-butyltin oxide in methanol [6] afforded (16) which in the presence of an excess of benzyl bromide in DMF solution under reflux gave (17) in 55% yield (oil,$[\alpha]_D$-14°).Methylation of (17) followed by debenzylation gave methyl 6-deoxy-2,4-di-O-methyl-β-D-galactopyranoside (5)(mp. 106-107°,$[\alpha]_D$-17°;lit.[7]mp. 111°, $[\alpha]_D$-20.9°), 3-O-acetate (6)(oil,$[\alpha]_D$+20° ;lit.[7]oil,$[\alpha]_D$+21°).Acid hydrolysis of (5) afforded in quantitative yield labilose (18)[8]the carbohydrate component of the antibiotic labilomycin [7].



(1) R = H

(2) R = $COCH_3$

The ^{13}C nmr shift contrast between the anomeric carbon of the D-subunit of (1) and (2) and between that of the model compounds (3) and (4) and the shift identity between the anomeric carbon of model compounds (5) and (6) demonstrate unambiguously that the glycosidic linkage between the C and D-subunits of (1) is of 1→3 type.Thus only the stereochemistry of the two orthoester linkages and the configuration at two centres of subunit G of the antibiotic remain unknown (1) [1] . Tentative ^{13}C nmr chemical shift assignment based on published models [9] , on the acetylation effect, on $J_{^{13}C-^{12}C}$ and $J_{^{13}C-^{12}C-^{12}C-^{1}H}$ couplings from the high resolution spectrum is presented in Table 1 for (1) and for its hexaacetate (2).



(7) R_1=OH ;R_2=H

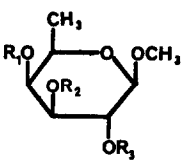
(8) R_1=OTs;R_2=H

(9) R_1=$SCH_2CH_2CH_3$;R_2=H

(10) R_1= R_2 = H

(11) R_1=H ;R_2=Bn

(12) R_1=H ;R_2=CH_3



(3) R_1=R_2=CH_3;R_3=H

(4) R_1=R_2=CH_3;R_3=Ac

(5) R_1=R_3=CH_3;R_2=H

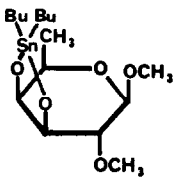
(6) R_1=R_3=CH_3;R_2=Ac

(13) R_1=R_2=H ;R_3=Bn

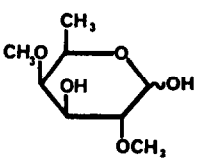
(14) R_1=R_2=H ;R_3=CH_3

(15) R_1=R_2=CH_3;R_3=Bn

(17) R_1=H;R_2=Bn;R_3=CH_3



(16)



(18)

Bn = benzyl;Bu = n-butyl

Ac = acetyl;Ts = tosyl

TABLE 1.

carbon	(1)	(2)[t]		(1)	(2)		(1)	(2)		(1)	(2)
G-4-S[(a)]	210.5	210.3	E-1	95.6	95.1	G-5	72.0[f]	72.0[f]	D-4-OMe	60.9[h]	60.9[i]
F-2-S	174.8	174.8	F-1	94.2	94.3	E-5	71.6[f]	70.4[f]	E-2-OMe	60.7[h]	60.4[i]
A-4-S	165.6	164.5	B-4	86.2	81.8	C-2	71.5	72.2	E-6-OMe	58.3	58.5
n	151.7	145.4	D-3	82.4	81.0	E-6	70.5	69.6	B-2	40.5	(b)
p	151.3	151.6	C-3	81.8	78.5	D-5	69.9	70.5	A-2	39.9	(b)
l	132.5	132.9	C-4	80.4	80.9	D-2	69.7	71.6	F-2-S	33.2	33.2
m	122.1	128.3[c]	G-4	80.3[d]	79.1[d]	A-3	69.3	70.1	G-4-S	27.4	27.4
B-1	119.6[c]	119.3[c]	G-3	79.3[e]	79.3[d]	C-5	69.3[g]	69.5[g]	F-2-S	18.7	18.2
G-1	118.6[c]	118.6[c]	E-2	79.2[e]	80.0[e]	F-2	69.3[g]	69.3[g]	F-2-S	18.6	18.2
k	118.5	124.4	A-4	78.5[e]	75.4[e]	B-5	68.7[g]	68.7[g]	C-6	18.4[i]	18.7[j]
o	113.9	120.2	E-4	78.2[e]	73.4[e]	A-5	67.4	67.5[h]	B-6	18.2[i]	18.0[j]
D-1	103.3	100.4	D-4	77.8[d]	77.8[d]	B-3	67.4	68.7[h]	A-6	17.5[i]	17.6[j]
C-1	101.1	98.4	G-2	77.5[d]	77.6[d]	E-3	67.4	70.0[h]	C-3-Me	17.3[i]	17.4[j]
A-1	99.8	99.0	F-4	74.4[d]	74.4[d]	F-5	62.3	62.3	D-6	17.2[i]	16.9[j]
G-2/3-S	96.0	96.0	F-3	73.9[d]	73.9[d]	r	61.8	62.3	s	16.0[i]	16.2[j]
									G-6	13.5	13.5

Tentative ^{13}C nmr chemical shift assignment (DMSO-d_6 solution, ppm, TMS=0) for flambamycin (1) and for its hexaacetate (2). $^1J_{^{13}C-^1H}$ coupling constants for the A-1 C-1, D-1 and E-1 sites are 163 Hz and for F-1 the value is 177 Hz.(a) "S" means that the signal represents a Substituent directly attached to the subunit or involved in a side chain.(b) signal hidden by the solvent. Carbon signals in DMSO-d_6 solution for the model methyl 4-O-benzoyl-2,6-dideoxy-β-D-arabino-hexopyranoside recently synthesised by us are the following: 165.6, 133.3, 130.0, 2x129.4, 2x128.7, 100.2, 78.5, 69.2, 67.7, 55.7 and 17.7.
c,d-j signals within the same vertical column may be interchanged. [t] acetate signals : six between 19.8-20.7 and six between 167.0-169.6.

TABLE 2.

carbon[a.]	(3)	(4)	(1)	(2)	(5)	(6)
C-1	104.4	101.9	103.3	100.4	103.9	103.3
C-2	69.7	71.4	69.7	71.6	81.0	78.1
C-3	84.2	82.1	82.4	81.0	73.3	75.0
C-4	77.8	78.0	77.8	77.8	82.0	79.2
C-5	69.7	70.8	69.9	70.5	69.5	69.2
C-6	16.7	17.1	17.2	16.9	16.5	16.0
OMe	56.0	56.7	-	-	55.9	56.0
OMe	57.6	58.0	-	-	60.0	60.0
OMe	61.0	61.8	60.9	60.9	61.5	61.3
$-O\underline{C}OCH_3$	-	170.9	-		-	170.0
$-OCO\underline{C}H_3$	-	21.6	-		-	21.0

Assignment of ^{13}C chemical shifts (ppm, TMS=0) in DMSO-d_6 solution for model compounds (3),(4),(5) and (6) and the corresponding signals in the spectrum of flambamycin (1) and its hexaacetate (2)(Table 1).
a. these carbons correspond to subunit-D in the antibiotic.

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8. Satisfactory spectroscopic and analytical data have been obtained for all the new compounds described. $[\alpha]_D$ values were measured in $CHCl_3$ solution.
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10. After submitting this manuscript for publication, Professor Ollis kindly informed us that his group has recently revised the nature of this glycosidic linkage.The corresponding results will appear shortly in Tetrahedron in three papers.

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