THE STRUCTURE OF LIENOMYCIN, A PENTAENE MACROLIDE ANTITUMOR ANTIBIOTIC

II. THE LOCATION OF THE PENTAENE CHROMOPHORE AND OF SIX ISOLATED DOUBLE BONDS. THE COMPLETE STRUCTURE OF THE ANTIBIOTIC

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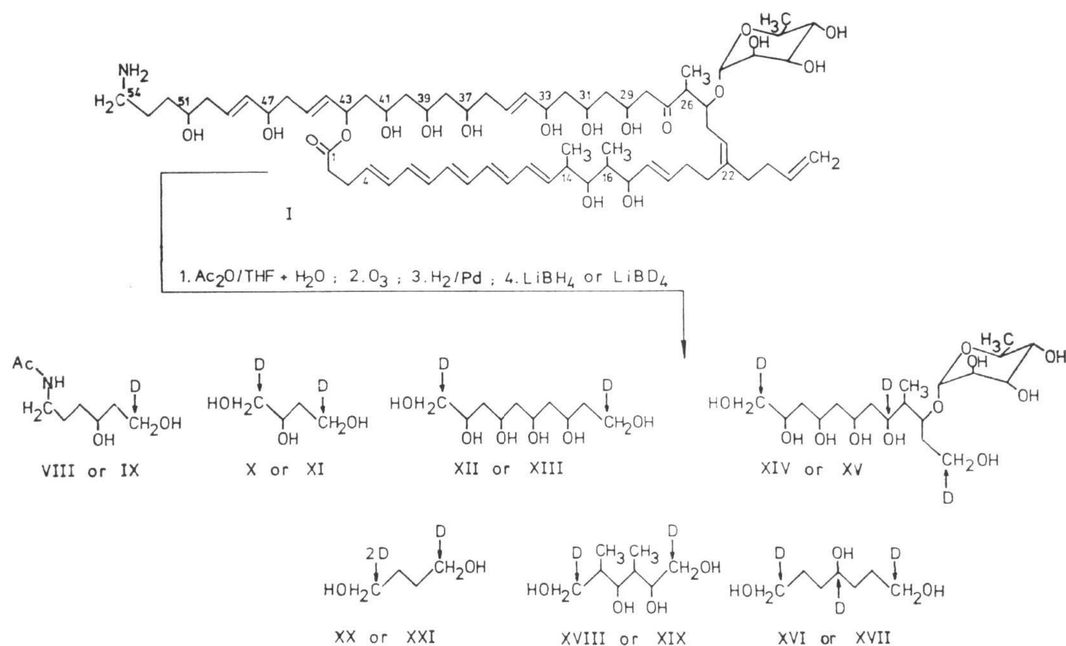
The locations of the isolated double bonds and of the pentaene chromophore in lienomycin (I) were established by chemical degradation of I followed by MS and ^{1}H NMR examination of the products. The complete structure of the antibiotic is proposed.

The results of the structure elucidation studies of the antibiotic lienomycin, as presented in part I[1], assigned the structure of docosahydrolienomycin, a lienomycin derivative with all double bonds hydrogenated. The evidence for the location of six isolated double bonds and of the pentaene chromophore in the lienomycin molecule (I, Fig. 1), as obtained from MS and ^{1}H NMR analysis of degradation products of I, is presented in this report.

The location of six isolated double bonds and of the pentaene chromophore in I was derived from the

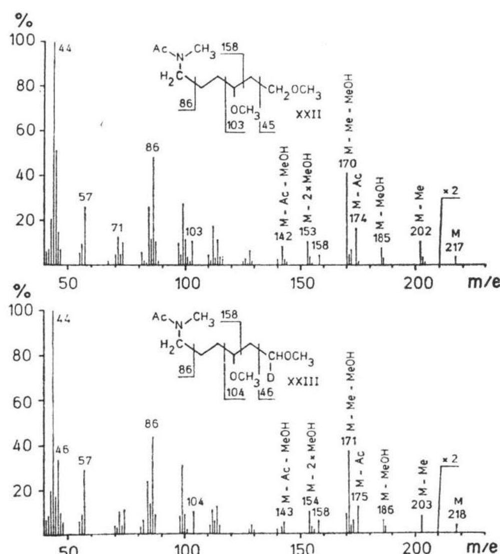
Fig. 1. The degradation of the double bonds of lienomycin (I) and the structures of the resulted products (VIII~XXI).



structures of the compounds **VIII**~**XXI** (Fig. 1) obtained from **I** by the following reaction sequence: (1) acetylation of the amino group, (2) ozonolysis of the double bonds, (3) hydrogenation of ozonides in the presence of palladium, (4) reduction with lithium borohydride or lithium borodeuteride of the carbonyl groups formed in the reactions 1~3, as well as of the keto group and the lactone bond existing in the native antibiotic. The indicated deuterated analogues **IX**, **XI**, **XIII**, **XV**, **XVII**, **XIX** and **XXI** (Fig. 1) were obtained with lithium borodeuteride as a reducing agent. The chemical structures of the compounds **VIII**~**XXI** (Fig. 1) were established by MS analysis of their volatile methyl or trimethylsilyl polyethers.

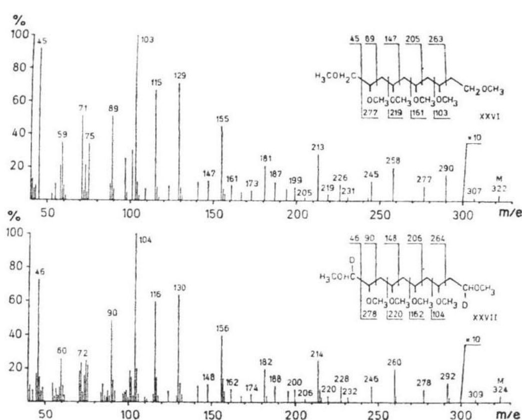
The mass spectra of di-O-methyl-N-methyl-6-acetamido-1,3-diol (**XXII**) and of its 1-deuterio analogue (**XXIII**) show Fig. 2 for **XXII** or **XXIII** the molecular ions at *m/e* 217 or 218, respectively, three series of elimination ions: (a), $[M-n\times MeOH]^+$ with *m/e* values: 185 or 186 (n=1) and 153 or 154 (n=2); (b), $[M-Me-n\times MeOH]^+$ with *m/e* values: 202 or 203 (n=0) and 170 or 171 (n=1); (c), $[M-Ac-n\times MeOH]^+$ with *m/e* values: 174 or 175 (n=0) and 142 or 143 (n=1). The indicated fragment ions (Fig. 2), formed by cleavage of the carbon-carbon bond next to the methoxy group or N-methylacetamide moiety, locate in **XXII** and **XXIII** both the methoxy groups at C-1 and C-3, the N-methylacetamido group at C-6, and one deuterium atom at C-1 in **XXIII**.

Fig. 2. The EI mass spectra of di-O-methyl-N-methyl-6-acetamido-hexane-1,3-diol (**XXII**) and its 1-deuterio analogue (**XXIII**).



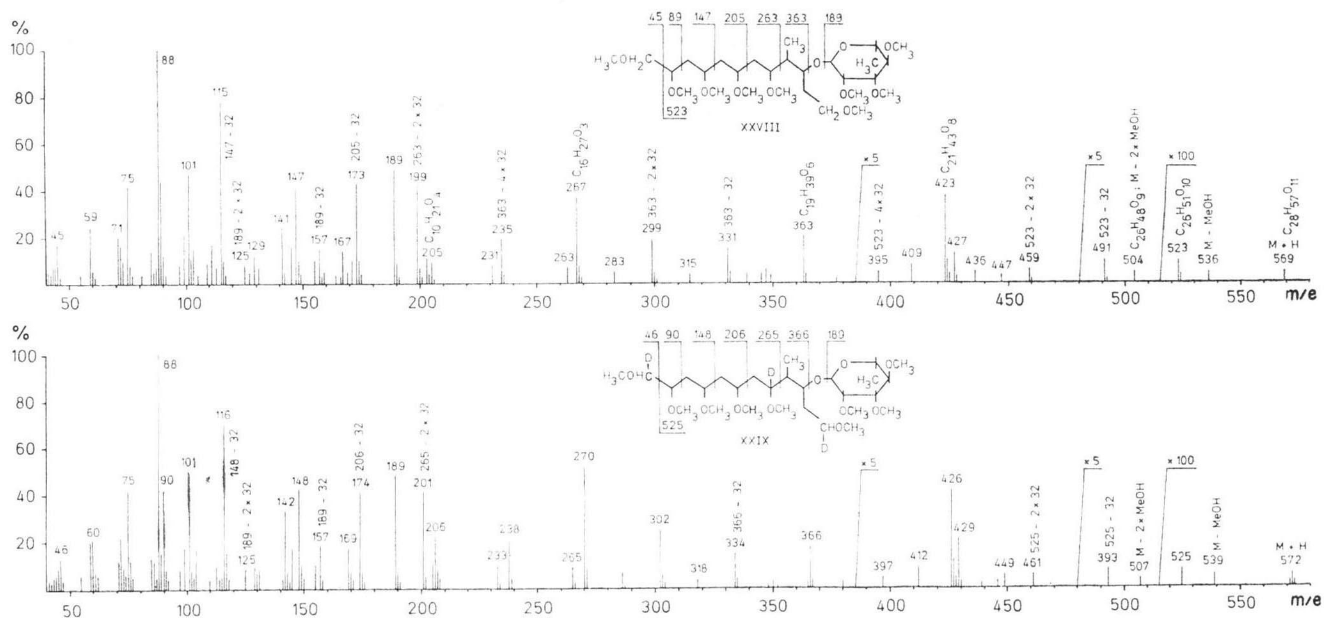
The mass spectra of tri-O-(trimethylsilyl)-butane-1,2,4-triol (**XXIV**) and of its 1,4-dideuterio analogue (**XXV**) show (see Experimental) for **XXIV** or **XXV** ions at *m/e* 307 or 309 of the $[M-Me]^+$ type, elimination ions at *m/e* 232 or 234 of the $[M-Me_3SiOH]^+$ type and fragment ions formed by an ether-type cleavage at *m/e* 103 or 104, 205 or 206, 219 or 220, and provide evidence thereby for the structures of both compounds.

Fig. 3. The EI mass spectra of hexa-O-methyl-decane-1,2,4,6,8,10-hexaol (**XXVI**) and its 1,10-dideuterio analogue (**XXVII**).



The mass spectra of hexa-O-methyldecane-1,2,4,6,8,10-hexaol (**XXVI**) and of its 1,10-dideuterio analogue (**XXVII**) are given in Fig. 3. Diagnostic for the structure of both compounds are the molecular ions at *m/e* 322 or 324, respectively, the elimination ions at *m/e* 290 or 292 and the indicated fragment ions (Fig. 3) formed by the cleavage of the carbon-carbon bonds next

Fig. 4. The EI mass spectra of nona-O-methyl-9-methyl-10-L-rhamnopyranosyloxy-dodecane-1,2,4,6, 8,12-hexaol (**XXVIII**) and its 1,8,12-trideuterio analogue (**XXIX**).



to the methoxy groups.

The mass spectra of nona-O-methyl-9-methyl-10-L-rhamnopyranosyloxy-dodecane-1,2,4,6,8,12-hexaol (**XXVIII**) and of its 1,8,12-trideuterio analogue (**XXIX**) provide evidence (Fig. 4), respectively, for **XXVIII** or **XXIX** with the ions at *m/e* 569 or 572 of the $[M+H]^+$ type as well as the elimination ions of the $[M-n\times MeOH]^+$ type at *m/e* 536 or 539 (n=1) and at *m/e* 504 or 507 (n=2). The mass spectra of **XXVIII** or **XXIX** show also respective fragment ions: at *m/e* 423 or 426 formed by the fragmentation of glycosidically bound tri-O-methylrhamnose without cleavage of the glycosidic bond[2]. The ions at *m/e* 363 or 366 either resulted from the elimination of methyl formate molecule[2] from the ions at *m/e* 423 or 426, or were due to the elimination of tri-O-methylrhamnosyloxy radical from the respective molecular ions. The fragment ions indicated (Fig. 4) as formed by an ether-type cleavage, locate the methoxy groups and glycosidically bound tri-O-methylrhamnose in **XXVIII** and **XXIX**, as well as provide evidence for the three deuterium atoms at C-1, C-8 and C-12 in **XXIX**. Elemental composition of the following ions: 89.06038 ($C_4H_9O_2$), 147.10228 ($C_7H_{15}O_3$), 189.11242 ($C_9H_{17}O_4$), 205.14425 ($C_{10}H_{21}O_4$), 263.18583 ($C_{13}H_{27}O_5$), 267.19564 ($C_{16}H_{27}O_3$), 363.27442 ($C_{19}H_{39}O_6$), 423.29584 ($C_{21}H_{43}O_8$), 504.32947 ($C_{26}H_{48}O_9$), 523.34762 ($C_{26}H_{51}O_{10}$), 569.38964 ($C_{28}H_{57}O_{11}$), as determined by HRMS, is in agreement with the proposed fragmentation pattern.

The mass spectra of tri-O-methylheptane-1,4,7-triol (**XXX**) and of its 1,4,7-trideuterio analogue (**XXXI**) show (see Experimental) respectively for **XXX** or **XXXI** the following ions diagnostic for the structures of both compounds: elimination ions of the $[M-n\times MeOH]^+$ type at *m/e* 158 or 161 (n=1) and 126 or 129 (n=2) and the fragment ions at *m/e*: 45 or 46, 117 or 119 and 145 or 147.

The mass spectra of tetra-O-methyl-2,4-dimethyl-hexane-1,3,5,6-tetraol (**XXXII**) and of its 1,6-dideuterio analogue (**XXXIII**) provide evidence for the structures of both compounds by the presence of the following ions (Fig. 5): ions at *m/e* 219 or 221 of the $[M-Me]^+$ type, elimination ions of the $[M-n\times MeOH]^+$ type at *m/e*: 202 or 204 (n=1) and 170 or 172 (n=2) and the fragment ions indicated (Fig. 5). The compound **XXXII** was examined by ^1H NMR and the spectrum shows two doublets for CH_3–CH groups at 0.87 and 0.9 ppm.

The mass spectrum for the compound **XXXIV** (see Experimental section) was identical with that for the reference di-O-(trimethylsilyl)butane-1,4-diol obtained by silylation of commercial butane 1,4-diol. Comparison of the mass spectra for **XXXIV** and for its deuterio analogue **XXXV** (see Experimental section) enabled to locate two and one deuterium atoms at C-1 and C-4, respectively in the latter compound.

The establishment of the structures of **VIII**~**XXI** (Fig. 1) and of docosahydrolienomycin (Part I[1]) enables one to locate in the lienomycin molecule (**I**, Fig. 1) the pentaene chromophore between C-4 and C-13 and six isolated double bonds between: C-18 and C-19, C-22 and C-23,

Fig. 5. The EI mass spectra of tetra-O-methyl-2,4-dimethyl-hexane-1,3,5,6-tetraol (**XXXII**) and its 1,6-dideuterio analogue (**XXXIII**).

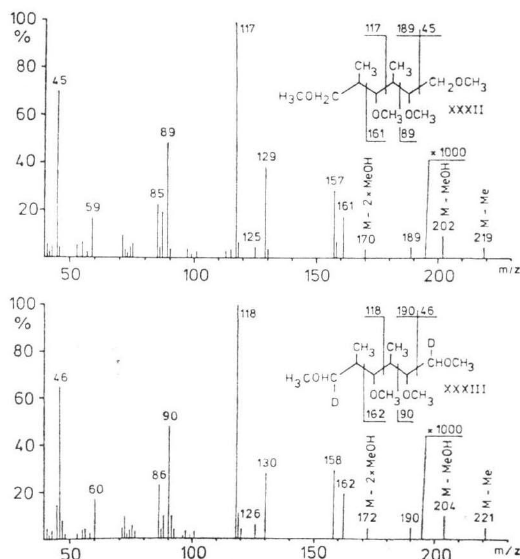


Table 1. The chemical shifts and coupling constants of the protons of the rhamnose moiety of 9-methyl-10-α-L-rhamnopyranosyloxy-decane-1,2,4,6,8,12-hexaol (**XIV**, Fig. 1) and of methyl-α-L-rhamnopyranoside.

	Chemical shifts (ppm)						Coupling constants (Hz)				
	H-1	H-2	H-3	H-4	H-5	H-6	$^3J(1, 2)$	$^3J(2, 3)$	$^3J(3, 4)$	$^3J(4, 5)$	$^3J(5, 6)$
Compound **XIV**	4.93	3.96	3.77	3.45	3.69	1.30	1.8	3.4	9.6	9.5	6.2
Methyl-α-L-rhamnopyranoside*	4.70	3.93	3.71	3.44	3.64	1.29	1.7	3.5	9.6	9.6	6.2

* The data from: DE BRUYN, A.; M. ANTEUNIS, R. DE GUSSEM and G. G. S. DUTTON: ^{1}H NMR study of L-rhamnose, methyl-α-L-rhamnopyranoside and 4-O-β-D-galactopyranosyl-L-rhamnose in deuterium oxide. Carbohydr. Res. 47: 159, 1976

C-3′ and C-4′, C-34 and C-35, C-44 and C-45, C-48 and C-49.

The conformation of glycosidically bound L-rhamnose was determined on the basis of ^{1}H NMR analysis of compound **XIV**. The values of chemical shifts and coupling constants of protons attached to the carbon skeleton of the rhamnose moiety of **XIV** and of methyl-α-L-rhamnopyranoside[3] are compared in Table 1, and providing evidence that L-rhamnose is in the α-L-rhamnopyranoside form in **XIV** and in the lienomycin.

The complete structure of the antibiotic lienomycin has been assigned, based upon all the evidence presented in the Part I[1] and in this report.

Experimental

Instrumental analysis

The electron impact mass spectra of compounds: **XXII**, **XXIII**, **XXVII**, **XXVIII**, **XXIX**, **XXX**, **XXXI**, **XXXII** and **XXXIII** were obtained on a Varian MAT 711 spectrometer by a direct introduction probe. The instrumental conditions were as follows: electron energy, 70 eV; emission current, 0.8 mA; accelerating voltage, 8 kV; ion source temperature, 250°C; resolution 1,000 (10,000 for exact mass determination). The field desorption mass spectrum of **XIV** was obtained with the following instrumental conditions: wire heating current, 18 mA; ion source temperature, 70°C; accelerating voltage, 8 kV; extraction voltage, −4 kV. The electron impact mass spectra of compounds **XXIV**, **XXV**, **XXXIV**, and **XXXV** were recorded on an LKB-9000 instrument combined with a gas chromatograph. For all separations a 3 m×0.3 cm column with 3% SE-30 on Chromosorb W, 80~100 mesh, and helium flow rate 40 ml/minutes were used. The ^{1}H NMR spectra of **XIV** and **XXXII** where obtained on a Varian HR 300 MHz and Tesla BS-487 80 MHz instruments, respectively. The electronic spectrum was recorded on a UV-VIS Zeiss-Jena spectrophotometer.

Methylation (procedure a)

Compounds **VIII**, **IX**, **XII**, **XIII**, **XIV**, **XV**, **XVI**, **XVII**, **XVIII** and **XIX** (Fig. 1) were methylated by the procedure (a) described in Part I[1].

Silylation (procedure b)

Compounds **X**, **XI**, **XX** and **XXI** (Fig. 1) (10 mg), dissolved in pyridine (0.1 ml), reacted overnight at room temperature with sequentially added hexamethyldisilazane (0.03 ml) and trimethylchlorosilane (0.015 ml), *n*-heptane (5 ml) and water (5 ml) were added, the organic phase was washed with water, dried $MgSO_4$ and evaporated to dryness. The residue was dissolved in *n*-heptane (0.2 ml) and examined by GC-MS.

N-Acetyllienomycin

Lienomycin (1.2 g), dissolved in THF - H_2O (5: 1, v/v; 60 ml), reacted (10 min, 5°C) with acetic anhydride (0.11 ml), water (50 ml) and 1-butanol (50 ml) were added, and the organic phase was con-

centrated to 20 ml. The product was precipitated from the butanol solution with ethyl ether; yield, 1.1 g of N-acetyllienomycin, $E^{1\%}_{1cm}=1,100$.

Degradation of the double bonds of lienomycin and isolation of the resulting products

N-Acetyllienomycin (1.1 g), dissolved in methanol (150 ml), was ozonized at −78°C until the pentaene chromophore disappeared. The excess of ozone was removed by a flow of nitrogen and the ozonide was hydrogenated (2 hours, room temperature) over palladium on asbestos (300 mg). The catalyst was removed and the solvent was evaporated. The residue was suspended in THF (30 ml) and added dropwise into $LiBH_4$ solution (220 mg in 60 ml THF) to react (2 hours) at the boiling temperature of THF.

The reaction mixture was ice-cooled, excess of $LiBH_4$ was decomposed, the lithium ions were removed with Dowex 50W×8 (H^+) and the solvents was evaporated to dryness. The residue was twice dissolved in methanol (25 ml) and evaporated to dryness; yield, 1.3 g of an oily mixture of compounds **VIII**, **X**, **XII**, **XIV**, **XVI**, **XVIII** and **XX** (Fig. 1). The deuterated analogues **IX**, **XI**, **XIII**, **XV**, **XVII**, **XIX** and **XXI** were obtained with lithium borodeuteride instead of lithium borohydride.

The products were separated by column chromatography in chloroform - methanol (5: 2, v/v). The eluate was collected in four fractions: fraction A with compounds **X**, **XVI** and **XX**, fraction B containing **VIII** and **XVIII**, fraction C with compound **XII** (143 mg) and fraction D with **XIV** (200 mg). The latter fraction was examined by MS and ^1H NMR (10% solution in D_2O), and the field desorption mass spectrum shows ion at *m/e* 443, $(M+H)^+$. The compounds **X**, **XVI** and **XX** (fraction A) were separated by column chromatography in benzene - ethyl acetate - ethanol (10: 10: 1, v/v); yield, 42 mg of **X**, 60 mg of **XVI** and 36 mg of **XX**. Compounds **VIII** and **XVIII** (fraction B) were separated by column chromatography in benzene - ethyl acetate - ethanol (5: 5: 1, v/v); yield, 70 mg of **VIII** and 89 mg of **XVIII**.

Di-O-methyl-N-methyl-6-acetamido-hexane-1,3-diol (**XXII**) and its 1-deuterio analogue (**XXIII**)

6-Acetamido-hexane-1,3-diol (**VIII**) or its 1-deuterio analogue (**IX**) were methylated by the procedure (a), purified by column chromatography in benzene - acetone (3: 2, v/v) and examined by MS (Fig. 2).

Tri-O-(trimethylsilyl) butane-1,2,4-triol (**XXIV**) and its 1,4-dideuterio analogue (**XXV**)

Butane-1,2,4-triol (**X**) or its 1,4-dideuterio analogue (**XI**) were silylated by procedure (b) and examined by GC-MS (retention time 7 minutes at 100°C). The major ions observed in the mass spectra of both compounds were as follows *m/e* (abund.): 1) in the mass spectrum of **XXIV** at: 73 (91%), 103 (100%), 129 (12%), 142 (8%), 147 (38%), 189 (10%), 205 (2%), 219 (40%), 232 (5%) and 307 (0.16%); 2) in the mass spectrum of **XXV** at: 73 (92%), 104 (100%), 130 (13%), 144 (9%), 147 (39%), 190 (9%), 206 (2%), 220 (41%), 234 (6%) and 309 (0.2%).

Hexa-O-methyldecane-1,2,4,6,8,10-hexaol (**XXVI**) and its 1,10-dideuterio analogue (**XXVII**)

Decane-1,2,4,6,8,10-hexaol (**XII**) and its 1,10-dideuterio analogue (**XIII**) were methylated by procedure (a), purified by column chromatography in benzene - ethyl acetate - ethanol (20: 20: 1, v/v) and examined by MS (Fig. 3).

Nona-O-methyl-9-methyl-10-L-rhamnopyranosyloxy-dodecane-1,2,4,6,8,12-hexaol (**XXVIII**) and its 1,8,12-trideuterio analogue (**XXIX**)

9-Methyl-10-L-rhamnopyranosyloxy-dodecane-1,2,4,6,8,12-hexaol (**XIV**) and its 1,8,12-trideuterio analogue (**XV**) were methylated by procedure (a), purified by column chromatography in benzene - ethyl acetate - ethanol (40: 40: 3, v/v) and examined by MS (Fig. 4).

Tri-O-methylheptane-1,4,7-triol (**XXX**) and its 1,4,7-trideuterio analogue (**XXXI**)

Heptane-1,4,7-triol (**XVI**) and its 1,4,7-trideuterio analogue (**XVII**) were methylated by procedure (a), purified by column chromatography and examined by MS. The major ions observed in the mass spectra of both compounds were as follows: (1) in the mass spectrum of **XXX** at *m/e* (abund.): 45 (48%), 55 (38%), 71 (14%), 85 (100%), 101 (18%), 113 (62%), 117 (5%), 126 (30%), 145 (13%) and 158 (10%); 2) in the mass spectrum of **XXXI** at *m/e* (abund.): 46 (35%), 55 (30%), 71 (22%), 87 (100%), 101 (18%), 115 (60%), 119 (6%), 129 (29%), 147 (14%), and 161 (11%).

Tetra-O-methyl-2,4-dimethylhexane-1,3,5,6-tetraol (**XXXII**) and its 1,6-trideuterio analogue (**XXXIII**)

2,4-Dimethylhexane-1,3,5,6-tetraol (**XVIII**) and its 1,6-dideuterio analogue (**XIX**) were methylated by procedure (a), purified by column chromatography in benzene - ethyl acetate (4: 1, v/v) and were examined by MS (Fig. 5) and ^{1}H NMR (10% solution of **XXXII** in $CDCl_3$).

Di-O-(trimethylsilyl)butane-1,4-diol (**XXXIV**) and its 1,1,4-trideuterio analogue (**XXXV**)

Butane-1,4-diol (**XX**) and its 1,1,4-trideuterio analogue (**XXI**) were silylated by procedure (b) and examined by GC-MS (retention time 4 minutes at 100°C). The major ions observed in the mass spectra of both compounds were as follows: (1) in the mass spectrum of **XXXIV** at *m/e* (abund.); 73 (37%), 75 (13%), 101 (12%), 103 (11%), 116 (48%), 147 (100%), 177 (14%), 219 (9%) and 234 (0.5%); (2) in the mass spectrum of **XXXV** at *m/e* (abund.); 73 (37%), 75 (12%), 101 (12%), 104 (6%), 105 (6%) 116 (24%), 117 (28%), 147 (100%), 178 (7%), 179 (7%), 222 (8%) and 237 (0.6%).

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