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STUDIES ON THE STRUCTURE OF OLIVOMYCIN A AND MITHRAMYCIN BY ^{1}H AND ^{13}C NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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Abstract— Extensive ^{1}H and ^{13}C NMR studies confirmed the gross structure of olivomycin A except for a revision with respect to a saccharide linkage. The structure of mithramycin was elucidated similarly, resulting in a substantial revision of structure for its carbohydrate moiety, including interglycosidic bonds.

Among the cytostatically-active antibiotics, the olivin oligosaccharide glycosides represent a closely related structural subgroup. Of marked therapeutic importance[1] are chromomycin (**1**, toyomycin®), olivomycin A **2**, and mithramycin (**3**, mithracin®). Doubtless, the former assignment[2-4] of their complex carbohydrate structures based on hydrolysis studies as well as on the application of Klyne's rule[5] is questionable with respect to the interglycosidic linkages and the sequence of the saccharides. Thus, a recent extensive spectroscopic investigation has led to a structural revision of chromomycin A_3 **1** concerning the saccharide components.[6] As a sound background for synthetic projects under way in our laboratories it was essential to perform corresponding studies with the other two cytostatic agents olivomycin A **2** and mithramycin **3**.

Olivomycin A

By chemical degradation experiments and application of Klyne's rule the structure of **2**, produced by *Streptomyces olivoreticuli*, was shown to be rather similar to that of **1**, produced by *Streptomyces griseus*.[3] Instead of a C-4E acetoxy group in the sugar components of **1** in **2** at C-4E an isobutyroxy group is found. Furthermore the C-7 methyl group in the chromophore of **1** is replaced by a hydrogen atom in olivomycin A **2** thus the aglycon olivin. Based on the structure revision for **1** consequently for **2** the question concerning the interglycosidic bond in the disaccharide unit B-A is raised.

The ^{1}H NMR spectrum of **2** shows largely agreement with that of **1**. Like in chromomycin A_3 **1** the H-4A in **2** is essentially shifted downfield in contrast to H-4B {δ(H-4A) = 5.14 and δ(H-4B) = 3.19}. On the contrary there is observed only a minor difference in the chemical shifts of H-3A and H-3B ($\Delta\delta$ = 0.06). Thus a structural revision of **2** also seems to be necessary.

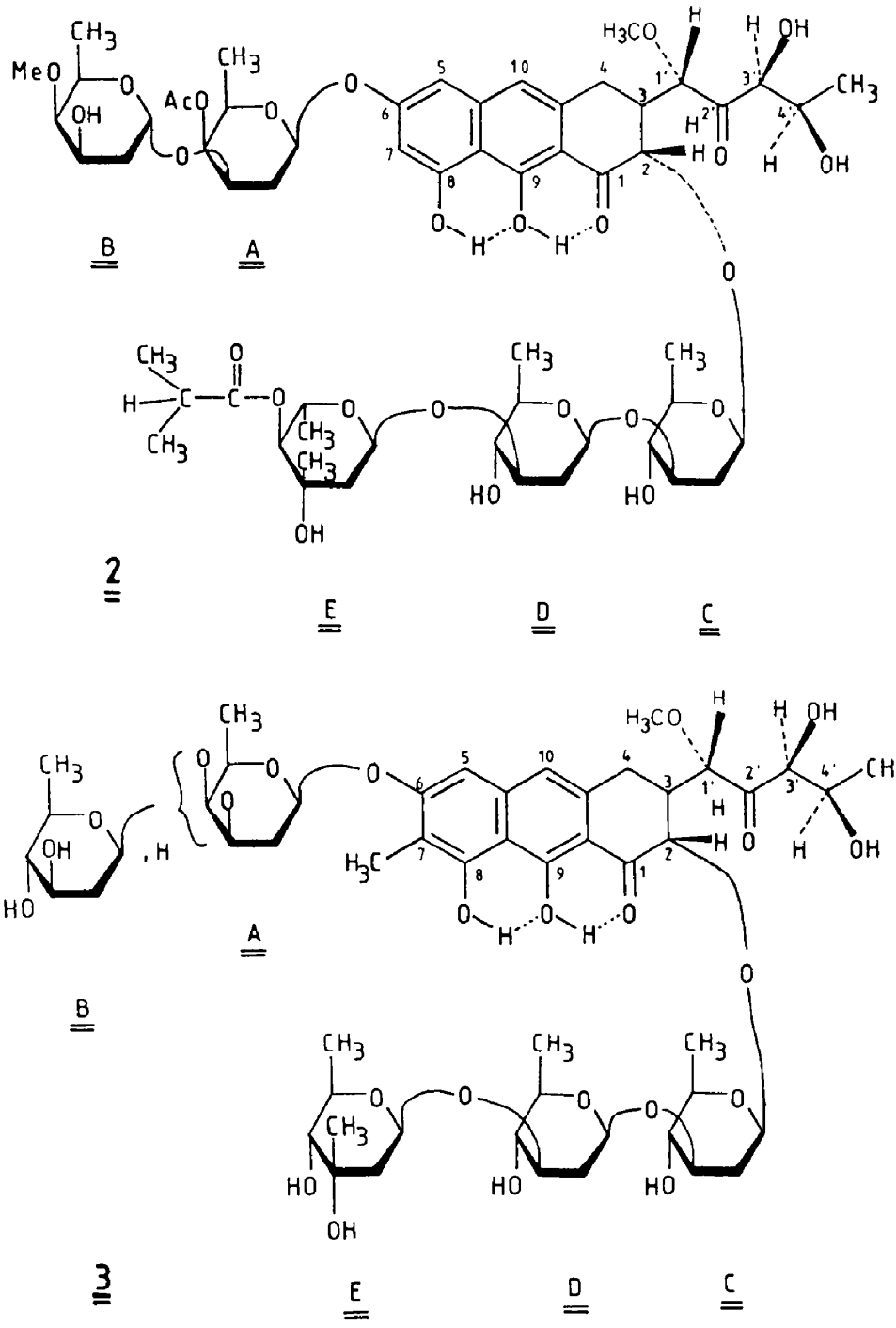
For the aglycon olivin the ^{1}H NMR spectrum confirms the proposed structure.[3] Both H-5 and H-7 are observed as double doublets (δ = 6.64 and/or 6.51) with a typical meta-coupling constant J(5, 7) = 2.2 Hz. The remaining chemical shifts and coupling constants in the aglycon of **2** exhibit only minor deviations from the data of chromomycinon in **1**.[6] Surprisingly the coupling constant J(3′, 4′) = 4.7 Hz turned out to be larger than that in **1** which can be attributed to the purity of **2**. Remaining traces of water may be responsible for a slightly varied conformation of the complex side chain of olivin in **2**.

Directly after dissolution of **2** in deuterochloroform the H-1A signal is observed as a double doublet with the coupling constants J(1A, 2aA) = 6.9 and J(1A, 2eA) = 5.2 Hz. After some hours at room temperature the shift of the signal remained constant, however, the value of the coupling constants are changed to J(1A, 2aA) = 9.0 and J(1A, 2eA) = 2.0 Hz as expected for a β-glycosidically-linked dideoxy unit A. Whereas the other protons in saccharide A do not show any alterations, this effect can possibly be attributed to a slow conformational change in the saccharide A with respect to the aglycon.

For the saccharide B the α-glycosidic linkage is evident from the coupling constants J(1B, 2aB) = 3.5 and J(1B, 2eB) = 2.4 Hz. All the other ring proton signal assignments are consistent with those observed in **1**.[6] The same applies to the dideoxy saccharide units C and D both of which clearly show β-linked arabino configurations. Similarly, the structure of saccharide E having a C-methyl branch at C-3 is in accord with an α-arabino configuration. Furthermore, there is consistent agreement with a C-4E acyloxy substitution as seen in the chemical shift of the adjacent proton δ(H-4E) = 4.59; and the signals of the isobutyroxy group are found at δ = 2.63 (methin, septet) and δ = 1.189 and 1.193 (methyl, doublets) (see Table 1).

The noise decoupled ^{13}C NMR spectrum of **2** is essentially similar to that of chromomycin A_3 **1**. Apart from some variations in the aglycone signals, for instance the expected high field shift of C-7 (δ(C-7) = 102.2) in olivin, the mean chemical shift deviation of the carbohydrate carbon atoms of **1** and **2** amount to only $\overline{\Delta\delta}$ = 0.2. Further confirmation of the saccharide configurations at the interglycosidic centers can again be deduced from the ^{1}J(C-1, H-1) coupling constants[7] which are consistent with those measured in **1** (see Table 2).

Thus the structure of olivomycin A is revised with respect to the interglycosidic linkage between the saccharides B and A to be: (3R)-[(3S), (4R)-dihydroxy-(1S)-methoxy-2-oxopentyl]-3,4-dihydro-8,9-



dihydroxy-1-oxo-2H-anthracen-6-yl-[4-O-acetyl-2,6-dideoxy-3-O-(2,6-dideoxy-4-O-methyl-α-D-lyxo-hexopyranosyl)-β-D-lyxo-hexopyranoside]-(2S)-yl-{2,6-dideoxy-3-O-[2,6-dideoxy-3-O-(2,6-dide-4-O-isobutyryl-3-C-methyl-α-L-arabino-hexopyranosyl)-β-D-arabino hexopyranosyl]-β-D-arabino-hexopyranoside}.

Mithramycin

In contrast to the chromomycins and the olivomycins, mithramycin (which was shown to be identical with aureolic acid[8]) represents a structurally and chemically markedly different compound. According to former chemical degradation studies[4] 3 comprises the aglycone chromomycinon and five 2,6-dideoxy sugars, none of which carried ether or ester functions. Three of these were assigned to be 2,6-dideoxy-D-arabino hexopyranoses, one to be a 2,6-dideoxy-D-hexopyranose, and one a 2,6-dideoxy-3-C-methyl-D-ribo-hexopyranose. At first glance only minor structural variations in comparison to **1** and **2**, these caused a drastic change in the solubility of **3**.

Table 1. ^{1}H NMR data of olivomycin A **2**[a]

Hydrogen atom	$\delta(^1H)$[b]		$J(H_1)$ [Hz][c]
Aglycone			
H-2	4.74	d	11.4(3)
H-3	2.62	m	-
H-4a	3.09	ddd	-15.0(4e),-1.0(10),12.0(3)
H-4e	2.69	m	-
H-5 [d]	6.64	d	2.2(7)
H-7 [d]	6.51	d	2.2(5)
H-10	6.80	d	-1.0(4a)
OH-8	9.60	s	-
OH-9	15.68	s	-
H-1'	4.71	d	1.5(3)
H-3'	4.23	d	4.7(4')
H-4'	4.36	br	-
CH_3-5'	1.34	d	6.4(4')
OH-3'	3.96	s	-
OH-4'	4.39	s	-
Carbohydrate A			
H-1A	5.27	dd	2.0(2e),9.0(2a)
H-2aA H-2eA	2.05	m	-
H-3A	3.99	m	-
H-4A	5.14	dd	0.9(5),3.1(3)
H-5A	3.81	dq	0.9(4),6.5(6)
CH_3-6A	1.23	d	6.4(5)
Carbohydrate B			
H-1B	5.03	dd	2.4(2e),3.5(2a)
H-2aB H-2eB	2.02	m	-
H-3B	3.93	m	-
H-4B	3.19	dd	0.9(5),2.9(3)
H-5B	3.86	dq	0.9(4),6.6(6)
CH_3-6B	1.24	d	6.6(5)
Carbohydrate C			
H-1C	5.09	dd	1.7(2e),9.4(2a)
H-2aC	1.65	ddd	9.6(1),-12.2(2e),12.0(3)
H-2eC	2.49	ddd	1.7(1),5.1(3),-12.2(2a)
H-3C	3.58	m	-
H-4C	3.07	dd	9.0(3),9.0(5)
H-5C	3.30	dq	9.0(4),6.2(6)
CH_3-6C	1.33	d	6.2(5)
Carbohydrate D			
H-1D	4.61	dd	2.0(2e),9.8(2a)
H-2aD	1.70	ddd	9.6(1),-12.3(2e),11.8(3)
H-2eD	2.29	ddd	1.8(1),5.1(3),-12.3(2a)
H-3D	3.53	m	-
H-4D	3.10	dd	9.4(3),9.2(5)
H-5D	3.38	dq	9.2(4),6.3(6)
CH_3-6D	1.35	d	6.3(5)
Carbohydrate E			
H-1E	5.07	dd	2.5(2a),2.5(2e)
H-2aE	1.66	m	-
H-2eE	2.00	m	-
H-4E	4.59	d	9.4(5)
H-5E	4.00	dq	9.4(4),6.3(6)
CH_3-6E	1.20	d	6.3(5)
CH_3-31E	1.35	s	-
Functional groups			
OCH_3-1'	3.49	s	-
OCH_3-4B	3.56	s	-
OAc-4A	2.15	s	-
CH_3-i-Bu-1E	1.19	d	7.2(CH-i-Bu)
CH_3-i-Bu-2E	1.18	d	7.2(CH-i-Bu)
CH-i-Bu-E	2.63	qq	7.2(CH_3-i-Bu-1),7.2(CH_3-i-Bu-2)

a) c=0.023 mol·l^{-1} in $CDCl_3$,270 MHz.Total data memory 32 K,spectral width 4 424.8 Hz,T=298 K.
b) Chemical shifts downfield from Me_4Si ($\delta(CHCl_3)$=7.27) and multiplicity of signals.
c) Coupling constants,coupled hydrogen atoms given in parenthesis.
d) Uncertain assignment.

Table 2. ^{13}C NMR data of **2**[a]

Carbon atom	$\delta(^{13}C)$[b]		J(C,H) [Hz]
Aglycone			
C-1	202.2	s	-
C-2	76.6	d	148.0
C-3	43.6	d	128.0
C-4	43.9	t	129.0
C-5	103.2	d	163.0
C-6	161.1	s	-
C-7	102.2	d	163.0
C-8	165.9	s	-
C-9	159.8	s	-
C-10	117.1	d	160.0
C-4a[c]	141.0	s	-
C-8a[d]	108.2	s	-
C-9a[d]	108.6	s	-
C-10a[c]	136.1	s	-
C-1'	79.6	d	145.0
C-2'	210.9	s	-
C-3'	78.3	d	145.0
C-4'	67.9	d	142.0
Carbohydrate A			
C-1A	97.5	d	162.0
C-3A	82.1	d	140.0
C-4A	67.6	d	146.0
C-5A	70.4	d	139.0
$OCOCH_3$	170.7	s	-
$OCOCH_3$	20.6	q	129.0
Carbohydrate B			
C-1B	97.3	d	168.0
C-3B	66.1	d	144.0
C-4B	81.7	d	143.0
C-5B	70.6	d	142.0
Carbohydrate C			
C-1C	100.4	d	161.0
C-3C	82.0	d	140.0
C-4C[e]	75.4	d	142.0
C-5C[f]	72.5	d	140.0
Carbohydrate D			
C-1D	99.7	d	162.0
C-3D	80.5	d	141.0
C-4D[e]	75.3	d	142.0
C-5D[f]	72.3	d	144.0
Carbohydrate E			
C-1E	95.7	d	169.0
C-3E	70.7	s	-
C-4E	67.2	d	139.5
C-5E	66.9	d	143.0
$OCOCH(CH_3)_2$	177.3	s	-
$OCOCH(CH_3)_2$	34.3	d	130.0
$OCOCH(CH_3)_2$-1	18.0	q	129.0
$OCOCH(CH_3)_2$-2	18.9	q	129.0
Remaining carbon atoms[g]			
CH_2	37.6	t	132.0
CH_2	37.2	t	133.0
CH_2	33.6	t	131.0
CH_2	33.1	t	131.0
CH_2	27.1	t	131.0
CH_2	23.1	q	127.0
CH_3	20.4	q	127.0
CH_3	18.0	q	128.0
CH_3	17.9	q	128.0
CH_3	17.8	q	128.0
CH_3	17.7	q	128.0
CH_3	17.2	q	128.0
CH_3	16.7	q	127.0
OCH_3-4B	59.6	q	143.0

[a] c=0.053 mol·l^{-1} in $CDCl_3$, 67.89 MHz. Total data memory 32 K, spectral width 16 129.0 Hz, T=303 K. [b] Chemical shifts downfield from Me_4Si ($\delta(CDCl_3)$=77.0) and multiplicity of signals. [c]-[f] Pairwise uncertain assignment. [g] Not assigned.

Despite its insolubility in dichloromethane-d_2, chloroform-d, and in benzene-d_6, its poor solubility in acetone-d_6 was sufficient for the ^1H NMR studies.

There were observed only slight differences in the ^1H NMR data of the aglycone chromomycinone in **3** compared to that in **1**. With the presumption of the formerly established threo configuration at C-3′ and C-4′[9] hydrogen bond bridging with the carbonyl function at C-2′ is likely to occur and leads to torsion angles Ø(HCOH) ≃ 180° which is reflected in the remarkably large HCOH coupling constants J(3′,OH-3′) = 8.0 and J(4′,OH-4′) = 7.6 Hz.

In the carbohydrate region of the ^1H NMR spectrum readily five signals for the anomeric protons δ(H-1A) = 5.31, δ(H-1B) = 4.70, δ(H-1C) = 5.11, δ(H-1D) = 4.69, and δ(H-1E) = 4.96 with coupling constants J(1,2a) = 9.6 9.8 and J(1,2e) = 1.9–2.3 Hz are observed. Thus, in this case these belong to the five saccharides, all of which are β-glycosidically linked to the preceeding saccharide unit or the aglycon, respectively. This finding is in contrast to the described α-linked lyxo unit in the original assignment.[4]

The pulsed INDOR spectroscopy technique[10] turned out to be of essential advantage in the assignment of the ^1H NMR spectrum of **3**. On the one hand the H-1 signals exhibit fairly different chemical shifts, and on the other hand because of the large coupling constants J(1, 2a) one half of the signal can be selectively inverted and allows the observation of an INDOR reply in the axial 2-deoxy protons. Consequently, all the different equatorial and axial 2-deoxy hydrogens can be assigned. In addition to this by the same procedure the hydroxyl signals can be easily attributed to their adjacent ring protons.

In agreement with the formerly established gross carbohydrate composition by hydrolysis studies[4] the present ^1H NMR results are in accord with three 2,6-dideoxy-arabino-, one 2,6-dideoxy-lyxo-, and one 2,6-dideoxy-3-C-methyl-ribo-hexopyranoside(s). By detailed double resonance and pulsed INDOR experiments further assignments could be achieved.

The carbohydrate unit B proved to be a terminal unit because both C-3B and C-4B carry hydroxyl groups evident from the coupling constants J(3B,OH-3B) = 5.8 and J(4B,OH-4B) = 4.2 Hz. Similarly, the sugar E represents another terminal unit in agreement with its unsubstituted C-4E hydroxyl group (J(4E,OH-4E) = 7.7 Hz) assuming a linkage via tertiary hydroxyl group to be rather unlikely.[11] Both the sugar components C and D with arabino configuration exhibit hydroxyl groups at C-4 with coupling constants J(4C,OH-4C) = 0.8 and J(4D,OH-4D) = 0.6 Hz respectively. This can be understood by hydrogen bond bridging to the neighbouring interglycosidic oxygens, which results in torsion angles Ø(HCOH) ≃ 70–90°, reflected in these comparatively small values.

As exemplified by the considerable downfield shift of the anomeric proton (δ(H-1A) = 5.31) the 2,6-dideoxy-lyxo compound A proves to be directly bound to the aglycon in contrast to the former assignment. The terminal saccharide unit B shows 2,6-dideoxy-arabino configuration, and its linkage to another saccharide is confirmed by the chemical shift of H-1B (δ = 4.70). However, its exact attachment site in unit A (at C-3A or C-4A) cannot be demonstrated by ^1H NMR spectroscopy because the remaining

Table 3. ^{1}H NMR data of mithramycin 3[a]

Hydrogen atom	$\delta(^1H)$[b]		$J(H_1)$ [Hz][c]
Aglycone			
H-2	4.78	d	11.5(3)
H-3	2.82	dddd	11.0(4a),11.5(2),3.0(4e),1.7(1
H-4a	2.97	ddd	11.0(3),-15.8(4e),-0.7(10)
H-4e	2.65	dd	3.0(3),-15.8(4a)
H-5	6.87	s	-
H-10	6.85	d	-0.7(4a)
OH-8	9.86	s	-
OH-9	15.95	s	-
H-1'	4.84	d	1.7(3)
H-3'			
H-4'	4.26	m	-
CH_3-5'	1.30	d	6.2(4')
OH-3'	3.80	d	8.0(3')
OH-4'	4.12	d	7.6(4')
CH_3-71	2.13	s	-
OCH_3	3.44	s	-
Carbohydrate A			
H-1A[d]	5.31	dd	9.8(2a),2.2(2e)
H-2aA[d]	1.82	ddd	9.8(1),11.6(3),-12.4(2e)
H-2eA[d]	2.38	dddd	2.2(1),5.2(3),-12.4(2a),0.7(4)
H-3A	3.70	ddd	3.3(4),5.2(2e),11.6(2a)
H-4A	3.23	ddd	3.3(3),1.0(5),0.7(2e)
H-5A	3.63	m	-
CH_3-6A[e]	1.26	d	6.3(5)
Carbohydrate B			
H-1B	4.70	dd	2.0(2e),9.7(2a)
H-2aB	1.61	ddd	12.2(3),-12.2(2e),9.7(1)
H-2eB	2.18	ddd	2.0(1),5.1(3),-12.2(2a)
H-3B	3.58	m	-
H-4B	2.98	ddd	9.4(3),9.4(5),5.2(OH-4B)
H-5B	3.30	dq	9.4(4),6.2(6)
CH_3-6B	1.30	d	6.2(5)
OH-4B	4.21	d	4.6(4)
OH-3B	4.10	d	5.8(3)
Carbohydrate C			
H-1C[d]	5.11	dd	9.6(2a),1.9(2e)
H-2aC[d]	1.63	ddd	-12.6(2e),9.6(1),9.8(3)
H-2eC[d]	2.53	ddd	-12.6(2a),5.2(3),1.9(1)
H-3C	3.66	m	-
H-4C	3.03	ddd	9.0(3),9.0(5),0.8(OH-4)
H-5C	3.49	dq	9.0(4),6.2(6)
CH_3-6C[f]	1.28	d	6.2(5)
OH-4C[f]	4.61	d	0.8(4)
Carbohydrate D			
H-1D	4.69	dd	2.3(2e),9.8(2a)
H-2aD	1.78	ddd	-11.6(2e),12.0(3),9.8(1)
H-2eD	1.94	ddd	2.3(1),4.8(3),-11.8(2a)
H-3D	3.89	ddd	4.8(2e),9.4(4),12.0(2a)
H-4D	2.95	ddd	9.2(5),9.4(3),0.6(OH-4)
H-5D	3.38	dq	9.2(4),6.3(6)
CH_3-6D[f]	1.30	d	6.3(5)
OH-4D[f]	4.65	d	0.6(4)
Carbohydrate E			
H-1E	4.96	dd	9.6(2a),2.2(2e)
H-2aE	1.54	dd	-13.4(2e),9.6(1)
H-2eE	1.88	dd	2.2(1),-13.4(2e)
H-4E	2.94	dd	9.6(5),7.4(OH-4)
H-5E	3.64	dq	9.6(4),6.3(6)
CH_3-6E	1.22	d	6.3(5)
CH_3-31E	1.21	s	-
OH-4E	3.88	d	7.7(4)

a) c=4.8 mmol·l^{-1} in CD_3COCD_3, 270 MHz, Total data memory 32 K, spectral width 4 424.8 Hz, T=296 K.
b) Chemical shifts downfield from Me_4Si ($\delta(CD_3COCD_3)$=2.04) and
c) multiplicity of signals.
d)-f) Coupling constants, coupled hydrogen atoms given in parenthesis. Pairwise uncertain assignment.

Table 4. ^{13}C NMR data of **3**[a]

Carbon atom	$\delta(^{13}C)$[b]		$^1J(C-H)$ [Hz]
Aglycone			
C-1	204.2	s	-
C-2	78.4	d	140.0
C-3	43.4	d	134.0
C-4	45.2	t	128.0
C-5	102.4	d	162.0
C-6	160.3	s	-
C-7	112.1	s	-
C-8	165.1	s	-
C-9	156.8	s	-
C-10	118.1	d	163.0
C-4a	139.7	s	-
C-8a	109.3	s	-
C-9a	108.8	s	-
C-10a	137.0	s	-
CH_3-71	8.5	q	127.0
C-1'	83.2	d	146.0
C-2'	213.3	s	-
C-3'	80.0	d	150.0
C-4'	69.4	d	144.0
Carbohydrate A			
C-1A	99.0	d	160.0
C-5A[c]	72.1	d	141.0
Carbohydrate B			
C-1B	100.2	d	160.0
C-4B[d]	78.2	d	142.0
C-5B[e]	73.7	d	139.0
Carbohydrate C			
C-1C	102.1	d	160.0
C-3C	81.2	d	143.0
C-4C[d]	77.9	d	140.0
C-5C[e]	73.4	d	140.0
Carbohydrate D			
C-1D	100.2	d	160.0
C-3D	81.2	d	140.0
C-4D[d]	76.7	d	142.0
C-5D[e]	73.4	d	140.0
Carbohydrate E			
C-1E	97.7	d	161.0
C-3E	71.8	s	-
C-4E[c]	72.1	d	141.0
C-5E	70.4	d	144.5
Remaining carbon atoms[f]			
CHO	77.5	d	140.0
CHO	76.3	d	143.0
CHO	72.0	d	141.0
OCH_3	59.6	q	142.0
CH_2	40.8	t	130.0
CH_2	38.3	t	131.5
CH_2	38.0	t	131.5
CH_2	33.2	t	132.0
CH_2	28.1	t	130.0
CH_3	27.3	q	126.0
CH_3	19.8	q	126.0
CH_3	18.7	q	127.0
CH_3	18.6	q	126.5
CH_3	18.5	q	126.5
CH_3	18.1	q	126.5
CH_3	17.0	q	127.0

a) c=0.042 mol·l^{-1} in CD_3OD, 67.89 MHz, Total data memory 16 129.0 Hz, T=303 K.
b) Chemical shifts downfield from Me_4Si ($\delta(CD_3OD)$=49.0) and multiplicity of signals.
c)-e) Pairwise uncertain assignment.
f) Not assigned.

hydroxyl signal (OH-3A or OH-4A) is obscured by the HDO signal.

A close inspection of the coupling constants in C and D readily show the all trans-axial arrangement of the ring protons H-1, H-3, H-4 and H-5, and thus β-arabino configurations. In accord with the chemical shift of the anomeric proton H-1C (δ = 5.11) C represents a unit directly linked to the aglycon. From the above mentioned OH-4C signal an interglycosidic linkage to the saccharide D via C-3C is ensued. By a corresponding argument owing to the OH-4D signal the terminal saccharide unit E has to be linked via C-3D. The anomeric proton H-1E resonates at comparatively low field (δ = 4.96) which is attributed to a 1,3-diaxial interaction with the tertiary hydroxyl group at C-3E.

With regard to the unsatisfactory solubilities the ^{13}C NMR spectrum of **3** had to be recorded in methanol-d_4. However, since no resolved ^1H NMR spectrum was obtained in this solvent the complete assignment of all the carbon signals by selective double resonance experiments[6] could not be acchieved. For an extensive interpretation some successful selective double resonance experiments and comparison with the ^{13}C NMR spectral data of **1**[6] and **2** could be used.

The difference for the chemical shift of chromomycinone of **3** and of **1** amounts to $\Delta\delta$ = 3.7, whereas the other deviations are between $\Delta\delta$ = 0 and 2.4. Owing to the protonic solvent methanol-d_4 the side chain obviously adopts a different conformation than in deuterochloroform.[6] As demonstrated before the B-linkages of all the saccharides are clearly confirmed by the chemical shifts of the anomeric carbon atoms as well as by the ^1J(C-1, H-1) coupling constants around 160 Hz.[7] A further proof for the glycosidic linkages to the C-3 positions in both the units C and D could be obtained by selective double resonance studies and comparision with the ^{13}C NMR spectra of **1**[6] and **2**. The terminal position of the saccharide unit E is also evident by the chemical shift of C-3E (δ = 71.8) and C-4E (δ = 72.1) which unequivocally exclude glycosidic linkages to this unit.

Because a clear-cut assignment of C-3A and C-4A was not feasible by ^{13}C NMR spectroscopy the binding site of the unit B to the unit A remains uncertain. Finally, a definite spectroscopic evidence for the exact positions of the units A and C with respect to the aglycon cannot be obtained. With slight reservations owing to the above mentioned uncertainties, which are to be settled conclusively by synthetic studies in progress, these results show mithramycin (**3**) to be (3R)-[(3S),(4R)-dihydroxy-(1S)-methoxy-2-oxopentyl]-3,4-dihydro-8,9-dihydroxy-7-methyl-1-oxo-2H-anthracen-6-yl-[2,6-dideoxy-3- (or 4-)-O-(2,6-dideoxy-β-D-arabino-hexopyranosyl)-β-D-lyxo-hexopyranoside]-(2S)-yl-{2,6-dideoxy-3-O-[2,6-dideoxy-3-O-(2,6-dideoxy-3-C-methyl-β-D-ribo-hexopyranosyl)-β-D-arabino-hexopyranosyl]-β-D-arabino-hexopyranoside}.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded on a Bruker WH 270 spectrometer (^1H: 270 MHz and ^{13}C: 67.89 MHz). Generally a 90° measuring pulse was applied. In homonuclear pulsed INDOR experiments a selective 180° pulse of 0.15 s and a non-selective 40° pulse were applied. The delay between two 180° pulses was 4 s, between the selective and the non-selective pulses 0.01 s.

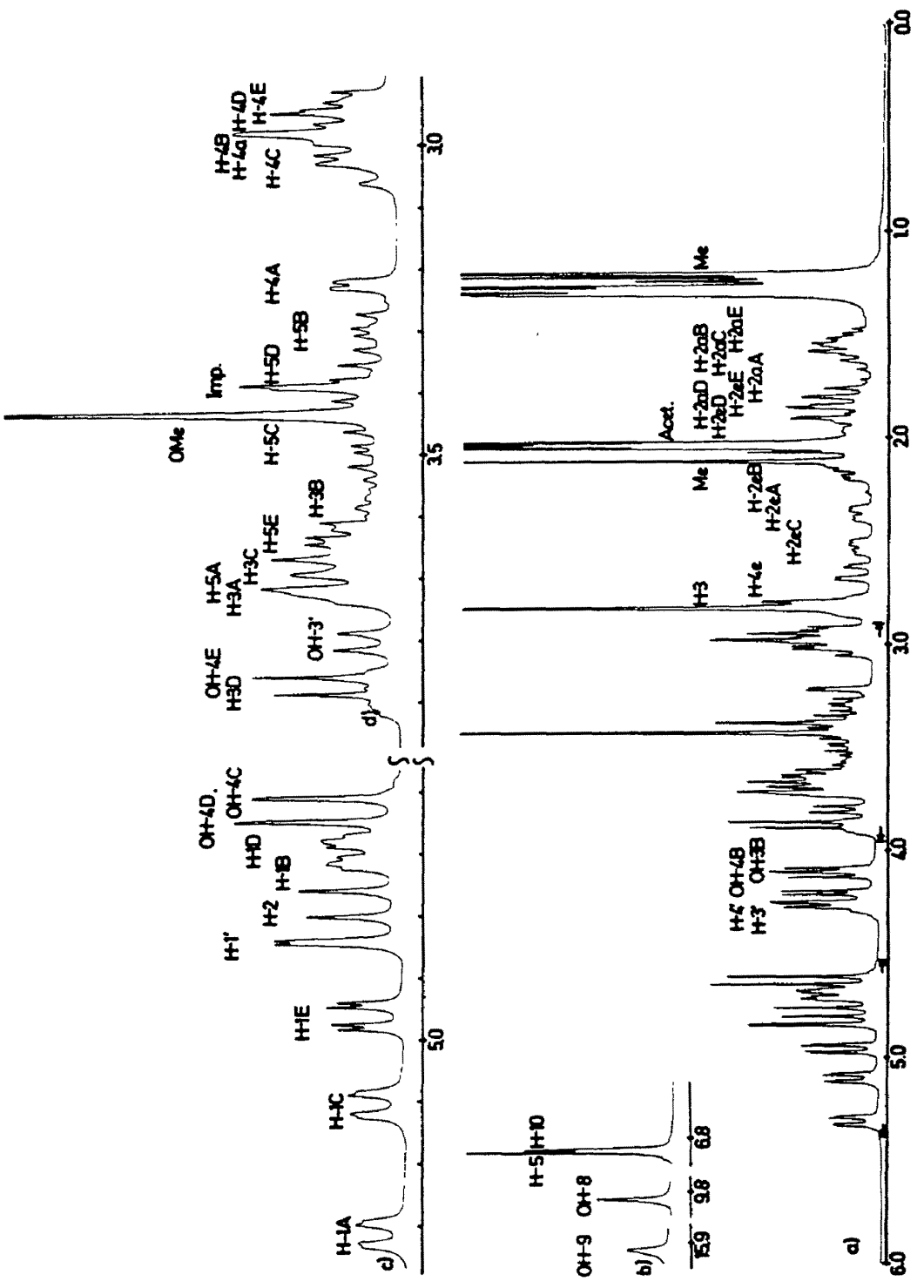


Fig. 1. ^{1}H NMR spectrum of mithramcyin (**3**) in acetone-d_6 (270 MHz). (a) normal spectrum, (b) downfield signals (normal spectrum); (c) and (d) selected expanded proton regions.

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