Tetrahedron Vol 39, No 9, pp 1583 to 1591, 1983
Printed in Great Britain

0040-4020/83/091583-09$03.00/0
Pergamon Press Ltd

MOENOMYCIN A: FURTHER STRUCTURAL STUDIES AND PREPARATION OF SIMPLE DERIVATIVES

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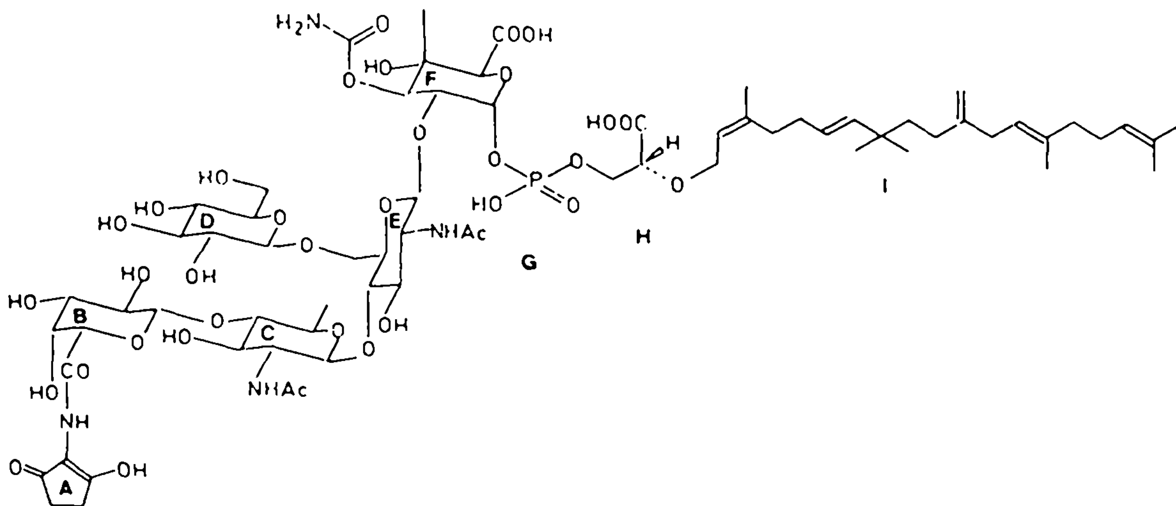
(*Received in Germany 9 August 1982*)

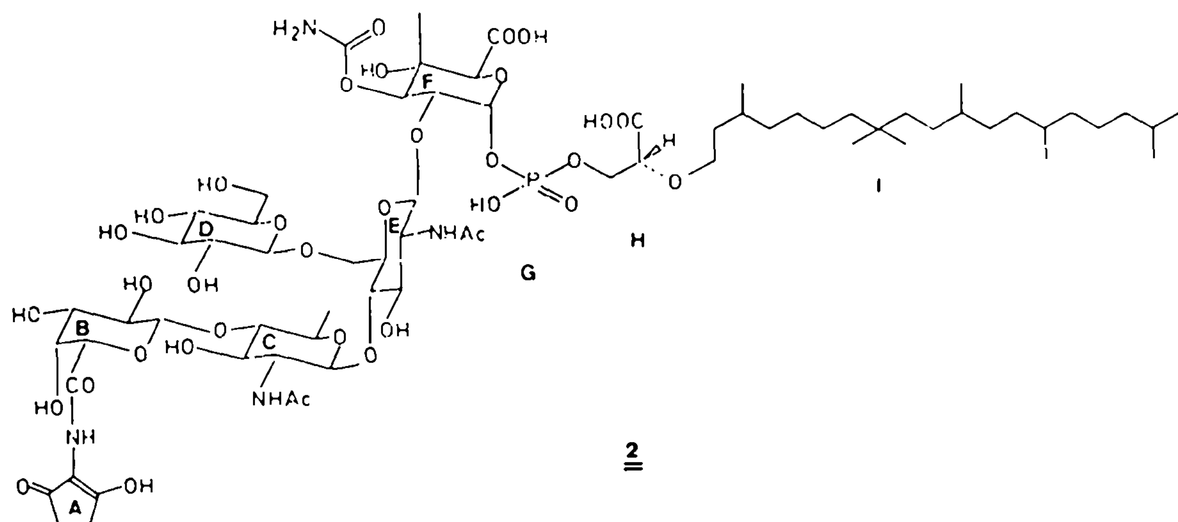
Abstract— Preparation of the moenomycin A derivatives **2–5** is reported. Structure **1** proposed for moenomycin A is confirmed by ^{13}C NMR and fast atom bombardment mass spectrometry data. Compounds **2** and **3** are antibiotically active whereas methyl derivatives **4** and **5** lack activity.

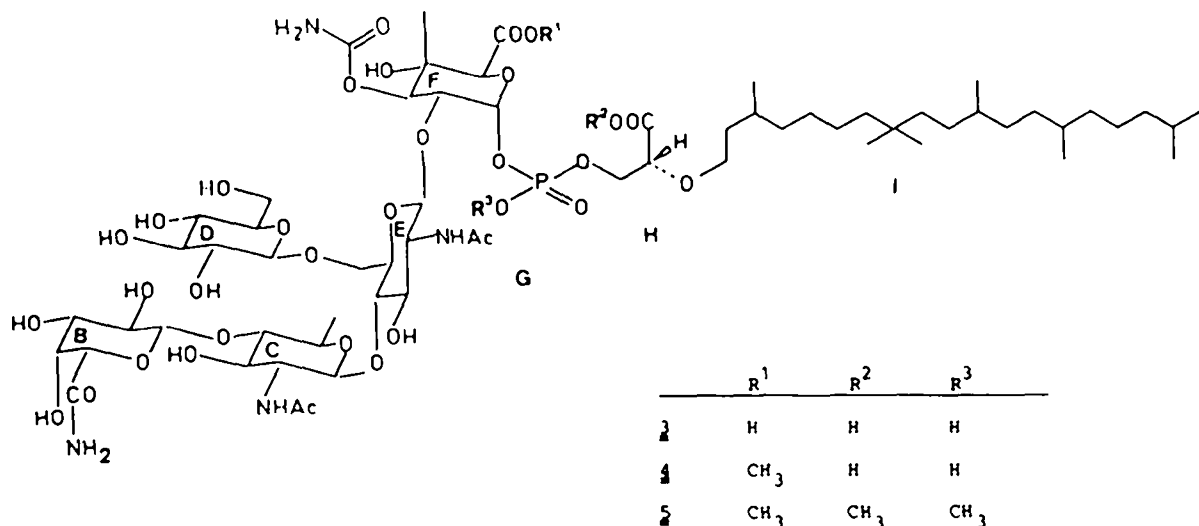
The antibiotic moenomycin A is the chief constituent of the trade product flavomycin® which is employed in animal nutrition.[1] It belongs to the most efficient inhibitors of the biosynthesis of bacterial cell wall peptidoglycan. Although the mechanism of this action is not completely understood, recent work by von Heijenoort *et al.* has demonstrated that the moenomycin-type antibiotics interact with the enzyme(s) catalyzing the transglycosylation step in which a disaccharide unit is transferred from a phospholipid intermediate to the growing linear peptidoglycan chain.[2] Recently, we proposed structure **1** for moenomycin A.[3] In this paper we wish to report on some spectral properties of **1** and on the formation of its simple derivatives **2**, **3**, **4** and **5**. Our work is directed towards defining the structural basis of the antibiotic action of **1**.

Moenomycin A (**1**) *and decahydromoenomycin A* (**2**)

Moenomycin A (**1**) is obtained from the flavomycin® complex by silica gel chromatography followed by reversed phase chromatography. The structure of **1** had to be deduced on the basis of degradation experiments and the spectroscopic properties of the degradation products. **1** does not crystallize and its structure turned out to be too complicated to be established solely by the spectroscopic methods available at the time when most of the structural work was done. Powerful new spectroscopic methods recently introduced confirm our earlier proposal. Using Barber's technique of fast atom bombardment mass spectrometry (FAB-MS)[4] for the first time mass spectra of **1** became available providing molecular weight information. Excellent positive ion spectra were obtained fully in accord







with structure 1. The ^{13}C-NMR spectra also confirm many of the structural details previously established. They will be later discussed in detail (*vide infra*). At this point we shall concentrate on the C-1, C-2 and C-3 signals of the "chromophore" unit (A) of 1 which were assigned by comparison with those of model compound 6[5] (see Tables 1 and 3). C-4 and C-5 of 6 give rise to only one signal. Similarly, only one signal is observed for C-1 and C-3 of 6 and the chromophor unit (A) of 1. This finding is in accord with the ^{1}H-NMR spectra of 2 in which the protons of the two ring methylene groups show only one averaged signal a fact which has been explained by rapid proton exchange in associated forms.[5] Interestingly, in the proton noise decoupled spectrum the C-1/C-3 signal of 6 and that of the amide CO are broadened. We believe this to be caused by a slow rotation process around the amide N–CO bond.[6] In the spectrum of 1 this broadening of the C-1/C-3 (A) signal was not observed. The moenocinol (unit I) signals were assigned by Coates.[7]

Hydrogenation of 1 in methanolic solution over Adam's catalyst yielded the decahydro-derivative 2.[8] From the FAB mass spectrum and the ^{13}C-NMR spectrum it was obvious that all the double bonds in the lipid part had been saturated. The chromophore

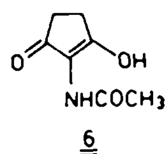
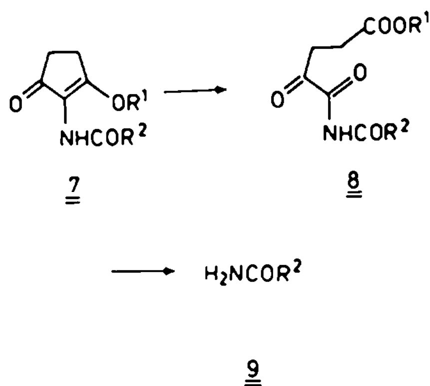


Table 1. [13]C spectral data for 6 (in [D_6] DMSO)

δ	185.7	171.7	114.8	28.4	21.7
assignment	C-1, C-3	NH$\underline{C}$OCH$_3$	C-2	C-4, C-5	NHCO$\underline{C}$H$_3$

unit (A) is inert under these conditions.[9] 2 is much more stable to acid treatment than 1 because it lacks the very labile allyl ether linkage between units I and H.

As well as providing molecular ions of 1 and 2 (Fig. 1), the FAB spectra show several fragments of 1 and 2 which are entirely consistent with the proposed structures. Fragmentation takes place as shown in Fig. 2, thus providing structural information about the various moieties of the molecules. For example, the GHI fragment is 10 mass units larger in 2 than in 1, while the ABCDEFG fragment has the same mass in both 1 and 2, thus confirming that the reduction has taken place in the lipid sidechain of the antibiotic (exclusively).



Ozonolysis product 3

We have previously shown that compounds of type 7 react with ozone to give N,N-diacyl amines 8. From 8 the α-ketoacid residue is removed selectively by solvolysis to given primary amides 9.[5,10] Making use of this procedure we transformed 2 into 3.

In the positive ion FAB spectrum of 3 both intense molecular ions (M) and fragments (BCDEF, BCDE, GHI) confirm that only the desired degradation of the chromophore (A) had taken place. This was corroborated by simple degradation reactions. From our previous work it is known that the glycosidic bonds of the 2-acylamino-2-deoxy sugar units (C and E) in 1 can be cleaved rather selectively with trifluoroacetic acid.[11] Neighboring group participation of the amide groups operates and intermediate oxazolinium ions are formed. Interestingly, and probably for similar reasons, these same bonds are selectively labile in positive ion FAB fragmentations (Fig. 2).

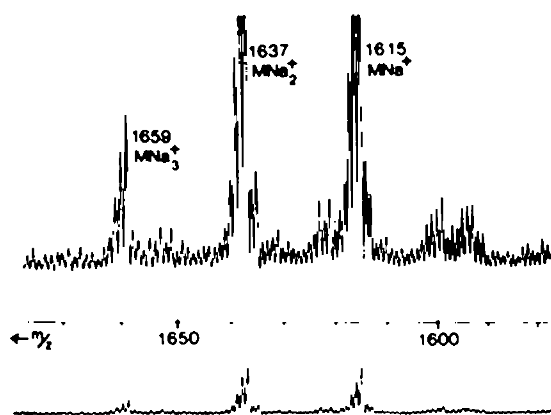


Fig. 1. FAB-MS spectrum of 2 after ion-exchange.

On the basis of these results we expected 2 to give 10 and 12 on trifluoroacetic/water treatment. After reduction of the cleavage products with sodium borohydride, Hakomori methylation,[12] and Sephadex LH-20 purification, the gas chromatogram (see Experimental for conditions) showed two main peaks corresponding to 14 and 15. Reference samples of 14 and 15 were prepared from the known moenomycin A degradation products 11[10] and 13[13] by sodium borohydride reduction and methylation.

When subjected to combined gas chromatography/mass spectrometry (GC/MS) analysis, 14 and 15 gave mass spectra fully consistent with the proposed structures. The most diagnostic fragments are depicted in formulae 14 and 15. The mass spectrum of 14 clearly shows the expected amide group in the galacturonic

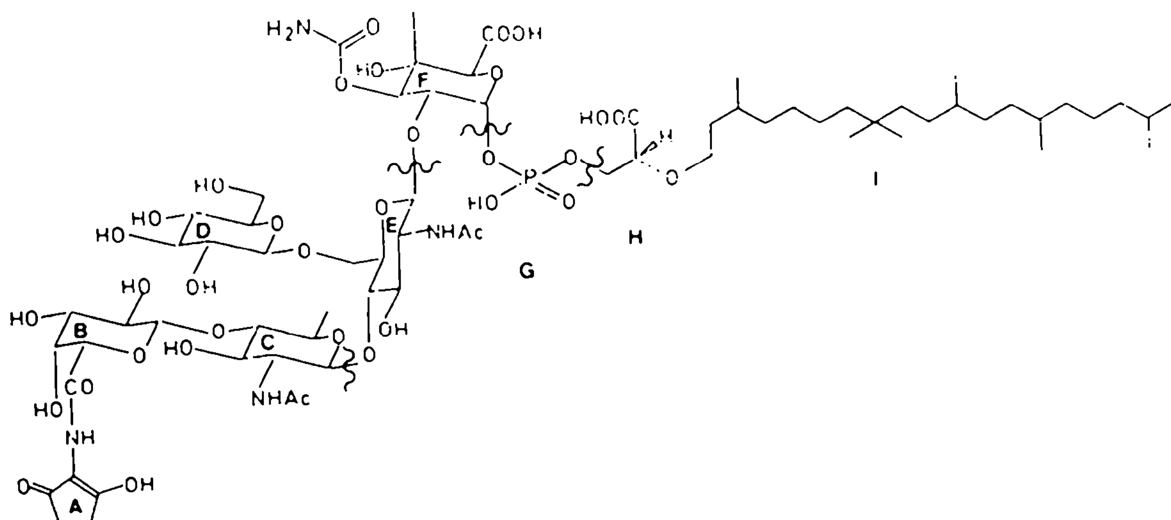
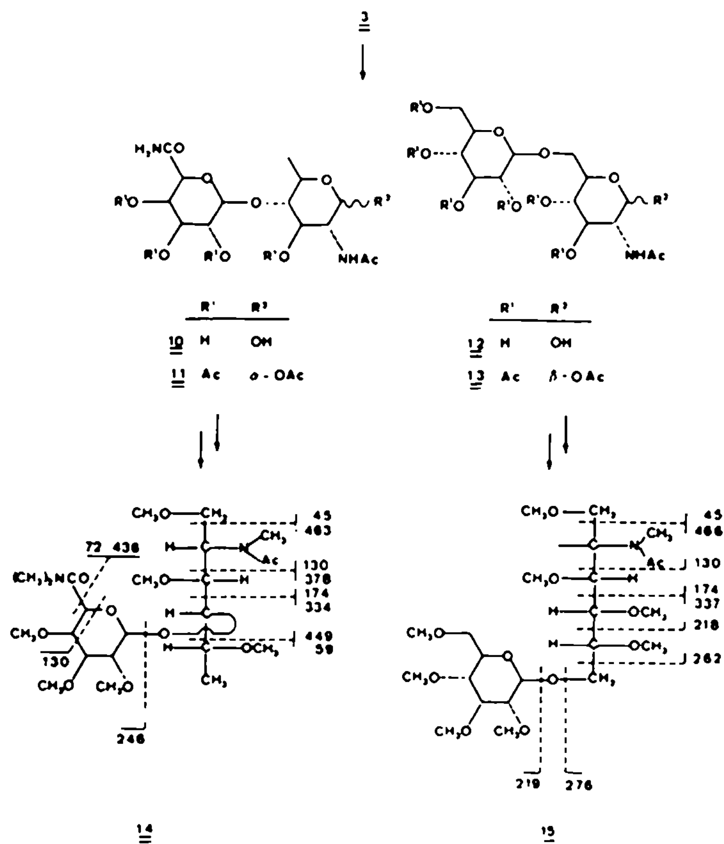


Fig. 2. FAB-MS fragmentation in 2.

Table 2. ^{13}C spectral data for **16** (in [D$_6$] DMSO)

δ	104.3	70.4*	73.4	69.2*	75.2	170.9	56.3
assignment	C-1	C-2	C-3	C-4	C-5	C-6	OCH_3

* Assignment interchangeable



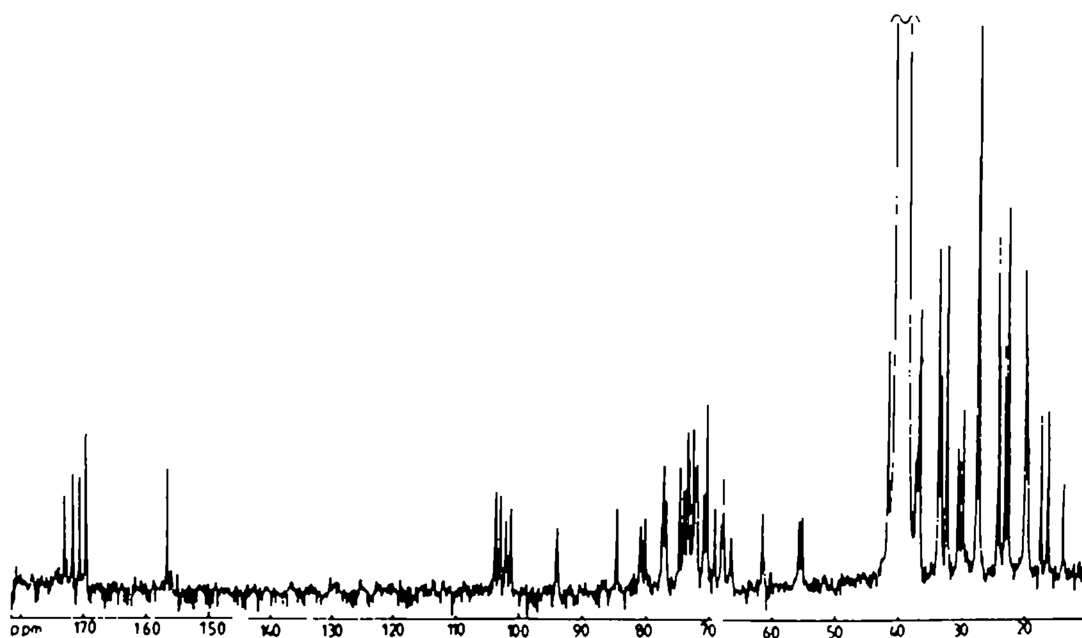


Fig. 3. The 62.9 MHz ^{13}C spectrum of 3 (in [D$_6$] DMSO).

Table 3. ^{13}C spectral data (δ-values) for units A–H of compounds 1–5 (in $[D_6]$ DMSO)

Moenomycin A (1)	Decahydro-moenomycin A (2)	Ozonolysis product (3)	Monoester (4)	Triester (5)	Assignment: Carbon number	Assignment: Unit	Method of assignment (model compound, reference)
192.8	191.9				C-1, C-3	A	6
173.0	173.3	173.1	172.1	170.1	C-1	H	ref.[15]
171.3	171.6	171.7	171.7	169.8	C-6	F	ref.[15]
167.4	167.6	170.6	170.7	169.3	C-6	B	16
170.0	169.8		169.5			C	
		169.7		169.0	NHCOCH3		ref.[10]
169.4	169.7		169.2			E	
156.8	156.6	156.5	156.4	156.2	O-C(=O)NH2	F	ref.[15]
110.0	110.6				C-2	A	6
*)	*)	103.6	103.7	103.6	C-1	D	ref.[18]
		102.9	102.9	103.3	C-1	B	16
		101.9	102.2	102.4	C-1	E	ref.[19]
		101.2	101.4	101.1	C-1	C	ref.[19]
93.0	93.7	93.7	93.8	96.3	C-1	F	ref.[15]
83.9	84.0	84.4	84.4	84.5	C-4	C	ref.[20,21]
		80.7	80.7	79.7	C-4	E	ref.[19]
80.0	ca. 80.2						
		80.0	78.6	77.1	C-2	H	ref.[22]
					C-2	F (?)	
		77.2	77.4	77.0	C-3	D	ref.[18]
	ca. 77.0						
		77.1	77.0	76.8	C-5	D	ref.[18]
	76.5	76.7	76.9	76.4	C-3	F	ref.[15]
	*)	74.6	74.6	74.6	C-5	B,E	16 ref.[19]
		74.0	73.9	73.6	C-2	D	ref.[18]
		73.4	73.5	73.5	C-3	B	16
*)	73.3	73.2	73.2	73.2	C-5	F	ref.[15]
	72.6	72.6	72.7	72.3	C-4	F	ref.[15]
	72.5	72.3	72.0	72.0	C-3	C	ref.[19]
		72.0	72.0	71.8	C-3	E	ref.[19]
		71.9	71.7	70.7	C-6	E	ref.[19]
		70.8	70.9	70.4	C-2	B	16
	*)	70.3	70.4	70.2	C-4	B	16
		69.0	69.1	69.0	C-4	D	ref.[18]
		67.9	68.4	68.7	C-5	C	ref.[21]
		67.6	68.0	67.6	C-1	I	ref.[22]
65.0	66.4	66.4	65.1	67.1	C-3	H	ref.[22]
61.1	61.4	61.4	61.4	61.3	C-6	D	ref.[18]
55.4		55.5	55.6	55.4	C-2	E	ref.[19]
	ca. 55.6						
54.2		55.0	54.8	55.0	C-2	C	ref.[19]
				54.5	COOCH3	F	ref.[23]
				54.3	POCH3	G	ref.[24]
			51.8	52.1	COOCH3	H	17
ca. 23.0	23.0	23.1	23.2	23.1	NHCOCH3	E	ref.[19]
	22.5	22.5	22.7	22.6	NHCOCH3	C	ref.[19]
17.5	17.5	17.3	17.3	17.2	CH3-6	C	ref.
16.3	16.2	16.2	16.4	16.1	CH3-4	F	ref.[15]

*) Unresolved spectral region

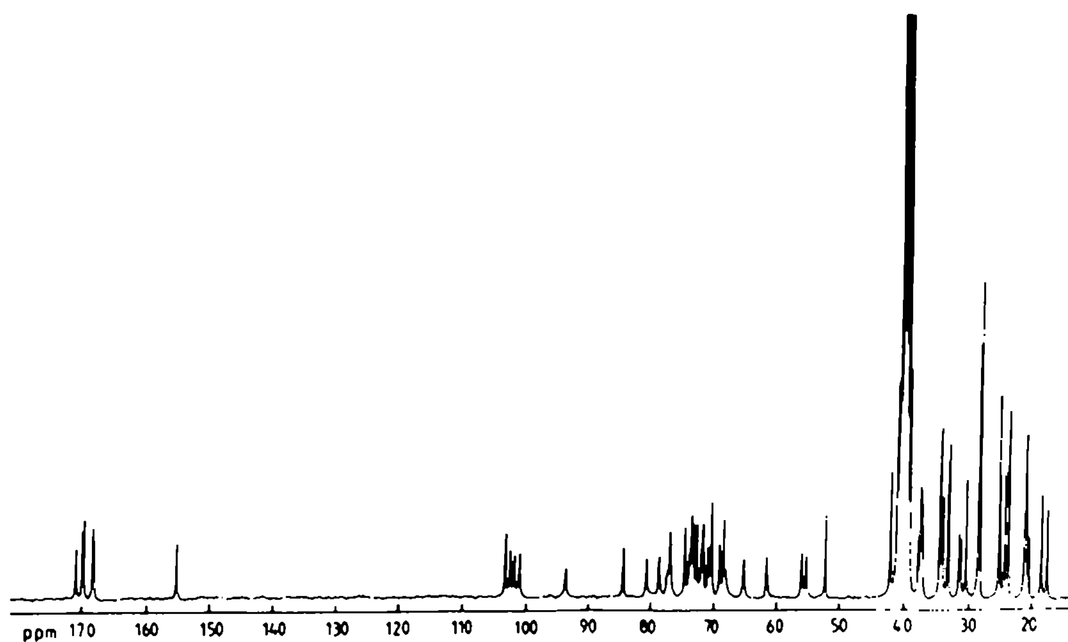


Fig. 5. The 62.9 MHz ^{13}C spectrum of 5 (in [D_6] DMSO).

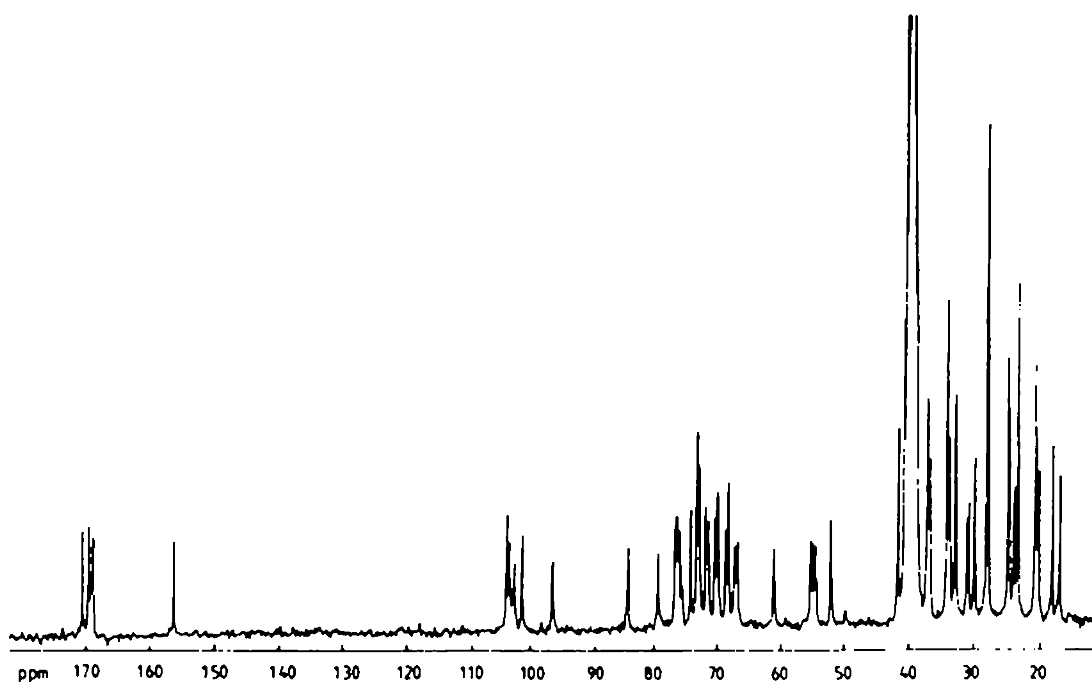
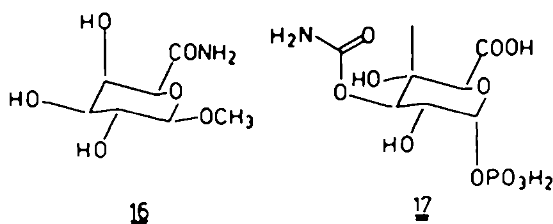


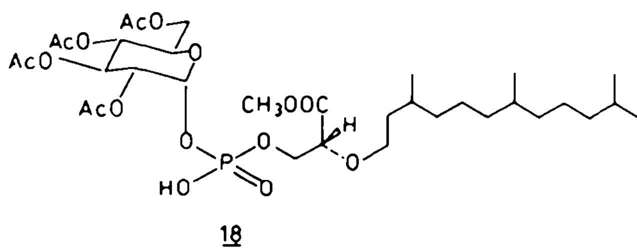
Fig. 4. The 62.9 MHz ^{13}C spectrum of 4 (in [D_6] DMSO).

acid part (ions m/z 72 and 436). Fragments m/z 246 and m/z 219 in the spectra of **14** and **15** and the ions resulting from cleavages of the carbon carbon bonds in the alditol portions allowed unequivocal determination of the 1→4 and 1→6 linkages in **14** and **15**, respectively.[14] Ion m/z 130 in the spectrum of **14** needs some comment. It is probably formed by repture of the C-2 C-3 bond in the alditol part as well as from the C-4, C-5 and C-6 part of the galacturonic acid moiety. A peak at m/z 130 was also found in the methylation product of model compound **16**.



From **3** a beautiful and very informative ^{13}C-NMR spectrum was obtained (see Fig. 3). For almost all units of **3** at least one signal could be assigned with certainty (C-4 (C), C-2 (C), C-4 (E), C-2 (E), C-6 (D), H_2N CO O (F), C-1 (F), C-2 (H), C-3 (H), C-2 (J). The assignments were made on the basis of resonance multiplicities (where possible) and by comparison with model compounds (see Table 3).

A rather difficult task in the structure elucidation of **1** has been to decide whether the glucosamine unit (E) is attached to the 2- or to the 4-position of the moenuronic acid moiety (F).[15] We have reinvestigated



this problem using a new ^{13}C-NMR technique. The C-4 signal of degradation product **17** appears at $\delta = 74.1$ (D_2O solution).[15] This signal should be slightly upfield shifted (~ 1 ppm by a small δ-effect) in the spectrum of a compound having unit E attached to the 2-position whereas substitution in the 4-position would cause a large downfield shift (8–10 ppm) by the β-effect of the 4-O-substituent.[16] In principle, the C-4 signal of unit F can be differentiated from all other near-by signals by an off-resonance proton decoupling experiment since it is the only one in this part of the spectrum to yield a singlet. Unfortunately, this region in the spectrum of **3** contains so many resonances that coupling information could not be retrieved from it. Recently, an improved method was introduced allowing recognition of one-bond C H coupling multiplicities by a simple excitation sequence (J-modulated spin-echos).[17] Applying this techniques to the analysis of the spectrum of **3**, the C-4 (F) signal could easily be recognized. After a delay time of $\tau = 1/J$, when quaternary carbon (and CH_2) resonances are positive and methine carbon (and CH_3) resonances negative, the $\delta = 65$-80 region in the spectrum of **3** showed only one positive signal at $\delta = 72.6$. This was confirmed performing an experiment with a delay time of $\tau = 1/2J$. Under these conditions only quaternary carbon resonances contribute significant intensity. In the $\delta = 65$ 80 region only one (positive) signal appeared: at $\delta = 72.6$. These results clearly demonstrate that the C-4 (F) signal is at $\delta = 72.6$, hence the glucosamine unit (E) must be attached to the 2-position of the moenuronic acid part (F) in **1**.

Methyl esters **4** *and* **5**

After treatment of **2** (in methanolic solution) with ethereal diazomethane TLC showed 4 reaction products. Two of them could be isolated in pure form in 22 and 25% yield, respectively. One of the compounds was a monomethylester as could be deduced from the positive ion FAB mass spectrum showing strong MNa^+, MK^+ and MNa_2^+ peaks. In the very informative ^{13}C-NMR spectrum (see Table 3 and Fig. 4) the OCH_3 signal appeared at $\delta = 51.8$. The glyceric acid moiety (H) is esterified, and the compound has structure **4**. This is suggested by comparison with model compound **18** ($\delta_{OCH_3} = 52.4$)[18], and confirmed by FAB MS. The fragment GHI containing the glyceric acid moiety (H) is observed at 14 mass units higher in **4** than in **3**, while the BCDEF fragment has the same mass in both derivatives.

The second methylation product was triester **5**. Its ^{13}C-NMR spectrum (Fig. 5, Table 3 showed 3 OCH_3-signals. One of them was split due to ^{13}C ^{31}P-coupling.

Antibiotic activity of **2**, **3**, **4**, and **5**

2, **3**, **4** and **5** were assayed for antibiotic activity using TLC followed by bioautography with *S.aureus* and *E.coli*. Active compounds were submitted to a screening with 24 gram-positive and gram-negative test organisms. **1** and **2** were similar in their activity whereas **3** showed somewhat diminished activity. Interestingly, methyl derivatives **4** and **5** did not show any antibiotic activity under these experimental conditions.

EXPERIMENTAL

The instrumentation used was ^{1}H-NMR: WP-80 (Bruker), WH-250 (Bruker), ^{13}C-NMR: WH-250 (Bruker), GC/EI-MS: glass capillary column (OV 17) coupled directly to a Varian MAT CH-5 mass spectrometer. FAB mass spectra were run in the positive ion mode on a Kratos MS-50 instrument equipped with a high mass magnet (mass range ca 3500 daltons at 8 kV). Samples were dissolved in glycerol on a copper probe tip and bombarded with 4-6 keV xenon atoms from a Kratos FAB source. Typical operating source

pressure was 10^{-5} torr, and spectra were scanned at 30-300 sec/decade.

Cation-exchange of purified **1** and **2** was accomplished by dissolving a small sample in 10 mM ammonium formate buffer (pH 3.1) and passing the solution through a small column of SP-Sephadex C-25 (NH_4^+ form) in the same buffer. This treatment resulted in improved FAB mass spectra.

Medium pressure LC was performed using 31.0 cm × 2.5 cm glass tubes, silica gel 60 (Merck), Duramat pump (CfG), UV detector Chromatochord III (Serva). GC was performed on a Pye 104 (Philips).

Moenomycin A (**1**)

Flavomycin® (58g) was dissolved in water (500 ml) and adsorbed on silica gel (200g, Merck, 0.06-0.2 mm) by evaporation of the solvent. The dry powder was placed on top of a column which was packed from a slurry of silica gel (2 kg) in 2-propanol–water (9:2). The column was eluted with 2-propanol-2M NH_3 (9:2, 5l), 2-propanol-2M NH_3 (8:2, 6l), and 2-propanol-2M NH_3 (8:3, 15l). Fractions containing **1** (TLC solvent system: conc NH_3–2-propanol (65:35) were collected. After evaporation and lyophylization almost pure **1** (15g) was obtained. 3g of this material were dissolved in water (15 ml) and separated by prep HPLC (Waters prep LC 500 with two C18 prep pack cartridges; mobile phase: methanol-acetonitrile–water (4:1:5), flow rate: 100 ml/min; detector: UV (275 nm)). Only the central fractions of the eluting peak were collected to give after evaporation and lyophilization pure **1** (540 mg). ^{13}C-NMR: see Table 3; FAB–MS: 1681 ($MNaK_2^+$), 1659 (strong, MK_2^+), 1643 (strong, $MNaK^+$), 1621 (MK^+), 1563, 1229 (strong, ABCDEFG-K_2^+), 1183, 1095 (ABCDEF-K^+), 902, 864 (FGHI-K_2^+), 642 (DEF-NaK^+), 604 (DEF-Na^+); after ion-exchange: 1627 (MNa_2^+), 1605 (MNa^+), 1078 (strong, ABCDEF–Na^+), 846 (strong, ABCDE-Na^+), 549 (GHI-Na^+).

Decahydromoenomycin A (**2**)

Pure **1** (516.6 mg, 0.33 mmol) and PtO_2 (106 mg) were stirred in methanol (52 ml) for 110 h at 22° under hydrogen (atmospheric pressure). The mixture was then filtered and the catalyst washed four times with methanol. The combined filtrates were evaporated and the crude white product was at 35° dissolved in a methanol–acetonitrile–water mixture and loaded on a small precolumn (10g silylated silica gel, Merck, 0.063–0.200 mm). Medium pressure LC (RP-8 silica gel, Merck, 40–63 μm), methanol–acetonitrile–water 6:3:1) gave **2** (381 mg, 74%). ^{13}C-NMR: see Table 3; FAB-MS: 1697 (strong, MNa_3K^+), 1681 (MNa_4^+), 1659 (strong, MNa_3^+), 1426 (strong), 1388, 1267 (strong, ABCDEFG–K_2^+), 902, 874 (FGHI-K_2^+); after ion-exchange: 1659 (MNa_3^+), 1637 (strong, MNa_2^+) 1615 (strong, MNa^+), 1155 (DEFGHI–K^+), 1139 (strong, DEFGHI–Na^+, 1078 (strong, ABCDEF-Na^+), 862 (ABCDE–K^+), 846 (ABCDE-Na^+).

2 - *O* - {2 - *Acetylamino* - 4 - *O* - [2 - *acetylamino* - 4 - *O* - ((5*S*) - 5 - *carbamoyl* - *β* - *L* - *arabinopyranosyl*) - 2,6 - *dideoxy* - *β* - *D* - *glucopyranosyl*] - 2 - *deoxy* - 6 - *O* - *β* - *D* - *glucopyranosyl* - *β* - *D* - *glucopyranosyl*} - 3 - *O* - *carbamoyl* - 1 - *O* - {[(*S*) - 2 - *carboxy* - 2 - (3,8,8,11,14,18 - *hexamethyl* - *nonadecyloxy*) - *ethoxy*] - *hydroxyphosphoryl*} - 4 - *C* - *methyl* - *α* - *D* - *glucopyranuronic acid* (**3**)

2 (1.051 g, 0.66 mmol) was dissolved in dry methanol (200 ml) and at −78° ozonized (10 l/h = 0.1 mmol O_3/min). When **2** could no longer be detected by TLC, the mixture was allowed to warm up to room temp. and was then flushed with O_2 to remove all O_3. Dimethylsulfide (0.65 ml, 13 mmol) was added and allowed to react at room temp overnight. After evaporation of methanol, addition of water, lyophilization, and medium pressure LC (chloroform methanol water 18:11:2.7) **3** (647 mg, 60%) was obtained. IR (Nujol): 3350 (broad, OH), 1700-1600 cm^{-1} (CO); ^{13}C-NMR: see Table 3 and Fig 3; FAB–MS: 1573 (strong, MK_2^+), 1557 (strong, $MNaK^+$), 1541 (MNa_2^+), 1535 (strong, MK^+), 1519 (strong, MNa^+), 998 (BCDEF-K^+), 982 (BCDEF–Na^+), 873, 857, 835, 766 (BCDE–K^+), 750 (BCDE-Na^+), 613 (GHI-K_2^+), 597 (GHI-NaK^+), 575 (GHI-K^+), 559 (strong, GHI–Na^+), 547.

Degradation of **3** *and identification of* **14** *and* **15**

3 (20 mg) was treated with trifluoroacetic acid for 5 h at 60°. The trifluoroacetic acid was then removed by evaporation under a stream of argon. The residue was dissolved in water (2 ml) and reduced with sodium borohydride (60 mg) overnight at room temperature. Excess borohydride was decomposed by addition of acetic acid. The solution was passed through a small Sephadex G-25 column and the column washed with water. After solvent evaporation under a stream of argon, the borate ions were removed as methyl borate by repeated addition and evaporation of methanol. The residue was permethylated by the Hakomori procedure.[12] The permethylated material was passed through a Sephadex LH-20 column using ethanol as solvent. After evaporation of the solvent, the residue was analyzed by GC and combined GC/MS. Reference samples of **14** and **15** were prepared from **11** and **13**, respectively, by reaction with sodium borohydride and subsequent Hakomori methylation.[14] GC conditions: 5 m × 0.25 mm i.d. OV-17 glass capillary column. Column: 210°, injector: 230°; FID: 280°. 0.9 bar hydrogen carrier.

Methyl-β-D-galactopyranosiduronamide (**16**)

16 was prepared from its 2,3,4-tri-O-acetyl derivative[20] by reaction with methanolic NH_3. M.p. 194-196° (from acetone). ^{1}H-NMR (80 MHz, [D_6] DMSO): δ = 3.45 (s, OCH_3), 4.58 (d, $J_{1,2}$ = 5.2 Hz, 1-H); ^{13}C-NMR: see Table 2.

Reaction of **3** *with diazomethane*

The acid form of **3** was obtained by passing an aqueous solution through an ion exchanger column (Dowex 50, H^+-form) and lyophylization of the effluent. 496 mg of this material were dissolved in methanol (42 ml) and at room temperature treated with ethereal diazomethane until a yellow colour persisted. Excess diazomethane was destroyed by addition of acetic acid. Evaporation of the solvent, addition of water, lyophilization, and medium pressure LC (chloroform methanol water 16:9:1.5) gave **4** (109 mg, 22%) and **5** (124 mg, 25%). Traces of silica gel were removed by passing methanolic solutions of **4** and **5** through short Diaion HP-20 resin (Mitsubishi) columns.

2 - *O* - {2 - *Acetylamino* - 4 - *O* - [2 - *acetylamino* - 4 - *O*((5*S*) - 5 - *carbamoyl* - *β* - *L* - *arabinopyranosyl*) - 2,6 - *dideoxy* - *β* - *D* - *glucopyranosyl*] - 2 - *deoxy* - 6 - *O* - *β* - *D* - *glucopyranosyl* - *β* - *D* -*glucopyranosyl*} - 3 - *O* - *carbamoyl* - 1 - *O* - {[(*S*) - 2 - *methoxycarbonyl* - 2 - (3,8,8,11,14,18 - *hexamethyl* - *nonadecyloxy*) - *ethoxy*] - *hydroxyphosphoryl*} - 4 - *C* - *methyl* - *α* - *D* - *glucopyranuronic acid* (**4**)

^{13}C-NMR: see Table 3 and Fig. 4; FABS-MS: 1555 (strong, MNa_2^+), 1549 (strong, MK^+), 1533 (strong, MNa^+), 982 (BCDEF-Na^+), 849, 833, 750 (BCDE–Na^+), 728 (BCDE-H^+), 681, 589 (strong, GHI-K^+), 573 (strong, GHI-Na^+), 551 (strong, GHI-H^+), 363.

Methyl 2 - *O* - {2 - *acetylamino* - 4 - *O* - [2 - *acetylamino* - 4 - *O* - ((5*S*) - 5 - *carbamoyl* - *β* - *L* - *arabinopyranosyl*) - 2,6 - *dideoxy* - *β* - *D* - *glucopyranosyl*] - 2 - *deoxy* - 6 - *O* - *β* - *D* - *glucopyranosyl* - *β* - *D* - *glucopyranosyl*} - 3 - *O* - *carbamoyl* - 1 - *O* - {[(*S*) - 2 - *methoxycarbonyl* - 2 - (3,8,8,11,14,18 - *tetramethyl* - *nonadecyloxy*)*ethoxymethoxyphosphoryl*} - 4 - *C* - *methyl* - *α* - *D* - *glucopyranuronate* (**5**)

^{13}C-NMR: see Table 3 and Fig. 5.

Acknowledgements Financial support from Hoechst AG, the Fonds der Chemischen Industrie, and the Science

and Engineering Research Council, UK is gratefully acknowledged.

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