MODIFICATIONS OF A MACROLIDE ANTIBIOTIC MIDECAMYCIN (SF-837)

I. SYNTHESIS AND STRUCTURE OF 9,3''-DIACETYLMIDECAMYCIN

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9,3''-Diacetylmidecamycin (**12**) was synthesized from 4''-depropionyl-9,2',4''-triacetylmidecamycin (**8**) by heating the latter with propionic anhydride in pyridine followed by removal of 2'-acetyl group, with or without 18-enolpropionyl group. Direct acetylation of midecamycin (**1**) led to the formation of the 3'',4''-positional isomer (**6**).

The structure of **12** was determined by mass, NMR and chemical degradation. The location of 3''-acetyl group was shown by the stereospecific 3→1 acetyl migration catalyzed by a base of 3-O-acetyl-4-O-propionyl-L-mycarose (**13**), and comparison of NMR and mass fragmentation with the 3,4-positional isomer (**15**). The latter's structure was independently supported by the nuclear OVERHAUSER effect between methyl and propionyl group at C-3.

The intramolecular 4→3 acyl shift that was taken place in the forced acylation of the mycarose moiety was found to be affected by the anomeric configuration, nature of aglycones and reaction temperature. Reverse 3→4 acyl migration occurred in acidic hydrolysis.

Midecamycin (**1**), a clinically useful 16-membered macrolide antibiotic, is a fermentation product of *Streptomyces mycarofaciens*.[1,2] The antibiotic **1** contains five hydroxy groups, of which two at C-3 and C-4'' are propionylated biogenetically. During our structure-activity studies with midecamycin, a large number of acyl derivatives of the remaining three hydroxy groups at C-9, C-2' and C-3'' were chemically synthesized, with the aim of obtaining derivatives having improved bioactivity and pharmaceutical properties such as taste. In particular, our effort has been focused on acyl derivatives of the tertiary hydroxy group at C-3'' which is far less reactive than the secondary alcohols at C-9 and C-2'.

In this paper we describe the synthesis and structure proof of 9,3''-diacetylmidecamycin (**12**). The biological properties of **12** will be reported elsewhere.

Synthesis of 9,3''-Diacetylmidecamycin (12)

Initial attempts to prepare **12** focused on direct acetylation of midecamycin (**1**). Treatment of **1** with acetic anhydride in pyridine at room temperature gave the known 9,2'-diacetate (**2**),[3] but at elevated temperature around 100°C, the triacetate (**3**) together with a tetraacetate (**4**) was obtained. Selective removal of 2'-acetyl group from **3** gave a diacetate (**6**), which turned out to be a positional isomer of **12**, as described later. Partial hydrolysis of **4** yielded the 18-enolacetate (**5**).

Subsequently, an alternative route to **12** was investigated *via* 4''-depropionylmidecamycin (**7**). Compound **7**, which was easily obtained from **1** or its 4''-congeners by microbial deacylation,[3,4] was reacted with acetic anhydride and pyridine at room temperature to give 4''-depropionyl-9,2',4''-triacetylmidecamycin (**8**).[4] Heating **8** with propionic anhydride in pyridine at 90~100°C for 1~2 days

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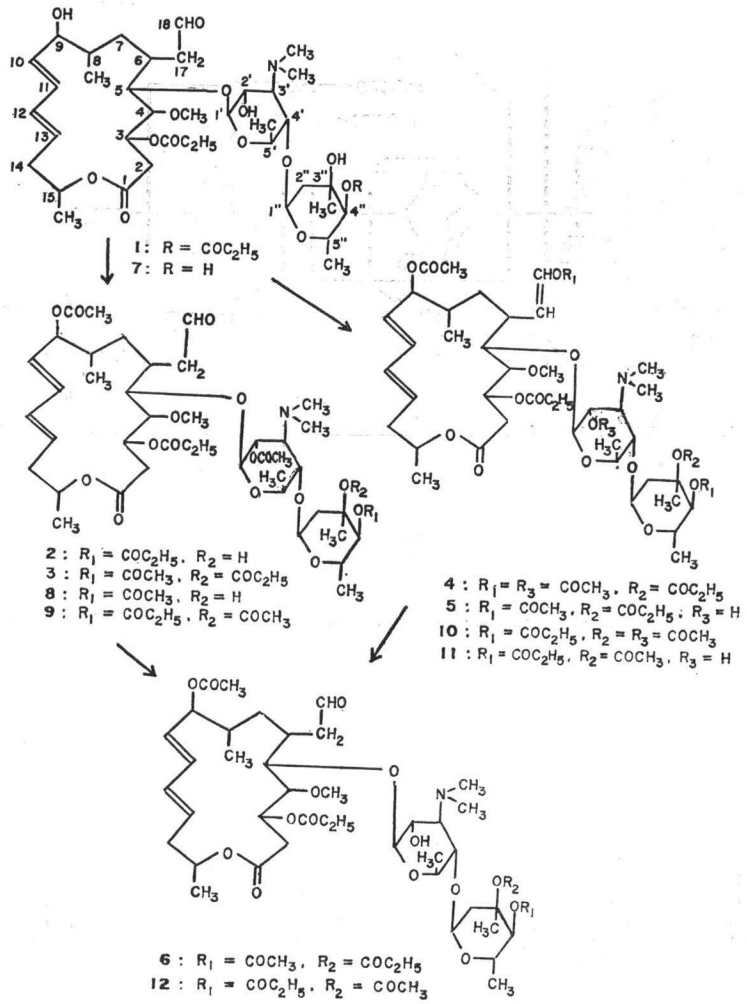
Chart 1.



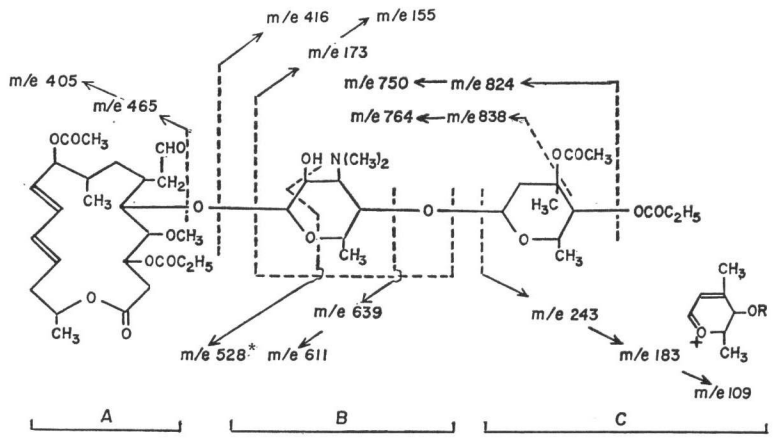
gave a propionate **9** predominantly, and in lesser extent **10**. Of the two reaction products, the major **9** afforded, on partial hydrolysis of 2′-acetyl group in aqueous methanol utilizing the intramolecular basic catalyst at C-3′, the desired 9,3″-diacetylmidecamycin (**12**).

The minor product **10** contained two new propionyl groups, an extra one being introduced on the enolic hydroxy of 18-formyl group. The 18-enolpropionate was stable to neutral hydrolysis, and was converted to 9,3″-diacetyl-18-enolpropionate (**11**), which could be hydrolyzed by the addition of a base like triethylamine to afford **12**.

Structure of 9,3″-Diacetylmidecamycin (12)

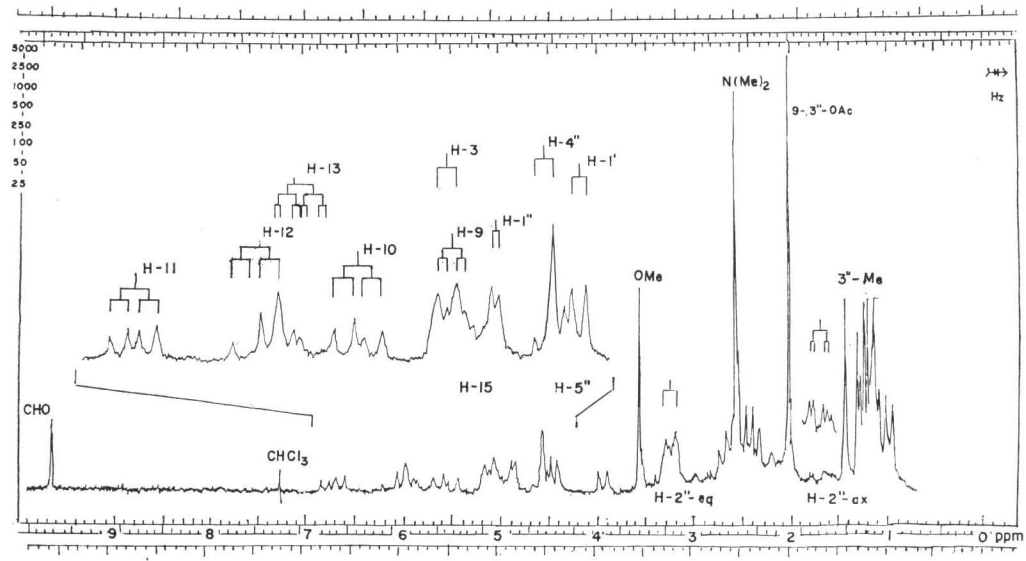
The gross structure of **12** was supported by mass fragmentation data summarized in Chart 2 and ^{1}H-NMR (proton nuclear magnetic resonance) data shown in Fig. 1. Based on the known fragmentation pattern of **1** and related compounds,[3,4] characteristic fragment ions were assigned as shown. Thus, a molecular ion (M^+) of **12** appeared at *m/e* 897, with the expected mass increase of *m/e* 84 ($2CH_3CO$ minus 2) over that of **1**. Fragments containing a macrocyclic lactone (*A*) and mycarose (*C*) moieties each showed mass increments of *m/e* 42, as compared to the corresponding ions of **1**,

Chart 2. Electron-impact mass fragmentation of 9,3″-diacetylmidecamycin (**12**) ($M^+ = m/e$ 897)



* Fragmentation involving transfer of a hydroxy group was supported by high-resolution mass data, reasonable mass increase upon deuteration of active hydrogens, and marked decrease in intensity by 2′-acylation.

Fig. 1. 100 MHz ^{1}H-NMR spectrum of 9,3″-diacetylmidecamycin (**12**) in deuteriochloroform.



with no mass increase for the mycaminose fragments (*B*). This clearly indicated the location of the acetyl group in portions *A* and *C*.

The ^{1}H-NMR spectrum of **12** as a whole was quite similar to that of the parent antibiotic (**1**), but differences were recognized in appearance of an acetyl signal at 2.02 ppm, and the marked down-field shift (*ca* 1 ppm) of the H-9 signal, which fixed the acetyl group introduced to *A* at C-9 (Table 1).

Determination of an acetyl group in the mycarose moiety (*C*) was not simple. The paramagnetic shift of H-2″$_{eq}$ (1.2 ppm) and 3″-CH_3 (0.3 ppm) (Table 1) suggested acylation at C-3″, but did not always imply acetylation. Midecamycin (**1**) shows a diagnostic (M^+ minus propionyloxy) ion at *m/e* 740, which arises from rupture of a propionyloxy group at C-4″.[3] An (M^+ minus propionyloxy) ion of **12** appeared at *m/e* 824, however, compared well with an (M^+ minus acetyloxy) ion at *m/e* 838,

Table 1. Proton chemical shifts and coupling constants of **12** and related compounds*1

	H-2 dd*2	H-3 d	H-9 dd	H-10 dd	H-11 dd	H-12 dd	H-13 ddd	H-17 d	H-18 d	9-CH_3CO s	18-CH_3CO s	OCH_3 s	CHO s	2′-CH_3CO s	$N(CH_3)_2$ s
1	2.80	5.16	4.08	5.62	6.65	6.07	5.78			1.99	—	3.53	9.64	—	2.52
3	*ca* 2.7	5.10	*ca* 5.1	5.56	6.71	6.04	5.89			2.01	—	3.48	9.62	2.02	2.44
4	2.7	5.10	5.26	5.56	6.70	6.07	5.78	5.52	7.01	1.99	2.08	3.47	—	2.04	2.46
5	2.8	5.10	5.24	5.56	6.71	6.06	5.76	5.52	7.08	1.97	2.10	3.56	—	—	2.34
6	2.7	5.10	5.08	5.56	6.74	6.07	5.91			2.01	—	3.56	9.60	—	2.56
9	2.7	5.10	*ca* 5.1	5.56	6.74	6.07	5.91			2.02	—	3.48	9.64	2.02	2.45
10	2.7	5.10	5.26	5.54	6.74	6.08	5.77	5.47	7.01	1.97	—	3.46	—	2.00	2.46
11	2.8	5.13	5.26	5.58	6.72	6.08	5.77	5.56	7.10	1.99	—	3.59	—	—	2.56
12	2.7	5.10	5.08	5.56	6.74	6.08	5.89			2.02	—	3.54	9.66	—	2.36

	H-1′ d	H-2′ dd	H-1″ d	H-2″$_{ax}$ dd	H-2″$_{eq}$ d	H-4″ d	3″-CH_3 s	3″-CH_3CO s	4-CH_3CO s	$J_{2,3}$	$J_{8,9}$	$J_{9,10}$	$J_{10,11}$	$J_{11,12}$	$J_{12,13}$
1	4.42	3.53	5.07	1.85	2.00	4.63	*ca* 1.1	—	—	10	3.2	9.4	14.3	9.4	14.4
3	4.55	*ca* 4.9	4.79	1.68	3.20	4.52	1.42	—	2.15	10	3.2	9.2	14.4	9.2	14.2
4		4.9	4.82	1.68	3.21	4.59	1.44	—	2.14	9.7	4.2	*ca* 9	14.2	9.3	14.2
5			4.82	1.68	3.23	4.59	1.42	—	2.14	10	3.3	9.0	14.3	9.2	14.2
6	4.42	3.3	4.86	1.70	3.25	4.59	1.44	—	2.14	9.2	3.2	9.6	14.4	9.2	14.0
9	4.59	4.9	4.80	1.67	3.19	4.59	1.42	2.02	—	10	3.2	9.2	14.4	9.2	14.4
10			4.82	1.65	*ca* 3.2	5.59	1.41	2.02	—	10	4.2	9.3	14.0	9.2	14.1
11			4.86	1.70	3.23	4.59	1.43	2.03	—	9.8	3.5	9.3	14.4	9.4	14.1
12	4.42	3.3	4.85	1.70	3.24	4.58	1.42	2.02	—	9.4	3.2	9.0	14.4	9.2	14.4

	$J_{6,17}$	$J_{17,18}$	$J_{1',2'}$	$J_{1'',2''_{ax}}$	$J_{2''_{ax},2''_{eq}}$	$J_{4'',5''}$
1			8.0	3.2	14.8	10.3
3			7.0	3.7	14.2	9.0
4	10.0	12.0		4.1	14.0	*ca* 9
5	9.7	12.0		3.6	14.4	8
6			7.4	4.0	14.2	8.6
9			7.4	3.6	14.2	8.6
10	10.0	12.0		3.5	14.4	8.5
11	9.9	12.1		3.0	14.0	*ca* 9
12			7.0	3.7	14.0	9.0

*1 Compound **1**: midecamycin
3: 4″-depropionyl-3″-propionyl-9,2′,4″-triacetylmidecamycin
4: 4″-depropionyl-17,18-enol-3″-propionyl-9,18,2′,4″-tetraacetylmidecamycin
5: 4″-depropionyl-17,18-enol-3″-propionyl-9,18,4″-triacetylmidecamycin
6: 4″-depropionyl-9,4″-diacetyl-3″-propionylmidecamycin
9: 9,2′,3″-triacetylmidecamycin
10: 17,18-enol-18-propionyl-9,2′,3″-triacetylmidecamycin
11: 9,3″-diacetyl-17,18-enol-18-propionylmidecamycin
12: 9,3″-diacetylmidecamycin

*2 Abbreviations: s, singlet; d, doublet; dd, double-doublet; ddd, triple-doublet.

giving no definite clue to the positions of acetyl and propionyl residues. Analysis of smaller fragments of *C*, in particular strong intensity of the *m/e* 183 ion ($R=COC_2H_5$) relative to the weak *m/e* 169 ion ($R = COCH_3$) (Table 2) favored 3''-acetyl-4''-propionylation, but there still remained the possibility of admixture of 4''-acetyl-3''-propionylation.

Table 2. Electron-impact mass fragmentation data of **12** and related compounds*

Fragment	**6** and **12**	**3** and **9**	**4**	**5**	**10**	**11**
M^+ $(A+B+C)^\dagger$	897 (2%, 1%)	939 (2%, 1%)	981 (1%)	939 (1%)	995 (0.8%)	953 (0.8%)
$M-CH_3COO$	838 (29, 14)	880 (22, 9)	922 (19)	880 (11)	936 (12)	894 (8)
$M-CH_3COO-C_2H_5COOH$*2	764 (9, 7)	806 (6, 6)	848 (8)	806 (9)	862 (8)	820 (6)
$M-C_2H_5COO$	824 (30, 18)	866 (20, 10)	908 (14)	866 (12)	922 (15)	880 (10)
$M-C_2H_5COO-C_2H_5COOH$*2	750 (6, 4)	792 (1, 4)	834 (3)	792 (7)	848 (4)	806 (5)
$A+B$	639 (5, 4)	681 (11, 4)	723 (36)*3	681 (4)	737 (39)*5	695 (2)
$A+B-CO$	611 (18, 16)	653 (36, 16)	695 (1)	653 (1)	709 (0.6)	667 (0.4)
$A+OCH(OH)_2$	528 (24, 27)	528 (0.4, 0.4)	570 (0.7)	570 (28)*4	584 (1)	584 (45)*6
A	465 (14, 12)	465 (6, 4)	507 (4)	507 (1)	521 (2)	521 (0.9)
$A-CH_3COOH$	405 (17, 15)	405 (2, 1)	447 (0.5)	447 (1)	461 (0.7)	461 (1)
$B+C$	416 (10, 8)	458 (30, 28)	458 (32)	416 (9)	458 (27)	416 (4)
B	173 (23, 22)	216 (6, 6)	216 (11)	173 (49)	216 (8)	173 (37)
$B-CH_3COOH$ $(B-H_2O)$	155 (14, 6)	155 (100, 81)	155 (94)	155 (41)	155 (100)	155 (31)
C	243 (9, 5)	243 (6, 4)	243 (8)	243 (19)	243 (8)	243 (5)
$C-CH_3COOH$	183 (3, 24)	183 (4, 21)	183 (7)	183 (9)	183 (28)	183 (45)
$C-C_2H_5COOH$	169 (40, 5)	169 (30, 2)	169 (41)	169 (82)	169 (7)	169 (11)
$C-CH_3COOH-C_2H_5COOH$	109 (100, 100)	109 (82, 100)	109 (100)	109 (100)	109 (68)	109 (100)

*1 Compound **6**: 4''-depropionyl-9,4''-diacetyl-3''-propionylmidecamycin
12: 9,3''-diacetylmidecamycin
3: 4''-depropionyl-3''-propionyl-9,2',4''-triacetylmidecamycin
9: 9,2',3''-triacetylmidecamycin
4: 4''-depropionyl-17,18-enol-3''-propionyl-9,18,2',4''-tetraacetylmidecamycin
5: 4''-depropionyl-17,18-enol-3''-propionyl-9,18,4''-triacetylmidecamycin
10: 17,18-enol-18-propionyl-9,2',3''-triacetylmidecamycin
11: 9,3''-diacetyl-17,18-enol-18-propionylmidecamycin

*2 Thermally degraded ions.

*3 *m/e* 663 (15%) (*m/e* 723–CH_3COOH); *m/e* 680 (15%) (*m/e* 723–CH_3CO).

*4 *m/e* 465 (23%) (*m/e* 570–$OCH(OH)_2$–CH_3CO).

*5 *m/e* 694 (11%) (*m/e* 737–CH_3CO); *m/e* 677 (10%) (*m/e* 737–CH_3COOH); *m/e* 663 (14%) (*m/e* 737–C_2H_5CO).

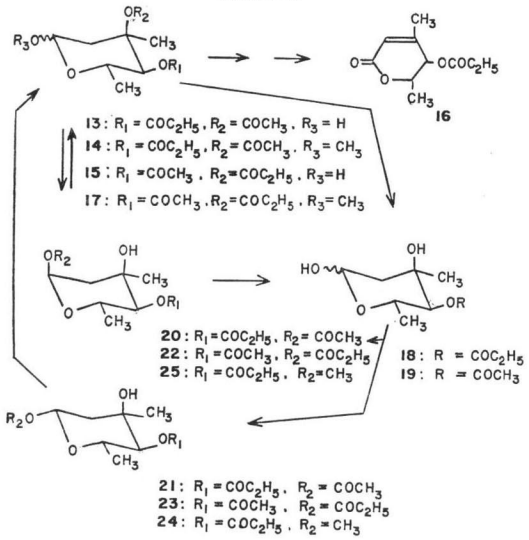
*6 *m/e* 479 (15%) (*m/e* 584–$OCH(OH)_2$–CH_3CO).

† *A*, *B*, and *C* represent macrocyclic lactone, mycaminose and mycarose moieties, respectively.

In order to obtain conclusive evidence on the location of an acetyl group in the mycarose moiety, compound **12** was hydrolyzed with Amberlyst 15 in aqueous dioxane-methanol, and the liberated mycarose fragments were chromatographed over silica gel to give crystalline diacylmycarose (**13**) together with its methyl glycoside (**14**). Similarly acidic hydrolysis of **6** gave syrupy diacylmycarose (**15**).

Independent of our work, JARET *et al.*[5] obtained gummy 3-O-acetyl-4-O-propionyl-L-mycarose (**13**) from the 14-membered macrolide, megalomicin C_2, and the structure assigned was based mainly on conversion by bromine oxidation and subsequent acidic β-elimination, to 4-O-propionyl-α,β-un-

Chart 3.



saturated lactone (**16**). They further showed that the acetyl signal of methyl 3-O-acetyl-4-O-propionyl-α and β-L-mycaroside (**14**) resonated at higher field (0.15 ppm for α, 0.03 ppm for β) than the 4-O-acetyl-3-O-propionyl isomer (**17**).

Comparison of ^{1}H-NMR of **13** and **15** suggested 3-O-acetyl-4-O-propionyl substitution in **13**, since the acetyl signals of **13** appeared at higher field (0.12 ppm for α, 0.04 ppm for β) than those of **15**. In accordance with this, mild basic hydrolysis of **13** gave 4-O-propionyl-L-mycarose (**18**). It was found however that acidic hydrolysis of **12** yielded, together with **13**, 4-O-acetyl-L-mycarose (**19**) but little **18**. If the 3″-acetyl-4″-propionyl structure was correct for **12**, there must be 3″→4″ acetyl migration under acidic condition, following the initial cleavage of 4″-propionyl group. This result rendered some ambiguity on the previous proof[5] of **13**, because compound **16** may also be formed from **6** *via* 3″→4″ propionyl migration followed by dehydration of a tertiary hydroxy group. Indeed, acidic hydrolysis of **6** gave 4-O-propionyl-L-mycarose (**18**), in addition to **15**. Therefore, it seemed to us that more evidence was desirable to establish the structure of **12**.

Careful examination of the alkaline hy-

Table 3. ^{13}C-Chemical shifts* of methyl 3-O-acetyl-4-O-propionyl-α- and β-L-mycarosides (**14**) and methyl 4-O-acetyl-3-O-propionyl-α- and β-L-mycarosides (**17**) in deuteriochloroform

Compound	C-1	C-2	C-3	C-4	C-5	6-CH_3	3-CH_3	OCH_3	$\underline{C}H_3CH_2$	$CH_3\underline{C}H_2$ CO	$\underline{C}H_3CO$	$C_2H_5\underline{C}O$	$CH_3\underline{C}O$
α-**14**	97.8	36.0	78.3	77.7	62.6	17.4	22.5	55.0	9.3	27.6	22.3	174.0	170.9
α-**17**	97.8	36.1	78.1	78.1	62.6	17.4	22.5	55.0	8.9	28.8	20.7	174.0	170.6
β-**14**	99.0	39.3	81.0	77.4	68.1	17.6	22.2	56.4	9.2	27.6	22.2	173.9	169.9
β-**17**	99.0	39.4	80.6	77.7	68.1	17.6	22.2	56.3	9.2	28.7	20.7	173.2	170.3

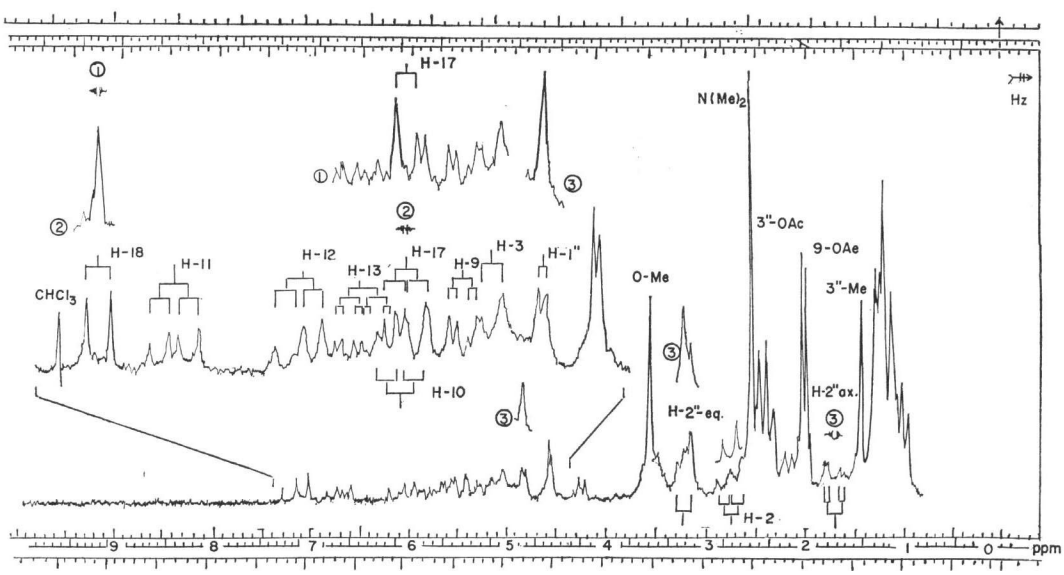
* The assignment was supported by partial decoupling.

drolysis of **13** revealed the formation of an immediate precursor of **18**. The structure of this compound was determined to be 1-O-acetyl-4-O-propionyl-α-L-mycarose (**20**) by ^{1}H-NMR spectroscopy (see Experimental), and synthesis from **18**. 1-O-Acetylation of **18** with acetic anhydride gave **20** as a minor product, together with the β-L-anomer (**21**) as a major. In contrast to the synthesis, exclusive formation of the α-L-anomer (**20**) without any β-L-anomer in the basic hydrolysis was best explained by the stereospecific acetyl migration between 1,3-*cis*-diaxial hydroxy groups, with the intact 4-O-propionyl group. Formation of **20** from **15** was highly unprobable, since it involved 4→1 acetyl migration followed by 3→4 propionyl transfer.

Quite similarly, compound **15** gave 1-O-propionyl-4-O-acetyl-α-L-mycarose (**22**), and subsequently 4-O-acetyl-L-mycarose (**19**) on reaction with a base. The structure of **15** was independently supported, after conversion to its methyl glycoside (**17**), by the nuclear OVERHAUSER effect between $\underline{CH_3}$ and $CH_3\underline{CH_2}CO$ at C-3. Since the ^{1}H-chemical shifts of both groups seriously overlapped, the respective chemical shifts were separated utilizing a lanthanide shift reagent ($Eu(fod)_3$). When the 3-C-methyl was irradiated, the methylene protons of 3-O-propionyl group increased 14% in intensity, indicating close proximity. Space interaction between the 3-C-methyl and 4-O-propionyl group was highly unlikely, since they are *trans*-diequatorial.

The structures of other synthetic intermediates (compounds **3**, **4**, **5**, **9**, **10** and **11**) were determined by mass and ^{1}H-NMR. Mass fragmentations summarized in Table 2 were consistent with the structures assigned, though the relative abundance of *A* and *B* fragments were considerably affected by 2′- and 18-acyl substitutions. The ^{1}H-NMR data shown in Table 1 were also consistent with the proposed structures. As represented in Fig. 2, the 18-enolpropionate (**11**) showed the newly formed olefinic protons at 5.56 ppm (double-doublet, H-17) and 7.10 ppm (doublet, H-18). The coupling of H-17 and H-18 was confirmed by the double resonance indicated*, and the magnitude of

Fig. 2. 100 MHz ^{1}H-NMR spectrum of 9,3″-diacetyl-17,18-enol-18-propionylmidecamycin (**11**) in deuteriochloroform.



* The coupling pattern involved H-6, H-17 and H-18 in Fig. 2 provided additional ^{1}H-NMR evidence for the original CH–CH_2–CHO structure, where the formyl signal is exceptionally a singlet in stead of a triplet.[6]

coupling constant (12 Hz) was suggestive of a *trans* configuration. Additional evidence in support of the enol structure was the disappearance of a formyl signal that appears usually at 9.6 ppm in the derivatives with the intact formyl group.

Intramolecular 4~3 Acyl Migration in the Mycarose Moiety

Though the tertiary 3''-hydroxy group was least reactive of the three in **1**, it could be acylated more easily than common tertiary alcohols. The ease of 3''-acylation was undoubtedly due to intramolecular 4''→3'' acyl migration, as suggested by JARET *et al.*[5] for methyl 3,4-di-O-acyl-L-mycarosides (**14**, **17**). However, in contrast to JARET's result,* we have found that the rate of 4→3 acyl migration was dependent to the anomeric configuration, nature of aglycones and reaction temperature, and that direct 3-O-acylation became significant in the β-L-anomer at lower reaction temperature.

Relative amounts of the acyl-migrated and directly substituted products were estimated from intensities of the ^{13}C signals of $\underline{C}H_3CO$ and $CH_3\underline{C}H_2CO$ groups which were most sensitive to the position of substitution as shown in Table 3, and the results are summarized in Table 4. Methyl 4-O-

Table 4. Relative amount (%) of the 4→3 acyl-migrated and directly 3-substituted 3,4-diacylmycarosides from 4-O-propionyl (or 4-O-acetyl) mycarosides by treatment with acetic anhydride (or propionic anhydride) in pyridine for 48 hours

Starting material	React. temp.	3,4-Diacylmycaroside	
		Acyl-migrat.	Direct.-subst.
Methyl 4-O-propionyl-β-L-mycaroside	90°C	50%	50%
Methyl 4-O-propionyl-β-L-mycaroside	100°C	75	25
Methyl 4-O-propionyl-α-L-mycaroside	100°C	85	15
Compound **2**	100°C	>98	<2
Methyl 4-O-acetyl-β-L-mycaroside	95°C	55	45
Methyl 4-O-acetyl-α-L-mycaroside	100°C	85	15
Compound **8**	95°C	>98	<2

propionyl-β-L-mycaroside (**24**) gave an equal mixture of the propionyl-migrated (**17**) and non-migrated 3,4-di-O-acylate (**14**), when reacted with acetic anhydride in pyridine at 90°C for 48 hours, and relative amounts of the migrated product (**17**) increased as temperature raised to 100°C (75: 25). On the other hand, the α-L-anomer (**25**) favored the rearranged product (**17**), with ratio of 85: 15 at 100°C, and the α-L-mycaroside of macrocyclic lactonyl-mycaminose (**2**) gave almost exclusively the rearranged product (**4**) at 90~100°C. A similar conclusion was derived from the propionylation of methyl 4-O-acetyl-α- and β-L-mycarosides and **8**. Apparently, close proximity of 3-hydroxy and glycosidic oxygen in the α-L-anomers seemed to promote 4→3 acyl shift. This may be due to intramolecular 3→1 hydrogen bonding[7] which favors acyl shift, and steric hindrance which acts against direct 3-O-substitution.

As described already, mild acidic hydrolysis of **12** gave 4-O-acetyl-L-mycarose (**19**), the formation of which could be explained by initial cleavage of 4''-propionyl group followed by 3''→4'' acetyl migration. So far, attempts to isolate 3-O-acylmycarose have been unsuccessful, as *cis*-migration either to the 1- or 4-hydroxy groups occurs spontaneously.

* They reported exclusive 4→3 acyl shift regardless to the anomeric configuration in the acylation of methyl 4-O-acyl-α- and β-L-mycarosides under a similar condition.

Experimental

General methods

Melting points are uncorrected. Optical rotations were determined with a Perkin-Elmer model 141 polarimeter. UV and IR spectra were recorded on Hitachi model 323 UV and model 215 IR spectrometers. NMR spectra were determined in $CDCl_3$ using a Varian XL-100 spectrometer (^{1}H-NMR) or a Varian CFT-20 spectrometer (^{13}C-NMR=carbon-thirteen nuclear magnetic resonance), with TMS as an internal standard. Chemical shifts given on the ppm scale from TMS were determined by the double resonance or 300 MHz spectra, if necessary. Mass spectra were obtained with a JMS-01SG double-focussing mass spectrometer at 75 eV. Column chromatography was performed on Mallinckrodt Silic AR (200~325 mesh). Analytical TLC plates were Merck silica gel F_{254}, and spots were detected by spraying sulfuric acid.

Synthesis of 9,3''-diacetylmidecamycin (12)

A solution of midecamycin (**1**, 100 g) and wet mycelia of *Mucor spinescens* (1 kg) and tartaric acid (10 g) in 0.1 M disodium hydrogen phosphate (9.5 liters) was kept at 32°C for 22 hours under agitation, and then extracted with ethyl acetate (30 liters). Evaporation of solvent from the extract gave 4''-depropionylmidecamycin (**7**, 95 g). Yield, 100%.

This was dissolved in a mixture of acetic anhydride (70 ml) and pyridine (200 ml), and the solution was allowed to react at room temperature overnight. Methanol (100 ml) was added, and after standing for 1 hour, the mixture was concentrated, and poured into water (1 liter). Extraction with ethyl acetate (600 ml) followed by evaporation of solvent gave 4''-depropionyl-9,2',4''-triacetylmidecamycin (**8**, 100 g), which, without further purification, was treated with propionic anhydride (300 ml) in pyridine (100 ml) at 95°C for 48 hours. After addition of methanol (100 ml), the solution which contained a (2: 1) mixture of 9,2',3''-triacetylmidecamycin (**9**) and its 18-enolpropionate (**10**) was concentrated, and were added 90% ethanol (450 ml) and triethylamine (10 ml).

The mixture was kept at 65°C for 5 hours, and evaporated to dryness. The residue was dissolved in benzene (1 liter), washed successively with 1% potassium hydrogen sulfate, 1% sodium hydrogen carbonate and water, and evaporated to dryness. The residue was crystallized from *iso*propanol (400 ml) to give **12**, 87 g. Yield, 80%. This was recrystallized from the same solvent, after decolorizing with active carbon (8 g). Yield, 79 g, total yield from **1**, 71%. mp, *ca* 220°C (with coloration), $[\alpha]_D^{25}$ −53° (*c* 1.0, $CHCl_3$), $[\alpha]_D^{20}$ −74° (*c* 1.0, MeOH), UV maximum in methanol, 231 nm ($E_{1cm}^{1\%}$ 342), IR bands in KBr, 3520, 2990, 2950, 1755sh, 1740, 1720, 1370, 1263, 1163 and 1090 cm^{-1}. ^{1}H-NMR and mass data are shown in Tables 1 and 2.

Anal. Calcd. for $C_{45}H_{71}NO_{17}$ (898.06): C, 60.19; H, 7.97; N, 1.56.
Found: C, 60.04; H, 7.91; N, 1.45.

Isolation of 9,2',3''-triacetylmidecamycin (9), 17,18-enol-18-propionyl-9,2',3''-triacetylmidecamycin (10) and 9,3''-diacetyl-17,18-enol-18-propionylmidecamycin (11)

A mixture (5.0 g) of **9** and **10** prepared by the procedure described above was chromatographed on a column of silica gel (4.5×20 cm), developing with a mixture of benzene and acetone (15: 1). Effluents were collected in 13 g fractions, and evaporation of solvent from fractions 35~50 afforded a glassy powder of **9** (920 mg) that was homogeneous on TLC. mp 127~128°C, $[\alpha]_D^{24}$ −87° (*c* 1.0, $CHCl_3$).

Anal. Calcd. for $C_{47}H_{73}NO_{18}$ (940.11): C, 60.05; H, 7.83; N, 1.49.
Found: C, 60.20; H, 7.52; N, 1.31.

Fractions 25~27 were combined, and evaporated to give a chromatographically homogeneous **10** (800 mg). mp, 98°C, $[\alpha]_D^{20}$ −122° (*c* 1.0, $CHCl_3$).

Anal. Calcd. for $C_{50}H_{77}NO_{19}$ (996.18): C, 60.29; H, 7.79; N, 1.41.
Found: C, 60.12; H, 7.51; N, 1.27.

A solution of **10** (500 mg) in methanol (30 ml) was kept at room temperature overnight, and evaporated to dryness. The residue was purified by a column chromatography of silica gel developing with benzene - acetone (10: 1), to give **11**(315 mg) as a glassy powder. mp, 98~101°C, $[\alpha]_D^{22}$ −95°

(*c*, 1.0, $CHCl_3$).

Anal. Calcd. for $C_{48}H_{75}NO_{18}$ (954.14): C, 60.42; H, 7.92; N, 1.47.
Found: C, 60.12; H, 7.86; N, 1.25.

Rf Values of **12** and related compounds on TLC (benzene - acetone, 3: 1) were 0.51 (**12**), 0.57 (**11**), 0.66 (**9**), 0.71 (**10**) and 0.16 (**1**).

Synthesis of 4''-depropionyl-9,4''-diacetyl-3''-propionylmidecamycin (**6**)

A solution of **1** (200 g) and acetic anhydride (600 ml) in pyridine (1.4 liters) was heated at 100°C for 24 hours, and the reaction mixture was poured into ice-water (5 liters), neutralized with sodium hydrogen carbonate (100 g), and extracted with benzene (2 liters). The benzene extract was washed successively with 5% potassium hydrogen sulfate (500 ml), saturated sodium hydrogen carbonate (500 ml) and water. The organic layer was dried over sodium sulfate, and evaporated to a (1: 1) mixture of 4''-depropionyl-3''-propionyl-9,2',4''-triacetylmidecamycin (**3**) and 4''-depropionyl-17,18-enol-3''-propionyl-9,18,2',4''-tetraacetylmidecamycin (**4**). Yield, 228 g.

A solution of a mixture of **3** and **4** (182 g) in 80% methanol (1 liter) was decolorized with active carbon (18 g), and heated at 60°C for 8 hours. Concentration of the reaction mixture afforded crystals of **6** (66 g). The dried mother liquor (99 g) was dissolved in 80% ethanol containing triethylamine (40 ml), and, after decoloration with active carbon (10 g), the solution was heated at 65°C for 8 hours. Evaporation of ethanol gave further crystals of **6** (63 g). Total yield, 129 g (75% from **1**). Recrystallization of this crystals (28 g) from *iso*propanol (500 ml) gave an analytical sample (25 g). mp, 227~230°C (with coloration), $[\alpha]_D^{25}$ −60° (*c* 1.0, $CHCl_3$), $[\alpha]_D^{20}$ −76° (*c* 0.62, MeOH), UV maximum in methanol, 231 nm ($E_{1cm}^{1\%}$ 343), IR bands in KBr, 3500, 2975, 2940, 1740, 1730, 1710, 1375, 1240, 1155 and 1045 cm^{-1}.

Anal. Calcd. for $C_{45}H_{71}NO_{17}$ (898.06): C, 60.19; H, 7.97; N, 1.56.
Found: C, 60.12; H, 8.02; N, 1.36.

Isolation of 4''-depropionyl-3''-propionyl-9,2',4''-triacetylmidecamycin (**3**), 4''-depropionyl-17,18-enol-3''-propionyl-9,18,2',4''-tetraacetylmidecamycin (**4**) and 4''-depropionyl-17,18-enol-3''-propionyl-9,18,4''-triacetylmidecamycin (**5**)

A mixture (5.0 g) of **3** and **4** obtained by acetylation of **1** as described above, was dissolved in hot *iso*propanol (30 ml), and the solution was allowed to stand at room temperature, whereupon crystals of **3** were deposited. Yield, 2.0 g. Analytical sample recrystallized from the same solvent showed mp, 218~220°C (decomp.), $[\alpha]_D^{20}$ −91° (*c* 1.0, $CHCl_3$), −128° (*c* 1.0, EtOH).

Anal. Calcd. for $C_{47}H_{73}NO_{18}$: C, 60.05; H, 7.83; N, 1.49.
Found: C, 60.12; H, 7.95; N, 1.55.

The mother liquor was concentrated, and applied on a column of silica gel (2.5×20 cm). The column was developed with a mixture of benzene - acetone (15: 1), and effluents were collected in 8-g fractions. Evaporation of solvent from fractions 13~15 yielded a glassy **4** (250 mg) that was homogeneous on TLC. Further **4** of inferior purity (1.2 g) was recovered from fractions 16~25. mp, 118~122°C, $[\alpha]_D^{20}$ −128° (*c* 1.0, $CHCl_3$).

Anal. Calcd. for $C_{49}H_{75}NO_{19}$ (982.15): C, 59.92; H, 7.70; N, 1.43.
Found: C, 59.42; H, 7.63; N, 1.28.

Partial hydrolysis of 2'-acetyl group of **4** by the procedure applied for **11** yielded **5** as a glassy powder. mp, 120~124°C, $[\alpha]_D^{20}$ −125° (*c* 1.0, EtOH).

Anal. Calcd. for $C_{47}H_{73}NO_{18}$: C, 60.05; H, 7.83; N, 1.49.
Found: C, 59.81; H, 7.68; N, 1.25.

Isolation of 3-O-acetyl-4-O-propionyl-L-mycarose (**13**) and its methyl glycoside (**14**)

A solution of **12** (13 g) in water (200 ml), methanol (300 ml), and dioxane (100 ml) was treated with Amberlyst 15 (70 ml) at 40°C for 5 hours. Resins were removed by filtration, and the filtrate was concentrated to a syrupy residue (4.5 g). This was chromatographed over silica gel (4×25 cm), developed with benzene - ethyl acetate (4: 1). Effluents were collected in 10-g fractions, and evaporation of solvent from fractions 90~160 gave crystalline mass of **13** (1.3 g). mp, 81°C, $[\alpha]_D^{20}$ −98°

(*c* 1.0, EtOH). Lit.[5] $[\alpha]_D^{25}$ −78.3° (EtOH). IR bands in KBr, 3460, 1745, 1710, 1180 and 1040 cm^{-1}. ^{1}H-NMR*[1], α-L-anomer: 1.12 ppm (d, 5-CH_3), 1.19 (t, 4-CH_3CH_2CO), 1.44 (s, 3-CH_3), 1.68 (ddd, H-2_{ax}), 2.03 (s, 3-CH_3CO), 2.42 (q, 4-CH_3CH_2CO), 3.10 (d, 1-OH), 3.16 (dd, H-2_{eq}), 4.42*[2] (dq, H-5), 4.60 (d, H-4), 5.23 (dd, H-1), $J_{1,2ax}$ 4.0 Hz, $J_{1,2eq}$ 1.0, $J_{2ax,2eq}$ 15.0, $J_{4,5}$ 9.3, $J_{5,6}$ 6.1, $J_{1,OH}$ 3.0. β-L-anomer: 1.16 ppm (d, 5-CH_3), 1.18 (t, 4-CH_3CH_2CO), 1.48 (s, 3-CH_3), 1.65 (dd, H-2_{ax}), 2.07 (s, 3-CH_3CO), 2.41 (q, 4-CH_3CH_2CO), 3.10 (dd, H-2_{eq}), 3.97 (dq, H-5), 4.59 (d, H-4), 4.93 (ddd, H-1), 3.74 (d, 1-OH), $J_{1,2ax}$ 9.6 Hz, $J_{1,2eq}$ 1.9, $J_{2ax,2eq}$ 13.9, $J_{4,5}$ 9.3, $J_{5,6}$ 6.1, $J_{1,OH}$ 6.3. Mass, *m/e* 259w (M^+ −1), 243w (M^+–OH), 216 m (M^+–1–CH_3CO), 200 m (M^+–CH_3COOH), 186 m, 183 m, 172 m, 156s, 127s, 126s, 114 m and 109 m.

Anal. Calcd. for $C_{12}H_{20}O_6$ (260.29): C, 55.37; H, 7.75.
Found: C, 55.30; H, 7.71.

From fractions 26~44 was obtained a syrupy mixture of methyl 3-O-acetyl-4-O-propionyl-α- and β-L-mycaroside (**14**) (1.0 g)*[1]. $[\alpha]_D^{21}$ −115° (*c* 1.0, EtOH), ^{1}H-NMR, 3.31 ppm (s, α-OCH_3), 3.47 (s, β-OCH_3), mass, *m/e* 273 w (M^+–1), 243 w (M^+–1–OCH_3), 214 m (M^+–CH_3COOH), 200 m (M^+–C_2H_5COOH), 183 m, 170 s, 154 s, 141 s, 140 s, 130 m, 114 s and 109 s. ^{13}C-NMR data are shown in Table 3.

Anal. Calcd. for $C_{13}H_{22}O_6$ (274.32): C, 56.92; H, 8.09.
Found: C, 56.74; H, 7.95.

Isolation of 4-O-acetyl-L-mycarose (**19**) from **12**

A solution of **12** (10 g) in a mixture of 1 N hydrochloric acid (500 ml) and dioxane (50 ml) was stood at room temperature for 3 hours. The reaction mixture was extracted three times with ether (each 200 ml). Ether extracts were combined, and evaporated to an oily residue. This was dissolved in a small amount of benzene, and chromatographed on a column of silica gel (1.5×20 cm), developing with benzene - ethyl acetate (4: 1). Effluents were collected in 7-ml fractions, and evaporation of solvent from fractions 68~82 gave crystalline 4-O-acetyl-L-mycarose (**19**, 43 mg). mp, 101°C, $[\alpha]_D^{20}$ −72° (*c* 1.9, EtOH). Lit.[5] $[\alpha]_D^{25}$ −69.9° (EtOH). The ^{1}H-NMR showed predominantly α-L-anomer. 5.20 ppm (dd, H-1), $J_{1,2ax}$ 3.6 Hz, $J_{1,2eq}$ 1.2, 2.17 ppm (s, 4-CH_3CO). Mass, *m/e* 205w ($M+1)^+$, 203w (M^+-1), 187s (M^+–OH), 169m (M^+–OH–H_2O), 142s, 116s and 109s.

Anal. Calcd. for $C_9H_{16}O_5$ (204.22): C, 52.93; H, 7.90.
Found: C, 52.95; H, 7.78.

From fractions 20~32 were recovered 257 mg of **13**.

Isolation of 4-O-acetyl-3-O-propionyl-L-mycarose (**15**) and 4-O-propionyl-L-mycarose (**18**) from **6**

A solution of **6** (10 g) in a mixture of 0.75 N hydrochloric acid (500 ml) and dioxane (30 ml) was stood at room temperature for 4 hours, then concentrated to 100 ml, and the concentrate was extracted twice with ether (each 600 ml). The ether extracts (845 mg) were worked out analogously to "Isolation of **13** and **14** from **12**". Compound **15** was obtained as a syrup (243 mg). $[\alpha]_D^{22}$ −88° (*c* 1.0, $CHCl_3$), ^{1}H-NMR, α-L-anomer: 1.13 ppm (d, 5-CH_3, t, 4-CH_3CH_2CO), 1.45 (s, 3-CH_3), 1.68 (dd, H-2_{ax}), 2.15 (s, 3-CH_3CO), 2.34, (q, 4-CH_3CH_2CO), 3.17 (dd, H-2_{eq}), 4.41 (dq, H-5), 4.60 (d, H-4), 5.24 (d, H-1), $J_{1,2ax}$ 3.9 Hz, $J_{1,2eq}$ 0.7, $J_{2ax,2eq}$ 14.8, $J_{4,5}$ 9.2, $J_{5,6}$ 6.0; β-L-anomer: 1.15 ppm (t, 4-CH_3CH_2CO), 1.17 (d, 5-CH_3), 1.49 (s, 3-CH_3), 1.56 (dd, H-2_{ax}), 2.03 (s, 3-CH_3CO), 2.35 (q, 4-CH_3CH_2CO), 3.11 (dd, H-2_{eq}), 3.97 (dq, H-5), 4.59 (d, H-4), 4.91 (dd, H-1), $J_{1,2ax}$ 9.3 Hz, $J_{1,2eq}$ 1.9, $J_{2ax,2eq}$ 14.0, $J_{4,5}$ 9.2, $J_{5,6}$ 6.0. Mass, *m/e* 261 w ($M+1)^+$, 259 w (M^+-1), 243 m (M^+–OH), 200 m (M^+–CH_3COOH), 183 w, 169 m, 142s and 127s.

Anal. Calcd. for $C_{12}H_{20}O_6$: C, 55.37; H, 7.75.
Found: C, 55.25; H, 7.54.

4-O-Propionyl-L-mycarose (**18**) was obtained in crystalline state. Yield, 214 mg. mp, 108°C, $[\alpha]_D^{22}$ −39° (*c* 1.0, $CHCl_3$), −60° (*c* 1.0, H_2O). Lit.[3] mp, 110~112°C, $[\alpha]_D^{20}$ −60° (H_2O). Mass, *m/e* 217 w (M^+-1), 201 m (M^+–OH), 200 m (M^+–H_2O), 156 m, 154m, 143 m, 130 s and 114s. The IR and

*[1] Ratio of α- and β-L-anomers of **13** and **14** in $CDCl_3$ were 11: 16 and 23: 19, respectively.
*[2] JARET *et al.*[5] assigned a signal at 3.37 ppm for H-2_{eq} of the α-L-anomer.

^{1}H-NMR of this compound were superimposable to those of the authentic sample.

In addition to **15** and **18**, formation of a trace amount of **19** was detected.

Basic degradation of 3-O-acetyl-4-O-propionyl-L-mycarose (**13**)

A solution of **13** (310 mg) in 0.5 M sodium carbonate (20 ml) and dioxane (3 ml) was stood at 5~15°C for 3 hours, then neutralized under ice-cooling, and extracted three times with ether (each, 20 ml). Ether extracts (318 mg) were chromatographed over silica gel (1.5×25 cm), developed with benzene - ethyl acetate (2: 1). Effluents were collected in 7-g fractions, and from fractions 17~35 were obtained 166 mg of syrupy 1-O-acetyl-4-O-propionyl-α-L-mycarose (**20**). $[\alpha]_D^{20}$ −138° (*c* 1.0, EtOH), IR in KBr, 3500, 2990, 1750, 1725sh, 1385, 1190 and 1165 cm^{-}.[1] ^{1}H-NMR, 1.16 ppm (d, 5-CH_3), 1.18 (s, 3-CH_3), 1.20 (t, 4-CH_3CH_2CO), 2.00 (dd, H-2_{ax}), 2.09 (dd, H-2_{eq}), 2.12 (s, 1-CH_3CO), 2.44 (q, 4-CH_3CH_2CO), 2.72 (bs, OH), 4.19 (dq, H-5), 4.71 (d, H-4), 6.16 (dd, H-1), $J_{1,2ax}$ 3.4 Hz, $J_{1,2eq}$ 1.9, $J_{2ax,2eq}$ 14.0, $J_{4,5}$ 10.0, $J_{5,6}$ 6.1. Mass, *m/e* 259 w (M^+−1), 242 w (M^+–H_2O), 201 s (M^+–CH_3COO), 186 m, 183 m, 174 w, 143 s, 130 s, 126 m, 114 s and 109 m.

Anal. Calcd. for $C_{12}H_{20}O_6$: C, 55.37; H, 7.75.
Found: C, 55.05; H, 7.90.

Prolonged treatment of **20** with sodium carbonate gave **18** and then L-mycarose.

Evaporation of solvent from fractions 38~50 gave crystalline 4-O-propionyl-L-mycarose (**18**, 50 mg), mp, 104~106°C, $[\alpha]_D^{20}$ −67° (*c* 1.0, EtOH). The IR, ^{1}H-NMR and Rf values were almost identical with those of the authentic sample.

Rf Values of **13** and its basic hydrolyzates on TLC (benzene - ethyl acetate, 2: 1) were 0.56 (**13**), 0.58 (**21**), 0.42 (**20**), 0.31 (**18**) and 0.08 (L-mycarose).

Basic degradation of 4-O-acetyl-3-O-propionyl-L-mycarose (**15**)

A solution of **15** (673 mg) in 0.5 M sodium carbonate (70 ml) was stood at room temperature for 25 minutes, neutralized with 1 N hydrochloric acid and then extracted twice with ethyl acetate (each, 30 ml). The organic extracts were purified by a chromatography similar to that employed for the hydrolyzate of **13**, and there were obtained 67 mg of syrupy 4-O-acetyl-1-O-propionyl-α-L-mycarose (**22**), 132 mg of crystalline 4-O-acetyl-L-mycarose (**19**) and 5 mg of 4-O-propionyl-L-mycarose (**18**).

Compound **22** showed $[\alpha]_D^{22}$ −136° (*c* 1.0, $CHCl_3$), ^{1}H-NMR, 1.17 ppm (d, 5-CH_3), 1.18 (t, 1-CH_3CH_2CO, s, 3-CH_3), 1.98 (dd, H-2_{ax}), 2.10 (bd, H-2_{eq}), 2.16 (4-CH_3CO), 2.39 (q, 4-CH_3CH_2CO), 4.17 (dq, H-5), 4.70 (d, H-4), 6.19 (dd, H-1), $J_{1,2ax}$ 3.0 Hz, $J_{1,2eq}$ 2.0, $J_{2ax,2eq}$ 14.0, $J_{4,5}$ 9.7, $J_{5,6}$ 6.0. Mass, *m/e* 260 w (M^+), 243 w (M^+–OH), 203 w (M^+–C_2H_5CO), 187 s (M^+–C_2H_5COOH), 143 m ,127 m, 114 s and 109 m.

Anal. Calcd. for $C_{12}H_{20}O_6$: C, 55.37; H, 7.75.
Found: C, 55.15; H, 7.72.

Compound **19** showed mp, 101°C, $[\alpha]_D^{22}$ −65° (*c* 1.0, $CHCl_3$), −46° (*c* 1.0, H_2O). This compound was identical in IR, ^{1}H-NMR, mass and Rf values with that derived from acidic hydrolysis of **12**.

Rf Values of **15** and its basic hydrolyzates on TLC (benzene - ethyl acetate, 2: 1) were 0.61 (**15**), 0.52 (**23**), 0.43 (**22**) and 0.23 (**19**).

Synthesis of 1-O-acetyl-4-O-propionyl-α- and β-L-mycarose (**20** and **21**)

A mixture of 4-O-propionyl-L-mycarose (**18**, 500 mg), acetic anhydride (3 ml) and sodium acetate (300 mg) was heated at 50°C for 3 hours, and concentrated. This was extracted four times with chloroform (each 10 ml), and the chloroform extracts were chromatographed over silica gel (2.5× 13 cm), developed with benzene-ethyl acetate (3: 1). Effluents were collected in 5-ml fractions, and evaporation of solvent from fractions 46~69 gave syrupy **20** (45 mg). $[\alpha]_D^{20}$ −137° (*c* 1.0, EtOH). The IR, ^{1}H-NMR and Rf values of this compound were indistinguishable from those of **20** obtained from the basic degradation of **13**.

From fractions 16~45 were obtained 410 mg of crystalline **21**. mp, 66~67°C, $[\alpha]_D^{21}$ −29° (*c*, 0.8, EtOH). ^{1}H-NMR, 1.17 ppm (d, 5-CH_3), 1.21 (t, 4-CH_3CH_2CO, s, 3-CH_3), 1.74 (H-2_{ax}), 2.05 (dd, H-2_{eq}), 2.10 (s, 1-CH_3CO), 2.43 (q, 4-CH_3CH_2CO), 4.02 (dq, H-5), 4.65 (dd, H-4), 5.08 (bs, 3-OH), 6.04 (dd, H-1), $J_{1,2ax}$ 9.7 Hz, $J_{1,2eq}$ 2.4, $J_{2ax,2eq}$ 13.0, $J_{4,5}$ 9.6, $J_{5,6}$ 5.9.

Anal. Calcd. for $C_{12}H_{20}O_6$: C, 55.37; H, 7.75.
Found: C, 55.12; H, 7.81.

Synthesis of 1-O-propionyl-4-O-acetyl-α- and β-L-mycarose (**22** and **23**)

A solution of 4-O-acetyl-L-mycarose (**20**, 120 mg) and propionic anhydride (0.5 ml) in pyridine (5 ml) was stood at room temperature overnight, and evaporated to dryness. The residue was shaken with water and ether, and the ether layer was worked out similarly to that described for **20** and **21**. Syrupy **22** (3 mg) obtained was identical in IR, mass and Rf values with that derived from **15**.

Crystalline **23** (59 mg) showed mp, 97~98°C, $[\alpha]_D^{22}$ −18° (*c* 1.0, $CHCl_3$). ^{1}H-NMR, 1.15 ppm (t, 1-CH_3CH_2CO), 1.17 (d, 5-CH_3), 1.19 (s, 3-CH_3), 1.74 (dd, H-2_{ax}), 2.06 (dd, H-2_{eq}), 2.15 (s, 4-CH_3CO), 2.38 (q, 1-CH_3CH_2CO), 4.01 (dq, H-5), 4.64 (d, H-4), 6.07 (dd, H-1), $J_{1,2ax}$ 9.4 Hz, $J_{1,2eq}$ 2.4, $J_{2ax,2eq}$ 13.2, $J_{4,5}$ 9.3, $J_{5,6}$ 6.0. Mass, *m/e* 260 w (M^+), 259 w (M^+-1), 243 w, 242 w, 216 w, 203 m, 200 m, 187 s, 169 m, 158 m, 126 s and 116 s.

Anal. Calcd. for $C_{12}H_{20}O_6$: C, 55.37; H, 7.75.
Found: C, 55.54; H, 7.65.

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