MODIFICATIONS OF A MACROLIDE ANTIBIOTIC MIDECAMYCIN. II*

REACTION OF MIDECAMYCIN AND 9-ACETYLMIDECAMYCIN WITH DIMETHYLSULFOXIDE AND ACETIC ANHYDRIDE

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Treatment of 9,2′-diacetylmidecamycin (**2**) with DMSO and acetic anhydride afforded 3″-methylthiomethyl derivative (**3**) preferably in the presence of pyridine. Reaction of midecamycin (**1**) with DMSO and acetic anhydride gave 2′-acetyl-9-dehydro-3″-methylthiomethyl derivative (**9**) indicating that the three hydroxyl groups reacted in a different way to the reagent. When compound **2** was reacted with DMSO and acetic anhydride in the presence of CCl_4, 3″-acetoxymethyl derivative (**13**) was a major product, which was formed *via* **3** through the PUMMERER rearrangement. The structures of **3**, **9** and **13** were confirmed by examining NMR and mass spectra of these compounds and their deuterio analogues. They showed antimicrobial spectra similar to **1** but superior *in vivo* activity.

In the course of our studies on the structure-activity relationship of midecamycin (**1**), in particular with regards to the derivatives of the 3″-tertiary hydroxyl group, we have reported the synthesis of 9,3″-diacetylmidecamycin[1]. Reaction of midecamycin (**1**) with dimethylsulfoxide (DMSO) and acetic anhydride was originally used to prepare 9-dehydromidecamycin, but the product obtained was found to be 3″-methylthiomethylated-9-dehydro derivative. This reaction was later modified to prepare 3″-acetoxymethyl derivatives. Thus, two new series of biologically active 3″-alkylated derivatives were provided.

The synthesis and structure proof of these derivatives are the main subject of this paper. Biological data are briefly summarised.

Preparation of 3″-Methylthiomethyl Derivatives of Midecamycin

9,2′-Diacetylmidecamycin (**2**) prepared from **1** by treatment with acetic anhydride and pyridine was allowed to react at room temperature with DMSO and acetic anhydride. TLC of the reaction mixture revealed that a spot of higher Rf value than **2** appeared after several hours, and became dominant within a couple of days, with almost complete disappearance of **2**. A new product (**3**) with higher Rf value was isolated by chromatography over silica gel, followed by crystallization from aqueous alcohol.

The ^{1}H-NMR spectrum of **3** showed two new signals that were absent in **1**; a singlet at 2.02 and an AB quartet at 4.52 and 4.66***. Other spectral feature of **3** was little changed from that of **2**, as

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*** It was difficult to find this AB quartet directly from the ^{1}H-NMR of **2** because of severe overlapping, however, the comparison of the spectra of **2** and its d_5-analogue prepared by the use of DMSO-d_6 revealed these signals.

shown in Fig. 1. Chemical shifts and intensities of new signals suggested the introduction of a methylthiomethyl group ($-OCH_2SCH_3$), as is frequently formed in the reaction of DMSO and acetic anhydride with an alcohol[2]. In order to confirm the location of the methylthiomethyl group on the mycarose moiety, an attempt was made by treating **3** with Amberlyst-15 in methanol, to isolate methyl mycaroside bearing a methylthiomethyl group. It was found, however, that the methylthiomethyl group was hydrolysed during methanolysis, and products isolated were methyl 4-O-propionyl-α,β-L-mycarosides (**4**). Reaction of methyl 4-O-propionyl-β-L-mycaroside (**4**β) with DMSO and acetic anhydride gave

Fig. 1. ^{1}H-NMR Spectrum of 9,2′-diacetyl-3″-methylthiomethylmidecamycin (**3**) (100 MHz, $CDCl_3$).

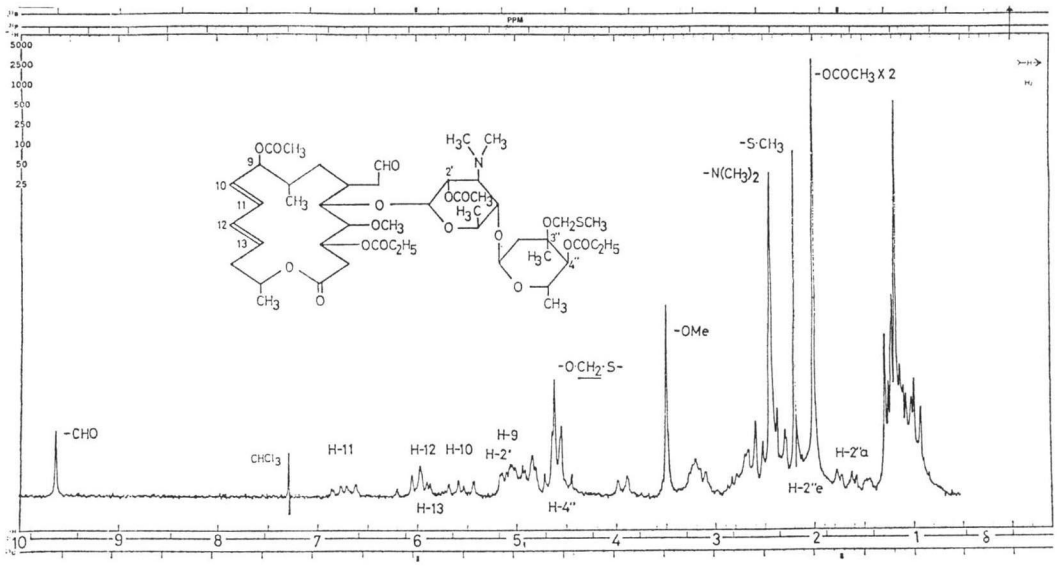


Fig. 2. ^{1}H-NMR Spectrum of methyl 4-O-propionyl-3″-O-methylthiomethyl-β-L-mycaroside (**5**β) (100 MHz, C_6D_6).

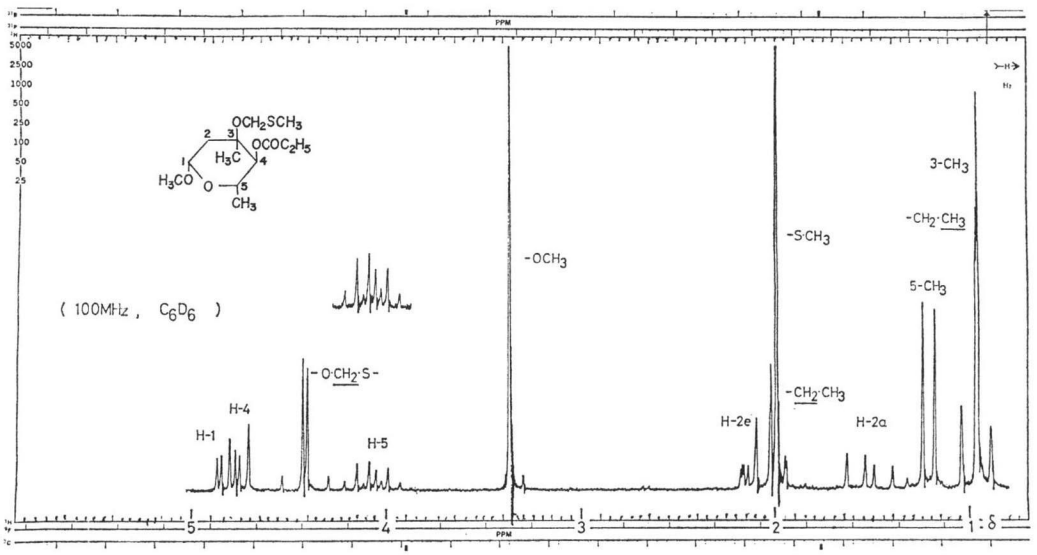
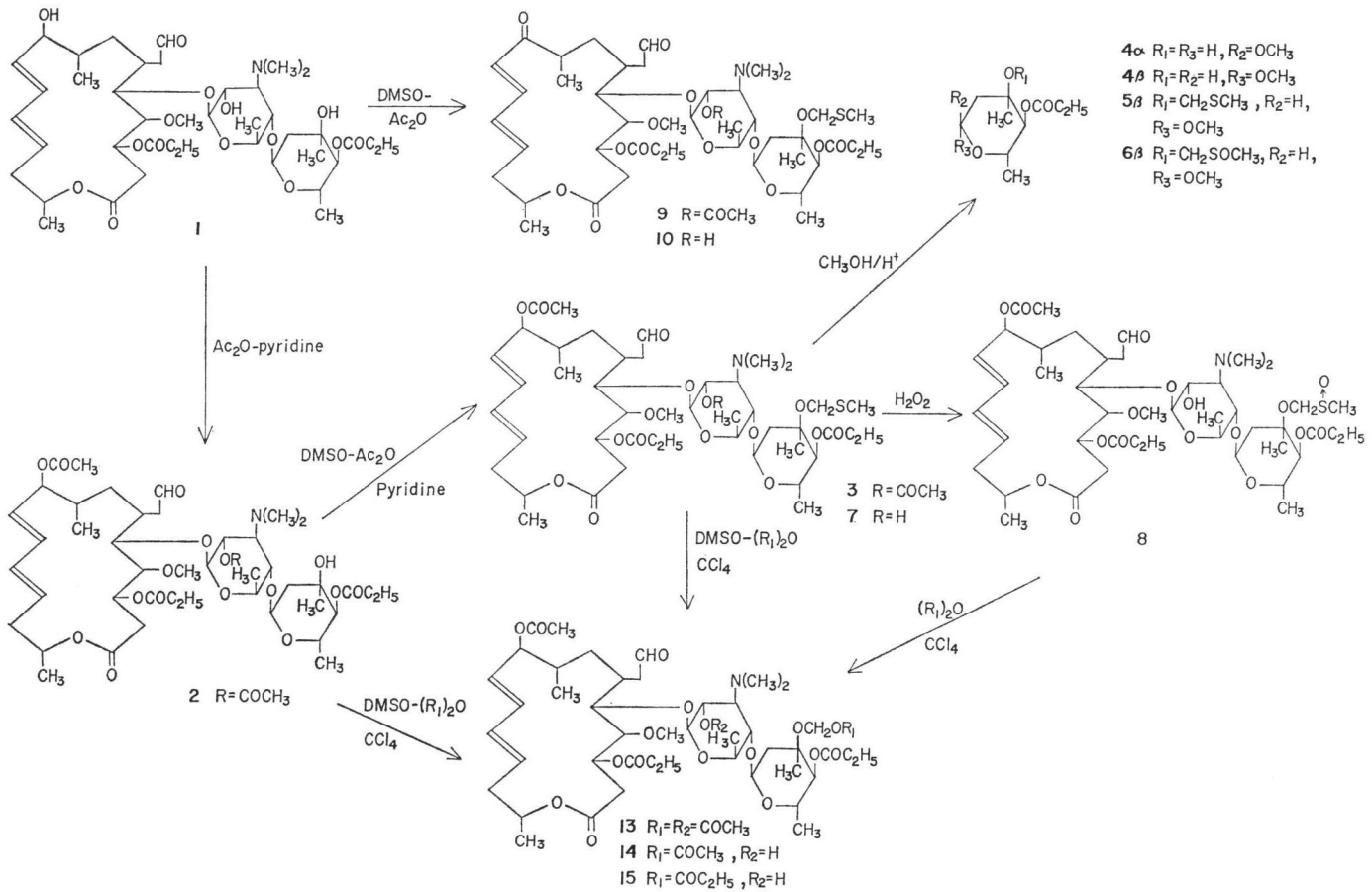


Chart 1.


DMSO-Ac2O
Ac2O-pyridine
DMSO-Ac2O
Pyridine
CH3OH/H+
H2O2
DMSO-(R1)2O
CCl4
(R1)2O
CCl4
DMSO-(R1)2O
CCl4
1
2 R=COCH3
3 R=COCH3
7 R=H
8
9 R=COCH3
10 R=H
13 R1=R2=COCH3
14 R1=COCH3, R2=H
15 R1=COC2H5, R2=H
4α R1=R3=H, R2=OCH3
4β R1=R2=H, R3=OCH3
5β R1=CH2SCH3, R2=H, R3=OCH3
6β R1=CH2SOCH3, R2=H, R3=OCH3

methyl 3-O-methylthiomethyl-4-O-propionyl-β-L-mycaroside (**5**β) in good yield, supporting indirectly the structure **3** proposed. The ^{1}H-NMR spectrum of **5**β showed, like **3**, a new singlet at 2.00 (SCH_3) and an AB quartet at 4.39 and 4.46 due to the magnetically inequivalent methylene of the $-OCH_2S-$ group, and other signals were assigned as shown in Fig. 2. Hydrogen peroxide oxidation of **5**β gave the corresponding sulfoxide (**6**β).

Partial hydrolysis of the 2′-acetyl group of **3** by treatment with 90% methanol gave quantitatively 9-acetyl-3″-methylthiomethylmidecamycin (**7**), which was crystallized from 70% methanol. The structures of **3** and **7** were further confirmed by the mass fragmentation pattern summarized in Chart 3 and Table 1, which indicated a mass increment of 60 due to $-CH_2SCH_3$ in the mycarose moiety. As compared to the fragmentation of **1** and **2**, that of **3** and **7** was complicated, especially, fragmentation involving the mycarose portion. Though a (M minus propionyloxy) ion was dominant in **1** and **2**, the corresponding ion at *m/e* 884 or 842 in **3** and **7** was surpassed in intensity by an ion at *m/e* 808 or 766 that arose from release of both propionyloxy radical and C_2H_4OS molecule. Other principal peaks were assigned as shown in Chart 3. The assignment was supported by high resolution mass data (Table 1) and the mass spectrum of the pentadeuteriomethylthiomethyl derivative (**3**-d_5), which was synthesized by the use of DMSO-d_6 in place of DMSO. Most interesting was the McLAFFERTY shift of a deuterium from $-OCD_2S-$ to C-4″, which occurred simultaneously or after the rupture of a propionyloxy radical.

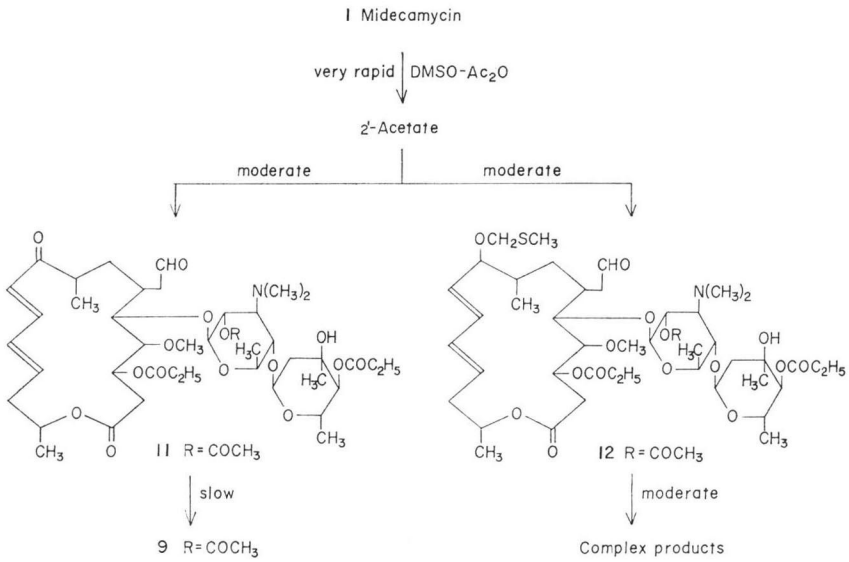
Table 1. High-resolution mass spectra for **7**.

Composition	Calcd. mass	Found mass
a. $C_{42}H_{68}NO_{14}S$	842.4381	842.4369
b. $C_{40}H_{64}NO_{13}$	766.4373	766.4373
c. $C_{37}H_{58}NO_{11}$	692.4006	692.3992
d. $C_{32}H_{58}NO_{10}$	611.3666	611.3679
e. $C_{25}H_{37}O_8$	465.2486	465.2513
f. $C_{20}H_{36}NO_7S$	434.2210	434.2212
g. $C_{23}H_{33}O_6$	405.2275	405.2278

Hydrogen peroxide oxidation of **3** and **7** in methanol gave the sulfoxide (**8**), with partial hydrolysis of the 2′-acetyl group in the latter. The structure of **8** was again confirmed by mass spectroscopy.

Reaction of midecamycin (**1**) itself with DMSO and acetic anhydride at room temperature gave a complex mixture at an early stage of the reaction, but gradually accumulated a main product (**9**), which was isolated after silica-gel chromatography. It exhibited a UV maximum at 280 nm, indicative of $\alpha,\beta,\gamma,\delta$-unsaturated carbonyl function[3]. ^{1}H-NMR in $CDCl_3$ showed new peaks at 2.03 ($COCH_3$), 2.18 (SCH_3) and 4.60 (OCH_2). The mass spectrum showed a dehydrolactone fragment at *m/e* 421, acetylmycaminose fragment at *m/e* 216 and methylthiomethylmycarose fragment at *m/e* 261. These results were fully consistent with the 2′-acetyl-9-dehydro-3″-methylthiomethylmidecamycin (**9**). It was of interest to see that each of the three hydroxyl groups in **1** reacted in a different way to the DMSO-acetic anhydride reagent, that is, oxidation of 9-allylic alcohol, acetylation of 2′-hydroxyl group catalyzed by an adjacent dimethylamino group, and methylthiomethylation of the 3″-hydroxyl group. Selective ester hydrolysis of **9** in aqueous methanol gave 9-dehydro-3″-methylthiomethylmidecamycin (**10**). According to TLC analysis, the first step of the reaction was very rapid (*ca* 10 minutes) acetylation of the 2′-hydroxyl group followed by oxidation. Methylthiomethylation of the 3″-hydroxyl group was the slowest reaction. It was found further that as the reaction proceeded, the 2′-acetate gradually changed to two compounds, 2′-acetyl-9-dehydromidecamycin (**11**) and 2′-acetyl-9-methylthiomethylmidecamycin (**12**) within a couple of hours. As the reaction progressed, compound **11** was converted

Chart 2.



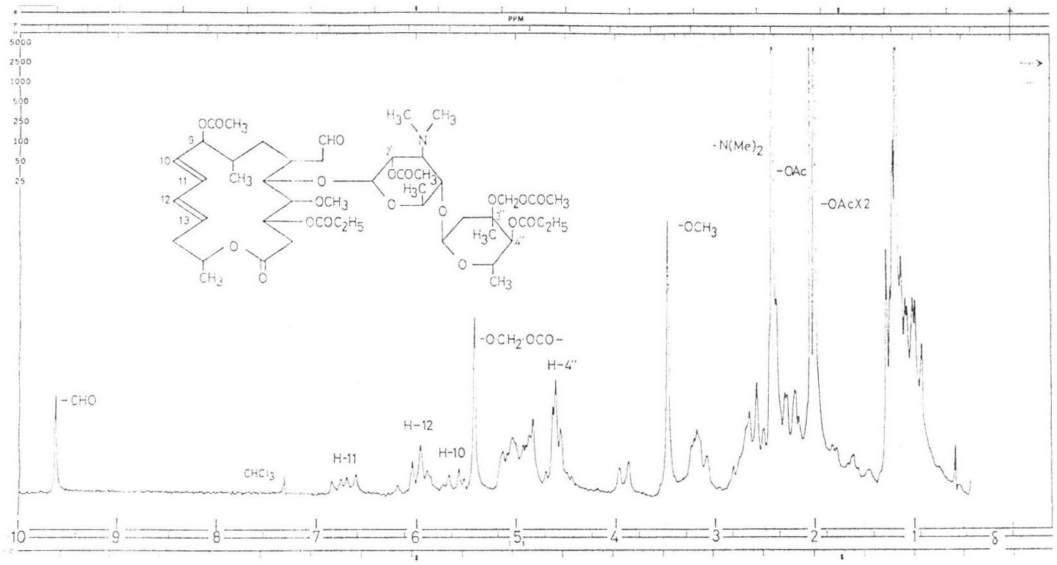
to a final product **9**, however, compound **12** changed to an extremely complex reaction mixture followed by decomposition at the end. These reactions are summarized in Chart 2. The formation of the 9-methylthiomethyl derivative resulted in great reduction of the yield of **9**. Therefore, if 9-dehydromidecamycin itself is required in quantity, a reagent other than acetic anhydride should be used in combination with DMSO. Of these reactions, particularly noteworthy was the ease of alkylation of a tertiary hydroxyl group, because it is relatively inert to substitution reactions[1]. So far as examined, other sulfoxides such as dibenzylsulfoxide do not participate in analogous reactions.

Preparation of 3″-Acyloxymethyl Derivatives of Midecamycin

During large scale synthetic work on **3** in a few cases a new product (**13**) was obtained together with **3**, by treatment of **2** with DMSO and acetic anhydride. Careful examination of the formation conditions for this unexpected product revealed that the residue of carbon tetrachloride that was used as a crystallizing solvent of **2** favoured the formation of a new product (**13**), whereas pyridine used in the acetylation reaction accelerated the formation of the methylthiomethyl derivative (**3**). Subsequently, yield of **13** was improved by the addition of carbon tetrachloride to the reaction mixture. Carbon tetrachloride was the best catalyst so far examined among many halogeno compounds.

^{1}H-NMR of the new product (**13**) showed an extra acetyl signal at 2.04 and a singlet at 5.42, with simultaneous disappearance of signals due to a methylthiomethyl group at 2.21, 4.53 and 4.66. (Fig. 3) Chemical shifts and intensities of these new signals could be best explained by the replacement of a methylthiomethyl group by an acetoxymethyl group at C-3″. This was supported by the mass spectrum of **13**, which showed an ion at *m/e* 244 assignable to the (acetoxymethylmycarose minus CHO) fragment, as well as an acetyllactone fragment at *m/e* 465 and an acetylmycaminose fragment at *m/e* 216. The dominant *m/e* 244 ion arose from rupture of the mycarose ring. This was in sharp contrast to the selective cleavage of the mycarose-mycaminose bond in **3** and **7**. Partial hydrolysis of **13** in 70% methanol gave 3″-acetoxymethyl-9-acetylmidecamycin (**14**).

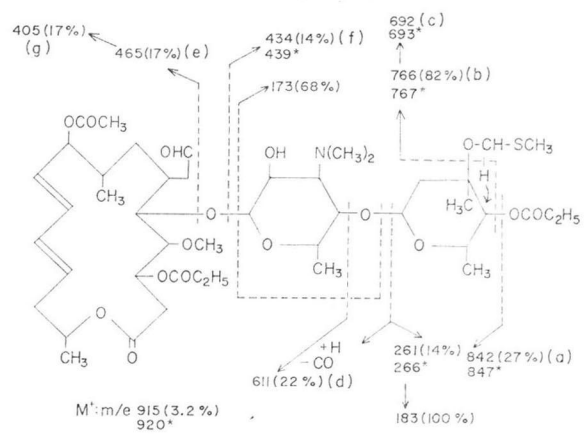
Fig. 3. ^{1}H-NMR Spectrum of 3″-acetoxymethyl-9,2′-diacetylmidecamycin (**13**) (100 MHz, $CDCl_3$).



As was expected, the acetoxymethyl derivatives (**13, 14**) were relatively unstable, and hydrolyzed partially to 9-acetylmidecamycin by lengthy heating at 60°C in 75% methanol. Methanolysis of **14** by refluxing with Amberlyst-15 in methanol gave methyl-4-O-propionyl-α and β-L-mycaroside (**4**), losing an acetoxymethyl group.

In order to investigate the mode of formation of **13**, 9-acetyl-3″-methylthiomethylmidecamycin (**7**) was brought into reaction with DMSO and acetic anhydride in the presence of carbon tetrachloride, yielding **13**, identical with that directly prepared from the 9,2′-diacetate (**2**). Use of acetic anhydride-d_6 or propionic anhydride in the reaction of **7** gave the trideuterioacetoxymethyl derivative (**13**-d_3, $-OCH_2OCOCD_3$) or propionyloxymethyl derivative (**15**) respectively. They showed M^+ at *m/e* 972 or 983 in the mass spectra. On the other hand, use of the DMSO-d_6-acetic anhydride reagent gave **13** with no mass increment, but did give the dideuterio derivative (**13**-d_2, OCD_2OCOCH_3) when reacted with the 9,2′-diacetate (**2**). These results indicated that compound **13** was derived from **3** by replacement of a methylthio group by an acetoxy group. The mass spectra of the partially deuterated derivatives (**13**-d_3, **13**-d_2) gave supporting evidence for the fragmentation of **13** as shown in Chart 4. Of particular interest was unambiguous confirmation of a McLAF-

Chart 3. Electron-impact mass fragmentation of 9-acetyl-3″-methylthiomethylmidecamycin (**7**).

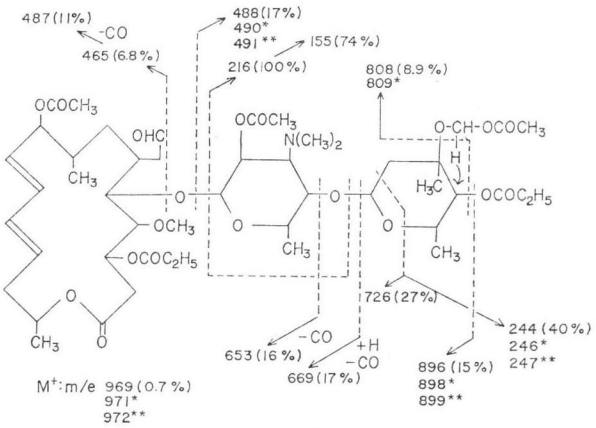


1) Figures in parenthesis indicate relative abundance based on the peak at *m/e* 183 (100%).
*: Fragments of **7**-d_5 (3″-OCD_2SCD_3)

FERTY shift of methylene deuterium of 3″–OCD_2OCOCH_3 to C-4″, as indicated in Chart 4.

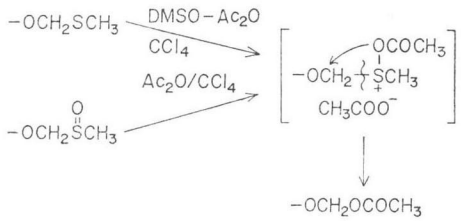
Further study revealed that the methylsulfoxidomethyl derivative (**8**) obtained by peroxide oxidation of **7** gave, upon reaction with acetic anhydride and carbon tetrachloride, the acetoxymethyl derivative (**13**) as a major product. DMSO was not necessary for this conversion reaction. Chart 5 summarizes the sequence of reactions to form an acetoxymethyl group from a methylthiomethyl group. This reaction can be considered to be a PUMMERER type rearrangement[4], in which C-S bond cleavage occurred predominantly. It was shown that the role of DMSO is not only as an oxygen donor but also as a methylene supplier in the direct formation of **13** from **2**. The role of carbon tetrachloride was not clear, but seemed to play some role in the fission of C-S bond.

Chart 4. Electron-impact mass fragmentation of 3″-acetoxymethyl-9,2′-diacetylmidecamycin (**13**).



1) Figures in parenthesis indicate relative abundance based on the peak at *m/e* 216 (100%).
*: Fragments of **13**-d_2 (3″–OCD_2OCOCH_3)
: Fragments of **13-d_3 (3″–OCH_2OCOCD_3)

Chart 5.



Antimicrobial Activity

Compounds **7**, **10**, **14** and **15** showed antimicrobial spectra similar to **1**. It is noted that 3″-methylthiomethyl derivatives are active against macrolide-resistant *Mycoplasma* (Table 2).

Protective effects of **7**, **14** and **15** against intraperitoneal infection with *Staphylococcus aureus* in the mouse were 2~4 times stronger than **1**. Among these, 9-acetyl-3″-acetoxymethylmidecamycin (**14**) showed the highest activity as seen in Table 2.

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 a Hitachi model 323 UV and a model 215 IR spectrometers. NMR spectra were determined in $CDCl_3$ unless otherwise noted using a Varian XL-100 spectrometer with TMS as an internal standard. Chemical shifts given on the ppm scale from TMS were determined by the double resonance if necessary. EI mass spectra were determined at 75 eV. Column chromatography was performed on Mallinckrodt Silic AR (200~325 mesh) or Wakogel C-300. Analytical TL plates were Merck silica gel F_{254}, and spots were detected by spraying with 10% sulfuric acid. Rf values of major compounds are listed in Table 3.

9-Acetyl-3″-methylthiomethylmidecamycin (7)

A solution of 9,2′-diacetylmidecamycin (**2**) (1.0 g) in DMSO (30 ml) and acetic anhydride (3 ml)

was kept at room temperature for 3 days. The mixture was poured into ice-water (500 ml) and extracted with benzene (100 ml). The benzene layer was washed thrice with water and concentrated to dryness. The residue, dissolved in a small amount of benzene, was charged on a column of silica gel (2.7×16 cm), and developed with a mixture of benzene - acetone (9: 1). Effluents were collected in 8 g fractions, and evaporation of solvent from fractions 11~14 gave 9,2′-diacetyl-3″-methylthiomethylmidecamycin (**3**) (662 mg), yield 62%, which was crystallized from cyclohexane. m.p. 179.5~181°C, $[\alpha]_D^{22}$ −85.7° (*c* 1.2, EtOH), MS; *m/e* 957 (M^+), ^{1}H-NMR; 2.02 ($CH_3CO \times 2$), 2.21 (CH_3SCH_2O–), 4.52, 4.66 (AB quartet, O–CH_2SCH_3).

Anal. Calcd. for $C_{47}H_{75}NO_{17}S$:
C, 58.91; H, 7.89; N, 1.46; S, 3.55.
Found:
C, 58.70; H, 7.80; N, 1.20; S, 3.00.

A large-scale preparation of **3** (44.7 g) was more easily accomplished by reacting **2** (60 g) with DMSO (300 ml) and acetic anhydride (200 ml) at 38°C for 2 days, followed by concentration and crystallization by addition of iso-propanol. Further crops of **3** (5.3 g) were recovered from the mother liquor by addition of hexane (300 ml), total yield, 78%.

To a solution of **3** (725 mg) in methanol (300 ml) was added water (3 ml). The mixture was kept at room temperature overnight, then poured into ice-water, and was extracted with benzene, after the pH was adjusted to 8 with sodium hydrogen carbonate. The benzene extract gave, after evaporation of solvent, 9-acetyl-3″-methylthiomethylmidecamycin (**7**) (690 mg), which was crystallized from 70% methanol. m.p. 188~189°C, $[\alpha]_D^{24}$ −65.9° (*c* 1.1, EtOH), MS; *m/e* 915 (M^+), ^{1}H-NMR; 2.01 (CH_3CO), 2.19 (CH_3SCH_2O–), 4.53, 4.65 (AB quartet, OCH_2SCH_3).

Anal. Calcd. for $C_{45}H_{73}NO_{16}S$: C, 59.00; H, 8.03; N, 1.53; S, 3.50.
Found: C, 58.85; H, 7.98; N, 1.40; S, 3.30.

Table 2. *In vitro* and *in vivo* antimicrobial activities of 3″-methylthiomethyl and acetoxymethyl derivatives of midecamycin.

In vitro activity

Organism	MIC (mcg/ml)				
	1	**7**	**10**	**14**	**15**
Staph. aureus 209P JC-1	0.78	0.78	1.56	1.56	1.56
Staph. aureus Smith	0.39	0.78	1.56	0.78	0.78
Staph. aureus Terajima	1.56	0.39	0.78	3.13	3.13
Strept. haemolyticus Cook	0.19	0.19	0.19	0.19	0.19
Strept. pneumoniae Type I	0.19	0.39	0.19	0.19	0.19
B. subtilis ATCC 6633	0.39	0.39	0.39	0.78	0.78
E. coli NIH TC-2	>100	>100	>100	>100	>100
Salm. typhi 0-901-W	>100	>100	>100	>100	>100
*Mycoplasma gallisepticum** T-4AT	>100	12.5	12.5	—	—

* Resistant to macrolide antibiotics.

In vivo effect against *Staphylococcus aureus* Smith S-242

Dose (oral administration)	Survival ratio of mice (%)*			
	1	**7**	**14**	**15**
400 mg/kg	70	100	100	100
200 mg/kg	33	90	100	100
100 mg/kg	0	20	70	11
PD_{50} (mg/kg)	275	131	*ca* 85	*ca* 140

* ICR male mice on an average weight 20 g were intraperitoneally challenged with a $100 \times LD_{50}$ dose (3.5×10^7 cells/mouse).

Table 3. Rf Values of midecamycin derivatives on silica gel TLC developed with a mixture of benzene - acetone (4 : 1).

Midecamycin derivative	Rf value
9,2′-Diacetyl- (**2**)	0.44
9,2′-Diacetyl-3″-methylthiomethyl- (**3**)	0.60
9-Acetyl-3″-methylthiomethyl- (**7**)	0.18
9-Acetyl-3″-methylsulfoxidomethyl- (**8**)	0.00
9-Dehydro-2′-acetyl-3″-methylthiomethyl- (**9**)	0.46
9-Dehydro-3″-methylthiomethyl- (**10**)	0.07
9,2′-Diacetyl-3″-acetoxymethyl- (**13**)	0.52
9-Acetyl-3″-acetoxymethyl- (**14**)	0.09
9-Acetyl-3″-propionyloxymethyl- (**15**)	0.14

Isolation of methyl-4-O-propionyl-mycaroside (**4**) and its conversion to the 3-O-methylthiomethyl derivative (**5**)

A mixture of 9-acetyl-3″-methylthiomethylmidecamycin (**7**) (10 g) and Amberlyst-15 (100 ml) in methanol (300 ml) was refluxed under stirring for 3 hours, and then filtered. The filtrate was concentrated to a syrup (6.1 g), which was chromatographed over silica gel (4×22 cm), developing with benzene - acetone (13: 1). Methyl 4-O-propionyl-α-L-mycaroside (**4α**) (400 mg) and β-L-mycaroside (**4β**) (1.0 g) were successively eluted and crystallized.

A solution of **4β** (168 mg) in a mixture of DMSO (20 ml) and acetic anhydride (10 ml) was kept at 35°C overnight. Water was added, and the mixture was extracted with chloroform. A crude product was precipitated from benzene-petroleum ether, and the precipitate (230 mg) was subjected to silica gel chromatography, (developer, benzene - acetone=20: 1) to give methyl 3-O-methylthiomethyl-4-O-propionyl-β-L-mycaroside (**5β**) (180 mg, 85%) as a colorless syrup. $[\alpha]_D^{20}$ −13° (*c* 1.4, $CHCl_3$), MS; *m/e* 292 (M^+), ^{1}H-NMR (C_6D_6, 100 MHz); δ, 0.96 (s, 3″-CH_3), 0.97 (t, CH_3CH_2CO, J=7.5 Hz), 1.21 (d, 6-CH_3, J=6.1 Hz), 1.51 (dd, H-2_{ax}, J=9.5 Hz, 13.8 Hz), 1.99 (s, CH_3S–), 2.05 (q, CH_3CH_2CO, J=7.5 Hz), 3.37 (s, CH_3O), 4.07 (dq, H-5, J=6.1, 9.7 Hz), 4.39 and 4.46 (AB quartet, CH_3SCH_2–J=10.0 Hz), 4.75 (d, H-4, J=9.7 Hz), 4.81 (dd, H-1, J=2.3 Hz, 9.6 Hz).

Anal. Calcd. for $C_{13}H_{24}O_5S$: C, 53.40; H, 8.27; S, 10.97.
Found: C, 53.49; H, 8.36; S, 11.16.

Crude **5β** (180 mg) was dissolved in methanol (20 ml), and 30% hydrogen peroxide (0.2 ml) was added. The mixture stood at room temperature overnight, and the crude product was chromatographed over silica gel, developing with chloroform - methanol (30:1). Methyl 3-O-methylsulfoxidomethyl-4-O-propionyl-β-L-mycaroside (**6β**) (133 mg) was obtained as a syrupy solid. $[\alpha]_D^{20}$ −40.8° (*c* 1.0, $CHCl_3$).

9-Acetyl-3″-methylsulfoxidomethylmidecamycin (**8**)

To a solution of 9-acetyl-3″-methylthiomethylmidecamycin (**3**) (250 mg) in methanol (30 ml) was added 30% aqueous hydrogen peroxide (0.5 ml), and the mixture was kept at room temperature for 4 hours. MnO_2 (100 mg) was added, and the filtered solution was evaporated to dryness. The residue was reprecipitated from benzene - cyclohexane to give 9-acetyl-3″-methylsulfoxidomethylmidecamycin (**8**) (230 mg, yield 95%) as a white powder. It reacted positively with the NaI-HCl reagent for sulfoxide. m.p. 126~133°C (decomp.), MS; *m/e* 931 (M^+), $[\alpha]_D^{20}$ −73° (*c* 0.9, EtOH). ^{1}H-NMR; 2.03 (CH_3CO–), 2.61 (–SCH_3), 2.58 ($N(CH_3)_2$).

Anal. Calcd. for $C_{45}H_{73}NO_{17}S$: C, 58.39; H, 7.99; N, 1.48; S, 3.38.
Found: C, 58.51; H, 8.16; N, 1.29; S, 3.32.

9-Dehydro-3″-methylthiomethylmidecamycin (**10**)

A solution of midecamycin (**1**) (5.0 g) in a mixture of DMSO (100 ml) and acetic anhydride (20 ml) was kept at room temperature for 3 days. Benzene (500 ml) and excess $NaHCO_3$ were added, and the benzene extract, after washing three times with water, was concentrated to dryness. The residue was dissolved in a small amount of benzene, and applied to a column of silica gel (2.7×16 cm), which was developed with a mixture of benzene - acetone (9: 1). Effluents were collected in 8-g fractions, and evaporation of solvent from fractions 24~27 gave 2′-acetyl-9-dehydro-3″-methylthiomethylmidecamycin (**9**) (1.92 g, yield 34%) as an amorphous powder. A further **9** of inferior purity (1.49 g) was recovered from the fractions 22~23 and 28~41. m.p. 108~112°C. $[\alpha]_D^{20}$ −42.2° (*c* 0.5, EtOH). MS; *m/e* 913. ^{1}H-NMR: δ 2.18 (SCH_3), *ca* 4.6 (OCH_2SCH_3), 2.03 ($COCH_3$).

Anal. Calcd. for $C_{45}H_{71}NO_{16}S$: C, 59.13; H, 7.83; N, 1.53; S, 3.51.
Found: C, 58.95; H, 7.50; N, 1.43; S, 3.30.

A solution of **9** (1.92 g) in 90% methanol (100 ml) was kept at room temperature overnight, and after concentration, was poured into ice-water. The mixture was neutralized with sodium hydrogen carbonate, and extracted with benzene. The benzene layer was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness to give 9-dehydro-3″-methylthiomethylmidecamycin (**10**) (1.62 g) as an amorphous powder. m.p. 108~112°C. $[\alpha]_D^{20}$ −40.4° (*c* 0.96, EtOH). ^{1}H-NMR: 2.19 (SCH_3).

Anal. Calcd. for $C_{43}H_{69}NO_{15}S$: C, 59.22; H, 7.98; N, 1.61; S, 3.68.
Found: C, 59.00; H, 8.20; N, 1.50; S, 3.44.

2′-Acetyl-9-dehydromidecamycin (**11**) and 2′-acetyl-9-methylthiomethylmidecamycin (**12**)

A solution of midecamycin (**1**) (400 mg) in a mixture of DMSO (10 ml) and acetic anhydride (3 ml) was kept at room temperature for 5 hours. Benzene (30 ml) and excess of sodium hydrogen carbonate were added and diluted with 50 ml of water. The benzene extract, after washing with water and drying over anhydrous sodium sulfate, was concentrated to dryness. The residue was purified on a column of silica gel (1.5 cm × 30 cm) which was developed with a mixture of benzene - acetone (10: 1). Effluents were collected in 5-g fractions, and from fractions 56～90, 2′-acetyl-9-dehydromidecamycin (**11**, identical with monoacetyl-SF-837 A_3[5]) was obtained (80 mg) as colourless crystals; m.p. 181°～183°C, $[\alpha]_D^{22}$ −61.9° (*c* 1, $CHCl_3$), MS; *m/e* 853 (M^+): and from fractions 20～40, 2′-acetyl-9-methylthiomethylmidecamycin (**12**, 102 mg) was obtained as a glass; $[\alpha]_D^{22}$ −59.9° (*c* 1.5, $CHCl_3$), MS; *m/e* 915 (M^+), ^{1}H-NMR, 2.03 (s, 2′-CH_3CO), 2.12 (s, 9-CH_3SCH_2-).

Anal. Calcd. for $C_{45}H_{73}NO_{16}S$: C, 59.00; H, 8.03; N, 1.53; S, 3.50.
Found: C, 58.85; H, 7.98; N, 1.40; S, 3.30.

3″-Acetoxymethyl-9-acetylmidecamycin (**14**)

(a) From 9,2′-diacetylmidecamycin (**2**)

A solution of **2** (3.0 g) that was free from pyridine in a mixture of DMSO (60 ml), acetic anhydride (15 ml) and carbon tetrachloride (7.5 ml) was allowed to react at 28°C for 6 days, and then poured into a large volume of ice-water containing excess of sodium hydrogen carbonate. The mixture was extracted twice with benzene (200 ml each), and the benzene layer was washed twice with water, dried over anhydrous sodium sulfate, and concentrated to dryness. The residue was dissolved in a small amount of benzene and placed on a column of silica gel (2.8 × 18 cm), which was developed with a mixture of benzene - acetone (10: 1). Effluents were collected in 8-g fractions, and concentration of fractions 11～28 gave 3″-acetoxymethyl-9,2′-diacetylmidecamycin (**13**) (2.1 g, yield 65%) that was reprecipitated from benzene - cyclohexane. m.p. 103～109°C. $[\alpha]_D^{20}$ −87° (*c* 1.0, $CHCl_3$). MS; *m/e* 969 (M^+). ^{1}H-NMR: δ 2.00 ($COCH_3 \times 2$), 2.04 ($COCH_3$), 5.42 (OCH_2OCOCH_3).

Anal. Calcd. for $C_{48}H_{75}NO_{19}$: C, 59.43; H, 7.79; N, 1.44.
Found: C, 59.15; H, 7.58; N, 1.52.

Three grams of **13** was dissolved in methanol (100 ml), and the solution was allowed to react at room temperature overnight. Evaporation of solvent and crystallization from 75% aqueous methanol gave 3″-acetoxymethyl-9-acetylmidecamycin (**14**) (2.5 g). m.p. 174～176°C (sintered), $[\alpha]_D^{24}$ −68.8° (*c* 1.1, EtOH). MS; *m/e* 927 (M^+). ^{1}H-NMR: δ 2.00, 2.04 ($COCH_3 \times 2$), 5.40 (OCH_2OCOCH_3).

Anal. Calcd. for $C_{46}H_{73}NO_{18}$: C, 59.53; H, 7.93; N, 1.51.
Found: C, 59.71; H, 7.68; N, 1.72.

(b) From 9-acetyl-3″-methylthiomethylmidecamycin (**7**)

A solution of **7** (500 mg) in a mixture of carbon tetrachloride (32 ml), acetic anhydride (8 ml) and DMSO (0.5 ml) was allowed to stand at 28°C for 5 days, and concentrated to dryness. The residue, dissolved in benzene, was chromatographed over silica gel (1 × 25 cm), developing with a mixture of benzene - acetone (10: 1). Effluents containing **13** were combined, and evaporated to give 3″-acetoxymethyl-9,2′-diacetylmidecamycin (**13**) (290 mg). This was dissolved in 90% ethanol (30 ml). After standing at room temperature overnight, the solution was evaporated to give **14** (245 mg).

(c) From 9-acetyl-4″-methylsulfoxidomethylmidecamycin (**8**)

A solution of **8** (120 mg) in a mixture of carbon tetrachloride (6 ml) and acetic anhydride (1.5 ml) were reacted at 28°C for 70 hours, and evaporated to dryness. The residue was chromatographed over silica gel (0.8 × 15 cm), using a mixture of benzene - acetone (10: 1) as a developer. Effluents containing **13** were collected and concentrated to give **13** (65 mg), which upon treatment with aqueous methanol, was converted to **14** (50 mg).

9-Acetyl-3″-propionyloxymethylmidecamycin (**15**)

To a solution of **3** (10 g) in carbon tetrachloride (320 ml) were added propionic anhydride (80 ml)

and DMSO (5 ml), and the mixture was allowed to stand at 50°C for 70 hours, and then repeatedly concentrated with addition of toluene. The residual syrup was chromatographed over silica gel, developing with benzene - acetone (13: 1). Effluents containing 9,2′-diacetyl-3″-propionyloxymethylmidecamycin were combined and evaporated to dryness. The residue, upon partial hydrolysis in 80% methanol, gave **15** (5.9 g). A sample recrystallized from 75% methanol showed m.p. 178~179°C (sintered), $[\alpha]_D^{24}$ −72.2° (*c* 1.0, EtOH), MS; *m/e* 941 (M^+).

Anal. Calcd. for $C_{47}H_{75}NO_{18}$: C, 59.52; H, 8.02; N, 1.49.
Found: C, 59.28; H, 8.35; N, 1.25.

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