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# Metabolism of 9,3″-Diacetylmidecamycin. II.[1] The Structures of Several Metabolites of 9,3″-Diacetylmidecamycin

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The structures of several metabolites of 9,3″-diacetylmidecamycin were studied by means of nuclear magnetic resonance (NMR) and mass spectroscopy. It was shown that these metabolites are a pair of 14-hydroxylated compounds which differ in the stereochemistry at carbon-14 of the macrocyclic lactone ring, and the corresponding 9-deacetylated products.

**Keywords**——macrolide antibiotics; 9,3″-diacetylmidecamycin; biological oxidation; drug metabolism; diastereoisomers; NMR; mass spectra; allylic long-range coupling; *γ-gauche* effect

9,3″-Diacetylmidecamycin (**1**)[2] is a derivative of a macrolide antibiotic, midecamycin (**2**),[3] which is a glycoside of a 16-membered lactone belonging to the leucomycin family. These compounds have similar *in vitro* activity and toxicity. It has been shown, however, that **1** is 2—10 times more effective than **2** in the treatment of experimentally infected mice.[4] In earlier papers[5] we studied the metabolism of **1** and **2** with the aim of clarifying the reason for the improved *in vivo* activity (in mice) of **1**. The present paper deals with the structures of several metabolites of **1**; the results are interesting in relation to the stereochemical features of 16-membered lactone antibiotics.

## Results and Discussion

Figure 1 shows a chromatographic profile of a mixture of the metabolites of **1** for a representative case (recovered from the urine of a man). The structures of these compounds (**1**—**8**) are given in Fig. 2. Full details regarding the biological treatments of **1**, the procedure for purification of the metabolites, and the metabolic pathways of **1** and **2** will appear in a separate paper.[1] The present report deals solely with the stereochemical features of the hydroxylated metabolites, Mb-3 (**4**), Mb-5 (**5**), Mb-9a (**7**), and Mb-9b (**8**), which we encountered during the study of the metabolism of **1**.

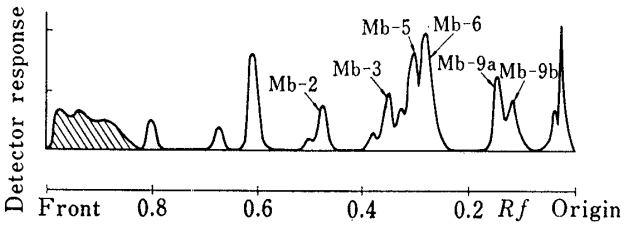


Fig. 1. Chromatographic Profile of a Mixture of the Drug Metabolites Recovered from the Urine of a Man

Mb's refer to the metabolic designations of the compounds[1] (*cf.* Fig. 2).

By spectral comparisons, compounds **6** and **7** were found to be identical with the substances $M_1$ and $M_2$, respectively, which we described in a previous paper[6] as metabolites of **2**. It was shown then that $M_1$ (**6**) is 4″-depropionylmidecamycin and that $M_2$ (**7**) is a hydroxylated product of **6**; the hydroxyl group was demonstrated to be at position 14 of the lactone ring.[6] The present materials (**3**—**8**), in mass spectral analysis, gave rise to peaks corresponding to their molecular ions ($M^+$), *m/e* 799, 815, 815, 757, 773, and 773, respectively, for **3**, **4**, **5**, **6**, **7**, and **8**.

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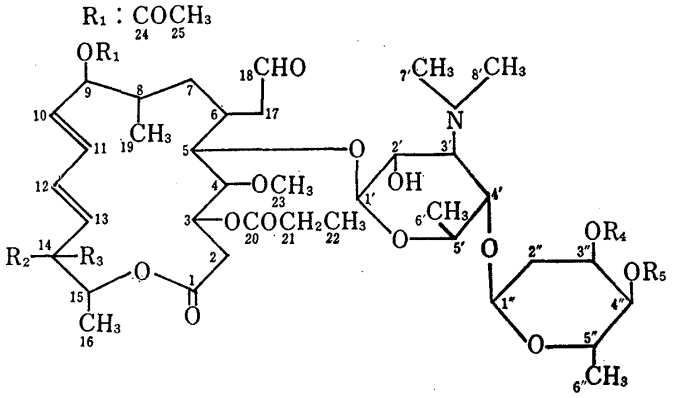

| Compound | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| 1 | $COCH_3$ | H | H | $COCH_3$ | $COCH_2CH_3$ |
| 2 | H | H | H | H | $COCH_2CH_3$ |
| 3 (Mb-2) | $COCH_3$ | H | H | H | H |
| 4 (Mb-3) | $COCH_3$ | OH | H | H | H |
| 5 (Mb-5) | $COCH_3$ | H | OH | H | H |
| 6 (Mb-6) | H | H | H | H | H |
| 7 (Mb-9a) | H | OH | H | H | H |
| 8 (Mb-9b) | H | H | OH | H | H |

Fig. 2. The Structures of the Antibiotics **1** and **2**, and of the Metabolites **3**—**8**

Examination of the mass spectra of these compounds suggests that **3** is an acetyl derivative of **6**. Thus, the fragment ions characteristic of a macrocyclic lactone (*m/e* 423 and 486[6]) are shifted upward by 42 mass units in **3** as compared to those in **6**. Comparisons of the fragmentation patterns suggest that **4** and **5**, and **7**, and **8** are hydroxylated products of **3** and **6**, respectively; the molecular as well as several fragment ion peaks of the hydroxylated metabolites are shifted by 16 mass units relative to the corresponding peaks in the non-hydroxylated materials.[6]

Tables I and II summarize the $^1H$ nuclear magnetic resonance (NMR) parameters of the metabolites. The corresponding values for **1** and **2** are included in the tables for comparison. It should be noted that the signal appropriate to the acetyl protons is absent in the spectra of **6**, **7**, and **8**, whereas for **3**, **4**, and **5** it appears at 2.02 ppm (3H). The peaks attributed to H-9 are shifted upfield (by *ca.* 1 ppm) in **6**, **7**, and **8**, relative to those in **3**, **4**, and **5**. This indicates that the acetyl group in **3**, **4**, and **5** is at position 9 of the lactone ring. The signals ascribed to H-14 are found at 4.42, 4.02, and 4.42 ppm, respectively, for **5**, **7**, and **8** (the position is unclear in the case of **4**, due to overlapping of the peaks). These values are con-

Table I. $^1H$ Chemiacl Shifts[a] of the Metabolites

| Compound | H-9 | H-10 | H-11 | H-12 | H-13 | H-14 | H-15 | $CH_3CO$ |
|---|---|---|---|---|---|---|---|---|
| 1[b] | 5.08 | 5.56 | 6.74 | 6.08 | 5.89 | [d] | [d] | 2.02(6H) |
| 2 | 4.08 | 5.62 | 6.65 | 6.07 | 5.78 | [d] | [d] | — |
| 3 | [d] | 5.58 | 6.73 | 6.07 | 5.89 | [d] | [d] | 2.02(3H) |
| 4 | 5.08 | 5.69 | 6.77 | 6.23 | 5.91 | [d] | 4.73 | 2.02(3H) |
| 5 | 5.08 | 5.69 | 6.81 | 6.42 | 6.11 | 4.42 | 5.08 | 2.02(3H) |
| 6[c] | 4.08 | 5.62 | 6.67 | 6.10 | 5.74 | 2.30 | [d] | — |
| 7 | 4.08 | 5.74 | 6.65 | 6.22 | 5.80 | 4.02 | 4.75 | — |
| 8 | 4.08 | 5.71 | 6.74 | 6.42 | 5.98 | 4.42 | 5.08 | — |

*a*) ppm downfield from internal TMS in $CDCl_3$.
*b*) Data for **1** and **2** are from Table I of ref. 2.
*c*) *cf.* ref. 7 for NMR data of **6** ($M_1$) and **7** ($M_2$).
*d*) Not determined due to complexity of the spectra.

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TABLE II. Coupling Constants in the $^1H$ NMR Spectra of the Metabolites (in Hz)

| Compound | $J_{9,10}$ | $J_{10,11}$ | $J_{11,12}$ | $J_{12,13}$ | $J_{13,14}$ | $^4J_{12,14}$ |
|---|---|---|---|---|---|---|
| **1**[a] | 9.0 | 14.4 | 9.2 | 14.4 | [b] | — |
| **2**[a] | 9.4 | 14.3 | 9.4 | 14.4 | [b] | — |
| **3** | 9 | 15 | 9 | 14 | [b] | — |
| **4** | 10 | 15 | 10 | 15 | 9 | — |
| **5** | 10 | 15 | 10 | 15 | 2 | 2 |
| **6** | 10 | 15 | 10 | 15 | [b] | — |
| **7** | 10 | 15 | 10 | 15 | 9 | — |
| **8** | 10 | 14 | 10 | 15 | 2 | 2 |

*a*) From Table I of ref. 2.
*b*) Not determined.

siderably lower than those observed for non-hydroxylated materials (2.30 ppm for **6**). This indicates that the position of the OH group in these metabolites is at C-14.

The vicinal coupling constants for the olefinic protons are similar among the metabolites (Table II); this suggests that the molecular geometries do not differ significantly among these compounds. A remarkable difference in the splitting patterns, however, was observed between **4** and **5** (and **7** and **8**), for peaks ascribed to H-13. Figures 3 and 4 show the $^1H$ NMR spectra of **4** and **5**. An interesting feature is that H-13 in **4** gives rise to a double doublet with coupling constants of 15 and 9 Hz, whereas H-13 in **5** appears as a doublet ($J$=15 Hz); the top of each signal split into two peaks ($J$ *ca.* 2 Hz). The signals assignable to H-12 appear as double doublets in both compounds but in **5** the peaks are broad, suggesting the presence of a long-range coupling. Irradiation of the signal at 4.42 ppm (assigned as H-14; see Fig. 5) resulted in the sharpening of the peaks at 6.42 (H-12) and 6.11 ppm (H-13), while the signals attributed to H-10 and H-11 remained unchanged.

The above results can be well understood by considering that **4** and **5** are epimers at C-14. Thus, the splitting of the H-12 signal ($J$ *ca.* 2 Hz) is a consequence of the allylic long-range interaction with H-14. The constant for allylic long-range coupling ($^4J_{HH}$) is known to be largest (*ca.* 2 Hz) when the relevant C–H groups are oriented perpendicular to each other and smallest when they lie in the same plane.[7] Therefore it is likely that H-14 in **5** is positioned out of the $\pi$-plane of the conjugated double-bond system, whereas in **4** the C–H (14) group lies approximately in this plane.

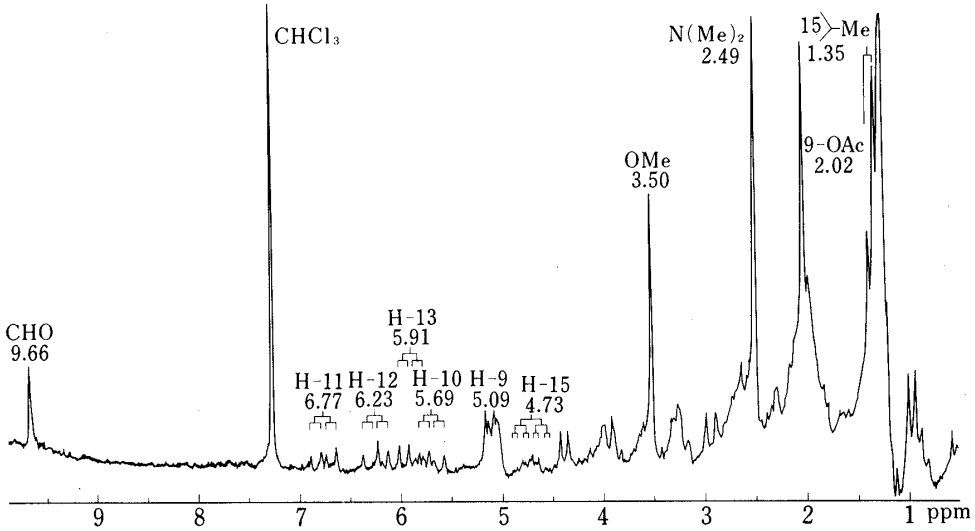


Fig. 3. The Proton NMR Spectrum of **4** determined in $CDCl_3$ Solution

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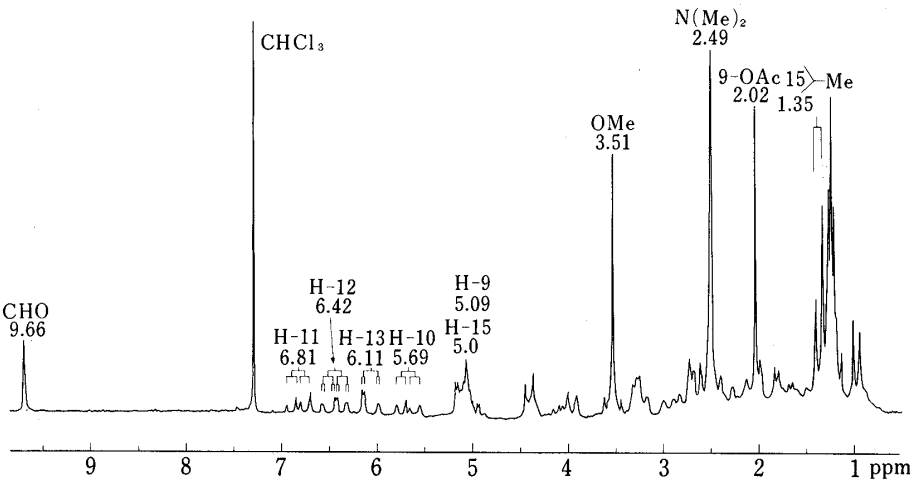


Fig. 4. The Proton NMR Spectrum of **5** determined in $CDCl_3$ Solution

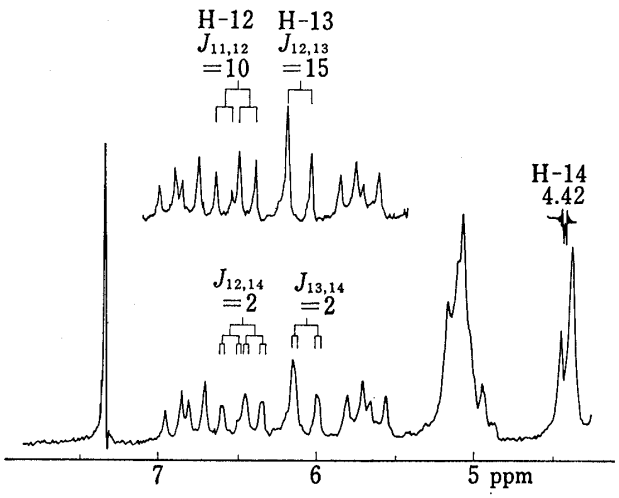


Fig. 5. Parts of the NMR Spectrum of **5** showing the Effect of Irradiation at 4.42 ppm

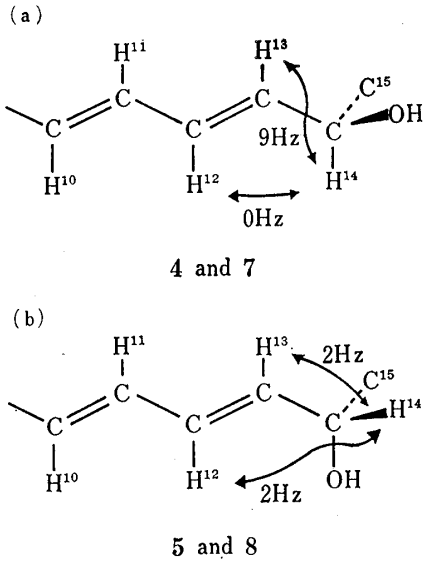


Fig. 6. The Stereochemical Relationships of H-14 with Respect to the Adjacent Double-Bond System

(a) **4** and **7**, (b) **5** and **8**

The difference observed in the splitting patterns of H-13 can also be understood on a similar basis. The coupling constant common to both cases (15 Hz) is attributed to the interaction with an olefinic proton, H-12, which is oriented *trans* to H-13. The other splittings (9 Hz in **4**, 2 Hz in **5**) are therefore ascribed to the interaction with H-14. In view of the Karplus relationship for vicinal coupling constants[8] ($^3J$ is largest when the relevant nuclei are oriented *anti* or eclipsed, and smallest when the torsional angle of these nuclei is *ca.* 90°), it is reasonable to consider that H-14 in **4** lies approximately in the plane of the diene system, whereas H-14 in **5** is about *gauche* with respect to H-13. This is consistent with the foregoing discussion regarding the long-range couplings. A similar relationship also holds in the NMR spectra of **7** and **8**. It follows therefore that **4** and **7** (and **5** and **8**) have the same configuration at carbon 14. The results are illustrated in Fig. 6.

Another interesting feature of the $^1H$ NMR data is the difference of the chemical shifts for H-12, H-13, and H-15 observed between the stereoisomers. For **5** and **8**, these protons appear at lower magnetic fields (*ca.* 0.2—0.3 ppm) relative to those in **4** and **7**. This effect

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Table III. $^{13}C$ Chemical Shifts of the Metabolites 3—8

| Carbon | Compound | | | | | |
|---|---|---|---|---|---|---|
| | 3 | 4 | 5 | 6 | 7 | 8 |
| 1 | 169.5 | 169.1 | 169.6 | 169.6 | 169.1 | 170.2 |
| 2 | 37.3 | 37.3 | 37.2 | 37.0 | 36.6 | 36.9 |
| 3 | 71.6 | 71.6[d] | 71.6[d] | 71.5 | 71.2[d] | 71.7[d] |
| 4 | 77.6 | 77.2 | 77.4 | 77.4 | 76.8 | 77.3 |
| 5 | 84.7 | 84.8 | 84.7 | 84.7 | 84.4 | 84.8 |
| 6 | 28.7[a] | 28.7[a] | 28.6[a] | 28.8[a] | 28.4[a] | 28.6[a] |
| 7 | 30.2[a] | 30.0[a] | 30.0[a] | 30.1[a] | 30.0[a] | 30.3[a] |
| 8 | 31.3 | 31.4 | 31.5 | 33.5 | 33.1 | 33.6 |
| 9 | 76.1 | 75.9 | 75.7 | 72.8 | 72.2 | 73.2 |
| 10 | 122.0 | 125.1 | 123.3 | 127.5 | 129.8 | 128.5 |
| 11 | 137.8 | 136.8 | 136.8 | 135.3 | 134.9 | 135.4 |
| 12 | 133.8 | 132.3 | 126.9 | 132.3 | 132.0 | 127.3 |
| 13 | 131.2 | 135.8 | 136.6 | 131.8 | 134.4 | 134.7 |
| 14 | 40.8 | 72.6[d] | 72.3[d] | 40.8 | 71.5[d] | 72.0[d] |
| 15 | 68.7[b] | 68.2[b] | 69.4[b] | 68.9[b] | 68.3[b] | 69.4[b] |
| 16 | 20.3 | 17.6 | 15.9 | 20.2 | 17.1 | 15.9 |
| 17 | 41.9 | 41.9 | 42.0 | 42.3 | 41.5 | 42.6 |
| 18 | 200.8 | 200.8 | 200.8 | 201.0 | 201.0 | 200.8 |
| 19 | 15.0 | 15.2 | 15.0 | 14.7 | 14.2 | 14.5 |
| 20 | 173.4 | 173.7 | 173.8 | 173.6 | 173.9 | 174.2 |
| 21 | 27.5 | 27.5 | 27.5 | 27.5 | 27.2 | 27.6 |
| 22 | 8.9 | 8.5 | 8.9 | 8.8 | 8.9 | 8.6 |
| 23 | 62.2 | 62.3 | 62.3 | 62.2 | 62.2 | 62.4 |
| 24 | 169.9 | 170.1 | 170.1 | — | — | — |
| 25 | 21.3 | 21.3 | 21.3 | — | — | — |
| 1′ | 103.8 | 103.9 | 103.7 | 103.6 | 103.8 | 103.9 |
| 2′ | 69.1[b] | 69.6[b] | 68.7[b] | 69.4[b] | 69.6[b] | 68.7[b] |
| 3′ | 68.5[b] | 68.9[b] | 68.7[b] | 68.7[b] | 69.6[b] | 68.7[b] |
| 4′ | 74.8 | 75.0 | 74.8 | 74.8 | 75.1 | 74.8 |
| 5′ | 72.9 | 73.2[d] | 72.9[d] | 72.9 | 73.1[d] | 73.0[d] |
| 6′ | 19.0[c] | 19.2[c] | 18.9[c] | 19.0[c] | 19.0[c] | 19.0[c] |
| 7′ | 41.9 | 42.1 | 41.9 | 42.0 | 42.0 | 42.0 |
| 8′ | 41.9 | 42.1 | 41.9 | 42.0 | 42.0 | 42.0 |
| 1″ | 96.3 | 96.5 | 96.3 | 96.3 | 96.5 | 96.3 |
| 2″ | 40.8 | 41.0 | 40.8 | 40.9 | 41.0 | 41.0 |
| 3″ | 69.3[b] | 69.6[b] | 68.7[b] | 69.4[b] | 69.6[b] | 69.4[b] |
| 4″ | 76.2 | 76.5 | 76.3 | 76.3 | 76.5 | 76.3 |
| 5″ | 65.9 | 66.2 | 65.9 | 65.9 | 66.1 | 66.0 |
| 6″ | 18.2[c] | 18.4[c] | 18.1[c] | 18.3[c] | 18.3[c] | 18.3[c] |
| 7″ | 25.4 | 25.6 | 25.3 | 25.4 | 25.4 | 25.4 |

$^{13}C$ chemical shifts: ppm downfield from internal TMS in $CDCl_3$.
*a*), *b*), *c*), *d*), Assignments within any vertical column may be reversed. The prime symbols refer to the mycaminose and double prime symbols to the mycarose carbons.

can be regarded as a result of the anisotropic effects of the hydroxyl group at C-14 on the neighboring protons.

Table III lists the $^{13}C$ NMR chemical shifts of **3**—**8**. The assignments of the signals were made by comparisons with those reported for related macrolide antibiotics[9] and also by means of mutual comparisons of the spectra of these metabolites. It is clear that the peaks ascribed to C-14 are remarkably shifted (by *ca.* 32 ppm) in **4**, **5**, **7**, and **8**, relative to those in **3** and **6**. Further, the peaks attributed to C-12 and C-16 are at considerably higher magnetic fields for the hydroxylated metabolites as compared to those in **3** and **6**. This can be understood in terms of the $\gamma$-effect; carbons 12 and 16 are situated $\gamma$ to the hydroxylated

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position. It is well known that the carbon chemical shift moves upfield, by several ppm, on substitution of a hydrogen at the $\gamma$-position with a functional group, X (X=$CH_3$, OH, $NH_2$, *etc.*).[10]

The most interesting feature is the differences in the $\gamma$-effect observed between the stereoisomers. Thus, the high-field shifts of carbons 12 and 16 are greater in **5** and **8** than in **4** and **7**. The magnitude of the $\gamma$-*gauche* effect is known to be larger when the relevant atoms or groups (C *vs.* X) come close to each other, and smaller when they are oriented apart.[11] It is therefore very likely that the difference in the $\gamma$-effect between the stereoisomers reflects a different geometrical disposition of the hydroxyl group with regard to carbons 12 and 16. It follows that the 14-hydroxyl group is positioned closer to these carbons in **5** and **8** than in **4** and **7**. This is illustrated in Fig. 7.

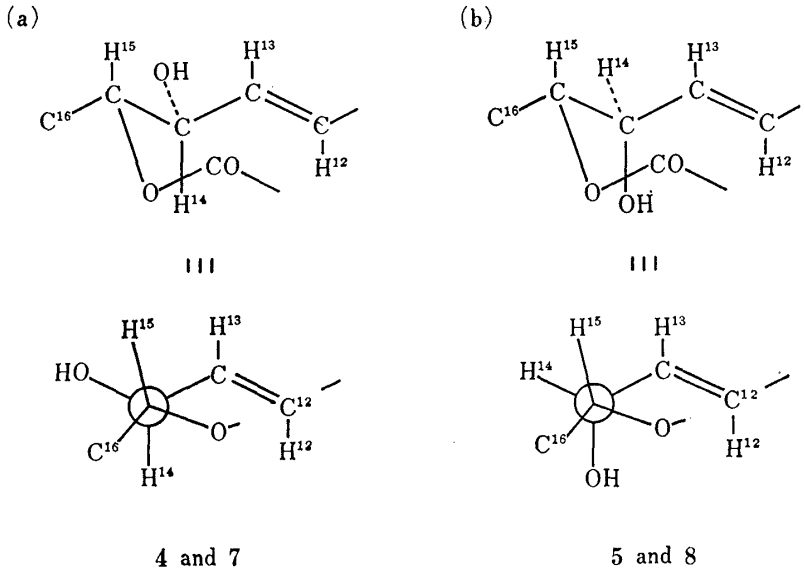


Fig. 7. Plausible Structures (Configuration and Conformation) of the Hydroxylated Metabolites

(a) **4** and **7**, (b) **5** and **8**.

In view of the above considerations, and on the basis of the absolute configuration and the conformation of leucomycin $A_3$[12] (midecamycin is known to have the same stereochemistry as leucomycins), we suggest that the configuration at C-14 of **4** and **7** is *S*. The compounds **5** and **8** are therefore suggested to have the *R*-configuration (Fig. 7). All of the data presented in this paper are consistent with these structures (the conformation is assumed to remain the same for these isomers). This conclusion, however, should be regarded as tentative and requires confirmation, *e.g.*, by crystallographic studies. Apart from the clarification of this point, it is interesting that hydroxylated metabolites with differing stereochemistries at the same carbon are produced simultaneously in the drug metabolism. This problem will be discussed in a forthcoming paper.

## Experimental

Compounds **1** and **2** were obtained as reported elsewhere.[2,3] The metabolites **3**—**8** were obtained from urine and/or bile of doped animals; procedures for the biological treatments and purifications of the metabolites are reported in separate papers.[1,5] The purities of the compounds studied in the present work were checked by thin-layer chromatography (TLC) and various spectral analyses.

**Urinary Metabolites of Man**——Tablets of **1** (MOM) were administered orally at 600 mg/man. Twenty-four hour urine was extracted twice with 2 volumes of EtOAc under alkaline conditions (pH=8—9 with $NaHCO_3$). TLC was carried out on 0.25 mm silica gel F-254 plates. $CHCl_3$: MeOH=10: 1 was used for

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development. TLC spots were visualized with 10% $H_2SO_4$ and densitograms were obtained by the use of a TLC scanner (Shimadzu CS-910).[5]

**MS Measurements**——Mass spectra were obtained with a JEOL 01SG high-resolution spectrometer at an ionizing potential of 75 eV.

**NMR Measurements**——NMR spectra were determined on a Varian XL-100 instrument for $CDCl_3$ solutions. The chemical shifts are given in ppm from internal tetramethylsilane ($\delta=0$) and are accurate to $\pm 0.01$ ppm for $^1H$ and $\pm 0.04$ ppm for $^{13}C$ resonances. The coupling constants are reported in Hz and are accurate to $\pm 0.5$ Hz.

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