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# STEREO-CHEMICAL STUDIES ON THE SULFOXIDE OF 5,6-*cis*-CARBAPENEM ANTIBIOTICS, C-19393 COMPONENTS

SETSUO HARADA*, SHIGETOSHI TSUBOTANI, SUSUMU SHINAGAWA and MITSUKO ASAI
Applied Microbiology Laboratories, Central Research Division, Takeda Chemical Industries, Ltd., Yodogawa-ku, Osaka 532, Japan

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**Abstract**—The absolute configuration at the sulfoxide of 5,6-*cis*-carbapenem antibiotics is discussed on the basis of chemical reactions and the CD spectral data. Some stereoisomers at the side chain were synthesized from C-19393 $H_2$ by the combination of hydrogenation, oxidation and *Z,E*-isomerization. The CD spectral studies revealed that the Cotton effects of stereoisomers at the sulfoxide indicated clear opposite signs at both 260–275 and 280–300 nm regions. Further CD spectral studies on the derivatives elucidated that these Cotton effects may reflect two chromophores; –CH–S(O)–CH=CH–NH– for the former region and $-CH_2-S(O)-\underset{|}{C}=\underset{|}{C}-COO^-$ for the latter. In conclusion, these naturally occurring 5,6-*cis*-carbapenem antibiotics have been shown to possess the *R*-configuration at the sulfoxide.

The similar structures excluding the absolute configuration at the sulfoxides have recently been reported for C-19393 $H_2$ (**1**)[1a] and carpetimycin A.[2] However, the specific rotations of **1**, $[\alpha]_D^{24}$ – 141° ($c$ = 0.5, $H_2O$),[1a] and carpetimycin A, $[\alpha]_D^{24}$ – 27° ($c$ = 1.7, $H_2O$),[2a,c,d] are quite different, although the other physico-chemical properties including the CD spectra[1a,2b] are almost the same. Furthermore, the specific rotations and CD spectra in C-19393 $S_2$ (**2**)[1a] and carpetimycin B[2] indicate similar data. This report deals with the chemical reactions of C-19393 derivatives and CD spectral studies on the sulfoxide at the side chain of 5,6-*cis*-carbapenem antibiotics as shown in Fig. 1.

At first, the antibiotics, **1** and **2**, were re-purified in order to confirm the physico-chemical data. The specific rotations in the purest samples of **1** and **2** were measured to be $[\alpha]_D^{24}$ – 200° ($c$ = 0.515, $H_2O$) and $[\alpha]_D^{24}$ – 175° ($c$ = 0.5, $H_2O$), respectively. The specific rotations of the hydrolysate of **2** (**1**) was also measured to be $[\alpha]_D^{24}$ – 199° ($c$ = 0.522, $H_2O$).

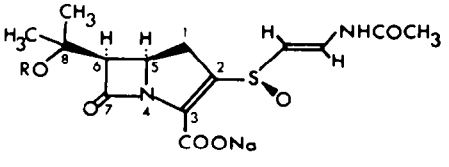
Whether **1** and carpetimycin A are the same or different compounds should be based on the absolute configuration at the sulfoxide because the structure of **1** has already been confirmed by the total synthesis of (±) C-19393 $H_2$.[3]

†A part of this report has been presented at the 222nd Meeting of Japan Antibiotics Research Association, Tokyo, 23 Jan. 1981.

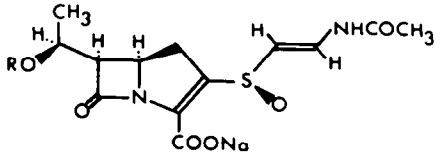
The antibiotic, **1**, was hydrogenated over 10% Pd-C to give a deoxy compound, **3**†, which was oxidized by *m*-chloroperbenzoic acid or hydrogen peroxide to afford **1** and its stereoisomer at the sulfoxide (C-19393 iso-$H_2$, **4**) in the ratio of 1:1.4 as shown in Fig. 2. The specific rotations of these compounds were measured to be $[\alpha]_D^{23}$ – 195° ($c$ = 0.527, $H_2O$) for the synthesized **1** and $[\alpha]_D^{23}$ – 125° ($c$ = 0.515, $H_2O$) for **4**, which demonstrated a distinct difference from that of carpetimycin A. These stereoisomers, **1** and **4**, showed clearly different retention times on HPLC (Fig. 3) and the CD spectral patterns.

However, from the X-ray analysis of C-19393 $H_2$ *p*-bromo-benzyl ester, the absolute configuration was unambiguously determined to be the *R*-configuration as shown in Fig. 1.

Interesting relationships between the configurations at the sulfoxide and the CD spectral patterns were found from the following studies. When **3** was kept in a solution saturated with hydrogen in the presence of 10% Pd-C (or by mercuric chloride), a *Z*-isomer of the side chain, **5**,[4] PMR: 5.72 (d, J = 8, *cis* coupling, S–CH=) and 7.17 (d, J = 8, N–CH=), was obtained besides **3** in the ratio of 1:1 (Fig. 2). The compound, **5**, was oxidized by *m*-chloroperbenzoic acid to give a *Z*-isomer of **1** (C-19393 *Z*-$H_2$, **6**)[4] and its stereoisomer at the sulfoxide (C-19393 *Z*-iso-$H_2$, **7**). The compounds, **5** and **6**, were also synthesized by the new *Z,E*-isomerization method[4] consistent with the configuration of the sulfoxide directly from the compounds, **3** and **1**, respectively. These reaction pathways from **1** to **7** were also applied to the




C-19393 $H_2$ (1): R=H
C-19393 $S_2$ (2): R=$SO_3Na$




C-19393 $E_5$ (23): R=H
MM 4550: R=$SO_3Na$

Fig. 1.

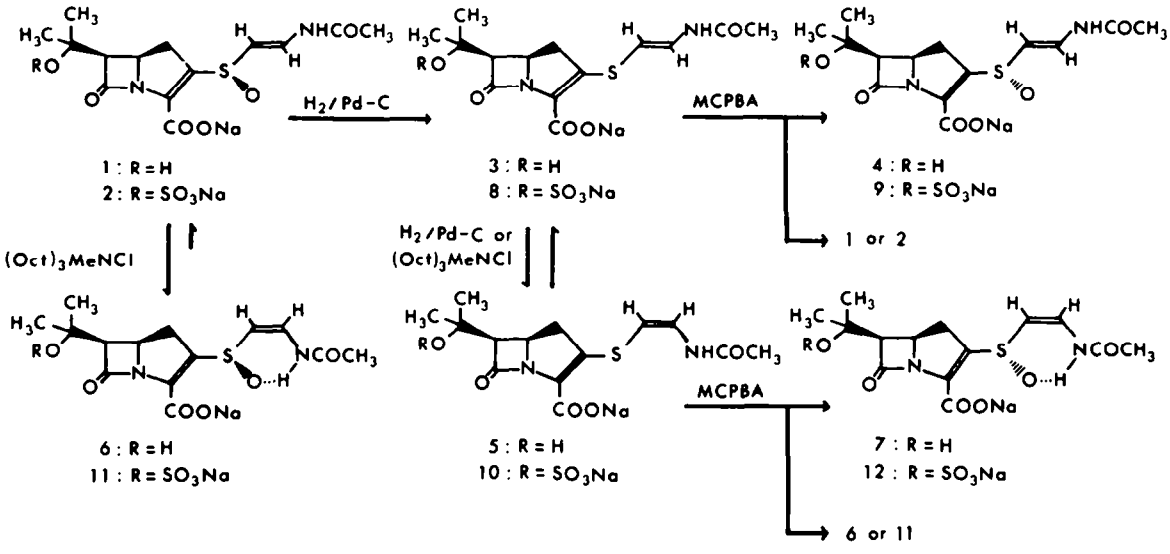



Fig. 2.

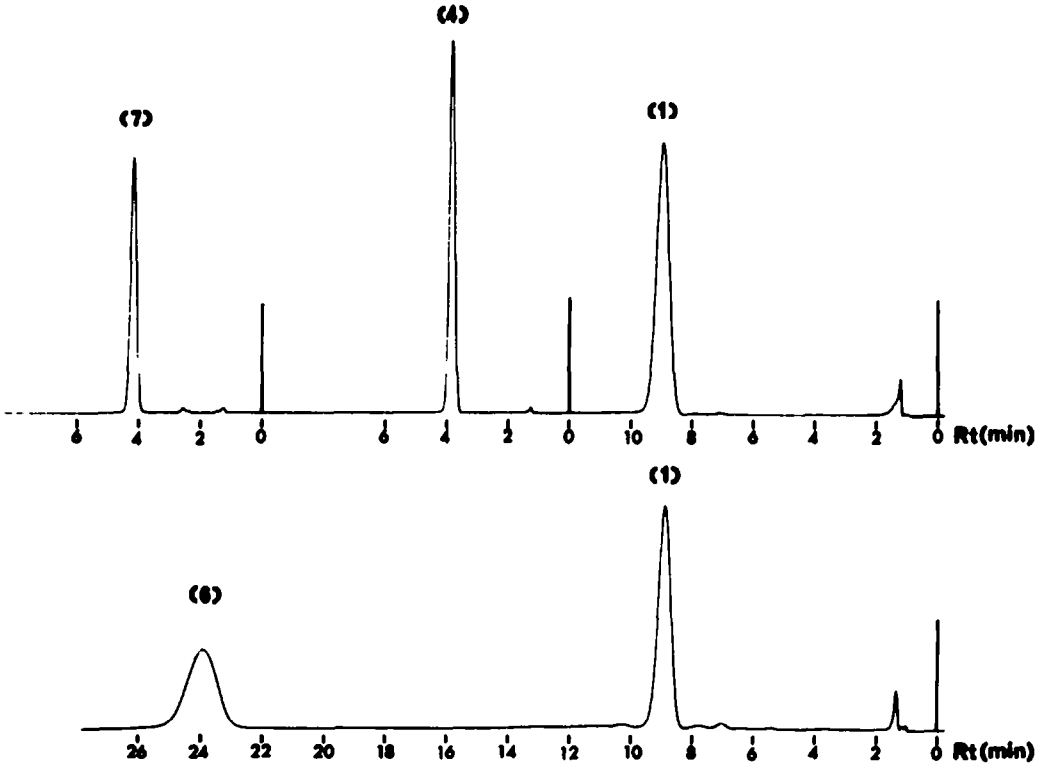



Fig. 3.

sulfonyloxy compounds in order to confirm the spectral data described below (Fig. 2).

Table 1 and Fig. 4 show the specific rotations and the UV and CD spectra of these compounds. The CD spectral data give important information: (1) the primary Cotton effects at 220–240 nm (Column I) show uncertain patterns by overlapping with the Cotton effect of $\beta$-lactam carbonyl according to variation of stereo-structures at the side chain and (2) the secondary and tertiary Cotton effects at 260–275 (Column II) and at 280–300 nm (Column III) indicate definite opposite signs between **1**, **6** and their stereoisomers, **4**, **7**. Specific rotations also show apparent contrast especially between **6** and **7**. Obvious differences in the CD spectra and specific rotations may be ascribable to enhancement of a symmetry at the chiral center which is derived from rigidity of the side chain by the presence of an H-bond between sulfoxide and acid amide groups.[4]

Two other results contributed to a more detailed analysis of the CD spectra. The first data obtained from

Table 1. Physico-chemical properties of the compounds, 1–12

| Compound | | 8-Hydroxy group. (3) | (1) | (4) | (5) | (6) | (7) | 8-Sulfonyloxy group. (8) | (2)[1a] | (9) | (10) | (11) | (12) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $[\alpha]_D$ (C) | | -38° (0.46) | -200° (0.515) | -125° (0.515) | -17.1° (0.51) | -357° (0.5) | +316° (0.493) | -179° (0.525) | -175° (0.5) | -12.8° (0.515) | +7.1° (0.495) | -281° (0.5) | +258° (0.415) |
| UV: $\lambda_{max}^{nm}$ | | 230 (15500) | 243 (16300) | 250 (18400) | 232 (15700) | 241 (14300) | 237 (15600) | 229 (16300) | 244 (16900) | 250 (14400) | 233 (13100) | 241 (16100) | 237 (13300) |
| ($\varepsilon$) | | 310 (14900) | 290 (13900) | 287 (14600) | 308 (14500) | 293 (9970) | 290 (10000) | 309 (16800) | 288 (13600) | 288 (13800) | 308 (12800) | 291 (11200) | 292 (9050) |
| CD: $[\theta]_{nm}$ | I | 224 (-32200) | 236 (+39000) | 240 (-63000) | 220 (-35600) | | 234 (-20200) | 230 (-40200) | 233 (+27700) | 240 (-56900) | 220 (-32100) | 220 (-21500) | 232 (-12900) |
| | II | | 265 (-37500) | 273 (+15100) | | 260 (-24500) | 260 (+28500) | | 263 (-46200) | 270 (+18500) | | 273 (-38600) | 272 (+25100) |
| | III | | 295 (-33000) | 285 (+10800) | 295 (+11100) | 300 (-28900) | 286 (+35800) | | 300 (-43100) | 290 (+32900) | 292 (+11900) | 292 (-32600) | 282 (+31400) |

The data were measured at 23 - 26° in $H_2O$.

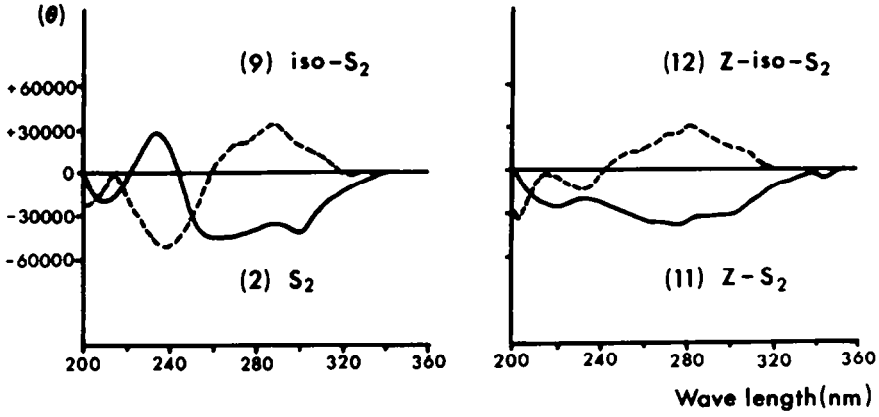



Fig. 4. CD Spectra of four stereoisomers at the side chain.

the reaction pathways are shown in Fig. 5. The compound, **2**, was hydrolyzed by refluxing in the tri-octyl methyl ammonium chloride/toluene solution saturated with sodium bicarbonate and phosphate buffer[4] to give desulfated compound, **13**, CD $[\theta]_{nm}$236 (+53100), 280 (−111000) and 325 (−34300). When **8** was hydrolyzed in sodium bicarbonate and phosphate buffer (pH 7.0) at 60°, a similar desulfation occurred to give **3** and its 6-dehydro-8-dehydroxy compound, **14**. On refluxing **13**† in methanol, methyl esters (**15** and **16**) were obtained as diastereomers at the position 2. These esters in water reached equilibrium in a ratio of about 1:1 after standing on at room temperature for one week. The separated diasteromers showed strong negative Cotton effects at about 260 nm such as $[\theta]_{nm}$230 (+18900) and 258 (−67000) in **15** and $[\theta]_{nm}$222 (+22800) and 262 (−79440) in **16** as shown in Fig. 6. The data revealed that the secondary Cotton effect at 260–275 nm could originate from the chromophore; –CH–S(O)–CH=CH–NH–.

†The compound is extremely unstable in cold water.

The second result was obtained when the side chain comprised an alkyl group as shown in Fig. 7. The deoxy compound, **8**, was further hydrogenated by 10% Pd-C to give a saturated compound at the side chain, **17**, CD: $[\theta]_{nm}$210(−15500) and 288 (+6200), as a minor product and a hydrogenolysis compound, **18**, CD $[\theta]_{nm}$288 (−17500) and 254 (+9300) as a main product. The compound, **17**, was oxidized by *m*-chloroperbenzoic acid to afford two enantiomers at the sulfoxide, **19** and **20**. Their CD spectra indicate the simple opposite signs of Cotton effect at 280–290 nm as $[\theta]_{nm}$230 (−15200) and 290 (−28100) in **19** and $[\theta]_{nm}$218 (−62500) and 280 (+24100) in **20** (Fig. 6). The data revealed that the tertiary Cotton effect at 280–300 nm may reflect the chromophore; $-CH_2-S(O)-C=C-COO^-$.

It was concluded from these findings that naturally occurring compounds (*R*-configuration) have negative Cotton effects at two regions of 260–275 nm and 280–300 nm, and their stereoisomers at the sulfoxide (*S*-configuration) have positive Cotton effects at the same regions.

Mislow *et al.* have reported on the correlation between

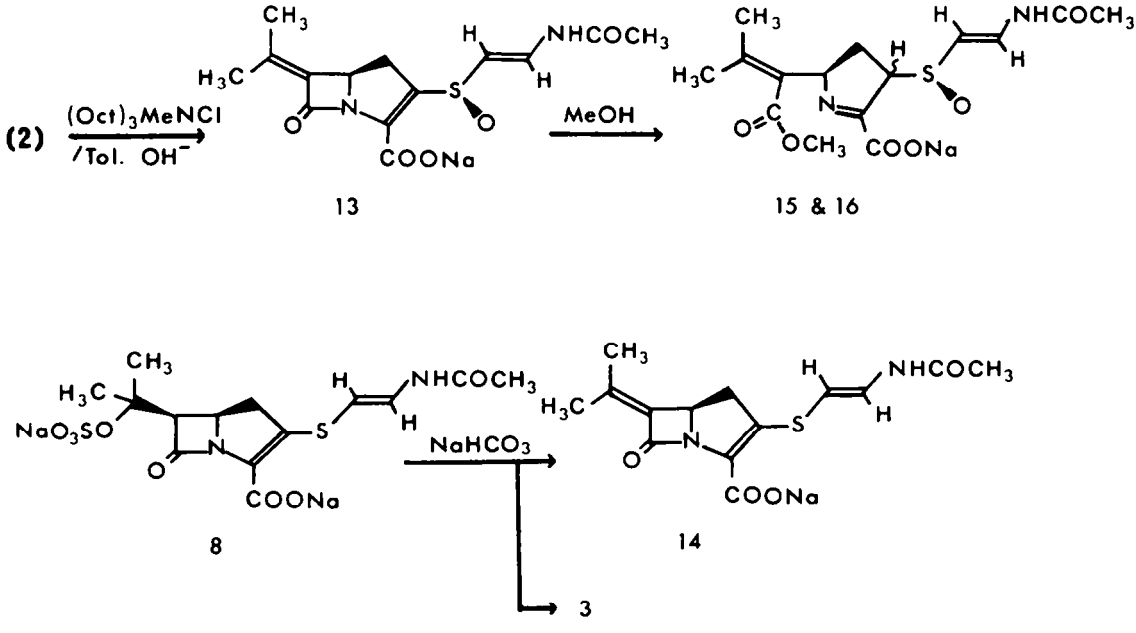



Fig. 5.

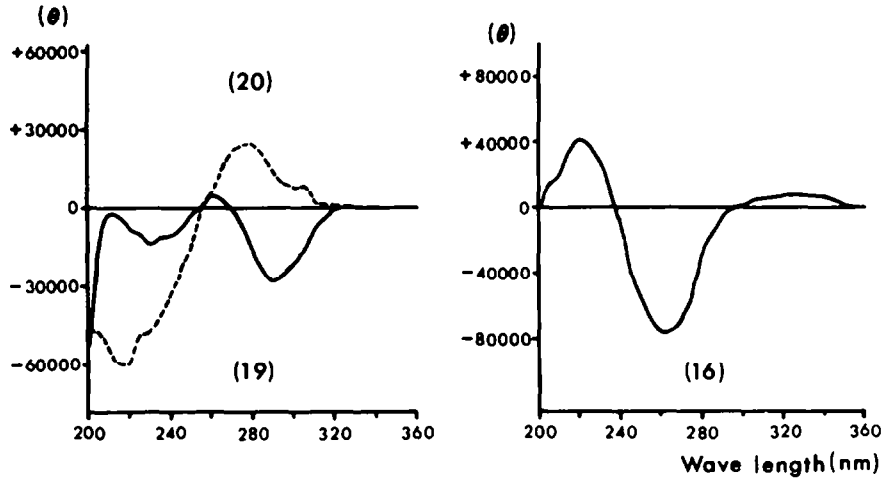



Fig. 6. CD Spectra of the compounds, **16**, **19** and **20**.

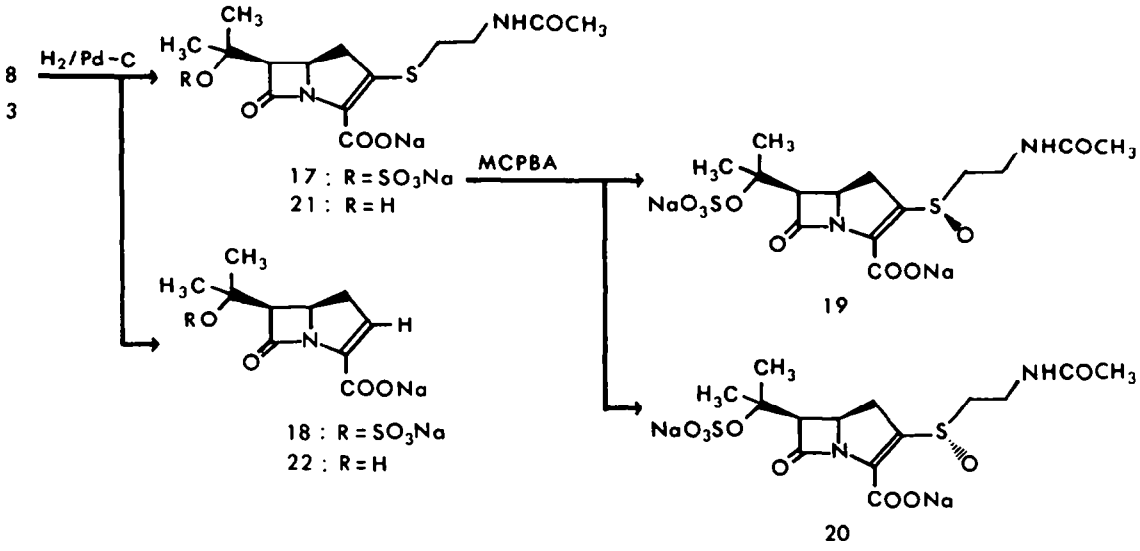



Fig. 7.

the absolute configuration of the dialkyl, alkyl aryl or diaryl sulfoxides and their CD spectra.[5a] However, the report has indicated that application of this empirical rule to alkyl allyl or diallyl sulfoxides is difficult.[5b] The results we obtained may be applicable in the case of diallyl or alkyl allyl sulfoxides.

These CD spectral studies are useful for elucidation of the absolute configuration in other related antibiotics. The CD spectrum of C-19393 $E_5$, **23**, and its stereoisomer at the sulfoxide **24**,[6] revealed that **23** has the *R*-configuration at the sulfoxide. Oxidation of MM13902 by *m*-chloroperbenzoic acid gave MM4550[7] and its stereoisomer, **25**, CD:$[\theta]_{nm}$233 (−62000) and 278 (+19400). MM 4550 also has the *R*-configuration at the sulfoxide (Fig. 1), Recently isolated asparenomycins A and C[8] could be shown to have the *R*-configuration by comparing the CD spectra with those of **13** and **14**, respectively.

It was observed that regularities exist in the sequence of retention time on HPLC and in strength of antimicrobial activity upon the *R*-isomers and the corresponding S-isomers as shown in Table 2.

The biological activities of these derivatives described above will be reported in the near future.[4,9]

## EXPERIMENTAL

The specific rotations, UV and CD spectra (220–340 nm) were measured at 23–26° in water. The IR spectra were measured in KBr pellet. The δ value in the PMR (100 MHz) spectra using XL-100 (Varian) were recorded in ppm down-field from TMS. All spectra were measured in $D_2O$ unless otherwise stated. The HPLC data were obtained by using Waters equipment, Model ALC/GPC 202/660. The analytical conditions were as follows: Radial Pak A/MeOH-0.02 M phosphate buffer (pH 6.3) (p.b.), flow rate, 2 ml/min, UV absorbance, 254 nm. Retention times (Rt) are expressed by minutes.

*Purification of* **1** *and* **2**

(a) A soln of the powdered **1** (150 mg) in 0.02M phosphate buffer (pH 6.3) (75 ml) was loaded on QAE-Sephadex A-25 ($Cl^-$ type, 30 ml) and the column was washed with the same buffer (100 ml). The antibiotic was eluted with 0.02M NaCl-$H_2O$ (150 ml). The active fractions after addition of NaCl (2.5 g) were applied to Diaion HP-20 (100–200 mesh, 30 ml) pretreated with 5% NaCl-$H_2O$ (60 ml) and the column was washed with 5%

Table 2. Relationships among the absolute configurations at the sulfoxide, retention times on HPLC and antimicrobial activities

| compound | 2 | 9 | 11 | 12 | 23 | 24 | MM4550 | 25 |
|---|---|---|---|---|---|---|---|---|
| Configuration*1 | R | S | R | S | R | S | R | S |
| Rt(min) on HPLC*2 | 3.3 | 1.6 | 5.9 | 1.8 | 5.3*3 | 3.7*3 | 4.7*4 | 2.7*4 |
| Activity*5 | 19.5 | 0 | 18.0 | 0 | 27 | 21 | 25*6 | 17*6 |

*1 Absolute configuration at the sulfoxide, *2 8% MeOH -phosphate buffer (pH 6.3, 2 ml/min), *3 4% MeOH, *4 0% MeOH, *5 Diameter (mm) on the diffusion assay using E. coli LD-2 (100 μg/ml), *6 1000 μg ml.

NaCl-$H_2O$ (90 ml). The antibiotic was eluted and fractionated with $H_2O$ (120 ml). The active fractions were freeze-dried to give 1 (68 mg) as a pure white powder.

(b) A soln of the powdered 2 (100 mg) in $H_2O$ (100 ml) was extracted with 1% tri-n-octyl methyl ammonium chloride in $CH_2Cl_2$ (100 ml). The extract was re-extracted with 0.75% NaI-$H_2O$ (25 ml). The concentrated aqueous layer was chromatographed on HP-20 (100 ml) by elution with $H_2O$ (200 ml). The active fractions were freeze-dried to afford 2 (82 mg) as a pure white powder: (1), HPLC; Rt, 8.9 (8% MeOH-p.b.). (Found, C, 44.34; H, 5.20; N, 7.02; S, 7.97. Calc for $C_{14}H_{17}N_2O_6SNa \cdot H_2O$ (382.38): C, 43.97; H, 5.01; N, 7.33; O, 29.29; S, 8.39; Na, 6.01%). CMR; δ 23.2 (q. $COCH_3$), 27.6, 29.3 (q. each, 8-$(CH_3)_2$), 29.4 (t., $C_1$), 54.7 (d., $C_6$), 63.3 (d., $C_5$), 71.4 (S., $C_8$), 112.1 (d., S-ÇH=), 134.4 (d., N-ÇH), 138.1, 140.9 (s. each, $C_2$ or $C_3$), 165.8 (S., $C_7$), 172.9 (S., NHÇO), 178.8 (S., ÇOONa).

Compound 2, HPLC; Rt. 6.7 (4% MeOH-p.b.). (Found, C, 33.65; H, 3.91; N, 5.27; S, 13.31. Calc for $C_{14}H_{16}N_2O_9S_2Na_2 \cdot H_2O$ (484.43), C, 34.71; H, 3.75; N, 5.78; O, 33.03; S, 13.24; Na, 9.49%) CMR; δ 23.2 (q., $COCH_3$), 24.7, 25.9 (q. each, 8-$(CH_3)_2$), 29.4 (t., $C_1$), 54.6 (d., $C_6$), 63.6 (d., $C_5$), 84.5 (S., $C_8$), 112.4 (d., S-ÇH=), 134.5 (d, N-ÇH=), 139.0, 140.3 (s. each, ($C_2$ or $C_3$), 165.9 (S., $C_7$), 173.1 (S., NHÇO), 177.4 (S., ÇOONa).

*Deoxygenation of* 1 *or* 2

A mixture of 2 (1.98 g) and 10% Pd-C (2.3 g) in 10% MeOH-$H_2O$ (230 ml) was hydrogenated at room temp for 1 h. After filtration the concentrated aqueous soln was chromatographed on HP-20 (1 l) with $H_2O$. The active fractions detected by HPLC were freeze-dried to give 8 (1.18 g) as a white powder: HPLC; Rt, 8.3 (8% MeOH-p.b.), IR; 1755 (CO), 1690 (NHAc), 1620, 1250 (br), 1050 $cm^{-1}$, PMR; δ 1.62, 1.69 (8-$CH_3$), 2.08 ($NHCOCH_3$), 3.04, 3.82 (1-$CH_2$), 6.11, 7.20 (CH=). By the similar method, 1 (480 mg) gave 3 (171 mg): (3) HPLC, Rt, 12.4 (10% MeOH-p.b.) IR; 1755 (CO), 1680 (NHAc), 1620, 1290 $cm^{-1}$, PMR; δ 1.30, 1.44 (8-$CH_3$), 2.09 (NHAc), 3.02, 3.74 (1-$CH_2$), 3.72 ($H_6$), 6.08, 7.18 (CH=).

*Isomerization of* 8 *or* 3

(a) A mixture of 8 (4 mg) and 10% Pd-C (15 mg) in 20% MeOH-$H_2O$ (15 ml) was saturated with $H_2$ and allowed to stand at 4° for 12 h. After filtration of the mixture, the filtrate was concentrated. The concentrate was loaded on prep. HPLC using Lichrosorb RP-18 (Merck AG.) and eluted with 8% MeOH/0.02M phosphate buffer (pH 6.3). The fractions giving the single peak by HPLC were collected and desalted with HP-20 (20 ml). Pure

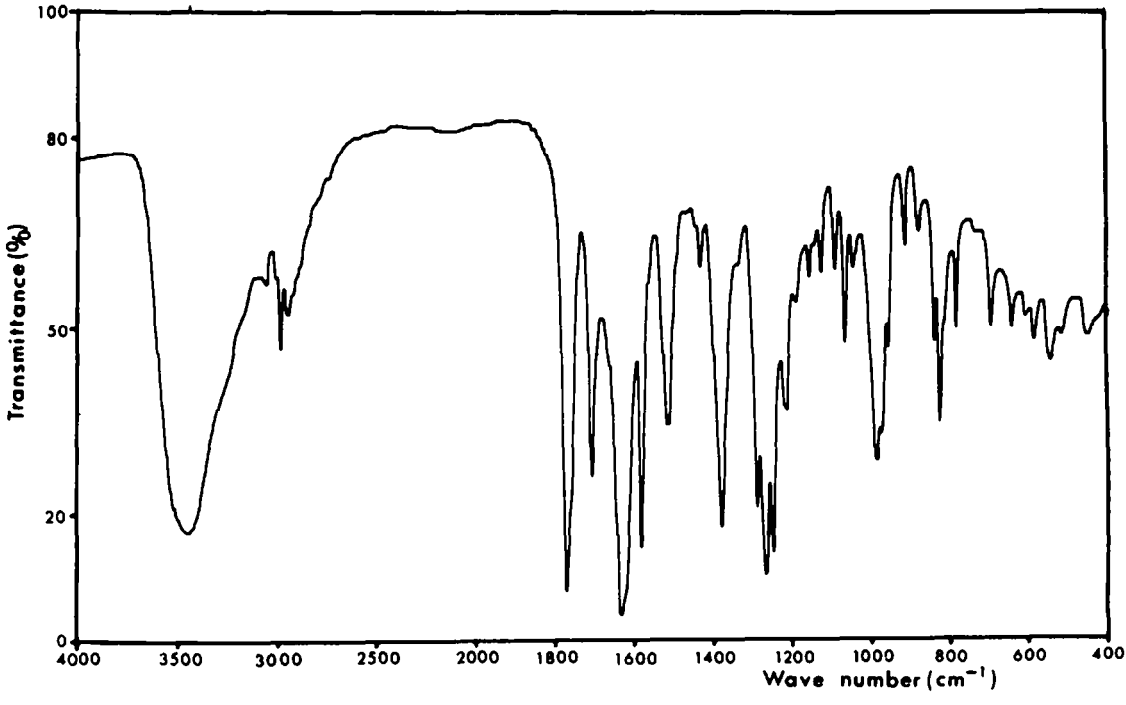



Fig. 8. IR Spectrum of C-19393 $H_2$ (KBr).

fractions were concentrated and freeze-dried to give white powder of **10**[4] (1 mg).

(b) To a soln of **3** (4 mg) in 50% MeCN-$H_2O$ (4 ml) was added $HgCl_2$ (1 mg) and the mixture was stirred at room temp for 30 min. By treatment similar to (a), the compound, **5**,[4] was obtained as a white powder (1 mg).

*Oxidation of* **8** *or* **3**

(a) To a soln of **8** (100 mg) in MeOH (50 ml) was added *m*-chloroperbenzoic acid (78 mg) and the mixture was stirred at 0–5° for 30 min. After addition of 0.02M phosphate buffer (pH 6.3, 100 ml), the mixture was concentrated. The concentrate (50 ml) was washed with AcOEt (50 ml). The aqueous soln was chromatographed on HP-20 (100 ml) pretreated with 5% NaCl-$H_2O$ by the solvent system of MeOH: 5% NaCl-$H_2O$ (5:95). Two fractions detected by HPLC were individually desalinated by activated carbon chromatography to give **2** (26 mg) and **9** (37 mg) freeze-dried as a white powder: (**9**), HPLC: Rt, 2.4 (4% MeOH-p.b.), IR; 1760 (CO), 1700 (NHAc), 1255, 1050 $cm^{-1}$, PMR; $\delta$ 1.66, 1.73 (8-$CH_3$), 2.15 (NHCOC$H_3$), 3.19, 3.91 (1-$CH_2$), 6.44, 7.65 (CH=).

(b) To a soln of **8** (2 mg) in MeCN (6 ml) was added 12% $H_2O_2$ (4 ml) and the mixture was stirred at room temp for 2 h. The mixture contained 48% of **2**, 22% of **9** and 28% of **8** by HPLC detection.

(c) To a soln of **3** (76 mg) in MeOH (38 ml) was added m-chloroperbenzoic acid (70 mg) and the mixture was stirred at 0–5° for 30 min. After treatment similar to (a) the aqueous soln was chromatographed on HP-20 (100 ml) and elution with $H_2O$. Two fractions detected by HPLC were individually concentrated to give **4** (25 mg) and crude **1** (39 mg). The crude compound of **1** was purified with QAE-Sephadex and HP-20 columns to afford pure **1** (12.5 mg): (**4**), HPLC, Rt, 3.8 (8% MeOH-p.b.), IR; 1770 (CO), 1710 (NHAc), 1270, 1010 $cm^{-1}$, PMR; $\delta$ 1.34, 1.45 (8-$CH_3$), 2.15 (NHCOC$H_3$), 3.14, 3.83 (1-$CH_2$), 6.31, 7.56 (CH=).

(d) By treatment similar to (a) or (c) the sulfoxide derivatives were obtained as follows.

| Starting material | (mg) | Chromatography | Product | (mg) |
|---|---|---|---|---|
| **10** | (33) | HP-20 (30 ml) | **11**[4], | (9.2) |
| | | | **12**, | (19.9) |
| **5** | (52) | HP-20 (50 ml) | **6**[4], | (21.0) |
| | | | **7**, | (18.0) |
| **17** | (36.5) | HP-20 (40 ml)/ Carbon (5 ml | **19**, | (8.1) |
| | | | **20**, | (15.4) |
| MM13902 | (70) | QAE-Sephadex A-25 (20 ml)/HP-20 (50 ml)/Carbon (10 ml) | MM4550, | (20.3) |
| | | | **25**, | (13.2) |

(**7**), HPLC; Rt, 4.2 (8% MeOH-p.b.), IR; 1765 (CO), 1705 (NHAc), 1270, 1020 $cm^{-1}$, PMR; $\delta$ 1.32, 1.41 (8-$CH_3$), 2.15 (NHCOC$H_3$), 5.71, 7.27 (CH=).

(**12**), HPLC; RT, 1.8 (8% MeOH-p.b.), IR; 1765 (CO), 1700 (NHAc), 1260, 1050 $cm^{-1}$, PMR; $\delta$ 1.65, 1.68 (8-$CH_3$), 3.26, 3.92 (1-$CH_2$), 5.85, 7.30 (CH=).

(**19**), HPLC; Rt, 2.3 (2% MeOH-p.b.), UV; $\lambda_{max}$ 287 nm ($\epsilon$9090), IR; 1760 (CO), 1620 (br), 1230 (br), 1050 $cm^{-1}$, PMR; $\delta$ 1.65, 1.74 (8-$CH_3$), 2.03 (NHCOC$H_3$).

(**20**), HPLC; Rt, 5.6 (2% MeOH-p.b.), UV; $\lambda_{max}$ 289 nm ($\epsilon$9190), IR; 1760 (CO), 1620 (br), 1230 (br), 1050 $cm^{-1}$ PMR; $\delta$ 1.64, 1.72 (8-$CH_3$), 2.02 (NHCOC$H_3$), (25), HPLC; Rt, 2.0 (2% MeOH-p.b.), UV; $\lambda_{max}$ 251 nm ($\epsilon$20000) and 285 (15700), IR; 1775 (CO), 1630, 1400, 1230–60 (br), 1020 $cm^{-1}$.

(**25**), HPLC; Rt, 2.0 (2% MeOH-p.b.), UV; $\lambda_{max}$ 251 nm ($\epsilon$20000) and 285 (15700), IR; 1775 (CO), 1630, 1400, 1230- 60 (br), 1020 $cm^{-1}$.

*Desulfonation of* **2** *or* **8**

(a) A soln of **2** (861 mg) in 0.02M phosphate buffer (pH 7.0) and 0.6% $NaHCO_3$ (1:1, 1liter) was extracted with 1% tri-n-octyl methyl ammonium chloride/toluene (1 l.) and the extract was warmed at 60° for 30 min. The mixture was re-extracted with 1.5% NaI (400 ml) and the aqueous layer was adjusted at pH 8 by dil $NH_4OH$. The concentration aqueous layer was loaded on HP-20 (500 ml) and eluted with dil $NH_4OH$ (pH 8.5). Active fractions were concentrated and freeze-dried to give **13** (297 mg) as a white powder: HPLC; Rt, 7.8 (15% MeOH-p.b.), UV; $\lambda_{max}$ 242 nm ($\epsilon$28100), 280 (sh) and 315 (sh), IR; 1760 (CO), 1710 (NHAc), 1635, 1260 $cm^{-1}$, PMR; $\delta$ (DMSO-$d_6$) 1.75, 1.98 (8-$CH_3$), 1.95 (NHCOC$H_3$), 2.74 (d like, J = 9, 1-$CH_2$), 4.6 (m, $H_5$), 6.22 (d, J = 14, S-CH=), 7.27 (q, J = 14, 11, N-CH=), 9.45 (d, J = 11, NHCO).

(b) A soln of **8** (1.05 g) in 0.1M phosphate buffer (pH 7.0) and 0.5% $NaHCO_3$ (1:1, 1 l.) was warmed at 65° for 3 h. The mixture was applied to HP-20 (0.5 l.) and eluted with $H_2O$ and followed by 5% MeOH-$H_2O$. The active fractions detected by HPLC were separately concentrated and freeze-dried to give **3** (129 mg) and **14** (181 mg) as white powders: (**14**), HPLC; Rt, 5.6 (25% MeOH-p.b.), UV; $\lambda_{max}$ 236 nm ($\epsilon$27100) and 297 (13900), CD; $[\theta]_{nm}$ 243 (−24400) and 272 (−20100), IR; 1755 (CO), 1710 (NHAc), 1630, 1265, 1220, 1055 $cm^{-1}$, PMR; $\delta$ 1.84, 2.06 (8-$CH_3$), 2.09 (NHCOC$H_3$), 3.08 (q like, 1-$CH_2$), 6.04, 7.15 (CH=).

*Methanolysis of* **13**

A soln of **13** (19.8 mg) in MeOH (10 ml) was refluxed for 1 h. The mixture was added into 0.02M phosphate buffer (pH 7, 10 ml) and the organic solvent was removed. The aqueous soln was chromatographed on HP-20 (10 ml) and elution with 5% MeOH-$H_2O$. The fractions detected by HPLC were divided to two groups. They were individually concentrated and freeze-dried to give **15** (10.7 mg) and **16** (7.6 mg) as white powders. The powders, **15** and **16**, contained 8% of **16** and 15% of **15** as impurities, respectively: (**15**), HPLC; Rt, 8.5 (20% MeOH-p.b.), UV; $\lambda_{max}$ 245 nm ($\epsilon$ 25000), IR; 1720 (COOC$H_3$), 1620 $cm^{-1}$, PMR; $\delta$ 1.94 (3H × 2, s, 8-$CH_3$), 2.16 (NHCOC$H_3$), 3.75 (COOC$H_3$), 6.25, 7.61 (CH=). (**16**), HPLC; Rt, 15.9 (20% MeOH-p.b.), UV; $\lambda_{max}$ 245 nm ($\epsilon$ 20400), IR; 1715 (COOC$H_3$), 1620 $cm^{-1}$, PMR; $\delta$ 1.95 (3H × 2, s, 8-$CH_3$), 2.14 (NHCOC$H_3$), 3.73 (COOC$H_3$), 6.12, 7.52 (CH=).

*Hydrogenation of* **8**

A mixture of **8** (360 mg) and 10% Pd-C (1.8 g) in $H_2O$ (360 ml) was hydrogenated at room temp for 3 h. Additional catalyst (360 mg) was added and hydrogenation was successively continued for 2 h. After filtration of the mixture, the filtrate was concentrated. The concentrate (200 ml) was chromatographed on HP-20 (180 ml) with 0–5% of gradient by MeOH/5% NaCl-$H_2O$. The fractions were analysed by HPLC to divide 2 groups containing **17** and **18**. The divided fractions were individually desalinated by activated carbon chromatography to give **17** (10.3 mg) and **18** (39.7 mg) as white powders: (**17**), HPLC, Rt, 7.3 (4% MeOH-p.b.), UV; $\lambda_{max}$ 301 nm ($\epsilon$9070), IR; 1740 (CO), 1660 (NHAc), 1230, 1040 $cm^{-1}$, PMR; $\delta$ 1.63, 1.69 (8-$CH_3$), 2.01 (NHCOC$H_3$), (**18**), HPLC; Rt, 2.6 (4% MeOH-p.b.), UV; $\lambda_{max}$ 263 nm ($\epsilon$6790), IR; 1750 (CO), 1595, 1240, 1045 $cm^{-1}$, PMR; $\delta$ 1.64, 1.72 (8-$CH_3$), 2.79, 3.55 (1-$CH_2$), 3.86 ($H_6$), 4.40 ($H_5$), 6.40 ($H_1$).

*Hydrolysis of* **17** *or* **18**

The sulfonated compounds, **17** or **18**, were hydrolyzed to give the corresponding hydroxy compounds (**21** or **22**) by a method similar to that in Ref. 1*a*: (**21**), HPLC; Rt, 6.3 (10% MeOH-p.b.), UV; $\lambda_{max}$ 300 nm ($\epsilon$10700), CD; $[\theta]_{nm}$ 238 (+6100), 290 (+6100) and 320 (−5500), PMR; $\delta$ 1.32, 1.42 (8-$CH_3$) and 2.02 (NHCOC$H_3$), (**22**), HPLC; Rt, 3.2 (10% MeOH-p.b.), UV $\lambda_{max}$ 263 nm ($\epsilon$6890), CD; $[\theta]_{nm}$ 230 (−27300) and 253 (+23100), IR; 1750 (CO), 1585, 1260 $cm^{-1}$, PMR; $\delta$ 1.32, 1.44 (8-$CH_3$), 2.74, 3.48 (1-$CH_3$), 3.73 ($H_6$), 4.35 ($H_5$) and 6.33 ($H_1$).

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