THE STRUCTURE OF A NEW ANTIBIOTIC, TERPENTECIN

Sir:

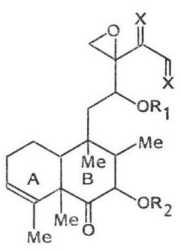
In a previous paper, the isolation and physicochemical properties of a new antitumor antibiotic, terpentecin (**1**, Fig. 1) was reported[1]. We now wish to report its structure.

The field desorption mass spectroscopy (FD-MS) of **1** showed a pseudo-molecular ion at *m/z* 365 (M+1), but **1** gave complicated ^{1}H NMR spectra in various solvents. The FD-MS of its 2,4-dinitrophenylhydrazone (**2**) showed the molecular ion at *m/z* 724 and indicated that **1** should have at least two carbonyl groups.

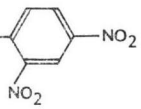
Treatment of **1** with (carbethoxymethylene)-triphenylphosphorane in benzene at 20°C for 18 hours gave **3**, EI-MS *m/z* 504 (M). The ^{1}H NMR spectra of **3** indicated the transformation from **1** to **3** containing a β-substituted diethyl *trans-trans* (or *cis-trans*)-muconate [δ 1.32 and 1.33 (3H, t, J=7.0 Hz), 4.23 and 4.26 (2H, q, J=7.0 Hz), 6.17 (1H, s), 6.36 (1H, d, J=16.4 Hz) and 8.46 (1H, d, J=16.4 Hz) a long range coupling between signals of δ 6.17 and δ 8.46]. These findings suggested the presence of an α-keto aldehyde in the structure of **1**. In addition, acetylation of **3** with acetic anhydride and pyridine (50°C, 5 hours) gave diacetate (**4**) indicating that **3** had two hydroxyl groups. The ^{1}H NMR spectrum of **4** showed two lower-shifted signals (δ 5.16, dd, J=2.0 Hz, 9.0 Hz and δ 5.37, d, J=14.0 Hz) with acetyl signals (δ 2.02 and δ 2.18). The two hydroxyl signals (δ 2.20, br and δ 3.65, d, J=4.0 Hz) of **3** disappeared in the spectrum of **4**. The two signals at $\sim\delta$ 2.2 of **4** could not be assigned. In order to clarify the spectrum at this region, compound (**6**) was prepared. The treatment of **1** with (carbomethoxymethylene)triphenylphosphorane in the manner as described above gave **5** which was treated with *p*-bromobenzoyl chloride in pyridine at 20°C for 3 hours to give **6** [HR-MS *m/z* 660.1707 ($C_{33}H_{39}O_9Br$*) and 658.1768 ($C_{33}H_{39}O_9Br$); UV λ_{max}^{MeOH} nm (ε) 247 (27,700) and 268 (17,100); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 2950, 1720 and 1590; $[\alpha]_D^{20}$ −66.5° (*c* 0.2, $CHCl_3$)]. Although crystallization of **6** was unsuccessful, its NMR and high resolution mass spectra were useful for structure determination of **1**. The molecular formula of **1** was thus determined to be $C_{20}H_{28}O_6$.

The ^{1}H and ^{1}H-^{13}C shift correlation spectra

Fig. 1. Structures of terpentecin and its derivatives.



Terpentecin (**1**) X = O
$R_1 = R_2 = H$

2 X = NNH– 

$R_1 = R_2 = H$

3 X = CHCOOEt
$R_1 = R_2 = H$

4 X = CHCOOEt
$R_1 = R_2 = Ac$

5 X = CHCOOMe
$R_1 = R_2 = H$

6 X = CHCOOMe
$R_1 = H$
R_2 = pBrBz

pBrBz = *p*-Bromobenzoyl

along with the gated decoupled ^{13}C-spectrum of **6** revealed the partial structures A, B, C, D, E, F, G and H (Fig. 2). The linkages from C-5 to C-8, C-2 to C-3 and C-11 to C-12 were shown by the ^{1}H shift correlation spectrum. And the allylic coupling observed between C-9-CH_3 and H-8 extended the linkage of C-5 ~ C-8 to C-9 as shown in the structure A. The characteristic $^1J_{C-H}$ value in the ^{13}C-gated decoupled spectrum (δ 49.1, dt, ^1J=175 Hz, ^3J=4.0 Hz) indicated the existence of an epoxymethylene group (partial structure G) in the molecule.

Partial structures A, B, E and H could be connected as follows. The long range ^{1}H-^{13}C shift correlation spectrum showed that carbons at δ 207.8 (C-1), 137.8 (C-9), 41.1 (C-5) and 51.8 (C-10) were coupled with CH_3 group on C-10 (δ 1.39). ^{1}H-^{13}C long range selective proton decoupling (LSPD) experiments, irradiating at δ 1.39 and 2.91 (H-5) confirmed the above results. Furthermore, the ^{1}H NMR spectrum of the triacetyl derivative which was prepared from **5** by hydride reduction ($NaCNBH_3$, THF - AcOH, 0 ~ 20°C, 3 hours) followed by acetylation (Ac_2O, pyridine, 50°C, 4 hours) clearly revealed the linkage from C-1 to C-3 as shown in Fig. 3. The linkages of partial structures B, C and F were established in a manner similar to that described above. Thus, the long range ^{1}H-^{13}C shift correlation spectrum showed that carbons at δ 40.2 (C-11), 41.1 (C-5), 41.4 (C-3) and 38.9 (C-4) were coupled with CH_3 on C-4 (δ 1.04).

Fig. 2. Partial structures of **6**.

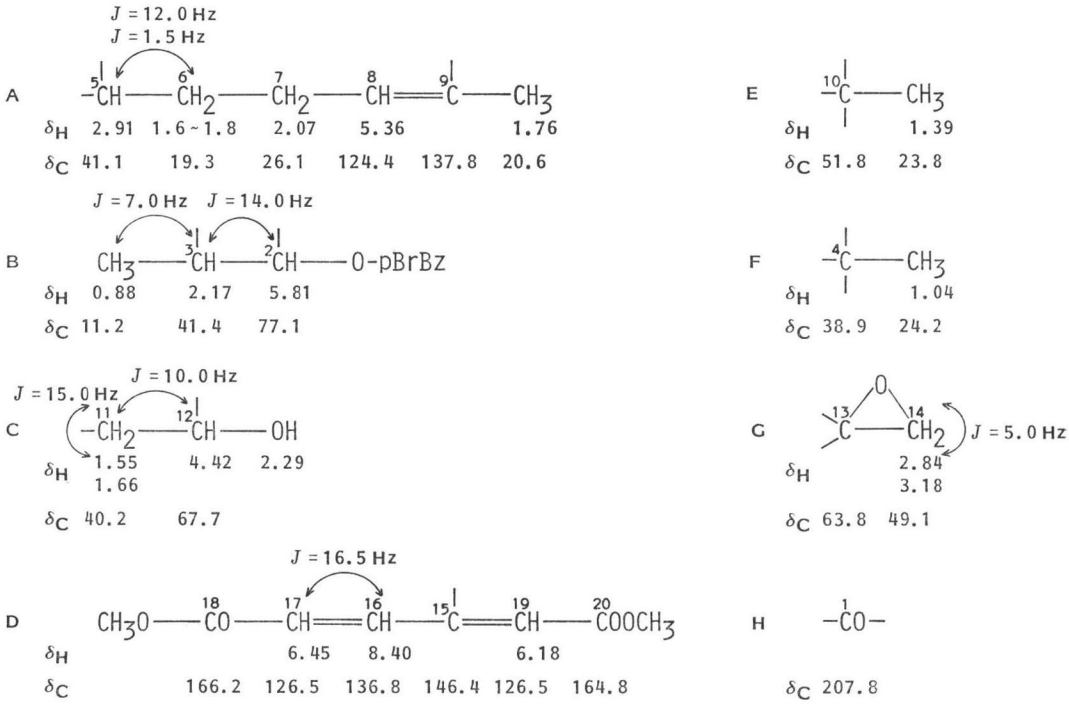


Fig. 3.

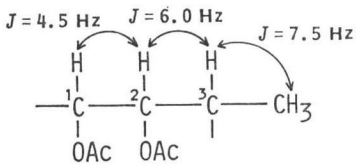
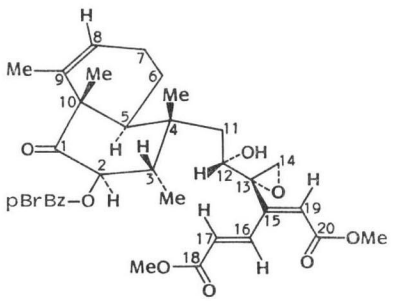


Fig. 4. Relative configuration of **6**.



And the LSPD experiment [irradiating H-12 (δ 4.42)] gave a sharp triplet signal of the epoxymethylene carbon at δ 49.1 (C-14) showing the connection of C-12 and C-13. The linkage between C-13 and C-15 was determined by the long range coupling between carbon at δ 63.8 (C-13) and protons at 8.40 (H-16) and 6.18 (*H-19*). From the above results, the structure of **6** was determined as shown in Fig. 1.

Configurational studies were accomplished by 2-dimensional spectroscopy of nuclear Overhauser effect (NOE) as follows. The coupling constant of H-5 and H-6,6′ (J=1.5 Hz and 12.0 Hz) showed that **6** had a *trans* Decalin configuration that was confirmed by the NOE between H-3 and methyl protons at δ 1.04 and 1.39, having no NOE between H-3 and H-5. From above results, it was indicated that two methyl groups in partial structures E and F were on the same side as of H-3 proton and counter side of H-5. The NOE between H-2 and H-5 suggested that the ring B took a boat form that was confirmed by the large coupling constant between H-2 and H-3 (J=14.0 Hz). It is impossible to take a chair form having H-5 and H-2 on the same side when the dihedral angle between H-2 and H-3 was 0 or 180 degrees. It was indicated by the NOE between H-19 and H-14 (δ 2.84) that partial structure D was β-substituted *cis-trans*-muconate having H-19 and C-14 on the same side. In addition, the NOE among protons H-2, H-5, H-12 and H-17 and

that between hydroxyl proton (C-12-OH) and H-14′ (δ 3.18) indicated that they were closely located. From all these findings, the relative configuration of **6** was suggested as illustrated in Fig. 4. The similar structure has been found in clerocidin[2].

Acknowledgments

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KUNIO ISSHIKI
TSUYOSHI TAMAMURA
YOSHIKAZU TAKAHASHI
TSUTOMU SAWA
HIROSHI NAGANAWA
TOMIO TAKEUCHI
HAMAO UMEZAWA

Institute of Microbial Chemistry
3-14-23 Kamiosaki,
Shinagawa-ku, Tokyo 141,
Japan

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