TERRECYCLIC ACID A, A NEW ANTIBIOTIC FROM *ASPERGILLUS TERREUS*

II. STRUCTURE OF TERRECYCLIC ACID A

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The structure of a new antibiotic, terrecyclic acid A, from a strain of *Aspergillus terreus* Thom, was established as **I** on the basis of spectroscopic and chemical evidences, by comparison of spectroscopic data with quadrone, a known antitumor substance, and further by conversion of terrecyclic acid A to quadrone through pyrolysis.

In the preceding paper[1], we described the isolation procedure, biological activities, physicochemical properties, and some spectroscopic data of a new antibiotic, terrecyclic acid A (**I**), produced by *Aspergillus terreus* Thom No. 14. In this paper we wish to report our experimental results leading to structural elucidation of **I**.

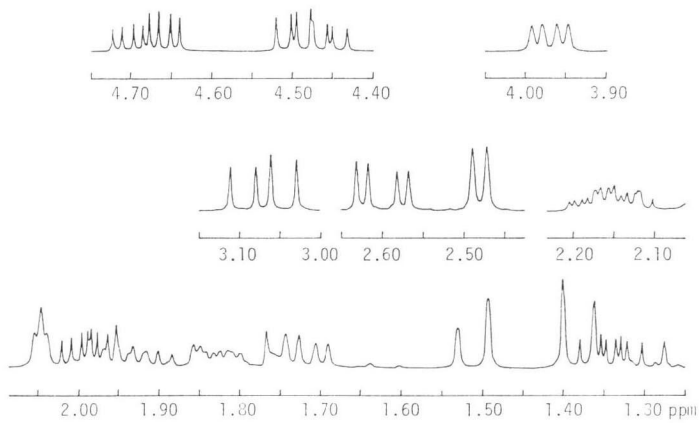
Structure of Terrecyclic Acid A (I)

Terrecyclic acid A (**I**), $C_{15}H_{20}O_3$, is an acidic substance and has a carboxyl group judging from IR spectrum (3300(br.), 3150(br.) and 1710 cm^{-1}), ^1H NMR spectrum (δ 10.0 ppm, br.s, 1H*) and ^{13}C NMR spectrum (δ 179.95 ppm, s). Since **I** was positive to 2,4-dinitrophenylhydrazine and no peak due to aldehyde was found in ^1H and ^{13}C NMR spectra, **I** was suggested to have a ketone group. The absorption at 1739 cm^{-1} in IR spectrum indicated the presence of a five-membered ketone and so one of three oxygens in **I** was a carbonyl group and others in a carboxyl group. In the ^1H NMR spectrum two singlets at δ 5.24 and 6.00 ppm were attributable to exomethylene and this assignment was supported by the ^{13}C NMR spectrum (δ 116.10 ppm (t) and 150.53 ppm (s)) and the IR spectrum (1630 cm^{-1}). Since by reaction with diazomethane **I** was converted into a diazomethane adduct of a methyl ester of **I** (**II**), which is precisely described later, **I** was established to have α-methylene cyclopentanone moiety. Furthermore the UV absorption (236 nm (ε 6,325)) supported this partial structure. According to the molecular formula, the total number of rings plus double bonds of **I** is six and so **I** has three rings.

I was reacted with diazomethane and the nicely crystalline substance (**II**), mp 122~123°C, was obtained from the neutral fraction of the reaction mixture. The high resolution mass spectrum of **II** indicated that the molecular formula of **II** was $C_{17}H_{24}N_2O_3$ (MS, M^+ m/z 304.1796), and the molecular ion easily lost N_2 and gave the ion of m/z 276 ($C_{17}H_{24}O_3$). Though olefinic proton was not observed in the ^1H NMR spectrum of **II**, a $COOCH_3$ signal at δ 3.50 ppm (3H, s) and signals due to pyrazoline[2]

* By addition of D_2O, this signal disappeared.

Fig. 1. 400 MHz ^{1}H NMR spectrum of **II** ($CDCl_3$, TMS).
Signals at δ 3.50 ppm (3H, s) and 1.16 (6H, s) are not depicted in this figure.



(δ 1.99 ppm (1H, m), 1.34 (1H, m), 4.48 (1H, m) and 4.68 (1H, m)) were shown. Accordingly cycloaddition of diazomethane to the exomethylene of **I** has occurred.

The ^{13}C NMR spectrum of **II** showed the presence of three methyl, six methylene, three methine, three quaternary and two carbonyl carbons. In the 400 MHz ^{1}H NMR spectrum signals were separated well enough to be decoupled (Fig. 1). Fifteen protons except nine from three methyl groups were tentatively named H_a, H_b, H_c, $\cdots$, H_n and H_o in order from lower magnetic field. H_a (δ 4.68 ppm) and H_b (δ 4.48 ppm) protons were assigned to methylene adjacent to nitrogen in a pyrazoline ring[2] and on irradiation of the H_a proton, both the H_i proton (δ 1.99 ppm, ddd) and the H_o proton (δ 1.34 ppm, ddd) collapsed to a double doublet. Since the H_i and H_o protons also collapsed to double doublet on irradiation of the H_b proton, the H_i and H_o protons were established to be in the methylene of a pyrazoline ring. Irradiation of the H_c proton at δ 3.97 ppm (dd) changed the H_d proton at δ 3.07 ppm (dd) and the H_e proton at δ 2.60 ppm (dd) to doublets (J=20.0 Hz), and sharpened the H_m proton at δ 1.51 ppm (d). Considering the chemical shifts of these three protons, the H_d and H_e protons were assigned to methylene protons adjacent to a ketone group and the H_c proton is on a methine carbon next to that methylene. As the result the partial structure **a** was proposed. (Fig. 2) Irradiation of the H_f proton at δ 2.48 ppm (d) collapsed the H_j proton at δ 1.93 ppm to an eight-line signals (ddd) and irradiation at δ 2.16 ppm (H_g proton, ddt) changed the H_h proton (δ 1.93 ppm) to a double doublet, and the H_i proton (δ 1.72 ppm, dd, J=14.5, 7.0 Hz) to a doublet (J=14.5 Hz), and changed the features of the H_k proton (δ 1.83 ppm, ddd). Besides, on irradiation of the H_i proton (δ 1.72 ppm, dd) the H_g proton collapsed to a double triplet and the H_j proton changed also to a double triplet.

The ^{1}H NMR data of **II** are summarized in Table 1 together with results of decoupling experiments above mentioned and the measured values of the coupling constants in the enlarged 400 MHz ^{1}H NMR spectrum. From these data partial structures of **II** could be derived as shown in Fig. 2.

During the elucidation of that structure by combining the partial structures and examination of known sesquiterpenes, we found **I** was similar in various data to quadrone, which was isolated as an antitumor substance from *Aspergillus terreus* and of which the structure had already been reported[3,4]. *dl*-Quadrone had also been synthesized[5,6,7].

From the comparison of the ^{1}H NMR and ^{13}C NMR spectra data of **I** with those of quadrone

Table 1. Summary of ^1H NMR spectrum of **II**.

Proton	Chemical shift (ppm) (multiplicity)	Coupling constant (Hz)
H_a	4.68 (ddd)	$J_{H_aH_b}=18.5$, $J_{H_aH_i}=4.5$, $J_{H_aH_o}=10.5$
H_b	4.48 (ddd)	$J_{H_aH_b}=18.5$, $J_{H_bH_i}=10.0$, $J_{H_bH_o}=7.5$
H_c	3.97 (dd)	$J_{H_cH_d}=12.5$, $J_{H_cH_e}=6.0$
H_d	3.07 (dd)	$J_{H_cH_d}=12.5$, $J_{H_dH_e}=20.0$
H_e	2.60 (dd)	$J_{H_cH_e}=6.0$, $J_{H_dH_e}=20.0$
H_f	2.48 (d)	$J_{H_fH_j}=7.0$
H_g	2.16 (ddt)	$J_{H_gH_h}=3.0$, $J_{H_gH_j}=13.0$, $J_{H_gH_k}=13.0$, $J_{H_gH_l}=7.0$
H_h	2.05 (t)	$J_{H_gH_h}=3.0$, $J_{H_hH_k}=3.0$
H_i	1.99 (ddd)	$J_{H_aH_i}=4.5$, $J_{H_bH_i}=10.0$, $J_{H_iH_o}=13.0$
H_j	1.93 (ddt)	$J_{H_fH_j}=7.0$, $J_{H_gH_j}=13.0$, $J_{H_jH_k}=7.0$, $J_{H_jH_l}=14.5$
H_k	1.83 (ddd)	$J_{H_gH_k}=13.0$, $J_{H_hH_k}=3.0$, $J_{H_jH_k}=7.0$
H_l	1.72 (dd)	$J_{H_gH_l}=7.0$, $J_{H_jH_l}=14.5$
H_m	1.51 (d)	$J_{H_mH_n}=15.5$
H_n	1.38 (d)	$J_{H_mH_n}=15.5$
H_o	1.34 (ddd)	$J_{H_aH_o}=10.5$, $J_{H_bH_o}=7.5$, $J_{H_iH_o}=13.0$
Others	3.50 (3H, s)	$-COOCH_3$
	1.16 (6H, s)	$CH_3>C<CH_3$

Fig. 2. Partial structures of **II**.

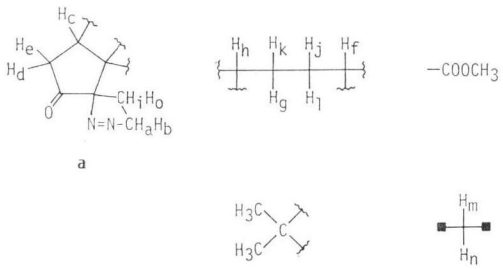
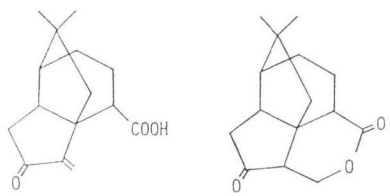


Fig. 3. Structures of terrecyclic acid A and quadrone.



Terrecyclic acid A (I) Quadrone

Table 2. ^{13}C NMR chemical shifts of **I**, **III** and quadrone. (ppm relative to TMS)

I	III	Quadrone (Ref.[8])
22.52 t	19.33 t	19.23 t
27.32 q	26.91 q	26.85 q
28.87 t	28.08 t	28.00 t
34.75 q	34.84 q	34.74 q
40.45 s	40.45 s	40.32 s
41.45 t	43.20 t	43.17 t
46.36 d	45.98 d	45.80 d
47.94 d	48.73 d	48.54 d
48.85 d	49.87 s	49.69 s
54.03 t	52.21 d	52.05 d
54.90 s	52.51 d	52.21 d
116.10 t	52.51 t	52.38 t
150.53 s	65.29 t	65.20 t
179.95 s	174.01 s	174.06 s
207.54 s	216.57 s	216.52 s

Abbreviations; s, d, t and q represent singlet, doublet, triplet and quartet, respectively.

(literature), **I** was assumed to be the final precursor of quadrone in the DANISHEFSKY synthesis[5,6] (Fig. 3). As shown in Table 2, the ^{13}C NMR spectrum of **I** was very similar to that of quadrone. Further, **I** was pyrolyzed at 190°C in an oil bath and a crystalline substance (**III**), mp 183~184°C, was obtained from the neutral fraction of the reaction mixture. The chemical shifts of fifteen carbons in the ^{13}C NMR spectrum of **III** were identical with those of quadrone[8] as shown in Table 2. Accordingly the

structure of terrecyclic acid A was established **I** as shown in Fig. 3.

Discussion

Terrecyclic acid A was established to be a new acidic antibiotic, a sesquiterpene with three rings, which would be a biological precursor of quadrone, an antitumor substance. As a natural product **I** is the second one which has this carbon skelton and **I** is interesting from the viewpoint of biosynthesis and biological activities. Though the producing microorganism of **I** was the same as that of quadrone, quadrone has not yet been found in the fermentation broth of No. 14 strain.

In the ^{1}H NMR spectrum of **II** the coupling constant of H_e and H_h, H_k and H_l, and H_f and H_l was very small or nothing. This is reasonable because the dihedral angle of the carbon-hydrogen bond would be close to 90°, a conclusion supported by Dreiding stereomodels.

Experimental

Melting points were determined on a microscope hot plate of Yanagimoto Co. and are reported uncorrected. The optical rotation was measured with a JASCO DIP-SL polarimeter. The IR spectra were recorded on a JASCO IRA-2 infrared spectrometer. The 100 MHz and 400 MHz ^{1}H NMR spectra were obtained with JNM-MH-100 and JNM-FX 400 spectrometers, respectively. The ^{13}C NMR (25 MHz) spectra were measured with a JEOL JMN-FX-100 spectrometer. Mass spectra and high resolution mass spectra were obtained with a Hitachi RMU-6M and a JEOL JMS D-300 mass spectrometers, respectively. UV spectra were recorded on a Shimadzu double-beam spectrophotometer UV-180.

Terrecyclic Acid A (**I**)

Mp 122°C; $[\alpha]_D^{20}$ +29.1° (*c* 4, EtOH); MS, M^+ *m/z* 248.1370 (Calcd. for $C_{15}H_{20}O_3$ 248.1411); IR_{max}^{KBr} cm^{-1}: 3100, 2930, 1738, 1710, 1630, 1450, 1410, 1322, 1180, 1165 and 940; UV_{max}^{EtOH} nm(ε): 236 (6,325); ^{1}H NMR (100 MHz, ppm, $CDCl_3$, TMS); 1.19 (3H, s), 1.23 (3H, s), 1.80 (2H, s), 1.8~2.2 (5H, m), 2.4~2.7 (2H, m), 2.8~3.1 (2H, m), 5.20 (1H, s), 5.98 (1H, s), 10.0 (1H, br.s); ^{13}C NMR (25 MHz, ppm, $CDCl_3$, TMS): 22.52 (t), 27.32 (q), 28.87 (t), 34.75 (q), 40.45 (s), 41.45 (t), 46.36 (d), 47.94 (d), 48.85 (d), 54.03 (t), 54.90 (s), 116.10 (t), 150.53 (t), 179.95 (s), 207.54 (s).

Diazomethane Adduct of Methyl Ester of **I** (**II**)

Through a solution (5 ml) of **I** (50 mg) in ethyl ether containing 10% methanol, diazomethane gas was bubbled using N_2 gas as a carrier gas at room temperature until yellow color of diazomethane did not disappear readily. The neutral fraction of reaction mixture was chromatographed by Wako gel C-200 (20 g) and **II** (40 mg) was eluted in hexane - ethyl acetate (85: 15). mp 122~123°C; $[\alpha]_D^{24}$ +349.2° (*c* 0.7, EtOH); MS, M^+ *m/z* 304.1796 (Calcd. for $C_{17}H_{24}N_2O_3$ 304.1786); IR_{max}^{KBr} cm^{-1}: 3500, 3450, 3000, 2950, 2910, 2880, 1750, 1725, 1550, 1460, 1435, 1410, 1380, 1370, 1350, 1300, 1260, 1220, 1190, 1170, 1155, 1090, 1055, 1035, and 1015; UV_{max}^{EtOH} nm(ε): 209.5 (2,934), 230 (1,277), 296 (sh.), 309 (261), 340 (460); ^{1}H NMR (400 MHz, ppm, $CDCl_3$, TMS); 1.16 (6H, s), 1.34 (1H, ddd), 1.38 (1H, d), 1.51 (1H, d), 1.72 (1H, dd), 1.83 (1H, ddd), 1.93 (1H, ddt), 1.99 (1H, ddd), 2.05 (1H, t), 2.16 (1H, ddt), 2.48 (1H, d), 2.60 (1H, dd), 3.07 (1H, dd), 3.50 (3H, s), 3.97 (1H, dd), 4.48 (1H, ddd), 4.68 (1H, ddd); ^{13}C NMR (25 MHz, ppm, $CDCl_3$, TMS): 18.28 (t), 23.46 (t), 26.76 (q), 28.40 (t), 33.84 (q), 39.34 (s), 40.34 (t), 43.23 (d), 44.87 (d), 50.28 (d), 50.63 (t), 50.98 (q), 57.62 (s), 77.69 (t), 111.65 (s), 175.01 (s), 209.76 (s).

Pyrolysis Product of **I** (**III**)

The test tube containing **I** (50 mg) was treated at 190°C in an oil bath for 7 minutes. The neutral fraction of reaction mixture was chromatographed by Wako gel C-200 (15 g) and **III** (10 mg) was eluted in benzene - ethyl acetate (95: 5). mp 183~184°C; $[\alpha]_D^{21}$ −44.6° (*c* 1.3, EtOH); MS, M^+ *m/z* 248; IR_{max}^{KBr} cm^{-1}: 3450, 2960, 1745, 1470, 1450, 1390, 1380, 1230, 1170, 1140, 1130, 1060 and 965; UV: end absorption; ^{1}H NMR (400 MHz, ppm, $CDCl_3$, TMS): 1.22 (3H, s), 1.28 (3H, s), 1.68 (1H, m),

1.75~2.00 (3H, m), 1.90 (1H, d), 2.01 (1H, t), 2.08 (1H, d), 2.36 (1H, dd), 2.42 (1H, d), 2.44 (1H, dd), 2.67 (1H, dd), 2.75 (1H, d), 4.21 (1H, dd), 4.63 (1H, d). ^{13}C NMR (25 MHz, ppm, $CDCl_3$, TMS): 19.33 (t), 26.91 (q), 28.08 (t), 34.84 (q), 40.45 (s), 43.20 (s), 45.98 (d), 48.73 (d), 49.87 (s), 52.21 (d), 52.51 (d), 52.51 (t), 65.29 (t), 174.01 (s), 216.57 (s).

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