

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

III.  $^{13}\text{C}$  NMR SPECTRUM OF TERRECYCLIC ACID A

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Assignment of the fifteen carbons of terrecyclic acid A,  $\text{C}_{15}\text{H}_{20}\text{O}_3$ , a new sesquiterpene antibiotic, in the  $^{13}\text{C}$  NMR spectrum was performed by  $^{13}\text{C}$ - $\{^1\text{H}\}$  selective proton decoupling experiments, comparison with spectra of its derivatives and chemical shifts.

Terrecyclic acid A (I),  $\text{C}_{15}\text{H}_{20}\text{O}_3$ , is a sesquiterpene antibiotic from *Aspergillus terreus* Thom No. 14<sup>1,2)</sup> and has the same novel carbon skeleton as does quadrone, an antitumor substance from *A. terreus*<sup>3,4)</sup> (Fig. 1). *A. terreus* No. 14 also produces several substances related to I<sup>5,6)</sup>. We are much interested in the biosynthesis of I and of related compounds.

Assignments of all carbons of I in  $^{13}\text{C}$  NMR spectrum are necessary not only for studying biosynthesis but also for characterizing related compounds which will be isolated in the future.

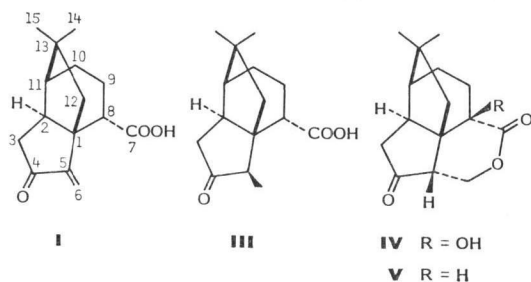
The  $^{13}\text{C}$  NMR spectral data for I are shown in Table 1. Since there are two signals in the region of the carbonyl carbons, chemical shifts readily assigned the C-7 and 4 carbons at  $\delta$  179.95 and 207.54, respectively. Also from the chemical shift the singlet at  $\delta$  150.53 must be due to C-5. The remaining

Table 1. Summary of  $^{13}\text{C}$  NMR spectral data of I, III, IV and V (25 MHz,  $\text{CDCl}_3$ ) ( $\delta$ , ppm, multiplicity).

Carbon	I	III	IV	V
1	54.90 (s)	54.93 (s)	54.49 (s)	49.87 (s)
2	46.36 (d)	45.02 (d) <sup>b</sup>	47.39 (d) <sup>c</sup>	45.98 (d) <sup>d</sup>
3	41.45 (t)	40.45 (t)	43.00 (t)	43.20 (t)
4	207.54 (s)	218.83 (s)	218.10 (s)	216.57 (s)
5	150.53 (s)	51.60 (d) <sup>b</sup>	52.13 (d) <sup>c</sup>	52.51 (d) <sup>d</sup>
6	116.10 (t)	8.57 (q)	66.98 (t)	65.29 (t)
7	179.95 (s)	181.24 (s)	172.49 (s)	174.01 (s)
8	47.94 (d)	48.03 (d) <sup>b</sup>	78.36 (s)	48.73 (d) <sup>d</sup>
9	22.52 (t)	22.17 (t)	28.72 (t) <sup>a</sup>	19.33 (t)
10	28.87 (t)	28.84 (t)	29.89 (t) <sup>a</sup>	28.08 (t)
11	48.85 (d)	49.32 (d) <sup>b</sup>	50.81 (d) <sup>c</sup>	52.21 (d) <sup>d</sup>
12	54.03 (t)	47.68 (t)	46.71 (t)	52.51 (t)
13	40.45 (s)	39.55 (s)	40.34 (s)	40.45 (s)
14	27.32 (q)	27.17 (q)	26.82 (q)	26.91 (q)
15	34.75 (q)	34.19 (q)	34.84 (q)	34.84 (q)

<sup>a</sup> Assignments may be reversed.

<sup>b, c, d</sup> Assignments may be changed.

Fig. 1. Structures of **I**, **III**, **IV** and **V**.

two quaternary carbons (C-1 and 13) were assigned mainly from the LINDEMAN-ADAMS rule<sup>7</sup>. According to this rule C-1 and 13 should resonate at  $\delta$  47.09 and 38.29, respectively. The difference between the calculated and found values comes from the  $\beta$ -deshielding effect, because the LINDEMAN-ADAMS rule applies to paraffin molecular structure. Consequently, C-1 appears at  $\delta$  54.90, while C-13 appears at  $\delta$  40.45.

There are three methine carbons and assignments of them could be made by  $^{13}\text{C}\{-^1\text{H}\}$  selective proton decoupling experiments. The 100 MHz  $^1\text{H}$  NMR spectrum of **I** has already

been reported, but the resolution was not complete<sup>1</sup>. That was improved in the 400 MHz  $^1\text{H}$  NMR spectrum (Fig. 2). Each proton was labeled according to that in the diazomethane adduct of the methyl ester of **I** (**II**), the  $^1\text{H}$  NMR spectrum of which was completely analyzed<sup>2</sup>. Assignment of the protons necessary for  $^{13}\text{C}\{-^1\text{H}\}$  selective proton decoupling experiments was made as follows\*.

By comparison of the spectra of **I** and **II**, the signals at  $\delta$  3.01 (1H, d) and at  $\delta$  1.97 (1H, dd) are attributable to the  $\text{H}_r$  and  $\text{H}_h$  protons, respectively. Since the  $\text{H}_r$  proton is affected by the carboxyl group, it is deshielded in the  $^1\text{H}$  NMR spectrum. From coupling constants the  $\text{H}_e$ ,  $\text{H}_d$  and  $\text{H}_g$  protons are assigned as shown in Table 2, but the assignment of  $\text{H}_d$  and  $\text{H}_g$  protons may be reversed.

Using the results of these assignments  $^{13}\text{C}\{-^1\text{H}\}$  selective proton decoupling experiments were conducted. On irradiation at  $\delta_{\text{H}}$  2.90 the signal at  $\delta_{\text{C}}$  46.36 was sharpened, and on the other hand, irradiation at  $\delta_{\text{H}}$  3.00 sharpened the  $\delta_{\text{C}}$  47.94 resonance. Thus the  $\delta_{\text{C}}$  46.36 and 47.94 resonances are due to C-2 and 8, respectively. The remaining methine carbon, C-11, was assigned as the resonance at  $\delta$  48.85.

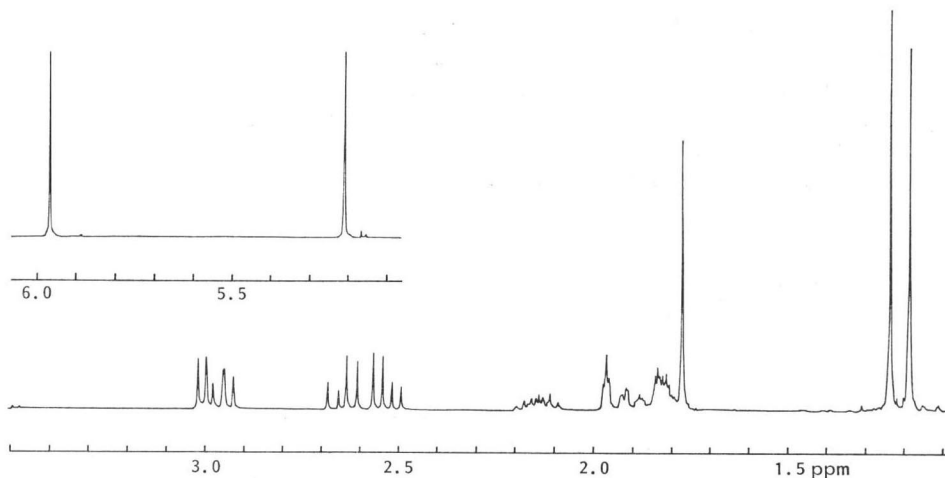
There exist five methylene carbons (C-3, 6, 9, 10 and 12) in **I** and the  $\delta$  116.10 resonance can readily be attributed to C-6, an olefinic carbon. Assignment of C-3 was made by  $^{13}\text{C}\{-^1\text{H}\}$  selective proton

Table 2. Summary of  $^1\text{H}$  NMR spectrum of **I**.

Proton	Chemical shift ( $\delta$ , ppm, multiplicity)	Coupling constant (Hz)
$=\text{CH}_2$	{ 5.96 (1H, s) 5.21 (1H, s)	
$\text{H}_r$	3.01 (1H, d)	$J=8.0$
$\text{H}_e$	2.95 (1H, dd)	$J=10.0, 11.0$
$\text{H}_d^*$	2.65 (1H, dd)	$J=11.0, 19.5$
$\text{H}_g^*$	2.53 (1H, dd)	$J=10.0, 19.5$
$\text{H}_g$	2.14 (1H, m)	
$\text{H}_h$	1.97 (1H, dd)	$J=2.8, 2.8$
$\text{H}_k$	1.90 (1H, m)	
$\text{H}_j$ and $\text{H}_l$	1.78 ~ 1.85 (2H, m)	
$\text{H}_m$ and $\text{H}_n$	1.77 (2H, s)	
$\text{H}_3\text{C}$	{ 1.24 (3H, s) 1.18 (3H, s)	

\* Assignment may be reversed.

\* Assignment of other protons was made as follows. Two singlets at  $\delta$  5.96 and 5.21 are readily assigned to the exomethylene protons. Judging from the characteristic shape of signals in the spectra of **I** and **II** the multiplet at  $\delta$  2.14 is assigned to the  $\text{H}_g$  proton and the multiplet at  $\delta$  1.90 to the  $\text{H}_k$  proton. In the  $^1\text{H}$  NMR spectrum of **II** the  $\text{H}_m$  and  $\text{H}_n$  protons show typical AB type doublets ( $J=15.5$  Hz), but in that of **I** these two protons are equivalent and appear as a singlet at  $\delta$  1.77. On the other hand the two methyl groups are non-equivalent, giving a 3H-singlet at  $\delta$  1.24 and a 3H-singlet at  $\delta$  1.18, while in **II** they are equivalent. Accordingly, the remaining 2H overlapped multiplet at  $\delta$  1.78 ~ 1.85 is assigned to the  $\text{H}_j$  and  $\text{H}_l$  protons. The results are summarized in Table 2.

Fig. 2. 400 MHz  $^1\text{H}$  NMR spectrum of **I** ( $\text{CDCl}_3$ , TMS).

decoupling experiment. Since on irradiation of  $\delta_{\text{H}}$  2.60 the signal at  $\delta_{\text{C}}$  41.45 was sharpened, the C-3 carbon was assigned as  $\delta$  41.45.

By catalytic hydrogenation of **I** over palladium-charcoal, the reduced product (**III**),  $\text{C}_{13}\text{H}_{22}\text{O}_3$ , was obtained in high yield<sup>9)</sup>. The relative stereochemistry of **I** has already been determined<sup>5)</sup>, and the new methyl group of **III** should be on the same side as the C-12 methylene carbon, because catalytic hydrogenation occurs on the side of less steric hindrance.

The  $^{13}\text{C}$  NMR spectral data for **III** are shown in Table 1. There are four methylene carbons (C-3, 9, 10 and 12) and comparison of the chemical shifts of the methylene carbons in  $^{13}\text{C}$  NMR spectra of **I** and **III** indicates the carbon signals at  $\delta$  22.52, 28.87, 41.45 and 54.03 in **I** shift upfield to  $\delta$  22.17, 28.84, 40.45 and 47.68 in **III**, respectively. Since C-12 in **III** is most affected by the steric compression effect of a new methyl group (C-6), C-12 appears at  $\delta$  47.68 in **III** and appears at  $\delta$  54.03 in **I**.

Furthermore, we have isolated some compounds related to **I** from the ethyl acetate neutral fraction of the culture filtrate of *A. terreus* No. 14 and have elucidated their structures<sup>9)</sup>. One of them is 8-hydroxyquadrone (**IV**), whose  $^{13}\text{C}$  NMR spectrum is shown in Table 1. There also exist five methylene carbons (C-3, 6, 9, 10 and 12) in quadrone (**V**) and **IV**. It is presumed by comparison to the  $^{13}\text{C}$  NMR spectrum of **I** that the remaining two methylene carbons (C-9 and 10) resonate at  $\delta$  19.31 and 28.08 in **V** and at  $\delta$  28.72 and 29.89 in **IV**. In 8-hydroxyquadrone the hydroxyl group shifts the C-9 resonance about 8 ppm downfield by its  $\beta$ -deshielding effect. Accordingly, C-9 resonates at  $\delta$  19.31 in **V** and at  $\delta$  22.52 in **I**.

Chemical shifts of the two methyl carbons in **I** are  $\delta$  27.32 and 34.75. According to the Dreiding stereomodels, C-14 exists spatially near C-9, 10, 12 and 15, and on the other hand C-15 exists near C-3, 12 and 14. Judging from the steric compression effect the C-14 resonance can be assigned as  $\delta$  27.32.

Assignments of all carbons in the  $^{13}\text{C}$  NMR spectrum of terrecyclic acid **A** are shown in Table 1, and the carbons of **III**, **IV** and **V** are also assigned, but assignments of the latter three compounds are in some cases tentative.

Terrecyclic acid **A** seems to be a sesquiterpene antibiotic with an abnormal carbon skeleton and now we are studying the biosynthesis of **I** on the basis of the data reported in this paper.

### Experimental

Melting points were determined on a microscope hot stage of Yanagimoto Co. and are uncorrected. The optical rotation was measured with a Jasco DIP-SL polarimeter. The IR spectra were recorded on a Jasco IRA-2 infrared spectrophotometer. The  $^1\text{H}$  NMR spectra were obtained with Jeol JNM-FX-400 and FX-100 spectrometers. The  $^{13}\text{C}$  NMR spectra were measured with a Jeol JNM-FX-100 spectrometer. The mass spectra and high resolution mass spectrum were obtained with a Hitachi RMU-6M and a Jeol JMS D-300 spectrometer, respectively.

#### Reduction Product from I (III)

Reaction conditions have been reported in detail<sup>5)</sup>. Physico-chemical properties of III are as follows: mp 128~129°C;  $[\alpha]_{\text{D}}^{28} -27^\circ$  (*c* 0.4, EtOH); MS *m/z* (relative abundance) 250 ( $\text{M}^+$ , 8.1), 235 (9.5), 232 (2.0), 193 (100), 163 (10.7), 121 (10.1); IR  $\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$  3150 (br), 2940, 1710 (br), 1450, 1380, 1290, 1240, 1190, 1160, 1060, 1040, 855.

#### 8-Hydroxyquadrone (IV)

The isolation procedure will be reported in detail<sup>6)</sup>. Physico-chemical properties of IV are as follows: mp 136~137°C;  $[\alpha]_{\text{D}}^{24} -51^\circ$  (*c* 0.3, EtOH); MS *m/z* 264.1378 ( $\text{M}^+$ , 264.1361 calcd for  $\text{C}_{15}\text{H}_{20}\text{O}_4$ ), 248 ( $\text{M}-16$ ), 236 ( $\text{M}-28$ ), 220 ( $\text{M}-44$ ); IR  $\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$  3450, 1740, 1730.

#### Quadrone (V)

Quadrone was obtained by pyrolysis of I<sup>2)</sup>: mp 183~184°C,  $[\alpha]_{\text{D}}^{21} -44.6^\circ$  (*c* 1.3, EtOH).

### Addendum in Proof

Quite recently the study on the biosynthesis of terrecyclic acid A using the results of this paper has been reported by the present authors<sup>8)</sup>.

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