TERRECYCLIC ACID A, A NEW ANTIBIOTIC
FROM ASPERGILLUS TERREUS

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

AKIRA HIROTA, MASAHIRA NAKAGAWA, HEIICHI SAKAI

Department of Agricultural Chemistry, University of Osaka Prefecture,
Sakai, Osaka 591, Japan

and AKIRA ISOGAI

Department of Agricultural Chemistry, The University of Tokyo,
Bunkyo-ku, Tokyo 113, Japan

(Received for publication August 29, 1983)

Assignment of the fifteen carbons of terrecyclic acid A, $C_{15}H_{26}O_3$, a new sesquiterpene antibiotic, in the $^{13}$C NMR spectrum was performed by $^{13}$C-$^1$H selective proton decoupling experiments, comparison with spectra of its derivatives and chemical shifts.

Terrecyclic acid A (I), $C_{15}H_{26}O_3$, is a sesquiterpene antibiotic from Aspergillus terreus Thom No. 14$^{1,2}$ and has the same novel carbon skeleton as does quadrone, an antitumor substance from A. terreus$^{3,4}$ (Fig. 1). A. terreus No. 14 also produces several substances related to I$^5,8)$. We are much interested in the biosynthesis of I and of related compounds.

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

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

$^a$ Assignments may be reversed.
$^b,c,d$ Assignments may be changed.
two quaternary carbons (C-1 and 13) were assigned mainly from the LINDEMAN-ADAMS rule\(^7\). 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 \(^{13}\)C-{\(^1\)H} selective proton decoupling experiments. The 100 MHz \(^1\)H NMR spectrum of I has already been reported, but the resolution was not complete\(^3\). That was improved in the 400 MHz \(^1\)H NMR spectrum (Fig. 2). Each proton was labeled according to that in the diazomethane adduct of the methyl ester of I (II), the \(^1\)H NMR spectrum of which was completely analyzed\(^2\). Assignment of the protons necessary for \(^{13}\)C-{\(^1\)H} selective proton decoupling experiments was made as follows*.

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

Using the results of these assignments \(^{13}\)C-{\(^1\)H} selective proton decoupling experiments were conducted. On irradiation at \(\delta_f 2.90\) the signal at \(\delta_o 46.36\) was sharpened, and on the other hand, irradiation at \(\delta_{tt} 3.00\) sharpened the \(\delta_o 47.94\) resonance. Thus the \(\delta_o 46.36\) 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 \(^{13}\)C-{\(^1\)H} selective proton

---

* 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 \(\text{H}_e\) proton and the multiplet at \(\delta 1.90\) to the \(\text{H}_k\) proton. In the \(^1\)H NMR spectrum of II the \(\text{H}_m\) and \(\text{H}_n\) protons show typical AB type doublets \((J=15.5\text{ Hz})\), 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 \(\delta 1.78 \sim 1.85\) is assigned to the \(\text{H}_j\) and \(\text{H}_i\) protons. The results are summarized in Table 2.
decoupling experiment. Since on irradiation of $\delta_{H} 2.60$ the signal at $\delta_{C} 41.45$ was sharpened, the C-3 carbon was assigned as $\delta 41.45$.

By catalytic hydrogenation of I over palladium-charcoal, the reduced product (III), $\text{C}_{11}\text{H}_{22}\text{O}_3$, was obtained in high yield. The relative stereochemistry of I has already been determined, 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 $^{13}$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 $^{13}$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. One of them is 8-hydroxyquadrone (IV), whose $^{13}$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 $^{13}$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 19-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 $^{13}$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 $^1$H NMR spectra were obtained with Jeol JNM-FX-400 and FX-100 spectrometers. The $^{13}$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. Physico-chemical properties of III are as follows: mp 128-129°C; $[\alpha]_D^{25} -27^\circ$ (c 0.4, EtOH); MS m/z (relative abundance) 250 (M+, 8.1), 235 (9.5), 232 (2.0), 193 (100), 163 (10.7), 121 (10.1); IR $\nu_{\text{IR}}^{\text{max}}$ 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. Physico-chemical properties of IV are as follows: mp 136-137°C; $[\alpha]_D^{14} -51^\circ$ (c 0.3, EtOH); MS m/z 264.1378 (M+, 264.1361 calcd for C$_{15}$H$_{12}$O$_{4}$), 248 (M-16), 236 (M-28), 220 (M-44); IR $\nu_{\text{IR}}$ cm$^{-1}$ 3450, 1740, 1730.

Quadrone (V)

Quadrone was obtained by pyrolysis of I: mp 183-184°C, $[\alpha]_D^{14} -44.6^\circ$ (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.

Acknowledgments

We wish to express our thanks to Mr. K. Furihata of the Institute of Applied Microbiology, The University of Tokyo, for measurement of the 400 MHz $^1$H NMR spectrum. We are also grateful to Dr. H. Hirota of Department of Chemistry, The University of Tokyo, for measurement of the high resolution mass spectrum. This work was supported by a Grant-in-aid awarded by the Agricultural Chemical Society of Japan.

References