A New N-Methy1tetrahydroprotoberberine Alkaloid from Tinospora hainanensis

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Received October 6, 1998; accepted November 13, 1998

Several species of the genera Tinospora (Menispermaceae) are widely distributed over Asia and Africa and are well-known for their medicinal properties. From the stems of Tinospora hainanensis, a new N-methy1tetrahydroprotoberberine alkaloid, N-methy1tetrahydrocolumbamine, was isolated and characterized on the basis of NMR, MS and X-ray.

Key words Tinospora hainanensis; Menispermaceae; tetrahydroprotoberberine; N-methy1tetrahydrocolumbamine; crystal structure

Tinospora (T.) hainanensis H. S. Lo et Z. X. Li (Menispermaceae) is found on Hainan island, China. The stems of this plant have been used as an anti-inflammatory for the treatment of arthritis. Tinospora, a genus of deciduous woody climbers is distributed in the tropics of Asia, Africa, and Australia. 1,2 In China the roots of T. sagittata have been used for treating coughs and other throat conditions. 1) And the stems and leaves of T. crispa and T. sinensis have been used for their anti-inflammatory (antirheumatic), febrifugal or antiperiodic (antimalarial), and antibacterial properties. 1,3,4) Tinospora species are widely acclaimed sources of remedies for many different complaints in many other countries: Java, Bali, Malaya, Philippine, Thailand, the Indian subcontinent and West Africa. 1,2) There has been a steady interest in the chemical nature of the active principles contained in Tinospora species. They appear to be protoberberine alkaloids and related bases. 1,5,6) And the occurrence of quaternary alkaloids in Tinospora species has been reviewed. 1,2) The present paper deals with the isolation and structural elucidation of a new tetrahydroprotoberberine alkaloid, together with five known compounds from T. hainanensis.

After drying and pulverization, the stems were extracted with ethanol. From the ethanolic extract, one new alkaloid N-methy1tetrahydrocolumbamine (1) was isolated along with one known alkaloid, columbamine (2), 6,7) three known ecdysteroids, makisterone A (3), 8—11) 24-epi-makisterone A (4), 11,12) 20-hydroxyecdysone (5), 9—13) and syringin (6). 14—16) Some ecdysteroids and phenolic compounds have been previously isolated from Tinospora species. 2,10,16,17)

The 1H-NMR chemical shift values of 1 are given in Table 1. The presence of two proton doublets at $\delta$ 6.95 and 7.04 ($J$=9 Hz) due to H-11 and H-12 indicates the substitution of ring D. In addition, the 1H-NMR spectrum displayed two proton singlets at $\delta$ 6.91 and 6.75 in the aromatic region which were assigned to H-1 and H-4 of ring A. 20) In the 13C-NMR spectrum four methylene signals were observed at $\delta$ 23.8, 28.9, 61.7 and 61.8 due to C-5, C-13, C-6 and C-8 (Table 1). These results indicate that 1 is a 2,3,9,10-tetrasubstituted-N-methy1tetrahydroprotoberberine. 21,22)
The N-methyl group and the three methoxyl group assignments were confirmed by hetronuclear multiple bond connectivity (HMBC), the methyl group was attached to N-7, and the methoxyl groups were present at C-3, C-9 and C-10, and correlation peaks were observed from the following pairs: H-OMe/C-3, H-OMe/C-9, H-OMe/C-10, and H-NMe/C-6.

The absolute structure of I was confirmed by single crystal X-ray diffraction, which completely supports the above structural elucidations by NMR and MS for I (Fig. 1).

### Experimental

#### General Procedures

NMR spectra were recorded on a JEOL JNM-A500 spectrometer in CDCl₃ with tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on a JEOL JMS-DX300 spectrometer. Optical rotations were measured with a JASCO DIP-4 digital polarimeter.

**Plant Material**  The stems of *T. hainanensis* H. S. Lo et Z. X. Li (Menispermaceae) were collected from Hainan island, China. The medicinal plant was botanically identified by Professor Yi Zhong who works in the Department of Biology, Hainan Normal University.

**Extraction and Isolation**  Dried stems of *T. hainanensis* (20 kg) were extracted with 92% ethanol. The alcoholic solution obtained was concentrated and dried to afford alcoholic extract (1.1 kg). The alcoholic extract was partitioned between water and ether, water and chloroform, water and ethyl acetate, water and butanol to give ether extract (283 g), chloroform extract (3 g), ethyl acetate extract (56 g) and butanolic extract (126 g). 80 g of butanolic extract was subjected to column chromatography on silica gel (900 g) and eluted with a solvent mixture of chloroform and methanol (95 : 5). Four fractions (I—IV) were collected according to TLC analysis.

Fraction I (5 g) was rechromatographed on a silica gel column to give 4 (0.2 g) and 6 (0.3 g). Fraction II (1.6 g) was rechromatographed on a silica gel column which was eluted with a solvent mixture of chloroform and methanol (95 : 5). Four fractions (I—IV) were collected according to TLC analysis.

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#### Chart 2

- **(1)** cyclization
- **(2)** hydroxylation
- **(3)** retro Diels-Alder reaction

**Table 1.** 1H- and 13C-NMR Spectral Data for Compound I in CDCl₃

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>H</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>111.5</td>
<td>6.91 (s)</td>
</tr>
<tr>
<td>2</td>
<td>145.7</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>147.4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>111.2</td>
<td>6.75 (s)</td>
</tr>
<tr>
<td>5</td>
<td>112.3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>121.6</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>113.5</td>
<td>6.95 (d, J=9 Hz)</td>
</tr>
<tr>
<td>8</td>
<td>123.4</td>
<td>7.04 (d, J=9 Hz)</td>
</tr>
<tr>
<td>9</td>
<td>121.4</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>113.5</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>6.95 (d, J=9 Hz)</td>
<td>3.77 (dd, J=5, 18 Hz)</td>
</tr>
<tr>
<td>12</td>
<td>6.95 (d, J=9 Hz)</td>
<td>3.77 (dd, J=5, 18 Hz)</td>
</tr>
<tr>
<td>14</td>
<td>66.0</td>
<td>5.19 (s)</td>
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<tr>
<td>14a</td>
<td>121.6</td>
<td></td>
</tr>
<tr>
<td>3-O Me</td>
<td>56.3</td>
<td>3.90 (s)</td>
</tr>
<tr>
<td>9-O Me</td>
<td>61.8</td>
<td>3.97 (s)</td>
</tr>
<tr>
<td>10-O Me</td>
<td>56.0</td>
<td>3.87 (s)</td>
</tr>
<tr>
<td>7-N Me</td>
<td>39.4</td>
<td>3.06 (s)</td>
</tr>
</tbody>
</table>

*a, b) Assignments may be interchanged. c) Overlapped with other signals.*
nates and thermal parameters, and individual bond lengths and angles will be available on request to the authors.

Crystal Data

C_{21}H_{26}O_4N·CH_3OH · I, M.W. = 515.4. Orthorhombic, \( a = 7.578(1) \), \( b = 15.350(2) \), \( c = 19.406(4) \) Å, \( V = 2257.1(11) \) Å\(^3\), space group \( P2_12_12_1 \), \( Z = 4 \), \( R = 0.042 \), \( D_e = 1.517 \) g·cm\(^{-3}\), \( 0.33 \leq \alpha \leq 0.36 \leq 0.48 \) mm, \( R(Mo-K\alpha) = 14.32 \) cm\(^{-1}\).

References