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ISOLATION OF A NEW ALKALOID (−)-*O*-ACETYLBAPTIFOLINE AND THE ABSOLUTE STEREOCHEMICAL RELATIONSHIPS OF LUPINE ALKALOIDS IN *THERMOPSIS CHINENSIS*

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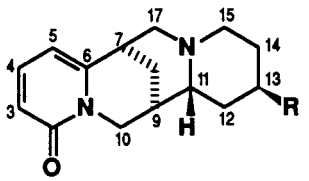
ABSTRACT.—A new lupine alkaloid, (−)-*O*-acetylbaptifoline [**3**], was isolated from the roots of *Thermopsis chinensis* together with seven known (−)-anagyrine-type (7*R*,9*R*,11*R*) alkaloids. The structure of **3** was confirmed by spectroscopic methods and by chemical transformations. We also isolated (+)-lupanine [**9**] and (−)-sparteine [**10**] (6*R*,7*S*,9*S*,11*S*) from this plant. These results indicate that *T. chinensis* has the biosynthetic ability to biosynthesize both (7*R*,9*R*,11*R*) and (7*S*,9*S*,11*S*) alkaloids.

Plants of the genus *Thermopsis* (Leguminosae) are known to contain a large amount of lupine alkaloids (1); they were occasionally used as sources of traditional oriental medicines (2). We have previously reported the isolation of seven lupine alkaloids from the aerial parts and roots of *Thermopsis chinensis* Benth. (3,4), an herbaceous plant distributed in the subtropical and temperate zones of eastern Asia.

In the present paper, we report the isolation and structural elucidation of a new alkaloid, (−)-*O*-acetylbaptifoline [**3**], and of (+)-lupanine [**9**] from *T. chinensis*. We also discuss the absolute stereochemical relationships of lupine alkaloids contained in *Thermopsis* species.

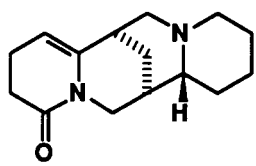
RESULTS AND DISCUSSION

From the 75% MeOH extract of the dry roots of *T. chinensis*, (−)-*O*-acetylbapti-



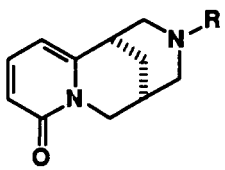
(7*R*,9*R*,11*R*)

1 R=H
2 R=OH
3 R=O-C(=O)-CH$_3$ (18 19)

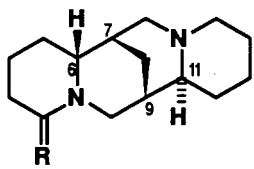


(7*R*,9*R*,11*R*)

4



5 R=H
6 R=Me
7 R=CHO
8 R=CH$_2$-CH$_2$-CH=CH$_2$



(6*R*,7*S*,9*S*,11*S*)

9 R=O
10 R=H$_2$

foline [**3**], a colorless oil, $[\alpha]_D$ −101°, was isolated in a yield of 0.005% of the dry wt by Si gel chromatography and by preparative hplc. The molecular formula of **3** was determined as $C_{17}H_{22}N_2O_3$ by hrms. The presence of an α-pyridone moiety in the molecule was indicated by the fragment ions at *m/z* 160 and 146 in the eims spectra and by the ^{1}H signals at δ 5.97 (1H, dd, J = 6.9 and 1.1 Hz, H-5), 6.44 (1H, dd, J = 9.1 and 1.1 Hz, H-3), 7.27 (1H, dd, J = 9.1 and 6.7 Hz, H-4), 3.91 (1H, dd, J = 15.4 and 6.6 Hz, H-10α) and 4.12 (1H, d, J = 15.4 Hz, H-10β). These data are characteristic for anagyrine-type alkaloids (3,4). The presence of an acetyl group at C-13 was suggested by the ^{13}C signals at δ 170.5 (s, C=O, C-18), 21.4 (q, -Me, C-19), and 69.2 (d, C-13) and by ir bands at 1740, 1245, and 1230 cm^{-1} (ester). In the ^{1}H-nmr spectrum, the signals at δ 2.08 (3H, s, -Me) and 5.20 (1H, t, J = 2.8 Hz, H-13α) also indicated the presence of an acetyl group at position 13β (axial). Thus, **3** was assumed to be *O*-acetylbaptifoline.

The final confirmation of the structure of **3** including absolute configuration was made by chemical interconversion between **2** and **3**. The compound **3** was easily hydrolyzed to (−)-baptifoline [**2**] with 1 N NaOH. Reversibly, **3** was synthesized from **2** by acetylation with Ac_2O and pyridine. In the cd spectrum, **3** showed a negative Cotton effect at 307 nm ($[\theta]_{307}$ −22000) and positive effects at 257 nm ($[\theta]_{257}$ +3200) and at 232 nm ($[\theta]_{232}$ +29000). These are the same Cotton effects as those of **2** ($[\theta]_{307}$ −20000, $[\theta]_{257}$ +2700, $[\theta]_{234}$ +21000). Furthermore, the synthetic **3** from **2** showed the same Cotton effects as those of natural **3**. These results confirmed that the absolute configuration of **3** is 7*R*, 9*R*, 11*R*, the same as that of (−)-baptifoline [**2**]. In the literature, the occurrence of **3** was briefly reported in a few species of *Anagyris* and *Baptisia* (5,6). However, these reports identified this alkaloid only tentatively and only by gc-ms. The present study is the first full characterization of **3** in a plant.

We also isolated from the dry roots of *T. chinensis* seven known (−)-anagyrine-type alkaloids, (−)-anagyrine [**1**], (−)-baptifoline [**2**], (+)-5,6-dehydrolupanine [**4**], (−)-cytisine [**5**], (−)-*N*-methylcytisine [**6**], (−)-*N*-formylcytisine [**7**], and (−)-rhombifoline [**8**]. They were identified by their physicochemical properties (mp, $[\alpha]_D$, ir, ms, ^{1}H nmr, ^{13}C nmr) and chromatographic behaviors (hplc and gc).

We have already found that *Thermopsis lupinoides* contains both (+)-lupanine-type (7*S*,9*S*,11*S*) and (−)-anagyrine-type (7*R*,9*R*,11*R*) alkaloids (7,8). The alkaloids of these two types have opposite absolute configurations. To prove that this is also the case in *T. chinensis*, we have determined the absolute configuration of lupanine and sparteine isolated from *T. chinensis*. Lupanine [**9**] was isolated from the fresh leaves collected in July, the content of **9** being highest in this season. The purified **9** had the value of $[\alpha]_D$ +54.0° and gave a positive Cotton effect, $[\theta]_{223}$ +15000. These results indicate that (+)-lupanine [**9**] (6*R*,7*S*,9*S*,11*S*) was produced in *T. chinensis* as well as in *T. lupinoides*. (−)-Sparteine [**10**] (6*R*,7*S*,9*S*,11*S*) was additionally isolated from the roots of *T. chinensis*.

Okuda *et al.* (9) have generalized from their extensive stereochemical studies of lupine alkaloids that the alkaloids having the same absolute configuration of the methylene bridge (C-7 and C-9) occur together in a plant and that (7*S*,9*S*) and (7*R*,9*R*) alkaloids generally do not. They also pointed out a few exceptional cases of these generalizations; for example, *Lupinus caudatus* and *Spartium junceum*. Our present findings show that plants in the genus *Thermopsis* produce both (7*S*,9*S*) and (7*R*,9*R*) alkaloids and thus constitute an exceptional case. The α-pyridone-type alkaloids such as **1–8** are always in (7*R*,9*R*) series in every plant species examined. However, the alkaloids, sparteine, lupanine, and related compounds, are in both antipodal configurations. It is interesting to clarify the biosynthetic relations between the (7*S*,9*S*) and (7*R*,9*R*) alkaloids in *Thermopsis* plants.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The high and low resolution eims were measured at 70 eV. ^{1}H-nmr and ^{13}C-nmr spectra were recorded at 500 and 67.8 MHz, respectively. TMS was used as internal standard in $CDCl_3$. Tlc was carried out on Si gel plates in CH_2Cl_2-MeOH-28% NH_4OH (90:9:1). Analytical hplc was performed as described previously (10). Preparative hplc was performed on a Licrosorb SI-100 5 μm, (0.8 × 50 cm) column with monitoring by a uv detector at 220 or 310 nm.

EXTRACTION AND ISOLATION OF **3**.—The roots of *T. chinensis* were collected in early June in Okinoerabu island in Kagoshima prefecture, Japan. The voucher specimen has been deposited in the Herbarium of Faculty of Pharmaceutical Sciences, Chiba University, Japan. The total alkaloidal fraction from 75% MeOH extracts of the dry roots was obtained in yields of 0.6% of the dry wt by the method reported previously (3). The mixture of bases (5.0 g) was chromatographed on Si gel column (Merck, type 60, 230–400 mesh, 130 g, 2.5 × 54 cm) with the solvent systems of 3% MeOH in CH_2Cl_2-28% NH_4OH (500:1) and 5% MeOH in CH_2Cl_2-28% NH_4OH (500:2). The **3**-rich fractions (250 mg) were eluted together with **1** and **6**. The pure **3** (45 mg) was obtained from these rich fractions by preparative hplc with 4% MeOH in CH_2Cl_2-2.5% NH_4OH (500:1).

(−)-*O*-ACETYLBAPTIFOLINE [**3**].—Colorless oil, $[\alpha]^{15}D$ −101° (c = 0.74, EtOH); hrms m/z (%) $[M]^+$ 302.1632 (42) (calcd for $C_{17}H_{22}N_2O_3$, 302.1631), 243 (40), 160 (12), 146 (23), 96 (100); ir ν max (CCl_4) cm^{-1} 2800 (Bohlmann band), 1660 (pyridone C=O), 1740, 1245, 1230 (ester); ^{1}H nmr δ 7.27 (1H, dd, J = 9.1, 6.7, H-4), 6.44 (1H, dd, J = 9.1, 1.1, H-3), 5.97 (1H, dd, J = 6.9, 1.1, H-5), 5.20 (1H, t, J = 2.8, H-13α), 4.12 (1H, d, J = 15.4, H-10β), 3.91 (1H, dd, J = 15.4, 6.6, H-10α), 3.32 (2H, m, H-17β), 3.10 (1H, ddd, J = 14.3, 14.3, 2.8, H-15α), 2.49 (2H, m, H-17α), 2.08 (3H, s, -Ac); ^{13}C nmr 163.5 (s, C-2), 116.8 (d, C-3), 138.7 (d, C-4), 104.5 (d, C-5), 151.5 (s, C-6), 35.3 (d, C-7), 22.8 (t, C-8), 31.8 (d, C-9), 48.4 (t, C-10), 56.6 (d, C-11), 26.2 (t, C-12), 69.2 (d, C-13), 20.6 (t, C-14), 51.4 (t, C-15), 52.2 (t, C-17), 170.5 (s, C-18), 21.4 (q, C-19); cd (c = 0.00046, MeOH) $[\theta]^{20}$ (nm) −22000 (307) (negative maximum), +3200 (257), +29000 (232) (positive maximum).

HYDROLYSIS OF **3** TO **2**.—Compound **3** (5 mg) was hydrolyzed with 1 N NaOH at room temperature for 1.0 h. Preparative hplc separation of the products gave **2** with a yield of 88% (4 mg).

SYNTHESIS OF **3** FROM **2**.—(−)-Baptifoline [**2**] (110 mg) was dissolved in 5 ml of pyridine. Ac_2O (4 ml) was added gradually to the solution. After stirring for 24 h at room temperature the products were extracted and purified by cc on Si gel with the solvent system CH_2Cl_2-MeOH-28% NH_4OH (100:0.5:0.1). Compound **3**, $[\alpha]^{20}D$ −92.0° (c = 0.78, EtOH), was obtained with a yield of 78% (100 mg).

(+)-LUPANINE [**9**].—Compound **9**, an oil, $[\alpha]^{24}D$ +54.0° (c = 0.04, EtOH), was isolated by repeated chromatography as described (7) in a yield of 0.07% of the fresh wt from the total alkaloidal fraction obtained from the leaves of *T. chinensis* harvested in July. Compound **9** was identified with the authentic compound (7) by eims, ^{1}H nmr, hplc, and gc.

ISOLATION AND IDENTIFICATION OF OTHER KNOWN ALKALOIDS.—The total alkaloidal fractions (10 g, 0.6% of the dry wt) from the roots of *T. chinensis* were chromatographed on a Si gel column with the solvent system of CH_2Cl_2-MeOH-28% NH_4OH as described previously (4,7,8) to yield the compounds as follows: (−)-anagyrine [**1**] (137 mg), oil, $[\alpha]D$ −163° (c = 0.84, EtOH); (−)-baptifoline [**2**] (70 mg), mp 208, $[\alpha]D$ −138° (c = 0.70, EtOH); (+)-5,6-dehydrolupanine [**4**] (8 mg), oil, $[\alpha]D$ +34.1° (c = 0.08, EtOH); (−)-cytisine [**5**] (2.1 g), mp 153°, $[\alpha]D$ −115° (c = 1.57, EtOH); (−)-*N*-methylcytisine [**6**] (38 mg) mp 137°, $[\alpha]D$ −221° (c = 0.38, EtOH); (−)-*N*-formylcytisine [**7**] (93 mg), oil, $[\alpha]D$ −229° (c = 0.92, EtOH); (−)-rhombifoline [**8**] (10 mg), oil, $[\alpha]D$ −216° (c = 0.10, EtOH); (−)-sparteine [**10**] (112 mg), oil, $[\alpha]D$ −16.9° (c = 1.12, EtOH). These alkaloids were identified with the authentic compounds by eims, ir, ^{1}H nmr, ^{13}C nmr, hplc, and gc.

ACKNOWLEDGMENTS

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ERRATA

The authors have requested the following corrections for the paper entitled "New Methods of Analyzing Tannins," *J. Nat. Prod.*, **52**, 1 (1989):

Page 9, (A) and (B) in Figure 5 should read: (A) in the shade (4 h); (B) under direct sunshine (3 days).

Page 12, line 49: (a) and (b) of "hemiacetal form (a) and *gem*-diol form (b)" should be deleted.

Page 15, line 5: The mass numbers of $[M + Na]^+$ ion peaks in the fabms of chebulinic acid [**26**] and chebulagic acid [**27**] should be *m/z* 979 and 977, respectively.

Page 22: in the spectrum of gemin D in Figure 10, the carbon number of the signal at δ 71.3 should be β-4, not β-3 as shown.

Page 26: The compound number of the cd spectra in Figure 12 should be (*R* -48 (solid line) and (*S*)-48 (dotted line).

The authors have requested the following correction for the paper entitled, "Synthesis of (±)-Neorautane," *J. Nat. Prod.*, **52**, 502 (1989). The nomenclature for compound **4** should read as 8,8-dimethyl-2,3,6,7-tetrahydro-4*H*,8*H*-benzo-(1,2*b*:5,4*b*')-dipyran-4-one.