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(−)-3β,13α-DIHYDROXYLUPANINE FROM *CYTISUS SCOPARIUS*

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Key Word Index—*Cytisus scoparius*; Leguminosae; lupin alkaloid; quinolizidine alkaloid; (−)-3β,13α-dihydroxylupanine; (+)-lupanine; (+)-13-hydroxylupanine; (−)-sparteine; tyramine; variation of alkaloid content.

Abstract—A new lupin alkaloid, (−)-3β,13α-dihydroxylupanine was isolated from *Cytisus scoparius* together with five known sparteine-type lupin alkaloids and tyramine. The absolute structure of the new alkaloid was confirmed by comparison of the natural product with the synthetic sample derived from (+)-13-hydroxylupanine. It was also shown that the alkaloid constituents of *C. scoparius* differed considerably in the aerial parts, flowers and seeds.

INTRODUCTION

Cytisus scoparius is a deciduous shrub native to Europe and is grown widely in Japan as an ornamental plant. The plant accumulates both sparteine-type lupin alkaloids, such as (−)-sparteine, (+)-lupanine, (+)-13-hydroxylupanine, etc., and phenethylamines, such as tyramine, hydroxytyramine, epinine and salsolidine, as the basic constituents [1]. The major alkaloid (−)-sparteine has been used as an oxytocic drug and also is of medical interest because of its antiarrhythmic effect [2]. In the course of our investigations on the lupin alkaloids of leguminous plants mainly growing in Japan [3–5], we have studied the alkaloidal constituents in the various organs of *C. scoparius*. A new lupin alkaloid (**1**) was isolated from the mature seeds, together with five known sparteine-type lupin alkaloids and tyramine. This paper describes the structural determination of the new alkaloid (**1**) as (−)-3β, 13α-dihydroxylupanine and the distribution of the alkaloids in fresh flowers, seeds, leaves and stems of this plant.

RESULTS AND DISCUSSION

The freshly harvested aerial parts of *C. scoparius* which were collected in April (flowering period) were separated into leaves, stems and flowers. The seeds were collected in August. Each part was treated as described previously [6] to give alkaloid mixtures in yields of 0.10, 0.20, 0.21 and 0.59% fr. wt, respectively. The alkaloid mixtures obtained were separated by repeated silica gel column chromatography followed by prep. HPLC. Table 1 shows the alkaloids isolated from the various parts.

The new alkaloid (**1**) was isolated from the seeds in a yield of 0.18% fr. wt, together with (−)-sparteine, (+)-lupanine, (+)-13-hydroxylupanine and (−)-17-oxosparteine. Compound **1**, $[\alpha]_D^{15}$ −7.4° (ethanol), mp 194–196°, formed colourless needles from dichloromethane–*n*-hexane. The molecular formula was determined by high resolution mass spectrometry as $C_{15}H_{24}N_2O_3$ ($[M]^+$ *m/z* 280.1778, calc. 280.1788). Its mass spectrum ($[M]^+$ *m/z* 280, 96%) showed fragment ions at *m/z* 263 (32), 262 (34) and 245 (8) corresponding to

Table 1. Distribution of lupin alkaloids and tyramine in various parts of *Cytisus scoparius*

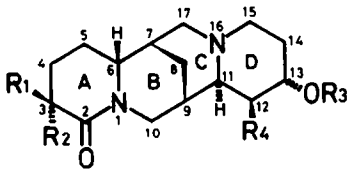
Alkaloids	Alkaloid content*			
	Leaves	Stems	Flowers	Seeds
(−)-Sparteine	0.15	0.075	0.004	0.03
α-Isosparteine†	tr	tr	—	—
(−)-17-Oxosparteine	0.015	0.004	—	0.008
(+)-Lupanine	0.004	0.002	—	0.20
(+)-13-Hydroxylupanine (**2**)	0.006	tr	—	0.11
(−)-13β,13α-Dihydroxylupanine (**1**)	—	—	—	0.18
Tyramine	0.016	0.013	0.13	—
Total alkaloids	0.10	0.20	0.21	0.59

*Alkaloid content were estimated by HPLC. Percent by weight of fresh plant material.

†Optical rotation has not been obtained due to the shortage of material.
tr, Trace.

$[M-OH]^+$, $[M-H_2O]^+$ and $[M-H_2O-OH]^+$, respectively, indicative of the presence of two hydroxyl groups in the molecule. The other main fragment ions at *m/z* 165 (43), 152 (100) and 134 (35) were very similar to those of 13-hydroxylupanine (**2**) [7] but not those of calpurmenine, 12,13-dihydroxylupanine (**6**) which exhibited significant fragment ions at *m/z* 168, 150, 132 and 112 [8]. The ^{1}H NMR spectrum ($CDCl_3$) of **1** also resembled that of 13-hydroxylupanine (**2**): **1**, δ4.30 (1H, *dt*, J = 13 and 2.5 Hz, 10α-H), 3.35 (1H, *m*, 6-H), 4.10 (1H, *quintet*, J = 3 Hz, 13β-H); **2**, δ4.49 (1H, *dt*, J = 13 and 2.5 Hz, 10α-H), 3.28 (1H, *m*, 6-H), 4.08 (1H, *quintet*, J = 3 Hz, 13β-H). The spectrum of **1** exhibited one more isolated signal at δ4.02 (1H, *dd*, J = 11.5 and 5.5 Hz) which was assigned to an axial proton on a methine carbon bearing a hydroxyl group because of its low chemical shift and coupling characteristics. These results suggested that **1** is a hydroxyl derivative of 13-hydroxylupanine (**2**) in which the second hydroxyl group was on either ring A or ring B and oriented equatorially.

In the ^{13}C NMR spectrum of **1**, the signals corresponding to C-6–C-17 on rings B, C and D were all coincident with those of **2** within 1 ppm differences (Table 2). The remaining ^{13}C NMR signals at δ68.2 (*d*), 26.3 (*t*) and 24.5 (*t*) were assigned by considering the substituent effects of a hydroxyl group on the basis of the ^{13}C NMR assignments of **2**. This result indicates that the most reasonable position of the hydroxyl group is C-3. Therefore, the new alkaloid was presumed to be (−)-3β,13α-dihydroxylupanine (**1**).



1 R_1 = OH, R_2 = R_3 = R_4 = H
2 R_1 = R_2 = R_3 = R_4 = H
3 R_1 = R_3 = R_4 = H, R_2 = OH
4 R_1 = OAc, R_2 = R_4 = H, R_3 = Ac
5 R_1 = R_4 = H, R_2 = OAc, R_3 = Ac
6 R_1 = R_2 = R_3 = H, R_4 = OH

Table 2. ^{13}C NMR spectra of (−)-3β,13α-dihydroxylupanine (**1**) and (+)-13-hydroxylupanine (**2**) in $CDCl_3$

Carbon No.	1	2*	Δδ(1 − 2)
2	173.8 (*s*)		
3	68.2 (*d*)	32.9 (*d*)	+35.3
4	26.3 (*t*)	19.6 (*t*)	+6.7
5	24.5 (*t*)	26.6 (*t*)	−2.1
6	61.6 (*d*)	60.8 (*d*)	+0.8
7	33.9 (*d*)	34.2 (*d*)	−0.3
8	27.4 (*t*)	27.3 (*t*)	+0.1
9	32.2 (*d*)	31.6 (*d*)	+0.6
10	47.8 (*t*)	46.8 (*t*)	+1.0
11	57.3 (*d*)	57.0 (*d*)	+0.3
12	39.6 (*t*)	39.9 (*t*)	−0.3
13	64.3 (*d*)	64.0 (*d*)	+0.3
14	31.5 (*t*)	32.4 (*t*)	−0.9
15	49.3 (*t*)	49.2 (*t*)	+0.1
17	52.3 (*t*)	52.4 (*t*)	−0.1

*Data from ref. [10].

The structure of **1** including absolute stereochemistry was determined by comparison of the natural product with a synthetic sample obtained by hydroxylation of (+)-13-hydroxylupanine (**2**) isolated from the same plant. An application of Wasserman's method for α-hydroxylation of tert. amides [9] to (+)-13α-hydroxylupanine (**2**) gave a mixture of diastereoisomeric 3β,13α-dihydroxylupanine (**1**) and 3α,13α-dihydroxylupanine (**3**), which were difficult to separate. The mixture was acetylated and then separated by silica gel column chromatography to yield the corresponding two diacetoxylupanines **4** and **5**. One of the two diastereomers agreed with (−)-3β,13α-diacetoxylupanine (**4**) derived from the natural product **1** (^{1}H NMR, IR, mass spectrometry and $[\alpha]_D$). The ^{1}H NMR spectrum of **4** was very similar to that of the other diacetoxylupanine (**5**) except for the signal due to the acetoxymethine proton at the 3-position. The acetoxymethine proton signal in **4** was observed at δ5.33 as a double doublet (J = 11 and 5.5 Hz) having coupling characteristics similar to that of the original dihydroxylupanine **1**, while the methine proton signal in **5** resonated at δ5.28 as a multiplet with $W_{h/2}$ = 8 Hz. This indicates that the orientation of the acetoxymethine proton 3-H is axial in **4** and equatorial in **5**, which is consistent with the above stereochemical assignment of **1**. Thus, the structure of the new base was determined to be (−)-3β,13α-dihydroxylupanine (**1**) (3*S*:6*R*:7*S*:9*S*:11*S*:13*S*).

The distribution of the lupin alkaloids and tyramine in the leaves, stems, flowers and seeds of *C. scoparius* is listed in Table 1. (−)-Sparteine comprised over 70% of the alkaloid mixture in the leaves and stems, while it was a minor constituent in the flowers and seeds. In the flowers, the phenethylamine-type alkaloid tyramine was a major component and only one lupin alkaloid, sparteine, was found as a very minor constituent. The seeds were a rich source of the oxidized sparteine-type alkaloids such as (+)-lupanine, (+)-13-hydroxylupanine (**2**), (−)-3β,13α-dihydroxylupanine (**1**) and (−)-17-oxosparteine. Thus, the alkaloid constituents and their distribution in *C. scoparius* was different in the aerial parts, flowers and seeds, like those in *Sophora franchetiana* [11], *S. tomentosa* [12], *S. flavescens* [6, 13], *S. mollis* [14], *Echinosophora koreensis* [15, 16], *Thermopsis lupinoides* [4] and *Sophora chrysophylla* [3] reported recently.

EXPERIMENTAL

General. Mps were uncorr. High and low resolution MS were measured at 70 eV using a direct inlet system. ^{1}H NMR (100 and 270 MHz) and ^{13}C NMR (25 MHz) spectra were recorded using TMS as int. standard. TLC were carried out on silica gel 60 plates (0.25 mm). Analytical and prep. HPLC were performed on LiChrosorb SI 100 (Merck, 10 μm, 0.3 × 50 cm for analytical and 0.8 × 50 cm for preparative) column, using a UV detector.

Plant material. Cytisus scoparius Link. was identified by Prof. I. Murakoshi, Faculty of Pharmaceutical Sciences, Chiba University. Voucher specimens have been deposited in the

Herbarium of the Faculty of Pharmaceutical Sciences, Chiba University, Chiba, Japan.

Extraction and isolation of alkaloids. Aerial parts and seeds of *C. scoparius* were collected at the suburb of Chiba-city in April (flowering period) and in August, respectively, and the former was separated into leaves, stems and flowers. Fresh plant material was homogenized in 80% EtOH and extracted with the same solvent several times at room temp. The EtOH extracts obtained from the leaves, stems, flowers and seeds were treated as described previously [3, 6, 11–16] to give crude alkaloid mixtures in yields of 0.10, 0.20, 0.21 and 0.59% fr. wt, respectively. (i) *Isolation of alkaloids from leaves.* Alkaloid mixture (4.6 g) was applied to a silica gel (Merck, 70–230 mesh, 420 g) column and developed successively with CH_2Cl_2 (1 l.), CH_2Cl_2–MeOH–28% NH_4OH (90:9:1, 2 l.), CH_2Cl_2–MeOH–28% NH_4OH (43:6:1, 2 l.) and MeOH (1 l.), 15 ml fractions being collected. Prep. HPLC separation of fractions 4–8 (142 mg) with 15% MeOH in Et_2O–2.5% NH_4OH (50:1) gave (−)-17-oxosparteine [112 mg, mp 83°, $[\alpha]_D^{20}$ −19.3° (*c* 0.25; EtOH)]. Fractions 144–166 (33 mg) were separated by prep. HPLC with 15% MeOH in Et_2O–H_2O–25% NH_4OH (500:10:3) to yield (+)-lupanine [25 mg, oil, $[\alpha]_D^{12}$ +62.8° (*c* 1.66; Me_2CO)]. Prep. HPLC separation of fractions 167–218 (163 mg) using 15% MeOH in Et_2O–H_2O–25% NH_4OH (500:20:15) gave α-isosparteine (2 mg, mp 101–105°), which was identical with a synthetic sample derived from (−)-sparteine [17, 18] (co-TLC, co-HPLC, IR, MS). Fractions 219–234 (145 mg) were subjected to prep. HPLC with 25% MeOH in Et_2O–H_2O–25% NH_4OH (500:20:15) to yield (+)-13-hydroxylupanine [**2**, 90 mg, oil, $[\alpha]_D^{20}$ +45.6° (*c* 0.20; EtOH)]. Tyramine (163 mg, mp 164–165°) was obtained from fractions 251–275 (176 mg) by prep. HPLC purification using 15% MeOH in Et_2O–H_2O–25% NH_4OH (500:10:3). Purification of the MeOH fraction (2.69 g) by silica gel CC (Merck, 70–230 mesh, 300 g) with 15% MeOH in Et_2O–H_2O–25% NH_4OH (500:20:15) gave 2.3 g of (−)-sparteine (oil, $[\alpha]_D^{33}$ −35.9° (*c* 0.29; Me_2CO). (ii) *Isolation of alkaloids from stems.* Alkaloid mixture (2.5 g) was separated by silica gel CC as described above to give (−)-17-oxosparteine (96 mg), (+)-lupanine (47 mg), α-isosparteine (3 mg), (+)-13-hydroxylupanine (5 mg), tyramine (303 mg) and (−)-sparteine (1.7 g) in that order of elution. (iii) *Isolation of alkaloids from flowers.* Alkaloid mixture (865 mg) was subjected to silica gel CC (Merck, 70–230 mesh, 100 g) using CH_2Cl_2–MeOH–28% NH_4OH (90:9:1) to yield tyramine (253 mg) and (−)-sparteine (6.5 mg), in that order of elution. (iv) *Isolation of alkaloids from seeds.* Alkaloid mixture (2.2 g) was applied to a silica gel column (Merck, 230–400 mesh, 150 g) and developed with Et_2O–MeOH–28% NH_4OH (40:2:1), 40 ml fractions being collected. The first effluent (fractions 7–10, 148 mg) of the column was a mixed fraction of two alkaloids, which were separated by prep. HPLC using 15% MeOH in Et_2O–H_2O–25% NH_4OH (500:10:3) to give (−)-17-oxosparteine (21 mg) and (−)-sparteine (93 mg). (+)-Lupanine (0.70 g), (+)-13-hydroxylupanine (**2**, 0.41 g) and the new alkaloid (−)-3β,13α-dihydroxylupanine (**1**, 0.63 g) were eluted as an almost pure form in fractions 13–25, fractions 40–53 and fractions 58–80, respectively.

Compound **1**. Colourless needles from CH_2Cl_2–*n*-hexane, mp 194–196°, $[\alpha]_D^{15}$ −7.4 (*c* 0.59; EtOH). MS *m/z* (rel. int.): 280.1778 ($[M]^+$, calc. for $C_{15}H_{24}N_2O_3$, 280.1788, 96), 263 $[M-OH]^+$ (32), 262 $[M-H_2O]^+$ (34), 245 $[M-H_2O-OH]^+$ (8), 166 (37), 165 (43), 152 (100), 134 (35).

Diacetylderivative **4**. Colourless oil, $[\alpha]_D^{18}$ −16.6° (*c* 0.69; EtOH); MS *m/z* (rel. int.): 365 $[M+1]^+$ (3), 364 $[M]^+$ (8), 363 $[M-1]^+$ (5), 322 (24), 321 (25), 305 (21), 245 (28), 244 (84), 146 (40), 132 (100). ^{1}H NMR (100 MHz, $CDCl_3$): δ5.33 (1H, *dd*, *J* = 11 and 5.5 Hz, 3α-H), 5.03 (1H, *quintet*, *J* = 3 Hz, 13β-H), 4.37 (1H, *dt*, *J* = 11.5 and 2 Hz, 10α-H), 3.33 (1H, *m*, 6β-H), 2.17 (3H, *s*, Ac), 2.08 (3H, *s*, Ac).

Hydroxylation of (+)-*13-hydroxylupanine* (**2**). To a soln of diisopropylamine (48.5 mg, 0.48 mmol) in THF (1 ml) was added 0.3 ml (0.48 mmol) of 1.6 M *n*-BuLi in hexane at 0° with stirring. After several min, a soln of **2** (52.8 mg, 0.20 mmol) in THF (0.5 ml) was added to the LDA soln at 0° and stirred for 15 min. The reaction mixture was allowed to pass through dry oxygen at 0°.

The solvent was removed from the reaction mixture *in vacuo* and an aq. soln of Na_2SO_3 was added to the residue. The reaction mixture was stirred for 10 min, made alkaline with K_2CO_3 and extracted with CH_2Cl_2. The extract was dried over K_2CO_3 and evaporated *in vacuo*. The residue was subjected to silica gel CC (Merck, 230–400 mesh, 40 g) to yield a mixture (25 mg) of the dihydroxylupanines **1** and **3** and the starting material (**2**, 17 mg). The mixed fraction of **1** and **2** was acetylated with pyridine–Ac_2O at 20–25° and the mixture of the diacetyl derivatives obtained separated by prep. HPLC using 4% MeOH in Et_2O–25% NH_4OH (500:2) to give **4** (15 mg) and **5** (7 mg); compound **4**: colourless oil, $[\alpha]_D^{18}$ −16.2° (*c* 0.72; EtOH), identical to the diacetylcompound (**4**) derived from natural **1** (HPLC, TLC, ^{1}H NMR, MS); compound **5**: colourless crystals from *n*-hexane, mp 182°, $[\alpha]_D^{18}$ +34.7° (*c* 0.15; EtOH), MS *m/z* (rel. int.): 365 $[M+1]^+$ (4), 364 $[M]^+$ (5), 363 $[M-1]^+$ (3), 321 (15), 305 (17), 245 (18), 244 (70), 146 (36), 132 (100). ^{1}H NMR (100 MHz, $CDCl_3$): δ5.28 (1H, *m*, $W_{h/2}$ = 8 Hz, 3β-H), 5.06 (1H, *quintet*, *J* = 3 Hz, 13β-H), 4.54 (1H, *dt*, *J* = 13 and 2.5 Hz, 10α-H), 3.40 (1H, *m*, 6-H), 2.14 (3H, *s*, Ac), 2.09 (3H, *s*, Ac).

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