Phytochemistry, Vol 26, No 1, pp 301–303, 1987
Printed in Great Britain

0031 9422/87 $3.00 + 0.00

STEROIDAL ALKALOID GLYCOSIDES FROM *LILIUM CORDATUM*

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(*Revised received* 27 *June* 1986)

Key Word Index *Lilium cordatum*; Liliaceae; glycoalkaloid; 22,26-epiminocholestane-type steroidal alkaloid; cordatine A; cordatine B.

Abstract—Two new steroidal alkaloid glycosides named cordatine A and B were isolated from the petals of *Lilium cordatum*. Their structures were elucidated by spectral data as (25*R*)- and (25*S*)-22,26-epimino-5α-cholest-22(*N*)-en-3β,6β-diol *O*(3)-β-D-glucopyranoside.

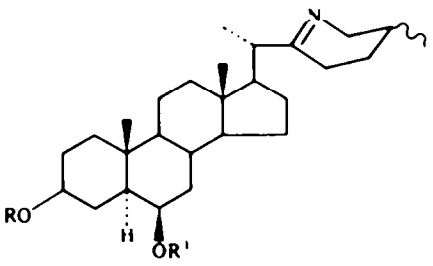
INTRODUCTION

The petals of *Lilium cordatum* (Thunb.) Koidz. (Liliaceae) are used as a folk medicine in a mountain region of Kochi prefecture. However, no detailed chemical investigation appears to have been done on this plant. We now report the isolation and structure elucidation of two new steroidal glycoalkaloids named cordatine A and B, the structures of which were established by spectral data.

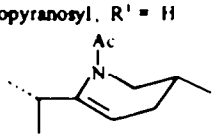
RESULTS AND DISCUSSION

The methanolic extract of the petals of this plant, on repeated chromatographic purification, gave two new steroidal alkaloids, cordatine A and B.

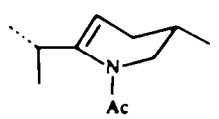
Cordatine A (**1**) showed absorptions in the IR spectrum due to an azomethine function [1] (1650 cm^{-1}) as well as the hydroxyl groups (3500 cm^{-1}). Acidic hydrolysis of **1** yielded both the aglycone (**3**) and glucose (on TLC). The EIMS spectrum of **3** showed a molecular ion peak at *m/z* 415.3475 (calcd for $C_{27}H_{45}NO_2$; 415.3500) suggesting that **3** should be a steroidal alkaloid, and two fragments at *m/z* 125.1209 (calcd for $C_8H_{15}N$; 125.1214) and 111.1054 (base peak, calcd for $C_7H_{13}N$; 111.1060) which are typical for a $\Delta^{22(N)}$-unsaturated side chain moiety [2, 3] of **3**.



1 (25*R*), R = β-D glucopyranosyl, R' = H
3 (25*R*), R = R' = H
3a (25*R*), R = R' = Ac,



2 (25*S*), R = β-D-glucopyranosyl, R' = H
4 (25*S*), R = R' = H
4a (25*S*), R = R' = Ac



Since the ^{13}C NMR spectrum of **3** exhibited a signal at δ55.7 (doublet) assignable to C-9 [4, 5], the configuration of the A/B ring junction in **3** was determined to be 5α. The ^{13}C NMR chemical data of **3** were compared with those of solacongestidine isolated from *Solanum congestiflorum* (Solanaceae) [6]. Thus, compound **3** showed two signals at δ71.4 (*d*), one of which should be assigned to C-3, and four methyl carbon signals at δ12.3 (C-18), 16.1 (C-19), 17.8 (C-21) and 18.1 (C-27). The C-19 signal shifted downfield (3.8 ppm) compared with that of solacongestidine because of a 1,3-diaxial interaction [7] with a β-axial hydroxyl group at C-2, C-4, C-6 or C-11. Since the signals ascribable to C-5, C-6, C-7 and C-8 disappeared, the remaining signal at δ71.4 could be assigned to C-6 by comparison with the chemical shifts of 6β-hydroxyandrostanol [4].

The ^1H NMR spectrum of **3** showed two singlets (3H each) at δ0.78 and 1.03, indicating the presence of C-18 and C-19 angular methyl groups, two doublets (3H each, J = 7 Hz) at 0.76 and 1.00 attributable to two secondary methyl groups at C-27 and C-21, two multiplets centred at 3.50 (1H, $W_{1/2}$ = 20 Hz) and 3.60 (1H, $W_{1/2}$ = 7 Hz) which could be assigned to the α-hydrogen adjacent to the β-hydroxyl groups at C-3 and C-6 (both signals shifted downfield to δ4.70 and 4.92 on acetylation, respectively, and these signals did not change by decoupling).

The configuration of C-25 was assumed to be 25*R* because of a positive Cotton effect at λ_{max} 255 nm [8]. Accordingly, the structure of the aglycone of cordatine A is regarded as (25*R*)-22,26-epimino-5α-cholest-22(*N*)-en-3β,6β-diol (**3**).

The FAB-MS spectrum of **1** gave two ion peaks at *m/z* 578 $[M+H]^+$ and 600 $[M+Na]^+$. Thus, cordatine A was considered to be a monoglucoside. The ^{13}C NMR spectrum of **1** exhibited a significant glycosylation shift [9, 10] on the C-3 signal of the aglycone (**3**). It also gave signals assigned to a β-D-glucopyranosyl moiety.

Based on the above-mentioned evidence, the structure of cordatine A was determined to be (25*R*)-22,26-epimino-5α-cholest-22(*N*)-en-3β,6β-diol *O*(3)-β-D-glucopyranoside (**1**).

Cordatine B (**2**) showed an IR spectrum exhibiting azomethine (1650 cm^{-1}) and strong hydroxyl (3500 cm^{-1}) absorptions.

The FAB-MS spectrum gave the highest ion peak at *m/z* 600 which was ascribable to $[M+Na]^+$. Acid hydrolysis of **2** yielded the aglycone (**4**) and glucose (on TLC).

The EIMS spectrum of **4** showed peaks at *m/z* 415.3480 (calcd for $C_{27}H_{45}NO_2$; 415.3510), 125.1202 (calcd for $C_8H_{15}N$; 125.1202) and 111.1065 (base peak, calcd for $C_7H_{13}N$; 111.1082) identical with that of **3**. The ^1H NMR spectrum of **4a** exhibited a similar pattern to that of **3a** except for the signals between δ 2.8 and 3.6 due to the C-26 methylene. Thus, **3a** showed 26-H_2 signals at δ 2.89 (*t*, *J* = 11 Hz) and 3.67 (*dd*, *J* = 2, 11 Hz), whereas **4a** exhibited them at 3.22 (*dd*, *J* = 7, 11 Hz) and 3.51 (*dd*, *J* = 4, 11 Hz). Moreover, compound **4** was observed to have a negative Cotton effect at λ_{max} 255 nm. Accordingly, compound **4** was assumed to be the 25*S* isomer of **3**.

Table 1. ^{13}C NMR chemical shifts of compound **3**, cordatine A (**1**) and cordatine B (**2**) in pyridine-d_5

Carbon No.		Compound 3	Cordatine A (1)	Cordatine B (2)
Aglycone	1	38.5	38.6	38.9
	2	32.5	30.1	30.2
	3	71.4	77.8	77.8
	4	36.9	32.8	32.8
	5	48.4	47.9	47.9
	6	71.4	71.0	71.0
	7	39.2	39.0	40.3
	8	31.0	31.0	31.0
	9	53.9	53.9	53.9
	10	36.0	36.1	36.0
	11	21.3	21.4	21.4
	12	40.6	40.8	40.7
	13	42.8	43.0	42.9
	14	56.0	57.2	56.4
	15	24.1	24.4	24.7
	16	27.4	27.6	27.7
	17	54.8	54.8	54.8
	18	12.3	12.1	12.4
	19	16.1	16.1	16.0
	20	48.4	47.9	47.9
	21	17.8	18.2	18.6
	22	173.4	173.5	174.8
	23	26.9	26.7	30.2
	24	27.2	28.0	28.1
	25	27.4	28.3	27.7
	26	55.7	56.1	56.9
	27	18.1	19.6	19.3
C-3 glc	1′		102.0	102.1
	2′		75.3	75.3
	3′		78.5*	78.5*
	4′		71.8	71.9
	5′		78.6*	78.4*
	6′		63.0	63.0

*Assignments may be interchanged in each column.

The signals in the ^{13}C NMR spectrum of **2** were assigned as shown in Table 1. Consequently, cordatine B was shown to be (25*S*)-22,26-epimino-5α-cholest-22(*N*)-en-3β,6β-diol *O*(3)-β-D-glucopyranoside (**2**).

Cordatine A and B are the first compounds to be isolated from a natural source which are 6β-hydroxy-22,26-epiminocholestane derivatives.

EXPERIMENTAL

Extraction and separation of cordatine A and B. The fresh petals (7.7 kg) from flowers of *Lilium cordatum* (Thunb.) Koidz. which were collected during August in Kamiyama (Tokushima) were extracted 3× with hot MeOH for 6 hr and the extract was concentrated *in vacuo*. The residue (284 g) was partitioned between BuOH and H_2O. The BuOH layer was concentrated under red. pres. to afford a brown powder (58 g), which was subjected to CC over silica gel using $CHCl_3$–MeOH–H_2O (9:1:0.1) followed by Sephadex LH-20 CC using 50% MeOH to give cordatine A (**1**, 275 mg) and cordatine B (**2**, 235 mg).

Cordatine A (**1**). R_f 0.41 ($CHCl_3$–MeOH–H_2O, 7:3:0.5), pale red powder, $[\alpha]_D^{29}$ −3.5° (MeOH; *c* 0.85), Dragendorff reagent positive, IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1650 (C=N). FAB-MS (*m/z*): 600 $[M+Na]^+$, 578 $[M+H]^+$. EIMS (*m/z*): 577 $[M]^+$, 415 $[C_{27}H_{45}NO_2]^+$, 400, 151, 125 $[C_8H_{15}N]^+$, 111 $[C_7H_{13}N]^+$ (base peak). ^1H NMR (pyridine-d_5): δ 0.77 (3H, *s*, 18-H_3), 0.84 (*d*, *J* = 7 Hz, 27-H_3), 0.89 (3H, *d*, *J* = 7 Hz, 21-H_3), 1.02 (3H, *s*, 19-H_3).

Hydrolysis of compound **1**. A soln of **1** (100 mg) in 2 N HCl–MeOH was refluxed for 2 hr. The reaction mixture was neutralized with 3% KOH–MeOH and evaporated to dryness under red. pres. The residue was subjected to Sephadex LH-20 CC using MeOH to give an aglycone **3** (27 mg) pale red powder, $[\alpha]_D^{27}$ +19.8° (MeOH; *c* 0.97), ORD (MeOH; *c* 0.11): $[M]_{255}^{25}$ +545°, IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1650 (C=N). EIMS (*m/z*): 415 $[M]^+$ ($C_{27}H_{45}NO_2$), 400, 125 $[C_8H_{15}N]^+$, 111 $[C_7H_{13}N]^+$. ^1H NMR (CD_3OD): δ 0.76 (3H, *d*, *J* = 6 Hz, 27-H_3), 0.78 (3H, *s*, 18-H_3), 1.00 (3H, *d*, *J* = 6 Hz, 21-H_3), 1.03 (3H, *s*, 19-H_3), 2.95–3.13 (2H, *m*, 26-H_2), 3.50 (1H, *m*, 3-H), 3.60 (1H, *m*, 6-H), and methyl glucoside, R_f 0.31 ($CHCl_3$–MeOH–H_2O, 7:3:0.5).

Acetylation of compound **3**. Compound **3** (19 mg) was acetylated with Ac_2O–pyridine (each 1 ml) for 24 hr at room temp. The reaction mixture was poured into ice-water and the ppt. was collected by filtration, dried and purified by CC on silica gel using hexane–Me_2CO (3:1) to afford a triacetate (**3a**, 4 mg), pale red powder, $[\alpha]_D^{25}$ −15.9° (dioxane; *c* 0.11). EIMS (*m/z*): 541 $[M]^+$, 167, 125. ^1H NMR ($CDCl_3$, 30°): δ 0.61 (3H, *s*, 18-H_3), 0.98 (3H, *d*, *J* = 7 Hz, 27-H_3), 1.00 (3H, *s*, 19-H_3), 1.14 (3H, *d*, *J* = 7 Hz, 21-H_3), 2.02, 2.04 (each 3H, *s*, OAc), 2.16 (3H, *s*, NAc), 2.89 (1H, *t*, *J* = 11 Hz, 26-H_a), 3.67 (1H, *dd*, *J* = 2, 11 Hz, 26-H_e), 4.72 (1H, *m*, $W_{1/2}$ = 20 Hz, 3-H), 4.92 (1H, *br s*, 6-H), 5.13 (1H, *br s*, 23-H).

Cordatine B (**2**). R_f 0.37 ($CHCl_3$–MeOH–H_2O, 7:3:0.5), pale red needles, mp 187–190°, $[\alpha]_D^{29}$ 11.2° (MeOH; *c* 1.07). Dragendorff reagent positive, IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1650 (C=N). FAB-MS (*m/z*): 600 $[M+Na]^+$, 578 $[M+H]^+$. EIMS (*m/z*): 577 $[M]^+$, 415, 400, 151, 125 $[C_8H_{15}N]^+$, 111 $[C_7H_{13}N]^+$ (base peak).

Hydrolysis of compound **2**. Compound **2** (100 mg) was hydrolysed in the same way as **1** to afford an aglycone (**4**, 28 mg), pale red powder, $[\alpha]_D^{27}$ +11.0° (MeOH; *c* 0.91), ORD (MeOH; *c* 0.04) $[M]_{255}^{25}$ −5.0°, IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1650 (C=N). EIMS (*m/z*): 415 $[M]^+$ ($C_{27}H_{45}NO_2$), 400, 125 $[C_8H_{15}N]^+$, 111 $[C_7H_{13}N]^+$, and methyl glucoside.

Acetylation of compound **4**. Compound **4** (10 mg) was acetylated in the same manner as **3** to afford a triacetate (**4a**, 5 mg), pale red powder, $[\alpha]_D^{25}$ + 33.3° (dioxane; *c* 0.15), EIMS (*m/z*): 541 $[M]^+$. ^{1}H NMR ($CDCl_3$): δ0.70 (3H, *s*, 18-H_3), 0.93 (3H, *d*, *J* = 7 Hz, 27-H_3), 1.02 (3H, *s*, 19-H_3), 1.13 (3H, *d*, *J* = 7 Hz, 21-H_3), 2.02, 2.04 (each 3H, *s*, OAc), 2.16 (3H, *s*, NAc), 3.22 (1H, *dd*, *J* = 7, 11 Hz, 26-H_a), 3.51 (1H, *dd*, *J* = 4, 11 Hz, 26-H_e), 4.70 (1H, *m*, $W_{1/2}$ = 20 Hz, 3-H), 4.93 (1H, *br s*, 6-H), 5.16 (1H, *t*, *J* = 2 Hz, 23-H).

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