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STEROIDAL ALKALOIDS FROM *SOLANUM CAPSICASTRUM**

CHUN-NAN LIN, MEI-ING CHUNG and SON-YUAN LIN

School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan 80731, Republic of China

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Key Word Index—*Solanum capsicastrum*; Solanaceae; steroidal alkaloid glycoside; 22,26-epiminocholestene; etioline; isoteinemine; capsicastrine; (22*R*,25*S*)-*O*(3)-β-D-galactopyranosyl-22,26-epiminocholest-5-ene-3β,16α-diol.

Abstract—From the root bark of *Solanum capsicastrum*, in addition to etioline and isoteinemine, a new 22,26-epiminocholestene glycoside named capsicastrine was isolated and its structure elucidated as isoteinemine *O*(3)-β-D-galactopyranoside by physical and chemical methods.

INTRODUCTION

According to the literature [1, 2], Formosan *Solanum* plants have been used as a folk medicine in Taiwan. While searching for the active constituents of Formosan *Solanum* plants, we have isolated a new steroidal alkaloid glycoside, capsicastrine (1), and two known steroidal alkaloids, etioline (3) and isoteinemine (5), from the root bark of *Solanum capsicastrum* Link. This plant was investigated previously by Schreiber and Ripperger [3] and solanocapsine was isolated. To our knowledge, this is the first report of a *Solanum* species that contains etioline (3) and isoteinemine (5) in a non-glycosylated form. The isolation of etioline (3) and isoteinemine (5) from natural sources by hydrolysis of glycoside mixtures has been reported [4, 5].

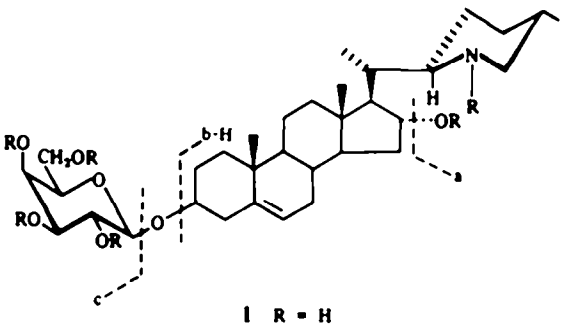
Although various data on etioline (3) and isoteinemine (5) were available, no detailed ^{13}C NMR data appeared to have been reported. In this paper, we report the structure elucidation of capsicastrine as 1 by spectral data. We also deal with the application of ^{13}C NMR spectroscopy to the characterization of etioline (3) and isoteinemine (5).

RESULTS AND DISCUSSION

Al_2O_3 chromatography of the tertiary base fraction obtained from the ethanol extracts of root bark yielded the new glycoside, capsicastrine (1), etioline (3) and isoteinemine (5).

Compound 1, $C_{33}H_{55}NO_7$, recrystallized from acetone as colourless needles, mp 220–221°. Its IR spectrum (KBr) showed the presence of hydroxyl groups at 3300 cm^{-1}, but no absorption of C=N was observed at 1660–1640 cm^{-1}. Acidic hydrolysis of 1 yielded isoteinemine (5) and galactose, the latter being detected by TLC.

The electron-impact (EI) mass spectrum of 1 showed no molecular ion but an $[M-H_2O]^+$ peak (559) besides typical ions at *m/z* 560 $[M-OH]^+$ 398 $[b-H]^+$, 397 $[M-galactose]^+$, 380 $[M-galactose-OH]^+$ and 18 $[a+H]^+$. A base peak at *m/z* 98, the same as that of isoteinemine (5) [5], appeared as the result of C-20–C-22



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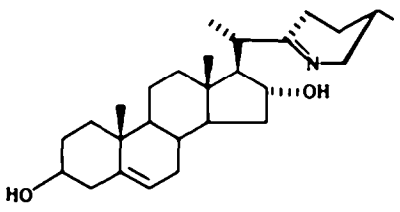
2 R = Ac



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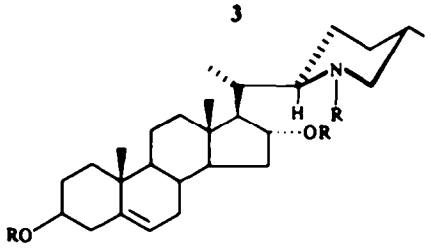

5 R = H
6 R = Ac

* Part II in the series "Studies on the Constituents of Formosan *Solanum* Species". For Part I see Hsu, P.-M. and Tien, H.-J. (1974) *J. Taiwan Pharm. Assoc.* **26**, 28.

bond fission [7]. The ion at m/z 162 [c]$^+$ indicates the sugar moiety [8].

The monoglycosidic nature of **1** was also confirmed by its ^{13}C NMR data (Table 1), in which all 33 carbon atoms were assigned by means of H^1-decoupling and H^1-coupling spectra and comparison with the published data of related compounds.

The shift values of the ring A and ring B carbon atoms were in agreement with the corresponding data of the aglycone of cholesteryl β-D-galactopyranoside and havanine [9, 10]. A characteristic signal shift is observed at the C-3 position of the hydroxyl group in which the glycosylation takes place [11]. The shift values for C-11 to C-27 corresponded very well to those of the ring C, D and F carbon atoms of isoteinemine (Table 1). These correlations indicated clearly that the galactosyl function is located at the 3β-position, whereas a hydroxyl group is present at C-16α.

Table 1. ^{13}C NMR chemical shift values and assignments ($CDCl_3$, TMS as internal standard)*

Carbon No.	1	2*	3	5
C-1	37.4	37.2	37.1	37.1
C-2	29.6	28.8	31.3	31.6
C-3	78.6	79.4	71.4	71.6
C-4	38.6	37.4	42.2	42.3
C-5	142.0	140.5	140.3	140.3
C-6	121.1	122.5	121.3	121.4
C-7	32.1	32.1	31.7	31.6
C-8	31.8	31.8	31.6	31.6
C-9	50.6	50.1	49.9	49.8
C-10	36.8	36.8	36.5	36.4
C-11	20.8	21.2	20.8	20.8
C-12	40.0	41.0	40.0	40.0
C-13	43.3	44.0	43.9	44.9
C-14	54.1	54.1	53.4	54.4
C-15	35.2	35.0	35.1	35.3
C-16	74.7	79.6	76.5	75.0
C-17	64.7	55.3	63.4	64.3
C-18	13.3	13.6	13.8	13.3
C-19	19.4	19.3	19.4	19.4
C-20	38.8	39.3	44.4	39.7
C-21	19.4	19.3	18.9	19.6
C-22	64.7	53.9	177.1	64.6
C-23	32.2	31.4	29.7	27.0†
C-24	33.4	31.6	27.9	34.3
C-25	32.0	31.1	27.3	26.8†
C-26	53.7	53.7	55.7	53.5
C-27	19.4	19.3	19.1	19.4
C-1′	101.4	99.9		
C-2′	74.8	71.0		
C-3′	75.0	72.2		
C-4′	71.2	69.2		
C-5′	77.9	73.6		
C-6′	62.3	62.5		
$\underline{C}H_3CO$		20.6		
Me$\underline{C}$O		169.7		
		169.8		
		170.3		
		170.5		

*See refs. [9, 10, 12].
†Interchangeable.

Acetylation of **1** afforded **2**, an *O,N*-hexaacetate, $C_{45}H_6 \cdot NO_{13}$, showing a base peak at m/z 140, a molecular ion peak at m/z 829 and sugar fragments at m/z 169 and 331, respectively, in the EI mass spectrum. The ^1H NMR spectrum of **2** showed two singlets (3H each) at δ0.94 and 1.22 for C-18 and C-19 angular methyl groups, two doublets (3H each, J = 7.0 Hz) at δ0.90 and 1.05 corresponding to two secondary methyl groups at C-21 and C-27 or C-27 and C-21, signals at δ1.96, 1.98 and 2.04 corresponding to five acetyl groups, a singlet at δ2.10 corresponding to the N Ac group, a doublet at δ4.55 (J = 7.5 Hz) corresponding to the α-hydrogen at C-3 and an unresolved triplet at δ5.06 and a broad singlet at δ5.28 corresponding to the β-hydrogen at C-16 and the vinylic H-6, respectively. A doublet at δ4.15 (J = 7.5 Hz) for the anomeric H-1 sugar proton indicated the β-configuration of the galactosidic linkage [10]. The IR spectrum of **2** showed absorption bands at 1730 (OAc) and 1640 cm^{-1} (N Ac).

All these data suggested the glycoalkaloid capsicastrine to be (22*R*,25*S*)-22,26-epiminocholest-5-ene-3β,16α-diol *O*(3)-β-D-galactopyranoside (**1**). The ^{13}C NMR spectrum of **2** (Table 1) also supports the structure elucidated as **1**.

In the ^{13}C NMR spectrum of **3** (Table 1), the shift values for C-1 to C-10 and C-19 corresponded very well to those of C-1 to C-10 and C-19 of (25*R*)-22,26-epiminocholest-5,22(*N*)-diene-3β-ol (25-isoverazine) and C-11 to C-18 and C-20 to C-27 corresponded very well to those of C-11 to C-18 and C-20 to C-27 of (25*S*)-22,26-epimino-5α-cholest-22(*N*)-ene-3β,16α-diol (25-isosolafloridine) [12]. The above data suggested **3** to be (25*S*)-22,26-epiminocholest-5,22(*N*)-diene-3β,16α-diol (**3**) (etioline), and **3** was identical with authentic etioline (**3**) by mmp, TLC and IR spectral comparison.

In the ^{13}C NMR spectrum of **5**, the shift values for C-1 to C-12 and C-19 corresponded very well to those of the corresponding carbons of **3** and C-13 to C-18 and C-20 to C-27 corresponded very well to those of the same carbons of (22*R*,25*S*)-22,26-epimino-5α-cholestane-3β,16α-diol [11, 12]. Reduction of **3** with sodium borohydride in methanol yielded a product, mp 226 [13], identical with **5** by mmp and IR spectral ($CHCl_3$) comparison.

All these data suggested **5** to be isoteinemine [(22*R*,25*S*)-22,26-epiminocholest-5-ene-3β,6α-diol] (**5**) [5, 6].

EXPERIMENTAL

All mps are uncorr.

Extraction and separation. Air-dried root bark of *Solanum capsicastrum* (10 kg) was collected at Tainan, Taiwan, in March 1970, and chipped and extracted several times with EtOH. The combined EtOH extracts were concentrated to dryness under red. pres. and the residue was dissolved in 3% HOAc. The 3% HOAc dissolved fraction was extracted with $CHCl_3$ and the $CHCl_3$ extracts were chromatographed on Al_2O_3. The column was eluted with $CHCl_3$ to afford **3** from the former fraction. The latter fraction, eluted with $CHCl_3$, was rechromatographed on Al_2O_3 and the Et_2O-C_6H_6 (4:1) eluted fraction was recrystallized from MeOH to give **5**. After the acid phase was extracted with $CHCl_3$, it was made alkaline with NH_3 and extracted with $CHCl_3$. The Me_2CO-insoluble part of the former $CHCl_3$ extracts was separated by TLC on silica gel (Merck) (cyclohexane EtOAc MeOH, 1:1:2). The spot at R_f 0.7 was rechromatographed on Fractogel (Merck) to yield **1**.

Capsicastrine (1). Colourless needles (Me_2CO), mp 220–221°, $[\alpha]_D^{21}$ −25.5° (*c* 0.1; $CHCl_3$). (Found: C, 68.55; H, 9.63; N, 2.51. $C_{33}H_{55}NO_5$ requires: C, 69.60; H, 9.59; N, 2.42%.) MS *m/z* (rel. int.): 560 (17), 559 (29), 398 (9), 397 (18), 380 (13), 98 (100) and 18 (35). IR ν_{max} cm^{-1}: 3300 (OH), 1090 and 1065. Further spectral data are given in the text.

Hydrolysis of 1. Compound 1 (100 mg) was refluxed for 6 hr with 10 ml 2 M HCl–MeOH, the mixture was cooled, MeOH was evaporated, and the residue was dissolved with H_2O, made alkaline with NH_3 and extracted with $CHCl_3$. The $CHCl_3$ extract was chromatographed on Al_2O_3 (Merck). Elution with Et_2O–C_6H_6 (4:1) gave isoteinemine (5), mp 220°, $[\alpha]_D^{25}$ −54° (*c* 0.5; $CHCl_3$), identical with an authentic sample. The aq. soln was neutralized with 3% KOH–MeOH. The product was examined by TLC (solvent $CHCl_3$–MeOH–Me_2CO–H_2O, 3:3:3:1) to detect methyl galactopyranoside (R_f 0.54).

Capsicastrine acetate (2). Acetylation of 1 in the usual manner gave a colourless powder (C_6H_6), mp 139–140°. (Found: C, 65.05; H, 8.19; N, 1.83. $C_{45}H_{69}NO_{13}$ requires: C, 65.12; H, 8.14; N, 1.69%.) MS *m/z* (rel. int.): 829 [M]$^+$ (2), 331 (16), 169 (35) and 140 (100). Further spectral data are given in the text.

Etioline (3). Colourless needles (Me_2CO), mp 149–151°, $[\alpha]_D^{24}$ −4.26° (*c* 0.94; $CHCl_3$). (Found: C, 77.95; H, 10.40; N, 3.14. $C_{27}H_{43}NO_2$ requires: C, 78.40; H, 10.48; N, 3.39%.) MS *m/z* (rel. int.): 413 [M]$^+$ (3), 394 (9), 385 (2), 380 (16), 163 (8), 162 (24), 148 (17), 138 (53), 125 (100), 98 (60). IR ν_{max}^{KBr} cm^{-1}: 3350, 1650 (C=N), 1050. ^{1}H NMR ($CDCl_3$): δ0.72 (3H, *s*, 18-H_3), 0.92 (3H, *d*, *J* = 6 Hz, 27-H_3), 1.01 (3H, *s*, 19-H_3), 1.12 (3H, *d*, *J* = 6 Hz, 21-H_3), 3.5–4.0 (2H, *m*, 3α-H and 16β-H), 5.36 (1H, *br s*, H-6). Further spectral data are given in the text. The compound was identified as etioline (3) by mmp and comparison of its IR spectrum ($CHCl_3$) with that of an authentic sample.

Etioline acetate (4). Acetylation of 1 in the usual manner gave colourless needles, mp 197–198°. MS *m/z* (rel. int.): 539 [M]$^+$ (10), 496 (5), 479 (11), 464 (20), 436 (12), 205 (24), 204 (16), 167 (28), 166 (46), 165 (42), 163 (21), 162 (16), 150 (20), 43 (52), 28 (100). IR ν_{max}^{KBr} cm^{-1}: 1640 (C=C–N–Ac), 1735 (O–Ac). ^{1}H NMR ($CDCl_3$): δ1.99 (3H, *s*, OAc), 2.01 (3H, *s*, O–Ac), 2.13 (3H, *s*, N–Ac), 5.13 and 5.35 (2 × olefinic protons).

Isoteinemine (5). Colourless needles (MeOH), mp 226°, $[\alpha]_D^{25}$ −54° (*c* 0.5; $CHCl_3$). (Found: C, 77.67; H, 10.79; N, 3.59. $C_{27}H_{45}NO_2$ requires: C, 78.09; H, 10.92; N, 3.37%.) MS *m/z* (rel. int.): 415 [M]$^+$ (1), 140 (6), 99 (8), 98 (100). IR ν_{max}^{KBr} cm^{-1}: 1050, 3300. ^{1}H NMR ($CDCl_3$): δ0.73 (3H, *s*, 18-H_3), 0.81 (3H, *d*, *J* = 6 Hz, 27-H_3), 0.99 (3H, *s*, 19-H_3), 1.04 (3H, *d*, *J* = 6 Hz, 21-H_3), 3.52 (1H, *m*, 3α-H), 4.05 (1H, *m*, 16β-H), 5.35 (1H, *d*, *J* = 6 Hz, H-6). Further spectral data are given in the text. The compound was identified as isoteinemine (5) by mmp and comparison of its IR spectrum ($CHCl_3$) with that of the reduction product of 3.

Isoteinemine acetate (6). Acetylation of 3 in the usual manner gave colourless needles, mp 178°. (Found: C, 73.96; H, 9.49; N, 2.59. $C_{33}H_{51}NO_5$ requires: C, 73.27; H, 9.44; N, 2.70%.) MS *m/z* (rel. int.): 541 [M]$^+$ (1), 140 (100), 99 (5), 98 (43), 43 (9). IR ν_{max}^{KBr} cm^{-1}: 1735 (O–Ac), 1605 (N–Ac). ^{1}H NMR ($CDCl_3$): δ1.99 (3H, *s*, OAc), 2.03 (3H, *s*, O–Ac), 2.01 (3H, *s*, N–Ac).

Reduction of 3 *with sodium borohydride*. Compound 3 (100 mg) was dissolved in MeOH and $NaBH_4$ added. The soln was stirred at room temp. for 12 hr. NH_4OH soln was added and the aq. phase was extracted with $CHCl_3$. The washed, dried (Na_2SO_4) extract indicated two components by TLC (cyclohexane–EtOAc–MeOH, 1:1:2, on silica gel). On Al_2O_3 chromatography and recrystallization from MeOH it gave isoteinemine (5) as colourless needles, mp 226° (45 mg).

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