Phytochemistry, Vol. 28, No. 12, pp. 3507–3509, 1989.
Printed in Great Britain.

0031 9422/89 $3.00 + 0.00

7β-HYDROXYHYOSCYAMINE FROM *DUBOISIA MYOPOROIDES–D. LEICHHARDTII* HYBRID AND *HYOSCYAMUS ALBUS*

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(*Received in revised form* 27 *April* 1989)

Key Word Index—*Duboisia myoporoides*; *D. leichhardtii*; *Hyoscyamus albus*; Solanaceae; *Agrobacterium rhizogenes*; tropane alkaloid; 7β-hydroxyhyoscyamine; 6β-hydroxyhyoscyamine.

Abstract—A new tropane alkaloid, 7β-hydroxyhyoscyamine was isolated, together with 6β-hydroxyhyoscyamine, hyoscyamine, norhyoscyamine, scopolamine and tropine, from the leaves of *Duboisia* hybrid M-II-8-6 (crossbred between *D. myoporoides* and *D. leichhardtii*) and the hairy roots of *Hyoscyamus albus*.

INTRODUCTION

Solanaceous plants, regarded as rich sources of various alkaloids, have produced tropane alkaloids such as hyoscyamine (**3**), norhyoscyamine (**4**) and scopolamine (**5**). Recently 6β-hydroxyhyoscyamine (**2**) [1–3], a very interesting compound as an intermediate in the conversion of **3** to **5**, has been isolated from several species of Solanaceae [4–5]. But the existence of 7β-hydroxyhyoscyamine (**1**), the structural isomer of **2**, in nature, has not been reported yet. In the course of our systematic chemical studies on tropane alkaloids and tissue culture products of solanaceous plants [6], we have now isolated **1** together with **2**–**5** and tropine (**6**) from the leaves of *Duboisia* hybrid M-II-8-6 (crossbred between *D. myoporoides* and *D. leichhardtii*) and the hairy roots of *Hyoscyamus albus*. The structural assignment of **1** was confirmed unequivocally by the comparison of its physicochemical data with that of the authentic sample prepared from (−)-scopolamine HBr (**5'**).

RESULTS AND DISCUSSION

The dry leaves of *Duboisia* hybrid M-II-8-6 were extracted with methanol–28% aqueous ammonia (99:1) and the extract, after concentration, was poured into chloroform and extracted with 0.25 M sulphuric acid. The aqueous solution was adjusted to pH 10 with 28% aqueous ammonia and extracted with chloroform. The chloroform layer, after evaporation to dryness, was applied to a combination of Fuji-gel ODS–G3 and Bondapak C_{18} porasil B column chromatography and preparative TLC to afford compounds **1**–**6**.

The hairy roots of *Hyoscyamus albus* were induced by the leaf disc method co-cultured with *Agrobacterium rhizogenes* strain 15834 and cultured on Murashige and Skoog medium [7] containing antibiotic to remove the bacteria. The axenic hairy roots were maintained on Woody Plant (WP) medium [8] (pH 5.7) containing 3% sucrose without phytohormones. The hairy roots were transferred into hormone-free WP liquid medium (pH 5.7) containing 5% sucrose and cultured for one month. The hairy roots, after lyophilization, were extracted with chloroform–methanol–28% aqueous ammonia (15:5:1) and the extract, followed by a similar chromatographic separation as mentioned above, gave compounds **1**, **2** and **5**. Compounds **2**–**6** were identified as 6β-hydroxyhyoscyamine, hyoscyamine, norhyoscyamine, scopolamine and tropine, respectively, by comparisons of their physical and spectral data with those of authentic samples.

Compound **1** was positive to Dragendroff's reagent (a reddish brown colouration). The ^{1}H NMR spectrum of **1** (Table 1) showed one methyl (δ2.44), three methylene (δ1.33, 2.03; δ1.40, 2.11; δ1.73, 2.31) and four methine signals (δ2.80, 3.17, 3.70 and 5.00) whose chemical shifts and coupling constants were closely correlated to those of **2**. The ^{13}C NMR spectrum of **1** (Table 2), also similar to that of **2**, revealed the presence of a tropane diol skeleton (δ28.4, 30.1, 36.4, 40.2, 58.0, 66.6, 67.9, 75.5) and one phenylhydroxypropionic acid moiety (δ54.4, 64.0, 127.9, 128.1, 129.0, 135.5, 172.1). Furthermore, the FAB mass spectrum of **1** with the prominent $[M+H]^+$ peak at *m/z* 306 indicated the same molecular mass as that of **2**. From these spectral data, **1** was presumed to be the structural isomer of **2**.

Acid hydrolysis of **1** with 10% hydrochloric acid gave the hydrolysate (**1a**) and tropic acid (**7**). The ^{1}H NMR spectrum of **1a** was completely identical with that of 3α, 6β-dihydroxytropane HCl (**2a**) [9] which was prepared in a similar way from **2**. This spectral evidence indicated **1a** to be the enantiomer of **2a**, namely, 3α, 7β-dihydroxytropane HCl. This presumption was supported by the EI mass spectrum of **1a** with the prominent $[M-HCl]^+$ ion peak at *m/z* 157. The negative sign of $[\alpha]_D$ of **7** (−65.1°) indicated that the configuration of the C-2' position of **1** was the same as that of (−)-tropic acid.

The ^{1}H NMR spectrum of **1** (Table 1) revealed the H-3 signal shifted downfield at δ5.00, showing that the tropic acid moiety was linked to this position. A methine signal assignable to H-7, observed relatively upfield (δ3.70) as compared with the H-6 signal (δ4.31) in **2**, indicated that there is an anisotropic interaction between the C-7 proton and the substituent (tropic acid moiety).

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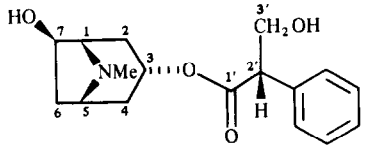
Final structural confirmation of **1** was obtained by preparing **1** from (−)-scopolamine HBr (**5′**) using Raney nickel (W4) in water at 20° at atmospheric pressure [10], which afforded **1** together with equal amounts of **2** and **3**. From the various spectroscopic and chemical evidence described above, **1** was characterized as 7β-hydroxyhyoscyamine. We have also demonstrated the production of **1** and **2**, which were very interesting compounds in the biosynthesis of tropane alkaloids, in the plants and in the tissue (adventitious root and/or hairy root) cultures of other solanaceous plants (*Scopolia lurida* and *S. tangutica* etc.) by means of the HPLC system (unpublished results).

EXPERIMENTAL

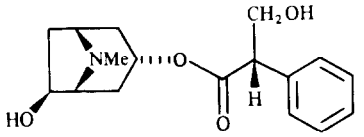
^1H and ^{13}CNMR spectra were measured at 270 and 67.5 MHz, respectively. TLC was conducted on silica gel and spots were visualized by spraying Dragendroff's reagent and 10% H_2SO_4.

Plant material. Duboisia hybrid M-II-8-6 and *Hyoscyamus albus*, were cultivated in Tsukuba Medicinal Plant Research Station. The leaves of *Duboisia* hybrid were collected in August 1988. The hairy roots of *Hyoscyamus albus* were induced by the leaf disc method co-cultured with *Agrobacterium rhizogenes* strain 15834 harboring Ri plasmid (pRi 15834) grown on YEB agar medium [11]. The opines (agropine and mannopine) of the hairy roots were extracted and detected by the method of ref. [12]. The hairy roots were cultured on hormone-free Murashige and Skoog medium containing antibiotic to remove the bacteria. The axenic hairy roots, thus obtained, were maintained on hormone-free WP medium containing 3% sucrose. The hairy roots were transferred into WP liquid medium (pH 5.7) containing 5% sucrose, without phytohormones, in the dark at 25° in 2 l air-lift type fermenters (×5) and harvested after 1 month of culture. Voucher specimens are deposited at the Herbarium of Breeding and Physiology Lab. in this Research Station.

Extraction and isolation. From the leaves of *Duboisia* hybrid M-II-8-6: dried leaves (940 g) of *Duboisia* hybrid M-II-8-6 were



1



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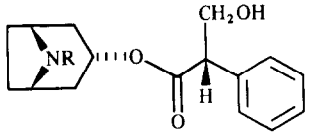
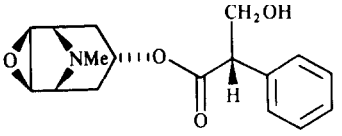


3 R = Me
4 R = H



5

6

Table 1. ^1H NMR spectral data of compounds **1**, **2**, **1a** and **2a** at 270 MHz (δ values)

H	1*	2*	1a†	2a†
1	2.80 (*br s*)	3.05 (*m*)	3.73 (*br s*)	4.02 (*m*)
2	1.33 (*br d*, *J* = 16.1)	1.20 (*br d*, *J* = 16.1)	2.06 (*br d*, *J* = 17.3)	1.90 (*br d*, *J* = 14.4)
	2.03 (*ddd*, *J* = 16.1, 4.6, 4.5)	2.01 (*dt*, *J* = 16.1, 4.5)	2.39 (*m*)	2.39 (*m*)
3	5.00 (*t*, *J* = 5.4)	5.01 (*t*, *J* = 5.4)	3.96 (*t*, *J* = 4.5)	3.96 (*t*, *J* = 4.5)
4	1.40 (*br d*, *J* = 16.1)	1.53 (*br d*, *J* = 16.1)	1.90 (*br d*, *J* = 14.4)	2.06 (*br d*, *J* = 17.3)
	2.11 (*dt*, J = 16.1, 4.5)	2.11 (*ddd*, *J* = 16.1, 4.6, 4.5)	2.39 (*m*)	2.39 (*m*)
5	3.17 (*m*)	2.92 (*br s*)	4.02 (*m*)	3.73 (*br s*)
6	1.73 (*br dd*, *J* = 13.6, 7.4)	4.31 (*dd*, *J* = 7.0, 2.4)	2.14 (*dt*, *J* = 13.6, 1.4)	4.93 (*dd*, *J* = 7.8, 2.9)
	2.31 (*dd*, *J* = 13.6, 7.0)		2.92 (*dd*, *J* = 13.6, 7.8)	
7	3.70 (*dd*, *J* = 7.0, 2.4)	1.55 (*br d*, *J* = 13.6)	4.93 (*dd*, *J* = 7.8, 2.9)	2.14 (*dt*, *J* = 13.6, 1.4)
		1.76 (*dd*, *J* = 7.0, 13.6)		2.92 (*dd*, *J* = 13.6, 7.8)
2′	3.79 (*m*)	3.78 (*m*)		
3′	3.79 (*m*)	3.78 (*m*)		
	4.16 (*dd*, *J* = 9.7, 7.6)	4.14 (*dd*, *J* = 9.7, 7.6)		
Ph	7.23–7.38 (5H, *m*)	7.22–7.36 (5H, *m*)		
N–Me	2.44 (3H, *s*)	2.43 (3H, *s*)	2.98 (3H, *s*)	2.98 (3H, *s*)

*In $CDCl_3$.
†In acetone-d_6 + D_2O.

mashed and extracted at room temp. with MeOH–28% NH_4OH (99:1) (7.3 l). The extract, after concn under red. pres. to *ca* 300 ml, was poured into $CHCl_3$ (900 ml) and extracted × 5 with 0.25 M H_2SO_4. The aq. soln was adjusted to pH 10 with 28% NH_4OH and extracted × 7 with $CHCl_3$. The $CHCl_3$ layer (dried with Na_2SO_4), after evapn to dryness, was applied to Fuji-gel ODS–G3 CC (20–40% MeOH) to afford **3** (*ca* 30 g) and the mixture of tropane alkaloids. The mixture was chromatographed over Bondapak C_{18} Porasil B [MeOH–10 mM Na 1-heptanesulphonate (pH 4 with HOAc) 3:17–1:3] to give **4** (1 g), **6** (2.3 g) and fractions 1 and 2. Fraction 1 was separated on prep. TLC ($CHCl_3$–Me_2CO–MeOH–28% NH_4OH 73:10:15:2) to give **1** (7 mg) and **5** (117 mg). Fraction 2 was chromatographed over Fuji-gel ODS–G3 (10–30% MeOH) to give **2** (50 mg) and **5** (550 mg). From the hairy roots of *Hyoscyamus albus*: lyophilized hairy roots (204 g) of *H. albus* were mashed and extracted × 2 with $CHCl_3$–MeOH–28% NH_4OH (15:5:1)(1 l and 0.8 l). The combined extract, followed by a similar chromatographic separation as mentioned above give **1** (9 mg), **2** (145 mg) and **5** (776 mg).

7β-Hydroxyhyoscyamine (**1**). Colourless needles (MeOH), mp 67° $[\alpha]_D^{25}$ +1.2° (Me_2CO; *c* 1.9), ^{1}H NMR: see Table 1, ^{13}C NMR: see Table 2, FABMS *m/z* (rel. int.): 306 $[M+H]^+$ (100), 140 (51), EIMS (high resolution) *m/z* (rel. int.): 305.1627 $[C_{17}H_{23}O_4N]^+$ (99), 261.1371 $[C_{15}H_{19}O_3N]^+$ (100).

Acid hydrolysis of **1**. A mixture of **1** (50 mg) and 10% HCl (3 ml) was heated (90°) for 17 hr. The reaction mixture, after cooling, was extracted with Et_2O. The aq. soln, after concn, was poured into Et_2O to give **1a** (33 mg). The Et_2O layer (dried with Na_2SO_4) was evapd to dryness and crystallized from H_2O to yield **7** (13 mg). Compound **7**: colourless needles, mp 123° (mp 126° for the authentic (−)-tropic acid purchased from Aldrich), $[\alpha]_D^{25}$ −65.1° (Me_2CO; *c* 0.22), ^{1}H NMR (Me_2CO–d_6) δ4.05 (1H, *t*, *J* = 8.5 Hz, H-3′), 4.66–4.78 (2H, *m*, H-3′, 2′), 7.21–7.34 (5H, *m*, Ph-H), ^{13}C NMR: see Table 2.

3α, *7β-Dihydroxytropane* HCl (**1a**). An off-white amorphous powder, $[\alpha]_D^{25}$ −1.8° (H_2O; *c* 1), ^{1}H NMR: see Table 1, ^{13}C NMR: see Table 2, EIMS *m/z* (rel. int.): 157 $[M-HCl]^+$ (21), 113 (100), 96 (63).

6β-Hydroxyhyoscyamine (**2**). An off-white amorphous powder, $[\alpha]_D^{25}$ −13.0° (Me_2CO; *c* 2.4), ^{1}H NMR: see Table 1, ^{13}C NMR: see Table 2, FABMS *m/z* (rel. int.): 306 $[M+H]^+$ (99), 140 (100), EIMS (high resolution) *m/z* (rel. int.): 305.1628 $[C_{17}H_{23}O_4N]^+$ (100), 261.1414 $[C_{15}H_{19}O_3N]^+$ (69).

Acid hydrolysis of **2**. A mixture of **2** (50 mg) and 10% HCl (3 ml) was treated as mentioned above to give **2a** (29 mg) and **7** (11 mg). Compound **7**: colourless needles, mp 123°, $[\alpha]_D^{25}$ −68.6° (Me_2CO; *c* 0.26).

3α, *6β-Dihydroxytropane* HCl (**2a**). An off-white amorphous powder, $[\alpha]_D^{25}$ −1.0° (H_2O; *c* 0.7), ^{1}H NMR: see Table 1, ^{13}C NMR: see Table 2, EIMS *m/z* (rel. int.): 157 $[M-HCl]^+$ (22), 113 (100), 96 (62).

Preparation of **1–3**. A solution of **5′** (1 g) in H_2O (20 ml) was treated with Raney Ni (W4) (1.5 g) at 20° for 19 hr with stirring under H_2. After removal of the catalyst by filtration, the filtrate was adjusted to pH 10 with 28% NH_4OH and extracted × 7 with $CHCl_3$. The $CHCl_3$ layer (dried with Na_2SO_4), after evapn to dryness, was applied to CC over Fuji-gel ODS-G3 (30%–40% MeOH) to afford **3** (290 mg) and the mixture of **1** and **2**. The mixture was separated on CC over Bondapak C_{18} Porasil B [MeOH–10 mM Na 1-heptanesulphonate (pH 4 with HOAc) 3:17–1:3] to afford **1** (150 mg) and **2** (150 mg).

Table 2. ^{13}C NMR spectral data of compounds **1**, **2**, **1a** and **7** at 67.5 MHz (δ values)

C	1*	2*	1a†	7‡
1	66.6	57.9	65.0	
2	28.4	29.8	37.4[a]	
3	67.9	67.9	72.3	
4	30.1	28.5	37.7[a]	
5	58.0	66.7	62.1	
6	40.2	75.8	41.4	
7	75.5	39.9	73.1	
1′	172.1	172.1		174.5
2′	54.4	54.4		55.7
3′	64.0	64.1		65.6
Ph	127.9	127.8		128.5
	128.1	128.1		129.7
	129.0	129.0		129.8
	135.5	135.5		138.4
N–Me	36.4	36.3	36.6	

*In $CDCl_3$.
†In acetone-d_6 + D_2O.
‡In acetone-d_6.
[a]Signals may be interchanged.

Acknowledgements—The authors thank Prof. S. Natori and Mr M. Hirose (Meiji College of Pharmacy) for the measurement of EIMS. They are also indebted to Dr Y. Yamakawa (Department of Agricultural Chemistry, University of Tokyo) for the measurement of FABMS. This work was supported in part by Special Cooperation Funds for Promoting Science and Technology (Basic Research Core System) from Science and Technology Agency, Japan.

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