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ALKALOIDS FROM *HYPECOUM LEPTOCARPUM*

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Abstract—Two new alkaloids, named isohyperectine and leptocarpine, were isolated from the whole plants of *Hypecoum leptocarpum*, along with the eight known alkaloids, dihydrosanguinarine, 8-acetonyldihydrosanguinarine, 8-methoxydihydrosanguinarine, oxohydrastinine, allocryptopine, protopine, hyperectine and corydamine. All structures were determined by spectroscopic methods.

INTRODUCTION

Hypecoum leptocarpum, a herbaceous plant growing in south west and north west China [1], is used in Tibetan medicine as an antipyretic, analgesic and anti-inflammatory remedy. It is employed in the treatment of colds, hepatitis, pneumonia, cholecystitis and food poisoning [2]. In previous studies of this species, several alkaloids were described [3, 4]. In the present investigation, besides the known alkaloids dihydrosanguinarine (**1**), 8-acetonyldihydrosanguinarine (**2**), 8-methoxydihydrosanguinarine (**3**), oxohydrastinine (**4**), allocryptopine (**5**), protopine (**6**), hyperectine (**7**) and corydamine (**10**), two new alkaloids, leptocarpine (**8**) and isohyperectine (**9**), have been identified by spectroscopy.

RESULTS AND DISCUSSION

The molecular formula $C_{21}H_{21}NO_6$ for leptocarpine (**8**) was established from the peak at *m/z* 383.1389 in its HR-EI mass spectrum. From its ^{1}H and ^{13}C NMR spectra, together with C,H-COSY and C,H-COLOC experiments, the following substructures were recognized. One phenyl ring bearing a methylèndioxy group appearing at δ5.80 (*s*, 2H, 2,3-OCH$_2$-O–) and two *para* protons at δ5.50 (*s*, 1H, 1-H) and δ6.50 (*s*, 1H, 4-H) (Ring A); another phenyl ring bearing two methoxy groups with δ3.90 (*s*, 3H, 2′-OMe) and δ3.88 (*s*, 3H, 3′-OMe), as well as two *ortho*-protons at δ7.25 (1H, *d*, J = 8 Hz, 4′-H) and δ7.70 (1H, *d*, J = 8 Hz, 5′-H) (ring D); a *N*-methyl group at δ3.00; two methine groups at δ6.55 (*s*, 1H, 9-H) and δ5.05 (*s*, 1H, 11-H); and a moiety –CH$_2$CH$_2$COO–, giving rise to signals at δ3.37 and 3.05 in the ^{1}H NMR spectrum, respectively. In the NOE difference experiments, effects were observed for the 11-H with the 5′-H, and the 9-H with the 2′-OMe. Interactions were also observed between the 9-H and the *N*-Me, and between the 11-H and the *N*-Me. Accordingly, the *N*-Me is situated between C(11)H and C(9)H, and C(11) and C(9) are attached to ring D. Thus, the ring C is assigned. Ring B was connected on the basis of NOE effects between the 1-H and 11-H, and from the NOE observed between the 5-H and 4-H. The following cross-signals in the C,H-COLOC experiment confirm further the structure of alkaloid **8**: C-4 (108.5 ppm), → 5-H (3.05 ppm); C-1 (102.1 ppm) and also C-5′ (112.2 ppm) → 11-H (5.05 ppm), as well as both the *N*-Me (40.2 ppm) and C-2′ (148.1 ppm) → 9-Me. This structure is a new skeleton with a nine-membered lactone. On the basis of the positive NOE effects between the *N*-Me and the 9-H, the 11-H and the *N*-Me, the relative configuration could be deduced as described in Fig. 1.

The mother liquor from the recrystallization of hyperectine (**7**) showed only one spot on TLC. Its mass and UV spectra were almost the same as those of hyperectine. However, in its ^{13}C NMR spectrum all signals were doubled. Therefore, this sample was a mixture of diastereomers. From the integrations in the ^{1}H NMR of the **7**/**9** complex, the ratio of **7**:**9** was estimated as *ca* 3:1. The mixture could neither be separated on TLC impregnated with Al_2O_3, nor with RP-18 or silica gel, and not even by using different eluants at different pH values. Compound **7** was identified as a mixture of enantiomers [5]. Because the **7**/**9** complex did not show any optical activity a mixture of two pairs of enantiomers, namely (±)-hyperectine (**7**) and its diastereomer (±)-isohyperectine (**9**), having the configurations 8*R*, 14*R* and 8*S*, 14*S*, appeared most probable.

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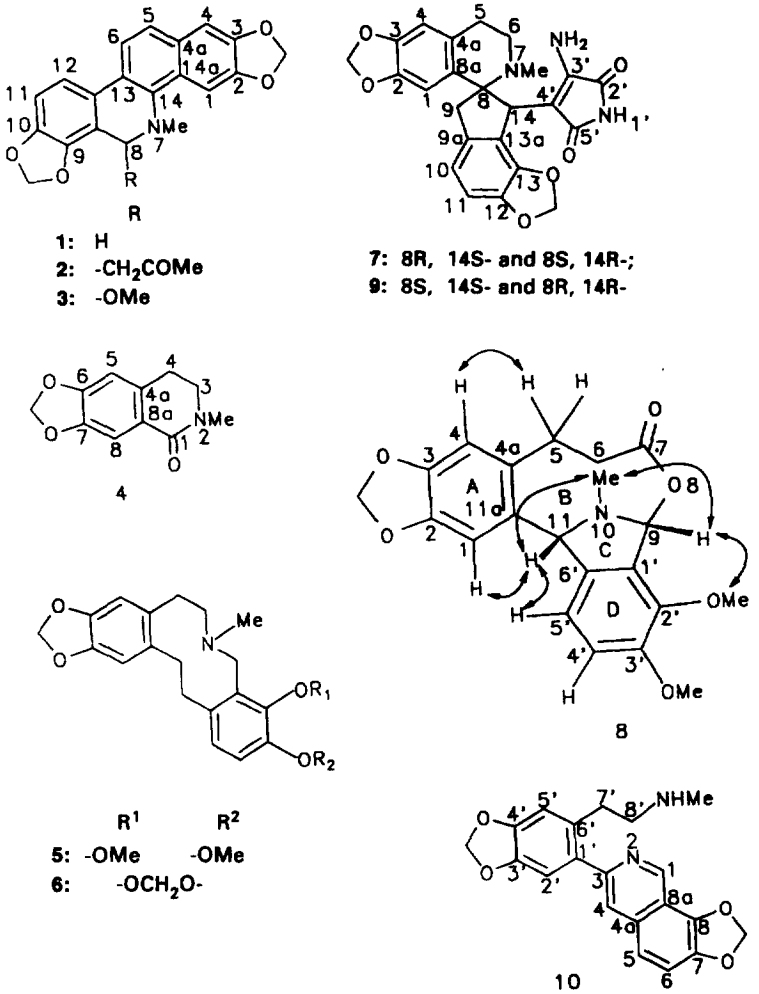

Fig. 1. Structures of alkaloids **1**–**10**.

EXPERIMENTAL

General. Mps: uncorr. NMR: 400 MHz for ^1H and 100 MHz for ^{13}C. MS: at 70 eV. CC: silica gel (Merck).

Plant material. Whole plants of *Hypecoum leptocarpum* Hook. f. et Thomas were collected in Rou Er Gai County (Sichuan Province, China), in September, 1990, and identified in the Chengdu Institute of Biology, Academia Sinica, where a voucher specimen is deposited.

Extraction. A sample of powdered dried whole plants (2.4 kg) was soaked in 95% EtOH (3 × 10 l). The EtOH extract was evapd under red. pres. The residue obtained was dissolved in EtOAc (2 l) and the EtOAc soln extracted with 5% aq. HCl (5 × 150 ml). The acidic soln was neutralized with 25% NH_3 to pH 9–10 and exhaustively extracted with $CHCl_3$. After drying (Na_2SO_4), the comb. $CHCl_3$ extracts were evapd to yield an alkaloid extract (14.7 g).

Isolation. The alkaloid extract was divided into 4 frs by CC with $CHCl_3$–MeOH (40:1). Fr. 1 was subjected to further CC ($CHCl_3$–petrol, 1:2) to yield **1** (20 mg) and **2** (50 mg), as well as the subfrs A and B. From subfr. A, alkaloids **3** (30 mg) and **4** (120 mg) were obtained by CC ($CHCl_3$–MeOH, 20:1). By purification of subfr. B by CC (CH_2Cl_2–EtOAc, 4:1), alkaloid **8** (80 mg) was obtained. Fr. 2 was sepd into 2 subfrs. C and D. Subfr. C was purified by CC (Al_2O_3, $CHCl_3$–MeOH–Et_2O, 40:4:1) and recrystallized from MeOH to obtain **7** (7 mg). Its mother liquor contained **7** and **9** (together 11 mg). Alkaloids **5** (100 mg) and **6** (1.1 g) were isolated from subfr. D by CC ($CHCl_3$–MeOH, 5:1). Alkaloid 10 was obtained by CC purification of fr. 3 (CH_2Cl_2–MeOH–Me_2CO, 4:1:1).

Dihydrosanguinarine (**1**). Amorphous powder. Mp 190–191° ($CHCl_3$–MeOH) (lit.: 189–190° [6]. UV, IR [6], ^1H NMR and MS identical to those reported [7]. ^{13}C NMR ($CDCl_3$): δ100.1 (C-1), 147.6 (C-2), 148.2 (C-3), 104.4 (C-4), 130.9 (C-4a), 124.0 (C-5), 120.4 (C-6), 41.7 (*N*-Me), 48.5 (C-8), 113.7 (C-8a), 144.7 (C-9), 147.2 (C-10), 107.3 (C-11), 116.3 (C-12), 127.3 (C-12a), 124.5 (C-13), 142.6 (C-14), 126.6 (C-14a), 101.4 and 101.1 (2 × $-OCH_2O-$).

8-*Acetonyldihydrosanguinarine* (**2**). Needles ($CHCl_3$–MeOH). Mp 195–197° UV, IR, ^{1}H NMR and MS same as those reported [8].

8-*Methoxyldihydrosanguinarine* (**3**). Mp, UV, and MS [9]. ^{1}H NMR ($CDCl_3$): δ7.70 (*s*, 1H, 1-H), 7.13 (*s*, 1H, 4-H), 7.49 (1H, *d*, J = 9 Hz, 5-H), 7.77 (1H, *s*, J = 9 Hz, 6-H), 2.70 (3H, *s*, *N*-Me), 5.40 (1H, *s*, 8-H), 3.45 (3H, *s*, 8-OMe), 6.94 (1H, *d*, J = 8 Hz, 11-H), 7.42 (1H, *d*, 12-H), 6.13 and 6.07 (each 1H, *d*, J = 1 Hz, $-OCH_2O-$), 6.06 and 6.04 (each 1H, *d*, J = 1 Hz, $-OCH_2O-$). ^{13}C NMR ($CDCl_3$): δ100.70 (C-1), 148.0 (C-2), 147.5 (C-3), 104.7 (C-4), 130.2 (C-4a), 123.8 (C-5), 120.3 (C-6), 41.0 (*N*-Me), 85.9 (C-8), 54.5 (8-OMe), 109.0 (C-8a), 144.0 (C-9), 147.3 (C-10), 108.9 (C-11), 116.4 (C-12), 125.0 (C-12a), 122.8 (C-13), 138.4 (C-14), 126.8 (C-14a), 109.9 and 100.6 ($2\times -OCH_2O-$).

Oxohydrastinine (**4**). Plates ($CHCl_3$). Mp 90–93° (lit.: 98–100° [2]). ^{1}H NMR, IR, MS and UV identical to those reported [2]. ^{13}C NMR ($CDCl_3$): δ164.1 (C-1), 34.8 (–NMe), 47.8 (C-3), 27.6 (C-4), 123.1 (C-4a), 106.8 (C-5), 149.9 (C-6), 146.4 (C-7), 108.0 (C-8), 133.2 (C-8a), 101.2 ($-OCH_2O-$).

Allocryptopine (**5**). Mp 153–155° (from MeOH, lit. 155–157° [10]), UV, IR and ^{1}H NMR identical to those reported [10].

Protopine (**6**). Mp, MS, UV, ^{1}H and ^{13}C NMR identical to those reported [10, 11].

Hyperectine (**7**). Orange powder. Mp 238° (decomp., from MeOH). UV [5]. IR ν_{max}^{KBr} cm^{-1}: 3460, 3360, 2890, 1760, 1710, 1650, 1480, 1460, 1350, 1240, 1120, 1060, 1040, 920 ($-OCH_2O-$), 760. ^{1}H NMR ($CDCl_3$): δ6.80 (*s*, 2H), 6.50 (2H), 5.90, 5.80, 5.77 and 5.75 (each 1H, *s*, $2\times -OCH_2O-$), 4.65 (1H, *s*, 14-H), 3.50 and 3.42 (each 1H, *d*, J = 16 Hz, 9-H), 2.30 (3H, *s*, –*N*-Me), 2.52 (1H, *dd*, J = 16, 4 Hz, 5-H), 3.0 (1H, *ddd*, J-16, 8, 4 Hz, 5-H), 2.85 (1H, *dd*, J = 16, 4 Hz, 6-H), 3.60 (1H, *ddd*, J = 16, 8, 4 Hz, 6-H). ^{13}C NMR ($CDCl_3$): δ172.0 (C=O), 167.4 (C=O), 147.0, 146.3, 145.2, 143.2, 143.1 (C-2, C-3, C-12, C-13), 139.5, 136.8 (C-3′, C-4′), 131.0, 129.3, 121.6, 108.5 (C-4a, C-8a, C-9a, C-13a), 116.4, 108.7, 106.7, 102.0 (C-1, C-4, C-10, C-11), 75.3 (C-8), 47.2, 46.9, 44.1, 38.9 (C-6, *N*-Me, C-9, C-14), 26.8 (C-5), 100.8 ($2\times -OCH_2O-$). ^{1}H and ^{13}C NMR data in DMSO-d_6 in Table 1.

Table 1. NMR data of hyperectine (**7**) and isohyperectine (**9**) in DMSO-d_6

^{1}H NMR			^{13}C NMR		
H-Atom	Hyperectine	Isohyperectine	C-Atom	Hyperectine	Isohyperectine
1	6.49 *s*	6.47 *s*	2′	168.6	168.4
			5′	173.6	172.4
4	6.52 *s*	6.50 *s*	2	146.4[a]	146.0[a]
			3	145.6[a]	145.4[a]
5	2.37 *dd*, J = 16, 4 Hz	2.47 *dd*, J = 16, 4 Hz	12	144.8[a]	144.8[a]
			13	144.0[a]	144.2[a]
	2.65 *dd*, J = 16, 8 Hz	2.75 *dd*, J = 16, 8 Hz	4a	131.6[b]	132.8[b]
			8a	128.8[b]	127.8[b]
6	2.85 *dd*, J = 16, 4 Hz	3.15 *dd*, J = 16, 4 Hz	9a	122.0[b]	123.4[b]
	3.43 *dd*, J = 16, 8 Hz	3.30 *m*	13a	107.3	107.5
			1	107.2[c]	107.4[c]
9	3.49 *d*		4	101.1	101.0
	3.23 *d*, J = 16 Hz	3.30	10	108.4[c]	108.0[c]
			11	116.0	115.6
10	6.79 *d*, J = 8 Hz	6.68 *d*, J = 8 Hz	5	27.2	27.0
			6	46.6	46.6
11	6.85 *d*, J = 8 Hz	6.72 *d*, J = 8 Hz	8	74.8	75.2
			13	38.8	38.6
NMe	2.12 *s*	2.22 *s*	14	47.0	47.8
OCH_2O	5.90 *s*	5.70 *s*	—NMe	43.4	43.6
	5.87 *s*		OCH_2O	100.8	100.6
	5.83 *s*			100.8	100.7
	5.80 *s*		3′	142.6	142.8
14	4.40 *s*	4.43 *s*	4′	137.6	137.4

[a,b,c] Tentative assigments in the same column may be interchangeable.

Leptocarpine (**8**). Powder. Mp 166–168°. ($CHCl_3$–MeOH). $[\alpha]_D^{25} = -94.3°$ (*c* 0.6, MeOH). UV λ_{max}^{MeOH} (log ε): 296 (3.84). IR ν_{max}^{KBr} cm^{-1} 2840, 1765, 1500, 1450, 1420, 1390, 1270, 1105, 1030, 980, 930, 910, 870, 830, 750, 740. MS *m/z* (rel. int.): 383.1389 ($[M]^+$, 0.03, $C_{21}H_{21}NO_6$, cal.: 383.1369), 382.1292 ($[M-1]^+$, 0.2), 381.1218 ($[M-2H]^+$, 0.2), 191 (25), 190 (100). ^1H NMR ($CDCl_3$): δ5.50 (1H, *s*, 1-H), 6.50 (1H, *s*, 4-H), 3.05 (2H, *m*, 5-H), 3.37 (2H, *m*, 6-H), 6.55 (1H, *s*, 9-H), 7.25 (1H, *d*, *J* = 8 Hz, 4′-H), 7.70 (1H, *d*, *J* = 8 Hz, 5′-H), 5.05 (1H, *s*, 11-H), 5.80 (2H, *s*, $-OCH_2O-$), 3.00 (3H, *s*, *N*-Me), 3.90 (3H, *s*, 2′-OMe), 3.88 (3H, *s*, 3′-OMe). ^{13}C NMR ($CDCl_3$): δ107.1 (C-1), 146.1 (C-2), 148.5 (C-3). 108.5 (C-4), 125.2 (C-4a), 21.9 (C-5), 46.0 (C-6), 166.5 (C-7), 78.9 (C-9), 66.3 (C-11), 117.5 (C-9a), 117.6 (C-1′), 148.1 (C-2′), 153.0 (C-3′), 119.4 (C-4′), 117.7 (C-5′), 138.1 (C-6′), 101.3 ($-OCH_2O-$), 40.2 (*N*-Me), 62.2. (2′-OMe), 56.6 (3′-OMe).

Isohyperectine (**9**). ^1H and ^{13}C NMR data in Table 1.

Corydamine (**10**). Yellow powder. Mp. 63–66°. (from MeOH). UV and MS same as those in lit. [12]. IR ν_{max}^{KBr} cm^{-1} 3450, 2960, 2920, 2780–2700, 2450, 1580, 1440, 1380, 1365, 1330, 1280, 1265, 1220, 1210, 1180, 1100, 1040, 980, 940, 920, 880, 860.^1H NMR ($CDCl_3$): δ9.30 (1H, *s*, 1-H), 7.70 (1H, *s*, 4-H), 6.42 (2H, *s*, 5-H and 6-H), 6.92 (1H, *s*, 2′-H), 6.87 (1H, *s*, 5′-H), 2.94 (2H, *t*, *J* = 7 Hz, 7′-H), 3.40 (2H, *t*, *J* = 7 Hz, 8′-H), 2.53 (3H, *s*, *N*–Me), 6.25 (2H, *s*, $-OCH_2O-$), 6.02 (2H, *s*, 3′,4′,7,8–OCH_2O-). ^{13}C NMR ($CDCl_3$): δ144.7 (C-1), 142.4 (C-3), 121.4 (C-4), 132.4 (C-4a), 120.5 (C-5), 116.2 (C-6), 145.4 (C-7), 148.8 (C-8), 113.8 (C-8a), 133.4 (C-1′), 110.6 (C-2′), 147.2 (C-3′), 148.6 (C-4′), 110.2 (C-5′), 129.0 (C-6′), 30.0 (C-7′), 51.0 (C-8′), 34.5 (–*N*–Me), 103.0 (7,8–OCH_2O-), 101.7 (3′,4′–OCH_2O-).

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