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Five alkaloids from *Hypecoum leptocarpum*

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Abstract

Five new alkaloids, leptocarpinine, leptopine, leptopinine, leptopidine and leptopidinine were isolated, together with protopine, isohyperectine and oxohydrastinine, from *Hypecoum leptocarpum*. The structures were elucidated by spectral evidence.

Keywords: Hypecoum leptocarpum; Fumariaceae; Alkaloids; NMR

1. Introduction

Six of the 18 species of the genus *Hypecoum* L. are distributed from Southwest to North China (How, 1984). Phytochemical studies have led to the isolation of many alkaloids from about half of the 18 species (Yakhontova, Komarova, Perel'son, Blinova, & Tolkachev, 1972; Hussain, Gözler, Shamma, & Gözler, 1982; Yakhontova, Tolkachev, Komarova, & Shreter, 1984; Táborská, Vězník, Slavík, Sedmera, & Simánek, 1988; Saad, 1995). In previous investigations of *H. leptocarpum* some alkaloids belonging to the benzo[c]phenanthridine, isoquinoline and spiroisoquinoline types were identified (Chen & Fang, 1985; Slavík & Slavíkova, 1961; Zhang, Rücker, Breitmaier, & Mayer, 1995). In this reinvestigation of this plant five new alkaloids leptocarpinine (**1**), leptopine (**2**), leptopinine (**3**), leptopidine (**4**) and leptopidinine (**5**), together with protopine (**6**), isohyperectine (**7**) and oxohydrastinine (**8**), were isolated. Their structures were determined predominantly by UV, MS and NMR including C,H-COSY and HMBC. Alkaloid **2** was determined not to be an alternative form of **5** in different solution at various pH values.

2. Results and discussion

The molecular ion peak of leptocarpinine (**1**) at *m/z* 394.1284 in its HR-EIMS suggested the molecular formula $C_{22}H_{20}NO_6$, corresponding to its ^{13}C NMR (DEPT) and ^{1}H NMR spectra. In its ^{13}C NMR spectrum, the twenty-two signals are derived from eleven quaternary C-atoms, four methines, two methyl groups and five methylenes belong to two methylenedioxy, one $-CH_2CH_2-$ (C-9 and C-10) and one terminal methylene ($CH_2=$, C-14). From the HMBC correlation of the quaternary C-atom at δ 171.68 ppm and the methyl group at δ 3.34 ppm a methoxycarbonyl could be deduced. Considering the molecular formula and the presence of two methylenedioxy resonances at δ 6.06 and 5.97 (each s, 2H) in the ^{1}HNMR spectrum and methoxycarbonyl group, the other methyl group at δ 3.72 (s, 3H) could only be determined as an *N*-methyl group. The chemical shifts of H-10 and H-9 from the C,H-COSY correlations δ 4.10 (br, 2H, H-10)/53.55 ppm (t) and 3.15 (br, 2H, H-9)/26.64 ppm (t) showed that the moiety $-CH_2CH_2-$ (C-9 and C-10) is also connected to an N-atom. From the following cross signals in the C,H-COSY two phenyl rings could be determined: δ 6.78 (d, 1H, $J = 8$ Hz, H-7)/108.74 ppm, 6.90 (d, 1H, $J = 8$ Hz, H-8)/123.68 ppm, 6.91 (s, 1H, H-3′)/109.27 ppm, and 7.25 (s, 1H, H-6′)/113.19 ppm. The signal at δ 174.28 ppm (C-2) for a quaternary C-atom could be assigned to a C-atom connected to an N-atom with a double bond upon the HMBC

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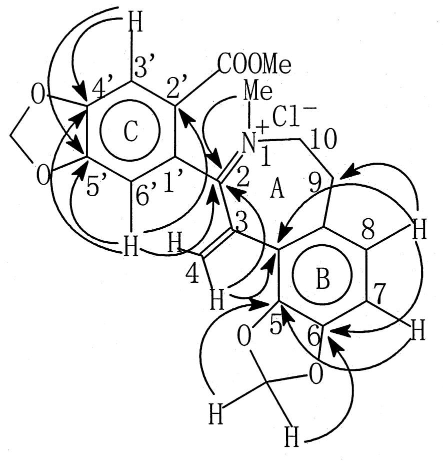

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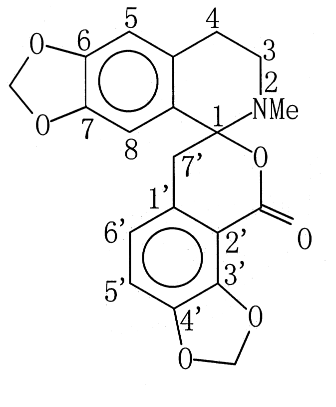

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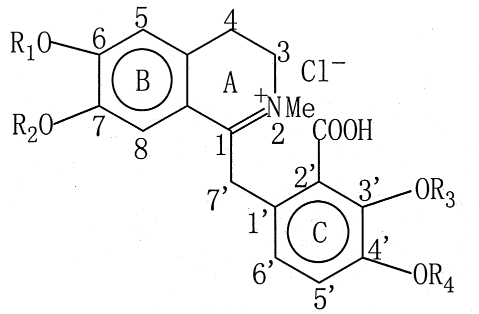
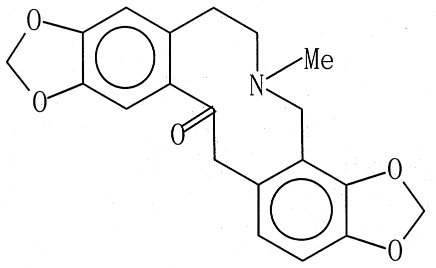


2. $R_1R_2{=}R_3R_4{=}CH_2$;
3. $R_1{=}R_2{=}H$, $R_3{=}R_4{=}Me$;
4. $R_1R_2{=}CH_2$, $R_3{=}H$, $R_4{=}Me$



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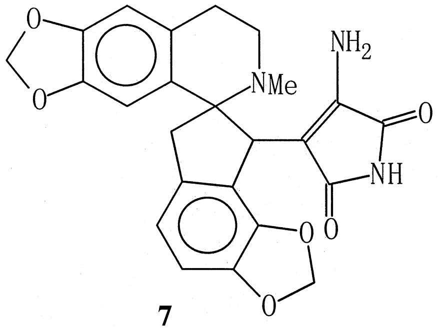

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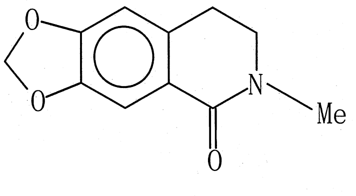

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correlation of the N–Me with C-2. In the HMBC the mutually correlating partners of C-2 are the *N*-methyl group, the protons (H-4) of the terminal methylene CH_2= resonating at δ 5.73 and 6.48 (each br, 1H) and H-6′. Confirmation of the structures rings A and B was achieved by the HMBC cross signals: H-4, H-7 and the protons of 5,6–OCH_2O–/C-5 at δ 146.19 ppm, and H-8 with C-9 at δ 26.64 ppm. Because of the *para* location of H-3′ and H-6′ on ring C, another methylenedioxy could be only assigned to C-4′ and C-5′. Therefore, the structure of **1** could be elucidated as that shown.

Leptopine (**2**) was obtained as yellow cubic crystals. Twenty signals were observed in its ^{13}C NMR (DEPT) spectrum, of which sixteen signals are above 100 ppm. Two methylenedioxy groups were ascribed by the C,H-COSY cross signals δ 104.45 (6,7–OCH_2–)/6.06 (s, 2H) and 102.75 (3′,4′–OCH_2O–)/5.92 ppm (s, 2H). A carboxyl group was supported by the ^{13}C NMR signal at δ 171.78 ppm and by IR absorptions at 2400–3400 and 1639 cm^{-1}. This conclusion was further confirmed by the molecular formula $C_{20}H_{18}NO_6$ (HREIMS ion peak at 368.1134) and the base peak at *m/z* 322 $([M-CO_2-2H]^+$. The *N*-methyl group was revealed by the ^{1}H NMR signals at δ 3.77 (s, 3H) and the ^{13}CNMR signal at δ 44.97 (q) ppm. Two phenyl rings were recognized from the ^{1}H NMR signals at δ 6.91 (s, 1H, H-5), 7.42 (s, 1H, H-8), 6.65 (d, 1H, $J = 8$ Hz, H-5′), and 6.49 (d, 1H, $J = 8$ Hz, H-6′) ppm. The moiety $=N–CH_2CH_2–$ could be determined by the NMR signals at δ 3.16 and 4.07 (each t, $J = 7.5$ Hz, H-3 and H-4), and 26.83 (t, C-4) and 53.73 (t, C-3). On the basis of the following correlations in the HMBC the isoquinoline rings A and B were deduced: H-8, N–Me and H-3/C-atom at δ 176.72 (s, C-1) ppm, H-6/C-4 and H-4/C-6. The benzyl moiety was confirmed by the HMBC cross signals of H-6′ with C-7′, C-2′ and C-4′, and of H-5′ with C-1′ and C-3′. Thus, the structure of **2** could be assigned as that shown.

The UV spectrum of leptopinine (**3**) (λ_{max} at 245, 310 and 353) resembled that of **2**. In its ^{13}C NMR (DEPT) twenty signals were observed. In the C,H-COSY an *N*-methyl group at δ 3.70 ppm and two methoxy groups at δ 3.83 (s, 3H, 3′-OMe) and 3.65 (s, 3H, 4′-OMe) could be recognized. Four aromatic protons appearing at δ 6.78 (s, 1H, H-5), 7.21 (s, 1H, H-8), 7.01 (d, 1H, $J = 8.5$ Hz, H-5′) and 6.58 (d, 1H, $J = 8.5$ Hz, H-6′) suggested the presence of two phenyl rings. The HMBC cross signals of 3′-OMe and H-5′ with C-3′ at δ 152.17 ppm, and of 4′-OMe and H-6′ with C-4′ at δ 141.60 ppm supported the assignments of 3′- and 4′-OMe. In the HMBC the H-6′ also correlates with C-7′ (37.00 ppm) and C-2′ (117.49 ppm). The following HMBC correlations defined the isoquinoline moiety: H-8, 2-Me and H-3 with C-1 (178.91 ppm), and H-5 with C-4 (26.30 ppm). The EIMS ion peak at *m/z* 327.1471 should be $[M-COOH]^+$ ($C_{19}H_{21}NO_4$, calcd.: 327.1471) because of the existence of a carboxyl group (IR: 2400–3400 and 1678 cm^{-1}, ^{13}C NMR: 172.48 ppm for a quaternary C-atom). Thus, two aromatic hydroxyl groups could be assumed, which was supported by the bathochromic shift of its UV absorption to λ_{max} at 271, 315 and 410 nm in KOH/MeOH. These hydroxyl groups should be located at C-5 and C-6 in view of the HMBC cross signals of H-8 and H-5 with C-6 (156.62 ppm) and C-7 (148.50 ppm). Consequently, the structure of **3** could be determined.

The UV spectrum of leptopidine (**4**) resembled closely those of **2** and **3**, indicating that **4** is also a 1,2-dehydrobenzylisoquinoline alkaloid. Comparing its ^{13}C and ^{1}H NMR spectra with those of **2** and **3**, it could be concluded that the substitution pattern of **4** is identical to those of **2** and **3**. From the ^{1}H NMR spectrum an *N*-methyl group at δ 3.70 (s, 3H) was postulated. A methoxy group resonated at δ 3.80 (s, 3H), and a methylenedioxy group, appeared at δ 5.98 (s, 2H) ppm. IR absorptions at 2400–3400 and a ^{13}C NMR signal at 174.99 provided evidence of a carboxyl group. Besides the substituents mentioned above, a hydroxyl group was concluded, according to the IR absorption at 3449 cm^{-1} and the molecular formula suggested by the EIMS ion peak at *m/z* 370.1291 ($C_{20}H_{20}NO_6$). The methoxy group and the hydroxyl group were located at C-4′ and C-3′ by a group of HMBC cross signals: –OMe, H-6′ (6.50 ppm, d, $J = 7.5$ Hz) with C-4′ (149.42 ppm), H-5′ (6.90 ppm, d, $J = 7.5$ Hz) with C-3′ (154.54 ppm) and H-6′ with C-7′ (38.08 ppm). In the HMBC the protons of the methylenedioxy group, together with H-5 (6.82 ppm, s, 1H) and H-8 (7.11 ppm, s, 1H), correlated with C-6 (148.61 ppm) and C-7 (155.11 ppm). Thus, the structure of **4** was elucidated.

The EI–MS ion peak of leptopidinine (**5**) at *m/z* 367.0953 indicated the molecular formula $C_{20}H_{17}NO_6$, corresponding to the twenty ^{13}C NMR (DEPT) signals for three aliphatic C-atoms, two methylenedioxy groups, one *N*-methyl group, four aromatic methines and ten quaternary C-atoms. Two aromatic rings were recognized from the ^{1}H NMR signals at δ 6.63 (s, 1H, H-5), 7.89 (s, 1H, H-8), 7.70 and 7.16 (each d, 1H, $J = 8$ Hz, H-5′ and H-6′). Two methylenedioxy groups resonate at δ 6.20 (s, 2H) and 5.96 (s, 2H) ppm. Due to the IR absorption at 1762 cm^{-1}, the ^{13}C NMR signal at δ 163.94 ppm and the molecular formula, the compound should be a lactone. The ^{1}HNMR signal at δ 2.73 (s, 3H) could be assigned to the *N*-methyl group. By comparing the NMR data with those of torulosine and torulosinine (Rücker, Breitmaier, Zhang, & Mayer, 1994), the structure of **5** could be determined as a secoberbine alkaloid.

It seems that alkaloids **2** and **5** could be interconverted. However, alkaloids **2** and **5** in solutions of NaOH (aq. or MeOH) or of diluted HCl, no identical UV behavior was observed. Comparing the TLC of **2** and **5** in H_2O or MeOH, and **2** and **5** in NaOH (aq.) or in diluted HCl, no identical spots were recognized. Therefore, it could be concluded that **2** is not an alternative form of **5**.

3. Experimental

3.1. General

Mp: uncorr. NMR: in CD_3OD, 500 MHz for ^{1}H and 125 MHz for ^{13}C. MS: at 70 eV. CC: silica gel, 200–300 mesh. IR: KBr discs. UV: in MeOH. Cl^- was identified by aqueous solution of $AgNO_3$ and KBr.

3.2. Plant material

Whole plants of *H. leptocarpum* were collected in September, 1997 in Roergai county, Sichuan Province, China, and identified by Prof. S.C. Xiao of the in Chengdu Institute of Biology, Chinese Academy of Sciences, where a voucher specimen is deposited.

3.3. Extraction

A sample of the cut, dried whole plants (13 kg) was soaked with 95% ethanol (3 × 100 l). After concentration in vacuum ca. 2.2 kg of the extract was obtained. This extract was dissolved in chloroform (3.5 l) and extracted with 7% HCl (6 × 1.2 l). The acidic solution was washed with petroleum ether (3 × 0.8 l), then the aqueous layer was treated with conc. ammonia to pH 9.4, the precipitate was filtered (F). The chloroform and petroleum ether layers were combined to give fraction H. The basified solution was first extracted with chloroform (6 × 0.8 l) to yield extract I (51 g), and the solution was then adjusted with NaOH to pH 12.3 to give a precipitate G. The solution extracted with *n*-butanol gave extract B (215 g). Extract I was dissolved in chloroform (300 ml) and extracted with 2% NaOH. From the chloroform layer extract A (29 g) was obtained.

3.4. Isolation

Extract A was divided into eight fractions FrA1–8 by CC with ethyl acetate. Alkaloid **8** was isolated from FrA1 by CC (200–300 mesh, $CHCl_3$–acetone 20:1). FrA8 was crystallized from chloroform to give alkaloid **6**. The extract B was subjected to CC eluting with $CHCl_3$–MeOH–EtOAc 4:1:8 to give twenty-four fractions FrB1–24. FrB1 to FrB5 and FrB7 were cooled to about 4°C to give alkaloids **5** and **7**, respectively. FrB15 was separated by CC with $CHCl_3$–MeOH–EtOAc–H_2O 4:4:4:1 to yield alkaloid **4**. Alkaloids **1**, **2** and **3** were isolated from FrB18 by CC with $CHCl_3$–MeOH–EtOAc–H_2O 4:4:4:1.

3.5. Leptocarpinine (*1*)

Amorphous powder. Mp > 350°C (MeOH). UV λ_{max} (log ε): 259 (4.23), 303 (4.01), 376 (3.94). IR ν_{max} (cm^{-1}): 1700, 1620, 1589, 150, 1444, 1393, 1345, 1287, 1251, 1039, 918. MS *m*/*z* (rel. int.): 394.1286 ([M]$^{+}$, 4, $C_{22}H_{20}NO_6$, calcd.: 394.1291), 393 ([M − 1]$^{+}$, 19), 349 (73), 348 (31), 347 (100), 337 (65), 335 (59), 334 (69), 333 (45), 322 (86), 318 (30). ^{1}H NMR: δ 3.72 (s, 3H, N–Me), 5.73 and 6.48 (each br, 1H, H-4), 5.97 (s, 2H, 5,6–OCH_2O–), 6.78 (d, 1H, $J = 8$ Hz, H-7), 6.90 (d, 1H, $J = 8$ Hz, H-8), 3.15 (br, 2H, H-9), 4.10 (br, 2H, H-10), 3.34 (s, 3H, –COOMe), 6.91 (s, 1H, H-3′), 6.06 (s, 2H, 4′,5′–OCH_2O–), 7.25 (s, 1H, H-6′). ^{13}CNMR: δ 46.30 (N–Me), 174.28 (C-2), 140.27 (C-3), 124.18 (C-3a), 146.19 (C-5), 150.18 (C-6), 108.74 (C-7), 123.68 (C-8), 128.02 (C-8a), 26.64 (C-9), 53.55 (C-10), 121.90 (C-1′), 138.36 (C-2′), 109.27 (C-3′), 148.86 (C-4), 156.32 (C-5′), 113.19 (C-6′), 103.15 (5,6–OCH_2O–), 104.44 (4′,5′–OCH_2O–).

3.6. Leptopine (*2*)

Yellow cubic crystals. Mp. 158–161°C (MeOH). UV λ_{max} (log ε): 248 (4.25), 300 (3.84), 365 (3.94); λ_{max} (MeOH + HCl): 247, 305, 363; λ_{max} (MeOH + NaOH): 247, 290, 358. IR ν_{max} (cm^{-1}): 2400–3400 (COOH), 1639 (C=O), 1600, 1590, 1509 (phenyl), 1444, 1399, 1375, 1325, 1290, 1253, 1223, 1039, 919 (–OCH_2O–). MS *m*/*z* (rel. int.): 368.1134 ([M]$^{+}$, 4, $C_{20}H_{18}NO_6$, calcd.: 368.1134), 367 (20), 336 (90), 322 (100), 190 (65). ^{1}H NMR: δ 3.77 (s, 3H, 2-Me), 4.07 (t, 2H, $J = 7.5$ Hz, H-2), 3.16 (t, 2H, $J = 7.5$ Hz, H-4), 6.91 (s, 1H, H-5), 6.06 (s, 2H, 6,7–OCH_2–), 7.42 (s, 1H, H-8), 5.92 (s, 2H, 3′,4′–OCH_2O–), 6.65 (d, 1H, $J = 8$ Hz, H-5′), 6.49 (d, 1H, $J = 8$ Hz, H-6′). ^{13}C NMR: δ 176.70 (C-1), 44.97 (N–Me), 53.73 (C-3), 26.83 (C-4), 137.58 (C-4a), 109.14 (C-5), 149.20 (C-6), 155.90 (C-7), 110.86 (C-8), 122.25 (C-8a), 125.21 (C-1′), 124.78 (C-2′), 146.58 (C-3′), 149.82 (C-4′), 108.60 (C-5′), 122.12 (C-6′), 35.41 (C-7′), 104.45 (6,7–OCH_2–), 102.75 (3′,4′–OCH_2O–), 171.78 (COOH).

3.7. Leptopinine (*3*)

Yellow powder. Mp. 207–210°C (MeOH). UV λ_{max} (log ε): 245 (4.22), 310 (3.97), 353 (3.97); λ_{max} (MeOH + HCl): 247, 311, 362; λ_{max} (MeOH + NaOH): 271, 315, 410. IR ν_{max} (cm^{-1}): 2400–3400 (–COOH), 1678 (C=O), 1627, 1583, 1524, 1438, 1268, 1200, 1061. MS *m*/*z* (rel. int.): 355 ([M–OH]$^{+}$, 20), 341 ([M–OMe]$^{+}$, 70), 327.1471 ([M–COOH]$^{+}$, 100, $C_{19}H_{20}NO_4$, calcd.: 327.1471), 313 (74), 312 (84), 204 (2), 190 (18), 177 (10). ^{1}H NMR: δ 3.70 (s, 3H, N–Me), 4.03 (t, 2H, $J = 7.5$ Hz, H-3), 3.06 (t, 2H, $J = 7.5$ Hz, H-4), 6.78 (s, 1H, H-5), 7.21 (s, 1H, H-8), 3.83 (s, 3H, 3′-OMe), 3.65 (s, 3H, 4′-OMe), 7.01 (d, 1H, $J = 8.5$ Hz, H-5′), 6.58 (d, 1H, $J = 8.5$ Hz, H-6′). ^{13}C NMR: δ 178.91 (C-1), 44.45 (N–Me), 53.88 (C-3), 26.30 (C-4), 135.42 (C-4a), 115.06 (C-5), 156.62 (C-6), 148.50 (C-7), 119.54 (C-8), 120.85 (C-8a), 122.71 (C-1′), 117.49 (C-2′), 152.17 (C-3′), 149.60 (C-4′), 115.83 (C-5′), 120.85 (C-6′), 37.00 (C-7′), 56.74 (3′-OMe), 56.85 (4′-OMe), 174.99 (COOH).

3.8. Leptopidine (*4*)

Yellow powder. Mp. 235–239°C (MeOH). UV λ_{max} (log ε): 243 (5.41), 305 (4.05), 352 (4.05); λ_{max}

(MeOH + HCl): 249, 305, 365; λ_{max} (MeOH + NaOH): 241, 304, 356. IR ν_{max} (cm^{-1}): 3449 (–OH), 2400–3400 (–COOH), 1636 (C=O), 1594, 1477 (phenyl), 1369, 1252, 1039, 930 (–CH$_2$O–). MS *m/z* (rel. int.): 370.1291 ([M]$^+$, 2, $C_{20}H_{20}NO_6$, calcd.: 370.1291), 337 (8), 325 (17), 324 (8), 310 (16), 191 (14), 190 (100). ^{1}H NMR: δ 3.70 (s, 3H, N–Me), 4.01 (t, 2H, $J = 7.5$ Hz, H-3), 3.09 (t, 2H, $J = 7.5$ Hz, H-4), 6.82 (s, 1H, H-5), 5.98 (s, 2H, 6,7–OCH$_2$–), 7.11 (s, 1H, H-8), 3.80 (s, 3H, 4′-OMe), 6.90 (d, 1H, $J = 7.5$ Hz, H-5′), 6.50 (d, 1H, $J = 7.5$ Hz, H-6′). ^{13}C NMR: δ 178.03 (C-1), 44.55 (N–Me), 53.54 (C-3), 26.69 (C-4), 137.17 (C-4a), 108.92 (C-5), 148.61 (C-6), 155.11 (C-7), 110.48 (C-8), 122.27 (C-8a), 129.87 (C-1′), 118.20 (C-2′), 154.54 (C-3′), 149.42 (C-4′), 114.17 (C-5′), 120.01 (C-6′), 38.08 (C-7′), 104.16 (6,7–OCH$_2$–), 58.39 (4′-OMe).

*3.9. Leptopidinine (**5**)*

Yellow needles (MeOH–CHCl$_3$). Mp. 345–350°C (CHCl$_3$). $[\alpha]_D^{20}$ 0°C (MeOH, *c* 1.5). UV λ_{max} (log ε): 240 (3.96), 307 (3.66), 330 (3.65), 410 (3.88); λ_{max} (MeOH + HCl): 220 (sh), 250 (sh), 315, 384: λ_{max} (MeOH + KOH): 240 (sh), 302, 330, 410. IR ν_{max} (cm^{-1}): 1762 (C=O), 1482, 1249, 1230, 1029. MS *m/z* (rel. int.): 367.0953 ([M]$^+$, 4, $C_{20}H_{17}NO_6$, calcd.: 367.1056), 366 (22), 365 (100), 350 (8), 309 (12), 308 (61), 207 (10), 279 (14), 182 (19). ^{1}H NMR: δ 2.73 (s, 3H, N–Me), 3.18 (br, 2H, H-3), 2.82 (s, 2H, H-4), 6.63 (s, 1H, H-5), 5.96 (s, 2H, 6,7–OCH$_2$–), 7.89 (s, 1H, H-8), 6.20 (s, 2H, 3′,4′–OCH$_2$O–), 7.16 (d, 2H, $J = 8$ Hz, H-5′), 7.70 (d, 1H, $J = 8$ Hz, H-6′). ^{13}C NMR: δ 106.01 (C-1), 42.76 (N–Me), 51.46 (C-3), 22.66 (C-4), 137.56 (C-4a), 108.19 (C-5), 146.58 and 148.10 (C-6 and C-7), 110.59 (C-8), 123.48 (C-8a), 131.51 and 133.21 (C-1′ and C-2′), 144.51 (C-3′), 147.49 (C-4′), 114.66 (C-5′), 117.90 (C-6′), 29.68 (C-7′), 101.13 and 102.97 (6,7–OCH$_2$– and 3′,4′–OCH$_2$O–), 163.94 (C=O).

*3.10. Protopine (**6**)*

Mp, MS, UV, ^{1}H and ^{13}C NMR data were identical to those reported (Lu, Wang, & Su, 1971; Balawant, Haider, & Pelletier, 1990).

*3.11. Isohyperectine (**7**) and oxohydrastinine (**8**)*

Mp, MS, UV, ^{1}H and ^{13}C NMR data were identical to those reported (Rücker et al., 1994).

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