Alkaloids from *Porcelia macrocarpa*[1]

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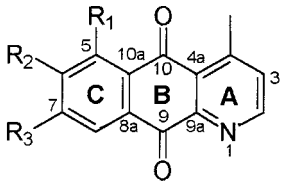
Three new azaanthracene alkaloids, 6,7-dimethoxycleistopholine (**3**), 5-hydroxy-6-methoxycleistopholine (**4**), and 5-hydroxy-6,7-dimethoxycleistopholine (**5**), along with 14 known alkaloids, including the new natural product 6-methoxycleistopholine (**2**), were isolated from a CH_2Cl_2 extract of the branches of *Porcelia macrocarpa*.

In continuation of our chemical studies on *Porcelia macrocarpa* (Warm.) R.E. Fries *(*Annonaceae),[2,3] we have undertaken the analysis of alkaloids extracted from the branches of this plant. The ^{1}H NMR spectrum of a basified CH_2Cl_2 extract indicated the presence of azaanthracene and azafluorene alkaloids, in addition to benzylisoquinoline and aporphine alkaloids, as indicated by signals of H-α (*δ* 8.20−8.96, d, *J* = 4.8−5.3 Hz) and H-*β* (*δ* 6.64−7.59 d, *J* = 4.8−5.3 Hz) of the pyridine ring and aromatic methyl protons (*δ* 2.50−2.95, s). This observation led us to attempt extraction of the alkaloids with two acidic aqueous solutions, at pH 2.0 and pH 0.4, successively. The more basic benzylisoquinoline, aporphine, and proaporphine alkaloids were extracted at pH 2.0. The oxoaporphine, azaanthracene, and azafluorene alkaloids were extracted at pH 0.4.

The known alkaloids reticuline,[4,5] 4′-methylcoclaurine,[4] coclaurine,[4] norjuziphine,[6] asimilobine,[7] (−)-norushinsunine (= michelalbine),[8] stepharine,[5,7,8] liriodenine,[7,10,11] onychine,[11,12] 6-methoxyonychine,[11,12] 7-methoxyonychine,[11] and 6,7-dimethoxyonychine[11] were identified by comparing their NMR and MS data with those reported. 7-Methoxyonychine has been described only as a synthetic product.[11]

Cleistopholine (**1**), the simplest member of the azaanthracene alkaloids, was identified by its NMR and MS data.[13,14] Analysis of the MS, ^{13}C NMR, and COLOC spectra of **2** identified this alkaloid as 6-methoxycleistopholine, a compound previously reported as a synthetic intermediate.[15]



1 R_1=R_2=R_3=H

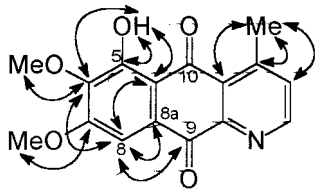
2 R_1=R_3=H; R_2=OMe

3 R_1=H; R_2=R_3=OMe

4 R_1=OH; R_2=OMe; R_3=H

5 R_1=OH; R_2=R_3=OMe

The ^{1}H NMR spectrum of **3** showed two singlets in the aromatic region, two singlets corresponding to two methoxyl groups, and other signals of the ring A protons of an azaanthracene alkaloid. These observations led to the identification of **3** as 6,7-dimethoxycleistopholine. The ^{13}C NMR, EIMS, and HRFABMS data were in agreement with structure **3**.



5

Figure 1. Important ^{1}H−^{13}C couplings observed in the COLOC spectrum of **5**.

The observation that the carbonyl group at C-10 showed a more intense signal than that of C-9 in the ^{13}C NMR spectra of **2** and **3**, due to the nuclear NOE effect of the methyl protons,[14] was very useful in the structural determination of azaanthracene alkaloids **4** and **5**. The ^{1}H NMR spectrum of **4** showed a hydroxyl signal at *δ* 12.82, a methoxyl singlet, and two doublets that were assigned to two *ortho* aromatic protons. These observations placed the hydroxyl group at C-5 or C-8 and the methoxyl *ortho* or *para* to this group. The *para*-substitution was eliminated because it would give almost the same chemical shifts for H-6 and H-7 as recorded in the literature.[16] The chelation effect in a C=O chemical shift is ca. 5.5 ppm.[17] The observation that the more intense carbonyl signal in the ^{13}C NMR spectrum is deshielded by 5.5 ppm in relation to C-10 in cleistopholine[14] enabled the hydroxyl group to be placed at C-5. The methoxyl group could then be placed at C-6. The assignments of the ^{13}C NMR data of **4** were based on data obtained for cleistopholine (**1**) and 1-hydroxy-2-methoxyanthraquinone.[17] The EIMS and HRFABMS were consistent with structure **4**.

The ^{1}H NMR of **5** gave signals for one chelated hydroxyl, two methoxyls, and one aromatic proton and the signals for ring A protons of an azaanthracene alkaloid. The ^{13}C NMR spectrum indicated that one methoxyl group is hindered and that the chelated carbonyl is at C-10. Comparison of its ^{1}H and ^{13}C NMR data with literrature values[14,18] placed the proton at C-8 and suggested the structure as 5-hydroxy-6,7-dimethoxycleistopholine (**5**). A COLOC spectrum confirmed the structure, by the observation of long-range couplings of H-8 with C-9, C-6, C-8a, and C-10a (Figure 1). The EIMS and HRFABMS were consistent with structure **5**.

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The co-occurrence of azapolycyclic alkaloids with liriodenine in *P. macrocarpa* reinforces their biogenetic origin as degradative natural products from oxoaporphine alkaloids.

Experimental Section

General Experimental Procedures. LREIMS was performed on a Finnigan MAT 90. HRFABMS was performed on a JEOL HX-110, FAB ionization (Xe gun), matrix: glycerol−thioglycerol−*m*-nitrobenzoyl alcohol (2:1:1) + 1% TFA. All of the ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 instrument in $CDCl_3$ or $CDCl_3$−CD_3OD, using TMS as internal standard. The IR spectra were recorded on a Nicolet FT-IR 510 instrument, and the UV spectra were recorded on a HP 8452 A diode array spectrophotometer. All chromatographic separations were performed using Merck Si gel 60 (40−63 μm), PF_{254}, and Al_2O_3 GF_{254}.

Plant Material. The branches of *P. macrocarpa* (Warm.) R.E. Fries were collected at the Instituto de Botânica de São Paulo, São Paulo, in June 1991. A voucher specimen is deposited in the herbarium of the Instituto de Botânica, São Paulo, Brazil, under reference number SP76791.

Extraction and Isolation. Dried powdered branches (1.1 kg) were extracted with hexane (40−60 °C) for 1 h. The air-dried marc was basified with NH_4OH and extracted with CH_2Cl_2. The CH_2Cl_2 concentrated solution was successively extracted with HCl at pH 2.0 and 0.4. The aqueous layers were made alkaline and re-extracted with CH_2Cl_2, with the organic solvent was removed under reduced pressure to afford mixtures of crude alkaloids.

The alkaloidal fraction from extraction at pH 2.0 (490 mg) was separated by flash chromatography on Si gel, eluting with $CHCl_3$−MeOH−NH_4OH (98:2:0.5)/hexane (1:1), $CHCl_3$−MeOH−NH_4OH (98:2:0.5), and (96:4:0.5). Additional preparative TLC on Si gel of the fractions eluted with $CHCl_3$−MeOH−NH_4OH (99:1:0.5), $CHCl_3$−MeOH−NH_4OH (98:2:0.5), and $CHCl_3$−MeOH−NH_4OH (90:10:0.5), respectively, afforded liriodenine[7,10,11] (2 mg) and 4′-methylcoclaurine[4] (12 mg, $[\alpha]^{25}_D$ +11°, *c* 0.45 MeOH), stepharine[5,7,8] (6 mg, $[\alpha]^{25}_D$ +40° *c* 0.13, MeOH), asmilobine[7] (5 mg, $[\alpha]^{25}_D$ −68°, *c* 0.24, $CHCl_3$), norushinsunine[8] (20 mg, $[\alpha]^{25}_D$ −93°, *c* 0.15 EtOH), reticuline[4,5] (6 mg, $[\alpha]^{25}_D$ +85° *c* 0.92, EtOH), norjuziphine[6] (7 mg, $[\alpha]^{25}_D$ +7°, *c* 0.45, MeOH), and coclaurine[4] (19 mg, $[\alpha]^{25}_D$ +23°, *c* 0.92, MeOH).

The alkaloidal fraction from extraction at pH 0.4 (477 mg) was separated by flash chromatography on Si gel and eluting with CH_2Cl_2−Me_2CO (98:2), giving three fractions, A, B, and C. Fraction A was purified by preparative TLC on Si gel (petroleum ether−EtOAc, 1:1) to afford cleistopholine[13,14] (**1**, 14 mg), 6-methoxyonychine[11,12,9] and 7-methoxyonychine[11] (3 mg), and onychine[11,12] (5 mg). Fraction B was purified by preparative TLC on Si gel (CH_2Cl_2−Me_2CO, 95:5) to afford liriodenine[7,10,11] (12 mg). Fraction C was purified by preparative TLC on Si gel (C_6H_6−Me_2CO, 7:3) to yield 5-hydroxy-6-methoxycleistopholine (**4**, 10 mg), 5-hydroxy-6,7-dimethoxycleistopholine (**5**, 20 mg), and fraction D. Fraction D was purified by preparative TLC on Si gel (CH_2Cl_2−Me_2CO, 95:5) to give 6,7-dimethoxycleistopholine (**3**, 4 mg) and fraction E. Fraction E was separated by preparative TLC on Al_2O_3 GF_{254} (petroleum ether−EtOAc, 6:4) to afford 6,7-dimethoxyonychine (**3**, 1 mg) and fraction F. Fraction F was purified by preparative TLC on Si gel (petroleum ether−EtOAc−MeOH−HOAc, 60:20:2:0.5) to yield cleistopholine (**1**, 11 mg) and 6-methoxycleistopholine (**2**, 21 mg).

6-Methoxycleistopholine (**2**): amorphous powder; UV (MeOH) λ_{max} (log ϵ) 234 (3.41), 262 (3.44), 302 (3.46). 366 (3.22) nm; IR (KBr) ν_{max} 1687, 1661, 1598, 1578, 1309 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.84 (1H, d, J = 4.8 Hz, H-2), 7.46 (1H, d, J = 4.8 Hz, H-3), 7.72 (1H, d, J = 2.7 Hz, H-5), 7.27 (1H, dd, J = 8.7, 2.7 Hz, H-7), 8.17 (1H, d, J = 8.7 Hz, H-8), 4.01 (3H, s, OCH_3), 2.88 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 152.9 (d, C-2), 131.3 (d, C-3), 151.4 (s, C-4), 129.1 (s, C-4a), 109.6 (d, C-5), 164.3 (s, C-6), 122.2 (d, C-7), 129.6 (d, C-8), 127.5 (s, C-8a), 183.8 (s, C-9), 150.2 (s, C-9a), 182.0 (s, C-10), 134.6 (s, C-10a), 56.1 (q, OCH_3), 22.9 (q, CH_3-4); EIMS *m/z* 253 [M]$^+$ (100), 252 (24), 239 (9), 225 (31), 224 (12), 223 (11), 210 (13), 197 (4), 196 (5), 195 (8), 182 (13), 154 (12); HRFABMS [M + H]$^+$ *m/z* 254.0814 (calcd for $C_{15}H_{12}NO_3$, 254.0817).

6,7-Dimethoxycleistopholine (**3**): amorphous powder; UV (MeOH) λ_{max} (log ϵ) 208 (3.76), 234 (3.79), 282 (3.91), 374 (2.95) nm; IR (KBr) ν_{max} 1675, 1661, 1582, 1513, 1316 cm^{-1}; ^{1}H NMR ($CDCl_3$, 50 MHz) δ 8.84 (1H, d, J = 4.8 Hz, H-2), 7.44 (1H, d, J = 4.8 Hz, H-3), 7.74 (1H, s, H-5), 7.65 (1H, s, H-8), 2,88 (3H, s, CH_3), 4.07, 4.05 (each 3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 153.0 (d, C-2), 131.0 (d, C-3), 151.2 (s, C-4), 129.0, 128.9 (each s, C-4a, C-10a), 108.4, 108.2 (each d, C-5, C-8), 154.2, 153.8 (each s, C-6, C-7), 127.6 (s, C-8a), 184.2 (s, C-9), 150.3 (s, C-9a), 181.1 (s, C-10), 56.7, 56.5 (each q, OCH_3), 22.9 (q, CH_3-4); EIMS *m/z* 283 [M]$^+$ (100), 282 (26), 268 (13), 255 (4), 254 (10), 252 (23), 240 (21), 227 (2), 226 (4), 225 (6), 224 (13), 212 (22), 169 (11), 141 (15), 77 (7); HRFABMS [M + H]$^+$ *m/z* 284.0916 (calcd for $C_{16}H_{14}NO_4$, 284.0916).

5-Hydroxy-6-methoxycleistopholine (**4**): amorphous powder; UV (MeOH) λ_{max} (log ϵ) 214 (2.43), 246 (3.65). 260 (3.49), 430 (2.75) nm; IR (KBr) ν_{max} 1662, 1636, 1606, 1579, 1301 cm^{-1}; ^{1}H NMR ($CDCl_3$, 50 MHz) δ 8.87 (1H, d, J = 4.8 Hz, H-2), 7.48 (1H, d, J = 4.8 Hz, H-3), 7.21 (1H, d, J = 8.5 Hz, H-7), 7.82 (1H, d, J = 8.5 Hz, H-8), 12.82 (1H, s, OH), 4.01 (1H, s, OCH_3), 2.89 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 153.1 (d, C-2), 131.7 (d, C-3), 151.7 (s, C-4), 129.7 (s, C-4a), 152.8 (s, C-5), 153.9 (s, C-6), 121.1 (s, C-7), 116.4 (d, C-8), 125.6 (s, C-8a), 183.1 (s, C-9), 150.0 (s, C-9a), 187.4 (s, C-10), 115.7 (s, C-10a), 56.4 (q, OCH_3), 23.0 (q, CH_3-4); EIMS *m/z* 269 [M]$^+$ (100), 268 (33), 255 (4), 241 (21) 240 (62), 239 (17), 213 (4), 212 (16), 211 (24), 198 (15), 195 (15), 183 (20), 154 (29), 141 (20), 77 (24); HRFABMS [M + H]$^+$ *m/z* 270.0700 (calcd for $C_{15}H_{12}NO_4$, 270.0766).

5-Hydroxy-6,7-dimethoxycleistopholine (**5**): amorphous powder; UV (MeOH) λ_{max} (log ϵ) 212 (4.30), 242 (4.27), 282 (4.35), 400 (3.80) nm; IR (KBr) ν_{max} 1669, 1641, 1581, 1509, 1332 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.87 (1H, d, J = 4.8 Hz, H-2), 7.47 (1H, d, J = 4.8 Hz, H-3), 7.42 (1H, s, H-8), 12.61 (1H, s, OH), 4.04 (3H, s, OCH_3-7), 4.03 (3H, s, OCH_3 -6), 2.86 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 153.1 (d, C-2), 131.4 (d, C-3), 151.7 (s, C-4), 129.0 (s, C-4a), 156.5 (s, C-5), 141.5 (s, C-6), 158.8 (s, C-7), 104.2 (d, C-8), 129.4 (s, C-8a), 183.4 (s, C-9), 150.1 (s, C-9a), 185.7 (s, C-10), 112.0 (s, C-10a), 61.1 (q, OCH_3-6), 56.5 (q, OCH_3 -7), 22.9 (q, CH_3-4); EIMS *m/z* 299 [M]$^+$ (49), 298 (15), 284 (100), 282 (29), 271 (4), 270 (15), 268 (15), 185 (17) 141 (10), 77 (9); HRFABMS [M + H]$^+$ *m/z* 300.0863 (calcd for $C_{16}H_{14}NO_5$, 300.0872).

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References and Notes

(1) Chaves, M. H. Estudo Químico de *Porcelia macrocarpa*. Ph.D. Thesis, Universidade de São Paulo, São Paulo, São Paulo, Brazil, 1996.
(2) Chaves, M. H.; Roque, N. F. *Phytochemistry* **1997**, *44*, 523−528.
(3) Chaves, M. H.; Roque, N. F. *Phytochemistry* **1997**, *46*, 879−881.
(4) Bick, I. R. C.; Sévenet, T.; Sinchai, W.; Skelton, B. W.; White, A. H. *Aust. J. Chem.* **1981**, *34*, 195−207.
(5) Shamma, M.; Hindenlang, D. M. *Carbon-13 NMR Shift Assignments of Amines and Alkaloids*; Plenum Press: New York, 1979; p 303.
(6) Kashiwaba N.; Morooka, S.; Ono, M.; Toda, J.; Suzuki, H.; Sano, T. *Chem. Pharm. Bull.* **1997**, *45*, 545−548.
(7) Guinaudeau, H.; Leboeuf, M.; Cavé, A. *Lloydia* **1975**, *38*, 275−338.
(8) Leboeuf, M.; Cavé, A.; Tohami, M. E.; Pusset, J.; Forgacs, P.; Provost, J. *J. Nat. Prod.* **1982**, *45*, 617−623.
(9) Hughes, D. W.; MacLean, D. B. In *The Alkaloids*; Rodrigo, R. G. A., Ed.; Academic Press: New York, 1981; Vol. 18, Chapter 3, pp 217−262.
(10) Guinaudeau, H.; Leboeuf, M.; Cavé, A. *J. Nat. Prod.* **1983**, *46*, 761−835.
(11) Guinaudeau, H.; Leboeuf, M.; Cavé, A. *J. Nat. Prod.* **1994**, *57*, 1033−1135.
(12) Guinaudeau, H.; Leboeuf, M.; Cavé, A. *J. Nat. Prod.* **1988**, *51*, 389−474.

(13) Waterman, P. G.; Muhammad, I. *Phytochemistry* **1985**, *24*, 523−527.
(14) Tadic, D.; Cassels, B. K.; Leboeuf, M.; Cavé, A. *Phytochemistry* **1987**, *26*, 537−541.
(15) Kitahara, Y.; Onikura, H.; Shibano, Y.; Watanabe, S.; Mikami, Y.; Kubo, A. *Tetrahedron* **1997**, *53*, 6001−6010.
(16) Konoshima, T.; Kozuka, M.; Koyama, J.; Okatani, T.; Tagahara, K.; Tokuda, H. *J. Nat. Prod.* **1989**, *52*, 987−995.
(17) Berger, Y.; Castonguay, A.; Brassard, P. *Org. Magn. Reson.* **1980**, *14*, 103−108.
(18) Wu, T. S.; Jong, T. T.; Tien, H. J.; Kuoh, C. S.; Furukawa, H.; Lee, K. H. *Phytochemistry* **1987**, *26*, 1623−1625.
(19) Bracher, F. *Pharm. Ztg. Wiss.* **1993**, *5*, 109−117.

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