Isoquinoline Alkaloids and Lignans from *Rollinia mucosa*

Yuan-Yng Chen, Fang-Rong Chang, and Yang-Chang Wu*

Graduate Institute of Natural Products, Kaohsiung Medical College, Kaohsiung 807, Taiwan, Republic of China

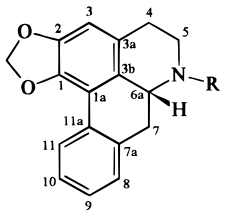
Received November 2, 1995[⊗]

Romucosine (**1**), a novel aporphinoid alkaloid possessing a *N*-(methoxycarbonyl) group, along with 12 known compounds including eight isoquinoline alkaloids, anonaine (**2**), glaucine, purpureine, liriodenine, oxoglaucine, oxopurpureine, berberine, and tetrahydroberberine, and four lignans, yangambin, magnolin, eudesmin, and membrin, have been isolated and characterized from the fresh unripe fruits of *Rollinia mucosa* (Annonaceae). The structure of **1** was established by chemical and spectroscopic means.

The genus *Rollinia* (Annonaceae) consists of about 65 species, some of which have been investigated for their chemical composition. One species, *Rollinia mucosa* Baill., has been used in folk medicine in the West Indies and in Indonesia for the treatment of tumors.[1]

The antineoplastic property may be due to the presence of acetogenins such as rolliniastatin 1 isolated in 1987 from the seeds of *R. mucosa* collected in French Guyana.[2] The alkaloidal extract of a sample collected in Brazil was shown to exhibit antimicrobial and antifungal activities.[3] Four aporphinoid alkaloids and one morphinandienone were isolated from its bark.[3] Three furofuranic lignans isolated from samples collected in Brazil and Peru were also reported to be effective in enhancing the toxicity of a wide variety of insecticides.[4]

As part of our continuing search for the bioactive compounds, e.g., antiplatelet aggregation, antitumor activity, and anti-HIV activity of Formosan Annonaceous plants, a new aporphinoid alkaloid, romucosine (**1**), along with a number of known compounds, anonaine (**2**), glaucine, purpureine, liriodenine, oxoglaucine, oxopurpureine, berberine, tetrahydroberberine, yangambin, magnolin, eudesmin, and membrin, were obtained by systematic extraction and isolation from the fresh unripe fruits of *R. mucosa*. In addition to **1**, the alkaloids glaucine, purpureine, oxoglaucine, oxopurpureine, berberine, tetrahydroberberine, eudesmin, and membrin were also found for the first time in this plant and genus.



Romucosine (**1**) was crystallized as colorless needles from $CHCl_3$. High-resolution MS revealed a $[M]^+$ at *m/z* 323.1152 (calcd 323.1158), corresponding to the molecular formula $C_{19}H_{17}O_4N$. The UV spectrum of **1** showed intense absorption bands at λ 235, 275, 292 (sh), and 325 (sh) nm, which suggested that it possesses the typical 1,2-substituted aporphine skeleton.[5] The IR spectrum of **1** exhibited absorption bands at ν_{max} 1680, 1040, and 920 cm^{-1}, indicating carbonyl and methylenedioxy groups, respectively. The ^1H NMR spectrum of **1** presented a doublet at δ 8.11 (1H, d, J = 7.6 Hz), a multiplet at δ 7.54−7.27 (3H, m), and a singlet at δ 6.59 (1H, s) in the aromatic region, in addition to a singlet due to a methoxycarbonyl group at δ 3.77 (3H, s) and two singlets due to methylenedioxy protons at δ 6.10 and 5.97 (each 1H, d, J = 1.5 Hz), accounting for 10 protons. This ^1H NMR pattern accommodated a substitution where the methylenedioxy group is placed at the 1,2-position of the aporphine skeleton. The other proton signals were at δ 4.86 (1H, dd, J = 13.7, 4.4 Hz), δ 4.44 (1H, m), and δ 3.06−2.61 (5H, m) for the seven aliphatic protons. Two significant downfield signals at δ 4.86 (1H, dd) for H-6a and δ 4.44 (1H, m) for H-5a indicated an electron-withdrawing group bonded to the nitrogen atom. The complete assignments of the relative configuration of aliphatic and aromatic protons of **1** was established by $^1H-^1H$ COSY and $^1H-^1H$ NOESY (Figure 1) experiments.

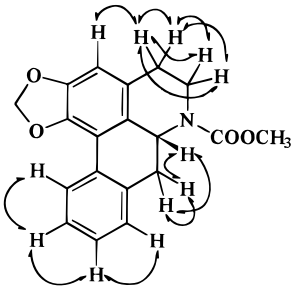


Figure 1. NOESY spectrum of **1**.

The significant correlation between H-6a and H-7, H-3, H-4, and H-5 as well as H-8, H-9, H-10, and H-11 was observed in the $^1H-^1H$ COSY and NOESY spectra. In particular, the protons of the carboxy methyl group at δ 3.77 did not correlate with any other protons. The ^{13}C NMR spectrum of **1**, which showed the chemical shift of carbonyl carbon at δ 155.80, 12 aromatic carbon atoms between δ 146.84 and 107.58, a methylenedioxy carbon atom at δ 100.89, as well as one carboxy methyl carbon atom at δ 52.66, provided the elucidation of this structure. The *N*-carbonyl and aromatic methoxy carbons in aporphines usually resonate at δ 161.7 and δ 56.0,[6] respectively, and in compound **1** these two signals occurred at δ 155.80 and 52.66. From the upfield shift of the resonance of the carboxy methyl carbon, the absence of functional group substitution in an aromatic moiety other than the methylenedioxy group, and

* To whom correspondence should be addressed. Tel.: 886-07-3114773. Fax: 886-07-3114773. E-mail: m825028 @cc.kmc.edu.tw.

[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1996.

S0163-3864(96)00414-4 CCC: $12.00

furthermore, on the basis of the chemical shift of H-6a and H-5a in the ^{1}H NMR, the chemical shift of the carbonyl carbon in the ^{13}C NMR and the absence of correlation of the methoxy group with any other proton in the NOESY spectrum, it was suggested that the methoxycarbonyl group was attached to the nitrogen atom. Although the spectral data supported the structure **1** for romucosine, the presence of an unprecedented *N*-(methoxycarbonyl) function could not be established unequivocally. Chemical evidence for the preparation of a *N*-(methoxycarbonyl) derivative was thus undertaken. Treatment of anonaine (**2**) with triethylamine and methyl chlorocarbonate gave a compound that had mp, UV, IR, TLC, and ^{1}H and ^{13}C NMR data identical to those of **1**. From the above discussion, romucosine (**1**) is a novel and the first aporphinoid alkaloid possessing an *N*-(methoxycarbonyl) group and had not been found before.

The identities of anonaine,[3,7] glaucine,[7] purpureine,[7] liriodenine,[7,9,10] oxoglaucine,[7] oxopurpureine,[7] berberine,[8] tetrahydroberberine,[8] yangambin,[4] magnolin,[4] eudesmin,[11] and membrin[12] were verified by comparison of the mp, IR, ^{1}H NMR, and/or ^{13}C NMR data with those published in the literature. Liriodenine, previously shown to be the main cytotoxic principle of *Thalictrum sessile*,[9] *Polyalthia longifolia*,[10] and *Artabotrys uncinatus*[13] was confirmed to have potent cytotoxicity against KB, A-549, HCT-8, P-388, and L-1210 cells, with ED_{50} values of 1.00, 0.72, 0.70, 0.57, and 2.33 μg/mL, respectively.[14] Moreover, liriodenine might act as a muscarinic receptor antagonist in isolated trachea ileum and cardiac tissue of guinea-pigs[15] and as a selective M3 receptor antagonist in canine tracheal smooth muscle.[16] Yangambin and magnolin showed antagonist activities against the platelet activating factor in the [^{3}H] PAF receptor binding assay.[17] Several furofuranic-type lignans are also active as cAMP phosphodiesterase inhibitors,[18] and some of them are effective in enhancing the toxicity of a wide variety of insecticides.[19]

Experimental Section

Plant Material. Fresh unripe fruits of *R. mucosa* were collected from Chia-Yi city, Taiwan, in June 1994. A voucher specimen is deposited in the Graduate Institute of Natural Products, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China.

Instrumentation and Chromatography. ^{1}H, ^{1}H−^{1}H COSY, and NOESY NMR spectra were recorded in $CDCl_3$ at 400 MHz and ^{13}C NMR spectra at 100 MHz on a Varian NMR spectrometer. Chemical shift values are shown in δ with tetramethylsilane (TMS) as an internal reference. All mass spectra (MS) and HRMS were taken under electron impact (EI) conditions using a JEOL JMS-SX/SX 102A mass spectrometer or Quattro GC/MS spectrometer having a direct inlet system. IR spectra were measured on a Hitachi 260-30 spectrophotometer, and UV spectra were obtained on a Hitachi 200-20 spectrophotometer in EtOH. Melting points were determined using a Yanagimoto micromelting point apparatus and were uncorrected. Si gel 60 (Macherey-Nagel, SIL G-25 UV254, 0.25 mm) and Sephadex LH-20 were used for open CC, and precoated Si gel glass plates (Macherey-Nagel, Alugram, SIL G/UV254, 0.25 mm) were used for preparative TLC.

Extraction and Isolation. Fresh unripe fruits of *R. mucosa* (11 kg) were extracted repeatedly with MeOH at room temperature for 7 days. The combined MeOH extracts were evaporated and partitioned to yield $CHCl_3$ and aqueous extracts. The $CHCl_3$ solution was extracted with 3% HCl to give a neutral $CHCl_3$ layer and an acidic aqueous layer. The latter was basified with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$-soluble fraction (35 g) gave a positive alkaloidal test with Dragendorff's reagent. The crude alkaloid fraction was chromatographed over Si gel (Macherey-Nagel, 1200 g) and eluted with increasing polarities of $CHCl_3$/EtOAc/MeOH mixtures. The eluted fractions (200 mL each) were monitored by TLC and combined into 18 fractions, out of which the fifth fraction afforded romucosine (**1**) (5.2 mg), anonaine (**2**) (3 mg), and berberine (23.6 mg) on elution with $CHCl_3$−MeOH (25:1), (30:1.5) and (80:5), respectively, while glaucine (4.5 mg) and tetrahydroberberine (31 mg) were isolated from the sixth fraction (Si gel 230−400 mesh) using $CHCl_3$−EtOAc−MeOH (10:2:1) and (20:4:1) as the eluting solvent system. Liriodenine (28.2 mg) was also eluted from the sixth alkaloidal fraction, while oxoglaucine (3 mg) was obtained from the ninth fraction on elution with $CHCl_3$−MeOH (85:15). Oxopurpureine (4.2 mg) and purpureine (6 mg) were eluted from the column using *n*-hexane−CH_2Cl_2−MeOH (2:7:2) and (3:5:1) as the solvent system in the twelfth fraction. Four lignans were isolated from the neutral $CHCl_3$ layer, which gave an amorphous residue (4 g). The crude residue was subjected to Si gel column chromatography using *h*-hexane−$CHCl_3$−MeOH as eluent. Further purification by chromatography on Sephadex LH-20 and preparative TLC yielded four lignans, yangambin [(52 mg), *n*-hexane−$CHCl_3$−MeOH (40:90:5)], magnolin [(35.6 mg), *n*-hexane−CH_2Cl_2−MeOH (30:95:5)], eudesmin [(35.6 mg), *n*-hexane−CH_2Cl_2−EtOAc (10:40:30)], and membrin [(5 mg), *n*-hexane−CH_2Cl_2−acetone (30:40:5)].

Romucosine (**1**) was obtained as colorless needles ($CHCl_3$): mp 152−153 °C; $[\alpha]^{25}_D$ −106.5° (*c* 0.04, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 235 (4.23), 275 (4.18), 292 (sh) (3.52), and 325 (sh) (3.41) nm; IR (Nujol) ν_{max} 1680, 1040, 920 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.11 (1H, d, J = 7.6 Hz, H-11), 7.54−7.28 (2H, m, H-9, 10), 7.27 (1H, m, H-8), 6.59 (1H, s, H-3), 6.10 and 5.97 (each 1H, d, J = 1.5 Hz, OC*H*$_2$O), 4.86 (1H, dd, J = 13.7, 4.4 Hz, H-6a), 4.44 (1H, m, H-5a), 3.77 (3H, s, N-COOC*H*$_3$), 3.06 (1H, m, H-7a), 2.99 (1H, m, H-5b), 2.91 (1H, m, H-7b), 2.82 (1H, m, H-4a), 2.61 (1H, m, H-4b); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.80 (s, N*C*OOCH$_3$), 146.84 (s, C-2), 143.01 (s, C-1), 135.76 (s, C-7a), 130.73 (s, C-11a), 128.67 (d, C-8), 127.79 (s, C-3a), 127.78 (d, C-9), 127.15 (d, C-10), 127.03 (d, C-11), 125.60 (s, C-3b), 117.31 (s, C-1a), 107.58 (d, C-3), 100.89 (t, O*C*H$_2$O), 52.66 (q, N-COO*C*H$_3$), 51.67 (d, C-6a), 39.15 (t, C-5), 34.53 (t, C-7), 30.35 (t, C-4); EIMS (70 eV) *m/z* [M]$^+$ 323 (98), 308 (28), 292 (5), 262 (20), 248 (21), 236 (81), 235 (100), 206 (17), 178 (27), 152 (3), 97 (4), 88 (17); HREIMS *m/z* [M]$^+$ 323.1152 (calcd for $C_{19}H_{17}O_4N$, 323.1158).

Preparation of *N*-(Methoxycarbonyl)anonaine (Romucosine (1)). Anonaine (22 mg) in dry CH_2Cl_2 (10 mL) was treated with triethylamine (3 μL), with stirring at 0 °C, for 10 min, and then methyl chlorocarbonate (2 mL) was slowly added. The reaction mixture was stirred for 10 min, and H_2O was added to quench excess reagent. The mixture was partitioned with $CHCl_3$ and then passed through a disposable pipet (0.6

× 6 cm) containing silica gel (230−400 mesh) and eluted with 5 mL of $CHCl_3$. Elution with $CHCl_3$ afforded colorless needles (7 mg) that were identified by comparison with **1** (mixed mp, co-TLC, UV, IR, ^1H and ^{13}C NMR).

Acknowledgment. This investigation was supported by a grant from the National Science Council of the Republic of China (NSC 83-0208-M-037-009) awarded to Y.-C.W.

References and Notes

(1) Hartwell, J. L. *Plants used against cancer*; Quarterman Publications, Inc.: Lawerence, MA, 1982; p 379.
(2) Pettit, G. R.; Cragg, G. M.; Polonsky, J.; Herald, D. L.; Goswami, A.; Smith, C. R.; Moretti, C.; Schmidt, J. M.; Weisleder, D. *Can. J. Chem.* **1987**, *65*, 1433−1435.
(3) Caetano, L. C.; Dadun, H. *J. Nat. Prod.* **1987**, *50*, 330.
(4) Paulo, M. D. Q.; Kaplan, M. A. C.; Laprevote, O.; Roblot, F.; Hocquemiller, R.; Cavé, A. *Fitoterapia* **1991**, *62*, 150−152.
(5) Shamma, M. *The Isoquinoline Alkaloids*; Academic Press: New York, 1972; p 221.
(6) Pachaly, P.; Adnan, A. Z.; Will, G. *Planta. Med.* **1992**, *58*, 184−187.
(7) Guinaudeau, H.; Leboeuf, M.; Cavé, A. *Lloydia* **1975**, *38*, 275−338.
(8) Shamma, M. *The Isoquinoline Alkaloids*; Academic Press: New York, 1972; pp 269−314.
(9) Wu, Y. C.; Lu, S. T.; Chang, J. J.; Lee, K. H. *Phytochemistry* **1988**, *27*, 1563−1564.
(10) Wu, Y. C.; Duh, C. Y.; Wang, S. K.; Chen, K. S.; Yang, T. H. *J. Nat. Prod.* **1990**, *53*, 1327−1331.
(11) Greger, H.; Hofer, O. *Tetrahedron* **1980**, *36*, 3551−3558.
(12) Saez, J.; Sahpaz, S.; Villaescusa, L.; Hocquemiller, R.; Cavé, A.; Cortes, D. *J. Nat. Prod.* **1993**, *56*, 351−356.
(13) Wu, Y. C.; Chen, C. H.; Yang, T. H.; Lu, S. T.; McPhail, D. R.; McPhail, A. T.; Lee, K. H. *Phytochemistry* **1989**, *28*, 2191−2195.
(14) Wu, Y. C.; Liou, Y. F.; Lu, S. T.; Chen, C. H.; Chang, J. J.; Lee, K. H. *Planta. Med.* **1989**, *55*, 163−165.
(15) Lin, C. H.; Chang, G. J.; Su, M. J.; Wu, Y. C.; Teng, C. M.; Ko, N. F. *Br. J. Pharmacol.* **1994**, *113*, 275−281.
(16) Lin, C. H.; Yang, C. M.; Ko, F. N.; Wu, Y. C.; Teng, C. M. *Br. J. Pharmacol.* **1994**, *113*, 1464−1470.
(17) Pan, J.-X.; Hensens, O. D.; Zink, D. L.; Chang, M. N.; Hwang, S.-B. *Phytochemistry* **1987**, *26*, 1377−1379.
(18) Massanet, G. M.; Pando, E. F.; Luis, R.; Zubia, E. *Fitoterapia* **1989**, *60*, 3−35.
(19) Mac Rae, W. D.; Towers, G. H. N. *Phytochemistry* **1984**, *23*, 1207−1220.

NP960414Z