Cerasodine and Cerasonine: New Oxoprotoberberine Alkaloids from *Polyalthia cerasoides*

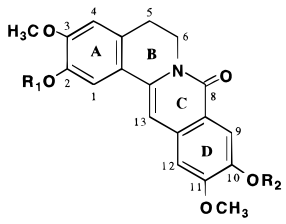
M. Carmen González, M. Carmen Zafra-Polo, M. Amparo Blázquez, Angel Serrano, and Diego Cortes*

Departamento de Farmacología, Farmacognosia y Farmacodinamia, Facultad de Farmacia, Universidad de Valencia, 46100 Burjassot, Valencia, Spain

Received August 12, 1996

Two new 7,8-dihydro-8-oxoprotoberberine alkaloids, cerasodine (**1**) and cerasonine (**2**), were isolated from the stem bark of *Polyalthia cerasoides* (Annonaceae).

Polyalthia cerasoides (Roxb.) Bedd. (Annonaceae) is a medium-sized tree occurring mainly in Asiatic and Oceanic areas.[1] In a previous study on *P. cerasoides* several prenylated benzopyran derivatives were isolated.[2−4] We discuss here the isolation and structure elucidation of two new oxoprotoberberine alkaloids, cerasodine (**1**) and cerasonine (**2**), from the EtOAc extract of *P. cerasoides* stem bark. Previous phytochemical studies on *Polyalthia* species had led to the isolation of several tetrahydroprotoberberine alkaloids,[5−8] some of which presented an *ortho*-diphenolic substitution on ring D, and exhibited cytotoxic, antioxidant, and dopaminergic activity.[9−11]



1: $R_1 = R_2 = H$
1a: $R_1 = R_2 = Ac$
2: $R_1 = H$; $R_2 = Me$
2a: $R_1 = Ac$; $R_2 = Me$

Results and Discussion

The EtOAc extract of *P. cerasoides* stem bark was fractionated by column chromatography on Si gel yielding alkaloids **1** and **2**, which were isolated as optically inactive oils. Compound **1** showed strong blue UV fluorescence and gave a blue spot with the Dragendorff spray reagent, characteristic of 8-oxoprotoberberines in general.[12] The IR spectrum displayed lactam carbonyl group (1636 cm^{-1}) and aromatic (1577 and 1508 cm^{-1}) absorption bands. The ^{1}H-NMR spectrum of **1** exhibited two 2H triplets at δ 2.88 and 4.33 ($J = 5.7$ Hz) assigned to protons of the [$-CH_2-CH_2-$] bonded to a pyridone.[12,13] In addition, a carbon resonance at δ 161.81 (aromatic lactam carbonyl) was observed in the ^{13}C-NMR spectrum. Two methoxyl groups at δ 3.99 and 4.01 and five singlets resonances between δ 6.79 and 7.95 were also observed. These spectral data, together with a $[M]^+$ at *m/z* 339 in the EIMS, suggested that **1** is a $C_{19}H_{17}NO_5$ compound with a 7,8-dihydro-8-oxoprotoberberine skeleton, substituted by two methoxyl and two hydroxyl groups, in agreement with the observed IR absorption band at 3343 cm^{-1} (OH) and the 19 carbon signals observed in the ^{13}C-NMR spectrum.

Compound **1** was transformed into its diacetyl derivative (**1a**) using Ac_2O in pyridine. The $[M]^+$ at *m/z* 423 in the EIMS and the two acetyl groups at δ 2.34 (3H, *s*) and 2.35 (3H, *s*) observed in the ^{1}H-NMR spectrum of **1a** confirmed the presence of two hydroxyl groups in **1**.

It remained to define the ^{1}H-NMR chemical shift assignments and the location of the oxygenated substituents on rings A and D. Although mass spectral evidence has been used repeatedly for structure assignment in the tetrahydroprotoberberine series, for example,[14] information on the fragmentation of 8-oxoprotoberberines is sparse. We initially had recourse to selective irradiations and NOE difference spectroscopy experiments. In both experiments, by irradiating the signal at δ 2.88 (H-5), the triplet at δ 4.33 (H-6) collapsed to a singlet, while the proton singlet at δ 6.80 was enhanced, thus indicating that this last signal belonged to H-4.

Assuming that in alkaloids with an 8-oxoprotoberberine skeleton the singlet signal resonance at δ 7.95 is characteristic of H-9, which is deshielded by the presence of a carbonyl group at C-8,[15] a hydroxy group must be located at C-10, because no NOE effect was observed after irradiation at δ 7.95. In addition, when the ^{1}H NMR was obtained in pyridine-d_5, a paramagnetic shift (0.58 ppm) of the proton resonance at lowest field, now at δ 8.53, was observed, confirming the H-9 position for this proton, near to a carbonyl function and *ortho* to a phenolic hydroxyl group.[16]

The ^{1}H-NMR assignments of the three remaining aromatic protons were confirmed by NOEDIFF experiments. The reciprocating enhancements of the resonance at δ 6.79 with the two other protons at δ 7.21 and 6.92, established δ 6.79 as the resonance of H-13. An NOE effect was also observed between the proton resonance at δ 6.92 and a methoxyl group at δ 4.01, in agreement with the chemical shift for H-12 in 7,8-dihydro-8-oxoprotoberberines with a OMe group at the C-11 position.[12] Thus, δ 7.21 was established as the resonance of H-1. Because a difference of δ 0.4 ppm is observed in values for H-1(δ 7.21) and H-4 (δ 6.80), a 2-OH, 3-OCH_3 substitution is proposed for the ring A,[17] also confirmed by the paramagnetic shift (0.42 ppm) observed for H-1 in pyridine-d_5 solution.

According to the ^{1}H NMR assigned δ values for **1**, H-1 (δ 7.21) appears downfield from H-13 (δ 6.79). These chemical shift assignments agree with those reported for 8-oxocoptisine (a 7,8-dihydro-8-oxoprotoberberine),[13] but are at variance with those made for other 8-oxoprotoberberine alkaloids, for example, 8-oxypseudopal-

* To whom correspondence should be addressed. Phone: (346) 386-49-75. Fax: (346) 386-49-43 E-mail: dcortes@uv.es.
⊗ Abstract published in *Advance ACS Abstracts,* February 1, 1997.

S0163-3864(96)00578-2 CCC: $14.00

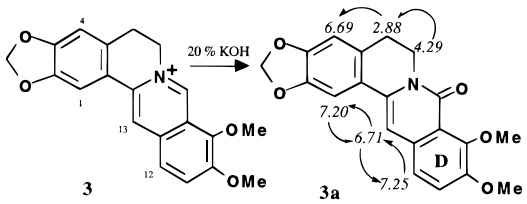
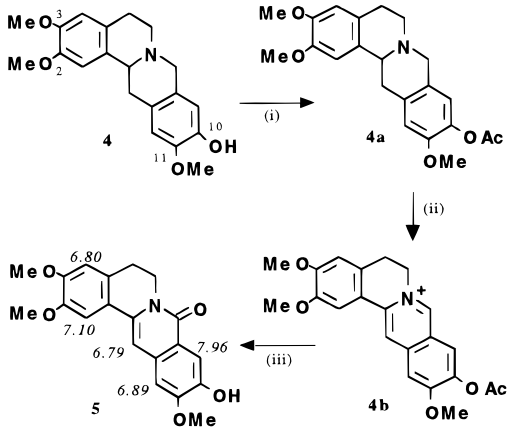


Figure 1. Preparation of oxyberberine (**3a**) from berberine chloride (**3**); and NOE's effects observed for **3a** ($CDCl_3$, 400 MHz).



(i) Ac_2O/Pyr; (ii) Iodine, reflux; (iii) 20 % KOH, reflux

Figure 2. Preparation of the 8-oxoprotoberberine (**5**) from (-)-10-demethylxylopinine (**4**).

matine, oxyberberine (**3a**), and oxypalmatine.[12,18] To clarify these differences, oxyberberine (**3a**) was semisynthesized from commercially available berberine chloride (**3**) by treatment with 20% aqueous KOH.[19] The ^{1}H-NMR chemical shift assignments for **3a** were confirmed by a detailed observation of NOE effects (Figure 1). In particular, irradiation at the frequency of the signal resonance at δ 6.71 (H-13) enhanced the signals at δ 7.20 (H-1) and 7.25 (H-12), similar to the enhancements observed for **1**. Our results corroborate the assignments made for **1** and show that the previously assigned ^{1}H-NMR chemical shifts for H-1 and H-13 in oxoberberine alkaloids need to be revised.

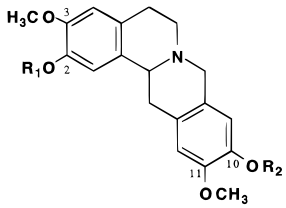
In order to confirm a 2,3,10,11-tetrasubstitution pattern in **1**, we have undertaken the semisynthesis of the 8-oxoberberine derivative (**5**) from (−)-10-demethylxylopinine (**4**), a known 2,3,10,11-tetrahydroprotoberberine isolated from *Guatteria ouregou*.[20]

As indicated in Figure 2, the hydroxyl group of **4** was first protected with an acetyl group (Ac_2O in pyridine) to give the monoacetate **4a**, which was then submitted to a controlled oxidation by iodine[21,22] to afford **4b** as an intermediate. Treatment of **4b** with 20% aqueous KOH led to the 7,8-dihydro-8-oxoprotoberberine (**5**), with simultaneous deacetylation.

Compound **5** showed UV fluorescence and ^{1}H-NMR spectral data similar to those of **1**, confirming the tetrasubstitution at C-2,C-3,C-10, and C-11 positions. Thus, **1** is 7,8-dihydro-2,10-dihydroxy-3,11-dimethoxy-8-oxoprotoberberine, a new natural compound to which the trivial name cerasodine has been proposed.

Compound **2** showed a $[M]^+$ peak at *m/z* 353 in the EIMS, 14 mass units greater than that for **1**. The obtention of a monoacetylated derivative (Ac_2O−Pyr) (**2a**) and the presence of an extra methoxyl group in the ^{1}H-NMR spectrum indicated that **2** is a monophenolic 8-oxoprotoberberine with molecular formula $C_{20}H_{19}NO_5$. Five aromatic proton resonances as singlets (similar to **1**) suggested a tetrasubstituted skeleton, again at positions 2,3,10, and 11. Allocation of the additional methoxyl group to ring D was clearly preferred because the H-9 proton resonance was shifted upfield from δ 7.95 in **1** to δ 7.80 in **2**, similar to the chemical shifts for this proton in 7,8-dihydro-10-methoxy-8-oxoprotoberberines, for example, 8-oxypseudopalmatine,[12] and the coincidence of the ^{1}H-NMR signals of the ring A of **2** with those of **1**. Thus, **2** is 7,8-dihydro-2-hydroxy-3,10,11-trimethoxy-8-oxoprotoberberine, a new natural compound to which the trivial name cerasonine has been proposed.

Our in vitro results duplicate the in vivo oxidation of a tetrahydroprotoberberine to a 7,8-dihydro-8-oxoprotoberberine *via* a protoberberine alkaloid, which had been reported previously.[23] In fact, a highly stereoselective and reversible enzyme system catalyzing the oxidation of tetrahydroprotoberberines to quaternary alkaloids is well known.[24] Because govadine (**6**) and the quaternary base of govanine (**7**) have been isolated from natural sources,[25,26] the biogenetic precursors of cerasodine (**1**) and cerasonine (**2**) should be govadine (**6**) and govanine (**7**), respectively.



6: $R_1 = R_2 = H$
7: $R_1 = H$; R_2 = OMe

Experimental Section

General Experimental Procedures. Optical rotations were determined with a Perkin-Elmer 241 polarimeter. IR spectra (film) were run on a Perkin-Elmer 843 spectrometer. UV spectra were taken on a Perkin-Elmer Lambda 15 UV/vis spectrophotometer in MeOH solutions. EIMS were determined on a VG Auto Spec Fisons spectrometer. ^{1}H- (250 or 400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Bruker AC-250 or a Varian Unity-400 instruments, using the solvent signal as reference ($CDCl_3$ at δ 7.26 and δ 77.0). Multiplicities of ^{13}C-NMR resonances were determined by DEPT experiments. Preparative TLC was carried out using precoated plates (Merck 7730) with CH_2Cl_2−MeOH (96:4) as the solvent system.

Plant Material. *P. cerasoides* (Annonaceae) was collected in December 1992, in the National Park of Varirata, located in the Central Province of Papua New Guinea, and was authenticated by P. Wanganigi and M. Kuduk (Department of Biology, Papua New Guinea University). A voucher specimen was deposited in the Herbarium of the University of Papua New Guinea.

Extraction and Isolation. Dried and powdered stem bark of *P. cerasoides* (550 g) was macerated with MeOH at room temperature. The concentrated MeOH extract (A) was first partitioned between H_2O and CH_2Cl_2[2] and then between H_2O and EtOAc (Extract B, 82 mg). The extract B was applied to a Si gel 60 H (Merck 7736) column chromatography and eluted with CH_2Cl_2−MeOH (93:7); fractions 4−13 were combined, and the residue was subjected to preparative TLC with

CH_2Cl_2−MeOH (96:4), to afford compounds **1** (12 mg) and **2** (3.5 mg).

Cerasodine (1): $C_{19}H_{17}NO_5$; optically inactive oil; IR (EtOH) ν max 3343, 2923, 1636, 1577, 1508, 1465, 1408, 1334, 1278, 1234 cm^{-1}; UV (EtOH) λ max (log ϵ) 238 (3.61), 262 (3.62), 336 (3.60) nm; EIMS *m/z* $[M]^+$ 339 (100), 324 (68), 309 (8), 296 (9), 281 (4), 264 (5), 207 (11), 195 (3), 170 (10), 162 (4), 153 (4), 148 (5); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.95 (1H, s, H-9), 7.21 (1H, s, H-1), 6.92 (1H, s, H-12), 6.80 (1H, s, H-4), 6.79 (1H, s, H-13), 4.33 (2H, t, J = 5.7 Hz, H-6), 4.01 (3H, s, OMe), 3.99 (3H, s, OMe), 2.88 (2H, t, J = 5.7 Hz, H-5); ^1H NMR (pyridine-d_5, 250 MHz) δ 2.73 (2H, t, J = 5.9 Hz, H-5), 3.84 (3H, s, OMe-3), 3.90 (3H, s, OMe-11), 4.45 (2H, t, J = 5.9 Hz, H-6), 7.04 (1H, s, H-13), 7.12 (1H, s, H-4), 7.22 (1H, s, H-12), 7.61 (1H, s, H-1), 8.53 (1H, s, H-9); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 27.93 (C-5), 39.78 (C-6), 56.03 (OCH_3), 56.20 (OCH_3), 101.12 (C-1), 105.37 (C-13), 106.96 (C-4), 111.69, 113.74 (C-9 and C-12), 119.48 (C-14), 122.23 (C-8a), 129.16 (C-4a), 131.82 (C-12a), 135.90 (C-14a), 145.49, 146.05, 146.75, 151.60 (C-2, C-3, C-10, and C-11), 161.81 (C-8).

Cerasonine (2): $C_{20}H_{19}NO_5$; optically inactive oil; IR (EtOH) ν max 3340, 2929, 2848, 1653, 1583, 1507, 1464, 1258, 1235 cm^{-1}; UV (EtOH) λ max (log ϵ) 229 (3.50), 260 (3.42), 333 (3.25) nm; EIMS *m/z* $[M]^+$ 353 (100), 338 (38), 323 (5), 281 (3), 239 (27) 208 (28), 207 (35), 189 (16), 177 (15), 176 (14), 175 (14), 164 (39), 163 (13); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.80 (1H, s, H-9), 7.23 (1H, s, H-1), 6.93 (1H, s, H-12), 6.81 (2H, s, H-4 and H-13), 4.34 (2H, t, J = 5.9 Hz, H-6), 4.01 (3H, s, OMe), 4.00 (3H, s, OMe), 3.99 (3H, s, OMe), 2.90 (2H, t, J = 5.9 Hz, H-5).

O-Acetylation of Cerasodine (1). Treatment of **1** (4.2 mg) with Ac_2O−Pyr, yielded **1a** (90%): IR (EtOH) ν max 2917, 2848, 1734, 1646, 1596, 1506, 1457, 1367, 1258, 1195, 1089 cm^{-1}; EIMS *m/z* $[M]^+$ 423 (31), 381 (39), 339 (100), 324 (23), 236 (6), 191 (6); ^1H NMR ($CDCl_3$, 250 MHz) δ 8.05 (1H, s, H-9), 7.33 (1H, s, H-1), 7.01 (1H, s, H-13), 6.97 (1H, s, H-12), 6.89 (1H, s, H-4), 4.35 (2H, t, J = 5.8 Hz, H-6), 3.95 (3H, s, OMe), 3.94 (3H, s, OMe), 2.92 (2H, t, J = 5.8 Hz, H-5), 2.35 (3H, s, OAc), 2.34 (3H, s, OAc).

O-Acetylation of Cerasonine (2). Treatment of **2** (2 mg) with Ac_2O−Pyr, yielded **2a** (92%): IR (EtOH) ν max 2920, 2850, 1734, 1646, 1595, 1457, 1368, 1257, 1094, 1017cm^{-1}; EIMS *m/z* $[M]^+$ 395 (46), 353 (100), 339 (19), 338 (71), 295 (9), 236 (4), 177 (4), 150 (5), 97 (4), 57 (8); ^1H NMR ($CDCl_3$, 250 MHz) δ 7.84 (1H, s, H-9), 7.33 (1H, s, H-1), 6.98 (2H, s, H-12 and H-13), 6.84 (1H, s, H-4), 4.36 (2H, t, J = 5.8 Hz, H-6), 4.02 (3H, s, OMe), 4.01 (3H, s, OMe), 3.92 (3H, s, OMe), 2.94 (2H, t, J = 5.8 Hz, H-5), 2.35 (3H, s, OAc).

Oxidation of Berberine (3). Berberine chloride (**3**) (50 mg, 0.134 mmol) was dissolved in 20% aqueous KOH (20 mL). The solution was refluxed for 6 h at 80 °C, when TLC indicated complete reaction. The reaction mixture was extracted with CH_2Cl_2 (3 × 15mL). The combined CH_2Cl_2 extracts were dried, filtered, and evaporated to yield oxyberberine (**3a**, 46.5 mg, 98.5%). For ^1H-NMR and NOE data, see Figure 1.

O-Acetylation of 10-Demethylxylopinine (4). Treatment of **4** (3.9 mg) with Ac_2O−Pyr yielded **4a** (4.2 mg, 96%): IR (EtOH) ν max 2926, 2848, 1761, 1636, 1609, 1510, 1457, 1365, 1259, 1228, 1200, 1095 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 2.30 (3H, s, OAc), 6.62, 6.71, 6.75 and 6.76 (1H each, s, aryl protons).

Oxidation of 4a. 10-Demethylxylopinine monoacetate, (**4a**) (4.2 mg, 0.011 mmol) was dissolved in MeOH (10 mL) with stirring, and iodine (7 mg, 0.028 mmol) was added. The mixture was refluxed for 4 h, cooled to room temperature, and several drops of saturated $Na_2S_2O_3$ were added to reduce excess iodine.[22] The reaction mixture was evaporated to eliminate the MeOH, and the aqueous portion was extracted with CH_2Cl_2 (3 × 15 mL). The combined CH_2Cl_2 extracts were concentrated to yield a yellow compound (**4b**) (2.5 mg, 0.0067mmol, 60.7%). To a solution of **4b** (2.5 mg) in MeOH (10 mL), a 20% aqueous KOH (10 mL) was added. The mixture was refluxed for 9 h at 80 °C. The reaction mixture was extracted with CH_2Cl_2 (3 × 15mL). The combined CH_2Cl_2 extracts were dried, filtered, and evaporated to yield **5** (0.8 mg, 0.0023 mmol, 34.3%): EIMS *m/z* $[M]^+$ 353 (100); ^1H NMR ($CDCl_3$, 250 MHz) δ 7.96 (1H, s, H-9), 7.10 (1H, s, H-1), 6.89 (1H, s, H-12), 6.80 (1H, s, H-4), 6.79 (1H, s, H-13).

Acknowledgment. This research was supported by the Spanish DGICYT under grant PB 93-0682. One of us (M. C. G.) wishes to thank Conselleria d'Educació i Ciència, Generalitat Valenciana, for the award of a Researchship. The authors also thank Dr.K. S. Rao, for providing the plant material.

References and Notes

(1) Fries, R. E. In *Die Natürlichen Pflanzenfamilien*; Engler, A., Prantl, K. Eds.; Dunker et Humblot: Berlin, 1960; 17 a II, pp 93−94.
(2) González, M. C.; Serrano, A.; Zafra-Polo, M. C.; Cortes, D.; Rao, K. S. *J. Nat. Prod.* **1995**, *58*, 1278−1284.
(3) González, M. C.; Sentandreu, M. A.; Rao, K. S.; Zafra-Polo, M. C.; Cortes, D. *Phytochemistry* **1996**, *43*, 1361−1364.
(4) Zafra-Polo, M. C.; González, M. C.; Tormo, J. R.; Estornell, E.; Cortes, D. *J. Nat. Prod.* **1996**, *59*, 913−916.
(5) Guinaudeau, H.; Ramahatra, A.; Leboeuf, M.; Cavé, A. *Plant. Méd. Phytother.* **1978**, *12*, 166−172.
(6) Zarga, M. H. A.; Shamma, M. *J. Nat. Prod.* **1982**, *45*, 471−475.
(7) Jossang, A.; Leboeuf, M.; Cabalion, P.; Cavé, A. *Planta Med.* **1983**, *49*, 20−24.
(8) Lavault, M.; Guinaudeau, H.; Bruneton, J.; Sévenet, T.; Hadi, H. A. *Phytochemistry* **1990**, *29*, 3845−3847.
(9) Dai, J. R; Chai, H.; Pezzuto, J. M.; Kinghorn, A. D.; Tsauri, S.; Padmawinata, K. *Phytother. Res.* **1993**, *7*, 290−294.
(10) Misík, V.; Bezáková, L.; Máleková, L.; Kostálová, D. *Planta Med.* **1995**, *61*, 372−373.
(11) Cortes, D.; Arbaoui, J.; Protais, P. *Nat. Prod. Lett.* **1993**, *3*, 233−238.
(12) Patra, A.; Montgomery, C. T.; Freyer, A. J.; Guinaudeau, H.; Shamma, M.; Tantisewie, B.; Pharadai, K. *Phytochemistry* **1987**, *26*, 547−549.
(13) Atta-ur-Rahman; Ahmad, S.; Bhatti, M. K.; Choudhary, M. I. *Phytochemistry* **1995**, *40*, 593−596.
(14) Chen, C. Y.; MacLean, D. B. *Can. J. Chem.* **1968**, *46*, 2501−2506.
(15) Zhang, J. S.; Le Men-Olivier, L.; Massiot, G. *Phytochemistry* **1995**, *39*, 439−442.
(16) Ricca, S. G.; Casagrande, C. *Gazz. Chim. Ital.* **1979**, *109*, 1−6.
(17) Ohiri, F. C.; Verpoorte, R.; Svendsen, A. B. *Planta Med.* **1983**, *49*, 162−164.
(18) Pinho, P. M. M.; Pinto, M. M. M.; Kijoa, A.; Pharadai, K.; Díaz, J. G.; Herz, W. *Phytochemistry* **1992**, *31*, 1403−1407.
(19) Shamma, M.; Smeltz, L. A. *Tetrahedron Lett.* **1976**, *18*, 1415−1418.
(20) Leboeuf, M.; Cortes, D.; Hocquemiller, R.; Cavé, A. *Planta Med.* **1983**, *48*, 234−245.
(21) Dyke, S. F.; Tiley, E. P. *Tetrahedron* **1975**, *31*, 561−568.
(22) Suau, R.; Silva, M. V.; Valpuesta, M. *Tetrahedron* **1991**, *47*, 5841−5846.
(23) Shamma, M.; Rahimizadeh, M. *J. Nat. Prod.* **1986**, *49*, 398−405.
(24) Bauer, W.; Zenk, M. H. *Tetrahedron Lett.* **1991**, *32*, 487−490.
(25) Mehra, M. K.; Garg, H. S.; Bhakuni, D. S.; Khanna, N. H. *Indian J. Chem.* **1976**, *14B*, 216−217.
(26) Moulis, C.; Gleye, J.; Stanislas, E. *Phytochemistry* **1977**, *16*, 1283−1287.

NP960578F