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PUNTARENINE: AN UNUSUAL ISOQUINOLINE ALKALOID

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Abstract: The novel alkaloid (±)-puntarenine (2) has been isolated from Berberis empetrifolia Lam. (Berberidaceae), where it co-occurs with the known alkaloids berberine (1) and (±)-chilenine (9).

The approximately 120 naturally occurring berbines occupy a focal node within the biogenetic progression represented by the isoquinoline alkaloids. Berbines may be formed in vivo from a variety of tetrahydrobenzylisoquinolines,[1] while they themselves may act as precursors to the protopines, the phthalideisoquinolines, the benzophenanthridines, and even to some of the aporphines.[2] A typical berbine alkaloid is berberine (1) which is present in all plants belonging to the genus Berberis (Berberidaceae).

We describe here the novel, colorless, base puntarenine (2), $C_{21}H_{19}O_6N$, which is probably derived from berberine (1) in a series of steps, one of which involves the acquisition of an extra carbon atom.

Work-up of 20 kg of the stems and above ground wood of Berberis empetrifolia Lam. collected near the town of Punta Arenas, in Chilean Patagonia, yielded 46 mg of the racemic, crystalline, alkaloid, mp 214-216° C (MeOH), ν max $CHCl_3$ 1642 (amide) and 1690 cm^{-1} (conj. ketone), λ max MeOH 231, 278, 316 nm (log ε 3.93, 3.45, 3.44). The 360 MHz (FT) NMR spectrum of puntarenine in $CDCl_3$ has been summarized around expression 2. Of particular import are the two-proton doublet of doublets at δ 3.00 and 3.88 (J_{gem} 19 Hz) representing the C-9 protons, and the one-proton singlet at δ 5.16 due to H-14.[3] Noteworthy also are the chemical shifts of the two adjacent aromatic protons of ring D which are separated by only δ 7.07 - 6.90 = 0.17 ppm. This simple observation serves to

eliminate from consideration alternate expression 3 for puntarenine since it is known that the chemical shifts for two adjacent aromatic protons in a ring conjugated to a ketone, as in partial structure 4, are separated by about 0.5 ppm.[4]

The ^{13}C NMR spectrum of puntarenine in $CDCl_3$ displays peaks at 169.0 ppm (amidic C-8) and 198.9 ppm (ketonic C-15), and has been indicated in expression 2a.

The mass spectrum of puntarenine (2) shows molecular ion peak m/z 381 (50), and base peak m/z 352 $(M - CO - H)^+$. Particularly relevant in support of structure 2 for puntarenine are peaks m/z 205 (28) and 190 (21). The former is due to fragment 5, and the latter to loss of a methyl group from 5.

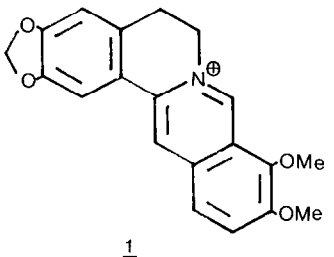
Acetylation of 2 with acetic anhydride in pyridine at room temperature led to puntarenine enol acetate (6), $C_{23}H_{21}O_7N$, ν max $CHCl_3$ 1655 (amide) and 1755 cm^{-1} (enol acetate), λ max MeOH 210, 318 nm (log ε 4.03, 3.80), whose NMR spectrum is marked by the absence of the δ 5.16 singlet present in the spectrum of 2.

Sodium borohydride in methanol reduction of 2 led to dihydropuntarenine (7), $C_{21}H_{21}O_6N$, ν max $CHCl_3$ 1635 (amide) and 3610 cm^{-1} (hydroxyl); λ max MeOH 212, 283 nm (log ε 4.46, 3.65). The base peak in the mass spectrum is now m/z 205, corresponding again to ion 5.

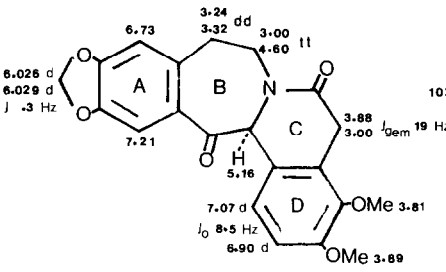
The most salient feature of the NMR spectrum of 7 is that H-14 and 15 appear as singlets at δ 4.63 and 4.73, even though they lie on adjacent carbon atoms. Molecular models show that species 7 exists in fairly rigid conformation 7a in which ring C is nearly planar, while the dihedral angle between H-14 and 15 is close to 90°, thus accounting for the zero coupling constant. Acetylation of 7, using acetic anhydride in pyridine, provided dihydropuntarenine acetate (8), $C_{23}H_{23}O_7N$, whose NMR spectrum displays singlets for H-14 and 15 at δ 4.81 and 5.69, respectively.

Conclusive proof for the structure of puntarenine was furnished by an NMR NOE study of 8, which connected the key hydrogen atoms present in an interlocking grid. This study has been summarized in expression 8a. Irradiation of the core C-14 proton (δ 4.81) caused NOE's of 10% for H-6 (δ 2.84), 8.1% for H-15 (δ 6.59), and 15% for H-13 (δ 6.97). The H-13 absorption also shows a 5.7% enhancement upon irradiation of H-12 (δ 6.88). It follows that the two methoxyl substituents in the bottom ring are located at C-10,11 rather than at C-12,13. Another significant result is that irradiation of the C-11 methoxyl singlet (δ 3.88) led to a 16% NOE of H-12 (δ 6.88).

The closest known natural analog of (±)-puntarenine (2) is (±)-chilenine (9) which is found in the same plant.[5] Chilenine (9) is probably formed in nature from berberine (1), and in fact can be readily derived from berberine by *in vitro* chemical oxidation.[6] An intriguing possibility is



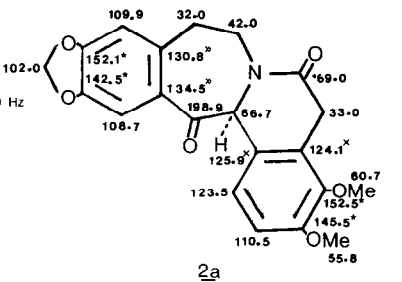
1



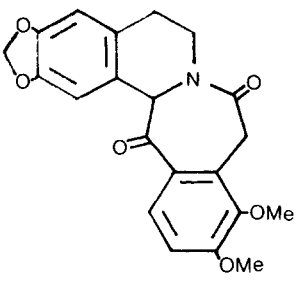
2

H-5 : J_1 8.0, J_2 13.0 Hz.

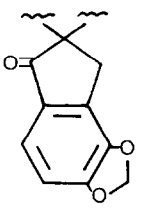
H-6 : J_1 7.0, J_2 19.0 Hz.



2a



3



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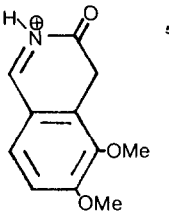
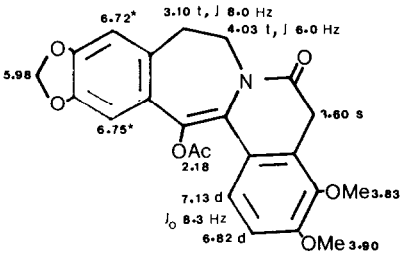


5



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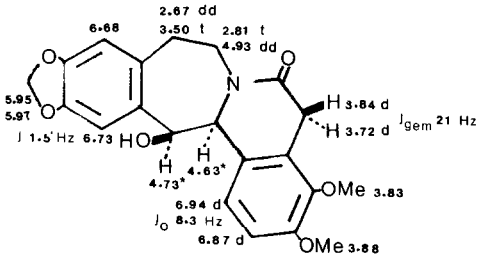


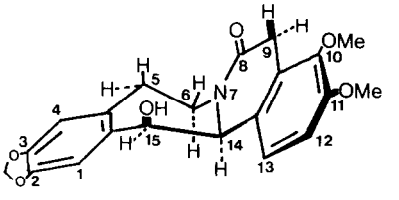
7

H-5α : J_1 5.0, J_2 14.0 Hz.

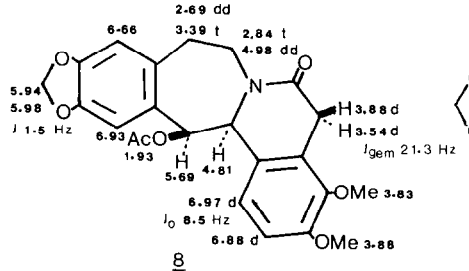
H-5β : J 12.0 Hz.

H-6α : J 12.5 Hz.

H-6β : J_1 5.0, J_2 14.0 Hz.



7a



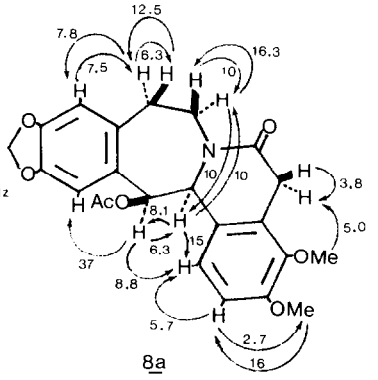
8

H-5α : J_1 5.5, J_2 15.7 Hz.

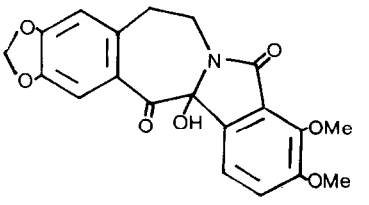
H-5β : J 12.2 Hz.

H-6α : J 12.2 Hz.

H-6β : J_1 6.4, J_2 15.4 Hz.



8a



9

that chilenine (9) or one of its close analogs may undergo in vivo homologation to provide puntarenine. If such were the case, we would be witnessing an interesting new biogenetic pathway from a berbine alkaloid.

TABLE: Mass Spectral Properties of Puntarenine and Derivatives

Puntarenine (2): ms m/z 381 $(M)^+$ (50), 353 (44), 352 (100), 322 (58), 294 (12), 206 (15), 205 (28), 190 (21), 176 (44), 175 (45), 148 (25).

Puntarenine Enol Acetate (6): ms m/z 423 $(M)^+$ (33), 380 (100), 352 (38), 322 (12), 205 (7), 175 (20), 147 (14).

Dihydropuntarenine (7): ms m/z 383 $(M)^+$ (29), 365 (6), 354 (9), 220 (14), 207 (55), 206 (72), 205 (100), 193 (11), 192 (85), 178 (35), 176 (55), 163 (18), 149 (36), 148 (33), 135 (27).

Dihydropuntarenine Acetate (8): ms m/z 425 $(M)^+$ (29), 382 (59), 248 (12), 220 (10), 218 (15), 217 (11), 206 (29), 205 (81), 192 (16), 191 (17), 177 (100).

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References and Footnotes

1. Berbines are formed in nature not only from (+)-reticuline and its close analogs, but also from tetrahydrobenzylisoquinolines originating from the breakdown of berbines. See V. Preininger, V. Šimanek and F. Šantavý, Tetrahedron Lett., 2109 (1969).
2. S.F. Hussain, M.T. Siddiqui, G. Manikumar and M. Shamma, Tetrahedron Lett., 21, 723 (1980).
3. It is difficult to tell if the "extra" carbon that is intercalated biogenetically is C-8 or C-9.
4. S.-T. Lu, T.-L. Su, T. Kametani, A. Ujiie, M. Ihara and K. Fukumoto, J. Chem. Soc., Perkin I, 63 (1976).
5. V. Fajardo, V. Elango, B.K. Cassels and M. Shamma, Tetrahedron Lett., 23, 39 (1982).
6. J.L. Moniot, D.M. Hindenlang and M. Shamma, J. Org. Chem., 44, 4343 (1979); and G. Manikumar and M. Shamma, Heterocycles, 14, 827 (1980).
7. Compounds with unspecified melting points are amorphous. All NMR spectra are at 360 MHz in $CDCl_3$. NMR chemical shifts with identical superscripts are interchangeable.

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