BERBERINE AND COPTISINE IN LIQUID AMMONIA

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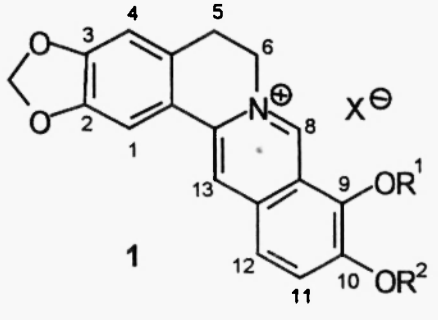
Abstract: The reactions of the quaternary protoberberine alkaloids berberine and coptisine with liquid ammonia were investigated. 8-Amino-8*H*-berberine and 8-amino-8*H*-coptisine were obtained as crystals directly from the reaction mixture. Their structures were determined by X-ray analysis and mass spectrometry.

Introduction

Yellow berberine (1a) and orange coptisine (1b) are quaternary protoberberine alkaloids derived from 5,6-dihydrodibenzo[*a,g*]quinolizinium system. They were found in many plant species of the *Berberidaceae*, *Fumariaceae*, *Papaveraceae*, *Rutaceae*, *Ranunculaceae* and other families. In Europe, probably the best source of berberine is root bark of *Berberis vulgaris* L. (1). Coptisine is the main alkaloid of *Chelidonium majus* L. (2-4). Protoberberine alkaloids are interesting for their diverse biological and pharmacological activities, e.g. antimicrobial, antiprotozoal, fungicidal, herbicidal, anti-inflammatory, anti-diarrhoeal (5-9). The chemical reactivity of the quaternary alkaloids relates to the positive charge on the nitrogen atom N7. The typical reaction of iminium cations 1 is a nucleophilic attack on the activated double bond $C=N^+$ (10-12). The products are usually unstable species, especially in acidic environment. The adducts with carbon-nucleophiles are more stable than oxygen- or nitrogen-nucleophile adducts. In this communication, we describe the conversions of berberine and coptisine in liquid ammonia.



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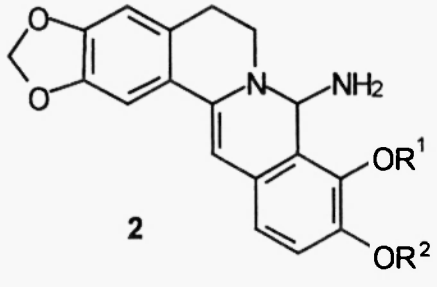

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a $R^1 = R^2 = CH_3$ *b* $R^1 + R^2 = CH_2$

Experimental

Melting points were determined on a Kofler hot-stage and are uncorrected. Berberine hydrogensulfate and coptisine chloride were donated by Professor Jiri Slavík (Department of Biochemistry, Faculty of Medicine, Masaryk University Brno). Mass spectra were recorded on a FISONS TRIO 1000 quadrupole spectrometer in electron impact mode (20 eV). Diffraction data were collected on a KUMA KM-4 κ-axis diffractometer using either ω-2θ (**2a**) or ω scan mode (CCD camera, **2b**). Both structures were solved by direct methods and refined by full-matrix least-squares methods using SHELXTL program package (13). The hydrogen atoms were positioned geometrically and refined as riding.

8-Amino-8H-berberine (2a)

Berberine hydrogensulfate (**1a**, X = HSO_4, 12 mg) was placed into a glass tube (2 mm i.d.). The tube was evacuated and cooled down to –70 °C. Liquid ammonia (0.5 ml) was condensed into the tube. The reaction mixture was allowed to stand at ambient temperature and then shaken thoroughly. Ammonia was slowly evaporated (within 5 hrs). A few colourless crystals of **2a** (1.4 mg) were found on the walls of the tube. M.p. 145–151 °C; MS-EI, m/z (%): 352 (M^+, 1), 350 (12), 337 (96), 336 (M – NH_2, 97), 335 (58), 322 (45), 320 (58), 307 (30). X-ray: Table 1, 2; Fig. 1, 3.

8-Amino-8H-coptisine (2b)

Coptisine chloride (**1b**, X = Cl, 9 mg) was placed into a tube and treated like **1a**. After two weeks, a few red crystals of **2b** (7 mg) were found on the bottom of the test tube. M.p. >161 °C, decomposition; MS-EI, m/z (%): 336 (M^+, 0.5), 334 (45), 320 (M – NH_2, 100), 305 (13), 290 (14), 262 (10). X-ray: Table 1,2; Fig. 2,4.

Results and Discussion

The first experiments were carried out with berberine in the tubes of 10 mm i.d. where ammonia was condensed. Under these conditions we observed no crystal formation. The product was white amorphous material that after exposing to ambient atmosphere instantly changed its colour to yellow. TLC and NMR analysis of the yellow product proved quaternary berberine (**1a**). Therefore a special approach to the conversion was required. Ammonia was condensed into a tube of very small internal diameter with berberine hydrogensulfate (**1a**, X = HSO_4) and the vessel was tightened up. After some time at ambient temperature the excess of ammonia was very slowly evaporated through a reduction valve. On the walls of the tube there were found small crystals of **2a**, relatively stable under laboratory conditions. The same procedure was applied for the conversion of coptisine chloride (**1b**, X = Cl). Unlike berberine, coptisine chloride was well soluble in liquid ammonia and afforded larger crystals of aminoadduct **2b** (Table 1).

Both products were analysed by NMR spectroscopy, mass spectrometry, and X-ray analysis. However, NMR measurements failed because the aminoadducts **2** immediately decomposed in the solvents used ($CDCl_3$, DMSO-d_6). Mass spectrometry could supported the structures only if the low electron potential (20 eV) was used for the ionisation. The molecular ions of the compounds **2** had relatively low intensities. This finding points to low stability of a structure with aminal moiety.

All bond lengths and bond angles in **2a** and **2b** are within normal range. Selected geometrical parameters are listed in Table 2. The conformation of partially hydrogenated ring C4a-C5-C6-N7-C13a-C13b resembles a distorted half-chair with atoms C5 and C6 significantly deviated from the plane of adjacent aromatic ring C1-C2-C3-C4-C4a-C13b (Fig. 1, 2). Aminogroups in both compounds are in axial positions with respect to ring conformations.

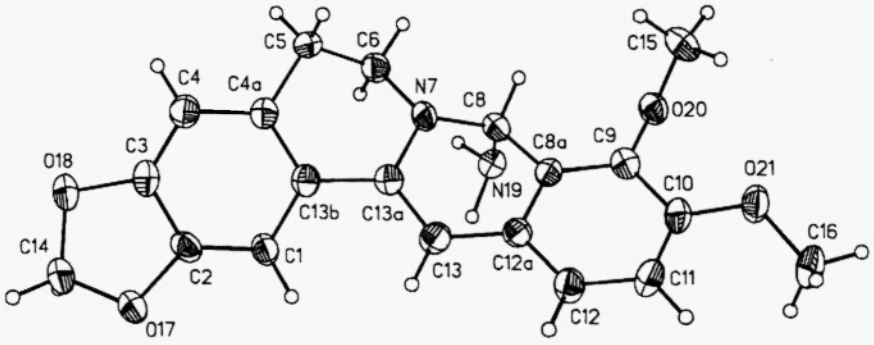

Fig. 1. A perspective view of the compound **2a**. Ellipsoids are drawn at the 50% probability level.

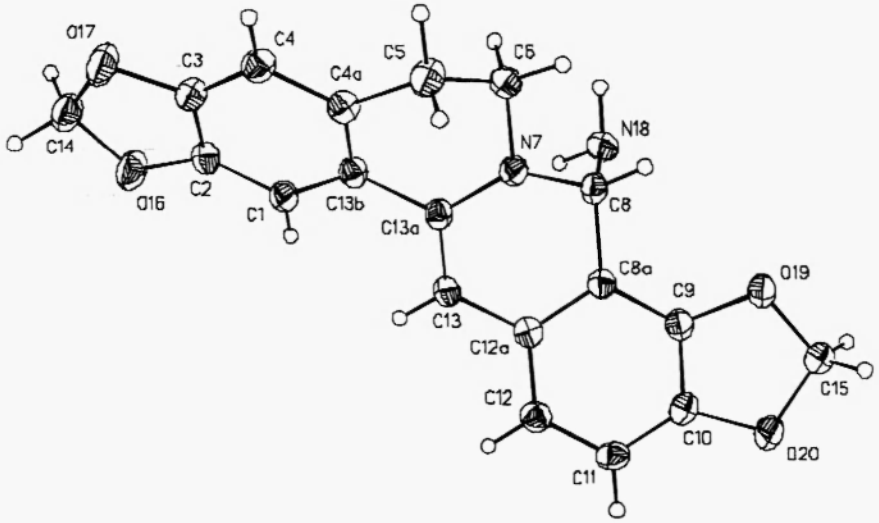

Fig. 2. A perspective view of the compound **2b**. Ellipsoids are drawn at the 50% probability level.

The C10-OMe methoxy group in the aminoadduct **2a** lies almost in the plane of adjacent aromatic ring; the corresponding torsion angles are: C11-C10-O21-C16 (14.5 °) and C9-C10-O21-C16 (-164.0 °). The neighbouring methoxyl at C9 is nearly perpendicular to the same plane as it follows from the torsion angles C8a-C9-O20-C15 (103.0 °), C10-C9-O20-C15 (-81.6 °).

Table 1. Crystal data of the compounds **2a** and **2b**.

Parameter	8-Amino-8*H*-berberine (**2a**)	8-Amino-8*H*-coptisine (**2b**)
Empirical formula	$C_{20}H_{20}N_2O_4$	$C_{19}H_{16}N_2O_4$
Formula weight	352.38	336.34
Temperature	150(2) K	150(2) K
Wavelength	0.71073 Å	0.71073 Å
Crystal system, space group	Monoclinic, *P2(1)/a*	Monoclinic, *P2(1)/c*
Unit cell dimensions	a = 16.397(3) Å α = 90 °	a = 11.245(2) Å α = 90 °
	b = 4.7430(10) Å β = 95.60(3) °	b = 18.916(4) Å β = 106.91(3) °
	c = 21.594(4) Å γ = 90 °	c = 7.3610(10) Å γ = 90 °
θ range for data collection	3.77 to 25.00 °	3.66 to 30.88 °
Reflections coll. / Unique / Observed	9406 / 2861 / 1924	10784 / 3803 / 3097
Data / Restraints / Parameters	2861 / 0 / 236	3803 / 0 / 226
Volume	1671.4(6) Å^3	1498.1(5) Å^3
Z, Calculated density	4, 1.400 Mg/m^3	4, 1.491 Mg/m^3
Absorption coefficient	0.099 mm^{-1}	0.106 mm^{-1}
Crystal size	0.15 × 0.10 × 0.08 mm	0.3 × 0.2 × 0.2 mm
Final R indices [$I > 2\sigma(I)$]	R1 = 0.0594, wR2 = 0.1170	R1 = 0.0572, wR2 = 0.1380
R indices (all data)	R1 = 0.1007, wR2 = 0.1508	R1 = 0.0704, wR2 = 0.1567

Table 2. Selected geometrical parameters of the compounds 2a and 2b.

Bond	Lenghts (Å)	
	2a	**2b**
C(4A)-C(13B)	1.401(4)	1.397(2)
C(4A)-C(5)	1.496(4)	1.504(2)
C(5)-C(6)	1.510(4)	1.515(3)
C(6)-N(7)	1.462(4)	1.452(2)
N(7)-C(13A)	1.406(4)	1.395(2)
N(7)-C(8)	1.477(4)	1.468(2)
C(8A)-C(9)	1.377(4)	1.377(2)
C(8)-N(19)	1.461(4)	-
C(9)-C(10)	1.402(4)	1.377(2)
N(18)-C(8)	-	1.465(2)
C(8)-C(8A)	1.509(4)	1.502(2)
C(8A)-C(12A)	1.407(4)	1.411(2)
C(12A)-C(13)	1.442(4)	1.447(2)
C(13)-C(13A)	1.361(4)	1.357(2)
C(13A)-C(13B)	1.476(4)	1.477(2)
C(10)-C(11)	1.386(4)	1.374(2)
C(11)-C(12)	1.382(4)	1.393(2)

Bonds	Angles (°)	
	2a	**2b**
C(4A)-C(5)-C(6)	109.6(3)	109.1(2)
N(7)-C(6)-C(5)	109.6(2)	109.8(2)
C(13A)-N(7)-C(6)	116.3(2)	119.0(2)
C(13A)-N(7)-C(8)	117.0(2)	119.0(2)
C(6)-N(7)-C(8)	113.4(2)	115.4(2)
N(19)-C(8)-N(7)	115.1(2)	-
N(18)-C(8)-N(7)	-	116.5(2)
N(19)-C(8)-C(8A)	107.2(2)	-
N(18)-C(8)-C(8A)	-	108.6(2)
N(7)-C(8)-C(8A)	109.9(2)	108.9(2)
N(7)-C(13A)-C(13B)	117.9(3)	117.4(2)
C(4A)-C(13B)-C(13A)	120.3(3)	119.7(2)
C(13)-C(13A)-C(13B)	123.0(3)	123.1(2)
C(8A)-C(12A)-C(13)	118.4(3)	118.0(2)
C(13A)-C(13)-C(12A)	121.7(3)	118.0(2)
C(13)-C(13A)-N(7)	119.0(3)	119.4(2)
C(4)-C(4A)-C(5)	120.5(3)	121.0(2)

Table 3. Hydrogen bond parameters for the compounds **2a** and **2b** (see Figures).

Compound / D..A	N-H [Å]	H..A [Å]	N..A [Å]	N-H..A [°]	Symmetry [1]
2a / N19..N19	1.02	2.53	3.176 (5)	121.0	-x, -y+1, -z
2a / N19..N7	1.08	2.34	3.252(4)	141.6	x, y-1, z
2b / N18..O19	0.96	2.46	3.357 (2)	154.5	-x, 0.5+y, 0.5-z

[1] Symmetry operators added to generate equivalent acceptor (A) atoms.

The mean deviation of the C14 atom from the least-squares plane calculated for the atoms C2, C3, O17, and O18 in **2a** is -0.360(5) Å, whilst both dioxolane rings in **2b** are reasonably planar. This indicates remarkable flexibility of dioxolane rings in such systems.

The crystal structure of **2a** comprizes an extensive system of H-bonds. Four molecules of **2a** are linked by six H-bonds which involve H atoms of aminogroups, aminogroup-nitrogens, as well as heterocycle-nitrogens (Fig. 3). The relevant data are listed in Table 3.

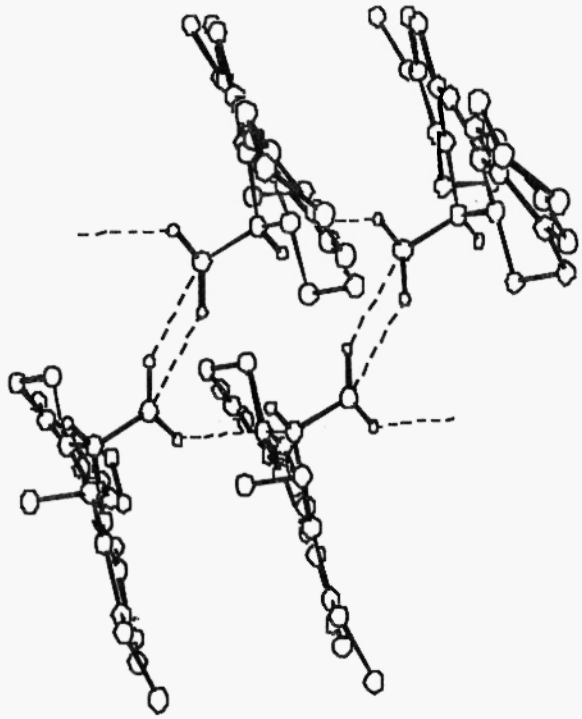

Fig. 3. Hydrogen bonds in **2a**.

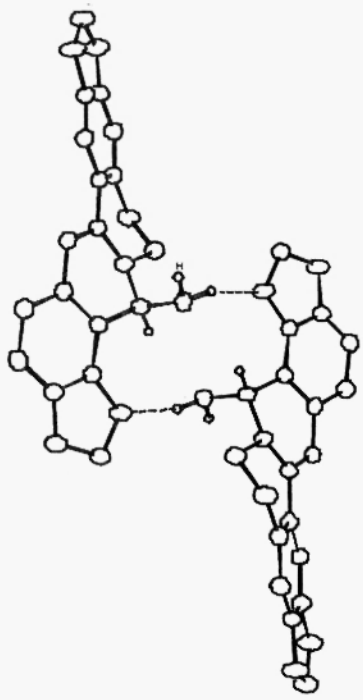

Fig. 4. Hydrogen bonds in **2b**.

The sum of three valence angles around the nitrogen N7 is 346.7 ° (**2a**) and 354.4 ° (**2b**) which perfectly correlates with H-bonds pattern. The anchoring of N7 as H-bond acceptor in **2a** reflects in much more evident pulling out from the heteroring plane.

In contrast, two molecules in **2b** are linked via N-H···O hydrogen bonds involving dioxolane oxygen atoms (Fig. 4). There are no other especially short intermolecular contacts in both structures. The investigations of crystal packing reveals the molecules in sandwich-like arrangement in both **2a** and **2b** crystal structures.

Acknowledgements

The authors thank Professor Jiri Slavík for kind donation of berberine and coptisine. The work was supported by the grants FRVS 403/99 and CEZ:J07/98:143100011 (Ministry of Education, Czech Republic) and 203/99/0067 (Grant Agency of Czech Republic).

References

1. J. Slavík and L. Slavíková, *Collect. Czech. Chem. Commun.* **60**, 1034 (1995).
2. J. Slavík and L. Slavíková, *Collect. Czech. Chem. Commun.* **42**, 2686 (1977).
3. E. Táborská, H. Bochoráková, H. Paulová and J. Dostál, *Planta Med.* **60**, 380 (1994).
4. G. Fulde and M. Wichtl, *Dtsch. Apoth. Ztg.* **134**, 1031 (1994).
5. S. Simeon, J.L. Rios and A. Villar, *Plant. Med. Phytother.* **23**, 202 (1989)
6. K. Iwasa, H.S. Kim, Y. Wataya and D.U. Lee, *Eur. J. Med. Chem.* **33**, 65 (1998).
7. B.K. Sarma, V.B. Pandey, G.D. Mishra and U.P. Singh, *Folia Microbiol.* **44**, 164 (1999).
8. T. Schmeller, B. Latz-Brüning and M. Wink, *Phytochemistry* **44**, 257 (1997).
9. J.P. Hu, N. Takahashi and T. Yamada, *Oral Diseases* **6**, 297 (2000).
10. M. Shamma, *The Isoquinoline Alkaloids*, Academic Press, New York, 1972, pp. 268-314.
11. D.S. Bhakuni and S. Jain, *The Alkaloids*, A. Brossi (Ed.), **28**. 95 (1986).
12. K.W. Bentley, *The Isoquinoline Alkaloids*, Harwood Academic Publishers, Amsterdam, 1998, pp. 219-263.
13. SHELXTL Version 5.1, Bruker AXS, GmbH, Karlsruhe, Germany, 1998.

Received on April 3, 2001