Chemistry of Natural Compounds, Vol. 41, No. 1, 2005

ASSOCIATE OF BETONICIN WITH UREA FROM *Phlomis regelii*

N. A. Razzakov, B. Tashkhodzhaev,
S. F. Aripova, and A. A. Tozhibaev

UDC 547.944/945+548.737

The structure of a base isolated from the aerial part of Phlomis regelii *as a solvate of the pyrrolidine alkaloid betonicin with urea in a 1:1 ratio was solved. A three-dimensional framework of intermolecular H-bonds was formed by the betonicin and urea molecules in the crystal. The inclusion of a urea molecule in the complex results in stable heteromolecular associates that persist during isolation of the alkaloid.*

Key words: *Phlomis,* alkaloids, betonicin, x-ray structure analysis.

In continuation of research on alkaloids of plants from the central Asian flora, we investigated for the first time the aerial part of *Phlomis regelii* (Lamiaceae, Labiatae) collected near Zhuduruk (western Tyan-Shan) [1]. A study of alkaloids from this plant revealed acute toxicity and even death in grazing herds after ingestion of *P. regelii.*

The air-dried plant was extracted repeatedly with CH_3OH. Solvent was removed from the combined extracts. The water-soluble part of the dry extract was chromatographed over a column of Al_2O_3. The $CHCl_3$:CH_3OH fractions yielded **1**, mp 235-236°C, $[\alpha]_D$ -24° (H_2O).

The IR spectrum of **1** contains absorption bands for stretching vibrations of a bonded OH (3400 cm^{-1}) and stretching and deformation vibrations of CH_3-, CH_2-, and CH-groups and an amide carbonyl (1635 cm^{-1}). The mass spectrum exhibits a peak for the molecular ion with *m/z* 159 $[M]^+$ and for fragment ions with *m/z* 132, 120, 107, 100, 84, 82, 70, 60(2), 58, 44, and 42.

The PMR spectrum has two 3H singlets at 3.12 and 3.38 ppm for two methyls bound to N. A broad signal (hump) at 4.54 ppm belongs to an OH. A multiplet (2H) in the range 2.30-2.65 ppm is observed for methylene protons (C3); 3.88-4.08 ppm, a 2H quartet (J_1 and J_2 = 6 Hz) for CH_2–N (C5); 4.16-4.36 ppm, a 1H quartet (J_1 = 8 Hz, J_2 = 2 Hz) for CH–CO_2 (C2). The proton of the CH–O group appears as a 1H multiplet at 3.40-3.46 ppm.

The spectral properties and water solubility of the isolated compound indicate that the base is a betaine (zwitterion).

However, the spectral data and properties as a whole do not unambiguously establish the structure of the isolated base. Therefore, we performed a single-crystal x-ray structure analysis (XSA) of **1**.

The XSA results show that the isolated compound is a complex of the pyrrolidine alkaloid betonicin with urea in a 1:1 ratio. Betonicin (*trans-N,N*-dimethyl-4-hydroxy-L-proline) is known in the literature and was isolated for the first time from *Achillea millefolium* [2] and *Betonica officinalis* [3]. The molecular structure of its hydrochloride was established previously by an XSA [4]. Betonicin could not be identified by spectral methods because it was isolated from the plant as the stable associate with urea, which retains this structure even during isolation of the alkaloid from the extract. This hypothesis was confirmed by the crystal packing of the molecules.

Figure 1 shows that the molecular structure of the alkaloid is a zwitterion. The pyrrolidine five-membered ring has the envelope conformation (within ±0.023 Å). Atom N1 deviates from the plane of the remaining four atoms by 0.610 Å. The substituents on C4 (OH) and C2 (COO^-) are oriented α- and β-equatorially, respectively. The plane of the COO^- (±0.009 Å) is twisted relative to the plane of the five-membered ring. The C3-C2-C6-O2 torsion angle, which characterizes this twist, is 46.0° whereas in betonicin hydrochloride this angle is 54.8° [4]. The C–O bonds in the carboxyl resonate so that the C6–O2 and C6–O3 bonds are averaged at 1.245(6) and 1.234(6) Å (Table 1), respectively, the values of which are closer to double bonds than single C–O bonds [5].

S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 46-48, January-February, 2005. Original article submitted October 4, 2004.

0009-3130/05/4101-0056 ©2005 Springer Science + Business Media, Inc.

TABLE 1. Bond Lengths (r, Å) and Angles (ω, deg) in **1**

Bond	r	Angle	ω
N1-C8	1.493 (6)	C8-N1-C5	110.8 (4)
N1-C5	1.499 (6)	C8-N1-C7	110.7 (4)
N1-C7	1.499 (6)	C5-N1-C7	109.8 (4)
N1-C2	1.532 (6)	C8-N1-C2	112.2 (4)
C2-C3	1.501 (7)	C5-N1-C2	101.5 (3)
C2-C6	1.537 (7)	C7-N1-C2	111.5 (4)
C3-C4	1.543 (7)	C3-C2-N1	104.2 (4)
C4-O1	1.409 (6)	C3-C2-C6	116.9 (4)
C4-C5	1.525 (7)	N1-C2-C6	115.2 (4)
C6-O3	1.234 (6)	C2-C3-C4	106.3 (4)
C6-O2	1.245 (6)	O1-C4-C5	106.7 (4)
O-C	1.238 (6)	O1-C4-C3	113.4 (5)
C-N3	1.337 (6)	C5-C4-C3	104.3 (4)
C-N2	1.342 (7)	N1-C5-C4	106.4 (4)
		O3-C6-O2	126.2 (5)
		O3-C6-C2	120.0 (5)
		O2-C6-C2	113.7 (5)
		O-C-N3	122.8 (5)
		O-C-N2	121.6 (5)
		N3-C-N2	115.6 (4)

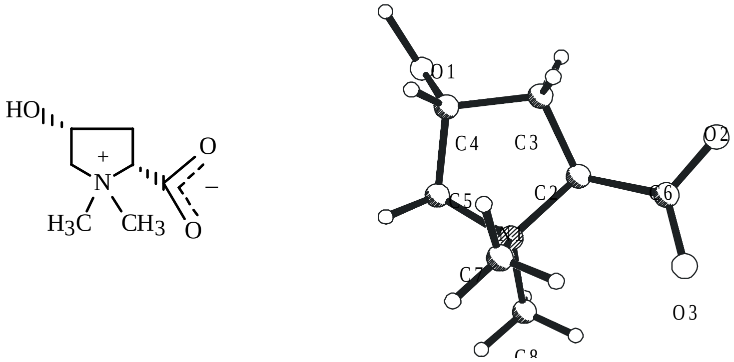

Fig. 1. Structure and atomic numbering in **1**.

The crystal packing (Fig. 2) shows that the urea solvate and betonicin form in the crystal a rather complicated system of H-bonds. The two NH$_2$ groups of the urea solvate are paired with the two O atoms of the alkaloid carboxyl. The parameters of these N–H...O H-bonds are N2...O2, N3...O3 and H21...O2, H32...O3 = 2.949, 2.954 and 2.07, 1.99 Å, respectively. The N2–H21...O2 and N3–H32...O3 bond angles that characterize the directions of these H-bonds are 172.0 and 170.2°. The other two H atoms of the two NH$_2$ groups form H-bonds (N2–H22...O and N3–H31...O) with O atoms of the next urea molecule related by a 2_1 screw axis (0.5 + x, 0.5 - y, -z). The parameters of these H-bonds are 2.89, 2.05 Å, 171.7° and 2.96, 2.12 Å, 174.3°. As a result, an infinite ribbon arises along the *b* axis that is linked with others through O1–H...O2 H-bonds (-x, 0.5 + y, 0.5 - z) (2.684, 1.60 Å, 148.6°). This forms a complicated three-dimensional framework, a part of which is shown in Fig. 2. This framework is evidently partially retained in solution, as a result of which the urea solvate is not removed during isolation of **1**.

TABLE 2. Crystallographic Data, Experimental Conditions, and Refinement Parameters for **1**

Empirical formula	$C_7H_{13}NO_3 \cdot C(NH_2)_2O$
Molecular weight	292.33
Exposure temperature, K	293
Space group	$P2_12_12_1$, Z = 4
A, Å	7.3640 (15)
B, Å	11.093 (2)
C, Å	13.257 (3)
V, $Å^3$	1082.9 (4)
ρ, g/cm^3	1.345
Absorption coefficient, μ (Mo) mm^{-1}	0.108
Crystal dimensions, mm	1.00×0.46×0.10
Range of θ, deg	2.39 on 24.99
Total number of reflections	1121
Number of reflections [$I>2\sigma(I)$]	865
R-factor [$I>2\sigma(I)$]	R1 = 0.0519, wR2 = 0.1136
R-factor (whole data set)	R1 = 0.0796, wR2 = 0.1340
S	1.088
Difference electron-density peaks	0.20 and -0.18 $eÅ^{-3}$

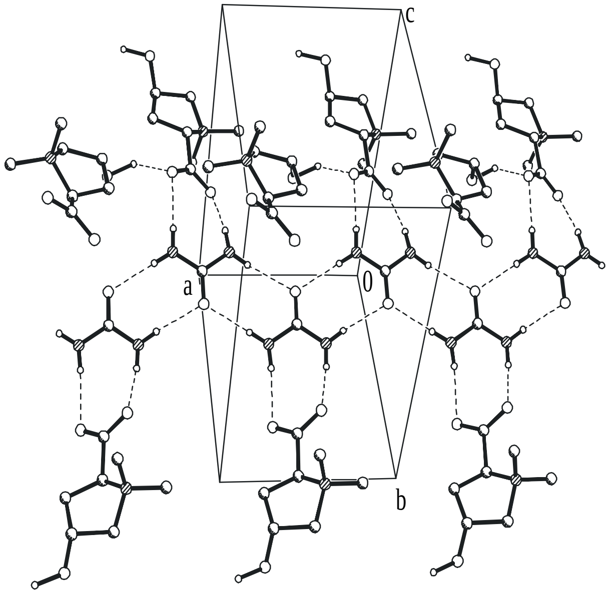

Fig. 2. Packing diagram of **1** showing intermolecular H-bonds (H atoms involved in H-bond formation are shown).

EXPERIMENTAL

IR spectra were recorded on a Perkin—Elmer Model 2000 Fourier-spectrometer in KBr disks; mass spectra, in an MX-1310 spectrometer equipped with a direct probe into the ion source. NMR spectra were recorded on a Tesla spectrometer at working frequency 100 MHz for protons. Column chromatography used Al_2O_3 (neutral, 100/16 μm). TLC was performed on Al_2O_3 plates (5/40 μm) using $CHCl_3$:CH_3OH:HCl (conc.) (5:5:0.2, 1) and $CHCl_3$:CH_3OH (4:1, 2).

Isolation of the Betonicin Associate with Urea. The ground and air-dried aerial part of *P. regelii* (7.5 kg) was extracted with CH_3OH, renewing the solvent daily (7 total). The combined CH_3OH extracts were condensed in vacuum until the solvent was removed. The solid was dissolved in water. The aqueous solution was purified of resins, filtered, and evaporated to dryness on a boiling-water bath. The solid was chromatographed over an Al_2O_3 column with elution by $CHCl_3$. The $CHCl_3$:CH_3OH eluates yielded **1** (3 g), mp 235-236°C, R_f 0.3 (system 1), R_f 0.1 (system 2).

X-ray Structure Analysis. Single crystals of **1** were grown from a solution of **1** in acetone by slow evaporation at room temperature. The crystals were transparent elongated prisms. The unit-cell constants and intensities of reflections were determined on a STOE Stadi-4 four-circle diffractometer ($\theta/2\theta$-scanning) using Mo $K\alpha$-radiation (graphite monochromator). Absorption corrections were not applied. Table 2 gives the principal crystallographic data and experimental conditions for the XSA.

The structure was solved by direct methods using the SHELXS-97 programs and refined by full-matrix isotropic-anisotropic least-squares (LS) methods using the SHELXL-97 programs. All nonhydrogen atoms were refined by full-matrix anisotropic LS (over F^2). The positions of H atoms were found geometrically and refined with fixed isotropic thermal parameters $U_{iso} = nU_{eq}$, where n = 1.5 for methyls and 1.2 for others and U_{eq} is the equivalent isotropic thermal parameter of the corresponding C atom. H atoms of the OH and NH_2 groups were found in a difference electron-density synthesis and refined isotropically.

Data from the XSA were deposited as a CIF file in the Cambridge Cystallographic Database (CCDC252168).

REFERENCES

1. *Flora of Uzbekistan* [in Russian], Izd. Akad. Nauk UzSSR, Tashkent (1961), Vol. V, p. 348.
2. F. M. Miller and L. M. Chow, *J. Am. Chem. Soc.*, **76**, 1353 (1954).
3. A. Schulze and A. Trier, *Helv. Chim. Acta*, **76**, 258 (1911).
4. G. P. Jones, B. P. Naidu, L. G. Paleg, and E. R. T. Tiekink, *Acta Crystallogr. Sect. C: Cryst. Struct. Commun.*, **44**, 2208 (1988).
5. F. H. Allen, O. Kennard, D. G. Watson, L. Brammer, A. G. Orpen, and R. Taylor, *J. Chem. Soc. Perkin Trans. II*, S1 (1987).