A New Pyrroloquinazoline Alkaloid from *Linaria vulgaris*

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A new alkaloid, 1,2,3,9-tetrahydropyrrolo(2,1-*b*)quinazolin-1-carboxylic acid (1), together with eight known compounds, 7-hydroxy vasicine (2), benzyl alcohol *β*-D-(2′-*O*-*β*-xylopyranosyl)glucopyranoside (3), benzyl alcohol *O*-*β*-D-glucopyranoside (4), benzyl alcohol *O*-*β*-D-primveroside (5), 3,5-dimethyl-4-hydroxy benzaldehyde (6), gluco-syringic acid (7), syringin (8), and liriodendrin (9), were isolated from the plants of *Linaria vulgaris*. Their structures were established by spectroscopic methods.

Key words *Linaria vulgaris*; Scrophulariaceae; pyrroloquinazoline alkaloid; 1,2,3,9-tetrahydropyrrolo(2,1-*b*)quinazolin-1-carboxylic acid

Linaria vulgaris MILL. is a grassy plant that occurs in northeast China. The plant is used in traditional folk medicine for the treatment of coughs and asthma and as an expectorant.[1)] Previous phytochemical investigations of the plant have revealed the alkaloids, flavonoids, triterpenoids, steroids, and iridoid glucosides.[2—9)] From the ethanol extract of the plants of *L. vulgaris*, we isolated a new compound **1** and eight known compounds. By comparison of physical and spectroscopic properties (mp, IR, MS, ^{1}H- and ^{13}C-NMR spectra), the known compounds were identified as 7-hydroxy vasicine (**2**),[10)] benzyl alcohol *β*-D-(2′-*O*-*β*-xylopyranosyl)-glucopyranoside (**3**),[11,12)] benzyl alcohol *O*-*β*-D-glucopyranoside (**4**),[13)] benzyl alcohol *O*-*β*-D-primveroside (**5**),[14)] 3,5-dimethyl-4-hydroxy benzaldehyde (**6**), gluco-syringic acid (**7**),[15,16)] syringin (**8**),[17)] and liriodendrin (**9**).[18)] In this article, we present the isolation and structural determination of the new compound and give the ^{1}H- and ^{13}C-NMR data of **2**, which have not been reported previously.

Results and Discussion

Compound **1**, called linarinic acid, was obtained as colorless needles, and the molecular formula was determined to be $C_{12}H_{12}O_2N_2$ by high resolution electron impact (HR-EI)-MS. The IR spectrum indicated the presence of a carboxyl group (1666 cm^{-1}) and aromatic ring (1614, 1586, 1500 cm^{-1}). The ^{1}H-NMR spectrum indicated the presence of an *ortho*-disubstituted benzene [δ 7.25 (ddd, J=7.8, 7.5, 1.9 Hz), 7.16 (ddd, J=7.8, 7.5, 1.0 Hz), 7.13 (dd, J=7.8, 1.9 Hz), 6.97 (dd, J=7.8, 1.0 Hz)] and AB system of CH_2 [4.92, 4.68 (d, J=15.2 Hz)]. It gave signals at δ 2.91 (m, 2H), 2.21 (m, 1H), 2.53 (m, 1H), and 4.21 (dd, J=9.0, 4.5 Hz), which were assigned to the structural fragment –CH_2–CH_2–CH– by ^{1}H–^{1}H correlation spectroscopy (COSY). The ^{1}H- and ^{13}C-NMR spectra suggested **1** to be 1,2,3,9-tetrahydropyrrolo[2,1-*b*]quinazoline alkaloid.[19)] The EI-MS peak at *m*/*z* 171 (M^+−COOH) and ^{13}C-NMR signal at 175.63 showed the presence of a carboxyl group. In the heteronuclear multiple bond connectivity (HMBC) spectrum, the long-range coupling between the H-1 proton at δ 4.21 and the carbon signals at δ 175.63 (COOH), 165.03 (C-3a), 46.44 (C-9), 30.41 (C-3), and 25.57 (C-2), suggested that the carboxyl group was linked with C-1. Thus compound **1** was assigned to be 1,2,3,9-tetrahydropyrrolo(2,1-*b*)quinazolin-1-carboxylic acid, which was confirmed by single-crystal X-ray diffraction.

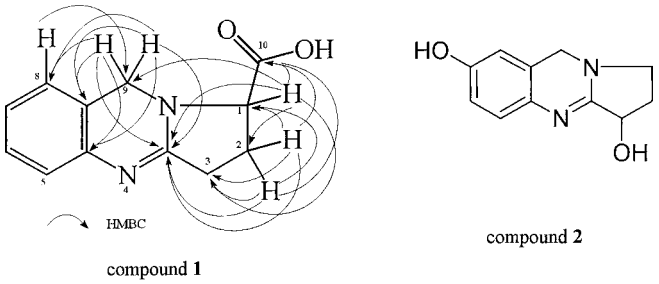

Fig. 1. HMBC Correlations Observed for Compound **1** and the Structure of Compound **2**

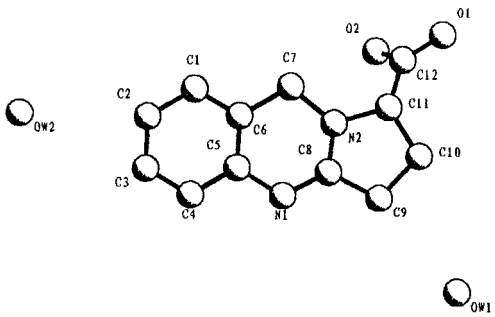

Fig. 2. PLUTO Drawing of Compound **1**

Experimental

General Experimental Procedures Melting points were uncorrected. Optical rotations were measured on a Perkin-Elmer 241MC polarimeter. The UV spectra were obtained with a Shimadzu UV-260 spectrophotometer. The IR spectra were recorded on a Bruker IR S-55 instrument. ^{1}H- and ^{13}C-NMR spectra were measured on a Bruker AC(E)-300 instrument. EI-MS and HR-MS were obtained on a VG-70SE mass spectrometer. Chromatography was performed with D 101 macroporous resin and silica gel (200—300 mesh).

Plant Material The plants of *L. vulgaris* (MILL.) were collected in Heilongjiang Province, People's Republic of China, in August 1995 and were identified by Professor Shiwen Su, Department of Chinese Traditional Medicine, Shenyang Pharmaceutical University. A voucher specimen (LV 960801) has been deposited at the Department of Natural Products Chemistry, Shenyang Pharmaceutical University.

Extraction and Isolation The air-dried aerial parts of plant (10 kg) were extracted with 95% ethanol under reflux (80×3 l) for 2 h each time. The alcohol extract was concentrated and successively extracted with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH. The $CHCl_3$-soluble fraction (76 g) was chromatographed over a silica gel (700 g) column eluted with $CHCl_3$ and $CHCl_3$–MeOH (9 : 1) to yield **6** (8 mg). The *n*-BuOH-soluble fraction (200 g) was chromatographed over a D 101 macroporous resin column with 0%, 20%, 40%, 60% and 95% EtOH in H_2O as eluants (5000 ml of each eluant). The fraction (129 g) eluted with H_2O was subjected to silica gel (1000 g) column chromatography eluted with a solvent system of $CHCl_3$–MeOH (9 : 1) to afford **7** (6 mg). The combined fractions (26 g) eluted with

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$CHCl_3$–MeOH (8 : 2) were isolated on a silica gel (250 g) column eluted with an EtOAc–MeOH (92 : 8, 6 : 4) gradient to afford **2** (14 mg) and **1** (25 mg). The fraction (47 g) eluted with 20% EtOH was separated on column chromatography over 500 g silica gel with a gradient (5000 ml of each eluant) of $CHCl_3$–MeOH (9 : 1, 88 : 12, 85 : 15) to give **4** (100 mg), **5** (25 mg), **3** (34 mg), **8** (14 mg) and **9** (20 mg).

Linarinic Acid (**1**): Colorless needles (MeOH). mp 218 °C (dec.); $[\alpha]_D^{18}$ −217° (c=0.01, l=0.2, MeOH). UV λ_{max} (MeOH) nm: 281, 211. IR ν_{max} (KBr) cm^{-1}: 3331, 2915, 2863, 2759, 1666, 1614, 1586, 1500, 1460, 1385, 1291, 764. ^{1}H-NMR (CD_3OD, 300 MHz) δ: 2.21 (1H, m, H-2), 2.53 (1H, m, H-2), 2.91 (2H, m, H-3), 4.21 (1H, dd, J=9.0, 4.5 Hz, H-1), 4.68, 4.92 (1H each, d, J=15.0 Hz, H-9), 6.97 (1H, dd, J=7.8, 1.0 Hz, H-5), 7.13 (1H, dd, J=7.8, 1.9 Hz, H-8), 7.16 (1H, ddd, J=7.8, 7.5, 1.0 Hz, H-7), 7.26 (1H, ddd, J=7.8, 7.5, 1.9 Hz, H-6); ^{13}C-NMR (CD_3OD, 75 MHz) δ: 25.57 (t, C-2), 30.41 (t, C-3), 46.44 (t, C-9), 69.71 (d, C-1), 118.99 (d, C-5), 127.43 (d, C-7), 128.05 (d, C-8), 130.01 (d, C-6 and s, C-8a), 134.65 (s, C-4a), 165.03 (s, C-3a), 175.63 (s, C-10); EI-MS m/z (rel. int.) 216 $[M]^+$ (94), 215 (100), 214 (37), 171 (97), 144 (96.5); HR-EI-MS m/z 216.0900 (Calcd for $C_{12}H_{12}O_2N_2$, 216.0899).

7-Hydroxy Vasicine (**2**): Colorless needles (MeOH). mp 260 °C (dec.). $[\alpha]_D^{18}$ −35.5° (c=0.013, l=0.2, MeOH). UV λ_{max} (MeOH) nm: 292, 204. ^{1}H-NMR (CD_3OD, 300 MHz) δ: 2.08 (1H, m, H-2), 2.62 (1H, m, H-2), 3.67 (2H, m, H-1), 4.72, 4.80 (1H each, d, J=15.6 Hz, H-9), 5.08 (1H, t, J=8.0 Hz, H-3), 6.60 (1H, d, J=2.4 Hz, H-8), 6.72 (1H, dd, J=8.7, 2.4 Hz, H-6), 6.98 (1H, d, J=8.7 Hz, H-5). ^{13}C-NMR (CD_3OD, 75 MHz) δ: 30.94 (t, C-2), 47.51 (t, C-9), 51.63 (t, C-1), 72.32 (d, C-3), 114.44 (d, C-8), 116.89 (d, C-6), 119.57 (s, C-8a), 119.64 (d, C-5), 124.06 (s, C-4a), 158.22 (s, C-7), 165.51 (s, C-3a). EI-MS m/z (rel. int.): 204 (M^+, 68), 203 (100), 175 (12), 147 (14), 38 (6.5), 36 (19.6).

Single-Crystal X-Ray Structure Determination Crystal data for **1**: $C_{12}H_{12}O_2N_2 \cdot 2H_2O$, MW=252.11, orthorhombic, space group $P2_12_12_1$, a=7.240(1) Å, b=8.051(2) Å, c=21.127(5) Å, V=1231.5(5) Å^3, Z=4, Dc=1.355 g/cm^3. The crystal of **1** with approximate dimensions 0.15×0.2×0.4 mm was selected for X-ray crystallographic analysis. The X-ray intensity data were measured on a MAC DIP2030K X-ray diffractometer with MoKα radiation. The detector-to-crystal distance was 100 mm. A total of 1137 unique reflections was recorded, of which 895 reflections were considered observed on the basis $|F|^2 > 8.0\sigma|F|^2$. The structure was solved by direct methods with the use of the SHELX-86 program. All the hydrogen atoms were located from a difference Fourier map and hydrogen parameters were refined. Nonhydrogen atoms were given anisotropic thermal parameters. Final R-factors were R=0.075 and Rw=0.073.

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