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N-ALLYLISONITRARINE AND NARCISSIN FROM PLANTS OF THE *Nitraria* GENUS

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The new alkaloid N-allylisonitrarine was isolated from the aerial part of Nitraria schoberi *L. Its structure was established using spectral data and chemical transformations. Leaves of* Nitraria komarovii *contain the flavonoid narcissin. Its ^{1}H and ^{13}C NMR spectra were studied.*

Key words: *Nitraria, Nitraria schoberi, Nitraria komarovii,* alkaloid N-allylisonitrarine, flavonoid narcissin.

In continuation of the investigation of the aerial part of *Nitraria schoberi* L. [1], an optically inactive base **1** with mp 257-258°C (ethanol), molecular weight 347 (mass spectrum), and formula $C_{22}H_{29}N_3$ was isolated by column chromatography from the $CHCl_3$ fraction of the total bases.

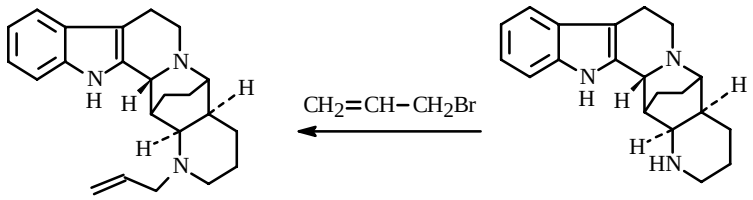
The UV spectrum of **1** contains the following absorption maxima (λ_{max}, C_2H_5OH): 223, 266-271, 280 (sh), 389 nm (log ε 4.74, 3.83, 3.80, and 3.48). These are characteristic of a nonconjugated indole chromophore [2].

The IR spectrum of **1** contains absorption bands characteristic of an *o*-disubstituted benzene ring (743 cm^{-1}), a substituted indole (1451, 1473, 1572, and 1523 cm^{-1}), and an isolated double bond (1640 cm^{-1}), etc.

The PMR spectrum exhibits signals typical of an allyl (3.31 ppm, d, 2H, J = 9.5 Hz; 5.24, m, 2H; 5.86, m, 1H) and aromatic and alicyclic protons.

The spectral data indicate that **1** is an indole derivative. The alkaloid N-allylnitrarine with the identical formula and molecular weight was isolated from this genus but differs in melting point [3]. Therefore, we supposed that **1** is an isomer of this alkaloid. Apparently, **1** isolated by us is N-allylisonitrarine, an epimer of nitrarine at C-3. The N-allyl derivative of isonitrarine synthesized by us is identical to **1**.

Thus, **1** is a new alkaloid and has the structure N-allylisonitrarine.



1 **2**

We investigated the components of leaves of *Nitraria komarovii* Iljin et Lava [3, 4] collected in June 1987 near Turkmenbashi (Krasnovodsk, Republic of Turkmenistan) during flowering.

Alkaloids were extracted from *N. komarovii* by a modified method (described in Experimental).

Chromatography of the ethylacetate fraction over a silica-gel column with $CHCl_3$—CH_3OH gradient elution afforded **3**.

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TABLE 1. ^{13}C NMR Chemical Shifts of Narcissin (**3**) and Isorhamnetin (**4**)

C atom	Narcissin			Isorhamnetin
	Flavone	D-Glucose	L-Rhamnose	
C-2	156.6s			147.4s
C-3	133.2s			135.7s
C-4	177.5s			175.9s
C-5	156.6s			156.2s
C-6	98.9d			98.4d
C-7	164.2s			164.2s
C-8	94.2s			93.6d
C-9	161.8s			160.6s
C-10	104.2s			103.1s
C-1′	121.2s	101.3*d	100.9*d	120.3s
C-2′	113.5d	74.4d	4.4d	111.9d
C-3′	147.1s	76.5d	70.7**d	147.4s
C-4′	149.6s	70.4d**	71.9d	149.0s
C-5′	115.4d	76.5d	68.4d	115.6d
C-6′	122.5d	66.9t	17.8q	121.7d
O-CH_3	55.8q			55.8q

*, **Signals marked with the same symbol may be reversed.

Compound 3. Yellow crystals, $C_{28}H_{32}O_{16}$, mp 201-203°C. The UV spectrum contains the following absorption maxima (λ_{max}, C_2H_5OH): 208, 256, 270, 362 nm (log ε 4.56, 3.97, 3.85, 3.74) that change upon basicification (λ_{max}, C_2H_5OH + OH^-): 210, 276, 422 nm [5, 6].

The PMR spectrum recorded in a CD_3OD—$CDCl_3$ (3:1) mixture exhibits signals (δ, ppm, J/Hz): 1.07 (3H, d, J = 6, CH_3 of rhamnose); 3.2-3.7 and 4.55-5.00 (carbohydrate); 4.48 (1H, s, anomeric proton of α-L-rhamnose), 5.05 (1H, d, J = 7.5, anomeric proton of β-D-glucose), 3.90 (3H, s, Ar-OMe), 6.19 (1H, d, J = 2.5, H-6), 6.35 (1H, d, J = 2.5, H-8), 6.88 (1H, d, J = 9.5, H-5′), 7.61 (1H, dd, J = 9.5, J = 1.5, H-6′), and 7.90 (1H, d, J = 1.5, H-2′).

Acid hydrolysis of **3** afforded isorhamnetin [6, 7], $C_{16}H_{12}O_7$, mp 306-308°C, $[M]^+$ 316. UV spectrum (λ_{max}, C_2H_5OH): 208, 257, 270, 372 nm.

PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 3.87 (3H, s, Ar-OMe), 6.09 (1H, d, J = 2.5, H-6), 6.44 (1H, d, J = 2.6, H-8), 6.85 (1H, d, J = 8.5, H-5′), 7.55 (1H, d, J = 2.5, H-2′), 7.62 (1H, dd, J = 8.5, J = 2.5, H-6'). We also identified D-glucose and L-rhamnose.

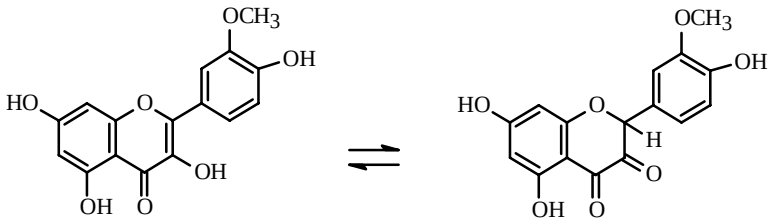
Acetylation of **3** by acetic anhydride in pyridine produced the nonaacetyl derivative of formula $C_{46}H_{50}O_{25}$ with mp 120-122°C. The mass spectrum contains a peak for the molecular ion (1002) and strong peaks for fragment ions acetylated biose with *m*/*z* 561 and terminal rhamnose with *m*/*z* 273, 213, and 153 [8].

Partial hydrolysis of **3** by acetic acid (10%) produced the 3-O-β-D-glucopyranoside of isorhamnetin (**5**), $C_{22}H_{22}O_{12}$, mp 171-173°C. UV spectrum (λ_{max}, C_2H_5OH): 208, 256, 271, 365 nm [9].

The location of the carbohydrates in the studied flavonoid was determined by comparing the ^{13}C NMR spectra of **3** and its hydrolysis product, isorhamnetin (**4**). Signals were assigned based on literature data [10, 11], the results of which are listed in Table 1.

Signals for C-2, C-3, and C-4 appear in the ^{13}C NMR spectrum of **3** at 156.6, 133.2, and 177.5 ppm, respectively; in that of its hydrolysis product, at 147.4, 135.7, and 175.9 ppm. The chemical shifts of these C atoms that are induced by glycosylation (ICS) are +9.2, +2.5, and -1.6 ppm, respectively. These same values have been reported [10, 11]. The anomalous shift induced for C-2 is explained by the partially olefinic nature of the C-2–C-3 bond.

This anomaly is evidently caused by keto—enol tautomerism in flavon-3-ols:



6 **7**

If the inversion occurs rapidly, the ^{13}C NMR spectrum exhibits signals that are averaged over the two tautomers (**6** and **7**), i.e., signals of the equilibrium state of **4** appear. Such a situation is impossible for **3**. This probably explains the rather large ICS for C-2 because of the lack of a contribution from tautomer **7** with an sp^3-hybridized C-2.

Molecular mechanics (MM2) and semi-empirical PM3 calculations showed that the energy of enol **6** is 84.92 kcal/mol; of the keto tautomer **7**, 85.46 kcal/mol, i.e., the difference between them is only 0.44 kcal/mol. At room temperature, one form can freely transform into the other. As a result, the NMR spectrum reflects the equilibrium state of the two rapidly interconverting forms **6** and **7**.

Thus, **3** is narcissin (isorhamnetin-3-O-rutinoside) [5]. It is isolated for the first time from this genus.

The alkaloids komavine and acetylkomavine [4], schoberine, nitramarine, dehydroschoberine, nitraraine, tetramethylenetetrahydro-β-carboline, nitraramine, desoxyvasicinone, nitraroxine, nitraramine N-oxide, and komarovine [12a-j, respectively] were isolated by column chromatography by a modified method from the ether fraction of the total alkaloids of *N. komarovii*.

The $CHCl_3$ fraction of the total bases gave by column chromatography nitrarine, isonitrarine, nitramidine, schoberidine, and nitraramine.

EXPERIMENTAL

UV spectra were recorded in alcohol on a Perkin—Elmer Lambda 16 spectrometer; mass spectra, in an MX-1310 spectrometer and a Kratos MS 25RF GC-MS; IR spectra, on a Perkin—Elmer System 2000 FT-IR in KBr pellets; ^1H and ^{13}C NMR spectra, in a $CDCl_3$—CD_3OD mixture on a Tesla BS 567A spectrometer. Chemical shifts were measured relative to HMDS (^1H) and TMS (^{13}C) standards.

The purity of the compounds was checked by TLC using silica gel (KSK and L 5/40) and Silufol UV-254 plates.

The following solvent systems were used for chromatography: $CHCl_3$—CH_3OH (8:1), $CHCl_3$—C_2H_5OH (8:1), $CHCl_3$—$OC(CH_3)_2$—CH_3OH (5:4:1), $CHCl_3$—CH_3OH (4:1), $CHCl_3$—C_2H_5OH—NH_4OH (8:2:0.1), $CHCl_3$—CH_3OH—NH_4OH (8:2:0.1), $CHCl_3$—$OC(CH_3)_2$—C_2H_5OH—NH_4OH (5:4:1:0.1), *n*-butanol—pyridine—water (6:4:3). Dragendorff's solution and iodine and ammonia vapor were used as developers.

Sugar was detected by paper chromatography (PC) using FN-13 paper (Germany) with spraying by acid anilinium phthalate and subsequent heating for 3-5 min at 90-100°C.

The extraction and isolation of the total bases from *N. schoberi* L. has been described in detail [1].

N-Allylisonitrarine. Chromatographic fractions 18-24 were combined and crystallized from ethanol to give **1**, 63 mg, mp 257-258°C.

Mass spectrum, *m/z* (I_{rel}, %): 347 $[M]^+$ (45), 320 (10), 319 (11), 318 (12), 306 (59), 289 (10), 277 (12), 263 (28), 243 (38), 237 (43), 225 (98), 224 (100), 222 (83), 221 (94), 169 (54), 168 (62), 144 (72).

IR spectrum (KBr, ν_{max}, cm^{-1}): 743, 773, 852, 892, 924, 937, 951, 1009, 1122, 1143, 1232, 1334, 1445, 1468, 1640, 2744, 2814, 2863, 2937, 3046.

PMR spectrum (CD_3OD + $CDCl_3$, δ, ppm, J/Hz): 1.62 (m), 2.08 (m), 2.75 (m), 3.12 (m), 3.31 (2H, d, J = 9.5), 4.38 (m), 4.68 (1H, br.s), 5.24 (2H, m), 5.86 (2H, m), 7.11 (2H, m), 7.42 (2H, m).

Alkylation of Isonitrarine by Allylbromide. N-Allylisonitrarine. A solution of isonitrarine (**2**, 0.2 g) in ethanol (5 mL) was treated with freshly distilled allylbromide (2 mL) and refluxed for 5 h. After the reaction was finished the solvent was removed, water was added, and the solution was basicified with KOH (10%) and extracted with $CHCl_3$. The $CHCl_3$ was removed. The solid was separated by chromatography over a silica-gel column with elution by $CHCl_3$—C_2H_5OH (5:1) to give

N-allylisonitrarine, 0.13 g, mp 257-258°C (ethanol).

Extraction of *N. komarovii* Leaves. Finely ground leaves (2.3 kg) were extracted with ethanol (10 times). All extracts were combined and condensed. The concentrated solution was washed with hexane (5 times) and diluted with an equal volume of H_2SO_4 (5%). The aqueous alcoholic solution was washed with ethylacetate and *n*-butanol to give ethylacetate (9.3 g) and *n*-butanol (16.8 g) fractions of total compounds. The overall yield was 26.1 g or 1.13% of the air-dried mass.

The acidic solution was basicified with NaOH (10%). Bases were extracted by ether and then $CHCl_3$, adding NH_4Cl. The phenolic fraction was extracted by $CHCl_3$. The following alkaloid fractions were obtained: nonphenolic part, ether 2.17 g, $CHCl_3$ 9.61 g; phenolic part, $CHCl_3$ 0.32 g. Overall yield 12.10 g or 0.53% of the air-dried mass.

Narcissin. The ethylacetate fraction (9.3 g) was chromatographed over a silica-gel column with elution by $CHCl_3$—CH_3OH mixtures (50:1, 20:1, 10:1, 5:1, and 4:1). Fractions of 120-150 mL were collected. Fractions 37-45 were evaporated and crystallized from CH_3OH to give **3**, 0.31 g, mp 201-203°C.

IR spectrum (KBr, ν_{max}, cm^{-1}): 854, 712, 806, 832, 922, 1033, 1061 (glycoside C–O), 1125, 1167, 1181, 1206, 1359, 1431, 1455, 1504, 1557, 1603 (aromatic C=C), 1656 (γ-pyrone C=O), 2920 (O–CH_3), 3277 (OH).

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 0.95 (3H, d, J = 5.5), 2.88-3.75 (D-glucose and L-rhamnose), 3.83 (3H, s, Ar-OMe), 4.41 (1H, s, L-rhamnose anomeric H), 5.38 (1H, d, J = 7.5, D-glucose anomeric H), 6.92 (1H, d, J = 1.5, H-6), 6.43 (1H, d, J = 1.5, H-8), 6.90 (1H, d, J = 9, H-5′), 7.53 (1H, dd, J = 9, J = 1.5, H-6′), 7.84 (1H, d, J = 1.5, H-2′). Properties of the PMR spectrum of **3** in CD_3OD + $CDCl_3$ are given in the text.

REFERENCES

1. T. S. Tulyaganov and O. M. Nazarov, *Khim. Prir. Soedin.*, 323 (2000).
2. A. W. Sangster and K. L. Stuart, *Chem. Rev.*, **65**, 69 (1965).
3. T. S. Tulyaganov and O. E. Makhmudov, *Khim. Prir. Soedin.*, 325 (2000).
4. T. S. Tulyaganov, O. M. Nazarov, M. G. Levkovich, and N. D. Abdullaev, *Khim. Prir. Soedin.*, 56 (2001).
5. M. P. Yuldashev, E. Kh. Batirov, and V. M. Malikov, *Khim. Prir. Soedin.*, 609 (1996).
6. T. J. Mabry, K. R. Markham, and M. B. Thomas, *The Systematic Identification of Flavanoids*, Springer-Verlag, Berlin (1970), p. 298.
7. M. S. Luk′yanchikov *Khim. Prir. Soedin.*, 139 (1992).
8. A. Sakushimoto, H. Hasado, Y. Ogihara, and S. Nishibe, *Chem. Pharm. Bull.*, **28**, 1219 (1980).
9. D. Dungerdorzh and V. V. Petrenko, *Khim. Prir. Soedin.*, 272 (1973).
10. P. K. Agrawall and R. P. Rostogi, *Heterocycles*, **5**, 2181 (1981).
11. K. R. Markham and B. Ternai, *Tetrahedron*, **32**, 2607 (1976).
12. R. Shakirov, M. V. Telezhenetskaya, I. A. Bessonova, S. F. Aripova, I. A. Israilov, M. N. Sultankhodzhaev, V. I. Vinogradova, V. I. Akhmedzhanova, T. S. Tulyaganov, B. T. Salimov, and V. A. Tel′nov, *Khim. Prir. Soedin.*, a) 1014, b) 673, c) 417, d) 674, e) 978, f) 675, g) 422, h) 775, i) 490, j) 460 (1996).