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ALKALOIDS OF THE *Nitraria* GENUS. KOMAVINE AND ACETYLKOMAVINE

T. S. Tulyaganov,[1] O. M. Nazarov,[2] M. G. Levkovich,[1] and N. D. Abdullaev[1]

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*Two new alkaloids, komavine (**1**) and acetylkomavine (**2**) were isolated for the first time from the aerial part of* Nitraria komarovii. *Their ^1H and ^{13}C NMR spectra were studied. Komavine was synthesized by condensation of tryptamine with cyclohexanone. These same alkaloids were also isolated from* Nitraria schoberi.

Key words: *Nitraria komarovii*, *Nitraria schoberi*, komavine, acetylkomavine.

In continuation of the investigation of alkaloids from the aerial part of *Nitraria komarovii* Iljin et Lava [1, 2], we isolated two alkaloids, **1** and **2**, from the benzene fraction of the total extract by column chromatography.

Compound **1**, $C_{16}H_{20}N_2$, M^+ 240, is called komavine. The UV spectrum has absorption maxima at 225, 282, and 291 (sh) nm, which are characteristic for chromophores of tetrahydro-β-carboline compounds [3]. The IR spectrum contains absorption bands for vibrations of *o*-disubstituted benzene (747), substituted indole (1441, 1464, 1580, 1617), saturated C–H bonds (2841, 2921), and active H (band centered at 3396 cm^{-1}).

The PMR spectrum of **1** exhibits two 2H triplets at 2.62 and 3.06 ppm from neighboring methylenes, the protons of which are spin—spin coupled to each other with an average SSCC J = 6.0 Hz owing to the conformational fluxionality of the corresponding CH_2–CH_2–N< moiety in komavine. A broad 10H signal appears in the region of methylene protons of a saturated spiro six-membered ring at 1.3-1.8 ppm. Aromatic protons (4H) resonate at 7.0-7.5 ppm as a complicated four-spin system. The NH proton gives a broad singlet at 7.68 ppm.

Compound **2**, $C_{18}H_{22}N_2O$, has absorption maxima in the UV spectrum at 225, 282, and 291 (sh) nm, which are typical of an unconjugated indole chromophore [3]. The IR spectrum contains the following absorption bands: 742 (*o*-disubstituted benzene), 1452, 1465, and 1620 (indole), 1642 (N–C=O), 2853 and 2927 (saturated C–H), 3057 (Ar-OH), and 3982 cm^{-1} (N–H).

The mass spectrum of **2** gives a molecular-ion peak (M^+ 282) and has a fragmentation pattern indicative of loss of methyl [267, $(M - 15)^+$] and acetyl [239, $(M - 43)^+$] groups. Otherwise, the mass spectra of **1** and **2** are similar.

The presence in the PMR spectrum of a 3H singlet at 2.21 ppm also indicates that an acetyl group is present.

The spectral data indicated that **1** and **2** are indole alkaloids. Acetylation of **1** by acetic anhydride in pyridine produced its N-acetylated derivative which is identical to **2**. The reverse reaction occurs upon hydrolysis of **2** in acidic medium. Thus, **2** is the N-acetyl derivative of **1**.

The PMR spectrum of **2** has signals of neighboring methylenes C-3 and C-4 that are shifted to weak field and appear as triplets at 2.77 and 3.72 ppm with J = 6.0 Hz. The signals of two of the ten protons of the cyclohexane ring, which form a complicated multiplet at 1.3-1.8 ppm, undergo a paramagnetic shift and resonate at 3.0 ppm.

Table 1 lists chemical shifts and SSCC J_{CH} for individual C atoms in the ^{13}C NMR spectra of **1** and **2**. A comparison indicates that, as expected, changing from **1** to **2** changes the chemical shift of mainly the spiro-C atom (+8.4 ppm) and C-3 (+4.6 ppm) in addition to the α-C (C-2′ and C-6′) and β-C (C-3′ and C-5′) of the saturated six-membered ring. Otherwise, the ^{13}C NMR spectra of **1** and **2** are similar.

1) S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75; 2) Fergana State University. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 56-58, January-February, 2001. Original article submitted December 11, 2000.

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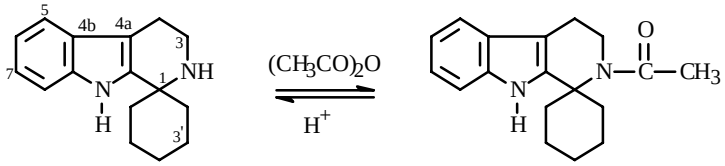
TABLE 1. ^{13}C NMR Data for Komavine (**1**) and Acetylkomavine (**2**)

Atom	**1**			**2**
	δ	$^1J_{CH}$	$^2J_{CH}$, $^3J_{CH}$	δ
1	52.3	-	13.0 6.5	60.7
3	39.2	136.2	-	43.7
4	23.1	128.2	-	22.2
4a	108.1	-	7.2	107.2
4b	135.4**	-	3.8	135.4
5	110.7*	158.8	7.2	111.1
6	118.2*	158.2	7.0	117.9
7	119.4*	159.2	7.0 1.5	119.6
8	121.5*	159.1	7.8 1.6	121.7
8a	141.2	-	3.2	141.0
9a	127.6**	-	6.0 3.5	126.5
2′ 6′	36.7	125.8	-	34.6
3′ 5′	21.4	125.6	9.0	23.6
4′	25.8	125.0	-	24.5
CH_3	-	-	-	26.1
C=O	-	-	-	171.9

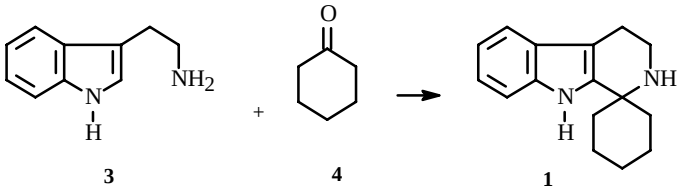
*,** Signals may be interchanged.

We propose the structure below as the most probable for komavine based on the above data:



1 **2**

A compound with this structure was synthesized by condensation of tryptamine (**3**) with cyclohexanone (**4**) by the Pictet—Spengler method [4].



3 **4** **1**

The resulting product **1** had properties that were identical in all respects to those of the natural alkaloid komavine. The physicochemical properties and structures of **1** and **2** and the synthetically known compounds are identical. We provide the spectral properties because they have not been reported [5, 6].

Komavine and acetylkomavine were isolated from the benzene fraction of the total extract of *Nitraria schoberi* L. collected in June 1995 near Ayakagitma, Bukhara district, Republic of Uzbekistan.

Histrionicotoxin alkaloids, which were isolated from the poisonous skin of the tropical treefrog *Dendrobates histrionicus* [7], have been found in nature. These are based on 1-azaspiro-(5,5)-undecane with allene and acetylene substituents in the side chain [8, 9].

The 1-azaspiro-(5,5)-undecane system in komavine and acetylkomavine is condensed with indole. Dimeric

proaporphine-tryptamine alkaloids contain such a system [10-12].

Thus, komavine and acetylkomavine are isolated from a natural source for the first time. The structure of komavine is 2,3,4,9-tetrahydrospiro[cyclohexan-1′,1-β-carboline].

EXPERIMENTAL

UV spectra were recorded in alcohol on a Lambda-16 (Perkin—Elmer) spectrometer; IR spectra, on UR-20 (Zeiss Jena) and FT-IR Model-2000 (Perkin—Elmer) spectrometers in KBr pellets; mass spectra, in an MX-1310 spectrometer equipped with a direct probe (ionizing potential 60/70, temperature 100-170°C); ^{1}H and ^{13}C NMR spectra, on a Tesla BS-567A instrument at working frequency 100 MHz (H) in $CDCl_3$ with and without full suppression of H coupling for ^{13}C and HMDS (H) and TMS (C) internal standards. KSK and L5/40 silica gel (Czech Rep.) in addition to Silufol UV-254 plates were used for TLC. Chromatography used the solvent systems: 1) C_6H_6—CH_3OH (4:1), 2) $CHCl_3$—$(CH_3)_2CO$—CH_3OH (5:4:1), 3) $CHCl_3$—CH_3OH (5:1), 4) $CHCl_3$—C_2H_5OH (4:1), 5) $CHCl_3$—$(CH_3)_2CO$—C_2H_5OH (5:4:1), 6) $CHCl_3$—C_2H_5OH (8:1), 7) $CHCl_3$—CH_2OH (9:1). Dragendorff's solution and iodine vapor were used as developers.

Extraction and separation of total alkaloids from *Nitraria komarovii* have been described in detail [13, 14].

The benzene fraction (28.67 g) of the total extract was boiled with petroleum ether. The solution was separated. The solvent was removed. The petroleum-ether fraction of the total extract (7.97 g) was separated by chromatography on a silica-gel column with elution by $CHCl_3$—CH_3OH in various ratios (20:1, 10:1, and 4:1). Fractions of 15-20 mL were collected. The following compounds were isolated from individual fractions:

Acetylkomavine (2). Fractions 8-13 were combined and rechromatographed on a silica-gel column with elution by $CHCl_3$—C_2H_5OH (10:1). Fractions of 10-12 mL were collected. Fractions 3-9 were combined. The solvent was removed. Crystallization from isopropanol gave **2**, 0.113 g (0.00067% of the air-dried plant mass), mp 162-163°C.

Mass spectrum, *m/z* (%): M^+ 282 (94), 267 (41), 240 (19), 239 (81), 225 (66), 223 (53), 211 (40), 210 (63), 197 (100), 184 (27), 183 (36), 168 (39), 167 (37), 155 (35), 154 (38), 144 (32), 130 (32), 129 (33), 95 (30), 93 (27), 91 (27), 81 (41).

IR spectrum (KBr, ν_{max}, cm^{-1}): 742 (*o*-disubstituted benzene), 1010, 1037, 1154, 1209, 1297, 1400, 1452, 1465, 1620 (indole), 1640 (amide carbonyl), 2853, 2927 (–CH_2– and –CH_3), 3057 (Ar-H), 3287 (N–H).

UV spectrum (EtOH, λ_{max}, nm): 225, 282, 291 (sh) (log ε 4.62, 4.03, 3.96).

PMR (δ, ppm, J, Hz): 1.30-1.80 (8H, m), 2.21 (3H, s, –CH_3), 2.77 (2H, t, J = 6.0), 2.85-3.12 (2H, m), 3.72 (2H, t, J = 6.0), 7.08 (2H, m), 7.23 (1H, m), 7.42 (1H, m), 8.26 (1H, br. s, N–H).

Komavine (1). Fractions 17-22 were combined. The solvent was removed. The solid was separated by chromatography on a silica-gel column with elution by system 4. Fractions of 12-15 mL were collected. Fractions 13-19 were combined. Solvent was removed. The solid was crystallized from hexane. Yield of **1**, 0.167 g (0.001% of the dry plant mass), mp 131-132°C (lit. 132-132.5°C [6]), mp of hydrochloride 279-281°C (dec.).

Mass spectrum, *m/z* (%): M^+ 240 (43), 212 (10), 211 (17), 198 (22), 197 (100), 184 (19), 168 (13), 167 (14), 155 (16), 154 (15), 98 (13), 85 (9), 81 (8), 78 (9).

IR spectrum (KBr, ν_{max}, cm^{-1}): 747 (*o*-disubstituted benzene), 835, 1026, 1157, 1234, 1295, 1345, 1368, 1441, 1464, 1580, 1617 (indole), 2841, 2921 (–CH_2–), 3050 (Ar-H), 3296 (N–H).

UV spectrum (EtOH, λ_{max}, nm): 225, 282, 291 (log ε 4.69, 4.14, 4.03).

PMR (δ, ppm, J, Hz): 1.30-1.80 (10H, m), 2.62 (2H, t, J = 6.0), 3.06 (2H, t, J = 6.0), 7.08 (2H, m), 7.18 (1H, m), 7.42 (1H, m), 7.68 (1H, br. s, N–H).

Acetylation of Komavine. N_2-Acetylkomavine. A mixture of **1** (0.05 g), pyridine (1.0 mL), and acetic anhydride (1.0 mL) was left for 3 d at room temperature. The excesses of the anhydride and pyridine were removed under vacuum. The dry solid was treated with distilled water (2.0 mL), basicified with conc. aqueous NH_3, and extracted with ether. The ether extract was dried over anhydrouse Na_2SO_4 and evaporated. Crystallization from isopropanol gave **2**, 0.043 g, mp 162-163°C, M^+ 282.

Hydrolysis of 2. Compound **2** (0.06 g) was dissolved in HCl (1 N, 5 mL) and heated at 80°C for 2 h. The product was decomposed with KOH solution (10%) and extracted by $CHCl_3$. The solvent was removed. Crystallization from hexane gave **1**, 0.038 g, mp 131-132°C, M^+ 240.

Synthesis of Komavine. A mixture of tryptamine·HCl (1 g, 0.005 mol) in water (25 mL), H_2SO_4 (2 N, 3 mL), and cyclohexanone (3 mL) was gradually heated to 100°C on a sand bath, held at this temperature for 5 h, cooled, and washed with

ether. The product was decomposed with KOH solution (10%) and extracted by $CHCl_3$. The solvent was removed. The solid was crystallized from hexane. Yield of **1**, 0.92 g (76.7%), mp 131-132°C (lit. 132-132.5°C [6]), M^+ 240.

Extraction and separation of the total extract of *Nitraria schoberi* L. has been described in detail [15].

The benzene fraction (14.26 g) of the total extract was separated by chromatography on a silica-gel column with elution by $CHCl_3$—C_2H_5OH in various ratios (20:1, 15:1, 10:1, 5:1, and 4:1).

Acetylkomavine. Mother liquors of fractions 9-15, 16-21, and 22-29 were combined after isolation of nitraraine, deoxyvasicinone, and tetramethylenetetrahydro-β-carboline and rechromatographed on a silica-gel column with elution by $CHCl_3$—C_2H_5OH (6:1). Fractions of 15-20 mL were collected. Fractions 6-13 were combined. The solvent was removed. The solid was crystallized from isopropanol. Yield of **2**, 63 mg, mp 162-163°C.

Komavine. Mother liquors of subsequent fractions 14-21 were separated by chromatography on a silica-gel column with elution by $CHCl_3$—CH_3OH (10:1) after isolation of dihydronitraraine. Fractions of 10-15 mL were collected. Fractions 14-23 gave **1**, which was crystallized from hexane. Yield, 52 mg, mp 131-132°C.

REFERENCES

1. T. S. Tulyaganov, *Khim. Prir. Soedin.*, 780 (1994).
2. T. S. Tulyaganov and N. D. Abdullaev, *Khim. Prir. Soedin.*, 95 (1995).
3. A. W. Sangster and K. L. Stuart, *Chem. Rev.*, **65**, 69 (1965).
4. V. M. Whaley and T. R. Govindachari, *Organic Reactions*, Vol. 6, R. Adams, ed., John Wiley & Sons, New York (1951).
5. *Analgesics*, Imperial Chemical Industries, Ltd., Neth. Pat. Appl. 6,512,087, March 17, 1966; Brit. Appl., Sept. 16, 1964 and Aug. 23, 1965; *Chem. Abstr.*, **65**, 3844d (1966).
6. G. Bobowski, *J. Heterocycl. Chem.*, **18**, No. 6, 1179 (1981).
7. J. W. Daly, J. Karle, C. W. Myers, T. Tokuyama, J. A. Waters, and B. Witkop, *Proc. Natl. Acad. Sci. USA*, **68**, 1870 (1971).
8. B. Witkop and E. Gossinger, "Amphibian alkaloids," in: *The Alkaloids, Vol. 21: Chemistry and Pharmacology*, A. Brossi, ed., Academic Press, New York (1983), p. 139.
9. J. W. Daly, *Prog. Chem. Org. Nat. Prod.*, **41**, 205 (1982).
10. B. Gosler, A. J. Freyer, and M. Shamma, *J. Nat. Prod.*, **53**, 675 (1990).
11. J. Podlaha, J. Podlahova, J. Symersky, F. Turecek, V. Hanus, Z. Koblicova, J. Trojanek, and J. Slavik, *Phytochemistry*, **28**, 1779 (1989).
12. B. Gosler, A. J. Freyer, and M. Shamma, *Tetrahedron Lett.*, **30**, 1165 (1989).
13. T. S. Tulyaganov, *Khim. Prir. Soedin.*, 39 (1993).
14. T. S. Tulyaganov and N. N. Shorakhimov, *Khim. Prir. Soedin.*, 560 (1990).
15. T. S. Tulyaganov and O. M. Nazarov, *Khim. Prir. Soedin.*, 323 (2000).