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FOLIPIDINE, A NEW TYPE QUINOLINE ALKALOID FROM PLANTS OF THE *Haplophyllum* GENUS

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The new quinoline alkaloid folipidine, the structure of which was established by chemical transformations and spectral data (UV, IR, mass, NMR) using APT, 2D ^{1}H—^{1}H COSY, NOESY, and ^{1}H—^{13}C HSQC, HMBC, was isolated from two plants of the Haplophyllum *genus. Folipidine is the first representative of a new type of quinoline alkaloids that contain a heteroaromatic skeleton of [3,4-b] conjugated pyrrole and quinoline fragments. The total alkaloids of these plants exhibit antitumor activity. Folipidine does not possess such activity.*

Key words: *Haplophyllum foliosum*, *H. pedicellatum*, Rutaceae, new quinoline alkaloid folipidine, chemical transformations, spectral data (UV, IR, mass, NMR, 2D ^{1}H—^{1}H COSY, NOESY, ^{1}H—^{13}C HSQC, HMBC), antitumor activity.

Plants of the *Haplophyllum* A. Juss (Rutaceae) genus are rich sources of quinoline alkaloids of various structures [1]. Many of them possess a broad spectrum of pharmacological activity [2]. At present more than 70 alkaloids have been isolated from central Asian *Haplophyllum* species [3a], for which the structures of 50 have been established. The isolated bases are quinoline derivatives. Only two of them are acyl derivatives of putrescin [4]. Many isolated alkaloids were the first representatives of unique quinoline derivatives with a terpene component [1], a new class of furanoquinoline glycoalkaloids [5, 6], new modifications of the furanoquinoline skeleton [3b], and dimeric [7] and acylated [8] quinoline alkaloids.

The new alkaloid folipidine (**1**), $C_{22}H_{22}N_2O_4$, is an unusual quinoline alkaloid from *Haplophyllum* plants. Its composition was established using elemental analysis and high-resolution mass spectrometry. The optically active alkaloid ($[\alpha]_D$ -46°) was isolated from two plants of this genus, *H. foliosum* Vved. and *H. pedicellatum* Bge. It is poorly soluble in polar and nonpolar organic solvents and soluble in pyridine and DMSO. The solution acquires a bright yellow color upon dissolution in mineral acids. It is insoluble in bases.

Analysis of the functional groups found that **1** does not contain methoxyls. Therefore, the 3H singlet at 4.24 ppm in its ^{1}H NMR spectrum belongs to protons of an N–CH_3 (Table 1).

The IR spectrum of **1** has a broad absorption band for active H with a maximum at 3330 cm^{-1}. Acetylation of **1** by acetic anhydride in pyridine gave the tetraacetyl derivative (**2**), mp 70°C, $C_{30}H_{30}N_2O_8$, 546 $[M]^+$, ν_{CO} 1750 cm^{-1}. Therefore, all four O atoms in **1** are hydroxyls. The ^{1}H NMR spectrum of **2** gives signals for three methine protons (5.33, 6.08, 6.88 ppm) and one methylene (3.60-4.10 ppm). Their multiplicity and weak-field shifts in the spectrum of **2** compared with those of the corresponding signals in the spectrum of **1** indicate that **1** contains a hydroxylated side chain of the following structure:

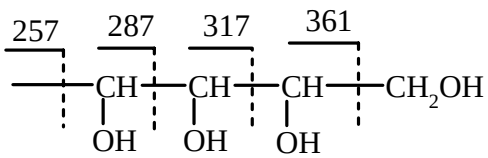

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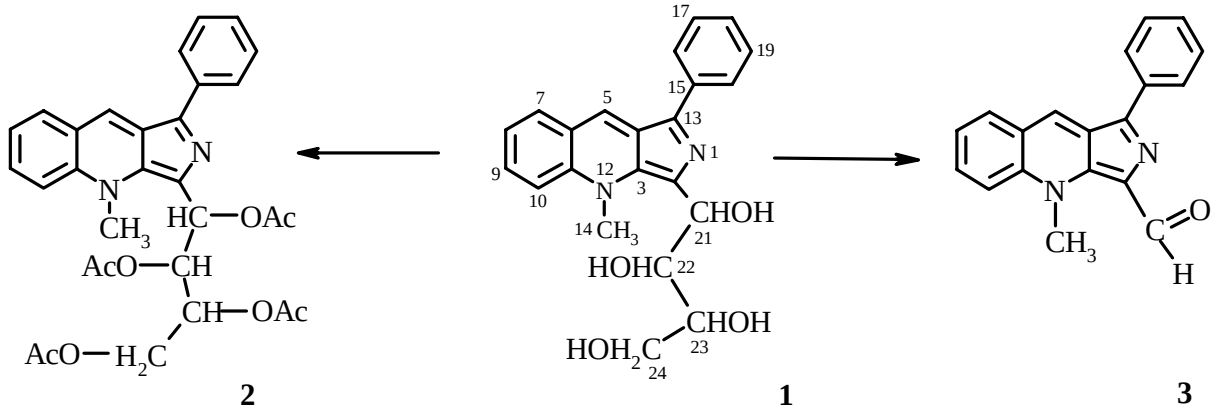
TABLE 1. Chemical Shifts of Protons and C Atoms in **1** (Py-d_5, δ, ppm, TMS internal standard = 0.00 ppm)

Proton	Chemical shift, multiplicity[a]	C atom	Chemical shift
		C-11	143.3
H-10	7.47 d	C-10	110.4
H-9	7.64 t	C-9	128.8
H-8	7.37 t	C-8	120.2
H-7	8.36 d	C-7	121.7
		C-6	140.5
H-5	8.63 s	C-5	111.2
		C-4	131.5
		C-3	134.3
		C-2	146.1
		C-13	145.0
		C-15	121.8
H-16,20	8.25 d	C-16,20	127.0
H-17,19	7.46 t	C-17,19	129.1
H-18	7.38 t	C-18	128.0
H-14	4.24 s	C-14	32.5
H-21	6.52 br.s[b]	C-21	68.8
H-22	4.94 m[b]	C-22	75.4
H-23	4.93 m[b]	C-23	72.7
H-24,24′	4.66 dd; 4.51 dd[b]	C-24	65.3

[a]s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. [b]Subspectrum 500 MHz ^{1}H NMR for polyol fragment of **1** in $CDCl_3$ +CD_3OD (2:1): H-21, br.s, δ 5.915, $J_{21,22}$ < 2; H-22, br.d, δ 4.30, $J_{22,23}$ = 8.4; H-23, ddd, δ 4.11, $J_{23,24a}$ = 3.1; H-24a, dd, δ 4.005, $J_{24a,24b}$ = 11.3; H-24b, dd, δ 3.85, $J_{24b,23}$ = 5.4 Hz.



2 **1** **3**

The ^{1}H NMR spectrum of **1** in DMSO-d_6 exhibits signals for hydroxyls as three 1H doublets at δ 4.93, 5.12, and 5.55 ppm for secondary hydroxyls and one 1H triplet at 4.39 ppm for the primary alcohol proton, which disappears upon deuteration. The mass spectrum of **1** has peaks for ions formed by successive loss of the aliphatic hydroxylated chain. These data confirm the structure of the side chain of **1**.

Oxidation of **1** by sodium periodate gave the aldehyde folipidinal (**3**), mp 165-166°C, $C_{19}H_{14}N_2O$ (286 $[M]^+$, ν_{CO} 1708 cm^{-1}, δ_{CHO} 10.12 ppm).

The frequency of the carbonyl absorption in the IR spectrum of **3** indicates that its aldehyde and, therefore, the side chain in the starting base are bound to the skeleton through a C–C bond and not an N–C bond.

TABLE 2. Correlation Peaks in the HMBC Spectrum of **1**

Proton	Correlation peaks observed due to SSCC through two or three bonds to C atoms
H-10	C-6, C-8, C-9
H-9	C-7, C-10, C-11
H-8	C-6, C-7, C-10
H-7	C-9, C-11
H-5	C-3, C-6, C-7, C-13
H-16,20	C-13, C-16, 20; C-17, 19; C-18
H-17,19	C-15, C-16,20; C-17,19
H-18	C-16, 20
H-14	C3, C-11
H-21	C-2, C-3
H-22	C-21, C-23
H-23	C-22, C-24
H-24	C-22
H-24′	C-23

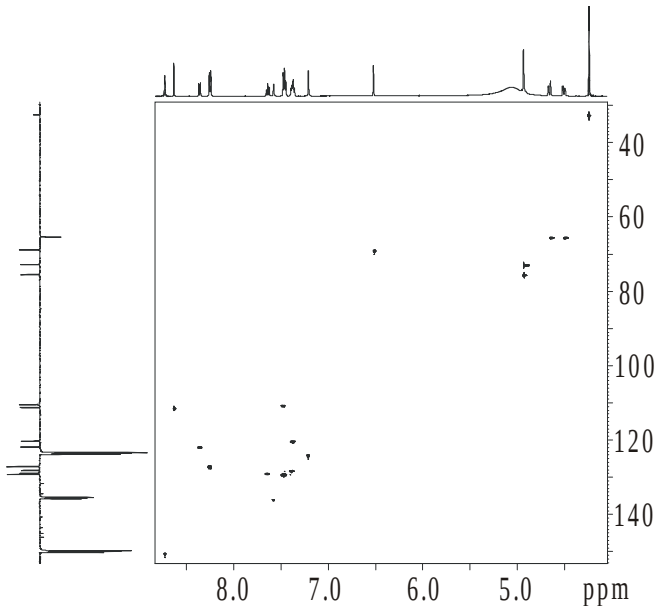


Fig. 1. HSQC spectrum of **1** in Py-d_5. Projections: ^{1}H NMR spectrum (above) and ^{13}C APT (left).

The ^{1}H NMR of **1** (Table 1) clearly exhibits in the aromatic region signals for a singly substituted benzene ring as a 2H doublet at 8.25 ppm, a 2H triplet at 7.46 ppm, and a 1H triplet at 7.38 ppm. The APT spectrum [9] revealed signals for 10 methines and 7 quaternary atoms in the ^{13}C region of sp^2-hybridized C atoms; three methines and one methylene, for sp^3-hybridized C atoms bound to one O atom; and a signal for a methyl on N.

The data in Table 1 indicate that **1** has the following structural formula: $C_{11}H_5N_2(NCH_3)(C_6H_5)(CHOH–CHOH–CHOH–CH_2OH)$. Based on a calculation of the number of rings and double bonds, it can be assumed that **1** is based on a tricyclic heteroaromatic skeleton consisting of condensed quinoline and pyrrole rings. The structure of the heteroaromatic system and the position of the substituents were unambiguously determined using two-dimensional (2D) NMR spectroscopy.

The COSY spectrum showed five closed proton spin systems: 1) four protons with δ 7.47, 7.64, 7.37, and 8.36 and splitting characteristic of *ortho*-disubstituted benzene (doublet, triplet, triplet, and doublet, respectively); 2) five protons of a singly substituted benzene ring with δ 8.25, doublet, 2H; δ 7.46, triplet, 2H; and δ 7.38, triplet, 1H; 3) five protons on C atoms bound to one O atom with δ 6.52, br. singlet; δ 4.93 and 4.94, two unresolved multiplets; and δ 4.51 and 4.66, two doublets of doublets; 4) an isolated proton with δ 8.63, singlet; and 5) a 3H singlet for NCH_3 with δ 4.24.

The NOESY spectrum revealed the spatial proximity of the proton with δ 8.63 to one of the protons of the first spin system (δ 8.36) and to two equivalent protons of the singly substituted benzene ring (δ 8.25) and the proximity of the methyl protons (δ 4.24) to the proton of the first spin system with δ 7.47 and the methylene protons of the third spin system (δ 6.52 and 4.93).

Signals of the protonated C atoms in the 1D ^{13}C NMR spectrum were assigned using the 2D ^{1}H—^{13}C HSQC spectrum (Fig. 1). This enabled correlation peaks in the 2D ^{1}H—^{13}C HMBC spectrum to be interpreted unambiguously (Table 2), assignments of the quaternary C atoms to be made, and a structural formula of **1** to be proposed that corresponds with all data in the 1D and 2D NMR spectra.

The total alkaloids of these plants exhibited antitumor activity in *in vitro* experiments on Hela and HCT 116 cancer cells. Folipidine does not possess such activity.

EXPERIMENTAL

General Comments. Column chromatography used KSK silica gel; TLC, the same silica gel with 5% added gypsum and solvent systems toluene:ethylacetate:formic acid (5:4:1) and benzene:methanol (4:1). The developer was Dragendorff's solution.

NMR spectra of **1** were recorded on a Bruker DRX-500 spectrometer at working frequency 500 MHz for ^{1}H and 125 MHz for ^{13}C at 30°C. 2D spectra were recorded using standard Bruker methods. The relaxation time in the NOESY experiment was 500 ms. The HMBC experiment was optimized for SSCC $J_{H,C}$ 8 Hz. UV, IR, and mass spectra were recorded in EPS-3T (Hitachi, EtOH), UR-20 (KBr disks), and MX-1310 instruments, respectively. The last had a direct probe into the ion source. ^{1}H NMR spectra of triacetylfolipidine and folipidinal were recorded on a JNM-4H-100/100 MHz spectrometer.

Isolation of 1 from *H. foliosum*. The air-dried aerial part of *H. foliosum* Vved. (50 kg) that was collected during flowering in the mountains of the Vakhshskii ridge (800-900 m above sea level) near the village Alimtai of Dangarinskii region in Tadzhikistan was extracted with CH_3OH. The solvent was removed. The extract was distributed between aqueous H_2SO_4 (10%) and $CHCl_3$ layers. The mixture was placed in a separatory funnel. Ether (1:10) was added to improve layering. The acid solution was washed with ether, made basic with cooling using conc. NH_4OH, and extracted successively with ether and $CHCl_3$. The condensed ether solution was treated with aqueous KOH (4%), washed with distilled water, dried over Na_2SO_4, and filtered. Condensation of the ether solution separated successively dubinidine (5.15 g), mp 132-133°C (acetone) and skimmianine (10.53 g), mp 175-176°C (methanol).

The residual (18.05 g) after removal of ether was treated with acetone (30 mL) and $CHCl_3$ (5 mL) and left overnight at 5°C. A polycrystalline solid precipitated and was not separated. The mixture was treated with acetone (25 mL) and left for 8 h at 5°C. The solid was filtered off and divided into two parts by selecting crystals of different shape. The first part consisted of skimmianine crystals as needles (1.2 g); the second, a mixture of crystals (0.98 g) that was shaken with hot acetone (200 mL) and filtered. The crystals were recrystallized from alcohol after the acetone treatment to give **1** (0.37 g). Crystals that precipitated from the condensed acetone solution at 5°C were separated (0.24 g). They were identical to **1**. The mother liquors were combined and recrystallized from alcohol to give additional **1** (0.12 g). The yield of **1** was 0.73 g.

Isolation of 1 from *H. pedicellatum*. The air-dried aerial part of *H. pedicellatum* (2.35 kg) that was collected during flowering near the village Khan-Khauz of Turkmenistan Republic was extracted with CH_3OH. The dry extract was distributed between aqueous H_2SO_4 (10%) and ether:$CHCl_3$ (1:2) layers. The acid solution was cooled and made basic with conc. NH_4OH. The alkaloids were extracted first with ether (4.29 g) and then $CHCl_3$ (7.95 g). The dry ether fraction was dissolved in $CHCl_3$ and treated with aqueous KOH (4%). Crystals precipitated at the interface of the basic solution and $CHCl_3$. These were separated, washed with acetone, and recrystallized from alcohol to give **1** (0.04 g). The basic solution was washed with $CHCl_3$, saturated with NH_4Cl, and extracted successively with ether and $CHCl_3$. Crystals precipitated from the condensed ether solution upon cooling (0.44 g). These were separated and washed with ether. Recrystallization from alcohol afforded **1** (0.20 g). The yield of **1** was 0.24 g.

Folipidine (1), mp 242-243°C (alcohol), $[\alpha]_D$ -46° (*c* 0.667, pyridine). Found, %: C 67.78, H 5.23, N 7.10. $C_{22}H_{22}N_2O_4$. Calc., %: C 69.82, H 5.87, N 7.40.

UV spectrum (nm): λ_{max} 230, 248, 278, 292 (inflection), 305 (infl.), 356, 370; λ_{min} 212, 240, 259, 330, 362.

IR spectrum (ν_{max}, cm^{-1}): 3330 (OH), 1625, 1560, 1460, 1420, 1320, 1300, 1280, 1260, 1220, 1140, 1100, 1090, 1055, 1030, 890, 880, 840, 800, 780, 750, 740.

Mass spectrum, *m*/*z* (%): 378 (5) $[M]^+$, 361 (4), 317 (6), 289 (10), 288 (55), 287 (100), 286 (11), 271 (13), 258 (20), 257 (42), 243 (13).

Acetylation of 1. A mixture of **1** (40 mg), acetic anhydride (1 mL), and pyridine (4 drops) was heated with stirring (1 min), left for 2 d, and evaporated. The solid was chromatographed over a silica-gel column with elution by ether. Crystals precipitated from the first eluates and were recrystallized from ether.

Tetraacetylfolipidine (2), mp 70°C (ether), $C_{30}H_{30}N_2O_8$.

IR spectrum (ν_{max}, cm^{-1}): 1750 (O–C=O), 1625, 1560, 1465, 1430, 1380, 1220, 1140, 1090, 1050, 960, 840.

Mass spectrum *m*/*z* (%): 546 (70) $[M]^+$, 503 (13), 487 (40), 427 (13), 401 (70), 385 (26), 357 (7), 344 (10), 330 (17), 288 (23), 287 (100), 271 (10), 257 (20).

^1H NMR spectrum ($CDCl_3$, 0 = HMDS, δ, ppm, J/Hz): 1.75, 1.82, 1.86, 2.01 (3H each, s, 4 × OAc), 3.60-4.10 (2H, m, CH_2–OAc), 4.16 (3H, s, N–CH_3), 5.33 (1H, m, CH–OAc), 6.08 (1H, t, J = 6, CH–OAc), 6.88 (1H, d, J = 6, CH–OAc), 7.00-7.54, 7.88-8.14 (5H and 4H, respectively, 9 × Ar–H), 8.24 (1H, s, Ar–H).

Periodate Oxidation of 1. A solution of **1** (82 mg) in CH_3OH (15 mL) was treated with stirring with sodium periodate (0.5 g) in water (3 mL). The mixture was stirred at room temperature for 10 h. The resulting solid was separated and recrystallized from acetone to give **3** (33 mg).

Folipidinal (3), mp 165-166°C (acetone), $C_{19}H_{14}N_2O$.

UV spectrum (nm): λ_{max} 224, 278, 292 sh, 305 sh, 356, 372, 406; λ_{min}, 250, 340, 360, 386.

IR spectrum (ν_{max}, cm^{-1}): 2835, 1708, 1630, 1628, 1598, 1588, 1495, 1470, 1430, 1375, 1323, 1292, 1261, 1228, 1167, 1140, 1118, 1090, 1050, 1022, 1015, 940, 913, 785, 738.

Mass spectrum *m*/*z* (%): 286 (62) $[M]^+$, 258 (25), 257 (100), 242 (6), 241 (5), 179 (5), 128 (4).

^1H NMR spectrum ($CDCl_3$, 0 = HMDS, δ, ppm): 4.01 (3H, s, N–CH_3), 7.33 (6H, m, Ar–H), 7.94 (3H, m, Ar–H), 8.23 (1H, s, Ar–H), 10.12 (1H, s, HC=O).

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