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GERPHYTINE, A NEW FURANOQUINOLINE ALKALOID FROM *Haplophyllum griffithianum*

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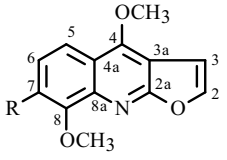
A series of known alkaloids and the new base gerphytine were isolated from total alkaloids from the aerial part of Haplophyllum griffithianum *growing in Surkhandar′ya Oblast, Republic of Uzbekistan. Gerphytin was identified based on spectral data and an x-ray crystal structure analysis as a furanoquinoline derivative and had the structure 7-*O*-allyl-4,8-dimethoxyfuranoquinoline.*

Keywords: *Haplophyllum griffithianum*, Rutaceae, new furanoquinoline alkaloid gerphytine, structure.

The plant genus *Haplophyllum* (Rutaceae) has for a long time been used in folk medicine as an anesthetic and wound-healing agent for burns and tumors, toothache, and stomach and skin diseases and as a treatment for respiratory organs and several types of cancer [1–3]. Extracts of several *Haplophyllum* species exhibit cytotoxic activity [4].

We reported earlier on the isolation from the aerial part and roots of *H. griffithianum* Boiss. collected during flowering (Nilu village, Surkhandar′ya Oblast) of the quinoline alkaloids skimmanine, dictamnine, dubinine, dubinidine, dubamine, and *N*-methylhaplofoline [5]. In continuation of studies of the alkaloid composition of the $CHCl_3$ total alkaloids, we isolated from the aerial part of the plant a new base with mp 93–95°C whose physicochemical properties and spectral data differed from quinoline bases that were previously reported and which we called gerphytine.

Gerphytine (**1**) had formula $C_{16}H_{15}NO_4$ ($[M]^+$ 285), was soluble in $CHCl_3$, poorly soluble in acetone and alcohol, and insoluble in water and base.



1, 2

1: R = $OCH_2^{9}CH^{10}=CH_2^{11}$

2: R = $OCH_2CHC(CH_3)_2$ (HO, OH)

The IR spectrum of the base exhibited absorption bands at 3139 and 3166 cm^{-1} due to a furan ring. Absorption bands of hydroxyl and carbonyl groups were missing.

The UV spectrum showed two principal absorption maxima including a strong (short-wavelength) band at 249.64 nm (log ε 4.7) and a weaker band at 318.91 nm (log ε 3.8). The long-wavelength band was split into three peaks and was separated from the first maximum by a deep minimum at 278.0 nm (log ε 3.00). This was characteristic of furanoquinoline alkaloids [6, 7].

The aromatic region of the PMR spectrum of gerphytine had two resonances for protons of a benzene ring at 7.87 and 7.11 ppm (1H each, d, J = 9.3 Hz) and resonances for protons of a furan ring at 7.48 (1H, d, J = 2.8 Hz, H-2) and 6.93 ppm (1H, d, J = 2.8, H-3) that were typical of furanoquinoline-type alkaloids.

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TABLE 1. Chemical Shifts of C and H Atoms, Data for DEPT Experiments of Gerphytine (**1**) ($CDCl_3$, δ, ppm, J/Hz, 0 = HMDS) and Evoxine (**2**) (DMSO-d_6) [9]

C atom	**1**			**2**
	δ_H	DEPT	δ_C	δ_C
2	7.48 (d, J = 2.8)	CH	143.11	143.6
3	6.93 (d, J = 2.8)	CH	104.75	105.3
2a		C	164.42	163.8
3a		C	115.27	114.1
4		C	157.23	156.7
4a		C	102.25	101.7
5	7.87 (d, J = 9.4)	CH	118.05	117.6
6	7.11 (d, J = 9.4)	CH	114.33	113.9
7		C	141.77	141.7
8		C	142.90	140.8
8a		C	151.23	151.6
4-OCH_3	4.06 s	CH_3	59.09	59.3
8-OCH_3	4.31 s	CH_3	61.72	60.8
9	4.70 (dt, J = 5.3, 1.5)	CH_2	70.82	71.6
10	6.05 (dtt, J= 17.3, 10.5, 5.3)	CH	133.66	76.1
11	5.38 (dq, J = 17.3, 1.5) 5.22 (dq, J = 10.5, 1.5)	CH_2	117.83	70.9
2 × CH_3		2 × CH_3		24.3, 27.3

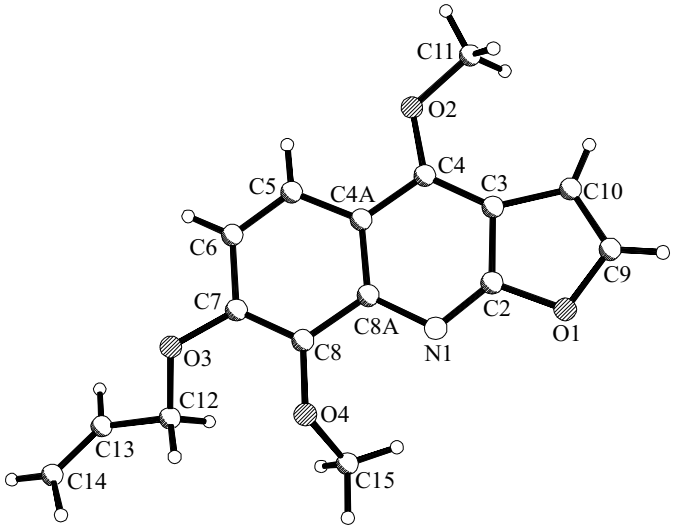

Fig. 1. Molecular structure and atomic numbering of **1**.

The PMR spectrum of **1** also exhibited two singlets at 4.31 and 4.06 ppm (3H each, s). Protons of C-4 methoxyls in spectra of furanoquinoline alkaloids resonated at weaker field of 4.30–4.46 ppm than protons of other methoxyls that appeared as singlets at 3.70–4.10 ppm [8]. Hence it followed that the resonance at 4.31 ppm was due to a C-4 OCH_3 group whereas the resonance of the C-5 proton situated in the *peri*-position to the C-4 methoxyl was observed in furanoquinoline-type alkaloids at weaker field than resonances of other aromatic protons [8]. Therefore, the resonance of H-5 was observed at 7.87 ppm and the multiplet and SSCC of the resonance at 7.11 ppm were due to H-6. This meant that the benzene ring of gerphytine had *O*-containing substituents in the C-7 and C-8 positions.

The ^{13}C NMR spectrum of **1** contained resonances for 16 C atoms. A DEPT experiment found that they belonged to two methyls, two methylenes, five methines, and seven quaternary C atoms. Table 1 presents the assignments of these resonances and their characteristics. The chemical shifts (CSs) of most gerphytine and evoxine (**2**) C atoms [9] were similar with the exception of C-10 and C-11. This indicated that gerphytine had the same heterocyclic skeleton as evoxine and contained a C-8 OCH_3 group. They differed in the nature of the C-7 substituent.

The CS of C-9 (70.82 ppm) suggested that C-9 was bonded to an O atom. The CSs of C-10 (133.66) and C-11 (117.83) and their multiplicities indicated that a –CH=CH_2 bond was present. Therefore, gerphytine differed from evoxine by the presence of a C-7 –OCH_2–CH=CH_2. This was confirmed by the proton spectrum of gerphytine that showed resonances at 4.70 (2H, dt, J = 5.3, 1.5, 2H-9), 6.05 (1H, dtt, J = 17.3, 10.5, 5.3, H-10), 5.38 (dq, J = 17.3, 1.5, H-11a), and 5.22 (dq, J = 10.5, 1.5, H-11b).

Thus, gerphytine had the structure 7-*O*-allyl-4,8-dimethoxyfuranoquinoline.

Figure 1 shows the molecular structure of gerphytine from an x-ray crystal structure analysis (XSA). The furanoquinoline fragment was planar with mean-square deviation 0.044 Å. All O atoms of the substituents lay in the plane of the furanoquinoline ring. Methoxyls on C-4 and C-8 were twisted relative to this plane by 11.85 and 62.92°, respectively. These orientations were stabilized by weak inter- and intramolecular H-bonds of the C–H…O and C–H…N types. The intramolecular C-15–H…N1 H-bond had the parameters 0.96(3), 2.42(3), 2.993(2) Å and 118(2)°. The geometry of the intermolecular C-11—H…O4^i H-bond in the crystal structure had the values 0.98(2), 2.52(2), 3.491(2) Å and 172(2)° (where $i = x, y, z - 1$).

EXPERIMENTAL

General Comments. Total alkaloids were separated and purified over columns packed with silica gel (L 100/160 μm; KSK 70-100 μm) using solvent systems benzine, benzine:EtOAc (1:1, 1:6, 1:10), EtOAc, and EtOAc:MeOH (25:1). TLC used L 5/40 silica gel (Czech. Rep.) containing gypsum (13%) and Silufol and the solvent systems MeC_6H_5:EtOAc:HOAc (1, 5:4:1), $CHCl_3$:MeOH (2, 8:1), EtOAc (3), EtOAc:MeOH (4, 8:1). Plates were detected in UV light and then sprayed with Dragendorff's solution.

UV spectra were measured in EtOH in a 1-cm quartz cuvette at concentrations $5\cdot10^{-5}$–$5\cdot10^{-4}$ M on a Perkin–Elmer Lambda-16 spectrometer. IR spectra were recorded in KBr pellets on a Model 2000 IR-Fourier spectrometer. PMR and ^{13}C NMR spectra were taken from $CDCl_3$ solutions with HMDS as an internal standard for PMR spectra on a Varian Unity 400 plus spectrometer (δ, ppm). CSs in ^{13}C NMR spectra are given relative to the resonance of $CDCl_3$ C atoms (CS = 77.23 ppm vs. TMS). DEPT experiments were carried out using the spectrometer standard method.

Isolation and Separation of Total Alkaloids. Dry ground raw material (580 g) was extracted with MeOH until alkaloids were totally extracted. The evaporated MeOH extract was distributed between $CHCl_3$ (A) and H_2SO_4 (5%). The $CHCl_3$ solution was shaken with H_2SO_4 (5%) and then base solution (4%). The combined acidic solutions were made basic. Alkaloids were extracted by $CHCl_3$ (basic fraction 2.32 g). Distillation of $CHCl_3$ from solution A produced neutral fraction B (24.57 g).

Separation of Basic Fraction. Work up of the $CHCl_3$ alkaloids (2.32 g) with acetone separated dubinine (0.32 g). The mother liquor (2.0 g) was chromatographed over a column of silica gel. Elution by hexane:$CHCl_3$ (7:1, 2:1, 1:1) isolated dictamnine (0.02 g), dubinine (0.8 g), dubinidine (0.08 g), *N*-methylhaplofoline (0.1 g), skimmianine (0.7 g), and a mixture of crystals (0.21 g).

The mixture of crystals (0.21 g) was chromatographed over a column of silica gel with elution by EtOAc. The first effluents contained gerphytine (0.05 g); the later ones, dubamine (0.12 g).

Gerphytine (1), crystals, mp 93–95°C (acetone), green in UV light in system 1, R_f 0.57. UV spectrum (λ_{max}, nm): EtOH: 249.64, 318.91; EtOH + OH^-: 249.65, 319.01; EtOH + H^+: 249.61, 318.88. IR spectrum (ν, cm^{-1}): 3166, 3139, 3081, 3021, 2952, 2872, 1625, 1609, 1583, 1505, 1480, 1456, 1399, 1367, 1322, 1296, 1237, 1090, 1036, 991, 963, 929, 824, 790, 751, 722.

X-ray Crystal Structure Analysis. Crystals of **1** were obtained from EtOH solution by slow evaporation at room temperature. Single crystals were transparent elongated prisms with $a = 11.0921(5)$, $b = 14.6218(5)$, $c = 8.9933(4)$ Å, $\beta = 111.966(5)°$, $V = 1352.7(1)$ Å^3, $\rho_{calc} = 1.406$ g/cm^3, space group $P2_1/c$, $Z = 4$. Unit cell constants were determined and refined from crystals of size $0.2 \times 0.3 \times 0.5$ mm on a CCD Xcalibur Ruby diffractometer (Oxford Diffraction) using Cu K_α-radiation (300 K, graphite monochromator). A three-dimensional dataset of reflections was measured on the same diffractometer. Absorption corrections were applied semi-empirically using the SADABS program [10]. The structure was solved by direct methods using the SHELXS-97 programs and refined using the SHELXL-97 program [11]. All nonhydrogen atoms were refined by anisotropic full-matrix least-squares methods (over F^2). Coordinates of H atoms of methyl groups and vinyl of the allyloxy group were found experimentally. Coordinates of other H atoms were found geometrically and refined

with fixed isotropic thermal factors $U_{iso} = nU_{eq}$, where n = 1.2 and U_{eq} was the equivalent isotropic thermal factor of the corresponding C atom. The final agreement factors (R) were 0.045 over 2208 reflections [$I > 2\sigma(I)$] (wR2 = 0.124) and 0.057 over all 2770 reflections (wR2 = 0.133). Data from the XSA experiment were deposited in the Cambridge Crystallographic Data Centre (CCDC 843089).

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