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Alkaloids of *Bongardia chrysogonum*

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Abstract

Two new pyridine alkaloids were isolated from the tubers of *Bongardia chrysogonum*, together with a new lupine alkaloid. The structures of the new alkaloids were characterized by spectroscopic analysis and the stereochemistry was confirmed by applying Horeau's method. © 1998 Published by Elsevier Science Ltd. All rights reserved.

Keywords: *Bongardia chrysogonum*; Leonticeae; Tubers; Pyridine alkaloids; Bongardine; *N*-acetyl bongardine; Bongardamine; Lupine alkaloids; Horeau's method

1. Introduction

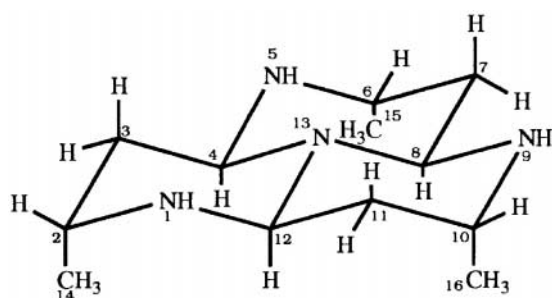
In Turkish folk medicine, the tubers of *Bongardia chrysogonum* are used for the treatment of haemorrhoids and prostate hypertrophy. The plant is widely spread in Turkey and Afghanistan. Previously, we have reported lupine and isoquinoline bases isolated from this species (Atta-ur-Rahman, Shahwar, Perveen, & Choudhary, submitted). In this paper, we report three new alkaloids, bongardamine (**1**), bongardine (**2**) and *N*-acetyl bongardine (**3**).

2. Results and discussion

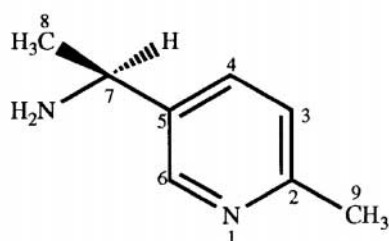
Bongardamine (**1**) showed a $[M]^+$ at m/z 224 in the EI mass spectrum. HREI mass spectrometry established the molecular formula as $C_{12}H_{24}N_4$ (m/z 224.2005). The 1H NMR spectrum (Table 1) was characterized by the presence of well-resolved signals at δ 1.15 (9H, d, $J = 8.3$ Hz), 1.25 (3H, dd, $J = 12.8$, 2.7 Hz), 1.88 (3H, dt, $J = 12.8$, 3.0 Hz), 2.87 (3H, ddq, $J = 8.3$, 3.0, 3.0 Hz) and 3.18 (3H, dd, $J = 10.6$, 3.9 Hz). The analysis of the COSY 45° spectrum of **1** showed the presence of a $-CH(CH_3)CH_2CH-$ frag-

ment (fragment I) in this molecule. The ^{13}C NMR spectrum of **1** displayed only four carbon signals. An HMQC experiment showed that the carbon at δ 41.2 (CH_2) was linked to the proton at δ 1.25 and 1.88. The signal at δ 21.5 was coupled with the methyl protons at δ 1.15. Another carbon signal at δ 50.5 was linked to the proton at δ 2.87, while the downfield signal at δ 73.4 was connected with the proton at δ 3.18. The molecular formula of **1** indicated 12 carbon atoms, while the ^{13}C NMR spectrum displayed only four carbon signals; therefore, it was suggested that fragment I was repeated three times in the molecule. The presence of fragment I was further confirmed on the basis of the HMBC spectrum. The methyl carbon at δ 21.5 exhibited shift correlations with the protons at δ 1.25 ($^3J_{CH}$) which were, in turn, coupled with the carbon at δ 50.5 ($^2J_{CH}$) and 73.4 ($^2J_{CH}$). Another $^2J_{CH}$ interaction was observed between the proton at δ 1.88 and the carbon at δ 73.4. The NOE measurements were used to probe the orientation of the different protons. Irradiation at δ 1.15 (CH_3) resulted in 4.4% enhancement of the signal at δ 3.18 (CH), which suggested a 1,3-diaxial orientation of methyl and methine protons at δ 3.18. This conclusion was further confirmed by irradiating the signal at δ 3.18, which showed 2.5% enhancement for δ 1.15. No such effect was observed at δ 2.87, which suggested the axial orientation of the methyl groups. The ring junction

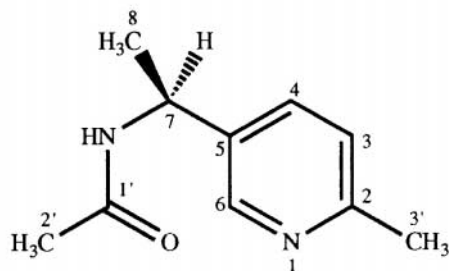
* Corresponding author.



Bongardamine (1)



Bongardine (2)



N-Acetylbongardine (3)

was determined to be *trans* by the presence of Bohlmann bonds ($2980\text{--}2900\text{ cm}^{-1}$) in the IR spectrum. These data suggested structure **1** for bongardamine.

Bongardine (**2**) was isolated from the crude butanolic extract of *B. chrysogonum* as a white amorphous solid. The IR spectrum (CHCl_3) exhibited stretching vibrations at 3325 and 3450 (primary amine) and 1590 ($\text{C}=\text{C}$, aromatic) cm^{-1} . The compound, when subjected to EI and FD mass spectrometry experiments, failed to exhibit a $[\text{M}]^+$. However, a positive ion FAB mass spectrum in a thioglycerol matrix indicated the

pseudomolecular ion $[\text{M} + 1]^+$ at m/z 137. The molecular formula was determined to be $\text{C}_8\text{H}_{12}\text{N}_2$ (136.0980 mu). The four degrees of unsaturation were accounted for by the pyridine ring.

The ^1H NMR spectrum of **2** (Table 2) was characterized by three one-proton signals at δ 7.35 (d, $J = 8.1$ Hz), 7.80 (dd, $J = 8.1, 2.4$, Hz) and 8.45 (d, $J = 2.4$ Hz), two methyl signals at δ 1.60 (d, $J = 6.8$ Hz) and 2.50 (s), and a one-proton quartet at δ 4.40 ($J = 6.8$ Hz). The COSY 45° spectrum disclosed the $^1\text{H}\text{--}^1\text{H}$ connectivities. The signal at δ 7.35 showed *ortho*-coupling with δ 7.80 which, in turn, was *meta*-coupled with a signal at δ 8.45. The doublet at δ 1.60 (3H) was coupled with the quartet at δ 4.40 (1H), indicating the connectivity of a methyl with a methine proton.

The ^{13}C NMR spectrum of **2** showed eight carbon signals, including two methyl, four methine and two quaternary carbons. The presence of quaternary carbons at δ 160.2 and 134.1, and methine carbons at 125.1, 136.7 and 148.5, suggested a disubstituted pyridine ring (Shamma, 1979). In order to establish the location of the substituents, HMBC and NOE experiments were carried out (Table 1). The downfield signal at δ 8.45, which could be assigned to C-6 of a pyridine ring, showed connectivity with the carbons at δ 49.8 ($^3J_{\text{CH}}$), 136.7 ($^3J_{\text{CH}}$) and 160.2 ($^3J_{\text{CH}}$) in the HMBC spectrum. The methine proton at δ 4.40 showed $^2J_{\text{CH}}$ coupling with the quaternary carbon at δ 134.1, which confirmed the location of the substituent at C-5. The methyl singlet at δ 2.50 showed interactions with the aromatic protons at δ 7.35 ($^3J_{\text{CH}}$) and the quaternary carbon at δ 160.2 ($^2J_{\text{CH}}$). These data established the position of one methyl group at C-2 and the methine proton (δ 7.35) at C-3. The substitution pattern in **2** was further confirmed by the NOE difference spectra. Irradiation at δ 7.80 (H-4) resulted in 16.6% enhancement at δ 7.35 (H-3), which confirmed the *ortho*-position of the two protons. Another enhancement was observed at δ 1.60, which established the location of this methyl at C-7. The proximity of the methyl group (δ 1.60) with the methine proton (δ 4.40) was indicated by 10.4% enhancement of the signal at δ 4.40.

The location of a methyl group at C-2 was confirmed by irradiating this signal (δ 2.50) which resulted in 7.6% enhancement at δ 7.35 (H-3). The presence of a NH_2 group in the side-chain was deduced by the presence of a downfield methine at δ 49.8, together with a fragment ion in the mass spectrum at m/z 94.0631 ($\text{C}_6\text{H}_8\text{N}$) which was formed by the loss of the side-chain from the $[\text{M}]^+$ of compound **2**. The stereochemistry of the side-chain of **2** was determined by Horeau's method (Horeau & Kagan, 1964), which established the *S*-configuration at C-7. These data established structure **2** for bongardine.

N-Acetylbongardine (**3**) was obtained as a white amorphous powder. The molecular formula was deter-

Table 1
¹H and ¹³C NMR data for bongardamine (1)

Carbon No.	δ _C	δ _H , J (Hz)	HMBC	NOE
2	50.5	2.87 ddq, 3.0, 3.0, 8.3	H-14, H-3	H-3α, H-3β, H-14
3	41.2	1.25 (H-3β) dd, 2.7, 12.8, 1.88 (H-3α) dt, 3.0, 12.8	H-4, H-14	H-4, H-2, H-3α, H-14, H-2, H-4
4	73.4	3.18 dd, 3.9, 10.6	H-3α, H-3β	H-14, H-3α, H-3β
6	50.5	2.87 ddq, 3.0, 3.0, 8.3	H-15, H-7	H-7α, H-7β, H-15
7	41.2	1.25 (H-7β) dd, 2.7, 12.8, 1.88 (H-7α) dt, 3.0, 12.8	H-8, H-15	H-8, H-6, H-7α, H-15, H-6, H-8
8	73.4	3.18 dd, 3.9, 10.6	H-7α, H-7β	H-15, H-7α, H-7β
10	50.5	2.87 ddq, 3.0, 3.0, 8.3	H-16, H-11	H-11α, H-11β, H-16
11	41.2	1.25 (H-11β) dd, 7, 12.8, 1.88 (H-11α) dt, 3.0, 12.8	H-12, H-16	H-12, H-10, H-11α, H-16, H-10, H-12
12	73.4	3.18 dd, 3.9, 10.6	H-11α, H-11β	H-16, H-11α, H-11β
14	21.5	1.15 d, 8.3	H-2	H-2, H-4
15	21.5	1.15 d, 8.3	H-6	H-6, H-8
16	21.5	1.15 d, 8.3	H-10	H-10, H-12

mined to be C₁₀H₁₄N₂O by HREI mass spectrometry, differing from the molecular formula of **2** by addition of an acetyl group (CH₃CO). The fragment ion at *m/z* 135.0917 corresponding to C₈H₁₁N₂ [M – 43]⁺ further confirmed the presence of an acetyl group in **3**. The acetyl group was suggested to be attached to the nitrogen atom in the side-chain, because the ¹³C NMR spectrum showed a quaternary carbon at δ 170.5. Comparison between spectral data of **3** with those of **2** revealed that the only difference was that in compound **3**, one of the hydrogens of the NH₂ group was replaced by an acetyl group. The connectivity of the COSY and HMBC experiments supported the assumed structure of **3**. The stereochemistry at C-7 was determined by comparing the sign of the optical rotation of *N*-acetyl bongardine (–56°) with its deacetylated analogue bongardine (–48°), which showed that the absolute stereochemistry at C-7 is *S*. The substitution pattern was determined by the experiments and found to be the same as that in **2**. Therefore, structure **3** was established for *N*-acetylbongardine.

Table 2
¹H and ¹³C NMR data for bongardine (2) and *N*-acetylbongardine (3)

Carbon No.	2		3	
	δ _C	δ _H , J (Hz)	δ _C	δ _H , J (Hz)
2	160.2	–	151.4	–
3	125.1	7.35 d, 8.1	126.8	7.25 d, 8.1
4	136.7	7.80 dd, 2.4, 8.1	139.1	7.70 dd, 2.3, 8.1
5	134.1	–	142.8	–
6	148.2	8.45 d, 2.4	144.6	8.35 d, 2.3
7	49.8	4.40 q, 6.8	46.2	4.85 q, 7.1
8	20.8	1.60 d, 6.8	21.6	1.45 d, 7.1
9	23.5	2.50 s	–	–
1'			170.5	–
2'			23.1	1.81 s
3'			19.2	2.49 s

3. Experimental

3.1. General

CC was performed on silica gel 60 (70–230 mesh). TLC was carried out on precoated silica gel 60 F₂₅₄ plates (Merck). NMR were recorded in CD₃OD at 500 MHz for ¹H and 125 MHz for ¹³C. IR were measured in CHCl₃ and in KBr. FABMS were recorded using MeOH as solvent and glycerol and thioglycerol as the matrix.

3.2. Plant material

Tubers of *B. chrysogonum* (L.) Griseb. were collected in the vicinity of Gaziantep, Turkey. The plant was identified at the Department of Pharmacy, Anadolu University, Eskisehir, Turkey, where a voucher specimen is deposited.

3.3. Extraction and isolation

Air-dried tubers (15 kg) were soaked in EtOH. After removing solvent under red. pres., 113 g of residue was obtained. This was dissolved in H₂O (1 l) and extracted with CHCl₃ at pH 9. The fr. obtained was subjected to CC. The fr. eluted with CHCl₃ was further purified by TLC in CHCl₃–MeOH (9.9:0.1). Bongardine (**2**) was obtained as a pale yellow amorphous solid (19.8 mg, 1.32 × 10^{–6}% yield). Another fr. eluted with CHCl₃–MeOH (99:1), on purification by TLC (CHCl₃–MeOH, 9:1), yielded *N*-acetyl bongardine (**3**) as a white amorphous solid (3 mg, 2 × 10^{–7}% yield).

The EtOH-insol. residue was dissolved in H₂O and extracted with *n*-BuOH. The BuOH-sol. fr. was subjected to CC. On elution with CHCl₃–MeOH (19:1) compound **1** was obtained. Recrystallization from Me₂CO resulted in colorless needle-like crystals of bongardamine (**3**) (15.2 mg, 1 × 10^{–6}% yield).

3.4. Bongardamine (1)

Needle-like crystals, mp 203°. $[\alpha]_{\text{D}}^{25^{\circ}\text{C}}$: -7.7° (*c* 0.31, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ (nm) (log ϵ): 205 (2.57). IR ν_{max} (KBr, cm^{-1}): 3400 (sec. amine), 2980–2900 (Bohlmann bands), 1625 (NH, deformation), 1380, 1310, 1182 (C–N stretchings). EIMS m/z (rel. int.): 224 (38), 223 (37), 181 (90), 139 (52), 111 (95), 70 (100).

3.5. Bongardine (2)

Pale yellow powder. $[\alpha]_{\text{D}}^{29^{\circ}\text{C}}$ = -48° (*c* 0.36, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ (nm) (log ϵ): 265 (5.5), 205 (6.1). IR ν_{max} (CHCl_3 , cm^{-1}): 3325, 3450 (primary amine), 1590 (C=C, aromatic). EIMS m/z (rel. int.): 135 (8), 121 (100), 94 (25), 65 (10), 51 (10).

3.6. N-Acetylbongardine (3)

White amorphous powder. $[\alpha]_{\text{D}}^{25^{\circ}\text{C}}$ = -56° (*c* 0.1, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ (nm) (log ϵ): 280.1 (4.5), 200.3 (7.2). IR ν_{max} (CHCl_3 , cm^{-1}): 3450 (CONH), 1628 (CO), 1605 (C=C, aromatic). EIMS m/z (rel. int.): 178 (45), 163 (10), 135 (42), 121 (100), 94 (12), 65 (6).

3.7. Determination of configuration at C-7 of bongardine (2)

Compound **2** (3 mg, ca. 0.022 mmol) was added to a soln. of racemic 2-phenylbutanoic anhydride (0.2 ml) in dry $\text{C}_5\text{H}_5\text{N}$ (ca 0.1 ml). The resulting mixt. was left overnight at room temp. The reaction mixt. was dild. with H_2O (0.2 ml) and allowed to stand for 30 min. 0.1 M NaOH was then added dropwise until the pH became 9 and the soln. was then extracted with C_6H_6 (10 ml). The C_6H_6 extract was concd. to 1 ml. The optical rotation of the resulting 2-phenylbutanoic acid in soln was found to be negative establishing the *S*-configuration at C-7.

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