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STRUCTURE OF IRNIINE, A PYRROLIDINE ALKALOID FROM *ARISARUM VULGARE*

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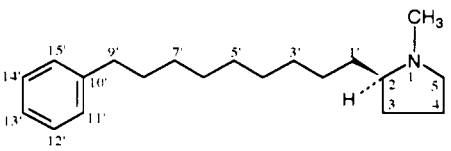
ABSTRACT.—A toxic pyrrolidine alkaloid, irniine [**1**], was isolated from *Arisarum vulgare* tubers, and its structure was elucidated by 2D COSY spectroscopic methods.

Arisarum vulgare Targ.-Tozz. (Araceae), is widespread in Morocco and is named "irni"; the starch-containing tubers were, in the past, eaten by people during periods of scarcity. The consumption of large amounts induced several toxicologic manifestations, such as irritation of the mucous membranes, gastro-enteritis, and allergic symptoms, mainly dermatitis and pruritis, and sometimes led to death (1,2). Previous investigations on this plant report the presence of lipids, proteins, and non-specified alkaloids (2,3). We investigated the toxic components in the tubers of *A. vulgare* by using a bioassay employing the larvae of brine shrimp *Artemia salina* (4). The alkaloid fraction of the MeOH extract of the tubers demonstrated toxicity in this bioassay. We report here the isolation and structure elucidation of the main alkaloidal, toxic, component which is new and which is named irniine.

RESULTS AND DISCUSSION

The MeOH extract of the ground fresh tubers was made basic with aqueous NH_3 and was extracted with CH_2Cl_2. The extract containing alkaloids was toxic to the brine shrimp. The usual way of purifying alkaloids failed, due to the lipophilic nature of the alkaloids. The toxic fraction was further purified by repetitive cc monitored by the bioassay. The toxic compound, irniine [**1**], was isolated as a colorless optically active oil, $[\alpha]^{21}D$ −35° (c = 0.2, CH_2Cl_2).



1

The eims of **1** exhibited a molecular ion $[M]^+$ at *m/z* 287, and the hrms was consistent with the molecular formula $C_{20}H_{33}N$ (*m/z* 287.2595, calcd 287.2613). The ^{13}C nmr displayed signals for twenty carbons, which were distributed into six aromatic carbon atoms (five methines and one quaternary), one methine at δ 66.41, twelve methylenes, one at δ 57.11 being linked to an heteroatom, and one Me group at δ 40.21. The ^{13}C chemical shifts of this Me, the methylene δ 57.11, and the methine groups suggested that they were bound to the nitrogen atom.

The ^1H-nmr spectrum showed 33 non-exchangeable protons, among which five were aromatic protons, and the *N*-Me group appeared as a singlet at δ 2.28. Analysis of the ^1H-^1H COSY and ^1H-^{13}C COSY spectra allowed us to define the following structural subunits: a monosubstituted benzene ring, a linear chain of methylenes -$(CH_2)_9$-, and a 2-substituted pyrrolidine ring. The methylene proton at δ2.57 (H_2-9′) was coupled to a methylene multiplet at δ 1.58 (H_2-8′) which was further coupled to a methylene at δ 1.31 (H_2-7′). The non-equivalent protons of the methylene at δ 3.06 and

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2.11 (H_2-5) were vicinal to a methylene at δ 1.75 and 1.60 (H_2-4) which was further coupled to a methylene at δ 1.90 and 1.40 (H_2-3). The *N*-methine at δ 1.95 (H-2) was coupled to this later methylene and to another one having non-equivalent protons at δ 1.62 and 1.20 (H_2-1′) and which in turn showed a cross peak in the ^1H-^1H COSY with a methylene at δ 1.26 (H_2-2′).

These structural subunits were connected on the basis of the long range heteronuclear ^1H-^{13}C COSY optimized for a coupling constant $J_{CH} = 7$ Hz. The aromatic carbon atoms at δ 142.74 (C-10′) and δ 128.22 (C-11′, -15′) showed ^2J and ^3J connectivities, respectively, with the -CH_2- group at δ 2.57 (H_2-9′) which was also correlated with carbon atoms at δ 31.40 (C-8′, ^2J) and δ 29.19 (C-7′, ^3J). The carbon atom at δ 26.58 (C-2′) was correlated with the methylene at δ 1.62 and 1.20 (H_2-1′). The methine carbon at δ 66.41 (C-2) showed ^3J connections with the protons of the *N*-Me at δ 2.28 and the methylene at δ 3.06 (H_a-5) and, finally, the carbon at δ 21.60 (C-4) with the protons at δ 1.95 (H-2), 1.90, and 1.40 (H_2-3). The results agreed with an *N*-methyl, 2-substituted-pyrrolidine ring system.

The eims spectrum exhibited a base peak at *m/z* 84 corresponding to $[C_5H_{10}N]^+$. This fragment arose from the cleavage of the chain, β to the nitrogen of the *N*-methylpyrrolidine ring, as expected. From these data, structure **1**, *N*-methyl-2-(9′-phenylnonyl)-pyrrolidine, was proposed for irniine. The absolute configuration of the asymmetric center was not determined. Nevertheless, 2-alkylpyrrolidine (*n*-butyl to heptyl) prepared by different enantioselective synthesis (5–7) display negative rotations (taken in CH_2Cl_2 or $CHCl_3$) for the *R* absolute configuration at their unique chiral center, and positive for *S* enantiomers. It is therefore suggested that the absolute configuration at C-2 of irniine is *R* due to its negative optical rotation value.

Irniine was toxic in the brine shrimp bioassay, showing an LC_{50} 1.0 μg/ml. This structure has some similarities with the alkyl pyrrolidine active constituents of the venom of several species of ants (8). Other toxic substances from the same plant and the cellular target of irniine are under investigation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded at 300 MHz for ^1H and 75 MHz for ^{13}C on a Bruker AC 300 spectrometer. Ei and ci mass spectra were obtained with a Nermag Sidar V 3.0 mass spectrometer and the hrms with a V.G. Analytical ZAB-HF mass spectrometer. The ir spectrum was registered on a Perkin-Elmer 881 ir spectrophotometer. Optical rotation was measured on a Perkin-Elmer 141 polarimeter.

PLANT MATERIAL.—*A. vulgare* was collected at Ain Sfâa, Oujda region, Morocco, in September 1989 and identified by Dr. El Ismaili. A voucher specimen was deposited at the Herbarium of the "Muséum national d'Histoire naturelle" (No. 21-1992 Laboratory of Phanerogamy) Paris, France.

EXTRACTION AND ISOLATION.—The fresh tubers (5.94 kg) of *A. vulgare* were ground and extracted with MeOH. The red crude extract was made basic with aqueous NH_3, and the solution was extracted with CH_2Cl_2. The organic phase yielded a residue (14.4 g) which was purified by repetitive cc on Sephadex LH 20 [MeOH-CH_2Cl_2 (7:3)], and the alkaloid fraction (2.6 g) was further chromatographed on Si gel [MeOH-CH_2Cl_2 (1:9)] to give irniine [**1**] (30 mg).

IRNIINE [**1**].—Colorless oil: $[\alpha]^{21}D$ −35° ($c = 0.2$, CH_2Cl_2); ir (KBr) ν max cm^{-1} 3024, 2928, 2856, 2777, 1596, 1457, 1359, 1248, 1165, 1120, 1040, 747, 700; eims (70 eV, 200°) *m/z* (rel. int.) $[M]^+$ 287 (75), 252 (7), 210 (6), 186 (15), 134 (22), 121 (95), 110 (64), 104 (60), 84 (100); cims (NH_3) *m/z* (rel. int.) $[M+H]^+$ 288 (100), 91 (5), 84 (56); hrms *m/z* $[M]^+$ 287.2595, calcd for $C_{20}H_{33}N$, 287.2613; ^1H nmr ($CDCl_3$, 300.13 MHz) 7.24 (H-12′ and H-14′, m), 7.15 (H-11′ and H-15′, m), 7.14 (H-13′, m), 3.06 (H_a-5, ddd, 7.0, 7.0, 2.3), 2.57 (H_2-9′, t, 7.2), 2.28 (*N*-Me, s), 2.11 (H_b-5, ddd, 8.9, 7.0, 7.0), 1.95 (H-2, m), 1.90 (H_a-3, m), 1.75 (H_a-4, m), 1.62 (H_a-1′, m), 1.64 (H_b-4, m), 1.58 (H_2-8′, m), 1.40 (H_b-3, m), 1.31 (H_2-7′, m), 1.26 (H_2-2′ to -6′, m); 1.20 (H_b-1′, m) (overlapping systems have been measured on 2D spectra); ^{13}C nmr ($CDCl_3$, 75.47 MHz) 142.74 (C-10′), 128.22 (C-11′ and C-15′), 128.05 (C-12′ and C-14′), 125.38 (C-13′),

66.41 (C-2), 57.11 (C-5), 40.21 (*N*-Me), 35.85 (C-9′), 33.48 (C-1′), 31.40 (C-8′), 30.59 (C-3), 29.87, 29.49, 29.40, 29.36, 29.19 (C-7′), 26.58 (C-2′), 21.60 (C-4).

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