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Isolation and X-ray Crystal Structure of Tetrahydroisoquinoline Alkaloids from *Calycotome Villosa* Subsp. *intermedia*

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Abstract: Two tetrahydroisoquinoline alkaloids were extracted from the alkaloid fraction of a methanol extract of the seeds of *Calycotome Villosa* Subsp. *intermedia*. Their structures were established as (R)-1-hydroxymethyl-7-8-dimethoxy-1,2,3,4-tetrahydro-isoquinoline (**1**) and (S)-7-hydroxymethyl-2-3-dimethoxy-7,8,9,10-tetrahydroisoquinoline chloride (**2**) by spectroscopic techniques and X-ray diffraction analysis.

Keywords: *Calycotome villosa* (Poiret) Link Subsp. *Intermedia*, alkaloids, tetrahydroisoquinoline, spectroscopy, X-ray crystal structure determination.

Introduction

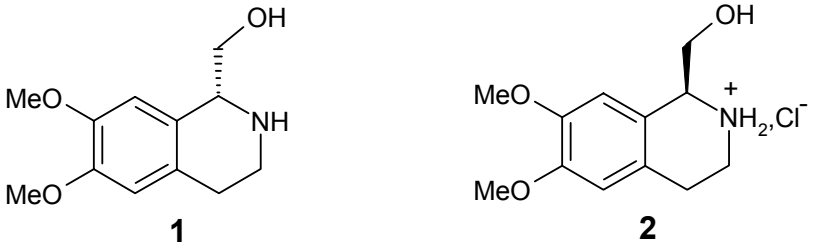
The isoquinoline alkaloids form one of the largest groups of compounds found in a variety of plant families [1-4]. With more than 50 different compounds found in nature, 1,2,3,4-tetrahydroisoquinoline

(THIQ) and especially its derivatives with alkoxy substituents on the aromatic rings constitute the largest group within the simple isoquinoline alkaloids. These natural alkaloids are generally optically active compounds possessing important clinical applications such as analgesics, antihypertensives, smooth or skeletal muscle relaxants, antispasmodics, antitussives, antimalarials, narcotics and antipyretics [5]. It is worth noting that 1-substituted-THIQs, in which C-1 is a quaternary stereogenic centre, have been reported to display very interesting biological and pharmacological properties [3,6,7]. For instance, 1-methyl- and 1-phenyltetrahydroisoquinoline are involved in the treatment of Parkinson's and other nervous system diseases [8-10]. Previous phytochemical studies resulted in the isolation of two flavone glucosides from the flowers and leaves of *Calycotome villosa* Subsp. *intermedia* [11]. In continuation of our chemical investigations on this plant, we describe in this paper the isolation and unambiguous structure elucidation of two alkaloids, with the chemical formulae $C_{12}H_{17}NO_3$ and $C_{12}H_{18}NO_3Cl$ and here labelled **1** and **2**, respectively.

Results and Discussion

The seeds of *Calycotome villosa* (Poiret) Link Subsp. *Intermedia* were extracted with hot methanol. The methanolic extract was purified in the usual way for the isolation of alkaloids [12]. Fractionation and silica gel column chromatography yielded the known compound **1** as the main constituent as well as the alkaloid **2** (see Scheme 1). Compound **1** was the most polar of all alkaloids found in the methanolic extract of *Calycotome villosa*. Alkaloids **1** and **2** were isolated in a crystalline form and completely characterized.

Scheme 1.



Alkaloid **1** was isolated as colorless crystals after recrystallization from methanol. It showed UV maxima at 235 and 278 nm, IR bands at 3346, 2938, 2936, 1613, 1521, 1465, 1367, 1269, 1225, 1135 and 1055 cm^{-1}. The ESI mass spectra afforded a molecular ion peak at m/z 224 $[M + H]^+$, calculated for $C_{12}H_{17}NO_3$. Its ^{1}H-NMR spectrum showed aromatic protons as two singlets at δ 6.55 (1H, s) and 6.56 (1H, s). The two methoxy groups protons showed two singlets at δ 3.84 (3H, s) and 3.85 (3H, s). The four saturated cyclic ring protons of isoquinoline ring moiety ($NC\underline{H}_2C\underline{H}_2$) showed signals as multiplets located around δ 2.63-2.69 and 2.96-3.13. Its ^{13}C-NMR spectrum showed four quaternary carbons, three methines, three methylenes and two methyl groups. Comparison of this data with the literature values [13-19] allowed identification of alkaloid **1** as the known compound (R)-1-hydroxy-methyl-7-8-dimethoxy-1,2,3,4-tetrahydroisoquinoline (named calycotomine).

Confirmation of the structure was obtained from X-ray diffraction of single crystals of **1** grown by slow evaporation from methanol. As expected, **1** has a tetrahydrosubstituted isoquinoline structure [20-23]. The molecular structure and numbering scheme are shown in Figure 1. All bond lengths and angles are reasonable within experimental error (Table 1).

Figure 1. ORTEP view of **1** showing the atomic labeling scheme, with H atoms removed for clarity. Thermal ellipsoids drawn at 50% probability levels.

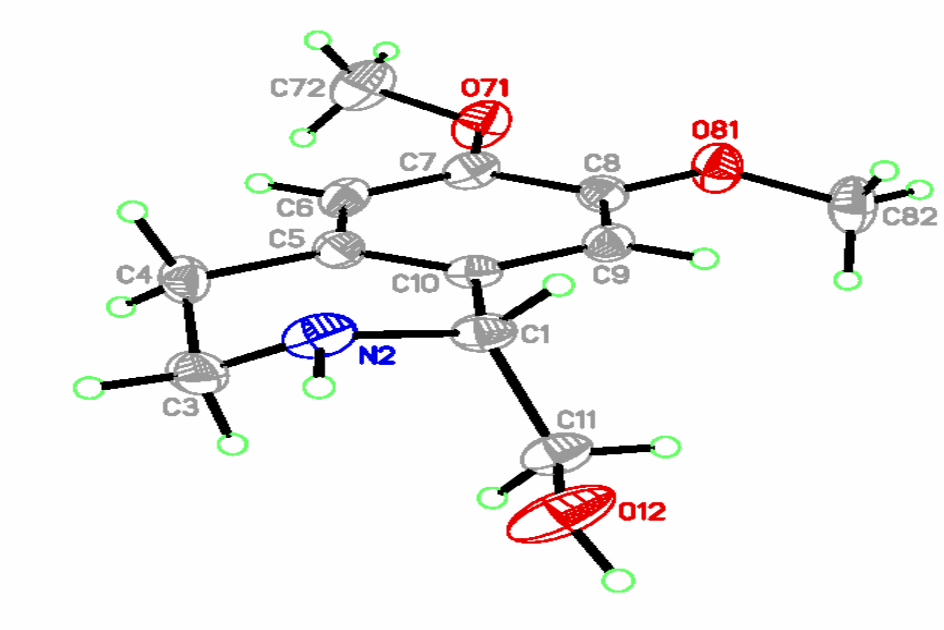


Table 1. Selected bond lengths and angles of **1**.

Bond lengths [Å]			
C1 C10	1.5200(16)	N2 C3	1.4712(19)
C1 C11	1.5306(17)	C3 C4	1.5206(19)
C1 N2	1.4761(16)	C4 C5	1.5170(16)
Bond angles [°]			
N2 C1 C10	110.36(11)	C5 C4 C3	110.70(11)
N2 C1 C11	112.29(10)	C10 C5 C4	120.70(11)
C10 C1 C11	111.91(10)	N2 C3 C4	108.33(10)
C3 N2 C1	112.61(10)	C5 C10 C1	122.56(10)
Torsion angles [°]			
C11 C1 N2 C3	-77.74(14)	C1 N2 C3 C4	-69.21(14)
N2 C3 C4 C5	51.84(14)	C3 C4 C5 C10	-19.79(16)
C10 C1 N2 C3	47.86(14)	C4 C5 C10 C1	0.44(16)
N2 C1 C10 C5	-13.40(15)	C11 C1 C10 C5	112.42(13)

The C5-C10 aromatic ring is planar (r.m.s. deviation = 0.0094 Å). The methoxy groups at C7 and C8 atoms are slightly rotated around the C7-O71 and C8-O81 bonds (Figure 1), the dihedral angles between the plane of the aromatic ring and the planes defined by atoms C7, O71, C72 and C8, O81, C82 being respectively 12.7° and 7.5°. The C4 atom lies almost in the plane of the aromatic ring, whereas atom C1 is slightly displaced from it (the deviation of atoms C4 and C1 from the ring plan are 0.003 and 0.045 Å, respectively). The C1-C10 and C4-C5 bonds are in the plane of the ring (angles with the normal to the plane are 91.0 and 90.6°, respectively), whereas the two remaining bonds, C8-O81 and C7-O71, are slightly out of the plane (angles with the normal to the plane are 91.4 and 88.6°, respectively). As expected, the heterocyclic ring of tetrahydroisoquinoline adopts a half chair conformation.

Alkaloid **2** was also obtained as colorless crystals. It showed UV maxima at 234 and 270 nm, IR bands at 3420, 3334, 2984, 2900, 1611, 1525, 1457, 1333, 1263, 1227, 1126 and 1067 cm^{-1} and MS fragment ions at m/z 207, 192, 189, 179, 175, 165, 158, 150 and 147, besides the molecular ion (m/z 224). Its ^{1}H- and ^{13}C-NMR spectral features were very similar to those of calycotomine (**1**), indicating the presence of the isoquionoline moiety in the molecule. Carbon resonances at δ 55.5, 55.6 and 61.5 confirm the presence of two methoxy groups and one methylene group.

However, the low basicity of **2**, its low solubility in apolar organic solvents and its high solubility in water led us to think that alkaloid **2** was a salt. In an attempt to confirm this, an X-ray crystallographic study was performed. Suitable crystals for X-ray studies were obtained from methanol by slow evaporation. A view of **2** is shown in Figure 2, while selected molecular dimensions are reported in Table 2. Bond lengths and angles values fall in the expected range [23]. The independent unit of the crystal consist of the protonated main molecule and Cl^- anion. As previously found for alkaloid **1**, the main skeleton of **2** is formed by a tetrahydroisoquinoline moiety. The chloride ion Cl^- interacts with the tetrahydroisoquinoline by means of weak H-bonds, with the strongest one [(N8-H8A…Cl1)] at 3.091 Å.

Figure 2. ORTEP view of (**2**) showing the atomic labelling scheme, with H atoms omitted for clarity. Thermal ellipsoids drawn at 50% probability levels.

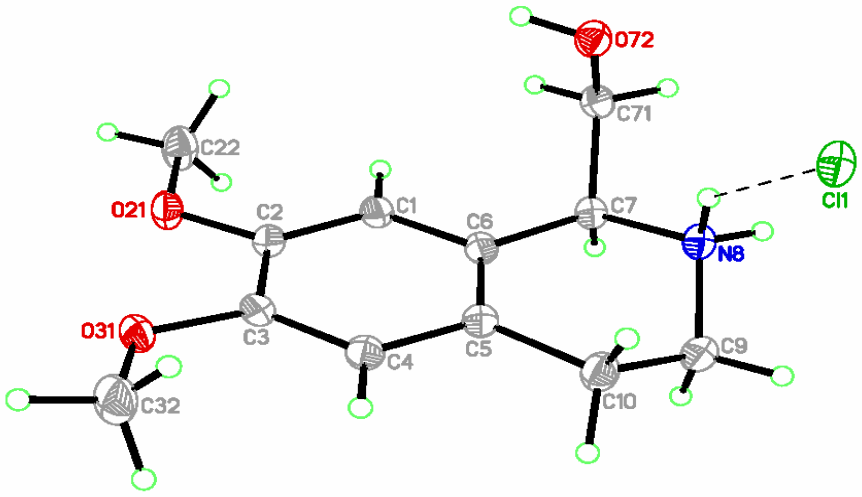


Table 2. Selected bond lengths and angles of **2**.

Bond lengths [Å]			
C6 C7	1.5200(12)	C7 N8	1.4983(11)
C7 C71	1.5275(12)	N8 C9	1.4883(12)
C5 C10	1.5123(12)	C9 C10	1.5195(13)
Bond angles [°]			
N8 C7 C6	109.89(7)	N8 C9 C10	108.46(7)
N8 C7 C71	107.23(7)	C5 C10 C9	111.10(8)
C9 N8 C7	112.95(7)	C6 C7 C71	114.65(7)
C5 C6 C7	122.27(8)	C6 C5 C10	122.02(8)
Torsion angles [°]			
C71 C7 N8 C9	173.06(7)	C6 C7 N8 C9	47.85(9)
C10 C5 C6 C7	-1.13(13)	C5 C6 C7 N8	-13.15(11)
C7 N8 C9 C10	-68.14(10)	C5 C6 C7 C71	-133.99(8)
N8 C9 C10 C5	49.53(10)	C6 C5 C10 C9	-17.45(12)

The crystallographic data obtained for the alkaloid **2** confirmed the previous spectroscopic attributions and the structure was assigned as (S)-7-hydroxymethyl-2-3-dimethoxy-7,8,9,10-tetrahydroisoquinoline chloride.

Conclusions

Two alkaloids were isolated from the seeds of *Calycotome Villosa* Subsp. *intermedia*. Their structures were solved by X-ray analysis. Isolation and characterization of other alkaloids from this plant are currently under investigation.

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Experimental

General

Melting points were measured in open capillary tubes in a Büchi 530 apparatus and are uncorrected. UV-visible spectra were obtained on a Varian Cary 3E spectrophotometer, and IR spectra

were recorded on a Pye Unicam Perkin-Elmer spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 on a Bruker (Wiessembourg, France) AM 300 spectrometer (300 and 75 MHz, for ^{1}H- and ^{13}C-NMR, respectively) and chemical shifts are given as δ values with TMS as an internal standard. $[\alpha]^{20}_{D}$ values were measured using an ADP 220 polarimeter (Bellingham + Stanley LDT). ESI-MS data were obtained on a Quattro II tandem quadripole mass spectrometer (Micromass, Manchester, UK) fitted with an electrospray ionisation. Silica gel GF_{254} was used for TLC. Spots on chromatograms were detected under UV light (254 nm) and by Dragendorff's reagent. Column chromatography (CC) was carried out on silica gel 60 (70-230 mesh).

Plant material

Seeds of *Calycotome Villosa* Subsp. *intermedia* were collected from the aerial part of the plant in June 2001 and again in June 2002 from Zrireg valley, plateau of Tazzeka, area of Taza, Morocco.

Extraction and isolation

The powdered seeds (100 g) were first extracted with hexane for 24 h and then with methanol for 48 h using a Soxhlet apparatus. The methanolic solution was evaporated to dryness and the resulting crude extract was dissolved in a 5% hydrochloric acid solution and extracted first with hexane and then with CH_2Cl_2. The aqueous solution was made basic to pH 10 with concentrated ammonia and extracted three times each with 200 mL of CH_2Cl_2. The collected organic phases were dried over anhydrous sodium sulfate and evaporated under reduced pressure to give 1.75 g of the crude alkaloid extract. This was extracted with acetone and the residual product was dissolved in methanol (25 mL) and kept at room temperature overnight to give a crystalline powder. The precipitate obtained was filtered off and washed three times with CH_2Cl_2 to yield 0.15 g of a purified alkaloid **2**. The combined organic extract (CH_2Cl_2 + CH_3COCH_3) was concentrated and chromatographed on silica gel. The fraction eluted with 10% methanol in CH_2Cl_2 was concentrated to dryness to give pure compound **1** as colorless crystals (0.5 g). These products were recrystallized from methanol to afford crystals suitable for X-ray analysis.

(R)-1-hydroxymethyl-7-8-dimethoxy-1,2,3,4-tetrahydroisoquinoline [(R)-Calycotomine, **1**]. Colorless crystals (0.55 g), mp 154-156 °C; $[\alpha]^{20}_{D}$ +46.1 (c 0.13, CH_3OH); UV-visible λ_{max} ($CHCl_3$) nm: 235, 278; ^{1}H-NMR ($CDCl_3$): 2.63-2.69 (3H, m), 2.96-3.13 (2H, m), 3.62 (1H, dd, *J* = 9.2, 10.8 Hz), 3.75 (1H, dd, *J* = 4.2, 10.8 Hz), 3.84 (3H, s), 3.85 (3H, s), 3.95 (1H, dd, *J* = 4.2, 9.2 Hz), 6.55 (1H, s), 6.56 (1H, s); ^{13}C-NMR ($CDCl_3$): 29.1 (C_4), 38.9 (C_3), 55.8 (CH_3), 56.0 (CH_3), 56.1 (C_1), 64.1 (CH_2), 109.2 (C_9), 112.0 (C_6), 127.1 (C_5), 127.7 (C_{10}), 147.4 (C_8), 147.7 (C_7); ESI-MS m/z = 224 $[M + H]^{+}$.

(S)-7-hydroxymethyl-2-3-dimethoxy-7,8,9,10-tetrahydroisoquinoline chloride (**2**). Colorless crystals (0.15 g); mp 202-204 °C; $[\alpha]^{20}_{D}$ +29.2 (c 0.24, CH_3OH); UV-visible λ_{max} (MeOH) nm: 234, 270; ^{1}H-NMR (DMSO-d_6) : 2.85-2.95 (2H, m), 3.13-3.28 (2H, m), 3.70 (3H, s), 3.71 (3H, s), 3.77 (1H, dd, *J* = 8.0, 10.8 Hz), 3.96 (1H, dd, *J* = 3.5, 10.8 Hz), 4.33 (1H, dd, *J* = 3.5, 8.0 Hz), 6.78 (1H, s), 6.85

(1H, s), 9.49 (2H, s); ^{13}C-NMR (DMSO-d_6) : 24.5 (C_{10}), 38.4 (C_9), 55.5 (CH_3), 55.6 (CH_3), 55.7 (C_7), 61.5 (CH_2), 109.9 (C_1), 111.8 (C_4), 121.4 (C_5), 124.7 (C_6), 147.5 (C_2), 148.3 (C_3); ESI-MS m/z = 224 $[M - Cl^-]^+$.

X-ray Crystal Structure Determination [24]

Colorless single crystals of **1** and **2** suitable for X-ray structure analysis were obtained by slow evaporation of a methanol solution. Table 3 summarizes the crystal and experimental data. The data for both crystals have been collected on a STOE IPDS II two-circle-diffractometer using a $Mo_{K\alpha}$ radiation (λ= 0.71073 Å). An empirical absorption correction was applied [25]. The structures were solved with direct methods [26] and refined with full-matrix least squares techniques [27]. All non-H atoms were refined with anisotropic displacement parameters. H atoms were located in an electron difference map and refined using a riding model. No decay of intensity was observed.

Table 3. Crystal and experimental data of **1** and **2**

	(**1**): $C_{12}H_{17}NO_3$	(**2**): $C_{12}H_{18}ClNO_3$
Measurement Temp. (K)	173(2)	100(2)
M (g/mol)	223.27	259.72
Symmetry (S. G., Z)	Monoclinic ($P2_1$, 2)	Monoclinic ($P2_1$, 2)
Cell parameters (Å, °)	a = 5.9832(6) b = 10.4332(12) c = 9.4578(9) β = 102.876(7) V = 575.55(10) Å^3	a = 7.6210(6) b = 7.7365(5) c = 11.0750(9) β = 92.487(7) V = 652.37(9) Å^3
$d_{cal.}$ (g·cm^{-3})	1.288	1.322
Crystal dimensions (mm)	0.47∗0.17∗0.11	0.38∗0.34∗0.14
F(000)	240	276
$\mu_{(Mo\ K\alpha)}$(mm^{-1})	0.092	0.290
$T_{min.}/T_{max.}$	0.9579 / 0.9899	0.8979 / 0.9606
Total measured reflexions / Refined [I>2∗σ(I)]	9590 / 2341	14717 / 3870
R / Rw (on F^2)	0.0303 / 0.0724	0.0234 / 0.0632

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24. CCDC 223477 and 223478 contain the supplementary crystallographic data for compounds **1** and **2**. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.htlm (or from the CCDC, 12 Union Road Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).
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Sample availability: Samples of compounds **1** and **2** are available from the authors and MDPI.