7-*O*-6′-*O*-Malonylcachinesidic Acid, a New Macrocyclic Iridoid Ester of Malonic Acid from the Tunisian Plant *Ajuga pseudoiva*

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A new iridoid glucoside, 7-*O*-6′-*O*-malonylcachinesidic acid (**1**), was isolated from the leaves of *Ajuga pseudoiva* and characterized as its triacetyl derivative (**1a**). Its structure was established by spectroscopic methods and showed an unusual feature, in which an alcoholic function of the iridoid moiety and the primary alcohol of a glucopyranose unit form a 13-membered heterocycle with malonic acid.

As part of our ongoing investigations on the secondary metabolites of Tunisian plants, we have studied the constituents of the aerial parts of *Ajuga pseudoiva* Rob. (Labiatae).[1] This Mediterranean plant, widely distributed in Tunisia, is a yellow flowering species.[2] Called "Chendgourâ" by inhabitants of North Africa, it has been used in folk medicine for its antifebrile and anthelminthic properties[2b] and its hypoglycemic effect.[3] Previously, its insect antifeedant[4] as well as antibacterial[5] activities have been studied. Earlier chemical investigations have led to the isolation of clerodane diterpenoids,[1,6] ecdysteroids,[3,5b,c,7] flavonoids,[3] iridoids,[3,8] and triglycerides.[9] The current study describes the isolation and structure determination of a new iridoid glucoside, 7-*O*-6′-*O*-malonylcachinesidic acid (**1**), a macrocyclic malonic ester of 8-hydroxy-8-*epi*-loganic acid, characterized as its triacetyl derivative (**1a**).

A concentrated methanolic extract of the aerial parts of *A. pseudoiva* was submitted repeatedly to silica gel chromatography. Compound **1a** was crystallized from acetone after acetylation of the crude polar acid. The FABMS of **1a** exhibited a sodiated molecular ion $[M + Na]^+$ at *m*/*z* 609, while an ammoniated molecular ion at *m*/*z* 604 $[M + NH_4]^+$ was observed in the positive CIMS. Both the negative and positive ESIMS were recorded and showed ions at *m*/*z* 585 $[M - H]^+$ and at *m*/*z* 609 $[M + Na]^+$, 625 $[M + K]^+$, and 1195 $[2M + Na]^+$, respectively. All these data are compatible with the molecular formula $C_{25}H_{30}O_{16}$ ($M_w = 586$), which was also confirmed by HRFABMS and was in good agreement with the 25 carbon atom resonances observed in the ^{13}C NMR spectrum.

The presence of an acetylated β-glucopyranose unit was suggested by the resonances at 72.3, 74.0, 71.4, 72.7, and 63.5 ppm in the ^{13}C NMR spectrum as well as the resonance at 98.9 ppm, consistent with an anomeric carbon atom. The HSQC spectrum was in agreement with this attribution and permitted the assignments of the protons attached to C-1′ (5.20 ppm (d, $J = 8$ Hz, β-anomer), C-2′, C-3′, C-4′, and C-5′. However, the unusual pattern of the protons at C-6′ and the presence of only three acetyl ester signals were quite intriguing (Table 1). The complete interpretation of the remaining NMR data was undertaken and established on the results of conclusive TOCSY and HMBC experiments.

Table 1. NMR Data of Compound **1a**

position	δ_H[a]	δ_C[b]	HMBC (C→H)
1	5.40, (d, $J = 10$)	97.3	7.53, 3.40, 2.00
3	7.53, (s)	152.6	3.40
4		112.6	7.53, 3.40, 1.45
5	3.40, (m)	34.2	1.45, 1.68, 2.08
6	b 1.45, (m) a 3.04 (ddd, $J = 16.\ 10.\ 7.5$)	40.8	3.40, 2.00
7	4.92, (d, $J = 7.5$)	83.3	3.04, 1.45
8		83.0	2.00, 1.45
9	2.00, (d, $J = 10.\ 7$)	51.3	4.92, 3.40, 3.04, 1.45
10	1.45, (s)	21.8	
11		170.6	7.53
12		166.5	4.92, 3.44, 3.49, 3.59, 3.64
13	3.44, 3.49, 3.59, 3.64 (AB, $J = 15.5$)	42.7	
14		167.9	3.94, 5.05, 3.44, 3.49, 3.59, 3.64
1′	5.20 (d, $J = 8$)	98.9	5.44, 5.08
2′	5.08 (dd, $J = 9.\ 8$)	72.3	
3′	5.44 (t, $J = 9$)	74.0	
4′	5.00 (t, $J = 9$)	71.4	
5′	4.07 (td, $J = 9.\ 3$)	72.7	
6′	3.94 (dd, $J = 11.5.\ 3$), 5.05 (m)	63.5	
acetyl	2.09 (s), 2.12 (s), 2.13 (s)	20.4, 20.5, 20.6 171.1, 171.4, 171.5	

[a] Measured at 400 MHz (*J* values in parentheses). [b] Measured at 100.6 MHz.

Two methylene resonances could be detected at δ 40.8 and 42.7 and were easily distinguished through the pattern of the attached protons in the ^1H NMR spectrum. Thus, four signals of an AB system at δ 3.44, 3.49, 3.59, and 3.64 corresponded to the carbon resonance at 42.7 ppm and a more complex system at δ 1.45 and 3.04 ppm for the protons attached to the carbon atom at 40.8 ppm (Table 1). The signal at 3.04 ppm showed three coupling constants and was then chosen for a selective excitation procedure in a TOCSY 1D experiment.[10] A selective excitation during a spinlock of 10 ms showed three signals. One was observed at δ 1.45 ppm (geminal proton) and the two others at δ 3.40 ppm (dd) and δ 4.92 ppm (d) (vicinal protons) (Figure 1). A spinlock of 60 ms indicated two long-range correlated protons at δ 2.00 (dd) and 5.40 ppm (d). These results permitted the establishment of the connectivities as depicted in Figure 1. In addition, the low-field resonances of the terminal proton and carbon atom [δ 5.40 (97.3); 4.92

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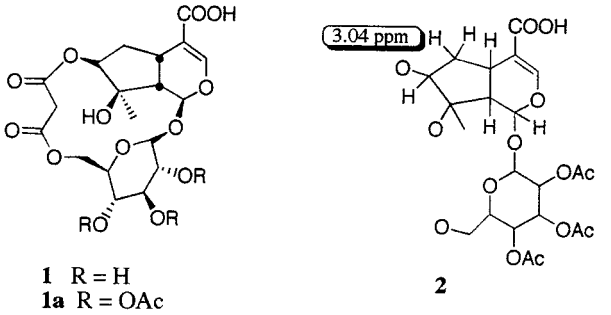

Figure 1. Structures of compounds **1** and **1a** and partial structure **2** deduced from the TOCSY experiments.

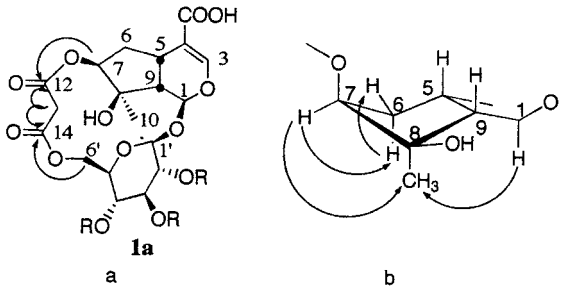

Figure 2. Important correlations for **1a**: (a) HMBC; (b) dipolar couplings observed through NOE-difference experiments.

(83.3)] suggested the presence of two oxymethine carbons. All these data were in favor of an iridoid framework, where 3.04 ppm is the resonance of H-6, characterized by a β-anomeric carbon atom C-1 at 97.3 ppm with a *trans* relationship between H-1 and H-9 (H-1, 5.40 ppm, d, J = 10 Hz), a conjugated acid function at 170.6 ppm, a double bond (C-3 at 152.6 ppm, C-4 at 112.6 ppm), a tertiary hydroxy-bearing carbon C-7, a quaternary carbon C-8 at 83.0 ppm most probably attached to a hydroxyl group, and a C-10 methyl group at 21.8 ppm. These data suggested the partial formula **2** for this new iridoid (Figure 1), with the glucoside attachment being confirmed by a correlation between H-1/H-1′ in the NOESY spectrum.

The HMBC data of **1a** allowed the assignment of the remaining proton (an AB system centered at 3.5 ppm) and carbon resonances. Accordingly, the protons of the AB system were correlated with the two remaining carbonyl resonances at 166.5 and 167.9 ppm. A careful examination of the spectrum indicated an additional correlation of these two carbonyl resonances with H-7 at δ 4.92 and the C-6′-methylene protons of the glucopyranose unit at δ 3.94 and 5.05, respectively. These data were in full agreement with the esterification of the hydroxyl groups at C-7 and C-6′ by malonic acid and were consistent with the molecular formula $C_{25}H_{30}O_{16}$. Although the occurrence of malonic acid is quite rare in plants, a diterpene malonate[11] and a C-6′ glucoside malonate[12] have already been isolated and described. It seems reasonable to propose the structure of 7-*O*-6′-*O*-malonylcachinesidic acid (**1**) for the natural product, although late esterification by malonic acid present in the plant during a lengthy treatment at elevated temperature cannot be totally excluded. On the other hand, the formation of a 13-membered ring is not thermodynamically favored.

The determination of the relative stereochemistry of the chiral centers was finally ascertained through NOESY and NOE difference experiments (Figure 2), leading to formula **1a**. A correlation between H-5 and H-9 observed in the NOESY spectrum confirmed the usual $\beta\beta$-*cis*-fused cyclopentanopyran ring system of most iridoids. The use of acetone-d_6 as a solvent in the NOE difference experiments was necessary for unambiguous conclusions, resulting in a much better resolution of the H-1 (δ 5.38) and H-7 (δ 4.88) proton signals. The dipolar coupling between H-1 and the methyl group at C-10 indicated that these protons are on the same side (α) of the iridoid skeleton and that the tertiary hydroxyl group at C-8 is β. Furthermore, dipolar couplings (acetone-d_6) between the C-10 methyl group and H-6 at δ 2.95 and H-7 at δ 4.88 were indicative of the α orientation proposed. Hence, the hydroxyl groups at C-7 and C-8 were assigned as β, and 7-*O*-6′-*O*-malonylcachinesidic acid (**1**) can therefore be related to 8-hydroxy-8-*epi*-loganic acid.[13]

The 7β,8β-dihydroxy-8α-methyl iridoid skeleton is well known and has already been extracted from several plants[14] such as campsiside[15] and cachinesides III−V[16] from *Campis chinensis*, 8-*epi*-caryptoside[17] from *Barleria prionitis*, and stegioside II[18] from *Phytostegia virginiana*. However, 7-*O*-6′-*O*-malonylcachinesidic acid (**1**) possesses an original feature due to the rare malonate esterification of the iridoid and glucoside moiety leading to a macrocyclic structure.

Experimental Section

General Experimental Procedures. Melting points were measured with a melting point apparatus (Leica Galen III). Optical rotations were determined at room temperature with a Perkin-Elmer 141 MC polarimeter and are referenced to the D-line of sodium. IR spectra were recorded on a Nicolet spectrometer FTIR 510 using KBr pellets. ^{1}H, ^{13}C, and 2D NMR spectra of acetone-d_6 or CD_3OD solutions were recorded on a Bruker Avance-400 spectrometer as well as on a Bruker AM 300 spectrometer operating at 400 and 100.6 MHz, and 300 and 75.5 MHz, respectively. A ZAB Spec/T mass spectrometer was used in the LSIMS experiments.

Plant Material. *Ajuga pseudoiva* was collected at Zaghouan, Tunisia, in February 1997. Voucher specimens (no. 105) of the plant were deposited in the Herbarium of the École Supérieure d'Horticulture de Chott Meriem, Sousse, Tunisia.

Extraction and Isolation. Dried and powdered leaves of *A. pseudoiva* (500 g) were extracted with methanol in a Soxhlet apparatus for 7 days. The crude residue (35 g) obtained after filtration and evaporation of the solvent was extracted with EtOAc at room temperature. The extract (17 g) was purified by silica gel column chromatography (Merck 7734, petroleum ether/EtOAc/MeOH). Four main fractions were collected. The more polar of those (6.1 g) was rechromatographed on silica gel using petroleum ether/AcOEt/MeOH. Elution with AcOEt/MeOH, 9:1, furnished 500 mg of a crude residue, which was treated with ice cold acetone to give 311 mg of a precipitate. This solid was then chromatographed on silica gel with a mixture of AcOEt/MeOH. Five fractions were collected. Elution with AcOEt/MeOH, 7:3, gave 107 mg of a solid, which was finally purified by preparative TLC (AcOEt/MeOH, 60:40). The crude polar compound (85 mg) was acetylated (Ac_2O/pyridine, room temperature, 12 h) to give 30 mg of a derivative **1a** after crystallization from acetone. Pure 7-*O*-6′-*O*-malonylcachinesidic acid triacetate **1a** was obtained after recrystallization from MeOH, mp 253−257 °C; $[\alpha]_D^{20}$ −54 ° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3472, 3413, 1754, 1739, 1693, 1625, 1262, 1219 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; CIMS (NH_3) *m*/z 604 $[M + NH_4]^+$ (100); LRFABMS *m*/z 609 $[M + Na]^+$ (100); positive-ion ESIMS *m*/z 1195 $[2M + Na]^+$ (10), 625 $[M + K]^+$ (34), 609 $[M + Na]^+$ (100); negative-ion ESIMS *m*/z 585 $[M - H]^-$ (100); HRFABMS *m*/*z* $[M + H]^+$, 586.5040, calcd for $C_{25}H_{30}O_{16}$ 586.5036.

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Supporting Information Available: ^{1}H, ^{13}C, TOCSY 1D, HMBC, and NOE-difference NMR spectra of **1a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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