SHORT REPORT

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Carboxy Methyl and Carboxy Analogs Argaminolics B and C

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Abstract: Two new analogs, a carboxy methyl (Argaminolic B) and a carboxy derivative (Argaminolic C), of a recently reported amino phenolic, Argaminolic A, isolated from the fruit of the argan tree, *Argania spinosa* (Skeels L.), are described. Argaminolic B exhibits facile hydrolysis of its methyl ester to yield Argaminolic C, which then undergoes a remarkably facile decarboxylation to the previously described Argaminolic A.

Keywords: Amino phenolics; *Argania spinosa* (Skeels L.); structural elucidation; spectroscopic analyses.

1. Plant Source

Within Morocco, the argan tree, *Argania spinosa* (Skeels L.), ranks second only to oak in terms of forest acreage [1,2]. The oleaginous tree belongs to the Sapotaceae family containing eight genera with the genus *Argania* consisting of a single species endemic to Morocco. Fruits of the argan tree used in this study were harvested from Essaouira, Morocco.

2. Previous Studies

There is much evidence for beneficial effects for human health from the products of argan tree, *A. spinosa* (Skeels L.). In our search for bioactive compounds and potential drugs for the treatment and prevention of cancer, we have previously examined extracts of the oil [2–4] and the fruit [5] of this tree for bioactivity and identified various active components. Recently, we described [6] a new compound, now dubbed Argaminolic A (**1**, Figure 1), that we had isolated from the fruit. Argaminolic A belongs to an unusual and relatively obscure class of natural products, the amino phenolics.

3. Present Study

The aims of this work were to search for, isolate, and describe additional new compounds from the fruit of the argan tree, particularly those with potential for high bioactivity and also belonging to the unusual and under-reported amino phenolic class. The natural compounds reported here consist of a carboxy methyl analog, dubbed Argaminolic B (**2**), and a carboxy analog, dubbed Argaminolic C

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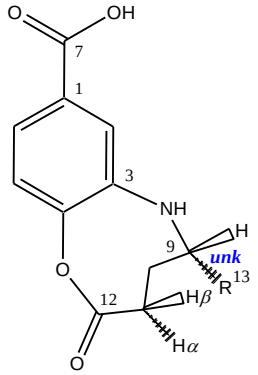
(**3**), of the unusual Argaminolic A (**1**) containing a fused bicyclic ring structure (Figure 1). One of the interesting features of **2** is that it is particularly prone to hydrolysis of its methyl ester functionality to yield the dicarboxylic acid **3**, which then surprisingly undergoes facile decarboxylation to yield the originally reported compound **1** [6].



Argaminolic A (**1**), R = H
Argaminolic B (**2**), R = CO_2CH_3
Argaminolic C (**3**), R = CO_2H

Figure 1. Argaminolics A–C (**1**–**3**, respectively), amino phenolics isolated from argan fruit. The indicated relative stereochemistry follows the system outlined recently [7]; the chiral states of **2** and **3** are undetermined.

Fruit flesh, after separation from the pits, was cut into small pieces, freeze-dried using a Christ lyophilizer (Osterode, Germany), pulverized into a fine powder, and then extracted with a Soxhlet apparatus using *n*-hexane for 3 h to remove lipids followed by extraction with MeOH (× 3, 3 h). The combined MeOH extracts were taken to dryness, suspended in absolute EtOH and then immobilized on Sephadex LH-20 (5.0 g) before being deposited on the top of a column of Sephadex LH-20 in EtOH. Fractionation was conducted by elution with increasing amounts of MeOH. After dissolution in 2% AcOH (2.0 mL), further fractionation was performed using solid-phase extraction on C18 columns eluting with increasing concentrations of MeOH in 2% AcOH. Final purification was accomplished by semi-preparative HPLC using a HP 1100 liquid chromatograph (Agilent Technologies, Waldbronn, Germany) fitted with a similar RP-C18 column (10 mm i.d.) for analytical HPLC-ESI analysis as previously described [8].

Compounds **1**–**3** were well separated chromatographically and yet in the isolated sample of **2**, both **3** and **1** were present testifying firstly to the facile hydrolysis of the methyl ester **2** to yield the dicarboxylic acid **3**, and then subsequently to the facile decarboxylation of compound **3** to yield **1**.

HPLC-ESI-MS was conducted on an Agilent 1100 HPLC system coupled to an Agilent single-quadrupole mass-selective detector (HP 1101; Agilent Technologies, Waldbronn, Germany). Chromatography was conducted using an RP-C18 column (250 mm, 4 mm i.d., 5 µm; Phenomenex, Aschaffenberg, Germany) eluted with a gradient of 2% AcOH in doubly distilled water (A) and AcCN (B): initially 95% A/10 min; to 90% A/1 min; to 60% A/9 min; to 80% A/10 min; to 60% A/10 min; to 0% A/5 min; and finally 0% A. UV absorbance was monitored at 257, 278, and 340 nm. Negative-ion mass spectra were acquired with fragmentor voltage, 200 V and capillary voltage, −2500 V; positive-ion mass spectra were acquired with fragmentor voltage, 100 V and capillary voltage, +1500 V. Parameters common to both modes: nebulizer pressure, 30 psi; drying gas temperature, 350 °C; and *m*/*z* scan range, 100–1500 Da.

The structural determination of **2** followed readily from the standard application of 1D and 2D NMR techniques and its striking similarity to many aspects of the NMR spectra for **1** (Table 1), *e.g.* a 1,2,4-trisubstituted benzene ring with oxygen bound to C-4 as inferred by the δ_Cs and $J_{H,H}$s. However, instead of three contiguous methylene groups, the aliphatic spin system consisted of two contiguous methylene groups and an adjacent methine group. From the MS (Table 2), a likely formula of $C_{13}H_{13}NO_6$ was inferred by a molecular mass of 279 Da indicated from both positive- and negative-ion analyses, thereby implying the presence of an additional carboxy group and a methyl group relative to **1**. The presumption was therefore that **2** was an analog of **1** with an additional carboxy group, with

either it or the carboxy group located at C-1 methylated given the presence of an O-methyl group observed in both the ^{1}H and ^{13}C NMR. The similarity of many of the NMR aspects of **2** to **1** confirmed this and permitted the rapid realization of the structure for **2**, for example a newly introduced group must be attached to the previously *n*-propyl chain in **1**. In negative-ion MS (Table 2), the loss of a fragment of 44 Da from the pseudomolecular ion, in similar fashion to **1** [6], indicated the presence of a carboxy moiety, while the loss of a fragment of 32 Da for methanol further cemented the premise of a methyl ester being present.

Table 1. ^{1}H and ^{13}C NMR data for Argaminolics B and C (**2** and **3**, respectively) in CD_3OD at 303 K.

Atom pos.	Argaminolic B (**2**)			Argaminolic C (**3**)		
	δ (ppm)[a]		^{1}H mult.: $J_{H,H}$ (Hz)	δ (ppm)[a]		^{1}H mult.: $J_{H,H}$ (Hz)
	^{13}C	^{1}H		^{13}C	^{1}H	
1	124.5	–		126.4	–	
2	131.9	7.88	d: J_{H6}, 2.1	132.1	7.80	d: J_{H6}, 1.8
3	127.6	–		127.9	–	
4	157.1	–		159.1	–	
5	116.6	6.90	d: J_{H6}, 8.5	118.3	6.92	d: J_{H6}, 8.5
6	131.7	7.83	dd: J_{H5}, 8.5; J_{H2}, 2.1	132.3	7.87	dd: J_{H5}, 8.5; J_{H2}, 1.8
7	172.0	No	–	171.3	No	–
8	–	No	–	–	No	–
9β	63.1	4.81	dd: $J_{H10\beta}$, 8.9; $J_{H10\alpha}$, 3.4	67.4	4.37	dd: $J_{H10\beta}$, 9.1; $J_{H10\alpha}$, 2.0
10α	24.1	2.24	ol m	26.6	2.24	ol m
10β		2.60	ol m		2.63	ol m
11α	30.5	2.52	$d_{AB}d_{AB}d$: $J_{H11\beta}$, −16.2; $J_{H10\beta}$, 9.5; $J_{H10\alpha}$, 3.8	30.8	2.43	ho m
11β		2.69	ol m		2.66	ol m
12	177.8	–		178.4	–	
13	173.6	–		173.5	no	–
OMe	52.7	3.69	s, 3 H	–	–	

Legend: d, doublet; d_{AB}, doublet with AB character; ho, higher order; m, multiplet; no, not observed; ol, overlapped; s, singlet. [a] ^{1}H and ^{13}C nuclei chemical shifts are reported relative to TMS (δ = 0 ppm for both ^{1}H and ^{13}C) using the solvent signals as secondary internal references (δ_{CHD2OD} = 3.31 ppm for ^{1}H and δ_{CD3OD} = 49.05 ppm for ^{13}C).

The loss of 60 Da, ostensibly HCO_2CH_3, was also consistent with the presence of a carboxy methyl substituent and yielded an ion of 220 Da representing the $[M - H]^-$ pseudomolecular ion for **1**. Correlations from HMBC spectra positioned the new carboxy group at C-9 and the O-methyl group observed in the ^{1}H and ^{13}C NMR as being attached to this C-9 carboxy group and not the C-1 carboxy group.

Given the close similarity of the NMR data between **3** to **2** (Table 1), with only the methyl group lacking in the former in both the ^{1}H and ^{13}C NMR spectra and some notable differences in the chemical shifts of H-9 and C-9 and scalar couplings in the former (*vide infra*), it was readily ascertained that **3** must be the hydrolysis product of **2** by loss of the methoxy group. This was also apparent from the MS (Table 2) with an indicated molecular mass of 265 Da from both positive- and negative-ion analyses. In negative-ion mode, the loss of a fragment of 44 Da, in similar fashion to **2** and **1** [6], indicates the presence of a carboxy moiety (and hence yielding an ion of 220 Da representing the $[M - H]^-$ pseudomolecular ion for **1**), while an additional loss of a second 44 Da fragment implies the presence of two carboxy moieties.

Table 2. Analytical HPLC-ESI-MS data for Argaminolics A–C (**1**–**3**, respectively).

	UV max (nm)	Formula	R_t (min)	HPLC-ESI-MS[a] ions (Da)			
				Negative-ion mode		Positive-ion mode	
				$[M-H]^-$	Neutral fragment losses	$[M+H]^+$	Adducts
1	255	$C_{11}H_{11}NO_4$	22.91	220.1	176.1, $-CO_2$, 59%	222.2	244.2, $+Na^+$, 5.5% 260.2, $+K^+$, 5.0%
2	255	$C_{13}H_{13}NO_6$	23.47	278.1	246.0, $-CH_3OH$, 20% 218.1, $-HCOOCH_3$, 100% 174.1, $-CO_2$, 82%	280.3	302.3, $+Na^+$, 21% 318.2, $+K^+$, 6.1%
3	255	$C_{12}H_{11}NO_6$	12.46	264.1	220.1, $-CO_2$, 90% 176.1, $-2\times CO_2$, 100%	266.2	288.3, $+Na^+$, 22% 304.2, $+K^+$, 6.6%

[a] Online HPLC-ESI-MS with negative- (fragmentor voltage, 200 V) and positive-ion (fragmentor voltage, 100 V) detection.

It is noteworthy that **2** undergoes facile hydrolysis during fractionation and isolation to yield **3**, which, quite remarkably, undergoes facile decarboxylation to yield **1**. The reason for compound **2** having such a propensity to undergo hydrolysis and lose the methyl group is unclear as the isolation conditions are not considered unduly harsh, and, in any event, the other ester functionality present in the molecule remains intact and ring opening was never observed.

The facile conversion of **3** to **1** is highly interesting and unprecedented. Decarboxylation evidently must be due to the α-amino group and facilitated no doubt due to conjugation with and/or activation by the aromatic ring. A plausible mechanism for the process is proposed in Scheme 1.

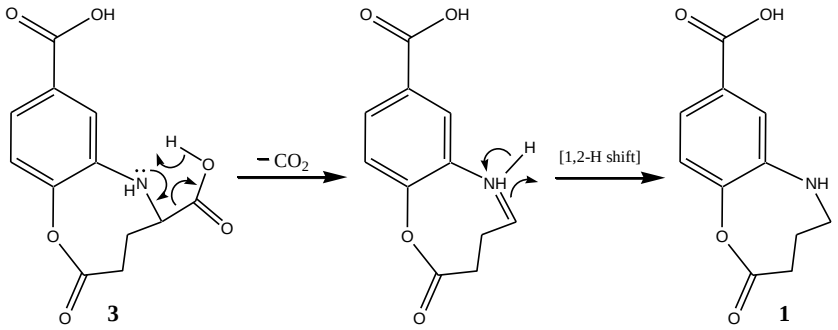


Figure 1. Proposed mechanism of decarboxylation of **3** to **1**.

Despite these facile transformations, we can clearly state that all three metabolites **1**–**3** are present in argan fruit. Compound **3** was only present in low concentration initially but its content increased during purification, which was subsequently followed by decarboxylation to **1**. The facile decarboxylation accounts for the strong presence of the 220 Da ion representing $[M-H]^-$ for **1** detected in negative-ion MS (Table 2) for both **2** (following loss of methanol or loss of methyl formate) and **3** and the observation of **1** in the solution-state NMR spectrum of **2**. The facile hydrolysis of **2** is revealed by the presence of **3** in the solution-state NMR spectrum of **2** under what is considered to be benign conditions during purification. Thus, the presence of **1** and **3** in isolates of **2** occurs despite notable differences in their R_ts, *e.g.* more than 11 min for **3** for the conditions applied herein.

Closer scrutiny of the NMR data for **2** and **3** reveals that there is a sizeable change in the chemical shifts of H-9 (4.81 to 4.37 ppm) and C-9 (63.1 to 67.4 ppm) upon hydrolysis of the ester to the free acid, perhaps therefore indicating a change in the heterocyclic ring conformation from **2** to **3** by way of the inherent heterocyclic ring anisotropy perturbing the chemical shifts. This conformational change cannot be as large as initially anticipated based on the relatively small change in the coupling constants of H-9 (8.9 and 3.4 to 9.1 and 2.0 Hz), the fact that the heterocyclic ring ηs were very similar for the two compounds **2** and **3**, and the fact that other chemical shifts in the aliphatic chain were not as perturbed in comparison (Table 1). The chemical shift changes of H-9 and C-9 therefore can be attributed as much to the slight changes in spatial disposition arising from the change in heterocyclic ring conformation of H-9 and C-9 with respect to the aromatic ring and its

associated anisotropy and/or to intramolecular hydrogen bonding changes of the amine nitrogen with the carboxy methyl/carboxy group and the consequent inductive effects associated with such a change.

The origin of the unexpected change in heterocyclic ring conformation may well be the postulated change in intramolecular hydrogen bonding of the amine nitrogen, either by the loss in **3** of the amine group acting as a proton donor in comparison to **2** by way of hydrogen bonding to the carbonyl oxygen of the ester functionality, or the taking up of the role of proton acceptor by the amine group in **3** to the free hydroxyl of the acid group, or the occurence of both of these interaction changes.

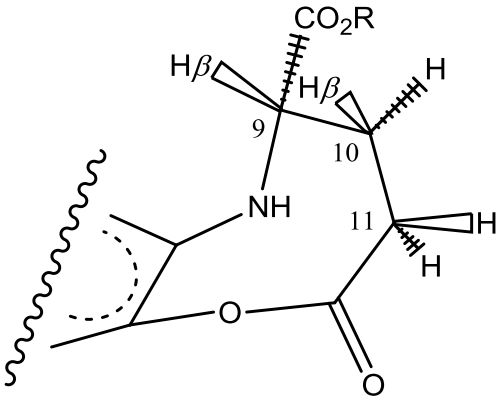


Figure 2. The approximate heterocyclic ring conformation of amino phenolics **2** and **3**. The H-9β and H-10β hydrogens are eclipsed/near eclipsed while H-10α is *gauche* to each of the H-11 pair.

The very sizeable change in retention time between **2** and **3** is surprising given that it is merely hydrolysis of the methyl ester to the free acid which differentiates the two compounds, and a change in hydrogen bonding may contribute to the large R_t difference observed between **2** and **3**. Also surprisingly, the change in heterocyclic ring conformation and/or intramolecular hydrogen bonding was evident too in the perturbation of the aromatic proton chemical shifts, *e.g.* H-6 and H-2 swap over in their order (Table 1). A depiction of the approximate heterocyclic ring conformation is portrayed in Figure 2 based on the sizes of the coupling constants and the observed ηs.

Thus, in concert with the stated aims of the work to isolate and describe new compounds, particularly those with potential for high bioactivity and also belonging to the unusual and under-reported amino phenolic class, two new metabolites, carboxy methyl, **2**, and carboxy, **3**, analogs of a recently reported amino phenolic, **1** [6], have been described. These compounds are all present naturally in argan fruit extracts but the levels of **1** and **3** increase during the purification of **2** implicating their facile interconversion, particularly the remarkable decarboxylation of **3** to **1**. Nevertheless it raises the question whether **1** and **3** are desired secondary metabolites in that the plant specifically produces them either as intended targets or as intermediates on the way to, for example, **2**, or if they are extraneous species present in the plant due to incidental hydrolysis of **2** to yield **3**, and/or then followed by decarboxylation to yield **1**. These transformations may assist in deconvoluting the biosynthetic pathway that leads to these compounds. In any event, as these compounds belong to an unusual and relatively obscure class of polyphenolics in plants, the amino phenolics. The potential for any health benefits of these amino phenolics is worth exploring–an intended future course of endeavor. It would also be interesting to determine the chiral state of **2** and **3**–either, for example, by chiral HPLC or by application of a CDA and analysis by NMR–as these compounds represent good subjects for chiral studies, though this is beyond the present scope of the work. In addition, other stereochemical aspects such as the complex conformational behavior of these compounds also represent a potentially rich subject for possible future study. Finally, this family of amino phenolics appear to be unique to argan fruits and thus represent a *bona fide* chemotaxonomic marker for this species. Therefore they could potentially replace the current, and not very specific, markers quercitrin, myricitrin, hyperoside, and myricetin-3-*O*-galactoside [9].

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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