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TRITERPENES FROM *AMARACUS DICTAMNUS*

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Key Word Index—*Amaracus dictamnus*; Labiatae; triterpenes; oleanolic, ursolic and 21α-hydroxyoleanolic acids; uvaol.

Abstract—From the aerial parts of *Amaracus dictamnus* several triterpenes were isolated: oleanolic and ursolic acids, uvaol, the rare 21α-hydroxyoleanolic acid and a new 21α-hydroxyursolic acid.

INTRODUCTION

We reported recently [1, 2] on the investigation of *Amaracus akhdarensis* and *A. pampaninii* (family Labiatae), from which four isopimarane diterpenoids were isolated. The work was aimed at finding a possible chemotaxonomical differentiation of the genus *Origanum* and restoring the genus *Amaracus*.

In this paper we report our results on extracts of *A. dictamnus* Bentham (syn *Origanum dictamnus* L.), collected in the island of Crete (Greece). Stems and leaves, and flowers were examined separately.

RESULTS AND DISCUSSION

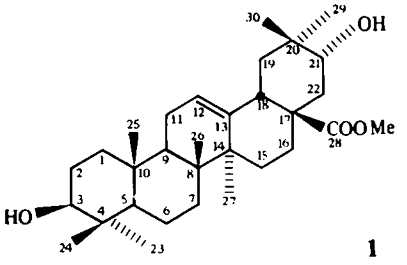
Usual work-up of the acetone extract of stems and leaves gave no diterpene derivatives. Oleanolic and ursolic acids were isolated as major components. After diazomethane methylation, methyl oleanolate and methyl ursolate were removed by chromatography and a small, more polar fraction was isolated. It contained two triterpene methyl esters.

The products proved to be isomers, both having the $C_{31}H_{50}O_4$ formula. Their mass spectra, very similar, showed peaks at m/z 486 $[M]^+$, 468, 278, 260, 219, 218 and 201. The peak at m/z 278 corresponded to the typical retro-Diels–Alder cleavage of an oleanene or ursene skeleton [3] with a carbomethoxy group on C-17 and a hydroxy group on ring D or E. This was confirmed by the peak at m/z 201, arising from the loss of 59 (COOMe) and 18 (H_2O) from the peak at m/z 278.

Careful examination of the ^{13}C NMR and ^{1}H NMR spectra of the first product (see Table 1 and Experimental) indicated a methyl oleanolate derivative [4–6] with a further hydroxy group, that was identified as 21α-hydroxy, as depicted in **1**.

Methyl 3β,21α-dihydroxy-olean-12-en-28-oate (**1**) has been previously prepared as a semisynthetic product [7]. Thereupon, **1** was isolated as a natural product only once [8], from the roots of *Olax dissitiflora* Oliv. (Olacaceae) where it occurs as a saponin of the free acid. Mass spectral data were reported only for **1** and ^{1}H NMR data only for its diacetyl derivative. This paper reports ^{1}H and complete ^{13}C NMR assignments for **1**. Physical data (mp and [α]) of our product are in agreement with previous data [7, 8].

The ^{13}C NMR and ^{1}H NMR spectra of the second product led to the identification of a methyl ursolate derivative [4–6] with a further hydroxy group. The



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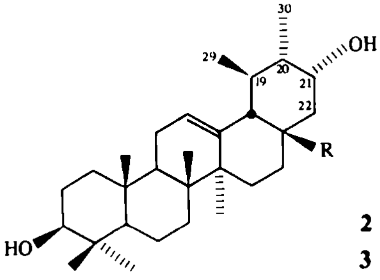

2 R = COOMe

3 R = CH_2OH

Table 1. ^{13}C NMR spectral data for compounds **1**, **4**, **2** and **5** (25.2 MHz, TMS as internal standard)*

C	1	4	2	5
1	38.6 *t*	38.1 *t*	38.8 *t*	38.8 *t*
2	27.1 *t*	27.1 *t*	27.3 *t*	27.3 *t*
3	77.8 *d*	78.7 *d*	79.0 *d*	78.8 *d*
4	38.6 *s*	38.7 *s*	38.8 *s*	38.8 *s*
5	55.3 *d*	55.2 *d*	55.4 *d*	55.4 *d*
6	18.3 *t*	18.3 *t*	18.4 *t*	18.4 *t*
7	32.8 *t*	32.6 *t*	33.2 *t*	33.0 *t*
8	39.1 *s*	39.3 *s*	39.5 *s*	39.6 *s*
9	47.5 *d*	47.6 *d*	47.7 *d*	47.5 *d*
10	36.9 *s*	37.0 *s*	37.1 *s*	37.0 *s*
11	23.3 *t*	23.1 *t*	23.4 *t*	23.3 *t*
12	122.1 *d*	122.1 *d*	125.9 *d*	125.5 *d*
13	143.8 *s*	143.4 *s*	137.9 *s*	138.0 *s*
14	41.8 *s*	41.6 *s*	42.3 *s*	42.0 *s*
15	27.9 *t*	27.7 *t*	28.5 *t*	28.2 *t*
16	26.4 *t*	23.4 *t*	27.1 *t*	24.3 *t*
17	46.6 *s*	46.6 *s*	48.2 *s*	48.1 *s*
18	41.2 *d*	41.3 *d*	53.7 *d*	52.8 *d*
19	40.8 *t*	45.8 *t*	32.0 *d*	39.1‡ *d*
20	35.0 *s*	30.6 *s*	42.8 *d*	38.8‡ *d*
21	72.9 *d*	33.8 *t*	71.8 *d*	30.7 *t*
22	39.0 *t*	32.3 *t*	42.8 *t*	36.7 *t*
23	28.3 *q*	28.1 *q*	28.2 *q*	28.2 *q*
24	15.9† *q*	15.6† *q*	15.5† *q*	15.5† *q*
25	15.3† *q*	15.3† *q*	15.7† *q*	15.7† *q*
26	16.8 *q*	16.8 *q*	16.8 *q*	16.9 *q*
27	27.6 *q*	26.0 *q*	23.2 *q*	23.6 *q*
28	177.6 *s*	177.9 *s*	177.6 *s*	177.7 *s*
29	25.3 *q*	33.1 *q*	17.0 *q*	16.9 *q*
30	24.8 *q*	23.6 *q*	17.2 *q*	21.2 *q*
OMe	51.2 *q*	51.3 *q*	51.5 *q*	51.4 *q*

*The spectra of **2**, **4** and **5** were taken in $CDCl_3$, while **1** was dissolved in $CDCl_3$ by adding a small amount of DMSO-d_6. Data for **4** and **5** are from ref. [6] including also the revised [14] assignments for C-11, C-27 and C-29.

†‡Assignments can be reversed.

presence of a multiplet at δ3.84 ($J = 3.5$, 3 and 2.5 Hz) was indicative of an equatorial proton geminal to an axial hydroxy group and coupled with three other protons. From the interpretation of its ^{13}C NMR spectrum it appeared that the product is methyl 3β,21α-dihydroxy-urs-12-en-28-oate (**2**). It has never been previously described and is therefore a new natural product.

In Table 1, comparison of **1** with methyl oleanolate (**4**) and of **2** with methyl ursolate (**5**) showed the predictable shifts attributable to β, γ and δ effects. The ^{13}C NMR data are consistent with the two chairs conformation of the *cis*-fused D and E rings [9, 10] for **1** as for **4**. The same discussion, based on inspection of the Dreiding models, renders probable the existence of chair–chair conformations for rings D and E in both **2** and **5**, and questions the earlier conformational assignment of **5** [6].

Parallel examination of the acetone extract of flowers gave similar results. No diterpene derivatives were found. Methyl oleanolate and methyl ursolate occurred as main components with a more polar fraction containing a mixture of methyl 21α-hydroxy-oleanolate (**1**) and methyl 21α-hydroxy-ursolate (**2**). Another fraction gave pure uvaol (**3**); this diol is absent in the extract of stems and leaves. Uvaol had been detected in *A. pampaninii* [1].

EXPERIMENTAL

Plant material was identified and collected in the island of Crete by Dr. G. Vourexakis, Rethymnon, in spring 1983. Samples are deposited in the collection of this Institute of Organic Chemistry.

Extraction and fractionation. Stems and leaves (800 g) were extracted with Me_2CO (3 l.) at room temp for 5 days. The residue (58 g) after evapn of solvent was chromatographed on a column of silica gel treated with 15% H_2O. After defatting with petrol, elution with petrol–EtOAc (4:1) gave a mixture (3 g) of oleanolic and ursolic acids, that was purified by CH_2N_2 methylation and CC on silica gel. Elution with petrol–EtOAc (7:3) yielded a small fraction, that was treated with CH_2N_2 and chromatographed on silica gel, giving the flavone 7-*O*-methylacacetin (70 mg) [11] and a mixture of **1** and **2** (200 mg). This mixture was resolved by fractional crystallization from $CHCl_3$ to give the less soluble pure **1** (110 mg) and a pure sample of the more soluble **2** (24 mg). The flavone was identified by conventional methods (mp, MS, NMR, UV); Me oleanolate and Me ursolate were identified by GC [12].

Flowers (50 g) were extracted in the same way. Elution with petrol–EtOAc (4:1) gave oleanolic and ursolic acids, purified and identified as Me esters by GC. The petrol–EtOAc (7:3) eluate was treated with CH_2N_2 and rechromatographed. After the mixture of **1** and **2** (15 mg), uvaol [13] (10 mg) was isolated and identified by comparison with an authentic sample (mp, MS, NMR); no 7-*O*-methylacacetin was detected.

Methyl 3β,21α-dihydroxy-olean-12-en-28-oate (**1**). Mp 275°–276° ($CHCl_3$), $[\alpha]_D^{21°}$ + 65° ($CHCl_3$); lit. mp 274°–275° [7], 273° [8], $[\alpha]_D^{27°}$ + 64° [7], + 66° [8]. ^{13}C NMR: see Table 1.

^{1}H NMR (100 MHz, CDCl$_3$): δ0.77 (3H, *s*, *t*-Me), 0.79 (3H, *s*, *t*-Me), 0.92–1.02 (12 H, *br s*, 4 *t*-Me), 1.17 (3H, *s*, *t*-Me), 2.12 (1H, *t*, J = 14 Hz, 19α-H), 2.44 (1H, *td*, J = 14 Hz and 4 Hz, 6β-H), 2.97 (1H, *dd*, J = 14 Hz and 4 Hz, 18β-H), 3.24 (1H, *dd*, J = 9 Hz and 6.5 Hz, 3α-H), 3.51 (1H, *dd*, J = 3.5 Hz and 3 Hz, 21β-H), 3.62 (3H, *s*, COOMe), 5.34 (1H, *t*, J = 3.8 Hz, H-12), 1.70 and 1.92 (2 × 1H, *dd* each, J_{gem} = 14 Hz, 22β-H and 22α-H: upon irradiation at δ3.51 these double-doublets collapse to an AB quartet, J_{gem} = 14 Hz).

Methyl 3β,21α-dihydroxy-urs-12-en-28-oate (**2**). Mp 214° (CHCl$_3$), $[\alpha]_D^{21°}$ + 24° (CHCl$_3$). MS (70 eV) *m/z*: 486, 468, 278, 260, 219, 218, 207, 201, 189. ^{13}C NMR: see Table 1. ^{1}H NMR (100 MHz, CDCl$_3$): δ0.77 (3H, *s*, *t*-Me), 0.80 (3H, *s*, *t*-Me), 0.85 (3H, *d*, J = 6.5 Hz, *s*-Me), 0.95 (3H, *s*, *t*-Me), 0.99 (3H, *s*, *t*-Me), 1.02 (3H, *d*, J = 6.5 Hz, *s*-Me), 1.11 (3H, *s*, *t*-Me), 2.31 (1H, *d*, J = 10.5 Hz, 18β-H), 2.47 (1H, *td*, J = 14 Hz and 4 Hz, 6β-H), 3.23 (1H, *dd*, J = 9 Hz and 7 Hz, 3α-H), 3.61 (3H, *s*, COOMe), 3.84 (1H, *m*, J = 3.5 Hz, 3 Hz and 2.5 Hz, 21β-H), 5.29 (1H, *t*, J = 3.8 Hz, H-12), 1.69 and 1.93 (2 × 1H, *dd* each, J_{gem} = 14 Hz, 22β-H and 22α-H; upon irradiation at δ3.84 they collapse to an AB quartet, J_{gem} = 14 Hz).

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