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AMMOLACTONE, A GUAIANOLIDE FROM A MEDICINAL PLANT, *AMMODAUCUS LEUCOTRICHUS*

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Key Word Index—*Ammodaucus leucotrichus*; Umbelliferae; monoterpene; sesquiterpene lactone; guaianolide; γ-decalactone; 12-nonacosanone.

Abstract—*Ammodaucus leucotrichus* afforded, in addition to common monoterpenoids, a new guaianolide ammolactone-A. Treatment with one equivalent of base and relactonization is a good means of purification. Treatment with excess alkali gave ammolactone-B with a different ring closure. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Ammodaucus leucotrichus Coss. & Dur. (Arabic: *al kamûne es sûfi*) is a monotypic genus belonging to the Umbelliferae (Apiaceae), distributed throughout north and west Africa in the Saharan and sub-Saharan countries. The seeds are used in traditional medicine for cold and fever. Surprisingly, no chemical analysis of this plant has previously been reported in the literature.

RESULTS AND DISCUSSION

This paper reports the isolation from the ethereal extract of the seeds of *A. leucotrichus*, of a new guaianolide lactone, ammolactone-A (**1**), together with (+)-limonene, (+)-perillaldehyde, (the two major peaks in gas chromatography), a new monoterpenoid ((+)-3-hydroxyperillaldehyde (**4**)), (−)-methylperillate, (+)-borneol angelate and (+)-γ-decalactone.

Kugelrohr, high vacuum distillation of the crude ethereal extract followed by chromatography on silica gel, furnished first a blue fraction of chamazulene indicating the presence of guaiane derivatives. γ-Decalactone was contained in the next fraction; this has long been known as a synthetic product, and has been found as a component in numerous fruit aromas. Within the Umbelliferae it was first isolated from *Daucus carota* seed oil [1], and later from *Angelica* [2, 3] and *Bupleurum* [4] species. The presence of the γ-lactone ring was best recognized from the mass spectra with a base peak at m/z 85, and confirmed by IR and NMR.

Ammolactone-A (**1**) was crystallized from the more polar fractions in a large quantity. The attribution of the relative stereochemistry is proposed on the basis of decoupling and nuclear Overhauser enhancement experiments. As is generally the case for guaianolides, the hydroazulene skeleton is *cis* fused [5]. However, the γ-lactone ring is closed in a *cis* manner, which is rather rare. The absolute configuration proposed here for carbon atoms 1, 5, 6 and 7 is that of grilactone, another component of Umbelliferae [6], whose structure was established by X-ray analysis [7]. An important nuclear Overhauser effect between H-7 and H-11 led us to assume that C-11 has the (*S*) configuration.

An interesting feature is that saponification led to a trihydroxycarboxylate which, on acidification, relactonizes with the 8-OH rather than the 6-OH group, leading to ammolactone-B (**2**). This is obvious from the ^{1}H NMR spectrum, in which the H-8 signal is shifted to δ 4.66 and the H-6 signal to δ 3.86. Mild saponification with an equimolar quantity of potassium hydroxide in methanol opens the lactone ring first, giving the acid **3**. The acid **3** relactonizes only slowly. Ammolactone-B is not present in the plant. The configuration proposed for 3-hydroxyperillaldehyde (**4**) comes from the assumption that the configuration of C-4 is the same as in perillaldehyde and from the *trans*-diaxial arrangement of the H-3 and H-4 protons indicated by their high coupling constant (J = 9.6 Hz). Small pieces of aerial

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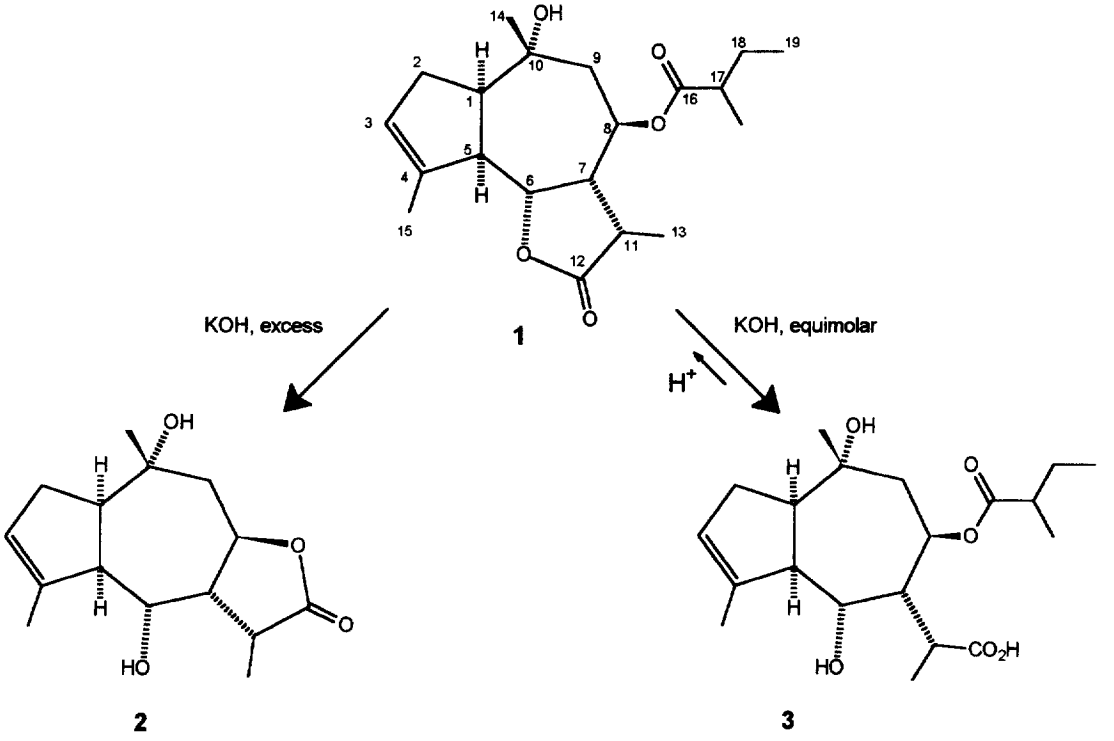
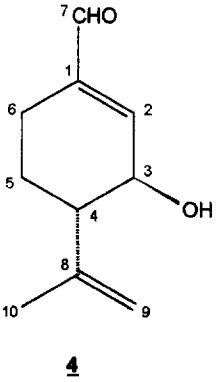


Scheme 1.



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parts of the plants are also present in the commercial drug. The GC-mass spectrometry analysis of these parts shows the absence of **1** but the presence of 12-nonacosanone. This long-chain ketone is reported to be present in *Thapsia villosa* [8]. As most of the sesquiterpenic lactones possess high biological activity, it is reasonable to assume that ammolactone-A is the active principle of the drug, but this remains to be established.

EXPERIMENTAL

Mps: uncorr. CC: Merck Kieselgel 60. All steps were monitored by GC-MS (HP 5890A-5970) using a 25 m BPX5 GC column.

Plant material. Seeds of *A. leucotrichus* were bought in a local market at Casablanca (Morocco) and were identified by one of the authors (J.-P.R.)

Extraction and isolation. Crushed seeds (2 kg) were extracted with Et_2O at room temp. The Et_2O extract was concentrated, then distilled in a Kugelrohr apparatus and subjected to CC on silica gel with mixtures of petrol and EtOAc as eluent. The fraction eluted with an 8:2 mixture gave 3.594 g of **1**.

8-(2-*Methylbutanoyl*-)-10-*hydroxy*-3-*guaien*-12,6-*olide* (**1**). Crystals, mp 149°; IR ν_{max} (CCl_4) cm^{-1}: 3536 (OH), 3045 (C=C), 1720 (C=O), 1770 (γ-lactone); MS *m/z*: 332 $[M-H_2O]^+$ (1), 293 $[M-C_4H_7]^+$ (3), 249 (13), 248 (73), 233 (13), 230 (30), 205 (40), 190 (32), 175 (46), 169 (21), 167 (47), 159 (39), 157 (35), 156 (11), 145 (38), 133 (64), 132 (100), 120 (93), 107 (65), 106 (39), 85 (54), 80 (47); $[\alpha]_D$ +22 ($CHCl_3$; $c = 0.006$). The fraction eluted with a 1:1 mixture gave **4**, further purified by CC on silica gel/alumina (19 mg).

3-*Hydroxyperillaldehyde* (**4**). Oil; IR ν_{max} ($CHCl_3$) cm^{-1}: 3510 (OH), 1683 (C=O); MS *m/z*: 166 $[M]^+$ (21), 161 (3), 151 $[M-CH_3]^+$ (8), 148 $[M-H_2O]^+$ (11), 138 $[M-CO]^+$ (9), 137 (76), 133 (13), 123 (16), 199 (22), 109 (12), 105 (16), 98 $[M-C_5H_8]^+$ (71), 91 (23), 81 (21), 79 (21), 77 (22), 69 (100), 68 $[M-C_5H_6O_2]^+$ (33), 67 (48), 55 (19), 53 (19), 41 (30); $[\alpha]_D$ −36 ($CHCl_3$; $c = 0.004$). ^{13}C NMR ($CDCl_3$): δ 19.0 (C-10); 21.6 (C-5); 25.3 (C-6); 50.4 (C-4); 68.7 (C-3); 113.0 (C-9); 141.3 (C-2); 145.3 (C-1); 150.1 (C-8); 193.8 (C-7). ^1H NMR ($CDCl_3$): δ 6.72 (*dt*, H-2); 4.38 (*ddt*, H-3); 2.37 (*dddd*, H-4); 1.91 (*dddd*, H-5); 2.01 (*m*; H-5′); 2.45 (*dddt*, H-6); 2.19 (*ddddd*, H-6′); 9.51 (*s*, H-7); 4.97 (*quintuplet*, H-9); 4.90 (*dq*, H-9′); 1.84 (3H, *dd*, H-10).

Table 1. ^{1}H NMR (250 MHz, $CDCl_3$) data for compounds **1–3**

H	δ **1**	**2**	**3**
1	2.37 *dddd*	2.96 *dq*	2.78 *dq*
2	2.12 *ddd*	2.39 *dddd*	2.40 *ddt*
2′	2.05 *ddd*	2.05 *dddd*	2.05 *ddd*
3	5.51 *td*	5.43 *ddt*	5.39 *d* (large)
5	2.55 *dd*	2.72 *dm*	2.91 *dm*
6	4.56 *dd*	3.86 *d*	3.95 *d*
7	3.04 *ddd*	1.56 *ddd*	2.11 *dd*
8	5.30 *ddd*	4.66 *ddd*	5.44 *dt*
9	2.04 *ddd*	2.29 *ddd*	2.07 *ddd*
9′	1.75 *dd*	1.98 *dd*	1.88 *dd*
11	2.68 *dq*	2.85 *dq*	2.85 *dq*
13	1.30 *d*	1.17 *d*	1.26 *d*
14	1.22 *s*	1.37 *s*	1.13 *s*
15	1.86 *dt*	1.70 *dt*	1.66 *dt*
17	2.34 *sextuplet*		2.31 *sextuplet*
18	1.47 *dquintuplet*		1.46 *dquintuplet*
18′	1.64 *dquintuplet*		1.73 *dquintuplet*
19	0.91 *t*		0.91 *t*
20	1.14 *d*		1.22 *d*

J (Hz)

Compound **1**: $J_{1,2} = 6.8$; $J_{1,2'} = 12$; $J_{1,5} = 5.6$; $J_{1,9} = J_{2,15} = J_{2',3} = J_{3,15} = 1.6$; $J_{2,2'} = 15$; $J_{2,3} = 3.2$; $J_{2',15} = 2.4$; $J_{5,6} = 11.4$; $J_{6,7} = 9$; $J_{7,8} = 11.2$; $J_{7,11} = 9.3$; $J_{8,9'} = 9.4$; $J_{8,9} = 0.8$; $J_{9,9'} = 14.6$; $J_{11,13} = 7.8$; $J_{17,18} = J_{17,18'} = 7.2$; $J_{18,18'} = 13.6$; $J_{18,19} = J_{18',19} = 7.2$

Compound **2**: $J_{1,2} = J_{1,2'} = J_{1,5} = 9.6$; $J_{1,9} = 1.2$; $J_{2,2'} = 16.2$; $J_{2,3} = 2.7$; $J_{2',3} = J_{3,5} = 1.8$; $J_{2',5} = 3.4$; $J_{2,15} = J_{3,15} = 1.6$; $J_{2',15} = 2.4$; $J_{5,6} = J_{5,7} = 1.5$; $J_{5,15} = 1.4$; $J_{7,11} = 12.4$; $J_{7,8} = 11$; $J_{8,9} = 4.8$; $J_{8,9'} = 11.5$; $J_{9,9'} = 13.5$; $J_{11,13} = 7$

Compound **3**: $J_{1,2} = J_{1,2'} = J_{1,5} = 10$; $J_{1,9} = 1.2$;$J_{2,2'} = 16$; $J_{2,3} = J_{2,5} = J_{5,6} = 2.3$; $J_{2',5} = 0.8$; $J_{7,8} = J_{8,9'} = 11$; $J_{7,11} = 3.2$; $J_{8,9} = 4.8$; $J_{9,9'} = 13.8$; $J_{11,13} = 7$; $J_{17,18} = J_{17,18'} = J_{17,20} = 7.2$; $J_{18,18'} = 13.6$; $J_{18,19} = J_{18',19} = 7.2$

Table 2. ^{13}C NMR data (62.9 MHz, $CDCl_3$) for compounds **1–3**

C	δ **1**	**2**	**3**
1	45.5	40.1	41.4
2	32.2	35.9	36.1
3	125.2	125.5	125.1
4	147.0	139.0	139.5
5	50.1	57.6	57.4
6	80.7	74.5	69.7
7	41.1	51.4	43.8
8	67.2	67.0	68.0
9	43.2	51.9	42.2
10	77.4	74.4	73.8
11	36.2	38.9	40.7
12	179.2	178.9	180.8
13	13.4	12.3	11.5
14	31.1	32.7	32.1
15	18.6	15.2	16.6
16	177.1		176.0
17	55.5		50.9
18	26.6		26.4
19	11.5		11.5
20	16.4		15.2

J (Hz): $J_{2,3} = 1.2$; $J_{2,6} = J_{2,6'} = 2.4$; $J_{3,4} = 9.6$; $J_{3,6} = J_{3,6'} = 2.4$; $J_{4,5} = 2.6$; $J_{4,5'} = 12.6$; $J_{4,9} = J_{4,9'} = J_{9,10} = 1.6$; $J_{5,6} = 2.4$; $J_{5,5'} = 13.6$; $J_{5,6'} = 1.2$; $J_{5',6} = 5.6$; $J_{5',6'} = 11.4$; $J_{6,6'} = 18$; $J_{9',10} = 0.8$.

Full saponification of **1**. Compound **1** was dissolved in 50% aq. MeOH and then KOH (excess) added. After the mixture had been stirred for 24 hr at room temp., H_2O was added and the reaction mixture was extracted with Et_2O. The result of evaporation was chromatographed on a column of silica gel and eluted with a petrol/EtOAc mixture. The fraction eluted with a 1:1 mixture gave **2**.

Ammolactone-B (**2**). Oil which slowly crystallizes on standing, mp 123–125°. IR ν_{max} (CCl_4) cm^{-1}: 3798 (OH), 3045 (C=C), 1770 (γ-lactone); $[\alpha]_D + 5$ ($CHCl_3$; $c = 0.01$).

Half saponification of **1**. Compound **1** was dissolved in 50% aq. MeOH and then KOH (1 eq.) added. After the mixture had been stirred for 24 hr at room temp., H_2O was added and the reaction mixture was extracted with Et_2O. The result of evaporation was subjected to CC on silica gel. The fraction eluted with EtOAc give dihydroxy acid **3**. Crystals, mp 150°. IR ν_{max} ($CHCl_3$) cm^{-1}: 3350 (OH), 1720 (C=O), 1709 (C=O); $[\alpha]_D + 5$ ($CHCl_3$; $c = 0.01$).

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