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DITERPENES FROM THE GENUS *AMARACUS*

SALVATORE PASSANNANTI, MARIAPIA PATERNOSTRO, FRANCO PIOZZI,*

Institute of Organic Chemistry, University of Palermo, Palermo, Italy

and CLAUDIA BARBAGALLO

Institute of Botany, University of Catania, Catania, Italy

Continuing our work on the terpenoids occurring in plants of the Labiatae family, we report now on the diterpenoids isolated from *Amaracus akhdarensis* (Ietswaart et Boulos) Brullo et Furnari (syn. *Origanum akhdarensis*) and *Amaracus pampanini* Brullo et Furnari, both growing in Libya.

The genus *Amaracus*, previously regarded in the eighteenth century as a separate genus, was later assimilated into the related genus *Origanum*. The recent treatise "Flora Europaea" (1) maintains this incorporation, but recognizes the occurrence of a section Amaracus inside the genus *Origanum*; so several species are known by both names. It has been suggested (2) that the genus should be restored and considered apart from *Origanum*; so a chemical investigation was undertaken on the secondary metabolites for the purpose of bringing support to a correct taxonomic classification.

From the Me_2CO extract of the aerial part of *A. akhdarensis* we isolated three diterpenes: akhdarenol (**1**), akhdardiol (**2**), and akhdartriol (**3**).

The pmr data of akhdarenol (**1**) suggested the occurrence of one primary hydroxyl, one vinyl, three tertiary methyl groups. A methine signal (multiplet at δ 5.42) indicated a trisubstituted double bond. This information, together with the molecular formula $C_{20}H_{32}O$, suggested that **1** could be a diterpenoid of the pimarane type. By treatment with Ac_2O and pyridine, acetylakhdarenol (**4**) was obtained. The chemical shift of CH_2OH and CH_2OAc proved (3) an axial configuration for this group. The cmr spectrum (Table 1) of acetylakhdarenol was consistent with structure (**4**) and in full agreement with data of similar products (4,5). Thus, akhdarenol was assigned the structure (**1**) of isopimara-7,15-dien-19-ol. A product with the same structure and the same physical data was quite recently isolated (6) from the fungus *Acremonium luzulae* (Fuckel) Gams: the direct comparison proved the products to be identical, thus confirming also the absolute stereochemistry of akhdarenol (*normal* series).

The second product, akhdardiol (**2**), had a molecular formula $C_{20}H_{34}O_2$. Its pmr spectrum showed signals for one primary hydroxyl, one vinyl, and three tertiary methyl groups; no other olefinic proton occurred. Treatment with Ac_2O and pyridine at room temperature gave monoacetylakhdardiol (**5**), whose ir spectrum still showed hydroxyl bands; thus, the second oxygen atom was shown to be a tertiary hydroxy group.

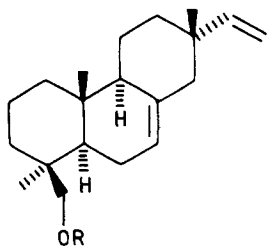
The cmr spectrum of akhdardiol (Table 1) was in good agreement with those of known 8-hydroxy-pimarane diterpenes (7,8). Both pmr and cmr data were consistent with structure (**2**) of isopimar-15-en-8β,19-diol. The axial 8β-OH configuration proceeded from the value of the axial 13β-CH_3 (9).

A product with this stereostructure was described some years ago (10); however, this product is probably isopimar-15-en-8β,20-diol, described elsewhere (11). So (**2**) occurs as a new natural product.

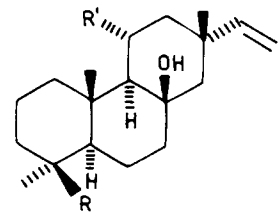
The occurrence of a primary hydroxy group was confirmed by careful CrO_3 oxidation of **2**, that yielded a mixture of products. Hplc separation gave the aldehyde (**6**) and the acid (**7**), character-

TABLE 1. Cmr Spectra, Registered at 25.2 MHz (ppm from TMS, $CDCl_3$ solvent)

Carbon	Compound		
	4	**2**	**8**
C-1	39.73 t	39.59 t	40.74 t
C-2	18.40 t	18.07 t	18.22 t
C-3	36.19 t	35.74 t	35.91 t
C-4	35.31 s	38.68 s	38.76 s
C-5	51.22 d	57.21 d	56.61 d
C-6	23.10 t	18.35 t	17.62 t
C-7	121.29 d	43.99 t	44.10 t
C-8	135.38 s	72.49 s	74.19 s
C-9	52.21 d	58.21 d	59.09 d
C-10	29.74 s	36.43 s	36.62 s
C-11	20.38 t	17.18 t	70.45 d
C-12	36.42 t	38.13 t	44.17 t
C-13	36.73 s	37.20 s	37.39 s
C-14	46.10 t	51.57 t	51.31 t
C-15	150.01 d	151.59 d	150.05 d
C-16	109.18 t	108.57 t	108.92 t
C-17	21.49 q	24.28 q	25.87 q
C-18	27.42 q	27.08 q	27.77 q
C-19	66.77 t	65.25 t	66.90 t
C-20	16.03 q	16.21 q	16.63 q
CH_3 acetate	20.81 q		20.91 q
			21.90 q
CO acetate	170.92 s		169.86 s
			171.13 s



1 R=H
4 R=Ac



2 $R=CH_2OH$ $R'=H$
3 $R=CH_2OH$ $R'=OH$
5 $R=CH_2OAc$ $R'=H$
6 R=CHO $R'=H$
7 R=COOH $R'=H$
8 $R=CH_2OAc$ $R'=OAc$

ized by their pmr, ir, and ms (see Experimental section).

The third diterpenoid, akhdartriol (**3**) $C_{20}H_{34}O_3$, showed a pmr spectrum very similar to that of akhdardiol (**2**). The main difference was a triplet of doublets at δ 4.45 (J=10 Hz and 4 Hz), corresponding to an axial C*H*OH hydrogen coupled with two axial and one equatorial proton. Only positions 6 and 11 could account for such a system. The occurrence of a doublet of multiplets at δ 2.48, attributed to a proton at position 1 (10), indicated that the hydroxy group is at position 11; therefore, the presence of an equatorial 11α-OH group is proved. Further support arose from the cmr spectrum (Table 1) of diacetylakhdartriol (**8**) by comparison with that of akhdardiol (**2**); C-11 appeared to be shifted from 17.18 to 70.45 ppm and C-12 from 38.13 to 44.17 ppm. All the data were

consistent with the structure and with the chemical shifts reported for similar products (7,8). Therefore, akhdartriol can be formulated as isopimar-15-en-8β,11α,19-triol (**3**).

The extract of *A. akhdarensis* revealed also the occurrence of the triterpenes, oleanolic acid and ursolic acid, and of the known flavonoid cirsimaritin (5,4′-dihydroxy-6,7-dimethoxyflavone).

From the aerial part of *A. pampanini* we isolated small amounts of akhdardiol (**2**) as the only diterpene. Also the triterpenes oleanolic acid, ursolic acid, and uvaol were detected, together with the known flavonoids 7-*O*-methyl-acacetin (5-hydroxy-7,4′-dimethoxyflavone) and 5-*O*-desmethyl-nobiletin (5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone).

As far as we are aware, no literature report quotes the occurrence of diterpenoids in *Origanum* species. No diterpene was found by us in *Origanum heracleoticum* L., widespread in Sicily. These findings seem to support a difference between *Amaracus* and *Origanum* and a need to separate these genera.

EXPERIMENTAL

PLANT MATERIAL.—*A. akhdarensis* and *A. pampanini* were collected and identified by Prof. F. Furnari, (Institute of Botany, University of Catania), in North Cyrenaica, Libya, on the Gebal el Akhdar, in May 1981. Specimens are deposited in the Herbarium of the above institute.

EXTRACTION AND CHROMATOGRAPHY OF *A. AKHDARENSIS*.—Air-dried aerial part (260 g) was powdered and extracted twice at room temperature with Me_2CO for 1 week, then the Me_2CO extract was evaporated to dryness under reduced pressure. The residue was chromatographed over silica gel (150 g) deactivated by 15% H_2O. The elution gave in the order: petroleum ether, hydrocarbons, fats, waxes (rejected); petroleum ether-EtOAc (90:10) akhdarenol (120 mg), petroleum ether-EtOAc (80:20) akhdardiol (1.2 g), petroleum ether-EtOAc (70:30) oleanolic and ursolic acid (2 g), petroleum ether-EtOAc (60:40) akhdartriol (70 mg), and petroleum ether-EtOAc (50:50) cirsimaritin.

As the first chromatographic separation was not clear-cut, purifications on smaller columns were required; final purification of akhdartriol required hplc [Waters, μ-Porasil 30 cm×3.9 mm column, eluent cyclohexane-EtOAc (1:1) 2 ml/min].

EXTRACTION AND CHROMATOGRAPHY OF *A. PAMPANINI*.—The plant material (300 g) was treated as described for *A. akhdarensis*. The chromatography gave in elution order: petroleum ether, hydrocarbons, fats, waxes (rejected); petroleum ether-EtOAc (80:20) akhdardiol (100 mg) and uvaol (50 mg), petroleum ether-EtOAc (70:30) oleanolic and ursolic acid (2 g), petroleum ether-EtOAc (60:40) 7-*O*-methyl-acacetin (20 mg), and petroleum ether-EtOAc (50:50) 5-*O*-desmethyl-nobiletin (60 mg).

The separation of akhdardiol from uvaol required semipreparative hplc [Waters, μ-Porasil 30 cm×7.8 mm column, eluent cyclohexane-EtOAc (70:30) 2 ml/min].

AKHDARENOL (**1**).—Mp 82°-83° (from EtOAc), lit. (6) mp 88°; ms *m/z* 288 (M^+), 273, 257, 109; hrms M^+ 288.240 (calcd. for $C_{20}H_{32}O$, 288.245) Calcd. C 83.27, H 11.18. Found C 83.04, H 11.11; ir ($CHCl_3$) 3350, 1630, 990, 910, 830 cm^{-1}; pmr $CDCl_3$, 60 MHz) δ 0.83 (6H, s, 2 *t*.CH_3), 0.96 (3H, s, *t*.CH_3), 3.51 and 3.95 (2H, q_{AB}, J=10.5 Hz, CH_2OH), 4.90 and 4.95 (2H, eight lines pattern, J=17.5, 10.5, 1.5 Hz, $CH=CH_2$), 5.42 (1H, m W½ 10 Hz, C=C*H*), 5.90 (1H, dd, J=17.5, 10.5 Hz, $CH=CH_2$). Glc comparison with an authentic specimen (6) gave the identical T_R also on co-injection (FID, 2 m×⅛ in. column, 3% OV-1 on Chromosorb, T 200°, carrier gas N_2 20 ml/min).

ACETYLAKHDARENOL (**4**).—Prepared by Ac_2O-pyridine treatment at room temperature. Oily, homogeneous on tlc; $[\alpha]^{20}D$ −19.2° ($CHCl_3$, c, 0.0114); ir ($CHCl_3$) 1725, 1630, 1200, 980, 910 cm^{-1}; ms *m/z* 330 (M^+, $C_{22}H_{34}O_2$ 330) 315, 303, 301, 255, 109; pmr ($CDCl_3$, 300 MHz) δ 0.86 (3H, s, *t*.CH_3), 0.87 (3H, s, *t*.CH_3), 0.96 (3H, s, *t*.CH_3), 2.02 (3H, s, CH_3COO), 3.94 and 4.36 (2H, q_{AB}, J=10.5 Hz, CH_2OAc), 4.83 and 4.89 (2H, eight lines pattern, J=17.5, 10.5, 1.5 Hz, $CH=CH_2$), 5.32 (1H, m W½ 10 Hz, C=C*H*), 5.81 (1H, dd, J=17.5, 10.5 Hz, $CH=CH_2$).

AKHDARDIOL (**2**).—Mp 117°-118° (from EtOH), $[\alpha]^{20}D$ −15.6° ($CHCl_3$, c, 0.0046); ms *m/z* 306 (M^+) 291, 288, 275, 257, 109; hrms M^+ 306.251 (calcd. for $C_{20}H_{34}O_2$, 306.255). Calcd. C 78.38, H 11.18. Found C 78.26, H 11.31; ir (nujol) 3500, 3400, 1630, 985, 912 cm^{-1}; pmr ($CDCl_3$, 200 MHz) δ 0.96 (6H, s 2 *t*.CH_3), 1.20 (3H, s, *t*.CH_3), 3.48 and 3.78 (2H, q_{AB}, J=10.5 Hz, CH_2OH), 4.82 and 4.85 (2H, eight lines, J=17.5, 10.5, 1.5 Hz, $CH=CH_2$), 5.72 (1H, dd, J=17.5, 10.5 Hz, $CH=CH_2$).

MONOACETYLAKHDARDIOL (**5**).—Prepared by Ac_2O-pyridine treatment at room temperature. Oily, homogeneous on tlc, $[\alpha]^{20}D$ −4.8° ($CHCl_3$, c, 0.0038); ir (CCl_4) 3600, 3520, 3080,

1730, 1630, 985, 912 cm^{-1}; ms *m/z* 348 (M^+, $C_{22}H_{36}O_3$ 348) 333, 330, 288, 270, 257, 192, 109; pmr ($CDCl_3$, 60 MHz): δ 0.95 (3H, s, *t*.CH_3), 1.00 (3H, s, *t*.CH_3), 1.22 (3H, s, *t*.CH_3), 2.05 (3H, s CH_3COO), 3.95 and 4.35 (2H, q_{AB}, J=10.5 Hz, CH_2OAc), 4.83 and 4.90 (2H, eight lines, J=17.5, 10.5, 1.5 Hz, $CH=CH_2$), 5.83 (1H, dd, J=17.5, 10.5 Hz, $CH=CH_2$).

CHROMIC ACID OXIDATION OF AKHDARDIOL (**2**).—Akhdardiol (30 mg) in Me_2CO (5 ml) was treated at 0° with a few drops of Jones reagent until yellow color persisted. Usual work-up gave a mixture (two main spots on tlc) that was resolved by hplc [Waters, μ-Porasil 30 cm×7.8 mm column, eluent cyclohexane-EtOAc (70:30) 2 ml/min].

8β-Hydroxy-isopimar-15-en-19-al (**6**) was an oil; ms *m/z* 304 (M^+, $C_{20}H_{32}O_2$ 304) 289, 286, 275, 257, 109; ir ($CHCl_3$) 3580, 2720, 1710, 1630, 990, 915 cm^{-1}; pmr ($CDCl_3$, 60 MHz) δ 0.80 (3H, s, *t*.CH_3), 0.92 (3H, s, *t*.CH_3, 1.20 (3H, s, *t*.CH_3), 4.80 and 4.84 (2H, eight lines, $CH=CH_2$), 5.72 (1H, dd, $CH=CH_2$), 9.82 (1H, s, *CHO*).

8β-Hydroxy-isopimar-15-en-19-oic acid (**7**), amorphous solid, could not be crystallized. Ms *m/z* 320 (M^+, $C_{20}H_{32}O_3$ 320) 305, 302, 275, 257, 109; ir ($CHCl_3$) 3580, 3500, 1680, 1630, 985, 910 cm^{-1}; pmr ($CDCl_3$, 60 MHz) δ 0.82 (3H, s, *t*.CH_3), 1.20 (6H, s, 2 *t*.CH_3), 4.80 and 4.84 (2H, eight lines, $CH=CH_2$), 5.72 (1H, dd, $CH=CH_2$).

AKHDARTRIOL (**3**).—Mp 115° (gel amorphous, from all solvents). Ms *m/z* 322 (M^+) 304, 273, 255, 109; hrms M^+ 322.246 (calcd. for $C_{20}H_{34}O_3$, 322.250). Calcd. C 74.49, H 10.63. Found C 74.24, H 10.45; pmr ($CDCl_3$, 60 MHz) δ 0.99 (3H, s, *t*.CH_3), 1.18 (3H, s, *t*.CH_3), 1.27 (3H, s, *t*.CH_3), 3.55 and 3.90 (2H, q_{AB}, J=10.5 Hz, CH_2OH), 4.45 (1H, td, J=10, 4 Hz, *CH*OH), 4.93 and 4.97 (2H, eight lines, J=17.5, 10.5, 1.5 Hz, $CH=CH_2$), 5.83 (1H, dd, J=17.5, 10.5 Hz, $CH=CH_2$), 2.48 (1H, dm, proton on C-1).

DIACETYLAKHDARTRIOL (**8**).—Prepared by Ac_2O-pyridine treatment at room temperature. Mp 141°-142° (from EtOH); $[\alpha]^{20}D$ −61.3° ($CHCl_3$, c, 0.0152); ms *m/z* 406 (M^+, $C_{24}H_{38}O_5$ 406) 388, 346, 328, 315, 273, 109; ir ($CHCl_3$) 3550, 1725, 1630, 1200, 990, 910 cm^{-1}; pmr ($CDCl_3$, 80 MHz) δ 0.96 (3H, s, *t*.CH_3), 1.05 (3H, s, *t*.CH_3), 1.29 (3H, s, *t*.CH_3), 1.98 and 2.02 (s, 3H each, 2 CH_3COO), 3.92 and 4.10 (2H, q_{AB}, J=10.5 Hz, CH_2OAc), 4.80 and 4.84 (2H, eight lines, J=17.5, 10.5, 1.5 Hz, $CH=CH_2$), 5.49 (1H, td, J=10, 4 Hz, *CH*OAc), 5.72 (1H, dd, J=17.5, 10.5 Hz, $CH=CH_2$).

MINOR CONSTITUENTS FROM *A. AKHDARENSIS*.—The mixture of oleanolic and ursolic acid was methylated with CH_2N_2 in Et_2O solution. Glc (FID, 1.5 m×3 mm column packed with 3% OV-1 on Chromosorb at 260°, detector and injector temp. 300°, carrier gas N_2 20 ml/min) revealed the occurrence of methyl oleanolate and methyl ursolate, identified (12) by co-injection with authentic samples.

Cirsimaritin (5,4′-dihydroxy-6,7-dimethoxyflavone) was identified (13) by mp, ir, uv, ms, and pmr spectra.

MINOR CONSTITUENTS FROM *A. PAMPANINI*.—The mixture of oleanolic and ursolic acid was examined as reported above.

Uvaol (3β,28-dihydroxy-urs-12-ene) was identified (14) by mp, ms, and pmr spectra and comparison with an authentic specimen.

7-*O*-Methyl-acacetin (5-hydroxy-7,4′-dimethoxyflavone) was identified (15-17) by mp, ir, uv, ms, and pmr spectra.

5-*O*-Desmethyl-nobiletin (5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone) was identified (18-20) by mp, ir, uv, ms, and pmr spectra, and by transformation into its acetylderivative.

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LITERATURE CITED

1. V.H. Heywood and I.B.K. Richardson, "Flora Europaea," vol. 3, Cambridge: University Press Cambridge, 1972, p. 171.
2. S. Brullo and F. Furnari, *Webbia*, **34**, 439 (1979).
3. A. Gaudemer, J. Polonsky, and E. Wenkert, *Bull. Soc. Chim. France*, 407 (1964).
4. E. Wenkert and B.L. Buckwalter, *J. Am. Chem. Soc.*, **94**, 4367 (1972).
5. R.C. Cambie, I.R. Burfitt, T.E. Goodwin, and E. Wenkert, *J. Org. Chem.*, **40**, 3789 (1975).
6. N. Cagnoli, P. Ceccherelli, M. Curini, G.F. Madruzza, and M. Ribaldi, *J. Chem. Res. (s)*, 254 (1982).
7. A. Matsuo, S. Uto, M. Nakayama, S. Hayashi, K. Yamasaki, R. Kasai, and O. Tanaka, *Tetrahedron Lett.*, 2451 (1976).
8. A.C. Pinto and C. Borges, *Phytochemistry*, **22**, 2011 (1983).
9. F. Bohlmann and C. Zdero, *Phytochemistry*, **21**, 1697 (1982).
10. F. Bohlmann, G. Weickgenannt, and C. Zdero, *Chem. Berichte*, **106**, 826 (1973).

11. F. Bohlmann, M. Wallmeyer, J. Jacupovic, and J. Ziesche, *Phytochemistry*, **22**, 1645 (1983).
12. S. Passannanti, M. Paternostro, and F. Piozzi, *Phytochemistry*, **22**, 1044 (1983).
13. M.M. Rao, D.G.I. Kingston, and T.D. Spittler, *Phytochemistry*, **9**, 227 (1970).
14. C. von Carstenn-Lichterfelde, B. Rodriguez, and S. Valverde, *Phytochemistry*, **12**, 3002 (1973).
15. K.H. Bauer and H. Dietrich, *Chem. Ber.*, **66**, 1053 (1933).
16. C.H. Brieskorn and H.J. Dömling, *Arch. Pharm.*, **300**, 1042 (1967).
17. M. Silva, J.M. Mundaca, and P.G. Sammes, *Phytochemistry*, **10**, 1942 (1971).
18. P.S. Sarin and T.R. Seshadri, *Tetrahedron*, **8**, 64 (1960).
19. J. Pinkas, D. Lavie, and M. Chorin, *Phytochemistry*, **7**, 169 (1968).
20. J.H. Tatum and R.E. Berry, *Phytochemistry*, **11**, 2283 (1972).

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