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TRITERPENES OF *CENTAUREA PTOSIMOPAPPOIDES**

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Key Word Index—*Centaurea ptosimopappoides*; Compositae; triterpenes; sesquiterpene lactones; coumarin; steroids.

Abstract—The roots of *Centaurea ptosimopappoides* afforded two new triterpenes; a new hopane derivative, 17β,21β-epoxy-16α-ethoxyhopan-3β-ol and a new baccharane type triterpene, 3β-acetoxy-17,24-dioxo-baccharane. The known compounds, 17β,21β-epoxyhopan-3β-ol, 3β-acetoxyhop-17(21)-ene and 3β-acetoxy-erythrodiol were also isolated. In addition, the aerial parts yielded scopoletin, 7-oxositosterol, stigmasterol, α-amyrin and common sesquiterpene lactones of the genus *Centaurea*, 11,13-dihydro-desacetylcynaropicrin and cynaropicrin. The structures of the new compounds were determined by high field spectroscopic methods including 2D NMR techniques. © 1997 Elsevier Science Ltd

INTRODUCTION

The genus *Centaurea* has been the subject of many chemical investigations due to the diversity of its chemical constituents. Acetylenes [1] and sesquiterpene lactones [2] are the main secondary metabolites of *Centaurea* species. In our continuing research on the chemistry of *Centaurea* species, we have investigated *Centaurea ptosimopappoides* Wagenitz, a plant endemic to Turkey, which is widely distributed in the southern part of Anatolia. We report herein the isolation and structure determination of two new triterpenes, a new hopane derivative, 17β,21β-epoxy-16α-ethoxyhopan-3β-ol (**1**), a new baccharane type triterpene, 3β-acetoxy-17,24-dioxo-baccharane (**2**), and the known compounds, 3β-acetoxyhop-17(21)-ene (**3**) [3], 17β,21β-epoxyhopan-3β-ol (**4**) [4], 3β-acetoxy-erythrodiol (**5**) [5] from the roots of *Centaurea ptosimopappoides*. In addition, from the aerial parts, 11,13-dihydro-desacetylcynaropicrin (**6**), cynaropicrin (**7**), 7-oxositosterol (**8**) [6], scopoletin, stigmasterol and α-amyrin were also isolated. The occurrence of baccharane type triterpenes in plants is rare. Baccharane oxide was the first example isolated from *Baccharis halimifolia* (Compositae) by Anthonsen *et al.* [7, 8]. Following this, only three papers describing the presence of baccharanes in *Impatiens balsamita* [9], *Actinostemma lobatum* [10] and *Lemmaphyllum microphyllum* [11] were reported, and there is no report of the presence of baccharane type triterpenes in the genus *Centaurea*. This is the first report for the occurrence of baccharane type triterpenes in this genus which might have chemotaxonomic importance.

RESULTS AND DISCUSSION

From the spectral properties, compound **3** was deduced as 3β-acetoxyhop-17(21)-ene [3]. The spectral data (^{1}H, ^{13}C NMR, ^{1}H-^{1}H COSY, HETCOR and mass spectroscopy) of compound **4** were identical with those of 17β,21β-epoxyhopan-3β-ol which has been previously isolated from *Euphorbia supina* [4] and its acetyl derivative, 3β-acetoxy-17β,21β-epoxyhopane, has been reported from *Centaurea chilensis* [12].

Compound **1** had a molecular ion peak in EI-mass spectrum at *m/z* 486 corresponding to a molecular formula $C_{32}H_{54}O_3$. The ^{1}H NMR spectrum (benzene-d_6) displayed six tertiary (δ 0.75, 0.79, 1.00, 1.01, 1.03 and 1.19), two secondary (δ 0.98, 1.04), one primary methyl groups (δ 1.11), two sets of proton resonances at δ 3.36 and 3.15 as a symmetrical doublet of quartets, and two carbinolic methine protons at δ 3.01 (*dd*, J = 5.5 and 10 Hz) and 3.55 (*dd*, J = 2 and 4 Hz). On acetylation, the *dd* at δ 3.01 was shifted downfield to δ 4.57 and assigned to H-3α (axial), while the *dd* at δ 3.55 (H-16) was not shifted and therefore was assigned to an oxymethine proton arising from an ethylenic linkage. The doublet of quartets at δ 3.36 (J = 7 and 9 Hz) and 3.15 (J = 7 and 9 Hz) were attributed to an oxymethylene group (-OCH_2-) which appeared at δ 64.2 in the ^{13}C NMR spectrum. This was further

Dedicated to Professor Ayhan Ulubelen (University of Istanbul, Turkey) on the occasion of her sixty-fifth birthday.

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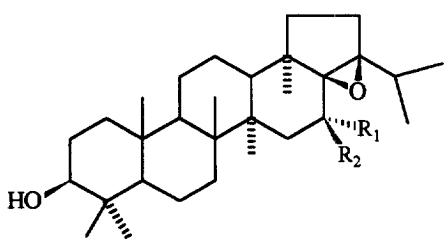
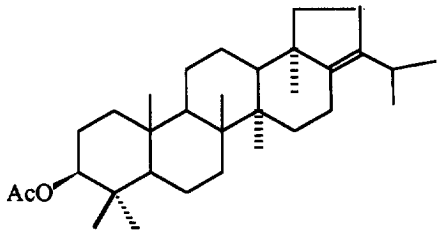


1 R_1=OCH_2CH_3 R_2=H

4 R_1=R_2=H



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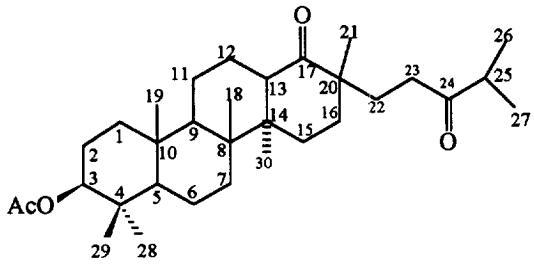

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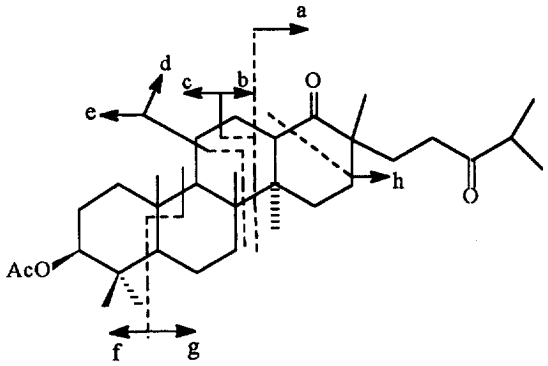

2

proved by spin-decoupling experiments. Irradiation of the *dq*s at δ 3.15 and 3.36, respectively, collapsed the methyl triplet (δ 1.11, *t*, J = 7 Hz) to a doublet with a vicinal coupling J = 7 Hz. On the contrary, irradiation of methyl resonance at δ 1.11, the *dq* at δ 3.36 (J = 7 and 9 Hz) and *dq* at δ 3.15 (J = 7 and 9 Hz) collapsed into doublets with geminal couplings J = 9 Hz, confirming the presence of an ethoxyl group in the molecule. The ^{13}C NMR spectrum of **1** was similar to that of **4**. The differences were the presence of an additional signal at δ 77.8 (-CHO-) along with the signals at δ 64.2 and 15.7 corresponding to $-OCH_2CH_3$ group in compound **1** and the lack of the resonance δ 20.1 (C-16) which was present in compound **4**. The EI mass spectrum showed the fragment ion peaks due to the cleavage of ring C at *m/z* 207 and 189 and, the peaks at *m/z* 471, 457, 441 originated from the molecular ion peak by the loss of the methyl, ethyl and ethoxyl moieties, respectively. From these fragments, it was obvious that the ethoxyl moiety could only be at rings D/E. The splitting pattern of the signal at δ 3.55 (*dd*, J = 2, 4 Hz, H-16) indicated that the ethoxyl group was between a methylene group and a quaternary carbon atom and in the equatorial α-position. A COLOC spectrum showing cross peaks between H-16 (δ 3.55) and C-14 (δ 42.5), and C-18 (δ 42.8) and C-15 (δ 34.2) indicating that the ethoxyl group was at C-16. Compound **1** was designated 17β,21β-epoxy-16α-ethoxyhopan-3β-ol.

Compound **2** was obtained as an amorphous powder, gave a positive Liebermann–Buchard test and a bright pink colouration with cerium sulphate, indicating its terpenic nature. The HREI mass spectrum showed a molecular ion peak at *m/z* 500.3845 (calcd. 500.3865) corresponding to a molecular formula $C_{32}H_{52}O_4$. The prominent fragment ions at *m/z* 482, 440 and 457 originated from the molecular ion peak by the loss of water, acetic acid and isopropyl group, respectively, and an another ion at *m/z* 425 represented the simultaneous loss of a methyl group and acetic acid. The IR spectrum gave strong absorbances of ketone (1705 cm^{-1}) and acetyl (1735 cm^{-1}) groups. The ^1H NMR spectrum displayed six tertiary (δ 0.82, 1.03, 1.01, 0.81, 0.86 and 1.00) and two secondary methyl groups (δ 1.06 and 1.07), an acetoxymethyl singlet (δ 2.04) and a carbinolic methine proton (δ 4.47, *dd*, J = 6 and 10 Hz, H-3α). A methine septet at δ 2.59 (J = 7 Hz, H-25) along with methyl doublets at δ 1.06 (J = 7 Hz) and 1.07 (J = 7 Hz) suggested the presence of an isolated isopropyl group in the molecule, this was further proved by spin-decoupling and ^1H-^1H-COSY experiments. The ^{13}C NMR and DEPT spectra revealed 32 carbon atoms consisted of nine methyl, ten methylene, five methine, and eight quaternary carbons of which were two ketones (δ 217.4 and 214.7) and one ester carbonyl (δ 171.0). The proton-bearing carbons were assigned by HETCOR and HMQC spectra and especially the methine protons (H-5, H-9, H-13 and C-25) and their respective carbons were easily deduced. All of these data and the molecular ion peak at *m/z* 500 and seven degrees of unsaturation (no olefinic carbon appeared in the ^{13}C

NMR spectrum) suggested that the presence of a tetracyclic triterpene skeleton having a side-chain with a terminal isopropyl group. Moreover, the four methine carbons at δ 55.4 (C-5), 50.7 (C-9), 44.4 (C-13), 41.0 (C-25) and five quaternary carbons at δ 38.5 (C-4), 37.0 (C-10), 41.9 (C-8), 42.0 (C-14) and 49.9 (C-20) were also indicative of a tetracyclic triterpene containing a side-chain. Compound **2** was first presumed to be a dammarane-type triterpene. However, its spectral data were not fully in agreement with the C/D rings, particularly the lack of the oxygenated quaternary carbon at C-20 which is characteristic for dammaranes. Hence, all spectral data could only be accommodated with a baccharane skeleton which was also established by comparison of its spectral properties with the data given in the literature for baccharanes [9–11]. In order to determine the location of the ketone groups, spin-decoupling and ^{1}H-^{1}H-COSY experiments were carried out. Most of the vicinal protons were analysed by ^{1}H-^{1}H COSY spectrum. The observed correlations led to the sequences of H-3 to H-1 and H-5 to H-7. Further correlations of H-9 (δ 1.29) with the signals at δ 1.57 and 1.38 led to the assignment of C-11 protons and then C-12 protons (δ 1.48 and 2.39). The location of the ketone resonating at δ 214.7 was easily deduced at C-24, by irradiation of methyl doublets at δ 1.06 and 1.07, the septet at δ 2.59 turned to a sharp singlet, and on the contrary, by irradiation of the septet, the methyl doublets collapsed into singlets. The HMBC spectrum unambiguously supported this assignment by showing correlations of C-24 to the methine at δ 2.59 and methyls at δ 1.06 and 1.07. The location of the second ketone group was deduced by the combination of ^{1}H-^{1}H COSY, EIMS and HMBC spectra. The fragment ions at *m/z* 249 (*e*) and corresponding ion due to the loss of water at *m/z* 189 which represented rings A and B, were indicative of the absence of a ketone function at rings-A/B and in addition, from the ^{1}H-^{1}H COSY spectrum it was obvious that the second ketone could not be at C-11 or C-12. Finally, the fragments at *m/z* 237 (*b*) and 223 (*a*) clearly indicated that the second ketone was on ring-D. Further information was provided by HMBC spectrum in which the observed significant cross-peaks between C-17 (δ 217.4) and the protons resonated at δ 1.68 (H-22a), 1.87 (H-22a), 1.01 (H-21) and 1.89 (H-16) confirmed that the second ketone had to be at C-17. The proposed structure was previously reported as a cleavage product of lupeol derivatives with lead acetate by Adhikary *et al.* [13]. The given IR and mass spectral data were similar to those of **2**. All the spectra data led to the conclusion that **2** was 3β-acetoxy-17,24-dioxo-baccharane.

EXPERIMENTAL

Instruments. ^{1}H NMR, 200 MHz, ^{13}C NMR 50.32 MHz, Bruker 200 AC L; Jeol JNM Ex-400 MHz spectrometer for HMBC and HMQC spectra; IR, Perkin-Elmer 1615 FT; MS, VG Zabspec GC-MS instrument; Optical rotations on Opt. Act. Ltd. AA-5 polarimeter.

Material. Centaurea ptosimopappoides Wagenitz was collected from Turkey (Karsanti-Adana) in July 1991. A voucher specimen is deposited in the Herbarium of Faculty of Pharmacy, University of Istanbul (ISTE: 43290).

Extraction and Isolation. The roots (1.5 kg) were extracted with petrol–Et_2O–EtOH (1:1:1) at room temp. for 48 hr and evapd *in vacuo* at 30–40° to dryness giving 94 g of extract. The extract was chromatographed on a silica gel column (500 g, 70–230 mesh) and eluted with petrol and gradient of petrol–Et_2O (0–100%) followed by MeOH. Nine frs of 200 ml were collected and monitored by TLC. Frs 5–8 were combined and refractioned on silica gel to afford compounds **1**–**4**. The aerial parts (1 kg) were worked up in the same way, and 22 g of crude extract gave compounds **5**–**8**. The single compounds were purified by prep. TLC. The yields were: **1** (16 mg), **2** (21 mg), **3** (28 mg), **4** (13 mg), **5** (11 mg), **6** (9 mg), **7** (9 mg), **8** (7 mg).

17β,21β-*Epoxy*-16-*ethoxy-hopan*-3β-*ol* (**1**). Mp 185–89°, $[\alpha]_D = +1.9°$ ($CHCl_3$; *c* 2.7), IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3462, 2944, 2870, 1090, 1042, 1011. ^{1}H NMR ($CDCl_3$): δ 3.20 (*dd*, J = 6, 10 Hz, H-3α), 3.27 (*dq*, J = 7, 9 Hz, H-1′a), 3.50 (*dq*, J = 7, 9 Hz, H-1′ b), 1.12 (*t*, H-2′), 3.49 (*s br*, H-16), 0.75 (*s*, Me-24), 0.81 (*s*, Me-25), 0.96 (*s*, Me-23, 26), 1.00 (*s*, Me-27), 1.26 (*s*, Me-28), 1.02 (*d*, J = 7 Hz, Me-29), 1.05 (*d*, J = 7 Hz, Me-30). ^{1}H NMR (C_6D_6): 3.02 (*dd*, J = 6, 9.5 Hz, H-3α), 3.15 (*dq*, J = 7, 9 Hz, H-1′a), 3.36 (*dq*, J = 7, 9 Hz, H-1′ b), 3.52 (*dd*, J = 2,4 Hz, H-16β), 1.00 (*s*, Me-23), 0.75 (*s*, Me-24), 0.79 (*s*, Me-25), 1.01 (*s*, Me-26), 1.03 (*s*, Me-27), 1.19 (*s*, Me-28), 0.98 (*d*, J = 7 Hz, Me-29), 1.04 (*d*, J = 7 Hz, Me-30), 1.11 (*d*, J = 7 Hz, Me-2′). EIMS *m/z*: $C_{32}H_{54}O_3$, 486 $[M]^+$ (77), 471 $[M-CH_3]^+$ (23), 457 (14), 441 $[M-OC_2H_5]^+$ (85), 425 $[M-HOAc]^+$ (17), 400 (16), 388 (100), 369 (15), 359 (11), 341 (6), 300 (8), 277 (13), 265 (10), 251 (29), 233 (10), 219 (36), 207 (46), 197 (75), 189 (44), 175 (28), 163 (37), 147 (72), 132 (60), 121 (49), 109 (40), 97 (46), 69 (42).

3β-*Acetoxy*-17,24-*dioxo-baccharane* (**2**). Mp 191–93°, $[\alpha]_D = +27.9°$ ($CHCl_3$; *c* 0.98), IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 2928, 1732, 1700, 1706, 1465, 1368, 1245. ^{1}H NMR ($CDCl_3$) δ: 0.85 (H-1a), 1.72 (H-1b), 1.04 (H-2a), 1.64 (H-2b), 4.47 (*dd*, J = 5.5 and 11 Hz, H-3α), 0.80 (H-5), 1.52 (H-6a), 1.43 (H-6b), 1.38 (H_2-7), 1.29 (H-9), 1.38 (H-11a), 1.57 (H-11b), 2.39 (H-12a), 1.48 (H-12b), 1.96 (H-13), 1.39 (H_2-15), 1.65 (H-16a), 1.89 (H-16b), 0.82 (*s*, H-18), 1.03 (*s*, H-19), 1.01 (*s*, H-21), 1.68 (H-22a), 1.87 (H-22b), 2.26 (H-23a), 2.37 (H-23b), 2.59 (*septet*, J = 7 Hz, H-25), 1.06 (*d*, J = 7 Hz, H-26), 1.07 (*d*, J = 7 Hz, H-27), 0.81 (*s*, H-28), 0.86 (*s*, H-29), 1.00 (*s*, H-30), 2.04 (*s*, OAc). HREIMS *m/z* 500.384550, $C_{32}H_{50}O_4$ (calcd 500.386561). EIMS *m/z* (rel. int): 500 $[M]^+$ (16), 482 $[M-H_2O]^+$ (43), 457 $[M-H_2O-CH_3]^+$ (7), 440, $[M-OAc]^+$ (55), 425 $[M-OAc-CH]^+$ (65), 401 $[M-C_6H_{11}O]^+$ (67), 397 (87), 384 (6), 371 (13), 358 (28), 342 (24), 327 (5), 299

Table 1. ^{13}C NMR spectral data of compounds **1**–**4** (50.32 MHz, $CDCl_3$)

	1(C_6D_6)	**2**	**3**	**4**
C-1	38.9 CH_2	37.8 CH_2	38.5	38.7
C-2	27.9 CH_2	23.6 CH_2	23.8	27.4
C-3	78.5 CH	80.7 CH	80.9	79.0
C-4	39.0* C	38.5* C	37.8	38.6
C-5	55.6 CH	55.4 CH	55.3	55.1
C-6	18.7 CH_2	18.0 CH_2	18.3	18.3
C-7	32.8 CH_2	33.1 CH_2	33.4	33.2
C-8	42.2 C	41.9 C	42.0	41.8
C-9	49.8 CH	50.7 CH	50.9	50.4
C-10	37.3* C	37.0* C	37.1	37.1
C-11	21.4 CH_2	21.2 CH_2	21.4	21.0
C-12	23.5 CH_2	23.8 CH_2	24.0	23.2
C-13	43.9 CH	44.4 CH	49.3	43.2
C-14	42.5 C	42.0 C	41.6	42.1
C-15	32.4 CH_2	30.8 CH_2	31.8	29.2
C-16	77.8 CH	33.4 CH_2	19.8	20.1
C-17	75.6 C	217.4 C	136.1	75.8
C-18	42.8 C	16.4 CH_3	49.8	43.3
C-19	36.0 CH_2	16.2 CH_3	41.6	34.5
C-20	23.3 CH_2	49.9 C	27.5	23.3
C-21	73.4 C	16.7 CH_3	139.9	74.2
C-22	28.5 CH	35.7 CH_2	26.4	28.5
C-23	28.3 CH_3	35.4 CH_2	28.0	28.0
C-24	15.7 CH_3	214.7 C	16.5	15.3
C-25	16.5 CH_3	41.0 CH	16.3	15.9
C-26	16.9 CH_3	18.3 CH_3	16.3	16.6
C-27	16.1 CH_3	18.3 CH_3	15.0	15.9
C-28	17.5 CH_3	16.5 CH_3	19.0	17.9
C-29	19.1 CH_3	27.9 CH_3	21.3	19.3
C-30	19.0 CH_3	19.9 CH_3	21.9	18.4
OAc	—	21.3	21.3	—
		171.0	—	170.1
OCH_2CH_3	64.2			
	15.7			

*Assignments may be interchanged. Multiplicities were determined by DEPT spectra.

(7), 271 (7), 261 (8), 249 (d) (11), 237 (b) (9), 229 (34), 223 (a) (14), 217 (18), 203 (35), 189 (100), 173 (39), 161 (38), 153 (h) (12), 149 (53), 135 (93), 121 (78), 107 (76), 97 (81), 85 (84), 71 (91). CIMS *m/z* (rel. int.): 501 $[M]^+$ (7), 483 (18), 469 (23), 457 (11), 441 (33), 423 (65), 409 (76), 395 (10), 381 (6), 331 (100), 313 (4), 289 (14), 271 (23), 255 (16), 229 (19), 213 (64), 195 (60), 183 (44), 169 (67), 153 (36), 137 (18), 123 (19), 109 (24), 95 (38), 85 (75), 71 (68).

3β-Acetoxyhop-17(21)-ene (**3**). Mp 261–63°, $[\alpha]_D = +50.1°$ ($CHCl_3$; *c* 1.10), IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 2945, 1726, 1442, 1374, 1249. ^1H NMR ($CDCl_3$): δ 4.48 (*dd*, $J = 5.5$ and 10 Hz, H-3α), 2.64 (*septet*, $J = 7$ Hz, H-22), 0.88 (*s*, Me-23), 0.82 (*s*, Me-24), 0.83 (*s*, Me-25,28), 0.90 (*s*, Me-26), 1.02 (*s*, Me-27), 0.92 (*d*, $J = 6.9$ Hz, Me-29), 0.99 (*d*, $J = 6.9$ Hz, Me-30). CIMS *m/z*: $C_{32}H_{52}O_2$, 467 $[M-1]^+$, 441, 425 $[M-43]^+$, 409 $[M-OAc]^+$, 395, 365, 217, 205, 191, 149, 121, 109, 95, 79.

17β,21β-Epoxyhopan-3β-ol (**4**): Mp. 273–74°, $[\alpha]_D = +19.4°$ ($CHCl_3$; *c* 0.99), IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3500, 2944, 2870, 1045, 1011. ^1H NMR ($CDCl_3$): δ 3.20 (*dd*, $J = 5.5$ and 10 Hz, H-3α), 0.97 (*s*, Me-23), 0.76 (*s*, Me-24), 0.82 (*s*, Me-25, 28), 1.02 (*s*, Me-26), 1.04 (*s*, Me-27), 0.94 (*d*, $J = 7$ Hz, Me-29), 1.06 (*d*, $J = 7$ Hz, Me-30). CIMS *m/z*: $C_{30}H_{50}O_2$, 442 $[M]^+$, 425 $[M-OH]^+$, 424 $[M-H_2O]^+$, 407, 291, 279, 257, 221, 205, 177, 157, 137, 119, 95, 81.

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