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NEW KAURANE DITERPENOIDS FROM THE AERIAL PARTS OF *DISTICHOSELINUM TENUIFOLIUM*

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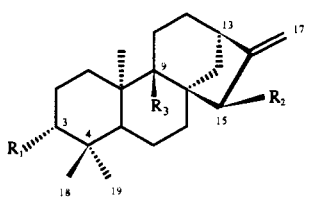
ABSTRACT.—The neutral components of the hexane extract from aerial parts of *Distichoselinum tenuifolium* (= *Elaeoselinum tenuifolium*) were identified as *ent*-kaurene, *ent*-kauren-3β-ol, *ent*-15α-angeloyloxykaur-16-en-3β-ol [**3**], the main secondary metabolite previously isolated from the roots of this plant, and two new natural kaurene derivatives, *ent*-15-α-angeloyloxykaur-16-ene-3β,9-diol [**4**] and *ent*-kaur-16-ene-3β,15α-diol [**5**].

As a continuation of our phytochemical studies on plants endemic to Comunidad Valenciana (East Spain), we have examined the chemical constituents of the aerial parts of *Distichoselinum tenuifolium* (Lag.) García Martin & Silvestre (Umbelliferae), also known as *Thapsia tenuifolia* Lag., *Elaeoselinum lagascae* Boiss., *Elaeoselinum tenuifolium* (Lag.) Lange, and *Laserpitium tenuifolium* (Lag.) Calest. (1,2). The name *Distichoselinum tenuifolium*, proposed recently for the plant under study, has been assigned on the basis of taxonomic characters (1) and is also supported by phytochemical studies: the essential oils and terpenoids from *D. tenuifolium* and other *Elaeoselinum* species are different (3–13).

In our first study on the components from the roots of the same plant (then named *E. tenuifolium*), we isolated the new diterpene alcohol **3** and its 16β-epoxy derivative (3). In this paper we now report the isolation of *ent*-kaurene, *ent*-kauren-3β-ol, *ent*-15α-angeloyloxykauren-3β-ol and the new diterpenoids *ent*-15α-angeloyloxykaur-16-ene-3β,9-diol [**4**] and *ent*-kaur-16-ene-3β,15α-diol [**5**]. This last substance had been described as the hydrolysis product from **3** (3).

The hexane extract from the aerial parts of *D. tenuifolium* (2%, dry wt) in Et_2O was extracted with 4% aqueous NaOH. The neutral fraction (96%) was composed of waxes and other straight-chain components as well as five kaurane derivatives, the known kaurene **1** (14), 3β-kaurenol [**2**] (15), *ent*-15α-angeloyloxykaur-16-en-3β-ol [**3**] (3), and the new natural alcohols **4** and **5**.

Compound **4**, $C_{25}H_{38}O_4$ ($[M]^+$ *m/z* 402), showed ir absorption bands for a hydroxyl group (3560, 3100 br cm^{-1}), a conjugated ester (1710 cm^{-1}), and a methylidene group (3060, 1655, 870 cm^{-1}). The ^1H-nmr spectrum was rather similar to that of **3** previously isolated from the same plant (3): both substances showed nearly the same type of signals slightly shifted. The kaurane skeleton assigned to **4** was confirmed by the signal of the allylic methine proton at δ = 2.71 ppm, characteristic of



1 $R_1=R_2=R_3=H$
2 $R_1=OH$, $R_2=R_3=H$
3 $R_1=OH$, $R_2=OAng$, $R_3=H$
4 $R_1=R_3=OH$, $R_2=OAng$
5 $R_1=R_2=OH$, $R_3=H$
6 $R_1=OAc$, $R_2=OAng$, $R_3=OH$

kaurane and phyllocladane skeletons (9). The allylic proton H-12 in atisanes generally resonates at ca. δ 2.3 ppm.

From the molecular formula and the preceding spectral data we concluded that **4** would have to have the same skeleton as **3** but with an additional hydroxyl group. This OH group must be tertiary because the ^{1}H-nmr spectrum does not show new signals corresponding to protons geminal to oxygen. The ^{13}C-nmr spectrum also showed a singlet carbon signal at δ 78.9 ppm in agreement with the expected shift for a carbinol carbon. The presence of the tertiary hydroxyl group was confirmed by acetylation of **4**, which gave a monoacetylated substance **6** with one free hydroxyl group not esterified (ir 3580 cm^{-1}).

The tertiary hydroxyl group in a kaurane skeleton can be placed on C-5, C-13, or C-9, but the C-5 and C-13 positions were ruled out on the basis of ^{1}H- and ^{13}C-nmr spectra: the nmr signals assigned to the A and D ring atoms of **3** and **4** are nearly identical.

The ^{13}C chemical shifts of **4** also confirm the proposed structure and were assigned from their multiplicities (DEPT experiments), by comparison with literature spectra, and by 2D δH/δC correlations. The δ values were also calculated by adding the increments due to introduction of an -OH group at C-9 (16,17) to the δ values of compound **3** (3). The observed and calculated δ values for the carbons of rings A and B match within 1 ppm, but for atoms C-9, C-15, C-12, and C-14, deviations of −8, −8, +3, and −3 ppm, respectively, were observed. These deviations could be explained if the C-9 hydroxyl group were associated by a hydrogen bond with the oxygen of the ester group at C-15, which would induce a conformational change in the molecule. Deviations between the predicted and observed δ values for some of the carbon atoms of ring B and D in *ent*-7α,15α-dihydroxykaurenoic acid have been also attributed to a change in the conformation of these rings (17). These conformational changes can also explain the deshielding of H-14β, observed as a doublet at 2.35 ppm, with $J = 12.7$ Hz (H-14, H-13 angle, ca. 90°).

Compound **5** was isolated from the most polar fractions. The mp, optical rotation, and spectral data of this compound were identical to those of the diol obtained from **3** by alkaline hydrolysis (3). As far as we know, compounds **4** and **5** have been isolated as new natural products for the first time.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Spectra were recorded with the following instruments: ir, Pye Unicam SP 2000; nmr, Bruker WP 200 (^{1}H, 200 MHz; ^{13}C, 50.3 MHz) and Varian EM 360L (60 MHz) recorded in $CDCl_3$ with TMS as internal standard (scale δ in ppm); ei mass spectra, VG TS-250 with direct inlet probe at 70 eV; ci mass spectra, HP-5988A (CH_4/0.6 torr); optical activity, AA-100 polarimeter.

EXTRACTION AND ISOLATION.—The plant was collected at Cabo de las Huertas, Alicante, Spain, and voucher specimens were deposited in the herbarium of the Department of Botany, University of Salamanca (SALA 49218) and in the herbarium of the Faculty of Pharmacy, University of Sevilla (SEVF). The air-dried aerial parts of *D. tenuifolium* (7.53 kg) were extracted with hexane in a Soxhlet apparatus (250 g, 5 h each). The hexane extract (151.8 g, 2% weight of dried plant) in Et_2O was extracted with 4% NaOH and divided into neutral (140 g, 96%) and acid 5.4 g, 4%) fractions.

The neutral fraction was chromatographed on Si gel (Merck art. 7733, 500 g) in a column packed with hexane using hexane-EtOAc (95:5) as eluent, and twelve fractions of 250 ml each were collected. Fractions 4 and 5 (13 g) contained compounds **3** (*ent*-15α-angeloyloxykaur-16-en-3β-ol) and **2** (3β-kaurenol), contaminated by straight-chain ketones, fatty esters, and β-sitosterol. The crystallization of both fractions in hexane afforded crude **3** (11.2 g), and the mother liquor (1.66 g) was subjected to chromatography by cc on Si gel (112 g) with hexane-EtOAc (95:5). Compounds **3** (0.6 g) and **2** (0.3 g) were isolated and purified by treatment with urea to eliminate the straight-chain inclusion complexes (clathrates) followed by crystallization in hexane. Compound **4** (*ent*-15α-angeloyloxykaur-16-ene-

3β,9-diol) (0.9 g), the main component of the original fraction 7, was isolated by cc on Si gel and purified by crystallization in hexane. Compound **1** (kaurene) (50 mg) was isolated from the less polar fractions, 1 to 3, and *ent*-kaur-16-ene-3β,15α-diol [**5**] (0.3 g) was also isolated by cc on Si gel with hexane-EtOAc (8:2).

ent-15α-Angeloyloxykaur-16-ene-3β,9-diol [**4**].—R_f 0.32 [hexane-EtOAc (7:3)]; mp 126° (hexane); $[\alpha]_D$ −27.2° ($CHCl_3$, c = 1.8); ir max (KBr) 3560, 3100 br, 3060, 2920, 2850, 1710, 1655, 1635, 1445, 1360, 1280, 1215, 1175, 1125, 975, 930, 870, 830, 800, 775, 740 cm^{-1}; ^1H nmr (200 MHz) δ 6.16 (1H, qq, J = 7.3 and 1.5 Hz, H-3′), 5.45 (1H, t, J = 2.4 Hz, H-15), 4.96 (1H, dd, J = 3.4 and 1.46 Hz, H-17a), 4.83 (1H, dd, J = 2.4 and 1.46 Hz, H-17b), 3.26 (1H, dd, J = 5.9 and 10.3 Hz, H-3), 2.71 (1H, m, H-13), 2.32 (1H, d, J = 12.7 Hz, H-14β), 2.03 (3H, dq, J = 6.8 and 1.5 Hz, H-4′), 1.94 (3H, quint, J = 1.5 Hz, H-5′), 1.12 (3H, s, H-20), 0.99 (3H, s, H-18), 0.77 (3H, s, H-19); ^{13}C nmr (assignments with asterisks may be reversed) δ 168.4 (s, C-1′), 152.2 (s, C-16), 139.9 (d, C-3′), 126.8 (s, C-2′), 105.4 (t, C-17), 84.2 (d, C-15), 78.9 (s, C-9), 78.4 (d, C-3), 50.0 (s, C-8), 46.2 (d, C-5), 44.4 (s, C-10), 39.3 (d, C-13), 38.7 (s, C-4), 36.5 (t, C-14), 35.5 (t, C-12), 34.7 (t, C-1), 30.8* (t, C-11), 29.7* (t, C-7), 28.4 (q, C-18), 27.3 (t, C-2), 20.7 (q, C-5′), 19.8 (t, C-6), 19.3 (q, C-20), 15.9 (q, C-4′), 15.6 (q, C-19); cims (CH_4) *m/z* (%) $[M]^+$ 402 (1), $[M-H_2O]^+$ 384 (5), $[384-Me-H]^+$ 368 (17) $[MH-AngOH]^+$ 303 (17), $[MH-H_2O-AngOH]^+$ 285 (100), $[285-H_2O]^+$ 267 (27); eims *m/z* (%) 384 (3), 368 (2), 302 (3), 301 (6), 284 (19), 269 (3), 241 (10), 229 (5), 215 (6), 199 (2), 185 (3), 161 (21), 148 (17), 135 (16), 123 (45), 107 (18), 93 (24), $[Ang]^+$ 83 (68), 69 (32), $[Ang-CO]^+$ 55 (100).

Acetate **6**.—The diol **4** (200 mg) in pyridine (1 ml) and Ac_2O (2 ml) was left overnight at room temperature. After usual workup, acetate **6** was isolated: mp 112–114°; $[\alpha]_D$ −58.4° ($CHCl_3$, c = 1.7); ir max (KBr) 3580, 2940, 2860, 1715, 1670, 1440, 1365, 1250, 1220, 1135, 990, 950, 875, 835, 780, 760 cm^{-1}; ^1H nmr (60 MHz) δ 6.15 (1H, br q, J = 6.5 Hz, H-3′), 5.40 (1H, t, J = 2.5 Hz, H-15), 4.95 (1H, br d, J = 2.5 Hz, H-17a), 4.85 (1H, m, $W_{1/2}$ = 5 Hz, H-17b), 4.50 (1H, t, J = 7 Hz, H-3), 2.70 (1H, m, H-13), 2.35 (1H, d, J = 12 Hz, H-14β), 2.05 (3H, s, MeCO), 2.05 (3H, br d, J = 6.5 Hz, H-4′), 2.00 (3H, br s, H-5′), 1.15 (3H, s, H-20), 0.85 (6H, s, H-18 and H-19).

ent-Kaur-16-ene-3β,15α-diol [**5**].—R_f 0.17, mp 167–168° (C_6H_6); $[\alpha]_D$ 61° ($CHCl_3$, c = 1.4); ir max (KBr) 3430, 3350, 3060, 2960, 2920, 2830, 1660, 1440, 1280, 1180, 1080, 1060, 1025, 990, 880, 865 cm^{-1}; ^1H nmr (200 MHz) δ 0.78 (3H, s, H-19), 0.98 (3H, s, H-18), 1.03 (3H, s, H-20), 2.70 (1H, m, H-13), 3.21 (1H, dd, J = 9 and 7 Hz, H-3), 3.75 (1H, t, J = 2.4 Hz, H-15), 4.95 (1H, d, J = 2.8 Hz, H-17a), 5.09 (1H, m, $W_{1/2}$ = 5 Hz, H-17b); ^{13}C nmr (assignments with the same superscript may be reversed) δ 158.6 (s, C-16), 104.8 (t, C-17), 82.4 (d, C-15), 79.0 (d, C-3), 54.6 (d, C-5), 46.5 (d, C-9), 45.7 (s, C-8), 40.2 (d, C-13), 38.9 (s, C-10*), 38.9 (t, C-14**), 38.8 (t, C-1**), 38.8 (s, C-4*), 36.5 (t, C-7), 33.3 (t, C-12), 28.4 (q, C-18), 27.5 (t, C-2), 19.7 (t, C-6), 18.2 (t, C-11), 16.7 (q, C-20), 15.5 (q, C-19); eims *m/z* (%) $[M]^+$ 304 (35), $[M-Me]^+$ 289 (22), $[M-H_2O]^+$ 286 (33), $[286-Me]^+$ 271 (58), $[271-H_2O]^+$ 253 (20), 246 (55), 203 (28), 173 (18), 164 (16), 147 (33), 121 (45), 107 (55), 91 (63), 84 (70), 83 (20), 81 (57), 67 (50), 55 (70), 43 (57), 41 (100).

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ERRATUM

For the paper by Lin and Cordell entitled "^{13}C-nmr Assignments of Camptothecine and 10-Hydroxycamptothecine," *JNP* 53, 186 (1990), the authors have requested the following correction: The chemical shift of H-11 in camptothecine should be 7.87 ppm and not 7.31 ppm as indicated in the paper. The authors apologize for any inconvenience caused.