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SESQUITERPENE LACTONES, WAXES AND VOLATILE COMPOUNDS FROM *ARTEMISIA HERBA-ALBA* SUBSPECIES *VALENTINA*

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Key Word Index—*Artemisia herba-alba* subsp. *valentina*; Compositae; structure elucidation: sesquiterpene lactones; eudesmanolides; essential oils; terpene hydrocarbons; waxes; dihydroreynosin; torrentin.

Abstract—The structures of two sesquiterpene lactones isolated from *Artemisia herba-alba* subsp. *valentina* have been determined by spectroscopic methods. One of these was dihydroreynosin, the other was a new compound assigned the name torrentin. The chemical compositions of a wax and a hydrocarbon fraction from the essential oil have also been investigated.

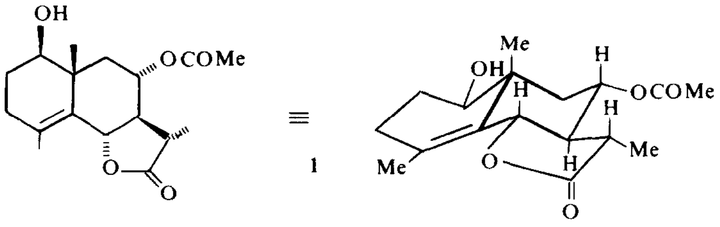
INTRODUCTION

Artemisia herba-alba subsp. valentina Asso, is a dwarf shrub, endemic in Valencia country and often used in folk medicine. It was classified by Dr. Mansanet, Prof. of Botany at Valencia University. Previous work on *A. herba-alba* has been published. Khafagy *et al.* [1] isolated α-santonin from the flowering branches. Segal *et al.* [2, 3] reported the isolation and structural elucidation of three new sesquiterpene lactones with germacranolide structure, named herbolides A, B and C, and of a new flavonoid. Also some components of the essential oil have been investigated [4]. We now wish to report the isolation and structure elucidation of two sesquiterpene lactones with a eudesmanolide structure. One was found to be a new compound, named torrentin by us, to which structure 1 has been assigned. The other one was identified with dihydroreynosin 2, previously described by Pathak *et al.* [5] as a product obtainable from dihydrocostunolide and also isolated by Ogurva *et al.* [6] from *Michelia compressa*. We have also studied the chemical composition of a wax and a hydrocarbon fraction from the essential oil with the aid of GC–MS.

RESULTS AND DISCUSSION

Cooling of a petrol extract of *A. herba-alba* subsp. *valentina* and filtration yielded a waxy product. Steam distillation of the filtrate yielded an oil. IR examination of the wax showed it to contain aliphatic long-chain acids, alcohols, esters and probably hydrocarbons. Alkaline extraction of this wax enabled the separation of the acids; column chromatography of the neutral part permitted the separation of the other types of compounds. The hydrocarbon fraction was directly analysed by GLC; the alcohols and acids were first transformed into their acetates and methyl esters, respectively. The esters were saponified and the acidic and alcoholic fractions analysed as above by GLC with reference compounds [7]. The following results were obtained: saturated straight-chain hydrocarbons (homologue, rel. amount %), C_{27} 7.7, C_{29} 67.7, C_{31} 24.2; free 1-alkanols, C_{16} 24.7, C_{18} 1.3, C_{20} 10.3, C_{21} 1.7, C_{22} 32.9, C_{23} 3.1, C_{24} 23.0, C_{26} 3.0; free saturated acids, C_{22} 1.4, C_{24} 5.0, C_{25} 1.1, C_{26} 8.6, C_{27} 4.8, C_{28} 35.2, C_{29} 9.6, C_{30} 26.5, C_{31} 2.3, C_{32} 5.0; bound acids (from esters), C_{17} 3.0, C_{18} 13.2, C_{19} 31.4, C_{20} 34.9, C_{21} 12.5, C_{22} 4.6 $>$ C_{23} less than 1%; bound alcohols (from esters), C_{16} 4.2, C_{18} 3.0, C_{19} 1.7, C_{20} 5.7, C_{21} 2.2, C_{22} 28.0, C_{23} 3.3, C_{24} 50.2, C_{26} 1.5.

The volatile oil was chromatographed on Si gel; elution with hexane gave hydrocarbons, elution with Et_2O gave oxygenated compounds. Only the former were studied by GC–MS and the following substances identified: *p*-cymene, 1-methyl-4-isopropenylbenzene, α-copaene, aromadendrene, α-guaiene, β-bisabolene, γ-



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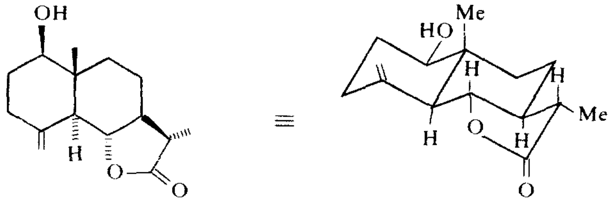

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cadinene, calamenene, calacorene, cadalene, *n*-heptadecane, *n*-octadecane, *n*-eicosane, α-curcumene.

An ethanol extract of this plant yielded, on column chromatography, three crystalline products, A, B and C. The first eluted, compound A, mp 169–70°, $[\alpha]_D^{20}$ −173° (MeOH), was easily identified as α-santonin by comparison with an authentic specimen. Compound B, mp 150–1°, was obtained in very low yield. Structure **1** was assigned on the following spectral evidence: the IR spectrum showed bands for γ-lactone (1778 cm^{-1}), hydroxyl (3450, 1030 cm^{-1}) and ester functions (1710, 1250 cm^{-1}), and also for double bonds (1662 cm^{-1}). The MS gave the molecular weight, M = 308, which, together with the IR findings, suggested the molecular formula $C_{17}H_{24}O_5$. Isotopic peaks confirmed this formula. No known compounds have been described [8] with these properties. The presence of hydroxyl and acetoxyl groups was evidenced by the peaks at M − 18, M − 42 and M − 60.

The ^{1}H NMR spectrum confirmed the eudesmanolide structure; it showed a three-proton singlet at δ 1.20 (C-10 Me), a slightly broad three-proton singlet at 1.82 (C-4 Me, homoallylically coupled with H-6) and a three-proton doublet at 1.24 (J = 8 Hz), assigned to the C-11 Me group. The signal of the acetoxyl Me group was easy recognizable as a sharp three-proton singlet at 2.10. At lower fields, signals for three H atoms were observed and assigned to protons α to an oxygen function: a double doublet (J = 12.5, 4.5 Hz) at 3.60 from H-1 (α to an OH group), a broad doublet (J = 9 Hz) at 4.65 from H-6 (α to lactone oxygen) and a broad triplet (J = 8.5 Hz) at 5.39 from H-8 (α to an acetoxyl group). The coupling constants measurable in the signals of H-1, H-6 and H-8 showed their axial position and hence the equatorial nature of the hydroxyl and acetoxyl groups. The axial–axial relationship of H-6 and H-7 showed also the *trans*-junction between the lactone and cyclohexane rings. In order to elucidate the stereochemistry at C-11, we have made use of the method of Narayanan *et al.* [9]. By comparison of the chemical shifts of the C-11 Me groups in $CDCl_3$ and C_6D_6 solutions, one should be able to infer the stereochemistry at C-11, provided that the stereochemistry of the ring-junction is known. In the case of lactones with the 6βH-7αH configuration, an upfield shift of about 0.20–0.30 ppm in the signals of α-Me groups is observed by changing $CDCl_3$ for C_6D_6 as solvent, while substantially greater shifts are observed when the C-11 Me group is β-oriented. The ^{1}H NMR spectrum of compound B in C_6D_6 solution showed for C-11 Me a doublet at δ 0.97 ($\Delta^{C_6D_6}_{CDCl_3}$ = 0.27 ppm), thus confirming the α-orientation. In view of this spectral data, we propose for this new compound the name torrentin and the structure **1**.

Compound C was obtained as needles, mp 139–40°, $[\alpha]_D^{20}$ + 146° (MeOH) and shown to be dihydroreynosin by the following spectral information: the IR spectrum showed bands for a γ-lactone (1775 cm^{-1}) and a hydroxyl group (3450, 1040 cm^{-1}) and also a methylene unit (1665, 900 cm^{-1}). The main MS feature was a molecular peak at m/e 250, which, together with isotopic peaks and IR findings, suggested the molecular formula $C_{15}H_{22}O_3$. Peaks at m/e 232 (M − H_2O), indicative of a hydroxyl group, and at m/e 217 (M − H_2O − Me) were also visible. The ^{1}H NMR spectrum showed a three-proton singlet at δ 0.8 (C-10 Me) and a three-proton doublet (J = 8 Hz) at 1.22 (C-11 Me). At lower fields, signals for four protons were observed and assigned to two olefinic protons and two protons α to an oxygen function: a double doublet (J = 11.5, 5.5 Hz) at 3.50 from an axial proton at C-1 (α to an OH group), a sharp triplet (J = 10.5 Hz) at 4.27 from H-6 (α to lactone oxygen), with an axial–axial relationship to protons at H-5 and H-7. Finally, two broad singlets at 4.84 and 4.96 were assigned to the two methylene protons at C-15. Comparison of these spectra with those of reynosin and santamarin [8,10] showed that they belong to dihydroreynosin, first described by Pathak *et al.* [5], not as a natural product but as an intermediate in a series of transformation products from dihydrocostunolide. Recently Ogura *et al.* [6] have also isolated dihydroreynosin from *Michelia compressa*. Structure **2** was hence assigned to compound C.

EXPERIMENTAL

IR spectra were recorded in KBr discs and ^{1}H NMR spectra (90 MHz) in $CDCl_3$ soln with TMS as internal standard. GC–MS analysis was performed at 70 eV. MS of the sesquiterpene lactones were obtained using a direct inlet system. Optical rotations were determined in MeOH soln. Mps (uncorr.) were recorded on a Reichert apparatus. CC was performed on Si gel (0.063–0.200 mm).

Extraction. Air-dried stems, leaves and flowers (4 kg) of *A. herba-alba*, subsp. *valentina* were Soxhlet-extracted with petrol (bp 50–70°) and then with EtOH. By concentrating the petrol extract and cooling to room temp., a crude wax was pptd. By repeated crystallization from EtOH, a white product (9.3 g) was obtained. This was dissolved in Et_2O and exhaustively extracted with 10% aq. NaOH. The aq. layer was acidified and re-extracted with Et_2O. Carboxylic acids (2.98 g) were obtained The non acidic fraction was chromatographed on neutral Al_2O_3 and eluted successively with hexane (hydrocarbons, 2.15 g), hexane–Et_2O, 10:1 (esters, 2.58 g) and hexane–Et_2O, 1:1 (alcohols, 1.57 g). All these compounds were then analysed as described in the text. After precipitation of the wax, the petrol extract was steam-distilled, the aq. layer extracted several times with Et_2O and the solvent removed *in vacuo*. A yellowish oil (10 g) was obtained. This oil was chromatographed on Si gel and eluted successively with hexane and Et_2O. Hexane elution gave hydrocarbon compounds, elution with Et_2O gave oxygenated compounds. Analysis of the hydrocarbon fraction by GC–MS enabled the identification of the compounds described in the results.

The EtOH extract was concd *in vacuo*, warm H_2O was then added and the resulting soln filtered and continuously extracted with Et_2O. The solvent was then removed and the extract (20 g) chromatatographed on Si gel with hexane–Et_2O (2:1) to obtain sesquiterpene lactones. Three compounds were successively eluted: α-santonin, mp 169–70°, $[\alpha]_D^{20} - 173°$ (*c*, 0.2; MeOH), identified by comparison with an authentic sample. *Torrentin*, (0.020 g), mp 150–1° (from hexane–Et_2O, 1:1); IR ν_{max} cm^{-1}: 3450, 1030 (OH), 1778 (γ-lactone), 1710 (ester), 1662 (C=C), 1378, 1250, 1240, 1175, 985; MS (probe), *m/e* (rel. int.): 308 M^+ (2), 290 (M^+ − 18, 1), 266 (M^+ − 42, 3), 248 (M^+ − 60, 100), 233 (M^+ − 60 − 15, 18), 230 (M^+ − 60 − 18, 10). *Dihydroreynosin* (0.050 g), mp 139–40° (from hexane–Et_2O, 1:1), $[\alpha]_D^{20} + 146°$ (*c*, 0.4; MeOH); IR ν_{max} cm^{-1}: 3450, 1040 (OH), 1775 (γ-lactone), 1655, 900 ($C=CH_2$), 1380, 1215, 998, 960, 730; MS (probe), *m/e* (rel. int.): 250 M^+ (12), 232 (M^+ − 18, 100), 217 (M^+ − 18 − 15, 8).

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