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A SESQUITERPENE δ-LACTONE FROM *ZINNIA JUNIPERIFOLIA**

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Key Word Index—*Zinnia juniperifolia*; Compositae; Zinninae; sesquiterpene lactones; elemenolides; δ-lactones.

Abstract—Juniperin, a constituent of *Zinnia juniperifolia*, is a sesquiterpene δ-lactone with an elemane skeleton. Its structure and stereochemistry are based on chemical and spectroscopic studies.

INTRODUCTION

Chemical investigations of the genus *Zinnia* (tribe Heliantheae) have resulted in the detection of sesquiterpene lactones, mainly of the elemenolide type [1–3]. In the present paper we report the isolation and structure determination of the novel δ-elemenolide juniperin from *Z. juniperifolia* (D.C.) A. Gray.

RESULTS AND DISCUSSION

Juniperin (**1**), $C_{24}H_{30}O_9$, mp 118–119°, $[\alpha]_D + 32.66°$, showed UV absorption at λ_{max} 219 nm ($\epsilon = 20068$). The IR spectrum exhibited bands at 3450 cm^{-1} (hydroxyl), 1735, 1720 and 1690 (esters, lactone and aldehyde, respectively) and 1655, 1650 and 1645 cm^{-1} (double bonds). In the ^1H NMR spectrum were observed two doublets of quartets at 1.82 (3H) and 1.92 ppm (3H), which together with the signal at 6.17 ppm (1H) are typical for protons of an angelate ester. Two equivalent methyl groups attached to a carbon atom bearing a single bonded oxygen group appeared as a singlet at 1.56 ppm; a singlet at 3 ppm (1H) disappeared on equilibration with D_2O indicating the presence of a hydroxyl group. Since it is the only hydroxyl group in the molecule, it was inferred that together with the two equivalent methyl groups it is part of an α-hydroxyisobutyrate. This assumption was corroborated by mass spectral peaks at *m/z* 358 ($M^+ -$ α-hydroxyisobutyrate), 83 (100%, C_5H_7O), 363 ($M^+ -$ ang) and 86 (C_4H_5O). An α,β-unsaturated aldehyde was indicated by a broad singlet at 9.35 ppm and two singlets at 6.25 (1H) and 6.71 ppm (1H) for the geminal vinylic protons of the conjugated system. Moreover, at 3.08 ppm (1H) appeared a doublet of doublets and a multiplet at 2.5 ppm (2H), the two signals corresponding to the protons of a monosubstituted epoxide. The presence of these two groups strongly supports an elemene skeleton for juniperin.

The remaining signals could also be clearly assigned; a doublet at 3.43 ppm ($J = 3$ Hz, 1H) corresponded to H-5 and a partially superimposed multiplet at 3.32 (1H) to H-7. The low field region had two signals (1H each) at 5.94 and 6.76 ppm of the lactonic exocyclic methylene protons. These signals disappeared on chemical reduction (see below). The signals for the protons on the carbon atoms bearing the ester and the lactone groups appeared as a doublet of doublets at 5.47 ($J = 3.5$ and 2.2 Hz), 4.96 ($J = 4$ and 3 Hz) and 4.62 ($J = 2$ and 2.2 Hz), the signal at highest field being attributed to the lactonic proton. The IR band [1720 cm^{-1}_{film} 1715 cm^{-1} (KBr, $CHCl_3$)] indicated a δ-lactone ring and not the common γ-lactone, present in almost all the known sesquiterpene lactones, thus suggesting lactonization to C-9. The assignments of H-6 and H-8 were done by spin-spin decoupling experiments: irradiation at 3.32 ppm (H-7 and H-5 partially superimposed) collapsed the doublet doublets at 5.47 and 4.62 (H-9) to doublets ($J = 2.2$ Hz) and the signal at 4.96 to a broad singlet. When the H-7 and H-5 frequencies were simultaneously irradiated this last signal changed into a singlet; therefore this signal must be attributed to H-6 and the one at 5.47 to H-8. The multiplicity of H-9 must be due to the interaction with H-8 and long-range coupling (M or W) with H-7 ($J = 2$Hz) as was demonstrated in the preceding irradiation experiment. Furthermore, on irradiation of the H-8 signal the H-9 signal was transformed into a doublet ($J = 2$ Hz, 4 σ coupling).

As an elemenolide, juniperin must have an H-7-α, H-5-α and C-10-β-methyl group. Since $J_{7,9} = 2$ Hz, H-9 must be α and both protons equatorial, which is a necessary condition for the 4σ interaction [4]. The assignment of the stereochemistry for H-6 and H-8 was based on the observed coupling constants.

When juniperin was reduced with $NaBH_4$ the IR spectrum of the major product (**2**) had bands for hydroxyl (3450 cm^{-1}) and a saturated δ-lactone (1730 cm^{-1}). In the ^1H NMR spectrum the signals for the aldehydic proton and the α-hydroxyisobutyrate ester did not appear. The vinylic protons 3 and 3′ gave a singlet at 5.25 ppm. At 4.05 appeared an AB system which corresponds to the 15 and 15′ protons. H-6 shifted to a higher field (3.96 ppm; *dd*, $J = 3$ and 4 Hz). These facts established that the α-hydroxyisobutyrate must be attached to C-6. The new doublet

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(1.44 ppm, $J = 7$ Hz, 3H) indicated reduction of the exocyclic methylene of the lactone.

Oxidation of the diol (**2**) yielded the derivative (**3**) whose ^{1}H NMR spectrum had two doublets at 5.32 (H-3) and 5.48 ppm (H-3′) ($J = 1$ Hz) and a singlet at 5.58 ppm for the hemiacetal proton H-15. The IR spectrum showed hydroxyl absorption at 3440 cm^{-1}. More vigorous oxidation conditions transformed the lactol (**3**) into the dilactone (**4**). This product had IR absorption for an α,β-unsaturated γ-lactone (1780 cm^{-1}). δ-lactone (1730 cm^{-1}) and ester (1940 cm^{-1}). The ^{1}H NMR spectrum showed two singlets at 6.37 and 5.86 ppm for the exocyclic methylene protons conjugated with the newly formed γ-lactone ring. The H-6 signal was shifted downfield appearing at 4.48 ppm (*dd*, $J = 3$ and 6 Hz, 1 H). These reaction products, established unequivocally the structure and stereochemistry (except for C-1) of juniperin, the first natural δ-lactone with an elemane skeleton.

EXPERIMENTAL

Mps are uncorr. Optical rotations were measured in $CHCl_3$ soln. IR spectra as film. ^{1}H NMR (100 MHz) in $CDCl_3$ with TMS as internal reference; chemicals shifts are in ppm. Mass spectrum (70 eV) values are in *m/z* units (% relative abundance). TLC and column chromatography separations were performed in Si gel 60-F 254 and Si gel 40 Merck.

Extraction and isolation. Z. juniperifolia was collected 50 km south of Saltillo (Coahuila, Mexico) in Sept. 1978 (MEXU-284793 on deposit in the Herbarium of the Instituto de Biología, U.N.A.M.). The dried plant (8.2 kg) was defatted with hexane. Further extraction with $CHCl_3$ gave 141 g of a syrup. Its fractionation by column chromatography (C_6H_6–EtOAc, 7:3) yielded 8.6 g of **1**.

Juniperin (**1**), mp 118–20° (Me_2CO–isopropyl ether). (Found: C, 62.12; H, 6.53; O, 31.23. $C_{24}H_{30}O_9$ requires: C, 62.32; H, 6.54; O, 31.41%).

Reduction of **1**. $NaBH_4$ (1 g) was added to a cold (0°) soln of **1** (443 mg) in MeOH (10 ml). After completion of the addition, the reaction mixture was left for 10 min and then acidified (HOAc) and worked up as usual. The mixture (two products) was separated by (×3) by prep. Si gel (EtOAc–hexane, 7:3 aq) giving the diol **2** (240 mg) and another unidentified product (20 mg).

Diol (**2**); mp 159–161° (Me_2CO–isopropyl ether). (Found: C, 62.89; H, 7.37; O, 29.59. $C_{20}H_{28}O_7$ requires: C, 63.14; H, 7.42; O, 29.44%.) UV λ_{max} nm: 221: ($\epsilon = 9193$); IR ν_{max} cm^{-1}: 3450, 1730, 1715, 1650, ^{1}H NMR: δ 2.62 (*d*, $J = 4$ Hz, 2H), H-2 and H-2′), 5.25 (*s*, 2H, H-3 and H-3′), 3.96, (*dd*, $J = 3$, $J = 4$ Hz, 1H, H-6), 5.74 (*dd*, $J = 3.6$, $J = 2$ Hz, 1H, H-8), 4.53 (*dd*, $J = 2$, $J = 2.5$ Hz, 1H, H-9), 1.14 (*s*, 3H, C-10-Me), 1.44 (*d*, $J = 7$ Hz, 3H, C-11-Me), 4.05 (*AB*, 2 H, H-15 and H-15′, 1.87 (*br d*, $J = 1.5$ Hz, 3H), 1.98 (*br d*, $J = 7$ Hz, 3H and 6.14 (*br q*, $J = 7$ Hz, 1H) angelate ester. MS: *m/z* 380 (M^+), 362 (M − 18), 281 (M − 99), 83 (100).

MnO_2 *oxidation of* **2**. To a stirred soln of **2** (32.5 mg) in $CHCl_3$ (10 ml) was added MnO_2-activated charcoal [5] (1.2 g). The mixture was left 3.5 hr at room temp. and filtered. The filtrate was evapd *in vacuo* to give the lactol **3** (23.5 mg), mp 181–182° ($CHCl_3$–hexane). (Found: C, 63.36; H, 6.96; O, 29.34. $C_{20}H_{26}O_7$ requires: C, 63.48; H, 6.93; O, 29.60.) IR ν_{max} cm^{-1} 3440, 1735, 1730, 1650. ^{1}H NMR: 3.18 (*t*, $J = 3.6$ Hz, 1H, H-1), 2.77 (*d*, $J = 3.6$ Hz, 2H, H-2 and H-2′), 5.32 (*d*, $J = 1$ Hz, 1H, H-3), 5.48 (*d*, $J = 1$ Hz, 1H, H-3′), 4.36 (*dd*, $J = 4$, $J = 3.5$ Hz, H-5), 5.58 (*m*, 2H, H-8 and H-15), 4.61 (*br s*, 1H, H-9), 0.9 (*s*, 3H, C-10–Me), 1.62 (*d*, $J = 7$ Hz, 3H, C-11–Me), 2.02 (*br d*, $J = 7$ Hz, 3H), 1.92 (*br d*, $J = 1.5$ Hz, 3H) angelate methyls. MS: *m/z* 378 (M^+), 360 (M–18), 279 (M − 99), 261 (M − 117), 83 (100).

Dilactone **4**. Further oxidation of lactol **3** under the same conditions (see above) provided the dilactone **4** in low yield. Syrup; IR ν_{max} cm^{-1} 1780, 1740, 1730, 1650. ^{1}H NMR: 3.1 (*t*, $J = 3$ Hz, H-1), 4.48 (*dd*, $J = 3$, $J = 6$ Hz, H-6), 4.65 (*t*, $J = 2$ Hz, 1H, H-9), 5.52 (*dd*, $J = 2$, $J = 4$ Hz, H-8), 5.86 (*s*, 1H, H-3), 6.37 (*s*, 1H, H-3′), 0.98 (*s*, 3H, C-10-Me), 1.57 (*d*, $J = 7$ Hz, 3H, C-11–Me). MS *m/z* 376 (M^+), 277 (M − 99), 83 (100).

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