SESQUITERPENE LACTONES OF *LAURUS NOBILIS* LEAVES

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ABSTRACT.—The leaves of *Laurus nobilis* (bay leaves) yielded five sesquiterpene lactones upon extraction, solvent partitioning, then column chromatography. The major constituent was identified as costunolide (**1**), while artemorin (**2**), verlotorin (**3**), santamarine (**4**) and reynosin (**5**) were obtained in lesser amounts. The identity of the isolated compound was established by a study of their spectral data and by direct comparison with authentic samples. Furthermore, sodium borohydride reduction of artemorin (**2**) yielded the known compound gallicin (**7**).

As a part of a research program dealing with the study of biogenetic-type synthesis of sesquiterpenes, it was desired to obtain a supply of desacetyllaurenobiolide (**6**) by isolation from bay leaves as previously described (1). This compound was reported to occur in this source in about 0.006% yield, along with traces of costunolide (**1**) and laurenobiolide (**8**) which were never isolated. When commercially available bay leaves (*Laurus nobilis*, family Lauraceae) were processed for the isolation of **6**, it was found to be lacking[1] in the collection of plant material examined; instead five other sesquiterpene lactones were found to occur in significant amounts. The isolation and characterization of these compounds are the subject of the present paper.

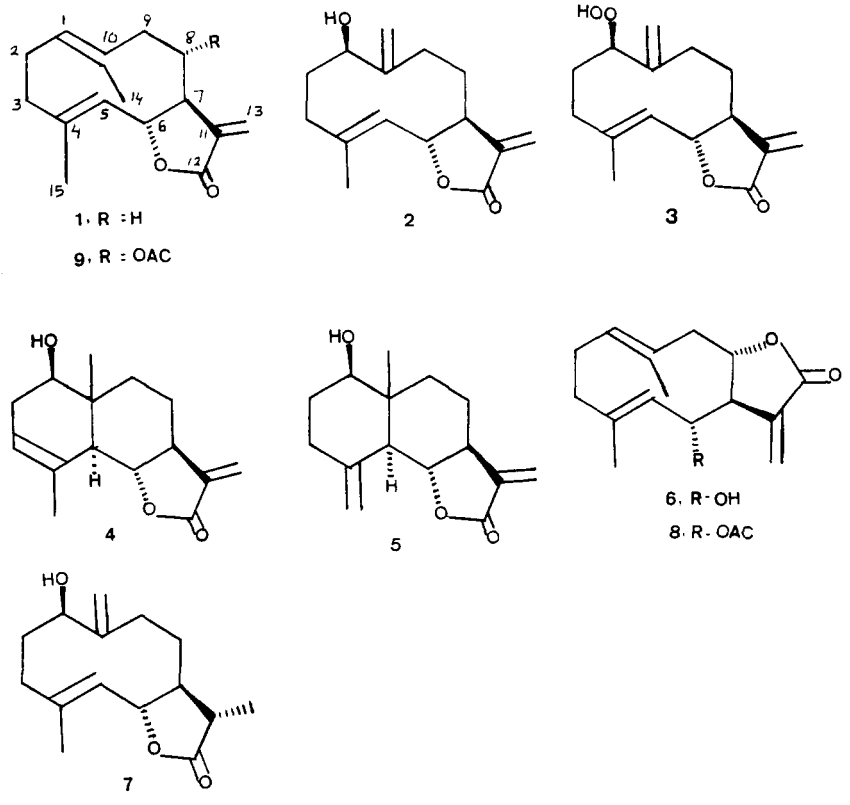
Commercially available bay leaves were extracted by cold percolation with 95% ethanol. The alcoholic extract was evaporated *in vacuo* at 40°, and the residue was partitioned between water and chloroform. The chloroformic phase was evaporated, and the residue was partitioned between 90% methanol and *n*-hexane. The methanol solubles were chromatographed on silica gel; chloroform was the solvent used.

The first column band contained mostly odoriferous monoterpenes (see Experimental), while the second band was essentially a mixture of costunolide (**1**) and verlotorin (**3**) (also known as peroxycostunolide). This was established when this band was subjected to thin layer chromatographic analysis on silica gel G plates with chloroform-isopropyl ether (1:4) as the solvent system. Costunolide (**1**) had an R_f value of 0.65, while verlotorin (**3**) ran with an R_f value of 0.25. Repeated chromatography of this band on silica gel with chloroform as solvent separated these two compounds, yielding pure costunolide (**1**) and pure verlotorin (**3**).

Costunolide (**1**) occurred in the form of colorless needles, $C_{15}H_{20}O_2$; mp 105–106°; $[\alpha]^{25}D+117°$ (*c*, 0.200 $CHCl_3$). Its ir spectrum ($CHCl_3$) showed the presence of an α,β-unsaturated-γ- lactone (1777 cm^{-1}), thus accounting for the two oxygen

[1]After it was discovered that **6** was lacking in the leaves, it was decided to isolate laurenobiolide (**8**) instead, from the roots of the same plant as previously reported (1). The plant material was collected in Tokyo, Japan, courtesy of Professor Y. Sashida for which we are grateful. The results were again disappointing as the plant material contained only costunolide (**1**) and tulipinolide (**9**), a structural isomer of laurenobiolide (**8**). Its identification was accomplished by comparison of its physical and spectral data with those in the literature (2). The ^{13}C nmr spectrum, which has not been reported before, was found to be in agreement with the structure. It exhibited two carbonyl signals at δ 169.8 and 169.7, six olefinic carbons at δ 142.1(s), 135.9(s), 131.9(s), 129.8(d), 127.5(d) and 124.8(t), two oxygenated carbons at δ 78.4(d) and 72.7(d), one methine carbon at δ 52.9, three methylenes at δ 48.9, 38.5 and 25.3 and three methyl signals at δ 21.1, 17.3, and 17.1.

atoms. The pmr spectrum exhibited signals characteristic of two vinylic methyl groups, an exocyclic olefinic methylene and an additional vinylic proton. These features suggested that the compound might be costunolide (**1**); its identity was confirmed by direct comparison with an authentic sample.[2]



Verlotorin (peroxycostunolide) (**3**) was obtained as colorless prisms, $C_{15}H_{20}O_4$, decomposing without melting near 141°; $[\alpha]^{25}D+171°$ (*c*, 0.2 acetone). The ir spectrum (KBr) indicated that the compound had an α,β-unsaturated-γ- lactone system as in costunolide (**1**), but in addition, it also exhibited a hydroxyl absorption band (see Experimental). The pmr (acetone-d_6) was remarkably similar to that of costunolide (**1**) except that it exhibited two exocyclic methylene signals, two protons on oxygenated carbons and an exchangeable one-proton singlet located at δ 10.51; a position characteristic for hydroperoxy protons (4). The structure of this compound was finally secured by comparison with an authentic sample of verlotorin (**3**).

The third column band was found to contain four different compounds when analyzed on silica gel G plates with acetone-isopropyl ether (1:4) as the solvent system. It showed four spots, R_f 0.53 which corresponded to costunolide (**1**) and 3 other spots of R_f values 0.35, 0.29 and 0.20. This mixture was separated by column charomatography on silica gel with the same solvent as eluent. The

[2]This sample was isolated from the root bark of *Magnolia grandiflora* L, as described in the literature (3).

early fractions contained costunolide (**1**) (identical with the material isolated from the second band). This was followed by the other compounds as follows:—

a) The compound with R_f value 0.35 was eluted next, and was obtained as colorless needles, $C_{15}H_{20}O_3$, mp 134–135°; $[\alpha]^{22}D+72.8°$ (*c*, 0.18 in absolute alcohol). Its ir spectrum ($CHCl_3$) suggested the presence of an α,β-unsaturated-γ-lactone group, and, also, it exhibited a hydroxyl absorption band. The pmr ($CDCl_3$) spectrum, on the other hand, exhibited (see Experimental) signals due to a quaternary methyl, a vinylic methyl, and two protons of oxygenated carbons. The spectral and physical features of this compound were in agreement with those of the eudesmanolide sesquiterpene lactone santamarine (**4**)[2].

b) The compound with R_f 0.29 was eluted next and was obtained as colorless needles, $C_{15}H_{20}O_3$; mp 145°–146°; $[\alpha]^{22}D+122°$ (*c*, 0.100 in absolute alcohol). Its spectral data indicated that the compound was similar to santamarine (**4**) except that it possessed two exocyclic methylene groups. These features pointed to the santamarine isomer reynosin (**5**). Direct comparison established conclusively that the isolated material was indeed identical with an authentic sample[2] of reynosin (**5**).

c) The last compound to be eluted from the column had an R_f value of 0.20 on tlc (*vide supra*) and was obtained as colorless prisms, $C_{15}H_{20}O_3$; mp 120–121°; $[\alpha]^{25}D+89°$ (*c*, 0.10, $CHCl_3$). Its spectral data exhibited features common with some of the other compounds, such as an α,β-unsaturated-γ-lactone group and in being hydroxylated The nmr spectrum ($CDCL_3$), however, exhibited not only two exocyclic methylenes but also a vinylic methyl group, suggesting a germacranolide skeleton. The properties of this compound were in agreement with those reported for artemorin (**2**), and direct comparison with an authentic sample[4] confirmed its identity.

The last band to be eluted from the original column was found to be homogeneous on tlc, and provided, upon crystallization from ether-chloroform, an additional amount of artemorin (**2**).

Thus, it has been shown that bay leaves contain a respectable amount of costunolide (**1**) (1.8%). In fact, they can serve as a good source for this biologically active compound. Artemorin (**2**), reynosin (**5**), santamarine (**4**) and verlotorin (**3**) are found in successively smaller yields: 0.50%, 0.25%, 0.15% and 0.14%, respectively. No trace of desacetyllaurenobiolide (**6**) was detected in the material examined.

The presence of these compounds in bay leaves in such significant amounts could have some undesirable effects. For example, foods flavored with bay leaves could cause allergic symptoms to their eaters in veiw of the reported allerginicity of many α,β-unsaturated-γ-lactones (6). Furthermore, the presence of a hydroperoxy group in verlotorin (**3**), on the other hand, might provide a source of free radical initiators, thus contributing to the rapid spoilage of foods.

While costunolide (**1**), santamarine (**4**) and reynosin (**5**) have been reported in numerous plants and are often found along with each other, verlotorin (peroxycostunolide) (**3**) has been reported previously only twice (4). Artemorin (**2**), on the other hand, was reported only once before (4), but recently there have been

[3]This sample was isolated from *Magnolia grandiflora* L as previously described (4).

[4]This sample was obtained by reducing verlotorin (**3**) with triphenylphosphine as previously described (5).

two reports on the isolation of 11,13-dihydroartemorin. The first report dealt with the α-isomer which was named gallicin (**7**) and was reported (7) to occur in *Artemisia maritima*. The second report described the isolation and characterization of the β-isomer.[5] Sodium borohydride reduction of artemorin (**3**) obtained from bay leaves was found to produce, exclusively, the α-isomer (gallicin).[6]

EXPERIMENTAL[7]

PLANT EXTRACTION.—The leaves of *Laurus nobilis* (bay leaves) were purchased from Meer Corporation, lot #8-01000, in February, 1979. The groundplant material (2180 g) was extracted by cold percolation with 95 percent ethanol. The alcoholic extract was evaporated[8] *in vacuo* at 40° to leave 551 g of a dark green residue. This residue was subjected to solvent partitioning between 2000 ml of chloroform and three 600 ml portions of water. The organic phase was then dried over anhydrous sodium sulfate then evaporated to yield 499 g of a dark thick extract. This extract was further partitioned by dissolving in 2200 ml of 90% methanol, then extraction with three 600-ml portions of *n*-hexane. Evaporation of the methanolic phase left a residue weighing 138 g.

CHROMATOGRAPHIC SEPARATION OF THE SESQUITERPENE LACTONES.—The 90 percent methanol solubles obtained above (10 g) were chromatographed on a column, 76.0 x 6.2 cm, of 1250 g silica gel packed in chloroform. Fifty ml fractions were collected and each was analyzed by tlc. Fractions of the same composition were pooled. The first column fraction yielded 54 mg of a mixture of relatively nonpolar compounds and was not further examined. The second fraction (3.01 g) was essentially a mixture of costunolide (**1**) and verlotorin (**3**) as revealed by tlc on silica gel G with chloroform-isopropyl ether (1:4) as solvent. The former exhibited R_f value of 0.61, while the latter R_f value was 0.40. One g of this fraction was rechromatographed on 100 g of silica gel to yield:

a) *Costunolide* (**1**) (0.87 g) which when recrystallized from ether-hexane gave colorless needles, mp 105–106°, $[\alpha]^{22}D+117°$ (*c*, 0.200 in $CHCl_3$); uv end absorption at 210 nm, ($\log \epsilon=4.05$); ir ($CHCl_3$); ν max 1765, 1667, 1285, 1136, 995, and 930 cm^{-1}; pmr ($CDCl_3$): δ1.70 and 1.42 (each 3H, br s, C_4–CH_3 and C_{10}–CH_3), 4.57 (1H, t, $J=10$ Hz, C_6–H), 4.79 (1H, m, C_5–H), 4.90 (1H, m, C_1–H), 5.54 (1H, d, $J=3$ Hz, C_{13}–H), and 6.27 (1H, d, $J=4$ Hz, C_{13}–H) ppm; mass spectrum: M^+ at *m/e* 232 (57%).

Anal. Calc. for $C_{15}H_{20}O_2$: C, 77.55; H, 8.12. Found: C, 77.39; H, 8.45. The identity of this compound was confirmed by comparison with an authentic sample of costunolide (3) (same mp, mmp, and indistinguishable ir and pmr spectra).

b) *Verlotorin* (**3**) (0.066 g) which was recrystallized from absolute ethanol-ether to yield colorless prisms, decomposing near 141°: $[\alpha]^{25}D+171°$ (*c*, 0.20 acetone); uv end absorption (ϵ_{210} 9000); ir (KBr) bands at 3440 and 3350 (OH), 3090 (unconjugated $^1C=CH_2$), 1745 (lactone C=O); and 1660 cm^{-1} (C=C); chemical ionization mass spectrum (isobutane) *m/e* 265 (12%; MH^+).

Anal. Calcd. for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.25; H, 7.77. The identity of this compound was further confirmed by comparison with an authentic sample of verlotorin (5) (indistinguishable physical and spectral data).

The third band from the original column (2.27 g) yielded four spots, R_f values 0.53, 0.35, 0.29 and 0.20 when examined by tlc on silica gel G plates with acetone-isopropyl ether (1:4) as solvent. Separation was accomplished by chromatographing 1.0 g of this band on 100 g of silica G packed in the same solvent to give:

a) *Costunolide* (**1**) (94 mg) characterized by comparison with the sample obtained before.

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[5]Preliminary account of this work was presented by Professor L. Zalkow during the meeting of the American Society of Pharmacognosy, July, 1979.

[6]We are grateful to Professor L. Zalkow of Georgia Institute of Technology for providing us with copies of the spectral data of gallicin (7) and its C-11 epimer.

[7]All melting points were taken in capillaries on a Thomas Hoover Unimelt apparatus and are uncorrected. Uv spectra were taken on a Beckman Model Acta III recording spectrophotometer; ir spectra were determined on either a Beckman IR-33 recording infrared spectrophotometer or a Perkin-Elmer 257 infrared spectrophotometer; optical rotations were measured on a Perkin-Elmer 141 automatic polarimeter; pmr spectra were recorded on a JEOL model C-60 nuclear magnetic spectrometer at 60 MHz operating at room temperature with $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard with chemical shifts reported as δ(ppm) values; the ^{13}C nmr spectra were recorded on JNM-FX60 Fourier Transform nmr spectrometer at 15.03 MHz. Proton noise decoupled and/or off-resonance decoupled spectra were obtained with $CDCl_3$ as solvent and TMS as internal standards. High and low resolution mass spectra were taken on an E. I. Dupont de Nemours model 21-492 mass spectrometer. Elemental analyses were done by Scandinavian Microanalytical Laboratory in Herlev, Denmark. Spot detection on tlc plates was achieved by spraying with 0.5% aqueous $KMnO_4$ or by viewing under ultraviolet light.

[8]During the evaporation of the alcohol extract colorless crystals of a water soluble substance (14 g) deposited, mp 165.5–166°. It was found to be mannitol.

b) *Santamarine* (**4**) (96 mg) which was recrystallized from chloroform-ether to give colorless prisms, mp 134–135°, $[\alpha]^{22}D$ +72.8° (*c*, 0.18 in absolute ethanol); uv end absorption at 210 nm, log ϵ=4.30; ir (KBr): ν max 3380, 1773, 1673, 1128, and 995 cm^{-1}; pmr ($CDCl_3$), δ 0.86 (3H, s, C_{10}–CH_3), 1.85 (3H, s, C_4–CH_3), 3.70 (1H, dd, *J*=6.0 and 9.5 Hz, C_1–H), 3.98 (1H, t, *J*=10 Hz, C_6–H), 5.43 (1H, d, *J*=3.0 Hz, C_{13}–H), and 6.10 (1H, d, *J*=3.0 Hz, C_{13}–H) ppm; mass spectrum M^- at *m/e* 248 (64%).

Anal. Calc. for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found C, 72.45; H, 8.22.

The identity of **4** was further confirmed by comparison with an authentic sample of santamarine (3), the two compounds proved to be identical in all respects.

c) *Reynosin* (**5**) (195 mg), mp 145–146°, $[\alpha]^{22}D$ +122° (*c*, 0.100 in absolute ethanol); uv: end absorption at 210 nm, log ϵ=3.96; ir ($CHCl_3$): ν max 3610, 1772, 1653, 1410, 1127, and 1082 cm^{-1}; pmr ($CDCl_3$): δ 0.83 (3H, s, C_{10}–CH_3), 3.55 (1H, dd, *J*=4 and 10.5 Hz, C_1–H), 4.06 (1H, t, *J*=11.0 Hz, C_6–H), 4.90 and 5.03 (1H each, br s, C_4=CH_2), 5.45 (1H, d, J=3.0 Hz, C_{13}–H), and 6.12 (1H, *J*=3.5 Hz, C_{13}–H) ppm; mass spectrum: M^+ at *m/e* 248 (9%).

Anal. Calc. for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.51; H, 8.02.

Direct comparison with an authentic sample of reynosin (3) proved that the two compounds had indistinguishable physical and spectral data.

d) *Artemorin* (**2**) (235 mg) was obtained as colorless needles from ether, mp 120–121°, $[\alpha]^{25}D$ +89° (*c* 0.10, $CHCl_3$); ir ($CHCl_3$) bands at 3600 and 3500 (OH), 1760 (lactone C=O), 1670 and 1640 cm^{-1} (C=C); mass spectrum: M^- at *m/e* 248 (6%).

Anal. Calc. for $C_{15}H_{20}O_3$: C, 72.55, H, 8.12. Found C, 72.35, H, 7.92.

The identity of **2** was established by direct comparison with an authentic sample (5) (same mp, mmp, indistinguishable spectra).

The fourth and last band from the original column (270 mg) crystallized spontaneously and was found to be pure artemorin (**2**) by comparison with the substance isolated from the third band.

SODIUM BOROHYDRIDE REDUCTION OF ARTEMORIN (**2**) TO GALLICIN (**7**).—Artemorin (**2**) (51 mg) was dissolved in 2 ml of absolute ethanol, and the solution was stirred with 36 mg of sodium borohydride for 35 minutes. The reaction was worked up by the addition of 0.5 ml of 5% acetic acid, then evaporation under reduced pressure at 40° to get rid of the solvent. The residue was taken up in 125 ml of chloroform, which was then washed with three 25 ml portions of water. The chloroform phase was dried (anhydrous sodium sulfate) and evaporated to leave 42 mg of an oil that crystallized from ethylacetate-hexane to give colorless crystals, mp 111–112°, $[\alpha]^{25}D$ +118° (*c*, 0.29 in $CHCl_3$); ir ($CHCl_3$) ν max: 3600 (OH), 1765 (γ-lactone), 1670 and 1640 (c=c) cm^{-1}; pmr ($CDCl_3$): δ 1.22 (d, *J*=7 Hz, C_{11}–CH_3), 1.70 (d, *J*=2 Hz, C_4–CH_3), 3.90 (m, C_1–H), 4.40 (dd, *J*=9 and 10 Hz, C_6–H), 4.75 (br s, C_{15}–H), 5.15 (d, *J*=9 Hz, C_5–H), and 5.17 (br s, C_{15}–H); mass spectrum: M^- at *m/e* 250 (12%).

Anal. Calc. for $C_{15}H_{22}O_3$: C, 71.95; H, 8.85. Found: C, 72.11; H, 9.11.

The pmr spectrum of **7** taken in benzene-d_6 showed that the doublet due to the C_{11}–CH_3 was shifted upfield by 0.21 ppm, relative to the $CDCl_3$ spectrum. This shift was in agreement with its α-configuration (7, 8).

ACKNOWLEDGMENTS

This research was supported in part by the Research Institute of Pharmaceutical Sciences, School of Pharmacy; University of Mississippi, University, Mississippi. Some of the mass spectra were taken by Dr. O. Bouwsma to whom we are thankful.

Received 26 December 1979.

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