NEW GERMACRANOLIDES FROM *ARTEMISIA HERBA ALBA*. X-RAY CRYSTAL STRUCTURES OF 3β,8α-DIHYDROXY-6βH,7αH,11βH-GERMACRAN-4(14), 9(10)-DIEN-6,12-OLIDE AND THE CORRESPONDING 3-OXO-OLIDE[1]

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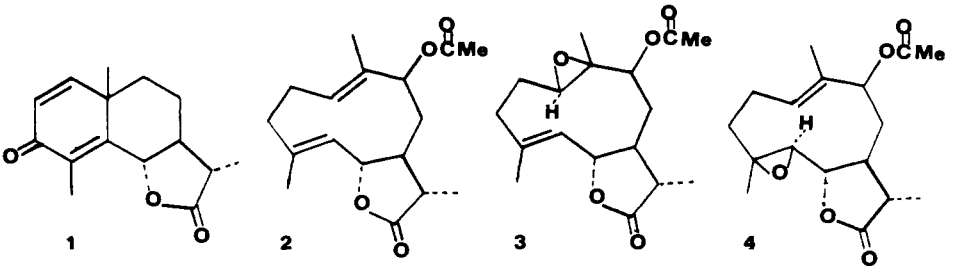
ABSTRACT.—The three new germacranolides 3β,8α-dihydroxy-6βH,7αH,11βH-germacran-4(14),9(10)-dien-6,12-olide (**10**), the corresponding 3-oxo-olide (**8**) and 1β-hydroxy-6βH,7αH,11αH-germacran-4(5),10(15)-dien-6,12-olide (**9**), together with 2,6,10-trimethyl-cis-7,10-oxido-dodeca-3E,11-dien-2-ol-5-one (**7**), obtained as a natural product for the first time, and thymol were isolated from the ethanol extract of *Artemisia herba alba* from the Sinai desert. The structures of germacranolides **8** and **10** were confirmed by single crystal X-ray analyses. Germacranolide **9** was shown to be the C-11 epimer of the previously reported gallicin. On preparative gas chromatography **9** was transformed into eudesmanolide **11**. The C-11 epimer of **11** has been previously prepared by treatment of gallicin with hydrochloric acid. Eudesmanolide **11** has been recently reported as a natural product.

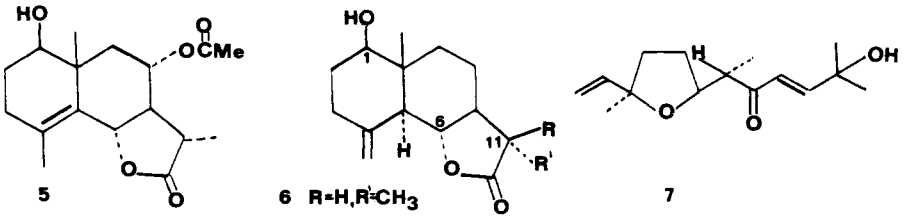
The genus *Artemisia* L. is one of the largest and most widely distributed of approximately 60 genera in the tribe Anthemideae of the Asteraceae (Compositae) (1). The sesquiterpene lactone data for the *Artemisia* have been compiled and reviewed categorically by subgenera (sections) in an attempt to use these chemical characteristics to better understand the phylogeny and systematics of the genus (1,2). *Artemisia herba alba* Asso. is a dwarf shrub which grows wild in areas of North Africa and the Middle East. It belongs to the subgenus Seriphidium. Classification by sesquiterpene lactone structural classes shows that most species within the Seriphidium produce eudesmanolides, although a few germacranolides, guaianolides and the single elemanolide, temisin, have been reported (1).

A. herba alba has been used by local populations in their native countries in folk medicine, in particular, as an anthelmintic (3,4). It is known by the Arabic name "Shih" (3,4). The essential oils of some varieties of *A. herba alba* have been known for over seventy-five years (5), but more extensive phytochemical investigations of this species have only been recently reported. In 1971 Khafagy *et al.* (3) reported the isolation of santonin (**1**), β-sitosterol and stigmasterol and an unidentified triterpenoid from the dried flowering branches of *A. herba alba* growing wild near Ras El-Hikmah, Egypt. These workers stated that earlier workers failed to find santonin in this plant, apparently arising from South Algeria or Morocco. Even more recently, Segal *et al.* (6) isolated the three new germacranolides, herbolide A (**2**), B (**3**) and C (**4**), from the chloroform extract of the flowers, small stems and leaves of *A. herba alba* collected near Sde Boker in the Negev desert of Israel. Segal (6) did not find santonin in her *A. herba alba*, but she did isolate three flavonoids from the non-glycosidic extract of the aerial parts, one of which was identified as 5,4'-dihydroxy-6,7,3'-trimethoxyflavone (4). Most

[1]Preliminary accounts of parts of this work were presented as follows: Leon H. Zalkow, Maureen M. Gordon, Charlotte Dickinson and Leslie Gelbaum, International Research Congress on Natural Products as Medicinal Agents, Strasbourg, France, July 6–11, 1980; L. H. Zalkow, M. M. Gordon and D. Van Derveer, 178th ACS National Meeting, Washington, D.C., September 10–14, 1979; L. H. Zalkow and M. M. Gordon, 20th Annual Meeting of the American Society of Pharmacognosy, Purdue University, July 29–August 3, 1979.

recently, Gomis *et al.* (7) examined the air-dried stems, leaves and flowers of *A. herba alba*, subsp. *Valentina* from the Valencia country of Spain and found, in addition to α-santonin (**1**), the new eudesmanolide torrentin (**5**) and the eudesmanolide dihydroreynosin (**6**), previously isolated as a natural product from *Michelia compressa* (8). These authors also identified *p*-cymene, 1-methyl-4-isopropylbenzene, α-copaene, aromadendrene, α-guaiene, β-bisabolene, γ-cadinene, calacorene, cadalene, n-hepatadecane, n-octadecane, n-eicosane and α-curcumene in the hydrocarbon fraction of the volatile oil by gc-ms.



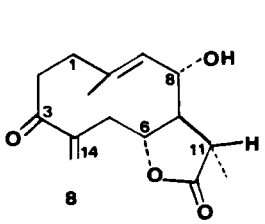


As part of our program to find new natural anti-tumor agents, we received dried samples of *A. herba alba* collected in the Santa Catherina area of the Sinai desert. In spite of the fact that the ethanol extract of the plant (NSC B837122) showed essentially no activity against P388 lymphocytic leukemia tumors, we decided to isolate the sesquiterpene lactones and submit them for anti-tumor screening. Our recent experiences with pyrrolizidine alkaloid bearing plants (9) had shown that active pyrrolizidine alkaloids could be isolated from apparently inactive plant extracts because the active compounds were present in small amounts and demonstrated activity only at large dosages.

The concentrated ethanol extract of *A. herba alba* was partitioned between chloroform and water. The chloroform layer, after removal of the solvent, was partitioned between hexane and aqueous methanol (1:9). After concentration, the aqueous methanol layer was washed with 5% aqueous sodium hydroxide and water, then dried and evaporated. The latter residue was chromatographed on silica gel; eluting was done with hexane containing increasing amounts of ethyl acetate. The 1:1 hexane-ethyl acetate fraction yielded a mixture from which thymol and 2,6,10-trimethyl-cis-7,10-oxido-dodeca-3E,11-dien-2-ol-5-one (**7**)

(hydroxydavanone) were obtained by chromatography on a silica gel packing with the Waters 500 preparative liquid chromatograph. Hydroxydavanone (**7**) was previously reported by Thomas and Dubini (10) as a minor product from the photosensitized oxygenation of the sesquiterpenoid davanone. Davanone itself had been previously isolated from *Artemisia pallens* Wall. (11), and simultaneously with this work Jork and Nachtrab (12) reported the isolation of davanone and hydroxydavanone from *Artemisia maritima* L. ssp. *maritima* L. Hydroxydavanone (**7**) was identified by comparison of its ir, ^{1}H nmr and mass spectra with those of an authentic sample.[2] Thymol was identified by comparison of its spectral data with those from the literature (13).

Three new germacronolides (**8**, **9**, **10**) were eluted from the original silica gel column with pure ethyl acetate and obtained in pure form by repeated chromatography on the Waters 500 preparative chromatograph with hexane-ethyl acetate as the eluent. The order of elution was 8α-hydroxy-3-oxo-6βH,7αH,11βH-germacran-4(14),9(10)-dien-6,12-olide (**8**), then 1β-hydroxy-6βH,7αH,11αH-germacran-4(5),10(15)-dien-6,12-olide (**9**) and finally 3β,8α-dihydroxy-6βH,7αH,11βH-germacran-4(14),9(10)-dien-6,12-olide (**10**). The mass spectrum and ^{1}H nmr spectrum of **9** suggested that it was closely related to gallicin (the C-11 epimer of **9**), but a direct comparison of the ^{1}H nmr spectrum with that of an authentic sample of gallicin showed that they differed in the signals due to the C-6 and C-11 protons (14).[3] Thus, the C-6 proton in **9** appeared as a triplet ($J=10$ Hz) at δ 4.59, whereas in gallicin the triplet ($J=10$ Hz) appeared at δ 4.40. The C-11 proton in **9** appeared as a quintet centered at δ 2.61 while in gallicin it appeared as an undefined multiplet centered at δ 2.47. In all other regards, the two nmr spectra were superimposable.



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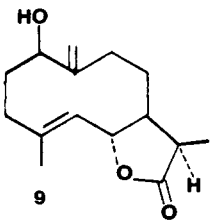

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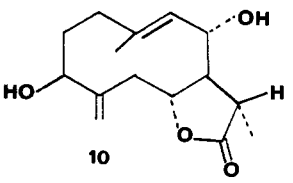

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On preparative gas chromatography, **9** was converted into eudesmanolide **11** which showed similar spectra to those of the C-11 epimer of **11** (6) prepared by Gonzales *et al.* (14) by treatment of gallicin with HCl.[3] Eudesmanolide (**6**) was first prepared by Pathak *et al.* from dihydrocostunolide (15) and then isolated shortly thereafter as a natural product from *Artemisia tridentata* (16). More recently, Farnsworth *et al.* reported its isolation from *Michelia compressa* (17). An examination of the C-6 and C-11 protons in the nmr spectra of **11** and **6**, as in the case of β and α-santonin, respectively (18), provides an unequivocal distinction between these structures. Thus, in our hands the C-11 proton in **11** appeared at δ 2.61 as a quintet as in β-santonin, while the C-11 methyl group showed an aromatic-induced solvent shift (deuterochloroform to deuterobenzene) of $\Delta\delta$ 0.44, characteristic of a pseudo axial methyl group (19). In the epimer, **6**,

[2]We thank Dr. Alan F. Thomas, Firmenich SA, Geneva, Switzerland, for copies of the ^{1}H nmr, mass and ir spectra of **7**.

[3]We thank Professor Antonio G. Gonzales, Director, Instiuto de Productos Naturales, Universidad de La Laguna, La Laguna, Tenerife, Spain, for the ir, mass and ^{1}H nmr spectra of gallicin and its cyclized eudesmanolide product.

the C-11 proton appears at higher field (δ 2.35), as in α-santonin (18). It shows an aromatic solvent induced shift of $\delta\Delta$ 0.27 (17), characteristic of a pseudo equatorial group (19). Gomis *et al.* (7) recently reported the isolation of a eudesmanolide from *Artemisia herba alba* subsp *valentina* endemic to the Valencia country of Spain and assigned it structure **6**. We believe this substance may in fact be **11** because the chemical shift of the C-6 proton is reported (7) to be δ 4.27, as in **11**, whereas the C-6 proton in **6** shows δ 4.02 (14, 16, 17).

The first germacranolide eluted from the chromatography column could be assigned structure **8** on the basis of its spectral properties. From the exact mass determination (mass spectrum), the formula $C_{15}H_{20}O_4$ could be assigned. On the basis of the ir and ^{1}H nmr spectra the molecule was seen to contain a γ-lactone, α,β unsaturated ketone, an allylic secondary hydroxyl group, exocyclic methylene group, a trisubstituted double bond containing a methyl group as one of the substituents and a secondary methyl group. These data suggested a germacranolide of structure **8**. While convincing arguments could be made for the structure of **8** with the relative configuration indicated, solely from analysis of the ^{1}H nmr spectrum, conclusive proof was obtained by a single crystal X-ray analysis.

A crystal of **8** with the approximate dimensions 0.6 x 0.3 x 0.3 mm was mounted on a glass fiber with epoxy cement in such a way that the longest crystal dimension was parallel to the fibre axis. Unit-cell parameters and the orientation matrix were determined on a Syntex $P2_1$ four-circle diffractometer equipped with a graphite monochromator using Mo-Kα radiation. Unit cell parameters obtained were $a=b=8.073(3)$Å,[4] $c=43.73(2)$Å and $V=2850(2)$Å^3. The observed density was 1.22 (calculated 1.23). The crystal belonged to the tetragonal system, and the structure solved for the space group $P4_32_12$ (No. 96) (20). By virtue of systematic absences, the space group $P4_12_12$ (No. 92) could also have been chosen, but a definite choice could not be made without knowledge of the absolute configuration. Intensity data were collected by θ-2θ scans with X-ray source and monochromator settings identical to those used for the determination of the unit cell parameters. No significant fluctuations were observed in the intensities of three standard reflections monitored every 97 reflections. From a total of 1602 unique reflections collected out to $2\theta=50°$; 1229 were accepted as statistically above background on the basis that F was greater than 3σ (F). Computations were performed according to standard programs.[5] For structure factor calculations, the scattering factors were taken from Cromer and Waber's tabulation (21). The agreement factors were defined in the usual way as $R=(\Sigma\ |F_o|-|F_c|\ )/(\Sigma\ F_o|)$ and $R_w=[\Sigma_w(|F_o|-|F_c|)\ (w)^{1/2}/\Sigma(\Sigma\ F_o|)\ (w)^{1/2}]$. In all least squares refinements, the quantity minimized was $w(|F_o|-|F_c|)^2$. A weighting scheme partially based on counting statistics $[w=3/[\sigma_F^2+0.0009F^2]$ was employed in the least squares refinement. All non-hydrogen atoms were located from an E-map based on phases generated by the direct methods section of SHELX-76. Hydrogen atoms were located from difference Fouriers or were calculated by means of the routine in SHELX-76 which fixes the C-H distance at 1.08Å and orients their positions so as to complete the sp^2 or sp^3 coordination about the carbon. Their temperature factors were refined isotropically. The final parameters varied included an overall scale factor, positional parameters for the oxygen and carbon atoms, and aniso-

[4]The numbers in parentheses here and elsewhere in this paper indicate estimated standard deviations in the least significant digit(s).

[5]Programs used were Sheldrick's SHELX-76, Johnson's ORTEP, Zalkin's FORDAP and Main, Germain and Wolfson's MULTAN.

tropic thermal parameters for the oxygen and carbon atoms (191 variables; 1229 observations). Hydrogen atoms had isotropic thermal parameters. The final R factor was 0.089 and $R_w = 0.090$.[6] A computer-generated picture of **8** appears in figure 1.

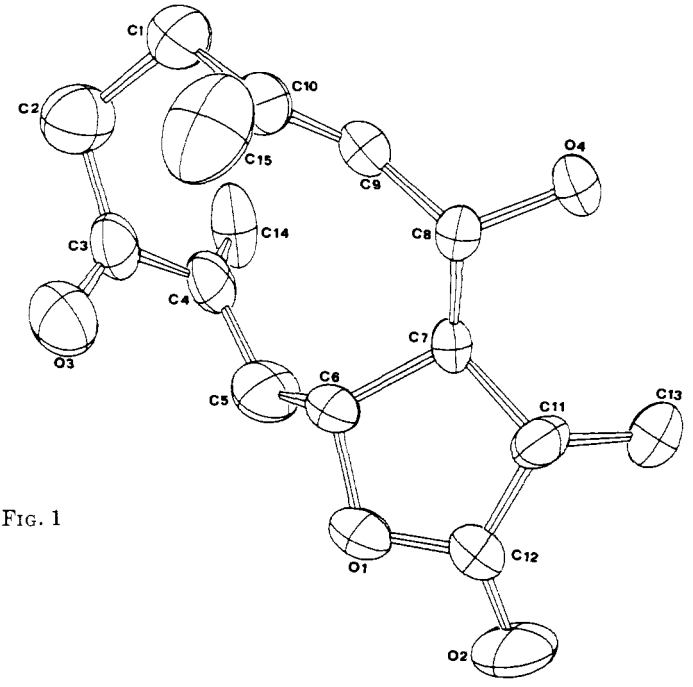


FIG. 1

The final germacranolide eluted by chromatography has been assigned structure **10** on the basis of spectral analysis. The structure and relative configuration were confirmed by single crystal X-ray analysis. Analyses of the ^{1}H nmr spectra of **10** in pyridine-d_5 and in acetone-d_6 were very instructive. Thus, the C–11 methyl group appeared farther downfield (δ1.69,d,J=7Hz) than the C–10 methyl group (δ1.61,d,J=1Hz) in pyridine-d_5 while in acetone-d_6 the C–11 methyl group appeared at δ1.13 (d,J=7Hz) and the C–10 methyl group appeared at δ1.59 (d,J=1Hz) as expected. Irradiation of the broad single proton signal at δ2.95 in pyridine-d_5 resulted in collapse of the δ1.69 doublet to a singlet thus allowing assignment of these signals to the C–11 and C–13 protons, respectively. The C–6 proton appeared as a broad triplet at δ4.65 in pyridine-d_5, while in acetone-d_6 it appeared as a doublet of triplets (J=10,3.7Hz) at δ4.40. These coupling constants showed that the lactone ring in **10** was *trans* and the axial C–6 proton in **10** was coupled to two *trans* axial protons (C-5α,C-7α) and an equatorial proton (C-5β).

A crystal of **10** with the approximate dimensions 0.8 x 0.6 x 0.3 mm was mounted and the orientation determined as above for **8**. Unit cell parameters obtained were a=8.058(3)Å, b=16.519(7)Å, c=10.934(3)Å and v=1455.5(9)Å^3.

[6]The strctuure factors and positional parameters are deposited with the editor.

The observed and calculated density were 1.21. This crystal belonged to the orthorhombic system and space group $P2_12_12_1$ (No. 19) by systematic absences. Intensity data were collected as above. From a total of 1507 reflections, 1397 were chosen as statistically above background and computations were performed as before using the same tabulations (20, 21). A weighting scheme based on counting statistics $w=86/[\sigma_F^2+0.0003F^2]$ was employed for calculating R_w. All non-hydrogen atoms were located from an E-map based on phases generated by the direct methods program MULTAN, while hydrogens were located as with **8**. The same final parameters were varied with 188 variables and 1397 observations, resulting in a final R factor of 0.061 and $R_w=0.057$.[6] A computer generated drawing of **10** appears in figure 2.

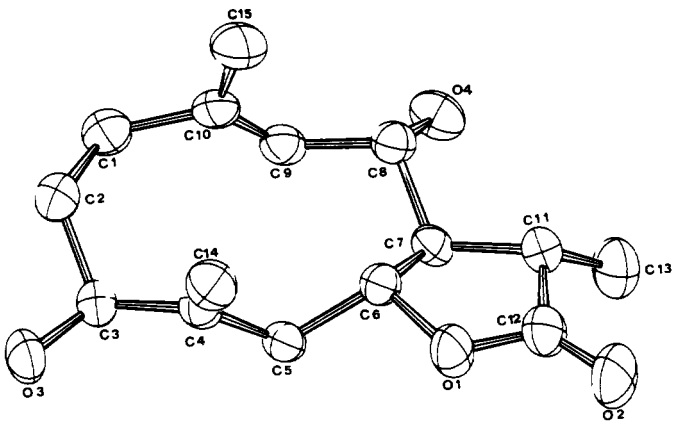
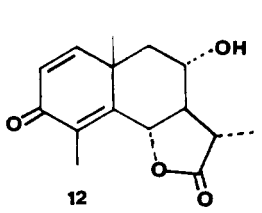


FIG. 2

In conclusion, it can be seen that the three germacranolides **8**, **9** and **10** isolated by us from *A. herba alba* are different from the three (**2**, **3**, **4**) found by Segal *et al.* (6). Neither of us found the eudesmanolide santonin, reported by earlier workers (3). This difference in content of secondary metabolites for the same species collected in different localities is not uncommon (1, 2) and probably reflects differences in stress conditions (22). *A. herba alba* has been placed in the subgenus Old World Seriphidium, most species of which produce identical or biosynthetically related sesquiterpene lactones, predominantly of the eudesmanolide class. Santonin (**1**), artemin (**12**) and tauremisin (**13**) are the most frequently encountered compounds. The only other species of this subgenus previously reported to produce germacranolides is *A. balchanorum* from which costunolide (**14**), hydroxycostunolide (**15**), balchanolide (**16**), hydroxybalchanolide (**17**) and isobalchanolide (**18**) have been isolated (1). On the other hand, the subgenus Tridentatae, also referred to as the New World Seriphidum, contains some species that produce only germacranolides (1). The germacranolides represent the simplest biosynthetic class and these are biosynthetically transformed into the more advanced classes such as the eudesmanolides, guaianolides, etc. We hope that our contributions presented here may have some phylogenetic value. We have not yet completed the antitumor evaluations of the germacranolides reported here.



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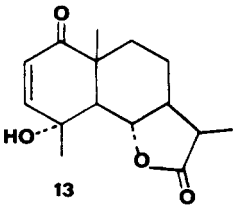

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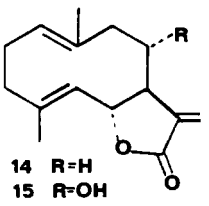

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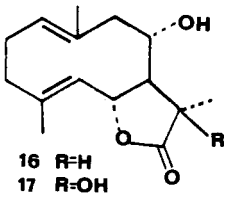
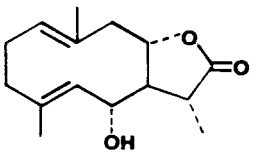
15 R=OH



16 R=H
17 R=OH



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EXPERIMENTAL[7]

EXTRACTION AND PRELIMINARY SEPARATION.—*Artemisia herba alba* was collected in the area of Santa Catherina Mountain in the Sinai Desert in October 1977. The dried whole plant (5 kg) was continuously extracted with 95% EtOH in a Soxhlet apparatus. Then the concentrated extract (600 g) was partitioned between chloroform and water (2 liters) to yield, after evaporation of the solvent, a chloroform soluble fraction (396 g) which was stirred with equal volumes (2 liters) of hexane and methanol-water (9:1). The methanol-water soluble fraction (326 g) was dissolved in ether (11 g were insoluble) and washed with cold 5% NaOH to yield after drying with $MgSO_4$, filtering and evaporation *in vacuo*, a neutral fraction (99 g). A portion (50 g) of this residue was chromatographed on silica gel (Grace, grade 923, 100–200 mesh) (1100 g); the eluent was hexane containing increasing amounts of ethyl acetate. The compounds presented in this paper were isolated from two of these fractions, the hexane-ethyl acetate (1:1) and the ethyl acetate.

2,6,10-TRIMETHYL-CIS-7,10-OXIDO-DODECA-3E,11-DIEN-2-OL-5-ONE (7).—The fraction (11.7 g) eluted with 1:1 hexane-ethyl acetate from the above chromatography was chromatographed on silica gel; the Waters 500 prep system was used eith hexane-ethyl acetate as the eluent (7:3). Glc analysis (3% SE 30, 192°) of the major fraction (5.4 g) showed that it basically consisted of one component (R_t 7 min 12 sec) along with several minor ones. After being chromatographed two more times as above, this compound (7, 3.1 g) was obtained pure, with bp 140°/0.05 mm (air-bath); mass spectrosopic molecular weight 237.1529 (Calc. for $C_{15}H_{24}O_3$ 237.1491); ν (film) 3450, 1680, 1660, 910 cm^{-1}; $[\alpha]^{25}D$ +43.7° (c, 1.65, $CHCl_3$); λ max (EtOH) 225 nm (ϵ=9650); ^1H nmr ($CDCl_3$) 1.00 (3 H, d, J=7), 1.25 (3 H, s), 1.37 (6 H, s), 3.00 (1H, p, J=7), 4.95 (1 H, d of d, J=10.2), 5.12 (1 H, d of d, J=16.2), 5.89 (1 H, d of d, J=16, 10), 6.39 (1 H, d, J=16), 6.93 (1 H, d, J=16); ^{13}C nmr ($CDCl_3$) 12.7 (q), 26.1 (q), 28.8 (q, t), 37.1 (t), 49.1 (d), 70.0 (s), 80.0 (d), 82.2 (s), 110.7 (t), 124.3 (d), 143.3 (d), 152.3 (d), 202.0 (s); *m/e* 237 (M^+, 4%), 113 (45), 111 (50), 93 (60), 85 (45), 65 (30), 50 (35), 43 (100). Comparison of the ir, ^1H nmr and mass spectra with those of authentic material verified the structure of 7.

THYMOL.—Glc analysis (3% SE 30, 192°) of one of the fractions from the above chromatographies showed that it contained 7 in addition to another component, R_t 3 min. This compound

[7]Mp's were taken on a Kofler hot-stage and are uncorrected. Ir spectra were recorded with a Perkin-Elmer 237 B spectrophotometer. ^1H nmr spectra were obtained with a Varian T-60 or JEOL-PFT-100 FT spectrometer with Me_4Si as an internal standard (δ 0); ^{13}C nmr spectra were also run on a JEOL-PFT-100 FT spectrometer. Mass spectra were run on a Hitachi RMU-7 spectrometer or Varian MAT 112S with SS200 data system. Liquid chromatography was done on a LDC, model 711 solvent delivery system and refractive index detector or Waters Prep LC System 500. Gas chromatography was done with a F&M Biomedical Gas Chromatograph, model 402 with 6' x ¼" columns. Rotations were performed on a Bendix Ericson Automatic Polarimeter.

was obtained pure by hplc on a Partisil M9 10/25 Whatman column with hexane-ether (2:3) as the eluant. The compound was identified as the monoterpene thymol by comparison of the following spectral data with those from the literature (2, 3): mass spectroscopic molecular weight 150.1029 (Calc. for $C_{10}H_{14}O$ 150.1045); ν (film), 3400, 1625, 1595, 1470, 1425, 1300, 920, 810 and 740 cm^{-1}; ^1H nmr ($CDCl_3$) 1.23 (6 H, d, $J=7$), 2.27 (3 H, s), 3.17 (1 H, p, $J=7$), 3.41 (1 H, s), 6.56 (1 H, br s), 6.71 (1 H, br d, $J=8$), 7.07 (1 H, br d, $J=8$); *m/e* 150 (M^+, 87%), 135 (100), 117 (30), 115 (52), 107 (45), 105 (20), 94 (20), 90 (60), 81 (18), 79 (23), 77 (28).

1β-HYDROXY-6βH,7αH,11αH-GERMACRAN-4(5)-10(15)-DIEN-6,12-OLIDE (**9**).—Then, the ethyl acetate fraction (7.2 g), described above was rechromatographed on silica gel by the Waters 500 prep system with hexane-ethyl acetate (3:2) as the eluent. Glc analysis (3% OV 17, 248°) of the nine fractions collected showed three that were less complex than others with one major component apiece: fraction 5 (0.48 g), R_t 13 min; fraction 6 (0.57 g), R_t 7 min and the ethyl acetate stripping, fraction 9 (2.86 g), R_t 13.5 min. The compound **9** corresponding to the major peak in fraction 6, was obtained pure by chromatography on a prepacked EM Reagents, silica gel 60 column (size B) and FM1 model RP-SY pump with hexane-ethyl acetate (3:2) as the eluent, followed by bulb to bulb distillation of fraction 19 (14 mg, 170°/0.05 mm) to yield 10 mg of **9**. It had a mass spectropic weight of M^+-H_2O of 232.1413 (Calc. for $C_{15}H_{20}O_2$ 232.1463); ν ($CHCl_3$) 3600, 3450, 1765, 1665, 1635, 1110, 1040, 965 and 900 cm^{-1}; $[\alpha]^{25}D+115°$ (c, 0.59, $CHCl_3$); ^1H nmr ($CDCl_3$) 1.19 (3 H, d, $J=7$), 1.69 (3 H, d, $J=1$), 2.61 (1 H, q, $J=7$), 3.80 (1 H, br t, $J=6$), 4.59 (1 H, t, $J=10$), 4.85 (1 H, d, $J=1$), 5.16 (1 H, d of d, $J=10.1$) and 5.19 (1 H, d, $J=1$); *m/e* 232 (M^+, 26.4%), 204 (26.4), 151 (44.2), 147 (25.9), 137 (30.6), 135 (34.9), 123 (46.1), 121 (45.7), 119 (39.5), 109 (51.9), 107 (58.5), 105 (37.8), 97 (39.0), 95 (64.1), 93 (59.8), 91 (49.9), 81 (81.9), 79 (71.6), 71 (43.2) 69 (53.1), 67 (52.6), 58 (100), and 55 (78.6). These spectral characteristics were similar to those reported by Gonzalez for gallicin, the C-11 epimer of **9** (14).

1β-HYDROXY-6βH,7αH,11αH-SELIN-4(14)-EN-12-OLIDE (**11**).—In an attempt to purify **9** by glc (3% OV 1, 200°), the major peak was collected, R_t 9 min; however, the physical and spectral properties presented below were not those of **9**, but similar to the selinolide, **6** (14), with mp 146–148°, mass spectroscopic molecular weight 250.1576 (Calc. for $C_{15}H_{22}O_3$ 250.1568); ν ($CHCl_3$) 3600, 3500, 1765, 1650, 995, 980, 960 and 900 cm^{-1}; $[\alpha]^{25}D+167°$ (C, 0.45, $CHCl_3$); ^1H nmr ($CDCl_3$) 0.82 (3 H, s), 1.20 (3 H, d, $J=7$), 2.61 (1 H, p, $J=7$), 3.51 (1 H, d of d, $J=10$, 6), 4.27 (1 H, t, $J=11$), 4.85 (1 H, d, $J=1$) and 4.97 (1 H, d, $J=1$); ^1H nmr (C_6D_6) 0.59 (3 H, s), 1.20 (3 H, d, $J=7$), 2.13 (1 H, p, $J=7$), 2.93 (1 H, d of d, $J=10$, 6), 3.94 (1 H, t, $J=11$), 4.91 (1 H, d, $J=1$), and 4.94 (1 H, d, $J=1$); *m/e* 250 (M^+, 10%), 232 (100), 217 (12), 193 (11), 191 (14), 177 (16), 165 (86), 159 (60), 158 (35), 151 (24), 147 (24), 133 (36), 123 (30), 121 (51), 119 (34), 110 (26), 109 (32), 107 (53), 105 (38), 95 (37), 91 (45), 81 (38), 79 (34), 77 (27), 67 (36), 55 (55), 53 (33), 43 (43), and 41 (47)

3β-HYDROXY-8-OXO-6βH,7αH,11βH-GERMACRAN-4(14),9(10)-DIEN-6,12-OLIDE (**8**).—Fraction 5 (9.48 g) from the chromatography on the Waters 500 which gave pure **8** was rechromatographed by hplc on a prepacked Partisil ODS-2 M9 column with methanol-water (2:3) as the eluent. The peak, which contained **8**, was collected; and the solvents were removed *in vacuo*. Final purification was affected by crystallization from ether-ethyl acetate (1:1); colorless square crystals of **8** (35 mg) when obtained, mp 155–156°; mass spectroscopic molecular weight 264.138 (calc. for $C_{15}H_{20}O_4$ 264.136); ν ($CHCl_3$) 3450, 1765, 1670 and 885 cm^{-1}; ^1H nmr ($CDCl_3$), 1.41 (3 H, d, $J=7.3$), 1.66 (3 H, d, $J=1$), 3.91 (1 H, m), 4.16 (1 H, t, $J=9.4$), 5.00 (1 H, br d, $J=9.4$) and 5.79 (2 H, br s), irradiation at δ 4.16 resulted in the collapse of the doublet at δ 5.00; *m/e* 264 (M^+, 3%), 246 (3), 218 (5), 191 (9), 181 (6), 173 (6), 167 (6), 163 (9), 145 (42), 127 (50), 121 (42), 109 (71), 97 (66), 95 (76), 93 (60), 83 (53), 81 (63), 71 (79), 69 (100), 67 (47), 58 (42) and 55 (92).

3β,8α-DIHYDROXY-6βH,7αH,11βH-GERMACRAN-4(14),9(10)-DIEN-6,12-OLIDE (**10**).—The ethyl acetate stripping, fraction 9 (2.86 g), from the above chromatography which gave pure **10** was then rechromatographed on the Waters 500 with hexane-ethyl acetate (2:3) as the eluent to yield fraction 4 (0.40), which had one major peak by glc analysis, (3% OV 17, 248°) R_t 13.5 min. The compound corresponding to this peak was obtained pure by crystallization from methanol to give colorless needles (50 mg) with mp 199–199.5°; mass spectroscopic molecular weight minus water 248.149 (Calc for $C_{15}H_{20}O_3$ 248.141); ν (nujol) 3300, 1770 and 910 cm^{-1}; ^1H nmr (pyridine-d_5) 1.61 (3 H, d, $J=1$), 1.69 (3 H, d, $J=7$), 2.95 (1 H, br m), 4.10 (2 H, br m), 4.65 (1 H, br t, $J=9.8$), 5.18 (1 H, br s), 5.53 (1 H, br d, $J=6$) and 5.68 (1 H, br s), irradiation at δ 2.95 resulted in the collapse of the doublet at δ 1.69; ^1H nmr (acetone-d_6) 1.31 (3 H, d, $J=7.1$), 1.59 (3 H, d, $J=1$), 4.40 (1 H, d of t, $J=10$, 4.2), 5.02 (1 H, d, $J=1.9$), 5.31 (1 H, br d, $J=9.6$) and 5.36 (1 H, d, $J=1.9$); *m/e* 248 (M^+, 3%), 220 (3), 193 (2), 175 (6), 174 (4), 165 (4), 163 (4), 157 (11), 151 (16), 135 (11), 133 (11), 124 (11), 123 (21), 121 (26), 109 (35), 107 (35), 105 (28), 97 (36), 95 (55), 93 (45), 91 (33), 85 (26), 82 (47), 81 (22), 80 (38), 78 (42), 76 (29), 71 (55), 70 (22), 69 (100), 67 (55), 55 (81) and 53 (42).

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