Pergamon

PII: S0031-9422(96)00225-7

Phytochemistry, Vol. 43, No. 1, pp. 67–69, 1996

0031-9422/96 $15.00 + 0.00

FUNGISTATIC SESQUITERPENOIDS FROM *PARTHENIUM*

GALAL T. MAATOOQ and JOSEPH J. HOFFMANN*

University of Arizona, College of Agriculture, Office of Arid Lands Studies, Bioresources Research Facility, 250 E. Valencia Road, Tucson, AZ 85706, U.S.A.

(*Received in revised form* 1 *February* 1996)

Key Word Index—*Parthenium argentatum* × *P. tomentosa*; Asteraceae; β-eudesmol; γ-eudesmol; partheniol; germacrene; guayulone; sesquiterpenes.

Abstract—β-Eudesmol, γ-eudesmol and partheniol were isolated from the derubberized resin of *Parthenium argentatum* × *P. tomentosa*; a new dinorsesquiterpenoid diketone (guayulone) was also isolated. Partheniol and guayulone demonstrated fungistatic activity. These compounds were identified by their spectroscopic and physical data. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

As part of our ongoing search for bioactive secondary metabolites from *Parthenium argentatum* × *P. tomentosa* (guayule hybrid), we reported the isolation of antifungal eudesmane sesquiterpenoids [1]. As a continuation of this study, we isolated β- and γ-eudesmol together with partheniol and guayulone, a methoxylated dinorsesquiterpenoidal diketone.

RESULTS AND DISCUSSION

Compounds **1** and **2** were characterized as β-eudesmol and γ-eudesmol, respectively. Their identity was based on the comparison of the reported EI-mass spectrometry, and ^{1}H and ^{13}C NMR data [2–5]. The high-resolution mass spectrum of compound **3** gave *m/z* 220.1793, for $C_{15}H_{24}O$ (calculated, 220.1828). It was found to be co-chromatographically identical with an authentic sample of partheniol (W. Schloman, University of Akron, OH, USA). Furthermore, the ^{1}H and ^{13}C-NMR data of compound **3** are comparable with the reported data [6].

Compound **4** gave a high-resolution EI-mass spectrum ion peak of *m/z* 234.1212, for $C_{14}H_{18}O_3$ (calculated, 234.1256). This indicated the presence of six double bond equivalents. The base peak of *m/z* 177 $[M - C_4H_9]^+$ suggested the presence of an isobutyl terminal group. The ^{13}C NMR spectrum displayed 13 carbon signals, one was assigned to two carbons (C-12 and C-13). The DEPT experiment discriminated the spectrum into three CH_3, one OCH_3, one aliphatic CH_2, three olefinic CH and five nonprotonated carbons, including two carbonyls and three olefinic carbons. These results indicated the presence of a single ring. The carbonyl signal at 201.96 ppm was assigned to an exocyclic α-β unsaturated ketone at the 8-position. The other carbonyl signal at 196.34 ppm was assigned to the quinone-like keto group at the 3-position. This was supported by the UV absorption at λ_{max} 245 nm, 268 nm and 298 nm (sh). The ^{1}H NMR spectrum integrated for 18 protons. The doublet at 0.96 ppm ($J = 7.0$ Hz) integrated for the six protons of the terminal isopropyl group. A COSY[45] experiment proved that these two methyls are coupled to the proton multiplet at 2.23 ppm, assigned for the CH of the isopropyl. This multiplet interacts with the doublet at 2.86 ppm ($J = 7.0$ Hz) and is assigned to a CH_2 at the 7-position. This CH_2 was correlated with the downfield shifted carbon signal at 52.58 ppm, suggesting its closeness to the exocyclic keto group. This was confirmed by a selective INEPT experiment (Fig. 1) [1], where 9 Hz of irradiation at 2.23 ppm (H-11) enhanced the carbon signals at 201.96 (C-8), 52.58 (C-7) and 22.66 ppm (C-12 and C-13). The same irradiation at 2.86 ppm (H-7) enhanced the carbon signals at 201.96 (C-8), 24.88 (C-11) and 22.66 ppm (C-12 and C-13). The downfield shift of the proton signal at 2.59 ppm, assigned to the C-15-methyl group, suggested the

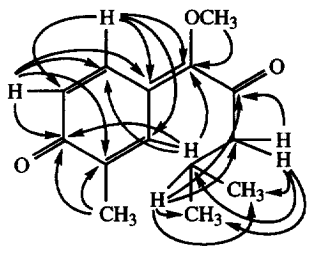
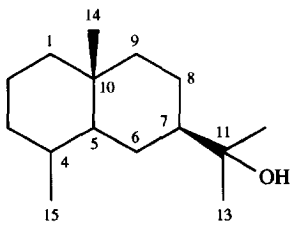
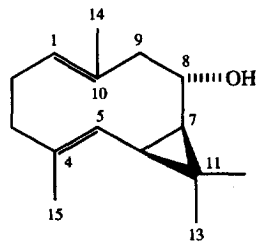


Fig. 1. Results of a selective INEPT experiment for compound **4**.

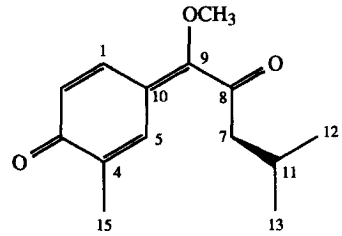
*Author to whom correspondence should be addressed.



$1 = \Delta^{4(15)}$
$2 = \Delta^{4}$



3



4

location of a double bond and an α-β keto system in this region [7]. The olefinic double doublet at 8.12 ppm (1H, $J = 7.0$, 1.5, H-1) was coupled to the doublet at 7.03 ppm (1H, $J = 7.5$ Hz, H-2) and meta coupled to the doublet at 8.22 ppm (1H, $J = 1.5$ Hz, H-5). The location of the methoxyl group at the 9-position and the full assignments were concluded with the help of a HETCOR experiment and the intensive use of the selective INEPT experiment (Fig. 1).

The presence of the known β-eudesmol and γ-eudesmol in *P. argentatum* × *P. tomentosa* is reported here for the first time. Partheniol was not isolated before from guayule except as its esters [8, 9]. Also, compounds **1** and **2** were found in the acetone extract of several fresh cultivar samples of *P. argentatum* Gray provided by Dr Dennis Ray (Plant Geneticist, University of Arizona, USA). Their identities were verified by TLC, GC and HPLC-PDA comparison.

The bioassay [1] revealed that compound **3**, at a concentration of 1.0 mg ml^{-1} inhibited the growth of *Aspergillus niger* cultures by 75% after 8 days. However, absolute inhibition of sporulation was observed, even after 6 months. At the same concentration compound **4** demonstrated a 40% growth inhibition.

EXPERIMENTAL

Instrumentation, isolation, bioassay and initial isolation procedures. Additional neutral material was fractionated as in ref. [1] to provide fr. A (23.5 g) and fr. B (5.2 g).

Fr. A was flash column chromatographed on silica gel (900 g, 40 μ, 6 × 60 cm column). Elution started with hexane, then with 5% Me_2CO (v/v) increments with each litre and collected frs were 500 ml each. This resulted in two main subfrs, fr. A_1 (8.5 g) eluted with 10% Me_2CO–hexane and A_2 (880 mg) eluted with 15–20% Me_2CO–hexane. Fraction A_1 was visualized as a grey spot with a red cap above on TLC after spraying with vanillin/sulphuric acid and heating for 5–10 s. with a heat gun. It was subjected to acetylation using 1:1 mixt. of C_5H_6N–Ac_2O followed by the usual work-up. The unacetylated fraction of the reaction mixture (5.8 g) was obtained after CC on silica gel (900 g, 40 μ, 6 × 60 cm) eluted with 5% EtOAc–hexane. A portion (1.0 g) of the unacetylated grey spot gave two compounds, **1** (96 mg) and **2** (123 mg) by HPLC chromatography (Hitachi, L4500 diode array detector, AS-4000 autosampler, D-6000 interface, L 6200 A pump, with 486 IBM PC compatible computer programmed with model D-6500 DAD system manager; the analytical column is Rainin Microsorb-MV, 10 cm long × 0.46 cm i.d., packed with C18, 3 μ, with pore size 100 Å. The preparative column was Alltech econosil, 50 cm long × 2.25 cm i.d., packed with C18, 10 μ, 60 Å. The eluant was CH_3CN-H_2O (65:35). Compound **1** was obtained as white needles, mp 75–76° (Lit. 74–76° [2]), while **2** was a yellow oil. Fr. A_2 demonstrated a fungal sporulation inhibitory activity against *A. niger* cultures. After repeated CC and prep. TLC, using hexane–EtOAc (80:20) and hexane–CH_2Cl_2 (20:80), 283 mg of compound **3** was recovered as white needles.

Partheniol; *8α-hydroxy-6-bicyclogermacrene*; *6-bicyclohumula-1(10)-4-dien-8α-ol* (**3**). Mp 126–127°, $[\alpha]_D^{20}$ +0.263 ($CHCl_3$; *c* 0.25), IR ν_{max}^{KBr} cm^{-1}, 3300, 2965, 1640, 1450, 1380, 1000, 850. EI-MS; 70 eV, *m/z* (relative intensity): 220 $[M]^+$ (8), 205 $[M - CH_3]^+$ (3), 202 $[M - H_2O]^+$ (4), 177 $[M - C_3H_7]^+$ (8), 161 $[M - CH_3 - C_3H_7]^+$ (3), 43 $[C_3H_7]^+$ (60).

Fr. B (3.4 g) was subjected to flash CC using silica gel 60 (40–60 μ, 300 g, 3.5 × 45 cm column). The elution profile was hexane–CH_2Cl_2 (1:1), hexane–CH_2Cl_2 (1:1) with 1% Me_2CO (v/v), hexane–CH_2Cl_2 (25:75) with 1% Me_2CO (v/v), CH_2Cl_2–Me_2CO 99:1, 97:3, 95:5, 92.5:7.5, 90:10, 87.5:12.5 and 85:15 (400 ml each)., Frs 6–8 (104 mg) eluted with hexane–CH_2Cl_2 (25:75 with 1% Me_2CO), were subjected to prep. TLC on 1 mm thick silica gel plates using 25% Me_2CO–hexane as a solvent (R_f, 0.88). This yielded 25 mg of compound **4** as a yellow oil.

Guayulone **4**. $[\alpha]_D^{25}$ −0.011 ($CHCl_3$; *c* 0.475), UV λ_{max} nm 245, 268 and 298 sh, IR ν_{max}^{KBr} cm^{-1}, 2985, 1685, 1605, 1265, 1180, 1020. EI-MS, 70 eV, *m/z* (relative intensity); 234 $[M]^+$ (8), 219 $[M - CH_3]^+$ (13), 203 $[M - OCH_3]^+$ (4), 191 $[M - C_3H_7]^+$ (6), 177 $[M - C_4H_9]^+$ (100), 162 $[M - C_4H_9 - CH_3]^+$ (1), 147 $[M - C_4H_9 - 2CH_3]^+$ (4). The HR EI-MS gave *m/z* 234.1212, for $C_{14}H_{18}O_3$ (calcd, 234.1256). ^1H NMR (250 MHz, $CDCl_3$, δ ppm, *J* Hz): 8.12 (1H, *dd*, $J = 7.0$, 1.5, H-1), 7.03 (1H, *d*, $J = 7.5$, H-2), 8.22 (1H, *d*, $J = 1.5$, H-5), 2.86 (2H, *d*, $J = 7.0$, H-7), 2.23 (1H, *m*, H-11), 0.96 (6H, *d*, $J = 7.0$, H-12 and H-13), 2.59 (3H, *s*, H-15), 3.98 (CH_3O-, *s*). ^{13}C NMR (62.5 MHz,

$CDCl_3$, δ ppm): 133.15 (C-1), 111.44 (C-2), 196.34 (C-3), 130.06 (C-4), 131.05 (C-5), 52.58 (C-7), 201.96 (C-8), 161.58 (C-9), 128.65 (C-10), 24.88 (C-11), 22.66 (C-12 and C-13), 26.40 (C-15), 55.88 (CH_3O-).

REFERENCES

1. Maatooq, G. T., Stumpf, D. K., Hoffmann, J. J., Hutter, L. K. and Timmermann, B. N. (1996) *Phytochemistry* **41**, 519.
2. Elgamal, M. H. and Wolf, P. (1987) *Planta Med.* **53**, 293.
3. Barton, D. H. R., Belocil, J., Billion, A., Boivin, J., Lallemand, J., Lelandais, P. and Mergui, S. (1989) *Helv. Chem. Acta* **70**, 2187.
4. El-Sayed, A. M., Al-Yahya, M. A. and Hassan, M. M. (1989) *Int. J. Crude Drug Res.* **27**, 185.
5. van Beek, T. A., Kleis, R., Posthumus, M. A. and van Veldhuizen, A. (1989) *Phytochemistry* **28**, 1909.
6. Paarodi, F. J., Nauman, M. and Fischer, N. H. (1987) *Spectroscopy Letters* **20**, 97.
7. Bohlmann, F., Zdero, C., Gupta, R. K., King, R. M. and Robinson, H. (1980) *Phytochemistry* **19**, 2695.
8. Proksch, P., Behl, H. M. and Rodriguez, E., (1981) *J. Chromatogr.* **213**, 345.
9. Schloman, W. W., Jr., Hively, H. A., Krishen, A. and Andrews, A. M. (1983) *J. Agric. Food Chem.* **31**, 873.