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NON-TRICHOTHECENES FROM *BACCHARIS MEGAPOTAMICA*

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Key Word Index—*Baccharis megapotamica*; Asteraceae; megapodiol.

Abstract—A large scale extract of *Baccharis megapotamic* has yielded small quantities of several known compounds (2,6-dimethoxybenzoquinone, scopoletin and clovandiol) and a new natural product, megapodiol.

INTRODUCTION

An extract of the Brazilian shrub, *Baccharis megapotamica* Spreng yielded a series of very active (*in vivo*) antileukaemic sesquiterpenes which were shown to be members of the trichothecene complex of antibiotics [1]. These compounds, called baccharinoids, are closely related in structure to the roridins which are fungal metabolites produced principally by *Myrothecium roridum* and *M. verrucaria* [2, 3]. We have presented evidence that the baccharinoids are the result of a plant–fungus interaction whereby fungal-produced roridins are taken up and metabolized by *B. megapotamica* to yield the baccharinoids found in the plant tissue. Because of the exceptionally high antileukaemic activity of the baccharinoids, a large collection (*ca* 3500 lbs) of *B. megapotamica* was made. Extraction of this material, initial fractionations (e.g. solvent partitions) and large scale chromatography were carried out under contract by the National Cancer Institute to Polyscience, Inc. The procedures were reported in preliminary from [4, 5], and the details will be reported elsewhere. Herein, we report the isolation of four non-trichothecene crystalline compounds from this large scale extract.

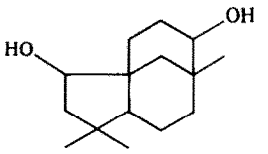
RESULTS AND DISCUSSION

After solvent partitioning, the initial crude extract (*ca* 30 kg) was reduced in weight to 4.7 kg. A large scale silica gel chromatography (hexane–CH_2Cl_2, CH_2Cl_2, MeOH–CH_2Cl_2 eluants) yielded ten fractions (fractions I–X). Trituration of fraction II (275 g) with ether gave a yellow crystalline material which upon recrystallization gave 7.0 g of 2,6-dimethoxybenzoquinone. Fractions III–V (*ca* 25 g each in weight) contained a compound which gave a highly fluorescent spot on the TLC plate. Trituration of these fractions with ether yielded a solid material which upon recrystallization yielded 1.9 g of scopoletin, the compound responsible for the fluorescent spot observed above on TLC.

The ether soluble fractions II–V proved to be difficult to purify further because of large amounts of intractable oily material. Washing with a 2.5% sodium hydroxide solution removed about half (by weight) of the material. Recovery of the base soluble material gave brown gums which upon TLC analysis showed nothing but streaks on the TLC plate. All attempts at isolating crystalline compounds from these gums were unsuccessful. The ether soluble fractions above upon extensive chromatographic procedures yielded small quantities of the less polar roridins and baccharinoids.

Further chromatography of the more polar fraction VII, gave a fraction which after washing with 2.5% aqueous sodium hydroxide and extensive chromatography gave (+)-clovan-2β,9α-diol (**1**), a compound previously isolated [6] and whose structure we have confirmed on the basis of NMR and mass spectral data. Also isolated from this fraction was a new compound, megapodiol (**2**).

Megapodiol is a colourless crystalline and optically active ($[\alpha]_D$ – 52.0°) compound with a molecular formula



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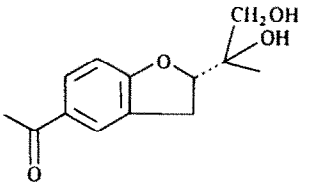

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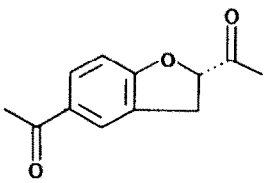

3

of $C_{13}H_{16}O_4$ (HR-MS). IR and UV spectral data suggested the presence of an arylketone. ^{1}H NMR spectroscopy showed the aromatic ring to be 1,2,4-trisubstituted in which a carbonyl group and ether oxygen atom appear to be *para* with respect to each other. Methyl resonances appear as singlets at δ1.20 and δ2.54; the latter signal is assigned to a methyl ketone. Diastereotopic protons (2H each) appear at $\sim\delta$3.3 and δ3.6. Upon acetylation (Ac_2O–pyridine), a monoacetate is formed, and the resonance at $\sim\delta$3.6 moves downfield to $\sim\delta$4.2 which indicates that the two proton AB pattern at δ3.6 is due to a hydroxymethylene group. The ABX multiplet at $\sim\delta$3.3 is due to benzylic protons adjacent to a chiral centre. Treatment of megapodiol with Jones' reagent gave diketone **3**, a compound previously reported and whose absolute stereochemistry has been established [7]. The reported optical activity for **3** is $[\alpha]_D -41°$. In our hands the $[\alpha]_D$ is $-20.5°$. Careful purification by HPLC and recrystallization did not alter this value. Since the sign rotation (−) is the same as reported earlier [6] we conclude that **3** has the absolute stereochemistry shown. The ^{13}C NMR spectrum and the mass spectral fragmentation pattern of **2** confirm the structure assignment.

The natural product tremetone has been oxidized (Ag_2O–I_2) to a diol having the overall structure of **2** [8] but with a specific rotation twice that observed for **2** ($-52°$ vs $-105°$). Since in our hands, ketone **3** also has a specific rotation just half that value reported by these same workers for **3**, we are unable to conclude whether our **2** has the same absolute configuration as that reported earlier for semisynthetic **2** [8]. In fact, from the synthesis of semisynthetic **2** from tremetone, it would appear that a mixture of diastereomers should have been formed, though no mention of this was made in the report [6]. A related congener of **2**, 12-acetoxytremetone, recently was isolated from *Baccharis tricuneata* [7].

Our experience with this extract of *B. megapotamica* has proven somewhat frustrating because of the high percentage of intractable oily and gummy material. By far and away the highest amount of crystalline material from this plant has proven to be the baccharinoids [8] which the plant has not even synthesized but has acquired from an external source.

EXPERIMENTAL

General methods. Mps (Fisher–Johns hot-stage apparatus): uncorr. NMR: IBM WP-200 SY, $CDCl_3$, TMS as int. standard. ^{13}C NMR chemical shift assignments were made by comparing proton decoupled spectra with spectra obtained in INEPT experiments and by use of structure-shift correlations/empirical additivity rules; IR: polystyrene absorption at 1601.8 cm^{-1} as calibration; TLC: silica gel plates (E. Merck or Analtech), visualization with short-wavelength UV light or H_2SO_4–EtOH–vanillin (20:3:1) spray; flash chromatography: silica gel 60 (230–400 mesh, E. Merck or Whatman LPS-2); MPLC: Whatman LPS-1 silica gel; prep. TLC: centrifugally accelerated, radial chromatotron model 7924 (Harrison Research, Palo Alto, CA) with plates coated with silica gel PF-254 (E. M. Merck No. 7749); microanalyses were determined by Dr. Franz Kasler, University of Maryland.

Isolation of 2,6-dimethoxybenzoquinone. Fraction II (275 g) was taken up in 1 l. of Et_2O, and the solids collected by filtration. Recrystallization yielded 7.0 g **1**, mp 256–257°, reported [9] mp 239–242°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1690, 1640, 1590, 1450, 1315, 1700, 860; ^{1}H NMR: δ3.89 (6H, *s*) (5.86) (2H, *s*).

Isolation of scopoletin. TLC analysis of fractions III (25.7 g), IV (22.5 g) and V (36.2 g) showed the presence of a highly fluorescent spot. Trituration of fractions IV and V with Et_2O gave solids which were collected by filtration and recrystallized from $CHCl_3$ to give 1.9 g **2**, mp 203–204°; reported [10] mp 203°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520, 1715, 1615, 1480, 1290, 1740, 860; ^{1}H NMR (Me_2CO-d_6): δ3.83 (3H, *s*), 6.21 (1H, *d*, J = 9.5 Hz), 6.79 (1H, *s*), 6.79 (1H, *s*) 7.21 (1H, *s*), 7.91 (1H, *d*, J = 9.5 Hz).

Isolation of clovan-2β,9α-diol (**1**). Fraction VII (1.1 kg), was subjected to silica gel chromatography (1–3% MeOH–CH_2Cl_2) to yield six fractions. The fifth fraction (39.2 g) was taken up in 200 ml CH_2Cl_2 and washed with 2 × 200 ml of 2.5% NaOH. Concn of the organic phase gave 17.3 g of gum which was triturated with Et_2O. The Et_2O soluble fraction (12.3 g) after solvent removal was subjected to filtration chromatography over 80 g alumina (neutral, activity II/III) with 20% *iso*-PrOH in hexane (1 l.), 100% *iso*-PrOH (1 l.) and MeOH (1 l.). The first fraction (10.0 g) was concd and applied to a Sephadex LH-20 column (200 g). Elution with CH_2Cl_2 gave **1** which upon recrystallization (Et_2O) gave 90 mg, mp 152–153°, reported [5] mp 152–153°; $[\alpha]_D^{25}$ + 3.6 (*c* 1.5; $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350, 1078 cm^{-1}; ^{1}H NMR: δ0.86, 0.97, 1.04 (each 3H, *s*, H-13, H-14, H-15), 1.10–1.60 (7H, *m*, H-5, H-6, H-7 and H-12), 1.60–1.75 (4H, *m*, H-3 and H-11), 2.01 (2H, *ddt*, J = 15, 5 and 3 Hz, H-10), 3.33 (1H, *s* (*br*), H-9), 3.79 (1H, *dd*, J = 10 and 6 Hz, H-2); ^{13}C NMR: δ20.6 (C-11), 25.3 (C-15), 26.0 (C-6 or C-10), 26.3 (C-10 or C-6), 28.3 (C-13), 31.4 (C-14), 33.1 (C-7), 34.7 (C-4), 35.5 (C-12), 37.1 (C-8), 44.2 (C-1), 47.5 (C-3), 50.5 (C-5), 75.0 (C-9), 80.0 (C-2); HR-MS: calc. for $C_{15}H_{16}O_2$: 238.1934. Found: 238.1932. Calc. for $C_{15}H_{16}O_2$: C, 75.55; H, 11.01. Found: C, 75.20; H, 11.00. Diacetate of **1**. ^{1}H NMR: δ0.83, 0.90, 1.03 (each 3H, *s*, H-13, H-14 and H-15), 1.10–1.60 (7H, *m*, H-5, H-6, H-7 and H-12), 1.60–1.85 (4H, *m*, H-3 and H-11), 2.03 (6H, *s*, OAc), 2.10–2.30 (2H, *m*, H-10), 4.53 (1H, *s* (*br*) H-9), 4.85 (1H, *t*, J = 8 Hz, H-2).

Isolation of megapodiol (**2**). The fourth fraction from the second large-scale chromatography (i.e. chromatography of fraction VII), was subjected to prep. HPLC (Waters 500 instrument) [4] to yield a number of fractions. From one of these fractions, after washing with 2.5% aqueous NaOH, filtration chromatography (alumina, neutral, activity II/III), flash and MPLC, prep. TLC and finally HPLC (silica gel, EtOAc–hexane, 3:1) was isolated 50 mg **2**, mp 135–136°; $[\alpha]_D^{25} -52°$ (*c* 1.0; MeOH); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400–3600 (br), 1680, 1610, 1595, 1490, 1440, 1370, 1290, 1275, 980; UV λ_{max}^{MeOH} nm: 226, 280, 286; ^{1}H NMR: δ7.83 (1H, *d*, $J_{4,6}$ = 1.2 Hz, H-4), 7.80 (1H, *dd*, $J_{4,6}$ = 1.2 Hz, $J_{6,7}$ = 8.2 Hz, H-6), 6.79 (1H, *d*, $J_{6,7}$ = 8.2 Hz, H-7), 4.91 (1H, *dd*, $J_{2,3\alpha} = J_{2,3\beta}$ = 9.3 Hz, H-2), 3.67 (2H, AB, J_{AB} = 11.0 Hz, H-11), 3.28 (2H, ABX, J_{AB} = 15.6 Hz, $J_{AX} = J_{BX}$ = 9.3 Hz, H-3), 2.54 (3H, *s*, H-14), 1.20 (3H, *s*, H-12); ^{13}C NMR: δ19.0 (C-12), 26.3 (C-14), 29.6 (C-3), 67.0 (C-11), 73.5 (C-10), 86.2 (C-2), 108.8 (C-7), 125.5 (C-6), 127.8 (C-4), 130.3 (C-9), 131.0 (C-5), 163.7 (C-8), 196.7 (C-13); HR-MS: calc. for $C_{13}H_{16}O_4$: 236.1049 Found: 236.1052 $[M]^+$.

Megapodiol monoacetate. ^{1}H NMR: δ7.81 (1H, *d*, $J_{4,6}$ = 1.2 Hz, H-4), 7.77 (1H, *dd*, $J_{4,6}$ = 1.2 Hz, $J_{6,7}$ = 8.3 Hz, H-6), 6.79 (1H, *d*, $J_{6,7}$ = 8.3 Hz, H-7), 4.83 (1H, *dd*, $J_{2,3\alpha} = J_{2,3\beta}$ = 9.3 Hz, H-2), 4.20 (2H, AB, J_{AB} = 11.3 Hz, H-11), 3.28 (2H, ABX, J_{AB} = 15.6 Hz, $J_{AX} = J_{BX}$ = 9.3 Hz, H-3), 2.52 (3H, *s*, H-14), 2.11 (3H, acetate), 1.23 (3H, *s*, H-12).

Jones oxidation of **2**. Jones' reagent (0.5 ml, prepared by dissolving 70.0 g CrO_3 in 500 ml H_2O and 61 ml H_2SO_4) was added dropwise to a stirred soln of megapodiol (**4**) (10 mg) in Me_2CO (2 ml) until the orange colour persisted for 5 min. After 10 min of further stirring, the Me_2CO soln was filtered. After removal of Me_2CO, the product was taken up in CH_2Cl_2 and purified on the Chromatotron (silica gel, EtOAc–hexane) to yield **3** (6.0 mg) as a colourless noncrystalline material $[\alpha]_D^{25} -20.5°$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 1675, 1605, 1595, 1360, 1290, 1260, 1110;

^{1}H NMR: δ2.29 (3H, *s*), 2.52 (3H, *s*), 5.03 (1H, J = 3.3, 5.0 Hz, H-2), 3.44 (1H, *dd*) 3.34 (1H, *dd*) 6.89 (1H, *d*, J = 8 Hz) 7.81 (2H, *m*); MS *m/z*: 204 $[M]^+$, 189, 161, 145, 118, 89, 63, 43 (100%).

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SESQUITERPENE LACTONES FROM *CENTAUREA CORONOPIFOLIA*

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Key Word Index—*Centaurea coronopifolia*; Compositae; sesquiterpene lactones; germacranolides; flavonoids.

Abstract—The aerial parts of *Centaurea coronopifolia* afforded three new germacronolides, two of which are closely related to balsamin. The third is a new derivative of stizolicin, which is also present. The aerial parts also yielded four 6-methoxylated flavonoids and the triterpene, α-amyrin. The structures of the compounds were determined by spectroscopic methods.

INTRODUCTION

In the course of our investigation on the genus *Centaurea* [1–4], we have isolated four sesquiterpene lactones from the leaves of *Centaurea coronopifolia* Lam. Although one of the lactones, stizolicin, was isolated from *C. coronopifolia* [5] under the syn. *Stizolophus coronopifolius*, the other three are new: 1(10)en-4α-5β-epoxy-9α-hydroxy germacranolides with 8α-(4-hydroxy senecioate) (**2**), 8α-senecioate (**3**) and 1(10)en-4α,5β-epoxy-8α-(4-hydroxy senecioate) (**4**) side chains.

In addition, we also isolated the 6-methoxylated flavonoids 6-methoxyapigenin (**6**), 6-methoxyluteolin (**7**), quercetagetin 3,6-dimethyl ether (**8**) and quercetagetin 3,6-dimethyl ether 7-*O*-glucoside (**9**). The last two flavonoids were isolated from a *Centaurea* species for the first time. The leaves also yielded large amounts of the well known triterpene, α-amyrin.

RESULTS AND DISCUSSION

Compound **2** had the molecular formula $C_{20}H_{26}O_7$ (EIMS). Its IR spectrum showed the presence of a γ-lactone (1745 and 1140 cm^{-1}), an α,β-unsaturated ester function (1705 and 1280 cm^{-1}) and hydroxyl groups (3400 cm^{-1}). Its structure was established by ^{1}H NMR spectroscopy.

The ^{1}H NMR spectrum of **2** (Table 1) contained doublets for an exocyclic methylene conjugated with a γ-lactone at δ6.29 and 5.76 (^4J = 3.5, 3 Hz). A doublet at δ2.62 (J = 9 Hz) for H-5 supported an epoxide group between C-4/C-5. This doublet collapsed to a singlet when the double doublet at δ4.33 (J = 6.5, 9 Hz) (H-6) was irradiated and, in addition, the signal at δ3.82 (*dddd*, H-7) was simplified. However, irradiation of H-7 did not affect the double doublet at δ2.62 (H-5) but converted the double doublet at δ4.33 to a doublet (J = 9 Hz), the