



BENZOFURANS, BENZOFURAN DIMERS AND OTHER CONSTITUENTS FROM *OPHRYOSPORUS CHARUA*

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Abstract—Aerial parts of *Ophryosporus charua* afforded several known and five new benzofuran derivatives, as well as two prenylated *p*-hydroxyacetophenone derivatives. The essential oil contained lavandulyl acetate, lavandulol and dehydrotremetone as the main constituents. © 1997 Elsevier Science Ltd

INTRODUCTION

Ophryosporus is a South American genus of nearly 40 species [1], eight of which have been investigated chemically [2–8]. Prenylated acetophenones, including tremetone derivatives, chromenes and chromanones, as well as labdanes, are common and, in three species, sesquiterpene lactones have been found [5, 6, 8]. The present report deals with *Ophryosporus charua*, a species of north western Argentina, the aerial parts of which are used in local traditional medicine as an antisyphilitic [9].

RESULTS AND DISCUSSION

The extract of the flowers and leaves furnished the benzofurans **1a** [10], **1b** [11], **1c**, **2a** [12, 13], **2b** (toxol) [14, 15] **2c** and **3** [2, 4], the prenylated chromanone (**4**), isolated earlier from *O. lorentzii* [8], and the prenylated *p*-hydroxyacetophenone (**5**) previously found in *O. macrodon* [7]. In addition, two stereoisomeric benzofuran ‘dimers’ (**6a** and **6b**) were isolated, together with small amount of a benzofuran derivative, to which we ascribe structure **7**.

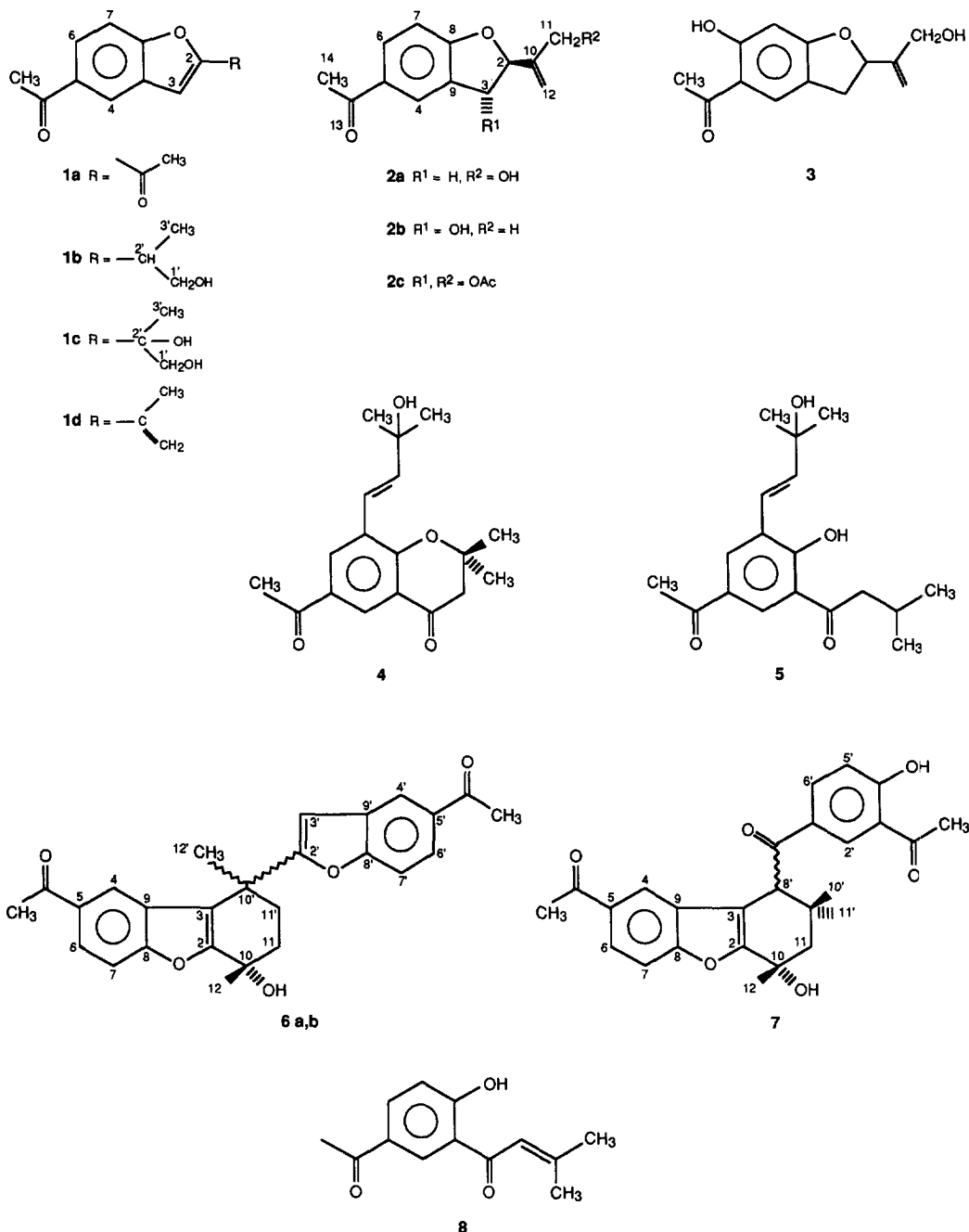
Structures of the new benzofurans **1c** and **2c** were obvious from the mass, ¹H and the ¹³C NMR spectra (see Experimental); in the case of **2c** the magnitude of *J*_{2,3} (2.5 Hz) indicated that the substituents on the five-membered ring were *trans* [15]. The 3-desacetyl analogue has been reported earlier from *Helichrysum italicum* but was correctly described as an ace-

toxytoxol (*J*_{2,3} = 3 Hz), although depicted as the 2,3-*cis* isomer [16].

The empirical formulae C₂₆H₂₄O₅ of two further constituents and their very similar but not identical ¹H and ¹³C NMR spectra (see Experimental), assisted by extensive decoupling indicated that they were formed by combination of two C₁₃H₁₂O₂ (dehydrotremetone) units (**1d**), plus an additional oxygen, such that the 2,3-double bond of one dehydrotremetone unit constituted the double bond of a 1,1,4,4-tetrasubstituted 2-cyclohexene ring, with two methyls, one hydroxyl and the benzofuran moiety of the second dehydrotremetone unit representing the four substituents in such a fashion so as to give rise to a *cis*- and *trans*- isomer, as in **6a,b**. The formation of such ‘dimers’ may involve an initial Diels–Alder-type reaction between two dehydrotremetone units, dehydrotremetone being an important constituent of the essential oil (*vide infra*), followed by allylic hydroxylation or hydroperoxidation of the initial adducts. Support for this mode of formation might also be found in the tendency of **1b** and **2c** to dimerize in the ion-source under the high pressure conditions prevailing during chemical ionization measurements; in the case of **2c** this occurs after initial loss of the elements of acetic acid. Somewhat similar ‘dimers’ accompanying their 2-vinylbenzofuran parents been isolated from the roots of *Ageratum houstonianum* [17].

Less than 1 mg was available for the study of a related substance C₂₆H₂₆O₆, to which we ascribe structure **7**, formed by a similar reaction of dehydrotremetone with dienophile **8**, the latter found, for example, in *O. chilca* [3]. In the ¹H NMR spectrum, the chemical shifts of the protons of the dehy-

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drotremetone parent on the left and the methyl group under the hydroxyl, paralleled those of **6a,b**, while the chemical shifts in the aromatic ring of the second partner were those of a 2-hydroxy-5-isovalerylacetophenone. The remaining signals were a low field singlet at δ 4.88 (H-8'), two mutually coupled doublets at δ 2.53 and 1.81 (H-11a,b) and two methyl singlets (H-10', H-11') at δ 1.39 and 1.01.

Details are given in the Experimental section of an investigation of the essential oil of *O. charua* by GC-mass spectrometric analysis. The principal constituents were lavandulyl acetate (55%), lavandulol

(2.5%) and dehydrotremetone (2.5%); the latter also separated from the oil on standing in the refrigerator.

EXPERIMENTAL

General. For sepn of mixts, HPLC with a differential refractometer was used. The column employed was a Beckman C-18 column (10 m i.d. $\times$ 25 cm). *R_s* were measured from the solvent peak.

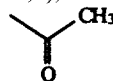
Plant material. Aerial parts of *O. charua* (Griseb.) Hieron. were collected at the flowering stage in September 1987 at Valle de la Sala, Tucumán Province,

Argentina. A voucher specimen (LIL 595246) is deposited in the herbarium of the Instituto Miguel Lillo, Tucumán.

Extraction and isolation. (A) Flowers and leaves (600 g) were extracted with CHCl_3 (2×6 l) at room temp. for 6 days to give 71.2 g (11.8%) of crude extract, which was suspended in EtOH (610 ml) at 55° , dild with H_2O (450 ml) and extracted successively with petrol (3×300 ml) and CHCl_3 (3×900 ml). The CHCl_3 extract, on evapn under red. pres. furnished a residue (18 g) which was chromatographed over silica gel (310 g) using CHCl_3 containing increasing amounts of Et_2O (0–50%) to give 82 frs which were monitored by TLC. Frs 33–56 (2.25 g) from the mother column were combined and rechromatographed over silica gel (120 g) using CHCl_3 containing increasing amounts of EtOAc (0–50%), 69 frs being collected. Frs 8–18 of the rechromatogram were combined (126 mg) and processed by HPLC ($\text{MeOH-H}_2\text{O}$ 2:1, 1.5 ml min^{-1}) to give 4.9 mg of **1a**, R_f 4.5 min, crude mp $128-132^\circ$, followed by 21.1 mg of **2c** as a gum, R_f 9.1 min. Frs 19–32 of the rechromatogram were combined (151 mg) and a portion (50 mg) was reprocessed by HPLC in the same manner to yield an additional 38 mg of **2c**. Frs 33–64 of the rechromatogram were combined (300 mg) and a portion (80 mg) was processed by HPLC ($\text{MeOH-H}_2\text{O}$ 7:3, 3 ml min^{-1}) to give 13.6 mg of **6a** as a gum, R_f 13.2 min, and 1 mg of a substance $\text{C}_{16}\text{H}_{14}\text{O}_5$, MS PCI (isobutane) m/z (rel. int.) 287 (100 [$\text{M} + \text{H}$]), crude mp $95-97^\circ$, R_f 31 min. Frs 65–69 of the rechromatogram were combined (460 mg) and a portion (250 mg) was processed by HPLC ($\text{MeOH-H}_2\text{O}$ 2:1, 2 ml min^{-1}) to give 6 peaks with R_f 's of 5.6, 9, 22.2, 28, 34.5 and 53.5 min, respectively. Rechromatography of peak 1 ($\text{MeOH-H}_2\text{O}$ 11:9, 2 ml min^{-1}) gave 4.5 mg of **3**, R_f 17.7 min, crude mp $56-60^\circ$, and 2.8 mg of **1b**, R_f 19.5 min. Rechromatography of peak 2 gave 9.7 mg of **4** as an amorphous solid, peak 3 gave 2.2 mg of **6a**, peak 4 gave 17.6 mg of **6b** as a gum, peak 5 gave 1.0 mg of **7**, and peak 6 gave 9.3 mg of **5**, crude mp $100-104.5$. Frs 57–71 of the mother column were combined (1.3 g). A portion (150 mg) was triturated with MeOH. The resulting solid (0.8 mg) was a mixt. of **2a** and **2b**; the supernatant soln was processed by HPLC ($\text{MeOH-H}_2\text{O}$, 51:49, 2.5 ml min^{-1}) to give 2 mg of **5**, R_f 6.7 min, 62.3 mg of **2a**, R_f 8 min, 18.8 mg of **3**, R_f 14 min, crude mp $55-58^\circ$ and 23.8 mg of **2b**, R_f 15.5 mg. Frs 72–77 of the mother column were combined (320 mg) and a portion (170 mg) was processed by HPLC in the same manner to give 15 mg of **1c**, R_f 4.7 min, and 9.2 mg of a complex mixt. (R_f 10.5 min) containing benzofurans of type **1** and **2**. Frs 78–82 of the mother column (90 mg) on HPLC afforded similar mixts. Known compounds were identified by MS and ^1H NMR.

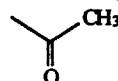
5-Acetyl-2-[1'-hydroxyisopropyl]-benzofuran. (1b). Although this substance has been isolated previously [11], no physical properties have been reported. Mp (without recrystallization) $80-85^\circ$. MS EI MS PCI (isobutane) m/z (rel. int.): 437 (100 [$2\text{M} + \text{H}^+$]), 419

(24.3 [$2\text{M} + \text{H-H}_2\text{O}$]) $^+$, 219 (56.6 [$\text{M} + \text{H}^+$]), 201 (35.0 [$\text{M} + \text{H}$]) $^+$. ^1H NMR (CDCl_3 , 500 MHz): δ 8.13 (d , $J = 2$ Hz, H-4), 7.89 (dd , $J = 9, 2$ Hz, H-6), 7.44 (d , $J = 9$ Hz, H-7), 6.55 (s , H-3), 3.87, (2p, brq , $J = 6.5$ Hz, H-1'a,b); 3.19 (s , $J = 6.5$ Hz, H-2'),



2.62 (s and 3p,), 1.39, (3p, d , $J = 6.5$ Hz, H-3').

5-Acetyl-2-(2-[1,2-dihydroxyisopropyl]-benzofuran (1c). Mp $130-132^\circ$ (without recrystallization). MS PCI (isobutane) m/z (rel. int.): 235 (100 [$\text{M} + \text{H}^+$]), 217 (32.2). ^1H NMR (CDCl_3 , 300 MHz): δ 8.15 (d , $J = 1.5$ Hz, H-4), 7.91 (dd , $J = 8.5, 1.5$ Hz, H-6), 7.46 (d , $J = 8.5$ Hz, H-7), 6.78 (s , H-3), 4.01 (d , $J = 11$ Hz, H-1'a), 3.70 (d , $J = 11$ Hz, H-1'b), 2.62



(s , 3p,), 1.60 (s , 3p, H-3). ^{13}C NMR (CDCl_3 , 75 MHz, assignments are tentative): δ 197.74 (s , C=O), 162.11 (s , C-8), 157.42 (s , C-10), 132.79 (s , C-5), 128.35 (s , C-9), 124.91 (d , C-6), 122.35 (d , C-4), 111.20 (d , C-7), 103.49 (d , C-3), 72.33 (s , C-10), 68.87 (t , C-11), 26.74 (q , CH_3 C=O), 23.49 (q , C-12).

(2S*,3S*)-5-Acetyl-2-(2-[1-acetoxy-2-propenyl]-3-acetoxybenzofuran (2c). Gum. MS EI m/z (rel. int.) 318 (19.2 [M^+]), 276 (11.5), 258 (94.4), 243 (100), 216 (95.6), 203 (30.3), 201 (33.2), 187 (25.6). The PCI MS (isobutane) indicated dimerization in the ion source, with 517 ($2 \times [\text{M} + \text{H} + 2 \text{HOAc}]^+$) as the peak of highest m/z . ^1H NMR (CDCl_3 , 500 MHz): δ 8.02 (d , $J = 2$ Hz, H-4), 7.98 (dd , $J = 8.5, 2$ Hz, H-6) 6.95 (d , $J = 8.5$ Hz, H-7), 6.21 (d , $J = 2.5$ Hz, H-3), 5.34 and 5.29 (both brs , H-12a,b), 5.21 (brd , $J = 2.5$ Hz, H-2), 4.66 and 4.60 (both d , $J = 13$ Hz, H-11a,b), 2.53 (s and 3p, CH_3 C=O), 2.09 and 1.93 (both s and 3p, acetates). ^{13}C NMR (CDCl_3 , 75 MHz, assignments are tentative): δ 196.15 (s , C-13), 170.48 and 170.25 (both s , acetate carbonyls), 164.56 (s , C-8), 139.35 (s , C-10), 137.90 (d , C-4), 131.65 (s , C-5), 128.11 (d , C-6), 124.71 (s , C-9), 117.01 (t , C-12), 110.21 (d , C-7), 88.88 and 77.34 (both d , C-2 and C-3), 63.72 (t , C-11), 26.44 (q , C-14), 21.01 and 20.64 (both q , Ac Me's).

Dimer 6a. Gum. MS PCI (isobutane) m/z (rel. int.): 417 (27.3 [$\text{M} + \text{H}^+$]), 399 (100 [$\text{M} + \text{H-H}_2\text{O}$]) $^+$. ^1H NMR (CDCl_3 , 500 MHz): δ 8.12 (d , $J = 2$ Hz, H-4 or H-4'), 8.03 (d , $J = 2$ Hz, H-4' or H-4), 7.94 (dd , $J = 8.5, 1.5$ Hz, H-6 or H-6'), 7.88 (dd , 8.5, 1.5 Hz, H-6' or H-6), 7.54 (d , $J = 9.5$ Hz, H-7 or H-7'), 7.44 (d , $J = 8.5$ Hz, H-7' or H-7), 6.21 (s , H-3'), 2.57 and 2.56 (both s and 3p, CH_3 C=O), 2.39 (ddd , $J = 14, 5.5, 3$ Hz, H-11 or H-11'a), 2.20 (ddd , $J = 14, 13, 3$ Hz, H-11b resp. H-11'b), 2.01 (ddd , $J = 14, 6, 3$ Hz, H-11'a resp. H-11a), 1.92 (ddd , $J = 14, 14, 3$ Hz, H-11'b resp. H-11b), 1.93 and 1.69 (both s and 3p, H-12 and H-12'). ^{13}C NMR (CDCl_3 , 75 MHz): δ 197.28 and 197.20 (both s , CH_3 -C=O), 163.43 (s , C-2'), 157.69, 157.44 and 157.26 (all s , C-2, C-8 and C-8'), 132.70 (s , C-5 and C-5'), 128.36 and 126.77 (both s , C-9 and C-9'), 125.18 and 124.48 (both d , C-6 and C-6'), 122.09

and 121.82 (both *d*, C-4 and C-4'), 118.28 (*s*, C-3), 111.59 and 110.91 (both *d*, C-7 and C-7'), 103.43 (*d*, C-3'), 66.83 (*s*, C-10), 38.11 (*s*, C-10'), 35.91 and 33.77 (both *t*, C-11 and C-11'), 26.86 and 25.86 (both *q*, C-12 and C-12'), 26.56 and 26.50 (both *q*, CH₃ C = 0).

Dimer 6b. Gum. MS PCI (isobutane) *m/z* (rel. int.): 417 (16.4 [M+H⁺]), 399 (100[M+H-H₂O]⁺). ¹H NMR (CDCl₃, 50 MHz): δ 8.09 (*d*, *J* = 1.5 Hz, H-4 or H-4'), 7.94 (*d*, *J* = 2 Hz, H-4' or H-4), 7.89 (*dd*, *J* = 8.5, 2 Hz, H-6' or H-6), 7.86 (*dd*, *J* = 9, 1.5 Hz, H-6 or H-6'), 7.51 (*d*, *J* = 9 Hz, H-7 or H-7'), 7.40 (*d*, *J* = 8.5 Hz, H-7' or H-7), 6.47 (*s*, H-3'), 2.59 and 2.48 (both *s* and 3p, CH₃ C = 0), 2.58 (*ddd*, *J* = 14, 8.5, 4 Hz), 2.08 (*m*, 2p), 1.95 (*ddd*, *J* = 14, 8.5, 3.5 Hz), the three preceding signals corresponding to H-11a,b and H-11'a,b 1.85 and 1.71 (both *s* and 3p, H-12 and H-12'); ¹³C NMR (CDCl₃, 75 MHz) δ 197.63 and 197.39 (both *s*, CH₃-C = 0), 163.89 (*s*, C-2'), 157.57, 157.47 and 157.33 (all *s*, C-2, C-8 and C-8'), 132.67 and 132.63 (both *s*, C-5 and C-5'), 128.42 and 126.70 (both *s*, C-9 and C-9'), 125.24 and 124.67 (both *d*, C-6, and C-6'), 122.06 and 121.74 (both *d*, C-4 and C-4'), 118.32 (*s*, C-3), 111.68 and 111.03 (both *d*, C-7, and C-7'), 103.49 (*d*, C-3'), 67.47 (*s*, C-10), 37.81 (*s*, C-10'), 35.97 and 34.47 (both *t*, C-11, and C-11'), 26.71 (three *q*, both CH₃ C = 0) and C-12 or C-12'), 24.72 (*q*, C-12' or C-12).

Compound 7. Gum. MS PCI (isobutane) *m/z* (rel. int.): 435 (20.3, [M+H⁺]), 417 (100 [M+H-H₂O]⁺). ¹H NMR (CDCl₃, 500 MHz): δ 12.67 (bonded OH), 8.92 (*d*, *J* = 2 Hz, H-2'), 8.14 (*dd*, *J* = 8, 1.5 Hz, H-6'), 7.89 (*dd*, *J* = 8.5, 2 Hz, H-6), 7.76 (*d*, *J* = 1.5 Hz, H-4), 7.50 (*d*, *J* = 8.5 Hz, H-7), 7.07 (*d*, *J* = 8.5 Hz, H-5'), 4.88 (*s*, H-8'), 2.67 and 2.48 (both *s* and 3p, two CH₃C = 0), 2.53 and 1.81 (both *d*, *J* = 14.5 Hz, H-11a,b), 1.77 (*s*, 3p, H-12), 1.39 and 1.01 (both *s* and 3p, H-10' and H-11').

(B) Steam distillation of 490 g of leaves and flowers for two days afforded 1.3 g (0.26%) of essential oil, which was analysed by capillary GC interfaced with MS. The following compounds were identified by comparison with published spectra and by coinjection of authentic material: α-thujene (0.07%), α-pinene (0.7%), sabinene (0.5%), β-pinene (1.4%), 3-carene (0.3%), *p*-cymene (0.09%), limonene (1.2%), lavandulol (2.6%), lavandulyl acetate (55%), β-elemene (0.6%), humulene (0.3%), δ-cadinene (0.1%) and dehydrotremetone (**1d**) (2.5%). The latter separated as a crystalline solid, mp 82–83°, after the oil was kept in the refrigerator for two weeks. The identify of dehydrotremetone, lavandulol and lavandulyl acetate,

which were also isolated by CC of the oil, was confirmed by ¹H NMR and IR; ca 30 peaks remained unidentified, most of them being sesquiterpenes.

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