

BENZOFURAN DERIVATIVES FROM *ENCELIOPSIS ARGOPHYLLA*

HERBERT BUDZIKIEWICZ, GABRIELA LAUFENBERG, CURTIS CLARK* and PETER PROKSCH†‡

Institut für Organische Chemie der Universität zu Köln, Greinstrasse 4, D-5000 Köln 41, West Germany, *Department of Biological Sciences, California State Polytechnic University, Pomona, 3801 West Temple Avenue Pomona, CA 91768, U.S.A., †Botanisches Institut der Universität zu Köln, Gyrhofstr 15, D-5000 Köln 41, West Germany

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Abstract—From the composite *Enceliopsis argophylla* several new benzofuran derivatives were isolated. The unusual structure of the skeleton comprises an isopropylidene group at C-2 and a keto function at C-3 of the furan ring. An organ specific analysis by HPLC showed the compounds being present in leaves, peduncles, bracts, ray and disc flowers. The absolute amounts, however, differed greatly being highest in leaves and bracts.

INTRODUCTION

The genus *Enceliopsis* (Gray) A. Nels (tribe Heliantheae, Compositae) comprises four species of herbaceous perennials that are found in arid parts of the south western USA [1]. *Enceliopsis* is a close relative of the genus *Encelia* Adams, it shares with *Encelia* the derived morphological features of flattened achenes coupled with caducous paleae, and intergeneric hybrids have been obtained by D. W. Kyhos [unpublished data]. *Encelia* comprises some 16 species of perennial shrubs growing in the warm arid regions of south western North America and arid areas of Chile, Peru and Argentina. The northern distribution of *Encelia* overlaps with the southern distribution of *Enceliopsis*.

Previously, we have shown that chromenes (benzopyrans) and benzofurans are among the characteristic constituents of *Encelia* [2–5] and are well suited for chemosystematic studies within this genus [Proksch, P. and Clark, C., in preparation]. Chromenes and benzofurans occur also in other genera of the Compositae and are mainly found in the tribes Astereae, Eupatorieae, Heliantheae, Inuleae and Senecioneae [6]. We have now extended our phytochemical analysis to the related genus *Enceliopsis* and wish to report the structure elucidation of five new benzofuran derivatives from *E. argophylla* (D. C. Eaton) A. Nels.

RESULTS AND DISCUSSION

The presence of lipophilic phenolic compounds in the leaves of *Enceliopsis argophylla* was indicated by several blue fluorescing spots (visible under UV_{366nm}) after TLC of the dichloromethane extract. The main components (1–5, Fig. 1) were isolated by combined CC on silica gel and on Sephadex LH-20 [2]. ¹H NMR spectra showed that the compounds have the same basic structure and differ only in the nature of their esterifying acids. Thus, the H-signals of the common skeleton can be found with identical chemical shifts in each case (Table 1). The

C-4 and C-7 protons of the benzene ring give rise to two singlets at δ 7.7 and 6.6, respectively, the downfield shift of the former being due to the vicinity of the carbonyl group at C-3 (evidenced by the IR band at 1695 cm⁻¹). In addition, an aromatic methoxyl (δ 3.9), the Ar-CH(OCOR)Me group (δ 6.2, q, 1H, δ 1.5 ppm, d, 3H) and two non-equivalent vinylic methyl groups (δ 2.0 and 2.3) could be recognized. These data agree well with those reported [7] for the free alcohol (6).

As esterifying acids propionic (1), isobutyric (2), angelic (4) and senecic acid (3) could be identified from the NMR data given in Table 1 which for the latter two agree completely with those obtained for the related flouresianol esters [11]. By GC/MS analysis of the crude mixture a further component could be detected whose [M]⁺ was 2 m.u. less than that of 2. The fragmentation pattern indicates an identical skeleton, although not enough material could be isolated for an NMR spectrum. It is very probable that dehydro-2 is actually the 6-methacrylate (5). The absolute stereochemistry of the esters could not be determined.

Compounds 1–5 show identical MS fragmentation. [M – OCOR]⁺ (m/z 231) is the main ion in the upper mass region accompanied by [M – COR]⁺ (m/z 247) of lesser abundance. The ions m/z 159, 215 and 216 are present in every spectrum. In the lower mass range the fragments stemming from the acid residue ([RCO]⁺, [R]⁺) can be recognized.

For further confirmation of the structure 2 was hydrogenated to give 7. Its ¹H NMR spectrum (see Table 1) shows a one-proton doublet at δ 4.4 (C-2) coupling with a multiplet at 2.5. The C-11 and C-12 methyl groups appear as non-equivalent doublets (δ 0.9 and 1.2). These data are in agreement with those reported for a 3-oxotremetone derivative [8]. The two diastereomers formed by hydrogenation (creation of a new chiral center of C-2) could be separated by GC/MS (ratio 2:1). The mass spectrum of 7 ([M]⁺ C₁₈H₂₄O₅ by exact mass measurement) is more complex than that of 2. The isopropyl group at C-2 is lost by a McLafferty rearrangement ([M – C₃H₆]⁺) thus establishing its vicinity to the keto function. For [M – COCH₃]⁺ (m/z 249) and [M – OCOCH₃]⁺ (m/z 233, C₁₄H₁₇O₃) see above. The latter ion loses C₃H₇

‡To whom correspondence should be addressed

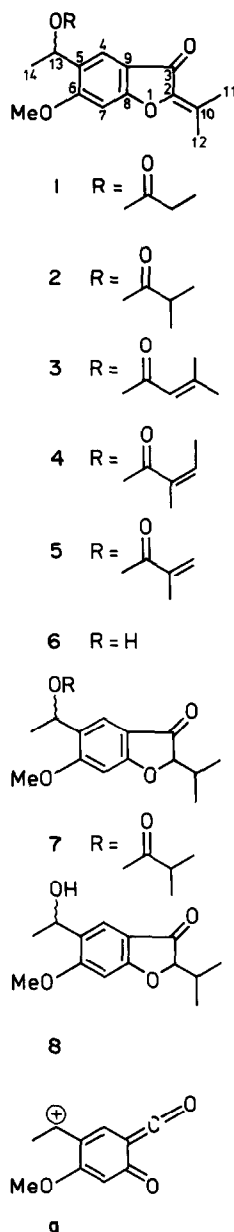


Fig 1 Structures of benzofuran esters 1–5 and derivatives 7 and 8 formed by chemical reactions

(m/z 190, $C_{11}H_{10}O_3$) and, somewhat unexpectedly, C_4H_8 (m/z 177, $C_{10}H_9O_3$, ion a)

Alkaline hydrolysis of 7 yielded the free alcohol 8. Its NMR spectrum differs from that of 7 only by the lack of the isobutyrate signals. The mass spectrum of 8 reflects the more facile loss from $[M]^+$ of CH_3 as compared with OH (in contrast to 7, where the loss of C_3H_7COO is more favored than that of CH_3) $[M - CH_3]^+$ (m/z 235) loses C_3H_6 (m/z 193) and C_4H_8 (m/z 179), m/z 179 then eliminates CO (m/z 151).

Only four benzofuran derivatives with the unusual ketone substituent at C-3 have been found before in the compositae [7–10]. Of the four species *Oyedaea boliviana*, *Clibadium armanii* and *Verbesina luetzelburgii* belong like

E. argophylla to the tribe Heliantheae. The benzofuran isolated from *Othonna arborescens* (tribe Senecioneae) differs in having a methoxy substituent instead of an acetyl group at C-5 [10]. Biogenetically these unusual benzofurans are probably formed via a 2-isopropylenebenzofuran by oxidation and double bond shift (Scheme 1). The concentration of compounds 1–4 were quantified by HPLC for leaves, peduncles, bracts, ray and disc flowers of *Enceliopsis argophylla* and found to be highest in leaves and bracts (Table 2). In comparison, stems and flowers showed a six-to-seven times lower total concentration. In all organs analysed, however, the angelic acid ester (4) was the main component. The high concentrations of the benzofurans in the leaves probably reflect their defensive role against herbivorous insects as could be demonstrated earlier for biogenetically related compounds [6].

EXPERIMENTAL

Plant material and extraction. *E. argophylla* (D. C. Eaton) A. Nels. was collected at the Lake Mead National Recreation Area, California, and identified by Dr. C. Clark (Cal State, Pomona). A voucher specimen is on file in the herbarium of Cal State, Pomona. The air-dried leaves were ground and extracted with CH_2Cl_2 . The extract was separated by CC on silica gel using CH_2Cl_2 as eluent followed by CC on Sephadex LH-20, using MeOH as eluent. Fractions containing 20 ml were monitored by TLC on silica gel, solvent CH_2Cl_2 , detection under UV_{366nm}. Compounds 1–5 appeared as blue fluorescent spots under UV.

Spectroscopic methods. IR film between KBr pellets, 1H NMR 90 MHz, 300 MHz, $CDCl_3$, TMS int. standard, δ -values. MS quadrupole instrument with data system, BP-1 capillary column (SGE), 70 eV, for GC/MS analyses (1–5, 7, 8).

Esters of 5-(1'- ξ -Hydroxyethyl)-2-isopropylidene-6-methoxy-3-oxo-2,3-dihydrobenzofuran (6). The IR spectra of the esters 1–5 are very similar: 1750 (ester CO), 1695 (Ar-CO-C=C-OR), 1655, 1615 cm^{-1} . For 1H NMR data see Table 1. MS m/z (rel. int.): 6-propionate (1) $[M]^+$ 304 (9), 247 (4) $[M - COC_2H_5]^+$, 231 (100) $[M - OCOC_2H_5]^+$, 230 (21), 216 (24), 215 (29), 159 (63), 6-isobutyrate (2) $[M]^+$ 318 (17), 247 (3), 231 (100), 230 (9), 216 (19), 215 (18), 159 (31), 6-methacrylate (?) (5) $[M]^+$ 316 (18), 247 (3), 231 (100), 230 (23), 216 (23), 215 (30), 159 (50), 6-angelicate (4) and 6-senecioate (3) (spectra practically identical) $[M]^+$ 330 (12), 247 (2), 231 (100), 230 (16), 216 (19), 215 (14), 259 (35).

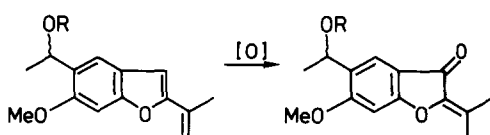
5-(1-Hydroxyethyl)-2-isopropyl-6-methoxy-3-oxo-2,3-dihydrobenzofuran isobutyrate (mixture of *rel-R,R* and *rel-R,S*) (7). This was obtained by hydrogenation (Pd) of 2 in EtOAc for 2 hr. 1H NMR see Table 1. MS m/z (rel. int.): $[M]^+$ 320 1627 (calc 320 1623 for $C_{18}H_{24}O_5$) (5), 278 (1) $[M - C_3H_6]^+$, 265 (1), 249 (3) $[M - COC_3H_7]^+$, 233 1170 (calc 233 117 for $C_{14}H_{17}O_3$) (55) $[M - OCOCH_3]^+$, 217 (6), 207 (3), 190 0643 (calc 190 0630 for $C_{11}H_{10}O_3$) (40) $[m/z$ 233 - $C_3H_7]^+$, 177 0556 (calc 177 0551 for $C_{10}H_9O_3$) (100) $[m/z$ 233 - $C_4H_8]^+$. The diastereomers could be separated by GC/MS. Their fragmentation patterns were identical.

5-(1-Hydroxyethyl)-2-isopropyl-6-methoxy-3-oxo-2,3-dihydrobenzofuran (mixture of *rel-R,R* and *rel-R,S*) (8). This was obtained by refluxing 7 for 1 hr dissolved in MeOH-H₂O (7:3) after addition of NaOH to give a 0.5 N soln. 1H NMR see Table 1. MS m/z (rel. int.): 250 (4), $[M]^+$, 235 (18) $[M - CH_3]^+$, 208 (10) $[M - C_3H_6]^+$, 193 (14) $[M - CH_3 - C_3H_6]^+$, 179 (100) $[M - CH_3 - C_4H_8]^+$, 151 (23), [179 - CO] $^+$.

HPLC analysis. Samples of 500 mg dried material were extracted with MeOH and the crude extracts injected directly

Table 1 ^1H NMR spectral data of compounds 1–8, δ (ppm), J (Hz) in parentheses

	1	2	3	4	7	8
H-2	—	—	—	—	<i>d</i> 4.4 (4.5)	<i>d</i> 4.4 (4.5)
H-4	<i>s</i> 7.7	<i>s</i> 7.7	<i>s</i> 7.7	<i>s</i> 7.7	<i>s</i> 7.7	<i>s</i> 7.7
H-7	<i>s</i> 6.6	<i>s</i> 6.6	<i>s</i> 6.6	<i>s</i> 6.6	<i>s</i> 6.6	<i>s</i> 6.6
H-10	—	—	—	—	<i>m</i> 2.5	<i>m</i> 2.4
H-11	<i>s</i> 2.3	<i>s</i> 2.3	<i>s</i> 2.3	<i>s</i> 2.3	<i>d</i> 1.2 (7.5)	<i>d</i> 1.1 (7.5)
H-12	<i>s</i> 2.0	<i>s</i> 2.0	<i>s</i> 2.1	<i>s</i> 2.1	<i>d</i> 0.9 (7.5)	<i>d</i> 0.8 (7.5)
H-13	<i>q</i> 6.2 (7.0)	<i>q</i> 6.2 (7.0)	<i>q</i> 6.2 (7.0)	<i>q</i> 6.2 (7.0)	<i>q</i> 6.2	<i>q</i> 5.1
H-14	<i>d</i> 1.5 (7.0)	<i>d</i> 1.5 (7.0)	<i>d</i> 1.5 (7.0)	<i>d</i> 1.5 (7.0)	<i>d</i> 1.5 (7.0)	<i>d</i> 1.5 (7.0)
OMe	<i>s</i> 3.9	<i>s</i> 3.9	<i>s</i> 3.9	<i>s</i> 3.9	<i>s</i> 3.9	<i>s</i> 3.9
OCOR	<i>t</i> 1.1 (7.5) <i>q</i> 2.3 (7.5)	<i>d</i> 1.1 (7.5) <i>d</i> 1.2 (7.5) <i>m</i> 2.5	<i>q</i> 2.15 (1.5) <i>q</i> 1.9 (1.0) <i>m</i> 5.75	<i>dq</i> 1.9 (1.5, 1.5) <i>dq</i> 2.0 (7.0, 1.5) <i>m</i> 6.1	<i>d</i> 0.9 (7.5) <i>d</i> 1.2 (7.5) <i>m</i> 2.5	

Scheme 1 Proposed biosynthesis of benzofuran derivatives in *Enceliopsis*Table 2 Concentrations of compounds 1–4 in $\mu\text{M/g}$ dry wt in different organs of *Enceliopsis argophylla* as quantified by HPLC

	1	2	3	4
Leaves	6.6	22.8	16.0	25.2
Peduncles	0.3	0.8	1.6	3.4
Bracts	2.6	6.5	15.2	34.4
Ray flowers	0.5	0.6	3.8	6.5
Disc flowers	0.4	0.1	1.1	2.3

into the HPLC Absorbance detector, column DuPont Zorbax C_{18} , 250×4.6 mm, pore size $5 \mu\text{m}$, solvent system 42% THF/58% H_2O isocratically, flow rate 1.2 ml/min, detection at

λ 350 nm) Identification and quantification of the peaks was done by coinjecting known amounts of compounds (1–4)

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