



BENZOQUINONES FROM *EMBELIA ANGUSTIFOLIA*

ANNE-KRISTINE LUND, JOHN LEMMICH,* ANNE ADSERSEN and CARL E. OLSEN†

Department of Medicinal Chemistry, Royal Danish School of Pharmacy, 2 Universitetsparken, DK-2100 Copenhagen, Denmark; †Department of Chemistry, Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

(Received in revised form 1 August 1996)

Key Word Index—*Embelia angustifolia*; Myrcinaceae; 2,5-dihydroxy-1,4-benzoquinones; embelin; angiotensin-converting enzyme; ACE.

Abstract—Three new 2,5-dihydroxy-3-alkyl-1,4-benzoquinones, (Z)-2,5-dihydroxy-3-(pentadec-8-enyl)-1,4-benzoquinone, (Z,Z)-2,5-dihydroxy-3-(heptadeca-8,11-dienyl)-1,4-benzoquinone and (Z)-2,5-dihydroxy-3-(heptadec-8-enyl)-1,4-benzoquinone and the known 2,5-dihydroxy-3-pentadecyl-1,4-benzoquinone were isolated from the leaves of *Embelia angustifolia*. Their structures have been established on the basis of spectral analysis and by chemical methods. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Embelia angustifolia (vernacular name *Liane savon*) is endemic to Réunion Island and Mauritius and is used locally in the treatment of urinary disorders, nephritis, cysts, colic and rheumatism [1]. In view of the fact that the diuretic effect might be related to angiotensin-converting enzyme (ACE) inhibition, a bioassay-directed fractionation of the ether-soluble components of the dried plant was undertaken, the *in vitro* ACE inhibitory activity being determined as described by Elbl and Wagner [2] and later modified by Hansen *et al.* [3]. This report deals with the isolation and structure elucidation of four ACE inhibitory 2,5-dihydroxy-3-alkyl-1,4-benzoquinones of which three are new compounds.

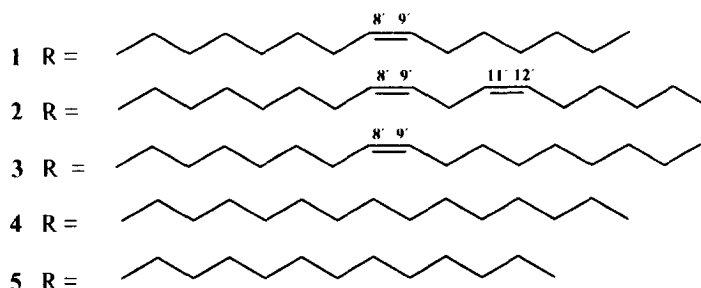
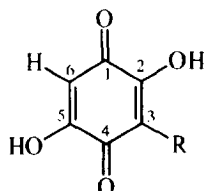
RESULTS AND DISCUSSION

The four ACE inhibitors (1–4) from *E. angustifolia* were isolated collectively by silica gel chromatography

but were readily separated by reversed phase HPLC on macroporous polystyrene (PLRP-S).

Compounds 1–4 are brick red and their UV spectra show the characteristic pattern for a 2,5-dihydroxy-1,4-benzoquinone, with absorption at about 288 nm and 417 nm [4]. The IR spectra are also characteristic of 2,5-dihydroxy-1,4-benzoquinones, with bands at about 3305 (OH) and 1610 ($>C=O$) cm^{-1} . This structural relationship was confirmed by comparison with authentic embelin (5) which is a common constituent in the family Myrcinaceae [5, 6]. It is noteworthy that 5 showed strong ACE inhibitory activity in the bio-assay employed.

The EI mass spectra of 1–4 were similar to that of 5, pointing to 2,5-dihydroxy-1,4-benzoquinone structures, each with a single side chain at position 3. This was evident from the occurrence in each case of a strong peak at m/z 154, formed by elimination of the side chain in a McLafferty rearrangement [7]. Differences in M_r , as observed from strong parent peaks,



could then be related to differences in the number of carbon atoms and in the number of double bonds in these side chains.

* Author to whom correspondence should be addressed.

Confirmation of this was found in the ^1H NMR spectra of 1–4, from which the side chains were determined to be unbranched. Thus compounds 1, 2 and 3 possessed pentadecenyl-, heptadecadienyl- and heptadecenyl- side chains, respectively, whereas 4, with a saturated pentadecyl side chain was the known compound homorapanone [8].

The configuration of the double bonds in 1–3 was established by means of ^1H - ^{13}C -correlated NMR spectra. In each case the δ -values for allylic carbons were found to be about 27 ppm. This corresponds to *cis* double bonds in an unbranched chain [9]. In the case of *trans* configurations 6–7 ppm higher δ -values would have been expected.

The relative position of the two double bonds in the side chain of 2 was determined by a comparison with (Z,Z)-9,12-octadecadienoic acid (linoleic acid). In both cases the ^1H NMR spectra show a pattern corresponding to the sequence $-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-$ with the methine groups at δ 5.3 and the methylene group as a triplet at δ 2.8.

The exact placement of the double bonds in the side chains was established by epoxidation followed by periodate cleavage [10]. The aldehydes formed from the terminal ends of the chains were then identified by GC-MS. In this way 1 was shown to be (Z)-2,5-dihydroxy-3-(pentadec-8-enyl)-1,4-benzoquinone, 2 was (Z,Z)-2,5-dihydroxy-3-(heptadeca-8,11-dienyl)-1,4-benzoquinone, and 3 was (Z)-2,5-dihydroxy-3-(heptadec-8-enyl)-1,4-benzoquinone.

EXPERIMENTAL

^1H and ^{13}C NMR: 200 MHz and 50.3 MHz respectively. Multiplicities in ^{13}C NMR spectra have been deduced from DEPT spectra; CC: Silica gel- KH_2PO_4 containing 6.2% KH_2PO_4 and 3% water. UV: MeOH–conc. HCl (999:1); GC-MS: HP5890 Series II gas chromatograph directly coupled to a Jeol JMS-AX505W mass spectrometer: SGE BPX5 column (25 m $\times$ 0.25 mm, 0.25 μm film thickness), oven temp programme 50° for 2 min, 50 to 200° at 5°/min, 200° for 10 min, head pressure 70 kPa, injection temp 250° with split injection. The spectrometer repetition rate was 1.0 scan/sec. The ion source was run in EI mode at 240°, 70 eV ionization energy. Identification of aldehydes was done by comparison of acquired mass spectra with spectra of the instruments library (NIST) and for hexanal also by comparison of the *R*_f with that of an authentic sample.

Plant material. Leaves of *Embelia angustifolia* (A.DC) DC in DC, were collected in February 1994, at Bélouve at Réunion Island in the Indian Ocean. A voucher specimen (A. & H. Adersen, 5433) is deposited in Herbarium C., Botanical Museum, Copenhagen, Denmark.

Extraction and isolation. The dried and powdered plant material (515 g) was extracted in a Soxhlet apparatus for 6 hr with Et_2O . The extract was partitioned in the system pentane–MeOH– H_2O (10:9:1)

and the polar phase evapd. CC of the residue (3.4 g) on silica gel- KH_2PO_4 with CH_2Cl_2 – EtOAc (9:1) $\rightarrow$ (4:1) afforded a mixture of 1–4 (0.71 g) which was separated by rev. phase prep. HPLC on macroporous polystyrene (PLRP-S) with AcOH – H_2O – CH_2Cl_2 – MeCN (2:27:5:66) as eluent. Yields (given in order of elution from PLRP-S): 1 40 mg, 2 150 mg, 3 10 mg and 4 50 mg. The separated compounds were finally purified by CC on macroporous polystyrene, XAD-2 with MeOH – CH_2Cl_2 – H_2O – AcOH (180:9:9:2) as eluent.

(Z)-2,5-Dihydroxy-3-(pentadec-8-enyl)-1,4-benzoquinone (1). Orange crystals (92% MeOH); mp 116–119°; UV $\lambda_{\text{max}}^{\text{MeOH-HCl}}$ nm (log ϵ): 288.0 (4.33) and 418.8 (2.48); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3305 (OH), 1610 ($>\text{C}=\text{O}$) and 1330 ($\text{C}-\text{O}$) cm^{-1} ; ^1H NMR (CDCl_3): δ 7.69 (2H, br, OH), 6.01 (1H, s, H-6), 5.35 (2H, m, H-8', H-9'), 2.45 (2H, t, $J = 7.4$ Hz, H-1'), 2.03 (4H, m, H-7' and H-10'), 1.46 and 1.28 (18H, m, H-2' and H-3' to H-6', H-11' to H-14' resp., overlapping), 0.89 (3H, t, $J = 7$ Hz, H-15'); ^{13}C NMR (CDCl_3): δ ca. 168 (very br C-1, C-2, C-4, C-5), 129.9 (d, C-8', C-9'), 117.0 (s, C-3), 102.2 (d, C-6), 32.0 (t, C-13'), 29.8–29.3 overlapping (triplets, C-3' to C-6', C-11', C-12'), 27.9 (t, C-2'), 27.2 (t, C-7'*), 26.9 (t, C-10'*), 22.5 (t, C-1'), 22.4 (t, C-14'), 14.0 (q, C-15'), assignments marked * are exchangeable; EIMS 70 eV *m/z* (rel. int.): 348 [M]⁺ (90), 154 [$\text{M} - \text{C}_{14}\text{H}_{26}$]⁺ (100).

(Z,Z)-2,5-Dihydroxy-3-(heptadeca-8,11-dienyl)-1,4-benzoquinone (2). Orange crystals (92% MeOH); mp 91–97°; UV $\lambda_{\text{max}}^{\text{MeOH-HCl}}$ nm (log ϵ): 287.0 (4.32) and 415.0 (2.52); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3300 (OH), 1610 ($>\text{C}=\text{O}$) and 1330 ($\text{C}-\text{O}$) cm^{-1} ; ^1H NMR (CDCl_3): δ 7.71 (2H, br, OH), 6.01 (1H, s, H-6), 5.37 (4H, m, H-8', H-9', H-11', H-12'), 2.77 (2H, br t, $J = 5.5$ Hz, H-10'), 2.45 (2H, t, $J = 7.4$ Hz, H-1'), 2.03 (4H, m, H-7' and H-13'), 1.47 (2H, m, H-2') overlapping with 1.31 (14H, m, H-3' to H-6' and H-14' to H-16'), 0.89 (3H, t, $J = 7$ Hz, H-17'); ^{13}C NMR (CDCl_3): δ ca 170 (br, C-1, C-2, C-4, C-5), 130.2, 130.1, 128.0 and 127.9 (d, C-8', C-9', C-11', C-12'), 116.9 (s, C-3), 102.2 (d, C-6), 31.5 (t, C-15'), 29.6–29.2 overlapping (triplets, C-3' to C-6' and C-14'), 27.9 (t, C-2'), 27.2 (t, C-7', C-13'), 25.6 (t, C-10'), 22.6 (t, C-16'), 22.5 (t, C-1'), 14.1 (q, C-17'); EIMS 70 eV *m/z* (rel.int): 374 [M]⁺ (75), 154 [$\text{M} - \text{C}_{16}\text{H}_{28}$]⁺ (100).

(Z)-2,5-Dihydroxy-3-(heptadec-8-enyl)-1,4-benzoquinone (3). Orange crystals (92% MeOH); mp 111–112°; UV $\lambda_{\text{max}}^{\text{MeOH-HCl}}$ nm (log ϵ): 288.2 (4.32) and 415.8 (2.51); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3305 (OH), 1615 ($>\text{C}=\text{O}$) and 1330 ($\text{C}-\text{O}$) cm^{-1} ; ^1H NMR (CDCl_3): δ 7.72 (2H, br, OH), 6.01 (1H, s, H-6), 5.35 (2H, m, H-8', H-9'), 2.45 (2H, t, $J = 7.5$ Hz, H-1'), 2.02 (4H, m, H-7' and H-10'), 1.47 (2H, m, H-2') overlapping with 1.30 (20H, m, H-2' to H-6' and H-11' to H-16'), 0.88 (3H, t, $J = 7$ Hz, H-17'); ^{13}C NMR (CDCl_3): δ ca 170 (br, C-1, C-2, C-4, C-5), 130.0 (d, C-8'*), 129.8 (d, C-9'*), 117.0 (s, C-3), 102.2 (d, C-6), 31.9 (t, C-15'), 29.8–29.2 overlapping (triplets, C-3' to C-6', C-11' to C-14'), 27.9 (t, C-2'), 27.2 (t, C-7'*), 27.2 (t, C-10'*), 22.7 (t, C-16'),

22.5 (*t*, C-1'), 14.1 (*q*, C-17'), assignments marked * are exchangeable; EIMS 70 eV *m/z* (rel.int): 376 [*M*]⁺ (100), 154 [*M* - C₁₆H₃₀]⁺ (92).

2,5-Dihydroxy-3-pentadecyl-1,4-benzoquinone (4). Not crystallized C₂₁H₃₄O₄; ¹H NMR (CDCl₃/DMSO-*d*₆ (2:1)): δ 5.76 (1H, *s*, H-6), 2.34 (2H, *t*, *J* = 7.0 Hz, H-1'), 1.25 (26H, *m*, H-2' to H-14'), 0.87 (3H, *t*, *J* = 6 Hz, H-15'); ¹³C NMR (CDCl₃/DMSO-*d*₆ (2:1)): δ 117.5 (C-3), 103.5 (C-6), 31.5 (C-13'), 29.3–27.8 overlapping (C-2' to C-12'), 22.3 (C-1'*), 22.2 (C-14'*), 14.0 (C-15'), assignments marked * are exchangeable; EIMS 70 eV *m/z* (rel.int): 350 [*M*]⁺ (100), 154 [*M* - C₁₄H₂₈]⁺ (100).

Epoxidation of 1. A mixture of **1** (4.9 mg), 70% 3-chloro-perbenzoic acid (5.1 mg), and CHCl₃ was left to stand at room temp for 9 hr. 30 μl of a 10% dimethylsulphide soln in MeOH was added to stop the reaction. The solvent was removed *in vacuo* and the residue chromatographed by rev. phase. prep. HPLC on PLRP-S with AcOH–water–CH₂Cl₂–MeCN (2:34:3:61) to yield epoxide **6** (3.6 mg).

Epoxide 6. ¹H NMR (CDCl₃): δ 6.01 (1H, *s*, H-6), 2.92 (2H, *m*, H-8', H-9'), 2.45 (2H, *t*, *J* = 7.4 Hz, H-1'), 1.49 (6H, *m*, H-2', H-7', H-10') overlapping with 1.41–1.30 (16H, *m*, H-3' to H-6', H-11' to H-14'), 0.92 (3H, *t*, *J* = 7 Hz, H-15').

Cleavage of epoxide 6. Epoxide **6** (0.18 mg) was dissolved in dry CHCl₃ (275 μl) and added to finely powdered HIO₂ (0.6 mg). The reaction mixture was stirred at 40° for 3 hr and then subjected to GC-MS. The aldehyde produced from the terminal end of the side chain was resolved and identified as heptanal by its MS.

Epoxidation of 2. A mixture of **2** (12.1 mg) was epoxidized approximately as described for **1**. Rev. phase. prep. HPLC on PLRP-S with AcOH–water–CH₂Cl₂–MeCN (4:80:5:111) yielded epoxide (**7**) (6.8 mg).

Epoxide 7. ¹H NMR (CDCl₃): δ 6.01 (1H, *s*, H-6), 3.12 and 3.00 overlapping (4H, *m*, H-8', H-9', H-11', H-12'), 2.45 (2H, *t*, *J* = 7.3 Hz, H-1'), 1.76 (2H, *m*, H-10'), 1.51 (6H, *m*, H-2', H-7', H-13') overlapping with 1.35 (14H, *m*, H-3' to H-6', H-14' to H-16'), 0.9 (3H, *t*, H-17'); ¹³C NMR (CDCl₃): mixture of diastereomers *a* and *b*, (2:1); δ 116.8 (C-3), 102.2 (C-6), 57.1 and 57.1 (C-9', C-11' *a*), 56.9 and 56.8 (C-9', C-11' *b*), 54.4 (C-8', C-12' *a*), 54.3 (C-8', C-12' *b*), 31.7 (C-15'), 29.3–27.8 overlapping (C-2' to C-6', C-14'), 27.2 (C-10' *a*),

26.9 (C-10' *b*), 26.4 and 26.1 (C-7', C-13' *a*), 26.5 and 26.3 (C-7', C-13' *b*), 22.6 (C-16'*), 22.4 (C-1'*), 14.0 (C-17'), assignments marked * are exchangeable.

Cleavage of epoxide 7. Method as described for **6**. The aldehyde produced from the terminal end of the side chain was identified as hexanal.

Epoxidation of 3. (5.1 mg) of **3** was epoxidized and subjected to HPLC as described for **1** to yield epoxide (**8**) (4.0 mg).

Epoxide 8. ¹H NMR (CDCl₃): δ 5.96 (1H, *s*, H-6), 2.91 (2H, *m*, H-8', H-9'), 2.44 (2H, *t*, H-1'), 1.48 (6H, *m*, H-2', H-7', H-10') overlapping with 1.27 (20H, *m*, H-3' to H-6', H-11' to H-16') and 0.88 (3H, *t*, H-17').

Cleavage of epoxide 8. Method as described for **6**. The aldehyde produced from the terminal end of the side chain was identified as nonanal.

Acknowledgements—The Velux Foundation and the Thorkil Steenbeck Foundation, the Ib Henriksen Foundation and the Danish Council for Technical Research are gratefully acknowledged for provision of the NMR instrument.

REFERENCES

1. Laverigne, R., *Tisaneurs et Plantes Medicinales Indigenes de L'Ile De La Reunion*, Editions Orphie 3 et 5 Avenue d'Orleans, Livry Gargan, 1990, p. 347.
2. Elbl, G. and Wagner, H., *Planta Medica*, 1991, **57**, 137.
3. Hansen, K., Nyman, U., Smitt, U. W., Adersen, A., Gudiksen, L., Rajaksekharan, S. and Pushpangadan, P., *Journal of Ethnopharmacology*, 1995, **48**, 43.
4. Ogawa, H. and Natori, S., *Phytochemistry*, 1968, **7**, 773.
5. Merian, M. and Schlittler, E., *Helvetica Chimica Acta*, 1948, **31**, 2237.
6. Bøgh, H. O., Andreassen, J. and Lemmich, J., *Journal of Ethnopharmacology*, 1996, **50**, 35.
7. Chow, P. W., Sim, K. Y., Lim, P. L. and Chung, V. C., *Bull. Singapore Natl. Inst. Chem.*, 1991, **19**, 87.
8. Khurana, S. K., Krishnamoorthy, V. and Seshadri, T. R., *Curr. Sci.*, 1972, **41**, 331.
9. Dorman, D. E., Jautelat, M. and Roberts J. D., *J. Org. Chem.*, 1971, **36**, 2757.
10. Fukuyama, Yo., Okino, J. and Kodama, M., *Chem. Pharm. Bull.*, 1991, **39**, 1877.