

THE CONSTITUENTS OF *ECBALLIUM ELATERIUM* L.—XXII¹ PHENOLICS AS MINOR COMPONENTS

M. M. RAO^a and D. LAVIE*

Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot, Israel

(Received in the UK 25 February 1974; Accepted for publication 29 May 1974)

Abstract—Chromatographic separation of the residue left after the separation of cucurbitacins D, E and I from the ethereal extracts of *Ecballium elaterium* L. (Cucurbitaceae) yielded five crystalline phenolics characterised as hydroquinone, 4-hydroxy acetophenone, 4-hydroxy-3-methoxy acetophenone (acetovanillone), 2-nitroquinol and 4-hydroxyphenyl-bisepoxy lignan. Evidence leading to the assignment of structures to the last two compounds is presented.

The isolation of cucurbitacins D, E and I from the fruit juice of the squirting cucumber *Ecballium elaterium* L. and their structure determination as well as the chemical transformations performed during their characterisation have been the subject of a number of publications²⁻⁴ which have been recently compiled in a review.⁵ The separation of these cucurbitacins from the crude ethereal extract by repeated crystallisations left a fairly large quantity of a brown semisolid residue. Analysis of this crude mixture indicated that cucurbitacin like compounds constitute the major fraction with aromatic components forming only a minor part. This paper deals with the latter.

Large scale fractionation of the crude mixture on a silica gel column afforded five phenolic compounds in a pure crystalline state. Through a combination of spectroscopic and mass spectrometric measurements, and by comparing with published data, three of the compounds were readily identified as hydroquinone, 4-hydroxy acetophenone (1) and 4-hydroxy-3-methoxy acetophenone (acetovanillone)⁶ (2). The fourth compound has been characterised as 2-nitroquinol (3) which, though known synthetically, constitutes the first report of its natural occurrence. The fifth compound was found to be a new lignan of the 3,7-dioxabicyclo[3.3.0]octane type and is assigned structure (4a). This is a particularly interesting compound since it is the first example of a naturally occurring lignan with monooxygenated aromatic rings.⁷ Evidence leading to the assignment of structures to the last two compounds is hereby presented.

Nitroquinol (3a). The orange red crystalline compound, M^+ 155 is an aromatic hydroxy compound with no CO or OMe groups (IR and NMR). It readily forms a diacetate (3b) (M^+ 239) (Ac_2O -

pyridine) indicating the presence of two OH groups. The presence of a nitro group is inferred from (a) the odd values of the molecular weights of the hydroxy compound and its diacetate, indicating the presence of one N atom, and (b) the strong IR absorption bands at 1525 and 1340 cm^{-1} in the spectrum of the hydroxy compound, and at 152 and 1342 cm^{-1} in that of the acetate.

1,2,4-Substitution of these three groups of benzene nucleus is shown by the NMR spectra analysis of 3b, an ABX pattern of the three aromatic proton signals confirmed by double resonance measurements.

Mass spectral fragmentation of the hydroxy compound gave peaks at m/e 138 (M^+-17), 12 (M^+-NO) and 109 (M^+-NO_2) as expected from an aromatic nitro compound having an *ortho* OH function⁸ leading to the formulation of the compound as in 3a. Final confirmation of the identity of the compound was obtained by a direct comparison of 3b with an authentic sample of nitroquinol diacetate prepared by the nitration of hydroquinone diacetate followed by acetylation.⁹

The fifth phenolic compound which is now named "ligballinol" (4a) analyses for $C_{18}H_{18}O_4$ (M 298) and is optically active. It has no CO, C-Me or O-Me groups (IR and NMR), but readily forms a diacetate (4b), $C_{22}H_{22}O_6$ (M^+ 382), revealing the presence of two OH groups. The absence of any other acylable group was confirmed by deuterium exchange (crystallisation of 4a from CD_3OD produced a product heavier by two μ as expected from a dihydroxy compound). The remaining two oxygens to be accounted for are, therefore involved in ether linkages.

The NMR spectrum of 4b is much simpler however, informative. It has only one signal (2:28) due to the acetate protons. The aromatic aliphatic and acetate protons are in the ratio 4:4:1 respectively, indicating, thereby that the molecule is a symmetrical dimer of a C_6-C_3 unit, with the two ether linkages in the aliphatic proton. The aromatic

^aOn leave of absence from the Department of Chemistry, Kurukshetra University, Kurukshetra (Haryana), India.

protons appear as A_2B_2 doublets (δ 7.0) and 7.37, $J = 8.5$ Hz), characteristic of a *para* disubstituted benzene ring. The signals in the aliphatic region appear as three distinct groups: (a) a doublet ($J = 4.0$ Hz) at δ 4.65, corresponding to 1H (b) a multiplet between 3.4–4.4, corresponding to 2H, and (c) a broad signal at δ 3.05 corresponding to 1H. The down field signal, obviously due to the benzylic proton, collapses to a singlet upon

irradiation at δ 3.05. Thus both the benzylic protons are identical. Such a situation is found in the bisepoxy lignan (+)-pinoresinol (**5A**) which shows a similar pattern of signals in the aliphatic region of its NMR spectrum (Table 1).

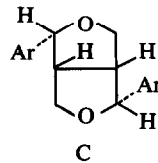
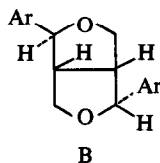
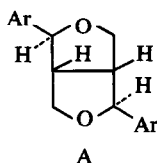
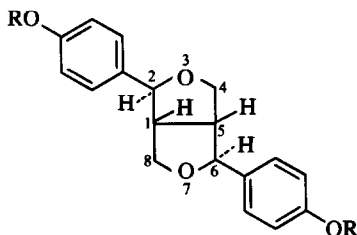
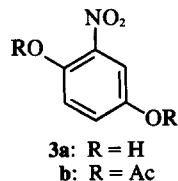
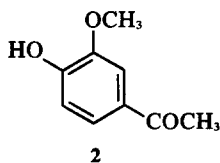
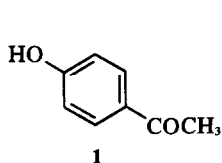
It thus became evident that ligballinol (**4a**) is a bisepoxy lignan. Of the three possible stereoisomeric structures A, B, C the unsymmetrical structure is ruled out on the basis of the

Table 1. NMR signals in the aliphatic region of ligballinol and some related bisepoxy lignans (δ units)^a

Protons	Compounds					
	4a ^b	4b	5A ¹²	6A ¹⁰	6B ¹⁰	6C ¹⁰
H-1 } H-5 }	3.05m	3.10m	3.09m	3.15m	2.9m 3.3m	3.15m
H-2 } H-6 }	4.65d(4.0)	4.81d(4.0)	4.72d(4.0)	4.75d(4.0)	4.95d(5.5) 4.45d(7.0)	4.90d(5.0)
H-4 ax } H-8 ax }	3.77(dd) (4.0 and 8.0)	3.91dd (3.5 and 9.0)	3.85dd (3.8 and 9.2)	3.8–4.0m	3.25–3.45 (1H) 3.7–3.9(2H) 4.1–4.4(1H)	3.3–3.65m
H-4 eq } H-8 eq }	4.18m (complex)	4.2m (complex)	4.24q (7.0 and 9.2)	4.2–4.4m		

^a Coupling constants in parentheses.

^b In CD_3OD .



- 5A:** Pinoresinol Ar = 4-OH-3-OMe-phenyl
6A: Eudesmin **6B:** epi-Eudesmin **6C:** dia-Eudesmin
 Ar = 3,4-(OMe)₂-phenyl
7A: Sesamin **7B:** epi-Sesamin **7C:** dia-Sesamin
 Ar = 3,4-(–OCH₂O–)-phenyl

observation that both the benzylic protons are identical. It was shown¹⁰ from a study of the NMR spectra of the three stereoisomers of eudesmin (6) and sesamin (7), the configurations of which were arrived at by other methods as well, that in the diequatorial lignans (A) the resonances of the methylene protons lie between δ 4.70 and 3.57 and in the diaxial lignans (C) they are between δ 4.0 and 3.3. Following this observation, ligballinol can be formulated in the diequatorial orientation¹¹ as shown in 4a.

The mass spectra of ligballinol (4a) and its diacetate (4b) supported the assigned structure. The fragmentation of bisepoxy lignans, discussed in detail in two papers,^{13,14} is usually minimal, being confined to benzylic cleavages and practically unaffected by stereoisomerism or variations in the ring substitution. However, the molecular ion is usually the base peak only in fully methylated compounds.

The mass spectrum of 4a exhibits all the major fragments of 5 but shifted to lower mass numbers—in the case of the molecular ion by 60 mu and in the fragments with only one aromatic ring by 30 mu. This is illustrated in Chart 1. The corresponding peaks are also seen in the mass spectrum of 4b showing as well two successive losses of 42 mu (ketene) from M^+ .

widely distributed as a glycoside, was isolated as an aglycone from species belonging to Salicaceae, Pinaceae, Rosaceae and Solanaceae whereas the occurrence of 4-hydroxy-3-methoxy acetophenone (acetovanillone) was reported in Apocynaceae, Cactaceae, Amaryllidaceae and Scrophulariaceae.

Acetovanillone is one of the products of "ethanolysis" of the lignin. It has been observed that monocots produce lignin with *p*-hydroxyphenyl derivatives and biosynthetic studies showed the presence of enzymes in grasses which incorporate tyrosine or *p*-hydroxyphenylpyruvic acid into lignin. Degradative studies of gymnosperm lignin also showed the presence of *p*-hydroxyphenyl derivatives, though in small quantities. Nevertheless no lignans derived from *p*-coumaryl alcohol have so far been reported in Nature.

In view of the isolation of 2-nitroquinol it may be interesting to report the strong smell of oxides of nitrogen as well as of brown vapours noticed when the containers of the fruit juice of *Ecballium elaterium* were opened following overnight storage in glass containers.

EXPERIMENTAL

Ms were taken on a Fisher-Johns apparatus and are uncorrected. UV spectra were recorded on a Cary 14 instrument (EtOH as solvent). IR spectra were recorded

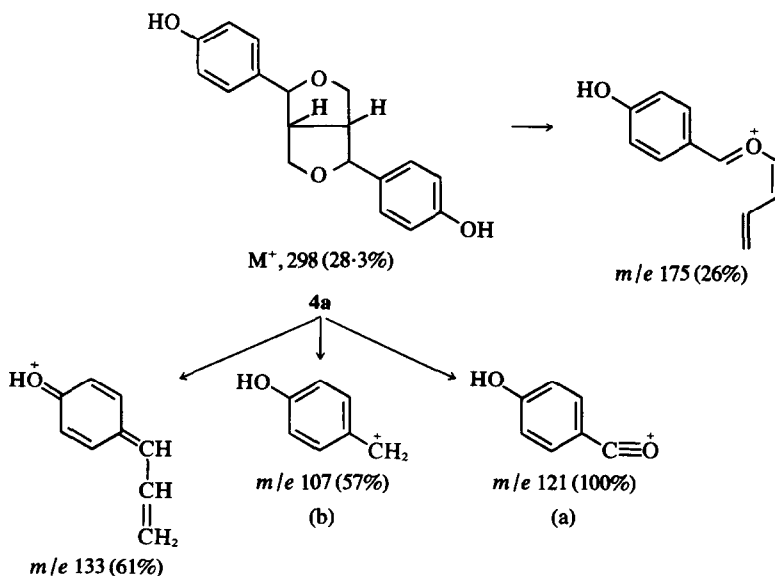


CHART 1. Mass-spectral fragmentation of 4a.

DISCUSSION

Hydroquinone, the most widely distributed simple phenol, was previously isolated from six families—Ericaceae, Rosaceae, Proteaceae, Compositae, Labiateae and Bignoniaceae and is reported to be of some systematic interest, especially in Rosaceae. 4-Hydroxy acetophenone, though

on a Perkin-Elmer infracord model 137 spectrophotometer equipped with a NaCl prism and refer to KBr pellets NMR spectra were recorded on a Varian A-60 spectrometer and refer to 5-10% solns in $CDCl_3$, unless otherwise stated, using TMS as internal standard. Decoupling experiments were carried out on a Bruker HFX-10 90 MHz spectrometer by Mr. M. Greenberg. MS were recorded on an Atlas CH4 instrument under the direction

of Dr. Z. V. I. Zaretskii and the relative intensities of the peaks, given in parentheses, are reported with reference to the most intense peak taken as 100%. Optical rotation was recorded with an automatic Perkin-Elmer 141 polarimeter in MeOH solution. Analyses were performed in the microanalytical laboratory of the Institute under the direction of Mr. R. Heller. Silica gel 60 (E. Merck) 70–230 mesh was used for column chromatography and TLC were carried on chromatoplates of silica gel G (E.M.). Acetylations were carried out with Ac₂O/pyridine at room temp. for 20 h and worked up by removal of the reagents under reduced pressure on a hot water bath.

Isolation procedure. Fruit juice of *Ecballium elaterium* freshly collected was continuously extracted with ether. From the ether extract elatericin A and B were obtained as previously described.² The resinous residue (125 g) left after evaporation of the mother liquor under reduced pressure was redissolved in EtOAc (250 ml) mixed with silica gel (200 g) and evaporated to dryness. The dry mass is placed on the top of a dry column of silica gel (2.2 kg) and eluted with n-hexane followed by mixtures of n-hexane with increasing quantities of ether. Fractions of about 250 ml each were collected and were monitored by TLC. The fractions from which the various phenolics were obtained are given below.

Fraction No.	Eluent	Compound
57–86	Ether hexane (3:7)	2-nitroquinol (56 mg)
87–114	Ether hexane (3:7)	hydroquinone (150 mg)
158–192	Ether hexane (3:7)	4-OH-3-OMe-acetophenone (125 mg)
193–240	Ether hexane (3:7)	4-OH-acetophenone (105 mg)
374–400	Ether hexane (3:2)	ligballinol (70 mg)

Hydroquinone. M.p. 170° (from CHCl₃); MS *m/e* 110 M⁺ (100), 109 (M-1) (5.9), 82 (8.6), 81 (18.8), 55 (5.9) and 53 (4.3). (Found: M⁺ 110. C₆H₆O₂ requires: M.wt. 110.11).

4-OH-3-OMe-Acetophenone (2). M.p. 114° (from aq. EtOH) (lit. 115°); MS *m/e* 166 M⁺ (45), 151 (M-CH₃) (100), 136 (8.9), 123 (M-CO) (18.8), 108 (M-COCH₃-CH₃) (9.4), 95 (2.3), 93 (2.6), 79 (4.8), 77 (6.6), 67 (4.0), 65 (8.7), 63 (5.5), 55 (5.0), 53 (6.6), 52 (14) and 43 (20.8); NMR, δ 2.57 (3H, s, ArCO.CH₃), 3.95 (3H, s, O-CH₃), 6.98 (1H, d, J = 9 Hz), 7.60 (1H, d, J = 2 Hz) and 7.57 (1H, dd, J = 2 and 9.0 Hz). (Found: M⁺ 166. C₉H₁₀O₃ requires: 166.17).

4-OH-Acetophenone (1). M.p. 109° (from aq. EtOH) (lit. 109°); δ 2.59 (3H, s, Ar-COCH₃), 3.95 (3H, s, OCH₃), 6.97 (2H, d, J = 9.0 Hz) and 7.96 (2H, d, J = 9.0 Hz); MS *m/e* 136 M⁺ (47.7), 121 (M-15) (100), 93 (M-COCH₃) (31.4). (Found: M⁺ 136. C₈H₈O₂ requires: 136.16).

2-Nitroquinol (3a). Fractions 57–86 were combined and evaporated to dryness. The residue upon trituration with CHCl₃ left **3a** as an insoluble residue. It was crystallised from MeOH-CHCl₃ as orange red crystals, m.p. 131–133° (lit. 133–134°). λ_{max} (EtOH) 250, 280 and 405 nm; ν_{max} 3445 (OH), 1581, 1491 (Ar), 1525 and 1340 (Ar-NO₂), 1230, 1193, 1167, 1125, 1071, 945, 861, 833, 789 and 758 (all very strong) cm⁻¹; MS *m/e* 155 M⁺ (90.5), 97 (39.7), 81 (52.6), 80 (38), 69 (37.1), 53 (79.2), 52 (100). (Found: M⁺ 155. C₈H₇O₃N requires: M.wt. 155.1).

Diacetate (3b). Crystallised from aq. EtOH m.p. 84–85° (lit. 86°). λ_{max} 255 and 297 (sh) nm; ν_{max} 1765, 1520, 1365, 1342, 1217, 1183, 1133, 1017, 910 and 815 cm⁻¹; MS *m/e* 239 M⁺ (2.6), 197 (M-42) (26), 181 (0.29), 155 (M-2COCH₃), (55.4), 139 (1.7), 109 (1.9), 107 (1.8), 97

(2.1), 81 (1.3), 79 (1.7), 63 (1.4), 53 (58.7), 43 (100). NMR δ 7.87, d, J = 2.5 Hz 3-H, 7.35, dd, J = 9 and 2.5 Hz 5-H and 7.19, d, J = 9 Hz 6-H.

Synthesis of 3b. Diacetylhydroquinone (m.p. 123°) (0.5 g) was added in small quantities to conc HNO₃ (d, 1.45, 2.5 ml) at 0°. The mixture was poured on crushed ice and the ppt was collected by filtration and washed with a dilute soln of NaOAc followed by water. The residue was extracted with a dilute Na₂CO₃ aq which was then acidified with AcOH. The yellow crystalline ppt was filtered off, washed with water and dried. The crude product was acetylated and the acetate thus obtained was crystallised from aq. EtOH to yield crystals, m.p. 85°, identical with **3b** (m.p. mixture m.p. IR and NMR).

Ligballinol (4a). Crystallised from CHCl₃ as colourless needles, soluble in warm MeOH and insoluble in ether, m.p. 264–266°; [α]_D²⁰ = -7.1° (c, 1.61); ν_{max} 3260–3300 (br), 2975, 2867, 1613, 1599, 1511, 1450, 1395, 1270, 1230, 1171, 1100, 1045, 1030, 950, 930 and 832 cm⁻¹ (all strong); MS *m/e* 298 M⁺ (28.3), 280 (1.2), 267 (10.4), 204 (6.5), 179 (9.1), 175 (26), 166 (7.8), 161 (10.4), 147 (15.6), 133 (61), 121 (100), 107 (57), 94 (15.6), 77 (18.2), 65 (18.2) and 54 (15.6). (Found: C, 72.53; H, 6.24; M⁺ 298. C₁₈H₁₈O₄ requires: C, 72.47; H, 6.08%; M.wt. 298.32).

Diacetate (4b). M.p. 94° (from ether-hexane); ν_{max} 2940,

2860 (br), 1755, 1500, 1374, 1225, 1200, 1165, 1075, 1050, 1017, 916 and 839 cm⁻¹ (all strong). MS *m/e* 382 M⁺ (33.9), 351 (7.9), 340 (9.0), 323 (5.6), 309 (9.6), 297 (3.4), 280 (2.1), 267 (6.8), 246 (10.7), 233 (5.5), 217 (3.2), 204 (8.3), 191 (6.6), 175 (45.2), 163 (28.2), 159 (12.4), 147 (19.2), 133 (80), 121 (100), 107 (96.6), 94 (13.6), 77 (20.9), 65 (18.6) and 54 (15.3). (Found: C, 69.15; H, 6.0; M⁺ 382. C₂₂H₂₂O₆ requires: C, 69.10; H, 5.80% M.wt. 382.40).

Acknowledgement—M. M. Rao is grateful to the Deutscher Akademischer Austauschdienst for a Fellowship.

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