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EIGHT 1,4-NAPHTHOQUINONES FROM *JUGLANS*

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Key Word Index—*Juglans nigra*, *Juglans regia*, Juglandaceae, walnut, volatile 1,4-naphthoquinones.

Abstract—Eight volatile 1,4-naphthoquinones from acetone extracts of unripe black walnut (*Juglans nigra*) and English walnut (*Juglans regia*) fruit were identified by gas chromatography-mass spectrometry assisted by ^{1}H NMR. Compounds not previously reported in walnut are 2-methyl-1,4-naphthoquinone, 2,3-dihydro-5-hydroxy-2-methyl-1,4-naphthalenedione (β-hydroplumbagin), 5-hydroxy-2-methyl-1,4-naphthoquinone (plumbagin), 5-hydroxy-3-methyl-1,4-naphthoquinone and 2,3-dimethyl-5-hydroxy-1,4-naphthoquinone The latter two compounds are here first reported as natural products

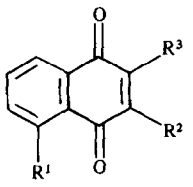
INTRODUCTION

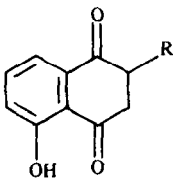
While investigating volatile compounds from walnut husks that might be attractive to the walnut husk fly, *Rhagoletis completa* (Cresson), we identified eight 1,4-naphthoquinones of which only three were previously reported to be present in walnut These three compounds are juglone (5-hydroxy-1,4-naphthoquinone) (**3**), β-hydrojuglone (2,3-dihydro-5-hydroxy-1,4-naphthalenedione (**7**) and 1,4-naphthoquinone (**1**). Juglone was initially thought to be in walnut tissues only in a combined form [1–3] that was shown to be a glucoside of 1,4,5-trihydroxynaphthalene (α-hydrojuglone) [2–5]. Recent work shows that juglone is also present in free form [6–10]. After hydrolysis of glucosides from unripe walnut husks, Mylius [1,11] isolated both α- and β-hydrojuglone. However, work by Thomson [12] indicated that the method of extraction would convert part of the α-hydrojuglone into the β-form. He subsequently reported that β-hydrojuglone did occur in free form in *Lomatia tinctoria* seeds [13] and it was more recently found in an extract of *Juglans regia* [14]. 1,4-Naphthoquinone, an intermediate in juglone biosynthesis, was isolated from aerial parts of young English walnut plants [14,15].

RESULTS AND DISCUSSION

Volatile compounds, including 1,4-naphthoquinones, were separated from nonvolatile components of the acetone extract of husk peelings of unripe walnuts by steam distillation. After recovery from the steam distillate, these compounds were analysed by gas chromatography-mass spectrometry.

Identifications of compounds were based on the correspondences of GC retention index and mass spectrum with those of authentic compounds obtained either commercially or by synthesis. The compounds listed in Table 1 all display an $[M-28]$ peak and, except for **8**, have $[M]^+$ as base ion. Juglone (**3**) and **4**–**8** with a similarly substituted hydroxyl group show ions at m/z 120, 92 and 63. For **4**–**6** and **8**, there is an $[M-15]$ ion for loss of a methyl group.





1 $R^1 = R^2 = R^3 = H$
2 $R^1 = R^3 = H$, $R^2 = Me$
3 $R^1 = OH$, $R^2 = R^3 = H$
4 $R^1 = OH$, $R^2 = H$, $R^3 = Me$
5 $R^1 = OH$, $R^2 = Me$, $R^3 = H$
6 $R^1 = OH$, $R^2 = R^3 = Me$

7 R = H
8 R = Me

Their ^{1}H NMR spectra helped identification of **5**–**8**. The component of the walnut husk volatiles with R_I 1568 (Table 1) was purified by chromatography on silica gel. Its ^{1}H NMR spectrum matched that of synthetic **5** [16] and was significantly different from the spectrum of **4** in the δ7.5–7.7 region and near δ12 (OH proton). Substituting a methyl group in **3** causes a change in the chemical shift of the hydroxy proton that depends on the position of the substituent [17]. Although the mass spectrum of the compound with R_I 1694 seemed to indicate structure **6**, the possibility of an ethyl derivative of **3** could not be excluded. However, the NMR spectrum of a sample obtained by preparative GLC showed a single peak at δ2.18 that represented six protons, which confirms structure 6. Volatiles recovered from freeze-drying of black walnuts contained a major amount of the compound with R_I 1515. In the NMR spectrum of a sample crystallized from hexane, a symmetrical signal centred at δ3.09 for four protons was evidence for structure **7** and the spectrum matched that of synthetic **7** prepared by $SnCl_2$–HCl reduction of **3** [12]. Reduction of **4** by the same method gave **8** whose NMR spectrum was the same as that of a sample of the compound with R_I 1550 isolated by chromatography on silica gel.

It is not clear that **7** and **8** are present in walnut husks. Hydrolysis of α-hydrojuglone glucoside yields 1,4,5-tri-

Table 1 Kováts indices of walnut husk compounds and their concentrations in peelings

Compound	R_I*	Concentration in μg/g *J nigra*	*J regia*
1,4-Naphthoquinone **1**	1356	0 19	0 54
5-Hydroxy-1,4-naphthoquinone **3**	1452	18 2	1 7
2-Methyl-1,4-naphthoquinone **2**	1466	0 24	0 09
2,3-Dihydro-5-hydroxy-1,4-naphthalenedione **7**	1515	3 2	3 2
2,3-Dihydro-5-hydroxy-2-methyl-1,4-naphthalenedione **8**	1550	17 8	2 1
5-Hydroxy-2-methyl-1,4-naphthoquinone **4**	1559	27 1	2 4
5-Hydroxy-3-methyl-1,4-naphthoquinone **5**	1568	19 7	1 8
2,3-Dimethyl-5-hydroxy-1,4-naphthoquinone **6**	1694	11 4	0 41

*Kováts Index [24] on 60 m DB-1 column

hydroxynaphthalene that could be oxidized to **3** or isomerized to the more stable form **7** as a result of the work-up procedures [11, 12, 18]. Analogously, hydroplumabagin glucoside, identified in *Drosera rotundifolia* L. [19] but not in *Juglans*, might yield **4** and **8**. However, it seems likely that **7** does occur in the husk because it was previously found in a walnut extract [14] and in our work was also obtained from the condensate of volatiles recovered from freeze-drying *J nigra* fruit.

Three compounds with mass spectra that strongly indicated them to be 1,4-naphthoquinone derivatives were not identified. A compound with R_I 1653 showed $[M]^+$ at *m/z* 202 and an [M−29] ion but not an [M−15] ion. It seems likely to be an ethyl derivative of **3**. The $[M]^+$ of a compound with R_I 1666 is also at *m/z* 202, but its MS is very similar to that of **6** The third compound had R_I 1722 and M^+ at *m/z* 216 All were present in trace amounts

The concentrations listed in Table 1 of **1–8** in husk peelings should not be considered as typical for the whole fruit When an extract of the entire husk of *J. nigra* was taken, **7** constituted 56% and **3** 26% of the mixture **1–8**. A study of α-hydrojuglone glucoside content in walnuts revealed a concentration gradient in the fruit [20]. There is also great seasonal variation in content of **3** and α-hydrojuglone glucoside [6, 20, 21]

Compounds **1–4** and **7–8** were known to occur in plants, but 5-hydroxy-3-methyl-1,4-naphthoquinone **(5)** and 2,3-dimethyl-5-hydroxy-1,4-naphthoquinone **(6)** were not previously found as natural products. From husks of *Juglans mandshurica maxim*, 5,8-dihydroxy-1,4-naphthoquinone was isolated [22] and 4,8-dihydroxy-1-tetralone was found in stem-bark of *J. regia* [23] We do not find evidence of these compounds in our samples These naphthoquinones and other walnut volatile compounds are being tested as attractants for the walnut husk fly

EXPERIMENTAL

Plant material Unripe *J nigra* and *J. regia* variety Hartley fruit were picked in a grove near Woodland, California on 3 July 1988 A sample of *J nigra* fruit was also picked on 31 July 1988 and freeze-dried

Extraction and isolation of volatile compounds. Peelings of the outermost layer of walnut husks (164 g from 1164 g *J nigra* and 184 g from 1293 g *J regia*) were cut with a vegetable peeler and dropped immediately into Me_2CO in a Waring Blendor Me_2CO was removed from the extract under red pres The residue was steam dist under vacuum The distillate was satd with salt and extracted with peroxide-free ether The ether solution was dried and then concd to 2 ml. Prep GC on a 2 7 m × 2 mm column of 2% OV-17 was used to isolate **6** Chromatography on acid-washed silica gel was used to purify **5** and **8**.

Analytical procedures R_Is (Table 1) were determined by use of a 60 m × 0 32 mm DB-1 fused silica column (J & W Scientific) operated with head pressure 24 psi He and temp program 50–240° at 4°/min An equivalent column was used for GC/MS with a Finnigan MAT 4500 system (ionizing energy 70 eV) GC of a measured mixture of walnut volatiles plus tetradecane allowed calculation of **1–8** concns Correction for FID response was made ^{1}H NMR (200 MHz) spectra were obtained for $CDCl_3$ solutions with TMS as int standard Some chemical shift values were obtained by computer simulation of the spectra

1,4-Naphthoquinone **(1)** MS *m/z* (rel int) 158$[M]^+$(100), 130 (34), 104 (46), 102 (44), 76 (34), 50 (14)

2-Methyl-1,4-naphthoquinone **(2)** MS *m/z* (rel int) 172$[M]^+$ (100), 144 (23), 116 (34), 115 (46), 104 (54), 76 (38), 50 (14)

5-Hydroxy-1,4-naphthoquinone **(3)** MS *m/z* (rel int) 174$[M^+]$(100), 146 (11), 120 (28), 118 (40), 92 (24), 63 (17)

5-Hydroxy-2-methyl-1,4-naphthoquinone **(4)** MS *m/z* (rel int) 188$[M]^+$ (100), 173 (27), 160 (21), 145 (5), 132 (22), 131 (40), 121 (17), 120 (27), 92 (25), 63 (14)

5-Hydroxy-3-methyl-1,4-naphthoquinone **(5)** MS *m/z* (rel int) 188$[M]^+$ (100), 173 (9), 160 (15), 145 (4), 132 (25), 131 (29), 121 (9), 120 (36), 92 (25), 63 (13) ^{1}H NMR δ2 19 (3H, *d*, J = 1 6 Hz, Me-3), 6 81 (1H, *q*, J = 1 6 Hz, H-2), 7 24 (1H, *dd*, J = 8, 2 Hz, H-6), 7 59 (1H, *dd*, J = 8, 2 Hz, H-8), 7 61 (1H, *t*, J = 8, 8 Hz, H-7) and 12.05 (1H, *s*, −OH).

2,3-Dimethyl-5-hydroxy-1,4-naphthoquinone **(6)** MS *m/z* (rel int) 202$[M]^+$(100), 187 (8), 174 (27), 159 (20), 145 (20), 131 (32), 121 (22), 120 (31), 92 (33), 63 (17) ^{1}H NMR δ2 18 (6H, *s*, 2Me), 7.22 (1H, *dd*, J = 7 5, 2.0 Hz, H-6), 7 57 (1H, *t*, J = 7 5, 7 5 Hz, H-7), 7 62 (1H, *dd*, J = 7 5, 2 0 Hz, H-8) and 12 19 (1H, *s*, −OH)

2,3-Dihydro-5-hydroxy-1,4-naphthalenedione **(7)** MS *m/z* (rel int) 176$[M]^+$(100), 148 (18), 147 (15), 121 (29), 120 (83), 92 (41), 63 (16) ^{1}H NMR δ3 0–3.2 (4H, AA′BB′ *m*, 2H-2 and 2H-3), 7 26 (1H, *dd*, J = 8 2, 1 4 Hz, H-6), 7 55 (1H, *dd*, J = 7 4, 1 4 Hz, H-8), 7 66 (1H, *dd*, J = 8 2, 7 4 Hz, H-7) and 12 12 (1H, *s*, −OH)

2,3-Dihydro-5-hydroxy-2-methyl-1,4-naphthalenedione **(8)** MS *m/z* (rel. int) 190$[M]^+$(83), 175 (100), 162 (20), 147 (22), 120 (59), 92 (38), 63 (15) ^{1}H NMR· δ1 34 (3H, *d*, J = 6 4 Hz, Me), 2 88 (1H, *dd*, J = 17, −12 Hz, H-3), 3 05–3 25 (2H, *m*, H-2 and H-3), 7 25 (1H, *dd*, J = 7 8, 1 4 Hz, H-6), 7 54 (1H, *dd*, J = 7 8, 1 4 Hz, H-8), 7 65 (1H, *t*, J = 7 8, 7 8, H-7) and 12 06 (1H, *s*, −OH)

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