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PHENOLIC GLUCOSIDES AND LIGNANS FROM *EHRETIA OVALIFOLIA*

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Key Word Index—*Ehretia ovalifolia*; Boraginaceae; bark; neolignan; sesquilignan; 2-methoxyhydroquinone; vanillic acid; syringic acid; ehlethianols A–D.

Abstract—Four new constituents comprising two 2-methoxyhydroquinone glucosides, a sesquilignan and a neolignan were isolated from the bark of *Ehretia ovalifolia*, together with four known lignans. The structures of the new compounds were elucidated by chemical and spectral methods as 1-*O*-[(6-vanilloyl)-β-D-glucopyranosyl]-4-*O*-[(6-syringoyl]-β-D-glucopyranosyl] 2-methoxyhydroquinone, 1,4-*bis* *O*-(6-vanilloyl)-β-D-glucopyranosyl-2-methoxyhydroquinone, guaiacylglycerol-larisiresinol ether and 2-{2-[*O*-β-D-glucopyranosyl]-3-methoxy-5-[1-(*E*)-propene-3-al]-phenyl}-3-(4-hydroxy-3-methoxyphenyl-propanol).

INTRODUCTION

Ehretia ovalifolia Hassk., a deciduous tree, 20 m high, distributed from the tropics to the temperate regions, is used as a structural wood or an apparatus material [1]. The bark of this species is also used as a dyestuff in Japan because it contains tannin [1]. The present paper reports the isolation of compounds from *E. ovalifolia* and their characterization. Eight phenolic compounds have so far been isolated and identified. These are the known compounds icariside E_5 (**1**) [2], 1-(4-hydroxy-3-methoxyphenyl)-2-{2-methoxy-4-[1-(*E*) propen-3-ol]-phenoxy}-propane-3-diol(*erythro*) (**2**) [3, 4], 1-(4-hydroxy-3-methoxyphenyl)-2-{2-methoxy-4-[1-(*E*) propen-3-ol]-phenoxy}-propane-1,3-diol (*threo*) (**3**) [3, 4] and buddlenol B (**4**) [5], and four novel phenolic compounds. Structures were elucidated by chemical and spectral methods, especially 2D-NMR techniques.

RESULTS AND DISCUSSION

Careful separation of the polar fraction of *E. ovalifolia* afforded eight polar compounds. The four known compounds were identified as **1**–**4** by comparison with published data [2–5].

Ehletianol A (**5**), $C_{36}H_{42}O_{20}$, was assigned an M_r of 794 by the observation of $[M - H]^-$ ion peak at *m/z* 793 in the negative FAB mass spectrum. The IR absorption bands at 1695, 1600 and 1515 cm^{-1} and the signals at δ166.8 and 166.6 in the ^{13}C NMR spectrum suggested the presence of a pair of benzoyl esters. Acid hydrolysis of **5** afforded 2-methoxyhydroquinone (**9**), vanillic acid (**10**), syringic acid (**11**) and D-glucose confirmed by specific rotation using chiral detection by HPLC. The ^1H and ^{13}C NMR spectra of **5** indicated that it was composed of 1 mol each of 2-methoxyhydroquinone, syringic acid and vanillic acid, and 2 mol of β-glucose. The β-anomeric configuration for glucose was judged from its large $^3J_{H1,H2}$ coupling constant ($J = 7.8$ Hz). A ^{13}C NMR spectral comparison of **5** with **9** showed glycosylation shifts [6, 7] by + 2.0 ppm at the C-1 signal and 1.5 ppm at the C-4 signal, demonstrating sugar linkages to be located at the C-1-OH and C-4-OH of 2-methoxyhydoquinone. This was further confirmed by HMBC and ROESY experiments (Fig. 1). Long-range correlations were seen between H-1′ (δ5.531) (Glc-1) and C-1 (δ143.1), and between H-1′ (δ5.526) (Glc-2) and C-4 (δ154.0) in the HMBC spectrum, and NOEs between H-1′ (δ5.531) (Glc-1) and H-6 (δ7.38) and between H-1′ (δ5.526) (Glc-1) and H-3 (δ6.83)/H-5 (δ6.93). The connectivity between syringic acid, vanillic acid and two glucose residues was also established from the acylation shifts. In the ^{13}C NMR spectrum of **5**, the C-6′ and C-5′ position of Glc-1 and the C-6′ and C-5′ position of Glc-2 were shifted to δ64.9 and 75.4, and to δ65.2 and 75.7, respectively, by acylation, suggesting that the C-6′ of each glucose should be acylated. A HMBC experiment revealed that H_2-6′ (δ5.26 and 4.98) of Glc-1 coupled to the carbonyl (δ166.6) of the vanilloyl group and H_2-6′ (δ5.47 and 4.99) of Glc-2 coupled to the carbonyl (δ166.8) of the syringoyl group, establishing the existence of vanilloyl at the C-6′ of Glc-1 and syringoyl at the C-6′ of Glc-2. Hence, **5** was formulated as 2-methoxyhydoquinone-1-*O*-(6-vanilloyl)-β-D-glucopyranosido-4-*O*-(6-syringoyl)-β-D-glucopyranoside.

Ehletianol B (**6**), $C_{35}H_{40}O_{19}$, was assigned an M_r of 764 by the observation of a $[M - H]^-$ ion peak at *m/z* 763 in the negative FAB mass spectrum, i.e. 30 amu lower than that of **5**. The IR absorption bands at 1695, 1600

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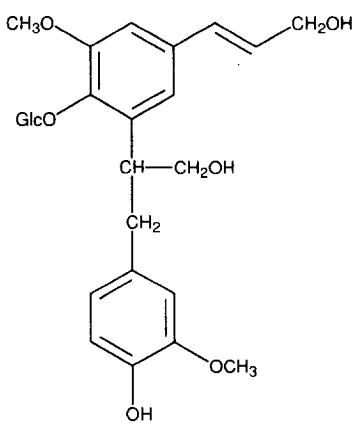

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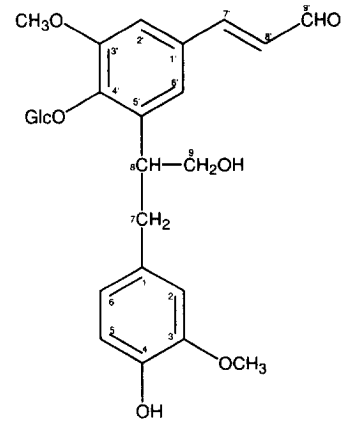

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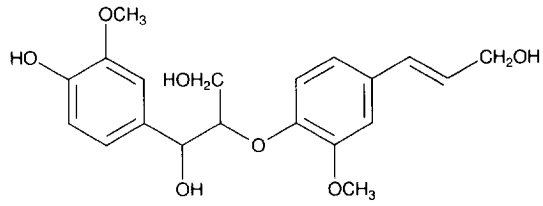
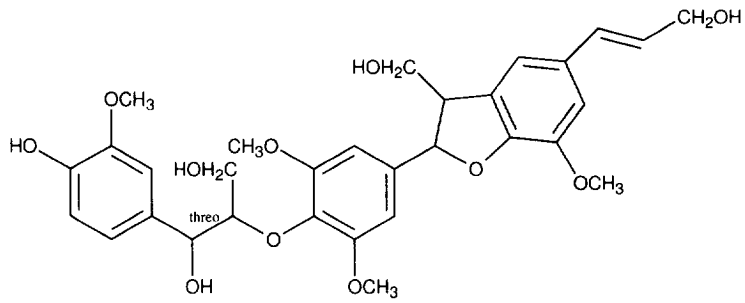


2 *threo*
3 *erythro*



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and 1515 cm^{-1} and the signals due to two carbons at δ166.6 showed the presence of a pair of benzoyl esters. Acid hydrolysis of **6** afforded **9**, **10** and D-glucose confirmed by specific rotation using chiral detection by HPLC. The ^1H and ^{13}C NMR spectra of **6** indicated that it was composed of 2 mol each of vanillic acid and β-glucose, and 1 mol of 2-methoxyhydoquinone. A ^{13}C NMR spectral comparison of **6** with **9**, showed glycosylation shifts by + 2.0 ppm at the C-1 signal and 1.5 ppm at the C-4 signal, demonstrating sugar linkages at the C-1–OH and C-4–OH of 2-methoxyhydoquinone. This was further confirmed by HMBC and ROESY experiments (Fig. 2). Long-range correlations were seen between H-1′ (δ5.53) (Glc-1) and C-1, and between H-1′ (δ5.52) (Glc-2) and C-4 in the HMBC spectrum, and NOEs between H-1′ (δ5.52) (Glc-1) and H-3 (δ7.40) and between H-1′ (δ5.53) (Glc-1) and H-5 (δ6.91). The connectivity between two vanillic acids and two glucose residues was also established from the acylation shifts. In the ^{13}C NMR spectrum of **6**, the C-6 and C-5 positions of the two glucoses were shifted to δ64.9 and 75.6 (each two carbon), respectively, by acylation, showing that the two vanilloyl groups must be linked to C-6′ of each glucose. This was further confirmed by an HMBC experiment (Fig. 2). Long-range correlations were seen between H-6′ (δ5.34 and 5.02) of Glc-1 and the carbonyl (δ166.6) of the vanilloyl group, and between H-6′ (δ5.32 and 4.97) of Glc-2 and the carbonyl (δ166.6) of the vanilloyl group. Hence, **6** was formulated as 2-methoxyhydoquinone-1,4 bis-*O*-(6-vanilloyl)-β-D-glucopyranoside.

Ehletianol C (**7**), $[\alpha]_D^{20}$ − 10.6° (MeOH) revealed a $[M - H]^-$ at *m/z* 555 in the negative FAB mass spectrum, suggesting the molecular formula, $C_{30}H_{36}O_{10}$. Absorption maxima at 230 and 281 nm in the UV spectrum

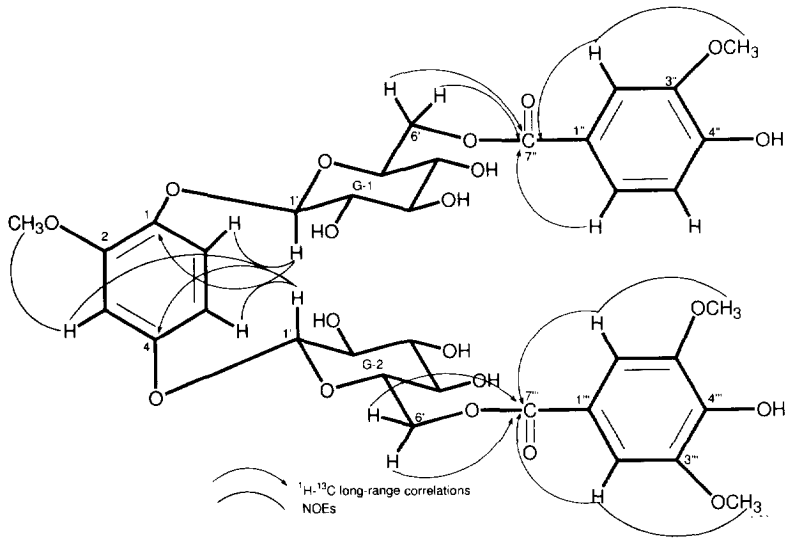


Fig. 1. Most significant correlations observed in HMBC and NOEs of **5**.

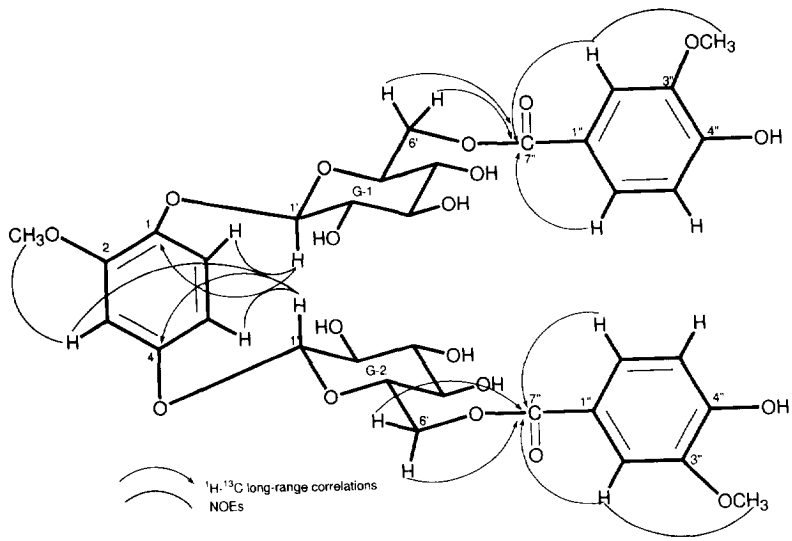


Fig. 2. Most significant correlations observed in HMBC and NOEs of **6**.

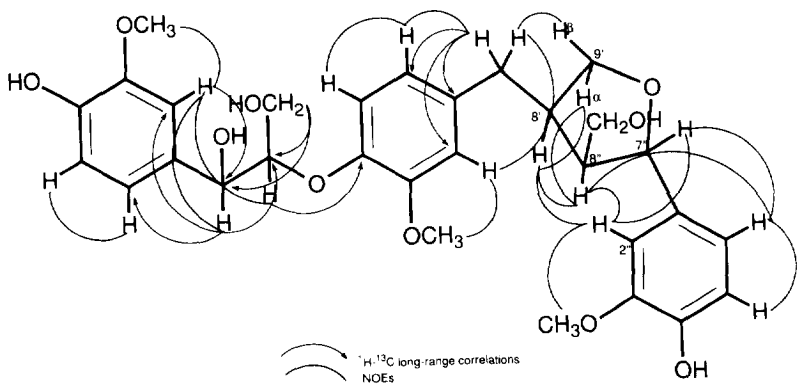
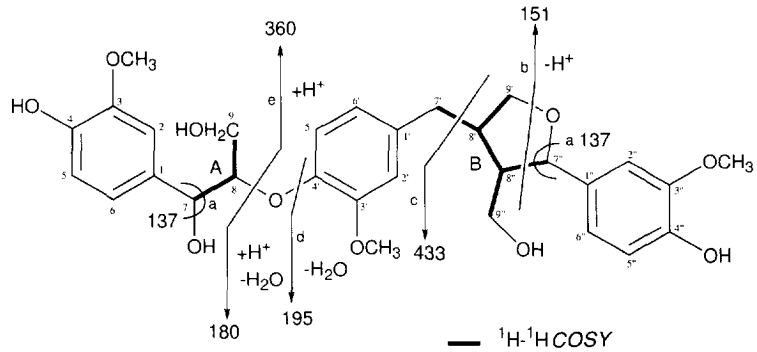


Fig. 3. Most significant correlations observed in HMBC and NOEs of **7**.



Scheme 1. Mass spectral fragmentation and ^1H–^1H COSY of **7**.

Table 1. ^1H and ^{13}C NMR spectral data of **5** and **6** (600/150 MHz, CD_3CD)

Position	**5** ^{13}C	**5** ^1H	**6** ^{13}C	**6** ^1H
1	143.1	—	143.1	—
2	150.7	—	150.7	—
3	103.6	6.83 *d* (2.7)	103.6	6.81 *d* (1.5)
4	154.0	—	154.0	—
5	107.7	6.93 *dd* (9.0, 2.7)	107.8	6.91 *dd* (8.8, 1.5)
6	116.9	7.38 *d* (9.0)	117.2	7.40 *d* (8.8)
O-Me	55.7	3.48 *s*	55.7	3.48 *s*
G-1				
1′	102.9	5.531 (7.8)	102.9	5.53 (7.6)
2′	74.9	4.34 (8.8, 7.8)	75.0	4.35 (8.5, 7.6)
3′	78.3	4.43 (9.5, 8.8)	78.3	4.44 (9.3, 8.5)
4′	71.8	4.21 (9.5, 9.0)	71.8	4.25 (9.3, 9.3)
5′	75.4	4.42 *m*	75.6	4.47 *m*
6′	64.9	5.26 *dd* (11.5, 1.5)	64.9	5.34 *dd* (11.0, 1.5)
		4.98 *dd* (11.5, 7.1)		5.02 *dd* (11.5, 7.0)
G-2				
1′	102.8	5.526 *d* (7.8)	102.9	5.52 *d* (7.6)
2′	75.1	4.35 *dd* (8.8, 7.8)	74.9	4.34 *dd* (8.5, 7.6)
3′	78.3	4.45 *dd* (9.5, 8.8)	78.3	4.42 *dd* (9.4, 8.5)
4′	71.9	4.24 *dd* (9.5, 8.8)	71.8	4.21 *dd* (9.5, 8.8)
5′	75.7	4.51 *m*	75.6	4.43 *m*
6′	65.2	5.47 *dd* (11.5, 1.5)	64.9	5.32 *dd* (11.0, 1.5)
		4.99 *dd* (11.5, 7.1)		4.97 *dd* (11.6, 7.2)
1″	121.7	—	121.6 × 2	—
2″	113.6	7.79 *d* (2.0)	113.6 × 2	7.82 *d* (1.5), 7.81 *d* (1.5)
3″	148.3	—	148.3 × 2	—
4″	153.3	—	153.3 × 2	—
5″	116.3	7.26 *d* (8.3)	116.2 × 2	7.26 *d* (8.5), 7.25 *d* (8.5)
6″	124.8	7.87 *dd* (8.3, 1.7)	124.9 × 2	7.91 *dd* (2H, 8.5, 1.5)
7″	166.6		166.6 × 2	
O-Me	55.8	3.64 *s*	55.7 × 2	3.63 *s*
1‴	120.2	—		
2‴	108.4	7.69 *s*		
3‴	148.7	—		
4‴	143.1	—		
5‴	148.7	—		
6‴	108.4	7.69 *s*		
7‴	166.8			
7‴	166.8			
O-Me	56.3 × 2	3.73 *s*		

and absorption bands at 1600 and 1515 cm^{-1} in the IR spectrum suggested the presence of a non-conjugated aromatic ring. The ^{13}C NMR spectrum of **7** showed 27 carbon signals except for the three methoxy signals, indicating **7** to be a sesquilignan (3 × 9). The ^1H and ^1H–^1H COSY spectra showed the presence of three sets of an ABX pattern in the aromatic region and a glycerol (part A in Scheme 1) and a tetrahydrofuran (part B). The EI mass spectrum showed a small $[M]^+$ peak at *m/z* 556 and the relatively high relative abundances of the peaks at *m/z* 538 $[M - H_2O]^+$, 520 $[M - 2H_2O]^+$, 508 $[M - H_2O–CH_2O]^+$, 490 $[M - 2H_2O–CH_2O]^+$ and 478 $[M - 2H_2O–2CH_2O]^+$. The relatively high relative abundances of peaks at *m/z* 360, 330 $[360-CH_2O]^+$ and 180 (path e, Scheme 1) and of peaks at *m/z* 151 and 121 $[151 - CH_2O]^+$ by splitting of the tetrahydrofuran ring (path b) [8] indicated the presence of a guaiaepoxylignan. The relatively high relative abundances of peaks at *m/z* 195, 177 $[195 - H_2O]^+$ and 165 $[195 - CH_2O]^+$ (path d) indicated the presence of guaiacylglycerol. The gross structure of **3** was determined by analysis of NMR data including ^1H–^1H COSY, HMQC, HMBC and ROESY and reference to the data of **2**, **3** and lariciresinol [9, 10]. The C-7 signal in the ^{13}C NMR spectrum of **7** appeared at lower field by 1.3 ppm than that of **3**, demonstrating that **7** is the *threo*-isomer. The stereochemistry of the tetahydrobenzofuran part was elucidated by a ROESY experiment. The NOEs between H-8″ and H-9′/H-8′/H-2″ indicated a *cis*-configuration of 8′/8″ and a *trans*-configuration of 7″/8″. Ehletianol C is therefore considered to have the structure **7**.

Ehletianol D (**8**), $C_{26}H_{32}O_{11}$, was an amorphous powder. The negative FAB mass spectrum showed peaks at *m/z* 519 $[M - H]^+$ and 357 $[M - H–C_6H_{10}O_5]^+$, i.e. 2H less that of **1**. Absorption maxima at 234 and 303 nm in the UV spectrum and absorptions at 1670, 1600 and 1515 cm^{-1} in the IR spectrum **8** suggested the presence of an aromatic propenal side-chain conjugated system. On acid hydrolysis, **8** afforded several unresolved components for aglycone, besides D-glucose confirmed by chiral detection by HPLC. A ^{13}C NMR spectral comparison of **8** with **1**, showed that **8** differs structurally from **1** only in

Table 2. ^1H and ^{13}C NMR spectral data of **7** (600/150 MHz) and **8** (200/50 MHz)

Position	**7** (CD_3OD) ^{13}C	**7** (CD_3OD) ^1H	**8** (pyridine-d_5) ^{13}C	**8** (pyridine-d_5) ^1H
1	133.8	—	132.5	—
2	111.7	7.01 *d* (1.5)	111.3	6.92 *d* (1.8)
3	148.8		148.8	—
4	147.2	—	146.4	—
5	111.5	6.74 *d* (8.0)	111.6	7.03 *d* (8.0)
6	120.7	6.85 *dd* (8.0, 1.5)	122.5	6.90 *dd* (8.0, 1.8)
7	74.1	4.87 *d* (6.0)	39.0	3.45 *d* (14.3, 7.2)
				3.17 *d* (14.3, 8.3)
8	87.5	4.24 *ddd* (6.0, 5.2, 4.0)	42.5	4.79 *m*
9	61.9	3.71 *dd* (12.0, 4.0)	66.5	4.34 *bd s* (2H)
		3.46 *dd* (12.0, 5.2)		
1′	136.7	—	131.7	—
2′	114.2	6.87 *d* (1.5)	110.7	7.11 *br s*
3′	151.8	—	153.6	—
4′	147.9		147.8	—
5′	119.5	6.97 *d* (8.0)	140.3	—
6′	122.4	6.71 *ddd* (8.0, 1.5, 1.5)	122.5	7.15 *br s*
7′	33.7	2.95 *dd* (13.0, 5.0)	154.3	7.53 *d* (16.2)
		2.52 *ddd* (13.0, 11.5, 1.5)		
8′	43.8	2.73 *dddd* (11.5, 6.4, 6.0, 6.0, 5.0)	128.7	6.94 *d* (16.2, 7.1)
9′	73.5	3.97 *dd* (8.0, 6.4)	195.0	9.83 *d* (7.1)
		3.70 *ddd* (8.0, 6.0, 1.0)	Glc	
1″	135.7	—	105.3	5.63 *d* (6.5)
2″	110.6	6.90 *d* (1.5)	76.4	
3″	149.0	—	78.6	
4″	147.1	—	71.4	
5″	116.0	6.75 *d* (8.0)	78.4	
6″	119.8	6.76 *dd* (8.0, 1.5)	62.5	
7″	84.0	4.74 *dd* (6.8, 1.0)		
8″	54.1	2.37 *dddd* (8.0, 6.8, 6.4, 6.0)		
9″	60.4	3.82 *dd* (11.0, 8.0)		
		3.63 *dd* (11.0, 6.4)		
O-Me	56.3	3.80 *s*	56.3	3.68 *s*
	56.4	3.81 *s*	56.6	3.70 *s*
	56.5	3.81 *s*		

its propenal side-chain, though the same sugar unit is also affixed to C-4'. Reduction of **8** with lithium borohydride gave **1**. Ehletianol D is therefore considered to have structure **8**.

EXPERIMENTAL

General. Mps uncorr. NMR: recorded on Varian UNITY 600 or 200 spectrometers in C_5D_5N soln using TMS as int. standard. NMR expts included ^1H–^1H COSY, ^1H–^{13}C COSY, DEPT, TOCSY, ROESY and HMBC (512 × 1024 data matrix size, 128 scans, recycle delay = 1.16 sec). Coupling constants (*J* values) are given in Hz. FAB-MS (Xe gun, 10 kV, *m*-nitrobenzyl alcohol as matrix). For CC, silica gel 60 (40–63 μm, Merck) and for TLC, silica gel 60F-254 (Merck) were used.

Isolation of saponins. Fresh powdered bark (5 kg) was extracted with EtOH at room temp. for 2 weeks. The EtOH extract (100 g) was subjected to CC on silica gel eluting with CH_2Cl_2–MeOH–H_2O (25:4:0.1–25:10:0.5) to afford frs 1–4. Fr. 22 (30 g) was further eluted with CH_2Cl_2–MeOH–EtOAc–H_2O (4:2:4:1, upper layer) to give frs I (4.5 g), II (3.5 g), III (2.0 g), and IV (3.0 g). Frs II and III were purified by prep. HPLC (YMC, ODS, S-5, 5–15% MeCN) to afford icariside E_5 (**1**, 800 mg), **2** (13 mg), **3**, (6 mg), buddlenol B (**4**, 6 mg), ehletianol C (**7**, 6 mg) and D (**8**, 200 mg). Fr. IV was purified by prep. HPLC (Nomura, phA, S-5, 1–2% MeCN) to give ehletianol A (**5**, 15 mg) and B (**6**, 47 mg).

Ehletianol A (**5**). Amorphous powder. $[\alpha]_D^{20}$ − 14.9° (pyridine; *c* 3.5). FABMS *m/z* 793 $[M - H]^-$. IR ν_{max} (film) cm^{-1}: 3425, 1695, 1600, 1520, 1455, 1295, 1220, 1075. UV λ_{max}^{MeOH} nm (log ε): 204 (4.55), 220 (4.52), 263 (4.16). For ^1H and ^{13}C NMR data, see Table 1.

Ehletianol B (**6**). Amorphous powder. $[\alpha]_D^{20}$ − 6.6° (pyridine *c* 1.7). FABMS *m/z* 763 $[M - H]^-$. IR ν_{max} (film) cm^{-1}: 3420, 1695, 1600, 1515, 1455, 1290, 1220, 1075. UV λ_{max}^{MeOH} nm (log ε): 204 (4.65), 219 (4.57), 261 (4.31). For ^1H and ^{13}C NMR data, see Table 1.

Ehletianol C (**7**). Amorphous powder. $[\alpha]_D^{20}$ − 10.6° (MeOH; *c* 0.7). FABMS *m/z* 555 $(M - H]^-$. IR ν_{max} (film) cm^{-1}: 3400, 1600, 1520, 1455, 1280, 1075. UV λ_{max}^{MeOH} nm (log ε): 230 (4.71), 281 (4.32). EIMS *m/z* (rel. int.): 556 (2), 538 (6), 520 (9), 508 (41), 490 (16), 478 (19), 360 (27), 330 (10), 490 (16), 285 (10), 236 (13), 205 (24), 195 (10), 193 (25), 177 (8), 175 (32), 165 (12), 163 (16), 151 (42), 137 (84). For ^1H and ^{13}C NMR data, see Table 2.

Ehletianol D (**8**). Amorphous powder. $[\alpha]_D^{20}$ − 165° (MeOH; *c* 2.5). FABMS *m/z* 519 $[M - H]^-$. 357 $[M - H\text{-}C_6H_{10}O_5]^-$. IR ν_{max} (film) cm^{-1}: 3400, 1670, 1600, 1515, 1465, 1270, 1080. UV λ_{max}^{MeOH} nm (log ε): 234 (4.29), 303 (4.22). EIMS *m/z* (rel. int.): 358 (28), 326 (4), 204 (24), 175 (4), 151 (3), 137 (100), 122 (5), 91 (3), 73 (6). For ^1H and ^{13}C NMR data, see Table 2.

Acid hydrolysis of ehletianol A (**5**) *and B* (**6**). A soln of each compound (3 mg) in 5% H_2SO_4 was heated at 100° for 2 hr. The reaction mixt. was passed through a column of Amberlite IR-45 on Mitsubishi Daiaon HP-20. From the H_2O eluate of **5** and **6**, D(+)-glucose was detected using RI and chiral detection (Shodex OR-1) by HPLC (Shodex RSpak DC-613, 75% MeCN, 1 ml min^{-1}, 70°) and comparison with authentic sugars (10 mM of D-Glc). From the MeOH eluate of **5**, **9**–**11** were detected by TLC [EtOAc–EtOH–50% HOAc, 100:10:1)]: R_f; 0.60 (2-methoxyhydroquinone), 0.47 (vanillic acid) and 0.39 (syringic acid). From the MeOH eluate of **6**, **9** and **10** were detected by TLC [EtOAc–EtOH–50% HOAc, 100:10:1)]: R_f; 0.61 (2-methoxyhydroquinone), and 0.48 (vanillic acid).

Acid hydrolysis of ehletianol D (**8**). A soln of **8** (3 mg) in 5% H_2SO_4 was heated at 100° for 2 hr. The reaction mixt. was extracted with Et_2O. The aq. layer was neutralized with Amberlite IR-35 and evapd *in vacuo* to dryness. Sugars were identified in the same way as described for **5** and **6**. The sugar part gave D-Glc.

$LIBH_4$ *reduction of ehletianol D* (**8**). A soln of **8** (10 mg) and $LiBH_4$ (10 mg) in MeOH (3 ml) was stirred for 1 hr at room temp. and worked-up as usual. The reaction mixt. (10 mg) was purified by HPLC (YMC, ODS S-5, 10% MeCN) to afford **1** (8 mg).

REFERENCES

1. Shibata, K. (1957) in *A Cyclopedia of Used Plants and Plant Products*, enlarged and revised Edn, p. 441. The Hokuryukan, Tokyo.
2. Miyase, T., Ueno, A., Takizawa, N., Kobayashi, H. and Oguchi, H. (1989) *Phytochemistry* **28**, 3483.
3. Miki, K., Takehara, T., Sasaya, T. and Sakakibara, A. (1980) *Phytochemistry* **19**, 449.
4. Sawabe, A., Mathubara, Y., Kumamoto, H., Iizuka, Y. and Okamoto, K. (1986) *Nippon Nogeikagaku Kaishi* **60**, 593.
5. Houghton, P. J. (1985) *Phytochemistry* **24**, 819.
6. Kasai, R., Okihara, M., Asakawa, J., Mizutani, K. and Tanaka, O. (1979) *Tetrahedron* **35**, 1427.
7. Tori, K., Yoshimura, Y., Arita, H. and Tomita, Y. (1979) *Tetrahedron Letters* 179.
8. Weinges, K. and Spaening, R. (1967) in *Oxidative Coupling of Phenol*, Vol. 1 (Taylor, W. I. and Battersby, A. R, eds), p. 23. Marcel Dekker, New York.
9. Fonseca, S. F., Campello, J. P., Barata, L. E. S. and Ruveda, E. A. (1978) *Phytochemistry* **17**, 499.
10. Abe, F. and Yamauchi, T. (1989) *Phytochemistry* **28**, 1737.