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FLAVONOIDS FROM THE CULTURED CELLS OF *GLYCYRRHIZA ECHINATA**

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Key Word Index—*Glycyrrhiza echinata*; Leguminosae; callus culture; chalcone; dibenzoylmethane derivative; flavone; ^{1}H NMR of keto–enol tautomeric mixture; retrochalcone biosynthesis; echinatin; licodione; licoflavone A; 7,4′-dihydroxy-8-prenylflavone.

Abstract—Constituents of the cultured cells of *Glycyrrhiza echinata* have been investigated. Echinatin (4,4′-dihydroxy-2-methoxychalcone), a biosynthetically unique retrochalcone, and licodione (1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)-1,3-propanedione), a dibezoylmethane derivative, which is the possible precursor of echinatin, were obtained. The structures were determined by spectroscopic methods and syntheses. ^{1}H NMR of licodione revealed new features in chemical shifts of protons of diketonic and keto–enolic forms. 7,4′-Dihydroxyflavone, two of its prenyl derivatives and formononetin were also isolated. A discussion on retrochalcone biosynthesis is presented.

INTRODUCTION

The dried roots of several *Glycyrrhiza* species are widely used as the crude drug licorice. As well as triterpenoid saponins the main sweetening principle, flavonoid components of licorice roots have been thoroughly studied since the antigastric ulcer effect of flavonoid-rich fractions was recognized [1, 2]. The callus culture of *G. echinata* L. has been derived in our laboratory and chemical investigations on its constituents have resulted in the isolation of biosynthetically unique flavonoids. This paper deals mainly with their structure determinations. Related ^{1}H NMR studies of a compound existing in a tautomeric mixture and biosynthetic consideration are also presented. Parts of this work have been published in preliminary form [3, 4].

RESULTS

The thin-layer chromatogram of the EtOAc-soluble fraction of the MeOH extract of *G. echinata* static callus on White's medium revealed an intense green fluorescent and a minor dark orange spot under 365 nm light. These spots, which were not found in the extract of the root of the original plant, were due to a new chalcone, echinatin (**1**), and a new dibenzoylmethane, licodione (**2**), respectively. Several blue fluorescent spots corresponding to flavone and isoflavone derivatives were also observable both in callus and plant extracts. The components of the callus were separated by repeated column chromatography on Si gel.

Echinatin (**1**), yellow needles, $C_{16}H_{14}O_4$, was shown to be a chalcone from its UV spectrum (EtOH) λ_{max} 237, 312 and 370 nm. The UV spectrum also suggested a phenolic hydroxy group at C-4 by the bathochromic shift of the high wavelength band accompanying the increase of intensity to 435 nm when alkali was added and the absence of hydroxyls at C-2′ and C-6′ as there was no shift on addition of aluminium chloride. The ^{1}H NMR spectrum ($(CD_3)_2CO$) of **1** showed methoxy and *trans*-olefinic protons in addition to aromatic protons. Three of the aromatic protons appeared as a degraded ABX pattern at δ 6.54 *dd* (J = 2, 9 Hz, H-5), 6.58 *br. s* (H-3) and 7.71 *br. d* (J = 9 Hz, H-6) and others as A_2B_2 signals at δ 6.98 *d* (A_2, J = 9 Hz) and 8.06 *d* (B_2, J = 9 Hz), the very low chemical shift of the B_2 part implying that these protons are attached to C-2′ and C-6′ bearing the *ortho*-carbonyl group. The appearance of a fragment ion at *m/e* 121 **2b** in the mass spectrum of **1** also supported the assumption that ring A bears no oxygen functional group at C-2′ and C-6′. The base peak at *m/e* 239 should be due to the fragment ion $M^+ - OMe$, characteristic of 2-methoxychalcones and *ortho*-methoxydibenzoylmethanes [5, 6]. Thus **1** was elucidated as 4,4′-dihydroxy-2-methoxychalcone. Synthesis of echinatin was carried out by the alkaline condensation of *p*-hydroxyacetophenone and 2-methoxy-4-hydroxybenzaldehyde in the usual manner, and a synthetic sample was identical to **1**.

Licodione (**2**), deep yellow needles, $C_{15}H_{12}O_8$, also revealed its polyhydroxychalcone nature by its UV spectrum (MeOH) λ_{max} 285, 376 nm and, with NaOMe, 242, 342, 415 nm. The predominant peaks in the mass spectrum, *m/e* 137.023 ($C_7H_5O_3$, 37%, **2a**) and 121.030 ($C_7H_5O_2$, 100%, **2b**), suggested mono- and dihydroxybenzoyl groups in the structure. Thus, a trihydroxydibenzoylmethane skeleton was assumed for **2**, and the positive colour reaction with Mg–HCl and blue fluorescent spot on TLC after spraying with H_2SO_4 and heating were explained by the formation of a flavone derivative. The IR bands ν 1625 sh and 1600 cm^{-1} indicated the tautomeric form of a β-diketone (C(OH)=CH—C=O). When **2** was treated with concentrated HCl it easily

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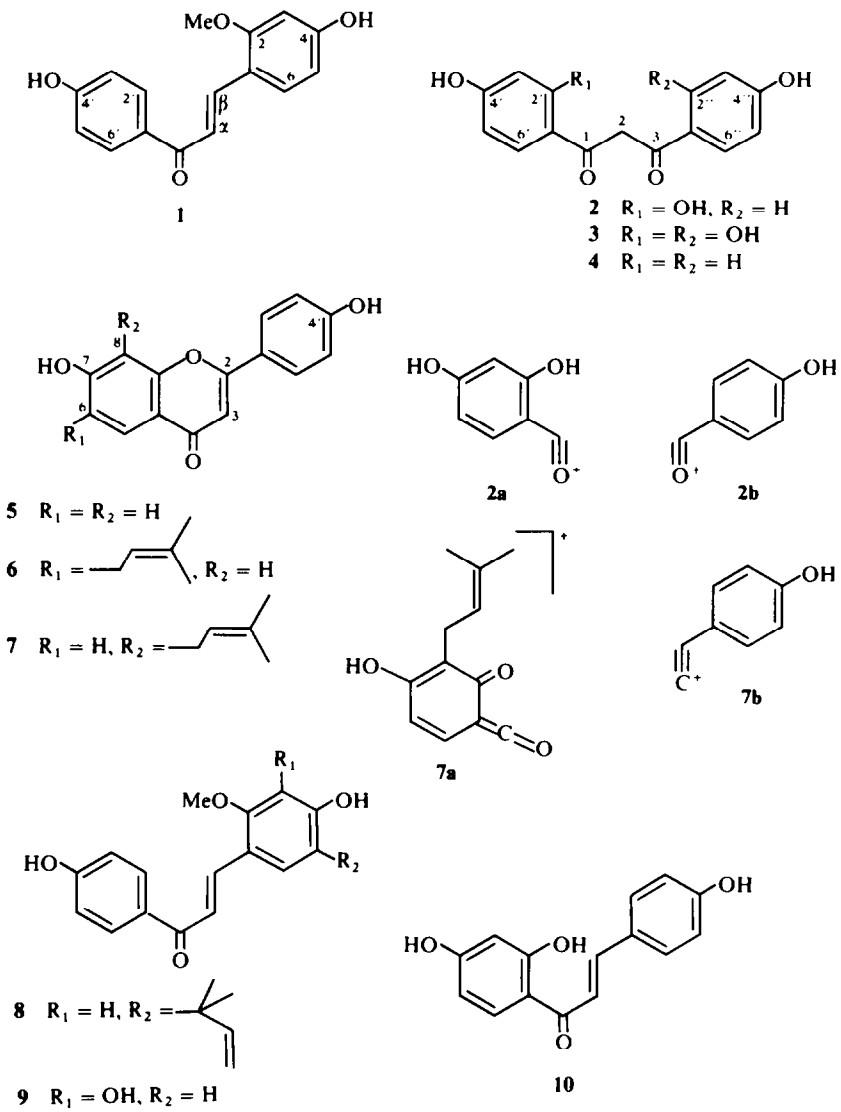

cyclized with loss of water to give 7,4′-dihydroxyflavone (**5**). These observations showed licodione to be 1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)-1,3-propanedione.

The ^{1}H NMR spectrum ($(CD_3)_2CO$) of licodione revealed a composite feature implying its existence in solution as an equilibrium mixture of tautomeric forms. A singlet signal at δ 4.6 which integrates for 0.6 protons was assigned to diketonic methylene protons agreeing with the reported 60 MHz spectra of some *o*-hydroxydibenzoylmethanes [7]. However, assignments of signals at δ 4.4–4.7 to shielded vinyl protons of a keto–enol form and signals at 3.2–4.0, which do not appear in the spectrum of **2**, to methylene protons of a diketonic form in naturally occurring dibenzoylmethanes bearing methoxy groups have been reported [6, 8, 9]. Furthermore, the possibility that licodione exists in the 2-hydroxyflavanone structure [10] (see Discussion) was also possible. Thus, a correct assignment of the ^{1}H NMR signals was desired to clarify the nature of tautomerism. We then synthesized model compounds, 1,3-bis(2,4-dihydroxyphenyl)-1,3-propanedione (**3**) and 1,3-bis(4-hydroxyphenyl)-1,3-propanedione (**4**), and their ^{1}H NMR spectra were compared to the spectrum of **2** (Table 1). It is reasonably assumed that **3** exists in pure diketonic form because of intramolecular hydrogen bonding in accordance with the 2H integration for the singlet signal at δ 4.65, while in **4** this signal (4.56) reduces to *ca* 0.3 H indicating that **4** exists as *ca* 85% keto–enolic form. Methylene protons in the spectra of **2** and **3** diminish as soon as D_2O is added, while singlet signals at δ 6.97 (**2**) and 6.98 (**4**), which were assigned to vinyl

Table 1. ^{1}H NMR data of **2**, **3** and **4** (100 MHz, $(CD_3)_2CO$, TMS as internal standard)

Compound	3′-H	5′-H	6′-H	2″,6″-H	3″,5″-H	2-H
	6.35 *d*	6.45 *dd*	7.96 *d*	7.98 *d*	6.96 *d*	6.97 *s*
Keto-enolic	(0.7 H)	(0.7 H)	(1.4 H)	(1.4 H)	(0.7 H)	(0.7 H)
2	*J* = 2	*J* = 2, 10	*J* = 9	*J* = 9	*J* = 9	
	6.33 *d*	6.44 *dd*	7.80 *d*	7.98 *d*	6.94 *d*	4.62 *s*
Diketonic	(0.3 H)	(0.3 H)	(0.3 H)	(0.6 H)	(0.6 H)	(0.6 H)
	J = 2	*J* = 2, 10	*J* = 9	*J* = 9	*J* = 9	
	6.34 *d*	6.46 *dd*	7.85 *d*			4.65 *s*
3	(2 H)	(2 H)	(2 H)	—		(2 H)
	J = 2	*J* = 2, 9	*J* = 9			
				2′,6′-H	3′,5′-H	4.56 *s* (0.3 H,
4				8.01 *d*	6.95 *d*	diketonic)
	—	—	—	(4 H)	(4 H)	and
				J = 9	*J* = 9	6.98 *s* (0.85 *H*,
						keto enolic)

Coupling constants in Hz.

protons of keto–enolic form, slowly diminish and completely disappear in 1–3 days. The aromatic proton signals of **2** showed mostly an overlapped pattern of signals of **3** and **4**, but additional minor peaks appeared. The unique feature is an isolated signal at δ 7.80 *d* (J = 9 Hz, 0.3 H) which was assigned to 6′-H of the diketonic form. Thus, the assignment of signals in ^{1}H NMR of licodione was tentatively achieved as shown in Table 1, and the existence in the mixture of *ca* 70% keto–enolic and 30% diketonic and none of 2-hydroxyflavanone form was indicated. This is the first report of the difference in chemical shifts of aromatic protons between diketo and keto–enolic forms of natural dibenzoylmethanes. Further investigation by the means of ^{13}C NMR is in progress. Synthesis of licodione was performed by the Baker–Venkataraman method from 2-hydroxy-4-benzyloxyacetophenone and *p*-benzyloxybenzoyl chloride followed by hydrogenolytic deshielding of the benzyl group, and the synthetic sample was identified with the material from callus (co-TLC, IR, NMR).

Formononetin (7-hydroxy-4′-methoxyisoflavone), a common isoflavone of Leguminosae, 7,4′-dihydroxyflavone (**5**) and two additional flavones (**6** and **7**, both $C_{20}H_{18}O_4$) were also obtained. The close similarity of their UV and IR spectra to **5** (see Experimental) suggested that they are *C*-alkyl derivatives of **5**, and the group attached was shown to be γ,γ-dimethylallyl (prenyl) from the ^{1}H NMR spectra. The substituted positions of prenyl in **6** and **7** were determined by comparison of their aromatic region signals in the ^{1}H NMR spectrum with those of **5**. Aside from the A_2X_2 signals of the B-ring protons in every compound, **6** shows singlet protons at δ 7.09 (H-8) and 7.83 (H-5), and **7** represents AX pattern of H-5 and H-6 (δ 7.83 and 7.03, *d*, J = 9 Hz, each), while in **5**, H-5, H-6 and H-8 show signals at δ 7.85 *d* (J = 8.5 Hz), 6.91 *d* (J = 8.5 Hz, overlapping on 2′ and 6′ protons) and 6.94 *s*, respectively. Thus **6** was elucidated as the 6-prenylated and **7** as the 8-prenylated derivative of **5**. Saitoh *et al.* have isolated **6** from commercial Shinkiang licorice and named it licoflavone A, which was compared by TLC and found to be identical to **6** from callus. Synthesis of licoflavone A was recently reported by Jain *et al.* [11]. The reported data almost agreed with **6**; however, their assignment of ^{1}H NMR signals of H-3 and H-8 is contrary to ours. Following their assignment, **7** should have the alternative structure of the 3-prenyl derivative, which does not fit with the peaks derived from retro-Diels–Alder fragmentation in MS of **7**, *m/e* 204 (RDA fragment **7a**, 6%), 189 (**7a** − Me, 43%), 149 (**7a** − C_4H_7, 49%), 118 (RDA fragment **7b**, 20%) and the absence of expected fragment peaks for the alternative structure at *m/e* 186, 165, 136, 125.

DISCUSSION

Echinatin (**1**), as well as licochalcones A (**8**) and B (**9**) from Shinkiang licorice [5], have the unusual substitution pattern of oxygen functional groupings and were designated as retrochalcones, in which the origins of two aromatic rings was different from that of normal flavonoids. This hypothesis was proved by feeding experiments with isotopically labelled cinnamates and isoliquiritigenin (**10**) using suspension culture of *G. echinata* callus [12]. Further examples of flavonoids having reversed A and B-rings have been reported by Dreyer *et al.* [13, 14]. The co-occurrence of licodione (**2**) and **1** in the same tissue strongly suggests its intermediacy in the biosynthesis of **1**, and cell-free studies on enzymatic methylation to **2** clearly supported this assumption [15]. Licodione may be generated in the cells directly from **10** through oxygenated chalcone, epoxide or peroxide [16]. However, the failure to detect **8**, in contrast to the existence of 7,4′-dihydroxyflavone (**5**) in the callus, may point to another possibility, i.e. hydration to 2,3-double bond of **5** resulting in 2-hydroxyflavanone structure, which is one of the tautomeric forms of dibenzoylmethane [10]. As support for this view, a similar enzymatic hydration of flavonol to 2-hydroxyflavanol in *Mentha* cell cultures was reported by Frey-Schröder and Barz [17].

EXPERIMENTAL

Callus cultures. Glycyrrhiza echinata callus was derived from the seedlings in 1965 and subcultured on White's agar medium containing 2,4-D (0.1 ppm) and yeast extract (0.1%). The callus

was maintained under dark at 26° and transferred to freshly prepared medium every 5–6 weeks. During this time interval, *ca* 2 g of callus grew to *ca* 10 g.

Extraction procedures. Homogenized callus (fr. wt 17.0 kg, dry wt 0.29 kg) was extracted with cold and then hot MeOH, and after evapn the residue was distributed between EtOAc and H_2O. Evapn of the EtOAc layer gave 20.6 g of a deep brown gum, which was chromatographed on Si gel (2.2 kg) and roughly separated by elution with $CHCl_3$–MeOH (96:4 → 92:8) into fractions, $F_1 \sim F_5$ (4 l. each). Each fraction was rechromatographed on Si gel with C_6H_6–EtOAc as solvent, and formononetin (10 mg) from F_1 and 7,4′-dihydroxyflavone (50 mg) from $F_{4,5}$ were obtained. F_2 afforded licodione (54 mg, eluted with C_6H_6–EtOAc, 4:1) and licoflavone A (20 mg, 3:2) and F_3 afforded echinatin (300 mg, 3:2) and 7,4′-dihydroxy-8-prenylflavone (6 mg, 2:3).

Echinatin (**1**). Recrystallization from EtOH–H_2O gave yellow needles, mp 209.5–212° (dec.). (Found: C, 70.6; H, 5.10; M^+, 270.0896. $C_{16}H_{14}O_4$ requires: C, 71.1; H, 5.22%; M^+, 270.0892). Positive to $FeCl_3$ (orange) and diazo reagent (deep yellow). UV λ_{max}^{EtOH} nm (log ε): 237 (3.79), 312 (3.94), 370 (4.20); + NaOEt, 252 (3.79), 271 (3.80), 435 (4.41). ^{1}H NMR [$(CD_3)_2CO$]: δ 3.92 (3 H, s, $OC\underline{H}_3$), 7.70 and 8.07 (1 H each, AB*q*, *J* = 16 Hz, $C\underline{H}$=$C\underline{H}$) and others presented in the text. MS *m/e* (rel. int.): 270 (11), 240 (18), 239 (100), 121 (26), 55 (11).

Licodione (**2**). Recrystallization from EtOH–H_2O gave yellow needless, mp 152–153° (dec). (Found: C, 63.4; H, 4.57; M^+ 272.0692. $C_{15}H_{12}O_5 \cdot \frac{1}{2}H_2O$ requires: C, 64.1; H, 4.66%; $C_{15}H_{12}O_5$ requires: M^+ 272.0685). Positive to $FeCl_3$ (green) and diazo reagent (red). UV λ_{max}^{MeOH} nm (log ε): 285 (4.28), 376 (4.55); + NaOMe, 242 (4.10), 342 (4.69), 415 (4.19). MS *m/e* (rel. int.): 272 (M^+, 30), 255 (8.5), 254 (18), 137.0232 ($C_7H_5O_3$, 37), 121.0304 ($C_7H_5O_2$, 100).

Licoflavone A (**6**). Recrystallization from EtOH–H_2O gave pale yellow needles, mp 217°. (Found: M^+ 322.1199. $C_{20}H_{18}O_4$ requires: M^+ 322.1205). Positive to $FeCl_3$ (deep yellow) and diazo reagent (brown). UV λ_{max}^{MeOH} nm (log ε): 250 sh (4.00), 320 sh (4.44), 331 (4.47); + NaOMe, 255 (4.25), 265 (4.24), 330 (4.24), 392 (4.57). ^{1}H NMR [$(CD_3)_2CO + D_2O$]: δ 1.74 (6 H, s, 2 × $C\underline{H}_3$), 3.36 (2 H, *d*, *J* = 8 Hz, $C\underline{H}_2$–CH=C), 5.38 (1 H, *br. t*, *J* = 8 Hz, CH_2–$C\underline{H}$=C), 6.64 (1 H, *s*, 3-H), 7.00 (2 H, *d*, *J* = 8 Hz, 3′, 5′-H), 7.09 (1 H, *s*, 8-H), 7.83 (1 H, *s*, 5-H), 7.87 (2 H, *d*, *J* = 8 Hz, 2′,6′-H). MS *m/e* (rel. int.): 322 (M^+, 75) 307 (M^+ − Me, 72), 279 (25), 267 (100), 239 (12), 149 (57), 118 (34).

7,4′-Dihydroxy-8-prenylflavone (**7**). Recrystallization from EtOH–H_2O gave pale yellow needles, mp 240–241° (dec.) (Found: M^+ 322.1027. $C_{20}H_{18}O_4$ requires: M^+ 322.1205). Positive to $FeCl_3$ (deep yellow) and diazo reagent (orange). UV λ_{max}^{MeOH} nm (log ε): 250 (4.21), 258 (4.22), 313 sh (4.36), 329 (4.41); + NaOMe, 272 (4.40), 337 (4.18), 390 (4.49). ^{1}H NMR [$(CD_3)_2CO$]: δ 1.69 and 1.85 (3 H each, *s*, 2 × $C\underline{H}_3$), 3.70 (2 H, *d*, *J* = 7 Hz, $C\underline{H}_2$–CH=C), 5.34 (1 H, *br. t*, *J* = 7 Hz, CH_2–$C\underline{H}$=C), 6.60 (1 H, *s*, 3-H), 7.03 (3 H, *d*, *J* = 9 Hz, 6, 3′,5′-H), 7.83 (1 H, *d*, *J* = 9 Hz, 5-H), 7.87 (2 H, *d*, *J* = 9 Hz, 2′,6′-H). MS *m/e* (rel. int.): 322 (M^+, 70), 267 (100), 204 (6), 189 (43), 149 (49), 118 (20).

Synthesis of echinatin (**1**). *p*-Hydroxyacetophenone (0.55 g) and 2-methoxy-4-hydroxyacetophenone (0.5 g) were dissolved in 8 ml 50% KOH and heated on a boiling H_2O bath for 10–15 min, then poured into ice-H_2O followed by the slow addition of dil HCl. The mixture was left to stand overnight, ppts were collected by filtration, and recrystallized from EtOH–H_2O. Yellow needles (0.30 g) were obtained, which were identical to **1** in all aspects.

Synthesis of 7,4′-dihydroxyflavone (**5**) from **2**. To licodione (40 mg) in EtOH (4 ml), conc HCl (5 drops) was added and stirred for 2 hr (room temp.). H_2O (10 ml) was added and the resulting ppts were recrystallized from MeOH. Pale yellow needles were obtained, mp > 300°. (Found: C, 70.6; H, 4.12. $C_{15}H_{10}O_4$ requires: C, 70.9; H, 3.96%). UV λ_{max}^{MeOH} nm (log ε): 255 sh (3.79), 314 sh (4.10), 330 (4.15); + NaOMe: 253 (4.05), 264 (4.06), 330 (3.91), 387 (5.50). ^{1}H NMR (DMSO-d_6): δ 6.66 (1 H, *s*, 3-H), 6.91 (3 H, *d*, *J* = 8.5 Hz, 6,3′,5′-H), 6.94 (1 H, *s*, 8-H), 7.85 (1 H, *d*, *J* = 8.5 Hz, 5-H), 7.87 (2 H, *d*, *J* = 9 Hz, 2′,6′-H). MS *m/e* (rel. int): 254 (M^+, 100), 226 (33), 137 (58), 136 (13), 118 (34).

Synthesis of licodione (**2**). *p-Benzyloxybenzoyl chloride* (**11**). To *p*-benzyloxybenzoic acid (2 g), which was prepared from ethyl *p*-hydroxybenzoate by the usual benzylation procedure with benzyl chloride, K_2CO_3 and KI in DMF (followed by alkaline hydrolysis), thionyl chloride (4 ml) and DMF (1 drop) were added. The mixture was stirred for 30 min at 60°, and resulting clear solution was evapd under red. pres. Recrystallization of the residue from CCl_4 gave 1.3 g colourless needles which were immediately used for the next reaction. Mp. 105–106° MS *m/e* (rel. int.): 248 (M^+, 0.8), 246 (M^+, 2.4), 211 (6), 91 (100).

1-(2-Hydroxy-4-benzyloxyphenyl)-3-(4-benzyloxyphenyl)-1,3-propanedione (**12**). 4-*O*-Benzylresacetophenone (0.8 g) [18] and **11** (0.8 g) were dissolved in dry Me_2CO (6 ml) and refluxed with dry K_2CO_3 (2.5 g) for 9 hr. H_2O (50 ml) was added to the mixture and extracted with EtOAc (120 ml), the EtOAc layer was concd to *ca* 50 ml and stirred with 5% $Cu(OAc)_2$ at room temp. The resulting Cu complex of **12** (**13**) was collected, suspended in EtOAc and shaken with 0.5 N HCl until the solid dissolved. The EtOAc layer was washed with H_2O, 5% K_2CO_3 and brine, and evapn yielded a yellow solid, **12** (0.8 g). The filtrate EtOAc layer, after collection of **13**, was washed with 1 N HCl, 5% K_2CO_3, brine and evapd, then the residue was suspended in dry pyridine (20 ml), stirred with KOH (1.3 g) at 80° for 1 hr. The mixture was poured into ice–HCl (1:1, total 50 ml) and extracted with EtOAc. Work-up as above gave a further 0.8 g of **12**. Recrystallization from C_6H_6–EtOH gave yellow plates, mp 140–142°. MS *m/e* (rel. int.): 452 (M^+, $C_{29}H_{24}O_5$, 14), 434 (3), 227 (3), 211 (23), 91 (100). ^{1}H NMR (DMSO-d_6): δ 4.65 (0.5 H, *s*, CO—$C\underline{H}_2$—CO), 5.11 and 5.15 (2 H each, *s*, $C\underline{H}_2$Ph) 6.50 (1 H, *d*, *J* = 3 Hz, 3′-H), 6.53 (1 H, *m*, 5′-H), 7.08 (2 H, *dd*, *J* = 2 and 9 Hz, 3″,5″-H), 7.10 (1 H, *s*, CO—$C\underline{H}$=C—OH), 7.2–7.5 (10 H, *m*, 2 × $CH_2C_6\underline{H}_5$), 7.7–8.1 (3 H, *m*, 6′,2″,6″-H).

Synthetic licodione. **12** (0.8 g) in EtOH–ethylene glycol monomethyl ether (1:1, 40 ml) was hydrogenated over 10% Pd–C (0.2 g) at room temp. After separation of Pd–C by filteration, the solvent was evapd and crystallization from EtOH–H_2O gave yellow needles (0.34 g), which were identical to the material from callus in all aspects.

1,3-Bis(2,4-dihydroxyphenyl)-1,3-propanedione (**3**). 2,4-Dibenzyloxybenzoic acid (0.8 g), which was prepared from 2,4-dihydroxybenzoic acid through benzylation of its methyl ester with benzyl chloride in DMF, was treated with $SOCl_2$ (1.6 ml) and DMF (1 drop) to give chloride as a pale yellow oil. 4-*O*-Benzylresacetophenone (0.5 g), K_2CO_3 (0.4 g) and 18-crown-6 (0.1 g) in MeCN (10 ml) were stirred at room temp. for 15 min, then the chloride was added and stirred at 50° for 2 hr. The product was taken up in EtOAc, and after evapn to dryness, suspended in dry pyridine (5 ml), stirred with 1 g KOH at 80° for 5 min. 1-(2,4-Dibenzyloxyphenyl)-3-(2-hydroxy-4-benzyloxyphenyl)-1,3-propanedione (**13**, 0.37 g) was isolated from the reaction mixture through Cu complex, mp 133.5–134.5°. MS *m/e* (rel. int.): 558 (M^+, $C_{36}H_{30}O_6$, 1), 540 (2.4), 332 (3.5), 317 (3.5), 242 (4.5), 227 (3.5), 200 (4.9), 91 (100). Hydrogenolysis of **13** (0.30 g) over Pd–C in EtOH–ethylene glycol monomethyl ether (15 ml) gave **3** as pale yellow plates (90 mg), mp 200–202° (dec.) (EtOH–H_2O). (Found: M^+ 288.0628. $C_{15}H_{12}O_6$ requires: M^+ 288.0633). UV λ_{max}^{EtOH} nm (log ε): 231 (4.23), 283 (4.45), 325 (4.28), 383 (3.89), 400 (3.89): + NaOEt, 281 (3.99), 324 (4.26), 356 (4.19),

417 (4.72). MS *m/e* (rel. int.): 288 (M^+, 18) 270 (30), 137 (100).

1,3-*Bis*(4-*hydroxyphenyl*)1,3-*propanedione* (**4**). *p*-Benzyloxyacetophenone (1 g), ethyl *p*-benzyloxybenzoate (2 g) and NaOEt, freshly prepared from 0.8 g of Na and 0.4 ml of EtOH, in dry Et_2O (20 ml) were refluxed for 8 hr. The mixture was poured into ice–HCl, extracted with EtOAc and work-up as above yielded 0.43 g of the dibenzyl ether of **4**(**14**), mp 170–171° (EtOH–H_2O), MS *m/e* (rel. int.): 436 (M^+, $C_{29}H_{24}O_4$, 11), 211 (11), 91 (100), 65 (75). Hydrogenolysis of **14** (0.4 g) over Pd–C (80 mg) in EtOH–ethylene glycol monomethyl ether gave 0.20 g of **4** as yellow needles, mp 220.5–222.5° (EtOH–H_2O). (Found: M^+ 256.0743. $C_{15}H_{12}O_4$ requires: M^+ 256.0735). UV λ_{max}^{EtOH} nm (log ε): 227 (4.18), 292 (4.12), 364 (4.71): + NaOEt, 239 (4.14), 345 (4.65), 425 (4.53). MS *m/e* (rel. int.): 256 (M^+, 98), 255 (50), 135 (42), 121 (100).

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