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BIOSYNTHESIS OF A RETROCHALCONE, ECHINATIN: INVOLVEMENT OF *O*-METHYLTRANSFERASE TO LICODIONE*

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Key Word Index—*Glycyrrhiza echinata*; Leguminosae; cell culture; cell free extract; retrochalcone biosynthesis; enzyme; *O*-methyltransferase; echinatin; licodione; *O*-methyl-licodiones.

Abstract—In order to clarify the *O*-methylation step in the biosynthesis of a retrochalcone, echinatin(4,4′-dihydroxy-2-methoxychalcone), methyl transfer from *S*-adenosyl-L-methionine (SAM) to licodione (1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)-1,3-propanedione) in the cell-free extract of the cultured cells of *Glycyrrhiza echinata* was examined. Time course of methyl transferring activity during culture cycle in 4 strains was correlated to echinatin content. The enzyme catalysing this reaction, licodione *O*-methyltransferase (LMT), was purified 135-fold. Substrate specificity studies implied that the hydroxy group *ortho* to the C_3 linkage in licodione was methylated in this reaction. *O*-Methyl-licodiones were synthesized for comparison and the sole product of LMT-catalysed reaction was identified as 2′-*O*-methyl-licodione. A possible scheme for the last steps of echinatin biosynthesis is proposed.

INTRODUCTION

Echinatin (**1**), found first in the cell culture of *Glycyrrhiza echinata* [1], and licochalcones A(**2**) and B(**3**), isolated together with **1** from commercial Shinkiang licorice [2, 3], have been proposed to be biosynthetical retrochalcones. The tracer experiments using suspension culture of *G. echinata* established that the origins of two phenolic rings of **1** are reversed to hitherto known flavonoids [4]. The isolation of a new dibenzoylmethane, licodione (**4**) [5], from the same cell culture led us to assume that **4** may be an intermediate in **1** biosynthesis. The steps from **4** to **1** are expected to consist of the removal of one of the ketonic oxygens and the methylation to the hydroxy group *ortho* to the C_3 linkage. *In vitro* studies on this process using cell free extracts have been the subject of our investigation.

This paper describes the enzymatic methylation to **4** in the cells of *G. echinata*. Most biological *O*-methylations are known to be mediated by *O*-methyltransferases (OMTs) with *S*-adenosyl-L-methionine (SAM) as methyl donor. OMTs of higher plants extensively investigated so far have been mainly those specific to catechol-type substrates including lignin precursors [6–10] and flavonoids [11–13]. OMTs catalysing *para O*-methylation of isoflavonoid [14] and a chalcone [15] have also been reported. In contrast, the OMT toward **4** in our cell culture was anticipated to be unique in its substrate specificity and the reaction catalysed by it may be significant in retrochalcone formation in cells. The results are discussed in relation to the role of the OMT in echinatin biosynthesis.

*Part 34 in the series "Studies on Plant Tissue Cultures". For Part 33, see ref. [16].

RESULTS AND DISCUSSION

Detection and partial purification of licodione O-methyltransferase

The strain of *G. echinata* callus mainly used in this study was grown and maintained in darkness on White's medium and was coloured pale yellow (W strain). Since in the previous feeding experiment, isotope-labelled isoliquiritigenin (**5**) had been incorporated into echinatin (**1**) [4], the methylation of **5** as well as of **4** was examined. The crude extract (1000 *g* supernatant) of the callus with [$^{14}CH_3$]-SAM and phenolic substrate was incubated and after acidification the mixture was extracted with EtOAc. A certain amount of conversion of radioactivity (maximum *ca* 50%) into the EtOAc layer was observed when **4** was used as phenolic substrate, but the conversion ratio was very low with **5** as methyl acceptor. Time course of activity change during culture was examined in W strain and additional 3 strains cultured on media containing various concentrations of auxins and kinetin under different light conditions. Strains (DK, KN) on media containing high concentration of kinetin turned green under illumination. The methyl transferring activity to **4** in each strain was found to change to various degrees within culturing days, but generally exhibited a pattern of two peaks. A typical case is shown in Fig. 1 together with echinatin content determined by UV absorption on TLC of an EtOAc extract of the callus. In spite of a remarkable variation in **1** content between four strains, a correlation of the pattern of activity and content curves in the 3 strains except DK was observable; **1** content curves also have two peaks and they appear *ca* 5 days after the peaks of methyltransferase activity. This result implied that the methyltransferase detected here is involved in **1** biosynthesis.

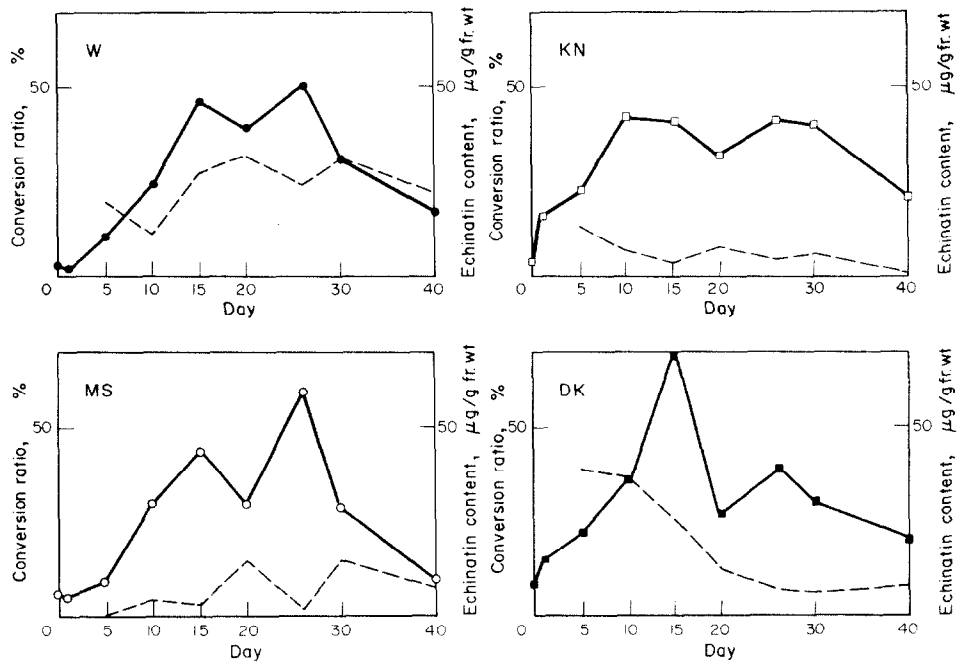


Fig. 1. Relationship between LMT activity and echinatin content in four strains of *G. echinata* cell culture. LMT activity in W (●——●), MS (○——○), KN (□——□) and DK (■——■) strains is expressed as the conversion ratio of radioactivity of [$^{14}CH_3$]-SAM to the EtOAc-soluble fraction of the incubation mixture. Echinatin content (- - -) was determined by UV absorption on TLC. For details, see Experimental.

The most active cells of *G. echinata* W (5 week culture) were collected, frozen and stored in a freezer until used for the purification. Frozen cells, 100 g, were suspended in an equal volume of 0.1 M phosphate buffer (pH 8) containing 14 mM mercaptoethanol and subjected to the purification steps described in the Experimental. The procedure and yield in the purification of the enzyme are summarized in Table 1. By means of $(NH_4)_2SO_4$ fractionation, Sephadex G-25, DEAE-Sephadex A-50 and Sephadex G-200 column chromatography, the enzyme was purified 135-fold.

The purified enzyme should be designated as *S*-adenosyl-L-methionine: licodione *O*-methyltransferase (LMT). The pH optimum of LMT reaction was 8.0. K_m values for licodione and SAM were 1.1×10^{-5} and 2.8×10^{-6} M respectively determined from Lineweaver–Burk plots. The reaction proceeded at the same rate without Mg^{2+} and addition of EDTA caused a

Table 1. Purification of SAM: licodione *O*-methyltransferase from *G. echinata* cell culture

Fraction	Total protein (μg/ml)	Total activity (dpm/ml)	Specific activity (dpm/μg protein)	Purification (fold)
Crude extract	940	20,094	21.4	1.0
Sephadex G-25 eluate*	1000	37,964	38.0	1.8
DEAE-Sephadex eluate*	42	36,158	860.9	40.2
Sephadex G-200 eluate*	4	11,550	2887.5	134.9

*Assay condition was same as described in Experimental (*Assay with crude enzyme*) but containing 0.5 ml of each eluate instead of crude extract.

Table 2. Substrate specificity of the *O*-methyltransferase from *G. echinata* cell culture

Substrate	Relative %
Licodione (**4**)	100
2″-OH-licodione (**6**)	38.0
2′-DeOH-licodione (**7**)	1.4
Isoliquiritigenin (**5**)	10.7
Dihydro-**5** (**8**)	7.2
Echinatin (**1**)	4.0
2,4-DiOH-benzoic acid (**9**)	4.5
Resacetophenone (**10**)	2.6

slight increase (*ca* 5%) of activity. Activity decreased to *ca* 20% and *ca* 70% of the maximum with Cu^{2+} and Zn^{2+} respectively at 0.4 mM.

Substrate specificity of LMT toward phenolic compounds structurally related to **4** was tested and the results are listed in Table 2. A notable feature is the difference in reactivity of 2″-hydroxy (**6**) and 2′-deoxy (**7**) licodiones which were recently synthesized by us [16]. The relatively high ratio of **6** and the very low reactivity of **7** can be explained if hydroxy groups only at *ortho* positions to the C_3 linkage of dibenzoylmethanes are methylated in the LMT-catalysed reaction. Other substances examined, especially compounds having one benzene ring, were poor methyl acceptors.

Identification of the product of the LMT-catalysed reaction

From the time-course studies and substrate specificity investigation, the involvement of LMT in echinatin biosynthesis was strongly suggested, therefore, product(s) characterization was thought to be essential to clarify the role of LMT in the cells. A TLC radiochromatogram of an EtOAc extract of the incubation mixture revealed only one methylated product. The R_f value of this product was close to **1**, however, repeated crystallization of the EtOAc extract with cold **1** as carrier caused a decrease in radioactivity.

Syntheses of several methylated licodiones were attempted for comparison with the product. The Baker–Venkataraman method yielded *ortho* hydroxyl derivatives, 4′-*O*-methyl (**11**), 4″-*O*-methyl (**12**) and 4′,4″-di*O*-methyl (**13**)-licodiones, and direct condensation of acetophenone derivative and ester with NaOEt gave 2′-*O*-methyl-licodione (**14**). Throughout the syntheses reactive hydroxyl groups of reactants were protected with a benzyl group. Satisfactory spectral data of **11**–**14** have been obtained (see Experimental). As shown in Table 3, the comparison of R_f values of **11**–**14** with the reaction product suggested that it was strongly **14**. However, TLC using silica gel, cellulose and polyamide plates with various combined solvents did not give good separation of **1** and **14**, making it difficult to judge whether radioactive **1** was also formed to any extent in LMT-catalysed reaction. A silica gel plate treated with $Cu(OAc)_2$ was then employed. On that plate, dibenzylmethanes did not move from the origin while chalcones gave the same R_f values as on the plate without Cu^{2+} treatment. Thus, a complete separation of **1** and **14** on TLC was achieved and the presence of no detectable radioactivity in **1** was confirmed. Furthermore, repeated recrystallization of the EtOAc extract added with cold **14** gave constant radioactivity. From these experiments, it was concluded that the reaction catalysed by LMT gives almost exclusively 2′-*O*-methyl-licodione (**14**).

Table 3. R_f values of the product of LMT-catalysed reaction, echinatin (**1**), licodione (**4**) and *O*-methylated licodiones (**11**–**13**)

Compound	R_f*
LMT reaction product	0.25–0.35
Echinatin (**1**)	0.28
Licodione (**4**)	0.35
4′-*O*-Me-licodione (**11**)	0.47
4″-*O*-Me-licodione (**12**)	0.47
4′,4″-Di*O*-Me-licodione (**13**)	0.56
2′-*O*-Me-licodione (**14**)	0.30

*Si gel GF_{254} (Merck).
Solvent system: C_6H_6–EtOAc–MeOH–petrol (bp 40–50°), 6:4:1:3 (BEMP).

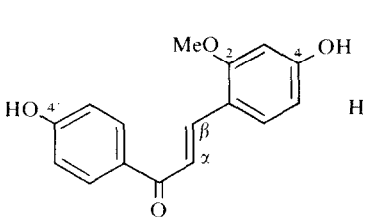
Biosynthesis of echinatin

The results obtained here point to some new features about retrochalcone biosynthesis. It is apparent from the observation of low activity of methylation of isoliquiritigenin (**5**) in the crude extract and with purified enzyme that the methyl transfer to **5** is not an important process in **1** biosynthesis. Although 2′-*O*-methyl-licodione (**14**) has not yet been detected in the callus, the close structural similarity of **14** and **1** suggests that **14** is the direct precursor of **1**. It is very likely that the last steps of echinatin biosynthesis are the reduction of **14** to hypothetical keto-alcohol (**15**) and subsequent enzymatic or non-enzymatic elimination of H_2O from **15**. In support of this hypothesis, the existence of β-hydroxydihydrochalcones (**16**, **17**) in nature has been reported by Bohlmann *et al.* [17] and more recently by Jurd's group [18].

EXPERIMENTAL

Cell cultures. Culture condition of *G. echinata* callus on White's medium (W) has been described [16]. Other strains (MS, DK, KN) were derived from W callus and subcultured on Murashige and Skoog's agar medium added with 2,4-D(1 ppm) and kinetin (0.1 ppm) for MS strain, 2,4-D(1 ppm) and kinetin (0.5 ppm) for DK strain and NAA (0.1 ppm) and kinetin (5 ppm) for KN strain. All strains were maintained at 26° in darkness (W, MS) and under diurnal 12 hr light (8000 lx) and 12 hr dark condition (DK, KN). Culture cycles were 6 weeks (W, MS) and 4 weeks (DK, KN).

Enzyme preparation and purification. Each 2 g of cultured cells was suspended in an equal vol. of 0.1 M Pi buffer (pH 8) containing 14 mM mercaptoethanol and homogenized in a Teflon homogenizer. After centrifuging at 1000 *g* for 10 min, the supernatant was treated with 1 g of Dowex 1-X2 (OH^- form) for 10 min. The filtrate through a glass filter was used as the crude enzyme prepn in this expt. The crude enzyme soln obtained from 100 g of frozen cells according to the same procedure as above, except using 10 g of Dowex 1-X2, was subjected to $(NH_4)_2SO_4$ fractionation by precipitating between 30 and 80% satn. The ppt was dissolved in 0.02 M Pi buffer containing 1.4 mM mercaptoethanol and column chromatography on Sephadex G-25 was carried out. The first protein fractions eluted from the column were chromatographed with a continuous gradient from 0.02 to 0.2 M Pi buffer (pH 8) on DEAE-Sephadex A-50 equilibrated with 0.02 M Pi buffer. Five fractions (50 ml) eluted with 0.06–0.07 M were concentrated with a collodion bag (Sartorius-membrane filter GmbH) to 3 ml. The soln was then



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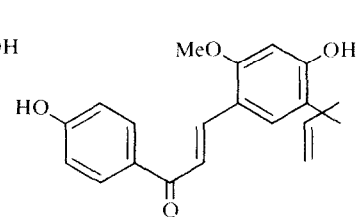

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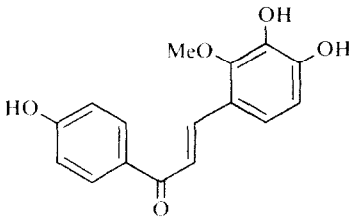

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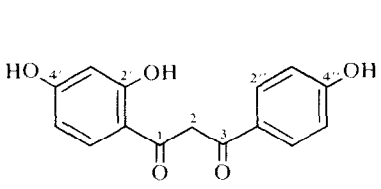

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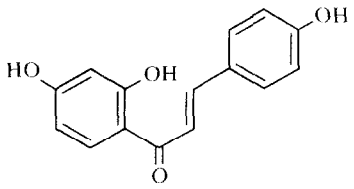

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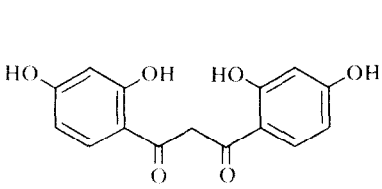

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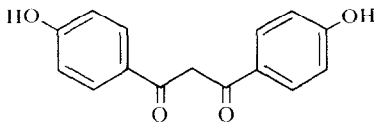

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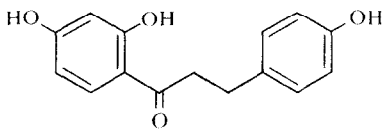

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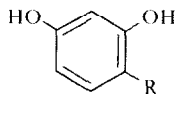

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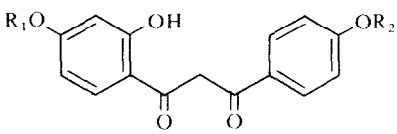
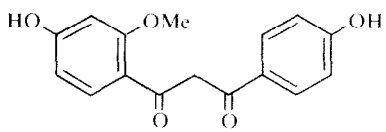
10 R = COMe



11 R_1 = Me, R_2 = H
12 R_2 = H, R_2 = Me
13 R_1 = R_2 = Me



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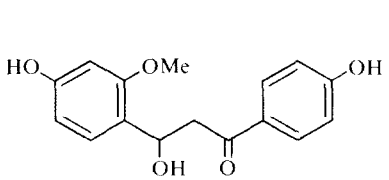

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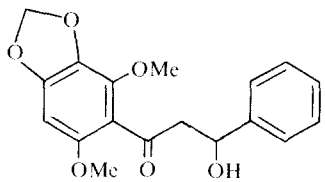

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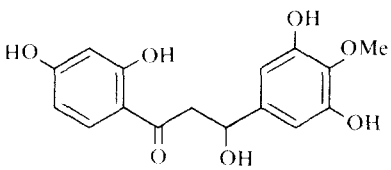

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applied to a column of Sephadex G-200 equilibrated with 0.02 M Pi buffer (pH 8). The fractions eluted subsequent to the first peak of protein were concentrated as above and finally lyophilized and stored at −20°. All steps in this purification were carried out at 4°.

Methyltransferase assay. (a) *Assay with crude enzyme.* A soln of 5.7 μmol of phenolic substrate (licodione or isoliquiritigenin), crude enzyme (0.5 ml), $MgCl_2$ (0.1 μmol), [$^{14}CH_3$]-SAM (*ca* 100 000 dpm) and 0.1 M Pi buffer (pH 8) containing 14 mM mercaptoethanol in a total vol of 1.45 ml was incubated at 30° for 2 hr. After termination of the reaction by the addition of 0.05 ml of ethylene glycol monomethyl ether (EGMM) and 0.02 ml of HOAc, the mixture was extracted with 4 ml of EtOAc. Radioactivity in a portion of the EtOAc layer was measured by liquid scintillation counting. Control expts without phenolic substrate were run each time to correct the value of radioactivity transferred into the EtOAc layer. In an expt using Pi buffers of pH 7 and 8.5 in addition to 8, the following counts were obtained: with isoliquiritigenin as phenolic substrate, dpm (at pH); 850 (7), 3680 (8), 1020 (8.5), with licodione as phenolic substrate; 18 700 (7), 23 120 (8), 5240 (8.5). (b) *Assay with purified enzyme.* Assay soln consisted of 20 μl of 10 mM soln of phenolic substrate in EGMM 20 μl of 10 mM $MgCl_2$, 100 μl of enzyme (0.1–0.2 mg) in 0.1 M Pi buffer (pH 8 with 14 mM mercaptoethanol) and 20 μl of [$^{14}CH_3$]-SAM (*ca* 120 000 dpm) in H_2O and 0.4 ml of the same buffer. Reaction was started by the addition of SAM. After incubation at 30° for 2 hr, 20 μl of HOAc was added and the mixture extracted with 1 ml of EtOAc. Radioactivity in the EtOAc layer was measured and corrected with the activity of a control run without phenolic substrate. In the expt of product identification, the EtOAc extract was evapd to dryness, dissolved in a few drops of MeOH and applied to TLC, then developed together with authentic samples.

TLC plate treated with Cu^{2+} was prepared by spraying with 2% EtOH soln of $Cu(OAc)_2$ to 20 × 20 cm plate partially covered at the lower quarter (5 × 20 cm) with glass. The sample was first developed at Cu^{2+} untreated area once with BEMP and then developed twice with the same solvent to the dimension of Cu^{2+} treated area.

Determination of echinatin content in callus. Lyophilized callus (fr. wt 2g) was extracted with 5 ml of MeOH (reflux, 2 hr) and the MeOH extract was distributed between H_2O (1 ml) and EtOAc (3 ml × 5). The EtOAc layer was evapd and the residue dissolved in 0.1 ml of MeOH. Part of this soln (5 μl) was applied to Si gel TLC and after development with BEMP, A at 350 nm at the spot corresponding to echinatin was measured with a dual-wavelength chromatoscanner.

Synthesis of O-methylated licodiones. 4′-O-*Methyl-licodione* (**11**). To an Me_2CO soln (10 ml) of 4-*O*-methyl-resacetophenone (0.3 g) and *p*-benzyloxybenzoyl chloride (0.4 g), K_2CO_3 (0.5 g) was added and refluxed for 5 hr. The reaction was stopped by the addition of H_2O and extracted with EtOAc. The EtOAc layer after evapn to dryness was suspended in dry pyridine (3 ml) and treated with KOH (0.2 g) at 100° for 1 hr. The resulting mixture was acidified with ice-HCl and extracted with EtOAc. The EtOAc layer after concn to *ca* 20 ml was stirred with 10 ml of 5% $Cu(OAc)_2$ aq. and the Cu complex of **11** benzyl ether (**18**) collected by filtration, resuspended in EtOAc and shaken with 1N HCl until the solid dissolved into EtOAc layer, which was subsequently washed with H_2O, dried (Na_2SO_4) and evapd to dryness. **18** (50 mg) as yellow needles was obtained, mp 111.5–112.5° (EtOH–H_2O). MS *m/e* (rel. int.): 376 (M^+, 21), 211 (21), 151 (14), 91 (100). Hydrogenolysis of **18** (0.1 g) over Pd–C in EtOH–EGMM (1:1 10 ml) and purification by prep. TLC (Si gel, BEMP) gave **11** as yellow plates (0.02 g), mp 119–120° (EtOH–H_2O), (Found: C, 66.4; H, 4.93. $C_{16}H_{14}O_5$ requires: C, 67.1; H, 4.93%). UV λ_{max}^{EtOH} nm (log ε): 283 (4.07), 378 (4.52), 390 sh. (4.49); + NaOEt, 383 (4.51). IR ν_{max}^{KBr} cm^{-1}: 3400, 1625, 1610, 1580, 1510, 1285, 1240, 1210, 1150. MS *m/e* (rel. int.): 286 (M^+, 25). 269 (4), 151 (36), 121 (100), ^{1}H NMR (100 MHz, $(CD_3)_2CO$))*: δ 3.89 (3H, *s*, C$\underline{H}_3$), 4.67 (0.5H, *s*, CO–C$\underline{H}_2$–CO), 6.49 (1H, *br. s*, 3′-H), 6.53 (1H, *dd*, *J* = 2, 9 Hz, 5′-H), 6.99 (2H, *d*, *J* = 9 Hz, 3″,5″-H), 7.02 (0.6H, *s*, CO–C$\underline{H}$=C–OH), 7.87 (0.3 H, *d*, *J* = 8 Hz, 6′-H of diketonic form), 8.01 (2H, *d*, *J* = 9 Hz, 2″, 6″-H), 8.04 (0.7H, *d*, *J* = 8 Hz, 6′-H of keto–enolic form).

4″-O-*Methyl-licodione* (**12**). Benzyl ether of **12** (**19**) was prepared from 4-*O*-benzylresacetophenone (0.2 g) and anisoyl chloride (0.4 g), using the same method described above, and yielded 0.12 g yellow plates, mp 118.5–119.5° (EtOH–H_2O). MS *m/e* (rel. int.): 376 (M^+, 30), 135 (84), 91 (100). Benzyl group of **19** was removed over Pd–C under H_2 and 0.04 g of **12** was obtained as fine yellow plates, mp 139–142° (EtOH–H_2O). (Found: C, 67.0; H, 4.88. $C_{16}H_{14}O_5$ requires: C, 67.1; H, 4.93%). UV λ_{max}^{EtOH} nm (log ε): 269 sh. (4.05), 283 (4.12), 376 (4.56), 388 sh. (4.52); + NaOEt, 256 (4.12), 290 sh. (3.99), 388 (4.71) IR ν_{max}^{KBr} cm^{-1}: 3340, 1625, 1600, 1595, 1570, 1505, 1430, 1340, 1255, 1235, 1210, 1175. MS *m/e* (rel. int.): 286 (M^+, 26), 269 (3), 137 (18), 135 (100). ^{1}H NMR (100 MHz, $(CD_3)_2CO$): δ 3.91 (3H, *s*, C$\underline{H}_3$), 4.69 (0.6H, *s*, CO–C$\underline{H}_2$–CO), 6.40 (1H, *d*, *J* = 2 Hz, 3′-H), 6.49 (1H, *dd*, *J* = 2, 9 Hz, 5′-H) 7.04 (0.7H, *s*, CO–C$\underline{H}$=C–OH), 7.07 and 7.09 (total 2H, *d*, *J* = 2 Hz, two doublet signals are overlapping, 3″,5″-H of both forms), 7.85 (0.4H, *d*, *J* = 8 Hz, 6′-H of diketonic form), 8.04 (0.6H, *d*, *J* = 9 Hz, 6′-H of keto–enolic form), 8.07 and 8.10 (total 2H, *d*, *J* = 9 Hz, two signals overlapping, 2″,6″-H of both forms).

4,4′-*Di-O-methyl-licodione* (**13**). 4-*O*-Methyl-resacetophenone (0.3 g) and anisoyl chloride (0.4 g) were condensed and rearranged as above. Recrystallization from EtOH–H_2O gave 0.21 g of **13** as fine yellow needles, mp 111.0–112.0° (Found: C, 67.8; H, 5.32. $C_{16}H_{14}O_5$ requires: C, 68.0; H, 5.37%). UV λ_{max}^{EtOH} nm (log ε): 270 sh. (4.16), 282 (4.20), 376 (4.70), 387 sh (4.66); + NaOEt, 263 (4.24), 372 (4.67). IR ν_{max}^{KBr} cm^{-1}: 3400, 1610 br., 1575, 1505, 1360, 1285, 1240, 1175, 1150. MS *m/e* (rel. int.): 300 (M^+, 66), 151 (12), 135 (100). ^{1}H NMR (100 MHz, $(CD_3)_2CO$); δ 3.89 and 3.93 (3H each *s*, C$\underline{H}_3$), 4.72 (0.5H, *s*, CO–C$\underline{H}_2$–CO) 6.44 (1H, *br. s*, 3′-H), 6.50 (1H, *dd*, *J* = 2, 9 Hz, 5′-H), 7.03 (0.8H, *s*, CO–C$\underline{H}$=C–OH), 7.06 (2H, *d*, *J* = 9 Hz, 3″,5″-H), 7.87 (0.3H, *d*, *J* = 8 Hz, 6′-H of diketonic form), 8.06 (0.7H, *d*, *J* = 9 Hz, 6′-H of keto–enolic form), 8.08 (2H, *d*, *J* = 9 Hz, 2″,6″-H).

2′-O-*Methyl-licodione* (**14**). Methyl 2-methoxy-4-benzyloxybenzoate (**20**) was prepared from 2,4-dihydroxybenzoic acid through benzylation of its Me ester with an equimolar amount of benzyl chloride followed by methylation with excess MeI and K_2CO_3 in the presence of 18-crown-6. *p*-Benzyloxyacetophenone (0.5 g) and **20** (1 g) in dry Et_2O (10 ml) were refluxed for 5 hr over NaOEt freshly prepared from 0.5 g of Na. The mixture was poured into ice-H_2O (100 ml), acidified with HCl and extracted with Et_2O. The Et_2O layer was evapd to a small vol. 5% $Cu(OAc)_2$ soln added and worked up in the same way as above to give 80 mg of dibenzyl ether of **14** (**21**), mp 133.0–134.0° (C_6H_6–EtOH), MS *m/e* (rel. int.): 466 (M^+, 10), 435 (12), 241 (14), 211 (7), 91 (100). Hydrogenolysis of **21** in EtOH–EGMM (1:1, 8 ml) over Pd–C gave **14** as colorless needles (46 mg), mp 176.5–178° (EtOH–H_2O). (Found: M^+, 286.0830. $C_{16}H_{14}O_5$ requires: M^+, 286.0841). UV λ_{max}^{EtOH} nm (log ε): 275 (4.20), 308 (4.20) 368 (4.66); + NaOEt, 242 (4.13), 339 (4.65), 428 (4.08). IR ν_{max}^{KBr} cm^{-1}: 3460, 3300, 1655, 1645, 1600, 1570, 1430, 1325, 1280, 1200, 1170, 1155. MS *m/e* (rel. int.): 286 (M^+, 38), 255 (62), 151 (100), 124 (21), 121 (47). ^{1}H NMR (100 MHz, $(CD_3)_2CO$): δ 3.61 (0.5H, *s*, C$\underline{H}_3$ of diketonic form), 3.97 (2.4H, *s*, C$\underline{H}_3$ of keto–enolic form), 4.45 (0.3H, *s*, CO–C$\underline{H}_2$–CO), 6.56 (1H, *d*, *J* = 9 Hz, 5′-H), 6.60 (1H, *s*, 3′-H), 6.95 (2H, *d*, *J* = 9 Hz, 3″,5″-H), 7.23 (0.8H, *s*, CO–C$\underline{H}$=C–OH), 7.89 (1H, *d*, *J* = 8 Hz, 6′-H), 7.92 (2H, *d*, *J* = 9 Hz, 2″,6″-H).

*^{1}H NMR spectra of **11**–**14** show a mixture of keto–enolic and diketo forms. Similar case was shown in ^{1}H NMR of licodione [16]. Assignment of Me signals in **14** was tentatively made from ^{13}C NMR data (Ayabe, S. and Furuya. T. (1980) *Tetrahedron Letters*, in press).

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