BIOCHEMICAL ACTIVITIES OF THE DERIVATIVES OF DEHYDRODICAFFEIC ACID DILACTONE

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Activities of derivatives of dehydrodicaffeic acid dilactone (DDCAD) to inhibit catechol-O-methyltransferase (COMT), cyclic AMP phosphodiesterase (PDE) and DOPA decarboxylase (DDC) were examined. Among those tested, 2,6-bis-(5′,6′-dibromo-4′-hydroxy-3′-methoxyphenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione was found to be the strongest inhibitor of both COMT and PDE. There were no derivatives which showed a stronger inhibition against DDC than the original compound, DDCAD.

As reported in a previous paper[1], dehydrodicaffeic acid dilactone (DDCAD) which inhibits catechol-O-methyltransferase (COMT) and DOPA decarboxylase (DDC) has been obtained from a cultured mushroom, *Inonotus* sp. K-1410. Studying the various activities of this compound, we found that DDCAD also inhibited cyclic AMP phosphodiesterase (PDE) prepared from rat brain. In order to obtain more potent inhibitors, we synthesized its structural analogs and examined their inhibitory activities on COMT, PDE and DDC, and found that 2,6-bis-(5′,6′-dibromo-4′-hydroxy-3′ methoxyphenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione showed the strongest inhibition against both COMT and PDE.

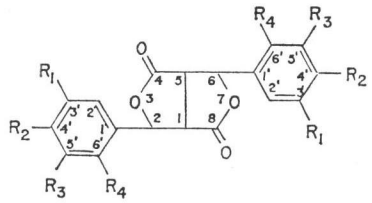
In this paper, the inhibitory activities of the DDCAD analogs against COMT, PDE and DDC are reported.

Structure-activity Relationships

Activities of a series of compounds structurally related to DDCAD to inhibit COMT, PDE and DDC were tested and compared. As shown in Tables 1 and 2, the replacement of the 3′-hydroxyl group of DDCAD by a methoxyl group caused an increase of the activity to inhibit COMT. The substitution of the 3′-hydroxyl group with the ethoxyl group or the substitution of 3′ and 4′-hydroxyl groups with the methoxyl and acetoxyl groups or the ethoxyl and acetoxyl groups reduced or eliminated the COMT-inhibiting activity. The presence of the 4′-hydroxyl group was shown to be essential for the inhibitory activity on COMT. In a series of the 3′-methoxyl derivatives, the bulky groups such as halogens and methoxyl group on C-5′ increased the activity to inhibit COMT. Particularly, the 5′,6′-dibromo derivative showed the strongest activity.

On the other hand, with PDE inhibition by these derivatives, no such structure-activity relationship was observed. There was not a significant difference in the inhibitory activity against PDE between the 3′-methoxyl and the 3′-ethoxyl derivatives. The 5′-substitution with bulky groups increased the activity to inhibit PDE. The 5′,6′-dibromo derivative also showed the strongest activity to inhibit PDE.

Table 1. Inhibition of COMT, PDE and DDC by the methoxyl derivatives of DDCAD



Compound	R_1	R_2	R_3	R_4	ID_{50}*1 for COMT ($\times 10^{-5}$ M)	ID_{50} for low *Km* PDE ($\times 10^{-5}$ M)	ID_{50} for DDC ($\times 10^{-5}$ M)
D-I	OCH_3	OH	H	H	3.6	17	NI*2
II	OCH_3	OH	OCH_3	H	2.2	1.0	〃
III	OCH_3	OH	Cl	H	0.73	5.1	〃
IV	OCH_3	OH	Br	H	0.59	4.8	15
V	OCH_3	OH	I	H	0.49	2.0	14
VI	OCH_3	OH	CH_2SCH_3	H	4.0	9.9	NI
VII	OCH_3	OH	Br	Br	0.057	0.19	3.4
VIII	OCH_3	OCH_3	H	H	NI	NI	NI
IX	OCH_3	$OCOCH_3$	H	H	〃	〃	〃
X	OCH_3	$OCOCH_3$	OCH_3	H	〃	〃	〃
XI	OCH_3	OCH_3	OCH_3	H	〃	21	〃

*1 ID_{50}: Inhibitor concentration for 50% inhibition.
*2 NI: No inhibition was observed at 100 μg/ml.

Table 2. Inhibition of COMT, PDE and DDC by the ethoxyl derivatives of DDCAD

Compound	R_1	R_2	R_3	R_4	ID_{50} for COMT ($\times 10^{-5}$ M)	ID_{50} for low *Km* PDE ($\times 10^{-5}$ M)	ID_{50} for DDC ($\times 10^{-5}$ M)
D-XII	OC_2H_5	OH	H	H	NI	16	NI
XIII	OC_2H_5	OH	Cl	H	17	2.0	〃
XIV	OC_2H_5	OH	Br	H	NI	4.4	16
XV	OC_2H_5	OH	I	H	9.6	1.1	10
XVI	OC_2H_5	OH	CH_2SCH_3	H	NI	1.8	NI
XVII	OC_2H_5	OCH_3	H	H	〃	NI	〃
XVIII	OC_2H_5	$OCOCH_3$	H	H	〃	〃	〃
(+)-DDCAD	OH	OH	H	H	3.9	15	2.1
(−)-DDCAD	OH	OH	H	H	5.0	18	1.3
Pyrogallol					7.5	—	—
Papaverine					—	2.0	—
Theophylline					—	37	—
α-Methyl DOPA					—	—	34

All the derivatives and analogs of DDCAD synthesized showed weaker activities against DDC than DDCAD. The catechol structure was suggested to be necessary for the inhibition of DDC.

Kinetic Studies on COMT

Kinetics of inhibition by (+)-DDCAD, D-I, D-V and D-VII were studied. *Km* and *Ki* were

Table 3. Inhibition of COMT by the derivatives of DDCAD

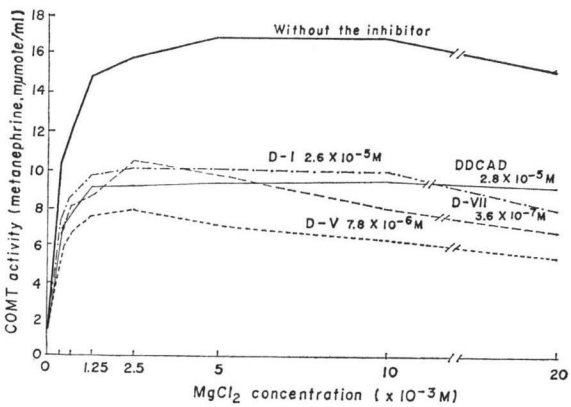
Compound	Substrate			
	Epinephrine ($Km=3.7\times10^{-4}$ M)		S-Adenosylmethionine ($Km=8.3\times10^{-5}$ M)	
	Type of inhibition	*Ki*	Type of inhibition	*Ki*
(+)-DDCAD	mixed	9.2×10^{-6} M	non-competitive	6.3×10^{-5} M
D-I	non-competitive	3.9×10^{-5} M	〃	6.7×10^{-5} M
D-V	〃	5.6×10^{-6} M	〃	5.6×10^{-6} M
D-VII	〃	4.3×10^{-7} M	〃	5.7×10^{-7} M

obtained from LINEWEAVER-BURK plots and DIXON plots, and the results are shown in Table 3. O-Methylation of catechols by COMT is known to occur mainly on the *meta* position[2]. D-I ($R_1=OCH_3$, $R_2=OH$, $R_3=R_4=H$) corresponds to the O-methylated product of DDCAD. ID_{50} values of DDCAD and O-methylated DDCAD (D-I) were nearly equal, although the type of inhibition by the former was mixed and that by the latter was non-competitive versus epinephrine. DDCAD which contained the 3′,4′-dihydroxyl groups, that is, the catechol moiety, was expected to be methylated by COMT. In fact, DDCAD was methylated, and the mixed type of inhibition shown by this compound is thought to be due to the production of the O-methylated DDCAD during the enzyme reaction. In addition, D-V and D-VII which had the 3′-methoxyl groups showed non-competitive type of inhibition against epinephrine and S-adenosylmethionine.

Effect of the Magnesium Ion Concentration

Since the O-methylation by COMT requires the presence of Mg^{++}[3], the effect of the inhibitors was examined in the presence of various concentrations of Mg^{++}. As shown in Fig. 1, the maximal activity of COMT was observed at 5×10^{-3} M of Mg^{++}. Inhibitions by DDCAD, D-I, D-V and D-VII were not reduced by an excess concentration of Mg^{++}. This result indicates that the inhibition of COMT by these agents is not due to the removal of Mg^{++} from the enzyme reaction system.

Fig. 1. Relationship between magnesium ion concentration and inhibition by the derivatives of DDCAD on COMT (average of the duplicate test)



Inhibition of COMT *In Vivo*

The effect of D-VII, the most potent inhibitor of COMT among the derivatives of DDCAD, on COMT activity of mouse liver was studied. After intraperitoneal injection of D-VII, mouse liver was homogenized and activity of the homogenate to methylate epinephrine was determined. Fig. 2 shows the time course of the inhibition of COMT of mouse liver after a single i.p. injection of D-VII (100 mg/kg). The maximum inhibition was observed within 15 minutes and the activity returned to the normal values 4 hours after the injection. A dose response curve for *in vivo* inhibition of COMT by

Fig. 2. Time course of the inhibition of mouse liver COMT after a single i.p. injection of D-VII

Inhibitor in 0.1 M phosphate buffer (pH 7.0) containing 5% DMSO was administered to mice (3 animals) by i.p. injection (100 mg/kg). Each point indicates the mean value±S.D. of the percent inhibition to the control mice liver COMT activity. Control animals were injected the same buffer without inhibitor.

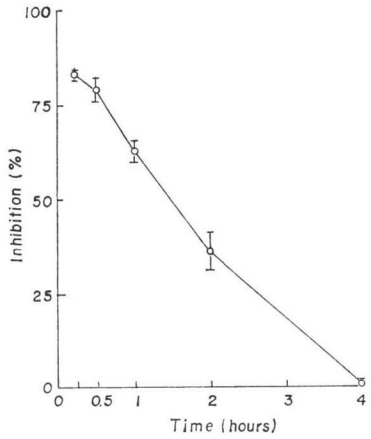
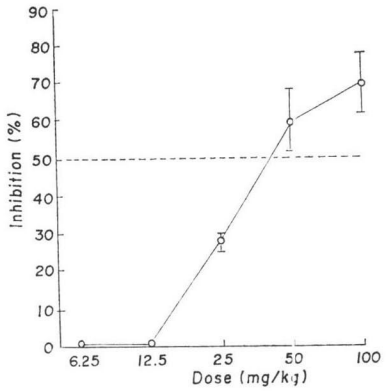


Fig. 3. Dose response curve for *in vivo* inhibition of COMT by D-VII

D-VII was administered to mice (3 animals) by i.p. injection 30 minutes prior to sacrifice. Each point indicates the mean value±S.D. of the percent inhibition.



D-VII at 30 minutes after a single i.p. injection is shown in Fig. 3. From this curve, the ED_{50} value was calculated to be 40 mg/kg.

Experimental

IR spectra were taken as KBr disks by Hitachi Infrared spectrophotometer EPI-G3. NMR spectra were taken by Varian HA-100D and Varian A-60D and chemical shifts were given in parts per million (δ) down field from the internal standard, TMS ($\delta=0.00$ ppm). Abbreviations: s=singlet, t=triplet, q=quartet, m=multiplet, bs=broad singlet.

Purification of COMT

Extraction and purification were carried out at 0~5°C. The assay of COMT was carried out by the method described in a previous paper[1]. COMT was purified from rat liver by the following method. Rat liver (74.3 g) was homogenized with 3 volumes of cold isotonic KCl solution containing 20 mM phosphate buffer (pH 6.9) and centrifuged at 105,000 *g* for 30 minutes. To the supernatant (200 ml), ammonium sulfate (35.2 g) was added and the precipitate was removed by centrifugation at 10,000 *g* for 20 minutes. Ammonium sulfate (39.6 g) was added to the supernatant and then it was centrifuged at 10,000 *g* for 20 minutes. The precipitate was dissolved in a minimal volume (50 ml) of 20 mM phosphate buffer (pH 6.9) and subjected to Sephadex G-100 column (3.5×105 cm) chromatography developed with the same buffer. The effluent was cut into 17-ml fractions, and active fractions (from 25th to 31th fractions, 120 ml) were pooled and dialyzed against 5 liters of 5 mM phosphate buffer containing 0.2 mM $MgCl_2$ and 1 mM dithiothreitol. This dialyzed solution was subjected to an affinity column[4] (1.1×12 cm) to which caffeic acid was bound as the ligand. The column was equilibrated with the same buffer used in the dialysis. The enzyme was eluted with a 500-ml linear gradient of NaCl from 0 to 1 M in the same buffer (15-ml fractionation). Most of the activity was eluted in five tubes (from 15th to 19th fractions, 75 ml). About 115-fold purification was accomplished with 14% yield by this method. This purified solution was employed as the enzyme solution.

Assay of PDE

The activity of PDE from rat brain was measured by the method described by FURUTANI *et al.*[5]. The reaction mixture (total volume 0.1 ml) consisted of 0.01 ml of 80 mM Tris-HCl (pH 7.5), 0.01 ml of 70 mM $MgSO_4$, 0.01 ml of 0.1 mM adenosine 5′-monophosphate (5′-AMP), 0.01 ml of 0.01 mM

^{14}C-cAMP (2.2×10^{-3} μCi), 0.01 ml of a test sample solution, 0.01 ml of the enzyme solution and 0.04 ml of H_2O. The reaction was carried out at 30°C for 20 minutes and stopped by heating in a boiling water bath for 3 minutes. The reacted solution was diluted with 0.4 ml of distilled water and passed through a dry alumina (neutral, Merck) column (0.5×2.7 cm, 0.5 g). The unhydrolyzed ^{14}C-cAMP was eluted from the column with 2 ml of 10 mM Tris-HCl (pH 7.5). The effluent and eluate were collected directly into counting vials and 8 ml of BRAY's scintillation fluid was added. The radioactivity was determined by a liquid scintillation counter.

Assay of DDC

The assay of DDC was carried out by the method described in a previous paper[1].

Preparation of Crude Liver COMT

Mice were sacrificed by cervical fracture. Livers were frozen immediately on a block of dry-ice and weighed. They were homogenized in 2 volumes of cold isotonic KCl with a motor-driven glass pestle. Taking 50 μl of the crude homogenate, the enzyme activity was determined.

General Procedure for the Preparation of the Derivatives and Analogs of DDCAD

Dilactone derivatives were prepared from the corresponding cinnamic acid derivatives by the modified method described by CARTWRIGHT and HAWORTH[6]. The solution of a cinnamic acid derivative in methanol or N,N-dimethylformamide (DMF) was added dropwise to a solution of ferric chloride in water and stirred by a rapid stream of air. The precipitate gradually appeared. The air was passed for 8 hours and the reaction mixture was allowed to stand overnight. The precipitate formed was collected as a paste, suspended in water (250 ml), heated for 10~15 minutes on a steam bath and acidified with 12 N sulfuric acid (50 ml) with vigorous shaking. After cooling, the precipitate was collected, washed and crystallized from the solvent systems described below.

2,6-Bis-(4'-hydroxy-3'-methoxyphenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione (D-I)

D-I was prepared by the method of CARTWRIGHT *et al.*[6] from ferulic acid (20 g) to yield 6 g (30%), m.p. 210~213°C (dec). Anal. Found: C, 62.27; H, 4.70; O, 33.01. Calcd. for $C_{20}H_{18}O_8$: C, 62.17; H, 4.70; O, 33.13. IR ν cm^{-1}: 3470 (OH), 1790 (lactone). NMR (DMSO-d_6) δ 9.20 (2H, s), 7.10~6.70 (6H, m), 5.77 (2H, bs), 4.22 (2H, bs), 3.81 (6H, s).

2,6-Bis-(4'-hydroxy-3',5'-dimethoxyphenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione (D-II)

D-II was prepared by the method of AHMED *et al.*[7] from sinapic acid (3 g) to yield 2.4 g (80%), m.p. 216~218°C. Anal. Found: C, 58.94; H, 4.94; O, 35.87. Calcd. for $C_{22}H_{22}O_{10}$: C, 59.19; H, 4.97; O, 35.84. IR ν cm^{-1}: 3450, 1770. NMR (DMSO-d_6) δ 8.48 (2H, s), 6.65 (4H, s), 5.68 (2H, bs), 4.22 (2H, bs), 3.77 (12H, s).

2,6-Bis-(5'-chloro-4'-hydroxy-3'-methoxyphenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione (D-III)

A solution of 5-chloro-4-hydroxy-3-methoxycinnamic acid (20 g) in DMF (500 ml) was added during 3 hours to a solution of ferric chloride (40 g) in water (1.5 liters) at 30°C, stirred by a rapid stream of air. The precipitate was treated as in the general procedure, washed by methanol and crystallized from acetone-methanol to yield 1.8 g (9.0%), m.p. 225~227°C. Anal. Found: C, 53.14; H, 3.51; O, 28.80; Cl, 15.85. Calcd. for $C_{20}H_{16}O_8Cl_2$: C, 52.76; H, 3.54; O, 28.12; Cl, 15.58. IR ν cm^{-1}: 3470, 1780. NMR (DMSO-d_6) δ 9.60 (2H, bs), 7.18~7.00 (4H, m), 5.80 (2H, bs), 4.29 (2H, bs), 3.90 (6H, s).

2,6-Bis-(5'-bromo-4'-hydroxy-3'-methoxyphenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione (D-IV)

D-IV was prepared from 5-bromo-4-hydroxy-3-methoxycinnamic acid (20 g) by the same procedure applied to D-III, yielding 2.6 g (13%), m.p. 250~253°C. Anal. Found: C, 43.78; H, 3.04; O, 24.61; Br, 29.06. Calcd. for $C_{20}H_{16}O_8Br_2$: C, 44.14; H, 2.96; O, 23.52; Br, 29.37. IR ν cm^{-1}: 3430, 1780. NMR (DMSO-d_6) δ 9.64 (2H, bs), 7.30~7.00 (4H, m), 5.80 (2H, bs), 4.28 (2H, bs), 3.90 (6H, s).

2,6-Bis-(4'-hydroxy-5'-iodo-3'-methoxyphenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione (D-V)

D-V was prepared from 4-hydroxy-5-iodo-3-methoxycinnamic acid (20 g) by the same procedure applied to D-III, yielding 2.8 g (14%), m.p. 250~260°C. Anal. Found: C, 37.77; H, 2.52; O, 20.32; I, 39.32. Calcd. for $C_{20}H_{16}O_8I_2$: C, 37.64; H, 2.53; O, 20.06; I, 39.78. IR ν cm^{-1}: 3380, 1770. NMR (DMSO-d_6) δ 9.70 (2H, bs), 7.37 (2H, m), 7.05 (2H, m), 5.78 (2H, bs), 4.24 (2H, bs), 3.86 (6H, s).

2,6-Bis-(4′-hydroxy-3′-methoxy-5′-thiomethoxymethylphenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione (D-VI)

D-VI was prepared from 4-hydroxy-3-methoxy-5-thiomethoxymethylcinnamic acid (20 g) by the same procedure applied to D-III, yielding 6.4 g (32%), m.p. 178~179°C. Anal. Found: C, 57.19; H, 5.19; O, 24.96; S, 13.22. Calcd. for $C_{24}H_{26}O_8S_2$: C, 56.90; H, 5.17; O, 25.27; S, 12.66. IR ν cm^{-1}: 3400, 1780, 1760. NMR (DMSO-d_6) δ 8.89 (2H, s), 6.95 (4H, bs), 5.78 (2H, bs), 4.20 (2H, bs), 3.85 (6H, s), 3.68 (4H, bs), 2.00 (6H, s).

2,6-Bis-(5′,6′-dibromo-4′-hydroxy-3′-methoxyphenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione (D-VII)

A solution of 5,6-dibromo-4-hydroxy-3-methoxycinnamic acid (20 g) in DMF (300 ml) was added during 2.5 hours to a solution of ferric chloride (40 g) in water (1.5 liters) at 80°C and stirred by a rapid stream of air. The precipitate was treated as described in the general procedure, washed with DMF and crystallized from DMF - *n*-hexane - acetone to yield 0.9 g (4.5%), m.p. 290~300°C (dec). Anal. Found: C, 34.62; H, 2.06; O, 17.81; Br, 45.19. Calcd. for $C_{20}H_{14}O_8Br_4$: C, 34.22; H, 2.01; O, 18.23; Br, 45.54. IR ν cm^{-1}: 3400, 1780. NMR (DMSO-d_6) δ 10.15 (2H, b), 6.94 (2H, bs), 6.15 (2H, bs), 4.25 (2H, bs), 3.89 (6H, s).

2,6-Bis-(3′,4′-dimethoxyphenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione (D-VIII)

D-VIII was prepared by the method of CARTWRIGHT *et al.*[6] from D-I (5 g) to yield 3.6 g (67%), m.p. 204~205°C. Anal. Found: C, 63.52; H, 5.37; O, 31.10. Calcd. for $C_{22}H_{22}O_8$: C, 63.76; H, 5.35; O, 30.89. IR ν cm^{-1}: 1770. NMR ($CDCl_3$) δ 6.90~6.75 (6H, m), 5.87 (2H, bs), 3.89 (6H, s), 3.87 (6H, s), 3.58 (2H, bs).

2,6-Bis-(4′-acetoxy-3′-methoxyphenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione (D-IX)

Acetic anhydride (150 ml) and pyridine (150 ml) were added to D-I (3 g), the mixture was warmed for 5 minutes and stirred overnight at room temperature. The precipitate produced on the addition of crushed ice was collected, dissolved in acetone and crystallized from aqueous acetone to yield 2.95 g (81%), m.p. 226~227°C. Anal. Found: C, 60.95; H, 4.88; O, 33.89. Calcd. for $C_{24}H_{22}O_{10}$: C, 61.27; H, 4.72; O, 34.01. IR ν cm^{-1}: 1780 (lactone and ester). NMR (DMSO-d_6) δ 7.32~7.08 (6H, m), 5.90 (2H, bs), 4.30 (2H, bs), 3.82 (6H, s), 2.25 (6H, s).

2,6-Bis-(4′-acetoxy-3′,5′-dimethoxyphenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione (D-X)

D-X was prepared from D-II (3 g) by the same procedure applied to D-IX, yielding 3.15 g (88%), m.p. 234~235°C. Anal. Found: C, 58.71; H, 4.88; O, 37.09. Calcd. for $C_{26}H_{26}O_{12}$: C, 58.86; H, 4.94; O, 36.19. IR ν cm^{-1}: 1770 (lactone and ester). NMR (DMSO-d_6) δ 6.85 (4H, s), 5.89 (2H, bs), 4.36 (2H, bs), 3.84 (12H, s), 2.27 (6H, s).

2,6-Bis-(3′,4′,5′-trimethoxyphenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione (D-XI)

An ethereal solution (200 ml) of diazomethane was added to a solution of D-II (3 g) in acetone (150 ml). After 24 hours, the excess of diazomethane was decomposed by addition of a few drops of concentrated hydrochloric acid. Most of the ether and acetone was removed, the residue was dissolved in chloroform and after washed with water and diluted sodium bicarbonate solution, the chloroform solution was evaporated and dried. D-XI crystallized from acetone in pale yellowish plates, 0.5 g (16%), m.p. 195~196°C. Anal. Found: C, 61.43; H, 5.38; O, 32.88. Calcd. for $C_{24}H_{26}O_{10}$: C, 60.75; H, 5.52; O, 33.72. IR ν cm^{-1}: 1780. NMR (DMSO-d_6) δ 6.72 (4H, s), 5.79 (2H, bs), 4.22 (2H, bs), 3.80 (12H, s), 3.67 (6H, s).

2,6-Bis-(3′-ethoxy-4′-hydroxyphenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione (D-XII)

D-XII was prepared from 3-ethoxy-4-hydroxycinnamic acid (20 g) by the same procedure applied to D-I, yielding 2.6 g (13%), m.p. 175~177°C. Anal. Found: C, 64.36; H, 5.30; O, 30.24. Calcd. for $C_{22}H_{22}O_8$: C, 63.76; H, 5.35; O, 30.89. IR ν cm^{-1}: 3410, 1780. NMR (DMSO-d_6) δ 9.08 (2H, bs), 7.10~6.82 (6H, m), 5.75 (2H, bs), 4.19 (2H, bs), 4.10 (4H, q, J=7 Hz), 1.34 (6H, t, J=7 Hz).

2,6-Bis-(5′-chloro-3′-ethoxy-4′-hydroxyphenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione (D-XIII)

D-XIII was prepared from 5-chloro-3-ethoxy-4-hydroxycinnamic acid (20 g) by the same procedure applied to D-III, yielding 1.7 g (8.5%), m.p. 230~233°C. Anal. Found: C, 54.61; H, 4.45; O, 26.81; Cl, 14.86. Calcd. for $C_{22}H_{20}O_8Cl_2$: C, 54.67; H, 4.17; O, 26.49; Cl, 14.67. IR ν cm^{-1}: 3450, 1760.

NMR (DMSO-d_6) δ 9.40 (2H, bs), 7.15~6.85 (4H, m), 5.78 (2H, bs), 4.25 (2H, bs), 4.15 (4H, q, J= 7 Hz), 1.37 (6H, t, J=7 Hz).

2,6-Bis-(5'-bromo-3'-ethoxy-4'-hydroxyphenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione (D-XIV)

A solution of 5-bromo-3-ethoxy-4-hydroxycinnamic acid (20 g) in DMF (1 liter) was added during 3 hours to a solution of ferric chloride (40 g) in water (1.5 liters) and stirred by a rapid stream of air. The precipitate was treated as described in the general procedure, washed by ethanol and crystallized from acetone-methanol to yield 3.5 g (18%), m.p. 233~235°C. Anal. Found: C, 46.63; H, 3.31; O, 21.93; Br, 27.72. Calcd. for $C_{22}H_{20}O_8Br_2$: C, 46.18; H, 3.52; O, 22.37; Br, 27.93. IR ν cm^{-1}: 3350, 1760. NMR (DMSO-d_6) δ 9.42 (2H, bs), 7.30~7.00 (4H, m), 5.78 (2H, bs), 4.24 (2H, bs), 4.15 (4H, q, J=7 Hz), 1.38 (6H, t, J=7 Hz).

2,6-Bis-(3'-ethoxy-4'-hydroxy-5'-iodophenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione (D-XV)

D-XV was prepared from 3-ethoxy-4-hydroxy-5-iodocinnamic acid (20 g) by the same procedure applied to D-III, yielding 5.1 g (26%), m.p. 252~256°C. Anal. Found: C, 40.17; H, 2.98; O, 20.63; I, 37.97. Calcd. for $C_{22}H_{20}O_8I_2$: C, 39.66; H, 3.03; O, 19.21; I, 38.10. IR ν cm^{-1}: 3350, 1765. NMR (DMSO-d_6) δ 9.48 (2H, bs), 7.38 (2H, m), 7.05 (2H, m), 5.76 (2H, bs), 4.21 (2H, bs), 4.15 (4H, q, J= 7 Hz), 1.36 (6H, t, J=7 Hz).

2,6-Bis-(3'-ethoxy-4'-hydroxy-5'-thiomethoxymethylphenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione (D-XVI)

D-XVI was prepared from 3-ethoxy-4-hydroxy-5-thiomethoxymethylcinnamic acid (20 g) by the same procedure applied to D-III, yielding 5.5 g (28%), m.p. 190~191°C. Anal. Found: C, 59.08; H, 5.69; O, 24.32; S, 12.23. Calcd. for $C_{26}H_{30}O_8S_2$: C, 58.41; H, 5.66; O, 23.94; S, 12.00. IR ν cm^{-1}: 3400, 1770. NMR (DMSO-d_6) δ 8.70 (2H, s), 6.94 (4H, bs), 5.77 (2H, bs), 4.20 (2H, bs), 4.14 (4H, q, J=7 Hz), 3.70 (4H, bs), 2.02 (6H, s), 1.36 (6H, t, J=7 Hz).

2,6-Bis-(3'-ethoxy-4'-methoxyphenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione (D-XVII)

D-XII (2.7 g) was methylated to D-XVII by the same procedure described in the preparation of D-XI, yielding 0.8 g (28%), m.p. 139~140°C. Anal. Found: C, 65.05; H, 5.89; O, 28.81. Calcd. for $C_{24}H_{26}O_8$: C, 65.15; H, 5.92; O, 28.93. IR ν cm^{-1}: 1780. NMR (DMSO-d_6) δ 7.02 (6H, bs), 5.80 (2H, bs), 4.19 (2H, bs), 4.08 (4H, q, J=7 Hz), 3.78 (6H, s), 1.30 (6H, t, J=7 Hz).

2,6-Bis-(4'-acetoxy-3'-ethoxyphenyl)-3,7-dioxabicyclo-[3,3,0]-octane 4,8-dione (D-XVIII)

D-XVIII was prepared from D-XII (3 g) by the same procedure described in the preparation of D-IX, yielding 3.5 g (97%), m.p. 118~120°C. Anal. Found: C, 62.87; H, 5.30; O, 31.52. Calcd. for $C_{26}H_{26}O_{10}$: C, 62.64; H, 5.26; O, 32.10. IR ν cm^{-1}: 1770 (lactone and ester). NMR (DMSO-d_6) δ 7.30~7.08 (6H, m), 5.89 (2H, bs), 4.28 (2H, bs), 4.11 (4H, q, J=7 Hz), 2.26 (6H, s), 1.29 (6H, t, J=7 Hz).

References

1) KUMADA, Y.; H. NAGANAWA, H. IINUMA, M. MATSUZAKI, T. TAKEUCHI & H. UMEZAWA: Dehydrodicaffeic acid dilactone, an inhibitor of catechol-O-methyltransferase. J. Antibiotics 29: 882~889, 1976
2) SENOH, S.; J. DALY, J. AXELROD & B. WITKOP: Enzymatic *p*-O-methylation by catechol O-methyltransferase. J. Am. Chem. Soc. 81: 6240~6245, 1959
3) AXELROD, J. & R. TOMCHICK: Enzymatic O-methylation of epinephrine and other catecholes. J. Biol. Chem. 233: 702~705, 1958
4) KUMADA, Y.; T. TAKEUCHI & H. UMEZAWA: Purification and properties of a dehydrodicaffeic acid dilactons-forming enzyme from a mushroom, *Inonotus* sp. K-1410. Agric. Biol. Chem. 41: 869~876, 1977
5) FURUTANI, Y.; M. SHIMADA, M. HAMADA, T. TAKEUCHI & H. UMEZAWA: Reticulol, an inhibitor of cyclic adenosine 3',5'-monophosphate phosphodiesterase. J. Antibiotics 28: 558~560, 1975
6) CARTWRIGHT, N. J. & R. D. HAWORTH: The constituents of natural phenolic resins. XIX. The oxidation of ferulic acid. J. Chem. Soc. 1944: 535~537, 1944
7) AHMED, R.; M. LEHRER & R. STEVENSON: Synthesis of thomasic acid. Tetrahedron 29: 3753~3759, 1973