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Inhibition of Adenosine 3′,5′-Cyclic Monophosphate Phosphodiesterase by Lignan Glucosides of *Eucommia* Bark[1)]

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Studies were conducted on adenosine 3′,5′-cyclic monophosphate (cyclic AMP) phosphodiesterase inhibition by lignan glucosides from bark of *Eucommia ulmoides* OLIV. (Eucommiaceae). Various lignan diglucosides, (+)-syringaresinol di-*O*-β-D-glucopyranoside (**1c**), (+)-medioresinol di-*O*-β-D-glucopyranoside (**2c**), (+)-pinoresinol di-*O*-β-D-glucopyranoside (**3c**) and (+)-1-hydroxypinoresinol 4′,4″-di-*O*-β-D-glucopyranoside (**4d**), showed strong inhibitory activity.

The structure–inhibitory activity relationships of the aglycone and its glucosides are discussed. The order of inhibitory effect was diglucoside ≧ aglycone > monoglucoside.

Keywords——cyclic AMP phosphodiesterase; inhibitor; *Eucommia ulmoides*; lignan glucoside; (+)-syringaresinol di-*O*-β-D-glucopyranoside; (+)-medioresinol di-*O*-β-D-glucopyranoside; (+)-pinoresinol di-*O*-β-D-glucopyranoside; (+)-1-hydroxypinoresinol 4′,4″-di-*O*-β-D-glucopyranoside; structure–inhibitory activity relationship

The adenosine 3′,5′-cyclic monophosphate (cyclic AMP) phosphodiesterase inhibition test provides a useful means for the screening of biologically active compounds contained in medicinal plants. Nikaido *et al.* reported on cyclic AMP phosphodiesterase inhibitors contained in various medicinal plants.[2)] In the previous papers,[3)] Nishibe *et al.* isolated a series of lignans from barks of *Olea europea* L., *O. africana* MILL., *O. capensis* L., *Fraxinus japonica* BLUME and *F. mandshurica* RUPR. var. *japonica* MAXIM. (Oleaceae), and reported on the inhibition of cyclic AMP phosphodiesterase by these lignans.[2j)]

Recently Deyama *et al.* isolated a series of lignans from bark of *Eucommia ulmoides* OLIV. (Japanese name: tochu) (Eucommiaceae), which is one of the longest-known tonic drugs in China.[4)]

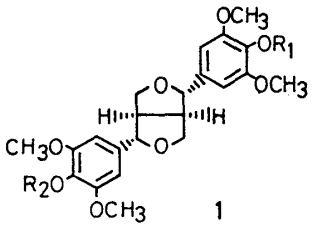
As a continuation of our studies on cyclic AMP phosphodiesterase inhibition by lignans contained in medicinal plants, this paper deals with the inhibitory effect of lignan glucosides from *Eucommia* bark. The structure–inhibitory activity relationships of the aglycone and its glucosides are also discussed.

Results and Discussion

Lignan glucosides from *Eucommia* bark were tested for inhibitory activity against beef

heart cyclic AMP phosphodiesterase using the method reported in the previous papers.[2] The assay consisted of a two-step isotopic procedure. Tritium-labelled cyclic AMP was hydrolyzed to 5′-AMP by phosphodiesterase and the 5′-AMP was then further hydrolyzed to adenosine by snake venom nucleotidase. The hydrolyzate was treated with an anion-exchange resin to adsorb all charged nucleotides and to leave [^{3}H]adenosine as the only labelled compound to be counted.

TABLE I. Inhibitory Activity of Lignan Glucosides on Cyclic AMP Phosphodiesterase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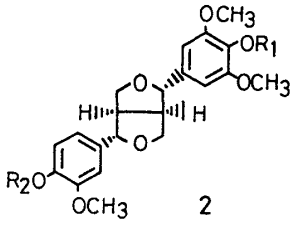
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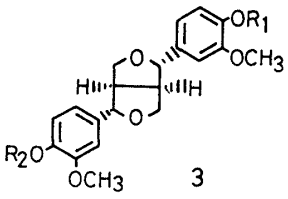
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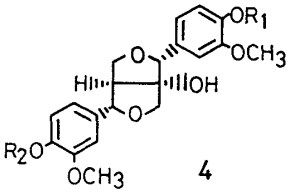
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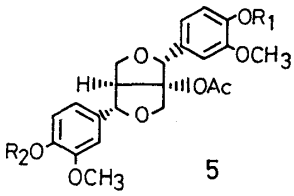
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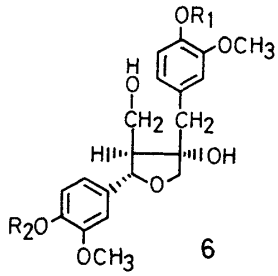
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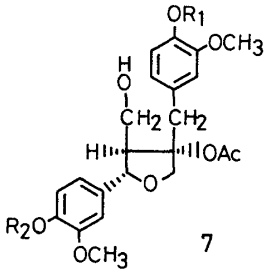
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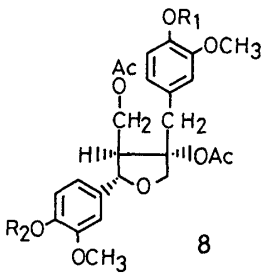
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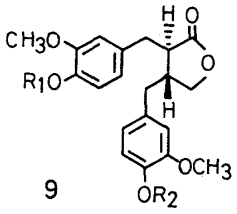
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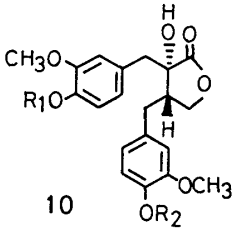
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Compound No.	R_1	R_2	IC_{50} ($\times 10^{-5}$ M)	Compound No.	R_1	R_2	IC_{50} ($\times 10^{-5}$ M)	Compound No.	R_1	R_2	IC_{50} ($\times 10^{-5}$ M)
1a	H	H	17.5	**4a**	H	H	21.3	**7a**	H	H	>50
1b	Glc	H	>50	**4b**	Glc	H	28.6	**7b**	Glc	Glc	16.5
1c	Glc	Glc	12.7	**4c**	H	Glc	33.2				
				4d	Glc	Glc	10.0	**8a**	H	H	>50
2a	H	H	12.1								
2b	Glc	H	29.7	**5a**	H	H	3.2	**9a**	H	H	9.8
2c	Glc	Glc	6.3	**5b**	Glc	H	4.4	**9b**	Glc	H	>50
				5c	Glc	Glc	1.1	**9c**	Glc	Glc	11.1
3a	H	H	7.5								
3b	Glc	H	14.2	**6a**	H	H	20.1	**10a**	H	H	19.5
3c	Glc	Glc	8.9	**6b**	Glc	H	35.8	**10b**	Glc	H	>50
				6c	H	Glc	40.1	**10c**	Glc	Glc	14.3
				6d	Glc	Glc	23.7				

IC_{50} ($\times 10^{-5}$ M) value of papaverine as a reference inhibitor: 3.0. All the lignan samples were isolated from *Eucommia* bark except the following samples: **5a** and **5b**, isolated from *Olive* bark[3]; **5c**, prepared from **4d**; **7a** and **8a**, prepared from **6a**; **7b**, prepared from **6d**; **9a**—**9b** and **10a**—**10c**, isolated from *Trachelospermum asiaticum* var. *intermedium*.[5]

Major lignan diglucosides, (+)-syringaresinol di-*O*-*β*-D-glucopyranoside (**1c**), (+)-medioresinol di-*O*-*β*-D-glucopyranoside (**2c**), (+)-pinoresinol di-*O*-*β*-D-glucopyranoside (**3c**) and (+)-1-hydroxypinoresinol 4′,4″-di-*O*-*β*-D-glucopyranoside (**4d**), showed strong inhibitory effects.

Previous studies on lignans having a 2,6-diarylated 3,7-dioxabicyclo[3.3.0]octane ring indicated that the presence of two *p*-hydroxyl groups is essential for the phosphodiesterase inhibitory activity, and both *O*-methylation and *O*-glucosylation of free hydroxyl groups generally decrease the activity.[2b,j]

In the case of the lignan diglucosides of *Eucommia* bark, **1c**, **2c**, **3c**, **4d** and **6d**, no decrease in the inhibitory effect, compared with that of their aglycones, **1a**, **2a**, **3a**, **4a** and **6a**, was observed. The structure–inhibitory activity data for the aglycone and its glucosides are summarized in Table I. The order of inhibitory effect was shown to be diglucoside ≧ aglycone > monoglucoside. Similar relationships were also observed among lignan diglucosides having a 2,3-dibenzylated butyrolactone ring, **9c** and **10c**, isolated from stems of *Trachelospermum asiaticum* NAKAI var. *intermedium* NAKAI (Apocynaceae).[5] These results suggested that lignan diglucosides derived from aglycones which have strong inhibitory activity might be potent cyclic AMP phosphodiesterase inhibitors.

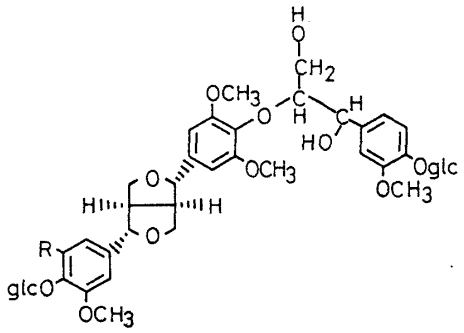
From this point of view, (+)-1-acetoxypinoresinol 4′,4″-di-*O*-*β*-D-glucopyranoside (**5c**) was newly prepared from **4d** as described in Experimental.

As expected, **5c** showed strong inhibitory activity. The newly prepared lignan diglucoside **7b** also showed inhibitory activity.

However, the sesquilignan and neolignan diglucosides, **11a**, **11b** and **12b**, showed no inhibitory effect (Table II).

Weinryb *et al.* reported that a considerable number of therapeutic agents used as antipsychotics, antianxiety agents, antihypertensives and the like showed strong inhibitory effects on phosphodiesterase *in vitro*, though it is not necessarily the case that the pharmacological activity is due to some alteration of cyclic AMP metabolism.[6] Recently it was reported that **1c** and **3c** protect animals from stress-induced decreases in sexual activity and in rectal temperature, stress-induced decreases in exploratory and spontaneous movements, and stress-induced failure of memory retrieval.[7] Thus, there may be some correlation

TABLE II. Inhibitory Activity of Sesquilignan and Neolignan Glucosides on Cyclic AMP Phosphodiesterase



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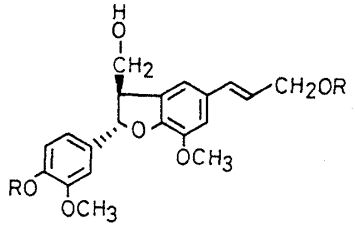

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Compound No.	R	IC_{50} ($\times 10^{-5}$ M)	Compound No.	R	IC_{50} ($\times 10^{-5}$ M)
11a	H	>50	**12a**	H	>50
11b	OCH_3	>50	**12b**	Glc	>50

IC_{50} ($\times 10^{-5}$ M) value of papaverine as a reference inhibitor: 3.0.

between the pharmacological effect and the cyclic AMP phosphodiesterase-inhibitory activity of these lignan diglucosides. Therefore, it is noteworthy from a medicinal viewpoint that major lignan diglucosides from *Eucommia* bark, used as a tonic, were shown to be strong cyclic AMP phosphodiesterase inhibitors.

Experimental

The following instruments were used: optical rotations, Yanaco OR-50D; ultraviolet (UV) spectra, Shimadzu UV 210; infrared (IR) spectra, Hitachi 270-30; proton nuclear magnetic resonance (^{1}H-NMR) spectra, JEOL JNM-FX 60 equipped with a JEC-980 computer with tetramethylsilane (TMS, $\delta=0$) as an internal reference; mass spectrum (MS), JEOL JMS-DX 303 and Shimadzu LKB-9000. The abbreviations used are as follows: s, singlet; m, multiplet. Precoated thin-layer chromatography (TLC) plates, Silica gel $60F_{254}$ (Merck), were used for TLC and preparative TLC. The spots were detected under UV (254 nm) illumination as dark, absorbing spots or by spraying the plates with 10% H_2SO_4 solution and heating.

Assay Method for Cyclic AMP Phosphodiesterase——Samples were tested for cyclic AMP phosphodiesterase activity in duplicate by the method described in the previous papers.[2] All the inhibitors were added as solutions in dimethylsulfoxide (DMSO). The presence of DMSO in the assay medium at up to 2% concentration is known to have no effect on the enzyme activity. The IC_{50} value is the concentration of a compound required to give 50% inhibition of cyclic AMP phosphodiesterase activity.

Enzymes and Chemicals——Beef heart phosphodiesterase was purchased from Boehringer. Snake venom nucleotidase and cyclic AMP were obtained from Sigma, and [^{3}H]cyclic AMP from the Radiochemical Centre. Papaverine, a reference inhibitor, was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo).

(+)-1-Acetoxypinoresinol 4′,4″-Di-*O*-*β*-D-glucopyranoside (5c)——**4d** (50.6 mg) was acetylated with acetic anhydride–pyridine in the usual way. The crude acetate was purified by preparative TLC using $CHCl_3$–AcOEt (1 : 2) as a developer to give (+)-1-acetoxypinoresinol 4′,4″-di-*O*-*β*-D-glucopyranoside octaacetate (**5d**) (36.9 mg) as an amorphous powder. ^{1}H-NMR (in $CDCl_3$) δ: 1.67 (3H, s, tertiary alcoholic $OCOCH_3$), 2.03, 2.07 (24H, each s, 8 × alcoholic $OCOCH_3$), 3.81, 3.84 (6H, each s, 2 × OCH_3), 6.80—7.20 (6H, m, arom. H).

5d (36.9 mg) was deacetylated with ammonia in methanol. The crude product was purified by preparative TLC using the lower layer of $CHCl_3$–MeOH–H_2O (65 : 35 : 10) as a developer to give **5c** (9.9 mg) as an amorphous powder. $[\alpha]_D^{24}$ −25.9° ($c=0.33$, MeOH). UV λ_{max}^{EtOH} nm: 229.5, 277. IR ν_{max}^{KBr} cm^{-1}: 3436 (OH), 1740 (C=O), 1596, 1514 (aromatic ring). FAB-MS *m/z*: 763 $[M(C_{34}H_{44}O_{18})+Na]^+$. ^{1}H-NMR (in CD_3OD) δ: 1.66 (3H, s, tertiary alcoholic $OCOCH_3$), 3.86, 3.88 (6H, each s, 2 × OCH_3), 6.80—7.20 (6H, m, arom. H).

(−)-Olivil Monoacetate (7a) and (−)-Olivil Diacetate (8a)——**6a** (94.6 mg) was acetylated with acetic anhydride–pyridine in the usual way. The crude acetate was purified by preparative TLC using $CHCl_3$–AcOEt (1 : 1) as a developer to give (−)-olivil tetraacetate (**8b**) (27 mg) as an amorphous powder. ^{1}H-NMR (in $CDCl_3$) δ: 1.82 (3H, s, tertiary alcoholic $OCOCH_3$), 2.07 (3H, s, alcoholic $OCOCH_3$), 2.29, 2.30 (6H, each s, 2 × phenolic $OCOCH_3$), 3.79, 3.82 (6H, each s, 2 × OCH_3), 6.60—7.10 (6H, m, arom. H).

8b (27 mg) was deacetylated with ammonia in methanol to give a mixture of **7a** and **8a** (22.5 mg). Separation and purification of the products by TLC afforded **7a** (7.2 mg) as an amorphous powder and **8a** (13.9 mg) as an amorphous powder.

7a: $[\alpha]_D^{24}$ −49.0° ($c=0.08$, MeOH). UV λ_{max}^{MeOH} nm: 223, 280.5. IR ν_{max}^{KBr} cm^{-1}: 3444 (OH), 1732 (C=O), 1606, 1516 (aromatic ring). MS *m/z*: 418 (M^+, $C_{22}H_{26}O_8$). ^{1}H-NMR (in $CDCl_3$) δ: 1.97 (3H, s, tertiary alcoholic $OCOCH_3$), 3.86, 3.88 (6H, each s, 2 × OCH_3), 6.50—7.00 (6H, m, arom. H).

8a: $[\alpha]_D^{24}$ −45.5° ($c=0.32$, MeOH). UV λ_{max}^{MeOH} nm: 229, 281. IR ν_{max}^{KBr} cm^{-1}: 3444 (OH), 1732 (C=O), 1608, 1516 (aromatic ring). MS *m/z*: 460 (M^+, $C_{24}H_{28}O_9$). ^{1}H-NMR (in $CDCl_3$) δ: 1.90 (3H, s, tertiary alcoholic $OCOCH_3$), 2.04 (3H, s, alcoholic $OCOCH_3$), 3.86, 3.87 (6H, each s, 2 × OCH_3), 6.50—7.00 (6H, m, arom. H).

(−)-Olivil 4′,4″-Di-*O*-*β*-D-glucopyranoside Monoacetate (7b)——**6d** (58.2 mg) was acetylated with acetic anhydride–pyridine in the usual way. The crude acetate was purified by preparative TLC using $CHCl_3$–AcOEt (1 : 3) as a developer to give (−)-olivil 4′,4″-di-*O*-*β*-D-glucopyranoside decaacetate (**8c**) (35.6 mg) as an amorphous powder. ^{1}H-NMR (in $CDCl_3$) δ: 1.87 (3H, s, tertiary alcoholic $OCOCH_3$), 2.03, 2.07 (27H, each s, 9 × alcoholic $OCOCH_3$), 3.79 (6H, s, 2 × OCH_3), 6.50—7.20 (6H, m, arom. H).

8c (35.6 mg) was deacetylated with ammonia in methanol. The crude product was purified by preparative TLC using the lower layer of $CHCl_3$–MeOH–H_2O (65 : 35 : 10) as a developer to give **7b** (4.1 mg) as an amorphous powder. $[\alpha]_D^{24}$ −64.3° ($c=0.13$, MeOH). UV λ_{max}^{MeOH} nm: 226, 278. IR ν_{max}^{KBr} cm^{-1}: 3428 (OH), 1730 (C=O), 1596, 1512 (aromatic ring). FAB-MS *m/z*: 765 $[M(C_{34}H_{46}O_{18})+Na]^+$. ^{1}H-NMR (in CD_3OD) δ: 1.83 (3H, s, tertiary alcoholic $OCOCH_3$), 3.84 (6H, s, 2 × OCH_3), 6.70—7.20 (6H, m, arom. H).

References and Notes

1) This paper forms Part XIV of "Inhibitors of Cyclic AMP Phosphodiesterase in Medicinal Plants." Part XIII: T.

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