Inhibitors of Adenosine 3′,5′-Cyclic Monophosphate Phosphodiesterase from *Schisandra chinensis* and the Structure Activity Relationship of Lignans[1)]

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The structure activity relationship was studied in analogous lignans from *Schisandra chinensis* and their derivatives. These compounds were tested for cyclic adenosine 3′,5′-monophosphate (cAMP) phosphodiesterase inhibition. An inhibitor, nordihydroguaiaretic acid (13), was isolated from this plant, so we discussed this compound and a derivative, nordihydroguaiaretic acid tetramethyl ether, using molecular mechanics involving three-dimensional modeling and minimization of the structure using the MM2PP program. As a result, it was found that the structure of nordihydroguaiaretic acid tetramethyl ether and papaverine (30) (positive control) shared a similar low energy conformation. This fact suggested that these compounds inhibited cAMP phosphodiesterase by a similar mechanism.

Keywords lignan; nordihydroguaiaretic acid; *Schisandra chinensis*; cAMP phosphodiesterase; inhibitor; structure activity relationship; molecular mechanic; 3D MOL

Since Sutherland found adenosine 3′,5′-cyclic monophosphate (cAMP) phosphodiesterase (PDE) as a second messenger inside cells, compounds that act to alter cAMP metabolism have been the subject of studies not from a biochemical point of view but with the aim of development of new medicinal drugs. Screening studies aimed at finding inhibitors of cAMP PDE have shown that a variety of natural products and synthetic compounds have inhibitory effects on this enzyme.[2-7)] We have demonstrated that measurement of cAMP PDE inhibition can be used as a screening method to detect biologically active compounds contained in medicinal plants used in traditional medicines. We have already reported on cAMP PDE inhibitors contained in various medicinal plants.[8)] This time we attempted to research cAMP PDE inhibitors from the fruit of *Schisandra chinensis* BAILLON, which is one of the most important Chinese herbs. As a result, we isolated several known lignans, which had potent cAMP PDE inhibitory activity, and one more potent inhibitor. These compounds and their derivatives were examined in order to elucidate the structure activity relationship, and the results are discussed by using molecular graphics and molecular mechanics calculation.

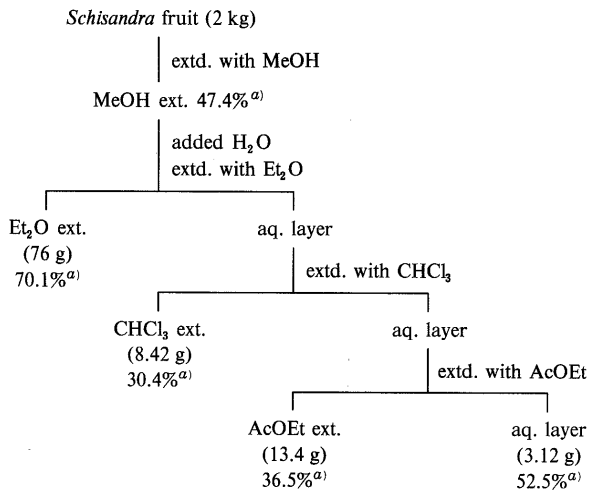


Chart 1. Extraction and Fractionation of *Schisandra* Fruit

The number inside parentheses shows the weight of extract. *a*) Inhibitory activity (%) of each extract.

Results and Discussion

Chemistry A hot methanol extract of the commercial fruit of *Schisandra chinensis* was fractionated as shown in Chart 1. The ether-soluble fraction, which showed high inhibitory activity, was fractionated repeatedly by column chromatography, preparative thin-layer chromatography (TLC), medium pressure liquid chromatography (MPLC) and high performance liquid chromatography (HPLC) with monitoring for inhibitory activity against cAMP PDE. One of the most active compounds, (±)-γ-schizandrin (**1**), was isolated from this fraction. Since the main compounds in the fraction were lignans, which have been isolated from this plant by Ikeya *et al.*,[9)] these isolated compounds (**1**—**12**) were tested for inhibition of cAMP PDE. A further high polar fraction was fractionated by several column chromatographies with monitoring for inhibitory activity against cAMP PDE. Then, the most active compound (**13**) was isolated from this fraction. The compound (**13**) was identified as nordihydroguaiaretic acid (NDGA) by comparison with an authentic sample. NDGA was already isolated from *Larrea divaricata*.[10)] But this was first isolated from *Schisandra chinensis*.

TABLE I. Inhibitory Activity of Lignans on cAMP Phosphodiesterase

R type	IC_{50} ($\times 10^{-5}$ M)	*S* type	IC_{50} ($\times 10^{-5}$ M)
(±)-γ-Schizandrin (**1**)	1.9	Wuweizisu C (**2**)	>500
Gomisin A (**3**)	55.2	Gomisin B (**5**)	47.0
Schizandrin (**4**)	>500	Gomisin C (**6**)	451.6
(±)-Gomisin M_1 (**8**)	4.5	Gomisin G (**7**)	340.7
(+)-Gomisin M_2 (**9**)	3.9	Gomisin D (**11**)	>500
Deoxyschizandrin (**10**)	12.8	Gomisin J (**20**)	13.6
Benzoylgomisin H (**12**)	8.7	Gomisin N (**21**)	1.1
(±)-Dibromo-γ-schizandrin (**14**)	0.4	Deangeloylgomisin B (**22**)	66.5
Dibromoschizandrin (**15**)	30.4	Angeloylgomisin Q (**23**)	>500
Dibromogomisin A (**16**)	8.0	Dimethylgomisin J (**24**)	8.3
Gomisin H (**18**)	>500	Schisantherin D (**25**)	366.2
Deoxygomisin A (**19**)	1.4	(−)-Gomisin L_1 Methyl ether (**26**)	1.5
NDGA (**13**)	0.1	Papaverine (**30**)	3.0
Meso-nordihydroguaiaretic acid (**27**)	0.3		
Pregomisin (**17**)	5.3		
NDGA tetramethyl ether (**28**)	5.4		
Machilin A (**29**)	>500		

Structure Activity Relationship In order to investigate the structure activity relationship, these isolated compounds and their derivatives were tested for cAMP PDE inhibitory activity. The results are summarized in Table I. The inhibitory activity of (±)-γ-schizandrin and gomisin N was stronger than deoxyschizandrin and dimethylgomisin J, respectively. This result showed that the presence of a methylenedioxy group at the one side ring of biphenyl is required for inhibitory activity. (±)-γ-Schizandrin and deoxyschizandrin showed higher activity than gomisin A and schizandrin. This fact indicates that the presence of a hydroxyl group at the 7-position weakened the inhibitory activity. Although these lignans have both *R*-type and *S*-type, a difference in activity of the enantiomers was not shown. The medicines having halogen inside a molecule, such as diazepam and hydrochloroquine, were strong inhibitors against cAMP PDE.[11] And we reported that halogenized *p*-benzoquinone and anthraquinone also have high inhibitory activity on cAMP PDE.[8h,i] In order to investigate the effect of halogen against cAMP PDE, three lignans (**1**, **3** and **4**) having different activity were derived from three bromides (**14**, **16** and **15**), respectively. As a result, the inhibitory activity of the lignans was ascended from the bromination. It is clear that bromine is one of the active factors in this skeleton.

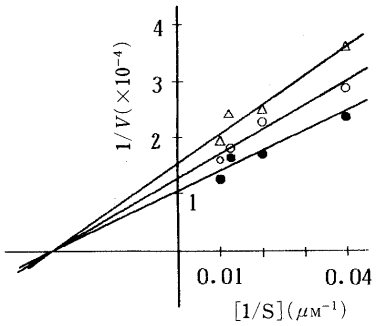


Fig. 1. Double Reciprocal Plots of Gomisin A
●, no inhibitor; ○, 50 μg/ml; △, 100 μg/ml.

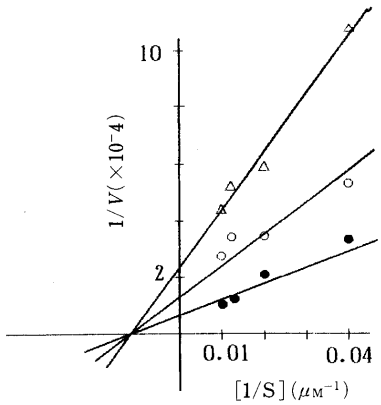


Fig. 2. Double Reciprocal Plots of NDGA
●, no inhibitor; ○, 50 μg/ml; △, 100 μg/ml.

The inhibitory activity of meso-dihydroguaiaretic acid (**27**),[12] which is the dimethyl ether of NDGA, is equal to that of NDGA. Similiary, the tetramethyl ether (**28**) of NDGA showed lower activity than meso-dihydroguaiaretic acid (**27**). But machilin A (**29**), having no hydroxyl group, does not show any inhibitory activity. In this case it is suggested that hydroxyl groups in 3′ and 3″ are an important factor in the inhibitory activity.

The kinetics of the effects of gomisin A on cAMP PDE were also analyzed. Gomisin A showed a non-competitive (K_m=33.3) inhibition pattern in Lineweaver–Burk plots

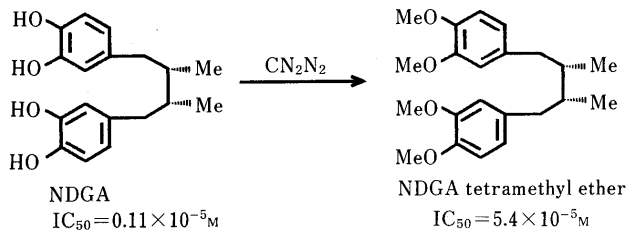


Chart 2. Methylation of NDGA

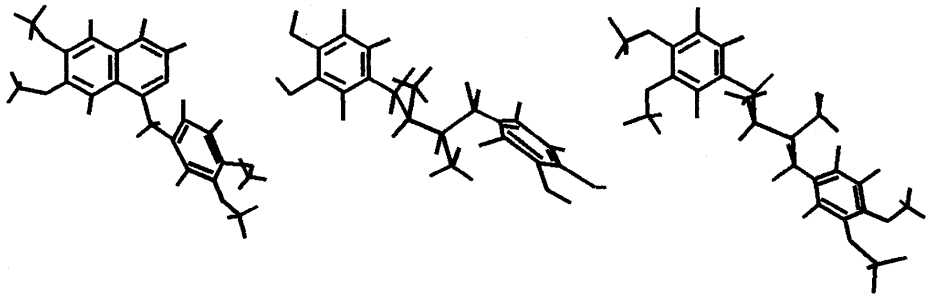


Fig. 3. Low Energy Conformation of Papaverine (Left), NDGA (Center) and NDGA Tetramethyl Ether (Right)

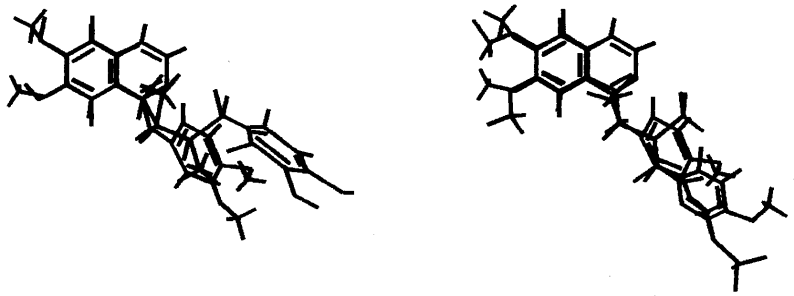
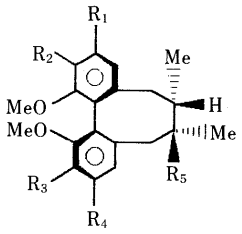
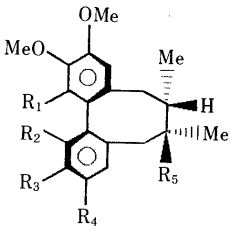


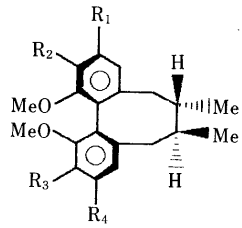
Fig. 4. Superimposition of Papaverine with NDGA (Left) and NDGA Tetramethyl Ether (Right)



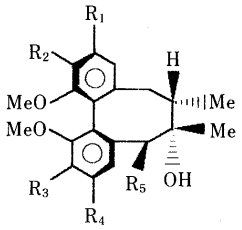
	R_1	R_2	R_3	R_4	R_5
(±)-γ-schizandrin (**1**)	$-OCH_2O-$		OMe	OMe	H
gomisin A (**3**)	$-OCH_2O-$		OMe	OMe	OH
schizandrin (**4**)	OMe	OMe	OMe	OMe	OH
deoxyschizandrin (**10**)	OMe	OMe	OMe	OMe	H
deoxygomisin A (**19**)	$-OCH_2O-$		OMe	OMe	H



	R_1	R_2	R_3	R_4	R_5
(±)-gomisin M_1 (**8**)	OH	OMe	$-OCH_2O-$		H
(+)-gomisin M_2 (**9**)	OMe	OH	$-OCH_2O-$		H
benzoylgomisin H (**12**)	OBz	OMe	OMe	OMe	OH
gomisin H (**18**)	OH	OMe	OMe	OMe	OH

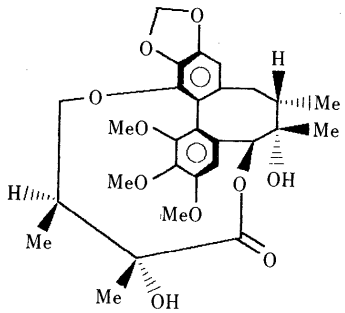


	R_1	R_2	R_3	R_4
wuweizisu C (**2**)	$-OCH_2O-$		$-OCH_2O-$	
gomisin J (**20**)	OH	OMe	OMe	OH
gomisin N (**21**)	$-OCH_2O-$		OMe	OMe
dimethylgomisin J (**24**)	OMe	OMe	OMe	OMe
(−)-gomisin L_1 methyl ether (**26**)	OMe	OMe	$-OCH_2O-$	

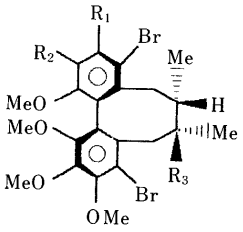


	R_1	R_2	R_3	R_4	R_5
gomisin B (**5**)	$-OCH_2O-$		OMe	OMe	OAng
gomisin C (**6**)	$-OCH_2O-$		OMe	OMe	OBz
gomisin G (**7**)	OMe	OMe	$-OCH_2O-$		OBz
deangeloylgomisin B (**22**)	$-OCH_2O-$		OMe	OMe	OH
angeloylgomisin Q (**23**)	OMe	OMe	OMe	OMe	OAng
schisantherin D (**25**)	$-OCH_2O-$		$-OCH_2O-$		OBz

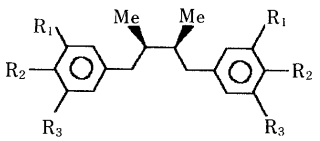
Ang : angeloyl, Bz : benzoyl



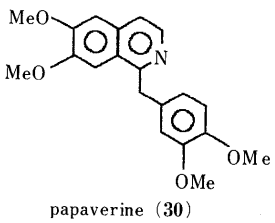
gomisin D (**11**)



	R_1	R_2	R_3
(±)-dibromo-γ-schizandrin (**14**)	$-OCH_2O-$		H
dibromoschizandrin (**15**)	OMe	OMe	OH
dibromogomisin A (**16**)	$-OCH_2O-$		OH



	R_1	R_2	R_3
NDGA (**13**)	OH	OH	H
meso-dihydroguaiaretic acid (**27**)	OH	OMe	H
pregomisin (**17**)	OH	OMe	OMe
NDGA tetramethyl ether (**28**)	OMe	OMe	H
machilin A (**29**)	$-OCH_2O-$		H



papaverine (**30**)

Chart 3

(Fig. 1).

Molecular Modeling We discussed the relationship between the new inhibitor, NDGA, and cAMP PDE. The kinetics of the effects of NDGA on cAMP PDE were analyzed. NDGA showed a non-competitive ($K_m = 90.9$) inhibition pattern in Lineweaver–Burk plots similar to papaverine[4] (Fig. 2). Thereupon, NDGA was treated in three-dimensional modeling by molecular processes; 3D-MOL Ver. 2.5 (Toray system center). This structure was minimized with MM2PP in MM2PPkit (Toray system center) and is shown in Fig. 3.[13] We used papaverine as a positive control for inhibitory activity on cAMP PDE. The minimumized structure of papaverine was calculated using the MM2PP program and is shown in Fig. 3. The comparison of these molecular graphics showed that they had a similar conformation (Fig. 4). The results suggested that the inhibitory activity on cAMP PDE was effected by the conformation of these two compounds. So the four hydroxyl groups of NDGA were exchanged for methoxyl groups using 3D-MOL. The minimumization of the NDGA tetramethyl ether was calculated using the MM2PP program (Fig. 3). The low energy conformation of NDGA tetramethyl ether was more similar to the conformation of papaverine than NDGA (Fig. 4). In fact, the methylation of NDGA was performed using diazomethane, and NDGA tetramethyl ether was synthesized. This compound was assayed, and its IC_{50} is shown in Chart 2. Although the potency of NDGA tetramethyl ether as an inhibitor was lower than NDGA, the IC_{50} of NDGA tetramethyl ether was near papaverine. These facts suggest that papaverine, NDGA and NDGA tetramethyl ether inhibit cAMP PDE by a similar mechanism.

Experimental

Materials and Methods All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. The ultraviolet (UV) spectra were recorded with a Hitachi 340 spectrophotometer and the infrared (IR) spectra with a Hitachi 260-30 unit. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Hitachi R-900 spectrometer or a JEOL GX-400 spectrometer for the 90- and 400-MHz ^{1}H-NMR spectra, respectively, with tetramethylsilane (TMS) as an internal standard: chemical shifts were given on the δ (ppm) scale and signal is quoted as s (singlet), d (doublet), or m (multiplet). The coupling constants are given in Hz. Mass spectra (MS) were measured with a JEOL JMS-D300 mass spectrometer. The specific rotation was measured with a JASCO DIP-4 unit. Column chromatography was carried out on silica gel (Fuji-Davison BW-820 MH) and ion exchange resin (Diaion HP-20, Mitsubishi Chemical Industry Ltd.). TLC and preparative TLC were performed on precoated Silica gel 60F plates (Merck) and Silica gel 60GF (Merck), respectively, and detection was achieved by illumination with a UV lamp or by spraying 10% H_2SO_4 followed by heating.

Assay Method for Inhibition of cAMP PDE cAMP PDE activity was assayed by the method of Thompson and Brooker as modified in the previous paper.[8]

Enzymes and Chemicals Beef heart PDE was purchased from Boehringer. Snake venom nucleotidase, cAMP and NDGA were obtained from Sigma, and [^{3}H]cAMP from Radiochemical Center. Papaverine, a reference inhibitor, was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo).

Extraction and Separation The dried fruit of *Scisandra chinensis* (2 kg, purchased from Uchida Pharmacy for Oriental Medicine, Tokyo) were extracted with hot MeOH (4 l) for 2 h three times. The extract was evaporated to dryness. The residue was suspended in H_2O and extracted with Et_2O, and the Et_2O layer was concentrated to dryness (Et_2O soluble fraction, 76 g). The aqueous layer was extracted with $CHCl_3$, and the $CHCl_3$ layer was concentrated to dryness ($CHCl_3$ soluble fraction, 8.4 g). The aqueous layer was extracted with AcOEt, and the AcOEt fraction was concentrated to dryness (AcOEt soluble fraction, 13.4 g). The aqueous layer was concentrated to dryness (3.1 g). These fractions were tested for their inhibitory effect on cAMP PDE (Chart 1). The Et_2O soluble fraction was most active in these fractions, so this fraction was chromatographed on silica gel with *n*-hexane, benzene, $CHCl_3$ and MeOH as eluents: each eluent was fractionated with monitoring by TLC and inhibitory activity against cAMP PDE. These fractions were further separated by silica gel column chromatography, preparative TLC, MPLC and HPLC. (±)-γ-Schizandrin (**1**), wuweizisu C (**2**), gomisin A (**3**), schizandrin (**4**), gomisin B (**5**), gomisin C (**6**), gomisin G (**7**), (±)-gomisin M_1 (**8**), (+)-gomisin M_2 (**9**), deoxyschizandrin (**10**), gomisin D (**11**), benzoylgomisin H (**12**) and NDGA (**13**) were isolated from these fractions. Compounds **1**, **2**, **3**, **4**, **7**, **8**, **9**, **11** and **12** were identified by IR, UV, ^{1}H-NMR and melting points in comparison with reported data.[9] Compounds **5**, **6**, **10** and **13** were identified by IR, ^{1}H-NMR and melting points in direct comparison with the authentic sample.[9]

(±)-Dibromo-γ-schizandrin (14) Bromine (0.1 ml) was added to a solution of (±)-γ-schizandrin (31.9 mg) in CCl_4 saturated with H_2O and the mixture was stirred at 19 °C for 3 h. After decomposition of excess bromine with 10% $Na_2S_2O_3$, the reaction mixture was diluted with H_2O and then extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried over Na_2SO_4 and concentrated under reduced pressure to give gum, which was purified by MPLC using *n*-hexane–acetone (9 : 1) to give bromide (15.3 mg) as colorless prisms, mp 156 °C, $[\alpha]_D^{24}$ 0° ($c = 0.22$, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 2940, 1612, 1460, 1440, 1406, 1394, 1210, 1094, 1070, 1032, 1005, 960. ^{1}H-NMR (δ in $CDCl_3$): 6.00 (2H, s), 3.92 (6H, s), 3.73 (3H, s), 3.54 (3H, s), 1.03 (3H, d, $J = 6.3$ Hz), 0.83 (3H, d, $J = 6.3$ Hz). MS *m/z* (%): 560 (53), 559 (27), 558 (100), 557 (14), 556 (52).

Dibromoschizandrin (15) Dibromoschizandrin was synthesized from schizandrin (36.7 mg) using the same method as above. The reaction mixture was purified by preparative TLC using $CHCl_3$–acetone (10 : 1) to give bromide (10.7 mg) as colorless plates, mp 109—111 °C, $[\alpha]_D^{24}$ −92.8° ($c = 0.67$, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3520, 2940, 1550, 1460, 1400, 1310, 1100, 1090, 1035. ^{1}H-NMR (δ in $CDCl_3$): 3.94 (6H, s), 3.90, 3.79, 3.50, 3.03 (each 3H, s), 1.14 (3H, s), 0.97 (3H, d, $J = 6.3$ Hz). MS *m/z* (%): 592 (54), 591 (28), 590 (100), 589 (16), 588 (57).

Dibromogomisin A (16) Dibromogomisin A was synthesized from gomisin A (27.7 mg) using the same method as above. The reaction mixture was purified by preparative TLC using $CHCl_3$–acetone (10 : 1) to give bromide (12.3 mg) as colorless plates, mp 182—184 °C, $[\alpha]_D^{24}$ −103.1° ($c = 0.72$, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3518, 2940, 1610, 1460, 1440, 1410, 1270, 1215, 1095, 1080, 1040, 1000, 980, 945. ^{1}H-NMR (δ in $CDCl_3$): 6.07 (2H, s), 3.94, 3.90, 3.78, 3.75 (each 3H, s), 1.12 (3H, s), 1.04 (3H, d, $J = 6.3$ Hz). MS *m/z* (%): 576 (54), 575 (23), 574 (100), 573 (18), 572 (51).

NDGA Tetramethylether (28) An ether–MeOH solution of NDGA (185.2 mg) was treated with diazomethane etherate and the mixture was allowed to stand 3 d. The solvent was evaporated off to give gum (216.9 mg), which was purified by MPLC using $CHCl_3$–acetone (50 : 1) to give NDGA tetramethyl ether (4.5 mg) as colorless plates, mp 88—89 °C, $[\alpha]_D^{22}$ 0° ($c = 0.2$, MeOH). IR ν_{max}^{KBr} cm^{-1}: 2960, 1515, 1468, 1452, 1260, 1240, 1155, 1140, 1027. ^{1}H-NMR (δ in $CDCl_3$): 0.85 (6H, d, $J = 6$ Hz), 1.74 (2H, m), 2.02—2.83 (4H, m), 3.83 (12H, s), 6.70 (6H, m). MS *m/z* (%): 358 (M^+, 60), 151 (100).

Meso-dihydroguaiaretic Acid (27) and Machilin A (29) These authentic samples have been isolated from the bark of *Machilus thunbergii*.[11]

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References and Notes

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