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Obovatol and Obovatal, Novel Biphenyl Ether Lignans from the Leaves of *Magnolia obovata* THUNB.[1)]

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Two new biphenyl ether lignans, named obovatol (**1**) and obovatal (**2**) were isolated from the leaves of *Magnolia obovata* THUNB. (Magnoliaceae) and their chemical structures were determined to be 4′,5-diallyl-2,3-dihydroxybiphenyl ether and 3,4-dihydroxy-5-(*p*-allylphenoxy)cinnamic aldehyde by means of chemical and spectral studies.

The former substance showed antibacterial activity against a cariogenic bacterium, *Streptococcus mutans*, but was less active than magnolol (**5**) and honokiol (**6**).

Keywords——*Magnolia obovata*; Magnoliaceae; lignans; biphenyl ethers; obovatol; obovatal; obovaaldehyde; magnolol; *Streptococcus mutans*; antibacterial activity

Previously, two biphenyl type lignans, magnolol (**5**)[2)] and honokiol (**6**)[3)] were isolated from the bark of *Magnolia obovata* THUNB. (a Japanese crude drug, "Wakoboku"). In the previous paper of our series of studies on this plant,[4)] several alkaloids from the dried leaves were reported. We have now reinvestigated the acidic and neutral components of *M. obovata*, as a part of our continuing studies on a series of magnoliaceous plants.

This paper describes the structure elucidation of two new biphenyl ether lignans, named obovatol (**1**) and obovatal (**2**), and a metabolic oxidative product of **1** or **2** (obovaaldehyde (**3**)), which were isolated from the fresh leaves of this plant.

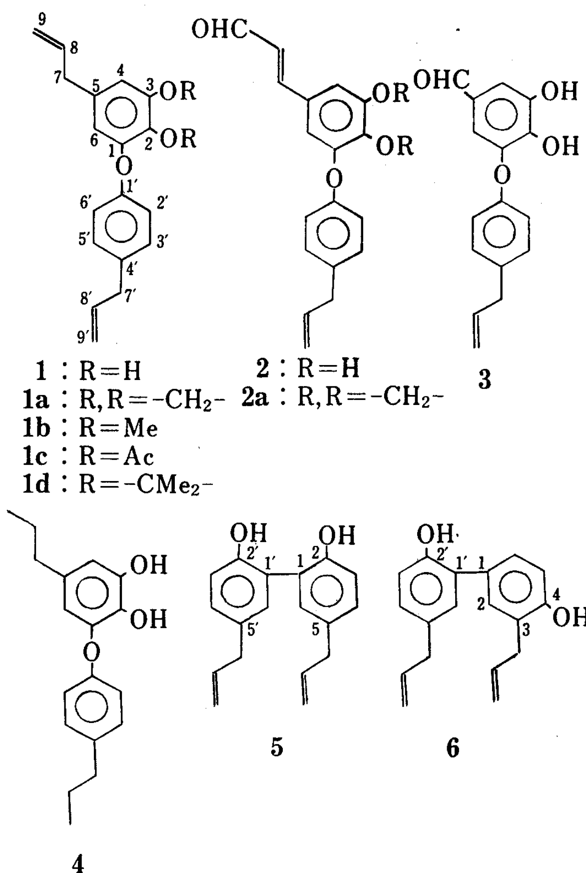

Chart 1

Obovatol (**1**), $C_{18}H_{18}O_3$, a colorless oil and the main constituent of the fresh leaves of *M. obovata*, gave a bluish color with ethanolic ferric chloride and a brown color with titanium trichloride in methanol–pyridine.[5)] These test results suggest that **1** is a phenolic compound with catecholic character.

The infrared absorption (IR) spectrum ($CHCl_3$) of **1** showed the presence of hydroxyl at 3600 cm^{-1}, phenyl at 1610 and 1500 cm^{-1} and vinyl groups at 1645 cm^{-1}. The proton nuclear magnetic resonance (^1H NMR) spectrum (δ in $CDCl_3$) showed the following signals: 3.17, 3.35

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(4H, each d, J=7 Hz, aryl-C$\underline{H}_2$-CH=), 4.9—5.2 (4H, m, two -CH=C$\underline{H}_2$), 5.5 (2H, br s, two OH), 5.66—6.16 (2H, m, two -C$\underline{H}$=CH$_2$), 6.28 (1H, d, J=2 Hz, aromatic H), 6.56 (1H, d, J=2 Hz, aromatic H), 6.9 (2H, d, J=8 Hz, aromatic H), 7.14 (2H, d, J=8 Hz, aromatic H). The spin-spin coupling constants in the aromatic region were due to *meta*- (δ 6.28, 6.56) and *ortho* coupling (δ 6.9, 7.14).

Furthermore, the peaks at m/z: 281 (M^+-1) and 133 (M^+-149) of the mass spectrum (MS) support the presence of the allylphenoxy linkage in **1**.[6]

Acetylation of **1** with acetic anhydride and pyridine afforded a diacetate (**1c**), which showed two acetyl signals at δ 2.14 and 2.24 in its ^{1}H NMR spectrum. On methylenation with dichloromethane and sodium hydroxide in dimethylsulfoxide,[7] **1** gave a methylenedioxy derivative (δ 5.72, 2H, s) (**1a**), and acetonidation of **1** with dimethylacetal and *p*-toluenesulfonic acid in benzene,[8] afforded an acetonide (**1d**).

These results and the ^{13}C NMR spectrum (Table I)[9] indicate that **1** is a biphenyl ether lignan possessing an allyl group and a *p*-allylphenoxy group on the catechol ring.

Table I. ^{13}C NMR Data (ppm) for Obovatol (**1**), Magnolol (**5**) and Honokiol (**6**)[9]

Carbon	1	5	6
1	144.9 s	124.5 s	126.5 s
1′	155.2 s		127.8 s
2	135.0 s	151.0 s	130.3 d
2′	117.8 d		150.7 s
3	144.0 s	116.8 d	128.8 s
3′	129.8 d		116.8 d
4	111.1 d	129.8 d	153.9 s
4′	133.3 s		129.6 d
5	132.5 s	133.3 s	116.5 d
5′	129.8 d		132.4 s
6	111.5 d	131.4 s	128.6 d
6′	117.8 d		131.1 d
7	{39.4 t	39.4 t	{35.0 t
7′	{39.5 t		{39.4 t
8	{137.2 d	137.6 d	{136.1 d
8′	{137.4 d		{137.8 d
9	{115.7 t	115.8 t	{115.6 t
9′	{115.8 t		{115.6 t

Measured in CDCl$_3$ at 25 MHz with TMS as an internal standard.
s, singlet; d, doublet; t, triplet.

The positions of these substituent groups in **1** were identified as mentioned below. On treatment with methyl iodide and potassium carbonate in acetone, **1** was converted to the dimethyl ether (**1b**). The cleavage of **1b** with metallic potassium in liquid ammonia at −70°C, followed by purification by silica gel chromatography afforded the non-phenolic product (**7**) and the phenolic product (**8**).

The non-phenolic product (**7**) was identical with 3,4-dimethoxypropylbenzene, which was obtained by catalytic reduction of *O*-methyleugenol. On the other hand, the phenolic product (**8**) was identical with *p*-allylphenol on the basis of MS and ^{1}H NMR spectral comparisons.

Thus, from the results of potassium cleavage of dimethylobovatol (**1b**), the location of the phenolic hydroxyl groups in obovatol was determined. Therefore, the structure of (**1**) was established to be 4′,5-diallyl-2,3-dihydroxybiphenyl ether. Obovatol (**1**) is the first example of a new type of compounds, which may be termed biphenyl ether lignans, in the magnoliaceous plants.[6]

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The second lignan, obovatal (2), $C_{18}H_{16}O_4$ (M^+ 296), mp 160.5—162°C (acetone), showed in its IR spectrum absorption bands due to hydroxyl group at 3530 cm^{-1}, an α,β-unsaturated aldehyde at 1665 cm^{-1}, olefin at 1630 cm^{-1} and aromatic system groups at 1595, 1500 and 1440 cm^{-1}.

Formation of a methylenedioxy group with CH_2Cl_2 and NaOH showed the presence of an *ortho* diphenolic group in obovatal, as well as in obovatol (**1**).

The ^{1}H NMR spectrum in d_6-acetone, with a doublet at δ 9.49 (1H, d, J=8 Hz), a doublet at δ 7.38 (1H, J=16 Hz) and a double doublet centered at δ 6.42 (1H, J=8, 16 Hz) indicated the presence of a –CH=CH–CH=O moiety. On the other hand, mass and ^{1}H NMR spectral analysis showed that **2** possessed a *p*-allylphenoxy group on the cinnamic aldehyde moiety. In addition, in view of the presence of *meta*- coupling (δ 6.75, 6.98, each d, J=2 Hz) in the ^{1}H NMR spectrum, the structure of **2** was assumed to be 3,4-dihydroxy-5-(*p*-allylphenoxy)cinnamic aldehyde (**2**).

In order to confirm the structure **2** for obovatal, the following experiments were carried out as shown in Chart 2.

Oxidation of **1a** with selenium dioxide in aqueous dioxane at 90°C[10] afforded compound **9** (yield 5%), $C_{19}H_{18}O_4$ (M^+ 310), which showed an absorption band at 3590 cm^{-1} due to the hydroxyl group in the IR spectrum, and a methylene proton signal (δ 4.24, t, J=6 Hz, 2H) due to the allyl-alcohol function in the ^{1}H NMR spectrum. Compound **9** was transformed by oxidation with active manganese dioxide in dichloromethane[11] to **2a** (yield 67%), $C_1{}^9H_{16}O_4$ (M^+ 308), which was identical with the methylenation product derived from **2** on the basis of IR (–CHO at 1670 cm^{-1}) and ^{1}H NMR (δ 9.59, d, J=8 Hz, 1H) spectral comparisons. Thus, the structure of obovatal was elucidated as **2**.

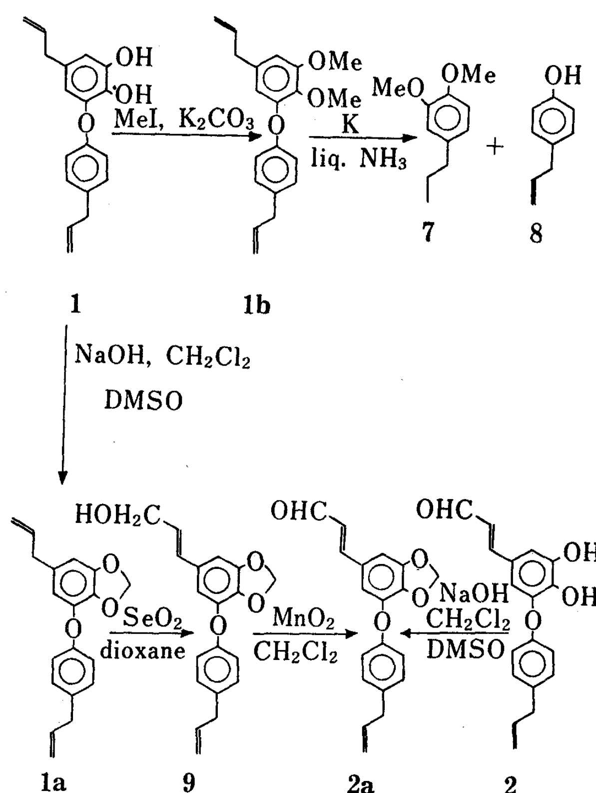

Chart 2

Obovaaldehyde (3) was obtained as colorless prisms (from acetone), $C_{16}H_{14}O_4$ (M^+ 270), mp 162—163°C. The IR spectrum of **3** showed a strong hydroxyl absorption at 3350 cm^{-1} and the band of an aromatic aldehyde group at 1645 cm^{-1}, and the ^{1}H NMR spectrum showed an aromatic aldehyde proton signal at δ 9.68 (1H, s). In addition, **3** showed *ortho*- and *meta*- coupling signals in the aromatic region of its ^{1}H NMR spectrum, and an allylphenoxy fragment in its mass spectrum, as did **1** and **2**.

These spectral data for **3** showed that it possess the same skeleton as **2**, except for the presence of the (Ar)–CH=CH–CH=O moiety. Thus, the structure of **3** was assumed to be 3,4-dihydroxy-5-(*p*-allylphenoxy)benzaldehyde. This compound **3** is an abnormal lignan on the basis of its chemical formula, $C_{16}H_{14}O_4$. It can be inferred that **3** is a metabolic oxidation product of **1** or **2**.

Furthermore, we have undertaken screening tests of obovatol (**1**) and its derivatives (**1b**, **4**) for antibacterial activity against the cariogenic bacterium, *Streptococcus mutans*, as part of a search for caries-preventing agents.[12,13] Table II compares the antibacterial activity of these

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substances against *S. mutans* with those of magnolol and honokiol, as determined by the paper disc method. Magnolol and honokiol showed very strong antibacterial activity against all the serotypes of *S. mutans*, while obovatol (**1**) and tetrahydroobovatol (**4**) had rather strong activity, but dimethylobovatol (**1b**) showed no antibacterial activity.

Table III lists the minimal inhibitory concentrations (MIC) of the antibacterial agents against *S. mutans* MT5091 (serotype c) and OMZ176 (serotype d) as determined by the tube dilution technique. Since types c and d are most frequently detected in Japanese dental caries[14,15] and they differ genetically from each other,[16] the MIC test was carried out by using serotypes c and d of *S. mutans*. Magnolol and honokiol inhibited the bacterial growth at the concentration of 6.25 μg/ml. On the other hand, obovatol and tetrahydroobovatol showed the same growth inhibition (MIC 50 μg/ml) as berberine hydrochloride.

Table II. Comparison of Antibacterial Activities against *S. mutans*

Compound	Serotypes of *S. mutans*						
	a	b	c	d	e	f	g
Obovatol	+++[a]	++	+++	+++	+++	++	+++
Tetrahydroobovatol	++	++	+++	+++	+++	++	+++
Dimethylobovatol	—	—	—	—	—	—	—
Magnolol	++++	++++	++++	++++	++++	++++	++++
Honokiol	++++	++++	+++++	+++++	++++	++++	++++

a) Inhibitory zones were formed by adding 10 μg (+++++), 20 μg (++++), 40 μg (+++), 60 μg (++), and 100 μg (+) per disc.

Table III. Values of Minimal Inhibitory Concentration against *S. mutans* (μg/ml)

Compound	MT5091 c	OMZ176 d
Obovatol	50	50
Tetrahydroobovatol	50	50
Dimethylobovatol	>100	>100
Magnolol	6.25	6.25
Honokiol	6.25	6.25
Berberine hydrochloride	50	50

Obovatol (**1**) is a rare compound bearing a C–O phenol-coupling skeleton, and may be biosynthesized from *p*-allylphenol and 4-allylcatechol. It is very interesting that the C–O phenol-coupling compound obovatol (**1**) was isolated in large amounts from *Magnolia obovata*, although C–C phenol-coupling lignans such as magnolol (**5**) and honokiol (**6**) have been found hitherto in magnoliaceous plants. This may be significant in relation to the chemotaxonomy and biosynthetic activities of magnoliaceous plants.

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus (a hot-stage type) and are uncorrected. The UV spectra were taken with a JASCO UVIDEC-410 digital spectrophotometer and the IR spectra with a JASCO A-3 spectrophotometer. The ^{1}H NMR spectra were recorded with a JEOL PS-100 spectrometer and the ^{13}C NMR spectra with a JEOL FX-100 spectrometer using tetramethylsilane (TMS) as an internal standard. MS were measured with a Hitachi M-52 double-focusing mass spectrometer. Precoated thin layer chromatography (TLC) plates (Kieselgel 60 F_{254}, Kieselgel 60 F_{254} Silanisiert and Alumi-

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niumoxid 60 F_{254} (Type E), Merck) were used for TLC and prep. TLC. The spots were detected under UV light (254 nm). Silica gel (Kieselgel 70—230 mesh, Merck) was used for column chromatography.

Extraction and Separation of Compounds——The MeOH extract of fresh leaves (8.3 kg) of *Magnolia obovata* collected in June 1981 at Takayama, Gifu Prefecture, was divided into *n*-hexane-soluble (1.6 g) and $CHCl_3$-soluble (50 g) fractions. The former fraction gave only an α-amyrin cinnamate (100 mg), and the latter was chromatographed on a silica gel column, using C_6H_6 with gradually increasing proportions of AcOEt as the eluent. Further purification was performed on prep. TLC. The first fraction (C_6H_6) afforded obovatol (**1**, 21.5 g) and magnolol (**5**, 100 mg), and second fraction (C_6H_6–AcOEt 5: 1) gave obovatal (**2**, 3 mg) and obovaaldehyde (**3**, 3 mg).

Obovatol (1)——Viscous oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1645, 1610, 1500. UV λ_{max}^{MeOH} nm: 206, 272. MS *m/z*: 281 (M^+-1, 100%), 149 (49), 133 (10), 117 (56). ^1H NMR ($CDCl_3$) δ: 3.17, 3.35 (4H, each d, $J=7$ Hz, 7,7′-H), 4.9—5.2 (4H, m, 9,9′-H), 5.5 (2H, br s, Ar–OH), 5.66—6.16 (2H, m, 8,8′-H), 6.28 (1H, d, $J=2$ Hz, 6-H), 6.56 (1H, d, $J=2$ Hz, 4-H), 6.9 (2H, d, $J=8$ Hz, 3′,5′-H), 7.14 (2H, d, $J=8$ Hz, 2′,6′-H). ^{13}C NMR: Table I.

Obovatal (2)——Colorless prisms from acetone, mp 160.5—162.0°C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3530, 1665, 1630, 1595, 1500, 1440. UV λ_{max}^{MeOH} nm: 210, 235 (sh), 336. MS *m/z*; 296 (M^+, 32%), 163 (8), 134 (22), 117 (100). ^1H NMR (acetone-d_6) δ: 3.30 (2H, d, $J=7$ Hz, 7′-H), 3.5—4.5 (2H, br s, Ar–OH), 4.88, 5.1 (2H, m, 9-H), 6.42 (1H, dd, $J=8$, 16 Hz, 8-H), 6.75 (1H, d, $J=2$ Hz, 6-H), 6.78 (2H, d, $J=9$ Hz, 3′,5′-H), 6.98 (1H, d, $J=2$ Hz, 4-H), 7.07 (2H, d, $J=9$ Hz, 2′,6′-H), 7.38 (1H, d, $J=16$ Hz, 7-H), 9.49 (1H, d, $J=8$ Hz, 9′-H).

Obovaaldehyde (3)——Colorless prisms from acetone, mp 162—163°C. IR ν_{max}^{KBr} cm^{-1}: 3350, 1645, 1615. UV λ_{max}^{MeOH} nm: 203, 232, 335. MS *m/z*: 272 (M^++2, 43%), 134 (9), 117 (100). ^1H NMR (acetone-d_6) δ: 3.34 (2H, d, $J=6$ Hz, 7-H), 4.9—5.10 (2H, m, 9′-H), 5.8—6.1 (1H, m, 8′-H), 6.85 (2H, d, $J=9$ Hz, 3′,5′-H), 6.96 (1H, d, $J=2$ Hz, 6-H), 7.14 (2H, d, $J=9$ Hz, 2′,6′-H), 7.18 (1H, d, $J=2$ Hz, 4-H), 9.68 (1H, s, 7′-H).

Magnolol (5)——Viscous oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1645, 1610. UV λ_{max}^{MeOH} nm: 213, 290. MS *m/z*: 266 (M^+, 100%), 225, 184, 133. ^1H NMR ($CDCl_3$) δ: 3.34 (4H, d, $J=7$ Hz, 7,7′-H), 4.98 (2H, s, Ar–OH), 5.12 (4H, dd, $J=2$, 4 Hz, 9,9′-H), 5.76—6.16 (2H, m, 8,8′-H), 6.86—7.16 (6H, m, Ar–H). ^{13}C NMR: Table I.

Obovatol Diacetate (1c)——A mixture of obovatol (60 mg), acetic anhydride (1 ml) and pyridine (1 ml) was allowed to stand overnight at room temperature. A few pieces of crushed ice were added and the solution was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water, dried over anhydrous sodium sulfate and concentrated. The residue was purified by silica gel column chromatography using $CHCl_3$ to afford a diacetate (**1c**) (77 mg). Viscous oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1775, 1640, 1610, 1595, 1500. MS *m/z*: 366 (M^+, 5%), 324 (28), 282 (100), 240 (20), 133 (6), 117 (32). ^1H NMR ($CDCl_3$) δ: 2.14, 2.24 (6H, each s, $2\times OCOC\underline{H}_3$), 3.3 (4H, t, $J=7$ Hz, 7,7′-H), 4.92—5.2 (4H, m, 9,9′-H), 5.68—6.20 (2H, m, 8,8′-H), 6.68 (1H, d, $J=2$ Hz, 6-H), 6.80 (1H, d, $J=2$ Hz, 4-H), 6.95 (2H, d, $J=8$ Hz, 3′,5′-H), 7.15 (2H, d, $J=8$ Hz, 2′,6′-H).

Dimethylobovatol (1b)——Methyl iodide (10 ml) and anhydrous potassium carbonate (3 g) were added to a solution of obovatol (720 mg) in abs. acetone (72 ml) and the mixture was refluxed overnight. The reaction mixture was filtered and concentrated. The residue was dissolved in $CHCl_3$, and the solution was washed with water then dried over anhydrous sodium sulfate. Concentration of this solution gave dimethylobovatol (**1b**) (782 mg). Viscous oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1640, 1610, 1590, 1500. MS *m/z*: 310 (M^+, 100%), 295 (34), 254 (100), 177 (58), 133 (33), 117 (73). ^1H NMR ($CDCl_3$) δ: 3.3 (4H, t, $J=7$ Hz, 7,7′-H), 3.8, 3.87 (6H, each s, $2\times OC\underline{H}_3$), 4.96—5.2 (4H, m, 9,9′-H), 5.72—6.19 (2H, m, 8,8′-H), 6.45 (1H, d, $J=2$ Hz, 6-H), 6.57 (1H, d, $J=2$ Hz, 4-H), 6.92 (2H, d, $J=8$ Hz, 3′,5′-H), 7.15 (2H, d, $J=8$ Hz, 2′,6′-H).

Cleavage of Dimethylobovatol (1b) by Metallic Potassium in Liquid Ammonia——A solution of dimethylobovatol (**1b**) (214 mg) in abs. ether (5 ml) was added to a solution of metallic potassium (1 g) in liquid ammonia (50 ml) at −62°C. The mixture was stirred for 1.5 h at −70°C. When the reaction was completed, MeOH and aqueous ether was added and the mixture was extracted with ether. The aqueous layer was acidified with 5% hydrochloric acid and extracted with ether. The combined solution was washed with water, dried over anhydrous sodium sulfate and then concentrated. The residue was purified by column chromatography on silica gel using $CHCl_3$ to afford a non-phenolic product (**7**) (42 mg) and a phenolic product (**8**) (60 mg). **7**: Viscous oil. IR ν_{max}^{film} cm^{-1}: 1610, 1590, 1510. ^1H NMR ($CDCl_3$) δ: 0.94 (3H, t, $J=8$ Hz, 9-H), 1.45—1.8 (2H, m, 8-H), 2.52 (2H, t, $J=8$ Hz, 7-H), 3.86, 3.88 (6H, each s, $2\times$OMe), 6.68—6.86 (3H, m, Ar–H). **8**: Viscous oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3610, 3370 (br), 1640, 1610, 1595, 1510. ^1H NMR ($CDCl_3$) δ: 3.29 (2H, d, $J=7$ Hz, 7′-H), 4.9—5.2 (2H, m, 9′-H), 5.78—6.30 (1H, m, 8′-H), 6.77 (2H, d, $J=8$ Hz, 3′,5′-H), 7.07 (2H, d, $J=8$ Hz, 2′,6′-H).

Treatment of Obovatol (1) with Dimethylacetal——*p*-Toluenesulfonic acid (2 mg) and dimethylacetal (1 ml) were added to a solution of obovatol (50 mg) in benzene (5 ml) and the solution was refluxed for 2 h. The solution was cooled, and washed successively with 5% potassium hydroxide and water. The organic layer was dried over anhydrous sodium sulfate and concentrated. The residue was purified by alumina prep. TLC using *n*-hexane to afford an acetonide (**1d**) (35 mg). Viscous oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1635, 1610, 1505. ^1H NMR ($CDCl_3$) δ: 1.66 (6H, s, $2\times$Me), 3.20, 3.32 (4H, each d, $J=7$ Hz, 7,7′-H), 4.92—5.16 (4H, m, 9,9′-H), 5.68—6.14 (2H, m, 8,8′-H), 6.3 (1H, d, $J=2$ Hz, 6-H), 6.40 (1H, d, $J=2$ Hz, 4-H), 6.90 (2H, d, $J=9$ Hz, 3′,5′-H), 7.10 (2H, d, $J=9$ Hz, 2′,6′-H).

Mtehylenation of Obovatol (1)——NaOH powder (550 mg) and abs. CH_2Cl_2 (22 ml) were added to a solution of obovatol (**1**) (1.13 g) in abs. DMSO (33 ml) and the mixture was heated to 120°C for 2.5 h under a

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nitrogen atmosphere. After being cooled and diluted with water, the reaction mixture was extracted with $CHCl_3$ and the extract was washed with water, then dried over anhydrous sodium sulfate. After removal of the solvent, the residue was purified by column chromatography on silica gel using $CHCl_3$ to afford a methylenedioxy compound (**1a**) (743 mg). Viscous oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1630, 1605, 1500. ^1H NMR (CCl_4) δ: 3.14, 3.22 (4H, each d, $J=7$ Hz, 7,7′-H), 4.81—5.1 (4H, m, 9,9′-H), 5.56—6.00 (2H, m, 8,8′-H), 5.72 (2H, s, $-OC\underline{H}_2O-$), 6.22 (1H, d, $J=2$ Hz, 6-H), 6.30 (1H, d, $J=2$ Hz, 4-H), 6.70 (2H, d, $J=8$ Hz, 3′,5′-H), 6.90 (2H, d, $J=8$ Hz, 2′,6′-H).

Oxidation of 1a with Selenium Dioxide——Powdered selenium dioxide (150 mg) was dissolved in water (1 ml) with heating. Dioxane (9 ml) and **1a** (200 mg) were added all at once to the warm stirred solution. The mixture was quickly heated to 90°C with rapid stirring for 30 min, then cooled. It was filtered, diluted with water, and extracted with $CHCl_3$. The $CHCl_3$ extract was washed first with 10% aqueous sodium hydrogen carbonate then with brine, and dried over anhydrous sodium sulfate. The solution was concentrated, and the residue was purified by prep. TLC to afford the starting material (**1a**) (21 mg) and (**9**) (10 mg). **9**: Viscous oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590, 3430, 1630, 1600, 1500. MS *m/z*: 310 (M^+, 100%), 178 (7), 133 (11), 117 (19). ^1H NMR ($CDCl_3$) δ: 3.31 (2H, d, $J=8$ Hz, 7′-H), 4.24 (2H, t, $J=6$ Hz, 9-H), 4.9—5.16 (2H, m, 9′-H), 5.82 (2H, s, $-OC\underline{H}_2O-$), 6.20 (1H, dt, $J=6$, 16 Hz, 8-H), 6.48 (1H, d, $J=2$ Hz, 6-H), 6.69 (1H, d, $J=2$ Hz, 4-H), 6.87 (2H, d, $J=8$ Hz, 3′,5′-H), 6.96 (1H, d, $J=16$ Hz, 7-H), 7.27 (2H, d, $J=8$ Hz, 2′,6′-H).

Oxidation of 9 with Active Manganese Dioxide——A solution of **9** (3 mg) in CH_2Cl_2 (2 ml) was added to a suspension of active manganese dioxide (7 mg) in CH_2Cl_2 (2 ml) and the mixture was allowed to stand overnight at room temperature. The mixture was then filtered and the filtrate was passed through a short silica gel column and evaporated to dryness to give **2a** (2 mg). The IR and ^1H NMR spectra and TLC behavior of this compound were identical with those of a sample (**2a**) derived from obovatal (**2**).

Methylenation of Obovatal (2)——NaOH powder (1 mg) and abs. CH_2Cl_2 (1 ml) were added to a solution of obovatal (**2**) (3 mg) in abs. DMSO (2 ml), and the mixture was heated to 115°C for 2.5 h under a nitrogen atmosphere. After being cooled and diluted with water, the reaction mixture was extracted with $CHCl_3$ and the $CHCl_3$ extract was washed with water, then dried over anhydrous sodium sulfate. Removal of the solvent gave a residue, which was dissolved in $CHCl_3$. The solution was filtered through a short silica gel column and evaporated to dryness to give **2a** (1.5 mg). Viscous oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1670, 1600, 1500. MS *m/z*: 308 (M^+, 100%), 280 (24), 175 (20), 134 (16), 117 (70). ^1H NMR ($CDCl_3$) δ: 3.38 (2H, d, $J=7$ Hz, 7′-H), 4.94—5.2 (2H, m, 9′-H), 6.06 (2H, s, $-OC\underline{H}_2O-$), 5.84—6.20 (1H, m, 8′-H), 6.49 (1H, dd, $J=8$, 16 Hz, 8-H), 6.75 (1H, d, $J=2$ Hz, 6-H), 6.88 (1H, d, $J=2$ Hz, 4-H), 6.95 (2H, d, $J=9$ Hz, 3′,5′-H), 7.16 (2H, d, $J=9$ Hz, 2′,6′-H), 7.28 (1H, d, $J=16$ Hz, 7-H), 9.59 (1H, d, $J=8$ Hz, 9-H).

Tetrahydroobovatol (4)——A mixture of obovatol (**1**) (1.150 g), 10% palladium-charcoal (100 mg) and MeOH (50 ml) was stirred under a hydrogen atmosphere at room temperature, and allowed to stand overnight. The mixture was then filtered and the filtrate was evaporated to dryness to give tetrahydroobovatol (**4**) (1.156 g). Viscous oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 1600, 1500. ^1H NMR ($CDCl_3$) δ: 0.87, 0.95 (6H, each t, $J=7$ Hz, 9,9′-H), 1.35—1.81 (4H, m, 8,8′-H), 2.38, 2.5 (4H, each t, $J=7$ Hz, 7,7′-H), 5.51, 5.59 (2H, each s, Ar–OH), 6.27 (1H, d, $J=2$ Hz, 6-H), 6.55 (1H, d, $J=2$ Hz, 4-H), 6.89 (2H, d, $J=7$ Hz, 3′,5′-H), 7.11 (2H, d, $J=7$ Hz, 2′,6′-H).

Antibacterial Assay——About 20 ml of BHI agar was overlaid with 0.6 ml of the seed BHI broth (turbidity of the cell suspension: 0.07 OD at 550 nm units/ml) which had been inoculated with precultured *Streptococcus mutans* cells to yield a lawn of growth. Thereafter, filter paper discs (8 mm in diameter, 1.5 mm in thickness, Toyo filter paper discs) containing various amounts of samples were carefully placed on the seeded Petri dishes. Cultivation was then carried out at 37°C until extensive and uniform colony growth was evident (15—18 h). Antibacterial activity was measured in terms of the size of inhibitory zones around the paper discs. The zone diameter was measured directly in four directions using a transparent scale.

Determination of Minimal Inhibitory Concentration (MIC)——A test compound (1 mg) was dissolved in a minimum amount of methanol and a series of diluted solutions was prepared. Next, 0.1 ml of the solution was added to 1.9 ml of sterile liquid BHI broth containing *ca.* 10^6 cells/ml of *S. mutans* in a test tube. The tube was mixed throughly and incubated at 37°C for 48 h. The minimal inhibitory concentration was determined by visually judging the bacterial growth in the series of test tubes.

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