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Lignans of *Trachelospermum asiaticum* var. *intermedium*. I.[1] Isolation and Structures of Arctiin, Matairesinoside and Tracheloside

Isao Inagaki, Sueo Hisada, and Sansei Nishibe

Faculty of Pharmaceutical Sciences, Nagoya City University[2]

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As the components of the stems of *Trachelospermum asiaticum* Nakai var. *intermedium* Nakai, arctiin(I), matairesinoside(VI) and tracheloside(VIII) were isolated. VI was elucidated as 4,4′-dihydroxy-3,3′-dimethoxy-lignan-olid(9,9′)-4′-β-D-glucopyranoside.

In addition the formula of VIII established by T. Takano, *et al.* as $C_{36}H_{50}O_{18}$ was reexamined and revised to $C_{27}H_{34}O_{12}\cdot 1/2H_2O$.

VIII gave trachelogenin(X), $C_{21}H_{24}O_7$, mp 139—141° and D-glucose by acid or emulsin hydrolysis. From chemical and physical data structure of X was proposed as 4′,8′-dihydroxy-3,4,3′-trimethoxy-lignan-olid(9,9′)(Xa) or 4,8′-dihydroxy-3,3′,4′-trimethoxy-lignan-olid(9,9′)(Xb). In this paper structure of methyltrachelogenin(XI) with absolute configuration was determined to be 8(*S*),8′(*S*)-8′-dihydroxy-3,4,3′,4′-tetramethoxy-lignan-olid(9,9′).

Now we suggest two possible structures for VIII; 8(*S*),8′(*S*)-4′,8′-dihydroxy-3,4,3′-trimethoxy-lignan-olid(9,9′)-4′-β-D-glucopyranoside(VIIIa) or 8(*S*),8′(*S*)-4,8′-dihydroxy-3,3′,4′-trimethoxy-lignan-olid(9,9′)-4-β-D-glucopyranoside(VIIIb).

The dried leaves and stems of *Trachelospermum asiaticum* Nakai var. *intermedium* Nakai (Teikakazura in Japanese) are one of chinese crude drug called "lo shin" and the usages for the edema, wound and low back pain *etc.* are written in literatures.[3]

As one of components of stems, the isolation of tracheloside and its structural elucidation as $C_{36}H_{50}O_{18}$, VIIIc, were already reported by T. Takano, *et al.*[4] VIIIc has a unique structure as a natural product, which gave us the interest to investigate the presence of the related components.

From the preliminaly investigation of methanol extract of the stems by thin-layer chromatography (TLC), the presence of several components was found. The separation of components was treated as described in experimental section. TLC of both chloroform and ethyl acetate extracts respectively revealed similar four spots (*Rf* values of 0.54, 0.45, 0.37, and 0.28).

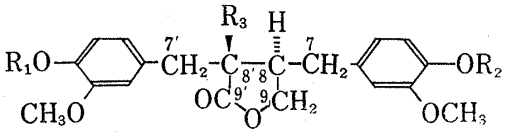
The chloroform extract was column chromatographed on silica gel and eluted by chloroform–ethanol (4: 1). Two components were separated. One of them (*Rf* 0.54) was recrystallized from ethyl acetate containing a small amount of water to give colorless crystalline powder (I), $C_{27}H_{34}O_{11}\cdot H_2O$, mp 110—112°, $[\alpha]_D^{27}$ −51.5 (ethanol). Infrared (IR) absorption of I at 1780 cm^{-1} suggested the presence of γ-lactone. The treatment of I with acetic anhydride in pyridine gave colorless powder (II), $C_{35}H_{42}O_{15}$, mp 66.5—68.5°, $[\alpha]_D^8$ −38.0 (ethanol). In the nuclear magnetic resonance (NMR) spectrum II displayed signals from three aromatic methoxyls (δ 3.75, 3.80, and 3.85, three singlets) and four aliphatic acetyls

1) A portion of this work was reported as preliminary communications: *a*) I. Inagaki, S. Hisada, and S. Nishibe, *Chem. Pharm. Bull.* (Tokyo), **16**, 2307 (1968); *b*) I. Inagaki, S. Hisada, and S. Nishibe, *Phytochemistry*, **10**, 211 (1971).

2) Location: *Tanabe-dori, Mizuho-ku, Nagoya, 467, Japan.*

3) T. Kariyone and K. Kimura (ed.), "Yakuyoshokubutu Daijiten," Hirokawa Publishing Co., Tokyo, 1963, p. 229; K. Akamatu, "Wakanyaku," Ishiyaku Publishing Co., 1970, p. 148.

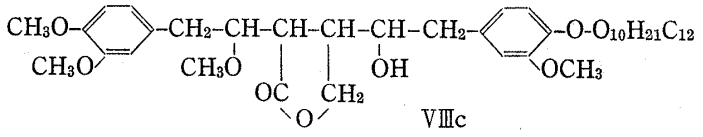
4) M. Miyazaki, H. Watanabe, and T. Takano, *Yakugaku Zasshi*, **78**, 879, 882, 885 (1958); T. Takano, *ibid.*, **79**, 443, 447, 1449 (1959).



I: R_1=glucosyl, R_2=CH_3, R_3=H
III: R_1=H, R_2=CH_3, R_3=H
IV: R_1=CH_3, R_2=CH_3, R_3=H
V: R_1=C_2H_5, R_2=CH_3, R_3=H
VI: R_1=glucosyl, R_2=H, R_3=H
VII: R_1=H, R_2=H, R_3=H

VIIIa : R_1=glucosyl, R_2=CH_3, R_3=OH
VIIIb : R_1=CH_3, R_2=glucosyl, R_3=OH
Xa : R_1=H, R_2=CH_3, R_3=OH
Xb : R_1=CH_3, R_2=H, R_3=OH
XI : R_1=CH_3, R_2=CH_3, R_3=OH
XIIa : R_1=C_2H_5, R_2=CH_3, R_3=OH
XIIb : R_1=CH_3, R_2=C_2H_5, R_3=OH

Chart 1



VIIIc

Chart 2

(δ 2.03 and 2.07, two singlets). Acid hydrolysis of I gave aglycone (III), $C_{21}H_{24}O_6$, mp 100—101°, $[\alpha]_D^{24}$ —37.7 (ethanol) and D-glucose. The properties of III agreed well to that of arctigenin (4′-hydroxy-3,4,3′-trimethoxy-lignan-olid(9,9′)[5]) in literature.[6]

Concequently I was presumed to be arctiin (4′-hydroxy-3,4,3′-trimethoxy-lignan-olid (9,9′)-4′-β-D-glucopyranoside), which was identified with an authentic sample, $C_{27}H_{34}O_{11}\cdot 1.5\ H_2O$, mp 105—106.5°, $[\alpha]_D^{27}$ —52.2 (ethanol), isolated from the seeds of *Arctium lappa* L. (compositae) after the procedure of Omaki.[6]

Another (*Rf* 0.37) was recrystallized from ethyl acetate to amorphous colorless powder (VI), $C_{26}H_{32}O_{11}\cdot H_2O$, mp 93°, $[\alpha]_D^{12}$ —46.0 (ethanol). The IR spectrum of VI resembled that of I, indicative of a closely related lignan glycoside. The ultraviolet (UV) spectrum of VI showed absorption maxima at 229 and 281 nm, giving a bathochromic shift with sodium ethoxide, which revealed the presence of free phenolic hydroxyl group. Acid hydrolysis of VI afforded aglycone (VII), $C_{20}H_{22}O_6$, mp 117—119°, $[\alpha]_D^{8}$ —40.0 (ethanol) and D-glucose. In the NMR spectrum, VII was suggested to be matairesinol and identified with an authentic sample. The methylation of VI with excess diazomethane gave I. Therefore VI was elucidated as 4,4′-dihydroxy-3,3′-dimethoxy-lignan-olid(9,9′)-4′-β-D-glucopyranoside (matairesinol-4′-β-D-glucoside) and named matairesinoside.[7]

The ethyl acetate extract was column chromatographed on silica gel in a similar manner as used to the chloroform extract.

Two components were separated. One of them (*Rf* 0.54) was identical to I and another (*Rf* 0.45) was recrystallized from ethanol to give colorless grains (VIII), mp 168—170°, $[\alpha]_D^{20}$ —60.0 (ethanol). The component of showing *Rf* 0.28 spot on TLC could not be separated as crystalls.

The IR spectrum of VIII showed absorption bands at 3450 (hydroxyl), 1770 (γ-lactone C=O), 1610, 1590, 1515 (aromatic) cm^{-1} and the UV spectrum showed absorption maxima at 230 and 280 nm.

These data indicated for VIII to be tracheloside,[4] which was identified with Takano's sample. Here we had doubt to the structure of tracheloside elucidated as VIIIc. For the isolated I and VI are the normal lignan components consisting of phenylpropane units, but

5) In preliminary communication,[1] a different numbering system was employed. In this paper the nomenclature of Freudenberg and Weinges was used.
6) T. Omaki, *Yakugaku Zasshi*, **55**, 816 (1935); *idem*, *ibid.*, **56**, 982, 985 (1936).
7) Authors presented the structure independently at the 89th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1969. At similar time the isolation of same glucoside from *Forsythia* species was reported by H. Thieme and H.J. Winkler (*Pharmazie*, **23**, 519 (1968)).

VIIIc is a sole unusual lignan-like component consisting of phenylbutane units. From the biogenetic point of view, tracheloside also might be one of series of normal lignan components.

Our analytical data of VIII was satisfactory for $C_{27}H_{34}O_{12}\cdot 1/2H_2O$. The treatment of VIII with acetic anhydride in pyridine gave colorless needle (IX), $C_{35}H_{42}O_{16}\cdot H_2O$, mp 98—102°, $[\alpha]_D^{20}$ −48.8 (ethanol). The molecular weight determination of IX by vapor pressure osmometry also agreed with that of the formula. The NMR spectrum of IX showed signals at δ 3.77, 3.85 (two singlets) due to three aromatic methoxyls and δ 2.02, 2.05 (two singlets) due to four aliphatic acetyls.

Acid or emulsin hydrolysis of VIII gave trachelogenin (X), $C_{21}H_{24}O_7$, mp 139—141°, $[\alpha]_D^{23}$ −43.3 (ethanol) and D-glucose. The shift of absorption maximum with sodium ethoxide in UV spectrum of X was similar to that of III. The methylation of X with excess diazomethane gave methyltrachelogenin (XI),[8] $C_{22}H_{26}O_7$, mp 97—98.5°, $[\alpha]_D^{28}$ −45.9 (ethanol) and alkaline permanganate oxidation of XI afforded 3,4-dimethoxybenzoic acid only. The ethylation of X with excess diazoethane yielded an amorphous mono-ethylate (XII), which on treatment with 1N NaOH afforded a hydroxy-acid, $C_{23}H_{30}O_8$, mp 114—115°. The gas-liquid chromatogram of the alkaline permanganate oxidation products of XII as the methyl ester showed the presence of both 3,4-dimethoxybenzoic acid and 3-methoxy-4-ethoxybenzoic acid in almost equal molar ratio. The fact was confirmed by the comparison with the chromatogram of oxidation products of arctigenin mono-ethylate (V).

The IR spectrum of XI indicated the presence of hydroxyl group and the absorption maxima at 231 and 281 nm in UV spectrum were not affected with sodium ethoxide. As the results, the presence of alcoholic hydroxyl group in XI was suggested. The hydroxyl group, resisted to usual acetylation, was presumed to be tertiary and attached to C-8 or C-8′. Further evidence of the absence of hydroxyl group attached to the benzylic carbons was provided by the lack of a color reaction with N-chloro-*p*-benzoquinoneimide.[9]

The position of tertiary hydroxyl group was suggested from the NMR spectral discussion. Burden[10] and Corrie,[11] *et al.* reported that J_{AB} for lactone methylene, *ca.* 8—9 cps, is approximately equal to $J_{8,9}(cis)$ and $J_{8,9}(trans)$, so the methylene signals appear as two triplets whose separation is markedly solvent-dependent. According to Corrie, *et al.*[11] the methylene protons at C-9 of lignans having no tertiary hydroxyl group (in $CDCl_3$) appear as multiplet at about δ 4.0 as an AB portion of an ABX pattern. According to Maclean, *et al.*,[12] the methylene protons of lignans having tertiary hydroxyl group at C-8′ (in $CDCl_3$) appear as a doublet centered near δ 4.0 with unresolved splitting indicative of the AB portion of an ABX pattern and the methylene protons of lignan having tertiary hydroxyl group at C-8 (in $CDCl_3$) appear as AB quartet at about δ_A 3.7 and δ_B 4.3 with J_{AB} value of 9 cps.

In the NMR spectra of X and XI both methylene protons appeared as doublet signals at δ 4.08 with separation of 6.0 cps. The methylene protons of VII, IV, and III, on the other hand, gave a multiplet in the range δ 4.0—4.4, 4.1—4.4, and 3.9—4.4, respectively. The methylene protons of hydroxythujaplicatin methyl ether, di-O-methylhydroxythujaplicatin methyl ether (XVI) and alcohol of helianthoidin, all having a hydroxyl group attached to C-8′, appeared as doublet signals at δ 4.05, 4.07, and 4.02 with a separation of 6.0 cps, respectively, in $CDCl_3$. The NMR spectra of X and XI thus clearly supported a hydroxyl group attached

8) Although we have not carried out the direct comparison of X with Takano's trachelogenin, it is clear that X is the same with Takano's trachelogenin from the properties described in literature.
On the other hand, sample and IR chart which were sent as methyltrachelogenin from Dr. T. Takano were those of dimethylmatairesinol. But here in order to avoid unnessary confusion we should like to use original names of "trachelogenin" and "methyltrachelogenin", respectively.

9) J. Gierer, *Acta Chem. Scand.*, **8**, 1319 (1954).

10) R.S. Burden, L. Crombie, and D.A. Whiting, *J. Chem. Soc.*, **1969**, 693.

11) J.E.T. Corrie, G.H. Green, E. Ritchie, and W.C. Taylor, *Aust. J. Chem.*, **23**, 133 (1970).

12) H. Maclean and K. Murakami, *Can. J. Chem.*, **44**, 1827 (1966).

to C-8′. As the results the possible structures for both X and XII were proposed as follows; 4′,8′-dihydroxy-3,4,3′-trimethoxy-lignan-olid(9,9′) (Xa) or 4,8′-dihydroxy-3,3′,4′-trimethoxy-lignan-olid(9,9′) (Xb) and 8′-hydroxy-4′-ethoxy-3,4,3′-trimethoxy-lignan-olid(9,9′) (XIIa) or 8′-hydroxy-4-ethoxy-3,3′,4′-trimethoxy-lignan-olid(9,9′) (XIIb), respectively. Further structure of XI was determined as 8′-hydroxy-3,4,3′,4′-tetramethoxy-lignan-olid(9,9′).

In regards to the absolute configurations of C-8 and C-8′, XI showed closely similar circular dichroism (CD) curve to IV of well established configuration.[13] So it was suggested that XI has the same configuration with IV.

In order to establish chemically the absolute configuration, the reduction of XI to triol (XIV) was tried. In this procedure it was found the reduction of XI with lithium aluminum hydride gave hemiacetal (XIII), $C_{22}H_{28}O_7$, mp 155—156°, when tetrahydrofuran[14] treated only with sodium metal was used as solvent. The NMR spectrum of XIII are as shown in Fig. 2.

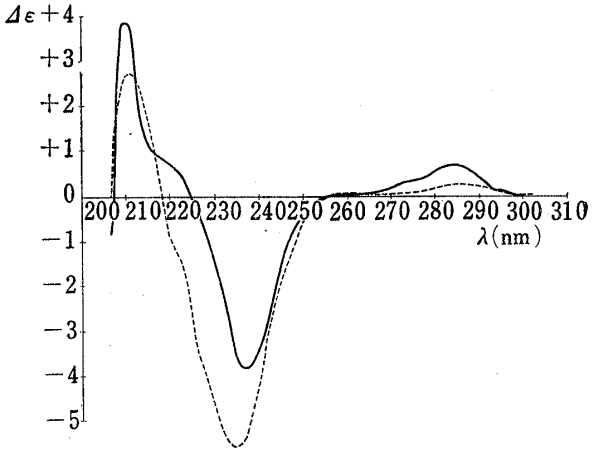


Fig. 1. Circular Dichroism Curves of Methyltrachelogenin (XI) (———) and Dimethylmatairesinol (IV) (-------) in Ethanol

The signals assigned to C-9′ protons were observed at δ 5.00 and 5.30 as separated two singlet signals. The similar signals were observed in hemiacetal (XVII) prepared from XVI of established structure. As the lignan having no tertiary hydroxyl group at C-8′ IV was treated in the same manner as for XI to give hemiacetal (XVIII), $C_{22}H_{28}O_6$, mp 140—141.5°, $[\alpha]_D^{27}$ −23.5 (ethanol), prepared for the first time, and diol (XIX)[15] $C_{22}H_{30}O_6$, mp 123—124°, $[\alpha]_D^{15}$ −31.4 (chloroform). The NMR spectrum of XVIII is as shown in Fig. 3. The signal assigned to C-9′ protons was observed at δ 5.20—5.45 as broad signal. The NMR spectrum

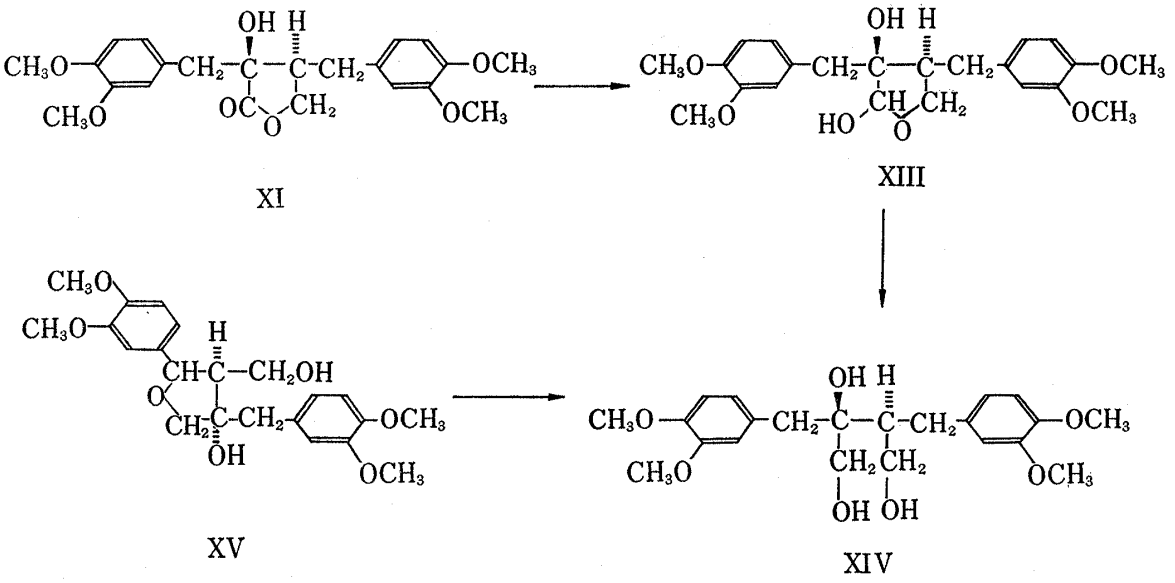


Chart 3

13) R.D. Haworth and D. Woodcock, *J. Chem. Soc.*, **1939**, 154; *idem*, *ibid.*, **1939**, 1054.
14) Tetrahydrofuran as solvent for reduction is usually purified by refluxing for several hours with, and distilling from, potassium hydroxide, then sodium, and finally lithium aluminum hydride.
15) This diol was identified with an authentic sample of *seco*-isolariciresinol.

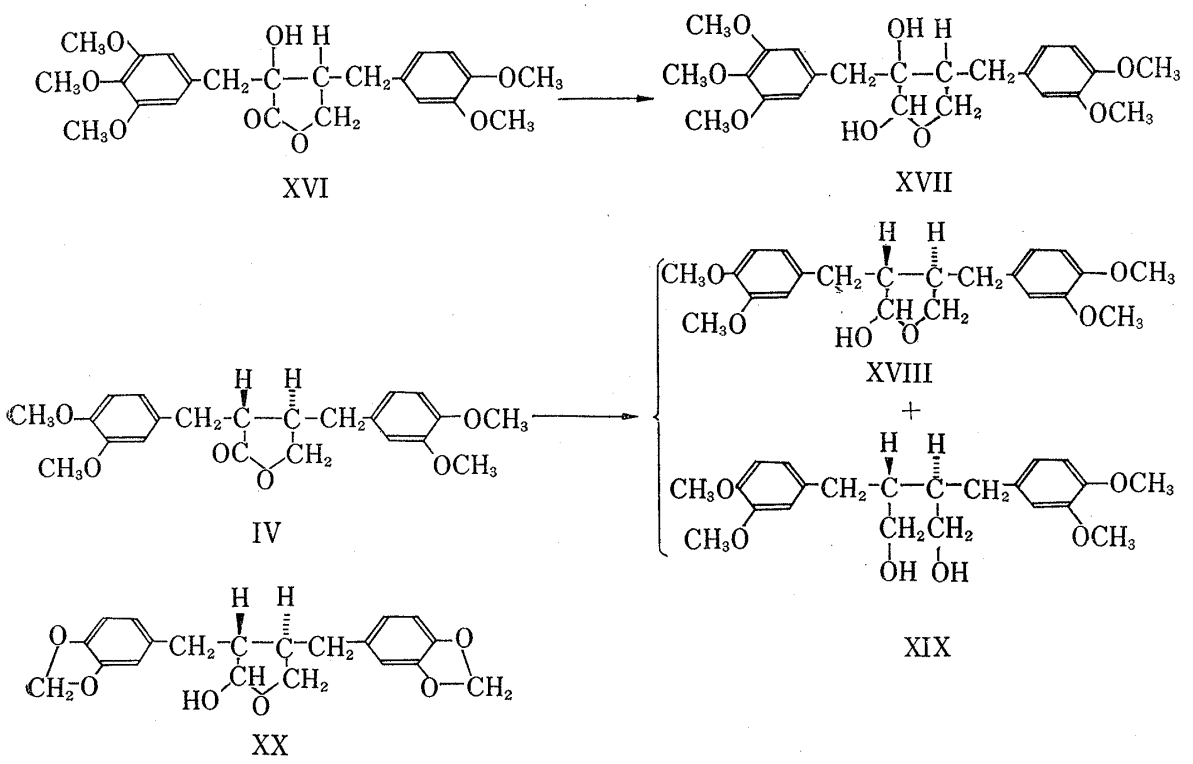


Chart 4

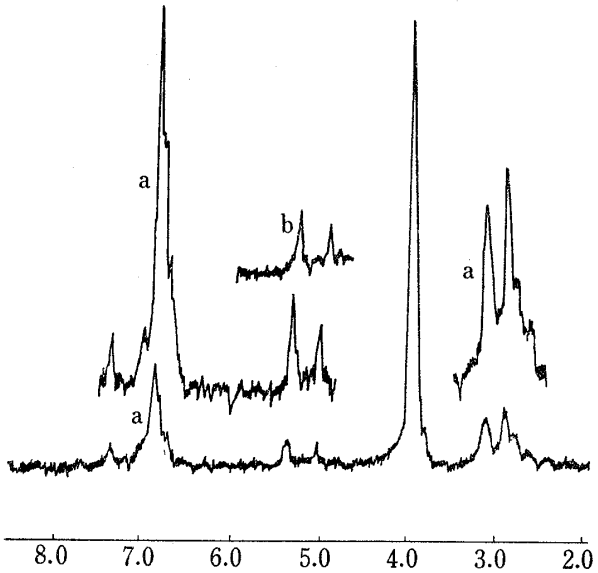


Fig. 2. Nuclear Magnetic Resonance Spectra of XIII and XVII in $CDCl_3$

a: hemiacetal (XIII) b: hemiacetal (XVII)

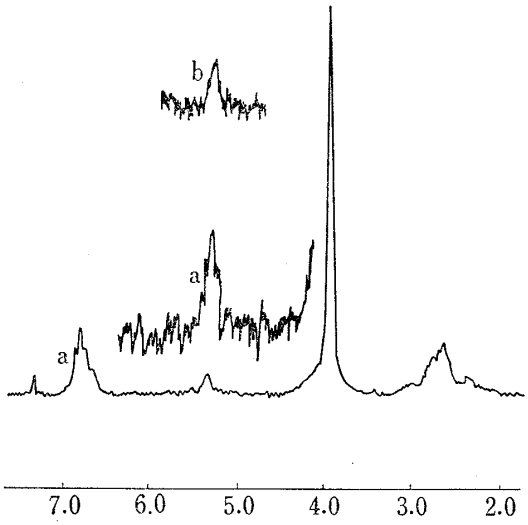


Fig. 3. Nuclear Magnetic Resonance Spectra of XVIII and XX in $CDCl_3$

a: hemiacetal (XVIII) b: cubebin (XX)

of cubebin (XX), a sole naturally occurring of hemiacetal, also showed a similar signal due to C-9′ protons as shown in Fig. 3. The NMR spectra suggested that lignans having hydroxyl group at C-8′ gave the mixture of diastereoisomer at C-9′. The fact was confirmed from that XIII showed one spot on TLC using chloroform–ethyl acetate (1:1) as solvent but showed two spots (*Rf* values of 3.4 and 4.0) on TLC using toluene–ethyl formate–formic acid (30:18:2) as solvent. Both of diastereoisomers give the same triol with the complete reduction. So here XIII without further separation was treated with lithium aluminum hydride in absolute ether to give triol (XIV), $C_{22}H_{30}O_7$, mp 132—133°, $[\alpha]_D^{18}$ −23.0 (chloroform), which

was identical to tetrahydrogmelinol[16] of established configuration, $C_{22}H_{30}O_7$, mp 132—133°, $[\alpha]_D^{15}$ −21.9 (chloroform), prepared from dimethylolivil (XV) by the metal-ammonia reduction according to the method as described by Birch, *et al.*[16] Therefore 8(*S*), 8′(*S*)-configuration has been determined for XI.

We now suggest two possible structures for VIII; 8(*S*),8′(*S*)-4′,8′-dihydroxy-3,4,3′-trimethoxy-lignan-olid(9,9′)-4′-*β*-D-glucopyranoside (VIIIa) or 8(*S*),8′(*S*)-4,8′-dihydroxy-3,3′,4′-trimethoxy-lignan-olid(9,9′)-4-*β*-D-glucopyranoside (VIIIb).

The establishment of structure of VIII will be reported in the following paper.

Experimental

All melting points were not corrected. The following equipment was used: IR spectra, Infrared Spectrophotometer IR-S and IR-E (JASCO); UV spectra, Hitachi Recording Spectrophotometer Model EPS-3T; CD curve, Spectrophotometer Model J-20 (JASCO); NMR spectra, JNM-MH-60 (JEOL) with tetramethylsilane (δ=O) as internal standard; Optical Rotation values, Direct Reading Polarimeter Model OR-10 (Yanagimoto); Molecular weight, Hitachi Perkin-Elmer 115 molecular weight apparatus with benzil as reference compound; Mass spectra, Hitachi Mass Spectrometer Model RMU-6C and JMS-01SG (JEOL); Gas-liquid chromatography (GLC), JGC-1100 (JEOL) with flame ionization detector.

The TLC values were obtained with Kieselgur G nach Stahl (Merck) as adsorbent; the spots were detected by spraying with 10% sulfuric acid and heating. For paper chromatography (PC) Toyo Roshi No. 51 (2 cm × 40 cm) was used. For column chromatography silica gel (100 mesh, Mallinckrodt) was used.

The abbreviation used are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; br. s, broad singlet; sh, shoulder.

Isolation of Arctiin(I), Matairesinoside(VI), and Tracheloside(VIII)——The air-dried and cut stems (8.5 kg) collected in April 1968 at Kushimoto were extracted four times with 20 liter each of hot MeOH. MeOH was evaporated to small volume under reduced pressure, diluted with water and filtered. The filtrate was extracted successively with petr. ether, ehter and $CHCl_3$. The $CHCl_3$ layer was evaporated to dryness. The aqueous layer was concentrated to syrup, which was extracted with hot AcOEt. The AcOEt layer was evaporated to dryness. The $CHCl_3$ extract (9.4 g) was column chromatographed and eluted by $CHCl_3$–EtOH (4: 1). Fractions (50 ml each) were monitored by TLC using $CHCl_3$–EtOH (4: 1) as a developer. The *Rf* 0.54 fraction was evaporated and recrystallized from AcOEt containing a small amount of water to give I (450 mg). The *Rf* 0.37 fraction was recrystallized from AcOEt to give VI (181 mg). The AcOEt extract (7.3 g) was column chromatographed and eluted by $CHCl_3$–EtOH (4: 1). Fractions (50 ml each) were monitored by TLC using $CHCl_3$–EtOH (4: 1) as developer. I (39 mg) was obtained from *Rf* 0.54 fraction. The *Rf* 0.45 fraction was evaporated and recrystallized from EtOH to give VIII (270 mg).

Properties of Arctiin(I)——Colorless crystalline powder, mp 110—112°. TLC *Rf*: 0.54 ($CHCl_3$–EtOH=4: 1). $[\alpha]_D^{27}$ −51.5 (c=2.0 in EtOH). UV λ_{max}^{EtOH} nm(log ε): 230 (4.26), 280 (3.63). IR ν_{max}^{KBr} cm^{-1}: 3280—3480 (OH), 1780 (γ-lactone C=O), 1593, 1510 (aromatic). *Anal.* Calcd. for $C_{27}H_{34}O_{11}\cdot H_2O$: C, 58.69; H, 6.57. Found: C, 58.63; H, 6.55.

Acetate of Arctiin(I)——I (100 mg) was dissolved in pyridine (0.2 ml) and acetic anhydride (0.2 ml) and left standing overnight at room temperature. The reaction product was added with stirring to ice water and extracted with ether. The ether solution was washed with water, dried and evaporated to dryness. The residue was column chromatographed with $CHCl_3$–AcOEt (1: 1) as eluent and recrystallized from dil. MeOH to give acetate(II) (73 mg) as colorless powder, mp 66.5—68.5°. $[\alpha]_D^8$ −38.0 (c=0.5 in EtOH). UV λ_{max}^{EtOH} nm(log ε): 228 (4.19), 280 (3.75). IR ν_{max}^{KBr} cm^{-1}: 1760 (C=O), 1595, 1515 (aromatic). *Anal.* Calcd. for $C_{35}H_{42}O_{15}$: C, 59.82; H, 6.03. Found: C, 60.12; H, 6.35. NMR ($CDCl_3$) δ: 6.36—6.96 (6H, m, arom. H), 4.85—5.31 (4H, m), 4.16 (3H, br. s), 3.86—4.08 (2H, m, C-9), 3.75, 3.80, 3.85 (9H, s, methoxyl), 2.80—3.00 (2H, br, C-8,8′), 2.43—2.66 (4H, br, C-7,7′), 2.03, 2.07 (12H, each s, acetyl).

Hydrolysis of Arctiin (I)——The solution of I (100 mg) in 10% H_2SO_4 was heated on a water bath for 2 hr. The oily product separated was extracted with $CHCl_3$. The $CHCl_3$ solution was washed with water, dried and evaporated to dryness. The residue was column chromatographed with $CHCl_3$–AcOEt (4: 1) as eluent. Recrystallization from MeOH-ether gave aglycone (III) as colorless prisms, mp 100—101°, yielding 36 mg. $[\alpha]_D^{24}$ −37.7 (c=1.03 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 231 (4.17), 282 (3.75). UV $\lambda_{max}^{EtOH+NaOH}$ nm (log ε): 253 (4.09), 288 (3.88), 299 (3.82). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1770 (γ-lactone C=O), 1605, 1590, 1515 (aromatic). *Anal.* Calcd. for $C_{21}H_{24}O_6$: C, 67.73; H, 6.50. Found: C, 67.79; H, 6.47. NMR (in $CDCl_3$) δ: 6.50—6.92 (6H, m, arom. H), 5.67 (1H, br. s, OH, quenched by deuterium exchange), 3.95—4.40 (2H, m, C-9), 3.87 (9H, s, methoxyl), 2.85—3.15 (2H, br. C-8,8′), 2.58 (4H, br. s, C-7,7′). Mass Spectrum *m/e*: 372 (M^+).

16) A.J. Birch and M. Smith, *J. Chem. Soc.*, **1964**, 2705.

After removal of the aglycone, mother liquor was neutralized with barium carbonate and evaporated to dryness. The PC of this residue (solvent: BuOH–AcOH–H_2O=4: 1: 1, color reagent: aniline hydrogen phthalate) showed the presence of D-glucose only.

Methylate of Aglycone (III)——III (70 mg) in MeOH was methylated with excess diazomethane as usual and the crude product was column chromatographed with $CHCl_3$–AcOEt (4: 1) as eluent. Recrystallization from MeOH gave the methylate (IV) (54 mg) as colorless needles, mp 126—127°. $[\alpha]_D^{24}$ —31.7 (c=1.07 in $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 231 (4.14), 281 (3.70). IR ν_{max}^{KBr} cm^{-1}: 1765 (γ-lactone C=O), 1605, 1590, 1515 (aromatic). *Anal.* Calcd. for $C_{22}H_{26}O_6$: C, 68.38; H, 6.78. Found: C, 68.13; H, 6.88. NMR (in $CDCl_3$) δ: 6.50—6.97 (6H, m, arom. H), 4.1—4.4 (2H, m, C–9), 3.99 (12H, s, methoxyl), 2.93—3.20 (2H, br. C–8,8′). 2.65 (4H, br, s, C–7,7′). Mass Spectrum *m/e*: 386 (M^+).

IV was identified with an authentic sample of dimethylmatairesinol by mixed melting point and IR spectral comparison.

Ethylate of Aglycone (III)——III (50 mg) in EtOH was ethylated with diazoethane. The treatment of product in the same manner as used to the above methylation afforded an amorphous ethylate (V) (36.5 mg). IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 1770 (γ-lactone C=O), 1590, 1510 (aromatic). NMR (in $CDCl_3$) δ: 6.40—6.90 (6H, m, arom. H), 4.1—4.4 (2H, m, C–9), 4.08 (2H, q, J=8 cps, ethoxyl-C$\underline{H}_2$-), 3.91 (9H, s, methoxyl), 2.80—3.17 (2H, br, C–8,8′), 2.63 (4H, br. s, C-7,7′), 1.52 (3H, t, J=8 cps, ethoxyl-C$\underline{H}_3$). Mass Spectrum *m/e*: 400 (M^+).

The solution of V (30 mg) in 1N NaOH (5 ml) was heated on water bath for the some time. The solution was neutralized with 10% H_2SO_4 and extracted with ether. The crude hydroxy-acid was recrystallized from ether to give colorless grains, mp 121—121.5°. UV λ_{max}^{EtOH} nm (log ε): 230.5 (4.24), 281 (3.79). IR ν_{max}^{KBr} cm^{-1}: 3360 (OH), 1685 (C=O), 1590, 1515 (aromatic). *Anal.* Calcd. for $C_{23}H_{30}O_7$: C, 66.01; H, 7.23. Found: C, 65.78; H, 7.21.

Isolation of Arctiin from *Arctium lappa* L.——Arctiin was isolated from the seeds (500 g) of *Arctium lappa* L. (Compositae) after the procedure of Omaki.[6] Colorless crystalline powder, mp 105—106.5°. TLC *Rf*: 0.54 ($CHCl_3$–EtOH=4: 1). $[\alpha]_D^{27}$ —52.2 (c=0.452 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 230 (4.29), 280 (3.63). IR ν_{max}^{KBr} cm^{-1}: 3480—3280 (OH), 1780 (γ-lactone C=O), 1593, 1510 (aromatic). *Anal.* Calcd. for $C_{27}H_{34}O_{11}\cdot 1.5\,H_2O$: C, 57.75; H, 6.60. Found: C, 57.74; H, 6.80.

I was identified with arctiin in all respects.

Properties of Matairesinoside (VI)——Colorless powder, mp 93°. TLC *Rf*: 0.37 ($CHCl_3$–EtOH=4:1). $[\alpha]_D^{12}$ —46.0 (c=0.5 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 229 (4.33), 281 (3.79). UV $\lambda_{max}^{EtOH+NaOH}$ nm (log ε): 248 (4.46), 281 (4.20), 297 (4.14). IR ν_{max}^{KBr} cm^{-1}: 3520—3280 (OH), 1760 (γ-lactone C=O), 1595, 1510 (aromatic). *Anal.* Calcd. for $C_{26}H_{32}O_{11}\cdot H_2O$: C, 57.98; H, 6.36. Found: C, 57.64; H, 6.41.

Hydrolysis of Matairesinoside (VI)——The solution of VI (120 mg) in 10% H_2SO_4 was heated on water bath for 3 hr. The aglycone was extracted with ether. Working up of the ether solution in the same manner as used to that of I afforded aglycone (VII), which was recrystallized from AcOH to give colorless needle (43 mg), mp 117—119°. $[\alpha]_D^{8}$ —40.0 (c=0.1 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 232 (4.06), 283 (3.74). UV $\lambda_{max}^{EtOH+NaOH}$ nm (log ε): 250 (4.27), 298 (4.00). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560 (OH), 1775 (γ-lactone C=O), 1610, 1510 (aromatic). *Anal.* Calcd. for $C_{20}H_{22}O_6$: C, 67.02; H, 6.19. Found: C, 67.23; H, 6.15. NMR (in $CDCl_3$) δ: 6.40—7.00 (6H, m, arom. H), 5.10—5.70 (2H, br, OH, quenched by deuterium exchange), 4.00—4.40 (2H, m, C-9), 3.95 (6H, s, methoxyl), 2.80—3.15 (2H, br, C-8,8′), 2.65 (4H, br. s, C-7,7′). Mass Spectrum *m/e*: 358 (M^+).

VII was identical with an authentic sample of matairesinol. The presence of D-glucose only was detected as the sugar moiety by PC.

Methylate of Matairesinoside (VI)——VI (50 mg) in MeOH was methylated with diazomethane and the crude methylate was purified by column chromatography. The methylate, mp 102—105° was identified with I by mixed melting point and IR spectral comparison.

Properties of Tracheloside (VIII)——Colorless grains, mp 168—170°. TLC *Rf*: 0.45 ($CHCl_3$–EtOH=4: 1). $[\alpha]_D^{20}$ —60.0 (c=0.5 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 230 (4.29), 280 (3.81). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1770 (γ-lactone C=O), 1610, 1590, 1515 (aromatic). *Anal.* Calcd. for $C_{27}H_{34}O_{12}\cdot 1/2H_2O$: C, 57.95; H, 6.31. Found: C, 57.90; H, 6.35.

VIII was identified with Takano's tracheloside by mixed melting point and IR spectral comparison.

Acetate of Tracheloside (VIII)——VIII (50 mg) was acetylated with acetic anhydride and pyridine in usual way. The crude acetate extracted with ether was subjected to column chromatography with $CHCl_3$–EtOH (49: 1) and recrystallized from water to give IX (38 mg) as colorless needles, mp 98—102°. $[\alpha]_D^{20}$ —48.8 (c=0.8 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 228 (4.23), 280 (3.78). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1770 (γ-lactone C=O), 1750 (acetyl C=O), 1595, 1515 (aromatic). *Anal.* Calcd. for $C_{35}H_{42}O_{16}\cdot H_2O$: C, 57.06; H, 6.02; O, 36.92; mol. wt. 736.7. Found: C, 57.39; H, 6.00; O, 36.32; mol. wt. (vapor pressure osmometry in $CHCl_3$) 756.5. NMR (in $CDCl_3$) δ: 6.45—7.00 (6H, m, arom. H), 4.85—5.40 (4H, m), 4.18 (3H, br. s), 4.03 (2H, d, separation of 6 cps, C-9), 2.2—3.2 (6H, m, C-7,8,7′ and OH), 2.02 2.05 (12H, each s, acetyl).

Hydrolysis of Tracheloside (VIII)——a) The solution of VIII (100 mg) in 10% H_2SO_4 (20 ml) was heated on a water bath for 2 hr. The crude aglycone extracted with ether was subjected to column chromatography with $CHCl_3$–AcOEt (4: 1) to obtain X (40.5 mg) as colorless needles, mp 139—141°. $[\alpha]_D^{23}$ —43.3 (c=0.25 in

EtOH). UV λ_{max}^{EtOH} nm (log ε): 231 (4.08), 282 (3.69). UV $\lambda_{max}^{EtOH+NaOH}$ nm (log ε): 249 (4.07), 287 (3.81), 297 (3.73). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560 (OH), 1775 (γ-lactone C=O), 1610, 1595, 1505 (aromatic). *Anal.* Calcd. for $C_{21}H_{24}O_7$: C, 64.93; H, 6.23. Found: C, 64.74; H, 6.19. NMR (in $CDCl_3$) δ: 6.6—7.0 (6H, m, arom. H), 5.50—6.05 (1H, br, OH, quenched by deuterium exchange), 4.08 (2H, d, separation of 6 cps, C-9), 3.95 (9H, s, methoxyl), 3.13 (2H, q, J_{AB}=15 cps, C-7'), 2.53 (1H, s, OH, quenched by deuterium exchange), 2.4—2.9 (3H, m, C-7,8). Mass Spectrum *m/e*: 388 (M$^+$).

The paper chromatogram of sugar moiety obtained in usual way (solvent: BuOH–AcOH–H_2O=4: 1: 1, color reagent: aniline hydrogen phthalate) showed only one spot of D-glucose. It was also confirmed as the trimethylsilyl ether by GLC (column: 3% SE-52 on Chromosorb W, 3 mm×2 m; column temperature 160°; carrier gas N_2 flow 0.5 kg/cm^2).

b) To VIII (30 mg) in 0.1M citrate buffer (10 ml) was added emulsin (10 mg) (Tokyo Chemical Industry Co.,) and the mixture was incubated at room temperature for 2 weeks. The mixture was extracted with ether and ether layer was dried and evaporated. The residue was identified as X by co-TLC (solvent: $CHCl_3$–AcOEt=1: 1) and IR spectral comparison. In the water layer the presence of D-glucose only was shown by PC.

Methylate of Trachelogenin (X)——X (50 mg) in MeOH was methylated with diazomethane as usual and the crude product was column chromatographed. The eluation with $CHCl_3$–AcOEt (4: 1) afforded the methylate which was recrystallized from MeOH to give XI (36 mg) as colorless needles, mp 97—98.5°. $[\alpha]_D^{28}$ −45.9 (c=0.51 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 231 (4.15), 281 (3.73). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560 (OH), 1778 (γ-lactone C=O), 1610, 1595, 1510 (aromatic). *Anal.* Calcd. for $C_{22}H_{26}O_7$: C, 65.66; H, 6.51. Found: C, 65.58; H, 6.39. NMR (in $CDCl_3$) δ: 6.6—7.0 (6H, m, arom. H), 4.08 (2H, d, separation of 6 cps, C-9), 3.95 (12H, s, methoxyl), 3.13 (2H, q, J_{AB}=14 cps, C-7), 3.03 (1H, sh, OH, quenched by deuterium exchange), 2.4—2.9 (3H, m, C-7,8). Mass Spectrum *m/e*: 402 (M$^+$).

Alkaline Permanganate Oxidation of Methyltrachelogenin (XI)——XI (50 mg) was dissolved in 1N NaOH (10 ml) and warmed on water bath for 2 hr. The solution was treated with 2% $KMnO_4$ in small portions at 35° to pink end point. The color was discharged with sodium bisulfite solution and the precipitate was filtered off. The filtrate, after being acidified with dil. H_2SO_4, was extracted with ether. The ether solution was evaporated to yield the oxidation product (18 mg) as colorless needles, mp 179—180°, which was identified with an authentic sample of 3,4-dimethoxybenzoic acid by mixed melting point and IR spectra.

Ethylate of Trachelogenin (X)——X (50 mg) in EtOH was ethylated with diazoethane. The treatment of product in the same manner as used to the above methylation gave an amorphous ethylate (XII) (38 mg). $[\alpha]_D^{18}$ −46.2 (c=1.9 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 231.5 (4.23), 281.5 (3.82). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3950 (OH), 1780 (γ-lactone C=O), 1605, 1595, 1515 (aromatic). NMR (in $CDCl_3$) δ: 6.60—6.97 (6H, m, arom. H), 4.15 (2H, q, J=8 cps, ethoxyl-CH_2-), 4.07 (2H, d, separation of 6 cps, C-9), 3.13 (2H, q, J_{AB}=15 cps, C-7'), 2.4—3.9 (4H, m, C-7,8 and OH), 1.45 (3H, t, J=8 cps, ethoxyl-CH_3). Mass Spectrum *m/e*: 416 (M$^+$).

The solution of XII (30 mg) in 1N NaOH (5 ml) was heated on water bath for the some time. The solution was neutralized with 10% H_2SO_4 and extracted with ether. The crude hydroxy-acid was recrystallized from ether to give colorless grains, mp 114—115°. IR ν_{max}^{KBr} cm^{-1}: 3240—3500 (OH), 1710 (C=O), 1610. 1595, 1515 (aromatic). *Anal.* Calcd. for $C_{23}H_{30}O_8$: C, 63.58; H, 6.96; O, 29.46. Found: C, 63.70; H, 7.04; O, 29.21.

GLC of Alkaline Permanganate Oxidation Products of Ethylate (XII)——XII was treated with alkaline permanganate in the same manner as for the oxidation of XI. The oxidation products which were extracted with ether were methylated with diazomethane for GLC. The chromatogram of the methyl ester showed that the oxidation of XII produced both 3,4-dimethoxybenzoic acid and 3-methoxy-4-ethoxybenzoic acid in almost equal mole ratio, which were confirmed by the comparison with the chromatogram of the oxidation products of V. GLC: column, 1.5% SE-30 on Chromosorb W. 3 mm×2 m; column temperature 170°; N_2 flow 15 ml/min; t_R (min) methyl 3,4-dimethoxybenzoate 3.6, methyl 3-methoxy-4-ethoxybenzoate 4.2.

$LiAlH_4$ Reduction of Methyltrachelogenin (XI), Di-O-methylhydroxythujaplicatin Methyl Ether (XVI) and Dimethylmatairesinol (IV)——a) XI (136 mg) in tetrahydrofuran (THF)[18] (5 ml) was added dropwise to the suspension of $LiAlH_4$ (136 mg) in THF (5 ml) and continued to stir at room temperature. After 8 hr the mixture was added to ice water and acidified carefully with 10% H_2SO_4. The product extracted with ether was subjected to column chromatography and eluted by $CHCl_3$–AcOEt (1: 1). Fractions were monitored by TLC using $CHCl_3$–AcOEt (1: 1) as developer. *Rf* 0.36 fraction was evaporated and recrystallized from EtOH to give hemiacetal (XIII) (42 mg) as colorless needles, mp 155—156°. $[\alpha]_D^{31}$ −23.0 (c=1.04 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 231 (4.22), 281 (3.75). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1605, 1590, 1510 (aromatic). *Anal.* Calcd. for $C_{22}H_{28}O_7$: C, 65.33; H, 6.98. Found: C, 65.14; H, 6.71. NMR (in $CDCl_3$) δ: 6.6—7.1 (6H, m, arom. H), 5.00, 5.30 (1H, s, C-9'), 3.92 (12H, s, methoxyl), 3.7—3.9 (2H, m, C-9), 3.08 (2H, br. s, OH), 2.80 (2H, s, C-7'), 2.5—3.1 (3H, m, C-7,8). Mass Spectrum *m/e*: 404 (M$^+$).

b) XVI (60 mg) in THF (2.5 ml)[18] was treated in the same manner as for XI. The purification by column chromatography gave XVII (20 mg) as syrup. UV λ_{max}^{EtOH} nm (log ε): 228.5 (4.27), 279 (3.61). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1590, 1505 (aromatic). NMR (in $CDCl_3$) δ: 6.5—6.8 (3H, m, arom. H), 6.40 (2H, s,

18) THF treated only with sodium metal was used as solvent.

arom. C-2′,6′), 5.21, 4.91 (1H, s, C-9′), 3.85 (15H, s, methoxyl), 3.6—3.9 (2H, m, C-9), 3.1—3.4 (2H, br, OH), 2.5—3.1 (5H, m, C-7,8,7′). Mass Spectrum *m/e*: 434 (M^+).

c) IV (250 mg) in THF (10 ml)[18] was added dropwise to the suspension of $LiAlH_4$ (306 mg) in THF (10 ml) and continued to stir at room temperature. After 3 hr the mixture was treated in the same manner as for XI. The column chromatography with $CHCl_3$–AcOEt (1: 1) as eluent gave two products, XVIII and XIX. XVIII (27 mg) as colorless needles, mp 140—141.5°. TLC *Rf*: 0.57 ($CHCl_3$–AcOEt=1: 1). $[\alpha]_D^{27}$ −23.5 (*c*=0.51 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 231 (4.24), 281 (3.79). IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1605, 1585, 1510 (aromatic). *Anal.* Calcd. for $C_{22}H_{28}O_6$: C, 68.02; H, 7.27. Found: C, 68.21; H, 6.95. NMR (in $CDCl_3$) δ: 6.5—7.0 (6H, m, arom. H), 5.20—5.45 (1H, br, C-9′), 3.95 (12H, s, methoxyl), 3.6—3.9 (2H, m, C-9), 2.5—3.2 (5H, m, C-7,7′ and OH), 2.10—2.45 (2H, br, C-8,8′). Mass Spectrum *m/e*: 388 (M^+). XIX (64 mg) as colorless needles, mp 122—123.5°. TLC *Rf*: 0.33 ($CHCl_3$–AcOEt=1: 1). $[\alpha]_D^{15}$ −31.4 (*c*=1.28 in $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 230 (4.12), 281 (3.67). IR ν_{max}^{KBr} cm^{-1}: 3520, 3400 (OH), 1605, 1590, 1515 (aromatic). *Anal.* Calcd. for $C_{22}H_{30}O_6$: C, 67.67; H, 7.74. Found: C, 67.57; H, 7.81. NMR (in $CDCl_3$) δ: 6.60—6.95 (6H, br. s, arom. H), 3.94 (12H, s, methoxyl), 3.40—3.75 (4H, m, C-9,9′), 3.71 (2H, br, OH, quenched by deuterium exchange), 2.87, 2.75 (4H, br. s, C-7,7′), 1.70—2.15 (2H, br, C-8,8′). Mass Spectrum *m/e*: 390 (M^+). XIX was identical with an authentic sample of *seco*-isolariciresinol.

$LiAlH_4$ reduction of Hemiacetal (XIII)——XIII (41 mg) in THF (1 ml) was added dropwise to the suspension of $LiAlH_4$ (80 mg) in absolute ether (10 ml) and continued to stir for 8 hr at room temperature. The mixture was treated in the same manner as for XI. Purification by column chromatography gave triol (XIV) (15 mg) as colorless needles, mp 132—133°. TLC *Rf*: 0.22 ($CHCl_3$–AcOEt=1: 1). $[\alpha]_D^{18}$ −23.0 (*c*=0.3 in $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 231 (4.28), 280 (3.82). IR ν_{max}^{KBr} cm^{-1}: 3550, 3450 (OH), 1605, 1585, 1510 (aromatic). *Anal.* Calcd. for $C_{22}H_{30}O_7$: C, 65.01; H, 7.44. Found: C, 64.74; H, 7.06. Mass Spectrum *m/e*: 406 (M^+).

Tetrahydrogmelinol was prepared from XV (100 mg) by the metal-ammonia reduction according to the method as described by A. J. Birch *et al.*[17] Tetrahydrogmelinol as colorless needles, mp 132—133°. $[\alpha]_D^{15}$ −21.9 (*c*=0.53 in $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3550, 3450 (OH), 1605, 1585, 1510 (aromatic). *Anal.* Calcd. for $C_{22}H_{30}O_7$: C, 65.01; H, 7.44. Found: C, 64.71; H, 7.57. NMR (in $CDCl_3$) δ: 6.60—6.95 (6H, br, arom. H), 3.91 (12H, s, methoxyl), 3.40—3.85 (4H, m, C-9,9′), 3.10—3.35 (2H, br, OH, quenched by deuterium exchange), 2.98 (2H, br. s, C-7′), 2.4—2.7 (2H, br, C-7), 1.9—2.2 (1H, br, C-8).

XIV was identical with tetrahydrogmelinol in all respects.

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