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Lignans of *Trachelospermum asiaticum* var. *intermedium*. II.[1] Structures of Tracheloside and Nortracheloside[2]

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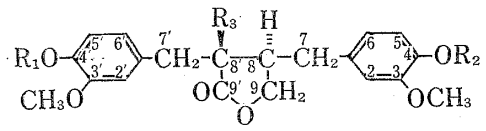
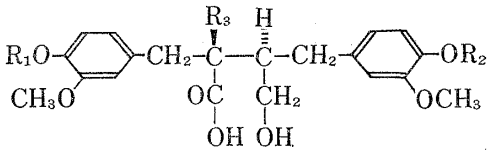
A new lignan glucoside named nortracheloside (IV), $C_{26}H_{32}O_{12}\cdot H_2O$, mp 95—100°, was isolated from the stems of *Trachelospermum asiaticum* Nakai var. *intermedium* Nakai (Apocynaceae). The lignan derivatives were prepared from tracheloside (III) and nortracheloside (IV). From the discussion of mass spectra of the lignan derivatives, the structures of III and IV were established as 8(*S*),8′(*S*)-4′,8′-dihydroxy-3,4,3′-trimethoxy-lignan-olid(9,9′)-4′-β-D-glucopyranoside and 8(*S*),8′(*S*)-4,4′,8′-trihydroxy-3,3′-dimethoxy-lignan-olid(9,9′)-4′-β-D-glucopyranoside, respectively.

In the previous paper[1] three lignan glucosides, arctiin (I), matairesinoside (II) and tracheloside (III) were isolated from the stems of *Trachelospermum asiaticum* Nakai var. *intermedium* Nakai and two possible structures for III; 4′,8′-dihydroxy-3,4,3′-trimethoxy-lignan-olid(9,9′)-4′-β-D-glucopyranoside or 4,8′-dihydroxy-3,3′,4′-trimethoxy-lignan-olid(9,9′)-4-β-D-glucopyranoside, were suggested along with the establishment of 8 (*S*), 8′ (*S*)-configuration.

This paper deals with the isolation of a new component named nortracheloside (IV) and the structural determinations of III and IV, respectively.



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



IXa : R_1=C_2H_5, R_2=CH_3, R_3=OH
Xa : R_1=CH_3, R_2=C_2H_5, R_3=OH

Chart 1

IV, $C_{26}H_{32}O_{12}\cdot H_2O$, mp 95—100°, $[\alpha]_D^{19}$ −47.9° (ethanol) was obtained from the ethyl acetate extract prepared by the procedure described in the previous paper.[1] IV is one of the four components detected by thin-layer chromatography (TLC). The infrared (IR) spectrum of IV showed the presence of γ-lactone, characteristic for lignans. The bathochromic shift of absorption maximum in ultraviolet (UV) spectrum of IV with sodium ethoxide was similar to that of II. The acid hydrolysis yielded aglycone (V) and D-glucose. V was

1) Part I: I. Inagaki, S. Hisada, and S. Nishibe, *Chem. Pharm. Bull.* (Tokyo), **20**, 2710 (1972).
2) A portion of this work was reported as preliminary communications: S. Nishibe, S. Hisada, and I. Inagaki, *Chem. Pharm. Bull.* (Tokyo), **19**, 866 (1971); S. Nishibe, S. Hisada, and I. Inagaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 2075 (1972).
3) Location: *Tanabe-dori, Mizuho-ku, Nagoya, 467, Japan.*
4) S. Nishibe, S. Hisada, and I. Inagaki, *Phytochemistry*, **10**, 2231 (1971).
5) The nomenclature of Freudenberg and Weinges was used.

identified with nortrachelogenin[4] which was isolated from the ether-soluble fraction and elucidated to be 4,4′,8′-trihydroxy-3,3′-dimethoxy-lignan-olid (9,9′).[5] In addition the properties of V and its derivatives were as follows.[6] V, $[\alpha]_D^{17}$ −16.8° (ethanol), although chromatographically pure, could not be crystallized. The bathochromic shift of absorption maximum in UV spectrum of V with sodium ethoxide indicated that V has the closely similar structure to matairesinol. The methylation of V with excess diazomethane gave methyltrachelogenin (VI). The ethylation of V with excess diazoethane afforded glassy diethylnortrachelogenin (VII), the nuclear magnetic resonance (NMR) spectrum of which showed signals assigned to aromatic protons (6.4—6.9 ppm, unresolved multiplet, 6H), methoxyl protons (3.87 ppm, singlet, 6H) and ethoxyl protons (CH_3- 1.43 ppm, triplet, J=8 cps, 6H; -CH_2- 3.95 ppm, quartet, J=8 cps, 4H). On the treatment with 1N sodium hydroxide VII gave hydroxy-acid, $C_{24}H_{32}O_8$, mp 113—114.5°. Alkaline permanganate oxidation of VII yielded only 3-methoxy-4-ethoxybenzoic acid. As the results, VII and V were elucidated as 8 (*S*), 8′ (*S*)-8′-hydroxy-3,3′-dimethoxy-4,4′-diethoxy-lignan-olid(9,9′) and 8 (*S*), 8′ (*S*)-4,4′,8′-trihydroxy-3,3′-dimethoxy-lignan-olid(9,9′), respectively.

On methylation with excess diazomethane IV gave III. Hence IV is mono-glucoside of V.

The method for determination of structures III and IV according to MacLean and Murakami[7] requires a few step reactions of low yield. So here the attempt was made to determine the structures by mass spectra as one of the more simple methods.

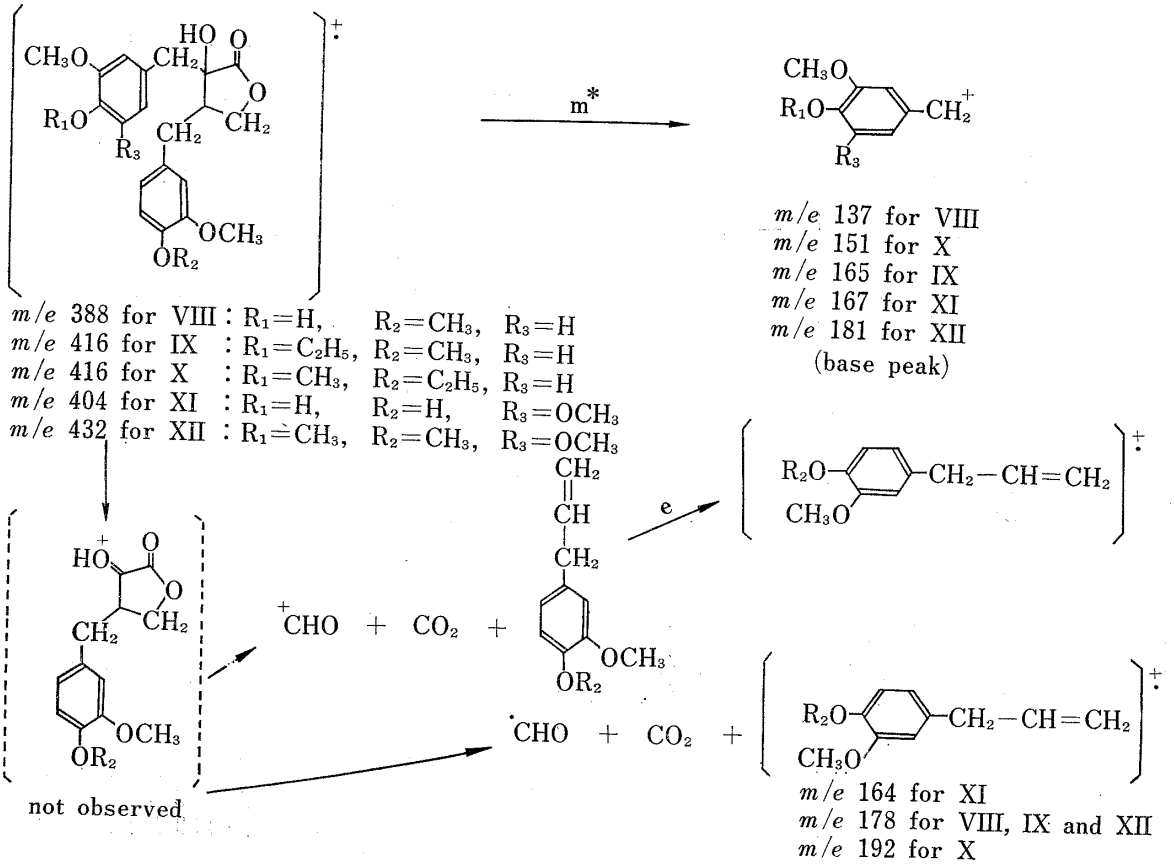


Chart 2

6) The structure of nortrachelogenin was presented as a short communication (S. Nishibe, S.Hisada, and I. Inagaki, *Phytochemistry*, **10**, 2231 (1971)). So in this paper we should like to report its properties in details.

7) H. MacLean and K. Murakami, *Can. J. Chem.*, **44**, 1827 (1966).

As the samples for mass spectral measurements, we prepared the compounds which have two different benzyl groups at γ-lactone; trachelogenin (VIII),[8] $C_{21}H_{24}O_7$, mp 139—141°, and ethyltrachelogenin (IX)[8] from III and ethylmethylnortrachelogenin (X) from IV. These preparations are described in experimental section. Although IX and X were obtained as amorphous powder, the formulas were confirmed by the NMR spectra and analytical data of crystals as hydroxy-acids; IXa, $C_{23}H_{30}O_8$, mp 114—115° and Xa, $C_{23}H_{30}O_8$, mp 115—116°.5, respectively. As the reference, mass spectra of established structures; hydroxythujaplicatin methyl ether (XI)[7] and di-O-methylhydroxythujaplicatin methyl ether (XII) were measured.

The fragmentation pathways of lignan derivatives could be interpreted as shown in Chart 2.

The presence of the metastable ion appropriate to a transition is denoted by m*. It has been reported that the fragmentation of the α-ketolactone ring occurs so readily that the ion is not observed.[9]

Mass spectra of acetylated derivatives; acetylmethyltrachelogenin (XIII), $C_{24}H_{28}O_7$, mp 101—102°, acetylethyltrachelogenin (XIV), $C_{25}H_{30}O_7$, mp 121—123°, acetylethylmethylnortrachelogenin (XV), $C_{25}H_{30}O_7$, syrup, as the samples and acetyl-di-O-methylhydroxythujaplicatin methyl ether (XVI) as the reference were also discussed. The fragmentation pathways of XIII, XIV and XV were sufficiently supported from those of XVI. Moreover similar fragmentation pathways have been reported on helianthoidin which has piperonyl ester at tertiary hydroxyl group.[10] These pathways were shown in Chart 3. The base peaks in these mass spectra were benzyl cation derived from β-position of γ-lactone.

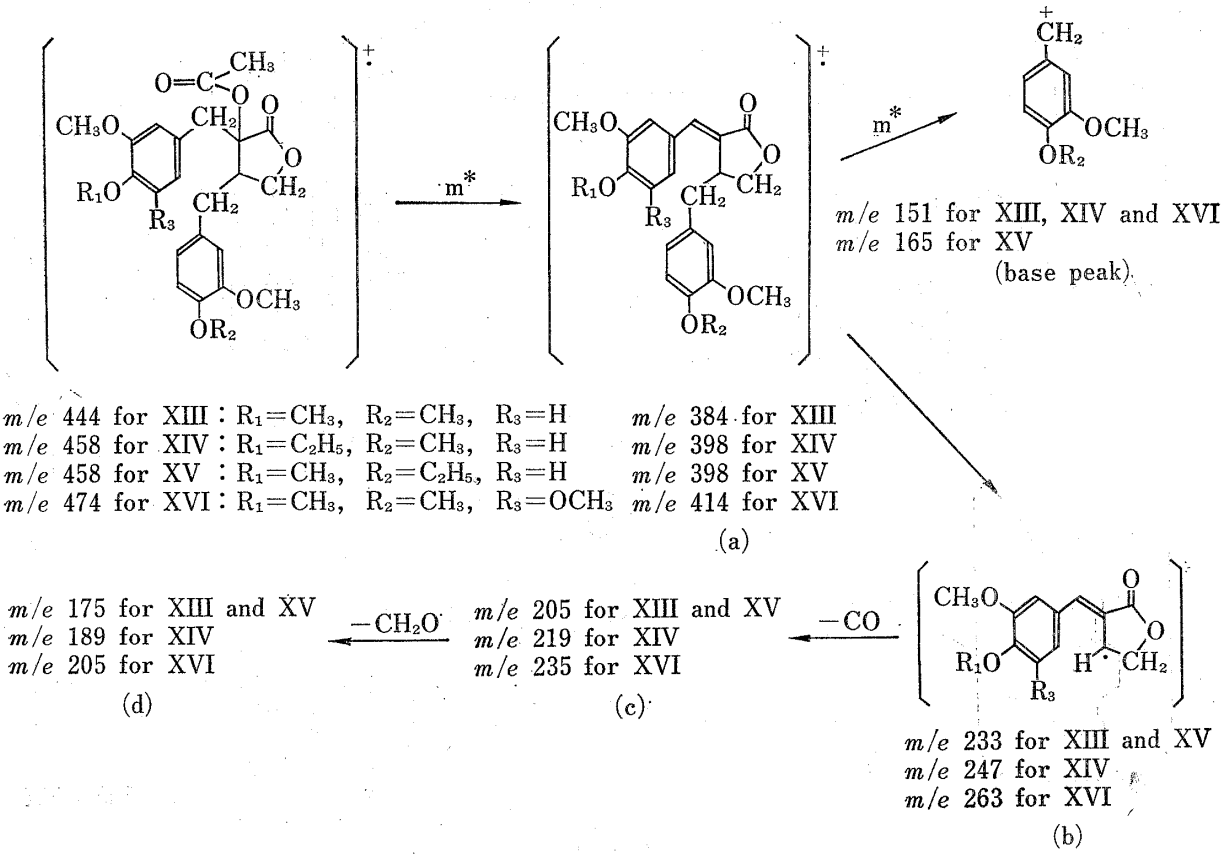


Chart 3

8) These compounds were reported in the previous paper.[1]
9) S. Yamamura and Y. Hirata, *Tetrahedron*, **19**, 1485 (1963).
10) R.S. Burden, L. Crombie, and D.A. Whiting, *J. Chem. Soc.* (*C*), **1969**, 693.

The displacing features of fragments assigned to ions (a), (b), (c) and (d) were in accord with difference of benzyl group at α-position of γ-lactone. These results from mass spectra of lignan derivatives shown in Charts 2 and 3 enabled the elucidations of structures III and IV.

Thus, III and IV have been established to be 8 (*S*), 8′ (*S*)-4′,8′-dihydroxy-3,4,3′-trimethoxy-lignan-olid(9,9′)-4′-β-D-glucopyranoside and 8 (*S*), 8′ (*S*)-4,4′,8′-trihydroxy-3,3′-dimethoxy-lignan-olid(9,9′)-4′-β-D-glucopyranoside, respectively.

Experimental

All melting points were not corrected. The following equipment was used: IR spectra, Infrared Spectrophotometer IR-S, IR-E and IRA-2 (JASCO); UV spectra, Hitachi Recording Spectrophotometer Model EPS-3T; NMR spectra, JNM-MH-60 (JEOL) with tetramethylsilane ($\delta=0$) as internal standard; Optical rotation values, Direct Reading Polarimeter Model OR-10 (Yanagimoto).

The mass spectra of the compounds were recorded on Hitachi Model RMU-6C mass spectrometer and JEOL JMS-O1SG mass spectrometer at 75 eV using in all cases a direct sample insertion into the ion source.

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 Nortracheloside (IV)——The air-dried and cut stems (25 kg) was extracted in the same manner as described in the previous paper.[1] The AcOEt extract (40 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. The *Rf* 0.28 fraction was evaporated and recrystallized from $CHCl_3$–EtOH to give IV (322 mg).

Properties of Nortracheloside (IV)——Amorphous colorless powder, mp 95—100°. TLC *Rf*: 0.28 ($CHCl_3$: EtOH=4: 1). $[\alpha]_D^{19}$ −47.9° (c=1.02 in Et OH). UV λ_{max}^{EtOH} nm(log ε): 229sh (4.07), 282 (3.70). UV $\lambda_{max}^{EtOH+NaOH}$ nm(log ε): 247 (4.03), 284 (3.74), 298 (3.73). IR ν_{max}^{KBr} cm^{-1}: 3560—3280 (OH), 1770 (γ-lactone C=O), 1605, 1515 (aromatic). *Anal.* Calcd. for $C_{26}H_{32}O_{12}\cdot H_2O$: C, 57.24; H, 6.10. Found: C, 57.58; H, 6.36.

Hydrolysis of Nortracheloside (IV)——The solution of IV (50 mg) in 10% H_2SO_4 was heated on a water bath for 2 hr. The oily product separated was extracted with ether. The ether solution was washed with water, dried and evaporated to dryness. The residue was column chromatographed with $CHCl_3$–AcOEt (4: 1) as eluent. V (21 mg), although chromatographically pure, could not be crystallized and was obtained as amorphous. V was identified with nortrachelogenin isolated from ether-soluble fraction by co-TLC using $CHCl_3$–AcOEt (1: 1) as developer and IR spectral comparison. After removal of the aglycone, mother liquor was neutralized with barium carbonate and evaporated to dryness. PC of this residue (solvent: BuOH: AcOH: H_2O=4: 1: 1, color reagent: aniline hydrogen phthalate) showed the presence of D-glucose only.

Properties of Nortrachelogenin (V)——Amorphous. $[\alpha]_D^{17}$ −16.8° (c=0.178 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 231 (4.09), 283 (3.75). UV $\lambda_{max}^{EtOH+NaOH}$ nm(log ε): 248 (4.29), 297 (3.96). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520 (OH), 1780 (γ-lactone C=O), 1610, 1510 (aromatic).

Dimethyl Ether of Nortrachelogenin (V)——V (30 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 dimethyl ether (VI) (18 mg) as colorless needles, mp 97—98°. VI was identical to methyltrachelogenin by a mixed melting point and IR spectra.

Diethyl Ether of Nortrachelogenin (V)——V (110 mg) in EtOH was ethylated with diazoethane. The treatment of product in the same manner as used to the above methylation afforded a glassy diethyl ether (VII) (86.3 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3530 (OH), 1780 (γ-lactone C=O), 1610, 1595, 1515 (aromatic). NMR (in $CDCl_3$) δ: 6.4—6.9 (6H, m, arom. H), 4.04 (2H, d, separation of 6 cps, C-9), 4.10 (4H, q, J=8 cps, ethoxyl-CH_2–), 3.87 (6H, s, methoxyl), 3.05 (2H, q, J_{AB}=14 cps, C-7′), 2.63 (1H, s, OH, quenched by deuterium exchange), 2.30—2.95 (3H, m, C-7,8), 1.43 (6H, t, J=8 cps, ethoxyl-CH_3). Mass Spectrum: Calcd. for $C_{24}H_{30}O_7$, 430.1991. Obsd., 430.1975.

The solution of V (50 mg) in 1N NaOH (20 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 (33.4 mg), mp 113—114.5°. UV λ_{max}^{EtOH} nm(log ε): 231 (4.35), 281 (3.86). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1705 (C=O), 1605, 1590, 1515 (aromatic). *Anal.* Calcd. for $C_{24}H_{32}O_8$: C, 64.27; H, 7.19; O, 28.54. Found: C, 64.05; H, 7.17; O, 28.59.

Alkaline Permanganate Oxidation of Diethylnortrachelogenin (VII)——VII (30 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 oxidative product (9.6 mg) as colorless needles, mp 196—197°. *Anal.* Calcd. for $C_{10}H_{12}O_4$: C, 61.21; H, 6.17. Found: C, 60.92; H, 6.29.

It was identified with an authentic sample of 3-methoxy-4-ethoxybenzoic acid by a mixed melting point and IR spectral comparison.

Methyl Ether of Nortracheloside (IV)——IV (50 mg) in MeOH was methylated with diazomethane and the crude methyl ether was purified by column chromatography and recrystallized from EtOH to give colorless grains. The methyl ether, mp 166—168.5° was identified with III by a mixed melting point and IR spectral comparison.

Ethylmethylnortrachelogenin (X)——a) IV (400 mg) in EtOH was ethylated with diazoethane. The crude ethyl ether was hydrolyzed with 10% H_2SO_4 and treated with the same manner as for III[1] to give 4-O-ethylnortrachelogenin (196 mg) as syrup. The syrup was used for the next reaction without crystallization. UV λ_{max}^{EtOH} nm(log ε): 231 (4.20), 282 (3.84). UV $\lambda_{max}^{EtOH+NaOH}$ nm(log ε): 248sh (4.11), 283 (3.87), 297sh (3.77). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560 (OH), 1775 (γ-lactone C=O), 1612, 1595, 1517 (aromatic). NMR (in $CDCl_3$) δ: 6.5—7.0 (6H, m, arom. H), 5.6—6.1 (1H, br, OH, quenched by deuterium exchange), 4.22 (2H, q, J=8 cps, ethoxyl-CH_2–), 4.15 (2H, d, separation of 6 cps, C-9), 3.94 (6H, s, methoxyl), 3.14 (2H, q, J_{AB}=14 cps, C-7′), 2.35—2.95 (4H, m, C-7,8 and OH), 1.48 (3H, t, J=8 cps, ethoxyl-CH_3).

On the treatment with 1N sodium hydroxide (10 ml), 4-O-ethylnortrachelogenin (30 mg) afforded hydroxy-acid, which was recrystallized from ether to give colorless grains (23 mg), mp 114—114.5°. IR ν_{max}^{KBr} cm^{-1}: 3420, 3200 (OH), 1695 (C=O), 1595, 1510 (aromatic). *Anal.* Calcd. for $C_{22}H_{28}O_8$: C, 62.85; H, 6.71. Found: C, 62.71; H, 6.84.

b) The above 4-O-ethylnortrachelogenin (160 mg) in MeOH was methylated with diazomethane as usual. The purification by column chromatography gave amorphous powder (134.2 mg). UV λ_{max}^{EtOH} nm (log ε): 231.5 (4.18), 281.5 (3.75). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560 (OH), 1770 (γ-lactone C=O), 1610, 1594, 1514 (aromatic). NMR (in $CDCl_3$) δ: 6.6—7.1 (6H, m, arom. H), 4.20 (2H, q, J=8 cps, ethoxyl-CH_2–), 4.12 (2H, d, separation of 6 cps, C-9), 3.94 (9H, s, methoxyl), 3.55 (1H, br.s, OH, quenched by deuterium exchange), 3.13 (2H, q, J_{AB}=14 cps, C-7′), 2.40—2.95 (3H, m, C-7,8), 1.45 (3H, t, J=8 cps, ethoxyl-CH_3). Mass Spectrum *m/e*: 416 (M^+).

On the treatment with 1N sodium hydroxide (20 ml), X (50 mg) afforded hydroxy-acid (Xa) (31.4 mg) as colorless grains, mp 115—116.5°. IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1705 (C=O), 1609, 1591, 1515 (aromatic). *Anal.* Calcd. for $C_{23}H_{30}O_8$: C, 63.58; H, 6.96. Found: C, 63.28; H, 6.96.

On heating Xa over melting point or dissolving in boiling methanol, and refluxing for 30 min, it was converted into the amorphous X, identified by TLC ($CHCl_3$: AcOEt=1: 1) and IR spectral comparisons with the above specimen.

Acetylmethyltrachelogenin (XIII)——The mixture of VI (40 mg) and *p*-toluenesulfonic acid (20 mg) in Ac_2O (0.3 ml) was heated at 100° for 1 hr. The solution was poured into ice water and extracted with ether. The ether solution was evaporated and the residue was subjected to column chromatography with $CHCl_3$–AcOEt (4: 1) as eluent. XIII (48.2 mg) was recrystallized from MeOH to give colorless needles, mp 101—102°. UV λ_{max}^{EtOH} nm(log ε): 232 (4.11), 280 (3.68). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no (OH), 1775 (γ-lactone C=O), 1740 (acetyl C=O), 1610, 1595, 1518 (aromatic). *Anal.* Calcd. for $C_{24}H_{28}O_8$: C, 64.85; H, 6.35. Found: C, 64.65; H, 6.30. NMR (in $CDCl_3$) δ: 6.30—6.95 (6H, m, arom. H), 4.05—4.40 (2H, m, C-9), 3.90 (12H, s, methoxyl), 3.12 (2H, q, J_{AB}=14 cps, C-7′), 2.27 (3H, s, acetyl), 2.10—2.95 (3H, m, C-7,8). Mass Spectrum *m/e*: 444 (2.9) (M^+), 384 (1.0), 233 (9.2), 205 (1.4), 175 (1.2), 151 (100%).

Acetylethyltrachelogenin (XIV)——The mixture of IX (100 mg) and *p*-toluenesulfonic acid (50 mg) in Ac_2O (0.8 ml) was treated in the same manner as the above XIII. XIV (65.5 mg) was recrystallized from EtOH to give colorless needles, mp 121—123°. UV λ_{max}^{EtOH} nm(log ε): 232 (4.29), 281 (3.90). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no (OH), 1775 (γ-lactone C=O), 1744 (acetyl C=O), 1612, 1596, 1517 (aromatic). *Anal.* Calcd. for $C_{25}H_{30}O_8$: C, 65.49; H, 6.60. Found: C, 65.45; H, 6.53. NMR (in $CDCl_3$) δ: 6.35—7.00 (6H, m, arom. H), 4.1—4.5 (2H, m, C-9), 4.15 (2H, q, J=8 cps, ethoxyl-CH_2–), 3.91 (9H, s, methoxyl), 3.13 (2H, q, J_{AB}=14 cps, C-7′), 2.28 (3H, s, acetyl), 2.10—2.95 (3H, m, C-7,8), 1.49 (3H, t, J=8 cps, ethoxyl-CH_3). Mass Spectrum *m/e*: 458 (10) (M^+), 398 (2.3), 247 (37), 219 (3.5), 189 (2.2), 165 (35), 151 (100%).

Acetylethylmethylnortrachelogenin (XV)——The mixture of X (80 mg) and *p*-toluenesulfonic acid (40 mg) in Ac_2O (0.6 ml) was treated in the same manner as the above XIII. XV (57 mg) was obtained as colorless syrup. Repeated attempts at crystallization in variety of solvents failed. UV λ_{max}^{EtOH} nm(log ε): 232 (4.19), 281 (3.76). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no (OH), 1770 (γ-lactone C=O), 1738 (acetyl C=O), 1609, 1590, 1510 (aromatic). *Anal.* Calcd. for $C_{25}H_{30}O_8$: C, 65.49; H, 6.60. Found: C, 65.50; H, 6.50. NMR (in $CDCl_3$) δ: 6.45—7.00 (6H, m, arom. H), 4.1—4.5 (2H, m, C-9), 4.20 (2H, q, J=8 cps, ethoxyl-CH_2–), 3.99 (9H, s, methoxyl), 3.18 (2H, q, J_{AB}=14 cps, C-7′), 2.32 (3H, s, acetyl), 2.1—3.0 (3H, m, C-7,8), 1.50 (3H, t, J=8 cps, ethoxyl-CH_3). Mass Spectrum *m/e*: 458 (17.7) (M^+), 398 (7.8), 233 (14.7), 205 (13.2), 175 (8.3), 165 (100), 151 (55.9%).

Acetyl-di-O-methylhydroxythujaplicatin Methyl Ether (XVI)——The mixture of XII (8 mg) and *p*-toluenesulfonic acid (4 mg) in Ac_2O (0.1 ml) was treated in the same manner as the above XIII. XVI (6 mg)

was purified by column chromatography for the instrumental measurements. UV λ_{max}^{EtOH} nm(log ε): 229sh (4.11), 279.5 (3.48). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no (OH), 1770 (γ-lactone C=O), 1738 (acetyl C=O), 1590, 1510 (aromatic). NMR (in $CDCl_3$) δ: 6.4—6.8 (5H, m, arom. H), 4.1—4.3 (2H, m, C-9), 3.86 (15H, s, methoxyl), 3.10 (2H, q, J_{AB}=14 cps, C-7′), 2.26 (3H, s, acetyl), 2.1—2.9 (3H, m, C-7,8). Mass Spectrum *m/e*: 474 (17.5) (M^+), 414 (1.9), 263 (61.3), 235 (1.1), 205 (1.0), 181 (80), 151 (100%).

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