[Chem. Pharm. Bull. 21(8)1734—1740(1973)]

UDC 547.814.5.02.05 : 581.192

Studies on the Constituents of *Lespedeza homoloba* Nakai. I. The Structure of Lespedeol A[1]

Akira Ueno, Masahiro Ichikawa, Toshio Miyase,[2a] Seigo Fukushima, Yasuhisa Saiki, and Kunio Morinaga

Shizuoka College of Pharmacy[2]

(Received January 12, 1973)

A new isoflavanone derivative named lespedeol A was isolated from the bark of *Lespedeza homoloba* Nakai. The structure was presumed to be 2′,4′,5,7-tetrahydroxy-6-geranylisoflavanone by spectral and chemical data, and this presumption was confirmed by the synthesis of 2′,4′,7-trimethoxy-5-hydroxy-6-tetrahydrogeranylisoflavone derived from lespedeol A.

Lespedeza homoloba Nakai (Japanese name; Tukushi-Hagi), which belong to Leguminosae plants, is a species of "Hagi" and is distributed in this country. In the course of our studies on the constituents of *Lespedeza homoloba* Nakai, a new isoflavanone derivative was isolated and the structure was studied.

The powdered bark was extracted with methanol and fractionated as shown in Chart 1.

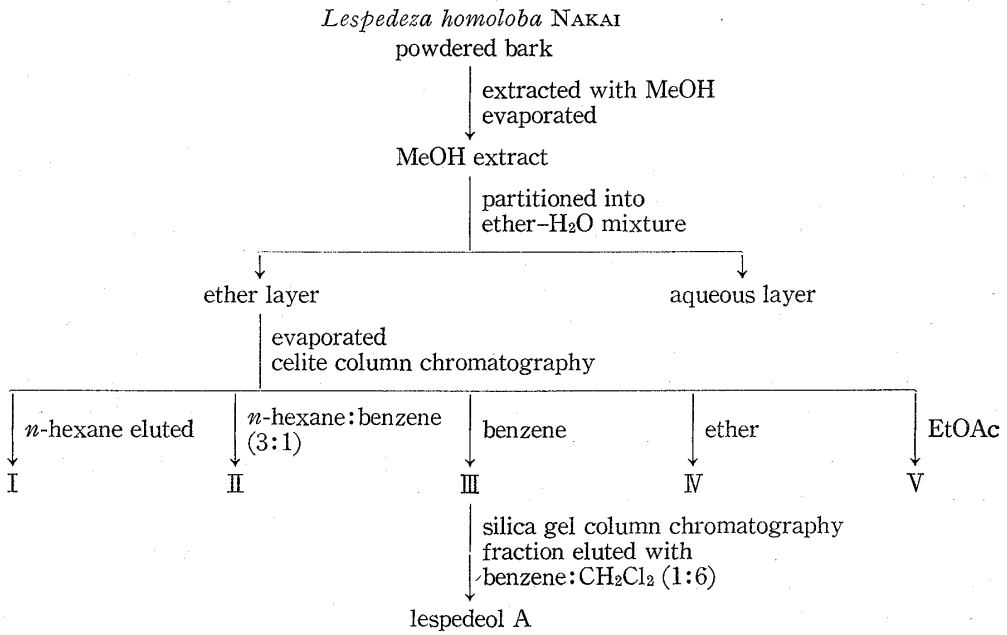


Chart 1. Isolation of Lespedeol A

From the fraction of a benzene–dichloromethane (1: 6) mixture, lespedeol A (I) was obtained as light yellow crystals; mp 146.5—150, $C_{25}H_{28}O_6$ (M^+=424). The infrared (IR) spectrum showed the presence of hydroxyl (3480, 3380, 3250 cm^{-1}), carbonyl (1620 cm^{-1}) and aromatic (1600 cm^{-1}) groups. The ultraviolet absorption (UV) spectrum (Fig. 1. λ_{max}^{EtOH} 294 mμ) suggested the presence of an isoflavanone nucleus in I. The nuclear magnetic resonance (NMR)

1) This work was reported at the 92nd Annual Meeting of the Pharmaceutical Society of Japan, Osaka, April 1972.

2) Location: *2-2-1, Oshika, Shizuoka*; *a*) Present address: *Pharmaceutical Institute, Tohoku University, Aoba-yama, Sendai.*

NII-Electronic Library Service

spectrum (C_5D_5N) showed three singlets at τ 8.45, 8.35, 8.06 (each 3H, =Ċ-CH_3), a broad singlet at τ 7.90 (4H, =Ċ-CH_2-CH_2-Ċ=), a doublet at τ 6.3 (2H, J=6.6 cps, =CH-CH_2-Ar), a broad triplet at τ 4.82 and a broad signal at τ 4.28 (each 1H, =CH-) due to a side chain. The mass spectrum showed a peak; *m/e* (%) 123 (100). These results show the presence of a geranyl or neryl group attached to the aromatic ring. On the NMR spectrum, a signal at τ 5.3 (3H, multiplet) was supported the presence of an isoflavanone nucleus and a singlet at τ −3.2 showed the presence of a hydrogen-bonded hydroxyl group assigned the 5 position of the isoflavanone nucleus.

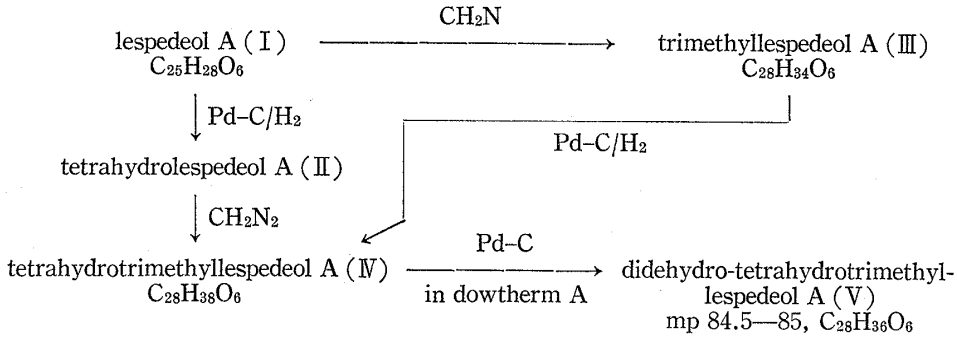


Chart 2

Lespedeol A was converted to their derivatives as shown in Chart 2. On the catalytic hydrogenation, I gave a tetrahydro derivative (II), $C_{25}H_{32}O_6$. On the methylation with diazomethane, II gave a tetrahydro trimethyl derivative (IV), $C_{28}H_{38}O_6$, which was dehydrogenated with 5% palladium on charcoal to give a compound (V) as pale yellow needles, mp 84—85°, $C_{28}H_{36}O_6$.

The UV spectra of I and IV (Fig. 1) are similar to that of 5,7,4′-trihydroxy-6,8-dimethylflavanone.[3] The UV spectrum of V (Fig. 2) is similar to that of 5-hydroxy-4′,7-dimethoxyisoflavone.[4] On the NMR spectrum of V, the peak at τ 5.3 (3H, multiplet) assigned to the

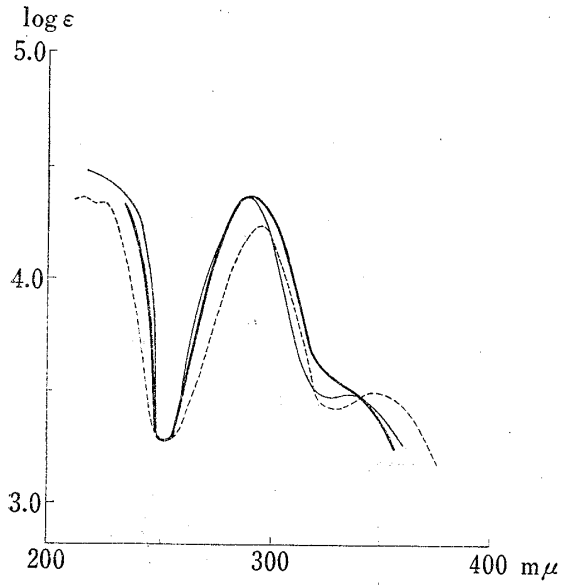


Fig. 1. The UV Spectra of I (——), IV (——) and 6,8-Dimethoxy-4′,5,7-trihydroxyisoflavanone (·····) in EtOH

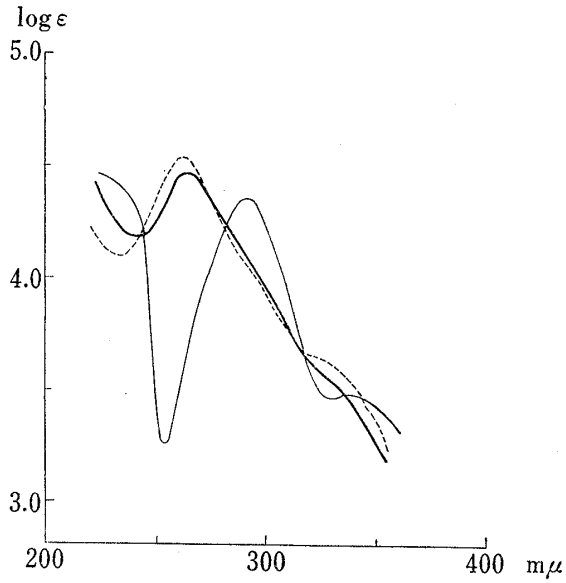


Fig. 2. The UV Spectra of IV (——), V (——) and 4′,7-Dimethoxy-5-hydroxyisoflavone (·····) in EtOH

3) T. Noro, S. Fukushima, Y. Saiki, A. Ueno, and Y. Akahori, *Yakugaku Zasshi*, **89**, 851 (1969).
4) R.M. Horowitz and L. Jurd, *J. Am. Chem. Soc.*, **26**, 2446 (1961).

NII-Electronic Library Service

protons of the 2 and 3 position of the isoflavanone in the case of I, disappeared and a singlet at τ 2.13 is newly found. Therefore, the compound V is assumed to be an isoflavone derivative and I is an isoflavanone derivative.

The mass spectrum of I (Chart 3) showed the following fragments; *m/e* (%) 301 (57.6), 165(71.2), 136(34.3), 123(100). The significant peak at *m/e* 301 shows the loss of *m/e* 123 (due to side chain) from the molecular ion. The charactristic peak at *m/e* 136 due to the retro Diels-Alder tipe fragmentation shows the presence of two hydroxyl groups at the ring B. The fragment of *m/e* 165 shows the presence of two hydroxyl groups and a geranyl (or neryl) group at the ring A.

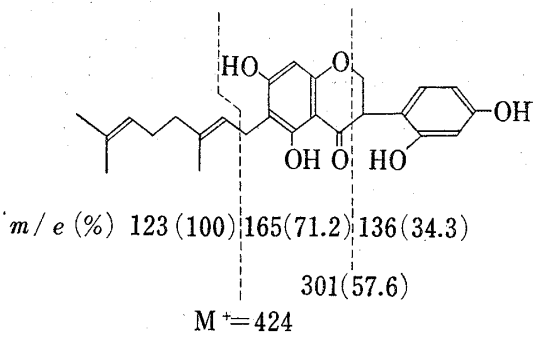
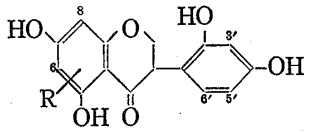


Chart 3. The Mass Spectrum of Lespedeol A

The aromatic protons of I, IV and V in the NMR spectra were assigned on the base of their chemical shift values and their ortho and meta coupling constants[5] as shown in Table I.

Table I. NMR Spectra of I, IV and V (Aromatic Protons)



Compound solvent position	I C_5D_5N	IV $CDCl_3$ Chemical shifts τ (J=Hz)	V $CDCl_3$
6 or 8	3.70 (s)	3.95 (s)	3.57 (s)
3	3.13 (d, J=2.3)	3.45 (d, J=2.5)	3.4 (2H, m)
5	3.20 (d-d, J=8.3, 2.3)	3.50 (d-d, J=8.3, 2.5)	
6	2.85 (d, J=8.3)	3.00 (d, J=8.3)	2.7 (d, J=8.8)

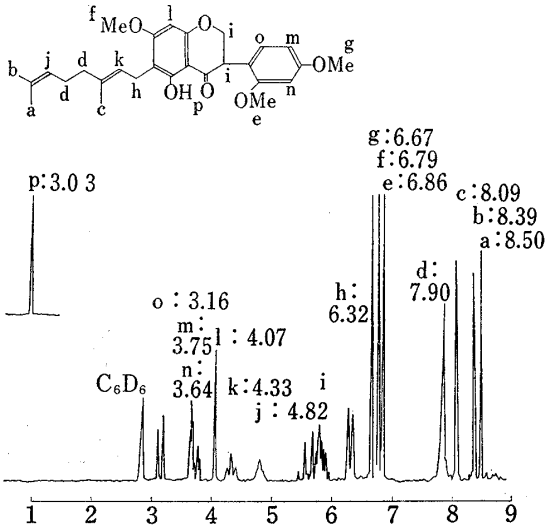


Fig. 3. NMR Spectrum of III in C_6D_6 (Chemical shift: τ, 100 MHz), (h: J=7; k: J=7; m: J=2.3; o: J=8 Hz)

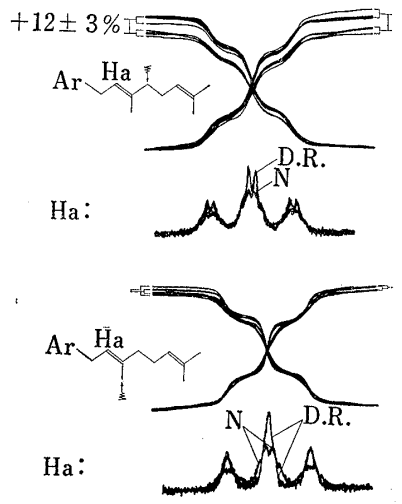


Fig. 4. Nuclear Overhauser Effects Observed in III

5) L. Crombie and J.W. Lown, *J. Chem. Soc.*, **1962**. 775.

NII-Electronic Library Service

Furthermore, all proton signals in the NMR spectrum of III were assigned as shown in Fig. 3. Therefore, the structure of I is presumed to be 2′,4′,5,7-tetrahydroxyisoflavanone having a geranyl or neryl group at the 6 or 8 position. The possibility of the neryl group is excluded by the nuclear Overhauser effect (NOE) observed in III.

As shown in Fig. 4, irradiation of the higher methylene signal (τ 7.88) increased the area (+12±3%) of the proton attached to the double bond (τ 4.33), while no effect showed by that of the lowest methyl signal (τ 8.09). Between the end methyl (τ 8.39) and the proton attached to the double bond (τ 4.82), the NOE was also observed (+12±4%). These observations make clear the relative position of the double bond and the partial structure has a trans conformation (geranyl group).

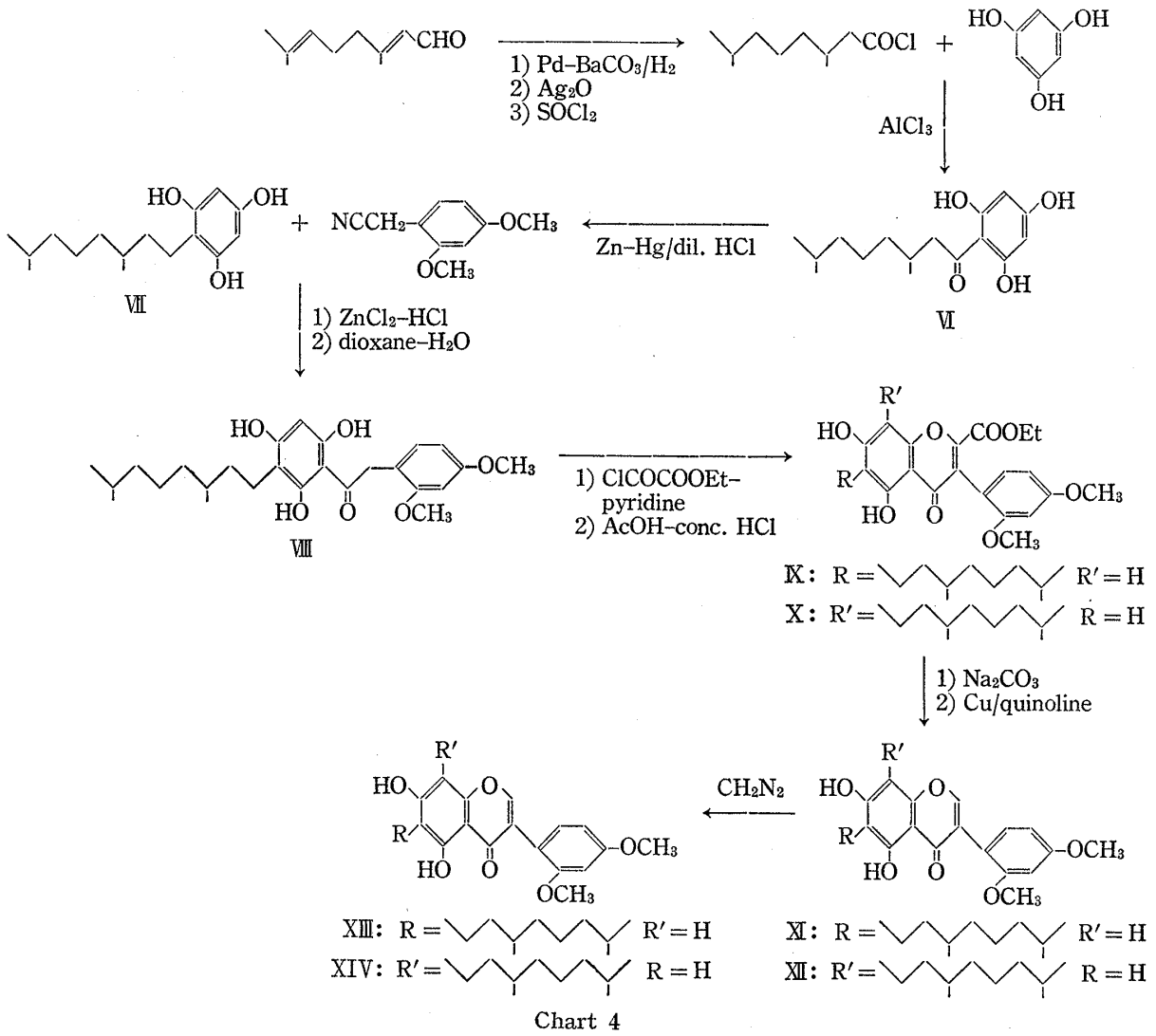


Chart 4

In order to decide the position of the geranyl group (6 or 8 position) in I, we synthesized 6-(3,7-dimethyloctyl)-2′,4′,7-trimethoxy-5-hydroxyisoflavone (XIII; 2′,4′,7-trimethoxy-5-hydroxy-6-tetrahydrogeranylisoflavone) and its isomer, 8-(3,7-dimethyloctyl)-2′,4′,7-trimethoxy-5-hydroxyisoflavone (XIV) *via* the routes as shown in Chart 4. Namely, citral was hydrogenated with 5% palladium on barium carbonate and the product was oxidized with silver oxide to give 3,7-dimethyloctanoic acid[6] which was converted to the acid chloride with thionyl chloride. The Friedel-Crafts reaction of phloroglucinol and the foregoing acid chloride

6) R.S. Cahn, A.R. Penford and J.L. Simonsen, *J. Chem. Soc.*, **1931** 3134.

NII-Electronic Library Service

gave (3,7-dimethyloctanoyl)phloroglucinol (VI) which was reduced with zinc amalgam and hydrochloric acid to give (3,7-dimethyloctyl)phloroglucinol (VII). By the Hoesh reaction, VII was condensed with 2,4-dimethoxybenzyl cyanide (mp 72°) prepared from resorcinol to give 2-(3,7-dimethyloctyl)-4-(2,4-dimethoxyphenylacetyl)phloroglucinol (VIII). VIII was treated with ethoxalyl chloride-pyridine and the product was cyclized with hydrochloric acid in glacial acetic acid to give the two products which were separated to 6-(3,7-dimethyloctyl)-2-ethoxycarbonyl-2′,4′-dimethoxy-5,7-dihydroxyisoflavone (IX) and 8-(3,7-dimethyloctyl)-2-ethoxycarbonyl-2′,4′-dimethoxy-5,7-dihydroxyisoflavone (X) by means of a column chromatography; the fraction eluted with benzene gave IX as pale yellow crystals (mp 99.5—100°), a benzene containing 5% acetone eluted X which was obtained as a yellowish orange oil. With 5% sodium carbonate solution, IX and X were separatedly hydrolized and the products were decarboxylated with copper in the quinoline solution to give the compound XI and the compound XII, respectively. The Gibbs test of XI showed positive, on the other hand, that of XII showed negative. This result shows that XI has a substituent at the 6 position of the isoflavone nucleus. Consequently, XI must be 6-(3,7-dimethyloctyl)-2′,4′-dimethoxy-5,7-dihydroxyisoflavone and XII is the isomer of XI having a 3,7-dimethyloctyl (tetrahydrogeranyl) group at the 8 position of the isoflavone nucleus.

On the methylation with diazomethane, XI and XII gave 2′,4′,7-trimethoxy-5-hydroxy-6-tetrahydrogeranylisoflavone (XIII), mp 84—85° and 2′,4′,7-trimethoxy-5-hydroxy-8-tetrahydrogeranylisoflavone (XIV), mp 84.5—85°, respectively. The UV and NMR spectra of XIII and XIV (Fig. 5, Fig. 6) are similar to each other.

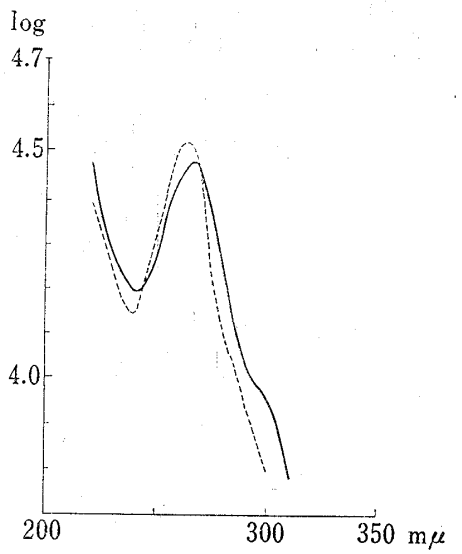


Fig. 5. The UV Spectra of XIII (------) and XIV (——) in EtOH

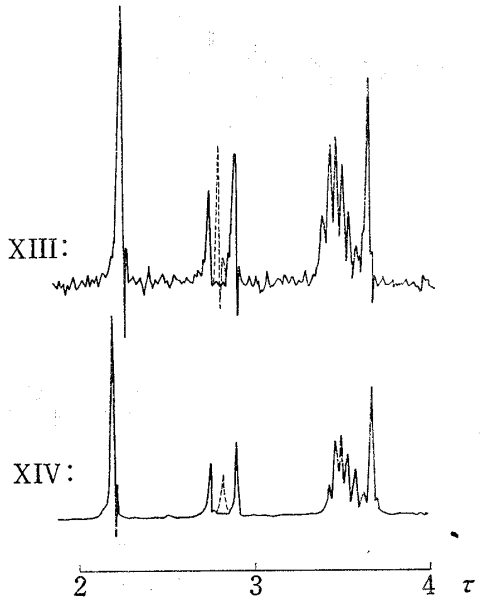


Fig. 6. The NMR Spectra of XIII and XIV in $CDCl_3$

The mixed melting point of XIII and V derived from the natural product (I) did not show depression of melting point (mixed mp 84—85°), on the other hand, that of XIV and V showed depression of melting point (mixed mp 62—71°). The IR, UV and NMR spectra of XIII agreed closely with that of V. These results show that the structure of V must be 2′,4′,7-trimethoxy-5-hydroxy-6-tetrahydrogeranylisoflavone. Therefore, the structure of lespedeol A is elucidated to be 2′,4′,5,7-tetrahydroxy-6-geranylisoflavanone.

It is the first case that an isoflavanone derivative having a C-10 side chain such as this lespedeol A is found from natural origin.

NII-Electronic Library Service

Experimental

All melting points are uncorrected. UV spectra were measured using a Hitachi recording spectrometer EPS-O32 type. IR spectra were determined on KBr disks or NaCl plates (liquid) using a Hitachi infrared spectrometer EPI-G21 type. NMR spectra were taken at 60 MHz with TMS as an internal standard using a JNM-C-60H high resolution NMR spectrometer. The chemical shifts were given in τ values. Abbreviation; s=singlet, d=doublet, t=triplet, m=multiplet, br=broad. Mass spectra were measured using a Hitachi RMU mass spectrometer.

Isolation of Lespedeol A (I)——The dried bark of *Lespedeza homoloba* NAKAI (powder 1.8 kg) was extracted two times with methanol at room temperature. The methanolic extracts were concentrated (476) and partitioned into an ether–H_2O mixture. The etherial layer was concentrated (121 g) and chromatographed on a celite column and separated to the fractions eluted with *n*-hexane (20 g), *n*-hexane–benzene (3: 1) (21 g), benzene (50 g), ether (20 g) and ethylacetate (2 g) as the solvents. The benzene fraction was rechromatographed on a silica gel column developing with benzene, benzene–CH_2Cl_2, CH_2Cl_2 and CH_2Cl_2–acetone successively. The eluate with benzene–CH_2Cl_2 was recrystallized from CH_2Cl_2 to give about 8 g of I as light yellow crystals, mp 146.5—150°. *Anal.* Calcd. for $C_{25}H_{28}O_6$: C, 70.74; H, 6.65. Found: C, 70.72; H, 6.73. IR ν_{max}^{KBr} cm^{-1}: 3480, 3380, 3250, 1620, 1600, 970, 820. UV (Fig. 1). MS (Chart 3).

Trimethyllespedeol A (III)——To an etherial solution of I (400 mg) containing a small amount of MeOH was added an etherial solution of CH_2N_2 under cooling. The reaction mixture was stirred at room temperature for two days and then concentrated. The residue was chromatographed on a silica gel column using benzene. The fraction first eluted, gave 233 mg of III as a pale yellow oil. *Anal.* Calcd. for $C_{28}H_{34}O_6$: C, 72.08; H, 7.34. Found: C, 72.09; H, 7.28. NMR (Fig. 3).

Tetrahydrolespedeol A (II)——I (500 mg) was hydrogenated with 5% Pd–C (50 mg) as a catalyst in EtOH (30 ml) at room temperature. The catalyst was filtrated off and the filtrate was concentrated to give II which was recrystallized from benzene to colorless powder, mp 104—106°. It was difficult to obtain as a state of the benzene free, but it showed a single spot (Rf=0.50) on a thin-layer chromatogram (TLC) of silica gel using benzene–EtOH (5:1). IR ν_{max}^{KBr} cm^{-1}: 3350, 1620, 1580, 800. NMR (C_5D_5N, τ) 7.00 (2H, br, t) Ar–CH_2–CH_2–.

Tetrahydrotrimethyllespedeol A (IV)——To an etherial solution of II (560 mg) containing a small amount of MeOH was added an etherial solution of CH_2N_2 under cooling. The reaction mixture was stirred at room temperature for two days and then concentrated. The residue was chromatographed on a silica gel column using benzene. The fraction first eluted, gave 430 mg of IV as a light yellow oil. *Anal.* Calcd. for $C_{28}H_{38}O_6$: C, 71.46; H, 8.14. Found: C, 71.46; H, 8.06. UV (Fig. 1). NMR ($CDCl_3$, τ): 7.45 (2H, t, J=7.5 cps) Ar–CH_2–CH_2–, 6.16 (3H, s) –OCH_3, 6.21 (6H, s) 2 –OCH_3.

This compound (IV) was also obtained on the hydrogenation of III by the same method described above in the formation of II.

Dehydrogenation of IV——To a solution of IV (425 mg) in Dowtherm A was added 5% Pd–C (200 mg). The mixture was refluxed for 6 hr and then chromatographed on a silica gel column. The eluate with a *n*-hexane–benzene (1: 2) mixture was recrystallized from *n*-hexane–benzene to give 186 mg of 2′,4′,7-trimethoxy-5-hydroxy-6-tetrahydrogeranylisoflavone (V) as pale yellow needles, mp 84—85°. *Anal.* Calcd. for $C_{28}H_{36}O_6$: C, 71.77; H, 7.74. Found: C, 71.46; H, 7.85. UV (Fig. 2).

(3,7-Dimethyloctanoyl)phloroglucinol (VI)——To a mixture of phloroglucinol (9.2 g), nitrobenzene (120 mg) and anhyd. $AlCl_3$ (11.5 g), 3,7-dimethyloctanoyl chloride (11.0 g, bp 100—101° (15 mmHg)) was added dropwise under cooling. The reaction mixture was stirred for 24 hr at room temperature and then treated with ice-dil. HCl and extracted with ether. After washing with H_2O, 1% Na_2CO_3 aq. and H_2O successively, the etherial extract was concentrated and steam-distilled. The residue was purified by means of chromatography to give 10.5 g of VI as a pale yellow oil, which was difficult to obtain a pure state. But it showed a single spot (Rf=0.45) on a TLC: silica gel using benzene.

(3,7-Dimethyloctyl)phloroglucinol (VII)——A mixture of VI (7.0 g), EtOH (100 ml), zinc amalgam (prepared from powdered Zn, 150 g and $HgCl_2$, 8 g) and 12% HCl aq. (280 ml) was allowed to stand overnight at room temperature. After addition of 12% HCl aq. (70 ml), the reaction mixture was refluxed for 2 hr. An additional portion of 12% HCl aq. (70 ml) was then added and refluxing was continued for 4 hr. The mixture was cooled and filtrated to remove inorganic substances and the filtrate was extracted with ether. The residue obtained by the concentration of the etherial extract was recrystallized from ligroine to give 5.2 g of VII as pale yellow powder, mp 99.5—100°. *Anal.* Calcd. for $C_{16}H_{26}O_3$: C, 72.14; H, 9.84. Found: C, 71.92; H, 9.75.

2-(3,7-Dimethyloctyl)-4-(2,4-dimethoxyphenylacetyl)phloroglucinol (VIII)——A solution of VII (3.5 g), 2,4-dimethoxybenzyl cyanide (3 g) and $ZnCl_2$ (1.21 g) in ether (75 ml) was saturated with dry HCl gas (for 25 min) under cooling and allowed to stand in a refrigerator for 2 days. A ketimine hydrochloride (6.1 g) was separated out from the reaction mixture as pale yellow crystals, mp 214—217° (decomp.).

A mixture of the ketimine hydrochloride (6 g), dioxane (30 ml), H_2O (30 ml) and conc. HCl (4 ml) was heated at 70° for 3 hr to give 4.8 g of VIII which was recrystallized from benzene-ligroine to yellowish brown crystals, mp 157—158°. *Anal.* Calcd. for $C_{26}H_{36}O_6$: C, 70.28; H, 8.16. Found: C, 70.39; H, 8.10.

NII-Electronic Library Service

6-(3,7-Dimethyloctyl)-2-ethoxycarbonyl-2′,4′-dimethoxy-5,7-dihydroxyisoflavone (IX) and 8-(3,7-Dimethyloctyl)-2-ethoxycarbonyl-2′,4′-dimethoxy-5,7-dihydroxyisoflavone (X)——To a solution of VIII (300 mg) in pyridine (1.5 ml), ethoxalyl chloride (0.4 ml) was added dropwise under cooling in an ice–water bath with stirring and stirring was continued for 2 hr at room temperature. Then the mixture was treated with ice-dil. HCl and extracted with ether. The etherial extract was concentrated and the resulting residue was dissolved in a glacial acetic acid (10 ml) containing 10% of conc. HCl. The solution was heated at 70° for 1 hr, diluted with H_2O, and extracted with ether to give two products which were separated by means of column chromatography using silica gel as the adsorbent. The fraction eluted with benzene recrystallized from *n*-hexane to give 95 mg of IX as yellow crystals, mp 99.5—100°. *Anal.* Calcd. for $C_{30}H_{38}O_8$: C, 68.48; H, 7.25. Found: C, 68.70; H, 7.15. IR ν_{max}^{KBr} cm^{-1}: 1723 (–COOEt). The fraction eluted with benzene containing 3% of acetone gave 203 mg of X as a yellowish orange oil. IR ν_{max}^{KBr} cm^{-1}: 1720 (–COOEt).

6-(3,7-Dimethyloctyl)-2′,4′-dimethoxy-5,7-dihydroxyisoflavone (XI)——A mixture of IX (450 mg), acetone (18 ml) and 8% Na_2CO_3 aq. (50 ml) was refluxed for 4 hr. After removal of acetone, the reaction mixture was acidified with dil. HCl and extracted with ether. The etherial extract was concentrated and the residue was recrystallized from benzene to give 436 mg of an acid as orange prisms, mp 135—136°. A mixture of the acid (350 mg), quinoline (1.5 ml) and powdered copper (150 mg) was heated at 160—170° until to cease the evolution of carbon dioxide. The reaction mixture was chromatographed on a silica gel column and the quinoline used as the solvent was eluted with *n*-hexane. The eluate with benzene–*n*-hexane was recrystallized from benzene–*n*-hexane to give 259 mg of XI as light yellowish needles, mp 159—160°. *Anal.* Calcd. for $C_{27}H_{34}O_6$: C, 71.34; H, 7.54. Found: C, 71.56; H, 7.65. Gibbs test showed a positive blue color.

8-(3,7-Dimethyloctyl)-2′,4′-dimethoxy-5,7-dihydroxyisoflavone (XII)——This compound was prepared in the same way as XI. From the ester X (655 mg), 430 mg of XII was obtained as light yellowish needles (from *n*-hexane–benzene), mp 161—161.5°. *Anal.* Calcd. for $C_{27}H_{34}O_6$: C, 71.34; H, 7.54. Found: C, 71.35; H, 7.51. Gibbs test gave no coloration.

6-(3,7-Dimethyloctyl)-2′,4′,7-trimethoxy-5-hydroxyisoflavone (XIII)——To an etherial solution of XI (110 mg) containing a small amount of MeOH was added an etherial solution of CH_2N_2. The reaction mixture was stirred for two days at room temperature and concentrated. The residue was chromatographed on a silica gel column to give 48 mg of XIII which was recrystallized from *n*-hexane–benzene to light yellowish needles, mp 84—85°. *Anal.* Calcd. for $C_{28}H_{36}O_6$: C, 71.77; H, 7.74. Found: C, 71.99; H, 7.63. The mixed melting point of XIII and V showed no depression (mixed mp 84—85°) and XIII gave completly superimposable IR, UV and NMR spectral curves with those of V.

8-(3,7-Dimethyloctyl)-2′,4′,7-trimethoxy-5-hydroxyisoflavone (XIV)——This compound (73 mg) was prepared from XII (160 mg) by the same way described in the formation of XIII as light yellow needles (from *n*-hexane–benzene), mp 84.5—85°. *Anal.* Calcd. for $C_{25}H_{36}O_6$: C, 71.77; H, 7.74. Found: C, 71.74; H, 7.81. The mixed melting point of XIV and V showed remarkable depression (mixed mp 62—71°).

Acknowledgement The authors wish to express their sincere thankness to Prof. A Tatematu of Faculty of Pharmacy, Meijo University for the measurements of the mass spectra, and to Dr. M. Kondo and Dr. H. Kuwano of Central Research Laboratories, Sankyo Co., Ltd. for the measurements of the NMR (100 MHz) and N.O.E.

They are also indebted to all the staffs of Analytical Center of this College for elemental analyses and spectral measurements.

NII-Electronic Library Service