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Studies on the Constituents of *Lespedeza homoloba* Nakai. III.[1] The Structures of Lespein and Lespedezin

Akira Ueno, Masahiro Ichikawa, Seigo Fukushima, Yasuhisa Saiki, Tadataka Noro, Kunio Morinaga[2a] and Harumitsu Kuwano[2b]

Shizuoka College of Pharmacy[2a] and Central Research Laboratories, Sankyo Co., Ltd.[2b]

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Two new coumaranochromane derivatives named lespein and lespedezin were isolated from the bark of *Lespedeza homoloba* Nakai. On the base of the chemical and spectral data, their structures were established as (−) lespein: 6*a*, 10-diisopentenyl-3,9-dihydroxy-(6*aR*, 11*aR*)pterocarpan and lespedezin: 10-geranyl-3,9-dihydroxypterocarpan.

In the previous paper, we reported the structures of lespedeol A[3] and lespedeol B,[1] which were isolated from the bark of *Lespedeza homoloba* Nakai. In our further studying on the constituents of this plant, two new coumaranochromane derivatives named lespein (I), $C_{25}H_{28}O_4$ (M=392) and lespedezin (II), $C_{25}H_{28}O_4$ (M=392) were isolated from the *n*-hexane–benzene (3: 1) fraction.

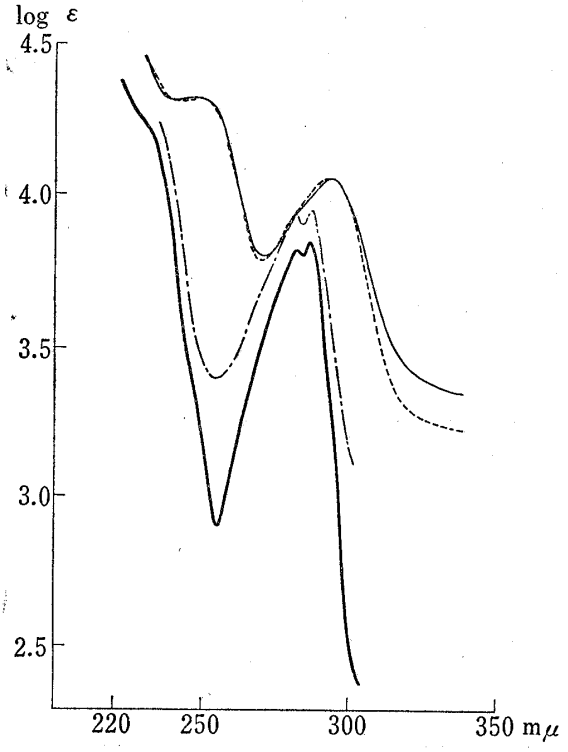


Fig. 1. The UV Spectra of Lespein (——— in EtOH; —— in Alkaline Solution) and Lespedezin (—·—·— in EtOH; ------ in Alkaline Solution)

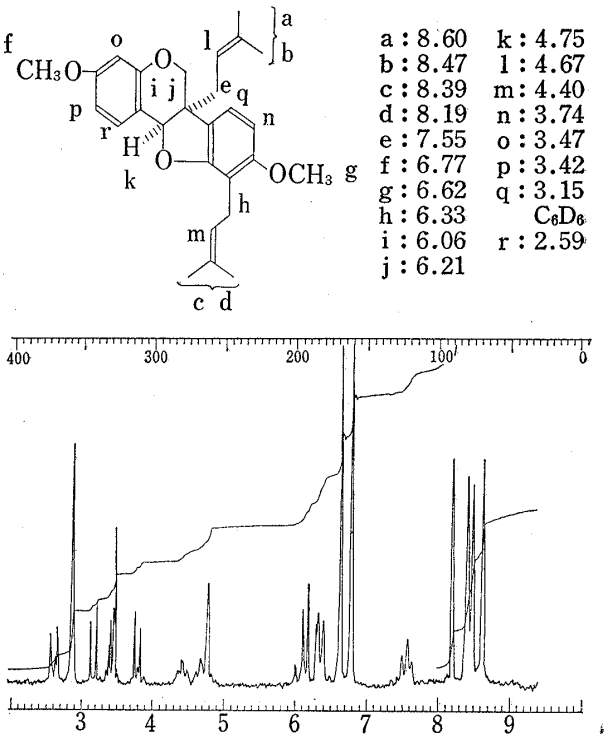


Fig. 2. The NMR Spectrum of Dimethyllespein (VI) in C_6D_6 (100 MHz; τ Values)

1) Part II: A. Ueno, M. Ichikawa, S. Fukushima, Y. Saiki and K. Morinaga, *Chem. Pharm. Bull.* (Tokyo), **21**, 2712 (1973).
2) Location: *a*) *2-2-1, Oshika, Shizuoka*; *b*) *Hiromachi 1-2-58, Shinagawa-ku, Tokyo.*
3) A. Ueno, M. Ichikawa, T. Miyase, S. Fukushima, Y. Saiki and K. Morinaga, *Chem. Pharm. Bull.* (Tokyo), **21**, 1734 (1973).

Both I and II gave a positive ferric chloride reaction, a negative Gibbs' test and a negative magnesium-hydrochloric acid reaction. The infrared (IR) spectra of I and II showed the presence of a hydroxyl group (3400 cm^{-1}) and an aromatic ring (1616, 1592 cm^{-1}), and were closely similar to each other. The ultraviolet (UV) spectra of I and II are similar to those of phaseollidin and demethylhomopterocarpin,[4] and show the same shift in the alkaline solution as shown in Fig. 1. These facts suggest that both I and II are coumaranochromane derivatives having two hydroxyl groups at the 3 and 9 position.

On the acetylation with acetic anhydride–pyridine, I and II gave diacetyllespein (III), $C_{29}H_{32}O_6$ and diacetyllespedezin (IV), $C_{29}H_{32}O_6$, respectively. On the catalytic hydrogenation with 5% palladium on charcoal, III gave tetrahydrodiacetyllespein (V), $C_{29}H_{36}O_6$. On the methylation with dimethyl sulfate and potassium carbonate, I and II gave dimethyllespein (VI), $C_{27}H_{32}O_4$ and dimethyllespedezin (VII), $C_{27}H_{32}O_4$, respectively. On the other hand, I gave monomethyllespein (VIII), on the methylation with diazomethane. On the catalytic hydrogenation with 5% palladium on charcoal, VIII gave tetrahydromonomethyllespein (IX), $C_{26}H_{34}O_4$.

The nuclear magnetic resonance (NMR) spectrum of VI (in hexadeuterobenzene; Fig. 2) showed four singlets at τ 8.60, 8.47, 8.39 and 8.19 (CH_3–C= 4), a multiplet at τ 7.55 (–CH_2–C=), a doublet at τ 6.33 (J=7 Hz, Ar–CH_2–C=), a signal at τ 4.67 (–CH=), and a broad triplet at τ 4.40 (–CH=), due to side chains which were presumed to be two isopentenyl groups. The multiplet at τ 7.55 was transformed into two broad doublets by the decoupling with the proton at τ 4.67 and was observed as AB in the protons of ABX splitting pattern. On the other hand, this methylene signal showed a doublet at τ 7.61 (2H, J=7 Hz) in the case of the carbon tetrachloride solution. This phenomenon is interpreted as a hindered of the free rotation of an isopentenyl group by the solvent effect under a condition of the hexadeuterobenzene solution. On the NMR spectrum of V, the signals of the side chains shifted to higher field than those of III, and a methylene signal adjacent to an aromatic ring was found at τ 7.60 (2H, br. t, J=7.6 Hz). On the base of the chemical shift and coupling constant values of two methylene signals, two isopentenyl groups were suggested to attach one to the aromatic ring and the other to a tertiary carbon atom.

The proton signals of the 6 and 11*a* position in pisatin[5] and kakkonein[6] have been assigned as shown in Table I. As the proton signals adapted these oxygen heterocyclic rings, the signals at τ 6.06 and 6.21 (each 1H, d, J=11.5 Hz) corresponded to the 6 position, and at

Table I. Chemical Shifts (τ-Values) and Coupling Constants of the Heterocyclic Ring Protons

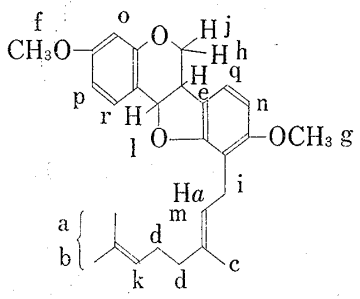
Compound	Position on carbon skeleton	
	11*a*	6
Pisatin[5]		6.01, 6.10 (each d, J=11.8 Hz)
Kakkonein[6]	4.74	5.86, 5.99 (each d, J=12 Hz)

τ 4.75 (1H, s) adjusted to the 11*a* position are found in the NMR spectrum of VI. These signals are similar to those of kakonein, especially with the splitting pattern. Therefore, it is considered that lespein is a pterocarpan derivative having two isopentenyl groups at

4) D.R. Perrin and C.P. Whittle, *Tetrahedron Letters*, **1972**, 1673.
5) D.D. Perrin and D.R. Perrin, *J. Am. Chem. Soc.*, **84**, 1922 (1962); D.R. Perrin and W. Bottomley, *ibid.*, **84**, 1919 (1962).
6) T. Takizawa, Y. Nishikawa and S. Shibata, "Symposium paper of the 90th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo," II, 1970, p. 206. The spectral data were presented by S. Shibata's private communication.

the 6*a* position and at an aromatic ring.

The NMR spectrum of dimethyllespedezin (VII; Fig. 3) showed three singlets at τ 8.56, 8.46 and 8.13 (3 CH_3–C=), a broad singlet at τ 7.96 (4H, =C–CH_2–CH_2–C=), a doublet at τ 6.34 (2H, d, J=7 Hz, Ar–CH_2–C=), a signal at τ 4.86 (–CH=) and a broad triplet at τ 4.36 (–CH=),



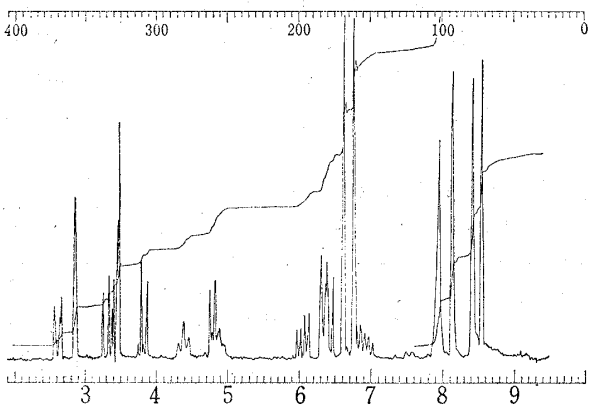
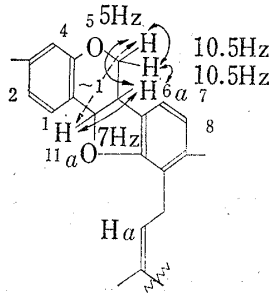


Fig. 3. The NMR Spectrum of Dimethyllespedezin (VII) in C_6D_6 (100 MHz; τ Values)



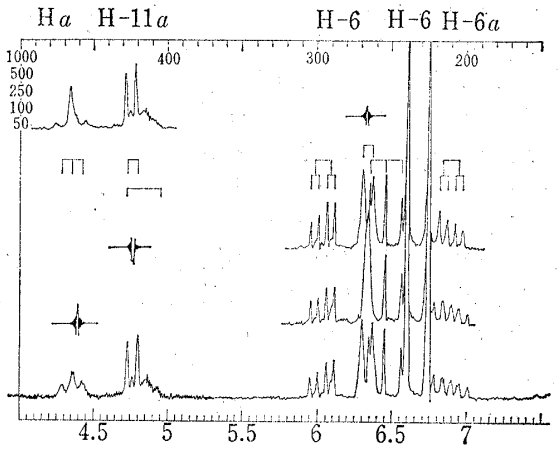


Fig. 4. The Proton Signals of 6, 6*a* and 11*a* position observed in the NMR Spectrum of Dimethyllespedezin (100 MHz; τ Values; in C_6D_6)

due to the side chain. The coupling constant between H*a* and methyl group is about 1 Hz, on the other hand, that of H*a* and methylene group (=C–CH_2–CH_2–CH=) is nearly zero. The chemical shifts of H*a*, methyl group and two methylene groups are closely similar to those of trimethyllespedeol A. Therefore, it is presumed that lespedezin has a geranyl group at an aromatic ring as the side chain.

Figure 4 shows the proton signals on the oxygen heterocyclic rings in the NMR spectrum of VII. The proton at the 6*a* position splitted clearly to a double-doublet (J=10.5, 5 Hz) by the decoupling with the proton at the 11*a* position (τ 4.77, d, J=7 Hz). These coupling constant values are nearly in agreement with those of pterocarpin and coumaranochromane derivatives.[7,8] Consequently, it is considered that lespedezin is a pterocarpan derivative having a geranyl group at the aromatic ring.

The peaks due to the retro Diels–Alder type fragmentation are either very weak or not observed at all in the mass spectra of pterocarpan derivatives, by which they are characterized.[9] Reasonably, such fragmentation could not be found in the mass spectra of lespein, lespedezin and their derivatives. On the mass spectrum of I (Fig. 5), the peak of *m/e* 323 (M^+-69) shows the loss of the isopentenyl group at the 6*a* position. The significant peak of *m/e* 267 (323—56) shows the benzyl-fission of the isopentenyl group attached to the aromatic

7) K.G.R. Pachler and W.G.E. Underwood, *Tetrahedron*, **23**, 1817 (1967).
8) K.K. Purushothaman, V.M. Kishore and V. Narayanswami, *J. Chem. Soc.*, (*C*), **1971**, 2420.
9) A. Pelter, P. Stainton and M. Barber, *J. Heterocyclic. Chem*, **2**, 262 (1965); *idem*, *ibid.*, **2**, 267 (1965).

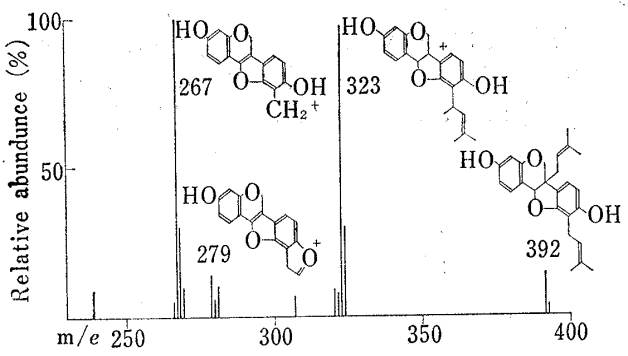


Fig. 5. The Mass Spectrum of Lespein

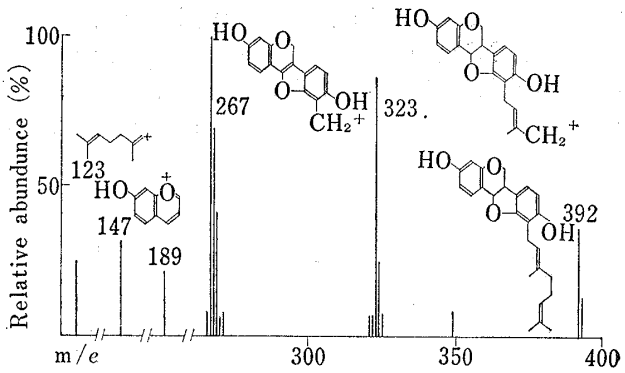


Fig. 6. The Mass Spectrum of Lespedezin

ring and the loss of the proton at the 11*a* position from the peak of *m/e* 323. Figure 6 shows the mass spectrum of lespedezin, and the molecular ion is found at *m/e* 392 (37%). The peak of *m/e* 323 shows the fragment of isopentenyl part in the geranyl group. The significant peak of *m/e* 267 shows the β-cleavage of the side chain (geranyl group) and the losses of two protons at the 6*a* and 11*a* position. The peak of *m/e* 123 shows the fragment due to β-cleavage of the side chain. These mass spectral findings supported the foregoing presumption on the structures of lespein and lespedezin.

Table II shows the NMR spectral data reported on the aromatic protons of coumarano-chromane derivatives having hydroxyl or methoxyl groups at the 3 and 9 position. The proton signal at the 1 position is found in the lower field than that of the 7 position because of the effect by the etherial oxygen atom at the 11 position. Table III shows the chemical shifts of the aromatic protons assigned on lespein, lespedezin and their derivatives in the NMR spectra. The proton signals are observed in order of lower field as H-1, H-7, H-2, H-4,

Table II. Aromatic Proton Signals of Pterocarpan Derivatives in the NMR Spectra (60 MHz; τ-Values, Solvent: $CDCl_3$)

Compound	Position					
	H-1	H-2	H-4	H-7	H-8	H-10
Phaseollidin[4]	2.63	3.47	3.60	3.07	3.64	
Phaseollin[4]	2.63	3.46	3.59	3.08	3.68	
Homopterocarpin[13]	2.64	3.44	3.60	2.95	3.95	3.63
Pterocarpin[13]	2.69	3.46	3.62	3.40		3.66

Table III. Aromatic Proton Signals of Lespein, Lespedezin and Their Derivatives in the NMR Spectra (60 MHz; τ-Values)

Compound	Solvent	Position				
		H-1	H-2	H-4	H-7	H-8
I	CCl_4	2.79	3.64	3.73	3.25	3.80
III	CCl_4	2.67	3.38	3.43	3.15	3.59
V	CCl_4	2.66	3.35	3.43	3.20	3.60
VI	CCl_4	2.71	3.50	3.68	3.20	3.75
VIII	CCl_4	2.63	3.43	3.61	3.19	3.76
II	CCl_4	2.69	3.58	3.66	3.14	3.71
IV	$CDCl_3$	2.63	3.37	3.45	3.12	3.62
VII	CCl_4	2.69	3.51	3.69	3.16	3.72
VII	C_6D_6	2.60	3.42	3.45	3.28	3.92

H-10 and H-8. A signal at τ 2.79 (d, J=8.5 Hz; H-1) corresponded to the 1 position in I shows the presence of a hydroxyl group at the 3 position and the coupling constant suggests that there is not any substituent at the *ortho* (2) position. A signal at τ 3.25 (d, J=8.0 Hz; H-7) in I exists in the lower field among the aromatic potons. The adaptable proton is considered to occupy the *meta* position in resorcinol type phenolic compound, whose aromatic proton signals move in shifting values of *meta*: −0.08, *ortho*: −0.17 and *para*: −0.30 ppm by the acetylation of the hydroxyl group.[10] The foregoing signals (H-1 and H-7) in I are no difference in the chemical shifts between those of the acetyl derivatives (III and V), and so these protons exist at the *meta* position in each resorcinol nucleus. The presence of proton signals at τ 3.64 (d-d, J=8.5, 2.5 Hz; H-2) and at τ 3.73 (d, J=2.5 Hz; H-4) in I suggests that lespein has not a side chain at the A ring. Two proton signals (H-7 and τ 3.80, d, J=8.0 Hz; H-8) in the D ring exist in coupling with *ortho* to each other. On the other hand, I gave a negative Gibbs' test. Therefore, two substituents in the D ring are considered to exist as a hydroxyl group at the 9 position and a isopentenyl group at the 10 position.

On the NMR spectrum of VI (Fig. 7), two pairs of the proton signals coupling with *ortho* to each other are found at τ 2.71 (H-1): 3.50 (H-2) and τ 3.20 (H-7): 3.75 (H-8). The Nuclear Overhauser effect (NOE) was observed at H-4, H-8 (about +14%) and at H-2 (+7%) by the irradiation of methyl signals of two methoxyl groups. By the assignment of these NMR

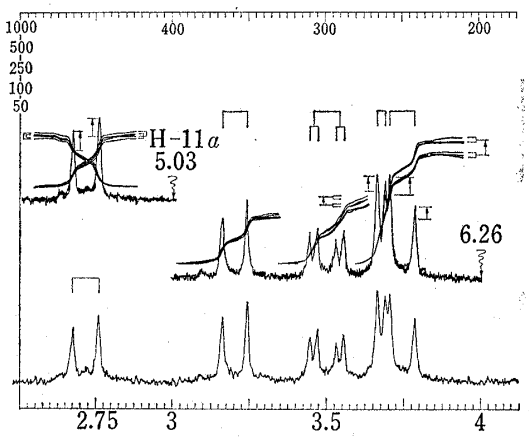


Fig. 7. The NOE of Aromatic Protons observed in the NMR Spectrum of VI (100 MHz; τ Values; in CCl_4)

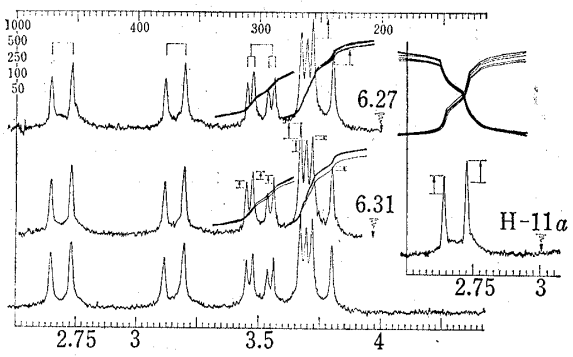


Fig. 8. The NOE of Aromatic Protons observed in the NMR Spectrum of VII (100 MHz; τ Values; in CCl_4)

spectra, the structure of lespein is deduced to be 6*a*, 10-diisopentenyl-3,9-dihydroxypterocarpan.

The aromatic proton signals of lespedezin and its derivatives are similar to those of lespein as shown in Table III. On the NMR spectrum of VII (Fig. 8), two pairs of the proton signals coupling with *ortho* to each other are found at τ 2.69 (H-1): 3.51 (H-2) and at τ 3.16 (H-7): 3.72 (H-8). Although these signals could not be divided clearly the signal of H-4 from that of H-8 because of neighborhood in their chemical shifts, the NOE was observed at H-2 (+5.7%) and at H-4 (+9.5%) by the irradiation of the signal of methyl group (OCH_3; τ 6.30), at H-8 (+4.4%) by that of the methyl group (OCH_3; τ 6.27), and at H-1 (+3.7%) by that of 11*a*-H.

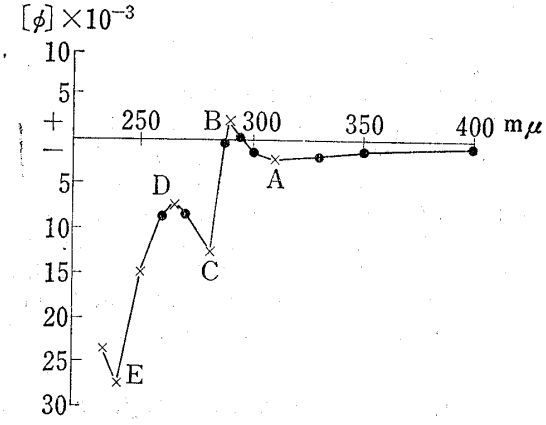


Fig. 9. ORD Curve for (−) Dimethyllespein in EtOH

A: − 2180 (310 mμ), B: +2340 (290 mμ), C: −12500 (282 mμ), D: −7200 (265 mμ), E: −27500 (240 mμ).

On the basis of the findings which are acquired from the chemical and spectral data of lespedezin and its derivatives, the structure of lespedezin is established as 10-geranyl-3,9-dihydroxypterocarpan.

Ordinarily, naturally occurring pterocarpan derivatives except (+) homopisatin and (+) sophojapnicin, have a large negative specific rotation. Lespedezin is optically inactive, and so it is considered to exist as a racemic compound. The absolute configuration at the 11*a* position in trifolirhizin[11] has been established as (*R*) and the juncture of B and C ring is able only *cis*, and so the configuration at 6*a* exists as (*R*). Numerous pterocarpan derivatives have been reported in the same absolute configuration on the base of their optical rotatory dispersion (ORD) curves.[12] As shown in Fig. 9, the ORD curve of dimethyllespein is similar to those[13] of (−) pterocarpin and (−) homopterocarpin. Therefore, the absolute configuration at 6*a* and 11*a* in (−) lespein is presumed to be 6*a* *R*, 11*a* *R*.

Experimental

All melting points are uncorrected. UV spectra were measured using a Hitachi recording spectrometer EPS-032 type. IR spectra were determined on NaCl plates (liquid) using a Hitachi infrared spectrometer EPI-G21 type. NMR spectra were taken at 60 MHz and 100 MHz with TMS as an internal standard using a JNM-C-60H and Varian HA-100 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 Lespein (I)——The dried bark (1.8 kg) of *Lespedeza homoloba* Nakai was extracted with MeOH and the extract (476 g) was treated by the method reported in the previous paper.[3] The *n*-hexane–benzene (3:1) fraction (21 g) was chromatographed on a silica gel column and the eluate with *n*-hexane–benzene (1:20) gave about 1.1 g of an oily substance. This was repeatedly purified by the thin-layer chromatography (TLC) (silica gel; $CHCl_3$: AcOEt=7:1) to give I of 200 mg as a colorless viscous oil which could not be crystallized. $[\alpha]_D^{23}=-71°$ ($CHCl_3$, $c=1.2$). $FeCl_3$ reaction (+), Mg+HCl reaction (−), Gibbs' test (−). *Anal.* Calcd. for $C_{25}H_{28}O_4$: C, 76.50; H, 7.19. Found: C, 76.66; H, 7.11. IR ν_{max}^{film} cm^{-1}: 3400, 1616, 1592. UV (Fig. 1). NMR (60 M; CCl_4, $c=20\%$): 8.49, 8.31, 8.28, 8.23 (each 3H, s), 7.60 (2H, d, $J=7.5$ Hz), 6.75 (2H, d, $J=7.5$ Hz), 6.45 (1H, d, $J=11$ Hz), 6.08 (1H, d, $J=11$ Hz), 5.03 (1H, s), 4.80 (2H, m).

Isolation of Lespedezin (II)——On the foregoing column chromatography of the *n*-hexane–benzene (3:1) fraction, the eluate (0.9 g) with benzene was repeatedly purified by the TLC (silica gel; $CHCl_3$: AcOEt=7:1) to give II of 200 mg as a colorless viscous oil which could not be crystallized. $[\alpha]_D^{23}=0$ ($CHCl_3$, $c=0.65$). $FeCl_3$ reaction (+), Mg+HCl reaction (−), Gibbs' test (−). *Anal.* Calcd. for $C_{25}H_{28}O_4$: C, 76.50; H, 7.19. Found: C, 76.59; H, 7.35. IR ν_{max}^{film} cm^{-1}: 3400, 1616, 1592. UV (Fig. 1). NMR (60 M; CCl_4, $c=15\%$): 8.45, 8.36, 8.27 (each 3H, s), 8.00 (4H, br. s), 6.70 (2H, d, $J=7.5$ Hz).

Diacetyllespein (III)——To a solution of I (60 mg) in pyridine (1 ml), acetic anhydride (1 ml) was added with stirring. After standing for 16 hr, the reaction mixture was treated with ice–water and extracted with ether. The etherial extract was concentrated and the residue was purified by the TLC (silica gel; benzene) to give 33.5 mg of III as a colorless viscous oil which showed a single spot of *Rf* 0.67 on the TLC (silica gel; benzene). IR ν_{max}^{film} cm^{-1}: 1750 (-OCOCH$_3$). NMR (60 M; CCl_4, $c=10\%$): 8.03 (6H, s, 2CH$_3$-CO).

Diacetyllespedezin (IV)——II (60 mg) was acetylated in the same way as I and the product was purified by the TLC (silica gel; benzene) to give 35 mg of IV as a colorless viscous oil. *Anal.* Calcd. for $C_{29}H_{32}O_6$: C, 71.66; H, 7.13. Found: C, 71.69; H, 6.92. IR ν_{max}^{film} cm^{-1}: 1755 (-OCOCH$_3$). NMR (60 M; $CDCl_3$): 8.49, 8.42, 8.33 (each 3H, s), 8.09 (4H, br. s), 7.85 (6H, s, 2CH$_3$-CO).

Tetrahydrodiacetyllespein (V)——III (30 mg) was hydrogenated with 5% Pd-C (50 mg) as a catalyst in EtOH (10 ml) at room temperature. The catalyst was filtered off and the filtrate was concentrated. The residue was purified by the TLC (silica gel; benzene) to give 19.6 mg of V as a colorless oil. *Anal.* Calcd. for $C_{29}H_{36}O_6$: C, 72.47; H, 7.55. Found: C, 72.72; H, 7.73. IR ν_{max}^{film} cm^{-1}: 1755 (-OCOCH$_3$). NMR (60 M; CCl_4, $c=27\%$): 9.07, 9.15 (12H, br. s), 7.80 (6H, s, CH$_3$-CO), 7.60 (2H, t, $J=7.6$ Hz). Mass Spectrum *m/e*: $M^+=480$; 438, 396, 367, 325, 267, 223, 205, 204, 105,

10) R.J. Highet and P.F. Hight, *J. Org. Chem.*, **30**, 902 (1967); K.G.R. Pachler and D.G. Roux, *J. Chem. Soc.*, (*C*), **1967**, 604.
11) H. Suginome and T. Iwadare, *Experimentia*, **18**, 163 (1962).
12) G.J.H. Rall, J.P. Eugelbrecht and A.J. Brink, *Tetrahedron*, **26**, 5007 (1970); H. Suginome, *Bull. Chem. Soc. Japan*, **39**, 1544 (1966).
13) A. Pelter and P.I. Amenechi, *J. Chem. Soc.*, **1969**, 887.

Dimethyllespein (VI)——A mixture of I (60 mg), K_2CO_3 (3 mg), Me_2SO_4 (0.7 mg) and dry acetone (20 ml) was refluxed for 5 hr and then filtered. The filtrate was concentrated and the residue was purified by the TLC (silica gel; benzene) to give 20 mg of VI as a colorless oil. *Anal.* Calcd. for $C_{27}H_{32}O_4$: C, 77.11; H, 7.67. Found: C, 76.94; H, 7.59. IR ν_{max}^{film} cm^{-1}: 1616, 1580, 1435, 1265, 1155, 1130, 785, 730.

Dimethyllespedezin (VII)——This compound was prepared in the same way as VI. From II (200 mg), 140 mg of VII was obtained as a colorless oil. *Anal.* Calcd for $C_{27}H_{32}O_4$: C, 77.11; H, 7.67. Found: C, 76.97; H, 7.50. IR ν_{max}^{film} cm^{-1}: 1616, 1580, 1440, 1265, 1155, 1130, 785, 752.

Monomethyllespein (VIII)——To an etherial solution of I (200 mg), was added an excess etherial solution of CH_2N_2. The reaction mixture was allowed to stand for 20 hr and then concentrated. The residue was purified by the TLC (silica gel; benzene) to give 106 mg of VIII as a light yellow oil which showed a single spot of *Rf* 0.40 on the TLC (silica gel; benzene). IR ν_{max}^{film} cm^{-1}: 3420, 1610, 1580, 825, 790. NMR (60 M; CCl_4, c=15%): 6.23 (3H, s, CH_3-O-).

Tetrahydromonomethyllespein (IX)——VIII (80 mg) was hydrogenated with 5% Pd-C (50 mg) as a catalyst in EtOH (20 ml) at room temperature. The catalyst was filtered off and the filtrate was concentrated. The residue was purified by the TLC (silica gel; benzene) to give 45 mg of IX as a colorless viscous oil. *Anal.* Calcd. for $C_{26}H_{34}O_4$: C, 76.06; H, 8.34. Found: C, 75.95; H, 8.25. NMR (60 M; CCl_4, c=8%): 6.20 (3H, s, CH_3-O-), 7.50 (2H, br. t, Ar-CH_2-). Mass Spectrum *m/e*: M^+=410; 339, 281.

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