

Oleanene-Type Triterpene Glycosides from *Puerariae Radix*. III.¹⁾ Three New Saponins from *Pueraria thomsonii*

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From the root of *Pueraria thomsonii* (Leguminosae), three new oleanane-type triterpene glycosides, named kudzusaponin B₁, acetyl-kaikasaponin III and acetyl-soyasaponin I were isolated, together with soyasaponin I (4) and subproside V (5). Their structures were determined to be 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-galactopyranosyl-(1 $\rightarrow$ 2)- β -D-glucuronopyranosyl kudzusapogenol B (1), 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-galactopyranosyl-(1 $\rightarrow$ 2)- β -D-glucuronopyranosyl sophoradiol 22-*O*-acetate (2) and 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-galactopyranosyl-(1 $\rightarrow$ 2)- β -D-glucuronopyranosyl soyasapogenol B 22-*O*-acetate (3), respectively.

Key words *Pueraria thomsonii*; Leguminosae; triterpene; saponin; kudzusaponin

Puerariae Radix, the root of *Pueraria lobata* OHWI or *Pueraria thomsonii* BENTHUM, is one of the most important Oriental crude drugs used as a perspiration, antipyretic and antispasmodic agent.²⁾ During the course of our studies on the constituents of these plants,³⁾ we had reported four new oleanene-type triterpene saponins from *P. lobata*.⁴⁾ As a continuing study on the ingredients of *Puerariae Radix*, we have examined the triterpenoidal constituents in the root of *P. thomsonii*. This paper deals with the isolation and structural elucidation of five triterpenoidal saponins.

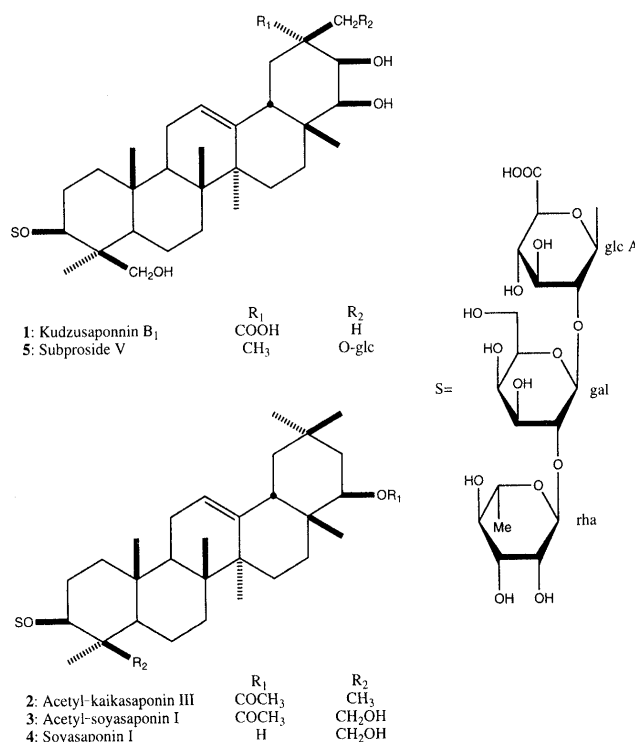
The methanolic extract of the root of *P. thomsonii* was partitioned between 1-BuOH and water. The 1-BuOH layer was concentrated and subjected to normal- and reversed-phase column chromatography to yield compounds 1–5. Compounds 4 and 5 were identified as soyasaponin I⁵⁾ and subproside V,⁶⁾ respectively, by comparison of the ¹H- and ¹³C-NMR spectral data (Tables 1 and 2).

Kudzusaponin B₁ (1) showed an [M+Na]⁺ ion at *m/z* 1011 in the positive ion FAB mass spectrum, which corresponds to the composition C₄₈H₇₆NaO₂₁ ([M+Na]⁺) given by the exact mass measurement under high resolution (HR). The occurrence of six tertiary methyl signals in the ¹H-NMR spectrum suggested 1 to be an oleanane derivative. The sapogenol obtained by acid hydrolysis of 1 was identified as kudzusapogenol B (1a)^{3a)} on TLC. A monosaccharide mixture revealed the presence of glucuronic acid, galactose and rhamnose. Their absolute configurations were determined to be D-form except for rhamnose (L-form), according to the procedure developed by Hara *et al.*⁷⁾ The ¹³C-NMR signals of the sugar part of 1 were identical with those of the β -fabatriosyl moiety⁸⁾ of 4 (Table 2), while the signals due to the aglycone part (Table 1) were in good accordance with those of 1a except for C-2 and -3, which were shifted downfield due to glycosylation.⁹⁾ Therefore, the structure of 1 was elucidated to be 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-galactopyranosyl-(1 $\rightarrow$ 2)- β -D-glucuronopyranosyl kudzusapogenol B. This is the first isolation of a saponin having kudzusapogenol B as an aglycone.

Acetyl-kaikasaponin III (2) showed an [M+Na]⁺ ion

(C₅₀H₈₀NaO₁₈) at *m/z* 991 in the positive ion FAB mass spectrum. The acid hydrolysate of 2 gave the same component sugars as those of 1. A genuine sapogenol (2a) was obtained by enzymatic hydrolysis. In the ¹H-NMR of 2a, an acetyl signal was observed at δ 2.03. Furthermore, the signal due to H-22 of 2a showed a downfield shift (+1.21 ppm) in comparison with that of sophoradiol (2b).¹⁰⁾ Therefore, 2a was concluded to be sophoradiol 22-*O*-acetate.¹¹⁾ Since the ¹³C-NMR signals of the sugar moiety of 2 were superimposable on those of kaikasaponin III (2c),^{12b)} 2 was determined to be 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-galactopyranosyl-(1 $\rightarrow$ 2)- β -D-glucuronopyranosyl sophoradiol 22-*O*-acetate.

Acetyl-soyasaponin I (3) showed an [M+Na]⁺ ion (C₅₀H₈₀NaO₁₉) at *m/z* 1007 in the positive ion FAB mass spectrum. On acid hydrolysis, 3 gave the same component sugars as those of 1 and 2. The aglycone obtained by acid



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Table 1. ^{13}C -NMR Data for Compounds **1**–**5**, **1a**, **2a**, and **2c** (Aglycone Moieties)

C	1	1a	2	2a	2c	3	4	5
1	38.5	38.9	38.8	39.1	38.8	38.5	38.5	38.8
2	26.5	28.4	26.3	28.1	26.5	26.2	26.5	26.3
3	91.1	80.1	89.8	78.1	89.9	91.1	91.2	91.4
4	43.8	43.2	39.7	39.4	39.6	43.6	43.9	43.9
5	55.9	56.3	55.8	55.7	55.8	56.1	56.1	56.2
6	18.5	19.1	18.6	18.7	18.5	18.5	18.5	18.6
7	32.8	33.1	33.3	32.1	33.2	32.9	33.3	33.3
8	40.1	40.3	39.7	40.1	39.9	40.0	39.9	40.0
9	47.6	48.0	47.8	47.9	47.9	47.7	47.8	47.8
10	36.4	37.0	36.5	37.2	36.8	36.6	36.5	36.4
11	24.1	24.2	23.8	23.8	23.7	24.0	24.0	24.0
12	123.1	123.8	123.4	122.9	122.5	122.8	122.4	123.2
13	143.9	143.6	144.2	144.1	144.7	144.1	144.9	144.1
14	42.0	42.0	41.9	41.8	42.3	41.9	42.4	42.3
15	26.5	26.5	26.5	26.2	26.4	26.6	26.5	26.3
16	27.4	27.4	29.9	28.7	28.6	30.0	28.5	28.5
17	39.0	38.9	37.4	36.6	37.9	36.4	38.0	37.9
18	42.8	42.7	44.7	44.8	45.3	44.7	45.2	44.8
19	42.5	42.4	46.1	46.2	46.7	46.1	46.8	42.1
20	49.4	44.9	30.5	30.5	30.8	30.1	30.9	35.0
21	70.8	70.5	38.5	38.5	42.2	38.5	42.4	37.0
22	79.2	79.1	77.7	78.3	75.5	77.7	75.6	75.5
23	22.9	23.5	28.4	28.7	28.6	23.0	22.7	22.9
24	63.6	64.5	15.6	15.8	15.6	63.6	63.6	63.4
25	15.7	16.2	16.8	16.6	16.7	15.8	15.7	15.9
26	16.8	16.9	16.9	17.0	17.1	16.8	17.0	17.1
27	26.5	26.5	26.2	26.3	25.7	26.3	25.7	25.9
28	22.2	22.1	27.1	27.2	28.3	27.1	28.7	21.0
29	181.0	178.7	33.6	33.6	33.2	33.6	33.3	28.7
30	17.0	16.5	21.2	21.2	21.1	21.2	21.2	77.5
C=O			170.0	170.1		170.1		
Me			21.2	21.0		21.2		

Chemical shifts (δ : ppm) were measured in pyridine- d_5 .

hydrolysis of **3** was identified as soyasapogenol B 22-*O*-acetate on TLC. The ^{13}C -NMR signals of the sugar moiety of **3** and rings A–D of the aglycone moiety were superimposable on those of **4**, except for the ring E signals, which were in accordance with those of **2**. Therefore, **3** was concluded to be 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-galactopyranosyl-(1 $\rightarrow$ 2)- β -D-glucuronopyranosyl soyasapogenol B 22-*O*-acetate.

Experimental

The root of *Pueraria thomsonii* was collected in the Hua-Nan region of China. TLC was performed on pre-coated Kieselgel 60F₂₅₄ plates (Merck). Column chromatography was carried out on Kieselgel 60 (70–230 mesh, and 230–400 mesh, Merck), Sephadex LH-20 (Pharmacia), Bondapak C₁₈ (Waters), Chromatorex ODS-DU 3050MT (Fuji Silysia) and MCI gel CHP 20P (Mitsubishi Chemical Ind.). The optical rotations were measured on a JASCO DIP-360 automatic digital polarimeter. ^1H - and ^{13}C -NMR spectra were measured on a JEOL JNM-EX 270 spectrometer and chemical shifts were given on a δ (ppm) scale with tetramethylsilane as an internal standard. FAB-MS were measured on a JEOL DX-300 HF spectrometer. HR FAB-MS were measured on a JEOL SX102A mass spectrometer (JEOL), which was controlled by a JEOL DA-7000 data system.

Extraction and Isolation The root (20 kg) of *P. thomsonii* was extracted with MeOH (60 l) once under reflux. The extract (430 g) was partitioned between 1-BuOH and H₂O. Removal of the solvent from each phase under reduced pressure gave the aqueous (267 g) and 1-BuOH (121 g) extracts. The 1-BuOH extract was subjected to MCI gel CHP 20P column chromatography using 0% $\rightarrow$ 100% MeOH to give fractions 1 to 4. Fractions 2 (9 g, 60% MeOH eluate) and 3 (15 g, 80% MeOH eluate) were further separated by Bondapak C₁₈ (20% $\rightarrow$ 100% MeOH), Chromatorex ODS (30% $\rightarrow$ 100% MeOH), silica gel (1-BuOH:AcOH:

Table 2. ^{13}C -NMR Data for Compounds **1**–**5** and **2c** (Sugar Moieties)

	1	2	2c	3	4	5
glc A-1	105.4	105.3	105.2	105.5	105.5	104.9
glc A-2	78.5	79.1	79.0	78.4	78.5	77.9
glc A-3	76.6 ^{a)}	76.2 ^{a)}	76.1 ^{a)}	76.6 ^{a)}	76.7 ^{a)}	76.8 ^{a)}
glc A-4	73.9	73.5	73.4	73.9	73.9	73.6
glc A-5	77.8 ^{b)}	77.4	77.2	77.7 ^{b)}	77.7 ^{b)}	77.2 ^{b)}
glc A-6	172.4	171.6	172.6	172.3	172.4	175.6
gal-1	101.7	102.0	101.9	101.7	101.7	101.7
gal-2	77.9 ^{b)}	78.8	78.5	77.7 ^{b)}	77.7 ^{b)}	77.8 ^{b)}
gal-3	76.5 ^{a)}	76.2 ^{a)}	76.0 ^{a)}	76.4 ^{a)}	76.5 ^{a)}	75.8
gal-4	71.1	70.5	70.3	71.1	71.1	70.9
gal-5	77.7 ^{b)}	76.6 ^{a)}	76.5 ^{a)}	76.6 ^{a)}	76.7 ^{a)}	76.4 ^{a)}
gal-6	61.5	61.9	61.8	61.6	61.6	61.8
rha-1	102.5	102.7	102.6	102.4	102.5	101.9
rha-2	72.4	72.4	72.2	72.4	72.4	71.9
rha-3	72.8	72.7	72.5	72.8	72.8	71.9
rha-4	74.4	74.3	74.2	74.4	74.4	73.8
rha-5	69.4	69.4	69.3	69.4	69.4	69.3
rha-6	19.0	18.9	18.5	19.0	19.0	18.6
glc-1						104.9
glc-2						74.9
glc-3						77.8 ^{b)}
glc-4						71.2
glc-5						77.8 ^{b)}
glc-6						62.3

a, b) In each vertical column, these may be interchanged.

H₂O=8:1:1) and silica gel (CHCl₃:MeOH:H₂O+0.1% AcOH=7:3:0.5+0.1% AcOH–6:4:1+0.1% AcOH) to provide compounds **1** (7 mg), **2** (13 mg), **3** (4 mg), **4** (14 mg), and **5** (7 mg).

Kudusaponin B₁ (1) A white amorphous powder, $[\alpha]_D^{25}$ –8.7° (c =0.66, pyridine:H₂O=1:1). HR positive ion FAB MS m/z : 1011.4824 (Calcd for C₄₈H₇₆NaO₂₁: 1011.4777). Positive ion FAB MS m/z : 1011 ([M+Na]⁺), 989 ([M+H]⁺), 865 ([M+Na–rha]⁺), 843 ([M+H–rha]⁺), 681 ([M+H–rha–gal]⁺). Negative ion FAB MS m/z : 987 ([M–H][–]), 841 ([M–H–rha][–]), 679 ([M–H–rha–gal][–]), 503 ([M–H–rha–gal–glc A][–]). ^1H -NMR (in pyridine- d_5): 0.71, 0.96, 1.29, 1.29, 1.41, 1.96 (each 3H, s, *tert*-Me $\times$ 6), 1.72 (3H, d, J =6 Hz, rha H-6), 5.33 (1H, brs, H-12), 5.68 (1H, d, J =8 Hz, gal H-1), 6.17 (1H, brs, rha H-1). ^{13}C -NMR: Tables 1 and 2.

Identification of the Saponin of 1 A sample of **1** (1 mg) was hydrolyzed with 2N HCl/MeOH (1 ml) and heated at 60°C for 2 h. The MeOH was evaporated under a N₂ stream. The aglycone was extracted with CHCl₃ and identified as kudusapogenol B methyl ester by TLC. R_f s, 0.35 (CHCl₃:MeOH=19:1), 0.16 (*n*-hexane:acetone=3:1).

Identification of the Sugars of 1–3 A sample of **1** (1 mg) was hydrolyzed with 2N HCl/H₂O (1 ml) and heated at 80°C for 2 h. After the partition between H₂O and CHCl₃, the aqueous layer was neutralized with 2N KOH/H₂O. The sugar mixture was subjected to TLC analysis [TLC, Kieselgel 60F₂₅₄ (Merck Art 5554), CHCl₃:MeOH:H₂O=6:4:1, R_f s: 0.09 (glucuronic acid), 0.28 (galactose), 0.56 (rhamnose). In the above manner, the sugars for **2** and **3** were also confirmed to be composed of the same units.

Determination of the Absolute Configuration of the Component Sugars of 1–3 A sample of **1** (1 mg) was methylated with ethereal CH₂N₂. To a solution of the methylated sample was added NaBH₄, and the mixture was kept at r.t. for 30 min. The reaction mixture was worked up with MCI gel CHP 20P. The MeOH eluate was evaporated and heated in 2N HCl/H₂O at 90°C for 3 h. The hydrolysate was partition between H₂O and CHCl₃. The H₂O layer was treated with Amberlite IRA-400 to give a sugar fraction. This fraction was dissolved in pyridine (0.1 ml), then the solution was added to a pyridine solution (0.1 ml) of L-cysteine methyl ester hydrochloride (0.1 mol/l) and warmed at 60°C for 2 h. The solvent was evaporated under a N₂ stream and dried *in vacuo*. The remaining syrup was trimethylsilylated with trimethylsilylimidazole (0.1 ml) at 60°C for 1 h. After the addition of *n*-hexane and H₂O, the *n*-hexane layer was removed and checked by GC. The retention times (t_R) of the peaks were 10.7 min (L-rhamnose), 15.6 min (D-glucose) and 16.6 min (D-galactose). In the above manner, the absolute configurations of component sugars for **2** and **3** were also the same form as those of **1**.

Acetyl-kaikasaponin III (2) A white amorphous powder, $[\alpha]_D^{25} + 3.6^\circ$ ($c=0.37$, pyridine). HR positive ion FAB MS m/z : 991.5225 (Calcd for $C_{50}H_{80}NaO_{18}$: 991.5242). Positive ion FAB MS m/z : 991 ($[M+Na]^+$). Negative ion FAB MS m/z : 967 ($[M-H]^-$), 821 ($[M-H-rha]^-$), 659 ($[M-H-rha-gal]^-$). 1H -NMR (in pyridine- d_5): 0.85, 0.92, 0.94, 1.07, 1.19, 1.23, 1.42, 1.57, (each 3H, s, *tert*-Me $\times$ 8), 1.77 (3H, d, $J=6$ Hz, rha H-6), 2.08 (3H, s, acetyl), 5.05 (1H, d, $J=7$ Hz, glc A H-1), 5.74 (1H, d, $J=7$ Hz, gal H-1), 6.34 (1H, s, rha-1). ^{13}C -NMR: Tables 1 and 2.

Identification of the Sapogenol of 2 To a solution of **2** (3 mg) in acetate buffer (1 ml) was added glycyrrhizin hydrolase (100 μ l), and the mixture was incubated at 40 $^\circ$ C for 2 d. The hydrolysate was filtered off to yield **2a** (0.5 mg). 1H -NMR (in $CDCl_3$): 0.80, 0.83, 0.90, 0.95, 0.99, 1.01, 1.16, 1.26 (each 3H, s, *tert*-Me $\times$ 8), 2.03 (3H, s, acetyl), 3.23 (1H, dd, $J=5$, 11 Hz, H-3), 4.65 (1H, m, H-22), 5.27 (1H, m, H-12).

Acetyl-soyasaponin I (3) A white amorphous powder, $[\alpha]_D^{25} - 9.7^\circ$ ($c=0.95$, pyridine). HR positive ion FAB MS m/z : 1007.5226 (Calcd for $C_{50}H_{80}O_{19}$: 1007.5192). Positive ion FAB MS m/z : 1007 ($[M+Na]^+$), 986 ($[M+H]^+$). Negative ion FAB MS m/z : 983 ($[M-H]^-$), 675 ($[M-H-rha-gal]^-$). 1H -NMR (in pyridine- d_5): 0.70, 0.93, 1.07, 1.25, 1.25, 1.25, 1.43 (each 3H, s, *tert*-Me $\times$ 7), 1.78 (3H, d, $J=6$ Hz, rha H-6), 2.08 (3H, s, acetyl), 5.27 (1H, s, H-12), 6.28 (1H, s, rha H-1). ^{13}C -NMR: Tables 1 and 2.

Identification of the Sapogenol of 3 A sample of **3** (1 mg) was hydrolyzed with 2 N HCl/MeOH (1 ml) and heated at 60 $^\circ$ C for 2 h. The MeOH was evaporated under a N_2 stream. The aglycone was extracted with $CHCl_3$ and identified to be soyasapogenol B 22-*O*-acetate (**3a**) by TLC. R_f s, 0.44 ($CHCl_3$: MeOH = 19:1), 0.42 (*n*-hexane: acetone = 3:1).

The Chemical Synthesis of 3a To a tetrahydrofuran solution of soyasapogenol B (200 mg/3 ml) was added α, α -dimethoxytoluene (140 μ l) and *p*-toluenesulfonic acid monohydrate (20 mg), and the mixture was kept at r.t. for 30 h. The reaction mixture was worked up with silica gel (*n*-hexane: AcOEt = 5:1) to obtain 3,24-*O*-benzylidene soyasapogenol B (166 mg). To a solution of 3,24-*O*-benzylidene soyasapogenol B (145 mg) in pyridine (2 ml) was added acetic anhydride (100 μ l) and 4-dimethylaminopyridine (0.5 mg), and the mixture was kept at 50 $^\circ$ C for 2 h. The reaction mixture was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ layer was concentrated to yield 3,24-*O*-benzylidene 22-*O*-acetyl soyasapogenol B. To a solution of 3,24-*O*-benzylidene 22-*O*-acetyl soyasapogenol B (5 mg) in acetic acid (400 μ l) was added H_2O (100 μ l), and the mixture was kept at 80 $^\circ$ C for 2 h. The reaction mixture was worked up with silica gel (*n*-hexane: AcOEt = 3:2) to obtain 22-*O*-acetyl soyasapogenol B (2 mg). 1H -NMR (in pyridine- d_5): 0.92, 0.96, 0.96, 0.98, 1.09, 1.22, 1.57 (each 3H, s, *tert*-Me $\times$ 7), 2.08 (3H, s, acetyl), 3.65 (1H, dd, $J=5$, 11 Hz, H-3), 3.72 (1H, d, $J=11$ Hz, H-24ax), 4.53 (1H, d, $J=11$ Hz, H-24eq), 4.88 (1H, m, H-22), 5.27 (1H, m, H-12). ^{13}C -NMR (in pyridine- d_5): 16.2 (C-25), 16.9 (C-26), 19.1 (C-6), 21.0 (C-30), 21.2 (CH_3CO), 23.6 (C-23), 24.1 (C-11), 26.3 (C-2, 27), 27.2 (C-15, 28), 28.4 (C-16), 30.6 (C-20), 33.2 (C-7), 33.6 (C-29), 36.6 (C-17), 37.0 (C-10), 38.5 (C-21), 38.9 (C-1), 40.2 (C-8), 41.9 (C-14), 43.2 (C-4), 44.8 (C-18), 46.2 (C-19), 48.0 (C-9), 56.3 (C-5), 64.6 (C-24), 78.4 (C-22), 80.1 (C-3), 122.9 (C-12), 144.1 (C-13), 170.1 (CH_3CO).

Soyasaponin I (4) A white amorphous powder, $[\alpha]_D^{25} - 7.9^\circ$ ($c=1.46$, pyridine). Negative ion FAB MS m/z : 941 ($[M-H]^-$), 796

($[M-H-rha]^-$), 633 ($[M-H-rha-gal]^-$), 458 ($[M-H-rha-gal-glcA]^-$). 1H -NMR (in pyridine- $d_5 + D_2O$): 0.71, 0.96, 1.00, 1.22, 1.29, 1.29, 1.44 (each 3H, s, *tert*-Me $\times$ 7), 1.79 (3H, d, $J=6$ Hz, rha-6), 4.99 (1H, d, $J=7$ Hz, glc A H-1), 5.30 (1H, s, H-12), 5.80 (1H, d, $J=8$ Hz, gal H-1), 6.28 (1H, s, rha H-1). ^{13}C -NMR: Tables 1 and 2.

Subprosidi V (5) A white amorphous powder, $[\alpha]_D^{25} - 6.6^\circ$ ($c=0.32$, pyridine: $H_2O=1:1$). Negative ion FAB MS m/z : 1119 ($[M-H]^-$), 973 ($[M-H-rha]^-$). 1H -NMR (in pyridine- $d_5 + D_2O$): 0.68, 0.86, 0.86, 1.16, 1.20, 1.42 (each 3H, s, *tert*-Me $\times$ 6), 1.79 (3H, d, $J=6$ Hz, rha-6), 5.33 (1H, s, H-12), 5.49 (1H, d, $J=8$ Hz, gal H-1), 5.97 (1H, s, rha H-1). ^{13}C -NMR: Tables 1 and 2.

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References and Notes

- Part I: see 3a) in these references; Part II: see 4) in these references; this report corresponds to part LI in a series of studies on leguminous plants.
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- 1H -NMR (in $CDCl_3$) for **2b**: 0.80, 0.88, 0.91, 0.95, 0.98, 1.00, 1.04, 1.12 (each 3H, s, *tert*-Me $\times$ 8), 3.23 (1H, dd, $J=5$, 11 Hz), 3.44 (1H, m, H-22), 5.27 (1H, m, H-12).
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