

## Notes

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Studies on the Constituents of *Trichosanthes* Species. I.<sup>1)</sup> On the Neutral Ether Extracts of the Dried Roots of *Trichosanthes japonica* REGEL, *Trichosanthes kirilowii* MAXIM. and *Trichosanthes cucumeroides* MAXIM.

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The ether-soluble neutral constituents of the dried roots of *Trichosanthes japonica* REGEL, *Trichosanthes kirilowii* MAXIM. and *Trichosanthes cucumeroides* MAXIM. were examined. Mixed glycerides,  $\Delta^7$ -stigmastenol (IV),  $\alpha$ -spinasterol (V), steryl (mixture of  $\Delta^7$ -stigmastenol and  $\alpha$ -spinasterol)- $\beta$ -D-glucopyranoside (VI, VII) and steryl (mixture of  $\Delta^7$ -stigmastenol and  $\alpha$ -spinasterol)-6'-fatty acyl (mixture of palmitic acid and (Z,Z)-9,12-octadecadienoic acid)- $\beta$ -D-glucopyranoside (VIII, IX, X, XI) were identified.

**Keywords**—*Trichosanthes japonica*; *Trichosanthes kirilowii*; *Trichosanthes cucumeroides*; Cucurbitaceae; dried root; neutral constituents;  $\Delta^7$ -stigmastenol;  $\alpha$ -spinasterol; steryl glucopyranoside; steryl-fatty acyl- $\beta$ -D-glucopyranoside

The Chinese drug "Karokon" (栝楼根) is used for regulation of water balance and for pyretolysis, and "Ohkakon" (王瓜根) is used for amelioration of a hypercoagulating state and as an anti-inflammatory in "She-nong-ben-caojing" (神農本草經). "Karokon"<sup>2)</sup> as described in the Japanese Pharmacopoeia 1981 is the dried root of *Trichosanthes japonica* REGEL (T.J), *Trichosanthes kirilowii* MAXIM. (T.K) and *Trichosanthes bracteate* VOIGT (Cucurbitaceae). "Ohkakon" is the dried root of *Trichosanthes cucumeroides* MAXIM (T.C) (Cucurbitaceae).

Earlier investigation of these drugs dealt with the water-soluble constituents and resulted in the isolation of citrulline and arginine<sup>3)</sup> from T.J, trichosanthin (protein)<sup>4)</sup> from T.K, and arginine and choline<sup>5)</sup> from T.C. As part of a program of studies on the constituents of *Trichosanthes* sp., we have examined the ether-soluble neutral constituents of the dried roots of T.J, T.K, and T.C, and compared their contents. The results are described herein.

The methanol extract of the dried root of T.J was separated as shown in Chart 1. The neutral ether extract was chromatographed on silica gel to give four substances which were tentatively named JE<sub>1</sub>, JE<sub>2</sub>, JE<sub>3</sub> and JE<sub>4</sub>.

JE<sub>1</sub> was assumed to be a glyceride from its infrared (IR) and nuclear magnetic resonance (NMR) spectra. Alkaline hydrolysis of JE<sub>1</sub> gave a mixture of fatty acids which consisted of palmitic acid (I), (Z,Z)-9,12-octadecadienoic acid (II), and (Z,Z,Z)-9,12,15-octadecatrienoic acid (III) in the ratio of approximately 2:4:3 as determined by gas chromatography (GC) of the methyl esters in comparison with authentic samples. Therefore, JE<sub>1</sub> is considered to be a mixture of mixed glycerides.

JE<sub>2</sub> showed a blue color in the Lieberman-Burchard (LB) reaction, indicating that JE<sub>2</sub> is a sterol. JE<sub>2</sub> acetate was suggested to be a mixture of two components by GC examination, and was chromatographed on silica gel impregnated with 20% AgNO<sub>3</sub> to give compounds (IV') and (V'), which were assumed to be  $\Delta^7$ -stigmasteryl acetate (IV') and  $\alpha$ -spinasteryl acetate (V') from their NMR and mass (MS) spectra. Alkaline hydrolysis of IV' and V' gave  $\Delta^7$ -stigmastenol (IV) and  $\alpha$ -spinasterol (V), which were identified by comparison with authentic samples [mixed mp, GC examination, IR (KBr), and MS]. From the result of GC analysis,

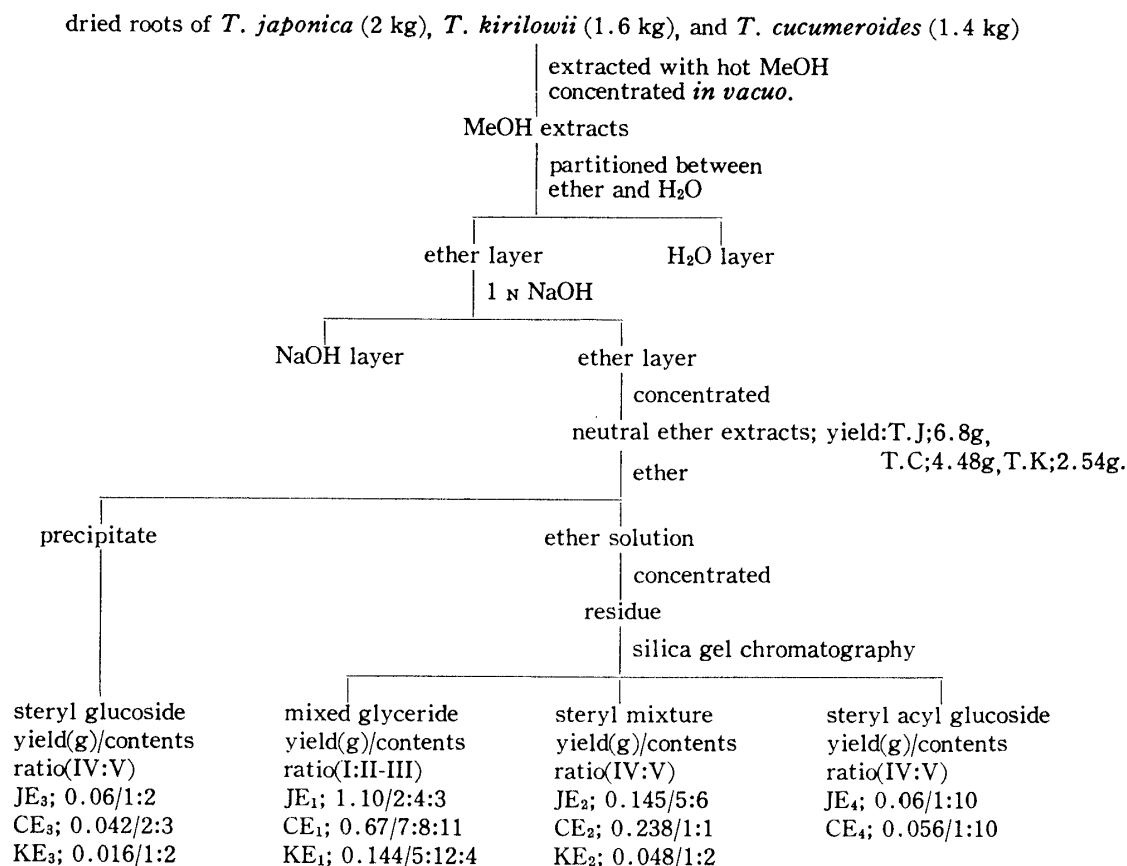


Chart 1

I; palmitic acid, II; *Z,Z*-9,12-octadecadienoic acid, III; (*Z,Z,Z*)-9,12,15-octadecatrienoic acid, IV;  $\Delta^7$ -stigmastenol, V;  $\alpha$ -spinasterol.

$JE_2$  consisted of IV and V in the ratio of approximately 5:6.

$JE_3$  showed a single spot in thin layer chromatography (TLC) and showed a purple-blue color in the LB reaction. Acetylation of  $JE_3$  gave a tetraacetate, which showed signals due to 18-CH<sub>3</sub> (singlet (s), 0.56), 19-CH<sub>3</sub> (s, 0.77) of sterol, four methyls (each s, 2.00, 2.02, 2.04, 2.08) of acetyls and the anomeric proton (doublet (d), 4.62,  $J=8$  Hz) and 6'-H (ddd, 4.20,  $J=12$  Hz, 5 Hz, 3 Hz) of the  $\beta$ -D-glucopyranosyl moiety. From the NMR and MS spectra of  $JE_3$ , it was assumed to be a steryl- $\beta$ -D-glucopyranoside. Acid hydrolysis of  $JE_3$  gave a D-glucose and a sterol mixture. The former was identical with D-glucose as regards  $R_f$  value on paper partition chromatography (PPC) and retention time on GC. After acetylation of the latter sterol mixture, it was chromatographed on AgNO<sub>3</sub>-silica gel in the same way as described for  $JE_2$  acetate to give  $\Delta^7$ -stigmasteryl acetate (IV') and  $\alpha$ -spinasteryl acetate (V'), which were identical with corresponding authentic samples by mixed mp examination, IR (KBr) and MS spectra comparisons. Therefore,  $JE_3$  was considered to be steryl (mixture of IV and V)- $\beta$ -D-glucopyranoside (VI and VII). The content ratio of IV and V in  $JE_3$  was approximately 1:2 from the result of GC analyses.

$JE_4$ , an amorphous powder, showed a single spot in TLC and a purple color in the LB reaction, and showed absorption due to hydroxy (3500—3300 cm<sup>-1</sup>) and carbonyl (1730 cm<sup>-1</sup>) functions in its IR(CHCl<sub>3</sub>) spectrum. The NMR spectrum of  $JE_4$  showed signals due to the methyls (0.56—0.9) of the sterol, the methylenes (1.27) of the fatty acid and the carbinol methines (3.30—3.70) of the glucose component. The IR and NMR spectra of  $JE_4$  were suggestive of the structure features of a fatty acid ester of steryl glucoside. Alkaline hydrolysis of  $JE_4$  gave a mixture of fatty acids and steryl glucosides. The former acids were identified as palmitic acid (I) and (*Z,Z*)-9,12-octadecadienoic acid (II) by direct GC comparisons.

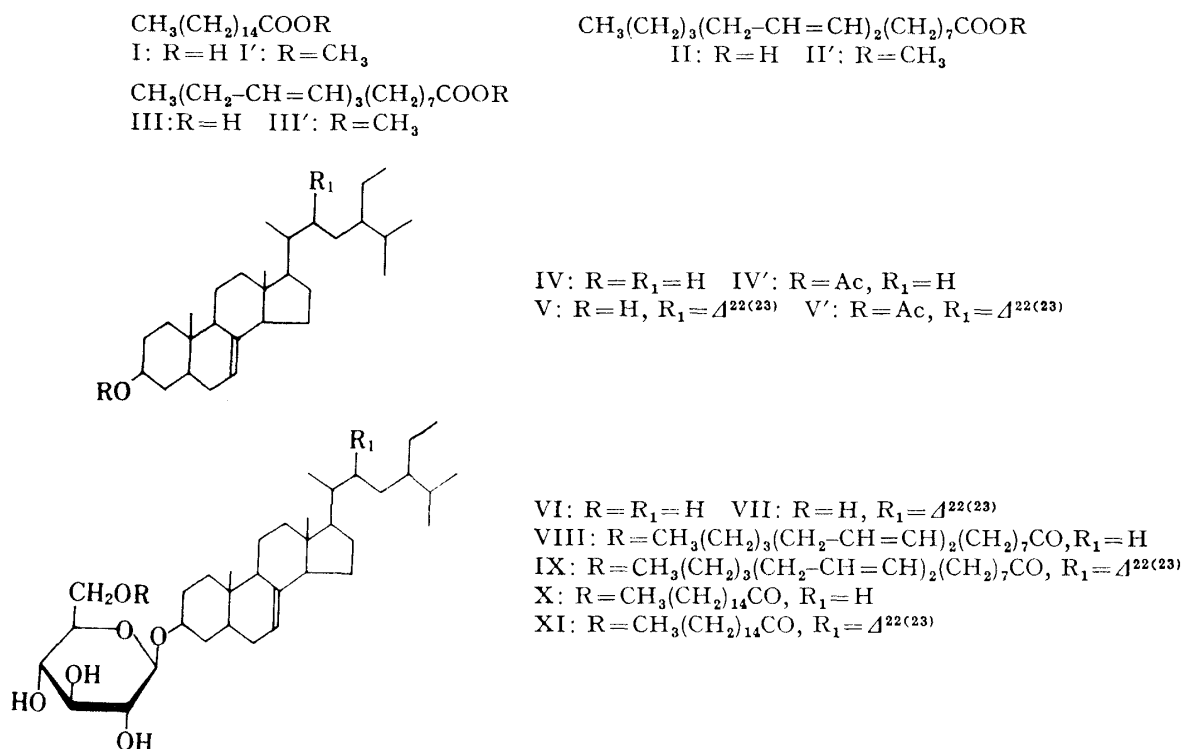


Chart 2

with authentic samples. The latter steryl glucosides were hydrolyzed in the manner described for  $\text{JE}_3$  to give  $\Delta^7$ -stigmasterol (IV),  $\alpha$ -spinasterol (V) and D-glucose which were assigned by GC analyses and  $R_f$  on PPC. Furthermore, the field desorption mass spectrum (FD-MS) of  $\text{JE}_4$  exhibited six high intensity peaks and four of them were assumed to be molecular ion peaks of the following compounds:  $m/z$  838 (VIII,  $\text{C}_{53}\text{H}_{90}\text{O}_7$ ); 836 (IX,  $\text{C}_{53}\text{H}_{88}\text{O}_7$ ); 814 (X,  $\text{C}_{51}\text{H}_{90}\text{O}_7$ ); 812 (XI,  $\text{C}_{51}\text{H}_{88}\text{O}_7$ ). The other two were fragment peaks which appeared at  $m/z$  576 (VI,  $\text{C}_{35}\text{H}_{60}\text{O}_6$ ) and 574 (VII,  $\text{C}_{35}\text{H}_{58}\text{O}_6$ ). As regards the site of linkage of the fatty acid residue to glucose, it was deduced that the fatty acid was bound at 6'-OH of the steryl glucosyl moiety, because the signals due to 2', 3'- and 4'-H appeared at 3.3–3.70 ppm, in the region of the methines adjacent to the free hydroxyl group of the glucosyl residue in the NMR spectrum.  $\text{JE}_4$  was finally considered to be steryl (mixture of IV and V)-6'-fatty acyl (mixture of I and II)- $\beta$ -D-glucopyranosides (VIII, IX, X and XI), which have not previously been found in *Trichosanthes* sp. The content ratio of IV and V in the steryl moiety of  $\text{JE}_4$  was approximately 1:10 from the results of GC analyses.

Next, we investigated the constituents of T.C and T.K in the same manner as described for T.J. Substances  $\text{CE}_1$ – $\text{CE}_4$  and  $\text{KE}_1$ – $\text{KE}_3$ , which corresponded to substances  $\text{JE}_1$ – $\text{JE}_4$ , were obtained from T.C and T.K, respectively. These substances were indicated by GC analyses to be mixture of the same components as those in substances  $\text{JE}_1$ – $\text{JE}_4$ , respectively, differing only in composition. The yields and the composition ratios of these substances are shown in Chart 1 together with those for  $\text{JE}_1$ – $\text{JE}_4$ . Substance  $\text{KE}_4$  was detected by TLC, but could not be isolated.

### Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 polarimeter. Spectra were obtained on the following machines: IR on a JASCO IR-2, NMR on Varian EM 390 and XL 200 (solvent:  $\text{CDCl}_3$ , internal standard:

tetramethylsilane, chemical shift:  $\delta$  (ppm)), EI- and FD-MS on a JEOL 300. GC analyses were done on a Shimadzu GC-6A with a flame ionization detector using 2 m glass columns (3 mm i.d.) packed with either 2% OV-17 on Gas-chrom Q (OV-17) or 3% SE-30 on Chromosorb W (SE-30). Nitrogen was employed as a carrier at a flow rate of 40 ml/min. Trimethylsilylimidazole was used as a reagent for trimethylsilylation. TLC was performed on precoated silica gel plates 0.25 mm thick (Kieselgel F<sub>254</sub>, Merck) and detection was achieved by UV irradiation (254 nm) or by spraying 1% Ce(SO<sub>4</sub>)<sub>2</sub> in 10% H<sub>2</sub>SO<sub>4</sub> followed by heating. PPC was carried out on Toyo filter paper No. 51 using aniline hydrogen phthalate detection. Column chromatography was performed on Kieselgel 60 (70–230 mesh, Merck), Mallinckrodt silica gel (100 mesh, Merck), and 20% AgNO<sub>3</sub>-silica gel.

**Materials**—The roots of *T. japonica* and *T. cucumeroides* were collected at Toyama in November 1979 and the dried root of *T. kirilowii* (Tianhuagen)<sup>2)</sup> was purchased at a Chinese drug store in Peking, China.

**(I) Extraction and Separation of Constituents of *T. japonica***—The dried and crushed root (2 kg) of *T. J.* was extracted with refluxing MeOH (3 l  $\times$  4). The MeOH extract was partitioned between ether (1.5 l) and H<sub>2</sub>O (100 ml), and the ether layer was washed with 1 N NaOH (50 ml). The organic layer was dried (MgSO<sub>4</sub>) and concentrated to give a neutral ether extract (6.8 g). The neutral ether extract was triturated with ether (20 ml) and the resulting insoluble substance (JE<sub>3</sub>, 60 mg) was filtered off and washed with MeOH (2 ml). The ethereal filtrate was chromatographed on silica gel (200 g) by stepwise elution with the following solvents to give substances JE<sub>1</sub>–JE<sub>4</sub>: JE<sub>1</sub> from 50% hexane–CH<sub>2</sub>Cl<sub>2</sub>, JE<sub>2</sub> from CH<sub>2</sub>Cl<sub>2</sub>, JE<sub>4</sub> from 10% MeOH–CH<sub>2</sub>Cl<sub>2</sub>.

**Treatment of JE<sub>1</sub>**—JE<sub>1</sub> (30 mg), oil, IR  $\nu_{\max}^{\text{film}}$  cm<sup>-1</sup>: 1730 (C=O), NMR: 1.00 (br triplet (t), CH<sub>3</sub>), 1.25 (–CH<sub>2</sub>–), 2.82 (=CH–CH=), 5.33 (–CH=CH–), 4.33 (–OCH<sub>2</sub>–), was boiled with 5% KOH–MeOH (3 ml) for 3 h, then diluted with H<sub>2</sub>O (6 ml) and extracted with ether. The aqueous layer was acidified with 4 N HCl and the mixture was extracted with ether. The ether solution was washed with H<sub>2</sub>O, dried (MgSO<sub>4</sub>) and concentrated *in vacuo*. The residue was methylated with diazomethane in ether. The resulting material (30 mg) was proved to be a mixture of methyl palmitate (I'), methyl (Z,Z)-9,12-octadecadienate (II') and methyl (Z,Z,Z)-9,12,15-octadecatrienate (III') by GC analyses. (column temperature, 180°C  $t_{\text{R min}}$  OV-17: 6.46 (I'), 14.2 (II'), 15.6 (III'). SE-30: 10.9 (I'), 19.85 (II'), 21.0 (III')).

**Treatment of JE<sub>2</sub>**—JE<sub>2</sub> (100 mg), colorless needles (from MeOH), was acetylated with acetic anhydride (1 ml) and pyridine (1 ml) overnight at room temperature. After usual work-up, the product was recrystallized from CH<sub>2</sub>Cl<sub>2</sub>–MeOH to give JE<sub>2</sub> acetate (90 mg), which was chromatographed on 20% AgNO<sub>3</sub>-silica gel (90 g) with ether–hexane (1: 49). The first fraction (35 mg) was  $\Delta^7$ -stigmasteryl acetate (IV'), colorless needles (from MeOH), mp 158–160°C,  $[\alpha]_D^{25} + 11.04^\circ$  ( $c = 0.96$ , CHCl<sub>3</sub>), NMR: 0.54 (3H, s, 18-CH<sub>3</sub>), 2.00 (3H, s, Ac), 4.70 (1H, m, 3-H), 5.18 (1H, br d, 7-H); MS  $m/z$ : 456 (M<sup>+</sup>, C<sub>31</sub>H<sub>52</sub>O<sub>2</sub>), 441, 396, 381, 315, 273, 255;<sup>6)</sup> Anal. Calcd for C<sub>31</sub>H<sub>52</sub>O<sub>2</sub>: C, 81.52; H, 11.48. Found: C, 81.27; H, 11.43. IV' (15 mg) was hydrolyzed with 5% KOH–MeOH (1 ml) under reflux for 3 h and the resulting precipitate was recrystallized from MeOH, to give  $\Delta^7$ -stigmastanol (IV), colorless needles, mp 149–150°C. MS  $m/z$ : 414 (M<sup>+</sup>, C<sub>29</sub>H<sub>50</sub>O), 399, 273, 255. Anal. Calcd for C<sub>29</sub>H<sub>50</sub>O: C, 83.99; H, 12.15. Found: C, 83.72; H, 11.94.

The second fraction (20 mg) eluted was  $\alpha$ -spinasteryl acetate (V') colorless needles (from MeOH), mp 179–180°C,  $[\alpha]_D^{25} - 3.5^\circ$  ( $c = 1.17$ , CHCl<sub>3</sub>), MS  $m/z$ : 454 (M<sup>+</sup>, C<sub>31</sub>H<sub>50</sub>O<sub>2</sub>), 439, 411, 394, 313, 273, 255. NMR: 0.56 (3H, s, 18-CH<sub>3</sub>), 0.82 (3H, s, 19-CH<sub>3</sub>), 2.01 (3H, s, Ac), 4.70 (1H, m, 3-H), 5.10 (3H, m, =CH  $\times$  3). Anal. Calcd for C<sub>31</sub>H<sub>50</sub>O<sub>2</sub>: C, 81.88; H, 11.08. Found: 81.74; H, 10.90. V' (10 mg) was hydrolyzed in the same manner as described for IV' to give  $\alpha$ -spinasterol (V), colorless needles (from MeOH), mp 170–172°C, MS  $m/z$ : 412 (M<sup>+</sup>, C<sub>29</sub>H<sub>48</sub>O), 397, 369, 271, 255.<sup>6)</sup> Anal. Calcd for C<sub>29</sub>H<sub>48</sub>O: C, 84.40; H, 11.72. Found: C, 84.21; H, 11.84. The content ratio of IV and V in JE<sub>2</sub> was determined by GC analyses (trimethylsilylation; column temperature, 270°C;  $t_{\text{R min}}$  OV-17, 18.35 (IV), 16.28 (V); SE-30, 15.16 (IV), 13.50 (V)).

**Treatment of JE<sub>3</sub>**—JE<sub>3</sub>, colorless needles (from MeOH), mp 277–279°C. MS  $m/z$ : 576 (M<sup>+</sup>, C<sub>35</sub>H<sub>60</sub>O<sub>6</sub> (VI)), 574 (M<sup>+</sup>, C<sub>35</sub>H<sub>58</sub>O<sub>6</sub> (VII)), 414 (IV, C<sub>29</sub>H<sub>50</sub>O), 412 (V, C<sub>29</sub>H<sub>48</sub>O), 399, 397, 255. JE<sub>3</sub> (10 mg) was acetylated with acetic anhydride (1 ml) and pyridine (1 ml) overnight at room temperature to give JE<sub>3</sub> tetraacetate (9 mg), colorless needles (from ether–MeOH), mp 170–171°C. JE<sub>3</sub> (30 mg) was hydrolyzed with 3% H<sub>2</sub>SO<sub>4</sub>–MeOH (30 ml) under reflux for 3 h. The resulting mixture was concentrated *in vacuo* after H<sub>2</sub>O (30 ml) had been added, and the residue was extracted with ether. The ether extract was washed with H<sub>2</sub>O, dried (MgSO<sub>4</sub>), and evaporated to dryness *in vacuo*. The residue was acetylated with acetic anhydride (0.3 ml) and pyridine (0.3 ml) in the usual manner. The acetylated product was chromatographed on 20% AgNO<sub>3</sub>-silica gel in the same way as described above for JE<sub>2</sub> acetate to give  $\Delta^7$ -stigmasteryl acetate (IV') and  $\alpha$ -spinasteryl acetate (V'), and each steryl acetate was shown to be identical with an authentic sample by IR (KBr), TLC and GC comparisons (column temperature, 270°C,  $t_{\text{R min}}$  OV-17, 29.46 (IV'), 26.26 (V'), SE-30, 17.53 (IV'), 15.48 (V')).

On the other hand, the water layer on ether extraction of acidic hydrolysate was neutralized with BaCO<sub>3</sub> and the precipitate was filtered off. The residue obtained by removal of water from the filtrate *in vacuo* was identified as D-glucose by PPC (development with *n*-BuOH–AcOH–H<sub>2</sub>O = 4: 1: 2, *R<sub>f</sub>*, 0.20; 75% phenol–H<sub>2</sub>O, *R<sub>f</sub>*, 0.46) and GC examination (trimethylsilylation; column temperature 170°C,  $t_{\text{R min}}$ : OV-17, 6.58; SE-30, 12.51). The content ratio of IV and V in JE<sub>3</sub> was determined by GC analyses.

**Treatment of JE<sub>4</sub>**—JE<sub>4</sub> was an amorphous powder,  $[\alpha]_D^{25} -10.5^\circ$  ( $c=1.2$ , CHCl<sub>3</sub>). JE<sub>4</sub> (30 mg) was refluxed with 5% KOH–MeOH (2 ml) for 4 h and the resulting precipitate was filtered off, washed with MeOH and then recrystallized from MeOH to give colorless needles (15 mg), mp 277–279°C, which were found to be identical with the mixture of steryl glucosides (JE<sub>3</sub>) by IR (KBr) and TLC comparisons. Hydrolysis of the mixture of steryl glucosides with 3% H<sub>2</sub>SO<sub>4</sub>–MeOH gave  $\Delta^7$ -stigmastenol (IV) and  $\alpha$ -spinasterol (V), which were identified by GC comparison with authentic samples. The content ratio of IV and V in JE<sub>4</sub> was determined by GC analysis.

On the other hand, the alkaline filtrate in the alkaline hydrolysis of JE<sub>4</sub> was concentrated *in vacuo*, and the residue was neutralized with 1 N HCl and extracted with ether. The ether extract was washed with NaCl aq., dried (MgSO<sub>4</sub>) and concentrated *in vacuo*. The residue was methylated with diazomethane in ether. The resulting material was proved to be a mixture of methyl palmitate (I') and methyl (Z,Z)-9,12-octadecadienate (II') by GC analyses.

**(II) Extraction and Separation of Constituents of *T. cucumeroides* and *T. kirilowii***—Dried and crushed roots of T.C (1.4 kg) and T.K (1.6 kg) were extracted with refluxing MeOH (3 l  $\times$  4) and the MeOH extracts were treated as shown in Chart 1 to afford neutral ether extracts. Yield: T.C, 4.48 g; T.K, 2.54 g.

Each neutral ether extract was triturated with ether (10–20 ml) and the resulting insoluble substance (CE<sub>3</sub>, 42 mg; KE<sub>3</sub>, 16 mg) was filtered off and washed with MeOH (2 ml). After removal of the solvent from the ether filtrate, the residue was chromatographed on silica gel, and stepwise elution with following solvents gave substances CE<sub>1</sub>–CE<sub>4</sub> and KE<sub>1</sub>–KE<sub>3</sub>: CE<sub>1</sub>, KE<sub>1</sub> from 50% hexane–CH<sub>2</sub>Cl<sub>2</sub>; CE<sub>2</sub>, KE<sub>2</sub> from CH<sub>2</sub>Cl<sub>2</sub>; CE<sub>4</sub> from 10% MeOH–CH<sub>2</sub>Cl<sub>2</sub>.

**Treatment of CE<sub>1</sub>–CE<sub>4</sub> and KE<sub>1</sub>–KE<sub>3</sub>**—(CE<sub>1</sub> and KE<sub>1</sub>): IR (film) and NMR spectra of CE<sub>1</sub> and KE<sub>1</sub> showed the same patterns as those of JE<sub>1</sub>. CE<sub>1</sub> and KE<sub>1</sub> were each hydrolyzed with 5% KOH–MeOH and methylated with diazomethane in the same manner as described for JE<sub>1</sub> to give a mixture of methyl palmitate (I'), methyl (Z,Z)-9,12-octadecadienate (II') and methyl (Z,Z,Z)-9,12,15-octadecatrienate (III') which were identified by GC analyses. The content ratios of I, II and III in CE<sub>1</sub> and KE<sub>1</sub> were 7: 18: 11 and 5: 12: 4, respectively.

(CE<sub>2</sub> and KE<sub>2</sub>): After acetylation of CE<sub>2</sub> and KE<sub>2</sub>, each product was chromatographed on 20% AgNO<sub>3</sub>–silica gel in the same manner as described for JE<sub>2</sub> to give  $\Delta^7$ -stigmasteryl acetate (IV') and  $\alpha$ -spinasteryl acetate (V'), whose identities were confirmed by mixed mp examination with authentic samples IR (KBr), NMR and MS analyses. The content ratios of IV and V in CE<sub>2</sub> and KE<sub>2</sub> were 1: 1 and 1: 2, respectively.

(CE<sub>3</sub> and KE<sub>3</sub>): IR (KBr) spectra of CE<sub>3</sub> and KE<sub>3</sub> showed a pattern quite similar to that of JE<sub>3</sub>. CE<sub>3</sub> and KE<sub>3</sub> were each hydrolyzed with 3% H<sub>2</sub>SO<sub>4</sub>–MeOH in the same manner as described for JE<sub>3</sub> to give a mixture of sterols (IV and V) and D-glucose. After trimethylsilylation of the sterol mixture, it was proved to be a mixture of  $\Delta^7$ -stigmastenol (IV) and  $\alpha$ -spinasterol (V) by GC analyses. The content ratios of IV and V in the steryl moieties of CE<sub>3</sub> and KE<sub>3</sub> were 2: 3 and 1: 2, respectively. The D-glucose was identified by PPC and GC analyses in comparison with an authentic sample.

(CE<sub>4</sub>): IR (CHCl<sub>3</sub>) and NMR spectra of CE<sub>4</sub> showed patterns quite similar to those of JE<sub>4</sub>. CE<sub>4</sub> was hydrolyzed with 5% KOH–MeOH in the same manner as described for JE<sub>4</sub> to give a mixture of steryl glucosides and a mixture of fatty acids. The mixture of steryl glucoside identical with that of CE<sub>3</sub> by comparison of IR (KBr) and TLC. Hydrolysis of the mixture of steryl glucoside with 3% H<sub>2</sub>SO<sub>4</sub>–MeOH gave D-glucose and a mixture of sterols (IV and V) which were identified by GC examination in comparison with authentic samples. The content ratio of IV and V in the steryl moiety of CE<sub>4</sub> was 1: 10. The mixture of fatty acids obtained by alkaline hydrolysis of CE<sub>4</sub> was methylated with diazomethane, and the resulting methyl esters were identical with methyl palmitate (I') and methyl (Z,Z)-9,12-octadecadienate (II') on the basis of GC analyses.

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## References and Notes

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