Notes

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Studies on the Constituents of *Trichosanthes* Species. I.[1] 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, Δ^7-stigmastenol (IV), α-spinasterol (V), steryl (mixture of Δ^7-stigmastenol and α-spinasterol)-β-D-glucopyranoside (VI, VII) and steryl (mixture of Δ^7-stigmastenol and α-spinasterol)-6′-fatty acyl (mixture of palmitic acid and (*Z*,*Z*)-9,12-octadecadienoic acid)-β-D-glucopyranoside (VIII, IX, X, XI) were identified.

Keywords——*Trichosanthes japonica*; *Trichosanthes kirilowii*; *Trichosanthes cucumeroides*; Cucurbitaceae; dried root; neutral constituents; Δ^7-stigmastenol; α-spinasterol; steryl glucopyranoside; steryl-fatty acyl-β-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-cao-jing" (神農本草経). "Karokon"[2] 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[3] from T.J, trichosanthin (protein)[4] from T.K, and arginine and choline[5] 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_1, JE_2, JE_3 and JE_4.

JE_1 was assumed to be a glyceride from its infrared (IR) and nuclear magnetic resonance (NMR) spectra. Alkaline hydrolysis of JE_1 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_1 is considered to be a mixture of mixed glycerides.

JE_2 showed a blue color in the Lieberman–Burchard (LB) reaction, indicating that JE_2 is a sterol. JE_2 acetate was suggested to be a mixture of two components by GC examination, and was chromatographed on silica gel impregnated with 20% $AgNO_3$ to give compounds (IV′) and (V′), which were assumed to be Δ^7-stigmastenyl acetate (IV′) and α-spinasteryl acetate (V′) from their NMR and mass (MS) spectra. Alkaline hydrolysis of IV′ and V′ gave Δ^7-stigmastenol (IV) and α-spinasterol (V), which were identified by comparison with authentic samples [mixed mp, GC examination, IR (KBr), and MS]. From the result of GC analysis,

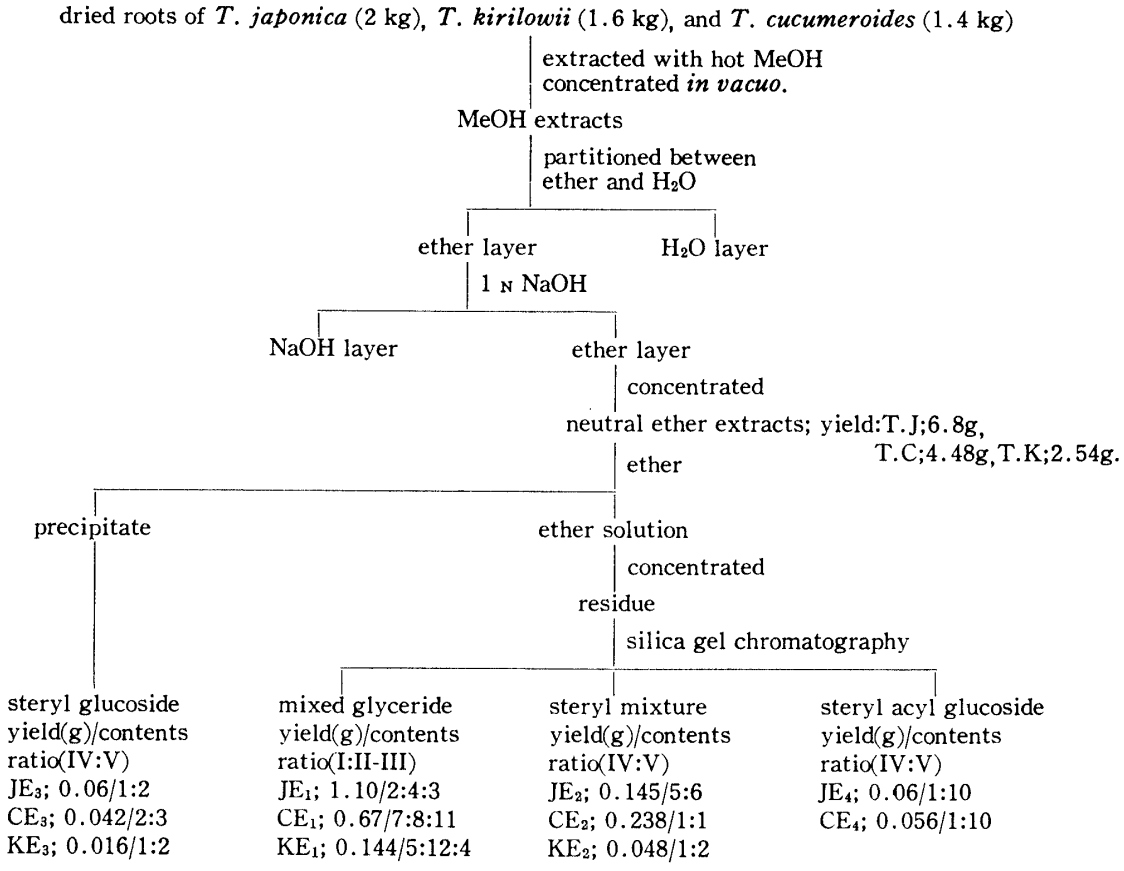


Chart 1

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

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

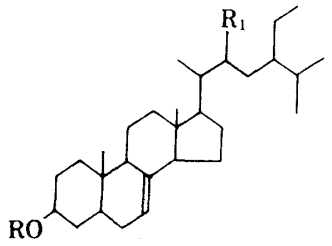
JE_3 showed a single spot in thin layer chromatography (TLC) and showed a purple-blue color in the LB reaction. Acetylation of JE_3 gave a tetraacetate, which showed signals due to 18-CH_3 (singlet (s), 0.56), 19-CH_3 (s, 0.77) of sterol, four methyls (each s, 2.00, 2.02, 2.04, 2.08) of acetyls and the anomeric proton (doublet (d), 4.62, J=8 Hz) and 6′-H (ddd, 4.20, J=12 Hz, 5 Hz, 3 Hz) of the β-D-glucopyranosyl moiety. From the NMR and MS spectra of JE_3, it was assumed to be a steryl-β-D-glucopyranoside. Acid hydrolysis of JE_3 gave a D-glucose and a sterol mixture. The former was identical with D-glucose as regards *Rf* value on paper partition chromatograpy (PPC) and retention time on GC. After acetylation of the latter sterol mixture, it was chromatographed on $AgNO_3$–silica gel in the same way as described for JE_2 acetate to give Δ^7-stigmastenyl acetate (IV′) and α-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)-β-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^{-1}) and carbonyl (1730 cm^{-1}) functions in its IR($CHCl_3$) 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

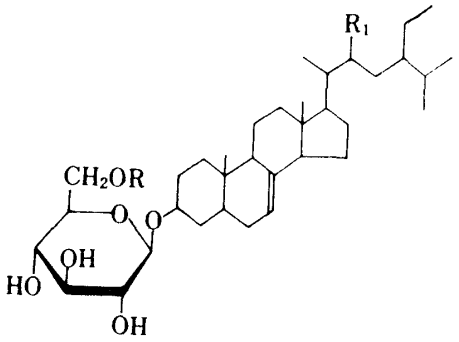
$CH_3(CH_2)_{14}COOR$
I: R=H I′: R=CH_3

$CH_3(CH_2{-}CH{=}CH)_3(CH_2)_7COOR$
III: R=H III′: R=CH_3

$CH_3(CH_2)_3(CH_2{-}CH{=}CH)_2(CH_2)_7COOR$
II: R=H II′: R=CH_3



IV: R=R_1=H IV′: R=Ac, R_1=H
V: R=H, $R_1=\Delta^{22(23)}$ V′: R=Ac, $R_1=\Delta^{22(23)}$



VI: R=R_1=H VII: R=H, $R_1=\Delta^{22(23)}$
VIII: R=$CH_3(CH_2)_3(CH_2{-}CH{=}CH)_2(CH_2)_7CO$, R_1=H
IX: R=$CH_3(CH_2)_3(CH_2{-}CH{=}CH)_2(CH_2)_7CO$, $R_1=\Delta^{22(23)}$
X: R=$CH_3(CH_2)_{14}CO$, R_1=H
XI: R=$CH_3(CH_2)_{14}CO$, $R_1=\Delta^{22(23)}$

Chart 2

with authentic samples. The latter steryl glucosides were hydrolyzed in the manner described for JE_3 to give Δ^7-stigmastenol (IV), α-spinasterol (V) and D-glucose which were assigned by GC analyses and *Rf* on PPC. Furthermore, the field desorption mass spectrum (FD-MS) of 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, $C_{53}H_{90}O_7$); 836 (IX, $C_{53}H_{88}O_7$); 814 (X, $C_{51}H_{90}O_7$); 812 (XI, $C_{51}H_{88}O_7$). The other two were fragment peaks which appeared at *m/z* 576 (VI, $C_{35}H_{60}O_6$) and 574 (VII, $C_{35}H_{58}O_6$). 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. JE_4 was finally considered to be steryl (mixture of IV and V)-6′-fatty acyl (mixture of I and II)-β-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 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 CE_1–CE_4 and KE_1—KE_3, which corresponded to substances JE_1—JE_4, were obtained from T.C and T.K, respectively. These substances were indicated by GC analyses 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 JE_1—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 JE_1—JE_4. Substance 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: $CDCl_3$, internal standard:

tetramethylsilane, chemical shift: δ (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_{254}, Merck) and detection was achieved by UV irradiation (254 nm) or by spraying 1% $Ce(SO_4)_2$ in 10% H_2SO_4 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% $AgNO_3$-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)[2] 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 × 4). The MeOH extract was partitioned between ether (1.5 l) and H_2O (100 ml), and the ether layer was washed with 1 N NaOH (50 ml). The organic layer was dried ($MgSO_4$) 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_3, 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_1—JE_4: JE_1 from 50% hexane–CH_2Cl_2, JE_2 from CH_2Cl_2, JE_4 from 10% MeOH–CH_2Cl_2.

Treatment of JE_1——JE_1 (30 mg), oil, IR ν_{max}^{film} cm^{-1}: 1730 (C=O), NMR: 1.00 (br triplet (t), CH_3), 1.25 ($-CH_2-$), 2.82 (=CH–CH=), 5.33 (–CH=CH–), 4.33 ($-OCH_2-$), was boiled with 5%KOH–MeOH (3 ml) for 3 h, then diluted with H_2O (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_2O, dried ($MgSO_4$) 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 $t_{R\ min}$ 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_2——JE_2 (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_2Cl_2–MeOH to give JE_2 acetate (90 mg), which was chromatographed on 20% $AgNO_3$-silica gel (90 g) with ether–hexane (1: 49). The first fraction (35 mg) was Δ^7-stigmastenyl acetate (IV′), colorless needles (from MeOH), mp 158—160°C, $[\alpha]_D^{26}$ +11.04° (c=0.96, $CHCl_3$), NMR: 0.54 (3H, s, 18-CH_3), 2.00 (3H, s, Ac), 4.70 (1H, m, 3-H), 5.18 (1H, br d, 7-H); MS *m/z*: 456 (M^+, $C_{31}H_{52}O_2$), 441, 396, 381, 315, 273 255;[6] *Anal.* Calcd for $C_{31}H_{52}O_2$: 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 Δ^7-stigmastenol (IV), colorless needles, mp 149—150°C. MS *m/z*: 414 (M^+, $C_{29}H_{50}O$), 399, 273, 255. *Anal.* Calcd for $C_{29}H_{50}O$: C, 83.99; H, 12.15. Found: C, 83.72; H, 11.94.

The second fraction (20 mg) eluted was α-spinasteryl acetate (V′) colorless needles (from MeOH), mp 179—180°C, $[\alpha]_D^{26}$ −3.5° (c=1.17, $CHCl_3$), MS *m/z*: 454 (M^+, $C_{31}H_{50}O_2$), 439, 411, 394, 313, 273, 255. NMR: 0.56 (3H, s, 18-CH_3), 0.82 (3H, s, 19-CH_3), 2.01 (3H, s, Ac), 4.70 (1H, m, 3-H), 5.10 (3H, m, =CH × 3). *Anal.* Calcd for $C_{31}H_{50}O_2$: 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 α-spinasterol (V), colorless needles (from MeOH), mp 170—172°C, MS *m/z*: 412 (M^+, $C_{29}H_{48}O$), 397, 369, 271, 255.[6] *Anal.* Calcd for $C_{29}H_{48}O$: C, 84.40; H, 11.72. Found: C, 84.21; H, 11.84. The content ratio of IV and V in JE_2 was determined by GC analyses (trimethylsilylation; column temperature, 270°C; $t_{R\ min}$ OV-17, 18.35 (IV), 16.28 (V); SE-30, 15.16 (IV), 13.50 (V)).

Treatment of JE_3——JE_3, colorless needles (from MeOH), mp 277—279°C. MS *m/z*: 576 (M^+, $C_{35}H_{60}O_6$ (VI)), 574 (M^+, $C_{35}H_{58}O_6$ (VII)), 414 (IV, $C_{29}H_{50}O$), 412 (V, $C_{29}H_{48}O$), 399, 397, 255. JE_3 (10 mg) was acetylated with acetic anhydride (1 ml) and pyridine (1 ml) overnight at room temperature to give JE_3 tetraacetate (9 mg), colorless needles (from ether–MeOH), mp 170—171°C. JE_3 (30 mg) was hydrolyzed with 3% H_2SO_4–MeOH (30 ml) under reflux for 3 h. The resulting mixture was concentrated *in vacuo* after H_2O (30 ml) had been added, and the residue was extracted with ether. The ether extract was washed with H_2O, dried ($MgSO_4$), 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% $AgNO_3$–silica gel in the same way as described above for JE_2 acetate to give Δ^7-stigmastenyl acetate (IV′) and α-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_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 $BaCO_3$ 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_2O=4: 1: 2, *Rf*, 0.20; 75% phenol–H_2O, *Rf*, 0.46) and GC examination (trimethylsilylation; column temperature 170°C, t_R min: OV-17, 6.58; SE-30, 12.51). The content ratio of IV and V in JE_3 was determined by GC analyses.

Treatment of JE_4——JE_4 was an amorphous powder, $[\alpha]_D^{26}$ −10.5° (c=1.2, $CHCl_3$). JE_4 (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_3) by IR (KBr) and TLC comparisons. Hydrolysis of the mixture of steryl glucosides with 3% H_2SO_4–MeOH gave Δ^7-stigmastenol (IV) and α-spinasterol (V), which were identified by GC comparison with authentic samples. The content ratio of IV and V in JE_4 was determined by GC analysis.

On the other hand, the alkaline filtrate in the alkaline hydrolysis of JE_4 was concentrated *in vacuo*, and the residue was neutralized with 1 N HCl and extracted with ether. The ether extact was washed with NaCl aq., dried ($MgSO_4$) 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×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_3, 42 mg; KE_3, 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_1—CE_4 and KE_1–KE_3: CE_1, KE_1 from 50% hexane–CH_2Cl_2; CE_2, KE_2 from CH_2Cl_2; CE_4 from 10% MeOH–CH_2Cl_2.

Treatment of CE_1—CE_4 and KE_1—KE_3——(CE_1 and KE_1): IR (film) and NMR spectra of CE_1 and KE_1 showed the same patterns as those of JE_1. CE_1 and KE_1 were each hydrolyzed with 5% KOH–MeOH and methylated with diazomethane in the same manner as described for JE_1 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_1 and KE_1 were 7: 18: 11 and 5: 12: 4, respectively.

(CE_2 and KE_2): After acetylation of CE_2 and KE_2, each product was chromatographed on 20% $AgNO_3$–silica gel in the same manner as described for JE_2 to give Δ^7-stigmastenyl acetate (IV′) and α-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_2 and KE_2 were 1: 1 and 1: 2, respectively.

(CE_3 and KE_3): IR (KBr) spectra of CE_3 and KE_3 showed a pattern quite similar to that of JE_3. CE_3 and KE_3 were each hydrolyzed with 3% H_2SO_4–MeOH in the same manner as described for JE_3 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 Δ^7-stigmastenol (IV) and α-spinasterol (V) by GC analyses. The content ratios of IV and V in the steryl moieties of CE_3 and KE_3 were 2: 3 and 1: 2, respectively. The D-glucose was identified by PPC and GC analyses in comparison with an authentic sample.

(CE_4): IR ($CHCl_3$) and NMR spectra of CE_4 showed patterns quite similar to those of JE_4. CE_4 was hydrolyzed with 5% KOH–MeOH in the same manner as described for JE_4 to give a mixture of steryl glucosides and a mixture of fatty acids. The mixture of steryl glucoside identical with that of CE_3 by comparison of IR (KBr) and TLC. Hydrolysis of the mixture of steryl glucoside with 3% H_2SO_4–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_4 was 1: 10. The mixture of fatty acids obtained by alkaline hydrolysis of CE_4 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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