Notes

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Plant Constituents Biologically Active to Insects. V.[1] Antifeedants for the Larvae of the Yellow Butterfly, *Eurema hecabe mandarina*, in *Osmunda japonica*. (1)

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Three compounds having antifeeding activities for the larvae of the yellow butterfly, *Eurema hecabe mandarina* DE L'ORZA, were isolated from *Osmunda japonica* THUNB. These compounds were identified as (4*R*,5*S*)-osmundalactone (II), (4*R*,5*S*)-5-hydroxy-2-hexen-4-olide (III) and succinic acid (V), of which the former is the main antifeedant in this plant. (4*R*,5*S*)-5-Hydroxyhexan-4-olide (I) and (3*S*,5*S*)-3-hydroxyhexan-5-olide (IV) were isolated in addition to the antifeedants, and various sterols, fatty alcohols and fatty acid esters were obtained as a mixture. This is the first report of the occurrence of I, II and III as natural products, though they have been artificially prepared.

Keywords——antifeedant; *Eurema hecabe mandarina*; Osmundaceae; *Osmunda japonica*; hydroxypentenolide; hydroxypentanolide; succinic acid; fatty alcohol; fatty acid ester; sterol

Previously we reported a survey of various plants having antifeeding activities against the larvae of the yellow butterfly, *Eurema hecabe mandarina* DE L'ORZA, and the isolation of an antifeedant from one of the antifeeding-active plants, *Arachniodes standishii* (MOORE) OHWI.[1] This paper describes the isolation and identification of (4*R*,5*S*)-osmundalactone (II), (4*R*,5*S*)-5-hydroxy-2-hexen-4-olide (III), and succinic acid (V) as antifeedants from *Osmunda japonica* THUNB. (Japanese name, Zenmai). A part of this work has been reported in a preliminary communication.[2]

Results and Discussion

The dried whole plants of *O. japonica* were extracted successively with hexane, ether, and methanol as shown in Chart 1. The feeding inhibitory activities of the resulting extracts and residues on *E. hecabe mandarina* larvae were tested according to the bioassay procedure described in the previous paper.[1] These results are shown in Table I.

Positive antifeeding responses were obtained with E_1, R_1, and E_2. Since the extract E_2 showed relatively strong activity, it was fractionated into water-soluble, basic, acidic, phenolic, and neutral fractions in the usual way. The water-soluble fraction E_4, showing the strongest feeding inhibition, was subjected to droplet countercurrent chromatography (DCC) using $CHCl_3$–MeOH–H_2O (5 : 5.7 : 3) mixture. Fractions 1—30 were repeatedly chromatographed on silicic acid to give oily materials I, III and IV, and a crystalline material II. Fractions 31—45 in the DCC afforded a crystalline material V, mp 185—186 °C, which was identified as succinic acid by direct comparison with an authentic specimen.

Compounds II (mp 80—82 °C) and III were both assigned the molecular formula

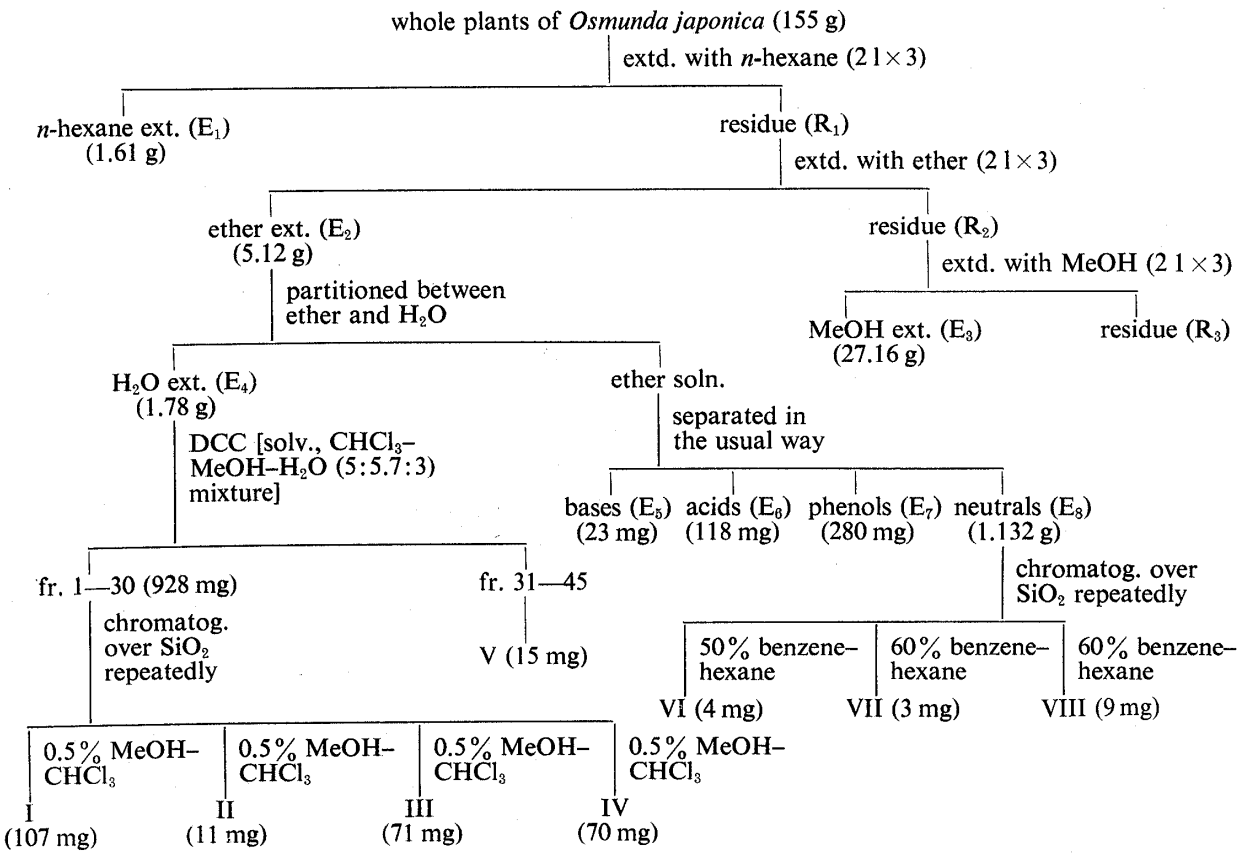

Chart 1

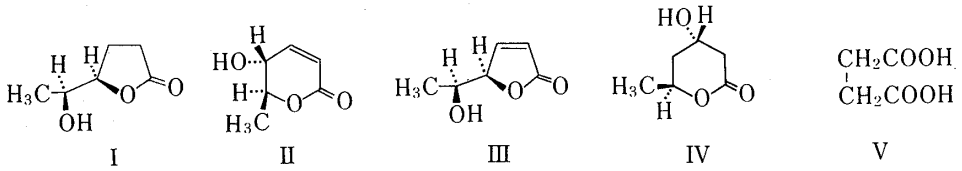

Chart 2

$C_6H_8O_3$ on the basis of the mass spectra (MS) and other spectral evidence. Their ultraviolet (UV), infrared (IR), and ^1H nuclear magnetic resonance (NMR) spectra showed that II and III are (4*R*,5*S*)-osmundalactone and (4*R*,5*S*)-5-hydroxy-2-hexen-4-olide, which have been obtained as the acid-hydrolysis products of osmundalin, a glucoside of osmundalactone, isolated from the same plant by Hollenbeak *et al.*[3]

Compound I was assigned the molecular formula $C_6H_{10}O_3$ on the basis of the MS and other spectral evidence. The IR and ^1H-NMR spectra showed I to be (4*R*,5*S*)-5-hydroxyhexan-4-olide, which was identified by spectral comparison with the saturated γ-lactone derived from III by catalytic hydrogenation. This compound has been obtained from II by catalytic hydrogenation followed by storage at room temperature for more than a year.[3]

Compound IV, mp 67—70 °C, had the molecular formula $C_6H_{10}O_3$ as determined from the MS and other spectral evidence. The specific rotation, IR and ^1H-NMR spectra showed IV to be (3*S*,5*S*)-3-hydroxyhexan-5-olide, which has been obtained as the aglycone of parasorboside, isolated from *Sorbus aucuparia* L. by Tschesche *et al.*[4] The ^1H-NMR spectra of this compound showed different patterns when measured in $CDCl_3$ and acetone-d_6. The signal of the methylene group attached to a carbonyl group appeared as a doublet (J=4 Hz)

TABLE I. The Feeding Inhibitory Activities of Some Fractions and Compounds

Sample	Amount of sample in the diet mg (concentration %)	Mean frass count	Feeding ratio[a] $\{(A)^{b)}-(B)/(C)-(B)\}\times 100$
Basal diet		2.5±0.4 (*B*)	
None (control)		23.3±2.4 (*C*)	
E_1	100 (7.75)	4.8±0.5	11.1
	50 (3.88)	6.4±0.9	18.8
	20 (1.55)	14.0±1.4	55.3
E_2	100 (7.75)	2.8±0.4	1.4
	50 (3.88)	5.2±0.6	13.0
	20 (1.55)	5.2±0.7	13.0
	10 (0.78)	6.0±0.4	16.8
E_3	30 (2.32)	16.6±0.9	67.8
R_1	100 (7.75)	7.4±0.9	23.6
R_2	100 (7.75)	19.2±1.1	80.3
R_3	100 (7.75)	21.6±2.4	91.8
E_4	10 (0.78)	6.6±1.0	19.7
	5 (0.39)	14.2±1.1	56.3
	1 (0.078)	14.2±1.9	56.3
E_5	10 (0.78)	13.8±1.2	54.3
E_6	30 (2.32)	10.4±2.0	38.0
	10 (0.78)	14.8±1.4	59.1
E_7	30 (2.32)	8.6±1.4	29.3
	10 (0.78)	17.6±3.0	72.6
E_8	30 (2.32)	10.8±1.8	39.9
	10 (0.78)	18.2±1.6	75.5
I	10.2 (0.8)	27.4±1.0	119.7
II	1.28 (0.1)	5.4±1.8	13.9
	0.64 (0.05)	5.8±2.0	15.9
	0.32 (0.025)	9.8±1.5	35.1
	0.128 (0.01)	16.6±2.7	67.8
III	6.4 (0.5)	7.0±2.3	21.6
	3.2 (0.25)	11.0±2.1	40.9
	1.28 (0.1)	15.5±1.3	62.5
IV	9.0 (0.7)	17.0±0.8	69.7
V	10.2 (0.8)	4.0±1.2	7.2
	5.1 (0.4)	6.4±2.4	18.8
	2.6 (0.2)	9.6±2.6	34.1
	1.28 (0.1)	18.4±3.3	76.4

a) Strong feeding inhibitory activity, 0—20%; slight, 20—50%; none, 50% and greater.
b) The mean frass count for test diets.

at δ2.66 in $CDCl_3$, while it appeared as multiplet at δ2.56 in acetone-d_6, as reported by Tschesche. Although these compounds I, II, III, and IV have been artificially prepared, this is the first report of their occurrence as natural products.

The feeding-inhibitory activities of the five compounds isolated herein are presented in Table I. Among them, II, III and V have antifeeding activities and their limiting concentrations for activity are 0.025, 0.25, and 0.2%, respectively. Compound II was therefore proved to be the main antifeedant in this plant.

Purification of the ether-extractable neutral fraction by repeated silicic acid column chromatography gave three crystalline materials, VI, VII and VIII, each showing a single spot in thin-layer chromatography (TLC). VIII, mp 114—122 °C, was proved to be a mixture of β-sitosterol and campesterol by gas-liquid chromatographic (GLC) analysis.

Compound VII, mp 78—81 °C, was presumed to be a mixture of fatty alcohols on the

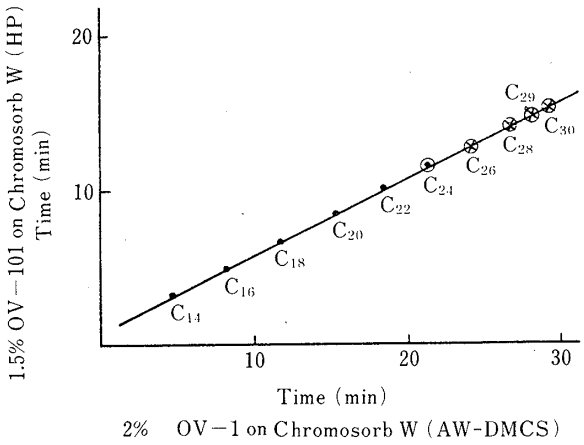

Fig. 1. Relationship between Retention Times and Carbon Numbers of Fatty Alcohols

●, fatty alcohol standards; ×, fatty alcohol (VII); ○, fatty alcohols from VI.

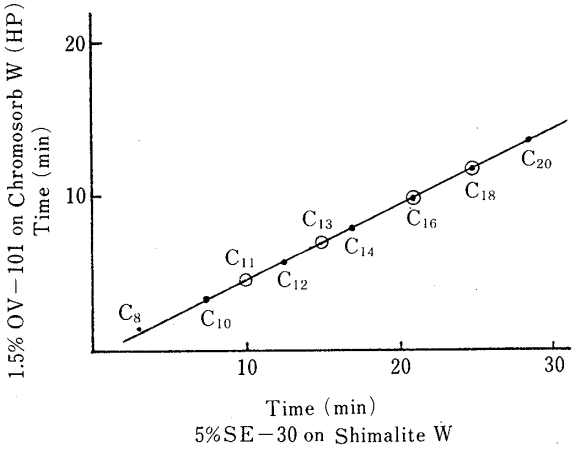

Fig. 2. Relationship between Retention Times and Carbon Numbers of Fatty Acids

●, fatty acid standards; ○, fatty acids from VI.

basis of the IR and ^{1}H-NMR spectra. Identification was done by a modification of the method of Kurono.[5] When the even-numbered fatty alcohol standards between C_{14} and C_{24} were analyzed by GLC using two phases, 2% OV-1 on Chromosorb W (AW-DMCS) and 1.5% OV-101 on Chromosorb W (HP), the plot of their retention times on OV-1 *vs.* those on OV-101 was linear (see Fig. 1). Application of the relationship between the retention times and the carbon numbers of the fatty alcohol standards showed VII to be a mixture of C_{26}, C_{28}, C_{29}, and C_{30} fatty alcohols, which comprised 27.8, 27.9, 6.1, and 38.2%, respectively, of the total alcohols, based on the peak areas on GLC using OV-101. This result was confirmed by the detection of four $M^+ - 18$ (H_2O) peaks, *m/z* 364, 392, 406, and 420, in the MS.

Compound VI, mp 74—76 °C, was presumed to be a mixture of fatty acid esters on the basis of the IR and ^{1}H-NMR spectra. On alkaline hydrolysis it gave acidic and alcoholic fractions. The latter was analyzed by the above-mentioned method and shown to be a mixture of C_{24}, C_{26}, C_{28}, C_{29}, and C_{30} fatty alcohols (see Fig. 1), which comprised 30.3, 29.6, 14.9, 3.0, and 22.1%, respectively, of the total alcohols. This result was confirmed by the detection of five $M^+ - 18$ peaks, *m/z* 336, 364, 392, 406, and 420, in the MS. The former fraction was analyzed by esterification to the corresponding methyl esters followed by GLC using two phases, 1.5% OV-101 on Chromosorb W (HP) and 5% SE-30 on Shimalite W. The plot of the retention times of the even-numbered fatty acid methyl ester standards between C_8 and C_{20} on OV-101 *vs.* those on SE-30 was linear (Fig. 2). Application of this relationship between the retention times and the carbon numbers showed the acidic fraction to be a mixture of C_{11}, C_{13}, C_{16}, and C_{18} fatty acids, which comprised 39.9, 42.4, 9.5, and 8.2%, respectively, of the total acids, based on the peak areas on GLC using OV-101. This result was supported by GLC comparison with authentic samples.

Based on the above-mentioned results and the MS (showing the molecular ions at *m/z* 522, 550, 578, 592, 606, 620, 648, 662, 676, 690), VI seems to be a mixture of esters from the following set of fatty acids and fatty alcohols, respectively: C_{11} and C_{24}, C_{11} and C_{26} (or C_{13} and C_{24}), C_{11} and C_{28}(or C_{13} and C_{26}), C_{16} and C_{24}, C_{11} and C_{30} (or C_{13} and C_{28}), C_{18} and C_{24} (or C_{16} and C_{26}), C_{16} and C_{28} (or C_{18} and C_{26}), C_{16} and C_{29}, C_{16} and C_{30} (or C_{18} and C_{28}), C_{18} and C_{29}.

Experimental

Spectral Measurement——All melting points were determined on a Yanagimoto micromelting point apparatus

and are uncorrected. The UV spectra were recorded with a Hitachi 323 spectrometer and the IR spectra with a Hitachi EPI-G2 spectrometer. The ^{1}H-NMR spectra were taken on a Hitachi R-40 spectrometer (90 MHz). Chemical shifts are given in parts per million (δ) downfield from tetramethylsilane (TMS) as an internal standard. The MS were taken on a Hitachi M-80 spectrometer. The optical rotatory dispersion (ORD) curves were measured on a JASCO ORD/UV-5 spectrometer.

Chromatography——GLC was run on a Hitachi 063 instrument equipped with a flame ionization detector. Sterols were analyzed on 1.5% OV-101 on 80—100 mesh Chromosorb W (HP) packed in a 2 m × 3 mm (i.d.) stainless steel column, operated at 260 °C. Fatty alcohols were analyzed on two phases, 1.5% OV-101 on 80—100 mesh Chromosorb W (HP) and 2% OV-1 on 80—100 mesh Chromosorb W (AW-DMCS), packed in 2 m × 3 mm (i.d.) stainless steel columns. Fatty acid methyl esters were analyzed on two phases, 1.5% OV-101 on 80—100 mesh Chromosorb W (HP) and 5% SE-30 on 60—80 mesh Shimalite W, packed in 2 m × 3 mm (i.d.) stainless steel columns. OV-101 was programmed from 100 to 300 °C at 10 °C/min with final hold, OV-1 from 150 to 300 °C at 5 °C/min with final hold, and SE-30 from 100 to 250 °C at 5 °C/min with final hold. The nitrogen carrier gas flow rate, injector and detector temperatures used for all the analyses were 30 ml/min, 340 and 350 °C, respectively. DCC was carried out with a chromatograph equipped with one hundred glass tubes (60 cm × 2 mm i.d.) packed with the upper layer (stationary phase) of $CHCl_3$–MeOH–H_2O (5 : 5.7 : 3). The flow rate of the moving phase (lower layer) was 0.21 ml/min and fractions of 15 ml were collected.

Extraction and Fractionation——The dried whole plants (155 g) of *O. japonica*, collected in the fields of Kawachinagano city, Osaka, were extracted with several solvents and the resulting extracts were fractionated as shown in Chart 1.

(4*R*, 5*S*)-5-Hydroxyhexan-4-olide (I)——A colorless oil. MS *m/z* (rel. intensity %): 131 ($M^+ + 1$, 4), 113 (1), 86 [$\cdot CH(OH)CH_2CH_2C \equiv O^+$, 100], 85 (87). ORD ($c = 0.79$, $CHCl_3$, 22 °C): $[\phi]_{589}$ −12.0, $[\phi]_{450}$ −21.2, $[\phi]_{350}$ −52.9, $[\phi]_{260}$ −125.7. This material had IR and ^{1}H-NMR spectra in accord with the published data,[3] and was identified by direct comparison with a sample derived from III by catalytic hydrogenation.

(4*R*, 5*S*)-Osmundalactone (II)——Colorless needles, mp 80—82 °C (from benzene). MS *m/z* (%): 129 ($M^+ + 1$, 40), 111 (7), 102 (10), 86 (23), 85 (81), 84 [$\cdot CH(OH)CH = CHC \equiv O^+$, 100]. UV $\lambda_{max}^{H_2O}$ nm: 197 (ε 10400). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3585, 3400 (OH), 1729 (C=O), 1628 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.50 (3H, d, $J = 6.5$ Hz, CH_3–CH), 2.52 (1H, d, $J = 7$ Hz, OH), 4.12—4.60 (2H, m, CH–OH and CHOCO), 5.98 (1H, dd, $J = 10.5$, 1.8 Hz, CH=CHCO), 6.85 (1H, dd, $J = 10.5$, 2.4 Hz, CH=CHCO). ORD ($c = 0.95$, $CHCl_3$, 24 °C): $[\phi]_{589}$ +10.8, $[\phi]_{450}$ +70.1, $[\phi]_{350}$ +431.2, $[\phi]_{280}$ +4203.8, $[\phi]_{270}$ +2910.3. The spectral data were in accord with the published values.[3]

(4*R*, 5*S*)-5-Hydroxy-2-hexen-4-olide (III)——A colorless oil. MS *m/z* (%): 129 ($M^+ + 1$, 23), 113 (6), 111 (4), 86 (28), 85 (94), 84 [$\cdot CH(OH)CH = CHC \equiv O^+$, 100]. UV $\lambda_{max}^{H_2O}$ nm: 203 (ε 6800). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590, 3400 (OH), 1781, 1758 (C=O), 1603 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.33 (3H, d, $J = 7$ Hz, CH_3–CH), 2.65 (1H, br s, OH), 4.05 (1H, d of quartet, $J = 6$, 5.6 Hz, CH–OH), 4.92 (1H, m, CHOCO), 6.17 (1H, dd, $J = 5.6$, 2 Hz, CH=CHCO), 7.57 (1H, dd, $J = 5.6$, 1.7 Hz, CH=CHCO). ORD ($c = 1.37$, $CHCl_3$, 22 °C): $[\phi]_{589}$ +112.4, $[\phi]_{450}$ +243.4, $[\phi]_{350}$ +599.3, $[\phi]_{300}$ +1198.5, $[\phi]_{250}$ +3033.8. The spectral data were in accord with the published values.[3]

Hydrogenation of III——III (28 mg) in methanol (10 ml) was shaken with Adams catalyst (80 mg) in a hydrogen atomosphere for 5 h. The filtered solution was evaporated to dryness *in vacuo* and the residue (27 mg) was passed through a silicic acid column. The fraction eluted with 0.5% MeOH–chloroform was concentrated to give I (14 mg) as an oil.

(3*S*, 5*S*)-3-Hydroxyhexan-5-olide (IV)——Colorless prisms, mp 67—70 °C (from ether). MS *m/z* (%): 131 ($M^+ + 1$, 42), 130 (M^+, 14), 115 (12), 113 (18), 102 (34), 97 (23), 88 (34), 86 [$\cdot CH_2CH(OH)CH_2C \equiv O^+$, 66], 84 (100), 73 (66), 71 (34). ^{1}H-NMR ($CDCl_3$) δ: 1.40 (3H, d, $J = 7$ Hz, CH_3–CH), 1.5—2.2 (2H, m, CH–CH_2–CH), 2.66 (2H, d, $J = 4$ Hz, OCOCH_2–CH), 2.77 (1H, s, OH), 4.36 (1H, quintet, $J = 4$ Hz, HOCH), 4.85 (1H, m, CO–OCH). ORD ($c = 1.57$, $CHCl_3$, 20 °C): $[\phi]_{589}$ −39.7, $[\phi]_{450}$ −79.5, $[\phi]_{350}$ −177.5, $[\phi]_{300}$ −291.5. The specific rotation, and IR and ^{1}H-NMR (measured in acetone-d_6) spectra were in accord with the published data.[4]

The acetate, prepared in the usual way and purified by silicic acid column chromatography with chloroform as the eluent, is a colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735 (acetate, δ-lactone). ^{1}H-NMR ($CDCl_3$) δ: 1.44 (3H, d, $J = 7$ Hz, CH_3–CH), 2.10 (3H, s, CH_3COO), 2.77 (2H, d, $J = 5$ Hz, CH–CH_2COO), 4.65 (1H, m, CHOCO), 5.25 (1H, m, $CHOCOCH_3$). MS *m/z* (%): 173 ($M^+ + 1$, 3), 128 (22), 113 (29), 112 ($M^+ - CH_3COOH$, 61), 97 (46), 87 (27), 86 (50), 85 (47), 84 (42), 69 (26), 68 (100).

Succinic Acid (V)——Colorless prisms, mp 185—186 °C (from AcOEt–MeOH). This compound was identified by direct comparison with an authentic sample.

Fatty Acid Ester (VI)——Colorless needles, mp 74—76 °C (from AcOEt). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730 (ester). ^{1}H-NMR ($CDCl_3$) δ: 0.91 (t, CH_3–CH_2), 1.26 (s, CH_2), 4.32 (t, CO–OCH_2). MS *m/z*: 522, 550, 578, 592, 606, 620, 648, 662, 676, 690 (each M^+). This material showed a single spot in silica gel TLC using benzene as a solvent.

Hydrolysis of VI——VI (20 mg) and 5% methanolic potassium hydroxide (3 ml) were refluxed for 3 h, then the mixture was concentrated under reduced pressure. The residue was diluted with water, and the solution was washed with chloroform, acidified with 10% aqueous hydrochloric acid, and extracted with ether. Evaporation of the ether gave the fatty acids (10 mg) as needles. Evaporation of the washings gave the fatty alcohols (8 mg) as an oil.

The fatty acids were methylated by treatment with dimethylformamide dimethylacetal and the resulting methyl esters were injected into the gas chromatograph. GLC showed peaks corresponding to C_{11}, C_{13}, C_{16} and C_{18} fatty acids, which were identified by GLC comparison with authentic samples. The fatty alcohols showed peaks corresponding to C_{24}, C_{26}, C_{28}, C_{29} and C_{30} fatty alcohols on GLC. MS *m/z*: 336, 364, 392, 406, 420 (each $M^+ - 18$).

Fatty Alcohol (VII)——Colorless needles, mp 78—81 °C (from AcOEt). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3450 (OH). ^{1}H-NMR ($CDCl_3$) δ: 0.90 (t, C$\underline{H}_3$–CH), 1.27 (s, CH_2), 3.67 (t, C$\underline{H}_2$OH). MS *m/z*: 364, 392, 406, 420 (each $M^+ - 18$). Silica gel TLC using benzene as a solvent showed a single spot. GLC showed peaks corresponding to C_{26}, C_{28}, C_{29} and C_{30} fatty alcohols.

Sterol (VIII)——Colorless needles, mp 114—122 °C (from *n*-hexane). A solution of the trimethylsilyl (TMS) ethers, prepared by heating with *N*-trimethylsilylimidazole (TMSI), was injected into the gas chromatograph. GLC showed two peaks corresponding to campesterol (t_R 14.8) and β-sitosterol (t_R 18.2).

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References and Notes

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