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Isolation of Ovulatory-Active Substances from Crops of Job's Tears (*Coix lacryma-jobi* L. var. *ma-yuen* STAPF.)[1)]

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Ovulatory-active substances were isolated from crops of Job's tears (*Coix lacryma-jobi* L. var. *ma-yuen* STAPF. Gramineae) with guidance by microscopic observation of oviducts of normal cycling female golden hamsters. These compounds were assigned as a 9 : 1 mixture of *trans*-feruloyl stigmastanol and *trans*-feruloyl campestanol (**1**), and a 9 : 1 mixture of their geometrical isomers (**2**) by chemical and spectral means. Synthesis of *trans*- and *cis*-feruloyl stigmastanol was also carried out. Substance **1** and the synthetic *trans*-feruloyl stigmastanol at 200 μg/d showed induction of ovulation and stimulation of ovarian follicular growth in female golden hamsters.

Keywords——ovulatory-active substance; ovarian follicular growth stimulation; *Coix lacryma-jobi* var. *ma-yuen*; Job's tears; *trans*-feruloyl stigmastanol; *trans*-feruloyl campestanol; *cis*-feruloyl stigmastanol; *cis*-feruloyl campestanol; photochemical conversion; feruloyl phytosterol

Fukuoka made the observation that a preparation containing crops of Job's tears (*Coix lacryma-jobi* L. var. *ma-yuen* STAPF. Gramineae) can improve disorders of the hypothalamus when given to patients with eugonadotropic hypogonadism.[2)] He also observed that oral administration of the Job's tears preparation to anovulatory patients caused the induction of ovulation based on the determination of luteinzing hormon-releasing hormon (LH-RH).[2)]

We now wish to report the isolation and structure determination of *trans*- and *cis*-feruloyl phytosterols which are the major ovulatory active principles of crops of Job's tears.

A preliminary examination showed that the hexane extract of Job's tears appears to cause ovarian stimulation, ovulation and sustained function of corpora lutea in normal cycling female golden hamsters. After the removal of triglycerides and acyloyl phytosterols (a mixture

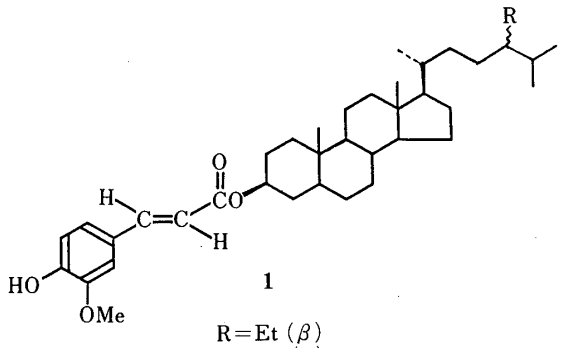
TABLE I. NMR Data for **1**, **2** and Their Acetates[a)]

	Signals due to phytosterol moiety C_3H		α-H	β-H	C_2H	C_5H	C_6H	OCH_3	OH	Ac
1	0.6—2.0	4.72 (m)	6.22 (d, $J=16$)	7.55 (d, $J=16$)	6.98 (br s)	6.86 (d, $J=9$)	7.04 (br d, $J=9$)	3.88	5.96	—
1 acetate	0.6—2.0	4.72 (m)	6.32 (d, $J=16$)	7.57 (d, $J=16$)	7.05 (br s)	7.00 (d, $J=9$)	7.10 (br d, $J=9$)	3.84	—	2.32
2	0.6—2.0	4.70 (m)	5.74 (d, $J=12$)	6.72 (d, $J=12$)	7.69 (d, $J=2$)	6.83 (d, $J=8$)	7.08 (dd, $J_1=8$, $J_2=2$)	3.88	5.80	—
2 acetate	0.6—2.0	4.70 (m)	5.88 (d, $J=12$)	6.80 (d, $J=12$)	7.48 (d, $J=2$)	6.95 (d, $J=8$)	7.12 (dd, $J_1=8$, $J_2=2$)	3.84	—	2.30

a) Run at 100 MHz in $CDCl_3$ solution; chemical shifts are given in ppm relative to tetramethylsilane as an internal standard.

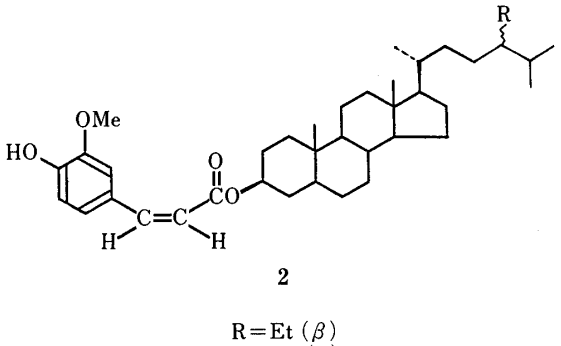
of palmitoyl β-sitosterol, stearoyl β-sitosterol, palmitoyl campesterol and stearoyl campesterol), two successive chromatographies of the hexane extract over silica gel and then alumina columns afforded a mixture of phenolic compounds together with triterpenoid I, 24-methylenecycloaltanol, β-sitosterol and campesterol (see Experimental). The phenolic mixture showed ovulatory activity as judged by microscopic observation on oviducts of experimental animals. After fractionation on a column of Lichroprep RP-18 and further purification by silica gel column chromatography, the phenolic mixture yielded substance **1**, colorless needles, mp 155—156 °C and substance **2**, colorless plates, melting from about 60 °C over a relatively wide range. Compound **1** showed broad hydroxyl and α,β-unsaturated carboxyl bands at 3120—3500 cm^{-1}, 1710 and 1625 cm^{-1} in the infrared (IR) spectrum. The nuclear magnetic resonance (NMR) spectrum of **1** exhibited signals at δ 6.22 and 7.55 (J = 16 Hz in each case), which were assigned to a *trans*-cinnamate system and, in addition, signals (Table I) that allowed assignment of **1** as a *trans*-feruloyl phytosterol.

When **1** was treated with sodium methoxide in methanol, *trans*-ferulic acid and a mixture of stigmastanol and campestanol (9 : 1) were obtained as reaction products. The latter was confirmed by gas chromatography-mass spectrometry (GC-MS) measurement after derivatization. These data show **1** to be a mixture of *trans*-feruloyl stigmastanol and *trans*-feruloyl campestanol (9 : 1). *trans*-Feruloyl stigmastanol has previously been isolated from corn germ oil[3] and wheat germ oil.[4]



1

R=Et (β)
R=Me (α)



2

R=Et (β)
R=Me (α)

The second phenolic compound **2** was isomeric with **1**, and its IR spectrum showed hydroxyl and α,β-unsaturated carboxyl bands at 3200—3500 cm^{-1}, 1710 and 1630 cm^{-1}. There were signals in the NMR spectrum of **2** associated with a phytosterol moiety (δ 0.6—2.0), a methoxy group (δ 3.88), a proton on an oxygen-bearing carbon (δ 4.70), two coupled (J = 12 Hz) one-proton doublets (δ 5.74 and 6.72), and three aromatic protons in an ABM system [δ 6.83 (d), 7.08 (dd) and 7.69 (d)] (Table I). The coupling constants of the doublets at δ 5.74 and 6.72 suggested that there is a *cis*-cinnamate system in **2**. Acetylation of **2** in the dark gave a monoacetyl derivative, mp 111 °C.

The structural relationship between **1** and **2** was proved by means of laser-Raman spectroscopy; the spectrum of **1** shows a *trans*-ethylenic bond at 1633 cm^{-1}, while that of **2** reveals a *cis*-ethylenic band at 1598 cm^{-1}. The structure of **2** was unequivocally confirmed by converting **2** to **1** under photochemical conditions. When a solution of **2** was irradiated with a photoflood lamp, an equilibrium mixture of **1** and **2** (3 : 1) was formed. Compound **1** was easily separated from the remaining **2** by silica gel column chromatography and its NMR spectrum was identical with that of **1** isolated from crops of Job's tears. Under identical conditions, **1** was converted in part to **2** in solution.

Finally, *trans*-feruloyl stigmastanol was synthesized and photo-conversion of this compound to *cis*-feruloyl stigmastanol was achieved. Stigmastanol[5] was added to a solution of *trans*-acetylferuloyl chloride in pyridine to afford the desired *trans*-acetylferuloyl stigmas-

TABLE II. Effect of Substance **1** on Ovulation in Normal Cycling Female Golden Hamsters

Substance **1** (μg)	Number of ova (M $\pm$ S.D.)
0	11 $\pm$ 1
200	18 $\pm$ 1[a]
1000	13 $\pm$ 2

a) Significantly different from the control at $p < 0.01$ by Student's t test.

TABLE III. Effects of Substance **1** on Ovarian and Pituitary Weights

Substance **1** (μg)	Ovarian weight (mg) (M $\pm$ S.D.)	Pituitary weight (mg) (M $\pm$ S.D.)
0	18.41 $\pm$ 2.18	5.03 $\pm$ 0.60
200	17.23 $\pm$ 1.67	5.02 $\pm$ 2.31
1000	16.32 $\pm$ 2.26	5.90 $\pm$ 1.43

tanol, mp 156 °C, in 62% yield. Deacetylation of the acetate with sodium borohydride at −2—−3 °C in a $CHCl_3$–MeOH solution provided *trans*-feruloyl stigmastanol, mp 155—156 °C, in 82% yield and this product was found to be pure *trans*-form from inspection of its NMR spectrum. Photolysis of *trans*-feruloyl stigmastanol in $CHCl_3$ afforded *cis*-feruloyl stigmastanol, colorless plates, mp *ca.* 60—80 °C, in 25% yield after chromatographic separation.

Although the *cis*-feruloyl phytosterol so far isolated could not be proved to be either a natural product or an artifact, the feruloyl phytosterol may occur as an equilibrium mixture of *trans*- and *cis*-form.

To examine the activity of **1** on the ovaries, an oral dose of 200 or 1000 μg per animal was administered daily to female golden hamsters for 3 weeks. With this treatment the normal cycling female golden hamsters underwent ovulation (Table II). The response at 200 μg/d was maximal, a larger dose (1000 μg/d) being less effective. Furthermore, the histological picture of the ovaries in the experimental groups showed excess follicular growth (data not shown). The estrous phase of the treated animals remained normal during the experiment. After administration for 3 weeks, the ovarian and pituitary weights of the treated animals were not significantly different from those of control groups (Table III). Analogous responses were observed on administration of synthetic *trans*-feruloyl stigmastanol.

These results suggested that the feruloyl phytosterols are the major ovulatory-active principle of crops of Job's tears.

Experimental

Melting points were taken on a Yamato MP-21 melting point apparatus and are uncorrected. NMR spectra were run on a JEOL JNM-FX-100 instrument using tetramethylsilane as an internal standard. Chemical shifts are given in δ units relative to tetramethylsilane. IR spectra were recorded on a JASCO A-100S grating infrared spectrophotometer. Low-resolution mass spectra (MS) and field desorption mass spectra (FD-MS) were taken on a JEOL JMS-01SG-2 mass spectrometer.

GC-MS was carried out by using a Hitachi M-52 spectrometer equipped with flame-ionization detector. The standard columns used included glass columns (1 m × 0.3 cm) packed with 2% or 1.5% OV-17 on Uniport HP (60—80 mesh).

Isolation——Powdered crops of Job's tears (*Coix lacryma-jobi* L. var. *ma-yuen* STAPF. Gramineae) (10 kg) were extracted three times with *n*-hexane at 50 °C. The *n*-hexane extract was evaporated under reduced pressure to give 500 g of an oily residue. The crude extract (300 g) was chromatographed over a column of silica gel (5 kg), which was eluted in the following order: 1, *n*-hexane; 2, *n*-hexane–AcOEt (100 : 1) (F-I; 4.35 g); 3, *n*-hexane–AcOEt (20 : 1) (F-II; 283 g); 4, AcOEt (F-III; 8.75).

The fraction eluted with *n*-hexane afforded a mixture of triglycerides.

Acyloyl Phytosterol——The F-I fraction was rechromatographed on silica gel using 1 : 1 *n*-hexane–benzene to give 1.32 g (0.022%) of acyloyl phytosterol, colorless plates, mp 65—67 °C. IR ν_{max}^{KBr} cm^{-1}: 1740 (–COO–). NMR ($CDCl_3$) δ: 0.6—2.0 (phytosterol moiety), 1.28 (s, $(-CH_2-)_n$), 2.25 (2H, t, J=7 Hz, $-COC\underline{H}_2-$), 4.60 (1H, m, $-O-C\underline{H}<$), 5.35 (1H, m, vinyl $\underline{H}$).

Hydrolysis of Acyloyl Phytosterol——A 5 ml portion of 10% sodium methoxide was added to a stirred solution of acyloyl phytosterol (1.0 g) in dioxane. The reaction mixture was refluxed for 4 h, then concentrated under reduced pressure, diluted with water (10 ml), and extracted with ether three times. The combined organic extracts were washed once with water and evaporated to give a pale yellow solid that was chromatographed over a column of silica gel to obtain 373 mg of a phytosterol as colorless plates. A solution of 10 mg of the phytosterol in 0.5 ml of CH_2Cl_2 was flushed with N_2, and 1 ml of hexamethyldisilazane–pyridine (TMSI-H) reagent was added. The reaction mixture was allowed to stand at room temperature for 1 h and subjected to GC-MS analysis (1 m OV-17 (2%) column at 257 °C). The chromatogram consisted of two peaks identified as β-sitosterol and campesterol in an 89 : 11 ratio.

The aqueous layer of the hydrolyzate was acidified with hydrochloric acid and extracted with ether. The ether extract was washed with water, dried (Na_2SO_4) and evaporated to give 195 mg of a brown oil. A solution of the oil (20 mg) in ether was treated with diazomethane. Analysis of the crude methyl ester by GC-MS on a 1 m OV-17 (1.5%) column showed an approximately 1 : 2 mixture of palmitic acid and stearic acid.

Terpenoid I——The F-II fraction (283 g) was chromatographed over a column of alumina (WAKO activated alumina, 300 mesh, 1.5 kg) with 20 : 1 *n*-hexane–AcOEt. Fractions of 50 ml were collected and monitored by thin layer chromatography (TLC). Fractions 11—16 gave a crystalline material. Recrystallization from MeOH afforded 128 mg of colorless needles, mp 201 °C. MS *m/z*: 426 (M^+). *Anal.* Calcd for $C_{30}H_{50}O$: C, 84.44; H, 11.81. Found: C, 84.35; H, 11.71. IR ν_{max}^{KBr} cm^{-1}: 3240—3360 (OH). NMR ($CDCl_3$) δ: 0.6—2.0 (triterpenoid moiety), 3.45 (1H, m, HO—$C\underline{H}<$), 5.61 (1H, m, vinyl $\underline{H}$). This compound remains unidentified at the present time.

Acetylation of Triterpenoid I——Triterpenoid I (10 mg) was acetylated in 1.4 ml of acetic anhydride–pyridine (1 : 2) overnight at room temperature and the reaction mixture was then evaporated under reduced pressure to give a brown syrup. The residue was chromatographed on a column of silica gel (1 g) to yield 8 mg of the acetate. Recrystallization from MeOH afforded colorless needles, mp 190 °C. MS *m/z*: 468 (M^+). IR ν_{max}^{KBr} cm^{-1}: 1740 (CH_2COO–). NMR ($CDCl_3$) δ: 0.6—2.0 (triterpenoid moiety), 2.0 (3H, s, $-COC\underline{H}_3$), 4.7 (1H, m, $>C\underline{H}$–OAc), 5.6 (1H, m, vinyl $\underline{H}$).

24-Methylenecycloaltanol——The subsequent eluate fractions containing 24-methylenecycloaltanol were combined and concentrated. Recrystallization from MeOH afforded pure 24-methylenecycloaltanol, mp 121—122 °C, which was identical with an authentic sample as judged by IR, NMR and MS comparisons.

β-Sitosterol and Campesterol——The subsequent eluate fractions, which showed a major spot of β-sitosterol, were combined. The residue was recrystallized from MeOH to give colorless plates (1.284 g). A solution of 20 mg of the crude β-sitosterol was treated with 1 ml of TMSI-H reagent as described above. GC-MS of the crude β-sitosteroyl trimethylsilylate on a 1 m OV-17 (2%) column showed a mixture of β-sitosterol and campesterol in a 9 : 1 ratio.

***trans*-Feruloyl Phytosterol (1)**——The subsequent eluate contained mainly material giving a positive ferric test. Upon removal of the solvent, a pale yellow solid (340 mg) was obtained. The crude solid was chromatographed on a column of Lichroprop RP-18 using a solvent mixture of AcOEt–EtOH (5 : 3) to give a phenolic material (280 mg). The phenolic portion was chromatographed on a column of silica gel (30 g) (solvent system: *n*-hexane–AcOEt (20 : 1)) to provide two phenolic compounds (**1** and **2**). The lower *Rf* (0.36) material (**1**) was recrystallized from MeOH to give colorless needles (210 mg, 0.0035%), mp 155—156 °C. FD-MS *m/z*: 578 $[(C_{38}H_{58}O_4)^+]$, 592 $[(C_{39}H_{60}O_4)^+]$. IR ν_{max}^{KBr} cm^{-1}: 3120—3500 (OH), 1710 (–COO–), 1640 (–COCH=CH–), 1600, 1510, 840, 805 (benzene ring), 1160, 1020 (–C–O–C–). NMR: Table I. UV $\lambda_{max}^{CHCl_3}$ nm (ε): 242 (10740), 295 (15200), 320 (20000). Raman ($CHCl_3$) cm^{-1}: 1633 ($\begin{matrix}-C\\H\end{matrix}\!\!>C=C<\!\!^{H}$).

Acetylation of 1——A solution of **1** (10 mg) in 0.5 ml of pyridine was added to 1.5 ml of acetic anhydride–pyridine (1 : 2). The reaction mixture was allowed to stand at room temperature for 10 h. The reaction mixture was evaporated under reduced pressure, and chromatography of the crude product on a column of silica gel (2 g) yielded 8 mg of *trans*-acetylferuloyl phytosterol, mp 155—156 °C. FD-MS *m/z*: 620 $[(C_{40}H_{60}O_5)^+]$, 634 $[(C_{41}H_{62}O_5)^+]$. IR ν_{max}^{KBr} cm^{-1}: 1770, 1710 (AcO), 1600, 1510 (benzene ring), 1180, 1080 (–C–O–C–). NMR: Table I.

Hydrolysis of 1——A solution of 30 mg of **1** in dioxane (1 ml) was added to an ice-cold 10% sodium methoxide solution (1 ml). The reaction mixture was refluxed for 3 h under an N_2 atmosphere, diluted with water, and extracted with ether three times. The combined ether extracts were washed once with water, dried over $MgSO_4$ and evaporated. The residue was treated with 1 ml of TMSI-H reagent. GC-MS of the crude phytosterol trimethylsilylate obtained

by work-up as described above showed a mixture of stigmastanol and campestanol in a 9 : 1 ratio.

The aqueous phase of the hydrolyzate was acidified with 10% hydrochloric acid and concentrated under reduced pressure. The residue was extracted repeatedly with AcOEt. The combined organic extracts were washed with water and evaporated to give a solid that was chromatographed on a column of silica gel (1 g) to give 6 mg of pale yellow needles, mp 173—174 °C. The identity of the product was confirmed by direct comparison with an authentic sample of *trans*-ferulic acid.

***cis*-Feruloyl Phytosterol (2)**——The higher *Rf* (0.52) material (**2**) was recrystallized from MeOH to afford colorless plates (90 mg, 0.0015%), mp from *ca.* 60 °C over a wide range. FD-MS *m/z*: 578 $[(C_{38}H_{58}O_4)^+]$, 592 $[(C_{39}H_{60}O_4)^+]$. IR ν_{max}^{KBr} cm^{-1}: 3200—3500 (OH), 1710 (–COO–), 1630 (–COCH=CH–), 1600, 1520 (benzene ring), 1170, 1030 (C–O–C). NMR: Table I. UV $\lambda_{max}^{CHCl_3}$ nm (ε): 242 (9300), 295 (12360), 320 (15420). Raman ($CHCl_3$) cm^{-1}: 1598 ($\overset{H}{\diagdown}C{=}C\overset{H}{\diagup}$).

Acetylation of 2——A solution of 10 mg of **2** in 1 ml of pyridine and 0.5 ml of acetic anhydride was allowed to stand overnight in the dark at room temperature. The reaction mixture was then evaporated to a brown syrup under reduced pressure. The residue was chromatographed on a column of silica gel (1 g) to afford 7 mg of *cis*-acetylferuloyl phytosterol, mp 111 °C. FD-MS *m/z*: 620 $[(C_{40}H_{60}O_5)^+]$, 634 $[(C_{41}H_{62}O_5)^+]$. IR ν_{max}^{KBr} cm^{-1}: 1770, 1720 (AcO), 1630 (–COCH=CH–), 1600, 1520 (benzene ring), 1170, 1070 (–C–O–C–). NMR: Table I.

Hydrolysis of 2——**2** (15 mg) was hydrolyzed with 10% sodium methoxide as described above. Work-up in the usual manner afforded 7 mg of a phytosterol mixture. Analysis by GC-MS (1 m OV-17 (2%) column at 270 °C) as described above indicated that the phytosterol contained 90% stigmastanol and 10% campestanol.

The aqueous phase on work-up gave a crystalline solid which consisted of *trans*- and *cis*-ferulic acid in a 3 : 1 ratio as judged by NMR analysis.

Photochemical Conversion of 2 to 1——A solution of 30 mg of **2** in $CHCl_3$ (10 ml) was irradiated with a photo-flood lamp (Toshiba, 375 W) for 2 h. Removal of the solvent under reduced pressure gave a white solid which consisted of **1** and **2** in a 3 : 1 ratio as judged by NMR analysis. Chromatography of the solid on a column of silica gel (10 g) in *n*-hexane–AcOEt (20 : 1) yielded the starting **2** (3 mg) and the desired compound **1** (17 mg). Recrystallization from MeOH gave 15 mg of **1** as colorless needles, mp 156 °C; this product was identical with **1** isolated from crops of Job's tears.

Synthesis of *trans*-Feruloyl Stigmastanol——Freshly distilled $SOCl_2$ (1.5 ml) was added to a solution of *trans*-acetylferulic acid (1.0 g) in dry $CHCl_3$ (10 ml) at 0 °C with vigorous stirring. After being stirred for 5 h, the reaction mixture was evaporated under reduced pressure. The residue (*trans*-acetylferuloyl chloride) was dissolved in 10 ml of pyridine, and a solution of 1.76 g of stigmastanol[5] in pyridine (15 ml) was added dropwise at 0 °C with stirring. The reaction mixture was allowed to stand overnight at room temperature and was then worked up in the usual manner, leaving 1.8 g of a crude product, which was subjected to silica gel (50 g) chromatography. Elution with *n*-hexane–AcOEt (20 : 1) gave 1.65 g (61.6%) of pure *trans*-acetylferuloyl stigmastanol, mp 156 °C, as colorless needles. *Anal.* Calcd for $C_{41}H_{62}O_5$: C, 77.56; H, 9.84. Found: C, 77.37, H, 9.83. FD-MS *m/z*: 620 $[(C_{40}H_{60}O_5)^+]$, 634 (M^+). IR ν_{max}^{KBr} cm^{-1}: 1770, 1710 (Ac), 1640 (–CH=CHCOO–), 1600, 1510 (benzene ring), 1180, 1080 (–C–O–C–). NMR ($CDCl_3$) δ: 0.6—2.0 (stigmastanol moiety), 2.32 (3H, s, –COC$\underline{H}_3$), 3.84 (3H, s, –OC$\underline{H}_3$), 4.70 (1H, m, –O–C$\underline{H}\lessdot$), 6.32 (1H, d, J=16 Hz, –COC$\underline{H}$=CH–), 7.00 (1H, d, J=9 Hz, $C_5\underline{H}$ of benzene ring), 7.05 (1H, br s, $C_2\underline{H}$ of benzene ring), 7.10 (1H, br d, J=9 Hz, $C_6\underline{H}$ of benzene ring), 7.60 (1H, d, J=16 Hz, –COCH=C$\underline{H}$–).

$NaBH_4$ (0.2 g, 5 mmol) was added portionwise to a solution of *trans*-acetylferuloyl stigmastanol (1.60 g, 2.5 mmol) in $CHCl_3$–MeOH (1 : 1, 30 ml) at −2—−3 °C with vigorous stirring, and stirring was continued for 30 min. The reaction mixture was then quenched by adding water and was extracted with ether. The combined organic layers were washed with water, dried over Na_2SO_4 and evaporated to afford a white solid. Chromatography of the solid on silica gel (70 g) in *n*-hexane–AcOEt (20 : 1) and recrystallization from MeOH provided 1.23 g (82%) of *trans*-feruloyl stigmastanol, mp 155—156 °C, as colorless needles. *Anal.* Calcd for $C_{39}H_{60}O_4$: C, 79.05; H, 10.14. Found: C, 79.17; H, 9.86. FD-MS *m/z*: 578 $[(C_{38}H_{58}O_4)^+]$, 592 (M^+). IR ν_{max}^{KBr} cm^{-1}: 3200—3500 (OH), 1710 (–COO–), 1640 (–COCH=CH–), 1600, 1520 (benzene ring), 1180, 1080 (–C–O–C–). NMR ($CDCl_3$) δ: 0.6—2.0 (stigmastanol moiety), 3.88 (3H, s, –OC$\underline{H}_3$), 4.72 (1H, m, –O–C$\underline{H}\lessdot$), 5.94 (1H, s, –O$\underline{H}$), 6.22 (1H, d, J=16 Hz, –COC$\underline{H}$=CH–), 6.86 (1H, d, J=9 Hz, $C_5\underline{H}$ of benzene ring), 7.00 (1H, br s, $C_2\underline{H}$ of benzene ring), 7.04 (1H, br d, J=9 Hz, $C_6\underline{H}$ of benzene ring), 7.55 (1H, d, J=16 Hz, –COCH=C$\underline{H}$–).

Photochemical Conversion of *trans*-Feruloyl Stigmastanol to *cis*-Feruloyl Stigmastanol——A solution of 200 mg in $CHCl_3$ (50 ml) was irradiated with a photo-flood lamp (Toshiba, 375 W) for 3 h. After removal of the solvent, chromatography of a white residue on silica gel (30 g) in *n*-hexane–AcOEt (20 : 1) gave 50 mg (25%) of *cis*-feruloyl stigmastanol as the first eluted material. Recrystallization from MeOH provided colorless plates, mp *ca.* 60—80 °C. *Anal.* Calcd for $C_{39}H_{60}O_4$: C, 79.05; H, 10.14. Found: C, 78.92; H, 10.09. FD-MS *m/z*: 592 (M^+). IR ν_{max}^{KBr} cm^{-1}: 3200—3500 (OH), 1710 (–COO–), 1630 (–CH=CH–COO–), 1600, 1520 (benzene ring), 1170, 1030 (C–O–C). NMR ($CDCl_3$) δ: 0.6—2.0 (stigmastanol moiety), 3.88 (3H, s, –OC$\underline{H}_3$), 4.70 (1H, m, –OC$\underline{H}\lessdot$), 5.74 (1H, d, J=12 Hz, –COC$\underline{H}$=CH–), 6.72 (1H, d, J=12 Hz, –COCH=C$\underline{H}$–), 6.83 (1H, d, J=8 Hz, $C_5\underline{H}$ of benzene ring), 7.08 (1H, dd, J_1=8 Hz, J_2=2 Hz, $C_6\underline{H}$ of benzene ring), 7.69 (1H, d, J=2 Hz, $C_2\underline{H}$ of benzene ring).

The subsequent eluate gave unchanged *trans*-feruloyl stigmastanol.

Ovulatory Activity

Animals——Female golden hamsters were obtained from the Shizuoka Laboratory Animal Center (Shizuoka, Japan) and maintained in rooms at 22 °C with a 14 h light period (8.00 am—10.00 pm). Golden hamsters (8weeks old) were used in the experiments only after three successive 4-d estrous cycles.

Assay of Ovulatory Activity——Groups of five golden hamsters were used for the assay of ovulatory activity. Substance **1** was suspended in 1 ml of 0.2% carboxy methyl cellulose (CMC) solution. Golden hamsters were given orally either 200 or 1000 μg on the day of estrus for 3 weeks. Control golden hamsters were given 1 ml of vehicle (0.2% CMC). The hamsters were sacrificed by injection of Nembutal on the last day of estrus, and the oviducts were removed and searched for ova by microscopic examination[6] (Table II). Ovaries were prepared for light microscopy by routine histological methods, sectioned serially at 7 μm, and stained with hematoxylin–eosin. The influence of **1** on ovary and pituitary weights was determined by direct weighing of the removed organs (Table III).

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References and Notes

1) A part of this study was presented at the 105th Annual Meeting of the Pharmaceutical Society of Japan, Kanazawa, April, 1985, Abstract of Papers, p. 457.
2) H. Fukuoka "On the ovulatory effect of MH-120 (a Job's tears preparation)," Circulation article, 1981.
3) J. Tamura, N. Sakaedani and T. Matsumoto, *Nippon Kagaku Zasshi*, **79**, 1011 (1958).
4) T. Tamura, T. Hibino, K. Yokoyama and T. Matsumoto, *Nippon Kagaku Zasshi*, **80**, 215 (1959).
5) The commercially available stigmastanol contained *ca.* 10% campestanol.
6) H. O. Burdick and R. Whitney, *Am. J. Physiol.*, **132**, 405 (1941).