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# Steroids from *Cucumis Melo* L. var. *Makuwa* Makino and *Cucumis sativus* L.

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A Chinese medicine, 'Melo Calyx' (Katei), calyxes of the fruits of *Cucumis Melo* L. var. *Makuwa* Makino, have been used for emetics.

Investigation of the bitter principles in these calyxes and the leaves of *Cucumis sativus* L. was undertaken.

During the isolation process of the components of them, two kinds of $\Delta^7$-sterol were obtained from both plants. It seemed that bitter tasts of the plants probably closely related to the contents of the steroids because the yields of the steroids varied in inverse ratio to the bitterness of the plants for extraction.

from *Cucumis sativus* L.

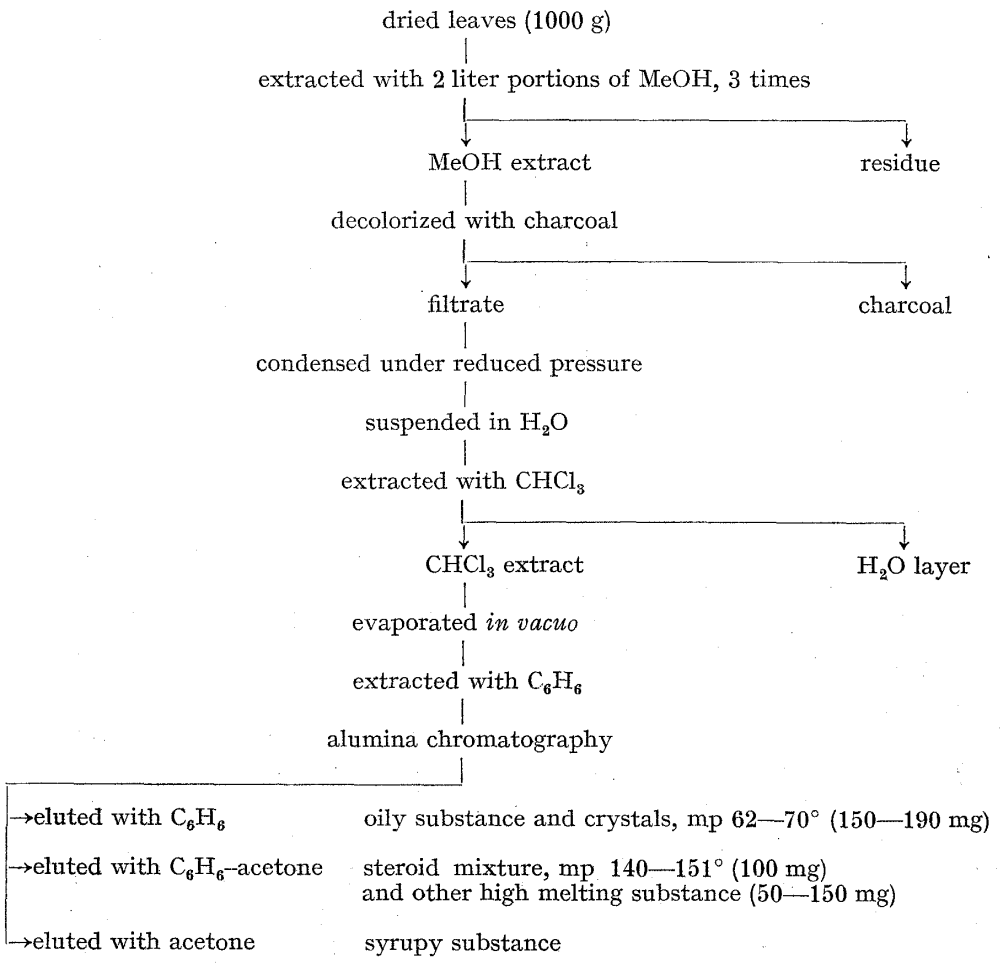



Fig. 1 (continue)

1) Location: *Kowakae, Higashi-Osaka, Osaka.*

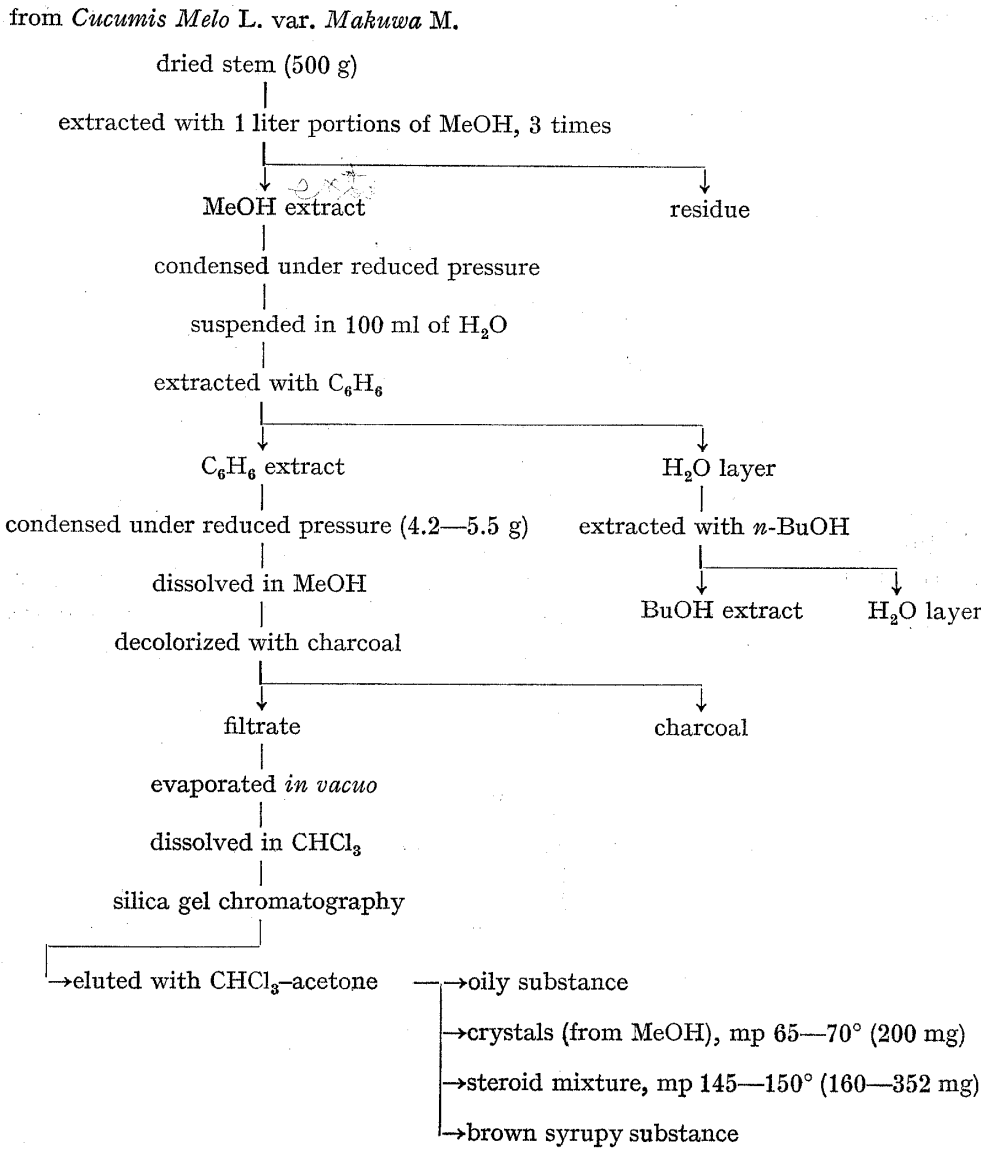



Fig. 1. Extraction of Steroids

For the calyxes of the fruits of *C. Melo* were difficult to collect, the stem was used for the extraction. Isolation of the components from the plants was illustrated in Fig. 1.

The steroid mixture which was obtained by column chromatography on silica gel was converted to a mixture of acetates. It was found that the mixture contains two kinds of steroid acetate by examinations using silver nitrate–impregnated thin-layer chromatography[2] and gas chromatography.

Isolation of two acetates was carried out throughing the mixture in a silver nitrate–impregnated silical gel column, and eluting with petroleum ether–ethyl ether. One of them (1b), mp 155—157°, $C_{31}H_{52}O_2$, $[\alpha]_D^{30}=+12.5°$ ($c=0.96$, chloroform), shows a parent peak in mass spectrum at *m/e* 456, and absorptions at 1665 $cm^{-1}$, 792 $cm^{-1}$ (>C=CH–), and 1736 $cm^{-1}$ ($CH_3COO$–) in the infrared spectrum. Methyl signals, 9.48 $\tau$ (3H, singlet), and 9.20 $\tau$ (3H, singlet) which were typical shifts assignable to $CH_3$-18, and $CH_3$-19 of sterol, respectively, and a signal of one olefinic proton at 4.92 $\tau$ were observed in the nuclear magnetic resonance spectrum.

2) J.M. Cubero, H.K. Mangold, *Microchem. J.*, 9, 227 (1965).

These results and identical properties described in literature, showing positive Liebermann–Buchard reaction (violet), and selenium dioxide test[3], strongly suggest that this sterol acetate is $\Delta^7$-stigmastenol-(3$\beta$) acetate.

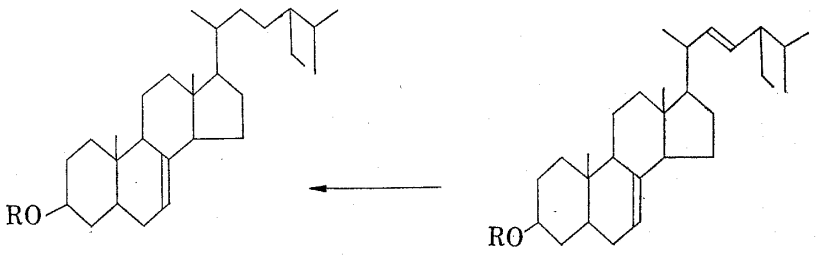
The acetate and the free sterol were identified with authentic $\Delta^7$-stigmastenol-(3$\beta$) acetate (1b),[4] and $\Delta^7$-stigmastenol-(3$\beta$)[5] (1a) by direct comparisons of silver nitrate–impregnated thin–layer chromatograms, infrared spectra, and the retention times of gas chromatography.

The other sterol acetate, mp 174—176°, $C_{31}H_{50}O_2$, $[\alpha]_D^{30}=-3.5°$ ($c$=1.17, chloroform), parent peak $m/e$ 454, shows absorptions at 1733 $cm^{-1}$ ($CH_3COO$–), and 970 $cm^{-1}$ (*trans* –CH=CH–) in the infrared region. The nuclear magnetic resonance spectrum of this compound showed chemical shifts at 9.48 $\tau$ (3H, singlet), and 9.20 $\tau$ (3H, singlet) which can be assign to $CH_3$-18 and $CH_3$-19 of sterol skeleton, respectively, and 4.80—5.40 $\tau$ (3H, multiplet, >C=CH–, and –CH=CH–). And it showed positive color tests of Liebermann–Buchard reaction (violet) and selenium dioxide.[3]

Above results apparently revealed that the compound belongs to $\Delta^7$– stigmastenol group, and it was completely identified with $\alpha$-spinasterol acetate (2b) by the comparisons of infrared, nuclear magnetic resonance spectra, *Rf* values of silver nitrate–impregnated thin–layer chromatography, and the retention times of gas chromatography.

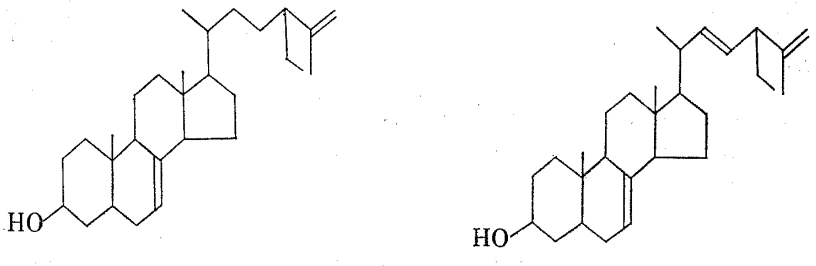
The additive proofs on the structures of these steroids were carried out by hydrogenation of the acetate, mp 174—176° ($\alpha$-spinasterol acetate (2b)) giving acetate, mp 155—157° ($\Delta^7$-stigmastenol-(3$\beta$) acetate (1b)).

Recently, Sucrow found $\alpha$-spinasterol (2a) as major $\Delta^7$-sterol from the fresh fruits of *Cucumis Melo* L. (melon), *Cucurbita Pepo* L., and *Citrullus vulgaris* SCHRAD., but only traces of $\Delta^{7.25}$-stigmastadienol-(3$\beta$) (3), $\Delta^{7.22.25}$-stigmastatrienol-(3$\beta$) (4), and no $\Delta^7$-stigmastenol-(3$\beta$) (1a), and the seeds of *C. Melo* L., *Cucurbita Pepo* L., and *Citrullus vulgaris* SCHRAD. contain ster-




1a : R=H–
1b : R=$CH_3CO$–

2a : R=H–
2b : R=$CH_3CO$–




3 4

Chart 1

3) L.F. Fieser, M. Fieser, "Reagents for Organic Synthesis," John Wiley and Sons, Inc., New York, London, Sydney, 1967, p. 998.
4) The sample was prepared by hydrogenation of $\alpha$-spinasterol acetate.
5) Presented by Prof. W. Sucrow, Berlin.

oids, 2a, 3, and 4 in moderate amounts and accompanied by very small amount of $\Delta^5$-sterol,[6] which were also found in the seeds of *Momordia charantia* L., and the fruits of *M. charantia*, 3, 4, $\Delta^{5,25}$-stigmastadienol(3$\beta$), and $\beta$-sitosterol.[7,8] Sucrow also reported the presence of 1a as a chief steroid in the root of *Coccinia indica* WIGHT which is a cucurbitaceae as well.[6]

On our experiments, no $\Delta^{7,22,25}$-stigmastatrienol (4), but 1a, and 2a were found in the stem of *C. Melo* L. var. *Makuwa* M. and the leaves of *C. sativus* L.

## Experimental

**Acetylation of the Steroid Mixture**——A mixture of $Ac_2O$ (1 ml), $C_5H_5N$ (0.8 ml), and the steroid mixture which was obtained from cucurbitaceae by the way described in scheme 1 (60 mg) was warmed on a water bath for 30 min. After cooling, the solution was poured into ice–water (10 ml). The separated solid was collected by filtration (65 mg), and recrystallized from MeOH.

**Thin-Layer Chromatography of the Acetate Mixture on Silver Nitrate-impregnated Plates**——The plates coated with "Kieselgel G nach Stahl" were activated for 2 hrs at 120°, and allowed to cool for 10 min at room temperature. The edges (3 mm) of the plates were soaked in 20% $AgNO_3$ solution under shielding of light, and made assend the solution to the top of the plates (about 5 min). After drying the plates at room temperature, they were activated for 1.5—2 hr at 110—120°. The prepared plates were used within 10 min.

Development of the spotted plates were carried out with a mixture of cyclohexane and isopropyl ether (9:1) and repeated for 4 times. $Rf$=0.52, 0.61.

**Isolation of the Acetate Mixture by Column Chromatography**——Silica gel (Mallinckrodt Chemical Works) (240 g), $AgNO_3$ (60 g), and distilled $H_2O$ (400 ml) were mixed and dried under occasional stirring for 5 hrs at 110—120°. The mixture was suspended in petroleum ether–ether (98:2) and packed in the column (4×43 cm).

The mixture of steroid acetate (419 mg) was subjected on the column. When the column was eluted with petroleum ether containing ether five crystalline fractions were obtained.

| fraction | *Rf* | | mp | weight |
|---|---|---|---|---|
| A | 0.61 | — | 143—145° | 57 mg |
| B | 0.61 | 0.52 (trace) | 150—152° | 52 mg |
| C | 0.61 | 0.52 | 164° | 160 mg |
| D | 0.61 (trace) | 0.52 | 173—175° | 92 mg |
| E | — | 0.52 | 162—164° | 102 mg |

**$\Delta^7$-Stigmastenol-(3$\beta$) Acetate (1b)**——i) The fraction A was recrystallized from 95% EtOH, and gave colorless needles, mp 154—156°, $[\alpha]_D^{30}$ =+12.5° ($c$=0.96, $CHCl_3$), (21 mg). *Anal.* Calcd. for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48. Found: C, 81.75; H, 11.37; C, 81.55; H, 11.33. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1736, 1665, 792. NMR 9.48 $\tau$ (3H, s.), 9.2 $\tau$ (3H, s.), 4.92 $\tau$ (1H). This compound completely identified with authentic sample by the comparisons of IR spectra and the retention times of gas chromatography.

ii) $\alpha$-Spinasterol acetate (2b) (57 mg) was hydrogenated in dioxane with reduced platinum (50 mg) for 1 hr. The solution was evaporated *in vacuo* to dryness and crystallized from 95% EtOH, mp 154—156°. The product was identical with authentic sample of 1b.

**$\alpha$-Spinasterol Acetate (2b)**——The fraction E was recrystallized from 95% EtOH to give colorless needles, mp 174—176°, $[\alpha]_D^{30}$ =−3.5° ($c$=1.17, chloroform) (72 mg). *Anal.* Calcd. for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08. Found: C, 81.68; H, 10.95. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1733, 970. NMR 9.48 $\tau$ (3H, s), 9.20 $\tau$ (3H, s), 4.80—5.40 $\tau$ (3H, mult). The IR spectrum, and the retention time of gas chromatography of this compound were completely identified with those of authentic sample.

**$\Delta^7$-Stigmastenol-(3$\beta$) (1a)**——The acetate (1b) was hydrolyzed with EtOH–KOH, and recrystallized from MeOH giving colorless crystals, mp 137—140°, $[\alpha]_D^{30}$ =+9.1° ($c$=0.95, chloroform).

The crystals were completely identical with authentic sample by the comparisons of IR spectra and the retention times of gas chromatography.

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6) Private communication of Prof. W. Sucrow and W. Sucrow, A. Reimerdes, *Z. Naturforschung*, **23b**, 42 (1968).
7) W. Sucrow, *Chem. Ber.*, **99**, 2765 (1966).
8) W. Sucrow, *Chem. Ber.*, **99**, 3559 (1966).