Phytochemistry, Vol. 22, No. 11, pp. 2621–2622, 1983.
Printed in Great Britain.

0031–9422/83 $3.00+0.00

4α-METHYL-5α-CHOLEST-8(14)-EN-3β-OL FROM THE SEEDS OF *CAPSICUM ANNUUM*

TARO MATSUMOTO, YOSUKE OSAWA and TOSHIHIRO ITOH

College of Science and Technology, Nihon University, 1–8, Kanda Surugadai, Chiyoda-ku, Tokyo, 101 Japan

(*Received* 8 *March* 1983)

Key Word Index—*Capsicum annuum*; Solanaceae; 4α-methylsterol; 4α-methyl-5α-cholest-8(14)-en-3β-ol; seeds.

Abstract—A 4α-methylsterol was isolated from the seeds of *Capsicum annuum* and was identified as 4α-methyl-5α-cholest-8(14)-en-3β-ol. This seems to be the first instance of the detection of a $\Delta^{8(14)}$-4α-methylsterol in a higher plant.

In a previous study on the constituents of 4α-methylsterols in the seeds of *Capsicum annuum* and some other Solanaceae plants, we have identified seventeen 4α-methylsterols of which four were new ones, but several minor constituents remained unidentified [1]. Our further study of the 4α-methylsterols of *C. annuum* seeds has now led to the isolation of a minor 4α-methylsterol and its identification as 4α-methyl-5α-cholest-8(14)-en-3β-ol (**1**).

The 4α-methylsterol fraction that was separated as described previously [1] from the unsaponifiable lipid of *C. annuum* seed oil was acetylated and the resulting acetate fraction (1.1 g) was separated into three bands by silver nitrate–silica gel TLC. The fraction (330 mg) recovered from the first band (R_f 0.84) down from the solvent front was subjected to reverse-phase HPLC which yielded a 4α-methylsterol (**1**) acetate (17 mg). GC had shown that this sterol comprised 5% of the total 4α-methylsterols. The mass spectrum of **1**-acetate (*m/z* 442, M^+, $C_{30}H_{50}O_2$) exhibited fragment ions at *m/z* 329 and 269 (loss of side chain and side chain plus acetic acid, respectively), and *m/z* 287 and 227 (loss of ring D and ring D plus acetic acid, respectively) showing that it was an acetate of a C_{28} 4α-methylsterol with a monounsaturated nucleus and a C_8 saturated side chain [2]. The ^{1}H NMR spectrum of **1**-acetate showed no olefinic protons indicating that the nuclear double bond could only be located at the $\Delta^{8(9)}$- or $\Delta^{8(14)}$-position. The double bond was established to be located at the $\Delta^{8(14)}$-position since **1**-acetate showed H-18 and H-19 methyl signals at δ 0.83 and 0.73, respectively, which were almost identical with those reported for 4α-methyl-5α-cholest-8(14)-en-3β-yl acetate (H-18, δ 0.84; H-19, δ 0.71) [3, 4]. The $\Delta^{8(9)}$-isomer, 4α-methyl-5α-cholest-8-en-3β-yl acetate, exhibited very different signals for these methyl group protons with H-18 at δ 0.61 and H-19 at δ 0.98 [1]. Thus the 4α-methylsterol had the structure 4α-methyl-5α-cholest-8(14)-en-3β-ol (**1**). The identification was confirmed by the direct comparison with authentic **1**-acetate which was synthesized from its Δ^7-isomer, lophenyl (4α-methyl-5α-cholest-7-en-3β-yl, **2**) acetate [1], by double bond isomerization (H_2–PtO_2, in AcOH) [5].

A minor 4α-methylsterol, which was detected by GC but remained unidentified in the previous study, of the 4α-methylsterol fractions from the seeds of further Solanaceae [1]: *Lycopersicon esculentum*, *Physalis alkekengi*, *Lycium chinense*, *Datura stramonium*, *D. metel*, *Nicotiana tabacum* and *Solanum melongena*, should now be considered to be **1**.

The occurrence of sterol with $\Delta^{8(14)}$-unsaturation in nature is rare. 5α-Stigmasta-8(14),22-dien-3β-ol in a higher plant, *Haplopappus heterophyllus* [6], and several $\Delta^{8(14)}$-4α-methylsterols have previously been detected in yeast [7], *Chlorella* [8, 9], a bacterium, *Methylococcus capsulatus* [3], and marine dinoflagellates [10, 11]. This study seems to be the first case for the idenfication of a $\Delta^{8(14)}$-4α-methylsterol, **1**, in a higher plant. $\Delta^{8(14)}$-Sterols have been suggested as intermediates arising during sterol biosynthesis as a consequence of the C-14 demethylation step [12].

EXPERIMENTAL

Mps are uncorr. HPLC was carried out on two Partisil 5 ODS-2 columns (Whatman, 10 mm i.d. × 25 cm; packed by Erma Optical Works, Tokyo) in series using a UV detector monitoring at 210 nm (mobile phase, MeOH). GC on an OV-17 SCOT glass capillary column was under the conditions already described [1]. RR_t in HPLC and GC were expressed relative to cholesteryl acetate. MS (70 eV) were taken with a direct inlet system. ^{1}H NMR spectra (100 MHz) were determined in $CDCl_3$ with TMS as internal standard. The seeds of *Capsicum annuum* were purchased from a seed merchant. Our general techniques have been described previously [1].

4α-Methyl-5α-cholest-8(14)-en-3β-yl (**1**) *acetate isolated from* C. annuum *seeds.* Mp 77–78° (lit. [3] mp 76.5–79°, [13] mp 78–78.5°) RR_t: 1.19 in HPLC, and 1.14 in GC. MS *m/z* (rel. int.): 442 $[M]^+$ (100), 427 (18), 382 (8), 367 (15), 329 (6), 302 (4), 287 (3), 269 (14), 243 (18), 227 (24). ^{1}H NMR: δ 0.83 (3H, *s*, H-18), 0.73 (3H, *s*, H-19), 0.92 (3H, *d*, J = 6.3 Hz, H-21), 0.86 (6H, *d*, J = 6.1 Hz, H-26, H-27), 0.85 (3H, *d*, J = 6.3 Hz, H-30), 4.41 (1H, *m*, $W_{1/2}$ = 26 Hz, H-3α).

Synthesis of **1**-*acetate from lophenyl* (**2**) *acetate by double bond isomerization.* A solution of **2**-acetate (13 mg) in AcOH (10 ml)

containing PtO_2 (40 mg) as catalyst was hydrogenated at atm. pres. and room temp. overnight. Usual work-up of the reaction product afforded **1**-acetate (10 mg, mp 78–80°). All the chromatographic and spectral data of the synthetic **1**-acetate were essentially identical with those of naturally occurring **1**-acetate.

Acknowledgement—We thank Drs. T. Takido and M. Aimi for the measurements of ^1H NMR and mass spectra. Our thanks are also due to K. Migita for technical assistance.

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Phytochemistry, Vol. 22, No. 11, pp. 2622–2624, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00
Pergamon Press Ltd.

OCCURRENCE OF 24-ETHYL-Δ^5- AND 24-ETHYL-Δ^7-STEROLS AS C-24 EPIMERIC MIXTURES IN SEEDS OF *CUCUMIS SATIVUS*

TARO MATSUMOTO, TOSHIFUMI SHIGEMOTO and TOSHIHIRO ITOH

College of Science and Technology, Nihon University, 1–8, Kanda Surugadai, Chiyoda-ku, Tokyo, 101 Japan

(*Received* 25 *April* 1983)

Key Word Index—*Cucumis sativus*; Cucurbitaceae; seeds; 24-ethylsterols; spinasterol; chondrillasterol; sitosterol; clionasterol; 22-dihydrospinasterol; 22-dihydrochondrillasterol.

Abstract—^1H NMR and ^{13}C NMR spectroscopy have demonstrated that the 24-ethyl-5α-cholesta-7, *trans*-22-dien-3β-ol, 24-ethylcholest-5-en-3β-ol and 24-ethyl-5α-cholest-7-en-3β-ol isolated from the seeds of *Cucumis sativus* are mixtures of both 24α- and 24β-epimers. This seems to be the first instance of the detection of 24β-ethylcholest-5-en-3β-ol in a higher plant.

24β-Ethyl-5α-cholesta-7,22,25(27)-trien-3β-ol (**1**) (all the sterols possessing Δ^{22}-double bond described here have a *trans*-configuration at C-22) and 24β-ethyl-5α-cholesta-7,25(27)-dien-3β-ol (**2**) together with 24α-ethyl-5α-cholesta-7,22-dien-3β-ol (spinasterol, **3**) and/or its 24β-epimer (chondrillasterol, **4**) are the major sterols in the seeds of some Cucurbitaceae [1]. We have shown recently that a minor 24-methylsterol isolated from the seeds of *Cucumis sativus* is 24α-methyl-5α-cholesta-7,22-dien-3β-ol (stellasterol, **5**) [2]. Our continuing study of the sterols of *C. sativus* seeds has now led to the isolation of three 24-ethylsterols, 24-ethyl-5α-cholesta-7,22-dien-3β-ol, 24-ethylcholest-5-en-3β-ol and 24-ethyl-5α-cholest-7-en-3β-ol, and the demonstration that these sterols are mixtures of the epimers at C-24, i.e. **3** and **4**, 24α-ethylcholest-5-en-3β-ol (sitosterol, **6**) and its 24β-epimer (clionasterol, **7**), and 24α-ethyl-5α-cholest-7-en-3β-ol (22-dihydrospinasterol, **8**) and its 24β-epimer (22-dihydrochondrillasterol, **9**), respectively. The other sterols identified were **1** and **2** in addition to **5**.

The sterol fraction that was separated from the unsaponifiable lipid of *C. sativus* seed oil was acetylated, and the resulting acetate fraction was separated into four bands (refered to as bands 1–4 in order of polarity, beginning with the least polar) by silver nitrate-silica gel TLC [2]. The fraction recovered from band 1 (R_f 0.66) was hydrolysed and the resulting free sterol fraction was subjected to reversed-phase HPLC (ODS-2 column), which yielded the mixture (RR_t 1.18 in HPLC) of 24-ethylcholest-5-en-3β-ol (sterol A, RR_t 1.61 in GC, **6**