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(22*E*,24*S*)-5α-ERGOSTA-7, 22-DIEN-3β-OL FROM THE SEEDS OF *CUCUMIS SATIVUS*

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Key Word Index—*Cucumis sativus*, Cucurbitaceae, seeds, 24-methyl-$\Delta^{7,22}$-sterol, (22*E*,24*S*)-5α-ergosta-7,22-dien-3β-ol, stellasterol

Abstract—(22*E*,24*S*)-5α-Ergosta-7,22-dien-3β-ol isolated from the seeds of *Cucumis sativus* was shown to be the 24*S*-epimer, i.e. stellasterol, by ^{1}H NMR (400 MHz) and ^{13}C NMR spectroscopy This seems to be the first instance of the detection of stellasterol in a higher plant

INTRODUCTION

Seeds of the family Cucurbitaceae contain the 24-ethyl-Δ^7-sterols, (22*E*,24*S*)-5α-stigmasta-7,22,25(27)-trien-3β-ol and (24*S*)-5α-stigmasta-7,25(27)-dien-3β-ol together with (22*E*,24*R*)-5α-stigmasta-7,22-dien-3β-ol (chondrillasterol) and/or its 24*S*-epimer (spinasterol), as the major sterols [1–4] Our further study of the sterols of Cucurbitaceae seeds has now led to the isolation and characterization of a minor 24-methylsterol, (22*E*, 24*S*)-5α-ergosta-7,22-dien-3β-ol (stellasterol, **1**), from the seeds of *Cucumis sativus* (cucumber)

RESULTS AND DISCUSSION

The sterol fraction that was separated from the unsaponifiable lipid of *C sativus* seed oil was acetylated and the resulting acetate fraction (6.4 g) was separated into four bands by silver nitrate–Si gel TLC The fraction (18 mg) recovered from the faint second band (R_f 0 56) from the solvent front was subjected to reverse-phase HPLC which yielded a steryl (**1**) acetate (7 mg). GC had shown that this sterol comprised 2.2 % of the total sterols. The mass spectrum of 1-acetate (*m/z* 440, M^+, $C_{30}H_{48}O_2$) exhibited the fragment ion at *m/z* 313 (base peak, loss of side chain with 2H transfer) indicating that it was an acetate of a C_{28}-sterol with two double bonds of which one was in the C_9 side chain and the other was probably located at C-7 [5, 6] The ^{1}H NMR spectrum displayed two methyl singlets at δ 0 542 (C-18) and 0.812 (C-19) accompanied by the other signals at δ 2 029 (3H, *s*, OCOMe), 4 69 (1H, *m*, C-3α) and 5 15 (1H, *m*, C-7) which were consistent with the nuclear structure of a 3β-acetoxy-5α-Δ^7-sterol [1, 7–9] The other signals associated with the side chain protons at δ0 821 (3H, *d*, *J* = 7 3 Hz, C-27), 0.839 (3H, *d*, *J* = 6.6 Hz, C-26), 0 912 (3H, *d*, *J* = 6 8 Hz, C-28), 1 007 (3H, *d*, *J* = 6 8 Hz, C-21) and 5.17 (2H, *m*, C-22, C-23) indicated that the sterol **1** had a (22*E*,24*S*)-24-methyl-Δ^{22}-cholestane side chain [7]. The possibility of the 24*R*-configuration was eliminated because such stereochemistry shifts the C-21 methyl signal to lower field (*ca* δ 0 01) [7] Thus, sterol **1** had the structure (22*E*,24*S*)-5α-ergosta-7,22-dien-3β-ol (stellasterol) This was substantiated by the ^{1}H NMR spectrum of its 24*R*-epimer, (22*E*,24*R*)-5α-ergosta-7,22-dien-3β-ol (5-dihydroergosterol, **2**), of which the acetate derivative gave the C-21 methyl doublet at δ1 016 The ^{13}C NMR spectrum afforded further evidence for the (24*S*)-stereochemistry of sterol **1**. The chemical shifts of the carbon signals of C-16 (δ_C 28.3), C-24 (43 0), C-26 (19 6), C-27 (20 0) and C-28 (17 9) of 1-acetate were consistent with those of a (22*E*,24*S*)-24-methyl-Δ^{22}-sterol [10] and differed enough from those of the corresponding signals of the 24*R*-epimer, **2**-acetate, i e C-16 (δ_C 28 0), C-24 (42 7), C-26 (19 9), C-27 (19 6) and C-28 (17.6), to allow differentiation between these diastereoisomeric 24-methylsterols

This seems to be the first record of the isolation and characterization of stellasterol (**1**) from a higher plant. This sterol has so far been detected only in some marine organisms, such as the starfish *Asteria rubens* [11] and *A amurensis* [12, 13], and the sponge *Axinella cannabina* [14] The biogenetic role of **1** in the seeds of *C sativus* remains as an open question

EXPERIMENTAL

Mp is uncorr HPLC was carried out on a Partisil 5 ODS-2 (Whatman, 25 cm × 8 mm i d) using a UV detector monitoring at 210 nm (mobile phase, MeOH–H_2O, 98 2) GC on an OV-17 SCOT glass capillary column was under the conditions already described [15] *RR*, in HPLC and GC were expressed relative to cholesteryl acetate MS (70 eV) were taken with a direct inlet system ^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (22 53 MHz) were determined in $CDCl_3$ with TMS as int standard Our general techniques have been described previously [16] The origin of the seeds of *C sativus* was reported previously [17] An authentic sample of 5-dihydroergosteryl (**2**) acetate was generously supplied by Dr H Yokokawa (Tachikawa College of Tokyo, Tokyo) and Dr S Endo (Tokyo Gakugei University, Tokyo)

Stellasteryl (**1**) *acetate* Mp 172–174° (lit [11] mp 173–175°) RR_t 1 345 in GC, and 0 86 in HPLC MS *m/z* (rel int): 440 3651 $[M]^+$ (47, $C_{30}H_{48}O_2$, calcd 440 3654), 425 (16), 397 (9), 380 (6), 365 (11), 342 (21), 337 (7), 313 (100), 288 (25), 273 (7), 255 (64), 253 (14), 241 (12), 229 (23), 213 (21) ^{13}C NMR C-1 (δ_C 36 8), C-2 (27 4), C-3 (73 4), C-4 (33 8), C-5 (40 0), C-6 (29 5), C-7 (117 2), C-8 (139 4), C-9 (49 3), C-10 (34 1), C-11 (21 4), C-12 (39 3), C-13

(43 2), C-14 (55.0), C-15 (22 9), C 16 (28 3), C-17 (55 9), C-18 (12.0), C-19 (12 8), C-20 (40 5), C-21 (21 1), C-22 (135.8), C-23 (131 9), C-24 (43.0), C-25 (33 2), C-26 (19 6), C-27 (20 0), C-28 (17 9), MeCO (170 5), MeCO (21 4) The carbon signals were assigned by comparison with those of related sterols in the lit [4, 10].

5-*Dihydroergosteryl* (**2**) *acetate* ^{1}H NMR. δ 0 541 (3H, *s*, C-18), 0.811 (3H, *s*, C-19), 1 016 (3H, *d*, J = 6.6 Hz, C-21), 0 836 (3H, *d*, J = 6 4 Hz, C-26), 0 820 (3H, *d*, J = 6.6 Hz, C-27), 0 913 (3H, *d*, J = 6 8 Hz, C-28), 4 69 (1H, *m*, C-3α), 5.15 (1H, *m*, C-7), 5 19 (2H, *m*, C-22, C-23) ^{13}C NMR those signals omitted from below are the same in their chemical shifts with the corresponding signals of 1-acetate, C-16 (δ_C 28 0), C-22 (135.6), C-23 (131.8), C-24 (42.7), C-25 (33 1), C-26 (19 9), C-27 (19 6), C-28 (17.6)

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PIGMENTS FROM *NECTRIA HAEMATOCOCCA*: ANHYDROFUSARUBIN LACTONE AND NECTRIAFURONE

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Key Word Index—*Nectria haematococca*, naphthoquinones, anhydrofusarubin lactone, nectriafurone

Abstract—The structures of anhydrofusarubin lactone and of nectriafurone, two new naphthoquinone pigments isolated from the fungus *Nectria haematococca*, are reported. Nectriafurone is the first natural isofuran naphthoquinone and is biogenetically related to fusarubin. In addition eight known *Fusarium* naphthoquinones were isolated. All the naphthoquinone pigments isolated from *N. haematococca* are produced in higher yields by selected mutant strains obtained from the wild strain

INTRODUCTION

The production of naphthoquinone pigments by fungi has been known for more than 40 years. Fusarubin (**3**) is a typical representative in this series [1–3] but more recently new antibiotics, possessing particular structural features, have also been reported, such as kalafungin [4] and the dimeric naphthoquinone lactones [5, 6] of the xanthomegnine type. Substances in this family are partly responsible for the biological activity of *Fusarium* on plants, as observed *in vitro* with tomato cuttings, as well as for cell division inhibition [7, 8].

Mutants were selected from a homothallic wild strain

This publication is dedicated to Professor Edgar Lederer on the occasion of his 75th birthday.