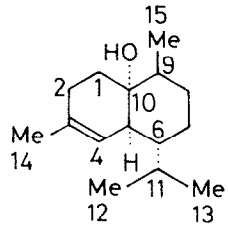
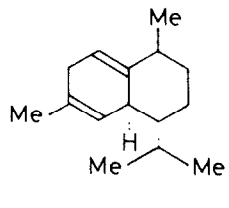


1



2

from *S. undulata* is a further example of the occurrence in liverworts of sesquiterpenoids enantiomeric to the corresponding compounds from higher plants.

EXPERIMENTAL

Isolation of (+)-ent-*epicubenol* (**1**). The essential oil (3 g) from *S. undulata* (leg. et det. S.H., GDR, Thuringian Forest, Kerngrund near Oberhof, 23.7.1980; this chemical race occurs widely in Scotland (Rycroft, D. S., unpublished work), prepared by steam distillation, was chromatographed on Si gel (80 g, with 5% H_2O). Elution with *n*-hexane (1.5 l.) and *n*-hexane–Et_2O (19:1) (500 ml) yielded an oily mixture. Subsequent elution with *n*-hexane–Et_2O (9:1) (500 ml) gave (+)-*ent*-epicubenol (**1**) as an oil with $[\alpha]_D^{24}$ +111.6° ($CHCl_3$; *c* 4.705), n_D^{24} 1.5027 and R_f 0.64 [Si gel PF, *n*-hexane–Et_2O–HCO_2H (30:25:6), AcOH + SO_3HCl, 150°, violet spot]. $C_{15}H_{26}O$ (222). MS *m/z* (rel. int.): 222 $[M]^+$ (48), 207 $[M - Me]^+$ (40), 205 (70), 204.1877 ($[M - H_2O]^+$, calc. 204.1877) (92), 189 $[M - Me - H_2O]^+$ (44), 179 $[M - Me{-}CH{-}Me]^+$ (86), 162 (76), 161 $[M - H_2O - Me\ CH{-}Me]^+$, 153 (35), 147 (46), 137 (58), 135 (56), 133 (52), 123 (76), 122 (75), 121 (75), 120 (82), 119 (100), 111 (74), 110 (74), 109 (79), 107 (77), 105 (95), 95 (88), 93 (90); IR ν_{max}^{film} cm^{-1}: 840, 854, 896, 924, 950, 968, 990, 1024, 1074, 1130, 1144, 1186, 1208, 1226, 1260, 1280, 1310, 1372, 1450, 2990, 3600; ^1H NMR (60 MHz, $CDCl_3$): δ 0.75, 0.85, 0.95 (3 × *sec* Me), 1.67 (3H, *s*, vinyl Me), 5.37 (1H, *d* (*br*), H-4); ^{13}C NMR ($CDCl_3$): δ 15.2 (*q*), 15.2 (*q*), 21.7 (*q*), 22.1 (*t*), 23.5 (*q*), 24.1 (*t*), 26.8 (*t*), 27.0 (*d*), 31.2 (*t*), 42.0 (*d*), 48.2 (*d*), 49.3 (*d*), 72.7 (*s*), 122.2 (*d*), 133.9 (*s*).

(+)-ent-*Cubenene* (**2**). To a soln of **1** (0.3 g) in pyridine (5 ml) was added $SOCl_2$ (1.5 ml) at 0°. After 30 min the mixture was poured into ice-cold aq. 10% $NaHCO_3$ and extracted with Et_2O. The ethereal soln was washed with 10% H_2SO_4, H_2O, $NaHCO_3$ soln and H_2O, dried with Na_2SO_4, the Et_2O removed *in vacuo* and the residue chromatographed on Si gel (10 g, with 10% $AgNO_3$). *n*-Hexane (400 ml) and *n*-hexane–Et_2O (46:1) (100 ml) eluted an oily mixture. Further elution with *n*-hexane–Et_2O (46:1) (100 ml) gave (+)-*ent*-cubenene (**2**) as an oil with $[\alpha]_D^{24}$ + 22.3° ($CHCl_3$; *c* 1.50) and R_f 0.60 [$AgNO_3$–Si gel (1:9), *n*-hexane–Et_2O (19:1), AcOH + SO_3HCl, 150°, violet spot]. $C_{15}H_{24}$ (204). MS *m/z* (rel. int.): 204.1880 ($[M]^+$, calc. 204.1878) (72), 189 $[M - Me]^+$ (15), 161 $[M - Me{-}CH{-}Me]^+$ (88), 147 (26), 133 (33), 121 (62), 120 (64), 119 (100), 105 (86), 93 (50), 92 (53), 91 (39); IR ν_{max}^{film} cm^{-1}: 790, 824, 852, 888, 944, 1030, 1112, 1170, 1272, 1364, 1380, 1450, 2900, 2960; ^1H NMR (CCl_4): δ 0.82, 0.87, 0.99 (3 × *sec* Me), 1.65 (3H, *s*, vinyl Me), 5.21 (1H, *m*, H-1), 5.41 (1H, *m*, H-4); ^{13}C NMR (CCl_4): δ 15.1 (*q*), 18.2 (*q*), 21.5 (*q*), 23.4 (*q*), 24.6 (*t*), 26.6 (*d*), 31.5 (*t*), 36.8 (*t*), 37.6 (*d*), 41.9 (*d*), 51.2 (*d*), 112.6 (*d*), 121.2 (*d*), 131.3 (*s*), 143.1 (*s*).

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STEROLS OF *CANDIDA TROPICALIS* GROWN ON *N*-ALKANES

D. SICA, L. BONIFORTI* and G. DI GIACOMO

Istituto di Chimica Organica e Biologica, Università di Napoli, via Mezzocannone 16, Napoli, Italy; * Istituto Superiore di Sanità, viale R. Elena, Roma, Italy

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Key Word Index—*Candida tropicalis*; Ascomycetes; fungi; ergosterol; ergost-7-en-3β-ol; (22*E*)-ergosta-7,22-dien-3β-ol; ergosta-7,24(28)-dien-3β-ol; cholesta-8,24-dien-3β-ol; (22*E*)-ergosta-5,7,9(11),22-tetraen-3β-ol.

Abstract—Six sterols isolated from the yeast *Candida tropicalis* were identified as ergosterol (major component), (22*E*)-ergosta-5,7,9(11),22-tetraen-3β-ol, ergost-7-en-3β-ol, (22*E*)-ergosta-7,22-dien-3β-ol, ergosta-7,24(28)-dien-3β-ol and cholesta-8,24-dien-3β-ol.

In continuation of our work on sterols [1, 2], we have now examined the sterol fraction of *Candida tropicalis*, a yeast that has attracted commercial interest for protein production by micro-organisms [3].

The unsaponifiable fraction of the crude extract of *C. tropicalis* was chromatographed on Si gel and the sterol fraction, after acetylation, was further fractionated on Si gel to give the 4-demethyl sterol fraction. Preliminary capillary GLC analysis on OV-101 of the steryl acetates indicated the presence of six sterols which were identified

Table 1. Sterol composition of *C. tropicalis*

TLC bands	Steryl acetates	Wt (mg)	RR_t*	Composition (%)
1a	Ergost-7-en-3β-ol (fungisterol)	9	1.44	4.0
1b	(22*E*)-Ergosta-7,22-dien-3β-ol (5-dihydroergosterol)	23	1.25	9.0
2	Cholesta-8,24-dien-3β-ol (zymosterol)	5	1.13	2.0
3	(22*E*)-Ergosta-5,7,9(11),22-tetraen-3β-ol	7	1.08	3.0
4	Ergosta-7,24(28)-dien-3β-ol (episterol)	15	1.41	6.0
5	(22*E*,24*R*)-Ergosta-5,7,22-trien-3β-ol (ergosterol)	191	1.22	76.0

* GLC retention time of steryl acetate relative to cholesteryl acetate (1.00) on OV-101.

after separation of the mixture into six bands by $AgNO_3$–Si gel TLC.

Bands 1a, 1b and 5 (Table 1) contained ergost-7-en-3β-yl acetate, (22*E*)-ergosta-7,22-dien-3β-yl acetate and (22*E*,24*R*)-ergosta-5,7,22-trien-3β-yl acetate respectively (comparison GLC, MS and NMR data with those of authentic steryl acetates [1, 4]). The 24*R* configuration of the C-24 methyl group of the ergosteryl acetate was based on the chemical shift of the C-21 doublet at δ 1.039. This signal appeared at δ 1.024 in the NMR spectrum of the 24*S* epimer, 24-epi-ergosteryl acetate [5]. Band **2** was identified as cholesta-8,24-dien-3β-yl acetate; ^{1}H NMR: δ 0.601 (*s*, H-18), 0.944 (*d*, J = 6.5 Hz, H-21), 0.958 (*s*, H-19), 1.601 and 1.680 (*s*, H-26 and H-27), 2.010 (*s*, $MeCO_2$–), 4.67 (*m*, H-3), 5.11 (*m*, H-24). The MS agreed with the literature [6]. Band 3 contained (22*E*)-ergosta-5,7,9(11),22-tetraen-3β-yl acetate. The UV spectrum showed absorption at 311, 325 and 341 nm and the MS contained ions at *m/z* 436 $[M]^+$, 376 $[M - HOAc]^+$, 251 $[M - HOAc$ and side-chain$]^+$, 249 $[M - HOAc$ and side-chain with 2 H transfer$]^+$ and 209 $[M - HOAc$ and ring D fission$]^+$ characteristic of a sterol with three double bonds in the ring system and one in the side-chain. A fragment ion at *m/z* 333 $[M - HOAc$ and terminal isopropyl group$]^+$ further indicated that the side-chain double bond was located at C-22 and C-23. The nuclear bonds were located in the $\Delta^{5,7,9(11)}$ positions by the presence of signals in the NMR spectrum at δ 0.580 and 1.256 for the C-18 and C-19 Me groups, respectively [7]. Furthermore, the presence of three-ring olefinic protons which were deshielded at δ 5.40, 5.51 and 5.69 was indicative of conjugation. This assignment was supported by direct comparison with an authentic sample which was prepared by acetylation of ergosta-5,7,9(11),22-tetraen-3β-ol [8]. Ergosta-5,7,9(11),22-tetraen-3β-ol was identified as a minor constituent in the fungus *Mucor rouxii* [9] and in the sponge *Biemma fortis* [8]. Band 4 contained ergosta-7,24(28)-dien-3β-yl acetate. The spectroscopic data were in agreement with the literature [10, 11].

C. tropicalis contains ergosterol as the predominant sterol accompanied by the closely related sterols cholesta-8,24-dien-3β-ol, ergosta-7,24(28)-dien-3β-ol, (22*E*)-ergosta-7,22-dien-3β-ol, ergost-7-en-3β-ol and (22*E*)-ergosta-5,7,9(11),22-tetraen-3β-ol. A previous investigation [12] of the sterol content of *C. tropicalis* grown on *n*-alkanes revealed the presence of ergosterol. Ergosterol has been identified as the major sterol in other *Candida* species, *C. utilis* [13, 14], *C. albicans* [14], *C. guilliermondii* [15] and *C. boidinii* [16].

EXPERIMENTAL

C. tropicalis was grown on purified *n*-alkanes by the industrial process of Liquichimica. UV, EtOH; IR, $CHCl_3$; NMR 270 MHz, $CDCl_3$, TMS as int. standard; GLC, glass capillary column (20 m) containing OV-101 at 248°; GC/MS 70 eV, capillary column (20 m) containing OV-101.

Extraction and separation. Lipids of *C. tropicalis* were extrd from the biomass (1 kg) first with petrol (bp 40–70) and then $CHCl_3$. The solvents were evapd and the extracts (30.7 g) were saponified under reflux for 2 hr with 10% KOH in 80% EtOH. The unsaponifiable lipid (11.6 g) was applied to a Si gel column which was eluted with CH_2Cl_2 and finally with CH_2Cl_2–MeOH (46:1). The sterol fraction (1.5 g) was acetylated (Ac_2O–C_5H_5N, 12 hr at room temp.) and the mixture of 4-demethyl steryl acetates (500 mg) separated on a Si gel column eluted with increasing concentrations of C_6H_6 in petrol. A part of the steryl acetates (300 mg) were separated into five bands (1–5 in order of decreasing mobility) by $AgNO_3$–Si gel TLC (0.5 mm thickness), developed twice with C_6H_6–hexane (1:1). Band 5 was detected by UV radiation and bands **1**–**4** were visualized under UV radiation after spraying with a soln of rhodamine B in EtOH. Band 1 exhibited two GLC peaks and was further separated into two bands (1a and 1b) on Ag^+–Si gel TLC (0.25 mm) developed twice with C_6H_6–hexane (1:1).

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ECDYSTEROIDS IN *SPINACIA OLERACEA* AND *CHENOPODIUM BONUS-HENRICUS*

M. BÁTHORY, I. TÓTH, K. SZENDREI and J. REISCH*

Department of Pharmacognosy, University Medical School, Szeged, H-6701 Pf. 121, Hungary; *Institute of Pharmaceutical Chemistry, Westfalian Wilhelm-University, D-4400 Münster/Westf. Hittorfstr. 58–62, West Germany

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Key Word Index—*Spinacia oleracea*; *Chenopodium bonus-henricus*; Chenopodiaceae; ecdysteroids; ecdysone, 20-hydroxyecdysone; polypodine B.

Abstract—The roots of *Chenopodium bonus-henricus* and the seeds of *Spinacia oleracea* contain 20-hydroxyecdysone and polypodine B. The seeds of *S. oleracea* also contain a compound with properties similar to those of 24(28)-dehydromakisterone-A and may contain small amounts of ecdysone.

The presence of ecdysone-type hydroxylated steroids have been demonstrated in a great number of plant families. Certain plant species (*Polypodium vulgare, Dacrydium intermedium, Cyanotis vaga, Vitex megapotamica, Ajuga japonica, Serratula inermis, Rhaponticum carthamoides*) can accumulate fairly large amounts (over 1% dry wt) of such compounds [1–5].

In spite of extensive screening programmes involving a few thousand plant species only very vague conclusions can be made on the occurrence of ecdysteroids in the Plant Kingdom. Nevertheless, certain taxonomic entities seem to emerge with a relatively high frequency of accumulation of such compounds, e.g. Polypodiaceae, Podocarpaceae, Taxaceae, Amaranthaceae, and the genera *Cyanotis, Vitex, Ajuga, Serratula* (see refs. [3, 5]). In view of the close relationship of the Chenopodiaceae to Amaranthaceae, one of the prominent ecdysteroid-bearing families, studies were undertaken on *Chenopodium bonus-henricus* L. and *Spinacia oleracea* L., two closely related species, both extensively used as vegetables and renowned for their roborant quality [6–8]. Since ecdysteroids had been reported to exert a specific anabolic effect on various animals [4, 9], it was expected that the alleged roborant activity could eventually be explained by the presence of ecdysteroids.

The present paper deals with the isolation and characterization of the major ecdysteroid-type components of the above species.

TLC and RIA experiments [10] demonstrated the presence of a series of ecdysteroid-type compounds in the herb and roots of both species as well as in the seeds of *S. oleracea*. Isolation was undertaken from the roots of *C. bonus-henricus* and from the seeds of *S. oleraceae*. The isolated ecdysteroids were identified on the basis of their physical constants and spectral characteristics as well as by direct comparison with authentic samples.

Polypodine B (**2**) and 20-hydroxyecdysone (**1**) were isolated from the roots of *C. bonus-henricus* and the seeds of *S. oleracea*. The latter also yielded an ecdysteroid showing similarities to 24(28)-dehydromakisterone A (**3**) [11]. The possible presence in *S. oleracea* of ecdysone (**4**) was demonstrated by RIA [12] and TLC.

Various genera of the Chenopodiaceae (*Spinacia, Atriplex, Beta, Chenopodium, Kochia, Anabasis*) have earlier been reported to contain saponins, but only a few of these saponins have as yet been characterized unequivocally. They were found with no exception to belong to the triterpene series having in most cases oleanolic acid as aglycone [13–15]. In addition, sterols and some sterol glycosides have frequently been indicated. To our knowledge the compounds **1–3** are the first isolated and characterized ecdysteroids from this family though one *Kochia* sp. was earlier reported to contain ecdysteroids [16]. The presence of the reported compounds in both vegetables provides a possible explanation of their roborant activity, although further work is needed to establish its mechanism and active principle(s).

EXPERIMENTAL

Plant material. C. bonus-henricus roots were collected from wild plants in the Bükk mountains, near Bánkut in Oct. 1980. Voucher specimens were deposited in the Herbarium of Szeged University. Seeds of *S. oleracea* var. Popeye were purchased in Szeged.