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SENITA CACTUS: A PLANT WITH INTERRUPTED STEROL BIOSYNTHETIC PATHWAYS

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Key Word Index *Lophocereus schottii*; Cactaceae; senita cactus; Δ^7-sterols; $\Delta^{8,14}$-sterols; nitrogenous inhibitors of sterol biosynthesis.

Abstract Locereol (4α-methylcholesta-8,14-dien-3β-ol) and 5α-cholesta-8,14-dien-3β-ol, not previously isolated from plants, 24-methylenelophenol, lathosterol, 5α-campest-7-en-3β-ol and spinasterol are present in senita cactus in addition to the lophenol and schottenol described previously.

INTRODUCTION

Senita cactus is a large multistemmed succulent plant of the Sonoran Desert [1, 2]. Its necrotic tissues are the sole breeding site for an endemic species of *Drosophila* which depends on the plant for a Δ^7-sterol to complete its life cycle [3, 4]. Djerassi and co-workers established the presence of unusual alkaloids [5-7] and lupeol, lophenol and schottenol [8] in senita. In a later study of the distribution of alkaloids, non-saponifiables and fatty acids over its range in Sonora, Mexico, the presence of three additional sterols was noted, two of which had attributes (IR, TLC) of 8,14-dienes [9]. In this paper we describe these plus three other sterols in the cactus.

RESULTS

Senita cactus lipids were saponified, the fatty acid and alkaloid fractions removed and the residue separated into three fractions on Si gel columns. The first contained lupeol, the second, lophenol and two other sterols and the third, schottenol and four other sterols. The second and third fractions were acetylated and separated on $AgNO_3$-Si gel columns.

Lophenol acetate, 24-methylenelophenol acetate and an unknown were isolated from the second fraction. The unknown was unstable to silver nitrate column chromatography but about 50 mg were obtained for analysis. Its chromatographic mobility (GLC and TLC), mp, UV, IR, NMR and MS showed it to be 4α-methyl-5α-cholesta-8,14-dien-3β-yl acetate which corresponded to unknown sterol B in the earlier work [9]. Its proposed trivial name, locereol, is derived from *Lophocereus*.

The third fraction contained 5α-cholesta-8,14-dien-3β-yl acetate (unknown sterol C [9]) and a mixture of Δ^7-steryl acetates that could not be readily separated. The mixture was analysed by $AgNO_3$-TLC and GC–MS and shown to be comprised of the acetates of lathosterol (unknown sterol A [9]), 5α-campest-7-en-3β-ol, schottenol and spinasterol. The absolute configuration of the alkyl group at C-24 in the last three cactus sterols was not determined.

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DISCUSSION

Identity of the cactus sterols was established by comparison of their properties to those of standards (lathosterol, 5α-campest-7-en-3β-ol, schottenol, spinasterol and 5α-cholesta-8,14-dien-3β-ol) and literature values (lophenol and 24-methylenelophenol). Preparations of both 4α- and 4β-methyl-5α-cholesta-8,14-dien-3β-ol have been reported [10, 11] but only in the latter report were data given for the acetate as well. Comparison of these with the corresponding values obtained by us for locereol acetate showed significant differences, e.g. mp 134.5–135° (4β), 92–94° (4α); MS *m/e* $M^+ = 440$, 100% (4β), 33% (4α); ^{1}H NMR, C_{30}, *d*, $\delta = 0.99$ (4β), 0.88 (4α), 3α-H, *m*, $\delta = 4.78$ (4β), 4.38 (4α).

Absence of Δ^5-sterols and presence of 4α-methyl, Δ^7- and $\Delta^{8,14}$-sterols as principal constituents of senita cactus lipids suggest that the later steps in the pathways to typical plant phytosterols (campesterol, sitosterol, stigmasterol) are either absent or inhibited in this plant. Δ^7-Sterols are not rare, they occur in alfalfa, cucurbits, spinach and green algae among others, but $\Delta^{8,14}$-derivatives, although well established as transitory intermediates during the biosynthesis of cholesterol and ergosterol [12, 13], have been reported only three times as constituents of undisturbed living systems. 5α-Stigmasta-8,14,24(28)*Z*-trien-3β-ol occurs in *Vernonia anthelmintica* seed [14], 5α-stigmasta-8,14-dien-3β-ol was detected by GLC as a minor (0.5%) component of rapeseed sterols early in the ripening process [15] and 5α-cholesta-8,14-dien-3β-ol-23-one is the major genin in saponins of the starfish *Echinaster sepositus* [16].

Sterol biosynthetic intermediates can be isolated when inhibitors are added to incubation media. The drug AY-9944, which inhibits reduction of the Δ^{14}-bond in 5α-cholesta-8,14-dien-3β-ol by rat liver homogenates [17], causes the build-up of 4α-methyl sterols, $\Delta^{8(9)}$-sterols, 5α-ergosta-8,14-dien-3β-ol and 5α-poriferasta-8,14-dien-3β-ol when added (4 ppm) to growing cultures of *Chlorella ellipsoida* [18, 19]. The same drug added to bramble cell cultures gave only $\Delta^{8(9)}$ and $\Delta^{8(14)}$-sterols [20], whereas fenarimol addition to the same cultures caused the build-up of 4α-methyl sterols, 5α-ergost-8-en-3β-ol and 5α-stigmast-8-en-3β-ol [21]. Replacement of ergosterol by 5α-ergosta-8,14-dien-3β-ol as the principal sterol when

yeast was grown with a D-homo-15-azasterol in the medium [22] was caused by inhibition of a Δ^{14}-reductase in the yeast by the azasterol [23].

This inhibition of Δ^{14}-reductase, Δ^{8}- → Δ^{7}-isomerase and build-up of 4α-methyl sterols by three nitrogenous compounds in systems where 4-desmethyl-Δ^{5}- and Δ^{7}-sterols are the common end-products of sterol biosynthesis suggest that a similar process may be operating in senita cactus. The high concentration [9] and unusual structure of senita cactus alkaloids, e.g. pilocereine [7], may inhibit the Δ^{14}-reductase, Δ^{5}-dehydrogenase and 4α-methyl-hydroxylase systems in the cactus. The considerable variability in content and composition of sterols in senita [9] suggest some kind of secondary control of their synthesis is operating.

EXPERIMENTAL

Mps *in vacuo*, corr. TLC on Si gel (Merck aluminum sheets), hexane–EtOAc (60:40), argentation TLC with Si gel plates dipped into 10% $AgNO_3$ in EtOH–H_2O (4:1), activated 20 min at 110° and developed 2 × in CH_2Cl_2, *R*ca (cholesteryl acetate) = 1.00. GLC with 2 m 5% OV-101 column, 250° *RR*$_t$c (cholesterol = 1.00) *RR*$_t$ca (cholesteryl acetate = 1.00), lit. values are from Patterson [24]. UV in 95% EtOH, IR in CS_2, ^{1}H NMR (270 MHz) in $CDCl_3$ and MS direct inlet. Lathosterol, 5α-campest-7-en-3β-ol, schottenol and spinasterol acetates were available from previous work [25, 26]; 5α-cholesta-8,14-dien-3β-yl acetate was prepared from 7-dehydrocholesteryl acetate [27].

Senita cactus was collected near Sonoyta, Son., Mexico, cut into strips, air-dried and extracted with $CHCl_3$–MeOH (2:1) in a blendor. The extract was saponified, the non-saponifiables extracted with Et_2O and alkaloids removed with 1 N HCl. The neutral constituents were separated on Si gel columns with hexane–C_6H_6 (9:1) into 3 fractions (TLC, Si gel): (1) lupeol, (2) lophenol and two other 4-methyl sterols, and (3) schottenol and four other 4-desmethyl sterols. Fractions 2 and 3 were acetylated and separated into their components on 20% $AgNO_3$-Si gel columns with hexane–C_6H_6 (7:3) and the 8,14-diene acetates subsequently purified on 5% $AgNO_3$-Si gel columns with hexane–C_6H_6 (4:1). Higher concentrations of $AgNO_3$ promoted decomposition of these dienes. The steryl acetates were crystallized from MeOH

Fraction 2 sterols. Lophenol, mp 147–149°, *RR*$_t$c 1.30, lit. [8] 149–151, *RR*$_t$c 1.32. *Acetate*, mp 116–118, *R*ca 1.21, *RR*$_t$ca 1.26, lit. [8] 119–121°, *RR*$_t$ca 1.27; NMR as reported by Sucrow [28]. *24-Methylenelophenol*, mp 166–167.5°, *RR*$_t$c 1.65, lit. [29] 166°, *RR*$_t$c 1.65. *Acetate*, mp 130–131°, *R*ca 0.21, *RR*$_t$ca 1.60, lit. [29] 133–135°, *RR*$_t$ca 1.60; IR 885 and 1648 cm^{-1} (CH_2=C<); MS as reported by Knights [30]. *Locereol acetate*, mp 92–94°, *R*ca 0.27, *RR*$_t$ca 1.16; λ_{max} 249, ε = 20 700; IR 800 cm^{-1} ($\Delta^{8,14}$); ^{1}H NMR: δ 0.82 (3H, *s*, C-18), 0.87 (6H, *d*, C-26, 27), 0.88 (3H, *d*, 4α-Me), 0.94 (3H, *d*, C-21), 1.03 (3H, *s*, C-19), 2.06 (3H, *s*, Ac), 4.38 (1H, *sextet*, 3α-H), 5.37 (1H, *m*, 15-H); MS *m/e* (rel. int.): 440 (M^+, 33), 425 (M − Me, 9), 380 (M − HOAc, 6), 365 (M − Me − HOAc, 100), 327 (M − C_8H_{17}, 13), 267 (M − C_8H_{17} − HOAc, 19), 252 (M − C_8H_{17} − HOAc − Me, 46), 237 (11), 225 (20), 213 (14), 211 (17); (Found: C, 81.56; H, 11.35. Calc. for $C_{30}H_{48}O_2$: C, 81.75; H, 10.98%).

Fraction 3 sterols. 5α-Cholesta-8,14-dien-3β-yl acetate, mp 97–98°, *R*ca 0.26, *RR*$_t$ca 1.03, lit. [27] 99–100°, *RR*$_t$ca 1.03; λ_{max} 249, ε = 15 900; TR 800 cm^{-1} ($\Delta^{8,14}$); ^{1}H NMR: δ 0.82 (3H, *s*, C-18), 0.87 (6H, *d*, C-26, 27), 0.94 (3H, *d*, C-21), 1.00 (3H, *s*, C-19), 2.03 (3H, *s*, Ac), 4.71 (1H, *m*, 3α-H), 5.37 (1H, *m*, 15-H), MS *m/e* (rel. int.): 426 (M^+, 36), 411 (M − Me, 9), 366 (M − HOAc, 8), 351 (M − Me − HOAc, 100), 313 (M − C_8H_{17}, 16), 253 (M − C_8H_{17} − HOAc, 5), 238 (M − C_8H_{17} − HOAc − Me, 25), 211 (9). *Lathosterol acetate*, *R*ca 1.14, *RR*$_t$ca 1.11, lit. *RR*$_t$ca 1.12, MS as reported [30]. *5α-Campest-7-en-3β-ol acetate*, *R*ca 1.12, *RR*$_t$ca 1.42, lit. *RR*$_t$ca 1.46, MS as reported [31]. *Schottenol acetate*, *R*ca 1.15, *RR*$_t$ca 1.78, lit. *RR*$_t$ca 1.78; MS *m/e* (rel. int.): 456 (M^+, 48), 441 (M − Me, 13), 396 (M − HOAc, 7), 381 (M − Me − HOAc, 12), 315 (M − $C_{10}H_{21}$, 12), 288 (M − $C_{10}H_{21}$ − C_2H_3, 10), 273 (M − $C_{10}H_{21}$ − C_3H_5 − H, 13), 255 (M − $C_{10}H_{21}$ − HOAc, 100), 229 (288 − OAc, 55), 228 (288 − HOAc, 19), 213 (273 − HOAc, 87), 201 (20), identical to that of an authentic sample [26]. *Spinasterol acetate*, *R*ca 1.05, *RR*$_t$ca 1.57, lit. *RR*$_t$ca 1.57, MS *m/e* (rel. int.): 454 (M^+, 18) 439 (M − Me, 4) 411 (M − C_3H_7, 8), 394 (M − HOAc, 4), 379 (M − Me − HOAc, 5), 351 (M − C_3H_7 − HOAc, 8), 342 (15), 313 (M − $C_{10}H_{19}$ − 2H, 100), 288 (M − $C_{10}H_{19}$ − C_2H_3, 4), 253 (313 − HOAc, 19), 241 (7), 211 (M − $C_{10}H_{19}$ − C_3H_5 − HOAc, 5), comparable to that of an authentic sample [26].

Nomenclature. Campesterol = campest-5-en-3β-ol, lathosterol = 5α-cholest-7-en-3β-ol, locereol = 4α-methyl-5α-cholesta-8,14-dien-3β-ol, lophenol = 4α-methyl-5α-cholest-7-en-3β-ol, 24-methylenelophenol = 4α-methyl-5α-ergosta-7,24(28)-dien-3β-ol, schottenol = 5α-stigmast-7-en-3β-ol, sitosterol = stigmast-5-en-3β-ol, spinasterol = 5α-stigmasta-7,22*E*-dien-3β-ol, stigmasterol = stigmasta-5,22*E*-dien-3β-ol.

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1,3,6,8-TETRAHYDROXYANTHRAQUINONE FROM *ASPERGILLUS VERSICOLOR*

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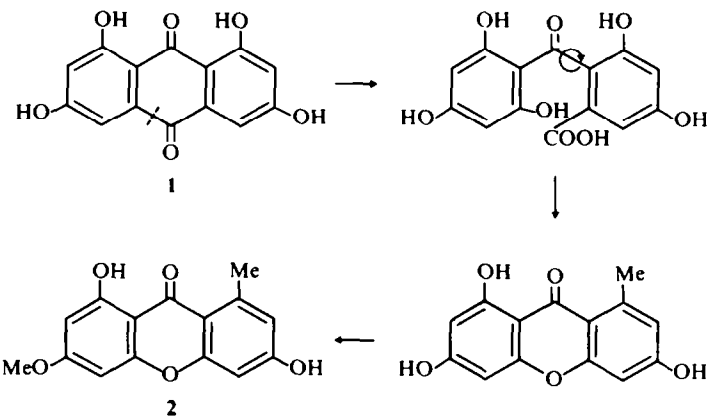
Key Word Index—*Aspergillus versicolor*; fungal metabolite; 1,3,6,8-tetrahydroxyanthraquinone; ^{13}C NMR studies.

Continuing our investigation [1–4] of *Aspergillus versicolor* (Vuillemin) Tiraboschi, we now report the isolation of 1,3,6,8-tetrahydroxyanthraquinone (**1**), a synthetic compound [5] isolated from a natural source for the first time. The identification of this compound from the mycelium of *A. versicolor* is particularly interesting biosynthetically. In fact, all anthraquinone metabolites previously isolated from this fungus are C_{18}- or C_{20}-anthraquinone derivatives, namely versicolorin A, B and C [6] and averufin [7]. Moreover, **1** is possibly the precursor of 3,8-dihydroxy-6-methoxy-1-methylanthrone (**2**) [8], since, on the basis of an aflatoxin biosynthesis [9–10], we can propose the following biosynthetic pathway (Scheme 1): the oxidative ring cleavage in **1** followed by reduction of CO_2H, recyclization and methylation, leads to **2**. The coupled ^{13}C NMR spectrum of **1** recorded in DMSO-d_6 after addition of a small amount of D_2O, reveals a wealth of fine structure. The observed long-range coupling constants confirm the assignments established on chemical grounds [12].



Scheme 1. Possible relationship between 1,3,6,8-tetrahydroxyanthraquinone and 3,8-dihydroxy-6-methoxy-1-methylanthrone.