EXPERIMENTAL

All mps are uncorr.

Extraction of Salvia lanata. *Ca* 1 kg air dried, finely powdered whole plant (aerial parts and roots) of *Salvia lanata* Roxb. was extracted with petrol (60–80°) in a Soxhlet for 48 hr. The extract was subjected to CC on 200 g Si gel (mesh 60–120). Fractions 178–187 were collected.

Isolation of 3-epi-ursolic acid (**1**). Fractions 178–187 yielded 3-epi-ursolic acid. It crystallized from EtOAC–petrol (40–60°; 1:3), yield 0.35 g, mp 240–245°, $[\alpha]_D + 98°$ ($CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2920, 1690, 1645, 1440, 1380, 1270, 1020, 985 and 660. ^1H NMR ($CDCl_3$) and MS: described in the text.

Acetylation of 3-epi-ursolic acid. 3-Epi-ursolic acid (50 mg) was dissolved in 5 ml Ac_2O and 0.5 ml C_5H_5N and the reaction mixture was kept at room temp. for 4 days. The reaction mixture was then poured into cold H_2O, extracted with Et_2O and dried when the amorphous acetate **2** was obtained (yield 56 mg) IR ν_{max}^{KBr} cm^{-1}: 2948, 1735 (acetyl), 1695 (carboxyl), 1640 (unsaturation). ^1H NMR (100 MHz, $CDCl_3$): described in the text.

Methyl ester of 3-epi-ursolic acid (**3**). 3-Epi-ursolic acid (50 mg) dissolved in MeOH and methylated with excess CH_2N_2 in Et_2O at 5°, crystallized from $CHCl_3$–MeOH (1:1), mp 185–190°. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1725 (ester carboxyl), 1645 (unsaturation).

Jones' oxidation of **3**. The methyl ester (**3**, 50 mg) was dissolved in 20 ml glacial HOAc and to it a soln of chromic acid (20 mg) in 5 ml glacial HOAc was added. The mixture was refluxed for nearly 2 hr at 50°, cooled, filtered and the filtrate was acidified with moderately conc. HCl in the cold. The ppt was dissolved in Et_2O, dried and the Et_2O removed to leave a crude solid which on repeated CC over 50 g Si gel (mesh 60–120) furnished the methyl ester of ursonic acid (**4**), mp 190–193°, $[\alpha]_D + 84°$ ($CHCl_3$) crystallized from $CHCl_3$–MeOH (1:1).

Alkaline hydrolysis of **4**. Methyl ursonate (**4**, 25 mg) was dissolved in 10 ml 20% ethanolic KOH and refluxed for 8 hr, solvent removed, H_2O added and the mixture filtered. The filtrate was acidified with HCl and extracted with Et_2O. The extract was washed with H_2O until free from acid, dried and the solvent distilled to leave ursonic acid, $C_{30}H_{46}O_3$ (**5**), mp 272–276° (d), $[\alpha]_D + 80°$ ($CHCl_3$) crystallized from MeOH.

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AMASTEROL, AN ECDYSONE PRECURSOR AND A GROWTH INHIBITOR FROM *AMARANTHUS VIRIDIS*

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Key Word Index—*Amaranthus viridis*; Amaranthaceae; sterol; ecdysone precursor; growth inhibitor.

Abstract—A sterol isolated from the roots of *Amaranthus viridis* has been assigned the structure 24-methylene-20-hydroxycholest-5,7-en-3β-ol.

INTRODUCTION

Several ecdysteroids have been isolated from members of the Amaranthaceae. The genus *Amaranthus* has been found to elaborate β-ecdysone [1] and inokosterone [2] while from *Cyathula*, amarasterone and amarasterone B [3] were isolated. While investigating the chemical constituents of Indian medicinal plants, our interest was directed to the genus *Amaranthus* which is cultivated in India as a crop. We now report the isolation of a new growth inhibitory steroid amasterol from the petroleum ether extract of the roots of *Amaranthus viridis L.*

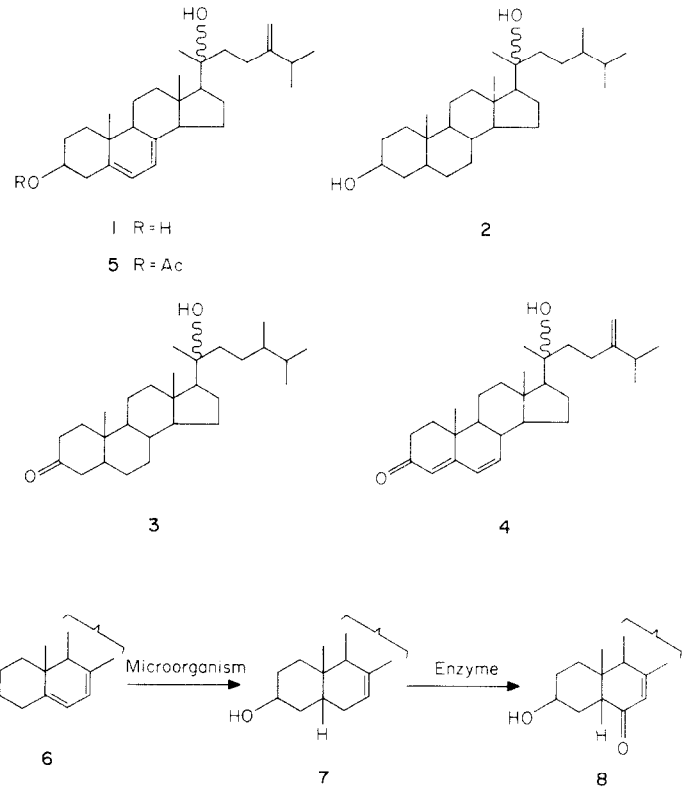

Table 1. NMR spectral data for amasterol and its acetate

Position of proton signals	Average chemical shift of proton signals (in δ values)	
	Amasterol	Amasterol acetate
Olefinic proton at C-6, C-7, C-24, C-25	5.2–5.1	4.3, 4.21
Tertiary proton at C-3	3.58–3.6 (α, a)	4.15–3.75 (α, a)
Deshielded protons at the side chain	1.3–1.82	1.1–1.55
Methyl protons at C-21	1.1(s)	0.9(s)
Methyl protons at C-19	0.58	0.45
Alicyclic methylene protons	0.6–1.0	0.6–0.78

RESULTS AND DISCUSSION

Amasterol, $C_{28}H_{44}O_2$ (1), mp 170° (M^+ 412), $[\alpha]_D^{CHCl_3}$ +42° was found to be homogeneous by TLC. The colour reaction suggested it to be an unsaturated sterol. The IR spectrum of the compound showed it to have a hydroxyl group (3400 cm^{-1}) and a terminal methylene group (885 cm^{-1}). The UV spectrum of amasterol showed absorption at λ_{max} 262, 271, 281, 294 nm with log ε 3.75, 3.94, 4.00, 3.73 which was very close to those of ergosterol and related homoannular dienes. So the presence of the homoannular diene in amasterol was readily discernible.

The NMR spectral data (60 MHz in $CDCl_3$) showed the presence of C-methyl groups at C_{18} and C_{19} (δ0.85 and 0.58) of a steroidal system and a gem dimethyl group (δ0.8) at the side chain. The singlet of the C-methyl proton at δ1.1 could account for a methyl group deshielded by a hydroxyl group. The one proton multiplet at δ3.58–3.6 could account for the proton at C-3 carrying a hydroxyl function. The complex signal at δ5.2–5.1 for four protons suggests that the terminal methylene and the two protons of the homodiene system have merged to give a signal of complex nature. Amasterol on hydrogenation (Pd–C,

H_2) gave a homogeneous hexahydro derivative (2), $C_{28}H_{50}O_2$, mp 139° (M^+ 418) which lacked the characteristic IR band for the terminal methylene group (885 cm^{-1}) and the UV maxima for the homodiene system (λ_{max} 262, 271, 281, 294).

The mass spectrum of amasterol showed a molecular ion at *m/z* 412 (97%) and another high intensity base peak at *m/z* 271 (100%) which in many steroids with a 20-hydroxy group arise by the cleavage of the C_{17}–C_{20} bond [4]. The fragment 271 accounted for the tetracyclic sterol nucleus in amasterol. In the hexahydro derivative the mass spectral peak for this fragment moved to *m/z* 275 further supporting the homoannular diene system in the tetracyclic fragment of amasterol. The mass spectral peak (*m/z* 141) for the side chain fragment moved to 143 in the hexahydro derivative, showing the presence of one double bond besides a hydroxyl group.

The hexahydro derivative (2) on Sarret oxidation gave a ketone (3), $C_{28}H_{48}O_2$, mp 176–177° (ν_{max}^{Nujol} cm^{-1}: 3400, 1720. On Oppenauer oxidation, amasterol furnished a conjugated cyclic ketone (4), $C_{28}H_{42}O_2$ mp 122° (ν_{max}^{Nujol} cm^{-1}: 3400, 1665 and λ_{max} 280 nm: 3.31) which showed the presence of a conjugated enone. This is due to the shift of the bond at $\Delta^{5,6}$ to $\Delta^{4,5}$ and its conjugation with ketone at the C-3 position formed during oxidation. This clearly establishes the presence of the homoannular diene system in **1**, as in ergosterol ($\Delta^{5,6}$ and $\Delta^{6,7}$ in ring B). On acetylation amasterol gave a monoacetate (**5**), $C_{30}H_{46}O_3$, mp 194° $[\alpha]_D^{CHCl_3}$ −87° (ν_{max}^{Nujol} cm^{-1}: 3400, 1740, 875). The presence of the hydroxyl band in **3–5** at 3400 cm^{-1} in the IR spectrum clearly indicated that amasterol contains one secondary and one tertiary hydroxy group. Interestingly enough, significant shifts of different proton signals were observed in the NMR spectrum of amasterol acetate (Table 1) as compared with that of amasterol. This shift and shape of the proton signals in the steroids may be due to various factors such as shielding, and deshielding, conformational and configurational changes [5]. The half band width (15 Hz) of the proton adjacent to a hydroxyl at δ3.58–3.6 or acetate at δ4.15–3.75 showed the hydroxyl at C-3 is β-equatorial. Thus structure **1** can be assigned to amasterol.

The isolation of amasterol from *A. viridis* is interesting from a biological view point. The Amaranthaceae has been found to elaborate ecdysones having the characteristic ring B enone. The biogenesis of the ring B enone is a matter of speculation. It may be mentioned here that 7-dehydrocholesterol may be converted to 5β-cholest-7-en-3β-ol (**6–8**) [6]. This sterol may thus be presented in the gut of certain insects and may serve as a precursor of ecdysones in such cases (**7–8**). Therefore a $\Delta^{5,7}$ diene may be considered to be a precursor of ecdysones. The presence of amasterol ($\Delta^{5,7}$ diene), spinosterol (Δ^7 sterol) in *A. viridis* and ecdysones in Amaranthaceae is suggestive of such a biosynthetic series.

Growth inhibitory activity of amasterol and its derivatives

Amasterol was found to be allelochemic in nature. The toxicity of the compound was tested on the seeds of lettuce (*Lactuca sativa* cv White). It caused inhibition of seed germination and growth of seedlings of the test material at different concentrations. The hexahydro derivative of amasterol and amasterol acetate were also found to be toxic to lettuce seeds. Further, amasterol was found to strongly inhibit the growth of *Helminthosporium oryzi*, a pathogenic fungus.

EXPERIMENTAL

All mps are uncorr. Petrol refers to light petroleum bp 60–80°. All solvent extracts were dried over Na_2SO_4. Optical rotations were measured in $CHCl_3$. Samples were analysed after drying in vacuum at 80° over P_2O_5 for 24 hr. The UV spectra were measured in 98% EtOH, IR spectra in Nujol, and NMR spectra in $CDCl_3$ with TMS as the int. standard. Si gel from Sarabhai Chemicals, India, was used as the absorbent for CC.

Isolation of amasterol (**1**). The neutral fraction of the petrol extract of dried and powdered plant of *Amaranthus viridis* L. was chromatographed over Si gel. The C_6H_6 and C_6H_6–$CHCl_3$ eluents on evaporation gave a solid material which on repeated crystallization from petrol–C_6H_6 furnished colourless homogeneous crystals of amasterol (**1**) mp 170°. NMR: δ5.2–5.1 (4H, complex *m*, *cis* olefinic protons at C-6 and C-7 and termial methylene protons at C-24), 3.58–3.6 (1H, *br*, H-3), 1.30–1.82 (22H, complex *m*, ring methylene protons, side chain methylene protons and methine protons at C-9, C-13, C-17, and C-25), 1.1 (3H, *s*, deshielded tertiary methyl at C-21), 0.8 (6H, *s*, gem dimethyl protons at C-26 and C-27), 0.85 (3H, *s*, Me-18), 0.58 (3H, *s*, Me-19). (Found: C, 81.54; H, 10.72. $C_{28}H_{44}O_2$ requires: C, 81.50; H, 10.75%.)

Hydrogenation of amasterol. An alcoholic soln of amasterol (100 mg) was hydrogenated in the presence of Pd–C (10%). After completion of the reaction, it was filtered, the solvent evaporated to dryness and the residue was crystallized from MeOH to give **2**, mp 139° (M^+ 418), IR ν_{max} cm^{-1}: 3400 (OH), 1380. (Found: C, 80.39; H, 11.8. $C_{28}H_{50}O_2$ requires: C, 80.38; H, 11.9%.)

Sarret oxidation of **2**. To a complex of chromic acid (60 mg) with C_5H_5N (4.0 ml) at −5°, a soln of **2** (75 mg) in C_5H_5N (2.0 ml) was added with stirring. After leaving overnight, the reaction mixture was poured into ice and extracted with Et_2O. After removal of the solvent, the residue was chromatographed over Si gel. The $CHCl_3$–EtOAc eluents (2:1) on evaporation gave a solid which on recrystallization from C_6H_6–$CHCl_3$ gave **3**, mp 176.77° (M^+ 416) UV λ_{max} nm: 279 (1.19); IR ν_{max} cm^{-1}: 3400 (OH), 1720 (keto carbonyl), 1385. (Found: C, 80.75; H, 11.58. $C_{28}H_{48}O_2$ requires: C, 80.71; H, 11.61%).

Oppenauer oxidation of amasterol. To a soln of amasterol (50 mg) in dry C_6H_6 (50 ml) and dry Me_2CO (40 ml) freshly prepared aluminium isopropoxide (30 mg) was added and the mixture was refluxed (12 hr) under anhydrous conditions. After cooling the reaction mixture was first washed with 30% H_2SO_4 then with H_2O. The solvent was removed under red. pres. the residue taken up in C_6H_6 and chromatographed over Si gel. The C_6H_6–$CHCl_3$ (1:1) and $CHCl_3$ eluents on evaporation gave a solid which on recrystallization yielded **4**, mp 122° (M^+ 410), UV λ_{max} nm: 280 (3.31). IR ν_{max} cm^{-1}: 3400 (OH), 1665 (conjugated ketone), 1385, 895 (terminal methylene). (Found: C, 81.88; H, 10.34. $C_{28}H_{42}O_2$ requires: C, 81.90; H, 10.31%.)

Amasterol acetate (**5**). This crystallized from MeOH, with mp 194° (M^+ 454), $[\alpha]_D$ −87°. UV λ_{max} nm: 292 (3.73), 281 (4.00), 270 (3.94), 262 (3.75); IR ν_{max} cm^{-1}: 3400 (OH) 1740

(acetoxy carbonyl), 1385, 875 (terminal methylene). NMR, δ 4.30 (2H, *d*, *cis* olefinic protons at C-6 and C-7, 4.21 (2H, *s*, terminate methylene protons at C-24), 4.15–3.75 (1H, *br*, H-3), 1.69 (3H, *s*, acetoxy methyl), 1.1–1.55 (22H, complex, *m*, ring methylene protons, side chain methylene protons and methine protons at C-9, C-13, C-17 and C-25), 0.70 (6H, *s*, gem dimethyl at C-26 and C-27), 0.83 (3H, *s*, Me-18), 0.9 (3H, *s*, deshielded tertiary methyl protons at C_{21}), 0.45 (3H, *s*, Me-19). (Found: C, 79.28, H, 10.24. $C_{30}H_{46}O_3$ requires: C, 79.25; H, 10.20%.)

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A DAMMARANE TRITERPENE FROM *COMMELINA UNDULATA**

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Key Word Index—*Commelina undulata*; Commelinaceae; dammarane triterpene; dammar-12, 25-dien-3β-acetate.

Abstract—The chemical investigation of *Commelina undulata* afforded, in addition to 2-heneicosanone, *n*-octacosanol, sitosterol and sitosterol-β-D-glucoside, a new triterpene characterized as dammar-12,25-dien-3β-acetate.

INTRODUCTION

In the course of our search for anti-cancer constituents in Indian plants, we have examined *Commelina undulata* which possessed anti-cancer activity against lymphoid leukaemia in mice (PS 388) in the screening programme of NIH. A new triterpene (**1**) of the dammarane series was isolated together with 2-heneicosanone, *n*-octacosanol, sitosterol and sitosterol-β-D-glucoside.

RESULTS AND DISCUSSION

Systematic fractionations of an alcoholic extract of the plant was carried out with hexane, benzene, ethyl acetate and *n*-butanol. The crystalline compound **1** was isolated by CC from the hexane fraction over Si gel.

The IR spectrum of compound **1** had absorption bands at 1740 (C = O); 1384, 1370 (gem methyls); 1245 (C–O–C), 1660, 1640, 985 and 885 cm^{-1} (C=CH and C=CH_2). The UV spectrum had λ_{max} at 240 nm (ϵ = 523). The compound exhibited a positive Liebermann–Burchardt test and gave a yellow colouration with tetranitromethane. The ^1H NMR spectrum showed five tertiary methyl signals at δ 0.73, 0.8, 0.82 (6H) and 0.88 and a signal at 0.95 (3H, *d*, J = 7 Hz), assigned to Me-20[1]. The methylene and methine protons were in the range δ1.55–1.08. A vinylic methyl appeared at 1.6 (3H, *s*) and an acetoxyl methyl singlet was at δ1.95. A multiplet was centered at 2.25 (4H, H-11 and H-24) and the signals at 4.6 and 4.48 (*d*, J = 2 Hz) were assigned to the C-26 methylene protons. The signal at 4.36 (1H, *dd*, $J_{ax,ax}$ = 10 Hz, $J_{ax,eq}$ = 6.5 Hz,) was assigned to the C-3α proton and an olefinic proton was observed at 5.1 (1H, *m*, H-12). The mass spectrum of this compound had the M^+ at *m/z* 468 and other ions at *m/z* 453 $[M-Me]^+$, 425 $[M-\overset{O}{\overset{\|}{C}}-Me]^+$ and 408 $[M-MeCO_2H]^+$. The presence of side chain and C-12 double bonds in the molecule was evident from the ion peak at *m/z*

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