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STIGMASTA-5,22,25-TRIEN-3β-OL : A NEW STEROL FROM *ALANGIUM LAMARCKII* THW[1].

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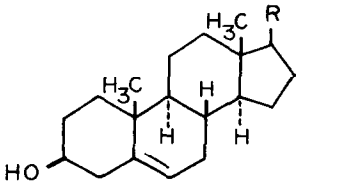
Recent report[2] of the structure elucidation of (24 S) -ethylcholesta-5,22,25-trien-3β-ol (I) prompts us to publish the isolation and characterisation of its hitherto unknown 24-epimer, stigmasta-5,22,25-trien-3β-ol (II), from the leaves of *Alangium lamarckii* Thw. (Alangiaceae) and also from the whole plant of *Enhydra fluctuans* Lour. (Compositae)[3]. Obtained from the petroleum ether extract of both the plants, it was purified from the closely associated stigmasterol through its acetate by chromatography over a column of silica gel impregnated with silver nitrate[4].

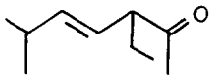
The acetate, m.p. 149-50°, $[\alpha]_D$ - 46.6° (c 0.815)[5]; ν_{max}^{Nujol} 1725, 1260 (C=O), 970 (-CH=CH- trans), 1645, 885 (CH_2=C<) and 800 (-CH=C<)cm^{-1}; δ^{CDCl_3} 5.1-5.5 (3H, m, -CH=C< and -CH=CH-), 4.73 (2H, bs, CH_2=C<), 4.4-4.9 (1H, m, >CH.O.Ac), 2.00(3H, s, -OCOCH_3), 1.63 (3H, bs, CH_3-C=C<), 1.05 (3H, s, 19-CH_3) and 0.67(3H,s, 18-CH_3) ppm; m/e 392(base peak, M^+ -60); yielded on saponification the new sterol, $C_{29}H_{46}O$, m.p. 151-52°, $[\alpha]_D$ - 44.0° (c 0.5); λ_{max}^{EtOH} 210 nm (ε = 3,100); m/e 410 (M^+), 395 (M-CH_3), 392 (M-H_2O), 381(M-C_2H_5), 377(M-CH_3-H_2O), 363(M-C_2H_5-H_2O), 314,300,271 (M-sidechain-2H), 255, 229, 213 (M-H_2O-side chain with a part of ring D), 159, 138, 137 [$C_{10}H_{17}^+$(side chain)], 109(base peak), 95, 81 and 55. It formed a benzoate, m.p. 138°, $[\alpha]_D$ - 14.9° (c 0.94).

Hydrogenation of the acetate in ethyl acetate solution in presence of Adam's catalyst afforded stigmastan-3β-ol acetate, m.p. 133-34°, $[\alpha]_D$ + 16.1° (c 0.87). The intense peaks at m/e 255, 271 and 300 in the mass spectrum of the sterol (II) indicated the presence of a monounsaturated ring system[6] with

additional double bonds in the side chain. NMR and IR evidences for a trans-$-CH=CH-$ and $CH_3-\overset{|}{C}=CH_2$ groupings located the unsaturations between $C_{22}-C_{23}$ and $C_{25}-C_{26}$. Finally, chemical shifts of the remaining olefinic proton, 19 -CH_3 and 18 -CH_3 are in agreement with the values recorded[7] for a Δ^5-3β-ol rather than a Δ^7-3β-ol structure[8].



III R =



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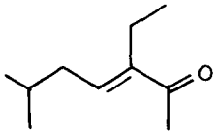

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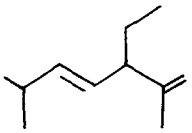

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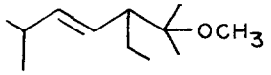

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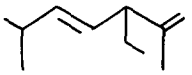

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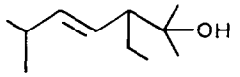

The locations of the side chain double bonds were confirmed as in the sequel. Hydrogenation of the sterol in dioxan solution using 5% Pd/C catalyst afforded a mixture from which stigmasterol could be isolated as its acetate tetrabromide, m.p. 193° d, $[\alpha]_D$ - 39.3° (c 0.28). Oxidation of sterol II with osmium tetroxide in benzene or potassium permanganate in aqueous tetrahydrofuran and cleavage of the resulting product with sodium periodate in pyridine yielded the ketone (III), m.p. 159-63°, $[\alpha]_D$ + 60.4° (c 0.48) ν_{max}^{Nujol} 1710, 965 and 795 cm^{-1}. The high positive molecular rotation difference (M_D keto sterol III - M_D sterol II = + 414) is comparable to that observed in stigmasta-7,22,25 -trien-3β-ol (ΔM_D = 418 for the acetate calculated from published values[8]) and can be attributed to the difference in contribution of the 24R carbon. The ketone isomerised in methanolic alkali (0.5 M) at room

temperature to the conjugated one (IV), ν_{max}^{Nujol} 1660, 965 and 795 cm^{-1}, λ_{max}^{EtOH} 232 nm; acetate (Ac_2O-Py), m.p. 143-44°, $[\alpha]_D$ - 46.7° (c 0.3), ν_{max}^{Nujol} 1725, 1665, 1615, 1235, and 795 cm^{-1}.

Upon refluxing with methanolic hydrochloric acid for 3 hr the sterol yielded two products, viz. sterol A (major), m.p. 157°, $[\alpha]_D$ - 50.4°(c 1.19); acetate, m.p. 166°, $[\alpha]_D$ - 56.6° (c 1.59); benzoate, m.p.161-63°, $[\alpha]_D$ - 25.8° (c 0.6) and sterol B, m.p. 171°, $[\alpha]_D$ - 71.7° (c 0.551); acetate (Ac_2O - Py), m.p. 163°, $[\alpha]_D$ - 76.3° (c 0.465). Again, sterol A under the same condition partly regenerated the original sterol and afforded sterol B as the minor product.

The IR and NMR spectra of both sterol A and sterol B acetates showed the absence of $CH_2{=}C<$ grouping. A new band at 1075 cm^{-1} in the IR spectrum and a NMR signal at δ 3.17 ppm (3H, s) clearly demonstrated the presence of a OCH_3 group in sterol A and the most intense peak at m/e 73 in its mass spectrum could be attributed to the ion $(CH_3)_2\overset{+}{C}{=}OCH_3$. Thus sterol A was assigned the 25-methoxystigmasta-5, 22-dien-3β-ol structure (V).

The addition of solvent to the terminal methylene of the sterol (II) was also evident from the structure of sterol B. The spectral data of the acetate showed the presence of a free hydroxy group $[\nu_{max}^{Nujol}$ 3400 cm^{-1} in the IR spectrum; δ 1.65 ppm (1H, s) in the NMR spectrum exchangeable with $D_2O]$. Like that of sterol A, the mass spectrum of sterol B did not show the molecular ion. Nevertheless, the base peak at m/e 59 assignable to the ion $(CH_3)_2\overset{+}{C}{=}OH$, supported the presence of a 1-hydroxyisopropyl group in the side chain, thus leading to the stigmasta-5,22-dien-3β,25-diol structure (VI) for sterol B. The structure was finally proved by its dehydration to the acetate of II with $POCl_3$ in pyridine at room temperature overnight. The formation of the possible conjugated diene in this process was also evident from the UV spectrum (λ_{max}^{EtOH} 237 sh, 242 nm) of the mother liquor though the compound could not be separated in a pure state.

From the leaves of A. lamarckii, in addition to β-sitosterol, stigmasterol and friedelin[9,10] we have also been able to isolate myristic acid and

three triterpenoids, viz. triterpene A, $C_{30}H_{48}(OH)_2$, m.p. 248-50°, $[\alpha]_D$+48.6° (c 0.741,Py), diacetate (M^+ 526), m.p. 235-36°, $[\alpha]_D$ + 59.0° (c 0.644); triterpene B, $C_{30}H_{50}(OH)_2$ (M^+ 444), m.p. 224-25°, $[\alpha]_D$ + 36.5° (c 0.631), monoacetate, m.p. 215°, $[\alpha]_D$ ± 0° (c 0.758); triterpene C, $C_{30}H_{50}(OH)_2$, m.p. 224°, $[\alpha]_D$ + 49.6° (c 0.403), monoacetate (M^+ 486), m.p. 262-63°, $[\alpha]_D$ + 39.4° (c 0.406), yet to be characterised.

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