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ISOLATION OF TWO NEW STEROLS FROM CLERODENDRUM CAMPBELLII

Loretta M. Bolger, H.H. Rees, E.L. Ghisalberti, L.J. Goad and T.W. Goodwin.

Department of Biochemistry, The University, P.O. Box 147, Liverpool L69 3BX.

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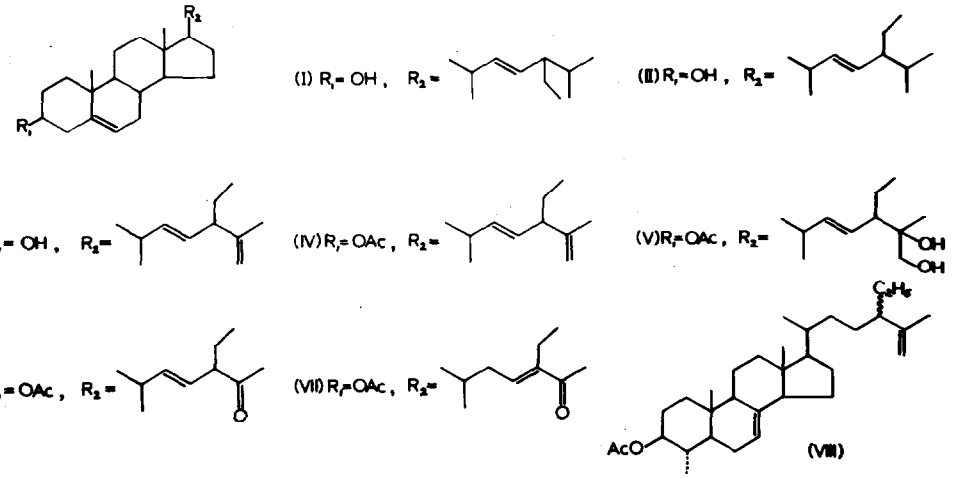
The occurrence of Δ^{25}-sterols has been reported[1-7] in a number of plants, including Clerodendrum species. The present communication reports the isolation and characterization of two new sterols, (24S)-ethyl-cholesta-5,22,25-trien-3β-ol and 24ξ-ethyl-4α-methyl-cholesta-7,25-dien-3β-ol, from Clerodendrum campbellii. Freeze-dried leaves of C. campbellii were extracted with ethanol, the lipid saponified and the 3β-hydroxy sterols precipitated with digitonin. The regenerated sterols were separated into 4,4-dimethyl,4α-methyl and 4-desmethyl sterols.

4-Desmethyl sterols. G.L.C. (QF-1 and SE-30) of this fraction showed a single peak of identical retention time to that of stigmasterol (I) and poriferasterol (II). Treatment with the Liebermann-Burchard reagent gave a blue-green colour, λ-max 620 nm, which reached a maximum intensity after 33 min. indicative of a Δ^5 bond. Recrystallization gave crystals of m.p. 146°C (acetate m.p. = 141°C); $[\alpha]_D^{25}$ =-37.8 ($CHCl_3$); I.R., νmax 890, 1645 cm^{-1} (terminal methylene), 965 cm^{-1} (trans disubstituted double bond), 800 cm^{-1} (trisubstituted double bond); M.S., m/e 410[M+], 395 [M-CH_3], 392 [M-H_2O], 381 [M-C_2H_5], 377 [M-CH_3-H_2O], 363, 314, 309, 300 [M-part of side chain], 271 [M-side chain plus two hydrogens], 255 [M-(H_2O + side chain)], 213 [M-(H_2O+ side chain plus part of ring D)]. N.M.R. ($CDCl_3$)[8], singlets at 0.69 (C-18 methyl), 1.00 (C-19 methyl), 1.56 (O-H), 1.64 (C-27 methyl) and 4.68 p.p.m. (2H, >C=CH_2); doublet centred at 1.00 p.p.m. (J=6.5 c.p.s., C-21 methyl); triplet centred at 0.82 p.p.m. (J=7 c.p.s., C-29 methyl); multiplets centred at 3.50 (C-3 proton), 5.21 (2H, -CH=CH-) and 5.34 p.p.m. (1H, C-6 proton). The chemical shifts for the C-18 and C-19 protons are in agreement with those reported for Δ^5 sterols, whereas in the case of Δ^7 sterols, the same protons are shifted to higher field.[2,3,9] Hydrogenation of the sterol with tris-(triphenylphosphin)-rhodium chloride[10] produced a dihydro derivative. M.S., m/e 412 [M+], 397 [M-CH_3], 379 [M-(CH_3+H_2O)], 369 [M-terminal isopropyl group], 351 [M-(terminal isopropyl + H_2O)], 300 [M-part of side chain], and 271 [M-(side chain +2H)]; I.R. and N.M.R. spectra indicated the absence of a

terminal methylene grouping. The dihydro compound co-chromatographed on G.L.C. (QF-1 and SE-30) with stigmasterol (I) and poriferasterol (II). M.p. 155-156°C, not depressed on admixture with poriferasterol (m.p. 155-156°C) but slightly depressed (153-154°C) on admixture with stigmasterol. The dihydro derivative and poriferasterol which has a 24S configuration showed identical O.R.D. curves which differed from that of stigmasterol, thus suggesting that the parent sterol has the 24S configuration. The combined data presented above suggest that the sterol has structure (III). Confirmation of the relative positions



of the two side chain double bonds was obtained in the following way. The steryl acetate (IV) was treated with osmium tetroxide[11] to produce two isomeric 25,26-diols (V) which were separable by t.l.c. on silica gel develped with 8% methanol in chloroform (R_f 0.66, m.p. 188-194°C and R_f 0.60, m.p. 194.5 - 196°C, respectively). Both diols had practically identical I.R. and mass spectra. The I.R. spectra showed the absence of a terminal methylene grouping but a very prominent band due to OH in the region 3,400 - 3,280 cm^{-1}. M.S. m/e, 455 [M-CH_2OH], 408 [M-(60+H_2O)], 395 [M-(CH_2OH+60)], 352 (base peak), 337, 283, 255 [M-(60+ side chain)], 231, 213 [M-(side chain +60 + part ring D)]. The diols were cleaved with sodium periodate to give identical 25-oxo compounds (VI); m.p. 142°C; M.S. m/e 394 [M-60], 379 [M-(60 + CH_3)], 351 [M-(60+CH_3CO-)], 283, 255 [M-(60+ side chain)], 213 [M-(side chain + 60 + part ring D)]; I.R. ν max 1710 (C=O), 965 cm^{-1} (trans disubstituted double bond). N.M.R. ($CDCl_3$) (cf. ref. 3), singlets at 0.68 (C-18 methyl), 1.01 (C-19 methyl), 2.00 (-OAc) and 2.08 p.p.m. (H_3C-CO); doublet centred at 1.01 p.p.m. (J= 6.5 c.p.s., C-21 methyl); triplet centred at 0.82 p.p.m. (J= 7.0 c.p.s., C-29 methyl); quartet centred at 2.84 p.p.m. (1H, C-24 proton); two sets of

multiplets (3H), one at 5.11 - 5.45 p.p.m. (-CH=CH-) and the other centred at 5.34 p.p.m. (C-6 proton); unresolved multiplet centred at 5.58 p.p.m. (1H, C-3 proton). Equilibration of the ketone (VI) with KOH in methanol-ethanol,[3] and reacetylation of the product, gave compound VII, m.p. 117-123°C; I.R. ν max 1665 cm^{-1} (C=O conjugated to double bond); U.V. (ethanol) λmax 229 nm;[3] M.S. m/e 454 [M+], 394 [M-60], 379 [M- (60+CH_3)], 366, 283, 253, 213. The foregoing data lead us to formulate the new 4-desmethyl sterol as (24S)-ethyl—cholesta-5,22, 25-trien-3β-ol (III).

4α-Methyl Sterols. G.L.C. analysis (QF-1 and SE-30) revealed the presence of four components, three of which corresponded to obtusifoliol (4α,14α-dimethyl-ergosta-8,24(28)-dien-3β-ol), 24-methylene lophenol (4α-methyl-ergosta-7,24(28)-dien-3β-ol) and cycloeucalenol (4α-methyl-9,19-cyclo-ergosta-24(28)-en-3β-ol). Separation of the acetates on $AgNO_3$-silica gel produced three bands corresponding to the acetates of (a) 24-methylene lophenol, (b) obtusifoliol and cycloeucalenol and (c) 24-ethylidene lophenol (4α-methyl-stigmasta-7,24(28)-dien-3β-ol). Band (a) corresponded to 24-methylene lophenol on G.L.C. (QF-1). I.R. ν max 885, 1640 cm^{-1} (terminal methylene); M.S. m/e 454 [M+], 439 [M-CH_3], 394 [M-60], 379 [M-(60 + CH_3)], 370 [M- (part of side chain)], 327 [M-(side chain + 2H)], 287 [M-(side chain + part of ring D)], 269 [M-(side chain + 60)], 267 [M-(side chain + 2H + 60)], 227 [M-(side chain + part of ring D + 60)]. This is in accord with the mass spectrum reported[12,13] for 24-methylene lophenyl acetate. Band (b) gave two peaks on G.L.C. (QF-1) co-chromatographing with the acetates of obtusifoliol and cycloeucalenol. Band (c) gave one peak on G.L.C. (QF-1 and SE-30) of slightly shorter retention time than 24-ethylidene lophenyl acetate; m.p. 142°C; I.R. ν max 890cm^{-1} (terminal methylene). It gave an immediate blue colour with the Liebermann-Burchard reagent, indicative of a Δ^7 bond. The U.V. spectrum (ethanol) had a high end absorption (ε_{225} = 1482) which was more characteristic of Δ^7 than of Δ^5 sterols[14] (ε_{225} for sterol III = 303). M.S. m/e 468 [M+], 453 [M-CH_3], 408 [M-60], 393 [M-(60 +CH_3)], 327 [M-(side chain + 2H)], 302,287, 269 [M-(side chain +60)], 267 [M-(side chain +2H+60)], 227 [M-(side chain + part of ring D)]. Only a very small peak was obtained at m/e 370, which is prominent in 24-ethylidene lophenyl acetate and corresponds to loss of part of the side chain.[12,13] N.M.R. ($CDCl_3$) (cf. ref. 2 & 3), singlets at 0.50 (C-18 methyl), 0.82 or 0.86 (C-19 methyl), 1.55 (C-27 methyl) and 2.02 p.p.m. (CH_3-CO-); probably centre of triplet at 0.80 p.p.m. (C-29 methyl); multiplets centred at 4.37 p.p.m. (1H, C-3 proton), 4.66 (2H, = CH_2) and 5.14 p.p.m. (1H, C-7 proton). The above data are in agreement with the formulation of the compound in band (c) as 24ξ-ethyl-4α-

methyl-cholesta-7,25-dien-3β-yl acetate (VIII).

4,4-Dimethyl Sterols. Cycloartenol and 24-methylene cycloartanol were identified by G.L.C., m.p., I.R. and mass spectra.

In summary, the sterols of Clerodendrum campbellii include (24S)-ethyl-cholesta-5,22,25-trien-3β-ol (III), cycloeucalenol, obtusifolid, 24-methylene lophenol, 24ξ-ethyl-4α-methyl-cholesta-7,25-dien-3β-ol, cycloartenol and 24-methylene cycloartanol. Observations on the biosynthesis of III are reported elsewhere.[15]

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