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Biosynthesis of 24-Ethylcholesta-5,22,25-trien-3β-ol, a New Sterol from *Clerodendrum campbellii*

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Sterols possessing Δ^{25}-bonds have previously been reported in a number of plants (Manzoor-i-Khuda, 1966; Sucrow, 1966*a*,*b*; Barua, Sanyal & Chakrabarti, 1967; Kawano, Miura & Kamo, 1967; Sucrow & Reimerdes, 1968). We have characterized the sterols of *Clerodendrum campbellii* as (24*S*)-ethylcholesta-5,22,25-trien-3β-ol (not previously reported as a natural product), cycloartenol, 24-methylenecycloartanol, cycloeucalenol, obtusifoliol, 24-methylenelophenol and 24-ethyl-4α-methylcholesta-7,25-dien-3β-ol (L. M. Bolger, H. H. Rees, E. L. Ghisalberti, L. J. Goad & T. W. Goodwin, unpublished work).

The major sterol (24*S*)-ethylcholesta-5,22,25-trien-3β-ol has the following characteristics: m.p. 146°C; $[\alpha]_D^{22}$ −37.8° (chloroform); i.r. spectrum (Nujol mull) $\nu_{max.}$ 890cm^{-1}, 1645cm^{-1} (terminal methylene), 965cm^{-1} (*trans* disubstituted double bond) and 800cm^{-1} (trisubstituted double bond); mass spectrum *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+2H)], 255 [$M-$(side chain+H_2O)] and 213 [$M-(H_2O+$side chain plus part of ring D)]; n.m.r. spectrum ($CDCl_3$) (chemical shifts are given in p.p.m. downfield from internal trimethylsilane standard) singlets at 0.69 (C-18 methyl), 1.00 (C-19 methyl), 1.56 (O–H), 1.64 (C-27 methyl) and 4.68p.p.m. (2H) (>C–CH_2), doublet centred at 1.00 (*J* 6.5Hz) (C-21 methyl), triplet centred at 0.82p.p.m. (*J* 7Hz) (C-29 methyl) and multiplets centred at 3.50 (C-3 proton), 5.34 (1H) (C-6 proton) and 5.21p.p.m. (2H) (–CH=CH–) (compare Sucrow, 1966*a*,*b*). We now report studies on the biosynthesis of (24*S*)-ethylcholesta-5,22,25-trien-3β-ol.

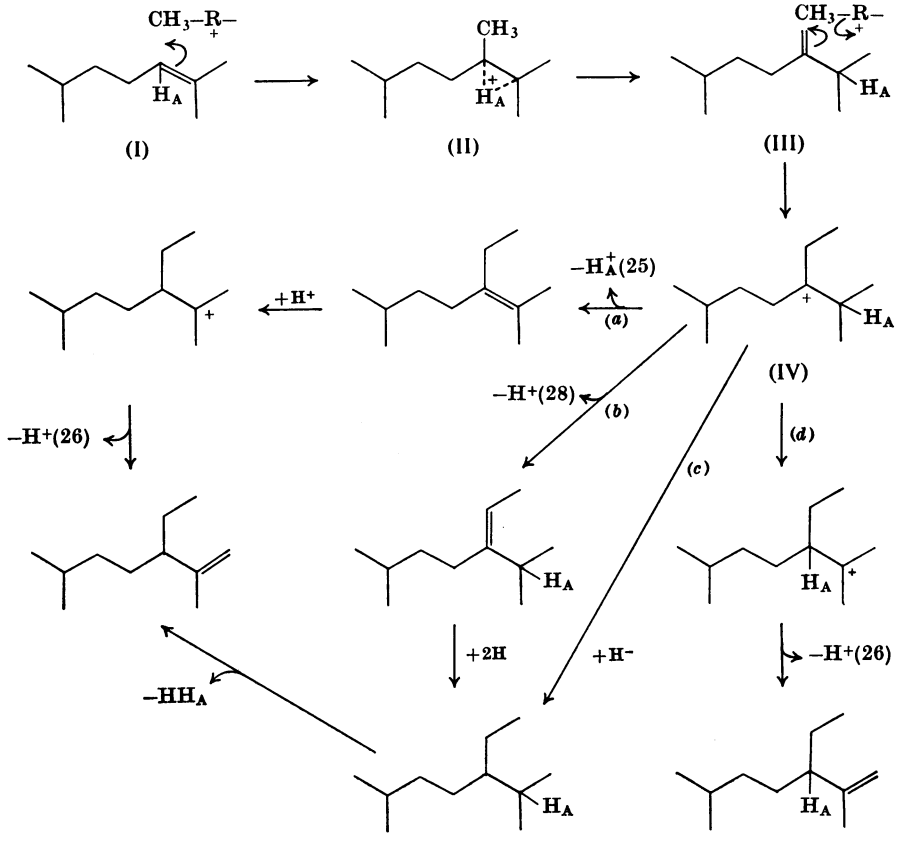
When [2-^{14}C,(4*R*)-4-$^3H_1$]mevalonate is used as substrate cycloartenol, the postulated phytosterol precursor, will be labelled with ^{3}H at the 3α-, 5α-, 8β-, 17α-, 20- and 24-positions and with ^{14}C at C-1, C-7, C-15, C-22, C-26 or -27 and C-30 (Rees, Goad & Goodwin, 1968). It has been demonstrated (Castle, Blondin & Nes, 1963; Bader, Guglielmetti & Arigoni, 1964; Villanueva, Barbier & Lederer, 1964) that the 24-ethyl group present in various phytosterols arises by two successive transmethylations from methionine, and that during the first alkylation at C-24 to give a 24-methylene compound there is a hydrogen migration from C-24 to C-25 (Scheme 1, I–III) (Akhtar, Hunt & Parvez, 1966; Raab, de Souza & Nes, 1968; Goad & Goodwin, 1969). Alkylation of structure (III) leads to the carbonium ion (IV), which can conceivably be stabilized in a number of ways to give the Δ^{25}-sterol (Smith, Goad, Goodwin & Lederer, 1967).

If one of pathways (*a*), (*b*) and (*c*) is operative the tritium atom originally at C-24 in the precursor (I) should be eliminated, whereas if pathway (*d*) is operative this tritium atom should be retained at C-24.

Experimental and results. [2-^{14}C,(4*R*)-$^3H_1$]-Mevalonate (containing 2.5μCi of ^{14}C and 25μCi of ^{3}H) was incubated with 3g of chopped leaf at room temperature for 15h. The non-saponifiable material (31mg; 716015d.p.m. of ^{14}C) was isolated and subjected to alumina column chromatography and t.l.c. on silica gel. Hexane was used as developing solvent for t.l.c. separation of squalene (R_F 0.42; 55637d.p.m. of ^{14}C), whereas chloroform was used for separation of 4,4-dimethyl sterols (R_F 0.27; 146646d.p.m. of ^{14}C), 4α-methyl sterols (R_F 0.22; 327395d.p.m. of ^{14}C) and 4-demethyl sterol (R_F 0.16; 660526d.p.m. of ^{14}C). The squalene was further purified by a second t.l.c. separation followed by formation of the thiourea adduct. 24-Methylenecycloartanol was isolated from the 4,4-dimethyl sterol fraction as the acetate by t.l.c. on $AgNO_3$-impregnated silica gel (42801 d.p.m. of ^{14}C), diluted with carrier material and recrystallized to constant specific radioactivity.

The 4-demethyl sterol fraction was diluted with carrier (24*S*)-ethylcholesta-5,22-25,trien-3β-ol, acetylated and purified by t.l.c. on $AgNO_3$-impregnated silica gel developed with 45% (v/v) benzene in hexane, to give the corresponding acetate (V) (R_F 0.13; 259550d.p.m. of ^{14}C). This was further diluted with carrier and recrystallized to constant specific radioactivity.

The purified sterol (V) was treated with osmium tetroxide in pyridine to give two isomeric 25,26-diols (VI), which were separated by t.l.c. on silica gel developed with 8% (v/v) methanol in chloroform: (i) R_F 0.66; 16.3mg; m.p. 188–194°C; (ii) R_F 0.60; 13.1mg; m.p. 194.5–196°C. The two diols



Scheme 1. Possible pathways for the biosynthesis of 24-ethylcholesta-5,22,25-trien-3β-ol. H_A becomes labelled from [2-^{14}C,(4*R*)-4-$^{3}H_1$]mevalonate.

had practically identical i.r. and mass spectra, and were shown in a preliminary experiment to yield the same ketone on cleavage. The i.r. spectra showed the absence of a terminal methylene grouping. The mass spectra showed *m/e* 455 ($M-CH_2 \cdot OH$), 408 [$M-(60+H_2O)$], 395 [$M-(CH_2 \cdot OH+60)$], 352 (base peak), 337, 283, 255 [$M-(60+$side chain)], 231 and 213 [$M-$(side chain+60+part ring D)]. The recombined diol was then dissolved in dioxan and, after the addition of carrier formaldehyde (20mg), was treated with sodium periodate and water (Baran, 1960). The mixture was steam-distilled into a solution of dimedone (300mg) in ethanol, and dimedone–formaldehyde complex collected. This was purified by t.l.c. on silica gel, yielding material cotaining negligible radioactivity.

The (24*S*)-24-ethyl-25-oxo-26-norcholesta-5,22-dien-3β-yl acetate (VII) was extracted from the reaction mixture, purified by t.l.c. on silica gel, developed with chloroform (R_F 0.41) and recrystallized to constant specific radioactivity. It had the following characteristics: m.p. 142°C; mass spectrum *m/e* 394 ($M-60$), 379 [$M-(60+CH_3)$], 351 [$M-60+CH_3 \cdot CO$)], 255 [$M-(60+$side chain)] and 213 [$M-$(side chain+60+part ring D)]; i.r. spectrum $\nu_{max.}$ 1710cm^{-1} (C=O) and 965cm^{-1} (–C=C–); n.m.r. spectrum ($CDCl_3$) singlet at 2.08p.p.m. (C27 methyl), quartet centred at 2.84p.p.m. (1H) (C-24 proton), multiplet at 5.11–5.45p.p.m. (–CH=CH–) and multiplet centred at 5.34p.p.m. (C-6 proton); integration of the region 5.0–5.5p.p.m. showed the presence of three protons. This material was dissolved in ethanol and equilibrated with methanolic KOH (Sucrow, 1966*b*), and the product reacetylated to give 24-ethyl-25-oxo-26-norcholesta-5,23-dien-3β-yl acetate (VIII), which was purified by t.l.c. on silica gel developed with chloroform (R_F 0.28; 2663d.p.m. of ^{14}C/mg). It had the following characteristics: m.p. 117–123°C; i.r. spectrum $\nu_{max.}$ 1665cm^{-1} (C=O conjugated to double bond); u.v. spectrum $\lambda_{max.}$ 229nm; mass spectrum *m/e* 454 (M^+), 394 ($M-60$), 379

[$M-(60+CH_3)$], 366, 283, 253 and 213. The $^3H/^{14}C$ ratios are given in Table 1.

Discussion. A $^3H/^{14}C$ ratio 3:5 for the (24*S*)-ethylcholesta-5,22,25-trien-3β-yl acetate (V) indicates that the tritium atom originally present at C-24 in cycloartenol is retained, also probably at C-24. It is reasonable to assume that the other two tritium atoms are located at C-17 and C-20, as demonstrated for cholesterol (Cornforth *et al.* 1965; Caspi & Mulheirn, 1969) and poriferasterol (Smith, 1969) biosynthesized from [2-^{14}C,(4*R*)-4-$^3H_1$]mevalonate. Since only negligible radioactivity (less than 0.0002% of the ^{14}C radioactivity of the diol) was recovered in the formaldehyde obtained by cleavage of the diol (VI) to the norketone, the methylene carbon atom of the original sterol (V) must be essentially devoid of label from C-2 of mevalonate. This is further substantiated if the reasonable assumption is made (E. L. Ghisalberti, unpublished work) that no tritium which may be α to the carbonyl group is lost on cleaving the diol (VI) to give compound (VII), in which case the $^3H/^{14}C$ radioactivity ratio in compound (VII) corresponds to an atomic ratio 3:5. Moreover, the loss of one tritium atom on equilibration of compound (VII) to compound (VIII) confirms the presence of a tritium atom at C-24 in compound (V). This therefore eliminates the possible operation of pathways (*a*), (*b*) and (*c*) in the biosynthesis of compound (V) and is in accordance with the operation of a pathway such as (*d*). A $^3H/^{14}C$ ratio 6:6 for 25-methylenecycloartanol is in agreement with previous reports (Akhtar *et al.* 1966; Raab *et al.* 1968; Goad & Goodwin, 1969; Rees *et al.* 1968) in which the tritium originally present at C-24 has been relocated at C-25. Therefore, in the biosynthesis of compound (V), a tritium migration back from C-25 to C-24 must have occurred during the second alkylation, with stabilization of the molecule by elimination of a proton from C-26 and formation of a Δ^{25}-bond. The present results should be considered in conjunction with those obtained for the introduction of the Δ^{25}-bond in cyclolaudenol (Ghisalberti, de Souza, Rees, Goad & Goodwin, 1969).

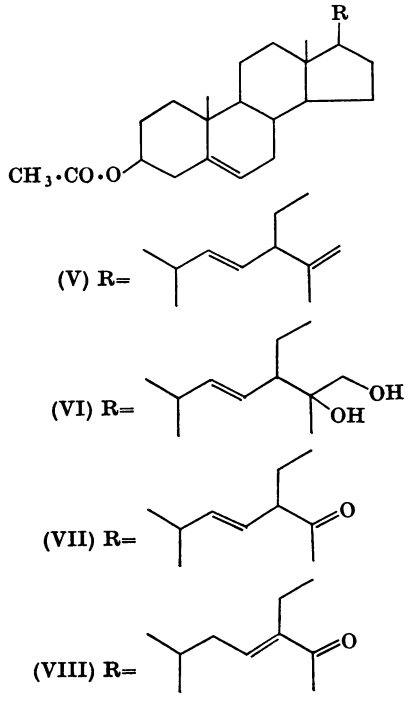


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Table 1. *Incorporation of* [2-^{14}C,(4*R*)-4-$^3H_1$]*mevalonate into* 24-*ethylcholesta*-5,22,25-*trien*-3β-*ol in* Clerodendrum campbellii

Compound	$^3H/^{14}C$ radioactivity ratio	$^3H/^{14}C$ atomic ratio (based on squalene)
[2-^{14}C,(4*R*)-4-$^3H_1$]Mevalonate	6.43:1.0	
Squalene	6.33:1.0	
(24*S*)-Ethylcholesta-5,22,25-trien-3β-yl acetate (V)	3.73:1.0	2.95:5
(24*S*)-Ethylcholesta-5,22-dien-3β,25ξ,26-triol 3-acetate (VI)	3.78:1.0	2.99:5
(24*S*)-24-Ethyl-25-oxo-26-norcholesta-5,22-dien-3β-yl acetate (VII)	3.72:1.0	2.96:5
24-Ethyl-25-oxo-26-norcholesta-5,23-dien-3β-yl acetate (VIII)	2.60:1.0	2.05:5
24-Methylenecycloartanyl acetate	6.30:1.0	5.98:6

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