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24-METHYLCHOLESTA-5,24(25)-DIEN-3β-OL: A NEW STEROL FROM WITHANIA SOMNIFERA

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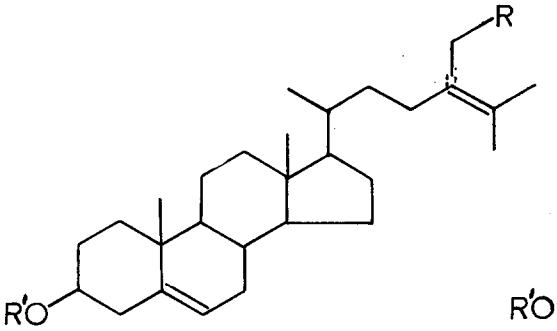
In connection with studies on the biosynthesis of the steroidal withanolides by Withania somnifera, the major sterols of the plant were investigated. We now report the isolation and characterisation of a new sterol (Ic) from this source. The 4-desmethyl sterols of Withania somnifera were isolated by extraction with methanol, saponification, column chromatography over alumina and t.l.c. on silica gel. The crude sterols were acetylated and the steryl acetates separated into four major bands by $AgNO_3$-silica gel t.l.c.. The major steryl acetate components of the various bands were identified by comparison of their g.l.c. retention times (OV-1 and OV-17 columns) and mass spectra with those of authentic steryl acetates (Table 1).

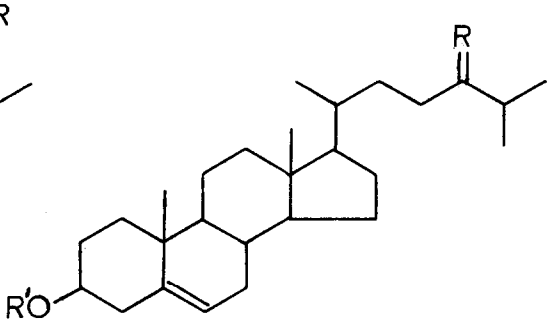
Table 1. Relative amounts of various steryl acetates separated by $AgNO_3$-silica gel/chloroform t.l.c.

BAND	APPROXIMATE R_f	MAJOR STERYL ACETATE(S)	APPROXIMATE %
1	0.43	24-ξ-methylcholest-5-en-3β-yl acetate	26
		24-ξ-ethylcholest-5-en-3β-yl acetate	19
2	0.24	24-ξ-ethylcholesta-5,22-dien-3β-yl acetate	9
3	0.16	Ia	7
		Ib	< 1
		Other uncharacterised steryl acetates	< 1
4	0.07	24-ethylcholesta-5,Z-24(28)-dien-3β-yl acetate	37

The mass spectrum of steryl acetate (1a) shows that it possesses a C_{28} skeleton with two double bonds [m/e. 380.345, 36%, M-HOAc; $C_{28}H_{44}$ requires m/e. 380.344] one of which is located in the side chain [peak at m/e. 253, 15%, M-HOAc-C_9H_{19}][1]. A prominent fragment ion at m/e. 296.251 [37%, M-HOAc-C_6H_{12}; $C_{22}H_{32}$ requires 296.250] further indicates that the side chain double bond is located either at C-24(28) or at C-24(25) in order to facilitate cleavage at the C-22-C-23 bond[1].

The C.A.T. accumulated N.M.R. spectrum[2] of Ia shows a six proton resonance at δ 1.61 and a three proton resonance at δ 1.50 corresponding to the presence of three vinylic methyl groups in the molecule. Further resonances at δ 2.26 (4βH), 2.30 (4αH) and 5.30 (W1/2 = 8Hz.;5H) are consistent with Δ^5 unsaturation, as are the positions of the C-18 and C-19 proton signals (δ 0.68 and 1.02, respectively)[3]. Other resonances are observed at δ 0.95 (C-21 protons, doublet J = 6Hz.), 2.01 (acetate protons) and 4.60 (3αH, multiplet). On the basis of the above data the steryl acetate was tentatively assigned the structure Ia.





(Ia) R=H, R'=CH_3CO
(Ib) R=CH_3, R'=CH_3CO
(Ic) R=H, R'=H

(IIa) R=O, R'=CH_3CO
(IIb) R=CH_2, R'=CH_3CO
(IIc) R=CH_2, R'=H

Synthetic 24-methylcholesta-5,24(25)-dien-3β-yl acetate (Ia) for comparison was prepared as follows. Wittig condensation between 3β-acetoxy-cholest-5-en-24-one (IIa) and methyltriphenylphosphonium iodide in the presence of n-butyllithium yielded a mixture of 24-methyl cholesta-5,24(28)-dien-3β-yl acetate (IIb) and the corresponding free sterol (IIc). Acetylation of this mixture gave IIb (36% yield from IIa). Isomerisation of IIb with iodine in benzene under reflux[4] yielded a mixture of dienes differing in the position of the side chain double bond. Separation of this mixture by $AgNO_3$-silica gel t.l.c. gave Ia, m.p. 144°

(30% yield from IIb). The synthetic and natural steryl acetates had identical retention times (RR_t[6]; OV-1 = 1.96, OV-17 = 2.16), mass spectra, I.R. spectra, and R_f values ($AgNO_3$-silica gel). In the N.M.R. spectrum of the synthetic steryl acetate (Ia; ca. 30mg./ml.) all three vinylic methyl groups resonate at δ 1.61. However, at lower concentrations (ca. 2mg/ml.) the spectrum is indistinguishable from that of the natural steryl acetate (Ia).

Since no isomerisation of IIb was observed under the chromatographic conditions used in our sterol extraction procedure, the possibility that Ia is an artefact[5] formed from IIb during isolation is unlikely.

Further g.l.c. analysis of band 3 showed the presence, in small amounts, of another steryl acetate with a longer retention time (RR_t[6]; OV-1 = 2.38, OV-17 = 2.51) than Ia. The material could not be separated by g.l.c. (OV-1, OV-17) from a sample of 24-ethylcholesta-5,24(25)-dien-3β-yl acetate prepared by isomerisation of fucosteryl acetate.[4] The steryl acetate may thus have the structure Ib.

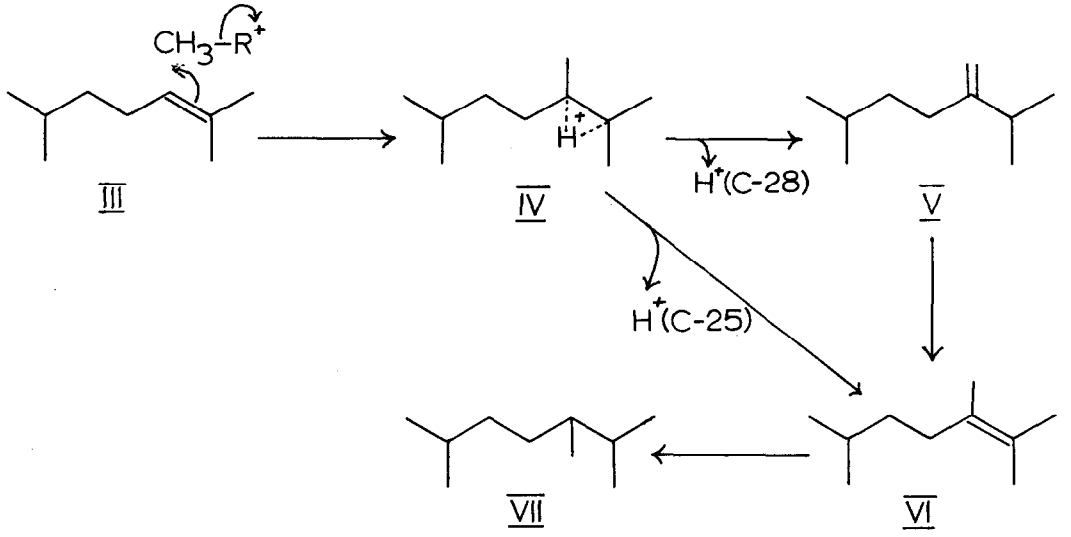


Fig.I

The only previous reported naturally occurring $\Delta^{24(25)}$ 24-alkyl sterol is 24-ethylcholesta-7,24(25)-dien-3β-ol, isolated from sunflower seed (Helianthus annuus) oil.[7] The occurrence of C-24 alkylated $\Delta^{24(25)}$ sterols is particularly noteworthy in view of their recent implication in certain mechanisms of C-24 alkylation during phytosterol biosynthesis (Fig. I).[8-11] Sterols possessing $\Delta^{24(25)}$ unsaturation may be precursors of C-24 alkyl sterols with

saturated side chains. Such $\Delta^{24(25)}$ unsaturation may arise by isomerisation of a $\Delta^{24(28)}$ bond (V ⟶ VI), as demonstrated for the corresponding C_{29} sterols in Hordeum vulgare.[11] Alternatively, it may arise directly by proton elimination from C-25 during stabilisation of the carbonium ion IV. If this route operates in vivo, the $\Delta^{24(25)}$ unsaturation is probably introduced at the 4,4-dimethyl sterol stage.[12] The 4,4-dimethyl and 4α-methyl sterols of Withania somnifera are currently under investigation. In the case of W. somnifera, it is possible that $\Delta^{24(25)}$ sterols may be precursors of the steroidal withanolides, many of which possess $\Delta^{24(25)}$ unsaturation.[13]

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