ISOLATION AND BIOGENESIS OF 24-ALKYLSTEROLS IN ALHAGI PSEUDALHAGI

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ABSTRACT

24-Alkyl sterols : 24-methyl cholest-5-en-3β-ol-24-ethyl cholest-5-en-3β-ol, 24-ethyl cholesta-5-22-dien-3β-cl, 24-ethyl cholesta-5-24 (28)-dien-3β-ol, 24-ethyl-5-cholest-7-en-3β-ol and Δ^7-avena sterol along with cholesterol have been isolated and characterised in the benzine extract of Alhagi Pseudalhagi. A probable biogenesis of these phytosterols in this plant has been suggested.

INTRODUCTION

ALHAGI Pseudalhagi is an annual herb, belongs to family Leguminoseae. The different parts of this plant are used to cure various ailments[1]. The present investigation was undertaken to isolate the phytosterols from the benzine extract of this plant and to suggest the probable biogenetic pathway to 24-alkyl sterols.

ISOLATION AND CHARACTERISATION

Benzine extract (1·8%) of the air-dried whole plant material was purified over a column of alumina and separated into four fractions over a column of silica gel. Rf values of these fractions were found to be 1·0, 0·90, 0·52 and 0·32 respectively using developer tetrachloromethane with 5% ethyl acetate. The last fraction (14·3%) of the purified extract was obtained on elution with light petroleum : benzene (1 : 4) which gave positive Liebermann-Burchard test and i.r. bands at 3400 cm^{-1} (OH), 1630 cm^{-1} (C=C) and (830 cm^{-1} C-trisubstituted), melting point ranges 125–135°C indicating it to be a sterol mixture. This sterol mixture was acetylated and spotted on a plate of silica gel impregnated with 20% silver nitrate solution and developed for five times in carbon tetrachloride : methylene chloride (5 : 1, v/v). This acetate was found to be a mixture of at least three sterols which were characterised as 24-methyl cholest-5-en-3β-ol (Rf. —0·7) 24-methyl cholesta-5-3β-ol (Rf. —0·65) and 24-ethyl cholesta-5-22-dien-3β-ol (Rf. 0·5) on the basis of co-argentative T.L.C. and was resolved by GLC for further characterisation and quantitative evaluation.

G.L.C. of the sterol acetate mixture was performed by a Shimadsu GC-4 gas chromatograph equipped with a flame ionisation detector. The chromatograph was fitted with a 30 m ScoT glass capillary column, 0·3 mm inside diameter packed with OV-17 on gas chrom-Z, 80–100 mesh. The column was operated at 260°C with nitrogen at 80 ml/min as carrier gas and split ratio 100 : 1, detector and injection temperature was 280°C. The cholesterol acetate was injected along with the sample and its retention time 5·47 min was taken as 1·0.

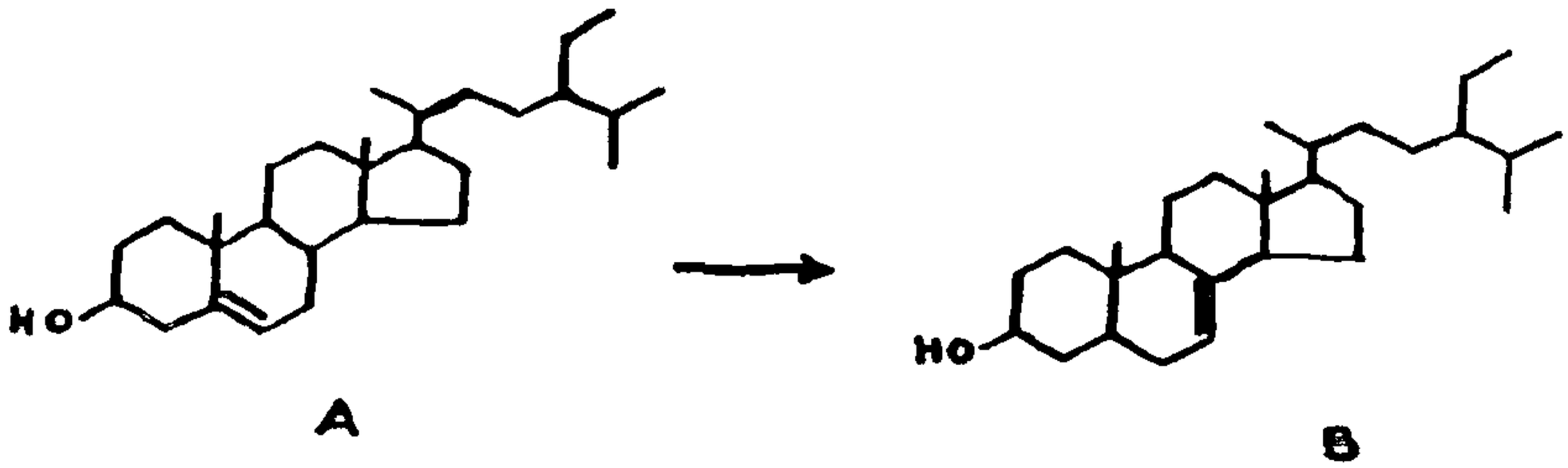
G.L.C. analysis revealed that this sterol was a mixture of seven sterols [24-methyl cholest-5-en-3β-ol, 24-ethyl cholest-5-en-3β-ol, 24-ethyl cholesta-5-22-dien-3β-ol, 24-ethyl cholesta-5-24 (28)-dien-3β-ol, 24-ethyl-5-cholest-7-en-3β-ol, Δ^7-avena sterol and cholesterol] identified as acetate on the basis of relative retention time (Table I) and comparison with the GLC graph of authentic samples run under similar conditions.

TABLE I

Sterols from benzine extract of Alhagi Pseudalhagi

Sl. No.	Sterol as acetate	RT[a]	RRT[b]	%
1.	24-methyl cholest-5-en-3β-ol	7·15	1·31	16·05
2.	24-ethyl cholest-5-en-3β-ol	8·96	1·64	36·32
3.	24-ethyl cholesta-5-22-dien-3β-ol	7·88	1·44	45·91
4.	24-ethyl cholesta-5-24 (28)-dien-3β-ol	9·96	1·82	1·58
5.	24-ethyl-5-cholesta-7-en-3β-ol	10·51	1·92	Tr
6.	Δ^7-avena sterol	11·80	2·16	Tr
7.	Cholesterol	5·47	1·0	Tr

[a] Retention time of sterol acetate.
[b] Retention time of cholesteryl acetate *ca.* 5·47 min was taken as 1·0.
[c] According to G.L.C. analysis.



Biogenesis

It is already known that three Δ^5 sterols : 24-ethyl cholest-5-en-3β-ol (β-sitosterol) [A], 24-methyl cholest-5-en-3β-ol and 24-ethyl cholesta-5-22-dien-3β-ol[2] form the major constituents of sterols in many plants along with cholesterol as minor component[3,4]. The presence of Δ^7 sterols : 24-ethyl cholest-7-en-3β-ol (stigmast-7-enol) [B] and Δ^7-avenasterol suggests the probable migration of double bond from Δ^5 to Δ^7 position.

Recently, it was reported that 24-alkylated $\Delta^{24[25]}$ sterols previously formed by isomerisation of $\Delta^{24[28]}$ sterols are reduced to 24-alkyl sterols in *Pinus pinea*[5]. But the occurrence of said biosynthetic precursors $\Delta^{24[28]}$ sterols [24-ethyl cholesta-5-22 (28)-dien-3β--ol (28-iso-fucosterol) [C] and Δ^7 avenasterol] and absence of $\Delta^{24[25]}$ sterols suggests that $\Delta^{24[28]}$ sterols are directly reduced to 24-alkyl sterols in this plant and *the finding seems to be of special interest from the viewpoint of the biogenesis of phytosterols.*

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