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DIRECT CYCLISATION OF SQUALENE TO 5α-STIGMAST-9(11)-EN-3β-OL VIA $\Delta^{9(11)}$LANOSTEROL IN *COSTUS SPECIOSUS* : A UNIQUE FINDING IN STEROL BIOSYNTHESIS

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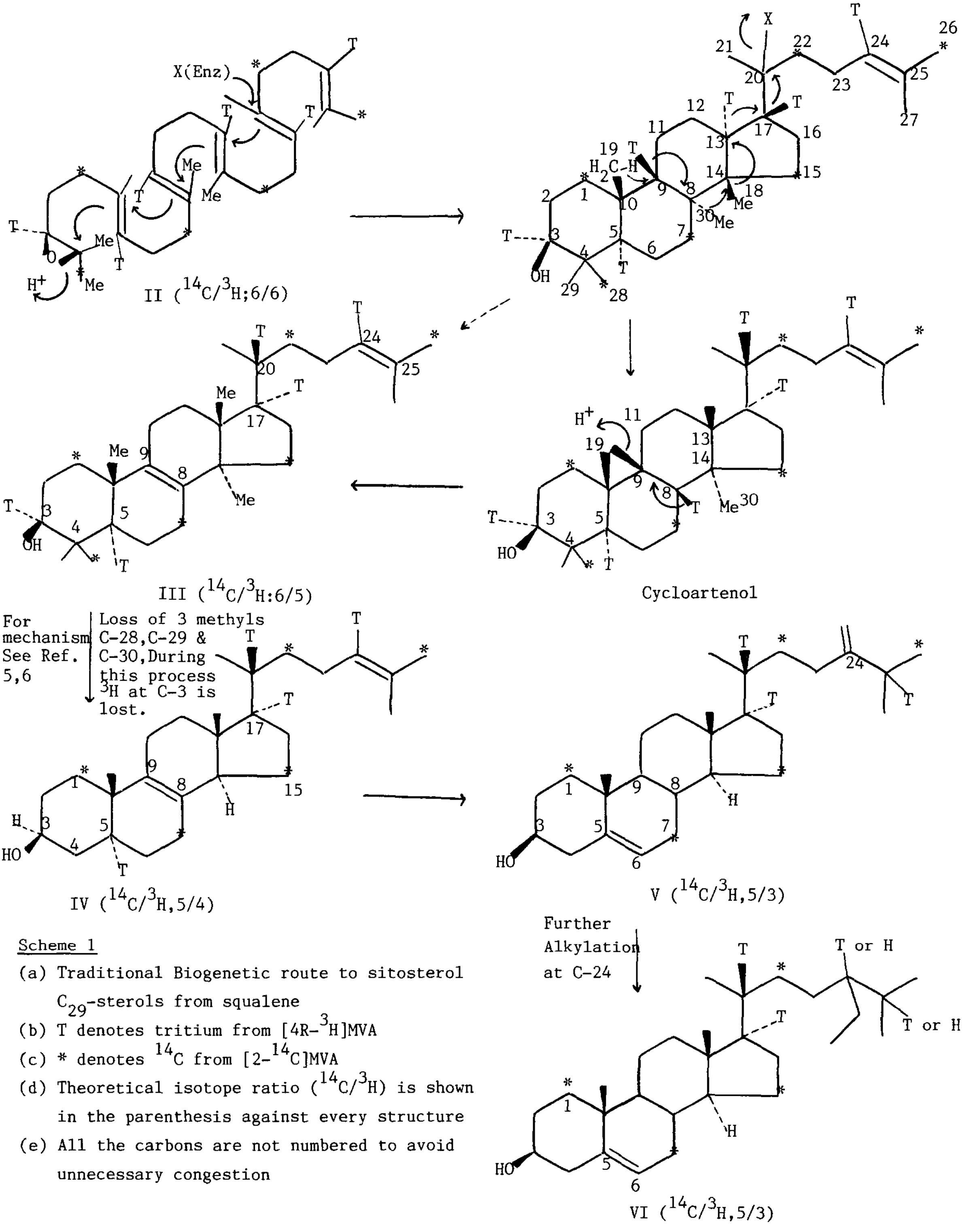
ABSTRACT : *Measurement of isotope ratios (^3H:^{14}C) in 5α-stigmast-9(11)-en-3β-ol (I), a C_{29}-sterol, biosynthesised in Costus speciosus from 3RS-mevalonate -[2-^{14}C,4R-^3H] have shown that squalene is possibly metabolised into I via $\Delta^{9(11)}$lanosterol (VII) rather than $\Delta^{8(9)}$lanosterol - - → zymosterol (IV) pathway (a regular feature of sterol biosynthesis).*

Earlier we have reported the occurrence of 5α-stigmast-9(11)-en-3β-ol(I) in C.speciosus[1] and the presence of $\Delta^{9(11)}$ bond generated interest in us to find out the biosynthetic mechanism of its formation. The presence of $\Delta^{9(11)}$ bond in C_{29}-sterols from higher plants is rarely encountered. There is one report of this type of compound i.e; indosterol from Seseli indicum[2].

The biosynthesis of C_{29}-sterols in higher plants has been studied by various workers[3,4] using mevalonic acid labelled with ^3H at different positions. However this is the first report on biosynthesis of a C_{29}-sterol having a $\Delta^{9(11)}$ bond. The normal biosynthetic pathway to various C_{29}-sterols is shown in Scheme 1[5,6] : squalene epoxide (II) is cyclised to $\Delta^{8(9)}$lanosterol (III) from where demethylation takes place (-3 methyls) to metabolize zymosterol (IV) ---→ 24-methylene cholesterol (V) which further alkylates to give sitosterol (VI) and various other sterols .

It is evident from the scheme 1 that ^3H from [2-^{14}C,4R-^3H]MVA (^{14}C/^3H; 1/1)[12] is incorporated at C-3, C-5, C-9, C-13, C-17 and C-24 whereas ^{14}C goes to C-1, C-7, C-15, C-22, C-26 and C-28 of squalene. It has been very well established that ^3H at C-3 and ^{14}C at C-28 are lost during demethylation of $\Delta^{8(9)}$lanosterol(III)[7]. The isotope ratio (^{14}C/^3H;1/0.996, crystalline derivative acetate m.p.99-101°, ketone m.p. 79-82°, specific activity 26 X 10^3 dpm/mmole) in I suggests that no ^{14}C or ^3H is lost from I. The only atoms which should be lost are ^{14}C at C-28 and ^3H at C-3 during the biosynthesis



Scheme 1

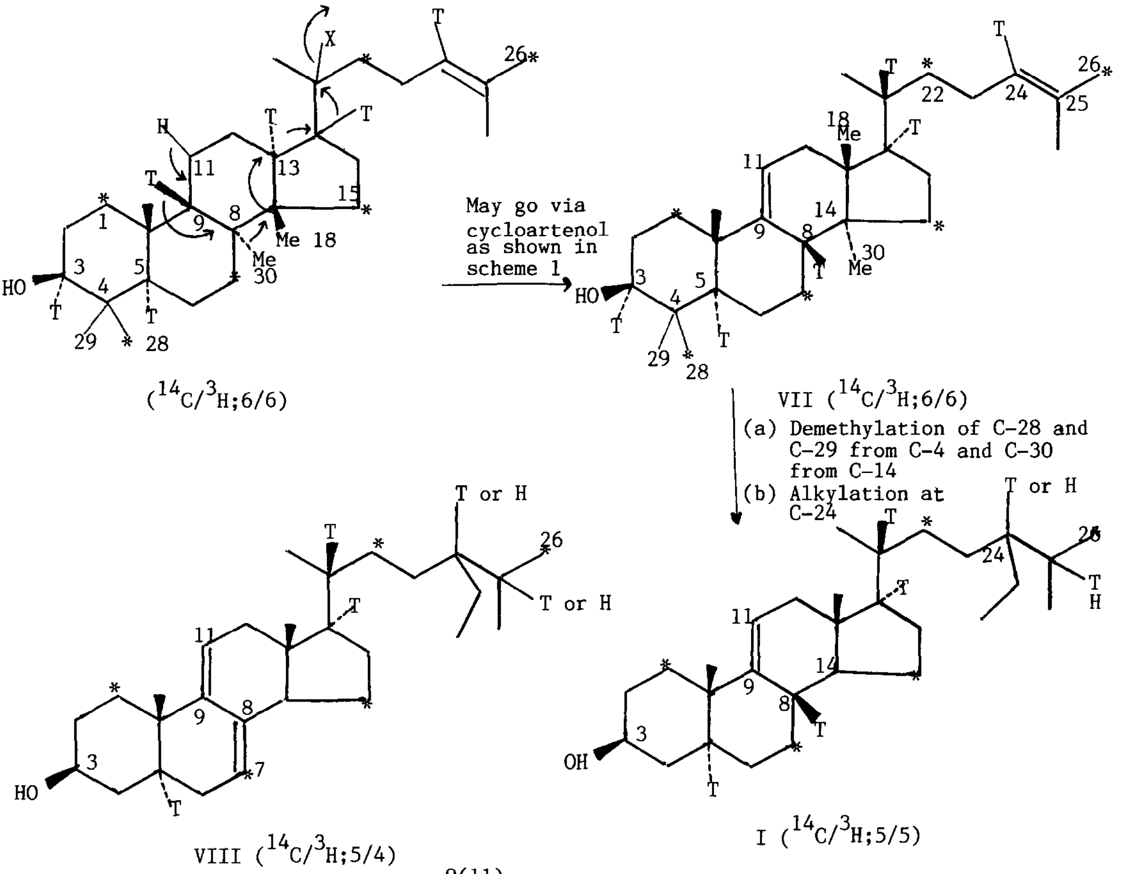
(a) Traditional Biogenetic route to sitosterol C_{29}-sterols from squalene

(b) T denotes tritium from [4R-^{3}H]MVA

(c) * denotes ^{14}C from [2-^{14}C]MVA

(d) Theoretical isotope ratio (^{14}C/^{3}H) is shown in the parenthesis against every structure

(e) All the carbons are not numbered to avoid unnecessary congestion



Scheme 2 : (a) Direct formation of $\Delta^{9(11)}$Lanosterol from squalene epoxide (II)

(b) T is ^{3}H from [4R-^{3}H]MVA and * is ^{14}C from [2-^{14}C]MVA

(c) Theoretical isotope ratio ($^{14}C/^{3}H$) is shown in the parenthesis against every structure

(d) Diene (VIII) is synthesised from I chemically to show the loss of ^{3}H from C-8

of this compound. Had it followed the route : II ⟶ III ⟶ IV ⟶ V ⟶ VI ⟶ ⟶ I, it would have definitely lost two or more ^{3}H atoms from C-3, C-5 or C-9. There was no ^{3}H at C-3 in I was confirmed by oxidising (Jone's oxidation) -OH at C-3 to carbonyl and the ketone thus obtained had the same isotope ratio ($^{14}C/^{3}H$;1/0.992) as in I. Had there been ^{3}H at C-3 it would have lost during oxidation. The most important finding which these results suggest is that the ^{3}H at C-9 is retained after being shifted to C-8. To confirm that ^{3}H at C-9 has shifted to C-8, I(25mg) was treated with $Hg(OAc)_2$(40mg) RT (stirring 48 h, prep.TLC C_6H_6 : Me_2CO ; 95 : 5) afforded diene[15](VIII) (sp.act. 754/12mg; $^{14}C/^{3}H$;1/0.813). This isotope ratio suggests loss of one ^{3}H from C-8 of I during the formation of VIII. This also suggests that the formation of $\Delta^{8(9)}$lanosterol (III) is very unlikely.

At the same time the ^{3}H at C-24 may or may not shift during the alkylation process and this needs further study[8]. But this is certain that ^{3}H at C-24 shall not be lost.After confirming by chemical means that ^{3}H at C-9 has shifted to C-8 this can be safely stated that the only mechanism possible for the biosynthesis of I (Scheme 2) is the loss of H from C-11 $\dashrightarrow$ formation of $\Delta^{9(11)}$bond $\dashrightarrow$ shifting of ^{3}H from C-9 to C-8 $\dashrightarrow$ shifting of CH_3 at C-8 to C-14 and so on in traditional fashion, finally giving rise to $\Delta^{9(11)}$lanosterol (VII) which after demethylation at C-4 and C-14 and alkylation at C-24 forms I.

For details of feeding methods, isolation of compounds, their derivatives, physical datas and radiochemical experiments see references[1,9,10,11,12,13,14,15,16]

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11. LKB Wallac Rack Beta II 1215/1216 Scintillation Counter was used for counting radioactivity. 40,000 disintegrations were accumulated to ensure that 2 was ± 1%.
12. MVA denotes mevalonic acid lactone which is hydrolysed to free acid before feeding. 3RS[2-^{14}C]MVA (sp.act. 53 mCi/mmole) and [3R,4R-$^{3}H_1$ + 3S,4S-$^{3}H_1$]MVA (sp.act. 1-3 Ci/mmole) (purchased from Radiochemical Centre, Amersham and BARC Bombay) was fed to the rhizomes of C.speciosus in appropriate amounts (5 µCi/100g of plant material; 3 plants were used; wt. of root/rhizome system 500 g) over 1 hr and the foliage was maintained on nutrient medium for 36-48 hrs before collection. The actual isotope ratio (^{3}H:^{14}C) in MVA fed was 1 : 1.013.
13. % incorporation of MVA was about 0.008 %.
14. The specific radioactivities in the text are expressed with respect to ^{14}C only.
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16. UV absorption of diene (VIII) λ_{max}^{nm} 232,238 and 248

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