Phytochemistry, Vol. 26, No. 10, pp. 2705–2707, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

1,2-HYDROGEN SHIFTS DURING THE BIOSYNTHESIS OF PATCHOULENES IN *POGOSTEMON CABLIN*

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(*Received* 10 *February* 1987)

Key Word Index—*Pogostemon cablin*; Labiatae; biosynthesis; sesquiterpenes; patchoulenes.

Abstract—The isotope ratio in α- and γ-patchoulenes in *Pogostemon cablin*, that has been fed with [2-^{14}C, 4*R*-$^{3}H_1$]MVA, suggests that a proton loss is followed by a 1,2-alkyl shift and two 1,2-hydrogen shifts during the biosynthesis of these two sesquiterpene hydrocarbons. Whereas isotope ratios in β- and δ-patchoulene suggests that a proton loss is followed by one 1,2-hydrogen shift in β-patchoulene and two 1,2-hydrogen shifts in δ-patchoulene.

The herb *Pogostemon cablin* Benth. is cultivated extensively in Indonesia, Malaysia, China and Brazil. The yield of essential oil varies from 1.8 to 3.0% depending upon the quality and maturity of leaves [1]. The non-Indian sample contains α-patchoulene (5.7%), β-patchoulene (3.0%), γ-patchoulene (0.7%), δ-patchoulene (0.68%), patchouli alcohol (31.2%), pogostol (2.0%), seychellene (8.6%), α-bulnesene (17.2%), caryophyllene (3.6%), α-guaiene (14.6%) norpatchoulenol (0.6%) and trace amounts of other epoxy and ketonic compounds [2, 3, 4]. The essential oil species cultivated in India has a very similar composition. Any systematic study on the biosynthesis of sesquiterpenes of this plant has not been carried out so far. However some preliminary reports on biosynthesis of terpenes in *Pogostemon cablin* have appeared [5, 6].

The biosynthesis of α-, β-, γ- and δ- patchoulene in *P. cablin* probably involves the biogenetic equivalent of *cis*-farnesylpyrophosphate (FPP, **5**) which undergoes cyclization **5** → **6** → **7** (Scheme 1). It appears that **7** is the probable intermediate for these patchoulenes. Theoretically, one ^{3}H should be lost from C-8 of **7** during the biosynthesis of α-(**1**), γ-(**3**) and δ-(**4**) patchoulene whereas two ^{3}H should be lost during the biosynthesis of β-patchoulene (**2**) from C-8 and C-12 of **7**. We have made attempts to establish the specific rearrangements and hydrogen shifts involved during the biosynthesis of these patchoulenes by feeding suitable doubly-labelled precursor to this plant.

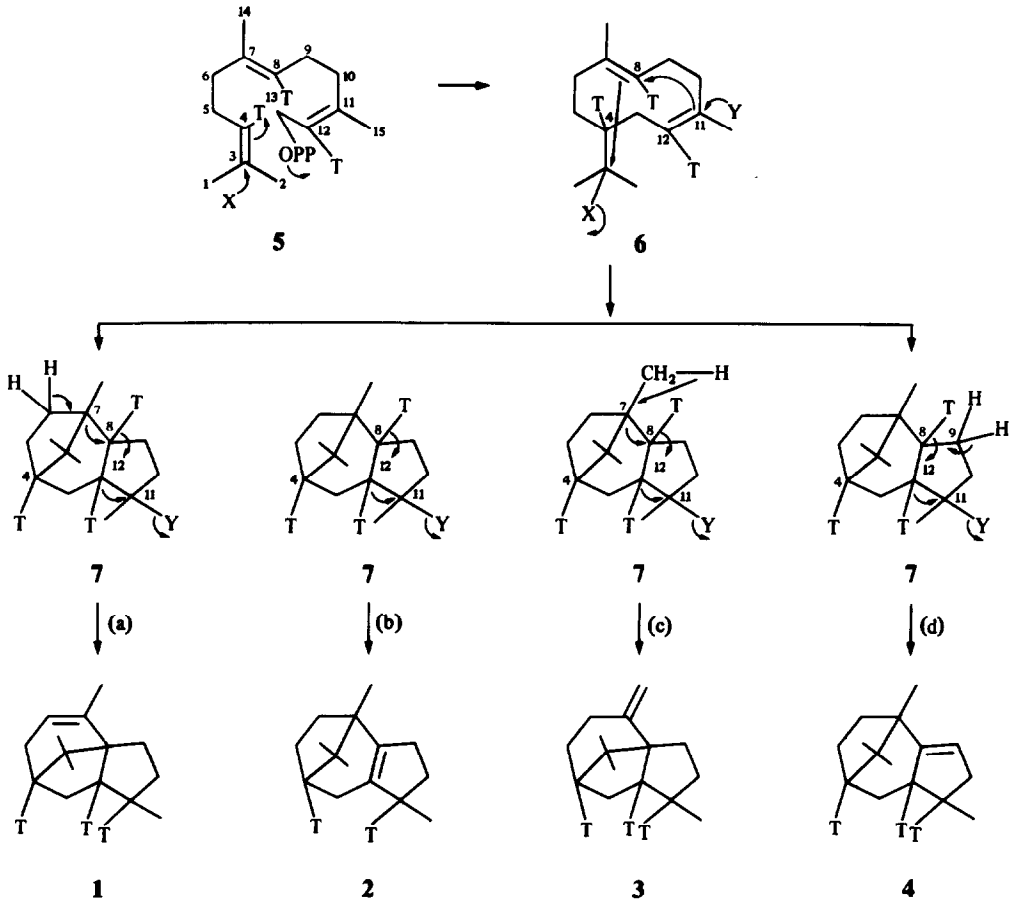
In general, *cis*-farnesylpyrophosphate (FPP, **5**) is the precursor for most of the bi- and tricyclic sesquiterpenes [7] and FPP is predominantly labelled at C-2, C-6 and C-10 when fed with [2-^{14}C]MVA in most of the reports available so far [8, 9]. Accordingly C-4, C-8 and C12 is labelled in FPP (**5**) if fed with mevalonic acid labelled at C-4 (Scheme 1). The reason for the particular choice of labelled precursor [4*R*-^{3}H, 2-^{14}C]MVA becomes apparent when a possible mechanism for the biosynthesis of patchoulenes is considered in *P. cablin* (Scheme 1).

The isotope ratio (^{3}H: ^{14}C; about 1:1; Table 1, Exps 1,3 and 4) in α-(**1**), γ-(**3**) and δ-patchoulene (**4**) suggests that no tritium is lost from any of the C-4, C-8 or C-12 carbons of FPP (**5**). This indicates that a 1,2-alkyl shift from C-7 to C-8 in **7** (route a) has taken place and ^{3}H from C-8 has shifted to C-12 consequently shifting ^{3}H from C-12 to C-11 and releasing the enzyme (Y) during the biogenesis of **1** (^{3}H: ^{14}C, 0.987:1; Table 1, Exp. 1) and **3**, route c (^{3}H: ^{14}C, 0.921:1, Table 1, Exp. 3). The proton at C-9 of **7** gets eliminated during the formation of $\Delta^{8(9)}$ (route d) and thus shifting ^{3}H at C-8 to C-12 and ^{3}H at C-12 to C-11 during the biogenesis of **4**. The isotope ratio (^{3}H: ^{14}C, 0.635:1) in β-patchoulene (**2**) suggests that one ^{3}H is lost either from C-8 or C-12 and this can be very well explained (route b, Scheme 1) if ^{3}H at C-8 is eliminated during the formation of $\Delta^{8(12)}$ consequently shifting ^{3}H at C-12 to C-11 and releasing the enzyme (Y) from C-11.

EXPERIMENTAL

The plants of *P. cablin* were grown in the experimental farm of CIMAP, Lucknow, India. DL[2-^{14}C]MVA lactone (sp. act. 53 mCi/mmol) and [3*R*, 4*R*-^{3}H + 3*S*, 4*S*-^{3}H]MVA lactone (sp. act. 1–3 Ci/mmol) were purchased from Radiochemical Centre, Amersham, England and BARC, Bombay. Young shoots of *P. cablin* were administered with labelled MVA [^{14}C and ^{3}H] using standard feeding methods [10, 11] and in each case 50 μCi of tracer was used. All incubations were carried out in April–May within 2–3 days. The leaf discs were steam distilled after 72 hr of administration of the radiosubstrate. Carrier essential oil (*ca* 1.5 ml) was added to the steam distilled material and it was subjected to column chromatography on Keiselgel 60 (Merck) columns to separate the hydrocarbon fractions.

The fractionation of hydrocarbons was carried out on $AgNO_3$–Keiselgel 60 (1:9) column (15 × 800 mm) packed in *n*-hexane. The column was eluted first with *n*-hexane (300 ml), then



Scheme 1. (a) X-group mechanism for the biosynthesis of various patchoulenes from a common intermediate 7. (b) X and Y represent enzymes or its biogenetic equivalent. (c) T denotes tritium from [4*R*-$^3H_1$]MVA.

with 200 ml each of 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60% and 75% Et_2O–*n*-hexane; Et_2O (200 ml) and MeOH (200 ml). Fractions of 10 ml each were collected. The fractions containing α, β, γ, and δ-patchoulenes were purified by prep. GLC: 10% polypropylene glycol sebacate (150 × 0.63 cm) column, temp. programmed 80–220° at 5°/min; argon 50 ml/min; inject. temp. 175°; detect. 240°. The purified compounds thus obtained were counted for their radioactivity by liquid scintillation spectrometry [10, 11].

Table 1. Incorporation of doubly-labelled mevalonate into patchoulenes by *P. cablin*

Exp. No.	Sesquiterpene	Precursor [4*R*-^{3}H, 2-^{14}C]MVA	Isotope ratio (^{3}H:^{14}C)* Patchoulenes
1.	α-Patchoulene (1)	1:1	0.987:1
2.	β-Patchoulene (2)	1:1	0.635:1
3.	γ-Patchoulene (3)	1:1	0.921:1
4.	δ-Patchoulene (4)	1:1	0.936:1

* Maximum incorporation of tracer (about 25×10^{-4} %) was observed after 48 hr of feeding.

Acknowledgements—We wish to thank Dr Akhtar Husain, Director, CIMAP for his keen interest during the course of this work. One of us (PKS) thanks CSIR, New Delhi for the award of fellowship.

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