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BIOSYNTHETIC RELATIONSHIPS OF PATCHOULI ALCOHOL, SEYCHELLENE AND CYCLOSEYCHELLENE IN *POGOSTEMON CABLIN*

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Key Word Index—*Pogostemon cablin*; Labiateae; biosynthesis; sesquiterpenes; patchouli alcohol; seychellene; cycloseychellene.

Abstract—The $^{3}H/^{14}C$ isotope ratios in patchouli alcohol seychellene and cycloseychellene isolated from *P. cablin* that was fed with [4–$^{3}H_1$ (4R)^{14}C]MVA suggests that these three sesquiterpenes are biosynthesized from a common intermediate and they are not interconvertible within the plant system. The results reveal that a stereospecific 1,2-methyl shift takes place during the formation of seychellene and cycloseychellene *in vivo*.

INTRODUCTION

The essential oil obtained from the herb *P. cablin* Benth. contains caryophyllene, α-, β-, γ- and δ- patchoulene, patchouli alcohol (**1**), pogostol, seychellene (**2**), cycloseychellene (**3**), α- and β-bulnesene, α- and δ-guaiene, norpatchoulenol and trace amounts of other epoxy and ketonic compounds [1–4]. In continuation to our studies [5] on the biosynthesis of sesquiterpenes present in the essential oil of this plant we now report our findings on **1–3**.

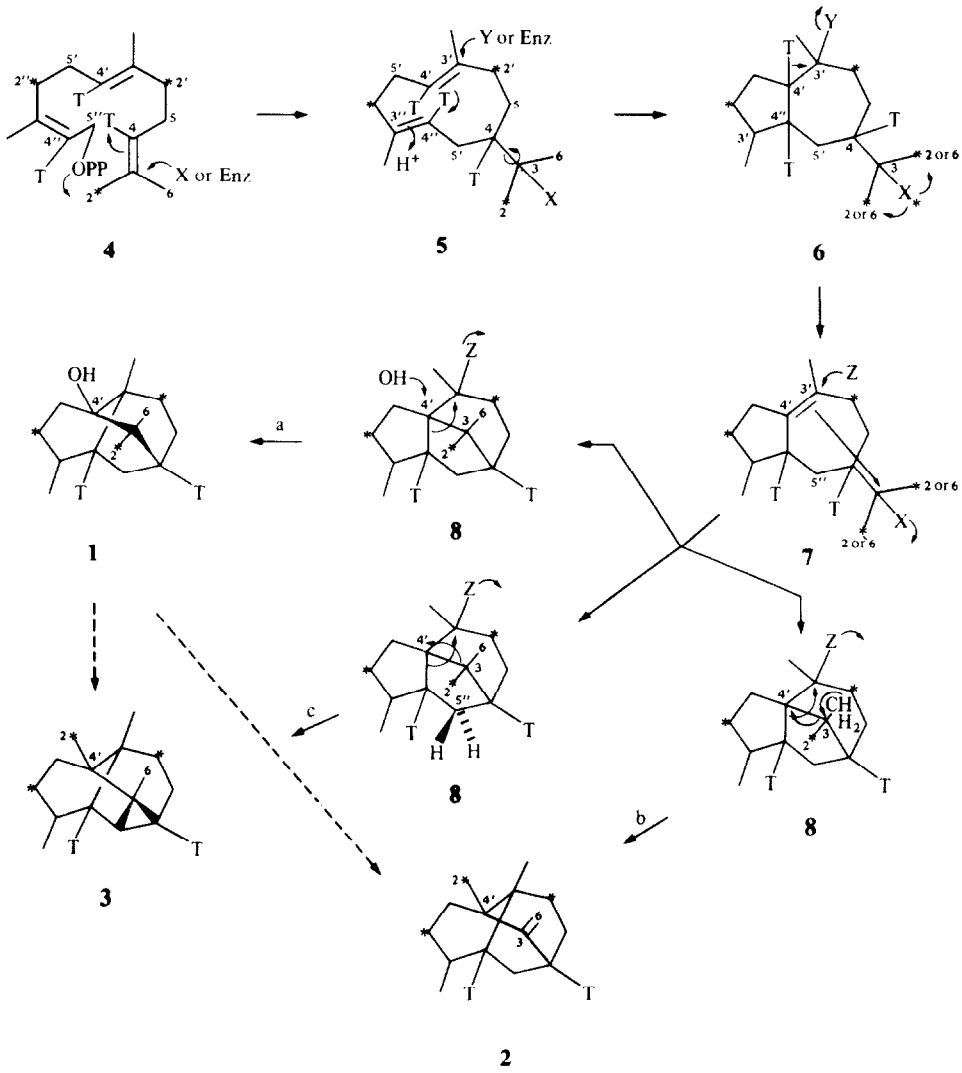
The biosynthesis of **1–3** probably involves the biogenetic equivalent of *cis*-farnesyl pyrophosphate (FPP, **4**, the 2*Z*-isomer) which undergoes cyclization **4**→**5** (Scheme 1). We have proposed below a biogenetic pathway from the enzyme bond intermediate (**5**) for the metabolism of **1**, **2** and **3**. Electrons from the $\Delta^{3'(4')}$ bond may attack at C-4″ and force the $\Delta^{3''(4'')}$ -bond to accept H^+ from the medium and give rise to **6** (Scheme 1). Later on through nucleophilic attack by enzymes, proton losses, intramolecular attacks by double bonds on electron deficient centres and rearrangements, **6** may cyclize to **8** (**6**→**7**→**8**), which could be the probable intermediate for **1**, **2** and **3**. This enzyme bonded intermediate (**8**) may metabolize **1**, **2** and **3** by separate routes (*a*, *b* and *c*, Scheme 1) or there may be some biosynthetic relationship between **1**, **2** and **3**. In order to elucidate this problem we have chosen [4-$^{3}H_1$(4*R*),2-^{14}C]MVA as the best precursor. The reason for choosing this precursor becomes apparent when possible mechanisms for the biosynthesis of **1–3** are considered.

RESULTS AND DISCUSSION

In general FPP (**4**) is labelled at C-2, C-2′, C-2″ (Scheme 1) and C-4, C-4′ and C-4″ when fed with MVA labelled at C-2 and C-4 respectively [6, 7]. There are some reports [8] that geranyl pyrophosphate (GPP) and FPP are asymmetrically labelled owing to the presence of a 'metabolic pool' of DMAPP and that the moeity of GPP and FPP derived from DMAPP gets appreciably diluted when [2-^{14}C]MVA is fed to the higher plants for studying terpene biosynthesis. In such a case C-2, C-3, C-4, C-5 and C-6 may not bear a tracer from exogenously supplied [2-^{14}C]MVA. In order to confirm this we have isolated α-guaiene (**9**, sp. act. 2172 dpm/mmol) β-bulnesene (**10**, 1976 dpm/mmol) and caryophyllene (**11**, 2456 dpm/mmol after feeding [2-^{14}C]MVA to the shoots of *P. cablin*. α-Guaiene (**9**) was degraded to **13** (not isolated) and formic acid (crystallized as *S*-benzyl isothiouronium salt, sp. act. 372 dpm/mmol, Scheme 2) which showed *ca* one-sixth of the sp. act, of **9** indicatng that some scrambling of *gem*-methyls has occurred and DMAPP derived from [2-^{14}C]MVA has been incorporated into FPP and at later stages of biosynthesis. These results were further supported when β-bulnesene (**10**, sp. act. 1976 dpm/mmol) was degraded to **14** (not isolated) and acetone (crystallized as semicarbazone, sp. act. 673 dpm/mmole) which possessed *ca* one-third of the total sp. act. in **10**. Caryophyllenic acid (**15**, sp. act. 832 dpm/mmol) from caryopheyllene (**11**, sp. act. 2456 dpm/mmol) also possessed about one-third of the total sp. activity of **11** (Table 1).

The $^{3}H/^{14}C$ isotope ratio of about 2/3 in compounds **1**, **2** and **3** (Expt. 1,2 and 3 respectively, Table 2) suggests that at least one ^{3}H is lost in all these three sesquiterpenes. This indicates that ^{3}H at C-4′ is lost either during the formation of the $\Delta^{3'(4')}$ -bond in **7** from **6** (this may be an enzyme bonded species) or by a nucleophilic attack at C-3 releasing the enzyme X and formation of **8**.

Compound **8** seems to be the common intermediate for **1**, **2** and **3**, which may be formed by three different mechanisms. (i) An OH^-(enzyme or its biogenetic equivalent) may attack at C-4′, giving rise to a skeleton rearrangement and releasing the enzyme Z, eventually giving rise to patchouli alcohol (**1**). (ii) A proton loss from methyl (C-2 or C-6, bridge methyls) leads to formation of a methylene group followed by a shift of the C-2 or C-6 methyl to C-4′ and skeleton rearrangement and formation of seychellene (**2**). (iii) Nucleophilic attack at C-3 by the electron cloud at C-5″ (Scheme 1) leads to a 1,2-methyl shift (C-2 or C-6, bridge methyls) from C-3 to



Scheme 1. X-Group mechanism for the biosynthesis of patchouli alcohol (**1**), seychellene (**2**) and cycloseychellene (**3**). X, Y, Z represent enzymes, phosphate esters or their biogenetic equivalents. T denotes tritium (^3H) from [4*R*-^3H]MVA; *denotes ^{14}C from [2-^{14}C]MVA

C-4′ and formation of cycloseychellene (**3**). All these three biogenetic routes will lead to a $^3H/^{14}C$ isotope ratio of about 2/3 in compounds **1**, **2** and **3**.

However, patchouli alcohol (**1**) may also act as a precursor for **2** and **3** (as shown in Scheme 1). In order to confirm this we had fed [^{14}C] patchouli alcohol (1415 dpm/mmol) to the plant and isolated **2** and **3** after an appropriate time [9] and found that radioactivity from **1** was not incorporated into **2** and **3**, thus eliminating the possibility of **1** being converted into **2** and **3** *in vivo*. At this stage one must take into consideration that authentic precursors, especially water insolubles such as patchouli alcohol, find difficulty in reaching the biosynthetic sites which are normally well compartmented. However, it can not be said with full certainty that this is why patchouli alcohol (**1**) is not converted into **2** or **3**.

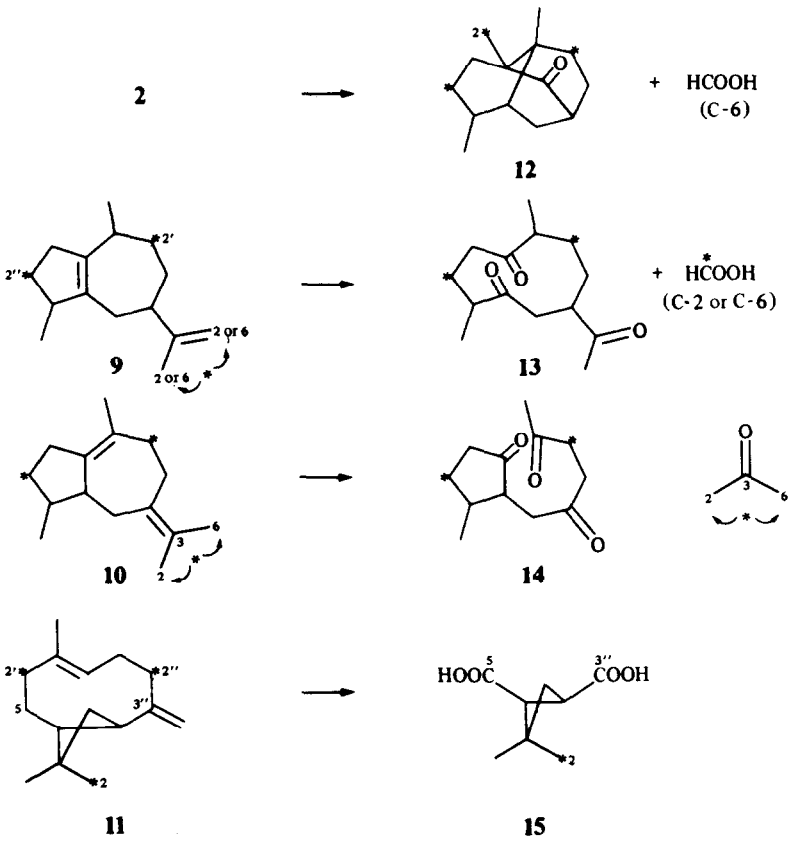
We have also carried out an additional experiment to check which of the bridge methyls (C-2 or C-6) is shifted to C-4′ during the formation of **2** and **3** from **8**. Seychellene (**2**, 1746 dpm/mmol) was oxidatively degraded to seychellenone (**12**, 1712 dpm/mmol, Scheme 2, Table 1) and the methylenic carbon thus cleaved as formic acid (*S*-benzyl isothiouronium salt 124 dpm/mmol) did not possess any radioactivity above the background count. This suggests that the methyl (i.e. the C-2 methyl) which possessed ^{14}C has exclusively shifted to C-4′ during the formation of **2** and possibly **3** and the *gem*-methyls of FPP (**4**) do not loose their identity during the cyclization process (**4** →**5** →**6** →**7** →**8**). It seems to be a spontaneous process and free rotation along the C-3/C-4 axis is not possible.

EXPERIMENTAL

The plants of *P. cablin* were grown in the experimental farm of CIMAP, Lucknow, India. DL[2-^{14}C]MVA lactone (sp. act

Table 1. Degradation products and their specific radioactivities in dpm/mmol from the sesquiterpene hydrocarbons obtained from *P. cablin* after feeding [2-^{14}C]MVA

Exp. no.	Sesquiterpene	Sp. act. dpm/mmol	Degraded product	Sp. act. dpm/mmol
1.	Seychellene (**2**)	1746	Seychellenone (**12**)	1712
			Formic acid (C-2 or C-6)	124
2.	α-Guaiene (**9**)	2172	**13** (not isolated)	—
			Formic acid (C-2 or C-6)	372
3.	β-Bulnesene (**10**)	1976	**14** (not isolated)	—
			Acetone (C-2 + C-3 + C-6)	673
4.	Caryophyllene (**11**)	2456	Caryophyllenic acid (**15**) (C-2 + C-3 + C-4 + C-5 + C-6 + C-3″ + C-4″ + C-5″)	832



Scheme 2. Degradation products from seychellene (**2**), α-guaiene (**9**), β-bulnesene (**10**) and caryophyllene (**11**).

Table 2. Incorporation of doubly-labelled mevalonate into patchouli alcohol (**1**), Seychellene (**2**) and Cycloseychellene (**3**) by *P. cablin*

Exp. no.	Sesquiterpene	Precursor [4-^{3}H(4R), 2-^{14}C]MVA	Isotope ratio (^{3}H:^{14}C)* Sesquiterpenes
1.	Patchouli alcohol (**1**)	0.987:1	0.632:1
2.	Seychellene (**2**)	0.987:1	0.643:1
3.	Cycloseychellene (**3**)	0.987:1	0.637:1

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

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 and BARC, Bombay. Young shoots of *P. cablin* were administered with labelled MVA [^{14}C and ^{3}H] using standard feeding methods [8, 9] and in each case 50 μCi of tracer was used. All incubations were carried out in April 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 CC on Keiselgel 60 (Merck) columns to separate the hydrocarbons **2**, **3**, **9**, **10** and **11**.

The fractionation was carried out on $AgNO_3$–Keiselgel 60 (1:9) column (15 × 800 mm) packed in *n*-hexane. The column was first eluted with *n*-hexane (300 ml), then with increasing amounts of Et_2O. Fractions of 10 ml each were collected [5]. Fractions containing seychellene (**2**), cycloseychellene (**3**), α-guaiene (**9**), β-bulnesene (**10**) and caryophyllene (**11**) were further purified by prep. GLC (10% polypropylene glycol sebacate (150 × 0.63 cm) column temp. programmed 80–200° at 5°/min; argon 50 ml/min.; inj. temp. 175°; detector 240°. The purified compounds possessed the same spectroscopic data as given in the literature [11–14]. After the elution of hydrocarbons, patchouli alcohol (**1**) was eluted with 10% benzene in petrol and crystallized (mp 38–39°) [3, 15]. Seychellene (**2**) was converted to seychellenone (**11**) by known methods and confirmed with spectral data (11, 16). The purified compounds thus obtained were radioassayed by liquid scintillation spectrometry [8, 10]. α-Guaiene and β-bulnesene were degraded with KIO_4–$KMnO_4$ or O_3 [17] to yield formic acid and Me_2CO, respectively. Compounds **13** and **14** thus formed were not isolated. Similarly caryophyllene (**11**) was degraded to caryophyllenic acid as described [18]. Acetone and formic acid were converted to semicarbazone and s-benzyl isothiouronium salt and crystallized as these solid derivatives. The samples for radioassay contained 2000–3000 dpm as ^{14}C and up to 20000 dpm as ^{3}H; 40000 disintegrations were accumulated to ensure that 2σ was ±1%. All the experiments were duplicated.

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