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1,2-HYDROGEN SHIFTS IN THE FORMATION OF THE SESQUITHUJANE SKELETON IN GINGER RHIZOMES

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Abstract—The isotope ratio [$^3H/^{14}C$] in sesquisabinene, sesquithujene, and *cis*-sesquithujene hydrate biosynthesized in ginger rhizomes from [2-^{14}C, 4*R*-^3H]MVA suggests that (a) the three sesquiterpenes have been formed from a common intermediate and (b) a 1,2-hydrogen shift takes place during the formation of the cyclopropane ring of the sesquithujane skeleton.

INTRODUCTION

The herbaceous perennial *Zingiber officinale* Rosc., is grown commercially in most tropical regions for its rhizomes, which are valued for their aroma and pungency. Steam distillation of semi-dried ginger rhizomes gives a pale yellow to light-amber oil in 1.5 to 3.0% yield depending upon the quality of the oil [1]. The oil consists of mainly mono- and sesqui- terpene hydrocarbons such as α- and β-zingiberene, β-bisabolene, ar-curcumene, α-selinene, β-elemene, β-farnesene, sesquithujene and sesquisabinene. In addition, it contains some oxygenated mono- and sesquiterpenes, one of which, *cis*-sesquithujene hydrate (**3**), is the subject of this communication [2-5]

The common structural features shared by compounds **1–3** with their monoterpene counterparts (sabinene, thujene and thujene hydrate respectively), in particular the presence of a cyclopropane ring, led us to determine if the biogenesis of **1–3** proceeds in a similar fashion as in their monoterpene counterparts [6]. Ruzicka's hypothetical scheme [7] for the biogenesis of the sesquithujene series of sesquiterpenes requires a 1,2-hydrogen shift (**5**→**6**, Scheme 1) to produce a cyclopropane ring Theoretically the scheme is correct in outline but a hydrogen shift has never been demonstrated for any sesquiterpene with this type of skeleton, although such 1,2-hydrogen shifts are well established in the formation of many other sesquiterpenoids [8, 9].

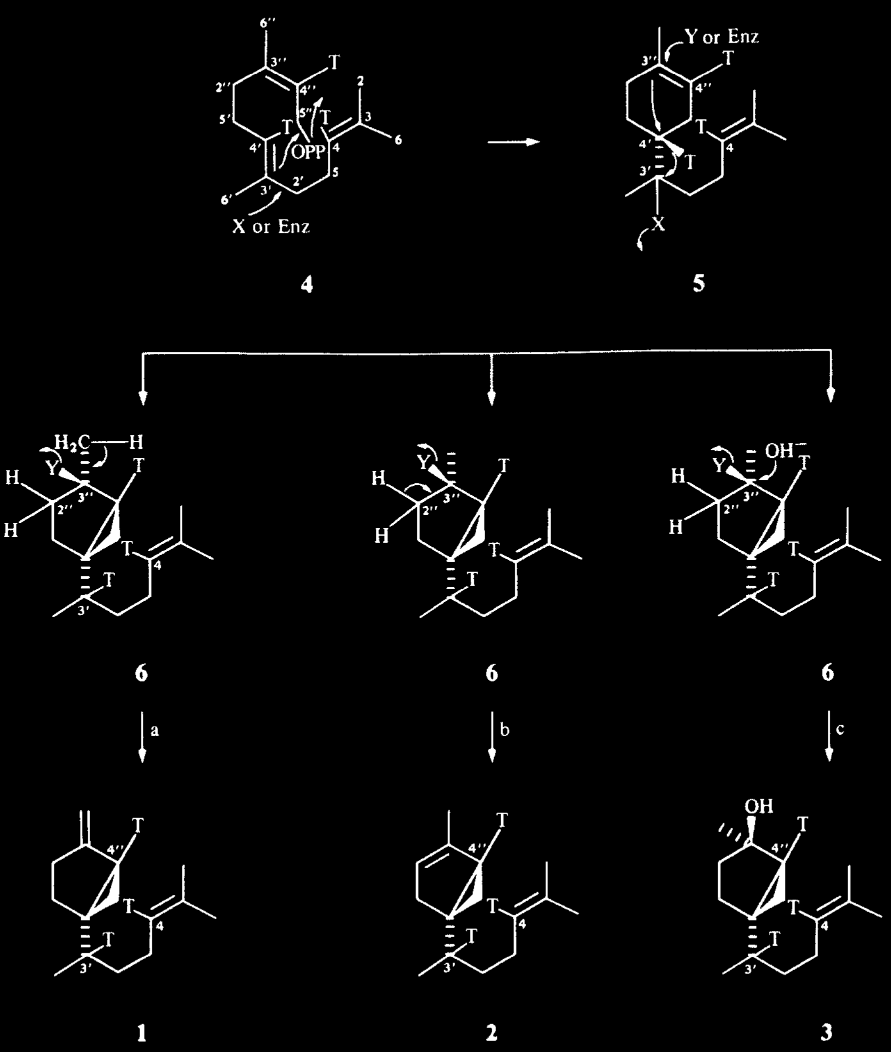
In this piece of work, we have tried to produce unequivocal evidence for such 1,2-hydrogen shifts. In general, *cis*-farnesyl pyrophosphate (FPP, **4**) is the notional precursor for most of the bi- and tricyclic sesquiterpenes. FPP is predominantly labelled at C-2, C-2′ and C-2″ when labelled biogenetically from [2-^{14}C]MVA, and at C-4, C-4′ and C-4″ when labelled from [4*R*-$^3H_1$]MVA [10, 11]. It followed therefore, that [2-^{14}C, 4*R*-$^3H_1$]MVA was the ideal precursor for studying the possible mechanism for the formation of the skeletons of compounds **1–3** (see Scheme 1).

RESULTS AND DISCUSSION

The atomic ratio ($^3H/^{14}C$) of about 1 (Table 1) found for compounds **1–3** biosynthesized from [2-^{14}C, 4*R*-$^3H_1$]MVA suggests that no tritium is lost in the course of their formation from FPP (**4**) and indicates that a 1,2-hydrogen shift has taken place from C-4′ to C-3′ (Scheme 1). A full biogenetic sequence can be suggested to account for this migration. A nucleophilic attack by the appropriate enzyme X on C-3′ of **4** initiates the process and cyclization of **4** to **5** takes place as shown in Scheme 1. Another enzyme Y attacks at C-3″ leading to formation of a C-4″–C-4′ bond, the migration of ^3H from C-4′ to C-3′ and the release of enzyme X. This gives an enzyme bound species (**6**) which seems to be the common intermediate for **1–3**. Loss of the proton from the methyl group attached to C-3″ with subsequent release of enzyme Y and formation of an exomethylene group produces **1**. A proton loss from C-2″ followed by release of enzyme Y and formation of a $\Delta^{2''(3'')}$ double bond gives **2**. Hydroxyl (or its biogenetic equivalent) attack at C-3″ followed by release of enzyme Y gives rise to **3**. All three sesquiterpenes thus produced retain the three ^3H atoms present in FPP.

EXPERIMENTAL

Z officinale was grown on the experimental farm of CIMAP, Lucknow, India (*R*,*S*) [2-^{14}C]MVA lactone (53 mCi/mmol) and [3*R*, 4*R*-^3H + 3*S*, 4*S*-^3H]MVA lactone (1-3 Ci/mmol) were purchased from the Radiochemical Centre, Amersham, England and Bhabha Atomic Research Centre, Bombay The shoots were *ca* 30 cm long and rhizomes weighed 50–100 g at the time of feeding. The plants were dug out of the ground, the rhizomes washed free of soil and immersed in an soln of radioactive MVA which had been earlier hydrolysed to its free acid with aq. $NaHCO_3$ by known methods [12, 13]. The rhizomes were left under natural conditions to allow the uptake of the substrate on sterile and bacteria free medium [14] In all 6 plants were taken and their



Scheme 1. Possible X-group mechanism for the formation of compounds **1**–**3** from FPP. T denotes ^3H from [2-^{14}C, 4*R*-$^3H_1$]MVA. The numbering system follows that of MVA. C-1 of MVA is lost during the formation of IPP.

Table 1. Incorporation of doubly labelled mevalonate into sesquiterpenes by ginger rhizomes

Substrate or sesquiterpene	^3H (dpm)	^{14}C (dpm)	Isotope ratio (^3H:^{14}C)	Atomic ratio (^3H:^{14}C)
[2-^{14}C,4*R*-$^3H_1$]MVA		-	6.23	1:1
Sesquisabinene (**1**)	1656	275	6.02	0.97:1
Sesquithujene (**2**)	1448	242	5.98	0.96:1
cis-Sesquithujene hydrate (**3**)	1236	201	6.13	0.98:1

Maximum incorporation of radioactivity (*ca* 25×10^{-4}%) was observed some 48 hr after administration of the labelled substrate.

rhizomes weighed about 225 g. 20 μCi as ^{14}C of a mixture of [2-^{14}C]- and [4*R*-$^3H_1$]- MVA was fed to each plant. After 48 hr the rhizomes were washed free of substrate, excised from the rest of the plant, semi-dried and steam distilled. Carrier essential oil (*ca* 1.5 ml) was added to the steam distilled material and the mixture subjected to CC on $AgNO_3$–Keiselgel 60 (1:9) (15 × 800 mm packed in *n*-hexane). The column was eluted with *n*-hexane (300 ml), then 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 sesquisabinene (**1**), sesquithujene (**2**) and *cis*-sesquithujene hydrate (**3**) were worked-up and pure compounds assayed for ^{14}C and ^{3}H by liquid scintillation spectrometry [12, 13].

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