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BIOSYNTHESIS OF BAKUCHIOL, A MEROTERPENE FROM *PSORALEA CORYLIFOLIA*

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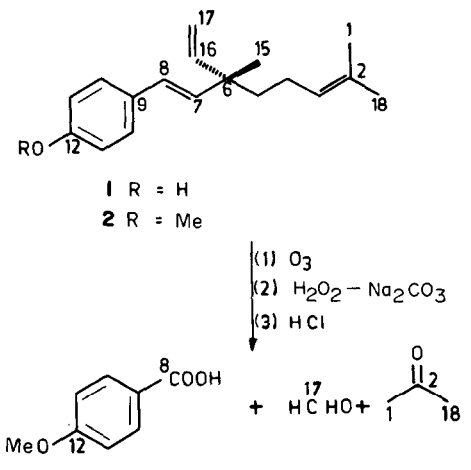
Key Word Index—*Psoralea corylifolia*; Leguminosae; bakuchiol; biosynthesis; meroterpene.

Abstract—Biosynthesis of bakuchiol was examined using phenylalanine and mevalonic acid as substrates. It has been demonstrated that bakuchiol is derived from one phenylpropane (with the loss of one carbon atom) and two isoprenoid (C_5) units.

INTRODUCTION

Many natural products are of mixed biogenetic origin and are derived by the condensation of isoprenoid units with non-terpenoidal moieties [1, 2]. Bakuchiol (**1**), isolated from the hexane extract of the medicinally important plant, *Psoralea corylifolia* (Leguminosae) belongs to this group [3]. In addition to reported antimicrobial activity [3, 4], bakuchiol also exhibits insect juvenile hormone (JH) properties and is more potent than the naturally occurring JH mimic, juvabione [5]. Inspection of structure **1** suggests that 10 (i.e. carbon atoms 1–6 and 15–18) out of 12 carbon atoms of the side chain are isoprenoid in nature. The aromatic ring, along with the two-carbon side chain (i.e. carbon atoms 7 and 8) may be considered to be derived from a phenylpropane unit or from a polyketide chain. However, oxygenation at C-12 would favour the former possibility. Thus, it was of interest to study the biogenetic origin of **1**. Biosynthesis of bakuchiol using phenylalanine and mevalonolactone as precursors is reported here.



RESULTS AND DISCUSSION

Though the chemical contents of the seeds of *P. corylifolia* have been examined extensively [6–8], not much work has been reported on other parts of the plant. In the present investigation, stem, leaves, seeds (both mature and immature) and roots have been examined separately for the presence of **1** and other components [9]. Results of our investigations show that **1** is present in all the parts of the plant and at all the stages of development. The content of **1** was maximum (5.45%) in the seeds while the roots contain only traces of **1**. Mature plants (8–10 weeks old) were used for the biosynthetic experiments. The optimum time for harvesting the treated plants (72 hr) was found by determining the incorporation efficiencies of sodium [2-^{14}C]acetate at different intervals of time. Bakuchiol (**1**) was isolated from the ether extract of the whole plant (excluding roots) by repeated prep. TLC. Bakuchiol (**1**) was transformed into its methyl ether (**2**) by the action of methyl iodide in the presence of sodium hydride. For the location of the labels in the radioactive products, **2** was ozonized and formaldehyde was collected. *p*-Anisic acid and acetone were obtained by the oxidative work-up of the ozonized product [3].

Bakuchiol methyl ether (**2**), prepared from biosynthesized **1** from L-[U-^{14}C]phenylalanine on degradation gave radioactive *p*-anisic acid which carried 87% of radioactivity of the parent compound. In the degradation of biosynthesized **2** to *p*-anisic acid, there is loss of one labelled carbon atom (i.e. C-7) out of eight labelled carbon atoms. Taking this into account it amounts to almost complete specific incorporation of the label. Other degradation products, namely formaldehyde and acetone, did not contain a significant amount of labels. The specific incorporation of phenylalanine into the aromatic ring and carbon atoms 7 and 8 has, thus, established the phenylpropanoid origin of the non-isoprenoid part of **1**. The carboxyl carbon of phenylalanine is presumably lost during the biosynthesis.

Loss of a carboxyl group during the biosynthesis of bakuchiol from phenylalanine has been shown by the experiments where DL-[4-^{3}H, 1-^{14}C] phenylalanine (^{14}C:^{3}H = 0.72:1) was used. The change in the ratio of ^{14}C and ^{3}H to 0.09:1 in **1** indicates a loss of 87% of radioactivity of ^{14}C. This indicates that the carboxyl carbon of phenylalanine is lost during the biosynthesis. Retention of ^{3}H-label from [4-^{3}H]phenylalanine in the bakuchiol molecule due to 'NIH shift' has been shown earlier [9].

Information about the biosynthesis of the side chain

Table 1. Specific activities of bakuchiol, its methyl ether and degradation products

Substrate (sp. act.)	Activity administered (dpm)	% incorporation in **1**	Bakuchiol (**1**) (dpm/mM)	Bakuchiol methyl ether (**2**) (dpm/mM)	Degradation products of **2** (dpm/mM)		
					Acetone semicarbazone	Formaldehyde dimedone	Anisic Acid
L-[U-^{14}C] Phenylalanine (432 mCi/mM)	2.2×10^8	0.01	7.45×10^4	7.67×10^4	—	—	6.71×10^4
(3*RS*)-[2-^{14}C]MVA (0.92 mCi/mM)	8.69×10^7	0.086	1.74×10^5	1.72×10^5	8.59×10^4	—	7.6×10^3
DL-[4-^{3}H,1-^{14}C]Phenylalanine	^{14}C 1.95×10^8	—	1.76×10^4	—	—	—	—
	^{3}H 2.72×10^8	—	2.01×10^5	—	—	—	—
	^{14}C:^{3}H 0.72:1	—	0.09:1	—	—	—	—

was obtained by using (3*RS*)-[2-^{14}C]MVA as a substrate. Degradation of **2**, obtained from biosynthesized **1**, gave acetone containing 47% of the total incorporated radioactivity. This indicates that two units of MVA are involved in the biosynthesis of **1**. *p*-Anisic acid and formaldehyde, the other isolable degradation products did not show significant labelling. In several cases of monoterpene biosynthesis it has been found that while the IPP derived moiety originates from MVA, the dimethylallyl pyrophosphate derived part of the molecule is biosynthesized from non-mevalonoid pathway [10, 11]. Since radioactive acetone is obtained from the DMAPP derived part of **1**, this experiment also establishes that the DMAPP derived moiety in the case of bakuchiol is also biosynthesized from MVA.

The results presented above establish that, in the biosynthesis of **1**, two pathways, namely phenylpropanoid and mevalonic acid pathways, are involved, thus supporting the meroterpenic origin [1].

EXPERIMENTAL

Determination of radioactivity was carried out by a liquid scintillation spectrophotometer. TLC analysis was carried out using Si gel G (Acme Synthetic Chemicals, Bombay) containing F_{254} fluorescent indicator. The identities of labelled compounds were established by direct comparison with authentic samples [9]. Radiochemical homogeneity was established by repeated prep. TLC or recrystallization to constant sp. act. The sp. act. of the radioactive samples are given in Table 1.

Radiochemicals. L-[U-^{14}C]Phenylalanine, DL-[1-^{14}C]phenylalanine, DL-[4-^{3}H]phenylalanine and sodium [2-^{14}C]acetate were obtained from the Isotope Division, Bhabha Atomic Research Centre, Bombay. (3*RS*)-[2-^{14}C]MVA was prepared by the one-step synthesis developed in this laboratory [12].

Isolation of bakuchiol. The substrates, dissolved in H_2O (1 ml) were fed to mature plants (8–10 weeks old) by the wick method. After 72 hr the plants were harvested. The whole plant (excluding the roots) was cut into small pieces and extracted by refluxing (× 3) with Et_2O. The concentrate of the combined Et_2O extracts was subjected to prep. TLC (solvent, C_6H_6). The band (R_f 0.5) corresponding to bakuchiol was separated and eluted with Et_2O. Crude bakuchiol (**1**) thus obtained contained psoralene and isopsoralene as impurities. It was further purified to constant sp. act. by prep. TLC by multiple developments (× 3; solvent, C_6H_6).

Bakuchiol methyl ether (**2**). Bakuchiol (80 mg) in dry THF (5 ml) was added to a stirred suspension of NaH (75 mg) in THF. MeI (460 mg) was added and the contents were refluxed for 6 hr. After usual work-up, bakuchiol methyl ether was isolated. It was purified to constant sp. act. by prep. TLC (solvent, C_6H_6).

Degradation of bakuchiol methyl ether (**2**). A soln of **2** (82 mg in EtOAc; 2 ml) was cooled to −25° and a stream of ozonized oxygen (0.55% O_3) was passed through it for 15 min. HCHO formed during the reaction was trapped by absorbing in a cooled dimedone soln (10% in EtOH). After removal of solvent, the ozonide was treated with Na_2CO_3 soln (16%; 2 ml) and H_2O_2 (30%, 1 ml) and heated on a water bath for 2 hr. Me_2CO formed was collected as the semicarbazone by distillation under water pump vacuum. The semicarbazone was purified to constant sp. act. by prep. TLC (solvent, $CHCl_3$–MeOH, 9:1). Acidification (6 N HCl) of the residue gave a crystalline solid (27 mg) which was identified as *p*-anisic acid by direct comparison (mp, mmp, IR, TLC) with an authentic sample. *p*-Anisic acid was purified to constant sp. act. by prep. TLC (solvent, $CHCl_3$–MeOH, 9:1) followed by recrystallization (Et_2O–hexane) and vacuum sublimation (145°, 0.2 mm).

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