Phytochemistry, Vol. 23, No. 8, pp. 1605–1606, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

BIOSYNTHESIS OF BAKUCHIOL FROM CINNAMIC AND *p*-COUMARIC ACIDS

ASOKE BANERJI* and GAJANAN J. CHINTALWAR

Bio-Organic Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085, India

(*Received* 1 *December* 1983)

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

Abstract—Specific incorporation of L-[U-^{14}C]phenylalanine, [U-^{14}C]cinnamic acid and *p*-[2-^{14}C]coumaric acid into bakuchiol has been observed in *Psoralea corylifolia*. Our findings show that the aromatic moiety along with two carbon atoms of the side chain are biosynthetically derived *via* phenylpropane pathway and not by the alternate pathway proposed earlier.

INTRODUCTION

Bakuchiol (**1**), isolated from the seeds of the Indian medicinal plant, *Psoralea corylifolia* L., is a novel phenolic compound with a monoterpene side chain [1]. In our earlier publication we have shown that **1** is biosynthetically derived from one phenylalanine (with the loss of the carboxyl carbon) and two isoprenoid units [2]. Thus, bukuchiol belongs to the rare group of meroterpenoids, the aromatic ring system of which is derived from a phenylpropane unit. In this communication, we describe experiments which elaborate the biosynthetic events between phenylalanine and **1**.

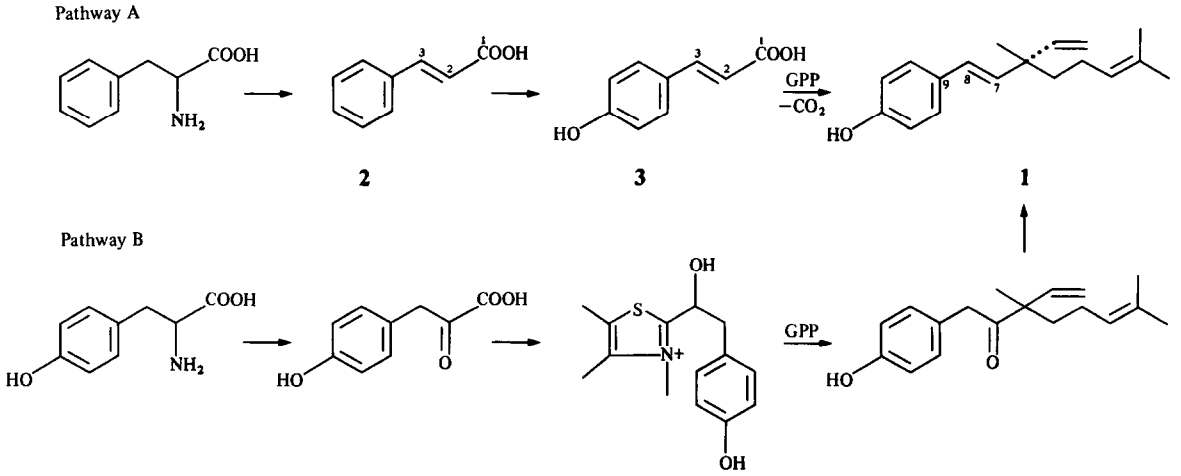
Scheme 1 shows two biosynthetic pathways, A and B, to bukuchiol. Pathway(A) represents well documented phenylalanine metabolism in which cinnamic and *p*-coumaric acids are formed *via* deamination, reduction, dehydration and aromatic hydroxylation [3]. According to an alternate pathway (B) suggested by Risinger *et al.* [4] tyrosine is the source of the aromatic moiety and the condensation of 2-(*p*-hydroxylphenyl)pyruvic acid (obtained by the deamination of tyrosine) with geranyl pyrophosphate is mediated by the coenzyme thiamine. With a view to finding which of the two pathways is operative, the following experiments were conducted.

*Present address: Department of Chemistry and Chemical Engineering, Stevens Institute of Technology, Hoboken, NJ, 07030, U.S.A.

RESULTS AND DISCUSSIONS

In parallel experiments L-[U-^{14}C]tyrosine and L-[U-^{14}C]phenylalanine were administered to 3-month-old plants of *Psoralea corylifolia* L.; compound **1** was isolated, purified, derivatized and degraded as described in our earlier publication [2]. Incorporation of tyrosine was found to be consistently lower by several folds as compared to that of phenylalanine.

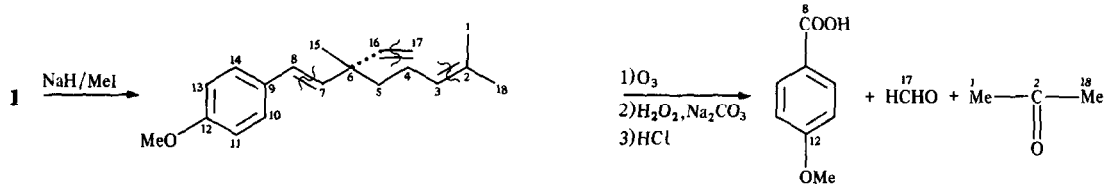
Since considerable variations have been observed in the



Scheme 1.

Table 1. Incorporation of substrates and specific activities of bakuchiol, its methyl ether and anisic acid

Substrates (sp. act.)	Activity administered (μCi)	% Incorporation	Bakuchiol (dpm/mmol)	Bakuchiol methyl ether (dpm/mmol)	Anisic acid (dpm/mmol from 1)
L-[U-^{14}C]Tyrosine (387 mCi/mmol)	(1) 100	0.005	4.76×10^4		
	(2) 100	0.006	5.07×10^4		
L-[U-^{14}C]Phenylalanine (360 mCi/mmol)	(1) 100	0.04	2.97×10^5		
	(2) 100	0.04	3.07×10^5		
	(3) 100	0.04	2.29×10^5	2.28×10^5	1.86×10^5
[U-^{14}C]Cinnamic Acid (324 mCi/mmol)	200	0.01	4.25×10^4	4.51×10^4	3.25×10^4
p-[2-^{14}C]Coumaric Acid (0.17 mCi/mmol)	10.6	0.08	4.78×10^4	4.88×10^4	3.34×10^3



4

Scheme 2.

biosynthetic activity of this plant [5], a competitive experiment was performed in which the same plant was administered with a mixture of L-[U-^{14}C]phenylalanine and L-[3,5-$^3H_2$]tyrosine with ^{3}H and ^{14}C ratio of 4.03. Determination of radioactivities due to ^{3}H and ^{14}C in the biosynthesized bakuchiol showed that the ratio of ^{3}H and ^{14}C had dropped to 0.3. These findings indicated that the incorporation efficiency of L-phenylalanine was 12 times higher than that of L-tyrosine; therefore, L-phenylalanine is the preferred precursor in the biosynthesis of **1**.

Cinnamic and *p*-coumaric acids are the obligatory intermediates between phenylalanine and **1** (Scheme 1, pathway A) [3]. Therefore, it was expected that use of these compounds as substrates would provide definite information about the biosynthesis of **1**. Feeding experiments were carried out using [U-^{14}C]cinnamic acid (**2**) and *p*-[2-^{14}C]coumaric acid (**3**) as substrates; both substrates were incorporated efficiently. Specificities of the incorporations were established by degradative experiments (Scheme 2) [2].

Anisic acid (**4**) obtained from the degradation of **1**, biosynthesized from [U-^{14}C]cinnamic acid retained 82% of theoretical radioactivity whereas other degradation products did not contain significant radioactivity. As expected, the degradation products, namely anisic acid, acetone and formaldehyde, obtained from radioactive **1**, biosynthesized from *p*-[2-^{14}C]coumaric acid (**3**), did not contain substantial activity.

The results presented above establish that bakuchiol is derived from phenylalanine *via* cinnamic and *p*-coumaric acids according to pathway A; the alternative pathway B starting from tyrosine is not favored.

EXPERIMENTAL

Radiochemicals. [U-^{14}C]Cinnamic acid was obtained by the enzymatic transformation of L-[U-^{14}C]phenylalanine as described by Pendharkar and Nair [6]. *p*-[2-^{14}C]Coumaric acid was prepared by the condensation of *p*-trimethylsilyloxybenzaldehyde and trimethylsilyl-[2-^{14}C]acetate. All the other radiochemicals were procured from the Isotope Division, Bhabha Atomic Research Centre, Bombay. Radioactivity measurements and purifications of the labelled compounds were carried out as described in our earlier publication [2].

Feeding experiments. The substrates, dissolved in water (1 ml) were fed to 3-month-old plants by wick method. The plants were harvested after 72 hr. Bakuchiol was extracted by refluxing with Et_2O. It was isolated and purified by prep. TLC (1 mm; solvent C_6H_6). Degradation was carried out by the ozonolysis of bakuchiol methyl ether following the procedure described earlier [2].

Acknowledgements—We are grateful to Drs. P. M. Nair and M. B. Pendharkar for their help in the preparation of *p*-[2-^{14}C]cinnamic acid.

REFERENCES

1. Mehta, G., Nayak, U. R. and Dev., S. (1973) *Tetrahedron* **29**, 1119.
2. Banerji, A. and Chintalwar, G. J. (1983) *Phytochemistry* **22**, 1945.
3. Hanson, K. R. and Havir, E. A. (1979) in *Biochemistry of Plant Phenolics* (Swain, T., Harborne, J. B. and Sumere, C. F. V., eds) p. 91. Plenum Press, London.
4. Risinger, G. E., Karimian, K., Jungk, St. and Simpson, J. B. (1978) *Experientia* **34**, 1121.
5. Banerji, A. and Chintalwar, G. J. (1981) *Symposium in Advances in the Chemistry of Natural Products and Their Biogenesis*, p. 7. Nainital.
6. Pendharkar, M. B. and Nair, P. M. (1981) *Indian J. Biochem. Biophys.* **18**, 356.