Phytochemistry, Vol. 24, No. 10, pp. 2259–2261, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00
Pergamon Press Ltd.

BIOSYNTHESIS OF NIMBOLIDE FROM [2-^{14}C, (4*R*)4-$^{3}H_1$]MEVALONIC ACID LACTONE IN THE LEAVES OF *AZADIRACHTA INDICA*

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(*Received* 13 *November* 1984)

Key Word Index—*Azadirachta indica*; Meliaceae: nimbolide; mevalonic acid lactone; euphol; butyrospermol; tirucallol.

Abstract—Nimbolide was biosynthesized from [2-^{14}C, (4*R*)4-$^{3}H_1$]mevalonic acid lactone in the leaves of *Azadirachta indica*. The nimbolide had a $^{3}H:^{14}C$ ratio of 3:5 which gives support to the suggestion of the involvement of a triterpenoid intermediate with a double bond at the $\Delta^{8(9)}$-position in the biosynthesis of nimbolide.

INTRODUCTION

In an earlier communication [1] we reported the results of feeding experiments with tritium labelled euphol, tirucallol, butyrospermol and Δ^7-tirucallol. Surprisingly the Δ^8-isomers were found to be more efficiently utilized than Δ^7-isomers for the biosynthesis of nimbolide in the leaves of *Azadirachta indica*. This result, which seemed to contradict the general expectation for the biosynthesis of the meliacin nucleus, was explained by the suggestion that because nimbolide is a ring C-*seco*-compound its biosynthesis might involve a $\Delta^{7,9(11)}$-diene which is easily formed from an 8α,9α-epoxide [2]. The Δ^7-function of the $\Delta^{7,9(11)}$-diene system would be readily oxidatively rearranged via its epoxide to give the 7α-hydroxy-apo skeleton found in the meliacins, while the $\Delta^{9(11)}$-function would activate the C-12 position towards eventual oxidation leading to ring C fission.

RESULTS AND DISCUSSION

We have now investigated the possible involvement of a $\Delta^{7,9(11)}$-system by feeding [^{3}H]8α,9α-epoxyeuphol, [^{3}H]8α,9α; 24,25 diepoxyeuphol and [^{3}H]euphol to *A. indica*. The results obtained (Table 1) agree with our earlier proposals. Both the mono- and the diepoxy euphol were found to be more efficiently utilized than euphol.

It has been unequivocally established [3] that the conversion of mevalonate to dimethylallyl pyrophosphate involves a stereospecific elimination of the (C-4) *pro-S* hydrogen. Therefore squalene biosynthesized from [2-^{14}C, (4*R*)4-$^{3}H_1$]mevalonate would be labelled as indicated in the scheme and would in turn afford lanosterol, euphol and butyrospermol with radioactivity distributed as shown. This has been demonstrated for lanosterol and may be presumed to be the case also in euphol and butyrospermol. Consequently if nimbolide is biosynthesized from [2-^{14}C, (4*R*)4-$^{3}H_1$]mevalonate it would have a $^{3}H:^{14}C$ ratio of 3:5 if the biosynthesis involved an intermediate such as euphol or a $\Delta^{7,9(11)}$-diene. On the other hand the ratio would be 4:5 if no intermediate with a double bond at C-9 was involved (Scheme 1). A mixture of DL-[2-^{14}C]mevalonic acid lactone and [(3*R*,4*R*)-4-$^{3}H_1$]mevalonic acid lactone equivalent to [2^{14}C, (4*R*)4-$^{3}H_1$]mevalonate was fed into the leaves of *A. indica* and the nimbolide isolated after 36 hr and crystallized to constant specific radioactivity. The results of the radioactivity measurements are given in Table 2.

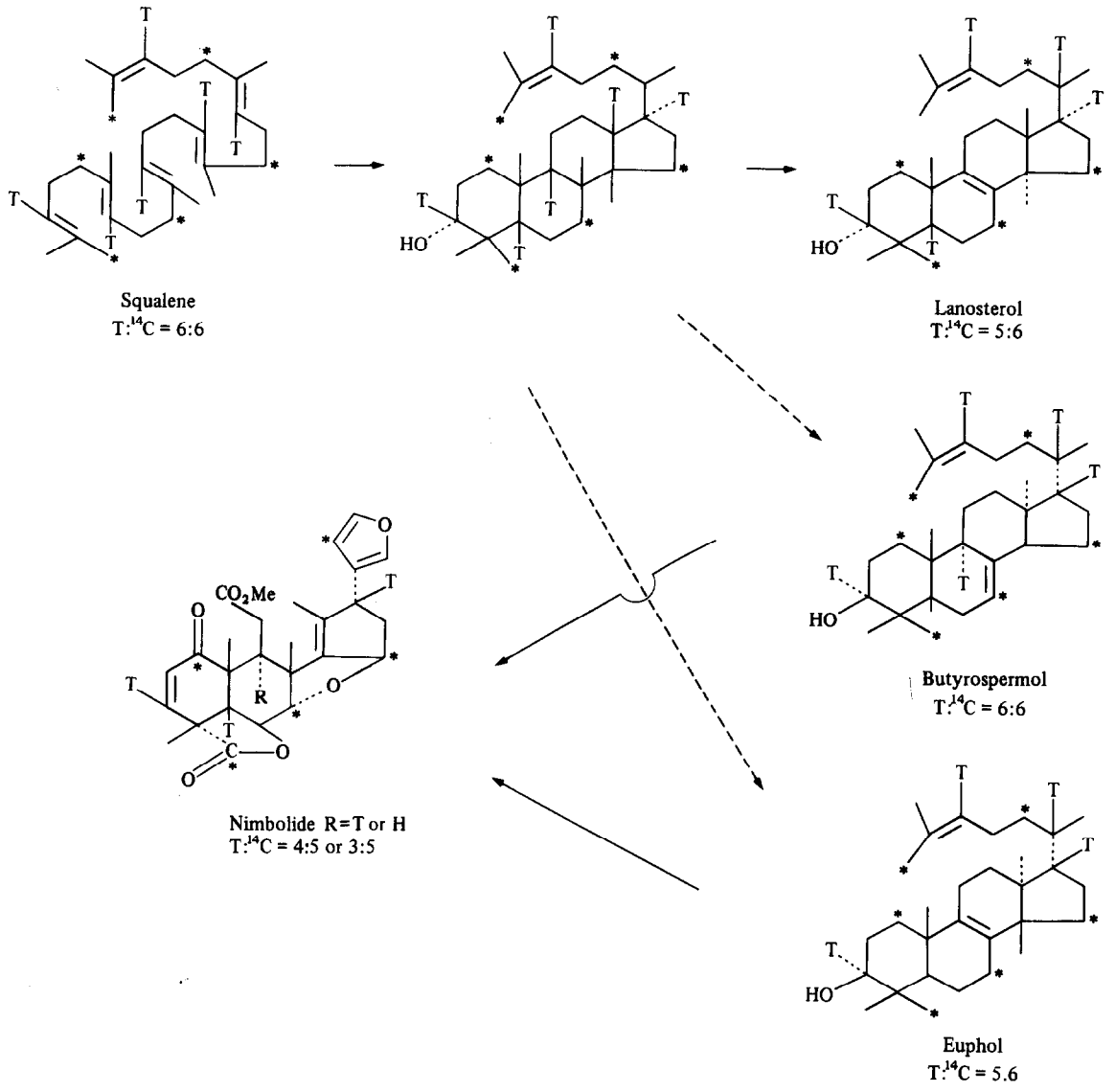
The $^{3}H:^{14}C$ ratio of 3:5 found supports the involvement of an intermediate with a double bond at C-9 in the biosynthesis of nimbolide.

The involvement of a euphol-type precursor was further investigated employing dilution techniques to isolate radioactive compounds corresponding to euphol or buryrospermol and by establishing the specificity of the label at C-3 in the nimbolide.

All the materials obtained from preparative TLC did not seem to indicate the presence of isolable euphol or butyrespermol type of intermediate. A faint yellow crystalline material of closely comparable polarity to euphol was obtained. This however had no infra-red absorption

Table 1. Incorporation of euphol and euphol derivatives into nimbolide by *A. indica* leaves

Precursor	Activity fed (cpm × 10^{-6})	Specific activity of nimbolide (cpm/mg)	Relative incorporation
Euphol	24.4	13.8	2.81
8α,9α-Epoxyeuphol	6.65	8.10	3.98
Diepoxyeuphol	8.1	17.5	5.94



Scheme 1.

band, nor melting point value to suggest that it is euphol or an allied compound. Further studies continue in this direction.

The specific location of ^3H at C-3 in the nimbolide was established by subjecting some of the nimbolide, mixed with unlabelled nimbolide, to reactions that resulted in the elimination of the ^3H atom at C-3 position. The nimbolide which had a specific activity of 54.2 cpm/mg was hydroxylated with osmium tetroxide and the product which retained the original radioactivity was subjected to *t*-butyl chromate oxidation in petrol at room temperature. The product, which did not crystallize, but was shown to be homogeneous by TLC, was found to have a specific activity of 39.1 cmp/mg. This result is interpreted to indicate removal of the ^3H atom at C-3 because this is the most vulnerable position to attack by the reaction.

Table 2. Incorporation of [2-^{14}C, (4*R*)4-$^3H_1$]mevalonate into nimbolide by leaves of *A. indica*

	^3H dpm	^{14}C dpm	^3H : ^{14}C
Mevalonic acid	658501	82492	7.983
Nimbolide	69.3	15.5	4.432*

*Theoretical value for a ratio 4:5 = 6.386; theoretical value for a ratio 3:5 = 4.788.

EXPERIMENTAL

Feeding. DL-[2-^{14}C]Mevalonic acid lactone (*ca* 0.1 mCi) was mixed with mevalonic acid (3*R*,4*R*-4-$^3H_1$ + 3*S*,4*S*-4-^3H) lactone (*ca* 20 mCi) in benzene and the benzene evaporated. The gum obtained was dissolved in methyl cellosolve and the soln painted on intact leaves of *A. indica*. The leaves were harvested after 36 hr and extracted with petrol (bp 60–80°). The extract on concn deposited nimbolide which was crystallized to constant sp. act. The ^3H:^{14}C ratio of the nimbolide was found to be 3:5 (Table 2).

Preparation of 3-keto-nimbolide. Radioactive nimbolide (100 mg) from the leaves fed with the double labelled mevalonic acid lactone was mixed with 200 mg of unlabelled nimbolide. The mixture (250 mg) was hydroxylated using OsO_4 and the gum obtained (100 mg) from the usual work up was dissolved in petrol (100 ml) and oxidised using *t*-butyl chromate soln in petrol. The work-up product was a gum which was found to have a specific activity of 39.1 cpm/mg (about 18% lower than the count for the hydroxyl compound). The products were checked by IR studies.

Acknowledgements—We are greatly indebted to Professor G. Ourisson and Dr. R. Brandt of the Centre de Recherches Nucleaires, Department des Application Biologique, Strassburg-Cronenbourg, France, for analysing the mixed mevalonic acid and the biosynthesized nimbolide. S. A. Ibiyemi is grateful for the University of Ilorin Senate Research Grant.

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