Biosynthesis of Echinulin. The Stereochemistry of Aromatic Isoprenylation

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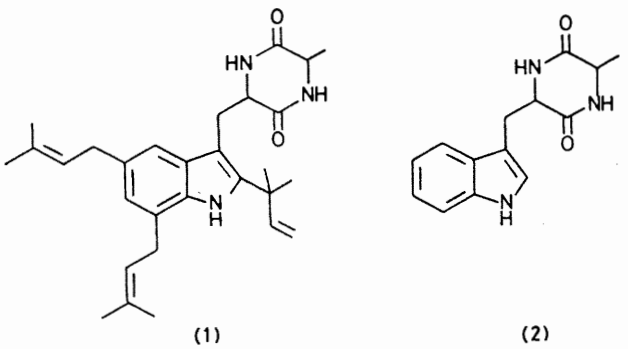
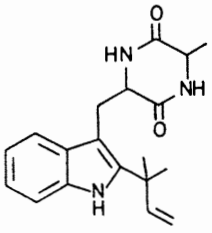
Summary The incorporation of [1,2-^{13}C]acetate into echinulin has shown that the aromatic isoprenylation at the 5- and 7-positions occurs without any change in stereochemistry around the double bond of the dimethylallyl group and chemical degradation of echinulin, obtained by feeding [5(*R*)-^{3}H]- and [5(*S*)-^{3}H]mevalonate, to isopentylamine and oxidation with pea seedling diamine oxidase has shown that the isoprenylation reactions at positions 5- and 7- proceed with inversion of configuration at the allylic pyrophosphate.

THE biosynthesis of echinulin (**1**) involves the multiple isoprenylation of the dioxopiperazine (**2**) and a crude enzyme preparation which converts (**2**) into (**3**) in the

presence of dimethylallyl pyrophosphate has been isolated from *Aspergillus amstelodami*.[1] The isoprenylation at the



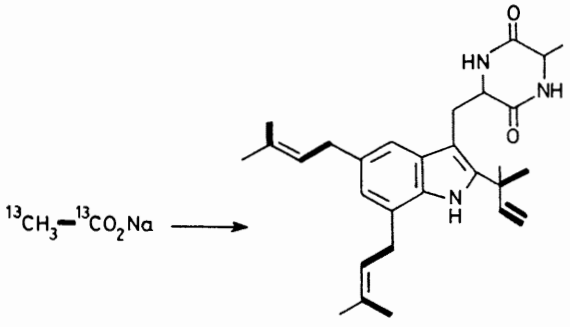
(1) (2)



(3)

5- and 7-positions of the indole ring involves the loss of the 5- and the 7-hydrogens.[2] We have investigated the stereochemistry of this isoprenylation at the 5- and 7-positions using [^{13}C] and [^{3}H] substrates.

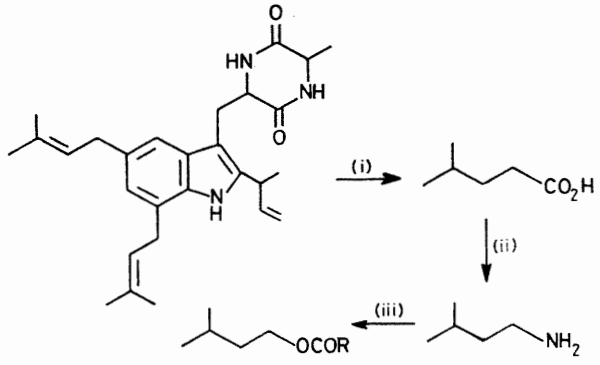
Feeding [1,2-^{13}C]acetate to *A. amstelodami* showed that the (*E*)-methyl groups of the dimethylallyl substituents were derived only from the 2-position of mevalonic acid. The (*E*)-methyl group was enriched but not coupled to the adjacent olefinic centre (Scheme 1). This establishes that



SCHEME 1

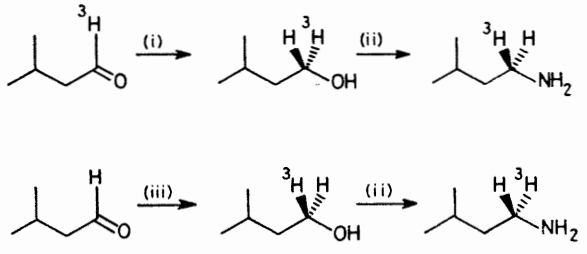
no change in stereochemistry has occurred around the double bond in the dimethylallyl groups of echinulin in the isoprenylation reactions at the 5- and 7-positions. This knowledge is necessary to draw definitive conclusions about the mechanism of incorporations of stereospecifically labelled [^{13}C]-leucines into echinulin.[3] We have observed the same stereochemistry of isoprenylation in the biosynthesis of the isoprenylated phenol flavoglaucin from [1,2-^{13}C]acetate.[4]

Feeding [(5*R*)-^{3}H]- and [(5*S*-^{3}H]-mevalonates to *A. amstelodami* gave incorporations of 0·1—0·5% into echinulin. The labelled echinulin was reduced with di-imide, the reduction products were oxidized with fuming nitric acid, and isocaproic acid was isolated in up to 5% overall yield. The isocaproic acid was converted into isopentylamine by conventional reactions (Scheme 1). The isopentylamine was oxidized with pea seedling diamine oxidase (E.C. 1.4.3.6.), the aldehyde produced was reduced with borohydride, and the resultant isopentyl alcohol was purified as its 3,5-dinitrobenzoate. The product obtained from the echinulin biosynthesized from [(5*R*)-^{3}H] mevalonic acid lost >90% of its tritium and that from [(5*S*)-^{3}H] mevalonic acid retained <10% of the tritium (Scheme 2).



SCHEME 2. Reagents: (i) a, di-imide, b, fuming HNO_3; (ii) a, CH_2N_2, b, NH_2NH_2, c, HNO_2, d, heat, Bu^tOH, e, HCl; (iii) a, pea seedling diamine oxidase, b, $NaBH_4$, c, 3,5-dinitrobenzoyl chloride.

Pea seedling diamine oxidase has been shown by Battersby[5] to be completely stereospecific in its action on [(1*R*)-^{3}H]-and [(1*S*)-^{3}H]-benzylamines and catalyses the removal of the *pro*-(*S*) hydrogen. We have confirmed this by oxidation of [(1*R*)-^{3}H]- and [(1*S*)-^{3}H]-isopentylamines prepared by standard methods (Scheme 3). The pea



SCHEME 3. Reagents: (i) liver alcohol dehydrogenase, NADH; (ii) a, toluenesulphonyl chloride, pyridine, b, N_3^-, aq. dimethyl formamide, c, Redal; (iii) liver alcohol dehydrogenase, NAD^{3}H.

seedling enzyme is completely stereospecific in its action and removes >95% of the tritium in the *pro*-(*S*) position.

The slight discrepancy shown in comparison of the oxidation of our synthetic substrates with those from degradation of echinulin we ascribe to the lengthly chemical degradation and errors in working with relatively low specific activities. These results however, clearly show that if the isolated isocaproic acid is derived from both

the substituents at the 5- and the 7-positions, then inversion of stereochemistry at the allylic centre has occurred in the reaction between (**2**) and dimethylallyl pyrophosphate. This is in complete accord with the stereochemistry observed for prenyl transferase[6] and in mycophenolic acid biosynthesis.[7] We therefore conclude that the substitution at the 5- and 7-positions of the indole ring in this series of dioxopiperazines is by direct electrophilic attack and does not occur by a rearrangement.

We thank the Australian Research Grants Committee for financial support.

(*Received, 12th December* 1978; *Com.* 1316.)

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