Synthesis of Tryptophan Stereoselectively Labelled with Tritium and Deuterium in the β-Methylene Group; the Steric Course of Hydroxylation in Sporidesmin Biosynthesis

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Summary The (3*R*)- and (3*S*)-forms of [3-^{3}H]tryptophan have been synthesised by the oxazolinone method and used to show that side-chain hydroxylation in the biosynthesis of sporidesmin A takes place with retention of configuration at the site of attack.

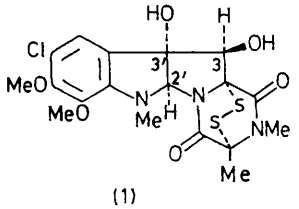
AROMATIC amino-acids labelled stereoselectively with deuterium or tritium in the β-methylene groups have proved valuable in determining the steric course of a variety of biosynthetic transformations.[1–3] In extending our work[1] on tyrosine and phenylalanine we have developed a synthesis of (3*R*)-[3-^{3}H]- and (3*S*)-[3-^{3}H]tryptophan and here illustrate their use in investigating the biosynthesis of sporidesmin A (**1**).

TABLE Incorporation of DL-tryptophan (**6**) into sporidesmin A (**1**) in *Pithomyces chartarum*.

Experiment	Labelling[a] pattern in (**6**)	Incorporation of ^{14}C (%)	Retention of tritium (%) in (**1**)
1	(3*S*)-[3-^{3}H, 1-^{14}C]	0·49	92
2	(3*S*)-[3-^{3}H, 2′-^{14}C]	0·37	90
3	(3*S*)-[3-^{3}H, 3-^{14}C]	0·43	92
4	(3*R*)-[3-^{3}H, 2′-^{14}C]	0·40	8
5	(3*R*)-[3-^{3}H, 3-^{14}C]	0·45	10
6	[2′-^{3}H, 3-^{14}C]	1·10	109

[a] ^{3}H:^{14}C ratios were typically 3·5:1

Treatment of anilinium 3-indolylglyoxylate with dioxan–D_2O (to replace NH by ND) followed by pyrolysis[4] of the deuteriated salt in anisole (160 °C) gave (after exchange of ND by NH) [*formyl*-^{2}H]-3-formylindole (**2**; R=H). N.m.r. spectroscopy [$(CD_3)_2SO$] showed that deuteriation had taken place predominantly in the formyl group and mass spectrometry revealed no substantial amounts of dideuteriated species. Tritiated 3-formylindole was prepared in analogous fashion; conversion into indole-3-carboxylic acid or 3-cyanoindole removed 99·4% of the tritium thus confirming the specificity of labelling. *N*-Acetyl-[*formyl*-^{2}H]-3-formylindole (**2**; R=Ac) condensed with *N*-acetylglycine in acetic anhydride containing anhydrous K_2CO_3 (1 h at 100 °C) to give the corresponding (*Z*)-oxazolinone which was hydrolysed (aqueous acetone then aqueous $KHCO_3$) to yield† [20% overall from (**2**)] the indolylacrylic acid (**3**). Catalytic hydrogenation (5% Pd–C in EtOH) of (**3**) produced the racemate (**4** + **5**). The n.m.r. spectrum of this product showed, as expected,[1,2] that hydrogenation had taken place *cis* with high (>95%) stereospecificity. The tritiated racemate (**4** + **5**; T in place of D) was prepared similarly. This was diluted with radioinactive *N*-acetyl-L-tryptophan and the mixture treated with (−)-1-phenylethylamine to afford[5] the corresponding diastereoisomeric salt which was obtained optically pure after one crystallisation with high (>80%) radiochemical yield. The mother-liquors from the resolution yielded *N*-acetyltryptophan enriched in the D-isomer (**5**; T in place of D). This, after dilution with radioinactive *N*-acetyl-D-tryptophan, was converted into an optically pure salt with (+)-1-phenylethylamine. The resolved samples of *N*-acetyl-[3-^{3}H]tryptophan were separately α-epimerised[6] then de-acetylated (H_2O–HCl) to give (3*R*)- and (3*S*)-[3-^{3}H]-DL-tryptophan suitable for comparative biosynthetic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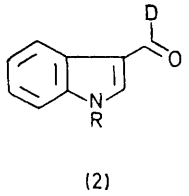
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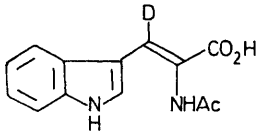
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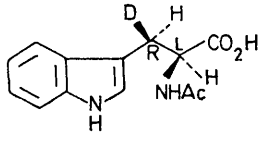
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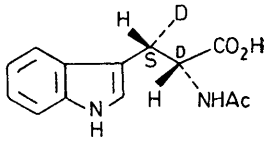
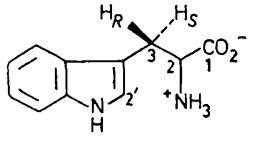

(5)


(6)

† Condensation did not go to completion under these mild conditions but prolonged heating produced tarry by-products. However the product (**3**) was readily separated from unchanged (**2**) by extraction into alkali. All other steps in the synthesis of labelled tryptophan proceeded in good yield.

experiments. Professor H. G. Floss (Purdue University) kindly compared our samples with specimens prepared[3] biochemically from stereospecifically tritiated 3-phosphoglyceric acid. Degradation *via* aspartic and malic acids to fumaric acid confirmed our assignments of configuration to the tritium labels, while biosynthetic incorporation into indolmycin established a configurational purity (at C-3) of *ca.* 94%.

Towers and Wright[7] have concluded tentatively that sporidesmin A (1), a toxic metabolite of the fungus *Pithomyces chartarum*, is derived biosynthetically from tryptophan, alanine, and methionine. Using their culture methods we have observed good incorporation (see Table) of DL-tryptophan into sporidesmin A (purified by t.l.c. and crystallisation of the benzene solvate). No satisfactory method was available for the systematic degradation of small quantities of the metabolite, so evidence for intact incorporation of the carbon skeleton of tryptophan (**6**) was sought by tactical use of ^{3}H and ^{14}C labels. Essentially the same retention (90—92%) of tritium from (3*S*)-[3-^{3}H]-DL-tryptophan was observed (experiments 1—3 in the Table) when a ^{14}C reference label was placed in the side-chain (C-1 or C-3) or the nucleus (C-2′). Moreover, corresponding loss of tritium occurred (experiments 4 and 5) from (3*R*)-[3-^{3}H]-DL-tryptophan while tritium from DL-[2′-^{3}H, 3-^{14}C]-tryptophan[8] was completely retained (experiment 6). These results appear consistent only with controlled incorporation of tryptophan involving, at some stage, replacement of a methylene hydrogen (or tritium) atom by OH with retention of configuration.

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