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# FATE OF THE BENZENE HYDROGENS IN ERGOT ALKALOIDS BIOSYNTHESIS

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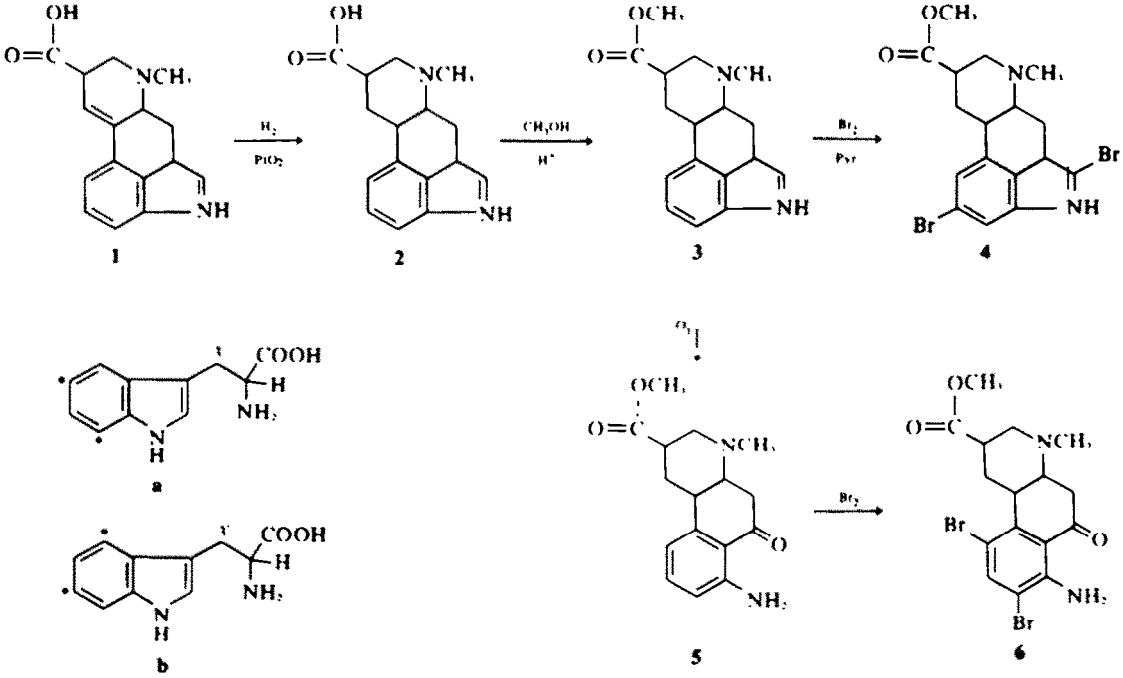
**Abstract**—Feeding experiments with specifically labelled precursors showed that in ergot alkaloids biosynthesis, the isoprenylation of tryptophan occurs without intermediate hydroxylation.

Many biosynthetic pathways[1] were proposed and investigated in the last decades to explain the insertion mechanism of the isoprenic unit in the 4 position of the tryptophan nucleus in the biogenesis of the ergot alkaloids. Hitherto no definite result was obtained about this subject.

The possible activation by hydroxylation of the benzene ring in the 5 position of the indole system was suggested by various authors,[2] but the experiments of Baxter[3] and Plieninger[4] did not agree with this hypothesis: Baxter could not obtain incorporation of 5-hydroxytryptophan into ergot alkaloids, whereas Plieninger observed the incorporation of 5- and 6-deuterotryptophan into clavines, but did not examine the deuterium distribution in the mould metabolites.

Since these results were not conclusive we undertook a general study to verify the hypothesis of the hydroxy-intermediate in order to examine possible shifts in the benzene nucleus during the biogenetic prenylation in the 4 position of the tryptophan. We investigate the fate of all the benzene aromatic hydrogens of the tryptophan unit during ergot alkaloid biosynthesis. Data were collected by measuring tritium retentions in lysergic acid biosynthesized from tryptophan samples carrying $^3H$ labels in 5 and 7 or 4 and 6 positions respectively. *Claviceps paspali* incorporated doubly labelled (5,7-$^3H_2$; 3'-$^{14}$C)tryptophan **a** and (4,6-$^3H_2$; 3'-$^{14}$C) tryptophan **b** into lysergic acid with 102% and 52% tritium retentions respectively. Labelled positions in the ergot alkaloids were detected by the reaction series described in the Scheme.

The lysergic acid **1** obtained by basic hydrolysis of the crude lysergamides, was purified by chromatography, hydrogenated to dihydrolysergic acid **2** and methylated to give the product **3** which was brominated to the




Scheme 1.

2,13-dibromomethyldihydrolisergate **4**.† This compound retained 95% of tritium in the case of tryptophan **a** incorporation. In contrast, all the tritium labelling was lost when the precursor was the tryptophan **b**. A portion of the product **3** was ozonized to the product **5** which was treated with bromine to give the dibromoderivative **6**. The product **6** showed 3% tritium retention with tryptophan **a** as precursor and 47% retention with tryptophan **b** (see Table 1). Therefore, no change occurred at the position 5, 6 and 7 of tryptophan during the biosynthetic pathway, while the hydrogen at position 4 was lost.

We can now definitely exclude the intermediancy of hydroxylated tryptophan derivatives and in the meantime any hydride shift of the kind observed in the biogenetic hydroxylation of tryptophan.[4]

Table 1. $^3H$ molar activities

| Product | Precursor: tryptophan **a** | Tryptophan **b** |
|---|---|---|
| Lysergic acid **1** | 102 | 52 |
| Hydrolysergic acid **2** | 98 | 48 |
| Methylderivative **3** | 97 | 48 |
| Bromoderivative **4** | 95 | 1 |
| Bromoderivative **6** | 3 | 47 |

Experiments to determine whether *N*-isopentenyl-(3'-$^{14}C$)-tryptophan is incorporated into lysergic acid, in accordance with the catalytic[6] or thermal[7] rearrangement of the *N*-isopentenylindole system, were negative.

## EXPERIMENTAL

Radioactivity assays were taken with a Beckman L.S. liquid scintillation counter; $^3H$-hexadecane and $^{14}C$-hexadecane were added as internal standards. NMR data were obtained on a Varian A.60 and were given in ppm downfield from TMS. Mass spectra were determined on a Hitachi RMU 6D spectrometer at 70 eV. M.ps are uncorrected.

*Synthesis of the precursors*

5,7-$^3H_2$-Tryptophan and 4,6-$^3H_2$-tryptophan were synthetized from 2,4,6-$^3H_3$-aniline and 3,5-$^3H_2$-aniline respectively through Fischer cyclization by known procedure.[8] 3'-$^{14}C$-Tryptophan was commercially available (Radiochemical Centre, Amersham U.K.).

*Feeding experiments and isolation of the metabolites*

Tryptophan samples (50 mg, 0.1 mc, $^3H/^{14}C$: 5) were dissolved in medium B inoculated with a *Clariceps paspali* strain in 4 shaken flasks (100 ml each).[9] After 14 days the culture medium was filtered and extracted with $CHCl_3$–MeOH (4:1); extracted labelled alkaloids were saponified with 40% aq NaOH at 80° (8 h) under nitrogen. The crude lysergic acid recovered (400 mg) by ion exchange column ($H^+$, IR 120), was purified by TLC (silica gel plate) in solvent system (ethylacetate, 20% $NH_3$, isopropanol: 45, 20, 35 v/v) to increase the radiochemical purity up to 95%. The incorporation values of tryptophan were about 5%.

*Labelling pattern of the metabolites*

300 mg of labelled lysergic acid **1** were dissolved in $NH_3$ (10 ml, 2%) and catalytically hydrogenated to dihydrolysergic acid **2** (25°, 30 atm, 0.2 g of $PtO_2$), checking the reaction by UV spectra. After 10 h the solutions was filtered to remove the catalyst and the product **2** was crystallized from cold water and dissolved in dry MeOH (10 ml) and sulphuric acid (0.5 g) to give in 1 h the methyldihydrolisergate **3** (230 mg, m.p. 185° from ether). 100 mg of **3** were dissolved in AcOH (2.0 ml) and $CHCl_3$ (2.0 ml) containing dry sodium acetate (200 mg), and treated with pyridinium perbromide (250 mg) under vigorous stirring (0°, 1 h). The reaction mixture was poured into ice-water and potassium carbonate, then evaporated to dryness. The bromoderivative **4** was extracted with $CHCl_3$, purified by TLC ($SiO_2$, ethylacetate), and crystallized from acetone (50 mg, m.p. 258°). The NMR spectrum ($CD_3CO_2D$, 60 MHz, $Me_4Si$ standard), showed in the aromatic region d 7.07 and 7.38 (1H each, AX, $J_{AX}$: 0.5 Hz) mass spectra, *m/e*: 442. Another portion of the methyldihydrolisergate **3** (100 mg) was diluted 2:1 with inactive material and ozonized in MeOH (30 ml) to give product **5** which was directly brominated in AcOH (2.5 mol equiv. of bromine, 5°, 1 h, under vigorous stirring). The reaction mixture was then poured into ice-water and ammonia and extracted with $CHCl_3$. The bromoderivative **4** was purified by TLC ($SiO_2$, ethylacetate) and crystallized from MeOH (70 mg, m.p. 192–194°); NMR data ($CD_3CO_2D$, 60 MHz, $Me_4Si$ standard) were: d 7.81 (s, 1H) only. Mass spectra, *m/e*: 446.

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†Tritium retentions refer to labelled tryptophans **a** and **b** and were determined from variations of $^3H/^{14}C$ ratio. All the reactions described had been proved on unlabelled lysergic acid in deuteriated solvents and no appreciable hydrogen exchange was detected in the benzene unit.

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