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SHORT COMMUNICATION

THE BIOSYNTHESIS OF 6-HYDROXYKYNURENIC ACID IN *NICOTIANA TABACUM*

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Abstract—Tryptophan-1-^{14}C was shown to be incorporated into 6-hydroxykynurenic acid in *Nicotiana tabacum* and decarboxylation showed that virtually all the label was incorporated into the carboxyl group.

INTRODUCTION

THE RECENT isolation of 6-hydroxykynurenic acid (6-HKA) (IV) from *Nicotiana tabacum* and its presence in a number of dicotyledons[1] is the first finding in plants of one of the mammalian breakdown products of tryptophan. Such quinoline derivatives which are excreted in animals[2] are formed from intermediates in the conversion of tryptophan to nicotinic acid. The significance of 6-HKA in *Nicotiana* is not known as the nicotinic acid which is involved in the biosynthesis of nicotine is formed from a C_3 unit and aspartic acid and not from tryptophan.[3,4]

In *N. tabacum* tryptophan-1-^{14}C is converted to carboxyl-labelled 6-HKA (Table 1) and at least twelve unidentified compounds as indicated by an autoradiogram of a two-dimensional chromatogram.

TABLE 1. INCORPORATION OF DL-TRYPTOPHAN-1-^{14}C INTO 6-HKA

	Specific activity (cpm/μmole)
DL-Tryptophan-1-^{14}C†	16×10^6
6-HKA-carboxy-^{14}C	970
$^{14}CO_2$ (as $Ba^{14}CO_3$) from 6-HKA-^{14}C	0·95*

* 6-HKA was diluted with carrier 1:950 before decarboxylation.
† 0·25 μmole fed.

The steps whereby tryptophan is converted to 6-HKA are not obvious. Tryptophan pyrrolase does not appear to exist in plants[1] and an alternative pathway for the cleavage of the indole ring probably occurs. For example, electrophilic substitution by oxygen at position 3 will form an imine (I) which could be readily hydrolysed to an α-hydroxyaldehyde (II) which by a reverse aldol reaction could form kynurenine (III).

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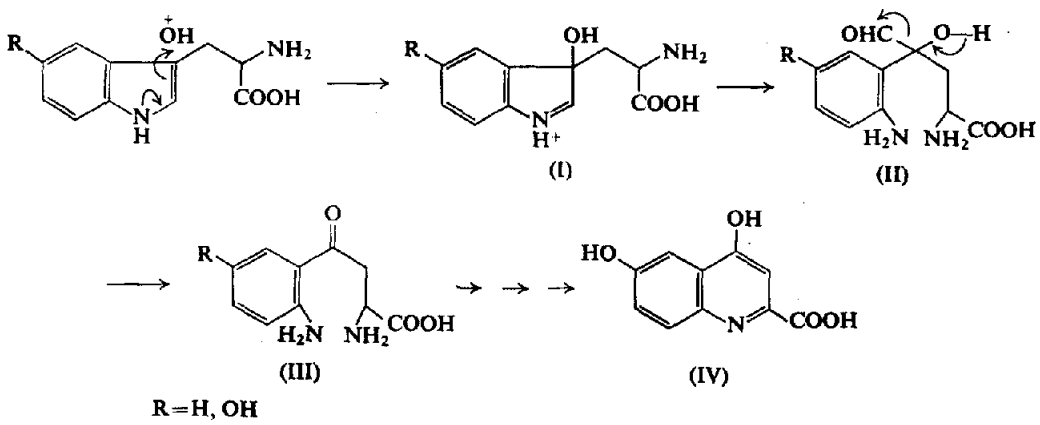

Witkop[5] suggests a similar initial step in the chemical oxidation of indoles under physiological conditions. This type of reaction would be aided by an electron-donating group in the 5 position (R=OH). The biosynthesis of the *Cinchona* alkaloids which are derived from tryptophan[6] and the 2,4-dihydroxyquinoline alkaloids[7] probably involves an analogous scheme.

EXPERIMENTAL

Feeding Experiments

Nicotiana tabacum plants about 30–40 cm high were fed dl-tryptophan-1-^{14}C (Calbiochem, L.A., California) (0·3 ml) by means of a cotton wick threaded through the base of the stem. Both ends of the wick were inserted into the flask containing the precursor, diluted with a small amount of water. Uptake was rapid (2–3 hr) and quantitative. Water was added to the flask after uptake to wash off any precursor on the wick. Plants were grown in a temperature-controlled greenhouse for 7 days at 80–85°F.

Extraction of 6-HKA

N. tabacum leaves (65 g, fresh weight) were homogenized in a Waring Blendor and exhaustively extracted with hot 70 per cent methanol. After removing chlorophyll with light petroleum the extract was taken to small volume *in vacuo* and chromatographed on Whatman No. 3 paper in butanol:pyridine:water (14:3:3). The 6-HKA was eluted with methanol and rechromatographed in isopropanol:formic acid:water (5:1:94), again eluted with methanol and recrystallized to constant, specific activity from 20 per cent HCl after the addition of synthetic[8,9] carrier 6HKA.HCl (62 mg). Estimation in 0·1 M NaOH on an Aminco Bowman Spectrophotofluorimeter (excitation wavelength 390 mμ, emission wavelength 530 mμ) showed 6-HKA to be present at a level of 0·004 μmole/g tissue wet weight.

Decarboxylation of 6-HKA

6-HKA.HCl (16 mg) was decarboxylated by heating to 300° under nitrogen. The gas evolved was washed in 0·1 M $AgNO_3$/1 M HNO_3 and trapped in a saturated $Ba(OH)_2$ solution. The $BaCO_3$ collected was dried, weighed and counted in a Packard Tri-Carb Scintillation spectrometer, Model 3375, using 15 ml of scintillant (PPO 4 g, POPOP 100 mg, toluene 1 l). 6-HKA.HCl (0·6 mg) was suspended in the scintillant with the aid of 500 mg of Packard Thixotropic gel powder. 6 per cent quenching occurred when quantities greater than 0·2 mg of 6HKA.HCl were counted.

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