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# BIOSYNTHESIS OF AN ISONITRILE ACID FROM TRICHODERMA HAMATUM

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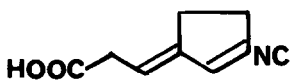
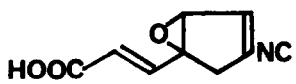
Abstract: The amino acid tyrosine has been shown to be the specific precursor of the isonitrile acid 1 produced by Trichoderma hamatum (Bon.) Bain. aggr.

The fungus Trichoderma hamatum (Bon.) Bain. aggr. has been reported[1] to produce the unstable isonitrile acids 1 and 2. We report here experiments showing that the isonitrile acid 1 is derived from the amino acid tyrosine in a novel manner.




1




2

Administration of [U-$^{14}$C]-L-tyrosine to a culture of T. hamatum growing for one day in production medium gave, after two days, the radioactive acid 1 that was isolated and purified as its benzyl ester 3 (Table, Expt. 1). Ozonolysis of 3 followed by reductive workup (see (Scheme I) yielded the benzyl ester 4. The ester 4 was diluted with carrier[2], purified by chromatography, and converted in two steps to the crystalline carboxylic acid 5. Acid 5 was further degraded to the amine 6, which was purified as its hydrochloride salt. The specific activity of 6 was found to be precisely 67% of the specific activity of 5. This result indicated that the [U-$^{14}$C]-L-tyrosine had been specifically incorporated into 1 without randomization of the label. A clue to the mode of incorporation was provided by administration of [carboxyl-$^{14}$C]-L-tyrosine to Trichoderma (Table, Expt. 2). In this experiment, the benzyl ester 3 was radioactive, but degradation to the carboxylic acid 6 proved that no radioactivity was present in the sidechain. This result suggested that the sidechain of 1 might be derived from the ring carbon atoms of tyrosine. This hypothesis was vindicated by feeding [2,6-$^{3}$H, U-$^{14}$C]-L-tyrosine (Expt. 3). Degradation in the usual way yielded a sample of the radioactive acid 5. The tritium to carbon-14 ratio for this acid is within experimental error for the value computed on the basis of the assumption that the three-carbon sidechain carries three-ninths of the carbon-14 label of the precursor and one-half of the tritium label. Furthermore, the position of the tritium label was determined by conversion of benzyl ester 4 into the acrylate ester 7 followed by osmate-periodate cleavage of 7 to yield formaldehyde that was trapped as its dimedone adduct.

No tritium loss accompanied the conversion of 4 to 7 and the tritium to carbon-14 ratio of the dimedone-formaldehyde was within experimental error of the value calculated on the basis of the assumption that the formaldehyde contains one-ninth of the carbon label of the precursor and one-half of the tritium label. One can therefore conclude that one of the ortho carbon atoms of the aromatic ring of tyrosine corresponds to C-3 of the isonitrile acid 1.

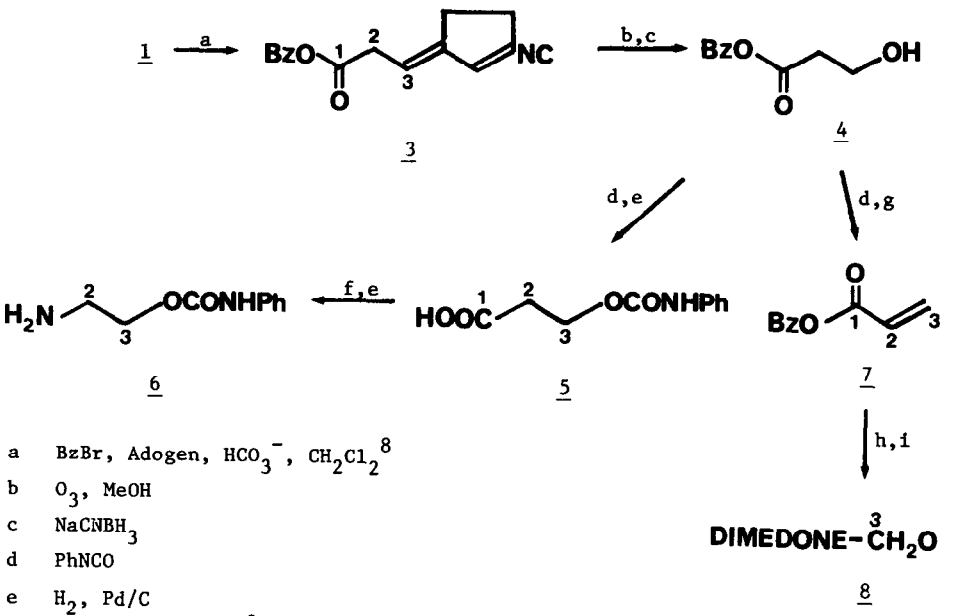
On the basis of the above results, it appeared likely that carboxyl group of 1 would be derived from the para-carbon atom of the aromatic ring of tyrosine. In order to evaluate this possibility, [p-$^{14}$C]-DL-tyrosine was synthesized from [1$^{14}$C]-p-nitrophenol.[3] The ring-labeled phenol was converted to [4-$^{14}$C]-p-anisidine by treatment with diazomethane followed by catalytic hydrogenation (10% Pd/C). The [4-$^{14}$C]-p-anisidine was then diazotized and converted to [4-$^{14}$C]-p-methoxy-α-bromohydrocinnamic acid via the Meerwein arylation reaction.[4] Ammonolysis of the bromoacid[4] and demethylation with 48% hydrobromic acid yielded [p-$^{14}$C]-DL-tyrosine. Administration of the ring-labeled tyrosine to Trichoderma gave the results shown in the Table (Expt. 4). Degradation of 1 to the acid 5 and the amine 6 clearly shows that the carbon-14 label of [p-$^{14}$C]-DL-tyrosine resides in the carboxyl group of 5 and hence the para carbon atom of tyrosine corresponds to C-1 of the isonitrile acid 1.

The labeling pattern observed in these experiments can be rationalized by the biosynthetic hypothesis outlined in Scheme II. The isonitrile acid 1 therefore joins the novel family of natural products including the pyrrolo[1,4]benzodiazepine antibiotics,[5] lincomycin,[6] and the betacyanins[7] that are formed by oxidative cleavage of the aromatic ring of tyrosine followed by recyclization.

Table. Precursor Incorporation Experiments with T. Hamatum

| Expt. No. | Precursor [$^{3}$H/$^{14}$C] | % Incorpn. (2) | Distribution of Radioactivity |
|---|---|---|---|
| 1 | [U-$^{14}$C]-L-tyrosine | ca. 0.8 | Specific activity of 6 = 67% of specific activity of 5 |
| 2 | [carboxyl-$^{14}$C]-L-tyrosine | 0.80 | 0% in 5 |
| 3 | [2,6-$^{3}$H,U-$^{14}$C]-L-tyrosine[4.80] | 1.0 | $^{3}$H/$^{14}$C for 5 = 7.25 (calc. = 7.20)<br>$^{3}$H/$^{14}$C for 7 = 7.29 (calc. = 7.20)<br>$^{3}$H/$^{14}$C for 8 = 21.7 (calc. = 21.6) |
| 4 | [p-$^{14}$C]-DL-tyrosine | 0.43 | Specific activity of 6 = < 1% of specific activity of 5 |

Scheme I




a BzBr, Adogen, $HCO_3^-$, $CH_2Cl_2$[8]
b $O_3$, MeOH
c $NaCNBH_3$
d PhNCO
e $H_2$, Pd/C
f $(PhO)_2PON_3$, $Et_3N$[9]
g DBU
h $OsO_4$, $NaIO_4$
i Dimedone[10]

Scheme II


Acknowledgments

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