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N-ACETYL-L-TRYPTOPHAN IN *CLAVICEPS PURPUREA* PRL 1980

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Key Word Index—*Claviceps purpurea* PRL 1980; Clavicipitaceae; ergot; amino acid derivatives; *N*-acetyl-L-tryptophan; clavine alkaloids.

Abstract—*Claviceps purpurpea* PRL 1980 converts L-tryptophan to *N*-acetyl-L-trytophan. There is little acetylation of D-tryptophan. Added *N*-acetyl-L-trypotophan. has no effect on alkaloid production. L-Tyrosine addition results in production of a compound which is probably *N*-acetyltyrosine and also causes accumulation of 4-γ,γ-dimethylallyltryptophan.

INTRODUCTION

N-Acetyltryptophan is found in plants [1] and is produced in several fungi [2–6]. Generally, D-tryptophan is acetylated whereas the L isomer is not acetylated or acetylation is very limited [1–6]. We report here the formation of *N*-acetyl-L-tryptophan in *Claviceps purpurea* PRL 1980.

RESULTS

Identification of N-*acetyl-*L-*tryptophan*

The major Van Urk's positive components in the BuOH extract from the mother liquor of *C. purpurea* PRL 1980 observed by 2D TLC were tryptophan, 4-γ,γ-dimethylallyltryptophan (DMAT), and clavicipitic acid. A fourth major component migrated on TLC close to indoleacetic acid, but had a slightly different R_f value. This compound was subsequently found to be the major component of the acid-ether fraction. After purification by preparative-TLC the MS of the compound was determined. The major peaks at 220°, 20 eV, were *m/e* (rel. intensity) 45(13), 130(100), 131(22), 187(71), 188(9), 228(4.1), 246(21); the compound was identified as *N*-acetyltryptophan [7].

The specific rotation of the isolated *N*-acetyltryptophan was $[\alpha]_D^{15} = +24°$(*c* 0.558) compared to the reported value for *N*-acetyl-L-tryptophan of $[\alpha]_D^{15} = +25°$ [8]. The compound from *C. purpurea* PRL 1980 therefore is the L isomer.

Production of N-*acetyl-*L-*tryptophan and alkaloids*

In Table 1 the effects of various additions to the culture medium of *C. purpurea* PRL 1980 are compared. As expected addition of 0.5 g/l. L-trytophan to the medium increased the production of *N*-acetyltryptophan, which was shown from the specific rotation to be entirely the L isomer. Less *N*-acetyltryptophan was produced when the equivalent amount of D-tryptophan was added to the culture medium. The specific rotation of the *N*-acetyltryptophan produced with added D-tryptophan was not determined but it is likely that much of it is *N*-acetyl-L-tryptophan derived from L-tryptophan synthesized by the cells or formed by racemization of D-tryptophan. About one-fifth of the added *N*-acetyl-L-tryptophan was recovered at the end of the 6-day incubation period. This indicates a moderately slow rate of metabolism of the compound.

When L-tyrosine was added, the production of *N*-acetyltryptophan was less than with no additions. A major compound was found in the acid-ether extract which had an R_f value slightly below that of *N*-acetyltryptophan in the CMA solvent and which gave a positive Millon's test. The R_f value and positive Millon's test are consistant with *N*-acetyltyrosine. The reduced production of *N*-acetyltryptophan could be attributable to competition between added L-tyrosine and endogenous L-tryptophan for the acetylating enzyme.

Neither *N*-acetyl-L-tryptophan, D-tryptophan nor L-tyrosine stimulated alkaloid production (Table 1).

2DTLC of C. purpurea *PRL1980 cultures*

The amounts of tryptophan, DMAT and clavicipitic acid in the mother liquor from cultures supplemented with various compounds were estimated from the size of the spot on 2D TLC (Table 1). There was some increase in the amounts of tryptophan, DMAT and clavicipitic acid with *N*-acetyl-L-tryptophan added compared to the culture with no addition. This suggests that there was conversion of *N*-acetyl-L-tryptophan to tryptophan. With addition of L-tryosine the amount of tryptophan was higher and the amount of DMAT was markedly higher than the control. There was no clavicipitic acid spot with added tyrosine. D-Tryptophan addition did not increase the amounts of DMAT or clavicipitic acid in the mother liquor.

DISCUSSION

Conversion of D-tryptophan to *N*-acetyl-D-tryptophan with minimal conversion of L-tryptophan has been reported in several plant and fungal systems [1–6]. *C. purpurea* PRL 1980 appears to be the first species to demonstrate preferential formation of *N*-acetyl-L-tryptophan. The formation of *N*-acetyl-L-tryptophan is an alternate fate for L-tryptophan, most of which, under slow or stationary growth conditions, is converted to DMAT and then to the clavine alkaloids.

The apparent acetylation of L-tyrosine suggests that *C. purpurea* PRL 1980 cultures can acetylate other L-amino acids in addition to L-tryptophan. The increase

Table 1. Effect of various additions on production of tryptophan derivatives

Additions*	*N*-AcTry†(mg/l.)	Alkaloid	Tryptophan‡	DMAT‡	Cl. Acid†‡
—	0.92	70	—	±	± ±
L-Tryptophan	3.34	293	+ +	+	+ +
N-AcTry	110	67	±	+	+ +
N-AcTry + L-try	65	200	+	+	+ +
D-Tryptophan	1.52	66	+ + +	±	— —
L-Tyrosine	0.57	41	+	+ +	— —

* Amount was 0.5 g/l except 0.25 g/l. each of *N*-AcTry and L-tryptophan in the flask with both compounds added.
† *N*-AcTry = *N*-acetyl-L-tryptophan; Cl. acid = clavicipitic acid.
‡ relative intensity of spot on two-dimensional TLC. ± = trace.

in amount of DMAT when tyrosine was added may involve both an increase in the rate of synthesis of DMAT from L-tryptophan and a decrease in the rate of conversion of DMAT to the alkaloids. Since tyrosine and trytophan are formed from a common intermediate, chorismate, addition of tyrosine may spare chorismate for L-tryptophan biosynthesis. This would in turn increase the rate of DMAT biosynthesis. Increased tryptophan was observed by 2D TLC when tyrosine was added to the medium. Although the level of DMAT was higher in 6-day cultures supplemented with L-tyrosine than in cultures supplemented with L-tryptophan, alkaloid production was very low. L-Tyrosine addition may inhibit the conversion of DMAT to the clavine alkaloids.

EXPERIMENTAL

Culture conditions. The inoculum of *C. purpurea* PRL 1980 was in growth medium as described previously [9]. The cells were washed with H_2O and added to the mannitol–tryptophan-succinic acid medium [10] (60 ml growth medium per 100 ml alkaloid-production medium). Cultures were shaken for 6 days at 24–26°

Isolation of N-*acetyltryptophan.* Six-day cultures were filtered and the mother liquor adjusted to pH 11 with 30% NH_3 and extracted with $CHCl_3$ to remove alkaloids. The aq. layer was adjusted to pH 2 with 6 MHCl and extracted with Et_2O The Et_2O extracted was concd to dryness on a rotary evaporator. The residue was dissolved in EtOH and purified successively with MeOAc–*iso*-PrOH–30% NH_3 (9 : 7 : 4) (MIA) on Si gel G, $CHCl_3$–MeOH–HOAc (15 : 4 : 1) (CMA) on Si gel G and 80% HCO_2H–H_2O (1 : 2) on Cheng-Chin polyamide sheets. The purified compound was then used for optical rotation measurements and MS.

2D TLC. The mother liqour was extracted (after removal of alkaloids with basic $CHCl_3$) with *n*-BuOH at pH 7. The BuOH extract was evapd to dryness and redissolved in EtOH. The sample was then separated on Si gel G TLC plate in the first dimension with MIA and in the second dimension with CMA The prominant Van Urk's positive spots were identified by MS and by comparison with reference compounds. 2D TLC was carried out in 6-day cultures with various additions replacing *L*-tryptophan and the intensity and size of the major spots were compared.

Quantitative determination of N-*acetyltryptophan.* Six-day cultures (100 ml each) with various additions were adjusted to pH 11 with 30% NH_3 homogenized and extracted with Et_2O. An aliquot of the Et_2O extract was extracted with 1% H_2SO_4. The H_2SO_4 extract was then used for the determination of total alkaloids with Van Urk's reagent [11]. The aq. layer from the basic Et_2O extraction was adjusted to pH 2–3 with 6 MHCl and extracted with Et_2O. The Et_2O extract was concd to dryness on the rotary evaporator and dissolved in EtOH. The sample was separated on Si gel G with CMA solvent. *N*-Acetyltryptophan was eluted with 95% EtOH and concd to dryness The concn of *N*-acetyltryptophan was determined with Van Urk's reagent. *N*-Acetyl-L-tryptophan (Sigma Chemical Company) was used as reference.

Optical measurements of N-*acetyltryptophan.* The concn of *N*-acetyltryptophan was determined from the *A* at 280 nm in 95% EtOH. The extinction coefficient of reference *N*-acetyl-L-tryptophan was 23 mg^{-1} ml cm^{-1}. The optical rotation was measured at the D line of Na (589 nm) at room temp.

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REFERENCES

1. Rosa, N. and Neish, A. C. (1968) *Can. J. Biochem.* **46** 797.
2. Siehr, D. J., Chang, C.-K. and Cheng, H. L. (1969) *Phytochemistry* **8**, 397
3. Höregott, H. (1973) *Biochem. Physiol. Pflanzen* **164**, 500.
4. Zenk, M. H. and Schmitt, J H. (1965) *Biochem. Z.* **342**, 54.
5. Glombitza, K.-W. and Hartmann, T. (1965) *Planta* **69**, 135.
6. Hagemann, F. (1964) *Arch. Mikrobiol.* **49**, 150.
7. Heyns, K. and Grützmacher, H.-F (1963) *Ann. Chem.* **667** 194
8. Weast, R. C. (1973) *Handbook of Chemistry and Physics* 54th edn, p. C-528 CRC Press, Cleveland.
9. Saini, M. S., Cheng, M. and Anderson, J. A (1976) *Phytochemistry* **15**, 1497.
10. Taber, W. A. (1963) *Develop. Ind. Microbiol.* **4**, 295
11. Allport, N. L. and Cocking T. T. (1932) *Quart. J. Pharm. Pharmacol.* **5**,341.