Phytochemistry, 1965, Vol. 4, pp. 511 to 515. Pergamon Press Ltd. Printed in England

5-HYDROXYINDOLE-3-ACETIC ACID AS A METABOLIC PRODUCT OF INDOLE-3-ACETIC ACID PRODUCED BY ERGOT FUNGUS

GISELA TEUSCHER and EBERHARD TEUSCHER

Laboratory of Pharmacognosy, Department of Botany, and Department of Microbiology, University of Greifswald, Greifswald, G.D.R.

(*Received* 15 *November* 1964)

Abstract—5-Hydroxyindole-3-acetic acid was shown to be the major product obtained after feeding indole-3-acetic acid (IAA) to an alkaloid-producing strain of ergot fungus (*Claviceps purpurea* (Fries) Tulasne) in saprophytic culture. Non-alkaloid-producing strains did not hydroxylate IAA. Unlike IAA itself, the 5-hydroxy derivative had no influence on the production of alkaloids by the fungus, and is probably a detoxication product.

IN A great number of micro-organisms and higher plants indole-3-acetic acid (IAA) is known to be a metabolic product of tryptophan. Its occurrence in the ergot fungus (*Claviceps purpurea* (Fries) Tulasne) in saprophytic culture after feeding tryptophan was first reported by Yamano *et al.*[1] We have confirmed these findings for our ergot strain SD 58[2] and also found that indole-3-pyruvic acid is an intermediate on the pathway from tryptophan to IAA.[3] After feeding tryptamine no IAA was found. If IAA is added, it is rapidly metabolized. The metabolic rate is about 1 mg/hr/g dry weight of mycelium.[2] The present report describes the isolation and identification of 5-hydroxyindole-3-acetic acid (5-OIAA) as the main product found after feeding the ergot fungus with unlabelled and ^{14}C-carboxyl labelled IAA.

RESULTS AND DISCUSSION

During an investigation of the function of IAA as a precursor of ergot alkaloids we have incubated ^{14}C-carboxyl labelled IAA with ergot mycelium under submerged conditions.[4] IAA dissolved in a phosphate buffer, pH 5·9, was the only organic compound added. Although after 3 days of fermentation 82·5 per cent of the radioactivity could be recovered from the medium no IAA was detectable by thin-layer chromatography. This shows that the first step of IAA-metabolism in our ergot strain cannot be decarboylation as has usually been observed in studies of other micro-organisms or higher plants.

Of the radioactive metabolic products of IAA, 91·8 per cent were extractable from the medium adjusted to pH 3·0 by shaking three times with an equal amount of peroxide-free ether. At pH 10·0 only 0·14 per cent was obtained. Autoradiography of the acidic fraction after separation by thin-layer and paper chromatography demonstrated that except for traces at the starting point the bulk of the radioactivity was located in one compound.

[1] T. YAMANO, K. KISHINO, S. YAMATODANI and M. ABE, *Ann. Rep. Takeda Res. Lab.* **21**, 83 (1962).
[2] E. TEUSCHER, *Phytochem.* **4**, 341.
[3] E. TEUSCHER, In preparation.
[4] E. TEUSCHER and D. GRÖGER, *Arch. Pharm.*, In press.

The above-mentioned observations showed that the unknown substance must be an acid having at least two C-atoms in position 3 of the indole nucleus. After treatment of the substance for 1 hr with 1 N HCl or 3 N $Ba(OH)_2$ in a boiling water bath no further spots other

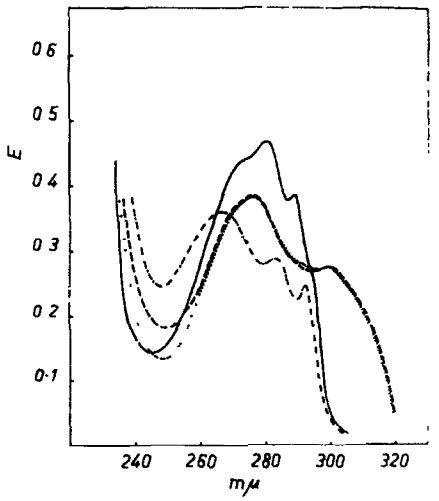

FIG. 1. ULTRAVIOLET ABSORPTION SPECTRA FROM IAA (———), THE METABOLIC PRODUCT OF IAA (– – – –), 5-HYDROXY-TRYPTAMINE (.....) AND 4-HYDROXY-TRYPTOPHAN (–·–·–) IN 80% ETHANOL.

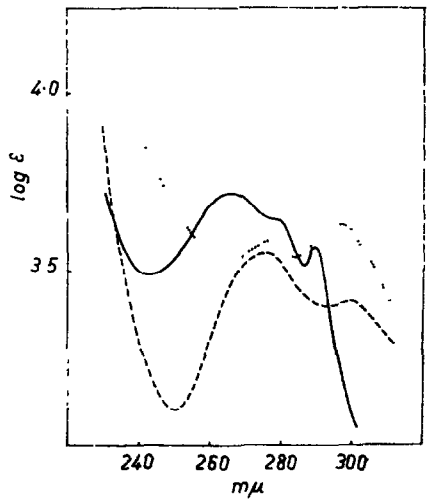

FIG. 2. ULTRAVIOLET ABSORPTION SPECTRA FROM 5-OIAA (– – – –), 7-HYDROXY-IAA (———) AND 6-HYDROXY-TRYPTAMINE (.....) (FROM REF. 5).

than that of the starting substance were detected on paper or thin-layer chromatograms sprayed with Salkowski's reagent. The isoelectric point of the compound was comparable with that of IAA, that is, near pH 2·85. The chromatographic behaviour and colour reactions of the compound indicated that it was probably a derivative of indoleacetic acid hydroxylated in the benzene ring of the indole nucleus.

Hydroxyindoles show characteristic u.v.-spectra which are unaffected by the structure

of the side chain, but are greatly influenced by the position of the hydroxy-group. 5-Hydroxy-indole, 5-hydroxy-tryptophan, 5-hydroxy-tryptamine and 5-OIAA give similar spectra.[5] Comparison of the spectra of IAA, of 4-hydroxy-tryptophan, of 5-hydroxy-tryptamine, and of the unknown product measured in 80% ethanol after thin-layer chromatography demonstrates that the latter compound is a 5-hydroxylated derivative (Fig. 1). The spectrum of the unknown metabolic product of IAA did not resemble the spectra of 6-hydroxy-tryptamine or 7-hydroxy-IAA,[5] but is identical with that of 5-OIAA (Fig. 2).

TABLE 1. R_f VALUES OF THE METABOLIC PRODUCT OF IAA AND OF 5-OIAA

Solvent system	Material	Metabolic product (R_f measured)	5-OIAA (R_f reported)	References
Isopropanol:ammonia:water (8:1:1)	Paper	0·23	0·20, 0·27	6, 7
			0·24, 0·22	8, 9
Isopropanol:ammonia:water (10:1:1)	Paper	0·18	0·17, 0·18	9, 10
Propanol:ammonia (1 N) (5:1)	Paper	0·22	0·27	11
20% KCl	Paper	0·47	0·49, 0·49	6, 12
			0·51	7
n-Butanol:acetic acid:water (4:1:5)	Paper	0·80	0·75, 0·73	6, 12
Chloroform:acetic acid: (95%) (95:1)	Silica gel: $CaSO_4$ (4:1)	0·13	0·17	13
Chloroform:acetic acid:methanol (95:5:10)	Silica gel: $CaSO_4$ (4:1)	0·77	—	—

TABLE 2. COLOUR REACTIONS OF THE METABOLIC PRODUCT OF IAA AND OF 5-OIAA

Reagent	Metabolic product	5-OIAA (reported)	References
Ehrlich's reagent	Golden→ dark blue	Golden→ blue	14, 15
		Blue	10
Ammonia alkaline $AgNO_3$	Immediate dark	Immediate dark	16
Udenfriend's nitroso-naphthol reagent	Deep violet	Violet	11
Salkowski's reagent	Pink, in higher conc. yellowish-green with mauve border, later grey	Pink	10
Gibbs' reagent	Violet	Blue	15
Molybdene phosphoric acid	Blue	Smalt blue	17
Vanillin–conc. HCl	Grey→ violet	Violet	14
Fluorescence after spraying with N HCl	Yellow	Golden	14

[5] H. NASU and Y. SAKAMOTO, *J. Biochem. (Tokyo)* **52**, 351 (1962).
[6] J. KIMMING, W. STICKERLING, R. TSCHECHE and H. G. URBACH, *Hoppe-Seylers Z. physiol. Chem.* **311**, 234 (1958).
[7] M. D. ARMSTRONG, N. F. K. SHAW, M. J. GORTATOWSKI and H. SINGER, *J. Biol. Chem.* **232**, 17 (1958).
[8] A. B. DURKEE and J. C. SIRVIS, *J. Chromatog.* **13**, 173 (1964).
[9] S. P. SEN, in *Papierchromatographie in der Botanik* (Edited by H. P. LINSKENS), Springer-Verlag, p. 248, Berlin (1959).
[10] J. A. BENTLEY, in *Handbuch der Pflanzenphysiologie* (Edited by W. RUHLAND), Vol. 14, p. 491, Springer-Verlag, Berlin 1961.
[11] S. UDENFRIEND, E. TITUS and H. WEISSBACH, *J. Biol. Chem.* **216**, 499 (1955).
[12] B. T. CHADWICK and J. H. WILKINSON, *Biochem. J.* **76**, 102 (1960).
[13] E. STAHL, *Dünnschichtchromatographie*, p. 534, Springer-Verlag, Berlin (1962).
[14] R. CLOTTEN and A. CLOTTEN, *Hochspannungselektrophorese*, p. 184, Thieme-Verlag, Stuttgart (1962).
[15] W. M. MCISAAC and J. H. PAGE, *J. Biol. Chem.* **234**, 858 (1959).
[16] J. A. BENTLEY, *Methods of Biochem. Anal.* **9**, 75 (1962).
[17] L. REIO, *J. Chromatog.* **1**, 330 (1958).

Confirmation that the metabolic product of IAA is identical with 5-OIAA is shown by comparison of the R_f values of the unknown substance with those of 5-OIAA reported in the literature (Table 1), and also by colour reactions with various reagents on paper chromatograms (Table 2). The absorption maximum of the coloured reaction product of the metabolic product with α-nitroso-β-naphthol was identical with that reported for 5-OIAA (540 mμ) by Udenfriend and co-workers.[11]

5-OIAA is well known as a breakdown product of serotonin (5-hydroxy-tryptamine) in mammals, but there is also evidence that 5-OIAA could be formed in animals by direct hydroxylation of IAA.[18] 5-OIAA exists in higher plants together with serotonin,[19] but we could not find any reference concerning the direct hydroxylation of IAA in micro-organisms or in plants. Commonly IAA is metabolized along other pathways in such organisms. The first step in its degradation seems to be an oxidative decarboxylation.[20] Less is known about the products formed: indolealdehyde was not isolated in large amounts.[20] It has been postulated, however, that dihydroxymethylindole[21] and derivatives of *o*-aminoacetophenone[22] are metabolic products of IAA. Yamano and co-workers[1] isolated indole-3-isopropionic acid from saprophytic ergot cultures after feeding tryptophan or IAA. This compound could not be detected in strain SD 58.

5-OIAA seems to be a detoxication product of IAA, derived from the breakdown of tryptophan by the ergot fungus or by the host plant. In our ergot strain 5-OIAA is only slowly further metabolized leading to the formation of a red-coloured product. Whether this step is enzymatically catalysed or not is uncertain. The great physiological activity of IAA in the fungus is lost after hydroxylation. Thus IAA enhances the alkaloid production of the fungus under optimal conditions about fifteen times, whereas 5-OIAA, isolated by thin-layer chromatography, had no influence. Growth-promoting activity of 5-OIAA on higher plants should be investigated.

An investigation of nine other strains of ergot showed that only strain SD 58 was capable of metabolizing IAA in measurable amounts and of forming 5-OIAA. The other strains were also unable to produce alkaloids under saprophytic conditions. Though it has been shown by feeding 5-deuterotryptophan that 5-hydroxylated compounds could not be on the main pathway of ergot alkaloid biosynthesis,[23] it is, nevertheless, possible that there exists a connexion between alkaloid biosynthesis and capability of hydroxylation. We plan to investigate the specificity of the hydroxylating enzyme and its capability of hydroxylating the indole nucleus in other than in the 5-position.

EXPERIMENTAL

We carried out the experiments utilizing the replacement technique with 7-day-old submerged grown cultures of strain SD 58 of *Claviceps purpurea*, which is capable of producing clavine-type alkaloids in saprophytic culture. The substances to be tested were dissolved to give 3·0 mM solutions in 80 ml phosphate buffer, pH 5·9, containing 1·3 g NaCl and after inoculation with 5 g (fresh weight) of the washed mycelium the flasks were placed for 3 days at 25° on a rotary shaker.

[18] K. RYSANEK and V. VITEK, *Experientia* **15**, 217 (1959).
[19] S. UDENFRIEND, E. TITUS, H. WEISSBACH and R. E. PETERSON, *J. Biol. Chem.* **219**, 335 (1956).
[20] R. E. STUTZ, *Plant Physiol.* **32**, 31 (1957).
[21] P. M. RAY, *Arch. Biochem. Biophys.* **64**, 193 (1956).
[22] D. T. MANNING and A. W. GALSTON, *Plant Physiol.* **30**, 225 (1955).
[23] H. PLIENINGER, R. FISCHER, G. KEILICH and H. D. ORTH, *Liebigs Ann. Chem.* **642**, 214 (1961).

For isolation of IAA and its acidic metabolic products the medium was acidified (pH 3·0) with tartaric acid and extracted with 3 × 100 ml peroxide-free ether. The ether extract was dried with Na_2SO_4 and the ether removed *in vacuo*. The residue was dissolved in 2 ml ether for chromatography. For solvent systems and reagents see references in Table 1 and 2.

Quantitative estimation of IAA was carried out according to the method of Gordon and Weber[24] after extraction from 1 ml acidified (pH 3·0) medium with 3 × 1 ml ether. To 2 ml of the dried ether extract, 3 ml Salkowski's reagent (50 ml $HClO_4$ (35%) + 1 ml 0·5 M $FeCl_3$) were added, and the red colour was measured at 530 mμ after standing 1 hr in the darkness. The values were corrected for 5-OIAA. 5-OIAA was determined according to Udenfriend *et al.*[11] directly in the replacement medium without separation from IAA.

The u.v.-absorption spectra were measured with a Zeiss-spectrophotometer after extraction of the substances with 80% ethanol from the thin-layer plate.

Acknowledgements—The authors wish to thank Professors H. Borriss and F. Mach for supporting this work.

[24] S. A. Gordon and L. G. Paleg, *Physiol. Plantarum* **10**, 39 (1957).