

Zusammenfassung. Es ist gelungen, *Nemalion multifidum*, eine marine Floridee, in axenische Kultur zu bekommen. Die Alge wurde in einer synthetischen Nährlösung gezüchtet. Wenn sie ohne Vitamine 2 Monate kultiviert wurde, konnte ein Zusatz einer Vitaminmischung den Zuwachs verdoppeln. Die wirksamen Vitamine waren am Anfang B₁₂ oder B₆; wenn die Alge aber längere Zeit in Hungerkultur gezüchtet worden war, konnte nur die B₁₂-Analoge, Factor Ib, den Zuwachs erhöhen.

LISBETH FRIES

Institute of Physiological Botany, University of Uppsala (Sweden), November 21, 1960.

6-Hydroxylation: An Important Metabolic Route for α -Methyltryptamine

D,L- α -methyl tryptamine (α MT) has been found to produce LSD₂₅ like effect in normal volunteers¹. We have found that the effect of 20 mg α MT is comparable in intensity to the psychotomimetic effect of about 60 mg N,N-diethyltryptamine (DET) in man with the difference that the perceptual distortions are more and the sympathomimetic vegetative changes are less pronounced than those produced by DET². Since DET has been found to produce the behavioral changes through the 6-hydroxylated metabolite³, the question arises whether or not α MT also forms 6-hydroxylated metabolites.

Although the biochemical effects of α MT on serotonin metabolism has been extensively studied⁴⁻⁶, very little is known about the metabolism of the compound itself. Since the side chain of α MT is identical with the side chain of amphetamine ($-\text{CH}_2\cdot\text{CH}\cdot\text{NH}_2$), the compound was expected to undergo the same microsomal deamination as amphetamine⁷.

Incubating α MT with rat liver microsomes, soluble supernatant fraction, TPN and Mg⁺⁺ at 37°C resulted in the formation of 3-indolylacetone as evidenced by the paper chromatographic identification of the metabolite in the neutral ether extract with synthetically prepared 3-indolylacetone⁸. Both the metabolite and the synthetic compound gave the same Rf value in three different solvent systems and produced the same purple color reaction with Ehrlich's *p*-dimethylaminobenzaldehyde. The chromatograms of the neutral ether extract also showed another new compound which gave a fast developing blue reaction with Ehrlich's reagent and instant red color with diazotized sulfanilic acid in HCl specific for 6-hydroxy indole derivatives⁹. The Rf values of this new compound (0.83 in *n*-butanol-acetic acid and 0.90 in isopropanol-ammonia) and the color reactions were identical with those of the single metabolite obtained from synthetic 3-indolylacetone in the same microsomal enzyme system. Therefore, this compound is most probably 6-hydroxy-3-indolylacetone.

The pH of the enzymatic reaction mixture (previously extracted with ether) was then adjusted to 9.5 and extracted with *n*-butanol. When the butanol extract was shaken with dilute acetic acid, the aqueous layer contained, besides unchanged α MT¹⁰, a new apparently basic indole derivative (Rf 0.48 in butanol-acetic acid, 0.66 in butanol-ammonia) which gave the color reactions characteristic for 6-hydroxy indoles. It is most likely that this compound is 6-hydroxy- α -methyltryptamine (6-HO- α MT).

Additional evidence for the identity of 6-HO- α MT was obtained by an experiment in which the isolated compound was again incubated with the microsomal enzyme system and yielded 6-hydroxy-3-indolylacetone by deamination.

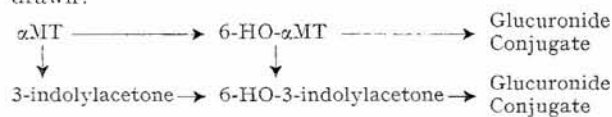
Thus the three major enzymatic products were tentatively identified as 3-indolylacetone, 6-HO- α MT and 6-hydroxy-3-indolylacetone.

The same metabolites have been found to be formed in intact animals and excreted in the urine mainly as glucuronide conjugates.

Male albino rats were injected with α MT (5 mg/kg) intraperitoneally and the urines were collected for 48 h in metabolic cages. Aliquots of the urine samples were incubated with bacterial β -glucuronidase (Sigma) in order to hydrolyze the glucuronide conjugates.

The urine samples were chromatographed without previous extraction. The chromatograms of the samples not treated with β -glucuronidase showed the presence of some unchanged α MT, a spot corresponding to 3-indolylacetone, a small spot corresponding to 6-HO- α MT and a large presumably double spot with low Rf values (0.04 to 0.20 in butanol-acetic acid and 0.20-0.45 in isopropanol-NH₃) and with typical 6-hydroxy-indole color reactions. On the chromatograms of the glucuronidase treated urine samples, this large double spot with low Rf values was absent but the spot corresponding to 6-HO- α MT was tremendously increased and another compound appeared which proved to be identical with the enzymatically prepared 6-hydroxy-3-indolylacetone.

From the evidence described above the following tentative scheme for the metabolism of α MT may be drawn:



Since 6-hydroxylated indole derivatives are more potent pharmacologically than the non-hydroxylated parent compounds¹¹, it is possible that one or both of the 6-hydroxy metabolites play a part in the behavioral effect of α MT.

Zusammenfassung. Der Stoffwechsel der psychotropischen Verbindung α -Methyltryptamin wurde untersucht. Die drei hauptsächlichsten Metabolite wurden *in vitro* und *in vivo* als 6-Hydroxy- α -methyltryptamin, 3-Indolylacetone und 6-Hydroxy-3-indolylacetone identifiziert.

S. SZARA

Clinical Neuropharmacology Research Center, Washington (D.C.), October 4, 1960.

- H. B. MURPHREE, JR., E. H. JENNEY, and C. C. PFEIFFER, *The Pharmacologist* 2, 64 (1960).
- S. SZARA, unpublished observation.
- S. SZARA, E. HEARST, and F. PUTNEY, *Fed. Proc.* 19, 23 (1960).
- W. G. VAN METER, G. F. AYALA, E. COSTA, and H. E. HIMWICH, *Fed. Proc.* 19, 265 (1960).
- M. E. GREIG, R. A. WALK, and A. J. GIBBONS, *J. Pharm. exp. Therap.* 127, 110 (1959).
- A. YUWILER, E. GELLER, and S. EIDUSON, *Arch. Biochem. Biophys.* 80, 162 (1959).
- J. AXELROD, *J. biol. Chem.* 214, 753 (1955).
- J. B. BROWN, H. B. HENBEST, and E. R. H. JONES, *J. chem. Soc.* 1952, 8172.
- S. SZARA and J. AXELROD, *Exper.* 15, 216 (1959).
- Since we used D, L- α MT (synthesized according to the method of E. H. P. YOUNG, *J. chem. Soc.* 1958, 3496), it is very probable that the bulk of the unchanged compound is the L-isomer not attacked by the stereospecific enzyme (see Ref. 7).
- E. HEARST and S. SZARA, *Amer. Psychologist* 15, 476 (1960).