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Dephosphorylation of Psilocybin to Psilocin by Alkaline Phosphatase. (26228)

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A number of indolic compounds have gained pharmacological and biochemical interest because of their ability to produce psychotic states in human subjects. Thus, compounds such as bufotenin, N, N-dimethyltryptamine and harmine(1) have been reported to produce behavioural changes in man and various central nervous system effects in experimental animals. The most recent additions to this class of compounds are the substances found in the *Psilocybe mexicana* Heim mushroom, psilocybin and psilocin. Chemically, these compounds are 4-phosphoryl-N, N-dimethyltryptamine and 4-hydroxy-N, N-dimethyltryptamine, respectively. Their chemical structures are shown below.

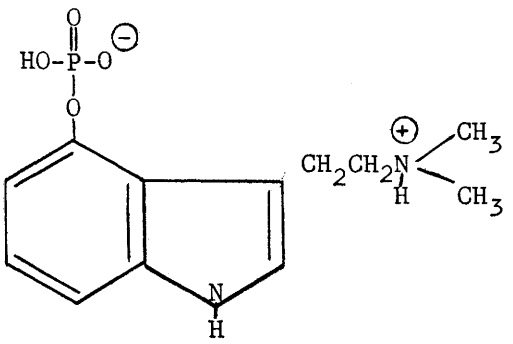
According to Troxler *et al.*(2) psilocybin and psilocin are the first indole compounds of natural sources possessing substitutions on the 4-position of the indole ring. Psilocin differs from bufotenin only by having its –OH group located on the 4- rather than the 5-position.

The present report is concerned with hydrolysis of the phosphate group of psilocybin *in vitro* by a purified preparation of calf intestinal phosphatase. These studies were initiated because of the possibility that psilocybin might serve as a substrate for the phosphatase enzyme. This appeared reasonable since other aromatic compounds with a phosphate group are excellent substrates of acid and alkaline phosphatases(3).

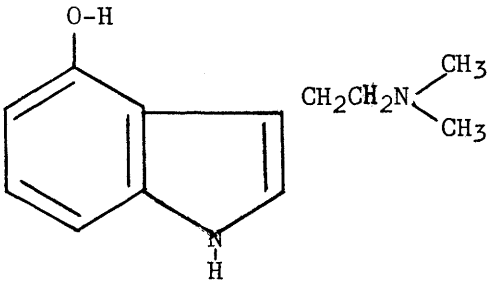
Methods. All experiments in this study were carried out using purified calf intestinal phosphatase containing 15,000 units per mg.* Psilocybin and psilocin were generously supplied by Mr. Harry Althouse of Sandoz Pharmaceuticals, San Francisco, Calif.

Inorganic phosphate was determined according to a modified method of Fiske and Subbarrow(4). Psilocin was determined by adapting the colorimetric analysis for serotonin as described by Udenfriend *et al.*(5). All steps in the procedure were similar to that for serotonin. The final reaction between the nitrosonaphthol reagent and psilocin in an acid medium resulted in formation of a brownish-orange color as compared to the bright violet color of serotonin or bufotenin. The color with psilocin exhibited maximum absorption at wave lengths of 540 and at 430 mμ as read on the spectrophotometer. Determinations of psilocin were made at 430

* Purified alkaline phosphatase preparation was purchased from Mann Research Labs., New York.



Psilocybin



Psilocin

mμ, although in some instances they were also checked at 540 mμ. Colors developed from standard solutions of pure psilocin were found to follow Beer's Law within the concentration range of 0.02 and 1.0 μmoles/ml.

Psilocybin also reacted with the nitrosonaphthol reagent but only at much higher concentrations. It exhibited a light yellow color, but this did not interfere with the psilocin readings at 430 mμ. Psilocybin, however, is not extracted into the butanol phase, and the final acid extract did not give any color reaction with the nitrosonaphthol reagent. This permits a selective extraction of psilocin by the butanol and prevents interference by any psilocybin that might be present.

The incubation mixture consisted of the following: 0.4 ml of intestinal phosphatase (1500 units/ml) and 5.0 ml of psilocybin (2 μmoles/ml) dissolved in Veronal buffer (pH 9.2). The psilocybin was pre-incubated for 5 minutes in order to equilibrate to 37°C. The enzyme solution was then added, and at varying times, tubes were removed and assayed for psilocin and inorganic phosphate. Duplicate assays were performed for each tube.

Descending paper chromatography of the incubation mixtures was used as a further identification of psilocin. A butanol-acetic acid-water (4:1:1) mixture acted as the developing solvent, and the spots were detected with a cinnamaldehyde-HCl reagent(6).

Results. The ability of purified intestinal alkaline phosphatase to dephosphorylate psilocybin with liberation of psilocin and inorganic phosphate is illustrated graphically in Fig. 1. A rapid dephosphorylation is evident in the initial 15-30 minutes and thereafter proceeds at a slower linear rate. Under the conditions described here it appears that about 5 x 10^{-4} μmoles of psilocin was liberated per unit of enzyme per hour. In contrast, dephosphorylation of disodium monophenylphosphate by alkaline phosphatase liberates about twice as much phenol and inorganic phosphate. In all instances liberation of inorganic phosphate follows closely the appearance of psilocin during incubation period.

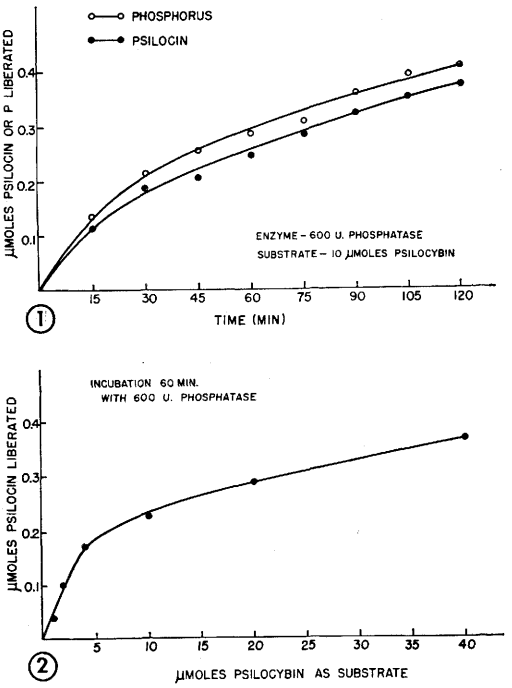


FIG. 1. Rate of phosphorus and psilocin liberation from action of purified alkaline phosphatase acting on psilocybin. Incubation in veronal buffer, pH 9.2 at 37°C.

FIG. 2. Effect of psilocybin concentration on liberation of psilocin by purified alkaline phosphatase. Incubations were carried out in veronal buffer, pH 9.2 at 37°C for 60 min.

That psilocin was liberated from psilocybin by phosphatase was evident not only from the colorimetric tests but also from the Rf values by paper chromatography. Development of the paper by spraying with the cinnamaldehyde-HCl reagent revealed only one spot identical in position with the standard psilocin strip. Average Rf value of both standard and unknown strips was 0.74 (average value of 4 strips). Psilocybin did not react with the reagent to give distinctive spot test.

The effect of psilocybin concentration on formation of psilocin is shown in Fig. 2. In these instances the incubation was carried out for one hour with 600 units of alkaline phosphatase. Concentrations of 1 μmole to 40 μmoles of psilocybin were employed as the substrate. A linear relationship between psilocybin concentrations and psilocin formation was noted up to about 4 μmoles of substrate. Beyond this concentration, rate of formation of psilocin approached a plateau, indicating saturation of the enzyme with excess substrate and attainment of a zero-order reaction.

Since the Mg^{++} ion is known to activate alkaline phosphatase its effect was also studied with psilocybin as substrate. It was found that at concentrations of 0.35% $MgSO_4$, psilocin and inorganic phosphate liberated increased about 30% above controls.

Discussion. Psilocybin is readily dephosphorylated by intestinal phosphatase to form inorganic phosphate and psilocin. This raises the question of whether the central nervous system actions of psilocybin are produced by its dephosphorylated congener, since many mammalian tissues as well as serum contain a variety of phosphatases. Experiments in progress indicate that both mammalian tissue and plasma are capable of rapidly dephosphorylating psilocybin.

It would appear reasonable that psilocin might produce central nervous system effects of psilocybin since the latter would be highly ionized and less likely to penetrate the blood-brain barrier. Determinations of the solubilities of these 2 compounds in organic solvents and in Tyrode solution at pH 7.4 showed psilocin to be over 100 times as soluble in chloroform than was psilocybin (unpublished results).

Liberation of psilocin from psilocybin was readily determined by specific assay methods. Although closely related in structure, these 2 compounds reacted quite differently to various color reagents. We are currently exploring the possibility of utilizing the spectrophotofluorimeter as a means of identifying small amounts of both of these compounds.

Summary. Incubation of psilocybin with purified intestinal phosphatase resulted in liberation of psilocin and inorganic phosphate. Psilocin formed was determined both quantitatively by a specific colorimetric method employing the nitrosonaphthol reagent and qualitatively by paper chromatography. Some characteristics of the phosphatase action on psilocybin are described. The possibility of a similar reaction occurring in the intact animal is discussed.

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