Occurrence of 5-Hydroxytryptamine and 5-Hydroxytryptophan in *Panaeolus sphinctrinus*

Sir:

The status of *Panaeolus sphinctrinus* (Fr.) Quél. as a hallucinogenic mushroom is currently a matter of dispute. Specimens of this species collected in Mexico by Schultes approximately 25 years ago were said to be employed there as psychotomimetic agents (1). The possibility of such application was seemingly verified when Heim and Hofmann reported the isolation of psilocybin from cultivated sporocarps of the fungus (2). However, their recent investigations of other samples of *P. sphinctrinus* failed to detect psilocybin (3). In neither case did the authors present experimental details or sources of the fungi examined, and herbarium reference specimens were apparently not retained.

An investigation of nine species and forms of *Panaeolus* by Tyler and Smith (4) revealed the presence of 5-substituted tryptamine derivatives but no 4-substituted derivatives. Because of its relative scarcity, *P. sphinctrinus* was not included in that study, but the possibility that it might contain psilocybin or psilocin appeared remote on chemotaxonomic grounds. Investigation of an authentic sample of the fungus was clearly required to settle the ethnological, phytochemical, and chemotaxonomic problems raised by this apparent discrepancy in the type of tryptamine derivatives reported to be contained in a single species of the genus *Panaeolus*.

Recently, we obtained through the courtesy of Dr. R. Singer[1] a small sample of *P. sphinctrinus* (Fr.) Quél. collected in Argentina (collection No. M 3514) and determined by Singer to be the same species as the Mexican material originally collected by Schultes (1). The powdered specimen (95 mg.) was extracted with 5 ml. of methanol and 100–150-μl. quantities of the extract subjected to circular chromatography on S. and S. 2043-bm. filter paper in three solvent systems essentially as described by Benedict, *et al.* (5). Spraying the chromatograms with *p*-dimethylaminobenzaldehyde solution (PDAB) or with Pauly's reagent revealed two principal zones (violet-PDAB, reddish orange-Pauly's) with R_f values in all three solvent systems corresponding to reference samples of 5-hydroxytryptophan (5-HTP) and 5-hydroxytryptamine (5-HT). The average R_f values of the zones are listed in Table I. Urea was also identified by its R_f value and distinctive yellow with PDAB.

TABLE I.—R_f VALUES OF INDOLE DERIVATIVES IDENTIFIED IN *P. sphinctrinus* EXTRACT AND OF REFERENCE COMPOUNDS

Compd. Spotted	R_f Values			
	5-HTP	5-HT	Psilocybin	Psilocin
	n-Butanol:Acetic Acid:Water (4:1:1)			
P. sphinctrinus extract	0.31	0.51	...	...
Reference	0.33	0.53	0.45	0.71
	n-Butanol (Water-saturated)			
P. sphinctrinus extract	0.18	0.34	...	...
Reference	0.19	0.33	0.09	0.54
	n-Propanol:1*N* Ammonium Hydroxide (5:1)			
P. sphinctrinus extract	0.21	0.59	...	...
Reference	0.22	0.59	0.16	0.90

The methanol extract was then concentrated to 2 ml., 200–400 μl. quantities were applied as 2-cm. streaks on thin-layer plates prepared with Merck silica gel G (according to Stahl), and the chromatograms developed twice in a solvent mixture composed of chloroform: 5% methanol, saturated with concentrated ammonium hydroxide (6). After spraying with PDAB, the chromatograms showed two principal violet spots with mobilities equivalent to 5-HTP and 5-HT and which failed to separate from these compounds when spotted in combination with them. Several other spots giving bluish colors with PDAB were noted on the chromatograms but did not prove to be identical with available reference compounds. None of the spots corresponded to psilocybin or psilocin.

From these observations in four different chromatographic systems, it was concluded that the sample of *P. sphinctrinus* examined contained neither psilocybin or psilocin. In agreement with the numerous species of this genus previously analyzed (4), it contained relatively large amounts of 5-HTP, 5-HT, and urea. The reported isolation of psilocybin from *P. sphinctrinus* sporocarps by Heim and Hofmann may be

[1] The authors gratefully acknowledge the valuable contribution made to this study by Dr. R. Singer, Department of Botany, University of Buenos Aires, Buenos Aires, Argentina.

attributed to the existence of different chemical races of the species; however, this possibility now appears remote. A more likely conclusion is that that report, like numerous others attributing hallucinogenic properties to *Panaeolus* species, was based on a misidentification of the specimens examined.

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Received January 7, 1964.
Accepted for publication January 31, 1964.

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Dielectric Constants and Solubility

Sir:

A recent publication of Sorby, Bitter, and Webb (1) reporting the dielectric constants of the water-ethanol-glycerin and water-ethanol-propylene glycol systems has prompted this communication. Since we are also presently engaged in studies of this nature, we wish to indicate briefly some additional observations concerning the possible relation between dielectric constant and the solubility of nonelectrolytes. These observations will be the subject of detailed reports at a later date.

Our studies have led us to believe that the dielectric constant principle of solvent blending outlined by Moore (2) is fortuitous. Implicit in Moore's suggestion is the presumption that solvent systems of approximately the same dielectric constant will show the same solvent properties for given drugs. Goyan (3) has recently pointed out the limitations of this assumption. Sorby, *et al.* (1), have suggested that Moore's method may fail in practice because of the approximations made in computing rather than directly measuring dielectric constants of complex mixtures. In a previous communication (4) we had pointed out that Moore had not considered cosolvency phenomena in his treatment.

A detailed examination of one of Moore's examples, the solubility of phenobarbital as a function of dielectric constant, proves illustrative. Figure 1 shows the solubility data of phenobarbital in several binary systems which were originally reported by Krause and Cross (5) and Peterson and Hopponen (6). The peak solubilities in different blends are seen to fall within a narrow range of dielectric constant (27–30). This observation led to our suggestion (4) that a dielectric requirement (DR) for solubility exists, *i.e.*, the dielectric constant at which peak solubility is observed in a solvent blend. At constant temperature, this requirement should be independent of the actual nature of the solvents in the blend and dependent only upon the nature of the drug. The existence of such a requirement has been confirmed in the cases of salicylic acid (DR: ~15) and theobromine (DR: ~20 and ~42) in binary systems of widely differing composition (7).

If Moore's technique is valid, then its application should not critically depend upon the selection of the solvent which determines the value of the approximate dielectric constant (A.D.C.)

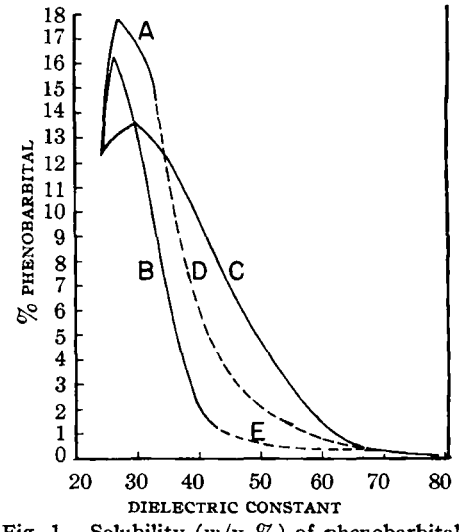

Fig. 1.—Solubility (w/v %) of phenobarbital in various binary systems at 25° plotted as a function of dielectric constant. Key: A, propylene glycol-ethanol; B, glycerin-ethanol; C, water-ethanol; D, propylene glycol-water; E, glycerin-water.