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# PAPER CHROMATOGRAPHIC DETECTION AND COLORIMETRIC DETERMINATION OF SOME 5-O-SUBSTITUTED TRYPTAMINES (3-(2-AMINOETHYL)INDOLES), UTILIZING THE FORMATION OF XANTHYLIUM SALTS*

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SUMMARY

A method is described which allows the rapid and precise quantitative determination, either directly in expressed plant juice, or in crude alkaloid extracts of 5-hydroxytryptamine (serotonin), N,N-dimethyl-5-hydroxytryptamine (bufotenine), 5-methoxytryptamine, and N,N-dimethyl-5-methoxytryptamine in the presence of tryptophan, tryptamine, N,N-dimethyl-5-methyltryptamine, N,N-dimethyltryptamine, 3-(2-dimethylaminoethyl)-indole (gramine), *p*-(2-dimethylaminoethyl)-phenol (hordenine), or 3-indolylacetic acid. The absorption spectra, effect of variable concentrations in the composition of the developing reagent, and conditions for the determination are reported. The concentrations of N,N-dimethyl-5-methoxytryptamine found in *Phalaris tuberosa* leaves are given.

## INTRODUCTION

Indolealkylamines are widely distributed in the plant kingdom[1]. GALLAGHER AND KOCH[2] recently implicated certain of these biogenic amines in a neurological syndrome of grazing animals. The possible involvement of indolealkylamines in the toxicity of range grasses precipitated our investigation of the effects of nutritional and environmental factors on the occurrence and biosynthesis of these amines in range plants. The more toxic of the tryptamines of *Phalaris tuberosa* (Hardinggrass) have been reported to be the 5-O-substituted tryptamine derivatives[2]. Methods for the quantitative determination of these compounds were the primary requirement for subsequent investigation of factors affecting the formation and accumulation of these alkaloids in plants.

One of the many color reactions of indoles is that with xanthydrol. Several methods have been proposed for determination of some tryptamine derivatives by formation of colored xanthylindole salts[3–5]. These methods were tested, but were not entirely suitable. Some of the procedures could not be replicated. Most of the methods investigated did not include the several tryptamines of interest. A method based on

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modifications of these procedures, combining paper chromatography and colorimetry, was developed and tested on ten indole compounds. All the 5-O-substituted tryptamines studied formed xanthylindole salts which had the same absorption maximum. The color formed was proportional to the concentration of the tryptamines. These could be quantitatively determined rapidly and precisely. The method allows the determination, either directly in expressed plant juice or in crude alkaloid extracts of plant material, of 5-hydroxytryptamine (serotonin), N,N-dimethyl-5-hydroxytryptamine (bufotenine), 5-methoxytryptamine, and N,N-dimethyl-5-methoxytryptamine in the presence of tryptophan, tryptamine, N,N-dimethyl-5-methyltryptamine, N,N-dimethyltryptamine, 3-(2-dimethylaminoethyl)-indole (gramine), *p*-(2-dimethylaminoethyl)-phenol (hordenine), or 3-indolylacetic acid.

## MATERIALS AND METHODS

### *Preparation of samples*

Fresh plant leaves were exposed to diethyl ether in a closed container for 3–5 min. Then the juice was expressed by the technique of MCCOMB AND RENDIG[6], and a quantity estimated to contain 10–100 $\mu$g of tryptamine derivatives was spotted on Whatman No. 1. chromatographic paper.

The tryptamines were isolated from dried leaf material by a procedure similar to that of KEFELI AND TURETSKAYA[7]. A weighed sample (40 mesh) was extracted with absolute methanol in a Soxhlet apparatus for approximately 7 h. The methanol was then evaporated, and 3–4 volumes of water were added to the liquid residue. After acidification with 0.1 *N* HCl, the mixture was extracted twice with an equal volume of toluene, then made alkaline with $NH_4OH$, and extracted three times with an equal volume of diethyl ether. The ether was evaporated and the residue made to volume with methanol. Samples estimated to contain 10–100 $\mu$g of tryptamine derivatives were spotted on the chromatographic paper.

### *Paper chromatography*

Included on each chromatogram were known tryptamine compounds. These were dissolved either in absolute methanol or in acidified (HCl) aqueous solution for spotting.

The chromatogram was irrigated with butanol–acetic acid–water (12:3:5, v/v) for 16 h, dried and then dipped in fresh xanthydrol reagent (1 % xanthydrol and 10 % trichloroacetic acid in absolute methanol). After drying, the 5-O-substituted tryptamine derivatives appeared as blue areas on a pale pink background.

### *Colorimetric procedure*

Those blue areas which had an $R_F$ value corresponding to the known were cut out and placed in spectrophotometer tubes. Five milliliters of methanol were added and the stoppered tubes were inverted a few times. Then 0.5 ml of concentrated HCl was added and the tubes were shaken again. The pieces of chromatographic paper were removed, and the percent transmittance was read at 600 m$\mu$. Areas of the paper similar in size and near the excised spots were treated in the same way and used to set the spectrophotometer at 100 % transmittance. The color was stable for at least 1 h. The amount of tryptamine in the sample was determined from a curve prepared from a chromatogram of the known compound (0–100 $\mu$g).

VARIABLES AND LIMITATIONS

The absorption spectra of the colors produced by the tryptamines and related compounds were determined, and the data for the compounds which produced xanthydrol-colored complexes are shown in Fig. 1. 5-Hydroxytryptamine, N,N-dimethyl-5-hydroxytryptamine, 5-methoxytryptamine, and N,N-dimethyl-5-methoxytryptamine produced curves almost identical in shape except for intensity. The

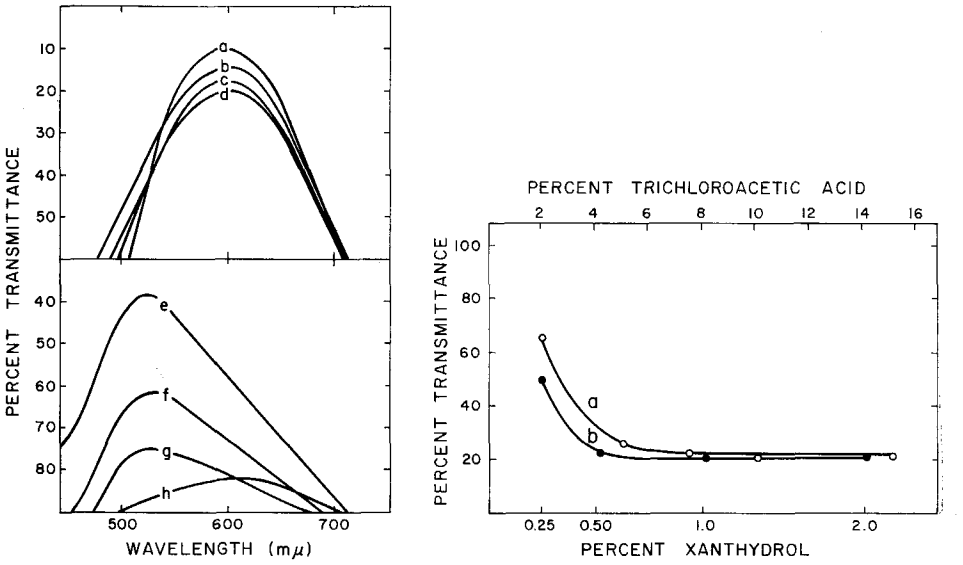


Fig. 1. Absorption maxima. a = 5-Hydroxytryptamine; b = N,N-dimethyl-5-hydroxytryptamine; c = 5-methoxytryptamine; d = N,N-dimethyl-5-methoxytryptamine; e = 3-indolylacetic acid; f = tryptamine; g = N,N-dimethyltryptamine; and h = N,N-dimethyl-5-methyltryptamine.

Fig. 2. Effect of reagent concentrations on transmittance. a = Trichloroacetic acid; b = xanthydrol.

absorption maximum in all instances occurred near 600 mμ. The absorption maximum for 3-indolylacetic acid, tryptamine, and N,N-dimethyltryptamine was near 525 mμ. N,N-Dimethyl-5-methyltryptamine failed to give a distinct maximum in the range of 500–700 mμ.

Initially a concentration of 15 % trichloroacetic acid, and xanthydrol concentrations of 0.25, 0.5, 1, and 2 % were tested. Maximum transmittance occurred with 1 and 2 % xanthydrol (Fig. 2).

Using 1 % xanthydrol 5, 7.5, 10, and 15 % trichloroacetic acid concentrations were tested. Maximum transmittance was produced with 10 % trichloroacetic acid (Fig. 2).

The xanthydrol reagent must be prepared directly before use, as maximum color development is not obtained when the reagent has been prepared longer than 2 h.

Plots of the log of the transmittance against the concentration over the range of 25–100 μg of the 5-O-substituted tryptamines gave straight lines (Fig. 3).

## DISCUSSION

Of the eleven tryptamines and related compounds which might be expected to occur in plant material[1], only 5-hydroxytryptamine, N,N-dimethyl-5-hydroxytryptamine, 5-methoxytryptamine, and N,N-dimethyl-5-methoxytryptamine produced a xanthylium·HCl salt the color of which was proportional to the concentration of the indole at 600 mμ. Gramine, hordenine, and tryptophan did not form colored complexes. 3-Indolylacetic acid, tryptamine, and N,N-dimethyltryptamine produced similar purple colors, all of which had absorption peaks at 525 mμ. No distinct absorption maximum was found for the color produced by N,N-dimethyl-5-methyltryptamine.

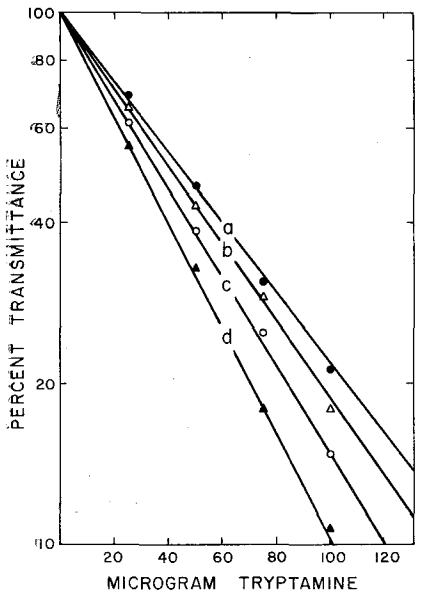


Fig. 3. Log of percent transmittance *vs.* μg tryptamine. a = N,N-Dimethyl-5-methoxytryptamine; b = 5-methoxytryptamine; c = N,N-dimethyl-5-hydroxytryptamine; and d = 5-hydroxytryptamine.

TABLE I

N,N-DIMETHYL-5-METHOXYTRYPTAMINE IN *Phalaris tuberosa* LEAVES

| *Leaf age (days)* | *Leaf condition* | *Tryptamine concentration (% dry weight)* |
|---|---|---|
| 7 | fresh | 0.236 |
| 9 | fresh | 0.105 |
| 21 | fresh | 0.077 |
| 21 | frozen (3 days) | 0.076 |
| 21 | dried | 0.071 |

Substitution of trichloroacetic acid for the acetic acid recommended by SCHREIER AND GAEDTKE[3] produced an intense color directly after the chromatogram was dipped and dried. Exposure to HCl fumes was not required. Because 9-substituted xanthylium salts are converted by alcohols to colorless ethers, it was necessary to add HCl to reform the colored indole-xanthylium·HCl complex. A more intense color was produced when the HCl was added after, and not with, the methanol. The color developed instantly. These results are in contrast to those obtained by the method of WILLIAMS[5], in which the color development time is critical.

The content of N,N-dimethyl-5-methoxytryptamine in leaves of *Phalaris tuberosa*, as determined by the proposed method, is shown in Table I.

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