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SEPARATION OF SOME INDOLYLALKYLAMINES FROM THEIR METHO CATION DERIVATIVES USING NEUTRAL POLYSTYRENE RESIN

APPLICATION TO THE ALKALOIDS OF *PHALARIS TUBEROSA* (*GRAMINEAE*)

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SUMMARY

It is shown that some indolylalkylamines —N,N-dimethyltryptamine, 5-methoxy-N,N-dimethyltryptamine and the related bases, 2-methyl-1,2,3,4-tetrahydro-β-carboline and its 6-methoxy derivative— are each cleanly separable from their respective metho cation derivatives on a column of the neutral polystyrene resin, Porapak Q, using methanol–0.2 *M* ammonium hydroxide (1:1) as the eluent. The method was applied to the isolation of the metho cations of N,N-dimethyltryptamine and 5-methoxy-N,N-dimethyltryptamine occasionally extracted from the pasture grass *Phalaris tuberosa*, together with the parent tertiary bases and others which are common constituents of the grass at all stages of its growth cycle. The metho cation of 5-methoxy-N,N-dimethyltryptamine was identified by paper electrophoresis and that of N,N-dimethyltryptamine by mass spectrometry.

INTRODUCTION

The value of the neutral polystyrene resin, Porapak Q, for the rapid extraction, desalting and fractionation of indole derivatives has recently been demonstrated by Niederwieser and Giliberti[1]. We have found that a modified technique can be used for the preparative isolation of the metho cations of some indolylalkylamines when these occur in admixture with their parent bases, and have used it in our studies of the alkaloids of the pasture grass *Phalaris tuberosa*. Most strains of this grown in Australia contain tryptamine alkaloids[2,3]. N,N-Dimethyltryptamine (DMT) is usually the most abundant of these and it is almost always accompanied by 5-methoxy-N,N-dimethyltryptamine (5MDMT) and gramine, together with smaller quantities of the related bases, 2-methyl-1,2,3,4-tetrahydro-β-carboline (2MTC) and its 6-methoxy derivative (6M2MTC)[3].

These are all tertiary amines, but several quaternary ammonium compounds are also detectable in extracts of *P. tuberosa* made at certain stages of its growth cycle, notably when it is rapidly regenerating following the first substantial rains of autumn or early winter. Two of these quaternary compounds have been identified as the

respective metho cations of DMT and 5MDMT and they can be isolated by the methods described here.

EXPERIMENTAL

Materials and reagents

Porapak Q (80–100 mesh) was obtained from Waters Ass., Framingham, Mass., U.S.A.

Commercial samples of DMT and 5MDMT (Aldrich, Milwaukee, Wisc., U.S.A.) were used without further purification. 2MTC and 6M2MTC were laboratory preparations[4], as were the methiodides of all four amines. These were prepared by dissolving each amine in a small volume of chloroform, adding excess methyl iodide and gently refluxing the mixtures for 2 h. The precipitated salts were obtained by filtration and recrystallized from ethanol.

A solution of sodium carbonate (0.05 *M*; pH 10.2) was used as the electrolyte for paper electrophoresis.

Xanthydrol (0.1% in ethanol containing 5% syrupy phosphoric acid) was used as the spray reagent for the indolylalkylamines on dried pherograms.

Other reagents were commercial samples of analytical grade.

Apparatus

Fractions from the column were automatically collected with a Paton Model LDT fraction collector (Paton Industries Pty. Ltd., Stepney, South Australia.)

A Unicam SP 800 UV spectrophotometer was used to monitor column effluents.

Paper electrophoresis was conducted in the enclosed-strip apparatus described in detail elsewhere[5], using Whatman No. 4 paper in strips 13.5×61 cm, with 45 cm under pressure and cooled. Ice-water was circulated through the coils of the cooling-plate and maintained the temperature of the paper at about 3°.

Mass spectrometry was performed with a Hitachi Perkin-Elmer Model RMU 6D instrument and fluorometric analysis with an Aminco-Bowman spectrofluorometer.

Procedures

Fractionation of the mixtures. This is illustrated with the mixture DMT (1.5 mg) and DMT methiodide (1.8 mg). The mixture also contained KNO_3 (10 mg), KCl (11 mg) and Na_2SO_4 (17 mg) to demonstrate the desalting procedure.

Porapak Q was suspended in acetone for 30 min, washed thoroughly with freshly-boiled water and allowed to settle into a glass column (1 cm I.D.) to a bed-depth of about 3 cm. (The column size may be scaled up to accommodate larger quantities of mixtures, if required.) The resin was equilibrated with ammonium hydroxide (0.1 *M*) and the test mixture, dissolved in 6 ml of ammonium hydroxide, was applied to the top of the column where the indole bases became firmly adsorbed.

Elution of Fraction I, inorganic salts. Irrigation of the column with ammonium hydroxide (50 ml; 0.1 *M*) effectively desalted the mixture. The weight (39 mg) of dried residue from this fraction indicated that the inorganic ions, including iodide, were recovered quantitatively.

Fraction II, DMT metho cation. The column was then eluted with methanol–

0.2 *M* ammonium hydroxide (1:1) at about 0.4 ml/min. DMT metho cation was preferentially desorbed and eluted from the column (as its hydroxide) by the passage of about 90 ml of eluent. The course of the elution was followed by recording the Ultraviolet (UV) spectrum of each 5 ml of eluate in succession. (DMT metho cation has $\lambda_{max.}$ 218 and 278 nm with a shoulder at λ 288 nm.)

Fraction III, DMT. The DMT was recovered by eluting the column with 50 ml of methanol–water (3:1), the course of the elution again being followed by UV spectroscopy. (The UV spectrum of DMT is very similar to that of its metho derivative.)

The column was cleared with absolute methanol and reequilibrated with ammonium hydroxide in preparation for the next experiment.

Paper electrophoresis. The eluting solvents were removed from the respective fractions under reduced pressure and the residues each dissolved in 3 or 4 drops of 0.05 *M* acetic acid. These solutions were transferred with a platinum loop delivering 1 μl to the starting-line of a paper strip impregnated with the sodium carbonate electrolyte. Solutions (0.02 *M*) of DMT and DMT methiodide were also applied to the starting-line as standards and electrophoresis was allowed to proceed for an hour or more. The papers were dried, sprayed with the xanthydrol reagent and heated for 7 min at 110° to reveal the indole bases as purple spots.

Fraction I was thus shown to be devoid of the bases, Fraction II to consist exclusively of DMT metho cation (mobility: 4.4 $cm \cdot h^{-1} \cdot kV^{-1}$ of applied voltage) and Fraction III to consist of DMT (mobility: 1.6 $cm \cdot h^{-1} \cdot kV^{-1}$).

Mass spectrometry. The mass spectrum of DMT metho cation isolated from *P. tuberosa* was identical with that of an authentic sample. Samples were introduced in the heated inlet system of the mass spectrometer as the hydroxides, but as expected, there was no trace of a molecular ion (220) because N-quaternary N,N,N-trimethylamino derivatives undergo Hofmann degradation under these conditions and liberate trimethylamine whose spectrum on electron bombardment is superimposed on those of the other components[6].

Trimethylamine (59) gives rise to a major peak (58) by loss of a hydrogen radical. The other product of the Hofmann degradation, 3-indolyl ethylene (143), constitutes the base peak of the spectrum and this loses ethylene to form the indolyl ion (115) which also appears as a major peak.

The distribution coefficients of DMT and of DMT metho cation between Porapak Q and methanol–0.2 M ammonium hydroxide (1:1). The static method described by Inczédy[7] was used for the determinations, the load being equivalent to about 30 μmoles of each base per gram of resin. The analyses of the solutions after setting up the equilibria were performed fluorometrically[8] (λ excitation 275 nm; λ emission 350 nm) and the weight distribution coefficients (*D*) calculated according to the definition:

$$D = \frac{\text{wt. of alkaloid/g of resin}}{\text{wt. of alkaloid/ml of solution}}$$

RESULTS AND DISCUSSION

The indolylalkylamines of *P. tuberosa* are usually extracted into chloroform from aqueous solutions of the grass contents made alkaline with ammonia or sodium

carbonate[2,3] and although the quaternary ammonium compounds sometimes present are salt-like in character they, too, are extracted into the chloroform layer, apparently quantitatively. Many quaternary ammonium compounds are known to form ion-pairs with suitable anions and to be extractable as such into organic solvents from aqueous media[9,10]. It is possible that the quaternary compounds of *P. tuberosa* are similarly extracted into chloroform as ion-pairs with anions, organic or inorganic, which occur naturally in the grass.

The charge on quaternary ions cannot be suppressed even in highly alkaline media (unless they are chemically changed) and they usually exhibit characteristic cationic mobilities when subjected to paper electrophoresis using sodium hydroxide as the electrolyte. By this means, the quaternary compounds extracted from *P. tuberosa* were readily identified as such, and were easily separated from the accompanying tertiary bases, which have their ionization completely suppressed in sodium hydroxide. Separations were also achieved in more mildly alkaline media such as sodium carbonate, as described in the Experimental section.

The evidence presented by Niederwieser and Giliberti[1] shows that an electric charge on the molecule of an indole derivative diminishes its adsorption on Porapak Q. It seemed that the preparative-scale separation of metho cations from the parent tertiary amines should be possible under alkaline conditions which would suppress the ionization of the latter and favour their adsorption on a column of the resin while the charged metho cations were eluted. Contrary to this expectation, however, it was found in tests with mixtures of DMT and its metho cation that both substances were strongly adsorbed on Porapak Q from dilute aqueous solutions of sodium or ammonium hydroxides. The passage of relatively large volumes of the alkaline solutions completely failed to remove the bases and this indicated that any desalting of the mixtures which might be necessary would present no problems.

Selective desorption of the bases with less polar alkaline solvents was then tried and a 1:1 methanol–0.2 *M* ammonium hydroxide mixture proved to be the eluent of choice. The distribution coefficients of the bases (DMT, 143; DMT metho

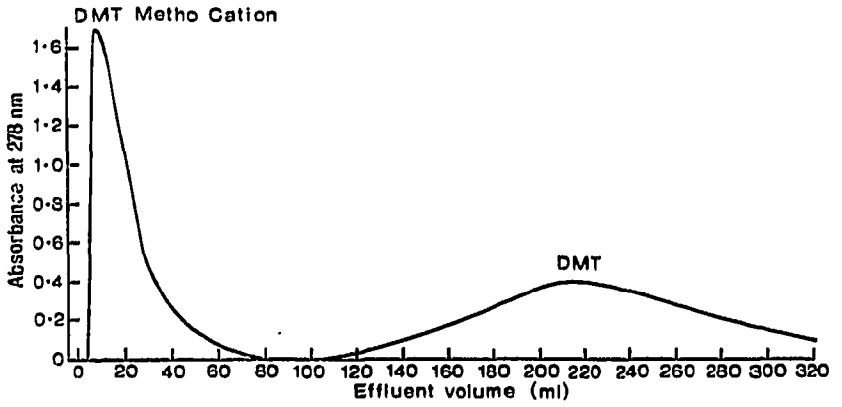

Fig. 1. Separation of DMT metho cation (5.5 μmole) and DMT (8 μmole) on a column (10 × 30 mm) of Porapak Q in methanol–0.2 *M* ammonia (1:1). Flow-rate: 0.4 ml/min. The eluate was monitored by UV spectroscopy at 278 nm; $\varepsilon = 6000$ for both compounds.

cation, 62) are consistent with the elution pattern obtained with this solvent and this is shown in Fig. 1.

As the curve indicates, complete recovery of the DMT metho cation is possible before any DMT emerges from the column. The DMT may also be recovered quantitatively with the same eluent, but an inconveniently large volume is required. It is more easily removed from the column as a relatively sharp peak by omitting the ammonia from the eluent and increasing the proportion of methanol as indicated in the Experimental section.

It has been found that the metho derivatives of 5MDMT, 2MTC and 6M2MTC can likewise be separated from their parent tertiary bases on a column of Porapak Q, but the method is not applicable to gramine and its metho derivative because the latter is rapidly decomposed in the alkaline solutions.

On rare occasions the metho derivative of DMT was the only quaternary compound detectable in extracts of *P. tuberosa*. It was therefore conveniently separated from the tertiary bases by the above procedure and obtained sufficiently pure to establish its identity by mass spectrometry. When it occurred in the grass during phases of active growth, the metho derivative was found to constitute up to 5% of the total isolated alkaloids, the content of these often reaching 0.08% of the dry weight of the grass. Other compounds behaving as quaternary bases sometimes occurred in the grass extracts, but of these only 5MDMT metho cation has tentatively been identified by UV spectroscopy and paper electrophoresis in several different electrolytes. It seems, on the basis of paper electrophoretic comparison with authentic samples, that the remaining compounds are not the metho derivatives of 2MTC, 6M2MTC or gramine.

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