Journal of Chromatography, 245 (1982) 155–157
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,984

Note

Separation of tryptophan derivatives in *Phalaris aquatica* by thin-layer chromatography

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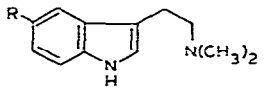
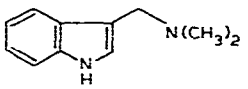
(Received April 26th, 1982)

The simple alkaloids N,N-dimethyltryptamine (I; R = H), 5-methoxy-N,N-dimethyltryptamine (I; R = OCH_3), gramine (II) and closely related alkaloids have been found in many plants, particularly among the Gramineae[1].



I



II

For example, the grass *Phalaris aquatica* produces all three alkaloids[2,3] together with several other indoles. Our attempts to resolve this complex mixture by gas–liquid chromatography or high-performance liquid chromatography were unsuccessful, but the mixture could be separated by two dimensional thin-layer chromatography (TLC) into 22 spots reacting with the Van Urk–Salkowski reagent (see ref. 4); fifteen compounds could be identified by comparison with standards and estimated by the Van Urk–Salkowski reagent.

EXPERIMENTAL

Methods

Standards for TLC were purchased from Sigma (St. Louis, MO, U.S.A.) with the exception of 3-aminomethylindole and 3-methylaminomethylindole which were synthesised[5].

Plant extracts

Seedlings (10-20 g) of *P. aquatica* cv Australian Commercial grown as previously described[6] were extracted with ethanol (2 × 20 ml). The ethanol extract was filtered, evaporated to dryness and the residue dissolved in 0.5 ml of 0.01 *M* hydrochloric acid.

TLC

Two-dimensional TLC was carried out on silica gel plates (Merck cat. No. 5715; 20 × 20 cm). The first dimension was developed in chloroform–methanol–

0021-9673/82/0000–0000/$02.75

0.088 ammonia (80:15:1) and the second dimension in *n*-butanol–ethanol–0.088 ammonia (20:1:1). Aliquots (5–20 μl) of standards (0.2–4.0 nmol) or of plant extracts were applied to the plates. After developing, the plates were sprayed evenly with Van Urk–Salkowski reagent[4] until the gel layer was transparent. The plates were heated in an oven at 105°C for 6 min and were then washed in distilled water three times to remove the acid. The position and colour of the spots on the dried plate were noted and the spots were scanned with a RFT Transidyne 2955 scanning densitometer.

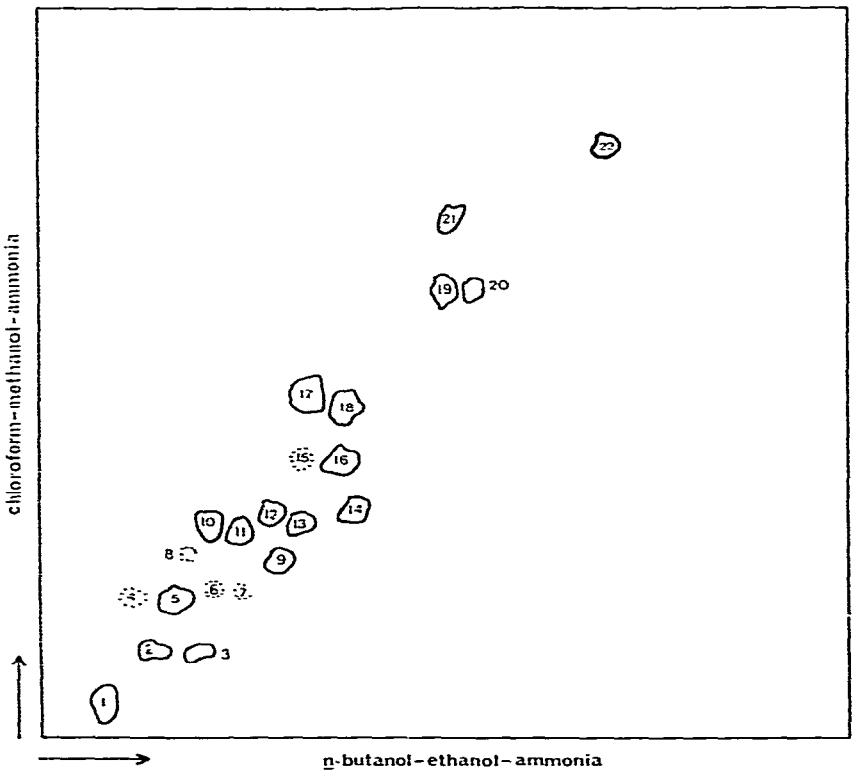


Fig. 1. Separation of tryptophan derivatives in ethanol extract of 7-day old *P. aquatica* seedlings by two-dimensional TLC. The identification of the numbered spots is indicated; the colour given by Van Urk–Salkowski reagent is shown in brackets as a colour region, colour name and page number from the *Horticultural Colour Chart*[7]. Only the colour for compounds not included by Ehmann[4] is given. 1 = Tryptophan, 5-methoxytryptophan; 2 = 5-hydroxy-N-methyltryptamine (blue, Sea Blue, 119); 3 = 5-hydroxytryptamine; 4 = unknown (blue, Princes Blue, 98); 5 = 3-methylaminomethylindole (violet, Campanula Violet, 37); 6 = unknown (red, Rose Madder, 23); 7 = unknown (yellow, Majolica Yellow, 102); 8 = unknown (blue, Princes Blue, 98); 9 = 3-aminomethylindole (violet, Campanula Violet, 37); 10 = 5-methoxy-N-methyltryptamine (blue, Princes Blue, 98); 11 = N-methyltryptamine (blue, Princes Blue, 98); 12 = 5-methoxytryptamine; 13 = tryptamine; 14 = 5-hydroxy-N,N-dimethyltryptamine; 15 = unknown (mauve, Dauphin's Violet, 117); 16 = gramine; 17 = unknown (yellow-grey, Yellow Ochre, 101); 18 = unknown (blue-grey, Wisteria Blue, 154); 19 = 5-methoxy-N,N-dimethyltryptamine (blue, Cornflower Blue 164); 20 = N,N-dimethyltryptamine (blue, Princes Blue, 98); 21 = unknown (purple, Magnolia Purple, 114); 22 = indole-3-aldehyde.

RESULTS AND DISCUSSION

A typical chromatogram obtained with an extract from 7-day old seedlings is shown in Fig. 1. The fifteen compounds identified by comparison with standards and the eight unidentified spots are indicated in the legend to Fig. 1. Clean separations were obtained with all the compounds, with the exception of tryptophan and 5-methoxytryptophan which could not be resolved. Only two of the eight unidentified spots seemed to be major components. These were spots 17 and 18 which gave a yellow-grey and a blue-grey colour, respectively, with the reagent. These colours are similar to those given by 2-methyl-1,2,3,4-tetrahydro-β-carboline and its 6-methoxy derivative. These compounds have been found in other strains of *P. aquatica*[3] but have different chromatographic properties than spots 17 and 18.

The intensity of the colour of the reference compounds versus their concentration was linear between 0.2 and 2.0 nmol and could be used as a quantitative measure of these compounds in plant extracts. For example, in 7-day old seedlings, the concentrations of the identified compounds ranged from 2 nmol/100 seedlings for 3-aminomethylindole to 300 nmol/100 seedlings for N,N-dimethyltryptamine, the major alkaloid in the plant extract. The Van Urk–Salkowski reagent has two advantages: it is very sensitive, detecting as little as 0.2 nmol, and it gives specific colours with different groups of indole metabolites. Thus compounds related to gramine, such as 3-aminomethylindole and its methyl derivative, 3-methylaminomethylindole, as well as gramine itself, give a purple colour; most of the tryptamine derivatives give a strong blue colour.

The widespread distribution of these alkaloids suggests that this method should have a wide application.

ACKNOWLEDGEMENTS

We thank Dale Lambert for synthesising 3-aminomethylindole and 3-methylaminomethylindole and J. L. Frahn and R. Illman of C.S.I.R.O., Division of Nutritional Biochemistry, Adelaide for gifts of 2-methyl-1,2,3,4-tetrahydro-β-carboline and 2-methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline.

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