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ANALYSIS OF PLANTS FOR FLUOROACETIC ACIDS

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Abstract—A paper chromatographic separation of mono-, di- and tri-fluoroacetic acids applicable to plant analysis is described. Improvements to the thioindigo method for the qualitative detection of the monofluoroacetate ion are presented. The improved method was used to detect this ion in *Dichapetalum heudelotii* (Planch. ex Oliv.) Baill. (Dichapetalaceae).

THE MONOFLUOROACETATE ION (MFA) is found naturally in *Dichapetalum toxicarium*[1] and *D. cymosum*.[2] There is also strong, but not conclusive, evidence for its presence in other *Dichapetalum* species: *D. michelsonii*,[3] *D. guineense*,[4] *D. venenatum*,[5] *D. braunii*,[6] *D. macrocarpum*,[7] *D. ruhlandii*,[7] and *D. stuhlmanii*.[7] In addition MFA is known to be present in *Acacia georginae*,[8] *Gastrolobium grandiflorum*,[9] and *Palicouria marcgravii*.[10]

While the natural synthesis of organofluorine compounds is intrinsically interesting, it is also important that the extremely poisonous MFA should be detected in plants, since plants containing it have been responsible for the deaths of many livestock.[3,11–13]

Lovelace *et al.*[14] have shown that MFA is produced in *Glycine max* if the plant is grown in a high fluoride environment. Other workers have investigated fluorine uptake by plants, particularly with respect to its toxicity.[15–17]

A number of methods have been used to detect MFA in plants: monofluoroacetic acid or a derivative have been isolated and unequivocally characterised,[2,8,9] while other workers have deduced the presence of MFA either from the deaths of animals,[3,7] or by the A.O.A.C. method[18] of qualitative analysis.[1,13] We have found that, after aqueous extraction of the plant tissue, this last method and the related quantitative method of Kawashiro *et al.*[19]

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are only successful if the tissues contain little or no iron, and only small amounts of red–brown pigments. Both of these interfere in the last stage of the analysis when the red thioindigo is produced if MFA is present. In the A.O.A.C. method[18] separation of MFA from interfering substances is effected either by adsorption of the latter on charcoal, or by partition chromatography on silicic acid. We have found that these separations are not successful with many plant extracts, but that separation is readily carried out by codistillation of the fluoroacids with water from acidic aqueous solution. When using the normal A.O.A.C. method,[18] iron is extracted together with monofluoroacetic acid into ether from aqueous sulphuric acid solution, presumably in the form of complexes with compounds which are naturally present in the plant.

Using the distillation method of separation, we have detected small amounts of MFA in *Dichapetalum heudelotii* (Planch. ex Oliv.) Baill. (Dichapetalaceae) where we could not detect it using the standard method, because of interference from both plant pigments and iron compounds.

MFA is present in comparatively large amounts in the young leaves of *Dichapetalum toxicarium*,[1] and it seemed possible that the difluoroacetate (DFA) and trifluoroacetate (TFA) ions might also be present. Neither DFA nor TFA have yet been found in plants. To our knowledge the only method so far published which describes the separation of MFA, DFA and TFA is that of Ramsey.[20] This method, which uses column chromatography, is applicable to the separation of 1–15 mg of these ions. Since we needed a method which was more sensitive than this, our first attempts at the separation of MFA, DFA and TFA were by the PC method of Bergmann and Segal[21] which is capable of separating acetate and MFA as the hydroxamates. This method, we discovered, gave a streak rather than spots with a mixture of MFA, DFA and TFA after development. With an aqueous solution from the young leaves of *D. toxicarium* which was known to contain MFA, again only a streak could be observed. This result was probably obtained because of the complex nature of the solution. After a number of trials we found that MFA, DFA and TFA could be readily separated as their acids by ascending PC using ethanol–ammonia–water–pyridine as the mobile phase and developing with Nile Blue. This method does not detect either formic acid or acetic acid, salts of which are known to be present in *D. cymosum*.[2] As with the modified thioindigo method we found that an initial distillation of the volatile acids was necessary in plant analysis. Only 20 μl of a solution containing 1 mg/ml of MFA, DFA or TFA was needed for the detection of these ions.

When this PC method was applied after distillation from an aqueous acidic extract of the young leaves of *D. toxicarium* only one spot was observed whose R_f was identical with that of pure MFA. DFA and TFA were therefore absent.

We would suggest that as it is known[14] that plants grown in a high fluoride environment can synthesise MFA, other workers in this field would do well to analyse their plants to determine if MFA, DFA or TFA can be synthesised under these conditions.

EXPERIMENTAL

Preparation of plant extract. This is applicable to either the thioindigo qualitative method,[18] or to the PC method.

The plant tissue was digested with H_2O at 90° for 1 hr. This digestion is known to remove MFA quantitatively.[1] After filtration the solution was made distinctly alkaline and evaporated to *ca.* 10 ml. After acidification with H_2SO_4, the solution was distilled down to *ca.* 1 ml. Further distillation caused the distillate

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to become coloured with decomposition products. The distillate was used directly for the A.O.A.C. qualitative method. A minimum of 0·5 mg MFA should be present at this stage. If only trace amounts of MFA, DFA or TFA were suspected the solution was made alkaline, concentrated, re-acidified and then used for the PC separation.

PC separation of MFA, DFA and TFA. 20 μl of solutions containing 1 mg/ml MFA, DFA or TFA were spotted separately on to Whatman No. 1 paper. A further 20 μl containing 1 mg/ml each of MFA, DFA and TFA was spotted onto the same paper. The mobile phase was EtOH–conc. NH_4OH–pyridine–H_2O (95:3:1:1). The separation was by ascending PC. Development was by spraying with Nile Blue solution which with MFA, DFA and TFA gave well defined blue spots on a brown background. R_fs at 28° were 0·65, 0·83 and 0·91 respectively. Neither HOAc nor HCO_2H could be detected under these conditions. The Nile Blue reagent was prepared by dissolving 0·4 g of the compound in 100 ml EtOH, adding triethanolamine until the blue colour changed to purple, and then adding a further 100 ml EtOH.

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