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Note

Simultaneous methylation and elution of organic acids from thin-layer chromatograms

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In the determination of acidic plant hormones such as abscisic acid, it is customary to elute appropriate zones of thin-layer chromatograms using alcohol–water or water-saturated ethyl acetate. Our experience is that low concentrations of organic acids, when adsorbed on relatively large quantities of silicic acid, are not eluted quantitatively by these procedures. Elution with 2% sodium bicarbonate solution is quantitative but leads to much additional work.

Methylation of organic acids decreases their adsorption to silicic acid. Hence methylation prior to elution is desirable. Some workers have methylated before the thin-layer chromatographic procedure[1] but suitable chromatographic solvents may not be available.

Chalvardjian[2] esterified fatty acids by refluxing thin-layer zones with methanol–benzene–sulphuric acid mixtures. This procedure, however, is not suitable for abscisic acid. We now report that distillation of diazomethane into a suspension of thin-layer absorbent results in quantitative methylation and elution of abscisic acid. The simplicity of the technique recommends itself for use with other organic acids.

Extracts containing plant organic acids were obtained by the usual procedures of partitioning between diethyl ether and aqueous solutions of appropriate pH. These were applied to aluminium foil-backed thin-layer sheets (Kieselgel F_{254}; Merck, Darmstadt, G.F.R.; Cat. No. 5554), which were then developed with diisopropyl ether–formic acid (95:5) and subsequently dried overnight in darkness. To validate the technique, replicate 100-μg spots of abscisic acid were chromatographed in the same way as the plant extracts. Abscisic acid-containing zones were identified with the help of a UV lamp, and then carefully cut out with ordinary scissors. The zones were folded to fit inside disposable polyethylene test tubes. (Other studies showed that silicic acid can be scraped off conventional glass plates and transferred into the test tubes, without changing results appreciably.)

Two alternative solvent systems were used. If adequate amounts of abscisic acid were present in the extract, then 5 ml of ethyl acetate–methanol (9:1) was added. Diazomethane was distilled into the sample until a persistent yellow colour formed[3]. After 10 min, excess diazomethane was destroyed with a few drops of 5% acetic acid in diethyl ether. Since silicic acid does not form a suspension in this solvent, 5-

or 10-μl aliquots were removed for gas chromatography without any preliminary filtration. Abscisic acid was determined on a Varian 1800 gas chromatograph with electron-capture detector (Varian, Palo Alto, Calif., U.S.A.) using a column of 3% OV-17 on Chromosorb W-DMCS at 215°. Recovery of the standard solutions averaged 101.5%, standard deviation 6.4% ($N=4$); this is within the limits of accuracy of our equipment.

Some samples did not contain enough abscisic acid to use a 5-ml dilution. In such cases, methylation and elution were performed with 5 ml of diethyl ether–methanol (9:1) but the solvent was then taken to dryness using a warm water-bath and a stream of nitrogen. It was not usually necessary to remove the thin-layer chromatography medium before drying. The residue was shaken with 0.5 ml ethyl acetate, and 5- or 10-μl aliquots were then removed with care to prevent needle blockage. Although additional steps were involved, recovery of standard abscisic acid using an ethereal methylation procedure averaged 98.3%, with a standard deviation of 2.0% ($N=6$).

This system may prove useful even with organic acids that can be eluted quantitatively as free acids, since the combination of methylation with elution is quite convenient.

REFERENCES

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