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Note

Separation of rotenoids by high-pressure liquid chromatography

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For many years rotenone has had biochemical application as an enzyme inhibitor specific for cellular respiration. As such, it has been extensively used to study the uncoupling of mitochondrial oxidative phosphorylation. Rotenone has also enjoyed widespread use as an insecticide, as it has been considered less of a toxic hazard to man than the widely used organochlorine and organophosphorous insecticides. However, Gosalvez and Merchan[1] have recently reported the induction of mammary tumors in rats exposed to rotenone. In order to confirm the findings of Gosalvez and Merchan, the study must be repeated using a rotenone sample of known purity.

While the literature contains reports of rotenoid separation by thin-layer chromatography[2,3] and gas chromatography[4,5], neither method can provide a quick and reproducible separation of at least six rotenoids as required for a purity analysis. High-pressure liquid chromatography (HPLC) has also been used to separate rotenoids, but produced less than satisfactory separations[6].

This report describes an HPLC method for the complete separation of six rotenoids, using a reversed-phase gradient system.

EXPERIMENTAL

Chromatographic system

A DuPont Model 830 high-pressure liquid chromatograph was used containing a UV detector operating at 254 nm and having gradient elution capability. The chromatograph was equipped with a DuPont Zorbax ODS column (25 cm × 2.1 mm I.D.), particle size 6–10 μm. The oven temperature was maintained at 34° and the column pressure, at 2000 p.s.i.

Chemicals

Reference samples of rotenone, tephrosine, rotenolone, deguelin, dehydrorotenone and dehydrodeguelin were the generous gift from Dr. Norman E. Delfel, Industrial Crops Laboratory, U.S. Department of Agriculture. All of the organic solvents used were of analytical grade.

Analytical method

Initially, each of the rotenoids was separately chromatographed to determine their retention time and order of elution in our chromatographic system. A mixture of the six rotenoids was then prepared to confirm the chromatographic peak resolution obtained for the individual chemicals.

The rotenoids were separated by gradient elution. The initial solvent composition was methanol–water–phosphoric acid (60:39.9:0.1). The final solvent composition was methanol–water–phosphoric acid (85:14.9:0.1). A convave hyperbolic gradient system was used that fits the equation, $\overline{X} = \overline{Y}^2$. The gradient rate was 1%/min, going from 10 to 100% in 90 min. The flow-rate was approximately 0.8 ml/min.

RESULTS AND DISCUSSION

Fig. 1 shows a chromatogram of a rotenoid mixture. The order of resolution is rotenolone, tephrosin, rotenone, deguelin, dehydrorotenone and dehydrodeguelin. Total separation of the six compounds takes approximately 55 min.

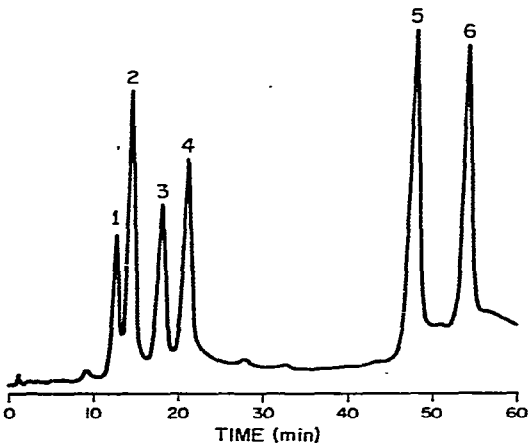

Fig. 1. Chromatogram showing the separation of a mixture of rotenoids on a DuPont Zorbax ODS microparticulate column, using gradient elution [initial solvent composition, methanol–water–phosphoric acid (60:39.9:0.1); final solvent composition, methanol–water–phosphoric acid (85:14.9:0.1)]. The gradient rate was 1%/min, going from 10 to 100% in 90 min. The flow-rate was *ca.* 0.8 ml/min. 1 = Rotenolone; 2 = tephrosin; 3 = rotenone; 4 = deguelin; 5 = dehydrorotenone; 6 = dehydrodeguelin.

The rapid and complete separation of the rotenoid mixture makes this procedure practical for industrial quality control application. It would also be useful in measuring the shelf life of commercially available pesticide preparations containing rotenone as the active ingredient. While quantitation was not pursued in this study, it could easily be accomplished by determining the absorptivity for each rotenoid at 254 nm, and then performing peak height measurements.

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