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Determination of Rotenoids in Soil and Crops by High-performance Liquid Chromatography*

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Using high-performance liquid chromatography, a method for the determination of seven rotenoids (rotenone, *O*–demethylrotenone, dehydrorotenone, 8′–hydroxy–6aα,12aα–rotenolone, 6aβ,12aβ–rotenolone, 6aβ,12aα–rotenolone and 8′–hydroxyrotenone) in soil and crops has been investigated. Successful separation was achieved by reversed-phase chromatography (column packed with JASCO, SC–02). The limits of detection were 0.02–0.08 ppm for all seven rotenoids. The mean recoveries of rotenone added to apples, tomatoes and soil were greater than 95% in all samples and those with the other six rotenoids were also satisfactory for quantitative residue analysis.

INTRODUCTION

Rotenone [6aβ,12aβ–4′,5′–tetrahydro–2,3–dimethoxy–5′β–isopropenylfurano–(3′,2′ : 8, 9)–6H–rotoxen–12–one] has widely been used as a botanical insecticide which permits to use without any significant toxic hazard to man. Until now, only the several methods to determine pesticide formulation levels of rotenone and rotenoids such as chromatographic procedures *i.e.*, gas liquid chromatography (*glc*)[1] and high-performance liquid chromatography (HPLC)[2–4] have been reported. However, these methods have not succeeded in complete separation of rotenoids, and any analytical method available to determine residue levels of rotenone and rotenoids has not yet been established. Recently, M.C. Bowman, *et al.*[5] reported a HPLC method to determine four rotenoids in animal tissues.

In this paper, a simple method is described for determination of the seven rotenoids in soil and crops by reversed-phase HPLC.

EXPERIMENTAL

1. *Chemicals and Reagents*

Rotenone and related compounds used in the study are shown in Fig. 1. All of them were provided by Prof. Dr. I. Yamamoto in Tokyo University of Agriculture. All other reagents were of analytical grade. Silica gel for column chromatography was Merck silica gel 60, 0.063–0.200 mm (70–230 mesh).

2. *Apparatus*

TRI ROTAR (JASCO) was employed for high-performance liquid chromatography. Rotenone and rotenoids were determined by the absorbance at 294 nm. JASCO, SC–02 reversed-phase column (4.2 mm i.d. × 25 cm) was employed and operated at an ambient temperature. The mobile phase was consisted of methanol–water (70 : 30 v/v,) and the flow rate was 2 ml/min (120 kg/cm^2). Chart speed was 0.5 cm/min and a sensitivity of the UV detector was setted at 0.04 A.U.F.S. in most instances.

3. *Determination of Rotenoids in Field Samples*

Homogenized apples and tomatoes (100 g) were extracted by shaking with acetone (150 ml) at room temperature for 30 min. The acetone extract was separated by filtration, and the residue was further extracted with the same solvent (50 ml) and filtered. After the concentration of the filtrate, saturated sodium chloride solution (150 ml) was added.

* Most part of this study was presented at Pesticide Science Society, Japan, 1979.

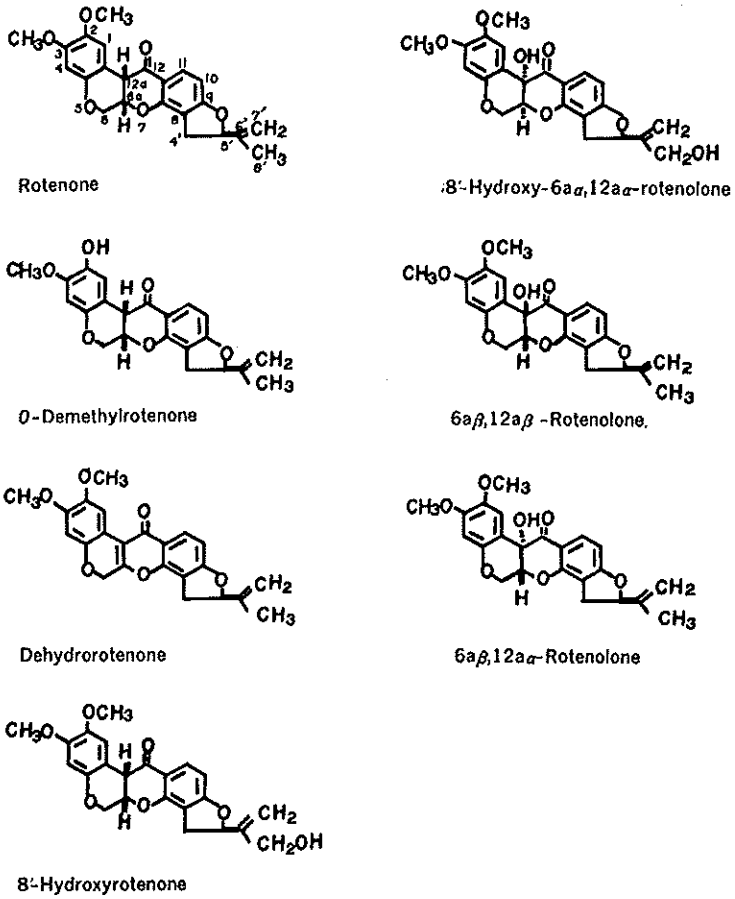

Fig. 1 Structure of rotenoids.

Then, the rotenoids were extracted twice with dichloromethane (100 ml) and evaporated to dryness *in vacuo*. The residue was dissolved in a little volume of *n*-hexane and passed through Silica gel column (10 g; 15 mm i.d.× 125 mm). After the successive washing of the column with 20% ethyl acetate–*n*-hexane (100 ml) and 30% ethyl acetate–*n*-hexane (10 ml), it was eluted with 30% ethyl acetate–*n*-hexane (160 ml) and ethyl acetate (100 ml), in this order. The effluents of 30% ethyl acetate–*n*-hexane and ethyl acetate were individually concentrated *in vacuo*. The each residues were dissolved in 10 ml of methanol, and the aliquots (10–50 μl) were injected into HPLC.

Soil was extracted by shaking with acetone–water (3 : 1, 200 ml) at room temperature for 30 min. And the extract was analyzed in the same procedure as described for crops.

RESULTS AND DISCUSSION

The wavelengths of maximal absorption were 294 nm for 8'-hydroxy-6aα,12aα-rotenolone, *O*-demethylrotenone, 6aβ,12aβ-rotenolone, 6aβ,12aα-rotenolone and rotenone, 292 nm for 8'-hydroxyrotenone and 279 nm for dehydrorotenone. To determine all the compounds tested, the absorption at 294 nm was selected for monitoring the eluate throughout the experiments.

For residue analysis of rotenone by HPLC, a variety of columns were tested and SS-05 (silica gel) with mobile phase dichloromethane–*n*-hexane (80 : 20 v/v) and SC-02 (ODS) were found to be suitable. However, for complete separation of rotenoids, SC-02 column was the most suitable. High-performance liquid chromatography on SC-02 column of the soil and crops extracts showed a complete separation of seven rotenoids. The chromato-

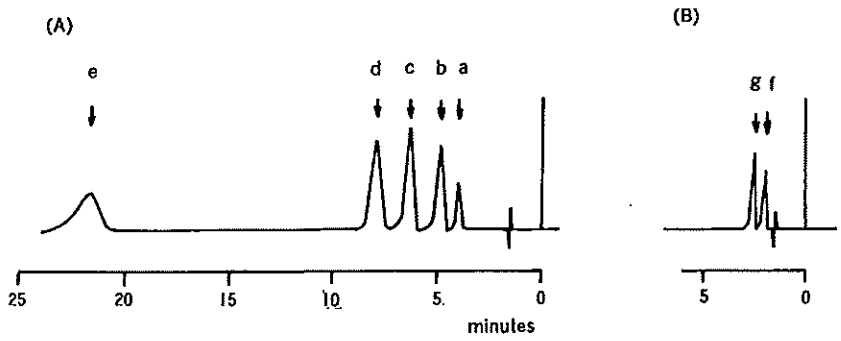

Fig. 2 HPLC of rotenoids extracted from soil.
Conditions: see experimental
A: 30% ethyl acetate–*n*–hexane fraction
B: ethyl acetate fraction
Peak a) *O*–Demethylrotenone, b) 6aβ,12aβ–Rotenolone, c) Rotenone, d) 6aβ,12aα–Rotenolone, e) Dehydrorotenone, f) 8′–Hydroxy–6aα,12aα–rotenolone, g) 8′–Hydroxyrotenone

Table 1 Recovery of rotenoids.

Rotenoids	Fortified (ppm)			Recovery* (%)		
	Soil	Tomato	Apple	Soil	Tomato	Apple
8′–Hydroxy–6aα,12aα–rotenolone	1.25	1.25	1.25	73.6	85.4	80.2
8′–Hydroxyrotenone	4.0	2.0	2.0	91.5	93.6	94.1
O–Demethylrotenone	5.0	2.5	2.5	70.0	92.7	85.9
6aβ,12aβ–Rotenolone	5.0	2.5	2.5	94.8	99.5	99.2
Rotenon	5.0	2.5	2.5	94.9	97.7	98.3
6aβ,12aα–Rotenolone	5.0	2.5	2.5	97.2	100.0	96.8
Dehydrorotenone	10.0	5.0	5.0	81.6	102.2	82.2

* Results are the means of duplicate determinations.

gram on the soil extract is shown in Fig. 2 as an example. The effluent of 30% ethyl acetate–*n*–hexane contained *O*–demethylrotenone, 6aβ,12aβ–rotenolone, rotenone, 6aβ,12aα–rotenolone and dehydrorotenone. And 8′–hydroxy–6aα,12aα–rotenolone and 8′–hydroxyrotenone were eluted in ethyl acetate.

The retention times for 8′–hydroxy–6aα,12aα–rotenolone, 8′–hydroxyrotenone, *O*–demethylrotenone, 6aβ,12aβ–rotenolone, rotenone, 6aβ,12aα–rotenolone and dehydrorotenone were 2.04, 2.50, 4.12, 4.92, 6.34, 7.92 and 21.66 min, respectively.

Recoveries of rotenoids (rotenone, 6aβ,12aβ–rotenolone and 6aβ,12aα–rotenolone) added to apples, tomatoes and soil were greater than 95% as shown in Table 1. And those with other rotenoids were about 80$\pm$10%.

Table 2 shows the limits of detection, which were 0.02–0.08 ppm for all seven rotenoids.

Table 2 Limit of detection. (ppm)

Rotenoids	Soil	Tomato	Apple
8′–Hydroxy–6aα,12aα–rotenolone	0.04	0.06	0.03
8′–Hydroxyrotenone	0.05	0.08	0.04
O–Demethylrotenone	0.04	0.02	0.02
6aβ,12aβ–Rotenolone	0.04	0.02	0.02
Rotenone	0.03	0.02	0.02
6aβ,12aα–Rotenolone	0.04	0.02	0.02
Dehydrorotenone	0.08	0.04	0.04

Therefore, this method appears to be suitable for quantitative residue analysis.

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要　　約

高速液体クロマトグラフィーによる土壌および作物中の Rotenoids の定量法

小林裕子，俣野修身，後藤真康

Rotenone および rotenoids の残留分析法を確立する目的で土壌および作物について，UV 検出器付高速液体クロマトグラフィーによる同時同定および定量を行なった．本実験に用いた rotenoids は rotenone, *O*-demethylrotenone, dehydrorotenone, 8′-hydroxy-6aα, 12aα-rotenolone, 6aβ,12aβ-rotenolone,6aβ,12aα-rotenolone, 8′-hydroxyrotenone の7種である．これら7種の rotenoids の検出限界は 0.02-0.08 ppm である．Rotenone の平均回収率は，リンゴ，トマト，土壌いずれの場合でも 95% 以上であり，他の rotenoids についても，残留分析をおこなうに十分な回収率が得られた．