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Note

The identification of iridoids of *Mentzelia decapetala* by two-dimensional paper chromatography

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Iridoid glucosides can be detected in plant parts in many ways¹. A rapid darkening often occurs on collection or processing^{2,3}. Field tests involving chemical color tests include heating with dilute hydrochloric acid⁴ or acetous copper sulfate⁵. Color reactions in conjunction with one-dimensional paper or thin-layer chromatography, as developed by Wieffering⁶ and Kooiman⁷, are currently used⁸⁻¹¹. We recently reported the structure elucidation of two iridoid glucosides obtained from *Mentzelia decapetala*^{12,13}. The purpose of this communication is to report a sensitive two-dimensional paper chromatographic method with application of diagonal chromatography for the detection of iridoid glucosides in crude extracts of *M. decapetala*, which could be utilized as a general screening procedure.

EXPERIMENTAL

The R_F values were measured on Whatman No. 1 paper using the ascending technique in 1-butanol-acetic acid-water (63:10:27, v/v). An aqueous solution (3.3%, w/v) obtained from the previously reported¹² extraction of *M. decapetala* was spotted (10 μ l). After the solvent had advanced *ca.* 20 cm (18 h) the papers were removed and air dried. After spraying lightly with an aqueous suspension (3%, w/v) of β -glucosidase, and leaving for 8 h in a horizontal position between glass plates, the chromatograms were developed using the same solvent in a second direction for *ca.* 10 cm. For the control, water was used as the spray instead of the enzyme. After air drying the chromatograms, the spots were visualized by heating at 100° for *ca.* 3 min with a spray reagent. Used was either a solution of vanillin (1 g) and concentrated hydrochloric acid (3 ml) in methanol (150 ml)¹⁴ or *p*-anisidine hydrochloride (4 g) in water (10 ml) and 1-butanol (90 ml).

RESULTS AND DISCUSSION

Application of the two-dimensional paper chromatographic technique allowed the detection of five iridoid glucosides, as shown in Table I (Nos. 4-8). The high R_F

TABLE I

 R_F VALUES OF IRIDOID GLUCOSIDES IN EXTRACTS OF *Mentzelia decapetala*Mean R_F values of three experimental results, measurements being made to the front of the spots.

No.	One-dimensional	Two-dimensional		
		Direction one	Direction two	
			Control	Enzyme
1	0.82	1.00	1.00	1.00
2	0.76	0.84	0.91	0.90
3	0.64	0.74	0.80	0.81
4	0.56	0.63	0.68	0.70, 0.95
5*	0.46	0.54	0.56	0.62, 0.93
6**	0.37	0.45	0.46	0.45, 0.81
7	0.24	0.30	0.29	0.26, 0.62
8	0.17	0.23	0.23	0.19, 0.50

* Mentzeloside.

** Decaloside.

values of Nos. 1–3 and the similarity between these values and those of the aglycones produced by the hydrolysis of compounds with one-dimensional R_F values of 0.56, 0.46 and 0.37 suggested that these are free aglycones. The significant increases in all the two-dimensional R_F values as compared to the one-dimensional, presumably caused by the diffusion of compounds during the hydrolysis period, necessitated the control.

Two of the compounds have been structurally identified as new iridoids, No. 5 being mentzeloside¹² and No. 6 being decaloside¹³. During the initial work these and No. 4 were present in large amounts, but extraction of plant parts obtained the following year from the same location at the same stage of development did not reveal the latter iridoid. This can be explained by climatic variance in iridoid metabolism. The compounds with R_F values of 0.24 and 0.17 gave only faint spots. That these compounds are present in only trace amounts has been subsequently verified by column chromatography.

The detection of free iridoid aglycones is very unusual. The only definite proven natural occurrence is that of genipin in *Genipin americana*¹⁵. Thus Nos. 1–3 may be formed during the fixation and extraction of the plant material. The faintness of these spots would also be indicative of this.

Both spray reagents were routinely used. The free reducing groups present in both of the hydrolysis products were detected with the *p*-anisidine reagent, while vanillin does not react with sugars but is equally affected by iridoid aglycones or glycosides. The R_F values in Table I were obtained using the vanillin reagent, which gave a detection limit of ca. 1 μ g for each compound. There has been no previous mention in the literature with respect to the sensitivity of spray reagents for iridoids. Distinctive coloration was also obtained; for example, mentzeloside and decaloside gave pink and violet spots, respectively. The *p*-anisidine reagent was useful for showing that glucose was the only carbohydrate formed. Hence the use of these spray reagents in conjunction with two-dimensional paper chromatography is a sensitive technique that allows the detection of both glycosides and aglycones of iridoids.

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