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Ultraviolet-induced isomerization of β-D-glucosyl o-hydroxycinnamic acid on filter paper*

Coumarinic acid glucoside (β-D-glucosyl *cis-o*-hydroxycinnamic acid) and *o*-coumaric acid glucoside (β-D-glucosyl *trans-o*-hydroxycinnamic acid) are readily detected as absorbing areas on filter paper chromatograms exposed to ultraviolet light at wavelengths near 260 mμ. Long wavelength ultraviolet radiation is frequently used to detect fluorescent compounds closely related to these two glucosides. The foregoing facts prompted this investigation concerning the influence of both long and short wavelength ultraviolet light on small amounts of coumarinic acid glucoside and *o*-coumaric acid glucoside, air-dried on filter paper strips. Ultraviolet-induced interconversion of these two isomers in aqueous solutions is well known[1].

Procedure

The two glucosides were isolated from hot water extracts of sweetclover leaves by paper chromatography. The solvent consisted of 2% acetic acid[2]. In this system R_F values for coumarinic acid glucoside and *o*-coumaric acid glucoside are 0.90 and 0.66, respectively. The glucosides were detected on test strips cut from chromatographic sheets; this prevented exposure of the entire chromatograms to ultraviolet light. Bands representing the two glucosides were cut out and eluted with water; eluates were assayed[3] and then diluted with water to a final concentration of 1 μmole/ml.

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A 0.1-ml aliquot of the coumarinic acid glucoside solution was applied to each of thirty-six 1 × 11-in. strips of Whatman No. 1 filter paper, along a 9-in. line marked in the center of the strip. Similarly, *o*-coumaric acid glucoside was applied to 36 filter paper strips. All strips were air-dried, after which they were irradiated at a distance of approximately 12 in., with either a Mineralight model R53* lamp (peak intensity near 254 mμ) or a Gates MR4* lamp equipped with the TF8 tube (peak intensity near 360 mμ). Strips were irradiated in duplicate for the times indicated in Fig. 1.

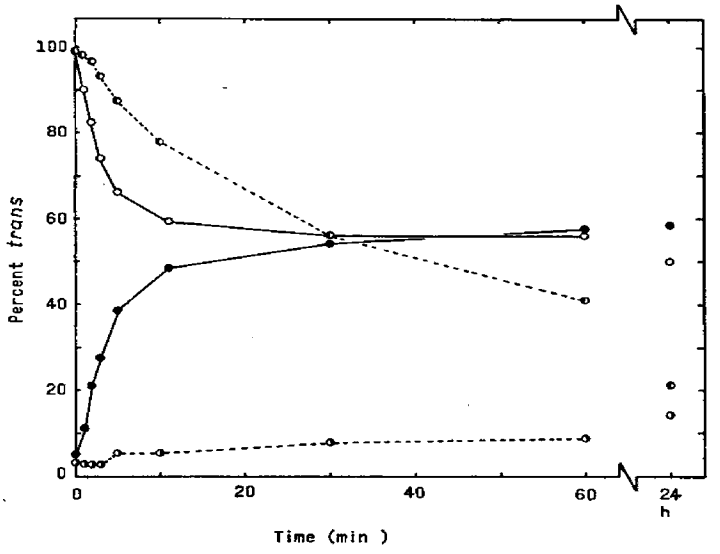

Fig. 1. Influence of short (peak near 254 mμ) and long (peak near 360 mμ) wavelength ultraviolet light upon coumarinic acid glucoside (*cis*) and *o*-coumaric acid glucoside (*trans*) dried on filter paper strips. See text for description of conditions used. Isomer at start: *cis*, ●———● (254 mμ), ◑ – – – ◑ (360 mμ); *trans*, ○———○ (254 mμ), ◐ – – – ◐ (360 mμ).

After treatment, strips were placed immediately in a freezer where they were stored for subsequent elution and assay. For parts of the study requiring more than 0.1 μmole of compound per strip, successive 0.1-ml applications of the glucoside solutions were made, with drying between applications, until the desired quantities had been applied.

All treated strips were eluted with water in a descending chromatography apparatus until 0.5 ml of eluate was collected from each. Eluates were diluted to 2.0 ml with water and were then assayed for the glucosides of coumarinic and *o*-coumaric acids as previously indicated. All work was done either in a dark room or in the laboratory under subdued light.

Results and discussion

As shown in Fig. 1, both sources of ultraviolet light were effective in interconverting the *cis* and *trans* isomers of β-D-glucosyl *o*-hydroxycinnamic acid on filter paper. Under the conditions used, the Mineralight source effected a much more rapid interconversion of the two isomers than did the longer wavelength Gates lamp. With the Mineralight, extensive isomerization occurred with exposures of 2 min or less.

* Mention of specific instruments is for identification only and does not imply endorsement by the U. S. Department of Agriculture.

Thus, unless precautions are taken, appreciable isomerization is likely to occur during routine examination of chromatograms with short wavelength ultraviolet light.

The equilibrium point of the isomerization differed with the wavelengths of the ultraviolet light used. Thus, with the Mineralight lamp, at equilibrium approximately 55% of the glucoside was present as the *trans* isomer. Although equilibrium apparently was not reached during the 24 h of treatment with the Gates lamp, available evidence indicates that at equilibrium, between 15% and 20% of the compound would be in the *trans* form.

As might be expected, extended irradiation with the Mineralight lamp was highly destructive to β-D-glucosyl *o*-hydroxycinnamic acid. Recovery measurements indicated that only about 40% of the compound remained intact at the end of the 24-h treatment. No significant destruction was observed as a result of irradiation with the Mineralight source for 30 min or less, or from any of the treatments with the Gates lamp. The glucosides did not appear to be hydrolyzed by irradiation.

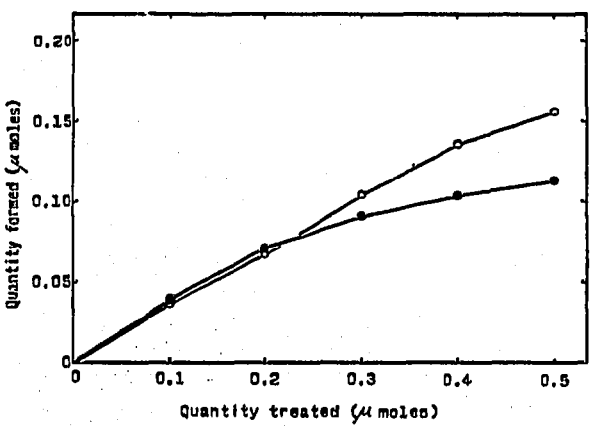

Fig. 2. Influence of concentration on extent of isomerization of β-D-glucosyl *o*-hydroxycinnamic acid dried on filter paper. Light source: Mineralight lamp (peak near 254 mμ). Treatment duration: 5 min. O———O *trans* to *cis* conversion; ●———● *cis* to *trans* conversion.

The extent of isomerization was influenced by the concentration of glucoside present on the paper when a 5-min duration of Mineralight irradiation was used (Fig. 2). The concentration effect was more pronounced in the case of coumarinic acid glucoside, indicating a difference in the spectral properties of the two glucosides in the dried condition. In aqueous solution, coumarinic acid glucoside is known to absorb maximally at 254 mμ, and *o*-coumaric acid glucoside at 270 mμ[2].

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