

1959

Influence of Spectral Composition of Light on *cis-trans* Interconversion of *o*- Hydroxycinnamic Acid

Francis A. Haskins

University of Nebraska - Lincoln, fhaskins@neb.rr.com

H. J. Gorz

University of Nebraska - Lincoln

Follow this and additional works at: <http://digitalcommons.unl.edu/agronomyfacpub>



Part of the [Plant Sciences Commons](#)

Haskins, Francis A. and Gorz, H. J., "Influence of Spectral Composition of Light on *cis-trans* Interconversion of *o*- Hydroxycinnamic Acid" (1959). *Agronomy & Horticulture -- Faculty Publications*. 169.
<http://digitalcommons.unl.edu/agronomyfacpub/169>

This Article is brought to you for free and open access by the Agronomy and Horticulture Department at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Agronomy & Horticulture -- Faculty Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Influence of Spectral Composition of Light on *cis-trans* Interconversion of *o*-Hydroxycinnamic Acid^{1,2}

F. A. Haskins and H. J. Gorz

From the Department of Agronomy, University of Nebraska, Lincoln, Nebraska

Received August 1, 1958

INTRODUCTION

In a recent report (1) from this laboratory, an improved fluorometric assay for coumarin in sweetclover is described. Briefly, the method consists of opening the lactone ring of coumarin by treatment with alkali, after which the resulting nonfluorescent *cis-o*-hydroxycinnamic acid is partially converted, by means of ultraviolet irradiation, to the fluorescent *trans* isomer. Measurements of fluorescence are then made. In the interval between ultraviolet treatment and measurement of fluorescence, the solutions to be assayed must be protected from the usual light of the laboratory, since exposure to such light results in loss of fluorescence, indicating a reconversion of some of the *trans* isomer to the *cis* form. The experiments reported here were designed to investigate, by fluorometric and spectrophotometric determinations, the influence of quality of light upon interconversion of the *cis* and *trans* isomers of *o*-hydroxycinnamic acid. Careful studies [e.g., (2-4)] have been made of the influence of ultraviolet irradiation upon *cis-trans* interconversion of various compounds, including *o*-hydroxycinnamic acid. A limited amount of information also is available concerning the influence of sunlight upon this compound (3), but no reports were found in the literature which deal specifically with the influence of quality of visible light upon the interconversion.

MATERIALS AND METHODS

Chemicals

Coumarin was obtained from the Eastman Kodak Company, and *o*-coumaric acid from the K and K Laboratories. Both compounds were recrystallized from water

¹ Published with the approval of the Director as paper No. 878, Journal Series, Nebraska Agricultural Experiment Station.

² Cooperative investigations of the Crops Research Division, Agricultural Research Service, U. S. Dept. of Agriculture, and the Nebraska Agricultural Experiment Station, Lincoln, Nebraska.

prior to use in these experiments. Quinine sulfate (U.S.P. grade) from Merck and Company was used as a standard in fluorometric determinations.

Light Source

A bank of twelve 48-in., 40-w. Standard Cool White fluorescent tubes (Amplex Corporation) was used as the light source. Distance from the light to the surface of the solutions receiving treatment was approximately 45 cm. Intensity of the nonfiltered light at the position of treatment was approximately 930 ft.-candles as measured with a Weston model 756 light meter. According to information received from the Amplex Corporation, the radiant-energy distribution of the Standard Cool White lamps is as shown in Table I.

Color Filters

The Corning glass color filters used in these studies are partially characterized in Table II. The ultraviolet-transmitting and blue filters transmit small percentages of certain wavelengths in the red which are not mentioned in the table, and all of the filters transmit to some extent in the infrared region of the spectrum. Colorless Pyrex glass was used as the colorless filter.

Procedure

In a darkroom with a Kodak Safelight (Wratten series OA filter) as a source of light for working, 4.4×10^{-3} *M* aqueous solutions of coumarin and *o*-coumaric acid were diluted 50-fold with 0.1 *N* NaOH to provide 8.8×10^{-5} *M* solutions of the *cis* and *trans* isomers, respectively, of *o*-hydroxycinnamic acid. Fifty-milliliter portions of these solutions were transferred to Pyrex Petri dishes 95 mm. in diameter which were contained in metal cans 100 mm. in diameter and 65 mm. deep. After removal of an initial 5-ml. sample, each can was covered with the appropriate filter and placed beneath the bank of fluorescent lamps. Solutions were irradiated at laboratory temperature, which, under the bank of lamps, ranged from 25 to 28.5°C. Following irradiation times of 0.25, 0.5, 1, 2, 4, and, in most instances, 6 hr., the filter-covered cans were moved to the darkroom where 5-ml. samples were removed.

TABLE I
Radiant-Energy Distribution of Amplex Standard Cool White Fluorescent Lamps^a

Color	Band	Radiant energy
	<i>mμ</i>	<i>% of total</i>
Violet	380-430	11.89
Blue	430-490	17.58
Green	490-560	26.84
Yellow	560-590	19.19
Orange	590-630	16.74
Red	630-680	7.76
		100.00

^a Personal communication from Mr. E. B. Saul, Director of Engineering, Amplex Corporation, Brooklyn, New York.

TABLE II
Partial Transmission Characteristics of the Corning Glass Color Filters Used^a

Filter designation		Transmission
Name	Color specification number	
Ultraviolet-transmitting	7-60	Zero below 290 $m\mu$, rises to 70% at 355 $m\mu$, falls to zero at 410 $m\mu$
Blue	5-60	Zero below 350 $m\mu$, rises to 65% at 415 $m\mu$, falls to zero at 530 $m\mu$
Green glass	4-74	Zero below 330 $m\mu$, rises to 68% at 490 $m\mu$, falls to zero at 700 $m\mu$
Green	4-65	Zero below 450 $m\mu$, rises to 63% at 545 $m\mu$, falls to 15% at 665 $m\mu$, then rises again
Yellow, sharp cut-off	3-69	Zero below 510 $m\mu$, rises to 88% at 565 $m\mu$, maintains that level throughout longer wavelengths of the visible spectrum
Red, sharp cut-off	2-58	Zero below 620 $m\mu$, rises to 88% at 690 $m\mu$, maintains that level throughout longer wavelengths of the visible spectrum

^a Summarized from "Glass Color Filters," a bulletin of the Corning Glass Works, copyright 1948.

All samples were stored in stoppered test tubes in the dark until determinations of ultraviolet absorption and of fluorescence could be made. In no case did the duration of storage exceed 8 hr. A Beckman model DU spectrophotometer equipped with the fluorescence attachment was used for the determinations. Absorption spectra were determined over the range from 240 to 390 $m\mu$. For determinations of fluorescence, a quinine sulfate standard was used as previously described (1). The $8.8 \times 10^{-5} M$ solutions were used without dilution for spectral readings, and were diluted 20-fold with 0.1 *N* NaOH for readings of fluorescence.

RESULTS AND DISCUSSION

It can be seen from the absorption spectra of *cis*- and *trans*-*o*-hydroxycinnamic acid (Fig. 1) that the *trans* isomer in base has prominent absorption peaks at about 280 and 360 $m\mu$, while the *cis* isomer has relatively low absorption at these wavelengths. Readings at these wavelengths were used, therefore, in calculating the percentage of the compound present as the *trans* isomer. For the 280- $m\mu$ readings, this equation was used:

$$0.932x + 0.251(100 - x) = \text{optical density}_{280 \text{ m}\mu} \times 100.$$

In the equation, x is the percentage of the compound present in the *trans* form, and 0.932 and 0.251 are the optical densities at 280 $m\mu$ of solutions which are $8.8 \times 10^{-5} M$ with respect to the *trans* and *cis* isomers, respectively. Graphs in which the 280- $m\mu$ -based percentage values were plotted

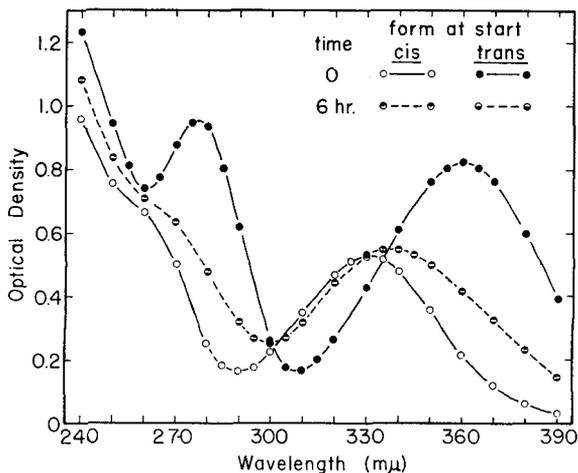


Fig. 1. Ultraviolet absorption spectra of $8.8 \times 10^{-5} M$ *o*-hydroxycinnamic acid in $0.1 N$ NaOH. Spectra are shown for the *cis* isomer, the *trans* isomer, and the equilibrium mixture resulting from exposing a basic solution of either isomer to blue light for 6 hr.

against duration of irradiation under the various filters are shown in Figs. 2 and 3. Agreement between the $360\text{-m}\mu$ -based values and the plotted values was excellent, differences in most instances amounting to less than 1%. Percentage values based upon determinations of fluorescence were generally a little lower (by as much as 5% in a few instances) than values based upon spectra. However, the conclusions permitted by the two types of determination do not differ appreciably.

It is clear from the data that not all wavelengths of light were equally effective in interconverting the *cis* and *trans* isomers. From the results with the green, yellow, and red filters, one can conclude that wavelengths longer than approximately $450\text{ m}\mu$ are without effect. It follows that probably no more than 15% of the radiant energy supplied by the fluorescent lamps is of wavelengths which are effective in bringing about the interconversion.

Exposure of the basic solutions to light of effective wavelengths eventually resulted in the establishment of equilibria between the *cis* and *trans* isomers as indicated by the graphs in Figs. 2 and 3. Within the range of effective wavelengths, the shorter the wavelength the higher was the percentage of *trans* isomer in the equilibrium mixture. Experiments utilizing an ultraviolet lamp whose energy peak is near $360\text{ m}\mu$ indicated that this trend continues into the ultraviolet. The equilibrium mixture resulting from exposure to this lamp was found to contain approximately 76% *trans* isomer and 24% *cis* isomer (Fig. 3).

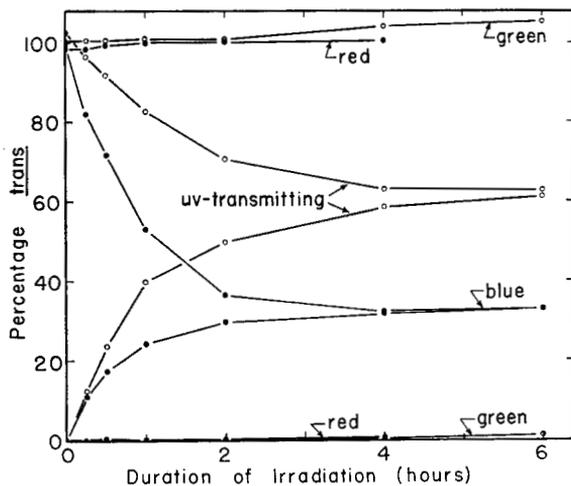


FIG. 2. Influence of light quality upon *cis-trans* interconversion in basic solutions of *o*-hydroxycinnamic acid. Initially the solutions were pure with respect to either the *cis* or *trans* form. Results are shown for ultraviolet-transmitting, blue, green, and red filters.

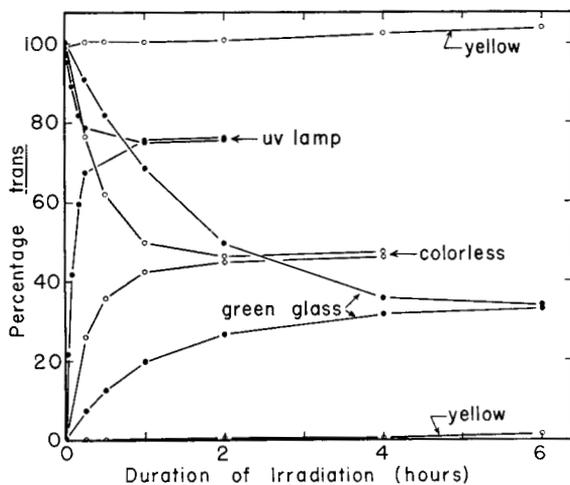


FIG. 3. Influence of light quality upon *cis-trans* interconversion in basic solutions of *o*-hydroxycinnamic acid. Initially the solutions were pure with respect to either the *cis* or *trans* form. Results are shown for colorless, green glass, and yellow filters and for an ultraviolet lamp.

Further evidence regarding the establishment of equilibria between the two isomers was obtained from an examination of absorption spectra throughout the range of 240–390 $m\mu$. After 6 hr. of irradiation under the blue filter, solutions which initially contained exclusively either the *cis* or the *trans* isomer had absorption spectra which were almost identical (Fig. 1). A similar situation was noted in solutions irradiated under the ultraviolet-transmitting filter, the green glass, and colorless glass, or under the ultraviolet lamp.

A comparison of results obtained with the blue filter and with the green glass demonstrates the influence of light intensity upon the time required for the attainment of equilibrium. These two filters have qualitatively similar transmissions in the region of the spectrum below 450 $m\mu$. The degree of transmission in this region, however, is somewhat greater for the blue filter than for the green glass (Table II). At equilibrium, approximately 32% of the compound irradiated under these filters was in the *trans* form, but the equilibrium point was attained in considerably less time under the blue filter than under the green glass (Figs. 2 and 3).

The possibility of destruction of *o*-hydroxycinnamic acid during the irradiation treatments should be considered. Polarographic evidence against such destruction has been presented by Patzak and Neugebauer (3) who state that when alkaline solutions of coumarin are irradiated, the quantity of coumaric acid formed is equivalent to the loss in coumarin. To obtain further evidence on this point, artificial mixtures were prepared to contain *cis*- and *trans*-*o*-hydroxycinnamic acid in the same proportions as the equilibrium mixtures resulting from irradiation of solutions under the ultraviolet-transmitting, blue, green glass, and colorless filters. In each case, the absorption spectrum and fluorescence of the equilibrium mixture resulting from irradiation were very similar to the spectrum and fluorescence of the comparable, nonirradiated artificial mixture. It is reasonable to conclude, therefore, that there was no appreciable destruction of the *o*-hydroxycinnamic acid molecule by the irradiation treatments. Furthermore, changes in absorption and fluorescence resulting from irradiation of the artificial mixtures under the appropriate filters were not appreciable. This observation adds further support to the conclusion that equilibrium had been attained in the irradiated solutions.

SUMMARY

Spectrophotometric and fluorometric determinations were made of the influence of light transmitted by various filters upon *cis-trans* interconversion in basic solutions of *o*-hydroxycinnamic acid. Wavelengths longer than 450 $m\mu$ were found to be without effect in interconverting the two isomers. Exposure of basic solutions to light of effective wavelengths

resulted in the establishment of equilibria between the *cis* and *trans* isomers. Within the range of effective wavelengths, decreases in wavelength were associated with increases in percentage of *trans* isomer in the equilibrium mixture.

REFERENCES

1. HASKINS, F. A., AND GORZ, H. J., *Agron. J.* **49**, 493 (1957).
2. LEWIS, G. N., MAGEL, T. T., AND LIPKIN, D., *J. Am. Chem. Soc.* **62**, 2973 (1940).
3. PATZAK, R., AND NEUGEBAUER, L., *Monatsh. Chem.* **82**, 662 (1951).
4. VAIDYA, B. K., *Proc. Roy. Soc. (London)* **A129**, 299 (1930).