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SHORT COMMUNICATION

TRANS-CIS-EQUILIBRIUM OF HYDROXYCINNAMIC ACIDS DURING IRRADIATION OF AQUEOUS SOLUTIONS AT DIFFERENT pH

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Abstract—*Trans-p*-coumaric acid, -caffeic acid, -ferulic acid and -sinapic acid in $0{\cdot}5 \times 10^{-3}$ molar aqueous solutions or in solutions of different pH were treated with 45,000, 15,000 and 5000 lx from a mercury vapour lamp in a rotating pyrex glass flask. Only 1·5–2 per cent of the total energy received from the lamp was effective for *trans-cis*-conversion. Solutions reached a constant *trans-cis*-equilibrium after 10, 30 and 90 min according to the three intensities of irradiation. The highest conversion to *cis* was found between pH 5·0–7·0 and the four *trans* acids were converted to 80, 42, 71, and 63 per cent *cis* respectively. Solutions of pH 3·5 contained only 62, 21, 37 and 32 per cent in the *cis* form. Sinapic acid and *p*-coumaric acid solutions of pH 6, prepared with four different buffers, all behaved similarly.

INTRODUCTION

Application of pure *trans*-sinapic acid solutions to leaves of *Brassica napus* var. *oleifera*, var. Lembkes Sommerraps delayed shoot and flower development for 1–4 weeks while treatment with the *cis*-compound hastened the development.[1] Besides other factors,[1] the *trans-cis*-equilibrium in the applied solutions seemed to be important in determining the reaction of the plants. This led to investigating how far the *trans*-compound is conversed to *cis in vitro* by light under different conditions.

Earlier workers have shown that different compounds show distinct equilibria, that there is a solvent effect and a concentration effect on the *trans-cis*-equilibrium,[2] and that bound cinnamic acids have a different equilibrium than the free acids.[2–4] Another factor is the pH of the cell sap, which can differ between plant species and during development. This paper reports an investigation of the *trans-cis*-equilibrium of phenylpropane derivatives in distilled water, in aqueous solutions of different pH, and in solutions of the same pH prepared with different buffers.

RESULTS

Intensity of Light and Trans-cis-*Conversion*

Aqueous solutions of *trans*-sinapic acid ($0{\cdot}5 \times 10^{-3}$ molar) irradiated with 45,000, 15,000 and 5000 lx showed nearly the same equilibrium after 10, 30 and 90 min treatment (Fig. 1).

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[1] G. Kahnt, *Naturwissenschaften.* In press.
[2] G. Kahnt, *Biol. Zb.* In press.
[3] F. A. Haskins and H. J. Gorz, *Agr. J.* **41,** 493 (1957).
[4] F. A. Haskins, L. G. Williams and H. J. Gorz, *Plant Physiol.* **39,** 777 (1964).

The equilibrium therefore is not influenced by the intensity of light in the selected region of energy. However in earlier experiments with intensities of 1500 lx and less this specific equilibrium characteristic was not attained even after 4 hr irradiation. Thus the conversion rate is a time-, light intensity-, and concentration-dependent reaction.

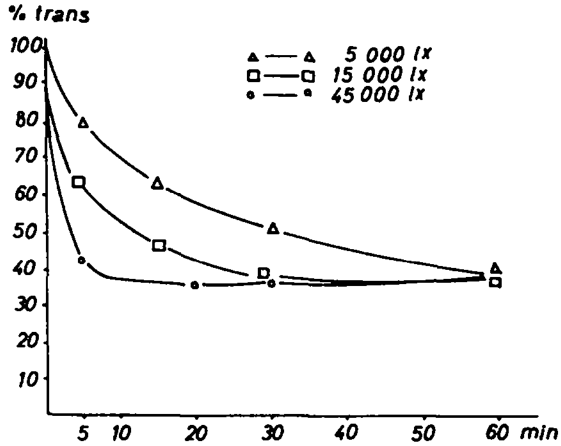

FIG. 1. *Trans* TO *cis* CONVERSION RATE OF AQUEOUS *trans*-SINAPIC ACID SOLUTIONS DURING IRRADIATION WITH DIFFERENT LIGHT INTENSITIES.

pH of the Solvent and Trans-cis-*Equilibrium*

15,000 lx treatment of *trans*-sinapic acid solutions, $0{\cdot}5 \times 10^{-3}$ molar, pH 3·5–pH 7·8, showed also an equilibrium after 30–35 min (Fig. 2), but it was very different in the acid-, neutral-, and basic region. Similar results were obtained with *p*-coumaric, ferulic and caffeic acids (Fig. 3). All compounds investigated showed maximal conversion to *cis* within the pH range from 5·0–7·0, while the least conversion to *cis* occurred at pH 3·5 and 7·8. Decreasing the concentration from $0{\cdot}5 \times 10^{-3}$ to 10^{-4} molar resulted in a 3–7 per cent increase

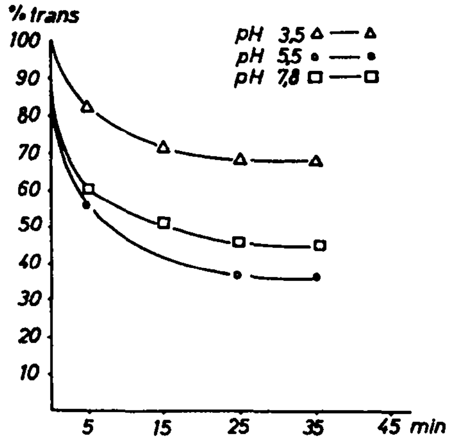

FIG. 2. *Trans* TO *cis* CONVERSION RATE OF *trans*-SINAPIC ACID SOLUTIONS OF DIFFERENT pH DURING IRRADIATION (15,000 LX, BUFFER: CITRIC ACID/Na_2HPO_4).

in the *cis*-form. Similar results have been found in earlier investigations using alcoholic solutions. Consequently *trans-p*-coumaric acid can be converted quantitatively to *cis* only at $0{\cdot}5 \times 10^{-6}$ molar and the other compounds at concentrations some powers lower.

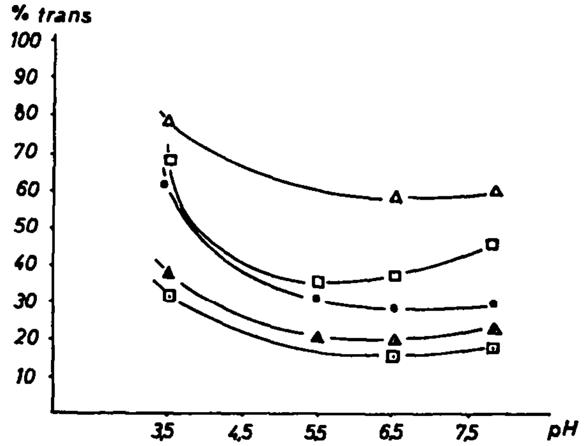

FIG. 3. *Trans-cis*-EQUILIBRIUM OF CAFFEIC (△—△), FERRULIC (○—○), SINAPIC (□—□) AND *p*-COUMARIC ACID (▲—▲, □—▣) AFTER 35 MIN LIGHT TREATMENT $0{\cdot}5 \times 10^{-3}$ MOLAR, □ = 10^{-4} MOLAR, BUFFER: CITRIC ACID/Na_2HPO_4, pH 6·0; 15,000 LX.

Type of Buffer and Trans-cis-*Equilibrium*

There was no difference in *trans-cis*-equilibrium of sinapic acid solutions of pH 6 prepared with four different buffers and only a small difference in *p*-coumaric acid solutions (Table 1).

TABLE 1. *Trans-cis*-EQUILIBRIA OF AQUEOUS *trans* SOLUTIONS AT DIFFERENT pHs AFTER 35 MIN LIGHT TREATMENT (15,000 LX, 250 W OSRAM MERCURY VAPOUR LAMP)

Buffer and pH (see Experimental)		Sinapic acid	*p*-coumaric acid	Caffeic acid	Ferulic acid
			($0{\cdot}5 \times 10^{-3}$ molar)		
			(% *cis*)		
I	3·5	32	62	21	37
	5·5	64	79	40	69
	6·5	63	80	42	72
	7·8	54	77	40	70
II	6·0	62	84	—	—
III	6·0	63	81	—	—
IV	6·0	62	78	—	—
Aqu. soln.	—	65	63	18	44
			(10^{-4} molar)		
I	3·5	—	67	—	—
	6·5	66	83	49	77
	7·8	—	79	—	—

DISCUSSION

From studying the *trans-cis*-equilibrium of *trans-p*-coumaric acid, -ferulic acid, -caffeic acid and -sinapic acid solutions after irradiation, it is clear that the equilibrium is effected by the compound itself, the molar concentration and the pH of the solvent. It is of interest that the highest *cis*-percentage was found at pH 5·0–7·0, a region within the range of the physiological pH of cell sap of many plants. So it is very probable, that the equilibrium found *in vitro* is nearly the same as that *in vivo*. This was found to be true in an earlier experiment using chlorogenic acid *in vitro* and leaf extracts from potato plants grown under short and long day conditions for the *in vivo* experiments.[5] With regard to the equilibrium of free and bound compounds, the analysis of Haskins *et al.*[4] showed for light treated *o*-coumaric acid ($3{\cdot}5 \times 10^{-6}$ molar in 0·25 N NaOH) 40% *cis* (coumarin), and for *o*-hydroxycinnamic acid glucoside ($1{\cdot}47–1{\cdot}10 \times 10^{-2}$ molar in water) 94% *cis*. Free caffeic acid (10^{-4} molar) in aqueous solution showed 45% *cis* and 3-caffeoylquinic acid only 20%. Thus the type of linkage is an important factor in determining the *trans-cis*-equilibrium. Furthermore, the compounds analysed occur in plants free or bound in a molar concentration ranging from 10^{-5} to 10^{-2} and their solubility in water must also be involved.

EXPERIMENTAL

Substances and Light Treatment

Aqueous solutions of authentic *trans-p*-coumaric acid, -ferulic acid, -caffeic acid and -sinapic acid (obtained from the firm of Roth, Karlsruhe) $0{\cdot}5 \times 10^{-3}$ molar were treated with 45,000, 15,000 and 5000 lx from a 250 W Osram mercury vapour lamp, and aqueous solutions of different pH were exposed to 15,000 lx. Only 1·5–2·0 per cent of the total energy received from this lamp was effective for *trans-cis*-conversion. During irradiation the 50 ml pyrex glass flask containing 10 ml of the *trans*-solutions was rotated.

Separation and Measurement of Isomers

This was done as earlier[5] on paper chromatograms by separation in 1% acetic acid for 2 hr. Solutions prepared with buffer IV were chromatographed for 3 hr in order to separate the buffer substance from the *cis*-spot.

Buffers Utilized[6]

I. citric acid/Na_2HPO_4 (pH 3·5, pH 5·5, pH 6·0, pH 6·5, pH 7·8); II. Sodium citrate/NaOH (pH 6·0); III. KH_2PO_4/Na_2HPO_4 (pH 6·0); IV. TRIS-maleate/NaOH (pH 6·0).

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[5] G. Kahnt, *Angew. Botan.* **39**, 177 (1966).

[6] M. H. Rauen, *Biochemisches. Taschenbuch*, Springer, Berlin (1965).