(a) Dans la molécule de β-alanine, une substitution sur le carbone β par un groupement méthyle ou phényle; la substitution sur le même atome de carbone par un groupement carboxylique, que réalise la passage de la β-alanine à l'acide aspartique, ne gêne cependant pas la formation de l'ester, mais l'acide aspartique s'est montré toutefois plus lentement estérifiable que l'acide glutamique.

(b) Dans la molécule d'acide glutamique, l'introduction d'un substituant en position γ inhibe l'estérification.

(c) Le remplacement du groupement carboxylique par un groupement acide sulfonique, dans les molécules d'acide aspartique et glutamique, empêche également la formation d'esters.

Remerciements

Nous remercions Mlle M. L. KORNGUTH et MM. BROCKMANN, ISENBERG ET FOWDEN, qui nous ont aimablement procuré certains acides aminés utilisés dans ce travail.

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Reçu le 30 juillet 1965

J. Chromatog., 21 (1966) 355–357

Paper chromatographic separation and behaviour of the *cis*- and *trans*-isomers of cinnamic acid derivatives

In a previous publication[1] it was shown that the *cis* and *trans* isomers of a number of cinnamic acid derivatives could be separated by paper chromatography using an aqueous 2 % v/v acetic acid solvent. With alcoholic and phenolic solvents the isomers were always unresolved. It was deduced that the spot of higher R_F in any given case represented the *cis* isomer, and that of lower R_F the *trans* isomer. Prior to this, CARTWRIGHT AND ROBERTS[2] examined the effect of aqueous acetic acid solvents upon the mobilities of *trans* cinnamic acid derivatives on paper chromatograms. They found that plain water, free from the slightest trace of acid, caused chlorogenic, *o*- and *p*-coumaric acids and caffeic acid to run very closely together near the solvent front.

The presence of only a trace of acetic acid caused a general lowering of R_F values together with considerable improvement in separation of the spots. Subsequent increase in concentration of acetic acid over the range 2% to 10% caused only a small general increase of R_F values with attendant small decrease in degree of separation.

In a later publication[3] we have described a procedure for the isolation of milligram quantities of the *cis* and *trans* isomers of *p*-coumaric acid by chromatography on thick paper, the separated isomer bands being cut out and eluted. It was found that solutions of the separated isomers could be preserved as such by keeping in total darkness. The U.V. spectra of the respective isomers in aqueous and alcoholic media were determined and the changes in the form of the spectra, after ionization, were interpreted by means of a derivative method[4,5]. It is the purpose of this communication to examine in more detail the chromatographic behaviour and U.V. spectra of the *cis* and *trans* isomers of a number of cinnamic acid derivatives.

Experimental

1% w/v solutions of cinnamic, *p*-coumaric, sinapic, caffeic, chlorogenic and ferulic acids, ethyl *p*-coumarate and a naturally occurring *p*-coumaroyl-O-glucose, all in ethanol, were sealed in Pyrex test tubes. These were irradiated for 24 h at a distance of about 6 in. under a Hanovia U.V. lamp with the Wood's glass filter attached to screen off excess visible light whilst allowing the most powerful band of radiation around 366 mμ to pass through. Paper chromatography of the irradiated solutions, in the absence of light, showed that in each case the *cis* spot of higher R_F was markedly increased in intensity as judged by fluorescence under the U.V. lamp.

The irradiated solutions were spotted onto Whatman No. 1 sheets in subdued light, and the sheets developed in total darkness. A glass tank with a glass trough was employed, and for the first run plain water was used with precautions taken to ensure the complete absence of traces of acid. Subsequent runs were made with 0.01, 0.1, 0.2, 0.4, 1, 2, 5, 10, 20 and 50% v/v glacial acetic acid–deionized water. After air-drying the sheets were examined under the U.V. lamp and the spots made visible by virtue of their fluorescence (if necessary in the presence of ammonia vapour) were marked. The isomers of cinnamic acid itself, which did not fluoresce with radiation of 366 mμ, were located as dark violet absorption spots under a Hanovia Chromatolite which emitted radiation mainly in the 254 mμ region. R_F values for all the respective *cis* and *trans* isomers were measured and plotted against the function $\log_{10}(c + 1)$ where c = conc. of acetic acid expressed as % v/v. This function was employed purely as a matter of convenience in presentation of the results.

The isomers of *p*-coumaric and ferulic acids and ethyl *p*-coumarate were isolated in ethanol solution by a procedure described previously[3]. The spectra of these were measured on a Unicam S.P. 500 spectrophotometer with precautions taken to minimise exposure to light during manipulation of the solutions.

Results and discussion

When chromatograms on which the *cis* and *trans* isomers had been resolved were inspected under the U.V. lamp it was observed that the *cis* isomers of caffeic, ferulic and chlorogenic acids were not immediately visible as blue fluorescent spots. After a few sec. exposure to U.V. light a weak blue fluorescence appeared, increasing rapidly in strength; after about half a minute, the *cis* band had the same appearance

as the *trans* band had all the time. A similar effect was found with *p*-coumaric acid; the *cis* isomer, unlike the *trans*, did not show an immediate violet fluorescence in the presence of ammonia vapour. Exposure of the chromatogram to daylight will also make the *cis* spot fluorescent; it seems clear that the *cis* isomers are not fluorescent but become visible after exposure to light by partial conversion to the fluorescent *trans* forms. A similar difference has been observed by HASKINS AND GORZ[6] between the *cis* and *trans* *o*-coumarate ions in basic solution.

The R_F values as plotted in Fig. 1–2 show clearly that the *cis* isomers of the free acids are, in general, considerably less sensitive to the presence of small amounts of acid in the developing solvent than are the *trans* isomers. This result might be expected in view of the greater planarity and lower solubility of the *trans* isomers[7] generally; the adsorptive properties of the cellulose must be considerably modified by the pres-

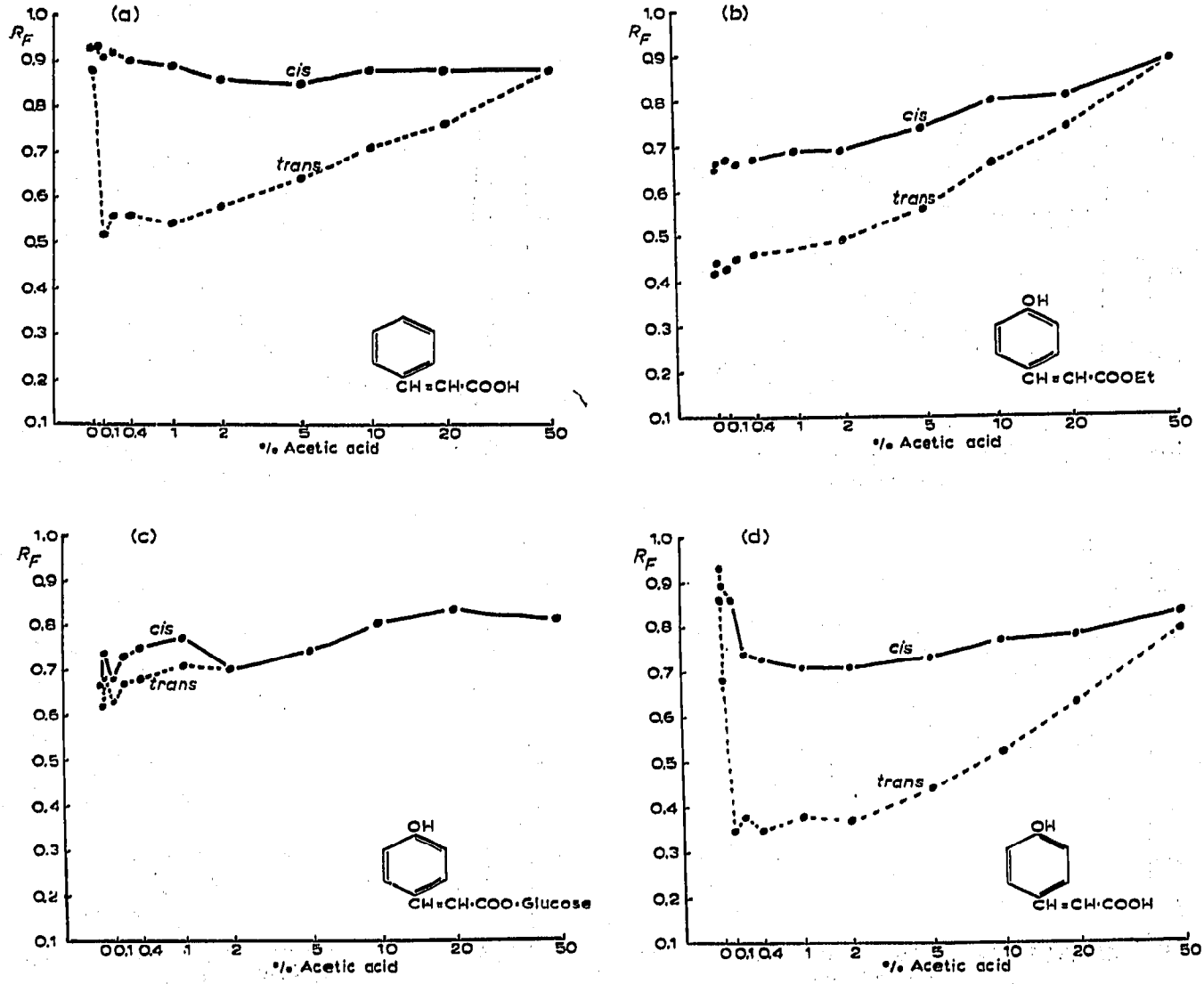

Fig. 1. Results of plotting the R_F values of the *cis* and *trans* isomers of a number of cinnamic acid derivatives against the concentration of acetic acid in the solvent; this being actually plotted as the function $\log_{10}(c + 1)$, where c = % v/v acetic acid in water. (a) Cinnamic acid. (b) Ethyl *p*-coumarate. (c) Glucose ester of *p*-coumaric acid. (d) *p*-Coumaric acid.

ence of very small amounts of acid and these variations affect the *trans* isomer to a greater extent than the *cis* isomer. The fact that the isomers of ethyl *p*-coumarate (Fig. 1b) do not show a lowering of R_F value by small amounts of acid suggests that a free carboxyl grouping is necessary for the phenomenon as observed with, for example, free *p*-coumaric acid (Fig. 1d). The glucose ester of *p*-coumaric acid (Fig. 1c) behaves in a similar manner to the ethyl ester, except that its greater solubility reduces the separation between the two isomers; concentrations of acetic acid above 2 % cause a failure of the isomers to resolve, whereas at lower concentrations they just separate.

CARTWRIGHT AND ROBERTS[2] recorded that phloroglucinol had identical R_F values in pure water and 2 % acetic acid solvents. We have found that phenolic acids such as *p*-hydroxy-benzoic and 3,4-dihydroxy-benzoic acids behave in a similar manner to *trans* *p*-coumaric and caffeic acids respectively. The reduced analogues of the latter cinnamic acids were found to behave more as the *cis* isomers of *p*-coumaric

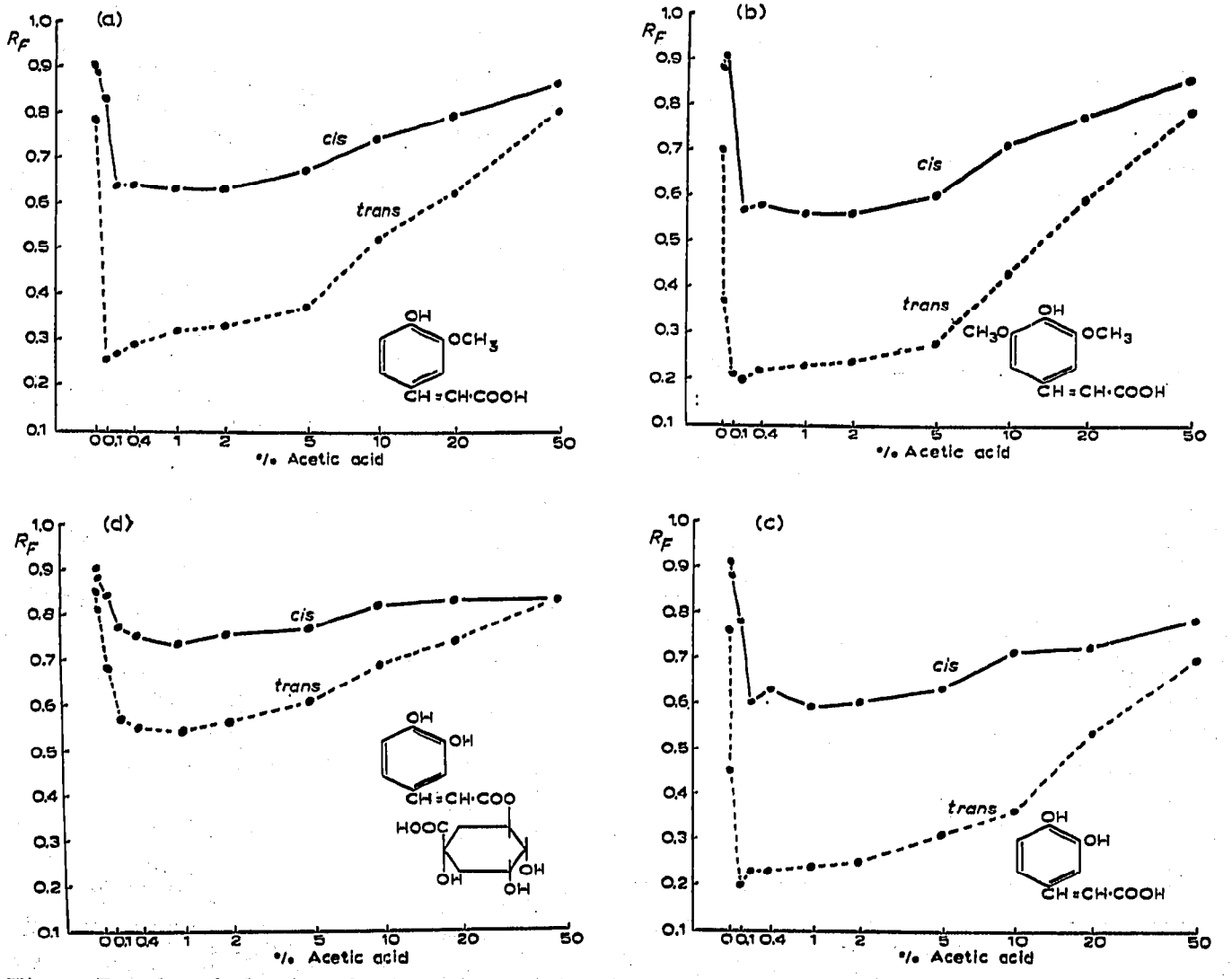

Fig. 2. Results of plotting the R_F values of the *cis* and *trans* isomers of a number of cinnamic acid derivatives against the concentration of acetic acid in the solvent; this being actually plotted as the function $\log_{10}(c + 1)$, where c = % v/v acetic acid in water. (a) Ferulic acid. (b) Sinapic acid. (c) Caffeic acid. (d) Chlorogenic acid.

and caffeic acids. It is evident that the extreme sensitivity of R_F values to small amounts of acid in the solvent occurs only with compounds possessing a free carboxyl function, the effect being independent of the presence or absence of a double bond between ring and carboxyl group. It will be observed also that the presence of a phenolic hydroxyl is not necessary for maintenance of the difference in behaviour between isomers with regard to variation in acid content of the solvent. This is demonstrated by Fig. 1a, where cinnamic acid itself has been studied. In general, concentrations of acetic acid below about 0.2 % caused tailing of the spots to occur, principally with the *trans* isomers. This made the accurate measurement of R_F values difficult; the spot centres were located in the region of maximum fluorescence.

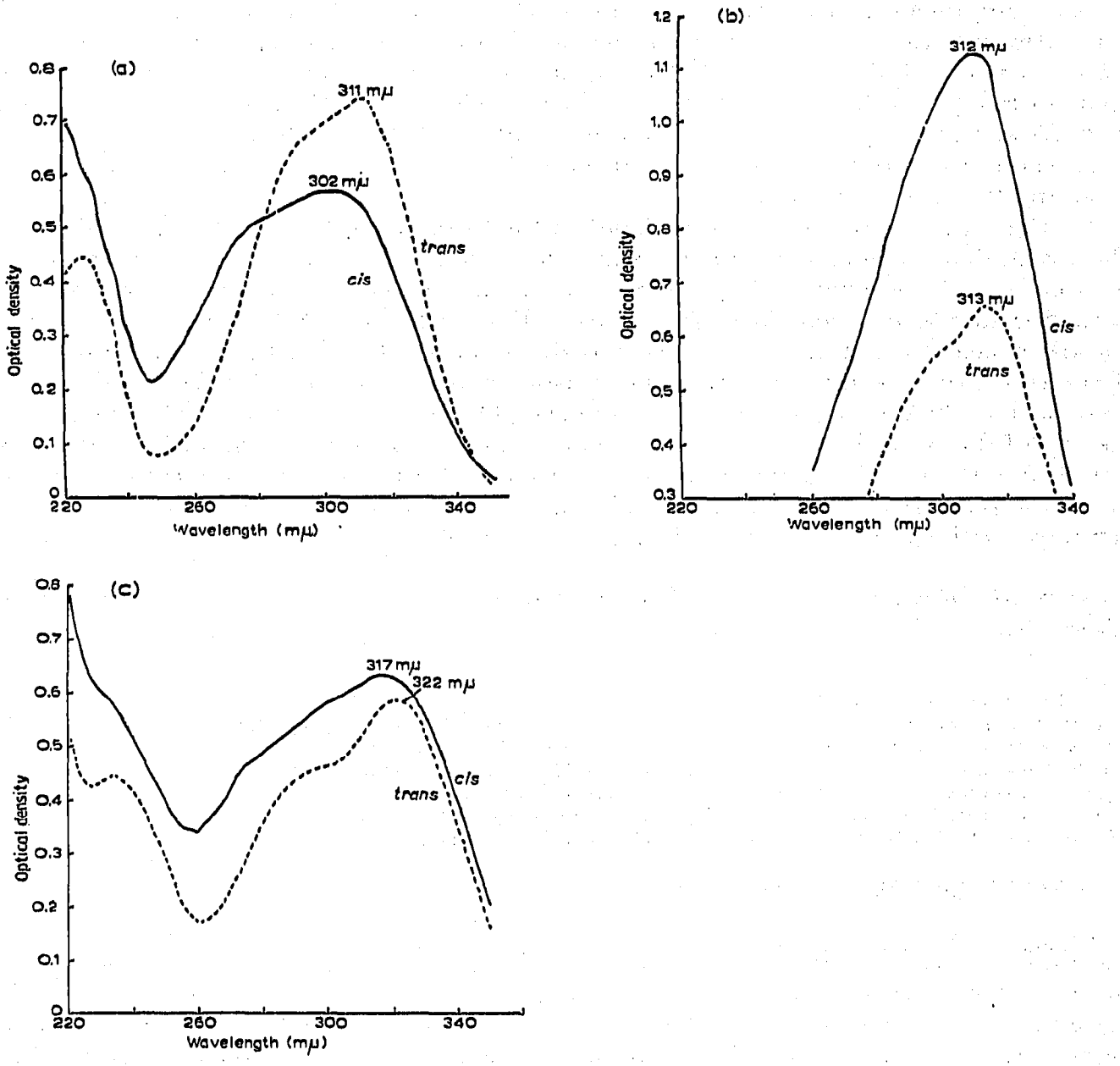

Fig. 3. U.V.spectra in ethanol of the isolated *cis* and *trans* forms of a number of cinnamic acid derivatives. (a) *p*-Coumaric acid. (b) Ethyl *p*-coumarate. (c) Ferulic acid.

A comparison of Figs. 1d, 2a and b (*p*-coumaric, ferulic and sinapic acids respectively) shows that the introduction of a methoxyl group at position 3 in *p*-coumaric acid to give ferulic acid causes greater lowering of the R_F values of both *cis* and *trans* isomers in response to the presence of small amounts of acid in the solvent. Addition of a further methoxyl at position 5 to give sinapic acid causes still greater lowering of R_F values.

Figs. 2c and d represent caffeic and chlorogenic acids respectively. It will be observed that the esterification of caffeic acid by quinic acid has considerably reduced but not inhibited the lowering in R_F values of both isomers in response to the presence of acid; the carboxyl group of the quinic acid moiety gives the expected effect here.

As a general effect, increase in concentration of acetic acid up to 50% in the solvent employed gives higher R_F values accompanied by diminishing degrees of separation between the isomers. Examination of Figs. 1b and d shows that the *cis* and *trans* spots of ethyl *p*-coumarate have quite similar R_F values to the corresponding spots of free *p*-coumaric acid for the 2% acetic acid solvent. In plain water, however, both *cis* and *trans* spots of the free acid move ahead of the corresponding spots of the ethyl ester; this would enable a separation of the four components of a mixture of the ester and free acid to be made.

Figs. 3a, b and c show comparisons of the form of the U.V. spectra in ethanol of the *cis* and *trans* forms of *p*-coumaric acid, ethyl *p*-coumarate and ferulic acids, respectively. Except for ethyl *p*-coumarate, the differences are sufficiently marked to permit their use for identification purposes. ROTH AND STOERMER[7] have determined the dissociation constants of the *cis* and *trans* forms of a number of cinnamic acid derivatives; their results show that the *cis* isomers have consistently greater degrees of dissociation than those of the *trans* isomers. These differences are reflected in the spectra of the *cis* and *trans* forms in aqueous solution; the ionized and unionized forms have different absorption maxima and spectra in aqueous solution could thus also be of value for purposes of identification. This has been found to be so in the case of *p*-coumaric acid, which has been studied in some detail[3].

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Received August 16th, 1965