Note

Effect of ultraviolet light on substituted cinnamic acids and the estimation of their *cis* and *trans* isomers by gas chromatography

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The substituted cinnamic acids, *p*-coumaric (PCA), caffeic (CA), ferulic (FA) and sinapic (SA), are widely distributed in plants and are found in various combined forms, for example as glycosides or as sugar esters[1–3]. These acids can occur in *cis* or *trans* forms owing to the presence of a vinyl group in the side chain. Various workers (*e.g.* Neish[4] and Kahnt[5]) have shown that the *trans* form can be partially converted to *cis* by ultraviolet (UV) light, and that conversion was maximized at between pH 5.0 and 7.0. Our interest in these compounds arises from recent work showing that *cis* and *trans* forms of PCA and FA are bound by ester links to carbohydrate in the cell walls of *Lolium multiflorum*[6–8]; the *trans* form was shown to be the most abundant[8].

Earlier workers[9–11] have separated the trimethylsilyl (TMS) ether derivatives of the four substituted cinnamic acids by gas–liquid chromatography (GLC), but there is confusion in the literature as to whether the *cis* or *trans* isomers were being separated. It is likely that both *cis* and *trans* isomers were present in these mixtures as no precautions were taken to prevent *cis–trans* isomerization due to the effect of light.

In the present work, the effects of both UV light and daylight have been investigated on solutions of the *trans* acids and on their TMS ether derivatives. Methods have been evolved for the separation and estimation of the *cis* and *trans* isomers of the acids by separation of their TMS ether derivatives by GLC.

EXPERIMENTAL

It was found necessary to carry out all manipulations of solutions of *cis* and *trans* phenolic acids and their TMS ether derivatives in "white" fluorescent light to prevent isomerization. *trans*-PCA, -FA and -SA were obtained from Koch-Light (Colnbrook, Great Britain) and *trans*-CA from Nipa Laboratories (Pontypridd, Great Britain); although the FA, SA and CA were not listed as either *cis* or *trans*, melting points[12] and paper chromatography[13] showed they were *trans*.

Preparation and GLC estimation of TMS ethers of PCA, CA, FA and SA

Solutions of each acid (0.5 mg in 0.1 ml of methanol) were evaporated in a stream of nitrogen and 0.2 ml of N,O-bis(trimethylsilyl)trifluoroacetamide (Pierce,

Rockford, Ill., U.S.A.) were added. The mixture was heated at 40° for 30 min with occasional shaking to form the TMS ether derivatives. The solutions were stored in sealed containers at 4° in darkness.

The TMS ether derivatives were separated using a Pye Unicam Series 104 chromatograph with a flame ionization detector and a 2.75-m glass column (I.D. 4 mm) containing 5% OV-25 on 80–100 mesh Diatomite CQ. The flow-rate of argon carrier gas was 55 ml/min and the inlet pressure $2.7 \cdot 10^5$ Nm^{-2} (gauge). The column oven temperature was maintained at 195° and the detector oven at 245°. An inlet heater was not employed, the samples being injected directly into the column packing using an 11-cm needle. A Kent Chromalog 3 integrator was used to determine peak areas.

Quantitative calibration. The TMS ether derivative of 2,4-dihydroxybenzoic acid was used as internal standard (retention time, t_R, 9.4 min). A calibration graph of peak area against weight was obtained for each of the four *trans* TMS ether derivatives in the range 1–200 μg of acid. A linear relationship was found in each case. Mixtures of *cis* and *trans* TMS ether derivatives obtained by exposure of each of the four *trans* acids (in methanol) to UV light for 3 h gave a total peak area similar to the original *trans* isomer within the range 1–200 μg.

The TMS ether derivatives were also separated by a similar technique using a 1.52-m column containing 3% SE-30 on Diatomite CQ. The flow-rate of argon was 50 ml/min. The column oven was maintained at 175° and the detector oven at 225°.

Exposure of trans-PCA, -CA, -FA and -SA and their TMS ethers to UV light

trans-PCA, -CA, -FA and -SA (5.0 mg of each in 1 ml of methanol) and their TMS ethers were exposed to UV light for varying periods of time. The solutions were irradiated in glass tubes using a 125-W Thorn mercury-vapour-discharge lamp, type MBW, with reflector, having 95% of the radiation at 365 nm. The bottom of the lamp was 20 cm from the surface of the solution.

Separation of cis and trans-PCA, -CA, -FA and -SA by thin-layer chromatography and their characterization by combined GLC–mass spectrometry (MS)

Solutions of each of the *trans* acids in methanol were exposed to UV light for 3 h and the suspected *cis* and unchanged *trans* isomers separated by preparative thin-layer chromatography (TLC) using Schleicher and Schüll cellulose plates (F1440) and formic acid–water (96:4) as solvent. The R_F values of the isomers were as follows: *cis*-PCA 0.67; *trans*-PCA 0.34; *cis*-CA 0.57; *trans*-CA 0.20; *cis*-FA 0.59; *trans*-FA 0.24; *cis*-SA 0.53; *trans*-SA 0.17. Both *cis* and *trans* isomers gave the expected colour reactions with *p*-nitraniline reagent[14,15]. *cis* Acids were obtained by preparative TLC, converted to their TMS ethers and each shown to give a single peak by GLC, as did the corresponding *trans* derivatives.

The TMS ethers of the suspected acids were prepared and submitted to combined GLC–MS using the GLC conditions described above but with the OV-25 column at 220°, detector oven at 270° and helium gas flow-rate 40 ml/min. Mass spectra were recorded at 70 eV, 8 KV accelerating voltage, with the source at 200° (AEI MS9 Instrument).

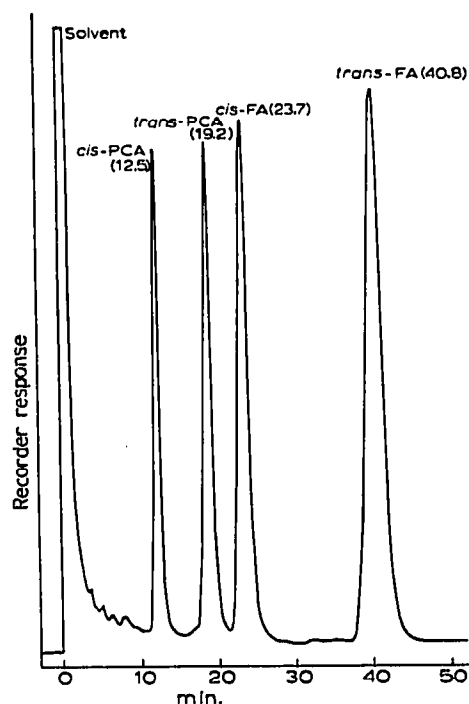The TMS ether of 2,4-dihydroxybenzoic acid was prepared and submitted to GLC–MS by the same methods.

Fig. 1. Separation of the *cis* and *trans* TMS ether derivatives of PCA and FA. GLC conditions given in text (OV-25 stationary phase). Retention times (min) in parenthesis.

RESULTS AND DISCUSSION

The *trans* isomers of PCA, CA, FA and SA, unlike the *cis* isomers, are commercially available. The *cis* isomers were obtained by exposure to UV light and their identities confirmed by TLC and by GLC–MS.

Separation of the *cis* and *trans* isomers of the TMS ether derivatives of the acids using OV-25 stationary phase are shown in Figs. 1 and 2. This method was shown to be suitable for quantitative estimation of the acids using the TMS derivative of 2,4-dihydroxybenzoic acid as internal standard. *cis*-CA and *cis*-FA were not completely separated by this system. In mixtures where these two acids occur the same derivatives can be separated using SE-30 as stationary phase. t_R Values (min) using SE-30 were as follows: *cis*-PCA 5.1; *trans*-PCA 9.1; *cis*-CA 11.6; *trans*-CA 22.4; *cis*-FA 8.4; *trans*-FA 16.7; *cis*-SA 14.5; *trans*-SA 31.0. By comparison with Figs. 1 and 2 it can be seen that this separation gave a reversal in the order of elution of several of the acids. Such behaviour is of use for identification of the acids in mixtures of unknown composition.

Results from the exposure to UV light of the *trans* acids dissolved in methanol are shown in Table I. *trans*-FA and -SA were less stable than the other acids. *trans*-PCA was the most stable but was more labile than its TMS ether derivative.

UV light was also found to have a considerable effect on *trans* TMS ether derivatives (Table I). Exposure for 30 min caused conversion from *trans* to *cis* in the
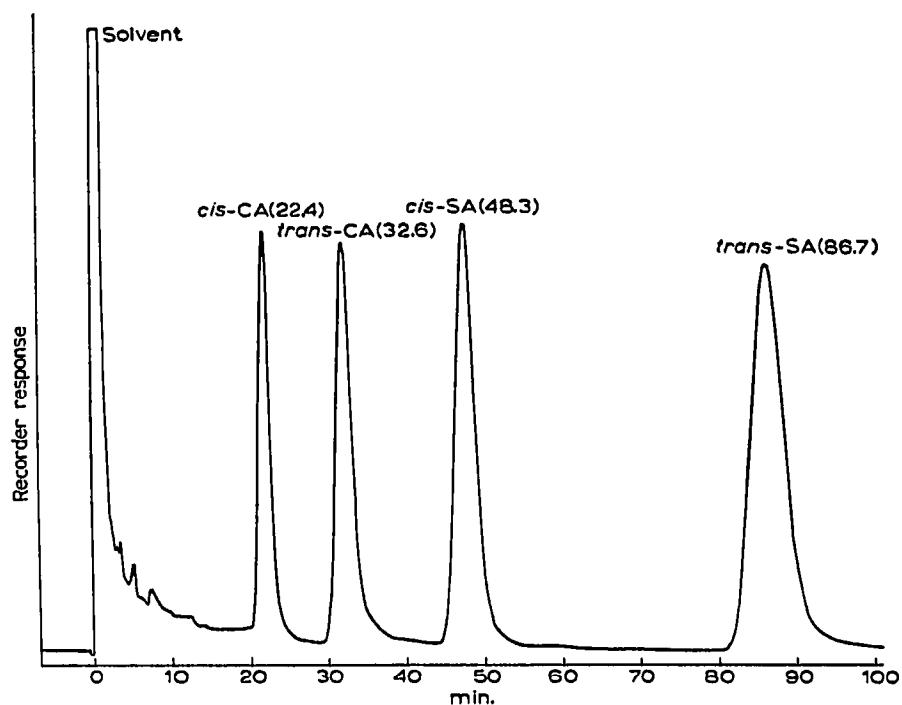
Fig. 2. Separation of the *cis* and *trans* TMS ether derivatives of CA and SA. GLC conditions given in text (OV-25 stationary phase). Retention times (min) in parenthesis.

TABLE I

EFFECT OF UV LIGHT ON SUBSTITUTED CINNAMIC ACIDS

Compound	Ratio of trans:cis isomers of TMS ether derivatives after exposure to UV light*	
	For 30 min	For 3 h
trans-p-Coumaric acid**	9.26:1	2.30:1
trans-Caffeic acid**	7.54:1	1.46:1
trans-Ferulic acid**	3.86:1	1.18:1
trans-Sinapic acid**	4.39:1	1.12:1
TMS ether derivatives of:		
trans-p-Coumaric acid	76.3 :1	11.3 :1
trans-Caffeic acid	3.14:1	2.70:1
trans-Ferulic acid	1.66:1	0.85:1
trans-Sinapic acid	6.21:1	2.53:1

* Ratio of peak areas from GLC (OV-25 stationary phase, conditions in text).
** After exposing to UV light, mixture of *cis* and *trans* acids converted to TMS ether derivatives in the absence of UV and daylight.

TABLE II

PARENT IONS AND MAJOR IONS IN THE MASS SPECTRA OF TMS ETHER DERIV-
ATIVES OF SUBSTITUTED CINNAMIC ACIDS

TMS ether derivatives (cis or trans)	Parent ion (m/e)	Other major ions (m/e)
p-Coumaric acid	308	219, 293, 249, 179
Caffeic acid	396	219, 381, 307, 191
Ferulic acid	338	308, 249, 323, 219, 293
Sinapic acid	368	338, 353, 249, 279
2,4-Dihydroxybenzoic acid	370	355, 281

order FA > CA > SA > PCA. Additional conversion occurred over a further $2\frac{1}{2}$ h and again FA showed the greatest change and PCA the least.

Results similar to those described above were obtained for the acids and their TMS ether derivatives when UV light was replaced by bright sunlight in the laboratory.

Mass spectrometry of the *cis* and *trans* TMS ether derivatives of any of the four acids gave very similar spectra (Table II). In each case the parent ion had *m/e* values agreeing with the calculated molecular weight of the fully silylated form of each acid, *i.e.* all phenolic hydroxyl and carboxyl groups had been silylated. The base peaks of CA, FA and SA were also the parent ions, but the base peak of PCA had an *m/e* value of 219. The TMS ether derivative of 2,4-dihydroxybenzoic acid, used as an internal standard in GLC quantitative work, gave a mass spectrum in which the parent-ion peak corresponded to the molecular weight of the fully silylated compound. The base peak had an *m/e* value of 355.

A major conclusion from the above work is that extraction of substituted cinnamic acids from plants and the preparation of their TMS ether derivatives should be carried out in the absence of UV and daylight to avoid *cis–trans* isomerisation. Quantitative analysis will be less reliable if these conditions are not observed.

ACKNOWLEDGEMENTS

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