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Note

Isomerization of some cinnamic acid derivatives

T. W. FENTON, M. M. MUELLER and D. R. CLANDININ
Department of Animal Science, University of Alberta, Edmonton, Alberta (Canada)
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During the investigation of polyphenolic compounds in rapeseed meal by gas–liquid chromatography (GLC) of the trimethylsilyl (TMS) derivatives of phenolic acids, we found some peaks which did not correspond to co-chromatographed standards. It was also observed that these unknown peaks increased with the length of time that the samples were stored. When pure phenolic acid standards were treated the same as the rapeseed meal extracts, the same unknown peaks developed at the expense of the original phenolic acid peaks. These additional peaks were observed to occur with *p*-coumaric, caffeic, ferulic, dimethoxycinnamic, and sinapic acids whereas with the corresponding benzoic acids like *p*-hydroxybenzoic, protocatechuic, vanillic, gallic, and syringic acids no such additional peaks were found.

Hartley and Jones[1] attributed these peaks to *trans–cis*-isomerism which occurs under UV irradiation as shown earlier by other workers (Kahnt[2] and Challice and Williams[3]). Because of large differences in GLC retention times of the isomers, we considered the possibility of ring formation in the *cis* form. We were also interested in the amount of *cis* formation that occurred under practical analytical conditions without applied UV radiation. Infrared (IR) spectra of *cis*- and *trans*-sinapic acids as TMS derivatives were also studied to further characterize the isomers.

EXPERIMENTAL

Caffeic (J. T. Baker, Phillipsburgh, N.J., U.S.A.), ferulic (Aldrich, Milwaukee, Wisc., U.S.A.), and sinapic (Aldrich) acids were dried over phosphorous pentoxide in a vacuum desiccator for several days. Their respective purities as determined by GLC were 99.2, 98.7 and 99.3%.

TMS derivatives were prepared by reacting 0.5 to 2 mg of the dry compounds with 0.2 to 0.5 ml of N,O-bis-(trimethylsilyl)-acetamide (BSA) (Pierce, Rockford, Ill., U.S.A.).

Gas chromatograms were done on a Bendix 2500 chromatograph equipped with flame ionization detectors. The column was 3 m × 2 mm I.D. glass tubing packed with 3.5% OV-1 on 80–100 mesh Chromosorb W. The oven was kept isothermal at 210° and the carrier gas (nitrogen) flow-rate was 40 ml/min.

Thin-layer chromatography (TLC) for separation of *cis–trans*-cinnamic acid derivatives was done on 5 × 5-cm plates using circular development. The layer was high-performance TLC (HPTLC) silica gel 60 F_{254} (Merck, Darmstadt, G.F.R.). The

solvent system used was diethyl ether–hexane–chloroform–acetic acid (12:38:50:0.5). Development time was approximately 3 min. The bands were detected by their fluorescence under long wave (360 nm) UV light.

The mass spectra were obtained from a Hewlett-Packard combined 5710A GLC–5980A mass spectrometry system.

To obtain IR spectra, the two peaks resulting from sinapic acid were collected separately on an Aerograph Series A90-P3 gas chromatograph with a thermal conductivity detector. The collecting traps were glass tubes filled with glass wool. The collected peaks were eluted from the traps with pentane, concentrated, found to be 98% pure by GLC and submitted to the Spectral Services Laboratory, Department of Chemistry, University of Alberta, for IR scans.

To follow the rates at which the additional peaks developed, two solutions were prepared. (1) Standard A: 1.051 mg ferulic acid + 1.913 mg caffeic acid + 3.144 mg sinapic acid per g methanol. (2) Standard B: 2.977 mg ferulic acid + 2.047 mg caffeic acid + 1.043 mg sinapic acid per g methanol. The solutions were sampled at various time intervals. These samples were evaporated to dryness under vacuum in a rotary evaporator and derivatized with BSA as mentioned above. The initial samples were taken immediately after preparation of the solutions and after evaporation were stored under BSA for analysis at various time intervals. A sample of each of the three acids was also stored under BSA. The samples were all stored on the laboratory bench at room temperature, exposed to artificial laboratory lighting and daylight from the windows, but never exposed to direct sunlight.

RESULTS AND DISCUSSION

A typical gas chromatogram of some cinnamic acid derivatives is shown in Fig. 1. Mass spectra were run on peaks b, c, e and f as a means of identification. The

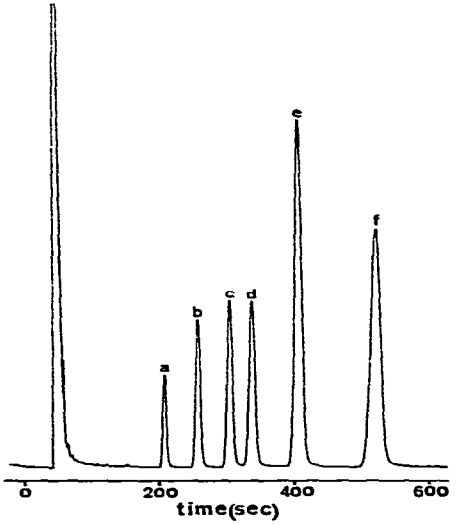

Fig. 1. Gas chromatogram of silylated phenolic acids using 3.5% OV-1 on Chromosorb W. Retention times (sec) at 210° are: a = *cis*-ferulic, 206; b = *cis*-caffeic, 256; c = *cis*-sinapic, 304; d = *trans*-ferulic, 336; e = *trans*-caffeic, 407; f = *trans*-sinapic, 522.

TABLE I

MASS SPECTRAL DATA OF SILYLATED PHENOLIC ACIDS

Characteristic ion		*Relative abundances of fragment ions*		
		cis-Caffeic acid	*trans-Caffeic acid*	
396	(M)	100	100	
381	(M−15)	26	31	
307	(M−89)	18	23	
249	(M−147)	26	32	
219	(M−177)	267	384	
191		36	51	
147		11	16	
73		147	460	
		cis-Sinapic acid	*trans-Sinapic acid*	*Sinapic acid**
368	(M)	100	100	100
353	(M−15)	53	60	51
338	(M−30)	118	118	104
323	(M−45)	42	39	30
279	(M−89)	29	30	30
249	(M−119)	22	23	36
73		62	70	200

* Values calculated from ref. 4.

relative abundances of their ion fragments are listed in Table I. The *cis* and *trans* isomers gave the same major fragments with essentially the same relative abundances.

The IR spectra of the TMS derivatives of *cis*- and *trans*-sinapic acids (Fig. 2)

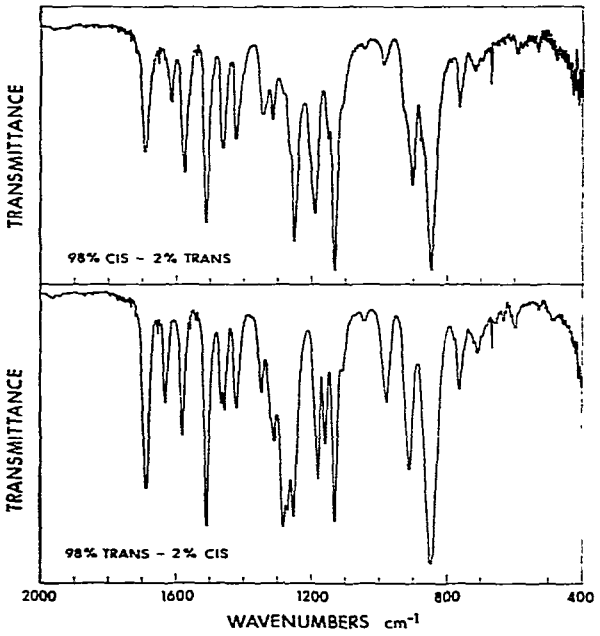

Fig. 2. Infrared spectra of the TMS derivatives of *cis*- and *trans*-sinapic acids.

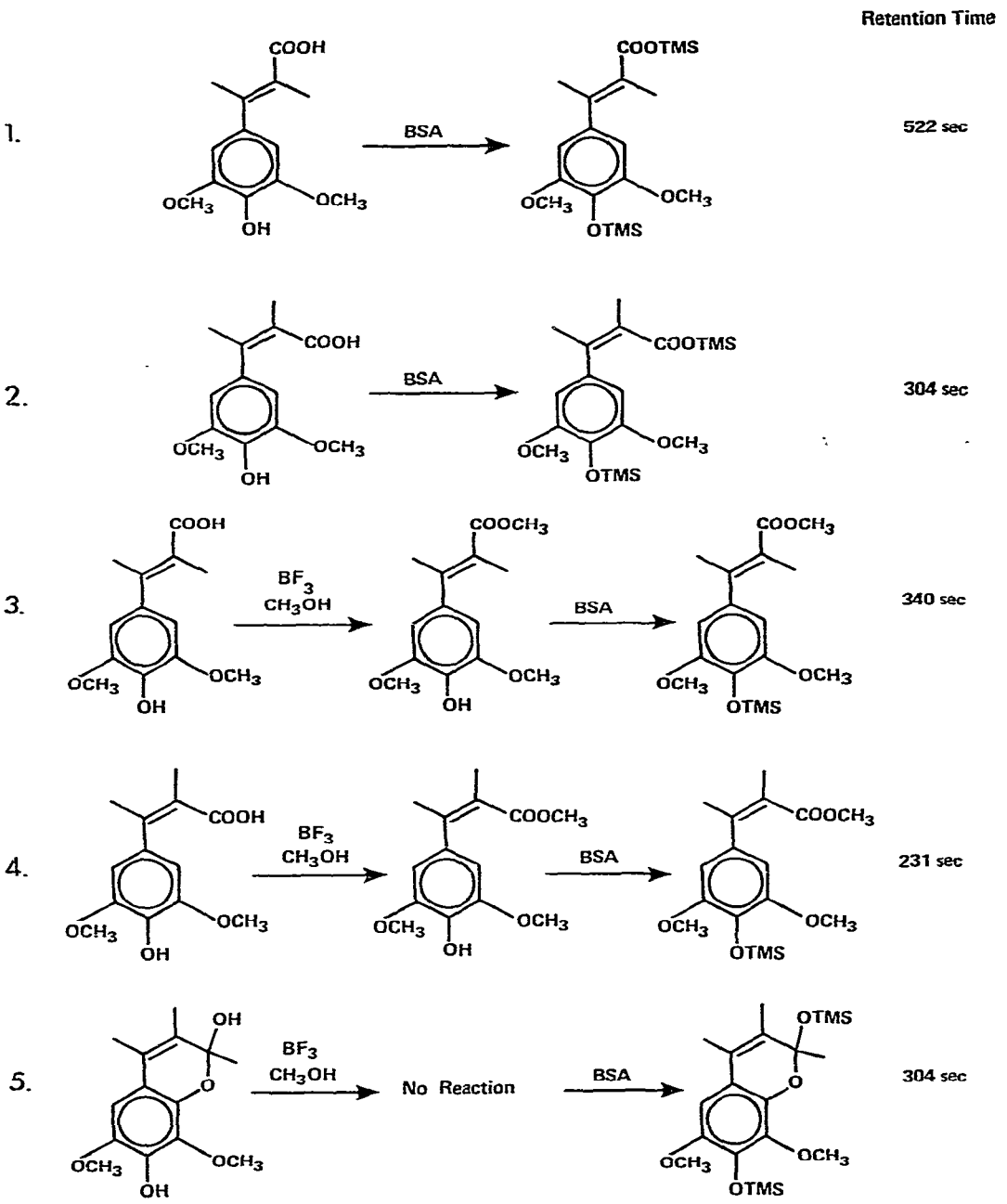

Fig. 3. Derivative formation of sinapic acid and retention times of the products.

showed major absorption differences at 980 and 1280 cm^{-1}, these bands being absent in the *cis* isomer.

A molecular model of sinapic acid suggests that the *cis* form would be a considerably less polar molecule than the *trans* form. The model also suggests that

if the *ortho* positions are very reactive the formation of a six-membered ring might be possible from the *cis* form without any change in molecular weight, also resulting in a less polar molecule. Both possibilities would agree with the GLC behavior. Ring formation was shown not to occur by consideration of the reactions and retention times of the products on the GLC as shown in Fig. 3.

Reaction 3 gave a peak at 340 sec corresponding to the methyl ester-TMS derivative. A *cis–trans* mixture (reactions 3 and 4) gave major peaks at both 340 and 231 sec corresponding to methyl ester-TMS derivatives, but no peak at 304 sec. If ring formation had occurred we would expect no methylation of the *cis* isomer and a major peak should have been found at 304 sec instead of 231 sec.

Challice and Williams[3] reported separation of *cis* and *trans* isomers of ferulic, caffeic and sinapic acids by paper chromatography. The *trans* isomer was immediately visible under UV light, while the *cis* isomer required several seconds of irradiation before it became visible. We were able to separate each of the three acids that were stored in methanol into two bands by TLC. The bands corresponding to the co-chromatographed *trans* isomers were immediately visible under UV light, while the others became visible after a few seconds of irradiation. This method seems to be very suitable for a fast check on *cis–trans* isomerism (3 min development). The R_F values for *cis*- and *trans*-caffeic, sinapic and ferulic acids are: 0.18, 0.27, 0.43, 0.55, 0.56 and 0.63. The *cis* isomers have lower R_F values than *trans* isomers with this system in contrast to those by paper chromatography as reported by Challice and Williams[3]. The use of HPTLC plates is a requirement for this separation as use of silica gel G layers results in no separation of the isomers.

To determine the rate of formation of these *cis*-phenolic acids under practical analytical conditions a time-course study was undertaken. As can be seen in Fig. 4, sinapic acid was most readily isomerized. Hartley and Jones[1] found after irradiation

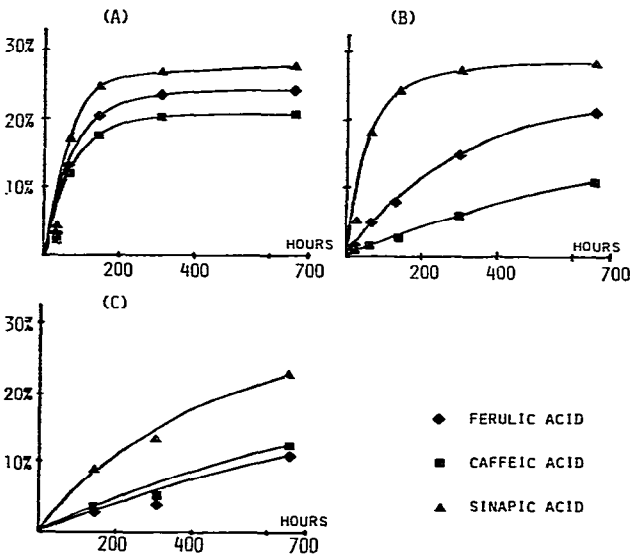

Fig. 4. The formation of the *cis* isomers of ferulic, caffeic and sinapic acids. (A) Acids stored individually in BSA; (B) acids stored together in BSA; (C) acids stored together in methanol.

of *trans*-TMS derivatives a conversion of *trans* to *cis* in the order ferulic > caffeic > sinapic acid. In the present study, when the samples were kept under normal laboratory conditions the order was sinapic > ferulic > caffeic acid (Fig. 4a). The curves also indicate the *trans–cis* equilibrium is more towards *trans* than reported elsewhere[1,2]. When sinapic acid was stored together with ferulic and caffeic acids in BSA the sinapic acid was isomerized at about the same rate as when stored alone (Fig. 4b), whereas the isomerization of ferulic and caffeic acids appeared to be inhibited by the presence of sinapic acid. Storing in methanol showed a much lower rate of conversion to the *cis* isomers compared to storing in BSA, but again sinapic acid appeared to inhibit the isomerization of the other two acids. It is speculated that free radical reaction, initiated by the small amount of UV in the normal room lighting, is a step in this isomerization process and the compound which is converted most rapidly acts as an inhibitor to the isomerization of the other compounds.

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REFERENCES

1 R. D. Hartley and E. C. Jones, *J. Chromatogr.*, 107 (1975) 213.
2 G. Kahnt, *Phytochemistry*, 6 (1967) 755.
3 J. S. Challice and A. H. Williams, *J. Chromatogr.*, 21 (1966) 357.
4 H. Morita, *J. Chromatogr.*, 71 (1972) 149.