Journal of Chromatography, 281 (1983) 326–329
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,209

Note

High-performance liquid chromatography separation of the *cis-trans* isomers of cinnamic acid derivatives

Ultraviolet and electrochemical detection

E. J. CONKERTON* and D. C. CHAPITAL
Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 19687, New Orleans, LA 70179 (U.S.A.)
(Received August 3rd, 1983)

Cinnamic acid derivatives, *p*-coumaric (*p*-COU), caffeic (CAF), ferulic (FER) and sinapic (SIN), are ubiquitous in plants and are found free and in various combined forms[1]. Because of the presence of a vinyl group in the side chain, these acids can occur in *cis* or *trans* forms. Kahnt[2] showed that UV light partially converts the *trans* forms of *p*-COU, CAF, FER and SIN to their respective *cis* forms. Kahnt[3] also demonstrated that the *cis–trans* ratio of chlorogenic acid in leaves of the potato plant varied as the light-dark cycle times during growth varied. Therefore, in any study of cinnamic acid derivatives in plant materials, it is essential to have an accurate, rapid method for the determination of the *cis* as well as the *trans* forms.

Several methods have been reported for the separation of certain *cis-trans* isomer, *i.e.*, paper chromatography[4], thin-layer chromatography[5], and gas chromatography[6–8]. Hoverman *et al.*[9] used normal phase high-performance liquid chromatography (HPLC) with UV detection at 270 nm to separate the *cis-trans* isomers of FER and *p*-COU acids. Caccamese *et al.*[10] used reversed-phase HPLC with UV detection at 290 nm to separate the *cis-trans* isomers of FER and SIN acids. Hartley and Buchan[11] also used reversed-phase HPLC for the separation of the *cis-trans* isomers of FER, SIN, CAF, and *p*-COU acids, but UV detection was at 275 nm. Although UV absorption is the most popular detection mode for the HPLC determination of phenolic acids, Roston and Kissinger[12] cite several characteristics that electrochemical detection (ED) possesses for phenolic acid determinations. Two of the more important factors are good selectivity and superior detection limits, typically in the picomole range.

This paper reports the separation of *cis-trans* isomers of *p*-COU, CAF, FER, SIN, and *o*-coumaric (*o*-COU) acids by reversed-phase HPLC using UV and electrochemical detection in series.

EXPERIMENTAL*

Cinnamic acid derivatives were obtained in the *trans* form from Aldrich, (Mil-

* Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.

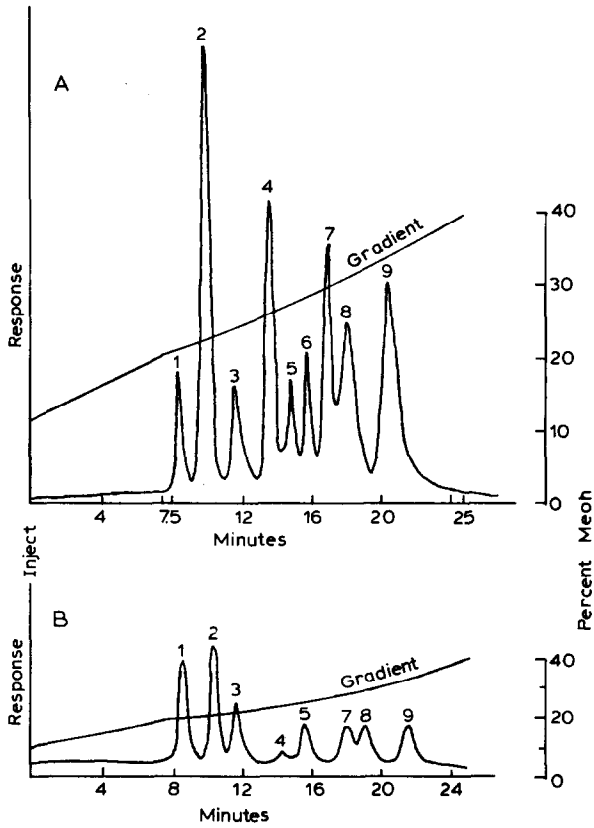

Fig. 1. Tracings from HPLC of a mixture of *cis-trans* cinnamic acids. Column, μBondapak C_{18}, 30 cm × 3.9 mm I.D. Solvents, pump A: water–acetic acid (99:1); pump B: methanol. Program, initial 90% A, 10% B, then linear to 80% A, 20% B (0–7.5 min), then concave to 60% A, 40% B (7.5–25 min). Flow-rate, 2 ml/min. (A) UV detection at 320 nm; (B) ED at 0.9 V. Peaks: 1 = *cis*-caffeic, 2 = *trans*-caffeic, 3 = *cis-p*-coumaric, 4 = *trans-p*-coumaric, 5 = *cis*-ferulic, 6 = *cis-o*-coumaric, 7 = *trans*-ferulic, 8 = *cis*-sinapic, 9 = *trans*-sinapic.

waukee, WI, U.S.A.). Using 1-ml borosilicate glass serum vials, CAF, *o*-COU, *p*-COU, FER, and SIN acids were dissolved separately in methanol (1 mg/ml). A mixture of the *trans*-acids was also made up in methanol (1 mg/ml). To verify the presence of only *trans* isomers, 5-μl aliquots were removed and analyzed under the chromatographic conditions described below. Solutions were exposed in a growth chamber, using fluorescent, PLANT-GRO, 15 watt bulbs, in 12-h intervals until *cis-trans* equilibrium was reached.

A Waters Assoc. liquid chromatograph equipped with a variable-wavelength detector, two 6000A pumps, and a systems controller was used. The eluent from the variable-wavelength detector was passed through an amperometric electrochemical detector (Bioanalytical Systems, Model LC-4B). The column was 30 cm × 3.9 mm I.D. packed with μBondapak C_{18}, 10 μm. Solvents were filtered using a glass Millipore system with a 0.45-μm filter and degassed at ambient temperature under vacuum with magnetic stirring. The elution solvent was water–acetic acid (99:1) from pump

TABLE I

ABSORPTION MAXIMA OF *cis-trans* ISOMERS OF CINNAMIC ACID DERIVATIVES

cis-trans isomers collected as they eluted from the HPLC analysis.

	trans-Absorption maxima (nm)		*cis-Absorption maxima (nm)*	
Caffeic	232	322	230	321
o-Coumaric	230, 258, 288	324	217, 273	310
p-Coumaric	230	308	227	292
Ferulic	228	320	228	317
Sinapic	228	321	227	319

A and methanol from pump B. Flow-rate was 2 ml/min with the gradient as indicated in Fig. 1. Retention times and peak areas from the UV detector at 254 and 320 nm were determined by a Waters Data Module while those from the electrochemical detector at 0.9 and 1.2 V were determined by a Hewlett-Packard laboratory automation system. Peak areas of each of the *cis-trans* isomers were also measured at 0.7, 0.8, 1.0 and 1.1 V using the electrochemical detector.

Identification of the *cis-trans* isomers of each acid was accomplished by collecting each peak from a minimum of 5 replicate injections of the individual conversions. The combined eluent was concentrated to 2.5 ml and the UV absorption spectra were measured on a Hewlett-Packard 8450 spectrophotometer.

RESULTS AND DISCUSSION

All samples reached *cis-trans* equilibrium after 24 h exposure in the plant growth chamber. The *cis*-form represented 40–49% of the total for all acids except *o*-COU. After 24 h exposure, *trans-o*-COU was 99% converted to the *cis*-form.

With UV detection, under the chromatographic conditions used, baseline separation of the *cis-trans* forms of CAF, *p*-COU, FER and SIN acids was obtained.

TABLE II

PEAK AREAS OF *cis-trans* ISOMERS OF CINNAMIC ACID DERIVATIVES OVER A RANGE OF APPLIED POTENTIALS (COUNTS × 1000)

A 3-μl amount of methanol solution, after maximum conversion, injected for each sample except that 2 μl of *trans-o*-coumaric were used.

	0.7 V	*0.8 V*	*0.9 V*	*1.0 V*	*1.1 V*	*1.2 V*
Caffeic, *cis-*	106.9	640.9	542.6	598.7	637.8	626.4
trans-	167.2	652.5	453.4	559.3	690.6	723.5
o-Coumaric, *cis-*	–	–	–	–	–	–
trans-	–	–	30.2	132.6	197.5	208.1
p-Coumaric, *cis-*	–	0.3	7.7	85.5	193.0	250.9
trans-	–	0.3	7.6	69.1	154.7	211.2
Ferulic, *cis-*	0.3	25.1	161.9	260.6	310.2	420.2
trans-	0.3	24.8	162.5	258.4	309.6	422.5
Sinapic, *cis-*	59.8	142.1	125.7	166.9	254.9	328.2
trans-	48.9	121.8	108.1	159.8	238.0	350.3

While baseline separation of the isomers of *o*-COU was not obtained the separation was adequate for accurate integration of peak areas.

In all cases, the *cis*-isomer eluted before the *trans*-isomer. This was verified by comparison of the UV spectra of the individual peaks with reported spectra of pure *cis-trans* isomers[10,13]. Absorption maxima for each peak are noted in Table I.

Since the oxidation of phenols is dependent on a number of factors, including electrode potential[11], the response of these *cis-trans* isomers was determined over a range of applied potentials, 0.7–1.2 V. The response factors, as indicated by peak areas, are noted in Table II. No significant response at the lower potentials was observed except for CAF and SIN acids. When the response is very intense, adjustments in the amounts of sample injected may have to be made in order to obtain reproducible areas.

From the data collected in this study, it is obvious that the UV and ED response varies for each isomeric set of acids. Therefore, using both detectors in series, the ED/UV ratio can be calculated for each cinnamic acid derivative. From a series of 5 replicates, using 0.9 V and 320 nm, the following ED/UV ratios were obtained:

	cis	*trans*
CAF	0.363 ± 0.029	0.130 ± 0.008
o-COU	–	0.017 ± 0.006
p-COU	0.038 ± 0.007	0.010 ± 0.002
FER	0.115 ± 0.009	0.045 ± 0.003
SIN	0.351 ± 0.016	0.131 ± 0.006

Since these ratios are specific for each of these *cis-trans* isomers, they could be used to verify the purity of peaks with similar retention times in samples.

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