Fluorometric Determination of *p*-Coumaric Acid in Beer

Francisco García-Sánchez,* Cristóbal Carnero, and Antonio Heredia

A new spectrofluorometric method to determine *p*-coumaric acid in beer has been developed. After the pH of the samples was adjusted to 2, impurities were extracted into hexane. Phenolics were then extracted into ethyl acetate, dried, and redissolved in water. Aliquots of the solutions were adjusted to pH 10.7, and the fluorescence intensities were measured at λ_{exc} = 330 nm and λ_{em} = 435 nm. Recovery data, detection limits, and also the corresponding analyses by standard addition method of endogenous *p*-coumaric acid in commercial beer are reported.

Because of their diverse activities in plants, the biological phenolic compounds have received considerable attention (Schneider and Wightman, 1974; Kefeki, 1978; Friend, 1979) as some of them cause adverse tastes and color changes in food products or decrease protein nutritive value (Sosulski, 1979).

Brewers employ many biological, chemical, and physical analyses for process and quality control, but it is a subjective quality, flavor, that is the most important of several criteria determining product acceptability and client satisfaction. Consequently, a sensitive analytical method permitting the evaluation of substances that may adversely affect beer flavor would have great practical interest, particularly if it was simple, quick, and objective.

Hydroxycinnamic acids are usually analyzed together with other phenolic compounds by one of several chromatographic methods: paper and thin-layer chromatographies (Hiller, 1965; Van Sumere, 1965; Mossor-Pietraszewska et al., 1981b), column liquid chromatography (Linser and Mascheck, 1953), gel filtration (Lattanzio and Marchesini, 1981) gas chromatography (Keith and Powers, 1966; Julkumen-Tiitto, 1985), and HPLC (Wulf and Nagel, 1976; Murphy and Stutte, 1978; Mossor-Pietraszewska et al., 1981a; Hagerman and Nicholson, 1982; Hardin and Stutte, 1980; Roston and Kissinger, 1981; Billet et al., 1981; Charpentier and Cowles, 1981; Price et al., 1979).

However, most of the analytical methods previously published concern the determination of phenolic acids in plant extracts. McMurrough et al. (1984) report the HPLC estimation of ferulic, vanillic, and *p*-coumaric acids in a number of British, American, and German beers. Okamura et al. (1981) and Roston and Kissinger (1981) describe the determination of *p*-coumaric acid in white wines by HPLC.

The present work describes a rapid assay for *p*-coumaric acid extracted from beer.

Reproducibility was examined by testing standard compounds. Comparative data of the results of determinations of *p*-coumaric acid in beer made by the standard addition method are also given.

EXPERIMENTAL METHODS

Solvents and Reagents. All chemicals used were analytical reagent grade. The water was both distilled and demineralized. Carbonate buffer (pH 11.5) was prepared by mixing appropriate volumes of 1 M sodium hydrogen carbonate and 1 M sodium hydroxide. All the acids, *p*-coumaric, caffeic, vanillic, protocatechuic, and syringic, were obtained from Sigma Chemical Co. Stock solutions were prepared in ethanol and stored in amber bottles at 4 °C. Dilute aqueous solutions were prepared daily from these solutions.

Apparatus. Fluorescence measurements were made with a Perkin-Elmer MPF-43A spectrofluorometer equipped with a 150-W Osram XBO xenon lamp, excitation and emission monochromators, 1 × 1 cm quartz cells, and a Perkin-Elmer 023 recorder. Instrument sensitivity was adjusted daily with a Rhodamine B bar as reference standard.

Extraction Procedure. The beer sample was degassed by agitation and moderate heating (between 30 and 35 °C), and the pH was adjusted to 2 (1 M HCl). From this acidified sample, 25 mL was taken and added to 25 mL of *n*-hexane. The resulting two phases were separated, and the aqueous phase was again extracted with 25 mL of ethyl acetate. The ethyl acetate extract was evaporated to dryness under reduced pressure at 25 °C, then deionized water was added to give 25 mL, and the resultant mixture was shaken until dissolved. Aliquots of this solution were then analyzed by both the proposed analytical procedure and the standard addition method.

Standard Addition Method. The standard addition method was applied to a series of five aliquots of 0.5 mL and four aliquots of 1.5 and 6.0 mL of aqueous extract, to which increasing amounts of *p*-coumaric acid had been added.

Analytical Procedure. The appropriate volume of *p*-coumaric acid aqueous working solution (0.1 mM) was introduced into a 25-mL standard flask to give a final concentration between 0.12 and 0.65 μg/mL. Of this buffer solution (pH 11.5) 0.25 mL was added and diluted to the mark with deionized water. The fluorescence intensity (λ_{ex} = 330 nm, λ_{em} = 435 nm) was measured against a reagent blank.

Recoveries. To determine recoveries, synthetic beer samples were prepared and spiked with *p*-coumaric acid. To a 100-mL volumetric flask were transferred 7 mL of ethanol (d = 0.79 g/mL) and 0.4 mL of 0.01 M *p*-coumaric acid, and the resultant mixture was diluted to the mark with deionized water.

RESULTS AND DISCUSSION

Pure *p*-coumaric acid in aqueous solution (0.394 μg/mL) at pH 10.7 has its emission maximum at 435 nm when excitation is locked at 330 nm. However, the pH of the medium must be precisely controlled because it affects both spectral shape and relative fluorescence intensity. Excitation and emission wavelengths suffer red shift as pH changes from acidic to basic (Figure 1). The fluorescence intensity is maximal at basic pH.

Fluorometric titration of *p*-coumaric acid carried out at two sets of λ_{ex}, λ_{em} values coinciding with the maxima of the prototropic spectral shifts (Figure 1) showed that there

Departments of Analytical Chemistry (F.G.-S., C.C.) and General Chemistry (A.H.), Faculty of Sciences, The University, 29071 Málaga, Spain.

0021-8561/88/1436-0080$01.50/0

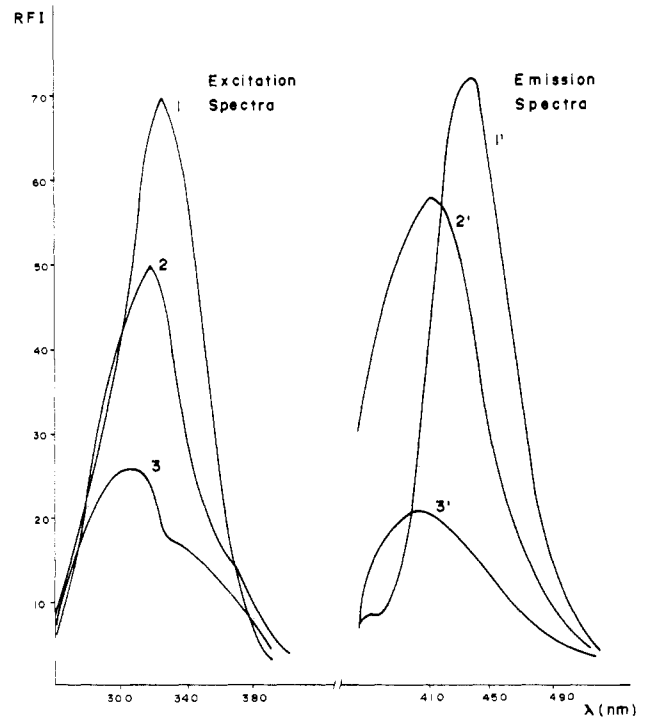

Figure 1. Fluorescence excitation and emission spectra of 10 μM aqueous p-coumaric acid at several pH values: 1, pH 10.70; 2, pH 6.15; 3, pH 1.80.

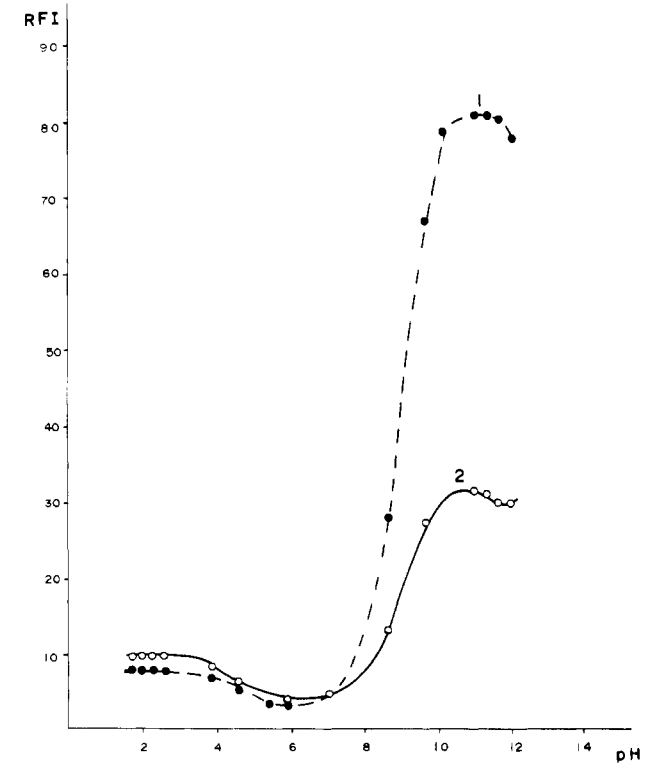

Figure 2. Effect of pH on fluorescence intensity of 10 μM aqueous solution of p-coumaric acid. λ_{exc} = 330 nm. λ_{em}: curve 1, 435 nm; curve 2, 405 nm.

was a suitable pH range between pH 10.5 and pH 11.0 to carry out the analytical method (Figure 2). Satisfactory results were be obtained by buffering the solution at pH 10.7.

The degradation of p-coumaric acid in both ethanol (stock solution) and aqueous ethanol (working solution) was studied by measuring the fluorescence emission change with storage time for each set of excitation and emission

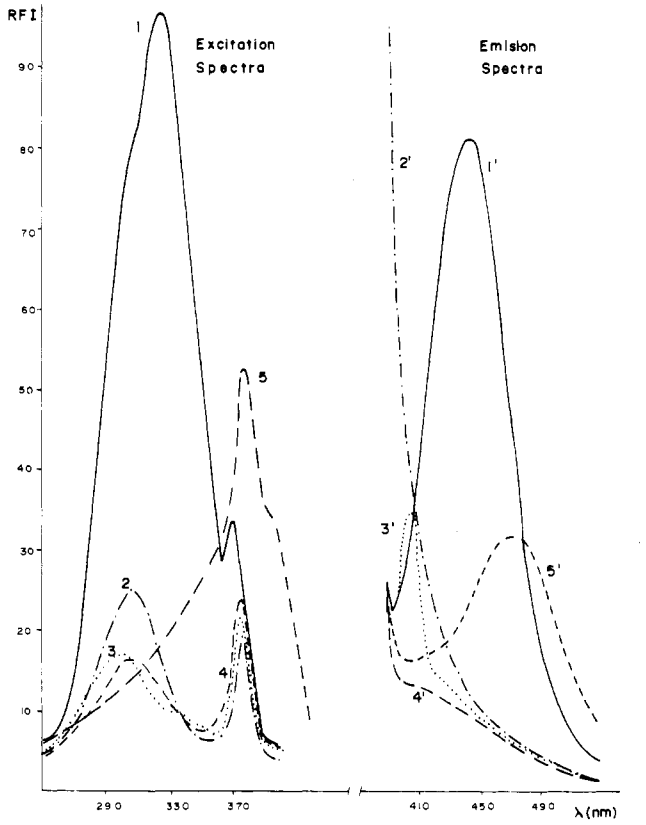

Figure 3. Excitation and emission spectra of 3.2 μM phenolic acids at pH 10.70 (λ_{exc} = 330 nm; λ_{em} = 435 nm): (1, 1′), p-coumaric acid; (2, 2′) syringic acid; (3, 3′) vanillic acid; (4, 4′) protocatechuic acid; (5, 5′) caffeic acid.

wavelengths. In aqueous solution, 10 μM p-coumaric acid gave constant fluorescence readings for at least 1 h. Ethanolic solutions of p-coumaric acid (0.01 M) stored in the dark at 4 °C are stable for at least 7 days.

Calibration Graphs, Sensitivity, and Precision. The calibration graph prepared by plotting fluorescence intensity readings against standard p-coumaric acid concentrations was linear for 0–0.65 μg/mL. The equation obtained by the least-squares method was $I = 168.3c + 2.57$, where $r = 0.995$, c is the p-coumaric acid concentration (μg/mL), and I is the relative fluorescence intensity.

The precision of the method was determined by measuring the fluorescence intensity of eight separate samples, each containing 0.328 μg/mL of p-coumaric acid and gave a relative error ($P = 0.05$) of 7.3% and relative standard deviation of 6.3%.

The sensitivity of the method is expressed as analytical sensitivity $S_A = S_S/m$, where S_S is the standard deviation of the analytical signal and m is the slope of the calibration graph (García-Sánchez and Cruces, 1986).

The detection limit, C_L, and determination limit, C_Q, are reported as defined IUPAC (1983). C_Q is employed to establish the inferior limit of the linear dynamic range. The results show that S_A = 0.027 μg/mL, C_L = 0.035 μg/mL, and C_Q = 0.12 μg/mL and the linear dynamic range is 0.12–0.65 μg/mL.

Interference Study. Test were made with appropriate concentrations of several phenolic acids that actually occur in beer extracts to check the selectivity of the method. Figure 3 shows the spectral shape of these phenolic acids under the same experimental conditions and instrumental parameters that show that a small contribution to the overall signal from the species tested may be expected.

Assuming that only a deviation of the fluorescence signal above $\pm tS_S$ (t, Student's t for 95% confidence; S_S, standard

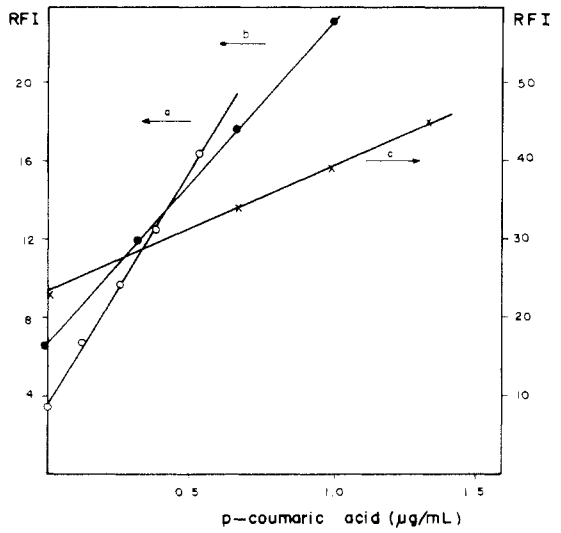

Figure 4. Determination of *p*-coumaric acid in beer extracts by the standard addition method: (a) 0.5 mL of extract; (b) 1.5 mL of extract; (c) 6.0 mL of extract. Arrows indicate the corresponding ordinate scale.

deviation of analytical signal) of that expected for *p*-coumaric acid will produce interference, the molar tolerance ratio for vanillic, protocatechuic, and syringic acids is 2:1 and for caffeic acid it is 0.5:1.

Recovery Assay. In order to simulate a beer matrix, an ethanol–water mixture (7%, v/v) solution containing 6.57 μg/mL of *p*-coumaric acid was prepared. Three aliquots were separately submitted to the extraction procedure and analyzed by the proposed method. The results showed that 5.39 ± 0.3 μg/mL of *p*-coumaric acid was extracted, which gave a recovery percentage of 82.2 ± 4.6%.

Analysis of Beer Samples. Although phenolic acids and other substances in the beer extracts may produce slight interference, *p*-coumaric acid can be quantified by the standard addition method.

The results of the standard addition method in the analyte determinations of three samples of different extracts of Victoria Spanish beer treated by the technique explained in the Experimental Section are shown in Figure 4. As shown, the graphs are linear with 0.5, 1.5, and 6.0 mL of extract. The change in slope as the sample volume increases reveals the existence of a matrix effect for high extract contents. To compensate for this effect, the standard addition method gives satisfactory results. This is expected, because changes in slope proceed from multiplicative interferences, and this type of interference is effectively accomplished by application of standard addition method (O'Haver, 1976).

The linear standard addition graphs (Figure 4) have intercepts corresponding to 0.14, 0.36, and 1.67 μg/mL, for aliquots of beer extracts of 0.5, 1.5, and 6.0 mL, respectively. Table I summarizes the results of the ordinary fluorometric procedure and those of the standard addition method to samples of extracts from a commercial beer. The results obtained agreed well with those reported by McMurrough et al. (1984) (1.1–4.6 mg/L).

Table I. Determination of *p*-Coumaric Acid in Beer

sample	vol of extr, mL	*p*-coumaric acid found, μg/mL	
		normal fluorometric[a]	std addn
1	0.5	6.66 ± 0.30	6.87
2	1.5	5.84 ± 0.37	6.01
3	6.0	5.19 ± 0.10	6.97

[a] Mean ± standard deviation (three measurements).

CONCLUSION

The usefulness of spectrofluorometric analysis has been shown to be a practical method for the determination of *p*-coumaric acid by using its characteristic intrinsic fluorescence at basic pH and is simple, rapid, and reproducible.

Combining the proposed method with the standard addition method, precise determination of concentrations in beer at microgram/milliliter levels were made.

Recovery values from synthetic beer samples were measured to evaluate the extraction procedure and gave satisfactory results.

Registry No. *p*-Coumaric acid, 7400-08-0.

LITERATURE CITED

Billet, E. E.; Grager-Barkmeijer, R. J.; Johnson, C. B.; Harborne, J. B. *Phytochemistry* **1981**, *20*, 1259–1263.
Charpentier, B. G.; Cowles, J. R. *J. Chromatogr.* **1981**, *208*, 132–136.
Friend, J. *Recent Adv. Phytochem.* **1979**, *12*, 557.
García-Sánchez, F.; Cruces, C. *Anal. Chem.* **1986**, *58*, 73–76.
Hagerman, A. E.; Nicholson, R. L. *J Agric. Food Chem.* **1982**, *30*, 1098–1102.
Hardin, J.; Stutte, C. A. *Anal. Biochem.* **1980**, *102*, 171–175.
Hiller, K. *Pharmazie* **1965**, *20*, 353–356.
IUPAC *Anal. Chem.* **1983**, *55*, 712A.
Julkunen-Tiitto, R. *J. Agric. Food Chem.* **1985**, *33*, 213–217.
Kefeki, V. T. *Natural Plant Growth Inhibitors and Phytohormones*; Junk Publishers: The Hague, 1978.
Keith, E. S.; Powers, J. J. *J. Food Sci.* **1966**, *31*, 971–979.
Lattanzio, V.; Marchesini, A. *J. Food Sci.* **1981**, *46*, 1907–1909.
Linser, H.; Maschek, F. *Planta* **1953**, *41*, 567–588.
McMurrough, I.; Roche, G. P.; Cleary, K. G. *J. Inst. Brew.* **1984**, *90*, 181–187.
Mossor-Pietraszewska, T.; Pienka, H.; Schramm, R. W. *Bull. Soc. Amis Sci. Lett.* **1981a**, *21*, 13–18.
Mossor-Pietraszewska, T.; Pienka, H.; Schramm, R. W. *Acta Phys. Plant.* **1981b**, *3*, 69–75.
Murphy, j. B.; Stutte, C. A. *Anal. Biochem.* **1978**, *86*, 220–228.
O'Haver, T. C. In "*Trace Analysis. Spectroscopic Methods for Elements*"; Winefordner, J. D., Ed.; Wiley: New York, 1976.
Okamura, S.; Watanabe, M. *Agric. Biol. Chem.* **1981**, *45*, 2063–2070.
Price, W. P.; Edens, R.; Hendrix, D. L. Deming, S. N. *Anal. Biochem.* **1979**, *93*, 233–237.
Roston, S. A.; Kissinger, P. T. *Anal. Chem.* **1981**, *53*, 1695–1699.
Schneider, E. A.; Wightman, F. *Annu. Rev. Plant Physiol.* **1974**, 487.
Sosulski, F. W. *JAOCS, J Am. Oil Chem. Soc.* **1979**, *56*, 711.
VanSumere, C. F.; Wolf, G.; Tenchy, H.; Kint, J. *J. Chromatogr.* **1965**, *20*, 48–60.
Wulf, L. W.; Nagel, C. W. *J. Chromatogr.* **1976**, *116*, 271–279.

Received for review March 5, 1986. Revised manuscript received November 20, 1986. Accepted September 3, 1987.