Process for the Purification of \textit{cis-}p-Coumaric Acid by Cellulose Column Chromatography after the Treatment of the \textit{trans} Isomer with Ultraviolet Irradiation

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A methanolic solution of \textit{trans-}p-coumaric acid was exposed to ultraviolet radiation and a mixture solution of the \textit{trans} and \textit{cis} isomers was subjected to cellulose column chromatography, eluting with an aqueous 0.1% trifluoroacetic acid solution containing methanol (90:10, v/v). Separation of the \textit{trans} and \textit{cis} isomers was achieved. The identity of the \textit{cis} isomer was confirmed by TLC, HPLC, and NMR. Since both the support and eluent are inexpensive, the \textit{cis} isomers can be obtained economically on both the laboratory and industrial scales.

Keywords \textit{cis-}p-Coumaric acid, isolation, hydroxycinnamic acid

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Introduction

Hydroxycinnamic acids are known to exist as both \textit{trans} and \textit{cis} isomers in plants; however, the \textit{cis} isomers tend to be less stable, and they are also present in smaller quantities.\textsuperscript{1} In addition, the \textit{cis} isomers of hydroxycinnamic acids play different roles from the \textit{trans} isomers. For example, \textit{cis} isomers are currently being actively studied for their roles in controlling phenylpropanoid biosynthesis\textsuperscript{2} and in the maturation of plants.\textsuperscript{3} However, although the \textit{trans} isomers of cinnamic acids can be easily obtained by chemical syntheses or from plant materials, the \textit{cis} isomers, whose isolation and preparation are more problematic,\textsuperscript{4,5} recently, Salum \textit{et al.} reported a method for the preparation of the \textit{cis} isomers of hydroxycinnamic acids by ultraviolet (UV) irradiation-treatment of \textit{trans} isomer-organic amine ion liquids.\textsuperscript{2,6} This process results in the establishment of a stoichiometric equilibrium between the \textit{trans} and \textit{cis} isomers. Subsequent treatment of this solution then allows separation and purification of the two isomers. However, this method requires the use of expensive solvents, and so the isolation of the \textit{cis} isomers of hydroxycinnamic acids would be a rather costly process.

Thus, we herein report a novel process for the preparation, separation, and purification of the different \textit{cis-trans} isomers of hydroxycinnamic acids, such as \textit{p}-coumaric acid (PC), caffeic acid (CA), and ferulic acid (FA), \textit{via} a low-cost UV irradiation and liquid chromatographic procedure using a cellulose column.

Experimental

Reagents

For this study, \textit{trans-}PC (Sigma-Aldrich), \textit{cis-}PC (Toronto Research Chemicals: TRC), and CA (Sigma-Aldrich) were employed in this study. FA was donated by Tsuno Co., Ltd. All chemicals were either of analytical- or HPLC-grade purity.

\textit{UV irradiation}

The UV lamp (Sankyo G15F8E, Sankyo Denki Co., Ltd.) employed herein emitted UV-B irradiation of wavelengths \(\geq 280\) nm and a peak wavelength of 306 nm. For irradiation of the samples, \textit{trans-}PC was dissolved in methanol (1000 \(\mu\)g/mL), and the resulting solution was placed in a glass vial and exposed to UV-B irradiation at an intensity of 0.75 mW/cm\(^2\) \(\pm 10\%\) for the desired time.

\textit{Cellulose column chromatography}

For cellulose column chromatography, microcrystalline cellulose (Merck) was used as the stationary phase and was suspended in a solvent mixture containing an aqueous solution of 0.1% trifluoroacetic acid (TFA) and methanol (90:10, v/v). The resulting suspension was poured into a glass column (\(650\) mm \(\times\) \(300\) mm, Asahi, Glass Inc.) to give a packed height of 230 mm. The above solvent was also used as the mobile
phase for separation at a flow rate of 4.7 mL/min. The resulting eluate was collected in 10 mL fractions, which were measured by UV absorption at 280 nm.

**Thin layer chromatography (TLC)**

One-dimensional TLC separation of the phenolic acids was carried out using a method previously reported by Kowalski.\(^7\) A 20 × 20 cm cellulose TLC plate (Merck) was employed, along with methanol:1% acetic acid in water (1:10, v/v) as a developing solution.

**UV spectroscopy**

UV spectra were collected on a Shimadzu 1800 UV/VIS spectrometer. Calibrated 1 cm cells were used throughout. Readings were taken at intervals of 1 nm at 20°C

**High performance liquid chromatography (HPLC)**

HPLC was carried out using a Shimadzu LC-2010 equipped with a HydroSphere C18 column (ϕ4.6 mm × 250 mm, YMC, Kyoto, Japan). The column was eluted using the following solvent mixtures at a flow rate of 1.0 mL/min: A = 0.1% TFA in water; B = methanol, 0 – 10 min; A:B = 80:20, 10 – 80 min; a linear gradient system was applied to give a final composition of A:B = 25:75. All analyses were carried out at 30°C and were monitored by UV absorption at 280 nm.

**Nuclear magnetic resonance spectroscopy (NMR)**

The samples for NMR analysis were prepared by dissolving the isolated sample in either deuterated methanol-\(d_4\) or deuterated dimethylsulfoxide (DMSO)-\(d_6\). The \(^1\)H NMR spectra were then recorded using a JEOL JNM-ECA 400 FT NMR spectrometer (\(4\), 400 MHz). Chemical shifts are reported as \(\delta\) values (in ppm) relative to a tetramethylsilane (TMS) standard, and all coupling constants (\(J\)) are quoted in Hertz.

**Results and Discussion**

**Discrimination between trans-PC and cis-PC**

Kowalski et al. previously reported the separation of trans-PC and cis-PC by TLC.\(^6\) We therefore employed the reference compounds cis-PC and trans-PC to reproduce their results. As shown in Fig. 1, the retardation factor (Rf) values for cis- and trans-PC using the solvent system described above were 0.62 and 0.26, respectively. In addition, HPLC using the previously described conditions resulted in the elution of cis-PC with a retention time (RT) of 32.784 min and trans-PC with a RT of 33.692 min (Fig. 2).

**UV irradiation of trans-PC**

As described in the Experimental section, trans-PC was dissolved in methanol and the resulting solution was placed in a glass vial (ϕ8 mm × 35 mm) for exposure to UV-B irradiation (≥280 nm) for 96 h. Samples were collected at various time intervals, and the production of the cis isomer was monitored and confirmed by both TLC and HPLC. As shown in Fig. 3, following exposure to UV irradiation, a sharp decrease in the peak area corresponding to trans-PC was observed, which was accompanied by the formation of a peak corresponding to cis-PC. Under these conditions, equilibrium was reached after exposure for ∼150 min.

**Separation of cis-PC acid and trans-PC using a cellulose column**

A methanolic solution of the trans-PC isomer (1000 \(\mu\)g/mL) was placed into a glass vial (ϕ35 mm × 78 mm), and exposed to UV irradiation of the same intensity for 72 h as one example. The resulting solution was then concentrated to dryness using a rotary evaporator. Subsequently, the obtained solid was redissolved in a 90:10 (v/v) mixture of 0.1% aqueous TFA and methanol to give a mixed solution of the trans and cis isomers (TCM) with a final concentration of 5000 \(\mu\)g/mL.

The TCM mixture (5 mL) was applied to the cellulose column and eluted using a 90:10 (v/v) mixture of 0.1% aqueous TFA and methanol. The resulting eluate fractions were then analyzed by UV absorption at 280 nm to detect the presence of cis- and trans-PC. As shown in Fig. 4, two peaks were present in the chromatogram, and so the fractions corresponding to each peak were collected, then evaporated to dryness using a rotary evaporator. Upon comparison of the developed TLC plate containing this dry material and the cis-PC and trans-PC reference products, it was evident that the first peak corresponded to cis-PC, while the second peak corresponded to trans-PC (Fig. 1). As indicated, a trace level spot with an Rf value comparable to that of trans-PC was present in the TLC plate corresponding to the product obtained from the first peak fractions (i.e., the fractions containing cis-PC). In addition, when this concentrated material was analyzed by HPLC, the main peak of RT 31.581 min was consistent with the cis-PC RT of 31.587 min obtained for the reference product. However, a small peak was also present at a RT of 32.745 min, which corresponded to that of trans-PC (Fig. 5). These results therefore indicate a cis-PC purity of 98.72%. It should also be noted that in addition to the main peak corresponding to cis-PC, a trace peak corresponding to trans-PC (RT 32.752 min) was
also observed in the reference product, giving a cis-PC purity of 99.2%. The absorption spectrum of the cis-PC reference products was almost consistent with that of the cis-PC prepared compound (Fig. 6). The shift toward shorter wavelength in cis-PC has been seen in comparison with trans-PC.

The separation and purification of the respective cis isomers of CA and FA could be also achieved using the above irradiation-separation procedure. Upon separation by cellulose column chromatography, the UV-irradiated CA and FA samples also produced two clear signals by HPLC (Fig. 2). In the case of CA, fractions numbered 40 – 50 were collected and the solvent evaporated (Fig. 7). The resulting dry material was redissolved in methanol and analyzed by HPLC. In this case, a single peak (peak X) was detected at RT 23.674 min. More specifically, trans-CA was observed at a RT of 24.520 min, while the peak X at RT 23.674 min should correspond to cis-CA (Fig. 2). Similar results were also observed for the UV-irradiated FA sample. As shown in Fig. 7, fractions of 40 – 50 were collected and analyzed. A single peak was also observed at a RT of 36.898 min, which was exactly equal to peak Y in Fig. 2. This peak should correspond to cis-FA.
Analysis by NMR spectroscopy
Following elution from the cellulose column, the fractions containing cis-PC were collected and concentrated to dryness using a rotary evaporator. The obtained powder was then analyzed by $^{1}$H NMR spectroscopy to confirm its identity as cis-PC. $^{1}$H NMR data for cis-PC: δ12, 26 (s, 1H), 9.85 (s, 1H), 7.65 – 7.62 (m, 2H), 6.80 – 6.72 (m, 3H), 5.74 (d, 1H, $J = 12.0$ Hz). $^{1}$H NMR data for trans-PC: δ12, 20 (s, 1H), 9.98 (s, 1H), 7.52 – 7.47 (m, 3H), 6.80 – 6.77 (m, 2H), 6.31 (d, 1H, $J = 16.0$ Hz).

Conclusions
As mentioned above, Salum et al. reported a method for the preparation of the cis isomers of hydroxycinnamic acids by ultraviolet (UV) irradiation treatment of trans isomer-organic amine ion liquids. Their process consisted of five steps: 1) ionic liquid compound preparation, 2) dilution of ionic liquid by acetonitrile, 3) UV treatment of ionic liquid compound in solution, 4) recovery of solid precipitate (cis isomer-organic amine ion liquids) by filtration, and 5) separation of ion liquids from cis isomer-organic amine ion liquids by ion exchange chromatography, or extraction of dichloromethane and removal of the solvent by evaporation. On the other hand, our process consisted of three steps; 1) UV treatment of trans isomer in...
methanol, 2) separation of trans and cis isomer by cellulose column chromatography, and 3) removal of the solvent by evaporation. From the above results, we could therefore conclude that our process for the preparation and isolation of the cis isomer of PC from the UV irradiation of the trans isomer appears suitable for both laboratory and industrial applications. As the cellulose column support and the methanol/aqueous TFA eluting solution are inexpensive, our process will allow for low-cost production of the cis isomers of hydroxycinnamic acids when compared to previous ionic liquid approaches. Furthermore, our process allows separation and purification of the individual geometric isomers of different hydroxycinnamic acids (i.e., CA and FA) through simple liquid chromatographic techniques, thus permitting the isolation of the cis isomers of cinnamic acids in large quantities. As it has been reported that the cis isomers of cinnamic acids exhibit stronger antimicrobial action than the trans isomers,8 we expect that our system will contribute to studies on the physiological activities of these compounds, as they can now be prepared and purified on a larger scale at lower cost.

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