Synthesis and radical scavenging activity of cinnamic acid esters

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Cinnamic and hydroxycinnamic acid esters (α, β-unsaturated esters), functional derivatives of cinnamic acids (cinnamic, ferulic, sinapic, caffeic) are secondary plant metabolites derived from phenylpropanoid pathway. Cinnamates, of both natural and synthetic origin, continue to elicit great interest due to diversity of biological activities they possess, such as: antioxidant, antimicrobial, anticancer, anti-inflammatory, anti-tyrosinase and etc.

INTRODUCTION

Cinnamic (3-phenyl propenoic) acid and its patterns with different hydroxylated and methoxylated phenyl moiety: p-coumaric (4-hydroxy cinnamic acid), ferulic (4-hydroxy-3-metoxy cinnamic acid), caffeic (3,4-dihydroxycinnamic acid), sinapic (3,5-dimetoxy-4-hydroxy-cinnamic acid) acids, belong to a diverse group of phenolic compounds. These secondary plant metabolites are biosynthesized via shikimate pathway that is involved in plant adaptation to environmental stress (e. g. microbial pathogens, mechanical wounding, UV irradiation, salinity) [1].

Keywords: N-protected amino alcohols, hydroxycinnamates, N-hydroxycinnamoyl amino acid amides, DPPH scavenging activity

Cinnamic and hydroxycinnamic acid esters are known with numerous bioactive properties, mostly related to their antioxidant activity [5-7].

Besides antioxidant activity, cinnamates have been regarded as photoprotectors, antimicrobials, and effective as anticancer, anti-inflammatory, analgesic, antimicrobial and antithrombotic agents [8-11].

Whereas hydroxycinnamates with alcohols, phenols, saccharides and flavonoids are common phytochemical constituents, those with the participation of the OH group of the side chain of amino acids are very scanty. There are only few reports of such metabolites - caffeic acid esters, derived from insects: O-caffeoyltirosine from Aonidiella aurantii (California red scale), O-caffeoylserine from Phenacoccus herreni (Cassava mealybug) [12, 13] and from plant origin: L-O-caffeoylhomoserine [14]. In addition, O-caffeoylserine has been also synthetically obtained [15].

The revealed pharmacological activities of hydroxycinnamic acid esters and their small quantities in plants, evoked the interest of organic researchers to design their synthetically analogues.

Ester functionalization of cinnamic acids comprises classical procedures-accomplished via cinnamoylchloride [16], N,N'-dicyclohexyl-carbodiimide (DCC) [17] or BOP [18] as coupling agents. Moreover, those compounds can also be obtained using Wittig reaction under different
conditions [19-22] and as well as green esterification procedures [23-27]. Considering the importance of phenolic compounds, e.g. hydroxycinnamic acid esters for removal of oxidative stress, and thus to prevent lifestyle-related diseases such as cancer, diabetes or heart diseases, herein we prepared hydroxycinnamic acid esters and tested them as scavengers against DPPH radical.

**EXPERIMENTAL**

**General information**

All amino acid derivatives, ferulic (3-methoxy-4-hydroxy-cinnamic, FA), sinapic (3,5-dimethoxy-4-hydroxy-cinnamic, SA) acids, as well as isobutyl chloroformate (IBCF), 4-methylmorpholine (NMM), 4-(dimethylamino)pyridine (DMAP), dicyclohexylcarbodiimide (DCC), NaBH₄, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH*) were purchased from Sigma Aldrich (FOT, Bulgaria). Tetrahydrofuran was obtained from Fisher Chemical (Bulgaria) and further was distilled over LiAlH₄ and stored under argon. All other solvents were of reagent grade and used without further purification.

Thin-layer chromatography (TLC) was conducted on precoated Kieselgel 60F₂₅₄ plates (Merck, Germany). Separation of the compounds by preparative thin layer chromatography with silica gel 60 GF₂₅₄ (Merck, Bulgaria).

The NMR experiments were recorded on Bruker Avance III 600 or Bruker Avance III 400 spectrometer, operating at 600.13 and 400.15 MHz for protons respectively. The measurements in CDCl₃ solutions were carried out at ambient temperature (300 K) and tetramethylsilane (TMS) was used as an internal standard. The UV spectra of the compounds were measured with an “Agilent 8453” UV-vis spectrophotometer. Electrospray Ionisation (ESI) and EI mass spectra were recorded corresponding on an Esquire 3000 and MAT 8230. Attenuated total reflectance infrared spectroscopy (ATR-IR) measurements were performed using Thermo Scientific Nicolet iS10 FT-IR device with ID5 ATR accessory (diamond crystal).

**General procedures**

**Synthesis of 2a, b (a modified method reported by Kokotos [28])**. A solution of 1.6 mmol N-protected amino acid (1a, b) in 10 ml dry THF is cooled to −15°C and added 0.18 ml (1.6 mmol) N-methylmorpholine. Isobutyl chloroformate (0.24 ml, 1.7 mmol) is added dropwise to make sure that the internal temperature does not rise above -10 °C. About 5-8 minutes later, to the white suspension of formed isobutylcarboxylic acid mixed anhydrides[29] is added 0.104 g (2.74 mmol) NaBH₄ in 10 ml THF, and for a period of 10 min abs.CH₃OH (5 ml) is added dropwise. Stirring is continued and the progress of the reaction is monitored by thin layer chromatograms (hexane:ethylacetate=1:1).

At the completion of the reduction (2h) the solvent is evaporated under reduced pressure. The residue is dissolved in ethylacetate and is washed twice consequently with 5% NaHSO₃, 5% NaHCO₃ and finally with saturated sodium chloride solution. The organic phase is dried over Na₂SO₄, filtered and the solvent removed in vacuo. The obtained crude product is purified by preparative thin layer chromatography (hexane:ethylacetate= 3:1).

S-Benzyl-N-(tert-butoxycarbonyl)-L-cysteinol (Boc-Cys(Bzl)-ol) (2a) white crystal, 61% yield;


N-(tert-butoxycarbonyl)-L-valinol (Boc-Val-ol) (2b) yellow oil, 53% yield;

**1H NMR** (CDCl₃, δ ppm): 0.9 (dd, 6H, -CH(CH₃)₂), 1.2 (s, 9H, -C(CH₃)₃), 1.8-1.84 (m, 1H, -CH(CH₃)₂), 3.4-3.6 (m, 3H, -CH₂OH, -NH-CH<), 4.8 (br. s, 1H, NH): **EI-MS**: 57.1, 73.1, 116.1, 130, 172.1, 203 [M⁺]; **IR (ATR)max**: 3318.1, 3189.5, 2978.6, 1679.7, 1366.5, 1289.4, 1150.7, 1011.8, 908.9, 772.0, 699.4 cm⁻¹;

**Esterification of cinnamic acids with N-protected amino alcohol [30]**. Cinnamic acids (1.5 mmol), DCC (1.5 mmol) and DMAP (0.0224 mmol) are dissolved in 10 mL of dry THF. The reaction mixture is stirred under argon at 0°C and then, after 10 min N-protected amino alcohol (0.6 mmol) is added. The mixture is kept under vigorous stirring and cooling (0°C) for 60 min and then is allowed to stand at room temperature overnight. The residue of dicyclohexylcarbamide is filtered and washed with cold ethylacetate. The combined solutions are evaporated under vacuum and the residue is purified by column chromatography on silica.

**Ferulate of Boc-Val-ol, 35% yield.** UV (C₃H₅OH) λ max = 203, 218, 236, 328 nm; **IR (ATR)max**: 3335.5, 2959.6, 2932.1, 1707.2, 1627.4, 1592.3, 1510.9, 1365.5, 1269.7, 1246.7, 1157.4, 1119.8, 1029.32, 978.9 cm⁻¹; **1H NMR** (CDCl₃)/600 MHz/ δ = 0.9 (dd, 6H, -CH(CH₃)₂), 1.2 (s, 9H, -C(CH₃)₃), 1.8 (m, 1H, -CH(CH₃)₂), 3.7 (s, 2H, -OCH₂), 3.8 (s, 3H, -OCH₃), 4.1 (m, 1H, -NH-CH<), 5.0 (br. s, 1H, NH), 6.2 (d, 1H, J=15.5 Hz, -CH=CH-
Sinapate of Boc-Val-ol, 49 % yield. UV (C₄H₅OH) λ max = 203,229, 329 nm; IR (ATR)υmax: 3376.3, 2936.4, 2844.2, 1704.6, 1632.7, 1594.5, 1456.2, 1419.11, 1383.1, 1275.2, 1204.2, 1111.6, 980.1, 822.8 cm⁻¹; ¹H-NMR (CDCl₃) /600 MHz/ δ = 0.9 (dd, 6H, -CH(CH₃)₂), 1.8 (m, 1H, -CH(CH₃)₂), 3.7 (s, 2H, -OCH₂), 3.8 (s, 6H, 2 x-OCH₃), 4.2 (m, 1H, NH-CH), 5.6 (br. s, 1H, NH), 6.5 (d, 1H, J = 15.5 Hz, -CH=CH₂), 7.5 (s, 2H, Ar-H), 7.5 (d, 1H, J = 15.5 Hz, -CH=CH₂); EI-MS: 57.1, 207.1, 308, 238.1, 336.2, 353.2, 409.2 [M⁺].

Ferulate of Boc-Cys(Bzl)-ol, 20 % yield. UV (C₄H₅OH) λ max = 203,217, 236, 327 nm; IR (ATR)υmax: 3343.4, 1679.7, 1526.6, 1364.7, 1340.9, 1311.3, 1284.1, 1163.2, 1076.9, 1003.9, 698.6 cm⁻¹; ¹H NMR (DMSO-d₆, ppm): δ 1.42 (s, 9H, -C(CH₃)₃), 3.21 (dd, J = 14.2, 5.2 Hz, 1H, CHCH₂), 3.4 (dd, J = 14.2, 4.6 Hz, 1H, CHCH₂), 3.66 (d, J = 5.6 Hz, 2H, -S-CH₂-Ph), 3.73 (s, 3H, OCH₃), 4.39 (d, 2H, -CH₂-O - ), 4.87 (ddd, J = 7.0, 5.2, 4.6 Hz, 1H, CHCH₂), 5.82 (br. s, 1H, OH), 6.28 (d, J = 7.0 Hz, 1H, NH), 6.32 (d, J = 15.6 Hz, 1H, -CH=CH₂), 6.93 (d, J = 6.7 Hz, 1H, m-ArH), 7.01 (d, J = 1.6 Hz, 1H, o-ArH), 7.06 (dd, J = 8.0, 1.6 Hz, 1H, o-ArH), 7.24 (m, 5H, Ar-H), 7.57 (d, J = 15.6 Hz, 1H, -CH=CH₂); EI-MS: 57.1, 91.0, 177.1, 473.3 [M⁺].

Sinapate of Boc-Cys(Bzl)-ol, yield 25 %

UV (C₃H₇OH) λ max = 203,228, 330 nm; IR (ATR)υmax: 3392.8, 1705.1, 1397.5, 1507.1, 1456.8, 1418.8, 1108.3, 870.4, 659.0 cm⁻¹; ¹H NMR (DMSO-d₆, ppm): δ 1.42 (s, 9H, -C(CH₃)₃), 3.71 (dd, J = 14.2, 5.2 Hz, 1H, CHCH₂), 3.4 (dd, J = 14.2, 4.6 Hz, 1H, CHCH₂), 3.68 (d, J = 5.6 Hz, 2H, -S-CH₂-Ph), 3.73 (s, 6H, 2 x OCH₃), 4.51 (d, 2H, -CH₂-O - ), 4.63 (ddd, J = 7.0, 5.2, 4.6 Hz, 1H, CHCH₂), 5.72 (s, 1H, OH), 6.28 (d, J = 7.0 Hz, 1H, NH), 6.32 (d, J = 15.6 Hz, 1H, =CH), 6.7 (s, 2H, Ar-H), 7.28-7.51 (m, 5H, Ar-H), 7.59 (d, J = 15.6 Hz, 1H, =CH); EI-MS: 57.1, 91.0, 207.1, 238.1, 266.1, 386.3, 447.1, 503.3 [M⁺].

RESULTS AND DISCUSSION

Herein, in order to elucidate the antiradical activity of cinnamic acid esters, we firstly obtained synthetically the amino alcohols (used as intermediates).

By applying a modified method [28], the NaBH₄ reduction of protected amino acids (Scheme1; 1a,b) into corresponding amino alcohols (2a, b) was occurred by means of in situ formed isobutyric acid mixed anhydrides in THF. However, the establishment that the reducing power of NaBH₄ in THF increases when methanol is added drop-wise [28] enforces us to accomplish the reaction in the same manner. The expected amino alcohols were isolated on silica gel by preparative thin layer chromatography (hexane:ethylacetate) in moderate yields.

The hydroxycinamic acid esters (Table 1, 3a-d) were prepared by esterification of sinapic- (SA) and ferulic (FA) acids with compounds 2a, b using DCC/DMAP method [30]. The structures of desired hydroxycinnamates were elucidated by UV, IR, ESI(EI)-MS and ¹H-NMR spectroscopic analyses.

Scheme 1. i) IBCF/NMM, THF, -15°C ii) NaBH₄, CH₃OH
The values of the proton-proton vicinal coupling constants ($J_{HH}$, about 15.5 Hz) measured for the olefinic protons of feruloyl- and sinapoyl moieties define $E$ configuration of the double bond of the studied compounds (3a-d).

Highlighting the valuable role of hydroxycinnamic acid derivatives as antioxidants, the search for new, more effective and better radical scavengers is a major challenge.

$1,1$-Diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity

Being stable and commercially available organic nitrogen radical, DPPH is often used for primary assessment of antioxidant activity. DPPH method gives rapid and highly reproducible results, therefore we applied this method to estimate and compared the radical scavenging abilities of the synthesized hydroxycinnamic acid derivatives (esters and amides).

As shown in Table 1, % RSA values of hydroxycinnamic acid (sinapic and ferulic) and their derivatives are presented for 20-min reaction period (3.6 mM), as proposed by Nenadis et al. [31].

The DPPH scavenging activity of hydroxycinnamates (3a-d) was compared with those of corresponding previously synthesized amides (4a-d). Results obtained indicated that amide derivatives (4a-d) were found to be more potent than corresponding hydroxycinnamoyl esters (3a-d). The established increase of antiradical activity in hydroxycinnamoyl amino acid amides may be due to the presence of other hydrogen-donating amide group. By comparison of DPPH activity of studied esters and amides with their corresponding free cinnamic acids the higher radical scavenging ability of the parent acids was established. Actually, $N$-sinapoyl amide of cysteine (SA-Cys(Bzl)-OEt (4b)) showed similar DPPH scavenging activity as ferulic acid was [32]. Moreover, our results are in a good correlation with those presented elsewhere, that

### Table 1. Structures of hydroxycinnamic derivatives studied and their DPPH*-scavenging activity.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>RSA %</th>
<th>3.6 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>R'</td>
</tr>
<tr>
<td></td>
<td>20'</td>
<td></td>
</tr>
<tr>
<td>FA ester of BocCys(Bzl)ol (3a)</td>
<td>H</td>
<td>-</td>
</tr>
<tr>
<td>SA ester of BocCys(Bzl)ol (3b)</td>
<td>OCH$_3$</td>
<td>-</td>
</tr>
<tr>
<td>FA ester of BocVal-ol (3c)</td>
<td>H</td>
<td>-</td>
</tr>
<tr>
<td>SA ester of BocVal-ol (3d)</td>
<td>OCH$_3$</td>
<td>-</td>
</tr>
<tr>
<td>FA-Cys(Bzl)-OEt* (4a)</td>
<td>H</td>
<td>C$_2$H$_5$</td>
</tr>
<tr>
<td>SA-Cys(Bzl)-OEt* (4b)</td>
<td>OCH$_3$</td>
<td>C$_2$H$_5$</td>
</tr>
<tr>
<td>FA-Val-OCH$_3$ (4c)</td>
<td>H</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>SA-Val-OCH$_3$ (4d)</td>
<td>OCH$_3$</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>Sinapic acid (SA)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ferulic acid (FA)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

% RSA—percent radical scavenging activity; % RSA = [Abs$_{516nm(t = 0)}$ - Abs$_{516nm(t = t')}$] x 100/Abs$_{516nm(t = 0)}$; sinapic and ferulic acids were used as standards.

*The RSA of hydroxycinnamoylamides were previously reported [32] and were used for comparison.
introduction of additional methoxyl group in an ortho-position to a hydroxyl group (such as in sinapic acid series, Table 1) is an important for the radical scavenging activities of phenolic acids.

CONCLUSION

In our study N-protected amino alcohols were chemically obtained and further used as intermediate analogues for synthesis of hydroxycinnamates.

The sinapic and ferulic acid derivatives (esters and amides) were tested and compared for their in vitro antiradical activity towards DPPH radical. It was found that N-hydroxycinnamamoyl amino acid amides showed better radical scavenging activity than the corresponding hydroxycinnamates, whereas the free hydroxycinnamic acids (used as standards) remained the most active ones in this test.

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**Synthesis and radical scavenging activity of cinnamic acid esters**

M. Chochkova et al.: Synthesis and radical scavenging activity of cinnamic acid esters

СИНТЕЗ И ИЗСЛЕДВАНЕ НА РАДИКАЛ-УЛАВЯЩА АКТИВНОСТ НА ЕСТЕРИ НА КАНЕЛЕНИТЕ КИСЕЛИНИ

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(Резюме)

Канелената, хидроксиканелените киселини (ферулова, синапова, кафеена) и техните естери представляват вторични растителни метаболити, биосинтезирани от фенилпропаноидния метаболитен път. Природните хидроксицинамати и техните синтетични аналоги привличат вниманието на изследователите поради широкия спектър от биологични активности като: антиоксидантна, антимикробиална, противотуморна, противовъзпалителна, тирозиназно-инхибиторна и др.

В настоящото изследване е разгледана редукция на карбоксилната група на защитени аминокарбоксилни киселини на Nα-място и в страничната верига. След естерифициране на получените аминоалкохоли с хидроксиканелени (синапова и ферулова) киселини, новосинтезираните производни са подложени на изследване за радикалуклавяща активност спрямо 1,1-Дифенил-2-пикрилхидразилов радикал (DPPH•). Резултатите от антирадикалова активност на хидроксикнинаматите са сравнени с тези на съответните хидроксицинамоиламиди с аминокарбоксилни киселини. Като стандарти антиоксиданти са използвани свободните хидроксицинамени киселини. Установено е, че хидроксицинамоиламидите показват по-ниска антирадикалова активност от съответните свободни хидроксицинамени киселини, но по-висока от тази на хидроксицинаматите.