Hydroxycinnamic Acid Amides with Oxazole-Containing Amino Acid: Synthesis and Antioxidant Activity

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Three hydroxycinnamic acid derivatives conjugated with glycine-containing oxazole were synthesized. The prepared compounds were tested for their antioxidant activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\cdot}$) test. Among the tested hydroxycinnamic acid amides the highest DPPH scavenging activity has been found for the sinapic acid amide.

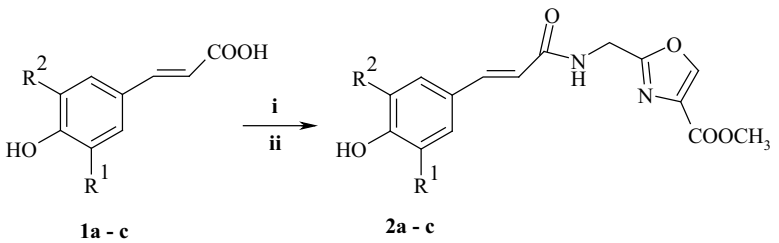
Key words: Hydroxycinnamoyl Amides, Oxazole, Radical Scavenging Activity

Introduction

Cinnamic acids and their derivatives (esters, amides and glycosides) attract attention in biology and medicine because of their antiviral (Burke *et al.*, 1995), antioxidant (Moon and Terao, 1998; Perez-Alvarez *et al.,* 2001; *Castelluccio et al.*, 1996; Lee *et al.,* 2007; Hensel *et al.,* 2007), anti-inflammatory (Sudina *et al.*, 1993) and antimutagenic properties (Namiki, 1990). Previously, we reported that hydroxycinnamic acid amides behave as good antioxidants in bulk phase lipid autoxidation (Spasova *et al.*, 2007). The highest antioxidant activity was found for the compounds (*E*)-*N*-(feruloyl)-L-phenylalanine *t*-butyl ester and (*E*)-*N*-(sinapoyl)-L-phenylalanine *t*-butyl ester. Actually, information on the radical scavenging activity of hydroxycinnamic acid of peptide mimetics is very limited (Stankova *et al.*, 2008). Our search for potent radical scavengers is continued with substituted cinnamic acids containing different peptide mimetics.

Results and Discussion

The synthetic rout for the preparation of *p*-coumaric, ferulic and sinapic acid amides is shown in Fig. 1. The synthesis of oxazole-containing glycine was done according to Videnov *et al.* (1996). A solution of sinapic (**1c**), *p*-coumaric (**1a**), and ferulic (**1b**) acids in dimethylformamide (DMF) was treated with triethylamine and TFA-2-aminomethyl-oxazole-4-carboxylic acid methyl ester, using the coupling agent *N*-ethyl-*N*'-(3-dimethyl-



1a - c **2a - c**

1 a) *p*-Coumaric acid; R^1, R^2 = H
1 b) Ferulic acid; $R^1 = OCH_3$; R^2 = H
1 c) Sinapic acid; R^1, $R^2 = OCH_3$

(**i**) TFA - 2-aminomethyl-oxazole-4-carboxylic acid methyl ester; (**ii**) EDC/ DMAP.

Fig. 1. Synthesis of hydroxycinnamic acid amides of 2-aminomethyl-oxazole-4-carboxylic acid methyl ester.

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aminopropyl) carbodiimide hydrochloride (EDC) and 4-(dimethylamino)-pyridine (DMAP) as a catalyst, to produce the amide derivates **2a–c**.

It is well accepted that the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging by antioxidants is attributed to their hydrogen-donating ability (Chen and Ho, 1995). The radical scavenging activities of the hydroxycinnamic acid amides **2a–c** were determined by the DPPH assay according to the method, proposed by Pekkarinen *et al.* (1999). The results obtained for the antioxidative potential of the synthesized amides against DPPH$^{\cdot}$ are shown in Table I. The synthesized hydroxycinnamic acid amides were found to be weak radical scavengers. Among them; compound **2c** showed the highest antioxidant activity, but it was lower than those of the standards α-tocopherol, ferulic and sinapic acids.

These results demonstrate that modification of hydroxycinnamic acid with peptide mimetics (oxazole, thiazole) does not lead to an antioxidative effect compared to natural amino acids.

Material and Methods

General

The amino acid derivatives and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma, DMAP and EDC were purchased from Merck. All other chemicals were from Fluka (Buchs, Switzerland).

The NMR spectra were obtained on a Bruker Avance DRX-250 spectrometer.

Mass spectra were mecesured using an API triple quadrupole mass spectrometer equipped with an electrospray ion sourse at atmospheric pressure (Sciex, Thornhill, Canada); electrospray ionization mass spectra were recorded in the positive ion mode.

The UV spectra were measured with a Specord UV-VIS spectrophotometer. An "Agilent 8453" spectrophotometer was used for the measurement of the reduction of DPPH$^{\cdot}$ absorbance at 516 nm.

Synthesis of amides

The phenolic acid (*p*-coumaric, ferulic or sinapic) (1 mM) was dissolved in 2 ml DMF. The solution was cooled in an ice-water bath and EDC (1 mM) was added. After 8 min TFA-2-aminomethyl-oxazole-4-carboxylic acid methyl ester (1 mM), triethylamine (1 mM), and DMAP (1 mM) were added. The reaction mixture was stirred for 18 h at room temperature. The mixture was poured into 5% $NaHCO_3$, extracted with CH_2Cl_2 (5 times), washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by TLC on Kieselgel 60 F_{254} (Merck) using the solvent system hexane/EtOAc (4:5).

p-Coumaric acid amide of 2-aminomethyl-oxazole-4-carboxylic acid methyl ester (**2a**): Yield

Table I. Radical scavenging activity (RSA) of hydroxycinnamic acid amides **2a–c** toward DPPH$^{\cdot}$.

Compound	RSA (%)					
	0.9 mM		1.8 mM		3.6 mM	
	Reaction time [min]					
	10	20	10	20	10	20
Sinapic acid (**1c**)	16.1	17.2	26.5	31.9	69.0	69.6
Sinapic acid amide of 2-aminomethyl-oxazole-4-carboxylic acid methyl ester (**2c**)	6.0	7.1	6.5	10.1	11.7	14.1
D,L-α-Tocopherol	15.5	15.9	34.9	38.4	53.0	58.1
Boc-2-aminomethyl-oxazole-4-carboxylic acid methyl ester	1.9	2.5	2.1	2.6	2.1	2.5
Ferulic acid (**1b**)	12.0	13.8	21.0	25.1	36.7	44.3
Ferulic acid amide of 2-aminomethyl-oxazole-4-carboxylic acid methyl ester (**2b**)	4.7	6.3	6.6	8.4	10.0	12.6
p-Coumaric acid (**1a**)	2.1	2.9	3.7	4.7	4.5	6.1
p-Coumaric acid amide of 2-aminomethyl-oxazole-4-carboxylic acid methyl ester (**2a**)	3.0	3.6	3.6	4.5	3.9	4.6

% RSA was determined as proposed by Pekkarinen *et al.* (1999); sinapic, ferulic, *p*-coumaric acids and α-tocopherol were used as standards.

0.258 g (85%). – UV (EtOH): λ_{max} = 208, 261 nm. – ^{1}H NMR (250 MHz, $CDCl_3$): δ = 3.86 (s, 1H, OC**H**$_3$), 4.62 (d, 2H, C**H**$_2$), 5.06 (br.s, 1H, O**H**), 6.57 (d, 1H, C**H**=), 6.75 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.31 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.61 (d, 1H, C**H**=), 7.92 (t, 1H, NH), 8.22 (s, 1H, CH_{Oxa}). – ^{13}C NMR (250 MHz, $CDCl_3$): δ = 166.0, 162.3, 160.8, 161.4, 146.8, 142.2, 133.4, 131.1, 126.7, 116.6, 114.7, 52.2, 38.0. – ESI-MS: *m/z* = 304 ([M + H]$^+$).

*Ferulic acid amide of 2-aminomethyl-oxazole-4-carboxylic acid methyl ester (***2b***)* : Yield 0.262 g (82%). – UV (EtOH): λ_{max} = 205, 279 nm. – ^{1}H NMR (250 MHz $CDCl_3$): δ = 3.77 (s, 3H, OC**H**$_3$), 3.83 (2H, d, CH_2), 3.91 (s, 1H, OC**H**$_3$), 5.68 (br.s, 1H, O**H**), 6.65 (d, 1H, C**H**=), 6.91 (d, 1H, Ar-**H**), 7.07 (d, 1H, Ar-**H**), 7.51 (d, 1H, C**H**=), 7.99 (t, 1H, NH), 8.23 (s, 1H, CH_{Oxa}). – ^{13}C NMR (250 MHz $CDCl_3$): δ = 166.8, 161.4, 162.3, 147.9, 146.6, 145.4, 144.2, 133.4, 126.7, 123.1, 114.7, 114.6, 109.8, 55.9, 52.6, 38.0. – ESI-MS: *m/z* = 321 ([M + H]$^+$).

*Sinapic acid amide of 2-aminomethyl-oxazole-4-carboxylic acid methyl ester (***2c***)* : Yield 0.289 g (80%). – UV (EtOH): λ_{max} = 206, 280 nm. – ^{1}H NMR (250 MHz, $CDCl_3$): δ = 3.16 (s, 6H, 2 x OCH_3), 3.84 (s, 3H, OCH_3), 4.62 (d, 2H, CH_2), 5.71 (br.s, 1H, O**H**), 6.65 (d, 1H, C**H**=), 6.75 (s, 2H, Ar-**H**), 7.99 (d, 1H, C**H**=), 8.03 (s, 1H, NH), 8.2 (s, 1H, CH_{Oxa}). – ^{13}C NMR (250 MHz; $CDCl_3$): δ = 169.1, 162.3, 161.4, 149.4, 147.2, 142.2, 139.6, 133.4, 126.6, 115.7, 106.9, 52.2, 38.0. – ESI-MS: *m/z* = 363 ([M + H]$^+$).

Estimation of the radical scavenging activity (RSA) by the DPPH· test

The radical scavenging activity determination of the new compounds was based on the method of Pekkarien *et al.* (1999). For each compound and concentration tested (0.9 mM, 1.8 mM and 3.6 mM), the reduction of the DPPH· radical was followed by monitoring the decrease of absorbance at 516 nm. The absorption was monitored at 10 and 20 min. The results are expressed as

$$\% \text{ RSA} = [\text{Abs}_{516\text{ nm (t = 0)}} - \text{Abs}_{516\text{ nm (t = t')}}] \cdot 100/\text{Abs}_{516\text{ nm (t = 0)}},$$

as proposed by Pekkarien *et al.* (1999).

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