Synthesis of Lipophilic Clovamide Derivatives and Their Antioxidative Potential against Lipid Peroxidation

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Some *N*-(hydroxycinnamoyl)-L-tyrosine and L-DOPA alkyl esters were synthesized and evaluated as a variation of the clovamide (*N*-caffeoyl-L-3,4-dihydroxyphenylalanine) structure, a known antioxidant found in red clover. The amides were prepared in good yields starting from methyl and dodecylesters of L-tyrosine and L-DOPA by reacting with the *N*-hydroxysuccinimidyl esters of ferulic, sinapic, and acetyl-protected caffeic acid, respectively. In the DPPH• (2,2-diphenyl-1-picrylhydrazyl) and superoxide radical quencher assays they showed radical scavenging activity equal to or higher than those of the standard antioxidants ascorbic acid and tocopherol. The antioxidative potentials of the clovamide derivatives against bulk lipid oxidation, as determined by the accelerated autoxidation of oils, were equal to or higher than those of the standard antioxidants; some of the compounds were able to protect an emulsion of linoleic acid/β-carotene against oxidation. *N*-Caffeoyl L-tyrosine methyl ester and the *N*-cinnamoyl L-DOPA alkyl esters especially were potent antioxidants in bulk lipids and moderate protectants in emulsions.

KEYWORDS: Antioxidants; clovamide; *N*-(hydroxycinnamoyl)-L-tyrosine alkyl esters; *N*-(hydroxycinnamoyl)-L-DOPA alkyl esters

INTRODUCTION

In food, autoxidation damages many constituents, mainly lipids, proteins, colorants, and flavor compounds. Frequently this process leads to negative nutritive and sensoric value of many food products. To diminish oxidative damage antioxidants are added, primarily to inhibit the autoxidation of highly unsaturated lipids or flavor compounds (*1*). In particular, the autoxidation of unsaturated lipids generates typical flavors and frequently off-flavors. As an example, the long-chain polyunsaturated fatty acids are degraded to different kinds of aldehydes such as *n*-alkanals (e.g., 1-hexanal), related α,β-unsaturated aldehydes including *E*-2-alkenals or 2,4-alkadienals (e.g., [2*E*]-hexenal, [2*E*]-nonenal, [2*E*,4*E*]-2,4-nonadienal), and 4-hydroxy-2-alkenals (e.g., 4-hydroxy-2-nonenal) (*2*). The unsaturated medium-chain aldehydes cause the characteristic rancid and metallic flavor of oxidized fats and oils (*3*). Some of the α,β-unsaturated fatty aldehydes and their oxygenated metabolites show biological activity as alkylation agents and are suspected to be genotoxic (*4*). In addition, oxidized lipids can be absorbed by the normal pathways in mammals and are incorporated into the lipoproteins, such as chylomicrons and LDL (*5*). They are cytotoxic and can negatively influence lipid metabolism. For example, high levels of LDL containing oxidized fatty acids, which are generated by ingestion of oxidized fats and oils, are considered a very important inducer for atherosclerosis (*6*). Therefore, it is important to protect food that contains unsatur-

ated lipids by purification of raw materials and addition of highly active antioxidants.

In our efforts to develop new antioxidants for cosmetic or food use, we have synthesized some *N*-(hydroxycinnamoyl)-L-tyrosine and L-DOPA alkyl esters as new clovamide derivatives and evaluated their antioxidative potential against lipid peroxidation using different in vitro test systems. The occurrence of *E*- and *Z*-clovamides (**1a** and **b**, *N*-caffeoyl-L-DOPA, **Figure 1**) in red clover (*Trifolium pratense*) was first described in 1974 by Yoshihara et al. (*7, 8*). Since then some further sources were found: from a fraction of African blackwood (*Dalbergia melanoxylon*) some clovamides were isolated (*9*), and Sanbongi et al. found clovamide (**1a**) and the deoxyclovamide **1c** in cocoa liquor (*Theobroma cacao*) (*10*). The same authors described the antioxidative potential of clovamides for the first time. After incubation of red clover seedlings with jasmonic acid, the biosynthesis of the clovamides *N*-caffeoyl-L-DOPA (**1a**, most abundant), *N*-*p*-coumaroyl-L-tyrosine (**1c**), *N*-caffeoyl-L-tyrosine (**1d**), and *N*-*p*-coumaroyl-L-DOPA (**1e**) was strongly induced (*11*). Other clovamides such as *N*-caffeoyl-L-tyrosine (**1d**) were found in green robusta coffee beans (*12*).

The structure of clovamides is very similiar to that of rosmarinic acid (**2**), which mainly occurs in the plant family Lamiaceae (*13, 14*), especially in rosemary and sage varieties. The antioxidative potential of rosmarinic acid is very well known (*15*).

MATERIALS AND METHODS

Materials. Squalene was obtained from E. Merck (Darmstadt, Germany), and soybean oil was obtained from Henry Lamotte (Bremen,

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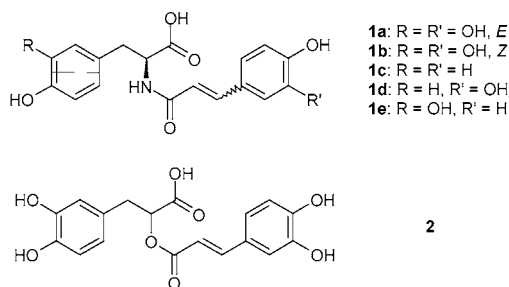
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Figure 1. Structures of different clovamides and of rosmarinic acid.

Germany). A sample of clovamide **1a** was supplied by Bayer AG, Germany. L-Tyrosine methyl ester hydrochloride (**3a**) and L-3,4-dihydroxyphenylalanine methyl ester hydrochloride (**3b**) were purchased from Sigma-Aldrich (Deisenhofen, Germany). The *N*-hydroxysuccinimidylesters of ferulic acid **4a**, sinapic acid **4b**, and 3,4-di-*O*-acetylcaffeic acid **4c** were prepared according to the literature (*16*). 2,2-Diphenyl-1-picrylhydrazyl (DPPH•), *N,N*′-azodiisobutyramidine dihydrochloride (ADIBA), horseradish peroxidase (HRPO), luminol, and all other chemicals were obtained from Sigma-Aldrich. 1,4-Dioxane was dried using activated molecular sieve (0.4 nm).

Instrumentation. Flash chromatography was performed using the Flash 40 system (Biotage, Charlottesville, VA). NMR spectra were recorded using a Varian VXR400S (^{1}H: 400 MHz) spectrometer (Varian, Darmstadt, Germany) at 25 °C using tetramethylsilane as internal standard. LC−MS spectra were recorded using the LCQ HPLC system Finnigan MAT HP1100 (Finnigan MAT, Egelsbach, Germany; APCI, atmospheric pressure chemical ionization). High-resolution mass spectra were recorded using a Finnigan MAT 8200 by flash evaporation of samples after dissolving them in methanol (resolution >8000). The oxidative stability of bulk lipids was measured using the Rancimat 679 (Deutsche Metrohm GmbH, Filderstadt, Germany). Chemiluminescence was determined using a Berthold LB96P (Perkin-Elmer GmbH, Überlingen, Germany). Photometric determinations were conducted using a Lambda 12 UV/Vis spectrophotometer (Perkin-Elmer); microplates were read using a Biotek EL808 microplate photometer (Bio-Tek Instruments GmbH, Bad Friedrichshall, Germany).

Syntheses. *L-Tyrosine Dodecyl Ester Hydrochloride (3c).* Thionyl chloride (1.8 g, 13.2 mmol) was added to dodecanol (30 g, 161 mmol) at room temperature in a nitrogen atmosphere. After the mixture was stirred for 10 min, L-tyrosine (2.5 g, 13.8 mmol) was added and the mixture was stirred at 90 °C for 19 h. After cooling to room temperature the resulting suspension was diluted by diethyl ether (75 mL) and filtered. The raw material was dissolved in a small amount of methanol and crystallized from diethyl ether. Yield: 5.04 g, colorless solid (95%). ^{1}H NMR (400 MHz, CD$_3$OD, δ): 7.04 (2H, d, *J* = 9.6 Hz, H-3, H-5), 6.75 (2H, d, *J* = 9.6 Hz, H-2, H-6), 4.13 (2H, t, *J* = 6.4 Hz, H-1′), 3.98 (1H, m, H-8), 3.00 (2H, t, *J* = 7.2 Hz, H-7), 1.59 (2H, m, H-2′), 1.31−1.27 (18H, m, H-3′−H-11′), 0.89 (3H, t, *J* = 6.9 Hz, H-12′). HRMS: M$^+$ calcd. for C$_{21}$H$_{35}$NO$_3$, 349.2611; found, 349.2617.

L-3,4-Dihydroxyphenylalanine Dodecyl Ester Hydrochloride (3d). Ester **3d** (*17*) was prepared analogously to ester **3c** from L-DOPA (7.5 g). Yield: 13 g, colorless crystals (85%). ^{1}H NMR (400 MHz, CD$_3$OD, δ): 6.74 (1H, d, *J* = 8.0 Hz, H-5), 6.66 (1H, d, *J* = 2.2 Hz, H-2), 6.55 (1H, dd, *J* = 8.0 Hz, *J* = 2.2 Hz, H-6), 4.20 (2H, t, *J* = 6.6 Hz, H-1′), 4.17 (1H, dd, *J* = 7.3 Hz, *J* = 6.4 Hz, H-8), 3.07 (1H, ABX, *J* = 14.3 Hz, *J* = 6.3 Hz, H-7), 3.02 (1H, ABX, *J* = 14.3 Hz, *J* = 7.3 Hz, H-7), 1.68−1.58 (2H, m, H-2′), 1.35−1.26 (18H, m, H-3′−H-11′), 0.89 (3 H, t, *J* = 7.0 Hz, H-12′) ppm. ^{13}C NMR (100 MHz, CD$_3$OD) δ: 170.63 (C, C-9), 146.91 (C, C-3 or C-4), 146.26 (C, C-3 or C-4), 126.46 (C, C-1), 121.77 (CH, C-6), 117.34 (CH, C-2), 116.77 (CH, C-5), 67.55 (CH$_2$, C-1′), 55.52 (CH), 37.32 (CH$_2$), 33.09 (CH$_2$), 30.78 (CH$_2$), 30.73 (CH$_2$), 30.62 (CH$_2$), 30.49 (CH$_2$), 30.35 (CH$_2$), 29.54 (CH$_2$), 26.90 (CH$_2$), 23.75 (CH$_2$, C-11), 14.45 (CH$_3$, C-12) ppm. MS (EI): *m/z* = 365 (free amine, M$^+$, 4%), 243 (68%), 242 (21%), 152 (72%), 124 (37%), 123 (60 5), 75 (100%), 74 (60%), 57 (48%), 43 (39%), 36 (22%), 28 (43%). HRMS: M$^+$ calcd. for C$_{21}$H$_{35}$NO$_4$, 365.2566; found, 365.2556.

N-(4-Hydroxy-3-methoxy-E-cinnamoyl)-L-tyrosine Methyl Ester (5a). To a solution of L-tyrosine methyl ester hydrochloride (**3a**) (398 mg, 1.72 mmol) and ferulic acid *N*-hydroxysuccinimidyl ester (**4a**) (500 mg, 1.72 mmol) in 1,4-dioxane (20 mL) and water (20 mL) under nitrogen atmosphere was added NaHCO$_3$ (144 mg, 1.72 mmol). The solution was stirred at room temperature for 16 h and extracted by ethyl acetate (3 × 30 mL). The combined organic phases were washed using 5% H$_2$SO$_4$ and brine, dried by Na$_2$SO$_4$, and filtered, and the filtrate was concentrated in vacuo. The resulting oil was chromatographed on silica gel 60 using the eluent ethyl acetate/*n*-hexane, 2:1 (v/v). Yield: 621 mg, light-yellow foam (97%, purity 96%, HPLC). ^{1}H NMR (400 MHz, CD$_3$OD, δ): 7.42 (1H, d, *J* = 15.6 Hz, H-7″), 7.12 (1H, d, *J* = 1.9 Hz, H-2″), 7.03 (2H, AA′XX′, H-2, H-6), 7.02 (1H, ddd, *J* = 8.1 Hz, *J* = 1.9 Hz, *J* = 0.4 Hz, H-6″), 6.79 (1H, d, *J* = 8.1 Hz, H-5″), 6.70 (2H, AA′XX′, H-3, H-5), 6.47 (1H, d, *J* = 15.6 Hz, H-8″), 4.71 (1H, dd, *J* = 8.3 Hz, *J* = 5.9 Hz, H-8), 3.88 (3H, s, Ar−O−CH$_3$), 3.69 (3H, s, H-1′), 3.08 (1H, dd, *J* = 14.0 Hz, *J* = 5.9 Hz, H-7), 2.94 (1H, dd, *J* = 14.0 Hz, *J* = 8.3 Hz, H-7) ppm. ^{13}C NMR (100 MHz, CD$_3$OD) δ: 173.80 (C, C-9), 168.95 (C, C-9″), 157.45 (C, C-4), 150.03 (C, C-3″), 149.32 (C, C-4″), 142.90 (CH, C-7″), 131.24 (2 × CH, C-2, C-6), 128.77 (C, C-1), 128.18 (C, C-1″), 123.45 (CH, C-6″), 118.05 (CH, C-8″), 116.49 (CH, C-5″), 116.31 (2 × CH, C-3, C-5), 111.61 (CH, C-2″), 56.42 (CH$_3$, Ar−OCH$_3$), 55.78 (CH, C-8), 52.68 (CH$_3$, C-1′), 37.88 (CH$_2$, C-7) ppm. HRMS: M$^+$ calcd. for C$_{20}$H$_{21}$NO$_6$, 371.1369; found, 371.1353.

N-(3,5-Dimethoxy-4-hydroxy-E-cinnamoyl)-L-tyrosine Methyl Ester (5b). Analogous to **5a**, **5b** was prepared starting from L-tyrosine methyl ester hydrochloride (**3a**) (181 mg, 0.78 mmol) and sinapic acid *N*-hydroxysuccinimidyl ester (**4b**) (250 mg, 0.78 mmol). The resulting oil was chromatographed on silica gel 60 using the eluent ethyl acetate/*n*-hexane/MeOH 5:5:1, (v/v/v). Yield: 223 m, off-white solid (71%, purity 98%, HPLC). ^{1}H NMR (400 MHz, CD$_3$OD, δ): 7.41 (1H, d, *J* = 15.7 Hz, H-7″), 7.04 (2H, AA′XX′, H-2, H-6), 6.85 (2H, s, H-2″, H-6″), 6.71 (2H, AA′XX′, H-3, H-5), 6.50 (1H, d, *J* = 15.7 Hz, H-8), 4.72 (1H, dd, *J* = 8.3 Hz, *J* = 5.8 Hz, H-8), 3.87 (6H, s, Ar−OCH$_3$), 3.70 (3H, s, H-1′), 3.09 (1H, dd, *J* = 13.9 Hz, *J* = 5.8 Hz, H-7), 2.94 (1H, dd, *J* = 13.9 Hz, *J* = 8.3 Hz, H-7) ppm. ^{13}C NMR (100 MHz, CD$_3$OD) δ: 173.77 (C, C-9), 168.84 (C, C-9″), 157.46 (C, C-4), 149.46 (2 × C, C-3″, C-5″), 143.10 (CH, C-7″), 139.07 (C, C-4″), 131.24 (2 × CH, C-2, C-6), 128.74 (C, C-1), 127.1 (C, C-1″), 118.49 (CH, C-8″), 116.31 (2 × CH, C-3, C-5), 106.55 (2 × CH, C-2″, C-6″), 56.81 (2 × CH$_3$, Ar−OCH$_3$), 55.78 (CH, C-8), 52.68 (CH$_3$, C-1′), 37.88 (CH$_2$, C-7) ppm. MS (EI): *m/z* = 401 (M$^+$, 25%), 223 (58%), 222 (100%), 208 (16%), 207 (81%), 192 (11%) 175 (24%), 119 (13%), 107 (36%).

N-(3,4-Dihydroxy-E-cinnamoyl)-L-tyrosine Methyl Ester (5c). By analogy with **5a**, **5c** was prepared starting from L-tyrosine methyl ester hydrochloride (**3a**) (321 mg, 1.39 mmol), 3,4-di-*O*-acetylcaffeic acid *N*-hydroxysuccinimidyl ester (**4c**) (500 mg, 1.39 mmol), and NaHCO$_3$ (355 mg, 4.24 mmol). The resulting oil was chromatographed on silica gel 60 using ethyl acetate. Yield: 247 mg, pale-yellow solid (46%, purity 93%, HPLC). ^{1}H NMR (400 MHz, CD$_3$OD, δ): 7.36 (1H, d, *J* = 15.7 Hz, H-7″), 7.02 (2H, AA′XX′, H-2, H-6), 6.99 (1H, d, *J* = 2.1 Hz, H-2″), 6.89 (1H, ddd, *J* = 8.2 Hz, *J* = 2.1 Hz, *J* = 0.5 Hz, H-6″), 6.75 (1H, d, *J* = 8.2 Hz, H-5″), 6.70 (2H, AA′XX′, H-3, H-5), 6.40 (1H, d, *J* = 15.7 Hz, H-8″), 4.70 (1H, dd, *J* = 8.3 Hz, *J* = 6.0 Hz, H-8), 3.69 (3H, s, H-1′), 3.07 (1H, AX, d, *J* = 6.0 Hz, H-7), 2.93 (1H, AX, d, *J* = 8.1 Hz, H-7) ppm. ^{13}C NMR (100 MHz, CD$_3$OD) δ: 173.85 (C, C-9), 169.14 (C, C-9″), 157.48 (C, C-4), 143.21 (CH, C-7″), 131.24 (2 × CH, C-2, C-6), 128.82 (2 × C, C-1, C-1″), 122.35 (CH, C-6″), 117.42 (CH, C-8″), 116.50 (CH, C-5″), 116.31 (2 × CH, C-3, C-5), 114.91 (CH, C-2″), 55.83 (CH, C-8), 52.65 (CH$_3$, C-1′), 37.90 (CH$_2$, C-7) ppm. MS (EI): *m/z* = 357 (M$^+$, 10.4%), 180 (39.3%), 179 (55.7%), 178 (55.5%), 163 (100%), 147 (17.7%), 135 (17.6%), 117 (11.9%), 107 (48.4%), 89 (20.5%), 77 (12.9%). HRMS: M$^+$ calcd. for C$_{19}$H$_{19}$NO$_6$, 357.1213; found, 357.1192.

N-(4-Hydroxy-3-methoxy-E-cinnamoyl)-L-tyrosine Dodecyl Ester (5d). Analogous to **5a**, **5d** was prepared starting from L-tyrosine dodecyl ester hydrochloride (**3c**) (666 mg, 1.73 mmol) and ferulic acid *N*-hydroxysuccinimidyl ester (**4a**) (500 mg, 1.72 mmol). The resulting oil was chromatographed on silica gel 60 using the eluent ethyl acetate/

n-hexane, 1:1 (v/v). Yield: 728 mg, white solid (80%, purity 97%, HPLC). ¹H NMR (400 MHz, CDCl₃, δ): 7.42 (1H, d, *J* = 15.6 Hz, H-7″), 7.11 (1H, d, *J* = 2.0 Hz, H-2″), 7.06−7.00 (3H, m, H-2, H-6, H-6″), 6.78 (1H, dd, *J* = 8.2 Hz, *J* = 0.6 Hz, H-5″), 6.72−6.69 (2H, m, H-3, H-5), 6.47 (1H, dd, *J* = 15.6 Hz, *J* = 0.6 Hz, H-8″), 4.68 (1H, dd, *J* = 8.2 Hz, *J* = 6.2 Hz, H-8), 4.08 (2H, t, *J* = 4.1 Hz, H-1′), 3.88 (3H, s, Ar−OCH₃), 3.06 (1H, dd, *J* = 13.9 Hz, *J* = 6.2 Hz, H-7), 2.95 (1H, dd, *J* = 13.8 Hz, *J* = 8.2 Hz, H-7), 1.61−1.54 (2H, m, H-2′), 1.33−1.24 (18H, m, H-3′−H-11′), 0.89 (3H, t, *J* = 6.9 Hz, H-12′) ppm. ¹³C NMR (100 MHz, CDCl₃, δ): 173.5 (C, C-9), 169.0 (C, C-9′), 157.5 (C, C-4), 150.6 (C, C-3″), 149.5 (C, C-4″), 143.0 (CH, C-7″), 131.24 (2 × CH, C-2, C-6), 128.7 (C, C-1 or C-1″), 127.9 (C, C-1 or C-1″), 123.5 (CH, C-6″), 117.8 (CH, C-8″), 116.6 (CH, C-5″), 116.3 (2 × CH, C-3, C-5), 111.7 (CH, C-2″), 66.4 (CH₂, C-1′), 56.4 (CH₃, Ar−OCH₃), 56.0 (CH₂, C-8), 37.9 (CH₂, C-7), 33.1 (CH₂), 30.81 (CH₂), 30.79 (CH₂), 30.74 (CH₂), 30.70 (CH₂), 30.5 (CH₂), 30.4 (CH₂), 29.7 (CH₂), 27.1 (CH₂), 23.8 (CH₂, C-11′), 14.5 (CH₃, C-12′) ppm. MS (APCI+): *m/z* = 526.5 (100%, [M + H]⁺), 527.24 (23%, [M + 2H]⁺). HRMS: M⁺ calcd. for C₃₁H₄₃NO₆, 525.3090; found, 525.3093.

N-(3,5-Dimethoxy-4-hydroxy-E-cinnamoyl)-ʟ-tyrosine Dodecyl Ester (5e). By analogy with **5a**, **5e** was prepared starting from ʟ-tyrosine dodecyl ester hydrochloride (**3c**) (301 mg, 0.78 mmol) and sinapic acid *N*-hydroxysuccinimidyl ester (**4b**) (250 mg, 0.78 mmol). In contrast to **5a** the reaction mixture was heated at 80 °C for 6 h prior to workup. The resulting oil was chromatographed on silica gel 60 using the eluent ethyl acetate/*n*-hexane, 2:1 (v/v). Yield: 358 mg, white solid (64%, purity 94%, HPLC). ¹H NMR (400 MHz, CD₃OD, δ): 7.41 (1H, d, *J* = 15.6 Hz, H-7″), 7.05−7.03 (2H, m, H-2, H-6), 6.85 (2H, s, H-2″, H-6″), 6.72−6.69 (2H, m, H-3, H-5), 6.51 (1H, d, *J* = 15.6 Hz, H-8″), 4.67 (1H, dd, *J* = 7.6 Hz, *J* = 5.8 Hz, H-8), 4.08 (2H, t, *J* = 6.5 Hz, H-1′), 3.87 (6H, s, Ar−OCH₃), 3.07 (1H, dd, *J* = 14.1 Hz, *J* = 6.0 Hz, H-7), 2.95 (1H, dd, *J* = 14.0 Hz, *J* = 7.7 Hz, H-7), 1.61−1.55 (2H, m, H-2′), 1.33−1.23 (18H, m, H-3′ − H-11′), 0.89 (3H, t, *J* = 6.8 Hz, H-12′) ppm. ¹³C NMR (100 MHz, CD₃OD, δ): 173.5 (C, C-9), 168.8 (C, C-9″), 157.5 (C, C-4), 149.5 (2 × C, C-3″, C-5″), 143.1 (CH, C-7″), 139.1 (C, C-4″), 131.2 (2 × CH, C-2, C-6), 128.7 (C, C-1), 127.1 (C, C-1″), 118.5 (CH, C-8″), 116.3 (2 × CH, C−C-3, C-5), 106.6 (2 × CH, C-2″, C-6″), 66.4 (CH₂, C-1′), 56.8 (2 × CH₃, Ar−OCH₃), 56.0 (CH, C-8), 37.9 (CH₂, C-7), 33.1 (CH₂), 30.81 (CH₂), 30.79 (CH₂), 30.74 (CH₂), 30.70 (CH₂), 30.50 (CH₂), 30.4 (CH₂), 29.7 (CH₂), 27.1 (CH₂), 23.8 (CH₂, C-11′), 14.5 (CH₃, C-12′) ppm. MS (EI): *m/z* = 555.2 (M⁺, 13%), 224.1 (17%), 223.1 (99.5%), 222.0 (100%), 208.0 (14%), 207.0 (84%), 175.0 (19%), 147.0 (11%), 107.0 (20%), 43.0 (12%). HRMS: M⁺ calcd. for C₃₂H₄₅NO₇, 555.3196; found, 555.3184.

N-(4-Hydroxy-3-methoxy-E-cinnamoyl)-ʟ-3,4-dihydroxyphenylala-nine Methyl Ester (5f). Analogous to **5a**, **5f** was prepared starting from ʟ-3,4-dihydroxyphenylalanine methyl ester hydrochloride (**3b**) (200 mg, 0.808 mmol) and ferulic acid *N*-hydroxysuccinimidyl ester (**4a**) (235 mg, 0.808 mmol). In contrast to **5a** the reaction mixture was heated at 50 °C for 6 h prior to workup. The resulting oil was chromatographed on silica gel 60 using the eluent ethyl acetate/*n*-hexane, 2:1 (v/v). Yield: 240 mg, white solid (70%, purity 91%, HPLC). ¹H NMR (400 MHz, CD₃OD, δ): 7.42 (1H, d, *J* = 15.7 Hz, H-7″), 7.13 (1H, d, *J* = 1.9 Hz, H-2″), 7.03 (1H, dd, *J* = 8.1 Hz, *J* = 1.9 Hz, H-6″), 6.79 (1H, d, *J* = 8.1 Hz, H-5″), 6.67 (1H, d, *J* = 8.0 Hz, H-5), 6.64 (1H, d, *J* = 2.1 Hz, H-2), 6.52 (1H, dd, *J* = 8.0 Hz, *J* = 2.1 Hz, H-6), 6.48 (1H, d, *J* = 15.6 Hz, H-8″), 4.70 (1H, dd, *J* = 8.0 Hz, *J* = 6.0 Hz, H-8), 3.88 (3H, s, Ar−OCH₃), 3.70 (3H, s, H-1′), 3.02 (1H, dd, *J* = 13.9 Hz, *J* = 6.1 Hz, H-7), 2.90 (1H, dd, *J* = 13.9 Hz, *J* = 8.0 Hz, H-7) ppm. ¹³C NMR (100 MHz, CD₃OD, δ): 173.81 (C, C-9), 168.96 (C, C-9″), 150.08 (C, C-4″), 149.35 (C, C-3″), 146.34 (C, C-3), 145.37 (CH, C-4), 142.89 (CH, C-7″), 129.45 (C, C-1), 128.20 (C, C-1″), 123.47 (CH, C-6″), 121.63 (CH, C-6), 118.10 (CH, C-8″), 117.26 (CH, C-2), 116.51 (CH, C-5″), 116.37 (CH, C-5), 111.62 (CH, C-2″), 56.43 (CH₃, Ar−OCH₃), 55.78 (CH, C-8), 52.67 (CH₃, C-1′), 38.12 (CH₂, C-7) ppm. HRMS: M⁺ calcd. for C₂₀H₂₁NO₇, 387.1318; found, 387.1305.

N-(3,5-Dimethoxy-4-hydroxy-E-cinnamoyl)-ʟ-3,4-dihydroxyphenyl-alanine Methyl Ester (5g). By analogy with **5f**, **5g** was prepared starting

from ʟ-3,4-dihydroxyphenylalanine methyl ester hydrochloride (**3b**) (200 mg, 0.808 mmol) and sinapic acid *N*-hydroxysuccinimidyl ester (**4b**) (260 mg, 0.808 mmol). The resulting oil was chromatographed on silica gel 60 using ethyl acetate. Yield: 230 mg, white solid (62%, purity 90%, HPLC). ¹H NMR (400 MHz, CD₃OD, δ): 7.41 (1H, d, *J* = 15.6 Hz, H-7″), 6.85 (2H, s, H-2″, H-6″), 6.67 (1H, d, *J* = 8.1 Hz, H-5), 6.65 (1H, d, *J* = 2.1 Hz, H-2), 6.52 (1H, dd, *J* = 8.1 Hz, *J* = 2.1 Hz, H-6), 6.51 (1H, d, *J* = 15.6 Hz, H-8″), 4.71 (1H, dd, *J* = 8.0 Hz, *J* = 5.9 Hz, H-8), 3.87 (6H, s, Ar−OCH₃), 3.71 (3H, s, H-1′), 3.02 (1H, dd, *J* = 13.8 Hz, *J* = 5.7 Hz, H-7), 2.90 (1H, dd, *J* = 13.8 Hz, *J* = 8.0 Hz, H-7) ppm. ¹³C NMR (100 MHz, CD₃OD, δ): 173.80 (C, C-9), 168.85 (C, C-9″), 149.51 (2 × C, C-3″, C-5″), 146.35 (C, C-3), 145.39 (C, C-4), 143.09 (CH, C-7″), 129.43 (C, C-1), 127.13 (C, C-1″), 121.62 (CH, C-6), 118.53 (CH, C-8″), 117.26 (CH, C-2), 116.37 (CH, C-5), 106.58 (CH, C-2″, C-6″), 56.83 (2 × CH, Ar−OCH₃), 55.76 (CH, C-8), 52.68 (CH₃, C-1′), 38.12 (CH₂, C-7) ppm. MS (EI): *m/z* = 417 (M⁺, 19.5%), 224 (14.4%), 223 (38.4%), 222 (77.1%), 208 (20.7%), 207 (100%), 175 (23.3%), 147 (11.1%), 123 (24.4%), 119 (10.1%). HRMS: M⁺ calcd. for C₂₁H₂₃NO₈, 417.1424; found, 417.1390.

N-(4-Hydroxy-3-methoxy-E-cinnamoyl)-ʟ-3,4-dihydroxyphenylala-nine Dodecyl Ester (5h). Analogous to **5e**, **5h** was prepared starting from ʟ-3,4-dihydroxyphenylalanine dodecyl ester hydrochloride (**3d**) (690 mg, 1.72 mmol) and ferulic acid *N*-hydroxysuccinimidyl ester (**4a**) (500 mg, 1.72 mmol). The resulting oil was chromatographed on silica gel 60 using the eluent ethyl acetate/*n*-hexane, 2:1 (v/v). Yield: 639 mg, pale-yellow solid foam (62%, purity 90%, HPLC). ¹H NMR (400 MHz, CD₃OD, δ): 7.43 (1H, d, *J* = 15.6 Hz, H-7″), 7.12 (1H, d, *J* = 1.9 Hz, H-2″), 7.03 (1H, ddd, *J* = 8.1 Hz, *J* = 2.0 Hz, *J* = 0.5 Hz, H-6″), 6.79 (1H, d, *J* = 8.1 Hz, H-5″), 6.67 (1H, d, *J* = 8.0 Hz, H-5), 6.66 (1H, d, *J* = 2.1 Hz, H-2), 6.53 (1H, dd, *J* = 8.0 Hz, *J* = 2.2 Hz, H-6), 6.49 (1H, d, *J* = 15.6 Hz, H-8″), 4.66 (1H, dd, *J* = 8.0 Hz, *J* = 6.4 Hz, H-8), 4.09 (2H, t, *J* = 6.4 Hz, H-1′), 3.88 (3H, s, Ar−O−CH₃), 3.00 (1H, dd, *J* = 13.8 Hz, *J* = 6.7 Hz, H-7), 2.91 (1H, dd, *J* = 13.8 Hz, *J* = 8.2 Hz, H-7), 1.62−1.55 (2H, m, H-2′), 1.33−1.23 (18H, m, H-3′ − H-11′), 0.89 (3H, t, *J* = 6.8 Hz, H-12′) ppm. ¹³C NMR (100 MHz, CD₃OD, δ): 173.5 (C, C-9), 169.0 (C, C-9″), 150.1 (C, C-4″), 149.3 (C, C-3″), 146.3 (C, C-3), 145.4 (C, C-4), 142.9 (CH, C-7″), 129.4 (C, C-1), 128.2 (C, C-1″), 123.5 (CH, C-6″), 121.6 (CH, C-6), 118.1 (CH, C-8″), 117.3 (CH, C-2), 116.5 (CH, C-5), 116.3 (CH, C-5″), 111.6 (CH, C-2″), 66.4 (CH₂, C-1′), 56.4 (CH₃, Ar−OCH₃), 56.0 (CH, C-8), 38.2 (CH₂, C-7), 33.1 (CH₂), 30.82 (CH₂), 30.80 (CH₂), 30.76 (CH₂), 30.71 (CH₂), 30.5 (CH₂), 30.4 (CH₂), 29.7 (CH₂), 27.1 (CH₂), 23.8 (CH₂, C-11′), 14.5 (CH₃, C-12′) ppm. MS (APCI+): *m/z* = 542.27 (100%, [M + H]⁺), 543.06 (49%); HRMS: M⁺ calcd. for C₃₁H₄₃NO₇, 541.3047; found, 541.3070.

N-(3,5-Dimethoxy-4-hydroxy-E-cinnamoyl)-ʟ-3,4-dihydroxyphenyl-alanine Dodecyl Ester (5i). By analogy with **5e**, **5i** was prepared starting from ʟ-3,4-dihydroxyphenylalanine dodecyl ester hydrochloride (**3d**) (624 mg, 1.56 mmol) and sinapic acid *N*-hydroxysuccinimidyl ester (**4b**) (500 mg, 1.56 mmol). The resulting oil was chromatographed on silica gel 60 using the eluent ethyl acetate/*n*-hexane, 2:1 (v/v). Yield: 582 mg, pale-yellow solid foam (65%, purity 99%, HPLC). ¹H NMR (400 MHz, CD₃OD, δ): 7.41 (1H, d, *J* = 15.5 Hz, H-7″), 6.85 (2H, s, H-2″, H-6″), 6.68 (1H, d, *J* = 8.1 Hz, H-5), 6.66 (1H, d, *J* = 2.1 Hz, H-2), 6.54 (1H, dd, *J* = 8.0 Hz, *J* = 2.1 Hz, H-6), 6.51 (1H, d, *J* = 15.6 Hz, H-8″), 4.67 (1H, dd, *J* = 8.0 Hz, *J* = 6.3 Hz, H-8), 4.09 (2H, dd, *J* = 6.7 Hz, *J* = 6.3 Hz, H-1′), 3.88 (6H, s, Ar−OCH₃), 3.04−2.88 (2H, AB, H-7), 1.62−1.55 (2H, m, H-2′), 1.33−1.23 (18H, m, H-3′− H-11′), 0.89 (3H, t, *J* = 6.9 Hz, H-12′) ppm. ¹³C NMR (100 MHz, CD₃OD, δ): 173.5 (C, C-9), 168.8 (C, C-9′), 149.5 (2 × C, C-3″, C-5″), 146.3 (C-3), 145.4 (C, C-4), 143.1 (C, C-7″), 139.1 (C, C-4″), 129.4 (C, C-1), 127.1 (C, C-1″), 121.6 (CH, C-6), 118.5 (CH, C-8″), 117.3 (CH, C-2), 116.3 (CH, C-5), 106.6 (2 × CH, C-2″, C-6″), 66.4 (CH₂, C-1′), 56.8 (2 × CH₃, Ar−OCH₃), 56.0 (CH, C-8), 38.2 (CH₂, C-7), 33.1 (CH₂), 30.82 (CH₂), 30.79 (CH₂), 30.75 (CH₂), 30.71 (CH₂), 30.5 (CH₂), 30.4 (CH₂), 29.7 (CH₂), 27.1 (CH₂), 23.8 (CH₂, C-11′), 14.5 (CH₃, C-12′) ppm. MS (EI): *m/z* = 571.3 (9%), 224.1 (19%), 223.1 (39%), 222.1 (46%), 208.0 (17%), 207.0 (100%), 175.0 (14%), 123.1 (11%), 43.1 (8%), 28.0 (10%). MS (APCI+): *m/z* = 572.32 (100%, [M + H]⁺), 573.16 (23%). HRMS: M⁺ calcd. for C₃₂H₄₅NO₈, 571.3145; found, 571.3145.
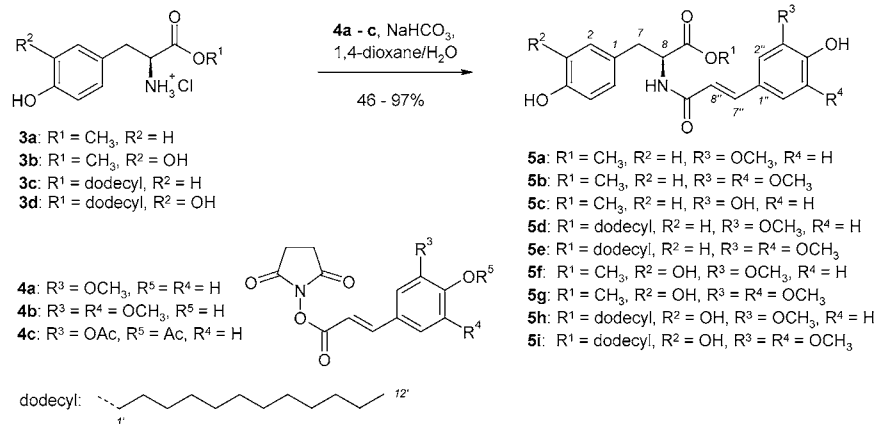
Figure 2. Synthesis and structures of *N*-(hydroxycinnamoyl)-L-tyrosine and L-DOPA alkyl esters **5a** to **5i**.

DPPH• Assay. The relative ability of test compounds to abstract hydrogen radicals was evaluated using the DPPH• assay according to the procedures described earlier (*18, 19*). The concentration-dependent remaining DPPH• was calculated from absorptions *A* at steady state by

$$\text{remaining DPPH (\%)} = \frac{A_{\text{test at steady state}}}{A_{\text{control at } t_0}} \quad (1)$$

The remaining DPPH• (%) in a series of dilutions of one test compound was used to calculate the effective relative concentration EC_{50} (based on the starting concentration of DPPH•, $EC = c_{\text{test}}/c_{\text{DPPH at t0}}$), at which 50% of DPPH• has been removed. The assay was performed in triplicate and the absorptions were averaged before calculation. α-Tocopherol and L-ascorbic acid were used as standards.

Superoxide Anion Radical Scavenging Assay (*16*). The test compounds were diluted starting from a dimethyl sulfoxide stock solution (10 mmol/L) using phosphate buffered saline (PBS). Each well of a 96-well white polystyrene plate was subsequently charged with 50 μL of luminol (200 μmol/L in PBS), 50 μL of horseradish peroxidase (HRPO, 1 U/mL in PBS), and 50 μL of the test solutions of different concentrations (e.g., 40 μmol/L). The mixtures were incubated at 37 °C for 5 min. Finally, 50-μL aliquots of H_2O_2 (40 μmol/L) were added and the chemiluminescence was recorded immediately for 10 s. The areas below the curves for blank (without test solution) and the test solutions were integrated (as relative light units, RLU) and the relative depletion of chemiluminescence was calculated by

$$\text{relative depletion (\%)} = \frac{\text{RLU}_{\text{test}}}{\text{RLU}_{\text{blank}}} \quad (2)$$

The relative depletion was plotted against log c of test compounds. The superoxide scavenging activity was calculated as inhibitor concentration IC_{50}, at which 50% of the generated superoxide was depleted. L-Ascorbic acid was used as standard.

Rancimat Assay. Rancimat determinations were performed as described earlier using purified soybean oil and squalene (*19*). The test compounds were dissolved in methanol (15 mg/mL for soybean oil and 1.5 mg/mL for squalene) and 100 μL of these solutions were added to a previously made 3-g lipid sample in the reaction vessels of the Rancimat apparatus. Pure solvent was added to control samples. The induction periods (IP) were calculated automatically by the Rancimat. All tests were run in duplicate and averaged before calculation. The following equation was used to calculate the antioxidative index (AOI):

$$\text{AOI} = \frac{\text{IP}_{\text{test}}}{\text{IP}_{\text{control}}} \quad (3)$$

α-Tocopherol was used as standard.

Carotene Bleaching Assay. A mixture of 1 mL of a solution of 2 mg/mL β-carotene in chloroform, 0.2 mL of linoleic acid, and 2 mL of Tween 20 was homogenized using an Ultraturrax mixer. The solution was transferred to a round-bottom vessel (brown glass) and the chloroform was evaporated by a constant stream of nitrogen for 1 h. Air-sparged double-distilled water (20 mL) was added to the mixture and vigorously mixed using the Ultraturrax mixer until a clear solution was formed. The test compounds were diluted by methanol. A 96-well microtiter plate (polystyrene) was charged by 25 μL of test solution and 200 μL of the emulsion. After the solution was shaken, 25 μL of an ADIBA solution (0.3 mol/L in double-distilled water) was added and the plate was immediately read at 450 nm and ambient temperature (20−23 °C) for 2 h. Control vials without test solution and additionally without test and ADIBA solutions were used. All dilutions and measurements were done in triplicate.

The ln *A* was plotted against time. The inhibition of β-carotene degradation after 1 h was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\ln A_{\text{test,1h}} - \ln A_{\text{test,0h}}}{\ln A_{\text{carotene+ADIBA,1h}} - \ln A_{\text{carotene+ADIBA,0h}}} \quad (4)$$

The inhibition of carotene degradation (%) after 1 h at 450 nm was plotted against concentration. The activity was calculated as inhibitor concentration IC_{50}, at which 50% of the β-carotene remained. Statistical significance was examined using Student's *t* test with Microsoft Excel 97 functions.

RESULTS AND DISCUSSION

The synthesis of clovamide ester derivatives **5a**−**5i** was performed according to the method described by Roblot and Wylde (*20*). Instead of coupling free cinnamic acids using benzotriazoyloxy *tris*-dimethylaminophosphonium phosphate we used the *N*-hydroxysuccinimidyl active esters of the appropriate hydroxycinnamic acid for preparation of the amides, as described earlier for other polyhydroxylated phenolic acid amides (*16*). The amino acid esters **3a**−**3d** were reacted with activated cinnamic acids **4a**−**4c** in a 1,4-dioxane/water mixture at pH > 8 to yield amides **5a**−**5i** (46−97%, **Figure 2**). The dodecyl esters **3c** and **3d** of L-tyrosine and L-DOPA were synthesized by a variation of the standard procedure for amino acids (*20*): the amino acid was added to a pre-made mixture of dodecanol and thionyl chloride. After refluxing of the mixture, **3c** and **3d** were isolated in high yields by simple precipitation using diethyl ether.

In the case of **4c** the *O*-acetylated caffeic acid was used to supress extended *O*-acylation as side reaction. Using acetyl-protected activated caffeic acid, *N*-acetyl-L-amino acid esters were produced as side products. All products were purified prior to analysis and evaluation by chromatographic methods. Methyl ester **5c** prepared according to the above-mentioned method is known (*20, 21*), but the other clovamide derivatives are all new.

Table 1. Radical Scavenging Action Determined Using DPPH• and SO Scavenging Assay, Antioxidative Activity against Accelerated Autoxidation of Bulk Lipids, and Action against β-Carotene Bleaching in Emulsions of *N*-(Hydroxycinnamoyl)-L-tyrosine and L-DOPA Alkyl Esters **5a** to **5i** Compared to L-DOPA, L-DOPA Dodecyl Ester (**3d**), Clovamide **1a**, Rosmarinic Acid (**2**), and Standards Ascorbic Acid and α-Tocopherol

			Rancimat AOI		
compound	DPPH• EC$_{50}$ (mol/mol)	SO IC$_{50}$ (nmol/L)	100 °C, soybean oil, 500 ppm	80 °C, squalene, 50 ppm	β-carotene IC$_{50}$ (mmol/L)
5a	0.38 ± 0.01	n.d.[a]	2.0 ± 0.1	3.4 ± 0.4	>0.6
5b	0.36 ± 0.01	n.d.	2.6 ± 0.3	4.8 ± 0.5	n.d.
5c	0.083 ± 0.002	75	10.0 ± 1.2	12.0 ± 1.3	0.04 ± 0.01
5d	0.38 ± 0.01	n.d.	1.5 ± 0.1	n.d.	>0.3
5e	0.341 ± 0.007	n.d.	2.3 ± 0.3	8.7 ± 0.6	0.19 ± 0.11
5f	0.115 ± 0.003	210	6.4 ± 0.3	18.9 ± 1.8	0.26 ± 0.1
5g	0.125 ± 0.003	130	7.3 ± 0.2	15.4 ± 1.7	0.13 ± 0.08
5h	0.23 ± 0.01	>10 000	5.3 ± 0.3	25 ± 2	>0.3
5i	0.245 ± 0.005	1200	5.0 ± 0.3	46 ± 0.9	0.23 ± 0.1
clovamide (**1a**)	0.050 ± 0.001	60	4.0 ± 0.1	2.9 ± 0.2	0.02 ± 0.01
rosmarinic acid (**2**)	0.058 ± 0.001	95	9.3 ± 0.1	21.1 ± 1.4	n.d.
L-DOPA	0.095 ± 0.002	200	3.3 ± 0.2	n.d.	>1.1
3d	0.053 ± 0.001	2500	1.6 ± 0.6	37 ± 1	0.03 ± 0.01
α-tocopherol	0.25 ± 0.02[b]	>10 000[b]	5.1 ± 0.1[b]	19 ± 1[b]	0.08 ± 0.05[c]
ascorbic acid	0.27 ± 0.02[b]	700[b]	1.2[b]	0.7[b]	>0.9

[a] n.d., Not determined. [b] Data from ref 19. [c] From repeated experiments ($n = 2$, tocopherol 4).

Especially in solution, the products are sensitive toward oxidation. Therefore, precautions during reaction and isolation from oxygen, such as degassing of solvents and the use of inert gas, are essential. The purity of the amides was determined by HPLC and for the chromatographed products it was higher than 90%, established by UV-detection by DAD and identification by LC−MS. The clovamide esters **5a**−**5i** were identified by ^{1}H- and ^{13}C NMR, and LC−MS or MS. All signals in ^{1}H NMR and ^{13}C NMR spectra corresponded to the proposed structures. The double bond geometry of the cinnamoyl moieties in all cases was *E*, because the coupling constants J^3 between the α and β protons were 15−16 Hz. For all new compounds satisfactory HRMS were measured. The difference between calculated and observed molecular weight was lower than 3×10^{-3} mass units.

To determine the antioxidant and radical scavenging action we used several physicochemical assays which cover different aspects of radical species and mechanistic details. For radical scavenging measurement we chose modified 2,2-diphenyl-1-picrylhydrazyl (DPPH•) scavenging and superoxide radical anion (SO) quenching assays, for antioxidative power in lipid systems we chose the accelerated autoxidation of bulk oils using the Rancimat, and the β-carotene bleaching assay was used in linoleic acid containing emulsions. The SO assay is applicable only for relatively hydrophilic antioxidants. **Table 1** gives a summary of the results obtained with the different assays.

The *N*-(hydroxycinnamoyl)-DOPA alkyl esters **5f**−**5i** were more powerful radical scavengers compared to the standards α-tocopherol and ascorbic acid, as determined by the DPPH• assay; in addition **5c**, **5f**, and **5g** were good SO scavengers. Despite the fact that **5h** and **5i** were catechols they showed no or only weak activity as SO scavengers; the derivatives were probably too lipophilic for the assay. The *N*-(hydroxycinnamoyl)tyrosine alkyl esters **5a**−**5e** showed slightly decreased activity in the DPPH• assay compared to that of standards with the exception of caffeoyl derivative **5c**, which scavenged DPPH• radicals much more effectively than the standards and was comparable to the highly active clovamide (**1a**), rosmarinic acid (**2**), L-DOPA, and L-DOPA dodecyl ester (**3d**). Therefore, the catechol moiety in **5c** and **5f**−**5i** is very important for an excellent radical scavenging activity, as has been shown in many investigations (*16*, *21*, *22*).

For determination of the antioxidative potential against lipid autoxidation, we have used the accelerated autoxidation of bulk oils by air with or without test compound (*16, 23*) and in an emulsion model monitored by β-carotene bleaching (*24*). Oxidation of the unsaturated lipids proceeded only very slowly for the induction period (IP) and then suddenly increased in the propagation period. The oxidation of bulk lipids was carried out in the Rancimat apparatus with stripped soybean oil or squalene. Squalene was chosen as a highly oxidizable terpenoid found in human sebum and olive oil, for example. Squalene showed an IP of 0.8−1 h at 80 °C and soybean oil had an IP of 2−3 h at 100 °C. IP determinations by the Rancimat method correlated well with other established measurements of oxidative stability of bulk oils, e.g., chemiluminescence, peroxide value by iodometric titration, and shift of double bond (*25*); Gordon and Mursi (*26*) showed that the IP values obtained at 100 °C correlated very well with oil stability as measured by peroxide value during storage at 20 °C.

The investigated clovamide esters **5a**−**5i** were fair to very good antioxidants in bulk lipid systems. The derivatives with one catechol moiety (**5c** and **5f**−**5i**) showed the same potential as α-tocopherol at 500 ppm in soybean oil at 100 °C. The more lipophilic esters **5d**, **5e**, **5h**, and **5i** were slightly less active than their methyl ester counterparts **5a**, **5b**, **5f**, and **5g**. However, in squalene, the situation changed: the more lipophilic clovamide derivatives showed much higher activities compared to that of the more polar methyl esters. The L-DOPA dodecyl esters **5h** and **5i** were much more potent than the lipophilic α-tocopherol. The exception was *N*-caffeoyl L-tyrosine methyl ester (**5c**), which showed high activity in soybean and moderate antioxidative potential in squalene. L-DOPA dodecyl ester (**3d**) was a fair antioxidant in soybean oil and very active in squalene. As shown in **Figure 3**, the ferulic acid amides **5a** and **5d** were not able to protect a β-carotene/linoleic acid emulsion against oxidative degradation. Sinapic acid amide **5e** showed only moderate activity, but surprisingly, the more hydrophilic clovamide **1a** and the *N*-caffeoyl L-tyrosine methyl ester (**5c**) were very good antioxidants in the tested emulsion. The *N*-(hydroxycinnamoyl)-DOPA alkyl esters **5i**−**5h** were only moderate protectants in the emulsion system compared to α-tocopherol (**Figure 4**). Again, the L-DOPA dodecyl ester (**3d**) was able to

Lipophilic Clovamide Derivatives as Antioxidants

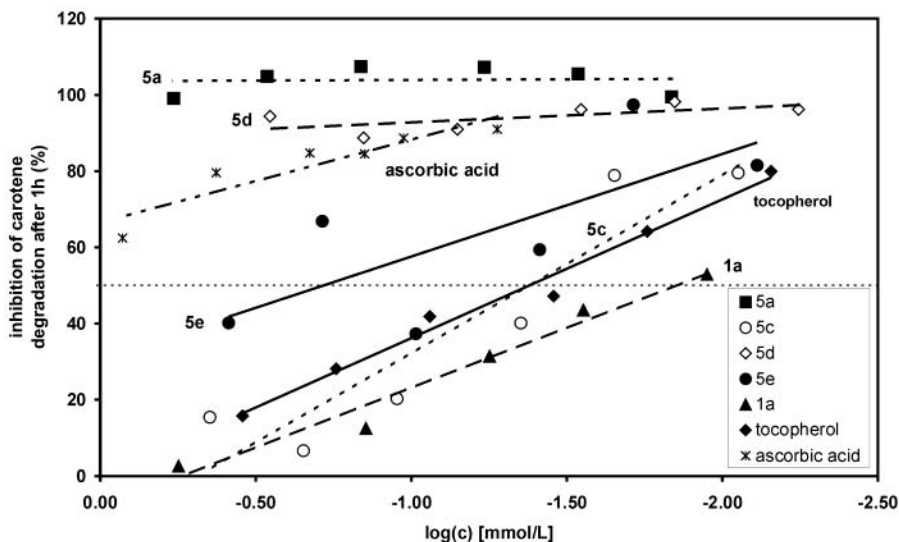*J. Agric. Food Chem.,* Vol. 51, No. 16, 2003   **4601**

Figure 3. Concentration-dependent inhibition of ADIBA-induced carotene bleaching in a linoleic acid emulsion by *N*-(hydroxycinnamoyl)-L-tyrosine alkyl esters **5a** to **5e** compared to that of clovamide **1a**, α-tocopherol, and ascorbic acid.
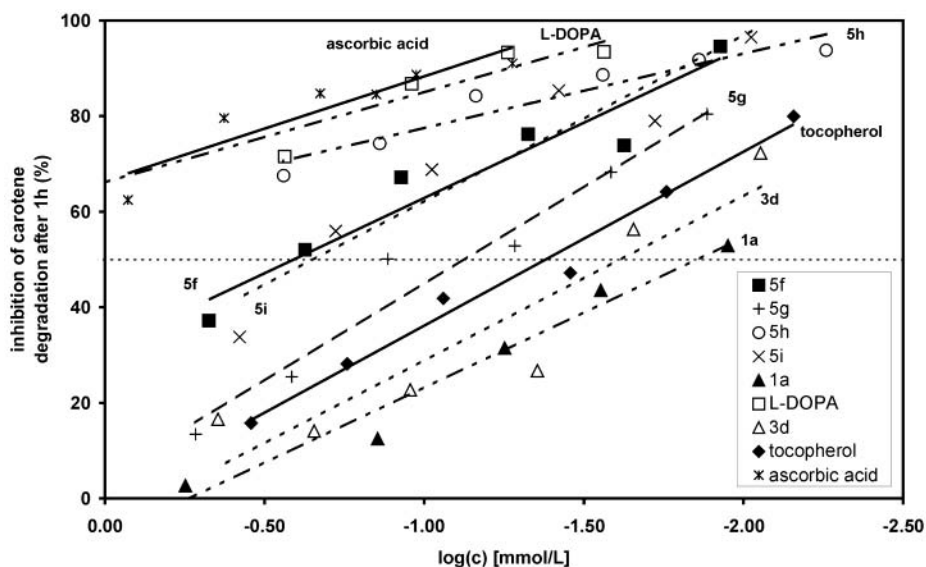

Figure 4. Concentration-dependent inhibition of ADIBA-induced carotene bleaching in a linoleic acid emulsion by *N*-(hydroxycinnamoyl)-L-DOPA alkyl esters **5f** to **5i** compared to that of clovamide **1a**, L-DOPA, L-DOPA dodecyl ester (**3d**), α-tocopherol, and ascorbic acid.

protect the model emulsion very effectively compared to α-tocopherol; this effect can be explained by the amphiphilic character of **3d**, which is perfectly incorporated in lipid bilayers and can act as radical scavenger near the lipid double bonds. However, it is not sufficient to introduce a long chain in the molecules to improve antioxidant activity in emulsions, because the hydrophilic head of the molecules is too bulky to orient in the right place.

Recently, the antioxidative activity of *N*-(3,4-dihydroxy-*E*-cinnamoyl)-L-tyrosine methyl ester (**5c**) was described by Rajan et al. (*21*) as part of a study on different caffeic acid amides. The activity of **5c** in a microsomal lipid peroxidation using the color reaction with thiobarbituric acid as read out was very good and comparable to our own results in emulsions.

In conclusion, the clovamide esters **5a**−**5i** can be prepared in fair to good yields from relatively cheap starting materials and are all good radical scavengers and moderate to good antioxidants compared to ascorbic acid and α-tocopherol. Especially *N*-caffeoyl tyrosine methyl ester (**5c**) and the *N*-cinnamoyl DOPA alkyl esters **5f**−**5i** are potent antioxidants in

bulk lipids; in contrast to these results they show only a moderate activity as protectants in emulsions compared to clovamide and α-tocopherol. Further investigations such as toxicological studies and stability testing on the most active clovamide derivatives **5c** and **5f**−**5i** are necessary to evaluate the potential of these compounds as food or cosmetic antioxidants.

Abbreviations Used: ADIBA, *N,N′*-azodiisobutyramidine dihydrochloride; AOI, antioxidative index; L-DOPA, 3,4-dihydroxy-L-phenylalanine; DPPH•, 2,2-diphenyl-1-picrylhydrazyl; EC, effective concentration; HRPO, horseradish peroxidase; IC, inhibitor concentration; IP, induction period; PBS, phosphate buffered saline; RLU, relative light units; SO, superoxide radical anion.

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