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Synthesis and Evaluation of Caffeic Acid Amides as Antioxidants

Padinchare Rajan,[a] Irina Vedernikova,[a] Paul Cos,[b] Dirk Vanden Berghe,[b] Koen Augustyns[a] and Achiel Haemers[a,*]

[a]*Department of Medicinal Chemistry, University of Antwerp (UIA), Universiteitsplein, 1, B-2610 Antwerp, Belgium*
[b]*Department of Pharmaceutical Microbiology, University of Antwerp (UIA), Universiteitsplein, 1, B-2610 Antwerp, Belgium*

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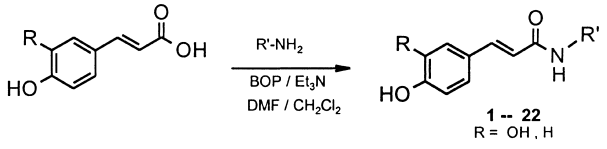
Abstract—A series of amides of caffeic acid has been synthesised and their antioxidant properties evaluated as lipid peroxidation inhibitors. Anilides of caffeic acid were found to be very efficient antioxidants with IC_{50}'s of 0.3 μM.

The biochemical properties of polyphenolic secondary plant metabolites such as flavonoids, chalcones and caffeic acid esters attract much attention in biology and medicine. These compounds show antiviral, antibacterial, vasoactive, antiatherogenic, antiproliferative and antiinflammatory properties.[1] These activities are at least partially related to their antioxidant properties.

Propolis is the substance that honeybees use to reduce the size of the entrance and seal holes in their hives and has been used as antiinflammatory medicine for centuries. In a research programme on the biological activity of caffeic acid esters isolated from propolis we found that the caffeic acid ester of 3-methylbut-2-enol (**23**) showed interesting antioxidant activities. The same activity is already reported for caffeic acid and several of its esters.[2] Caffeic acid derived antioxidant compounds occur in various beverages and foodstuffs and the atherosclerosis preventive activity attributed to these foodstuffs is due to the presence of these compounds and analogous polyphenols.[3] Lipid peroxidation is indeed an initial step in the atherosclerosis pathology. Hence, these compounds are candidates to be used as drugs in preventive cardiovascular medicine. Their use is however compromised by the low metabolic stability of esters. Although literature data on caffeic esters are scarce and mainly concern the metabolism of the phenolic groups, it is clear that the ester group is metabolically very labile.[4] This prompted us to investigate the properties of a series of corresponding amides **1**–**21**. The antioxidant activity was determined measuring the inhibition of the microsomal lipid peroxidation,[5] being the most biomimetic antioxidant test. The results were compared with those obtained with the corresponding *p*-coumaric amide **22**, the corresponding ester **23**, caffeic acid **24**, *p*-coumaric acid **25** and standard antioxidants such as trolox **26** and quercetine **27**.

The amides were synthesized from the free acid and the amine using BOP as coupling reagent (Scheme 1).[6]



Scheme 1. Synthesis of amides of caffeic acid (R = OH) and *p*-coumaric acid (R = H).

The caffeic acid amides show a pronounced antioxidant activity in the microsomal lipid peroxidation test (Table 1). Amides with aliphatic amines (**1**–**9**,**18**–**21**) possess IC_{50}'s in the low micromolar range comparable with the values obtained for standard antioxidants such as trolox **26**. Caffeic acid (**24**) and ester **23** appeared as active as amide **1**, proving that the caffeoyl group is the most important moiety for the antioxidant activity in this series. *p*-Coumaric acid amides are about 10 times less active as shown by compound **22** and *p*-coumaric acid is only poorly active.

Amides with aromatic amines (**10**–**17**) are more active and show IC_{50}'s in the higher nanomolar range and are more active than quercetin **27** a well known antioxidant

*Corresponding author. Tel.: +32-3-8202717; fax: +32-3-8202739; e-mail: haemers@uia.ua.ac.be

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flavonoid. Most interesting compounds are anilides **10**–**13**. A phenolic group (**11**–**13**) seems less important. The relatively high activity, although lower than the anilides, of the dopamine amide **16** can be explained by the presence of two catechol moieties.

Antioxidant activity and, in particular, inhibition of lipid peroxidation is a multifactorial event. Propensity of radical formation and stabilisation, ability of metal complexation and lipophilicity are important factors for this inhibitory activity. We focused on the radical stabilisation properties.

The radical scavenging moieties of polyphenolic compounds are the hydroxyl groups. The presence of *p*-substituted conjugated side chains and of *o*-substituted electron donating groups increase the stability of the radical and hence, the antioxidative activity. In our previous study,[9] we found that the formation of an internal H-bond in catechol moieties results in a high propensity to electron transfer and stabilisation of radical species. This bond facilitates the delocalisation of the unpaired electrons in the considered radical and leads to their overall stabilisation. The H-bond energy ($\Delta E_{\text{H-bond}}$) of non-substituted catechol, as the total energy difference between the corresponding conformers with and without H-bond, was calculated to be 3.6 kcal/mol in the neutral state.

We compared the importance of this internal hydrogen bond in the catechol moiety of the *N*-methylamide **4a** and the *N*-anilide **10a**, the latter being 10 times more active. The conformational behaviour of both amides with and without the H-bond was investigated and minimal conformers are denoted in Scheme 2.[10]

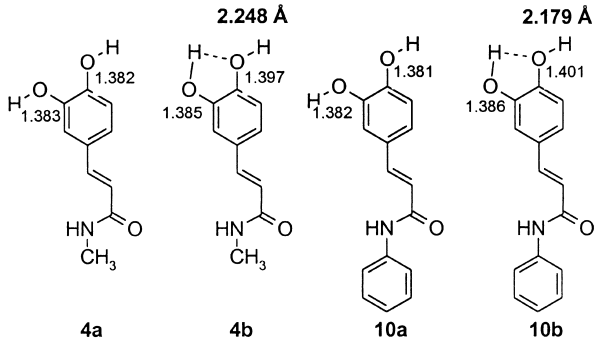
The DFT calculation using deMon-KS shows that the H-bonded *N*-methyl **4b** and *N*-phenyl **10b** compounds are lower than the non-bonded conformers by 3.8 kcal/mol, corresponding to a medium strength interaction.

In the corresponding radicals **4c** and **10c**, the donating C–O bond of *N*-methylamide and *N*-anilide is shortened relative to the neutral state from 1.379 to 1.264 Å and from 1.404 to 1.263 Å, respectively. The H-bond length decreases from 2.248 to 2.045 Å in the case of **4c** and from 2.179 to 2.041 Å in the case of **10c**. The radical stability ΔE_{R} was 75.3 kcal/mol and 75.6 kcal/mol respectively for the radicals, **4c** and **10c**. This value for catechol is 78.0 kcal/mol. This reflects the influence of the cinnamoyl group on the radical stabilisation.[11] The ΔE_{R} in the *N*-methyl compound with a non-hydrogen bonded radical was 80.7 kcal/mol. For the conformer

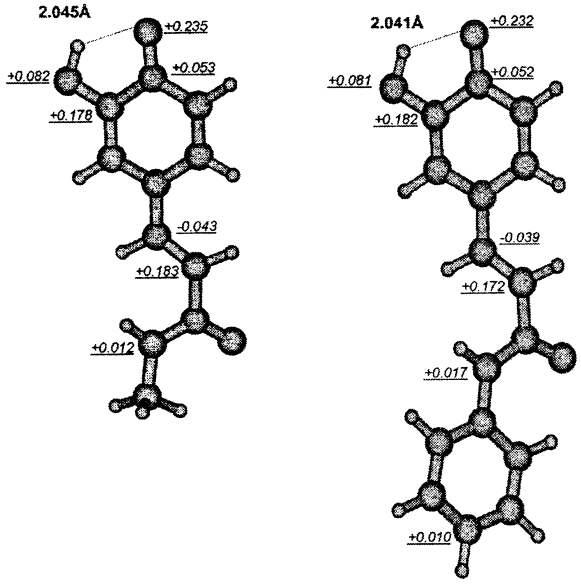
Table 1. Lipid peroxidation inhibitory activity[7] of caffeic acid amides and related compounds

	Acid	Amine (R′)	IC_{50}
1	Caffeic	3-Methylbut-2-enyl amine	3.4 μM±0.02
2	Caffeic	Ammonia	2.2 μM±0.07
3	Caffeic	Hydroxylamine	2.1 μM±0.3
4	Caffeic	Methylamine[d]	6.0 μM±0.09
5	Caffeic	Ethylamine	2.7 μM±0.2
6	Caffeic	Isopropylamine	3.9 μM±0.2
7	Caffeic	Isobutylamine	2.2 μM±0.07
8	Caffeic	Isopentylamine	1.4 μM±0.15
9	Caffeic	Allylamine	2.2 μM±0.02
10	Caffeic	Aniline[d]	0.38 μM±0.01
11	Caffeic	2-Aminophenol	0.29 μM±0.01
12	Caffeic	3-Aminophenol	0.37 μM±0.03
13	Caffeic	4-Aminophenol	0.63 μM±0.01
14	Caffeic	Benzylamine	1.02 μM±0.08
15	Caffeic	Phenethylamine	0.85 μM±0.007
16	Caffeic	Dopamine	0.59 μM±0.08
17	Caffeic	Tyrosine-OCH_3	3.2 μM±0.06
18	Caffeic	Diethylamine	4.1 μM±0.06
19	Caffeic	Pyrrolidine	2.4 μM±0.09
20	Caffeic	Piperidine	3.6 μM±0.04
21	Caffeic	Morpholine	6.1 μM±0.2
22	*p*-Coumaric	3-Methyl-2-butenyl amine	29.1 μM±3.7
23[a]	Caffeic[b]	3-Methyl-2-butenol	3.5 μM±0.2
24	Caffeic acid[c]	—	3.3 μM±0.08
25	*p*-Coumaric acid[c]	—	100 μM±0.15
26	Trolox[c]	—	2.8 μM±0.15
27	Quercetin[c]	—	0.95 μM±0.04

[a]**23**–**27** for comparison.
[b]Ester.
[c]Commercial products.
[d]NMR data: ref 8.



Scheme 2. Minimal conformers of *N*-methylamide **4** and the *N*-anilide **10**.



Scheme 3. Spin delocalisation in the *N*-methylamide (**4c**) and the *N*-anilide (**10c**) caffeoyl radical minimal conformers.

with the radical on the *m*-hydroxyl group we found 83.2 and 87.0 kcal/mol, respectively, for the hydrogen and non-hydrogen bonded radical, indicating clearly the importance of **4c** in the radical formation.

The spin population defined as $N_S(A) = N_{A\uparrow} - N_{A\downarrow}$ illustrates the effect of the *N*-substituent on the caffeoyl group on the unpaired spin density distribution showing an additional mesomeric effect with the second aromatic ring offering a positive spin density to the C4′-nucleus and responsible for the more effective spin delocalisation and the higher activity of the *N*-phenyl compound **10** (Scheme 3).

This study shows that caffeic anilides are strong inhibitors of lipid peroxidation. Further investigation is needed to evaluate their complete antioxidant profile, safety and detailed structure–activity relationship.

References and Notes

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6. The hydroxycinnamic acid (10 mmol) is dissolved in 20 mL of DMF and 1.4 mL (10 mmol) of triethylamine. The solution is cooled in an ice–water bath and 10 mmol of the amine are added followed by a solution of 10 mmol of BOP in 20 mL of CH_2Cl_2. The mixture is stirred at 0 °C for 30 min and then at room temperature for 2 h. CH_2Cl_2 is removed under reduced pressure and the solution is diluted with 150 mL of water. The products are extracted with ethyl acetate. The extract is washed successively with 1 N HCl, water, 1 M $NaHCO_3$ and water, dried over $MgSO_4$, filtered and evaporated. The residue is purified on a silica gel column (eluent: ethyl acetate–petroleum ether). (Yields are between 65 and 85%.)
7. The antioxidant activity of each compound was expressed as IC_{50} value, i.e. the concentration in μm necessary to inhibit TBARS formation by 50%, and was calculated from the corresponding log-dose inhibition curve.
8. ^{1}H NMR (400 MHz) spectral data for **4** and **10**, respectively: 2.83 (3H, s, CH_3), 6.20 (1H, d, $J = 20$ Hz, vinyl), 6.70–7.00 (3H, m, arom), 7.30 (1H, d, J 20 Hz); 6.43 (1H, d, $J = 22$ Hz, vinyl), 6.80–7.50 (8H, m, arom), 7.53 (1H, d, $J = 22$ Hz, vinyl).
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10. The electronic structure and geometry parameters of the compounds in their neutral and radical state were calculated using the linear combination of Gaussian type orbitals (LGTCO) Kohn–Sham (KS) DFT program deMon-KS3 (St-Amant, A.; Salahub, D. R. *Chem. Phys. Lett.* **1990**, 169). Orbital basis sets of DZVP quality with added polarisation functions were used (621/41/1*) for C and O atoms and (41/1*) for H atoms. The auxiliary basis used to fit the exchange-correlation potential were as follows: (5,2;5,2) for C, (4,4;4,4) for O and (5,1;5,1) for H atoms. The geometries were optimised until both the norm and the local energy gradient and the norm of the maximal individual gradient fell below 0.0003 a.u. using the kinetic-energy-density dependent exchange-correlation scheme PLAP3.
11. The radical stability ΔE_R was calculated as the difference between the total energy of the neutral compound and the sum of the total energy of the related phenolic radical and the H-radical.