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Short Communication

Antioxidant activity of some ascorbic and cinnamic acids derivatives

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Abstract

Some 4-benzoyl 3-hydroxy furan-2 (*5H*) ones (**3a–d**) and 2-amino 3-hydroxymethyl 4-aryl 4-oxo 2-butenoic acids (**4a–h**) have been synthesized. Compound **3c** with an isobutyl substituent in the 5-position of the furan ring was the most effective ($IC_{50} = 8.69 \times 10^{-4}$ M) in scavenging the superoxide anion. In vivo, **3c** was also protective against reperfusion injury.

Keywords: Ascorbic acid derivatives; Cinnamic acid derivatives; Furan-2-ones derivatives; Antioxidant activity

1. Introduction

Reactive oxygen species are regarded as merely pernicious according to their critical role in atherogenesis and reperfusion injury [1]. In the development of antioxidant therapies, synthetic analogues of ascorbic acid and cinnamates constitute a group of derivatives active as radical scavengers [2–4].

Ongoing our program related to the investigation of these classes of compounds as antioxidants [5], we now report the results obtained with new 3-hydroxy furan-2-ones and 2-amino 3-hydroxymethyl 4-aryl 4-oxo buten-2-oic acids.

2. Chemistry

Furanones **3** and α-amino butenoic acids **4** were synthesized from esters **2** (Scheme 1). As previously described [5], preparation of furanones **3** involved a Claisen condensation between methylketones **1** and diethyl oxalate followed by action of aldehydes on **2** and cyclization. Formation of butenoic acids **4** was accomplished in ethanol by reaction between derivatives **3** and primary amines. Compounds **3** and **4** are listed in Table 1. Their structures were established by means of their IR, ^1H and ^{13}C NMR spectral data. Two characteristic $\nu C{=}O$ bands appear at about 1750 and 1660 cm^{-1} for furanones **3**. Chemical shift of the carbon related to the lactone function in ^{13}C NMR is observed at 168–169 ppm. According to their IR and ^1H NMR spectra, derivatives **4** occur as Zwitterionic species involving protonation of the amino group. Thus, the ^1H resonance of the COOH group appears in the aromatic region (δ 7–8.5) and not between δ 10–13. In addition the value for the ^{13}C resonance of the carboxylic C atom of δ 170–175 confirms the resonance of a carboxylate group attached to a double bond.

3. Experimental

3.1. Chemistry

3.1.1. Material and methods

Melting points were determined on a Reichert apparatus and are uncorrected. The IR spectra obtained with a Beckman 4240 spectrophotometer and NMR spectra recorded on a Bruker AC 400 spectrometer were in agreement with the assigned structures. Elemental analyses (C, H, N, O within ±0.4% of the theoretical values) were performed at the Service Central d'Analyses, Centre National de la Recherche Scientifique, 69390 Vernaison, France.

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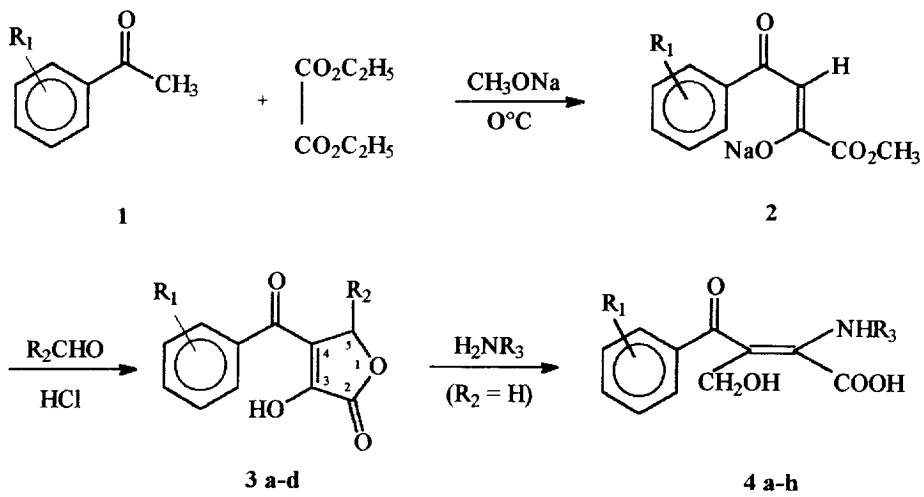

Scheme 1.

As typical examples the preparations of derivatives **3** and **4** are given.

*3.1.2. 4-Benzoyl 3-hydroxy 5-isobutyl furan-2 (5H) one (**3c**)*

To an aqueous solution (100 ml) of sodium bicarbonate (3 g) was added isovaleraldehyde (1.72 g, 0.02 mol) and then ester **2** [5] (3.42 g, 0.015 mol). The reaction mixture was vigorously stirred at 0°C for 2 h. Then the mixture was acidified to pH = 3 with 50% aqueous hydrochloric acid. The precipitate formed was filtered off and washed with ice water. The crude product was recrystallized from ethanol–water (50:50) to yield 1.0 g (26%) of **3c**.

IR (KBr, cm^{-1}): ν 3260 (OH), 1750 (C=O lactone), 1660 (C=O), 1640 (C=C).

^1H NMR: δ 0.9 (d, 6H, 2 CH_3), 1.5 (m, 2H, CH_2), 1.8 (m, 1H, CH), 5.4 (m, 1H, H-5), 7.5–7.9 (m, 5H, C_6H_5), 7.8 (s, 1H, OH).

^{13}C NMR: δ 21.2–22.9 (2 CH_3), 24.3 (CH), 41.5 (CH_2), 77.6 (C_5), 124.3 (C_4), 127.9–128.2–132.7 (C arom), 137.0 (C_3), 145.0 (C arom *ipso*), 168.5 (C=O lactone), 189.4 (C=O).

*3.1.3. 3-Hydroxymethyl 4-oxo 4-phenyl 2-phenylamino buten-2-oic acid (**4a**)*

To a solution of furanone **3a** (2.04 g, 0.01 mol) in 50 ml chloroform was added aniline (0.93 g, 0.01 mol). The reaction mixture was stirred for 12 h at room temperature. The product which separated out was filtered off and recrystallized from ethanol–water (50:50) to yield 0.62 g (21%) of **4a**.

IR (KBr, cm^{-1}): ν 3600–3400 (OH), 2600 (NH_2^+), 1710 (COO^-), 1660 (C=O), 1620 (C=C).

^1H NMR: δ 5.0 (s, 2H, CH_2), 6.5–8.0 (m, 13H, $2C_6H_5 + NH_2^+ + OH$).

^{13}C NMR: δ 68.0 (CH_2), 115.2–117.3–128.1–128.8–132.6 (C arom), 119.7 (C_3), 137.6 (C_2), 146.3–146.6 (C arom *ipso*), 170.4 (COO^-), 189.0 (C=O).

3.2. Pharmacological studies

3.2.1. Superoxide anion scavenging assay

The technique of Slater and Eakins [7] utilizing the interactions of NADPH, phenazine methosulfate (PMS), molec-

Table 1
Physical data for compounds **3** and **4**

Compound	R^1	R^2	R^3	Formula	Yield	T (°C)
3a	H	H		$C_{11}H_8O_4$	21	148 (Lit. 157 [6])
3b	OCH_3	H		$C_{12}H_{10}O_5$	16	155 (Lit. 155 [6])
3c	H	$CH_2CH(CH_3)_2$		$C_{15}H_{16}O_4$	26	182
3d	OCH_3	$CH_2CH(CH_3)_2$		$C_{16}H_{18}O_5,H_2O$	22	260
4a	H		C_6H_5	$C_{17}H_{15}NO_4$	21	138
4b	OCH_3		C_6H_5	$C_{18}H_{17}NO_5$	31	163
4c	H		CH_3	$C_{12}H_{13}NO_4$	89	136
4d	OCH_3		CH_3	$C_{13}H_{15}NO_5$	77	191
4e	H		$CH_2C_6H_5$	$C_{18}H_{17}NO_4$	87	125
4f	OCH_3		$CH_2C_6H_5$	$C_{19}H_{19}NO_5$	48	158
4g	H		4-$OMeC_6H_4$	$C_{18}H_{17}NO_5$	38	132
4h	OCH_3		4-$OMeC_6H_4$	$C_{19}H_{19}NO_6$	67	140

ular oxygen and nitro blue tetrazolium (NBT) was used for evaluating superoxide anion scavenging. The NADP/PMS/O_2/NBT system involves the intermediate formation of the superoxide anion radical ($O_2^{\cdot -}$) from the interaction of reduced PMS with O_2; the superoxide anion radical then reduces NBT to the highly colored formazan. The reaction can be followed by measuring the absorbance of formazan at 578 nm. The incubation mixture contained disodium hydrogen phosphate buffer (200 μl, 76 mM, pH = 7.4), PMS (200 μl, 10.8 mM), NBT (200 μl, 172 μM) and NADH + H^+ (200 μl, 360 μM). Each assay, repeated 5 times, was performed after 5 min incubation with several effector concentrations.

3.2.2. Acute toxicity in mice

Compound **3c** was administered intraperitoneally in saline (0.9% NaCl) at doses of 200, 400, 600 and 800 mg/kg. Swiss male mice purchased from Depre (Saint-Doulchard, France) weighing 18–22 g were used. Mice were kept in groups of ten in a temperature controlled room with a 12 h light/dark cycle. Food and water were available ad libitum during the time of the experiment. The animals were observed for 8 days in order to detect any signs of toxicity.

3.2.3. Evaluation of free radical production in an ischaemia-reperfusion model [8]

Twenty male New Zealand rabbits, 2500 g, were divided randomly into two groups. One group was subjected to experimental ischaemia by means of a tourniquet and received 10 ml of a solution containing 30 mg of **3c**, 200 μl NaOH 1 N, 5 ml disodium hydrogen phosphate buffer and 5 ml glucose in water (5%); the other did not receive drug and was the control group. Preparation of samples was carried out under the same conditions as previously described [8].

A catheter was placed in the marginal vein of the right ear of the rabbits after vasodilatation with chloroform. A first blood sample was collected 25 min before application of tourniquet on the upper right fore limb of the rabbits. A second blood sample was taken 5 min before release of the tourniquet. Third and fourth samples were taken, respectively, 1 and 10 min after release of the tourniquet. The lipoperoxides formed by peroxidation of the free radicals are converted into malondialdehyde, which reacts with thiobarbituric acid to form a colored complex. Malondialdehyde was assayed by HPLC with fluorimetric detection. The excitation wavelength was set at 520 nm and the emission wavelength at 550 nm.

3.2.4. Determination of IC_{50} and LD_{50} statistical analysis

IC_{50} and LD_{50} values were obtained by graphic estimation from log concentration–response curves. The Student' *t*-test was used to assess the statistical significance between the means of unpaired data compared to the control group. $p < 0.05$ was statistically significant. Thus, all percentage inhibition values given in Table 2 for each compound are statistically significant unless otherwise indicated.

Table 2
Superoxide anion scavenging activity of compounds **3** and **4**

Compound	Inhibition at 1 mg/ml [a,b] (%)
3c	85 ± 4 ($IC_{50} = 8.69 \times 10^{-4}$ M)
4a	19 ± 2 (NS)
4c	18 ± 2 (NS)
4d	29 ± 3
4f	7 ± 2 (NS)
4g	0
4h	12 ± 1 (NS)
Ascorbic acid	24 ± 3

[a] Percent inhibition of formazan absorbance (see Section 3).
[b] The values are the mean ± SD of five separate determinations.
NS: not significantly different from the control.

4. Results and discussion

Compounds **3** and **4** were evaluated as superoxide anion scavengers and compared with ascorbic acid (Table 2). Compounds **3a, 3b, 3d, 4b, 4e** cannot be tested due to their poor solubility in the biological reaction mixture. The antioxidant effects of cinnamic acid derivatives as well as vitamin C were very low or absent at the concentration of 1 mg/ml. In contrast, furanone **3c** exhibited a potent activity with an IC_{50} value of 8.69×10^{-4} M. This result corroborates preceding reports [5,9,10] showing the necessity of modifying ascorbic acid by introduction of lipophilic moieties on the furan ring for marked antioxidant properties.

However substitution of the furan nucleus by a benzoyl moiety at the 4-position and an isobutyl group at the 5-position enhanced acute toxicity of **3c** ($LD_{50} = 72.9$ mg/kg i.p.) with regard to ascorbic acid ($LD_{50} > 800$ mg/kg i.p.). From 100 mg/kg i.p. **3c** caused the death of all the animals. Therefore **3c** was tested at the maximum dose of 10 mg/kg i.v. for the study of protective effects against reperfusion injury in rabbits. The results summarized in Table 3 indicated that there was a significant increase in free radicals only with the control group during the reperfusion phase immediately following removal of the tourniquet. In contrast, the weak variation in

Table 3
Malondialdehyde assay in rabbit plasma after application of a tourniquet for 30 min

Sampling time	Control	Experimental (compound **3c**)
Before application of tourniquet	2.37 ± 0.43	1.31 ± 0.57
After 25 min	2.01 ± 0.42	1.13 ± 0.34
1 min after removal	2.49 ± 0.70 [a]	1.42 ± 0.59
10 min after removal	2.23 ± 0.68 [b]	1.32 ± 0.69

[a] $p < 0.01$ compared with 25 min sample.
[b] $p < 0.04$ compared with 1 min sample.
No other significant statistical difference was observed between different plasma concentrations before tourniquet application and before or after its removal.

levels of free radicals observed when **3c** was administered to rabbits was not statistically significant.

This protective effect of derivative **3c** against reperfusion injury confirms the interest in lipophilic synthetic analogues of ascorbic acid as antioxidant drugs.

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