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Synthesis and Properties of 11-(3,5-Di-*tert*-butyl-2-hydroxyphenylcarbamoyl)undecanoic Acid, a New Amphiphilic Antioxidant

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Abstract—Based on the membrane addressing concept, designing and synthesis of 11-(3,5-di-*tert*-butyl-2-hydroxyphenylcarbamoyl)undecanoic acid have been carried out. Antioxidant properties of the prepared compound were investigated in comparison with its non-amphiphilic analogues.

Experimental and clinical data accumulated thus far provide evidence of a substantial role of free-radical oxidation processes taking place in membranes[1] in the development and progression of cancer, atherosclerosis and ischaemic diseases, as well as in inflammation and degenerative processes, in particular, those of aging.[2] In this connection, intense activities are deployed within the recent decades aimed at finding synthetic free-radical process inhibitors, which are considered as potential drug substances for prevention and treatment of the named pathologies.[3] In the area of medicinal chemistry, the major part of such compounds is found among structural analogues of natural antioxidants and sterically hindered phenols.[4]

An important approach to the design of synthetic inhibitors of lipid peroxidation (LPO) is, along with variation of sterical and stereoelectronic parameters of the radical-acceptor center of the molecule, localization of their activity sites in biological membranes. Earlier, it was achieved by introducing hydrophobic aliphatic substituents,[5] or by forming a lipidomimetic phosphatidyl group within the antioxidant structure.[6] In the work aimed at elucidation of the structure–function relationship within the series of 3-*O*-alkylascorbic acids[7] and hydroquinone monoalkyl ethers,[8] the principal means for improving anti-radical activity was optimization of hydrophobicity of precursor compounds using the side chain homologation method.

The concept of our study is, in its turn, based on localization of the antioxidant molecule in a specified area of the membrane. This is accomplished by introducing into the terminal part of aliphatic chain a hydrophilic carboxylic group. The latter, being substantially deprotonized at physiological pH values, is located in the aqueous phase and plays the role of an 'anchor' preventing bulk diffusion of antioxidant molecules into the lipid bilayer. Quite naturally, this leads to an increase in the lipophilic antioxidant concentration at a definite area of hydrophobic layer of the membrane. Localization of radical-acceptor groups is controlled by means of selecting the side-chain length to fit the conditions imposed. In the course of LPO in biosystems, radical species are generated, in particular $HO^{\bullet}$, which enter the lipid bilayer mainly from the aqueous phase. The preferential site of radical attack at a polyunsaturated fatty acid molecule (PUFA) is the methylene group of a 1,4-pentadiene moiety.[2b] Hence, to achieve the highest LPO-inhibiting activity, the sterically hindered OH group of the antioxidant should probably be situated between the phase interface and the first methylene group of a 1,4-pentadiene moiety, with respect to the carboxyl group of PUFA (i.e., C_7 for arachidonic acid, C_{11} for linolic and linolenic acids).

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With the intent of obtaining the required structure, we fixed upon derivatives of 2-amino-di-*tert*-butylphenol (**1**). The 2-amino-di-*tert*-butylphenol derivatives are supposed to be efficient antioxidants,[9] and the presence of an aromatic primary amino group provides favourable opportunities for structural modifications of the molecule. Dodecanedioic acid (**2**) has been chosen as a membrane-directing agent, because ten methylene groups in the structure of the acid **2** are approximately equivalent to seven methylene and two methyne groups of the Z-double bond present in linolic and linolenic acid moieties.

Assemblage of antioxidant **3** molecule was performed using the amino group of aminophenol **1** and one of the carboxyl groups of dicarboxylic acid **2**. Simultaneously, in order to elucidate the role of terminal carboxyl group in manifestation of antioxidant activity in the membrane, a group of compounds having similar structures has been obtained (**4**–**7**, Fig. 1).

Synthesis of the amide **3** was performed as follows. By treatment the readily available catechol (**8**) with *tert*-butanol and concentrated sulphuric acid for 12 h, a di-*tert*-butylated product **9** has been obtained,[10,11] which was further oxidized into a quinone **10** by reaction with $NaNO_2$ in AcOH.[10,11] The quinone **10**, after reaction with NH_3 aq in MeOH and subsequent addition of powdered $NaBH_4$, gave the aminophenol **1**[12] in a yield of 91%.[13] Selective hydrolysis of the dodecanedioic acid dimethyl ester was performed by reaction in the system KOH–MeOH–MeCN–Et_2O[14] during 52 h at 0 °C, which gave dodecanedioic acid monomethyl ester (**11**) in a good yield.[13] The monoester **11** was quantitatively converted into the respective 11-chlorocarbonyl-undecanoic acid methyl ester (**12**) by reaction with $(COCl)_2$ in dry toluene. The aminophenol **1** was regioselectively acylated at the amino group by reaction with the obtained acyl chloride in THF at 0 °C in the presence of an equivalent of Et_3N. A subsequent treatment with LiOH in aqueous methanol gave the amide **3**[13] in a high yield (Scheme 1). The compounds **4**–**7**[13] were obtained from the respective carboxylic acids and aminophenol **1** according to the scheme realized for the synthesis of the compound **3**.

Radical-inhibiting activity of the compounds **3**–**7** was evaluated by studying their effects on the free-radical oxidation reaction of lipids in multilamellar liposomes[15] formed of phosphatidyl choline freshly isolated from egg yolk.[16] The LPO processes were followed by recording the accumulation of primary products-conjugated diene products (CDP),[18] as well as secondary products—2-thiobarbituric acid reacting substances (TBARS).[19] The experimental results are presented in Tables 1 and 2.

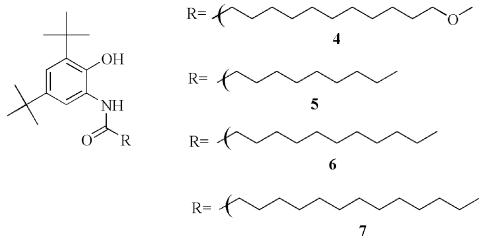
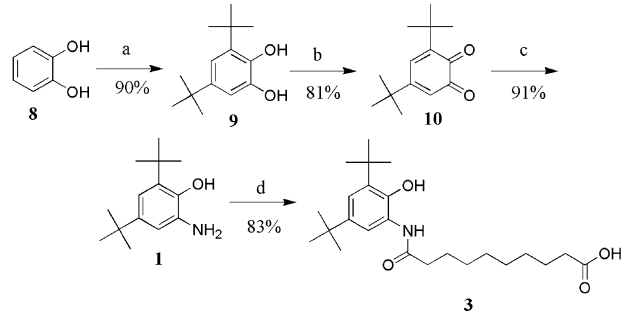


Figure 1. Analogues of the compound **3**.

Hydrophobicity of compounds **3**–**7** was evaluated using the HPLC method. The capacity coefficient k'[8] of title compounds was used as a hydrophobicity-characterizing parameter (Table 3). As seen from the table, the compound **3** has the lowest hydrophobicity of the whole group of compounds tested.

After having analyzed the data of Tables 1 and 2, one can come to the conclusion that compounds **3**–**7** suppress considerably the formation of LPO products. In the series of compounds **4**–**7**, a higher inhibitory activity is displayed by the more lipophilic analogues, because of an increased antioxidant concentration in the membrane. On the other hand, due to restricted linear dimensions considerable of the bilayer, an increase in the side chain length possibly creates obstacles in packing the antioxidant molecule into the hydrophobic phase. Thus, compound **7**, which has the highest hydrophobicity of the compound group studied, is inferior to compound **6** in anti-radical activity. This is in accordance with the conclusions made in reports[7,8] concerning the presence of an optimal hydrophobicity level, above and below which the antioxidant activity in a homologue series comes down. Compounds **4** and **5**, which are close as to hydrophobicity, are different in their capability of inhibiting peroxidations in the liposomal model. It can be supposed that this is probably due to the terminal methoxy group in the structure **4**, which favours to some extent an oriented arrangement of the antioxidant in the membrane, although its effect is feebly marked.

The amphiphilic compound **3**, in spite of its relatively low hydrophoby, possess considerable inhibitory activity. It is noteworthy that the compound **3** is much more active than compounds **4**–**7** of similar structures, and is on average 3.17 times superior to α-tocopherol as to suppressing the LPO product formation initiated by γ-irradiation in the liposomal model ($I_3/I_{\alpha\text{-tocopherol}} = 3.17$). This can be due to an oriented arrangement of compound **3** molecules in the lipid bilayer owing to the presence of an ionogenic group in the



Scheme 1. Reagents and conditions: (a) *t*BuOH, H_2SO_4, rt, 12 h; (b) $NaNO_2$, AcOH, rt, 1 h; (c) (i) NH_3 aq, MeOH, rt, 10 min; (ii) $NaBH_4$; (d) (i) **12**, Et_3N, THF, 0 °C→rt, 30 min; (ii) LiOH, MeOH/H_2O, 5 °C, 20 h.

Table 1. Inhibiting effects of compounds 3–7 on conjugated diene products formation

Dose kGy	[AO] mM[a]	Inhibition of CDP formation, %[b]					
		3	4	5	6	7	α-T
0.9	0.4	85.7±6.4	67.0±5.0	48.7±9.7	68.1±4.8	63.7±7.0	63.7±6.4
1.1	0.1	86.0±3.4	26.7±7.9	53.3±7.5	46.7±9.1	60.0±6.9	27.8±7.1

[a]The phosphatidyl choline concentration was 20 mM.
[b]Per cent inhibition with respect to a blank experiment, without antioxidant. The confidence interval was calculated for $n=3$, $P=0.95$ using the Student's t-distribution.

Table 2. Inhibiting effects of compounds 3–7 on TBARS formation

Dose kGy	[AO] mM[a]	Inhibition of TBARS formation, %[b]					
		3	4	5	6	7	α-T
0.9	0.4	92.9±1.9	80.7±2.9	65.5±4.4	81.0±3.3	71.5±4.3	78.7±4.1
1.1	0.1	83.1±3.8	58.8±6.3	73.7±4.2	72.3±4.7	69.5±7.3	66.5±6.6

[a]The phosphatidyl choline concentration was 20 mM.
[b]Per cent inhibition with respect to a blank experiment, without antioxidant. The confidence interval was calculated for $n=3$, $P=0.95$ using the Student's t-distribution.

Table 3. Hydrophobicity of the compounds 3–7

Compd	k'[a]
3	0.38
4	6.39
5	7.13
6	12.60
7	25.20

[a]HPLC was conducted with Restek Ultra Aqueous C18 column; the mobile phase consisted of MeOH/H_2O (85/15).

terminal part of the side chain. The oriented arrangement of molecules leads to an increase in local concentration of the antioxidant at a definite distance from the phase interface. The 'anti-radical shield' thus formed protects the membrane from attacks with radical species coming from the aqueous phase more effectively than anti-oxidant molecules being diffusely distributed through the lipid bilayer.

Because of high efficacy of the antioxidant 3, we intend to prepare its analogues with various terminal ionogenic groups. The following study will also include preparation of a series of compound 3 homologues, and elucidation of the role of alkyl spacer in manifestation of antioxidant activity.

Thus, synthesis and inhibiting properties are described in this paper of a new antioxidant 3 and its analogues having a non-amphiphilic structure. The importance of the forming of an amphiphilic domain within the phenolic antioxidant molecule is shown for enhancement of the efficacy in suppressing the LPO processes, possibly due to a localization of the radical-inhibiting moieties within the membrane.

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13. 3: ^{1}H NMR (200 MHz, $CDCl_3$) δ 11.91 (s, 1H), 8.52 (br, 1H), 7.42 (br, 1H), 7.22 (s, 1H), 6.78 (s, 1H), 2.40 (m, 4H), 1.74 (m, 4H), 1.44 (s, 9H), 1.26 (m, 21H), MS m/z 433.40 (M^+), 221.25 ($M-C_{12}H_{20}O_3)^+$, mp 143 °C. **4**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.52 (br, 1H), 7.42 (br, 1H), 7.22 (s, 1H), 6.78 (s, 1H), 3.30 (m, 5H), 2.30 (t,

$J=7.1$ Hz, 2H), 1.74 (q, $J=8.2$ Hz, 2H), 1.42 (s, 9H), 1.26 (m, 25H), MS m/z 434.50 $(M+1)^+$, 433.50 (M^+), 221.22 $(M-C_{13}H_{24}O_2)^+$, mp 65 °C. **5**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.52 (br, 1H), 7.42 (br, 1H), 7.20 (s, 1H), 6.78 (s, 1H), 2.22 (t, $J=7.0$ Hz, 2H), 1.74 (q, $J=8.4$ Hz, 2H), 1.42 (s, 9H), 1.26 (m, 21H), 0.92 (t, $J=8.2$ Hz, 3H), MS m/z 376.40 $(M+1)^+$, 375.41 (M^+), 221.25 $(M-C_{10}H_{18}O)^+$, mp 116 °C. *6*: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.52 (br, 1H), 7.42 (br, 1H), 7.20 (s, 1H), 6.77 (s, 1H), 2.22 (t, $J=7.0$ Hz, 2H), 1.74 (q, $J=8.3$ Hz, 2H), 1.42 (s, 9H), 1.26 (m, 25H), 0.92 (t, $J=8.2$ Hz, 3H), MS m/z 404.45 $(M+1)^+$, 403.45 (M^+), 221.25 $(M-C_{12}H_{22}O)^+$, mp 102 °C., *7*: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.52 (br, 1H), 7.42 (br, 1H), 7.22 (s, 1H), 6.78 (s, 1H), 2.22 (t, $J=7.0$ Hz, 2H), 1.74 (q, $J=8.1$ Hz, 2H), 1.42 (s, 9H), 1.26 (m, 29H), 0.90 (t, $J=8.0$ Hz, 3H), MS m/z 432.50 $(M+1)^+$, 431.50 (M^+), 221.24 $(M-C_{14}H_{26}O)^+$, mp 52 °C.

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