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Antioxidant Activity of Natural Allylpolyalkoxybenzene Plant Essential Oil Constituents

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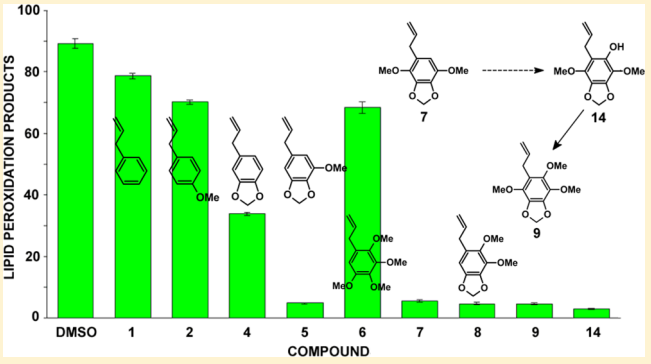
Supporting Information

ABSTRACT: Free-radical-scavenging capacity antioxidant and membrane-protective properties of natural and related synthetic allylpolyalkoxybenzenes with different numbers of alkoxy/methoxy groups in the aromatic ring were evaluated using several in vitro models. These included the DPPH assay, inhibition of lipid peroxidation products accumulation, inhibition of H_2O_2-induced hemolysis, and oxidation of oxyhemoglobin. A synthetic protocol for the synthesis of natural nothoapiol (**9**) from a parsley seed metabolite, apiol (**7**), was developed. A structure−activity relationship study revealed that both the methylenedioxy fragment and methoxy groups in the aromatic ring are favorable for antioxidant activity. Hydroxyapiol (**14**), containing a hydroxy group in the aromatic core, was identified as the most potent compound. The pentaalkoxy-substituted nothoapiol (**9**) showed antioxidant activity in mouse brain homogenates, whereas in mouse erythrocytes it exhibited a marked pro-oxidant effect. Despite their low free-radical-scavenging capacity, allylpolyalkoxybenzenes can contribute to the total antioxidant potencies of plant essential oils.



Plant extracts and essential oils (EOs) are attracting a growing interest as natural antioxidants that are relatively less damaging to human and animal health and the environment, suggesting their potential application in medicine and the food industry. A considerable number of EOs have been reported to possess substantial antioxidant potencies, attenuating free-radical oxidative reactions detrimental to macromolecules, cells, and organisms.[1−4] Although the chemical composition and antioxidant properties of EOs from various plants have been studied extensively, data on the antioxidant potential of individual components of EOs are rather scarce. Generally, phenolic compounds of both the terpenoid and phenylpropanoid families are considered to be the principal antioxidative constituents of EOs due to their high reactivity with peroxyl radicals.[3−6] Literature data suggest that allylpolyalkoxybenzenes (Figure 1) exhibit antioxidant activity. A number of EOs with potent antioxidant properties contain diverse allylpolyalkoxybenzenes as major components (Table S1, Supporting Information).[7−21] A commercial EO from parsley, *Petroselinum sativum* Hoffm. (Apiaceae), seeds, containing myristicin (**5**) (44%) and apiol (**7**) (12.1%) as the predominant components, showed discernible activity in 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical-scavenging and malonaldehyde/gas chromatography assays.[1] Also, the EO extracted from aerial parts of English parsley, *P. crispum* (Mill.) Nym. ex A.W. Hill, with myristicin (**5**) (36.15%) and apiol (**7**) (20.97%) as major constituents, demonstrated antioxidant activity in both the DPPH and ferric reducing antioxidant power tests, although exhibiting less potency than that of butylhydroxytoluene (BHT).[22] In turn, EO from the stems of striped hemlock ("Coscoll"), *Molopospermum peloponnesiacum* W.D.J. Koch (Apiaceae), containing 60.1% dillapiol (**8**), exhibited relatively strong radical-scavenging capacity in the DPPH test.[18] There is some evidence that individual allylpolyalkoxybenzenes may be considered as compounds contributing to the total antioxidant potency of an EO. Thus, myristicin (**5**)- and apiol (**7**)-containing fractions separated from the commercial crude EO of English parsley displayed good DPPH free-radical-scavenging capacity, and apiol (**7**) was assumed to be the major contributor to the antioxidant effect of its EO.[23] Thin-layer chromatography analysis of the EO from the aerial parts of *Psammogeton canescens* Vatke (Apiaceae) revealed spots related to apiol (**7**) and dillapiol (**8**) with DPPH radical-scavenging activity compared to that of butylated hydroxyanisole and ascorbic acid, but with little impact on the total antioxidant effect of the EO.[24] In contrast,

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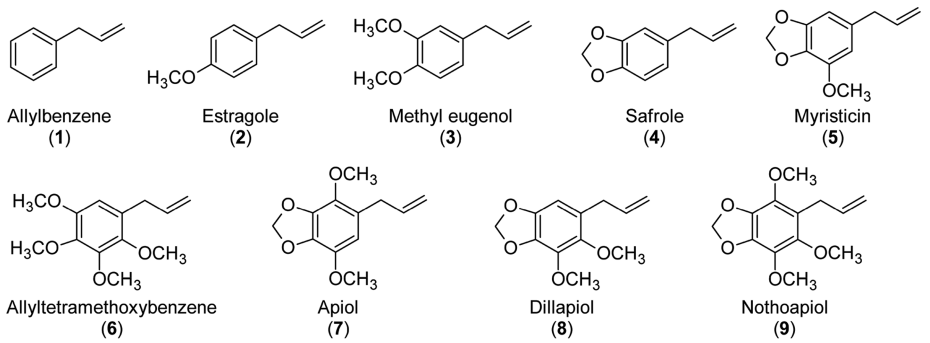
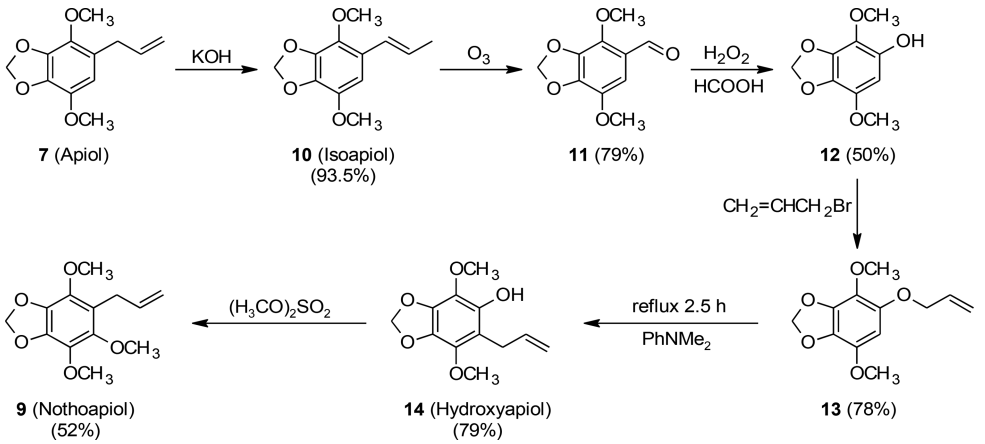


Figure 1. Structures of allylpolyalkoxybenzenes.

Scheme 1. Synthesis of Nothoapiol (9) from Apiol (7)



it was found that dillapiol (**8**) at 50 μM concentration acted as a pro-oxidant, promoting the generation of reactive oxygen species in MDA-MB-231 human breast adenocarcinoma cells.[25] The antioxidant effectiveness of a number of pure EO components was evaluated using a modified thiobarbituric acid reactive substance (TBARS) assay and measurement of induced linoleic acid autoxidation rate.[26] However, of the molecules tested, only one allylpolyalkoxybenzene, estragole (**2**), was studied, showing substantially less antioxidant potency than that of α-tocopherol. Similarly, EO from aerial parts of sweet basil, *Ocimum basilicum* L. (Lamiaceae), with estragole (**2**) as the main component was reported to exhibit free-radical-scavenging activity, but estragole itself was inactive.[19] Recently, the EO obtained from aerial parts of tarragon, *Artemisia dracunculus* L. (Asteraceae), and its major constituent, estragole (**2**), were evaluated by two different antioxidant activity tests, TBARS and β-carotene bleaching, with BHT and vitamin E as reference compounds. Both assays identified the EO and estragole (**2**) as weaker antioxidants than BHT and vitamin E, and compound **2** was less active than the EO.[20]

In addition to the beneficial antioxidant properties, there are data, although contradictory, that allylpolyalkoxybenzenes exhibit genotoxic effects. Estragole (**2**), methyl eugenol (**3**), safrole (**4**), myristicin (**5**), apiol (**7**), and dillapiol (**8**) were reported to form DNA adducts in HepG2 human hepatoma cells, which may cause mutations, eventually leading to carcinogenesis. This damaging DNA-targeting capacity decreases with an increase of the number of alkoxy substituents in the aromatic core: estragole (**2**) > methyl eugenol (**3**) > safrole (**4**) ≈ myristicin (**5**) > dillapiol (**8**) > apiol (**7**).[27] Conversely, further studies showed the absence of both cytotoxic and genotoxic effects of estragole (**2**) in HepG2 cells up to a 0.57 mM concentration[28] as well as no toxicity of apiol (**7**) and dillapiol (**8**) on sea urchin embryos up to a 100 μM concentration.[29] The above data encouraged special attention to be paid to the most oxygenated and pentasubstituted nothoapiol (**9**). Nothoapiol (**9**) is not as common as allylpolyalkoxybenzenes **2**–**8**, although it has been identified in a number of EOs from plants of the families Apiaceae and Lamiaceae, as well as in a small amount in the brown alga *Spatoglossum variabile* Figari & De Notaris (Table S2, Supporting Information).[8,13,16,30−36]

Preparative isolation of nothoapiol (**9**) was challenging due to its low concentration in plants and the use of column chromatography unsuitable for a large-scale separation. Nothoapiol is a major component of EOs extracted from roots and aerial parts of *Carum montanum* (Coss. et Dur.) Benth. et Hook. (Apiaceae) (Table S2, Supporting Information). However, only 1 mL of EO could be separated from 1 kg of *C. montanum* dry roots.[36] Moreover, this plant is an endemic species growing in hard-to-reach areas in mountain regions of Eastern Algeria at 1500 m above sea level and is not cultivated as a crop.[36] Until now, a synthetic procedure for compound **9** has not been elaborated.

Considering the above literature evidence, the aim of the present study was to assess antioxidant properties of natural and related synthetic allylpolyalkoxybenzenes featuring an increasing number of alkoxy substituents for the aromatic ring and to develop a synthetic protocol for nothoapiol (**9**) from a natural building block.

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RESULTS AND DISCUSSION

Synthesis of Nothoapiol (9). A facile synthesis of nothoapiol (**9**) based on a parsley seed essential oil extracted from cultivated in Russia parsley *Petroselinum sativum* varieties has been developed. The oil is a versatile source of apiol (**7**) (65−70%)[37] and can be isolated by liquid CO_2 extraction followed by high-efficiency large-scale distillation (up to 40 kg of apiol).[14] A combination of the specialized ozonolysis equipment and optimized protocols described earlier[37] allowed for preparation of the targeted aldehyde **11** in yields up to 80% on a 100 g scale (Scheme 1). Oxidation of apiol aldehyde (**11**) under Bayer−Villiger rearrangement conditions at room temperature afforded phenol **12**,[38] which was further alkylated by allyl bromide to ether **13**. Aromatic Claisen rearrangement of **13** to hydroxyapiol (**14**) followed by alkylation with dimethyl sulfate afforded the target nothoapiol (**9**). The compounds obtained were characterized by their ^{1}H and ^{13}C NMR spectra and by elemental analysis.

Antioxidant Activity. Free-radical-scavenging capacity and antioxidant properties of natural products **2** and **4**−**9** and synthetic allylpolyalkoxybenzenes **1** and **14** were evaluated in in vitro models that have been used successfully for natural phenolic compounds and their derivatives.[39−43] A majority of compounds showed negligible radical-scavenging capacity in the DPPH test even at a 1 mM concentration (Table 1). In contrast, hydroxyapiol (**14**), a hydroxy analogue of nothoapiol (**9**), was identified as a strong free-radical-scavenging agent comparable in potency with the standards, BHT and trolox, suggesting that a hydroxy substituent in an aromatic ring is essential for interaction with persistent DPPH radicals. An electron-donating methoxy group in an *ortho*-position contributed to the radical-scavenging activity of **9**, as reported for phenolic products of lignin depolymerization,[44] whereas the second *ortho*-methoxy group could increase the activity even more.[45,46] Low free-radical-scavenging activity of allylbenzenes with a methylenedioxy fragment (**4**, **5**, and **7**−**9**) and methoxy groups (**2**, **3**, and **6**) as compared to hydroxyapiol (**14**) correlated well with literature data. As an example, hydroxy-substituted coumaperine derivatives were found to be 10−100 times more potent than the respective methylenedioxy- and methoxy-containing analogues.[47]

Table 1. DPPH Radical Scavenging Capacity of Allylpolyalkoxybenzenes at 0.1 and 1 mM Concentrations

	radical-scavenging capacity (%)[a]	
compound	0.1 mM	1 mM
1	1.01 ± 0.16	4.29 ± 0.42
2	0.25 ± 0.13	1.16 ± 0.19
4	1.26 ± 0.21	10.6 ± 0.22
5	1.31 ± 0.13	5.25 ± 0.25
6	0.10 ± 0.06	1.47 ± 0.15
7	0.05 ± 0.05	0.30 ± 0.14
8	0.38 ± 0.20	3.94 ± 0.44
9	0.91 ± 0.45	4.30 ± 0.80
14	88.9 ± 0.42	90.2 ± 0.21
BHT	72.0 ± 4.0	ND[b]
trolox	94.9 ± 0.2	ND[b]

[a]The values are expressed as the means ± SE from four independent experiments. [b]ND: not determined.

Likewise, in a system containing mouse brain lipids, hydroxyapiol (**14**) was as potent as the standard antioxidants, BHT and trolox (Figure 2; Table S3, Supporting Information). The ability of hydroxyapiol (**14**) to prevent completely the initiated oxidation of animal lipids at a low concentration (0.1 mM) may be explained by its high radical-scavenging activity due to the presence of a phenolic hydroxy and a methoxy substituent in an *ortho*-position. All compounds tested showed a concentration-dependent ability to reduce the formation of TBARS in this assay model. At a 0.1 mM concentration, myristicin (**5**), apiol (**7**), dillapiol (**8**), and nothoapiol (**9**) exhibited weak antioxidant properties, allylbenzene (**1**), safrole (**4**), and tetramethoxybenzene (**6**) were inactive, and estragole (**2**) caused a pro-oxidant effect. In contrast, at a 1 mM concentration, all allylpolyalkoxybenzenes inhibited statistically significantly the induced oxidation of mouse brain lipids. Figure 2 shows that compounds **5** and **7**−**9**, featuring both a methylenedioxy moiety and methoxy substituents on the aromatic ring, demonstrated pronounced activity and inhibited both induced and spontaneous autoxidation, in comparison with blank (intact) samples. Safrole (**4**), without any methoxy group, was less potent, and molecules **1**, **2**, and **6**, lacking a methylenedioxy fragment, displayed moderate activity. Notably, apiol (**7**) and dillapiol (**8**) at a 1 mM concentration were determined to be considerably more potent than their analogue, tetramethoxybenzene (**6**), with four methoxy groups.

It has been proposed that compounds with a methylenedioxy fragment can exhibit antioxidant effects due to their enzymatic transformation, particularly cytochrome P450-catalyzed oxidative demethylenation, to form the respective catechol metabolites.[48−53] The resulting OH groups could be responsible for further interaction with free radicals and oxidative chain break. In addition, the free-radical-scavenging capacity of the metabolites is facilitated by the electron-donating methoxy substituents in an *ortho*-position to hydroxy groups.[44,45] Notably, P450 inactivation was reported to reduce the ability of methylenedioxybenzenes to prevent ferric nitrilotriacetate-induced TBARS generation in rat hepatocytes.[49] To confirm the above mechanism of the antioxidant activity of allylbenzenes, we studied the effects of compounds featuring the methylenedioxy group, **5**, **7**−**9**, and **14**, after pretreatment of mouse brain homogenates by a P450 inhibitor, isopropanol.[49] It was found that preincubation with 0.5% isopropanol during 30 min increased markedly TBARS formation in the presence of allylpolyalkoxybenzenes **5**, **7**−**9**, and **14** (Figure 3), suggesting the value of enzymatic hydrolysis of methylenedioxybenzenes for their antioxidant effect.

Induced oxidative hemolysis of erythrocytes is used widely to assess the antioxidant potential of natural products.[54−56] It was found that allylbenzenes **4**, **5**, **7**, and **8** at a 0.1 mM concentration induced hemolysis of up to 10−12% of red blood cells (RBCs). In addition, at this concentration, compounds **1**, **2**, **4**, **6**, and **7** showed noticeable pro-oxidant activity in the H_2O_2-induced mouse RBC hemolysis assay. A statistically significant antioxidant effect was observed only for myristicin (**5**) and to a lesser extent for dillapiol (**8**) (Table S4, Supporting Information). Therefore, further studies of biological activity in the RBC model were carried out at a 0.01 mM concentration (Figures 4−6). As for the mouse brain homogenates, hydroxyapiol (**14**) suppressed strongly the accumulation of secondary lipid peroxidation products in RBCs, and its effect was comparable to that of BHT (Figure 4; Table S5, Supporting Information). Other allylbenzenes

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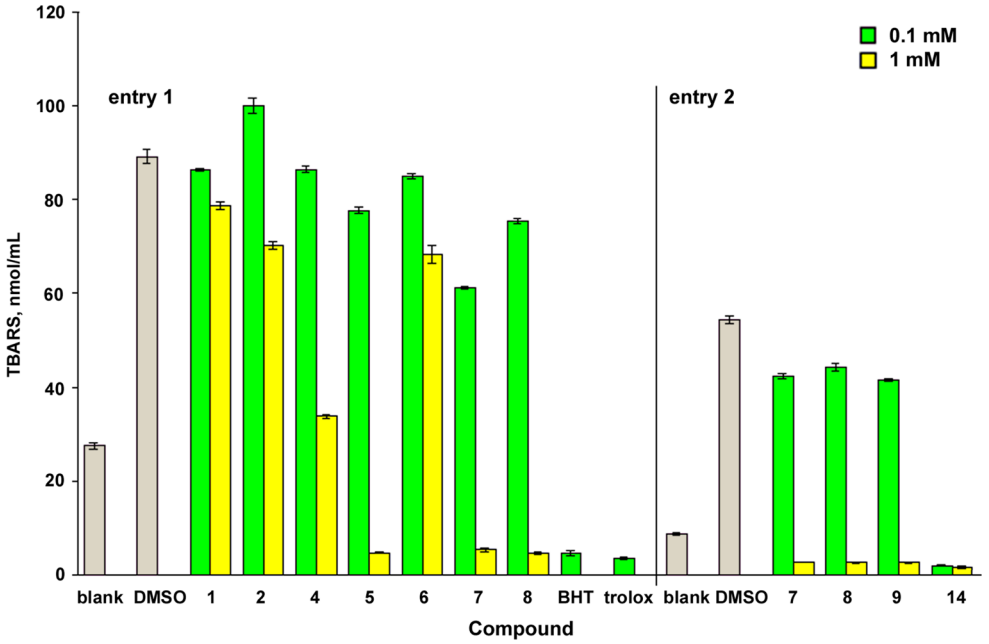

Figure 2. Effects of allylpolyalkoxybenzenes **1**, **2**, and **4**–**8** on TBARS accumulation in mouse brain homogenates. Data of two independent experiments (entries 1 and 2). TBARS concentrations were measured at 1 h after the initiation of lipid peroxidation by Fe^{2+}/ascorbate. Blank: Intact sample without initiation of oxidation. Vertical bars: means ± SE (n = 4).

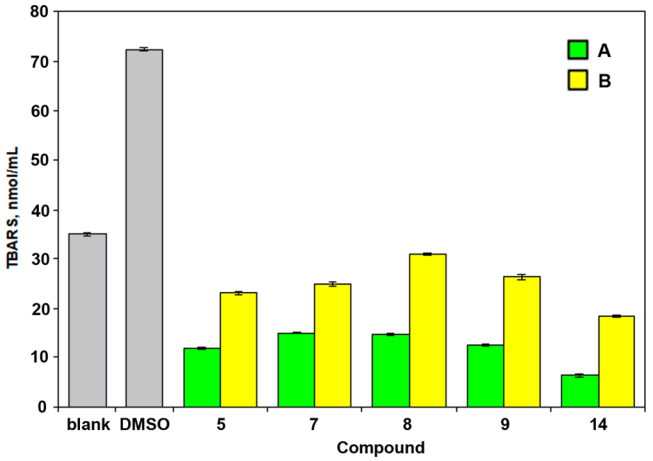

Figure 3. Effects of allylpolyalkoxybenzenes **5**, **7**–**9**, and **14** (0.5 mM) on TBARS accumulation in mouse brain homogenates. Data of two independent experiments. (A) Intact brain homogenates. (B) Brain homogenates, preincubated with isopropanol for 30 min, 0.5% v/v. TBARS concentrations were measured at 1 h after the initiation of lipid peroxidation by Fe^{2+}/ascorbate. Blank: Intact sample without initiation of oxidation. Vertical bars: means ± SE (n = 4).

displayed marginal antioxidant properties, with the notable exception of nothoapiol (**9**), containing trimethoxy-methylenedioxy substituents in the aromatic ring. This molecule, at a 0.01 mM concentration, did exhibit a pronounced pro-oxidant effect, but it increased both TBARS accumulation and RBC hemolysis (Figures 4 and 5; Table S5, Supporting Information). Similarly, dillapiol (**8**) at a 0.05 mM concentration also enhanced the generation of reactive oxygen species in MDA-MB-231 human breast adenocarcinoma cells.[25] This ambiguity in the definition of pro- and antioxidant properties of the tested allylpolyalkoxybenzenes as well as of other natural modulators of free-radical processes may be related to the experimental model, the primary target of oxidation initiator, the availability of catalytic metal ions, and the ratio between an antioxidant concentration and the amount of oxidation material.[57−62]

Allylpolyalkoxybenzenes **1**, **2**, **4**–**8**, and **14** decreased statistically significantly RBC death caused by H_2O_2-initiated oxidative stress during the whole incubation time (5 h) (Figure 5; Table S5, Supporting Information). Hydroxyapiol (**14**) showed the best membrane protective activity at 1 and 3 h of incubation, whereas at 5 h its effect was similar to that of other compounds. As described above, the methylenedioxy moiety and methoxy substituents in the aromatic ring were favorable, since myristicin (**5**) was more active than safrole (**4**), and apiol (**7**) and dillapiol (**8**) displayed higher membrane-protective properties than tetramethoxybenzene (**6**) (Figure 5; Table S5, Supporting Information). All molecules tested inhibited statistically significantly both methemoglobin and ferrylhemoglobin formation (Figure 6; Table S5, Supporting Information), although no structure−activity correlation was found. Dillapiol (**8**) was identified as the most potent inhibitor of the accumulation of hemoglobin oxidation products. Notably, the same effect on metHB/oxyHB ratio was observed for dillapiol and BHT.

To summarize, the evaluation of natural allylpolyalkoxybenzenes both in RBC-based and brain lipid-containing models shows that these molecules may be considered biological antioxidants, despite their low free-radical-scavenging capacity. Along with phenolic compounds, allylpolyalkoxybenzenes can contribute to the total antioxidant potencies of EOs. The antioxidant activities of the allylpolyalkoxybenzenes differed depending on the assay and the source of lipid-containing substrate. The pentaalkoxy-substituted nothoapiol (**9**) was identified as an antioxidant in mouse brain homogenates, whereas in an RBC model, this molecule exhibited a pronounced pro-oxidant activity. Generally, the most potent allylbenzenes featured both a methylenedioxy moiety and different numbers of alkoxy/methoxy groups in the aromatic ring. Their antioxidant effects were related likely to

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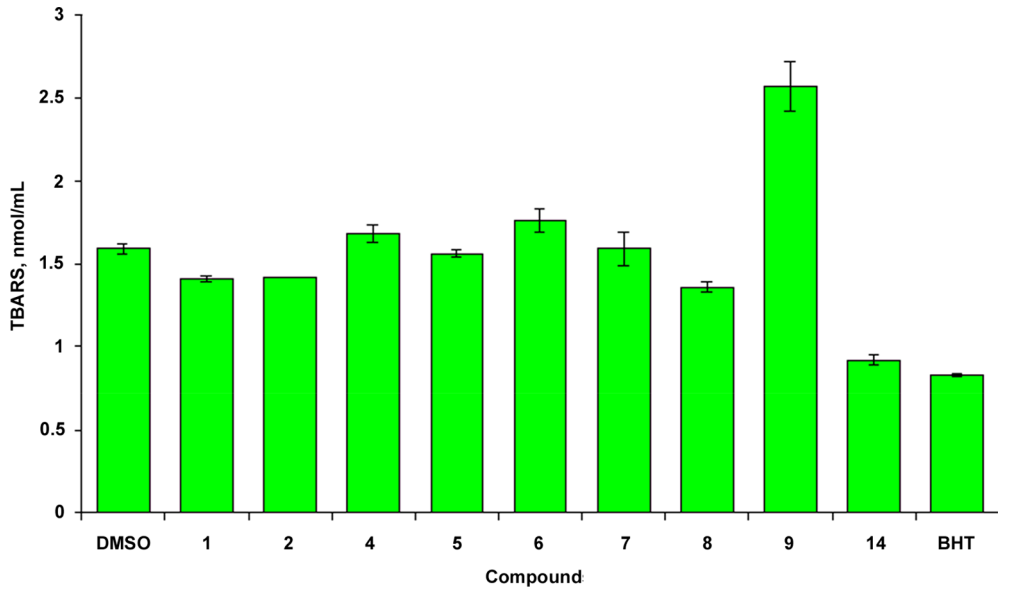

Figure 4. Effect of allylpolyalkoxybenzenes **1**, **2**, **4**–**9**, and **14** and BHT (0.01 mM) on TBARS accumulation in mouse RBCs under H_2O_2-induced oxidative stress. TBARS concentration was measured at 4 h after adding H_2O_2 to the RBC suspension. Vertical bars: means ± SE (n = 4–10).

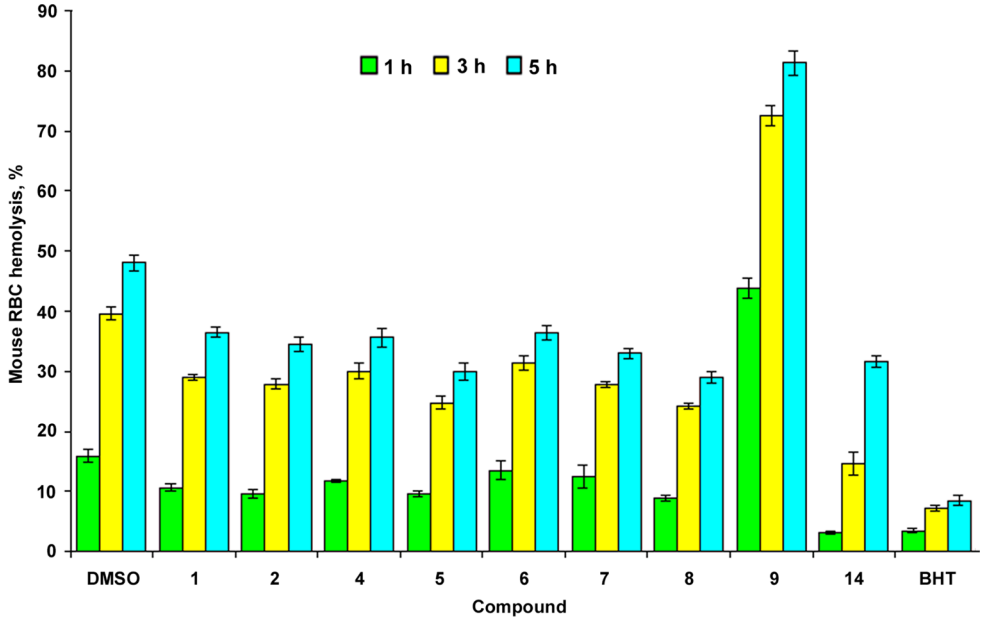

Figure 5. Effect of allylpolyalkoxybenzenes **1**, **2**, **4**–**9**, and **14** and BHT (0.01 mM) on mouse RBC hemolysis under H_2O_2-initiated oxidative stress. Vertical bars: means ± SE (n = 4–10).

oxidative demethylenation of the methylenedioxy fragment to yield catechol metabolites. Introduction of a hydroxy group into the benzene ring markedly enhanced the antioxidant effect, as exemplified by hydroxyapiol (**14**).

EXPERIMENTAL SECTION

General Experimental Procedures. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-500 instrument [working frequencies of 500.13 MHz (^{1}H) and 125.76 MHz (^{13}C)]. Chemical shifts were stated in parts per million (ppm) and referenced to the appropriate NMR solvent peaks. Spin–spin coupling constants (J) were reported in hertz (Hz). Elemental analysis was performed on an automated PerkinElmer 2400 CHN microanalyzer. Flash chromatography was carried out on silica gel (Acros, 0.035–0.070 mm, 60 Å). TLC was performed on Merck 60 F_{254} plates. Solvents and allyl bromide, allylbenzene (**1**), estragole (**2**), and safrole (**4**) at the highest commercial quality were purchased from Acros Organics (Belgium) and used as received. 4,7-Dimethoxy-1,3-benzodioxol-5-ol (**12**) was synthesized according to a published procedure.[38] Ozonolysis was conducted using a custom-designed apparatus (Science and Technology Park, St. Petersburg State Polytechnic University, Russia) equipped with an IR detector of O_3 concentration (Japan) and an automated shut-down circuit. The device allowed for the controlled generation of ozone, with a maximal capacity of 10 g of O_3 per hour from O_2.

Isolation of Plant Allylpolyalkoxybenzenes 5–8. Liquid CO_2 extraction of parsley and dill seeds was carried out by the company Karavan Ltd. (Krasnodar, Russia).[14,37] Allylpolyalkoxybenzenes **5**–**8** with 98–99% purity were obtained by high-efficiency distillation using a pilot plant device at N.D. Zelinsky Institute of Organic Chemistry RAS (Moscow, Russia). The seed essential oils of parsley varieties cultivated in Russia contained 40–46% myristicin (**5**) (var. Astra),

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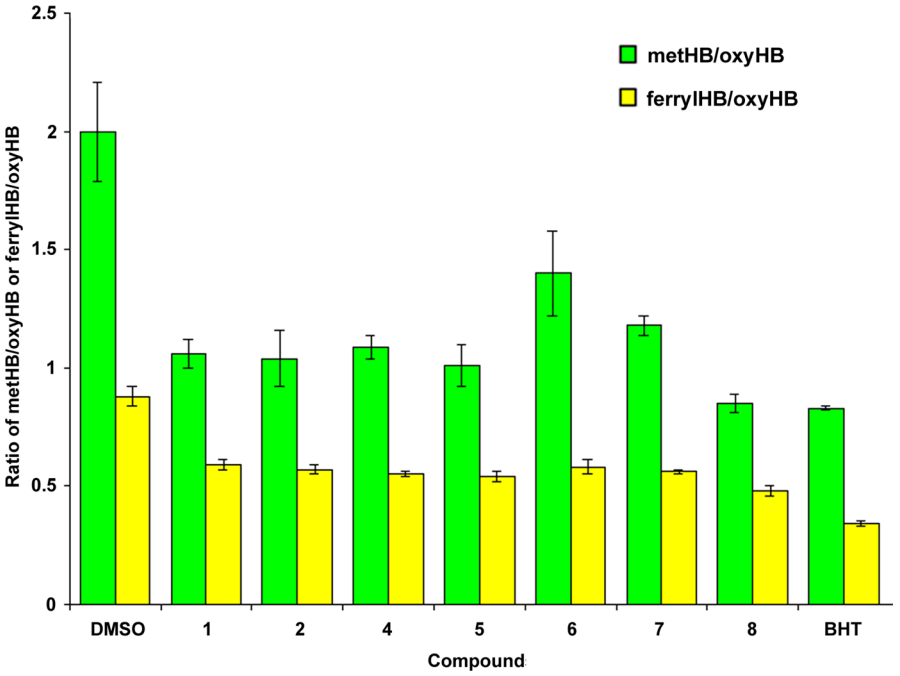

Figure 6. Effect of allylpolyalkoxybenzenes **1**, **2**, and **4**–**8** and BHT (0.01 mM) on methemoglobin (metHB) and ferrylhemoglobin (ferrylHB) formation in mouse RBCs under H_2O_2-induced oxidative stress after a 5 h incubation. Vertical bars: means ± SE (n = 4–10).

21% allyltetramethoxybenzene (**6**) (var. Slavyanovskaya), and 70–75% apiol (**7**) (var. Sakharnaya). Indian dill seeds were purchased from Vremya & Co. (St. Petersburg, Russia). The dill seed essential oil contained 30–33% dillapiol (**8**).

Synthesis of Isoapiol (10). A suspension of apiol (**7**) (666.6 g, 3 mol) and finely powdered KOH (118 g, 2.1 mol) was heated for 2.5 h at 85–95 °C in a rotary-film evaporator. The product was distilled from the evaporator at 136–138 °C/1–2 mmHg to afford pure isoapiol (**10**) as a mixture of *trans*- (ca. 80–85%) and *cis*-isomers (15–20%).

Synthesis of 2,5-Dimethoxy-3,4-methylenedioxybenzaldehyde (11). Pure O_3 (9 g, 0.188 mol) was bubbled through isoapiol (**10**) (30 g, 0.135 mol) in a mixture of $CHCl_3$ (120 mL)–MeOH (30 mL)–pyridine (9 mL) for 1 h at −20 to −8 °C. The resulting solution was concentrated in vacuo at 20 °C. The residue was treated with 150 mL of H_2O, and the pH of the slurry was adjusted to ca. 3 with HCl (18%). The resulting solid was filtered, crystallized from EtOH, and dried to afford the desired aldehyde **11**. Yield: 22.5 g (79%); white crystals, mp 99–101 °C (lit.[63] 102 °C).

Synthesis of 5-Allyloxy-4,7-dimethoxy-1,3-benzodioxol (13). A solution of 4,7-dimethoxy-1,3-benzodioxol-5-ol (**12**)[38] (279 mg, 1.41 mmol) and powdered NaOH (60 mg, 1.5 mmol) in dimethylformamide (DMF) (2 mL) was stirred for 20 min at room temperature, and then allyl bromide (188 mg, 1.55 mmol) was added. The mixture was stirred for 1 h, diluted with water (10 mL), neutralized with HCl, and extracted with CH_2Cl_2 (2 × 5 mL). The combined extracts were dried, and the solvent was removed to yield 261 mg (78%) of the product as a yellowish oil, which slowly solidified. ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.30 (1H, s, H-6), 6.02 (1H, m, =CH), 5.93 (2H, s, OCH_2O), 5.39 (1H, dm, J = 17.3 Hz, $=CH_2$) (*trans*), 5.25 (1H, dm, J = 10.5 Hz, $=CH_2$) (*cis*), 4.51 (2H, dm, OCH_2, J = 5.3 Hz), 3.77 (3H, s, OCH_3), 3.75 (3H, s, OCH_3); anal. C 60.83; H 5.80%; calcd for $C_{12}H_{14}O_5$, C 60.50; H 5.92%.

Synthesis of 6-Allyl-4,7-dimethoxy-1,3-benzodioxol-5-ol (14). Allyloxybenzene (**13**) (261 mg, 1.10 mmol) was dissolved in *N*,*N*-dimethylaniline (3 mL) and refluxed for 2.5 h. After cooling, the mixture was diluted with toluene (10 mL), washed with 20% HCl (2 × 5 mL), then with water (2 × 5 mL), dried, and evaporated. Flash chromatography of the residue (silica gel, eluent heptane–EtOAc, 1:3) afforded 206 mg (79%) of **14** as a brownish-yellow oil: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.45 (1H, s, OH), 5.89 (2H, s, OCH_2O), 5.83 (1H, m, =CH), 4.87–4.92 (2H, m, $=CH_2$), 3.78 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.20 (2H, m, CH_2); ^{13}C NMR (125.76 MHz, DMSO-d_6) δ 140.6, 136.9, 136.0, 131.4, 126.8, 116.6, 114.2, 110.6, 100.9, 60.2, 60.1, 27.7; anal. C 60.67; H 5.99%; calcd for $C_{12}H_{14}O_5$, C 60.50; H 5.92%.

Synthesis of 6-Allyl-4,5,7-trimethoxy-1,3-benzodioxol (Nothoapiol, 9). A solution of the allylphenol **14** (209 mg, 0.88 mmol) and powdered NaOH (36 mg, 0.90 mmol) in DMF (3 mL) was stirred for 20 min at room temperature, and then dimethyl sulfate (122 mg, 0.97 mmol) was added. The mixture was stirred for 2 h and diluted with water (10 mL), and then the pH was adjusted to 2.0 with HCl. The resulting mixture was extracted with CH_2Cl_2 (2 × 5 mL), the combined extracts were dried, and the solvent was removed. Flash chromatography of the residue (silica gel, eluent heptane–EtOAc, 1:3) afforded 115 mg (52%) of nothoapiol (**9**) as a yellowish oil: ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.97 (2H, s, OCH_2O), 5.85 (1H, m, =CH), 4.89–4.96 (2H, m, $=CH_2$), 3.83 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 3.65 (3H, s, OCH_3), 3.24 (2H, dt, J = 6.1, J = 1.6 Hz, CH_2). The TLC data and the ^{1}H and ^{13}C NMR spectra of synthesized nothoapiol (**9**) matched those of natural nothoapiol.[36]

Biology: Materials and Methods. Experiments were conducted using the equipment of the Center of Collective Usage "Molecular Biology", Institute of Biology, Komi Scientific Center, Ural Branch of RAS. Mice were obtained from the scientific collection of experimental animals at Institute of Biology, Komi Scientific Center, Ural Branch of RAS (http://www.ckp-rf.ru/usu/471933/) and were kept under bioethical conditions (Protocol No. 1, dated 24.01.2017, approved by Academic Council of the Institute of Biology of the Komi Scientific Center of the Ural Branch of the RAS). Absorption was measured using a Thermo Spectronic Genesys 20 instrument (USA). Absorption spectra of hemolysates were analyzed on a Fluorat-02-Panorama spectrofluorimeter (Russian Federation). Mouse RBCs were incubated in a thermostated Biosan ES-20 shaker (Latvia), with a 0.1% v/v DMSO–ethanol mixture (1:9) used as a negative control. BHT and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) served as reference compounds. Each experiment was repeated 4–10 times. Statistical analysis was conducted using Microsoft Office Excel 2007 software packages. Reagent grade and pure grade $FeSO_4$ (Reachim Ltd., Moscow, Russian Federation), 3% H_2O_2 (Lekker, St. Petersburg, Russian Federation), isopropanol (Vekton, Russian Federation), phosphate-

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buffered saline (PBS, pH 7.4) (Sigma-Aldrich, USA), DPPH (Alfa Aesar, USA), trichloroacetic acid (Sigma-Aldrich, Germany), thiobarbituric acid (Alfa Aesar, UK), BHT (Alfa Aesar, UK), trolox (Sigma-Aldrich, Germany), and ascorbic acid (ICN Biomedicals, USA) were used.

Free-Radical-Scavenging Capacity (RSC). DPPH Test. Stock solutions of compounds **1**, **2**, **4**–**9**, and **14** were prepared in DMSO at 10 mM concentration. Compounds **1**, **2**, **4**–**9**, and **14** at final concentrations of 0.1 and 1 mM were added to a 0.0015% DPPH solution in MeOH (1% v/v). Then, each mixture was shaken vigorously and kept in the dark at room temperature for 30 min. The absorption decrease was measured at λ 517 nm. RSC was calculated as a percentage of DPPH discoloration using the following equation: RSC, % = 100 × (1 − A_t/A_c), where A_t is the absorbance of the sample containing the test compound and A_c is the absorbance of the control sample with all reagents except for the compound.[64]

Antioxidant Activity. TBARS Assay. The antioxidant activity of allylpolyalkoxybenzenes **1**, **2**, **4**–**9**, and **14** was evaluated in vitro as inhibition of accumulation of secondary lipid peroxidation products in mouse brain homogenates.[65,66] The brain was homogenized in physiological saline (pH 7.4) (10% v/v) and centrifuged at 3000 rpm for 10 min. The low-speed supernatant containing water, proteins, DNA, RNA, and lipids (cholesterol, galactolipids, individual phospholipids, and gangliosides) was separated.[65,67] The test compounds were added to the supernatant at final concentrations of 0.1 and 1 mM (1% v/v); then in 30 min lipid autoxidation was initiated by adding a freshly prepared solution of $FeSO_4$ (final concentration 3 μM) and ascorbic acid (final concentration 300 μM) (1% v/v).[68,69] For cytochrome P450 inhibition, homogenates were preincubated with isopropanol (0.5% v/v, 30 min). Samples were stirred gently for 1 h at 37 °C, and then the reaction was stopped by adding 1 mL of trichloroacetic acid (20%), 1 mL of 2-thiobarbituric acid (0.7%), and 0.8 mL of H_2O to 0.2 mL of substrate. The reaction mixture was heated in a boiling water bath for 15 min. After cooling, the precipitate was removed by centrifugation (3000 rpm, 10 min). The concentration of secondary lipid peroxidation products reacting with TBARS was determined at λ 532 nm using the extinction coefficient of 1.56 × 10^5 $M^{-1}\cdot cm^{-1}$.[66,70,71]

Mouse RBC Test for Toxicity and Antioxidant and Membrane-Protective Activity. The toxicity and antioxidant and membrane-protective activities of allylpolyalkoxybenzenes **1**, **2**, **4**–**9**, and **14** were evaluated in 0.5% (v/v) suspension of mice RBCs in PBS (pH 7.4). Toxicity was assessed by RBC hemolysis after 5 h of incubation with the test compounds (0.1% v/v, final concentration 0.1 mM). Antioxidant and membrane-protective activities were determined by inhibition of H_2O_2-induced hemolysis, inhibition of lipid peroxidation products accumulation (TBARS assay), and oxidation of oxyhemoglobin in RBCs. After addition of the test compound solutions (0.1% v/v, 0.01 mM final concentration), the suspension of RBCs was incubated for 30 min, and then hemolysis was initiated by addition of H_2O_2 (2% v/v, final concentration 1.8 mM). The reaction mixture was shaken gently for 5 h at 37 °C. An aliquot was taken from the incubation medium each hour and centrifuged for 5 min at 3000 rpm (1600*g*). Hemolysis was determined as hemoglobin content in the supernatant at λ 524 nm.[72] The percentage of hemolysis was calculated relative to complete hemolysis of the sample, where it was triggered by addition of distilled water. The concentration of secondary lipid peroxidation products in the RBC hemolysate was assayed using the formation of malondialdehyde as an indicator as described above. The reaction was stopped by adding trichloroacetic and 2-thiobarbituric acids. Samples were heated in a boiling water bath for 15 min, followed by centrifugation for 10 min at 1600*g* to remove the precipitated protein. The adduct of 2-thiobarbituric acid with malondialdehyde in the supernatant was measured by the spectrophotometric method at λ 532 nm with an extinction coefficient of 1.56 × 10^5 $M^{-1}\cdot cm^{-1}$.[70,71] To assess the accumulation of hemoglobin oxidation products, the absorption spectrum of the hemolysate was analyzed at λ values of 540–640 nm. The content of oxyhemoglobin (oxyHb), methemoglobin (metHb), and ferrylhemoglobin (ferrylHB) was calculated using corresponding extinction coefficients and eqs 1–3.[73]

$$[\text{oxyHb}] = -472A_{560} + 536A_{577} - 104A_{630} \quad (1)$$

$$[\text{metHb}] = -784A_{560} + 430A_{577} + 1500A_{630} \quad (2)$$

$$[\text{ferrylHb}] = 976A_{560} - 536A_{577} - 476A_{630} \quad (3)$$

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.8b00878.

Content of allylpolyalkoxybenzenes **2** and **4**–**9** in plant EOs, antioxidant and membrane-protective properties of allylpolyalkoxybenzenes; Tables S1–S5 (PDF)

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Notes

The authors declare no competing financial interest.

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