ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2011, Vol. 37, No. 5, pp. 614–618. © Pleiades Publishing, Ltd., 2011.
Original Russian Text © E.V. Buravlev, I.Yu. Chukicheva, O.G. Shevchenko, K.Yu. Suponitsky, A.V. Kutchin, 2011, published in Bioorganicheskaya Khimiya, 2011, Vol. 37, No. 5, pp. 685–689.

Separation of Racemic *ortho*-Isobornylphenol into Enantiomers and Evaluation of Their Antioxidant Activity

E. V. Buravlev[a, 1], I. Yu. Chukicheva[a], O. G. Shevchenko[b], K. Yu. Suponitsky[c], and A. V. Kutchin[a]

[a] *Institute of Chemistry, Komi Research Center, Ural Branch, Russian Academy of Sciences, ul. Pervomaiskaya 48, Syktyvkar, GSP-2, 167982 Russia*

[b] *Institute of Biology, Komi Research Center, Ural Branch, Russian Academy of Sciences, Syktyvkar, 167000 Russia*

[c] *Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, ul. Vavilova 28, Moscow, GSP-1, 119991 Russia*

Received February 9, 2011; in final form March 24, 2011

Abstract—Racemic *ortho*-isobornylphenol was separated into enantiomers through diastereomeric camphanates. The absolute configuration of chiral centers of the isolated products was determined by X-ray studies. Antioxidant activity and membrane-protective properties of the enantiomers were studied on the model of H_2O_2-induced hemolysis of erythrocytes.

Keywords: ortho-isobornylphenol, enantiomers, diastereomers, antioxidant activity, membrane-protective properties

DOI: 10.1134/S1068162011050049

INTRODUCTION

The biological activity of chiral phenols (for example, α-tocopherol [1], *para*-nonylphenols [2], and Δ^8-tetrahydrocannabinols [3]) is known to be determined by the configuration of their chiral centers. Isobornylphenols have a chiral alkyl fragment in their structure and are analogues of natural phenol antioxidants. In this connection, we pioneered the separation of racemic *ortho*-isobornylphenol (**I**) into enantiomers and the evaluation of their antioxidant properties.

RESULTS AND DISCUSSION

The separation method of racemic compounds through their diastereomeric derivatives remains important for modern organic chemistry. For example, several racemic phenol compounds were separated via their camphanates [4, 5], camphor-10-sulfonates [6, 7], and amides [8]. In this study, we have separated the *rac*-(**I**) racemate through the corresponding diastereomeric esters (**II**) and (**III**), which were prepared with the use of (1*S*)-camphanic acid chloride. The mixture of camphanates (**II**) and (**III**) was fractionated by the column chromatography on silica gel. These compounds were hydrolyzed, and isomers (+)-(**I**) and (–)-(**I**) were obtained with >95% purity. The diastereomeric purity of derivatives (**II**) and (**III**) was determined by ^{1}H NMR spectroscopy. The enantiomeric purity of the separated phenols was evaluated by HPLC (scheme).

The absolute configuration of compound (**II**) was determined on the basis of configuration of the starting (1*S*)-camphanic acid chloride and data on the relative configuration, which was evaluated by X-ray diffraction analysis (Fig. 1). The (1*R*,2*S*,4*S*) configuration was attributed to the chiral centers of the isobornyl fragment of camphanate (**II**) and phenol (+)-(**I**) that was prepared from it. Hence, camphanate (**III**) and phenol (–)-(**I**) had the opposite (1*S*,2*R*,4*R*) configuration.

The structure of the terpene fragment of compound (**II**) in a crystal is analogous to that of the aminomethyl derivatives of *ortho*-isobornylphenols that we observed previously [9]. The value of the C13–C12–C17–C22 torsion angle was –19.8(3) deg, and the fragment of the ester group was almost perpendicular to a plane of the aromatic ring [85.32(11) deg]. Thus, the molecular structure of compound (**II**) was mainly determined by intramolecular rather then intermolecular interactions.

Abbreviations: AP-TBA, active products of 2-thiobarbituric acid; BHT, butylhydroxytoluene or 2,6-di-*tert*-butyl-4-methylphenol (phenolic antioxidant); DMAP, 4-dimethylaminopyridine; ferrylHb, ferrylhemoglobin; LPO, lipid peroxidation; metHb, methemoglobin; oxyHb, oxyhemoglobin; PBS, phosphate buffered saline.

[1] Corresponding author: phone: (8212)21-99-16; fax: (8212)21-84-77; e-mail: buravlev-ev@chemi.komisc.ru.

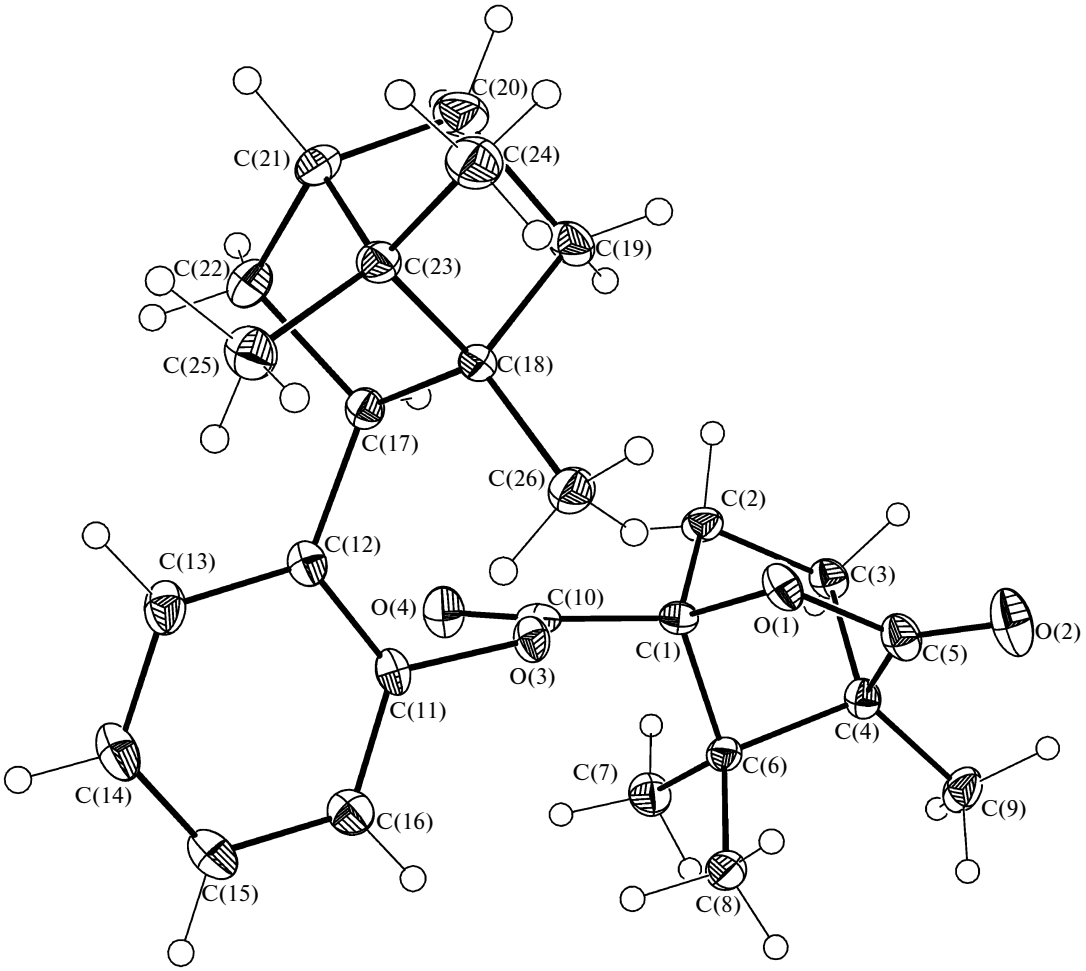
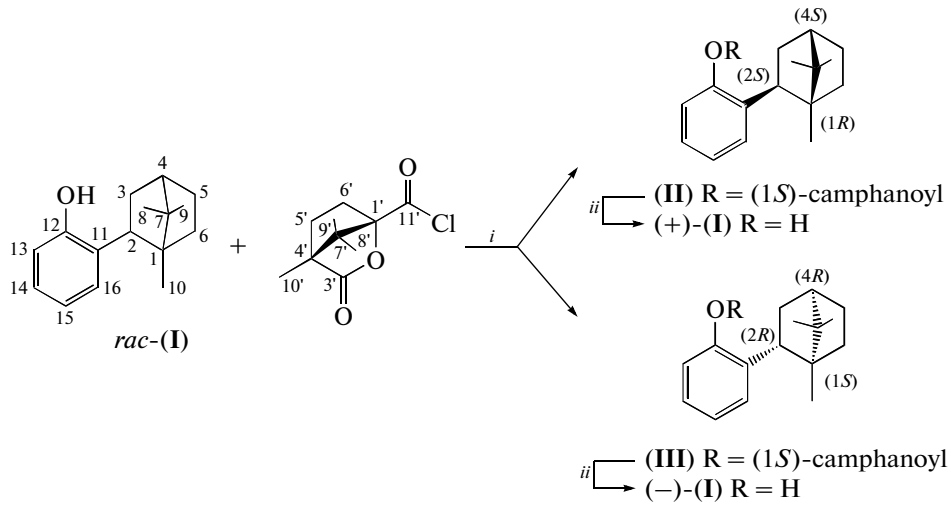


Fig. 1. General view of molecule (**II**) where the atoms are presented as thermal ellipsoids of atomic displacements with 50% probability.



Scheme. The reaction conditions: (*i*) Triethylamine, DMAP, PhMe, reflux 5 h, chromatography on silica gel; (*ii*) KOH/tetrahydrofuran, room temperature, 15 h.

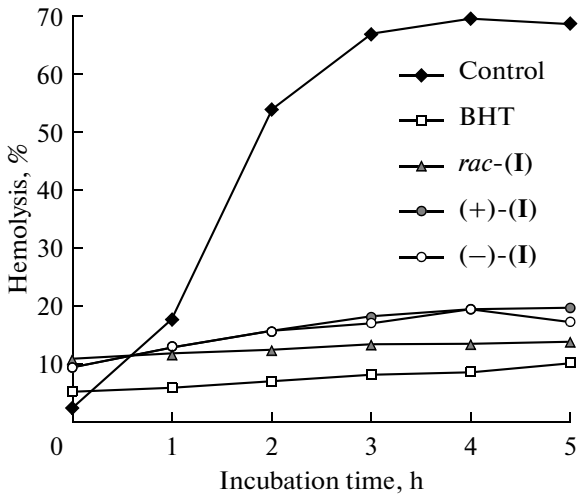


Fig. 2. Inhibition of the H_2O_2-induced hemolysis of erythrocytes by *rac*-(**I**), (+)-(**I**), and (−)-(**I**) (50 μM) in comparison with BHT. Every point is an average value of 2–4 repetitions.

The antioxidant activity and membrane-protective properties were determined for the different forms of *ortho*-isobornylphenol (**I**). The method of the induced erythrocyte hemolysis was a suitable model for studies of these properties in vitro [10–12]. Both racemic phenol *rac*-(**I**) and its enantiomeric phenols (+)-(**I**) and (−)-(**I**) were shown to exhibit a pronounced hemolytic effect in the concentration of 100 μM and have no membrane-protective properties under the H_2O_2-induced oxidative stress. Earlier, hemolytic activity was observed for high concentrations of new hybrid spatially hindered phenols, ichfanes [13]. Compounds *rac*-(**I**), (+)-(**I**), and (−)-(**I**) effectively inhibited the H_2O_2-induced hemolysis of erythrocytes in the concentration of 50 μM (Fig. 2). Analysis of the content of the secondary products of LPO 4 h after the incubation with the examined substances demonstrated the significant decrease in the AP-TBA content in the erythrocyte suspension.

An inhibiting effect of the tested compounds on the H_2O_2-induced oxidation of oxyHb, metHb, and ferrylHb was found. The antioxidant and membrane-protective activities of *ortho*-isobornylphenol and its enantiomers in the concentration of 50 μM were comparable to those of BHT (the comparative substance) (table). We found no differences in the biological activity of enantiomers (+)-(**I**) and (−)-(**I**) under these experimental conditions.

EXPERIMENTAL

The ^{1}H NMR and ^{13}C NMR spectra (δ, ppm; *J*, Hz) were recorded on a Bruker Avance II 300 spectrometer (Germany) in $CDCl_3$ at a working frequency of 300 and 75.5 MHz, respectively. Chloroform was used as an internal standard. The IR spectra were recorded on a Shimadzu IR Prestige 21 Fourier spectrometer (Japan) in tables with KBr. The specific rotation was measured on a Kruss Optronic P3002RS polarimeter (Germany). Melting points were determined on a Koffler table. We previously synthesized the racemic *ortho*-isobornylphenol, *rac*-(**I**) [14]. Chloride of (1*S*)-camphanic acid, triethylamine, and 4-dimethylaminopyridine (Alfa Aesar, Great Britain and Sigma-Aldrich, United States) were used for the syntheses of derivatives (**II**) and (**III**). The reactions were monitored by TLC on Sorbfil plates. The substances were detected by the treatment of the plates with the solution of Bromocresol Purple. Silica gel 60 (70/230 μ, Alfa Aesar) was used for the column chromatography.

Synthesis of derivatives (II) and (III). Phenol *rac*-(**I**) (1.16 g, 5.0 mmol), chloride of (1*S*)-camphanic acid (1.19 g, 5.5 mmol), triethylamine (0.77 ml, 5.5 mmol), and DMAP (0.06 g, 0.5 mmol) were refluxed in toluene (50 ml) upon stirring in the argon atmosphere for 5 h. The reaction mixture was cooled to room temperature, and the quaternary ammonium salt was filtered off. The residue was evaporated and fractionated on a column with silica gel (PhH was used as an eluent). Compounds (**II**) and (**III**) were obtained with yields of 0.47 g (23%) and 0.64 g (31%) and diastereomeric purity of >98% and >95%, respectively.

Parameters of H_2O_2-induced hemolysis of erythrocytes and their changes after the action of *rac*-(**I**), (+)-(**I**), and (−)-(**I**) in the concentration of 50 μM in comparison with BHT

Variation	Parameters		
	metHb/oxyHb (5 h)	ferrylHb/oxyHb (5 h)	AP-TBA, nmol/ml (4 h)
Control	0.91 ± 0.09	0.32 ± 0.01	2.95 ± 0.10
BHT	0.305	0.238	1.93 ± 0.07
rac-(**I**)	0.25 ± 0.04	0.20 ± 0.01	1.87 ± 0.08
(+)-(**I**)	0.48 ± 0.13	0.23 ± 0.02	1.73 ± 0.06
(−)-(**I**)	0.33 ± 0.03	0.21 ± 0.001	1.81 ± 0.12

2-((1*R*,2*S*,4*S*)-1,7,7-Trimethylbicyclo[2.2.1]hept-2-yl)phenyl(1*S*,4*R*)-4,7,7-trimethyl-3-oxo-2-oxabicyclo [2.2.1]heptane-1-carboxylate (II) was prepared as colorless crystals; mp. 127–128°C. R_f 0.28 (Sorbfil, benzene); $[\alpha]_D^{22}$ –6.3 (*c* 0.39, $CHCl_3$); IR (KBr, ν, cm^{-1}): 2953, 2878 (CH_2, Me), 1792, 1748 (C=O), 1260 (=C–O–C), 1094, 1053 (C–O–C); ^{1}H NMR: 0.81 (3 H, s, H10), 0.85 (3 H, s, H9), 0.90 (3 H, s, H8), 1.15 (6 H, s, H8', H9'), 1.17 (3 H, s, H10'), 1.24–1.46 (2 H, m, H3, H4), 1.59–1.70 (1 H, m, H5), 1.73–1.90 (3 H, m, 2H6 + H5'), 1.95–2.04 (1 H, m, H5'), 2.11–2.26 (2 H, m, H3, H6'), 2.52–2.62 (1 H, m, H6'), 2.94 (1 H, t, *J* 8.8, H2), 6.99–7.05, 7.17–7.25, and 7.45–7.50 (each of them 1 H, m, H13, H14, H15, H16); ^{13}C NMR: 9.71 (C10'), 12.69 (C10), 16.76, 16.95 (C8', C9'), 20.61 (C9), 21.37 (C8), 27.36 (C5), 28.91, 31.06 (C5', C6'), 34.51 (C3), 40.19 (C6), 45.55 (C2), 46.16 (C4), 47.97 (C7), 50.09 (C1), 54.60, 54.90 (C4', C7'), 90.67 (C1'), 121.72, 125.89, 126.42, 128.91 (C13, C14, C15, C16), 135.55, 149.69 (C11, C12), 166.32, 177.94 (C3', C8').

Found, (%): C 76.06 and H 8.35 for $C_{26}H_{34}O_4$ (*M* 410.55). Calcd., (%): C 76.11 and H 8.30.

2-((1*S*,2*R*,4*R*)-1,7,7-Trimethylbicyclo[2.2.1]hept-2-yl)phenyl(1*S*,4*R*)-4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carboxylate (III) was prepared as colorless crystals; mp 123–124°C. R_f 0.25 (Sorbfil, PhH); $[\alpha]_D^{22}$ –3.1 (*c* 0.49, $CHCl_3$); IR (KBr, ν, cm^{-1}): 2953, 2878 (CH_2, Me), 1792, 1767 (C=O), 1261 (=C–O–C), 1105, 1047 (C–O–C); ^{1}H NMR: 0.76 (3 H, s, H10), 0.85 (3 H, s, H9), 0.91 (3 H, s, H8), 1.12 and 1.17 (both 3 H, s, H8', H9'), 1.20 (3 H, s, H10'), 1.25–1.46 (2 H, m, H3, H4), 1.57–1.67 (1 H, m, H5), 1.75–1.88 (3 H, m, 2H6 + H5'), 1.97–2.07 (1 H, m, H5'), 2.12-2.29 (2 H, m, H3, H6'), 2.56–2.65 (1 H, m, H6'), 2.99 (1 H, t, *J* 8.8, H2), 6.99–7.05, 7.17–7.25, and 7.45–7.51 (1 H, all m, H13, H14, H15, H16); ^{13}C NMR: 9.69 (C10'), 12.81 (C10), 16.98, 17.01 (C8', C9'), 20.55 (C9), 21.45 (C8), 27.34 (C5), 29.10, 31.13 (C5', C6'), 34.47 (C3), 39.99 (C6), 45.54 (C2), 45.88 (C4), 48.00 (C7), 50.03 (C1), 54.51, 54.87 (C4', C7'), 90.76 (C1'), 121.77, 125.94, 126.44, 129.04 (C13, C14, C15, C16), 135.49, 149.62 (C11, C12), 166.11, 177.74 (C3', C8').

Found, (%): C 76.17 and H 8.22 for $C_{26}H_{34}O_4$ (*M* 410.55). Calcd., (%): C 76.11 and H 8.30.

Hydrolysis of the ester group of derivatives (II) and (III). Compounds (**II**) or (**III**) (0.3 g, 7.3 mmol) were dissolved in tetrahydrofuran (10 ml), 5 M aqueous solution of KOH (3 ml) was added, and the reaction mixture was stirred for 15 h at room temperature. The organic layer was separated, washed with the saturated solution of NaCl (2 × 10 ml), and dried over anhydrous Na_2SO_4. The solvent was evaporated at a reduced pressure. The residue was purified by column chromatography in the mixture of light petroleum and diethyl ether (35 : 1). The enantiomeric purity was determined by HPLC on a Chiralcel OD-H column eluted with the mixture of hexane and isopropanol (99 : 1) at a flow rate of 1 ml/min at λ 219 mn. Retention times of (+)-(**I**) and (–)-(**I**) were 17.3 and 19.5 min, respectively. The spectral characteristics of the enantiomers corresponded to those described in the literature for *rac*-(**I**) [14].

2-((1*R*,2*S*,4*S*)-1,7,7-Trimethylbicyclo[2.2.1]hept-2-yl)phenol(+)-(I) was obtained as colorless crystals with the yield of 0.16 g (95%); mp 97–98°C; the enantiomeric purity 98.5%; $[\alpha]_D^{23}$ +31.9 (*c* 0.55, $CHCl_3$).

2-((1*S*,2*R*,4*R*)-1,7,7-Trimethylbicyclo[2.2.1]hept-2-yl)phenol(–)-(I) was obtained as colorless crystals with the yield of 0.16 g (95%); mp 96–97°C; the enantiomeric purity 96.4%; $[\alpha]_D^{23}$ –32.1 (*c* 0.72, $CHCl_3$).

X-ray analysis of compound (II). Monocrystals of compound (**II**) suitable for an X-ray experiment were prepared by slow evaporation from a hexane solution. The crystals of compound (**II**), $C_{26}H_{34}O_4$ were rhombic at 100 K. Their space group was $P2_12_12_1$: a = 7.3272(10) Å, b = 10.1382(13) Å, c = 29.778(4) Å, V = 2212.0(5) Å^3, Z = 4, M = 410.53, d_{calc} = 1.233 g/cm^3, μ = 0.081 mm^{-1}. Experimental intensities of 13873 reflections were measured on a SMART APEX2 difractometer (λ(Mo-K_α) = 0.71073 Å, graphitic monochromator, ω-scanning, 2θ < 29°). The starting massive of measured intensities was processed using the SAINT and SADABS programs from the APEX2 program software [15]. After averaging the equivalent reflections, 3332 independent reflections (R_{int} = 0.0656) was obtained. They were used for deciphering and refinement. The structure was resolved by a direct method. All the nonhydrogen atoms were localized in differential syntheses of the electron density and refined by F^2_{hkl} in anisotropic approximation. All the hydrogen atoms were placed in geometrically calculated positions and took into account during refinement in the "riding" model [U_{iso}(H) = nU_{eq}(C), where n = 1.5 for carbon atoms of methyl groups and n = 1.2 for the rest of carbon atoms that are bound to the corresponding hydrogen atoms]. The final values of invalidation factors were R_1 = 0.0415 [it was calculated from F_{hkl} for 2913 reflections with $I > 2\sigma(I)$], wR_2 = 0.1284 (it was calculated from F^2_{hkl} for all the reflections), and GOF = 1.059. All the calculations were performed using the SHELXL-97 program complex [16]. coordinates of the atoms and the temperature factors are deponed in the Cambridge bank of structural data, no. 827025.

Investigation of the antioxidant and membrane-protective properties. The 1% suspension of mouse erythrocytes in PBS (pH 7.4)[2] was used in the experiments.

[2] The phosphate-salt buffer was prepared by dissolution of one pellet of PBS (Sigma-Aldrich, United States) in 200 ml of deionized water. The resulting solution contained 0.01 M phosphate buffer, 0.0027 M KCl, and 0.137 M NaCl (pH 7.4) at 25°C.

The solutions of compound (**I**) in ethanol were added to the erythrocyte suspension and incubated for 10 min at 37°C. The control samples contained no examined compounds. The alcohol solution of BHT (antioxidant, 2,6-di-*tert*-butyl-4-methylphenol) was used as a comparative compound. The amount of ethanol that was added to the erythrocyte suspension was no higher than 0.1% of the total volume of the incubation mixture. Hemolysis was initiated by the solution of hydrogen peroxide. The reaction mixture was incubated at slow stirring at 37°C. Aliquots were systematically taken and centrifuged at 3000 rpm for 5 min. The degree of hemolysis was determined on a spectrophotometer at λ 541 nm according to the method [10]. Hemolysis was calculated from the optical density of the supernatant (A) relative to the complete hemolysis of the sample (B). The percentage of hemolysis was calculated by the formula A/B × 100. The content of the secondary products of LPO that reacted with 2-thiobarbituric acid was determined on a spectrophotometer [17]. Accumulation of the products of the hemoglobin oxidation was evaluated by analysis of the absorption spectrum in the interval of wave lengths from 500 to 710 nm. The content of various hemoglobin forms (oxyHb, metHb, and ferrylHb) was calculated subject to the corresponding extinction coefficients [18].

ACKNOWLEDGMENTS

This study was supported by the Russian Foundation for Basic Research, project no. 10-03-01129-a.

REFERENCES

1. Burton, G.W., Traber, M.G., Acuff, R.V., Walters, D.N., Kayden, H., Hughes, L., and Ingold, K.U., *Am. J. Clin. Nutr.*, 1998, vol. 67, pp. 669–684.
2. Saito, H., Uchiyama, T., Makino, M., Katase, T., Fujimoto, Y., and Hashizume, D., *J. Health Sci.*, 2007, vol. 53, pp. 177–184.
3. Lu, D., Guo, J., Duclos, Jr.R.I., Bowman, A.L., and Makriyannis, A., *J. Med. Chem.*, 2008, vol. 51, pp. 6393–6399.
4. Jiang, B. and Zhao, X.-L., *Tetrahedron: Asymmetry*, 2004, vol. 15, pp. 1141–1143.
5. Zhuravsky, R., Starikova, Z., Vorontsov, E., and Rozenberg, V., *Tetrahedron: Asymmetry,* 2008, vol. 19, pp. 216–222.
6. Chow, H.-F., Wan, C.-W., and Ng, M.-K., *J. Org. Chem.*, 1996, vol. 61, pp. 8712–8714.
7. Buckley, B.R., Bulman, Page P.C., Chan, Y., Heaney, H., Klaes, M., McIldowie, M.J., KcKee, V., Mattay, J., Mocerino, M., Moreno, E., Skelton, B.W., and White, A.H., *Eur. J. Org. Chem.*, 2006, pp. 5135–5151.
8. Nikiforov, G.A., Nabiev, O.G., Chervin, I.I., and Kostyanovsky, R.G., *Mendeleev Commun.*, 2010, vol. 20, pp. 291–292.
9. Buravlev, E.V., Chukicheva, I.Yu., Suponitskii, K.Yu., and Kuchin, A.V., *Russ. J. Gen. Chem.*, 2008, vol. 78, pp. 1411–1417.
10. Ajila, C.M. and Prasada, RaoU.J.S., *Food Chem. Toxicol.*, 2008, vol. 46, pp. 303–309.
11. Tabart, J., Kevers, C., Pincemail, J., Defraigne, J.-O., and Dommes, J., *Food Chem.*, 2009, vol. 113, pp. 1226–1233.
12. Takebayashi, J., Chen, J., and Tai, A., in *Advanced Protocols in Oxidative Stress II, Methods in Molecular Biology,* Armstrong, D., Ed., New York: Humana Press, 2010, pp. 287–296.
13. Parshina, E.Yu., Gendel, L.Ya., and Rubin, A.B., *Biophysics*, 2009, vol. 54, pp. 706–708.
14. Chukicheva, I.Yu., Spirikhin, L.V., and Kuchin, A.V., *Russ. J. Org. Chem.*, 2008, vol. 44, pp. 62–66.
15. *APEX2 and SAINT*, Madison, WI, USA, 2005.
16. Sheldrick, G.M., *Acta Cristallogr. A*, 2008, vol. A64, pp. 112–122.
17. Asakawa, T. and Matsushita, S., *Lipids*, 1980, vol. 15, pp. 137–140.
18. van den Verg, J.J.M., Op den Kamp, J.A.F., Lubin, B.H., Roelofsen, B., and Kuypers, F.A., *Free Radical Biol. Med.*, 1992, vol. 12, pp. 487–498.