Chemistry of Natural Compounds, Vol. 48, No. 4, September, 2012 [Russian original No. 4, July–August, 2012]

MACROMOLECULAR ANTIOXIDANTS BASED ON POLYSACCHARIDES AND 2,6-DIISOBORNYL-4-METHYLPHENOL DERIVATIVES

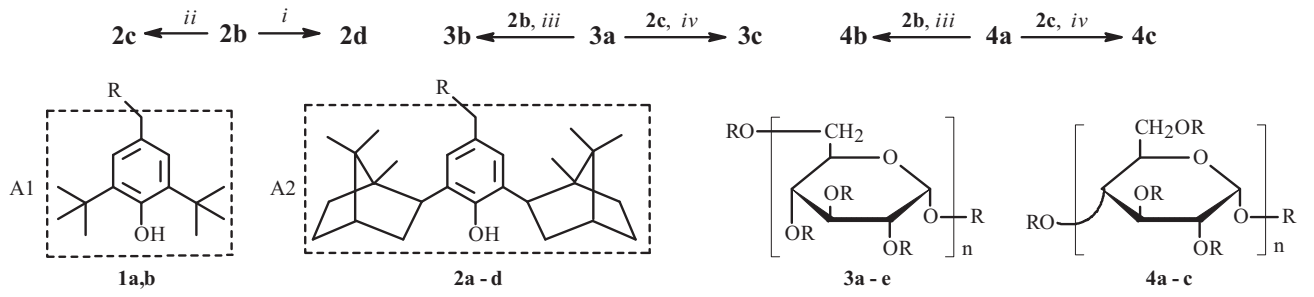
P. S. Vlasov,[1]* O. Yu. Sergeeva,[1] N. S. Domnina,[1] I. Yu. Chukicheva,[2] E. V. Buravlev,[2] and A. V. Kuchin[2]

UDC 547.562.1+547.458.61+542.9 43′78

*New macromolecular antioxidants that were conjugates of dextran or hydroxyethylated starch with functionalized derivatives synthesized from 2,6-diisobornyl-4-methylphenol were prepared. Their antiradical activity compared with derivatives of 2,6-di-*tert*-butyl-4-methylphenol was studied in a model reaction with 2,2-diphenyl-1-picrylhydrazyl. The studied conjugates exhibited greater activity than sterically hindered phenols not bonded to a polymer chain. The synthesized isobornyl derivatives were more active than previously studied* tert*-butyl analogs.*

Keywords: 2,6-diisobornyl-4-methylphenol, terpenoids, dextran, hydroxyethylated starch, antioxidants, 2,2-diphenyl-1-picrylhydrazyl.

In addition to the well studied derivatives of 2,6-di-*tert*-butylphenols (**1a** and **1b**) [1], the sterically hindered phenol 2,6-diisobornyl-4-methylphenol (**2a**) is also a promising antioxidant. Synthetic methods for preparing derivatives of isobornylphenols containing aminomethyl and amidomethyl groups [2, 3] and heterocyclic substituents [4] have now been developed. The structural similarity of the isobornyl substituents to natural terpenoids is responsible for the variety of biological activity of these compounds. Isobornylphenols and their derivatives typically exhibit hemorheological and anti-aggregant effects [5, 6] and anti-inflammatory activity [7, 8]. Insufficient solubility of isobornylphenols in aqueous media limits their broad use in medical practice. A possible method for increasing the water-solubility of this class of compounds is the synthesis of conjugates with hydrophilic polymers, e.g., natural and semisynthetic polysaccharides [9].



1a: R = H, **1b:** R = CH_2COOH; **2a:** R = H, **2b:** R = Br, **2c:** R = SCH_2CH_2COOH, **2d:** R = OMe; **3a:** R = H, **3b:** R = H or A2, **3c:** R = H or $C(O)CH_2CH_2SA2$, **3d:** R = H or A1, **3e:** R = H or $C(O)CH_2A1$; **4a:** R = H or CH_2CH_2OH, **4b:** R = H, or CH_2CH_2OH, or CH_2CH_2OA2; **4c:** R = H, or CH_2CH_2OH, or $CH_2CH_2OC(O)CH_2CH_2SA2$

i. MeOH/MeCN (2:1, v/v), 60°C; *ii*. $HSCH_2CH_2COOH$ (2 equiv.), CH_2Cl_2, 35°C; *iii*. DMSO or DMSO/MeCN (2:1, v/v), 35°C; *iv*. DCC, DMAP, DMSO, 20°C

1) Chemistry Department, St. Petersburg State University, 198504, St. Petersburg, Universitetskii Pr., 26, Russia, fax: +7(812) 428 67 33, e-mail: petr_vlasov@mail.ru; 2) Institute of Chemistry, Komi Scientific Center, Ural Branch, Russian Academy of Sciences, 167982, Syktyvkar, Ul. Pervomaiskaya, 48, fax: +7(8212) 21 84 77, e-mail: kutchin-av@chemi.komisc.ru.
Translated from *Khimiya Prirodnykh Soedinenii*, No. 4, July–August, 2012, pp. 481–484. Original article submitted January 31, 2012.

TABLE 1. Polymer Modification Conditions and Conjugate Properties, χ-Content of Bonded Modifier Fragments, mass%

Expt. No.	Conjugate	Modifier × 100/polymer**		χ, mass%	Water solubility, 1 g/L
		reaction mixture	conjugate		
		DMSO			
1	**3b-1**	1	0.7	1.6	+
2*	**3b-2**	5	1.5	3.3	+
		DMSO + MeCN (2:1, v/v)			
3*	**3b-3**	5	1.1	2.5	+
4	**3b-4**	3	2.2	4.9	+
5	**3b-5**	9	4.4	9.3	–
6	**3b-6**	15	6.7	13.5	–
7	**3b-7**	21	7.8	15.7	–
8	**3b-8**	27	9.7	18.7	–
		DMSO			
9	**3c**	25	4.2	10.8	–
10	**4b-1**	1	0.5	1.1	+
		DMSO + MeCN (2:1, v/v)			
11	**4b-2**	1	0.6	1.4	+
12	**4b-3**	5	0.5	1.1	+
		DMSO			
13	**4c**	25	3.7	9.6	–

*Experimental temperature was 15°C. **Amount of modifier per 100 monomer units.

The goal of the present work was to modify chemically dextran (**3a**) and hydroxyethylated starch (**4a**) using functionalized derivatives of 2,6-diisobornylphenol. Two approaches were used to synthesize the conjugates. These were the reaction of polymer hydroxyls with 4-bromomethyl-2,6-diisobornylphenol (**2b**) and activated esterification via the reaction of a carboxylic acid (**2c**) in the presence of *N*,*N*′-dicyclohexylcarbodiimide. Such methods were used previously to prepare conjugates of dextran and derivatives of 2,6-di-*tert*-butylphenols (**1a** and **1b**) that showed rather high antiradical activity [10–12].

The schemes by which the conjugates were prepared are shown below. Table 1 presents the results for modification of dextran and hydroxyethylated starch.

The content of bonded fragments (χ, mass%) A1 and A2 in the conjugates was determined using absorption maxima at λ = 283–286 nm in electronic spectra that corresponded to π–π^* transitions of substituted phenols **2d** and **2c**. The purity of the conjugates was monitored using PMR spectroscopy.

Compound **2b** had limited solubility in dimethylsulfoxide (DMSO), in which the majority of dextran modification reactions are usually carried out [13]. Under the conditions of experiment No. 2* at 15°C, **2b** at the start of the reaction did not dissolve completely so only 31% modifier was added to the reaction mixture. The process occurred under homogeneous conditions upon addition of acetonitrile (MeCN) to the mixture. However, the degree of modification did not increase (experiment No. 3). Complete dissolution of the reagents even without the addition of MeCN and bonding of 70% of the modifier was observed upon reducing the amount of **2b** taken for the reaction and increasing the synthesis temperature to 35°C (experiment No. 1).

Syntheses in which the amount of modifier in the reaction mixture was varied over wide ranges (experiments Nos. 4–8) were performed in the mixed solvent at 35°C. Regardless of the amount of **2b** added to the reaction mixture, only half of the starting amount was bonded to the polymer. These results agreed with those obtained previously for the reaction of dextran with 4-bromomethyl-2,6-di-*tert*-butylphenol [14]. The conditions of experiment No. 4 were optimal. For these, 74% of the used modifier was bonded to the polymer. Nevertheless, increasing the concentration of **2b** enabled conjugates containing >14 mass% bonded fragments to be prepared.

However, conjugates based on dextran that contained >5 mass% modifier fragments were no longer soluble in water. Dextran-based conjugates **3d** with modifier content ~12 mass% that were prepared by us earlier using 4-bromomethyl-2,6-di-*tert*-butylphenol were water-soluble [10]. Thus, conjugates with isobornyl fragments had higher hydrophobicity than the *tert*-butyl analogs.

Modification of hydroxyethylated starch by derivative **2b** under the conditions proposed for dextran occurred very poorly. Thus, 61% of the modifier was bonded at a polymer:modifier ratio of 100:1 (experiment No. 11). Increasing the concentration of **2b** by five times (experiment No. 12) did not increase the content of A2 fragments in the product.

The result was possibly related to side reactions of **2b** that occurred in the used solvent. NMR spectroscopy found that **2b** was unstable upon standing in DMSO and formed a mixture of products, one of which was 4-hydroxy-3,5-diisobornylbenzaldehyde. According to the literature, DMSO is the preferred solvent for chemical modification of polysaccharides despite the fact that it can act as an oxidant [13].

The low degree of modification of starch (experiments Nos. 11 and 12) provided the impetus to synthesize derivative **2c**, which was stable in DMSO. Furthermore, the presence of a S atom in **2c** could potentially increase the antiradical activity. Conjugates in which the contents of bonded fragments of **2c** were ~10 mass% regardless of the used polymer were obtained from experiments Nos. 9 and 13. As suggested, use of this method for hydroxyethylated starch produced a conjugate with a much higher content of bonded A2 fragments than if bromide **2b** was used.

Thus, use of 4-bromomethyl-2,6-diisobornylphenol (**2b**) and 3-(4-hydroxy-3,5-diisobornylbenzylthio)propionic acid (**2c**) produced conjugates based on dextran and hydroxyethylated starch. The solubility of the conjugates in aqueous dioxane enabled their antiradical activity against the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) to be determined [11]. The reaction rate constants (k, L/mol·s) of the free radical with low-molecular-weight antioxidants and the conjugates were used as criteria for assessing the activity. The results were compared with literature data [12, 14] for previously studied conjugates **3d** and **3e**, which contained *tert*-butyl substituents instead of isobornyl fragments. Thus, isobornyl derivatives **2c** (40.0 ± 1.5), **3b–4** (66.0 ± 2.5), and **3c** (48.0 ± 2) were more active than *tert*-butyl analogs **1b** (3.4 ± 0.2), **3d** (25 ± 2), and **3e** (12.5 ± 0.8). It was shown earlier that the reaction rate of DPPH with conjugates containing A1 fragments depended strongly on the medium [11], which was natural for reactions occurring by the sequential proton-loss electron-transfer (SPLET) mechanism [15]. Therefore, the question of the influence of alkyl substituents in the *ortho*-position of the aromatic ring of the bonded fragments (A1 and A2) on the activity of the conjugates in various solvents requires further study. Like for *tert*-butyl derivatives **1b** and **3e** [14], conjugate **3c** exhibited higher activity than the starting modifier **2c** (**1b**, **2c**) although the relative gain from bonding the antioxidant to the polymer chain was lower. Compound **2d** was poorly soluble in aqueous dioxane so that reliable values of the rate constant could not be obtained.

Thus, the antiradical activity of the conjugates exceeded that of the low-molecular-weight antioxidants. The comparison of the rate constants with those obtained earlier by the analogous method [10–14] for conjugates that were derivatives with *tert*-butyl substituents in the *ortho*--positions of the phenol ring indicated that the new conjugates obtained in the present work are promising.

EXPERIMENTAL

Materials and Methods. UV spectra were recorded in H_2O:dioxane (1:1, v/v) on a UV-1700 spectrophotometer (Shimadzu). The amount of added A2 fragments was calculated using the molar extinction of model compounds **2d** and **2c**. NMR spectra were recorded on a Bruker DPX-300 instrument. Antiradical activity of the conjugates was assessed from the rate constants (k) with DPPH in aqueous dioxane (1:1, v/v) at 20°C by the reported method [11]. Analytical data for **2c** and **2d** agreed with those calculated.

4-Bromomethyl-2,6-di(1,7,7-trimethylbicyclo[2.2.1]heptan-*exo*-2-yl)phenol (2b) [16] was synthesized by the literature method [17]. Dextran (MW 40,000, **3a**) and hydroxyethylated starch (MW 200,000, degree of hydroxyethylation 0.5, **4a**) were isolated by dialysis with subsequent lyophilization from the pharmacopoeic preparations Reopoliglyukin (Eskon, RF) and Infukol (Serum Werk, Germany), respectively.

MeCN was dried by distillation from P_2O_5; DMSO, by storage over 4-Å molecular sieves. 3-Mercaptopropionic acid was purified by vacuum fractionation. Other solvents were distilled before use. 4-Dimethylaminopyridine (DMAP, Fluka) and *N*,*N*′-dicyclohexylcarbodiimide (DCC, Aldrich) were used without additional purification.

4-Methyloxymethyl-2,6-di(1,7,7-trimethylbicyclo[2.2.1]heptan-*exo*-2-yl)phenol (2d). Compound **2b** (150 mg, 326 μmol) was dissolved in MeCN (2 mL), treated with MeOH (4 mL), heated under Ar at 60°C for 4 h, poured into H_2O, and extracted with CH_2Cl_2. The organic phase was washed with soda solution (3%, 3×) and H_2O (3×) and dried over Na_2SO_4 for 24 h. The solvent was removed in vacuo to afford a yellow oil (126 mg), crystallization of which from MeOH gave the product (105 mg). Yield 79%, mp 158–161°C, $C_{28}H_{42}O_2$. UV spectrum (dioxane:H_2O, 1:1 v/v, λ_{max}, nm, ε): 283 (2300 L/mol·cm).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.81 (6H, s, CH_3), 0.84 (6H, s, CH_3), 0.90 (6H, s, CH_3), 1.40 (4H, m, CH_2), 1.63 (4H, m, CH_2), 1.86 (4H, m, CH_2), 2.28 (2H, m, CH), 3.00 (2H, t, J = 8.7, CH), 3.33 (3H, s, OCH_3), 4.37 (2H, s, CH_2O), 4.84 (1H, s, OH), 7.12 (2H, =CH). ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 12.53, 20.19, 21.43, 27.56, 33.98, 40.01, 45.44, 46.12, 48.19, 49.96, 57.64, 75.40, 125.47, 128.24, 128.37, 153.70.

3-(4-Hydroxy-3,5-di(1,7,7-trimethylbicyclo[2.2.1]heptan-*exo*-2-yl)benzylthio)propionic Acid (2c). A solution of **2b** (161 mg, 350 μmol) in anhydrous CH_2Cl_2 (2 mL) was treated with 3-mercaptopropionic acid (90 mg, 848 μmol) in CH_2Cl_2 (1 mL). The mixture was held at 35°C for 4 h, treated with CH_2Cl_2 (5 mL), washed with H_2O (5–10 mL, 6×), and dried over Na_2SO_4. The solvent was removed. Crystallization from MeOH afforded the product (126 mg) as colorless crystals. Yield 74%, mp 181–185°C, $C_{30}H_{44}O_3S$. UV spectrum (dioxane:H_2O, 1:1, v/v, λ_{max}, nm, ε): 286 (2300).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.80 (6H, s, CH_3), 0.84 (6H, s, CH_3), 0.89 (6H, s, CH_3), 1.40 (4H, m, CH_2), 1.63 (4H, m, CH_2), 1.88 (4H, m, CH_2), 2.25 (2H, m, CH), 2.50 (2H, m, C$\underline{H}_2$CH$_2$), 2.61 (2H, m, CH$_2$C$\underline{H}_2$), 2.99 (2H, t, J = 8.7, CH), 3.69 (2H, s, SC$\underline{H}_2$Ar), 4.82 (1H, br.s, OH), 7.09 (2H, =CH–), 9–12 (1H, COOH). ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 12.53, 20.13, 21.42, 25.46, 27.53, 33.98, 34.42, 36.73, 40.01, 45.40, 46.07, 48.16, 49.96, 126.04, 127.78, 128.62, 153.12, 178.00.

***O*-(4-Hydroxy-3,5-di(1,7,7-trimethylbicyclo[2.2.1]heptan-*exo*-2-yl)benzyl)-(1→6)-α-D-glucan (3b).** Dextran (162 mg, 1 mmol) was dissolved in DMSO (2 mL), treated with the required amount of **2b** in MeCN (1.5 mL), purged with Ar, and left for 4 h at 35°C. The product was isolated by precipitation from *i*-PrOH:$CHCl_3$ (3:1), separated by centrifugation, washed repeatedly with the precipitant, purified by reprecipitation from DMSO, and dried in vacuo at room temperature. Yield 60–80%.

***O*-(4-Hydroxy-3,5-di(1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl)benzyl)oxy)ethyl)-*O*-(2-hydroxyethyl)-(1→4)-α-D-glucan (4b)** was prepared analogously to **3b**. Yield 70%.

***O*-(4-Hydroxy-3,5-di(1,7,7-trimethylbicyclo[2.2.1]heptan-*exo*-yl)benzylthio)propionyl-(1→6)-α-D-glucan (3c).** Compound **2c** (112 mg, 230 μmol), DCC (48 mg, 115 μmol), and DMAP (28 mg, 23 μmol) were dissolved in DMSO (0.75 mL), stirred for 40 min on a magnetic stirrer at room temperature, and treated with dextran (150 mg, 920 μmol) with agitation by a stream of Ar. The precipitate of dicyclohexylurea was filtered off after 24 h. The product was isolated by precipitation in *i*-PrOH and reprecipitation from DMSO. Yield 140 mg (58%).

***O*-(4-Hydroxy-3,5-di(1,7,7-trimethylbicyclo[2.2.1]heptan-*exo*-2-yl)benzylthio)propionyloxy)ethyl)-*O*-(2-hydroxyethyl)-(1→4)-α-D-glucan (4c)** was prepared analogously to **3c**. Yield 63%.

REFERENCES

1. V. V. Ershov, G. A. Nikiforov, and A. A. Volod′kin, *Sterically Hindered Phenols* [in Russian], Khimiya, Moscow, 1972.
2. E. V. Buravlev, I. Yu. Chukicheva, K. Yu. Suponitskii, and A. V. Kuchin, *Zh. Obshch. Khim.*, 1177 (2008).
3. E. V. Buravlev, I. Yu. Chukicheva, and A. V. Kuchin, *Khim. Prir. Soedin.*, 31 (2010).
4. E. V. Buravlev, I. Yu. Chukicheva, and A. V. Kuchin, *Khim. Prir. Soedin.*, 367 (2008).
5. M. B. Plotnikov, V. I. Smol′yakova, I. S. Ivanov, A. V. Kuchin, I. Yu. Chukicheva, E. V. Buravlev, and E. A. Krasnov, *Khim.-farm. Zh.*, **44** (10), 9 (2010).
6. M. B. Plotnikov, E. A. Krasnov, V. I. Smol′yakova, I. S. Ivanov, A. V. Kuchin, I. Yu. Chukicheva, and E. V. Buravlev, RF Pat. No. 2,347,561 (2009); *Byull.*, No. 6 (2009).
7. I. Yu. Chukicheva, I. V. Fedorova, E. V. Buravlev, A. E. Lumpov, Yu. B. Vikharev, L. V. Anikina, V. V. Grishko, and A. V. Kuchin, *Khim. Prir. Soedin.*, 402 (2010).
8. E. V. Buravlev, I. Yu. Chukicheva, K. Yu. Suponitsky, Yu. B. Vikharev, V. V. Grishko, and A. V. Kutchin, *Lett. Org. Chem.*, **8**, 301 (2011).
9. E. F. Panarin and N. P. Kuznetsova, *Nauka v Rossii*, **2**, 18 (2003).
10. D. V. Arefjev, N. S. Domnina, E. A. Komarova, and A. Y. Bilibin, *Eur. Polym. J.*, **35**, 279 (1999).
11. D. V. Arefiev, N. S. Domnina, E. A. Komarova, and A. Y. Bilibin, *Eur. Polym. J.*, **36**, 857 (2000).
12. D. V. Aref′ev, "Synthesis of Covalent Conjugates of Dextran and Sterically Hindered Phenols and the Study of Their Antiradical Activity" [in Russian], Dissertation, St. Petersburg St. Univ., St. Petersburg, 2000.
13. T. Heinze, T. Liebert, B. Heublein, and S. Hornig, *Adv. Polym. Sci.*, **205**, 199 (2006).
14. D. V. Aref′ev, I. S. Belostotskaya, V. B. Bol′eva, N. S. Domnina, N. L. Komissarova, O. Yu. Sergeeva, and R. S. Khrustaleva, *Izv. Akad. Nauk, Ser. Khim.*, **56** (4), 751 (2007).
15. G. Litwinienko and K. U. Ingold, *Acc. Chem. Res.*, **40**, 222 (2007).
16. I. Yu. Chukicheva, E. V. Buravlev, and A. V. Kuchin, Appl. No. 2011127776/04, prior. Jul. 6, 2011.
17. G. M. Coppinger and T. W. Campbell, *J. Am. Chem. Soc.*, **75**, 734 (1953).