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ANTIOXIDANT AND CHOLESTEROL LOWERING PROPERTIES OF 2,6-DI-*t*-BUTYL-4-[(DIMETHYLPHENYLSILYL)METHYLOXY]PHENOL AND DERIVATIVES: A NEW CLASS OF ANTI-ATHEROGENIC COMPOUNDS.

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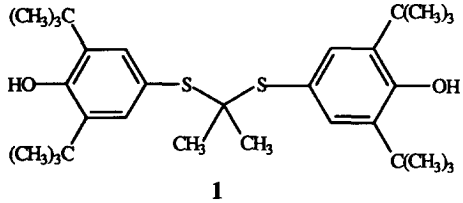
Abstract: Di-*t*-butylphenol derivatives were synthesized and evaluated as antioxidants and cholesterol lowering agents. When evaluated in cholesterol-fed rabbits, the compounds were found to exhibit both properties. Of special interest was the finding that several of the compounds elevated HDL cholesterol levels.

Introduction

Treatment and/or prevention of coronary heart disease by decreasing blood cholesterol levels through the use of drugs or diet is now an established therapy.[1a-c] The observation that oxidation of low density lipoprotein (LDL) is an important early step in atherosclerosis[2a,b] suggests the use of antioxidants in the prevention of atherogenesis.[3a-d] Probucol (**1**), an antioxidant which is useful as an antiatherogenic drug, has been shown to be a cholesterol lowering agent as well as an antioxidant.[4] Mao et al[5] have reported a probucol analogue without cholesterol lowering activity which demonstrates an effect on atherosclerosis in a rabbit progression model, suggesting that both activities contribute to the antiatherogenic activity of probucol. A property of probucol which impedes its clinical acceptance is lowering of high density lipoprotein (HDL) levels. Thus, the combination of antioxidant and cholesterol lowering activities would provide a more useful therapy if the effect on HDL could be circumvented. In this paper we report a series of di-*t*-butylphenol derivatives (**2**) with these properties. We demonstrate that in a rabbit model, several of these compounds significantly lower total cholesterol levels and increase HDL levels.



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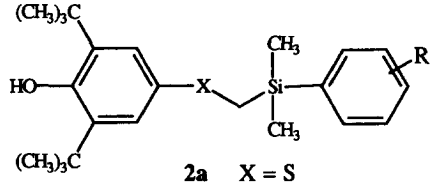
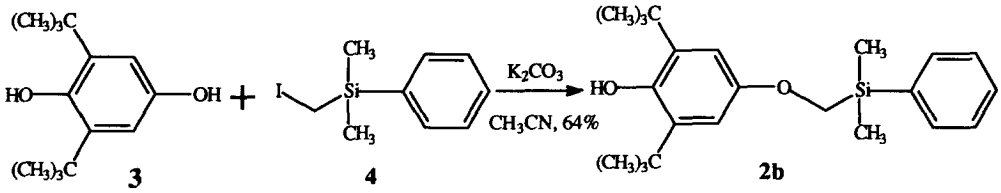


2a X = S
b-e X = O

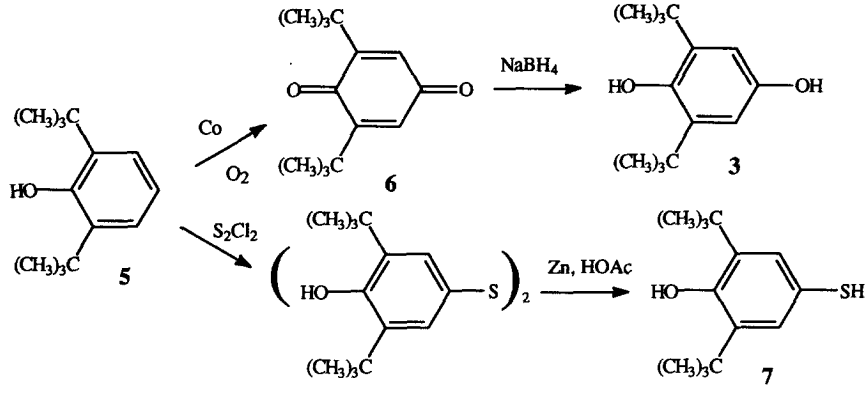
Chemistry

The compounds were prepared by treatment of 2,6-di-*t*-butylbenzhydroquinone (**3**) with a halomethylsilane (**4**) (Scheme 1). The benzhydroquinone is commercially available or can be prepared in two steps from 2,6-di-*t*-butylphenol by a cobalt-catalyzed oxidation of di-*t*-butylphenol to give the benzoquinone (**6**) followed by a borohydride reduction of the benzoquinone (Scheme 2).[6] The thiophenol (**7**) was prepared from di-*t*-butylphenol (5)

by treatment with sulfur monochloride, followed by reduction of the disulfide intermediate with zinc/acetic acid (Scheme 2).[7] The chloromethylsilanes were prepared by published procedures.[8]



Scheme 1



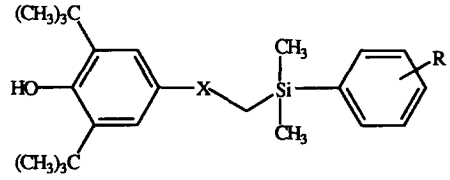
Scheme 2

Results and Discussion

The compounds in Table 1 were evaluated in a cholesterol fed rabbit model. New Zealand white rabbits were provided a diet which contained 0.2% cholesterol with or without 0.4% compound. After one week, serum total cholesterol levels were measured and are given as percent of control (Table 1). The cholesterol values were further differentiated into LDL and HDL cholesterol[9] and these values are also given as % control. The products formed on lipid oxidation, such as malonaldehyde, can be measured directly or indirectly to ascertain the antioxidant ability of test compounds. One of the indirect procedures used is to measure the products formed from lipid oxidation by incubation of the serum with thiobarbituric acid, which reacts with these substances. This measure of antioxidant activity, serum thiobarbituric acid reactive substances (TBARS)[10] is reported as per cent of control values, where values <100% indicate less lipid oxidation (antioxidant activity). In all cases, food intake was 100 - 102% of control. The data show that compounds within the series lower cholesterol levels up to 50% in this model. With compounds **2c** and **2d** an elevation of HDL cholesterol was also observed. This is of importance because the HDL/LDL ratio has been proposed as a clinically significant parameter in atherogenesis.[11] It should be noted that there was no correlation between antioxidant and cholesterol lowering activities; these activities involve

different mechanisms. All compounds were antioxidants in this test, as measured by inhibition of copper-induced TBARS .

Table 1 Structures and activity of di-*t*-Butylphenols[12], cholesterol fed rabbit[a]



			Cholesterol				
Cpd. #	X	R	Total, mg/dL	Total,%C	LDL,%C	HDL,%C	TBARS, %C
2a	S	H	119 ± 18	52	42	75	17
2b	O	H	165 ± 31	72	69	81	28
2c	O	4-OCH_3	153 ± 60	54	66	123	60
2d	O	2-OCH_3	235 ± 86	77	61	217	75
2e	O	4-Cl	247 ± 75	87	89	109	57

a)New Zealand White rabbits (female, 5 per group, ≈2.5 kg) were fed a control diet (100g/day) containing 0.2% cholesterol or 0.2% cholesterol + 0.4% drug. Initial cholesterol levels were in the range of 73± 10 mg/dL. After 7 days, rabbits were sacrificed by iv pentobarbital, plasma or serum was collected, cholesterol levels were determined by assay using a Cobas MIRAS autoanalyzer with Roche Diagnostic reagents and TBARS were calculated. Typical control levels for cholesterol were 325±43 mg/dL at the 7-day termination. Treated values are given as % control. Probucol in this test at 1% of diet gave total cholesterol, LDL and HDL values of 64, 62 and 58 %C respectively.

In summary, we report a novel series of di-*t*-butylphenol derivatives and show data from an animal model in which these compounds exhibit both antioxidant and cholesterol lowering activities. While the mechanism for the cholesterol lowering has not been definitively established, there was inhibition of cholesterol and bile acid absorption as evidenced by higher levels of both in the feces of treated rabbits. In addition, several of the compounds elevated HDL levels, which greatly improved the clinically relevant HDL/LDL ratio.

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10. 50 μL of rabbit serum was incubated with 450 μL of 4 mM $CuSO_4$ at 37 °C for 5 h. The reaction was stopped by addition of 25 μL of 10% EDTA in phosphate buffered saline of pH 7.4. TBARS was measured as described in Mao, S. J. T.; Yates, M. T.; Parker, R. A.; Chi, E. M.; Jackson, R. L. *Arteriosclerosis and Thromb.* **1991**, *11*, 1266.

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12. **2a**. A mixture of 2,6-di-*t*-butyl-4-mercaptophenol (2.4 g, 10 mmol), potassium carbonate (1.4g, 10 mmol) and chloromethyldimethylphenylsilane (1.9 g, 10 mmol) in DMF (50 mL) was stirred for 18 h at ambient temperature (argon). The mixture was diluted with water/ether, the ether layer was dried and evaporated. Unreacted starting materials were removed by kugelrohr (160 - 170 °C @0.1 mm Hg). The residue was chromatographed ($CCl_4/CHCl_3$, 1/1) to give **1** as a white waxy solid (2.3 g, 59%). Anal. calc. for $C_{23}H_{34}OSSi$: C, 71.44; H, 8.86; S, 8.29. Found: C, 71.14; H, 8.86; S, 7.98. **2b**. A mixture of 2,6-di-*t*-butylbenzhydroquinone (5.4 g, 24.4 mM), iodomethyldimethylphenylsilane (7.4 g, 26.8 mM) and potassium carbonate (3.7 g, 26.8 mM) in acetonitrile (125 mL) was heated at reflux (nitrogen atmosphere) for 18 h. The mixture was filtered and evaporated, the residue was redissolved in EtOAc (100 mL) and the solution was washed with water. The organic layer was isolated, dried and evaporated. The residue was kugelrohred to 135 °C @ 0.1 mM Hg to remove unreacted starting materials. Distillation of the residue at 155 - 165 °C @ 0.1 mM Hg gave an oil which crystallized on standing. Recrystallization from MeOH gave 5.8 g (64 %) of a white solid, mp 90 - 93 °C. Anal. calc. for $C_{23}H_{34}O_2Si$: C, 74.54; H, 9.25. Found: C, 74.51; H,9.28. **2c** mp 122 - 123 °C. Anal. calc. for $C_{24}H_{36}O_3Si$: C, 71.95; H, 9.06. Found: C, 71.80; H, 9.00. **2d** mp 89 - 90 °C. Anal. Calcd for $C_{24}H_{36}O_3Si$: C, 71.95; H, 9.06. Found: C, 71.84; H, 9.05. **2e** mp 102 - 105 °C. Anal. calc. for $C_{23}H_{33}ClO_2Si$: C, 68.20; H, 8.21; Found: C, 68.20; H, 8.13.

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