Phosphorylation of 3',4',5,7-Tetramethyldihydroquercetin

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Abstract—3',4',5,7-Tetramethyldihydroquercetin was phosphorylated with phosphorous acid derivatives, and similar results were obtained with phosphorochloridites and phosphoramidites. The P^{III} derivatives obtained were subjected to oxidation. The structures of the products were determined by NMR spectroscopy.

Dihydroquercetin is a widely occurring natural flavanoid, which is of large importance for medicine and food industry [1–5]. Molecules of this compound contain reactive carbonyl and hydroxy groups. However, the reactivity of dihydroguercetin has been studied inadequately. It was only shown that this compound can be efficiently methylated with dimethyl sulfate to form 3',4',5,7-tetramethyldihydroquercetin I [6]. Attempted complete methylation of the pentaol resulted in breakdown of the flavanoid system [7]. Recently, we initiated a study on phosphorylation of dihydroquercetin and obtained phosphites, which were formed by replacement of hydrogen atoms in the phenolic hydroxyls of the flavanoid [8]. In this work we examined the possibility and pathway of dihydroquercetin phosphorylation at the alcoholic hydroxy groups. As the starting compound we chose 3',4',5,7-tetramethyldihydroquercetin I.

The phosphorylation was performed using two different procedure: halophosphite [with 5,5-dimethyl-2-chloro-1,3,2-dioxaphosphorinane (neopentylene phosphorochloridite)] and amidophosphite (with a phosphoramidite or -diamidite). In the first case, the reaction was performed with continuous stirring at 20°C for 2 h in anhydrous benzene using triethyl-amine as HCl acceptor.

In the amidophosphite procedure, 5,5-dimethyl-2dimethylamino-1,3,2-dioxaphosphorinane (neopentylene dimethylphosphoramidite) was used as a phosphorylating agent. The reaction also occurred in anhydrous benzene, but required heating to 80°C for 6 h in the presence of catalytic amounts of diethylamine hydrochloride.



After complete phosphorylation, the singlets in the ³¹P NMR spectrum with chemical shifts of 146 and 144 ppm, characteristic of the starting compound, disappear, and a singlet at 120 ppm appears; its position is typical of complete cyclic phosphites. The compounds obtained by the above two procedures are identical. They are white crystalline substances with mp 142–144°C. Comparison of the two phosphorylation

procedures shows that the yields of the target compounds are comparable. However, in the chlorophosphite method, the target compound contains a small amount of triethylamine hydrochloride. The phosphorus derivatives isolated were characterized by ¹³C NMR spectroscopy. The spectrum of neopentylene dihydroquercetin-3-yl phosphite (Table 1) contains signals of all the carbon atoms of the dihydroquercetin

Comp. no.	C ²	C^2 C^3		C ⁴	C ⁴ C ⁵		C ⁶		C ⁷		C ⁸		C ⁹		C ¹⁰		C ¹		C ²	
I	83.6	72.5		189.7	16	163.7		93.8		165.7		.1	161.7		103.7		' 129.9		112.5	
II	82.3	77.6	(6.7)	189.3	16	53.7		4.4 1		56.7 9		.2	162.7		104.2		128.3		112.4	
IV	81.7	77.3	(6.4)	189.1	16	53.8 9		4.1	16	6.6 93		.3 162.8		104.1		127.9		112.1		
V	82.8	77.05	(6.6)	189.2	16	163.9		94.2		166.7		.3	162.9		104.2		2 128.5		112.2	
VI	82.8	77.9	(6.9)	185.1	16	164.3		93.9		166.2		.4	162.7		105.0		128.4		112.1	
VII	82.7	78.7	(6.9)	185.0	16	64.5			166.2		93.5		162.7		105.1		128.4	1	12.1	
VIII	82.1	77.8	(5.5)	189.3	16	53.8	9	4.2	16	66.6	93	.3	162.8		104.1		128.2	112.3		
IX	82.1	77.8	(6.7)	189.4	16	53.8	94.6		16	66.5	93	.1	162.9	9 104.4		4	128.3		12.4	
X	82.2	77.7	(6.5)	188.9	16	53.8	9	4.6	166.6		93.3		162.8		104.4		128.5		112.6	
		L	I		L	4			L		L					L_				
Comp no		C ³		C ⁵	⁵ C		OC		H ₃	C^1			C ¹²	2 C ¹³		C ¹⁴		C ¹⁵		
I	148.9		149.	5 112	2.3	120.6		55.9		_		_		_		_				
II	14	149.3		1 111	1.8 12		1.5 56		.5 73.5		(5.4) 31.9		(6.5)	22	22.4		21.1		-	
IV	14	149.2		3 111	.8	121.2		56.3		73.6 (5.		31.8 (6.6)		24.3		20.0		-		
\mathbf{V}	14	149.3		3 111	1.8 121		.3 56.		.3	73.5	(5.4) 32.		(6.6)	21	21.4		20.4		-	
VI	14	149.9		7 111	.7 121		.7 55		.5	5 40.3		1	13.8		13.9		63.4 (5.0)		(8.6)	
VII	VII 1		50.1 150		.4	121.7		55.	.6	40.2	(5.0)		13.9		13.9		63.3 (5.1)		15.6 (8.5)	
VIII	14	149.4		3 111	.9 121		.3 56		.3 73.4		(5.4) 31		31.7 (6.8)		22.2		21.2		_	
IX	14	149.5		3 111	1.9 121		.6 56		.7 73.3		(5.3) 31.		7 (6.7)	22	22.1		21.4		—	
X	۲ ۱۰		9.5 150.2		.9	121.6		56.6		73.2	(5.3) 32.		(6.7)	22	22.2		21.3		_	

¹³C NMR data [δ_C , ppm (² J_{CP} , Hz)] for the compounds obtained^a

^a Solvents: $CDCl_3$ for I; C_6D_6 for II, VI, VII, IX, and X; and $[(CD_3)_2N]_3PO$ for IV, V, and VIII.

tetramethyl ether and neopentylene moieties. The signal of the C³ atom in dihydroquercetin tetramethyl ether is a doublet with the coupling constant ${}^{2}J_{CP}$ 6.7 Hz.

In the next step, we studied phosphorylation of dihydroquercetin tetramethyl ether with ethyl tetraethylphosphorodiamidite.



The reaction occurs in refluxing absolute toluene in 8 h in the presence of catalytic amounts of diethylamine hydrochloride. The reaction completion was judged from TLC and ³¹P NMR data. In the ³¹P spectrum, the singlet at 134.2 ppm characteristic of the starting phosphorylating reagent is absent, and two singlets of equal intensity, belonging to the diastereomers of ethyl dihydroquercetin-3-yl diethylphosphoramidite **III**, appear at 151.02 and 151.90 ppm. The yield of the target compound can be estimated at 75–80% from the integral intensities of the ³¹P NMR signals.

Thus, we showed that trivalent phosphorus acid chlorides and amides are convenient and promising agents for preparing phosphorylated derivatives of partially protected dihydroquercetin.

We also studied oxidation of phosphite II and amidophosphite III. The reactions of II with sulfur and selenium were carried out in anhydrous benzene: with sulfur, for 2 h at 50°C with a catalytic amount of triethylamine, to obtain O-3',4',5,7-tetramethyldihydroquercetin-3-yl O,O'-neopentylene phosphorothioate IV, and with selenium, for 8 h at the same





RUSSIAN JOURNAL OF GENERAL CHEMISTRY Vol. 73 No. 11 2003

temperature without catalyst, to obtain O-3',4',5,7-tetramethyldihydroquercetin-3-yl O,O'-neopentylene phosphoroselenoate V. Compounds IV and V spontaneously precipitated from the reaction mixtures.

Compound **III** was brought into reactions with sulfur and selenium without isolation because of its high lability. The reaction was performed at 20°C with continuous stirring. In the first case, O-3',4',5,7tetramethyldihydroquercetin-3-yl *O*-ethyl diethylphosphoramidothioate **VI** was formed in 8 h, and in the second case O-3',4',5,7-tetramethyldihydroquercetin-3-yl *O*-ethyl diethylphosphoramidoselenoate **VII** was obtained after stirring for 10 h.



X = S (VI), Se (VII).

Compounds **VI** and **VII** were isolated by column chromatography on silica gel, but we failed to separate the diastereomers.

We also prepared *O*-3',4',5,7-tetramethyldihydroquercetin-3-yl *O*,*O*'-neopentylene phosphate **VIII** by passing an oxygen flow through a solution of phosphite **II** in benzene at external UV irradiation. Due to its low solubility in benzene, compound **VIII** also spontaneously crystallizes and precipitates from the reaction mixture.

The completion of all the above reactions was judged from disappearance of the signals the starting phosphites in the ³¹P NMR spectra. The reactions occur with virtually quantitative yields.



We studied the reaction of phosphite **II** with some transition metal derivatives, with the aim to obtain metal complexes promising as catalysts in enantioselective processes. Phosphite **II** was brought into reactions with copper monobromide and (cyclooctadiene)platinum dichloride. With the platinum derivative, we obtained the *cis* complex with *O*-3',4',5,7-tetramethyldihydroquercetin-3-yl *O*,*O*'-neopentylene phosphate (**IX**), which showed doublets at $\delta_{\rm P}$ 66.13 and 64.66 ppm ($J_{\rm PPt}$ 5940.95 and 5904.79 Hz) [9], and with copper, an *O*-3',4',5,7-tetramethyldihydroquercetin-3-yl *O*,*O*'-neopentylene phosphate complex **X** with a typical broad signal at $\delta_{\rm P}$ 103.65 ppm.



The structure of all the synthesized compounds was confirmed by 13 C NMR spectroscopy and elemental analysis. As a rule, in going from three- to four-cordinate phosphorus derivatives, no significant carbon shift was observed. The data obtained are listed in the table.

Thus, we synthesized the first 3',4',5,7-tetramethyldihydroquercetin derivatives by phosphorylation at the alcoholic hydroxy groups. We found that the complete phosphites and their derivatives are relatively stable in storage.

EXPERIMENTAL

The ¹³C NMR spectra were recorded on a Bruker AC-200 instrument at operating frequency of 50.32 MHz, external reference TMS. The ³¹P NMR spectra were obtained on a Bruker WP-80 instrument at operating frequency of 32.4 MHz, external reference 85% phosphoric acid. Optical rotation angles were measured in an EPO polarimeter. Thin-layer chromatography was performed on Silufol plates in 1:1 (A) and 3:1 (B) benzene–dioxane systems; the plates were developed with iodine vapor and by calcination. Absorption column chromatography was performed on silica gel L 100/160 µm.

All syntheses were performed under argon, using absolute solvents.

3',4',5,7-Tetramethyldihydroguercetin-3-yl neopentylene phosphite II. a. To a solution of 1 g of I in 20 ml of benzene, 0.47 g of neopentylene phosphorochloridite in 10 ml of benzene and 0.28 of freshly distilled triethylamine in 10 ml of benzene were added simultaneously with vigorous stirring, and the mixture was stirred for 2 h at 20°C. The mixture was filtered, and 150 ml of hexane was added to the filtrate. In so doing, a colorless precipitate of II formed, which was then filtered off, washed with hexane $(2 \times 30 \text{ ml})$, and dried in a vacuum. Yield 0.96 g (70%), mp 142–144°C, R_f 0.82 (A), 0.6 (B), $[\alpha]_{D}^{18}$ 11.3° (c 0.01, benzene). ³¹P NMR spectrum: δ_{P} 120.10 ppm, s. Found, %: C 57.37; H 6.57; O 30.68; P 5.38. C₂₄H₉O₉P. Calculated, %: C 58.54; H 5.89; O 29.27; P 6.30.

b. To a solution of 1 g of **I** in 10 ml of benzene, 0.46 g of neopentylene dimethylphosphoramidite in 5 ml of benzene and a catalytic amount of diethylamine hydrochloride were added with vigorous stirring. The mixture was refluxed for 6 h. Compound **II** was isolated as described above. Yield 1.1 g (80%), mp 142–144°C. The other characteristics correspond to those of the compound obtained by method *a*.

3',4',5,7-Tetramethyldihydroquercetin-3-yl ethyl diethylphosphoramidite III. To a solution of 0.5 g of I in 20 ml of toluene, 0.3 g of ethyl tetraethylphosphorodiamidite in 10 ml of toluene was added with vigorous stirring. The reaction mixture was refluxed for 10 h. The reaction completion was judged from the ³¹P NMR spectra of the reaction mixture. Amidophosphite III was not isolated because of its high lability. The integral intensities of the peaks in the ³¹P NMR spectra showed that the target compound was formed in 75–80% yield, R_f 0.45 (A), 0.65 (B). ³¹P NMR spectrum: δ_p 151.02 and 151.90 ppm, d.

O-3',4',5,7-Tetramethyldihydroquercetin-3-yl *O*,*O*'-neopentylene phosphorothioate IV. To a solution of 0.3 g of II in 10 ml of benzene, 0.02 g of crystalline sulfur was added with vigorous stirring. The reaction was carried out for 2 h at 50°C with addition of a catalytic amount of triethylamine. The phosphorothioate precipitated spontaneously. The precipitate was filtered off, washed with benzene and hexane (2 × 50 ml), and dried in a vacuum. Yield 0.31 g (96%), mp 200–201°C, R_f 0.83 (A), 0.58 (B), $[\alpha]_D^{26}$ 7° (*c* 0.17, hexamethylphosphoramide). ³¹P NMR spectrum: δ_P 59.2 ppm. Found, %: C 55.29; H 5.77; O 27.38; P 5.75; S 5.81. C₂₄H₂₉O₉PS. Calculated, %: C 54.96; H 5.53; O 27.48; P 5.92; S 6.11. *O*-3',4',5,7-Tetramethyldihydroquercetin-3-yl *O,O*'-neopentylene phosphoroselenoate V. To a solution of 0.3 g of II in 10 ml of benzene, 0.05 g of selenium was added with vigorous stirring.The reaction was performed at 50°C for 8 h. The phosphoroselenoate precipitated spontaneously. Compound V was isolated similarly to IV. Yield 0.39 g (95%), mp 206–207°C, R_f 0.81 (A), 0.58 (B), $[\alpha]_D^{26}$ 4° (*c* 0.16, hexamethylphosphoramide). ³¹P NMR spectrum: δ_P 65.9 ppm (J_{PSe} 992.01 Hz). Found, %: C 50.71; H 5.28; O 25.32; P 4.94; Se 13.75. C₂₄H₂₉O₉PSe. Calculated, %: C 50.44; H 5.08; O 25.22; P 5.43; Se 13.83.

O-3',4',5,7-Tetramethyldihydroquercetin-3-yl *O*-ethyl diethylphosphoramidothioate VI. To a solution of 0.87 g of III in 15 ml of toluene, 0.0544 g of crystalline sulfur was added with vigorous stirring over a period of 3 h at 20°C in the presence of a catalytic amount of triethylamine. The solution was filtered, the solvent was removed in a vacuum, and the residue was chromatographed on a column packed with silica gel (eluent B). The fraction with R_f 0.77 was collected, the solvent was removed, and the product was dried in a vacuum. Yield 0.8 g (85%), yellow sirupy substance, R_f 0.57 (A), 0.77 (B), [α]_D¹⁸ 41° (c 0.01, benzene). ³¹P NMR spectrum: δ_P 75.58 and 76.9 ppm, d. Found, %: C 55.32; H 6.57; N 2.78; O 24.19; P 5.51; S 5.63. C₂₅H₃₄NO₈PS. Calculated, %: C 55.66; H 6.31; N 2.60; O 23.74; P 5.75; S 5.94.

O-3',4',5,7-Tetramethyldihydroquercetin-3-yl *O*-ethyl diethylphosphoramidoselenoate VII. To a solution of 0.3 g of III in 15 ml of toluene, 0.1343 g of selenium was added over a period of 5 h with vigorous stirring at 20°C. Phosphoroselenoate VII was isolated similarly to VI. Yield 0.6 g (60%), yellow sirupy substance, R_f 0.59 (A), 0.79 (B), $[\alpha]_D^{18}$ 45° (*c* 0.01, benzene). ³¹P NMR spectrum: δ_P 78.86 and 81.27 ppm, d (J_{PSe} 995.71 Hz). Found, %: C 51.39; H 5.61; N 2.59; O 22.02; P 5.15; Se 13.24. C₂₅H₃₄NO₈PSe. Calculated, %: C 55.66; H 6.31; N 2.60; O 23.74; P 5.75; Se 5.94.

3',4',5,7-Tetramethyldihydroquercetin-3-yl neopentylene phosphate VIII. Through a solution of 0.3 g of II in 10 ml of benzene in a fused quartz tube, an oxygen flow was passed for 6 h at external UV irradiation. The reaction product spontaneously precipitated as a colorless substance. Compound VIII was filtered off, washed with benzene and hexane $(2 \times 50 \text{ ml})$, and dried in a vacuum. Yield 0.30 g (98%), mp 209– 211°C, R_f 0.84 (A), 0.57 (B), $[\alpha]_D^{26}$ 8° (*c* 0.17, hexamethylphosphoramide). ³¹P NMR spectrum: δ_P –9.98 ppm, s. Found, %: C 56.20; H

RUSSIAN JOURNAL OF GENERAL CHEMISTRY Vol. 73 No. 11 2003

6.08; O 2.01; P 5.71. $C_{24}H_{29}O_{10}P$. Calculated, %: C 56.69; H 5.71; O 31.50; P 6.10.

3',4',5,7-Tetramethyldihydroquercetin-3-yl neopentylene phosphite platinum complex IX. To a solution of 0.162 g of II in 5 ml of methylene chloride, 0.06 g of (cyclooctadiene)platinum(II) dichloride in 5 ml of methylene chloride was added. The reaction mixture was vigorously stirred for 1 h. The metal complex solution was poured into hexane (50 ml), and a white precipitate formed. The precipitate was filtered off, washed with hexane (2 × 30 ml), and dried in a vacuum. Yield 0.18 g (87%), mp 171–173°C (decomp.), R_f 0.64 (A), 0.54 (B), $[\alpha]_D^{18}$ –58.99° (*c* 0.007, methylene chloride). ³¹P NMR spectrum: δ_P 63.8 and 64.2 ppm, d (J_{ppt} 5940.95, J_{ppt} 5904.79 Hz).

3',4',5,7-Tetramethyldihydroquercetin-3-yl 3-neopentylene phosphite copper complex X. Similarly to the synthesis of compound IX, complex X was obtained from 0.162 g of II and 0.048 g of copper(I) bromide. Yield 0.18 g (85%), mp 151–151°C (decomp.), R_f 0.7 (A), 0.33 (B), $[\alpha]_D^{18}$ 45.7° (*c* 0.007, methylene chloride). ³¹P NMR spectrum: δ_P 103.6 ppm, br.m.

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