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CHEMISTRY

Phosphates, Phosphinate, and Hydrophosphoryl Compound Derived from Flavonoid Dihydroquercetin

Corresponding Member of the RAS E. E. Nifant'ev, M. S. Krymchak, T. S. Kukhareva, and L. K. Vasyanina

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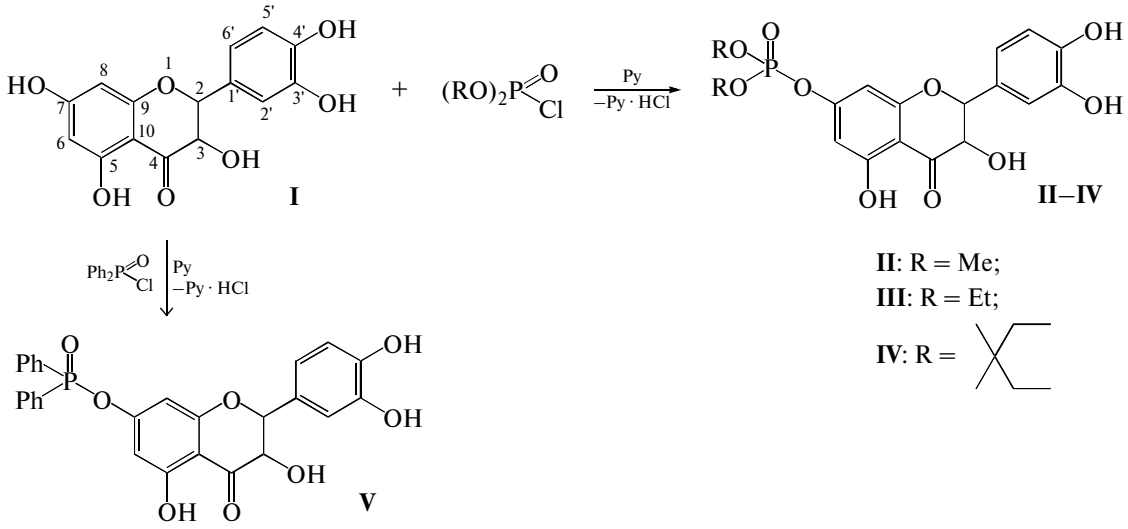
In recent years, there has been growing interest of organic chemists in plant-derived products. Thus, studies were carried out to convert flavonoid dihydroquercetin into a series of efficient pharmaceuticals [1, 2]. In this context, our aim was to develop the synthesis of organophosphorus compounds based on the above flavonoid using trivalent phosphorus derivatives. Thus, we obtained interesting results on the synthesis of modified dihydroquercetins containing residues of alkyl thiophosphoric and selenophosphoric acids [3]. It is significant that these compounds show high cytotoxic activity [4]. Nonetheless, this method of synthesis has certain limitations. For example, we failed to obtain dihydroquercetin phosphates and phosphinates in good yield. Therefore we studied the phosphorylation of dihydroquercetin (**I**) with dialkyl phosphorochloridates and diphenylphosphinic chloride.



Scheme 1.

The reactions were carried out in an anhydrous dioxane medium in a nitrogen atmosphere in the presence of anhydrous pyridine as a hydrogen chloride scavenger and a catalyst at an equimolar ratio of the reagents.

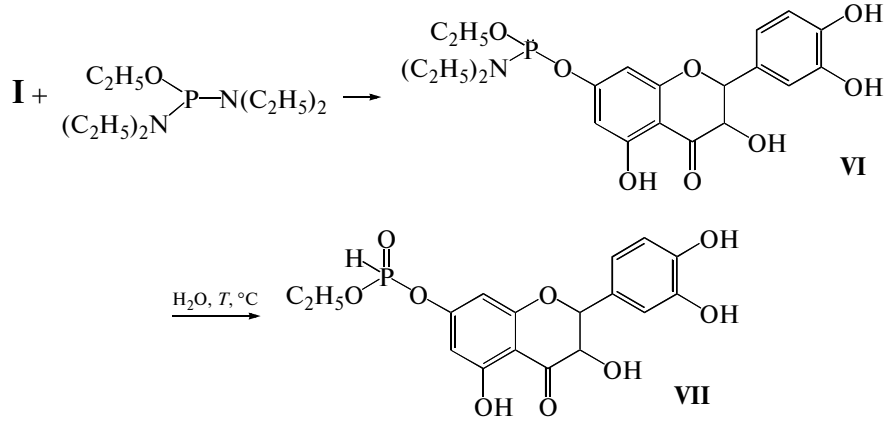
It should be noted that, in contrast to tris(diethylamino)phosphine that readily reacted with dihydroquercetin at the hydroxy groups at the 7-, 3'-, and 4'-positions of the flavonoid core [5], the phosphorylation with pentavalent phosphorus reagents proceeds only at the most reactive hydroxy group at the 7-position.

Phosphorylation with phosphorochloridates proceeded with low rates. However, the use of microwave radiation allows a considerable increase in reaction rate. At the same time, the phosphorylation with phosphinic acid chloride required a much shorter time.

Moscow State Pedagogical University,
ul. Malaya Pirogovskaya 1, Moscow, 119899 Russia

The phosphorylation course and reaction mixture composition were monitored by thin layer chromatography (TLC) and ^{31}P NMR spectroscopy. ^{31}P NMR spectra showed singlet signals with chemical shifts −9.4 ppm (**II**), −12.6 ppm (**III**), −20.0 ppm (**IV**), and 25.9 ppm (**V**). Compounds **II**–**V** were isolated by column chromatography. Their structure was confirmed by ^{1}H and ^{13}C NMR spectroscopy. The ^{1}H NMR spectra of these compounds show the lack of proton signal of the hydroxy group at the 7 position in the region of 10.8 ppm and show signals typical for flavonoid core and alkyl and aryl substituents with corresponding spin–spin coupling constants $^{3}J_{PH}$. ^{13}C NMR spectra fully confirm the structure of the above compounds (see Experimental).

One of important aims of our study was to obtain hydrophosphoryl compounds based on dihydroquercetin. Common hydrogen phosphites do not react with dihydroquercetin, therefore we used the hydrolysis of phosphoramidite **VI** prepared from dihydroquercetin (**I**):



Scheme 2.

Phosphoramidite **VI** without isolation from reaction mixture was hydrolyzed using an equimolar amount of water. Reaction course was monitored by TLC and ^{31}P NMR spectroscopy. When reaction mixture was kept at 40–50°C for 14 h, ^{31}P NMR spectrum showed the disappearance of the signal of compound **VI** at 145.1 ppm and appearance of a singlet at 2.9 ppm with $^{1}J_{PH} = 647$ Hz corresponding to compound **VII**. Increase in temperature above this range favored destructive processes and reduced the yield of compound **VII**.

The noted ethyl phosphite **VII** was isolated in pure state and characterized by ^{1}H and ^{13}C NMR spectroscopy. The ^{1}H NMR spectrum shows the lack of signal corresponding to hydroxy group at the 7' position and shows the proton signals of flavonoid core and phosphorus-containing substituent. ^{13}C NMR spectrum fully confirms the structure of compound **VII**.

Thus, we synthesized for the first time in satisfactory yield novel phosphorus-containing dihydroquercetins that are of interest as potential biologically active compounds.

EXPERIMENTAL

NMR spectra were recorded on a JNM-ECX 400 spectrometer (operating at 161.83 MHz for ^{31}P, 100.5 MHz for ^{13}C, and 400 MHz for ^{1}H) using TMS as an internal reference for ^{1}H and ^{13}C NMR spectra and 85% H_3PO_4 as an external reference for $^{31}P\{^{1}H\}$ NMR spectra. Elemental analyzes were made with a PerkinElmer 2400 analyzer. Certain syntheses were carried out using CEM Discover microwave reactor. All syntheses were performed in a dry nitrogen atmosphere in solvents dried using standard procedures.

TLC analysis was conducted with Silica gel 60 F_{254} plates using benzene–dioxane (3 : 1, A), benzene–dioxane (1 : 1, B), and hexane–dioxane (1 : 3, C) systems. Compounds were visualized with iodine vapor or by calcination.

Melting points were determined in sealed capillary tubes at heating rate of 1 K/min. The phosphorylating reagents were obtained by standard procedures, and their constants coincided with literature data.

2,3-Dihydroquercetin 7-*O*-(dimethyl phosphate) (II). Dimethyl phosphorochloridate (0.143 g) was added to a mixture of 0.304 g of dihydroquercetin (**I**) and 0.08 mL of pyridine dissolved in 50 mL of dioxane at ambient temperature under continuous stirring.

(1) The reaction mixture was kept for 48 h at ambient temperature.

(2) The reaction mixture was kept for 3 h at 25°C in the CEM Discover microwave reactor.

In the case of compounds **II**–**IV**, the reaction mixture was filtered. The solvent was removed in a vacuum. The solid residue was dissolved in dioxane and purified by column chromatography using system B as an eluent. All prepared compounds are yellow powders.

Compound **II**: yield 0.103 g (25%), mp. 66–68°C, R_f 0.38 (B), 0.69 (C).

^{31}P NMR (acetone-d_6, δ, ppm): –9.4 s.

^{1}H NMR (acetone-d_6, δ, ppm, J, Hz): 3.6 (d, 6H, $(C\underline{H}_3O)_2P$, $^{3}J_{PH}$ 11.04), 4.6 (d, 1H, C^3H, $^{3}J_{HH}$ 11.9), 4.97 (d, 1H, C^2H, $^{3}J_{HH}$ 11.9), 5.91 (d, 1H, C^8H, $^{4}J_{HH}$ 2.4), 5.96 (d, 1H, C^6H, $^{4}J_{HH}$ 2.4), 6.83 (s, 2H, $C^{5'}H$ and $C^{6'}H$), 7.07 (s, 1H, $C^{2'}H$), 8.69 (br s, 2H, $C^{3'}OH$ and $C^{4'}OH$), 11.5 (s, 1H, C^5OH).

^{13}C NMR (acetone-d_6, δ, ppm, J, Hz): 54.19 (d, CH_3, $^{2}J_{CP}$ 5.82), 72.35 (C^3), 83.64 (C^2), 95.38 (C^8), 96.41 (C^6), 100.63 (C^{10}), 115.7 ($C^{6'}$), 115.23 ($C^{5'}$), 120.07 ($C^{2'}$), 128.85 ($C^{1'}$), 145.84 ($C^{4'}$), 146.12 ($C^{3'}$), 163.25 (C^9), 164.07 (C^5), 167.3 (C^7), 197.27 (C^4).

For $C_{17}H_{17}O_{10}P$ anal. calcd. (%): C, 49.52; H, 4.16; P, 7.51.

Found (%): C, 49.34; H, 4.32; P, 7.82.

2,3-Dihydroquercetin 7-*O*-(diethyl phosphate) (III). Diethyl phosphorochloridate (0.173 g) was added to a mixture of 0.304 g of dihydroquercetin (**I**) and 0.08 mL of pyridine dissolved in 50 mL of dioxane at ambient temperature and continuous stirring.

(1) The reaction mixture was kept for 72 h at ambient temperature.

(2) The reaction mixture was kept for 3 h at 25°C in the CEM Discover microwave reactor.

Compound **III**: yield 0.1 g (22%), mp. 73–75°C, R_f 0.43 (B), 0.65 (C).

^{31}P NMR (acetone-d_6, δ, ppm): –12.6 s.

^{1}H NMR (acetone-d_6, δ, ppm, J, Hz): 1.21 (t, 6H, $C\underline{H}_3$, $^{3}J_{HH}$ 7.4), 3.99 (m, 4H, $C\underline{H}_2$, $^{3}J_{PH}$ 8.69, $^{3}J_{HH}$ 7.4), 4.60 (d, 1H, C^3H, $^{3}J_{HH}$ 11.4), 5.0 (d, 1H, C^2H, $^{3}J_{HH}$ 11.4), 5.96 (d, 1H, C^8H, $^{4}J_{HH}$ 1.8), 5.99 (d, 1H, C^6H, $^{4}J_{HH}$ 1.8), 6.8 (s, 2H, $C^{5'}H$ and $C^{6'}H$), 7.1 (s, 1H, $C^{2'}H$), 7.9 (br s, 2H, $C^{3'}OH$ and $C^{4'}OH$), 11.8 (s, 1H, C^5OH).

^{13}C NMR (acetone-d_6, δ, ppm, J, Hz): 15.36 (CH_3), 58.59 (d, CH_2, $^{2}J_{CP}$ 6.13), 71.73 (C^3), 84.43 (C^2), 94.27 (C^8), 95.16 (C^6), 101.87 (C^{10}), 116.41 ($C^{6'}$), 116.93 ($C^{5'}$), 121.29 ($C^{2'}$), 127.15 ($C^{1'}$), 146.24 ($C^{4'}$), 146.92 ($C^{3'}$), 162.91 (C^9), 164.62 (C^5), 167.49 (C^7), 197.74 (C^4).

For $C_{19}H_{21}O_{10}P$ anal. calcd. (%): C, 51.82; H, 4.81; P, 7.03.

Found (%): C, 51.78; H, 4.99; P, 7.30.

2,3-Dihydroquercetin 7-*O*-(neopentylene phosphate) (IV). Neopentylene phosphorochloridate (0.185 g) was added to a mixture of 0.304 g of dihydroquercetin (**I**) and 0.08 mL of pyridine dissolved in 50 mL of dioxane at ambient temperature and continuous stirring.

(1) The reaction mixture was kept for 96 h at ambient temperature.

(2) The reaction mixture was kept for 5 h at 30°C in the CEM Discover microwave reactor.

Compound **IV**: yield 0.11 g (24%), mp. 78–80°C, R_f 0.35 (B), 0.58 (C).

^{31}P NMR (acetone-d_6, δ, ppm): –20.0 s.

^{1}H NMR (acetone-d_6, δ, ppm, J, Hz): 0.85 and 1.25 (both s, 6H, $C\underline{H}_3$), 4.02 (m, 2H, CH^{e}_{2}, $^{3}J_{PH_3}$ 11.3, $^{2}J_{HH}$ 7.1), 4.48 (m, 2H, $C\underline{H}^{a}_{2}$, $^{2}J_{HH}$ 7.1), 4.58 (d, 1H, C^3H, $^{3}J_{HH}$ 11.0), 4.99 (d, 1H, C^2H, $^{3}J_{HH}$ 11.0), 5.90 (d, 1H, C^8H, $^{4}J_{HH}$ 2.3), 5.97 (d, 1H, C^6H, $^{4}J_{HH}$ 2.3), 6.8 (d, 1H, $C^{5'}H$, $^{3}J_{HH}$ 8.1), 6.87 (d, 1H, $C^{6'}H$, $^{3}J_{HH}$ 8.1), 7.04 (s, 1H, $C^{2'}H$), 8.1 (br s, 2H, $C^{3'}OH$ and $C^{4'}OH$), 11.6 (s, 1H, C^5OH).

^{13}C NMR (acetone-d_6, δ, ppm, J, Hz): 18.7 and 20.76 (CH_3), 31.88(C), 72.28 (C^3), 79.34 c, 83.66 (C^2), 95.25 (C^8), 96.18 (C^6), 100.65 (C^{10}), 115.05 ($C^{5'}$), 115.14 ($C^{2'}$), 120.00 ($C^{6'}$), 128.94 ($C^{1'}$), 141.47 ($C^{4'}$), 144.88 ($C^{3'}$), 163.32 (C^9), 164.94 (C^7), 163.89 (C^5), 197.32 (C^4).

For $C_{21}H_{24}O_{10}P$ anal. calcd. (%): C, 53.97; H, 5.18; P, 6.63.

Found (%): C, 53.69; H, 5.92; P, 6.38.

2,3-Dihydroquercetin 7-*O*-diphenylphosphinate (V). Diphenylchlorophosphinate (0.19 mL) was added to a mixture of 0.304 g of dihydroquercetin (**I**) and 0.08 mL of pyridine dissolved in 50 mL of dioxane at ambient temperature and continuous stirring. The reaction mixture was kept for 10 h at ambient temperature and filtered. The solvent was removed in a vacuum. The solid residue was dissolved in benzene and purified by column chromatography using system B as an eluent.

Compound **V**: yield 0.09 g (18%), mp. 75–77°C, R_f 0.42 (B), 0.64 (C).

^{31}P NMR (acetone-d_6, δ, ppm): 25.9 s.

^{1}H NMR (DMSO-d_6, δ, ppm, J, Hz): 4.56 (d, 1H, C^3H, $^3J_{HH}$ 11.4), 4.98 (d, 1H, C^2H, $^3J_{HH}$ 11.4), 5.94 (d, 1H, C^8H, $^4J_{HH}$ 1.8), 5.97 (d, 1H, C^6H, $^4J_{HH}$ 1.8), 6.82 (d, 2H, $C^{5'}H$ and $C^{6'}H$, $^3J_{HH}$ 12.4), 7.04 (s, 1H, $C^{2'}H$), 7.32–7.77 (m, 10H, Ph), 12.3 (s, 1H, $C^{5'}OH$).

^{13}C NMR (acetone-d_6, δ, ppm): 71.95 (C^3), 84.14 (C^2), 94.68 (C^8), 95.11 (C^6), 102.81 (C^{10}), 114.7 ($C^{6'}$), 117.53 ($C^{5'}$), 121.27 ($C^{2'}$), 128.93–131.6 (Ph), 132.65 ($C^{1'}$), 146.34 ($C^{4'}$), 147.52 ($C^{3'}$), 156.7 (C^9), 159.25 (C5), 163.06 (C^7), 196.98 (C^4).

For $C_{27}H_{21}O_8P$ anal. calcd. (%): C, 64.29; H, 4.20; P, 6.14.

Found (%): C, 64.67; H, 4.76; P, 6.25.

2,3-Dihydroquercetin 7-*O*-(*O*,*N*,*N*-triethylphosphoramidite) (VI). *O*,*N*,*N*,*N*',*N*'-Pentaethylphosphordiamidite (0.22 mL) was added to a mixture of 0.304 g of dihydroquercetin (**I**) and 0.08 mL of pyridine dissolved in 50 mL of dioxane at ambient temperature and continuous stirring. The reaction mixture was kept for 2 h at ambient temperature. The product was not isolated from the reaction mixture.

Compound **VI**: R_f 0.55 (A), 0.75 (B).

^{31}P NMR (dioxane, δ, ppm): 145.1 s.

2,3-Dihydroquercetin 7-*O*-(ethyl hydrogen phosphite) (VII). Water (0.018 mL) was added to a solution of 0.451 g of compound **VI** in 50 mL of dioxane and stirred at 40°C for 14 h. The reaction mixture was filtered. The solvent was removed in a vacuum. The solid residue was dissolved in dioxane. The reaction product (**VII**) was precipitated from dioxane with hexane.

Compound **VII**: yield 0.1 g (26%), mp. 62–64°C, R_f 0.64 (B), 0.72 (C).

^{31}P NMR (acetone-d_6, δ, ppm): 2.89 (d, $^1J_{PH}$ 647 Hz).

^{1}H NMR (acetone-d_6, δ, ppm, J, Hz): 1.14 (t, 3H, CH_3, $^3J_{HH}$ 7.2), 3.68 (m, 2H, CH_2, $^3J_{PH}$ 8.68, $^3J_{HH}$ 7.2), 4.44 (d, 1H, C^3H, $^3J_{HH}$ 11.74), 4.94 (d, 1H, C^2H, $^3J_{HH}$ 11.74), 5.83 (d, 1H, C^8H, $^4J_{HH}$ 2.8), 6.02 (d, 1H, C^6H, $^4J_{HH}$ 2.8), 6.55 (d, 1H, $^2J_{PH}$ 580), 6.74 (d, 1H, $C^{5'}H$, $^3J_{HH}$ 5.3), 6.78 (d, 1H, $C^{6'}H$, $^3J_{HH}$ 5.3), 6.84 (s, 1H, $C^{2'}H$), 9.2 (br s, 2H, $C^{3'}OH$, $C^{4'}OH$), 11.79 (s, 1H, $C^{5'}OH$).

^{13}C NMR (acetone-d_6, δ, ppm): 17.17 (s, CH_3), 58.69 (d, $C\underline{H}_2$, $^2J_{CP}$ 8.9), 72.05 (C^3), 83.56 (C^2), 95.57 (C^8), 96.56 (C^6), 100.83 (C^{10}), 115.66 ($C^{5'}$), 115.91 ($C^{2'}$), 119.8 ($C^{6'}$), 128.86 ($C^{1'}$), 145.52 ($C^{3'}$), 146.34 ($C^{4'}$), 163.04 (C^9), 163.83 (C^5), 167.82 (C^7, $^2J_{CP}$ 0.97 Hz), 198.23 (C^4).

For $C_{17}H_{17}O_9P$ anal. calcd. (%): C, 51.52; H, 4.32; P, 7.82.

Found (%): C, 51.58; H, 4.51; P, 7.81.

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