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NEW PHOTOLABILE PHOSPHATE PROTECTING GROUPS

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Abstract: The synthesis of phosphate, protected with various photolabile protecting groups, is described. Upon laser photolysis at 355 nm, inorganic phosphate is released, in yields of up to 85%.

The application of photolabile protecting groups in synthetic organic chemistry has been of some interest due to the neutral deprotection conditions which can be used[1]. A number of photolabile protecting groups have been developed, but the most commonly used for the protection of phosphate have been the 2-nitrobenzyl or α-methyl-2-nitrobenzyl phosphate esters[1,2,3]. The mechanism of cleavage of the latter upon laser photolysis has recently been reported[4]. Difficulties with these protecting groups arise on photolysis, however, due to the formation of o-nitrosocarbonyl compounds, which can lead to undersirable side reactions, and also due to the formation of the corresponding coupled azo intermediates, which act as internal light filters[1,5]. This photolytic deprotection approach has been successfully employed for the liberation of free phosphate during biochemical studies of glycogen phosphorylase[6], an important phosphorylating enzyme. Our interest in this area arose due to the need to find alternative phosphate protecting groups which might be rapidly and cleanly removed upon laser photolysis, and which did not produce highly reactive species that might lead to the inactivation of biological systems.

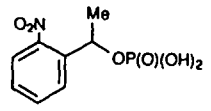
Earlier work[7,8] indicated that substituted benzoin esters could be readily cleaved to benzofuran derivatives and the corresponding acid upon photolysis. However, the analogous benzoin phosphate esters were unknown. Other literature reports suggested that (2,2'-dinitrodiphenyl) methyl[9] and p-methoxyphenacyl[10] phosphate esters would be useful candidates worthy of study, since the corresponding carboxylic esters had been shown to be easily photolysed to give the deprotected carboxylic acids.

Phosphates (1) - (5) were prepared from the corresponding alcohols and methyl dichlorophosphate, according to the method of Rubenstein and Patchornik[9]. The crude phosphates thus obtained were purified by ion-exchange chromatography and isolated as their triethylammonium salts[11]. Phosphate (6) was prepared by treating phenacyl bromide with silver phosphate[12,13]. Each phosphate was photolysed using laser irradiation at either 308 nm or 355 nm, and the production of inorganic phosphate determined colourimetically[14].

The concentration of liberated inorganic phosphate as a function of photolysis time is shown in the Figure below. These results indicate that phosphate (1) is readily photolysed to give inorganic phosphate in 28% yield. However, the dinitrophosphate (2) is much more efficiently photolysed, to give a yield of 85% of inorganic phosphate, although this advantage is offset by the more lengthy synthesis of the starting material. Benzoins (3), (4) and (5) are also efficiently photolysed to give reasonable yields (35-55%) of inorganic phosphate. The facile

photolytic cleavage of benzoin acetates has been previously reported[7] to give the corresponding benzofuran derivative in good yield, along with acetic acid. This same report described the greater ease of photolysis of (3,5-dimethoxy)benzoin acetates compared to the unsubstituted benzoin acetate, and the same effect was observed for the phosphates (3) and (5). However, this effect is not great, and the easier synthesis of phosphate (3) makes it a useful protecting group, especially in view of the fact that the benzofuran produced upon photolysis would be expected to be inert to biological systems. p-Methoxyacetophenone could be identified as a major product from the photolysis of phenacyl phosphate (6).

The application of these compounds to biochemical studies is currently in progress.



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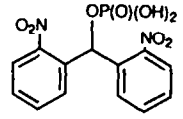

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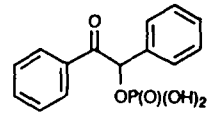

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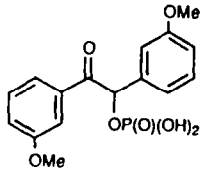

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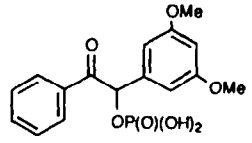

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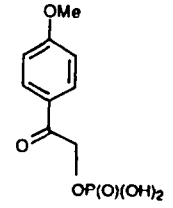

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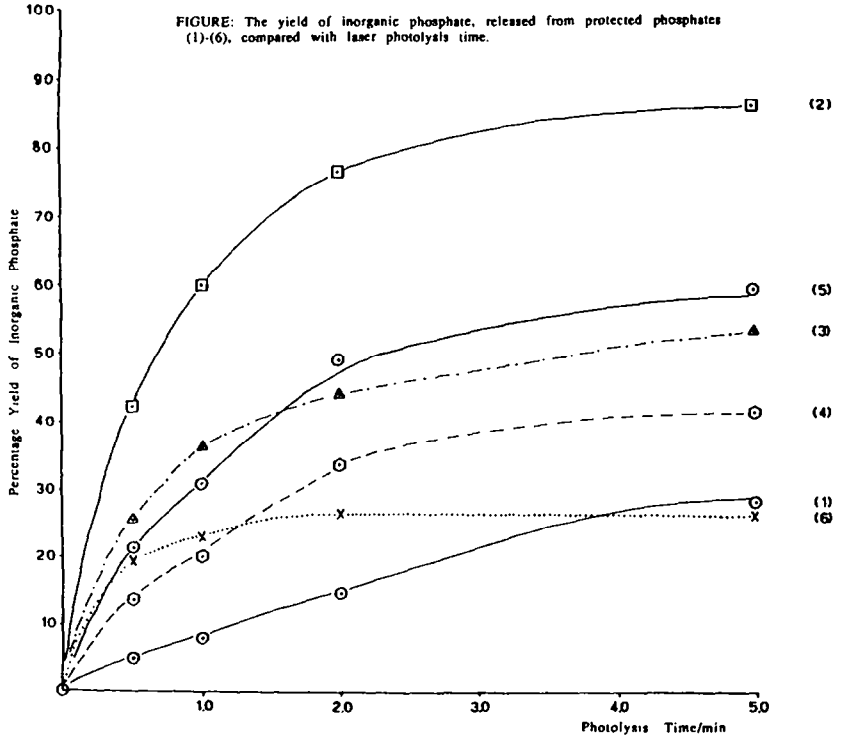

EXPERIMENTAL

^{31}P n.m.r. spectra were recorded at 101.3 MHz on a Bruker AM250 spectrometer. Mass spectra were recorded on a VG Analytical Ltd ZAB1F mass spectrometer, using either Ammonia Desportion Chemical Ionisation (DCI (NH_3)) or Positive Argon Fast Atom Bombardment (FAB).

Ion exchange purifications were carried out using DEAE "Sephacel" resin (Pharmacia) in a 20 x 3 cm glass column run under gravity. A gradient of 20-200 mM triethylammonium bicarbonate (prepared by dissolving triethylamine in distilled water, and bubbling carbon dioxide through the solution until the pH reached 7.0) was used, with 10 ml fractions collected by automatic fraction collector. After analysis of the fractions by UV absorption at 700 nm, all fractions containing the product were combined and freeze-dried.

The photolysis experiments were performed on a frequency-tripled neodymium-YAG laser at 355 nm (System 2000, SK lasers, Cambridge, U.K.), or on a Lambda Physik 201 XeCl Excimer Laser at 308 nm. Irradiations were performed in quartz cuvettes of 1 mm path length.

2,2'-Dinitrodiphenylmethanol was prepared by sodium borohydride reduction[15] of 2,2'-dinitrodiphenyl ketone[16], and obtained as a yellow solid in 71% yield, m.p. 120-122.5°C (lit.[17] m.p. 126°C). 1-(3,5-Dimethoxyphenyl)-2-phenyl-2-oxoethanol was prepared according to the method of Sheehan[7] and obtained as a colourless solid in 8.5% yield, m.p. 108-110°C (lit.[6] m.p. 110-111.5°C). 3,3'-Dimethoxybenzoin was prepared in 25% yield according to the method of Hodgson and Rosenberg[18].

Preparation of Phosphates (1) - (5)

To dry, distilled pyridine at 0°C was added methyl dichlorophosphate. After stirring for 15 min, when a precipitate of methylpyridinium dichlorophosphate had formed, the alcohol was added, and the mixture stirred overnight with careful exclusion of water. The solution was then worked-up as described below:

(a) 1-(2-Nitrophenyl) ethyl phosphate (1) was prepared from pyridine (6 ml), methyl dichlorophosphate (0.6 ml) and 1-(2-nitrophenyl) ethyl alcohol (0.77g). After stirring overnight, the mixture was poured into aqueous sodium bicarbonate (10%), the residue diluted with water (10 ml) and extracted with ether (2 x 10 ml). The aqueous phase was acidified with hydrochloric acid (2N) to pH 1, and extracted with chloroform, to give the crude product (100 mg). Purification by ion-exchange column gave the product as the triethylammonium salt. δ_H (200 MHz, CD_3DD). 1.25 (9H, t, J = 7.4 Hz, 3 x CH_2 $C\underline{H}_3$), 1.55 (3H, d, J = 3.9 Hz, CH_3), 3.05 (5H, q, J = 7.9 Hz, 3 x CH_2 $C\underline{H}_3$), 5.95 (1H, m, Ar $C\underline{H}$ 0), 7.4-8.0 (4H, m, ArH). ν_{max} (KBr disc) 3400(b), 1650(s), 1550(s), 1330(s), 1230(s) cm^{-1}. γ_{max} (H_20) 264 nm. m/z (DCI (NH_3)) 167 (8%), 150 (30), 134 (60), 102 (100), 86 (25), 74 (23).

(b) (2,2'-Dinitrodiphenyl) methyl phosphate[21] (2) was prepared from pyridine (10 ml), methyl dichlorophosphate (0.6 ml) and 2,2-dinitrodiphenylmethanol (0.5 g). The reaction mixture was extracted with chloroform/n-butanol (7:3), washed with water (20 ml) and the solvent removed. n-Butanol was removed by azeotroping with cyclohexane. On complete removal of the solvent, (2,2'-dinitrodiphenyl) methyl phosphate remained (0.36 g, 57%). δ_H (200 MHz, $CDCl_3/CD_3OD$) 7.45 (2H, m, ArH), 7.60 (4H, d, ArH), 7.75 (1H, d, $C\underline{H}$ OP (O) $(OH)_2$), 8.00 (2H, d, ArH); δc (62.5 MHz, CD_3OD), 72.5 (d,

CH OP (O) $(OH)_2$), 126.0 (d, ArCH), 130.6 (d, ArCH), 130.9 (d, ArCH), 134.5 (d, ArCH), 135.8 (s, ArC), 149.2 (s, ArC). δp (101.3 MHz, CD_3OD) - 5.19 (s). ν_{max} (Nujol) 1525 (s), 1345 (s), 1225 (m), 1185 (m), 1025 (br) cm^{-1}. λ_{max} ($CHCl_3/CH_3OH$) 254 nm (log ε 4.0), 235 (4.0). m/z (FAB) 399 ($M+ 2Na^+$, 100%), 377 ($M+ Na^+$, 96). m/z (DCI (NH_3)) 274 (71%), 257 (55), 241 (100), 196 (29), 80 (93).

This product (100 mg) was purified by ion-exchange chromatography, to give the corresponding triethylammonium salt (90 mg, 69%), δ_H (200 MHz, $CDCl_3/CD_3OD$) 1.27 (9H, t, J = 7.0 Hz, CH_2CH_3), 3.06 (6H, q, J = 7.0 Hz, CH_2CH_3), 7.39 (2H, m, ArH), 7.56 (2H, m, CH OP (O) $(OH)_2$ and ArH), 7.70 (2H, m, ArH), 7.92 (2H, m, ArH).

(c) 2-Oxo -1,2-diphenylethylphosphate (3) was prepared from pyridine (10 ml), methyl dichlorophosphate (1 ml) and 2-oxo-1,2-diphenylethanol (4.9 g). The reaction mixture was refluxed from 6 h, and worked-up as described in (b) above. The product thus obtained was dried over phosphorous pentoxide, to give the product (3) as a white solid (0.80 g, 29%), m.p. 78-82°C. δ_H (200 MHz, $CDCl_3$) 4.23 (br, OH), 6.59 (1H, d, J = 8.6 Hz, ArCH OP (O) $(OH)_2$), 7.24 (3H, m, ArH), 7.60 (1H, d, ArH), 7.87 (4H, m, ArH), 8.06 (2H, d, J = 6.6 Hz, ArH). δp (101.3 MHz, CD Cl_3) -3.05 (s). ν_{max} (Nujol) 1695(s), 1225 (m), 1065 (m), 980 (br) cm^{-1}. γ_{max} ($CHCl_3$) 252 nm (log ε 4.0). m/z (DCI (NH_3) 214 (18%), 197 (70), 122 (25), 105 (100), 80 (80), 78 (38).

This product was purified by ion-exchange chromatography, to give the bis (triethylammonium) phosphate salt (20 mg). δ_H (200 MHz, $CDCl_3$) 1.20 (18H, t, 6 x CH_2 CH_3), 3.00 (12H, q, 6 x CH_2 CH_3), 6.50 (1H, br d, Ar CH OP (O) O_2^{2-}), 7.30 (8H, m, ArH), 7.96 (2H, m, ArH). δ_C (250 MHz, $CDCl_3$) 8.49 (q, CH_2CH_3), 45.8 (t, CH_2 CH_3), 78.8 (d, Ar CH OP (O) O_2^{2-}), 127.7 (d, ArCH), 128.1 (d, ArCH), 128.4 (d, ArCH), 128.5 (d, ArCH), 129.3 (d, ArCH), 132.8 (d, ArCH), 135.2 (s, ArC), 136.8 (s, ArC), 196.9 (s, ArC(O)CHAr). m/z (FAB) 394 ($M+ Et_3NH$, 28%), 315 ($M+Na^+$, 59), 207 (25), 102 (Et_3NH^+, 100).

(d) 3,3'-Dimethoxybenzoin phosphate (4) was prepared from pyridine (20 ml), methyl dichlorophosphate (20 ml) and 3,3'-dimethoxybenzoin (2.5 g). After stirring overnight, the reaction mixture was poured into aqueous sodium bicarbonate, and the solution extracted with chloroform. The water layer was acidified to pH 1 with hydrochloric acid (2M), and then extracted with chloroform, to give the crude product (300 mg). Purification by ion-exchange chromatography gave the triethylammonium salt of 3,3'-dimethoxybenzoin phosphate (100 mg). δ_H (200 MHz, CD_3OD), 1.25 (9H, t, J = 8.4 Hz, 3 x CH_2CH_3), 3.15 (6H, q, J = 8.2 Hz, 3 x CH_2CH_3), 3.72 and 3.78 (6H, 2 x OCH_3), 6.4 (1H, d, J = 10.3 Hz, CHOP), 7.0 - 8.4 (8H, m, ArH). ν_{max} (KBr disc) 3400 (b), 3050 (b), 2900 (b), 2700 (b), 1690 (s), 1680 (s), 1580 (s), 1490 (s), 1460 (b), 1450 (b), 1430 (s), 1390 (s) cm^{-1}. γ_{max} (H_2O) 256 nm. m/z (DCI (NH_3)) 372 (23), 273 (35), 255 (80), 135 (45), 102 (100), 86 (30), 74 (17), 58 (9).

(e) 1-(3,5-Dimethoxyphenyl)-2-phenyl-2-oxoethylphosphate (5) was prepared from pyridine (10 ml), methyl dichlorophosphate (0.6 ml) and 1-(3,5-dimethoxyphenyl)-2-phenyl-2-oxoethanol (0.5 g). After stirring overnight, the mixture was worked-up as described in part (a) above, in 55% crude yield. δ_H (200 MHz, $CDCl_3$) 3.75 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 6.23 (1H, br s, CH OP (O) $(OH)_2$), 6.45 (2H, s,

ArH), 6.59 (1H, m, ArH), 6.96 (1H, m, ArH), 7.42 (2H, m, ArH), 8.20 (2H, m, ArH). δ_C (62.5 MHz, $CDCl_3$) 55.6, 55.9, 102.9, 108.5, 127.7, 129.4, 130.0, 133.5, 135.0, 145.6, 162.1. δ_P (101.3 MHz, $CDCl_3$) -3.15 (s), -18.70 (s). γ_{max} ($CHCl_3$) 255 nm (log ε 5.6). m/z (FAB) 375 ($M+ Na^+$, 26%), 334 (100), 255 (69).

This compound (160 mg) was purified by ion-exchange chromatography to give the bis (triethylammonium) salt of the phosphate (80 mg, 39%). δ_H (200 MHz, $CDCl_3$) 1.3 (18H, t, 6 x $CH_2\underline{CH_3}$), 3.1 (12H, q, 3 x $\underline{CH_2}CH_3$), 3.62 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 6.25 (1H, s, $\underline{CH}$ OP (O) O_2^{2-}), 6.50 (1H, s, ArH), 6.58 (2H, s, ArH), 7.02 (1H, s, ArH), 715 (1H, m, ArH), 7.38 (1H, m, ArH), 7.90 (2H, d, ArH). δ_P (101.3 MHz, $CDCl_3$) -2.91 (s). m/z (FAB) 397 ($M+ 2Na^+$, 2%), 375 ($M+ Na^+$, 8), 102 (100).

2-Oxo-2-(4-methoxyphenyl) ethylphosphate (6)

To 2-oxo-2-(4-methoxyphenyl) ethyl bromide (3.0 g, 0.013 mol) in dry acetonitrile (20 ml) was added silver dihydrogenphosphate (2.6 m, 0.013 mol)[12,13] and the mixture refluxed with vigorous stirring for 6 h. The mixture was cooled, the precipitated silver bromide filtered, and the residue washed with ethyl acetate. The solvent was removed in vacuo and the residual solid recrystallised from ethanol, to give a mixture of the desired phosphate (6) and the starting bromide. This crude product was extracted with diethyl ether at reflux several times to leave 2-oxo-(4-methoxyphenyl) ethylphosphate as a white solid (0.10 g, 13%), m.p. 82-89°C. δ_H (200 MHz, $CDCl_3$) 3.86 (3H, s, OCH_3), 5.52 (2H, d, J = 10.9 Hz, $\underline{CH_2}$OP (O) $(OH)_2$, 6.94 (2H, AA 'BB' system, $J_{AB} + J_{AB}$ = 8.7 Hz, ArH ortho - to OCH_3), 7.90 (2H, AA 'BB' system, $J_{AB} + J_{AB'}$ = 8.9 Hz, ArH Meta - to OCH_3). δ_P (101.3 MHz, $CDCl_3$) -3.61 (s). ν_{max} (Nujol) 1685 (s), 1260 (s), 1205 (s), 1020 (s) cm^{-1}. m/z (DCI (NH_3)) 246 (M^+, 4%), 231 (191, 229 (20), 151 (100, 135 (29).

General Photolysis and Inorganic Phosphate Assay Procedures

Inorganic phosphate was assayed according to the method of Pradines et. al.,[14] with the following reagents: Solution A (copper sulfate (0.25 g) and sodium acetate (4.5 g) in aqueous acetic acid (2M, 100 ml); Solution B (ammonium molybdate (5 g) in water (100 ml); Solution C (methyl amino-4-phenol sulfate (1 g) in water (50 ml) was dissolved in a solution of sodium sulfite (2.5 g) in water (50 ml).

Five separate samples (160 µl each) of phosphates (1) - (6) of known concentration in water (2-5 mM) were photolysed by laser irradiation for 0, 0.5, 1.0, 2.0 and 5.0 minute intervals. Identical blank samples, which contained no phosphate, were similarly irradiated. The photolysed solutions were diluted with water to (1.5 ml), and solution A (2.5 ml), then solution B (0.5 ml) and finally solution C (0.5 ml) were added. The absorption at 700 mm was measured after 30 min. A calibration plot was determined by measuring the absorbance of solutions of potassium orthophosphate in the range 0.0 - 1.0 mM using the same colourmetric method.

The results obtained for the photolysis of compounds (1) - (6) are shown in the Figure.

Photolysis of 2-oxo-2-(4-methoxyphenyl)ethyl phosphate (6)

A large scale photolysis of phosphate (6) (7.7 mg, 0.014 mmol) in dioxan (3 ml) was irradiated for 6 min. The solution was freeze-dried and the presence of p-methoxyacetophenone was confirmed by comparison with an authentic sample by t.l.c. ($CHCl_3$), and by spectroscopic techniques.

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