A Simple Preparation of *O*-Phospho-L-tyrosine

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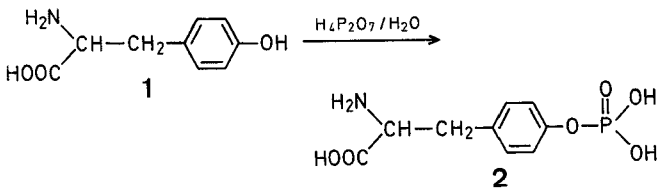
The phosphorylation of proteins is now widely recognised as an important regulatory mechanism for numerous physiological processes[1]. In living cells protein-phosphorylation occurs predominantly on serine and threonine residues. Recently, tyrosine-phosphorylation has been reported[2]. While less abundant than serine- or threonine-phosphorylation, tyrosine-phosphorylation is of great significance as it has been linked with the malignant transformation of cells by some RNA tumour viruses[3].

In view of the biological importance of tyrosine-phosphorylation, we have undertaken studies[4] on the chemistry of *O*-phospho-L-tyrosine **(2)** and synthetic peptides containing phosphorylated tyrosine. We outline a simple procedure for the preparation and isolation of optically pure **2.** While several syntheses of **2** have been published[5-11], complete characterisation in terms of spectral data is lacking and much of the physical data reported is conflicting[12]. Thus, the structure of **2** is heavily based on elemental analysis and mode of preparation. In most cases, the direct route of converting L-tyrosine **(1)** to *O*-phospho-L-tyrosine **(2)** has been followed.



The use of varying ratios of phosphorus pentoxide and phosphoric acid as the phosphorylating agent has been described[7,9,10] and product yields of 40–50% were reported. This route involved a 3 days reaction at 100 °C[13] and lengthy isolation by ion-exchange chromatography.

In a modification of the above procedures, we heated L-tyrosine in the presence of pyrophosphoric acid[8] at 80 °C and followed the conversion of **1** → **2** by H.P.L.C.[14]. After 24 h, almost quantitative conversion was obtained. This was considerably faster than previous syntheses and the high conversion obviated the inconvenient chromatography step; product **2** was precipitated from the reaction mixture using *n*-butanol.

The isolated solid was homogeneous by T.L.C., H.P.L.C., amino acid analysis, and paper electrophoresis. Its properties were identical to those described[5,6] while its structure as a phenolic monoester of phosphoric acid **(2)** was confirmed by ^{1}H-, ^{13}C-, and ^{31}P-N.M.R. spectroscopy and acid hydrolysis to tyrosine **(1)**. Its optical purity was found to be >99% by a H.P.L.C. modification of the Manning-Moore procedure[15].

***O*-Phospho-L-tyrosine (2):**

In a two-necked round bottomed 100 ml flask equipped with a magnetic stirrer/heater and a nitrogen inlet, fresh phosphorus pentoxide (10.0 g, 70.4 mmol) and 85% phosphoric acid (13.0 g) are placed. L-Tyrosine (**1**; 3.22 g, 17.8 mmol) is added and mixed with the aid of a vibramix. The reaction mixture is heated and stirred at 80 °C for 24 h; the conversion of **1** → **2** is followed by H.P.L.C.[14]. To the amber viscous liquid, water (30 ml) is added[16] and heating is continued for 30 min. The reaction mixture is cooled to room temperature, diluted with *n*-butanol (650 ml) and kept at 0 °C for 3 h. The fine white precipitate is filtered, washed successively with ice/water (2 × 20 ml), ethanol (2 × 20 ml), and ether (4 × 20 ml) to give a white powder. It is homogeneous by H.P.L.C.[14], T.L.C.[17], and paper electrophoresis[18]. Amino acid analysis gives a single peak immediately after cysteic acid while acid hydrolysis (5.7 molar hydrochloric acid, 24 h) results in complete conversion to L-tyrosine; yield[19]: 2.4 g (50%); m.p. 226–227 °C; $[\alpha]_D^{28}$: −7.8° (*c* 1, 2 molar hydrochloric acid)[20] [Ref.[7], m.p. 227 °C; $[\alpha]_D^{20}$: −8.8° (*c* 1, 2 molar hydrochloric acid)].

I.R. (Nujol): ν = 3300–2200 (OH); 1725 (C=O); 1250–1200 cm^{-1}.

U.V. (0.05 molar hydrochloric acid): λ_{max} = 265 nm (ε = 710).

^{1}H-N.M.R. (NaOD/D_2O/TMS_{ext}, pH 8.5): δ = 3.6–4.0 (m, 2H, CH_2); 4.4–4.7 (m, 1H, CH); 7.84 ppm (br. s, 4H_{arom}).

^{13}C-N.M.R. (D_2O/TMS_{ext}, pH 6.3): δ = 37.4; 57.9; 122.5 (d, J_{CP} = 4 Hz); 130.9; 132.0; 154.6 (d, J_{CP} = 6 Hz); 175.8 ppm.

^{31}P-N.M.R. (NaOD/D_2O/H_3PO_{4ext}, pH 8.5): δ = 0.0 ppm; (DCl/D_2O/H_3PO_{4ext}, pH 2.0): δ = −4.8 ppm.

Optical Purity of 2:

This is determined by a modification of the Manning-Moore procedure[15]. L-Leu-L-PTyr and L-Leu-D-PTyr are prepared and are separated on a RP18 column (0.1% triethylamine phosphate, pH 3.3/2% CH_3CN). No *O*-phospho-D-tyrosine is detected in the isolated **2.**

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1 P. Cohen, *Nature* **296,** 613 (1982).
2 B. M. Sefton, T. Hunter, K. Beeman, W. Eckhart, *Cell* **20,** 807 (1980).
3 R. Erikson, M. Collet, E. Erikson, A. Purchio, *Perspect. Virol.* **11,** 93 (1981).
4 P. Alewood, R. B. Johns, B. E. Kemp, R. Valerio, unpublished results.
5 P. Levene, A. Schormuller, *J. Biol. Chem.* **100,** 583 (1933).
6 R. H. Plimmer, *Biochem. J.* **35,** 461 (1941).
7 T. Posternak, S. Grafl, *Helv. Chim. Acta* **28,** 1258 (1945).
8 R. Ferrel, H. Olcott, H. Fraenkel-Conrat, *J. Org. Chem.* **70,** 2101 (1948).
9 H. Mitchell, K. Lunan, *Archiv. Biochem. Biophys.* **106,** 219 (1964).
10 P. Rothberg et al., *Proc. Natl. Acad. Sci. USA* **75,** 4868 (1978).
11 W. Eckhart, M. Hutchinson, T. Hunter, *Cell* **18,** 925 (1979).
12 Melting points have been reported ranging from 219 °C to 246 °C while rotations range from −2° to −9.2°.
13 Plimmer[6] reports the conversion of **1** → **2** under these conditions to be ca. 55%.

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[14] H.P.L.C. was carried out on a Water's C-18 25 cm analytical column using 0.1% H_3PO_4 as the mobile phase and monitoring at 214 nm.

[15] T. Takaya, Y. Kishida, S. Sakakibara, *J. Chromatogr.* **215**, 279 (1981).

[16] The quantity of water added is important for the successful precipitation of **2**.

[17] T.L.C. was run on silica gel plates using butanol/acetic acid/water (4 : 1 : 1).

[18] Compound **2** ran as a single ninhydrin positive spot at pH 1.9 (relative mobility to lysine, 0.07).

[19] Yields up to 91% have been achieved by standing 24 h at 0 °C. However, longer standing increases the risk of tyrosine contamination (1–3%) of **2**.

[20] As $[\alpha]_D$ values were found to be strongly dependent on temperature, solute concentration and acidity, the optical purity of **2** was determined via diastereomer separation[15].

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