Pergamon

Tetrahedron Letters, Vol. 36, No. 17, pp. 3055-3058, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)00425-4

The Chemistry of Castanospermine, Part III:[1,2] Castanospermine-6-phosphate, an Unusual Route to a Novel Compound.

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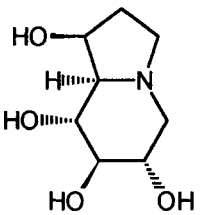
Abstract: Castanospermine-6-phosphate (**6**) is formed efficiently from 6-chloro-6-deoxycastanospermine (**2**) by an unusual nucleophilic displacement of chloride by a (di)hydrogenorthophosphate anion.

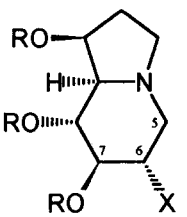
Castanospermine (**1**), a polyhydroxy alkaloid isolated from seeds of the trees *Castanospermum australe* and *Alexa leiopetala*[3,4], is a potent inhibitor of a number of mammalian and insect glycosidases[5]. It has anti-viral[6], -cancer[7], -malaria[8] and -diabetes[9] activities which are thought to arise from its glycosidase inhibitory properties. The diverse array of potentially useful activities has stimulated considerable interest in the synthesis of castanospermine and its stereoisomers and analogues[10].

We have been engaged in the synthesis of castanospermine analogues for structure - activity studies[1,2]. Having prepared 6-chloro-6-deoxycastanospermine (**2**), it was noticed that the glycosidase inhibition constants were changing as a function of time after dissolution. We then observed, by ^{13}C n.m.r. spectroscopy, that D_2O solutions of (**2**) decomposed to a small extent after several hours standing at room temperature. Prolonged standing did not result in further decomposition and we were unable to unquivocally identify the products by ^{13}C or ^{1}H n.m.r. However, we predicted, by analogy with displacement reactions of mesylates (**3**) and (**4**), that the hydrolysis product would be castanospermine (**1**), formed *via* intermediate aziridinium ion (**5**)[1]. This reaction would lower the solution pH and we reasoned that the reaction might proceed to completion if the solution was maintained at neutral pH. We therefore dissolved chloride (**2**) in 1M, pH 7 phosphate buffer. After 29 hr at room temperature, 50% of the chloride had been converted, not to castanospermine as expected, but to castanospermine-6-phosphate (**6**). After a further week at room temperature the chloride was almost entirely converted to a mixture of phosphate ester (**6**) and castanospermine in 5:1 ratio.

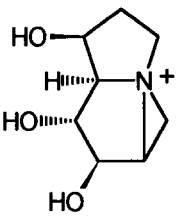
The phosphate ester (**6**) may be formed through nucleophilic displacement of chloride ion from (**2**) by (di)hydrogenorthophosphate, *via* aziridium ion (**5**), or through phosphorylation of castanospermine formed by hydrolysis of chloride (**2**). This second option was ruled out since castanospermine was inert under these reaction conditions.



(1)



(2) X=Cl, R=H
(3) X=OMs, R=Ac
(4) X=OMs, R=H
(6) X=$OPO(OH)_2$, R=H



(5)

Phosphate esters are most usually prepared synthetically through nucleophilic attack by an alcohol on a substituted phosphoryl chloride. The 'reverse' reaction, as observed in this case, whereby a nucleophilic phosphate anion attacks an alkyl chloride has been long known[11], but has found only limited synthetic use. It is exemplified by the formation of glycosyl phosphates from acyl glycosyl halides and silver dibenzyl phosphate[12], and by the reaction between tetraalkylammonium dialkylphosphates and alkyl halides in which alkyl phosphate esters are formed in competition with olefins[13]. However, the reaction we report here is unusual in that the nucleophile is unsubstituted phosphate ion and that it competes successfully with solvent water for the substrate.

The phosphate ester was purified chromatographically. The residue obtained by freeze - drying the reaction mixture (0.1 g, 0.48 mmol (**2**) in 5 ml of 1M pH 7 phosphate buffer) was taken up in water (1.0 ml) and applied to a column (60 x 1.8 cm) of Dowex X-2 cation exchange resin in the sodium form. Phosphate (**6**) was eluted with water, whilst residual chloride (**2**) and castanospermine (**1**) were retained on the column. Fractions containing phosphate (**6**) were lyophilised, redissolved in water (1.0 ml) and desalted on a column of Biogel P-2 (90 x 1.8 cm). The appropriate fractions (pH 5.4) were freeze dried to give pure phosphate (**6**) (0.025g, 0.09 mmol, 19%) as a colourless solid.

The structure of phosphate (**6**) was supported fully by both its accurate mass and its ^{1}H and ^{13}C n.m.r. spectra,[14] the resonances in which were assigned by ^{1}H ^{1}H- and ^{1}H ^{13}C- COSY experiments. In particular the equatorial disposition of the phosphate group at C-6 was clearly indicted by the large (9.2 and 11.3 Hz) diaxial coupling between H-6 and both H-7 and H-5_{ax}[1], and its presence confirmed by ^{13}C - ^{31}P coupling observed in the C-5, 6 and 7 resonances.

Acknowledgements. This work was partly funded by the ICI Corporate Carbohydrate Group (now part of Zeneca Pharmaceuticals PLC). The interest and support shown by Dr Mike Gravestock of Zeneca Pharmaceuticals has been much appreciated. We wish to thank Dr Daryl Rowan (The New Zealand Institute for Horticulture and Food Research Limited , Palmerston North) for FAB-MS and Dr Herbert

Wong for n.m.r. spectra.

REFERENCES

1. Furneaux, R.H.; Gainsford, G.J.; Mason, J.M.; Tyler, P.C. *Tetrahedron*, **1994**, *50*, 2131.
2. Furneaux, R.H.; Mason, J.M.; Tyler, P.C. *Tetrahedron Lett.*, **1994**, *35*, 3143.
3. Hohenschutz, L.D.; Bell, E.A.; Jewess, P.J.; Leworthy, D.P.; Price, R.J.; Arnold, E.; Clardy, J. *Phytochemistry*, **1981**, *20*, 811.
4. Nash, R.J.; Fellows, L.E.; Dring, J.V.; Stirton, C.H.; Carter, D.; Hegarty, M.P.; Bell, E.A. *Phytochemistry*, **1988**, *27*, 1403.
5. (a) Saul, R.; Chambers, J.P.; Molyneux, R.J.; Elbein, A.D. *Arch. Biochem. Biophys.*, **1983**, *221*, 593. (b) Elbein, A.D. *CRC Critical Reviews in Biochemistry*, **1984**, *16*, 21. (c) Winchester, B.G.; Cenci di Bello, I.; Richardson, A.C.; Nash, R.J.; Fellows, L.E.; Ramsden, N.G.; Fleet, G.W.J. *Biochem. J.*, **1990**, *269*, 227. (d) Cenci di Bello, I.; Mann, D.; Nash, R.; Winchester, B. in "Lipid Storage Disorder"; Salvayre, R.; Douste-Blazy, L.; and Gatt, S.; Ed.; Plenum: New York, **1988**; Vol. 150, pp 635. (e) Saul, R.; Ghoidoni, J.J.; Molyneux, R.J.; Elbein, A.D. *Proc. Natl. Acad. Sci. USA*, **1985**, *82*, 93. (f) Pan, Y.T.; Hori, H.; Saul, R.; Sandford, B.A.; Molyneux, R.J.; Elbein, A.D. *Biochemistry*, **1983**, 22, 3975. (g) Hori, H.; Pan, Y.T.; Molyneux, R.J.; Elbein, A.D. *Arch. Biochem. Biophys.*, **1984**, *228*, 525. (h) Furhmann, U.; Bause, E.; Ploegh, H. *Biochim. Biophys. Acta.*, **1985**, *825*, 95.
6. (a) Sunkara, P.S.; Bowlin, T.L.; Liu, P.S.; Sjoerdsma, A. *Biochem. Biophys. Res. Commun.*, **1987**, *148*, 206. (b) Schlesinger, S.; Koyama, A.H.; Malfer, C.; Gee, S.L.; Schlesinger, M.J. *Virus Research*, **1985**, *2*, 139. (c) Gruters, R.A.; Neefjes, J.J.; Tersmette, M.; De Goede, R.E.Y.; Tulp, A.; Huisman, H.G.; Miedema, F.; Ploegh, H.L. *Nature*, **1987**, *330*, 74. (d) Walker, B.D.; Kowalski, M.; Goh, W.C.; Kozarsky, K.; Krieger, M.; Rosen, C.; Rohrschneider, L.; Haseltine, W.A.; Sodroski, J. *Proc. Natl. Acad. Sci. USA*, **1987**. *84*, 8120. (e) Ruprecht, R.M.; Mullaney, S.; Anderson, J.; Bronson, R.J. *Acquired Immune Defic. Synd.*, **1989**, *2*, 149. (f) Tyms, A.S.; Berrie, E.M.; Ryder, T.A.; Nash, R.J.; Hegarty, M.P.; Taylor, D.L.; Mobberley, M.A.; David, J.M.; Bell, E.A.; Jeffries, D.J.; Taylor-Robinson, D.; Fellows, L.E. *Lancet*, **1987**, 1025. (g) Sunkara, P.S.; Taylor, D.L.; Kang, M.S.; Bowlin, T.L.; Liu, P.S.; Tyms, A.S.; Sjoerdsma, A., *Lancet*, **1989**, 1206. (h) Fleet, G.W.J.; Karpas, A.; Dwek, R.A.; Fellows, L.E.; Tyms, A.S.; Petursson, S.; Namgoong, S.K.; Ramsden, N.G.; Smith, P.W.; Son, J.C.; Wilson, F.; Witty, D.R.; Jacob, G.S.; Rademacher, T.W. *FEBS Lett.*, **1988**, *237*, 128. (i) Karpas, A.; Fleet, G.W.J.; Dwek, R.A.; Petursson, S.; Namgoong, S.K.; Ramsden, N.G.; Jacob, G.S.; Rademacher, T.W. *Proc. Natl. Acad. Sci. USA*, **1988**, *85*, 9229.

7. (a) Trugnan, G.; Rousset, M.; Zweibaum, A. *FEBS Lett.*, **1986**, *195*, 28. (b) Humphries, M.J., Matsomoto, K.; White, S.L.; Olden, K. *Cancer Res.*, **1986**, *46*, 5215. (c) Ahrens, P.B.; Ankel, H. *J. Biol. Chem.*, **1987**, *262*, 7575. (d) Dennis, J.W.; Laferte, S.; Wahorne, C.; Breitman, M.L.; Kerbel, R.S. *Science*, **1987**, *236*, 582. (e) Dennis, J.W. *Cancer Res.*, **1986**, *46*, 5131. (f) Ostrander, G.K.; Scribner, N.K.; Rohrschneider, L.R. *Cancer Res.*, **1988**, *48*, 1091.
8. Bitonti, A.J.; McCann, P.P. *Eur. Patent Appl.*, **1990**, EP423,728.
9. (a) Rhinehart, B.L., Robinson, K.M.; Payne, A.J.; Wheatley, M.E.; Fisher, J.L.; Liu, P.S.; Cheng, W. *Life Sci.*, **1987**, *41*, 2325. (b) Liu, P.S.; Rhinehart, B.L. *Eur. Patent Appl.* **1986**, EP202,661. (c) Liu, P.S.; Daniel, J.K.; Rhinehart, B.L. *Eur. Patent Appl.*, **1988**, EP297,534.
10. Burgess, K.; Henderson, I. *Tetrahedron*, **1992**, *48*, 4045 and references therein. Ina, H.; Kibayashi, C. *J. Org. Chem.*, **1993**, *58*, 52. Gerspacher, M.; Rapoport, H. *J. Org. Chem.*, **1991**, *56*, 3700. Lee, C.K.; Sim, K.Y.; Zhu, J. *Tetrahedron*, **1992**, *48*, 8541. Mulzer, J.; Dehmlow, H.; Buschmann, J.; Luger, P. *J. Org. Chem.*, **1992**, *57*, 3194. Burgess, K.; Chaplin, D.A.; Henderson, I.; Pan, Y.T.; Elbein, A.D. *J. Org. Chem.*, **1992**, *57*, 1103. Gradnig, G.; Berger, A.; Grassberger, V.; Stutz, A.E.; Legler, G. *Tetrahedron Lett.*, **1991**, *32*, 4889.
11. Emsley, J. and Hall, D. *The Chemistry of Phosphorus*, John Wiley and Sons, New York, 1976.
12. Ferrier, R.J. in *Carbohydrate Chemistry* (ed. John F. Kennedy). Oxford University Press, Oxford, 1988.
13. Sturtz, G. and Rio, M. *Bull. Soc. Chim. Fr.*, **1974**, (9-10, Pt.2), 2187-2192.
14. For (6). Accurate mass (FAB); calc. for $C_8H_{17}O_7NP$ (MH^+) 270.0743; obs. 270.0751. ^1H n.m.r. (500 MHz) (D_2O, acetone δ 2.217 as int. std) 4.62 (m, H-1) 4.18 (m, H-6) 3.86 (dd, J=10.5, and 9.8, H-8) 3.78 (dd, J=12.0, and 5.4, H-5_{eq}) 3.70 (m, H-3) 3.66 (t, J=9.2, H-7) 3.11 (dd, J=11.2, and 6.6, H-3') 3.06 (dd, J=10.5, and 3.4, H-8a) 2.94 (t, J=11.3, H-5_{ax}) 2.49 (m, H-2) 2.02 (m, H-2'). ^{13}C n.m.r. (125 MHz) (D_2O, acetone δ 33.17 as int. std.) 79.3 (d, J=5.2± 0.5 Hz, C-7) 74.3 (d, J=5.3± 0.5 Hz, C-6) 73.8 (C-8a); 70.6 (C-1); 69.3 (C-8); 55.0 (d, J=2.8± 0.5 Hz, C-5) 54.7 (C-3); 34.6 (C-2).

(*Received in UK* 17 *February* 1995; *accepted* 3 *March* 1995)