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Synthesis of an Artificial Phosphate Bio-isostere of Glucotropaeolin

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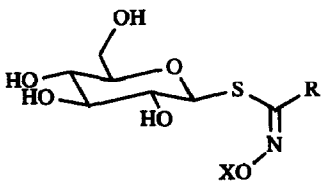
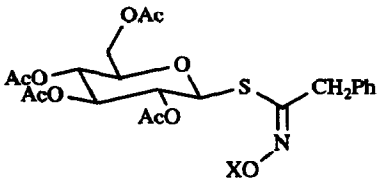
Key Words: *glucosinolates; myrosinase; thiohydroximate; phosphorylation.*

Abstract: A synthetic sequence was devised to produce phospho-glucotropaeolin **6**, the first representative of phosphate bio-isosteres of naturally-occurring glucosinolates with a view to enzymatic studies.

Glucosinolates (GSL) **1** are widespread secondary plant metabolites which occur mainly in the botanical family *Cruciferae*. These S-glucopyranosyl thiohydroximates play a key biological role in the metabolism and the catabolism of the many diverse vegetable species, notably in association with myrosinase (EC 3.2.3.1) - the enzyme controlling the degradation pathways of GSL.[1]

In order to carry out detailed investigations of myrosinase activity, modified GSL-like substrates are required : a wide range of sugar variants of natural GSL has thus already been synthetically elaborated in our laboratory.[2-4] On the other hand, the anionic site of GSL being critical in the recognition process by myrosinase,[5] replacement of the O-sulfate moiety by another anion, *viz.* O-phosphate, appeared of prime interest. Glucotropaeolin **2**, which has recently become commercially available for HPLC standardization, is a convenient model substrate for comparative enzymatic studies.





1 X = SO^-_3, > 100 different R

2 X = SO^-_3, R = CH_2Ph

6 X = $PO^{=}_3$, R = CH_2Ph

3 X = H

4 X = $PO(OCH_2Ph)_2$

5 X = $PO^{=}_3$, $(Et_3NH^+)_2$

The key intermediate (Z)-thiohydroximate **3** was elaborated through reaction of 1-thio-β-D-glucose tetraacetate with phenylacethydroximoyl chloride, following a modified version[6] of Benn's procedure.[7] Dibenzyl chlorophosphate - prepared[8] from dibenzyl phosphite - was used to effect the phosphorylation of **3** according to a protocol developed in our laboratory (Et_3N, benzene, -10 to 25°C, 24h)[9] to furnish phosphotriester **4** in 77% yield.[10]

Removal of the benzyl groups in **4** was achieved by catalytic hydrogenolysis (10% Pd/C, MeOH, Et_3N, 25°C, 1h)[11] to give a 60% yield of the phosphate **5** in the form of its bis(triethylammonium) salt.[12]

Finally, **5** was submitted to de-O-acetylation (saturated methanolic solution of NH_3, 25°C, 3h) to produce **6**, which was isolated in 90% yield in the form of its disodium salt[13] after elution from a Dowex 50X8 (Na^+ form) ion-exchange column and freeze-drying.

Preliminary enzymatic assays[14] indicate that **6**, when submitted to the action of myrosinase, undergoes hydrolysis albeit with modified kinetic parameters as compared with glucotropaeolin **2**. Extension of the synthetic sequence to other GSL isosteres is under development.

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References and notes

1. Fenwick, G.R.; R.K. Heaney, R.K.; Mullin, W.J. *CRC Critical Rev. in Food Sci. and Nutrition* **1983**, *18*, 123-201.

2. Blanc-Muesser, M.; Driguez, H.; Joseph, B.; Viaud , M.C.; Rollin, P. *Tetrahedron Lett.* **1990**, *31*, 3867-3868.

3. Gardrat, C.; Quinsac, A.; Joseph, B.; Rollin, P. *Heterocycles* **1993**, *35*, 1015-1027.

4. Joseph, B.; Rollin, P. *J. Carbohydr. Chem.* **1993**, *12*, 719-729.

5. Palmieri, S.; Iori, R.; Joseph, B.; Rollin, P. *Proceedings du Colloque Glucosinolates*, 13-14/01/1993, Ardon (France).

6. Brochard, L.; Joseph, B.; Viaud, M.C.; Rollin, P. *Synthetic Commun.*, in press.

7. Benn, M.H. *Can. J. Chem.* **1963**, *41*, 2836-2838.

8. Kenner, G.W.; Todd, A.R.; Weymouth, F.J. *J. Chem. Soc.* **1952**, 3675-3681.

9. Lazar, S.; Jabbouri, S.; Moisand, C.; Hocquet, S.; Meunier, J.C.; Ropars, C.; Guillaumet, G. *Eur. J. Med. Chem.* **1994**, *29*, 45-53.

10. Satisfactory spectral (^{1}H- and ^{31}P-NMR, MS) data were obtained for all new compounds reported. **4**: $[\alpha]_D$ +10 (*c* 1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ (ppm), J (Hz): 1.94, 1.97, 2.03, 2.11 (4s, 12H, Ac), 3.44-3.53 (m, 1H, H_5), 3.98 (dd, 1H, $J_{5,6b}$ 2.2, $J_{6a,6b}$ 12.5, H_{6b}), 4.04 (s, 2H, CH_2Ph), 4.15 (dd, 1H, $J_{5,6a}$ 5.1, H_{6a}), 4.75 (d, 1H, $J_{1,2}$ 10.3, H_1), 4.90-5.05 (m, 3H, H_2, H_3, H_4), 5.18 (d, 4H, $^3J_{H,P}$ 8.1, OCH_2Ph), 7.19-7.40 (m, 15H, H_{Ar}).

11. Srivastava, G.; Hindsgaul, O.; Palcic, M.M. *Carbohydr. Res.* **1993**, *245*, 137-144.

12. **5**: $[\alpha]_D$ -8 (*c* 1, MeOH); ^{1}H-NMR (DMSO-d_6) δ (ppm), J (Hz): 1.16 (t, 18H, $\underline{CH_3}CH_2N$), 1.92 (s, 6H, Ac), 1.95, 1.97 (2s, 6H, Ac), 3.01 (q, 12H, $CH_3\underline{CH_2}N$), 3.76 (dd, 1H, $J_{5,6b}$ 2.3, $J_{6a,6b}$ 12.5, H_{6b}), 3.85-3.98 (m, 3H, H_5, CH_2Ph), 4.03 (dd, 1H, $J_{5,6a}$ 6.0, H_{6a}), 4.85 (ft, 1H, J_{vic} 9.5, H_2), 4.92 (ft, 1H, J_{vic} 9.5, H_4), 5.29 (ft, 1H, J_{vic} 9.5, H_3), 5.32 (d, 1H, $J_{1,2}$ 9.6, H_1), 7.20-7.35 (m, 5H, H_{Ar}), 10.16 (bs, NH^+).

13. **6**: $[\alpha]_D$ -15 (*c* 1, H_2O); ^{1}H-NMR (D_2O) δ (ppm), J (Hz): 3.21-3.53 (m, 4H, H_2, H_3, H_4, H_5), 3.60-3.74 (m, 2H, H_{6a}, H_{6b}), 4.07 (s, 2H, CH_2Ph), 4.70 (d, 1H, $J_{1,2}$ 9.5, H_1), 7.23-7.53 (m, 5H, H_{Ar}).

14. Palmieri, S.; Iori, R., personal communication.

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