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THE FORMATION OF 2-HYDROXYBUT-3-ENYL CYANIDE FROM (2S)-2-HYDROXYBUT-3-ENYL GLUCOSINOLATE USING IMMOBILIZED MYROSINASE

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Abstract. Starting from (2S)-2-hydroxybut-3-enylglucosinolate (epi-progoitrin) isolated from *Crambe abyssinica* seeds the chiralic 2-hydroxy-3-butenyl cyanide was produced in pure form and acceptable yield, using immobilized myrosinase purified from *Sinapis alba* on a Nylon 6.6 membrane.

Natural glucosinolates (GLs) are well known secondary plant compounds, which, together with the enzyme myrosinase (thioglucoside glucohydrolase EC 3.2.3.1.), are particularly abundant in the seeds of the Cruciferae. The myrosinase catalyzes their hydrolysis to produce D-glucose, sulfate, and a series of isothiocyanates, thiocyanates, and nitriles, depending on both the substrate and the reaction conditions[1].

GLs and their aglycon derivatives are present in significant amounts in proteic, defatted meals from oil cruciferous seeds and are generally associated with endemic hypothyroidism and hepatotoxicity in humans and animals. However, recent results make these compounds interesting for new technological applications, one being the production of chiral cyanides starting from hydroxylated GLs.

The enzymatic reaction at neutral pHs of these GLs, e.g. (2R and 2S)-2-hydroxybut-3-enylglucosinolate (progoitrin and epi-progoitrin), does not produce the corresponding isothiocyanates, as are generally formed as the final products with other GLs. As reported in Fig. 1, they have a short life and generally cyclize to produce 5-vinyl-oxazolidine-2-thiones, whereas the formation of nitriles and

epithionitriles can be obtained at acidic pHs and in the presence of Fe^{2+}. In latter case, however, in addition to myrosinase, an epithiospecifier protein (ESP) is required.

Our aim in this paper is to point out (i) the great potential of progoitrin and epiprogoitrin, which can be obtained in large amounts as byproducts of the detoxication of defatted meals of cruciferous seeds with high erucic acid contents, such as oilseed rape (*Brassica napus*) and crambe (*Crambe abyssinica*) and (ii) the advantageous use of immobilized myrosinase for producing chiralic 2-hydroxy-3-butenyl cyanide (HBC).

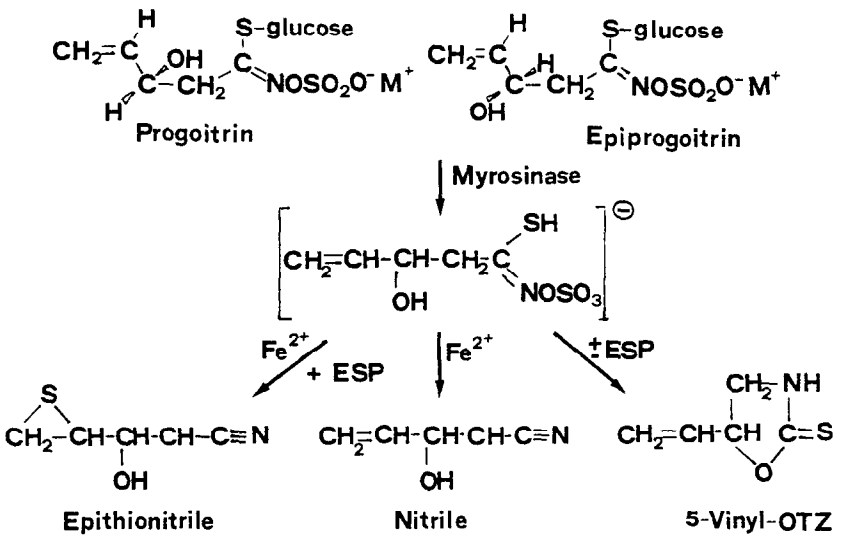

Fig.1 Pathway of the myrosinase-catalyzed hydrolysis of (epi) progoitrin to form 2-hydroxybut-3-enyl cyanide.

Epi-progoitrin (8.8 g) was isolated starting from seeds (400 g) of *Crambe abyssinica* cv. Belenzian following a procedure recently reported[2]. HPLC analysis of the GLs[3] in the final product, showed the predominance (ca. 90%) of epiprogoitrin.

Myrosinase from *Sinapis alba*[4] before immobilization on a Nylon 6.6 membrane[5] showed a specific activity of ca. 78 units/mg, whereas the final immobilized activity was more than 5 units/cm^2, which corresponds to 14 mg protein/g of membrane. Although the enzymatic reaction can be carried out continuously in a small bioreactor containing the immobilized myrosinase[6], ca. 5 mg of HBC were produced

in batch. This amount of HBC sufficied for the following analytical evaluation.

The enzymatic reaction was carried out with immobilized myrosinase on a 16.8 cm^2 Nylon 6.6 membrane at 37 °C, using a substrate concentration of 3.5 mg/ml in a total volume of 10 ml in 0.2 M MES pH 5.0, in the presence of 2.5 mM $FeSO_4$ and 5 mM cysteine[7]. The reaction was followed by monitoring the decrease of absorbance at 227nm due to glucosinolate hydrolysis and the formation of the 5-vynil oxazolidine-2-thione (VOT) by HPLC[8]. In these reaction conditions, the epi-progoitrin was completely hydrolyzed after about one hour. At the end of the hydrolysis the active membrane was removed and the reaction mixture loaded in a RSil C_{18} HL (Chemie Uetikon, RSL) column (1x10cm). This procedure completely removed the VOT, which was produced by cyclization of 2-hydroxy-3-butenyl isothiocyanate. The eluate was extracted by methylene chloride and then concentrated in a rotary evaporator.

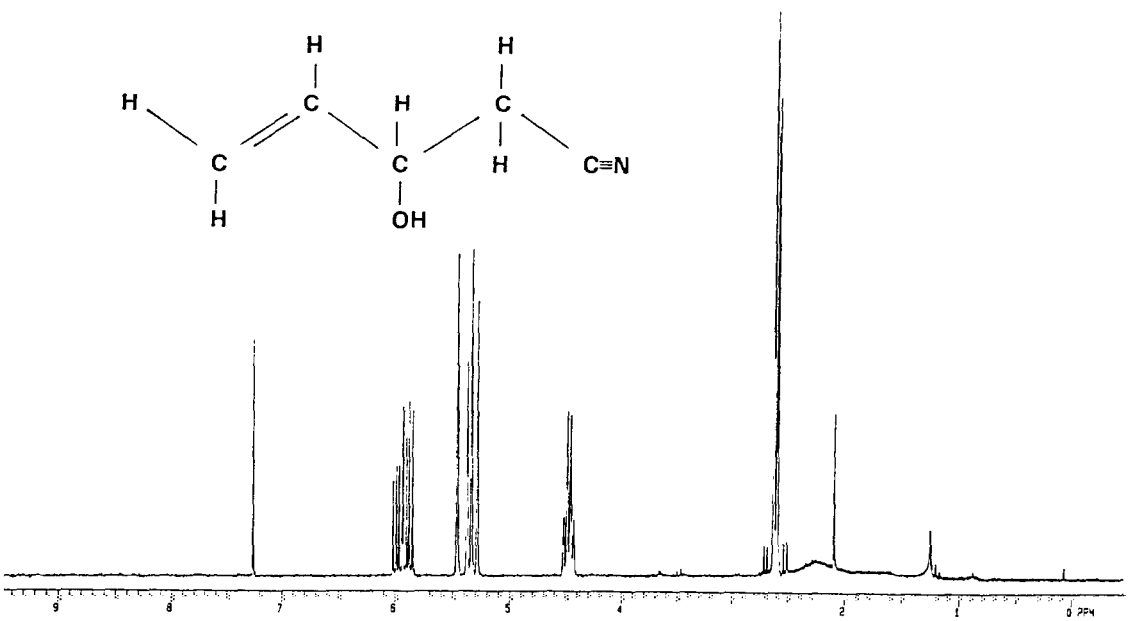

Fig.2. ^1H NMR spectrum of 2-hydroxybut-3-enyl cyanide obtained by the myrosinase-catalyzed hydrolysis of (2S)-2-hydroxybut-3-enyl glucosinolate.

The product was analyzed by TLC, comparing the Rf with an authentic HBC sample opportunely synthesized with standard procedures[9]. In addition, the sample was analyzed both by GLC using a

Rtx 2330 capillary column (Restek) for the qualitative and quantitative determination of the HBC and by HPLC using a C_8 RP column (Hewlett Packard) to evaluate the VOT. This procedure allows one to obtain HBC with a 15% yield, which is 65% of the theoretical yield.

The ^1H NMR spectrum (Fig. 2) confirms the structure of HBC, whose polarization index, $[\alpha]^{20}_D$, is -0.22°, (c =1.6, $CHCl_3$). The IR spectrum confirms the structure of Fig. 2. In fact, IR ν_{max} (liquid film): 3436 (OH), 2246 (C≡C), 1647 (C=C).

In conclusion, we wish to emphasize that HBC can be easily produced by tranforming (epi-)progoitrin, a natural, undesirable compound present in high amounts (ca. 10%) in the defatted high proteic meals of cruciferous seeds with high erucic-acid contents. HBC, like many other chiralic compounds, is an interesting chemical intermediate, potentially useful for producing new compounds for industrial and fine chemistry.

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References

1. Fenwick, R.G.; Heaney, R.K.; Mullin, W.J. *CRC Crit. Rev. Food Sci. Nutr.* **1983**, *18*, 123-201.
2. Visentin, M.; Tava, A.; Iori, R.; Palmieri, S. *J. Agric. Food Chem.* **1992**, *40*, 1687-1691.
3. Buchner, R. *Glucosinolates in Rapeseed: Analytical Aspects*; Wathelet J.P., Ed.; M, Nijhoff Publishers: Dordrecht, **1987**: pp. 76-79.
4. Palmieri, S.; Iori, R.; Leoni, O. *J. Agric. Food Chem.* **1986**, *34*, 138-140.
5. Leoni, O.; Iori, R.; Palmieri, S. *J. Agric. Food Chem.* **1991**, *39*, 2322-2326.
6. Leoni, O. et al. in preparation.
7. Uda, Y.; Kurata, T.; Arakawa, N. *Agric. Biol. Chem* **1986**, 50, 2741-2746
8. Quinsac, A.; Ribaillier, D.; Rollin, P.; Dreux, M. *JAOAC* **1992**, *75(3)*, 529-536
9. Das, N.B.; Torsell, K.B.G. *Tetrahedron* **1983**, *39*, 2247-2253.

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