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Preparation of (5R)-5-Vinyloxazolidine-2-thione from Natural Epi-Progoitrin Using Immobilized Myrosinase

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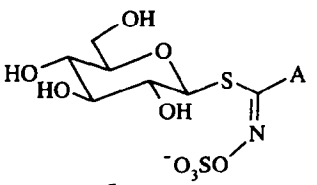
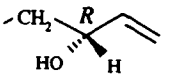
Abstract: Starting from (2S)-2-hydroxybut-3-enyl glucosinolate, i.e. epi-progoitrin **3**, isolated from *Crambe abyssinica* ripe seeds, (5R)-5-vinyloxazolidine-2-thione (VOT) **8** was produced in enantiomerically pure form and high yield using a small bioreactor containing myrosinase - purified from *Sinapis alba* - immobilized on 18-36 mesh granular Nylon 6.6.

Glucosinolates (GSL) **1** constitute a structurally homogeneous family of more than 100 miscellaneous secondary plant metabolites[1] which are contained, together with the enzyme myrosinase (thioglucoside glucohydrolase EC 3.2.3.1) in the seeds of Cruciferae. Myrosinase catalyzes the hydrolysis of GSL to D-glucose, sulfate ion, and a series of sulfur- and/or nitrogen-containing compounds such as thiocyanates, isothiocyanates, oxazolidinethiones, nitriles, etc...depending on the substrate and on the reaction conditions.[2]

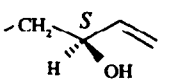


1

2 progoitrin

A = 

3 epi-progoitrin

A = 

GSL **1** and their breakdown products are often present in significant amounts in proteic, defatted meals which can be obtained from many cruciferous oil-bearing seeds. Whereas GSL in native form usually show low biological activity, their breakdown products often display antinutritional properties, generally associated with endemic hypothyroidism and hepatotoxicity in mammals.[3] On the other hand, recent papers indicate that many of these GSL-derived compounds can show therapeutical activities, particularly in the field of cancer prevention.[4-6]

One of our last papers[7] describes a biotechnological process for the production of a chiral hydroxy-nitrile from epi-progoitrin **3**, a GSL which can potentially be obtained in large amounts from crambe (*Crambe abyssinica*) seed meal. The use of the immobilized myrosinase-technology appears very attractive in the case of GSL **3** to produce either 2-hydroxybut-3-enyl cyanide **5**, epithionitrile **6** or (5R)-VOT **8** depending on the reaction conditions (Fig. 1).

In the last case, the isothiocyanate resulting from the Lossen rearrangement of the hydrolytic intermediate is not isolated as in most GSL cases, but rather undergoes a fast cyclization[8] into (5R)-VOT **8.**

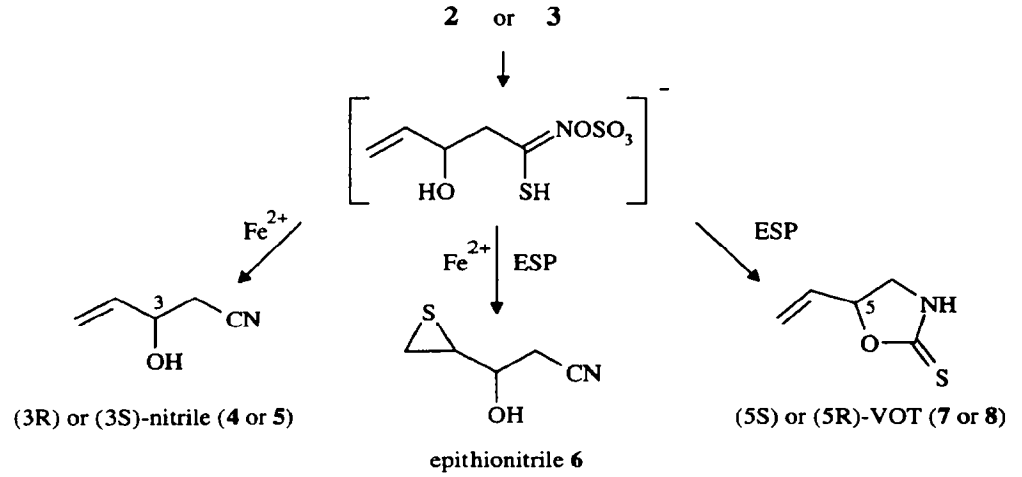


Fig. 1. Pathways of the myrosinase-catalyzed hydrolysis of progoitrin **2** or epi-progoitrin **3** (ESP : epithio-specifier protein).

With the present paper, we would like to:

- stress the high chemical potential of epi-progoitrin **3**, which could be obtained in large amounts as a by-product of crambe-oil production following the known processes in use for oil-seed rape.[9,10]
- point out the ease with which (5)-VOT can be produced continuously using a small bioreactor containing immobilized myrosinase.

Epi-progoitrin **3** (5.2 g) was isolated starting from seeds (400 g) of *Crambe abyssinica*, cv. Belenzian, following a reported procedure.[11] Polarographic determination of total GSL content[12] coupled with HPLC analysis of the end product[13] showed a GSL percentage of 75% (contamination by water and K_2SO_4), and a clear predominance of **3** (ca. 90%).over the other GSL.

Myrosinase was isolated from sound seeds of *Sinapis alba* and purified following a previously described protocol.[14] Initially effected on Nylon 6.6 membrane[15], immobilization of the enzyme was here achieved on granular Nylon 6.6 (18-36 mesh particles).[16,17] The specific activity of the enzyme was ca. 60 units/mg protein before immobilization, whereas the final immobilized activity reached 325 units/g solid.

The enzymatic reaction was carried out continuously in a thermostated (37°C) small column (Ø 2.6, L 2.8 cm), using a substrate concentration of 5 mg/ml in 0.1 M phosphate buffer pH 6.5, a total volume of 1000 ml and a ca. 40 ml/h flow-rate. The reaction was followed polarographically[12] and/or by monitoring the absorbance increase to 240 nm due to the formation of VOT. In such reaction conditions, epi-progoitrin **3** underwent complete hydrolysis. In contrast with the results reported[7] for the preparation of the corresponding hydroxy-nitrile **5**, no further purification was required to obtain high-grade VOT after the following work-up : *in vacuo*

evaporation of the eluate, solubilization of the residue in dichloromethane, filtration through anhydrous Na_2SO_4, final evaporation giving **8** in crystalline form (m.p. 48°C).[18]

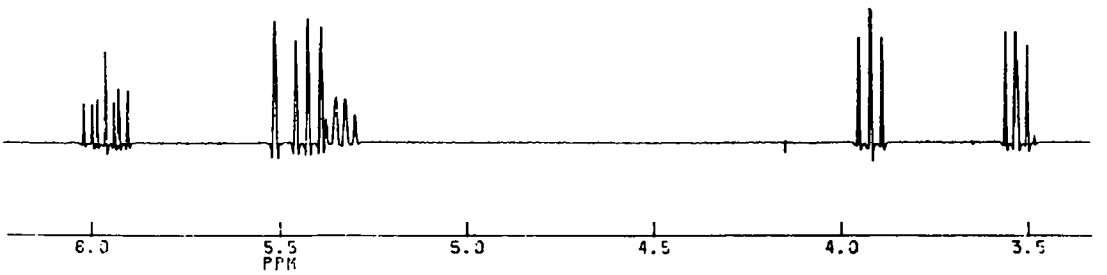


Fig. 2. 300 MHz ^{1}H-NMR ($CDCl_3$) spectrum of (5R)-5-vinyloxazolidine-2-thione **8** in the 3-6 ppm region.

HPLC analysis[19] (Hewlett-Packard mod. 1090L) using a C_8 RP column, shows the reaction product to be nearly homogeneous; the reaction yield, determined by UV spectrophotometry (ε 16,000 $M^{-1}.cm^{-1}$), was 90%. The IR-spectrum (KBr, ν_{max} (cm^{-1}) 3215 (NH), 1746, 1531 (C=S), 1171 (C-O stretching))[20] and the ^{1}H-NMR spectrum (Fig. 2)[21] confirm the structure of our compound, which displays a specific rotation $[\alpha]_D^{20}$ = + 72 (*c* 1.0, $CHCl_3$).[18]

Finally, mass spectrometry data (EI, VG-16F instrument) obtained were in agreement with earlier reported values.[22]

In summary, immobilized myrosinase has proven to be a very efficient and stable biocatalyst for producing several interesting chiral compounds, starting from GSL **1**. In this specific case, enantiomerically pure (5R)-VOT **8** can be easily prepared in high yield by hydrolyzing epi-progoitrin **3**, a natural, undesirable compound which is present in large amounts (ca. 10% w/w) in the defatted meals produced through the milling of several cruciferous seeds with high erucic acid content.

VOT is a structurally-striking representative of 2-thioxotetrahydro-1,3-O,N-heterocycles, an important class of heterocyclic compounds which have attracted unremitting attention from both chemical and pharmaceutical points of view. In particular, the use of oxazolidine-2-thiones as chiral auxiliaries has promoted the syntheses of optically pure, substituted derivatives of such templates.[23]

Depending on the naturally-occurring starting material involved - progoitrin **2** or epimeric **3** - the present methodology is well suited for the production of (5S)- and (5R)-VOT, respectively. Investigation of a broad array of stereocontrolled chemical modifications of both enantioforms is currently under way in our laboratories.

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21. 300 MHz ^{1}H-NMR ($CDCl_3$) δ ppm (J Hz) 3.53 (dd, 1H, $J_{4a,4b}$ 9.5, $J_{4b,5}$ 8.3, H-4b), 3.92 (ft, 1H, $J_{4a,5}$ 9.4, H-4a), 5.33 (ddd, 1H, H-5), 5.40 (d, 1H, $J_{6,7Z}$ 10.5, H-7Z), 5.48 (d, 1H, $J_{6,7E}$ 17.0, H-7E), 5.96 (ddd, 1H, $J_{5,6}$ 6.7, H-6), 7.40 (bs, 1H, NH); see also : Gardrat, C.; Latxague, L.; Picard, J.P. *J. Heterocycl. Chem.* 1990, **27**, 811-812.
22. Main fragmentation peaks : m/z 129 (M, 100), 68 (44.8), 57 (15.6), 43 (11.9), 42 (24.2), 41 (20.5), 39 (20.4), 29 (10.4), 28 (11.9), 27 (13.0); cf. Zenker, N.; Hubbard, L.S.; Wright, J. *J. Nat. Prod.* 1988, **51**, 862-865.
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