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FORMATION OF GLUCORAPHANIN BY CHEMOSELECTIVE OXIDATION OF NATURAL GLUCOERUCIN: A CHEMOENZYMATIC ROUTE TO SULFORAPHANE

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Abstract. A new semi-synthetic way to produce glucoraphanin (**2**), the bio-precursor of the potential anticarcinogen sulforaphane (**3**), has been developed. Starting from glucoerucin (**1**), isolated from ripe seeds of *Eruca sativa*, glucoraphanin was obtained through chemoselective oxidation. Controlled myrosinase-catalysed hydrolysis of this precursor quantitatively afforded sulforaphane.

Glucosinolates (GLs) and their hydrolysis products have so far mainly been studied with respect to their antinutritional effects - mostly hypothyroidism and hepatotoxicity - whereas some of them have been recently reevaluated with regard to their interesting biological activity.[1] Recent results demonstrate the importance of some GL-derived isothiocyanates (ITCs) for their role in cancer prevention. In particular, both natural and synthetic sulforaphane (**3**) were shown to be the most potent inducers of the anticarcinogenic marker phase II enzymes, such as glutathione S-transferase and quinone reductase,[2] which are involved in the detoxification of xenobiotic compounds assimilated with diet and environment. The application in pharmacology of **3** has also been patented.[3] In addition, it was recently demonstrated that a crude broccoli freeze-dried extract, in which glucoraphanin **2** was the main GL component, showed a protective role against mammary tumour formation in rats fed with the carcinogen 7,12-dimethylbenz(*a*)anthracene.[4] Debates concerning the effects of ITCs and GLs in the dietary implications are ongoing.[5] From these results immediately stands out the strong interest for pure intact GLs as inhibitors of cancer development.

In broccoli seeds or sprouts **2** represents 1-2% of dry weight, and it is associated with other GLs, *viz.* glucoiberin, glucoerucin (**1**), 4-hydroxy-glucobrassicin, progoitrin, glucoibervirin and glucobrassicin, which together represent the 40-50% of the total GLs content. Although the isolation of **2** from the GLs mixture can be achieved by HPLC,[6] this technique is not suitable for large-scale preparations.

Taking into account these findings, we have developed a new procedure for producing **2** in quantitative yield starting from **1**, the GL present mainly in nearly pure form and good amount (ca. 3%) in rocket (*Eruca sativa* Miller) ripe seeds. This procedure makes it possible to readily produce **2** on the gram-scale and it also

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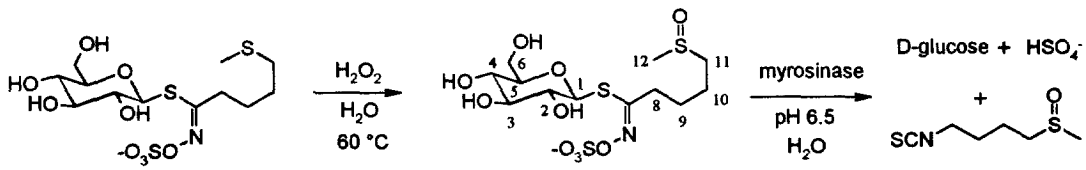
appears to be suitable for large-scale production, considering that **1** can be easily isolated from rocket seeds in almost homogeneous form following a well protocol developed in our laboratory.[7]

The conversion of **1** into **2** is based on the oxidation reaction of sulfides into the corresponding sulfoxides. This reaction did not affect the anomeric thiohydroximate function which reacts much slower under the fixed conditions.[8]



glucoerucin (**1**) glucoraphanin (**2**) sulforaphane (**3**)

As expected, the synthetic **2** produced is a 1:1 mixture of sulfoxide epimers (α_D = -15, *c* = *1* in H_2O) whose purity can be assessed by HLPC analysis according to the method ISO 9167-1. The high-field ^1H and ^{13}C NMR data (Bruker AMX 500 spectrometer operating at 500 and 125.7 MHz respectively) could not discriminate between the diastereomeric sulfoxides.[9]

The production of racemic **3** by myrosinase-catalysed hydrolysis (37 °C; pH 6.5 buffer) of synthetic **2** was also investigated. Compound **3** was analysed according to the method reported by Chiang *et al.*[10] In addition, the GC profile and MS spectrum confirmed the structure of **3** and its purity.

In conclusion, we wish to emphasize that **2** was easily produced oxidizing **1** and that this procedure is suitable for large-scale production of this GL,[11] which is important at least to try and make clear the mechanism of its potential anti-cancer protective ability.

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8. Typical procedure: To an aqueous solution of **1** (3g in 80 mL water) was added 3 mL of a hydrogen peroxide solution (35 wt % in water) and the mixture was maintained at 60°C for 30 min. Compound **2** was isolated following our standard method (see ref. 7).
9. ^1H NMR (D_2O) : 1.93 (m, 2 H, H-10), 1.98 (m, 2 H, H-9), 2.77 (m, 3 H, H-12), 2.88 (t, 2 H, $J_{8,9}$ = 6.8 Hz, H-8), 3.02 (m, 2 H, H-11), 3.54 (m, 2 H, H-2 and H-4), 3.65 (m, 2 H, H-3 and H-5), 3.78-3.97 (2 dd, 2 H, $J_{6a,6b}$ = 12.5 Hz, $J_{6a,5}$ = 1.7 Hz, $J_{6b,5}$ = 5.4 Hz, H-6a and H-6b), 5.12 (d, 1 H, $J_{1,2}$ = 9.5 Hz, H-1) ; ^{13}C NMR (D_2O) : 40.0 (C-10), 44.3 (C-9), 50.5 (C-8), 55.3 (C-12), 71.0 (C-11), 79.5 (C-6), 88.0 (C-4), 96.0 (C-3), 99.0 (C-5), 100.6 (C-1), 182.5 (C-7).
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