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Chemo-enzymatic preparation from renewable resources of enantiopure 1,3-oxazolidine-2-thiones

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Abstract

Chiral 1,3-oxazolidine-2-thiones were prepared in enantiopure form from renewable resources through an enzymatic process involving immobilized myrosinase (thioglucoside glucohydrolase E.C. 3.2.3.1).

1. Introduction

Chiral auxiliary methodologies are proving to be increasingly useful for the asymmetric synthesis of many classes of compounds. Chiral 1,3-oxazolidin-2-one[1] and 1,3-oxazolidine-2-thione[2] heterocyclic templates have proven to be remarkably versatile and efficient auxiliaries (Fig. 1).

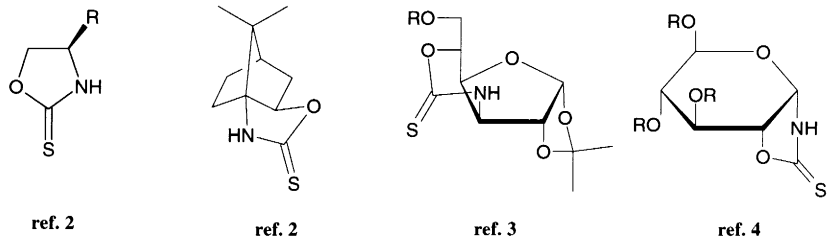

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Fig. 1.

The development of five membered 2-thioxo-*O*,*N*-heterocycles as chiral auxiliaries has motivated the search for enantiomerically pure representatives. As chiral auxiliaries, oxazolidine-2-thiones[5] show some advantages when compared to their 2-oxo-analogs: they exhibit a strong UV absorption and they have

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been claimed to be more efficient in aldol-type reactions involving complex molecules.[6] Moreover, they can be readily transformed into their oxo counterparts.[7]

In contrast with 1,3-oxazolidinones which require *N*-metallation prior to acylation, 2-thioxo-*O*,*N*-heterocyclic systems can be acylated using mild procedures.[8] An extra advantage of oxazolidinethiones consists in their easy removability under smooth conditions.[9]

Enantiomerically pure oxazolidine-2-thiones are generally prepared in basic medium from chiral α-aminoalcohols and carbon disulfide.[10] We herein report a chemo-enzymatic process to furnish miscellaneous enantiopure 1,3-oxazolidine-2-thiones from vegetal sources.

2. Results and discussion

Glucosinolates (GSL) are naturally occurring thio-sugars mainly found in *Brassicaceae*[11]-mustard, cabbage, broccoli, turnip, radish, rape, etc. More than 100 GSL have been identified to date, differing only in the nature of the aglyconic side chain A (Fig. 2).

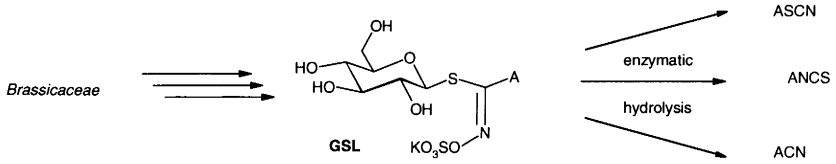

Fig. 2.

In the *Brassicaceae* family (and also in *Capparidaceae*), every plant species displays a characteristic individual 'GSL profile' which can be relatively simple in some cases. Enzyme catalysed hydrolysis of GSL by myrosinase (thioglucoside glucohydrolase EC 3.2.3.1) affords D-glucose, sulfate anion and a series of sulfur and nitrogen-containing compounds principally isothiocyanates, whose bioactivity is well documented.[12] In cases when the side-chain A carries a hydroxyl group in β-position to the isothiocyanate function, spontaneous cyclization occurs to yield enantiopure 1,3-oxazolidine-2-thiones (Fig. 3).

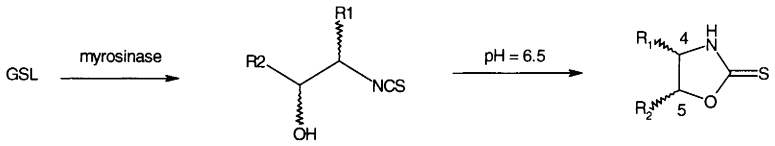

Fig. 3.

Using a bioreactor containing myrosinase immobilized on Nylon 6.6,[13] we have developed a simple and effective process to prepare different kinds of bioactive compounds starting from pure native GSL isolated from different cruciferous seeds. This process was shown to be very efficient for the production on a gram scale of diverse 1,3-oxazolidine-2-thiones in enantiopure form (see Table 1). Those molecules are being currently tested in our laboratory to perform stereoselective aldol reactions[6] and Michael additions.[14]

3. Experimental

Melting points were determined with a Tottoli apparatus (Büchi SMP 20) and are uncorrected. Specific rotations were measured at 20°C using a Perkin–Elmer polarimeter 141. ^{1}H and ^{13}C NMR spectra

Table 1

Glucosinolate Side chain A	Vegetable	1,3-Oxazolidine-2-thione	Reference
Progoitrin (2*R*)-2-hydroxybut-3-enyl	*Brassica napus*	**(-)-(5*S*)-5-vinyl- (1)**	17
***Epi*-progoitrin** (2*S*)-2-hydroxybut-3-enyl	*Crambe abyssinica*	**(+)-(5*R*)-5-vinyl- (2)**	18
Gluconapoleiferin (2*S*)-2-hydroxypent-4-enyl	*Brassica napus*	**(-)-(5*S*)-5-(prop-2-enyl)- (3)**	19
Glucobarbarin (2*S*)-(2-hydroxy-2-phenyl)ethyl	*Barbarea vulgaris*	**(-)-(5*R*)-5-phenyl- (4)**	19, 20
Glucosisymbrin (2*S*)-(2-hydroxy-2-methyl)ethyl	*Sisymbrium loeseli*	**(+)-(4*S*)-4-methyl- (5)**	21
Glucosisaustricin (2*R*)-(2-hydroxy-1-ethyl)ethyl	*Sisymbrium loeseli*	**(+)-(4*R*)-4-ethyl- (6)**	21
Glucocleomin (2*S*)-(2-hydroxy-2-methyl)butyl	*Cleome spinosa*	**(-)-(5*S*)-5-methyl-5-ethyl- (7)**	22
Glucoconringiin* (2-hydroxy-1,1-dimethyl)ethyl	*Conringia orientalis*	**5,5-dimethyl- (8)**	23

*not chiral

were recorded in $CDCl_3$ solution on a Bruker Avance DPX250 instrument operating at 250 MHz and 62.89 MHz, respectively. The coupling constants (J) are reported in Hz and the chemical shifts (δ) in ppm downfield from tetramethylsilane as the internal standard. IR spectra were measured using a Perkin–Elmer FT Paragon 1000 PC spectrometer. Mass spectra (MS) were obtained on a Perkin–Elmer SCIEX API 300 spectrometer (Ionspray® mode). HPLC profiles were recorded on a Hewlett Packard 1090 L with diode array detector, and GC–MS spectra were measured on a Hewlett Packard GCD 1800 apparatus.

3.1. General procedure for the preparation of enantiopure 1,3-oxazolidine-2-thiones

The bioreactor is constituted by a thermostated column (Ø 2.6 cm, L 30 cm, 37°C), loaded with 10 g of Nylon 6.6-supported myrosinase. This enzyme was purified from white mustard seeds[13] and immobilized on Nylon 6.6 granules (18–36 mesh particles).[15] The specific activity of the enzyme was ca. 65 units/mg protein before immobilization, whereas the final immobilized activity reached 325 opponent units/g solid. GSL were extracted from the ripe seeds of each plant species (see Table 1) homogenizing the seeds in boiling water. The centrifuged crude extract was loaded into a DEAE-Sephadex A25 ion exchange chromatographic column. After suitable washing of the column with water, the GSL were eluted by a 0.5 M K_2SO_4 aqueous solution. The GSL solution was concentrated, dried and solubilized with boiling methanol. Finally, the GSL were precipitated with ethanol at −20°C.[16] The isolation yields ranged from 40 up to 60%, depending on the starting seed species. The substrate was dissolved in phosphate buffer (pH=6.5) at the concentration of 10 mg/mL and passed through the column with a 2 mL/min flow. The reaction was followed polarographically and/or by increasing of UV absorption at 240 nm due to the formation of the 1,3-oxazolidine-2-thione and in all cases the transformation of the starting material was complete. The final products were purified on a RPC18 column using water as eluent; after freeze drying, the purity of the oxazolidine-2-thiones were assessed by HPLC, GC–MS and NMR (^1H and ^{13}C).

3.2. (5S)-5-Vinyl-1,3-oxazolidine-2-thione **1**

$[\alpha]_D$=−70 (*c* 1, $CHCl_3$);[17] mp=48°C; IR (KBr): 3202 (NH), 1532 (CS); ^{1}H NMR: 3.53 (dd, 1H, $J_{4b\text{-}5}$=8.3, H-4b), 3.92 (dd, 1H, $J_{4a\text{-}4b}$=9.5, H-4a), 5.33 (ddd, 1H, $J_{5\text{-}4a}$=9.4, H-5), 5.40 (d, 1H, $J_{7Z\text{-}6}$=10.5, H-7Z), 5.48 (d, 1H, $J_{7E\text{-}6}$=17.0, H-7E), 5.96 (ddd, 1H, $J_{6\text{-}5}$=6.7, H-6), 7.44 (s, 1H, NH); ^{13}C NMR: 49.5 (C-4), 83.8 (C-5), 121.0 (C-7), 133.4 (C-6), 189.7 (CS); MS: MH^+=130.

3.3. (5R)-5-Vinyl-1,3-oxazolidine-2-thione **2**

$[\alpha]_D$=+72 (*c* 1, $CHCl_3$);[18] mp=48°C; IR (KBr): 3215 (NH), 1531 (CS); ^{1}H NMR: 3.53 (dd, 1H, $J_{4b\text{-}5}$=8.3, H-4b), 3.92 (dd, 1H, $J_{4a\text{-}4b}$=9.5, H-4a), 5.33 (ddd, 1H, $J_{5\text{-}4a}$=9.4, H-5), 5.40 (d, 1H, $J_{7Z\text{-}6}$=10.5, H-7Z), 5.48 (d, 1H, $J_{7E\text{-}6}$=17.0, H-7E), 5.96 (ddd, 1H, $J_{6\text{-}5}$=6.7, H-6), 7.40 (s, 1H, NH); ^{13}C NMR: 49.5 (C-4), 83.8 (C-5), 121.0 (C-7), 133.4 (C-6), 189.7 (CS); MS: MH^+=130.

3.4. (5S)-5-(Prop-2-enyl)-1,3-oxazolidine-2-thione **3**

$[\alpha]_D$=−6 (*c* 1, $CHCl_3$); mp=60°C;[19] IR (KBr): 3188 (NH), 1537 (CS); ^{1}H NMR: 2.50–2.70 (m, 2H, H-6a and H-6b), 3.48 (dd, 1H, $J_{4b\text{-}4a}$=9.0, H-4b), 3.81 (dd, 1H, $J_{4a\text{-}5}$=8.9, H-4a), 5.00 (m, 1H, $J_{5\text{-}4b}$=7.7, H-5), 5.17–5.30 (m, 2H, H-8a and H-8b), 5.75 (m, 1H, H-7), 7.42 (s, 1H, NH); ^{13}C NMR: 38.4 (C-6), 48.1 (C-4), 82.1 (C-5), 120.1 (C-8), 130.6 (C-7), 189.8 (CS); MS: MH^+=144.

3.5. (5R)-5-Phenyl-1,3-oxazolidine-2-thione **4**

$[\alpha]_D$=−51 (*c* 1, $CHCl_3$);[20] mp=116°C;[19] IR (KBr): 3176 (NH), 1530 (CS); ^{1}H NMR: 3.75 (dd, 1H, $J_{4a\text{-}4b}$=9.6, H-4b), 4.17 (dd, 1H, $J_{4a\text{-}5}$=9.4, H-4a), 5.90 (t, 1H, $J_{5\text{-}4b}$=8.2, H-5), 7.37–7.46 (m, 5H, H-ar), 7.75 (s, 1H, NH); ^{13}C NMR: 51.2 (C-4), 83.9 (C-5), 125.8, 129.0, 129.4, 136.9 (C-ar), 189.7 (CS); MS: MH^+=180.

3.6. (4S)-4-Methyl-1,3-oxazolidine-2-thione **5**

$[\alpha]_D$=+23 (*c* 1, $CHCl_3$);[21] mp=60°C; IR (KBr): 3263 (NH), 1509 (CS); ^{1}H NMR: 1.36 (d, 1H, J_{vic}=6.0, CH_3), 4.17–4.29 (m, 2H, H-4 and H-5b), 4.77 (m, 1H, $J_{5a\text{-}4}$=6.8, $J_{5a\text{-}5b}$=11.5, H-5a), 7.90 (s, 1H, NH); ^{13}C NMR: 20.4 (CH_3), 52.7 (C-4), 76.9 (C-5), 189.9 (CS); MS: MH^+=118.

3.7. (4R)-4-Ethyl-1,3-oxazolidine-2-thione **6**

$[\alpha]_D$=+47 (*c* 1, $CHCl_3$);[21] mp=32°C; IR (KBr): 3199 (NH), 1526 (CS); ^{1}H NMR: 0.98 (t, 3H, J_{vic}=7.4, $\mathbf{CH_3}CH_2$), 1.68 (m, 2H, $\mathbf{CH_2}CH_3$), 4.02 (m, 1H, H-4), 4.32 (dd, 1H, $J_{5b\text{-}4}$=6.6, $J_{5b\text{-}5a}$=8.9, H-5b), 4.74 (dd, 1H, $J_{5a\text{-}4}$=8.7, H-5a), 7.73 (s, 1H, NH); ^{13}C NMR: 9.7 ($\mathbf{CH_3}CH_2$), 27.7 ($\mathbf{CH_2}CH_3$), 58.1 (C-4), 75.3 (C-5), 190.1 (CS); MS: MH^+=132.

3.8. (5S)-5-Ethyl-5-methyl-1,3-oxazolidine-2-thione **7**

$[\alpha]_D$=−23 (*c* 1, $CHCl_3$);[22] mp=43°C; IR (KBr): 3184 (NH), 1556 (CS); ^{1}H NMR: 0.99 (t, 3H, J_{vic}=7.5, $\mathbf{CH_3}CH_2$), 1.50 (s, 3H, CH_3), 1.79 (q, 2H, $\mathbf{CH_2}CH_3$), 3.43 (d, 1H, $J_{4b\text{-}4a}$=9.6, H-4b), 3.56 (d, 1H, H-4a),

7.93 (s, 1H, NH); ^{13}C NMR: 8.5 (**C**$\mathbf{H_3}$CH$_2$), 25.5 (CH$_3$), 33.4 (**C**$\mathbf{H_2}$CH$_3$), 54.3 (C-4), 92.0 (C-5), 189.6 (CS); MS: MH$^+$=146.

3.9. 5,5-Dimethyl-1,3-oxazolidine-2-thione ***8***

Mp=106°C;[23] IR (KBr): 3191 (NH), 1531 (CS); ^{1}H NMR: 1.57 (s, 6H, CH$_3$), 3.53 (s, 2H, H-4), 8.06 (s, 1H, NH); ^{13}C NMR: 26.7 (CH$_3$), 55.3 (C-4), 88.4 (C-5), 188.6 (CS); MS: MH$^+$=132.

Acknowledgements

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