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Synthesis of ^{14}C-Labelled Sulforaphane

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Summary

Sulforaphane (SFN) a naturally occurring isothiocyanate present in broccoli, shows strong evidence of anti-carcinogenic activity. The mechanism of action, absorption, distribution, metaboli^{i}sm and excretion of the compound is however still poorly understood and requires a radiolabelled version of the compound for further studies. The paper describes an optimised cold synthesis of SFN and a radiosynthesis of ^{14}C-labelled SFN.

Keywords: ^{14}C-labelling, isothiocyanates, anti-cancer compounds, phytochemicals

Introduction

The inverse relationship between vegetable consumption and the incidence of various cancers in humans is well established [1]. It is not only the high concentration of vitamins, minerals and fibres that are responsible for these effects but a group of compounds summarised as phytochemicals [2].

One major subgroup of phytochemicals are the glucosinolates (GLS) e.g. Glucoraphanin **1**. GLS per se are biologically inactive but following cell disruption, the GLSs undergo enzymatic or non-enzymatic hydrolysis [3-5] to give as major products isothiocyanates (e.g. sulforaphane), which are very likely to be responsible for the protective, anticarcinogenic effects of high *Brassica* vegetable consumption [6, 7]. As a result of a statement of the National Cancer Institute, sulforaphane **2** (SFN), the 4-methylsulfinylbutyl- isothiocyanate, is considered one of the 40 most promising anticarcinogens [8].

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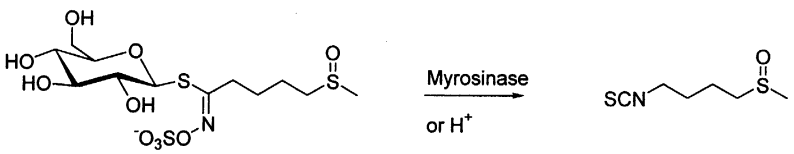
Based on epidemiological data [1] and results from numerous *in vivo* and *in vitro* studies on the biological activity of SFN [9-11], there is a great interest in this particular isothiocyanate mainly found in broccoli [12-13].



Glucoraphanin **1** Sulforaphane **2**

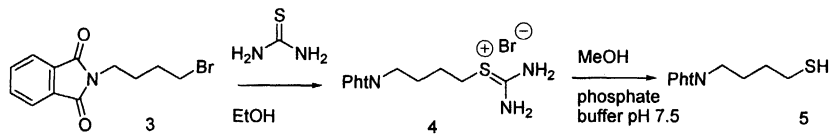
Using different models it has been shown that SFN is a very potent phase II enzyme inducer that does not activate phase I enzymes. In this way it alters the balance of phase I / phase II enzyme metabolism in such a way that the activation of carcinogens during phase I is relatively reduced and the rate of detoxification in phase II is increased. That is why SFN may play an important role in cancer prevention [14-16]. Recent results show that SFN is also able to induce cell cycle arrest and apoptosis in HT29 human colon cancer cells, which is further evidence for an anti-carcinogenic activity [17]. Thus SFN belongs to a small group of compounds enabling dual inhibitory action in carcinogenesis. However, the mechanisms of absorption and metabolism are poorly understood. In order to answer questions related to bioavailability and mechanism of biological effects there is a need for labelled SFN, which will enable us to:

1.) Identify possible metabolites and conjugates with biological (macro) molecules e.g. enzymes, peptides, amino acids.
2.) Obtain pharmacokinetic data on absorption and transport.
3.) Investigate the mechanism of action on signal transduction and enzyme induction.

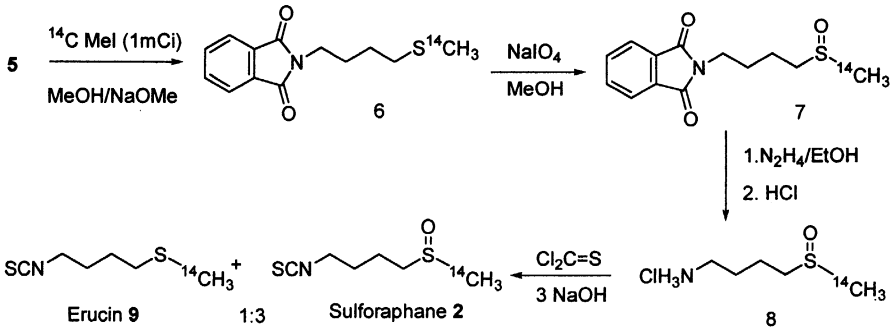
Results and Discussion

Three chemical syntheses of sulforaphane **2** have been reported to date [18-21]. Our synthetic strategy for the synthesis of 14-C labelled **2** follows the synthesis by Karrer et al. and represents an optimised and improved variation thereof [20]. The radiolabel is introduced by S-alkylation of a thiolate with $^{14}CH_3I$ [22].

The cold synthesis was optimised with the aim of avoiding any chromatographic or distillation work-up procedures, thus minimising the contamination of waste solvents. After optimisation the cold synthesis was reproduced five times on a scale identical to the radiosynthesis. With the exception of **5** all compounds have been described in the literature [19, 20]. However, no NMR or IR-spectroscopic data are given in the references. We therefore include them in our experimental section. Starting with phthalimido bromide **3** displacement of the bromide by thiourea gave the thiuronium salt **4** in high yields, which could be isolated as a stable intermediate. Hydrolysis of **4** turned out to be rather precarious, since an excess of base leads to cleavage of the imide [23]. Optimised conditions for the chemoselective hydrolysis were found using a 1:5 mixture of phosphate buffer at pH 7.5 and MeOH to give the thiol **5** in excellent yield.



Alkylation of thiol **5** with $^{14}CH_3I$ (Sigma 70 mCi/mmol, 1 mCi) gave the thioether **6** in good chemical but poor radiochemical yield (0.33 %). For the oxidation of **6** sodium periodate in MeOH was shown to be the condition of choice to yield the sulfoxide **7** in almost quantitative yield. Cleavage of the phthalimide using hydrazine was uneventful to give the amine **8**, which was isolated as its hydrochloride. For the final transformation to give sulforaphane thiophosgene and 3 equivalents of NaOH in a diethylether/water two phase solvent system gave the title compound in good overall chemical and radiochemical yield. It is worth noting that the final reaction also gives erucin **9**, which is a natural product found in a variety of other *brassicas* most notably rocket salad [24] as an unexpected side product. This observation is mechanistically suprising, since no evident reducing agent is present in the reaction mixture. Currently we have no satisfactory explanation for the formation of this side product. Similar deoxygenations in the absence of a reducing agent have been observed in the chemistry of co-ordinated sulfines [25, 26].



Analytically pure erucin could be obtained by Kugelrohr distillation. Pure SFN **2** (by 1-H-NMR and tlc) was obtained by aqueous extraction. The identity of sulforaphane was confirmed by comparison with authentic material and analytical data from the literature [18-20].

Conclusion

We have established a reliable and efficient synthesis of cold racemic sulforaphane. The reaction sequence is amenable to a radiosynthesis giving a good radiochemical yield of 59 % over the last 3 steps of the synthesis. Current work is concerned with addressing the optimisation of the S-alkylation step in the radiosynthesis and the synthesis of other labelled compounds related to the glucoraphanin-sulforaphane group of compounds. Moreover we will use the radiolabelled compounds in biological studies to find answers to the problems outlined in the introduction.

Experimental

1-Bromo-4-phthalimido-butane was prepared according to the literature method [20]. Thiophosgene was distilled prior to use. All solvents and other reagents were used without further purification. $^{14}CH_3I$ from Sigma at a specific activity of 70 mCi/mmol was used. NMR spectra were obtained on a Jeol GSX-270. Spectroscopic data refer to cold material. Radioactivities were determined with a Packard Tri Carb 1500 scintillation counter.

1-Sulfanyl-4-phthalimido-butane (5).

1-Bromo-4-phthalimido-butane (2.82 g , 10 mmol) and thiourea (760 mg, 10 mmol) were heated under reflux for 2 h in 50 ml ethanol. The solution was cooled to 0^0 C and the white precipitate collected by filtration. The precipitate was washed twice with 5 ml ethanol and dried under vacuum to give 3.5 g (98 %) of the thiuronium salt **4** as a white powder.

2 g (5.6 mmol) of the thiuronium salt **4** was heated under reflux in 20 ml of a 5:1 mixture MeOH and phosphate buffer (pH 7.5) for 40 minutes. The solvent was removed in vacuum and the residue suspended in 50 ml chloroform. The chloroform was washed twice with 20 ml water and once with 20 ml brine. The organic layer was dried over $MgSO_4$ and the solvent removed in vacuum. The product was recrystallised from chloroform/diethylether to give 1.18 g (90 %) of the thiol (**5**) as a white waxy solid. IR (Nujol) cm^{-1}: 2926 (s), 2349 (w, SH), 1695 (vs, C=O).- ^{1}H NMR ($CDCl_3$): 7.80 (dd, J = 3 Hz; 8.3 Hz, 2H, Ar), 7.68 (dd, J = 3 Hz, 8.3 Hz, 2H, Ar), 3.67 (t, J = 6.9 Hz, 2H, CH_2N), 2.55 (q, J = 7.4 Hz, 2H, CH_2S), 1.60-1.80 (m, 4H, CH_2), 1.33 (t, 1H, J = 7.0 Hz, SH).- ^{13}C NMR ($CDCl_3$): 168.6, 134.2, 132.7, 123.4, 37.5, 31.3, 27.5, 24.3.

1-Methylsulfanyl-4-phthalimido-butane (6).

Thiol **5** (35 mg, 1.5 mmol), NaOMe (10 mg, 1.8 mmol) and MeI (5 mg , 0.3 mmol diluted with ^{14} CH_3I (1 mCi)) were dissolved in 5 ml of MeOH and stirred for 2 h at 50 ^{0}C in a sealed tube. The reaction was cooled to 0^0 C and 17 mg (1.2 mmol) MeI were added and the reaction mixture again heated for 2 h at 50 ^{0}C in a sealed tube. The solvent was removed in vacuum and the residue dissolved in 30 ml Et_2O and extracted twice with 5 ml brine. The organic layer was dried over $MgSO_4$ and removed in vacuum to give the thioether **2** as a white solid (Chemical yield:variable between 85% and 70 %). The spectroscopic data are identical with the compound in reference [21]. Radiosynthesis: Yield 3.2 μCi (0.32%).

1-Methylsulfinyl-4-phthalimido-butane (7).

Thioether **2** (62 mg, 2.5 mmol) and $NaIO_4$ (60 mg, 2.8 mmol) were dissolved in 10 ml MeOH and stirred for 12 h at 0^0 C. The solvent was removed in vacuum

and the residue suspended in 30 ml $CHCl_3$. The salts were filtered off and washed with 20 ml $CHCl_3$. The combined organic solvents were removed in vacuum to give the sulfoxide **3** as a white solid. (Yield 95-99%).
Radiosynthesis: Yield 3.2 μCi (99 %).- m.p. 133 °C .- IR (Nujol) cm^{-1}: 2926 (s), 1697 (vs, C=O), 1462 (m), 1400 (m), 1376 (s), 1085 (s, S=O), 1012 (s), 718 (s).-^{1}H NMR ($CDCl_3$): 3.71 (t, J = 6.3 Hz, 2H, NCH_2), 2.99 (m, 2H CH_2SOMe), 2.32 (s, 3H, SOMe), 1.70-1.83 (m, 4H, CH_2 CH_2). ^{13}C NMR ($CDCl_3$): 165.6 (C=O), 134.2, 132.1, 123.5 (Ar), 53.9, 39.7, 37.2, 27.6, 23.1.

1-Methylsulfinyl-4-amino–butane hydrochloride (8).
The sulfoxide **3** (65 mg, 2.5 mmol) was dissolved in 10 ml of refluxing EtOH and 12.5 mg (2.5 mmol) of hydrazine hydrate was added. The solution was refluxed for 3 h. 20 ml 3 N HCl was added and the phthalhydrazide filtered off. The aqueous solvent was removed in vacuum to give the amine hydrochloride **8** as a colourless oil. (Yield 95 %). ^{1}H NMR (D_2O/5 % MeOH): 3.12 (br, 2H, CHN), 2.94 (br, 2H, CH_2SOMe), 2.70 (s, 3H, CH_3SO), 1.84 (br, 4H, CH_2CH_2).

1-Methylsulfinyl-4-isothiocyanato –butane (sulforaphane) (2).
The amine hydrochloride **8** (2.2 mmol) was dissolved in a 2 phase solvent system (5 ml water 10 ml Et_2O) at 0^0C. 6.6 mmol of NaOH (1.3 ml 5 N NaOH solution) was slowly added followed by 25.2 mg (2.2 mmol) of thiophosgene. The solution was stirred for 30 min. The crude NMR shows the presence of a 3:1 mixture of sulforaphane and erucin. The organic layer was separated and washed twice with 5 ml water. The aqueous layers were combined and reduced in vacuum to 5 ml. 1g NaCl is added to the aqueous layer, which was extracted with 3 times 15 ml DCM, dried over $MgSO_4$ and removed in vacuum to give the title compound (Sulforaphane **2**) as a yellow oil. (Yield (85-90 %). The diethylether phase contains a 60:40 ratio of erucin and sulforaphane.
Radiosynthesis: 1.1 μCi (33 %) pure sulforaphane and 0.9 μCi erucin: sulforaphane mixture (27 %), combined radiochemical yield over 2 steps 59%.
IR (Neat): 2994 (m), 2941 (s), 2180 (vs), 2108 (vs), 1435 (s), 1348, 1309 (m), 1057 (s), 952, 697.- ^{1}H NMR ($CDCl_3$): 3.58 (t, J = 6.4 Hz, 2H, $SNCCH_2$), 2.72 (m, 2H, CH_2SOMe), 2.60 (s, 3H, SOMe), 1.68-1.94 (m, 4H, CH_2 CH_2).

1-Methylsulfanyl-4-isothiocyanato–butane (Erucin) (9):

Analytically pure erucin was obtained in a cold synthesis on a 10 mmol scale using Kugelrohr distillation of the crude reaction mixture. Yield: 0.41 g (24%), yellow liquid, b.p. 63 °C (1 Torr).- IR (neat) cm^{-1}: 2946 (s), 2915 (s) (CH), 2183 (vs), 2107 (vs) (NCS), 1449 (m), 1347 (m), 1269 (w), 1070 (w), 957 (w), 770 (w), 686 (w), 637 (w).-^{1}H NMR ($CDCl_3$): 3.54 (t, J = 6.3 Hz, 2H, $SNCCH_2$), 2.51 (t, J = 6.8 Hz, CH_2SMe), 2.07 (s, 3H, SMe), 1.70-1.79 (m, 4H, CH_2 CH_2). ^{13}C NMR ($CDCl_3$): 137.9 (NCS), 44.8, 33.3, 28.9, 25.9, 15.5.

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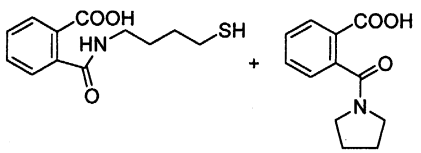
21. Note that suprisingly we found 5 more references to the synthesis of sulforaphane using various methods without an indication of how the material was prepared, spectroscopic data or indication of purity.

22. For a precedence of a 14-C labelled SMe compound see: Wheeler WJ, Kau DLK, Bach NJ, *J. Label. Comp. Radiopharm.* (1990), 28, 273-295.

23. Upon more basic hydrolysis conditions the following 2 products are obtained as side products or major products using 2N NaOH:



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