Stability of Benzylic-Type Isothiocyanates in Hydrodistillation-Mimicking Conditions

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INTRODUCTION

Natural essential oils, generally obtained from vegetable matter (flowers, leaves, bark, roots, wood, rhizomes), contain highly volatile and fragrant organic compounds. Since ancient times, they have been used for perfumes, foods, and medicinal purposes (aromatherapy). Various technologies were developed to obtain the best aromatic substances to fulfill the needs of the perfumer and flavorist and to respond to the pharmaceutical industry’s demands. Some authors consider the term “essential oil” as a generic term that encompasses natural extracts obtained by various processes (steam distillation, solvent extraction, supercritical fluid extraction, etc.) however, this term should be strictly applied to volatile extracts obtained from a raw material of plant origin by steam distillation, which can be volatile and pungent. In most cases, ITCs are constituted of benzyl isothiocyanate and benzyl cyanide. In a previous study by the authors, it was surmised that partial hydrolytic degradation of 4-methoxybenzyl isothiocyanate, one major expected compound, occurred during the hydrodistillation process of essential oil preparation. To probe this hypothesis, a selection of diversely substituted benzylic-type isothiocyanates was submitted to standard hydrodistillation-mimicking conditions. After extraction with dichloromethane, the reaction mixtures were analyzed using GC-MS. The aqueous phases resulting from liquid–liquid extraction were analyzed by HPLC and GC-MS. 2-Methoxybenzyl, 4-methoxybenzyl, 3,4-dimethoxybenzyl, and 3,4,5-trimethoxybenzyl isothiocyanates underwent conversion into 2-methoxybenzyl, 4-methoxybenzyl, 3,4-dimethoxybenzyl, and 3,4,5-trimethoxybenzyl alcohols, respectively, whereas benzyl, 3-methoxybenzyl, and 4-chlorobenzyl isothiocyanates were converted into the corresponding benzylamines.

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characterized and quantified according to the HPLC analysis of desulfo-GLs.\textsuperscript{13} Glucotropaeolin (benzyl GL), glucolinanthann (3-methoxybenzyl GL), and glucouabrietin (4-methoxybenzyl GL) were shown to be present in the root extract, whereas the seed mainly contained glucouabrietin. 3,4-Dimethoxybenzyl GL, glucobrassicin (indol-3-ylmethyl GL), and traces of glucotropaeolin were detected in the leaf extract. The predictions of the GL profile based on desulfo-GL analysis did not fit the hydrodistillation results. Various extraction and analysis techniques led to different profiles. In addition, the results apparently lacked consistency with data previously published.\textsuperscript{13,15} Those observations led us to study and compare the stability of some ITCs in aqueous solutions has been characterized and quantified according to the HPLC analysis of desulfo-GLs.\textsuperscript{13} Glucotropaeolin (benzyl GL), glucolinanthann (3-methoxybenzyl GL), and glucouabrietin (4-methoxybenzyl GL) were shown to be present in the root extract, whereas the seed mainly contained glucouabrietin. 3,4-Dimethoxybenzyl GL, glucobrassicin (indol-3-ylmethyl GL), and traces of glucotropaeolin were detected in the leaf extract. The predictions of the GL profile based on desulfo-GL analysis did not fit the hydrodistillation results. Various extraction and analysis techniques led to different profiles. In addition, the results apparently lacked consistency with data previously published.\textsuperscript{13,15} Those observations led us to study and compare the stability of some ITCs in aqueous solutions.\textsuperscript{\textsuperscript{13,15}} In this investigation, the essential oil, obtained after a 5 h hydrodistillation of the root, was constituted of 78% of BITC 5, 17% of benzyl cyanide, 0.1% of 4-methoxybenzyl ITC (4-MBITC, 2), and 0.2% of 4-methoxybenzyl alcohol.\textsuperscript{14} Therefore, this discrepancy prompted us to repeat the hydrodistillation process on a portion of the sample of \textit{P. brazzeana} root used for the quantification of GLs.\textsuperscript{13} The essential oil, obtained after a 5 h hydrodistillation at pH 5.9, was shown to contain 57% of BITC 5, 10% of benzyl cyanide, and only 5% of 2 (Figure 1).\textsuperscript{13} Several studies have shown that individual GLs and GLs in plant extracts are degraded under hydrodistillation-mimicking conditions.\textsuperscript{8,15–18} Benzyl cyanide and 5 are expected to result from the enzymatic decomposition of glucotropaeolin (benzyl GL).\textsuperscript{20} Despite the fact that the stability of glucouabrietin or the related ITC under hydrodistillation conditions has never been investigated, it is reasonable to ascribe the decrease of these compounds to thermal breakdown and leaching into the heating medium, during hydrodistillation. However, taking into account that glucouabrietin is by far the major GL present in \textit{P. brazzeana} root and that MBITC 2, the main degradation product originated from this GL, was found only as a minor component in the essential oil of \textit{P. brazzeana}, it had to be surmised that partial hydrolytic degradation of 2 occurred during the hydrodistillation process.

The stability of some ITCs in aqueous solutions has been investigated. In distilled water at 37 °C, allyl ITC decomposes into \textit{N,N′}-diallyltiourea, allyl allyldithiocarbamate, diallyl tetrasulfide, and diallyl pentasulfide.\textsuperscript{17} Allyl ITC isomerizes to allyl thiocyanate and decomposes into allylamine, allyl dithiocarbamate, diallyltiourea, carbon disulfide, diallylurea, and diallyl sulfide in buffer solutions (pH 4, 6, and 8) at 80 °C for 80 min.\textsuperscript{17} Within 1 h in boiling water, allyl ITC degrades to diallyl di-, tri-, and tetrasulfide, allyl thiocyanate, 3H-1,2-dithiolene, 2-vinyl-4H-1,3-dithiin, 4H-1,2,3,4-tetrasulfane, and \textit{N,N′}-diallyltiourea.\textsuperscript{21} In other respects, the thermal degradation of sulforaphane (4-methylsulfinylbutyl ITC), in aqueous solution at 100 °C, was shown to produce dimethyl disulfide, S-methyl methanethiosulfinate, S-methyl methanethiosulfonate, methyl (methylsulfanyl)methyl disulfide, 1,2,4-triiodioline, 4-isothiocyanato-1-(methylsulfinyl)-1-buten, 3-butenyl ITC, and \textit{N,N′}-di(4-methylsulfinyl)butyl thiourea.\textsuperscript{22} It has long been known that indol-3-ylmethyl ITC, resulting from enzymatic degradation of glucobrassicin, is spontaneously transformed into indol-3-ylmethanol.\textsuperscript{23} In addition, approximately 50% of phenylethyl ITC degrades after 4 h in a phosphate-buffered saline, at pH 7.4 and 37 °C, producing phenethylamine.\textsuperscript{24} Finally, 4-hydroxybenzyl ITC, resulting from enzymatic hydrolysis of glucosinolate, is unstable in aqueous media, producing 4-hydroxybenzyl alcohol under release of a thiocyanate ion.\textsuperscript{25–27}

Those observations led us to study and compare the stability of ITCs (2) and benzyl ITCs (5) corresponding respectively to glucouabrietin and glucotropaeolin, present in \textit{P. brazzeana} root, by mimicking hydrodistillation extraction conditions. Furthermore, to check the correlation of substituents on the benzyl moiety with the transformation of the benzylic-type ITCs under hydrolytic conditions, we chose to probe the stability of several diversely substituted ITCs in the same experimental conditions. Our major objective was to investigate and compare the stability of benzylic ITCs associated with known naturally occurring arylaliphatic GLs\textsuperscript{28} under hydrodistillation-mimicking conditions. Therefore, 2-methoxy (2-MBITC, 1), 3-methoxy (3-MBITC, 6), and 4-methoxybenzyl (4-MBITC, 2) ITCs (Figure 1) were tested to check the influence of the substituent’s location; 3,4-dimethoxy (3,4-DMBITC, 3) and 3,4,5-trimethoxybenzyl (3,4,5-TMBITC, 4) ITCs (Figure 1) were tested to evaluate a possible cumulative effect of substituents. Finally, non-natural 4-chlorobenzyl ITC (4-CIBITC, 7) (Figure 1) was tested to probe the deactivation effect of a chlorine atom in comparison with the electron-donating effect of a methoxy group on the benzyl moiety.

### MATERIALS AND METHODS

**Materials.** Benzyl ITC (5) was purchased from Fluka Chemie GmbH (Buchs, Switzerland). PE and EA (analytical grade) were purchased from Carlo Erba (France). DCM and acetonitrile (HPLC grade) were purchased from Sigma-Aldrich Chemie GmbH, (Steinheim, Germany). Ultrapure water (pH 5.0 ± 0.2) was obtained from a Milli-Q Gradient instrument (Millipore SAS, Molsheim, France) equipped with a Millipack filter 0.22 μm (Millipore, SAS). CDCI\textsubscript{3} was purchased from Eurisio-top (St-Aubin, France). \textsuperscript{\textsuperscript{1}}H NMR spectra were recorded at 250 MHz on a Bruker Avance DPX 250 spectrometer, δ values being referenced to residual CHCl\textsubscript{3} at 7.26 ppm. Mass spectra were recorded on a Perkin-Elmer Sciex API-300 spectrometer (electrospray, positive mode). Infrared spectra were recorded on an Attenuated Total Reflectance Thermo-Nicolet Avatar 320 AEKO200713 instrument.

**Syntheses of Arylaliphatic Isothiocyanates.** ITCs 1–4, 6, and 7 (Figure 1) were prepared from the corresponding amines following the standard procedure of Goodyer et al.\textsuperscript{19} 2-Methoxybenzyl isothiocyanate 1. Compound 1 was isolated in 89% yield as a yellow oil. R\textsubscript{f} 0.73 (EA/PE 1:3). IR (neat) 2165, 2072 (−N=S). \textsuperscript{1}H NMR (250 MHz, CDCl\textsubscript{3}) δ 7.32 (d, 2H, J = 7.5 Hz, H-4, H-6), 6.98 (td, 1H, J = 7.5, 1.0 Hz, H-5), 6.92 (td, 1H, J = 8.5, 1.0 Hz, H-3), 4.70 (s, 2H, CH\textsubscript{2}N), 3.86 (s, 3H, OMe). The spectroscopic data agree with the published values.\textsuperscript{13,30,31}

![Figure 1. Hydrolytic degradation of benzylic-type ITCs.](image-url)
3-Methoxybenzyl Isothiocyanate 6. Compound 6 was isolated in 96% yield as a yellow oil. Rf 0.8 (EA/PE 1:3). IR (neat) 2166, 2078 (−N=C=S). 1H NMR (250 MHz, CDCl3) δ 7.30 (1H, J = 7.8 Hz, H-5), 6.89 (m, 2H, H-4, H-6), 6.86 (s, 1H, H-2), 4.69 (s, 2H, CH2N), 3.83 (s, 3H, OMe). The spectroscopic data agree with the published values.13,31

4-Methoxybenzyl Isothiocyanate 7. Compound 7 was isolated in 96% yield as a yellow oil. Rf 0.7 (EA/PE 1:3). IR (neat) 2166, 2073 (−N=C=S). 1H NMR (250 MHz, CDCl3) δ 7.24 (d, 2H, J = 9.2 Hz, H-2, H-6), 6.91 (d, 2H, J = 9.2 Hz, H-3, H-5), 4.64 (s, 2H, CH2N), 3.82 (s, 3H, OMe). The spectroscopic data agree with the published values.13,31,32

3,4-Dimethoxybenzyl Isothiocyanate 3. Compound 3 was isolated in quantitative yield as a yellowish solid. Rf 0.44 (EA/PE 1:3). IR (neat) 2163, 2078 (−N=C=S). 1H NMR (300 MHz, CDCl3) δ 6.86 (m, 2H, Ar−H), 6.82 (m, 1H, Ar−H), 4.64 (s, 2H, CH2N), 3.91 (s, 3H, OMe), 3.89 (s, 3H, OMe). The spectroscopic data agree with the published values.3

3,4,5-Trimethoxybenzyl Isothiocyanate 4. Compound 4 was isolated in 94% yield as a yellow oil. Rf 0.32 (EA/PE 1:3). IR (neat) 2166, 2080 (−N=C=S). 1H NMR (250 MHz, CDCl3) δ 6.52 (s, 2H, Ar−H), 4.65 (s, 2H, CH2N), 3.88 (s, 6H, 2×OMe), 3.85 (s, 3H, OMe). The spectroscopic data agree with the published values.34

RESULTS AND DISCUSSION

The stability of the ITCs 1−7 (Figure 1) in water at 90 °C was studied according to the protocol described above and monitored by GC-MS and HPLC. All of the ITCs and degradation products were detected by GC-MS analysis in the DCM fractions obtained after liquid−liquid extraction of the aqueous samples. All tested ITCs were susceptible to hydrolysis at 90 °C. Each ITC afforded only a single detectable degradation product in the DCM fractions. Finally, checking...
the aqueous fractions by HPLC and GC-MS analyses allowed us to rule out the presence of any other degradation product.

Two types of degradation pathways were observed. The first type displayed hydrolytic conversion of the ITCs into the corresponding benzyl alcohols, in analogy with the case of glucosinolbin and 4-hydroxybenzyl ITC previously studied by Borek and Morra.27 In fact, 4-MBITC (2) was transformed into 4-methoxybenzyl alcohol (Figures 2 and 3) and 2-MBITC (1) into 2-methoxybenzyl alcohol at a slower rate compared with 2 (Figure 4) over the first hour under the experimental conditions used. Figure 3 shows the degradation of 4-MBITC (2) and the concomitant formation of 4-methoxybenzyl alcohol. The qualitative analyses of the degradations of the ITCs were carried out using normalized area as described under Materials and Methods. The peak area of 2 at time 0 min was set at the default value of 1, and the peak area of 4-methoxybenzyl alcohol at time 60 min was assigned the value of 1. The other peak areas in the sampling sequence are relative to the peak area at 0 min for 2 and to the peak area at 60 min for 4-methoxybenzyl alcohol. As a matter of fact, the initial area value for 4-methoxybenzyl alcohol are arbitrary. Even though we can exclude from our GC-MS and HPLC analyses the presence of any other degradation product apart from 4-methoxybenzyl alcohol, the trends reported in Figure 3 are only qualitative. The quantification in terms of percentage of degraded substrate and product formed could be affected by several factors such as a different solubilization in water or a different detector response for the two compounds. 3,4-DMBITC (3) was transformed into 3,4-dimethoxybenzyl alcohol, showing a rate of hydrolysis similar to the one observed for 2. The more substituted 3,4,5-TMBITC (4) underwent slower degradation in water at 90 °C (Figure 4). The second type displayed hydrolytic conversion of the ITCs into the corresponding benzylamines. Thereby, BITC (5) and 3-MBITC (6) were degraded in water at 90 °C to afford benzylamine and 3-methoxybenzylamine, respectively (Figure 4).

This dramatic difference observed in the behavior of benzylic ITCs could be rationalized in terms of electron delocalization within their aromatic frame. A proposed pathway for the formation of 4-methoxybenzyl alcohol from 2 is shown in Figure 5. The intermediacy of a labile pseudoquinone methide is anticipated, in analogy with Borek and Morra’s mechanism.

assumption for the conversion of 4-hydroxybenzyl ITC into 4-hydroxybenzyl alcohol: similarly to the hydroxyl group in sinalbin, the marked electron-donating character of the methoxy group in 2 facilitates the hydrolytic process through resonance stabilization of the transient pseudoquinone methide.35 As they both bear a p-methoxy group, 3 and 4 are assumed to produce the respective alcohols following the same pathway. However, whereas the hydrolys of 2 and 3 are closely comparable, complete conversion of 4 is more sluggish, owing probably to the conflicting effect of m-methoxy groups (Figure 4a). Finally, the observed conversion of 1 is slightly
slower than the hydrolysis of 2, probably because of a lesser stabilization in the transient o-quinone methide.\textsuperscript{35}

In contrast, other benzylic ITCs such as 5 and its meta-substituted derivative 6 are unable to generate or stabilize exomethylene intermediates to allow nucleophilic substitution of the ITC group. Therefore, a standard addition of water on the C\(-\)N bond occurs to generate an unstable thionocarbamic species, which degrades to give the primary amine under elimination of carbon oxysulphide (Figure 6).

\begin{align*}
\text{H}_2\text{O} & \xrightarrow{\text{N} \equiv \text{S}} \text{[} \text{H} - \text{C} \equiv \text{S} \text{]} \quad \text{O} \equiv \text{C} \equiv \text{S} \\
& \xrightarrow{\text{H}_2\text{N}} \\
\text{OH} & \quad \text{NH}_2
\end{align*}

Figure 6. Proposed pathway for the conversion of benzyli thiocyanate (BITC, 5) into benzyl amine.

Our hypothesis was supported by performing a comparative hydrolysis experiment on 4-CIBITC (7). Contrary to 2, this structural analogue displays a deactivating group in the para-position, which is expected to hamper the formation of the exomethylene reactive species and favor the nucleophilic addition of water onto the ITC function. When reacted with water at 90 °C, 7 was indeed readily converted into 4-chlorobenzylamine.

The above study clarifies why 2 was not detected in the essential oil obtained from the hydrodistillation of \textit{P. brazzeana} samples: the ITC would be totally hydrolyzed during the process. The study was extended to a family of methoxylated samples: the ITC would be totally hydrolyzed during the process. The present study indicates that benzylic ITCs are generally not stable in water at 90 °C. The sensitivity of those compounds to hydrolysis has important implications in the modes of extraction used to obtain essential oil not only from \textit{P. brazzeana} but from any other GL-containing plant. In our \textit{P. brazzeana} essential oil sample, 4-methoxybenzyl derivatives were not detected in substantial amounts, whereas benzyl derivatives were the major compounds in the mixture.\textsuperscript{33} This is somehow puzzling, considering that the parent GL glucobractein is the major GL in the plant in comparison to the minor glucotropaeolin. Other parameters such as the complexity of the plant matrix or the harsh hydrodistillation conditions should probably be considered as well to understand why 4-methoxybenzyl derivatives can hardly be detected in the hydrodistillate. It has to be noted that a similar observation has been made in a previous investigation of the essential oil of \textit{R. subintegri folia} root.\textsuperscript{34} In addition, the stability of glucobractein in hydrodistillation-mimicking conditions should be addressed in complementary studies to evaluate the formation of ITC 2 and other 4-methoxybenzyl derivatives during the process. Finally, the present paper allows for a better understanding of our preliminary observations on varying the hydrodistillation length of time for \textit{P. brazzeana} root to modulate the essential oil composition. The results of that study in progress in our laboratory will be described in a separate paper.

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\section*{Notes}
The authors declare no competing financial interest.

\section*{ABBREVIATIONS USED}
ITC, isothiocyanate; GL, glucosinolate; DCM, dichloromethane; EA, ethyl acetate; PE, petroleum ether; BITC, benzyl isothiocyanate; 2-MBITC, 3-MBITC, 4-MBITC, 2-methoxy-, 3-methoxy-, 4-methoxybenzyl isothiocyanate; 3,4,5-DIMTIC, 3,4,5- TMBITC, 3,4-dimethoxy-, 3,4,5-trimethoxybenzyl isothiocyanate; 4-CIBITC, 4-chlorobenzyl isothiocyanate

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