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Stability of Benzylic-Type Isothiocyanates in Hydrodistillation-Mimicking Conditions

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ABSTRACT: *Pentadiplandra brazzeana* Baillon (Pentadiplandraceae) is known to contain benzyl-, 3-methoxybenzyl-, 4-methoxybenzyl-, 3,4-dimethoxybenzyl-, and indole-type glucosinolates, and the essential oil obtained from its roots is mainly 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.

KEYWORDS: *Pentadiplandra brazzeana, glucoaubrietin, hydrodistillation, isothiocyanates, glucosinolates, thermal decomposition*

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, flavors, and medicinal purposes (aromatherapy). Various technologies were developed to obtain the best aromatic substances to fulfill the needs of the perfumer and flavorist[1] 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, mechanical processes, or dry distillation.

On the basis of the source material, two major types of distillation processes are used: water distillation (in which the plant material is immersed in water and boiled by an external heat source) and steam distillation (in which steam is pumped through the plant matrix to extract the oil).[2] Improvement in production technology is an essential element to ameliorate the overall yield and quality of the product.[3] Hydrodistillation, which is the most "traditional" method for aromatic plant treatment, is still used for the production of many commercial oils, especially in tropical countries, because it is a simple and cheap technology. Nevertheless, chemical changes are invariably associated with water or steam distillations.[4] Any nonoptimized distillation conditions can lead to hydrolysis of essential oil components; the thermal degradation of carbohydrates and proteins may also result in the formation of volatile molecules, which affect the acceptability and quality of freshly distilled oil.[5] Distillation over short periods of time helps in reducing hydrolysis, decomposition, and resinification.

In damaged plant cells, glucosinolates (GLs) are transformed by the endogenous myrosinase (β-thioglucoside glucohydrolase; EC 3.2.1.147) to produce isothiocyanates (ITCs), many of which can be volatile and pungent. In most cases, ITCs are sensitive to thermal degradation in aqueous medium.[6−9] Indeed, in our continuous program of chemical investigations on aromatic and medicinal plants from Central and West Africa, a great chemical variability was observed for essential oils obtained from *Rinorea subintegrifolia* O. Ktze (Violaceae family) roots and *Drypetes gossweileri* S. Moore (Euphorbiaceae family) bark.[10,11] The content of benzyl isothiocyanate (BITC, **5**) (Figure 1) decreased during the distillation process, whereas the relative proportion of benzyl cyanide increased, suggesting a chemical transformation of the ITCs.

Pentadiplandra brazzeana Baill. (Pentadiplandraceae family) is a climber from Cameroon, the berries of which are eaten and used as a sweetener of beverages.[12] Recently, the GLs present in root, seed, and leaf extracts of the plant *P. brazzeana* were

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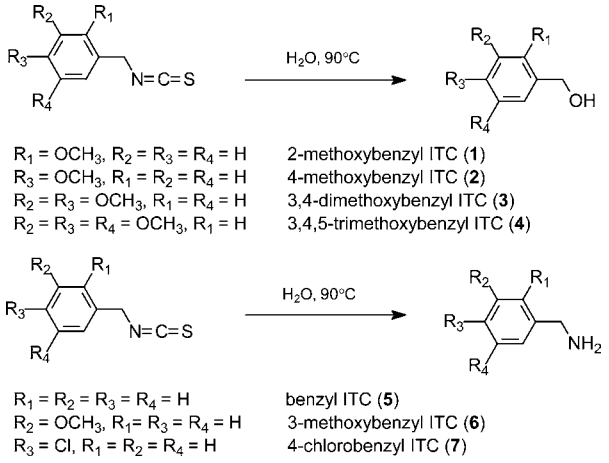

Figure 1. Hydrolytic degradation of benzylic-type ITCs.

characterized and quantified according to the HPLC analysis of desulfo-GLs.[13] Glucotropaeolin (benzyl GL), glucolimnanthin (3-methoxybenzyl GL), and glucoaubrietin (4-methoxybenzyl GL) were shown to be present in the root extract, whereas the seed mainly contained glucoaubrietin. 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 above results apparently lacked consistency with data previously obtained from hydrodistillation experiments.[14] 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.[14] Therefore, this discrepancy prompted us to repeat the hydrodistillation process on a portion of the sample of *P. brazzeana* root used for the quantification of GLs.[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).[13] Several studies have shown that individual GLs and GLs in plant extracts are degraded under hydrodistillation-mimicking conditions.[8,15−19] Benzyl cyanide and **5** are expected to result from the enzymatic decomposition of glucotropaeolin (benzyl GL).[20] Despite the fact that the stability of glucoaubrietin 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 glucoaubrietin is by far the major GL present in *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 *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 *N*,*N*′-diallylthiourea, allyl allyldithiocarbamate, diallyl tetrasulfide, and diallyl pentasulfide.[6] Allyl ITC isomerizes to allyl thiocyanate and decomposes into allylamine, allyl dithiocarbamate, diallylthiourea, carbon disulfide, diallylurea, and diallyl sulfide in buffer solutions (pH 4, 6, and 8) at 80 °C for 80 min.[7] Within 1 h in boiling water, allyl ITC degrades to diallyl di-, tri-, and tetrasulfide, allyl thiocyanate, 3*H*-1,2-dithiolene, 2-vinyl-4*H*-1,3-dithiin, 4*H*-1,2,3-trithiin, 5-methyl-1,2,3,4-tetrathiane, and *N*,*N*′-diallylthiourea.[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-trithiolane, 4-isothiocyanato-1-(methylsulfanyl)-1-butene, 3-butenyl ITC, and *N*,*N*′-di(4-methylsulfinyl)butyl thiourea.[22] It has long been known that indol-3-ylmethyl ITC, resulting from enzymatic degradation of glucobrassicin, is spontaneously transformed into indol-3-ylmethanol.[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.[24] Finally, 4-hydroxybenzyl ITC, resulting from enzymatic hydrolysis of glucosinalbin, is unstable in aqueous media, producing 4-hydroxybenzyl alcohol under release of a thiocyanate ion.[25−27]

Those observations led us to study and compare the stability in water at 90 °C of the major 4-methoxybenzyl (**2**) and benzyl ITCs (**5**) corresponding respectively to glucoaubrietin and glucotropaeolin, present in *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[28] under hydrodistillation-mimicking conditions. Therefore, 2-methoxy (2-MBITC, **1**), 3-methoxy (3-MBITC, **6**), and 4-methoxybenzyl (4-MBTIC, **2**) ITCs (Figure 1) were tested to check the influence of the substituent's location; 3,4-dimethoxy (3,4-DMITC, **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-ClBITC, **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). $CDCl_3$ was purchased from Euriso-top (St-Aubin, France). ^{1}H NMR spectra were recorded at 250 MHz on a Bruker Avance DPX 250 spectrometer, δ values being referenced to residual $CHCl_3$ 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 AEK0200713 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.[29]

2-Methoxybenzyl Isothiocyanate ***1***. Compound **1** was isolated in 89% yield as a yellow oil. R_f 0.73 (EA/PE 1:3). IR (neat) 2165, 2072 (−N═C═S). ^{1}H NMR (250 MHz, $CDCl_3$) 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_2N), 3.86 (s, 3H, OMe). The spectroscopic data agree with the published values.[13,30,31]

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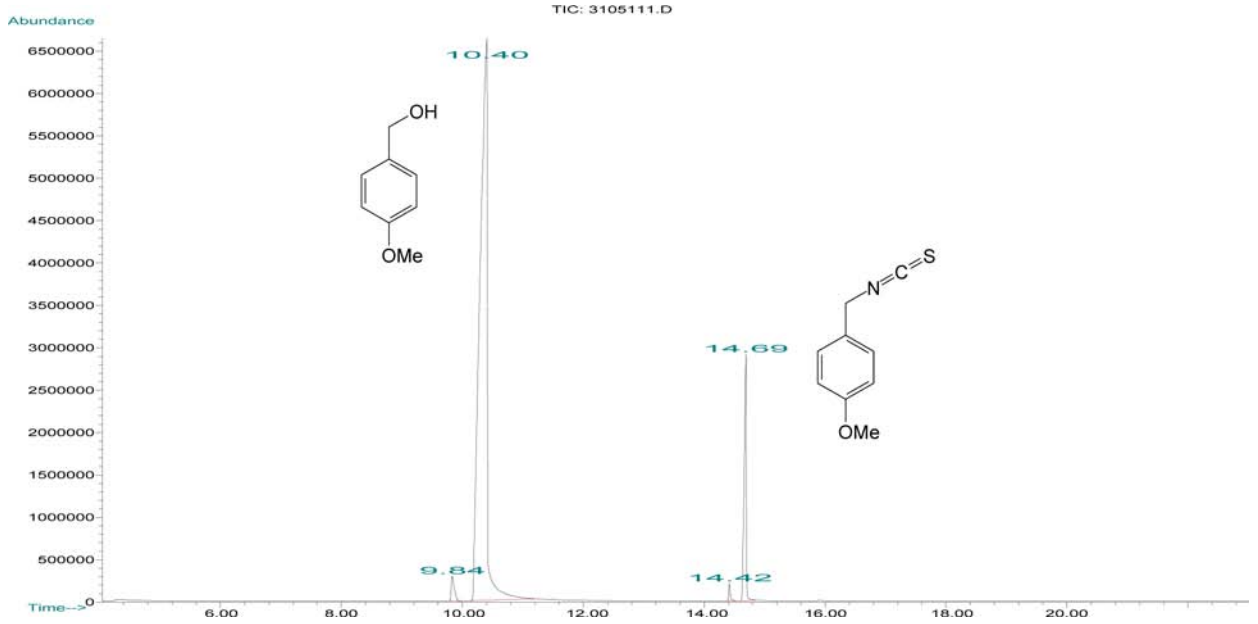

Figure 2. Gas chromatogram of the DCM fraction obtained after a 10 min degradation of 4-methoxybenzyl ITC (4-MBITC, **2**) in water at pH 5.0 ± 0.2 at 90 °C (minor peaks were present in the starting synthesized **2**).

3-Methoxybenzyl Isothiocyanate ***6***. Compound **6** was isolated in 96% yield as a yellow oil. R_f 0.8 (EA/PE 1:3). IR (neat) 2166, 2080 (−N═C═S). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.30 (t, 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, CH_2N), 3.83 (s, 3H, OMe). The spectroscopic data agree with the published values.[13,31]

4-Methoxybenzyl Isothiocyanate ***2***. Compound **2** was isolated in 92% yield as a yellow oil. R_f 0.7 (EA/PE 1:3). IR (neat) 2164, 2073 (−N═C═S). ^{1}H NMR (250 MHz, $CDCl_3$) 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, CH_2N), 3.82 (s, 3H, OMe). The spectroscopic data agree with the published values.[13,31,32]

4-Chlorobenzyl Isothiocyanate ***7***. Compound **7** was isolated in 96% yield as a yellow oil. R_f 0.73 (EA/PE 1:3). IR (neat) 2173, 2073 (−N═C═S). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.37 (d, 2H, J_{vic} = 8.6 Hz, H-3, H-5), 7.26 (d, 2H, J_{vic} = 8.6 Hz, H-2, H-6), 4.69 (s, 2H, CH_2N).[32]

3,4-Dimethoxybenzyl Isothiocyanate ***3***. Compound **3** was isolated in quantitative yield as a yellowish solid. R_f 0.44 (EA/PE 1:3). IR (neat) 2163, 2078 (−N═C═S). ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.86 (m, 2H, Ar−H), 6.82 (m, 1H, Ar−H), 4.64 (s, 2H, CH_2N), 3.91 (s, 3H, OMe), 3.89 (s, 3H, OMe). The spectroscopic data agree with the published values.[33]

3,4,5-Trimethoxybenzyl Isothiocyanate ***4***. Compound **4** was isolated in 94% yield as a yellow oil. R_f 0.32 (EA/PE 1:3). IR (neat) 2166, 2080 (−N═C═S). ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.52 (s, 2H, Ar−H), 4.65 (s, 2H, CH_2N), 3.88 (s, 6H, 2 × OMe), 3.85 (s, 3H, OMe). The spectroscopic data agree with the published values.[34]

Stability of Benzylic ITCs in Water. A sample of ca. 20 mg of ITC was weighed in a 25 mL flask to which 10 mL of ultrapure water (pH 5.0 ± 0.2) was added. The mixture was vortexed for 2 min, and an aliquot of 400 μL was transferred into a 1.5 mL glass vial and extracted with 400 μL of DCM by vortexing for 1 min. The aqueous phase was analyzed by HPLC and the DCM fraction by GC-MS to have the peak area at time 0 min. The flask was then fitted with a condenser and the mixture immediately kept under vigorous stirring in a water bath heated at 90 ± 2 °C. Aqueous samples of 400 μL were then withdrawn every 10 min during a 1 h heating period (and every successive hour over 7 h, in case of slower degradation) and extracted with the same volume of DCM as described above for the first point at time 0 min. Each aqueous fraction was then analyzed by HPLC, whereas each organic fraction was analyzed by GC-MS as described in the following two sections. After complete degradation, the water phase was analyzed by HPLC and freeze-dried. The resulting residue was dissolved in DCM and analyzed by GC-MS. Each degradation trial was performed in triplicate.

GC-MS Analyses of the Dichloromethane and Aqueous Fractions. The analyses were carried out using a Hewlett-Packard GCD System model G1800A equipped with a 30 m × 0.25 mm HP-5 ms capillary column. DCM extracts (1 μL) were manually injected in splitless mode. The flow rate of the carrier gas (He) was 1 mL min^{-1}. The column temperature was programmed from 60 °C (isocratic 2 min) to 220 °C (isocratic 1 min) at a rate of 10 °C min^{-1}. The temperatures of the injector and detector were 200 and 280 °C, respectively. The experiments were carried out with electron impact ionization (EI) mode at an electron energy of 70 eV. The degradation products were identified by matching the recorded mass spectra with the NBS75K.L (NIST, 1992 version) mass spectrum library of the GC-MS data system. The qualitative analyses of the degradations of the ITCs were carried out using the normalized area. The detector response for each eluted compound was set at the default value of 1, and the peak area of the ITC at time 0 min in the sampling sequence was assigned the value of 1. The successive peak areas at 10, 20, 30, 40, 50, 60, 120, 180, 240, 300, 360, and 420 min in the degradation trial were relative to the peak area at 0 min.

HPLC Analyses of the Aqueous Fractions. The analyses were performed on an HPLC Agilent 1100 system equipped with a photodiode array detector, on an Inertsil ODS-3 column (250 × 3.0 mm, 5 μm particle size) thermostated at 30 °C. The instrument was operated with a gradient of water (A) and acetonitrile (B) at a flow rate of 0.8 mL min^{-1} using the following program: 1 min, 10% B; 16 min, linear gradient up to 40% B; 3 min, linear gradient down to 10% B. Eluting peaks were detected by monitoring the absorbance at 226, 240, 254, and 280 nm.

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

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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 glucosinalbin 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**)

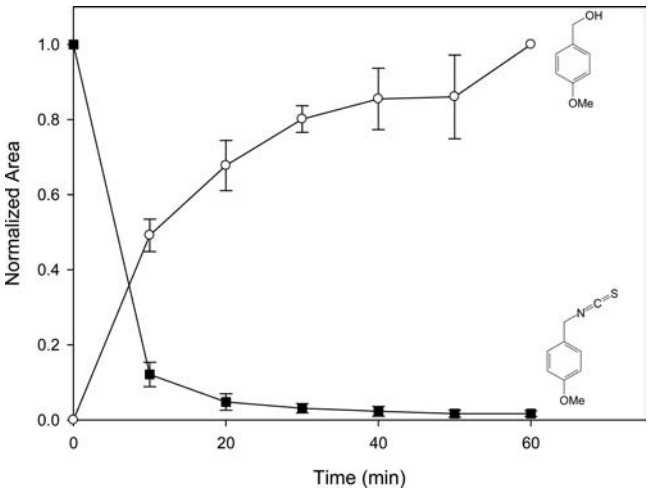

Figure 3. Degradation of 4-methoxybenzyl ITC (4-MBITC, **2**) and concomitant formation of 4-methoxybenzyl alcohol in water at pH 5.0 ± 0.2 at 90 °C.

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 **2** and the final one 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

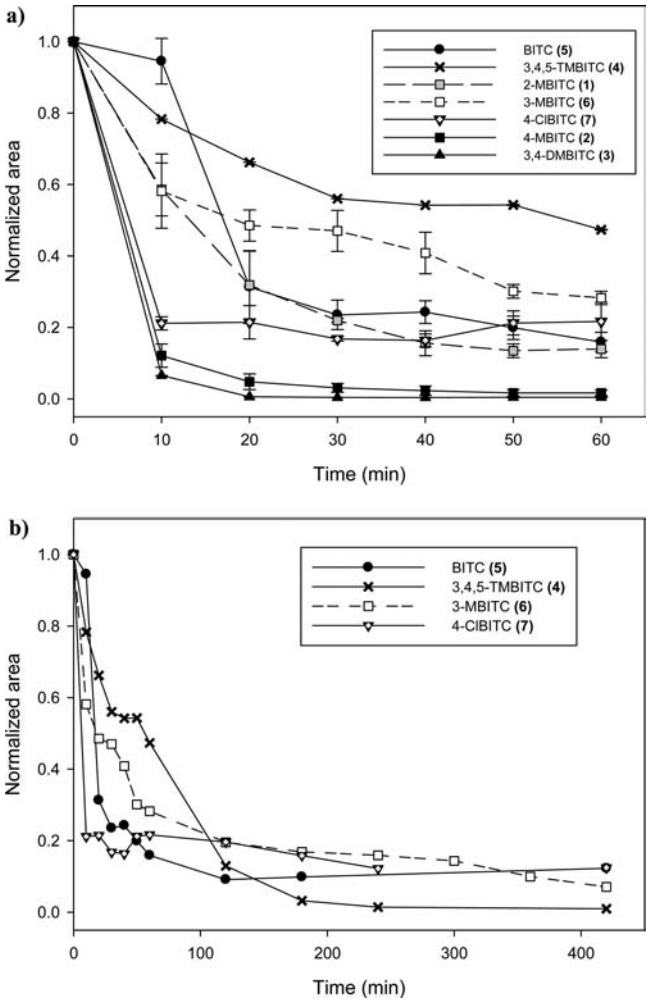

Figure 4. Time course degradation of benzylic-type ITCs in water at pH 5.0 ± 0.2 at 90 °C: (a) after 1 h; (b) after 7 h.

Figure 5. The intermediacy of a labile pseudoquinone methide is anticipated, in analogy with Borek and Morra's mechanistical

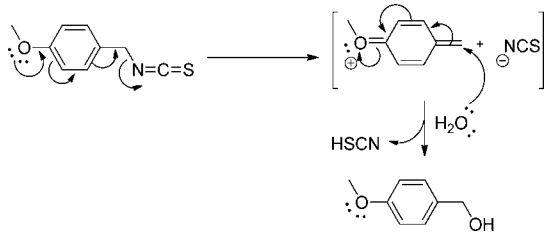

Figure 5. Proposed pathway for the conversion of 4-methoxybenzyl ITC (4-MBITC, **2**) into 4-methoxybenzyl alcohol.

assumption[27] 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 hydrolyses 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

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slower than the hydrolysis of **2**, probably because of a lesser stabilization in the transient *o*-quinone methide.[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 oxysulfide (Figure 6).

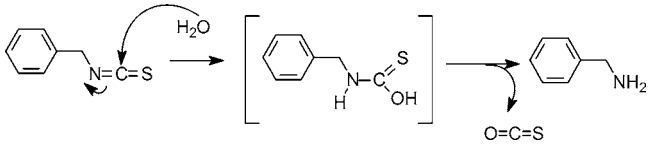

Figure 6. Proposed pathway for the conversion of benzyl isothiocyanate (BITC, **5**) into benzylamine.

Our hypothesis was supported by performing a comparative hydrolysis experiment on 4-ClBITC (**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 *P. brazzeana* samples: the ITC would be totally hydrolyzed during the process. The study was extended to a family of methoxylated benzyl ITCs derived from less common GLs: 2-methoxyglucotropaeolin,[30] glucolimnanthin,[36] 3,4-dimethoxyglucotropaeolin,[33] and 3,4,5-trimethoxyglucotropaeolin.[34] With regard to hydrolysis of ITCs, the position of the methoxy electron-donating group on the aromatic ring was shown to be critical. The related benzylic alcohol is formed only when a methoxy group is present in ortho- or para-position. In other cases, that is, when the methoxy group is in the meta-position, or without any substituent, the reaction shifts toward the formation of the benzylic amine.

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 *P. brazzeana* but from any other GL-containing plant. In our *P. brazzeana* essential oil sample, 4-methoxybenzyl derivatives were not detected in substantial amounts, whereas benzyl derivatives were the major compounds in the mixture.[13] This is somehow puzzling, considering that the parent GL glucoaubrietin 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 *R. subintegrifolia* root.[11] In addition, the stability of glucoaubrietin 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 *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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Notes

The authors declare no competing financial interest.

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-DMITC, 3,4,5-TMBITC, 3,4-dimethoxy-, 3,4,5-trimethoxybenzyl isothiocyanate; 4-ClBITC, 4-chlorobenzyl isothiocyanate

REFERENCES

(1) Meyer-Warnod, B. Natural essential oils. Extraction processes and applications to some major oils. *Perfum. Flavor.* **1984**, *9*, 93−104.

(2) Whish, J. P. M. A flexible distillation system for the isolation of essential oils. *J. Essent. Oil Res.* **1996**, *8*, 405−410.

(3) Singh, K. P. Challenges and opportunities in essential oil processing industries. *Res. Ind.* **1993**, *38*, 83−89.

(4) Baerheim Svendsen, A.; Scheffer, J. J. C. Isolation and analysis of essential oils. In *On Essential Oils*; Synthite Industrial Chemical Private Ed.: Kolenchery, India, 1986; pp 3−13.

(5) Coleman, W. M.; Lawrence, B. M.; Craven, S. H. The use of a non-equilibrated solid phase microextraction method to quantitatively determine the off-notes in mint and other essential oils. *J. Sci. Food Agric.* **2004**, *84*, 1223−1228.

(6) Kawakishi, S.; Namiki, M. Decomposition of allyl isothiocyanate in aqueous solution. *Agric. Biol. Chem.* **1969**, *33*, 452−459.

(7) Pecháček, R.; Velíšek, J.; Hrabcová, H. Decomposition products of allyl isothiocyanate in aqueous solutions. *J. Agric. Food Chem.* **1997**, *45*, 4584−4588.

(8) Bones, A. M.; Rossiter, J. T. The enzymic and chemically induced decomposition of glucosinolates. *Phytochemistry* **2006**, *67*, 1053−1067.

(9) Leoni, O.; Iori, R.; Palmieri, S. Hydrolysis of glucosinolates using nylon-immobilized myrosinase to produce pure bioactive molecules. *Biotechnol. Bioeng.* **2000**, *68*, 660−664.

(10) Eyele Mvé-Mba, C.; Menut, C.; Bessière, J.-M.; Lamaty, G.; Nzé Ekekang, I.; Denamganai, J. Aromatic plants of tropical central Africa. XXIX. Benzyl isothiocyanate as major constituent of bark essential oil of *Drypetes gossweileri* S. Moore. *J. Essent. Oil Res.* **1997**, *9*, 367−370.

(11) Agnaniet, H.; Mounzeo, H.; Menut, C.; Bessière, J.-M.; Criton, M. The essential oils of *Rinorea subintegrifolia* O. Ktez and *Drypetes gossweileri* S. Moore occurring in Gabon. *Flavour Fragrance J.* **2003**, *18*, 207−210.

(12) Tsopmo, A.; Ngnokam, D.; Ngamga, D.; Ayafor, J. F.; Sterner, O. Urea derivatives from *Pentadiplandra brazzeana*. *J. Nat. Prod.* **1999**, *62*, 1435−1436.

(13) De Nicola, G. R.; Nyegue, M.; Montaut, S.; Iori, R.; Menut, C.; Tatibouët, A.; Rollin, P.; Ndoyé, C.; Amvam Zollo, P.-H. Profile and quantification of glucosinolates in *Pentadiplandra brazzeana* Baillon. *Phytochemistry* **2012**, *73*, 51−56.

(14) Nyegue, M.; Ndoyé, F.; Amvam Zollo, P.-H.; Etoa, F.-X.; Agnaniet, H.; Menut, C. Chemical and biological evaluation of

essential oil of *Pentadiplandra brazzeana* (Bail.) roots from Cameroon. *Adv. Phytother. Res.* **2009**, 91−107.
(15) Chevolleau, S.; Gasc, N.; Rollin, P.; Tulliez, J. Enzymatic, chemical, and thermal breakdown of ^{3}H-labeled glucobrassicin, the parent indole glucosinolate. *J. Agric. Food Chem.* **1997**, *45*, 4290−4296.
(16) Slominski, B. A.; Campbell, L. D. Formation of indole glucosinolate breakdown products in autolyzed, steamed, and cooked brassica vegetables. *J. Agric. Food Chem.* **1989**, *37*, 1297−1302.
(17) Oerlemans, K.; Barrett, D.; Bosch Suades, C.; Verkerk, R.; Dekker, M. Thermal degradation of glucosinolates in red cabbage. *Food Chem.* **2006**, *95*, 19−29.
(18) Dekker, M.; Hennig, K.; Verkerk, R. Differences in thermal stability of glucosinolates in five *Brassica* vegetables. *Czech. J. Food Sci.* **2009**, S85−S88.
(19) Hanschen, F. S.; Rohn, S.; Mewis, I.; Schreiner, M.; Kroh, L. W. Influence of the chemical structure on the thermal degradation of the glucosinolates in broccoli sprouts. *Food Chem.* **2012**, *130*, 1−8.
(20) Hasapis, X.; MacLeod, A. J. Benzylglucosinolate degradation in heat-treated *Lepidium sativum* seeds and detection of thiocyanate-forming factor. *Phytochemistry* **1982**, *21*, 1009−1013.
(21) Chen, C.-W.; Ho, C.-T. Thermal degradation of allyl isothiocyanate in aqueous solutions. *J. Agric. Food Chem.* **1998**, *46*, 220−223.
(22) Jin, Y.; Wang, M.; Rosen, R. T.; Ho, C.-T. Thermal degradation of sulforaphane in aqueous solution. *J. Agric. Food Chem.* **1999**, *47*, 3121−3123.
(23) Agerbirk, N.; De Vos, M.; Kim, J. H.; Jander, G. Indole glucosinolate breakdown and its biological effects. *Phytochem. Rev.* **2009**, *8*, 101−120.
(24) Song, L.; Iori, R.; Thornalley, P. J. Purification of major glucosinolates from *Brassicaceae* seeds and preparation of isothiocyanate and amine metabolites. *J. Sci. Food Agric.* **2006**, *86*, 1271−1280.
(25) Kawakishi, S.; Muramatsu, K. Studies on the decomposition of sinalbin. Part I. The decomposition products of sinalbin. *Agric. Biol. Chem.* **1966**, *30*, 688−692.
(26) Kawakishi, S.; Namiki, M.; Wanatabe, H.; Muramatsu, K. Studies on the decomposition of sinalbin. Part II. The decomposition products of sinalbin and their degradation pathways. *Agric. Biol. Chem.* **1967**, *31*, 823−830.
(27) Borek, V.; Morra, M. J. Ionic thiocyanate (SCN^{-}) production from 4-hydroxybenzyl glucosinolate contained in *Sinapis alba* seed meal. *J. Agric. Food Chem.* **2005**, *53*, 8650−8654.
(28) Fahey, J. W.; Zalcmann, A. T.; Talalay, P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* **2001**, *56*, 5−51.
(29) Goodyer, C. L. M.; Chinje, E. C.; Jaffar, M.; Stratford, I. J.; Threadgill, M. D. Synthesis of *N*-benzyl- and *N*-phenyl-2-amino-4,5-dihydrothiazoles and evaluation as modulators of the isoforms of nitric oxide synthase. *Bioorg. Med. Chem.* **2003**, *11*, 4189−4206.
(30) Kjær, A.; Jensen, R. B. Isothiocyanates. XVII. *o*-Methoxybenzyl isothiocyanates and some derivatives. *Acta Chem. Scand.* **1956**, *10*, 141−142.
(31) Radulović, N. S.; Dekić, M. S.; Stojanović-Radić, Z. Z. Antimicrobial volatile glucosinolate autolysis products from *Hornungia petraea* (L.) Rchb. (Brassicaceae). *Phytochem. Lett.* **2012**, *5*, 351−357.
(32) Akinboye, E. S.; Rosen, M. D.; Denmeade, S. R.; Kwabi-Addo, B.; Bakare, O. Design, synthesis, and evaluation of pH-dependent hydrolysable emetine analogues as treatment for prostate cancer. *J. Med. Chem.* **2012**, *55*, 7450−7459.
(33) Ettlinger, M. G.; Kjaer, A.; Thompson, C. P.; Wagnières, M. Veratryl isothiocyanate, a new mustard oil from *Heliophila longifolia* DC. (Cruciferae). *Acta Chem. Scand.* **1966**, *20*, 1778−1782.
(34) Bach, E.; Kjaer, A. Methoxy-substituted benzyl isothiocyanates and *N*-benzyl thioureas. *Acta Chem. Scand.* **1971**, *25*, 2629−2634.
(35) Wan, P.; Barker, B.; Diao, L.; Fischer, M.; Shi, Y.; Yang, C. Quinone methides: relevant intermediates in organic chemistry. *Can. J. Chem.* **1996**, *74*, 465−475.
(36) Vaughn, S. F.; Berhow, M. A. Glucosinolate hydrolysis products from various plant sources: pH effects, isolation, and purification. *Ind. Crops Prod.* **2005**, *21*, 193−202.