



HAL
open science

Stability of Benzylic-Type Isothiocyanates in Hydrodistillation-Mimicking Conditions

Gina R de Nicola, Sabine Montaut, Patrick Rollin, Maximilienne Nyegue, Chantal Menut, Renato Iori, Arnaud Tatibouët

► **To cite this version:**

Gina R de Nicola, Sabine Montaut, Patrick Rollin, Maximilienne Nyegue, Chantal Menut, et al.. Stability of Benzylic-Type Isothiocyanates in Hydrodistillation-Mimicking Conditions. *Journal of Agricultural and Food Chemistry*, 2013, 61 (1), pp.137-142. 10.1021/jf3041534 . hal-04179231

HAL Id: hal-04179231

<https://hal.science/hal-04179231>

Submitted on 9 Aug 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Stability of benzylic-type isothiocyanates in
2 hydrodistillation-mimicking conditions

3 *Gina R. De Nicola,[†] Sabine Montaut,^{‡,*} Patrick Rollin,[§] Maximilienne Nyegue,^{||} Chantal Menut,[⊥]*

4 *R. Iori,[†] A. Tatibouët[§]*

5 [†] Agricultural Research Council – Industrial Crop Research Centre (CRA-CIN), Via di Corticella 133,
6 40128 Bologna, Italy.

7 [‡] Department of Chemistry and Biochemistry, Laurentian University, 935 Ramsey Lake Road, Sudbury,
8 ON, Canada P3E 2C6.

9 [§] Institut de Chimie Organique et Analytique, UMR-CNRS 7311, Université d'Orléans, B.P. 6759, F-
10 45067 Orléans Cedex 2, France.

11 ^{||} Department of Biochemistry, University of Yaoundé I, B.P. 812, Yaoundé, Cameroon.

12 [⊥] IBMM, UMR 5247 UM2-UM1, 15 avenue Charles Flahault, B.P. 14491, 34093 Montpellier, France.

13 AUTHOR EMAIL ADDRESS: smontaut@laurentian.ca

14 **RECEIVED DATE (to be automatically inserted after your manuscript is accepted if required**
15 **according to the journal that you are submitting your paper to)**

16 TITLE RUNNING HEAD: Stability of benzylic-type isothiocyanates.

17 CORRESPONDING AUTHOR FOOTNOTE: Sabine Montaut Department of Chemistry and
18 Biochemistry, Laurentian University, 935 Ramsey Lake Road, Sudbury, ON, Canada P3E2C6. TEL.:
19 +1(705)675-1151 x 2185, fax: +1(705)675-4844, email address: smontaut@laurentian.ca

20 ABSTRACT

21 *Pentadiplandra brazzeana* Baillon (Pentadiplandraceae) is known to contain benzyl-, 3-methoxybenzyl-,
22 4-methoxybenzyl-, 3,4-dimethoxybenzyl-, and indole-type glucosinolates, and the essential oil obtained
23 from its roots is mainly constituted of benzyl isothiocyanate and benzyl cyanide. In a previous study in
24 our group, we surmised that partial hydrolytic degradation of 4-methoxybenzyl isothiocyanate – one
25 major expected compound - occurred during the hydrodistillation process of essential oil preparation.
26 To probe this hypothesis, a selection of diversely substituted benzylic-type isothiocyanates was
27 submitted to standard hydrodistillation-mimicking conditions. After extraction with dichloromethane,
28 the reaction mixtures were analyzed using GC-MS. The aqueous phases resulting from liquid-liquid
29 extraction were analyzed by HPLC and GC-MS. 2-Methoxybenzyl-, 4-methoxybenzyl-, 3,4-
30 dimethoxybenzyl-, and 3,4,5-trimethoxybenzyl isothiocyanates underwent conversion into 2-
31 methoxybenzyl-, 4-methoxybenzyl-, 3,4-dimethoxybenzyl-, and 3,4,5-trimethoxybenzyl alcohols,
32 respectively, whereas benzyl-, 3-methoxybenzyl-, and 4-chlorobenzyl isothiocyanates were converted
33 into the corresponding benzylamines.

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

36
37
38
39
40

41 INTRODUCTION

42 Natural essential oils, generally obtained from vegetable matter (flowers, leaves, barks, roots, woods,
43 rhizomes...), contain highly volatile and fragrant organic compounds. Since ancient times, they are used
44 for perfumes, flavors, and for medicinal purposes (aromatherapy). Various technologies were developed
45 in order to obtain the best aromatic substances to fulfill the needs of the perfumer and flavorist¹ and to
46 respond to the pharmaceutical industries' demands. Some authors consider the term "essential oil" as a
47 generic term which encompasses natural extracts obtained by various processes (steam distillation,
48 solvent extraction, super critical fluid extraction etc.); however, this term should be strictly applied to
49 volatile extracts obtained from a raw material of plant origin either by steam distillation, mechanical
50 processes or dry distillation. According to the nature of the raw material, two major types of distillation
51 processes are used for aromatic plant materials: water distillation (in which the plant material is
52 immersed in water and boiled by an external heat source) and steam distillation (in which steam is
53 pumped through the plant charge to extract the oil).² Improvement in production technology is an
54 essential element to ameliorate the overall yield and quality of the product.³ Hydrodistillation, which is
55 the most "traditional method" for aromatic plant treatment, is still used for the production of many
56 commercial oils, specially in tropical countries, since it is a simple and cheap technology. Nevertheless,
57 chemical changes are invariably associated with water or steam distillations.⁴ Any non-optimized
58 distillation conditions can lead to hydrolysis of essential oil components; the thermal degradation of
59 carbohydrates and proteins may also result in the formation of highly volatile off-notes which affect the
60 acceptability and the quality of freshly distilled oil.⁵ Distillation over short periods of time helps in
61 reducing hydrolysis, decomposition, and resinification.

62 In damaged plant cells, glucosinolates (GLs) are transformed by the endogenous myrosinase (β -
63 thioglucoside glucohydrolase; E.C. 3.2.1.147) to produce mainly pungent volatile isothiocyanates (ITCs)
64 which are particularly sensitive to thermal degradation in aqueous medium.⁶⁻⁹ Indeed, in our continuous
65 program of chemical investigations on aromatic and medicinal plants from Central and West Africa, a
66 great chemical variability was observed for essential oils obtained from *Rinorea subintegrifolia* O. Ktze

67 (Violaceae family) roots and *Drypetes gossweileri* S. Moore (Euphorbiaceae family) bark.^{10,11} The
68 content of benzyl isothiocyanate (BITC, **5**) (**Figure 1**) decreased during the distillation process while the
69 relative proportion of benzyl cyanide increased, suggesting a chemical transformation of the ITCs.

70 *Pentadiplandra brazzeana* Baill. (Pentadiplandraceae family) is a climber from Cameroon, the berries
71 of which are eaten and used as sweetener of beverages.¹² Recently, the GLs present in root, seed, and
72 leaf extracts of the plant *P. brazzeana* were characterized and quantified by us according to the HPLC
73 analysis of desulfo-GLs.¹³ Glucotropaeolin (benzyl GL), glucolimnanthin (3-methoxybenzyl GL), and
74 glucoaubrietin (4-methoxybenzyl GL) were shown to be present in the root extract, whereas the seed
75 mainly contained glucoaubrietin. 3,4-Dimethoxybenzyl GL, glucobrassicin (indol-3-ylmethyl GL), and
76 traces of glucotropaeolin were detected in the leaf extract. During this investigation, the prediction of
77 the GL profile from desulfo-GL analysis and hydrodistillation products was shown to be rather tricky.
78 Varying extraction and analysis techniques led to different profiles. In addition, the above results
79 apparently lacked consistency with data previously obtained from hydrodistillation experiments.¹⁴
80 Therefore, this discrepancy prompted us to repeat the hydrodistillation process on a portion of the
81 sample of *P. brazzeana* root used for the quantification of GLs.¹³ The essential oil, obtained after a 5 h-
82 hydrodistillation at pH 5.9, was shown to contain 57% of BITC **5**, 10% of benzyl cyanide, and only 5%
83 of 4-methoxybenzyl ITC (4-MBITC, **2**) (**Figure 1**).¹³ Several studies have shown that individual GLs
84 and GLs in plant extracts are degraded under hydrodistillation-mimicking conditions.^{8,15-19} Benzyl
85 cyanide and **5** are expected to result from the enzymatic decomposition of glucotropaeolin (benzyl
86 GL).²⁰ Despite the fact that the stability of glucoaubrietin or its ITC under hydrodistillation conditions
87 has never been investigated, it is reasonable to ascribe the decrease of these compounds to thermal
88 breakdown and leaching into the heating medium, during hydrodistillation. However, taking into
89 account that glucoaubrietin is by far the major GL present in *P. brazzeana* root and that MBITC **2**, the
90 main degradation product originated from this GL, was only found as a minor component in the
91 essential oil of *P. brazzeana*, it had to be surmised that partial hydrolytic degradation of **2** occurred
92 during the hydrodistillation process.

93 The stability of some ITCs in aqueous solutions has been investigated. In distilled water at 37°C, allyl
94 ITC decomposes into *N,N'*-diallylthiourea, allyl allyldithiocarbamate, diallyl tetra-, and diallyl penta-
95 sulfide.⁶ Allyl ITC isomerizes to allyl thiocyanate and decomposes into allylamine, allyl
96 dithiocarbamate, diallylthiourea, carbon disulfide, diallylurea, and diallyl sulfide in buffer solutions (pH
97 4, 6 and 8) at 80°C for 80 min.⁷ Within 1 h in boiling water, allyl ITC degrades to diallyl di-, diallyl tri-
98 and tetrasulfide, allyl thiocyanate, 3*H*-1,2-dithiolene, 2-vinyl-4*H*-1,3-dithiin, 4*H*-1,2,3-trithiin, 5-methyl-
99 1,2,3,4-tetrathiane, and *N,N'*-diallylthiourea.²¹ In other respects, the thermal degradation of sulforaphane
100 (4-methylsulfinylbutyl ITC), in aqueous solution at 100°C, was shown to produce dimethyl disulfide, *S*-
101 methyl methanethiosulfinate, *S*-methyl methanethiosulfonate, methyl (methylsulfonyl)methyl disulfide,
102 1,2,4-trithiolane, 4-isothiocyanato-1-(methylsulfonyl)-1-butene, 3-butenyl ITC and *N,N'*-di(4-
103 methylsulfinyl)butyl thiourea.²² It has long been known that indol-3-ylmethyl ITC, resulting from
104 enzymatic degradation of glucobrassicin, is spontaneously transformed into indol-3-ylmethanol.²³ In
105 addition, approximately 50% of phenylethyl ITC degrades after 4h in a phosphate buffered saline, at pH
106 7.4 and at 37°C, producing phenethylamine.²⁴ Finally, 4-hydroxybenzyl ITC, resulting from enzymatic
107 hydrolysis of glucosinabin, is unstable in aqueous media, producing 4-hydroxybenzyl alcohol under
108 release of a thiocyanate ion.²⁵⁻²⁷

109 Those observations led us to study and compare the stability in water at 90°C of the major 4-
110 methoxybenzyl- (**2**) and benzyl ITCs (**5**) corresponding respectively to glucoabrietin and
111 glucotropaeolin, present in *P. brazzeana* root, by mimicking hydrodistillation extraction conditions.
112 Furthermore, in order to check the correlation of substituents on the benzyl moiety with the
113 transformation of the benzylic-type ITCs under hydrolytic conditions, we chose to probe the stability of
114 several diversely substituted ITCs in the same experimental conditions. Our major objective was to
115 investigate and compare the stability of benzylic ITCs associated with known naturally-occurring
116 arylaliphatic GLs²⁸ under hydrodistillation-mimicking conditions. So, 2-methoxy- (2-MBITC, **1**), 3-
117 methoxy- (3-MBITC, **6**), and 4-methoxybenzyl (4-MBTIC, **2**) ITCs (**Figure 1**) were tested to check the
118 influence of the substituent's location; 3,4-dimethoxy-, (3,4-DMITC, **3**) and 3,4,5-trimethoxybenzyl

243 oil composition. The results of that study in progress in our laboratory will be described in a separate
244 report.

245 ABBREVIATIONS USED

246 ITC: isothiocyanate, GL: glucosinolate, DCM: dichloromethane, EA: ethyl acetate, PE: petroleum
247 ether, BITC: benzyl isothiocyanate, 2-MBITC, 3-MBITC, 4-MBITC: 2-methoxy-, 3-methoxy-, 4-
248 methoxybenzyl isothiocyanate, 3,4-DMITC, 3,4,5-TMBITC: 3,4-dimethoxy-, 3,4,5-trimethoxybenzyl
249 isothiocyanate, 4-CIBITC: 4-chlorobenzyl isothiocyanate.

250 LITERATURE CITED

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

253 (2) Whish, J. P. M. A flexible distillation system for the isolation of essential oils. *J. Essent. Oil Res.*
254 **1996**, *8*, 405-410.

255 (3) Singh, K. P. Challenges and opportunities in essential oil processing industries. *Res. Ind.* **1993**,
256 *38*, 83-89.

257 (4) Baerheim Svendsen, A.; Scheffer, J. J. C. Isolation and analysis of essential oils. In *On essential*
258 *oils*, Synthite Industrial Chemical Private Ed., Kolenchery 68311, India, **1986**, pp. 3-13.

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

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

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

- 266 (8) Bones, A. M.; Rossiter, J. T. The enzymic and chemically induced decomposition of
267 glucosinolates. *Phytochemistry* **2006**, *67*, 1053-1067.
- 268 (9) Leoni, O.; Iori, R.; Palmieri, S. Hydrolysis of glucosinolates using nylon-immobilized myrosinase
269 to produce pure bioactive molecules. *Biotechnol. Bioeng.* **2000**, *68*, 660-664.
- 270 (10) Eyele Mvé-Mba, C.; Menut, C.; Bessière, J.-M.; Lamaty, G.; Nzé Ekekang, I.; Denamganai, J.
271 Aromatic plants of tropical central Africa. XXIX. Benzyl isothiocyanate as major constituent of bark
272 essential oil of *Drypetes gossweileri* S. Moore. *J. Essent. Oil Res.* **1997**, *9*, 367-370.
- 273 (11) Agnanié, H.; Mounzeo, H.; Menut, C.; Bessière, J.-M.; Criton, M. The essential oils of *Rinorea*
274 *subintegrifolia* O. Ktez and *Drypetes gossweileri* S. Moore occurring in Gabon. *Flavour Fragr. J.* **2003**,
275 *18*, 207-210.
- 276 (12) Tsopmo, A.; Ngnokam, D.; Ngamga, D.; Ayafor, J.F.; Sterner, O. Urea derivatives from
277 *Pentadiplandra brazzeana*. *J. Nat. Prod.* **1999**, *62*, 1435-1436.
- 278 (13) De Nicola, G. R.; Nyegue, M.; Montaut, S.; Iori, R.; Menut, C.; Tatibouët, A.; Rollin, P.; Ndoyé,
279 C.; Amvam Zollo, P.-H. Profile and quantification of glucosinolates in *Pentadiplandra brazzeana*
280 Baillon. *Phytochemistry* **2012**, *73*, 51-56.
- 281 (14) Nyegue, M.; Ndoyé, F.; Amvam Zollo, P.-H.; Etoa, F.-X.; Agnanié, H.; Menut, C. Chemical and
282 biological evaluation of essential oil of *Pentadiplandra brazzeana* (Bail.) roots from Cameroon. *Adv.*
283 *Phytotherapy Res.* **2009**, 91-107.
- 284 (15) Chevolleau, S.; Gasc, N.; Rollin, P.; Tulliez, J. Enzymatic, chemical, and thermal breakdown of
285 ³H-labeled glucobrassicin, the parent indole glucosinolate. *J. Agric. Food Chem.* **1997**, *45*, 4290-4296.
- 286 (16) Slominski, B. A.; Campbell, L. D. Formation of indole glucosinolate breakdown products in
287 autolyzed, steamed, and cooked brassica vegetables. *J. Agric. Food Chem.* **1989**, *37*, 1297-1302.

- 288 (17) Oerlemans, K.; Barrett, D.; Bosch Suades, C.; Verkerk, R.; Dekker, M. Thermal degradation of
289 glucosinolates in red cabbage. *Food Chem.* **2006**, *95*, 19-29.
- 290 (18) Dekker, M.; Hennig, K.; Verkerk, R. Differences in thermal stability of glucosinolates in five
291 Brassica vegetables. *Czech. J. Food Sci.* **2009**, S85-S88.
- 292 (19) Hanschen, F. S.; Rohn, S.; Mewis, I.; Schreiner, M.; Kroh, L. W. Influence of the chemical
293 structure on the thermal degradation of the glucosinolates in broccoli sprouts. *Food Chem.* **2012**, *130*,
294 1-8.
- 295 (20) Hasapis, X.; MacLeod, A. J. Benzylglucosinolate degradation in heat-treated *Lepidium sativum*
296 seeds and detection of thiocyanate-forming factor. *Phytochemistry* **1982**, *21*, 1009-1013.
- 297 (21) Chen, C.-W.; Ho, C.-T. Thermal degradation of allyl isothiocyanate in aqueous solutions. *J.*
298 *Agric. Food Chem.* **1998**, *46*, 220-223.
- 299 (22) Jin, Y.; Wang, M.; Rosen, R. T.; Ho, C.-T. Thermal degradation of sulforaphane in aqueous
300 solution. *J. Agric. Food Chem.* **1999**, *47*, 3121-3123.
- 301 (23) Agerbirk, N.; De Vos, M.; Kim, J. H.; Jander, G. Indole glucosinolate breakdown and its
302 biological effects. *Phytochem. Rev.* **2009**, *8*, 101-120.
- 303 (24) Song, L.; Iori, R.; Thornalley, P. J. Purification of major glucosinolates from *Brassicaceae* seeds
304 and preparation of isothiocyanate and amine metabolites. *J. Sci. Food Agric.* **2006**, *86*, 1271-1280.
- 305 (25) Kawakishi, S.; Muramatsu, K. Studies on the decomposition of sinalbin Part I. The
306 decomposition products of sinalbin. *Agric. Biol. Chem.* **1966**, *30*, 688-692.
- 307 (26) Kawakishi, S.; Namiki, M.; Wanatabe, H.; Muramatsu, K. Studies on the decomposition of
308 sinalbin Part II. The decomposition products of sinalbin and their degradation pathways. *Agric. Biol.*
309 *Chem.* **1967**, *31*, 823-830.

- 310 (27) Borek, V.; Morra, M. J. Ionic thiocyanate (SCN⁻) production from 4-hydroxybenzyl
311 glucosinolate contained in *Sinapis alba* seed meal. *J. Agric. Food Chem.* **2005**, *53*, 8650-8654.
- 312 (28) Fahey, J. W.; Zalcmann, A. T.; Talalay, P. The chemical diversity and distribution of
313 glucosinolates and isothiocyanates among plants. *Phytochemistry* **2001**, *56*, 5-51.
- 314 (29) Goodyer, C. L. M.; Chinje, E. C.; Jaffar, M.; Stratford, I.J.; Threadgill, M.D. Synthesis of N-
315 benzyl- and N-phenyl-2-amino-4,5-dihydrothiazoles and evaluation as modulators of the isoforms of
316 nitric oxide synthase. *Bioorg. Med. Chem.* **2003**, *11*, 4189-4206.
- 317 (30) Kjær, A.; Jensen, R. B. Isothiocyanates. XVII. *o*-Methoxybenzyl isothiocyanates and some
318 derivatives. *Acta Chem. Scand.* **1956**, *10*, 141-142.
- 319 (31) Radulović, N. S.; Dekić, M. S.; Stojanović-Radić, Z. Z. Antimicrobial volatile glucosinolate
320 autolysis products from *Hornungia petraea* (L.) Rchb. (Brassicaceae). *Phytochem. Lett.* **2012**, *5*, 351-
321 357.
- 322 (32) Ettlinger, M. G.; Kjaer, A.; Thompson, C. P.; Wagnières, M. Veratryl isothiocyanate, a new
323 mustard oil from *Heliophila longifolia* DC. (Cruciferae). *Acta Chem. Scand.* **1966**, *20*, 1778-1782.
- 324 (33) Bach, E.; Kjaer, A. Methoxy-substituted benzyl isothiocyanates and N-benzyl thioureas. *Acta*
325 *Chem. Scand.* **1971**, *25*, 2629-2634.
- 326 (34) Wan, P.; Barker, B.; Diao, L.; Fischer, M.; Shi, Y.; Yang, C. Quinone methides: relevant
327 intermediates in organic chemistry. *Can. J. Chem.* **1996**, *74*, 465-475.
- 328 (35) Vaughn, S. F.; Berhow, M. A. Glucosinolate hydrolysis products from various plant sources: pH
329 effects, isolation, and purification. *Ind. Crops Prod.* **2005**, *21*, 193-202.

330 **Funding**

331 We gratefully acknowledge financial support from the Natural Sciences and Engineering Research
332 Council of Canada (Discovery grant, S.M.), and from the Université d'Orléans (A.T. and P.R.).

333 **Notes**

334 The authors declare no competing financial interest.

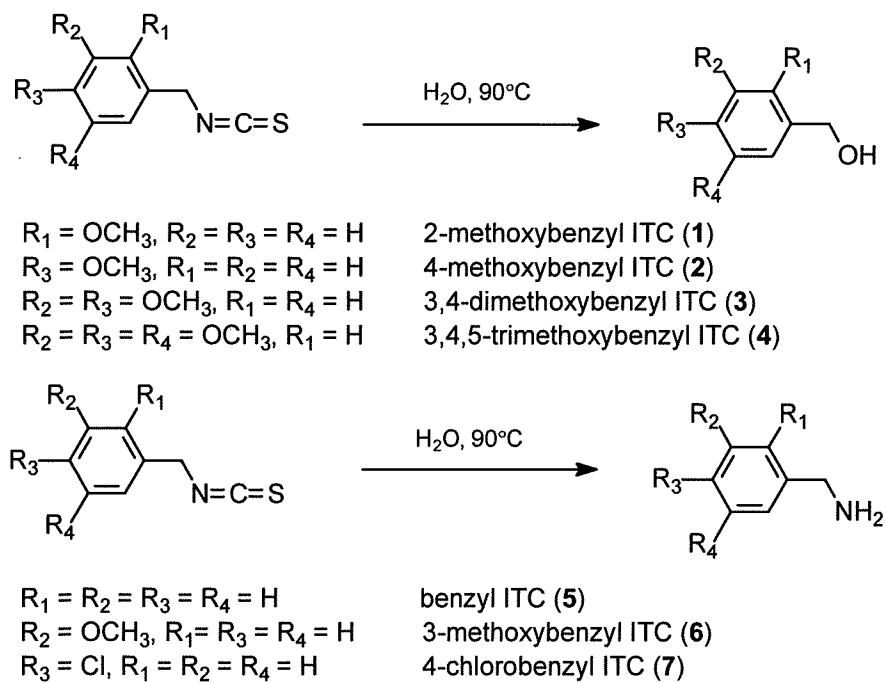


Figure 1. Hydrolytic degradation of benzylic-type ITCs.

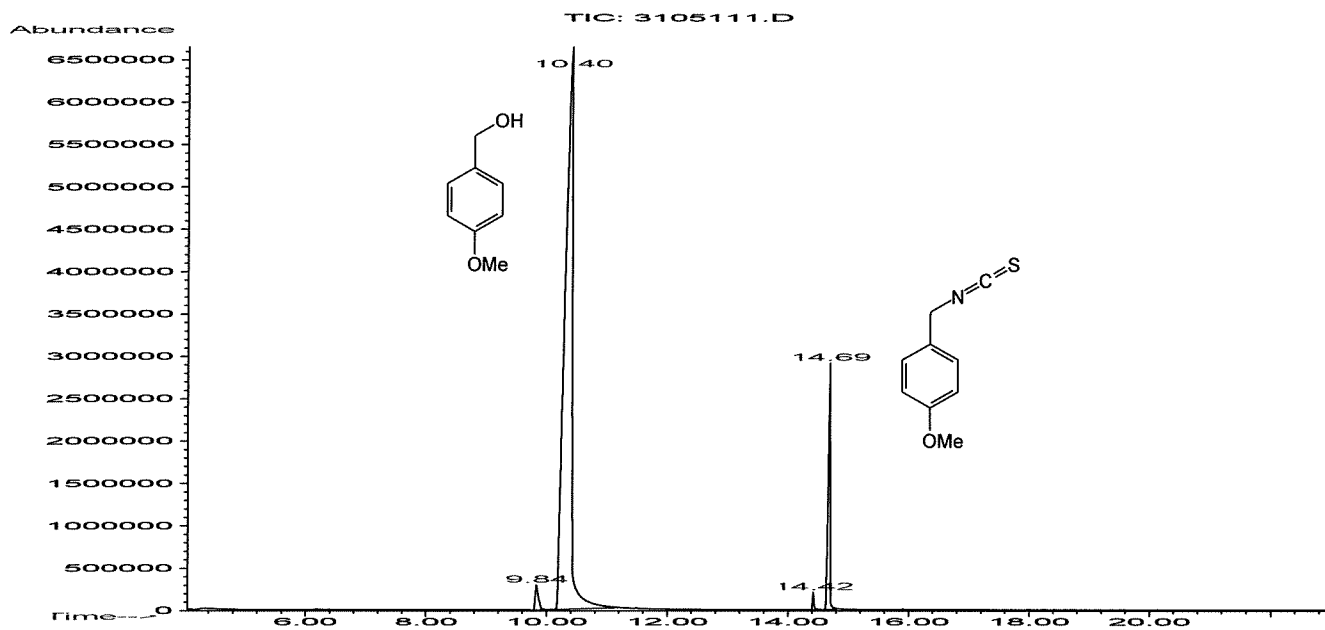


Figure 2. GC chromatogram of the DCM fraction obtained after a 10-min degradation of 4-methoxybenzyl ITC (4-MBITC, **2**) in water at pH 5.0 ± 0.2 at 90°C (minor peaks were present in the starting synthesized **2**).

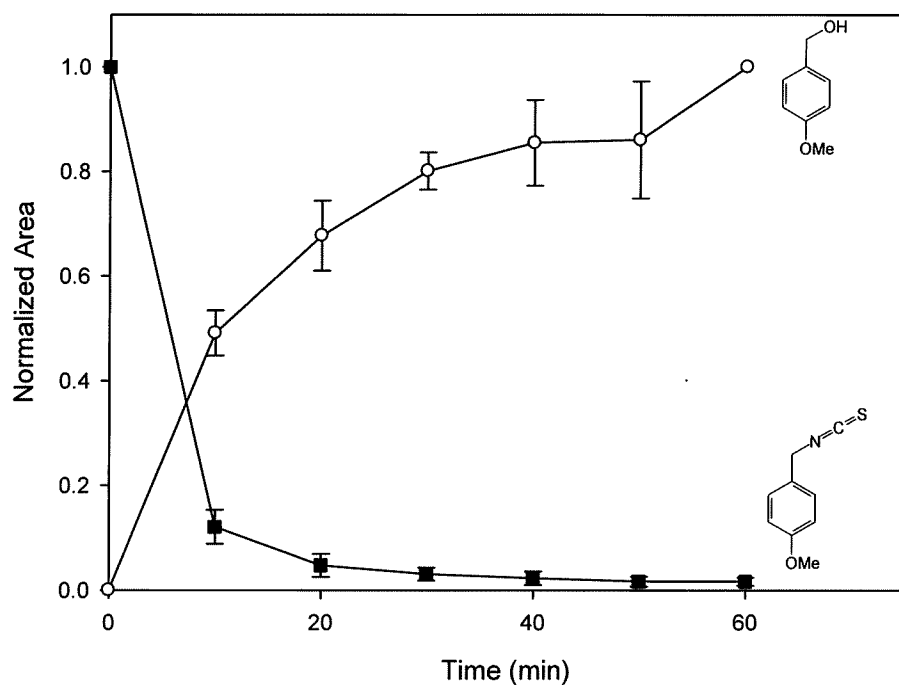
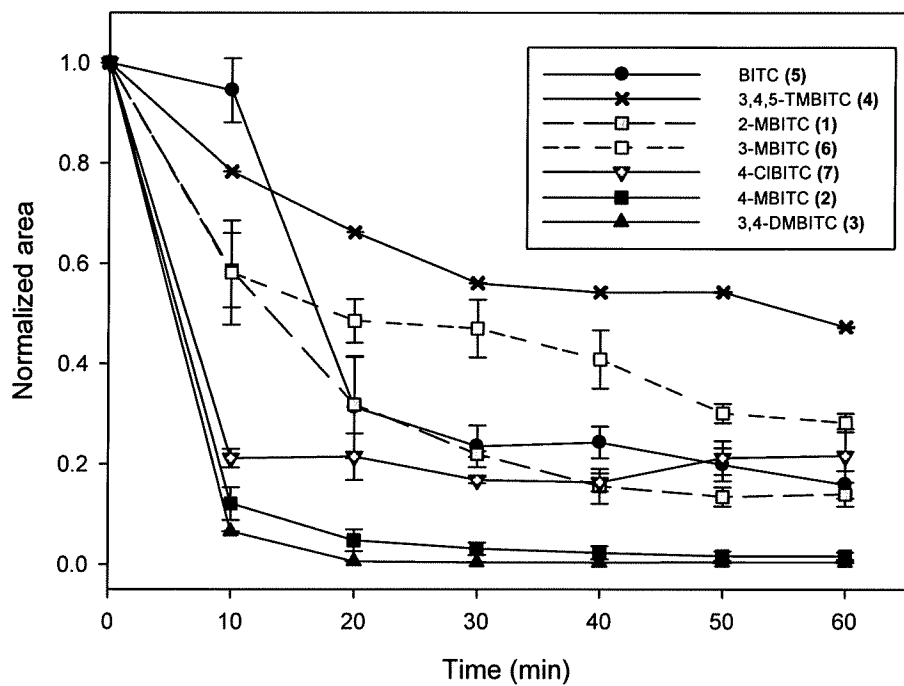


Figure 3. Degradation of 4-methoxybenzyl ITC (4-MBITC, **2**) and concomitant formation of 4-methoxybenzyl alcohol in water at pH 5.0 ± 0.2 at 90°C .

a)



b)

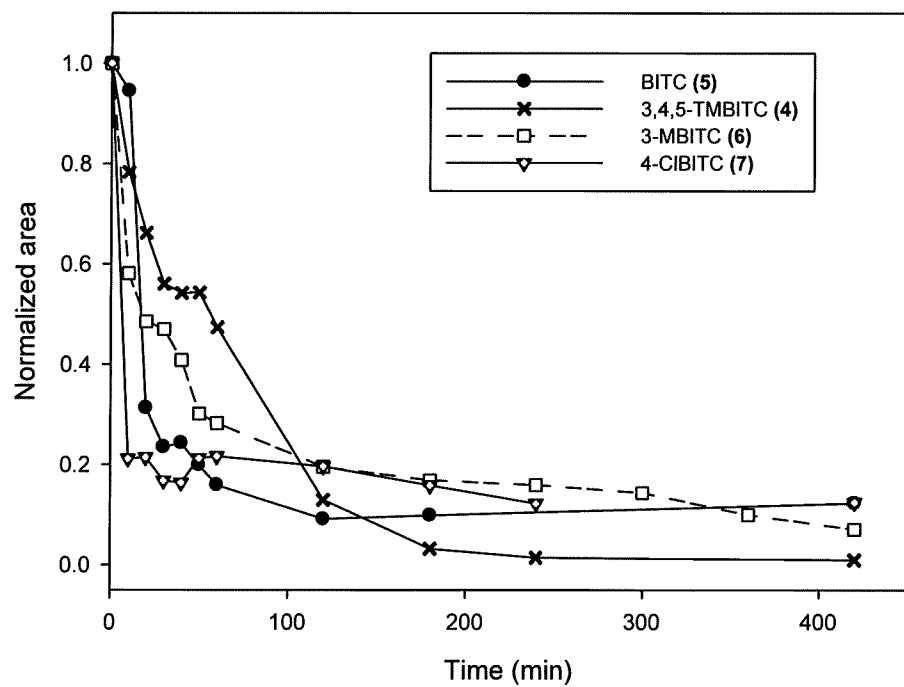


Figure 4. Time-course degradation of benzylic-type ITCs in water at pH 5.0 ± 0.2 at 90°C a) after 1 h, b) after 7 h.

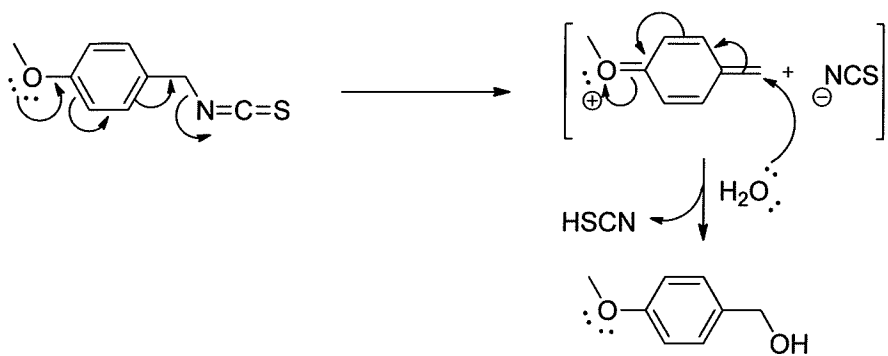


Figure 5. Proposed pathway for the conversion of 4-methoxybenzyl ITC (4-MBITC, **2**) into 4-methoxybenzyl alcohol

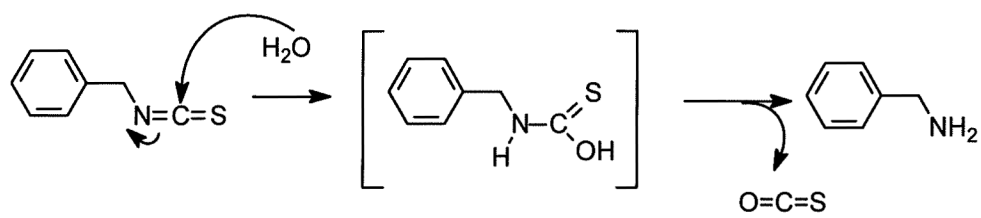


Figure 6. Proposed pathway for the conversion of benzyl isothiocyanate (BITC, **5**) into benzylamine

SYNOPSIS TOC

