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Ethyl 4-isothiocyanatobutyrate as a potential attractant for *Nysius cymoides* (Het.: Lygaeidae)

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ABSTRACT

Due to increasing attacks by *Nysius cymoides*, False Chinch Bug (FCB), in the canola fields of Iran, attempts have been made on its different control measures. Use of attractants might be a promising method in this relation. Behavioral effect of a synthesized isothiocyanate attractant was studied on FCB adults under laboratory condition. Ethyl 4-isothiocyanatobutyrate (ETCB) was synthesized via two-step reaction sequences from starting material of 4-aminobutyric acid with exposure to HCl gas, following the treatment of resulting ester with 1,1`-thiocarbonyldiimidazole. This technique is a newly modified procedure whose comparison with the commonly used technique is discussed. Although the synthetic attracted both sexes in a two-choice pitfall olfactometer, it was more efficient on the female FCBs.

Key words: Heteroptera, *Nysius cymoides*, False Chinch Bug, Isothiocyanate, Ethyl 4-isothiocyanatobutyrate, Attractant, Chemical synthesis, Olfactometry, Iran.

چکیده

به دلیل هجوم فزاینده‌ی سن *Nysius cymoides* در مزارع کلزای کشور، روش‌های مختلفی برای کنترل آن مورد بررسی قرار گرفته‌است. استفاده از ترکیبات جلب‌کننده یکی از روش‌های امیدبخش کنترل این حشره محسوب می‌شود. در این تحقیق با استفاده از یک ترکیب ایزوسیاناتی (اتیل ٤- ایزوتیوسیا نا توبوتیرات) اثر آن بر روی رفتار سن بذر خوار کلزا، در آزمایشگاه مورد بررسی قرار گرفت. اتیل ٤- ایزوتیوسیا نا توبوتیرات در طی دو مرحله سنتزی

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جداگانه از ماده اولیه ٤-آمینوبوتیریک اسید سنتز شد. ابتدا کربوکسیلیک اسید در معرض عبور جریان خشک گاز اسید کلریدریک در اتانول تولید استر نمود. سپس استر تولید شده با ۱و۱-تیوکربونیل دی ایمیدازول ماده نهایی اتیل ٤- ایزوتیوسیا نا توبوتیرات را تولید نمود. این تکنیک یک روش تغییر یافته از روش‌های قبلی می‌باشد که مقایسه بین آن‌ها مورد بحث قرار گرفته است. بررسی‌های رفتارشناسی با استفاده از یک بویایی‌سنج دو سوراخه نشان دادند که اتیل ٤-ایزوتیوسیا نا توبوتیرات سنتتیک برای افراد بالغ ماده و نر سن بذرخوار کلزا جلب‌کننده است. افراد بالغ ماده سن بذرخوار کلزا در مقایسه با افراد بالغ نر به اتیل ٤- ایزوتیوسیا نا توبوتیرات حساس‌تر بودند.

واژه‌های کلیدی: ناجوربالان، *Nysius cymoides*، سن بذرخوار کلزا، ایزوتیوسیاتات، اتیل ٤-ایزوتیوسیا نا توبوتیرات، جلب‌کننده، سنتز شیمیایی، بویایی‌سنجی، ایران*.

Introduction

Glucosinolates are amino acid-derived natural products that, upon hydrolysis, typically release isothiocyanates with a wide range of biological activities. Glucosinolates play a role in plant defence as attractants and deterrents against herbivores and pathogens, and have great potential for improvement of resistance to these agents (Mikkelsen & Halkier, 2003). The possible role of glucosinolates and their hydrolysis products, like allylisothiocyanate and benzylisothiocyanate, were tested as feeding stimulant as well as attractant in host-seeking in the cruciferous pest *Hellula undalis* (Lep.: Pyralidae). Application of 4-10 molar of these compounds to leaves significantly influenced the migration of the early instars. Low concentrations of the glucosinolates hydrolysis products, allyl-isothiocyanate and benzyl-isothiocyanate, induced anemotaxis in adults in the Y-olfactometer, while a high concentration of allyl-isothiocyanate was repellent (Mewis *et al*., 2001).

The role of the secondary plant compound allylisothiocyanate as a feeding attractant and ovipositing stimulant on the diamondback moth *Plutella xylostella* L. and the cabbage white *Pieris brassicae* L. was investigated by Gupta and Thorsteinson (1960).

The *Brassica* plant species synthesize large quantities of glucosinolates to produce

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active isothiocyanates and thiocyanates by the action of cell wall-bond enzyme myrosinase. Using nine isothiocyanates or mustard oil, showed that *Phyllotreta cruciferae* Goeze and *P. striolata* were more attracted to allylisothiocyanate than other compounds (Al-Doghairi, 1999).

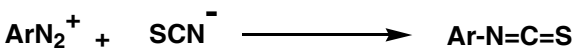
Selected isothiocyanates (mustard oils) were tested as attractants for adult *Nysius niger* Baker (Het.: Lygaeidae), a pest of cruciferous crops in the Canadian prairies, where the females were dominantly caught in the yellow boll-weevil traps baited with mustard oils. The methyl ester of Ethyl 4-isothiocyanatobutyrate (ETCB) in the seed of cruciferous genus *Erysium*, was strikingly attractive to the bug while the methyl ester itself was either less or completely not attractive to *N. niger* (Pivnick *et al.*, 1991). Using bait containing isothiocyanates and pheromone would efficiently attract both important pests of the genus *Phyllotreta* (Csonka *et al.*, 2006).

The ETCB was synthesized in accordance with Smith`s procedure (Smith and March., 2001) as follows:

1- Treatment of different primary amine with thiophosgene in the basic condition, after excluding HCl gas from the reaction would produce isothiocyanate.

2) The alkylation or acylation of thiocyanates by using alkylhalides and thiocyanato anion reacts with halide will produce isothiocyanate. The unwanted *S*-alkylation reaction is one of the limitations of the procedure.

3) Reaction of diazonium salt with thiocyanate anion produces an aromatic isothiocyanate.



4) Adding carbon disulphide into an amine in the presence of a base such as pyridine or dicyclohexylcarbodiimide would produce an anion of dithiocarbamic, which after removing H_2S gases, is converted to isothiocyanate (Nicolaou & Snyder, 2003).

In this study we intend to synthesize ETCB, using a modified technique to develop the previous techniques by Pivnick *et al.* (1991) and Nicolaou & Snyder (2003).

Material and methods

Chemical Synthesis. General: ^{1}H-NMR spectra were determined at 80 MHz, all NMR spectra were recorded in $CDCl_3$ and chemical shifts are expressed in ppm downfield from tetramethylsilane. Data are presented in order of hydrogen, multiplicity and coupling constant. IR values are in inverse centimeters. All substrates and reagents were obtained from Merck and Aldrich Chemical Co.

In addition to expanding synthetic utility, after many experiments, the conditions of the reaction were modified. Finally target of isothiocyanate was prepared by the following route:

For synthesizing 4-aminobutyrate, HCl gas was bubbled into a water-bath cooled slurry of 4-aminobutyric acid (4.0 g, 38.8 mmol) in absolute ethanol (50 ml). After being dissolved, the solution cooled in an ice bath, and was saturated with HCl gas. The solvent evaporated, followed by sequential addition and evaporation of aliquots of EtOH and ether (30 ml) to remove traces of HCl. The residue was dissolved in warm MeOH (10 ml), and product was precipitated as white crystals by slow addition of ether (50 ml). The crystals were filtered out, rinsed with ether, and air dried, giving a quantitative yield of ethyl 4-aminobutyrate (6.0 g as the hydrochloride salt).

To prepare ETCB, 1,1'-thiocarbonyldiimidazole (3.96 g, 90% pure, 20 mmol) was added to a solution of ethyl 4-aminobutyrate hydrochloride (3.36 g, 20 mmol) in 100 ml CH_3CN at 10^{o}C. The mixture warmed to room temperature while being stirring, giving a yellow solution, which subsequently precipitated imidazole by-product. The mixture was stirred at room temperature for 24 hours, later water was added (30 ml), as stirring continued for one more hour. The mixture was concentrated using a rotary evaporator and partitioned between water and ether. The ether layer was drained by water and brine and dried over anhydrous Na_2SO_4 to change into a brown oil, before distilled to the desired product ETCB.

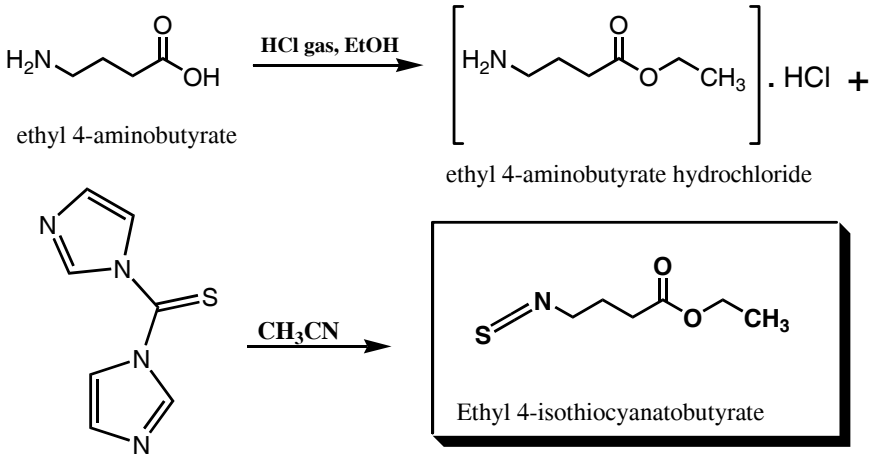


Fig. 1- Synthetic procedure of Ethyl 4-isothiocyanatobutyrate

Bioassay. The FCBs were from a strain in Faridan (Esfahan Province, Iran) and reared under condition described by Mohaghegh (2009) on canola seeds. The 40-day-old FCBs were kept unmated at about 25 °C and 65% humidity, in a 16: 8 hr light-dark regim in plastic boxes (5.5 cm high × 19.5 cm long × 13.5 cm wide) with a wire mesh cover. The day before the tests, insects were separated by sex and kept individually in cylindrical glass tubes (15 mm × 90 mm) without food and later transferred to the bioassay room and allowed to acclimate for at least one hour.

A two-choice pitfall olfactometer adapted from Pierce *et al.* (1981) were used. The olfactometer consisted of a circular arena of 9 cm (diameter) × 3 cm (height) connected to two glass jars of 1.5 cm (height) by 4.3 cm (length). The bottom of the arena was made of Plexiglass and covered with white paper to ease the movement of the bugs. The bioassays were run during the first half of the scotophases at 25 ± 1 °C under white light. The FCBs were transferred to the testing room in their individual tubes one hour prior to the experiment and singly released at the center of the olfactometer arena every 10 minutes.

Bioassays were conducted, using 20-28 FCBs in the following treatments: 1- control with two empty jars; 2- control with two jars containing a solvent (acetone, Merck, Germany, m 99.5%); 3- 10 ng of ETCB vs. solvent; 4- 100 ng of ETCB vs. solvent; 5- 1 µg of ETCB vs.

solvent; 6- 10 µg of ETCB vs. solvent. One microliter of acetone containing synthetic stimulus was applied to a filter paper (1 × 1 cm) placed into a glass jar just prior to the test. The proportion of the FCBs that responded after 10 minutes and the proportions that responded in presence of the stimulus were compared with those in the absence of the stimulus (control treatments), using the χ^2 test for two independent samples (Siegel, 1956). In addition, the number of FCBs choices for the stimulus jar was compared with the number of choices for the associated control jar, using the nonparametric binomial test with the null hypothesis of an equal probability of choosing the control or the stimulus (Siegel, 1956).

Results and Discussion

Chemical Synthesis. Literature Data Analysis: Kugelrohr distilled, bp ~80 ^{o}C (0.05 mm Hg), giving 1.61 g (93 %) of the isothiocyanate as an oil, 98% pure by capillary GC (DB-5, 20 m x 0.32 mm ID). ^{1}H-NMR (80MHz, $CDCl_3$) δ 4.14 (quartet, 2H, *J*= 7.1 Hz; $CH_3\underline{CH_2}O$), 3.60 (t, 2H, *J*= 6.5 Hz; CH_2-N), 2.43 (t, 2H, *J*= 7.1 Hz; CH_2COOR), 1.99 (quartet, 2H, *J*= 6.8 Hz; $CH_2\underline{CH_2}CH_2O$), 1.25 (t, 3H, *J*= 7.1 Hz; CH_3); IR: 2990 (m), 2945(m), 2190 (s), 2105 (s), 1730 (s), 1180 (s), 1030 (m) cm^{-1}. MS *m/z* (relative abundance): 175 (2; M + 2), 173 (37; M^+), 129 (64), 128 (61), 127 (54), 100 (51), 88 (33), 70 (46), 61 (64), 60 (100), 45 (46).

Synthetic sample: Kugelrohr distilled, bp ~120^{o}C (3 mm Hg), giving 0.86 g, 5 mmol (25 %) of the isothiocyanate as an oil plus most undistilled crude products; ^{1}H-NMR (80MHz, $CDCl_3$) δ 4.14 (quarted, 2H, *J* =7.12 Hz; $CH_3\underline{CH_2}O$), 3.60 (t, 2H, *J* =6.4 Hz; CH_2N), 2.43 (t, 2H, *J* =7.16 Hz; CH_2COOR), 1.99 (quintet, 2H, *J* =6.8 Hz; $CH_2\underline{CH_2}CH_2$) 1.25 (t,3H, *J* =7.11 Hz CH_3) (Fig. 2).

FT-IR (KBr) ν 2979 (m), 2934 (m), 2187 (m), 2111 (s), 1733 (s), 1652 (m), 1546 (s), 1178 (s), 1031 (m) cm^{-1}.

The ETCB synthesis was a modification of the procedure by Pivnick *et al.* (1991), Huh *et al.* (1996) and Nicolaou & Snyder (2003). We set the temperature of reaction between 25-28 ^{o}C while, HCl gas flew for 24 hours, and absolute ethanol was used (Fig. 1). The reaction sequences started from the commercially available 4-aminobutyric acid that was treated with a continuous stream of HCl gas. The reaction was stirred at room temperature overnight until the saturation of ethanolic solution with the gas flow. During the esterification temperature of the reaction was kept under 5 ^{o}C by an ice cool bath and later increased to 25-28 ^{o}C. After completing the reaction by checking the gas flow in the external tube of the reaction's flask,

the extra solvent was entirely drained under a reduced pressure to produce ethyl 4-aminobutyrate hydrochloride in shape of white crystals. The solid crystals were fully removed under a reduced pressure to let the extra hydrochloric acid gases out. After inserting an argon or nitrogen gas, the flask was refrigerated to prevent the product from being decomposed. In the next sequential experiment (Fig.1), the amine functional group of ethyl 4-aminobutyrate hydrochloride was treated with isothiocyanate of 1,1'-thiocarbonyldiimidazole in an isothiocyanation reaction (Nicolaou & Snyder, 2003; Paquette *et al*., 1992). In this procedure, using more solvent (100 ml) and increasing the reaction to 24 hours, resulted in the production of isocyanate. This step was done in an anhydrous acetonitrile (CH_3CN) and under argon condition to keep moisture off the experiment. The resulted crude attractant and white solid of diimidazole at the bottom of reaction flask was filtered out and washed with some more acetonitrile.

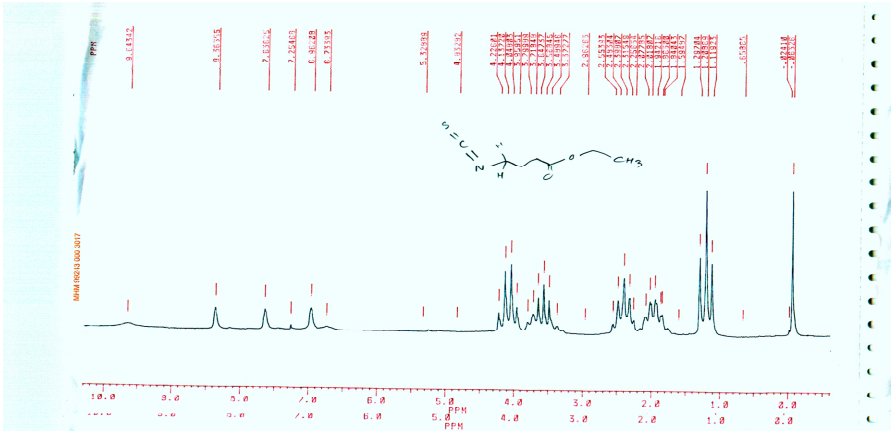


Fig. 2- NMR spectra of ethyl 4-isothiocyanatobutyrate (ETCB)

The combined acetonitrile solution was collected and the solvent evaporated under reduced pressure to form a yellow oil, which was later dissolved into ethylacetate and washed with water and brine to change into a brown colored oil distilled at 80 ^{o}C under a low pressure (0.05 mm Hg) to become a colorless oil. However, in place of using vacuum pump, we had to increase the temperature (Mayo *et al*., 2001). The heightened temperature of the bath of distillation flask up to ~120 ^{o}C with a 2-3 mm Hg pressure considerably decomposed the product and sharply decreased the reaction yield to 25% due to ETCB sensitivity to high temperature.

Table 1- *Nysius cymoides* responses to ethyl 4-isothiocyanatobutyrate (ETCB) in two-choice pitfall olfactometer after 10 minutes

Test No.	Choice 1	Choice 2	Sex	No. of tested bugs	No. of responses[a]	Percent response		Binomial probability
						stimulus	Control	Stimulus vs. control[b]
1	Empty	Empty	Female	11	2	50	50	0.5000
			Male	13	0	50	50	1.0000
			Total	24	2	50	50	0.5000
2	Acetone	Acetone	Female	13	2	50	50	0.5000
			Male	15	0	50	50	1.0000
			Total	28	2	50	50	0.5000
3	10 ng ETCB	Acetone	Female	9	0 ns	50	50	1.0000
			Male	11	1 ns	100	0	0.5000
			Total	20	1 ns	100	0	0.5000
4	100 ng ETCB	Acetone	Female	10	0 ns	50	50	1.0000
			Male	10	1 ns	100	0	0.5000
			Total	20	1 ns	100	0	0.5000
5	1 µg ETCB	Acetone	Female	11	4 ns	75	25	0.2500
			Male	10	2 ns	100	0	0.2500
			Total	21	6 ns	83	17	0.0938
6	10 µg ETCB	Acetone	Female	10	4*	100	0	0.0625
			Male	10	2 ns	100	0	0.2500
			Total	20	6*	100	0	0.0156
Total except the 1st and the 2nd tests			Female	40	9*	89	11	0.0313
			Male	41	6*	100	0	0.0156
			Total	81	15**	93	7	0.0009

a: Difference from the control ($\chi 2$ test) indicated by: ns: not significant, * $P < 0.05$, ** $P < 0.01$

b: Under the null hypothesis to respond to the stimulus and the control with an equal probability of ½.

Behavioral Bioassay. The rate of FCBs response was generally low for all tests (0-40%). Only a small number of FCBs responded to the control treatments (Table 1). The response to ETCB was at the highest dose (10 µg) ($P < 0.05$), that suggested the applied two-choice pitfall olfactometer without air flow was not sufficiently discriminative for *N. cymoides*. Our single-insect laboratory bioassay avoided any form of contact among the individuals to ensure the exactness of the results.

The FCB choice for the ETCB-baited jar was highly significant ($P < 0.001$) as both sexes responded efficiently ($P < 0.05$).

The rate of females′ response to 10 µg of ETCB was statistically greater than the males to the control treatments, and any stimulus doses (Table 1), so the effect of ETCB was found stronger in females than males. Our findings correspond to the results of the field trapping experiments on *C. niger*, using mustard oil baits (Pivnick *et al.*, 1991).

The results is to open new fields of investigation for the possible improvement of FCB control.

References

AL-DOGHAIRI, M. 1999. Pest management tactics for the western cabbageflea beetle (*Phyllotreta pusilla* Horn) on brassica crops. Ph.D. thesis, Colorado State University, FortCollins. 85 pp.

CSONKA, E., R. J. BARTELT, A. A. COSSE, B. W. ZILKOWSKI, I. UJVARY and M. TOTH, 2006. Similarities and differences in pheromonal and host-plant related chemical communication of flea beetles *Phyllotreta cruciferae* Goeze and *Phyllotreta vittula* Redtenbacher (Col.: Chrysomelidae). Abstracts of the International Society of Chemical Ecology Meeting. P. 211.

GUPTA, P. D. and A. J. THORSTEINSON, 1960. Food plant relationships of the diamondback moth (*Plutella maculipennis* (Curt.)). Entomol. Exp. et App. 3: 305-314.

HUH, N., A. REGE, B. YOO, T. P. KOGAN and H. KOHN, 1996. Design, synthesis, and evaluation of mitomycin-tethered phosphorothioate oligodeoxynucleotides; J. Org. Chem. 6: 659-669.

MAYO, D. W., R. M. PIKE and P. K. TRUMPER, 2001. Microscale techniques for the organic laboratory. John Willey & Sons, Inc., 319 pp.

MEWIS, I., C. ULRICHS and W. H. SCHNITZLER, 2001. Feeding behaviour and host-plant

finding of the cabbage webworm, *Hellula undalis* (F.) (Lep.: Pyralidae) in the context of secondary host-plant compounds. Mitteilungen der Deutschen Gesellschaft fur allgemeine und angewandte Entomologie. 13: 273-278.

MIKKELSEN, M. D. and B. A. HALKIER, 2003. Metabolic engineering of valine- and isoleucine-derived glucosinolates in Arabidopsis expressing CYP79D2 from cassava, Plant Physiology. 131: 773-779.

MOHAGHEGH, J. (2009). Demography of *Nysius cymoides* (Het.: Lygaeidae) fed on canola seeds under laboratory conditions. *Applied Entomology and Phytopathology* (in Persian with English summery) 76 (2): 67-80.

NICOLAOU, K. C. and S. A. SNYDER, 2003. Classics in total synthesis. Willey-VCH Verlag GmbH and Co. KGaA, Weinheim, 512-513.

PAQUETTE, L. A., H. KANG and C. S. RA, 1992. Stereocontrolled access to the most highly condensed penttalenolactone antibiotic. J. Am. Chem. Soc. 114: 7387-7395.

PIERCE, A. M., J. H. BORDEN and A. C. OEHLSCHLAGER, 1981. Olfactory response to beetle-produced volatiles and host-food attractants by *Oryzaephilus surinamensis* and *O. mercator*. *Canadian Journal of Zoology* 56: 1980-1990.

PIVNICK, K. A., D. W. REED, J. G. MILLAR and E. W. UNDERHILL, 1991. Attraction of northern false bug *Nysius niger* (Het.: lygaeidae) to mustard oils. J. Chem. Ecol. 5: 931-940.

SIEGEL, S. 1956. Nonparametric statistics for the behavioral sciences. McGraw-Hill, New York. 312 pp.

SMITH, M. and J. MARCH, 2001. March`s advanced organic chemistry, A Willey-Interscience Publication, John Willey & Sons. Fifth ed. 516pp.

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