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Synthesis and field examinations of the sex pheromone of the diamondback moth, Plutella xylostella Linnaeus (Lepidoptera: Plutellidae) in the Mekong Delta of Vietnam

Dinh Thi Chi[1,2] and Le Van Vang[1*]

[1]*Department of Plant Protection, College of Agriculture and Applied Biology, Can Tho University, Vietnam*

[2]*Department of Plant Protection, Soc Trang Community College, Vietnam*

**Correspondence: Le Van Vang (email: lvvang@ctu.edu.vn)*

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ABSTRACT

The diamondback moth (Plutella xylostella) is one of the most destructive pests of cruciferous vegetables in the Mekong Delta of Vietnam. In order to apply sex pheromones as tool for a sustainable management program, the sex pheromone components of P. xylostella, (Z)-11-hexadecenal (Z11-16:Ald), (Z)-11-hexadecenyl acetate (Z11-16:OAc) and (Z)-11-hexadecen-1-ol (Z11-16:OH) were synthesized using a Wittig reaction as a key step, and their attraction activities were evaluated in cabagge fields. The synthesis of the three C_{16} monoenyl compounds was started from commercialized 11-bromo-1-undecanol. This C_{11} bromohidrine was converted into the methoxymethyl (MOM) ether and heated with Ph_3P. The produced phosphonium salt was treated with $NaN(SiMe_3)_2$ to make the corresponding ylide, which was coupled with C_5 aldehyde to prepare an MOM ether of the C_{16} monoenyl alcohol. Its deprotection produced prospected Z11-16:OH (>96% geometric purity) in 42.9% overall yield. Following Pyridinium chlorochromate (PCC) oxidation and acetylation of Z11-16:OH gave Z11-16:Ald and Z11-16:OAc, respectively. In the field, traps baited with a synthetic mixture of Z11-16:Ald, Z11-16:OAc and Z11-16:OH in a ratio of 5:5:1 or 5:5:0.1 caught many P. xylostella males as well as with a virgin female. Furthermore, the field tests confirmed that Z11-16:Ald and Z11-16:OAc were essential components for the attraction, and Z11-16:OH played an auxilary role

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1 INTRODUCTION

The diamondback moth, *Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae), is one of the most serious defoliators of cruciferous vegetables in Southeast Asia and over the world (Talekar and Shelton, 1993; Waterhouse, 1993). Female moths lay their eggs on leaf surface. That newly hatching larvae feed on the leaf tissue causing of 30-50% yield loss (Nguyen Duc Khiem, 2006). According to the Arthropod Pesticide Resistance Database (APRD, 2016), *P. xylostella* has resisted to 95 active ingredients of insecticides. Due to high resistance, the effective control of *P. xylostella* by use of insecticides was difficult to achieve. Consequently, farmers had to use insecticides with high doses and short interval to control this species (Nguyen Van Huynh and Le Thi Sen, 2013). This affected

negatively the environment, causing the problems of insecticide residue on crops and promoting insecticide resistance development of *P. xylostella*.

The sex pheromone of *P. xylostella* was identified as a composition of (*Z*)-11-hexadecenal (Z11-16:Ald) and (*Z*)-11-hexadecenyl acetate (Z11-16:OAc) (Tamaki *et al.*, 1977). Then, the results of field examinations by Ando *et al.* (1979) supplemented (*Z*)-11-hexadecen-1-ol (Z11-16:OH) and (*E*)-11-hexadecenal (E11-16:Ald) as synergistic components. Synthetic sex pheromones have been effectively applied for monitoring the population dynamics, mass trapping and mating disruption of *P. xylostella* (Reddy and Urs, 1997; Reddy and Guerrero, 2000; Schroeder *et al.*, 2000). The application of the sex pheromone as a tool of integrated management programs is expected to be effective alternation for insecticide sprays in the control of *P. xylostella* in the Mekong Delta. Since the sex pheromone of the diamond back moth has not been commercialized in Vietnam, the chemical synthesis of its pheromone components for the utilization is necessary.

Z11-16:OH was synthesized by coupling of docec-11-yn-1-ol with *n*-butyl iodine to form firstly an 11-hexadecynyl compound and following by the partial reduction of the triple bond (Tamaki *et al.*, 1977). On the other hand, Nguyen Cong Hao *et al.* (1996) and Zong *et al.* (2011) used a Wittig reaction as a key step for the construction of C_{16} chain skeleton starting from 2-buten-1,4-diol or 10-undecen-1-ol. Since the Wittig reaction is useful to produce selectively a monoenyl compound with a *Z* configuration, we examined the simple synthetic route utilizing this coupling reaction between pentanal and a ylide derived from commercially available 11-bromo-1-undecanol. This report deals with a new synthesis of the three pheromone components of *P. xylostella* and their field evaluation against the male moths inhabiting the Mekong Delta.

2 MATERIALS AND METHODS

2.1 Chemicals

11-Bromo-1-undecanol, pyridinium chlorochromate (PCC), pentanal and sodium bis(trimethylsilyl)amide [$NaN(SiMe_3)_2$, 1.0 M solution in tetrahydrofuran (THF)] were purchased from Aldrich (America); dimethoxymethane (DMM), lithium bromide (LiBr), *p*-tolunenesulfonic acid monohydrate (*p*-TsOH), triphenylphosphine (PPh_3), sodiumsulfate (Na_2SO_4) were purchased from Wako (Japan); silica gel was a product of Kanto (Japan). Solvents including *n*-hexane, benzene, ethyl acetate, tetrahydrofuran (THF), methylene chloride (CH_2Cl_2), acetic anhydride, pyridine were products of Merck (Germany).

2.2 Synthetic route

(Z)-11-Hexadecenyl compounds, Z11-16:OH, Z11-16:Ald and Z11-16:OAc, were synthesized by a route using a Wittig reaction as a key step which was modified from the scheme described for another insect pheromone by Vang *et al.* (2008) (Fig. 1). Specifically, after protection of a hydroxyl group of 11-bromo-1-undecanol (**1**) by DMM, the forming MOM ether (**2**) was stirred with PPh_3 at 100°C for 24 hrs. to obtain a phosphonium salt (**3**). The salt (**3**) was converted to the corresponding ylide by the treatment with $NaN(SiMe_3)_2$ in THF, and then coupled with pentanal by the Wittig reaction to form MOM ether of Z11-16:OH (**4**). The protection group was removed by stirring (**4**) in 0.5N HCl methanol solution, and Z11-16:OH (geometric purity >96%) was obtained. PCC oxidation and acetylation of Z11-16:OH gave Z11-16:Ald and Z11-16:OAc, respectively.

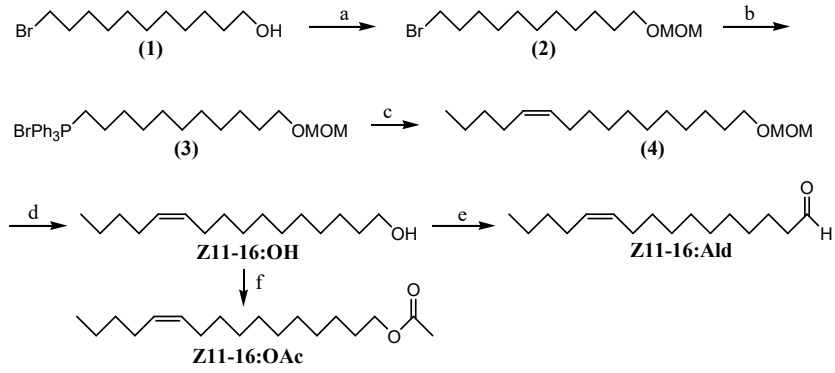

Fig. 1: Scheme for synthesis of Z11-16:OH, Z11-16:Ald and Z11-16:OAc, the sex pheromone components of P. xylostella. a = DMM/LiBr/p-TsOH; b = PPh3/1000C; c = 1) NaN(SiMe3)2/THF, 2) pentanal/THF; d = 0.5N HCl/MeOH; e = PCC/CH2Cl2; f = acetic anhydride /pyridine

^{1}H and ^{13}C NMR spectra of synthetic samples obtained by a Jeol Alpha 300 Fourier transform spectrometer (Nihondenshi, Tokyo, Japan) with the frequency at 300.4 MHz and 75.45 MHz were used for measurement, respectively. Deuterium chloroform ($CDCl_3$) and tetramethylsilane (TMS) were used as a solvent and an internal standard, respectively.

Mass spectra of synthetic samples were measured by a GC-MS Thermo system equipped with a TG-SQC capillary column (15 m x 0.25 mm x 0.25 µm; TraceGOLD™). Program temperature using for the analysis was starting at 60°C (keeping 2 minutes), ramping at 2°C/minute to 80°C (keeping 2 minutes), ramping at 8°C/minute to 210°C (keeping 2 minutes), ramping at 10°C/minute to 220°C and keeping at 220^0C for 10 minutes.

2.3 Field examination

The attraction to *P. xylostella* males by synthetic sex pheromones were tested at cabbage fields in My Xuyen district, Soc Trang province. The experiment was arranged as completely randomized block design with replications with three traps for each lure.

The lure was a rubber tube (1.0 mm x 5.0 mm) impregnated with synthetic pheromones. In the experimental field, a lure was placed at the center of a sticky board of the delta sticky trap which was hung on bamboo stakes at 0.5 m height (Fig. 2). Traps baited with blank rubber tubes and one virgin female were used as negative and positive controls. The number of *P. xylostella* captured by traps was counted weekly.

Fig. 2: Pheromone traps in the experimental cabbage field

3 RESULTS

3.1 Synthesis of sex pheromone components

3.1.1 (Z)-11-Hexadecen-1-ol (Z11-16:OH)

a. Protection of the hydroxyl group

A mixture of bromohydrin (**1**) (3 g, 11,9 mmol), LiBr (1,38g) and *p*-TsOH (190 mg) was stirred in DMM (60 ml) at room temperature for 24 hrs. The reaction was worked off by adding water (100 ml) and pouring into a separatory funnel to extract with *n*-hexane (100 ml x 3 times). The organic layers were combined and filtrated through Na_2SO_4 powders. After removing *n*-hexane and unreacted DMM by evaporation, the residue was purified by an open column chromatography on silica gel to give MOM-the desired ether (**2**) (3.03 g; 10.3 mmol) in 86.6% yield. GC-MS data: retention time (Rt) 31.37 minutes; Mass spectrum (MS, *m/z*): 45 (base), 61, 55, 69, 83, 95, 109, 123, 232 and 295. ^{1}H NMR (ppm): 1.28 (14H, broad), 1,54-1,64 (2H, tt, J = 6.9, 6.7 Hz), 1,80-1,90 (2H, tt, J = 7.1, 7.2 Hz), 3.34 (3H, s), 3.37-3.41 (2H, t, J= 6.9 Hz), 3.48-3.53 (2H, t, J= 6.6 Hz) and 4.61 (2H, s). ^{13}C NMR (ppm): 26.18, 28.14, 28.72, 29.38, 29.39, 29.43, 29.51, 29.71, 32.81, 34.01, 55.06, 67.84 and 96.35.

b. Wittig coupling reaction

A mixture of (**2**) (3.03 g; 10.3 mmol) and PPh_3 (3.42 g; 13 mmol) was stirred at 100°C until forimg a gum-like material (about 24 hrs.). After cooling down to room temperature, THF (50 ml) was added, and then $NaN(SiMe_3)_2$ (1.0 M THF soluttion, 12 ml) was dropwisely added. Waiting for the mixture in the flask dissolved absolutely (about 30 minutes), pentanal (1.0 g, 11.6 mmol) was dropwisely added. After stirring for 1 hr, the reaction mixture was poured into water and extracted with *n*-hexane (100 ml x 3 times). The *n*-hexane extract was successively washed with 1.0N HCl and a saturated aqueous solution of $NaHCO_3$, and chromatographed on a silica gel column to give MOM ether of Z11-16:OH [(**4**), 2.04 g, 7.2 mmol] in 69.9% yield. GC-MS data: Rt 32.09 minutes; MS (*m/z*): 45 (base), 55, 61, 69, 81, 95, 109, 223, 252 and 284.

c. Deprotection of the MOM ether

The MOM ether (**4**) (2.04 g, 7.2 mmol) was dissolved in a solution of 0.5N HCl in methanol (50

ml) and stirred at room temperature overnight. After that, the reaction mixture was poured into a separatory funnel with water (50 ml) and extracted with *n*-hexane (50 ml x 3 times). The *n*-hexane extract was washed with a saturated aqueous solution of $NaHCO_3$ and chromatographed on a silica gel column to give Z11-16:OH (1.22 g, 5.1 mmol) in 70.8% yield. The geometric purity of the product was >96% as checked by a GC-MS analysis. GC-MS data: Rt 30.65 min; MS (*m/z*): 55 (base), 69, 81, 96, 109, 124, 138, 166, 222 and 240. ^{1}H NMR (ppm): 0.86-0.91 (3H, t, *J*=7.05 HZ), 1.30-1.37 (22H, broad), 1.54-1.63 (2H, tt, *J*=6.7 Hz, 6.9 Hz), 2.0 (1H, s), 3.60-3.65 (2H, t, *J*=6.6 Hz) and 5.29-5.39 (2H, m). ^{13}C NMR (ppm): 14.0, 22.34, 25.73, 26.91, 27.18, 29.29, 29.43, 29.52, 29.55, 29.60, 29.76, 31.95, 32.78, 63.05, 129.86 and 129.88.

3.1.2 (Z)-11-Hexadecenal (Z11-16:Ald)

A mixture of Z11-16:OH (500 mg; 2.1 mmol) and PCC (679 mg; 3.15 mmol) was stirred in CH_2Cl_2 (50 ml) at room temperature for 3 hrs. After that, the reaction flask was connected to the rotatory evaporator to vapor all the reaction solvent. The mixture was then added 50 ml *n*-hexane and poured into a separatory funnel with water (50 ml) to shake and take the organic layer. The remain water was extracted again with *n*-hexane (50 ml x 2 times). The *n*-hexane extract was successively washed with 1.0N HCl and a saturated aqueous solution of $NaHCO_3$, and chromatographed on a silica gel column to give Z11-16:Ald (453 mg, 1.9 mmol) in 90.4% yield. GC-MS data: Rt 31.83 minutes, MS (*m/z*): 55 (base), 69, 83, 97, 98, 111, 123, 137, 192, 136, 218 và 236. ^{1}H NMR (ppm): 0.86-0.91 (3H, t, *J*=6.92 HZ), 1.28-1.31 (22H, broad), 1.58-1.64 (2H, tt, *J*=7.31 Hz, 7.52 Hz), 2.39-2.44 (2H, td, *J*=7.35 Hz, 1.85 Hz), 5.29-5.39 (2H, m) and 9.75-9.76 (1H, t, J=1.63 Hz). ^{13}C NMR (ppm): 14, 22.07, 22.34, 26.90, 27.16, 29.15, 29.25, 29.34, 29.39, 29.45, 29.73, 31.95, 43.91, 129.83, 129.89 and 203.04.

3.1.3 (Z)-11-Hexadecenyl acetate (Z11-16:OAc)

Z11-16:OH (500 mg, 2.1 mmol) was stirred with acetic anhydride (3.0 ml) in pyridine (15 ml) at room temperature for 3 hrs. then adding 50 ml water to work off the reaction. The mixture was poured into a separatory funnel and extracted with *n*-hexane (50 ml x 3 times). The *n*-hexane extract was successively washed with 1.0N HCl and a saturated aqueous solution of $NaHCO_3$, and chromatographed on a silica gel column to give Z11-16:OAc (415 mg, 1.5 mmol) in 71.4% yield. GC-MS data: Rt 31.64 minutes; MS (*m/z*): 55, 61, 67, 81 (base), 96, 110, 124, 138, 166, 194, 222 and 282. ^{1}H NMR (ppm): 0.86-0.90 (3H, t, *J*=6.93 HZ), 1.26-1.34 (22H, broad), 1.56-1.67 (2H, tt, *J*=6.27 Hz, 6.8 Hz), 2.0 (3H, s), 4.02-4.07 (2H, t, *J*=6.8 Hz) and 5.31-5.44 (2H, m). ^{13}C NMR (ppm): 13.95, 21.01, 22.12, 22.17, 25.90, 28.59, 29.12, 29.25, 29.47, 29.49, 29.52, 29.63, 31.82, 3227, 32.60, 64.67, 130.30, 130.32, 171.28.

3.2 Field attraction of *P. xylostella* by synthetic sex pheromones

Table 1: Field attraction of *P. xylostella* males by synthetic sex pheromones in My Xuyen district, Soc Trang province

Lure component (mg/tube)			males/trap/week[(1)]
Z11-16:Ald	Z11-16:OAc	Z11-16:OH	
Test 1[(2)]			
0.5	0.0	0.0	3.0 ± 2.0^{d}
0.0	0.5	0.0	3.3 ± 2.4^{d}
0.0	0.0	0.5	12.0 ± 8.7^{c}
0.25	0.25	0.0	35.7 ± 19.8^{b}
0.5	0.0	0.05	5.8 ± 3.5^{cd}
0.0	0.5	0.05	4.0 ± 3.7^{d}
0.25	0.25	0.05	52.8 ±19.3^{ab}
0.0	0.0	0.0	0.0 ± 0.0^{e}
01 virgin female[(4)]			96.2 ± 48.7^{a}
CV(%)			*29.6*
Test 2[(3)]			
0.25	0.25	0.005	116.1 ± 61.2^{a}
0.25	0.25	0.05	74.8 ± 46.3^{a}
0.25	0.25	0.5	31.7 ± 12.2^{b}
0.0	0.0	0.0	0.0 ± 0.0^{c}
01 virgin female[(4)]			84.5 ± 39.6^{a}
CV(%)			*15.9*

Mean ± SE. Values within each test followed by a different letter are significantly different at P<0.01 by Duncan Test; [(2)] Test was carried out at a cabbage field (2,500 m^2) at Thanh Don hamlet from 9th September to 11th October 2016; [(3)] Test was carried out at a cabbage field (1,500 m^2) at Thanh Don hamlet from 6th March to 27th March 2017; [(4)] replaced by a new emerging female weekly

Table 1 shows the results of field examinations of synthetic pheromones. Numbers of captured *P. xylostella* males in all traps baited with synthetic lures were significantly higher than those of the negative control (traps baited with blank tube), affirming the attraction of synthetic compounds to *P. xylostella* males. Among the tested lures, only the three-component lure attracted the males as strong as the positive control (traps baited with a virgin female) did. In the case of two-component lures, only traps baited with a mixture of Z11-16:Ald and Z11-16:OAc caught more *P. xylostella* males than the traps baited with single-component lures. The

attraction activities of the other combinations from two-component lures were not significantly different from those of single-component lures. These indicates that Z11-16:Ald and Z11-16:OAc are essential for the attraction while Z11-16:OH was an auxiliary component for the attraction of *P. xylostella* males. Test 2 shows the results investigating the dose response of Z11-16:OH (Table 1). The three-component lures mixed with Z11-16:OH from 1% - 10% attracted to *P. xylostella* males as strong as a virgin female did, while 50% significantly decreased the number of captured males.

4 DISCUSSION

The synthetic route using the Wittig reaction as a key step successfully prepared Z11-16:OH as shown in Fig. 1. The overall yield calculated from starting reagent was 42.9%. Z11-16:OH had been synthesized by an acetylene coupling reaction (Tamaki *et al.*, 1977) or a Wittig reaction (Nguyen Cong Hao *et al.*, 1996; Zong *et al*., 2011). While the synthetic route also uses the Wittig reaction, it has a unique point to start from a commercialized C_{11} bromohydrin. The MOM ether is stable against heat at the step of preparation of the phosphonium salt and also against a strong basic condition of the coupling step. The synthetic route of Z11-16:OH in Fig. 1, which consists of only four steps, is very simple compared to the previous syntheses. Furthermore, the Wittig reaction using sodium bis(trimethylsilyl)amide as a base for ylide formation instead of *n*-butyllithium produced predominantly *Z*-isomer; *i.e.,* the geometric purity was more than 96%. Since the addition of a trace amount of E11-16:Ald into the lure prepared from a mixture of Z11-16:Ald and Z11-16:OAc significantly increased number of captured *P. xylostella* males (Ando *et al*., 1979), the crude synthetic products could be directly used for preparation of lures without further geometric purification such as column chromatography with $AgNO_3$ impregnated silica gel.

Traps baited with lures prepared from synthetic pheromone components captured significantly more *P. xylostella* males than that control (trap baited with blank rubber tube) confirming the attraction of the synthetic compounds. Among them, only three-component lure attracted *P. xylostella* males as same as a virgin female, while one and two-component lures captured lower number (Test 1, Table 1). Otherwise, traps baited with lures prepared from both Z11-16:Ald and Z11-16:OAc captured significantly higher number of *P. xylostella* males than those of traps baited with lures which lacked one in two of these components (Test 1, Table 1). Sex pheromone of *P. xylostella* was identified as a composition of Z11-16:Ald and Z11-16:OAc at 2:3 ratio (Tamaki *et al*., 1977). Tests of the field attraction of Z11-16:Ald and Z11-16:OAc in Japan resulted in the ratio 1:1 attracted *P. xylostella* males as strong as that of virgin female (Koshihara *et al*., 1978). Following, the field evaluation of Ando *et al.* (1979) recorded that the addition of Z11-16:OH or E11-16:Ald at a mixing ratio of 1% into the lure increased significantly the number of captured *P. xylostella* males. The results of the field tests indicated that sex pheromone of *P. xylostella* inhabiting the Mekong Delta of Vietnam was also comprised of the same three components. Z11-16:Ald and Z11-16:OAc were essential active components, while Z11-16:OH was an auxiliary component. The optimum mixing ratio of Z11-16:OH in the lure was 1 - 10% (Test 2, Table 1), and this three-component lure would be applied in the Mekong Delta as a monitoring tool instead of the female moths.

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