

## Synthesis Of Pheromon Grapholitha Molesta Busck

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**Abstract.** This study aims to develop a new method for the synthesis of the pheromone Grapholitha molesta Busck based on local raw materials, to determine some of the physicochemical properties of the obtained substances, to manufacture various traps based on synthesized pheromones and to conduct tests in orchards in Uzbekistan. To date, scientists have developed several methods for the synthesis of the pheromone Grapholitha molesta Busck [1-3]. Together with scientists of the Institute of Bioorganic Chemistry in the conditions of the Republic, a mixture of the main components (E)-8-dodecyl and (Z)-8-dodecenylacetates was synthesized in a simpler, more economical and at the same time corresponding to the composition of insect pheromone.

**Keywords:** pheromone, Grapholitha molesta Busck, physicochemical constants, pheromone trap, (E)-8-dodecyl acetate, (Z)-8-dodecyl acetate.

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### Introduction

This scientific research contributes to the implementation of the Decree of the President of the Republic of Uzbekistan dated October 23, 2019 № PF-5853 "On approval of the Strategy of agricultural development of the Republic of Uzbekistan for 2020-2030", as well as other regulations related to this activity does.

In the process of radical reform of the agricultural sector, special attention is paid to the further development of horticulture, growing fruits and vegetables, melons and increasing exports.

Grapholitha molesta Busck, which is now widespread in Central Asia, is a dangerous pest of many fruit trees. This pest is widespread in East Asia (China, Korea, Japan), as well as in Central Asia, especially in

Uzbekistan (Samarkand, Tashkent and Fergana Valley). It causes great damage to the buds and fruits of various fruit trees every year, including almonds, peaches, apples, quinces and pears. *Grapholitha molesta* Busck is responsible for the loss of 50-100% of the annual crop [4-6].

The *Grapholitha molesta* Busck species found in Uzbekistan is known to produce 4-6 generations a year. The *Grapholitha molesta* Busck pest was first identified to give 1 generation in mid-March, which usually coincides with the beginning of the peach flowering period. The developmental period of the embryo is 6-12 days in spring, 3-6 days in summer and 12-16 days in autumn. This pest has been found to live up to 7 days in summer and up to 25 days in autumn. The upper-temperature limit for butterflies is around 36 °C and their reproduction process has been found to occur mainly at night. It has been observed that the low-temperature limit for laying eggs of a female butterfly is 13.1-16.5 °C, and the process of laying eggs is completely stopped when the relative humidity is less than 70% [7].

With this in mind, it is expedient to synthesize synthetic pheromones, which are similar in composition and properties to natural pheromones, and to study their composition and properties.

## Results and discussion

Various methods of physicochemical analysis were used to determine the individuality, purity and structure of the synthesized substances. In this case:

Silufol-254 (Czech Republic) was used to obtain thin-layer chromatography. The substances were separated into columns using silica gel L 40/100.

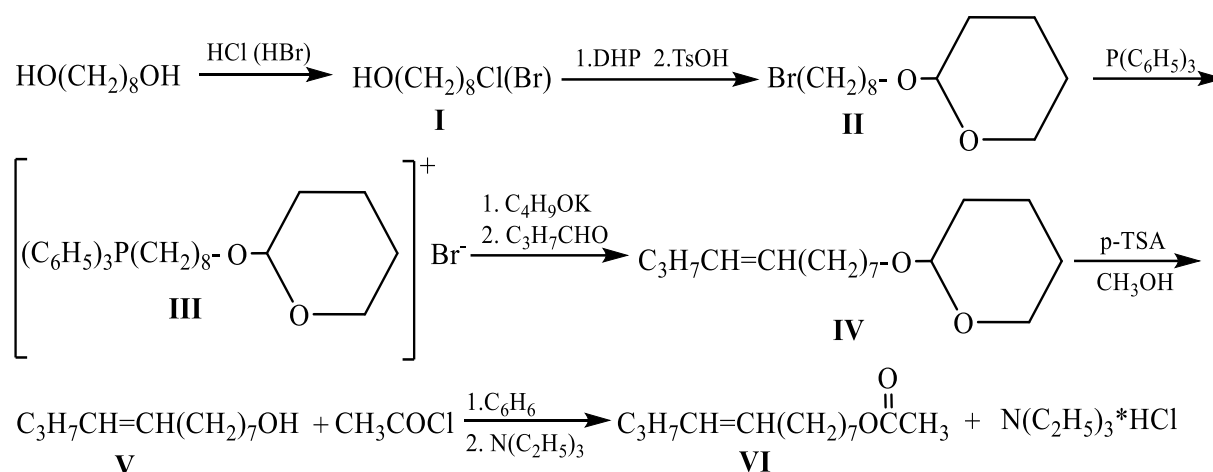
IR spectra were studied on an IRTracer-100 (SHIMADZU, 2017) Fure spectrometer in an area of 4000–400  $\text{cm}^{-1}$ .

To determine the individuality and composition of volatile substances, GC/ MSD (Agilent 5799B) was detected using a column 30 m long and 0.250 mm in diameter.

The liquefaction temperature of the substances was determined using the PTP TU 25-11-1144 device.

## Progress:

Obtaining the reaction product proceeds in a total of 6 steps. The reactions can be described using the following scheme:



In the first stage (I), octandiol-1,8 to 8-bromooctanol-1 were synthesized, and to determine the optimal conditions for synthesis, the nature of the reaction yield solvent, temperature, and time dependence were studied. The optimal solvent toluene was determined to be 2 hours and a temperature of 80-90 °C. The substance obtained was a colourless clear liquid that turned yellow when stored for a long time.

**Table-1** Dependence of the nature of the solvent on the reaction yield

No	Solvent	Time(h)	Temperature (°C)	Reaction yield (%)
1	Benzene	1	80-90	43,5
2	Heptan	1	80-90	53,0
3	Toluene	1	80-90	58,0

**Table-2** Dependence of time on reaction yield

No	Solvent	Time(h)	Temperature (°C)	Reaction yield (%)
1	Toluene	1	80-90	58,0
2	Toluene	2	80-90	70,0
3	Toluene	3	80-90	68,1

**Table-3** Temperature dependence on reaction yield

No	Solvent	Time(h)	Temperature (°C)	Reaction yield (%)
1	Toluene	2	40-50	35,0
2	Toluene	2	60-70	53,2
3	Toluene	2	80-90	70,0

In the second stage (II), the hydroxyl group was protected by 8-bromooctanol-1 with 3,4-dihydro-2H-pyran. To determine the optimal conditions for the synthesis of tetrahydro-2H-pyran ether of 8-bromooctanol-1, the

nature of the reaction yield solvent, time and temperature were studied. The optimal solvent chloroform was found to have a time of 4 hours and a temperature of 60 °C. The resulting 8-bromooctan-oxy-THP ether is a colourless liquid.

**Table-4** Dependence of the nature of the solvent on the reaction yield

Nº	Solvent	Time(h)	Temperature (°C)	Reaction yield (%)
1	Benzene	4 c	50	43,5
2	Chloroform	4 c	50	59,0
3	Tetrachloromethane	4 c	50	53,0

**Table-5** Temperature dependence on reaction yield

Nº	Solvent	Time(h)	Temperature (°C)	Reaction yield (%)
1	Chloroform	4	40	35,0
2	Chloroform	4	50	59,0
3	Chloroform	4	60	70,0

**Table-6** Dependence of time on reaction yield

Nº	Solvent	Time(h)	Temperature (°C)	Reaction yield (%)
1	Chloroform	2	60	53,8
2	Chloroform	4	60	70,0
3	Chloroform	6	60	65,2

**In the third stage (III)**, a complex salt of 8-bromooctane-oxy-THP with triphenylphosphine was obtained. To determine the optimal conditions for the synthesis of triphenylphosphine bromide complex salt, the nature of the reaction yield solvent, time and temperature dependence were studied. The optimal solvent was acetonitrile, the time was 8-10 hours and the temperature was 80 °C. The complex salt of triphenylphosphine bromide is a yellow oily liquid.

**Table-7** Dependence of the nature of the solvent on the reaction yield

Nº	Solvent	Temperature (°C)	Time(h)	Reaction yield (%)
1	Toluene	60	4	54,8
2	Acetonitrile	60	4	72,3
3	DMF	60	4	59,7

**Table-8** Dependence of time on reaction yield

No	Solvent	Temperature (°C)	Time(h)	Reaction yield (%)
1	Acetonitrile	60	4	72,3
2	Acetonitrile	60	8	75,1
3	Acetonitrile	60	12	75,4

**Table-9** Temperature dependence on reaction yield

No	Solvent	Temperature (°C)	Time(h)	Reaction yield (%)
1	Acetonitrile	60	8	60,4
2	Acetonitrile	70	8	67,1
3	Acetonitrile	80	8	75,1

In the fourth stage (IV), tetrahydropyranyl ether of 8-cis,trans-dodecanol was obtained from the complex salt of triphenylphosphine bromide. To determine the optimal conditions for the synthesis of tetrahydropyranyl ether of 8-cis, trans-dodecanol, the nature of the reaction yield solvent, time and temperature were studied. The optimal solvent THF was found to be 6 hours in time and 0-5 °C in temperature.

**Table-10** Dependence of the nature of the solvent on the reaction yield

No	Solvent	Temperature (°C)	Time(h)	Reaction yield (%)
1	DMSO/THF (1:1)	0	4	32,1
2	DMF	0	4	20,7
3	CH <sub>2</sub> Cl <sub>2</sub>	0	4	22,8

**Table-11** Temperature dependence on reaction yield

No	Solvent	Temperature (°C)	Time(h)	Reaction yield (%)
1	DMSO/THF (1:1)	0	4	32,1
2	DMSO/THF (1:1)	-5	4	33,7
3	DMSO/THF (1:1)	-10	4	32,4

**Table-12** Dependence of time on reaction yield

No	Solvent	Temperature (°C)	Time(h)	Reaction yield (%)
1	DMSO/THF (1:1)	-5	4	32,1

2	DMSO/THF (1:1)	-5	6	40,6
3	DMSO/THF (1:1)	-5	8	40,8

In the fifth stage (V), the nature and time dependence of the catalyst reaction of 8-cis,trans-dodecanol-1 from tetrahydropiranyl ether, 8-cis,trans dodecanol-1 in the presence of methyl alcohol was studied. It was found that p-TSA as a catalyst and the optimal time was 6 hours.

**Table -13** Dependence on the nature of the catalyst

No	Solvent	Temperature (°C)	Time(h)	Catalyst	Yield (%)
1	Methyl alcohol	65	6	Sulfuric acid	75
2	Methyl alcohol	65	6	p-TSA	83

**Table -14** Dependence of time on reaction yield (Sulfuric acid)

No	Solvent	Temperature (°C)	Time(h)	Catalyst	Yield (%)
1	Methyl alcohol	65	2	Sulfuric acid	68,7
2	Methyl alcohol	65	4	Sulfuric acid	75
3	Methyl alcohol	65	6	Sulfuric acid	78,2
4	Methyl alcohol	65	8	Sulfuric acid	76,1

**Table -15** Time dependence on reaction yield (p-TSA)

No	Solvent	Temperature (°C)	Time(h)	Catalyst	Yield (%)
1	Methyl alcohol	65	2	p-TSA	74,3
2	Methyl alcohol	65	4	p-TSA	83
3	Methyl alcohol	65	6	p-TSA	87,4
4	Methyl alcohol	65	8	p-TSA	81,2

In the sixth stage (VI), the reaction products were found to be 8-cis, trans dodecanol-1 to 8-cis, trans dodecanyl acetate obtaining reaction solvent CH<sub>2</sub>Cl<sub>2</sub>, CH<sub>3</sub>COCl as an acetylating reagent in the presence of sulfuric acid, optimal solvent C<sub>6</sub>H<sub>6</sub>.

**Table -16** Dependence of the acetylating reagent on the reaction yield

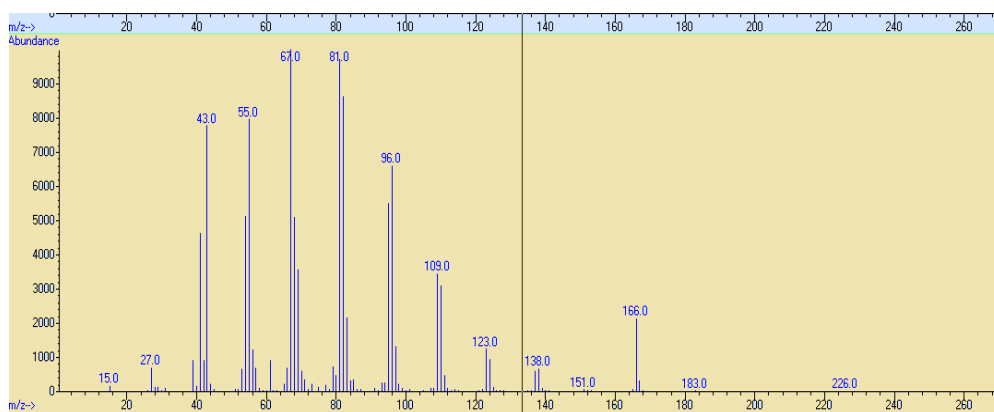
No	Acetylating reagent	Solvent	Temperature (°C)	Time(h)	Catalyst	Yield (%)
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1	CH <sub>3</sub> COOH	CH <sub>2</sub> Cl <sub>2</sub>	40	6	Sulfuric acid	68,3
2	(CH <sub>3</sub> CO) <sub>2</sub> O	CH <sub>2</sub> Cl <sub>2</sub>	40	6	Sulfuric acid	72,4
3	CH <sub>3</sub> COCl	CH <sub>2</sub> Cl <sub>2</sub>	40	6	Sulfuric acid	78,0

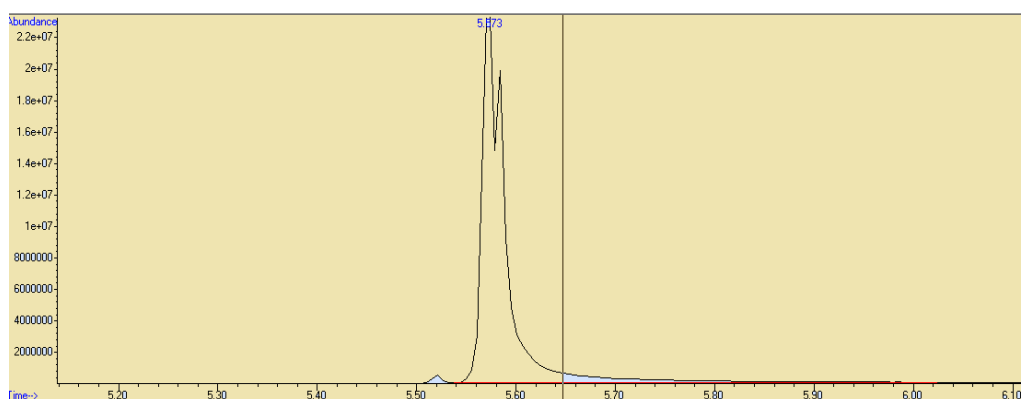
**Table -17** Dependence of the nature of the solvent on the reaction yield

No	Solvent	Temperature (°C)	Acetylating reagent	Time(h)	Catalyst	Yield (%)
1	ТГФ	65	CH <sub>3</sub> COCl	6	Sulfuric acid	83,0
2	CH <sub>2</sub> Cl <sub>2</sub>	40	CH <sub>3</sub> COCl	6	Sulfuric acid	77,3
3	C <sub>7</sub> H <sub>8</sub>	110	CH <sub>3</sub> COCl	6	Sulfuric acid	71,2

The purity level and individuality of the synthesized 8-cis, -trans dodecyl acetate were determined using gas chromatography-mass spectrometry (Agilent 5799B GC/MSD). The obtained spectra showed that 8-tsis corresponds to trans dodecyl acetate and that it consists of a mixture of two different isomers.



**Figure 1.** (E)-8 and (Z)-8-dodecyl acetate GC/MS spectr



**Figure 1.** (E)-8 and (Z)-8-dodecyl acetate GC/MS spectr

Based on the obtained substances, 3 different pheromone capsules were prepared and field experiments were tested in horticultural farms of the Fergana Valley region of the Republic of Uzbekistan [10,11].

The test results based on the experiments are shown in Figures 3 and Tables 18.



**Figure 3.** Field test results of pheromone traps prepared on the basis of synthesized substances



**Table -18** The results of observations carried out in the field plots of the farm "Abdullaev Halimzhan Nodir Oybek" Uzbekistan district of Fergana region (2020)

Product code	Returns	Number(pieces) of Grapholitha molesta Busck in one trap														Total
		June						July				August				
		25.05	29.05	7*.06	14.06	21.06	27**.06	4.07	11.07	18*.07	25.07	1.08	8**.08	15.08	23.08	
A-2	1	27	51	39	18	101	83	38	47	63	69	42	38	61	55	732
	2	19	39	41	23	88	47	43	39	58	51	49	27	49	33	606
	3	24	17	77	20	92	73	35	55	56	57	50	33	44	40	673
<b>Average</b>	-	<b>23</b>	<b>36</b>	<b>52</b>	<b>20</b>	<b>94</b>	<b>68</b>	<b>39</b>	<b>47</b>	<b>59</b>	<b>59</b>	<b>47</b>	<b>33</b>	<b>51</b>	<b>43</b>	<b>670</b>

Product code	Returns	Number(pieces) of Grapholitha molesta Busck in one trap														Total
		Июнь						Июль				Август				
		25.05	29.05	7*.06	14.06	21.06	27**.06	4.07	11.07	18*.07	25.07	1.08	8**.08	15.08	23.08	
A-3	1	22	40	70	44	105	104	56	44	85	79	69	51	77	55	901
	2	19	42	92	23	39	116	60	49	81	94	61	49	62	43	830
	3	33	71	88	38	99	125	58	32	62	108	55	36	58	39	902
<b>Average</b>	-	<b>25</b>	<b>51</b>	<b>83</b>	<b>38</b>	<b>81</b>	<b>115</b>	<b>58</b>	<b>42</b>	<b>76</b>	<b>94</b>	<b>62</b>	<b>45</b>	<b>66</b>	<b>46</b>	<b>878</b>

Product code	Returns	Number(pieces) of Grapholitha molesta Busck in one trap														Total
		June						July				August				
		25.05	29.05	7*.06	14.06	21.06	27**.06	4.07	11.07	18*.07	25.07	1.08	8**.08	15.08	23.08	

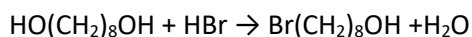
<b>A-4</b>	1	36	72	77	38	35	39	49	53	73	52	69	58	61	49	761
	2	29	66	69	43	42	52	51	49	68	67	57	33	59	41	725
	3	31	53	105	51	56	89	44	55	61	85	38	35	43	32	778
<b>Average</b>	-	<b>32</b>	<b>64</b>	<b>84</b>	<b>44</b>	<b>44</b>	<b>60</b>	<b>48</b>	<b>52</b>	<b>67</b>	<b>68</b>	<b>55</b>	<b>42</b>	<b>54</b>	<b>41</b>	<b>755</b>

**Note: \*** - day of replacement of the pheromone glue insert

**\*\*** - day of replacement of the pheromone capsule and insert

## Experimental part

### Synthesis of 8-bromooctanol-1.



15.4 g (0.105 mol) of octandiol-1,8, 2 g of tetrabutylammonium iodide, 18 ml of 48% bromic acid (0.15 mol) and 70 ml of toluene were taken into a two-mouth flask and cooled again at 80-90 °C for 2 h. The resulting 8-bromooctanol-1 was transferred to a solution of octandiol-1,8 toluene. The toluene solution was first washed in 40 ml of water and then with a 2% alkaline solution until the solution medium was neutral. It was then dehydrated using the Na<sub>2</sub>SO<sub>4</sub> salt. The remaining toluene solution was pumped at 75-78 °C/14 mm.Hg.ust using a vacuum pump. The product was obtained from 15.1 g (70%). R<sub>f</sub> = 0.46 (system:hexane-ether 2:1).

**IR spectral results:** 3650-3200 (-OH), 3000-2840 (-CH), 1475-1450 and 770-720 (-CH<sub>2</sub>), 1475-1315 (C-OH), 1080-1000 and 700-500 (C-Br) cm<sup>-1</sup>.

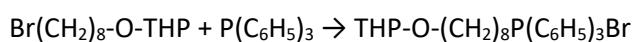
### Synthesis of 8-bromooctane-oxy-THP.



By connecting the two-mouth flask back to the refrigerator, 2 g of 8-bromooctanol-1 and 20 ml of chloroform, p-TSA, 0.81 g of 3,4-dihydropyran 60 °C were reacted for 6 h. The course of the reaction was checked using a thin layer chromatography method. Hexane: ether (4:1) was selected as the system. The solvent was removed from the reaction mixture, and the resulting 8-bromooctane-oxy-THP was separated in a column on the basis of a hexane-ether (4:1) system for pure separation. The product was obtained 1.82 g (65.2%). R<sub>f</sub> = 0.78 (system: hexane-ether 4:1).

**IR spectral results:** 3000-2840 (-CH), 1475-1450 and 770-720 (-CH<sub>2</sub>), 1310-1230 and 1055-870 (C-O-C), 1190-990 (C-O ), 1080-1000 and 700-500 (C-Br) cm<sup>-1</sup>.

### Synthesis of triphenylphosphine-bromide complex salt.

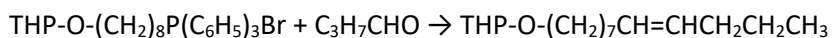


By connecting the one-mouth flask back to the refrigerator, 2 g of 8-bromooctane-oxy-THP and 20 ml of acetonitrile, 2.7 g of triphenylphosphine were reacted for 8 h at a temperature of 70 °C. The course of the reaction was checked using a thin layer chromatography method. Hexane: ether (3:1) was selected as the system. The solvent was removed from the reaction mixture, and the resulting triphenylphosphine bromide complex salt was separated in a column on the basis of a hexane-ether (3:1) system for pure separation. The product was obtained at 2.45 g (64.7%). R<sub>f</sub> = 0.0 (system: hexane-ether 3:1).

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**IR spectral results:** 3000-2840 (-CH), 1600-1575 (-C<sub>6</sub>H<sub>5</sub>), 1475-1450 and 770-720 (-CH<sub>2</sub>), 1340-1110 (P-C<sub>6</sub>H<sub>5</sub>), 1310-1230 and 1055- 870 (C-O-C), 1190-990 (C-O), 1080-1000 and 700-500 (C-Br) cm<sup>-1</sup> [8].

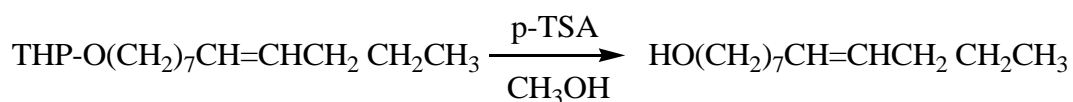
#### **Synthesis of tetrahydropiranyl ether of 8-cis, -trans dodecenol-1.**



Place the two-mouth flask in a magnetic stirrer, reconnect the refrigerant and the dropper funnel, dissolve 0.4 g of triphenylphosphine bromide complex salt in 5 ml of THF, then add 0.112 g of Butoxy-potassium salt and stir at 0-5 °C for 10 min. The 64.8 mg butanal was then dissolved in 3 ml of THF in a dropper funnel and added dropwise to the reaction mixture for 10 min and the reaction was carried out for 10 h. The course of the reaction was checked using a thin layer chromatography method. Hexane:ethyl acetate (5:1) was selected as the system. Pour 10 ml of water into the reaction mixture, then extracted 3 times in ether and dried. The solvent was then removed and the resulting tetrahydropiranyl ether of 8-cis, trans dodecenol-1 was separated in a column on the basis of a hexane-ethyl acetate (5:1) system for the separation of a colourless liquid in pure form. The product was obtained from 0.0743 g (40.3%). R<sub>f</sub> = 0.82.

**IR-spectral results:** 3000-2840 (-CH), 1600-1575 (-C<sub>6</sub>H<sub>5</sub>), 1690-1665 (cis-C=C), 1665-1635 (trans-C=C), 1475-1450 and 770- 720 (-CH<sub>2</sub>), 1462-1438 (-CH<sub>3</sub>), 1340-1110 (P-C<sub>6</sub>H<sub>5</sub>), 1260-1140 and 1055-870 (C-O-C), 1190-990 (-C-O), 1080 -1000 and 700-500 (C-Br) cm<sup>-1</sup>.

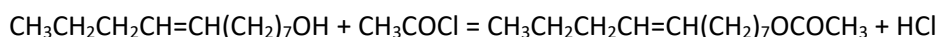
#### **Synthesis of 8-cis, -trans dodecenol-1.**



Place a single-mouth flask in a magnetic stirrer using a refrigerant, dissolve 0.1 g of in 5 ml of methanol from the tetrahydropiranyl ether of 8-cis, trans dodecenol and then stir the reaction in 0.01 g of p-TSA 65 °C for 6 h. The course of the reaction was checked using a thin layer chromatography method. Hexane: ethyl acetate (3:1) was selected as the system. The reaction mixture was washed in 1 ml of a 5% solution of NaHCO<sub>3</sub> until pH = 7, then washed 3 times in distilled water and dried 3 times in ether extracted in sodium sulfate. The solvent was then pumped out and the hexane-ethyl acetate (3:1) system was separated in a column to separate the 8-cis, -trans dodecenol-1 formed. The product was obtained from 0.060 g (83%). R<sub>f</sub> = 0.46. Clear colourless liquid.

**IR spectral results:** 3000-2800 and 1470-1360 (-CH-), 1600-1575 (-C<sub>6</sub>H<sub>5</sub>), 722 (cis-C=C), 1378, 1251, 1027, 972 (trans-C=C), 1475-1450 and 720-600 (-CH<sub>2</sub>), 1462-1438 (-CH<sub>3</sub>), 1340-1110 (P-C<sub>6</sub>H<sub>5</sub>), 1260-1140 and 1055-870 (C-O-C), 1152-990 (C-O), 1080-1000 and 700-500 (C-Br) cm<sup>-1</sup> [9].

### Synthesis of 8-cis, -trans dodecenyl acetate.



Place the three-mouth flask in a magnetic stirrer using a refrigerant, add 0.3 g of 8-cis, trans dodecenol, and 0.13 g of  $\text{CH}_3\text{COCl}$ , dissolve in 7 ml of  $\text{C}_6\text{H}_6$ , then add a 96% solution of sulfuric acid in a catalytic amount of HCl. 0.1 g of triethylamine was added for loss and the reaction was stirred for 4 h at 80 °C. The course of the reaction was checked using a thin layer chromatography method. Hexane: ethyl acetate (4: 1) was selected as the system. The reaction mixture was washed in 1 ml of a 5% solution of  $\text{NaHCO}_3$  until pH=7, then washed 3 times in distilled water and extracted 3 times in ether and dried in sodium sulfate. The solvent was then removed, and the resulting 8-cis, -trans dodecenyl acetate was separated in a column based on a hexane-ethyl acetate (4:1) system to separate trans dodecenyl acetate. The product was obtained from 0.296 g (75%).  $R_f = 0.68$ . The colourless liquid is pure.

**IR spectral results:** 3000-2800 and 1470-1360 (-CH), 723 (cis C=C), 1463, 1366, 1240, 1042, 970 (trans C=C), 1475-1450 and 720- 600 (-CH<sub>2</sub>), 1462-1438 (-CH<sub>3</sub>), 1260-1140 and 1055-870 (C-O-C), 1152-990 (C-O), 1750-1740 (C=O), 1240 (-COOCH<sub>3</sub>)  $\text{cm}^{-1}$  [9].

### Conclusions

1. For the first time in the Republic of Uzbekistan developed a method for the synthesis of 8-cis and 8-trans dodecenyl acetates, the main components of the pheromone *Grapholitha molesta* Busck from local raw materials.
2. Based on the synthesized 8-cis and 8-trans dodecenyl acetate, 3 different pheromone samples were prepared. They underwent initial field tests. As a result of field tests, the most optimal pheromone sample was selected.
3. Research has been conducted to optimize the synthesis stages. The effect of reagent concentration, solvent nature, reaction temperature and duration on process efficiency was investigated.
4. Based on the synthesized substances, 3 pheromone capsules with different compositions were prepared and tested in field conditions in horticultural farms of the Fergana Valley region of the Republic of Uzbekistan. The A-3 showed the best result according to the test results. This sample caught an average of 878 *Grapholitha molesta* Busck butterflies between June and August 2020.

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