proceedings

MDPI

Proceedings

A simple and sustainable synthetic lab-protocol for obtaining racemic dominicalure- aggregation pheromone of the grain beetle *Rhyzopertha dominica F.* (Coleoptera, Bostrichidae)

Juan Enrique Tacoronte Morales[1], María Teresa Cabrera Pedroso[2], Kenia Silvana Holguín García[3], Andrea Cevallos Camacho[4]

[1] Technical University of Esmeraldas, Faculty of Science & Engineering, Chemical Engineering Division, Esmeraldas, Campus Nuevos Horizontes, PC 080150, Ecuador; juan.tacaronte.morales@utelvt.edu.ec
[2] Faculty of Health Sciences, Universidad de Las Américas, UDLA, UDLA-Park, PC 170124 Quito, Ecuador; maria.cabrera@udla.edu.ec
[3] Technical University of Esmeraldas, Faculty of Science & Engineering, Chemical Engineering Division, Esmeraldas, Campus Nuevos Horizontes, PC 080150, Ecuador; kenia.holguin@utelvt.edu.ec
[4] Cannabis Research Group. Biotech & Physical Chemistry Division, Ecuador; crischemist@gmail.com

* Correspondence: juan.tacaronte.morales@utelvt.edu.ec; Tel.: +593-991702915;
Presented at The 24th International Electronic Conference on Synthetic Organic Chemistry; 15 November-15 December, 2020; Available online: https://ecsoc-24.sciforum.net/
Received: date; Accepted: date; Published: date

Abstract: The pheromones constitute a decisive component in the strategies for the conservation of stored foods. In Ecuador several studies are developing, under ecosustainable conditions, on synthetic pheromones and their applicability in the control of specie *Rhyzopertha dominica* which causes severe damages in stored grains. Previously reported information details the use of expensive reagents for carrying out the synthesis of the aggregation pheromone of this specie. Using propionic and isobutiric aldehydes as starting substrates were synthesized, by means of aldolic condensation, selective oxidation with Ag_2O-methanol of monoenic aldehydes, treatment with $SOCl_2$ and esterificatión with 2-pentanol, the respective components of the racemic aggregation pheromone of this species.

Keywords: *Rhyzoperta*, aggregation pheromone, synthesis, sec-amyl racemic esters.

1. Introduction

The grain beetle (Rhyzopertha dominica F. Coleoptera Bostrichidae) constitutes a devastating pest of stored grains, characterized by its cosmopolitan habits and wide geographic distribution. In Ecuador, it causes severe damage that affects the development of local food programs. This species has shown genetic variability and resistance to organophosphate insecticides [1,2] and synthetic pyrethroids, which makes its control difficult [3]. The use of repellants and natural toxic agents and essential oils [4] as well as biological control using toxins from *Bacillus thuringiensis* and natural parasitic predators [5,6] have been economically unsatisfactory. One of the methods of controlling the population dynamics of this harmful species considers the use of the aggregation pheromone secreted by these insects during the infestation stage. This pheromone was isolated and identified by Silverstein *et al*. [7] as a mixture of the S - (+) - isomers of isoamyl esters I and II (Figure 1) being called dominicalure. These components were synthesized from glutamic acid, natural amino acids, and crotonaldehyde using the Sharpless asymmetric epoxidation. [8] Cheskis and Schpiro, using the enantiomers of 2-pentanol and a co-catalytic mixture of Et_2CuLi-$ZnCl_2$ synthesized the chiral dominicalures with yields greater than 50% [9],

Razkin, Gil and Gonzalez [10] synthesized the dominicalures by esterifying α, β unsaturated acids with (S)-(+)-2-pentanol obtained by an asymmetric reduction of 3-penten-2-one. Rossi [11] *et al*. synthesized (S) -1-methylbutyl (E) -2-methyl-2-pentenoate, one of the components of dominicalure, from the palladium catalyzed reaction between ethyl-2-pentynoate and Bu_3SnH. These synthetic efforts are characterized by the use of rare and expensive reactants, in addition to using non-classical reaction conditions, generating significant amounts of contaminants and toxic residues at laboratory scale

Figure 1. Dominicalure I y II. **R = H (I) R = CH_3 (II)** Racemic Form

The objective of our communication is to report a synthetic pathway, operationally affordable, of the racemic form of both components of the pheromone of the species *Rhyzopertha dominica* F. (Coleoptera-Bostrichidae) with potential high attractant activity.

2. Materials and Methods

All reagents used were supplied by MERCK, Darmstadt, Germany, and were used without prior purification. The physico-chemical characteristics of the synthesized esters are in correspondence with the data reported in the literature. Boiling points were not corrected. The synthetic processes were controlled by thin-layer chromatography using silica 60G chromatoplates of 0.25 mm thickness, a mixture of ethyl acetate/n-hexane (AcOEt /Hex. 3:7 v/v) as a solvent and concentrated H_2SO_4 was used as chromogenic agent with subsequent heating up to 110 ^{0}C, and iodine chambers.

2.1. IR Spectroscopy.

The infrared spectra were recorded on a PHILIPS ANALYTICAL FTIR PU-9600 spectrophotometer, Germany; the samples were prepared in potassium bromide (KBr) tablets at 25 °C. Alternatively, the spectra were recorded in a JASCO-Canvas 4600, Japan system in CsBr tablets at 25 °C.

2.2. NMR Spectroscopy.

NMR spectra were recorded on a BRUKER AC-250 instrument, Germany, at 25 °C. The protonic chemical (δ) shifts are given in ppm, using tetramethylsilane as internal reference (TMS, δ = 0.0) and as a solvent $CDCl_3$. The chemical shifts (δ) for ^{13}C refers to the central peak of the $CDCl_3$ solvent at 77.03 ppm.

2.3. Experimental protocols.

2-Methylpent-2E-enal (IIIa).

Over 15 min, 24 g (0.4 mole) of propionaldehyde was added to 20 ml of a vigorously stirred 1 N aqueous solution of KOH (20 mmoles). After 5 min, the mixture, which had become hot, was cooled to 25 °C and was extracted with ether. The combined extract was neutralized with 5% HCI, washed with saturated NaCI solution, and dried with $MgS0_4$. The ether was evaporated off at 150 mm Hg, and the residue was distilled. This gave 13.7 g (70%) of the aldehyde (IIIa) with bp 50 °C (30 mm). n^{20} 1,4192. FTIR (ν, cm^{-1}, KBr): 2879 (C-H); 1369 (f, δs CH_3); 1658 (m, -HC=C-); 1669 (d, HC=C); 1710 y 2710 (HC=O). RMN-^{1}H ($CDCl_3$, δ, ppm): 9,49 (s, 1H, CHO); 6,38 (1H, CH=C), 2,26 (2H, -CH_2-);1,55 (d, 3H, CH_3-C=CH-); 0,96 (3H, CH_3-CH_2-). RMN-^{13}C ($CDCl_3$, δ, ppm) C1 (191.28); C2 (140,1); C3 (146,4); C4 (21,4); C5 (13,27); C6 (10,78).

Propylidene-tert-butylamine (II).

With stirring (600 rpm), 29 g (0.5 mole) of propionaldehyde was added over 20 min to 36.5 g (0.5 mole) of tert-butylamine, and then, at 5 °C, K_2C0_3 was added until the separation of water ceased. The organic layer was

separated off, dried with K_2CO_3, and distilled. The yield is 55 g (97%) of the azomethine (II) with bp 101-103 °C. RMN-^{1}H ($CDCl_3$, δ, ppm): 1,10 (m, 3H, CH_3-CH_2-CH=N); 1,29 (s, 9H, $(CH_3)_3$-C-N=); 2,38 (2H, -CH_2-CH=); 7,66 (CH=N). RMN- ^{13}C ($CDCl_3$, δ, ppm) C1 (160,45); C2 (28,10); C3 (11,37); C4 (56,08); C5, C6, C7 (31,4).

2,4-Dimethylpent-2E-enal (IIIb).

Over 15 min, a solution of 5.05 g (50 mmoles) of diisopropylamine in 6 ml of dry THF was added (by dropping) at -20 °C (Ar) to 50 ml of a 1 N solution of n-butyllithium (50 mmoles) in hexane. The reaction mixture was stirred at ~25 °C for 30 min and then at -15 ^{0}C for 20 min. A solution of 5.65 g (50 mmoles) of the azomethine (II) in 10 ml of THF and, after 40 min., at -20 °C (saline bath: NH_4SCN-NaCl 133/33 g in 150g ice), a solution of 3.6 (50 mmoles) of isobutyraldehyde in 5 ml of THF were added to it. The reaction mixture was heated to 25 °C over 1 h, and after 15 min. 100 ml of 20 % H_2SO_4 was added to the reaction mixture at 0°C. The resulting emulsion was stirred vigorously (800 rpm) for 40 min. and the aqueous layer was separated off and carefully extracted with ether. The combined extract was washed with saturated aqueous NaCI solution and dried with $MgSO_4$, the solvent was evaporated off at 150 mm Hg, and the residue was distilled. This gave 2.8 g (50%) of the aldehyde (IIIb), bp 45 °C (15 mm), n_D^{20} 1.4465. FTIR (ν, cm-1, KBr): 2870 (m, HC); 1383 y 1369 (f, doublet, HC$(CH_3)_2$-); 1715 (f, HC=O). RMN-^{1}H ($CDCl_3$, δ, ppm): 1.08 (d, J= 7Hz, 6H, CH_3-CH); 1,75 d (J= 1.5Hz, 3H, CH_3C=C); 2.38 m (1H, $CHCH_3$); 6.25 dq (J=10Hz and 1.5Hz 1H HC=C); 9.40 s (1H, CHO). RMN-^{13}C ($CDCl_3$, δ, ppm) C1 (196,21); C2 (140,45); C3 (160,23); C4 (29,33); C5 (24,15); C6 (24,20); C7 (10,19).

2-Methylpent-2E-enoyl Chloride (IVa).

In one portion, a solution of 0.98 g (10mmoles) of the aldehyde (IIIa) in 20 ml of MeOH was added to a solution of 3.6 g (20 mmoles) of $AgNO_3$ in 15 ml of H_2O and this was followed, with stirring, over 40 min, by 42 ml of a 1N aqueous solution of NaOH (42 mmoles). After 3 h, the precipitate was filtered off and was washed with hot water and with ether and this ether was used to extract the aqueous filter, which was then acidified with 20 ml of 10 % HCI and was carefully extracted with ether. The combined extract was dried with $MgSO_4$, the solvent was evaporated off in vacuum, and the residue was treated with 2.4 g (20 mmoles) of $SOCl_2$. The mixture obtained was heated at 60 °C for 1 h, and then the excess of $SOCl_2$ was evaporated in vacuum, and the residue was distilled. This gave 1.13 g (85 %) of the acid chloride (IVa), bp 52 °C (11 mm Hg). FTIR (ν, cm^{-1}, KBr): 1777 (f, Cl-C=O) RMN-^{1}H ($CDCl_3$, δ, ppm): 1.13 t (J = 7Hz, 3H, CH_3CH_2); 1,88 d (J=1.5Hz, 3H, CH_3-C=C); 2.29 q (J=7Hz, 2H, CH_2); 7.10 tq (J=7 y 1,5Hz 1H, CH). RMN- ^{13}C ($CDCl_3$, δ, ppm) (169,5); C2 (128,67); C3 (147,98); C4 (21,10); C5 (12,23); C6 (13,78)

2,4-Dimethylpent-2E-enoyl Chloride (IVb).

Similarly, 1.68 g (15 mmoles) of the aldehyde (IIIb) in 23 ml of MeOH, 5.1 g (30 mmoles) of $AgNO_3$ in 23 ml of H_20, 63 ml of a 1 N solution of NaOH (63 mmoles), and 3.57 g (30 mmoles) of $SOCl_2$ gave 1.8 g (82%) of the acid chloride (IVb) with bp 57 °C (9 mm Hg), n_D^{20} 1.4645. FTIR (ν, cm^{-1}, KBr): 1787(f, Cl-C=O). RMN-^{1}H ($CDCl_3$, δ, ppm): 1.08 d (J=7Hz, 6H, CH_3CH); 1.90 d (J=1.5 Hz, 3H, CH_3C=C);2.73 m (1H, $CHCH_3$); 7.00 dc (J=10and 1.5 Hz, 1H, HC=C). RMN-^{13}C ($CDCl_3$, δ, ppm) C1(168, 75); C2 (127,97); C3 (157,98); C4 (26,19); C5 (21,81); C6(21,84); C7(12,96).

l-Methylbutyl 2-Methylpent-2E-enoate (Ia).

Over 15 min, a solution of 0.8 g (6 mmoles) of the acid chloride (IVa) in 2 ml of ether was added to a stirred solution of 0.62 g (7mmoles) of sec-amyl alcohol in 4 ml of pyridine. After 2 h, the reaction mixture was diluted with 10 ml of ether, washed free from pyridine with 5 % HCI, washed additionally with a saturated aqueous solution of NaCI, and dried with $MgSO_4$. After the solvent had been driven off *in vacuo* and the residue had been distilled 1.05 g (94 %) of the ester (la) was obtained with bp 59-60 °C (3 mm Hg). n_D^{20} 1,4410. FTIR (ν, cm^{-1}, KBr): 1707 (m, C=O); 1290 (f, =C-C(=O)-O-); 1088 (m, O-C-C). RMN-^{1}H ($CDCl_3$, δ, ppm) : 0.90 t (J=7Hz, 3H, $CH_3CH_2CH_2$);

1,05 t (J=7,5 Hz, 3H, $CH_3CC=C$); 1,2-1,7 m (4H, CH_2CH_2); 1,23 d (J= 6,5 Hz 3H, CH_3CH); 1,82 s (3H, $CH_3C=C$); 2,18 q (J=7,5, 2H, $CH_2C=C$); 4,96 m (J=6, 1H, HC=C-C=O); 6,72 t (J= 7,5 Hz,1H, HC=C). RMN-^{13}C ($CDCl_3$, δ, ppm) acidic fragment C1(168,01); C2 (127,13); C3 (141,26); C4 (22,54); C5 (13,01); C6 (12,56); alcoholic fragment C7 (73,01); C8 (37,03); C9 (17,44); C10 (14,86); C11 (20,04).

l-Methylbutyl 2,4-Dimethylpent-2E-enoate (Ib).

Similarly, 1.6 g (10.9 mmoles) of the acid chloride (IVb) and 1.23 g (14 mmoles) of sec-amyl alcohol in 5 ml of pyridine gave 2.05 g (95%) of the ester (Ib) with bp 64-65 °C (2 mm). FTIR (ν, cm^{-1}, KBr): 1644 (m, C=O); 1284 (f, =C-C(=O)-O-); 1085 (m, O-C-C). RMN-^{1}H ($CDCl_3$, δ, ppm) 0,92 t (J= 7 Hz, 3H, CH_3CH_2); 1,02 d (J= 6,5 Hz ,6H, $CH_3CHC=C$); 1,25 d (J=6,5 Hz, 3H, $CH_3C=C$-); 1,2-1,7 m (4H,CH_2CH_2); 1,83 d (J=1,5,3H,$CH_3C=C$); 2,63 m (1H, CHC=C); 5,02 m (1H, HC=C-C=O); 6,55 dq (J=10 and 1,5 Hz, 1H, HC=C). RMN-^{13}C ($CDCl_3$, δ, ppm) acidic fragment C1(169,13); C2 (124,98); C3 (147,08); C4 (26,67); C5 (22,08); C6 (21,98); C7 (3,07); alcoholic fragment C8 (70,85); C9 (34,22); C10 (17,78); C11 (14,12); C12 (18,92)

3. Results and general discussion

The synthetic sequence is shown in Figure 2

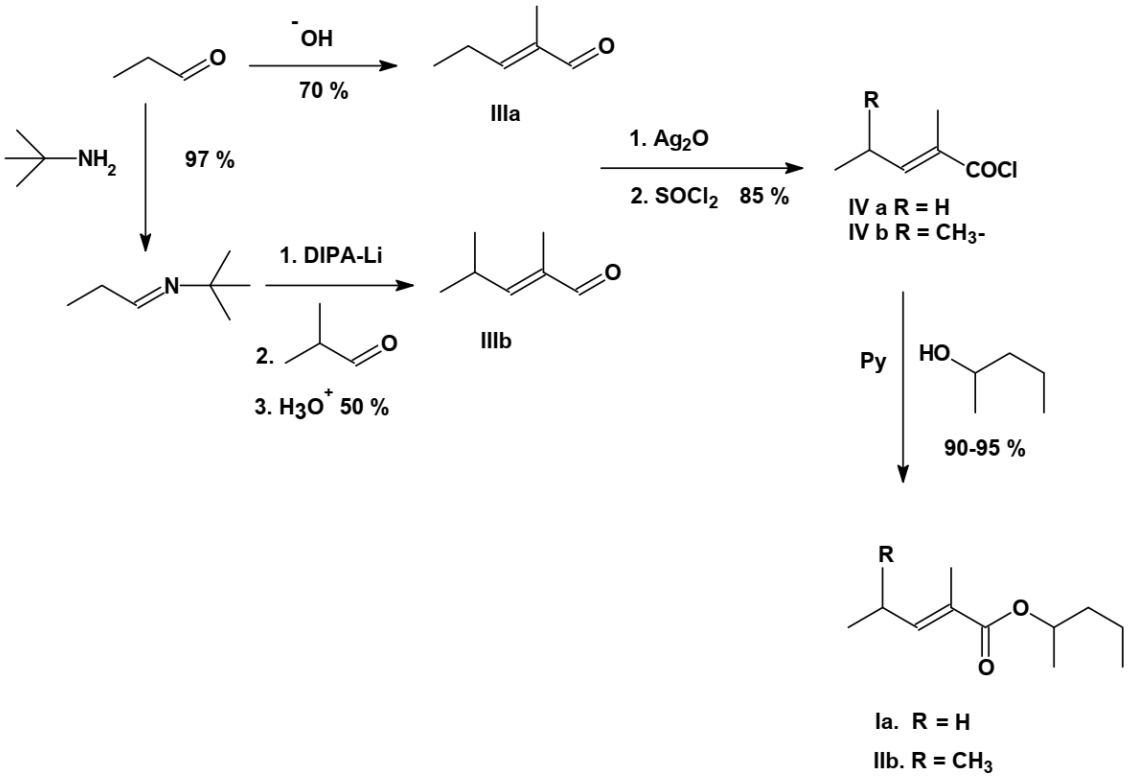

Figure 2. Synthetic sequence for obtaining racemic derivatives

The E-configuration of the unsaturated carbon skeleton of the acidic fragment is obtained from the aldol condensation of the propanal and isobutanal aldehydes. The unsaturated aldehydes are oxidized in a quantitative way with Ag_2O in their respective acids, which, *in situ*, are transformed into their chloro-anhydrides by $SOCl_2$. The esterification reaction with 2-pentanol was carried out in a classical way and allowed to obtain the pheromone components of aggregation of *Rhyzopertha dominica*.

Conclusion

The racemic pheromone, Dominicalure, was obtained from the species Rhyzopertha dominica F. using as starting substrates commercially available aldehydes and their aldol condensation. The synthetic methodology do not generate contaminants and does not need special laboratory conditions nor the use of complex purification techniques, allowing obtain the E-isomer of the components with yields greater than 98%. The process is simple and is developed in 3 synthetic steps, where one of the sequences (oxidation-formation of chloro-anhydrides) is achieved *in situ*, minimizing the operational risk and the loss of derivatives. This procedure does not require sophisticated technical equipment nor excessive energy costs, constituting a feasible protocol under laboratory conditions.

Author Contributions: JETM participated in the conceptualization of the methodology, investigation, structural elucidation and original draft preparation, review and editing; MTCP contributed with the development of experimental protocols at laboratory scale; KSHG contributed with the preparation of the manuscript; and ACC participated in the purification of synthetized derivatives and draft preparation of the document.

Funding: This research received no external funding but was developed under auspicious of Technical University of Esmeraldas, Ecuador.

Acknowledgments: The authors, to a great extent acknowledge the technical, and administrative, support from Technical University of Esmeraldas, Ecuador during 2019-2020.

Conflicts of Interest: The authors declare no conflict of interest.

References

1 Guedes R.N.C, Kambhampati S., and Dover B.A. Allozyme variation between Brazilian and US populations of *Rhyzopertha dominica* resistant to insecticides. *Entomol Experim et Applicata*, (**1997**) 84, 49-57.

2. Pires M. and Nogueira R. Damage caused by *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrichidae) in stored Brazil nuts. *Sci. Elec. Arch*. Vol. 11 (**2018**), 1, 57-61.

3. Arthur F.H. Differential effectiveness of deltamethrin dust against stored-product beetles. *J. Stored Prod. Res*. (**1997**), 33, 167-173.

4. Bekele A.J., Obengofori D. and Hassanali A. Evaluation of *Ocimum suave* Willd as a source of repellents, toxicants and protectants in storage against 3 stored-products insect pests. *Int. J. Pest Management*, 42 (**1996**), 139-142.

5. Beegle C.C. Efficacy of *Bacillus thurigiensis* against lesser grain borer, *Rhyzopertha dominica* (Coleoptera. Bostrichidae.) *Biocontrol Sci. & Technol*., l6 (**1996**), 15-2.

6. Flinn P.W., Hagstrum D.W. and McGaughey W.H. Suppression of beetles in stored wheat by augmentative releases of parasitic wasps. *Environm. Entomol*. 25 (**1996**), 505-511.

7. Silverstein W.H.J, Burkholder R.M, and Khorramshai W.E. Components of Aggregation Pheromone from Male Lesser Grain Borer *Rhyzopertha dominica* F. Coleoptera: Bostrichidae. *J. Chem. Ecol*., 7 (**1981**), 759-780.

8. Lin-Yu L., Guo-Qiang L. Enantiomeric synthesis of dominicalure aggregation pheromone of lesser grain borer. *J. Chem. Ecol*., 16 (**1990**), 1921-25.

9. Cheskis B.A, Schpiro H.A y Moiseenkov A.M. Síntesis efectiva de 2-pentanoles enantioméricos y dominicalures quirales. Zh. Organisch. Khim., 26 (**1990**), 1864-69.

10. Razkin J., Gil P. and Gonzalez A. Stereoselective synthesis of dominicalure-1 and dominicalure-2, components of aggregation pheromone from male lesser Grain Borer *Rhyzopertha dominica* (F). *J. Chem. Ecol*., 22 (**1996**), 673-80.

11. Rossi R., Carpita A., Cossi P. Synthetic Applications of Alkyl (E)-2-tributylstannyl-2-alkenoates. *Synth. Commun*. 23 (**1993**), 143- 52.