An Efficient Synthesis and Bio Efficacy Confirmation of Female Sex Pheromone of the Rice Moth, *Corcyra Cephalonica Stainton*

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**Abstract**

An efficient and simple protocol is developed for the synthesis of 6,10,14-trimethyl-2-pentadecanol, female sex pheromone of the rice moth. It has been achieved starting from farnesol employing Wittig reaction, hydrogenation and oxidation as key steps. The synthesized pheromone compound was found to cause significant electroantennogram (EAG) response from the male rice moth in electrophysiological assays in the laboratory and attracted a considerable number of male moths when baited with suitable traps in the mini rice storage warehouse during bio-evaluation studies.

**Keywords:** Rice moth; Sex pheromone; 6,10,14-Trimethyl-2-pentadecanol; Electroantennography (EAG); Wittig reaction; Hydrogenation; Oxidation

**Introduction**

Rice moth, *Corcyra cephalonica Stainton* (Lepidoptera: Pyralidae; Galleriinae) is a serious pest of stored paddy and other cereals. It nourishes well in humid climates and also attacks wheat, maize, sorghum, barley, millets, soybean and oilseeds like groundnuts. Its harm reaches a very unpleasant level in subtropical countries like India. The larva stage is mainly responsible for the damage. Young larva feeds on the broken grain or on the grains which already damaged by other insects. Besides polluting the grain with large quantities of frass and silken cocoons, webbing together the grains into large lumps occur and thus deflate the nutritional value of the grains. Using of insecticides to control storage pests led to contaminate the food grain which is not useful for human consumption. Insect pheromones play a major role in pest-control strategies and are considered to be potential tools in integrated pest management (IPM) [1], an eco-friendly and environmentally safe agricultural technique that is practiced worldwide.

The female emitted sex pheromone of the rice moth was isolated and identified as 6,10,14-trimethyl-2-pentadecanol (compound 1) (Figure 1) by Hall and co-workers [2]. They synthesized 6,10,14-trimethyl-2-pentadecanol as a racemic mixture and found it to be bioactive. However, electrophysiological bioassay of synthetic pheromone compound revealed that it (3.5 ng) causes a lower EAG response (1.23 mV) from the male rice moths than that to an equal amount of the natural pheromone (2.31 mV). The lower EAG responses to the synthetic compound suggest that the natural material consists of only one or a few of the possible isomers. Mori et al. [3] synthesized four of the eight possible stereoisomers of 6,10,14-trimethyl-2-pentadecanol [(2R,6R,10R)-, (2S,6R,10R)-, (2S,6S,10S)-, and (2R,6S,10S)] based on the hypothesis that the natural pheromone of the rice moth might be an isomer with two methyl groups at C6 and C10 in syn relative stereochemistry. Among these four synthetic stereoisomers of 6,10,14-trimethyl-2-pentadecanol, the (2R,6R,10R)-1 isomer alone was found to be at least twice as active as the diastereometric mixture of 6,10,14-trimethyl-2-pentadecanol both by EAG and behavioral bioassay. The natural pheromone was also reported to be twice as active as the diastereometric mixture of 6,10,14-trimethyl-2-pentadecanol and therefore, (2R,6R,10R)-1 was deduced to be the natural pheromone or at least the major component of the female blend.

![Figure 1: Structure of 6,10,14-trimethyl-2-pentadecanol.](image)

The female rice moth sex pheromone has attracted the attention of synthetic chemists due to its significant biological activity and unique structural features comprising secondary alcohol and three methyl branches: six total syntheses have been published till date. Shafikov et al. [4] have reported a short synthetic route (4 steps) to chiral alcohol 1 starting from chlorophyll, a natural product. However, it suffers from the low abundance of starting material, poor yields (12%) and poor optical purity (26% ee). It is noteworthy that the racemic pheromone compound can be used for trapping the male rice moths in fields instead of expensive chiral pheromone compound, since the racemic compound itself showed excellent catching ability. The synthetic strategies to 6,10,14-trimethyl-2-pentadecanol reported to date suffer from one or more of the drawbacks including lengthy reaction sequences [5,6], poor yields [2,4,7] and expensive starting materials and reagents [5,6]. In continuation of our interest in the synthesis of pheromones [8-10], we describe herein an efficient synthesis of 6,10,14-trimethyl-2-pentadecanol and its biological evaluation studies.
Biological evaluation

**Electroantennogram recording technique (EAG):**
Electroantennography (EAG), introduced by Schneider in 1957 [11], is an effective electrophysiological tool to evaluate and record the olfactory responses of an insect via their antennal receptors [10]. Insects perceive volatile organic compounds by means of olfactory receptors located on the antennae. EAG is essentially the record of the sum of many olfactory receptor potentials generated by a stimulus compound more or less simultaneously on the sensory epithelium of the antennal surface. Basically, it records the responses of many receptor neurons of the antenna to the presentation of a stimulus compound. The principle of EAG is to record voltage changes between the tip and the base of an antenna during stimulation by a volatile compound and the resulting EAG signal will be amplified and recorded with the help of data acquisition controller and software system (IDAC-2 EAG system from Syntech, The Netherlands) and displayed as electrical depolarization on the computer screen. The intensity of deflection is usually measured in milli Volts (mV). EAG recording experiments were conducted to confirm the bioactivity of synthesized pheromone compound 6,10,14-trimethyl-2-pentadecanol against 2-5 day old male *Corcyra cephalonica* in our laboratory utilizing established EAG facility.

**Preparation of stimulus pipettes:** EAGs were recorded directly by puffing the stimulus along with the humidified air on to the male moth antennal preparation. Stimulus cartridges were prepared by dispensing known amount (100 μg) of synthetic pheromone in n-hexane (50 μL) on to the filter paper. The solvent was allowed to evaporate for 30 sec before dispensing the puff.

**Antennal preparation:** The antenna of male moth was carefully excised along with the head and fixed between two probes of an electrode holder with the help of an electrically conducting gel. The antennal preparation was fixed on to the sensory epithelium of electrode holder with the help of an electrically conducting gel. The intensity of solution (8 mg (blend-1) and 10 mg (blend-2) of pheromone) is mixed blend by adding farnesol in 1:1 ratio to the active ingredient (4 mg 6,10,14-trimethyl-2-pentadecanol and 4 mg farnesol) with an pipette containing the test compound was inserted into the side hole of the tip and the base of an antenna during stimulation by a volatile compound and the resulting EAG signal will be displayed as electrical depolarization on the computer screen. The sum of many olfactory receptor potentials generated by a stimulus and the resulting EAG signal will be displayed as electrical depolarization on the computer screen. The intensity of stimulus is usually measured in milli Volts (mV).

**Preparation pheromone lures:** Consisting air, 1-hexanol and three continuous puffs of synthesized 6,10,14-trimethyl-2-pentadecanol against 2-5 day old male *Corcyra cephalonica* in our laboratory utilizing established EAG facility.

**Chemistry**

**Synthesis of (2E,6E)-3,7,11-trimethylododeca-2,6,10-trienal (compound 5)**
To a solution of farnesol (compound 4) (4 g, 18 mmol) in acetonitrile (40 mL) was added IBX (7.8 g, 27 mmol) slowly at room temperature. The flask was fitted with a condenser and refluxed under nitrogen atmosphere for 30 min. The reaction mixture was then allowed to cool to room temperature, precipitated solid was filtered through a pad of Celite and then the residue was washed with diethyl ether (2 mL × 50 mL). The filtrates were concentrated under reduced pressure and the residue was purified by column chromatography with the use of 10% EtOAc/hexane (v/v) as the eluent to furnish the aldehyde 5 (3.76 g, 17.1 mmol) in 95% yield as a pale yellow liquid. IR (neat): 2965, 2921, 2856, 1674, 1444, 1379, 1191, 1119, 838 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) (S 1): \(\delta\) 9.99 (d, J=8.0 Hz, 1H), 5.88 (dd, J=8.0, 1.1 Hz, 1H), 5.12-5.05 (m, 2H), 2.26-2.19 (m, 5H), 2.18-2.15 (m, 3H), 1.71-1.66 (m, 6H), 1.63-1.58 (m, 6H); MS (ESI): 243 [M + Na]\(^{+}\); GC MS: 220 [M]\(^{+}\). Rt=9.831 min (95.2% purity).

**Synthesis of ethyl-(2E,4E,8E)-5,9,13-trimetyltetradeca-2,4,8,12-tetraene-1-ol (compound 3)**
To a solution of aldehyde 5 (3.73 g, 16.9 mmol) in dichloromethane (56 mL) was added ethyl (triphenylphosphoranylidine) acetate (7.6 g, 27.9 mmol) in 95% yield as a viscous oil. IR (neat): 2968, 2925, 1713, 1635, 1446, 1370, 1149, 1039, 979, 885 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\)) (S 2): \(\delta\) 7.58 (dd, J=15.1, 11.5 Hz, 1H), 5.99 (d, J=11.5 Hz, 1H), 5.78 (d, J=15.1 Hz, 1H), 5.14-5.05 (m, 2H), 4.20 (q, J=7.1 Hz, 2H), 2.19-2.11 (m, 4H), 2.09-2.02 (m, 3H), 2.00-1.94 (m, 1H), 1.89 (s, 3H), 1.71-1.58 (m, 9H), 1.29 (t, J=7.1 Hz, 3H); MS (ESI): 313 [M + Na]\(^{+}\).

**Synthesis of (2E,4E,8E)-5,9,13-trimetyltetradeca-2,4,8,12-tetraen-1-ol (compound 6)**
To a solution of ethyl ester 3 (4.45 g, 15.34 mmol) in anhydrous dichloromethane (61 mL) cooled at -78°C was added DIBAL-H (1.4 M in toluene, 27.4 mL, 38.35 mmol) slowly under the nitrogen atmosphere. The reaction mixture was stirred at the same temperature for 30 min and then quenched by the slow addition of ice pieces. The precipitated solids were filtered with diethyl ether (2 mL × 50 mL). The combined filtrates were evaporated under reduced pressure and the residue was precipitated by column chromatography using 8% EtOAc/hexane (v/v) as the eluent to afford the ester 3 (4.45 g, 15.54 mmol) in 92% yield as a viscous oil. IR (neat): 2968, 2925, 1713, 1635, 1446, 1370, 1149, 1039, 979, 885 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\)) (S 3): \(\delta\) 6.52-6.42 (m, 1H), 5.88-5.82 (m, 1H), 5.78-5.67 (m, 1H), 5.16-5.06 (m, 2H), 4.22-4.14 (m, 2H), 2.22-2.15 (m, 1H), 2.15-2.02 (m, 6H), 2.01-1.94 (m, 1H), 1.80-1.75 (m, 3H), 1.70-1.58 (m, 9H); MS (ESI): 271 [M + Na]\(^{+}\); GC MS: 248 [M]\(^{+}\), Rt=12.691 min (98.1% purity).

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Synthesis of 5,9,13-trimethyltetradecan-1-ol (compound 2)

To a solution of allylic alcohol 6 (3.32 g, 13.39 mmol) in methanol (45 mL) was added Pd/C (330 mg, 10% by wt). The reaction mixture was evacuated, subsequently filled with H₂ and stirred for 20 h at atmospheric pressure. The solid catalyst was filtered through a small pad of Celite and the catalyst was washed with ethyl acetate (30 mL). Evaporation of the combined filtrates in vacuo afforded a crude product that was purified by column chromatography with use of 15% EtOAc/hexane (v/v) as the eluent to furnish the hydroxy compound 2 (2.88 g, 11.25 mmol) in 84% yield as a viscous oil. IR (neat): 3334, 2956, 2926, 2865, 1611, 1461, 1375, 1054, 734 cm⁻¹; ¹H NMR (300 MHz, CDCl3) (S 5): δ 9.77 (t, J=1.8 Hz, 1H), 2.43-2.38 (m, 2H), 1.71-1.47 (m, 3H), 1.44-1.19 (m, 10H), 1.18-1.01 (m, 6H), 0.91-0.81 (m, 12H); GC MS: 255 [M-H]⁺, Rt=21.94 min (99.6% purity).

Synthesis of 5,9,13-trimethyltetradecan-1-ol (compound 2)

To a solution of hydroxy compound 2 (2.85 g, 11.13 mmol) in acetone (28 mL) was added IBX (4.82 g, 16.7 mmol) slowly at room temperature. The reaction mixture was then allowed to cool to room temperature, precipitated solid was filtered through a short pad of Celite and the residue was washed with diethyl ether (2 mL × 30 mL). The combined filtrates were concentrated under reduced pressure and the residue thus obtained was puriﬁed by column chromatography with use of 5% EtOAc/hexane (v/v) as the eluent to furnish the aldehyde 7 (2.57 g, 10.13 mmol) in 91% yield as a viscous liquid. IR (neat): 3348, 2956, 2926, 2865, 1611, 1461, 1375, 1154, 1118, 937, 735 cm⁻¹; ¹H NMR (500 MHz, CDCl3) (S 6): δ 9.77 (t, J=1.8 Hz, 1H), 2.43-2.38 (m, 2H), 1.71-1.47 (m, 3H), 1.44-1.19 (m, 10H), 1.18-1.01 (m, 6H), 0.91-0.81 (m, 12H); GC MS: 279 [M + Na]+; GC MS: 255 [M-H]⁺, Rt=21.49 min (97.6% purity).

Synthesis of 5,9,13-trimethyltetradecan-1-ol (compound 2)

To a solution of hydroxy compound 2 (2.85 g, 11.13 mmol) in acetone (28 mL) was added IBX (4.82 g, 16.7 mmol) slowly at room temperature. The reaction mixture was then allowed to cool to room temperature, precipitated solid was filtered through a small pad of Celite and the residue was washed with diethyl ether (2 mL × 30 mL). The combined filtrates were concentrated under reduced pressure and the residue thus obtained was puriﬁed by column chromatography with use of 5% EtOAc/hexane (v/v) as the eluent to furnish the aldehyde 7 (2.57 g, 10.13 mmol) in 91% yield as a viscous liquid. IR (neat): 3348, 2956, 2926, 2865, 1611, 1461, 1375, 1154, 1118, 937, 735 cm⁻¹; ¹H NMR (500 MHz, CDCl3) (S 5): δ 9.77 (t, J=1.8 Hz, 1H), 2.43-2.38 (m, 2H), 1.71-1.47 (m, 3H), 1.44-1.19 (m, 10H), 1.18-1.01 (m, 6H), 0.91-0.81 (m, 12H); GC MS: 255 [M-H]⁺, Rt=21.94 min (99.6% purity).

Synthesis of 5,9,13-trimethyltetradecan-1-ol (compound 2)

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Synthesis of sex pheromone component of female rice moth

We began the synthesis from commercially available farnesol, which on oxidation with IBX in acetonitrile furnished aldehyde 5 [12,13]. Wittig olefination of aldehyde 5 with ethyl (triphenylphosphoranylidine) acetate in dichloromethane afforded the trans-ethyl ester 3 [14], which was chemoselectively reduced with DIBAL in dichloromethane to furnish allylic alcohol 6 [15]. Reduction of four oleﬁnic bonds in allylic alcohol 6 with palladium over carbon under an atmosphere of hydrogen in MeOH yielded the primary alcohol 2 [16]. The primary hydroxy group in 2 was oxidized without incident with use of IBX in acetonitrile under reﬂux to yield aldehyde 7. Finally, the reaction of aldehyde 7 with methyl lithium in diethyl ether [12] produced the desired racemic pheromone compound 1 (Scheme 2). The synthesized compound 1 had physical characteristics that were in excellent agreement with those reported in the literature [2].

Bio efficacy confimation of 6,10,14-trimethyl-2-pentadecan-1-ol, the female rice moth produced sex pheromone, in the laboratory

EAG recording experiments were conducted to conﬁrm the bioactivity of synthesized pheromone compound 6,10,14-trimethyl-2-pentadecan-1-ol against 2-5 day old male Corcyra cephalonica in our laboratory utilizing established EAG facility. The synthesized 6,10,14-trimethyl-2-pentadecanol elicited a significant EAG responses ranging from 1.28 mV to 1.40 mV from the male antenna of C. cephalonica (Any response measured from 0.5 mV and above is considered as good response). The representative EAG profiles caused due to solvent (n-hexane alone) and the synthesized pheromone are represented in Figures 2 and 3.
Efficacy of synthesized 6,10,14-trimethyl-2-pentadecanol in experimental mini rice storage warehouse at Agricultural Research Station of ANGRAU at Ananthapur, A. P., India

Electrophysiological (EAG) bioassay provides information only about activation of receptors. It does not provide information about the type of insect behavior the compound elicits; which could be as an attractant, repellent or another type of behavioral response. Volatiles proved positive in EAG experiments are typically taken on to further behavioral assays involving olfactometers/wind tunnels as the chemical identity and electrophysiological activity of a compound are of little value if no behavioral response associated with it is demonstrated. Since behavioral assays involving olfactory cues are often tedious and time-consuming, it is decided to evaluate the synthetic rice moth pheromone for its efficacy in the experimental warehouse of Agricultural Research Station of ANGRAU at Ananthapur, A.P.

| S No | Days/ Weeks | Number of male moths trapped |
|------|-------------|------------------------------|
|      |             | Blend-1 | Blend-2 | Blend-3 |
| 1    | 1           | 7       | 11      | 5       |
| 2    | 3           | 28      | 37      | 21      |
| 3    | 5           | 51      | 64      | 29      |
| 4    | 1 Week      | 76      | 81      | 41      |
| 5    | 10 Days     | 95      | 112     | 59      |
| 6    | 2 Weeks     | 134     | 145     | 73      |
| 7    | 3 Weeks     | 199     | 211     | 101     |
| 8    | 1 Month     | 253     | 266     | 121     |
| 9    | 6 Weeks     | 345     | 386     | 165     |
| 10   | 2 Months    | 449     | 493     | 189     |

*Note: Blend 1: 8 mg of synthetic pheromone in hexane with equal amount of BHT; Blend 2: 10 mg of synthetic pheromone in hexane with equal amount of BHT; Blend 3: 8 mg of synthetic pheromone and farnesol (1:1 ratio) in hexane with equal amount of BHT

Table 1: Male moth catches in different traps baited with C. cephalonica pheromone green rubber septa lures using different blends over a period of two months.

| Colour of dispenser | Number of male moths trapped |
|---------------------|------------------------------|
|                     | Blend-1 | Blend-2 | Blend-3 |
| Red                 | 478     | 514     | 196     |
| Green               | 449     | 493     | 189     |
| Yellow              | 395     | 458     | 221     |
| White               | 497     | 557     | 203     |

Table 2: Male moth catches in different traps baited with C. cephalonica pheromone different colors lures using different blends over a period of two months.

The pheromone lures were made with the synthesized compound and evaluated its efficacy at experimental rice storage warehouse,
Agriculture Research Station of ANGARAU at Ananthapur, India. The results of male moth catches recorded during evaluation of pheromone lures were displayed in Tables 1 and 2. These results revealed that the blend-1 and blend-2 have certainly shown excellent trapping ability irrespective of the color of the dispenser. And also it was observed that the blend-2 was attracted significantly more male moths than blend-1. However addition of farnesol in blend-3 depleted the trapping efficiency although we expected the synergistic effect of farnesol may improve the catching efficiency. It is concluded that the synthetic pheromone blend in combination of antioxidant BHT is good enough for better trapping.

Conclusion

In summary, we have designed an efficient synthetic route to 6,10,14-trimethyl-2-pentadecanol, employing Wittig reaction, hydrogenation, and oxidation as key steps. In contrast to many earlier syntheses, our synthetic strategy afforded high yields and would be useful for the synthesis of 1 in kilogram scale. The synthesized 6,10,14-trimethyl-2-pentadecanol is found to be active in both EAG response from the male moth and attracting male moths in the bio-evaluation field studies at warehouse.

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