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Synthesis and field testing of esters of (2*R*)-butan-2-ol and (2*S*)-butan-2-ol and monounsaturated fatty acids as sex attractants for the males of Procridinae species (Lepidoptera: Zygaenidae)

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Abstract

In the family Zygaenidae (Lepidoptera) with more than 1000 species a complete chemical analysis of the structure of female sex pheromones has been carried out for only three species of the subfamily Procridinae while sex attractants have been found for males of more than 60 species of two subfamilies: Procridinae and Zygaeninae. The synthesis of new sex attractants was performed from (*Z*)-dodec-5-enoic acid and *R*- and *S*-enantiomers of butan-2-ol at the Crimean Federal University in 2021. The biological activity of received esters was tested during fieldwork in the Crimean Peninsula. It has been proved that (2*R*)-butan-2-yl (*Z*)-dodec-5-enoate (named EFETOV-S-5) and (2*S*)-butan-2-yl (*Z*)-dodec-5-enoate (named EFETOV-S-S-5) are sex attractants for the males of *Jordanita* (*Tremewania*) *notata* (Zeller, 1847), while (2*S*)-butan-2-yl (*Z*)-dodec-5-enoate (EFETOV-S-S-5) is a sex attractant for *Adscita* (*Adscita*) *geryon* (Hübner, 1813). In addition, (2*R*)-butan-2-yl (*Z*)-hexadec-9-enoate and (2*S*)-butan-2-yl (*Z*)-hexadec-9-enoate were synthesised in 2022. However, these esters did not attract any of the Zygaenidae species which are common in the Crimea.

Keywords: Lepidoptera, Zygaenidae, Procridinae, *Adscita geryon*, *Jordanita notata*, EFETOV-S-5, EFETOV-S-S-5, sex attractant, Crimea.

Síntesis y pruebas de campo de ésteres de (2*R*)-butan-2-ol y (2*S*)-butan-2-ol y ácidos grasos monoinsaturados como atrayentes sexuales para los machos de especies de Procridinae (Lepidoptera: Zygaenidae)

Resumen

En la familia Zygaenidae (Lepidoptera) con más de 1.000 especies, se ha realizado un análisis químico completo de la estructura de las feromonas sexuales de las hembras para sólo tres especies de la subfamilia Procridinae, mientras que se han encontrado atrayentes sexuales para los machos de más de 60 especies de dos subfamilias: Procridinae y Zygaeninae. La síntesis de los nuevos atrayentes sexuales se llevó a cabo a partir del ácido (*Z*)-dodec-5-enoico y de los enantiómeros *R* y *S* del butan-2-ol en la Universidad Federal de Crimea en 2021. La actividad biológica de los ésteres recibidos se probó durante el trabajo de campo en la Península de Crimea. Se ha comprobado que el (2*R*)-butan-2-il (*Z*)-dodec-5-enoato (denominado EFETOV-S-5) y el (2*S*)-butan-2-il (*Z*)-dodec-5-enoato (denominado EFETOV-S-S-5) son atrayentes sexuales para los machos de *Jordanita* (*Tremewania*) *notata* (Zeller, 1847), mientras que el (2*S*)-butan-2-il (*Z*)-dodec-5-enoato (EFETOV-S-S-5) es atrayente sexual para *Adscita* (*Adscita*) *geryon* (Hübner, 1813). Además, en 2022 se sintetizaron (2*R*)-butan-2-il (*Z*)-hexadec-9-enoato y

(2*S*)-butan-2-il (*Z*)-hexadec-9-enoato. Sin embargo, estos ésteres no atrajeron a ninguna de las especies de Zygaenidae comunes en Crimea.
Palabras clave: Lepidoptera, Zygaenidae, Procridinae, *Adscita geryon*, *Jordanita notata*, EFETOV-S-5, EFETOV-S-S-5, atrayente sexual, Crimea.

Introduction

In recent years, there has been an increasing amount of literature on various approaches to the study of pheromone systems in Lepidoptera. Much of the current research on these attractant molecules pays particular attention to their chemical structure, isomerism and biosynthesis (El-Sayed, 2022). In the family Zygaenidae (Lepidoptera) which includes five subfamilies (Efetov, 1999, Efetov et al. 2014b; Efetov & Tarmann, 2017a) with more than 1000 species (Efetov, 1996a, 1996b, 1998, 1999, 2001, 2006, 2010; Efetov & Hayashi, 2008; Efetov et al. 2004, 2006, 2015a, 2019a, 2019c; Efetov & Knyazev, 2014; Efetov & Savchuk, 2009; Efetov & Tarmann, 1999, 2013a, 2013b, 2014a, 2014b, 2016a, 2016b, 2017a, 2017b; Knyazev et al. 2015) a complete chemical analysis of the structure of female sex pheromones has been carried out for only three species (all are pests) of the subfamily Procridinae (Efetov & Kucherenko, 2020; Subchev, 2014). There is a little more information on sex attractants (chemicals that are not naturally produced by the same species but found to be attractive in field or laboratory experiments) for Zygaenidae species. Sex attractants have been found for males of more than 60 species of two subfamilies Procridinae and Zygaeninae (Drouet et al. 2021; Efetov et al. 2010, 2011, 2014a, 2015b; Landolt et al. 1991; Priesner et al. 1984; Razov et al. 2017; Subchev, 2014; Subchev et al. 2010, 2012, 2013, 2016).

Previously, we had already synthesised (2*R*)-butan-2-yl dodec-2-enoate (named EFETOV-S-2, another name is (2*R*)-butyl 2-dodecenoate) and (2*S*)-butan-2-yl dodec-2-enoate (named EFETOV-S-S-2, another name is (2*S*)-butyl 2-dodecenoate). In different countries of the world field experiments with these esters (alone or in racemic mixture) have confirmed their attractive properties for almost 30 Zygaenidae species (Can et al. 2019; Can Cengiz et al. 2018; Efetov et al. 2014c, 2016, 2018, 2019b, 2020, 2022; Efetov & Kucherenko, 2021; Nahirnić-Beshkova et al. 2021; Vrenozi et al. 2019).

The aims of the present work were: 1) to synthesise esters of (2*R*)-butan-2-ol or (2*S*)-butan-2-ol and (*Z*)-dodec-5-enoic acid as well as (*Z*)-hexadec-9-enoic acid; 2) to test obtained esters as sex attractants for Zygaenidae species in the Crimean Peninsula.

Materials and methods

Synthesis of the target esters

Commercial chemicals (2*S*)-butan-2-ol, (2*R*)-butan-2-ol, (*Z*)-dodec-5-enoic acid were purchased from Sigma-Aldrich, USA and (*Z*)-hexadec-9-enoic acid from Toronto Research Chemicals, Canada. All chemicals were of 99% purity. Synthesis of (2*S*)-butan-2-yl (*Z*)-dodec-5-enoate and (2*R*)-butan-2-yl (*Z*)-dodec-5-enoate was performed in two stages: (1) synthesis of acyl chloride; (2) alcoholysis of acyl chloride.

1. Synthesis of (*Z*)-dodec-5-enoyl chloride. 5 g (0.025 mol) of (*Z*)-dodec-5-enoic acid was placed in a 50 ml round-bottom flask equipped with a water-cooled reflux condenser. 10 ml of anhydrous benzene, 0.5 ml of *N*,*N*-dimethylformamide and 2.2 ml (0.03 mol) of thionyl dichloride were added. The reaction mass was heated to boiling and maintained at boiling point for one hour. At the end of the reaction, the solvent (benzene) and excess of thionyl dichloride were removed under vacuum on a rotary evaporator. Acyl chloride was used at the stage of synthesis of the corresponding ester.

2. Synthesis of (2*S*)-butan-2-yl (*Z*)-dodec-5-enoate. 10 ml of anhydrous benzene, 3 ml (0.3 mol) of (2*S*)-butan-2-ol and 3 ml (0.03 mol) of pre-dried pyridine were added to a 50 ml round-bottom flask equipped with a water-cooled reflux condenser containing the previously synthesized (*Z*)-dodec-5-enoyl chloride and 0.5 ml of *N*,*N*-dimethylformamide. The reaction mass was heated to boiling and maintained at boiling point for one hour. After completion of the reaction, the pyridine hydrochloride was removed by filtration on a Schott filter under vacuum. The precipitate was washed with 20 ml of anhydrous benzene. The filtrate was washed on a separating funnel with two portions of 20 ml of 5% hydrochloric acid solution, separating the excess of pyridine. Then the filtrate was washed with two portions of 20 ml of 10% sodium hydroxide solution, with the aim of removing unreacted acid and alcohol. The benzene layer was separated. After removing the benzene on a rotary evaporator, 4.5 g of (2*S*)-butan-2-yl (*Z*)-dodec-5-enoate were obtained (85% purity according to high-performance liquid chromatography). Synthesis of (2*R*)-butan-2-yl (*Z*)-dodec-5-enoate (75% purity according to HPLC) was performed from (2*R*)-butan-2-ol by a similar procedure. As a result, two esters, viz. (2*S*)-butan-2-yl (*Z*)-dodec-5-enoate and (2*R*)-butan-2-yl (*Z*)-dodec-5-enoate, were obtained and called EFETOV-S-S-5 and EFETOV-S-5, respectively (Figures 1-2). EFETOV-S-S-5 was obtained two times and these samples were conventionally marked EFETOV-S-S-5(60) and EFETOV-S-S-5(85).

(2*S*)-butan-2-yl (*Z*)-hexadec-9-enoate and (2*R*)-butan-2-yl (*Z*)-hexadec-9-enoate were synthesised as described above but at the first stage (*Z*)-hexadec-9-enoyl chloride was obtained by treating (*Z*)-hexadec-9-enoic acid with thionyl dichloride.

Preparation of lures

Synthetic attractant candidate compounds were used without solvents in a dose of 50 µl for preparation each lure. Different types of esters were loaded on rubber dispensers which were fixed on cardboard rectangles after the attractant adsorption. All baits were marked as 'EFETOV-S-5' for (2*R*)-butan-2-yl (*Z*)-dodec-5-enoate, 'EFETOV-S-S-5' for (2*S*)-butan-2-yl (*Z*)-dodec-5-enoate, 'EFETOV-S-9-16' for (2*R*)-butan-2-yl (*Z*)-hexadec-9-enoate, and 'EFETOV-S-S-9-16' for (2*S*)-butan-2-yl (*Z*)-hexadec-9-enoate.

As a positive control, we used rubber caps impregnated with 50 µl of the sex attractant EFETOV-2 (the racemic mixture of (2*R*)-butan-2-yl dodec-2-enoate and (2*S*)-butan-2-yl dodec-2-enoate), which had been synthesised earlier according to the procedure published by Efetov et al. (2014c). The efficacy of baits with EFETOV-2 has been proven for seven species of Zygaenidae of the Crimean fauna (Efetov & Kucherenko, 2021). Empty rubber caps without any synthetic compounds were used as a negative control.

The lures with labelled cardboard holders were fixed in transparent plastic Delta traps containing removable sticky layers (Figure 3).

Field experiments

Attempts to observe the attraction of males of Zygaenidae species to synthetic esters were conducted in 2021 (with EFETOV-S-5 and EFETOV-S-S-5) and 2022 (with EFETOV-S-9-16 and EFETOV-S-S-9-16) at four localities of the Crimean Peninsula in biotopes corresponding to the preferred habitats of the target species (Efetov, 2005). The experimental and control traps were hung on bushes or trees at a height of 1.0-1.5 m above the ground. The distance between different types of traps was at least 10 meters. The traps were inspected on average once a week. Sometimes we just put the baits on rocks on the ground (the distance between the baits was not less than several meters). In this case, we collected attracted moths by catching them with a net near the rubber caps. All

specimens (captured and glued moths) were determined by examination of the genitalia by K. A. Efetov.

The list of the studied localities of the Crimean Peninsula and the time of observations are presented below (Figure 4).

Locality 1: vic. Belogorsk, Mt. Sary-Kaya, 230-239 m, grassy slope near steep calcareous edge of the mountain (Figure 5). Periods of observation: 15-V-2021 - 15-VII-2021, one trap with EFETOV-S-5, one trap with EFETOV-S-S-5(60), one trap with EFETOV-S-S-5(85), and one control trap (without attractant); 06-V-2022 - 02-VII-2022, one trap with EFETOV-S-9-16, one trap with EFETOV-S-S-9-16, and one control trap (without attractant).

Locality 2: vic. Simferopol, Bitak, 320 m, grassy slope near steep calcareous edge of the mountain. Periods of observation: 16-V-2021 - 08-VII-2021, one trap with EFETOV-S-5, one trap with EFETOV-S-S-5(85), and one control trap (without attractant); 06-V-2022 - 01-VII-2022, one trap with EFETOV-S-9-16, one trap with EFETOV-S-S-9-16, and one control trap (without attractant).

Locality 3: Mt. Chatyr-Dag, 514 m, grassy slope near deciduous forest (Figure 6). Periods of observation: 16-V-2021 - 08-VII-2021, one trap with EFETOV-S-5, one trap with EFETOV-S-S-5(60), one trap with EFETOV-S-S-5(85), and one control trap (without attractant); 23-V-2022 - 29-VII-2022, one trap with EFETOV-S-9-16, one trap with EFETOV-S-S-9-16, and one control trap (without attractant).

Locality 4: vic. Luchistoye, NE of Alushta, 376 m, abandoned vineyard near the lake. Periods of observation: 16-V-2021 - 24-IX-2021, one trap with EFETOV-S-5, one trap with EFETOV-S-S-5(60), one trap with EFETOV-S-S-5(85), one trap with EFETOV-2, and one control trap (without attractant); 23-V-2022 - 04-VIII-2022, one trap with EFETOV-S-9-16, one trap with EFETOV-S-S-9-16, one trap with EFETOV-2, and one control trap (without attractant).

Results and discussion

During our field screening tests the attraction of two Procridinae species to the baits with new synthetic compounds EFETOV-S-5 and EFETOV-S-S-5 was recorded, viz. *Adscita* (*Adscita*) *geryon* (Hübner, 1813) and *Jordanita* (*Tremewania*) *notata* (Zeller, 1847) (Figure 3). No specimens were attracted by the lures EFETOV-S-9-16 and EFETOV-S-S-9-16. In addition, the males of *Theresimima ampellophaga* (Bayle-Barelle, 1809) were found in sticky traps with the baits EFETOV-2, which were used as a positive control in our experiments in 2021-2022. Traps without attractant (the negative control) were empty in all studied localities. The list of attracted specimens, type of lures, and the time of observation are provided below.

A. geryon (Hübner, 1813)

Locality 1, trap with EFETOV-S-S-5(60), 1 ♂, 20-V-2021; 1 ♂, 04-VI-2021; trap with EFETOV-S-S-5(85), 2 ♂♂, 20-V-2021; 1 ♂, 04-VI-2021; locality 2, trap with EFETOV-S-S-5(85), 1 ♂, 22-V-2021; locality 3, trap with EFETOV-S-S-5(60), 2 ♂♂, 29-VI-2021; trap with EFETOV-S-S-5(85), 1 ♂, 31-V-2021; 1 ♂, 22-VI-2021; near rubber cap with EFETOV-S-S-5(85), 1 ♂, 07-VI-2021.

J. notata (Zeller, 1847)

Locality 1, trap with EFETOV-S-5, 12 ♂♂, 20-V-2021; 9 ♂♂, 27-V-2021; 3 ♂♂, 04-VI-2021; 1 ♂, 12-VI-2021; trap with EFETOV-S-S-5(60), 2 ♂♂, 20-V-2021; 6 ♂♂, 27-V-2021; 8 ♂♂, 04-VI-2021; near trap with EFETOV-S-S-5(60), 1 ♂, 27-V-2021; trap with EFETOV-S-S-5(85), 13 ♂♂, 20-V-2021; 3 ♂♂, 27-V-2021; near trap with EFETOV-S-S-5(85), 4 ♂♂, 27-V-2021.

Th. ampellophaga (Bayle-Barelle, 1809)

Locality 4, trap with EFETOV-2, 17 ♂♂, 15-VII-2021; 7 ♂♂, 24-VII-2021; 2 ♂♂, 29-VII-2021; 7 ♂♂, 14-VII-2022; 8 ♂♂, 22-VII-2022; 3 ♂♂, 29-VII-2022.

In total, 25 males of *J. notata* were attracted to the baits EFETOV-S-5 and 37 males came to lures with EFETOV-S-S-5 (17 males to EFETOV-S-S-5(60) and 20 males to EFETOV-S-S-5(85)), whereas all 11 males of *A. geryon* were attracted only to EFETOV-S-S-5 (4 males to EFETOV-S-S-5(60) and 7 males to EFETOV-S-S-5(85)). No specimens of *J. notata* and *A. geryon* were found in sticky traps with EFETOV-S-9-16 and EFETOV-S-S-9-16. However, at the same time some males of both species were caught by a net near the rubber caps with attractant EFETOV-2 when we put them on the rocks on the ground during inspection of the traps. Previously, attractiveness of EFETOV-2 has been proved for both of these species (Efetov et al. 2016). Thus, only esters of butan-2-ol and (*Z*)-dodec-5-enoic acid, but not of (*Z*)-hexadec-9-enoic acid, can be considered new sex attractants for males of *J. notata* and *A. geryon*.

It should be noted that 44 males of *Th. ampellophaga* were found in sticky traps with sex attractant EFETOV-2, that was used as a positive control in 2021-2022 in locality 4. However, the males of this species were absent in traps with newly synthesized esters. Besides this, some other Zygaenidae species (observed in the biotopes on the wing) did not respond to the tested baits. These species were *Rhagades* (*Rhagades*) *pruni* ([Denis & Schiffermüller], 1775), *Jordanita* (*Roccia*) *budensis* (Speyer & Speyer, 1858), *J.* (*Jordanita*) *graeca* (Jordan, 1907), *J.* (*J.*) *chloros* (Hübner, 1813), *J.* (*J.*) *globulariae* (Hübner, 1793), *J.* (*Solaniterna*) *subsolana* (Staudinger, 1862) (all Procridinae) and *Zygaena* (*Mesembrynus*) *purpuralis* (Brünnich, 1763), *Z.* (*Agrumenia*) *viciae* ([Denis & Schiffermüller], 1775), *Z.* (*Lictoria*) *loti* ([Denis & Schiffermüller], 1775), *Z.* (*Z.*) *filipendulae* (Linnaeus, 1758) (all Zygaeninae).

Previously, Efetov et al. (2015b, 2016, 2020), Efetov & Kucherenko (2021), Razov et al. (2017), Subchev et al. (2010) have shown that the *R*- and *S*-enantiomers of 2-butan-2-yl dodec-2-enoate and 2-butan-2-yl dodec-7-enoate are sex attractants for males of *A. geryon*, *J. notata* and some other species. These esters differ from each other and from the esters (2*R*)-butan-2-yl dodec-5-enoate and (2*S*)-butan-2-yl dodec-5-enoate by the position of the double bond in the acid radical, but this change affects the attractive properties of the esters in relation to males of the same species.

In our fieldwork, *A. geryon* were attracted to the *S*-enantiomer of 2-butan-2-yl dodec-5-enoate. This is in good agreement with the data which have been obtained earlier in Bulgaria, Crimea, Hungary and Italy (Efetov et al. 2015b; Subchev et al. 2010), where males of this species were mainly attracted to the *S*-enantiomer of 2-butan-2-yl dodec-7-enoate alone or in a mixture with the corresponding *R*-enantiomer. In the case of (2*R*)-butan-2-yl dodec-7-enoate and (2*S*)-butan-2-yl dodec-7-enoate, the presence of the *R*-enantiomer does not influence the attractiveness of the *S*-enantiomer. The males of *A. geryon* were not found in sticky traps with the racemic mixture of 2-butan-2-yl dodec-2-enoate and its enantiomers alone. The males only flew up at a close distance of about 20-50 cm to the rubber caps with EFETOV-2 and then flew away. The main catches of this species in field trials with the enantiomers of 2-butan-2-yl dodec-5-enoate and 2-butan-2-yl dodec-7-enoate were recorded in sticky traps.

The main catches of *J. notata* were recorded in traps baited with (2*R*)-butan-2-yl dodec-2-enoate, (2*R*)-butan-2-yl dodec-5-enoate, (2*R*)-butan-2-yl dodec-7-enoate or in traps with lures containing these compounds in mixtures with other substances (Efetov et al. 2015b, 2016, 2020; Efetov & Kucherenko, 2021; Razov et al. 2017; Subchev et al. 2010). The presence of the corresponding *S*-enantiomers does not inhibit the attractiveness of the *R*-enantiomers for *J. notata.* It is clear that the stereoisomerism of the ester molecules is more important for their recognition by the olfactory receptors of males of this species than the position of the double bond in acid radicals.

In the Crimea in many localities two species, viz. *A. geryon* and *A. albanica* (Naufock, 1926), are sympatric and syntopic. When we performed experiments with *R*- and *S*-enantiomers of 2-butan-2-yl dodec-7-enoate and 2-butan-2-yl tetradec-9-enoate not only the males of *A. geryon* were attracted mainly to left isomers, but two males of *A. albanica* were attracted to mixtures containing (2*S*)-butan-

2-yl dodec-7-enoate (one male was in a trap with *S*- and *R*-enantiomers of 2-butan-2-yl dodec-7-enoate in 2006 and one male was in a trap with *S*-enantiomers of 2-butan-2-yl dodec-7-enoate and 2-butan-2-yl tetradec-9-enoate in 2007) (Subchev et al. 2010). It means that (2*S*)-butan-2-yl dodec-7-enoate is a sex attractant for *A. albanica*.

Conclusion

New sex attractants for two Procridinae species, viz. *Adscita geryon* and *Jordanita notata*, were found. *A. geryon* males reacted to lures with (2*S*)-butan-2-yl (*Z*)-dodec-5-enoate (EFETOV-S-S-5), while *J. notata* males were attracted to the both enantiomers of 2-butan-2-yl (*Z*)-dodec-5-enoate (EFETOV-S-5 and EFETOV-S-S-5).

(2*R*)-butan-2-yl (*Z*)-hexadec-9-enoate and (2*S*)-butan-2-yl (*Z*)-hexadec-9-enoate did not attract Zygaenidae moths, at least those species inhabiting the Crimea.

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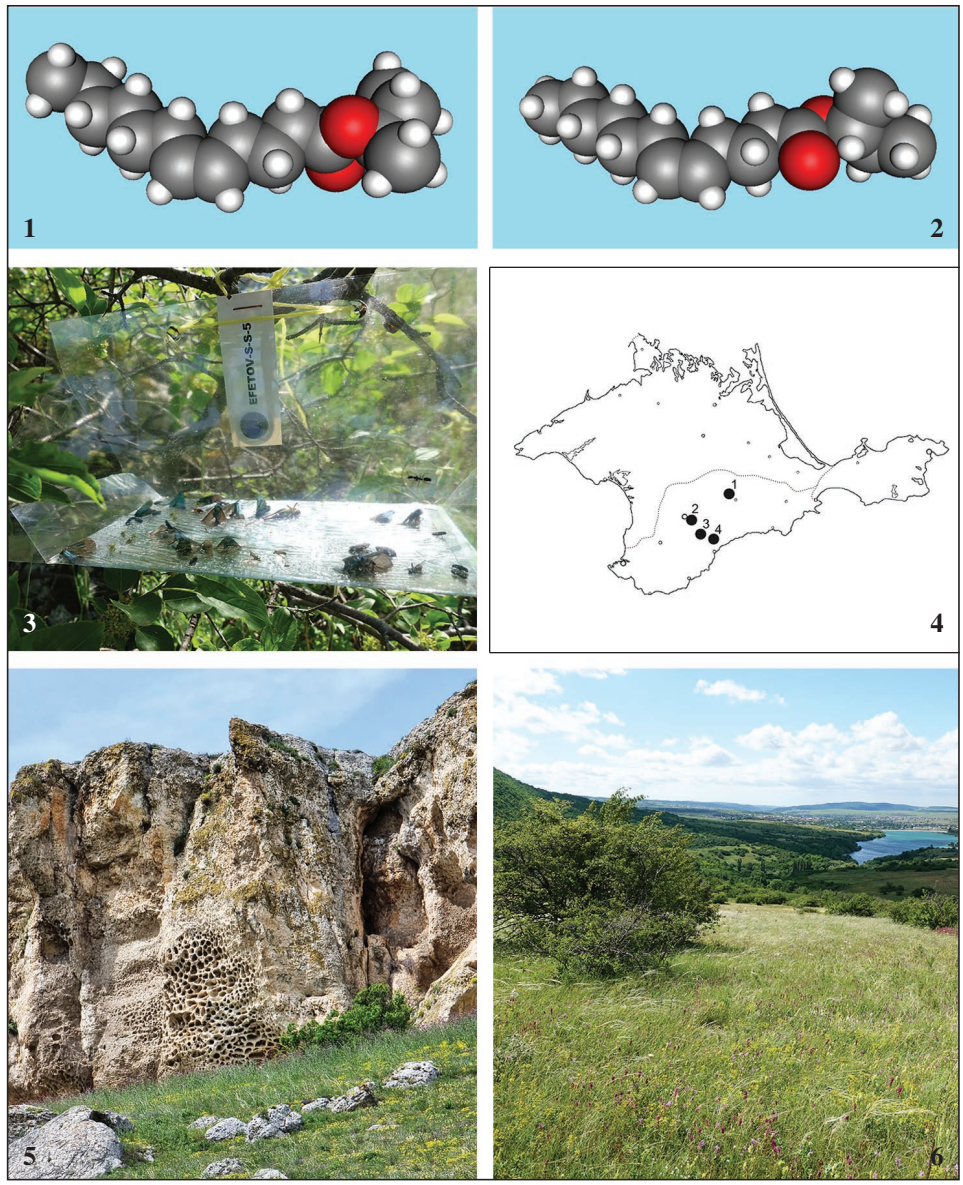

Figures 1-6. 1. (2*S*)-butan-2-yl (*Z*)-dodec-5-enoate (named EFETOV-S-S-5). **2.** (2*R*)-butan-2-yl (*Z*)-dodec-5-enoate (named EFETOV-S-5). Models were performed by the program 'Chemistry. Virtual Laboratory' (LLC 'Virtual Spaces', Yoshkar-Ola, RU). Oxygen atoms are marked in red. **3.** *J. notata* (13 ♂♂) and *A. geryon* (2 ♂♂) in sticky Delta trap with EFETOV-S-S-5 in locality 1, 20-V-2021. **4.** Map of the Crimean Peninsula showing the localities that were investigated using attractant traps: 1, vic. Belogorsk, Mt. Sary-Kaya; 2, vic. Simferopol, Bitak; 3, Mt. Chatyr-Dag; 4, vic. Luchistoye, NE of Alushta. **5.** Locality 1, Crimea, vic. Belogorsk, Mt. Sary-Kaya, photo: K. A. Efetov. **6.** Locality 3, Crimea, Mt. Chatyr-Dag, photo: K. A. Efetov.