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(5*R*,7*R*)-5-Methylheptadecan-7-ol: a novel sex pheromone component produced by a female lichen moth, *Miltochrista calamina*, in the family Arctiidae

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ABSTRACT

A methyl-branched heptadecanol was found in the pheromone gland extract of a female lichen moth, *Miltochrista calamina* (Arctiidae, Lithosiinae). GC–MS analyses of the alcohol and a hydrocarbon derived from it by subsequent treatments with methanesulfonyl chloride and $LiAlD_4$ in microscale reactions indicated 5-methylheptadecan-7-ol (**1**) as one possible structure. The four stereoisomers of **1** in a ratio of 4:4:1:1 were prepared from (*S*)-β-citronellol with 60% ee, and were separated by a combination of achiral and chiral HPLC columns. The absolute configuration of each isomer was determined by the comparison with the chromatographic behaviors of other samples synthesized by a different scheme, which applied the Jacobsen hydrolytic kinetic resolution of racemic 1,2-epoxydodecane to fix the configuration of the 7-hydroxy group. Only the (5*R*,7*R*)-isomer attracted male moths; thus, we concluded that *M. calamina* females secrete (5*R*,7*R*)-**1** as a sex pheromone, indicating a new chemical class of lepidopteran female sex pheromones.

Female moths secrete species-specific pheromones to attract males. Sex pheromones have been identified from adult females of more than 600 lepidopteran species, and are mainly composed of fatty alcohols and their derivatives (Type I pheromones) or polyenyl hydrocarbons and their epoxy derivatives (Type II pheromones).[1] In addition to these well-known components, many miscellaneous chemicals have been identified, such as secondary alcohols and methyl-branched hydrocarbons.[1,2] Recently, we found novel methyl-branched ketones from *Lyclene dharma dharma* in the subfamily Lithosiinae in the family Arctiidae.[3] Larvae of many Lithosiinae species feed on lichens and the adults are called lichen moths. The family Arctiidae is one of the most evolved groups in the Lepidoptera, and is additionally divided into two other subfamilies, Syntominae and Arctiinae. It is known that the females in these two subfamilies commonly utilize Type II pheromones.[1,2] However, information on sex pheromones of the lichen moths is still limited, and only the second identification from a lichen moth has been reported for *Eilema japonica japonica*. Unexpectedly, *E. j. japonica* females secreted unbranched polyenyl hydrocarbons, a typical Type II pheromone, to attract males.[4] We were interested to know whether or not only *L. d. dharma* had established an anomalous chemical communication system. Lithosiinae includes many species, with about 80 species recorded from Japan. To more thoroughly understand their mating communication, we examined a pheromone gland extract obtained from another lichen moth, *Miltochrista calamina*, when a single female was captured on one of our university campuses in a suburb of Tokyo (June, 2010). In analytical experiments, the sex pheromone was successfully determined to be a novel methyl-branched secondary alcohol. Furthermore, a field evaluation of all stereoisomers, prepared by stereoselective syntheses, determined the absolute configuration of the natural pheromone.

In a GC–MS analysis of an aliquot of the pheromone gland extract (0.2 female equivalent, FE) on a highly polar capillary column (DB-23),[5] only one abundant component was detected at 16.78 min. The mass spectrum, with characteristic ions at *m*/*z* 269 (M-1), 252 (M-18), 171, and 129 (Fig. 1A), suggested a C_{18} secondary alcohol structure possessing two alkyl groups (C_7H_{15} and $C_{10}H_{21}$), but the GC–MS data did not coincide with those of octadecan-8-ol (Fig. 1B), which was synthesized by Grignard coupling between octanal and decylmagnesium bromide. Because the t_R of the natural component was shorter than that of authentic octadecan-8-ol (17.84 min), a methyl-branched skeleton was assigned as a possible pheromone candidate. However, the position of the methyl group could not be determined from the mass spectrum. To determine the position, microscale reactions were carried out with an aliquot of the pheromone extract (0.4 FE). The secondary alcohol in the extract was reacted with methanesulfonyl chloride, and the mesylate produced was reduced with $LiAlD_4$.[6] The GC–MS analysis showed the production of a mono-deuterated hydrocarbon ($C_{18}H_{37}D$) with M^+ at *m*/*z* 255, and the mass spectrum showed characteristic fragment ions at *m*/*z* 198, 170, 85, and 57 (Fig. 1C), indicating a methyl branch at the 5-position. This result

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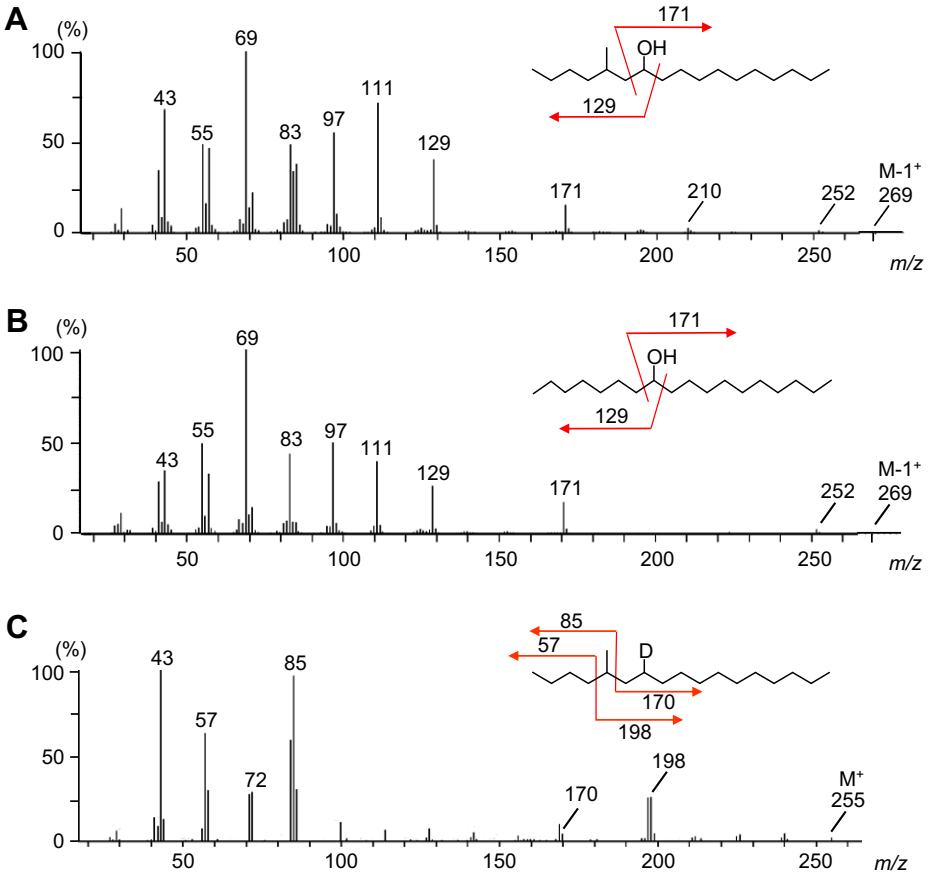

Figure 1. Mass spectra: (A) a pheromone candidate (**1**) extracted from a *M. calamina* female, (B) synthetic octadecan-8-ol, and (C) hydrocarbon derived from **1** by treatments with methanesulfonyl chloride and $LiAlD_4$. The spectra were measured by electron impact ionization (EI) using GC–MS with a DB-23 column (0.25 mm ID × 30 m). The column temperature program was 50 °C for 2 min, 10 °C/min to 160 °C, and 4 °C/min to 220 °C.

indicates two possible structures, 5-methylheptadecan-7-ol and 13-methylheptadecan-8-ol, for the parent alcohol. Although the use of $LiAlD_4$ did not reveal the position of the hydroxyl group, the deuteration contributed to the identification of the hydrocarbon derived from the alcohol, among many components in the crude product after two chemical reactions. To determine the exact structure of the alcohol, we had to approach it by synthesis because an insufficient gland extract was available for further analysis. The pheromone candidates included two chiral centers, and so we examined two synthetic routes to authentic compounds, fixing the configuration of one of the two centers in each synthesis.

First, (5*S*,7*S*)- and (5*S*,7*R*)-isomers of 5-methylheptadecan-7-ol (**1**) were synthesized from (*S*)-(−)-β-citronellol (**2**, 60% ee) as shown in Figure 2A. After protection of the hydroxyl group as a methoxymethyl (MOM) ether, the double bond was oxidized with Oxone to yield a diol (**3**),[7] which was cleaved to an aldehyde (**4**) by the treatment with $NaIO_4$. Wittig reaction between **4** and methylenetriphenylphosphorane produced the terminal olefin (**5**). By the catalytic hydrogenation and MOM-deprotection of **5**, (*S*)-3-methylheptan-1-ol (**6**) was obtained. IBX oxidation and subsequent Grignard coupling with decylmagnesium bromide yielded **1** as a mixture mainly composing of its (5*S*,7*S*)- and (5*S*,7*R*)-isomers. The diastereomers were separable by normal-phase HPLC with an achiral column (NO_2),[8] and two fractions (Fr. I, t_R 17.8 min; and Fr. II t_R 20.0 min) were obtained by preparative HPLC. GC–MS analysis of both diastereomers showed the same mass spectrum as the natural pheromone candidate, confirming its planar structure. The diastereomer in Fr. I (GC t_R 16.78 min) also had the same t_R as the natural component and was eluted later than the diastereomer in Fr. II (GC t_R 16.66 min) from the DB-23 column.[5,9] Furthermore, enantiomeric resolution by chiral HPLC was examined with the two fractions (Fig. 3). The analysis revealed that each fraction included two enantiomers in a ratio of about 4:1, as expected from the ee of **2** used as the starting material. The four stereoisomers of **1** had different chromatographic behaviors on a chiral HPLC column (Chiralpack AY-H); thus, the optical purity of each synthetic isomer was evaluated by chiral HPLC analysis. While the configurations of the two enantiomers in each fraction could not be determined, the analysis showed that each (5*S*)-isomer eluted faster than its antipode regardless of the configuration at the 7-position.

The elution order of the four stereoisomers from the chiral column was proven by comparing with analytical data of the samples prepared by another synthetic route, which fixed the configuration of the hydroxyl group at the 7-position, as shown in Figure 2B. Thus, 1-dodecene (**7**) was epoxidized with a peracid to yield a racemic mixture of 1,2-epoxydodecane (**8**), which was then subjected to a hydrolytic kinetic resolution with Jacobsen's catalyst.[10] Hydrolysis of **8** was monitored by GC–MS, and the optical purity of the unhydrolyzed epoxide was analyzed by chiral HPLC.[8] Grignard coupling between (*S*)-**8** (>98% ee) and 2-hexylmagnesium bromide produced a 1:1 mixture of (5*S*,7*S*)-**1** and (5*R*,7*S*)-**1**. The two diastereomers were separable by HPLC with the NO_2 column,[8] and two fractions (Fr. III and Fr. IV) were obtained. Similarly, another 1:1 mixture of (5*R*,7*R*)-**1** and (5*S*,7*R*)-**1** was synthesized from (*R*)-**8** (>98% ee), and two fractions (Fr. V and Fr. VI) were obtained. Chiral HPLC analyses of all four fractions revealed stereoisomers included in each fraction as follows: (5*S*,7*S*)-**1** in Fr. III (t_R 16.5 min),

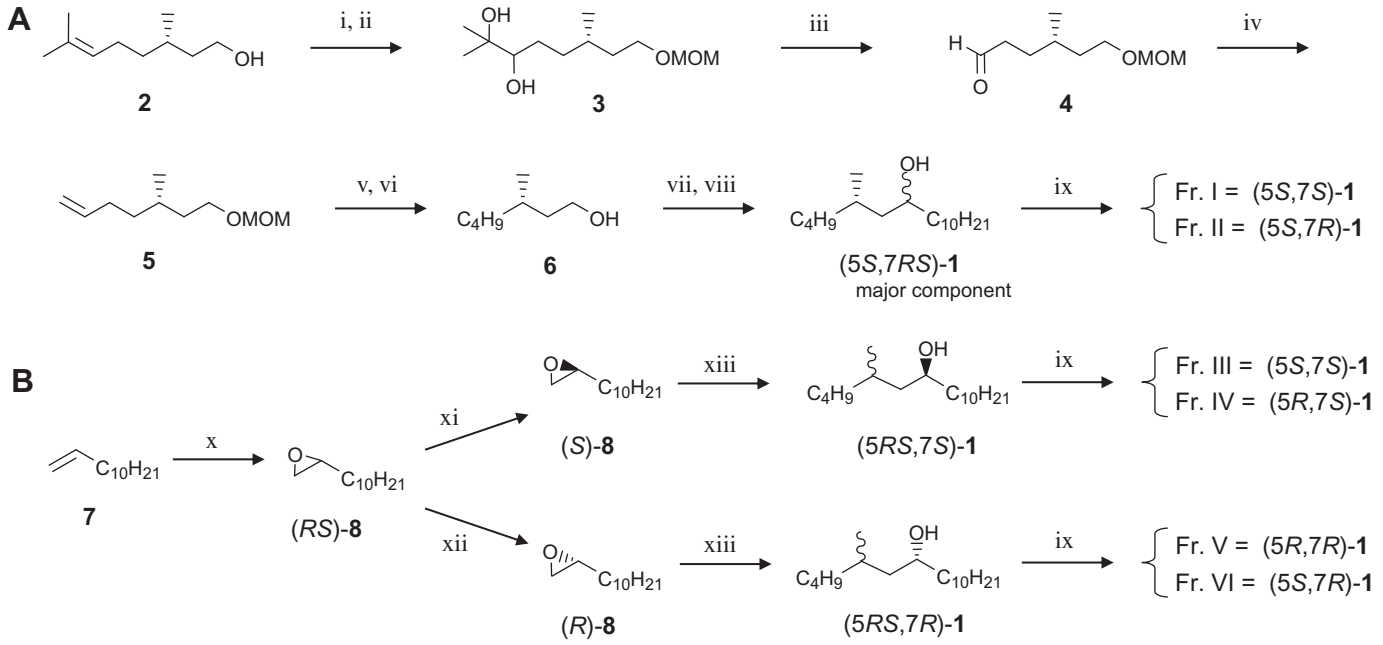

Figure 2. Synthetic routes to four stereoisomers of 5-methylheptadecan-7-ol (**1**): (A) synthesis starting from (*S*)-β-citronellol (**2**, 60% ee) and (B) synthesis of two diastereomeric mixtures utilizing the hydrolytic kinetic resolution of an epoxy intermediate (**8**). Reagents (yield): (i) DMM, *p*-TsOH, LiBr (91%); (ii) trifluoroacetone, Oxone, $NaHCO_3$, EDTA, CH_3CN, H_2O (87%); (iii) $NaIO_4$, Et_2O, H_2O (81%); (iv) $CH_2{=}PPh_3$, THF (65%); (v) H_2, Pd–C, MeOH (quant.); (vi) dry HCl, MeOH (90%); (vii) IBX, DMSO (62%); (viii) $C_{10}H_{21}MgBr$, THF (45%); (ix) HPLC (achiral NO_2 column); (x) MCPBA, CH_2Cl_2 (75%); (xi) (*S*,*S*)-(salen)Co(III)OAc, H_2O, dimethoxymethane (93%); (xii) (*R*,*R*)-(salen)Co(III)OAc, H_2O, dimethoxymethane (91%); (xiii) 2-hexylmagnesium bromide, THF, CuI (65%).

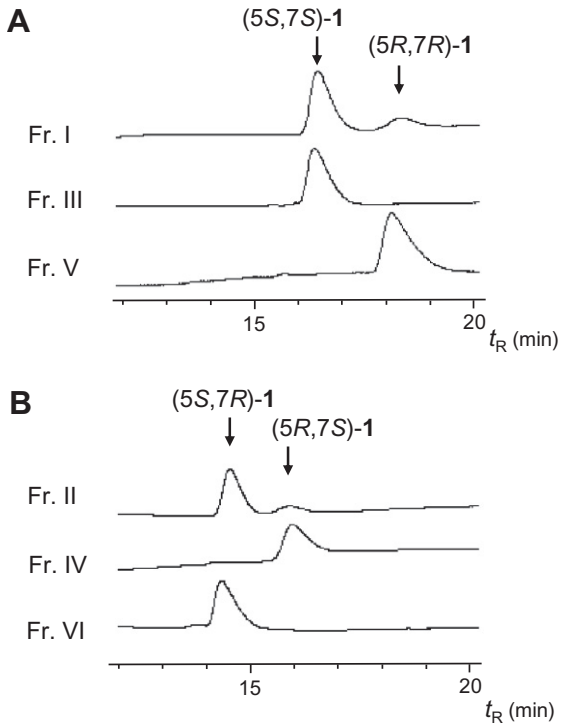

Figure 3. Chiral HPLC analyses of stereoisomers of synthetic 5-methylheptadecan-7-ol (**1**) to determine their absolute configurations. The HPLC was conducted with a Chiralpack AY-H column (4.6 mm ID × 25 cm) and an RI detector using 0.3% 2-propanol in hexane as an eluent (flow rate, 0.5 ml/min). Frs. I and II mainly include (5*S*,7*SR*)-isomers synthesized from (*S*)-β-citronellol (**2**, 60% ee), Frs. III and IV include (5*SR*,7*S*)-isomers derived from (*S*)-**8**, and Frs. V and VI include (5*SR*,7*R*)-isomers derived from (*R*)-**8**.

(5*R*,7*S*)-**1** in Fr. IV (t_R 14.5 min), (5*R*,7*R*)-**1** in Fr. V (t_R 18.0 min), and (5*S*,7*R*)-**1** in Fr. VI (t_R 15.9 min) (Fig. 3).[11] This result clarified the components in Fr. I; thus, the 5*S*,7*S* or 5*R*,7*R* configuration was assigned for the natural component.

The activity of the synthetic pheromone candidate was evaluated at coppices in the university campus from May, 2011. Each isomer (0.2 mg) was impregnated into a small rubber septum, which was placed in a sticky trap as a lure. Traps baited with (5*R*,7*R*)-**1** specifically attracted *M. calamina* males (about 30 males/trap/month), and no males were captured by traps baited with other isomers. Based on this result, we concluded that mating communication of *M. calamina* was mediated by (5*R*,7*R*)-**1**, a new natural product, although the possibility of 13-methylheptadecan-8-ol has not been eliminated. Further experiments with binary lures indicated that the other isomers neither synergized nor inhibited responses to (5*R*,7*R*)-**1**. The gland extract and synthetic diastereomers were also analyzed by GC equipped with an electroantennogram detector (GC–EAD)[12] using antennae from male lichen moths captured by the traps. The antennae clearly responded to the natural and synthetic (5*R*,7*R*)-**1** (Fig. 4). Details of the field and GC–EAD tests will be reported elsewhere.

Methyl-branched hydrocarbon pheromones have been identified from several species in some families of Lepidoptera, such as the Lyonetiidae and Geometridae. In the pheromone gland of an Arctiinae species, 5-methylheptadecane has been interestingly found together with Type II pheromone components, while the role in the mating communication has not been reported.[13] And, secondary alcohols with a short straight chain have been identified from species in the Ereiocraniidae.[1,2] However, this is the first identification of a methyl-branched secondary alcohol as a lepidopteran sex pheromone. Further studies will clarify the novelty or commonness of branched secondary alcohols among the lepidopteran sex pheromones. Identifications of the pheromones of many other lichen moths are expected. In addition to chain length, different positions and configurations of methyl and hydroxyl groups could provide a diversity of species-specific pheromones as do those of double bonds in Type I pheromones. Some ponerine ant species secrete methyl-branched secondary alcohols with a

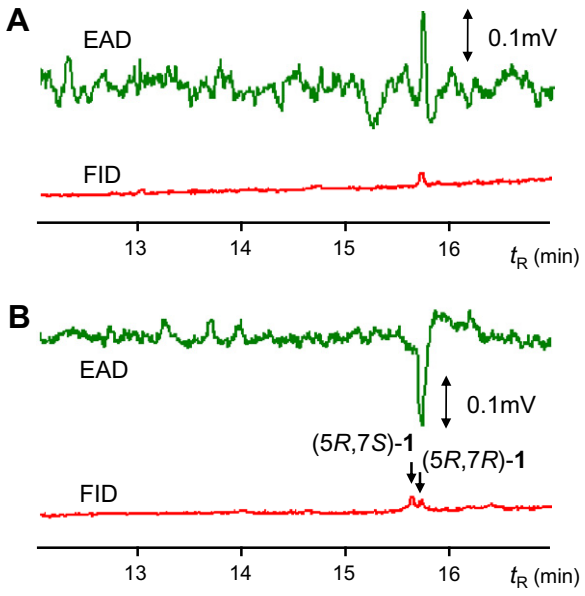

Figure 4. GC–EAD analyses: (A) a pheromone gland extract (0.05 FE) and (B) a mixture of synthetic (5*R*,7*S*)-**1** and (5*R*,7*R*)-**1** (1 ng each). The analysis was conducted with the same GC column and temperature program as for the GC–MS measurement.

C_7–C_{15} chain skeleton or their acetates as trail pheromones.[14] Pheromone biosynthesis in these ants has not been reported. The lichen moth and ants may have similar enzymes that produce these chemically similar structures. The biosynthetic pathways for these branched secondary alcohols will be an interesting subject for the follow-up research.

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5. Mass spectra were measured in EI mode (70 eV) with a GC–MS system equipped with a split/splitless injector and a DB-23 column (0.25 mm ID × 30 m, 0.25 μm film, J&W Scientific). The column temperature program was 50 °C for 2 min, 10 °C/min to 160 °C, and 4 °C/min to 220 °C. The carrier gas was He.
6. A crude gland extract (0.4 FE) dissolved in CH_2Cl_2 (100 μl) was mixed with triethylamine (7.5 mg), DMAP (1.0 mg), and methanesulfonyl chloride (33 mg). After stirring at 0 °C for 2 h and at room temperature for 1 day, a saturated $NaHCO_3$ solution (200 μl) was added to the mixture, which was extracted with hexane, dried over Na_2SO_4, and concentrated. The crude produced mesylate was dissolved in dry THF (100 μl) and treated with $LiAlD_4$ (5.5 mg) under an argon atmosphere. The reaction mixture was acidified with 1 N HCl after 3 h stirring and then extracted with hexane.
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8. All HPLC analyses were conducted with an RI detector. For the separation of diasteromers, a functionalized silica column (Senshu-pak NO_2-3151-N, 8 mm ID × 15 cm) was used. Eluent, 3% EtOAc in hexane; and flow rate, 1.0 ml/min. For the enantiomeric resolution of the alcohol (**1**), a Chiralpak AY-H column (4.6 mm ID × 25 cm, Daicel Chemical Industry, Osaka, Japan) was used. Eluent, 0.3% 2-propanol in hexane; and flow rate, 0.5 ml/min. For the enantiomeric resolution of the epoxide (**8**), a Chiralpak AS-H column (4.6 mm ID × 25 cm, Daicel Chemical Industry) was used. Eluent, 0.1% 2-propanol in hexane; and flow rate, 0.5 ml/min. t_R of (*S*)-**8**, 14.3 min; and t_R of (*R*)-**8**, 11.2 min.
9. NMR data of **1**: Isomers in Fr. I [(5*S*,7*S*)-**1** and (5*R*,7*R*)-**1**], ^{1}H NMR δ: 0.88 (6H, t, J = 6.4 Hz), 0.90 (3H, d, J = 6.4 Hz), ~1.16 (1H, m), ~1.26 (22H, m), ~1.42 (4H, m), 3.69 (1H, m). ^{13}C NMR δ: 14.14, 14.17, 19.30, 22.71, 23.00, 25.72, 29.19, 29.23, 29.36, 29.65 (×3), 29.74, 31.94, 37.63, 38.41, 45.04, 69.68. Isomers in Fr. II [(5*S*,7*R*)-**1** and (5*R*,7*S*)-**1**], ^{1}H NMR δ: 0.88 (3H, t, J = 6.5 Hz), 0.89 (3H, t, J = 6.6 Hz), 0.90 (3H, d, J = 6.4 Hz), ~1.16 (1H, m), ~1.26 (22H, m), ~1.42 (4H, m), 3.69 (1H, m). ^{13}C NMR δ: 14.14, 14.18, 20.43, 22.71, 23.05, 25.58, 29.11, 29.36, 29.58, 29.64 (×2), 29.67, 29.75, 31.94, 36.31, 37.81, 45.30, 70.04.
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11. $[\alpha]_D^{24}$: (5*S*,7*R*)-Isomer, −5.12 (*c* 1.45, $CHCl_3$); (5*R*,7*S*)-isomer, +5.59 (*c* 1.12, $CHCl_3$); (5*S*,7*S*)-isomer, +11.57 (*c* 0.69, $CHCl_3$); (5*R*,7*R*)-isomer, −10.92 (*c* 0.65, $CHCl_3$).
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