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CHARACTERIZATION OF (1*R*,4*S*,4a*R*,7*S*,7a*R*)-DIHYDRONEPETALACTOL AS A SEMIOCHEMICAL FOR LACEWINGS, INCLUDING *Chrysopa* spp. AND *Peyerimhoffina gracilis*

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Abstract—The enantiomerically pure diastereoisomers (1*R*,4*S*,4a*R*,7*S*,7a*R*)-(**1**) and (1*R*,4*R*,4a*R*,7*S*,7a*R*)-dihydronepetalactol (**2**) were synthesized diastereoselectively from a renewable resource, (4a*S*,7*S*,7a*R*)-nepetalactone (**3**), isolated as the main constituent of the essential oil of the catmint plant *Nepeta cataria*. The stereochemistry of the compounds was determined by NMR spectroscopy and X-ray crystallography, and the compounds were identified, respectively, as neomatatabiol and isoneomatatabiol, natural products from *Actinidia polygama*, for which the lactol stereochemistry was previously incompletely defined. Compound **1** was found to catch significant numbers of three species of lacewing in the field: in Korea, *Chrysopa cognata*, and in the United Kingdom, *Nineta vittata* and most notably *Peyerimhoffina gracilis*. All species caught in significant numbers were found more frequently in traps releasing **1** than **2**, while more *C. cognata*, *C. formosa*, and *C. phyllochroma* were found in traps releasing (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (**4**). The catch of *P. gracilis* with **1** is of particular interest as this lacewing has only recently been recorded in the United Kingdom.

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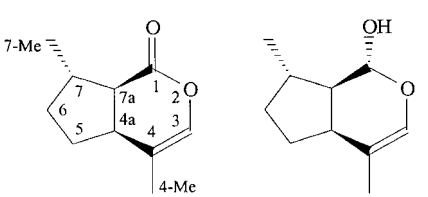
Where sexed, the lacewings of all species trapped were found to be male, implying a possible pheromonal role for these or structurally related compounds.

Key Words—*Nepeta cataria*, *Chrysopa cognata*, *Chrysopa formosa*, *Chrysopa phyllochroma*, *Nineta vittata*, *Peyerimhoffina gracilis*, lacewing, pheromone, dihydronepetalactol, neomatatabiol, isoneomatatabiol.

INTRODUCTION

The cyclopentanoids (4a*S*,7*S*,7a*R*)-nepetalactone (**3**) and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (**4**) (Figure 1) comprise the sex pheromones of a number of pest aphids (Aphididae), either as single compounds or as species-specific blends (Dawson et al., 1987, 1989). Aphid species including *Myzus persicae*, *Aphis fabae*, *Acyrthosiphon pisum*, *Sitobion fragariae*, *Tuberocephalus momonis*, and *Rhopalosiphum padi* have been trapped in the field using synthetic samples of these pheromone components (Dawson et al., 1990; Hardie et al., 1997; Boo et al., 2000). In addition, predatory species of lacewing such as *Chrysopa cognata* (Chrysopidae) are caught in traps releasing the same compounds, leading to the suggestion that, just as many aphid species use similar sex pheromones, lacewing predators may be using these compounds as kairomones in locating prey (Boo et al., 1998). Earlier literature reports revealed that the lacewing *Chrysopa septempunctata* was attracted to the vine *Actinidia polygama* (Actinidiaceae), a plant that generates two dihydronepetalactols previously named neomatatabiol and isoneomatatabiol (Hyeon et al., 1968), structurally related to **3** and **4**. The present work aimed to synthesize neomatatabiol and isoneomatatabiol and to determine unambiguously their stereochemistry. Studies were then undertaken to compare the potency of the aphid sex pheromone components **3** and **4** with the dihydronepetalactols related stereochemically to **3**, and to correlate lacewing catches in the field with the lactol stereochemistry not reported previously.



3 **4**

FIG. 1. Structures of aphid sex pheromone components (4a*S*,7*S*,7a*R*)-nepetalactone (**3**) and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (**4**).

METHODS AND MATERIALS

Experimental Chemistry

NMR spectra were obtained using the Bruker 500 Avance NMR spectrometer. GC-MS analysis employed the VG-Autospec (Fisons Instruments) with a siloxane column (50 m × 0.32 mm ID; 0.52 μm) operated from 30°C to 250°C at 5°C/min; EI at 70 EV, 250°C. GC analysis was on an HP-5 siloxane column (30 m × 0.32 mm ID; 0.25 μm) operated from 40°C to 150°C at 5°C/min and from 150°C to 250°C at 10°C/min. Compounds eluted at 16.42 (**2**), 16.93 (**1**), 31.08 (**11**), and 31.44 min (**10**).

(*1*R,*4*S,*4a*R,*7*S,*7a*R)*-Dihydronepetalactol (**1**).* The essential oil from *N. cataria* (10.0 g, 60.2 mmol) was dissolved in ethanol (250 ml) under a hydrogen balloon with 10% palladium on charcoal (600 mg) as a catalyst. The mixture was stirred overnight, filtered, and the solvent removed *in vacuo*. A portion of the crude product (2.0 g, 11.9 mmol) was dissolved in dry THF (30 ml) at –78°C under nitrogen. DIBAL (13.1 ml, 1 M in hexanes) was added slowly and the mixture warmed to room temperature over 1 hr. The reaction was quenched without acidification, extracted with diethyl ether, and the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by flash column chromatography (30% diethyl ether in petroleum ether) and recombination of the purest fractions gave **1** as a clear colorless oil (1.02 g, 50%). $[\alpha]_D^{20} - 1.1^\circ$ ($c = 9.0$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.41 (1H, d, $J = 9.1$ Hz, H-1), 3.66 (1H, dd, $J = 5.2$, 11.8 Hz, *pro-R* H-3), 3.28 (1H, t, $J = 11.8$ Hz, *pro-S* H-3), 3.02 (1H, br s, OH), 2.25 (1H, m, H-4a), 2.14 (1H, m, H-4), 2.06 (1H, m, H-7), 1.98 (1H, m, *pro-R* H-6), 1.59 (1H, m, *pro-R* H-5), 1.46 (1H, m, H-7a), 1.44 (1H, m, *pro-S* H-5), 1.17 (1H, m, *pro-S* H-6), 1.00 (3H, d, $J = 6.9$ Hz, 7-CH_3), 0.80 (3H, d, $J = 7.1$ Hz, 4-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 97.1 (C1), 67.3 (C3), 53.2 (C7a), 41.7 (C4a), 34.5 (C7), 32.3 (C6), 30.1 (C4), 23.9 (C5), 22.4 (7Me), 14.7 (4Me); $m/z = 170$ (0.16%, M^+), 81 (100%), 67 (96), 41 (77), 137 (65), 39 (58), 95 (55), 109 (50), 79 (44), 152 (42, M^+-18), 82 (40), 27 (39), 110 (37), 107 (36), 55 (36), 53 (34), 43 (26), 77 (26), 29 (25).

*(1*R,*4*R,*4a*R,*7*S,*7a*R)*-Dihydronepetalactol (**2**).* The essential oil from *Nepeta cataria* (10.0 g, 60.2 mmol) was dissolved in methanol (100 ml) at 0°C. A solution of sodium borohydride (2.51 g, 66.2 mmol) in ice cold methanol (100 ml) was added to the mixture by cannula, and the reaction mixture was stirred until complete. The pH was adjusted to 7 after the addition of water (250 ml), and the mixture was extracted with diethyl ether. The combined organic extracts were washed with water, dried ($MgSO_4$), and concentrated *in vacuo*. A portion of the crude mixture (3.0 g, 17.9 mmol) was dissolved in ethanol (75 ml) under a balloon of hydrogen with 10% palladium on charcoal (200 mg) as a catalyst. The mixture was stirred overnight, filtered, and the solvent evaporated to yield a colorless oil containing

a 91:9 mixture of diastereoisomers (2.91 g, 96%). Purification of 2.91 g of the mixture by flash column chromatography (30% ether in petroleum ether) and recombination of the purest fractions gave **2** as a colorless oil (1.85 g, 64%). $[\alpha]_D^{20} + 9.4^\circ$ ($c = 6.2$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.12 (1H, br s, H-1), 4.17 (1H, br s, OH), 3.60 (1H, t, $J = 11.0$ Hz, *pro-R* H-3), 3.42 (1H, dd, $J = 4.7$, 11.3 Hz, *pro-S* H-3), 2.00 (1H, m, H-7), 1.92 (1H, m, *pro-R* H-6), 1.88 (1H, m, H-4a), 1.68 (1H, m, *pro-R* H-5), 1.52 (1H, m, *pro-S* H-5), 1.46 (1H, m, H-4), 1.44 (1H, m, H-7a), 1.19 (1H, m, *pro-S* H-6), 1.01 (3H, d, $J = 6.3$ Hz, 7-CH_3), 0.79 (3H, d, $J = 6.6$ Hz, 4-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 92.8 (C1), 64.1 (C3), 51.1 (C7a), 41.5 (C4a), 33.6 (C7), 31.5 (C6), 30.9 (C4), 27.9 (C5), 20.0 (7Me), 15.5 (4Me); $m/z = 170$ (0.4%, M^+), 67 (100%), 81 (91), 137 (78), 41 (76), 95 (62), 39 (55), 109 (45), 152 (42, M^+-18), 79 (42), 27 (37), 110 (34), 53 (33), 55 (33), 82 (32), 107 (31), 77 (26).

*(1*R*,4*S*,4a*R*,7*S*,7a*R*)-Dihydronepetalactol Dimer (**10**).* Isomer **1** (420 mg, 2.47 mmol) was dissolved in diethyl ether (15 ml) and 6 N HCl (9 drops) and stirred overnight. The solvent was removed by rotary evaporation, and the residue was purified by flash column chromatography (10% diethyl ether in petroleum ether) to yield **10** as a clear colorless oil (300 mg, 75%). $[\alpha]_D^{20} - 25.5^\circ$ ($c = 10.2$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.51 (1H, d, $J = 7.4$ Hz, H-1), 3.65 (1H, dd, $J = 4.9$, 11.3 Hz, *pro-R* H-3), 3.27 (1H, t, $J = 11.3$ Hz, *pro-S* H-3), 2.25 (1H, m, H-4a), 2.22 (1H, m, H-4), 1.96 (1H, m, H-7), 1.94 (1H, m, *pro-R* H-6), 1.57 (2H, m, *pro-R* H-5, H-7a), 1.43 (1H, m, *pro-S* H-5), 1.12 (1H, m, *pro-S* H-6), 0.99 (3H, d, $J = 6.6$ Hz, 7-CH_3), 0.80 (3H, d, $J = 6.6$ Hz, 4-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 98.1 (C1), 66.9 (C3), 50.6 (C7a), 40.9 (C4a), 35.0 (C7), 32.9 (C6), 29.6 (C4), 24.3 (C5), 21.8 (7Me), 14.8 (4Me); $m/z = 322$ (0.012%, M^+), 81 (100%), 67 (83), 137 (80), 41 (76), 95 (71), 109 (58), 39 (55), 152 (51), 70 (49), 153 (47), 107 (47), 110 (46), 27 (39), 55 (37), 53 (36), 93 (31), 77 (28), 43 (27).

*(1*R*,4*S*,4a*R*,7*S*,7a*R*)-Dihydronepetalactol Dimer (**11**).* Isomer **2** (2.41 g, 14.2 mmol) was dissolved in a mixture of diethyl ether (100 ml) and 6 N HCl (4 ml) and stirred overnight. The solvent was removed by rotary evaporation and the crude residue purified by flash column chromatography (10% diethyl ether in petroleum ether) to yield **11** as a white solid (1.76 g, 77%). Mp 110–113°C; $[\alpha]_D^{20} - 39.1^\circ$ ($c = 5.8$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.02 (1H, br s, H-1), 3.36 (1H, dd, $J = 4.9$, 11.0 Hz, *pro-S* H-3), 3.29 (1H, t, $J = 11.0$ Hz, *pro-R* H-3), 2.01 (1H, m, H-7), 1.92 (1H, m, *pro-R* H-6), 1.79 (1H, m, H-4a), 1.68 (1H, m, *pro-R* H-5), 1.50 (1H, m, *pro-S* H-5), 1.44 (1H, m, H-4), 1.37 (1H, m, H-7a), 1.15 (1H, m, *pro-S* H-6), 1.00 (3H, d, $J = 6.4$ Hz, 7-CH_3), 0.75 (3H, d, $J = 6.4$ Hz, 4-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 92.7 (C1), 64.4 (C3), 50.8 (C7a), 41.8 (C4a), 33.6 (C7), 31.6 (C6), 31.1 (C4), 27.9 (C5), 19.9 (7Me), 15.4 (4Me); $m/z = 322$ (0.001%, M^+), 67 (100%), 137 (97), 81 (96), 41 (77), 95 (73), 153

(59), 39 (59), 109 (51), 152 (50), 79 (49), 110 (43), 107 (39), 27 (37), 53 (35), 55 (34), 77 (29), 93 (28), 91 (26).

X-Ray Crystallography

Crystals of **11** suitable for single crystal X-ray structure determination were grown by recrystallization from hexane. A single crystal of **11** was mounted on a glass fiber using high-vacuum grease. X-ray measurements were made with a Bruker SMART CCD area-detector diffractometer with Mo-K_α radiation ($\lambda =$ 0.71073 Å); structure solution SHELXTL program system version 5.1, 1998.

*Crystal Data for **11**.* $C_{20}H_{34}O_3$; $M = 322.47$, orthorhombic, space group *P2(1)2(1)2(1)*, $a = 8.7397(13)$ Å, $b = 9.4463(14)$ Å, $c = 22.898(3)$ Å, $U =$ 1890.4(5) Å^3, $Z = 4$, $D_c = 1.133$ Mg/m^3, μ(Mo-K_α) 0.074 mm^{-1}, $F(000) =$ 712, $T = 173(2)$ K, 3336 independent reflections with $2\theta < 50^\circ$. Refinement of 212 parameters converged at final $R_1 = 0.0434$, $wR_2 = 0.0806$.

Field Trapping in Korea

Field trapping experiments were carried out at the Suwon Agricultural Campus of Seoul National University and in the campus arboretum during July and August 2000. Wing trap dispensers (IMP Technology Inc.) and rubber septa (Sigma) were used. The traps were mounted 1.5 m above the ground and 2 m apart. The traps were checked and rotated 90° at 06:00 hr every morning. Analysis of trap catches was carried by using the GLIM statistics program (Crawley, 1993). The analysis was carried out declaring Poisson errors, with the catch for each replicate treated as independent.

Field Trapping in the United Kingdom

Lacewings were caught in clear water traps made of 14-cm-diam. × 2-cm-deep Petri dishes filled with a 1% clear odorless detergent solution. The traps were placed 2 m apart on 1.1-m-high poles. The volatiles were located in the center of the Petri dish above the detergent level, in dispensers made of amber glass vials [Chromacol 08-CPV(A)] with 1-mm holes drilled in the plastic lids. The vials were suspended below plastic shields to prevent rain entering and affecting release rates. Vials contained either the test compound (10 mg) in diethyl ether (50 μl) or ether alone. Traps were located at four woodland edge sites at Silwood Park, Ascot, Berkshire, with 16 traps at each site. In each trial, there were two traps for each treatment at each site. Traps were distributed so that identical treatments did not occur in adjoining traps. During the trial, which was run between May and August 2000, traps were rotated every four to five days. Traps were visited every two to three days, when all trapped lacewings were removed, identified, and

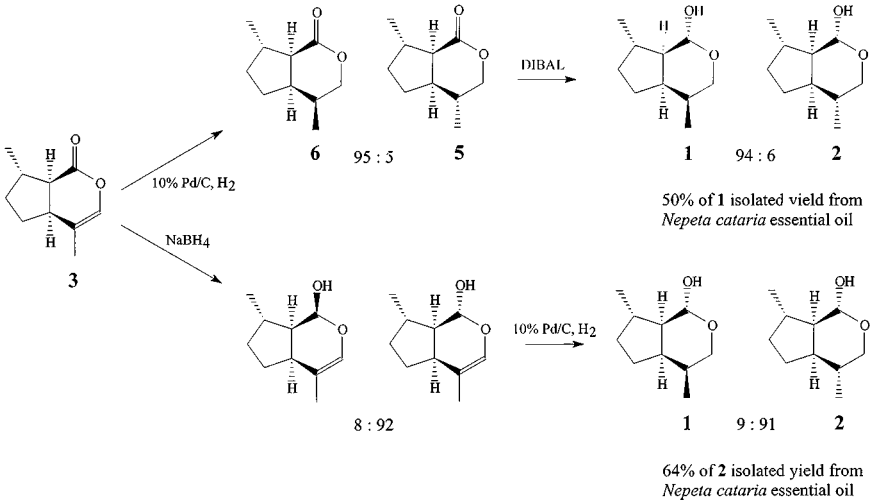
counted, and the solution in the traps was replaced. Analysis of water trap catches was carried with the GLIM statistics program (Crawley, 1993), with the trap totals for each treatment from each site being averaged, and each site treated as a block. The analysis was carried out declaring Poisson errors, with each site treated as an independent replicate.

Electrophysiology

Live adult male *Peyerimhoffina gracilis* were collected at Silwood Park, from traps made from clear plastic drinking bottles, during February and March 2001. Electroantennograms (EAGs) were obtained from excised antennae ($N = 7$), using Ag–AgCl glass electrodes filled with a saline solution. The signals generated by the antenna were passed through a high impedance amplifier (Syntech UN-06, Hilversum, The Netherlands), and data storage and processing were carried out with a PC-based interface and customised software package (Syntech). The system for stimulus delivery employed a filter paper strip in a disposable Pasteur pipet cartridge. Samples of test solutions (1 mg/ml in hexane, 10 μl applied) were applied to filter paper strips, and the solvent was allowed to evaporate for 30 sec before the paper strip was placed in the cartridge. A 2-sec stimulus (1 liter/min) was delivered into a purified airstream flowing continuously over the preparation. Results were expressed as a percentage of the response to a standard application of (*E*)-2-hexenal at the same concentration, and analysed for significant differences by Student's *t* test.

RESULTS AND DISCUSSION

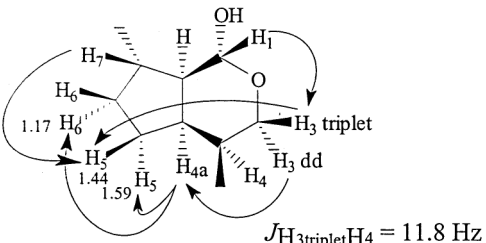
Synthesis and Structure Elucidation. (4a*S*,7*S*,7a*R*)-Nepetalactone **(3)** is found in the essential oil of many species of the *Nepeta* genus (Regnier et al., 1967; Eisenbraun et al., 1980; De Pascual Teresa et al., 1987) but, for high yield and purity, is obtained from *N. cataria* commercially by our collaborators (Botanix Limited) at approximately 90% of **3**. The essential oil was used here without further purification for the synthetic work. Compounds **1** and **2** were synthesized diastereoselectively from **3**, either by hydrogenation followed by lactone reduction in the case of **1**, or by lactone reduction followed by hydrogenation for **2**. Reduction of **3** with 10% palladium on carbon gave a 95:5 ratio of two products by GC, determined to be dihydronepetalactones by GC-MS (Scheme 1). The major component did not have the same spectroscopic properties as that reported for (4*R*,4a*R*,7*S*,7a*R*)-dihydronepetalactone (**5**) (De Pascual Teresa et al., 1987), but full characterization and stereochemical assignment by NMR spectroscopy was consistent with (4*S*,4a*R*,7*S*,7a*R*)-dihydronepetalactone **6**, formed by reduction from the less sterically hindered face. Interestingly, this is the opposite face selectivity to that reported when palladium on strontium carbonate is used as a



SCHEME 1. Divergent diastereoselective synthesis of **1** and **2** from (4a*S*,7*S*,7*R*)-nepetalactone (**3**).

hydrogenation catalyst (Regnier et al., 1967). In our hands, however, reduction with 2% $Pd/SrCO_3$ gave a different result from Regnier et al. (1967) and yielded the same major isomer **6** in a **6:5** ratio of 96:4 by GC. The crude mixture obtained from reduction with 10% palladium on carbon was then further reduced with DIBAL, and the crude product was analyzed by GC to reveal a 94:6 ratio of isomers **1:2**. By altering the order of chemical transformation, it was possible to synthesize **2** diastereoselectively. Borohydride reduction of the essential oil gave diastereomeric nepetalactols in the ratio 92:8, the stereochemistry of which was previously elucidated by X-ray crystallography of a derivative (Dawson et al., 1989). Hydrogenation of this crude mixture then gave a quantitative 91:9 mixture of **2:1** by GC. The diastereoselectivity of hydrogenation of nepetalactone can be explained on steric grounds, but, after borohydride reduction, the face selectivity of hydrogenation is reversed with comparable selectivity. This phenomenon has been observed previously when hydrogenation of *O*-ethoxyethyl protected (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (**4**) was reported to occur with the same facial selectivity (Kigawa et al., 1992).

The relative stereochemistry of compounds **1** and **2** was determined by 1D gradient NOE spectroscopy (GOESY) (Figure 2) (Stonehouse et al., 1994). In the case of isomer **1**, the H-1 proton gives a NOE to the triplet at H-3 that, in turn, shows a NOE with a proton at H-5. Due to the known *cis* ring junction of **3**, this is only possible if H-1, H-3 (triplet), and the H-5 proton are all on the same upper face. The other H-3 proton, a doublet of doublets, shows a NOE to proton H-4a that is



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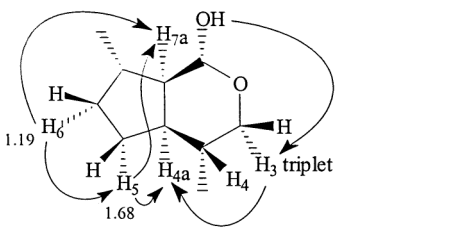

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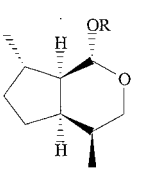
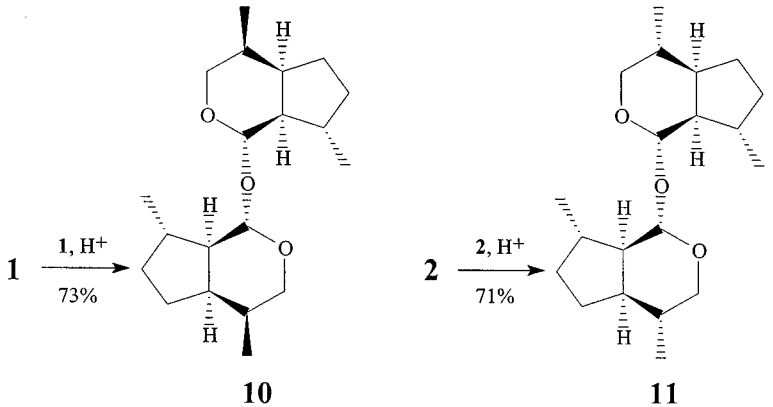
FIG. 2. Assignment of the stereochemistry of (1*R*,4*S*,4a*R*,7*S*,7a*R*)-dihydronepetalactol (**1**) and (1*R*,4*R*,4a*R*,7*S*,7a*R*)-dihydronepetalactol (**2**), showing the structurally revealing GOESY results.

known to be on the back face, verifying the structure. The methyl stereochemistry at C-4 is inferred by the large *trans* diaxial coupling constant of 11.8 Hz between the H-3 triplet and H-4. H-4a also shows a NOE to H-6 (1.17 ppm) and H-5 (1.59 ppm) and finally, a NOE from H-7 to H-5 (1.44 ppm) completes the assignment of the C-5 and C-6 protons. Several derivatives were made of **1** in an attempt to produce crystals for X-ray crystallography (Figure 3). None of compounds **7**–**9** was suitable for X-ray diffraction, but all showed the same NOEs between protons H-1, H-3 (triplet), and H-5, suggesting the same stereochemistry as presented in Figure 3. To ensure that the basic conditions used in generating **7**–**9** (treatment



7 : R = 3',5'-dinitrobenzoyl
8 : R = *p*-bromobenzoyl
9 : R = 3',5'-dinitrobenzyl

FIG. 3. Derivatives of **1** synthesized, which all revealed the same stereochemistry by GOESY spectroscopy.



SCHEME 2. Acid catalyzed dimerization of **1** and **2**.

of **1** with *n*-BuLi in anhydrous solvent followed by the acyl or alkyl chloride) was not epimerizing the lactol center, **7** was saponified in basic conditions and **1** reisolated with no lactol isomerization. An attempt to epimerize the lactol center of **1** using acid conditions was also made but instead of epimerization, **10** was isolated in good yield (Scheme 2). Infrared analysis of compound **10** showed it did not contain a hydroxyl group, and with one set of NMR peaks, it was characterized as a symmetric dimer. A very weak parent ion at *m/z* 322 was detected by electron impact mass spectroscopy. The ion at *m/z* 153 could be rationalized by loss of the $C_{10}H_{17}O_2$ group and the ion at *m/z* 152 by loss of dihydronepetalactol, analogous to loss of water from the monomeric compounds **1** and **2**. Again, NOE studies revealed the same relative stereochemistry as **1**, demonstrating the 1*R* stereochemistry to be the thermodynamically stable configuration as the reaction mechanism must involve equilibrium of **1** with an oxonium ion.

Despite purified **2** giving a single peak by three different GC columns (HP-1, HP-5, and β-cyclodextrin), its NMR spectrum in $CDCl_3$ showed it to be a mixture of two isomers, in a ratio of 90:10. GOESY studies on the major isomer **2** (Figure 2) revealed NOEs between the lactol OH and the H-3 triplet, which, in turn, gave a NOE to H-4a. As the ring junction stereochemistry is known, H-4a, H-3 (triplet), and the lactol OH must all be on the same back face. The stereochemistry at C-4 was assigned using the *trans* diaxial coupling of 11.0 Hz between H-3 (triplet) and H-4. Full assignment of the C-5 and C-6 protons was completed as the H-6 proton at 1.19 ppm shows a NOE to H-7a and the H-5 proton at 1.68 ppm, which in turn also relaxes H-4a and H-7a. GOESY studies on the minor isomer of the $CDCl_3$ solution of **2** revealed a NOE between H-1 and the H-3 triplet, demonstrating it to be the C-1 epimer. The treatment of **2** with acid generated a crystalline dimer, **11**. Mass spectrometry data for **11** were similar to those for **10**

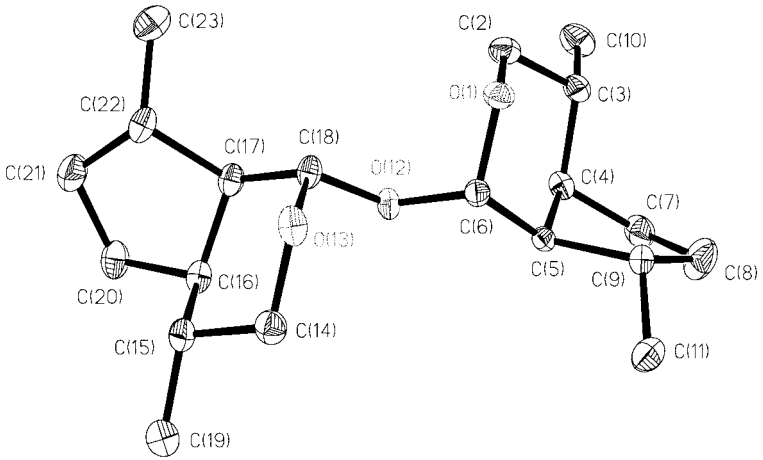


FIG. 4. Ortep diagram of **11**. Ellipsoids are drawn at the 30% probability level.

and crystals were obtained, suitable for X-ray crystallographic study (Scheme 2). The X-ray crystallographic results revealed the relative stereochemistry of **2** and based on the known chiral centers, stereochemistry at C-4 was verified as 4*R* and the thermodynamically stable lactol as 1*R* (Figure 4). By comparing the NMR data and synthetic methodology with the paper of Hyeon et al. (1968), structure **1**, (1*R*,4*S*,4a*R*,7*S*,7a*R*)-dihydronepetalactol, can be termed neomatatabiol and structure **2**, (1*R*,4*R*,4a*R*,7*S*,7a*R*)-dihydronepetalactol, can be termed isoneomatatabiol.

Field Results and Electrophysiology. Field trapping experiments in Suwon, Korea, and at Ascot, United Kingdom, caught several species showing varying preferences for the compounds described above. The initial study in Korea was performed between July 17 and 22, 2000. The traps releasing compound **1** caught significantly more *Chrysopa cognata* than did those releasing **2**. However, compound **4** was more effective than either, and also caught smaller but significant numbers of *Chrysopa formosa* and *Chrysopa phyllochroma* (Table 1). This study

TABLE 1. *Chrysopa* spp. CAPTURED IN SUWON, JULY 17–22, 2000[a]

Dispenser treatment	*C. cognata*	*C. formosa*	*C. phyllochroma*
1	171.3a	1.3ab	0.7a
2	63.7b	0.3b	0.3a
N. cataria extract containing **3**	20c	0b	0a
4	363.3d	17a	1.7a
Control	0e	0b	0a

[a] The insects were not sexed. Values are means. Means within a column followed by a different letter are significantly different at $P < 0.05$.

TABLE 2. *Chrysopa* spp. CAPTURED IN SUWON, AUGUST 2–6, 2000[a]

Dispenser treatment	*C. cognata*	*C. formosa* and *C. phyllochroma*
1	113a	1.3a
2	48b	0.7a
4	181.3c	3.7a
control	0d	0a

[a] All lacewings captured were male. Values are means. Means within a column followed by a different letter are significantly different at $P < 0.05$.

did not differentiate between male and female insects. The next study, which ran from August 2 to 6, 2000, caught fewer insects but they were all found to be male (Table 2). Although the catch means of *C. phyllochroma* (Table 1) and of *C. formosa* and *C. phyllochroma* (Table 2) for the different treatments did not differ significantly, analyses of variance showed the response to **4** to be significant at $P < 0.01$. The third trial studied the response of *C. cognata* to increasing release rates of **1** and **2** (Table 3).

In the United Kingdom, compounds **1** and **2** were initially tested for lacewing attraction in the field from May to July 2000 (Table 4). In this study, 11 specimens of *Nineta vittata* and 19 of *P. gracilis* were trapped, all of which were male. Further trials were performed during July and August 2000 (Table 5), with the addition of traps containing both **1** and **2** at equal concentrations and both at the same concentration as compounds in the other traps. Results showed compound **1** was

TABLE 3. *Chrysopa cognata* CAUGHT IN SUWON, AUGUST 6–10, 2000

Dispenser treatment	*C. cognata*/trap[a]
Compound **1**	
0.1 mg	8.7ab
1.0 mg	23.0a
10 mg	46.7c
50 mg	183.0d
Compound **2**	
2 0.1 mg	5.3b
2 1.0 mg	5.3b
2 10 mg	22.3a
2 50 mg	60.3c
Compound **4**, 10 mg	110.7e
Control	1.3b

[a] Values are means. means within a column followed by a different letter are significantly different at $P < 0.05$.

TABLE 4. MALE *Nineta vittata* AND *Peyerimhoffina gracilis* CAUGHT AT SILWOOD PARK, MAY–JULY 2000[a]

Dispenser treatment	*N. vittata*	*P. gracilis*
1	9	18
2	1	0
3	1	0
4	0	1
Control	0	0

[a] Catches with compound **1** are significant at $P < 0.001$.

highly effective at trapping lacewings compared to the other compounds tested and that isomer **2** did not influence the response to **1**. Of the 198 specimens of *P. gracilis* trapped in this study, only one was female.

Previous work (Hyeon et al., 1968) reported the compounds now defined as **1** and **2** to be attractants of adult male *C. septempunctata* and male *Chrysopa japonica*. We have demonstrated that the field response of adult male *P. gracilis* and *N. vittata* can be attributed to the 1*R*,4*S* stereochemistry of **1**. The preference of adult male *C. cognata* is for 1*R*,4*S* over 1*R*,4*R*, but the overall preference of *C. cognata*, *C. formosa*, and *C. phyllochroma* is for compound **4**.

Results of EAG analysis using male *P. gracilis* collected from the same trapping area showed compounds **1** and **2**, and the two aphid sex pheromone components, (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (**4**) and (4a*S*,7*S*,7a*R*)-nepetalactone (**3**), to have significant activity (difference from control, $P < 0.001$), the first eliciting a significantly higher response than the latter three ($P < 0.05$) (Table 6). The results demonstrate that the antennae of *P. gracilis* show a strong response to all the compounds tested in the traps, but the largest EAG response to **1** correlates with the finding that this compound is the most active in the field.

TABLE 5. MALE *Peyerimhoffina gracilis* CAUGHT AT SILWOOD PARK, JULY–AUGUST 2000[a]

Dispenser treatment	*P. gracilis*
1	82
2	1
3	5
4	10
1 + **2**	95[b]
Control	4

[a] Catches with compound **1** and **1** + **2** are significant at $P < 0.001$ and differ from the control at $P < 0.05$.
[b] One female was also caught in this treatment.

TABLE 6. EAG RESPONSES OF MALE *Peyerimhoffina gracilis* TO TEST COMPOUNDS (1 mg/ml, 10 μl APPLIED), EXPRESSED AS PERCENTAGE OF RESPONSE TO (*E*)-2-HEXENAL AT THE SAME CONCENTRATION

Stimulus	Response[a]
1	266 ± 30.52a
2	160 ± 16.38b
3	175 ± 26.09b
4	185 ± 13.42b
Hexane control	47 ± 7.29

[a] Numbers followed by a different letter are significantly different at $P < 0.05$; all treatments are significantly different from the hexane control at $P < 0.001$ ($N = 7$).

The trapping of large numbers of *P. gracilis* was of interest for several reasons. This species was not known to be native to the U.K. and recent surveys at Silwood Park did not reveal its presence (Hollier and Belshaw, 1992, 1993; see Donato et al., 2001). In addition, during the period of trapping, no specimens of *P. gracilis* were caught in malaise traps or a suction trap present in the same area. Only in the traps releasing cyclopentanoids were large numbers caught. This species is believed to be relatively sedentary (Duelli, 1984) and to have a particular preference for conifer trees, which were not present in the immediate vicinity of the field sites at Silwood Park. Each of the four sites used in this trial was at least 20 m from a tree of a species known to support *P. gracilis* and three sites were approximately 100 m away. This suggests that either the species is more mobile and less constrained by habitat than is currently thought or that these compounds are effective over a considerable distance.

The presence of lacewings in traps releasing aphid sex pheromones and closely related cyclopentanoids may suggest a kairomonal role for these compounds in prey location, as has previously been proposed (Boo et al., 1998). Lacewings are polyphagous and feed on a number of insect species, including aphids. The fact that many aphid species use the same sex pheromone components would allow their use as indicators of aphid prey. Lacewing larvae are insectivorous predators but only a minority of species is predatory as adults; the majority feed on honeydew, nectar, and pollen. Of the species recorded in this study, adults of the genera *Nineta* and *Chrysopa* are insectivorous; *Peyerimhoffina* adults are not. The compounds tested here, therefore, elicit a response from both predatory and nonpredatory species, so it is unlikely that food location by adults is the cause of the observed activity.

It is possible that lacewings are sensitive to these compounds as an aid to the location of suitable oviposition sites. However, aphids only produce their sex

pheromones during the autumn, as part of the sexual stage in the life cycle that culminates in the production of overwintering eggs. In the United Kingdom, lacewings usually overwinter as pupae. Of the British species reported here, *N. vittata* is typically recorded as an adult from May to August (Plant, 1994), and so would not normally be exposed to the aphid sex pheromones. Although *P. gracilis* is present as an adult in the autumn, studies suggest that its oviposition is stimulated by increasing day length (Grimal, 1988). Therefore, it might be expected to utilize prey-produced kairomonal cues to locate suitable oviposition sites during the spring, rather than during the autumn months. If these compounds were functioning as prey-location kairomones, it would be expected that the numbers trapped would either show little sex bias or would be biased towards females looking to oviposit near a food source, as is the case with *Praon* parasitoids of aphids (Hardie et al., 1991). However, only male insects were detected in these trials, suggesting that these compounds, or analogous structures, may play a pheromonal role in intraspecies sexual communication and that their similarity to aphid sex pheromones may be simply incidental.

The possible role played by pheromones in intraspecific communication within the Chrysopidae, and the Neuroptera as a whole, is poorly understood. The only compound identified to date as a lacewing secretion is (*Z*)-4-tridecene, which has recently been identified from *Chrysoperla carnea* and appears to have defensive functions (Zhu et al., 2000). Although close-range communication in the Chrysopidae involves species-specific substrate-borne songs (Henry, 1979), it is thought that sex pheromones are involved in the initial mate location process (Walker et al., 1994). However, no sex pheromones or glandular sources for pheromones have been found. In the related Myrmeleontidae, some pheromones with a role in sexual communication are known, but these species exhibit a lek mating system and the pheromones eliciting a behavioral response are male-produced. Instead of functioning as sex pheromones, they appear to act as aggregation pheromones, with females responding to groups of males rather than to individuals (Yasseri et al., 1998). Although members of the Chrysopidae appear to be solitary and do not lek, a possible role for these compounds as aggregation pheromones cannot be discounted. In the Myrmeleontidae, different species utilize different blends of related compounds, providing species-specific signals (Baeckstrom et al., 1989; Bergstrom et al., 1992). From the results obtained here, in particular the differences in response shown to the compounds by the different *Chrysopa* species, it would seem possible that a similar pattern might be found in chrysopid lacewings. The demonstration that *P. gracilis* antennae respond strongly to all the cyclopentanoids tested, and the behavioral response of males of some species to more than one cyclopentanoid structure, is consistent with the possibility that these, or similar structures, may be natural pheromones.

The reasons for the appearance of *P. gracilis* in the United Kingdom may be speculated as being due to accidental release, its presence previously being

overlooked, wind-borne arrival, or range expansion as a consequence of global warming (Donato et al., 2001). The identification of cyclopentanoid monoterpenes as semiochemicals for a number of lacewings will facilitate monitoring the changing status of the new species and may allow manipulation of natural populations of these beneficial insects in integrated pest control strategies. Further research into the ecological and possibly pheromonal roles of these and related compounds is ongoing.

Supplementary Data—The CIF file for the crystal structure has been deposited at the Cambridge Crystallographic Centre, 12 Union Road, Cambridge CB2 1EZ, UK (www.ccdc.cam.ac.uk) from which it is available on request. The deposition number is CCDC 165880.

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