Full Paper

Reduction Chemistry of Natural Pyrethrins and Preliminary Insecticidal Activity of Reduced Pyrethrins

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The natural extract pyrethrum is an insecticidal oil derived from *Tanacetum cinerariifolium* that is commonly used in domestic and agricultural pesticides. The major constituents of the extract are the Pyrethrins, six esters that provide pyrethrum with its insecticidal properties. These Pyrethrins readily degrade through several environmental means and as such, there can be significant Pyrethrin losses during processing and long-term storage of pyrethrum-based insecticides. This work attempts to alleviate the effect of these degradative processes through the pursuit of stabilised Pyrethrins by chemically removing oxidatively sensitive functionality. Several reduced Pyrethrin analogues were produced and a method to convert the more sensitive Pyrethrins present in the pyrethrum concentrate into their respective more stable jasmolin counterparts, as a mixture with the over-reduced tetrahydropyrethrins, was developed. All other reduction processes abolished insecticidal activity against *Lucilia cuprina* larvae, whereas some isomerised analogues showed comparable potency with the individual natural pyrethrin esters. This work has revealed new insights into the structure–activity relationships in this unique class of insecticide.

Introduction

Pyrethrum, the natural extract of *Tanacetum cinerariifolium* (pyrethrum daisies), has long been utilised as an insect repellent and insecticidal agent.[1] The extract is used widely throughout the domestic and agricultural sectors for pest control and crop protection respectively, particularly where low mammalian toxicity and minimal environmental impact are paramount. The pyrethrum concentrate contains six esters, commonly known as the Pyrethrins,[†] which are the source of its repellent and insecticidal activity. These esters (Fig. 1) comprise a chrysanthemic acid (Pyrethrins I, **1a–3a**) or pyrethric acid, also known as chrysanthemic diacid (Pyrethrins II, **1b–3b**), linked with hydroxycyclopentenones, known as rethrolones.[2] The individual esters differ by the hydrocarbon chains on the rethrolone moiety where the pyrethrins (**1a** and **1b**) contain a conjugated pentadienyl substituent, the jasmolins (**2a** and **2b**) are substituted with a *cis*-pentenyl chain, and the cinerins (**3a** and **3b**) have a shorter *cis*-butenyl alkyl group.[3]

The Pyrethrins are prone to degradation through several chemical routes, bestowing the concentrate with some attractive qualities, such as its short residence time in the environment and very limited propensity to induce insect resistance, which have allowed its continued widespread use. Despite these environmentally favourable qualities, the ready decomposition by thermal, photochemical, and oxidative means can detrimentally affect the long-term storage and applicability of pyrethrum.[2,4–6] Current measures in place to limit degradation include storage in dark, cool facilities and the presence of antioxidant additives like butylated hydroxytoluene (BHT). However, these preventative measures do not entirely alleviate the loss of Pyrethrins during the production and storage of the pyrethrum extract.

Previous synthetic chemical approaches aiming to reduce the rate of degradation of this class of insecticide led to the development of the pyrethroids. These fully synthetic analogues share structural similarities with the natural Pyrethrins and act on insects through similar modes.[7] However, the pyrethroids

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[†]The informal nomenclature used in the pyrethrin field is somewhat confusing. Collectively, the six esters shown in Fig. 1 are known as Pyrethrins, three of which are known as Pyrethrins I and the other three Pyrethrins II, but there are also individual esters that are known as pyrethrins – pyrethrin I (**1a**) and pyrethrin II (**1b**). The terms that refer to groups of compounds tend to be capitalised (e.g. Pyrethrins), whereas the terms that refer to the individual pyrethrin esters are not (e.g. pyrethin I).
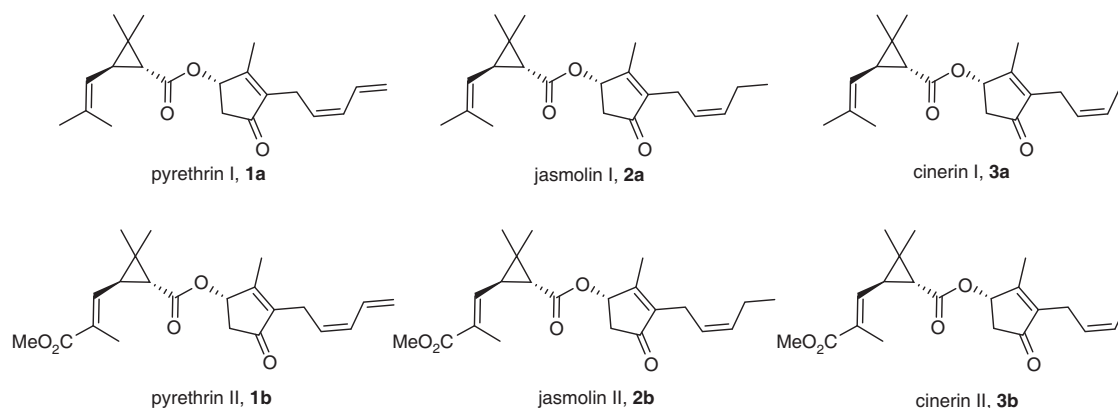
Fig. 1. The individual Pyrethrins found in the pyrethrum concentrate of *Tanacetum cinerariifolium.*

are accompanied by their own limitations such as increased environmental persistence and inducing insecticidal resistance.[8,9] The synthetic manipulation of the natural Pyrethrins still remains a fairly unexplored approach to increasing the stability of these environmentally sensitive natural products.

The pyrethrin scaffold is rich with chemical functionality (Fig. 2), which ultimately contributes to the instability and high reactivity of the pyrethrins under environmental conditions, but also provides avenues for synthetic manipulation.

Many of the functional groups within the pyrethrin structures contain unsaturated centres, which contribute to their photolytic and oxidative sensitivity.[2,5,10] Pyrethrins I (**1a**) and II (**1b**) are the predominant constituents of the concentrate, each contributing 30–40 % of the total Pyrethrin content. However, importantly, these two constituents are also more prone to the degradative processes than the minor constituents.[1,2] The jasmolins (**2a** and **2b**) and cinerins (**3a** and **3b**) exhibit greater stability to these chemical decomposition pathways but are present in much lower concentrations in the extract. As the jasmolins (**2a** and **2b**) differ from pyrethrins (**1a** and **1b**) through a single point of unsaturation, the selective transformation of the less stable pyrethrins (**1a** and **1b**) to the more stable jasmolins (**2a** and **2b**) through reductive chemistry may result in the desired stability increase while potentially maintaining many of the advantageous qualities of the original pyrethrum extract. Described herein is the exploration of several protocols for the chemo- and regioselective reduction of pyrethrins (**1a** and **1b**) and a preliminary evaluation of the insecticidal activity of the various reduction products using larvae of the Australian sheep blowfly (*Lucilia cuprina)* as a test organism. As such, the current knowledge of structure–activity relationships within this unique class of natural product has been expanded.

Experimental

General Methods

Pyrethrum concentrate (80 % Pyrethrins) was supplied by Botanical Resources Australia, reagents were purchased from Sigma–Aldrich, and solvents were purchased from Chem-Supply. THF was dried by distillation over sodium benzophenone ketyl and DCM was dried by distillation over calcium hydride. Thin-layer chromatography (TLC) was performed using Chem-Supply silica gel 60 F$_{254}$ TLC plates and visualised under UV light (254 nm). Column chromatography was undertaken using Sanpont silica gel of 230–400 mesh (0.040–0.063 mm) at atmospheric pressure unless otherwise stated.
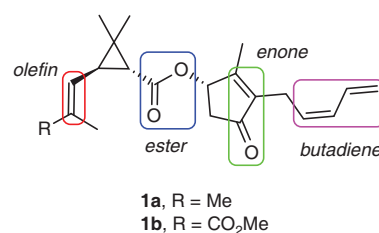

1a, R = Me
1b, R = CO$_2$Me

Fig. 2. The chemical structure of the pyrethrin scaffold highlighting the various reactive functionalities.

The pyrethrum concentrate was separated by dry column vacuum chromatography (DCVC) using silica gel of 230–400 mesh (0.040–0.063 mm) and a gradient elution from 1 to 25 % ethyl acetate in hexane to give the Pyrethrins I and Pyrethrins II subsets. The individual ester pyrethrin I (**1a**, 91 % purity) was obtained by subjecting the Pyrethrins I subset to column chromatography on a three-tiered glass column eluting in 8 % ethyl acetate in hexane and pyrethrin II (**1b**, 91 % purity) was obtained by repeated DCVC of the Pyrethrins II subset. Individual jasmolins (**2a** and **2b**) and cinerins (**3a** and **3b**) were provided by CSIRO in >98 % purity. For synthetic protocols, BHT was removed from pyrethrum concentrate by application to a short silica gel plug with hexane, then eluting the Pyrethrins off the plug with ethyl acetate.

All ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 600 MHz Avance III NMR spectrometer at 25°C using CDCl$_3$ as the solvent and internal lock. All spectra were referenced to the residual solvent peak (CDCl$_3$: ^{1}H 7.26 ppm; ^{13}C 77.0 ppm) and are recorded as follows: (1) chemical shift (ppm); (2) integration; (3) multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; dd, doublet of doublets; dt, doublet of triplets; ddd, doublet of doublet of doublets; m, multiplet; brs, broad singlet; *, multiplicity assigned based on prior literature,[11] signals overlap owing to two similar-magnitude coupling constants); (4) coupling constant (Hz). Stereochemistry was assigned through NOESY correlation.

High-performance liquid chromatography (HPLC) analysis was performed on an Agilent Technologies 1200 series system with a photodiode array detector at 223, 229, and 235 nm with a Phenomenex Phenosphere-Next C18 column (150 × 4.6 mm ID, 5 µm). Solvent A was 1 % acetic acid in water and solvent B was acetonitrile. The column was kept at 40°C with a constant flow rate of 0.8 mL min^{-1} and a 10 µL injection. The solvent program

was amended from Wang et al.[12] as follows: 50 % solvent A for 10 min followed by a linear gradient to 40 % solvent A over 5 min. Solvent A was held at 40 % for a further 10 min before a linear gradient to 35 % solvent A over 5 min; 35 % solvent A was held for 10 min after which a linear gradient to 20 % solvent A was undertaken over 5 min; 20 % solvent A was then held for an additional 5 min.

Liquid chromatography–mass spectrometry (LC-MS) analysis was conducted on a Shimadzu LCMS-2020 with a photodiode array detector at 254 nm and a single quadrupole mass spectrometer. Solvent A was 0.1 % formic acid in water and solvent B was 0.1 % formic acid in acetonitrile. The column was a Phenomenex Kinetex C18 column (100×2.1 mm ID, 2.1 µm) maintained at 30°C with a constant flow rate of 0.4 mL min^{-1} and a 1 µL injection. The solvent program was as follows: 30 % solvent A for 0.5 min before a linear gradient to 5 % solvent A over 5.5 min. Solvent A was held at 5 % for 2 min before a linear gradient to 30 % solvent A over 0.5 min. Solvent A was held at 35 % for an additional 3.5 min.

Ultraperformance convergence chromatography (UPCC) was used for chiral analysis on a Waters Acquity UPC^2 system equipped with photodiode array detection at 230 nm. The column was maintained at 40°C with a constant flow rate of 1.2 mL min^{-1}, a 1 µL injection, and convergence pressure of 13790 kPa. The solvent program was as follows: 97 % solvent A for 0.5 min followed by a linear gradient to 40 % solvent A over 2.5 min. Solvent A was held at 40 % for 3 min before a linear gradient to 97 % solvent A over 0.1 min. Solvent A was supercritical carbon dioxide (scCO$_2$) and solvent B was either ethanol/isopropanol/acetonitrile (1 : 1 : 1) with 20 mM ammonium acetate, methanol/isopropanol (1 : 1) with 0.2 % v/v formic acid, ethanol/acetonitrile (1 : 1) with 0.2 % v/v formic acid, or ethanol/isopropanol (1 : 1) with 0.2 % v/v formic acid. The column was either a Waters Trefoil AMY1 (50×2.1 mm ID, 2.5 µm), Waters Trefoil CEL1 (50×2.1 mm ID, 2.5 µm), or Waters Trefoil CEL2 (50×2.1 mm ID, 2.5 µm).

Fourier-transform infrared spectroscopy (FTIR) was recorded using a PerkinElmer Spectrum100 spectrometer with an attenuated total reflectance (ATR) diamond crystal attachment. All spectra are reported in wavenumbers (v, cm^{-1}).

High-resolution mass spectrometry (HRMS) was performed on a PerkinElmer AxION Direct Sample Analysis (DSA) with an AxION®2 time of flight (ToF) mass spectrometer using atmospheric pressure chemical ionisation (APCI) in positive ion mode.

Optical rotations were recorded on a PolAAR 21 polarimeter referenced to the sodium D line (589 nm) at 20°C.

Chemistry

Hydroboration–Protonolysis of Pyrethrins (1a and 1b) with 9-Borabicyclo[3.3.1]none. Pyrethrin I **1a** (117 mg, 0.357 mmol) was stirred in a solution of 9-borabicyclo[3.3.1]none (9-BBN) (0.65 mL, 0.5 M in THF, 0.3 mmol) under an atmosphere of nitrogen for 5 h. Acetic acid (200 µL, 3.49 mmol) was added and the resulting mixture refluxed for 1 h. Residual acid was quenched with 10 % sodium bicarbonate solution and the resulting mixture extracted with ethyl acetate. The solution was dried (Na$_2$SO$_4$) and solvent removed under vacuum, giving a pale yellow oil. The residue was purified by column chromatography (15 % ethyl acetate in hexane), yielding the allylic alcohol 4R-**4a** (18 mg, 15 %) and allylic ester **5a** (11 mg, 8 %, dr 4.0 : 1.0 (S : R)).

Pyrethrin II **1b** (79 mg, 0.212 mmol) was subjected to the above procedure using 9-BBN (0.6 mL, 0.5 M in THF,

0.3 mmol) and acetic acid (200 µL, 3.49 mmol). Column chromatography (15 % ethyl acetate in hexane) afforded the allylic alcohol 4R-**4b** (15 mg, 19 %) and allylic ester **5b** (8 mg, 9 %, dr 3.3 : 1.0 (S : R)).

Reduction of Pyrethins (1a and 1b) with Sodium Borohydride. Sodium borohydride (90 mg, 2.4 mmol) was added to a solution of pyrethrin I **1a** (310 mg, 0.95 mmol) in methanolic THF (10 % v/v, 5 mL) at 0°C. The resulting mixture was stirred for 3 h with continued cooling. Water was added and the aqueous mixture was extracted with ethyl acetate. The ethyl acetate was dried (Na$_2$SO$_4$) and the solvent removed under vacuum, giving a diastereomeric mixture of the allylic alcohol **4a** as a colourless oil (305 mg, 98 %, dr 5.5 : 1.0 (R : S)). The individual diastereomers were isolated by silica gel column chromatography (15 % ethyl acetate in hexane) giving 4R-**4a** (202 mg) and 4S-**4a** (45 mg).

(1S, 4R)-4-Hydroxy-2-methyl-3-((2Z)-penta-2,4-dien-1-yl)cyclopent-2-en-1-yl (1R,3R)-2,2-Dimethyl-3-(2-methylprop-1-enyl)cyclopropane-1-carboxylate 4R-4a

δ_H (600 MHz, CDCl$_3$) 6.75 (1H, ddd*, J 10.6, 16.9), 6.07 (1H, dd*, J 10.9), 5.42 (1H, dt, J 8.3, 9.7), 5.35 (1H, dd, J 3.4, 7.2), 5.23 (1H, d, J 16.7), 5.16 (1H, d, J 10.1), 4.90 (1H, d, J 7.7), 4.50 (1H, brs), 3.16 (1H, dd, J 8.5, 14.9), 3.05 (1H, dd, J 7.3, 14.9), 2.78 (1H, m), 2.04 (1H, m), 1.71 (3H, s), 1.70 (3H, s), 1.67 (1H, d, J 8.1), 1.57 (3H, s), 1.54 (1H, ddd*, J 3.7, 14.7), 1.39 (1H, d, J 5.3), 1.25 (3H, s), 1.12 (3H, s). δ_C (150 MHz, CDCl$_3$) 172.61, 141.65, 135.70, 134.83, 131.83, 130.55, 128.34, 121.26, 118.30, 79.34, 75.84, 40.72, 35.04, 32.63, 28.73, 25.69, 24.83, 22.32, 20.56, 18.63, 11.76. $[\alpha]_D^{20}$ 73.7 (c 0.6, CHCl$_3$).

(1S, 4S)-4-Hydroxy-2-methyl-3-((2Z)-penta-2,4-dien-1-yl)cyclopent-2-en-1-yl (1R,3R)-2,2-Dimethyl-3-(2-methylprop-1-enyl)cyclopropane-1-carboxylate 4S-4a

δ_H (600 MHz, CDCl$_3$) 6.75 (1H, ddd*, J 10.3, 16.8), 6.09 (1H, dd*, J 10.9), 5.69 (1H, d, J 6.1), 5.45 (1H, dt, J 8.0, 9.9), 5.24 (1H, d, J 16.7), 5.16 (1H, d, J 10.2), 4.88 (1H, d, J 7.7), 4.81 (1H, brs), 3.12 (1H, dd, J 8.5, 14.9), 3.03 (1H, dd*, J 7.3, 14.9), 2.15 (1H, ddd, J 3.2, 7.0, 14.9), 2.06 (1H, ddd, J 3.1, 7.0, 14.9), 2.03 (1H, m), 1.70 (3H, s), 1.69 (6H, s), 1.49 (1H, d, J 6.4), 1.36 (1H, d, J 5.3), 1.24 (3H, s), 1.11 (3H, s). δ_C (150 MHz, CDCl$_3$) 172.80, 142.08, 135.67, 135.32, 131.79, 130.68, 128.24, 121.24, 118.33, 81.11, 76.85, 41.23, 35.02, 32.60, 28.67, 25.67, 24.89, 22.30, 20.53, 18.60, 11.84. $[\alpha]_D^{20}$ 286.7 (c 0.5, CHCl$_3$). v_{max}/cm^{-1} 3424, 2921, 1719, 1421, 1378, 1282, 1195, 1158, 1081, 1023, 904, 852. m/z (HRMS) 313.2162; calc. for C$_{21}$H$_{29}$O$_2$ [M – OH]$^+$ 313.2162.

Pyrethrin II **1b** (330 mg, 0.89 mmol) was subjected to the same procedure using sodium borohydride (86 mg, 2.3 mmol). The diastereomeric mixture of the allylic alcohol **4b** (330 mg, 99 %, dr 4.5 : 1.0 (R : S)) was resolved by silica gel column chromatography (30 % ethyl acetate in hexane) giving 4R-**4b** (163 mg) and 4S-**4b** (40 mg).

(1S,4R)-4-Hydroxy-2-methyl-3-((2Z)-penta-2,4-dien-1-yl)cyclopent-2-en-1-yl (1R,3R)-3-((E)-3-Methoxy-2-methyl-3-oxoprop-1-enyl)-2,2-dimethylcyclopropane-1-carboxylate 4R-4b

δ_H (600 MHz, CDCl$_3$) 6.75 (1H, ddd*, J 10.7, 16.8), 6.46 (1H, d, J 9.7), 6.08 (1H, dd*, J 10.9), 5.42 (1H, dt, J 8.2, 9.7), 5.36 (1H, dd, J 3.1, 6.9), 5.54 (1H, d, J 16.8), 5.17 (1H, d, J 10.2), 4.51 (1H, brs), 3.73 (3H, s), 3.16 (1H, dd, J 8.6, 14.9), 3.05 (1H, dd, J 7.2, 14.9), 2.80 (1H, m), 2.20 (1H, dd, J 5.2, 9.6), 1.93

(3H, s), 1.72 (1H, d, J 5.2), 1.70 (3H, s), 1.69 (1H, d, J 7.4), 1.57 (3H, s), 1.54 (1H, ddd*, J 3.5, 14.7), 1.30 (3H, s), 1.22 (3H, s). δ_C (150 MHz, CDCl$_3$) 171.47, 168.36, 141.96, 139.64, 134.54, 131.79, 130.64, 129.59, 128.20, 118.41, 79.87, 75.77, 51.93, 40.65, 36.35, 32.70, 24.84, 22.79, 22.55, 20.59, 13.00, 11.75. $[\alpha]_D^{20}$ 117.3 (c 0.5, CHCl$_3$).

(1S,4S)-4-Hydroxy-2-methyl-3-((2Z)-penta-2,4-dien-1-yl)cyclopent-2-en-1-yl (1R,3R)-3-((E)-3-Methoxy-2-methyl-3-oxoprop-1-enyl)-2,2-dimethylcyclopropane-1-carboxylate 4S-*4b*

δ_H (600 MHz, CDCl$_3$) 6.75 (1H, ddd*, J 10.8, 16.8), 6.45 (1H, d, J 9.7), 6.10 (1H, dd*, J 10.9), 5.70 (1H, d, J 6.3), 5.45 (1H, dt, J 8.2, 9.6), 5.26 (1H, d, J 16.7), 5.17 (1H, d, J 10.2), 4.82 (1H, brs), 3.72 (3H, s), 3.12 (1H, dd, J 8.7, 14.9), 3.04 (1H, dd, J 7.1, 14.9), 2.17 (2H, m), 2.06 (1H, ddd, J 3.1, 7.1, 14.8), 1.93 (3H, s), 1.70 (4H, m), 1.54 (1H, d, J 6.7), 1.29 (3H, s), 1.21 (3H, s). δ_C (150 MHz, CDCl$_3$) 171.63, 168.36, 142.43, 139.65, 135.07, 131.74, 130.82, 129.58, 128.09, 118.48, 81.63, 76.86, 51.92, 41.20, 36.35, 32.69, 30.24, 24.91, 22.55, 20.57, 12.99, 11.85. $[\alpha]_D^{20}$ −177.5 (c 0.4, CHCl$_3$). ν_{max}/cm^{-1} 3489, 2924, 1713, 1642, 1434, 1261, 1222, 1176, 111, 1010, 940, 905, 831, 762. m/z (HRMS) 357.2077; calc. for C$_{22}$H$_{29}$O$_4$ [M − OH]$^+$: 357.2060.

General Procedure for the Acylation of Allylic Alcohols 4. Acetic anhydride (1 mL, 11 mmol) and triethylamine (1 mL, 7 mmol) were added to a solution of the allylic alcohol **4** in dry DCM (5 mL) under a nitrogen atmosphere. The mixture was heated under reflux for 4.5 h. The resulting solution was allowed to cool to room temperature and washed with water. The organic layer was collected and dried (Na$_2$SO$_4$). The solvent was removed under vacuum, yielding the allyl ester **5**.

(1S,4R)-4-Acetoxy-2-methyl-3-((2Z)-penta-2,4-dien-1-yl)cyclopent-2-en-1-yl (1R,3R)-2,2-Dimethyl-3-(2-methylprop-1-enyl)cyclopropane-1-carboxylate 4R-*5a*

Yield: 110 mg, 85 %. δ_H (600 MHz, CDCl$_3$) 6.64 (1H, ddd*, J 10.2, 16.8), 6.05 (1H, dd*, J 10.8), 5.48 (1H, m), 5.44 (1H, dd, J 3.5, 7.4), 5.35 (1H, dt, J 7.9, 10.6), 5.23 (1H, d, J 16.8), 5.14 (1H, d, J 10.2), 4.90 (1H, d, J 7.8), 3.04 (2H, m), 2.92 (1H, m), 2.05 (1H, m), 2.04 (3H, s), 1.74 (3H, s), 1.71 (3H, s), 1.70 (3H, s), 1.54 (1H, ddd*, J 3.7, 15.1), 1.41 (1H, d, J 5.3), 1.25 (3H, s), 1.13 (3H, s). δ_C (150 MHz, CDCl$_3$) 172.47, 170.88, 137.78, 137.55, 135.73, 131.64, 130.56, 127.77, 121.23, 118.27, 79.05, 77.68, 38.20, 34.93, 32.72, 28.79, 25.70, 24.99, 22.32, 21.33, 20.58, 18.62, 11.92. $[\alpha]_D^{20}$ −12.5 (c 0.4, CHCl$_3$).

(1S,4S)-4-Acetoxy-2-methyl-3-((2Z)-penta-2,4-dien-1-yl)cyclopent-2-en-1-yl (1R,3R)-2,2-Dimethyl-3-(2-methylprop-1-enyl)cyclopropane-1-carboxylate 4S-*5a*

Yield: 68 mg, 56 %. δ_H (600 MHz, CDCl$_3$) 6.65 (1H, ddd*, J 10.6, 16.9), 6.06 (1H, dd*, J 10.9), 5.72 (1H, d, J 6.9), 5.68 (1H, brs), 5.37 (1H, dt, J 8.2, 10.3), 5.22 (1H, d, J 16.8), 5.14 (1H, d, J 10.3), 4.88 (1H, d, J 7.8), 3.01 (2H, m), 2.20 (1H, ddd, J 3.7, 15.2), 2.12 (1H, ddd, J 3.4, 7, 15.2), 2.04 (1H, m), 2.01 (3H, s), 1.73 (3H, s), 1.71 (3H, s), 1.70 (3H, s), 1.38 (1H, d, J 5.3), 1.24 (3H, s), 1.12 (3H, s). δ_C (150 MHz, CDCl$_3$) 172.68, 171.11, 138.39, 138.09, 135.72, 131.66, 130.63, 127.61, 121.22, 118.22, 80.63, 79.39, 38.59, 34.95, 32.66, 28.83, 25.69, 25.06, 22.29, 21.32, 20.52, 18.62, 11.89. $[\alpha]_D^{20}$ 183.3 (c 0.6, CHCl$_3$). ν_{max}/cm^{-1} 2924, 1736, 1720, 1433, 1377, 1236, 1194, 1158, 1115, 1022, 997, 906. m/z (HRMS) 373.2382; calc. for C$_{23}$H$_{32}$O$_4$ [M + H]$^+$: 373.2373.

(1S,4R)-4-Acetoxy-2-methyl-3-((2Z)-penta-2,4-dien-1-yl)cyclopent-2-en-1-yl (1R,3R)-3-((E)-3-methoxy-2-methyl-3-oxoprop-1-enyl)-2,2-dimethylcyclopropane-1-carboxylate 4R-*5b*

Yield: 158 mg, 91 %. δ_H (600 MHz, CDCl$_3$) 6.64 (1H, ddd*, J 10.6, 16.8), 6.45 (1H, d, J 9.7), 6.05 (1H, dd*, J 10.8), 5.49 (1H, m), 5.44 (1H, m), 5.33 (1H, dt, J 7.9, 10.7), 5.23 (1H, d, J 16.8), 5.14 (1H, d, J 10.1), 3.72 (3H, s), 3.04 (2H, m), 2.93 (1H, m), 2.19 (1H, dd, J 5.3, 9.7), 2.04 (3H, s), 1.93 (3H, s), 1.73 (4H, m), 1.53 (1H, ddd*, J 3.8, 15.1), 1.29 (3H, s), 1.21 (3H, s). δ_C (150 MHz, CDCl$_3$) 171.30, 170.84, 168.32, 139.55, 138.13, 137.19, 131.58, 130.62, 129.62, 127.60, 118.34, 79.57, 77.58, 51.92, 38.17, 36.21, 32.73, 30.30, 24.98, 22.53, 21.30, 20.58, 12.98, 11.89. $[\alpha]_D^{20}$ 25.0 (c 0.4, CHCl$_3$).

(1S,4S)-4-Acetoxy-2-methyl-3-((2Z)-penta-2,4-dien-1-yl)cyclopent-2-en-1-yl (1R,3R)-3-((E)-3-Methoxy-2-methyl-3-oxoprop-1-enyl)-2,2-dimethylcyclopropane-1-carboxylate 4S-*5b*

Yield: 44 mg, 46 %. δ_H (600 MHz, CDCl$_3$) 6.65 (1H, ddd*, J 10.3, 16.8), 6.45 (1H, d, J 9.7), 6.07 (1H, dd*, J 10.9), 5.72 (1H, m), 5.67 (1H, m), 5.37 (1H, dt, J 7.9, 10.1), 5.23 (1H, d, J 16.8), 5.14 (1H, d, J 10.1), 3.72 (3H, s), 3.02 (2H, m), 2.19 (2H, m), 2.14 (1H, ddd, J 3.4, 7.1, 15.4), 2.02 (3H, s), 1.93 (3H, s), 1.72 (3H, s), 1.70 (1H, d, J 5.2), 1.29 (3H, s), 1.22 (3H, s). δ_C (150 MHz, CDCl$_3$) 171.55, 171.11, 168.34, 139.57, 138.49, 137.99, 131.61, 130.72, 129.61, 127.47, 118.30, 81.71, 79.29, 51.92, 38.57, 36.25, 32.72, 30.36, 25.04, 22.52, 21.31, 20.54, 12.99, 11.89. $[\alpha]_D^{20}$ −70.0 (c 0.4, CHCl$_3$). ν_{max}/cm^{-1} 3668, 2970, 2924, 1715, 1643, 1434, 1381, 1259, 1222, 1174, 1150, 1111, 1050, 904, 804. m/z (HRMS) 357.2062; calc. for C$_{22}$H$_{28}$O$_4$ [M − OAc]$^+$: 357.2062.

Stereoselective Reduction of Pyrethrins (1a and 1b) with L-Selectride. L-Selectride (1.0 M in THF, 0.5 mL, 0.5 mmol) was added to a solution of pyrethrin I **1a** (160 mg, 0.49 mmol) in dry THF 5 mL at 0 °C under an atmosphere of nitrogen. The mixture was stirred with continued cooling for an additional 1 h. The reaction was quenched with water and extracted with ethyl acetate. The ethyl acetate was dried (Na$_2$SO$_4$) and the solvent removed under vacuum, giving a yellow oil. The oil was subjected to column chromatography (15 % ethyl acetate in hexane), giving allylic alcohol 4R-*4a* (62 mg, 38 %).

Pyrethrin II **1b** (150 mg, 0.3 mmol) was subjected to the same procedure detailed above. The resulting oil was subjected to column chromatography (30 % ethyl acetate in hexane), giving allylic alcohol 4R-*4b* (30 mg, 20 %).

Catalytic Hydrogenation of the Pyrethrins (1a and 1b). Pyrethrin I **1a** (100 mg, 0.305 mmol) in THF (2 mL) was stirred in the presence of palladium on carbon (10 wt-% loading, 10 mg) under an atmosphere of hydrogen at room temperature. After 4 h, the mixture was filtered over Celite and the solvent removed under vacuum, yielding **7a** as a colourless oil (83 mg, 83 %).

(1S)-2-Methyl-4-oxo-3-pentylcyclopent-2-en-1-yl (1R,3R)-2,2-Dimethyl-3-(2-methylprop-1-enyl)cyclopropane-1-carboxylate 7a

δ_H (600 MHz, CDCl$_3$) 5.65 (1H, d, J 6.2), 4.89 (1H, d, J 7.7), 2.83 (1H, dd, J 6.3, 18.7), 2.21 (1H, dd, J 1.7, 18.7), 2.18 (2H, m), 2.08 (1H, m), 2.01 (3H, s), 1.71 (3H, s), 1.69 (3H, s), 1.40–1.27 (7H, m), 1.26 (3H, s), 1.13 (3H, s), 0.88 (3H, t, J 7.3). δ_C (150 MHz, CDCl$_3$) 204.73, 172.50, 164.67, 144.37, 136.02, 120.97, 73.09, 42.27, 34.72, 33.06, 31.91, 29.21, 27.89, 25.69,

23.17, 22.57, 22.25, 20.54, 18.63, 14.11 (two overlapping signals). v_{max}/cm^{-1} 3675, 2971, 2922, 1713, 1655, 1420, 1380, 1282, 1235, 1192, 1151, 1114, 1065, 995, 964, 906, 849. m/z (HRMS) 333.2439; calc. for $C_{21}H_{33}O_3$ $[M+H]^+$: 333.2424. $[\alpha]_D^{20}$ −27.4 (c 0.8, $CHCl_3$).

Pyrethrin II **1b** (136 mg, 0.366 mmol) was subjected to the procedure detailed above using palladium on carbon (1 wt-% loading, 12 mg). Tetrahydropyrethrin **7b** was obtained as a colourless oil (133 mg, 97 %).

(1S)-2-Methyl-4-oxo-3-pentylcyclopent-2-en-1-yl (1R,3R)-3-((E)-3-Methoxy-2-methyl-3-oxoprop-1-en-1-yl)-2,2-dimethylcyclopropane-1-carboxylate 7b

δ_H (600 MHz, $CDCl_3$) 6.45 (1H, d, J 9.6), 5.64 (1H, d, J 5.9), 3.72 (3H, s), 2.84 (1H, dd, J 6.3, 18.6), 2.22 (4H, m), 1.99 (3H, s), 1.84 (3H, s), 1.74 (1H, d, J 5.2), 1.38 (2H, m), 1.30 (3H, s), 1.27 (4H, m), 1.23 (3H, s), 0.87 (3H, t, J 6.9). δ_C (150 MHz, $CDCl_3$) 204.45, 171.41, 168.27, 164.22, 144.63, 139.18, 129.89, 73.59, 51.96, 42.20, 35.96, 33.02, 31.90, 30.46, 27.88, 23.18, 22.57, 22.47, 20.55, 14.11, 13.01. v_{max}/cm^{-1} 3675, 2954, 2928, 2872, 1710, 1649, 1435, 1385, 1260, 1221, 1173, 1148, 1055, 993, 831. m/z (HRMS) 377.2333; calc. for $C_{22}H_{31}O_5$ $[M+H]^+$: 377.2323. $[\alpha]_D^{20}$ 15.2 (c 1.3, $CHCl_3$).

Isomerism of Pyrethrins (1a and 1b) Under Catalytic Transfer Hydrogenation Conditions. Pyrethrin I **1a** (110 mg, 0.335 mmol) was heated under reflux in dry THF (2 mL) in the presence of palladium on carbon (10 wt-% loading, 15 mg) and formic acid (200 μL, 5.30 mmol) for 5 h under nitrogen. The mixture was allowed to cool, filtered over Celite, and the solvent removed under vacuum, yielding a pale yellow oil. The resulting mixture was purified by column chromatography (10 % ethyl acetate in hexane) giving *trans*-pyrethrin **8a** (10 mg, 9 %) and iso-pyrethrin **9a** as a mixture of diastereomers (17 mg, 15 %).

(1S)-2-Methyl-4-oxo-3-((E)-penta-2,4-dien-1-yl)cyclopent-2-en-1-yl (1R,3R)-2,2-Dimethyl-3-(2-methylprop-1-enyl)cyclopropane-1-carboxylate 8a

δ_H (600 MHz, $CDCl_3$) 6.27 (1H, ddd*, J 10.3, 16.9), 6.05 (1H, dd, J 10.3, 15.2), 5.67 (1H, d, J 6.1), 5.64 (1H, dt, J 6.8, 15.2), 5.12 (1H, d, J 17.0), 5.00 (1H, d, J 10.1), 4.90 (1H, d, J 7.7), 3.00 (2H, d, J 6.8), 2.87 (1H, dd, J 6.3, 18.7), 2.24 (1H, dd, J 1.32, 18.7), 2.08 (1H, m), 2.03 (3H, s), 1.72 (3H, s), 1.71 (3H, s), 1.40 (1H, d, J 5.3), 1.26 (3H, s), 1.14 (3H, s). δ_C (150 MHz, $CDCl_3$) 203.99, 172.46, 165.87, 141.67, 136.75, 136.08, 132.45, 129.64, 120.94, 116.22, 73.07, 42.19, 34.70, 33.14, 29.29, 26.18, 25.70, 22.25, 20.55, 18.64, 14.22. v_{max}/cm^{-1} 3675, 2972, 2924, 1715, 1655, 1420, 1380, 1282, 1235, 1193, 1152, 1114, 1065, 1003, 963, 901, 849. m/z (HRMS) 329.2122; calc. for $C_{21}H_{29}O_3$ $[M+H]^+$: 329.2111. $[\alpha]_D^{20}$ −44.7 (c 0.8, $CHCl_3$).

(S)-2-Methyl-4-oxo-3-(penta-1,3-dien-1-yl)cyclopent-2-en-1-yl (1R,3R)-2,2-Dimethyl-3-(2-methylprop-1-enyl)cyclopropane-1-carboxylate 9a

δ_H (600 MHz, $CDCl_3$) 7.64 (0.37H, dd, J 11.4, 15.5), 7.29 (0.63H, dd, J 10.7, 15.6), 6.09 (2H, m), 5.92 (0.63H, m), 5.67 (1.4H, m), 4.90 (1H, d, J 7.6), 2.90 (1H, m), 2.28 (1H, m), 2.09 (3H, s), 1.85 (2H, d, J 7.1), 1.80 (3H, d, J 6.7), 1.72 (3H, s), 1.71 (3H, s), 1.41 (2H, m), 1.26 (3H, s), 1.13 (3H, s) (some diastereomeric signals overlap). δ_C (150 MHz, $CDCl_3$) 203.42, 172.50, 163.92, 163.38, 137.94, 136.21, 136.05, 133.31, 132.60,

130.97, 130.36, 130.17, 120.96, 119.68, 117.74, 72.71, 42.95, 34.75, 33.14, 30.47, 29.28, 25.70, 22.25, 20.58, 18.65, 14.36, 14.06 (some diastereomeric signals overlap). v_{max}/cm^{-1} 2955, 2926, 1716, 1431, 1379, 1282, 1193, 1152, 1114, 994, 860. m/z (HRMS) 329.2122; calc. for $C_{21}H_{29}O_3$ $[M+H]^+$: 329.2111. $[\alpha]_D^{20}$ −179.2 (c 0.5, $CHCl_3$).

Pyrethrin II **1b** (108 mg, 0.290 mmol) was treated following the above procedure using palladium on carbon (10 wt-% loading, 12 mg) and formic acid (200 μL, 5.30 mmol). The isolated yellow oil was purified by column chromatography (20 % ethyl acetate in hexane) giving **8b** (15 mg, 14 %) and **9b** as a mixture of diastereomers (20 mg, 19 %).

(1S)-2-Methyl-4-oxo-3-((E)-penta-2,4-dien-1-yl)cyclopent-2-en-1-yl (1R,3R)-3-((E)-3-Methoxy-2-methyl-3-oxoprop-1-en-1-yl)-2,2-dimethylcyclopropane-1-carboxylate 8b

δ_H (600 MHz, $CDCl_3$) 6.46 (1H, d, J 9.6), 6.26 (1H, ddd*, J 10.3, 16.9), 6.05 (1H, dd, J 10.5, 15.1), 5.67 (1H, d, J 6.1), 5.64 (1H, dt, J 6.8, 15.2), 5.11 (1H, d, J 17.0), 4.99 (1H, d, J 10.3), 3.72 (3H, s), 3.01 (2H, d, J 6.8), 2.88 (1H, dd, J 6.2, 18.7), 2.23 (2H, m), 2.03 (3H, s), 1.94 (3H, s), 1.74 (1H, d, J 5.1), 1.30 (3H, s), 1.23 (3H, s). δ_C (150 MHz, $CDCl_3$) 203.71, 171.35, 168.25, 165.40, 141.92, 139.10, 136.69, 132.52, 129.92, 129.48, 116.30, 73.54, 51.96, 42.10, 35.91, 33.05, 30.71, 26.17, 22.46, 20.55, 14.20, 13.02. v_{max}/cm^{-1} 3662, 2952, 1709, 1649, 1435, 1383, 1340, 1260, 1221, 1173, 1147, 1111, 1055, 996, 904, 830. m/z (HRMS) 373.2022; calc. for $C_{22}H_{29}O_5$ $[M+H]^+$: 373.2010. $[\alpha]_D^{20}$ 17.8 (c 0.5, $CHCl_3$).

(S)-2-Methyl-4-oxo-3-(penta-1,3-dien-1-yl)cyclopent-2-en-1-yl (1R,3R)-3-((E)-3-Methoxy-2-methyl-3-oxoprop-1-en-1-yl)-2,2-dimethylcyclopropane-1-carboxylate 9b

δ_H (600 MHz, $CDCl_3$) 7.71 (0.33H, dd, J 11.5, 15.4), 7.35 (0.66H, dd, J 10.7, 15.6), 6.46 (1H, d, J 9.6), 6.09 (2H, m), 5.92 (0.66H, m), 5.66 (1.66H, m), 3.72 (3H, s), 2.90 (1H, m), 2.25 (2H, m), 2.08 (3H, s), 1.94 (3H, s), 1.84 (1H, d, J 7.2), 1.80 (2H, d, J 6.7), 1.74 (1H, m), 1.30 (3H, s), 1.24 (3H, s) (some diastereomeric signals overlap). δ_C (150 MHz, $CDCl_3$) 203.12, 171.37, 168.24, 163.34, 162.80, 139.13, 138.08, 136.37, 133.45, 132.54, 131.11, 130.30, 129.88, 119.55, 117.61, 73.17, 51.94, 42.85, 35.95, 33.02, 30.66, 22.45, 20.56, 18.60, 14.32, 14.04, 13.00 (some diastereomeric signals overlap). v_{max}/cm^{-1} 2952, 2928, 1710, 1643, 1435, 1385, 1260, 1221, 1173, 1147, 1111, 993, 830. m/z (HRMS) 373.2010; calc. for $C_{22}H_{29}O_5$ $[M+H]^+$: 373.2010. $[\alpha]_D^{20}$ −56.9 (c 1.0, $CHCl_3$).

Reduction of Pyrethrins (1a and 1b) by Diimide-Mediated Transfer Hydrogenation. Pyrethrin I **1a** (110 mg, 0.335 mmol) in THF (5 mL) was stirred vigorously open to air in the presence of hydrazine monohydrate (800 mg, 15.98 mmol), copper(II) sulfate pentahydrate (8.5 mg, 0.034 mmol, 10 mol-%), and acetic acid (20 mg, 0.33 mmol). The reaction was monitored by TLC (8 % ethyl acetate in hexane) until all of the pyrethrin starting material was consumed (typically 7–8 h). The reaction mixture was filtered and subsequently diluted with brine water. The resulting solution was extracted with ethyl acetate and dried (Na_2SO_4). Solvent was removed under vacuum, yielding a colourless oil (85 mg, 77 % mass recovery), which was determined by HPLC to be 68 % jasmolin I **2a** and 25 % tetrahydropyrethrin I **7a**. Characterisation was undertaken on the isolated mixture with signals assigned to jasmolin I by comparison with the natural ester.

Jasmolin I **2a**

δ_H (600 MHz, CDCl$_3$) 5.65 (1H, d, *J* 6.2), 5.42 (1H, dt, *J* 7.3, 17.8), 5.24 (1H, dt, *J* 7.3, 17.8), 4.90 (1H, d, *J* 7.7), 2.98 (2H, d, *J* 7.3), 2.85 (1H, dd, *J* 6.4, 18.7), 2.22 (1H, d, *J* 18.7), 2.15 (2H, m), 2.08 (1H, m), 2.03 (3H, s), 1.73 (3H, s), 1.71 (3H, s), 1.41 (1H, m) 1.26 (3H, s), 1.14 (3H, s), 0.99 (3H, t, *J* 7.5). δ_C (150 MHz, CDCl$_3$) 204.12, 172.47, 164.95, 142.89, 136.04, 133.30, 124.05, 120.95, 73.13, 42.21, 34.71, 33.08, 29.23, 25.69, 22.23, 21.35, 20.73, 20.54, 18.63, 14.22, 14.17. ν_{max}/cm^{-1} 3675, 2965, 2928, 1714, 1655, 1447, 1420, 1380, 1282, 1235, 1193, 1152, 1114, 1046, 995, 850. *m/z* (HRMS) 331.2256; calc. for C$_{21}$H$_{31}$O$_3$ [M + H]$^+$: 331.2268. Isolated mixture $[\alpha]_D^{20}$ −32.2 (*c* 0.9, CHCl$_3$) (natural jasmolin I $[\alpha]_D^{20}$ −56.0 (*c* 0.5, CHCl$_3$)).

Pyrethrin II **1b** (145 mg, 0.390 mmol) in THF (5 mL) was subjected to the same procedure as above using hydrazine monohydrate (270 mg, 5.4 mmol), copper(II) sulfate pentahydrate (11 mg, 0.044 mmol, 10 mol-%), and acetic acid (10 mg, 0.17 mmol). The reaction was monitored by TLC (25 % ethyl acetate in hexane) until all of the pyrethrin starting material was consumed (typically 7–8 h). A colourless oil was obtained (120 mg, 83 % mass recovery), which was determined by HPLC to be 66 % jasmolin II **2a** and 26 % tetrahydropyrethrin II **7b**. Characterisation was undertaken on the isolated mixture with signals assigned to jasmolin II by comparison with the natural ester.

Jasmolin II **2b**

δ_H (600 MHz, CDCl$_3$) 6.45 (1H, d, *J* 9.6), 5.64 (1H, d, *J* 5.4), 5.42 (1H, dt, *J* 7.26, 10.6), 5.23 (1H, dt, *J* 7.3, 10.6), 3.73 (3H, s), 2.97 (2H, d, *J* 7.2), 2.86 (1H, dd, *J* 6.4, 18.7), 2.23 (2H, m), 2.16 (2H, quin, *J* 7.4), 2.03 (3H, s), 1.94 (3H, s), 1.74 (1H, d, *J* 5.2), 1.30 (3H, s), 1.23 (3H, s), 0.99 (3H, t, *J* 7.5). δ_C (150 MHz, CDCl$_3$) 203.87, 171.39, 168.28, 164.51, 143.17, 139.19, 133.42, 129.91, 123.94, 73.63, 51.97, 42.20, 35.95, 33.03, 30.69, 22.47, 21.37, 20.75, 20.56, 14.23, 14.17, 13.02. ν_{max}/cm^{-1} 3675, 2956, 1712, 1648, 1435, 1383, 1324, 1261, 1221, 1174, 1148, 1111, 1056, 995, 830, 762. *m/z* (HRMS) 375.2162; calc. for C$_{22}$H$_{31}$O$_5$ [M + H]$^+$: 375.2166. Isolated mixture $[\alpha]_D^{20}$ 10.3 (*c* 1.1, CHCl$_3$) (natural jasmolin II $[\alpha]_D^{20}$ 8.0 (*c* 0.5, CHCl$_3$)).

Reduction of Pyrethrum Concentrate by Diimide-Mediated Transfer Hydrogenation. Pyrethrum concentrate (HPLC analysis: 43 % pyrethrin I **1a**, 36 % pyrethrin II **1b**, 4 % jasmolin I **2a**, 4 % jasmolin II **2b**) (500 mg, ~1.36 mmol) in THF (5 mL) was stirred vigorously open to air in the presence of hydrazine monohydrate (517 mg, 10.3 mmol), copper(II) sulfate (19 mg, 0.12 mmol, 10 mol-%), and acetic acid (10 mg, 0.17 mmol). The reaction was stirred at room temperature for 8 h before being filtered and diluted with brine. The resulting solution was extracted with ethyl acetate and dried (Na$_2$SO$_4$). Solvent was removed under vacuum, yielding a pale yellow oil (HPLC analysis: 3 % pyrethrin I **1a**, 2 % pyrethrin II **1b**, 29 % jasmolin I **2a**, 36 % jasmolin II **2b**, 7 % tetrahydropyrethrin I **7a**, 8 % tetrahydropyrethrin II **7b**) (400 mg, 80 % mass recovery).

Biology

Australian sheep blowfly (*Lucilia cuprina*) larvae were prepared and used in insecticidal activity assays as detailed in published procedures.[13,14] Specifically, a plug of cotton wool (~0.2 g) on top of three layers of filter paper (within a 70-mL plastic pot) was loaded with 4 mL of a solution of the compound in ethanol, and the solvent allowed to evaporate. Controls were prepared in the same manner, by loading the cotton wool plug with ethanol or a solution of BHT. On Day 0 of the assay, a sheep serum-based medium was added to the cotton wool and 50 freshly hatched *L. cuprina* larvae were added. The plastic pots containing the larvae were incubated at 28°C over a period of 4 days. The larvae were fed with 1 mL of nutrient medium on Day 1, and 2 mL on Days 2 and 3. Late on Day 4, the larvae were transferred to larger pots with a layer of sand (a medium for pupation) and allowed to incubate further. On Day 9, the resulting pupae were collected by sieving the sand and the bioactivity calculated by pupation rate: the number of collected pupae in assays with experimental compounds was expressed as a percentage of the average number of pupae in the control assays. All tested materials had a purity >90 % and were prepared as solutions in hexane, stabilised with 5 wt-% BHT and subsequently diluted for testing in ethanol. BHT controls exhibited no inhibition of pupation on *L. cuprina* larvae at concentrations up to 5 μmol per assay, 10 times the amount present in the individual samples. Each assay was performed in duplicate.

The pupation rate dose–response data were analysed with *GraphPad Prism®* software. Non-linear regression was used with the 'variable slope' option to determine the concentration of the test compound that gives 50 % of the normal pupation rate (IC$_{50}$), together with 95 % confidence intervals.

Results and Discussion

Borane and Borohydride Reducing Agents

Regioselective reduction of the terminal olefin of the penta-dienyl unit in pyrethrins **1a** and **1b** was a challenge that was thought could potentially be overcome by application of sterically hindered reagents that should only access the least hindered alkene. Hydroboration with 9-BBN, and subsequent protonolysis with acetic acid, were chosen owing to the steric encumbrance imposed by the 9-BBN and its propensity to undergo monohydroboration with conjugated dienes.[15] Rather than obtaining the desired reactivity of the terminal olefin, however, the carbonyl of the enone was reduced (Scheme 1), with the isolation of both the allyl alcohols (**4a** and **4b**) and allyl esters (**5a** and **5b**) via column chromatography.

The allyl alcohols **4a** and **4b** were produced in 15–19 % yield as the (4*R*)-stereoisomers, as determined by NOE correlation, whereas the allyl esters **5a** and **5b** were isolated as diastereomeric mixtures in yields of 8–9 %. This hydroboration–protonolysis procedure was quite inefficient, with small amounts of the intermediate organoborane and the starting ketone also being isolated. The allylic esters **5a** and **5b** were likely formed through esterification of the allyl alcohols **4a** and **4b** with the excess acetic acid under the forcing conditions employed for protonolysis. This esterification reaction appears to proceed more readily for the 4*S*-isomers, with the 4*S*-acetates dominating over the 4*R*-acetates, and no 4*S*-alcohol being observed. The propensity to reduce the ketone functionality observed here is consistent with other reported outcomes with 9-BBN, which has previously been used for carbonyl reduction in ketones, aldehydes, and their α,β-unsaturated counterparts, the latter selectively leading to allylic alcohols in the presence of other reducible functionalities.[16,17]

Given the observed reactivity of 9-BBN on the enone unit, sodium borohydride was employed in an attempt to optimise the yield of the allyl alcohols **4a** and **4b** and in so doing, provide sufficient quantities for bioactivity testing and ultimately allow the further exploration of structure–activity relationships. It was
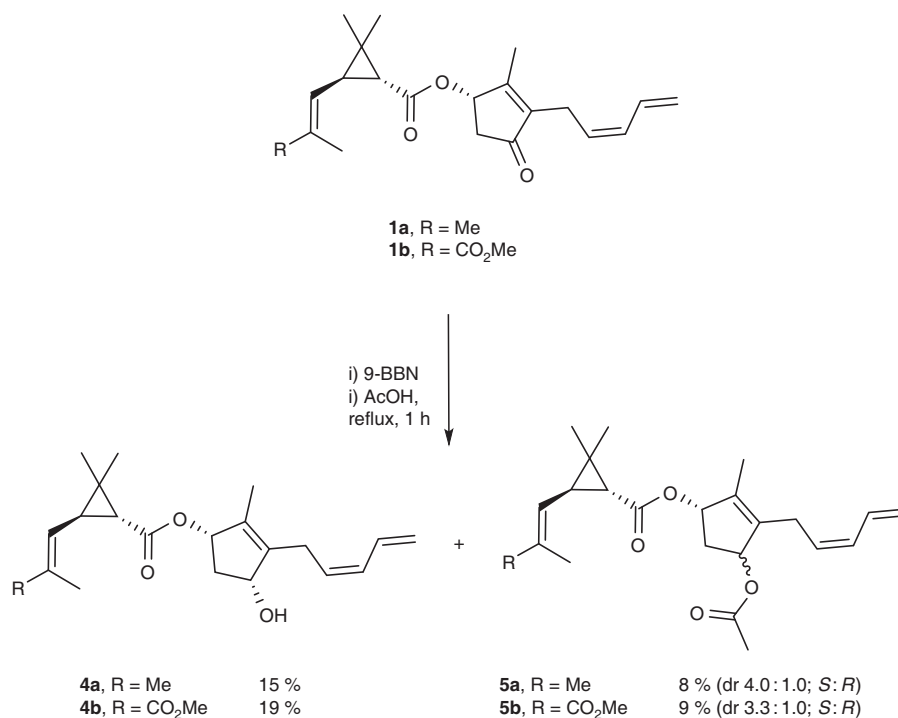
Scheme 1. Generation of allylic alcohols (**4a** and **4b**) and allylic esters (**5a** and **5b**) through hydroboration–protonolysis of pyrethrins (**1a** and **1b**) with 9-BBN/acetic acid.

found that subjecting pyrethrins **1a** and **1b** to reduction with sodium borohydride in a 1:10 methanol/THF solvent blend resulted in near-quantitative conversion to the allyl alcohols **4a** and **4b** as a mixture of diastereomers (Scheme 2).

The competing 1,4-enone reduction was not observed and its occurrence is likely limited by the hindrance enforced by both the methyl substituent on the cyclopentenone and the reactive sodium borohydride–methanol adduct employed.[18,19] Column chromatography of the diastereomeric mixture gave the individual diastereomers, which were in turn subjected to acetylation in acetic anhydride/triethylamine to give the individual diastereomers of the allyl esters **5a** and **5b** (Scheme 2), allowing investigation into the potential role of stereochemistry at the new stereocentre on insecticidal action. Single diastereomers of the allyl alcohols (4*R*-**4a** and 4*R*-**4b**) could be furnished from the pyrethrins **1a** and **1b** through reduction with the stereoselective borohydride L-selectride, albeit in low to moderate yields of 20 and 38 %, depending on the substituent on the chrysanthemic acid moiety of the starting pyrethrin.

Although the boron reagents did not selectively produce the jasmolins (**2a** and **2b**) from pyrethrins (**1a** and **1b**), the resultant allylic alcohols (**4a** and **4b**) and esters (**5a** and **5b**) were nonetheless subjected to preliminary insecticidal activity assays (see below).

Hydrogenation

Previous investigations into the reduction of pyrethrins (**1a** and **1b**) employed catalytic hydrogenation, leading to the tetrahydropyrethrins (**7a** and **7b**). It was reported that such analogues had significantly lower bioactivity than the natural Pyrethrins (**1–3**).[20–22] Attempts to regulate these hydrogenation procedures towards selective reduction of pyrethrins (**1a** and **1b**) to their jasmolin counterparts (**2a** and **2b**) has not previously been described.

In an effort to achieve selectivity in the reductive process, experiments with several catalyst loadings, ranging from 1 to 10 wt-% palladium on carbon, employing short reactions times and atmospheric pressure, were undertaken. Unfortunately, all of the conditions employed yielded the tetrahydropyrethrins (**7a** and **7b**; Scheme 3) exclusively. Optimised reaction conditions with the highest catalyst loading (10 wt-% Pd/C) furnished the tetrahydropyrethrins (**7a** and **7b**) in high yields of 83–97 %. The intermediate dihydropyrethrins (**6a** and **6b**; Scheme 3) were detected by ^{1}H NMR analysis of the reaction mixtures following 2-h hydrogen exposure with a 1 wt-% Pd/C catalyst loading, suggesting that these partially reduced compounds are intermediates in the full reduction of the pentadienyl side chains to give the tetrahydropyrethrins (**7a** and **7b**). The dihydropyrethrins **6a** and **6b** could not be separated from the tetrahydropyrethrins **7a** and **7b**, which were also present in these reaction mixtures, and as a result the bioactivity of the partially reduced pyrethrins **6a** and **6b** could not be explored.

Transfer Hydrogenation

The increased control provided by catalytic transfer hydrogenation was of interest to alleviate the selectivity issues encountered with the standard hydrogenation procedures.[23] Initial experiments made use of cyclohexene or cyclohexadiene as hydrogen transfer reagents; however, neither resulted in reduction. This lack of reactivity is likely due to the temperature dependence of the competition between hydrogen transfer and disproportionation of these transfer reagents.[24] This issue is usually overcome by substitution of the reaction solvent with a higher-boiling liquid; however, in this case this was not feasible owing to the thermal instability of the Pyrethrins (**1–3**).[4]

Experiments were then performed with formic acid as the hydrogen transfer reagent using similar reaction conditions to those previously employed. Interestingly, instead of the desired
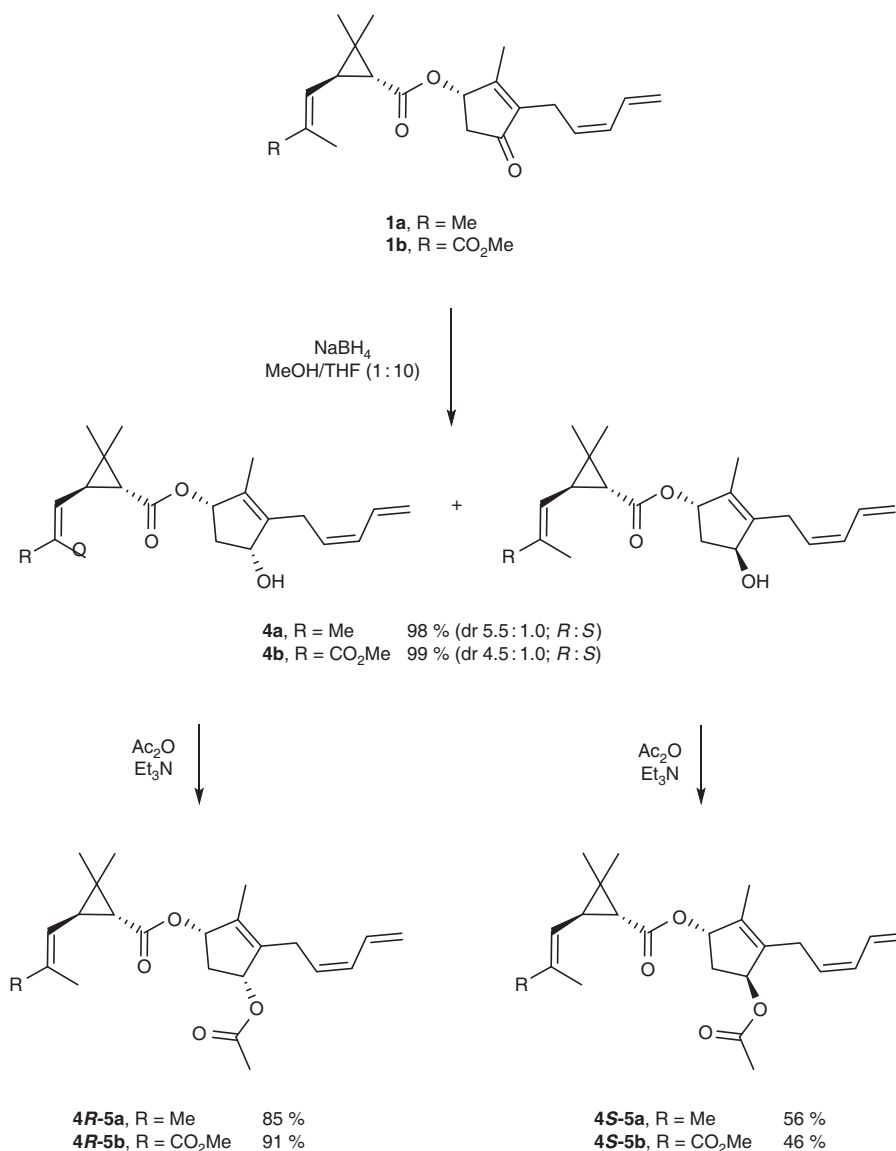
Scheme 2. Production of allyl alcohols (**4a** and **4b**) by 1,2-enone reduction with NaBH$_4$ followed by acetylation, giving individual allyl ester stereoisomers (**5a** and **5b**).

olefin reduction, the combination of formic acid and palladium on carbon at elevated temperatures resulted in both *cis* to *trans* isomerism and olefin migration in the pentadienyl rethrolone side chain (Scheme 4) of the pyrethrins (**1a** and **1b**). Such processes likely result from a palladium hydride, generated by the transfer process,[24–28] undergoing hydropalladation of the *cis*-olefin to give organopalladium species **10** (Scheme 4), and subsequent β-hydride elimination, giving the *trans*-pyrethrins **8** or double-migration isomers **9**, presumably via the partially conjugated isomer **11**. This isomerisation process was, however, ineffective at producing large quantities of these olefin isomers, with significant amounts of starting material recovered from reaction mixtures.

Diimide-Mediated Transfer Hydrogenation

Diimide (HN=NH) is a reducing agent that is known to have a high chemoselectivity towards unpolarised olefins and a high sensitivity to steric hindrance, with reactivity significantly decreasing with increasing substitution around the double

bond.[29] Reduction protocols that employ diimide use cheap, readily available materials and are operationally simple; the diimide is generated in situ from hydrazine in the presence of atmospheric oxygen, acetic acid, and catalytic quantities of copper(II). When pyrethrin I (**1a**) and II (**1b**) were individually subjected to these conditions, the desired jasmolins (**2a** and **2b**) were obtained as mixtures in 77 and 83 % mass recovery respectively (Scheme 5). The jasmolin components of these mixtures were found to be chemically identical to the natural jasmolins: in addition to being spectroscopically identical, analysis by UPCC found that the semisynthetic variants and the individual natural jasmolins (**2a** and **2b**) had very similar retention times. Further, the stereocentres present in the pyrethrins (**1a** and **1b**) appear to have been undisturbed under the conditions of the diimide reduction, as no evidence of the semisynthetic jasmolin signals resolving into more than a single peak could be found with a range of chiral columns and chromatographic conditions. Chromatographic analysis also revealed the presence of a single significant by-product in each
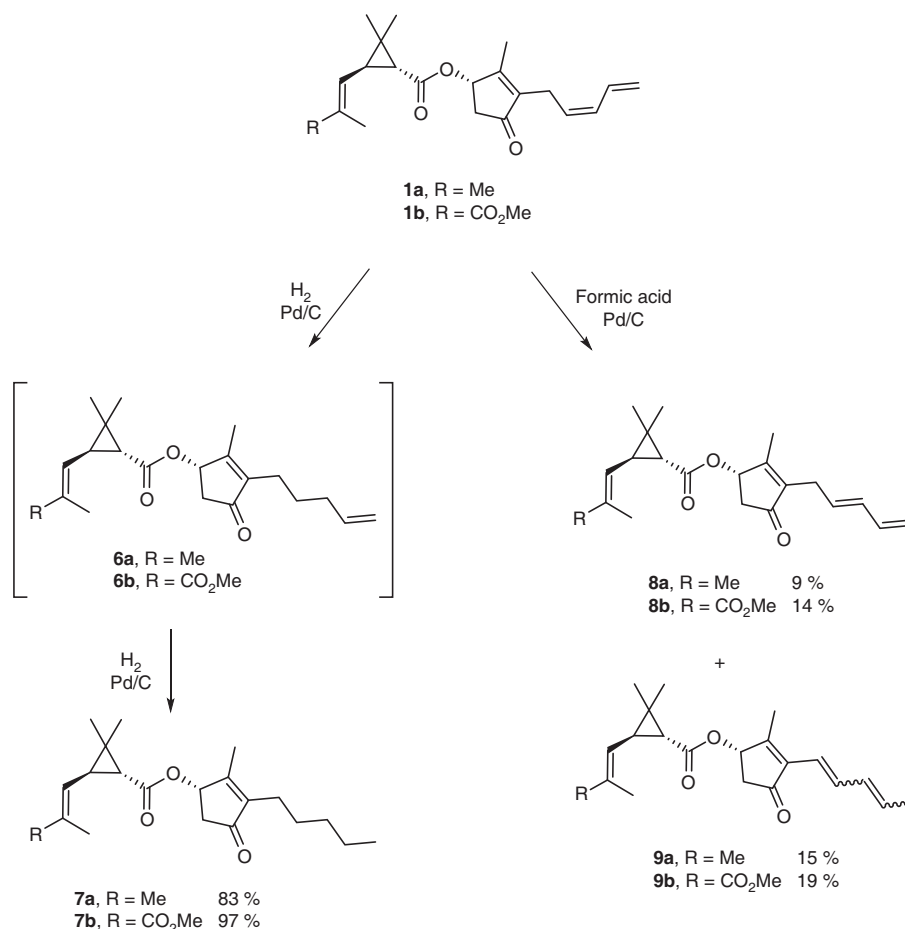
Scheme 3.   Palladium-catalysed reactions of pyrethrins (**1a and 1b**).

of the diimide reduction mixtures, constituting ~27 % of the product. Further analysis by LC-MS identified these by-products as the corresponding tetrahydropyrethrins **7a** and **7b**, which resulted from reduction of the semisynthetic jasmolins **2a** and **2b**.

This methodology could then be applied to the pyrethrum concentrate, where all six of the Pyrethrin esters (**1–3**) are present. It was found that pyrethrins I (**1a**) and II (**1b**) could be reduced to their respective jasmolins (**2a** and **2b**) in the presence of other minor pyrethrum constituents. HPLC analysis showed that very little pyrethrin I (**1a**) or II (**1b**) was detectable in the resulting mixture (Fig. 3b), with the jasmolins (**2a** and **2b**) constituting ~50 % of the treated concentrate, as opposed to 7 % in the original concentrate. HPLC analysis also indicated the presence of the two minor reaction by-products, with the elution of tetrahydropyrethrin II **7b** at 33.94 min and tetrahydropyrethrin I **7a** at 49.80 min (Fig. 3b) constituting ~15 % of the resulting mixture.

This process was found to be amenable to hydrazine levels as low as 10 equiv. before the rate of reduction started to be adversely affected and reaction times needed to be increased. This necessity for an excess of hydrazine stems from the readiness of diimide to undergo disproportionation to hydrazine and molecular nitrogen.[30] The protocol also showed promise under catalyst-free conditions, potentially alleviating the issues associated with heavy metal disposal in an industrial application. The copper-free reaction was generally significantly slower than the catalysed variant, however, and required

increased equivalents of hydrazine to combat the disproportion-ation process.[30] The copper-catalysed diimide-mediated reduction has shown promise up to gram scale with the pyre-thrum concentrate in the conversion of the more susceptible pyrethrins (**1a** and **1b**) and is potentially suitable for scale-up via large-scale continuous flow processes.[31]
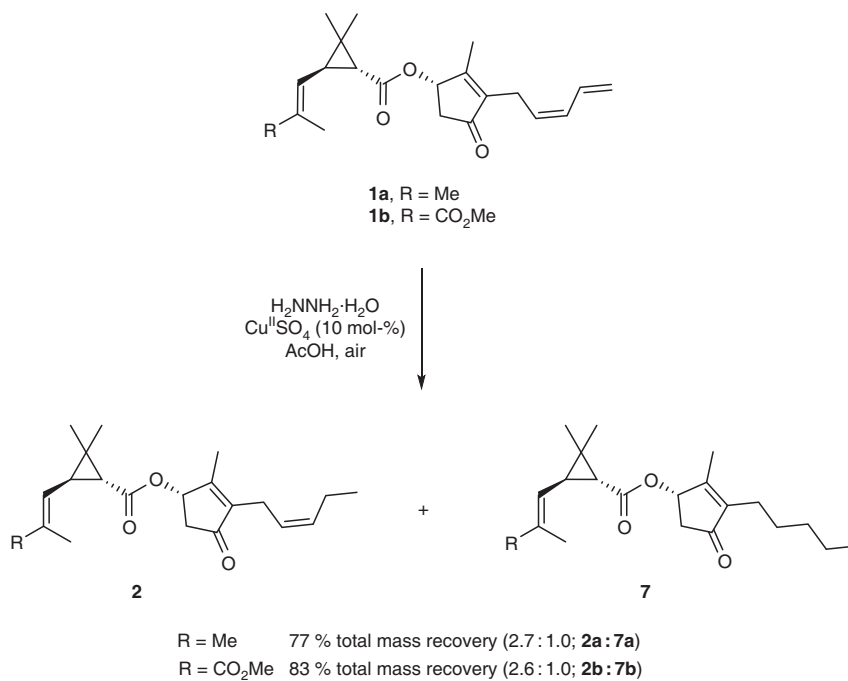
Preliminary Insecticidal Activity and Structure–Activity Relationships

Pyrethrins, and pyrethroids, act on the voltage-gated sodium ion channels that regulate nerve stimulation in the insect nervous system.[32] The binding of Pyrethrins and pyrethroids to these channels causes them to remain open for longer periods of time, allowing a greater influx of sodium ions, ultimately resulting in overexcitation of the nerve cell.[7,32] The major consequence of this is the 'knockdown' of the insect, where it is heavily inca-pacitated by paralysis and overstimulation. Eventually the overexcitation results in the death of the organism.[32] Much of the data on the mode of action of the natural Pyrethrins is extrapolated from that of the pyrethroids, with little data existing for both the action and insecticidal activity of the individual Pyrethrin esters. The in vitro inhibition by these natural products on cockroach sodium ion channels expressed in *Xenopus* oocytes has, however, been recently reported, and the results suggest that Pyrethrins are also able to interact with the closed form of the channels.[30]

In the current study, a preliminary screen of the insecticidal activity of the individual natural Pyrethrins was performed by
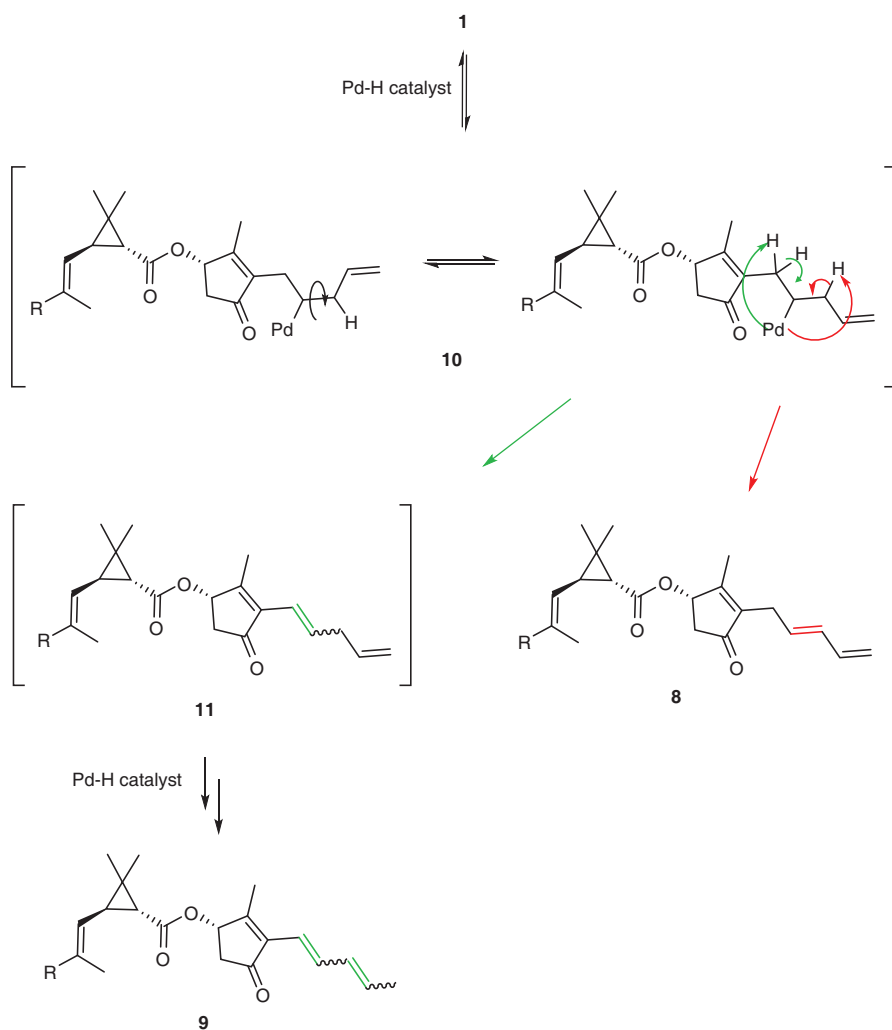
Scheme 4. Generation of the *trans*-pyrethrins **8** and olefin migration isomers **9** via intermediate palladium hydride species **10**.

1a, R = Me
1b, R = CO$_2$Me

H$_2$NNH$_2$·H$_2$O
Cu^{II}SO$_4$ (10 mol-%)
AcOH, air

2   +   **7**

R = Me   77 % total mass recovery (2.7 : 1.0; **2a** : **7a**)
R = CO$_2$Me   83 % total mass recovery (2.6 : 1.0; **2b** : **7b**)

Scheme 5. Diimide-mediated reduction of individual pyrethrins (**1a** and **1b**) to the corresponding jasmolins (**2a** and **2b**) and tetrahydropyrethrins (**7a** and **7b**).
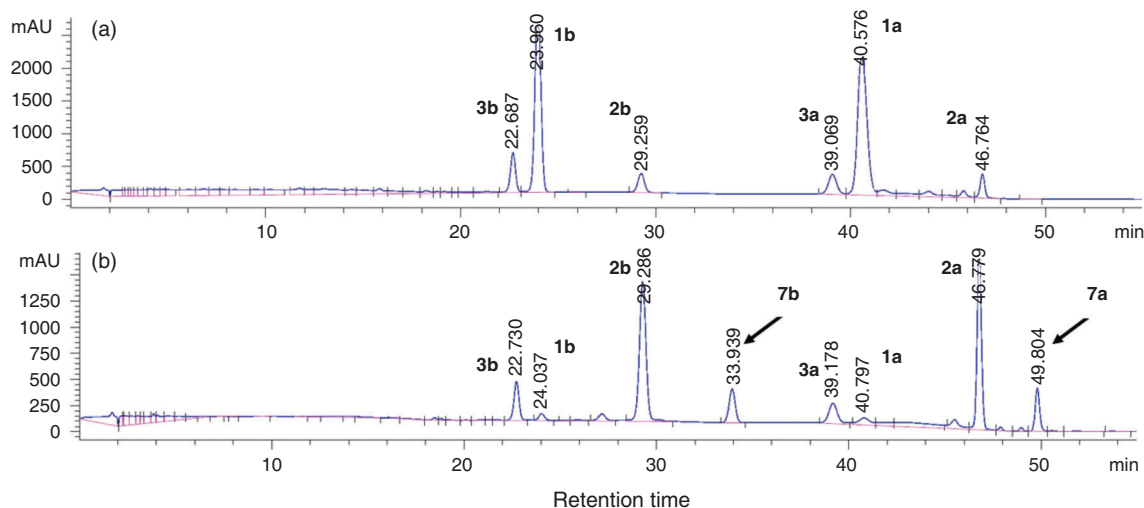
Fig. 3. HPLC traces of unaltered pyrethrum concentrate (a); and the pyrethrum concentrate after being subjected to diimide reduction (b).
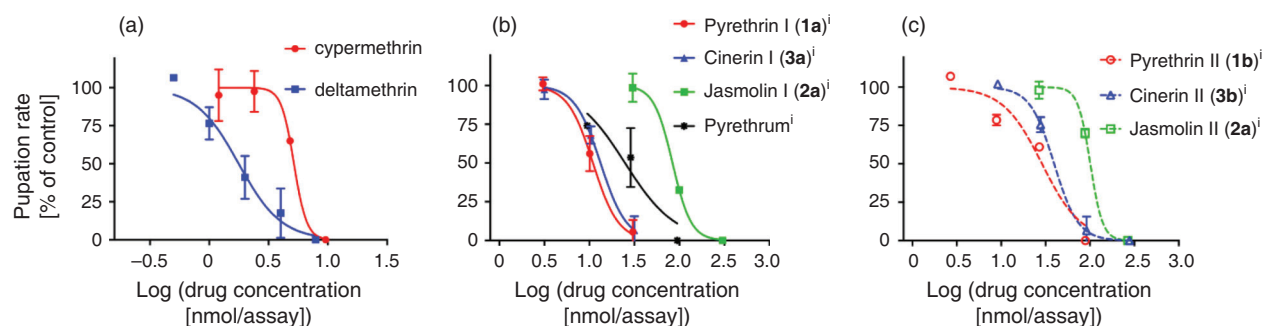

Fig. 4. Dose–response curves of the pupation rate of *Lucilia cuprina* larvae in response to the synthetic pyrethroid controls (a); the natural Pyrethrins I and pyrethrum concentrate (b); and the natural Pyrethrins II (c). Each data point represents mean ± s.e., $n = 2$ assays at each concentration. [i]Isolated from *Tanacetum cinerariifolium*.
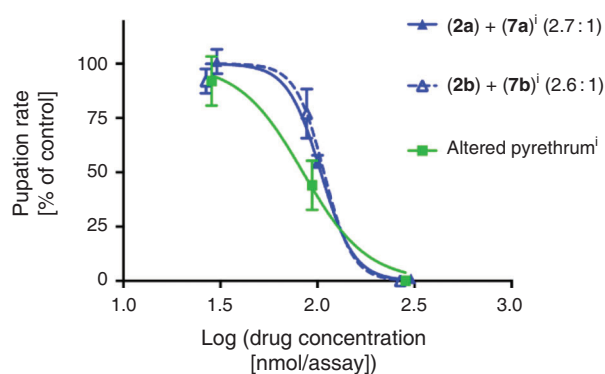

Fig. 5. Dose–response curves of the pupation rate of *Lucilia cuprina* larvae in response to mixtures produced by diimide-mediated reduction reactions of jasmolins and pyrethrum. Each data point represents mean ± s.e., $n = 2$ assays at each concentration. [i]Obtained by reduction under diimide-mediated conditions.

measuring their effects on the larval mortality of an economically important pest, *Lucilia cuprina* (Australian sheep blowfly), with the ability of larvae to develop to the pupal stage used as a measure of the larvicidal activity of the compounds. This is an operationally simple insecticidal assay, and assesses effects on larvae as this is the most relevant life stage for the

control of this important pest in the Australian context. Commercial synthetic pyrethroids, α-cypermethrin and deltamethrin, were used as positive controls. The chemically reduced and isomerised pyrethrin analogues (**4a**, **4b**, **5a**, **5b**, **7a**, **7b**, **8a**, **8b**, **9a**, and **9b**) were also tested, as well as products resulting from the diimide reduction reactions. Dose–response curves are shown in Figs 4 and 5 and IC_{50} data are shown in Table 1.

The individual Pyrethrins exhibited a range of toxicities towards *L. cuprina*, despite the minor differences between the acid moieties and/or the rethrolone side chain. Of the six isolated natural Pyrethrins, pyrethrin I **1a** and cinerin I **3a** were the most potent, with IC_{50} values for pupation inhibition of 11 and 13 nmol per assay respectively. Jasmolin I **2a** was significantly less active than the other two esters in the series, requiring ~6.5 times the concentration of cinerin I **3a** to inhibit pupation to 50 % (84 versus 13 nmol per assay). Pyrethrins II **1b**–**3b** also showed this trend across the series. However, the Pyrethrins II **1b** and **3b** were less active than their Pyrethrin I counterparts **1a** and **3a**, whereas **2a** and **2b** showed similar activity. The insecticidal activities of the isolated pyrethrins **1a** and **1b** and cinerins **3a** and **3b** have previously been reported, using the house fly (*Musca domestica*)[33] and mustard beetle (*Phaedon cochleariae*)[34] as test organisms. Of these four natural Pyrethrins, cinerin II **3b** was always found to be the least potent,[33,34] and the results with *L. cuprina* reported here are consistent with this finding. The other three more potent

Table 1. Bioactivity (IC$_{50}$) of the Pyrethrins and Pyrethrin analogues on the pupation rate of *Lucilia cuprina* larvae

a, IC$_{50}$; b, 95 % confidence interval

Compound	Structure	Activity [nmol per assay]		Compound	Structure	Activity [nmol per assay]	
α-Cypermethrin		a b	5.0 3.8–7.2	4*R*-**5a**		a	>200
Deltamethrin		a b	1.8 1.4–2.2	4*S*-**5a**		a	>200
Pyrethrum[A]	mixture	a b	25 14–46	4*R*-**5b**		a	>200
pyrethrin I **1a**[A]		a b	11 8–13	4*S*-**5b**		a	>200
pyrethrin II **1b**[A]		a b	29 19–43	**7a**		a	>200
jasmolin I **2a**[A]		a b	84 66–110	**7b**		a	>200
jasmolin II **2b**[A]		a b	100 57–180	**8a**		a b	46 35–60
cinerin I **3a**[A]		a b	13 11–17	**8b**		a b	71 37–140
cinerin II **3b**[A]		a b	39 33–47	**9a**		a	>200
4*R*-**4a**		a	>200	**9b**		a	>200
4*S*-**4a**		a	>200	**2a** + **7a** (2.7 : 1.0)[B]	Mixture	a b	100 93–120
4*R*-**4b**		a	>200	**2b** + **7b** (2.6 : 1.0)[B]	Mixture	a b	110 36–330
4*S*-**4b**		a	>200	Altered pyrethrum[B]	Mixture	a b	84 65–110

[A]Isolated from *T. cinerariifolium*.
[B]Obtained by reduction under diimide-mediated conditions.

Pyrethrins (**1a**, **1b**, and **3a**) were reported to have varied relative activities, depending on the test organism and mode of administration. The isolated jasmolins I **2a** and II **2b** have only very recently been tested on live insects (common mosquito, *Culex pipiens pallens*).[35] The reported toxicity of the jasmolins against the mosquito are consistent with the trend in activity

found with *L. cuprina*, being the weakest insecticides of the six natural Pyrethrin esters. This trend observed with the natural Pyrethrins (**1–3**) is also consistent with the in vitro electrophysiology activities with cockroach sodium channels recently reported by Dong and coworkers.[7] The individual semisynthetic jasmolin mixtures retained similar activities to that of their natural counterparts, likely due to the high jasmolin (**2a** and **2b**) content.

The pyrethrum concentrate exhibited a similar IC_{50} to that of pyrethrin II (**1b**), presumably due to the majority of the mixture comprising the more active pyrethrins (**1a** and **1b**), with only minor amounts of the less active esters being present. The altered pyrethrum concentrate obtained after diimide reduction retained insecticidal activity (IC_{50} 84 nmol per assay); however, as expected, it had a reduced potency, very similar to that of the jasmolins **2a** and **2b**.

All the reduced pyrethrin analogues synthesised (**4a**, **4b**, **5a**, **5b**, **7a**, and **7b**) via various reductive procedures were found to be inactive in the *L. cuprina* assay. Specifically, the ketone functionality appears to be vital for insecticidal activity. Consistent with this finding, acetylation of the allylic alcohols **4a** and **4b** to give **5a** and **5b** failed to restore biological activity. Unsaturation in the pentyl side chain of the Pyrethrins also greatly influenced insecticidal activity. The lack of observed activity of the tetrahydropyrethrins **7a** and **7b** is consistent with previous reports that indicated that these compounds had considerably less insecticidal activity than their natural, unsaturated analogues pyrethrin I and II (**1a** and **1b**).[19–21] It is also consistent with the in silico model of a cockroach sodium ion channel developed by Dong and coworkers, in which unsaturation in the sidechain was predicted to be important for channel binding and inactivation by pyrethrin II (**1b**).[7] The importance of the unsaturated sidechain is further highlighted by the measured activities of the butadiene isomers **8a**, **8b**, **9a**, and **9b**. The *trans*-pyrethrin isomers **8a** and **8b** showed significant inhibition of pupation, albeit slightly decreased from that of pyrethrins **1a** and **1b** bearing the natural *cis*-geometry. The migratory isomers **9a** and **9b**, however, were found to be inactive. This is again consistent with Dong's model, which requires the pyrethrin molecule to bend at the methylene that links the cyclopentenone ring and butadiene chain in order to make important contacts with particular protein helices of the channel. In the fully conjugated trienes **9a** and **9b**, the diene would be coplanar with the cyclopentenone ring and incapable of bending and making the required protein contacts.

Conclusion

In summary, several Pyrethrin analogues have been synthesised through the exploration of the reduction chemistry of the natural Pyrethrins. As a result, a procedure for the conversion of pyrethrins (**1a** and **1b**) to jasmolins (**2a** and **2b**) was developed, resulting in the removal of the more sensitive esters; however, some over-reduction was observed, resulting in the coproduction of the tetrahydropyrethrins **7a** and **7b**. The bioactivity of the synthesised analogues was assessed using the commercially important organism *Lucilia cuprina* and new structure–activity relationships relating to the ketone and unsaturated side chain functionalities have been revealed. From these assays, it was also found that the altered pyrethrum concentrate still exhibits significant insecticidal activity while being expected to have increased stability relative to the natural extract.[2]

Supplementary Material

NMR and IR spectra of all new compounds, NMR spectra of natural isolated jasmolins, NMR and IR spectra, and HPLC, LC-MS, and UPCC traces of mixtures produced from diimide reduction reactions and figures showing NOE correlations for epimers of **4a** and **4b** are available on the Journal's website.

Conflicts of Interest

The CSIRO has previously undertaken commercial research for Botanical Resources Australia, a major commercial producer of pyrethrum.

Acknowledgements

References

[1] J. E. Casida, *Environ. Health Perspect.* **1980**, *34*, 189. doi:10.1289/EHP.8034189

[2] J. A. Freemont, S. W. Littler, O. E. Hutt, S. Mauger, A. G. Meyer, D. A. Winkler, M. G. Kerr, J. H. Ryan, H. F. Cole, P. J. Duggan, *J. Agric. Food Chem.* **2016**, *64*, 7134. doi:10.1021/ACS.JAFC.6B02959

[3] L. Crombie, P. Hemesley, G. Pattenden, *J. Chem. Soc.* **1969**, 1016.

[4] B. L. Atkinson, A. J. Blackman, H. Faber, *J. Agric. Food Chem.* **2004**, *52*, 280. doi:10.1021/JF0304425

[5] M. J. Bullivant, G. Pattenden, *Pestic. Sci.* **1976**, *7*, 231. doi:10.1002/PS.2780070304

[6] E. V. Minello, F. Lai, M. T. Zonchello, M. Melis, M. Russo, P. Cabras, *J. Agric. Food Chem.* **2005**, *53*, 8302. doi:10.1021/JF0510992

[7] M. Chen, Y. Du, G. Zhu, G. Takamatsu, M. Ihara, K. Matsuda, B. S. Zhorov, K. Dong, *Pestic. Biochem. Physiol.* **2018**, *151*, 82. doi:10.1016/J.PESTBP.2018.05.002

[8] D. G. Beddie, A. W. Farnham, B. P. S. Khambay, *Pestic. Sci.* **1996**, *48*, 175. doi:10.1002/(SICI)1096-9063(199610)48:2<175::AID-PS456>3.0.CO;2-T

[9] F. Zhu, J. Wigginton, A. Romero, A. Moore, K. Ferguson, R. Palli, M. F. Potter, K. F. Haynes, S. R. Palli, *Arch. Insect Biochem. Physiol.* **2010**, *73*, 245.

[10] Y.-L. Chen, J. E. Casida, *J. Agric. Food Chem.* **1969**, *17*, 208. doi:10.1021/JF60162A036

[11] A. F. Bramwell, L. Crombie, P. Hemesley, G. Pattenden, *Tetrahedron* **1969**, *25*, 1727. doi:10.1016/S0040-4020(01)82745-9

[12] I.-H. Wang, V. Subramanian, R. Moorman, J. Burleson, J. Ko, *J. Chromatogr. A* **1997**, *766*, 277. doi:10.1016/S0021-9673(96)00969-7

[13] N. H. Bagnall, B. M. Hines, A. J. Lucke, P. K. Gupta, R. C. Reid, D. P. Fairlie, A. C. Kotze, *Int. J. Parasitol. Drugs Drug Resist.* **2017**, *7*, 51. doi:10.1016/J.IJPDDR.2017.01.001

[14] A. C. Kotze, N. H. Bagnall, A. P. Ruffell, R. Pearson, *Med. Vet. Entomol.* **2014**, *28*, 297. doi:10.1111/MVE.12041

[15] H. C. Brown, R. Liotta, G. W. Kramer, *J. Org. Chem.* **1978**, *43*, 1058. doi:10.1021/JO00400A007

[16] H. C. Brown, S. Krishnamurthy, N. M. Yoon, *J. Org. Chem.* **1976**, *41*, 1778. doi:10.1021/JO00872A025

[17] S. Krishnamurthy, H. C. Brown, *J. Org. Chem.* **1977**, *42*, 1197. doi:10.1021/JO00427A021

[18] R. S. Varma, G. W. Kabalka, *Synth. Commun.* **1985**, *15*, 985. doi:10.1080/00397918508076830

[19] G. R. Meyer, *J. Chem. Educ.* **1981**, *58*, 628. doi:10.1021/ED058P628

[20] W. A. Gersdorff, *J. Econ. Entomol.* **1947**, *40*, 878. doi:10.1093/JEE/40.6.878

[21] H. L. Haller, W. N. Sullivan, *J. Econ. Entomol.* **1938**, *31*, 276. doi:10.1093/JEE/31.2.276

[22] L. Crombie, M. Elliott, S. H. Harper, *J. Chem. Soc.* **1950**, 971. doi:10.1039/JR9500000971

[23] D. Wang, D. Astruc, *Chem. Rev.* **2015**, *115*, 6621. doi:10.1021/ACS.CHEMREV.5B00203

[24] G. Brieger, T. J. Nestrick, *Chem. Rev.* **1974**, *74*, 567. doi:10.1021/CR60291A003

[25] D. Gauthier, A. T. Lindhardt, E. P. K. Olsen, J. Overgaard, T. Skrydstrup, *J. Am. Chem. Soc.* **2010**, *132*, 7998. doi:10.1021/JA9108424

[26] R. Shen, T. Chen, Y. Zhao, R. Qiu, Y. Zhou, S. Yin, X. Wang, M. Goto, L.-B. Han, *J. Am. Chem. Soc.* **2011**, *133*, 17037. doi:10.1021/JA2069246

[27] E. H. P. Tan, G. C. Lloyd-Jones, J. N. Harvey, A. J. J. Lennox, B. M. Mills, *Angew. Chem. Int. Ed.* **2011**, *50*, 9602. doi:10.1002/ANIE.201103947

[28] J. Yu, J. B. Spencer, *J. Org. Chem.* **1997**, *62*, 8618. doi:10.1021/JO971602C

[29] C. Smit, M. W. Fraaije, A. J. Minnaard, *J. Org. Chem.* **2008**, *73*, 9482. doi:10.1021/JO801588D

[30] Y. Imada, H. Iida, T. Naota, *J. Am. Chem. Soc.* **2005**, *127*, 14544. doi:10.1021/JA053976Q

[31] B. Pieber, S. T. Martinez, D. Cantillo, C. O. Kapper, *Angew. Chem. Int. Ed.* **2013**, *52*, 10241. doi:10.1002/ANIE.201303528

[32] T. G. E. Davies, L. M. Field, P. N. R. Usherwood, M. S. Williamson, *IUBMB Life* **2007**, *59*, 151. doi:10.1080/15216540701352042

[33] R. M. Sawicki, M. Elliott, J. C. Gower, M. Snarey, E. M. Thain, *J. Sci. Food Agric.* **1962**, *13*, 172. doi:10.1002/JSFA.2740130307

[34] J. Ward, *Chem. Ind.* **1953**, 586.

[35] M. Kawamoto, M. Moriyama, Y. Ashida, N. Matsudo, Y. Tanabe, *J. Org. Chem.* **2020**, *85*, 2984. doi:10.1021/ACS.JOC.9B02767