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# Insect-antifeedant and antibacterial activity of diterpenoids from species of *Plectranthus*

Julia Wellsow, Renée J. Grayer, Nigel C. Veitch, Tetsuo Kokubun, Roberto Lelli, Geoffrey C. Kite, Monique S.J. Simmonds *

*Royal Botanic Gardens, Biological Interactions, Kew, Richmond, Surrey TW9 3AB, UK*

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Dedicated to Professor Rod Croteau on the occasion of his 60th birthday.

**Abstract**

Bio-assay guided fractionation of an acetone extract of leaf material from *Plectranthus saccatus* Benth. resulted in the isolation of a beyerane diterpenoid. This compound, characterised by spectroscopic methods as *ent*-3β-(3-methyl-2-butenoyl)oxy-15-beyeren-19-oic acid, showed insect antifeedant activity against *Spodoptera littoralis*. Known quinonoid abietane diterpenoids obtained from new sources included a mixture of the (4*R*,19*R*) and (4*R*,19*S*) diastereoisomers of coleon A from *P.* aff. *puberulentus* J.K. Morton, coleon A lactone from *P. puberulentus* J.K. Morton, and coleon U and coleon U quinone from *P. forsteri* 'Marginatus' Benth. These compounds, and the crude acetone extracts from the leaf surfaces of 11 species of *Plectranthus*, were tested for antifeedant activity against *S. littoralis*, antibacterial activity against *Bacillus subtilis* and *Pseudomonas syringae* and antifungal activity against *Cladosporium herbarum*. The coleon A mixture showed potent antifeedant activity against *S. littoralis*, whereas coleon U showed the greatest antimicrobial activity.


*Keywords:* *Plectranthus*; Lamiaceae; Diterpenoids; Insect antifeedant; Antimicrobial activity

## 1. Introduction

The genus *Plectranthus* (family Lamiaceae) consists of mainly tropical Old World herbs and shrubs. A recent molecular study of the genus revealed the presence of two major clades, the "Coleus" clade, which comprises most of the species which used to be called *Coleus*, and a second clade contains the remaining species of *Plectranthus* (Paton et al., 2004). Sixty-two species of *Plectranthus* are reported to be of economic and medicinal interest and some are grown as ornamental plants (Lukhoba et al., 2006). They are used in folk medicine for a variety of diseases including infectious conditions (Rivera Nuñez and Obón de Castro, 1992; Matu and van Staden, 2003). Diterpenoids are the most studied group of secondary compounds in *Plectranthus*, the majority being abietanes containing phenolic or quinonoid rings, in addition to some labdanes, *ent*-kauranes and *seco*-kauranes (Abdel-Mogib et al., 2002). Recently, several neoclerodane diterpenoids were detected in *P. ornatus* (Rijo et al., 2002). Interest in the isolation of diterpenoids continues to grow because of their broad spectrum of biological activities (Hanson, 2004). For example, abietane diterpenoids have attracted interest on account of their antibacterial (Batista et al., 1995; Dellar et al., 1996; Teixeria et al., 1997), antioxidant (Narukawa et al., 2001) and insect antifeedant activities (Kubo et al., 1984) as well as their inhibitory effects on different human cancer cell lines (Marques et al., 2002). In the present report, the results of a survey of insect-antifeedant and antimicrobial activities of a number of species of *Plectranthus* are described. Fractionation of the active extracts led to the isolation of a new beyerane diterpenoid (**1**), and four known quinonoid abietane diterpenoids from new sources

* Corresponding author. Tel.: +44 208 332 5328; fax: +44 208 332 5340.
*E-mail address:* m.simmonds@kew.org (M.S.J. Simmonds).

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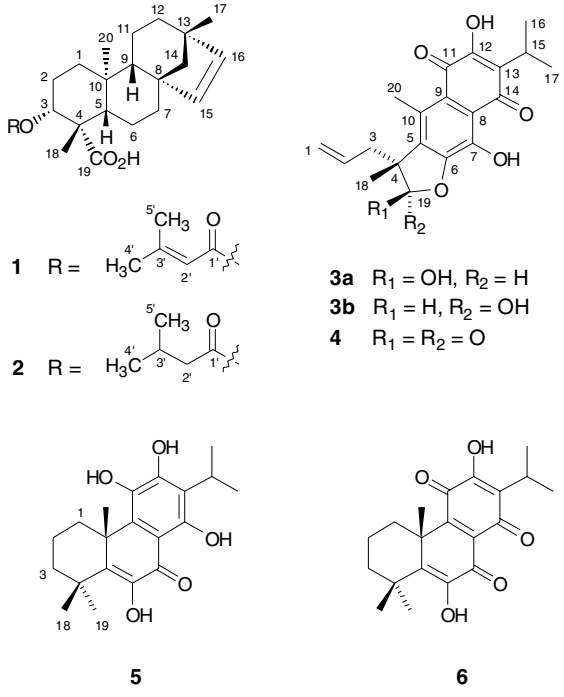


Fig. 1. Diterpenoids from species of *Plectranthus*.

(**3a**–**6**) (Fig. 1). This paper describes their identification by spectroscopic methods, and the determination of their activities.

## 2. Results and discussion

### 2.1. Identification of the diterpenoids

Fractionation of an acetone extract of the leaves of *Plectranthus saccatus* Benth. by silica gel column chromatography followed by semi-preparative HPLC yielded **1** as a colourless solid, for which a molecular formula of $C_{25}H_{36}O_4$ was assigned by HRESIMS. The structure of this compound was determined by analysis of 1D $^1$H, 1D $^{13}$C, 1D selective NOE, DEPT, DQF-COSY, HSQC and HMBC spectra acquired using standard pulse sequences and parameters. A full set of $^1$H and $^{13}$C NMR spectroscopic assignments is given in Table 1. Among the characteristic features that indicated a beyer-15-ene skeleton for **1** were three quaternary methyl groups at $\delta$ 1.29 (3H, *s*, $CH_3$-18), 1.01 (3H, *s*, $CH_3$-17) and 0.77 (3H, *s*, $CH_3$-20) together with a (*Z*)-disubstituted double bond at $\delta$ 5.67 (1H, *d*, $J = 5.7$ Hz, H-15, $\delta_C$ 134.6) and 5.47 (1H, *d*, $J = 5.7$ Hz, H-16, $\delta_C$ 136.8) linked directly to quaternary carbon atoms at $\delta_C$ 48.8 (C-8) and 43.7 (C-13), respectively (Grande et al., 1989). The presence of a carboxylic acid group was indicated by a broad exchangeable resonance in the $^1$H NMR spectrum at $\delta$ 10.46 (1H, *br s*, COOH-19) and the resonance at $\delta_C$ 179.2 in the $^{13}$C NMR spectrum. Long-range connectivities in the HMBC spectrum from both H-3 and H-5 to the carboxylic acid group at $\delta_C$ 179.2 and the quaternary methyl group at $\delta_C$ 24.1 ($\delta_H$ 1.29) confirmed that these were attached at C-4. As expected, a further correlation was observed between the quaternary methyl protons ($\delta_H$ 1.29) and $\delta_C$ 179.2 (Table 1). A subset of $^1$H and $^{13}$C resonances identified as those of a carbonyl group ($\delta_C$ 166.3), a trisubstituted double bond characterised by $\delta_H$ 5.70 (1H, *m*, H-2′, $\delta_C$ 116.4) and $\delta_C$ 156.9 (C-3′), and two methyl groups at $\delta_H$ 2.16 ($CH_3$-5′, $\delta_C$ 20.3) and 1.89 ($CH_3$-4′, $\delta_C$ 27.4) represented a 3-methyl-2-butenoyloxy side chain attached to the beyer-15-ene-19-oic acid skeleton. The location of this substituent was confirmed as C-3 from the connectivity observed in the HMBC spectrum between H-3 at $\delta$ 4.60 (1H, *dd*, $J = 12.3$, 4.4 Hz) and $\delta_C$ 166.3 (C-1′). These observations indicated that **1** was a 3-methyl-2-butenoyl derivative of 3-hydroxy-15-beyeren-19-oic acid.

Table 1
$^1$H and $^{13}$C NMR spectroscopic data for 1 ($\delta$ in $CDCl_3$ at 30 °C)

| Atom | Assignment ($\delta$ in ppm, $J$ in Hz) | | Long-range connectivities |
|---|---|---|---|
| | $\delta^{13}$C | $\delta^1$H | HMBC ($^2J$ and $^3J$) |
| 1 | 37.5 | 1.78 *dm* (13.1) | C-2, C-3, C-5, C-10, C-20 |
| | | 1.15 *m* | C-2, C-3, C-9, C-10 |
| 2α | 24.3 | 2.31 *m* | C-1, C-3, C-4 |
| 2β | | 1.71 *m* | C-1, C-3, C-10 |
| 3 | 78.2 | 4.60 *dd* (12.3, 4.4) | C-1, C-2, C-4, C-5, C-18, C-19, C-1′ |
| 4 | 48.2 | | |
| 5 | 56.6 | 1.18 *dd* (11.8, 2.1) | C-1, C-4, C-6, C-7, C-10, C-18, C-19, C-20 |
| 6α | 21.4 | 1.66 *m* | C-5 |
| 6β | | 1.86 *m* | C-7, C-8 |
| 7 | 37.3 | 1.69 *m* | C-5, C-6, C-8, C-9, C-15 |
| | | 1.32 *m* | C-6, C-8, C-14, C-15 |
| 8 | 48.8 | | |
| 9 | 52.4 | 0.97 *m* | C-8, C-10, C-11, C-15, C-20 |
| 10 | 37.7 | | |
| 11 | 20.5 | 1.54 *m* | |
| | | 1.28 *m* | |
| 12 | 33.0 | 1.28 *m* (2H) | C-14, C-16 |
| 13 | 43.7 | | |
| 14 | 60.9 | 1.48 *dd* (9.7, 1.9) | C-7, C-8, C-9, C-12, C-13, C-17 |
| | | 1.03 *d* (9.8) | C-8, C-9, C-12, C-15, C-16 |
| 15 | 134.6 | 5.67 *d* (5.7) | C-7, C-8, C-13, C-14 |
| 16 | 136.8 | 5.47 *d* (5.7) | C-8, C-13, C-14, C-17 |
| 17 | 24.8 | 1.01 *s* | C-12, C-13, C-14, C-16 |
| 18 | 24.1 | 1.29 *s* | C-3, C-4, C-5, C-19 |
| 19 | 179.2 | (10.46 *br s*, CO*OH*) | |
| 20 | 13.5 | 0.77 *s* | C-1, C-5, C-9, C-10 |
| 1′ | 166.3 | | |
| 2′ | 116.4 | 5.70 *m* | C-1′, C-4′, C-5′ |
| 3′ | 156.9 | | |
| 4′ | 27.4 | 1.89 *d* (1.3) | C-2′, C-3′, C-5′ |
| 5′ | 20.3 | 2.16 *d* (1.3) | C-2′, C-3′, C-4′ |

The relative configuration of **1** was investigated using 1D site selective NOE experiments (Fig. 2). NOE connectivities typical for beyerane diterpenoids were detected between $CH_3$-20 and both H-6 and H-15 (Zdero et al., 1989). Evidence for the axial position of H-3 included not only the NOEs to H-5 (1,3-diaxially related) and $CH_3$-18, but also the coupling constants for this resonance

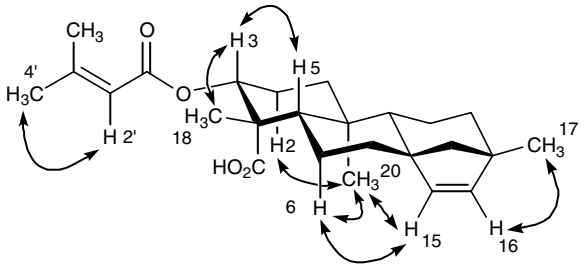


Fig. 2. Key NOE connectivities for **1** (shown in the *ent*-beyerane configuration).

of 12.3 and 4.4 Hz which can only be satisfied in terms of $^{3}J$(H-3$_{ax}$,H-2$_{ax}$) and $^{3}J$(H-3$_{ax}$,H-2$_{eq}$), respectively. This allowed the relatively bulky 3-methyl-2-butenoyloxy side chain to be placed in the equatorial position. The carboxylic acid group at C-19 lies in the axial position as expected (Liu et al., 2003). The absolute configuration of **1** was not determined experimentally, although it is assumed to belong to the *ent*-series of beyeranes. This is the most commonly observed configuration for this class of diterpenoids, and appears to be favoured on biogenetic grounds (Connolly and Hill, 1991; Macmillan and Beale, 1999). On this basis **1** was characterised as *ent*-3β-(3-methyl-2-butenoyl)oxy-15-beyeren-19-oic acid, the 3-methyl-2-butenoyl derivative of *ent*-3β-hydroxy-15-beyeren-19-oic acid (3α-hydroxystachen-19-oic acid), a constituent previously obtained from the aerial parts of *Nidorella anomala* Steetz (Asteraceae) (Bohlmann and Wegner, 1982). The partial set of $^{1}$H NMR spectroscopic assignments obtained for the methyl ester of the latter compound is in good agreement with the corresponding data for **1** in Table 1.

A consistent feature of samples of **1** purified from acetone extracts of *P. saccatus* leaves using HPLC was the presence of a minor component (typically between 5% and 30% of the sample, depending on the number of semi-preparative HPLC steps used; see Section 3.3) that co-eluted with the major beyerane diterpenoid component. ESI-MS (negative mode) gave deprotonated molecules at *m*/*z* 399 and 401 for the major and minor component, respectively, suggesting that the latter might be a dihydro derivative of **1**. The difference between the two components was traced to the side chain at C-3, based on correlations observed for the minor component in 2D spectra. In the $^{1}$H NMR spectrum, a minor component resonance corresponding to H-3 appeared at $\delta$ 4.56 (*dd*, $J = 12.2$, 4.5 Hz), slightly upfield of the corresponding major component resonance at $\delta$ 4.60 (*dd*, $J = 12.3$, 4.4 Hz). The former gave a $^{3}J$ correlation to $\delta_C$ 172.8 in the HMBC spectrum, a minor component resonance corresponding to C-1′. The remainder of the side chain was revealed from correlations in the DQF-COSY spectrum as $(CH_3)_2CHCH_2$-, with the minor component methylene resonance at $\delta$ 2.21 ($\delta_C$ 43.8) giving a $^{2}J$ correlation to $\delta_C$ 172.8 (C-1′). It was noted that resonances corresponding to the double bond at C-15 and C-16 were present for the minor component at chemical shift values almost coincident with those of the major component. These data indicated that the minor component (**2**) was likely to be *ent*-3β-(3-methylbutanoyl)oxy-15-beyeren-19-oic acid.

Fractionation of acetone extracts of several species of *Plectranthus* by silica gel column chromatography and semi-preparative HPLC yielded a series of known coleon diterpenoids as yellow to orange amorphous solids (see Section 3 for details). These were identified, using UV, MS and NMR spectroscopic data, as a mixture of the (4*R*,19*R*) and (4*R*,19*S*) diastereoisomers of coleon A (**3a** and **3b**, respectively) from *P.* aff. *puberulentus* J.K. Morton, coleon A lactone (**4**) from *P. puberulentus* J.K. Morton, and coleon U (**5**) and coleon U quinone (**6**) from *P. forsteri* 'Marginatus' Benth. Coleon A (**3**) was first described as a pigment of the leaves of *Coleus igniarius* Schweinf. (Karanatsios and Eugster, 1965), but Baxter et al. (1995) later showed that this compound exists as a mixture of diastereoisomers both in the solid and solution state. Using material isolated from leaves of *Coleus kilimandjari* Bally, they demonstrated the existence of a 3:1 mixture of the (4*R*,19*R*) and (4*R*,19*S*) diastereoisomers, respectively, in acetone-$d_6$ solution. NMR spectroscopic data obtained in $CDCl_3$ on a sample of coleon A (**3**) purified from leaves of *Plectranthus* aff. *puberulentus* during the present study indicated that a 7:3 mixture of the (4*R*,19*R*) and (4*R*,19*S*) diastereoisomers, respectively, was present under these solution conditions. The $^{1}$H NMR spectroscopic assignments for these diastereoisomers were in good agreement with the partial datasets given by Baxter et al. (1995). Sections 3.8.3 and 3.8.4 also give full sets of $^{13}$C NMR spectroscopic assignments for each diastereoisomer, which have not been reported previously. These assignments were confirmed from long-range correlations in the HMBC spectrum of the mixture of diastereoisomers. NMR data obtained for coleon A lactone (**4**) were in good agreement with those published previously (Künzle et al., 1987).

The $^{1}$H NMR spectrum of coleon U (**5**) was characterised by exchange-broadening, a feature also commented on by Miyase et al. (1977) in an earlier paper describing this compound from *Plectranthus myrianthus* Briq. The $^{13}$C NMR spectrum of our sample, isolated from leaves of *P. forsteri* 'Marginatus', was identical to that reported previously under the same solution conditions, but a few assignments required revision, based on 2D NMR spectroscopic correlations (Section 3.8.6). A complete assignment of the $^{1}$H NMR spectrum of coleon U, including the exchange-broadened resonances of $CH_2$-1, $CH_2$-2 and $CH_2$-3, and all hydroxyl protons, is also given in Section 3.8.6.

Coleon U quinone (**6**), which was also obtained from *P. forsteri* 'Marginatus', appears to be an oxidation product of coleon U, as it was not detected by HPLC in the crude extract of this species. The NMR data obtained for this compound were in good agreement with those in the literature (Alder et al., 1984). The $^{1}$H NMR spectroscopic assignments for $CH_2$-1, $CH_2$-2 and $CH_2$-3 of coleon U

quinone (**6**), which were missing from the earlier study, are included in Section 3.8.7.

### 2.2. Antifeedant activity

The acetone leaf-surface extracts of 11 species of *Plectranthus* were tested for antifeedant activity against *Spodoptera littoralis* and the results are presented in Table 2. None of the extracts elicited a dose-dependent antifeedant response from the larvae, so the $FI_{50}$ values were not calculated. The extract of *P. saccatus* was the only one to show significant antifeedant activity against larvae of *S. littoralis* at 100 ppm (Table 2). A bioassay-guided fractionation of the *P. saccatus* extract resulted in the isolation of diterpenoid **1**, which also showed significant antifeedant activity at 100 ppm and did not give a dose-dependent response (Table 3). The HPLC traces of some of the extracts from the other species of *Plectranthus* showed the presence of diterpenoids that were isolated and tested. A mixture of the diastereoisomers of coleon A (**3a** + **3b**) was isolated from a crude extract of *P.* aff. *puberulentus*. This extract was not significantly active at 100 ppm, although the feeding index had a positive value. This positive value could be explained by the presence of a low concentration of the coleon A mixture, as this showed significant antifeedant activity at 100 ppm and the estimated $FI_{50}$ was 87 ppm. Coleon A lactone (**4**) also showed antifeedant activity at 100 ppm, although it was not as active as the coleon A mixture (Table 3). Coleon A lactone was isolated from the crude extract of *P. puberulentus*, an extract that did not show significant antifeedant activity in the feeding bioassay, although like the extract of *P.* aff. *puberulentus* the feeding index was positive. Coleon U (**5**) was not active in the bioassay. It was isolated from an inactive extract of *P. forsteri* 'Marginatus'. An analysis of the HPLC traces of the other species of *Plectranthus* shows that coleon U was present in the crude extracts of *P. argentatus* and *P. madagascariensis*, extracts that stimulated feeding. Coleon U quinone (**6**) shows potent antifeedant activity at 100 ppm with a low $FI_{50}$ value of 91 ppm. Coleon U (**6**) quinone is an oxidation product of coleon U that only appeared in significant amounts during the isolation process. It cannot therefore be considered as a contributory factor towards the activity of the crude extracts.

Table 2
Effect of acetone extracts of *Plectranthus* species on insect feeding and bacterial growth

| Species | Kew Accession No. | Feeding index[a] Mean (SEM ) | Minimum inhibitory concentration (μg/ml) | |
|---|---|---|---|---|
| | | *Spodoptera littoralis* | *Bacillus subtilis* (Gram-positive) | *Pseudomonas syringae* (Gram-negative) |
| *P. argentatus* S.T. Blake | 1999-10 | −25 (14.5) | 40 | 1000 |
| *P. elegans* Britten | 1970-3417 | −15 (8.8) | 1000 | 1000 |
| *P. forsteri* 'Marginatus' Benth. | 1999-11 | 11 (15.2) | 8 | 40 |
| *P.* aff. *hadiensis* (Forssk.) Schweinf. ex Sprenger | 1970-3766 | −4 (9.8) | – | 1000 |
| *P.* aff. *hadiensis* (Forssk.) Schweinf. ex Sprenger | 1970-3770 | −3 (13.2) | – | 1000 |
| *P. madagascariensis* Benth. | 1980-3446 | −89 (7.8)** | 8 | 40 |
| *P. mutabilis* Codd | 1955-47101 | −38 (18.8) | – | – |
| *P.* aff. *puberulentus* J.K. Morton | 1970-3735 | 27 (14.5) | 200 | 200 |
| *P. puberulentus* J.K. Morton | 1970-3763 | 23 (14.8) | – | 40 |
| *P. pseudomarrubioides* R.H. Willemse | 1970-4305 | −37 (18.4) | 1000 | 1000 |
| *P. saccatus* Benth. | 1996-2730 | 38 (15.8)* | – | 1000 |
| *P. zuluensis* T. Cooke | 1992-2850 | 26 (11.4) | 1000 | 1000 |
| Chloramphenicol (pos. control) | | – | 6.25 | 6.25 |

**$p < 0.01$, *$p < 0.05$ Wilcoxon matched pairs test ($n = 8$–15 larvae per extract tested).
[a] Feeding index $((C - T)/(C + T))\%$ at 100 ppm (see Section 3.5 for details); −ve value = phagostimulant, +ve value = antifeedant.

Table 3
Effect of diterpenoids isolated from species of *Plectranthus* on insect feeding and bacterial growth

| Diterpenoid | *Spodoptera littoralis* | | Minimum inhibitory concentration (μg/ml) | |
|---|---|---|---|---|
| | Feeding index[a] Mean (SEM) | $FI_{50}$[b] | *Bacillus subtilis* (Gram-positive) | *Pseudomonas syringae* (Gram-negative) |
| Diterpenoid (**1**) | 38 (4.7)* | nd | – | – |
| Coleon A (**3a** + **3b**) | 64 (10.0)** | 87 | 25 | – |
| Coleon A lactone (**4**) | 48 (12.3)* | 165 | 25 | 50 |
| Coleon U (**5**) | 18 (21.7) | nd | 3.13 | 6.25 |
| Coleon U quinone (**6**) | 56 (5.6)* | 91 | 25 | 3.12 |
| Chloramphenicol (pos. control) | – | – | 6.25 | 6.25 |

**$p < 0.01$, *$p < 0.05$ Wilcoxon matched pairs test.
[a] Feeding index $((C - T)/(C + T))\%$ at 100 ppm (see Section 3.5 for details); +ve value = antifeedant.
[b] $FI_{50}$ = concentration (ppm) calculated to give a feeding index of 50%; nd = no dose-dependent response ($n = 8$–15 larvae per concentration tested).

### 2.3. Antimicrobial activity

The same extracts tested in the insect assay were investigated for their antibacterial activities against Gram-positive (*Bacillus subtilis*) and Gram-negative (*Pseudomonas syringae*) bacteria. The minimum inhibitory concentration (MIC) of each extract against the bacteria was determined and the results are presented in Table 2. The extracts of *P. madagascariensis* and of *P. forsteri* 'Marginatus' were active against both species of bacteria. The extract of *P. argentatus* was highly active only against Gram-positive bacteria. The extract of *P. puberulentus* was highly active against Gram-negative bacteria and that of *P.* aff. *puberulentus* showed moderate activity against both types of bacteria. Coleon A (**3a** + **3b**), present in the extract of *P.* aff. *puberulentus*, showed activity against *B. subtilis* and coleon A lactone (**4**), isolated from *P. puberulentus*, showed activity against both *B. subtilis* and *P. syringae* (Table 3). Of note were the activities of coleon U (**5**) and coleon U quinone (**6**), which were comparable with that of the positive control, chloramphenicol, against *B. subtilis* and *P. syringae*, respectively. The presence of coleon U (**5**) may explain the antibacterial activities of the extracts from *P. forsteri* 'Marginatus' , *P. madagascariensis* and *P. argentatus* as HPLC analysis of these extracts indicated the presence of relatively high amounts. Coleon U (**5**) is an interesting compound, as it has also proved active against cancer cells (Marques et al., 2002). All the diterpenoids tested only displayed bacteriostatic and not bactericidal activity.

None of the extracts *of Plectranthus* showed any antifungal activity. Of the three diterpenoids tested, only coleon U (**5**) showed moderate antifungal activity, with a MIC of 200 μg/ml.

## 3. Experimental

### 3.1. General

$^{1}H$ and $^{13}C$ NMR spectra were recorded in $CDCl_3$ on Bruker Avance 400 and Varian 600 MHz spectrometers. Chemical shift referencing was carried out using TMS as an internal standard. ESI-MS data were obtained using a quadrupole ion trap mass spectrometer (Thermo-Finnigan LCQ) in negative mode. High-resolution ESI-MS data were recorded on a Bruker Daltonics micrOTOF instrument operating in the positive mode. A Perkin–Elmer 141 polarimeter with a 10 cm light path cylindrical cell of 1 ml volume was used to measure optical rotation.

### 3.2. Plant material

All the species of *Plectranthus* studied were cultivated under glasshouse conditions at the Royal Botanic Gardens, Kew, and voucher specimens have been deposited in the herbarium at Kew (K). The Kew Accession Numbers of species for which crude extracts were made for antifeedant and antimicrobial bioassays are listed in Table 2.

### 3.3. Extraction and isolation

Extracts for activity tests were prepared by steeping small portions of fresh *Plectranthus* leaves in acetone for about two minutes at room temperature. This quick and easy method was used because the diterpenoids of interest are located in leaf glands on the leaf surface and can be extracted without having to cut or grind the leaves. The extracts were left to evaporate in a fume cupboard to give the dried plant extracts.

A dried extract (0.8 g) obtained after steeping fresh *P. saccatus* leaves (1.3 kg) in acetone and solvent evaporation was subjected to silica gel CC, eluting with a petroleum ether:chloroform:EtOAc gradient system from 100% petroleum ether to 100% EtOAc. A fraction selected on the basis of its activity in the insect bioassays (Section 3.5) gave 49 mg of a 68:32 mixture of **1**, and its dihydro derivative **2**, after semi-preparative HPLC using the method described in Section 3.4 ($t_R$ ca. 22 min). This sample, when subjected to a second semi-preparative HPLC step, afforded 7 mg of a 93:7 mixture of **1** and **2** as a white crystalline solid.

For the isolation of coleon A (**3a** + **3b**) and coleon A lactone (**4**), dried acetone extracts of *P.* aff. *puberulentus* and *P. puberulentus* were subjected to silica gel CC eluting with a petroleum ether:EtOAc gradient system (1:0 to 0:1). Brown and orange zones clearly separated on the column. These zones were collected to afford coleon-A (**3a** + **3b**) from *P.* aff. *puberulentus* and coleon-A-lactone (**4**) with a small impurity of coleon-A (**3a** + **3b**) from *P. puberulentus*. The dried extract of *P. forsteri* 'Marginatus' was subjected to preparative TLC (Merck, Kieselgel 60, 1 mm) with petroleum ether:EtOAc:$CHCl_3$ (19:4:4), to yield a distinct yellow-orange zone rich in coleon U (**5**) with varying amounts of coleon U quinone (**6**), which were further separated by semi-preparative HPLC.

### 3.4. Analytical and semi-preparative HPLC

The HPLC system consisted of a Waters LC 600 pump, 996 photodiode array detector, 717 autosampler and a computerized data station equipped with Waters Millennium software. LiChrospher 100RP-18 columns were used; 4.0 mm × 250 mm for analytical and 10.0 mm × 250 mm for semi-preparative applications. A gradient system based on two solvents (A and B) was employed. For analytical work, solvent A consisted of MeOH:$H_2O$:AcOH, 90:5:5 (vol/vol) and B was 2% acetic acid in $H_2O$. Initial conditions were 60% A and 40% B, with a linear gradient reaching 100% A at $t = 15$ min. This was followed by isocratic elution with 100% A to $t = 20$ min after which the programme returned to the initial solvent composition. For semi-preparative work, solvent A was MeOH with 0.1% trifluoroacetic acid and B was water with 0.08% trifluoro-

acetic acid. Initial conditions were set to 60% A and 40% B, with a linear gradient reaching 100% A at $t = 20$ min. This was followed by isocratic elution with 100% A to $t = 25$ min and a return to initial conditions at $t = 27$ min. The column temperature was maintained at 30 °C. Flow rates were 1.0 and 4.5 ml/min for analytical and semi-preparative work, respectively.

### 3.5. Antifeedant bioassay

A binary choice bioassay using sucrose treated glass-fibre discs (Whatman 2.1 cm diameter) was used to investigate whether extracts or compounds from the different species of *Plectranthus* influenced the feeding behaviour of final stadium larvae of *S. littoralis* (Simmonds et al., 1990). Larvae were placed singly in a petri dish with a control disc (*C*) and a disc treated with the test extract or test compound (*T*). The respective amounts eaten of each disc were used to calculate the feeding index $((C - T)/(C + T))\%$. Antifeedant activity is represented by a positive value, whereas phagostimulant activity is represented by a negative value. The extracts and the compounds were each tested at 3–4 concentrations (1, 10, 100 and 500 ppm). Each concentration was tested against 8–15 different larvae. The Wilcoxon matched-pairs test was used to analyse the data. Regression analysis was used to calculate the concentration (ppm) required to give a feeding index of 50% ($FI_{50}$). The feeding index for each extract at 100 ppm is presented so that the results can be compared with those from other studies.

### 3.6. Antibacterial assay

The crude acetone extracts of *Plectranthus* and the isolated compounds coleon A (**3**), coleon A lactone (**4**), coleon U (**5**) and coleon U quinone (**6**) were tested against *B. subtilis* (IMI347329) and *P. syringae* pv. *syringae* (IMI347448) in a medium containing peptone (1%), NaCl (0.5%), and yeast extract (0.3%). A 50%-microdilution method in 96-well plates was used. The final concentration range for the crude extracts was 1000–8 μg/ml and the test was performed with three replicates per concentration. The final concentration range for the pure compounds was 200–0.39 μg/ml and test was performed in four replicates per concentration. Chloramphenicol was used as positive control. The plates were incubated in the dark at 37 °C for 24 h. Following this 20 μl of *p*-iodonitrotetrazolium chloride solution (stock solution 1 mg/ml EtOH, diluted 10-fold in $H_2O$ immediately prior to use) were added and incubated for a further 60 min under the same conditions. The colour development was observed visually.

### 3.7. Antifungal assay

The antifungal activity of the crude extracts and the isolated compounds coleon A, coleon A lactone, and coleon U were tested against *Cladosporium herbarum* (IMI300461) suspended in a medium containing malt extract (2%), peptone (0.2%), and glucose (1%) using a 50%-microdilution method in 96-well plates. The final concentration range for the crude extracts was 1000-8 μg/ml and the test was performed with three replicates per concentration. The final concentration range for the pure compounds was 200–0.39 μg/ml and test was performed in four replicates per concentration. Nystatin was used as positive control and tested in the same concentration range as the pure compounds. The plates were incubated in the dark at 25 °C for 72 h and the concentration that gave complete growth inhibition was considered the active concentration.

### 3.8. Compounds

#### 3.8.1. *ent*-3β-(3-Methyl-2-butenoyl)oxy-15-beyeren-19-oic acid (**1**)

Colourless solid (MeOH); $[\alpha]_D^{22}$ −2.8° ($CHCl_3$; *c* 0.6) (note that the sample contained 7% of **2**): UV (MeOH) $\lambda_{max}$ nm: 214. For $^1H$ and $^{13}C$ NMR spectra, see Table 1; ESI-MS (negative mode) *m/z*: 399 $[M - H]^-$; HRESIMS *m/z*: 423.2516 $[M + Na]^+$ (calc. for $C_{25}H_{36}O_4Na$, 423.2506).

#### 3.8.2. *ent*-3β-(3-Methylbutanoyl)oxy-15-beyeren-19-oic acid (**2**)

Colourless solid (MeOH); $^1H$ NMR for C-3 side chain ($CDCl_3$): δ 4.56 (1H, *dd*, $J = 12.2$, 4.5 Hz, H-3), 2.21 (2H, *m*, $CH_2$-2′), 2.11 (1H, *m*, H-3′), 0.95 (6H, *d*, $J = 6.6$ Hz, $CH_3$-4′ and $CH_3$-5′); $^{13}C$ NMR for C-3 side chain ($CDCl_3$): δ 172.8 (C-1′), 79.0 (C-3), 43.8 (C-2′), 25.7 (C-3′), 22.4 ($CH_3$-4′ and $CH_3$-5′); ESI-MS (negative mode) *m/z*: 401 $[M - H]^-$.

#### 3.8.3. (4*R*,19*R*)-Coleon A (**3a**)

Orange solid (MeOH); UV (MeOH) $\lambda_{max}$ nm: 253, 313, 432; $^1H$ NMR ($CDCl_3$): δ 13.40 (1H, *s*, OH-7), 8.09 (1H, *s*, OH-12), 5.85 (1H, *br d*, $J = 3.9$ Hz, H-19), 5.61 (1H, *m*, H-2), 5.10 (2H, *m*, $CH_2$-1), 3.60 (1H, *d*, $J = 4.9$ Hz, OH-19), 3.35 (1H, *septet*, $J = 7.1$ Hz, H-15), 2.67 (3H, *s*, $CH_3$-20), 2.51 (2H, *m*, $CH_2$-3), 1.51 (3H, *s*, $CH_3$-18), 1.29 (6H, *d*, $J = 7.1$ Hz, $CH_3$-16 and $CH_3$-17); $^{13}C$ NMR ($CDCl_3$): δ 191.2 (C-14), 180.6 (C-11), 154.5 (C-12), 152.4 (C-6), 146.9 (C-7), 137.0 (C-5), 134.4 (C-10), 132.5 (C-2), 125.4 (C-13), 120.3 (C-9), 119.5 (C-1), 116.8 (C-8), 107.1 (C-19), 50.6 (C-4), 43.0 (C-3), 23.9 (C-15), 19.8 ($CH_3$-16, $CH_3$-17), 18.4 ($CH_3$-18), 17.4 ($CH_3$-20); ESI-MS (negative mode) *m/z*: 357 $[M - H]^-$.

#### 3.8.4. (4*R*,19*S*)-Coleon A (**3b**)

Orange solid (MeOH); UV (MeOH) $\lambda_{max}$ nm: 253, 313, 432; $^1H$ NMR ($CDCl_3$): δ 13.40 (1H, *s*, OH-7), 8.09 (1H, *s*, OH-12), 5.77 (1H, *m*, H-2), 5.72 (1H, *d*, $J = 6.6$ Hz, H-19), 5.13, 5.02 ($2 \times 1H$, $2 \times m$, $CH_2$-1), 3.85 (1H, *d*, $J = 6.8$ Hz, OH-19), 3.35 (1H, *septet*, $J = 7.1$ Hz, H-15), 2.65 (3H, *s*, $CH_3$-20), 2.77, 2.67 ($2 \times 1H$, $2 \times$ m, $CH_2$-3), 1.45 (3H, *s*, $CH_3$-18), 1.29 (6H, *d*, $J = 7.1$ Hz, $CH_3$-16 and $CH_3$-17); $^{13}C$ NMR ($CDCl_3$): δ 191.2 (C-14), 180.6 (C-11), 154.5

(C-12), 152.1 (C-6), 146.9 (C-7), 136.9 (C-5), 135.1 (C-2), 134.3 (C-10), 125.5 (C-13), 120.2 (C-9), 118.4 (C-1), 116.8 (C-8), 110.5 (C-19), 50.0 (C-4), 38.5 (C-3), 24.6 ($CH_3$-18), 23.9 (C-15), 19.8 ($CH_3$-16, $CH_3$-17), 17.6 ($CH_3$-20); ESI-MS (negative mode) $m/z$: 357 $[M - H]^-$.

### 3.8.5. Coleon A lactone (*4*)

Orange solid (MeOH); UV (MeOH) $\lambda_{max}$ nm: 253, 301, 419; $^1H$ NMR ($CDCl_3$): $\delta$ 13.34 (1H, *s*, OH-7), 7.96 (1H, *br s*, OH-12), 5.33 (1H, *m*, H-2), 5.05 (1H, *ddd*, $J = 16.9$, 2.7, 1.2 Hz, H-1), 4.95 (1H, *m*, H-1), 3.38 (1H, *septet*, $J = 7.1$ Hz, H-15), 2.84 (2H, *m*, $CH_2$-3), 2.69 (3H, *s*, $CH_3$-20), 1.66 (3H, *s*, $CH_3$-18), 1.29 (6H, *d*, $J = 7.0$ Hz, $CH_3$-16 and $CH_3$-17); $^{13}C$ NMR ($CDCl_3$): chemical shift values in agreement with the literature (Künzle et al., 1987), however, the original assignments for C-9 and C-10 should be interchanged; ESI-MS (negative mode) $m/z$: 355 $[M - H]^-$.

### 3.8.6. Coleon U (*5*)

Yellow-orange solid (MeOH); UV (MeOH) $\lambda_{max}$ nm: 259, 283, 331, 383; $^1H$ NMR ($CDCl_3$): $\delta$ 12.90 (1H, *br s*, OH-14), 6.98 (1H, *s*, OH-6), 6.05 (1H, *br s*, OH-12), 4.98 (1H, *s*, OH-11), 3.47 (1H, *br m*, H-15), 2.87, 1.84 ($2 \times 1H$, $2 \times br\ m$, $CH_2$-1), 1.99, 1.45 ($2 \times 1H$, $2 \times br\ m$, $CH_2$-3), 1.87, 1.67 ($2 \times 1H$, $2 \times br\ m$, $CH_2$-2), 1.66 (3H, *s*, $CH_3$-20), 1.46, 1.44 ($2 \times 3H$, $2 \times br\ s$, $CH_3$-18 and $CH_3$-19), 1.38 (6H, *br d*, $J = 6.9$ Hz, $CH_3$-16 and $CH_3$-17); $^{13}C$ NMR ($CDCl_3$): chemical shift values in agreement with the literature (Miyase et al., 1977), however, the original assignments for C-5 and C-6, should be interchanged, and those for C-1, $CH_3$-19 and $CH_3$-20 corrected to those of $CH_3$-20, C-1 and $CH_3$-19, respectively; ESI-MS (negative mode) $m/z$: 345 $[M - H]^-$.

### 3.8.7. Coleon U quinone (*6*)

Orange-brown solid (MeOH); UV (MeOH) $\lambda_{max}$ nm: 247, 275sh, 432; $^1H$ NMR ($CDCl_3$): $\delta$ 7.08 (1H, *s*, OH-6), 7.06 (1H, *s*, OH-12), 3.23 (1H, *septet*, $J = 7.0$ Hz, H-15), 2.65, 1.60 ($2 \times 1H$, $2 \times br\ m$, $CH_2$-1), 2.00, 1.47 ($2 \times 1H$, $2 \times br\ m$, $CH_2$-3), 1.90, 1.62 ($2 \times 1H$, $2 \times br\ m$, $CH_2$-2), 1.64 (3H, *s*, $CH_3$-20), 1.43, 1.42 ($2 \times 3H$, $2 \times br\ s$, $CH_3$-18 and $CH_3$-19), 1.26 (6H, *br d*, $J = 7.0$ Hz, $CH_3$-16 and $CH_3$-17); $^{13}C$ NMR ($CDCl_3$): chemical shift values in agreement with the literature (Alder et al., 1984), however, the original assignments for $CH_3$-20 and $CH_3$-18 should be interchanged; ESI-MS (negative mode) $m/z$: 343 $[M - H]^-$.

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## References

Abdel-Mogib, M., Albar, H.A., Batterjee, S.M., 2002. Chemistry of the genus *Plectranthus*. Molecules 7, 271–301.

Alder, A.C., Rüedi, P., Eugster, C.H., 1984. Drüsenfarbstoffe aus Labiaten: Identifizierung von 17 Abietanoiden aus *Plectranthus sanguineus* BRITTEN. Helv. Chim. Acta 70, 975–983.

Batista, O., Simões, M.F., Duarte, A., Valdeira, M.L., De la Torre, M.C., Rodríguez, B., 1995. An antimicrobial abietane from the root of *Plectranthus hereroensis*. Phytochemistry 38, 167–169.

Baxter, R.L., Blake, A.J., Gould, R.O., 1995. The absolute configuration of coleon A: a seco diterpenoid pigment from *Coleus* spp. Phytochemistry 38, 195–197.

Bohlmann, F., Wegner, P., 1982. *ent*-Beyer-15-ene derivatives from *Nidorella anomala*. Phytochemistry 21, 1175–1177.

Connolly, J.D., Hill, R.A., 1991Dictionary of Terpenoids, vol. 1. Chapman and Hall, London.

Dellar, J.E., Cole, M.D., Waterman, P.G., 1996. Antimicrobial abietane diterpenoids from *Plectranthus elegans*. Phytochemistry 41, 735–738.

Grande, M., Mancheño, B., Sanchez, M.J., 1989. Elasclepiol and elasclepic acid, beyerane diterpenoids from *Elaeoselinum asclepium*. Phytochemistry 28, 1955–1958.

Hanson, J.R., 2004. Diterpenoids. Nat. Prod. Rep. 21, 785–793.

Karanatsios, D., Eugster, C.H., 1965. Struktur von Coleon A. Helv. Chim. Acta 48, 471–508.

Kubo, I., Matsumoto, T., Tori, M., Asakawa, Y., 1984. Structure of plectrin, an aphid antifeedant diterpene from *Plectranthus barbatus*. Chem. Lett., 1513–1516.

Künzle, J.M., Rüedi, P., Eugster, C.H., 1987. Isolierung und Strukturaufklärung von 36 Diterpenoiden aus Trichomen von *Plectranthus edulis* (VATKE) T.T.AYE. Helv. Chim. Acta 70, 1911–1929.

Liu, G., Müller, R., Rüedi, P., 2003. Chemical transformations of phyllocladane (=13β-kaurane) diterpenoids. Helv. Chim. Acta 86, 420–438.

Lukhoba, C.W., Simmonds, M.S.J., Paton, A.J., 2006. *Plectranthus*: A review of ethnobotanical uses. J. Ethnopharmacol. 103, 1–24.

Macmillan, J., Beale, M.H., 1999. Diterpene biosynthesis. In: Barton, D., Nakanishi, K., Meth-Cohn, O. (Eds.), Comprehensive Natural Products Chemistry, vol. 2. Elsevier, Oxford, pp. 217–243.

Marques, C.G., Pedro, M., Simões, M.F.A., Nascimento, M.S.J., Pinto, M.M.M., Rodríguez, B., 2002. Effect of abietane diterpenes from *Plectranthus grandidentatus* on the growth of human cancer cell lines. Planta Med. 68, 839–840.

Matu, E.N., van Staden, J., 2003. Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. J. Ethnopharmacol. 87, 35–41.

Miyase, T., Rüedi, P., Eugster, C.H., 1977. Diterpenoide Drüsenfarbstoffe aus Labiaten: Coleone U, V, W und 14-*O*-Formyl-coleon-V sowie 2 Royleanone aus *Plectranthus myrianthus* BRIQ.; *cis*- und *trans*-A/B-6,7-Dioxoroyleanon. Helv. Chim. Acta 60, 2770–2779.

Narukawa, Y., Shimizu, N., Shimotohno, K., Takeda, T., 2001. Two new diterpenoids from *Plectranthus nummularius* Briq. Chem. Pharm. Bull. 49, 1182–1184.

Paton, A.J., Springate, D., Suddee, S., Otieno, D., Grayer, R.J., Harley, M.M., Willis, F., Simmonds, M.S.J., Powell, M.P., Savolainen, V., 2004. Phylogeny and evolution of basils and allies (Ocimeae, Labiatae)

based on three plastid DNA regions. Mol. Phylogenet. Evol. 31, 277–299.

Rijo, P., Gaspar-Marques, C., Simões, M.F., Duarte, A., Areda-Rojas, M.D., Cano, F.H., Rodríguez, B., 2002. Neoclerodane and labdane diterpenoids from *Plectranthus ornatus*. J. Nat. Prod. 65, 1387–1390.

Rivera Nuñez, D., Obón de Castro, C., 1992. The ethnobotany of Old World Labiatae. In: Harley, R.M., Reynolds, T. (Eds.), Advances in Labiate Science. Royal Botanic Gardens, Kew, pp. 455–473.

Simmonds, M.S.J., Blaney, W.M., Fellows, L.E., 1990. Behavioural and electrophysiological study of antifeedant mechanisms associated with polyhydroxyalkaloids. J. Chem. Ecol. 16, 3167–3196.

Teixeria, A.P., Batista, O., Simões, M.F., Nascimento, J., Duarte, A., De la Torre, M.C., Rodríguez, B., 1997. Abietane diterpenoids from *Plectranthus grandidentatus*. Phytochemistry 44, 325–327.

Zdero, C., Bohlmann, F., King, R.M., Robinson, H., 1989. Sesquiterpene lactones and other constituents from Australian *Helipterum* species. Phytochemistry 28, 517–526.