Stereoselective synthesis, natural occurrence and CB_2 receptor binding affinities of alkylamides from herbal medicines such as *Echinacea* sp.

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A divergent synthesis of (2*E*,4*E*,8*E*,10*E*)- and (2*E*,4*E*,8*E*,10*Z*)-*N*-isobutyldodeca-2,4,8,10-tetraenamides from pent-4-yn-1-ol allowed identification of the (2*E*,4*E*,8*E*,10*Z*)-isomer for the first time in *Echinacea* species. A short, stereoselective synthesis of the (2*E*,4*E*,8*E*,10*Z*)-isomer is also described which allowed further biological evaluation of this material, and the demonstration that this isomer does not occur in *Spilanthes mauritiana* as previously reported.

Introduction

Polyunsaturated isobutyl alkyl amides are found in a variety of plants that posses interesting biological activities. The two tetraene isomers **1** and **2** (Fig. 1), are the major lipophilic constituents found in the two commonly used *Echinacea* species (*E. purpurea* and *E. angustifolia*).[1] They have also been found in other medicinal plants such as *Salmea scandens*,[2] and *Asarum forbesii* Maxim.[3] *S. scandens* has various uses in traditional medicine, especially as an anaesthetic for treating toothache,[4] while *A. forbesii* Maxim has been used for its analgesic, antitussive, anti-allergic and diuretic effects.[5] The 8*E*,10*Z* tetraene isomer **3** has also been reported to occur in *Spilanthes mauritiana*, and the isolated compound was demonstrated to be a potent mosquito larvicide.[6]

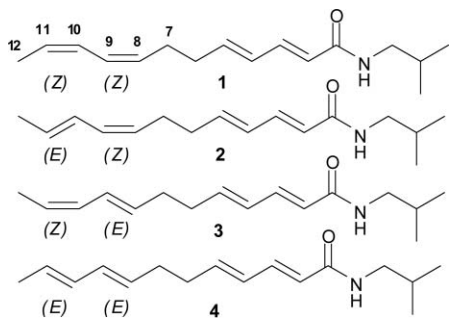

Fig. 1 Four geometric isomers about the 8,10 double bonds of isobutyl-dodeca-2(*E*), 4(*E*), 8, 10-tetraenamide are possible. Only **1**, **2** and **3** were identified in *E. purpurea* and *E. angustifolia.*

E. purpurea and *E. angustifolia* are the most widely used species in *Echinacea* derived herbal medicines and together form the basis of the *Echinacea* industry, on which an estimated $US500 million dollars was spent in the USA alone in 2002.[7] These preparations are popularly believed to act *via* modulating the cellular immune system and frequently taken for prevention or treatment of cold and flu like symptoms.[8] The phytochemical profile of *E. purpurea* and *E. angustifolia* is well documented, consisting of caffeic acid derivatives, mono and polysaccharides and a complex mixture of alkyl amides. It is still unclear exactly which components are responsible for the reported effects of *Echinacea* extracts, however recent publications have focused on the alkyl amide constituents found in commercial (mainly ethanolic) extracts. Although the effectiveness of *Echinacea* in preventing viral illness has recently been called into question,[9] it is clear that the alkyl amides do possess significant biological activities. The alkyl amides are the only components of *Echinacea* extracts found to cross the intestinal barrier,[10] and they have been reported to display anti-inflammatory activity.[11] Certain alkyl amides have also recently been shown to act as full agonists at the cannabinoid type-2 (CB_2) receptor.[12]

Analysis of the alkyl amides is typically performed using RP-HPLC, but this fails to completely resolve the predominant tetraenes **1** and **2**, which are thus reported as a mixture. GCMS analysis not only separates isomers **1** and **2**, but also reveals a third component (Fig. 2) with mass spectral fragmentation characteristic of tetraene alkyl amides.[13] This third component was suspected to be either **3** or **4**, *cis*/*trans* isomers of **1** and **2**. We identified tetraenes **1** and **2** by comparison to synthetic standards available in our laboratory but identification of the third component required the synthesis of **3** and **4**.

Results and discussion

The simplest approach for rapid access to both **3** and **4** in isomerically pure form was by a divergent synthesis that started from pent-4-yn-1-ol, available in two steps from commercial tetrahydrofurfural alcohol (Scheme 1).[14] THP protection, followed by treatment with butyl lithium and then paraformaldehyde gave propargyl alcohol **6**, which after treatment with $LiAlH_4$ cleanly provided the *E*-allyl alcohol **7**. Consecutive Swern and Wittig reactions resulted in a 1 : 1 mixture of **8a** and **8b** that could be separated into the pure isomers by chromatography utilising silver nitrate impregnated silica.[15] The stereochemistry of the dienes was

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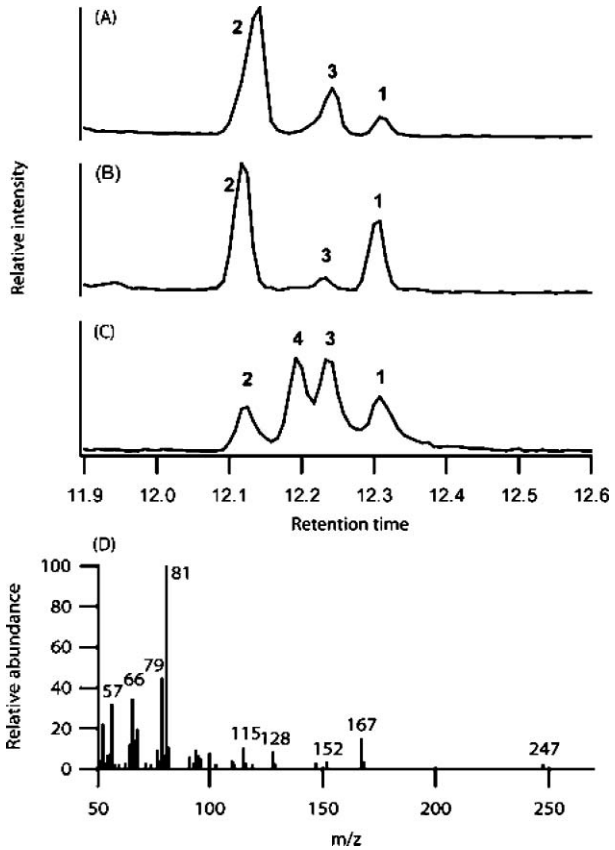
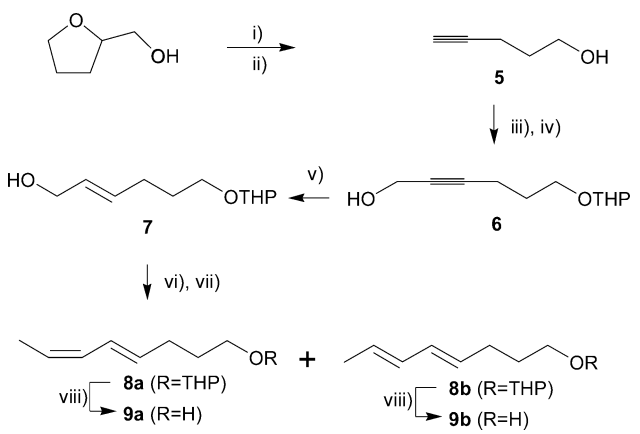


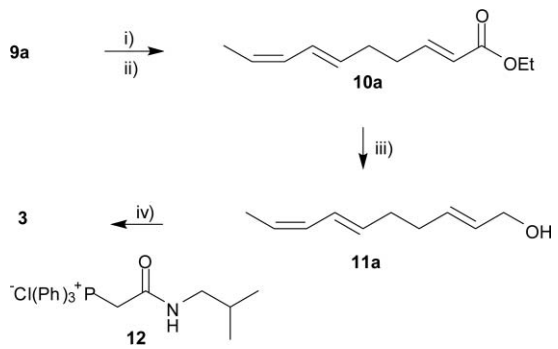
Fig. 2 GC/MS trace of *E. angustifolia* (**A**) and *E. purpurea* (**B**) root extracts and synthetic standards (**C**). Mass spectrum of **3** (**D**).



Scheme 1 *Reagents and conditions*: i) $SOCl_2$, pyridine, 84%; ii) $NaNH_2$, NH_3 liq, 91%; iii) DHP, TsOH, CH_2Cl_2, 88%; iv) a) ^{n}BuLi, THF, −78 °C; b) $(CHO)_n$, 84%; v) $LiAlH_4$, Et_2O, reflux, 85%; vi) a) DMSO, $(COCl)_2$, DCM, −78 °C, then Et_3N; b) $Ph_3P{=}CHCH_3$, Et_2O, −78 °C; vii) silica gel–$AgNO_3$ chromatography, 22% **8a**, 18% **8b**; viii) TsOH, MeOH, 91% **9a**, 89% **9b**.

readily ascertained by NMR. In the ^{1}H NMR of **8a** the two hydrogens belonging to the newly formed *Z*-double bond were easily distinguished ($CDCl_3$, δ 5.37, dq and δ 5.97, br t), and showed the 11 Hz coupling expected of a *cis* olefin. The hydrogens of the newly formed *E*-double bond of **8b** could only be clearly identified in C_6D_6, but displayed the 14 Hz coupling expected of a *trans* alkene.

Alcohol **9a**, obtained from subsequent THP deprotection of **8a**, was converted into tetraene **3** in four steps as illustrated in Scheme 2, with the final Wittig reaction employing phosphonium salt **12**,[16] completing the installation of the isobutyl *E*,*E*-dieneamide moiety. This provided **3** as a white crystalline solid (mp 78–79 °C), previously only reported as an oil.[17] The same methodology was also used to obtain tetraene **4** (mp 104–106 °C) from **9b**, and constituted the first reported complete synthesis of this tetraene isomer. Tetraene **4** has been previously prepared by isomerisation (I_2/UV) of a mixture of **1** and **2**, that were isolated from *Asiasarum heterotropoides*,[18] and the reported ^{13}C NMR data agrees with our synthetic compound.



Scheme 2 *Reagents and conditions*: i) DMSO, $(COCl)_2$, CH_2Cl_2, −78 °C, then Et_3N; ii) $(EtO)_2POCH_2COOEt$, NaH, Et_2O, 71%; iii) DIBAL, Et_2O, 87%; iv) a) DMSO, $(COCl)_2$, CH_2Cl_2, −78 °C, then Et_3N; b) **12**, KO*t*-Bu, THF, 0 °C, 63%.

When comparing our NMR data for **3** with the reported data for the naturally occurring compound isolated from *S. mauritiana*,[6] we noted obvious discrepancies in both the ^{13}C and ^{1}H spectra. In particular the ^{13}C chemical shift for C7 was reported as δ 26.97 ppm compared with δ 32.1 ppm in our synthetic standard. Upon comparison of the NMR data for all four synthetic tetraene isomers, it was found that the data for natural material isolated from *S. mauritiana* was in fact consistent with that of the *Z*,*Z* isomer **1**, and not the *E*,*Z* isomer **3** as the authors had claimed.[6] Inspection of the ^{13}C NMR data from our synthetic tetraene standards revealed that the stereochemistry of the 8,10-diene moiety can be readily assessed by observation of the chemical shifts of C7 and C12, as shown in Table 1. The shielding experienced by allylic carbons adjacent to the *cis* double bond caused them to resonate approximately 5 ppm upfield compared with their *trans* counterparts, which is in accordance with literature observations.[18]

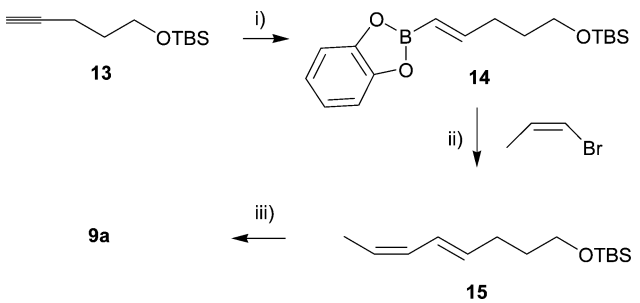
One other reported isolation of tetraene **3** from *Leucanthemum* sp. has appeared in the literature,[17] and although the ^{1}H NMR data reported matched our data for **3**, no ^{13}C NMR or other physical data was available for the natural compound. Thus this report[17]

Table 1 ^{13}C NMR chemical shift values of positions 7 and 12 of all four synthetic tetraene alkyl amides ($CDCl_3$)

Position	Tetraene **1** (ppm)	Tetraene **2** (ppm)	Tetraene **3** (ppm)	Tetraene **4** (ppm)
C7	26.7	26.9	32.1	31.8
C12	13.1	18.3	13.3	18.0

should be regarded as tentative and the ^{13}C NMR data for **3** is reported correctly for the first time in this communication.

A synthesis of **3** has also previously been reported,[19] but it contains no mention of stereochemical purity of the material obtained nor was ^{13}C data supplied. Additionally, the ^{1}H NMR data was stated to correlate with that of the incorrectly assigned material isolated from *S. mauritiana.* For this reason, and to supply a larger quantity of material for biological testing (*vide infra*), we undertook a stereospecific synthesis of **3**. We proposed that a Suzuki coupling[20] would provide the required stereocontrol in the preparation of the key alcohol intermediate **9a**. Thus, the boronate ester **14** was prepared in excellent yield from protected pentynol **13**. Upon coupling of **14** with *cis*-1-bromo propene under Suzuki conditions[20] (Scheme 3), the diene **15** was produced as the sole isolated product and was simply converted to the desired alcohol (**9a**). This was readily converted to **3** *via* the method already established (Scheme 2). In our hands, we found yields of the cross coupled product **15** to be very disappointing in the presence of a THP protecting group, but this was rectified by substitution with the TBS moiety.



Scheme 3 *Reagents and conditions*: i) catechol borane, 60 °C, 4 hr, 92%; ii) $Pd(PPh_3)_4$, NaOEt, benzene, 60 °C, 2 h, 77%; iii) MeOH, conc. HCl, 25 °C, 91%.

With standards of the various alkyl amides **1–4** in hand, GCMS analysis of *Echinacea* extracts, along with co-injections with either **3** or **4**, were undertaken. These studies revealed that the third tetraene present in these extracts (Fig. 1) was indeed the *E*,*Z* isomer **3** and that none of the *E*,*E* isomer **4** could be detected in any *Echinacea* sample examined. This prompted us to determine the relative proportions of the various tetraenes **1–4** in several *E. angustifolia* and *E. purpurea* root samples from Australia, Canada, Germany, New Zealand, USA and Kenya. Not surprisingly, the levels of tetraenes differed between the two species (Table 2). Unexpectedly, however, the relative abundance of **1–3** differed *within* a species, especially in *E. purpurea* (Table 2). However, not only did the levels of tetraenes in *E. purpurea* extracts differ greatly between samples grown in different locations but differences were found between plants grown in the same field! There did not appear to be any obvious geographical trends that could be correlated with the differences in respective tetraene levels.

Due to the different abundance of the three isomers and the variation between the two *Echinacea* species, we wished to investigate the effect, if any, that double bond geometry had upon biological activity of these isomers **1–4**. We chose to examine the binding affinity of each isomer for the CB_2-receptor, as a recent study has shown that the tetraene alkyl amide **1** binds more strongly to this receptor than do endogenous cannabinoids.[12]

These studies revealed that tetraene **1** is a high affinity binder for the CB_2 receptor as it competitively inhibited binding of the synthetic high affinity cannabinoid ligand [^{3}H]CP55,940 (Table 1). However, tetraenes **2** and **3**, whilst binding to CB_2 exhibited much lower affinities, possessing K_i values approximately two orders of magnitude higher than isomer **1**. Interestingly, tetraene **4**, which has not been reported to occur naturally, showed no binding affinity for the CB_2 receptor.

Conclusions

Concomitant use of GC/MS and pure synthetic tetraene alkyl amides, has allowed the positive identification of the (2*E*,4*E*,8*E*,10*Z*) tetraene isomer in *Echinacea* for the first time. The variation in the binding affinity for the CB_2 receptor observed for the tetraene alkyl amides **1–3** raises the possibility that some of the differences seen in the activity of *Echinacea* preparations may be linked to the species used (*E. purpurea* or *E. angustifolia*) and the fluctuating ratios of tetraenes that would be encountered. Reporting the total tetraene content of an *Echinacea* preparation may not be specific enough, and quantification of the individual isomers may be required to truly allow comparison of biological data with different extracts. It is expected that the short stereoselective synthesis of isomerically pure **3** and **4** reported here will aid in providing pure material as a reference for the standardization of *Echinacea* products.

Experimental

General conditions

All reactions involving air or moisture sensitive reagents were carried out under a nitrogen atmosphere in oven pre-dried glassware. Diethyl ether and THF were distilled from sodium–benzophenone prior to use; and dichloromethane was distilled from calcium hydride. $CDCl_3$ and C_6D_6 were purchased from

Table 2 Tetraene alkyl amide distribution in *E. angustifolia* and *E. purpurea*, and activity towards CB_2 receptor inhibition

	% in *E. angustifolia* [a] ($n = 6$)	% in *E. purpurea*[a] ($n = 10$)	K_i CB_2/nM ($n = 3$)
1	10 ± 3	32 ± 14	57 ± 9
2	80 ± 5	62 ± 12	9044 ± 2985
3	10 ± 2	6 ± 6	4535 ± 711
4	nd	nd	>100 000

[a] Expressed as a percentage of the total tetraene alkyl amide content. In *E. angustifolia* this was 18.8 ± 9.8 mg g^{-1} and in *E. purpurea* this was 4.4 ± 4.0 mg g^{-1}.

Cambridge Isotope Laboratories. NMR was performed on either a Bruker Avance 300 MHz, AV400 MHz or DRX500 MHz. Chemical shifts (δ) were referenced to internal solvent and are reported relative to $SiMe_4$. GC/MS analyses were performed using a Shimadzu-17A GC equipped with J & W Scientific DB5 column (internal diameter 0.2 mm/30 m) coupled to a Shimadzu QP5050 Mass Spectrometer (70 eV). *Echinacea* extracts obtained from Mediherb Pty. Ltd. were prepared for GC/MS analysis by the following general proceedure: 0.5 ml of solution was stripped of solvent under vacuum (<0.1 mm) without external heating. Once the solvent was removed (1–2 mins) the residue was re-dissolved in methanol (0.1 ml) and refrigerated until analysis. GC parameters were as follows: $t = 0$–2 mins oven temp = 100 °C; $t = 2$–11.375 mins oven temp = 100–250 °C, ramped at 16 °C/min; $t = 11.375$–21 mins oven temp = 250 °C; $t = 21$ mins end run. Injector temp = 250 °C; interface temp = 270 °C; flow rate = 76.9 ml min^{-1} helium; split ratio = 22; injection volume = 2 μl.

2-Octa-4(*E*),6(*E*)-dienyloxy-tetrahydro-pyran (8a) and 2-octa-4(*E*),6(*Z*)-dienyloxy-tetrahydro-pyran (8b). Oxalyl chloride (0.44 g, 3.5 mmol) was added to CH_2Cl_2 (25 ml) and the solution was cooled to −78 °C. DMSO (0.39 g, 5.0 mmol) was then added dropwise and after complete addition the solution was stirred a further 2 minutes before the dropwise addition of **7**,[21] (0.49 g, 2.45 mmol) dissolved in CH_2Cl_2 (3 ml). Stirring was continued at −78 °C for 30 minutes and then the reaction was warmed to −10 °C for 5 minutes. The mixture was then cooled to −78 °C and Et_3N (1.41 g, 14 mmol) was slowly added. After the mixture had returned to room temperature it was washed twice with water (20 ml). The organic layer was dried ($MgSO_4$) and the CH_2Cl_2 removed to give crude aldehyde (0.4 g), which was used in the next step without delay. δ_H(300 MHz; $CDCl_3$; Me_4Si): 1.46–1.89 (m, 8H), 2.46 (q, 2H, $J = 6.7$ Hz), 3.89–4.06 (m, 2H), 4.23–4.40 (m, 2H), 4.59 (t, 1H, $J = 3.9$ Hz), 6.16 (qt, 1H, $J = 1.5$, 7.8 Hz), 6.89 (dt, 1H, $J = 6.9$, 7.8 Hz), 9.52 (d, 1H, $J = 7.8$ Hz).

Butyllithium (2.46 ml, 3.2 mmol of a 1.3M solution in hexanes) was added dropwise to a suspension of ethyltriphenylphosphonium iodide (1.50 g, 3.6 mmol) in ether (20 ml) at room temperature. The resulting deep orange solution was stirred for a further 25 minutes, then cooled to 0 °C using an external ice-bath. Next the crude aldehyde prepared above was dissolved in ether (5 ml) and added dropwise over 2 minutes. After stirring a further 20 minutes at 0 °C, water (20 ml) was added and the mixture stirred vigorously for 5 minutes. The water was separated and the ether layer washed with brine, then dried ($MgSO_4$) and evaporated. Then the residue was taken up in ether–hexane (1 : 1, 50 ml) and passed through a short column of silica gel in order to remove Ph_3PO. The silica was rinsed well with solvent and the combined filtrates were evaporated to leave a colourless oil containing a roughly 1 : 1 mixture of **8a** and **8b** (220 mg) as evidenced by GC/MS. Separation of the isomers was achieved by column chromatography using silver nitrate impregnated silica gel prepared from $AgNO_3$ (2.9 g) and silica gel 60 (22 g). Elution with 5% ethyl acetate in hexanes afforded **8b** (92 mg, 18%) which eluted first, followed by **8a** (113 mg, 22%).

8b δ_H(300 MHz; $CDCl_3$; Me_4Si): 1.43–1.90 (m, 11H), 2.14 (q, 2H, $J = 7.8$ Hz), 3.33–3.53 (m, 2H), 3.69–3.90 (m, 2H), 4.58 (t, 1H, $J = 3.0$ Hz), 5.50–5.62 (m, 2H), 5.95–6.07 (m, 2H). δ_C(75 MHz; $CDCl_3$; Me_4Si): 17.9, 19.6, 25.6, 29.2, 29.5, 30.7, 62.2, 66.9, 98.8, 126.8, 130.7, 131.1, 131.6. δ_H(500 MHz; C_6D_6; Me_4Si): 1.20–1.41 (m, 3H), 1.51–1.81 (m, 5H), 1.58 (d, 3H, d, $J = 7.0$ Hz), 2.14 (q, 2H, $J = 7.5$ Hz), 3.31 (dt, 1H, $J = 6.5$, 9.5 Hz), 3.35–3.41 (m, 1H), 3.76–3.82 (m, 1H), 3.79 (dt, 1H, $J = 6.5$, 9.5 Hz), 4.56 (dd, 1H, $J = 3.0$, 3.0 Hz), 5.99–6.08 (m, 2H), 5.47 (dq, 1H, $J = 7.0$, 14.0 Hz), 5.51 (dt, 1H, $J = 7.0$, 14.0 Hz). HRMS EI(m/z): [M^+] calcd. for $C_{13}H_{22}O_2$, 210.1619; found, 210.1622.

8a δ_H(300 MHz; $CDCl_3$; Me_4Si): 1.42–1.87 (m, 11H), 2.20 (q, 2H, $J = 7.2$ Hz), 3.32–3.52 (m, 2H), 3.70–3.39 (m, 2H), 4.59 (t, 1H, $J = 3$ Hz), 5.38 (dq, 1H, $J = 7.2$, 11.1 Hz), 5.68 (dt, 1H, $J = 7.5$, 15.0 Hz), 5.98 (tq, 1H, $J = 1.2$, 11.1 Hz), 6.36 (ddq, 1H, $J = 1.2$, 10.8, 15 Hz). δ_C(75 MHz; $CDCl_3$; Me_4Si): 13.2, 19.6, 25.5, 29.4, 29.5, 30.7, 62.2, 66.9, 98.8, 124.0, 125.8, 129.4, 133.5. HRMS EI(m/z): [M^+] calcd. for $C_{13}H_{22}O_2$, 210.1619; found, 210.1618.

Octa-4(*E*),6(*E*)-dien-1-ol (9b). **8b** (82 mg, 0.39 mmol) was dissolved in MeOH (4 ml) and *p*-TsOH (6 mg, 0.03 mmol) was added. The mixture was stirred at room temperature for 2 hours then aq. NaOH (1 ml, 1M) solution was added followed by water (10 ml). The mixture was extracted repeatedly into ether, then the ether layers were washed with brine and dried ($MgSO_4$). The title product (43 mg, 87%) obtained after evaporation of the ether was sufficiently pure to carry on to the next step. δ_H(300 MHz; $CDCl_3$; Me_4Si): 1.62 (quint, 2H, $J = 6.6$ Hz), 1.71 (d, 3H, $J = 6.3$ Hz), 2.11 (q, 2H, $J = 7.0$ Hz), 2.22 (s, 1H), 3.60 (t, 2H, $J = 6.6$ Hz), 5.46–5.63 (m, 2H), 5.93–6.06 (m, 2H). δ_C(75 MHz; $CDCl_3$; Me_4Si): 17.9, 28.8, 32.3, 62.2, 127.1, 130.8, 130.9, 131.5. HRMS EI(m/z): [M^+] calcd. for $C_8H_{14}O$, 126.1045; found, 126.1042.

Octa-4(*E*),6(*Z*)-dien-1-ol (9a). Prepared in 91% yield from **8a** utilizing the procedure used for synthesizing **9b**. δ_H(400 MHz; $CDCl_3$; Me_4Si): 1.63 (quint, 2H, $J = 7.6$ Hz), 1.69 (dd, 3H, $J = 1.6$, 7.2 Hz,), 2.15 (q, 2H, $J = 7.6$ Hz), 2.26 (s, 1H), 3.56 (t, 2H, $J = 6.4$ Hz), 5.35 (dq, 1H, $J = 6.8$, 10.8 Hz), 5.61 (dt, 1H, $J = 6.8$, 15.2 Hz), 5.92 (tq, 1H, $J = 1.6$, 11.2 Hz), 6.32 (ddq, 1H, $J = 1.2$, 10.4, 11.2 Hz). δ_C(100 MHz; $CDCl_3$; Me_4Si): 13.2, 29.1, 32.2, 62.2, 124.3, 125.9, 129.2, 133.3. HRMS EI(m/z): [M^+] calcd. for $C_8H_{14}O$, 126.1045; found, 126.1048.

Deca-2(*E*),6(*E*),8(*E*)-trienoic acid ethyl ester (10b). Oxalyl chloride (64 mg, 0.5 mmol) was added to CH_2Cl_2 (12 ml) and the solution was cooled to −78 °C. DMSO (62 mg, 0.8 mmol) was then added dropwise and after complete addition the solution was stirred a further 2 minutes before the dropwise addition of **9b** (40 mg, 0.32 mmol). Stirring was continued at −78 °C for 30 minutes and then the reaction was warmed to −10 °C for 5 minutes. The mixture was then cooled to −78 °C and Et_3N (404 mg, 4.0 mmol) was added slowly. The reaction was allowed to return to room temperature, then washed once with water (5 ml), once with aq. HCl (5 ml, 1M) and then once more with water (5 ml). The organic layer was dried ($MgSO_4$) and the CH_2Cl_2 removed, leaving the crude aldehyde (35 mg) which was used in the next step without delay.

NaH (34 mg, 50% suspension in mineral oil, 0.7 mmol) was suspended in ether (5 ml) and cooled to −10 °C. Then (diethoxy-phosphoryl)-acetic acid ethyl ester (157 mg, 0.7 mmol) was added dropwise (H_2 evolution) with stirring. After complete addition the mixture was stirred a further 10 minutes and the crude aldehyde solution prepared above was added slowly. After stirring a further 10 minutes at −10 °C, water (10 ml) was added. The organic layer

was collected and the aqueous phase was extracted with hexanes. The combined organic layers were washed thoroughly with water and then once with brine. Drying ($MgSO_4$) and evaporation of the solvents left an oil which was purified by column chromatography (silica gel 60, 10% ether in pentane) to give the title compound (48 mg, 77% over 2 steps) as a colourless oil. δ_H(400 MHz; $CDCl_3$; Me_4Si): 1.23 (t, 3H, $J = 7.2$ Hz), 1.68 (d, 3H, $J = 6.4$ Hz), 2.13–2.28 (m, 4H), 4.13 (q, 2H, $J = 7.2$ Hz), 5.41–5.60 (m, 2H), 5.78 (dt, 1H, $J = 1.6$, 15.2 Hz), 5.91–6.02 (m, 2H), 6.90 (dt, 1H, $J = 7.2$, 15.2 Hz). δ_C(100 MHz; $CDCl_3$; Me_4Si): 14.1, 17.8, 30.8, 31.9, 59.9, 121.5, 127.5, 129.5, 131.2, 131.3, 148.1, 166.4. HRMS EI(m/z): [M^+] calcd. for $C_{12}H_{18}O_2$, 194.1307; found, 194.1307.

Deca-2(*E*),6(*E*),8(*Z*)-trienoic acid ethyl ester (10a). Prepared in 71% yield from **9a** utilizing the procedure used for synthesizing **10b**. δ_H(300 MHz; $CDCl_3$; Me_4Si): 1.24 (t, 3H, $J = 6.9$ Hz), 1.69 (dd, 1H, $J = 1.5$, 6.9 Hz), 2.20–2.30 (m, 4H), 4.14 (q, 2H, $J = 7.2$ Hz), 5.36 (dq, 1H, $J = 7.2$, 10.8 Hz), 5.58 (dt, 1H, $J = 6.3$, 15.0 Hz), 5.80 (dt, 1H, $J = 1.5$, 15.6 Hz), 5.87–5.97 (m, 1H), 6.33 (dd, 1H, $J = 10.8$, 13.5 Hz), 6.92 (dt, 1H, $J = 6.6$, 15.6 Hz). δ_C(100 MHz; $CDCl_3$; Me_4Si): 13.1, 14.1, 31.1, 31.9, 59.9, 121.7, 124.6, 126.3, 129.1, 131.9, 148.0, 166.4. HRMS EI(m/z): [M^+] calcd. for $C_{12}H_{18}O_2$, 194.1307; found, 194.1306.

Deca-2(*E*),6(*E*),8(*E*)-trien-1-ol (11b). DIBAL (67 mg, 0.46 mmol) was dissolved in ether (6 ml) and the solution cooled to 0 °C. Then **10b** (45 mg, 0.23 mmol) dissolved in ether (2 ml) was added dropwise. After stirring at 0 °C for one hour, the mixture was carefully quenched by the addition of water (0.2 ml), followed by aq. HCl (3 ml, 1M). The mixture was stirred for 30 minutes, then the organic layer was separated and the aqueous phase extracted with ether. The combined ether layers were washed with brine, dried ($MgSO_4$) and evaporated. Purification of the residue by column chromatography (silica gel 60, 20% ethyl acetate in hexane) gave the title compound (28 mg, 82%) as a colourless oil. δ_H(500 MHz; $CDCl_3$; Me_4Si): 1.71 (d, 3H, $J = 6.5$ Hz), 1.91 (s, 1H), 2.09–2.18 (m, 4H), 4.05 (d, 2H, $J = 5$ Hz), 5.47–5.70 (m, 4H), 5.95–6.03 (m, 2H). δ_C(125 MHz; $CDCl_3$; Me_4Si): 17.9, 31.9, 32.0, 63.5, 127.1, 129.3, 130.71, 130.74, 131.4, 132.2. HRMS EI(m/z): [M^+] calcd. for $C_{10}H_{16}O$, 152.1201; found, 152.1197.

Deca-2(*E*),6(*E*),8(*Z*)-trien-1-ol (11a). Prepared in 87% yield from **10a** utilizing the procedure used for synthesizing **11b**. δ_H(500 MHz; $CDCl_3$; Me_4Si): 1.72 (dd, 3H, $J = 1.5$, 7.0 Hz), 1.90 (s, 1H), 2.10–2.22 (m, 4H), 4.06 (d, 2H, $J = 4.5$ Hz), 5.37 (dq, 1H, $J = 7.0$, 14.0 Hz), 5.60–5.71 (m, 3H), 5.95 (dt, 1H, $J = 1.5$, 11.6 Hz), 6.33 (ddq, 1H, $J = 1.5$, 11.0, 15.0 Hz). δ_C(125 MHz; $CDCl_3$; Me_4Si): 13.2, 31.9, 32.3, 63.4, 124.3, 125.8, 129.2, 129.4, 132.1, 133.1. HRMS EI(m/z): [M^+] calcd. for $C_{10}H_{16}O$, 152.1201; found, 152.1201.

Dodeca-2(*E*),4(*E*),8(*E*),10(*E*)-tetraenoic acid isobutyl-amide (4). Oxalyl chloride (45 mg, 0.35 mmol) was added to CH_2Cl_2 (7.5 ml) and the solution was cooled to −78 °C. DMSO (55 mg, 0.7 mmol) was then added dropwise and after complete addition the solution was stirred a further 2 minutes before the dropwise addition of **11b** (30 mg, 0.2 mmol). Stirring was continued at −78 °C for 30 minutes and then the reaction was warmed to −10 °C for 5 minutes. The mixture was cooled to −78 °C and Et_3N (354 mg, 3.5 mmol) was added slowly. After returning to room temperature, the reaction was diluted with CH_2Cl_2 (8 ml), then washed once with water (5 ml), once aq. HCl (5 ml, 1M) and once more with water (5 ml). The organic layer was dried ($MgSO_4$) and the CH_2Cl_2 removed to give the crude aldehyde (26 mg), which was diluted with tetrahydrofuran (0.5 ml) and used in the next step without delay.

KO*t*-Bu (39 mg, 0.35 mmol) was added in one portion to a stirred suspension of **12**[16] (165 mg, 0.4 mmol) in THF (3 ml) at room temperature. Stirring was continued for a further 20 minutes at this temperature, then the mixture was cooled to 0 °C and the crude aldehyde solution was added in one portion. Stirring was continued at 0 °C for 30 minutes, then saturated ammonium chloride solution (7 ml) was then added and the organic phase was separated. The aqueous phase was extracted into ether, and the combine organic phases washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. Purification by column chromatography (silica gel 60, 20% ethyl acetate in hexane) yielded the title compound (19 mg, 40%) as a white solid. Mp = 104–106 °C. δ_H(500 MHz; $CDCl_3$; Me_4Si): 0.92 (d, 6H, $J = 6.8$ Hz), 1.72 (d, 3H, $J = 6.4$ Hz), 1.79 (sept, 1H, $J = 6.8$ Hz), 2.15–2.27 (m, 4H), 3.16 (dd, 2H, $J = 6.4$, 6.4 Hz), 5.46–5.64 (m, 3H), 5.76 (d, 1H, $J = 15.2$ Hz), 5.96–6.09 (m, 3H), 6.14 (dd, 1H, $J = 10.4$, 15.2 Hz), 7.17 (dd, 1H, $J = 10.4$, 15.2 Hz). δ_C(125 MHz; $CDCl_3$; Me_4Si): 18.0, 20.1, 28.6, 31.8, 32.8, 46.9, 122.1, 127.5, 128.6, 130.3, 131.1, 131.4, 141.1, 142.0, 166.3. Anal. Calcd: C, 77.68; H; 10.19; N, 5.66; Found C 77.46; H, 10.18; N, 5.84%.

Dodeca-2(*E*),4(*E*),8(*E*),10(*Z*)-tetraenoic acid isobutyl-amide (3). Prepared in 43% yield as a white solid from **11a** utilizing the procedure used for synthesizing **4**. Mp = 78–79 °C. δ_H(500 MHz; $CDCl_3$; Me_4Si): 0.92 (d, 6H, $J = 6.8$ Hz), 1.73 (dd, 3H, $J = 2.0$, 6.8 Hz), 1.78 (sept, 1H, $J = 6.8$ Hz), 2.20–2.30 (m, 4H), 3.15 (dd, 2H, $J = 6.8$, 6.8 Hz), 5.39 (dq, 1H, $J = 7.2$, 10.8 Hz), 5.57–5.66 (m, 2H), 5.77 (d, 1H, $J = 14.8$ Hz), 5.95 (dd, 1H, $J = 11.0$, 11.5 Hz), 6.05 (dt, 1H, $J = 6.7$, 14.8 Hz), 6.15 (dd, 1H, $J = 10.8$, 15.2 Hz), 6.34 (dd, 1H, $J = 10.8$, 15.2 Hz), 7.18 (dd, 1H, $J = 10.4$, 14.8 Hz). δ_C(125 MHz; $CDCl_3$; Me_4Si): 13.3, 20.1, 28.6, 32.1, 32.8, 46.9, 122.2, 124.6, 126.1, 128.7, 129.2, 132.6, 141.0, 141.8, 166.3. Anal. Calcd: C, 77.68; H, 10.19; N, 5.66; Found C 77.36; H, 10.13; N, 5.84%. MS (70 eV,%): m/z 247(M^+ 2.4), 167(15.3), 152(3.6), 128(2.0), 115(10.4), 81(100), 79(44.7), 66(34.7), 57(31.9).

***tert*-Butyldimethyl((4*E*,6*Z*)-octa-4,6-dienyloxy)silane (15).** Catechol borane (0.62 g, 5.2 mmol) and **13**,[22] (1.0 g, 5.07 mmol) were mixed together at 60 °C for 4 hours, by which time ^{1}H NMR analysis indicated complete consumption of the alkyne and formation of the (*E*)-benzodioxole alkenyl borane (**14**). δ_H(300 MHz; $CDCl_3$; Me_4Si): 0.05 (s, 6H), 0.90 (s, 9H), 1.72 (pent, 2H, $J = 6.6$ Hz), 2.35 (br q, 2H, $J = 6.6$ Hz), 3.67 (t, 2H, $J = 6.3$ Hz), 5.80 (dt, 1H, $J = 1.8$, 18.3 Hz), 7.0–7.24 (m, 5H). A solution consisting of $Pd(PPh_3)_4$ (117 mg, 0.05 mmol) and *cis*-1-bromopropene (0.605 g, 5.0 mmol) in benzene (8 ml) was prepared, and the solution was stirred for 30 minutes at 25 °C. Then the alkenyl borane prepared above was added in one portion followed by a solution of NaOEt in ethanol (5 ml, 2 M, 2 equivalents). The mixture was then stirred under reflux for 5 hours. After cooling to room temperature aq. NaOH (3 ml, 3M) was added followed by aq. H_2O_2 (0.3 ml, 30%) and the mixture was stirred at room temperature for 2 hours. Water (25 ml) was then added and the mixture was extracted into ether (2 × 15 ml). The combined ether layers were washed with brine, dried

($MgSO_4$) and evaporated. Flash chromatographic purification (5% ether in hexanes, silica gel 60) yielded the title compound (0.96 g, 80%) as a colourless oil. δ_H(400 MHz; $CDCl_3$; Me_4Si): 0.05 (s, 6H), 0.90 (s, 9H), 1.62 (pent, 2H, $J = 6.4$ Hz), 1.73 (dd, 3H, $J = 1.7$, 7.0 Hz), 2.18 (br q, 2H, $J = 7.2$ Hz), 3.62 (t, 2H, $J =$ 6.4 Hz), 5.38 (dq, 1H, $J = 7.0$, 10.8 Hz), 5.66 (dt, 1H, $J = 7.0$, 14.6 Hz), 5.98 (br t, 1H, $J = 11.2$ Hz), 6.35 (ddq, 1H, $J = 1.4$, 11.0, 15.0 Hz). δ_C(100 MHz; $CDCl_3$; Me_4Si): 5.3, 13.3, 18.3, 25.9, 29.1, 32.4, 62.5, 124.0, 125.7, 129.4, 133.8. Anal. Calcd: C, 69.93; H, 11.74. Found: C, 70.13; H, 11.63%.

Octa-4(*E*),6(*Z*)-dien-1-ol (9a). **15** (0.63 g, 2.6 mmol) was dissolved in MeOH (12 ml) and conc. HCl (0.3 ml) was added. The solution was stirred at 25 °C for 35 minutes, then saturated aq. $NaHCO_3$ (30 ml) was added and the mixture was extracted into ether (3 × 15 ml) the combined ether layers were washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatographic purification (20% EtOAc in hexanes, silica gel 60) yielded the title compound (290 mg, 90%) as a colourless oil. Spectroscopic data were identical to that for **9a** prepared above.

***Echinacea* sample preparation.** Dried *Echinacea* roots were obtained from several commercial sources. The roots were finely ground and a liquid preparation obtained by centrifugation after a 48 hour 1 : 5 extraction in 60% ethanol. The alkylamide fraction was separated from the caffeic acid fraction by diluting 1 : 100 with water and fractionation on a solid phase extraction cartridge (Strata C18-E; 55 μm, 70 Å; 500 mg 6 mL^{-1}; Phenomenex, USA) conditioned with 100% methanol (5 ml) then water (5 ml). The caffeic acids were eluted from the column with water and 25% methanol and then discarded. The alkylamide fraction was eluted using 100% methanol.

Radioligand displacement assays on CB_2 receptors. For the CB_2 receptor, binding experiments were performed in the presence of 0.08 nM of the radioligand [^{3}H]-CP55,940 at 30 °C in siliconized glass vials together with 3.8 μg of membrane recombinantly over-expressing CB_2, which was resuspended in 0.2 mL (final volume) binding-buffer (50 mM TRIS-HCl, 2.5 mM EGTA, 5 mM $MgCl_2$, 0.5 mg ml^{-1} fatty acid free BSA, pH 7.4). Test compounds were present at varying concentrations and the non-specific binding of the radioligand was determined in the presence of 10 μM CP55,940. After 90 min incubation, the suspension was rapidly filtered through 0.05% polyethylenimine pre-soaked GF/C glass fiber filters on a 96-well cell harvester and washed nine times with 0.5 mL ice-cold washing-buffer (50 mM TRIS-HCl, 2.5 mM EGTA, 5 mM $MgCl_2$, 2% BSA, pH 7.4). Radioactivity on filters was measured with a Beckman LS 6500 scintillation counter in 3 mL Ultima Gold scintillation liquid. Data collected from three independent experiments performed in triplicates were normalized between 100% and 0% specific binding for [^{3}H]-CP 55,940. These data were graphically linearized by projecting Hill-plots, which allowed the calculation of IC_{50} values. Derived from the dissociation constant (K_D) of [^{3}H]CP-55,940 and the concentration-dependent displacement (IC_{50} value), inhibition constants (K_i) of competitor compounds were calculated using the Cheng–Prusoff equation [$K_i = IC_{50}/(1 + L/K_D)$].

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