Isolation, structure elucidation and total synthesis of a cytotoxic dienone from *Echinacea pallida*†

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The isolation and structure characterization of a dienone from the roots of *Echinacea pallida*, namely (8*Z*,11*Z*)-pentadeca-8,11-dien-2-one, are described here. To assess the configuration of this secondary metabolite, the stereoselective total synthesis of the two isomeric forms, (8*Z*,11*Z*)- and (8*Z*,11*E*)-pentadeca-8,11-dien-2-one, was undertaken and the structure elucidation of the natural compound was unambiguously carried out. The cytotoxic activity of both isomers was also evaluated on a human T cell leukaemia cancer line (Jurkat cells). The results indicated that these compounds exert a dose-dependent cytotoxicity with a medium-level potency on the tested cell line.

Introduction

Polyacetylenes and polyenes are classes of natural products well known for their potent antifungal[1] and antibacterial[2] activity, as well as for their ability to inhibit a number of enzymes, such as cholesterol acyltransferase.[3] Furthermore, several experiments attested that these secondary metabolites exhibit antiallergenic[4] and anti-inflammatory[5] activities and have proven to be cytotoxic against a number of solid and leukemic cancer cell lines.[6]

In the search for cytotoxic compounds from plants of the genus *Echinacea*, the *n*-hexane extracts of *E. pallida* roots have shown higher inhibitory activity on cell proliferation than those obtained from *E. purpurea* and *E. angustifolia.*[7] This is in agreement with the different chemical composition of these species; in fact, *E. purpurea* and *E. angustifolia* have alkamides as the main lipophilic compounds,[8,9] whereas *E. pallida* contains polyacetylenes and polyenes as the typical hydrophobic constituents.[8,10–12] Up to now, nine compounds (**1–9**, Fig. 1) have been isolated by bioassay-guided fractionation from the roots of *E. pallida* and characterized by spectroscopic methods.[10,11] The purified constituents have been tested for their cytotoxic activity and (8*Z*,13*Z*)-pentadeca-8,13-dien-11-yn-2-one (**8**) has been found the most active, displaying a selective cytotoxic activity toward cancer cells and being able to cross the Caco-2 monolayer, which indicates a potential good absorption in humans after oral administration.[13] Due to the difficulty in purifying compound **8** from *E. pallida* roots, we recently performed the first total synthesis of this metabolite.[14]

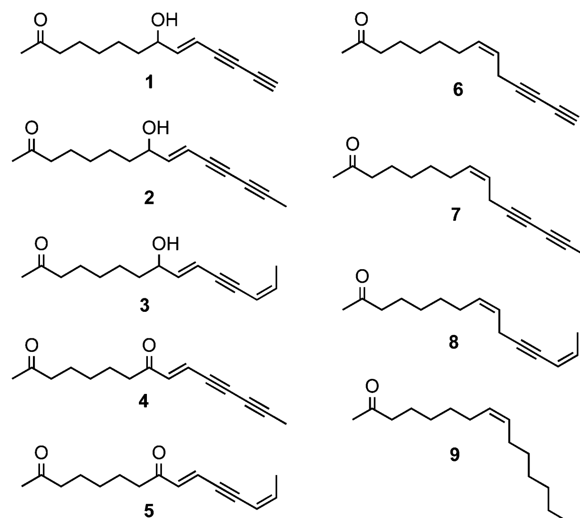

Fig. 1 Chemical structures of polyacetylenes and polyenes previously isolated from *E. pallida* roots.

The application of a suitable RP-HPLC method that has been developed and validated to quantify the content of the hydrophobic constituents in *E. pallida* roots and dietary supplements[11] indicated that the *n*-hexane root extracts contain other lipophilic compounds whose structures have not been previously elucidated due both to instability and difficult isolation from the root extracts.

As part of our ongoing research on bioactive metabolites from plants of the genus *Echinacea*, the isolation and structural characterization of a dienone from *E. pallida* roots, namely (8*Z*,11*Z*)-pentadeca-8,11-dien-2-one (**10**, Fig. 2) are described here. Although the structure of this secondary metabolite was reported by both Bauer and Baumann *et al.* in 1988,[8,15] to the best of our knowledge, its detailed isolation procedure, structural

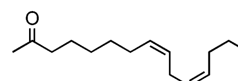

Fig. 2 Structure of the newly isolated polyene (**10**).

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characterization and biological activity have never been fully described in the literature.

Furthermore, to assign unambiguously the stereochemistry of this secondary metabolite, the synthesis of the two isomerically pure forms, (8*Z*,11*Z*)-pentadeca-8,11-dien-2-one (**10a**) and (8*Z*,11*E*)-pentadeca-8,11-dien-2-one (**10b**), was undertaken, thus allowing the assessment of the (8*Z*,11*Z*) configuration of **10**.

In view of the structural analogy of this metabolite with previously described cytotoxic polyacetylenes and polyenes isolated from the same plant material,[10,13] the cytotoxic activity of the two synthetic isomers was also evaluated on a human T cell leukaemia cancer line (Jurkat cells), indicating that these molecules possess a cytotoxic activity of the same order of magnitude as previously isolated compound **8**.

Results and discussion

Isolation and structural characterization

Previous studies on the composition of the extracts of *E. pallida* roots indicated that the main constituents are lipophilic compounds, identified as polyacetylenes and polyenes (Fig. 1).[10,11] The genuine secondary metabolites recovered from roots of *E. pallida* are fairly stable compounds, that are easily converted into the corresponding hydroxylated derivatives and eventually into the diketo-derivatives through allylic oxidation by molecular oxygen.[9,10] The RP-HPLC analysis of the crude *n*-hexane extract obtained from *E. pallida* roots indicated that the retention times (t_R) of these constituents clearly reflect their polarity (Fig. 3):[11,16] the hydroxylated compounds (**1–3**) eluted first, followed by the diketo-ones (**4–5**), whilst the genuine (*viz.* not oxidized) secondary metabolites (**6–9**) displayed higher retention times.

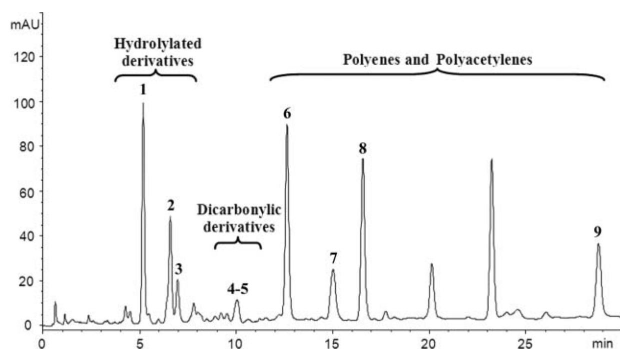

Fig. 3 Chromatogram of a *n*-hexane extract of *E. pallida* roots on a LiChrospher RP-18 column (125 mm × 4.0 mm i.d., 5 µm). For peak identification, see Fig. 1.

In the meantime, the RP-HPLC analysis of *E. pallida* roots showed the presence of two further peaks in the region of lipophilic metabolites, at 20.1 min (5.0%) and 23.2 min (13.1%), respectively, thus suggesting for these constituents a structural analogy with the less polar authentic polyacetylenes and polyenes previously isolated and characterized from *E. pallida* roots.[10,11]

The purification of the crude *n*-hexane extract of *E. pallida* roots by silica gel column chromatography, followed by reversed-phase column chromatography, led to the isolation of these constituents. A preliminary NMR and MS analysis indicated that the first eluted fraction (t_R = 20.1 min) was actually a mixture of at least three compounds (by NMR analysis) and was not pursued further, whilst the second eluted peak (t_R = 23.2 min) was present in the crude extract as a single metabolite (**10**).

The structural characterization of **10** was carried out by IR, UV, MS as well as NMR spectroscopy, including extensive homonuclear and heteronuclear techniques (COSY, HSQC, HMBC, NOESY).

The IR spectrum of the neat sample showed the presence of a carbonyl group (1719 cm^{-1}) as well as olefinic double bond(s) (1461 and 720 cm^{-1}), while the UV spectrum only displayed an absorption maximum at 190 nm, attesting the absence of a conjugated chromophore in the new isolated metabolite, thus suggesting a possible structural relation with the previously described (8*Z*)-pentadeca-8-en-2-one (**9**).[11]

By comparison of the EI-MS data with those of the above mentioned compound **9** ($C_{15}H_{28}O$, *m/z* 224), the presence of a molecular ion at *m/z* 222 would suggest the molecular formula $C_{15}H_{26}O$, accounting for an additional and not conjugated double bond. Furthermore, the identification of the peak matching $[M - 15(CH_3)]^+$ and $[M - 43(CH_3CO)]^+$ was consistent with the observation that all lipophilic constituents nowadays isolated from *E. pallida* extracts present a common aliphatic backbone ending with an acetyl moiety.[10,11]

The ^{1}H and ^{13}C NMR spectra, supported by bidimensional techniques, allowed the assignments reported in Table 1 for protons and carbons from C-1 to C-7. Comparison of these assignments with those previously obtained for related compounds **1–9**[10,11] displayed the expected strict correspondences. Furthermore, the ^{1}H NMR spectrum of **10**, supported by HSQC-DEPT data, showed the presence of four olefinic protons, as an unresolved multiplet in the region 5.32–5.47 ppm, that correlates with the corresponding olefinic carbons (δ_C 128.1, 128.3, 129.8 and 130.0 ppm), disclosing the presence of two double bonds in the molecule. ^{1}H–^{1}H COSY confirmed that these two double bonds were effectively not conjugated (as supposed on the basis of the UV data), displaying a correlation of the olefinic multiplet with a triplet at δ 2.81 ppm (2H, $^3J_{H-H}$ 5.9 Hz), whose deshielded chemical shift would account for a bis-allylic methylenic position of a 1,3-pentadienyl system. In addition, the olefinic protons also correlate with a multiplet at δ 1.96–2.13 ppm, integrating for 4H, possibly resulting from the overlapped resonances of the external allylic protons characterized by a similar magnetic environment. By analogy with the literature,[10,11] the first of the two double bonds was supposed to be located between carbons C-8 and C-9; the evidence of the presence of a 1,3-pentadienyl system allowed us to locate the second double bond between C-11 and C-12. Long-range HMBC correlation of the olefinic protons and carbons further corroborated this supposition and guided the assignment of the allylic positions: the deshielded triplet at δ 2.81 ppm (δ_C 25.7 ppm) accounts for the bis-allylic protons at C-10, while the multiplet at δ 1.96–2.13 ppm results from the overlapped resonances of protons at C-7 (δ_C 27.0 ppm) and C-13 (δ_C 29.3 ppm). Finally, through COSY and HSQC-DEPT data, it was also possible to individuate the presence of an additional terminal methyl group, besides the acetyl one at C-1, as a triplet at δ 0.95 ppm (J 7.4 Hz, δ_C 13.8 ppm). Homo- and heteronuclear correlations of the C-15 position proved it to belong to a propyl moiety located between C-13 and C-15. In fact, COSY and HMBC experiments allowed us to trace back the

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Table 1 ^{1}H and ^{13}C NMR spectral data [δ (ppm) and J (Hz)] of compounds **10**, **10a** and **10b** (400 MHz and 100 MHz respectively, $CDCl_3$, TMS as the reference)

Position	Natural compound **10**		Synthetic compound **10a**		Synthetic compound **10b**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	2.17 *s*	29.9	2.17 *s*	29.9	2.17 *s*	29.8
2	—	209.1	—	209.2	—	209.2
3	2.46 *t* (7.4)	43.8	2.46 *t* (7.4)	43.8	2.45 *t* (7.4)	43.7
4	1.62 *quint* (7.4)	23.8	1.62 *quint* (7.4)	23.8	1.61 *quint* (7.4)	23.7
5	1.28–1.48 *m*[a]	28.8	1.21–1.49 *m*[a]	28.8	1.27–1.48 *m*[a]	28.8
6	1.28–1.48 *m*[a]	29.4	1.21–1.49 *m*[a]	29.4	1.27–1.48 *m*[a]	29.4
7	1.96–2.13 *m*	27.0	2.00–2.14 *m*	27.0	2.03–2.13 *m*	26.9
8	5.32–5.47 *m*[a]	130.0[b]	5.32–5.47 *m*[a]	130.0[b]	5.32–5.52 *m*[a]	130.1[b]
9	5.32–5.47 *m*[a]	128.1[c]	5.32–5.47 *m*[a]	128.1[c]	5.32–5.52 *m*[a]	128.0[c]
10	2.81 *t* (5.9)	25.7	2.81 *t* (6.2)	25.7	2.76 *dd* (4.8;5.7)	30.5
11	5.32–5.47 *m*[a]	128.3[c]	5.32–5.47 *m*[a]	128.3[c]	5.32–5.52 *m*[a]	128.4[c]
12	5.32–5.47 *m*[a]	129.8[b]	5.32–5.47 *m*[a]	129.8[b]	5.32–5.52 *m*[a]	130.6[b]
13	1.96–2.13 *m*	29.3	2.00–2.14 *m*	29.3	1.95–2.03 *m*	34.7
14	1.28–1.48 *m*[a]	22.8	1.21–1.49 *m*[a]	22.8	1.27–1.48 *m*[a]	22.6
15	0.95 *t* (7.4)	13.8	0.95 *t* (7.4)	13.8	0.91 *t* (7.3)	13.8

[a] Signals are overlapped. [b] Signals are interchangeable. The attributions are consistent with HMBC data. [c] Signals are interchangeable. The attributions are consistent with HMBC data.

resonances of the adjacent methylene at C-14 as a multiplet at δ 1.28–1.48 ppm (δ_C 22.8 ppm), even though overlapped with C-5 and C-6, and also correlating with C-13 in the multiplet at δ 1.96–2.13 ppm (δ_C 29.3 ppm). HMBC data confirmed this hypothesis, attesting that the connection between the three fragments so far individuated was correct: in fact, a long range correlation of both C-6 and C-14 with olefinic protons was present, indicating that the 1,3-pentadienyl system was interposed between the alkyl chain and the propyl moiety.

Thus, compound **10** was identified as (8*Z*)-pentadeca-8,11-dien-2-one (Fig. 4).

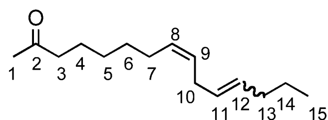
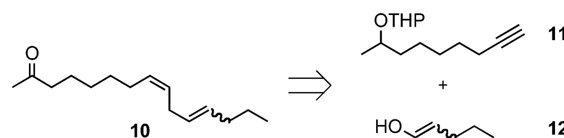


Fig. 4

NOESY experiments were then acquired in order to determine the configuration of the double bonds. A diagnostic correlation was observed between H-10 (δ 2.81 ppm) and H-7, H-13 multiplet (δ 1.96–2.13 ppm), assessing the *Z* configuration for at least one of the two double bonds, presumably the one located between C-8 and C-9, as suggested by analogy with other polyacetylenes and polyenes isolated from *E. pallida*.[10,11] Unfortunately, the overlap of both olefinic and allylic proton resonances precluded any further assignment by this technique. Non-decoupled HSQC experiments were also acquired with the aim of indirectly estimating the vicinal coupling constants ($^3J_{H-H}$) of double bonds:[17] however, ^{13}C satellite analysis of ^{1}H NMR signals indicated a borderline value of 13–15 Hz for all olefinic positions.

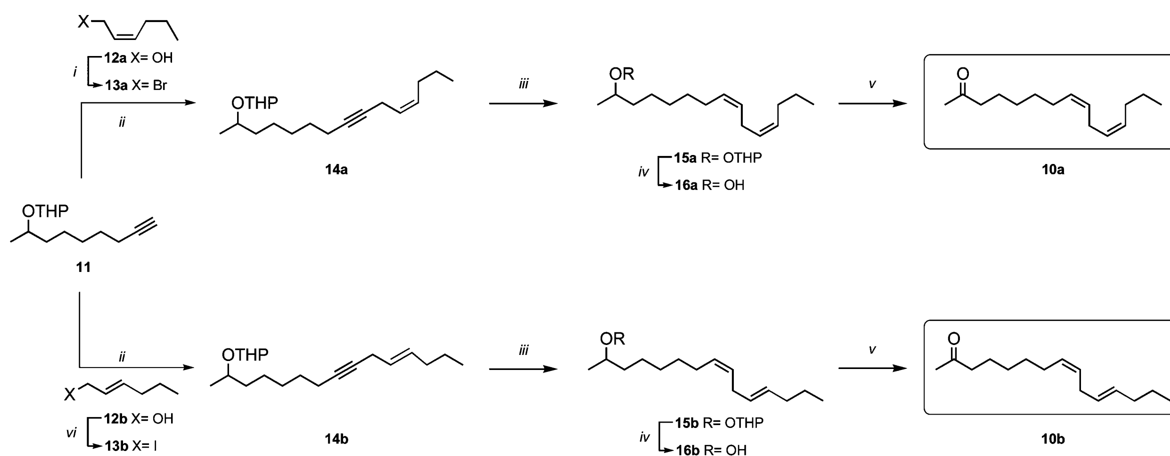
Synthesis

To assess the configurations of the two double bonds of this secondary metabolite (**10**) and to corroborate its constitution, the synthesis of the two isomerically pure forms (8*Z*,11*Z*)- and (8*Z*,11*E*)-pentadeca-8,11-dien-2-one (**10a** and **10b** respectively) was undertaken. By analogy to the total synthesis of related compounds,[14,18,19] the route depicted in Scheme 1 was envisaged as a facile access to the target molecules, which were retro-synthetically disconnected into fragments **11** and **12**.



Scheme 1 Retrosynthetic analysis of **10**.

Terminal alkyne **11** can be obtained in three steps from commercial 1-hexyne following literature methods,[14,20] while 2-hexenol is commercially available in both the isomeric *Z* and *E* forms (**12a** and **12b**, respectively) and can be easily converted into the corresponding halides **13a** and **13b**, through well trodden paths (Scheme 2).[21,22] Coupling of **11** with **13** was achieved in good yields (80–90%) by nucleophilic displacement of the alkynyllithium salt of **11** with the halide **13** in the presence of HMPA as a co-solvent, to obtain the stereoisomerically pure compound **14**.[23,24] The good outcome of the reaction was confirmed by ^{1}H NMR data, which displayed a diagnostic resonance around δ 2.9 ppm, corresponding to the newly inserted propargyl-allylic protons. In particular, in the case of **14b**, it was possible to measure the olefinic coupling constants ($J = 15.1$ Hz), whilst the overlap of olefinic resonances in **14a** prevented such determination. Controlled hydrogenation of **14** to **15** allowed the stereoselective reduction of the alkyne between C-8 and C-9 to a *Z*-configured double bond: the reduction of **14a** was easily accomplished with *in situ*-generated Ni catalyst and afforded the desired product **15a** in 98% yield.[18,25] Finally, removal of the THP group in acidic methanol gave **16a** in quantitative yield and subsequent oxidation of the hydroxyl moiety with PCC[18] (90% yield) led to isomer **10a** in 78% overall yield from **14a**. Following the same procedure, isomer **10b** was obtained from **14b** in 42% overall yield. It is worth noting that compound **10b** displayed a small percentage (15%) of **10a**, which probably arose from a partial *Z*/*E* isomerization of **12b** during its conversion into **13b**.[21]



Scheme 2 Synthesis of isomers **10a**, **b**: (*i*) $P(OPh)_3$, Br_2, TEA, CH_2Cl_2, −80 °C → 0 °C, 1 h; (*ii*) *n*-BuLi, HMPA, THF, −78 °C → rt, overnight; (*iii*) $Ni(OAc)_2$, $NaBH_4$, EtOH, H_2, rt, 2 h; (*iv*) PTSA, MeOH, 40 °C, 2 h; (*v*) PCC, CH_2Cl_2, rt, 30 min; (*vi*) I_2, TMSCl, CH_3CN, rt.

By comparison of the spectral NMR data reported in Fig. 5a, it appears that the olefinic resonances were not particularly indicative of the configuration: the only difference between the two isomers was a slight 0.03 ppm downfield shift of the unresolved olefinic multiplet. By contrast, allylic protons H-7, H-10 and H-13 (see Fig. 5b) were appreciably affected by the configuration of the double bond: moving from isomer **10a** to **10b**, the protons H-10 (around δ 2.80 ppm) showed a 0.06 ppm upfield shift, while protons H-7 and H-13 in the region around δ 2.05, completely overlapped in **10a**, became significantly different, with a 0.08 ppm upfield shift of the H-13 protons.

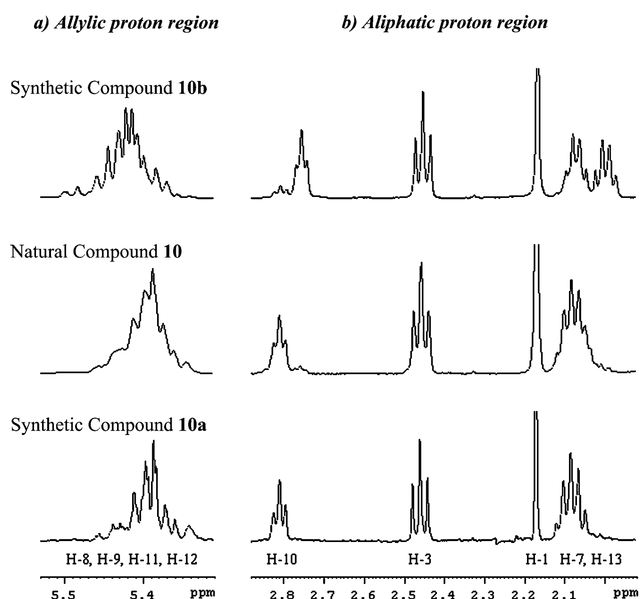


Fig. 5 Comparison of ^{1}H NMR spectra of natural and synthetic compounds: (a) allylic protons region, (b) aliphatic protons region.

By comparison of the NMR spectra of synthetic (8*Z*,11*Z*)-**10a** and (8*Z*,11*E*)-**10b** with that of the natural compound **10**, the (8*Z*,11*Z*) configuration of the natural dienone **10** was finally inferred.

Table 2

Compound	$IC_{50}/\mu M$
(8*Z*,11*Z*)-Pentadeca-8,11-dien-2-one (**10a**)	10.21 ± 2.06
(8*Z*,11*E*)-Pentadeca-8,11-dien-2-one (**10b**)	8.11 ± 1.87
(8*Z*,13*Z*)-Pentadeca-8,13-dien-11-yn-2-one (**8**)	9.61 ± 2.70
Doxorubicin	0.11 ± 0.03

Cytotoxicity

The two synthetic compounds (8*Z*,11*Z*)-**10a** and (8*Z*,11*E*)-**10b** induced a concentration-dependent decrease of cell viability on Jurkat cells after 48 h exposure, in a similar manner to that observed with the previously isolated compound **8**. In fact, no statistically significant difference was observed in the cytotoxicity values (IC_{50}) of all three structurally related compounds (Table 2). On the other hand, a significant difference was observed between these compounds and the classic anticancer agent doxorubicin (Fig. 6). The ability to induce apoptosis in cancer cells has been recently observed for compound **8**[13] and this mechanism may be reasonably hypothesized also for the two isomers evaluated for the first time in this study. Further investigations will clarify the exact nature of the cytotoxic activity of these molecules.

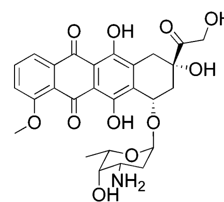


Fig. 6 Structure of doxorubicin.

Conclusions

In the present work, chromatographic and spectroscopic techniques, combined with total synthesis, were successfully applied to the isolation and structure elucidation of a dienone from *E. pallida* roots, namely (8*Z*,11*Z*)-pentadeca-8,11-dien-2-one (**10**). In particular, the stereoselective total synthesis gave access to

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the two isomeric forms (8*Z*,11*Z*)-pentadeca-8,11-dien-2-one (**10a**) and (8*Z*,11*E*)-pentadeca-8,11-dien-2-one (**10b**), whose NMR data allowed us to unambiguously assess the exact constitution of the natural dienone. Both isomers were tested for their cytotoxic activity on Jurkat cancer cells and displayed a concentration-dependent cytotoxicity with a medium-level potency on this cell line. Additional investigations will be performed to determine the exact mechanism of action of these molecules.

Experimental

General

IR spectra were obtained with a Bruker VERTEX 70 FT-IR instrument. UV spectra were recorded on-line by photodiode array detection in H_2O–CH_3CN mixtures. Elemental analyses were performed with a Carlo Erba Elemental Analyzer mod. 1110. Mono- and bidimensional NMR spectra, including ^1H, ^{13}C, COSY, HSQC-DEPT, HMBC and NOESY experiments, were acquired in $CDCl_3$ on a Bruker FT-NMR AVANCE 400 or a DPX200 spectrometer. Chemical shifts are reported as δ values (ppm) using TMS as reference. Coupling constants (J) are given in Hz. Mass spectra were obtained on a Finnigan MAT-SSQ 710A mass spectrometer (direct inlet), in EI mode with an ionization voltage of 70 eV, over the mass range 45–400 m/z. High-performance liquid chromatography was performed on an Agilent Technologies 1100 system, consisting of a vacuum degasser, a quaternary pump, an autosampler, a thermostatted column compartment and a photodiode array detector. Analyses were carried out on a Lichrospher RP-18 column (125 mm × 4 mm i.d., 5 μm, Agilent Technologies).[11] Silica gel chromatography was performed with Kieselgel 60 (40–63 μm, Merck, Darmstadt, Germany). Reversed-phase column chromatography was carried out with LiChroprep RP-18 (40–63 μm, Merck). Pre-coated glass supported Kieselgel 60 F254 plates (Merck) were used for TLC. Compounds were visualized by dipping the plates in potassium permanganate stain (1.5 g $KMnO_4$, 10 g K_2CO_3, and 1.25 mL 10% NaOH in 200 mL of water) followed by heating on a hot plate.

Water used for HPLC was purified using a Milli-Q PLUS 185 system from Millipore (Milford, MA, USA). All the reagents and the HPLC grade solvents were purchased from Sigma-Aldrich (Milan, Italy).

Extraction and purification

Authentic dried roots (1 kg) of *E. pallida* (Nutt.) Nutt. were kindly donated by Dr Federica Monti, Planta Medica s.r.l., Pistrino, Perugia, Italy, in January 2006. The plant material was kept in the dark, protected from high temperature and humidity, until required for extraction. The extraction of the powdered dried roots of *E. pallida* (1 kg, divided in three portions) was carried out with a Soxhlet apparatus for 8–10 h using *n*-hexane (2 L for each step) as the extraction solvent. All the extracts were combined and evaporated to dryness under vacuum to give a yellow oil (7.57 g). The *n*-hexane extract was then subjected to silica gel flash column chromatography and eluted with *n*-hexane–EtOAc (2 : 1, v/v), affording to 120 fractions of 15.5 ml. Each fraction was analyzed by RP-HPLC and combined into 7 fractions (A–G), according to the chromatographic profile. Compound **10** was isolated as a yellow oil from fraction A (2.82 g) by reversed-phase flash column chromatography on LiChroprep RP-18, with a gradient elution composed of H_2O–CH_3CN from initial 60 : 40 (v/v) to final 20 : 80 (v/v), to give 160 fractions of 10 mL, which were analyzed by RP-HPLC. The fractions 127–132 were combined and concentrated under reduced pressure to give an aqueous residue, which was extracted with $CHCl_3$ (2 × 20 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to afford a yellow oil (192 mg, 0.02% yield). The purified compound was stored under argon atmosphere at low temperature (–20 °C), protected from light and humidity. Found: 81.22; H, 11.53. $C_{15}H_{26}O$ requires: C, 81.02; H, 11.79%. δ_H (400 MHz, $CDCl_3$): 0.95 (3H, t, J = 7.4 Hz, H-15); 1.28–1.48 (6H, m, H-5, H-6, H-14); 1.62 (2H, quint, J = 7.4 Hz, H-4); 1.96–2.13 (4H, m, H-7, H-13); 2.17 (3H, s, H-1); 2.46 (2H, t, J = 7.4 Hz, H-3); 2.81 (2H, t, J = 5.9 Hz, H-10); 5.32–5.47 (4H, m, H-8, H-12, H-9, H-11). δ_C (100 MHz, $CDCl_3$): 13.8 (C-15), 22.8 (C-14), 23.8 (C-4), 25.7 (C-10), 27.0 (C-7), 28.8 (C-5), 29.3 (C-13), 29.4 (C-6), 29.9 (C-1), 43.8 (C-3), 128.1 (C-9/C-11), 128.3 (C-9/C-11), 129.8 (C-8/C-12), 130.0 (C-8/C-12), 209.1 (C-2). EI-MS: m/z 222 (M^+, 20%), 207 ($M^+ - CH_3$, 7), 179 ($M^+ - COCH_3$, 32), 164 (43), 135 (20), 121 (27), 109 (37), 95 (51), 81 (78), 79 (79), 67 (100), 55 (27). IR (neat): ν_{max}/cm^{-1} 3009, 2957, 2930, 2858, 1719, 1461, 1359, 1161, 720. UV: λ_{max} (CH_3CN–H_2O)/nm 190.

Synthesis

(2*Z*)-1-Bromo-2-hexene (13a). To a stirred solution of $P(OPh)_3$ (0.858 mL, 3.36 mmol) in dry CH_2Cl_2 (12 mL) were added sequentially, at –80 °C under argon, Br_2 (0.155 mL, 3.02 mmol), Et_3N (0.562 mL, 4.03 mmol) and a solution of **12a** (500 μL, 4.03 mmol) in dry CH_2Cl_2 (5 mL). The reaction mixture was stirred for 1 h at –80 °C, then quickly warmed to 0 °C in 45 min and finally quenched with saturated NH_4Cl (15 mL). Layers were separated and the organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography (eluant light petroleum) to afford **13a** as a colourless oil (221 mg, 45% yield). Found: 44.03; H, 6.62. $C_6H_{11}Br$ requires: C, 44.20; H, 6.80%. δ_H (400 MHz, $CDCl_3$): 0.98 (3H, t, J = 7.4 Hz, H-6), 1.42–1.54 (2H, m, H-5), 2.12–2.21 (2H, m, H-4), 2.14 (2H, d, J = 7.3 Hz, H-1), 5.59–5.70 (1H, m, H-3), 5.72–5.84 (1H, m, H-2). δ_C (100 MHz, $CDCl_3$): 13.7, 22.3, 27.4, 28.9, 125.4, 135.8. MS: m/z 162 (M^+, 6%), 164 ($M^+ + 2$, 6), 83 (100), 82 (10), 67 (11), 55 (100), 53 (18).

(4*Z*)-14-(2-Oxacyclohexyl)oxypentadeca-4-en-7-yne (14a). 8-(2-Oxacyclohexyl)oxy-1-nonyne (**11**)[14,20] (200 mg, 0.89 mmol) was dissolved in freshly distilled THF (4 mL) under argon and cooled to −78 °C. *n*-BuLi (1.6 M soln in hexane, 0.56 mL, 0.89 mmol), a solution of HMPA (0.604 mL, 3.47 mmol) in THF (1 mL) and finally a solution of **13a** (104 mg, 0.64 mmol) in THF (1 mL) were added in succession with 15 min intervals. The reaction mixture was allowed to warm to rt overnight, then was partitioned between saturated NH_4Cl (50 mL) and light petroleum (100 mL). Combined organic layers were washed with water (50 mL) and brine (50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford a yellow oil, which was purified by column chromatography (eluant 10 : 1 light petroleum–EtOAc). **14a** was obtained as a mixture of two diastereoisomers, clearly

detectable by NMR analysis (163 mg, 83% yield). Found: C, 78.59; H, 11.33. $C_{20}H_{34}O_2$ requires: C, 78.38; H, 11.18%. δ_H (400 MHz, $CDCl_3$): 0.94 (3H × 2, t, J = 7.4 Hz, H-1), 1.14 (3H, d, J = 6.1 Hz, H-15), 1.25 (3H, d, J = 6.3 Hz, H-15), 1.28–1.95 (16H × 2, m, H-3′ to H-5′, H-10 to H-13, H-2), 2.00–2.10 (2H × 2, m, H-3), (2H × 2, m, H-9), 2.93 (2H × 2, quint, J = 4.4 Hz, H-6), 3.47–3.57 (1H × 2, m, H-6′), 3.70–3.86 (1H × 2, m, H-14), 3.88–4.01 (1H × 2, m, H-6′), 4.64–4.69 (1H, m, H-2′), 4.71–4.76 (1H, m, H-2′), 5.41–5.25 (2H × 2, m, H-4, H-5). δ_C (100 MHz, $CDCl_3$): 13.8, 17.2, 18.7, 18.8, 19.1, 19.8, 20.1, 21.6, 22.6, 25.0, 25.56, 25.61, 29.0, 29.1, 29.2, 31.2, 31.3, 36.5, 37.5, 62.5, 62.8, 71.1, 73.9, 78.46, 78.53, 79.9, 80.0, 125.20, 125.23, 131.05, 131.07. MS: m/z 306 (M$^+$, <1%), 223 (1), 205 (1), 191 (1), 175 (3), 161 (4), 121 (8), 101 (9), 91 (13), 85 (100), 79 (13), 67 (17), 55 (16). IR (neat): ν_{max}/cm^{-1} 2963, 2933, 2860, 1455, 1375, 1133, 1022, 994.

(4*E*)-14-(2-Oxacyclohexyl)oxypentadeca-4-en-7-yne (14b). By analogy to the synthesis of **14a**, compound **14b** was obtained from **11** and **13b**[21] in 87% yield. Found: C, 78.47; H, 11.37. $C_{20}H_{34}O_2$ requires: C, 78.38; H, 11.18%. δ_H (200 MHz, $CDCl_3$): 0.85 (3H × 2, t, J = 7.2 Hz, H-1), 1.59 (3H, d, J = 6.1 Hz, H-15), 1.76 (3H, d, J = 6.3 Hz, H-15), 1.20–1.86 (16H × 2, m, H-3′ to H-5′, H-10 to H-13, H-2), 1.86–2.03 (2H × 2, m, H-3), 2.03–2.20 (2H × 2, m, H-9), 2.76–2.89 (2H × 2, m, H-6), 3.36–3.51 (1H × 2, m, H-6′), 3.58–3.96 (2H × 2, m, H-6′, H-14), 4.54–4.70 (1H × 2, m, H-2′), 5.35 (1H × 2, dtt, J = 15.1, 5.5, 1.2 Hz, olefinic proton), 5.62 (1H × 2, dtt, J = 15.1, 6.6, 1.6 Hz, olefinic proton). δ_C (50 MHz, $CDCl_3$): 13.6, 18.67, 18.71, 19.01, 19.7, 19.98, 21.5, 21.9, 22.4, 24.9, 25.3, 25.5, 25.6, 28.88, 28.97, 29.0, 31.16, 31.19, 34.3, 36.4, 37.4, 62.3, 62.6, 70.9, 73.8, 77.5, 77.6, 81.7, 81.8, 95.5, 98.6, 124.9, 131.4. MS: m/z 306 (M$^+$, <1%), 223 (2), 205 (1), 191 (1), 175 (4), 161 (5), 121 (9), 109 (8), 91 (16), 85 (100), 79 (16), 67 (20), 55 (19). IR (neat): ν_{max}/cm^{-1} 2962, 2935, 2859, 1465, 1376, 1134, 1022, 993, 967.

(4*Z*,7*Z*)-14-(2-Oxacyclohexyl)oxypentadeca-4,7-diene (15a). A solution of $NiAc_2 \cdot 4H_2O$ (21 mg, 0.084 mmol) in EtOH (1 mL) was treated with $NaBH_4$ (3 mg, 0.084 mmol) suspended in EtOH (1 mL) under H_2 and the reaction mixture turned from green to black. The so formed catalyst was poisoned with ethylendiamine (0.283 mL of a 0.60 M soln in EtOH, 0.17 mmol) and treated with **14a** in EtOH (2 mL). The mixture was stirred for 2.5 h at rt, the solvent was removed under reduced pressure and the crude product was purified by column chromatography (eluant 10 : 1 light petroleum–EtOAc) to give a pale yellow oil (**15a**, 127 mg, 98% yield) as a mixture of two diastereoisomers, clearly discernable in the NMR spectra. Found: C, 78.04; H, 11.90. $C_{20}H_{36}O_2$ requires: C, 77.87; H, 11.76%. δ_H (400 MHz, $CDCl_3$): 0.95 (3H × 2, t, J = 7.4 Hz, H-1), 1.14 (3H, d, J = 6.1 Hz, H-15), 1.26 (3H, d, J = 6.1 Hz, H-15), 1.27–1.94 (16H × 2, m, H-3′ to H-5′, H-10 to H-13, H-2), 1.98–2.15 (4H × 2, m, H-9, H-3), 2.82 (2H × 2, dd, J = 6.2, 6.3 H-6), 3.47–3.58 (1H × 2, m, H-6′), 3.70–3.87 (1H × 2, m, H-14), 3.87–4.02 (1H × 2, m, H-6′), 4.64–4.71 (1H, m, H-2′), 4.71–4.78 (1H, m, H-2′), 5.30–5.49 (4H × 2, m, H-4, H-5, H-7, H-8). δ_C (100 MHz, $CDCl_3$): 13.8, 19.1, 19.8, 20.1, 21.6, 22.8, 25.4, 25.56, 25.62, 25.66, 25.8, 27.19, 27.24, 29.3, 29.4, 29.7, 31.3, 36.5, 37.5, 62.4, 62.8, 71.1, 73.9, 95.6, 98.6, 128.02, 128.09, 128.14, 128.18, 129.93, 129.93, 130.05, 130.13. MS: m/z 308 (M$^+$, <1%), 206 (3), 177 (1), 163 (2), 123 (7), 110 (16), 95 (4), 85 (100), 81 (23), 67 (34), 55 (26). IR (neat): ν_{max}/cm^{-1} 3009, 2960, 2929, 2857, 1455, 1376, 1134, 1022, 994, 723.

(4*Z*,7*E*)-2-(14-Oxacyclohexyl)oxypentadeca-4,7-diene (15b). Following the same protocol described for the synthesis of **15a**, the diene **15b** was obtained from **14b** in quantitative yield. Found: C, 77.97; H, 11.88. $C_{20}H_{36}O_2$ requires: C, 77.87; H, 11.76%. δ_H (400 MHz, $CDCl_3$): 0.92 (3H × 2, t, J = 7.4 Hz, H-1), 1.13 (3H, d, J = 6.1 Hz, H-15), 1.23 (3H, d, J = 6.3 Hz, H-15), 1.27–1.94 (16H × 2, m, H-3′ to H-5′, H-10 to H-13, H-2), 1.95–2.03 (2H × 2, m, H-3), 2.03–2.13 (2H × 2, m, H-9), 2.76 (2H × 2, dd, J = 5.3, 5.6 Hz, H-6), 3.47–3.60 (1H × 2, m, H-6′), 3.69–3.88 (1H × 2, m, H-14), 3.88–4.01 (1H × 2, m, H-6′), 4.63–4.70 (1H, m, H-2′), 4.70–4.78 (1H, m, H-2′), 5.33–5.52 (4H × 2, m, H-4, H-5, H-7, H-8). δ_C (100 MHz, $CDCl_3$): 13.6, 19.1, 19.7, 20.1, 21.6, 22.6, 25.4, 25.61, 25.66, 25.8, 27.06, 27.11, 29.4, 29.6, 30.5, 31.2, 34.7, 36.5, 37.5, 62.4, 62.8, 71.1, 73.9, 95.6, 98.6, 127.79, 127.85, 128.50, 128.54, 130.30, 130.39, 130.56, 130.58. MS: m/z 308 (M$^+$, <1%), 290 (1), 222 (1), 206 (4), 177 (2), 163 (3), 149 (2), 123 (8), 110 (16), 95 (11), 85 (100), 81 (17), 67 (25), 55 (17). IR (neat): ν_{max}/cm^{-1} 3010, 2961, 2928, 2856, 1465, 1135, 1022, 994, 967, 725.

(8*Z*,11*E*)-Pentadeca-8,11-dien-2-ol (16a). A solution of **15a** (108 mg, 0.35 mmol) and *p*-toluenesulfonic acid (4 mg, 0.02 mmol) in MeOH (2.5 mL) was stirred for 1 h at rt. After the removal of the solvent under reduced pressure, the crude product was purified by chromatography on silica (eluant 10 : 1 light petroleum–EtOAc) to afford **16a** (70 mg, 89% conversion, 100% yield). Found: C, 80.06; H, 12.35. $C_{15}H_{28}O$ requires: C, 80.29; H, 12.58%. δ_H (400 MHz; $CDCl_3$): 0.95 (3H, t, J = 7.4 Hz, H-15), 1.23 (3H, d, J = 6.2 Hz, H-1), 1.21–1.57 (10H, m, H-3 to H-6, H-14), 1.90–2.16 (4H, m, H-7, H-13), 2.82 (2H, t, J = 6.0 Hz, H-10), 3.78–3.89 (1H, m, H-2), 5.33–5.48 (4H, m, H-8, H-9, H-11, H-12). δ_C (100 MHz, $CDCl_3$): 13.8, 22.8, 23.5, 25.7, 27.2, 29.30, 29.32, 29.6, 29.7, 39.3, 68.2, 128.1, 129.98, 130.01. MS: m/z 224 (M$^+$, 2%), 206 (7), 191 (2), 177 (4), 163 (6), 149 (9), 135 (13), 125 (13), 121 (21), 110 (41), 95 (38), 93 (34), 81 (84), 79 (69), 67 (100), 55 (46), 45 (35). IR (neat): ν_{max}/cm^{-1} 3343, 3009, 2959, 2926, 2855, 1462, 1376, 1126, 722.

(8*Z*,11*Z*)-Pentadeca-8,11-dien-2-ol (16b). By close analogy to the synthesis of **16a**, alcohol **16b** was obtained from **15b** (87% conversion, 86% yield). Found: C, 80.10; H, 12.41. $C_{15}H_{28}O$ requires: C, 80.29; H, 12.58%. δ_H (200 MHz, $CDCl_3$): 0.89 (3H, t, J = 7.3 Hz, H-15), 1.19 (3H, d, J = 6.2 Hz, H-1), 1.22–1.60 (10H, m, H-3 to H-6, H-14), 1.90–2.16 (4H, m, H-7, H-13), 2.74 (2H, dd, J = 4.9, 5.8 Hz, H-10), 3.70–3.91 (1H, m, H-2), 5.30–5.54 (4H, m, H-8, H-9, H-11, H-12). δ_C (50 MHz, $CDCl_3$): 13.7, 22.6, 23.5, 25.6, 27.0, 29.2, 29.6, 30.4, 34.7, 39.3, 68.1, 127.9, 128.5, 130.3, 130.6. MS: m/z 224 (M$^+$, 2%), 206 (8), 191 (2), 177 (4), 163 (7), 149 (9), 135 (14), 121 (20), 110 (40), 96 (42), 93 (38), 81 (89), 79 (72), 67 (100), 55 (50). IR (neat): ν_{max}/cm^{-1} 3350, 3010, 2962, 2929, 2857, 1464, 1375, 1133, 966, 725.

(8*Z*,11*Z*)-Pentadeca-8,11-dien-2-one (10a). A solution of **16a** (69 mg, 0.31 mmol) and PCC (137 mg, 0.62 mmol) in dry CH_2Cl_2 (2 mL) was stirred for 4 h at rt. The resulting black pitchy reaction mixture was directly loaded on a silica column and purified by chromatography (eluant 10 : 1 light petroleum–EtOAc) to afford **10a** as a yellow oil (62 mg, 89% yield). Found: 80.88; H, 11.60. $C_{15}H_{26}O$ requires: C, 81.02; H, 11.79%. δ_H (400 MHz, $CDCl_3$): 0.95 (3H, t, J = 7.4 Hz, H-15), 1.21–1.49 (6H, m, H-5, H-6, H-14), 1.62 (2H, quint, J = 7.4 Hz, H-4), 2.00–2.14 (4H, m, H-7, H-13), 2.17

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(3H, s, H-1), 2.46 (2H, t, $J = 7.4$ Hz, H-3), 2.81 (2H, t, $J = 6.2$ Hz, H-10), 5.32–5.47 (4H, m, H-8, H-9, H-11, H-12). δ_C (100 MHz, $CDCl_3$): 13.8, 22.8, 23.8, 25.7, 27.0, 28.8, 29.3, 29.4, 29.9, 43.8, 128.1, 128.3, 129.8, 130.0, 209.2. MS: m/z 222 (M^+, 9%), 207 (3), 179 (10), 164 (14), 151 (5), 147 (5), 135 (11), 121 (13), 109 (20), 95 (36), 93 (23), 91 (18), 81 (72), 79 (54), 67 (100) 55 (36). IR (neat): ν_{max}/cm^{-1} 3009, 2957, 2930, 2858, 1719, 1461, 1359, 1161, 720.

(8*Z*,11*E*)-Pentadeca-8,11-dien-2-one (10b). Following the procedure described for the oxidation of **16a** to **10a**, compound **10b** was obtained from **16b** in 91% yield. Found: 80.82; H, 11.55. $C_{15}H_{26}O$ requires: C, 81.02; H, 11.79%. δ_H (400 MHz; $CDCl_3$): 0.91 (3H, t, $J = 7.4$ Hz, H-15), 1.27–1.48 (6H, m, H-5, H-6, H-14), 1.61 (2H, quint, $J = 7.4$ Hz, H-4), 1.95–2.03 (2H, m, H-13), 2.03–2.13 (2H, m, H-7), 2.17 (3H, s, H-1), 2.45 (2H, t, $J = 7.4$ Hz, H-3), 2.76 (2H, dd, $J = 4.8$, 5.7 Hz, H-10), 5.32–5.52 (4H, m, H-8, H-9, H-11, H-12). δ_C (100 MHz, $CDCl_3$): 13.8, 22.6, 23.7, 26.9, 28.8, 29.4, 29.8, 30.5, 34.7, 43.7, 128.0, 128.4, 130.1, 130.6, 209.2. MS: m/z 222 (M^+, 13%), 207 (4), 193 (1), 179 (14), 164 (20), 151 (5), 147 (6), 137 (7), 135 (12), 125 (11), 123 (12), 121 (14), 109 (24), 95 (40), 93 (24), 91 (18), 81 (76), 79 (54), 67 (100), 55 (39). IR (neat): ν_{max}/cm^{-1} 3009, 2959, 2928, 2856, 1718, 1462, 1356, 1160, 967, 720.

Cytotoxicity assay

The newly synthesized compounds (**10a** and **10b**) were evaluated for their cytotoxic activity on the human T cell leukemia line (Jurkat) obtained from cell bank ICLC (Interlab Cell Line Collection) (Genova, Italy). Jurkat cells were maintained in RPMI 1640 medium supplemented with L-glutamine (2 mM), 15% fetal bovine serum and 1% of a 1 : 1 mixture of penicillin (50 IU ml^{-1}) and streptomycin (50 µg ml^{-1}) (Roche Molecular Biochemicals, Milan, Italy). Exponentially growing cells (2×10^4) were seeded into 96 well plates in serum-free medium and after 2 h incubation, they were exposed to the synthesized isomers **10a** and **10b** and, for comparison, to the analogous compound **8**, that previously demonstrated cytotoxic activity on two human cancer cell lines.[13] These compounds were dissolved in DMSO at 100 mM and diluted to working concentrations with RPMI 1640 medium (w/o serum); doxorubicin, used as standard anticancer agent, was dissolved in PBS at 1 mM and then diluted in RPMI medium (w/o serum) to working concentrations.

Cell viability was measured using a method based on the cleavage of the 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate (WST-1) to formazan by mitochondrial dehydrogenase activity (cell proliferation reagent WST-1; Roche, Milan, Italy). Following compound exposure, WST-1 was added to each well and after 60 min incubation at 37 °C, the absorbance at 450 nm was measured by a microplate reader (Wallac Victor II, Perkin-Elmer, MA, USA).

Cells were exposed to the investigated compounds in the concentration range 0.1–100 µM and to doxorubicin in the range 0.01–1 µM. Inhibition of cell viability was calculated, after 48 h exposure, by comparing the number of viable cells after treatment, to cells exposed to DMSO (controls). IC_{50} values represent the concentrations of drugs at which the absorbance data subtracted of relative blank was 50% of that in controls. All experiments were performed in triplicate and results are expressed as mean ± s.e. Statistical difference among IC_{50} values was evaluated by means of ANOVA analysis of variance and Bonferroni post-test. A P value ≤ 0.05 was considered to be significant.

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Notes and references

1 S. E. Binns, B. Purgina, C. Bergeron, M. L. Smith, L. Ball, B. R. Baum and J. T. Arnason, *Planta Med.*, 2000, **66**, 241–244.
2 C. A. Parish, J. Huber, J. Baxter, A. Gonzalez, J. Collado, G. Platas, M. T. Diez, F. Vicente, K. Dorso, G. Abruzzo and K. Wilson, *J. Nat. Prod.*, 2004, **67**, 1900–1902.
3 M.-C. Rho, H. S. Lee, S. W. Lee, J. S. Chang, O. E. Kwon, M. Y. Chung and Y. K. Kim, *J. Agric. Food Chem.*, 2005, **53**, 919–922.
4 N. Wang, X. Yao, R. Ishii and S. Kitanaka, *Chem. Pharm. Bull.*, 2001, **49**, 938–942.
5 Y. Chen, T. Fu, T. Tao, J. Yang, Y. Chang, M. Wang, L. Kim, L. Qu, J. Cassady, R. Scalzo and X. Wang, *J. Nat. Prod.*, 2005, **68**, 773–776.
6 V. M. Dembitsky, *Lipids*, 2006, **41**, 883–924; A. Siddiq and V. Dembitsky, *Anticancer Agents Med. Chem.*, 2008, **8**, 132–170.
7 A. Chicca, B. Adinolfi, E. Martinotti, S. Fogli, M. C. Breschi, F. Pellati, S. Benvenuti and P. Nieri, *J. Ethnopharmacol.*, 2007, **110**, 148–153.
8 R. Bauer, I. A. Khan and H. Wagner, *Planta Med.*, 1988, **54**, 426–430.
9 R. Bauer, P. Remiger and H. Wagner, *Phytochemistry*, 1988, **27**, 2339–2342; R. Bauer and P. Remiger, *Planta Med.*, 1989, **55**, 367–371; R. Bauer, P. Remiger and H. Wagner, *Phytochemistry*, 1989, **28**, 505–508.
10 F. Pellati, S. Calò, S. Benvenuti, B. Adinolfi, P. Nieri and M. Melegari, *Phytochemistry*, 2006, **67**, 1359–1364.
11 F. Pellati, S. Calò and S. Benvenuti, *J. Chromatogr., A*, 2007, **1149**, 56–65.
12 I. Oniga, H. Popescu, P. Verite and R. Oprean, *Clujul Medical*, 1997, **71**, 304–308.
13 A. Chicca, F. Pellati, B. Adinolfi, A. Matthias, I. Massarelli, S. Benvenuti, E. Martinotti, A. M. Bianucci, K. Bone, R. Lehmann and P. Nieri, *Br. J. Pharmacol.*, 2008, **153**, 879–885.
14 S. Morandi, F. Pellati, S. Benvenuti and F. Prati, *Tetrahedron*, 2008, **64**, 6324–6328.
15 F. Heinzer, M. Chavanne, J.-P. Meusy, H.-P. Maître, E. Giger and T. W. Baumann, *Pharm. Acta Helv.*, 1988, **63**, 132–136.
16 The correlation coefficient (r^2) between t_R and the calculated log octanol/water partition coefficients (CLogP, evaluated with ChemOffice 2002 software) was 0.876.
17 A. V. Buevich, T.-M. Chan, C. H. Wang, A. T. McPhail and A. K. Ganguly, *Magn. Reson. Chem.*, 2005, **43**, 187–199; K. Kamienska-Trela and J. Wojcik, *Nucl. Magn. Reson.*, 2006, **35**, 152–198.
18 G. A. Kraus, J. Bae and J. Schuster, *Synthesis*, 2005, **20**, 3502–3504.
19 N. Matovic, A. Matthias, J. Gertsch, S. Raduner, K. M. Bone, R. P. Lehmann and J. J. DeVoss, *Org. Biomol. Chem.*, 2007, **5**, 169–174.
20 A. P. Dobbs, A. Venturelli, L. A. Butler and R. J. Parker, *Synlett*, 2005, 1–3.
21 T. Kanai, Y. Kanagawa and Y. Ishii, *J. Org. Chem.*, 1990, **55**, 3274–3277.
22 D. G. Coe, S. R. Landauer and H. N. Rydon, *J. Chem. Soc.*, 1954, 2281–2288.
23 K. A. Tallman, B. Roschek, Jr. and N. A. Porter, *J. Am. Chem. Soc.*, 2004, **126**, 9240–9247; Y. Masuda and K. Mori, *Biosci., Biotechnol., Biochem.*, 2002, **66**, 1032–1038.
24 Coupling reaction catalyzed by CuI [R. J. Capon and R. A. Barrow, *J. Org. Chem.*, 1998, **63**, 75–83; L. W. Bieber and M. F. da Silva, *Tetrahedron Lett.*, 2007, **48**, 7088–7090] was found to proceed both through the direct (C-1) and conjugated (C-3) attack of the alkynyllithium on the allyl halide, as also observed by Tallmann (see ref. 21). This led to a mixture of two inseparable regioisomers, clearly discernible by NMR means.
25 C. A. Brown and V. K. Ahuja, *J. Org. Chem.*, 1973, **38**, 2226–2230.