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A hydroxy-β-caryophyllene from *Pterocaulon serrulatum*

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Abstract

A new caryophyllene, 4,5-epoxy-13-hydroxy-β-caryophyllene, has been isolated from *Pterocaulon serrulatum* using a bioassay-guided fractionation procedure. Several known compounds were also identified in the extracts, including 14-hydroxy-β-caryophyllene, three simple coumarins and a flavanone.

Keywords: *Pterocaulon serrulatum*; Asteraceae; 4,5-epoxy-13-hydroxy-β-caryophyllene; 14-hydroxy-β-caryophyllene; Coumarins; Pinocembrin

1. Introduction

Pterocaulon serrulatum (Montr.) Guillaumin is a herbaceous plant which is widespread across central and northern Australia and is usually found near rocky drainage lines or seasonal creeks. In inhabited areas it is considered to be a weed and commonly occurs wherever the ground is disturbed, such as at construction sites. In the Northern Territory, aboriginal communities use the plant in traditional bush medicine, both for the relief of congestion by inhaling the aromas from the crushed leaves and as an antiseptic wash. For the latter purpose, leaves and twigs are boiled in water and the strained liquid used as a wash to treat skin disorders such as scabies and ringworm as well as sores and cuts (Barr, 1988).

In this paper, we report the isolation and identification from *P. serrulatum* of three compounds (**4**–**6**), including a new β-caryophyllene, all of which showed activity in the bioassay-directed extraction procedure used, as well as three inactive coumarins (**1**–**3**).

2. Results and discussion

The air-dried aerial parts of *P. serrulatum*, collected in the Nitmiluk National Park, Katherine, Northern Territory, were first extracted with heptane. The inactive residue left after washing the dried heptane extract with diethyl ether contained a mixture of three coumarins **1**, **2** and **3**, which could be separated by flash column chromatography and purified by recrystallisation.

Mass spectrometry together with ^{1}H NMR and ^{13}C NMR spectroscopy established the structure of **1** as that of the known coumarin, ayapin (Ziegler, Möhler & Effenberger, 1987) and **2** as 5-methoxy-6,7-methylenedioxycoumarin (Maldonado, Hernandez & Ortega, 1992). Compound **3** was identified as 6,7,8-trimethoxycoumarin from its ^{1}H NMR and mass spectra and m.p. of 104–106° (King, Housley & King, 1954) which differed substantially from that of the two other possibilities, 5,6,7- and 5,7,8-trimethoxycoumarins (74–75° and 179–180°, respectively) (Wagner & Bladt, 1975; Dean, Costa, Harborne & Smith, 1978).

The diethyl ether extract of the residue from heptane extraction showed activity in the anti-bacterial bioassay. After two vacuum liquid chromatography (VLC) steps, a single active compound was isolated which was subsequently identified as 14-hydroxy-β-caryophyllene (**4**). The EI mass spectrum of **4** showed a weak M^{+} ion at m/z 220 which by accurate mass measurement corresponded to a molecular formula of $C_{15}H_{24}O$, suggesting that it was a sesquiterpene. Both the ^{1}H NMR and ^{13}C NMR spectra, however, contained too many signals to fit the above formula. HPLC and GC/MS confirmed its purity and mass spectrum, hence the only explanation for the doubling-up of signals in the NMR spectra was that it was a

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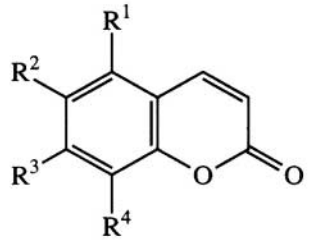
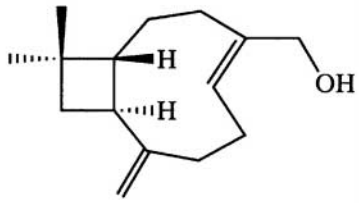


	R^1	R^2	R^3	R^4
1	H	$-OCH_2O-$		H
2	OMe	$-OCH_2O-$		H
3	H	OMe	OMe	OMe



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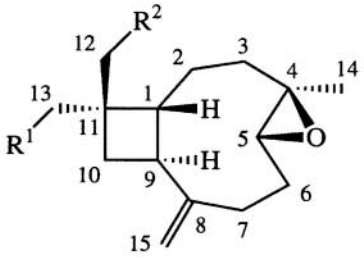
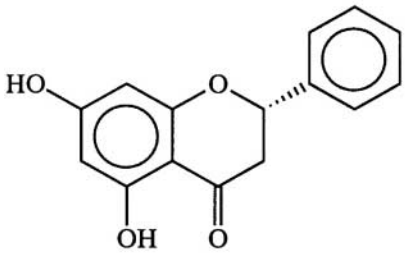


	R^1	R^2
6	OH	H
9	H	OH



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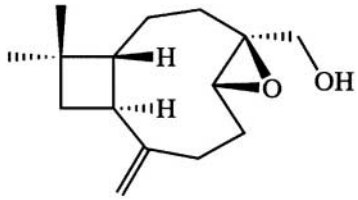

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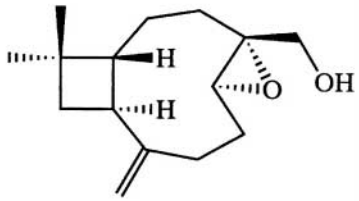

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mixture of two stable conformers which did not interconvert on the NMR timescale. The presence in the NMR spectrum of an exocyclic methylene group, coupled with an indication of a *gem*-dimethylcyclobutane ring from retro-[2 + 2] fragment ions at *m/z* 56 ($C_4H_8^+$) and 164 ($M^+-C_4H_8$) in its mass spectrum, suggested that **4** was a caryophyllene derivative. A previously reported compound with identical optical rotation and spectra data, which had been shown to be a mixture of conformers by variable temperature ^1H NMR experiments, was originally assigned the structure 14-hydroxy-9-epi-β-caryophyllene (Barrero, Sanchéz & Ferrol, 1990; Barrero, Oltra & Barragán, 1990). This was later shown to be in error and reassigned the correct structure, 14-hydroxy-β-caryophyllene (**4**) (Hinkley, Perry & Weavers, 1994; Barrero et al., 1995).

The methylene chloride extract of the plant material after heptane extraction gave rise to two bioactive compounds following several VLC steps. The first was readily identified as 5,7-dihydroxyflavanone (**5**) from its NMR and mass spectra, the latter containing characteristic retro Diels Alder fragment ions. Its m.p. and spectral data were identical to that reported for pinocembrin (Jaipeteh, Kanghae, Patrick & White, 1982; Talapatra, Deb & Talapatra, 1985). The second compound (**6**) showed an MH^+ ion at *m/z* 237 in its CI mass spectrum but no discernible M^+ in its EI

mass spectrum. The latter did, however, contain fragment ions at m/z 221 ($C_{14}H_{21}O_2$) and 205 ($C_{14}H_{21}O$) corresponding to losses of CH_3 and CH_2OH, respectively, from the molecular ion, confirming its composition as $C_{15}H_{24}O_2$.

The ^{1}H NMR spectrum of **6** showed similarities to that of **4** indicating that they were closely related. Two methyl singlets were present at δ1.06 and 1.20, while an AB system at δ3.52 and 3.68 and two one proton singlets at δ4.90 and 5.02 indicated the presence of a hydroxymethyl group on a quaternary carbon atom and an exocyclic methylene group, respectively. The caryophyllene skeleton was confirmed by a DQF-COSY experiment which showed two isolated spin systems: an eight proton system for H-10, 9, 1, 2 and 3 and a five-proton system for H-5, H-6 and H-7. The absence of a vinylic proton together with an extra oxygen atom in the molecular formula led to the assignment of a 4,5-epoxide. Because of its co-occurrence with **4**, the hydroxymethyl group was tentatively placed at C-4. However, both diastereomers of 4, 5-epoxy-14-hydroxy-β-caryophyllenes (**7** and **8**, respectively) have previously been prepared by partial synthesis (Barrero et al., 1995) and comparison of their ^{1}H NMR and ^{13}C NMR spectra with those of **6** ruled them out as possible structures. The possibility that **6** could be the 9-epi isomer of either **7** or **8**, which would explain the large difference ($\Delta\delta \sim 0.2$ ppm) in the chemical shift of the two methyl groups (Hinkley, Perry & Weavers, 1994; Backens et al., 1984), was excluded by a NOESY experiment. Also ruled out on the basis of the ^{1}H NMR and ^{13}C NMR spectra was the 12-hydroxy compound (**9**) which had been reported previously as a natural compound (Abraham, Ernst & Stumpf, 1990).

All of the observed NOESY interactions could be explained, however, on the basis of **6** being the 13-hydroxy derivative of 4,5-β-epoxy-β-caryophyllene. Observed NOEs for H-1 → 12Me → H-10β → H-10α → H-9 confirmed the *trans*-relationship of H-1/H-9 and H-1 and the C-13 hydroxymethyl group (Fig. 1). In addition, the methylene protons on C-13 showed an interaction with H-2α. The interaction of H-5 with H-3β and H-6β established that the epoxide was on the top face of the molecule.

Attempts to prepare the crystalline *p*-bromobenzoate and 4-bromo-3,5-dinitrobenzoate derivatives of **6** were unsuccessful. Since all β-caryophyllene derivatives with confirmed absolute configuration isolated from plants have been shown to possess the (1*R*,9*S*)-configuration, it can be assumed that **6** has the same absolute stereochemistry.

3. Experimental

3.1. General

M.p.'s: uncorr. ^{1}H NMR: 300 and 500 MHz, TMS int. standard. ^{13}C NMR: 75.5 MHz, solvent as internal standard. 2D NMR experiments were performed using standard Varian pulse sequences. MS: EI (70 eV), 7070F and ZAB-2SEQ; CI(NH_3), 7070F. TLC: Merck silica gel 60 PF254. VLC: Merck silica gel 60.

3.2. Plant material

The aerial parts of *P. serrulatum* were collected in the Nitmiluk National Park, Katherine, Northern Territory, Australia. A voucher specimen was deposited at the Northern Territory Herbarium, Darwin (voucher: G. M. Wightman 6122 and LLVW).

3.3. Bioassay

The micro-organisms used in the bioassay, *Bacillus subtilis* RSC1 and *Escherichia coli* RSC2, are stock test organisms continuously cultivated in the microbiological laboratory of the Research School of Chemistry. To carry out the assay, samples of extracts were dissolved in an appropriate solvent and aliquots equivalent to *ca.* 1 and 0.1 mg of dry sample, were used to wet filter paper disks (5 mm), together with a solvent blank. The disks were air-dried, placed on an inoculated agar plate and incubated at 28° overnight. Inhibition zones were measured after 18–24 h.

3.4. Extraction and isolation

The dried aerial parts of *P. serrulatum* (270 g) were macerated with heptane (1 l) in a blender, then left overnight. After filtering, extraction of the residue with heptane was repeated (1 l × 3), the extracts combined and the solvent removed under vacuum, leaving a

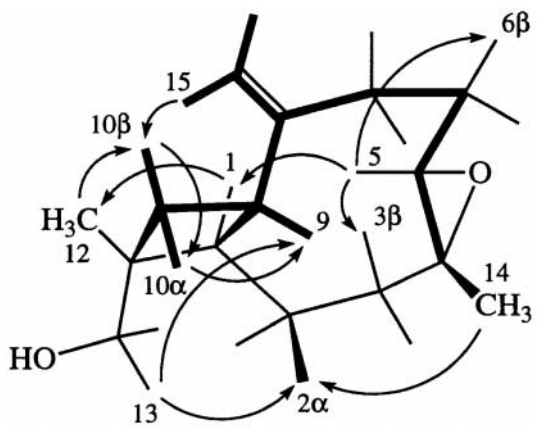

Fig. 1. Important observed NOEs for compound **6**.

solid residue (7.3 g). The heptane extract was suspended in Et_2O and filtered. The inactive solid residue (1.1 g), which showed three major fluorescent spots on TLC, was purified by flash CC on silica, eluting with CH_2Cl_2–EtOAc (2:1:1) to give the three known coumarins **1** (16 mg), colourless needles; m.p. 217–220° (lit. [2], m.p. 224°); **2** (76 mg), colourless needles; m.p. 200–202° (lit. [3], m.p. 200–202°); **3** (430 mg), colourless needles; m.p. 104–106° (lit. [4], m.p. 103–105°). The ethereal filtrate, after removal of solvent, was fractionated by VLC, using gradient elution with hexane–EtOAc (100:0 → 0:100). Three successive frs, which were active in the bioassay and showed similar TLC traces, were combined and further purified by VLC using a hexane–CH_2Cl_2–Et_2O gradient (7:2:1 → 0:3:2), followed by flash CC on silica impregnated with $AgNO_3$(5%), to give 14-hydroxy-β-caryophyllene (**4**) (165 mg), colourless oil; $[\alpha]_D$ −14.2° ($CHCl_3$, *c* 0.76) (lit. (Barrero, Oltra & Barragán, 1990), −13.5°).

The residual plant material after heptane extraction was re-extracted with CH_2Cl_2 (4 × 1 l, 24 h). After combining the extracts and removal of the solvent, the residue was fractionated by VLC using a CH_2Cl_2–EtOAc (100:0 → 0:100) gradient. The bioactive fr. (2.6 g) was suspended in Et_2O and filtered. Activity was present in the filtrate only, which after concentration was further purified by VLC, eluting with hexane–THF (9:1 → 0:100). The active fr. was again subjected to VLC, first eluting with CH_2Cl_2 to remove most of the fluorescent material, then with CH_2Cl_2–MeOH (100:0 → 95:5). A further VLC step, eluting with hexane–EtOAc (4:1 → 0:100), was necessary to separate the two active components. A final purification by flash CC on silica of the first fr., eluting with CH_2Cl_2–EtOAc (99:1 → 95:5) gave pinocembrin (**5**) (66 mg) as colourless needles, m.p. 200–202° (lit. [11], m.p. 200–201°). Flash CC of the second fr. using CH_2Cl_2–EtOAc (9:1) as eluent, gave **6** (41 mg).

3.5. 4,5-Epoxy-13-hydroxy-β-caryophyllene (6)

Colourless oil; $[\alpha]_D$ −64.1° ($CHCl_3$, *c* 0.86). HREIMS $[M–Me]^+$, found 221.1538. $C_{14}H_{21}O_2$ requires 221.1542. $[M–CH_2OH]^+$, found 205.1593. $C_{14}H_{21}O$ requires 205.1592.

^1H NMR (500 MHz, $CDCl_3$): δ 0.95 (1H, *ddd*, J = 5.5, 12.7, 13.3 Hz, H-3a), 1.06 (3H, *s*, H-12), 1.20 (3H, *s*, H-14), 1.33 (1H, *dddd*, J = 5.0, 7.5, 10.5, 12.4 Hz, H-6a), 1.54 (1H, *dddd*, J = 4.1, 10.3, 13.3, 14.8 Hz, H-2a), 1.60 (1H, *dd*, J = 11.0, 11.4 Hz, H-10a), 11.85 (1H, *dddd*, J = 1.3, 3.3, 5.5, 14.8 Hz, H-2b), 1.91 (1H, *ddd*, J = 1.3, 8.5, 10.3 Hz, H-1), 1.96 (1H, *dd*, J = 8.8, 11.4 Hz, H-10b), 2.08 (1H, *ddd*, J = 3.3, 4.1, 12.7 Hz, H-3b), 2.10 (1H, *ddd*, J = 0.8, 7.5, 13.0 Hz, H-7a), 2.24 (1H, *dddd*, J = 0.8, 4.5, 7.3, 12.4 Hz, H-6b), 2.36 (1H, *ddd*, 5.0, 7.3, 13.0 Hz, H-7b), 2.67 (1H, *ddd*, J = 8.5, 8.7, 11.0 Hz, H-9), 2.86 (1H, *dd*, J = 4.5, 10.5 Hz, H-5), 3.52 (1H, *d*, J = 10.8 Hz, H-13a), 3.68 (1H, *d*, J = 10.8 Hz, H-13b), 4.90 (1H, *d*, J = 1.6 Hz, H-15a), 5.02 (1H, *d*, J = 1.6 Hz, H-15b). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 16.9 (C-12), 24.9 (C-13), 26.2 (C-2), 29.8 (C-6), 30.1 (C-7), 35.2 (C-10), 38.6 (C-11), 39.3 (C-3), 48.3 (C-9), 51.4 (C-1), 59.7 (C-4), 63.6 (C-5), 66.9 (C-14), 113.0 (C-15). 151.7 (C-8). EIMS (probe) 70 eV, *m/z* (rel. int): 221 $[M–Me]^+$(1), 205 $[M–CH_2OH]^+$(7), 159(21), 147(28), 135(29), 119(60), 107(92), 84(100), 79(28), 55(23). CIMS (NH_3) *m/z* (rel.int): 237$[MH]^+$(19), 219$[MH–H_2O]^+$(58), 201$[MH–2H_2O]^+$.

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