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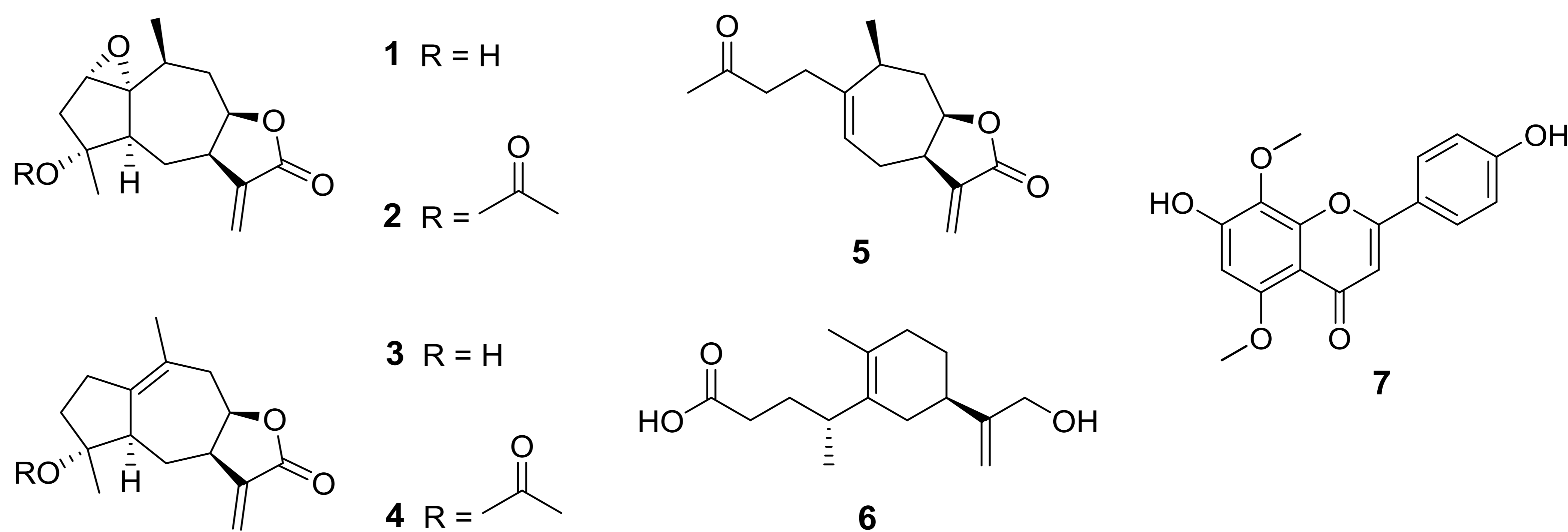
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INTRODUCTION

Leucophyta brownii Cass. (syn. *Calocephalus brownii* (Cass.) F. Muell.), a shrub of the Asteraceae family, is endemic to Australia, where it typically occurs in the southern coastal areas. *L. brownii* is commonly cultivated as an ornamental plant, and it has not been used in traditional plant medicine. Previous investigations of the resinous exudates of cushion bush resulted in the isolation of several 8,12-guaianolides and other sesquiterpenoids [1–3] of which several display cytotoxic and anti-inflammatory activity [1]. A reinvestigation of the resinous exudates of cushion bush resulted in the isolation of the 8,12-guaianolides **1–4**, the xanthanolide tomentosin (**5**), and the 1,10-seco-eudesmane leucophytalin C (**6**) [1], as well as new flavone 7,4'-dihydroxy-5,8-dimethoxyflavone (**7**). Compounds **1–6** were investigated for their cytotoxic activity towards human breast cancer (MCF-7) and colon cancer (HT-29) cells and their ability to induce apoptosis.



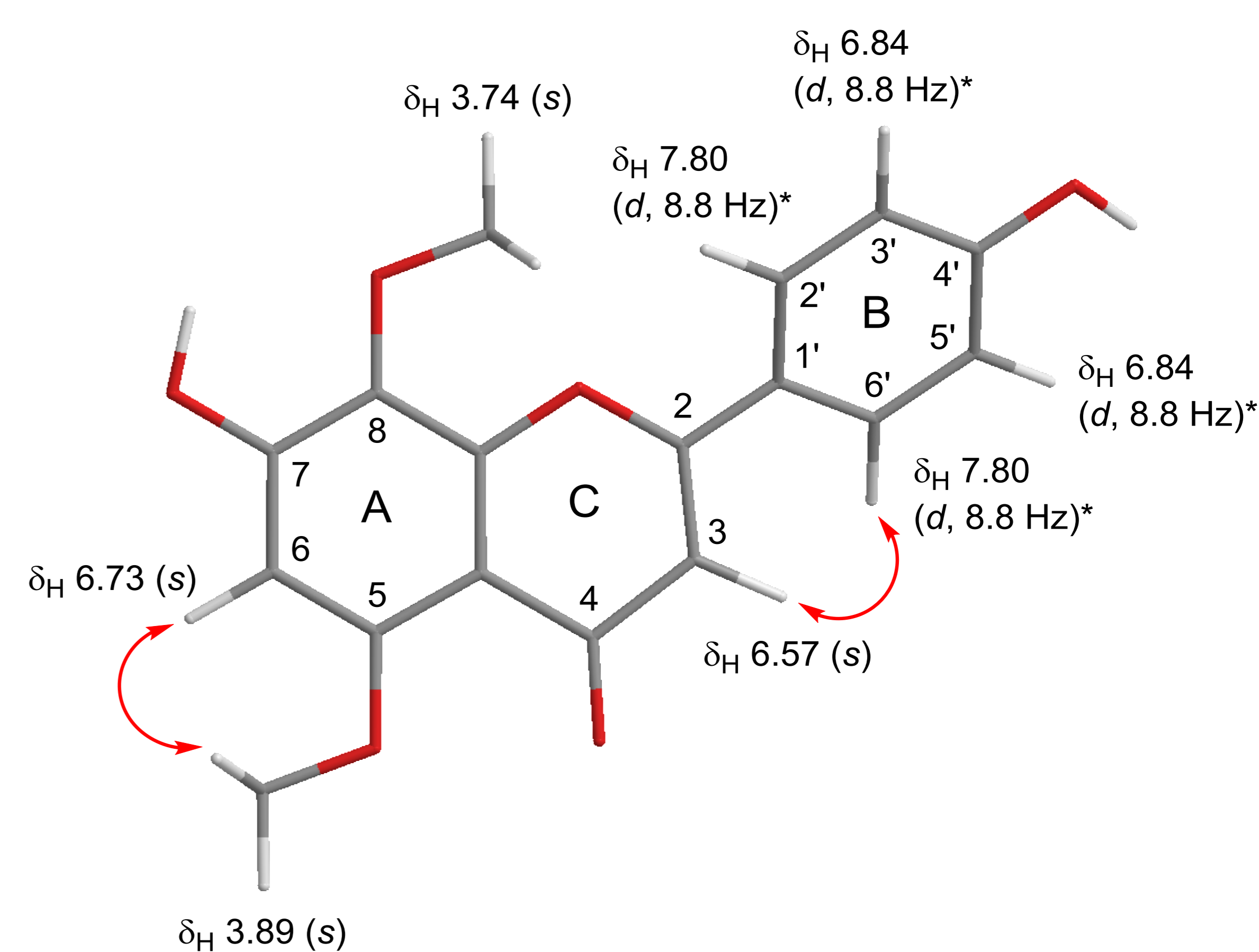
Leucophyta brownii



IDENTIFICATION OF A NEW FLAVONE

Fresh aerial parts of *L. brownii* (1.6 kg) were extracted with CH_2Cl_2 , and the extract was subjected to flash column chromatography over silica gel and semipreparative HPLC to yield the sesquiterpenoids **1–6** and a the flavone **7**. Compound **7** (2 mg) was isolated as a light yellow oil. Compound **7** showed UV absorptions characteristic of a flavone (λ_{max} (MeOH) 281, 305 nm) and was assigned the molecular formula $\text{C}_{17}\text{H}_{14}\text{O}_6$ on the basis of 1D NMR and HRESIMS data (m/z 315.0870 [$\text{M} + \text{H}$]⁺, calculated for $\text{C}_{17}\text{H}_{15}\text{O}_6$ 315.0869). The ¹H NMR spectrum (recorded in methanol-*d*₄) exhibited characteristic signals from two methoxy groups, a hydrogen proton in position 3 of ring C, an aromatic proton in position 6 of ring A and four aromatic protons in ring B forming a non-first order AA'BB'-system (Fig. 1). The latter was confirmed by the ¹H–¹H COSY and NOESY spectra. The NOESY spectrum revealed furthermore key correlations between H-3 and H-6', and H-6 and the methoxy group at C-5 (Fig. 1). Therefore the structure of **7** was established as 7,4'-dihydroxy-5,8-dimethoxyflavone.

The flavone **7** has previously been synthesized and the spectroscopic data were in accordance with literature values [4]. However to the best of our knowledge this is the first report of 7,4'-dihydroxy-5,8-dimethoxyflavone (**7**) from natural sources.



*Part of a non-first order AA'BB' system

Fig. 1. ¹H NMR data and key NOESY correlations (↔).

CYTOTOXICITY OF SESQUITERPENIDS

Sesquiterpene lactones that contain an α,β -unsaturated γ -lactone moiety are known for their anti-inflammatory and cytotoxic activity due to reactions with sulfhydryl groups of functional proteins via a Michael-type reaction [1]. Compounds **1–6** were investigated for their cytotoxic activity towards human breast cancer (MCF-7) and colon cancer (HT-29) cells and their ability to induce apoptosis. Compounds **1–4** reduced proliferation of HT-29 and MCF-7 cells between 60–90% at a concentration of 18.9, 16.3, 20.2 and 17.2 μM , respectively, with IC_{50} values < 10 μM . Tomentosin (**5**) showed less cytotoxicity with an IC_{50} value of > 20 μM for both cell lines. As expected leucophytalin C (**6**) did not show any significant cytotoxicity at a concentration of > 79 μM (Table 1).

No activation of caspase-3, -7 and -8 was observed in the tested cancer cell lines. This was also confirmed by gene expression studies, indicating that the observed cytotoxic effect of compounds **1–5** was not due to an apoptosis initiated mechanism.

Table 1. Antiproliferative activity of compounds **1–6** on cancer cells of breast (MCF-7) and colon (HT-29) origin

Compound	IC_{50} values in μM^a	
	MCF-7	HT-29
1	7.6	9.5
2	6.5	8.2
3	8.1	8.1
4	6.9	8.6
5	> 20	> 20
6	> 79	> 79

^aDetermined by a Resazurin metabolism assay as previously described [1].

REFERENCES

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