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Antimicrobial acylphloroglucinols and dibenzyloxy flavonoids from flowers of *Helichrysum gymnocomum*

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ABSTRACT

From the dichloromethane extract of the flowers of *Helichrysum gymnocomum* (Asteraceae) two known flavonoids, **4** and **5**, and a known acylphloroglucinol, **3B**, were isolated. In addition to **1** and **2**, the 4′,6′-dibenzyloxy-2′-hydroxy derivative of 2′,4′,6′-trihydroxychalcone and 5,7-dibenzyloxy derivative of pinocembrin, respectively, are reported in Nature for the first time. A compound **3A**, related to **3B** has the structure 2-methyl-1-[2,4,6-trihydroxy-3-(2-hydroxy-3-methyl-3-butenyl)phenyl]-1-propanone. Compounds **1, 2, 3A, 3B, 4** and **5** have MIC values below 64 μg/ml against a selection of pathogens, with **3B** having the highest sensitivity (6.3–45 μg/ml) for eight of the ten pathogens tested, including *Staphylococcus aureus* (6.3 μg/ml) and methicillin and gentamycin resistant strain of *S. aureus* (7.8 μg/ml). With the exception of **2**, the other compounds had notable activity (45–63 μg/ml) towards *Pseudomonas aeruginosa*.

1. Introduction

Some 600 species of *Helichrysum* (Asteraceae) occur in Africa, of which some 244 species are found in South Africa (Pooley, 2003). The plants occur as herbs and shrubs. Often the leaves and flowers are pleasantly scented and may be burnt by the indigenous people to fumigate a sick room or to invoke the goodwill of the ancestors (Pooley, 2003). Mixed with fat, the plant produces a soothing ointment. Other applications include the use of various parts of the plant to treat coughs and colds and particularly to combat the infection of wounds (Pooley, 2003; Bremner and Meyer, 2000). *Helichrysum gymnocomum*, the subject of this investigation, is a perennial herb which grows profusely in the KwaZulu-Natal Drakensburg. The flowers are pleasantly scented. The above properties, coupled with its long flowering season (February–July) (Hilliard, 1997) make the plants an obvious choice for medicinal and ritual purposes.

The chemistry of a large number of *Helichrysum* species from diverse areas of South Africa has been studied at length by Bohlmann and Mahanta (1979), Bohlmann and Abraham (1979), Bohlmann and Hoffmann (1979). More recent research on the genus has focused on the antimicrobial properties of the phloroglucinol derivatives in the genus (Meyer et al., 1997; Bremner and Meyer, 2000; Drewes et al., 2006).

On the whole researchers have utilized only the leaves, stems and roots of *Helichrysum* plants. Since flowers have long been known to be particularly rich sources of biologically active compounds (Swerdlow, 2000), this paper reports on the constituents in the flowers of *H. gymnocomum*. This decision was further reinforced by the findings of a recent paper (Appendino et al., 2007) in which the authors describe the isolation of arzanol, a phloroglucinol α-pyrone having anti-inflammatory and anti-HIV properties. Arzanol was extracted from the bright yellow flowerheads of *Helichrysum italicum* growing in Sardinia.

2. Results and discussion

2.1. Determination of structure

From the yellow flowers of *H. gymnocomum,* three known compounds were isolated. These were 3-methoxyquercetin **4** (Bouktaib et al., 2002), the 4′-*O*-glucose derivative of 2′-hydroxy-6′-methoxy chalcone **5** (Wright, 1976), and the acylphloroglucinol derivative **3B**, 3-[3′,3′-dimethylallyl-(1′)]-1-isobutyrylphloroglucinol (Bohlmann and Mahanta, 1979). Comparison of spectral data of our compounds with those published in the literature together with high resolution mass spectral analysis of **4, 5** and **3B** confirmed the identity of the isolated compounds (see Fig. 1).

The compounds **1** (2′-hydroxy-4′,6′-dibenzyloxychalcone) and **2** (5,7-dibenzyloxyflavanone) are new in Nature although their 'parent' compounds (2′,4′,6′-trihydroxychalcone and

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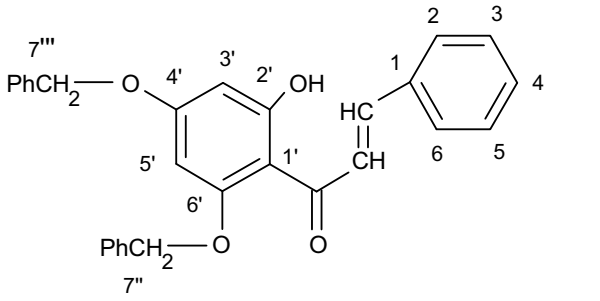
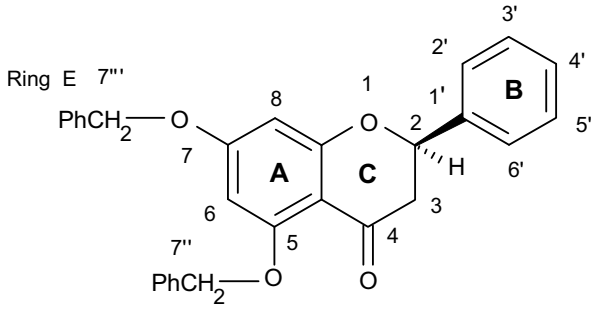


(1)



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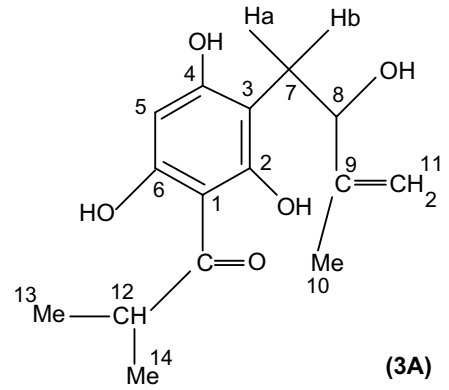
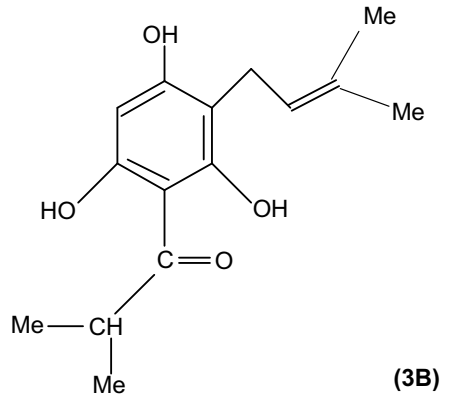


(3A)



(3B)

Fig. 1. Structures of compounds (**1**), (**2**), (**3A**) and (**3B**).

5,7-dihydroxyflavanone, respectively) are well known (Linstedt, 1951; Jurd and Horowitz, 1961). It is interesting to note that the recent findings on benzyloxy derivatives of a range of chalcones have shown them to be potent inhibitors of interleukin-5 (Yang et al., 2007). The NMR data for compounds **1** and **2** are shown in Table 1.

The new compound **3A** reported here using IU PAC nomenclature, is 1-[2,4,6-trihydroxy-3-(2-hydroxy-3-methyl-3-butenyl)phenyl]-1-propanone. It is close in structure to **3B**. The latter was first isolated from *H. gymnocomum* (Bohlmann and Mahanta, 1979) and later from *H. platypterum* (Bohlmann and Zdero, 1979). In both instances, leaves and stalks of the plants were extracted. A synthesis of **3B** was subsequently published (Kuhnke and Bohlmann, 1985). Accurate ^{1}H and ^{13}C NMR data have been reported by Bremner and Meyer (2000). Comparison of our detailed spectral data for **3B**, coupled with a high resolution mass analysis, leave no doubt that it is identical to the compound described in the literature as cited here.

Compound **3A** was only separated with great difficulty from **3B**. When pure, it crystallized in very fine needles, m.p. 133 °C. Its structure was established using ^{1}H and ^{13}C NMR techniques including COSY, DEPT, HSQC and HMBC programmes. Comparison of the proton spectra of **3A** and **3B** shows immediately that both compounds contain a phloroglucinol ring with the identical substituent (2-methylpropan-1-one) at C-1 and a singlet aromatic proton, C-5, at 5.89 ppm. The single difference is the replacement of the prenyl side chain at C-3 with a 2-hydroxy-3-methyl-3-butenyl moiety in **3A**. Since the methylene group at C-7 (using the "simpler" numbering of the side chain) is adjacent to the chiral centre at C-8, the two protons are diastereotopic, and give rise to a clear doublet of doublets (Table 2). For purposes of comparison the ^{13}C spectrum of **3B** is also included in the table. The isolation of **3A** neatly supplements the existence of a series of highly active antimicrobial phloroglucinol derivatives starting with **3B** (Bohlmann and Mahanta, 1979), followed by the acylphloroglucinols from *Hypericum foliosum* and *Hypericum beanii* (Gibbons et al., 2005; Shiu and Gibbons, 2006), and very recently the isolation of arzanol (Appendino et al., 2007) from *H. italicum*. Apart from all these compounds being based on phloroglucinol, they have other structural features of interest. Thus, the acyl phloroglucinol from *H. foliosum* (Gibbons et al., 2005) contains a five carbon epoxy side chain which chemically is not far removed from the CH_2–CH(OH)C-(Me)=CH_2, group present in **3A**. In fact, the one could be a precursor of the other.

2.2. The dibenzyloxy derivatives 1 and 2

The existence of the dibenzyloxy derivatives in the yellow flowers (and not in the leaves and stems) of *H. gymnocomum* was unexpected. While the benzyloxy group is a familiar protecting group for phenolic OH's, its existence in Nature is not recorded. The antimicrobial test results (Table 3) reveal high activity against several pathogens (Section 2.3). At this stage it is not known to what extent the existence of the benzyloxy group, as well as the presence of the unsubstituted B-ring in both **1** and **2**, play a role in influencing antimicrobial activity. It is noteworthy that Yang et al. (2007) reported that benzyloxy derivatives of some chalcones are potent inhibitors of interleukin-5.

2.3. Antimicrobial test

The antimicrobial activity of the crude *H. gymnocomum* extract exhibited a broad spectrum of activity with minimum inhibitory concentration (MIC) values ranging from 312 to 1000 μg/ml depending on the pathogen studied. According to Fabry et al. (1998), extracts having MIC values below 8000 μg/ml possess some antimicrobial activity. MIC values below 1000 μg/ml are considered noteworthy (Gibbons, 2004; Rios and Reico, 2005). Thus, the crude extract having activities of 1000 μg/ml or lower against all the pathogens studied demonstrated potential anti-infective properties.