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Amorfrutin-type phytocannabinoids from *Helichrysum umbraculigerum*

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ABSTRACT

Helichrysum umbraculigerum Less. has been reported to be a prolific producer of phytocannabinoids from the alkyl-, aralkyl-, normal-, and abnormal types. Investigation of an acetone extract from the aerial parts of the plant afforded two novel amorfrutin-type phytocannabinoids (**3b**, **4**) and the new geranylated phloroglucinol **5a**. The presence of cannabigerol (CBG, **1a**) and its acidic precursor (pre-CBG, CBGA, **1b**), previously reported from this plant, could not be confirmed, but the phenethyl analogue of CBG (Heli-CBG, **2a)** and the methyl ester of its carboxylated version (**2b**) were isolated. Heli-CBG (**2a**) was assayed against a series of metabotropic (CB_1 and CB_2)- and ionotropic (thermo-TRPs) targets of phytocannabinoids, comparing its profile with the one of cannabigerol (CBG). A decreased affinity for cannabinoid receptor was observed, along with substantial retention of the thermo-TRP profile. The biogenetic relationships between the isoprenylated phenolics from *H. umbraculigerum* are discussed, highlighting the relevance of this species for biogenetic investigations on phytocannabinoids

1. Introduction

As the result of the convergence of two modular pathways (polyketides and isoprenoids), phytocannabinoids occur in a variety of chemotypes, distinct on the basis of their derivation from an aliphatic or an aromatic ketide starter (alkyl- and aralkyl phytocannabinoids, respectively) and of the degree of oligomerization of the isoprenyl residue [1]. Although traditionally associated to cannabis (*Cannabis sativa* L.), phytocannabinoids have actually a broader, although point-like, distribution in Nature, encompassing not only higher plants, but also liverworts and fungi [1]. For this reason, and to avoid confusion with the pharmacological meaning of the name "cannabinoid" (modulator of the cannabinoid receptors CB_1 and CB_2), the name phytocannabinoids has been proposed in alternative to simply "cannabinoids", that overemphasizes the relationship with *C. sativa*[1]. At least in plants, a single species typically accumulates only a limited range of chemotypes. Thus, *C. sativa* and rhododendrons produce exclusively alkyl phytocannabinoids [1], while aralkyl phytocannabinoids are typical of some leguminous plants from the genera *Amorpha*, *Glycyrrhiza* and *Macherium*[1]. Phytocannabinoids have a pleiotropic bioactivity profile, as exemplified by the metabotropic cannabinoid receptors CB_1 and CB_2, the ionotropic thermo-TRP ion channels, and the transcription factors from the PPAR family [2]. Modulation of CBs is typical of the alkyl phytocannabinoid Δ^9 -THC, while the one of PPARs is the hallmark of amorfrutins, a class of bibenzyls belonging to the aralkyl phytocannabinoid chemotype [3]. Surprisingly, phytocannabinoids from both the alkyl- and the aralkyl series have been reported from the South-African plant *Helichrysum umbraculigerum* Less [4]. Although only non-narcotic compounds were isolated, some African *Helichrysum* species are used for ritual inebriating fumigations, a use strongly reminiscent of the ones described by the ancient authors for cannabis [5], and this folk use presumably underlies the trade of some South-African *Helichrysum* species for recreational narcotic purposes [6]. As part of our investigation on phytocannabinoids, we have phytochemically profiled an acetone extract from *H. umbraculigerum*, a plant reported to be the most abundant natural source of cannabigerol (**1a**) [4], the precursor of all members of the alkylcannabinoid family.

2. Results and discussion

An acetone extract from the aerial parts (leaves and flowers) of *H. umbraculigerum* was decarboxylated under the classic conditions used for phytocannabinoids (heating at 120 °C for 2 h) [7]. Fractionation by a combination of gravity column chromatography (GCC), semi-

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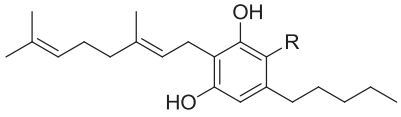
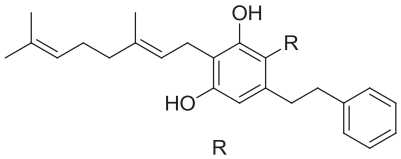
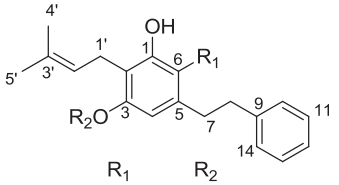
preparative and analytical HPLC afforded, overall, thirteen phenolics, sorted out into resorcinoids (**2a-2b, 3a-3d, 4**) and phloroglucinoids (**5a-5b, 6, 7a-7b, 8**). Interestingly, all resorcinoids are derived from an aromatic ketidic starter, and belong to the bibenzyl type of aralkyl phytocannabinoids. Conversely, with the exception of the flavanone glabranin (**8**), all phloroglucinoids derive from an aliphatic ketide starter. Thus, within *H. umbraculigerum* there seems to exist a clear distinction between the Claisen-type and the aldol-type aromatization mode of polyketides, with, respectively, formation of resorcinoids or phloroglucinoids depending on the aliphatic or aromatic nature of the starter, as highlighted by comparison of **2a** and **5a**.



	R
1a	H
1b	COOH

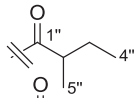


	R
2a	H
2b	COOMe

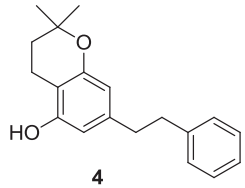


	R_1	R_2
3a	H	H
3b	H	MeBu
3c	COOH	MeBu
3d	COOH	iBu

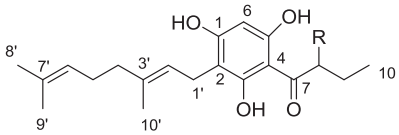
MeBu =



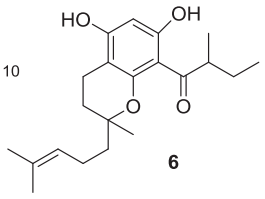
iBu =



4



	R
5a	H
5b	Me



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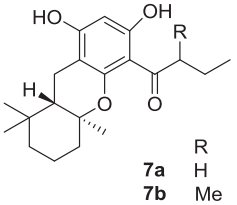
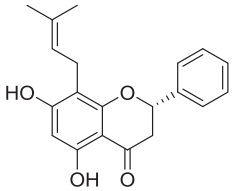


	R
7a	H
7b	Me



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The bibenzyls **2a,b**, and **3a,c,d** are known and had been previous isolated from *H. umbraculigerum*[4] and/or related species [8], while **3b** and **4** are new, as is the phloroglucinoid **5a**.

Compound **3b** was isolated as a colorless amorphous solid with molecular formula $C_{24}H_{30}O_3$ (positive ions HR-ESIMS). ^{1}H NMR spectrum of **3b** strongly suggested its bibenzyl resorcinoid nature, hallmarked by the five proton cluster at δ_H 7.35-7.15, assigned to a monosubstituted phenyl group, by the two methine resorcinyl resonances (δ_H 6.56 and 6.44, both bs) and by two overlapped methylene signals resonating at δ_H 2.83-2.85. The ^{1}H NMR spectrum of **3b** was completed by the typical resonances of a prenyl unit and of a 2-methylbutanoyl group. Once the last two spin systems were secured by means of the 2D COSY spectrum, and each proton had been associated to the directly bound carbon through the HSQC spectrum, we could use the 2D HMBC NMR to merge all these moieties. Thus, cross-peaks of H-7 with C-4, C-5, C-6 and C-9 confirmed the bibenzyl resorcinoid substructure, the network of cross-peaks of H-4 and H-6 allowed the carbon assignment of the tetrasubstituted phenyl ring and correlations of H_2-1′ with C-2, C-3, C-4 located the prenyl unit at C-2, while the 2-methylbutanoyl group could be located at either phenolic hydroxyls due to the symmetry of the compound. The esterified phenolic carbon experienced the expected ^{13}C NMR upfield shift (δ C: C-1 = 155.8, C-3 = 149.6).

Compound **4** ($C_{19}H_{22}O_2$ by HR-ESIMS) was found to be an analogue of **3b** lacking the 2-methylbutanoyl esterification and featuring cyclization of the prenyl unit to a chromane-type system. Accordingly, ^{1}H NMR resonances of the bibenzyl moiety of **4** were almost superimposable to parallel resonances of **3b**, while signals of the prenyl unit were replaced by two mutually coupled triplets (δ_H 2.60 and 1.80) and by a 6H singlet resonating at δ_H 1.34. Similarly to **3b**, the 2D HSQC spectrum associated the resonances of directly bound proton and carbon atoms, while the 2D HMBC showed key $^{2,3}J_{C,H}$ cross-peaks. In particular, H_2-1′ correlated with C-1, C-2, C-3 and the oxygenated unprotonated sp^3 carbon C-3′, while H_3-4′/H_3-5′ exhibited cross-peaks with C-2′ and C-3′. In agreement with the molecular formula and with the NMR resonance pattern of **4**, an oxygen bridge between C-1 and C-3′ should complete the structure of this metabolite. Compound **4** is a new natural product, but it had been obtained semisynthetically by Asakawa et al. [9] The (incomplete) spectral data reported are in agreement with ours.

Compound **5a** ($C_{20}H_{28}O_4$, negative ions HR-ESIMS) was characterized as a new phloroglucinol differing from **5b** for the ketone-bearing side chain. In particular, signals of a geranyl moiety were easily recognizable in the ^{1}H NMR spectrum of **5a**, including two sp^2 methines (δ_H 5.25 and 5.05), three allylic methylenes (δ_H 3.38, 2.10 and 2.08) and three allylic methyl groups (δ_H 1.80, 1.67 and 1.59, all bs). These signals were associated to those of corresponding carbons through the HSQC spectrum and then, via HMBC, the prenyl unit could be attached at the C-2 of a phloroglucinol moiety. In particular, cross-peaks of H_2-1′ with C-1, C-2 and C-3 and of H-6 with C-1, C-2, C-4 and C-5 completely defined this portion of the molecule. The structure of compound **5a** was completed by a linear C_4 unit embedding a ketone carbonyl (δ_C 205.9). Its attachment at C-4 was secured by HMBC cross-peaks of the deshielded H_2-8 with the ketone C-7 and C-4.

The acids **3c** and **3d** were surprisingly stable under the conditions of decarboxylation of pre-cannabinoids [7]. Both compounds are acylated at the *para*-hydroxyl, and this structural feature might be responsible for their surprising thermal stability. Decarboxylation of *o*-hydroxybenzoic acids occurs via their non-aromatic carbonyl tautomer (Fig. 1). A *p*-hydroxyl can stabilize by electron donation this tautomer, facilitating loss of carbon dioxide via the cyclic mechanism typical of β-ketoacids (Fig. 1). Acylation of the *p*-hydroyl removes the mesomeric contribution to the stabilization of the carbonyl tautomer, providing a possible explaination for the increased thermal stability associated to this maneuver.

The phenethyl analogue of CBG (Heli-CBG, **2a**) and its lower prenylogues **3a**, **3b** and **3c** were assayed against a series of metabotropic (CB_1 and CB_2) and ionotropic (thermo-TRPs) targets of