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Anti-*Helicobacter pylori* metabolites from *Heterotheca inuloides* (Mexican arnica)

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ABSTRACT

Preparations of the plant known as "Mexican arnica" (*Heterotheca inuloides*) have been traditionally used for the treatment of several ailments including stomach ulcers and for relief of colic. To find possible biologically active components against these ailments, we tested the acetone extract as well as different metabolites biosynthesized by this species, including cadinane-type sesquiterpenoids, triterpenes, phytosterols and flavonoids, against *Helicobacter pylori*. Among these compounds, sesquiterpenes 7-hydroxy-3,4-dihydrocadalene (**3**) and 7-hydroxy-cadalene (**4**) exhibited strong anti-*H. pylori* activity with MICs values of 1.95 and 3.91 μg/mL, respectively. HPLC-UV/DAD analyses revealed that both substances together with related compounds **7**, **8** and **9** were the major constituents of the acetone extract. Enzymatic assays and docking studies showed that unlike quercetin (**10**), a secondary metabolite also isolated from this species, compounds **3** and **4** did not inhibit the activity of urease, a potent virulence factor of *H. pylori*. Additionally, two new C_1-C_9 secocadinanes (**1** and **2**) were isolated from the aerial parts of this species. Their structures were determined by extensive NMR and HRESIMS spectroscopic data analyses.

1. Introduction

Heterotheca inuloides (*H. inuloides*) is a Mexican native plant belonging to the Asteraceae (Compositae) family which is frequently used as folk medicine in the treatment of a wide range of diseases involving inflammatory conditions. In Mexico, this species has been traditionally employed as teas, infusions, or decoctions to diminish the pain caused by rheumatism, contusions, stomach ulcers, topical skin inflammation, and colic [1,2]. Due its ethnomedical uses, phytochemical investigation of *H. inuloides* has focused on the identification of anti-inflammatory agents that could explain the effects described by people [3–5]. However, it is clear that some lesions and symptoms, such as stomach ulcers and colics, for which infusions of *H. inuloides* are frequently consumed, may have diverse etiological factors. While the beneficial effects traditionally observed after the oral consumption of preparations of this plant for the treatment of stomach disorders, may be a direct consequence of the presence of metabolites displaying anti-inflammatory properties, the therapeutic value may lie in the mitigation of factors which promote the appearance of these conditions. *Helicobacter pylori* (*H. pylori*), a bacterium affecting nearly half of the world's population, is the main known cause for gastritis, gastroduodenal ulcer disease, and gastric cancer [6,7]. The standard first-line treatment for infections caused by this microorganism consists in the administration of clarithromycin, amoxicillin, or metronidazole combined with a proton-pump inhibitor [8]. However, the emergence and spread of antibiotic resistance makes eradication of this bacterium increasingly difficult [9], so investigation of new anti-*H. pylori* substances is essential to determine the efficacy of future treatments. In this context, the exploration of non-antibiotic metabolites from natural sources appears to be an alternative that could cope with antibiotic resistance [10,11]. Considering this background, we evaluated the potential of the compounds present in *H. inuloides* as anti-*H. pylori* agents. The main metabolites biosynthesized by this species are encompassed in the following structural groups: cadinane-type sesquiterpenoids, triterpenes, phytosterols, and flavonoids [1]. Thus, a set formed by the major substances belonging to each one of these groups was tested.

Recent re-investigations of the constituents of *H. inuloides* led to the isolation of new cadinane-type sesquiterpenoids which had not been obtained from previously studied populations, suggesting an important metabolic variability of this species probably associated with

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Table 1
^{1}H and ^{13}C NMR data of compounds **1** and **2**.

	1[a]		**2**[b]	
Position	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)	δ_C, type
1		211.5, C		212.2, C
2	2.24, m	41.8, CH_2	2.25, m	42.5, CH_2
3	*α* 2.09, m *β* 1.71, m	26.9, CH_2	*α* 2.16, m *β* 1.84, m	27.2, CH_2
4	2.60, ddd (12.1, 8.7, 3.9)	44.3, CH	2.81, ddd (9.0, 8.2, 4.7)	45.8, CH
5	6.55, dd (3.0, 0.7)	114.7, CH	7.78, d (2.0)	131.7, CH
6		149.8, C		123.0, C
7	6.56, dd (9.4, 3.0)	113.7, CH	7.76, dd (8.3, 2.0)	130.3, CH
8	6.68, dd (9.4, 0.7)	117.0, CH	6.84, d (8.3)	115.6, CH
9		148.2, C		161.6, C
10		131.7, C		131.4, C
11	1.80, hept d (8.7, 6.7)	33.2, CH	1.95, hept d (6.7, 8.2)	33.7, CH
12	1.00, d (6.7)	21.3, CH_3	1.02, d (6.7)	21.5, CH_3
13	0.74, d (6.7)	20.9, CH_3	0.78, d (6.7)	21.1, CH_3
14	2.05, s	30.2, CH_3	2.05, s	29.9, CH_3
15				170.7, C

[a] ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) measured in $CDCl_3$.
[b] ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) measured in CD_3OD.

morphophysiological conditions [4]. Motivated by this observation, we examined the chemical composition of an additional population of this species. In this report, the purification and structural elucidation of two new 1,9-secocadinanes isolated from this population are discussed, along with the anti-*H. pylori* evaluation of *H. inuloides* metabolites.

2. Results and discussion

Two new compounds (**1** and **2**), in addition to the know metabolites (**3–18**), were afforded by successive chromatographic separations of the acetone extract of the aerial parts of *H. inuloides*. Structures of known substances were confirmed by comparing their spectroscopic data with those reported in the literature and by direct comparison with authentic samples, and the structures of the new compounds were elucidated by extensive spectroscopic analysis.

Compound **1** was isolated as a yellow oil and its molecular formula was determined to be $C_{14}H_{20}O_3$ on the basis of HRESIMS at *m/z* 237.14909 $[M + H]^+$ and ^{13}C NMR data. The ^{13}C NMR spectrum of compound **1** (Table 1) exhibited signals for 14 carbons, and were classified in accordance with DEPT experiments as three sp^2 methines (δ_C 117.0, 114.7, 113.7), two oxygenated sp^2 quaternary carbons (δ_C 149.8, 148.2), two sp^2 quaternary carbons (δ_C 131.7 and a carbonyl group at δ_C 211.5), two sp^3 methines (δ_C 44.3, 33.2), two sp^3methylenes (δ_C 41.8, 26.9) and three methyls (δ_C 30.2, 21.3, 20.9).

The ^{1}H NMR spectrum of compound **1** (Table 1) showed resonances signals of three benzenoid hydrogens with *ortho*, *meta* and *para* couplings: δ_H 6.68 (1H, dd, J = 9.4 Hz, J = 0.7 Hz), δ_H 6.56 (1H, dd, J = 9.4 Hz, J = 3.0 Hz), δ_H 6.55 (1H, dd, J = 3.0 Hz, J = 0.7 Hz). It was also observed a signal of an aliphatic proton at δ_H 2.60 (1H, ddd, J = 12.1 Hz, J = 8.7 Hz, J = 3.9 Hz) correlated with the carbon signal

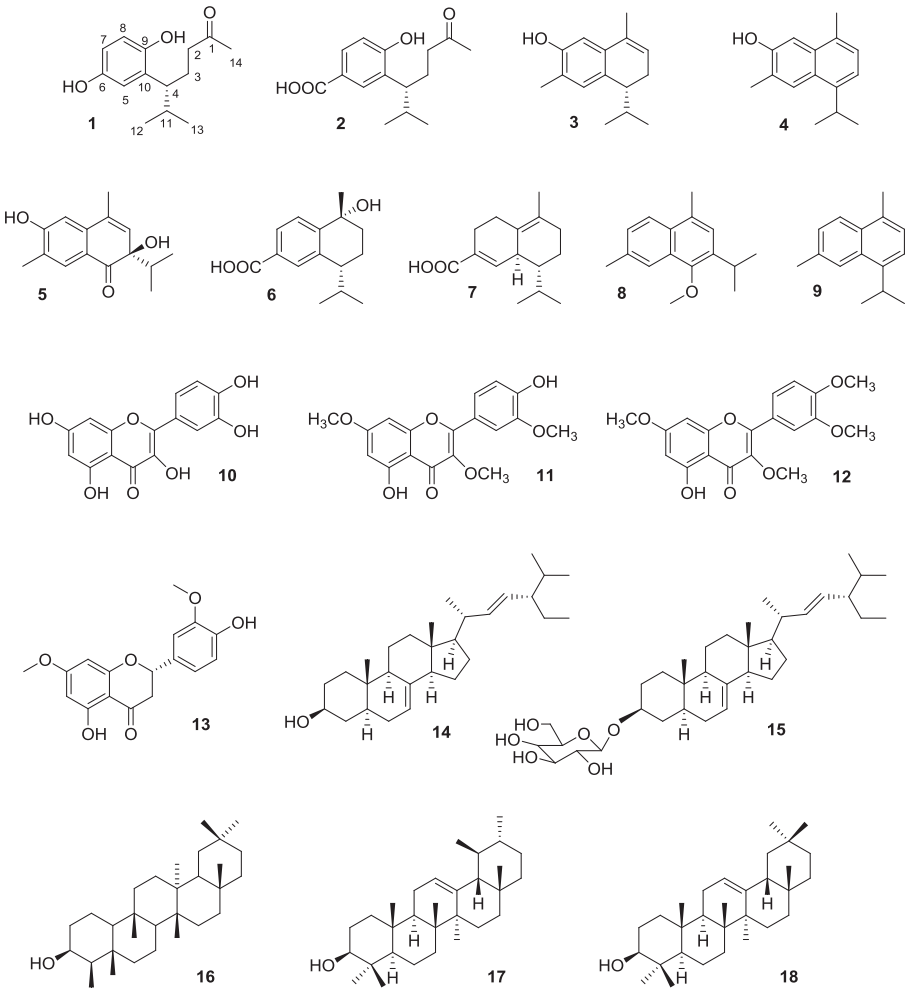

Fig. 1. Structures of compounds **1–18**.