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SHORT REPORTS

A PLANT GROWTH INHIBITORY SESQUITERPENOID FROM *HETEROTHECA INULOIDES*

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Key Word Index—*Heterotheca inuloides*; Compositae; sesquiterpene; inuloidin; plant growth inhibitory activity.

Abstract—A new sesquiterpenoid, inuloidin, isolated from the dried flower of *Heterotheca inuloides* has been characterized as 2,7-dihydroxy-β-calacoren or 7-hydroxy-5,6,7,8-tetrahydro-3-methyl-8-methylene-5-(1-methylethyl)-2-naphthalenol by means of spectroscopic method. It exhibited plant growth inhibitory activity in lettuce seedling assay.

INTRODUCTION

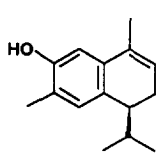
By bioassay directed fractionations, we have previously reported the isolation and identification of four sesquiterpenoids, β-caryophyllene, β-caryophyllene-4,5-α-oxide, 7-hydroxy-3,4-dihydrocadalin (**1**) and 7-hydroxycadalin [1], as antibacterial agents [2], and two flavonoids, quercetin and kaempferol, as mushroom tyrosinase inhibitors [3], from the dried flowers of *Heterotheca inuloides* Cass., a Mexican medicinal plant locally known as 'arnica' [4]. In our continuing search for biologically active substances from the same source, the methanol extract was found to exhibit plant growth inhibitory activity in lettuce seedling assay [5].

RESULTS AND DISCUSSION

The methanol extract was suspended in water and the suspension was successively partitioned with *n*-hexane, methylene chloride and ethyl acetate. Bioassay showed that the methylene chloride fraction retained the activity. The bioassay guided fractionation of the methylene chloride portion led to isolation of the active principle, a new sesquiterpenoid which was named 'inuloidin'. Its structure was elucidated by means of spectroscopic methods, in particular, the NMR spectra.

Inuloidin (**2**) was a pale yellow oil whose molecular formula, $C_{15}H_{20}O_2$, was established by EI-mass spectrometry in conjunction with NMR data. It gradually decomposed, more easily at higher temperature. The IR spectrum suggested the presence of hydroxyl groups at 3550 and 3350 cm^{-1} while the UV spectrum had λ_{max}^{MeOH} at 216, 255 and 306 nm. In addition, analysis of the NMR data showed close structural similarity to 7-hydroxy-3,4-dihydrocadalin (**1**). Inuloidin had one more oxygen than **1**. In the ^1H NMR spectrum, the olefinic proton at δ5.68 and the methyl protons at δ1.98 of **1** were replaced by signals of an exocyclic methylene at δ5.43 (*s*) and 5.19 (*s*), and a geminal methine proton to a hydroxy group at δ4.62 (*t*). This is consistent with ^{13}C signals at δ108.7 (*t*) and 146.5 (*s*) for the terminal methylene carbons (C-15 and C-8), and at δ70.1 (*d*) for the hydroxylated carbon (C-7). In addition, DQF (Double Quantum Filter Correlation) [6] in the ^1H NMR spectrum indicated the presence of partial structures ***a***, ***b*** and ***c***. The ^{13}C assignments were largely based on C–H COSY spectrum. This was further confirmed by NOESY (Fig. 1) and HMBC (Fig. 2) spectra of inuloidin (**2**).

The coupling constants of $J_{5,6}$ (6.3 Hz) and $J_{6,7}$ (5.4 Hz) established the OH-7 and isopropyls groups as *cis*. Therefore, the structure of inuloidin was established as 2,7-dihydroxy-β-calacoren or 7-hydroxy-5,6,7,8-tetrahydro-3-methyl-8-methylene-5-(1-methylethyl)-2-naphthalenol (**2**). The absolute configuration has not yet been determined.



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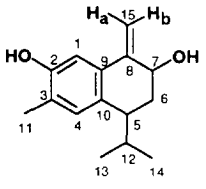

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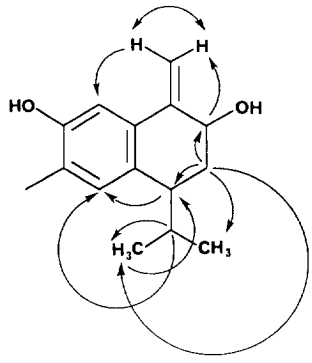

Fig. 1. NOESY correlation for inuloidin.

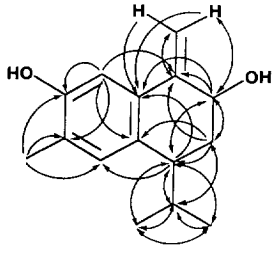

Fig. 2. Long-range correlations in HMBC spectrum of inuloidin.

Inuloidin was the only active substance isolated in minute amount from the flowers of *H. inuloides* when isolation was guided by lettuce seedling assay [5] at 500 $\mu g\,ml^{-1}$. Hence it can be expected to show potent activity. However, the further biological significance has not yet been investigated with the purified inuloidin because of its limited availability. Interestingly, its structurally related 7-hydroxy-3,4-dihydrocadalin (**1**) did not exhibit any activity in lettuce seedling assay up to 500 $\mu g\,ml^{-1}$. In contrast, inuloidin did not show any antibacterial activity up to 800 $\mu g\,ml^{-1}$, although 7-hydroxy-3,4-dihydrocadalin exhibited antibacterial activity [2].

EXPERIMENTAL

General. ^1H and ^{13}C NMR were taken in $CDCl_3$ at 500 MHz for ^1H and 125 MHz for ^{13}C. General procedures are the same as in previous work [7].

Plant material. The dried fluffy flowers of *H. inuloides* were purchased at market places in Guadalajara, Mexico. The plant was identified by Dr D. N. Pelaez, School of Biology, Universidad Autonoma de Guadalajara where a voucher specimen is deposited.

Extraction and isolation. The dried and pulverized flower of *H. inuloides* (2 kg) was extracted with MeOH ($\times 3$) at ambient temp. for 10 days. After conc. of the solvent under red. pres., the MeOH extract was suspended in H_2O and the suspension was successively partitioned into *n*-hexane-, CH_2Cl_2-, EtOAc- and H_2O-sol. frs. The CH_2Cl_2-sol. fr. (10 g) was chromatographed over a silica gel (100 g, 230–400 mesh) column using *n*-hexane, containing increasing quantities of EtOAc as eluent, to give 5 frs. Subsequent bioassay indicated fr. 4 to be active. Hence this bioactive fr. (3 g) was further chromatographed on silica gel eluting with *n*-hexane–EtOAc (6:4) followed by recycling HPLC [8] (ODS column, detection at 254 nm, flow rate 2.5 $ml\,min^{-1}$) eluted with MeOH to afford pure inuloidin (15 mg).

Inuloidin. A pale yellow oil. UV γ_{max}^{MeOH} nm: 216, 255, 306 (log ε 4.81, 4.45, 4.08); IR γ_{max}^{KBr} cm^{-1}: 3550, 3350, 2850, 1700, 1615, 1575, 1500, 1250; EIMS *m/z* (%); 232 $[M]^+$ (26), 189 (100), 171 (28), 161 (63), 146 (8), 115 (5), 91 (4); ^1H NMR ($CDCl_3$): δ0.76 and 0.98 (3H, *d*, J = 6.8Hz, Me-12), 1.93 (2H, *dd*, J = 6.3, 5.4 Hz, H-6), 2.20 (1H, *sep*, J = 6.8 Hz, H-12), 2.23 (3H, *s*, Me-3), 2.82 (1H, *dd*, J = 6.3 Hz, H-5), 4.62 (1H, *t*, J = 5.4 Hz, H-7), 4.90 (1H, *br s*, OH-2), 5.19 (1H, *s*, H_b-15), 5.43 (1H, *s*, H_a-15), 6.95 (1H, *s*, H-1), 6.97 (1H, *s*, H-4); ^{13}C NMR ($CDCl_3$): δ15.8 (C-11), 17.6 (C-14), 21.2 (C-13), 31.2 (C-12), 31.6 (C-6), 40.2 (C-5), 70.1 (C-7), 108.7 (C-15), 111.0 (C-1), 124.3 (C-3), 130.6 (C-4), 132.0 (C-10), 132.1 (C-9), 146.5 (C-8), 152.3 (C-2).

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