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A bioactive annuionone from sunflower leaves

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Abstract

From aqueous extract of *Helianthus annuus* L. cv. Suncross-42 leaves, a annuionone have been isolated. The structural elucidation of the compound is based on ^{1}H and ^{13}C NMR spectral studies. The potential of the compound to be used as natural herbicide template has been evaluated through laboratory bioassays against five weed species. Results proved annuionone H as a potent plant growth inhibitor that can be exploit for the development of a herbicide model.

Keywords: *Helianthus annuus* L.; Sunflower; Allelochemicals; Annuionone; Weeds

1. Introduction

Due to increased awareness about the risks involved in use of pesticides, much attention is being focused on the alternative methods of weed control. In past two decades, much work has been done on plant-derived compounds as environment friendly alternatives to synthetic herbicides for weed control (Vyvyan, 2002). Allelochemicals are plant secondary metabolites. The chemistry of these compounds may be used to develop newer herbicides with novel molecular sites of action. As very little overlap between the known molecular target sites of natural phytotoxins and those of commercial synthetic herbicides have been reported, these natural compounds can provide tools to combat the evolution of herbicide resistance in weeds.

Sunflower is well known for its allelopathic compounds. Several phenols and terpenes have been reported so far in various cultivars of sunflower (Spring et al., 1992; Macias et al., 2002). Present study was designed to evaluate the allelopathic potential of leaves of sunflower cv. Suncross-42 against weeds including *Chenopodium album* L., *Coronopis didymus* (L.) Sm., *Medicago polymorpha* L., *Rumex dentatus* L. *and Phalaris minor* Retz.

2. Experimental

2.1. General

^{1}H NMR and ^{13}C NMR spectra were recorded at 399.92 and 100.6127 MHz, respectively, on a Bruker AM-400 NMR spectrometer at 30 °C. Samples were dissolved in $CDCl_3$ and TMS was used as a primary reference. Positive ion-first-order MS were recorded using LC–MS (Thermo Finnigan LCQ) with an electrospray ionization (ESI) source. Atmospheric pressure chemical ionization mass spectra (negAPCI-MS) were acquired using a micromass LCT mass spectrometer calibrated with a PEG calibration solution (acetonitrile:water 50:50). For HPLC a Waters system consisting of a 600E pump, 717 autosampler and 996-photodiode-array detector with detection span from 200 to 700 nm was used. To determine the chemical profiles of extracts analytical HPLC was carried out on a Merck LiChrospher100 RP-18e column (250 mm × 4 mm i.d.) with a 5 μm particle

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size, and at flow rate of 1 ml min^{-1}. A linear solvent gradient of MeOH:H_2O 25:75 to MeOH:H_2O 100:0 over 40 min was used for separation.

2.2. Plant material

Leaves of *H. annuus* L. cv. Suncross-42 were collected during the third plant developmental stage (plants 1 m tall with flowers, 1 month before harvest) and then dried and powdered before extraction. A voucher specimen is deposited at the Herbarium of the Department of Mycology and Plant Pathology, University of the Punjab, Pakistan (MPPL/005/03).

2.3. Extraction

Dry leaves were extracted in water (1:3) after 24 h soaking at room temperature. Crude aqueous extract was then partitioned by dichloromethane (DCM-A). The solvent of organic layer was removed by reduced pressure evaporation and residue was dissolved in 1–2 ml of methanol for HPLC analysis. The DCM-A was further fractionated and various fractions were isolated through several runs of HPLC and then were bioassayed with selected weeds. Fraction found most effective against tested weeds was then checked for its chemical profile and was purified for structural elucidation. The isolated compound was rechecked against targeted weeds.

2.4. Bioassay

Weed seeds were sown in 9 cm diameter Petridishes moistened with 5 ml of isolated fractions, while control received distilled water in equal amount. Peteridishes were placed in dark at 20 ± 1 °C for 10 days. Each treatment was replicated thrice. Dry weights were recorded at the end. Data were statistically analysed using Duncan's multiple range test.

3. Results and discussion

3.1. Structure elucidation

Dry powdered leaves of *H. annuus* cv. Suncross-42 were extracted with water at room temperature for 24 h. The aqueous extract was then partitioned with dichloromethane. The dichloromethane fraction revealed rich profile of various compounds when analysed through high performance liquid chromatography (HPLC). Various peaks collected by several runs of HPLC were then checked against selected weeds. The isolated peak found most effective in reducing the weeds biomass was further purified for spectroscopic analysis. The spectroscopic data and comparison with the literature values led us to propose the structure (Fig. 1) for isolated compound.

Annuionone H was isolated as colourless oil. The molecular ion $M + H^+$ at *m/z* 281.9 in the LC/negACPI-MS spectrum along with the ^{13}C NMR data (Table 2) was in good agreement with the molecular formula $C_{16}H_{26}O_4$ (calculated mass 282.272). For ^1H NMR data see Table 1 and for ^{13}C data see Table 2.

The relative configuration to new compound was assigned on the basis of comparison of ^1H and ^{13}C NMR data with the previously known annuionones. ^1H NMR and ^{13}C NMR data showed that analyzed compound contains a similar basic cyclohexanone ring of annuionone, the apocarotenoids reported by Macias et al.,1998, 2002 and in 2004 with modified structures. ^1H NMR of H4 (δ 2.26 *d* $J = 16$ Hz), H4′ (δ 2.36 *d* $J = 16$ Hz) and H6 (δ 1.62 *ddd* $J = 6.2$, 6.2, 1.2 Hz) of annuionone H have almost same chemical shifts as of other reported annuionones. Furthermore in ^{13}C NMR of annuionone H, C-1 (δ 45.13), C-3 (δ 209.32), C-4 (δ 49.5), C-5 (δ 83.4) and C-6 (53.76) also have similar shift values indicating the similarity between cyclohexanone rings of annuionone H with previously known annuionones. The spectral data also confirmed the revised structure of annuionones A, B and E (Macias

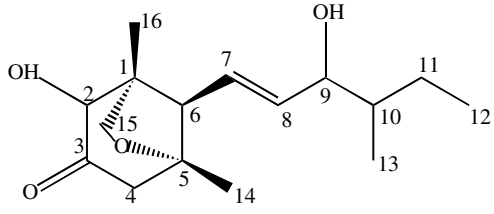

Fig. 1. Isolated annuionone H.

Table 1
^1H NMR data of annuionone H

H	δ ^1H in ppm
H2	5.69 *s*
H4	2.26 *d* ($J = 16$ Hz)
H4′	2.36 *d* ($J = 16$ Hz)
H6	1.62 *ddd* ($J = 6.2$, 6.2, 1.2 Hz)
H7	6.77 *d* ($J = 15.8$ Hz)
H8	6.38 *d* ($J = 15.8$ Hz)
H9	3.88 *m*
H10	1.04 *d* ($J = 6.7$ Hz)
H11	1.79 *m*
H11′	1.46 *d*
H12	1.25 *m*
H13	0.8 *d* ($J = 7.5$ Hz)
H14	1.31 *s*
H15	3.58 *dd* ($J = 8.1$, 2.8 Hz)
H15′	*3.63* *d* ($J = 7.9$ Hz)
H16	0.99 *s*

s, singlet; *d*, doublet; *dd*, doublet of doublets; *m*, multiplet 399.92 MHz, $CDCl_3$ signals.

Table 2
^{13} NMR data of annuionone H

C	δ^{13}C
C1	45.13
C2	77.02
C3	209.32
C4	49.50
C5	83.51
C6	53.76
C7	130.31
C8	150.48
C9	68.14
C10	48.76
C11	38.99
C12	28.208
C13	15.92
C14	24.57
C15	77.34
C16	20.87

100.6127 MHz, signals of $CDCl_3$.

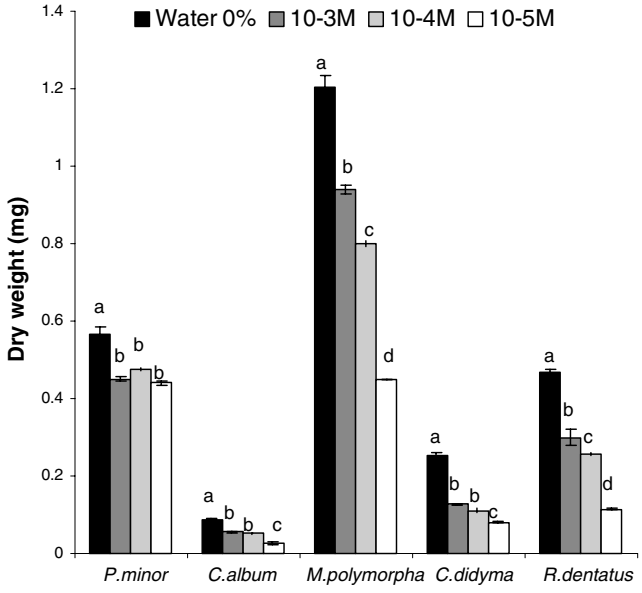

Fig. 2. Bioactivity of isolated annuionone H against five most problematic weeds of wheat.

et al., 2004). The data allowed elucidating that the structure has not an epoxide group between C-5 and C-14 as was reported by Macias et al. (2002); rather the oxygen atom is connected to the C-15 and C-5 forming a second five member cycle. This structure explains the unusual chemical shift values observed for carbons connected to oxygen (δ 83.4 and δ 77.3 ppm) as well as for the protons H-15 (δ 3.58 and δ 3.63 ppm), which are rather big to an epoxide group. The chemical shift values for both ^{1}H and ^{13}C at C-7 and C-8 appeared downfield suggesting the presence of double bond between the two carbons. This annuionone can be referred as dihydroxy-annuionone H as it contains two additional hydroxyl groups despite other similarities with previously known annuionones.

3.2. Bioassay results

The growth and hence dry biomass of all the five tested weeds was reduced significantly ($P < 0.01$) when treated with various concentrations of isolated annuionone H (10^{-3}, 10^{-4} and 10^{-5} M). The effect was found dose dependent (Fig. 2) as increase in concentration increased the phytotoxic effect. Among the five weeds tested, *P. minor* showed highest tolerance. Broadleaved weeds especially *R. dentatus* and *C. album* presented maximum dry weight losses, i.e., up to 75% as compared to the control.

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