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Evaluation of antidesmone alkaloid as a photosynthesis inhibitor

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ABSTRACT

Antidesmone, isolated from *Waltheria brachypetala Turcz.*, owns special structural features as two α,β-unsaturated carbonyl groups and a side alkyl chain that can compete with the quinones involved in the *pool* of plastoquinones at photosystem II (PSII). In this work, we showed that the alkaloid is an inhibitor of Hill reaction and its target was located at the acceptor side of PSII. Studies of chlorophyll (Chl) *a* fluorescence showed a J-band that indicates direct action of antidesmone in accumulation of Q_A^- (reduced plastoquinone A) due to the electron transport blocked at the Q_B (plastoquinone B) level similar to DCMU. *In vivo* assays indicated that antidesmone is a selective post-emergent herbicide probe at 300 μM by reducing the biomass production of *Physalis ixacarpa* plants. Furthermore, antidesmone also behaves as pre-emergent herbicide due to inhibit *Physalis ixacarpa* plant growth about 60%. Antidesmone, a natural product containing a 4(1H)-pyridones scaffold, will serve as a valuable tool in further development of a new class of herbicides.

1. Introduction

Increasing agricultural productivity without expansion of land for food production is a necessity for a growing world population. In this sense, avoid agricultural pests is a great concern. Since weeds are one of the major pests causing a continual reduction in the quantity and quality of crops worldwide, increasing agricultural yield is highly dependent on the use of synthetic herbicides to control weeds. The continuous use of herbicides has resulted in selective pressures, leading to the replacement of sensitive weeds by herbicide-resistant biotypes [1–3].

The major goal of modern weed management research is to discover new herbicides that control the widest possible range of weed species (*i.e.* can be sprayed at pre- and post-emergence), and at low application rates. Furthermore, selective herbicides need to be safe to the target crop, environment and humans. In this context, natural products fit well against these objectives, and become interesting alternatives for herbicides because they are non-toxic and have demonstrated good activity in weed control. To satisfy this demand, research in academia and industry has focused on natural products as a promising strategy for developing new herbicides. In the search of potential herbicides, several natural products, such as epifriedelinol [4], evolitrine [5] and ocotilone [6] (Fig. 1), have been studied as inhibitors of PSII with good prospects as natural inhibitors of plant growth.

Waltheria brachypetala Turcz (Malvaceae) is a plant with an endemic occurrence in semi-arid areas in Brazil [7]. Previous phytochemical studies showed that *W. brachypetala* leaves and stems contain cyclic peptide and quinolone alkaloids, such as antidesmone [8]. Looking at the chemical structure of antidesmone, this compound has a system of two α,β-unsaturated carbonyl groups and a side alkyl chain, similarly to the quinones involved in the pool of plastoquinones at PSII (Fig. 2). Our aims in this work were to evaluate and determine the effects of antidesmone on photosynthetic apparatus, germination, roots and stems growth and dry biomass experiments.

2. Material and methods

2.1. Antidesmone isolation

The ethanol extract of *W. brachypetala* stems and leaves (180 g) was subjected to liquid-liquid extraction with methanol:water (1:3) and hexane to provide the hexane fraction (12.0 g). Six grams from hexane fraction was subjected to chromatographic purification using silica gel (70–230 mesh) as the stationary phase. The mobile phase was

Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; Chl, chlorophyll; DBMIB, 2,5-dibromo-6-isopropyl-3-methyl-1,4-benzoquinone; DCBQ, 2,5-dichloro-1,4-benzoquinone; F_0, initial fluorescence; F_M, maximum fluorescence level; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; IC_{50}, (the half maximal inhibitory concentration); MV, methylviologen; OEC, oxygen evolving complex; PSI, photosystem I; PSII, photosystem II; SiMo, sodium silicomolybdate; Tris, (hydroxymethyl)-aminoethane.

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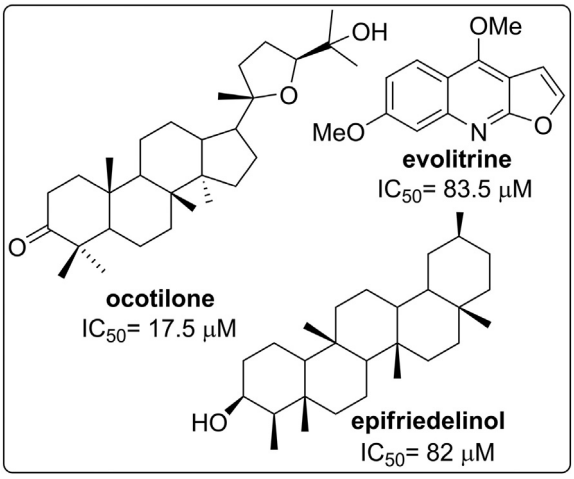

Fig. 1. Natural products that are ATP synthesis inhibitors of PSII.

composed of hexane/ethyl acetate in ascending order of polarity (9:1, 8:2, 7:3, 6:4, 1:1), followed by dichloromethane:methanol (1:0, 95:5, 9:1, 8:2, 7:3, 6:4, 0:1) to obtain 49 fractions. Fraction 42 was again subjected to new chromatographic purification using silica (70–230 mesh), eluting with solvents isocratic elution (dichloromethane: methanol (95:5)) to obtain 29 new fractions. Fractions 16–18 were combined and subjected to semipreparative HPLC (Shimadzu, SCL-10 A VP) using a Phenomenex propyl-ether-diol normal bond as stationary phase (300 × 8 mm, 10 μm) with hexane:ethanol (95:5) as eluting system at 1.4 mL/min flow rate, to afford the pure alkaloid antidesmone (0.03 g). The compound was identified by spectroscopic analysis and confirmed according to thorough comparisons with the found literature data for the isolated alkaloid [9]. The obtained signal assignments are described as it follows:

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.87 (t, J = 7.0 Hz, 2H, H-17), 1.25 (2H, H-17, overlapping), 1.25–1.80 (10H, H-12-16, overlapping) 1.45 (1H, H-11b, overlapping), 1.75 (1H, H-11a, overlapping), 2.08 (dddd, J = 14.7, 14.0, 4.5, 4.3 Hz, 1H, H-6_{ax}), 2.20 (dddd, J = 14.0, 5.3, 4.3, 2.4 Hz, 1H, H-6_{eq}), 2.36 (s, 3H, 2-$\underline{CH_3}$), 2.58 (ddd, J = 18.1, 4.4, 2.4 Hz, 1H, H-7_{eq}), 2.75 (ddd, J = 18.1, 14.7, 5.3 Hz, 1H, H-7_{ax}), 3.26 (m, 1H, H-5), 3.93 (s, 3H, O$\underline{CH_3}$), 8.73 (sl, 1H, *N*—H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0 (C-18), 14.6 (C2—$\underline{CH_3}$), 22.6 (C-17), 24.4 (C-6), 29.2–29.6 (C-12-16), 30.3 (C-5), 30.5 (C-11), 32.2 (C-7), 59.5 (OCH_3), 131.9 (C-9), 139.0 (C-2), 139.1 (C-10), 147.6 (C-3), 173.3 (C-4), 194.8 (C-8). MS (ESI+) m/z 320 [M + H]. $[\alpha]_D^{25}$: +26 (c 1.50, $CHCl_3$).

2.2. Chloroplast isolation and Chl determination

Intact chloroplasts were isolated from spinach leaves (*Spinacea oleracea* L.) as previously described [10]. The Chl concentration was measured spectrophotometrically through a chloroplast suspension in a solution of 400 mM sucrose, 5 mM $MgCl_2$, 10 mM KCl, 30 mM tricine-KOH and pH 8.0 [11].

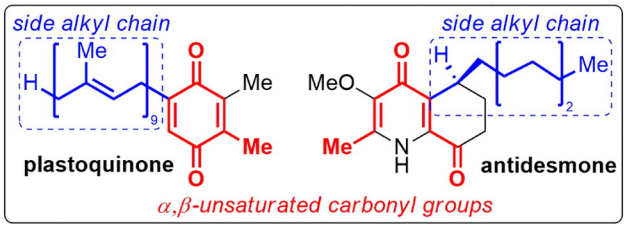

Fig. 2. Structure of plastoquinona and antidesmone.

2.3. Measurement of ATP synthesis

ATP synthesis was determined by titration using a microelectrode (Orion model 8103; Ross, Beverly, MA, USA) connected to a Corning potentiometer model 12 (Corning Medical, Acton, MA, USA) with an expanded scale and a Gilson recorder (Kipp & Zonen, Bohemia, NY, USA) [12,13].

A non-buffered solution was employed to break the intact chloroplasts by osmotic rupture, using 100 mM sorbitol, 10 mM KCl, 5 mM $MgCl_2$, 0.5 mM KCN, 50 μM MV, 1 mM Na^+-tricine, 1 mM of ADP, with the pH adjusted to 8.0 applying 50 mM of KOH. The mixture was illuminated for 1 min and the ATP synthesis was measured as micromoles of ATP per milligram of Chl per hour.

2.4. Measurement of non-cyclic electron transport rate

Light-induced non-cyclic electron transport activity from water to MV was determined polarographically using a Clark type electrode in the presence of 50 μM of MV [14]. Basal electron transport was measured by illuminating a solution of chloroplasts (20 μg Chl/mL) in 3 mL of 100 mM sorbitol, 10 mM KCl, 5 mM $MgCl_2$, 0.5 mM KCN, 15 mM tricine-KOH and 50 μM MV at pH 8.0 for 1 min. The phosphorylating non-cyclic electron transport rate was measured as for the basal electron transport from water to MV, adding 1 mM of ADP and 3 mM KH_2PO_4. Uncoupled electron transport was evaluated in the same solution used for basal electron transport with 6 mM NH_4Cl added as an uncoupler [10].

2.5. Uncoupled PSII electron flow determination

The electron flow activities were monitored with an oxygen monitor yellow spring instrument model 5300 A using a Clark type electrode. All reaction mixtures were illuminated with filtered light (filter of 5 cm of 1% $CuSO_4$ solution) from a projector lamp (GAF 2660) at room temperature. For each reaction, a blank experiment was performed with chloroplasts in the reaction medium. The IC_{50} value for each activity was determined from plots of the activity *versus* different concentrations of antidesmone.

The solution used for PSII reactions was the same medium used for basal electron transport measurements except MV. The uncoupled electron transport rate from water to DCBQ was measured by illuminating the chloroplasts for 1 min (20 μg Chl/mL) in a solution for PSII reactions, 100 μM of DCBQ, 1 μM of DBMIB and 6 mM NH_4Cl [15].

To determine the uncoupled partial reaction of PSII measured from water to SiMo, a solution of 200 μM of SiMo and 10 μM of DCMU were added to the solution used for the PSII reactions (3 mL), then chloroplasts (20 μg Chl/mL) were added and illuminated for 1 min [16].

2.6. Chl a fluorescence measurements on thylakoids

Chl *a* fluorescence transients were measured with a Handy-PEA (Plant Efficient Analyzer, from Hansatech, King's Lynn, Norfolk, UK), as previously described [5]. The maximum fluorescence yield from the sample was generated by illumination for two seconds with continuous light (650 nm wavelength, intensity equivalent of 2830 μmol photons m^{-2} s^{-1} 1 and gain of 0.7) from an array of three light-emitting diodes. The reaction medium employed was the solution used in PSII reactions. To monitor Chl *a* fluorescence transient, aliquots of dark-adapted thylakoids for 5 min containing 60 μg of Chl were transferred to filter paper by gravity with a dot-blot apparatus (Bio-Rad, United States) in order to ensure a homogeneous and reproducible distribution of thylakoids. The filter paper was dipped immediately in 3 mL of the medium with different concentrations of antidesmone in DMSO (150 and 300 μM) and the control was made with the medium plus DMSO.

Infiltrated chloroplasts with 10 μM of DCMU and chloroplasts previously treated with 0.8 M of Tris were used as a positive control. The