Journal of Photochemistry and Photobiology B: Biology 151 (2015) 213–220

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Mexican propolis flavonoids affect photosynthesis and seedling growth

Beatriz King-Díaz [a], Jessica Granados-Pineda [b,1], Mustapha Bah [c], J. Fausto Rivero-Cruz [b], Blas Lotina-Hennsen [a,*]

[a] Departamento de Bioquímica, Facultad de Química, Universidad Nacional Autónoma de México (UNAM), Ciudad Universitaria, 04510 México, DF, Mexico
[b] Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México (UNAM), Ciudad Universitaria, 04510 México, DF, Mexico
[c] Facultad de Química, Universidad Autónoma de Querétaro, Centro Universitario, Cerro de las Campanas, Querétaro 76010, Mexico

ARTICLE INFO

Article history:
Received 2 March 2015
Received in revised form 28 July 2015
Accepted 10 August 2015
Available online 15 August 2015

Keywords:
Acacetin
Chrysin
4′,7-dimethylnarangenin
Photosynthesis
Propolis
Seedling growth

ABSTRACT

As a continuous effort to find new natural products with potential herbicide activity, flavonoids acacetin (**1**), chrysin (**2**) and 4′,7-dimethylnarangenin (**3**) were isolated from a propolis sample collected in the rural area of Mexico City and their effects on the photosynthesis light reactions and on the growth of *Lolium perenne*, *Echinochloa crus-galli* and *Physalis ixocarpa* seedlings were investigated. Acacetin (**1**) acted as an uncoupler by enhancing the electron transport under basal and phosphorylating conditions and the Mg^{2+}-ATPase. Chrysin (**2**) at low concentrations behaved as an uncoupler and at concentrations up to 100 μM its behavior was as a Hill reaction inhibitor. Finally, 4′,7-dimethylnarangenin (**3**) in a concentration-dependent manner behaved as a Hill reaction inhibitor. Flavonoids **2** and **3** inhibited the uncoupled photosystem II reaction measured from water to 2,5-dichloro-1,4-benzoquinone (DCBQ), and they did not inhibit the uncoupled partial reactions measured from water to sodium silicomolybdate (SiMo) and from diphenylcarbazide (DPC) to diclorophenol indophenol (DCPIP). These results indicated that chrysin and 4′,7-dimethylnarangenin inhibited the acceptor side of PS II. The results were corroborated with fluorescence of chlorophyll *a* measurements. Flavonoids also showed activity on the growth of seedlings of *Lolium perenne* and *Echinochloa crus-galli*.

1. Introduction

Propolis, a natural substance collected by honeybees from buds and exudated by certain trees and plants is used in the beehives as a protective barrier against their enemies [1,2], and it has been reported to possess biological activities such as antibacterial [3,4], antiviral [4,5], anti-inflammatory [6,7], anticancer [8–12] and antifungal [13,14]. Moreover, several reports have referred to the phytoinhibitory and phytotoxic activities of propolis extracts. Aqueous extract of propolis has shown to be responsible for inhibiting germination and growth of lettuce seedling and rice grains [15,16]. Alcoholic extracts of propolis obtained from Russia inhibited the germination of *Cannabis sativa* seeds [17,18]. Recently, the composition and phytotoxic activity of the volatile fraction of Brazilian green propolis have been reported [19]. Despite the wide spectrum of biological activity of propolis, to our knowledge no studies have so far been carried out about phytotoxic properties of Mexican propolis and its constituents.

Propolis contains a variety of chemical compounds such as polyphenols (flavonoids, phenolic acids and their esters), terpenoids, steroids, and amino acids. However, the major constituents of propolis from most sources are flavones, flavonols and flavanones [13,20–23].

In previous works flavones as tephroleocarpin, glabranin and methylglabranin isolated from the *Tephrosia* species showed inhibition of different photosynthetic activities [24]. Pachypodol, isolated from the *Croton ciliatoglanduliferus*, behaves as a Hill reaction inhibitor of photosynthesis of isolated chloroplasts from spinach leaves [25]. Some flavonoids possess various clinically relevant properties such as anti-tumor, anti-platelet, anti-inflammatory, antimicrobial, and antioxidant activity [26–29]. In this work, we report the phytotoxic effect of the flavonoids acacetin (**1**), chrysin (**2**) and 4′,7-dimethylnarangenin (**3**) isolated from the ethanolic extract propolis collected in the rural area of Mexico City. The effects of the isolated compounds (**1**–**3**) were assayed on the photosynthesis light reactions of fresh lysed chloroplasts isolated from spinach leaves, and on the germination and growth of seedling of *Physlis ixocarpa*, *Lolium perenne* and *Echinochloa crus-galli*.

* Corresponding author.
E-mail address: blas@unam.mx (B. Lotina-Hennsen).
[1] Taken in part from the PhD thesis of J. Granados-Pineda.

http://dx.doi.org/10.1016/j.jphotobiol.2015.08.019
1011-1344/

2. Materials and methods

2.1. Propolis material

Raw propolis (54 g) was collected in November 2012 from beehives located in the "Acuexcomatl Environmental Education Center", San Luis Tlaxialtemalco, Xochimilco, Mexico, and was stored at −40 °C.

2.2. Isolation of flavonoids

The air-dried and powdered propolis (50 g) was extracted with ethanol by maceration, for one week. The resultant extract was concentrated *in vacuo*. A portion of ethanol-soluble extract (21.8 g) was subjected to silica gel vacuum column chromatography (VLC) and eluted with a gradient mixture of dichloromethane–acetone (1:0 → 0:1) to provide eight pooled fractions (F1–F8). Fraction F1 was chromatographed over a silica gel (100 g) column and eluted with dichloromethane–methanol (1:0 → 0:1) to yield **1** (980 mg). Fraction F4, eluted with dichloromethane–acetone (9:1), was chromatographed over silica gel column, using dichloromethane–acetone (1:0 → 0:1) as eluent, to give six fractions. Subfraction F4-3 (540 mg) was recrystallized to give **2** (315 mg). Fraction F8 was re-chromatographed on a silica gel column using dichloromethane-acetone (1:0 → 0:1) as a solvent to produce six sub-fractions. Sub-fraction F8-3 yielded crystals of **3** (980 mg).

Acacetin (**1**). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.89 (s, 1H, OH-5); 10.57 (s, 1H, OH-7); 7.99 (d, 2H, J = 8.0 Hz, H-2′, and H-6′); 7.09 (d, 2H, J = 8.0 Hz, H-3′, and H-5′); 6.83 (s, 1 H, H-3); 6.48 (s, 1H, H-8); 6.17 (s, 1H, H-6); 3.82 (s, 3H, MeO-4′). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.2 (C-4), 164.6 (C-2), 163.7 (C-7), 162.7 (C-5), 161.9 (C-4′), 157.8 (C-9), 128.7 (C-2′ and C-6′), 123.2 (C-1′), 115.0 (C-3′ and C-5′), 104.2 (C-10), 103.9 (C-3), 99.3 (C-6), 94.5 (C-8), 56.0 (MeO-4′).

Chrysin (**2**). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.60 (d, 2H, J = 8.0 Hz, H-2′, and H-6′); 7.56–7.57 (m, 2H, H-3′ and H-5′); 6.83 (s, 1 H, H-3); 6.52 (1H, d, 4.0, H-8); 6.22 (1H, d, 4.0, H-6). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 181.2 (C-4), 163.7 (C-7), 163.4 (C-2), 162.4 (C-4′), 161.7 (C-5), 157.5 (C-9), 128.2 (C-2′ and C-6′), 122.9 (C-1′), 114.5 (C-3′ and C-5′), 104.1 (C-10), 103.6 (C-3), 99.2 (C-6), 94.2 (C-8).

4′,7-dimethyl naringenin (**3**). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.0 (brs, 1H, OH-5); 7.38 (d, 2H, J = 8.0 Hz, H-2′, and H-6′); 6.96 (d, 2H, J = 8.0 Hz, H-3′, and H-5′); 6.06 (s, 1H, H-8); 6.03 (s, 1H, H-6); 5.35 (dd, 1 H, J = 9.0, 4.0 Hz, H-2); 3.82 (s, 3H, MeO-4′); 3.79 (s, 3H, MeO-7); 3.09 (dd, 1 H, J = 16.0, 9.0 Hz, H-3a); 2.78 (dd, 1 H, J = 16.0, 9.0 Hz, H-3b). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 196.0 (C-4), 167.9 (C-7), 164.2 (C-5), 162.9 (C-9), 160.0 (C-4′), 130.3 (C-1′), 127.7 (C-2′ and C-6′); 123.57 (C-1′), 114.2 (C-3′ and C-5′), 103.1 (C-10), 95.0 (C-6), 94.2 (C-8), 79.0 (C-2), 55.7 (MeO-4′), 55.3 (MeO-7).

2.3. Chloroplast isolation and chlorophyll determination

Intact chloroplasts obtained from spinach leaves (*Spinacia oleraceae* L.) purchased from the local market as previously described [30,31] were suspended in a small volume of the following solution: 400 mM sucrose, 5 mM $MgCl_2$, 10 mM KCl, and 30 mM of the buffer tricine–KOH (pH 8.0). They were stored as a concentrated suspension in the dark at 4 °C before used. The chlorophyll (Chl) concentration was measured according to Strain et al. [32].

2.4. ATP synthesis and electron flow determinations

ATP synthesis was determined titrimetrically as the pH rose from 8.0 to 8.1 during illumination of chloroplasts, using a microelectrode (Orion model 8103; Ross, Beverly, MA) connected to a potentiometer (Corning Medical, Acton, MA, model 12) with expanded scale and a Gilson recorder (Kipp & Zonen, Bohemia, NY) as previously reported [30,33]. Intact chloroplasts (20 μg of Chl/mL) were broken before each assay by osmotic rupture in 3 mL of the non-buffered solution containing: 100 mM sorbitol, 10 mM KCl, 5 mM $MgCl_2$, 0.5 mM KCN, and 1 mM tricine–KOH at pH 8.0 in the presence of 50 μM methylviologen (MV) and 1 mM adenosine diphosphate (ADP). The synthesized ATP was calculated as micromoles of ATP per milligram of Chl per hour. The reaction was calibrated by back titration with saturated HCl.

2.5. Measurements of noncyclic electron transport rate

Light induced non-cyclic electron transport activity from water to MV was performed using a Clark type electrode. Chloroplasts were efficiently lysed to yield free thylakoids prior to each experiment by incubating them in the following basal electron transport medium: 100 mM sorbitol, 10 mM KCl, 5 mM $MgCl_2$, 0.5 mM KCN, and 30 mM tricine (*N*-tris[hydroxymethyl]methylglicine) buffer (pH 8 with the addition of KOH). Twenty micrograms of chlorophyll per milliliter of medium were illuminated during 1 min. Phosphorylating non-cyclic electron transport was measured with the basal electron transport medium plus 1 mM ADP and 3 mM KH_2PO_4. Uncoupled electron transport was tested in the basal transport medium by adding 6 mM NH_4Cl as an uncoupler agent [30,34].

2.6. Uncoupled photosystem II (PS II) and uncoupled photosystem I (PS I) electron flow measurements

These experiments were performed in an uncoupled non-cyclic electron transport assay. The activities were monitored with an YSI (Yellow Spring Instrument) oxygen monitor, model 5300A, using a Clark-type electrode. Uncoupled PS II from water to DCBQ [30,35] was monitored using the basal electron transport medium, and 1 μM 2,5-dibromo-6-isopropyl-3-methyl-1,4-benzoquinone (DBMIB), 100 μM DCBQ, and 6 mM NH_4Cl were added. MV was omitted. DCBQ accepts electrons at the D1 protein.

Partial reaction of PS II electron transport from water to SiMo was determined using the same basal electron transport medium without MV as in PS II, 50 μM SiMo and 10 μM DCMU were added [36].

Uncoupled PS I electron transport was determined using the basal electron transport medium with MV, plus 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, which inhibits PS II at Q_B level), 100 μM of DCPIP reduced with 300 μM ascorbate and 6 mM NH_4Cl [37]. I_{50} value is the concentration producing 50% inhibition of the activity. The I_{50} value for each activity was calculated by linear regression analysis using SigmaStat 3.5 program and confidence intervals were calculated with a 95% confidence level using *t*-test.

2.7. Mg^{2+}-ATPase activity assays

Chloroplasts were isolated from 30 to 40 g of spinach leaves, which were ground in 160 mL of medium containing 350 mM sorbitol, 5 mM ascorbic acid and 20 mM 2-(*N*-morpholino) ethanesulfonic acid (MES) at pH 6.5. Chloroplasts were centrifuged at 3000 g for 60 s, washed once in 40 mL of grinding medium, and resuspended in 35 mM *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (HEPES) buffer (pH 7.6). The light-triggered Mg^{2+}-ATPase activity associated to thylakoid membranes was measured as previously described by Mills and collaborators, 1980 [31], and released inorganic phosphate was measured as reported by Sumner [38].