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Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

ISSN 2278-4136
ISSN 2349-8234
JPP 2013; 2 (3): 136-139

Received: 26-8-2013
Accepted: 10-9-2013

Mahmoud A. Mansour
Pharmacognosy Department, Faculty of Pharmacy, Egyptian Russian University, Badr city, Cairo, Egypt.

Mohamed R. Elgindi
Pharmacognosy Department, Faculty of Pharmacy, Egyptian Russian University, Badr city, Cairo, Egypt.

Abd ELnasser B. Sengab
Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

Correspondence:
Abd ELnasser B. Sengab
Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.
E-Mail: mrelgindi@yahoo.com

Sesquiterpenes Quinones from *Thespesia populnea* and Their Biological Studies

Abd ELnasser B. Sengab*, Mohamed R. Elgindi and Mahmoud A. Mansour

ABSTRACT

Five sesquiterpenoid quinones were isolated from the aerial parts of *Thespesia populnea* L (Malvaceae) cultivated in Egypt for the first time. Their structures were determined on the basis of spectral data. The Mansonones were identified as: Mansonones C (1), G (2), E (3), H (4) and 7-hydroxy-2,3,5,6-tetrahydro-3,6,9-trimethylnaphtho[l,8-b,c]pyran-4,8-dione (5).

Keywords: *Thespesia populnea,* Malvaceae, Aerial part, Mansonones.

1. Introduction

Thespesia is a genus of trees belonging to family Malvaceae, *Thespesia populnea* L (also called Milo or Portia tree) is fast growing, medium sized evergreen tree, up to 3 m tall grows in Cairo in El-Orman garden. *Thespesia populnea* L also cultivated in widespread in Hawaii, California and Florida, and also in Asia, Africa and the Caribbean Islands [1, 8].

Cotton plants (*Gossypium hirsutum*) produce various sesquiterpenes when they are attacked by pathogens. These compounds are often toxic to the pathogen and thus help protect the plant from attack. *T. populnea* is a rich source of sesquiterpenoid quinones including Mansonones [10]. Mansonones, highly oxidized sesquiterpenes containing a cadinane skeleton [1].

Some of these compounds possess significant cytotoxic [11, 2], antifungal [9] and antioxidative activities [6].

2. Results and discussion

Dichloromethane extract was prepared from aerial part of *T. populnea* L fractionated by silica gel column chromatography followed by PTLC which afford mansonones C, G, E, H and 7-hydroxy-2,3,5,6-tetrahydro-3,6,9-trimethylnaphtho[l,8-b,c]pyran-4,8-dione.

Mansonone (1)

The ^{1}H NMR spectrum of mansonone (1) displayed two ortho –coupled of aromatic protons at [δ 7.23 (1H, br, H-2) and 7.31 (1H, br, H-3)].One singlet signal of aromatic protons at [δ 7.71 (1H, s, H-5)]. In addition, the presence of two methyl groups at [δ 2.58 (3H, s, H-9) and 2.06 (3H, s, H-13)] and one isopropyl moiety at [δ 3.39 (1H, m, H-10), 1.38 (3H, d, J = 6.9 Hz, H-11) and 1.3 (3H, d, J = 6.9 Hz, H-12)]. The ^{13}C NMR spectrum displayed two carbonyl carbon at C-7 (δ_C 182.2) and C-8 (δ_C 182.9), suggesting an *o*-naphthoquinone cadinane skeleton. The comparison of these data with that reported [5] proved that mansonone 1 is mansonone C

Mansonone (2)

The^{1}H and ^{13}C NMR spectra of mansonone (2) were closely resembled to those of mansonone (1). In ^{1}H NMR spectrum, the *ortho*-coupled proton at δ 7.31 (1H, br, H-3) disappeared. The^{1}H spectrum of mansonone (2) displayed one aromatic proton at [δ 6.57 (1H, s, H-2)].The ^{13}C NMR spectrum displayed signal of sp^2 oxy-quaternary carbon C-3 (δ_C 162.4) instead of C-3 (δ_C 131.9) in mansonone (1), whose down field signal suggested a connection to a hydroxyl group. The comparison of these data with that reported proved that mansonone 2 is mansonone G [7, 10].

Mansonone (3)

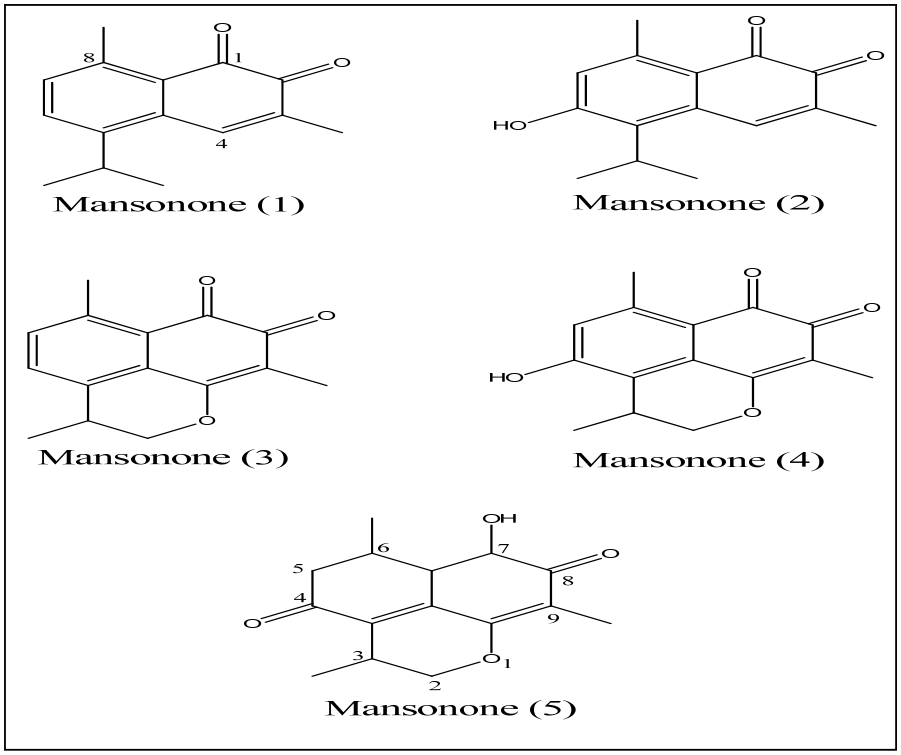
The ^{1}H and ^{13}C NMR spectra of mansonone (3) were related to those of mansonone (1), except for the olefinic proton signal on the quinone ring at [δ 7.71 (1H, S, H-5)] and one of methyl proton signal of the isopropyl at [δ 1.3 (3H, d, J = 6.9 Hz, H-12)] were absent in mansonone (3), being replaced by oxymethylene proton resonance at [δ 4.42 (1H, dd, J = 10, 4 Hz, H-12), 4.2 (1H, dd, J = 10, 4 Hz, H-12)]. The comparison of these data with that reported proved that mansonone 3 is mansonone E [6].

Mansonone (4)

The ^{1}H and ^{13}C NMR spectra of mansonone (4) were closely resembled to mansonone (3). In ^{1}H NMR spectrum, the *ortho*-coupled proton at [δ 7.42 (1H, br, H-3)] disappeared. The ^{1}H spectrum of mansonone (4) displayed one aromatic proton at [δ 6.4 (1H, s, H-2)]. The ^{13}C NMR spectrum displayed signal of sp^2 oxyquaternary carbon C-3 (δ_C 156.6) instead of C-3 (δ_C 132) in mansonone (3), whose down field signal suggested a connection to a hydroxyl group. The comparison of these data with that reported proved that mansonone 4 is mansonone H (Kim, *et al*., 1996).

Mansonone (5)

The ^{1}H and spectrum of mansonone (5) showed the presence of two secondary methyl groups at [δ 1.2 (3H, d, J = 6.8 HZ, H-9), 1.12 (3H, d, J = 6.8 HZ, H-11)]. Furthermore, signals of methane protons at [δ 3.6 (1H, dq, H-1), 3.21 (1H, m, H-10)] coupled to two pairs of methylene proton located at [δ 2.81 (1H, dd, H-2), 2.51 (1H, dd, H-2)] and [δ 4.26 (1H, dd, J = 10.3, 3 HZ, H-12), 4.38 (1H, dd, J = 10.3, 3 HZ, H-12)]. The comparison of these data with that reported proved that mansonone 5 is 7-hydroxy-2,3,5,6-tetrahydro-3,6,9-trimethylnaphtho[l,8-b,c]pyran-4,8-dione [8].



2.1 Cytotoxic activity

Using MTT assay, the effect of the extract on the proliferation of MCF-7 cells was studied after 48 h of incubation. The treatment with Total extract showed strong cytotoxic effect against MCF-7, as concluded from its IC_{50} value 107.8 µg/ml, as shown in figure 1.

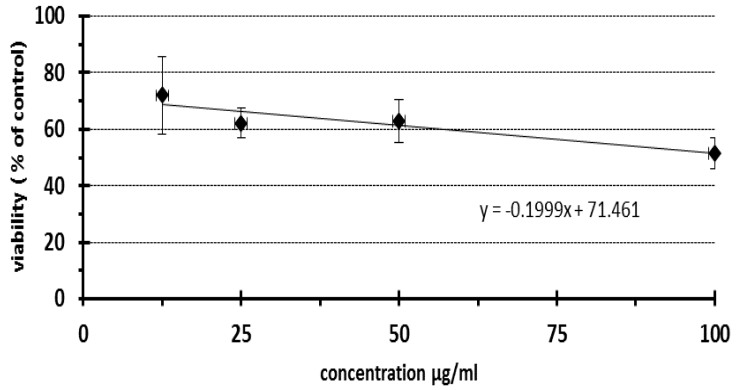


Fig 1: Cytotoxic effect of Methanolic extract against MCF-7 cells using MTT assay (n=4), data expressed as the mean value of cell viability (% of control) ± S.D.

2.2 Anti-inflammatory activity

Using Nitric oxide method, the results indicated that the inflammagen lipopolysaccharide (LPS 100 µg/ml) induced nitric oxide production up to 2 folds of the control, while that the potent anti-inflammatory Dexamethasone (50 ng/ml) inhibited nitric oxide production to 3.5 µ mole/ml compared to 6.5 µ mole/ml of that of the LPS with level of 97 % inhibition, very close to the negative control cells with 3.2 µmole/ml.

Methanolic extract (25 µg/ml); showed anti- inflammatory effect as shown in the amount of NO produced with a level of 4.025 µmole/ml, leading to 75% inhibition, in comparison to the potent anti-inflammatory drug Dexamethasone (97 % inhibition).

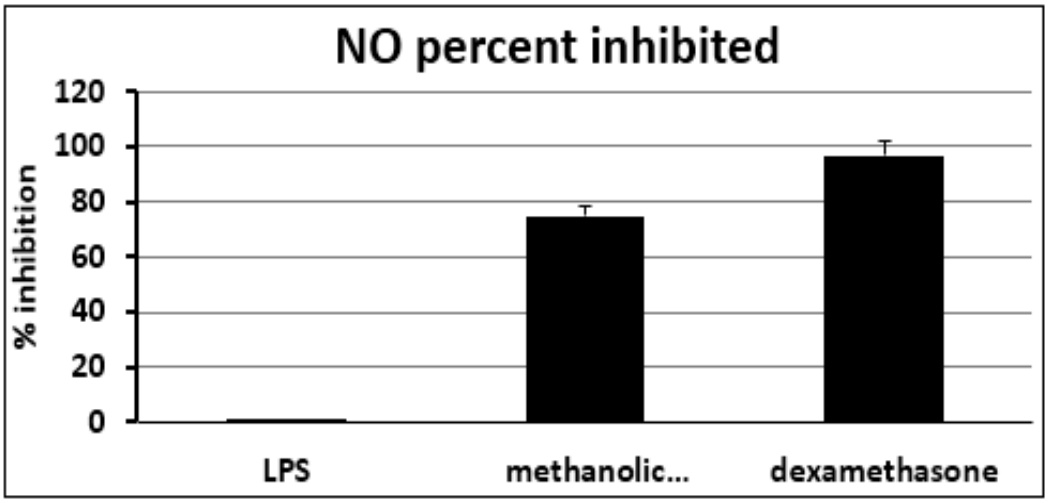


Fig 2: *In-Vitro* Anti-inflammatory by Nitric oxide method, data expressed as Percent inhibition of Nitric oxide of both the sample and Dexamethasone compared to the LPS- stimulated cells alone

3. Experimental

3.1 General

^{1}H and ^{13}C NMR spectra were recorded in DMSO on JEOL 300 MHZ. TLC was carried out on precoated silica gel 60 F_{254} (Merck) and spots were visualized by spraying with 10% Methanolic H_2SO_4. Kieselgel (40-60-µm, Merck) was used for CC.

3.2 Plant Material

Parts of the aerial part of *Thespesia populnea* were collected from the Egyptian Orman garden, Giza, Egypt from June 2010. The plant was identified by Agricultural Engineer Terese Labib, El Orman Botanical Garden

3.3 Extraction and Isolation

The air dried powdered aerial part of *T. populnea* (750 g) were macerated in 2 L 70% aqueous methanol with occasional stirring at room temperature for three days. The process was repeated three times till exhaustion. The combined methanol extract was concentrated and dried under vacuum. The dried residue (76 g) was diluted with H_2O and extracted with dichloromethane. The dichloromethane extract was separated and evaporate to concentrate. Extraction method repeated increase the yield (25 g). The dichloromethane extract were chromatographed on silica gel column (300 g silica). Elution was achieved with gradient of $CHCl_3$: MeOH (100% $CHCl_3$ to 100 % MeOH). Similar fractions were collected together according to their profiles on TLC silica gel G F_{245} plates. Fractions eluted from the main column was subjected to subcolumn silica gel column for further purifications. Elution was achieved with gradient of $CHCl_3$: MeOH. Fractions eluted from subcolumns were subjected to PTLC to obtain the five mansonones, mansonones 1, 2, 3, 4 and 5.

Mansonone 1 was isolated as an orange solid. The ^{1}H NMR (DMSO) spectrum of **1** showed δ 7.23 (1H, br, H-2), 7.31 (1H, br, H-3), 7.71 (1H, s, H-5), 2.58 (3H, s, H-9) 3.39 (1H, m, H-10), 1.38 (3H, d, J = 6.9 Hz, H-11), 1.3 (3H, d, J = 6.9 Hz, H-12), 2.06 (3H, s, H-13). The ^{13}C NMR (DMSO) data represented in table 1.

Mansonone 2 was isolated as orange solid. The ^{1}H NMR (DMSO) spectrum showed δ 6.57 (1H, s, H-2), 7.71 (1H, s, H-3), 2.56 (3H, s, H-9), 3.5 (1H, m, H-10), 1.25 (3H, d, J = 7 Hz, H-11), 1.29 (3H, d, J = 7 Hz, H-12), 1.94 (1H, s, H-13). The ^{13}C NMR (DMSO) data represented in table 1

Mansonone 3 was isolated as a reddish brown solid. The ^{1}H NMR (DMSO) spectrum of **3** showed δ 7.29 (1H, br, H-2), 7.37 (1H, br, H-3), 2.58 (3H, s, H-9), 3.1 (1H, m, H-10), 1.25 (3H, d, J = 6.8 Hz, H-11), 4.42 (1H, dd, J = 10, 4 Hz, H-12), 4.2 (1H, dd, J = 10, 4 Hz, H-12), 2.1 (1H, s, H-13). The ^{13}C NMR (DMSO) data represented in table 1.

Mansonone 4 was isolated as a reddish brown solid. The ^{1}H NMR (DMSO) spectrum of showed δ 6.4 (1H, s, H-2), 2.56 (3H, s, H-9), 3.31 (1H, m, H-10), 1.27 (3H, d, J = 6.8 Hz, H-11), 4.42 (1H, dd, J = 10.3, 3 Hz, H-12), 4.26 (1H, dd, J = 10.3, 3 Hz, H-12), 2.07 (1H, s, H-13); The ^{13}C NMR (DMSO) data represented in table 1.

Mansonone 5 was isolated as a reddish brown solid. The ^{1}H NMR (DMSO) spectrum of **5** showed δ 3.6 (1H, dq, H-1), 2.81 (1H, dd, H-2), 2.51 (1H, dd, H-2), 1.2 (3H, d, J = 6.8 Hz, H-9), 3.21 (1H, m, H-10), 1.12 (3H, d, J = 6.8 Hz, H-10) 4.26 (1H, dd, J = 10.3, 3 Hz, H-12), 4.38 (1H, dd, J = 10.3, 3 Hz, H-12), 2.09 (1H, s, H-13).

Table 1: ^{13}C NMR spectral data of Mansonone 1, Mansonone 2, Mansonone 3 and Mansonone 4

Position	1	2	3	4
	δ_C	δ_C	δ_C	δ_C
1	142.2	150.7	142	144.2
2	134.7	118.2	134.6	123.9
3	131.9	162.4	132	155.8
4	146	132.3	136.7	116.4
4a	132.4	134	126.4	128.3
5	137	136.2	162.4	165.8
6	134.9	137.3	117.1	114
7	182.2	180.7	179.9	180.8
8	182.9	182	183	182.1
8a	129	124	127	131.7
9	23.1	23.2	22.5	23.3
10	28.5	27.8	31.9	28
11	23.9	22.1	18	16.1
12	23.9	22.1	72.2	67.7
13	18	14.1	7.8	7.5

3.4 Cytotoxic Assay:

Cytotoxicity of the total extract was measured against MCF-7 cells using the MTT Cell Viability Assay. Cells (0.5X105 cells/ well), in serum-free media, were plated in a flat bottom 96-well microplate, and treated with 20 µl of different concentrations of the total extract for 48 h at 37 ºC, in a humidified 5% CO2 atmosphere. After incubation, media were removed and 40 µl MTT solution / well were added and Incubated for an additional 4 h. MTT crystals were solubilized by adding 180 µl of acidified isopropanol / well and plate was shacked at room temperature, followed by photometric determination of the absorbance at 570 nm using microplate ELISA reader. Triplicate repeats were performed for each concentration and the average was calculated. Data were expressed as the percentage of relative viability compared with the untreated cells compared with the vehicle control, with cytotoxicity indicated by <100% relative viability [4].

3.5 Anti-inflammatory Assay:

Anti-inflammatory assay by Nitric oxide method, in each well of a flat bottom 96 well- microplate, 40 µl freshly prepared Griess reagent was mixed with 40 µl cell supernatant or different concentrations of sodium nitrite ranging from 0-50 µ mole/ml. The plate was incubated for 10 min in the dark and the absorbance of the mixture at 540 nm was determined using the microplate ELISA reader [3].

A standard curve relating NO in µmole/ml to the absorbance is constructed, from which the NO level in the cell supernatant is computed by interpolation.

The NO level of each of the tested cell supernatant was expressed as NO level of the tested cell supernatant x100/ NO level of the control

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