Asian Journal of Chemistry Vol. 14, No. 1 (2002), 197–202

Iridoid Glycosides from *Citharexylum quadrangular*

TAHA I. KHALIFA†, OMAYMA D. EL-GINDI*, HASSAN A. AMMAR† and DINA M. El-NAGGAR
Department of Pharmacognosy, Faculty of Pharmacy for Girls Al-Azhar University, Nasr City, Cairo, Egypt.

The first isolation of biologically active iridoid glycoside phlomiol, 5-deoxy pulchelloside, lamiide, durantoside I and lamidoside from *Citharexylum quadrangular* has been done. A revision in some of the NMR spectral assignments has also been made to confirm the structure of these compounds. The biological screening of the 70% aqueous ethanolic extract revealed a significant antiulcer, antihypertensive and hepatoprotective effect of these plants.

Key words: Citharexylum quadrangular, verbenaceae, iridoid glycosides, phlomiol, 5-deoxy pulchelloside, lamiide, durantoside I, lamiidoside, isolation.

INTRODUCTION

The genus *Citharexylum* belongs to the subfamily Viticoideae and represents one of the largest genera of the family Verbenaceae. Several interesting iridoid glycosides have been reported from *Citharexylum fruticosum*[1,2] and *Citharexylum solanaceum*[3]. *Citharexylum quadrangular* Jacq. is widely distributed in many gardens in Egypt and used in traditional medicine as a diuretic, antipyretic and for treatment of liver disorders. The considerable importance of *Citharexylum quadrangular* in folk medicine and the paucity of reports on its phytochemical constituents prompted us to investigate the biologically active content of this species.

RESULTS AND DISCUSSION

Biological screening revealed that the 70% methanolic extract of *Citharexylum quadrangular* at the studied doses possesses a significant antiulcer, antihypertensive and hepatoprotective effect (Figs. 1–3).

Antiulcer effect: A significant decrease in the gastric ulcer area percentage in the treated group was noticed at the doses of 10 and 20 mg on rats. No increase in the antiulcer effect was observed with an increase in the dose.

Hypotensive effect: The systolic and diastolic blood pressure of normal cats was lowered after its administration with doses 40 mg and increased with the other higher concentrations till it reached its maximum effect at the concentration of 160 mg.

†Department of Pharmacognosy, Faculty of Pharmacy for Boys, Al-Azhar University, Nasr City, Cairo, Egypt.

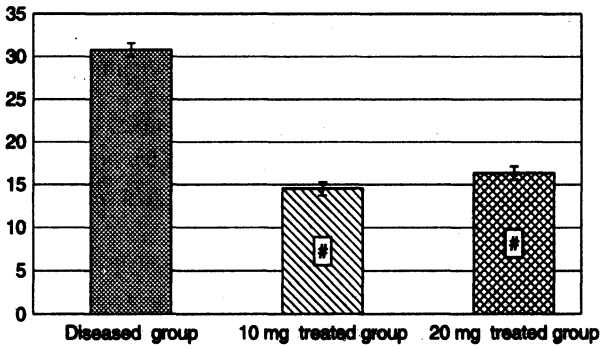

Fig. 1. Comparison between the diseased area percentage of the ulcer stomach diseased and the *Citharexylum quadrangular* treated groups (Mean ± S.E.).
Significant change from diseased group.

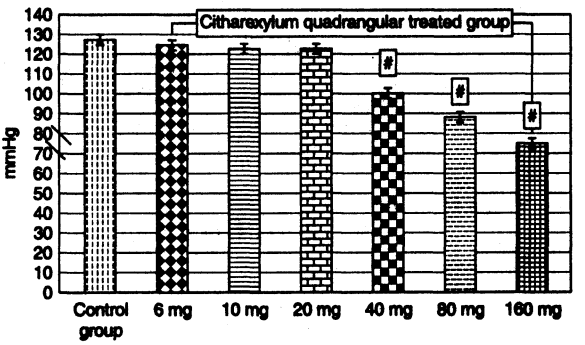

Fig. 2. Comparison between the systolic blood pressure of the control group and the *Citharexylum quadrangular* treated group with different doses (mean + S.E.).
Significant change trom other groups.

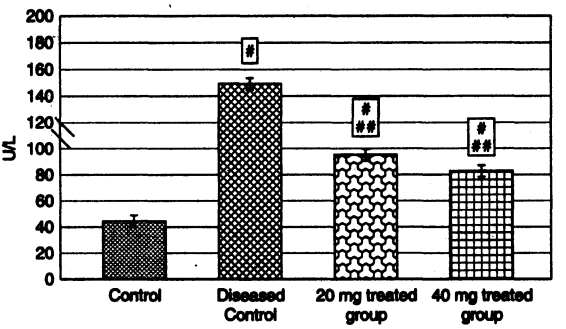

Fig. 3. Comparison between the control, control diseased, 20 and 40 mg *Citharexylum quadrangular* treated groups (after CCl_4 administration) according to serum live enzymes (AST or ALT or GGT) level (U/L) (mean + S.E.), # Significant change from the control group, ## Significant change from the diseased control group

Hepatoprotective effect: *Citharexylum quadrangular* extract in concentration of 20 and 40 mg showed a hepatoprotective activity. No superior effect was observed of the concentration 40 mg over lower one.

The isolated compounds are similar in the basic nucleus of phlomiol[1] (cyclopentanone glycosides), but differ in the snbstituents present in C-5, C-6, C-7 and C-8. Phlomiol **(5)** showed 17 resonances in its ^{13}C-NMR spectrum (Table-1) and was assigned the formula $C_{17}H_{26}O_{13}$ based on an m/z 429 $[M + Na]^+$ in its positive FAB-mass spectrum. These data, as well as a COSY and HMBC spectrum confirm the iridoid structure and the conjugation of the enol-ether, group[6]. The singlet of the H-3 proton at δ 7.5 is highly deshielded by the presence of a carbomethoxy group at C-4 (singlet at δ 3.75). The signals of an AB system arising from two protons geminal with hydroxyl functions located as doublet J = 4.5 Hz at δ 4.05 and doublet J = 4.5 Hz at δ 3.56 for C-6 and C-7 respectively. Deshelding of C-8 singlet at δ 1.1 assigned to the methyl group at C-8 tertiary OH at C-8. All the data are in agreement with the presence of four hydroxyl groups (as C-5, C-6, C-7, C-8), of a carbomethoxy group at C-4 and of a methyl group at C-8 in the aglycone moiety and therefore support the phlomiol structure, Lamiide-4: the positive FAB-mass spectrum, showed a peak at m/z 445 (M + Na]+ and established a molecular formula of $C_{17}H_{26}O_2$. The ^{1}H-NMR spectrum showed sharp singlet at δ 3.75 ppm and singlet at δ 7.45 ppm indicating the presence of 4-substituted enol-ether system of iridoids[6]. The methyl group at C-8 geminal to a tertiary OH was indicated by the sharp singlet at δ 1.1 ppm. This compound differs from phlomiol in the absence of substituents in C-6 and three hydroxyls only present at C-5, C-7 and C-8. The 2H-6 appeared as double-doublet at 2.21, 2.39 ppm. The proposed structure was in accordance with the ^{13}C-NMR spectrum. Proton and carbon resonances of the iridoid part were in agreement with those of lamiide[7, 8]; COSY experiment and HMBC were also done and confirmed the structure. This is the first isolation of lamiide from *Citharexylum quadrangular*. The positive FAB-mass spectrum of compound **3** showed a peak at m/z 407 which could be assigned to the $[M+H]^+$ peak compatible with the m.f. $C_{17}H_{26}O_{11}$. The peak at m/z 245 (oxonium ion of the aglycon), 244, 227, 226 and 208 indicated the presence of two free hydroxyl groups in the aglycon moiety which could be easily eliminated as water. The ^{1}H-NMR spectrum showed singlet at δ 7.45 and peaks at 153.8 and 111.2 ppm in ^{13}C-NMR which assigned to C-3 and C-4 respectively typical of a C-4 substituted iridoid glycoside with a carbomethoxy function at C-4[9]. The methyl signals at δ 1.1 ppm, multiplet at 2.24 $J_{8,9} = 10$ Hz, $J_{8,10} = 7$ Hz, broad doublet at δ 4.01 and dd at δ 3.53 indicate the absence of hydroxyl group at C-8, HMBC and COSY, which have not been reported before, confirm the identity of this compound as 5-deoxy pulchelloside[1]. The positive FAB-mass spectrum of compound **2** showed a peak at m/z 591 $[M+Na]^+$ corresponding to the m.f. $C_{26}H_{32}O_{14}$. The ^{1}H-NMR, showing that 2H-6 appear as dd at δ 2.4 and 2.45 ppm, are deshielded due to the presence of the *p*-substituted cinnamoyl moiety at C-7. For this reason the CH_3 at C-8 is a little more deshielded δ 1.18 ppm than Lamiide. The sharpness of the signal of the H-3 δ 7.4 ppm, and CH_3 at C-8 indicate the absence of protons on C-5 and C-8. This data is comparable to the data reported for Lamiidoside

(durantoside-4) which has been isolated from the seeds of *Phlomis fruticosa* (F. Labiatae)[10] which is more confirmed by HMBC. The positive FAB-mass spectrum of compound **1** showed a peak at m/z 575 $[M + Na]^+$ corresponding to the m.f. $C_{26}H_{32}O_{13}$. The ^{1}H-NMR and ^{13}C-NMR confirm the iridoid structure and the conjugation of the enol-ether group[6]. The singlet at δ 1.15 ppm was assigned to the methyl group at C-8 deshielded by an oxygenated function, probably a tertiary OH. ^{1}H-NMR spectrum showed the existence of one *cis*-olefinic system δ 6.1 and 7.11 ppm, J = 13H. The position of the *cis*-cinnamoyl moiety was determined by comparison of ^{1}H and ^{13}C-NMR spectra with those of the unsubstituted Lamiide[11]. The COSY and HMBC experiment confirmed that compound **1** is durantoside-1 which is isolated from *Duranta erecta* (F. Verbenaceae)[7].

TABLE (1)
^{13}C-NMR SPECTRAL DATA FOR IRIDOID GLYCOSIDES

C	1	3	4	5
C-1	93.99	96.05	94.38	94.10
C-3	152.10	153.80	152.30	154.10
C-4	115.50	111.20	115.30	114.30
C-5	68.87	38.77	69.16	68.76
C-6	45.37	78.31	46.73	71.63
C-7	80.31	79.97	79.05	78.61
C-8	78.52	39.50	78.30	76.33
C-9	58.09	40.34	57.96	57.94
C-10	21.30	13.91	21.23	21.35
C=O	167.20	169.10	167.90	168.2
OCH_3	51.66	51.73	51.60	51.86
glc-1′	99.55	99.78	99.55	99.68
glc-2′	71.65	74.57	74.30	76.11
glc-3′	77.37	77.53	77.34	77.33
glc-4′	68.93	71.60	71.58	74.34
glc-5′	72.30	77.97	77.79	78.39
glc-6′	62.79	62.81	62.74	62.79
C-1″	135.90			
C-2″, 6″	129.00			
C-3″, 5″	131.00			
C-4″	130.10			
α	120.60			
β	144.80			
C=O	166.90			

EXPERIMENTAL

Plant material: *Citharexylum quadrangular* (Jacq.) Verbenaceae was collected in January 1998 from the Agricultural Museum, Giza, Egypt. It was identified by Dr. Nabil El-Hadidi, Department of Botany, Faculty of Science, Cairo University, Giza, Egypt, and a voucher specimen was deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Girls' Al-Azhar University, Nasr City, Cairo, Egypt.

Extraction and isolation: Air-dried leaves (1 kg) were extracted with methanol. The MeOH was evaporated *in vacuo* to yield 85 g residue, which was dissolved in water and partitioned between water and petroleum ether. The H_2O was evaporated *in vacuo* to leave 60 g of residue, which was treated with MeOH. The MeOH-soluble part was evaporated *in vacuo* to give 50 g crude iridoid and/or glycoside residue. This residue was purified by dissolving in H_2O and fractionated on polyamide column (0–100% MeOH). The MeOH content was increased 5% in each of 100 mL Fr:

Fr. 1 (100% H_2O; 15.8 g): 300 mg **1** and 30 mg **2** after rechromatography on C-18 silica gel (H_2O-MeOH, gradient) and then sephadex LH-20 column.

Fr. 2 (5% MeOH; 870 mg): 116 mg **3** after rechromatography on normal phase silica gel ($CHCl_3$-MeOH gradient) and C-18 silica gel (H_2O-MeOH, gradient) and then sephadex LH-20 column.

Fr. 3 (50% MeOH; 300 mg): 31 mg **4** and 80 mg **5** after rechromatography on normal phase silica gel ($CHCl_3$-MeOH, gradient) and then sephadex LH-20 column.

Biological screening

Antiulcer effect: Twelve rats (200 g) were used to induce gastric erosion by cold immobilization stress method[12]. The drug treatment had been administered to another group (12 rats) before the ulcer induction for 10 days with different concentrations (10, 20 mg). The protective effect was tested and the area of the rat's ulcers was measured.

Hypotensive effect: Six cats (3 kg body weight) were used and anaesthetized by IV sodium pentobarbitone in a dose of 30 mg/kg body weight. then the cat was placed and secured to the side of the table. Arterial cannula was put in the right common carotid and connected *via* a pressure rubber tubing to a mercury manometer provided with a float recording lever writing on a slowly moving, smoked kymograph. The manometer was connected at the other end through a rubber tube to a glass reservoir filled with 2% Na citrate solution as anticoagulant. Heparin (200–500 IU) was also injected into the femoral cannula to guard against blood clotting[13]. After stabilization of the preparation the initial systemic blood pressure was recorded as control. *Citharexylum quadrangular* extract was then injected in gradually increasing doses (5, 10, 20, 40, 80 and 160 mg/3 kg) into the femoral cannula to demonstrate their effects on blood pressure.

Hepatoprotective effect: Four animal groups (each group containing six animals) were used in this experiment. The first group was the normal control group and the second was diseased control group (which administered CCl_4 only

for 36 h with a dose of 2.5 mL/kg body wt.). Blood samples were collected from the control group immediately and from the diseased control group after the end of 36 h. The third and the fourth groups received 20 and 40 mg of the extract respectively for 3 weeks and CCl_4 for 36 h. Blood samples were collected, serum separated by centrifugation at 2500 ppm for 15 min and analyzed for the biochemical parameters AST, ALT and GGT (by the use of the kits supplied by Diamond Biomedica Company, Cairo, Egypt) in order to assess the liver profile[14].

REFERENCES

1. S. Ganapaty, J. Heni, D.V. Rao and H. Rimpler, *Planta Med.*, **54**, 42 (1988).
2. S. Ganapaty and D.V. Rao, Fitoterapia, 44, 13 (1983).
3. S. Milz and H. Rimpler, Z. Naturforsch, **34**, 319 (1979).
4. W.C. Worsdell, "Index Londinensis" to Illustrations of Flowering Plants, Ferns and Fern Allies, Clarendon Press, Oxford. p. 41 (1941).
5. H. Wagner and P. Wolff, New Natural Products and Plant Drugs with Pharmacological, Biological or Therapeutic Activity, p. 231 (1977).
6. A. Bianco, M. Guiso, C. Iavarone and C. Trogolo, *Gazzetta Chimica (Italiana)*, **105**, 185 (1975).
7. Y. Takeda, Y. Morimoto, T. Matsumoto, C. Ogimi, E. Hirata, A. Takushi and H. Otsuka, *Phytochem.*, **39**, 829 (1995).
8. I.T.A. Schuquel, A. Malheiros, M.H. Sarragiotto, and G.J. Vidotti, *Phytochem.*, **49**, 2409 (1998).
9. A. Rathore, V. Srivastava, K.C. Srivastava and J.S. Tandon, *Phytochem.*, **29**, 1917 (1990).
10. A. Bianco, C. Bianco, M. Guiso, C. Iavarone and C. Trogolo, *Gazzetta Chimica (Italiana)*, **107**, 67 (1977).
11. P. Junior, *Planta Med.*, **51**, 229 (1985).
12. M. Robert, N. Nezamis and W. Philips, Gastroentrology, **55**, 481 (1968).
13. M.N. Ghosh, Fundamentals of Experimental Pharmacology, p. 70 (1971).
14. K.S. Rao and S.H. Mishra, *Fitoterapia*, **6**, 510 (1997).

(*Received*: 30 July 2001; *Accepted*: 3 October 2001) AJC-2476