Natural Product Sciences
2(2) : 96 ~ 101 (1996)

Chemical and Biological Investigations of the Constitutive Phenolics of Two Egyptian Folk-Medicinal Plants; A Novel Phenolic from the Galls of *Tamarix aphylla*

Heba H. Barakat* and Somaia A. Nada**

*Dept. of Tanning Materials & Proteins;
**Dept. of Pharmacology, National Research Center, Dokki, Cairo, Egypt.

Abstract – A new natural product, 3,4,8-trihydroxybenzopyranopyran-6,9-dione was isolated from the aqueous ethanolic gall extract of *Tamarix aphylla* (Tamaricaceae) along with the known phenolics, monodecarboxyellagic acid and brevifolin carboxylic acid as well. The structures have been established by ESI-MS, ^{1}H and ^{13}C NMR spectral analysis. Antiinflammatory, antipyretic and ulcerogenic activities determination for both plant (*Tamarix aphylla* and *Phragmites australis*) were carried out on aq. ethanolic of extracts.

Key words – *Tamarix aphylla*, Tamaricaceae; galls, Phenolic lactone, 3,4,8-trihydroxybenzopyranopyran-6,9-dione, *Phragmites australis*, Gramineae; flowers, C-glycosyl flavonoids, antiinflammatory, antipyretic, ulcerogenic activities.

Tamarix aphylla, a tree native to Egypt, provides galls which have been proven to be rich source of polyphenolics. Previous phytochemical investigations of gall extracts led to the isolation and structure elucidation of several polyphenolics including gallic, dehydrodigallic, dehydrotrigallic, ellagic, ferulic and isoferulic acids as well as the unique ellagitannin, tamarixellagic acid and the new gallotannins, 2,6- di-O-(α/β)-$^{4}C_{1}$-glucopyranose and 3,6-di-O-(α/β)-$^{4}C_{1}$-glucopyranose (Ishak, 1972; Nawwar, 1994a; Nawwar, 1994b). In the present communication, we report on the further isolation and structure determination of the new natural phenolic, 3,4,8-trihydroxybenzopyranopyran-6,9-dione (**I**) from the aqueous ethanolic gall extract of this plant. The known compounds monodecarboxyellagic acid (**II**) and brevifolin carboxylic acid (**III**) were also isolated and characterized. Compound (**I**) is another natural phenolic added to the well known biosynthetic transformation products of hexahydroxydiphenic acid such as monodecarboxyellagic acid (**II**), brevifolin carboxylic acid (**III**), (Nawwar, 1994c) and chebulic acid (Swain, 1977). Also it belongs to the 3,4-dihydroxycoumarin derivatives which are of rare natural occurrence (Pastor, 1987).

On the other hand, the flowers of the Egyptian wild plant, *Phragmites australis* have been proven, in a previous phytochemical study carried out by one of the authors (Nawwar, 1980), to be capable of synthesizing and accumulating large amount of C-glycosyl flavones and O-glycoside derivatives as well, whereby swertiajaponin, isoswertiajaponin together with the new 3'-O-glucoside and 3'-O-gentiobioside of the former have been isolated and identified along with some other known flavonol glycosides. It was therefore found reasonable to undertake a biological investigation in the present study, to deter-

* Author for correspondence.

mine the antiinflammatory, antipyretic and ulcerogenic activities of the aqueous ethanolic extracts of the galls of *T. aphylla* and that of the flowers of *P. australis*.

Experimental

General – NMR analysis. A JEOL GX 400 spectrometer, 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR, was used with superconducting magnet from Oxford and 5 mm Dual probehead for ^{1}H and ^{13}C-analysis. Data processing with LABONE-software from NMRi, Syracuse, New York. Typical conditions: spectral width = 6000 Hz for ^{1}H and 22000 Hz for ^{13}C, 32 K data points and a flip angle of 45°. The UV spectra were taken in MeOH, using a Shimadzu UV-240 spectrophotometer; IR spectra were measured on a Perkin-Elmer 2000 FTIR (Perkin-Elmer Ü berlingen, Germany) as standard KBr disks measured against a pure KBr reference disk in a sample shuttle. The sample, detector and interferometer were purged with dried CO_2 free air; ESI-MS (negative mode): the direct flow injection technique was applied, sample in MeOH was introduced (1.25 ml/min) together with MeOH sheath-liquid (5 ml/min) by a Harvard infusion pump 9 ml/min SF_6 sheath-gas into the ESI ion source of a Finnigan MAT 4600 spectrometer.

Plant Materials – Aq. EtOH extracts (3:1) of *T. aphylla* galls and *P. australis* flowers were individually worked up as described in (Nawwar, 1994a) and (Nawwar, 1980), respectively.

Extraction and isolation – PC was carried out on Whatman No. 1 paper using solvent systems: (1) H_2O; (2) HOAc-H_2O (3:50); (3) n-BuOH-HOAc-H_2O (4:1:5, upper layer); (4) C_6H_6 -n-BuOH-H_2O-pyridine (1:5:3:3, upper layer). Solvent 3 was used for prep. PC on Whatman No. 3 MM paper and solvents 3 and 4 for sugar analysis. The last fraction, eluted with ethanol from the aqueous ethanolic gall extract of *T. aphylla* (Nawwar, 1994a) was shown by 2D-PC under UV light to contain three fluorescent compounds **I-III**. Pure sample of each was separated by applying repeated Sephadex LH-20 column fractionation of the received ethanolic fraction, using n-butanol saturated with water as an eluent. One of the obtained compounds is new **I**. The remaining two compounds **II** and **III** are known and gave chromatographic, UV and IR spectral data identical with those of the monodecarboxyellagic acid, 3,4,8, 9,10-pentahydroxydibenzo[b,d]pyran-6-one, (**II**) and brevifolin carboxylic acid, (**III**). The structure of both compounds was then confirmed by EI-MS or -ve ESI-MS, ^{1}H and ^{13}C NMR spectral analysis (Table 1).

3,4,8-Trihydroxybenzopyranopyran-6,9-dione (**I**): R_f-values: 20(1), 26(2), 42(3). UV (MeOH) λ_{max}: 282 and 400 nm. IR (ν_{max} KBr): 3400, 2910, 2850, 1695, 1630, 1580 cm^{-1}; ESI-MS (-ve): m/z 261 [M-H]$^-$. Normal acid hydrolysis (25 mg in 10 ml aq. 2 N HCl, 100°, 3 hrs) **I** recovered unchanged. Normal alkaline hydrolysis (20 mg in 10 ml aq. NaOH, 5%, 100°C, $1^{1/2}$ hr followed by acidification and extraction with ethyl acetate) **I** recovered unchanged. Alkali-fusion (two fused KOH pellets added to 8 mg of **I**, 200-210°C, $\simeq 2^{1/2}$ min, followed by dilution, acidification and extraction with ethyl acetate), fusion product: R_f-values: 58(1), 73(2), 75(3); UV (MeOH) λ_{max}: 266 nm; ^{1}H NMR (400 MHz, DMSO-d_6) δppm: 6.22 (2H, d, J=7.5 Hz, H-3 and H-5), 6. 39 (1H, t, J=7.5 Hz, H-4). ^{1}H NMR of I: δ6.9 (1H, d, J=9 Hz, H-2), 8.32 (1H, d, J=9 Hz, H-1), 7.23 (1H, s, H-7). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: Table 1.

3,4,8,9,10-Pentahydroxydibenzo[b,d]pyran-6-one (II). R_f-values: 00(1), 16(2), 46(3). UV (MeOH) λ_{max}: 264 , 287 sh , 349 (inflection) nm. IR (ν_{max} KBr): 3400 , 3300, 2910, 2850, 1690, 1610 ,1590 cm^{-1}. EI-MS [m/z (rel. int.)]: 276 (100, [M]$^+$), 247(8), 248(10), 219(15), 191(10), 163(12), 115(10), 89(12), 73(15), 55(18). ^{1}H NMR (400 MHz , DMSO-d_6) δppm: 8.44 (1H, d, J=9 Hz, H-1), 6.8 (1H, d ,J=9 Hz , H-2), 7.46

Table 1. ^{13}C NMR data of polyphenolics **I**, **II**, and **III** (100 MHz, DMSO-d_6)

Carbon* No.	Compound I	Compound II	Compound III
1	116.90	118.40	173.40
2	112.80	112.10	41.70
3	149.30	151.50	37.40
4	132.50	133.10	194.30
4a	142.00	143.60	149.00
4b	123.60	112.10	115.40
6	160.15	161.60	160.70
6a	102.20	112.00	112.80
7	108.50	108.80	108.10
8	127.40	146.00	144.30
9	160.00	140.60	141.50
10	-------	146.00	146.10
10a	158.90	146.50	140.1

*Numbering is for convenience.

(1H, s, H-7). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: Table 1.

Brevifolin carboxylic acid (III). R_f-values: 55(1), 59(2), 62(3). UV (MeOH) λ_{max}: 278, 350, 362 sh nm. IR (ν_{max} KBr): 3440, 1705, 1625, 1605 cm^{-1}. FAB-MS: neg. ion: 291[M-H]$^-$, pos. ion: 293[M+H]$^+$. ^1H NMR (400 MHz, DMSO-d_6) δppm: 2.75 (2H, brs, $\Delta_v^{1/2}$=13 Hz, 2H-3), 4.35 (1H, t, J=7.5 Hz, H-2), 7.22 (1H, s, H-7). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: Table 1.

Biological test

Animals – Male albino rats (100 to 120 g b. wt.) were obtained from the Animal House Colony, NRC, Cairo, Egypt. They were housed 6 per cage and maintained under standard conditions of humidity, temperature and light. The animals were fed with standard laboratory diet and had free access to water.

Preparation of drugs – The extracts were dissolved in H2O and administered at two dose levels equivalent to 1/10 and 1/5 LD_{50}: 20 and 40 mg/kg for *T. aphylla*, 450 and 900 mg/g for P. australis.

Acute toxicity – Male and female albino rats (10 rats/group) were intraperitoneally (i. p.) injected with the test extracts at doses ranging from 0.1 to 0.4 g/kg for *T. aphylla* and 4.5 to 6 g/kg for *P. australis*. LD_{50} doses were determined for the two extracts by (Karber, 1931) method and a post - mortem examination was done on the dead animals.

Antipyretic activity – An increase in body temperature was induced in rats by intramuscular injection of a 15% yeast suspension in saline (10 ml/kg) according to (Roszkowski, 1971). The mean rectal temperature was measured at different intervals after i. p. administration of the plant extracts or acetylsalicylic acid (50 mg/kg).

Antiinflammatory effects – Acute antiinflammatory effect was studied according to (Winter, 1962) and the chronic effect by (Meier, 1950). Six groups of rats (6 rats/group) were injected i. p. with the tested extracts (1/10 and 1/5 LD_{50} doses). The mean percent paw oedema in rats and the mean gain in pellet's weight were calculated and compared with that of the non-treated and a group given a standard drug (indomethacine: 5 mg/kg b. wt., i. p.).

Ulcerogenic effect – Eight groups of male rats (6 rats/group) were fasted overnight and orally given the tested doses of the plant extracts. Five hrs later, they were killed. Their stomachs were removed, opened along the greater curvature and the number of ulcers were assessed according to (Corell, 1979). A group of animals were treated with acetylsalicylic acid (50 mg/kg) orally as a positive control.

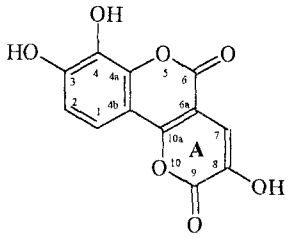
Statistical analysis – Data were analyzed using the Student's "t" test (Snedecor, 1967).

Results and Discussion

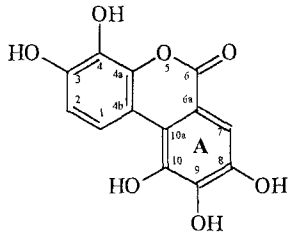
Compound **I** was obtained as a dark reddish amorphous powder. It gave a dull green $FeCl_3$ reaction and remained unchanged after normal acid (2 N HCl, 3 hrs) or alkaline (5% NaOH, $1^{1/2}$ hr) hydrolysis, but yielded pyrogallol on alkali-fusion (fused together with two pellets of KOH at 200-210 °C, 2-3 minutes, the product was identified by Co-PC, UV absorption and ^1H NMR). Compound I exhibited a UV absorption spectrum

with two maxima at 282 and 400 nm in methanol and showed an IR spectrum with strong absorption bands at 3400, 1695, 1630 and 1610 cm^{-1}, consistent with the presence of phenolic OH, α-pyrone carbonyl and benzenoid C=C groups, respectively. The ESI-MS (-ve mode) spectrum of **I** revealed an $[M-H]^-$ ion peak at m/z 261 and showed also a sequential loss of 28 mu fragments, a pattern of fragmentation which is typical for hydroxylated coumarins (Pastor, 1987). This and the above given data suggested compound **I** to be a 3,4-dihydroxy coumarin derivative (dull green and not intense blue $FeCl_3$ reaction, production of pyrogallol on alkali-fusion and its IR spectral data) which might be originated biosynthetically from the known pentahydroxydibenzopyran-6-one precursor (**II**), (molecular weight 276 mu). A comparative study of the ^1H NMR spectra of both compounds **I** and **II** lent a support to this view. The spectra showed closely similar pattern of three aromatic proton resonances two of which appeared as two doublets at δ 6.9 and 8.32 ppm in the spectrum of **I** and at δ 6.8 and 8.44 ppm in the spectrum of **II** assignable to two ortho aromatic protons (H-1 and H-2, in the case of compound **II**). The third proton resonance revealed its presence at δ ppm 7.23 of **I** and comparatively downfield at δ ppm 7.46 of **II**. This recognizable chemical shift difference is best interpreted in terms of the changes in the chemical environments surrounding this proton in the new compound **I** and support the previous assumption that **I** is most probably a biosynthetic transformation product originated from compound **II** through a loss of one of its ring A carbons (see formulae). The lost carbon is not certainly the protonated one. The ambiguity about the final structure of **I** was then unravelled through ^{13}C NMR spectral analysis. The received spectrum revealed the presence of 12 individual ^{13}C resonances, among which the two most downfield resonances located at δ 160.15 and 160.0 ppm were assigned to two distinct α-pyrone carbonyl carbons (α-pyrone carbonyl carbon of **II** was found resonating at δ 161.6 ppm and that of compound **III** at δ 160.7 ppm, Table 1). Comparison of the carbon resonances in the spectrum of both compounds **I** and **II** confirmed the presence of the 3, 4-dihydroxycoumarin moiety in both molecules. It also confirmed drastic changes in the chemical shift values of the resonances of ring A carbons and proved the transformation of this ring to an α-pyrone ring to produce the new compound **I** which is therefore identified to be 3,4,8-trihydroxybenzopyranopyran -6, 9-dione (Nawwar, 1994a).

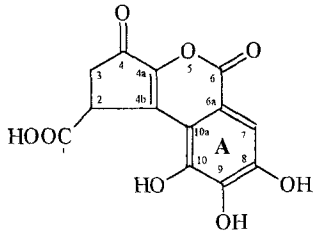
Biological Investigation – Aqueous ethanolic extracts of *Tamarix aphylla* and *Phragmites australis* given in rats by intraperitoneal route induced a dose dependent mortality with LD_{50} of 0.2 and 4.5 g/kg, respectively. Post mortum examination revealed



Compound I



Compound II



Compound III

Table 3. Antipyretic effect of the 75% EtOH extracts of *Tamarix aphylla* and *Phragmites australis*

Groups	0 time	30 min	1 hr	2 hrs	3 hrs
Control	38.32±0.07	38.23±0.12	38.27±0.06	38.25±0.09	38.07±0.08
T. aphylla (10 mg/kg)	38.00±0.19	38.32±0.22	38.70±0.24	39.08± 0.8**	38.95±0.16**
T. aphylla (20 mg/kg)	38.70±0.17	37.00±0.20**	36.83±0.16**	36.75±0.01**	36.90±0.14**
P. australis (450 mg/kg)	38.50±0.17	38.67±0.22	38.77±0.12**	38.72±0.09**	38.75±0.17**
P. australis (900 mg/kg)	38.63±0.24	38.77±0.08**	38.73±0.08**	38.77±0.12**	38.72±0.11**
Acetylsalicylic acid (50 mg/kg)	38.70±0.16	37.22±0.08**	36.97±0.07**	36.65±0.10**	36.78±0.10**

n=6; ** P<0.01 Student's "t" test.

Table 2. Antiinflammatory activity of the 75% EtOH extracts of *Tamarix aphylla* and *Phragmites australis*

Groups	Acute: Expressed as ml of oedema and % in extracts vs.	Chronic: granuloma weight mean ±SE (gm)
Control	43.78±2.490	0.700 ±0.0020
T. aphylla (10 mg/kg)	21.46±1.662**	0.0166±0.0020**
T. aphylla (20 mg/kg)	12.24±0.990**	0.0330±0.0020**
P. australis (450 mg/kg)	13.66±1.220**	0.0270±0.0022**
P. australis (900 mg/kg)	36.88±0.969**	0.0282±0.0031**
Indomethacine (5 mg/kg)	19.60±0.81**	0.0310±0.0020**

n=6;** P<0.01 Student's "t" test.

general abdominal congestion, dilatation of subcutaneous blood vessels engorged with blood and flabby heart muscles. The aqueous ethanolic extracts of *T. aphylla* and *P. australis* produced a significant dose dependent antiinflammatory effect at dose equivalent to 1/5 and 1/10 of their LD_{50} on cotton pellet and rat paw oedema techniques (Table 2). This antiinflammatory activity can be attributed to the contained phenolics in both extracts which are known to inhibit the increase in capillary permeability at the early phases of inflammation and they might inhibit some stages of the inflammatory reaction including granulation tissue formation and chronic arthritis (Rang, 1991). Both tested extracts were devoid of any ulcerogenic effect, while acetylsalicylic acid produced gastrointestinal erosions (12±1.54 ulcers of 50 mg/kg per Os). Concerning the antipyretic effect of *T. aphylla* extract, the highest doses used decreased signifi- cantly the mean rectal temperature of the hyperthermic rats. The effect of *T. aphylla* (20 mg/kg) was nearly of the same potency as that of 50 mg/kg acetylsalicylic acid. On the contrary, *P. australis* extract at such a low dose failed to produce any anti - pyretic effect (Table 3).

In conclusion, *Tamarix aphylla* and *Phragmites australis* were found to exert an antiinflammatory activity. Moreover, *Tamarix aphylla* has an antipyretic effect at 20 mg/kg.

Acknowledgment

This work was supported by a Deutsche Forschungsgemeinschaft project (Wi 383/6-1).

References

Corell, T., Jensen, K. M. and Splwinski, J. A new antiinflammatory derivative of imidazol which is less ulcerogenic than indomethacine in rats. *Acta Pharmacol. Toxicol.* **45**, 232 (1979).

Ishak, M. S., El-Sissi, H. I., El-Scherbieny, A. E. and Nawwar, M. A. M. Tannins and polyphenolics of the galls of *Tamarix aphylla*. Part I & II. *Planta Med.* **21** (4), 246-253 & 374-381 (1972).

Karber, G. Pharmacologische Methoden Zur Auffindung Von Arzneimittainund Gifter Wirkungsweise Vor. Dr. Med. Leopold Ther. Wissenschaftliche Varlage Gesse. Gesellschaft. M. B. H. Arch. *Exprtl. Pharmacol.* **162**, 480 (1931).

Meier, R., Schular, W. and Dasauiies, P. Zur frage des mechanismus der hemmung der bin bindegeubstums durch carisone. Experientia, **6**, 469 (1950).

Nawwar, M. A. M., El-Sissi, H. I. and Barakat, H. H. The flavonoids of Phragmites australis flowers. *Phytochemistry*, **19**, 1854 (1980).

Nawwar, M. A. M., Hussein, S. A. M., Buddrus, J. and Linscheid, M. Tamarixellagic acid, an ellagitannin from the galls of Tamarix aphylla. *Phytochemistry*, **35**(5), 1349 (1994a).

Nawwar, M. A. M. and Hussein, S. A. M. Gall polyphenolices of Tamarix aphylla. *Phytochemistry* **36** (4), 1035 (1994b).

Nawwar, M. A. M., Hussein, S. A. M. and Merfort, I. NMR spectral analysis of polyphenols from Punica granatum. *Phytochemistry* **36** (3), 793 (1994c).

Pastor, R. E., Fabron, J. and Combon, A. Étude en resonance magnétique nucléaire du ^{13}C des F-mé thyl-4-coumarines. *Can. J. Chem.* **65**, 1356 (1987).

Rang, H. P. and Dale, M. M. (eds). Pharmacology, 2nd (ed.), Churchill Living-stone, Longman Group, UK Ltd (1991).

Roszkowski, A., Rooks, W. H., Tonolonis, A. J. and Miller, L. M. Antiinflammatory and analgesic properties of Naproxien. *J. Pharmacol. Exp. Thera.* **179**, 1 (1971).

Snedecor, G. W. and Cochram, W. G. Statistical Methods, 6th ed., Thelowa State Univ. Press, Ames, Lowa, USA (1967).

Swain, T., Harborne, J. B. and Van-Sumer, C. F. (eds). Biochemistry of Plant Phenolics, Plenum Press, London (1977).

Winter, G. A., Rislfy, E. A. and Nuss, G. W. Carrageenin induced oedema in hind paw of rats as an assay for antiinflammatory drugs. *Proc. Soc. Exp. Biol. Med.* **111**, 121 (1962).

(Accepted September 8, 1996)