Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 63 (2003) 433–436
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Unique phenolic carboxylic acids from *Sanguisorba minor*

Nahla A. Ayoub*

Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt

Received in revised form 10 February 2003

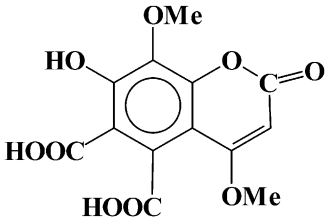
Abstract

The unique phenolic carboxylic acids, 4,8-dimethoxy-7-hydroxy-2-oxo-2H-1-benzopyran-5,6-dicarboxylic acid and 2-(4-carboxy-3-methoxystyryl)-2-methoxysuccinic acid were isolated and identified from the whole *Sanguisorba minor* plant. The known phenolics, gallic acid; ellagic acid; quercetin-3-*O*-(6″-galloylglucose); β-glucogallin; 2,3-hexahydroxydiphenoyl-(α/β)-glucose; 1-galloyl-2,3-hexahydroxydiphenoyl-α-glucose together with its β-isomer were also characterized. Structures were established by conventional methods of analysis and confirmed by NMR and ESI-MS spectral analysis.

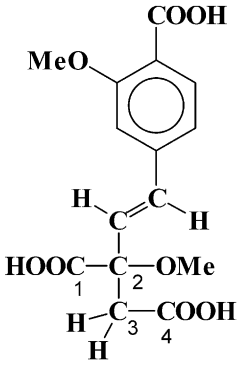
Keywords: *Sanguisorba minor*; Rosaceae; Phenolic acids; 4,8-Dimethoxy-7-hydroxy-2-oxo-2H-1-benzopyran-5,6-dicarboxylic acid; 2-(4-Carboxy-3-methoxystyryl)-2-methoxysuccinic acid

1. Introduction

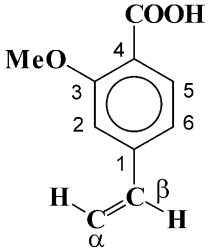
Sanguisorba minor Scop, is a perennial herb with pinnate leaves and reddish-green flowers (Täckholm, 1974). It grows wild in Sinai peninsula, Egypt and provides extracts which are used, in folk medicine for their hypoglycaemic activity (Boulos, 1983). On a continuing study on the phenolic constituents of rosaceous Egyptian medicinal plants (El Mousallami et al., 2000; El Mousallami, 1998; Souleman and El Mousallamy, 2000), I describe herein the isolation and structure elucidation of eleven phenolics from the aqueous ethanolic whole plant extract of *S. minor* which have not been subjected before to any phytochemical investigation. In the present study two unique natural products, namely, 4,8-dimethoxy-7-hydroxy-2-oxo-2H-1-benzopyran-5,6-dicarboxylic acid (**1**) and 2-(4-carboxy-3-methoxystyryl)-2-methoxysuccinic acid, or 5-(4-carboxy-3-methoxyphenyl)-3-methoxy-3-carboxy-4-pentenoic acid (**2**) were isolated and identified. The known compounds, 1-*O*-β-galloyl-glucose (**3**); 2,3-hexahydroxydiphenoyl-(α/β)-glucose (**4**); gallic acid (**5**); 1-galloyl-2,3-hexahydroxydiphenoyl-α-glucose (**6**); its β-isomer (**7**); quercetin-3-*O*-β-(6″-galloylgalactoside) (**8**); kaempferol (**9**), quercetin (**10**) and ellagic acid (**11**) were also isolated and characterized. Compound (**1**) is of special interest because it belongs, also to the coumarin class of natural products and represents therefore, the first coumarin dicarboxylic acid derivative occurring in nature. In general, coumarin carboxylic acids are of very rare natural occurrence.



Compound 1
$C_{13}H_{10}O_9$



Compound 2
$C_{15}H_{16}O_8$



3-methoxy-4-carboxy-styryl moiety (2a)

* Fax: +20-245-125-50.
E-mail address: ayoub.n@link.com.eg (N.A. Ayoub).

0031-9422/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0031-9422(03)00198-5

2. Results and discussion

The concentrated 75% aqueous ethanol extract from a homogenate of the dried whole *Sanguisorba minor* herbs was fractionated by column chromatography over a polyamide (6S Riedel-De Haen AG, Seelze-Hannover) columns and eluted with H_2O, followed by H_2O–MeOH mixtures of decreasing polarities, to yield eleven fractions (I–XI). Repeated Sephadex LH-20 column fractionation followed by preparative paper chromatography of the 10, 20, 30, 50 and 90% aqueous methanol column fractions afforded pure samples of compounds (**1**–**11**). The known compounds (**3**–**11**) showed chromatographic, UV absorption, hydrolytic, -ve ESI-MS, ^1H and ^{13}C NMR analytical data identical with those reported for 1-*O*-galloyl-β-glucopyranose **3** (Nawwar et al., 1984a,b), 2,3-di-*O*-hexahydroxydiphenoyl-(α/β)-glucopyranose **4** (Tanaka et al., 1985), gallic acid **5** (Nawwar et al., 1982), 1-*O*-galloyl-2,3-di-*O*-hexahydroxydiphenoyl-α-glucopyranose **6** (Nonaka et al., 1982), 1-*O*-galloyl-2,3-di-*O*-hexahydroxydiphenoyl-β-glucopyranose **7** (Nonaka et al., 1982), quercetin-3-*O*-β-(6″-galloylgalactopyranose) **8** (Souleman and El-Mousallami, 2000), Kaempferol **9** and quercetin **10** (Nawwar et al., 1984a,b), ellagic acid **11** (Nawwar et al., 1994).

Compound (**1**), isolated as a white amorphous powder, was found to possess chromatographic properties (fluorescent light blue spot under UV light, high R_f-values in aqueous solvents and low R_f-values in organic solvents), UV absorption data in methanol (λ_{max} : 252 (inflection), 310 and 323 nm) and result of normal aqueous acid hydrolysis (recovered unchanged after being refluxed with 2 N aqueous HCl, 100 °C, 3 h) which suggested that **1** is most probably a coumarin derivative. The compound exhibited, in −ve ESI-MS, a molecular ion $[M–H]^-$ at *m*/*z*: 309.3 corresponding to a molecular mass, M_r of 310. Direct measurement of the ESI-mass spectrum of **1**, at collision induced dissociation voltage (CID), resulted in the formation of fragment ions at *m*/*z*: 264.9 ([M–H]- CO_2), 221 ([M–H]- 2 × CO_2) and 193.1 (221- CO), a pattern of fragmentation which is consistent with a dicarboxycoumarin structure. This view is supported by IR spectral analysis which showed bands at *v*: 1685, 1700, 1720 and 3450 cm^{-1}, thus suggesting the presence of a coumarin bearing two carboxyl groups, one of which might be involved in a hydrogen bond formation (1685 cm^{-1}) (Hesse et al., 1997). The ^1H NMR spectrum (DMSO-d_6) of **1** disclosed 4 proton singlet resonances at δ 3.82, 3.99 (each integrated to three protons), 6.55 and 12.35 (each integrated to one proton), a pattern of resonances which confirms substitution at the coumarin position No. 4, thus leaving the H-3 proton uncoupled to produce the singlet resonance at δ 6.55 ppm. Obviously, the two upfield resonances at δ 3.82 and 3.99 ppm are attributable to the protons of two methoxyl groups, while the most downfield sharp singlet resonance at δ 12.35 ppm is assignable to a hydroxyl proton involved in a hydrogen bond formation. Consequently, compound **1** is a mono-hydroxy-di-methoxycoumarin-dicarboxylic acid, a structure which account for the high molecular mass (M_r 310) of the compound. The ambiguity about the site of attachment of the substituents to the coumarin nucleus of **1** have been unravelled by de-coupled and gated decoupled ^{13}C NMR spectral analysis. The spectra revealed the presence of two carboxyl carbon resonances at δ 172.8 (s) and 169.1 (s) ppm, the first of which belongs to carboxyl group bearing an *ortho*-hydroxyl substituent, thus forcing it to be involved in a hydrogen bridge formation and shifting therefore, its resonance downfield to δ 172.8 ppm. The resonances, in these spectra at δ 56.5 and 59.7 ppm (both as *q*, $J = 130$ Hz), are clearly, due to two methoxyl carbons, the second of which must bears an *ortho*-oxygenated function (OH or OMe group) from both sides (Hussein et al., 1997), thus causing its resonance to be shifted downfield in comparison with the resonance of the first methoxyl group. The spectra also, showed a lowfield carbon resonance at δ 163.3 ppm appearing as a doublet of small *J* value (1.5 Hz). This was attributed to a coumarin carbonyl carbon whose adjacent carbon (C-3) must be protonated to cause the measured small *J* value (across two bonds). The resonance of the protonated carbon (C-3) itself was recognized at δ 95.04 ppm (*d*, $J = 169.3$ Hz, one bond coupling), a chemical shift value which is similar to those reported for the corresponding resonance in the spectra of 3-unsubstituted-4-methoxy coumarin derivatives (Duddeck and Kaiser, 1982). On the other hand, the methoxylated carbon (C-4) was found resonating downfield as a doublet ($J = 1.7$ Hz) at δ 167.7 ppm. This ^{13}C NMR data when incorporated with the above given analytical data is best interpreted in terms of a 4,8-dimethoxy-7-hydroxy-2-oxo-2-H-1-benzopyran-5,6-dicarboxylic acid structure for compound **1**. The remaining six carbons (C-5 to C-10) exibited singlet resonances, except (C-10), which appeared as a doublet ($J = 9$ Hz, coupling across three bonds) resonance (see Experimental). The chemical shifts of these carbons agreed well with the achieved structure of **1**, as 4,8-dimethoxy-7-hydroxy-2-oxo-2H-1-benzopyran-5,6-dicarboxylic acid, which represents, to the best of my knowledge a new natural product.

The isolated new compound **2**, a minor constituent of a white amorphous nature was found to possess chromatographic properties [blue spot on paper chromatograms (PC) turning fluorescent blue when fumed with ammonia vapour under UV light], UV spectral data in methanol [λ_{max} (nm): 242 (inflection), 293, 324] and colour reaction [negative $FeCl_3$ test and reddish colour with anyline/xylose spray reagent, specific for carboxylic acids (Lindstadt, 1950)] similar to those reported for 3,4-disubstituted styryl derivatives, e.g. ferulic acid

(Nawwar et al., 1982). The compound resisted acid hydrolysis, but produced salicylic and oxalic acids, among other products, when fused with KOH at 200–205 °C [comparative paper chromatography (co-PC)].

The IR spectrum of **2** showed strong absorbtion at ν: 1710, 1700, cm^{-1}, consistent with free two carboxylic carbonyl groups. A study of the −ve ESI-M and CID spectra of compound **2** proved that it possesses a M_r of 324 {$[M–H]^-$: m/z: 322.93}, which produced fragment ions at m/z: 290.93, 278.93, 247.07 and 163.47, the former three of which were found in consistent with ([M–H]–[OCH_3 + H]), ([M–H]–CO_2) and (278.93–[OCH_3 + H] or 290.93–CO_2), respectively. The ^{1}H NMR spectral analysis of **2** led finally to the determination of its structure. The received spectra (DMSO-d_6) revealed, in the aromatic region a pattern of resonances attributable to a 3,4-disubstituted styryl moiety (**2a**), [δ 7.00 (*d*, J = 2.5 Hz, H-2); 6.90 (*d*,*d*, J = 7.5 and 2.5 Hz, H-6); 6.75 (*d*, J = 7.5 Hz, H- 5); 7.47 (*d*, J = 16 Hz, β-H); 6.20 (*d*, J = 16 Hz, α-H)], which bears a methoxyl [δ 3.9 ppm (s)] and a carboxyl [δ 8.55 (broad *s*)] substituents. The presence of **2a** in the molecule of compound **2** can also, be evidenced by the presence, in the CID-MS spectrum of the fragment ion at m/z 163.47, which corresponds to the M_r of **2a**, after the loss of a CH_3 group during the fragmentation process. This data when incorporated with the results of alkali-fusion (production of salicylic acid) showed that **2a** would possess a 4-carboxy-3-methoxystyryl structure. The 3-carboxy-4-methoxy positional isomer of this moiety, would give another chemical shifts sequence for the aromatic proton resonances, in which the resonance of the *meta*-coupled styryl H-2 proton will not be the most downfield in this sequence (Nawwar et al., 1984a,b). The ^{1}H NMR spectrum of **2** revealed in addition, the presence of a second methoxyl group [δ 3.80 ppm (*s*) methoxy protons, in 2-methoxysuccinic] together with a methylene group [δ 3.05 ppm (broad *s*) methylene protons, 2H-3, of the 2-methoxysuccinic]. This and the above given data, when incorporated with the facts that oxalic acid is produced by the alkali-fusion of **2**, and that the M_r of **2** is 324, while that of **2a** is 177, confirmed that the molecule of **2** is built up from a 2-methoxy succinyl moiety substituted at its 2 position by the styryl moiety **2a** to result in the 2-(4-carboxy-3-methoxystyryl)-2-methoxy-succinic acid structure of compound **2**, which has not been reported previously, as a natural product.

3. Experimental

NMR:. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AMX 400 spectrometer. ^{1}H chemical shifts were measured relative to TMS and ^{13}C, were measured at 100 MHz, relative to DMSO-d_6 and converted to TMS scale by adding 39.5. Typical conditions: spectral width = 6000 Hz for ^{1}H and 22,000 Hz for ^{13}C, 32 K data points and a flip angle of 45°, ^{1}H–^{13}C coupled NMR spectra were obtained by the gated decoupling technique. ESI-MS: micromass quattro-LC triple quadrupole mass spectrometer equipped with a "Z-spray" electrospray ion source. UV: UV/V Shimazu spectrometer. PC (descending): Whatman No. 1 paper, using solvent systems: (1) H_2O; (2) 15% HOAc; (3) BAW (*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). Solvents 3 and 4 were used for sugar analysis and solvent 3 for preparative paper chromatographic (PPC) isolation, on Whatman No. 3MM paper.

3.1. Plant material

Fresh herbs of *Sanguisorba minor* growing wild near El-Ariesh, north of Sinai proper, Egypt were collected in November, 2000 and authenticated by Dr. M. El Gibali, National Research Centre (NRC), Dokki, Cairo, Egypt. A voucher specimen has been deposited at the herbarium of Faculty of Pharmacy, Ain-shams University, Cairo, Egypt.

3.2. Isolation and identification

The collected plant specimen was exhaustively, extracted with 75% aqueous ethanol. The concd extract was applied to a polyamide (6S Riedel-De Haen AG, Seelze-Hannover) columns and eluted with H_2O, followed by H_2O–MeOH mixtures of decreasing polarities, to yield eleven fractions (I–XI). Compounds (**1** and **2**) were isolated from fraction II (eluted by 10% MeOH) by preparative paper chromatography (PPC), using BAW. Compounds (**3** and **4**) from fraction III (eluted by 20% MeOH) by repeated Sephadex LH-20 column fractionation using *n*-BuOH saturated with H_2O for elution. Compound (**5**) from fraction IV (eluted by 30% MeOH) by Sepadex LH-20 (Pharmacia) column using, H_2O–MeOH mixture (7:3) as an eluent, compounds (**6**, **7** and **8**) from fraction V (eluted by 40%) by sephadex LH-20 column fractionation, using H_2O–MeOH mixture (1:1) for elution. Compounds (**9**, **10** and **11**) were separated from fraction X (eluted by 90%) by PPC, using BAW.

3.3. 4,8-Dimethoxy-7-hydroxy-2-oxo-2H-1-benzopyran-5,6-dicarboxylic acid (*1*)

M_r 310, ESI-MS: negative ion: m/z: $[M–H]^-$: 309.3; CID fragments: m/z: 264.9, 221, 193.1. R_f- values: 0.66 (H_2O), 0.70 (HOAc), 0.30 (BAW). UV λ_{max}^{MeOH} (nm): 252 (inflection), 310, 320. Normal acid hydrolysis: recovered unchanged (Co-PC). IR ν (cm^{-1}): 3450, 3100, 2910, 1720, 1700, 1685. ^{1}H NMR (ppm): 3.82 (*s*, OMe-4); 3.99 (*s*, OMe-8), 6.55 (*s*, H-3), 12.35 (s, OH-7). ^{13}C NMR: δ

(ppm) 163.3 (*d*, C-2), 95.04 (*d*, C-3), 167.7 (*d*, C-4), 123.11 (*s*, C-5), 114.08 (*s*, C-6), 147.61 (*s*, C-7), 137.04 (*s*, C-8), 143.38 (*s*, C-9), 113.90, (*d*, C- 10), 56.5 (*q*, OMe - 4), 59.7 (*q*, OMe - 8), 169.1 (*s*, C5–COOH), 172.8 (*s*, C6–COOH).

3.4. 2-(4-Carboxy-3-methoxystyryl)-2-methoxysuccinic acid (2)

M_r 324, ESI-MS: negative ion: *m*/*z*: $[M–H]^-$: 322.93; CID fragments: *m*/*z*: 290.93, 278.93, 247.07. R_f- values: 0.69 (H_2O), 0.72 (HOAc), 0.32 (BAW). UV λ_{max}^{MeOH} (nm): 242 (inflection), 293, 324. Normal acid hydrolysis: recovered unchanged (co-PC). Fusion with KOH: 5 mg of **2** were fused at 200–205 °C for 3 min. The cold fusion product. dissolved in 10 ml dist. water was acidified (pH 2) by aqueous 2 N HCl then extracted with ethyl acetate: Salicylic and oxalic acids (co-PC). IR ν (cm^{-1}): 3450, 2910, 1710, 1700, 1695, 1400, 1220. ^1H NMR (ppm): 3.05 (broad *s*, 2H-3), 3.80 (*s*, OMe-in the methoxysuccinic moiety); 3.90 (*s*, OMe-in styryl moiety), 6.20 (*d*, $J = 16$ Hz, H-α), 6.75 (*d*, $J = 7.5$ Hz H-5), 6.90 (*d*,*d*, $J = 2.5$ and 5 HZ, H-6), 7.00 (*d*, $J = 2{,}5$ Hz, H-2), 7.47 (*d*, $J = 16$ Hz, H-β).

References

Boulos, L., 1983. Medicinal Plants of North Africa. Reference Publicationa, Inc., Michigan, USA. (p. 134).

Duddeck, H., Kaiser, M., 1982. ^{13}C NMR spectroscopy of coumarin derivatives. Org. Magnetic Resonance 20, 55.

El Mousallami, A.M.D., 1998. Chemical investigation of the constitutive flavonoid glycosides of the leaves of *Crataegus sinica*. J. Nat. Prod. 3, 53.

El Mousallami, A.M.D., Hussein, S.A.M., Merfort, I., Nawwar, M.A.M., 2000. Unusual phenolic glycosides from *Cotoneaster orbicularis*. Phytochemistry 53, 699.

Hesse, M., Meier, H., Zeeh, B., 1997. Spectroscopic Methods in Organic Chemistry. Georg Thieme Verlag Stuttgart, New York. (p.29).

Hussein, S.A.M., Barakat, H.H., Nawwar, M.A.M., Willuhn, G., 1997. Flavonoids from *Ephedra aphylla*. Phytochemistry 45, 1529.

Linstadt, G., 1950. Acta. Chem. Scand. 4, 448.

Nawwar, M.A.M., Buddrus, J., Bauer, H., 1982. Dimeric phenolic constituents from the roots of *Tamarix nilotica*. Phytochemistry 21, 1755.

Nawwar, M.A.M., Souleman, A.M.A., Buddrus, J., Linscheid, M., 1984a. Polyphenolic constituents of the flowers of *Tamarix nilotica*, the structure of nilocitin, a new digalloyl glucose. Tetrahedron Letters 25, 49.

Nawwar, M.A.M., Ishak, M., Michael, H.N., Buddrus, J., 1984b. Leaf flavonoids of *Ziziphus Spina-christi*. Phytochemistry 23, 2110.

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

Nonaka, G., Tanaka, T., Nishioka, I., 1982. Tannins and related compounds, part 3. A sanguisorbic acid dilactone, and three new ellagitannins, H-1, H-2 & H-3, from *Sanguisorba officinalis*. J. C. S. Perkin I 1067.

Souleman, A.M.A., El Mousallamy, A.M.D., 2000. Chemical investigation of the constitutive phenolics of *Rosa arabica*. J. Nat. Prod. 6, 82.

Täckholm, V., 1974. Student Flora of Egypt. Cairo. University Press, Egypt. p. 217.

Tanaka, T., Nonaka, G., Nishioka, I., 1985. Tannins and related commpounds, revision of the structure of sanguiins H-2 and H-3, and isolation and characterization of sanguiin H-11 a new tetraneric hydrolysable tannin and sanguiin related tannins, from *Sanguisorba officinalis*. J. Chem.Res. Mini print 6, 2001.