New Quinic Acid Derivatives from Hepatoprotective *Inula crithmoides* Root Extract

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Fractionation directed by hepatoprotective activity of *Inula crithmoides* L. root resulted in the isolation of two new quinic acid derivatives, 3,5-di-*O*-caffeoylquinic acid 1-methyl ether (**I**; caffeoyl = (*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl; quinic acid = 1,3,4,5-tetrahydroxycyclohexanecarboxylic acid) and 4,5-di-*O*-caffeoylquinic acid 1-methyl ether (**II**), in addition to the well-known hepatoprotective compound, 1,5-di-*O*-caffeoylquinic acid (**III**). The hepatoprotective effect was indicated by the significant decrease in the level of four measured serum biochemical parameters (SGOT, SGPT, ALP, and bilirubin) in experimental rats. The structures of the isolated compounds were determined by analyses of 1D- and 2D-NMR spectroscopic data.

Introduction. – Asteraceae is the largest family of the plant kingdom comprising approximately one-tenth of the flowering plants [1], and it is especially known for hepatoprototective constituents as those in *Cynara cardunculus* L. [2] and in *Silybum marianum* [3], which have been used in folk medicine for the treatment of chronic liver diseases [3][4]. *Inuleae*, a cosmopolitan tribe of the Asteraceae, is abundant in South Africa, Australia, and in the Mediterranean region and includes *ca.* 200 genera of which the genus *Inula* contains a selection of plants that have been known for their useful properties as herbal remedies [5]. A large number of *Inula* species were found to have many important therapeutic effects including antimicrobial [6], anti-inflammatory [7], antioxidant [8], and antihepatotoxic activities [9], which are mainly due to pharmacologically and biologically active compounds such as flavonoids, polyphenols, and quinic acid derivatives, abundantly present in these plants. These findings, in addition to the fact that there is a high incidence of hepatic problems in different areas of the world, prompted us to explore the hepatoprotective activity of *I. crithmoides*, a common plant belonging to genus *Inula* and growing wildly in Egypt. This work also involves a phytochemical study of the biologically active fractions aiming at isolation of the candidates responsible for the hepatoprotective activity.

Results and Discussion. – *Chemistry.* The phenolic nature of compounds **I**–**III** was evident from the olive green color observed upon spraying with $FeCl_3$ reagent, while

the effervescence occuring upon addition of Na_2CO_3 to compounds **I**–**III** referred to the presence of a free carboxylic group(s). The IR spectrum showed characteristic bands of CO, OH, and ether groups. UV Spectra of **I**–**III**, in MeOH, displayed absorption maxima characteristic for an unsaturated CO chromophore conjugated with an aromatic residue [10]. The bathochromic shift observed, after addition of $AlCl_3$, and its complete recovery by HCl indicated the presence of an *ortho*-dihydroxy substitution and provided a preliminary evidence for caffeoyl (=(*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl) moieties in **I**–**III** [11], as parts of their chemical structures. Positive-ion-mode FAB-MS of **I** showed the pseudomolecular-ion peak at *m*/*z* 531 ($[M+H]^+$), which suggested the molecular formula $C_{26}H_{26}O_{12}$. EI-MS displayed two prominent fragment-ion peaks at *m*/*z* 180 and 163, suggesting the presence of a free caffeic acid and a caffeoyl moiety, respectively. The ^{1}H-NMR spectrum (*Table 1*) of **I** displayed the signals of two sets of H-atoms in the form of two pairs of *doublet*s ($J = 16$), at δ(H) 6.20, 6.32, and 7.52, 7.61, due to two moieties each of which contains two (*E*)-olefinic H-atoms prior to a CO group [12], and two, almost identical, trisubstituted aromatic moieties, as revealed by the presence of a pair of *ABX* aromatic H-atom signals at δ(H) 7.08, 6.80 and 6.97, each integrating for two H-atoms, due to H–C(2′,2″)[1]), H–C(5′,5″), and H–C(6′,6″), respectively. The presence of two caffeoyl moieties as parts of the chemical structure of **I** was further supported by the ^{13}C-NMR spectrum (*Table 2*) which displayed 23 signals due to 25 C-atoms; of which 18 were of two caffeoyl moieties and seven of a quinic acid moiety. Quinic acid signals appeared as follows: two signals were observed at δ(C) 36.8 and 35.7 due to CH_2(2) and CH_2(6) groups respectively; three signals at δ(C) 69.8, 72.0, and 72.3 due to three O-bearing CH groups, CH(4), CH(3), and CH(5), respectively; one signal at δ(C) 74.7 due to an O-bearing quaternary C-atom, C(1); and one signal at δ(C) 175.7 due to C(7)OOH. Moreover, ^{1}H-NMR spectra showed also a sharp *singlet* at δ(H) 3.71 integrating for 3 H-atoms, along with the corresponding C-atom signal observed at δ(C) 53.0, indicating the presence of a MeO group, attached to an aliphatic residue [13]. The location of the two caffeoyl substituents on the quinic acid moiety were deduced from the comparative analysis of ^{1}H-NMR chemical shifts with those of the free quinic acid [11] and chlorogenic acid [14]. The observed chemical shift of H–C(5) (δ(H) 5.30–5.34) is very close to that of H–C(5) in chlorogenic acid (δ(H) 5.37), indicating the presence of one caffeoyl moiety at C(5), while the appearance of H–C(3) signal at δ(H) 5.36–5.48, shifted downfield by *ca.* 1.22 ppm, with respect to that of chlorogenic acid (δ(H) 4.20) [14], suggested that the second caffeoyl group is located at C(3). It was also concluded that the MeO group was located at C(1), based on the measurable upfield shift of C(1) in free quinic acid (δ(C) 74.7) with respect to the esterified C(1) (*ca.* δ(C) 81.0) [11]. Further evidence for the presence of the MeO group at C(1) was the magnetic equivalence of the two H-atoms at C(2) (δ(H) 2.10–2.28 (*m*)) [10]. The presence of the MeO group at C(1) was finally confirmed by the HMBC experiment (*Table 3*) which illustrated a strong long-range correlation between the MeO H-atoms signal resonating at δ(H) 3.71 with the carboxylic C-atom signal at δ(C) 175.7. ^{1}H-NMR and COSY spectra were very helpful in assigning chemical shifts to all the H-atoms of the

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[1]) C-Atom numbering as indicated in the Formulae. For systematic names, see the *Exper. Part.*

quinic acid moiety. Accordingly, **I** could be identified as 3,5-*O*-dicaffeoylquinic acid 1-methyl ether.

All of the observed spectral data indicated that **II** is a 1-methoxydicaffeoylquinic acid derivative with the same molecular formula $C_{26}H_{26}O_{12}$ as **I**. In the ^{1}H-NMR spectrum (*Table 1*), the downfield shift of H–C(4) (δ(H) 5.06–5.18) in comparison to that of chlorogenic acid (δ(H) 3.76), and the appearance of H–C(5) and H–C(3) signals at δ(H) 5.48–5.62 and 4.30–4.40, respectively, suggested a quinic acid derivative esterified at C(4) and C(5) with two caffeoyl moieties [14]. Further evidence to support this suggestion was the observed downfield shift of C(4) and C(5) signals appearing at δ(C) 75.0 and 69.1, respectively [11]. Thus, **II** could be identified as 4,5-*O*-dicaffeoylquinic acid 1-methyl ether. It is important to mention that both **I** and **II** seem to be new natural dicaffeoylquinic acid derivatives isolated for the first time from a natural source with a potential hepatoprotective activity.

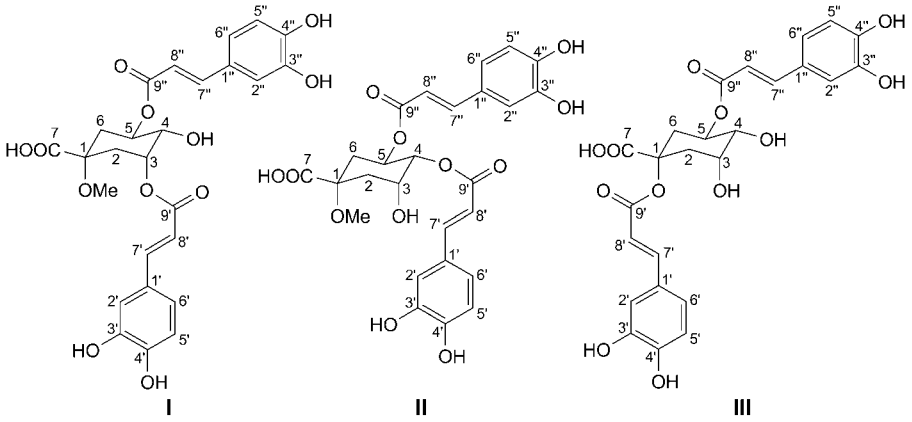

Table 1. *^{1}H-NMR Data of* **I**–**III**. Atom numbering as indicated in the *Formulae*.

	I	**II**	**III**
CH_2(2)	2.10–2.18 (*m*)	1.94–2.22 (*m*)	2.53 (*dd*, *J* = 4, 14), 2.42 (*dd*, *J* = 3, 14)
H–C(3)	5.36–5.48 (*m*)	4.30–4.40 (*m*)	4.25–4.35 (*m*)
H–C(4)	3.90–4.10 (*m*)	5.06–5.18 (*m*)	3.80 (*dd*, *J* = 3, 8.5)
H–C(5)	5.30–5.34 (*m*)	5.48–5.62 (*m*)	5.39–5.45 (*m*)
CH_2(6)	2.30–2.36 (*m*)	2.23–2.31 (*m*), 2.31–2.35 (*m*)	2.04–2.09 (*m*), 2.61 (*dd*, *J* = 3, 12)
H–C(2′,2″)	7.08 (*d*, *J* = 2)	7.02, 7.05 (2*d*, *J* = 2)	7.08 (br. *s*)
H–C(5′,5″)	6.80 (*d*, *J* = 8)	6.76, 6.78 (2*d*, *J* = 8)	6.80 (*d*, *J* = 8)
H–C(6′,6″)	6.97 (*dd*, *J* = 2, 8)	6.93 (*dd*, *J* = 2, 8)	6.97 (*dd*, *J* = 2, 8)
H–C(7′,7″)	7.52, 7.61 (2*d*, *J* = 16)	7.54, 7.60 (2*d*, *J* = 16)	7.60 (*d*, *J* = 16)
H–C(8′,8″)	6.20, 6.32 (2*d*, *J* = 16)	6.18, 6.29 (2*d*, *J* = 16)	6.30, 6.33 (*d*, *J* = 16)
MeO	3.71 (*s*)	3.74 (*s*)	–

Table 2. *^{13}C-NMR Data of* **I**–**III**. Atom numbering as indicated in the *Formulae.*

	I	**II**	**III**
C(1)	74.7	75.9	81.7
CH_2(2)	36.8	38.4	35.9
H–C(3)	72.0	68.7	69.8
H–C(4)	69.8	75.0	73.3
H–C(5)	72.3	69.1	71.7
CH_2(6)	35.7	38.7	37.3
C(7)	175.7	175.2	178.0
C(1′,1″)	127.7, 128.0	127.6, 127.7	127.3, 127.9
H–C(2′,2″)	115.3, 115.3	114.6, 114.8	115.3, 115.3
C(3′,3″)	146.8, 146.9	146.8, 146.8	146.8, 146.8
C(4′,4″)	149.5, 149.7	149.7, 149.7	149.6, 149.6
H–C(5′,5″)	116.6, 116.6	116.5, 116.5	116.6, 116.6
H–C(6′,6″)	123.0, 123.0	123.1, 123.1	123.0, 123.1
H–C(7′,7″)	147.1, 147.4	147.7, 147.7	147.2, 147.3
H–C(8′,8″)	115.0, 115.6	115.2, 115.2	115.3, 115.7
C(9′,9″)	168.0, 168.8	168.0, 168.5	168.2, 168.8
MeO	53.0	53.1	–

Table 3. *The HMBCs of* **I**–**III**

H-Atom	**I**	**II**	**III**
CH_2(2)	C(3), C(4), C(5), C(7)	C(7)	–
H–C(4)	C(9′)	–	C(3)
H–C(5)	–	–	C(9″)
CH_2(6)	C(3), C(5)	–	C(3), C(5)
H–C(2′,2″)	C(4′), C(6′)	C(4′), C(6′)	C(1′), C(3′), C(4′),C(5′), C(6′)
H–C(5′,5″)	C(1′), C(3′), C(4′)	C(1′), C(3′)	C(1′),C(2′), C(3′), C(4′), C(6′)
H–C(6′,6″)	C(2′),C(4′), C(8′)	C(2′), C(5′),C(7′)	C(2′), C(4′), C(7′)
H–C(7′,7″)	C(2′), C(6′), C(8′), C(9′)	C(5′), C(6′), C(9′)	C(1′), C(2′), C(6′), C(8′), C(9′)
H–C(8′,8″)	C(1′), C(9′)	C(1′)	C(1′), C(7′), C(9′)
MeO	C(7)	C(7)	–

Finally, all the observed spectral data of **III** are similar to those reported for 1,5-*O*-dicaffeoylquinic acid isolated from other plants [11], and previously isolated from the aerial parts of *Inula crithmoides* [15], but this is the first report for its isolation from the roots of the title plant.

Experimental Part

General. Column chromatography (CC): silica gel *GF-254* (SiO_2; *E. Merck*, D-Darmstadt). Prep. TLC: precoated *RPC-18 F-254* (*E. Merck*, D-Darmstadt). M.p.: *Stuart SMP* heating stage microscope, UK. UV Spectra: *Thermospectronic Helious α*, supported by vision 32 (England); λ_{max}(log ε), nm. IR Spectra: *Perkin-Elmer spectrum RXI-FT-IR* spectrophotometer in KBr pellets; $\tilde{\nu}_{max}$, cm^{-1}. ^{1}H- and ^{13}C-NMR (CD_3OD): *Bruker* spectrometer at 500 (^{1}H) and 125 MHz (^{13}C); δ in ppm, with TMS as internal standard, and *J* in Hz. EI-MS: *Finnigan-Mat SSQ 7000* at 70 eV. FAB-MS: *JEOL JMS-GC* mate in *m*/*z* (rel.%).

Plant Material. Inula crithmoides L. was collected in April 2006 during the flowering stage, from Rosette. The plant was identified by comparison with voucher sample verified by Dr. *Nabil El-Hadidy*, Professor of Plant Taxonomy, Faculty of Science, Cairo University. A voucher specimen with access No. IC202 is deposited with the Pharmacognosy Department, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt.

Extraction and Isolation. Treatment of hepatotoxicity-model rats with different fractions of the roots of *I. crithmoides* revealed that the most active fractions were the AcOEt and BuOH fractions (shown by decrease in the measured biochemical parameters) [16]. This necessitated phytochemical investigations of bioactive fractions aiming at isolation of compounds which could be used as natural hepatoprotective drugs.

Isolation of Two Compounds from the AcOEt Fraction (Bioactive Fraction). The residue (15 g) left after solvent evaporation of the AcOEt extract (25 g) was chromatographed on a SiO_2 column (600 g; 6 cm in diameter and 120 cm in length). Elution was performed with $CHCl_3$, and then polarity was increased by gradual addition of MeOH. Fractions (12% MeOH in $CHCl_3$) were subjected to prep. TLC (C_{18} silica gel plates; H_2O/MeOH 6:4). The zones were visualized by UV light, scraped off, and eluted with $CHCl_3$/MeOH 1:1. The zones yielded 10 and 12 mg of yellow amorphous powder, designated as compounds **I** and **II**, resp.

Isolation of One Compound from the BuOH fraction (Bioactive Fraction). The residue (15 g) left after solvent evaporation of the BuOH extract (60 g) was chromatographed on a SiO_2 column (600 g; 6 cm in diameter and 120 cm in length), using $CHCl_3$/MeOH mixtures as eluting solvents. Fractions (14–24% MeOH in $CHCl_3$) were subjected to CC (SiO_2 (150 g, 4 cm in diameter and 75 cm in length); MeOH/AcOEt). The fraction eluted with 2% MeOH in AcOEt was subjected to prep. TLC (fluorescent SiO_2 plates; AcOEt/MeOH/H_2O/AcOH 30:5:4:4 drops; double developments). The major zone was visualised by UV light, scraped off, and eluted with $CHCl_3$/MeOH 1:2 to give 25 mg of yellowish-white crystals identified as compound **III**.

3,5-Di-O-*caffeoylquinic Acid 1-Methyl Ether* (= *3,5-Bis[[(2*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-4-hydroxy-1-methoxycyclohexanecarboxylic Acid*; **I**). Obtained as a yellowish amorphous powder. UV (MeOH): 208, 330 (4.269). UV (MeOH + $AlCl_3$): 208, 357. UV (MeOH + $AlCl_3$ + HCl): 208, 330. IR: 3396 (O–H str.), 1690 (C=O str.), 1601 (C=C str.), 1114.5 (C–O str.), 1384.7 (C–H bending). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. FAB-MS (pos.): 531 ($[M+H]^+$). EI-MS: 180 (53, [free caffeic acid]), 163 (19, [caffeoyl moiety]).

4,5-Di-O-*caffeoylquinic Acid 1-Methyl Ether* (= *3,4-Bis[[(2*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-5-hydroxy-1-methoxycyclohexanecarboxylic Acid*; **II**). Obtained as a yellowish amorphous powder. UV (MeOH): 208, 332 (4.263). UV (MeOH + $AlCl_3$): 208, 355. UV (MeOH + $AlCl_3$ + HCl): 208, 331. IR: 3394 (O–H str.), 1690 (C=O str.), 1609 (C=C str.), 1116 (C–O str.), 1384 (C–H bending). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. FAB-MS (pos.): 531 ($[M+H]^+$). EI-MS: 180 (39, [free caffeic acid]), 163 (5, [caffeoyl moiety]).

1,5-Di-O-*caffeoylquinic Acid* (= *1,3-Bis[[(2*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-4,5-dihydroxycyclohexanecarboxylic Acid*; **III**). Obtained as yellowish-white crystals. M.p.: 178–180°. UV (MeOH): 218, 326 (4.096). UV (MeO + $AlCl_3$): 206, 359. UV (MeOH + $AlCl_3$ + HCl): 206, 328. IR: 3390 (O–H str.), 1692 (C=O str.), 1611 (C=C str.), 1116 (C–O str.), 1384 (C–H bending). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. FAB-MS (pos.): 517 ($[M+H]^+$). EI-MS: 180 (51, [free caffeic acid]), 163 (15, [caffeoyl moiety]).

REFERENCES

[1] J. B. Harborne, B. L. Turner, 'Plant Chemosystematics', Academic Press, London, 1984, p. 30.
[2] V. Lattanzio, P. A. Kroon, V. Linsalata, A. Cardinali, *J. Funct. Foods* **2009**, *1*, 131.
[3] C. Morishima, M. C. Shuhart, C. C. Wang, D. M. Paschal, M. C. Apodaca, Y. Liu, D. D. Sloan, T. N. Graf, N. H. Oberlies, D. Y.-W. Lee, K. R. Jerome, S. J. Polyak, *Gastroenterology* **2010**, *138*, 671.
[4] E. Speroni, R. Cervellati, P. Govoni, S. Guizzardi, C. Renzulli, M. C. Guerra, *J. Ethnopharmacol.* **2003**, *86*, 203.

[5] V. Heywood, J. Harborne, B. Turner, 'The Biology and Chemistry of Compositae', Volume I and II, Academic Press, London, New York, San Francisco, 1977.
[6] A. Deriu, S. Zanetti, L. A. Sechi, B. Marongiu, A. Piras, S. Porcedda, E. Tuveri, *Int. J. Antimicrob. Agents* **2008**, *31*, 588.
[7] V. Hernández, M. C. Recio, S. Máñez, R. M. Gíner, J. Rios, *Life Sci.* **2007**, *81*, 480.
[8] K. Kogure, I. Yamauchi, A. Tokumura, K. Kondou, N. Tanaka, Y. Takaishi, K. Fukuzawa, *Phytomedicine* **2004**, *11*, 645.
[9] Ş. Saygi, B. Konuklugil, O. Kutsal, I. T. Uzbay, G. Deniz, Z. Gören, *Phytother. Res.* **2003**, *17*, 683.
[10] P. Wenzl, A. L. Chaves, J. E. Mayer, I. M. Rao, M. G. Nair, *Phytochemistry* **2000**, *55*, 389.
[11] I. Merfort, *Phytochemistry* **1992**, *31*, 2111.
[12] J. Tan, P. Bednarek, J. Liu, B. Schneider, A. Svatoš, K. Hahlbrock, *Phytochemistry* **2004**, *65*, 691.
[13] B. N. Timmermann, J. J. Hoffmann, S. D. Jolad, K. H. Scharm, R. E. Klenck, R. B. Bates, *J. Nat. Prod.* **1983**, *46*, 365.
[14] A. Cheminat, R. Zawatzky, H. Becker, R. Brouillard, *Phytochemistry* **1988**, *27*, 2787.
[15] H. M. Hammoda, M.Sc. of Pharmaceutical Sciences, Faculty of Pharmacy, Alexandria University, Egypt, 1996.
[16] M. S. Abdel-Kader, S. I. Alqasoumi, *Alex. J. Pharm. Sci.* **2008**, *22*, 47.

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