Department of Pharmacognosy[1], Faculty of Pharmacy, Assiut University, Assiut, Egypt, and Tokyo University of Pharmacy and Life Science[2], Tokyo, Japan

Further quinoidal derivatives from *Rubia cordifolia* L.

H. A. HASSANEAN[1], Z. Z. IBRAHEIM[1], K. TAKEYA[2] and H. ITORAWA[2]

Two new quinoidal dimers, a new naphthohydroquinone anhydride, a known naphthoic acid ester derivative and two known benzoic acid derivatives were isolated from the chloroform fraction of a chloroform-methanol (1:1) extract of *Rubia cordifolia* L. The identification of these compounds was based on spectral analysis.

1. Introduction

The genus *Rubia* is highly reputed for its high content of quinoidal derivatives [1]. Due to their nucleophilic characters, these compounds exhibited cytotoxic and antimicrobial activities [2].

Reviewing the current literature concerning the constituents of *Rubia cordifolia* L. showed the presence of cytotoxic anthraquinones, naphthoquinones, naphthohydroquinones, their dimeric derivatives [1, 3–7] as well as antitumour bicyclic hexapeptides [8, 9]. The anticancer, antiviral, calcium channel blocking, or antibacterial activities of these constitutents have also been well documented [10–14].

In this study, the isolation of three new quinoidal compounds **2**–**4**, two known organic acids **5**, **6**, and a known naphthoic acid derivative **1** is reported.

2. Investigations, results and discussion

2.1. Identification of compounds 1, 5 and 6

The structure of **1** was analyzed by EIMS, ^{1}H- and ^{13}C NMR spectra (see Exp.). The compound has been previously reported from *R. cordifolia* [7]. However, we could not compare our results due to unavailable data.

Compounds **5** and **6** were identified as 5-hydroxy-2-methoxy benzoic acid and 4-hydroxy-3,5-dimethoxy benzoic acid (syringic acid), respectively, (Exp. section) and they are first reported in the genus *Rubia*.

2.2. Identification of compound 2

The molecular formula of **2** was deduced as $C_{19}H_{14}O_7$ (EIMS, ^{1}H NMR and DEPT ^{13}C NMR). The ^{1}H- and ^{13}C NMR spectra of **2** showed a close relationship with the data reported for mollugin and its derivatives [1, 6] revealing an AA′BB′ aromatic system and a chelated carbomethoxy function as always biogenetically encountered in all quinoidal derivatives reported from *R. cordifolia*. However, the DEPT ^{13}C NMR further showed two carbonyl groups at δ 166.3 confirmed by the IR bands at ν_{max}^{KBr} cm^{-1} 1763 and 1729 with the lower frequency band much stronger indicating the presence of a conjugated five membered cyclic anhydride moiety [15, 16]. In comparison with mollugin; this anhydride function must be located on C-11 and C-12 positions of the cyclized isoprenyl side chain due to appearance of two quaternary carbons at δ 136.1 and δ 147.7 ppm instead of the respective two CH signals of mollugin at these positions [17]. Hence, the structure of **2** was depicted as shown, this represents the first report of the presence of anhydride function in natural quinoidal compounds.

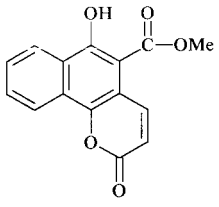
2.3. Identification of compound 3

The molecular formula of **3** was deduced as $C_{30}H_{18}O_8$ (^{1}H NMR, decoupled and DEPT ^{13}C NMR spectra, see Exp.). The ^{1}H NMR of **3** revealed a pair of AA′BB′ aromatic protons which were assigned by decoupling experiments, a downfield shifted aromatic singlet at δ 7.86 ppm, two methyl functions as sharp singlets at δ 1.51 and 1.82 ppm and one methoxyl group at δ 3.59 (3 H, s). A total of 30 carbon signals were detected in ^{13}C NMR spectra and DEPT experiments demonstrating one aliphatic methoxyl, two methyls, nine sp_2 CH and eighteen quaternary carbons of which four are quinoidal carbonyls at δ 187.4, 183.0, 180.8 and 179.7 ppm, two are anhydride carbonyls at δ 166.3, 166.4, eleven are sp_2 and one sp_3 quaternary oxygenated carbon at δ 88.5. These data are very valuable for suggesting that **3** is a dimeric quinoidal structure having a methoxy isopropyl function.

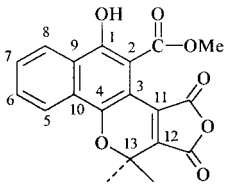
The first moiety was suggested to be a trisubstituted C ring of anthraquinone derivative at C-5, C-6, C-7 leaving the downfield shifted aromatic proton on C-8 at δ 7.86 ppm.

The symmetric signal pattern tentatively assigned for the quaternary carbons of C-6, C-7, C-9, C-10, C-11 and C-12 at δ 134.5, 135.2, 135.6, 136.5, 133.7, 133.7 respectively and the similar downfied shifts of C-9 and C-10 lead to the conclusion that the anhydride function is present on C-6 and C-7 of the anthraquinone moiety.

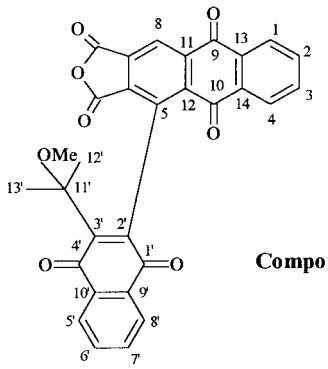
The second part showed ^{1}H NMR and ^{13}C NMR patterns very close to a 1,4 disubstituted naphthoquinone structure



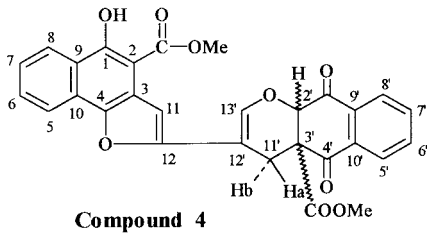
Compound 1



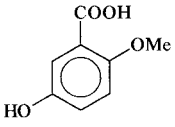
Compound 2



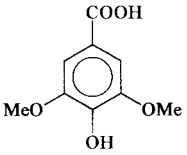
Compound 3



Compound 4



Compound 5



Compound 6

showing the absence of any quinoidal protons for H-2 or H-3 which are usually observed at about 6.87 ppm [18]. Consequently the methoxy isopropyl function is placed at either of these positions and the second left position is assigned to be connected with C-5 of the anthraquinone moiety leading to the tentative structure shown. Unfortunately FAB-MS trials had consumed the rest of this compound without giving any molecular ion peak.

2.4. Identification of compound 4

The molecular formula of **4** was established as $C_{29}H_{20}O_9$ (^{1}H NMR, decoupled and DEPT ^{13}C NMR, see Exp.). The ^{1}H NMR of **4** shows eight coupled aromatic protons of the AA′BB′ pattern confirmed by eight sp_2 CH signals in ^{13}C NMR. It also further showed signals which could be easily assigned to a chelated phenolic proton, two sp_2 proton singlets, an oxygenated sp_3 proton singlet and an isolated geminally coupled methelene function.

The broad band proton decoupled ^{13}C NMR spectrum revealed the presence of twenty nine carbons and DEPT spectra revealed two methoxyls, one CH_2, eleven CH comprising nine sp_2 CH, one oxygenated sp_2 CH and one oxygenated sp_3 CH and fifteen quaternary carbons of which four are carbonyl groups (two are quinoidal and two are ester carbonyls), two are oxygenated carbons, eight sp_2 carbons and one is an sp_3 quaternary carbon bearing a carbomethoxy at 56.8 [19].

These data are very important for considering **4** as a dimeric structure. The first moiety could be easily assigned to be a fingerprint of a C-12 substituted furanonaphthohydroquinone structure [1]. The second moiety was assigned to a naphthoquinoidal derivative, however the corresponding signals for C-2 and C-3 or their substituted derivatives were not detected in the spectra of 4; instead; one oxygenated sp_3 CH signal at δ 77.8 and a downfield shifted quaternary sp_3 carbon signals due to substitution with a carbomethoxy function was found. Hence the structure of the second moiety and consequently the structure of **6** were tentatively deduced as shown. Unfortunately as in the case of **3** the compound showed no peaks in FAB-MS even after addition of halide salts.

3. Experimental

3.1. Instruments

M.p.s were uncorrected, instruments for ^{1}H and ^{13}C NMR, MS were previously described [1]. TLC with precoated silica gel and precoated RP_{18}-sheets (aluminium foil, E. Merck). CC on a silica gel column (E. Merck, type 230–400 mesh) and MPLC on a 20 μm ODS column (Kusano Scientific Company, Tokyo, Japan). The homogeneity of the isolated compounds was checked by HPLC and TLC on precoated silica gel and RP-18 using different solvent systems.

3.2. Plant material

The roots of *Rubia cordifolia* L. used in this experiment were purchased in India. They were identified by Dr. Sang Rae Lee (Institute of Oriental Botanical Resources of Korea).

3.3. Extraction and isolation

The roots of *R. cordifolia* L. (20 kg) were extracted with $CHCl_3$/MeOH (1:1) three times. The concentrated extract was diluted with distilled H_2O and then fractionated successively and exhaustively with $CHCl_3$ and n-butanol. In a previous work [1], 20 g of the $CHCl_3$ fraction were used for isolation and further identification of the compounds cited therein [1]. However, compounds **1**–**6** herein reported could not be isolated in a sufficient yield. For enrichment, further quantities (65 g) of this fraction were grossely fractionated similarly over silica gel CC (1:25) and the target compounds were retraced. Known compounds were excluded using authentic samples.

Elution was proceeded using n-hexane/EtOAc mixtures in a manner of increasing polarity. Elution with hexane/EtOAc (98:2) afforded **1** in a mixture of quinoidal constituents from which **1** was isolated by repeated SiO_2 CC and then purified by MPLC on ODS column using MeOH/H_2O (90:10).

Further fractions eluted with n-hexane/EtOAc (90:10) gave a mixture of naphthohydroquinones reported earlier [1] from which **2** was isolated by repeated silica gel CC and finally with MPLC on ODS column using CH_3CN/H_2O (90:10).

Compound **3** was obtained from further fractions eluted with n-hexane/EtOAc (80:20) together with a mixture of other quinoidal derivatives. Final purification of **3** was achieved using Sephadex LH-20 eluted with MeOH followed by MPLC on ODS column using MeOH/H_2O (75:25).

Compound **4** was isolated from further fractions eluted with hexane/EtOAc (70:30) together with a mixture of other known naphthohydroquinone derivatives. The compound was finally purified using Sephadex LH-20 eluted with MeOH followed by MPLC on ODS column using MeOH-H_2O (70:30).

Compounds **5**, **6** were eluted with hexane/EtOAc (60:40) in a mixture and finally isolated by rechromatography on a silica gel column to give **5** followed by **6** using a benzene-chloroform system in a manner of increasing polarity.

Due to lack of 2D-NMR analysis all assignments given below are tentative and made by comparison with series of reported quinoidal derivatives.

3.4. Identification of 1

Yellowish powder (MeOH) 10 mg, EI-MS m/z (Rel. int.%): 270 (39), 237 (100), 210 (40), 180 (29), 154 (29), 126 (44), 105 (14). ^{1}H NMR ($CDCl_3$), δ ppm: 4.11 (3 H, s, OMe), 6.51 (1 H, d, J = 10.2 Hz, H-12), 7.71 (1 H, dt, J = 8.2, 1.2 Hz, H-7), 7.79 (1 H, dt, J = 8.2, 1.2 Hz, H-6), 8.47 (2 H, m, H-5, H-8), 8.76 (1 H, d, J = 10.2 Hz, H-11), 12.69 (1 H, sharp s, OH phenolic). ^{13}C NMR δ ppm: 53.1 (q, OMe), 114.0 (s, C-3), 116.1 (d, C-12), 122.6 (d, C-5), 124.6 (d, C-8), 126.1 (s, C-9), 127.8 (s, C-10), 129.2 (d, C-7), 131.2 (d, C-6), 143.3 (d, C-11), 143.3 (s, C-4), 160.2 (s, C-13), 160.2 (s, C-1), 172.2 (COO on C-2).

3.5. Identification of 2

Yellowish powder (MeOH) 11 mg, mp. 181–183 °C, IR ν_{max}^{KBr} cm^{-1} 3460, 3230, 2975, 2440, 1763, 1729, 1662, 1425, 1330, 1249, 1190, 1144, 1102, 1048, 803, 762, 690. EIMS, m/z (Rel. int.%): 354 [M$^+$] (100), 294 [M-COOMe-H$^+$] (18), 276 [M-COOMe-H$^+$-H_2O] (18), 165 (24). ^{1}H-NMR ($CDCl_3$), δ ppm: 1.81 (6 H, s, 2 × Me), 3.90 (3 H, s, OMe), 7.8 (2 H, m, H-6, H-7), 8.5 (2 H, m, H-5, H-8), 10.77 (1 H, sharp singlet; chelated phenolic OH). ^{1}H NMR pyridine-d_5, δ ppm: 1.78 (6 H, sharp singlet, 2 × Me), 3.98 (3 H, s, OMe), 7.73 (2 H, m, H-6, H-7), 8.40 (1 H, dd, J = 1.2, 7.5 Hz, H-5), 8.62 (1 H, dd, J = 1.5, 6.7 Hz, H-8). ^{13}C NMR ($CDCl_3$), δ ppm "C-1-C-13": 153.7 (s), 105.8 (s), 107.9 (s), 144.4 (s), 122.8 (d), 130.3 (d), 129.7 (d), 124.5 (d), 125.6 (s), 127.8 (s), 136.1 (s), 147.7 (s), 85.0 (s); COOMe on C-2 168.8 (s), 52.5 (q); anhydride function on C-11, C-12: 166.7 (s); two methyl functions on C-13: 24.7 (q). ^{13}C NMR pyridine-d_5, δ ppm "C-1-C-13": 155.9 (s), 101.4 (s), 107.0 (s), 143.4 (s), 122.9 (d), 130.9 (d), 129.8 (d), 125.6 (d), 125.9 (s), 126.7 (s), 136.8 (s), 147.7 (s), 86.2 (s); COOMe on C-2: 169.9 (s), 52.8 (q); anydride function on C-11, C-12: 166.3; two methyl groups on C-13: 24.5 (q), 25.9 (q).

3.6. Identification of 3

Yellowish powder 8 mg, ^{1}H-NMR ($CDCl_3$), δ ppm (assignments were made by decoupling experiments): 1.51, 1.82 (each 3 H, s, 2 × Me), 3.59 (3 H, s, OMe), 7.70 (1 H, dt, J = 7.6, 1.2 Hz, H-7′), 7.78 (1 H, dt, J = 7.6,

1.2 Hz, H-6′), 7.86 (1 H, s, H-8), 7.88 (2 H, m, H-5′ and H-2), 7.96 (1 H, dt, J = 7.8, 1.0 Hz, H-3), 8.13 (1 H, dt, J = 8.0, 1.1 Hz, H-8′), 8.35, 8.37 (2 H, m, H-1 and H-4). ^{13}C NMR anthraquinone moeity C-1-C-14: 128.2 (d), 135.0 (d), 135.7 (d), 128.4 (d), 129.4 (s), 134.5 (s), 135.2 (s), 127.4 (d), 183.0 (s), 187.0 (s), 135.6 (s), 136.5 (s), 133.7 (s), 133.7 (s); anhydride moeity on C-6, C-7: 166.3 (s), 166.4 (s); naphthoquinoidal moiety C-1′-C-13′: 179.0 (s), 138.2 (s), 142.5 (s), 180.0 (s), 127.1 (d), 134.9 (d), 134.3 (d), 127.2 (d), 131.8 (s), 132.8 (s), 86.5 (s), 26.6 (q), 26.6 (q); OMe on C-11: 54.3 (q).

3.7. Identification of 4

Yellowish powder (MeOH) 4 mg, ^{1}H NMR ($CDCl_3$), δ ppm: 2.55, 3.68 (each 1 H, d, J = 13.8 Hz, H-11′a, H-11′b), 3.18 (6 H, s, 2 × OMe groups), 5.3 (1 H, s, H-2′), 6.98 (1 H, s, H-11), 7.52 (1 H, dt, J = 7.7, 1.1 Hz, H-7′), 7.64 (1 H, dt, J = 8.3, 1.1 Hz, H-7), 7.72 (1 H, dt, J = 7.7, 1.1 Hz, H-6′), 7.74 (1 H, dt, J = 8.3, 1.0 Hz, H-6), 7.88 (1 H, brd, J = 7.7 Hz, H-5′), 8.0 (1 H, s, H-13′), 8.17 (1 H, brd, J = 8.3 Hz, H-5), 8.22 (1 H, brd, J = 7.8 Hz, H-8′), 8.43 (1 H, brd, J = 8.2 Hz, H-8), 12.29 (1 H, s, chelated phenolic group). ^{13}C NMR ($CDCl_3$), δ ppm C-1-C-12: 155.5 (s), 99.0 (s), 120.2 (s), 144.2 (s), 119.8 (d), 130.4 (d), 125.4 (d), 125.1 (d), 122.9 (s), 124.5 (s), 106.1 (d), 159.4 (s). C-1′-C-13′: 183.3 (s), 77.8 (d), 56.8 (s), 190.9 (s), 126.9 (d), 134.9 (d), 133.5 (d), 127.2 (d), 136.3 (s), 133.3 (s), 36.1 (t), 110.5 (s), 154.1 (d); COOMe on C-2: 168.2 (s), 52.7 (q); COOMe on C-3‘: 172.0 (s), 53.4 (q).

3.8. Identification of 5 and 6

The known compounds 5-hydroxy-2-methoxy benzoic acid **5** and 3,5-dimethoxy, 4-hydroxybenzoic acid (**6**, syringic acid) were identified from their ^{1}H and ^{13}C NMR spectra and by comparison of their mp, MS with literature data [20–22].

References

1 Itokawa, H.; Ibraheim, Z.Z.; Qiao, Y. F.; Takeya, K.: Chem. Pharm. Bull. **41**, 1869 (1993) and references cited therein
2 Bruneton, J.: Pharmacognosy, Phytochemistry, Medicinal Plants, p. 344, Intercept Limited, Andove, England 1995
3 Wang, S. X.; Hua, H. M.; Wu, L. J.; Li X.; Zhu, T. R.: Yao Hsueh Hsueh Pao **27**, 743 (1992)
4 Vidal-Tessier, A. M.; Delaveau, P.; Champion, B.: Ann. Pharm. Fr. **45**, 261 (1987)
5 Vidal-Tessier, A. M.; Delaveau, P.; Champion, B.: Ann. Pharm. Fr. **44**, 117 (1986)
6 Itokawa, H., Qiaw, Y. F.; Takeya, K.: Phytochemistry **28**, 3465 (1989)
7 Hua, H. M.; Wang, S. X.; Wu, L. J.; Li, X.; Zhu, T. R.: Yao Hsueh Hsueh Pao **27**, 279 (1992)
8 Takeya, K.; Yamamiya, T.; Morita, H.; Itokawa, H.: Phytochemistry **33**, 613 (1993) and references cited therein
9 Morita, H.; Yamamiya, T.; Takeya, K.; Itokawa, H.: Chem. Pharm. Bull. **40**, 1352 (1992)
10 Adwankar, M. K.; Chitnis, M. P.: Biomed. Pharmacother. **36**, 104 (1982)
11 Tripathi, Y. B.; Sharma, M.; Manickam, M.: Indian J. Bioch. Biophys. **34**, 302 (1997)
12 Ho, L. K.; Don, M. J.; Chen, H. C.; Yeh, S. F.; Chen, J. M.: J. Nat. Prod. **59**, 330 (1996)
13 Gilani, A. H.; Janbaz, K. H.; Zaman, M.; Lateef, A.; Suria, A.; Ahmed, H. R.: J. Pak. Med. Association **44**, 82 (1994)
14 Qiao, Y. F.; Wang, S. X.; Wu, L. J.; Li, X.; Zhu, T. R.: Yao Hsueh Hsueh Pao **25**, 834 (1990)
15 Kalinowski, H. O.; Berger, S.; Braun, S.: ^{13}C-NMR Spectroskopie, p. 182, George Thieme Verlag Stuttgart, New York 1984
16 Pretsch, E.; Seibl, J.; Clerc, T.; Simon, W.: Tabellen zur Strukturaufklärung Organischer Verbindungen mit Spectroskopischen Methoden. p. 1185, Springer-Verlag, Berlin Heidelberg, New York 1981
17 Harl, L.; De Buyck, L. F.; De Pooter, H. L.: Phytochemistry **30**, 1726 (1991)
18 El-Gamal, A. A.; Takeya, K.; Itokawa, H.; Halim, A. F.; Amer, M. M.; Saad, H-E. A.; Awad, S. A.: Phytochemistry **40**, 245 (1995)
19 Qiao, Y. F.; Takeya, K.; Itokawa, H.: Chem. Pharm. Bull. **38**, 2896 (1990)
20 Scott, K. N.: J. Magn. Reson. **2**, 361 (1970)
21 Scott, K. N.: J. Am. Chem. Soc. **94**, 8564 (1972)
22 Devon, T. K.; Scott, A. I.: Handbook of naturally occurring compounds, vol. 1, p. 13, Academic Press Inc. New York, San Francisco, London 1975

Received May 18, 1999
Accepted August 11, 1999

Dr. Hashem A. Hassanean
Department of Pharmacognosy
Faculty of Pharmacy
Assiut University
Assiut, 71526
Egypt