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MINOR CONSTITUENTS FROM *RUBIA CORDIFOLIA* L. ROOT

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بإعادة فحص جزء الكلوروفورم لخلاصة الميثانول : كلوروفورم (١:١) للجذور الجافة لنبات روبيا كوردفوليا ل. تم فصل مركب جديد من نوع ثنائى النافثوهيدروكينونات (١) بالإضافة إلى أربعة مركبات معروفة هى ٣ بيتا فريديلينول (٢) ، حمض الأتراريك (٣) ، حمض الفانيليك (٤) ، دى ٣ أ ميثوكسى كيروانوزيتول (٥). وقد تم التعرف على المركبات المفصولة باستخدام الطرق الفيزيائية والكيميائية والطيفية المختلفة.

*Reinvestigation of the chloroform soluble fraction of the chloroform- methanol (1:1) extract of the dried roots of Rubia cordifolia L. using different chromatographic techniques led to isolation of one new naphthohydroquinone dimer (**1**) and four known compounds identified as 3-β-friedelinol (**2**), atraric acid (**3**), vanillic acid (**4**) and D-3-O-methoxy-chiro-inositol (**5**). The identification of the isolated compounds was carried out using different physical, chemical and spectral methods of analysis.*

INTRODUCTION

Rubia cordifolia L. (Rubiaceae), is an important medicinal plant used for treatment of various diseases such as tumors[1-10], inflammations, urinary disorders, stress and as antimicrobial[10-21], hepatoprotective[7,22&23], radio-protective[24], hypoglycemic[7,25&26] and anti-psychotic[27]. It has been listed officially as a herbal medicine in Chinese Pharmacopeia for treatment of arthritis, dysmenorrhea, hematorrhea and hemostasis which are free radical related diseases[7,23,24&28-30]. It is used also as natural food colourant and as natural hair and colour dye[7].

Many classes of compounds were isolated from the roots of *R. cordifolia* L.[2,7,23&31-45], besides large numbers of cyclic hexa-peptides[1-4&23]. In the current study, a new naphthohydroquinone dimer (**1**) and four known compounds (**2**-**5**) were isolated and identified.

MATERIALS AND METHODS

Experimental

Melting points (uncorrected) were determined by electrothermal digital model 550. IR spectrum was recorded in KBr using Unicam SP 1025 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ (or C_5D_5N or CD_3OD) at 400 MHz and 100 MHz respectively, with TMS as an internal standard on Bruker AM-400 spectrometer. Mass spectra were carried out using Hitachi M-80 spectrometer. For CC, silica gel (E. Merck, Germany 70-230 mesh) and reversed phase (RP-18, ODS) were used. Precoated silica gel G_{60} F_{254} and RP-C18 F_{254} S (E-Merck) were used for TLC and 10% H_2SO_4 was used as spraying reagent followed by heating for 10 minutes. Final purification was carried out using Moderate Pressure Liquid Chromatography (MPLC) (22 mm, i.d. x 30 cm, Kusano Scientific co., Tokyo, Japan).

Plant material

The dried roots of *Rubia cordifolia* L. were purchased from India and identified by Dr. Sang Rae Lee (Institute of Oriental Botanical Resources of Korea). A voucher sample (No. 20103) was kept in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt as a reference material. The powdered roots were extracted and kept in refrigerator till used.

Extraction and isolation

The air-dried powdered root of *Rubia cordifolia* L. (20 Kg) was extracted with chloroform-methanol (1:1) till exhaustion (3x30 L). The combined extract was

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evaporated till dryness. The dried extract was suspended in distilled water and extracted with chloroform to give 1890 g of chloroformic extract.

Part of the dried chloroform fraction (60 g) was chromatographed over silica gel column (2 kg), eluted with hexane-EtOAc gradiently and finally the column was washed with EtOAc-MeOH (9:1). Fractions (300 ml, each) were collected and monitored using TLC and 10% H_2SO_4 as spraying reagent; similar fractions were pooled.

Fractions eluted with hexane-EtOAc (8:2) were subjected to silica gel CC using hexane-EtOAc (85:15), where sub-fractions 5-9 (containing similar spots) were rechromatographed over silica gel CC using CH_2Cl_2-acetone (95:5), then final purification was done using (MPLC) RP-18 CC and methanol-water (7:3) to afford compound **1** (7 mg) and compound **2** (8 mg).

Fractions eluted with hexane-EtOAc (7:3) were subjected to silica gel CC using hexane-EtOAc (75:25) followed by RP-18 CC (MPLC) using methanol-water (7:3). Upon repeated crystallization, sub-fractions 3-8 afforded compound **3** (6 mg, methanol) while sub-fractions 10-12 afforded compound **4** (12 mg, methanol).

Fractions eluted with EtOAc-MeOH (9:1) were rechromatographed over silica gel CC using $CHCl_3$-MeOH in a gradient elution technique. Fractions eluted with $CHCl_3$-MeOH (9:1) were chromatographed over silica gel CC, followed by RP-18 CC (MPLC) using CH_3CN-H_2O (25:75), where sub-fractions (16-20) were purified by re-crystallization from methanol to afford compound **5** (11 mg).

Compound **1**: yellowish-brown amorphous powder. IR (KBr): 3420, 1670, 1642, 1602 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$) (Table 1).

Compound **2**: white fine needles. m.p. 281-283°C. EI-MS *m/z* (% rel. int.): 428 $[M]^+$ (20) 413 (29), 275 (36), 248 (19), 231 (30), 220 (34), 205 (24), 177 (20), 165 (52), 125 (83), 95 (100, base peak), 69 (58) and 55 (56). ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 0.84 (3H, s, CH_3-25), 0.91 (3H, s, CH_3-29), 0.92 (3H, s, CH_3-26), 0.93 (3H, d, *J*= 8 Hz, CH_3-23), 0.97 (6H, s, CH_3-27 & 30), 0.98 (3H, s, CH_3-24), 1.15 (3H, s, CH_3-28), 3.72 (1H, br.s, H-3). ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C 16.10 (C-1, t), 36.30 (C- 2, t), 72.97 (C-3, d), 49.38 (C-4, d), 38.05 (C-5, s), 41.94 (C-6, t), 17.77 (C-7, t), 53.41 (C-8, d), 37.32 (C-9, s), 61.56 (C-10, d), 35.56 (C-11, t), 30.86 (C-12, t), 38.59 (C-13, s), 39.89 (C-14, s), 32.55 (C-15, t), 35.77 (C-16, t), 30.24 (C-17, s), 43.03 (C-18, d), 35.40 (C-19, t), 28.40 (C-20, s), 33.03 (C-21, t), 39.50 (C-22, t), 11.85 (C-23, q), 16.62 (C-24, q), 18.47 (C-25, q), 20.34 (C-26, q), 18.88 (C-27, q), 32.01 (C-28, q), 35.25 (C-29, q), 32.31 (C-30, q).

Compound **3**: pale yellow amorphous powder. EI-MS: *m/z* (% rel. int.): 196 $[M]^+$ (45), 164 (100, base peak) and 136 (60). ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 12.00 (1H, s, OH), 6.19 (1H, s, H-5), 3.90 (3H, s, OCH_3), 2.43 (3H, s, C-6 methyl), 2.08 (3H, s, C-3 methyl). ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C C-1-C-6: 105.37 (s), 163.36 (s), 108.75 (s), 158.37 (s), 110.77 (d), 140.32 (s); Me-C-3: 7.88 (q); Me-C-6: 24.31 (q); COOMe: 169.76 (s), 52.01 (q).

Compound **4:** faint brown needles, m.p. 211-213°C. CI-MS *m/z* (% rel. int.): 169 $[M+1]^+$ (100), 148 (30), 125 (26), 79 (23), 69 (41) and 51 (20). ^{1}H-NMR (400 MHz, C_5D_5N): δ 8.13 (1H, dd, *J*= 1.9, 8.2 Hz, H-6), 8.04 (1H, d, *J*= 1.9 Hz, H-2), 7.31 (1H, d, *J*= 8.2 Hz, H-5), 3.94 (3H, s, OCH_3).

Compound **5:** white crystals, m.p. 180-182°C. EI-MS *m/z* (% rel. int.): 194 $[M]^+$ (26), 163 (11) and 54 (100, base peak). ^{1}H-NMR (400 MHz, CD_3OD): δ 3.27 (1H, t, *J*= 9.2 Hz, H-3), 3.58 (1H, t, *J*= 9.2 Hz), 3.69 (1H, dd, *J*= 2.4, 10.0 Hz), 3.73 (1H, dd, *J*= 2.4, 10.0 Hz), 3.88 (2H, m). ^{13}C-NMR (100 MHz, CD_3OD): δ_C C-1-C-6: 72.34 (d), 70.62 (d), 83.51 (d), 72.91 (d), 71.13 (d), 72.06 (d); OMe: 59.39 (q).

RESULTS AND DISCUSSION

From the chloroform soluble fraction of the chloroform-methanol extract of *Rubia cordifolia*, five compounds were isolated (**1**-**5**).

Compound 1: The IR spectrum of compound **1** showed the presence aromatic CH stretching, hydroxyl and ester groups (experimental part). The molecular formula was established as $C_{34}H_{30}O_9$ from ^{1}H- and ^{13}C- including DEPT ^{13}C-NMR spectral data (Table 1). The ^{1}H-NMR spectrum showed the presence of ten protons in the aromatic region including a pair of AA`BB` type aromatic system in the region at

Table 1: ^{13}C- (100 MHz) and ^{1}H-NMR (400 MHz) data of compound **1** (in $CDCl_3$).

C	δ_C (m)	δ_H (m)	C	δ_C (m)	δ_H (m)
1	158.61 (s)	-	1`	158.78 (s)	-
2	98.47 (s)	-	2`	98.34 (s)	-
3	120.93 (s)	-	3`	120.80 (s)	-
4	142.86 (s)	-	4`	142.58 (s)	-
5	119.20 (d)	7.76 (1H, br. d, 8.1 Hz)	5`	119.14 (d)	7.84 (1H, br. d, 8.0 Hz)
6	129.53 (d)	7. 35-7. 43 (2H, m)	6`	129.50 (d)	7. 35-7. 43 (2H, m)
7	125.06 (d)		7`	125.06 (d)	
8	124.53 (d)	8.27 (1H, br. d., 8.1 Hz)	8`	124.30 (d)	8.30 (1H, br. d, 8.0 Hz)
9	122.07 (s)	-	9`	122.28 (s)	-
10	125.06 (s)	-	10`	125.06 (s)	-
11	104.12 (d)	6.16 (1H, s)	11`	104.05 (d)	6.35 (1H, s)
12	163.38 (s)	-	12`	160.89 (s)	-
13	72.87 (s)	-	13`	35.74 (s)	-
14	27.67 (q)	1.46 (3H, s)	14`	31.16 (q)	1.64 (3H, s)
15	54.66 (t)	2.79 d (1H, d, 14.5 Hz) 2.44 d (1H, d, 14.5 Hz)	15`	30.54 (q)	1.68 (3H, s)
16	171.43 (s)	-	16`	171.43 (s)	-
17	51.82 (q)	3.56 (3H, s)	17`	51.72(q)	3.59 (3H, s)
-OH		11.93 (1H, br. s)			11.87 (1H, br. s)

m = multiplicity

δ_H 7.53-8.30 (Table 1) and two singlet signals at δ_H 6.35 and 6.16, in addition to two singlet protons in the most shielded region at δ_H 11.93 and 11.87 for two chelated hydroxyl aromatic groups. Seventeen protons in the aliphatic region were observed as three singlet methyl signals at δ_H 1.46, 1.64, 1.68; two methoxy singlet signals at δ_H 3.56, 3.59 and two doublets (each equivalent to one proton) at δ_H 2.79 and 2.44. The identification of each proton was confirmed by proton decoupling experiment. The ^{13}C-NMR spectral data including DEPT mode measurement, revealed the presence of 30 carbon signals for 34 carbon atoms which include 10 methine, 1 methylene, 3 methyl, 2 methoxy and 18 quaternary atoms. The spectral data (Table 1) indicated that the two methoxy groups were observed at δ_c 51.72 and 51.82; the doublet signals at δ_H 2.79 and 2.44 in the ^{1}H-NMR (each, 1H, d, J= 14.5 Hz) with δ_C 54.66 indicated the presence of geminally coupled methylene proton. The aromatic protons with their ^{13}C-NMR data in addition to the quaternary carbon atoms at δ_C 171.43 and the two methoxy groups demonstrate the presence of two naphtho-hydroquinone moieties most probably C-12 substituted furomollugin[32]. The chemical shifts of 28 carbon atoms in the ^{13}C-NMR spectrum confirmed the presence of the two furomollugin moiety which are similar to that reported in the literature[32&33] while the remaining six carbons including two quaternary at δ_C 72.87 and 35.74; three methyls at δ_C 27.67, 30.54 and 31.16 and one methylene at δ_C 54.66 were interpreted as shown in figure 1.

The location of the methylene group must be between the two aliphatic quaternary carbons since its presence between any of the two furomollugin groups and any of aliphatic quaternary carbons will make it resonate in the upfield region in the ^{13}C-NMR spectra[46]. Many naphthohydroquinones and naphthoquinone dimers were isolated from the genus *Rubia* and their formation was done through condensation of furomollugin or its derivatives with prenylated hydroquinone derivatives by different ways of condensation[5,32,33,45&47]. The possible biosynthetic pathway of formation of this compound can be suggested by the oxidation with concomitant allylic trans-position of prenylated naphthoquinone [a] a known natural product previously isolated from family Rubiaceae which afford the reactive vinyl quinone [b] as a hypothetical biosynthetic intermediate that by acid-catalyzed or photo-chemical isomerization would yield furo-mollugin derivatives [c][5]. The condensation of two furomollugin derivatives with the removal of water molecule will give compound **1** as shown in figure 2. From all the above-mentioned data, compound **1** was identified as a new naphthohydroquinone dimer with the structure as shown in figure 1.

Compound 2: colourless needle crystals that gave positive colour with Liebermann-Burchard's test suggested its triterpenoidal nature. The molecular formula was deduced to be $C_{30}H_{52}O$ from ^{1}H- and ^{13}C- including DEPT ^{13}C-NMR and MS spectra. The ^{13}C-NMR spectrum showed the presence of signals for 30 carbon atoms which with the DEPT ^{13}C-NMR showed the presence of eight methyl, eleven methylene, four methine, one oxymethine and six quaternary carbon atoms confirming the triterpenoidal skeleton. Comparing the ^{1}H- and ^{13}C-NMR data of the compound with similar compounds[48], the data can be interpreted as follow: the ^{1}H-NMR showed the presence of one doublet methyl signal at δ_H 0.93 (J= 8 Hz) (δ_C 11.85) which assigned for C-23; seven singlet methyl signals at δ_H 1.15 (δ_C 32.01); 0.98 (δ_C 16.62); 0.97 ($2CH_3$) (δ_C 18.88 and 32.31); 0.92 (δ_C 20.34); 0.91 (δ_C 35.25) and 0.84 (δ_C 18.47) which assigned for C-28, C-24, C-27, C-30, C-26, C-29 and C-25, in addition to a broad singlet at δ_H 3.72 (δ_C 72.97) which assigned for C-3. The chemical shifts were found to be close to friedelane triterpene nucleus having an oxygenated carbon[48]. Comparing these data with the data reported for 3-β-friedelinol confirmed its identity[49]. The assignment of the structure was supported by the EI-MS, which exhibited beside the molecular ion peak at *m/z* 428 $[M]^+$, other peaks at *m/z* 413 (29), 275 (36), 248 (19), 231 (30) and 95 (100, base peak). Based on these evidences, the structure of compound **2** was deduced as 3-β-friedelinol (Fig. 1). Friedelinol type triterpene was previously isolated from the family Rubiaceae (*Paederia foetida*)[50] but this is the first report for its isolation from the genus *Rubia*.

Compound 3: The ^{13}C-NMR including DEPT spectra showed the presence of ten carbon signals including six quaternary, one methine, two methyls and one methoxy groups. The ^{1}H-NMR spectrum showed the presence of four singlet signals; one in the aromatic region at δ_H 6.19 (1H) and three in the aliphatic region at δ_H 2.08, 2.43 and 3.90 (3H, each), in addition to chelated hydroxyl group at δ_H 12.00. The aromatic proton and six carbons at δ_C 105.37 (C), 163.36 (C), 108.75 (C), 158.37 (C), 110.77 (CH), 140.32 (C) indicated the presence of penta-substituted benzene ring; the two signals at δ_C 7.88 and 24.31 were assigned for the two aromatic methyls and the two signals at δ_C 52.01 and 169.76 were assigned for the carbomethoxy group. The full identification of this compound was deduced by 2D NMR spectroscopy including HSQC and HMBC. The HMBC spectrum (Fig. 1) showed correlations between the proton at δ_H 6.19 with carbons at δ_C 105.37, 108.75 and 24.31; protons at δ_H 2.43 with carbons at δ_C 110.77 and 105.37; protons at δ_H 2.08 with carbons at δ_C 163.36 and 158.37 and finally the methoxy protons at δ_H 3.90 with the carbonyl carbon at δ_C 172.82. Final identification was supported by MS which showed $[M]^+$ at *m/z* 196 corresponding to the molecular formula $C_{10}H_{12}O_4$. Comparing these data with that reported for similar compounds indicated that this compound is atraric acid which isolated from many plants as *Pygeum africanum, Newbouldia laevis, Ekebergia pterophylla* and *Evernia prunastri*[51-54]. Although, many phenolic acid derivatives were isolated from the genus *Rubia*[33&55], this is the first report about the isolation of atraric acid from the family Rubiaceae.

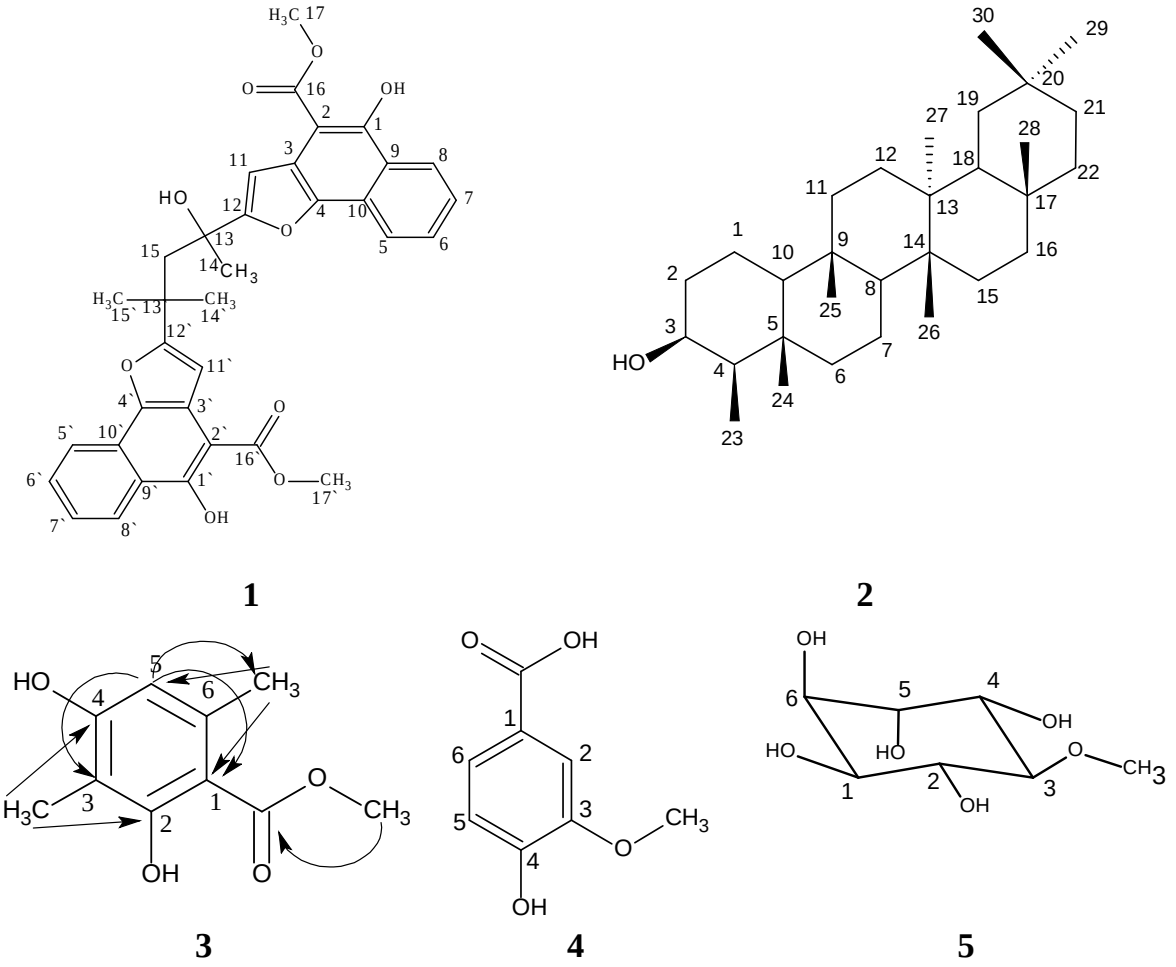

Fig. 1: Structures of compounds (**1-5**) and HMBC of **3**.

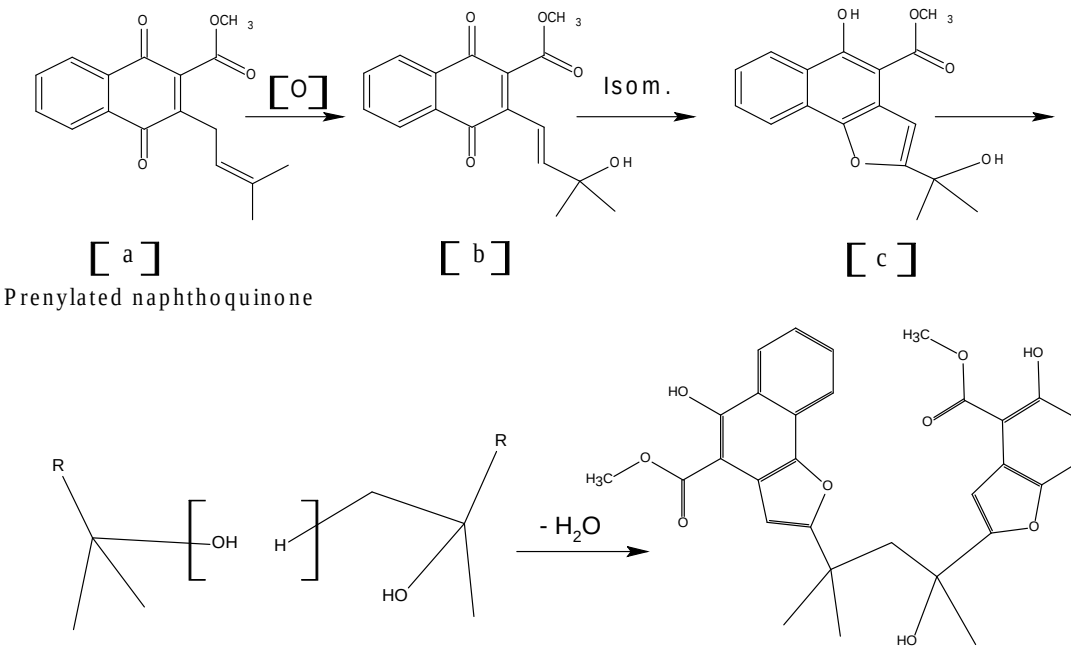

Fig. 2: The possible biosynthetic pathway of the compound **1**.

Compound 4: The ^{1}H-NMR spectrum of compound **4** showed the presence of three peaks in the aromatic region at δ_H 8.04 (1H, d, *J*= 1.9 Hz), 8.13 (1H, dd, *J*= 1.9, 8.2 Hz) and 7.31 (1H, d, *J*= 8.2 Hz) indicated the presence of trisubstituted benzene ring with ABX system while the singlet signal at δ_H 3.94 (3H) suggested the presence of methoxy group. This data with the aid of MS at *m/z* 169 $[M+1]^+$ corresponding to the molecular formula $C_8H_8O_4$ and physical properties (experimental part) in addition to the reported data indicated that compound **4** is vanillic acid which was previously reported from *Rubia yunnanensis*[55] but this is the first report about its isolation from this species.

Compound 5: The ^{13}C-NMR data of the compound showed the presence of seven carbon signals for seven carbons including one methoxy at δ_C 59.39 and six oxygenated methine atoms resonating at δ_C 70.62, 71,13, 72.06, 72.34, 72.91 and 83.51. The ^{1}H-NMR data revealed the absence of aromatic and presence of aliphatic groups as follow: δ_H 3.27 (1H, t, *J*= 9.2 Hz), 3.58 (1H, t, *J*= 9.2 Hz), 3.69 (1H, dd, *J*= 2.4, 10.0 Hz), 3.73 (1H, dd, *J*= 2.4, 10.0 Hz), 3.88 (2H, m). The chemical shifts of the oxymethine groups indicated the presence of inositol moiety[56] substituted with one methoxy group. Comparing the chemical shift with the reported inositol isomers[46] indicated that the compound is *chiro*-inositol substituted at C-3 with the methoxy group, thus the compound was identified as D-3-*O*-methoxy-*chiro*-inositol and this is the first report about its isolation from the genus *Rubia*.

REFERENCES

1- H. Morita, T. Yamamiya, K. Takeya and H. Itokawa, "New antitumor bicyclic hexapeptides, RA-XI, XII, XIII and XIV from *Rubia* cordifolia", Chem. Pharm. Bull., 40, 1352-1354 (1992).

2- J. Lee, Y. Hitotsuvanagi and K. Takeya, "Structures of cytotoxic bicyclic hexapeptides, RA-XIX, -XX, -XXI, and -XXII, from *Rubia cordifolia* L", Tetrahedron, 64, 4117-4125 (2008) and references cited in.

3- J. E. Lee, Y. Hitotsuyanagi, H. Fukaya, K. Kondo and K. Takeya, "New cytotoxic bicyclic hexapeptides, RA-XXIII and RA-XXIV from *Rubia cordifolia* L", Chem. Pharm. Bull., 56, 730-733 (2008).

4- J. E. Lee, Y. Hitotsuyanagi, I. H. Kim, T. Hasuda and K. Takeya, "A novel bicyclic hexapeptide, RA-XVIII, from *Rubia cordifolia*: structure, semi synthesis, and cytotoxicity", Bioorg. Med. Chem. Lett., 18, 808-811 (2008).

5- J. P. Lumb and D. Trauner, "Biomimetic synthesis and structure elucidation of Rubicordifolin, a cytotoxic natural product from *Rubia cordifolia*", J. Am. Chem. Soc., 127, 2870-2871 (2005).

6- M. Shoemaker, B. Hamilton, S. H. Dairkee, I. Cohen and M. J. Campbell, "*In-vitro* anticancer activity of twelve Chinese medicinal herbs", Phytother. Res., 19, 649-651 (2005).

7- N. Deshkar, S. Tilloo and V. Pande, "A comperhensive review of *Rubia cordifolia* L.", Pharmacognosy Reviews, 2, 124-134 (2008) and references cited in.

8- P. R. Patel, B. P. Raval, H. A. Karanth and V. R. Patel, "Potent antitumor activity of Rubia cordifolia", Int. J. Phytomedicine, 2, 44-46 (2010).

9- P. P. Gupta, R. C. Srimal, N. Verma and J. S. Tandon, "Biological activity of *Rubia cordifolia* and isolation of an active principle", Pharm. Biol., 37, 46-49 (1999).

10- S. Ghosh, M. Das Sarma, A. Patra and B. Hazra, "Anti-inflammatory and anticancer compounds isolated from *Ventilago madraspatana* Gaertn., *Rubia cordifolia* Linn. and *Lantana camara* Linn", J. Pharm. Pharmacol., 62, 1158-1166 (2010).

11- S. Basu, A. Ghosh and B. Hazra, "Evaluation of the antibacterial activity of *Ventilago madraspatana* Gaertn. *Rubia cordifolia* Linn. and *Lantana camara* Linn.: isolation of emodin and physcion as active antibacterial agents", Phytother. Res., 19, 888- 894 (2005).

12- R. Singh, A. Jain, S. Panwar, D. Gupta and S. K. Khare, "Antimicrobial activity of some natural dyes", Dyes and Pigments, 66, 99-102 (2005).

13- S. B. Kasture, V. S. Kasture and C. T. Chopde, "Anti-inflammatory activity of *Rubia cordifolia* roots", J. Nat. Rem., 1, 111-115 (2001).

14- P. Jaijesh, K. K. Srinivasan, K. P. Bhagath, G. Sreejith and A. M. Ciraj, "Anti-arthritic property of the plant *Rubia cordifolia* Lin", Pharmacologyonline, 1, 107-113 (2008).
15- S. Basu and B. Hazra, "Evaluation of nitric oxide scavenging activity, *in-vitro* and e*x-vivo*, of selected medicinal plants traditionally used in inflammatory diseases", Phytother. Res., 20, 896-900 (2006).
16- T. C. Shekhar, Y. M. Bahuguna and S. Vijender, "Anti-inflammatory activity of ethanolic stem extracts of *Rubia cordifolia* Linn. in rats", Int. J. Res. in Ayurveda and Pharmacy, 1, 126-130 (2010).
17- P. Kaur, S. Kaur, S. Kumar and P. Singh, "*Rubia cordifolia* L. and *Glycyrrhiza glabra* L. medicinal plants as potential source of COX-2 inhibitors", Am. J. Biomed. Sci., 2, 108-120 (2010).
18- K. Divakar, A. T. Pawar, S. B. Chandrasekhar, S. B. Dighe and G. Divakar, "Protective effect of the hydro-alcoholic extract of *Rubia cordifolia* roots against ethylene glycol induced urolithiasis in rats", Food Chem. Toxicol., 48, 1013-1018 (2010).
19- A. T. Pawar, D. Kalyani, S. B. Chandrasekar and G. Divakar, "Diuretic activity of root extract of *Rubia cordifolia* Linn.", Pharmacologyonline, 1, 597-603 (2009).
20- S. J. Rani, R. Nagarauk and P. Anuradha, "Antibacterial properties of extracts of Indian medicinal plants: *Syzygium alternifolium*, *Phyllanthus niruri* and *Rubia cordifolia*", Biomed. Pharmacol. J., 3, 123-128 (2010).
21- K. C. Naidu, R. Lalam and V. Bobbarala, "Antimicrobial agents from *Rubia cordifolia* and *Glycyrrhiza glabra* against phytopathogens of *Gossypium*", Int. J. Pharm.Tech. Res., 1, 1512-1518 (2009).
22- G. M. M. Rao, C. V. Rao, P. Pushpangadan and A. Shirwaikar, "Hepatoprotective effects of rubiadin, a major constituent of *Rubia cordifolia* Linn.", J. Ethnopharmacol., 103, 484-490 (2006).
23- R. Singh and S. M. Chauhan, "9,10-anthraquinones and other biologically active compounds from the genus *Rubia*", Chem. Biodivers., 1, 1241-1264 (2004) and references cited in.
24- A. K. Meena, B. Pal, P. Panda, R. Sannd and M. M. Rao, "A review on *Rubia cordifolia*: Its phyto-constituents and therapeutic uses", Drug Invention Today, 2, 244-246 (2010) and references cited in.
25- R. A. Patil, S. C. Jagdale and S. B. Kasture, "Antihyperglycemic, antistress and nootropic activity of roots of *Rubia cordifolia* Linn", Indian J. Exp. Biol., 44, 987-992 (2006).
26- R. Baskar, L. M. Bhakshu, G. V. Bharathi, S. S. Reddy, R. Karuna, G. K. Reddy and D. Saralakumari, "Antihyperglycemic activity of aqueous root extract of *Rubia cordifolia* in streptozotocin-induced diabetic rats", Pharm. Biol., 44, 475-479 (2006).
27- M. T. Harde, A. S. khairrar, A. S. Kasture and S. B. kasture, "Evaluation of antipsychotic and anti-diarrhoeal activities of ethanolic extract of roots of *Rubia cordifolia* Linn.", Oriental Pharmacy and Experimental Medicine, 8, 73-80 (2008).
28- A. K. Rawal, D. K. Nalh and S. K. Biswas, "Plausible mechanism of antioxidant action of *Fagonia cretica* Linn, *Rubia cordifolia* and *Tinospora cordifolia*, during ischematic reperfusion injury in rat hippocampus", Int. J. App. Res. Nat. Prod., 1, 16-25 (2008).
29- Y. B. Tripathi, S. Shukla, M. Sharma and V. K. Shukla, "Antioxidant property of *Rubia cordifolia* extract and its comparison with vitamin E and parabenzoquinone", Phytother. Res., 9, 440-443 (1995).
30- A. A. Joharapurkar, S. P. Zambad, M. M. Wanjari and S. N. Umathe, "*In-vivo* evaluation of antioxidant activity of alcoholic extract of *Rubia cordifolia* Linn. and its influence on ethanol-induced imm-unosuppression", Indian J. Pharmacol., 35, 232-236 (2003).
31- Y. Lu, R. Hu, Z. Dai and Y. Pan, "Preparative separation of anti-oxidative constituents from *Rubia cordifolia* by column-switching counter-current chroma-tography", J. Sepa. Sci., 33, 2200-2205 (2010.).
32- H. Itokawa, Z. Z. Ibraheim, Y. F. Qiao and K. Takeya, "Anthraquinones, naphtho-

hydroquinones and naphthohydroquinone dimers from *Rubia cordifolia* and their cytotoxic activity", Chem. Pharm. Bull., 41, 1869-1872 (1993).

33- H. A. Hassanean, Z. Z. Ibraheim, K. Takeya and H. Itokawa, "Further quinoidal derivatives from *Rubia cordifolia* L.", Pharmazie, 55, 317-319 (2000).

34- Z. Z. Ibraheim, "Triterpenes from *Rubia cordifolia* L.", Bull. Pharm. Sci. Assiut Univ., 25, 155-163 (2002).

35- Z. Z. Ibraheim, "Saponins, naphtho-hydroquinones and anthraquinone glycosides from *Rubia cordifolia* L", ibid., 25, 85-94 (2002).

36- Z. Z. Ibraheim and A. S. Ahmed, "Further constituents from *Rubia cordifolia* L. root", Bull. Fac. Pharm. Cairo Univ., 47, 233-243 (2009).

37- R. Liu, Y. Lu, T. Wu and Y. Pan, "Simultaneous isolation and purification of mollugen and two anthraquinones from *Rubia cordifolia* by HSCCC", Chromatographia, 68, 95-99 (2008).

38- M. Miyazawa and J. Kawata, "Identification of the key aroma compounds in dried roots of *Rubia cordifolia*", J. Oleo-Science, 55, 37-39 (2006).

39- N. P. Mishchenko, S. A. Fedoree, V. M. Bryukhano, Ya. F. Zverev, V. V. Lampatov, O. V. Azarova, Yu. N. Shkry and G. K. Chernaded, "Chemical composition and pharmacological activity of anthraquinones from *Rubia cordifolia* cell culture", Pharm. Chem. J., 41, 605-609 (2007).

40- D. Gupta, S. Kumari and M. Gulrajani, "Studies with hydroxyanthraquinones extracted from Indian madder. Part 1: Dyeing of nylon with purpurin", Coloration Technology, 117, 328-332 (2001).

41- Y. Lu, R. Liu, C. Sun and Y. Pan, "An effective high-speed countercurrent chromatographic method for preparative isolation and purification of mollugin directly from the ethanol extract of the Chinese medicinal plant *Rubia cordifolia*", J. Sepa. Sci., 30, 1313-1317 (2007).

42- W. Ma, Y. Lu, X. Dai, R. Liu, R. Hu and Y. Pan, "Determination of anti-tumor constitute mollugin from traditional Chinese medicine *Rubia cordifolia*: Comparative study of classical and microwave extraction techniques", Separation Science and Technology, 44, 995-1006 (2009).

43- X. Li, Z. Liu, Y. Chen, L. Wang, Y. Zheng, G. Sun and C. Ruan, "Rubiacordone A: A New Anthraquinone glycoside from the roots of *Rubia cordifolia*", Molecules, 14, 566-572 (2009).

44- M. S. Akhtar, M. Ali, M. Madhuriya, S. R. Mir and O. Singh, "New anthraquinones from *Rubia cordifolia* roots", Indian J. Chem., 45, 1945-1950 (2006).

45- Y. Qiao, K. Takeya, H. Itokawa and Y. Iitaka, "Three novel naphthohydroquinone dimers from *Rubia oncotricha*", Chem. Pharm. Bull., 38, 2896-2898 (1990).

46- E. Breitmaier and W. Voelter, "Crabon-13 NMR spectroscopy", VCH Verlag-sgesellschaft, Weinheim, Third, Completely Revised Edition (1987).

47- J. P. Lumb, K. C. Choong, and D. Trauner, "O*rtho*-quinone methides from *para*-quinones: Total synthesis of rubioncolin B", J. Am. Chem. Soc., 130, 9230-9231 (2008).

48- S. B. Mahato and A. P. Kundu, "^{13}C NMR spectra of pentacyclic triterpenoids - A compilation and some salient features. Review article", Phytochemistry, 37, 1517-1575 (1994).

49- G. C. M. Salazar, G. D. F. Silva, L. P. Duarte, S. A. Vieira Filho and I. S. Lula, "Two epimeric friedelane triterpenes isolated from *Maytenus truncata* Reiss: ^{1}H and ^{13}C chemical shift assignments", Magn. Reson. Chem., 38, 977-980 (2000).

50- C. P. Khare, "Indian Herbal Remedies: Rational Western Therapy, Ayurvedic, and Other Traditional Usage, Botany", Springer-Verlag Berlin Heidelberg New York, 2004, p. 347.

51- M. Papaioannou, S. Schleich, I. Prade, S. Degen, D. Roell, U. Schubert, T. Tanner, F. Claessens, R. Matusch and A. Baniahmad, "The natural compound atraric acid is an antagonist of the human androgen receptor inhibiting cellular

invasiveness and prostate cancer cell growth", J. Cell. Mol. Med., 13, 2210-2223 (2009).

52- R. Gormann, M. Kaloga, X-C. Li, D. Ferreira, D. Bergenthal and H. Kolodziej, "Furanonaphthoquinones, atraric acid and a benzofuran from the stem barks of *Newbouldia laevis*", Phytochemistry, 64, 583-587 (2003).

53- D. A. Mulholland, S. E. Iour1ne, D. A. H. Taylor and F. M. Dean, "Coumarins from *Ekebergia pterophylla*", ibid., 47, 1641-1644 (1998).

54- A. M. Ahad, Y. Goto, F. Kiuchi, Y. Tsuda, K. Kondo and T. Sato, "Nematocidal principles in oakmoss absolute and nematocidal activity of 2,4-dihydroxybenzoates", Chem. Pharm. Bull., 39, 1043-1046 (1991).

55- J. Tao, T. Morikawa, S. Ando, H. Matsuda and M. Yoshikawa, "Bioactive Constituents from Chinese Natural Medicines. XI. Inhibitors on NO Production and Degranulation in RBL-2H3 from *Rubia yunnanensis*: Structures of Rubianosides II, III and IV, Rubianol-g, and Rubianthraquinone", ibid., 51, 654-662 (2003).

56- C. Sridhar, A. V. Krishnaraju and G. V. Subbaraju, "Antiinflammatory constituents of *Teramnus labialis*", Indian J. Pharm. Sci., 68, 111-114 (2006).