Bull. Pharm. Sci., Assiut University, Vol. 25, Part 2, December 2002, pp. 155-163.

# TRITERPENES FROM *RUBIA CORDIFOLIA* L.

Zedan Zeid Ibraheim

*Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt*

من جزء الهكسان لخلاصة الميثانول-كلوروفورم (١:١) للجذور الجافة لنبات روبيا كورديفوليا ل. تم فصل ثلاثة مركبات جديدة بالاضافة الى خمسة مركبات معروفة من التربينات الثلاثية. والمركبات الجديدة هي ٣-بيتا اسيتوكسي اوليانين-١٢-اون ، و٣-بيتا-١٣-بيتا-١٥-الفا-ثلاثي هيدروكسي اوليانين-١٢-اون ، و٣-بيتا-١٩-الفا-داى هيدروكسي أربور-٩(١١)-ين والمركبات المعروفة هي ٣-بيتا-اسيتوكسي اوليانين-١٣-بيتا-أول-١٢-اون ، و٣-بيتا-اسيتوكسي اوليانين-١٣-بيتا-١٥-الفا-داى أول-١٢-اون ، وحمض الأوليانولك وخلاتة بالاضافة الى الهيدراجنين. وقد تم التعرف على المركبات المفصولة باستخدام الطرق الفيزيائية والكيميائية والطيفية المختلفة. وتم اجراء بعض التجارب لمعرفة تأثير بعض المركبات المفصولة على الخلايا السرطانية المعزولة.

*From the hexane fraction of the methanol-chloroform (1:1) extract of the dried roots of Rubia cordifolia L., three new and five known triterpenoidal compounds were isolated and identified. The new triterpenes are 3β-acetoxyoleanane-12-one; 3β,13β,15α-trihydroxyoleanane-12-one and 3β,19α-dihydroxy arbor-9 (11)-ene. The known triterpenes are 3β-acetoxyoleanane, 13β-ol-12-one; 3β-acetoxyoleanane-13β,15α-dihydroxyoleanane-12-one; oleanolic acid and its acetate and hederagenin. The identification of the isolated compounds was carried out using different physical, chemical and spectral methods of analysis. The cytotoxic activity of some of the isolated compounds was studied.*

## INTRODUCTION

*Rubia cordifolia* L. (Family Rubiaceae) is well known for its versatile medicinal uses. It is recommended for the treatment of hematorrhea, hematemesis, nose bleeding, traumatic bleeding, dysmenorrhea, and arthritis.[1] Many anthraquinones, naphthoquinones, naphthohydroquinones, naphthohydroquinones dimers,[2-12] triterpenes,[13-17] iridoids[18] and quinoidal derivatives[19] were isolated from the root of *R. cordifolia* L. in addition to cyclic hexapeptides[20-26] and polysaccharides.[27] The cytotoxic, anticancer, antibacterial, antifungal and some pharmacological activities of these compounds have been well-documented.[28-32] In a previous study on the butanol fraction of the $CHCl_3$-MeOH (1:1) extract of the dried roots, saponins, naphthohydroquinone and anthraquinone glycosides were isolated and identified.[33]

## EXPERIMENTAL

Melting points (uncorrected) were determined by electrothermal model 550. IR spectra were recorded in KBr using Unican Sp 1025 spectrophotometer. $^1$H- and $^{13}$C-NMR spectra were recorded in $C_5D_5N$ and $CDCl_3$, 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, type 70-230 mesh) and irregular reversed phase (R 18-37, 20 μm ODS, Kusano Scientific Co., Tokyo, Japan) were used. MPLC [CIG column system (22 mm., i.d. x 30 cm, Kusano Scientific Co., Tokyo, Japan)] was used for final purification. Precoated silica gel 60 $F_{254}$ and RP-$C_{18}$ $F_{254}$ S (E-Merck) were used for TLC and 10% $H_2SO_4$ was used as spraying reagent followed by heating.

Received in 30/6/2002 & Accepted in 16/10/2002

**Plant material**

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

**Extraction and isolation**

The air-dried powdered roots of *Rubia cordifolia* L. (20 kg) were exhaustively extracted with chloroform-methanol (1:1), (50 L x 3). The combined extract was evaporated till dryness under reduced pressure at temperature below 50° till constant weight (1.51 Kg). The dried extract was diluted with distilled water and successively extracted with n-hexane (fraction A, 184 g), chloroform (fraction B, 235 g), and lastly with butanol (fraction C, 455 g). Each fraction was subjected to cytotoxic screening using V-79 cell.

The dried hexane fraction (35 g, $IC_{50}$= 30 µg/ml) was column chromatographed over silica gel, elution was started with hexane and increasing the polarity with ethyl acetate gradiently. Fractions, 200 ml each, were collected and monitored using TLC and 10% $H_2SO_4$ as spraying reagent. Similar fractions were pooled together where six groups of fractions were obtained.

**Group 1**: Frs. 2-5 eluted with n-hexane, showed many inseparable spots.

**Group 2**: Frs. 6-9 eluted with n-hexane-EtOAc (95:5), ($IC_{50}$= 10 µg/ml) were injected in CIG column system. Elution was carried out with methanol-water (80:20) using RP-$C_{18}$ column where five sub-fractions were obtained, on repeated crystallization of sub-fraction 3 compound **I** was obtained (22 mg).

**Group 3**: Frs. 10-14 eluted with n-hexane-EtOAc (92.5:7.5), ($IC_{50}$= 10 µg/ml) were injected in CIG column system. Elution was carried out with methanol-water (80:20) using RP-$C_{18}$ column where five sub-fractions were obtained, on repeated crystallization of sub-fraction 4 compound **II** was obtained (32 mg).

**Group 4**: Frs. 15-19 eluted with n-hexane-EtOAc (90:10), ($IC_{50}$= 30 µg/ml). On repeated CC using CIG column system (silica gel prepacked column, using MPLC) and using n-hexane- EtOAc (85:15) isocratically. Sub-fractions 6-9 upon crystallization afforded compound **III** (28 mg) and sub-fractions 10-13 upon repeated crystallization afforded compound **IV** (36 mg).

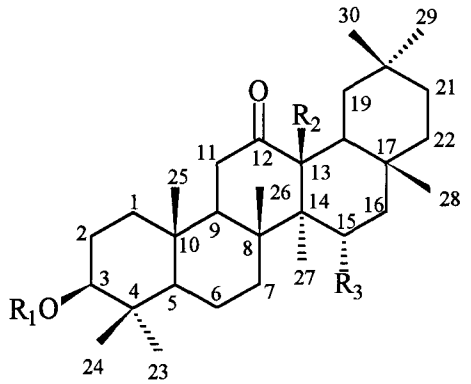
**Group 5**: Frs. 20-24 eluted with n-hexane-EtOAc (85-15) ($IC_{50}$= 10 µg/ml). Upon repeated CC using RP-$C_{18}$ column and $CH_3CN$-$H_2O$ (80:20) afforded compound **V** (sub-fractions. 3-6,18 mg) and compound **VI** (sub-fractions. 8-14, 22 mg).

**Group 6**: Frs. 25-30 eluted with n-hexane-EtOAc (80:20) ($IC_{50}$= 30µg/ml). Upon repeated CC using RP-$C_{18}$ column and MeOH-$H_2O$ (75:25) afforded compounds **VII** (sub-fractions. 3-6, 26 mg) and **VIII** (sub-fractions 7-11, 34 mg).

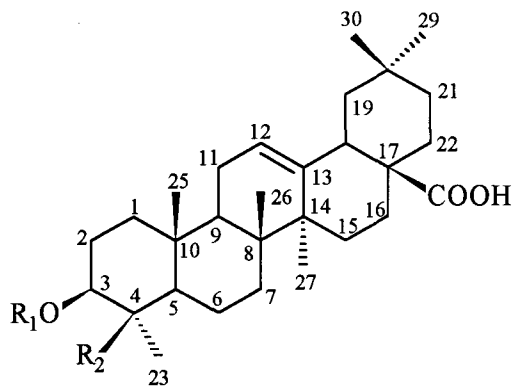
**Acetylation:** Each compound to be acetylated (5-10 mg) was dissolved in equal volume of pure pyridine and acetic anhydride (2 ml, each) mixed well and left for 24 hours at room temperature then diluted with water and extracted with $CHCl_3$ (10 ml x 3), the chloroform extract was dried over anhydrous $Na_2SO_4$, and evaporated to dryness under reduced pressure. The acetate obtained was crystallized from anhydrous chloroform.

**Compound (I)**: needles ($CHCl_3$-MeOH 1:1), m.p 223-225°. IR $\nu^{KBr}$; 2928, 1722, 1700 and 1468 $cm^{-1}$, EI-MS, m/z (% rel.int.) 484 [$M^+$] (21), 469 (8), 359 (9), 249 (12), 234 (100), 220 (67), 205 (20), 189 (78), 177 (60), 147 (18), 134 (81) and 121 (82). 400 MHz $^1$H-NMR ($CDCl_3$), δ 0.86 (6H, s, 2x$CH_3$, $CH_3$-24 and 27), 0.87 (3H, s, $CH_3$-23), 0.88 (3H, s, $CH_3$-29), 0.90 (3H, s, $CH_3$-25), 0.95 (3H, s, $CH_3$-30), 0.98 (3H, s, $CH_3$-28), 1.15 (3H, s, $CH_3$-26), 2.02 (3H, s, $\underline{CH_3}$-CO), 2.10 (1H, dd, *J*= 17.0 and 13.0 Hz, H-11a), 2.26 (1H, dd, *J*= 17.0 and 5.3 Hz, H-11b), 2.78 (1H, d, *J*= 3.2 Hz, H-13) and 4.48 (1H, dd, *J*= 11.0 and 5.1 Hz, H-3). 100 MHz $^{13}$C-NMR data ($CDCl_3$) are listed in Table 1.

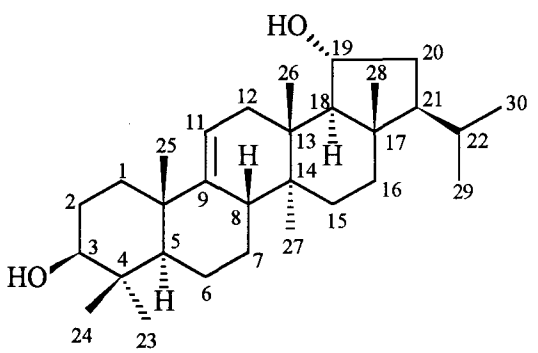
**Compound (II)**: (rubiprasin B): needles (MeOH), m.p 278-280°, IR $\nu^{KBr}$ 3458, 2920, 2875, 1722, 1693, 1478, 1460 and 1030 $cm^{-1}$, EI-MS, m/z (% rel. int.) 500 [$M^+$] (16), 338 (3),



| Compd. No. | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **I** | Ac. | H | H |
| **II** | Ac. | OH | H |
| **III** | Ac. | OH | OH |
| **V** | H | OH | OH |



| Compd. No. | $R_1$ | $R_2$ |
|---|---|---|
| **IV** | Ac. | $CH_3$ |
| **VII** | H | $CH_3$ |
| **VIII** | H | $CH_2OH$ |



**VI**

Triterpenoidal compounds isolated from the hexane fraction of *R. cordifolia* L.

248 (100), 233 (8), 220 (10), 208 (18), 203 (92) and 189 (33). 400 MHz $^1$H-NMR ($C_5D_5N$), δ 0.88 ($CH_3$-23), 0.91 ($CH_3$-24), 0.93 ($CH_3$-29), 0.95 ($CH_3$-25), 1.02 ($CH_3$-30), 1.42 ($CH_3$-27), 1.60 ($CH_3$-28), 1.85 ($CH_3$-26); (eight signals, 3H each, s), 1.88 (1H, dd, *J*= 14.0 and 3.0 Hz, H-18), 2.05 (3H, s, $\underline{CH_3}$-CO), 2.24 (1H, dd, *J*= 14.0 and 3.0 Hz, H-11α), 3.38 (1H, t, *J*= 14.0 Hz, H-11β) and 4.66 (1H, dd, *J*= 12.1 and 5.0 Hz, H-3). 100 MHz $^{13}$C-NMR data ($C_5D_5N$) are listed in Table 1.

**Compound (III)**: [rubiprasin A], needles ($CHCl_3$-MeOH 1:1), m.p >300. IR $\nu^{KBr}$; 3450, 3430, 2920, 1720, 1695, 1480, 1420 and 1025 cm$^{-1}$, EI-MS, m/z (% rel. int.) 516 [$M^+$] (8%), 498 (22), 266 (10), 235 (57), 207 (100) and 189 (82). 400 MHz $^1$H-NMR ($C_5D_5N$), δ 0.89 ($CH_3$-23), 0.92 ($CH_3$-24), 0.93 ($CH_3$-29), 0.94 ($CH_3$-25), 1.01 ($CH_3$-30), 1.42 ($CH_3$-27), 1.61 ($CH_3$-28), 1.85 ($CH_3$-26); (eight singlet signals, 3H each), 1.87 (1H, dd, *J*= 14.0 and 2.8 Hz, H-18), 2.04 (3H, s, $\underline{CH_3}$-CO), 2.25 (1H, dd, *J*= 14.0, 2.9 Hz, H-11α), 3.38 (1H, t, *J*= 14.0 Hz, H-11β), 4.68 (1H, dd, *J*= 12.0 and 5.0 Hz, H-3), 5.20 (1H, br.s, OH-15) and 5.22 (1H,dd, *J*= 4.6 and 11.4 Hz, H-15), 100 MHz $^{13}$C-NMR data ($C_5D_5N$) are listed in Table 1.

**Compound (IV)**: needles ($CHCl_3$-MeOH 1:1), m.p 265-268°, IR $\nu^{KBr}$; 3380, 2875, 1718, 1695, 1622, 1475, 1450 and 1030 cm$^{-1}$. 400 MHz $^1$H-NMR ($C_5D_5N$) δ 0.84, 0.89, 0.94, 0.97, 1.00, 1.03 and 1.29 (seven singlet signals, 3H each) 2.07 (3H, s, $\underline{CH_3}$CO), 4.31 (1H, dd, *J*= 4.0 and 14.0 Hz, H-3) and 5.48 (1H, br.s, H-12). 100 MHz $^{13}$C-NMR data ($C_5D_5N$) are cited in Table 1.

**Compound (V)**: fine needles (methanol), m.p 233-235°. IR $\nu^{KBr}$; 3460, 3440, 2931, 1690, 1482, 1422 and 1025 cm$^{-1}$, EIMS m/z (% rel. int.) 474 [$M^+$] (9), 456 (12), 266 (18), 235 (8), 207 (100) and 189 (65). 400 MHz $^1$H-NMR ($C_5D_5N$) δ 0.91 ($CH_3$-23), 0.99 ($CH_3$-24), 1.00 ($CH_3$-29), 1.02 ($CH_3$-25), 1.22 ($CH_3$-30), 1.39 ($CH_3$-27), 1.61 ($CH_3$-28) and 1.88 ($CH_3$-26); (eight singlet signals, 3H each), 2.28 (1H, dd, *J*= 14.0 and 2.9 Hz, H-11α), 3.40 (1H, t, *J*= 14.0 Hz, H-11β), 3.51 (1H, dd, *J*= 12.0 and 5.0 Hz, H-3), 5.21 (1H, br.s, OH-15) and 5.24

**Table 1:** $^{13}$C-NMR data of Compounds (I-VIII)*.

| C. No. | I* | II | III | IV | V | VI | VII | VIII |
|---|---|---|---|---|---|---|---|---|
| 1 | 37.75 | 38.09 | 38.42 | 38.21 | 38.34 | 36.66 | 38.99 | 38.82 |
| 2 | 23.45 | 23.88 | **24.02** | **23.70** | **28.53** | 28.77 | **28.13** | 27.68 |
| 3 | 80.66 | 80.51 | **80.55** | **80.76** | **77.91** | 78.14 | **78.14** | 74.08 |
| 4 | 37.82 | 38.03 | **37.96** | **37.90** | **39.44** | 39.76 | **39.40** | 42.84 |
| 5 | 55.83 | 55.39 | 55.20 | 55.55 | 55.46 | 52.96 | 55.87 | 48.22 |
| 6 | 18.25 | 18.19 | 18.65 | 18.49 | 18.96 | 21.94 | 18.83 | 18.61 |
| 7 | 31.87 | 31.72 | 38.17 | 33.22 | 39.16 | 27.23 | 33.33 | 32.95 |
| 8 | 41.81 | 42.65 | 44.86 | 39.72 | 44.95 | 41.22 | 39.81 | 39.80 |
| 9 | 49.31 | 46.20 | 46.20 | 47.91 | 46.19 | 148.88 | 48.10 | 48.55 |
| 10 | 36.81 | 37.32 | 37.88 | 37.18 | 38.08 | 40.00 | 37.43 | 37.31 |
| 11 | 38.45 | 39.08 | 39.14 | 23.77 | 39.16 | 114.97 | 23.85 | 23.79 |
| 12 | 212.08 | 210.34 | 210.44 | 122.39 | 210.71 | 37.32 | 122.59 | 122.58 |
| 13 | 49.85 | 82.95 | 85.82 | 144.67 | 85.48 | 37.69 | 144.87 | 144.82 |
| 14 | 43.83 | 45.23 | 51.10 | 42.19 | 51.10 | 38.55 | 42.06 | 42.04 |
| 15 | 34.68 | 22.96 | 66.68 | 28.31 | 66.68 | 30.01 | 28.36 | 28.34 |
| 16 | 25.51 | 33.86 | 43.98 | 23.70 | 43.97 | 36.65 | 23.79 | 23.71 |
| 17 | 38.94 | 34.16 | 35.04 | 46.67 | 35.02 | 44.15 | 46.54 | 46.67 |
| 18 | 38.80 | 51.16 | 51.58 | 41.99 | 51.85 | 59.08 | 42.23 | 42.19 |
| 19 | 36.98 | 35.37 | 35.18 | 46.47 | 35.27 | 70.25 | 46.14 | 46.47 |
| 20 | 30.93 | 29.99 | 31.81 | 30.97 | 32.24 | 41.92 | 30.98 | 30.94 |
| 21 | 31.33 | 34.57 | 34.54 | 34.25 | 34.58 | 57.82 | 34.29 | 33.23 |
| 22 | 34.55 | 39.62 | 39.58 | 33.27 | 39.62 | 30.70 | 33.27 | 32.99 |
| 23 | 27.95 | 27.95 | 28.04 | 28.16 | 28.23 | 28.96 | 28.80 | 68.12 |
| 24 | 16.52 | 16.63 | 16.68 | 15.39 | 16.23 | 16.57 | 16.54 | 13.11 |
| 25 | 15.34 | 15.80 | 15.98 | 16.97 | 16.05 | 22.47 | 15.57 | 15.91 |
| 26 | 16.23 | 20.06 | 20.42 | 17.37 | 20.45 | 17.53 | 17.47 | 17.50 |
| 27 | 20.91 | 18.67 | 14.48 | 26.18 | 15.39 | 16.82 | 26.18 | 26.15 |
| 28 | 32.41 | 31.40 | 32.01 | 180.15 | 32.00 | 15.96 | 180.13 | 180.14 |
| 29 | 33.58 | 32.04 | 32.19 | 32.04 | 32.14 | 22.28 | 33.32 | 33.21 |
| 30 | 23.58 | 25.75 | 24.86 | 25.75 | 25.83 | 23.21 | 23.79 | 23.77 |
| **<u>OC</u>-CH$_3$** | 170.83 | 170.53 | **170.55** | **170.58** | -- | -- | -- | --- |
| **OC-<u>CH$_3$</u>** | 21.18 | 21.10 | **21.11** | **21.11** | -- | -- | -- | --- |

*At 100 MHz, in $C_5D_5N$ except **I** in $CDCl_3$

(1H, dd, $J$= 4.7 and 11.2 Hz, H-15). 100 MHz $^{13}$C-NMR ($C_5D_5N$) as listed in Table 1.

**Compound (VI)**: fine needles (methanol), m.p 259-261°. IR $\nu^{KBr}$; 3440, 1635, 1520, 1504 and 1055 cm$^{-1}$. EI-MS m/z (% rel. int.), 442 [M$^+$] (57), 427 (37), 424 (83), 409 (66), 391 (27), 295 (26), 273 (30), 255 (40), 215 (24), 173 (31), 159 (44), 149 (78), 135 (75), 107 (98) and 103 (100). 400 MHz $^1$H-NMR ($C_5D_5N$) δ 0.87 (3H, d, $J$= 6.0 Hz, CH$_3$-29), 0.89 (3H, s, CH$_3$-28), 0.93 (3H, d, $J$= 6.0 Hz, CH$_3$-30), 0.99 (3H, s, CH$_3$-26), 1.08 (3H, s, CH$_3$-25), 1.13 (3H, s, CH$_3$-24), 1.16 (3H, s, CH$_3$-27), 1.29 (3H, s, CH$_3$-23), 2.38 (1H, d, $J$= 9.0 Hz, H-18), 3.51 (1H, dd, $J$= 10.4 and 6.0 Hz, H-3), 4.50 (1H, td, $J$= 9.0 and 2.0 Hz, H-19) and 5.45 (1H, br.d, $J$= 6.0 Hz, H-11). 100 MHz $^{13}$C-NMR data ($C_5D_5N$) are listed in Table 1.

**Compound (VII)**: needles (methanol), m.p >300°, IR $\nu^{KBr}$; 3340, 1690, 1605, 1520, 1480 and 1420 $cm^{-1}$. EI-MS m/z (% rel. int.) 456 [$M^+$] (8), 411, [$M^+$-COOH] (11), 248 (100) and 203 (82). 400 MHz $^1$H-NMR ($CDCl_3$): δ 0.711. 0.723. 0.852, 0.861, 0.883, 0.944, 1.08 (3H, s, each), 2.77 (1H, dd, *J*= 13.0 and 4.6 Hz, H-18), 3.27 (1H, dd, *J*= 11.5 and 4.2 Hz, H-3) and 5.23 (1H, br.s, H-12). 100 MHz $^{13}$C-NMR data ($C_5D_5N$) are listed in Table 1.

**Compound (VIII)**: needles (methanol), m.p >300°, IR $\nu^{KBr}$; 3345, 1703, 1620, 1520, 1504, 1150 and 1055 $cm^{-1}$. EI-MS m/z (% rel. int.), 470 [$M^+$] (27), 452 (13), 437 (41) and 427 (38). 400 MHz $^1$H-NMR ($C_5D_5N$): δ 0.94 (3H, s), 0.99 (3H, s), 1.02 (3H, s), 1.06 (3H, s), 1.07 (3H, s), 1.26 (3H, s), 3.37 (1H, dd, *J*= 14.0 and 4.0 Hz, H-3), 3.75 (1H, d, *J*= 10.7 Hz, H-24a), 4.23 (1H, d, *J*= 10.7 Hz, H-24b) and 5.53 (1H, br.s, H-12). 100 MHz $^{13}$C-NMR data ($C_5D_5N$) are cited in Table 1.

**Bio-assay of cytotoxic activity towards V-79 cells**:[10] V-79 cells was supplied by Dr. S. Tsukagoshi, Japan Foundation for Cancer Research, were maintained in medium containing kanamycin (100 μg/ml) and incubated at 37° in humidified atmosphere of 5 % $CO_2$. V-79 cells (3 x$10^2$ cells/well) were cultured in corning disposable 6-well plates containing 2 ml per well, PRMI-1640 medium (Nissui Pharm.Co. Ltd.) supplemented with 10 % fetal calf serum (Whittaker M.A. Bioproducts Inc.). Isolated compounds solutions of various concentrations (100 μl, 30 μl, 10 μl and 3 μl) in 0.3 % EtOH were added to the culture on day 1 after the cell-transplantation (day 0). On day 5, the colonies were fixed with 10 % HCHO solution (1.5 ml) for 30 min and stained with 0.05 % crystal violet (0.75 ml).

The cytotoxic activity of the drug was assessed by determining T/C % or $IC_{50}$ (drug concentration that inhibit the colonies growth by 50%) in drug containing-medium relative to the colonies growth in 0.3 % ethanol medium at the day 5 after treatment.

## RESULTS AND DISCUSSION

**Compound (I)**: was obtained as colorless needles ($CHCl_3$-MeOH, 1:1). The IR spectrum showed the presence of carbonyl and ester function groups (1722, 1700 $cm^{-1}$). $^1$H-NMR spectrum showed the presence of 8 singlet tertiary methyl signals and one acetyl signal, the signal at δ 4.48 was assigned for the H-3 proton where the large coupling constant (*J*= 11.0 Hz) clearly established an axial (α) configuration of this proton and β- hydroxyl group. The doublet signal at δ 2.78 (1H, d, *J*= 3.2 Hz) was assigned for H-13, while the two signals at δ 2.26 and 2.10 (1H, dd, each) were assigned for 2H-11 (see experimental) indicating that the position of the ketone was at C-12. $^{13}$C-NMR spectrum showed 32 carbon signals i.e. seven singlet, five doublet, ten triplet and eight quartet carbon signals in addition to the two carbon signals of acetyl group. The signals at δ 80.66 (d) and 212.08 (s) were assigned for C-3 and C-12, respectively, and the two signals at δ 170.83 (s) and 21. 18 (q) were assigned for the acetyl group. The assignments of each carbon signal are cited in Table 1. Comparing the chemical shifts of each carbon with those reported for rubiprasins A and B suggested that, the ketone group must be present at C-12.[15,34] The molecular formula of compound **1** was deduced to be $C_{32}H_{52}O_3$ from the MS, $^1$H-, $^{13}$C-NMR and DEPT. The MS spectrum showed [$M^+$] at m/z 484, other fragments at m/z 469 for the loss of methyl group, m/z 442 for the loss of acetyl group and the base peak appeared at m/z 234 for the upper fragment. As such compound **(I)** was identified as 3β-acetoxyoleanane-12-one. According to the available literature this is the first report for isolation of this compound from natural source.

**Compound (II)**: was obtained as colorless needle crystals (MeOH), m.p 278-280 °C, IR showed the presence of hydroxyl group(s) and Ketone group(s). The physical characters and spectral data [IR, $^1$H-NMR, $^{13}$C-NMR and MS] are identical to those reported for 3β-acetoxy-oleanane-13β-ol-12-one (rubiprasin B) isolated from *R. cordifolia* var. *pratensis* Maxim collected from China.[15] This is the first report for the isolation of this compound from *R. cordifolia* L.

**Compound (III)**: was obtained as colorless needles, IR showed the presence of hydroxyl group(s) and ketone group(s). $^1$H NMR

spectrum showed 8 singlet methyls and one acetyl signals (at δ 2.04). $^{13}$C-NMR spectrum showed 8 methyl signals, acetyl signals [δ 21.11 (q), 170.55 (s)] one ketonic carbon [δ 210.44, s] and three oxygenated carbons [δ 80.55 (d), 85.82 (s) and 66.68 (d)], other carbons are assigned as cited in Table (1). EI-MS showed molecular ion peak at m/z 516 calculated for $C_{32}H_{52}O_5$ and fragments at m/z 498 for the loss of one molecule of water and m/z 266 for [M-(207+Ac)]$^+$.

The physical characters and spectral data of compound **III** are identical to those reported for 3β-acetoxy, 13β, 15α-dihydroxyolean-12-one (rubiprasin A)[15] isolated from *R. cordifolia* var. *pratensis* Maxim collected from China.[15] Hence, compound **III** was identified as 3β-acetoxyoleanane-13β-15α-diol-12-one (rubiprasin A). This is the first report for isolation of this compound from *R. cordifolia* L.

**Compound (IV)**: was obtained as needles, its m.p is similar to that reported for oleanolic acid acetate.[34] IR showed absorption bands for ester and free carbonyl groups (1718 and 1695 cm$^{-1}$, respectively). $^1$H-NMR showed the presence of seven singlet methyl signals, acetyl signal (at δ 2.07), and two signals at δ 4.31 (1H, dd, *J*= 4.0 and 14.0 Hz) and 5.48 (1H, br.s) assigned for substituted H-3 and olefinic H-12 respectively. $^{13}$C-NMR spectrum showed 30 carbon signals similar to those reported for oleanolic acid[35] with additional two signals for acetate indicating that the hydroxyl group was acetylated and this was clear from the downfield shift of C-3 and upfield shift of both C-2 and C-4 (Table 1). When oleanolic acid was acetylated by the usual way (using pyridine / acetic anhydride), it gave compound **IV**. This is the first report for the isolation of this compound from *R. cordifolia* L.

**Compound (V)**. was obtained as fine needles, IR spectrum showed the presence of hydroxyl groups (3460 and 3440 cm$^{-1}$), and ketone group (1690 cm$^{-1}$). $^1$H-NMR spectrum showed the same pattern of compound **(III)** except the disappearance of the signal at δ 2.04 (3H, s) for acetyl group and the upfield shift of the signal at δ 3.51 (1H, dd, *J*= 12.0 and 5.0 Hz) assigned for H-3 indicating the position of the acetylation, most of the methyl signal position was downfield shifted and this is due to the removal of the acetyl group from C-3-OH.[14,15] The two signals at δ 2.28 (1H, dd, *J*= 14 and 2.9 Hz) and 3.40 (1H, t, *J*= 14.0 Hz) were assigned to 2H-11 forming a part of an ABX coupling pattern with H-9, these two signal were appeared more downfield than in compound **I** (measured in $CDCl_3$), since in this compound, $^1$H-NMR spectrum was measured in $C_5D_5N$. $^{13}$C-NMR spectrum showed 30 carbon signals i.e. eight singlet, five doublet, nine triplet and eight quartet carbon signals. Comparing the $^{13}$C-NMR data of compound **(V)** with those of compound **(III)** [Table 1], showed that the two signals appeared in the spectrum of compound **(III)** at δ 21.11 (q) and 170.55 (s) for the acetyl group were disappeared in the spectrum of compound **(V)**, also the signals for C-2 and C-4 were downfield shifted by 4.51 and 1.48 ppm, respectively, while the signal for C-3 was upfield shifted by 2.64 ppm confirming the removal of the acetate group from C-3 position of compound **(III)**.[35] The IR also showed only one carbonyl absorption band (at 1690 cm$^{-1}$) and the disappearance of the ester carbonyl band (at 1720 cm$^{-1}$) comparing with compound **III**. The MS showed M$^+$ at m/z 474 calculated for $C_{30}H_{50}O_4$ also confirm this finding. When compound **(V)** was acetylated with $Ac_2O$ and pyridine with usual way, it gave monorubiprasin A acetate as colorless needles with m.p 271-272° (reported 270-271°).[15]

From all the above mentioned data compound **(V)** was identified as 3β,13β,15α-trihydroxyoleanane-12-one [deacetylrubiprasin A]. According to the available literature this is the first report for the isolation of this compound from natural source.

**Compound (VI)**: was obtained as fine needles, the IR spectrum showed the presence of hydroxyl group(s) (3440 cm$^{-1}$) and unsaturated position (1635 cm$^{-1}$). $^1$H-NMR spectrum showed the presence of two doublet methyl signals at δ 0.93 and 0.87, (3H each, d, *J*= 6.0 Hz) assigned for C-29 and C-30 and six singlet methyl signals (3H each, s) indicating that compound **(VI)** is a triterpene with eight methyl groups, the position of the two doublet methyls (δ 0.87 and 0.93), a singlet methyl at δ 1.29 and an olefinic proton at δ 5.45 (br.d, *J*= 6.0 Hz) suggested that compound **(VI)** has an arbor-9 (11)-ene nucleus.[16,36-38] Arborane and fernane

triterpenoids have the same configuration at 5- and 10 position, but they have enantiomeric ones at the 8-, 13-, 14-, 17-, 18-, and 21-positions.[36-38] So the relationship between $C_{10}$-$CH_3$ and $C_8$-H is *syn*-1,3-diaxial in arborane, but *anti*-1,3-diaxial in fernane.[16,38] The signal at δ 3.51 (1H, dd, *J*= 10.4 and 6.0 Hz) was assigned for H-3 α, this indicates that the –OH group is present in β- configuration, while the signal at δ 4.50 (1H, td, *J*= 9.0 and 2.0 Hz) was assigned to H-19 β indicating that the –OH group at C-19 is present in α-configuration. The signal at δ 5.45 (1H, br.d, *J*= 6.0 Hz) was assigned to the olefinic proton (H-11) and the doublet signal at δ 2.38 (1H, d, *J*= 9.0 Hz) was assigned for H-18. Since one of the two-hydroxyl groups was deduced to be at C-3, the other hydroxyl must be at C-19 because H-18 appeared at δ 2.38 (1H, d, *J*= 9.0 Hz);[16] if the second hydroxyl group was present at C-7 as in case of some isolated compounds, the C-6 proton will appear at δ ≅ 2.20-2.31 as ddd (*J*= 13.0, 5.0, 2.0 Hz).[16]

$^{13}$C-NMR spectrum showed the presence of 30 Carbon signals, the two signals at δ 148.88 (s) and 114.97 (d) were assigned for the olefinic carbons (C-9 and C-11, respectively) and the two doublet signals at δ 78.14 and 70.25 were assigned for the two oxygen carrying carbons (C-3 and C-19, respectively). Comparing the chemical shifts of the two carbons and the chemical shifts of the neighboring carbons with those reported for the other arboranes deduced that the two hydroxyl groups must be present at C-3 and C-19, since in all the isolated arboranes the hydroxyl group at C-19 usually resonated at δ ≈ 70 ppm, but when it present at C-7 usually resonated at δ ≈ 72 ppm.[16,37] The other carbon signals are assigned as listed in (Table 1) using other arboranes as guidance.[16,37] The molecular formula of compound **VI** was deduced to be $C_{30}H_{50}O_2$ from the MS, $^1$H-, $^{13}$C-NMR and DEPT. EI-MS should $M^+$ at m/z 442, other peaks for loss of methyl group at m/z 427 and peak at m/z 424 for the loss of water were present. The other fragments suggesting that compound **VI** is a pentacyclic triterpene alcohol having a double bond at the 9 (11) position and the position of the hydroxy functions was deduced to be at C-3 and C- 19.[13,36,39]

From all the above mentioned data, compound **(VI)** was identified as 3β,19α-dihydroxy arbor-9(11)-ene. According to the available literature this is the first report for the isolation of this compound from natural source.

**Compound (VII)**: m.p, IR, $^1$H-NMR and $^{13}$C-NMR (Table 1) of compound **(VII)** are identical to those reported for oleanolic acid.[35,40] Further conformation was carried out using authentic sample. Acetylation gave acetate similar to compound **IV** (m.p, m.m.p and IR).

**Compound (VIII)**: m.p, IR, $^1$H-NMR and $^{13}$C-NMR (Table 1) of compound **(VIII)** are identical to those reported for hederagenin.[35,41,42] This is the first report for isolation of hederagenin from *R. cordifolia* L.

The results of cytotoxic activity (Table 2) showed that; non of the isolated compounds showed a significant cytotoxic activity on the chosen cell line (V-79 cells), compounds **I**, **III**, **IV** and **V** showed a marked cytotoxic activity, compound **VII** showed a weak activity while compound **VI** didn't showed any activity against Chinese hamster cells (V-79 cells). Since the total extract showed a marked cytotoxic activity similar to some of the isolated compounds and some of the isolated compounds showed less activity, the activity of the total fraction may be due to other compounds rather than the triterpenes such as quinones.[10]

**Table 2:** Cytotoxic activity of the isolated compounds.

| Compound No. | $IC_{50}$ (μg/ml) |
|---|---|
| **I** | 25 |
| **II** | ..* |
| **III** | 31 |
| **IV** | 18 |
| **V** | 25 |
| **VI** | >100 |
| **VII** | 58 |
| **VIII** | * |

* Not tried.

Many ursanes and fernanes triterpenes[13,43] were isolated from *Rubia* spices. Oleanane analogs bearing a ketonic carbonyl group at C-12 or hydroxyl group at C-13[44,45] have been reported independently. However, oleanane

types triterpenes having both functions at C-12 and C-13 were isolated from *Rubia cordifolia* var. *pratensis*, but here they were isolated for the first time from *Rubia cordifolia* L. The isolation of the rare arborane type triterpene from *Rubia cordifolia* L. is important from the taxonomic point of view, since they are reported once before in the genus *Rubia*.[16]

**Acknowledgment**

The author would like to thank Prof. Dr. H. Itokawa and Prof. Dr. K. Takeya, Tokyo University of Pharmacy and Life Science for providing the plant material and measuring the NMR and MS spectra and measuring the cytotoxic activity of the isolated compounds.

## REFERENCES

1- W. Tang and G. Eisenbrand, "Chinese Drugs of Plant Origin", Chemistry, Pharmacology, and Use in Traditional and Modern Medicine, Springer-Verlag, Berlin, Heidelberg (1992), pp 885-895, and references cited therein.

2- H. Itokawa, K. Mihara and K.Takeya, Chem. Pharm. Bull., 31, 2352 (1983).

3- H. Itokawa, Y.-F. Qiao and K.Takeya, Phytochemistry, 28, 3465 (1989).

4- H. Itokawa, Y.-F. Qiao and K. Takeya, Phytochemistry, 30, 637 (1991).

5- Y. F. Qiao, K. Takeya and H. Itokawa, Chem. Pharm. Bull., 38, 2896 (1990).

6- R. Wijnsma, R. Verpoorte, Th. Mulder-Krieger and A.B. Svendsen, Phytochemistry, 23, 2307 (1984).

7- R. Wijnsma, and R. Verpoorte, "Progress in the Chemistry of Organic Natural Products", Vol. 49, ed. By W. Herz, H. Griesebach, G. W. Kirby and Ch. Tamm, Springer-Verlag, Wien-New York (1986), pp.79-149, and references cited therein.

8- N. Warma, P. Painuly, S. C. Sharma and J. S. Tandon, Ind. J. Chem., 24B, 791 (1985).

9- Ch. Dosseh, A. M. Tessier and P. Delaveau, Planta Medica, 43, 141 (1981).

10- H. Itokawa, Z. Z. Ibraheim, Y.-F. Qiao and K. Takeya, Chem. Pharm. Bull., 41, 1869 (1993).

11- P. P. Gupita, R. C. Srimal, N. Verma and J. S. Tandon, Pharm. Biol., 37, 46 (1999).

12- L. C. Chang, D. Chavez, J. J. Gills,.H. H. S. Fang, J. M. Pezzuto and A. D. Kinghorn, Tetrahedron Lett., 41 (37), 7152 (2000)

13- S. K Talapatra, A. S. Sarkar and B. Talapatra, Phytochemistry, 20, 1923 (1981).

14- M. Arisawa, H. Ueno, M. Nimura, T., Hayashi and N. Morita, J. Nat. Prod., 49, 1114 (1986).

15- H. Itokawa Y. -F. Qiao K. Takeya and Y. Iitaka, Chem. Pharm. Bull, 37, 1670 (1989).

16- H. Itokawa, Y. -F. Qiao and K. Takeya, Chem. Pharm. Bull, 38, 1435 (1990).

17- H. Inouye, Y. Takeda, H. Nishimura, A. Kanomi, T. Okuda and C. Puff, Phytochemistry, 27, 2591 (1988).

18- A. Bianco, M. Guiso, C. Iavarone, P. Passacantilli and C. Trogolo, Gazz.Chim. Ital., 108, 13 (1978).

19- H. A. Hassanean, Z. Z. Ibraheim, K. Takeya and H. Itokawa, Pharmazie, 54, 12, 1999.

20- H. Morita, T. Yamamiya, K. Takeya and H. Itokawa, Chem. Pharm. Bull., 40, 1352 (1992).

21- H. Itokawa, T. Yamamiya, H. Morita and K. Takeya, J. Chem. Soc., Perkin Trans., 1, 455 (1992).

22- H. Itokawa, H. Morita, K. Takeya, N. Tomioka, A. Itai and Y. Iitaka, Tetrahedron, 47, 7007, (1991).

23- H. Itokawa, K. Takeya, N. Mori, T. Sonobe, S. Mihashi and T. Hamanaka, Chem. Pharm. Bull., 34, 3762 (1986).

24- H. Itokawa, K. Takeya, N. Mori, T. Hamanaka and K. Mihara, Chem. Pharm. Bull., 32, 284 (1984).

25- K. Takeya, T. Yamamiya, H. Morita and H. Itokawa, Phytochemistry, 33, 613 (1993).

26- H. Itokawa, K. Takeya, N. Mori, M. Takahashi, H. Yamamoto, T. Sonobe and S. Kidokoro, Jpn. J. Cancer Res. (Gann), 75, 929 (1984).

27- H. X. Wang and B. J. Wang, Chin. Tradit. Herb. Drugs, 29, 219 (1998).

28- M. K. Adwankar and M. P. Chitnis, Biomed. Pharmachother., 36, 104 (1982).

29- Y. B. Tripathi, M. Sharma and M. Manickam, Indian J. Biochem. Biophys., 34, 302 (1997).
30- L. K. Ho, M. J. Don, H. C. Chen, S. F. Yeh and J. M. Chen, J. Nat. Prod., 59, 330 (1996).
31- H. A. Galani, K. H. Janbaz, M. Zaman, A. Lateef, A. Suria and H. R. Ahmed, J. Pak. Med. Association, 44, 82 (1994).
32- Y. -F. Qiao, S. X. Wang, L. J. Wu, X. Li and T. R. Zhu, Yao Hsuch Hsuch Pao, 25, 834 (1990).
33- Z. Z. Ibraheim, Bull. Pharm. Sci. Assiut University, 25 (1), 85, (2002).
34- J.S. Glasby,"Encyclopaedia of the Terpenoids", A Wiley-Interscience Publication, John Wiley & Sons, Chichester. New York, Brisbane. Toronto. Singapore (1982), p. 1819.
35- S. B. Mahato and A. P. Kundu, Phytochemistry, 37, 1517 (1994).
36- K. Nishimoto, M. Ito and S. Natori, Tetrahedron, 24, 735 (1968) and references cited therein.
37- T. Akihisa, K. Yamamoto, T. Tamura, Y. Kumura, T. Iida, T. Nambara and F. Chang, Chem. Pharm. Bull., 40, 789 (1992).
38- K. Nakanishi, T. Goto, S. Ito, S. Natori and S. Nozoe, "Natural Product Chemistry" vol. 1, Kodansha, Tokyo, Academic Press, New York (1974), p. 365, 377, and references cited therein.
39- W. H. Hui and C. W. Lam, Phytochemistry, 4, 333 (1965).
40- M. Maillard, C. O. Adewunmi and K. Hostettman, Phytochemistry, 31, 1321 (1992).
41- H. Kizu and T. Tomimori, Chem. Pharm. Bull., 30, 3340 (1982).
42- V. U. Ahmad and Att-ur-Rahman "Handbook of Natural Products Data, II". Pentacyclic Triterpenoids. In: A. U. Rahman, editor. Amsterdam; Elsevier, 223 (1994).
43- D. M. Doddrell, P. W. Khond and K. G. Lewis, Tetrahedron Letters, 2381 (1974).
44- M. J. Begley, L. Crombie, W. M. L. Crombie and D. A. Whiting, J. Chem. Soc. Perkin Trans., 1, 1905 (1986).
45- R. Tschesche, S. Sepulveda and T. M. Braun, Chem. Ber., 113, 1754 (1980).