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Rubiasins A–C, new anthracene derivatives from the roots and stems of *Rubia cordifolia*

Leng Chee Chang,† Daniel Chávez, Joell J. Gills, Harry H. S. Fong, John M. Pezzuto and A. Douglas Kinghorn*

Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA

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Abstract

Three new anthracene derivatives, rubiasins A–C (**1**–**3**), were isolated from the combined roots and stems of *Rubia cordifolia*, and their structures were elucidated by spectroscopic analysis. Their absolute configurations were determined by Mosher ester methodology. A known compound, mollugin (**4**), was obtained as an active antiproliferative principle by bioassay-monitored fractionation using a human colon cancer (Col2) cell line.

The dried roots and rhizomes of *Rubia cordifolia* L. (Rubiaceae) are listed officially as a herbal medicine in the Chinese Pharmacopeia for the treatment of arthritis, dysmenorrhea, hematorrhea, and hemostasis, as a tonic, and for wound healing.[1] In addition, this plant has been used for menstrual pain, rheumatism, and urinary disorders in India.[2] Extracts of *R. cordifolia* have shown antibacterial, antineoplastic, antiviral, and hepatoprotective activities.[3–6] A number of constituents have been reported from *R. cordifolia* including several anthraquinones,[7,8] cyclic hexapeptides,[9,10] and triterpenoids.[11,12] As part of an ongoing project directed toward the discovery of novel naturally occurring cancer chemopreventive agents from plants, we report herein the isolation of three new inactive anthracene derivatives (**1**–**3**) from *R. cordifolia* of a rare structural type, as well as one active antiproliferative compound, the previously known naphthohydroquinone, mollugin (**4**).

* Corresponding author. Tel: 312 996-0914; fax: 312 996-7107; e-mail: kinghorn@uic.edu

† Current address: National Institutes of Health, Laboratory of Bioorganic Chemistry, NIDDK, Building 8, Room 1A-02, Bethesda, MD 20892-0820, USA.

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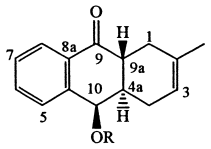
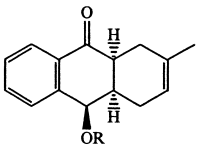


1 R = H
1s R = (*S*)-MTPA
1r R = (*R*)-MTPA



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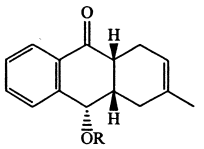
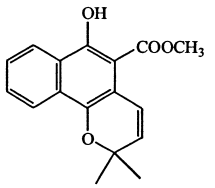


3 R = H
3s R = (*S*)-MTPA
3r R = (*R*)-MTPA



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A methanolic extract (90%) of the air-dried combined roots and stems of *R. cordifolia*[13] (9.8 kg) was partitioned with petroleum ether and EtOAc, respectively, to afford dried petroleum ether- (15 g) and EtOAc-soluble (450 g) residues. Activity-guided fractionation of the EtOAc-soluble residue using a human colon cancer (Col2) cell line, involving successive Si gel, Sephadex LH-20 ($CHCl_3$:MeOH, 1:1) and HPLC (YMC, Inc., ODS, MeCN:H_2O, 55:45) chromatographic steps, afforded three new compounds [**1**[14] (7.0 mg, 0.00007% w/w), **2**[14] (9.0 mg, 0.00009% w/w), and **3**[14] (2.5 mg, 0.00003% w/w)], along with six known constituents, mollugin[15] (**4**) (400 mg, 0.004% w/w), alizarin[16] (50.0 mg, 0.0005% w/w), 1,3-dihydroxy-2-ethoxymethyl-9,10-anthraquinone[16] (10.0 mg, 0.0001% w/w), 3-dihydroxy-2-methoxymethyl-9,10-anthraquinone[17] (16.0 mg, 0.00016% w/w), furomollugin[16] (53.0 mg, 0.0005% w/w), and 1-hydroxy-2-methyl-9,10-anthraquinone[18] (30.0 mg, 0.0003% w/w).

A molecular formula of $C_{15}H_{16}O_2$ was determined from the HRCIMS of compound **1**. Comparison of its ^1H and ^{13}C NMR data (Table 1) with literature values indicated that it is structurally related to the quinone class.[19,20] Compound **1** exhibited characteristic UV bands for a dihydronaphthoquinone nucleus (λ_{max} at 247 and 286 nm).[21] The functional groups present in the molecule of **1** could be assigned as an α,β-unsaturated carbonyl (ν_{max} 1610 and 1664 cm^{-1}; δ_C 199.9), a hydroxyl group (ν_{max} 3466 br cm^{-1}; δ_C 69.9), an olefinic proton (δ_C 119.7 and 131.3; δ_H 5.47), and a methyl functionality (δ_C 23.6; δ_H 1.74). The location of the hydroxyl group at C-10 was determined by analysis of the HMQC and HMBC (Table 1) spectral data, in which cross-peaks were observed between δ_H 4.72 (H-10) and δ_C 27.9 (C-4), δ_C 129.1 (C-5), and δ_C 143.9 (C-10a). The positions of the methyl at C-2 and the olefinic proton at C-3 were determined from HMBC correlations between δ_H 1.74 (Me-2) with δ_C 30.8 (C-1), δ_C 131.3 (C-2), and δ_C 119.7 (C-3), and between δ_H 5.47 (H-3) with δ_C 39.4 (C-4a), respectively, confirming that **1** possesses a pentahydroanthraquinone-type of nucleus. The relative stereochemistry of **1** was assigned based on a NOESY experiment, in which H-10 (δ_H 4.72) showed an NOE correlation with H-4a (δ_H 2.25), but not with H-9a (δ_H 2.99). Accordingly, the H-10 and H-4a protons were determined as having the same orientation in the molecule of **1**. In addition, no NOE interaction occurred between H-4a and H-9a, supporting the placement of these protons in a *trans* orientation. The absolute configuration of the stereogenic centers in **1** was determined using Mosher ester methodology.[22] Compound **1** was treated with (*R*)- and (*S*)-(–)α-methoxy-α-(trifluoromethyl)phenylacetyl chloride to obtain the (*S*)- (**1s**) and (*R*)-ester (**1r**) as C-10 analogues (see Experimental section).[23] Analysis of the $\Delta\delta_{H(S-R)}$ data (Table 2) showed a positive difference in chemical shift for protons in the cyclohexene moiety indicating that the absolute configuration at C-10 was *R*. Thereafter, the absolute configuration at C-4a and C-9a was deduced as *R* in both cases, because of the *cis* configuration for H-10/H-4a and the *trans* relationship of H-4a/H-9a. Thus, the structure of **1** was determined to be (–)-(4a*R*,9a*R*,10*R*)-1,4,4a,9a,10-pentahydro-10-hydroxy-2-methyl-2-en-anthracen-9-one, to which we have accorded the trivial name rubiasin A.

Table 1
NMR spectral data and HMBC correlations for **1**–**3**[a]

#	**1**			**2**			**3**		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1α	2.18 m	30.8 t	4a, 9a	2.16 m	29.4 t	4a, 9a	2.19 m	24.8 t	4a, 9a
1β	2.55 m		2, 3	2.86 m		2,3	3.01 m		2,3
2		131.3 s			131.0 s		5.45 br s	120.0 d	4a
3	5.47 s	119.7 d	4a	5.43 br s	118.9 d	4a		131.3 s	
4α	2.18 m	27.9 t	4a, 9a	1.90 m	21.6 t	4a,	1.71 m	26.7 t	4a,
4β	2.55 m		2, 3	2.16 m		2,3	2.15 m		2,3
5	7.41 dd (7.5, 1.0)	129.1 d	6, 7, 10	7.73 dd (7.8, 1.0)	126.9 d	6, 7, 10	7.73 dd (7.8, 1.0)	126.7 d	6, 7, 10
6	7.58 ddd (7.5, 7.4, 1.0)	134.2 d	7, 8, 10a	7.62 ddd (7.5, 7.4, 1.0)	134.2 d	8, 10a	7.63 ddd (7.7, 7.5, 1.3)	134.3 d	8, 10a
7	7.46 ddd (7.5, 7.4, 1.0)	129.4 d	5, 8	7.40 ddd (7.5, 7.4, 1.0)	127.9 d	5, 8, 8a	7.40 ddd (7.5, 7.4, 1.0)	128.1 d	5,6
8	8.06 dd (7.8, 1.0)	128.0 d	9, 8a, 10a	8.00 dd (7.8, 1.3)	127.9 d	6, 9, 10a	8.03 dd (7.8, 1.3)	127.1 d	8a, 10a
9		199.9 s			197.8 s			197.8 s	
10	4.72 br s	69.9 d	4, 5, 10a	5.43 br s	71.3 d	4, 10a	5.33 br s	71.3 d	4, 10a
8a		132.8 s			133.2 s			133.2 s	
10a		143.9 s			143.9 s			143.9 s	
9a	2.99 m	40.8 d	1, 4, 9, 4a	2.90 m	45.9 d	1, 4, 9, 4a	2.88 m	45.2 d	4a, 4
4a	2.25 m	39.4 d	4, 9a	2.76 m	40.6 d	4, 9, 9a, 10a,	2.85 m	41.2 d	4, 9a, 10, 10a
CH_3	1.74 s	23.6 q	1, 2, 3	1.73 s	23.8 q	1, 2, 3	1.61 s	23.9 q	2, 3,4

[a]500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR, $CDCl_3$. Figures in parentheses are coupling constants; carbon multiplicities were determined according to DEPT spectra.

Table 2
Partial ^{1}H NMR data of the (*S*)- and (*R*)-Mosher ester derivatives of compounds **1**–**3**[a]

	δ_H			δ_H			δ_H		
proton	**1s**	**1r**	$\Delta\delta_{S-R}$	**2s**	**2r**	$\Delta\delta_{S-R}$	**3s**	**3r**	$\Delta\delta_{S-R}$
1α	2.06	2.05	+0.01	2.16	2.15	+0.01	2.19	2.20	-0.01
1β	2.59	2.58	+0.01	2.85	2.84	+0.01	2.95	2.96	-0.01
2	-	-	-	-	-	-	5.36	5.40	-0.04
CH_3-2	1.71	1.69	+0.02	1.71	1.69	+0.02	-	-	-
3	5.40	5.34	+0.06	5.23	5.14	+0.09	-	-	-
CH_3-3	-	-	-	-	-	-	1.41	1.49	-0.08
4α	2.26	2.08	+0.18	1.89	1.72	+0.17	1.60	1.71	-0.11
4β	2.23	2.11	+0.12	1.80	1.72	+0.08	1.60	1.63	-0.03
4a	2.41	2.40	+0.01	2.97	2.96	+0.01	2.95	2.96	-0.01
9a	2.82	2.82	0	3.03	3.02	+0.01	3.00	3.02	-0.02
10	6.18	6.22	*R*[b]	6.65	6.63	*R*[b]	6.63	6.65	*S*[b]

[a]Obtained in $CDCl_3$ at 500 MHz.
[b]Absolute configuration.

Compound **2** was shown to possess a molecular formula of $C_{15}H_{16}O_2$ by HRCIMS. Analysis of its EIMS, ^{1}H and ^{13}C NMR data suggested that **2** is a stereoisomer of **1** (Table 1), with the only differences being the relative stereochemistry at H-10, H-4a, and H-9a for these anthracene derivatives, thereby affecting the chemical shifts observed. Downfield shifts for the ^{1}H NMR signals of **2** at H-4 (δ_H 1.90, m and 2.16, m), H-4a (δ_H 2.76, m), H-5 (δ_H 7.73, dd), and H-10 (δ_H 5.43, br s) (Table 1) were observed as compared with **1**. Moreover, a ^{13}C NMR downfield shift

was also observed for C-9a (δ_C 45.9). The relative stereochemistry at the H-10, H-4a, and H-9a positions was again investigated by applying a NOESY experiment. Cross-peaks between H-10 and H-4a, and between H-10 and H-9a confirmed that these pairs of protons are both in a *cis* arrangement in the molecule of **2**. The absolute configuration at C-10 of **2** was also determined by analysis of the ^{1}H NMR data of the (*S*)- and (*R*)-Mosher ester derivatives **2s** and **2r** (Table 2), in a similar manner to the procedure described for **1**. The positive values ($\Delta\delta_{S-R}$) obtained for the cyclohexene moiety showed that the absolute stereochemistry of the chiral center at C-10 was *R*. The absolute stereochemistry for C-4a and C-9a was deduced as *R* and *S*, respectively, because of the *cis* configurations for H-10/H-4a and H-4a/H-9a. Thus, rubiasin B (**2**) was assigned as (–)-(4a*R*,9a*S*,10*R*)-1,4,4a,9a,10-pentahydro-10-hydroxy-2-methyl-2-en-anthracen-9-one.

Compound **3** was shown to possess a molecular formula of $C_{15}H_{16}O_2$ by HRCIMS. Comparison of its ^{1}H and ^{13}C NMR data (Table 1) with **2** indicated that they were very closely related isomers. When the spectroscopic data of compound **2** and **3** were compared, the major differences for these isomers were due to the methyl and olefinic protons. HMBC data supported the assignment of the methyl group at C-3 and the olefinic proton (δ_H 5.45) at C-2 from observed correlations between δ_H 1.61 (Me-3) with δ_C 26.7 (C-4), δ_C 131.3 (C-3), and δ_C 120.0 (C-2). Thus, the HMBC data ruled out the possibility of the opposite locations of these two functionalities, as in **2**. The relative stereochemistry of the chiral centers of **3** was determined by a NOESY experiment, in which strong cross-peaks between H-9a and H-4a and H-10, and between H-4a with H-9a, respectively, indicated that these protons all have the same orientation. The absolute configuration of the stereogenic center at the C-10 position in **3** was determined in a similar manner to **1** and **2**. Analysis of the $\Delta\delta_H(_{S-R})$ data (Table 2) showed a negative difference in chemical shift for protons in the cyclohexene moiety indicating that the absolute configuration at C-10 was *S*. Therefore, rubiasin C (**3**) was assigned as (+)-(4a*S*,9a*R*,10*S*)-1,4,4a,9a,10-pentahydro-10-hydroxy-3-methyl-2-en-anthracen-9-one.

All of the isolates obtained in this investigation were evaluated for their antiproliferative activity using Col2 human colon cancer cells, according to an established protocol.[24] Compounds with IC_{50} (half maximal inhibitory concentration) values of $\leq$5 μg/mL are considered active.[25] However, in the present investigation, only mollugin (**4**) was detected as an active antiproliferative compound, with an IC_{50} value of 3.5 μg/mL (12.3 μM). Isolates **1**–**3** and the known compounds alizarin, 1,3-dihydroxy-2-ethoxymethyl-9,10-anthraquinone, 3-dihydroxy-2-methoxymethyl-9,10-anthraquinone, furomollugin, and 1-hydroxy-2-methyl-9,10-anthraquinone were inactive in this test system. Mollugin (**4**) has shown strong suppressive activity on hepatitis B surface antigen secretion in human hepatoma Hep3B cells,[26] and is being further investigated in our laboratory for cancer chemopreventive activity.

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13. The roots and stems of *Rubia cordifolia* were purchased in September, 1999 from Dhawan International, New Delhi, India. A voucher specimen (accession number PB0268) has been deposited at the University of Illinois Pharmacognosy Field Station.
14. Compound **1**: Colorless needles; mp 146–148°C; $[\alpha]_D^{20}$ –123.2 (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 213 (3.9), 247 (4.0), 286 (3.3) nm; CD (MeCN) nm $\Delta\varepsilon_{336}$ +3.05, $\Delta\varepsilon_{255}$ –11.0, $\Delta\varepsilon_{221}$ –6.3, $\Delta\varepsilon_{210}$ +13.6; IR (film) ν_{max} 3466, 1664, 1610, 990, 905 cm^{-1}; ^1H NMR and ^{13}C NMR ($CDCl_3$), see Table 1; EIMS *m*/*z* $[M]^+$ 228 (28), 209 (71), 195 (100), 165 (17), 105 (25); HRCIMS *m*/*z* $[M+H]^+$ 229.1228 [calcd for $C_{15}H_{17}O_2$, 229.1228]. Compound **2**: Colorless needles; mp 138–140°C; $[\alpha]_D^{20}$ –240.8 (*c* 0.03, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 214 (4.0), 246 (4.0), 290 (3.3) nm; CD (MeCN) nm $\Delta\varepsilon_{254}$ +1.35, $\Delta\varepsilon_{240}$ –0.14, $\Delta\varepsilon_{211}$ –21.8; IR (film) ν_{max} 3460, 1658, 990, 903 cm^{-1}, ^1H NMR and ^{13}C NMR ($CDCl_3$), see Table 1; EIMS *m*/*z* $[M]^+$ 228 (6), 210 (80), 195 (100), 165 (13), 119 (26), 105 (48); HRCIMS *m*/*z* $[M+H]^+$ 229.1227 [calcd for $C_{15}H_{17}O_2$, 229.1228]. Compound **3**: Colorless needles; mp 109–112°C; $[\alpha]_D^{20}$ +110.0 (*c* 0.03, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 (4.0), 244 (3.5), 286 (2.8) nm; CD (MeCN) nm $\Delta\varepsilon_{268}$ –0.30, $\Delta\varepsilon_{246}$ +0.13, $\Delta\varepsilon_{208}$ +17.7; IR (film) ν_{max} 3455, 1659, 990, 905 cm^{-1}; ^1H NMR and ^{13}C NMR ($CDCl_3$), see Table 1; HRCIMS *m*/*z* $[M+H]^+$ 229.1208 [calcd for $C_{15}H_{17}O_2$, 229.1228].
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23. Preparation of (*S*)- and (*R*)-MTPA ester derivatives of compounds **1**–**3**.[22] To solutions of compounds **1**–**3** (0.8 mg in a 0.5 mL of $CHCl_3$) were added sequentially pyridine (100 μL), 4-(dimethylamino)pyridine (0.5 mg), and (*R*)-(–)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (10 mg). Each mixture was heated at 50°C for 4 h under N_2 and then passed through a disposable pipet (0.6×5 cm) packed with silica gel and eluted with 5 mL of $CHCl_3$. The solvent was removed in vacuo, to obtain the respective *S*-Mosher ester **1s**, **2s**, and **3s**. Individual treatment of compounds **1**–**3** [0.8 mg with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride], as described above, yielded the *R*-Mosher esters **1r**, **2r**, and **3r**, respectively (^1H NMR data, Table 2).

24. Bioassay for antiproliferative activity. A human colon cancer cell line (Col2) was cultured in MEME medium containing 10% non-essential amino acid solution (NAA), 1×antibiotic-antimycotic (Gibco BRL, Grand Island, NY), plus 10% fetal bovine serum (FBS) at 37°C in a 5% CO_2 atmosphere. To perform the sulforhodamine B (SRB) assay, exponentially growing cells were added to 96-well microtiter plates containing test compounds dissolved in DMSO. Cells were allowed to grow at 37°C, and, after 3 days they were fixed with trichloroacetic acid and stained with 0.4% sulforhodamine B in 1% acetic acid. The bound dye was solubilized in 0.1 M tris and absorbance at A_{515} was measured. Percent growth was calculated from the formula: %growth = $(\text{Absorbance}_{\text{sample}}-\text{Absorbance}_{\text{day 0}})/(\text{Absorbance}_{\text{DMSO control}}-\text{Absorbance}_{\text{day 0}})\times 100$. For additional details, see Ref. 25.
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