REVIEW

9,10-Anthraquinones and Other Biologically Active Compounds from the Genus *Rubia*

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The secondary metabolites isolated from *Rubia* species, their biological activities, and colouration properties have been reviewed. Over 150 chemical constituents belonging to different classes of bioactive compounds such as anthraquinones and their glycosides, naphthoquinones and glycosides, terpenes, bicyclic hexapeptides, iridoids, and carbohydrates are listed together with their source(s) and corresponding references.

Contents

1. Introduction. – The genus *Rubia* belongs to the family Rubiaceae, which comprises *ca.* 450 genera and 6500 species and includes trees, shrubs, and, infrequently, herbs. *Rubia* has over 60 species distributed widely, including Africa, temperate Asia, and America. About 15 species are reported from India [1]. They are perennial, herbaceous climbing plants, with very long roots, cylindrical and flexuous, with a thin red bark. Stems often have a long, rough, grooved, woody base. The *Rubia* species have high commercial, economical, and medicinal importance. Plants belonging to this species are known to contain substantial amounts of anthraquinones, especially in the roots [2][3]. Economically, the *Rubia* species are important as the source of potential 'biocolorants' (natural dyes). They had long been used in India and elsewhere for imparting various shades of red, scarlet, coffee-brown, and mauve to cotton and woolen fabrics [3]. Medicinally, the *Rubia* species are reputed in the Indian Ayurvedic system of medicine. In India and neighboring countries, *Rubia* has long history in skin care and treatment, and has been used in disorders of the urinary tract [4][5].

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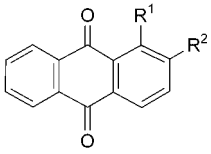
The chemistry of *Rubia* species has been widely investigated, and the phytochemical investigations from both hemispheres led to the isolation of a number of physiologically active compounds *viz.*, anthraquinones, naphthoquinones, terpenes, bicyclic hexapeptides, iridoids, and carbohydrates. The lack of a comprehensive review on this subject prompted us to gather information on the different classes of secondary metabolites isolated from various *Rubia* species with special emphasis on medicinal and biocoloration properties of the crude extracts and different classes of purified compounds.

2. Chemical Constituents of Genus *Rubia*. – Various compounds isolated from different *Rubia* species have been classified and discussed under five categories, *viz.* anthraquinones and their glycosides, naphthoquinones and their glycosides, terpenes, bicyclic hexapeptides, and miscellaneous, which includes iridoids, flavonoids, and carbohydrates, and are listed in *Tables 1–5*.

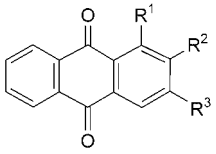
2.1. *Anthraquinones (AQs) and Their Glycosides.* Anthraquinones (=anthracene-9,10-diones, AQs) are common secondary metabolites occurring in bacteria, fungi, lichens, and higher plants [2][6–8]. In higher plants, they are found in a large number of plant families, including Verbenaceae [8], Bignoniaceae [9], Rhamnaceae [10], Polygonaceae [11], Leguminosae [12], and Rubiaceae [13]. It has been established that the AQs originate from a variety of different precursors and pathways [14][15]. There are two main biosynthetic pathways leading to AQs in higher plants: the polyketide pathway [16] and the chorismate/*o*-succinylbenzoic acid pathway [17], the latter occurs in the Rubiaceae.

The AQs, **1–50,** and their glycosides, **51–68**, found in genus *Rubia* differ in the nature of their substituents and the substitution patterns (*Table 1*). Most of the *Rubia* AQs are substituted in only one of the aromatic benzo rings. *Burnett* and *Thomson* isolated 19 AQs from the air-dried roots of mature *R. tinctorum* [18]. The presence of a glycoside of alizarin in *R. tinctorum* was first shown in 1847 by *Schunck*, who obtained an amorphous preparation, which he called 'rubian', but crystalline ruberythric acid (**60**) was isolated a few years later and was one of the first glycosides to be obtained pure [19]. *Murti et al.* later reported glycoside of lucidin, lucidin primeveroside (**61**) along with **60** from *R. tinctorum* [20] and *R. cordifolia* [21]. A complete list of AQs and their glucosides isolated from *Rubia* species together with their source(s) and references are listed in *Table 1*. The low content of biologically active substances in the genus *Rubia* has served as an incentive for the development of cell cultures. AQs such as munjistin (**17**) and purpurin (**29**) were major components of anthraquinone pigments produced by the callus cultures of *R. cordifolia* [22].

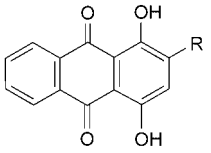
2.2. *Naphthoquinones and Their Glycosides.* Many plants of the family Rubiaceae have been found to be a rich source of anthraquinones, but the exhaustive investigation led also to the isolation of a few naphthoquinone derivatives, **69–99**. The first naphthoquinone to be isolated from *Rubia* species was 5- or 8-methoxy-3-(3-methylbut-2-enyl)-1,4-naphthoquinone (**82**) [45]. *Delavean* and co-workers isolated **82** from the EtOH extract of the roots of *R. cordifolia*, but they did not specify the position of MeO group [45]. The Et_2O-soluble fraction of MeOH extract of *R. cordifolia* also led to the isolation of 2-carbamoyl-3-hydroxy-1,4-naphthoquinone (**79**) and its 3-MeO derivative **80** [23]. The hexane-soluble fraction of the same extract gave



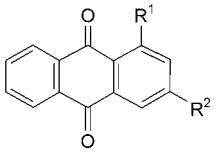
	R^1	R^2
1	H	Me
2	H	$HOCH_2$
3	H	MeO
4	H	OH
5	OH	H
6	OH	Me
7	OH	$HOCH_2$
8	OH	EtOOC
9	MeO	Me
10	OH	OH
11	OH	MeO
12	MeO	OH
13	MeO	MeO



	R^1	R^2	R^3
14	OH	Me	OH
15	OH	$HOCH_2$	OH
16	OH	CHO	OH
17	OH	HOOC	OH
18	OH	MeOOC	OH
19	OH	$EtOCH_2$	OH
20	OH	$MeOCH_2$	OH
21	MeO	$HOCH_2$	MeO
22	MeO	HOOC	MeO
23	OH	$PhCH_2$	OH
24	OH	OH	MeO
25	OH	MeO	MeO
26	OH	HOOC	MeO
27	MeO	$MeOCH_2$	OH
28	OH	OH	OH



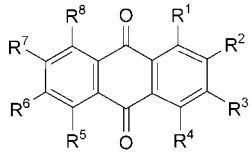
29 R = OH
30 R = H
31 R = $HOCH_2$
32 R = EtOOC
33 R = $EtOCH_2$
34 R = Me



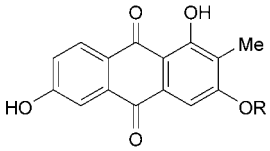
	R^1	R^2
35	OH	OH
36	OH	MeO
37	MeO	MeO
38	OH	MeOOC

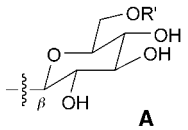
mollugin (**69**) and dehydro-α-lapachone (**84**) [23]. The $CHCl_3$-soluble fraction of MeOH extract of the roots of *R. oncotricha* also gave naphthoquinone and naphthohydroquinones [39]. The BuOH-soluble fraction of MeOH extract of the roots of *R. cordifolia* on column chromatography over *Amberlite XAD-2* and silica gel furnished **72**, **97**, and **98**. Rubinaphthins A – D (**91** – **94**) were isolated from the MeOH extract of the roots of *R. yunnanensis* [26]. Various naphthoquinones and their glycosidic derivatives, which have been isolated so far from *Rubia* species, are listed in *Table 2*.

2.3. *Terpenes and Their Glycosides.* Rubiacoumaric acid (**118**) and rubiafolic acid (**119**) were the first triterpene constituents isolated from the $CHCl_3$ extract of the defatted whole plant of *R. cordifolia* [70]. Later, three oleanolic type triterpenoids, rubiaprassin A – C (**115** – **117**), were isolated from the $CHCl_3$-soluble fraction of methanolic extract of the roots of *R. cordifolia* [71], and three arborinane-type triterpene glycosides, rubianosides II, III, and IV (**122** – **124**), and a triterpene,



	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8
39	OH	OH	HOOC	OH	H	H	H	H
40	OH	Me	H	OH	MeO	H	H	H
41	OH	Me	H	OH	H	H	H	MeO
42	OH	H	H	OH	H	Me	H	H
43	OH	Me	H	H	OH	H	H	H
44	OH	H	Me	H	H	MeO	H	OH
45	OH	Me	OH	H	H	OH	H	H
46	OH	H	H	OH	H	H	Me	H
47	H	Me	H	OH	OH	H	MeO	H
48	H	OH	Me	MeO	H	H	OH	H
49	H	Me	H	H	H	H	OH	H
50	H	HOOC	H	OH	H	H	H	H

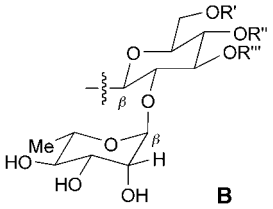




A

51 R = **A**, R' = H
52 R = **A**, R' = Ac

53 R = **B**, R' = R'' = R''' = H
54 R = **B**, R' = R'' = H, R''' = Ac
55 R = **B**, R' = Ac, R'' = R''' = H
56 R = **B**, R' = R''' = Ac, R'' = H
57 R = **B**, R' = H, R'' = R''' = Ac
58 R = **B**, R' = R''' = H, R'' = Ac



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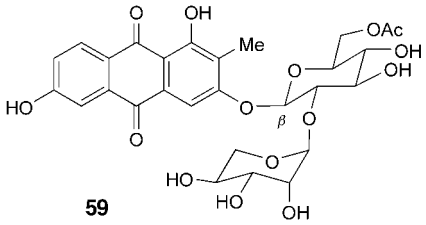

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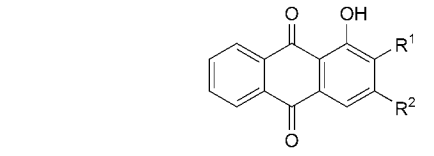
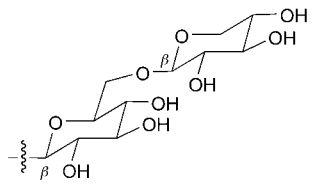
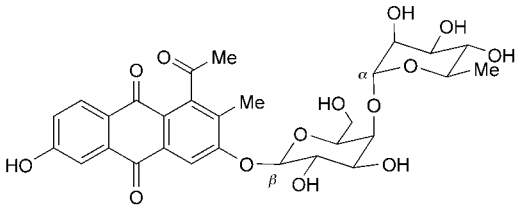


60 R^1 = RO, R^2 = H
61 R^1 = $HOCH_2$, R^2 = RO

R =





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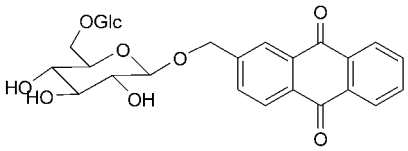

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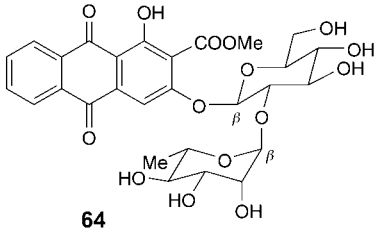

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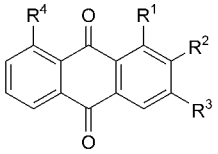


	R^1	R^2	R^3	R^4
65	H	$HOCH_2$	GlcO	H
66	H	$HOCH_2$	GlcO	OH
67	OH	GlcO	OH	H
68	OH	$HOCH_2$	GlcO	H

Glc = β-D-Glucopyranosyl

rubianol-a (**125**), were isolated from the roots of *R. yunnanensis* [59]. Six arborane-type triterpenoids, rubiarbonol A–F (**107**–**113**), were isolated from the MeOH extracts of *R. cordifolia* and *R. oncotricha* roots [72]. Various terpenes and their glycosidic derivatives, which have been isolated so far from *Rubia* species, are listed in *Table 3*.

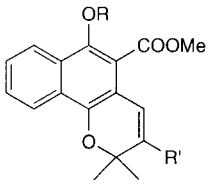
2.4. *Bicyclic Hexapeptides.* RA Series peptides are cyclic hexapeptides found in *Rubia* and are characterized by a bicyclic structure including a unique cycloisodityrosine unit. The bicyclic hexapeptides RA-III (**135**) to RA-VII (**138**) have been obtained from the benzene-soluble fraction of MeOH extracts of *R. cordifolia* and *R. alane* [78–80]. Similarly, RA-I (**133**) and RA-II (**134**) have been isolated from $CHCl_3$/MeOH 1:1 extract of *R. cordifolia* as minor constituents [81]. Further, the BuOH fraction of CH_2Cl_2/MeOH 1:1 extract of *R. cordifolia* gave RA-XI (**140**) and three unique bicyclic hexapeptide glucosides RA-**XII**–RA-**IV** (**141**–**143**) [82]. RA-IX and RA-X (**139**) from *R. cordifolia* were isolated from $CHCl_3$/MeOH 1:1 extract [83]. RA-XV (**144**) and RA-XVI were also isolated from CH_2Cl_2/MeOH 1:1 extract of *R.*

Table 1. *Anthraquinones* **1**–**50** *and Anthraquinone Glycosides* **51**–**68** *Isolated from Genus* Rubia

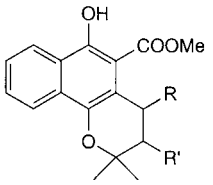
Compound No.	Name	Species
1	Tectoquinone (= 2-Methyl-AQ)	*R. cordifolia* [23], *R. oncotricha* [24], *R. tinctorum* [25]
2	2-(Hydroxymethyl)-AQ	*R. yunnanensis* [26], *R. tinctorum* [27]
3	2-Methoxy-AQ	*R. tinctorum* [18][28]
4	2-Hydroxy-AQ	*R. tinctorum* [18][28]
5	1-Hydroxy-AQ	*R. cordifolia* [29–31]
6	1-Hydroxy-2-methyl-AQ	*R. tinctorum* [18][28][32], *R. cordifolia* [23][33–38], *R. akane* [39] [40], *R. lanceolata* [39][41], *R. oncotricha* [39][24], *R. sylvatica* [39][42], *R. yunnanensis* [26]
7	1-Hydroxy-2-(hydroxymethyl)-AQ	*R. cordifolia* [24][43]
8	2-(Ethoxycarbonyl)-1-hydroxy-AQ	*R. akane* [39][40]
9	1-Methoxy-2-methyl-AQ	*R. tinctorum* [18]
10	Alizarin (= 1,2-Dihydroxy-AQ)	*R. akane* [44], *R. cordifolia* [21][37][38][45], *R. tinctorum* [18][20][27][44][46–48], *R. lanceolata* [39][40], R. iberica [49]
11	Alizarin 2-methyl ether (= 1-Hydroxy-2-methoxy-AQ)	*R. tinctorum* [18], *R. oncotricha* [24][39], *R. cordifolia* [33]
12	Alizarin 1-methyl ether (= 2-Hydroxy-1-methoxy-AQ)	*R. tinctorum* [18]
13	Alizarin 1,2-dimethyldiether (= 1,2-Dimethoxy-AQ)	*R. tinctorum* [18][20]
14	Rubiadin (= 1,3-Dihydroxy-2-methyl-AQ)	*R. tinctorum* [18], *R. cordifolia* [23][33][36], *R. lanceolata* [39][41], *R. yunnanensis* [26]
15	Lucidin (= 1,3-Dihydroxy-2-(hydroxmethyl)-AQ)	*R. cordifolia* [37], *R. tinctorum* [18][50], *R. iberica* [2][48]
16	Nordamnacanthal (= 1,3-Dihydroxy-2-formyl-AQ)	*R. cordifolia* [34][45], *R. iberica* [48], *R. tinctorum* [25]
17	Munjistin (= 1,3-Dihydroxy-2-carboxy-AQ)	*R. tinctorum* [18], *R. cordifolia* [36][51], *R. munjista* [52][53], *R. sikkimensis* [2][53], *R. chinensis* [2], *R. yunnanensis* [26]
18	1,3-Dihydroxy-2-(methoxycarbonyl)-AQ	*R. tinctorum* [25]
19	2-(Ethoxymethyl)-1,3-dihydroxy-AQ	*R. cordifolia* [37]
20	1,3-Dihydroxy-2-(methoxymethyl)-AQ	*R. cordifolia* [54]
21	Lucidin dimethyl ether	*R. lanceolata* [39][41]
22	Munjistin dimethyl ether (= 2-Carboxy-1,3-dimethoxy-AQ)	*R. cordifolia* [33]
23	2-Benzylxanthopurpurin	*R. tinctorum* [32]
24	Anthragallol 3-methyl ether	*R. tinctorum* [18]
25	Anthragallol 2,3-dimethyl ether	*R. tinctorum* [28]
26	2-Carboxy-1-hydroxy-3-methoxy-AQ	*R. cordifolia* [55]
27	3-Hydroxy-1-methoxy-2-(methoxymethyl)-AQ	*R. cordifolia* [54]
28	Anthragallol (= 1,2,3-Trihydroxy-AQ)	*R. tinctorum* [28]
29	Purpurin (= 1,2,4-Trihydroxy AQ)	*R. tinctorum* [18][20][46–48], *R. cordifolia* [2][21][29][31][45][51], *R. munjista* [2], *R. sikkimensis* [2], *R. tetragona* [2]
30	Quinizarin (= 1,4-Dihydroxy-AQ)	*R. cordifolia* [56], *R. tinctorum* [32][46]
31	1,4-Dihydroxy-2-(hydroxymethyl)-AQ	*R. cordifolia* [54], *R. yunnanensis* [26], *R. tinctorum* [46]
32	2-(Ethoxycarbonyl)-1,4-dihydroxy-AQ	*R. cordifolia* [55]
33	Christofin (= 2-(Ethoxymethyl)-1,4-dihydroxy-AQ)	*R. tinctorum* [46][47]

Table 1 (cont.)

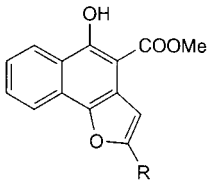
Compound No.	Name	Species
34	1,4-Dihydroxy-2-methyl-AQ	*R. cordifolia* [23][45], *R. lanceolata* [41]
35	Xanthopurpurin (= 1,3-Dihydroxy-AQ)	*R. tinctorum* [20][27][28], *R. cordifolia* [21][23][36][45], *R. oncotricha* [24][39], *R. yunnanensis* [26]
36	Xanthopurpurin 3-methyl ether (= 1-Hydroxy-3-methoxy-AQ)	*R. tinctorum* [28]
37	Xanthopurpurin dimethyl ether (= 1,3-Dimethoxy-AQ)	*R. tinctorum* [28]
38	1-Hydroxy-3-(methoxycarbonyl)-AQ	*R. lanceolata* [39][41], *R. oncotricha* [24][39], *R. cordifolia* [23]
39	Pseudopurpurin (= 3-(Carboxy)-1,2,4-trihydroxy-AQ)	*R. tinctorum* [27][28], *R. cordifolia* [2][45][57], *R. peregrina* [2]
40	1,4-Dihydroxy-2-methyl-5-methoxy-AQ	*R. cordifolia* [33]
41	1,4-Dihydroxy-2-methyl-8-methoxy-AQ	*R. cordifolia* [33]
42	1,4-Dihydroxy-6-methyl-AQ	*R. cordifolia* [34]
43	1,5-Dihydroxy-2-methyl-AQ	*R. cordifolia* [45]
44	Physcion (= 1,8-Dihydroxy-3-methoxy-6-methyl-AQ)	*R. cordifolia* [34]
45	2-Methyl-1,3,6-trihydroxy-AQ	*R. cordifolia* [31][36][37][43], *R. oncotricha* [24][39], *R. schumanniana* [39], *R. sylvatica* [39], *R. yunnanensis* [26][58]
46	1,4-Dihydroxy-7-methyl-AQ	*R. cordifolia* [45]
47	4,5-Dihydroxy-2-methoxy-7-methyl-AQ	*R. cordifolia* [45]
48	2,7-Dihydroxy-4-methoxy-3-methyl-AQ	*R. yunnanensis* [59]
49	2-Hydroxy-7-methyl-AQ	*R. tinctorum* [39][60]
50	2-Carboxy-4-hydroxy-AQ	*R. cordifolia* [54]
51	3-(β-D-Glucopyranosyloxy)-1,6-dihydroxy-2-methyl-AQ	*R. cordifolia* [31][36][38]
52	3-(6-*O*-Acetyl-β-D-glucopyranosyloxy)-1,6-dihydroxy-2-methyl-AQ	*R. cordifolia* [38]
53	3-[(2-*O*-6-Deoxy-α-L-mannopyranosyl-β-D-glucopyranosyl)oxy]-1,6-dihydroxy-2-methyl-AQ	*R. cordifolia* [36–38][61], *R. schumanniana* [62], *R. akane* [37], *R. yunnanensis* [26]
54	3-[(3-*O*-Acetyl-2-*O*-6-deoxy-α-L-mannopyranosyl-β-D-glucopyranosyl)oxy]-1,6-dihydroxy-2-methyl-AQ	*R. cordifolia* [36]
55	3-[(6-*O*-Acetyl-2-*O*-6-deoxy-α-L-mannopyranosyl-β-D-glucopyranosyl)oxy]-1,6-dihydroxy-2-methyl-AQ	*R. cordifolia* [36–39][61], *R. akane* [37], *R. yunnanensis* [26], *R. schumanniana* [62]
56	3-[(3,6-*O*-Diacetyl-2-*O*-6-deoxy-α-L-mannopyranosyl-β-D-glucopyranosyl)oxy]-1,6-dihydroxy-2-methyl-AQ	*R. cordifolia* [36]
57	3-[(4,6-*O*-Diacetyl-2-*O*-6-deoxy-α-L-mannopyranosyl-β-D-glucopyranosyl)oxy]-1,6-dihydroxy-2-methyl-AQ	*R. cordifolia* [36]
58	3-[(4-*O*-Acetyl-2-*O*-6-deoxy-α-L-mannopyranosyl-β-D-glucopyranosyl)oxy]-1,6-dihydroxy-2-methyl-AQ	*R. cordifolia* [63]
59	3-[(6-*O*-Acetyl-2-*O*-β-D-xylopyranosyl-β-D-glucopyranosyl)oxy]-1,6-dihydroxy-2-methyl-AQ	*R. cordifolia* [31]
60	Ruberythric acid (= 1-Hydroxy-2-[(6-*O*-β-D-xylopyranosyl-β-D-glucopyranosyl)oxy]-AQ)	*R. cordifolia* [21][37][61], *R. tinctorum* [20][27][64], *R. iberica* [49]
61	Lucidin primeveroside (= 1-Hydroxy-2-(hydroxymethyl)-3-[(6-*O*-β-D-xylopyranosyl-β-D-glucopyranosyl)oxy]-AQ)	*R. cordifolia* [37], *R. tinctorum* [20][27], *R. iberica* [49][65], *R. yunnanensis* [26]
62	1-Acetyl-3-[(4-*O*-6-deoxy-α-L-mannopyranosyl-β-D-glucopyranosyl)oxy]-6-hydroxy-2-methyl-AQ	*R. cordifolia* [66]
63	2-{[(6-*O*-β-D-Glucopyranosyl-β-D-glucopyranosyl)oxy]methyl}-11-hydroxy-AQ	*R. cordifolia* [63], *R. schumanniana* [62]
64	3-[(2-*O*-6-Deoxy-α-L-mannopyranosyl-β-D-glucopyranosyl)oxy]-1-hydroxy-2-(methoxycarbonyl)-AQ	*R. cordifolia* [63]
65	3-(β-D-Glucopyranosyloxy)-2-(hydroxymethyl)-AQ	*R. tinctorum* [44]
66	3-(β-D-Glucopyranosyloxy)-8-hydroxy-2-(hydroxymethyl)-AQ	*R. tinctorum* [44]
67	2-(β-D-Glucopyranosyloxy)-1,3-dihydroxy-AQ	*R. cordifolia* [67]
68	3-(β-D-Glucopyranosyloxy)-1-hydroxy-2-(hydroxymethyl)-AQ	*R. tinctorum* [44]



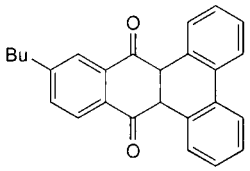
69 R = R' = H
70 R = H, R' = MeO
71 R = H, R' = OH



72 R = R' = H
73 R = R' = OH
74 R = OH, R' = MeO



75 R = H
76 R = MeOOC



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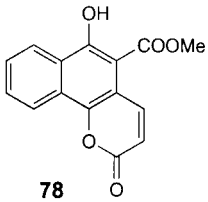

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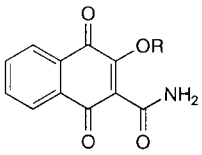
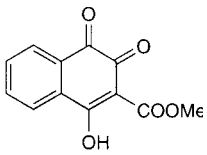


79 R = H
80 R = Me



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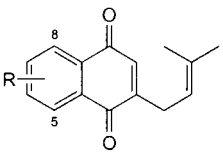
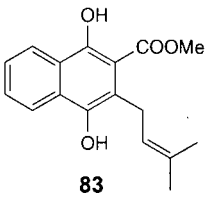


82 R = 5- or 8-MeO



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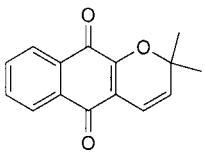

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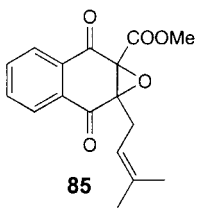

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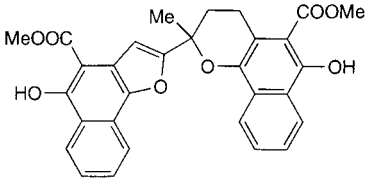

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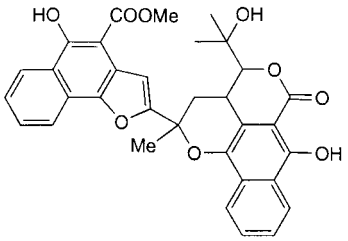
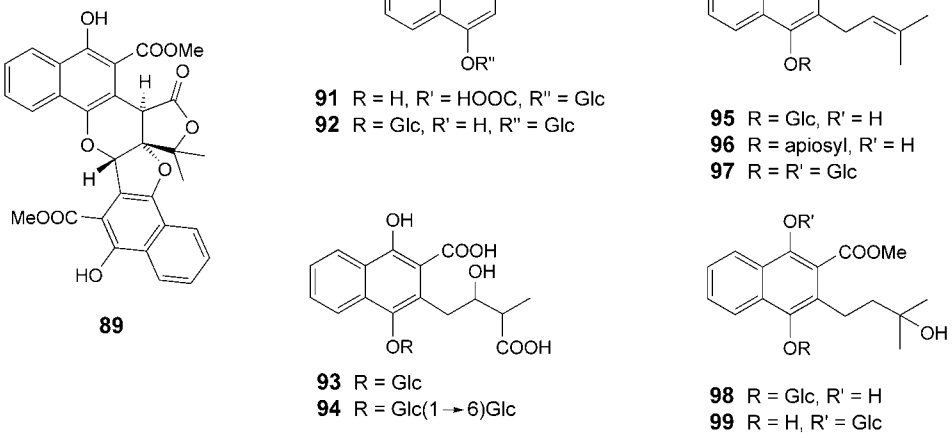


87

cordifolia [84]. The $CHCl_3$-soluble portion of MeOH extract from the roots of *R. cordifolia* gave bicyclic hexapeptide dimer RA-dimer A (**149**), in which two molecules of deoxybouvardin are linked together *via* an ether linkage [85]. The complete list of RA series isolated from various species of *Rubia* is summarized in *Table 4*.

2.5. *Miscellaneous Compounds.* Besides the above-mentioned groups of compounds, several iridoid glycosides, **153**–**157**, flavonoids, **160**–**162**, carbohydrates, **166**–



172, and other compounds have also been isolated from *Rubia* species (*Table 5*). The chemotaxonomic studies of Rubiaceous plants containing iridoid glycosides have been conducted for various species [86], but their presence in every *Rubia* species is yet to be established.

Rhamnose, sucrose, arabinose, xylose, mannose, glucose, and galactose were isolated from *R. cordifolia* [56][94]. Sucrose was also obtained from *R. tinctorum* [20].

The leaves and stems of *R. tinctorum* afforded rutin (**161**) whereas the flowers gave hyperoside (**162**) [95].

3. Biological Activities of Compounds from the Genus *Rubia*. – The biological investigations have shown that many of the medicinal properties claimed for the genus *Rubia* in the historical texts do, indeed, have sound scientific basis. Root extracts of *R. cordifolia* possess hepatoprotective activity and antineoplastic properties, and are considered to be useful for the disintegration and elimination of urinary stones [98]. *R. cordifolia* is an important component of the Ayurvedic system of medicine [4][5]. It has a variety of uses such as blood purifier, immunomodulant, anti-inflammatory and anti-PAF (platelet-activating factor) [98]. The root extract of *R. yunnanensis* has been found to enhance the quantity of ATP in the brain and heart, and to increase leukocytes and has been used to treat psoriasis [99].

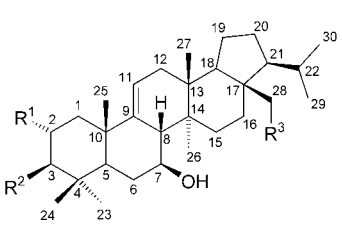
Each of the major biological effects, namely, antitumor activity, and antimicrobial and antithrombotic properties will be considered below.

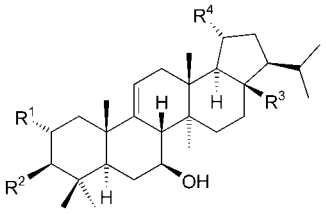
3.1. *Antitumor Activity.* The activity against P388 leukaemia of the MeOH and $CHCl_3$ extracts of the whole *R. cordifolia* plant [100] has initiated the research of genus *Rubia* with respect to antitumor activity [101].

In another attempt, antitumor activity of RC-18, a pure isolate from *R. cordifolia*, was repeatedly tested on murine tumors, *viz.* P388, L1210, L5178Y, B16 melanoma, *Lewis* lung carcinoma, and sarcoma-180. RC-18 exhibited significant increase in the life span of animals with ascites leukemia P388, L1210, L5178Y, and a solid-tumor B16

Table 2. Isolated Naphthoquinones and Their Glycosides

Compound No.	Name	Species
69	Mollugin (= Methyl 6-hydroxy-2,2-dimethyl-2*H*-naphto[1,2-*b*]pyran-5-carboxylate)	*R. cordifolia* [23][36][37][43], *R. lanceolata* [41], *R. oncotricha* [24]
70	3-Methoxymollugin	*R. cordifolia* [43]
71	3-Hydroxymollugin	*R. cordifolia* [43]
72	3,4-Dihydromollugin	*R. cordifolia* [36]
73	3,4-Dihydro-3,4-dihydroxymollugin	*R. cordifolia* [43]
74	3,4-Dihydro-4-hydroxy-3-methoxymollugin	*R. cordifolia* [43]
75	Furomollugin (= Methyl 5-hydroxynaphtho[1,2-*b*]furan-4-carboxylate)	*R. oncotricha* [24]
76	2-(Methoxycarbonyl)furomollugin	*R. oncotricha* [24]
77	11-Butyl-8b,14a-dihydrobenzo[*b*]triphenylene-9,14-dione	*R. cordifolia* [68]
78	Rubilactone (= Methyl 6-hydroxy-2-oxo-2*H*-naphtho[1,2-*b*]pyran-5-carboxylate)	*R. cordifolia* [68]
79	2-Carbamoyl-3-hydroxy-1,4-naphthoquinone	*R. cordifolia* [23]
80	2-Carbamoyl-3-methoxy-1,4-naphthoquinone	*R. cordifolia* [23]
81	Methyl 1,2-dihydro-4-hydroxy-1,2-dioxonaphthalene-3-carboxylate	*R. oncotricha* [24]
82	5-Methoxy- or 8-methoxy-3-(3-methylbut-2-enyl)-1,4-naphthoquinone	*R. cordifolia* [45]
83	Methyl 1,4-dihydroxy-3-(3-methylbut-2-enyl)-naphthalene-2-carboxylate	*R. sylvatica* [42]
84	Dehydro-α-lapachone	*R. cordifolia* [23]
85	Methyl 2,3-epoxy-1,2,3,4-tetrahydro-3-(3-methylbut-2-enyl)-1,4-dioxonaphthalene-2-carboxylate	*R. cordifolia* [43], *R. oncotricha* [24]
86	Naphthohydroquinone dimer A	*R. cordifolia* [43]
87	Naphthohydroquinone dimer B	*R. cordifolia* [43]
88	Rubiacolin A	*R. oncotricha* [69]
89	Rubiacolin B	*R. cordifolia* [43], *R. oncotricha* [69]
90	Rubiacolin C	*R. oncotricha* [69]
91	Rubinaphthin A (= 4-(β-D-Glucopyranosyloxy)-1-hydroxynaphthalene-1-carboxylic acid	*R. yunnanensis* [26]
92	Rubinaphthin B (= 1,4-Bis(β-D-glucopyranosyloxy)-naphthalene)	*R. yunnanensis* [26]
93	Rubinaphthin C (= 3-(4-Carboxy-2-hydroxybutyl)-4-(β-D-glucopyranosyloxy)-1-hydroxynaphthalene-2-carboxylic acid)	*R. yunnanensis* [26]
94	Rubinaphthin D (= 3-(4-Carboxy-2-hydroxybutyl)-4-[(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-1-hydroxynaphthalene-2-carboxylic acid)	*R. yunnanensis* [26]
95	Methyl 4-(β-D-glucopyranosyloxy)-1-hydroxy-3-(3-methylbut-2-enyl)naphthalene-2-carboxylate	*R. cordifolia* [63], *R. oncotricha* [24]
96	Methyl 4-(D-apiosyloxy)-1-hydroxy-3-(3-methylbut-2-enyl)naphthalene-2-carboxylate	*R. oncotricha* [24]
97	Methyl 1,4-bis(β-D-glucopyranosyloxy)-3-(3-methylbut-2-enyl)naphthalene-2-carboxylate	*R. cordifolia* [36][38][63]
98	Methyl 4-(β-D-glucopyranosyloxy)-1-hydroxy-3-(3-hydroxy-3-methylbutyl)naphthalene-2-carboxylate	*R. cordifolia* [36]
99	Methyl 1,4-bis(β-D-glucopyranosyloxy)-3-(3-hydroxy-3-methylbutyl)naphthalene-2-carboxylate	*R. cordifolia* [68]





Rubiarbonol A – F

		R^1	R^2	R^3
100	A	H	OH	$HOCH_2$
101	B	H	OH	Me
102	C	AcO	OH	Me
103	D	OH	AcO	Me
104	E	OH	OH	Me
105	F	OH	OH	$HOCH_2$

Glc = β-D-Glucopyranosyl

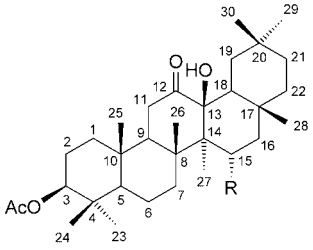
	R^1	R^2	R^3	R^4
106	H	OH	$HOCH_2$	AcO
107	H	=O	$HOCH_2$	AcO
108	H	=O	$HOCH_2$	OH
109	H	=O	AcO	OH
110	H	=O	Me	OH
111	H	=O	HOOC	OH
112	Ac	GlcO	Me	OH
113	OH	GlcO	$HOCH_2$	OH
114	H	$(Glc)_2O$	$HOCH_2$	OH

$(Glc)_2O$ = Glc(1→6)GlcO

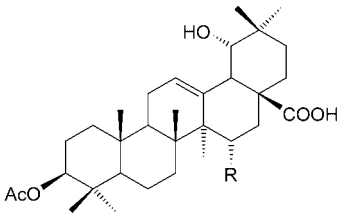
melanoma. However, it failed to show any inhibitory effect on solid tumors, *Lewis* lung carcinoma, and sarcoma 180. Promising results against a spectrum of experimental tumors suggest that RC-18 may lead to the development of a potential anticancer agent [93]. But the structure of RC-18 has not been established. In the course of a continuing search for antitumor substances from crude extract of *Rubia*, it was established that the bicyclic hexapeptides showed potent antitumor activities against various experimental murine tumors *in vivo* and cultured cells *in vitro*. The cyclic hexapeptides showed potent antitumor activity by binding to eukaryotic 80S ribosomes, resulting in inhibition of aminoacyl-tRNA binding and peptidyl-tRNA translocation, thus leading to the stopping of protein synthesis [88][102].

The treatment of human colon cancer DLD-1 cells with RA-VII (**138**) induces cell-growth inhibition associated with a partial G1 arrest and a rapid decrease (below 2 h) in the level of cyclin D1 protein, and, hence, RA-VII (**138**) or *O*-Me-deoxybouvardin is known to inhibit protein biosynthesis *in vitro* and *in vivo* [103]. Further, other compounds of RA series such as RA-I – RA-IV (**133** – **136**) [80][81], RA-XI – RA-IV (**140** – **143**) [82], RA-IX,RA-X (**139**) [83], RA-XV (**144**), RA-XVI (**145**) [84], and RA-dimer A (**149**) [85] have been tested for antitumor activities. A detailed study of the conformations of RA series under various conditions was performed to help in designing new derivatives on the basis of structure – activity relationships [80][83][104][105].

RA-V (**137**) and its hexyl ether showed significant effects against human nasopharynx carcinoma (KB), P388 lymphocytic leukemia, and MM2 mammary carcinoma cells. The activity values (log $1/IC_{50}$) of ether derivatives of RA-V (**137**) gave an upward parabolic or bilinear relationship when plotted against log P (P = partition coefficient determined with the octan-1-ol/H_2O system) as the number of the C-atoms of the side chain at the phenol moiety of RA-V (**137**) was increased, the optimum log P



115 R = OH
116 R = H



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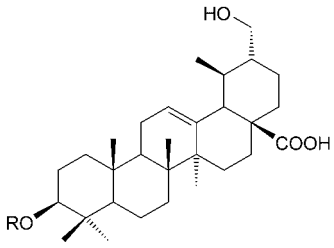
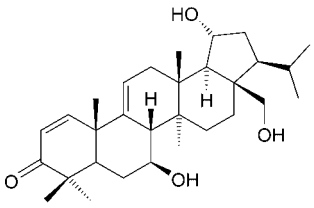


118 R = (*E*)-4-hydroxycinnamoyl
119 R = H



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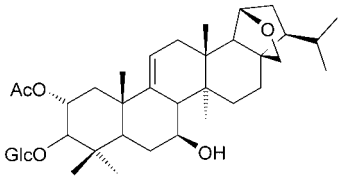

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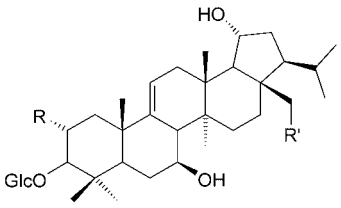
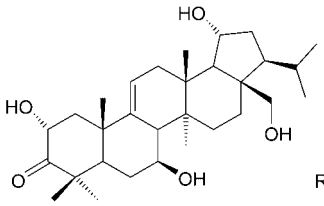


122 R = H, R' = H
123 R = H, R' = OH
124 R = OH, R' = OH



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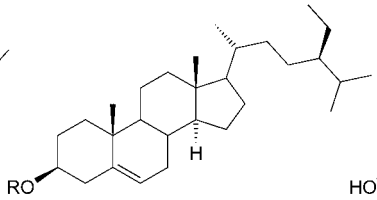
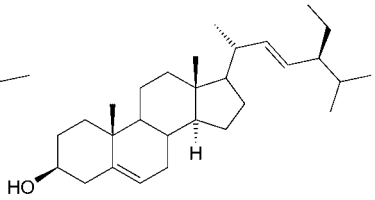


126 R = H
127 R = Glc



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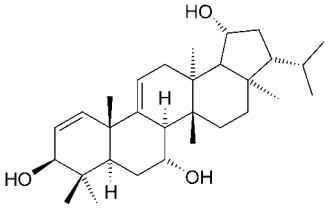

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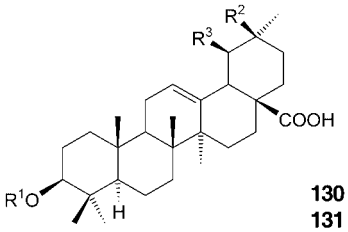


	R^1	R^2	R^3
130	H	Me	H
131	Ac	Me	H
132	H	H	Me

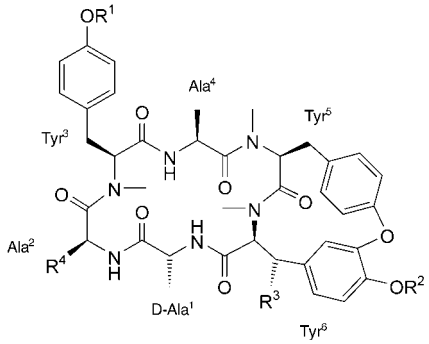
Table 3. *Isolated Terpenes and Their Glycosides*

Compound No.	Name	Species
100	Rubiarbonol A	*R. yunnanensis* [58][73–75], *R. oncotricha* [72], *R. cordifolia* [72]
101	Rubiarbonol B	*R. oncotricha* [72], *R. cordifolia* [72], *R. yunnanensis* [75]
102	Rubiarbonol C	*R. oncotricha* [72], *R. cordifolia* [72]
103	Rubiarbonol D	*R. oncotricha* [72], *R. cordifolia* [72]
104	Rubiarbonol E	*R. oncotricha* [72], *R. cordifolia* [72]
105	Rubiarbonol F	*R. oncotricha* [72], *R. cordifolia* [72], *R. yunnanensis* [75]
106	Rubiarbonol G	*R. yunnanensis* [58][73–75]
107	Rubiarbonone A	*R. yunnanensis* [58][73][74]
108	Rubiarbonone B	*R. yunnanensis* [73][75]
109	Rubiarbonone C	*R. yunnanensis* [75]
110	Rubiarbonone D	*R. yunnanensis* [75]
111	Rubiarbonone F	*R. yunnanensis* [75]
112	Rubiarboside A	*R. yunnanensis* [75]
113	Rubiarboside F	*R. yunnanensis* [75]
114	Rubiarboside G	*R. yunnanensis* [75]
115	Rubiaprassin A	*R. cordifolia* [71]
116	Rubiaprassin B	*R. cordifolia* [71]
117	Rubiaprassin C	*R. cordifolia* [71]
118	Rubiacoumaric acid	*R. cordifolia* [70]
119	Rubiafolic acid	*R. cordifolia* [70], *R. peregrina* [76]
120	Rubiarbonone E	*R. yunnanensis* [75]
121	Rubianoside I	*R. yunnanensis* [59]
122	Rubianoside II	*R. yunnanensis* [59]
123	Rubianoside III	*R. yunnanensis* [59]
124	Rubianoside IV	*R. yunnanensis* [59]
125	Rubianol-a	*R. yunnanensis* [59]
126	β-Sitosterol	*R. cordifolia* [29][38][55], *R. yunnanensis* [58][75], *R. peregrina* [76], *R. salvatica* [42]
127	Daucosterol	*R. cordifolia* [29], *R. salvatica* [42]
128	Stigmasterol	*R. yunnanensis* [75]
129	Rubiatriol	*R. cordifolia* [77]
130	Oleanolic acid	*R. peregrina* [76]
131	Oleanolic acid acetate	*R. cordifolia* [55]
132	Ursolic acid	*R. peregrina* [74]

values being in the range from 3.5 to 4.9. The ester derivatives showed a similar relationship, the optimum log *P* values being 6.3–6.7, which are higher than those of the ether derivatives. The lethal effect of RA-V (**137**) on KB cells was clearly different from that of mitomycin C, and RA-V (**137**) was concluded to be a 'time-dependent drug' like vinblastine [106].

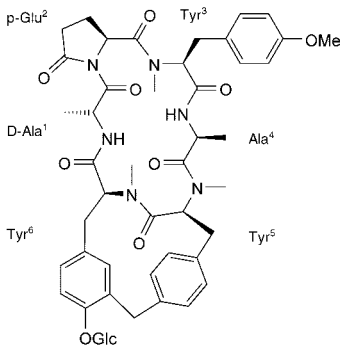
In mice implanted with P-388 cells, i.p. administration of cyclic hexapeptides obtained from *R. radix* inhibited tumor growth with *ED* ranges of 0.01–4.0 mg/kg for RA-VII (**138**) and 0.05–10.0 mg/kg for RA-V (**137**) [107]. The minimum *ED* for RA-IV (**136**) was 10 mg/kg and 0.05–0.5 mg/kg for RA-III (**135**) [107]. In another study, four bicyclic hexapeptides RA-III–RA-V and RA-VII (**135**–**138**) showed antineoplastic activity against leukemia P-388, L1210, B-16 melanoma, Colon 38, *Lewis* lung carcinoma, and *Ehrlich* carcinoma [108]. A number of RA derivatives were also synthesized and evaluated for cytotoxicity to P388 leukemia and KB cells *in vitro* [109].

Table 4. *Bicyclic Hexapeptides* **133**–**151** *Isolated from* Rubia. Glc = β-D-Glucopyranosyl.



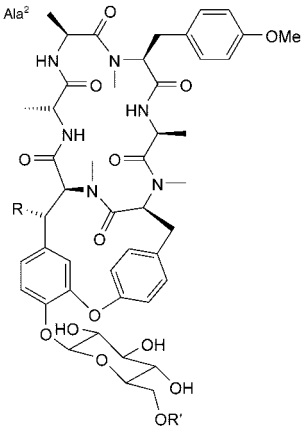
133 – **142**

Compound No.	Abbreviation	R^1	R^2	R^3	R^4	Species
133	RA-I	Me	H	H	CH_2OH	*R. cordifolia* [81]
134	RA-II	H	Me	H	Me	*R. cordifolia* [81]
135	RA-III	Me	Me	H	CH_2OH	*R. cordifolia* [78][79][81]
136	RA-IV	Me	Me	OH	Me	*R. cordifolia* [78][79][81]
137	RA-V	Me	H	H	Me	*R. cordifolia* [78][79][81], *R. akane* [78]
138	RA-VII	Me	Me	H	Me	*R. cordifolia* [78][79][87], *R. akane* [78]
139	RA-X	Me	Me	H	CH_2CH_2COOH	*R. cordifolia* [87]
140	RA-XI	Me	H	H	CH_2CH_2COOH	*R. cordifolia* [82]
141	RA-XII	Me	Glc	H	Me	*R. cordifolia* [82][88]
142	RA-XIII					*R. cordifolia* [82]

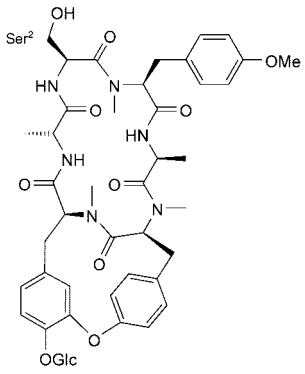


143 RA-XIV

Glc = β-D-glucopyranosyl



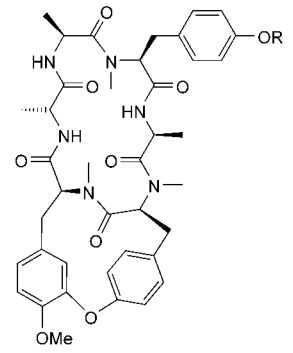
144 RA-XV R = H, R' = Ac
145 RA-XVI R = AcO, R' = H
146 R = R' = H



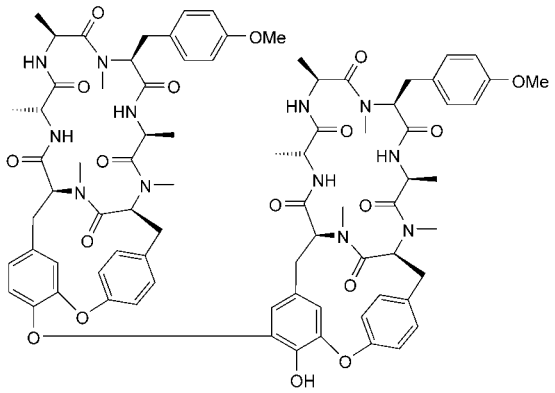
147

Table 4 (cont.)

Compound No.	Abbreviation	R^1	R^2	R^3	R^4	Species
143	RA XIV					*R. cordifolia* [82]
144	RA XV					*R. cordifolia* [84]
145	RA XVI					*R. cordifolia* [84]
146	RY-I					*R. yunnanensis* [89][90]
147	RY-II					*R. yunnanensis* [90]



148
R = β-D-glucopyranosyl



149 RA-Dimer

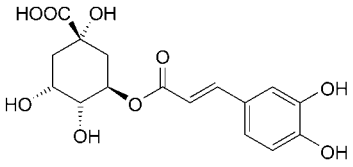
148	RY-III					*R. yunnanensis* [91]
149	RA-dimer A					*R. cordifolia* [85]
150	RA-700					*Rubia* roots [92]
151	RC-18					*R. cordifolia* [93]

3.2. *Antimicrobial Activity.* Madder-root powder, and the total anthraglycosides and total anthracene-aglycons isolated from the roots showed limited antifungal and antibacterial activity *in vitro* [110]. An anthraglycoside isolated from *R. cordifolia* showed certain antibacterial activity [38]. The anthraglycosides were active against *Shigella largei-sachsii* and the aglycons against all of the four species tested, *i.e.*, *Shigella largei-sachsii*, *Shigella ambigua*, *Staphylococcus aureus*, and *Staphylococcus haemolyticus*. The aglycons in Et_2O solution but not in oily solution were active against fungi and yeasts, especially *Candida albicans*, *Geotrichum louberi*, and *Saccharomyces cerevisiae*. The anthraglycosides showed no fungicidal activity, and the root powder was moderately fungicidal [110].

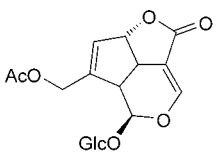
3.3. *Antithrombotic Activity.* Thrombus formation in humans can restrict blood flow to vital tissues or organs, and cause peripheral, cerebral, or coronary ischemia. Embolism of the thrombosis can be catastrophic, and it is a common cause of death. Platelet aggregation is considered to be a major pathogenic mechanism that leads to arterial thrombosis.

The physicians of the Indian System of Medicine clinically use *R. cordifolia* for the purification of blood. For the first time, the effect of the partially purified fraction of this whole plant has been studied on rabbit platelets. It inhibits the platelet aggregation

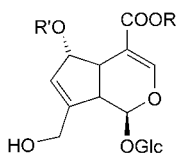
Table 5. *Miscellaneous Compounds* **152**–**165** *Isolated from* Rubia



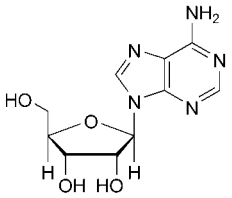
152



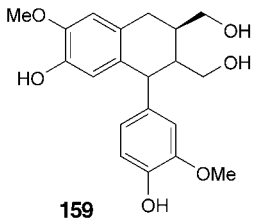
153



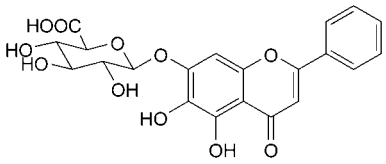
154 R = R' = H
155 R = Me, R' = Ac
156 R = Me, R' = H
157 R = H, R' = Me



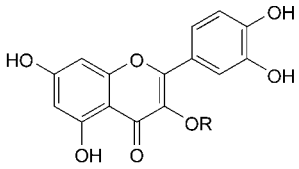
158



159



160



161 R = rutinosyl
162 R = Glc

Glc = β-D-glucopyranosyl

Compound No.	Name	Species
152	Chlorogenic acid	*R. tinctorum* [95]
153	Asperuloside	*R. tinctorum* [20][21]
154	Desacetyl asperulosidic acid	*R. tinctorum* [32][86], *R. cordifolia* [86]
155	Daphylloside	*R. tinctorum* [32]
156	10-Acetylscandoside	*R. tinctorum* [32]
157	6-Methoxygeniposidic acid	*R. cordifolia* [96]
158	Adenosine	*R. cordifolia* [63]
159	(+)-Isolariciresinol	*R. akane* [97]
160	Baicalin	*R. yunnanensis* [26]
161	Rutin	*R. tinctorum* [95]
162	Hyperoside	*R. tinctorum* [95]
163	6α-Epiferetoside	*R. tinctorum* [32]
164	Hederagenin-3-*O*-α-L-arabinopyranoside	*R. cordifolia* [63]
165	3-O-α-L-Arabinopyranosyl-hederagenin-28-O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside	*R. cordifolia* [63]

induced by PAF but not thrombin. It also inhibits the binding of 3 H-PAF to the platelets in the dose-dependent manner. Thus, it appears that *R. cordifolia* inhibits action of PAF at its receptor level either by blocking it or by desensitization [111].

Mollugin (**69**) showed strong inhibition of arachidonic acid (AA)-induced and collagen-induced platelet aggregation [112]. In contrast, 1,3,6-trihydroxyl-2-methyl-AQ (**45**), and xanthopurpurin (**35**) showed mainly strong inhibition of collagen-induced platelet aggregation [112].

3.4. *Ethnoveterinary Usage. R. cordifolia* is used in the treatment of liver fluke, dysentery, maggots, wounds, and intestinal worms in animals [113].

3.5. *Other Pharmacological Aspects.* The antiproliferative property of *R. cordifolia* extract has been studied on two different cell types, A-431 cells (epidermal carcinomoid cells) and 3T3 fibroblast cells. The extract significantly inhibited the incorporation of thymidine induced by fetal bovine serum in a dose-dependent manner [102]. It also inhibited the PMA (phorbol 12-myristate 13-acetate)-induced expression of c-fos genes in A-431 cells. It appears that inhibition of DNA synthesis underlies the mechanism for its antiproliferative properties [102]. The alcoholic extract of *R. cordifolia*, which has anti-PAF and anti-lipid peroxidative properties, inhibited potato lipoxygenase. Lipoxygenase catalyzes the interaction between oxygen and polyunsaturated fatty acid. Their products, mainly leukotrienes and hydroperoxycompounds, are involved in several diseases, like asthma, arthritis, inflammation *etc.* The effect was dose- and time-dependent. The AcOEt fraction was found to be the most active [114].

The antiperoxidative property of the solvent-free alcoholic extract of *R. cordifolia* has been studied in rat liver homogenate [115]. It prevents the cumene hydroperoxide-induced malondialdehyde formation in a dose- and time-dependent manner. This effect is accompanied by the maintained reduced glutathione level even in the presence of the above toxin [115]. Comparative inhibition studies of $FeSO_4$-induced lipid peroxidation in rat liver by alcoholic extract of *R. cordifolia* and its constituent **14** have also been conducted [116]. Both have been found to inhibit lipid peroxidation in a dose-dependent manner. Whereas the former shows both oxidizing and reducing properties with Fe^{2+} and Fe^{3+}, the latter shows oxidizing property only by converting Fe^{2+} to Fe^{3+}. The former inhibits the oxidation of reduced glutathione, while the latter does not [116].

Madder root, *R. tinctorum*, is a traditional herbal medicine used against kidney stones [117]. At pH > 7, free anthraquinone preparations formed colored, insoluble Ca and Mg complexes, which were deposited with urinary stones [118]. However, at pH 5 – 7, glycoside-bound anthraquinones formed soluble complexes, thus decreasing the amount of ionized Ca and Mg in the urine and preventing stone formation [118]. A preparation from the roots of *R. tinctorum* is able to dissolve oxalates, phosphates, and uric acid, which deposit in the kidneys and the urinary tract as stones and sand [119]. The addition of 'rubiateep' (20%, a preparation of *R. tinctorum*) to the diet of rat inhibits or even completely prevents the formation of $CaCO_3$ calculi but acts only slightly inhibitory toward the formation of oxamide calculi [120].

The growth-inhibiting influence of hydroxy-anthraquinone derivatives of root of *Rubia* in various Ca offerings was investigated by a foreign-body bladder calculus model in rabbits. The use of anthraquinone glycosides to prevent recurrence of Ca-containing urinary stones is recommended [121]. The anthraglycosidic fraction of *R.*

tinctorum showed greater anti-calculi activity in rats than a crude powder of the alcoholic extract. The aqueous extract and ruberythric acid (**60**) were the least effective. None of the fractions had any toxic or other side effects [122]. The crude extract of *R. cordifolia* was tested in isolated tissue preparations for its possible Ca-channel antagonistic activity [123]. The extract suppressed the spontaneous contractions of guinea pig atria, rabbit jejunum and rat uterus in a concentration-dependent manner (0.1 – 3 mg/ml). In rabbit aorta, it inhibited norepinephrine (10 μM) and KCl (80 mM) induced contractions [123].

Lucidin (**15**) has shown mutagenic activity on *Salmonella typhimurium TA 100* and *98* [124]. The formation of DNA adducts in tissue culture and mice after treatment with the derivative of **15** has also been studied [125]. The possible carcinogenicity of madder root have been studied by screening three groups of male and female ACI rats, which received either a normal diet or a diet supplemented with 1 or 10% drug for a total period of 780 days. Weight gain and morbidity were not different among the three groups. Non-neoplastic lesions related to the treatment were evident in the liver and kidneys of both sexes. Moreover, dose-dependent increases in benign and malignant tumor formation were observed in the liver and kidneys of treated animals. 32P-Post-labelling analysis showed an increase in the overall level of DNA adducts observed in the liver, kidney, and colon of rats treated with 10% madder root in the diet for 2 weeks. HPLC Analysis of 32P-labelled DNA adducts revealed a peak co-migrating with an adduct obtained after *in vitro* treatment of deoxyguanosine-3′-phosphate with lucidin. These observations indicate that the use of madder root for medicinal purposes is associated with a carcinogenic risk [125].

The anti-inflammatory, analgesic, antipyretic, and gastrolesive properties of petroleum ether extract of *R. cordifotia* roots have been evaluated [126]. An active triterpene responsible for the anti-inflammatory activity was separated. The compound exhibited anti-inflammatory activity in the carrageenan-induced edema, cotton pellet granuloma, and adjuvant-induced arthritis. The analgesic activity was studied using AcOH-induced writhing and radiant-heat analgesiometry, and antipyretic activity was assessed in yeast-induced hyperpyrexia in rats. The ulcerogenic potential was studied in pyloric-ligated rats. The compound possesses anti-inflammatory, analgesic, and antipyretic activity, and strong gastrolesive properties. The study justifies the use of *R. cordifolia* in the treatment of inflammation, pain, and fever [126].

4. Genus *Rubia* as Potential Source of Biodyes. – The dyes of the ancient world were derived from plant and earth pigments. These were mixed natural resins, animal fats, or drying oils to produce paints or boiled with or painted onto cloth as a dye. However, for most people, the colors available to them were limited because of geographical isolation, expense, or rarity of certain plant or mineral substances, and also due to lack of proper standarization and reproducibility of shades. The practices and techniques of dyeing have remained mostly unchanged until the mid 19th century but lost the race with synthetic dyes, invented in Europe by the pioneers of chemical science, *William Perkins* in England and *Adolph Baeyer* in Germany. Within a short time, these chemical dyes, though they were visually harsher, displaced everywhere the centuries-old traditions of natural dyeing. With the recent trend of higher preference for biodyes, fueled by increased public awareness about environmental

issues and actions like the German ban on the use of some synthetic dyes, prompted the phytochemists and related researchers to have a re-look at the potential of bio-dyes.

Among cosmetic and food additives, synthetic dyes have been receiving, in the past two decades, the largest number of adverse criticisms. As a matter of fact, more than a real technological tool (as, *e.g.*, antimicrobics and emulsifiers), they simply represent an answer of industry to market needs. The choice of biocolorants instead of synthetic ones is certainly due to health concerns, though real safety and healthiness of natural substances is not scientifically established yet. Toxicological investigations of food colors are already performed all over the world, and every year they result in revisions, and often reduction of their authorized maximum content. This kind of impulse, together with the modification of the requests from consumers, has caused a general lowering of industrial interest in synthetic dyes. Therefore, finding out and exploitation of naturally occurring dyes have strong motivations that range from the field of food additives to many other sectors, from cosmetics to clothing. The interest in all plants able to provide pigments to be used by industry is, therefore, increasing. Some of these plants are already known [127] (*Isatis*, *Rubia*, safflower), and, before the worldwide spreading of synthetic dyes, these were largely used to this purpose. The literature on this subject also quotes many other species as potentially useful [127].

The history of natural dyes from madder plant has been known for a long time. The Egyptian mummies have been found wrapped in cloths dyed from the madder plant. *Alexander the Great* is supposed to have deceived the Persians into thinking that his army was wounded by sprinking his soldiers with a red dye, probably madder juice, which contains the dye alizarin and hence, the use of *Rubia* species as a source of biocolorants is known to be as old as mankind and is being used nowadays also [128–134]. People have been using common madder (*R. tinctorum*) and other *Rubia* species for dyeing silk [135–138], cotton [139], jute [140], and other textiles [141][142], along as cosmetic and food additives [127][143].

The ancient Indian dyers used turmeric for yellow colors, madder from plants other than *R. tinctorum*, and indigo. They had the knowledge of dyeing plant fibers and of reducing the vat dye before dyeing with indigo. A high level of textile printing was achieved. However, all preservation techniques, except for mordant preservation, were of Chinese rather than Indian origin [139].

The story of alizarin (**10**), the main tinctorial component of madder root, occupies a unique place in 19th century chemistry as the first dye for which there was significant structural insight, which, in turn, prompted its successful synthesis [144][145].

Silk was dyed with a colorant extracted from *R. tinctorum* with highest dyeing capacity at pH 3.5 and 40° for 120 min, similar to results with **10** or purpurin (**29**) colorants. A change in hue from yellowish brown to red was observed in dyeings with aluminium mordanting [128][135]. A concentration of 6 g dye from *R. tinctorum* extracts per 100 ml provided the maximum optical density in the dye liquor. The best results for dyeing silk with *R. tinctorum* extracts have been determined with the mordant concentration for alum (25), copper sulfate (6), stannous chloride (8), ferrous sulfate (8), and potassium dichromate (8 g/100 g silk) [136]. Post-mordanting gave the best results with ferrous sulfate, copper sulfate, and chrome. Alum was best with

premordanting and stannous chloride with simultaneous mordanting [136]. Several studies have been done for the optimization of procedure for dyeing of silk and the effects of pigments obtained from various *Rubia* species [136–138][146].

Purpurin (**29**) is the major colorant present in the roots of *R. cordifolia* for dyeing nylon [147]. The aqueous extract of *R. iberica* was used for dyeing wool in the carpet industry [142]. Wool dyed with these natural dyes has good fastness to alkaline agents or sunlight [130][142]. Extraction of the rhizome of the madder *R. tinctorum* is used at various pH values of the media and in the presence of different mordants for dyeing wool yarn with various colors. The madder-dyed wool carpets had a high light fastness and resistance to soap solutions [148]. Bleached jute fibers were dyed with *R. cordifolia* using aluminium sulfate as mordant [140]. The effect of various inorganic acids and organic monocarboxylic acids on the color change for dyeing fibers from the extract of Indian madder was studied by means of the psychometric lightness and psychometric chroma coordinates [149].

The red color of candies is obtained with water or alcohol extract of *R. cordifolia* root, cultured cells of *R. cordifolia* and/or *R. cordifolia* root fermentation products, and applied with alum, organic acid salts, and carbonic acid salts [150].

The extract of *R. tinctorum* has been used for coloring chewing gums [151], noodles [152], and salted foods [153]. Jams are colored with red pigment components containing hydrolyzates of H_2O or aqueous alcohol extracts of root tissue of *R. tinctorum*, alum, organic acid salts, and carbonates. Roots of *R. tinctorum* were extracted with 70% alcohol at 80° for 10 h, and the extract was hydrolyzed with the enzyme Kokulase SS at 35° for 36 h to manufacture pigments [154].

A completely natural hair dye is obtained from *R. tinctorum* and used in shampoos, lotions, sprays, *etc.* [143]. This extract is nontoxic and has antibacterial properties. Other plants of the Rubiaceae family are also useful for the extraction of hair dyes [143]. The filtrate of the successive extraction of 1 kg of *R. tinctorum*, with 6 l and 4 l of MeOH acidified with 0.5% H_3PO_4 at reflux temperature for 1 h, was centrifuged, concentrated to 2 l, and evaporated in the presence of 2 l of propane-1,3-diol until MeOH was completely removed. The extract was clarified and standardized to contain 1% coloring matter for use in cosmetics [143].

Due to its importance in the dye industry, experiments are being conducted to make industrial extraction unit [155].

5. Conclusions. – The survey of phytochemical investigations of the genus *Rubia* has revealed a wide variety of chemical constituents, important classes, among others, being anthraquinones, naphthoquinones, terpenes, and bicyclic hexapeptides. Out of a total of 172 different compounds isolated from *Rubia* species, 68 are anthraquinones and their glycosides, 31 are naphthoquinones and their glycosides, 33 are terpenes, and 19 are bicyclic hexapeptides. These investigations were aimed to pinpoint the active components besides finding out new structural leads for future drugs.

Various crude fractions and purified compounds have shown potential medicinal and pharmacological activities. The use of different plant parts, especially the roots as coloring materials, is no longer a potential but very much a commercial reality. Their use in the food and fabrics industries is already extensive.

6. Future Prospects. – Taking into account the fact that there are 60 species belonging to this genus spread throughout the world, and only 25% of them have been phytochemically investigated, this genus still remains a potent one to work on.

Bioprospecting – prospecting of bioresources for economic development – has emerged as a new economic venture, and the genus *Rubia* is an asset to this new and emerging field, waiting for its full exploitation.

G. is grateful to *CSIR*, New Delhi, *R. S.* and *S. M. S. C.* are grateful to *DBT*, New Delhi, India, for grant of funds under the research project BT/012/10/NBDB/2000, for '*Prospecting for Natural Dyes from Bioresources – an All India Coordinated Research Project*'.

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Received April 19, 2004