

Isolation and synthesis of anthraquinones and related compounds of *Rubia cordifolia*

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Abstract: Anthraquinones and their glycosides, along with other compounds, have been isolated and characterized from the acetone:water (1:1) percolation of dried roots of *Rubia cordifolia*. Selected anthraquinones were synthesized using montmorillonite clays under solventless condition in 75 to 85 % yield.

Keywords: Anthraquinones, *Rubia cordifolia*, montmorillonite clays.

INTRODUCTION

The family Rubiaceae comprises about 450 genera and 6500 species and includes trees, shrubs and infrequently herbs. *Rubia cordifolia* is a perennial, herbaceous climbing plant, with very long roots, cylindrical, flexuous, with a thin red bark.¹ Stems often have a long, rough, grooved, woody base. Plants belonging to this family are known to contain substantial amounts of anthraquinones, especially in the roots.^{2,3}

The traditional therapeutic use of the plant has been for skin disorders and for anticancer activity.^{3–5} Furthermore, the anthraquinones of the Rubiaceae family exhibit some interesting *in vivo* biological activities, such as antimicrobial,⁶ antifungal,⁷ hypotensive,⁸ analgesic,⁸ antimalarial,^{5,9} antioxidant,¹⁰ antileukemic and mutagenic functions.^{11,12} Apart from its medicinal value, this plant has also been used as natural food colourants and as natural hair dyes.¹³ The interest in the isolation of natural dyes and colouring matters is increasing due to their applications in food, drugs and other human consumptions. The present paper describes the isolation of anthraquinones and related compounds from *R. cordifolia* and their solventless synthesis in the presence of montmorillonite clays.

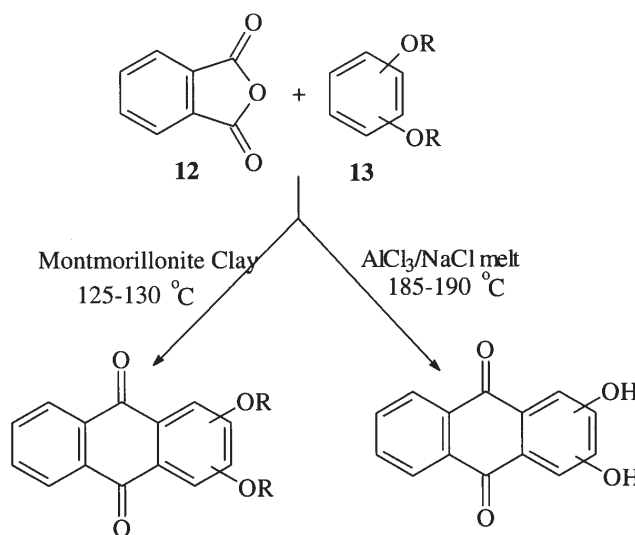
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ISOLATION OF ANTHRAQUINONES AND RELATED COMPOUNDS

The air dried roots of *R. cordifolia* were percolated with acetone:water (1:1) for 48 h at room temperature. The acetone was removed by simple distillation to give a brown-coloured precipitate. The water-soluble portion was further extracted successively with ethyl acetate and *n*-butanol. Each of the above fractions was separately dissolved in *n*-hexane and the undissolved portions were removed by filtration. The thin layer chromatography (TLC) profile of the hexane soluble portion was found to be same in all cases, varying only in the concentration of the compounds. All the above hexane fractions were mixed together and adsorbed on silica gel (60 – 120 mesh) and purified by column chromatography (CC). In addition, the ethyl acetate soluble fraction was also adsorbed on silica gel (60 – 120 mesh) and purified by CC. A naphthoquinone derivative, mollugin (**1**), anthraquinone derivatives (**2–9**) and anthraquinone glycosides (**10,11**) were obtained from various fractions and characterized by their various spectroscopic data and comparison with literature data (experimental section).

SYNTHESIS OF SELECTED ANTHRAQUINONES

Friedel–Crafts reactions between phthalic anhydride and substituted benzenes in the presence of an eutectic mixture of aluminium chloride and sodium chloride (2:1) melt have been used for the preparation of various anthraquinones.^{14–16} This approach to synthesize anthraquinones is unsatisfactory due to poor yields, tedious work-up, drastic conditions, and lack of regioselectivity.^{14–16} Furthermore, methoxy substituents are hydrolyzed during the course of the reaction. To overcome the above difficulties, some modifications have been made for the synthesis of anthraquinones *via* Friedel–Crafts reactions. The traditional reagent, an alumi-



Scheme 1.

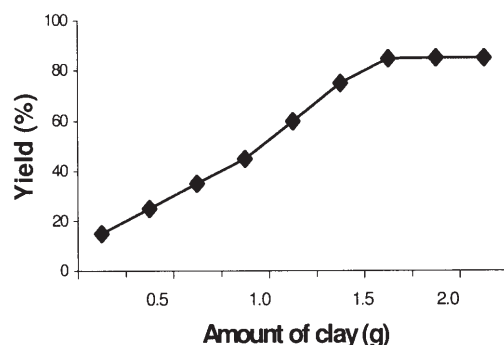


Fig. 1. Yield of anthraquinone as a function of amount of clay used.

um chloride/sodium chloride melt has been replaced by cheaper, non-corrosive, reusable and ecofriendly montmorillonite clays^{17,18} (Scheme 1). Phthalic anhydride (**12**) and the substituted benzene (**13**) were added to the activated montmorillonite clay (heated at 120–130 °C for 24 h) and the reaction continued for a further 25–30 min at the same temperature. The reaction mixture was extracted with ethyl acetate and filtered over celite to remove the clay. The removal of ethyl acetate gave the crude product, which was purified by column chromatography and recrystallization from the appropriate solvent system. To optimize the reaction, different amounts of the clay montmorillonite K10 were used for a fixed amount of reactants (Fig. 1). The use of montmorillonite clays also avoids the hydrolysis of the methoxy group in the benzene ring (Scheme 1). Montmorillonite clays K10 and KSF were used for the reaction and the montmorillonite K10 was found to be the better of the two clays (Table I). The reaction of **12** with 1,2-dihydroxybenzene (catechol, **13c**) in the presence of clay gave two products, 1,2-dihydroxyanthraquinone (alizarin, **5**) and 2,3-dihydroxyanthraquinone (hystazarone, **15**) in the ratio 45:55. This was determined on the basis of ¹H-NMR data of the crude mixture and by direct comparison with the assignments for each pure isomer.

TABLE I. Yield (%) of synthesized anthraquinones under various reaction conditions

Substituted benzene	Reagent	Product	Yield [#] /%
1,3-dihydroxy (13a)	Mont. KSF	1,3-dihydroxyanthraquinone (7)	77
	Mont. K10		81
	NaCl/AlCl ₃		35
1,4-dihydroxy (13b)	Mont. KSF	1,4-dihydroxyanthraquinone (14)	75
	Mont. K10		83
	NaCl/AlCl ₃		31
1,2-dihydroxy (13c)	Mont. KSF	1,2-dihydroxyanthraquinone (5) +	75
	Mont. K10	2,3-dihydroxyanthraquinone (15)	79
	NaCl/AlCl ₃		33

TABLE I. Continued

Substituted benzene	Reagent	Product	Yield [#] /%
1,3-dimethoxy (13d)	Mont. KSF	1,3-dimethoxyanthraquinone (16)	78
	Mont. K10		85
	NaCl/AlCl ₃	1,3-dihydroxyanthraquinone (7)	30

[#] Isolated yield

In conclusion, the extraction of dried roots of *R. cordifolia* with acetone:water (1:1) at room temperature was found to be a mild solvent system for the isolation of the maximum number of compounds, including anthraquinones and their glycosides. The use of montmorillonite clays in place of an AlCl₃-NaCl melt was found to be the best alternative reagent for the synthesis of anthraquinones with good to moderate yield under mild reaction conditions. The use of montmorillonite clays also avoids the hydrolysis of methoxy substituents during the course of reaction, which is the must when an AlCl₃-NaCl melt is employed. Montmorillonite K10 was found to be a better reagent for this type of synthesis than montmorillonite KSF.

EXPERIMENTAL

All the required solvents were purchased and distilled before use. Montmorillonite clays, KSF and K10 were purchased from Fluka. Phthalic anhydride and the substituted benzenes were purchased from s.d. Fine Chemicals. Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected.

Extraction of powdered roots of Rubia cordifolia with acetone:water (1:1)

The air-dried roots of *Rubia cordifolia* (1.5 kg) were percolated three times in acetone:water (1:1) (5 L) for 48 h at room temperature. The combined decanted solvent was distilled by simple distillation to remove the acetone. A brown colour solid (10.497 g) separated after removal of the acetone (Extract **I**). The aqueous portion was further extracted with ethyl acetate (3 L) (Extract **II**) and *n*-butanol (2.5 L) (Extract **III**). The solvents were removed under reduced pressure to obtain crude extracts **I–III**. All three extracts (**I**, **II** and **III**) were redissolved separately in hexane and the hexane soluble portions were collected.

Separation of the n-hexane soluble portion

The TLC of the *n*-hexane soluble portions was found to be same. The combined hexane extract was adsorbed on silica gel (60–120 mesh) for column chromatography. Elution of the column with *n*-hexane gave a low melting waxy compound. Further, elution with *n*-hexane-benzene (4:1) gave a yellow coloured compound, mollugin (**1**), yield: 25 mg, mp. 132 °C (lit¹⁹ mp. 132–134 °C).

Further elution of column with benzene gave a mixture of two compounds which were separated by preparative TLC using benzene:ethyl acetate (99:1) as the mobile phase to give 1-hydroxy-2-methylanthraquinone (**2**), yield: 12 mg, mp. 183 °C (lit¹⁹ mp. 181–183 °C), and 1,4-dihydroxy-2-methylanthraquinone (**3**), yield: 15 mg, mp. 177–178 °C (lit² mp. 175–176 °C). Further elution of column with benzene:chloroform (10:1) gave 2-methylanthraquinone (**4**), yield: 10 mg, mp. 175–176 °C (lit² mp. 178–179 °C). Elution of the column with chloroform gave 1,2-dihydroxyanthraquinone (alizarin, **5**) and a mixture of unidentified compounds.

Separation of the ethyl acetate soluble portion

The ethyl acetate was removed under reduced pressure and the residue was chromatographed over silica gel (60–120 mesh) using different solvent systems. The elution of the column with petroleum ether yielded **5**, yield: 89 mg, mp. 287–289 °C (lit² mp. 289–290 °C).

Further elution of the column with a mixture of petroleum ether–ethyl acetate in the ratio 4:1 gave 1,3-dihydroxy-2-methylanthraquinone (rubiadin, **6**), yield: 8 mg, mp. 243 °C (lit² mp. 244–245 °C). Further elution of the column with a mixture of petroleum ether–ethyl acetate in the ratio 3:1 gave 1,3-dihydroxyanthraquinone (xanthopurpurin, **7**), yield: 11 mg, mp. 266–269 °C (lit² mp. 266–268 °C). Elution of the column with a mixture of petroleum ether–ethyl acetate in the ratio 2:1 gave 1,2,4-trihydroxyanthraquinone (purpurin, **8**), yield: 12 mg, mp. 262–264 °C (lit² mp. 263 °C).

Finally, the column was eluted with methanol to give a mixture of compounds. This was again chromatographed over silica gel (60–120 mesh) with chloroform as the eluting solvent, whereby 1,4-dihydroxy-2-methyl-5-methoxyanthraquinone (**9**) was obtained, yield: 10 mg, mp. 163–164 °C (lit^{2,20} mp. 165 °C). Elution of the column with a mixture of chloroform–methanol in the ratio 4:1 gave ruberythric acid (**10**), yield: 17 mg, mp. 257–258 °C (lit²⁰ mp. 258–260 °C) and the primeveroside of lucidine (**11**), yield: 17 mg, mp. 225–226 °C (lit²⁰ mp. 227–228 °C).

General procedure for the synthesis of anthraquinones

AlCl₃:NaCl Melt method. A mixture of purified phthalic anhydride (33.78 mmol) (**12**) and substituted benzene (45.45 mmol) (**13**) was added in small portions to a molten mixture of AlCl₃–NaCl (2:1) at 180 °C and stirred for 30 min. The reaction mixture was cooled to room temperature. Crushed ice (100 g) and conc. HCl (20 ml) were added to the reaction mixture. The reaction mixture was extracted with ethyl acetate (2×100 mL). The ethyl acetate layer was dried over anhydrous Na₂SO₄ and concentrated to give a yellow solid. The residue was purified by column chromatography on silica gel (60–120 mesh) to give anthraquinones.

Activated montmorillonite clay method. Phthalic anhydride (**12**) (33.78 mmol) and substituted benzene (**13**) (45.45 mmol) were added to the activated montmorillonite clay (2 g) in an oil bath at 120–130 °C for 24 h and the reaction was allowed to continue for a further 30 min. The reaction mixture was cooled to room temperature and ethyl acetate (100 ml) was added. The clay was filtered over celite 545 and the filtrate was removed under reduced pressure to obtain crude anthraquinone, which was further purified by recrystallization from methanol. The physical and spectroscopic data of the compounds synthesized by both the methods and those isolated were comparable.

1,4-Dihydroxyanthraquinone (**14**): mp. 198 °C (lit²¹ mp. 200–203 °C); 2,3-dihydroxyanthraquinone (**15**): mp. >300 °C (lit²² mp. 393–394 °C); 1,3-dimethoxyanthraquinone (**16**): mp. 156 °C (lit² mp. 154–155 °C).

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ИЗВОД

ИЗОЛОВАЊЕ И СИНТЕЗА АНТРАХИНОНА И СЛИЧНИХ ЈЕДИЊЕЊА ИЗ *Rubia cordifolia*

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Изолувани су и карактерисани антрахинони и њихови гликозиди, као и друга једињења из сушеног корена *Rubia cordifolia* перколацијом смешом ацетон – вода (1:1). Поједини антрахинони су синтетизовани коришћењем монтморијонитске глине без растварача са 75 – 85 % приносом.

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