Phytochemistry, Vol. 31, No. 8, pp. 2907–2908, 1992
Printed in Great Britain.

0031-9422/92 $5.00+0.00

TWO NAPHTHOQUINONES FROM *RUBIA CORDIFOLIA*

JUNKO KOYAMA, TAMAKI OGURA, KIYOSHI TAGAHARA, TAKAO KONOSHIMA* and MUTSUO KOZUKA*

Kobe Women's College of Pharmacy, Kobe 658, Japan; *Kyoto Pharmaceutical University, Kyoto 607, Japan

(*Received in revised form* 21 *January* 1992)

Key Word Index—*Rubia cordifolia*; Rubiaceae; Rubiae Radix; naphthoquinone; anthraquinone.

Abstract—The methanol extract of *Rubia cordifolia* afforded two new naphthoquinones, 2-carbamoyl-3-methoxy-1,4-naphthoquinone and 2-carbamoyl-3-hydroxy-1,4-naphthoquinone, isolated along with a known naphthoquinone and naphthohydroquinone and six known anthraquinones. The structures of the new compounds were determined on the basis of spectroscopic data and direct comparisons with synthetic compounds.

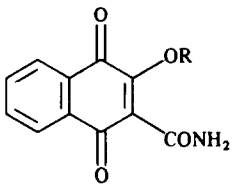
INTRODUCTION

Many plants of the Rubiaceae have been shown to be rich sources of anthraquinones. The reinvestigation of the constituents of *Rubia cordifolia* led us to isolate two new naphthoquinones (**1** and **2**) together with the known naphthoquinone, dehydro-α-lapachone (**3**) [1], and naphthohydroquinone, mollugin (**4**) [2], and six known anthraquinones, 3-carbomethoxy-1-hydroxyanthraquinone (**5**) [3], 1,4-dihydroxy-2-methylanthraquinone (**6**) [4], 1-hydroxy-2-methylanthraquinone (**7**) [5], tectoquinone (**8**) [6], 1-hydroxy-2-hydroxymethylanthraquinone (**9**) [7] and 1,3-dihydroxyanthraquinone (**10**) [8]. This paper deals with the structure elucidation of the new naphthoquinones isolated from this plant.

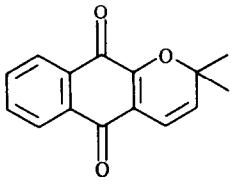
RESULTS

The methanol extract of *R. cordifolia* was dissolved in water and sequentially partitioned with *n*-hexane, ether, chloroform and ethyl acetate. Silica gel column chromatography of the *n*-hexane layer was used to isolate **3**–**8**. The residue from the ether-soluble layer was chromatographed on silica gel to yield **1**, **2**, **9** and **10**.

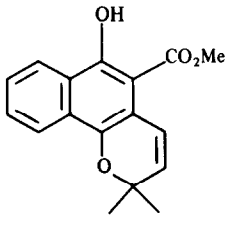
Compound **1**, $C_{12}H_9NO_4$, was obtained as yellow crystals. The ^1H NMR spectrum showed a signal for a methoxyl group [δ3.95 (3H, *s*)] and signals due to amide protons [δ7.04 (1H, *br s*) and 9.20 (1H, *br s*)]. The ^{13}C NMR spectrum showed 12 carbon signals consisting of one methoxyl carbon (δ52.2), four quaternary carbons (δ101.5, 129.5, 134.0, 169.4), four methyne carbons



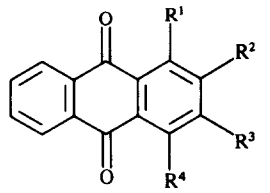
	R
1	Me
2	H



3



4



	R^1	R^2	R^3	R^4
5	OH	H	CO_2Me	H
6	OH	Me	H	OH
7	OH	Me	H	H
8	H	Me	H	H
9	OH	CH_2OH	H	H
10	OH	H	OH	H

(δ126.3, 127.4, 132.4, 135.9), a carbamoyl carbon (δ153.1) and two carbonyl carbons (δ179.0, 180.6). The IR spectrum suggested the presence of an amide group (3450, 3300, 1670 cm^{-1}) and carbonyl groups [1680 (sh), 1640 cm^{-1} (sh)]. The structure of **1** was determined by means of ^1H and ^{13}C NMR, HMBC (Fig. 1) and IR as 2-carbamoyl-3-methoxy-1,4-naphthoquinone. Finally, the structure was confirmed by a direct comparison of the physical data and TLC behaviour with a synthetic product, 2-carbamoyl-3-methoxynaphthoquinone, synthesized from 2-carbomethoxy-3-methoxynaphthoquinone [9] by ammonolysis.

Compound **2**, $C_{11}H_7NO_4$, was obtained as yellow crystals and characterized as 2-carbamoyl-3-hydroxy-1,4-naphthoquinone from the ^1H NMR spectrum, which resembled that of **1** with the methoxyl signal replaced by a hydroxyl signal [δ12.47 (1H, *s*)]. Demethylation of synthetic **1** with boron trichloride gave 2-carbamoyl-3-hydroxynaphthoquinone. The ^1H NMR spectral data and TLC behaviour of **2** were identical with those of synthetic 2-carbamoyl-3-hydroxynaphthoquinone.

EXPERIMENTAL

Mps: uncorr; ^1H NMR: 200 MHz, $CDCl_3$, TMS as int. standard; CC: silica gel (Mallinckrodt, AR) at amounts equivalent to 50 times the weight of the extract; prep. TLC: silica gel (Merck, $60F_{254}$; thickness 0.5 mm).

Plant material. The root of *Rubia cordifolia* (Rubiae Radix) was purchased in China (produced at Shisen Province).

Extraction and isolation. The roots (1 kg) of *R. cordifolia* were extracted with hot MeOH. After removal of the MeOH by evapn, the residue was dissolved in water and sequentially partitioned with *n*-hexane (residue 24.87 g), Et_2O (16.18 g), $CHCl_3$ (5.67 g) and EtOAc (6.79 g). The *n*-hexane-soluble portion was chromatographed on silica gel using as solvent systems *n*-hexane, benzene, benzene–$CHCl_3$, $CHCl_3$ and $CHCl_3$–MeOH. Elution with hexane–benzene gave yellow crystals of **4** (4.9 g). Elution with benzene gave a yellow residue which was subjected to prep. TLC on silica gel using benzene to give **6** (7 mg) and **7** (6 mg). Elution with benzene–$CHCl_3$ (10:1) gave a yellow oil, which on separation by prep. TLC gave **8** (2 mg). Elution with benzene–$CHCl_3$ (2:1) gave a yellow residue which was subjected to prep. TLC on silica gel using $CHCl_3$ to give **3** (8 mg) and **5** (3 mg). The Et_2O-soluble portion was also chromatographed on silica gel. Elution with benzene gave yellow crystals of **9** (5 mg). Elution with $CHCl_3$ gave a yellow residue which was subjected to prep. TLC using $CHCl_3$–Me_2CO (50:1) to give **10** (5 mg), **1** (10 mg) and **2** (1 mg).

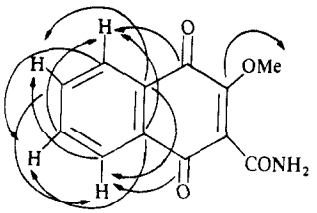


Fig. 1. CH long-range correlations in the HMBC spectrum of **2**.

2-Carbamoyl-3-methoxy-1,4-naphthoquinone (**1**). Yellow crystals, mp 150–151° (MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 3300, 1680 (sh), 1670, 1640 (sh), 1600, 1580; UV λ_{max}^{EtOH} nm (log ε): 225 (3.24), 270 (3.26); (+OH^-): 223 (sh) (3.28), 269 (3.24); ^1H NMR ($CDCl_3$): δ3.95 (3H, *s*, OMe), 7.04 (1H, *br s*, NH), 7.71 (1H, *td*, J = 8 and 1.5 Hz, H-6 or -7), 7.82 (1H, *td*, J = 8 and 1.5 Hz, H-7 or -6), 8.11 (1H, *dd*, J = 8 and 1.5 Hz, H-5 or -8), 8.25 (1H, *dd*, J = 8 and 1.5 Hz, H-8 or -5), 9.20 (1H, *br s*, NH); ^{13}C NMR ($CDCl_3$): δ52.2, 101.5, 126.3, 127.4, 129.5, 132.4, 134.0, 135.9, 153.1, 169.4, 179.0, 180.6; MS *m/z*: 231.0530 ([M]$^+$, calc. for $C_{12}H_9NO_4$, 231.0531).

Ammonolysis of 2-carbomethoxy-3-methoxy-1,4-naphthoquinone. A soln of naphthoquinone (0.5 g), NH_4OH (5 ml) and NH_4Cl (0.5 g) in MeOH (10 ml) was stirred for 2 hr at room temp. After evapn of the solvent, the residue was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water, dried ($MgSO_4$) and evapd to dryness to give amide **1**. Yield, 0.4 g (85%).

2-Carbamoyl-3-hydroxy-1,4-naphthoquinone (**2**). Yellow crystals, mp 200° (subl.) (MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3430, 3250, 1685, 1680 (sh), 1620, 1600; UV λ_{max}^{EtOH} nm (log ε): 212 (2.85), 251 (2.90), 275 (sh) (2.63), 317 (2.63); (+OH^-): 217 (sh) (2.91), 225 (sh) (2.89), 255 (3.03), 309 (2.44); ^1H NMR ($CDCl_3$): δ7.40 (1H, *br s*, NH), 7.80 (1H, *td*, J = 8 and 1.5 Hz, H-6 or -7), 7.90 (1H, *td*, J = 8 and 1.5 Hz, H-7 or -6), 8.21 (1H, *dd*, J = 8 and 1.5 Hz, H-5 or -8), 8.29 (1H, *dd*, J = 8 and 1.5 Hz, H-8 or -5), 9.75 (1H, *br s*, NH), 12.47 (1H, *s*, OH); MS *m/z*: 217.0371 ([M]$^+$, calc. for $C_{11}H_7NO_4$, 217.0374). Synthetic **3**. ^{13}C NMR ($CDCl_3$): δ127.4, 127.5, 129.7, 132.8, 133.9, 134.0, 136.5, 170.1, 173.4, 179.7, 184.5.

Demethylation of **1**. A soln of compound **1** (100 mg) and BCl_3 (200 mg) in CH_2Cl_2 (10 ml) was stirred at −78° for 24 hr. After evapn of the solvent, the crude product was separated by prep. TLC to give **2** (60 mg; 64%) and the starting material **1** (20 mg).

REFERENCES

1. Burnett, A. R. and Thomson, R. H. (1967) *J. Chem. Soc.* (*C*) 2100.
2. Schildknecht, H., Straub, F. and Scheidel, V. (1976) *Justus Liebigs Ann. Chem.* 1295.
3. Itokawa, H., Qiao, Y. and Takeya, K. (1991) *Phytochemistry* **30**, 637.
4. Dosseh, Ch., Tessier, A. M. and Delaveau, P. (1981) *Planta Med.* **43**, 141.
5. Itokawa, H., Mihara, K. and Takeya, K. (1983) *Chem. Pharm. Bull.* **31**, 2353.
6. Burnett, A. R. and Thomson, R. H. (1968) *Phytochemistry* **7**, 1421.
7. Wijnsma, R., Verpoorte, R., Mulder-Krieger, Th. and Svendsen, A. B. (1984) *Phytochemistry* **23**, 2307.
8. Burnett, A. R. and Thomson, R. H. (1968) *J. Chem. Soc.* (*C*) 854.
9. Hieber, W. and Lipp, A. (1959) *Chem. Ber.* **92**, 2071.