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THREE NOVEL NAPHTHOHYDROQUINONE DIMERS FROM *RUBIA ONCOTRICHA*

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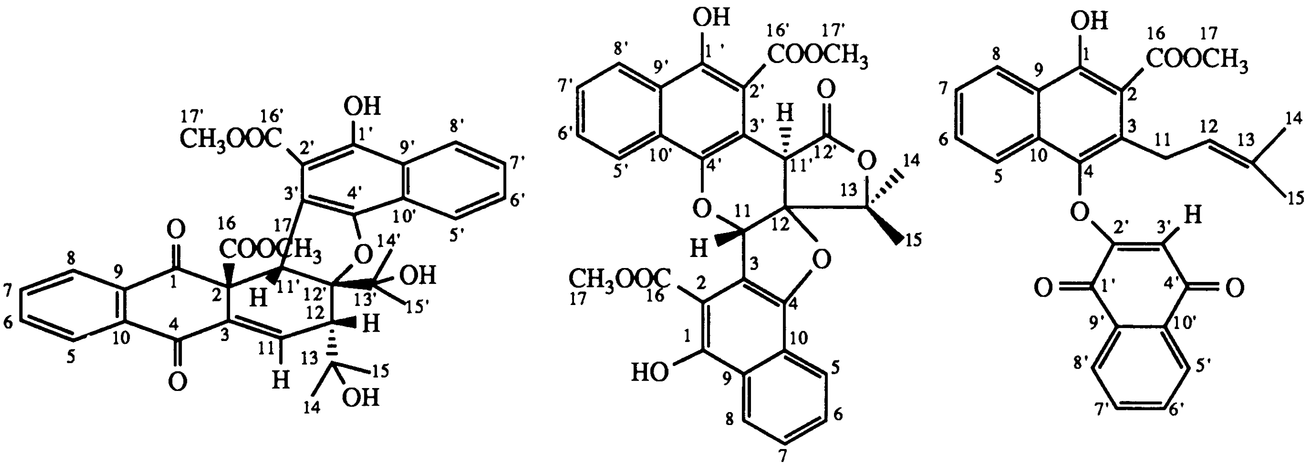
The structures of three novel dimers made up of naphthoquinone derivatives , rubioncolins A, B and C, from *Rubia oncotricha* Hand-Mazz, were determined by various spectroscopic methods and by X-ray diffraction.

KEYWORDS rubioncolin; *Rubia oncotricha*; Rubiaceae; naphthoquinone; naphthohydroquinone; dimer; ^{13}C-^{1}H COSY; COLOC; LSPD; X-ray diffraction

Variously substituted anthraquinones, naphthoquinones and naphthohydroquinones were obtained from species of *Rubia*, e.g. *R. cordifolia* and *R. akane*,$^{1)}$ *R. cordifolia* var. *pratensis*,$^{2)}$ *R. oncotricha*,$^{3)}$ etc. However dimers consisting of naphthoquinone derivatives were not known heretofore. This is the first report of the isolation and determination of three novel naphthohydroquinone dimers from *R. oncotricha*, also giving their proton and carbon signal assignments.

The methanol extract of the roots of *R. oncotricha* collected in China was partitioned between chloroform and water, then the chloroform-soluble fraction was separately column-chromatographed (Sephadex LH-20 and Silica gel) and rubioncolins A, B and C (Fig. 1) were isolated as yellowish needles, red-yellowish prisms and pale yellowish prisms respectively.

Rubioncolin A,$^{4)}$ mp 235-236°C, had a molecular formula $C_{34}H_{32}O_{10}$ determined from HREIMS (m/z: Calc. 600.1992, Found. 600.1992, $[M]^+$), and a pair of AA'BB' type aromatic protons (Table I) were identified by the ^{1}H NMR spectrum and ^{1}H-decoupling experiments at 400 MHz in $CDCl_3$. That was very important information for considering rubioncolin A to be made up of two naphthoquinone derivatives. The ^{1}H NMR spectrum also showed a singlet signal due to one proton, double doublet signals with a 5-Hz coupling constant due to two vicinal protons, one of them linked to a sp^2 carbon atom, as well as four methyl and two methoxyl groups (Table I). Based on the broad-band proton-decoupling ^{13}C NMR and DEPT spectra, it has two ketonic carbonyl groups, two methoxy carbonyl groups, two aromatic oxygenated-carbons and four sp^3 quarternary carbons (Table II). Also the ^{13}C-^{1}H COSY and long-range ^{13}C-^{1}H COSY (COLOC) experiments were invaluable for building up partial substructures (Fig. 2), and giving complete proton and carbon signal assignments (Table I and II). Two and three-bond correlations from H_3-14 and H_3-15 to C-13 and C-12, and from H-12, H_3-14' and H_3-15' to C-12' and C-13' were ascertained. The existence of α, β-unsaturated ketone was indicated by a correlation signal from H-11 to C-4 in the COLOC



A B C

Fig. 1. Structures of Rubioncolins A-C

spectrum. The location of C-11' were suggested by the correlations from C-3, C-2, C-4', C-3' and C-16, a methoxy carbonyl group, to H-11'. The remaining carbomethoxyl group was placed at C-2' by a lower-field signal due to a chelated hydroxyl proton. Another important three-bond coupling from HO-1' to C-9' appeared in the COLOC spectrum. This provided conclusive evidence for the proton and carbon signal assignments of the partial structure. Further NOE enhancements appeared between H_3-14' and H-11' (21%), H_3-15' and H-11' (14%), H_3-15' and H-12 (15%), H_3-14 and H-12 (12%), H_3-14 and H-11 (21%), and between H_3-15 and H-11 (16%) in the 1D-NOE spectra. So, the relative configurations of H-11' and C-13' were assumed to be cis-form, and C-13 and C-13' to be trans-form as shown in Fig. 1, and the assignments for the four methyl groups were specified as follows, the pro-S methyl groups were CH_3-14 and CH_3-14', and the pro-R methyl groups were CH_3-15 and CH_3-15'. According to the various spectral data, the structure of rubioncolin A was concluded to be as shown in Fig. 1, and that was confirmed by X-ray diffraction (Fig. 3).

Table I. ^1H NMR Spectral Data of Rubioncolins A-C (400 MHz, $CDCl_3$, Coupling Constants in Hz)

H-No.	HO-1	H-5	H-6	H-7	H-8	H-11	H-12	H_3-14	H_3-15	H_3-17
A		7.80 (brd, 8)	7.57 (brt, 8)	7.68 (brt, 8)	7.93 (brd, 8)	7.20 (d, 5)	2.82 (d, 5)	1.43 (s)	1.69 (s)	3.66 (s)
B	11.85 (s)	7.87 (brd, 8)	7.60 (brt, 8)	7.54 (brt, 8)	8.32 (brd, 8)	6.74 (s)		1.85 (s)	1.60 (s)	4.28 (s)
C	12.50 (s)	7.60 (brd, 8)	7.58 (brt, 8)	7.53 (brt, 8)	8.47 (brd, 8)	3.69 (2H, brs)	5.06 (m)	1.53 (d, 1.2)	1.57 (d, 1.2)	3.99 (s)
H-No.	**HO-1'**	**H-5'**	**H-6'**	**H-7'**	**H-8'**	**H-11'**	**H-3'**	**H_3-14'**	**H_3-15'**	**H_3-17'**
A	10.64 (s)	7.62 (brd, 8)	7.51 (brt, 8)	7.40 (brt, 8)	8.02 (brd, 8)	5.36 (s)		1.17 (s)	1.85 (s)	4.12 (s)
B	12.16 (s)	7.84 (brd, 8)	7.55 (brt, 8)	7.47 (brt, 8)	8.30 (brd, 8)	5.83 (s)				4.07 (s)
C		8.04 (m)a)	7.78 (m)	7.78 (m)	8.26 (m)a)		5.67 (s)			

a) Assignments may be exchangeable.

Table II. ^{13}C NMR Spectral Data of Rubioncolins A-C (100 MHz, $CDCl_3$)

C-No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
A	191.15	62.96	133.70	81.04	127.04	134.90	134.00	127.11	135.14	133.70	146.21	52.42	72.43	32.06	26.77	168.56	53.63
B	157.47	101.64	113.34	149.08	121.04	129.92	126.78	124.23	126.69	123.53	80.58	93.88	84.43	24.19	22.54	170.90	52.77
C	159.81	105.79	128.88	138.40	121.02	130.76	124.80	122.51	124.89	129.39	27.67	126.28a)	132.51b)	18.13	25.51	172.20	52.65
C-No.	**1'**	**2'**	**3'**	**4'**	**5'**	**6'**	**7'**	**8'**	**9'**	**10'**	**11'**	**12'**	**13'**	**14'**	**15'**	**16'**	**17'**
A	154.57	104.11	113.33	149.73	121.40	129.61	126.87	124.40	125.20	122.61	58.34	100.91	76.78	29.25	23.57	170.58	52.83
B	158.15	104.48	110.61	141.43	121.74	129.78	127.53	124.76	125.44	129.34	43.97	170.96				171.58	52.73
C	179.76	160.77	113.93	184.82	126.41a)	134.47c)	133.57c)	126.77a)	131.30b)	132.07b)							

a), b), c) Assignments may be exchangeable.

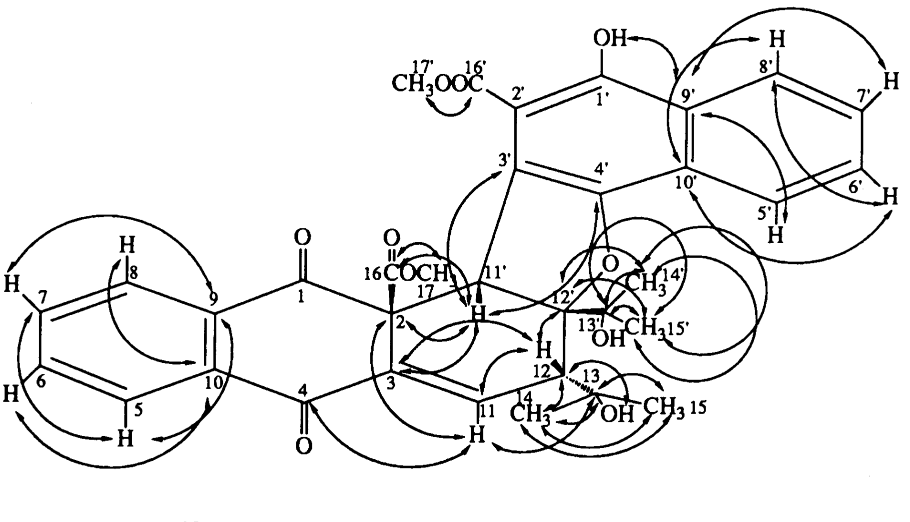
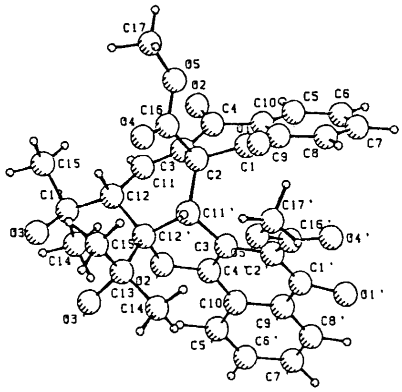


Fig. 2. ^{13}C-^1H Correlations of Rubioncolin A (COLOC)

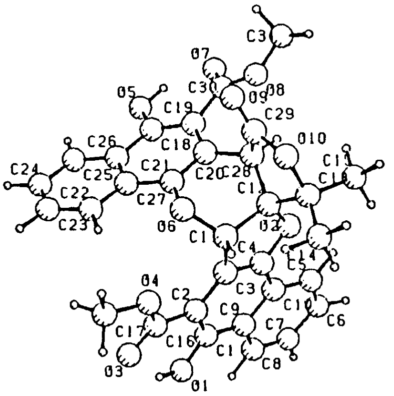
Rubioncolin B,5) mp 235-236°C, had a molecular formula $C_{31}H_{24}O_{10}$ determined from EIMS (m/z: 556 $[M]^+$), HREIMS (m/z: Calc. 556.1367, Found 556.1243, $[M]^+$) and NMR data. Comparing the two NMR spectra indicated that the skeleton of rubioncolin B was similar to that of rubioncolin A. In the aromatic region of the ^{13}C NMR spectrum, there were two oxygenated-carbons, eight quartemary carbons and two hydroxylated-carbons whose protons were chelated with ortho-positional carbomethoxyl groups respectively. That was suggested by the hydroxyl proton signals in lower-field of the ^1H NMR spectrum (Table I). It was assumed that the above data comprised two very similar partial structures, 2-carbomethoxy-1,4-naphthohydroquinone derivatives. In the IR spectrum, the 1784 cm^{-1} band showed five-membered ring lacton, whose location was confirmed by the two-bond correlations from H-11' to C-12', C-12 and C-3', and three-bond correlations from H-11' to C-11, C-2' and C-4' in the COLOC spectrum. Two singlets of methyl signals in the ^1H NMR spectrum were assigned to C-14 and C-15 respectively by a ^1H-^{13}C COSY experiment, and they were adjacent to the same quarternary carbon C-13 confirmed by two-bond correlations from H_3-14 and H_3-15. Two- and three-bond correlations to H-11 confirmed the location of C-3, C-4 and C-13. It was indicated that the relative configurations between H-11' and CH_3-14, and H-11 and CH_3-15 were in cis-form as shown in Fig. 1, because 21% and 29% NOE enhancements were found between them in the 1D-NOE spectra respectively. From the above results , the structure shown in Fig. 1 was derived, and it was established by the X-ray diffraction (Fig. 3). The proton and carbon signal assignments (Table I and II) were based on ^{13}C-^{1}H COSY, COLOC data and LSPD with Gated Decoupling experiments (all at 500 MHz, in $CDCl_3$). Two- and three-bond correlations to HO-1 and HO-1' distinguished the locations between C-1 and C-1', C-2 and C-2', and C-9 and C-9' in the COLOC spectrum. The carbon signals of C-5 to 8 and C-5' to 8' were assigned by the three-bond correlation from H-7' to C-9', and two methoxycarbonyl groups by the four-bond correlation from HO-1 to C-16 in the LSPD spectrum.

monoclinic
space group: Cc
z: 4
a = 16.647 (9) Å
b = 35.732 (19) Å
c = 9.884 (5) Å
v = 3177 Å^3
R = 0.048



rubioncolin A

monoclinic
space group: $P2_1/n$
z: 4
a = 19.244 (12) Å
b = 12.002 (7) Å
c = 11.470 (7) Å
v = 2548 Å^3
R = 0.058



rubioncolin B

Fig. 3. Perspective View of Rubioncolins A and B

Rubioncolin C,⁶⁾ mp 171-172°C, had a molecular formula $C_{27}H_{22}O_6$ determined from HREIMS (m/z: Calc. 442.1414, Found 442.1366 $[M]^+$), EIMS (m/z: 442 $[M]^+$) and NMR spectra. A pair of AA'BB' type protons also were found as in A and B in the ^{1}H NMR and ^{1}H-decoupling spectra, but there were some difference among their coupling patterns (Table I). A multiplet signal owing to one alkene proton H-12 had vicinal couplings with two protons H_2-11 and long-range couplings with two methyl protons linked to a sp^2 carbon was suggested by the ^{1}H-decoupling experiments. They were ascribed to a prenyl group. The ^{13}C NMR resonances of C-1', C-2' and C-4' comprised a 1,4-naphthoquionone oxygenated at C-2.⁷⁾ The location of carbomethoxyl group found in the ^{13}C NMR spectrum was shown to be linked to C-2 by a lower-field signal due to a chelated hydroxyl proton in the ^{1}H NMR spectrum. The remaining proton H-3' showed a singlet signal should be ascribed to C-3'. Also the important EIMS fragment of the 2-oxygen-1,4-naphthoquinone ion was indicated by m/z 269. In addition, a remaining carbon signal C-4 could be assigned to an oxygenated-carbon atom. So it had a structure as shown in Fig. 1.

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3) H. Itokawa, Y.-F. Qiao and K. Takeya, Phytochemistry in press.
4) UV (MeOH) nm (ε): 220 (40143), 240 (31422), 265 (32736), 380 (4301), IR ($CHCl_3$) cm^{-1}: 1748, 1739 (sh), 1684, 1672 (C=O), 1648, 1622, 1602 (C=C), $[\alpha]_D$ +40.6° (c 0.3, $CHCl_3$).
5) UV (MeOH) nm (ε): 221 (42485), 247 (20710), 253 (22249), 262 (20592), 362 (7337), IR ($CHCl_3$) cm^{-1}: 1784, 1664, 1658 (sh) (C=O), 1652, 1640, 1608 (C=C), $[\alpha]_D$ 0° (c 0.3, $CHCl_3$).
6) UV (MeOH) nm (ε): 216 (43033), 243 (44180), 248 (47049), 259 (36967), 275 (17295), 305 (5328), 338 (6885), IR (CCl_4) cm^{-1}: 1688, 1662, 1658 (sh) (C=O), 1632, 1618, 1600, 1580 (C=C).
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