A New Naphthopyrone Derivative from *Cassia quinquangulata* and Structural Revision of Quinquangulin and Its Glycosides

Xing-Cong Li,[†] D. Chuck Dunbar,[†] Hala N. ElSohly,[†] Melissa R. Jacob,[†] Alison C. Nimrod,[†] Larry A. Walker,[†,‡] and Alice M. Clark*[,†,§]

National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, Department of Pharmacology, and Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, Mississippi 38677

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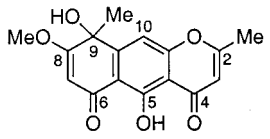
A novel naphthopyrone derivative, named quinquangulone (**1**), has been isolated from *Cassia quinquangulata*, along with the known compounds quinquangulin (**2**) and its two glycosides (**3** and **4**), rubrofusarin (**5**) and its two glycosides (**6** and **7**), nor-rubrofusarin (**8**) and its 6-*O*-glucoside (**9**), and three stilbenes (**10**−**12**). The structure of quinquangulone was established by spectral interpretation as 5,9-dihydroxy-8-methoxy-2,9-dimethyl-6-oxo-4*H*,6*H*,9*H*-naphtho-[2,3-*b*]pyran-4-one. Reinvestigation of the NMR spectra of quinquangulin led to revision of its structure as 5,6-dihydroxy-8-methoxy-2,9-dimethyl-4*H*-naphtho-[2,3-*b*]pyran-4-one (**2a**). The structures of two quinguangulin glycosides, **3** and **4**, were also revised accordingly. Compound **2a** exhibited activity against *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MIC, 3.125 and 6.25 μg/mL, respectively).

A previous chemical study of the Peruvian plant *Cassia quinquangulata* Rich. (Fabaceae) revealed the presence of two cytotoxic naphthopyrone compounds, quinquangulin (**2**) and rubrofusarin (**5**), as well as an anthraquinone compound, chrysophanol.[1] In a study on cancer chemoprevention, the stilbene-related compound resveratrol was isolated as a potential cancer chemopreventive agent from the same species.[2] In the course of our search for antimicrobial compounds from higher plants, we reinvestigated this plant. The present paper reports the isolation and structure elucidation of a new naphthopyrone derivative, quinquangulone (**1**), and the revision of the structure of quinquangulin (**2**) and its glycosides (**3** and **4**). The antimicrobial activity of the isolated compounds has also been evaluated.



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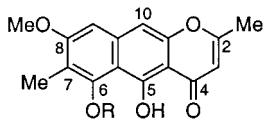
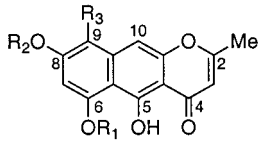
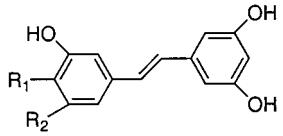


	R
2	H
3	Glc
4	Glc(6←1)Api



	R_1	R_2	R_3
2a	H	Me	Me
3a	Glc	Me	Me
4a	Glc(6←1)Api	Me	Me
5	H	Me	H
6	Glc	Me	H
7	Glc(6←1)Api	Me	H
8	H	H	H
9	Glc	H	H



	R_1	R_2
10	OH	H
11	OMe	OH
12	OH	OH

Results and Discussion

Repeated column chromatography of a chloroform-soluble portion of the ethanol extract of *C. quinquangulata* roots afforded compound **1** and 11 known compounds that were identified by their spectral data (NMR, MS, IR, UV) as quinquangulin (**2**),[1] quinquangulin-6-*O*-β-D-glucopyranoside (**3**),[3] quinquangulin-6-*O*-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside (**4**),[3] rubrofusarin (**5**),[1] rubrofusarin-6-*O*-β-D-glucopyranoside (**6**),[3] rubrofusarin-6-*O*-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside (**7**),[3,4] nor-rubrofusarin (**8**),[5] nor-rubrofusarin-6-*O*-β-D-glucopyranoside (**9**),[6] *trans*-3,3′,5,5′-tetrahydroxystilbene (**10**),[7] *trans*-3,3′,5,5′-tetrahydroxy-4-methoxystilbene (**11**),[3,8] and *trans*-3,3′,4,5,5′-pentahydroxystilbene (**12**).[9] The ^{1}H and ^{13}C NMR signals of **9** were assigned by a combination of DEPT and 2D NMR techniques (COSY, HMQC, HMBC) and are listed in Table 1 since these data are not available in the literature.

Compound **1** was obtained as colorless needles, mp 218−220 °C. The high-resolution ESIMS indicated its molecular formula to be $C_{16}H_{14}O_6$. Its IR spectrum showed a strong conjugated carbonyl absorption at 1663 cm^{-1} and aromatic absorptions at 1614 and 1457 cm^{-1}. Further support for the conjugated carbonyl was derived from the UV spectrum, which showed strong absorption bands at 224, 251, 306, and 341 nm. The ^{13}C NMR spectrum of **1** displayed 16 carbon resonances, while its ^{1}H NMR spectrum showed only eight singlets (Table 1). With the aid of an HMQC experiment, the presence of two methyls (δ_H 1.52/δ_C 31.5, δ_H 2.30/δ_C 19.4), one methoxyl (δ_H 3.83/δ_C 56.7), three aromatic (or olefinic) protons (δ_H 6.11/ δ_C 109.9, δ_H 5.65/ δ_C 99.7, δ_H 7.15/δ_C 105.3), and two hydroxyl protons (δ_H 6.22, 14.5) was confirmed. The HMBC technique was then

* To whom correspondence should be addressed. Tel: 662-915-1005. Fax: 662-915-1006. E-mail: ncnpr@olemiss.edu.
† National Center for Natural Products Research.
‡ Department of Pharmacology.
§ Department of Pharmacognosy.

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Table 1. NMR Data of Compounds **1**, **2a**−**4a**, and **9** (δ, ppm; J, Hz)

	1 (DMSO-d_6)		**2a** ($CDCl_3$)		**3a** (DMSO-d_6)		**4a** (DMSO-d_6)		**9** (DMSO-d_6)	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	165.6		169.1		169.0		168.9		168.7	
3	109.9	6.11 s	106.4	6.01 s	106.7	6.18 s	106.6	6.19 s	106.5	6.16 s
4	177.6		184.2		183.8		183.7		183.7	
4a	110.7		102.3		103.1		103.1		103.0	
5	162.5		162.9		162.4		162.4		162.1	
5a	109.3		106.2		106.6		106.6		106.9	
6	187.5		157.6		156.7		156.5		158.3	
7	99.7	5.65 s	96.5	6.63 s	97.9	7.09 s	97.6	7.01 s	101.2	6.69 d (2)
8	178.3		159.6		157.7		157.7		159.7	
9	69.4		108.9		109.9		110.0		101.6	6.73 d (2)
9a	154.3		138.5		138.2		138.2		140.5	
10	105.3	7.15 s	98.3	7.12 s	97.5	7.15 s	97.5	7.17 s	100.0	7.06 s
10a	159.3		152.2		152.2		152.6		152.3	
Me-2	19.4	2.30 s	21.2	2.40 s	20.1	2.38 s	20.1	2.39 s	20.2	2.37 s
Me-9	31.5	1.52 s	10.5	2.33 s	10.5	2.29 s	10.5	2.29 s		
MeO-8	56.7	3.83 s	56.1	3.93 s	55.9	3.91 s	56.0	3.92 s		
HO-5		14.5 br s[a]		16.0 br s		14.9 br s		14.9 br s		14.9 br s
HO-6				9.83 br s						
HO-8										10.3 br s
HO-9		6.22 br s[a]								
Glc-1					101.8	5.03 d (7.6)	101.5	5.07 d (8.0)	102.5	4.98 d (7.6)
2					73.5	3.43	73.4	3.43	73.5	3.42
3					76.6	3.30	75.8	3.65 t (8.0)	76.4	3.33
4					70.2	3.15	70.2	3.12	69.5	3.12
5					77.6	3.41	76.2	3.35	77.3	3.41
6					61.0	3.76 br d (8)	67.8	3.94 br d (11)	60.6	3.75 dd (13, 3)
						3.45		3.48		3.54 dd (13, 7)
Api-1							109.1	4.80 d (3.3)		
2							75.8	3.71 d (3.3)		
3							78.6			
4							73.2	3.85 d (10.3)		
								3.57 d (10.3)		
5							62.8	3.26		

[a] Disappeared after adding D_2O or when measured in CD_3OD.

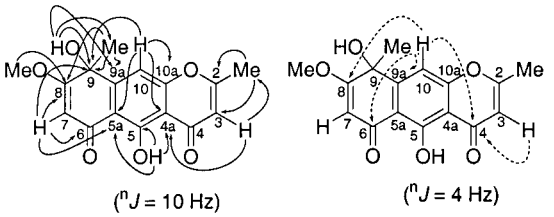

Figure 1. HMBC correlations of **1**.

employed to determine long-range H/C correlations. Using a delay time of 50 ms, corresponding to a J coupling of 10 Hz, all of the protons displayed three-bond correlations, as well as some *ortho*-oxygenated two-bond correlations (Figure 1). When the delay time was set to 250 ms (J coupling of 4 Hz), some important four-bond and two-bond H/C correlations were also observed (Figure 1, depicted with dashed lines).[10,11] On the basis of these two experiments, the molecular skeleton of **1** was readily established, and its structure was shown to be 5,9-dihydroxy-8-methoxy-2,9-dimethyl-6-oxo-4*H*,6*H*,9*H*-naphtho-[2,3-*b*]pyran-4-one. A NOESY experiment was also employed to confirm the structure shown. NOE correlations were observed between H-10/Me-9, H-3/Me-2, H-7/MeO-8, and Me-9/HO-9. Compound **1** is a naphthopyrone derivative similar to quinquangulin (**2**)[1] and was thus named quinguangulone. The stereochemistry of C-9 in **1** remains undetermined, but the optical rotation data of **1** {$[\alpha]^{25}_{\lambda}$ 0° (λ = 546, 589, 633 nm) (c 0.2, MeOH)} suggests it is a racemic mixture.

Quinquangulin (**2**) was first reported as a constituent of *Cassia quinquangulata* in 1977, and the structure was proposed based largely on interpretation of the ^{1}H NMR spectral data.[1] The assignment of the C-7 methyl group in quinquangulin (**2**) was based on empirical calculation of the ^{1}H NMR chemical shifts of its two mononitro dimethyl ethers, 9-nitro- and 10-nitroquinquangulin dimethyl ethers prepared by nitration of quinquangulin dimethyl ether with copper nitrate.[1] The observed ^{1}H NMR chemical shifts of the aromatic protons induced by the nitro group in each mononitrate were close to the calculated values for the effect of a nitro group on the chemical shifts of the aromatic protons in naphthalene derivatives, as reported by Wells.[12,13] However, quinquangulin is actually a multiply substituted naphthopyrone derivative, not a naphthalene derivative; thus, the 7-nitroquinquangulin dimethyl ether could coincidentally produce a nitro substitution effect similar to that of the 9-nitroquinquangulin dimethyl ether. In other words, one of the two nitration products, which was believed to be 9-nitroquinquangulin dimethyl ether in the previous study, could be 7-nitroquinquangulin dimethyl ether. Thus, the possibility of the C-9 methyl substitution in quinquangulin could not be excluded at that time.

Isolation of compound **1**, which possesses a C-9 methyl, prompted us to reconsider the structure of **2** since, from the biogenetic viewpoint, the location of this methyl group in **2** would reasonably be expected to be the same as in **1**, i.e., at C-9. Using an HMBC experiment, a methyl group on C-7 in structure **2** should show HMBC correlations to two *ortho*-oxygenated aromatic carbons (C-6 and C-8, both resonating around δ 160) (Figure 2). However, in the HMBC spectrum (J = 10 Hz) of quinquangulin (obtained for this study), the methyl protons at δ 2.33 correlated with only one *ortho*-oxygenated aromatic carbon at δ 159.6 (C-8). Instead, the aromatic proton at δ 6.63 correlated with two *ortho*-oxygenated aromatic carbons (C-6 and C-8) (Figure 2). This confirms that the methyl is located at C-9

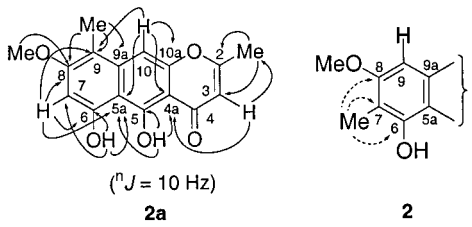

Figure 2. HMBC correlations of **2a** (and **2**).

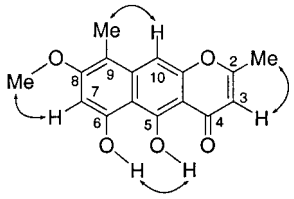

Figure 3. NOE correlations of **2a**.

and not at C-7. Detailed HMBC correlations are summarized in Figure 2, and complete assignments of the ^{1}H and ^{13}C NMR signals are listed in Table 1. Additional evidence for the assignment of the C-9 methyl group was obtained from the NOESY experiment in $CDCl_3$, which showed correlations between H-10/Me-9, H-7/MeO-8, H-3/Me-2, and HO-5/HO-6. The NOE correlations further indicated that quinquangulin adopted a predominant conformation form in $CDCl_3$, as shown in Figure 3. This also confirmed the presence of the intramolecular hydrogen bonds (HO-6/HO-5 and HO-5/O=C-4) in quinquangulin, which appeared as two relatively sharp peaks of the hydroxy protons (HO-5 and HO-10) in the downfield region of its ^{1}H NMR spectrum. Therefore, the structure of quinquangulin should be revised as 5,6-dihydroxy-8-methoxy-2,9-dimethyl-4*H*-naphtho[2,3-*b*]pyran-4-one (**2a**). The X-ray crystallographic structure of compound **2a**, which was named 9-methoxyrubrofusarin, was previously reported by De Gil et al.[14] However, the source of the compound was not identified. A search of literature since 1977 has revealed no other report on this compound. It is worthwhile to note that all the NOE evidence observed in our study for compound **2a** is consistent with the reported X-ray crystallographic data.

So far, only two glycosides, **3** and **4**, possessing a quinquangulin aglycon have been isolated from *Cassia pudibunda*.[3] In our study, the same two compounds were isolated in small quantities. Extensive 2D NMR studies were employed to confirm their structures. The HMBC spectra of **3** and **4** displayed the same correlation pattern as that in **2a**, supporting the location of the methyl group at C-9. In addition, the ^{1}H and ^{13}C NMR signals of these two compounds were assigned with the aid of COSY, HMQC, and HMBC experiments (Table 1). Therefore, the structures of quinquangulin-6-*O*-*β*-D-glucopyranoside (**3**) and quinquangulin-6-*O*-*β*-D-apiofuranosyl(1→6)-*β*-D-glucopyranoside (**4**) should also be revised as **3a** and **4a**, respectively.

Compounds **1**, **2a**, **4a**, and **5**−**12** were tested for antimicrobial activity against *Candida albicans, Cryptococcus neoformans, Aspergillus fumigatus, Staphylococcus aureus*, methicillin-resistant *S. aureus*, and *Mycobacterium intracellulare*, using methods previously described.[15−17] Compound **2a** was active against *S. aureus* and methicillin-resistant *S. aureus*, with MIC values of 3.125 and 6.25 μg/mL, respectively. The MIC values of two control drugs, rifampicin and erythromycin, for *S. aureus* were 0.005 and 1.25 μg/mL, respectively. Rifampicin also inhibited methicillin-resistant *S. aureus*, with an MIC value of <0.005 μg/mL.

Experimental Section

General Experimental Procedures. Melting points were measured on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Optical rotations were determined on an AutoPol IV automatic polarimeter. UV spectra were measured on a Hewlett-Packard 8453 spectrometer. NMR spectra were recorded on Bruker Avance DPX-300 (300 MHz), DRX-400 (400 MHz), or DRX-500 (500 MHz) NMR spectrometers. Chemical shifts are expressed relative to the internal standard TMS. COSY, HMQC, HMBC (*J*, 10 or 4 Hz), and NOESY (mixing time, 800 ms) NMR spectra were performed with standard pulse programs. ESI-FTMS were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron high-resolution HPLC-FT spectrometer by direct injection into an electrospray interface. Column chromatography was run using silica gel (40 μm, J. T. Baker), reversed-phase silica gel (RP-18, 40 μm, J. T. Baker), Sephadex LH-20 (Supelco), and polyamide (10−160 μm, Supelco). Semipreparative HPLC was conducted on an ODS (Prodigy) column (250 × 10 mm, 10 μm) using a UV detector at 254 nm. TLC was performed on silica gel sheets (Alugram Sil G/UV_{254}, Macherey-Nagel, Germany) and reversed-phase plates (RP-18 F_{254S}, Merck, Germany).

Plant Material. The roots of *C. quinquangulata* were collected by Mr. Manuel Rimachi in Porvenir, Lorento, Peru, in May 1994, and identified by Mr. M. Rimachi and Prof. Sidney McDaniel. A herbarium specimen of this plant is deposited at the Herbarium of Mississippi State University (Voucher No. IBE-MR 10936).

Extraction and Isolation. The dried roots (500 g) were ground to a coarse powder and percolated with 95% EtOH (4 L × 2). Removal of the solvent under vacuum at 45 °C yielded an EtOH extract (54.7 g). The EtOH extract was dissolved in MeOH−H_2O (9:1, 1.5 L) and defatted with hexane (1.2 L). To the MeOH−H_2O layer was added H_2O (300 mL), and the resulting solution was further partitioned with $CHCl_3$ (1.5 L). The $CHCl_3$ layer was evaporated to dryness in vacuo (45 °C) to give a red residue (8.6 g). The residue was chromatographed on Si gel using a stepwise gradient solvent system consisting of $CHCl_3$−MeOH (0% to 100%, a total of 8.7 L) to yield column fractions A−I. Part of fraction B (30 mg) was separated by HPLC (95% MeOH) to yield **2a** (8.8 mg) and **5** (18.9 mg). Fraction D (143 mg) was chromatographed over Sephadex LH-20 using EtOH to afford **8** (5.9 mg) and crude **1** (41.3 mg), which was further purified by HPLC (65% MeOH) to furnish pure **1** (25 mg). Fraction F (150 mg) was crystallized with MeOH to afford **9** (12.5 mg). The mother liquid of **9** was separated by HPLC (70% MeOH) to afford **3a** (1.2 mg) and **6** (3.2 mg). Fraction G (42 mg) was chromatographed on a polyamide column using MeOH to afford **10** (2.2 mg) and **11** (5.0 mg). Fraction H (90.5 mg) was chromatographed on reversed-phase silica gel using 50% MeOH to yield **12** (32 mg). Part of fraction I (15 mg) was separated by HPLC (70% MeOH) to give **4a** (2.5 mg) and **7** (6.8 mg).

Quinquangulone (1): colorless needles from MeOH, mp 218−220 °C (dec); $[\alpha]_\lambda^{25}$ 0° (λ = 546, 589, 633 nm) (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (4.41), 251 (4.44), 306 (3.94), 341 (4.05) nm; IR (KBr) ν_{max} 1663, 1614, 1457, 1375, 1229, 1136 cm^{-1}; NMR data, see Table 1; ESIMS *m*/*z* 303.0896 {calcd for [M($C_{16}H_{14}O_6$) + H], 303.0865}.

Quinquangulin (2a): brown-red powder, mp 196 °C (dec); UV (MeOH) λ_{max} (log ϵ) 227 (4.57), 254 (sh, 4.49), 282 (4.80), 430 (2.85) nm; IR (KBr) ν_{max} 1653, 1622, 1585, 1320, 1133, 830 cm^{-1}; NMR data, see Table 1; ESIMS *m*/*z* 287.0934 {calcd for [M($C_{16}H_{14}O_5$) + H], 287.0914}. Comparison of the UV, IR, and ^{1}H NMR data for this isolate with those reported in the literature[1] showed them to be the same. [The ^{1}H NMR signal for the hydroxyl proton (HO-5) was at δ 10.94 (1H, br s, OH) in ref 1; while in the present study, it was observed at δ 16.04 (1H, br s)].

Quinquangulin-6-*O*-*β*-D-glucopyranoside (3a): yellow powder, $[\alpha]_\lambda^{25}$ −131° (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (4.23), 256 (sh, 4.21), 280 (4.28), 405 (3.49) nm; IR (KBr) ν_{max} 3435 (br), 2918, 1650, 1622, 1586, 1076 cm^{-1}; NMR data, see Table 1; ESIMS *m*/*z* 449 $[M(C_{22}H_{24}O_{10}) + H]^+$. Identified by comparison of the UV, ^1H NMR, and MS data with literature values[3] (IR and ^{13}C NMR data were not reported in ref 3).

Quinquangulin-6-*O*-*β*-D-apiofuranosyl(1→6)-*β*-D-glucopyranoside (4a): yellow powder, $[\alpha]_\lambda^{25}$ −145° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 225 (4.16), 256 (sh, 4.20), 280 (4.31), 410 (3.59) nm; IR (KBr) ν_{max} 3417 (br), 2924, 1652, 1622, 1586, 1077 cm^{-1}; NMR data, see Table 1; ESIMS *m*/*z* 603.1652 {calcd for $[M(C_{27}H_{32}O_{14}) + Na]$, 603.1689}. Identified by comparison of the UV, ^1H, ^{13}C NMR, and MS data with literature values[3] (IR data were not reported in ref 3).

Antimicrobial Assay. *Staphylococcus aureus* ATCC 6538 and methicillin-resistant *S. aureus* ATCC 33591 were stored on Eugon (Difco, Detroit, MI) agar slants at 4 °C until needed. Susceptibility testing was performed using a modified version of the protocol of the National Committee on Clinical Laboratory Standards.[15] Cells were transferred to 5 mL of Eugon broth (BBL, Cockeysville, MD) and incubated at 37 °C. Overnight cultures were diluted with Eugon broth (1:50) for the antimicrobial assay. Test compounds were dissolved in DMSO, serially diluted using normal saline, and transferred to 96-well microtiter plates. The inoculum was added to achieve a final volume of 200 μL and final concentrations of 50−0.02 μg/mL. Drug [erythromycin and rifampicin at 5.0 to 0.002 μg/mL (Sigma, St. Louis, MO)] as well as growth and blank (media only) controls were added to each test plate. Plates were read turbidimetrically at 630 nm using the EL-340 Biokinetics Reader (Bio-Tek Instruments, Winooski, VT) prior to and after incubation (24 h at 37 °C). Percent growth was calculated and plotted versus concentration to afford the minimum inhibitory concentration (MIC) in which at least 80% growth was inhibited. The procedures used for *Candida albicans* ATCC 32354 (B311), *Cryptococcus neoformans* ATCC 52657, *Aspergillus fumigatus* ATCC 26934, and *Mycobacterium intracellulare* ATCC 23068 have been described in previous papers.[16,17]

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