Cytotoxicity of Rhamnosylanthraquinones and Rhamnosylanthrones from Rhamnus nepalensis

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · AUGUST 2001

Impact Factor: 3.8 · DOI: 10.1021/np010030v

Cytotoxicity of Rhamnosylanthraquinones and Rhamnosylanthrones from *Rhamnus nepalensis*

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Received January 26, 2001

An extract of the fruits of *Rhamnus nepalensis* collected in Hoa Binh Province, Vietnam, was cytotoxic to KB cells. A bioassay-guided fractionation led to the isolation of a series of known anthraquinones and anthrones, one new rhamnosylanthraquinone, 3′-*O*-acetylfrangulin A (**8**), several new rhamnosylanthrones, the prinoidin-emodin bianthrones (**9A**−**D**), the prinoidin bianthrones (**10A**,**B**), and the rhamnepalins (**11A**−**C**). A structure−cytotoxic activity relationship study was performed on these isolates and some semisynthetic derivatives.

The genus *Rhamnus* (Rhamnaceae), which is encountered both in temperate and in tropical countries, includes well-known medicinal species possessing various biological properties, for example *R. cathartica*,[1] *R. frangula*, and *R. purshiana*. Generally, *Rhamnus* species contain anthraquinones such as emodin[2−8] or chrysophanol,[3,6,8,9] their reduced forms, chrysophanol-anthrone[3] and emodin-anthrone,[3−5] dimers such as chrysophanol bianthrone,[9] emodin bianthrones,[3−5] and chrysophanol-emodin bianthrones[3,10] (unknown configuration), or their glycosides such as prinoidin,[4,5] while some others contain flavonoids.[2,5,7,8,12,13] Some of these anthraquinones have been found to have antileukemic, cytotoxic, laxative (or purgative), photosensitizing, and vasorelaxant properties.[8,14,15] In the course of our ongoing search for anticancer agents from natural sources, an ethyl acetate extract of the fruits of *Rhamnus nepalensis* Laws. (Rhamnaceae), collected in Vietnam, was found to be cytotoxic to the KB cell line. Previously, emodin and known flavones have been isolated from *R. nipalensis* Laws., collected in Pachmari, India,[2] a species presumably identical to *R. nepalensis* Laws. Bioassay-guided fractionation of an extract of fruit of *R. nepalensis* led to the isolation of 21 anthraquinones and anthrones, of which 10 are new. We report here the isolation of these compounds, the structure elucidation of the new compounds, and a study of the structure−cytotoxicity relationships in this series.

Results and Discussion

Dried and powdered fruits of *R. nepalensis* were first defatted with hexane, then extracted with EtOAc. Cytotoxicity-guided purification by column chromatography under medium-pressure TLC and HPLC on Si gel allowed the isolation of 11 known compounds, namely, chrysophanol[3,6,8,9] physcion,[2,4,6,8,9] emodin,[2,4,6−8] emodin-anthrone,[3−5,10] prinoidin (**1**),[4,5] 2′,3′-di-*O*-acetylfrangulin A (**2**),[5] 2′-*O*-acetylfrangulin A (**3**),[5] frangulin A peracetate (**4**),[5] chrysophanol bianthrones (**5A**,**B**),[9] two chrysophanol-emodin bianthrones **6** (stereochemistry at C10/C10′ unknown),[3,10] emodin bianthrones (**7A**,**B**), and 10 new compounds, 3′-*O*-acetylfrangulin A (**8**), four prinoidin-emodin bianthrones (**9A**−**D**), two prinoidin bianthrones (**10A**,**B**), and three rhamnepalins (**11A**−**C**) (Chart 1). The known compounds were readily identified by comparison of their spectroscopic data with those of reference samples or as described in the literature. However, the NMR spectrum of compound **5** was recorded on a mixture of *cis* and *trans* compounds. HPLC separation on an analytical chiral column clearly shows the presence of the three isomers (Table 1). The mixture of the compounds **5A** and **5B** was purified on a chiral column to give **5A** (*cis*) and **5B** (*trans*), which were used only for evaluation of the cytotoxicity.

Compound **8** exhibited a major peak $[M + H]^+$ at *m*/*z* 459.1291 (HRCIMS) which matched the molecular formula $C_{23}H_{23}O_{10}$. The ^1H and ^{13}C NMR spectra of **8** were similar to those of 2′-*O*-acetylfrangulin A (**3**) except that in the ^1H NMR spectrum of **8** the signal of H-2′ was shielded from δ 5.23 to 4.95, and H-3′ appeared at δ 5.98. Compound **8** was thus assigned as 3′-*O*-acetylfrangulin A, a regioisomer of **3** with the acetyl group at C-3′.

The four C-10, C-10′ diastereomers of prinoidin-emodin bianthrones **9A**−**D** were each isolated by preparative TLC on silica gel. These compounds gave a major peak $[M + H]^+$ at *m*/*z* 741.2183 (HRCIMS) corresponding to the molecular formula $C_{40}H_{37}O_{14}$. Their structures were deduced from a comparison of their NMR data with those of prinoidin (**1**), emodin, and emodin bianthrones. The signals of H-10 and H-10′ differed from **9A** to **9D**. In the ^1H spectrum of compounds **9A**, **9B**, and **9C**, they both appeared as two doublets ($J = 3$ Hz) at δ 4.18 and 4.10 (**9A**), δ 4/13 and 4.03 (**9B**), and δ 4.10 and 4.00 (**9C**). The ^1H NMR spectrum of **9D** revealed a 2H singlet at δ 4.21, corresponding to these two protons (Tables 2 and 3). NOESY correlations did not permit a determination of the stereochemistry at C-10 and C-10′ in compounds **9A**−**D**.

Prinoidin bianthrones **10A** and **10B** revealed a peak $[M + H]^+$ at *m*/*z* 971.2974 ($C_{50}H_{51}O_{20}$ by HRCIMS). The H-10 and H-10′ signals appeared as one 2H singlet at 4.41 ppm in the ^1H NMR spectrum of **10A**, while in **10B** these same protons in 10 and 10′ resonated at 4.32 ppm (d, $J = 3$ Hz, 1H) and 4.38 ppm (d, $J = 3$ Hz, 1H). This indicates that

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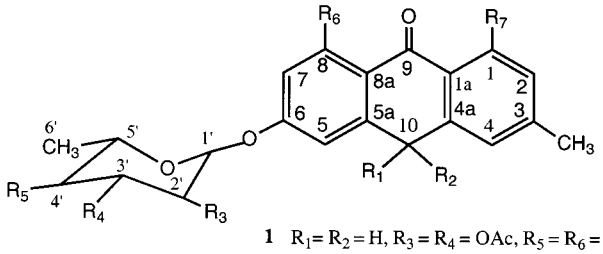
[⊥] To whom this work is dedicated. Deceased on March 10, 2000.

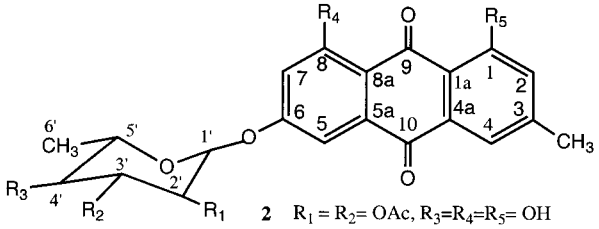
10.1021/np010030v CCC: $20.00
Published on Web 08/30/2001

Chart 1



1 $R_1 = R_2 = H$, $R_3 = R_4 = OAc$, $R_5 = R_6 = R_7 = OH$

13 $R_1 = H$, $R_2 = COCH_3$, $R_3 = R_4 = OAc$, $R_5 = R_6 = R_7 = OH$



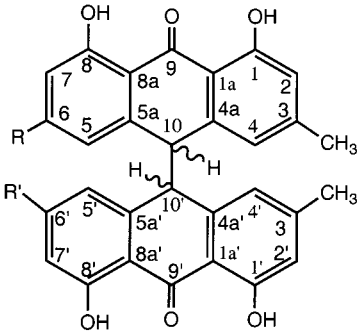
2 $R_1 = R_2 = OAc$, $R_3 = R_4 = R_5 = OH$

3 $R_2 = R_3 = R_4 = R_5 = OH$, $R_1 = OAc$

4 $R_1 = R_2 = R_3 = R_4 = R_5 = OAc$

8 $R_1 = R_3 = R_4 = R_5 = OH$, $R_2 = OAc$

12 $R_1 = R_2 = R_3 = OAc$, $R_4 = R_5 = OH$



5A R = R' = H; *meso* C_{10}/C_{10}

5B R = R' = H; C_{10}-αH, $C_{10'}$-βH or C_{10}-βH, $C_{10'}$-αH

6A R = H, R' = OH; C_{10}-αH, $C_{10'}$-αH

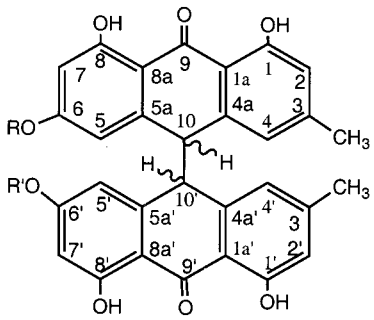
6B R = H, R' = OH; C_{10}-βH, $C_{10'}$-βH

6C R = H, R' = OH; C_{10}-αH, $C_{10'}$-βH

6D R = H, R' = OH; C_{10}-βH, $C_{10'}$-αH

7A R = R' = OH; *meso* $C_{10}/C_{10'}$

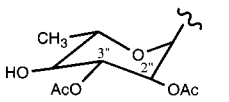
7B R = R' = OH; C_{10}-αH, $C_{10'}$-βH or C_{10}-βH, $C_{10'}$-αH



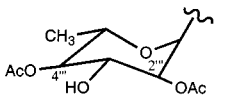
9A - D R = 2",3"-di-*O*-acetylrhamnose, R' = OH

10A, 10B R = 2",3"-di-*O*-acetylrhamnose
R' = 2",3"-di-*O*-acetylrhamnose

11A - C R = 2",3"-di-*O*-acetylrhamnose
R' = 2"',4"'-di-*O*-acetylrhamnose



2",3"-di-*O*-acetylrhamnose



2"',4"'-di-*O*-acetylrhamnose

Table 1. ^{1}H NMR Data of Compounds **5**−**7**

carbon	**5A**,**B**[a]	**6A**,**B**[b]	**6C**,**D**[b]	**7A**[c]	**7B**[c]
2	6.68 d (1.0)	6.64 brs	6.61 brs	6.30 brs	6.37 brs
4	6.00 d (1.0)	6.14 brs	5.82 s	6.68 brs	6.59 brs
5	6.68 dd (1.0, 8.0)	6.78 d (7.8)	6.91 d (7.8)	6.25 d (2.0)	6.33 d (2.0)
6	7.41 t (8.0)	7.28 d (7.8)	7.45 t (7.8)		
7	6.89 dd (1.0, 8.0)	6.35 d (7.8)	6.70 d (7.8)	6.15 d (2.0)	6.07 brs
10	4.49 s	4.37 d (3.0)	4.42 d (3.0)	4.55 s	4.55 s
OH-1	11.63 s			11.85 s	11.75 s
OH-8	11.85 s			11.97 s	12.05 s
CH_3-3	2.25 s	2.27 s	2.20 s	2.27 s	2.20 s
2′	6.60 d (1.0)	6.59 brs	6.59 brs	6.30 brs	6.37 brs
4′	5.70 d (1.0)	6.02 brs	5.62 s	6.68 brs	6.59 brs
5′	6.29 dd (1.0, 8.0)	6.19 d (2.0)	6.30 d (2.0)	6.25 d (2.0)	6.33 d (2.0)
6′	7.29 t (8.0)				
7′	6.81 dd (1.0, 8.0)	5.82 d (2.0)	6.20 d (2.0)	6.15 d (2.0)	6.07 brs
10′	4.51 s	4.23 d (3.0)	4.25 d (3.0)	4.55 s	4.55 s
OH-1′	11.58 s			11.85 s	11.75 s
OH-8′	11.75 s			11.97 s	12.05 s
CH_3-3′	2.15 s	2.21 s	2.17 s	2.27	2.20 s

[a] $CDCl_3$. [b] $CDCl_3$ + CD_3OD, 95:5; [c] Acetone d_6.

10A and **10B** are a mixture of C-10/C-10′ isomers, and these were not separable in the various HPLC conditions used (Tables 2 and 3).

Rhamnepalins (**11A**−**C**) gave a $[M + H]^+$ peak at *m*/*z* 971.2979 (HRFABMS), which matched the molecular formula $C_{50}H_{51}O_{20}$. In the ^{1}H NMR spectra, protons H-10 and H-10′ appeared as singlets at δ 4.33 (2H), 4.37 (2H), and 4.36 (2H), respectively (Tables 2 and 3). A fourth expected isomer has not been isolated. HMBC correlations allowed us to observe two different sequences for the rhamnose part of compounds **11A**−**C**, one being H-4″ (−OH), H-3″ (−OAc), H-2″ (−OAc) and the other H-4‴ (−OAc), H-3‴ (−OH), H-2‴ (−OAc), which means that compounds **11A**, **11B**, and **11C** are all prinoidin-2‴,4‴-di-*O*-acetylrhamnoside-emodin bianthrones. We propose the trivial name rhamnepalins for these compounds.

Some of the bianthrones isolated are possibly artifacts formed during the extraction and purification processes, as the treatment of prinoidin (**1**) by MeOH and SiO_2 under mild conditions led to a mixture of prinoidin bianthrones

Table 2. ^{1}NMR Data for Compounds **9**−**11**[a] (δ ppm, J Hz)

carbon	**9A**	**9B**	**9C**	**9D**	**10A**	**10B**	**11A**	**11B**	**11C**
2	6.61 s	6.58 s	6.55 s	6.70 s	6.85 s	6.74 s	6.62 s	6.75 s	6.76 s
4	6.12 s	5.60 s	5.55 s	6.18 s	6.82 s	6.83 brs	5.73 s	6.62 s	6.76 s
5	5.75 d (2.0)	6.30 s	6.30 d (2.0)	5.92 d (2.0)	5.16 d (2.3)	6.72 d (2.3)	6.49 s	6.70 s	6.63 s
7	6.55 d (2.0)	6.65 d (2.0)	6.69 d (2.0)	6.60 d (2.0)	6.45 d (2.3)	6.66 d (2.3)	6.68 d (2.1)	6.53 s	6.67 s
10	4.18 d (3.0)	4.13 d (3.0)	4.10 d (3.0)	4.21 s	4.41 s	4.38 d (3.2)	4.33 s	4.37 s	4.36 s
CH_3-3	2.27 s	2.10 s	2.12 s	2.43 s	2.45 s	2.43 s	2.21 s	2.40 s	2.43 s
2′	6.61 s	6.55 s	6.50 s	6.63 s	6.85 s	6.55 s	6.62 s	6.62 s	6.58 s
4′	6.02 s	5.45 s	5.40 s	6.00 s	6.82 s	6.72 s	5.71 s	5.61 s	5.58 s
5′	5.70 d (2.0)	6.28 d (2.0)	6.30 d (2.0)	5.89 d (2.0)	5.16 d (2.3)	5.32 d (2.3)	6.49 brs	6.50 brs	5.47 brs
7′	6.32 d (2.0)	6.42 d (2.0)	6.45 d (2.0)	6.40 d (2.0)	6.45 d (2.3)	6.42 d (2.3)	6.66 d (2.2)	5.52 s	6.47 s
10′	4.10 d (3.0)	4.03 d (3.0)	4.00 d (3.0)	4.21 s	4.41 s	4.32 d (3.2)	4.33 s	4.37 s	4.35 s
CH_3-3′	2.27 s	2.10 s	2.12	2.30 s	2.45 s	2.08 s	2.21 s	2.17 s	2.16 s
1″	5.50 d (2.0)	5.55 s	5.60 d (2.0)	5.50 brs	5.34 brs[b]	5.73 brs[b]	5.58 brs	5.37 s	5.61 s
2″	5.37 m	5.50 brs	5.55 s	5.55 d (3.0)	5.23 m[b]	5.47 brs[b]	5.48 brs	5.29 s	5.47 brs
3″	5.31 t (3.0)	5.40 d (3.0)	5.43 dd (3.5, 9.6)	5.38 dd (3.5, 9.0)	5.26 m[b]	5.38 dd (3.0, 10)[b]	5.34 dd (3.4, 9.7)	5.25 s	5.35 dd (3.4, 9.6)
4″	3.75 t (9.9)	3.92 m	3.85 m	3.80 t (9.6)	3.62 t (9.7)[b]	3.69 m[b]	3.75 t (9.7)	3.67 d (9.7)	3.75 t (9.6)
5″	3.85 m	3.88 m	3.85 m	3.85 m	3.73 m[b]	3.89 m[b]	3.82 m	3.79 m	3.83 m
6″	1.40 d (6.0)	1.40 d (6.0)	1.40 d (6.0)	1.42 d (6.0)	1.37 d (6.0)[b]	1.32 d (6.0)[b]	1.35 d (6.0)	1.41 d (6.0)	1.37 d (6.5)
1‴							5.68 s	5.72 s	5.39 s
2‴							5.31 brs	5.33 brs	5.17 s
3‴							4.26 dd (3.5, 9.6)	4.26 d (9.2)	4.15 m
4‴							4.96 t (9.8)	4.97 t (9.7)	4.92 t (9.8)
5‴							3.89 dt (3.5, 9.6)	3.92 dt (3.5, 9.6)	3.89 dt (3.4, 9.5)
6‴							1.21 d (6.1)	1.21 d (6.1)	1.23 d (6.0)
OH-1	11.80 s	11.60 s	11.55 s	11.80 s	12.00 s	11.58 s	11.60 s	11.60 s	11.80 s
OH-1′	11.75 s	11.62 s	11.60 s	11.79 s	12.00 s	11.75 s	11.60 s	11.80 s	11.60 s
OH-8	12.10 s	11.90 s	12.18 s	12.05 s	11.90 s	11.83 s	12.10 s	11.90 s	12.0 s
OH-8′	12.10 s	12.10 s	12.21 s	12.05 s	11.90 s	12.30 s	12.10 s	12.20 s	11.9 s
CH$_3$CO-2″	2.20 s	2.30 s	2.22 s	2.20 s	2.11 s[c]	2.20 s[c]	2.26 s	2.13 s	2.20 s
CH$_3$CO-2‴							2.20 s	2.16 s	2.20 s
CH$_3$CO-3″	2.12 s	2.20 s	2.18 s	2.12 s	2.15 s[c]	2.11 s[c]	2.14 s	2.24 s	2.18 s
CH$_3$CO-3‴							2.14 s	2.11 s	2.13 s
CH$_3$CO-4‴									

[a] $CDCl_3$. [b] Value given for 2H; value given for 6H

Table 3. ^{13}C Assignments for Compounds **9**−**11** (δ ppm)[a]

no.	9A	9B	9C	9D	10A	10B	11A	11B	11C
1	161.6	162.0	162.4	161.9	161.3	161.8	162.0	162.9	162.2
2	117.2	116.8	117.3	116.9	117.6	117.1	117.1	117.7	117.2
3	147.2	146.2	147.0	147.0	148.3	147.6	147.3	148.0	148.6
4	121.0	121.0	121.2	120.7	121.1	120.6	121.3	121.2	121.3
5	109.2	109.4	109.8	110.0	110.1	108.7	109.2	109.9	107.3
6	160.4	161.5	161.6	161.1	159.8	161.2	160.9	160.7	161.8
7	102.5	102.3	102.8	102.0	101.9	104.2	103.3	104.0	102.9
8	164.7	164.1	164.7	164.3	164.8	163.4	164.3	165.7	164.8
9	190.5	190.5	190.6	190.1	190.7	190.5	192.0	191.1	191.8
10	56.3	56.4	59.6	56.2	55.8	56.8	56.3	56.9	56.4
1a	114.0	114.0	113.6	114.9	115.4	115.5	115.8	115.6	115.1
4a	140.4	139.0	139.0	140.5	140.8	140.6	141.8	141.7	141.8
5a	143.5	144.2	144.7	144.0	142.3	144.9	144.8	144.9	145.0
8a	112.0	113.0	112.8	112.0	113.6	112.8	113.5	113.1	113.7
CH_{3-3}	22.1	21.6	22.1	22.9	22.4	22.5	22.1	22.7	22.8
1′	162.5	162.0	162.3	161.9	161.3	162.2	162.1	162.5	162.4
2′	117.2	116.8	117.3	116.9	117.6	117.1	117.1	117.7	117.2
3′	147.2	146.5	146.7	146.9	148.3	147.0	147.1	147.7	147.8
4′	121.0	121.0	121.5	120.7	121.1	121.8	120.5	122.0	120.6
5′	108.7	107.9	108.4	108.2	110.1	109.4	108.9	109.5	109.0
6′	161.8	162.2	163.0	162.5	159.8	159.6	160.9	162.2	160.5
7′	102.5	102.3	102.8	102.5	101.9	101.7	102.0	102.7	102.6
8′	164.4	164.1	164.5	164.3	164.8	164.4	163.7	164.3	165.0
9′	190.4	190.5	190.4	190.1	190.7	190.2	191.8	190.9	191.8
10′	56.3	56.4	56.9	56.2	55.8	56.7	56.0	56.7	56.2
1a′	114.0	114.0	113.6	114.9	115.4	113.3	115.6	114.1	113.8
4a′	140.4	139.0	139.2	140.0	140.8	138.1	141.5	139.4	139.8
5a′	142.9	144.5	145.2	143.5	142.3	142.2	145.0	142.2	142.4
8a′	111.0	112.0	111.7	111.0	113.6	111.1	112.8	111.9	111.8
$CH_{3-3'}$	22.1	21.6	22.1	22.9	22.4	21.0	21.8	22.5	22.5
1″	95.1	95.6	96.0	96.0	94.7	95.1	95.3	95.5	95.7
2″	69.9	69.4	70.1	69.4	71.4	71.4	69.8	70.4	69.6
3″	71.8	71.9	72.2	71.9	69.8	69.5	71.4	72.1	71.7
4″	71.2	70.6	71.1	70.6	71.3	70.8	71.0	71.7	70.9
5″	69.8	69.9	70.4	69.9	69.7	70.2	69.5	70.2	70.1
6″	17.7	17.4	17.9	17.5	17.6	17.5	17.4	18.1	18.1
1‴					94.7	94.7	94.8	96.0	95.0
2‴					71.4	72.0	71.9	72.5	71.9
3‴					69.8	69.8	68.2	68.8	68.3
4‴					71.4	71.0	74.0	74.7	74.3
5‴					69.7	70.2	67.5	68.1	67.2
6‴					17.6	17.6	17.4	18.1	17.8
CO-2″	171.0	170.5	170.8	171.0	170.9	171.0	169.7	171.2	170.2
CO-2‴					171.9	171.8	171.3	171.5	170.5
CO-3″	172.0	171.4	172.0	172.0	170.1	170.1	171.5	170.6	171.2
CO-3‴					170.1	170.1			
CO-4‴							172.6	172.0	172.0
CH_3CO-2′	21.2	20.7	21.4	21.0	21.1	21.8	20.8	21.6	21.8
CH_3CO-2‴					21.0	21.7	20.8	21.6	21.5
CH_3CO-3″	21.2	20.6	21.2	20.8	20.9	20.9	20.9	21.4	21.6
CH_3CO-3‴					20.9	20.9			
CH_3CO-4‴							21.0	21.4	21.8

[a] In $CDCl_3$

10A and **10B**. However, it should be noted that the monomeric 2′,4′-di-*O*-acetylrhamnoside-emodin-anthrone, one of the moieties of the rhamnepalins (**11A**−**C**), was not isolated in the course of this study.

To study the structure−cytotoxicity relationships in this series, acetylation of compounds **1** and **2** was carried out. Treatment of 2′,3′-di-*O*-acetylfrangulin A (**2**) with acetic anhydride in pyridine for 24 h led to the known fully acetylated compound **4**, which was identified after comparison with literature data.[5] The regioselective acetylation at C-4′ was carried out by treatment of **2** with acetic anhydride and DMAP and led to a new compound **12**. This compound gave a major peak $[M + H]^+$ at *m*/*z* 543.1493 (HRCIMS), corresponding to the molecular formula $C_{27}H_{27}O_{12}$. The ^1H NMR spectrum of compound **12** possessed the same characteristics as that of **2** but differed in terms of the presence of an acetyl group at C-4′ [(H-4′ at δ 5.17 (t, $J = 9.7$ Hz)]. Compound **12** has thus been identified as 2′,3′,4′-tri-*O*-acetylfrangulin A.

Acetylation of prinoidin **1** by acetic anhydride in the presence of catalytic amounts of pyridine led to the formation of a new compound, **13**, which corresponds to the molecular mass of prinoidin plus 84 amu. Its ^1H NMR spectrum showed a signal for an acetyl group at δ 1.82 ppm and a singlet at δ 5.05 ppm corresponding to H-10. Thus, these spectroscopic data led us to propose the structure of **13** as 4′-*O*-acetyl,10-*C*-acetylprinoidin, which comes from prinoidin (**1**) via the substitution at C-10 of an acylium group and acetylation at C-4′.

To prepare all the possible stereomers of the most biologically active natural dimers of chrysophanol bianthrones, emodin bianthrones, and chrysophanol-emodin bianthrones, synthetic work was carried out using both chrysophanol and emodin.[16,17] The two substances were

Table 4. Cytotoxicity for KB Cells of Compounds Isolated from *Rhamnus nepalensis* and Some Synthetic Derivatives ($n = 3$)

compound	IC_{50} (μM)	compound	IC_{50} (μM)
doxorubicin	0.2	**7A**	1.1
chrysophanol	inactive	**7B**	2.5
emodin	inactive	**8**	inactive
emodin anthrone	3.9	**9A**	1.3
physcion	inactive	**9B**	3.3
prinoidin (**1**)	0.045	**9C**	4.5
2	1.9	**9D**	0.8
3	inactive	**10A**	0.9
4	inactive	**10B**	2.5
5A	0.2	**11A**	0.8
5B	1.2	**11B**	1
6A	1.2	**11C**	1.2
6B	3.4	**12**	inactive
6C	1.8	**13**	0.07
6D	1.4		

first reduced to the corresponding anthrones by $SnCl_2$, then coupled using $FeCl_3$ in acidic conditions. Two diastereomers, **5A** (*meso*-isomer) and **5B** (racemic), identical to the natural compounds were obtained, together with the four diastereomers **6A**−**D** and the two diastereomers **7A** (*meso*-isomer) and **7B** (racemic). The diastereomeric pairs were separated by HPLC on Si gel, and the corresponding enantiomers have been observed by using an analytical chiral OD column. To date, only the racemic compounds of each *threo* and *meso* diastereomeric couples have been isolated and characterized. However, the free rotation around the C-10/C-10′ bond precluded any stereochemical assignment of these carbons by NMR techniques.

To specify the relative stereochemistry of C-10 and C-10′ in compounds **10A** and **10B**, it should have been possible to hydrolyze the sugar portion to obtain emodin bianthrones **7A** and **7B**, but epimerization of the two stereocenters occurred. Indeed, treatment of emodin bianthrone **7A** under acidic conditions led to compound **7B**. This precluded any chemical hydrolysis of one of the diastereomers of prinoidin bianthrone. Enzymic hydrolysis was also unsuccessful.

Table 4 summarizes the cytotoxic activities observed against KB cells for the *R. nepalensis* isolates and some of their semisynthetic derivatives. Prinoidin (**1**) was 4 times more potent than the standard, doxorubicin. Chrysophanol bianthrone **5A** was as active as doxorubicin, whereas its isomer (**5B**) was six times less active. In fact, compared to the selected standard, the various diastereoisomers of the bianthrones were observed to be significantly active but did not differ very much from each other in terms of cytotoxic potency. Consequently, a careful determination of the relative configuration of the dimers being impossible, it was also not possible to correlate the weak differences of activity with stereochemistry at C-10 and C-10′. Compound **13** was 2-fold less cytotoxic against KB cells (7×10^{-8} M) than prinoidin (**1**) (Table 4). Acetylation of the hydroxyl groups in the sugar part of compound **2** led to a loss of cytotoxicity.

When evaluated in vivo, prinoidin (**1**) was toxic when administered as a single intraperitoneal dose of 10 mg/kg to two mice grafted i.v. with P388 leukemia cells, with mice dying 2 days early after the injection.[18] The medium dose of 5 mg/kg allows the two mice to survive 4 days, and the lower dose of 2.5 mg/kg proved inactive, the mice surviving 7 days, which is the average of survival for the two mice grafted.

Finally, by comparing these natural and synthetic bianthrones with doxorubicin, it seems that anthraquinones can serve as model compounds to synthesize additional cytotoxic molecules. Doxorubicin and mitoxantrone, two well-known antitumor compounds, also contain anthraquinone moieties in the molecule.

Experimental Section

General Experimental Procedures. Optical rotations were measured at 25 °C on a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Shimadzu UV-161 UV−visible spectrophotometer and IR spectra on a Perkin-Elmer Spectrum BX FT-IR instrument. The NMR spectra were recorded on Bruker AC-200, AC-250, AC-300, and AM-400 spectrometers, using TMS as internal standard. The NMR assignments were based on 2D COSY, HMQC, and HMBC NMR spectra. CIMS and HRCIMS were obtained on a Kratos MS-9 mass spectrometer, and EIMS on a Kratos MS-50 mass spectrometer. Column chromatography was performed using Si gel 60H (Merck, Darmstadt, Germany). Purification of compounds **5A**,**B**, **6A**−**D**, **7A**,**B**, and **11A**−**C** was performed by semipreparative HPLC on Novapak Silica (4 μm, 150 × 3.9 mm) or on an analytical Chiralcell OD column (Daicel Europa GmbH, Dusseldorf, Germany).

Plant Material. Leaves of *Rhamnus nepalensis* were collected at Pà Co, Mai Chau, Hoa Binh Province, 150 km west of Hanoi, Vietnam, in November 1995. Identification was provided by one of us (V. D.) and Tran Ngoc Ninh (Institute of Ecology, NCST, Hanoi). Voucher specimens (VN 026) are deposited in the Herbarium of the Institute of Ecology and Biological Resources, NCST, Hanoi, Vietnam.

Extraction and Isolation. The dried ground fruits of *Rhamnus nepalensis* (460 g) were defatted by hexane, then extracted in a Soxhlet at room temperature with EtOAc, and the extract was evaporated under vacuum (60 g, yield 13%). Repeated column chromatography of the crude extract (4 g) and TLC and HPLC on silica gel afforded chrysophanol (32 mg, 0.8%, heptane−EtOAc, 7:3), physcion (12 mg, 0,3%, heptane−EtOAc, 7:3), chrysophanol bianthrones (**5A**,**B**) (36 mg, 0,8%, heptane−CH_2Cl_2, 6:4), emodin (440 mg, 11%, heptane−acetone, 5:5), emodin-anthrone (140 mg, 3.5%, heptane−acetone, 5:5), two chrysophanol-emodin bianthrones (**6**) (the first weighing 60 mg, 1.5%, heptane−EtOAc−acetic acid, 95:5:0.5, the second 48 mg, 1.2%, heptane−EtOAc−acetic acid, 95:5:0.5), emodin bianthrones (**7A**) (400 mg, 10%) and **7B** (400 mg, 10%), heptane−EtOAc−acetic acid, 95:5:0.5), then prinoidin (**1**) (480 mg, 12%, CH_2Cl_2−acetone, 95:5), 2′,3′-di-*O*-acetylfrangulin A (**2**) (600 mg, 15%, CH_2Cl_2−acetone, 9.5:0.5), prinoidin-emodin bianthrones **9A** (24 mg, 0.6%, CH_2Cl_2−acetone, 9.5:0.5), **9B** (28 mg, 0.7%, CH_2Cl_2−acetone, 9.5:0.5), **9C** (56 mg, 1.4%, CH_2Cl_2−acetone, 9.5:0.5), and **9D** (44 mg, 1.1%, CH_2Cl_2−acetone, 9.5:0.5), 2′-*O*-acetylfrangulin A (**3**) (52 mg, 1.3%, CH_2Cl_2−MeOH, 9:1) and 3′-*O*-acetylfrangulin A (**8**) (12 mg, 0.3%, CH_2Cl_2−MeOH, 9:1), prinoidin bianthrones **10A** (24 mg, 0.6%, CH_2Cl_2−MeOH, 8:2) and **10B** (24 mg, 0.6%, CH_2Cl_2−MeOH, 8:2), and a fraction containing rhamnepalins (CH_2Cl_2−acetone, 85:15), which were further purified by HPLC (CH_3CN−H_2O−acetic acid, 65:35:0.1) to give rhamnepalins **11A** (8 mg, 0.2%), **11B** (8 mg, 0.2%), and **11C** (9.6 mg, 0.24%).

3′-*O*-Acetylfrangulin A (8): amorphous powder; UV (EtOH) λ_{max} (log ϵ) 433 (4.27), 285 (4.38), 262 (4.53), 224 (4.77) nm; IR (KBr) ν_{max} 1750, 1625, 1605 (CO) cm^{-1}; ^1H NMR (300 MHz, C_5D_5N) δ 12.3 (2H, brs, OH-8, OH-1), 7.79 (1H, d, $J = 2$ Hz, H-5), 7.70 (1H, d, $J = 2$ Hz, H-4), 7.30 (1H, d, $J = 2$ Hz, H-7), 7.15 (1H, d, $J = 2$ Hz, H-2), 6.27 (1H, d, $J = 2$ Hz, H-1′), 5.98 (1H, dd, $J = 4.0$, 9.0 Hz, H-3′), 4.95 (1H, br s, H-2′), 4.58 (1H, t, $J = 9$ Hz, H-4′), 4.30 (1H, m, H-5′), 2.27 (3H, s, CH_3-3), 2.00 (3H, s, CH_3CO-3′); ^{13}C NMR (75 MHz, C_5D_5N) δ 191.2 (C-9), 181.7 (C-10), 171.0 (*C*OC-3′), 165.5 (C-8), 163.7 (C-1), 162.9 (C-6), 149.0 (C-3), 135.9 (C-5a), 133.8 (C-4a), 124.8 (C-2), 121.5 (C-4), 114.3 (C-1a), 112.2 (C-8a), 110.0 (C-5, C-7), 99.8 (C-1′), 75.7 (C-2′), 71.8 (C-3′), 70.4 (C-4′), 69.0 (C-5′), 21.9 (CH_3-3), 21.2 (*C*H_3CO-3″), 18.6 (*C*H_3-6′); CIMS m/z 459 $[MH]^+$ (35), 271 (100), 257 (40); HRCIMS [M + H^+] m/z 459.1286 (calcd for $C_{23}H_{23}O_{10}$ 459.1291).

Prinoidin-emodin bianthrone (9A): amorphous powder; $[\alpha]^{25}_D$ 0° (*c* 0.76, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 363 (4.46),

277 (4.39), 225 (4.68), 203 (4.85) nm; IR ($CHCl_3$) ν_{max} 3681, 3625 (OH), 1630 (CO) cm^{-1}; ^1H and ^{13}C NMR, Tables 2 and 3; FABMS *m/z* 747 (M + Li) (70), 492 (8), 313 (37), 256 (40), 160 (100); HRCIMS $[M + H]^+$ *m/z* 741.2171 (calcd for $C_{40}H_{37}O_{14}$ 741.2183).

Prinoidin-emodin bianthrone (9B): amorphous powder; $[\alpha]^{25}_D$ +6° (*c* 0.76, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 363 (4.46), 277 (4.39), 225 (4.68), 203 (4.85) nm; IR ($CHCl_3$) ν_{max} 3681, 3625 (OH), 1630 (CO) cm^{-1}; ^1H and ^{13}C NMR, Tables 2 and 3; FABMS *m/z* 747 (M + Li) (70), 492 (8), 313 (37), 256 (40), 160 (100); HRCIMS $[M + H]^+$ *m/z* 741.2171 (calcd for $C_{40}H_{37}O_{14}$ 741.2183).

Prinoidin-emodin bianthrone (9C): amorphous powder; $[\alpha]^{25}_D$ 0° (*c* 0.6, $CHCl_3$); ^1H and ^{13}C NMR, Tables 2 and 3; FABMS *m/z* 747 (M + Li) (70), 492 (8), 313 (37), 256 (40), 160 (100); HRCIMS $[M + H]^+$ *m/z* 741.2171 (calcd for $C_{40}H_{37}O_{14}$ 741.2183).

Prinoidin-emodin bianthrone (9D): amorphous powder; $[\alpha]^{25}_D$ −16.5° (*c* 0.4 $CHCl_3$); ^1H and ^{13}C NMR, Tables 2 and 3; FABMS *m/z* 747 (M + Li) (70), 492 (8), 313 (37), 256 (40), 160 (100); HRCIMS $[M + H]^+$ *m/z* 741.2171 (calcd for $C_{40}H_{37}O_{14}$ 741.2183).

Prinoidin bianthrone (10A): yellow crystals; mp 144−147 °C; $[\alpha]^{25}_D$ +133° (*c* 1.06, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 361 (4.56), 277 (4.49), 209 (4.89) nm; IR ($CHCl_3$) ν_{max} 3677, 3505 (OH), 1748 (ester), 1637, 1619, 1607 (CO) cm^{-1}; ^1H and ^{13}C NMR, Tables 2 and 3; CIMS *m/z* 993 $[M + Na]^+$ (100), 971 $[M + 1]^+$, 508 (50), 360 (25), 279 (27), 150 (70); HRCIMS $[MH]^+$ *m/z* 971.2975 (calcd for $C_{50}H_{51}O_{20}$ 971.2974).

Prinoidin bianthrone (10B): yellow crystals; mp 155−157 °C; $[\alpha]^{25}_D$ +127° (*c* 0.96, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 362 (4.44), 277 (4.39), 210 (4.75) nm; IR ($CHCl_3$) ν_{max} 3677, 3475 (OH), 1748 (ester), 1637, 1619, 1605 (CO) cm^{-1}; ^1H and ^{13}C NMR, Tables 2 and 3; CIMS *m/z* 993 $[M + Na]^+$ (100), 971 $[M + 1]^+$, 508 (50), 360 (25), 279 (27), 150 (70); HRCIMS $[MH]^+$ *m/z* 971.2975 (calcd for $C_{50}H_{51}O_{20}$ 971.2974).

Rhamnepalin (11A): yellow amorphous powder; mp 177−179 °C; $[\alpha]^{25}_D$ +47.2° (*c* 0.7, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 361 (4.46), 278 (4.32), 206 (4.82) nm; IR ($CHCl_3$) ν_{max} 3683, 3657 (OH), 1747 (ester), 1636, 1619, 1605 (CO) cm^{-1}; ^1H and ^{13}C NMR, Tables 2 and 3; CIMS *m/z* 969 [M − 1] (100), 682 (10), 516 (20), 485 (35); HRFABMS *m/z* 971.2979 $[M + H]^+$ (calcd for $C_{50}H_{51}O_{20}$ 971.2972).

Rhamnepalin (11B): yellow amorphous powder; mp 153−155 °C; $[\alpha]^{25}_D$ + 69° (*c* 0.96, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 360 (4.42), 277 (4.37), 207 (4.79) nm; IR ($CHCl_3$) ν_{max} 3680, 3657 (OH), 1747 (ester), 1636, 1619, 1605 (CO) cm^{-1}; ^1H and ^{13}C NMR, Tables 2 and 3; CIMS *m/z* 969 [M − 1] (100), 682 (10), 516 (20), 485 (35); HRFABMS *m/z* 971.2979 $[M + H]^+$ (calcd for $C_{50}H_{51}O_{20}$ 971.2972).

Rhamnepalin (11C): yellow amorphous powder; mp 167−170 °C; $[\alpha]^{25}_D$ +56.2° (*c* 1.12, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 361 (4.42), 277 (4.36), 206 (4.78) nm; IR ($CHCl_3$) ν_{max} 3680, 3657 (OH), 1747 (ester), 1636, 1619, 1604 (CO) cm^{-1}; ^1H and ^{13}C NMR, Tables 2 and 3; CIMS *m/z* 969 [M − 1] (100), 682 (10), 516 (20), 485 (35); HRFABMS *m/z* 971.2979 $[M + H]^+$ (calcd for $C_{50}H_{51}O_{20}$ 971.2972).

Acetylation of Compound 2. A solution of 2′,3′-di-*O*-acetylfrangulin A (**2**) (10 mg, 0.02 mmol) in $(Ac)_2O$ (1 mL) and pyridine (1 mL) was stirred for 24 h at room temperature. After addition of water, the reaction mixture was extracted by CH_2Cl_2. The combined organic phases were washed, dried (Na_2SO_4), and evaporated. The residue, after preparative TLC, gave compound **4** (10.2 mg, yield 81%) as an amorphous powder: $[\alpha]^{25}_D$ −80.8° (*c* 0.5, $CHCl_3$), and other data comparable with literature values.

Preparation of Compound 12. To a solution of 2′,3′-di-*O*-acetylfrangulin A (**2**) (10 mg, 0.02 mmol) in $(Ac)_2O$ (3 mL) and CH_2Cl_2 (3 mL) was added 4-DMAP (3 mL), and the reaction mixture was stirred for 30 min at room temperature. After the addition of water, the reaction mixture was extracted by CH_2Cl_2. The combined organic phases were washed, dried (Na_2SO_4), and evaporated. The residue, after preparative TLC, gave compound **12** (7.2 mg, yield 67%) as an amorphous powder: $[\alpha]^{25}_D$ −118° (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 432 (3.96), 299 (3.92), 288 (4.06), 261 (4.24), 225 (4.48) nm; IR ($CHCl_3$) ν_{max} 3690 (OH), 1755, 1628, 1609 (CO) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 12.5 (1H, s, OH-8), 12.2 (1H, s, OH-1), 6.68 (1H, brs, H-2), 6.70 (2H, brs, H-4, H-7), 6.55 (1H, brs, H-5), 5.55 (1H, brs, H-1′), 5.44 (1H, t, J = 3.8 Hz, H-2′), 5.40 (1H, m, H-3′), 5.17 (1H, t, J = 9.7 Hz, H-4′), 5.05 (1H, br s, H-10), 3.90 (1H, m, H-5′), 2.35 (3H, s, CH_3-3), 2.22 (3H, s, CH_3-CO-2″), 2.02 (6H, s, CH_3CO-3″, CH_3CO-4″), 1.82 (3H, s, CH_3-11), 1.25 (3H, d, J = 6 Hz, CH_3-6′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.0 (C-11), 191.6 (C-9), 171.2 (*C*OC-3′), 170.0 (*C*OC-2′, *C*OC-4′), 165.6 (C-8), 163.3 (C-1), 161.9 (C-6), 148.7 (C-3), 140.1 (C-4a), 137.5 (C-5a), 120.1 (C-4), 117.9 (C-2), 112.5 (C-1a), 110.5 (C-8a), 108.1 (C-5), 103.9 (C-7), 95.4 (C-1′), 70.6 (C-2′), 69.5 (C-3′), 69.0 (C-4′), 68.2 (C-5′), 59.1 (C-10), 22.2 (*C*H_3-3), 20.9 (*C*H_3CO-3″), 20.8 (*C*H_3CO-2″, *C*H_3CO-4″), 17.5 (*C*H_3-6′); EIMS *m/z* 542 $[M]^+$ (25), 498 (15), 273 (100), 241 (80); HRCIMS *m/z* 543.1493 $[M + H]^+$ (calcd for $C_{27}H_{27}O_{12}$ 543.1503).

Preparation of Compound 13. A solution of prinoidin (**1**) (20 mg, 0.04 mmol) in $(Ac)_2O$ (4 mL) and pyridine (25 μL) was stirred for 5 h at room temperature. After washing, the reaction mixture was dried (Na_2SO_4), and the solvent evaporated. The residue, after preparative TLC, gave compound **12** (17 mg, yield 76%) as an amorphous powder: UV (MeOH) λ_{max} (log ϵ) 357 (3.86), 271 (4.06), 221 (4.12) nm; IR ($CHCl_3$) ν_{max} 3693 (OH), 1755, 1628, 1609 (CO) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 12.2 (1H, s, OH-8), 12.0 (1H, s, OH-1), 7.58 (1H, d, J = 2 Hz, H-4), 7.45 (1H, d, J = 2 Hz, H-5), 7.05 (1H, d, J = 2 Hz, H-2), 6.87 (1H, d, J = 2 Hz, H-7), 5.57 (1H, d, J = 2 Hz, H-1′), 5.40 (2H, m, H-2′, H-3′), 5.10 (1H, t, J = 9.5 Hz, H-4′), 5.05 (1H, s, H-10), 3.85 (1H, m, H-5′), 2.37 (3H, s, CH_3-3), 2.05 (3H, s, CH_3CO-2′), 2.00 (6H, s, CH_3CO-3′, CH_3CO-4′), 1.82 (3H, s, COCH_3), 1.15 (3H, d, J = 6 Hz, CH_3-6′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202 (*C*HOCH$_3$), 191.2 (C-9), 181.7 (C-10), 170.5 (*C*OC-2′), 170.1 (*C*OC-3′, *C*OC-4′), 164.9 (C-8), 162.7 (C-1), 162.2 (C-6), 148.9 (C-3), 135.5 (C-5a), 133.1 (C-4a), 124.7 (C-2), 121.6 (C-4), 113.6 (C-1a), 111.7 (C-8a), 109.6 (C-5), 109.3 (C-7), 95.4 (C-1′), 70.6 (C-2′), 69.2 (C-3′), 69.7 (C-4′), 68.0 (C-5′), 24.7 (CHO*C*H$_3$), 22.3 (*C*H$_3$-3), 20.9 (*C*H$_3$CO-3′), 20.8 (*C*H$_3$CO-2′, *C*H$_3$CO-4′), 17.5 (*C*H$_3$-6′); EIMS *m/z* 570 $[M]^+$ (35), 528 (55), 298 (10), 273 (15), 256 (100); HRCIMS *m/z* 571.1805 $[M + H]^+$ (calcd for $C_{29}H_{31}O_{12}$ 571.1816).

Dimerization of Emodin Anthrone and Chrysophanol Anthrone: Compounds 5A,B, 6A−D, and 13A,B. A solution of emodin (108 mg, 0.4 mmol) or chrysophanol (108 mg, 0.4 mmol) in acetic acid (10 mL) was added to a solution of $SnCl_2$ (303 mg) in concentrated HCl (0.9 mL), and the reaction mixture was stirred for 5 h at 80 °C. After the addition of water, the reaction mixture was extracted by CH_2Cl_2. The organic phases were dried (Na_2SO_4) and evaporated to yield emodin anthrone (79 mg, yield 74%) or chrysophanol anthrone (98 mg, yield 94%). To a solution of emodin anthrone (72 mg) and chrysophanol anthrone (77 mg) in EtOH (35 mL) was added a solution of $FeCl_3$ (0.2 g) in EtOH (21 mL). The reaction mixture was stirred for 3 h under reflux, then, after addition of a solution (1 L) of 5% HCl, extracted by CH_2Cl_2. The combined organic phases were washed, dried (Na_2SO_4), and evaporated. The residue, after preparative TLC and HPLC of the isolated fractions on a chiral OD column (heptane−2-propanol−acetic acid 8:2:0.02), gave compounds **5A** (4.2 mg), **5B** (3 mg), **6A** (6 mg), **6B** (5.8 mg), **6C** (8.1 mg), **6D** (7 mg), **7A** (8.2 mg), and **7B** (10 mg).

In Vivo Bioassay of Prinoidin (1). Prinoidin (**1**) was injected intraperitoneally to two mice CDF_1 grafted i.v. with P388 leukaemia cells according to a published technique.[18]

KB Cytotoxicity Assay. The assays were performed according to a published technique.[19] The control used for comparison was doxorubicin (IC_{50} 0.058 μg/mL).

Acknowledgment. This study has been carried out in the framework of a program of cooperation between CNRS (France) and NCST (Vietnam). The authors acknowledge Mrs. M.-T. Adeline (HPLC) and Mrs. Gaspard (Service des Cultures Cellulaires, ICSN).

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NP010030V