

Cytotoxic and New Tetralone Derivatives from *Berchemia floribunda* (WALL.) BRONGN.

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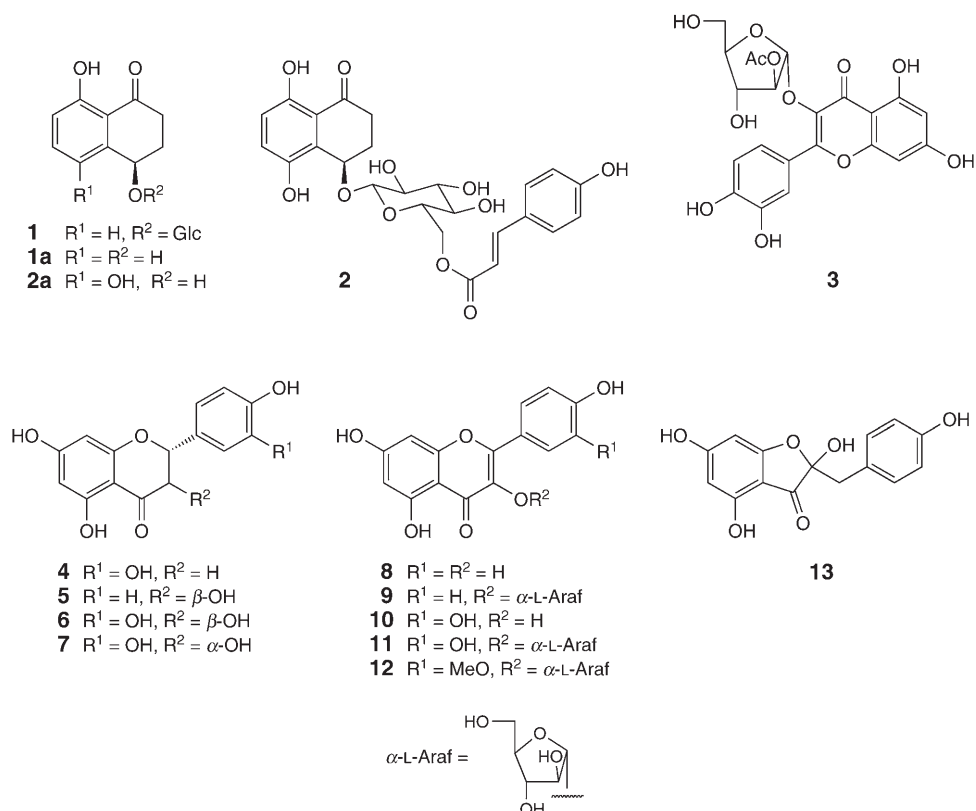
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Two new α -tetralone (= 3,4-dihydronaphthalen-1(2H)-one) derivatives, berchemiaside A and B (**1** and **2**, resp.), and one new flavonoid, quercetin-3-*O*-(2-acetyl- α -L-arabinofuranoside) (**3**), together with ten known flavonoids compounds, eriodictyol (**4**), aromadendrin (**5**), *trans*-dihydroquercetin (**6**), *cis*-dihydroquercetin (**7**), kaempferol (**8**), kaempferol-3-*O*- α -L-arabinofuranoside (**9**), quercetin (**10**), quercetin-3-*O*- α -L-arabinofuranoside or avicularin (**11**), quercetin 3'-methyl ether, 3-*O*- α -L-arabinofuranoside (**12**), and maesopsin (**13**), were isolated from the bark of *Berchemia floribunda*. Their structures were determined by various NMR techniques and chemical studies. Compounds **3–13** were tested for their cytotoxic activity against human leukemia cells. Among them, kaempferol (**8**) and maesopsin (**13**) showed significant inhibitory activities against human leukemia cells CCRF-CEM and its multidrug-resistant sub-line, CEM/ADR5000, with IC_{50} values of 14.0, 5.3, 10.2, and 12.3 μ M, respectively.

Introduction. – *Berchemia floribunda* (WALL.) BRONGN., which is widely distributed in China, was used for treatment of rheumatic arthritis, jaundice and contusions, and strains and dysmenorrhoea [1]. No work has been previously performed on the chemical constituents and biological activity. The AcOEt extract showed activity against human leukemia cells CCRF-CEM. So we undertook the study of bioactive constituents. From the AcOEt fraction of EtOH extracts of *B. floribunda*, two new α -tetralone (= 3,4-dihydronaphthalen-1(2H)-one) derivatives, **1** and **2**, and one new flavonoid, **3**, together with ten known flavonoid compounds, **4–13**, were obtained. Compounds **3–13** were examined for their biological activities against human leukemia cells CCRF-CEM and its multidrug-resistant sub-line, CEM/ADR5000. This paper mainly reports the isolation and structure elucidation of the new compounds, and the cytotoxicity of the tested compounds.

Results and Discussion. – *Structure Elucidation.* The bark of *Berchemia floribunda* (WALL.) BRONGN. was extracted with 70% EtOH. The EtOH extract was fractionated by petroleum ether, AcOEt, and BuOH. Only the AcOEt fraction showed the selective inhibition of human leukemia cells CCRF-CEM at a concentration 10 μ g/ml. The separation of the AcOEt fraction by silica gel gave 19 fractions. Further purification of these fractions by successive column chromatography (*Sephadex LH-20* and *RP-18* silica-gel columns) afforded three new compounds, **1–3**, in addition to ten known



flavonoids, **4–13**. The latter were identified as eriodictyol (**4**) [2], aromadendrin (**5**) [3], *trans*-dihydroquercetin (**6**) [4], *cis*-dihydroquercetin (**7**) [5], kaempferol (**8**) [6], kaempferol-3-*O*- α -L-arabinofuranoside (**9**) [6], quercetin (**10**) [6], quercetin-3-*O*- α -L-arabinofuranoside or avicularin (**11**) [7], quercetin 3'-methyl ether, 3-*O*- α -L-arabinofuranoside (**12**) [8], maesopsin (**13**) [9], by comparing their mass and NMR spectral data with those reported in the corresponding literature.

Compound **1**, obtained as a white powder, had the molecular formula $\text{C}_{16}\text{H}_{20}\text{O}_8$ based on analysis of negative-mode HR-FAB-MS (339.1084 ($[M - H]^+$; calc. 339.1079)). The ^{13}C -NMR (DEPT) spectra of **1** indicated 16 C-atoms (see Table 1). After hydrolysis of **1**, glucose was identified on a TLC plate by comparison with a reference sample. Except for a glucosyl group, ten C-atoms were observed in the ^{13}C -NMR spectral data, including a C=O C-atom at $\delta(\text{C})$ 205.3, two CH_2 C-atoms at $\delta(\text{C})$ 30.4 and 34.7, four CH and three quaternary C-atoms, indicating an oxygenated tetralone moiety in **1** [10]. The presence of this aglycone was further confirmed by its negative-mode FAB mass spectrum, which showed prominent fragment-ion peaks at m/z 177 ($[M - 162]^+$) and 159 ($[M - 162 - \text{H}_2\text{O}]^+$). In the ^1H -NMR spectrum, the coupling constant (3.8 Hz) of the signal at δ 5.17 (H-C(4)) indicated a H-atom in equatorial position based on the half-chair form of cyclohexenone [10]. The

Table 1. ^1H - and ^{13}C -NMR Data of Compounds **1** and **2** in (D_5)Pyridine. δ in ppm, J in Hz.

	1		2	
	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$
C(1)	–	205.3 (s)	–	206.0 (s)
H $_{\beta}$ –C(2)	3.04 (<i>ddd</i> , $J=18.1, 8.3, 5.8$)	34.7 (t)	3.32 (<i>ddd</i> , $J=18.0, 13.3, 5.1$)	33.7 (t)
H $_{\alpha}$ –C(2)	2.55 (<i>dt</i> , $J=18.1, 5.8$)		2.55 (<i>dt</i> , $J=17.2, 3.5$)	
CH $_2$ (3) or H $_{\beta}$ –C(3)	2.33 (<i>m</i>)	30.4 (t)	2.74 (<i>m</i>)	29.3 (t)
H $_{\alpha}$ –C(3)			2.18 (<i>m</i>)	
H–C(4)	5.17 (t, $J=3.8$)	73.9 (d)	5.88 (t, $J=2.8$)	69.7 (d)
C(4a)	–	144.0 (s)	–	127.8 (s)
H–C(5)	7.50 (d, $J=7.4$)	119.7 (d)	–	148.5 (s)
H–C(6)	7.42 (t, $J=8.0$)	136.8 (d)	7.34 (d, $J=9.0$)	126.7 (d)
H–C(7)	7.00 (<i>dd</i> , $J=8.3, 1.1$)	117.8 (d)	6.99 (d, $J=9.0$)	118.7 (d)
C(8)	–	163.1 (s)	–	156.3 (s)
C(8a)	–	116.4 (s)	–	116.5 (s)
H–C(1')	5.00 (d, $J=7.8$)	103.6 (d)	5.37 (d, $J=7.8$)	104.8 (d)
H–C(2')	4.10 (t, $J=8.1$)	75.4 (d)	4.09 (d, $J=8.4$)	75.5 (d)
H–C(3')	4.25 (<i>m</i>)	78.7 (d)	4.25 (<i>m</i>)	78.4 (d)
H–C(4')	4.25 (<i>m</i>)	71.8 (d)	4.20 (<i>m</i>)	71.7 (d)
H–C(5')	4.01 (<i>m</i>)	78.7 (d)	4.20 (<i>m</i>)	75.7 (d)
H $_{\alpha}$ –C(6')	4.43 (<i>dd</i> , $J=11.8, 5.8$)	62.9 (t)	5.16 (d, $J=11.4$)	64.6 (t)
H $_{\beta}$ –C(6')	4.64 (<i>dd</i> , $J=11.8, 2.2$)		5.01 (<i>dd</i> , $J=11.4, 5.5$)	
CO	–	–	–	167.6 (s)
H–C(α)	–	–	6.64 (d, $J=16.1$)	115.2 (d)
H–C(β)	–	–	7.98 (d, $J=16.1$)	145.4 (d)
H–C(1'')	–	–	–	126.1 (s)
H–C(2'',6'')	–	–	7.53 (d, $J=8.6$)	130.7 (d)
H–C(3'',5'')	–	–	7.15 (d, $J=8.6$)	116.9 (d)
H–C(4'')	–	–	–	161.5 (s)
OH	12.81 (br. s)	–	12.48 (s)	–

configuration of the anomeric H-atom of the glucose moiety was proposed to be β -oriented, based on the coupling constant (7.8 Hz) of the signal at $\delta(\text{H})$ 5.00. The signal of the C(1)-atom appeared unusually downfield at δ 205.3 because of a strong intramolecular H-bond with the OH group at C(8) as shown by *Kim et al.* [11]. In the ^1H , ^1H -COSY spectrum, the correlations of the signals at δ 5.17 (H–C(4)) with those at δ 2.33 (*m*, 2 H), 2.55 (*dt*, $J=18.1, 5.8, 1$ H), and 3.04 (*ddd*, $J=18.1, 8.3, 5.8, 1$ H) indicated the presence of one partial structure $[\text{C}]-\text{CH}_2-\text{CH}_2-\text{CH}(\text{OH})-[\text{C}]$. The sugar linkage was determined by HMBC correlations observed between the signals at $\delta(\text{H})$ 5.00 (H–C(1')) and $\delta(\text{C})$ 73.9 (C(4)). In the ROESY experiment (see *Fig. 1*), the correlations of H–C(4) with the H–C(5) and H–C(1') were observed; this was further confirmed by the axial position of the OH group at C(4).

To determine the absolute configuration of the stereogenic center C(4) in **1**, acid hydrolysis of **1** was performed to afford **1a** and glucose. Compound **1a** was determined to be 4,8-dihydroxy- α -tetralone by mass-spectral data, and it exhibited a negative optical-rotation value ($[\alpha]_D^{20} = -25$ ($c=0.4$, CHCl_3)), indicating (*R*)-configuration at C(4) in comparison with the reported data of natural tetralones, such as (4*R*)-

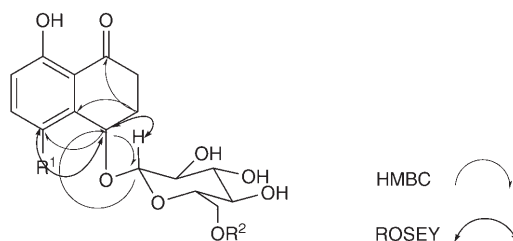


Fig. 1. Key HMBC and ROESY correlations observed in **1** and **2**

shinanolone ($[\alpha]_D^{21} = -22.8$ (CHCl_3) [12]), (4*S*)-isosclerone ($[\alpha]_D^{24} = +15.3$ and $[\alpha]_D^{15} = +19$ in CHCl_3) [13][14]. So, the structure of **1** was identified to be (*R*)-8-hydroxy- α -tetralone-*O*- β -D-glucopyranoside.

Compound **2**, a white solid, had the molecular formula $\text{C}_{25}\text{H}_{25}\text{O}_{11}$ based on the negative-mode HR-FAB-MS (501.1397 ($[M-H]^+$; calc. 501.1396)). The signals at $\delta(\text{H})$ 6.64 (*d*, $J = 16.1$, 1 H), 7.98 (*d*, $J = 16.1$, 1 H), 7.15 (*d*, $J = 8.6$, 2 H), and 7.53 (*d*, $J = 8.6$, 2 H) in the ^1H -NMR spectrum (see Table I) indicated the presence of a (*E*)-3-(4-hydroxyphenyl)prop-2-enoyl moiety, which was further supported by an intense fragment ion at m/z 147 in the FAB mass spectrum. Careful investigation of the ^1H - and ^{13}C -NMR spectral data of **2** revealed its structure to be very similar to **1**, except for a (*E*)-3-(4-hydroxyphenyl)prop-2-enoyl moiety and another OH group on the basis of its molecular weight. The correlations observed between the two signals at $\delta(\text{H})$ 5.16 (*d*, $J = 11.4$, $\text{H}-\text{C}(6')$) and 5.01 (*dd*, $J = 11.4$, 5.5, $\text{H}-\text{C}(6')$) with the ^{13}C signal ($\delta(\text{C})$ 167.6 ($\text{C}=\text{O}$ of coumaroyl group)) in the HMBC experiment confirmed that the coumaroyl moiety should be assigned to gluc- $\text{C}(6')$. The coupling constants (9.0 Hz) of the aromatic H-atoms at $\delta(\text{H})$ 7.34 and 6.99, and the correlations between the signal at $\delta(\text{H})$ 5.88 ($\text{H}-\text{C}(4)$) with the signal at $\delta(\text{C})$ 148.5 indicated another OH group at C(5). This was further confirmed by the correlations of $\text{H}-\text{C}(4)$ with only $\text{H}-\text{C}(1')$ in the ROESY experiment (see Fig. 1). Acid hydrolysis of compound **2** yielded 4,5,8-trihydroxy- α -tetralone (**2a**), 4-hydroxycinnamic acid, and glucose. Compound **2a** exhibited a negative optical-rotation value ($[\alpha]_D^{20} = -40$ ($c = 0.5$, CHCl_3)), indicating (*R*)-configuration at C(4) in comparison with the reported data of natural tetralones [15], and similar as the value of **1a**. Based on the above discussion, compound **2** should be (*R*)-5,8-dihydroxy- α -tetralone-*O*- β -D-[6-(4-hydroxycinnamoyl)] glucopyranoside.

Compound **3**, obtained as a yellow powder, had the molecular formula $\text{C}_{22}\text{H}_{20}\text{O}_{12}$ based on the negative-mode HR-FAB-MS (475.0858 ($[M-H]^+$; calc. 475.0876) and NMR spectral data. UV ($(\lambda_{\text{max}} [\text{nm}])$: 370 (MeOH)), and IR ((KBr): 3320 (OH), 1656 (α,β -unsaturated $\text{C}=\text{O}$), 1606, 1506, 1445, 1365, 956, 809) data indicated that **3** was a flavone derivative. Comparison of the ^1H - and ^{13}C -NMR data of **3** with those of avicularin (**11**) [7] showed that they had similar skeletons. The only difference was due to the presence of the Ac group in **3**. The correlations of the signals at $\delta(\text{H})$ 2.08 (*s*, MeCO) with the signals at $\delta(\text{C})$ 85.6 (*d*, C(2')) and 171.8 (MeCO) indicated the Ac group to be located at C(2) of the arabinofuranosyl group. Based on the above discussion, compound **3** should be quercetin-3-*O*-(2-acetyl- α -L-arabinofuranoside).

Biological Results. Using a growth inhibition assay, we tested compounds **3–13** at a single dose of 10 µg/ml. The amount of compounds **1** and **2** were too small to be tested in anticancer screening test systems. Two compounds, kaempferol (**8**) and maesopsin (**13**), strongly inhibited growth of CCRF-CEM human leukemia cells with growth rates below 30% compared to untreated controls.

These two compounds were further analyzed with six concentrations in a range between 0.1 and 30 µg/ml. We investigated the drug-sensitive CCRF-CEM parental cell line and its multidrug-resistant sub-line, CEM/ADR5000. The dose-response curves obtained were used to calculate the 50% inhibition concentration (IC_{50} , as shown in Fig. 2). The IC_{50} values for the two compounds were 14.0 and 5.3, and 10.2 and 12.3 µM, in CCRF-CEM and CEM/ADR5000 cells, respectively (Table 2). The degrees of resistance ranged from 0.74 to 2.3, indicating no or only minimal involvement of these substances in the multidrug resistance phenotype. A comparison with the standard cytostatic drugs showed that the CEM/ADR5000 cells revealed 1036-fold resistance to doxorubicin [16] (see Table 2). The degrees of cross-resistance of CEM/ADR5000 cells to vincristine and paclitaxel were 613-fold and 200-fold, respectively (see Table 2). Cross-resistance to other well-known drugs derived from traditional Chinese medicine (cantharidin, artesunate, berberine, and cephalotaxine) was also absent or minimal [17] (see Table 2).

Table 2. IC_{50} Values and Relative Resistance of Kaempferol (**8**) and Maesopsin (**13**) in Wild-Type CCRF-CEM and Multidrug-Resistant CEM/ADR5000 Cells Determined by Growth-Inhibition Assay, Including Standard Anti-Cancer Drugs (doxorubicin, vincristine, and paclitaxel) and Known Drugs from Traditional Chinese Medicine (cantharidin, artesunate, berberine, and cephalotaxine)

Compounds	IC_{50} Value ^a) [nM]		Degree of resistance ^b)
	CCRF-CEM	CEM/ADR5000	
Quercetin 3- <i>O</i> -(2-acetyl- α -L-arabinofuranoside) (3)	NA	NA	
Eriodictyol (4)	NA	NA	
Aromadendrin (5)	NA	NA	
<i>trans</i> -Dihydroquercetin (6)	NA	NA	
<i>cis</i> -Dihydroquercetin (7)	NA	NA	
Kaempferol (8)	14000 ± 1700	10200 ± 500	0.74
Kaempferol-3- <i>O</i> - α -L-arabinofuranoside (9)	NA	NA	
Quercetin (10)	NA	NA	
Quercetin-3- <i>O</i> - α -L-arabinofuranoside or avicularin (11)	NA	NA	
Quercetin 3'-methyl ether, 3- <i>O</i> - α -L-arabinofuranoside	NA	NA	
Maesopsin (13)	5300 ± 200	12300 ± 2600	2.3
Doxorubicin	11.8 ± 1.9	12.3 ± 2.6	1036
Vincristine	1.7 ± 0.1	1042.7 ± 145	613
Paclitaxel	3.7 ± 0.4	740.7 ± 137	200
Artesunate	1800 ± 1200	1200 ± 700	0.7
Cantharidin	19600 ± 2600	17700 ± 3100	0.9
Berberine	26000 ± 3300	158000 ± 9700	6.1
Cephalotaxine	15000 ± 6100	139900 ± 37200	9.3

^a) NA: No activity (no or minimal growth inhibition at a high concentration of 10 µg/ml). ^b) IC_{50} Value of CEM/ADR5000 divided by IC_{50} value of CCRF-CEM.

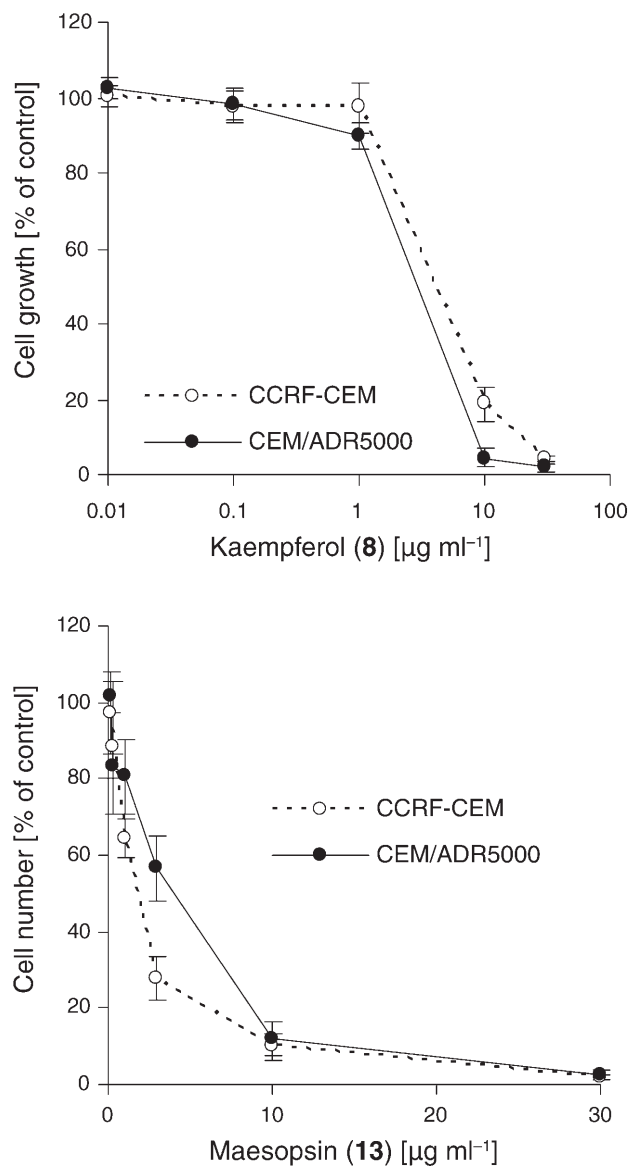


Fig. 2. Dose response curves of drug-sensitive CCRF-CEM and multidrug-resistant CEM/ADR5000 human leukemia cells after treatment with compounds

Conclusions. – In the present investigation, the multidrug resistance-conferring gene *MDR1* did not or minimally influence resistance to two compounds isolated from *Berchemia floribunda*, while high degrees of resistance were found to natural products used in standard tumour chemotherapy such as doxorubicin [16], vincristine, or paclitaxel. These results are in accordance with a previous report showing that the

MDRI-overexpressing CEM/ADR5000 cells were not or only minimally cross-resistant towards a panel of compounds derived from traditional Chinese medicine [17]. These findings allow speculation that natural products from traditional Chinese medicine might be helpful to treat refractory and otherwise drug-resistant tumors in the clinic.

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Experimental Part

1. *General*. Silica gel (200–300 mesh, or silica gel *H*, 10–40 μm) for column chromatography (CC) and *GF*₂₅₄ for TLC were obtained from the *Qindao Marine Chemical Factory*, Qindao, P. R. China. Optical rotation: *SEPA-300* polarimeter. UV: *Shimadzu* double-beam *210A* spectrophotometer; λ_{max} in nm. IR: *BioRad FTS-135* spectrometer; KBr pellets; $\bar{\nu}$ in cm^{-1} . ^1H -, ^{13}C -, and 2D-NMR: *Bruker AM-400* and *DRX-500* spectrometer; Me_4Si as internal standard; δ in ppm, *J* in Hz. MS: *VG AutoSpec 3000* spectrometers; *m/z* (rel. intensity).

2. *Plant Material*. The barks of *Berchemia floribunda* were collected in Kunming, Yunnan, P. R. China, in June 2003. The plant material was identified by Prof. *Wu Shugong*, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, P. R. China, where a voucher specimen (*Wu et al.* 2003101) is deposited.

3. *Extraction and Purification of Compounds*. The dried powdered plant material (7.8 kg) was extracted by percolation with EtOH at r.t. The combined extracts were concentrated under reduced pressure to yield 680 g of a brown residue. The AcOEt extract (120 g) was chromatographed over silica-gel column (78 cm \times 10 cm) eluting with a gradient mixture of $\text{CHCl}_3/\text{MeOH}$ from 95:5 to 70:30 to give five fractions (*A–E*). After repeated CC on a silica gel ($\text{CHCl}_3/\text{CH}_3\text{OH}$ from 9:1 to 8:2) and *Sephadex LH-20* (MeOH), *Fr. B* afforded compounds **4**, **5**, and **8**; *Fr. C* afforded compounds **6**, **7**, and **13**; *Fr. E* afforded compounds **11** and **12**. *Fr. D* was further purified over silica gel ($\text{CHCl}_3/\text{MeOH}$ 9:1), then by *RP18* silica-gel column eluting with 80% MeOH/ H_2O to afford compounds **1**, **2**, **3**, **9**, and **10**.

4. *Acidic Hydrolysis*. The compounds **1** and **2** (each 5 mg) were dissolved in a mixture of MeOH (1.0 ml) and 2M HCl (1.0 ml), and hydrolyzed by refluxing on a boiling water bath for 2 h. The hydrolysate was allowed to cool, diluted twofold with distilled H_2O , and partitioned between H_2O and AcOEt. The aq. layer was neutralized and concentrated *in vacuo* to give a residue. Glucose was identified from the residue by TLC comparison with an authentic sample with BuOH/AcOH/ H_2O 5:1:5 (upper layer).

8-Hydroxy- α -tetralone-4-O- β -D-glucopyranoside (= 4-(β -D-Glucopyranosyloxy)-3,4-dihydro-8-hydroxynaphthalen-1(2H)-one; **1**). $\text{C}_{16}\text{H}_{20}\text{O}_8$. White powder (MeOH). $[\alpha]_{\text{D}}^{27} = -76.67$ ($c=0.15$, MeOH). IR: 3396, 2926, 2877, 1709, 1641, 1604, 1579, 1452, 1339, 1254, 1163, 1034. ^1H - and ^{13}C -NMR: see Table 1. FAB-MS (neg.): 339 (86, $[M - \text{H}]^+$), 177 (27, $[M - 162]^+$), 159 (100, $[M - 162 - \text{H}_2\text{O}]^+$), 125 (42), 99 (65). HR-FAB-MS (neg.): 339.1084 ($[M - \text{H}]^+$, $\text{C}_{16}\text{H}_{19}\text{O}_8$; calc. 339.1079).

5,8-Dihydroxy- α -tetralon-4-O- β -D-[6-(4-hydroxycinnamoyl)]glucopyranoside (= 3,4-Dihydro-5,8-dihydroxy-4-[6-[(E)-3-(4-hydroxyphenyl)prop-2-enoyl]glucopyranosyloxy]naphthalen-1(2H)-one; **2**). $\text{C}_{25}\text{H}_{26}\text{O}_{11}$. White solid. $[\alpha]_{\text{D}}^{27} = -80.0$ ($c=0.2$, MeOH). IR: 3423, 2925, 2850, 1692, 1638, 1514, 1466, 1263, 1170, 1028. ^1H - and ^{13}C -NMR: see Table 1. FAB-MS (neg.): 501 (100, $[M - \text{H}]^+$), 339 (14, $[M - 162]^+$), 147 (55), 80 (5). HR-FAB-MS (neg.): 501.1397 ($[M - \text{H}]^+$, $\text{C}_{25}\text{H}_{25}\text{O}_{11}$; calc. 501.1396).

Quercetin-3-O-(2-O-acetyl- α -L-arabinofuranoside) (= 3-(2-O-Acetyl- α -L-arabinofuranosyloxy)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one; **3**). $\text{C}_{22}\text{H}_{20}\text{O}_{12}$. Yellow powder. IR: 3320 (OH), 1728, 1656, 1606, 1506, 1445, 1365, 1301, 1262, 1199, 1168, 956, 809. ^1H -NMR ((D_5) pyridine): 7.49 (*d*, $J=2.1$, H-C(2'')); 7.47 (*dd*, $J=8.4$, 2.1, H-C(6'')); 6.88 (*d*, $J=8.3$, H-C(5'')); 6.34 (*d*, $J=2.0$, H-C(8)); 6.17 (*d*, H-C(6)); 5.61 (*s*, H-C(1'')); 5.33 (*d*, $J=3.1$, H-C(2'')); 4.05 (*dd*, $J=6.3$, 3.1,

H–C(3''); 3.88 (*d*, *J* = 7.7, H–C(4'')); 3.52 (*dd*, *J* = 14.0, 3.5, H–C(5''a)); 3.48 (*dd*, *J* = 14.0, 4.9, H–C(5''b)); 2.08 (*s*, MeCOO). ¹³C-NMR ((D₅)pyridine): 179.6 (*s*, C(4)); 171.8 (MeCO); 165.9 (*s*, C(7)); 163.0 (*s*, C(5)); 159.2 (*s*, C(2)); 158.4 (*s*, C(9)); 149.8 (*s*, C(4')); 146.3 (*s*, C(3')); 134.7 (*s*, C(3)); 123.1 (*s*, C(1')); 122.9 (*d*, C(6')); 116.8 (*d*, C(2')); 116.4 (*d*, C(5')); 107.0 (*d*, C(1'')); 105.7 (*s*, C(10)); 99.8 (*d*, C(6)); 94.8 (*d*, C(8)); 87.3 (*d*, C(4'')); 85.6 (*d*, C(2'')); 76.7 (*d*, C(3'')); 61.9 (*t*, C(5'')); 20.7 (MeCO). FAB-MS (*neg.*): 475 (*[M – H]*⁺, 100), 302 (38). HR-FAB-MS: 475.0858 (*[M – H]*⁺, C₂₂H₁₉O₁₂; calc. 475.0876).

5. *Cytotoxicity Assays*. Standard anti-cancer drugs (doxorubicin, vincristine, and paclitaxel) and known drugs from traditional Chinese medicine (cantharidin, artesunate, berberine, and cephalotaxine) were obtained from commercial sources.

Human CCRF-CEM leukemia cells were maintained in RPMI medium supplemented with 10% fetal calf serum in a 5% CO₂ atmosphere at 37°. Cells were passaged twice weekly. All experiments were performed with cells in the logarithmic growth phase. The development of the multidrug-resistant sub-line CEM/ADR5000 has been described [18]. CEM-ADR5000 Cells were maintained in the absence of drug, and resistance was stabilized by drug treatment (5000 ng/ml doxorubicin), for 4 d every four weeks: the drug-resistant cells over-express the multidrug-resistance conferring P-glycoprotein (P-gp) and its encoding MDR1 gene.

The *in vitro* response to drugs was evaluated by means of a growth inhibition assay as described in [19]. Aliquots of 5 × 10⁴ cells/ml were seeded in 24-well plates, and drugs were added immediately at different concentrations. The compounds were used in a dose range from 0.3 to 10 µg/ml to allow calculation of inhibition concentration 50% (IC₅₀) values (see Fig. 2). Cells were counted 7 d after treatment with the drugs. The resulting growth data represent the net outcome of cell proliferation and cell death. Vehicle controls were included for DMSO as solvent.

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