New Rotenoids and Coumaronochromonoids from the Aerial Part of *Boerhaavia erecta*

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From the aerial part of *Boerhaavia erecta* L., three new rotenoids (3, 8, 10) and two new coumaronochromonoids (6, 11) were isolated, together with ten known compounds. The structure of the new compounds was established by one dimensional (1D)- and 2D-NMR spectroscopy, as well as high resolution-electrospray ionization (HR-ESI)-MS analysis. The absolute configuration of compound 11 was determined by UV circular dichroism spectroscopy. Compounds were evaluated for their cytotoxic activity against HeLa (human epithelial carcinoma), NCI-H460 (human lung cancer) and MCF-7 (human breast cancer) cell lines at the concentration of 100 μg/mL. Rotenoids 3, 4 and 5 showed a strong cytotoxic activity against HeLa cell line and rotenoids 5 and 8 showed good activity against MCF-7 cell line.

Key words *Boerhaavia erecta*; rotenoid; coumaronochromonoid; flavonoid

Boerhaavia is a genus of about 40 species mostly distributed in tropical and subtropical areas.[1] *Boerhaavia erecta* L. Nyctaginaceae, is a puberulous and erect annual herb which grows throughout the tropical regions of Africa and Asia.[2] In contrast with *B. diffusa* whose phytochemistry has been intensively studied,[3–6] to the best of our knowledge little phytochemical studies have been reported on *B. erecta*[7–10] even though these studies have evidenced an *in vivo* antimalarial activity for *B. erecta* extracts in mice parasitized with *Plasmodium berghei berghei*. Betacyanins and phenolics would be the metabolites responsible for this activity.[9]

As a part of an ongoing research program aimed at studying Vietnamese medicinal plants, we recently started the exhaustive phytochemical analysis of some *Boerhaavia* species. Preliminary chemical investigation of the aerial part of *B. erecta* has already led us to isolate 2,6-dimethoxybenzoquinone, (+)-catechin, isorhamnetin 3-*O*-β-D-glucopyranoside and rutin.[11] Herein, we report the isolation and identification of five new compounds from the ethyl acetate extract of the aerial part of *B. erecta*. Those compounds are three new rotenoids: 8-methoxy-10-demethoxycoccineone E (named boeravinone K, **3**), 6-demethylboeravinone G (named boeravinone M, **8**), and 10-demethylboeravinone C 11-*O*-β-D-glucopyranoside (named boeravinone N, **10**), and two new coumaronochromonoids: 10-demethylboeravinone J (named boeravinone L, **6**) and 10-demethyl-6a,12a-dihydroboerhavinone J 3-*O*-β-D-glucopyranoside (named boeravinone O, **11**). Cytotoxic activity against HeLa (human epithelial carcinoma), NCI-H460 (human lung cancer), and MCF-7 (human breast cancer) cell lines at the concentration of 100 μg/mL was determined.

Results and Discussion

Chemical examination of the ethyl acetate extract of *Boerhaavia erecta* aerial part led to the isolation of fifteen compounds. Among those ten were rapidly identified as boeravinone G (**1**),[12] boeravinone H (**2**),[12] boeravinone C (**4**),[13] 10-demethylboeravinone C (**5**),[14] boeravinone J (**7**),[15] cucumegastigmane (**9**),[16] isovitexin (**12**),[17] quercetin 3-*O*-β-D-glucopyranoside (**13**),[18] isorhamnetin 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**14**),[18] and kaempferol 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**15**)[18] (Fig. 1) by comparison of their spectral data with those reported in the literature. The five remaining unknown compounds included three rotenoids (compounds **3**, **8**, **10**) and two coumaronochromonoids (compounds **6**, **11**). Their structures were elucidated as follows.

Boeravinone K or 8-methoxy-10-demethoxycoccineone E (**3**) was isolated as pale yellow needles and its specific rotation was $[\alpha]_D^{23}$ −260 (c=0.001, MeOH). Its positive-ion electrospray ionization (ESI) mass spectrum showed a pseudomolecular ion peak at m/z 345.1 $[M+H]^+$. Its ^{1}H-NMR spectrum indicated the presence of 16 protons and its ^{13}C-NMR spectrum displayed resonances for 18 signals. Therefore, the molecular formula $C_{18}H_{16}O_7$ was assigned to compound **3**. In the one dimensional (1D)–^{1}H-NMR and ^{1}H–^{1}H correlation spectroscopy (COSY) spectra, an ABC signal system was observed [δ_H 4.68 (1H, dd, 9.0, 7.0 Hz, H-6a), 4.50–4.54 (1H, m, H-6β), 4.52 (1H, dd, 9.5, 3.5 Hz, H-6α)], indicating the presence of an isolated –O–CH–CH_2–O– system. The ^{1}H-NMR spectrum also showed four signals that were unambiguously assigned to the four aromatic CHs of a rotenoid A-ring at δ_H 8.33 (1H, dd, 8.0, 1.5 Hz, H-1), 7.07 (1H, dt, 8.0, 1.0 Hz, H-2), 7.33 (1H, dt, 7.5, 2.0 Hz, H-3) and 6.93 (1H, dd, 8.5, 1.0 Hz, H-4). Finally, the ^{1}H-NMR spectrum of **3** also displayed signals for one chelated hydroxyl proton (δ_H 11.72, s), one aromatic proton (δ_H 6.19, s), two methoxy groups [δ_H 3.92 (3H, s) and 3.83 (3H, s)], and one isolated hydroxyl proton (δ_H 2.82, s). The ^{13}C-NMR spectrum of **3** showed the presence of a ketone function (δ_C 192.9), seven aromatic quaternary carbons (δ_C 161.7, 161.4, 154.9,

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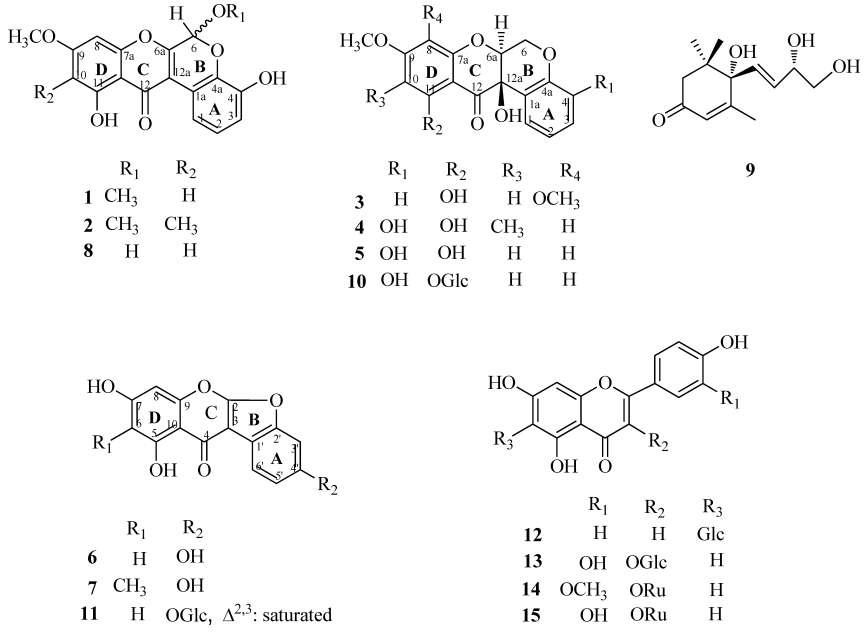

Fig. 1. Chemical Structures of Isolated Compounds

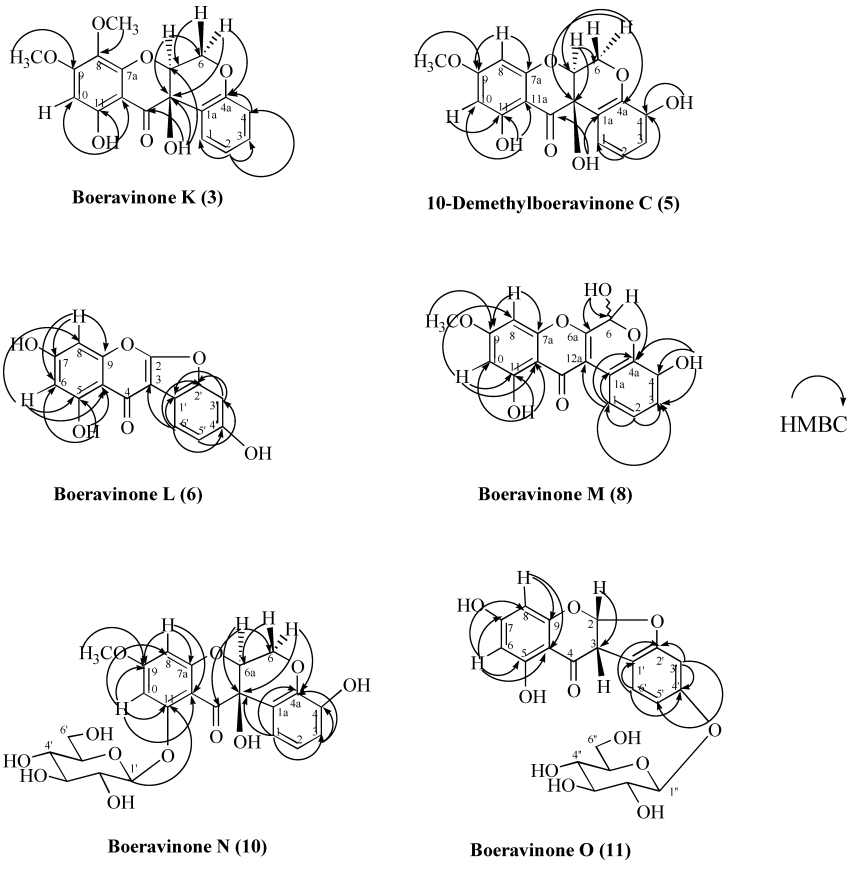

Fig. 2. Structures of **3**, **5**, **6**, **8**, **10**, and **11** with Key HMBC Correlations

152.1, 129.4, 118.6, 101.4), five aromatic methine carbons (δ_C 131.2, 131.1, 121.5, 117.5, 94.1), one aliphatic methine carbon (δ_C 76.4), one aliphatic quaternary carbon (δ_C 66.2), and one methylene carbon (δ_C 61.6). It also confirmed the presence of two methoxy groups (δ_C 61.5, 56.4).

The spectral pattern of **3** closely resembles that of coccineone E,[19] a compound in which positions 9 and 10 are substituted with a methoxy group and position 11 with an OH group. The heteronuclear multiple bond connectivity (HMBC) spectrum of **3** (Fig. 2) indicated that the chelated hydroxyl group (δ_H 11.72) is located at C-11 due to its correlations with signals at δ_C 161.4 (C-11), 101.4 (C-11a), 94.1 (C-10). The sin-

glet at δ_H 6.19 showed correlations with signals at δ_C 161.4 (C-11), 101.4 (C-11a), 129.4 (C-8). It was therefore unambiguously attributed to H-10.

The presence of a methoxy group at position C-8 influences the splitting patterns of protons H-6a and H-6. In coccineone E,[19] chemical shift and multiplicity values ($CDCl_3$) of these protons are δ_H 4.77 (dd, $10.0_{ax\text{-}ax}$, $5.5_{ax\text{-}eq}$ Hz) for H-6a, 4.48 (t, 10.0 Hz) for H-6β, and 4.44 (dd, 10.0_{gem}, $5.5_{eq\text{-}ax}$ Hz) for H-6α. In **3**, the chemical shift and multiplicity values ($CDCl_3$) of H-6a and H-6 protons are δ_H 4.68 (dd, 9.0, 7.0 Hz), 4.50–4.54 (m), and 4.52 (dd, 9.5, 3.5 Hz), respectively.

The circular dichroism (CD) and X-ray analyses are used to establish the absolute configuration of natural 12a-hydroxyrotenoids. However, a literature search showed that the geometry of the B/C-ring junction of 12a-hydroxyrotenoids could be assigned by analysis of their chemical shift value of H-1 in their ^{1}H-NMR spectra as well as the comparison of their optical rotation value with those of known compounds. Literature revealed that the chemical shift value of H-1 would be δ_H 6.6–7.0 for a *cis*-junction as in 4′,5′-dihydro-11,5′-dihydroxy-4′-methoxytephrosin,[20] or in (6aα,12aα)-12a-hydroxyelliptone,[21] and around δ_H 8.0 for a *trans*-junction as in boeravinone C.[13] Further it has been observed that natural *trans*-junction 12a-hydroxyrotenoids would be either 6aα,12aβ-configuration as in coccineone E[19] and abronione[22] or 6aβ,12aα-configuration as in (+)-12a-epimilletosin[23] and usararotenoid C.[24] Those with a 6aα,12aβ-configuration display negative specific rotation,[13,19,22] while those with a 6aβ,12aα-configuration display positive specific rotation.[23,24]

The chemical shift value for H-1 (δ_H 8.33) of **3** is strongly deshielded when compared to the value observed for rotenoids with *cis*-B/C ring junction indicating that the B/C ring junction in **3** has a *trans*-geometry. It exhibited negative optical rotation, therefore, from biogenetic considerations of 12a-hydroxyrotenoids of the family Nyctaginaceae,[13,19,22] the absolute configurations at C-6a and C-12a in **3** are likely to be as in boeravinone C, coccineone E and other related natural rotenoids. The structure of compound **3** was determined as (6aα,12aβ)-6a,12a-dihydro-11,12a-dihydroxy-8,9-dimethoxy[1]benzopyrano[3,4-*b*][1]benzopyran-12(6*H*)-one or Boeravinone K.

Boeravinone L or 10-demethylboeravinone J (**6**) was obtained as a white amorphous powder. Its molecular formula determined as $C_{15}H_8O_6$ through the pseudomolecular ion peak *m*/*z* 285.0383 $[M+H]^+$ in the high resolution (HR)-ESI-MS spectrum was supported by its ^{13}C-NMR spectrum in which fifteen carbons could be detected (see Table 1). The polarization transfer (DEPT) NMR spectra of **6** displayed five aromatic methine and ten aromatic quaternary carbons. The ^{1}H-NMR spectrum of **6** showed a singlet of a chelated hydroxyl proton at δ_H 12.97, five aromatic methine signals between δ_H 6.30 and 7.80. The two doublet signals at δ_H 7.80 (1H, *J*=8.0 Hz, H-6′), 7.12 (1H, *J*=2.0 Hz, H-3′) and one double doublet signal at δ_H 7.01 (1H, *J*=8.0, 2.0 Hz, H-5′) were assigned unambiguously to a 1,2,4-trisubstituted A-ring. The ^{13}C-NMR spectrum of **6** showed the presence of a conjugated carbonyl (δ_C 181.1), nine aromatic quaternary carbons (δ_C 165.8, 164.4, 164.1, 157.4, 156.2, 151.5, 115.3, 104.5, 98.4) and five aromatic methine carbons (δ_C 122.4, 114.7, 100.8, 99.6, 95.7). The spectral pattern of **6** closely resembles that of sophorophenolone, isolated from *Astragalus membranaceus*.[25] Only the methoxy group at the position C-7 in sophorophenolone was lacking in **6** suggesting its replacement by a hydroxyl group. The series of $^{2,3}J_{H-C}$ correlations detected in the HMBC spectrum of **6** (Fig. 2) confirmed the presence of a coumaronochromone skeleton and the location of the substituents. Thus **6** was identified as 5,7,4′-trihydroxycoumaronochromone or Boeravinone L or 10-demethylboeravinone J.

Table 1. NMR Spectroscopic Data of Compounds **6** and **11**

Position	Compound **6** (DMSO-d_6)		Compound **11** (C_5D_5N)	
	δ_H, *J* (Hz)	δ_C	δ_H, *J* (Hz)	δ_C
2	—	165.8	6.57 (d, 7.5)	104.0
3	—	98.4	4.38 (d, 7.5)	76.8
4	—	181.1	—	179.3
–OH	12.97 (s)	—	—	—
5	—	164.1	—	166.4
6	6.36 (d, 2.0)	100.8	6.75 (d, 2.0)	95.1
7	—	164.4	—	162.2
8	6.59 (d, 2.0)	95.7	6.73 (d, 1.5)	100.4
9	—	156.2	—	163.3
10	—	104.5	—	105.8
1′	—	115.3	—	116.7
2′	—	151.5	—	158.1
3′	7.12 (d, 2.0)	99.6	8.54 (d, 2.0)	114.9
4′	—	157.4	—	158.1
5′	7.01 (dd, 8.0, 2.0)	114.7	7.81 (dd, 8.5, 2.0)	122.5
6′	7.80 (d, 8.0)	122.4	7.27 (d, 8.5)	123.6
β-Glc				
1″	—	—	6.35 (d, 7.5)	104.6
2″	—	—	4.32–4.36 (m)	76.6
3″	—	—	4.38–4.41 (m)	79.0
4″	—	—	4.26–4.30 (m)	72.0
5″	—	—	4.04–4.09 (m)	79.5
6″	—	—	4.40–4.46 (m), 4.26–4.30 (m)	63.1

Boeravinone M or 6-demethylboeravinone G (**8**) was isolated as white needles. Its molecular formula was determined as $C_{17}H_{12}O_7$ through its pseudomolecular ion peak at *m/z* 351.0474 $[M+Na]^+$ in the HR-ESI-MS spectrum. This molecular formula is well consistent with the presence of 12 protons the ^{1}H-NMR spectrum and 17 signals in the ^{13}C-NMR spectrum of **8**. The ^{1}H-NMR spectrum of **8** (Table 2) showed ten signals: a chelated hydroxyl proton (δ_H 12.87, 11-OH), two isolated hydroxyl protons (δ_H 9.40, 4-OH and δ_H 8.14, 6-OH), six methine resonances (two double doublets, one triplet and three doublets) in the region between δ_H 6.20 ppm and 8.20 ppm and a methoxy signal at δ_H 3.88. The two *meta*-coupled protons at δ_H 6.73 and 6.46, each was a doublet with J=2.0 Hz, were attributed to H-8 and H-10, respectively in D-ring. The triplet at δ_H 6.92 (J=8.0 Hz, H-2) was *ortho*-coupled with both the doublet at δ_H 8.14 (J=8.0, 1.5 Hz, H-1) and the doublet at δ_H 6.85 (J=8.0, 1.5 Hz, H-3). The ^{1}H–^{1}H COSY spectrum of **8** confirmed that these three aromatic methines were arranged in a continuous sequence in A-ring. The remaining proton resonated at δ_H 6.22 (d, J=6.0 Hz). The heteronuclear single quantum coherence (HSQC) spectrum of **8** confirmed that this signal at δ_H 6.22 was the hemiacetal proton H-6 and its corresponding carbon resonated at δ_C 87.6 (DMSO-d_6). Such up-field shifted chemical shift value for C-6 was also observed in boeravinone B (δ_C 89.5 in pyridine-d_5).[5] The ^{13}C-NMR spectrum (Table 2) of **8** showed signals for a conjugated carbonyl (δ_C 180.0), nine aromatic quaternary carbons (δ_C 165.5, 161.8, 157.8, 156.3, 146.4, 136.8, 117.1, 109.0, 105.4), six aromatic methine carbons (δ_C 121.7, 116.6, 116.0, 98.5, 92.7, 87.6), and one methoxy group (δ_C 56.2). The HMBC spectrum of **8** showed correlations between the hemiacetal signal at δ_H 6.22 and signals at δ_C 136.8 (C-4a), 157.8 (C-6a) and 109.0 (C-12a), as well as between the signal of 6-OH at δ_H 8.14 (d, J=6.5 Hz) and signals at δ_C 87.6 (C-6), 157.8 (C-6a). Complete analysis of the HSQC and HMBC data (Fig. 2) for **8** resulted in its formulation as 4,6,11-trihydroxy-9-methoxy[1]benzopyrano[3,4-*b*][1]benzopyran-12(6*H*)-one or 6-demethylboeravinone G.

Compound **5** was identified as a 12a-hydroxyrotenoid derivative from mass and NMR spectroscopy data. ^{1}H–^{1}H COSY, HSQC, HMBC spectral data of **5** were similar to those of (−)-4,11,12a-trihydroxy-9-methoxyrotenoid {$[\alpha]_D^{23}$ −339 (c=1.1, MeOH)}, isolated from *B. diffusa*[14] and *B. coccinea*,[26] although there was a disparity in the optical rotation values. Based on the 2D-NMR data (Fig. 2), all chemical shift values for protons and carbons of **5** are precisely pointed out. It turned out that some signals (C-7a and C-11, C-4 and C-4a, C-8 and C-10) had been interchanged in literature.[26] Compound **5** was identified as (6a*S*,12a*R*)-4,11,12a-trihydroxy-9-methoxyrotenoid or 10-demethylboeravinone C.

Boeravinone N or 10-demethylboeravinone C 11-*O*-*β*-D-

Table 2. NMR Spectroscopic Data of Compounds **3**, **8**, and **10**

Position	Compound **3** ($CDCl_3$)		Compound **8** (DMSO-d_6)		Compound **10** (CD_3OD)	
	δ_H, J (Hz)	δ_C	δ_H, J (Hz)	δ_C	δ_H, J (Hz)	δ_C
1	8.33 (dd, 8.0, 1.5)	131.2	8.14 (dd, 8.0, 1.5)	116.6	7.40 (dd, 8.0, 1.5)	123.7
1a	—	118.6	—	117.1	—	121.4
2	7.07 (dt, 8.0, 1.0)	121.5	6.92 (t, 8.0)	121.7	6.86 (t, 8.0)	121.7
3	7.33 (dt, 7.5, 2.0)	131.1	6.85 (dd, 8.0, 1.5)	116.0	6.81 (dd, 8.0, 1.5)	116.9
–OH	—	—	9.40 (s)		—	—
4	6.93 (dd, 8.5, 1.0)	117.5	—	146.4	—	146.9
4a	—	154.9	—	136.8	—	144.5
–OH	—	—	8.14 (d, 6.5)		—	—
6	4.52 (dd, 9.5, 3.5) 4.50–4.54 (m)	61.6	6.22 (d, 6.0)	87.6	4.46 (dd, 9.5, 4.5) 4.39 (dd, 11.5, 10.0)	62.7
6a	4.68 (dd, 9.0, 7.0)	76.4	—	157.8	4.66 (dd, 11.5, 4.5)	77.8
7a	—	152.1	—	156.3	—	163.8
–OCH_3	3.83 (s)	61.5	—	—	—	—
8	—	129.4	6.73 (d, 2.0)	92.7	6.32 (d, 2.5)	97.3
–OCH_3	3.92 (s)	56.4	3.88 (s)	56.2	3.85 (s)	56.4
9	—	161.7	—	165.5	—	167.3
10	6.19 (s)	94.1	6.46 (d, 2.0)	98.5	6.63 (d, 2.5)	99.8
–OH	11.72 (s)	—	12.87 (s)	—	—	—
11	—	161.4	—	161.8	—	162.1
11a	—	101.4	—	105.4	—	107.5
12	—	192.9	—	180.0	—	190.5
–OH	2.82 (s)	—	—	—	—	—
12a	—	66.2	—	109.0	—	68.8
β-Glc						
1′	—	—	—	—	4.85 (d, 8.0)	104.5
2′	—	—	—	—	3.57 (dd, 9.0, 8.0)	74.9
3′	—	—	—	—	3.50 (t, 9.0)	77.4
4′	—	—	—	—	3.38 (dd, 10.0, 8.5)	71.5
5′	—	—	—	—	3.45–3.49 (m)	78.7
6′	—	—	—	—	3.92 (dd, 12.0, 2.5) 3.70 (dd, 12.0, 6.0)	62.6

glucopyranoside (**10**) was isolated as a yellow wax. The molecular formula of compound **10** was determined as $C_{23}H_{24}O_{12}$ through its pseudomolecular ion peak at *m/z* 515.1320 $[M+Na]^+$ in the HR-ESI mass spectrum. ^{1}H-NMR spectra of **10** and **5** exhibited a very similar pattern of a 12a-hydroxyrotenoid except for the lack of the signal of the chelated hydroxyl proton (11-OH) and the appearance of signals corresponding to a hexopyranosyl moiety. The presence of a *β*-D-glucopyranosyl moiety was demonstrated by the doublet signal of an anomeric proton at δ_H 4.85 (d, *J*=8.0 Hz, H-1′) which had COSY correlation chains with H-2′/H-3′/H-4′/H-5′/H-6′ in the zone from 3.3 ppm to 4.9 ppm. HMBC experiments confirmed the attachment of the *β*-D-glucopyranosyl moiety to the rotenone skeleton at the position C-11 due to the cross peak between H-1′ (δ_H 4.85) and C-11 (δ_C 162.1). HMBC experiments also showed that the methoxy group was attached to the aglycone at C-9.

The sugar moiety was identified as D-(+)-glucose by the acidic hydrolysis and using TLC to compare the hydrolysate with the authentic D-(+)-glucose. The B/C-ring junction of the 12a-hydroxyrotenoid aglycone was considered to be *trans* due to the chemical shift value of H-1 (δ_H 7.40 in CD_3OD) and the negative optical rotation value of the aglycone after the acidic hydrolysis. The absolute configurations at C-6a and C-12a in **10** were expected to be the same as those in **3** and **5**, as they were isolated from the same materials and possessing similar spectral data.[13,19,22] The structure of compound **10** was assigned as 10-demethylboeravinone C 11-*O*-*β*-D-glucopyranoside.

Boeravinone O (**11**) was obtained as a yellow wax. Its molecular formula was determined as $C_{21}H_{20}O_{11}$ through the pseudomolecular ion peak at *m/z* 471.0891 $[M+Na]^+$ in the HR-ESI-MS spectrum. The ^{13}C-NMR spectrum (Table 1) of **11** showed signals for a conjugated carbonyl (δ_C 179.3), seven aromatic quaternary carbons (δ_C 166.4, 163.3, 162.2, 158.1, 158.0, 116.7, 105.8), five aromatic methine carbons (δ_C 123.6, 122.5, 114.9, 100.4, 95.1), two acetal carbons (δ_C 104.6, 104.0) and 6 carbinol carbons in the region between δ_C 79 ppm and 63 ppm. The ^{1}H-NMR spectrum of **11** showed the presence of 1,2,4-trisubstituted and 1,2,3,5-tetrasubstituted aromatic rings: δ_H 8.54 (1H, d, *J*=2.0 Hz, H-3′), 7.81 (1H, dd, *J*=8.5, 2.0 Hz, H-5′), 7.27 (1H, d, *J*=8.5 Hz, H-6′), 6.75 (1H, d, *J*=2.0 Hz, H-6) and 6.73 (1H, d, *J*=1.5 Hz, H-8). The ^{1}H-NMR spectrum also showed signals for protons of a sugar moiety. This sugar was identified as *β*-D-glucopyranose by comparison of the ^{13}C-NMR data of **11** with those of other glucosides isolated in the same material.[16–18] The *β* configuration of the glucose residue was deduced from its anomeric proton at δ_H 6.35 (1H, d, *J*=7.5 Hz, H-1″) and its corresponding acetal carbon resonated at δ_C 104.6. The remaining doublet at δ_H 6.57 (1H, d, 7.5 Hz, H-2) correlated with the second acetal carbon at δ_C 104.0 in the HSQC spectrum of **11**. This characteristic carbon signal was assigned to the position C-2 of the coumaronochromone moiety. The ^{1}H–^{1}H COSY spectrum of **11** showed that proton H-2 at δ_H 6.57 was adjacent to another proton at δ_H 4.38 (1H, d, 7.5 Hz, H-3) whose carbon resonated at δ_C 76.8. The large downfield chemical shift value of this aliphatic methine carbon revealed that it was next to more than one electron–withdrawing group and was then assigned to the position C-3 of the aglycone.

Comparison of the 1D and 2D spectral data of **6** and **11** (Table 1) revealed that they both possessed a coumaronochromone partial structure in which C-5 and C-7 in the D-ring were oxygenated carbons and so was C-4′ in the A-ring. Further analysis showed that C-4′ was not linked to a free hydroxyl group but to an *O*-glucose group. This was proved by the chemical shift values of carbons C-3′, C-4′, C5′ were shifted down-field compared to those of **6**.

The coumaronochromone moiety of **11** possesses two chiral centers at C-2 and C-3, therefore, a possible configuration of compound **11** is (2*S*,3*S*) or (2*R*,3*R*). To determine the absolute configuration of **11**, ultraviolet CD spectroscopy was applied. The CD spectrum of compound **11** in methanol showed a strong positive peak at 251 nm, a weak negative at 269 nm and a broad negative band at around 340 nm. The former two bands were assigned to the $\pi\rightarrow\pi^*$ transitions and the later to the $n\rightarrow\pi^*$ transition of the acetophenone chromophore as observed in CD spectra of other flavonoids.[27] Because the sign of the $n\rightarrow\pi^*$ CD band is known to be insensitive to the substitution of phenyl ring,[28] it can be used for the determination of the absolute configuration at C2 position in acetophenone-containing molecule.[27,29] Compound **11** with 2*S* configuration possesses a conformation with M helicity so that it will exhibit a negative cotton effect at the $n\rightarrow\pi^*$ band.[28,29] Accordingly, the absolute configuration (2*S*,3*S*) was assigned to compound **11**.

Combined data allowed to identify compound **11** as (2*S*,3*S*)-5,7,4′-trihydroxy-2,3-dihydrocoumaronochromone 4′-*O*-*β*-D-glucopyranoside.

Cytotoxicity assay of nine compounds **3**–**6**, **8**–**11**, and **15** against the HeLa, MCF-7 and NCI-H460 cell lines was evaluated by using the Sulforhodamine B (SRB) method (Table 3). At a concentration of 100 *μ*g/mL, compounds **3**, **4**, and **5** showed the highest cytotoxic activity against HeLa. Inhibition reached 81 to 91 percent for compounds **5** and **8** against MCF-7 at the concentration of 100 *μ*g/mL. Poor solubility of **3**, **4**, **5**, and **8** in the biological medium prevented an accurate IC_{50} determination.

Our results confirm previous statements suggesting that plants of the family Nyctaginaceae are the source of simple rotenoids. During this study, we have isolated seven rotenoids from the aerial part of *B. erecta*, three dehydrorotenoids (**1**, **2**, **8**) and four 12a-hydroxyrotenoids (**3**, **4**, **5**, **10**). Five rotenoids do not possess a methyl group at the C-10 position (**1**, **3**, **5**, **8**, **10**) and two do (**2**, **4**), in contrast to the fact that almost all known natural rotenoids contain an isoprenoid-derived substituents usually at the C-8 position and occasionally at the C-10 position.[23] To the best of our knowledge, our study also constitutes the first report of a rotenoid glycoside (**10**) in the *Boerhaavia* genus. We also isolated three coumaronochromones, a small subclass of the isoflavonoids. Known natural coumaronochromones are often prenylated at C-6, C-8 and C-3′[23] and only three *O*-substituted coumaronochromones: boeravinone J in *B. diffusa*,[15] sophorophenolone in *Astragalus membranaceus*[25] and coccineone A in *B. coccinea*[30] were isolated before.

Experimental

General Experimental Procedures NMR spectra were recorded on a Bruker Avance III spectrometer using residual solvent signal as internal reference: chloroform-*d* δ_H 7.24, δ_C 77.23; methanol-d_4 δ_H 3.31, δ_C 49.15; DMSO-d_6 δ_H 2.50, δ_C 39.51; $(CD_3)_2CO$ δ_H 2.09, δ_C 206.31, 30.6; and pyridine-

Table 3. Cell Growth Inhibitory Effects of the Purified Compounds

No.	Compound[a)]	Inhibition of cell growth (%)		
		HeLa	NCI-H460	MCF-7
1	Boeravinone K (**3**)	91.14±0.17[b)]	54.73±6.70	30.53±2.30
2	Boeravinone C (**4**)	89.39±1.35	4.46±13.85	74.04±6.29
3	10-Demethylboeravinone C (**5**)	84.20±0.90	5.15±2.36	81.37±5.17
4	Boeravinone L (**6**)	7.76±1.64	7.19±4.72	8.00±0.97
5	Boeravinone M (**8**)	15.69±5.50	48.52±1.58	85.08±4.42
6	Cucumegastigmane (**9**)	5.23±8.41	2.55±5.37	0.46±8.25
7	Boeravinone N (**10**)	19.85±4.89	31.78±4.20	35.67±3.68
8	Boeravinone O (**11**)	7.43±6.02	7.27±2.69	5.83±12.60
9	Kaempferol 3-*O*-rutinoside (**15**)	−4.73±1.37	3.08±1.43	−6.15±1.49
	Camptothecin (positive control)[c)]	61.0±4.78	77.9±2.30	47.0±1.86

a) The compounds were tested at the concentration of 100 μg/mL. *b*) The presented data are means of three experiments±S.D. *c*) Camptothecin was tested at the concentration of 0.01 μg/mL for MCF-7 and NCI-H 460 and of 1 μg/mL for HeLa.

d_5 at δ_H 8.74, 7.58 and 7.22, δ_C 123.87, 135.91, 150.35. The HR-ESI-MS were recorded on a HR-ESI-MS MicroOTOF-Q mass spectrometer or on a LC-Agilent 1100 LC-MSD Trap spectrometer. Melting points were determined on Maquenne block and are uncorrected. Optical rotations (MeOH) were measured on a Kruss digital polarimeter. Absorption and CD spectra were measured on a JASCO V-570 spectrophotometer and on a JASCO J-820E spectropolarimeter, respectively. TLC was carried out on precoated Silica gel 60 F_{254} or Silica gel 60 RP-18 $F_{254}S$ (Merck). Spots were visualized by spraying with 10% aqueous H_2SO_4 or 5% ferric chloride solutions followed by heating. Gravity column chromatography was performed with Silica gel 60 (0.040–0.063 mm, Himedia).

Biological Assays[31,32)] Determination of cytotoxic activities against the HeLa (human epithelial carcinoma), MCF-7 (human breast cancer) and NCI-H460 (human lung cancer) cell lines of tested samples were performed at the concentration of 100 μg/mL using the antiproferative Sulforhodamine B (SRB) assay with camptothecin as the positive control. All cells were cultured in E'MEM medium (Eagle's Minimal Essential Medium) supplemented with 10% foetal bovine serum (FBS), 1% of 2 mM L-glutamine, 50 IU/mL penicillin, 50 μg/mL streptomycin and maintained at 37°C in a 5% CO_2 atmosphere with 95% humidity. Viable cells were counted and inoculated in 96-well plate with density of 10^4 cells/100 μL/well. After 24 h the cells were treated with pure compound while the control wells were added only by 100 μL medium. All experiments were in triplicate. The plates were incubated in an atmosphere of 5% CO_2, 95% humidity at 37°C for 48 h. Adherent cell cultures were fixed by adding 50 μL of cold 50% (w/v) trichloroacetic acid per well and incubated at 4°C for 1 h. The plates were washed five times with distilled water and air dried. Then a solution of 50 μL of SRB (0.4% w/v in 1% acetic acid) was added to each well and allow staining at room temperature for 30 min. The SRB solution was removed out of plates by rinsing 4 times with a 1% glacial acetic acid solution (200 μL/well). The plates were air-dried for 12–24 h. The bound SRB was solubilised to each well by adding 100 μL of 10 mM Tris Base (pH 10.5). The plates were shaken gently for 20 min and the optical density of each well was read using a scanning multiwall spectrophotometer at a test wavelength of 492 nm and a reference wavelength of 620 nm. The optical density (OD) of SRB in each well is directly proportional to the cell number. Cell survival was measured as the percentage absorbance compared to the control (non-treated cells).

Plant Material *Boerhaavia erecta* L. was collected at Thu Duc District, Ho Chi Minh City, Vietnam in March 2009. The material was identified by botanist Vo Van Chi. A voucher specimen (No US-A002) was deposited at the Herbarium of the Department of Organic Chemistry, Faculty of Chemistry, University of Science, National University, Ho Chi Minh City, Vietnam.

Extraction and Isolation Air-dried aerial parts of *B. erecta* (3.8 kg) were exhaustedly extracted with methanol by maceration at room temperature, then evaporated under reduced pressure to afford a residue (245 g). This latter was suspended in water and partitioned between petroleum ether then ethyl acetate to afford a petroleum ether extract (38.1 g), an ethyl acetate extract (25.1 g), and the remaining aqueous solution. The ethyl acetate extract was subjected to silica gel column chromatography using gradient elution of ethyl acetate–methanol (10 : 0–0 : 10) to give fractions EA.A to EA.F. Fraction EA.B (4.5 g) was subjected to silica gel column chromatography eluted with chloroform–methanol (98 : 2) to give sub-fractions B.1 and B.2. Sub-fraction B.1 was subjected to silica gel column chromatography eluted with chloroform–methanol (98 : 2) to give **1** (20 mg). Sub-fraction B.2 was chromatographed with C-18 silica gel eluted with water–methanol (6 : 4) to afford **2** (24 mg), **3** (8 mg), **4** (72 mg) and **5** (154 mg).

Fraction EA.D (7.1 g) was subjected to silica gel column chromatography eluted with chloroform–methanol (90 : 10) to give 3 sub-fractions D.1 to D.3. Sub-fraction D.1 was chromatographed with C-18 silica gel eluted with water–methanol (6 : 4) to afford **6** (76 mg). Sub-fraction D.2 was chromatographed with silica gel C-18 eluted with water–methanol (7 : 3) to afford **7** (22 mg), **8** (65 mg) and **9** (6 mg). Sub-fraction D.3 was chromatographed with silica gel C-18 eluted with water–methanol (75 : 25) to afford **10** (10 mg), **11** (32 mg) and **12** (20 mg). Fraction EA.E (20 g) was subjected to silica gel column chromatography eluted with chloroform–methanol–water (80 : 20 : 0.2) to give **13** (85 mg), **14** (1.4 g) and **15** (1.1 g).

Boeravinone K (**3**): Pale yellow needles, mp 244–247°C (chloroform). $[\alpha]_D^{23}$ −260 (c=0.001, MeOH). ESI-MS m/z 345.1 $[M+H]^+$ (Calcd for $C_{18}H_{16}O_7$+H: 345.097). ^{1}H- and ^{13}C-NMR ($CDCl_3$) see Table 2.

10-Demethylboeravinone C (**5**): White needles. mp 248°C (chloroform). $[\alpha]_D^{23}$ −513 (c=0.001, MeOH). HR-ESI-MS m/z 353.0602 $[M+Na]^+$ (Calcd for $C_{17}H_{14}O_7$+Na: 353.0637).

^{1}H-NMR (acetone-d_6), δ ppm: 11.85 (1H, s, 11-OH), 7.79 (1H, dd, J=6.0, 3.5 Hz, H-1), 6.85 (1H, t, J=8.0 Hz, H-2), 6.83 (1H, m, H-3), 6.11 (1H, d, J=2.5 Hz, H-10), 6.09 (1H, d, J=2.0 Hz, H-8), 5.86 (1H, br s, 4-OH), 4.81 (1H, dd, J=10.0, 6.0 Hz, H-6a), 4.48 (1H, t, J=10.0 Hz, H-6ax), 4.45 (1H, dd, J=10.0, 6.0 Hz, H-6eq), 3.88 (3H, s, 9-OCH_3). ^{13}C-NMR (acetone-d_6), δ ppm: 195.0 (C-12), 168.8 (C-9), 166.2 (C-11), 162.7 (C-7a), 146.6 (C-4), 143.8 (C-4a), 122.7 (C-1), 121.5 (C-2), 121.4 (C-1a), 116.7 (C-3), 102.9 (C-11a), 96.1 (C-10), 94.2 (C-8), 76.9 (C-6a), 66.8 (C-12a), 62.2 (C-6), 56.3 (OCH_3).

Boeravinone L (**6**): White amorphous powder. HR-ESI-MS m/z 285.0383 $[M+H]^+$ (Calcd for $C_{15}H_8O_6$+H, 284.0321). ^{1}H- and ^{13}C-NMR (DMSO-d_6) see Table 1.

Boeravinone M (**8**): White needles, mp 217°C (chloroform). $[\alpha]_D^{23}$ 0 (c=0.001, MeOH), HR-ESI-MS m/z 351.0474 $[M+Na]^+$ (Calcd for $C_{17}H_{12}O_7$+Na, 351.0481). ^{1}H- and ^{13}C-NMR (DMSO-d_6) see Table 2.

Boeravinone N (**10**): White amorphous powder, $[\alpha]_D^{23}$ −198 (c=0.001, MeOH), HR-ESI-MS: m/z 515.1320 $[M+Na]^+$ (Calcd for $C_{23}H_{24}O_{12}$+Na, 515.11654). ^{1}H- and ^{13}C-NMR (methanol-d_4) see Table 2.

Boeravinone O (**11**): Yellow wax, $[\alpha]_D^{23}$ +58 (c=0.001, MeOH), HR-ESI-MS: m/z 471.0891 $[M+Na]^+$ (Calcd for $C_{21}H_{20}O_{11}$+Na, 471.09032). ^{1}H- and ^{13}C-NMR (pyridine-d_5) see Table 1.

Acidic Hydrolysis of 10[33] Compound **10** (1 mg) was dissolved in 0.5 mL solution of 1 N HCl–methanol (1 : 1) and refluxed at 75°C for 90 min. The reaction solution was evaporated under reduced pressure, then the hydrolysate was extracted with ethyl acetate (1 mL×3). The aqueous fraction was neutralized with Ag_2CO_3 and then filtered. The filtrate was concentrated under reduced pressure to afford a residue. This residue was compared to the authentic D-(+)-glucose (Merck) by using TLC (ethyl acetate–methanol–water, 10 : 4 : 1), (Rf=0.17). The TLC result showed that the sugar of **10** was D-(+)-glucose. The ethyl acetate extract was determined to be 10-demethylboeravinone C by comparing it with the authentic sample (compound **5**) by TLC (chloroform–methanol, 98 : 2), (Rf=0.30).

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