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Constituents of the Roots of *Boerhaavia diffusa* L. I. Examination of Sterols and Structures of New Rotenoids, Boeravinones A and B[1)]

Shigetoshi KADOTA, Nzunzu LAMI, Yasuhiro TEZUKA, and Tohru KIKUCHI*

Research Institute for Wakan-Yaku (Oriental Medicines), Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan. *Received May 18, 1989*

Examination of the ether extract of the roots of *Boerhaavia diffusa* L. led to the isolation of three new rotenoid analogues along with fatty acids, glycerides, and sterol derivatives. The structures of two rotenoid analogues named boeravinone A and boeravinone B have been determined by means of nuclear magnetic resonance spectroscopy including two-dimensional incredible natural abundance double quantum transfer experiment (2D INADEQUATE) and ^{1}H–^{13}C long range correlation spectroscopy.

Keywords *Boerhaavia diffusa*; Nyctaginaceae; rotenoid; boeravinone A; boeravinone B; 2D NMR; 2D INADEQUATE; acylated steryl glucoside

Boerhaavia diffusa L. is a plant of the family of Nyctaginaceae and is widely used in traditional medicine: in Nepal as a remedy for back bone pain (root paste) and as a tonic (whole plant) in association with other plants[2)]; in Sri Lanka to treat rheumatism and snake bite; in India as a medicine with multiple actions (stomachic, diuretic, anti-asthmatic, diaphoretic, anthelminthic, febrifuge, antileprosy, antiscabies, anti-urethritis, *etc.*[3)]); in East Africa for scabies, abscesses, and boils[4)]; and in Zaire as a medicinal plant.[5)] Antitumor and antiviral effects of *B. diffusa* have also been reported.[6,7)] However, up to now, investigations on its chemical constituents have been limited to the isolation and structure elucidation of hentriacontane, ursolic acid, β-sitosterol, glucose, fructose,[6)] β-ecdysone,[8)] a new C-methyl-flavone,[9)] and punanarvine.[3)]

As a part of our chemical studies on Ayurvedic traditional medicines, we examined the constituents of the roots of this plant and found that the ether extract shows a mild growth-inhibitory activity against HeLa-S_3 cells, whereas the methanol extract shows an α_2-receptor binding activity. Thus, systematic chemical investigation of these extracts was undertaken. This paper describes the isolation and structure elucidation of sterol derivatives and two new rotenoid analogues named boeravinone A (**9**) and boeravinone B (**10**) from the ether extract.

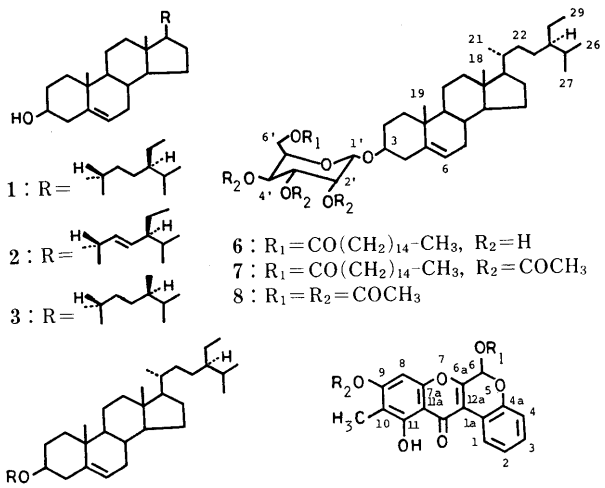

Chart 1

Air-dried roots of *B. diffusa*, collected at Manang, Nepal, in August 1983, were pulverized and extracted with ether at room temperature and then with hot methanol and hot water successively. The ether extract was concentrated to yield a white precipitate, which was determined to be a mixture of D-glucosides of sitosterol (**1**), stigmasterol (**2**), and campesterol (**3**) in a ratio of about 84:13:3 (see Experimental). The ethereal solution was then evaporated to dryness and the residue was divided into an acidic fraction and a neutral fraction in the usual manner. The acidic fraction gave a mixture of fatty acids, which was converted to a mixture of methyl esters. These esters were identified by means of gas chromatography (GC) and gas chromatography combined with mass spectrometry (GC-MS) as methyl palmitate, methyl heptadecyclate, methyl oleate, methyl stearate, methyl arachidate, and methyl behenate (see Experimental). The neutral fraction was separated by silica gel column chromatography to give fractions containing sitosterol esters, glycerol esters, sterols, rotenoid analogues, and an acylated sterol glucoside as shown in Chart 2.

Fraction 1 was further separated into two substances by preparative thin layer chromatography (TLC). The less polar substance, a colorless oil, showed a carbonyl absorption (1721 cm^{-1}) in the infrared (IR) spectrum and its proton nuclear magnetic resonance (^{1}H-NMR) spectrum suggested a sterol ester structure. On alkaline hydrolysis, it gave sitosterol (**1**) and an acidic substance, which was methylated with diazomethane to give a mixture of esters. These esters were identified as methyl palmitate (about 84%), methyl heptadecyclate, methyl stearate, methyl arachidate, and methyl behenate by GC-MS analysis. Thus, the less polar substance is concluded to be a sterol ester mixture containing sitosteryl palmitate (**4**) as the main component. On the other hand, the more polar substance, a colorless oil, $C_{47}H_{82}O_2$, showed IR absorptions at 1720 (CO) and 1669 (C=C) cm^{-1}. Its ^{1}H-NMR spectrum showed a pattern similar to that of sitosteryl palmitate (**4**), except for the appearance of a signal due to olefinic protons (δ_H 5.34, 2H, m). Alkaline hydrolysis of this substance gave a sterol and an acid. This sterol was identified as sitosterol (**1**), while the fatty acid moiety was identified as oleic acid by GC and GC-MS analyses. Thus, the more polar substance was determined to be sitosteryl oleate (**5**).

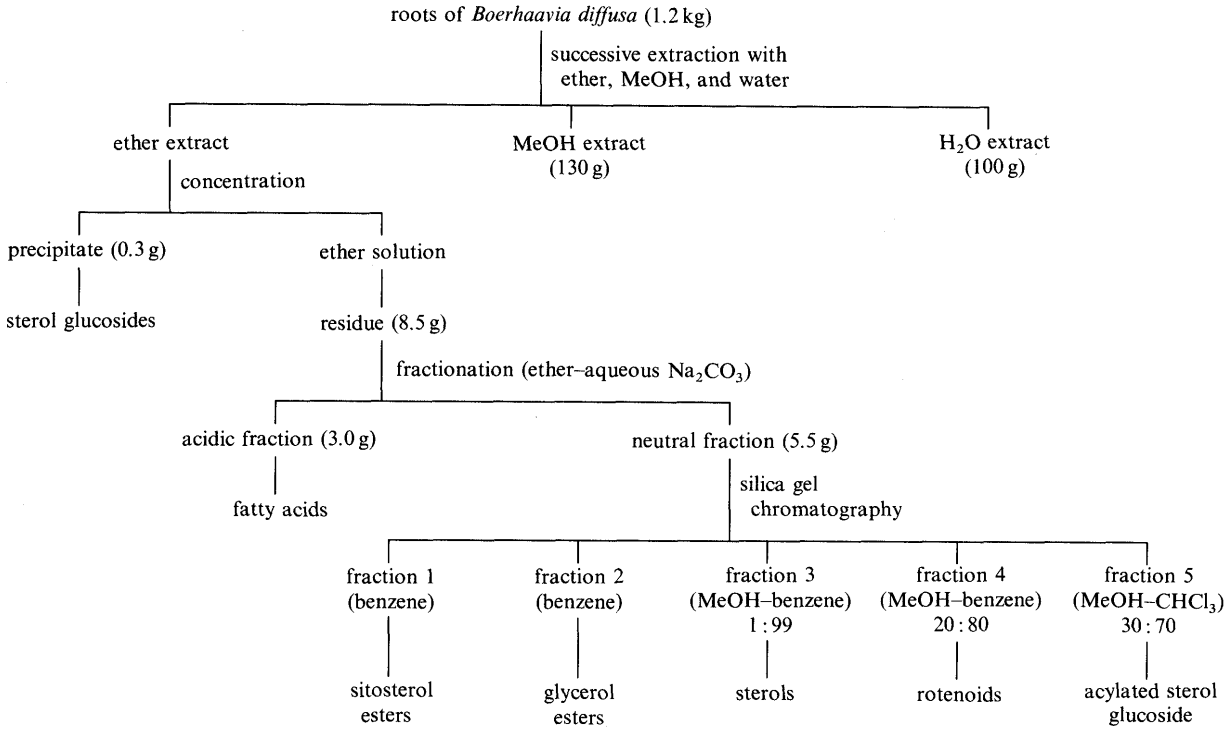

Chart 2

Fraction 3 gave a crystalline substance which was determined to be a mixture of sitosterol (**1**), stigmasterol (**2**), and campesterol (**3**) by GC-MS examination.

Fraction 5 was further separated by repeated preparative TLC to give an ester (**6**) as a yellow greasy substance, $[\alpha]_D$ $-47.7°$ ($CHCl_3$). The microanalytical data of **6** are consistent with the molecular formula $C_{51}H_{90}O_7$ and its IR spectrum showed a carbonyl band at 1733 cm^{-1}. The ^{1}H-NMR spectrum showed characteristic signals assignable to an anomeric proton at δ_H 4.38 (d, J=8.0 Hz), methylene protons geminal to an ester grouping at δ_H 4.26 (dd, J= 12.2, 2.0 Hz) and 4.49 (dd, J=12.2, 4.9 Hz), and three hydroxyl-bearing methine protons at δ_H 3.36 (t, J=8.0 Hz), 3.38 (t, J=9.0 Hz), and 3.57 (t, J=9.0 Hz), suggesting the presence of a 6-*O*-acylglucoside residue in **6**. Also the ^{1}H-NMR spectrum showed signals due to an olefinic proton (δ_H 5.36), two primary methyl groups (δ_H 0.85 and 0.88), three secondary methyl groups (δ_H 0.81, 0.84, and 0.92), and two tertiary methyl groups (δ_H 0.68 and 1.01). Acetylation of **6** afforded a triacetate (**7**), IR ν_{max} 1755 cm^{-1} whose ^{1}H-NMR spectrum showed singlets due to three acetoxyls (δ_H 2.00, 2.02, and 2.05), and the spectral pattern was closely similar to that of sitosteryl D-glucoside tetraacetate (**8**), except for the loss of one acetoxyl singlet and the appearance of a methylene signal (δ_H 2.35, t, J=7.6 Hz) and a primary methyl signal (δ_H 0.88, t, J=6.4 Hz). These spectral data indicated that **6** is a 6′-*O*-ester of sitosteryl D-glucoside.

Alkaline hydrolysis of **7** afforded a fatty acid, which was identified as palmitic acid by GC and GC-MS examinations of its methyl ester. Based on the foregoing findings, the structure of this ester was determined to be **6**.

Fraction 2 yielded a pale yellow oil. The IR spectrum of this substance showed a carbonyl band (1736 cm^{-1}) and the ^{1}H-NMR spectrum showed a pattern suggestive of a glycerol ester structure. On alkaline hydrolysis, it furnished palmitic acid, oleic acid, stearic acid, arachidic acid, and behenic acid in a ratio of about 16.6:20.0:6.1:1.5:5.5; these identifications were confirmed by GC and GC-MS analyses after methylation. Therefore, this substance is considered to be a mixture of fatty acid glycerol esters.

Fraction 4 from the silica gel column chromatography of the neutral fraction gave boeravinone B (**10**) as a yellow precipitate. The mother liquor was separated by preparative TLC to give boeravinone A (**9**) and another new compound named boeravinone C.[10]

Boeravinone A (**9**) was obtained as yellow needles, mp 215—217 °C, $[\alpha]_D$ 0° (acetone). The MS of **9** exhibited the molecular ion peak at m/z 326 and fragment ion peaks at m/z 295 ($M^+ - OCH_3$) and 267 ($M^+ - OCH_3 - CO$) and its molecular formula was determined to be $C_{18}H_{14}O_6$ by high-resolution MS measurement. It showed IR absorptions at 3600, 3250 (OH), 1650 (conjugated CO), 1620 (C=C), 1595, and 1495 (phenyl) cm^{-1}. In the ultraviolet (UV) spectrum it showed absorption bands at 217, 276, 300_{sh}, and 340_{sh} nm (log ε: 4.39, 4.37, 4.07, and 3.39 , respectively). On addition of 3 N NaOH (2 drops), bathochromic displacements of these absorptions were noticed to 219, 291, 348, and 354_{sh} nm (log ε: 4.96, 4.56, 3.99, and 3.96, respectively), suggesting that **9** may be a phenolic compound.

The ^{1}H-NMR spectrum of **9** (in pyridine-d_5), analyzed with the aid of ^{1}H–^{1}H shift correlation spectroscopy (COSY), showed signals due to *o*-disubstituted phenyl protons at δ_H 7.16, 7.20, 7.29, and 9.10, along with signals at δ_H 13.44 (1H, OH), 6.56 (1H, s, aromatic proton), 5.89 (1H, s, methine proton), 3.45 (3H, s, OCH_3), and 2.39 (3H, s, vinyl-CH_3). On the other hand, the ^{13}C-NMR spectrum indicated the presence of a carbonyl (δ_C 180.40) and five sp^2 methine carbons (δ_C 93.53, 117.48, 122.91, 127.48, and 129.06) together with a methoxy carbon (δ_C 55.73), a *tert*-

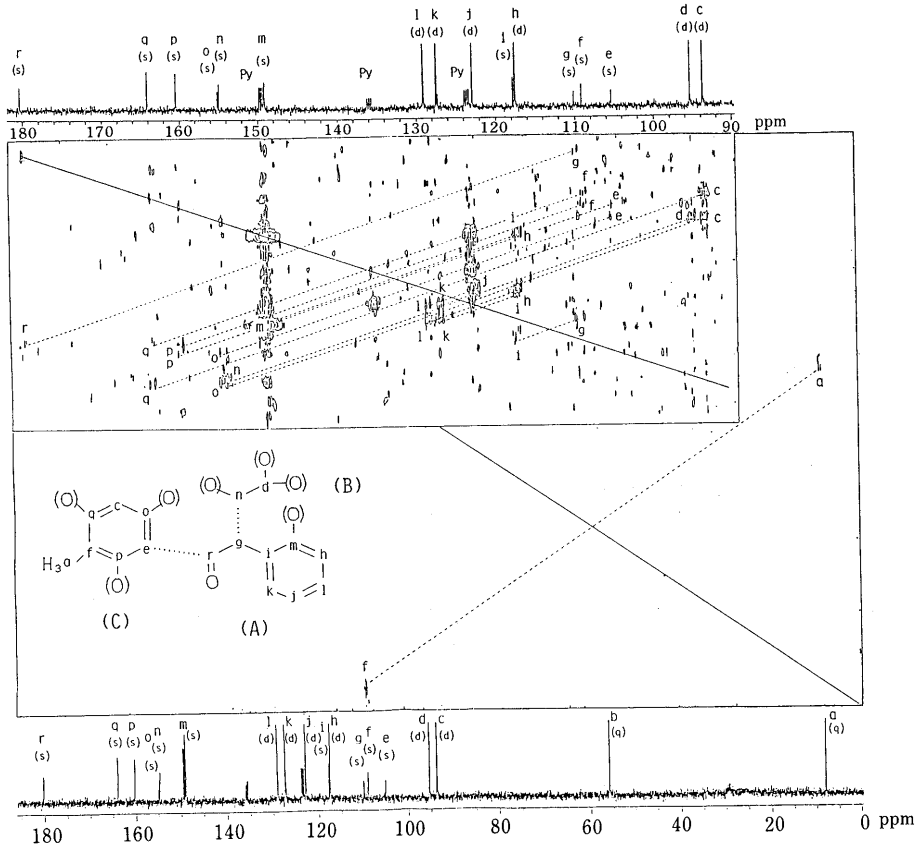

Fig. 1. 2D INADEQUATE Spectrum of Boeravinone A (**9**) in Pyridine-d_5

Sample, 115 mg; J_{CC}=60 Hz; 40 °C; 90 h run.

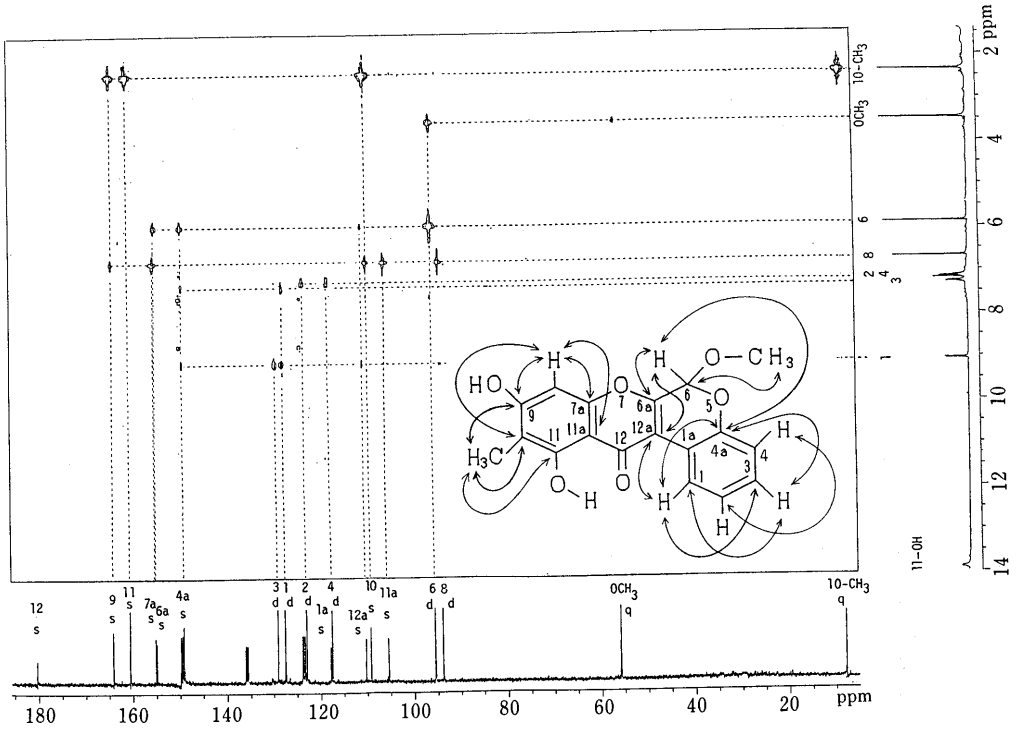

Fig. 2. ^1H–^{13}C Long-Range Shift Correlation Spectrum of Boeravinone A (**9**) in Pyridine-d_5 (J_{CH}=10 Hz)

methyl carbon (δ_C 7.92), an acetal carbon (δ_C 95.33), and nine quaternary carbons (see Table I). Each of these carbon signals, except for quaternary ones, was assigned based on the ^1H–^{13}C COSY.

Methylation of **9** with diazomethane gave a methyl ether (**11**), $C_{19}H_{16}O_6$ (amorphous powder). The ^1H-NMR spectrum of **11** showed a singlet due to a newly introduced methoxy group at δ_H 3.91 along with a singlet (1H, δ_H

12.94) which could be ascribed to a phenolic hydroxyl proton, strongly hydrogen-bonded with the carbonyl group (see Table I).

Since boeravinone A (**9**) has a number of quaternary carbons, the two-dimensional incredible natural abundance double quantum transfer experiment (2D INADEQUATE)[11] was applied to clarify the sequence of carbon atoms in the molecule, employing the pulse system: (90°)–τ–(180°)–τ–(90°)–$t_{1/2}$–(135°)–$t_{1/2}$–t_2. The result is reproduced in Fig. 1, in which carbon signals are marked with small letters a—r in the order of increasing δ value and coupled ^{13}C–^{13}C pairs are joined by slanting dotted lines. Figure 1 shows that carbon r (s) is directly connected with carbon g (s), carbon g (s) with carbon i (s), carbon i (s) with carbons m (s) and k (d), carbon k (d) with carbons i (s) and j (d), carbon j (d) with carbons k (d) and l (d), carbon l (d) with carbons j (d) and h (d), and carbon h (d) with carbons m (s) and l (d). Further, correlations were observed between each ^{13}C–^{13}C couple in carbon series a→f→p→e→o→c→q→f and n→d. This result indicated the presence of partial structures A, B, and C in boeravinone A as depicted in Fig. 1.

Next, we measured the ^1H–^{13}C long-range COSY[12] of **9** in order to clarify the connectivities of the partial structures and substituent groups. As shown in Fig. 2, the carbon signal at δ_C 110.12 (g, C-12a) is correlated with the protons at δ_H 5.89 (6-H) and 9.10 (1-H), and the signal at δ_C 95.33 (d, C-6) with the proton at δ_H 3.45 (OCH_3). On the other hand, the carbon signal at δ_C 149.05 (m, C-4a) is correlated with the proton signals at δ_H 5.89 (6-H), 7.29 (3-H), and 9.10 (1-H) and other long-range correlations observed are also shown here by arrows. Therefore, it is reasonable to conclude that the carbon g (C-12a) is connected with the carbon n (C-6a) and that the methoxyl group is bonded to the carbon d (C-6), and the latter is connected with the carbon m (C-4a) through an acetal linkage. Although connectivity could not be detected between the quaternary carbons r (C-12) and e (C-11a), there must be a chemical bonding between them because one of the two hydroxyl groups is internally hydrogen-bonded with the 12-ketone group. This hydroxyl group was proved to be linked to the carbon p (C-11) by the use of deuterium-induced isotope effect[13] under the slow OH–OD exchange condition: *viz.*, in the ^{13}C-NMR spectrum, the signal p at δ_C 160.54 was obviously broadened on addition of a 1 : 1 mixture of deuterium oxide and water, as shown in Fig. 3. Further, in view of the composition, an ether linkage should exist between quaternary carbons n (C-6a) and o (C-7a).

On the basis of the above findings, the structure of boeravinone A was determined to be racemic 6-methoxy-9,11-dihydroxy-10-methyl-6a,12a-dehydrorotenoid as represented by the formula **9**.[14]

Boeravinone B (**10**) was obtained as a yellow amorphous powder, $[\alpha]_D$ 0° (acetone), and was determined to have the molecular formula $C_{17}H_{12}O_6$ by high-resolution MS measurement. The IR spectrum of **10** showed absorption bands at 3250 (OH), 1650 (conjugated CO), 1595, and 1495 (phenyl) cm^{-1} and the UV spectrum showed absorptions at

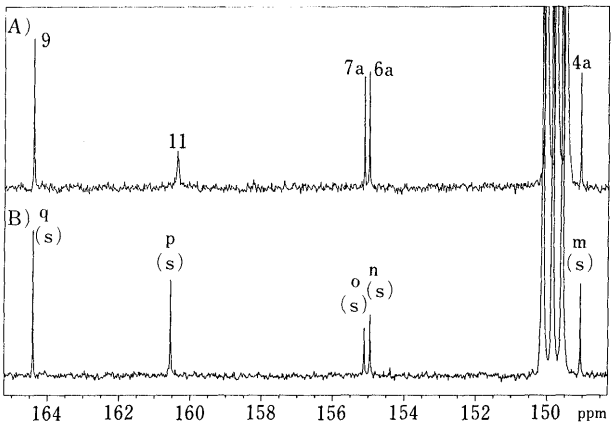

Fig. 3. ^{13}C-NMR Spectrum (Low-Field Region) of Boeravinone A (**9**) in Pyridine-d_5

TABLE I. 400 MHz ^1H- and 100 MHz ^{13}C-NMR Data for Boeravinone A (**9**), Boeravinone A Methyl Ether (**11**), and Boeravinone B (**10**)

Position	**9** (in pyridine-d_5)		**11** (in $CDCl_3$)		**10** (in pyridine-d_5)	
	δ_H [a]	δ_C [b,c]	δ_H	δ_C [d]	δ_H [a]	δ_C [b,c]
1	9.10 dd (8.0, 1.3)	127.48 d	8.85 dd (8.0, 1.5)	127.23	9.14 dd (8.0, 1.2)	127.44 d
1a	—	117.77 s	—	117.10	—	118.00 s
2	7.16 ddd (8.0, 7.6, 1.3)	122.91 d	7.17 ddd (8.0, 7.5, 1.5)	122.98	7.16 ddd (8.0, 6.0, 3.0)	122.53 d
3	7.29 ddd (8.0, 7.6, 1.3)	129.06 d	7.30 ddd (8.0, 7.5, 1.5)	128.96	7.30 m	128.96 d
4	7.20 dd (8.0, 1.3)	117.48 d	7.12 dd (8.0, 1.5)	116.97	7.30 m	118.00 d
4a	—	149.05 s	—	148.37	—	150.06 s
6	5.89 s	95.33 d	5.80 s	95.06	6.52 s	89.53 d
6a	—	154.82 s	—	154.40	—	155.19 s
7a	—	155.06 s	—	155.00	—	155.77 s
8	6.56 s	93.53 d	6.45 s	89.35	6.65 s	93.58 d
9	—	164.11 s	—	163.64	—	164.08 s
10	—	109.12 s	—	109.73	—	109.00 s
11	—	160.54 s	—	159.21	—	160.59 s
11a	—	105.32 s	—	106.16	—	105.28 s
12	—	180.40 s	—	180.28	—	180.69 s
12a	—	110.12 s	—	110.54	—	109.47 s
6-OCH_3	3.45 s	55.73 q	3.60 s	55.95	—	—
9-OCH_3	—	—	3.91 s	56.04	—	—
10-CH_3	2.39 s	7.92 q	2.12 s	7.37	2.42 s	8.08 q
11-OH	13.44 s	—	12.94 s	—	13.67 s	—

δ values in ppm and coupling constants in Hz. *a*) ^1H–^1H COSY was measured. *b*) Multiplicities of carbon signals were determined by means of the DEPT method and are indicated as s, d, t, and q. *c*) ^1H–^{13}C COSY was measured. *d*) Only the complete decoupling spectrum was measured.

217, 273, 300_{sh}, and 345_{sh} nm (log ε: 4.41, 4.40, 4.13, and 3.61, respectively). Its MS exhibited the M^+ peak at *m/z* 312 and fragment ion peaks at *m/z* 295 ($M^+ - OH$), 283 (base peak, $M^+ - CHO$),[15] and 267 ($M^+ - OH - CO$). The ^1H- and ^{13}C-NMR spectra of **10** showed very similar patterns to those of boeravinone A (**9**), respectively, except for the absence of signal due to the methoxy group, suggesting that the structure would be **10** (Table I).

Treatment of boeravinone B (**10**) with *p*-toluenesulfonic acid in dry methanol gave a methyl acetal, mp 207—209 °C, which was found to be identical with boeravinone A (**9**) by IR and ^1H-NMR comparisons. Therefore, the structure of boeravinone B was established to be racemic 6,9,11-trihydroxy-10-methyl-6a,12a-dehydrorotenoid (**10**).

The biological activities of **9** and **10** are currently under investigation.

Rotenoids[16] are a class of isoflavonoids having an extra carbon atom in an additional pyran ring and some of them are known to have insecticidal and piscidal activity[17] and other interesting biological activities.[18] Their occurrence in natural resources has hitherto been limited to the Leguminosae and Stemonaceae plants, but recently two new 12a-hydroxyrotenoids have been isolated from a Brazilian plant *Boerhaavia coccinea*,[19] and our present result provides the second example of isolation of rotenoids from *Boerhaavia* species. It should be mentioned that most of the known natural rotenoids contain an isoprenoid-derived substituent, usually at the C-8 position and occasionally at the C-10 position. In contrast, boeravinones A and B have a methyl group at the C-10 position and are interesting from a biogenetic viewpoint.[20] The distribution of rotenoids in other Nyctaginaceae plants and their biological activities would be worthy of investigation.

Experimental

Melting points were determined with a Kofler-type apparatus and are uncorrected. Optical rotations were measured in acetone or chloroform solutions on a JASCO DIP-4 automatic polarimeter at 20 °C. UV spectra were taken with a Shimadzu 202 UV spectrometer in EtOH solutions and IR spectra with a JASCO IRA-2 or a Nicolet DX FT-IR spectrometer in chloroform solutions unless otherwise noted. ^1H- and ^{13}C-NMR spectra were taken on a JEOL JNM-GX 400 spectrometer with tetramethylsilane as an internal standard, and chemical shifts are recorded in δ values. ^1H–^1H COSY, ^1H–^{13}C COSY, ^1H–^{13}C long-range COSY, and 2D INADEQUATE spectra were obtained with the JEOL standard pulse sequences and data processing was performed with the JEOL standard software. MS and high-resolution MS were obtained with a JEOL JMS-D 300 spectrometer (ionization voltage, 70 eV; accelerating voltage, 3 kV) using a direct inlet system. GC analyses were done on a Shimadzu gas chromatograph (model GC-6A) with a flame ionization detector using a 2% OV-17 column (on Gas-Chrom Q, 2 m × 3 mm i.d. glass tube) at a column temperature of 298—300 °C (2 °C/min) or a 3% OV-1 column (on Gas-Chrom Q, 2 m × 3 mm i.d. glass tube) at 180—300 °C (3 °C/min). Nitrogen was used as the carrier gas at flow rate of 35 ml/min. GC-MS analyses were done on a JEOL JMS-D 300 mass spectrometer using a GC injection system. Helium was used as the carrier gas (1 kg/cm^2). Column chromatography was done with Mallinkrodt silica gel. Preparative TLC was carried out on Merck Kieselgel GF_{254} plates and the plates were examined under UV light. Extraction of substances from silica gel was done with MeOH–CH_2Cl_2 (1 : 9 or 3 : 7) and solutions were concentrated *in vacuo*. TLC analyses were done on Merck Kieselgel GF_{254} plates developed with $CHCl_3$, MeOH–$CHCl_3$ (1 : 99, 6 : 94, 1 : 9), $CHCl_3$–hexane (15 : 85), and MeOH–benzene (1 : 9) and spots were detected by the use of 1% $Ce(SO_4)_2$–aqueous H_2SO_4 (10%) reagent. For drying organic solutions, anhydrous $MgSO_4$ was used.

Extraction and Separation of Constituents of *Boerhaavia diffusa* Air-dried roots of *B. diffusa* (1.2 kg), collected at Manang (Central Nepal) in August 1983, were pulverized and extracted three times (2 d each) with ether (1.5 l each) at room temperature. The plant material was further extracted successively with boiling methanol (2 l × 3) and with boiling water (2 l × 3) to give a methanolic extract (130 g) and a water extract (100 g).

The above ether solutions were combined and concentrated (about 200 ml) to yield a white precipitate, which was collected by filtration, giving sterol glucosides (300 mg).

The concentrated ether solution freed from the precipitate was evaporated to dryness. The yellow brownish residue (8.5 g) was dissolved again in ether and extracted with saturated aqueous Na_2CO_3. The aqueous layer was made slightly acidic by the addition of 5% HCl and extracted with ether. The combined ether layers were evaporated to give an acidic substance as an oil (3 g).

The remaining ether solution from the Na_2CO_3 treatment was washed with saturated NaCl, dried, and evaporated to give a greasy residue (5.5 g), which was subjected to column chromatography on silica gel (127.5 g). Elution with benzene (1050 ml), methanol–benzene (1 : 99, 3 : 97, 6 : 94, 10 : 90, 20 : 80, and 30 : 70; 500 ml each), and finally with methanol–chloroform (30 : 70, 1000 ml) gave fractions 1 (1 g), 2 (1.5 g), 3 (0.6 g), 4 (1.1 g), and 5 (0.3 g), containing sitosterol esters, glycerol esters, sterols, rotenoid analogues, and an acylated sterol glucoside, respectively.

Identification of Acids A portion of the acidic substance (195 mg) was separated by preparative TLC with MeOH–$CHCl_3$ (6 : 94) to yield a mixture of fatty acids (180 mg). IR ν_{max} cm^{-1}: 3000 (broad, OH) and 1710 (CO). This mixture (110 mg) was treated with an excess of diazomethane to give a mixture of methyl esters. GC and GC-MS (3% OV-1 column) analyses of this mixture revealed the presence of methyl palmitate, methyl heptadecyclate, methyl oleate, methyl stearate, methyl arachidate, and methyl behenate (intensity ratio 55 : 1 : 27 : 7 : 2 : 3).

Identification of Sterol Glucosides A portion (16 mg) of the white precipitate, IR ν_{max} cm^{-1} (KBr): 3400 (OH), from the ether extract was treated with acetic anhydride (1 ml) and pyridine (1 ml) at room temperature for 24 h. Addition of water to the reaction mixture afforded a precipitate, which was collected by filtration and washed with water. This was purified by preparative TLC with $CHCl_3$ to give a tetraacetate (14 mg). IR ν_{max} cm^{-1}: 1755 (CO). The ^1H-NMR of this tetraacetate was almost superimposable on that of authentic sitosterol D-glucoside tetraacetate (**8**). ^1H-NMR ($CDCl_3$) δ_H: 0.68 (3H, s, 18-H_3), 0.81 (3H, d, J = 7.0 Hz, 27- or 26-H_3), 0.84 (3H, d, J = 7.3 Hz, 26- or 27-H_3), 0.85 (3H, t, J = 7.6 Hz, 29-H_3), 0.92 (3H, d, J = 6.4 Hz, 21-H_3), 0.99 (3H, s, 19-H_3), 2.00—2.08 (each 3H, s, 4 × OAc), 3.49 (1H, m, 3-H), 3.68 (1H, ddd, J = 9.8, 4.8, 2.4 Hz, 5′-H), 4.11 (1H, dd, J = 12.2, 2.4 Hz, 6′-H), 4.26 (1H, dd, J = 12.2, 4.8 Hz, 6′-H), 4.59 (1H, d, J = 7.9 Hz, 1′-H), 4.96 (1H, dd, J = 9.5, 7.9 Hz, 2′-H), 5.08 (1H, t, J = 9.8 Hz, 4′-H), 5.20 (1H, t, J = 9.5 Hz, 3′-H), 5.36 (1H, m, 6-H).

A sample (6 mg) of the tetraacetate (**8**) was hydrolyzed with 5% HCl–EtOH (2 ml) under reflux for 2 h. After neutralization with aqueous Na_2CO_3, the reaction product was taken up with $CHCl_3$, and then purified by preparative TLC with MeOH–$CHCl_3$ (1 : 99) to yield a mixture of free sterols (4 mg). This was proved to be a mixture of sitosterol (**1**), stigmasterol (**2**), and campesterol (**3**) in a ratio of about 84 : 13 : 3 by GC (2% OV-17 column) and GC-MS analyses.

Identification of Sitosterol Esters Fraction 1 (1 g), obtained from the silica gel chromatography of the neutral fraction, was further separated by repeated preparative TLC with $CHCl_3$ and $CHCl_3$–hexane (1.5 : 8.5) into two zones. The less polar zone gave a mixture of sterol esters (80 mg), colorless greasy substance, IR ν_{max} cm^{-1}: 1721 (CO), while the more polar zone afforded sitosteryl oleate (**5**) (150 mg), colorless greasy substance. IR ν_{max} cm^{-1}: 1720 (CO), 1669 (C=C). ^1H-NMR ($CDCl_3$) δ_H: 0.68 (3H, s, 18-H_3), 0.81, 0.84 (each 3H, d, J = 7.0 Hz, 26- and 27-H_3), 0.85 (3H, t, J = 7.3 Hz, 29-H_3), 0.88 (3H, t, J = 6.7 Hz, 18′-H_3), 0.92 (3H, d, J = 6.7 Hz, 21-H_3), 1.02 (3H, s, 19-H_3), 4.61 (1H, m, 3-H), 5.34 (2H, m, 9′- and 10′-H), 5.37 (1H, br d, J = 4.5 Hz, 6-H). *Anal.* Calcd. for $C_{47}H_{82}O_2$: C, 83.12; H, 12.17. Found: C, 83.22; H, 12.05.

The less polar substance (10 mg) dissolved in tetrahydrofuran (THF) (0.5 ml) was hydrolyzed with 5% KOH–MeOH (2 ml) by refluxing for 4 h. After neutralization with 5% HCl, the mixture was concentrated *in vacuo* and extracted with $CHCl_3$. The product was separated by preparative TLC with MeOH–$CHCl_3$ (1 : 99) to give a free sterol (5 mg) and a mixture of carboxylic acids (3 mg).

The acidic fraction was esterified with diazomethane in ether. The product was proved to be composed of methyl palmitate, methyl heptadecyclate, methyl stearate, methyl arachidate, and methyl behenate (intensity ratio 84 : 2 : 6 : 2 : 3) by GC (3% OV-1 column) and GC-MS analyses. On the other hand, the sterol moiety was identified as sitosterol (**1**)

(over 93%) by GC (2% OV-17 column) and GC-MS analyses.

The more polar substance (**5**) (10 mg) was hydrolyzed in the same manner as above to give a free sterol (5 mg) and a fatty acid (3 mg). The sterol moiety was found to be identical with sitosterol (**1**) by GC and GC-MS analyses. The acidic moiety was derived to a methyl ester, which was identified as methyl oleate by GC-MS analysis.

Identification of Free Sterols On standing, fraction 3 (600 mg) yielded a crystalline mass which was recrystallized from acetone–methanol to afford colorless plates (12 mg). IR ν_{max} cm^{-1}: 3600 (OH). GC and GC-MS analyses (2% OV-17 column) of this substance revealed the presence of sitosterol (**1**), stigmasterol (**2**), and campesterol (**3**) (intensity ratio 80 : 16 : 4).

Identification of Palmitoyl Glucosyl Sitosterol (6) Fraction 5 (300 mg) was purified by repeated preparative TLC with MeOH–$CHCl_3$ (1 : 9) to yield palmitoyl glucosyl sitosterol (**6**) (60 mg), slightly yellow grease. $[\alpha]_D$ −47.7° (c=0.4, $CHCl_3$). IR ν_{max} cm^{-1}: 3595—3399 (OH), 1733 (CO). ^{1}H-NMR ($CDCl_3$) δ_H: 0.68 (3H, s, 18-H_3), 0.81 (3H, d, J=7.0 Hz, 27- or 26-H_3), 0.84 (3H, d, J=7.3 Hz, 26- or 27-H_3), 0.85 (3H, t, J=7.3 Hz, 29-H_3), 0.88 (3H, t, J=6.4 Hz, 16″-H_3), 0.92 (3H, d, J=6.7 Hz, 21-H_3), 1.01 (3H, s, 19-H_3), 2.35 (2H, t, J=7.6 Hz, 2″-H_2), 3.36 (1H, t, J=8.0 Hz, 2′-H), 3.38 (1H, t, J=9.0 Hz, 4′-H), 3.46 (1H, m, 3-H), 3.56 (1H, m, 5′-H), 3.57 (1H, t, J=9.0 Hz, 3′-H), 4.26 (1H, dd, J=12.2, 2.0 Hz, 6′-H), 4.38 (1H, d, J=8.0 Hz, 1′-H), 4.49 (1H, dd, J=12.2, 4.9 Hz, 6′-H), 5.36 (1H, br d, J=5.0 Hz, 6-H). *Anal.* Calcd for $C_{51}H_{90}O_7 \cdot 1/2H_2O$: C, 75.13; H, 11.13. Found: C, 75.59; H, 11.40.

Acetylation of Palmitoyl Glucosyl Sitosterol (6) A mixture of **6** (4.5 mg), acetic anhydride (0.2 ml) and pyridine (0.2 ml) was left to stand at room temperature for 45 h. The reaction mixture was worked up in the usual manner to give an amorphous substance, which was purified by preparative TLC with MeOH–$CHCl_3$ (0.5 : 99.5) to afford a triacetate (**7**) (4 mg). IR ν_{max} cm^{-1}: 1755 (CO). ^{1}H-NMR ($CDCl_3$) δ_H: 0.67 (3H, s, 18-H_3), 0.81 (3H, d, J=7.0 Hz, 27- or 26-H_3), 0.83 (3H, d, J=7.0 Hz, 26- or 27-H_3), 0.84 (3H, t, J=7.5 Hz, 29-H_3), 0.88 (3H, t, J=6.4 Hz, 16″-H_3), 0.92 (3H, d, J=6.7 Hz, 21-H_3), 0.99 (3H, s, 19-H_3), 2.00 (3H, s, -OCOCH_3), 2.02 (3H, s, -OCOCH_3), 2.05 (3H, s, -OCOCH_3), 2.32 (2H, t, J=7.5 Hz, 2″-H_2), 3.47 (1H, m, 3-H), 3.68 (1H, m, 5′-H), 4.13 (1H, dd, J=12.0, 2.5 Hz, 6′-H), 4.23 (1H, dd, J=12.0, 5.0 Hz, 6′-H), 4.59 (1H, d, J=8.0 Hz, 1′-H), 4.95 (1H, dd, J=12.0, 8.0 Hz, 2′-H), 5.05 (1H, t, J=12.0 Hz, 4′-H), 5.20 (1H, t, J=12.0 Hz, 3′-H), 5.35 (1H, m, 6-H).

Alkaline Hydrolysis of Palmitoyl Glucosyl Sitosterol (6) A solution of **6** (2 mg) in 5% KOH–MeOH (1 ml) was refluxed for 2 h. The reaction mixture was concentrated *in vacuo* and the residue was diluted with water, then extracted with $CHCl_3$, dried, and evaporated. The residue was methylated with diazomethane in ether. The resulting material was proved to be methyl palmitate by GC (3% OV-1 column) and GC-MS analyses.

Treatment of Glycerides Glycerol ester mixture was obtained as a pale yellow oil (1.5 g). IR ν_{max} cm^{-1}: 1736 (CO). ^{1}H-NMR ($CDCl_3$) δ_H: 0.88 (9H, t, J=6.7 Hz, $CH_3 \times 3$), 2.31 (4H, t, J=7.5 Hz, -OCOCH_2), 2.32 (2H, t, J=7.3 Hz, -OCOCH_2), 4.14 (2H, dd, J=11.9, 6.0 Hz, 1-H and 3-H), 4.29 (2H, dd, J=11.9, 4.5 Hz, 1-H and 3-H), 5.26 (1H, tt, J=6.0, 4.5 Hz, 2-H), 5.35 (about 2H, m, vinyl protons).

A portion of the glycerol ester mixture (1 mg) was refluxed with 5% KOH–MeOH for 3 h, and then neutralized with 5% HCl. After evaporation to dryness, a solution of CH_2N_2 in Et_2O was added to the crude residue. Concentration of the ether solution gave a mixture of methyl esters. GC-MS analysis (3% OV-1 column) revealed the presence of methyl palmitate, methyl oleate, methyl stearate, methyl arachidate, and methyl behenate in a ratio of about 16.6 : 20.0 : 6.1 : 1.5 : 5.5, respectively.

Isolation of Boeravinones A (9), B (10), and C Fraction 4 (1.1 g), obtained from the silica gel column chromatography of the ethereal neutral fraction, afforded an amorphous yellow precipitate from MeOH–CH_2Cl_2 (5 : 95). This was separated by filtration to give boeravinone B (**10**) (41 mg). On the other hand, the MeOH–CH_2Cl_2 solution was concentrated to dryness and the residue was separated by repeated preparative TLC with MeOH–$CHCl_3$ (5 : 95) and with MeOH–$CHCl_3$ (1 : 9) to give two yellow compounds. The less polar compound was recrystallized from MeOH–CH_2Cl_2 to afford boeravinone A (**9**) (125 mg) as yellow needles. The more polar compound was recrystallized from $CHCl_3$ to yield boeravinone C (129 mg) as pale yellow needles.

Boeravinone A (9) Yellow needles from MeOH–CH_2Cl_2, mp 215—217 °C, $[\alpha]_D$ 0° (c=0.13, acetone), IR ν_{max} cm^{-1}: 3600, 3250 (OH), 1650 (conj. CO), 1620 (C=C), 1595, 1495 (phenyl). UV λ_{max} nm (log ε): 217 (4.39), 276 (4.37), 300$_{sh}$ (4.07), 340$_{sh}$ (3.39); UV λ_{max} (2 drops of 3 N NaOH added) nm (log ε): 219 (4.96), 291 (4.56), 348 (3.99), 354$_{sh}$ (3.96). ^{1}H- and ^{13}C-NMR: see Table I. MS m/z: 326 (M^+), 295 (base peak, $M^+ - OCH_3$), 282, and 267 ($M^+ - OCH_3 - CO$). High-resolution MS m/z: Found 326.0757, Calcd for $C_{18}H_{14}O_6$ (M^+) 326.0790; Found 295.0584, Calcd for $C_{17}H_{11}O_5$ 295.0606; Found 282.0541, Calcd for $C_{16}H_{10}O_5$ 282.0529; Found 267.0645, Calcd for $C_{16}H_{11}O_4$ 267.0656.

Boeravinone A Methyl Ether (11) A solution of CH_2N_2 in ether was added to a solution of **9** (4 mg) in a small amount of MeOH and the mixture was allowed to stand for 40 h at room temperature. After evaporation of the solvent, the residue was purified by preparative TLC and then recrystallized from MeOH–CH_2Cl_2 to afford a monomethyl ether (**11**) as yellow needles (3 mg), mp 208—210 °C. IR ν_{max} cm^{-1}: 3400 (OH), 1655 (conjugated CO), 1590, 1495 (phenyl). UV λ_{max} nm (log ε): 217.5 (3.96), 275.5 (3.96), 309$_{sh}$ (3.69), 328.5$_{sh}$ (3.13). ^{1}H-NMR: see Table I. MS m/z: 340 (M^+), 309 ($M^+ - OCH_3$, base peak), 281 ($M^+ - OCH_3 - CO$), 266. High-resolution MS m/z: Found 340.0923, Calcd for $C_{19}H_{16}O_6$ (M^+) 340.0946; Found 309.0767, Calcd for $C_{18}H_{13}O_5$ 309.0763.

Boeravinone B (10) Yellow amorphous powder, $[\alpha]_D$ 0° (c=1, acetone). IR ν_{max} cm^{-1} (KBr): 3250 (OH), 1650 (conjugated CO), 1620 (C=C), 1595, 1495 (phenyl). UV λ_{max} nm (log ε): 217 (4.41), 273 (4.40), 300$_{sh}$ (4.13), 345$_{sh}$ (3.61); UV λ_{max} (2 drops of 3 N NaOH added) (log ε): 214 (4.81), 252 (4.16), 284.5 (4.51), 295$_{sh}$ (4.39), 343 (4.09). ^{1}H- and ^{13}C-NMR: see Table I. MS m/z: 312 (M^+), 295 ($M^+ - OH$), 283 ($M^+ - CHO$, base peak), 267 ($M^+ - OH - CO$). High-resolution MS m/z: Found 312.0645, Calcd for $C_{17}H_{12}O_6$ (M^+) 312.0634; Found 295.0623, Calcd for $C_{17}H_{11}O_5$ 295.0607.

Boeravinone B Methyl Acetal (9) A mixture of **10** (2.8 mg), absolute MeOH (4 ml), and *p*-TsOH (1 mg) was refluxed for 3 h. After neutralization with saturated Na_2CO_3 and evaporation of MeOH, the mixture was extracted with ether. The combined ether solutions were dried and concentrated. The residue was purified by preparative TLC (developed with MeOH–benzene, 1 : 9) and then recrystallized from MeOH–CH_2Cl_2 to give a methyl acetal (**9**) (2.7 mg), yellow needles, mp 207—209 °C. UV λ_{max} nm (log ε): 219 (4.16), 276 (4.21), 299$_{sh}$ (3.97), 338$_{sh}$ (3.45). The IR and ^{1}H-NMR spectra of this compound were superimposable on those of boeravinone A (**9**).

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References and Notes

1) A preliminary report of a part of this work has appeared: S. Kadota, N. Lami, Y. Tezuka, and T. Kikuchi, *Chem. Pharm. Bull.*, **36**, 834 (1988).
2) T. Namba (ed.), "Medicinal Resources and Ethnopharmacology in Sri Lanka and Nepal," Research Institute for Wakan-Yaku (Oriental Medicines), Toyama Medical and Pharmaceutical University, Toyama, 1983, pp. 20, 277, and 279.
3) A. K. Nadkarni, "Dr. K. M. Nadkarni's Indian Materia Medica," Vol. 1, A. K. Nadkarni Popular Prakashan Pvt. Ltd., Bombay, 1976, pp. 203—205.
4) J. O. Kokwaro, "Medicinal Plants of East Africa," General Printers Ltd., Nairobi, 1976, pp. 276—280.
5) J. M. Janzen, "The Quest of Therapy in Lower Zaire," University of California Press, Los Angeles, 1978, p. 246.
6) A. N. Misra and H. P. Tiwari, *Phytochemistry*, **10**, 3318 (1971).
7) H. N. Verna, L. P. Awasthi, and K. C. Saxena, *Can. J. Bot.*, **57**, 1214 (1979).
8) Suri O. P., R. Kant, R. S. Jamwal, K. A. Suri, and C. K. Atal, *Planta Medica*, **44**, 180 (1982).
9) D. R. Gupta and A. Bahar, *Indian J. Chem. Sect. B*, **23B**, 682 (1984).
10) A preliminary report of the structure elucidation of boeravinone C has appeared in *Chem. Pharm. Bull.*, **36**, 2289 (1988).
11) A. Bax, "2D NMR in Liquids," D. Reidel Publishing Co., Dordrecht, Holland, 1982, pp. 155—174; D. L. Turner, *J. Magn. Res.*, **49**, 175 (1982); *idem*, *ibid.*, **53**, 529 (1983).
12) C. Francisco, B. Banaigs, and J. Teste, *J. Org. Chem.*, **51**, 1115 (1986).
13) M. Sugita, T. Sasaki, K. Furihata, H. Seto, and N. Otake, *J. Antibiot.*, **35**, 1467 (1982); J. C. Christofides and D. B. Davies, *J. Chem. Soc., Chem. Commun.*, **1983**, 324.
14) The possibility that boeravinone A (**9**) is an artifact was excluded, because **9** was detected in the ether extract of the plant material by TLC.
15) D. M. Piatak, G. A. Flynn, and P. D. Sorensen, *Phytochemistry* **14**,

1392 (1975).
16) J. B. Harborne and T. J. Mabry (ed.), "The Flavonoids: Advances in Research," Chapman and Hall, London, 1982, pp. 564—576; J. B. Harborne (ed.), "The Flavonoids, Advances in Research Since 1980," Chapman and Hall, London, 1988, pp. 152—157.
17) H. Fukami and M. Nakazima, "Naturally Occurring Insecticide," ed. by M. Jacobson and D. G. Crosby, Marcel Dekker, Inc., New York, 1971, p. 71; T. J. Haley, *J. Environ. Pathol. Toxicol.*, **1**, 315 (1978).
18) J. Burgos and E. R. Redfearn, *Biochim. Biophys. Acta*, **110**, 475 (1965); M. Gosalvez, J. Diaz-Gil, J. Coloma, and L. Salganicoff, *Br. J. Cancer*, **36**, 243 (1977); A. M. Johnson-Flanagan and M. S. Spencer, *Plant Physiol.*, **68**, 1211 (1981). Also, *Mundulea sericea* (Leguminosae), which contains several rotenoids, has been reported to have a crocodile repellent activity; see N. Finch and W. D. Ollis, *Proc. Chem. Soc.*, **1960**, 176.
19) I. Messana, F. Ferrari, and A. E. Goulart Sant'Ana, *Phytochemistry*, **25**, 2688 (1986).
20) J. Crombie, *Natural Product Reports*, **1**, 3 (1984).