Crotalionosides A—C, Three New Megastigmane Glucosides, Two New Pterocarpan Glucosides and a Chalcone *C*-Glucoside from the Whole Plants of *Crotalaria zanzibarica*

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From a 1-BuOH-soluble fraction of the MeOH extract of the leaves of *Crotalaria zanzibarica* collected in the Okinawa Islands, three new megastigmane glucosides, named crotalionosides A—C, two new pterocarpan glucosides and a chalcone *C*-glucoside were isolated together with two known flavonoid glycosides and one known megastigmane glucoside. The structures of the new compounds were elucidated by a combination of spectroscopic analyses. The absolute configurations of allenic megastigmane glucosides were determined by application of the modified Mosher's method. Those of the allenic moieties were determined by interpretation of the circular dichroism (CD) spectra of the reduction products derived from citrosides A and B. The aglycones of pterocarpan glucosides were found to be melilotocarpan B and the absolute structure of the chalcone *C*-glucoside was determined by comparison of the CD spectral behavior with the reported values.

Key words *Crotalaria zanzibarica*; Leguminosae; megastigmane glucoside; crotalionoside; pterocarpan glucoside; chalcone *C*-glucoside

The *Crotalaria* genus comprises about 350 species, which are mainly found in tropical areas. *C. zanzibarica* BENTHAM (Leguminosae)[1] (syn. *C. usaramoensis* BAKER f.)[2] is a perennial shrubby herb and was introduced to the Okinawa Islands from Eastern Africa as a green manure crop. These days, this plant is widely found in the Okinawa Islands. It grows up to about 1.5 m in height and bears yellow flowers all year. Some pyrrolidine alkaloids, usaramine,[3] and usaramoensine,[4] have been isolated from *C. usaramoensis* (synonym of *C. zanzibarica*). A related pyrrolidine alkaloid, integerrimine, has also been isolated from *C. incana*,[4] and monocrotaline and trichodesmine from *C. sessiliflora*.[5] From a 1-BuOH-soluble fraction, new megastigmane glucosides, named crotalionosides A—C (**1**—**3**), two pterocarpane glucosides (**4**, **5**), and a chalcone *C*-glucoside (**6**) were isolated, together with three known compounds, 3-hydroxy-5,6-epoxy-β-ionol 9-*O*-β-D-glucopyranoside (**7**),[6] kaempferol 3-*O*-robinoside (**8**),[7] and robinin (**9**).[8] This paper deals with structural elucidation of the new compounds and stereochemical discussion of allene-possessing megastigmane aglycones.

Results and Discussion

Air-dried whole plants of *C. zanzibarica* were extracted with MeOH three times and the concentrated MeOH extract was partitioned with solvents of increasing polarity. The 1-BuOH-soluble fraction was separated by means of various chromatographic procedures including column chromatography (CC) on a highly porous synthetic resin (Diaion HP-20), and then normal silica gel and reversed-phase octadecyl silica gel (ODS) CC, droplet counter-current chromatography (DCCC), and high-performance liquid chromatography (HPLC), which afforded nine compounds (**1**—**9**). The details and yields are given under Experimental. The structures of the new megastigmane glucosides, crotalionosides A—C (**1**—**3**), pterocarpane glucosides (**4**, **5**), and chalcone *C*-glucoside (**6**) were elucidated on the basis of spectroscopic evidence, and those of the known compounds (**7**—**9**) were identified by comparison of spectroscopic data with those reported in the literature.

Crotalionoside A (**1**), $[\alpha]_D^{21}$ −18.9°, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{32}O_9$ by high resolution (HR)-electrospray ionization (ESI)-mass spectrometry (MS). The IR spectrum indicated the presence of hydroxyl (3367 cm^{-1}) and allenic groups (1959 cm^{-1}). The prominent ^{1}H-NMR resonances were assigned to three singlet methyls, one doublet methyl, one set of methylene protons [δ_H 1.38 (dd, J=12, 12 Hz) and 1.83 (dd, J=12, 5 Hz)], three oxymethine protons, one olefinic proton (δ_H 5.35), and an anomeric proton. In the ^{13}C-NMR spectrum, resonances for four methyls, one methylene, three oxymethines, two quaternary carbons and three olefinic carbons attributable to an allenic unit along with six signals assignable to a glucopyranose moiety were observed (Table 1). The above evidence indicates that crotalionoside A (**1**) is a derivative of megastigmane glucoside with an allenic unit and three hydoxyl groups, as shown in Fig. 1. Two proton sequences, –CH_2–CHO(H)–CHO(H)– and =CH–CHO(H)–CH_3, were observed in the ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum, and the protons involed were correlated with the carbon signals in the heteronuclear single quantum coherence (HSQC) experiment. The heteronuclear multiple bond connectivity (HMBC) correlations from methyl protons on two *gem*-dimethyl groups with a methylene carbon placed the methylene carbon at the 2-position, and since the anomeric proton (δ_H 4.62) showed a cross peak with one of the oxymethine carbons and the oxymethine proton (δ_H 3.26) on the oxymethine carbon (δ_C 91.7) showed a cross peak with the adjacent oxymethine proton (δ_H 4.22) (Fig. 2), the glucosidic linkage was placed on the hydroxyl group at the

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Table 1. ^{13}C-NMR Spectroscopic Data for Compounds **1**, **2** and **3** (CD_3OD, 100 MHz)

C	1	2	3
1	35.5	35.6	44.5
2	47.6	47.7	49.4
3	69.5	69.5	76.8
4	91.7	91.7	48.7
5	74.9	74.9	82.3
6	117.4	117.5	92.8
7	201.0	200.9	126.7
8	100.5	100.5	134.3
9	67.2	67.2	78.1
10	23.6	23.6	21.4
11	29.4	29.4	25.9
12	32.9	32.7	32.8
13	27.1	27.2	31.6
1′	106.1	106.1	102.9
2′	76.2	76.2	75.5
3′	78.3	78.3	78.2
4′	71.6	71.6	71.5
5′	78.2	78.1	78.0
6′	62.8	62.8	62.8

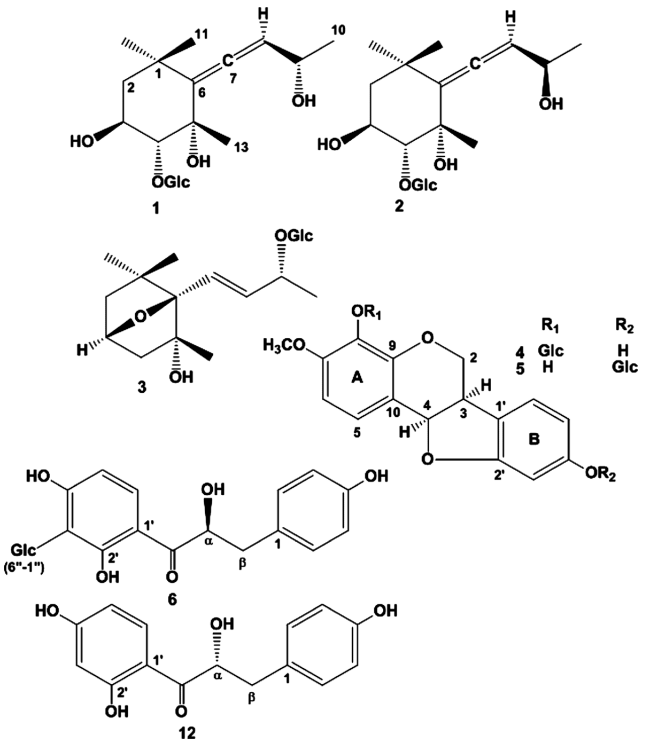

Fig. 1. Structures

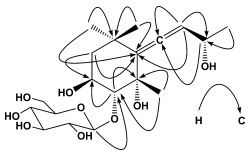

Fig. 2. HMBC Correlations of **1**

4-position. Thus, the planar structure of crotalionoside A (**1**) was elucidated to be megastima-6,7-diene-3,4,5,9-tetrol 4-*O*-glucopyranoside (Fig. 1). The proton–proton coupling constant (J=9 Hz) of H-3 and H-4 indicated that these protons were in axial positions, and the significant correlation between H-4 and H_3-13 observed in the phase-sensitive (PS)-nuclear Overhauser effect spectroscopy (NOESY) spectrum revealed that these protons were on the same side. Enzymatic hydrolysis of crotalionoside A (**1**) gave an aglycone (**1a**) and glucose, which was found to be of the D-series from its positive optical rotation sign, and thus the mode of the glucosidic linkage was determined to be β from the coupling constant of the anomeric proton (J=8 Hz). To the aglycone (**1a**), the modified Mosher's method was applied.[9] (*R*)- and (*S*)-α-Methoxy-α-trifluoromethylphenyacetic acid (MTPA) derivatives (**1b**, **1c**) were prepared from the aglycone (**1a**) using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and *N*,*N*-dimethyl-4-aminopyridine (4-DMAP), which afforded 3,9-diesters. As a result, the absolute configurations at the 3- and 9-positions were determined to 3*S* and 9*S*, respectively (Fig. 3, **1b**, **1c**). In turn, those at the 4- and 5-positions were *R* and *S*, respectively. The remaining stereochemical issue of the axis chirality in the allene part will be discussed later.

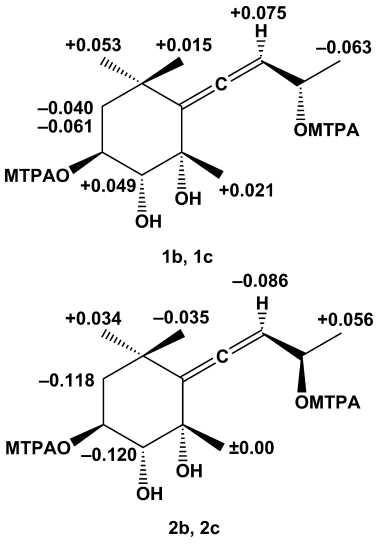

Fig. 3. Results of Modified Mosher's Method ($\Delta_{\delta S-\delta R}$)

Crotalionoside B (**2**), $[\alpha]_D^{27}$ −12.1°, was isolated as an amorphous powder and its elemental compsotion was found to be the same as that of crotalionoside A (**1**). The physicochemical properties of crotalionoside B (**2**) were almost identical to those of **1** (Table 1), except for the optical rotation value. Since crotalionosides A and B (**1**, **2**) exhibited different retention times in the same HPLC run, they were unambiguously different compounds. As there was a probable chiral probe, β-D-glucopyranose, at the hydroxyl group of the ring moiety, the stereochemistry of **2** must be the same as that of **1**, although the result of the modified Mosher's method, applied to its aglycone, did not show a regular distribution of signs. To clarify the stereochemsitry of the side chain, the aglycone (**2a**) of crotalionoside B (**2**), obtained by enzymatic hydrolysis, was analyzed by the modified Mosher's method, as shown in Fig. 3 (**2b**, **2c**). The absolute configuration at the 9-position was revealed to be *R*, which was opposite to that of crotalionoside A (**1**).

Compounds structurally related to crotalionosides A and B (**1**, **2**), citrosides A (**10**) and B (**11**), were first isolated from *Citrus unshiu*, and they are epimers as to the allene axis, 7*R* and 7*S*, respectively.[10] Citrosides A (**10**) and B (**11**),

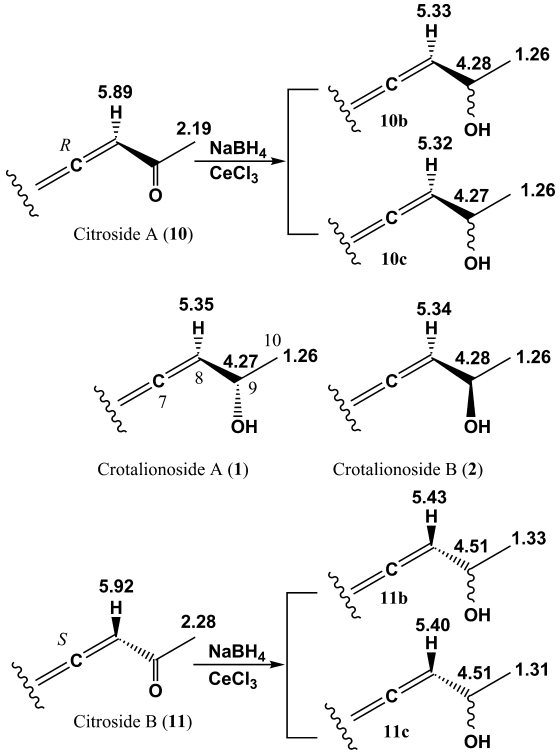

Fig. 4. Partial ^{1}H-NMR Chemical Shifts for **10**, **10b**, **10c**, **11**, **11b** and **11c**

used in this experiment, were independently obtained from *Elaeocarpus japonicus* SIEBOLD *et* ZUCCARINI[11] and *Sambucus chinensis* LINDLEY,[12] respectively. Citrosides A (**10**) and B (**11**) were reduced with $NaBH_4$ in the presence of $CeCl_3$ to give the corresponding alcohols (**10a**, **11a**).[13] In the circular dichroism (CD) spectra, an positive Cotton effect at 225 nm was observed for both alcohols, and the absorption position and sign of the Cotton effect were also the same as those of crotalionosides A (**1**) and B (**2**). Thus, CD spectral analyses were not effective for determining the absolute configuration of the allene axis. The alcohols (**10a**, **11a**) comprised *R* and *S* mixtures as to the 9-position and they were separated by HPLC, which yielded pure compounds **10b** and **10c**, and **11b** and **11c**, of which the absolute configurations at the 9-position remain uncertain. Figure 4 shows the ^{1}H-NMR spectral data for **10b** and **10c**, and **11b** and **11c**, the chemical shifts for H-8, 9 and 10 for 7*R* compounds being essentially the same for **10b** and **10c**, and those for 7*S* compounds being different from those for 7*R* compounds, but also essentially the same for **11b** and **11c**. These different chemical shifts solely depended upon the configurations at the 7-postition, that is, not upon those at the 9-positions. However, since determination of axis configurations of citrosides A (**10**) and B (**11**) still has some ambiguity,[10,14] the absolute configurations at the 7-positions of crotalionosides A (**1**) and B (**2**) were concluded to be the same as that of citroside A and tentatively assigned as 7*R*. Therefore, the structures of crotalionosides A (**1**) and B (**2**) were elucidated to be (3*S*,4*R*,5*S*,7*R*,9*S*)- and (3*S*,4*R*,5*S*,7*R*,9*R*)-megastigma-6,7-diene-3,4,5,9-tetrol 4-*O*-β-D-glucopyranosides, respectively, as shown in Fig. 1.

Crotalionoside C (**3**), $[\alpha]_D^{25}$ +15.8°, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{32}O_8$. The ^{13}C-NMR spectrum indicated the presence of 19 resonances, including six assignable to glucopyranose, and four methyl, two methylene, one *trans* double bond, two oxymethine, two oxygenated quarternary carbon and one quarternary carbon signals. The ^{1}H–^{1}H COSY spectrum exhibited two proton sequences, that is, A: –C(2)H_2–C(3)HO(H)–C(4)H_2– and B: –C(7)H=C(8)H–CH(9)O(H)–C(10)H_3. In the HMBC spectrum, geminal methyl proton signals showed long-range correlation with a methylene carbon, A: C(2) and an oxygen-bearing quaternary carbon (δ_C 92.8), to which two olefinic protons (δ_H 5.78, 5.80) also correlated (Fig. 5). From the degree of unsaturation, calculated from the elemental composition, other than the six-membered ring, crotalionoside C (**3**) was expected to possess one more cyclic system, whose presence was substantiated by the HMBC correlation cross peak between H-3 (δ_H 4.33) and C-6 (δ_C 92.8). The position of the sugar linkage was revealed to be at the hydroxyl group on the side chain by the HMBC spectrum and the mode of the linkage was determined to be β from the coupling constant of the anomeric proton (δ_H 4.36, J=8 Hz), together with the results of chirality analysis of glucose. From the above evidence, the structure of crotalionoside C (**3**) was assigned as megastagman-7-en-3,6-epoxy-5,9-diol 9-*O*-β-D-glucopyranoside. Enzymatic hydrolysis of **3** gave an aglycone, crotalionol C (**3a**), and application of the modified Mosher's method to **3a** established the absolute configuration at the 9-position to be *R* (Fig. 6). However, the ring region lacks a clue for determing the absolute configuration. The PS-NOESY correlation between H_3-13 (δ_H 1.19) and H-4eq (δ_H 1.95) revealed that the epoxy ring and H_3-13 were on the same side, namely the ring has a 3*S**,5*R**,6*R** relative configuration, as shown in Fig. 1.

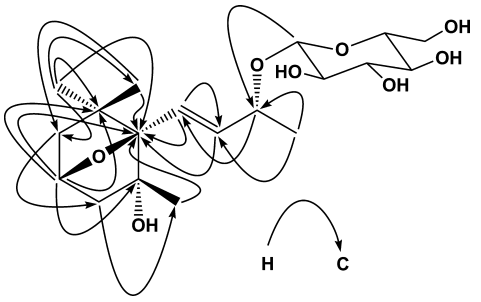

Fig. 5. HMBC Correlations of **3**

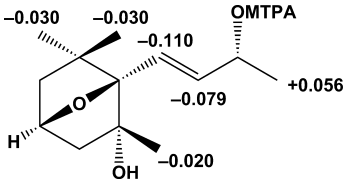

Fig. 6. Results of Modified Mosher's Method ($\Delta_{\delta S-\delta R}$)

Compound **4**, $[\alpha]_D$ −65.6°, was isolated as an amorphous powder and its elemental composition was determined to be $C_{22}H_{24}O_{10}$ by HR-ESI-MS. The IR spectrum indicated that compound **4** possessed aromatic rings (1615, 1505, 1470 cm^{-1}) with a glycosidic moiety (3388 cm^{-1}), the presence of the aromatic rings being also supported by the UV absorption at 282 nm. In the ^{1}H-NMR spectrum, four

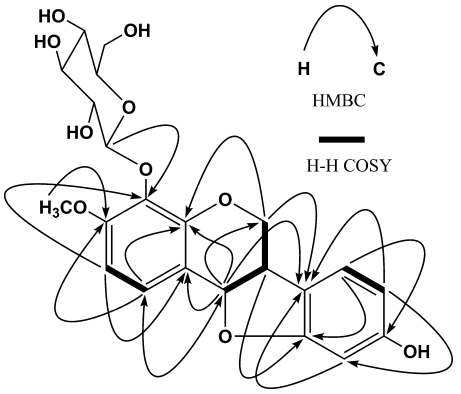

Fig. 7. H–H COSY and HMBC Correlations of **4**

aliphatic proton signals, two aromatic proton signals coupled in an AB doublet system [δ_H 6.78 (d, J=9 Hz) and 7.24 (d, J=9 Hz)], three aromatic proton signals coupled in an ABX system [δ_H 6.24 (d, J=2 Hz), 6.33 (dd, J=8, 2 Hz), and 7.09 (d, J=8 Hz)], one methoxy signal and an anomeric proton signal [δ_H 4.90 (d, J=8 Hz)] were observed. The ^{13}C-NMR spectrum exhibited 22 resonances, including six signals assignable to glucopyranose, 12 aromatic signals, and one methoxyl, one oxymethylene, one oxymethine and one methine signal. A sequence of oxymethylene, methine and oxymethine protons was observed in the ^{1}H–^{1}H COSY spectrum (Fig. 7). One of the aromatic rings was substituted with three oxygen atoms, and these substituted carbon atoms were correlated with methoxy, anomeric and oxymethylene protons in the HMBC spectrum. On irradiation of methoxyl protons, one (δ_H 6.78) of the aromatic protons was signifcantly enhanced in the difference NOE experiment and this proton showed cross peaks with δ_C 135.4 (C-8) and 116.6 (C-10). The HMBC correlation from the oxymethine proton to C-10 and other correlations shown in Fig. 7 revealed the structure of ring A. While the other trisubstituted aromatic ring possessed two oxygen atoms, and HMBC correlation cross peaks between oxymethylene protons and the quartenary carbon, δ_C 119.2, methine proton and oxygen bearing quartenary carbon, δ_C 160.0, together with other correlations suggested the structure of around B ring was as shown in Fig. 1. The above evidence substantiated that compound **4** was a 8-*O*-glucoside of pterocarpan, melilotocarpan B, isolated from *Melilotus alba*.[15] On enzymatic hydrolysis of **4**, melilotocarpan B (**4a**) was obtained. The optical rotation value and sign of **4a** showed good accordance with those reported in the literature.[15] Thus, compound **4** was concluded to have the 3*R*,4*R* configuration. The negative Cotton effect at 234 nm observed in the CD spectrum also supported the configuration. Sugar analysis, using a chiral detector, revealed that the glucose was of the D-series and the coupling constant (J=8 Hz) of the anomeric proton established the mode of sugar linkage was β.

Compound **5**, $[\alpha]_D$ −94.1°, was isolated as an amorphous powder and its elemental composition was found to be the same as that of compound **4**. Other spectroscopic data were similar to those for compound **4**. In the HMBC spectrum, the anomeric proton (δ_H 4.84) showed a cross peak with δ_C 160.4, to which was also correlated the aromatic proton, H-5′ [δ_H 6.64 (dd, J=8, 2 Hz)]. Sugar analysis also revealed that glucose was of the D-series. Thus, the structure of compound **5** was elucidated to be melilotocarpan 4′-*O*-β-D-glucopyranoside.

Compound **6**, $[\alpha]_D$ +61.5°, was isolated as an amorphous powder and its elemental composition was determined to be $C_{21}H_{24}O_{10}$. The ^{1}H- and ^{13}C-NMR spectroscopic data were essentially the same as those for coatline A, which was isolated from *Elysenhardtia polystachya* by Beltrami *et al.*[16] Although Beltrami *et al.* did not disscuss the stereochemistry of coatline A, compound **6** was expected to be a stereochemical isomer of coatline A ($[\alpha]_D$ −45.2), since their optical rotation signs and values were opposite to each other. Thus, they are diastereomers, however, their NMR spectra were identical due to the remoteness of the chiral centers, such as the sugar moiety and α-carbon. Augustyn *et al.* examined the CD spectra of enantioselectively synthesized α-hydroxydihydrochalcones.[17] A similar compound, (αR)-α,2′4′-trihydroxy-4-methoxydihydrochalcone, (**12**) exhibited negative Cotton effect at 313 nm ($[\theta]$ −12000) and a positive one at 245 nm ($[\theta]$ +4000), which were opposite to those of compound **6**. Thus, compound **6** has the α-*S* configuration and coatline A α-*R*. This conclusion is in a good accord with that made by Alvarez and Delgado on determination of the absolute configuration of related α-hydroxydihydrochalcone.[18]

Experimental

General Experimental Procedures Mp was measured with a Yanagimoto micromelting point apparatus and is uncorrected. Optical rotations and CD spectra were measured on JASCO P-1030 and JASCO J-720 polarimeters, respectively. IR and UV spectra were measured on Horiba FT-710 and JASCO V-520 UV/Vis spectrophotometers, respectively. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL JNM α-400 spectrometer at 400 MHz and 100 MHz, respectively, with tetramethylsilane as an internal standard. Positive-ion HR-MS were performed with an Applied Biosystem QSTAR XL system ESI-time-of-flight (TOF)-MS.

A highly-porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel CC and reversed-phase [octadecyl silica gel (ODS)] open CC were performed on silica gel 60 (Merck, Darmstadt, Germany) and Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) [Φ=50 mm, L=25 cm, linear gradient: MeOH–H_2O (1 : 9, 1 l)→(1 : 1, 1 l), fractions of 10 g being collected], respectively. Droplet counter-current chromatography (DCCC) (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns (Φ=2 mm, L=40 cm), and the lower and upper layers of a solvent mixture of $CHCl_3$–MeOH–H_2O–*n*-PrOH (9 : 12 : 8 : 2) were used for the stationary and mobile phases, respectively. Five-gram fractions were collected and numbered according to their order of elution with the mobile phase. HPLC was performed on an ODS column (Inertsil; GL Science, Tokyo, Japan; Φ=6 mm, L=25 cm), and the eluate was monitored with a UV detector at 254 nm and a refractive index monitor.

Emulsin was purchased from Tokyo Chemical Industries Co., Ltd. (Tokyo, Japan), and crude hesperidinase was a gift from Tokyo Tanabe Pharmaceutical Co., Ltd. (Tokyo, Japan). (*R*)- and (*S*)-α-MTPAs were the products of Wako Pure Chemical Industry Co., Ltd. (Tokyo, Japan).

Plant Material Whole plants of *C. zanzibarica* Bentham (Leguminosae) were collected in Kunigami-son, Kunigami-gun, Okinawa, Japan, in July 2003, and a voucher specimen was deposited in the Herbarium of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Hiroshima University (03-CZ-Okinawa-0630).

Extraction and Isolation Dried aerial parts of *C. zanzibarica* (18.9 kg) were extracted three times with MeOH (45 l) at 25 °C for one week and then concentrated to 6 l *in vacuo*. The extract was washed with *n*-hexane (6 l, 167 g) and then the MeOH layer was concentrated to a gummy mass. The latter was suspended in water (6 l) and then extracted with EtOAc (6 l) to give 260 g of an EtOAc-soluble fraction. The aqueous layer was extracted with 1-BuOH (6 l) to give a 1-BuOH-soluble fraction (228 g), and the remaining water-layer was concentrated to furnish 1.21 kg of a water-soluble fraction.

A portion (96.8 g) of the 1-BuOH-soluble fraction was subjected to a Diaion HP-20 column (Φ=60 mm, L=50 cm) using H_2O–MeOH (4 : 1, 4 l),

(2 : 3, 4 l), (3 : 2, 4 l), and (1 : 4, 4 l), and MeOH (4 l), 1 l fractions were being collected. The residue (15.4 g in fractions 3—6) of the 20—40% MeOH eluent was subjected to silica gel (500 g) CC (Φ=50 mm, L=50 cm), with elution with $CHCl_3$–MeOH [(9 : 1, 2 l), (4 : 1, 2 l), and (3 : 1, 2 l)], $CHCl_3$–MeOH–H_2O (35 : 15 : 2, 2 l), and MeOH (2 l), 200 ml fractions being collected. Combined fractions 18—23 (1.02 g) of the 20% MeOH eluate were purified by ODS CC to give 88.4 mg of **7** in fractions 118—124. Combined fractions 24—30 (735 mg) of the 20—30% MeOH eluate were separated by ODS CC to give two fractions (59.1 mg in fractions 122—133 and 32.6 mg in fractions 134—144). Purification of the former by repeated HPLC [MeOH–H_2O (7 : 13) and then (11 : 29)] afforded 0.5 mg of **2**. The latter was purified by HPLC [MeOH–H_2O (2 : 3)] to give 3.7 mg of **6** and 2.7 mg of **1** from the peaks at 6 min and 10 min, respectively. An aliquot (2.5 g) of combined fractions 31—40 (4.02 g) was subjected ODS CC and the residue (89.1 mg) in 139—161 was purified by DCCC. The residue (8.8 mg) in fractions 37—45 was finally purified by HPLC [MeOH–H_2O (2 : 3)], 0.5 mg of **2** and 1.8 mg of **1** being yielded from the peaks at 7.5 min and 9 min, respectively.

The residue (12.7 g) in fractions 7—10, obtained from the 40% MeOH eluate on Diaion HP-20 CC, was separated by silica gel (600 g) CC (Φ=58 mm, L=49 cm), with elution with $CHCl_3$–MeOH [(9 : 1, 3 l), (4 : 1, 3 l), and (3 : 1, 3 l)], $CHCl_3$–MeOH–H_2O [(35 : 15 : 2, 3 l) and (65 : 35 : 4, 3 l), and MeOH (3 l), 300 ml fractions being collected. Combined fractions 9—18 (2.23 g) were purified by ODS CC to give a residue (150 mg in fractions 142—152) and 65.8 mg of **4** in fractions 157—161. The residue was then purified by DCCC to give a residue (17.1 mg in fractions 80—88) and 40.6 mg of **5** in fractions 108—115. The residue was finally purified by HPLC (MeOH–H_2O, 37 : 63) to give 5.3 mg of **3** from the peak at 16 min. Combined fractions 19—26 (2.20 g) were purified by ODS CC to give a residue (384 mg in fractions 75—84) and 68.7 mg of **9**. The residue was separated by DCCC to give 28.8 mg of **6** in fractions 24—27. On partial evaporation of combined fractions 32—46 (5.70 g), 236 mg of **8** was obtained as a yellow precipitate.

Crotalionoside A (**1**): Amorphous powder; $[\alpha]_D^{21}$ −18.9° (c=0.19, MeOH): IR ν_{max} (film) cm^{-1}: 3367, 2926, 1959, 1649, 1454, 1371, 1076, 1029; UV λ_{max} (MeOH) nm (log ε): 208 (3.32); ^{1}H-NMR (CD_3OD, 400 MHz) δ: 5.35 (1H, d, J=6 Hz, H-8), 4.62 (1H, d, J=8 Hz, H-1′), 4.27 (1H, qd, J=6, 6 Hz, H-9), 4.22 (1H, ddd, J=12, 9, 5 Hz, H-3), 3.85 (1H, dd, J=12, 2 Hz, H-6′a), 3.69 (1H, dd, J=12, 5 Hz, H-6′b), 3.39 (1H, m, H-5′), 3.36 (1H, dd, J=9, 9 Hz, H-3′), 3.33 (1H, dd, J=10, 9 Hz, H-4′), 3.29 (1H, dd, J=9, 8 Hz, H-2′), 3.27 (1H, dd, J=10, 9Hz, H-3′), 3.26 (1H, d, J=9 Hz, H-4), 1.83 (1H, dd, J=12, 5 Hz, H-2a), 1.45 (3H, s, H_3-13), 1.38 (1H, dd, J=12, 12 Hz, H-2b), 1.31 (3H, s, H_3-11), 1.26 (3H, d, J=6 Hz, H_3-10), 1.06 (3H, s, H_3-12); ^{13}C-NMR (CD_3OD, 100 MHz): Table 1; CD $\Delta\varepsilon$ (nm): +2.46 (224) (c=3.01×10^{-5} M); HR-ESI-MS (positive-ion mode) m/z: 427.1935 $[M+Na]^+$ (Calcd for $C_{19}H_{32}O_9Na$: 427.1938).

Crotalionoside B (**2**): Amorphous powder; $[\alpha]_D^{27}$ −12.1° (c=0.03, MeOH): IR ν_{max} (film) cm^{-1}: 3363, 2925, 1959, 1453, 1370, 1074, 1034; UV λ_{max} (MeOH) nm (log ε): 209 (3.35); ^{1}H-NMR (CD_3OD, 400 MHz) δ: 5.34 (1H, d, J=6 Hz, H-8), 4.62 (1H, d, J=8 Hz, H-1′), 4.28 (1H, qd, J=6, 6 Hz, H-9), 4.21 (1H, ddd, J=12, 9, 5 Hz, H-3), 3.84 (1H, dd, J=12, 2 Hz, H-6′a), 3.69 (1H, dd, J=12, 5 Hz, H-6′b), 3.39 (1H, m, H-5′), 3.33 (1H, dd, J=10, 9 Hz, H-4′), 3.29 (1H, dd, J=9, 8 Hz, H-2′), 3.27 (1H, dd, J=10, 9 Hz, H-3′), 3.26 (1H, d, J=9 Hz, H-4), 1.83 (1H, dd, J=12, 5 Hz, H-2a), 1.44 (3H, s, H_3-13), 1.38 (1H, dd, J=12, 12 Hz, H-2b), 1.31 (3H, s, H_3-11), 1.26 (3H, d, J=6 Hz, H_3-10), 1.07 (3H, s, H_3-12); ^{13}C-NMR (CD_3OD, 100 MHz): Table 1; CD $\Delta\varepsilon$ (nm): +3.55 (225) (c=2.98×10^{-5} M); HR-ESI-MS (positive-ion mode) m/z: 427.1936 $[M+Na]^+$ (Calcd for $C_{19}H_{32}O_9Na$: 427.1938).

Crotalionoside C (**3**): Amorphous powder; $[\alpha]_D^{25}$ +15.8° (c=0.31, MeOH); IR ν_{max} (film) cm^{-1}: 3395, 2970, 1368, 1305, 1243, 1074, 1036; ^{1}H-NMR (CD_3OD, 400 MHz) δ: 5.80 (1H, d, J=16 Hz, H-7), 5.78 (1H, dd, J=16, 5 Hz, H-8), 4.39 (1H, qd, J=6, 5 Hz, H-9), 4.36 (1H, d, J=8 Hz, H-1′), 4.33 (1H, br dd, J=6, 6 Hz, H-3), 3.81 (1H, dd, J=12, 2 Hz, H-6′a), 3.66 (1H, dd, J=12, 5 Hz, H-6′b), 3.39 (1H, dd, J=9, 9 Hz, H-3′), 3.35 (1H, m, H-5′), 3.32 (1H, dd, J=9, 9 Hz, H-4′), 3.18 (1H, dd, J=9, 8 Hz, H-2′), 1.95 (1H, ddd, J=12, 6, 2 Hz, H-4a), 1.77 (1H, ddd, J=12, 6, 2 Hz, H-2a), 1.64 (1H, dd, J=12, 6 Hz, H-4b), 1.59 (1H, d, J=12 Hz, H-2b), 1.40 (3H, s, H_3-11), 1.30 (3H, d, J=6 Hz, H_3-10), 1.19 (3H, s, H_3-13), 0.58 (3H, s, H_3-12); ^{13}C-NMR (CD_3OD, 100 MHz): Table 1; HR-ESI-MS (positive-ion mode) m/z: 411.1993 $[M+Na]^+$ (Calcd for $C_{19}H_{32}O_8Na$: 411.1989).

Compound **4**: Amorphous powder; $[\alpha]_D^{25}$ −65.6° (c=0.16, MeOH); IR ν_{max} (film) cm^{-1}: 3388, 2897, 1615, 1505, 1470, 1288, 1099, 1027, 800; UV λ_{max} (MeOH) nm (log ε): 282 (3.56), 230sh (3.90), 212 (4.21); ^{1}H-NMR (CD_3OD, 100 MHz) δ: 7.24 (1H, d, J=9 Hz, H-5), 7.09 (1H, d, J=8 Hz, H-6′), 6.78 (1H, d, J=9 Hz, H-6), 6.33 (1H, dd, J=8, 2 Hz, H-5′), 6.24 (1H, d, J=2 Hz, H-3′), 5.51 (1H, d, J=7 Hz, H-4), 4.90 (1H, d, J=8 Hz, H-1″), 4.31 (1H, dd, J=10, 3 Hz, H-2a), 3.87 (3H, s, –OCH_3), 3.76 (1H, dd, J=12, 3 Hz, H-6″a), 3.65 (1H, dd, J=12, 5 Hz, H-6″b), 3.61 (1H, dd, J=10, 10 Hz, H-2b), 3.58 (1H, ddd, J=10, 7, 3 Hz, H-3), 3.47 (1H, dd, J=9, 8 Hz, H-2″), 3.42 (1H, dd, J=10, 9 Hz, H-4″), 3.40 (1H, dd, J=10, 9 Hz, H-3″), 3.19 (1H, m, H-5″); ^{13}C-NMR (CD_3OD, 100 MHz): Table 2; CD $\Delta\varepsilon$ (nm): +4.90 (285), −13.1 (234), −17.2 (213) (c=4.29×10^{-5} M); HR-ESI-MS (positive-ion mode) m/z: 471.1264 $[M+Na]^+$ (Calcd for $C_{22}H_{24}O_{10}Na$: 471.1261).

Compound **5**: Amorphous powder; $[\alpha]_D^{25}$ −94.1° (c=0.19, MeOH); IR ν_{max} (film) cm^{-1}: 3360, 2937, 1620, 1491, 1269, 1100, 1070, 707; UV λ_{max} (MeOH) nm (log ε): 280 (3.61), 233sh (3.92), 213 (4.25); ^{1}H-NMR (CD_3OD, 100 MHz) δ: 7.18 (1H, d, J=8 Hz, H-6′), 6.95 (1H, d, J=9 Hz, H-5), 6.69 (1H, d, J=9 Hz, H-6), 6.64 (1H, dd, J=8, 2 Hz, H-5′), 6.57 (1H, d, J=2 Hz, H-3′), 5.53 (1H, d, J=7 Hz, H-4), 4.84 (1H, d, J=8 Hz, H-1″), 4.30 (1H, dd, J=10, 3 Hz, H-2a), 3.84 (3H, s, –OCH_3), 3.87 (1H, dd, J=12, 2 Hz, H-6″a), 3.68 (1H, dd, J=12, 5 Hz, H-6″b), 3.61 (1H, dd, J=10, 10 Hz, H-2b), 3.58 (1H, dd, J=10, 7 Hz, H-3), 3.44 (1H, dd, J=9, 8 Hz, H-2″), 3.39 (1H, dd, J=9, 10 Hz, H-4″), 3.45 (1H, dd, J=9, 9 Hz, H-3″), 3.40 (1H, m, H-5″); ^{13}C-NMR (CD_3OD, 100 MHz): Table 2; CD $\Delta\varepsilon$ (nm): +2.96 (285), −13.9 (233), −11.1 (212) (c=4.17×10^{-5} M); HR-ESI-MS (positive-ion mode) m/z: 471.1254 $[M+Na]^+$ (Calcd for $C_{22}H_{24}O_{10}Na$: 471.1261).

Compound **6**: Amorphous powder; $[\alpha]_D^{21}$ +61.5° (c=0.27, MeOH); IR ν_{max} (film) cm^{-1}: 3367, 2928, 16180, 1514, 1444, 1396, 1249, 1077, 1028, 711; UV λ_{max} (MeOH) nm (log ε): 319sh (3.72), 283 (3.97), 239sh (3.86), 220 (4.17); ^{1}H-NMR (CD_3OD, 100 MHz) δ: 7.71 (1H, d, J=9 Hz, H-6′), 7.01 (2H, d, J=8 Hz, H-2, 6), 6.67 (2H, d, J=8 Hz, H-3, 5), 6.41 (1H, d, J=9 Hz, H-5′), 5.17 (1H, dd, J=7, 5 Hz, H-α), 4.94 (1H, d, J=10 Hz, H-1″), 4.05 (1H, dd, J=10, 10 Hz, H-2″), 3.86 (1H, dd, J=12, 2 Hz, H-6″a), 3.74 (1H, dd, J=12, 5 Hz, H-6″b), 3.49 (1H, dd, J=10, 10 Hz, H-4″), 3.48 (1H, dd, J=10, 10 Hz, H-3″), 3.42 (1H, m, H-5″), 3.04 (1H, dd, J=14, 5 Hz, H-βa), 2.86 (1H, dd, J=14, 7 Hz, H-βb); ^{13}C-NMR (CD_3OD, 100 MHz): Table 2; CD $\Delta\varepsilon$ (nm): +32.0 (325), +17.9 (293), −5.80 (254), [θ] nm +105600 (325), +59070 (293), −19140 (254) (c=3.00×10^{-5} M); HR-ESI-MS (positive-ion mode) m/z: 459.1236 $[M+Na]^+$ (Calcd for $C_{21}H_{24}O_{10}Na$: 459.1261).

Table 2. ^{13}C-NMR Spectroscopic Data for Pterocarpans (**4**, **5**) and Chalcone (**6**) (CD_3OD, 100 MHz)

C	**4**	**5**	**6**
1			129.3
2	68.2	67.9	131.6
3	41.0	41.2	116.2
4	79.8	80.2	157.2
5	127.8	122.1	116.2
6	107.7	107.1	131.6
7	154.6	150.0	
8	135.4	135.8	
9	150.7	145.6	
10	116.6	115.7	
1′	119.2	122.6	112.2
2′	160.0	161.9	165.4
3′	98.8	100.5	112.9
4′	162.0	160.4	165.6
5′	108.9	110.4	109.7
6′	126.1	126.0	133.2
1″	105.1	102.6	75.6
2″	75.8	75.0	72.9
3″	78.4	78.2	80.2
4″	71.5	71.5	71.8
5″	77.9	78.0	82.7
6″	62.7	62.6	62.8
–OCH_3	57.1	57.8	
α			74.4
β			42.2
>C=O			205.7

Enzymatic Hydrolysis of Crotalionoside A (1) Crotalionoside A (**1**) (4.2 mg) in 2 ml of H_2O was hydrolyzed with emulsin (5.2 mg) and crude hesperidinase (5.0 mg) for 24 h at 37 °C. The reaction mixture was evapo-

rated to dryness, and then the methanolic solution was adsorbed on silica gel and subjected to silica gel CC (20 g, Φ=22 mm, L=11 cm) with $CHCl_3$ (100 ml) and $CHCl_3$–MeOH (19 : 1, 100 ml, 9 : 1, 100 ml, 17 : 3, 100 ml, and 7 : 3, 300 ml), 12 ml fractions being collected. Crotalionol A (**1a**) (1.8 mg, 72%) and D-glucose (1.6 mg, 86%) were recovered in fractions 26—31 and 36—42, respectively. Crotalionol A (**1a**): an amorphous powder, $[\alpha]_D^{27}$ −34.2° (c=0.12, MeOH); ^{1}H-NMR (CD_3OD, 400 MHz) δ: 5.35 (1H, d, J=6 Hz, H-8), 4.27 (1H, qd, J=6, 6 Hz, H-9), 3.95 (1H, ddd, J=12, 9, 5 Hz, H-3), 2.99 (1H, d, J=9 Hz, H-4), 1.82 (1H, dd, J=12, 5 Hz, H-2a), 1.36 (3H, s, H_3-13), 1.34 (1H, dd, J=12, 12 Hz, H-2b), 1.30 (3H, s, H_3-11), 1.25 (3H, d, J=6 Hz, H_3-10), 1.06 (3H, s, H_3-12); ^{13}C-NMR (CD_3OD, 100 MHz) δ: 200.8 (C-7), 117.6 (C-6), 100.3 (C-8), 82.2 (C-4), 74.7 (C-5), 69.2 (C-3), 67.2 (C-9), 47.6 (C-2), 35.6 (C-1), 32.9 (C-12), 29.4 (C-11), 26.7 (C-13), 23.5 (C-10); HR-ESI-TOF-MS (positive-ion mode) m/z: 265.1409 $[M+Na]^+$ (Calcd for $C_{13}H_{22}O_4Na$, 265.1410). D-Glucose: $[\alpha]_D^{27}$ +35.2° (c=0.11, H_2O, 24 h after being dissolved in the solvent).

Preparation of Crotalionol A (*R*)- and (*S*)-MTPA 3,9-Diesters (1b, 1c) from 1a A solution of **1a** (1.1 mg) in 1 ml of dehydrated CH_2Cl_2 was reacted with (*R*)-MTPA (66 mg) in the presence of EDC (17 mg) and 4-DMAP (12 mg), the mixture being occasionally stirred at 25 °C for 6 h. After the addition of 1 ml of CH_2Cl_2, the solution was washed with H_2O (1 ml), 5% HCl (1 ml), $NaHCO_3$-saturated H_2O, and then brine (1 ml), successively. The organic layer was dried over Na_2SO_4 and then evaporated under reduced pressure. The residue was purified by preparative TLC [silica gel (0.25 mm thickness), being applied for 18 cm, development with $CHCl_3$–$(CH_3)_2CO$ (19 : 1) for 9 cm, and then eluted with $CHCl_3$–MeOH (9 : 1)] to furnish an ester, **1b** (0.5 mg, 29%). Through a similar procedure, **1c** (1.0 mg, 45%) was prepared from **1a** (0.8 mg) using (*S*)-MTPA (46 mg), EDC (17 mg), and 4-DMAP (12 mg). Crotalionol A 3,9-di-*O*-(*R*)-MTPA ester (**1b**) of **1a**: an amorphous powder; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.46—7.59 (4H, m, aromatic protons), 7.34—7.43 (6H, m, aromatic protons), 5.55 (1H, qd, J=6, 6 Hz, H-9), 5.46 (1H, ddd, J=12, 9, 5 Hz, H-3), 5.41 (1H, dd, J=6, 1 Hz, H-8), 3.59 (3H, s, –OCH_3), 3.54 (3H, s, –OCH_3), 3.29 (1H, d, J=9 Hz, H-4), 1.96 (1H, dd, J=12, 5 Hz, H-2a), 1.45 (1H, dd, J=12, 12 Hz, H-2b), 1.42 (3H, d, J=6 Hz, H_3-10), 1.37 (3H, s, H_3-11), 1.34 (3H, s, H_3-13), 0.96 (3H, s, H_3-12); HR-ESI-TOF-MS (positive-ion mode) m/z: 697.2209 $[M+Na]^+$ (Calcd for $C_{33}H_{36}O_8F_6Na$, 697.2206). Crotalionol A 3,9-di-*O*-(*S*)-MTPA ester (**1c**) of **1a**: an amorphous powder, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.48—7.67 (4H, m, aromatic protons), 7.34—7.42 (6H, m, aromatic protons), 5.53 (1H, qd, J=6, 6 Hz, H-9), 5.48 (1H, d, J=6 Hz, H-8), 5.47 (1H, ddd, J=12, 9, 5 Hz, H-3), 3.60 (3H, q, J=1 Hz, –OCH_3), 3.51 (3H, q, J=1 Hz, –OCH_3), 3.34 (1H, d, J=9 Hz, H-4), 1.90 (1H, dd, J=12, 5 Hz, H-2a), 1.41 (1H, dd, J=12, 12 Hz, H-2b), 1.38 (3H, s, H_3-11), 1.36 (3H, d, J=6 Hz, H_3-10), 1.36 (3H, s, H_3-13), 1.01 (3H, s, H_3-12); HR-ESI-TOF-MS (positive-ion mode) m/z: 697.2204 $[M+Na]^+$ (Calcd for $C_{33}H_{36}O_8F_6Na$, 697.2206).

Enzymatic Hydrolysis of Crotalionoside B (2) Crotalionoside B (**2**) (0.9 mg) was similarly hydrolyzed with emulsin (1.5 mg) and crude hesperidinase (1.5 mg). The reaction mixture was evaporated to dryness, and then the methanolic solution was adsorbed on silica gel and subjected to silica gel CC (7 g, Φ=10 mm, L=17 cm) with $CHCl_3$ (50 ml) and $CHCl_3$–MeOH (19 : 1, 50 ml, 9 : 1, 50 ml, 17 : 3, 50 ml, and 7 : 3, 150 ml), 5 ml fractions being collected. Crotalionol B (**2a**) (0.4 mg, 74%) and D-glucose (0.2 mg, 50%) were recovered in fractions 28—34 and 45—53, respectively. Crotalionol B (**2a**): amorphous powder; $[\alpha]_D^{25}$ +3.77° (c=0.038, MeOH); ^{1}H-NMR (CD_3OD, 400 MHz) δ: 5.35 (1H, d, J=6 Hz, H-8), 4.28 (1H, qd, J=6, 6 Hz, H-9), 3.94 (1H, ddd, J=12, 9 5 Hz, H-3), 3.00 (1H, d, J=9 Hz, H-4), 1.82 (1H, dd, J=12, 5 Hz, H-2a), 1.36 (3H, s, H_3-13), 1.35 (1H, dd, J=12, 12 Hz, H-2b), 1.30 (3H, s, H_3-11), 1.25 (3H, d, J=6 Hz, H_3-10), 1.06 (3H, s, H_3-12); ^{13}C-NMR (CD_3OD, 100 MHz) δ: 200.7 (C-7), 117.6 (C-6), 100.4 (C-8), 82.2 (C-4), 74.7 (C-5), 69.2 (C-3), 67.2 (C-9), 48.0 (C-2), 35.7 (C-1), 32.7 (C-12), 29.4 (C-11), 26.8 (C-13), 23.5 (C-10); HR-ESI-TOF-MS (positive-ion mode) m/z: 265.1408 $[M+Na]^+$ (Calcd for $C_{13}H_{22}O_4Na$, 265.1410).

Preparation of Crotalionol B (*R*)- and (*S*)-MTPA 3,9-Diesters (2b, 2c) from 2a From **2a** (0.2 mg each), **2b** (0.2 mg, 36%) and **2c** (0.3 mg, 54%) were prepared with the respective amounts of (*R*)- and (*S*)-MTPA (51, 41 mg), EDC (13, 15 mg), and 4-DMAP (10, 18 mg). Crotalionol B 3,9-di-*O*-(*R*)-MTPA ester (**2b**): an amorphous powder, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.49—7.58 (4H, m, aromatic protons), 7.36—7.43 (6H, m, aromatic protons), 5.58 (1H, qd, J=6, 6 Hz, H-9), 5.52 (1H, d, J=6 Hz, H-8), 5.46 (1H, ddd, J=12, 9, 5 Hz, H-3), 3.58 (3H, q, J=1 Hz, –OCH_3), 3.51 (3H, q, J=1 Hz, –OCH_3), 3.28 (1H, d, J=9 Hz, H-4), 2.00 (1H, dd, J=12, 5 Hz, H-2a), 1.39 (3H, s, H_3-11), 1.36 (3H, d, J=6 Hz, H_3-10), 1.32 (3H, s, H_3-13), 1.06 (3H, s, H_3-12), H-2b could not be assigned, due to overlapping signals; HR-ESI-TOF-MS (positive-ion mode) m/z: 697.2213 $[M+Na]^+$ (Calcd for $C_{33}H_{36}O_8F_6Na$, 697.2206). Crotalionol B 3,9-di-*O*-(*S*)-MTPA ester (**2c**): an amorphous powder, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.46—7.62 (4H, m, aromatic protons), 7.33—7.43 (6H, m, aromatic protons), 5.56 (1H, qd, J=6, 6 Hz, H-9), 5.43 (1H, d, J=6 Hz, H-8), 5.43 (1H, ddd, J=12, 9, 5 Hz, H-3), 3.61 (3H, q, J=1 Hz, –OCH_3), 3.56 (3H, q, J=1 Hz, –OCH_3), 3.16 (1H, d, J=9 Hz, H-4), 1.88 (1H, dd, J=12, 5 Hz, H-2a), 1.42 (3H, s, H_3-11), 1.42 (3H, d, J=6 Hz, H_3-10), 1.36 (3H, s, H_3-13), 1.02 (3H, s, H_3-12), H-2b could not be assigned, due to overlapping signals; HR-ESI-TOF-MS (positive-ion mode) m/z: 697.2204 $[M+Na]^+$ (Calcd for $C_{33}H_{36}O_8F_6Na$, 697.2206).

$NaBH_4$ Reduction of Citrosides A (10) and B (11) to the Corresponding Alcohols (10a, 10b) To a solution of citroside A (**10**) (10 mg) in 0.5 ml of MeOH was added 10 mg of $CeCl_3 \cdot 7H_2O$ and then 2 mg of $NaBH_4$, the reaction mixture being stirred for 5 min at 25 °C. Excess $NaBH_4$ was quenched by the addition of 100 ml of $(CH_3)_2CO$ and then the reaction mixture was evaporated and purified by preparative TLC (developed with $CHCl_3$–MeOH–H_2O, 15 : 6 : 1, and then eluted with $CHCl_3$–MeOH, 1 : 1) to afford 1.7 mg of **10a**. Citroside B (**11**) (1.5 mg) was similarly reduced to give 0.5 mg of **11a**. **10a**: CD $\Delta\varepsilon$ (nm): +2.32 (226) (c=4.90×10^{-5} M, MeOH). **11a**: CD $\Delta\varepsilon$ (nm): +1.29 (225) (c=5.15×10^{-5} M, MeOH).

HPLC Separation of 10a and 11a Compound **10a** was separated by HPLC (MeOH–H_2O, 2 : 3) to give 0.4 mg of **10b** and 0.2 mg of **10c** from the peaks at 11 min and 12 min, respectively. Compound **11a** was similarly separated by HPLC to give 50 μg of **11b** and 50 μg of **11c** from the peaks at 20 min and 23 min, respectively. **10b**: ^{1}H-NMR (CD_3OD, 400 MHz) δ: 5.33 (1H, d, J=6 Hz, H-8), 4.50 (1H, d, J=8 Hz, H-1′), 4.28 (2H, overlapped, H-3, 9), 3.79 (1H, dd, J=12, 3 Hz, H-6′a), 3.64 (1H, dd, J=12, 5 Hz, H-6′b), 3.18—3.36 (3H, m, H-3′, 4′, 5′), 3.12 (1H, dd, J=9, 8 Hz, H-2′), 2.41 (1H, ddd, J=13, 4, 2 Hz, H-4a), 1.84 (1H, ddd, J=13, 4, 2 Hz, H-2a), 1.41 (3H, s, H_3-13), 1.29 (3H, s, H_3-12), 1.26 (3H, d, J=6 Hz, H_3-10), 1.09 (3H, s, H_3-11), H-2b and 4b could not be assigned, due to overlapping signals; CD $\Delta\varepsilon$ (nm): +1.20 (226) (c=5.15×10^{-5} M, MeOH); HR-ESI-TOF-MS (positive-ion mode) m/z: 411.1985 $[M+Na]^+$ (Calcd for $C_{19}H_{32}O_8Na$, 411.1989). **10c**: ^{1}H-NMR (CD_3OD, 400 MHz) δ: 5.32 (1H, d, J=6 Hz, H-8), 4.50 (1H, d, J=8 Hz, H-1′), 4.27 (2H, overlapped, H-3, 9), 3.79 (1H, dd, J=12, 3 Hz, H-6′a), 3.64 (1H, dd, J=12, 5 Hz, H-6′b), 3.18—3.36 (3H, m, H-3′, 4′, 5′), 3.12 (1H, dd, J=9, 8 Hz, H-2′), 2.41 (1H, ddd, J=13, 4, 2 Hz, H-4a), 1.84 (1H, J=13, 4, 2 Hz, H-2a), 1.42 (3H, s, H_3-13), 1.29 (3H, s, H_3-12), 1.26 (3H, d, J=6 Hz, H_3-10), 1.08 (3H, s, H_3-11), H-2b and 4b could not be assigned, due to overlapping signals; CD $\Delta\varepsilon$ (nm): +1.65 (226) (c=5.15×10^{-5} M, MeOH); HR-ESI-TOF-MS (positive-ion mode) m/z: 411.1983 $[M+Na]^+$ (Calcd for $C_{19}H_{32}O_8Na$, 411.1989). **11b**: ^{1}H-NMR (CD_3OD, 400 MHz) δ: 5.43 (1H, d, J=6 Hz, H-8), 4.53 (1H, d, J= 8Hz, H-1′), 4.51 (1H, overlapped with HDO signal, H-9), 4.30 (1H, m, H-3), 3.80 (1H, dd, J=12, 2 Hz, H-6′a), 3.65 (1H, dd, J=12, 5 Hz, H-6′b), 3.12—3.36 (4H, m, H-2′, 3′, 4′, 5′), 2.41 (1H, m, H-4a), 1.38 (1H, m, H-2a), 1.38 (3H, s, H_3-13), 1.33 (3H, d, J=6 Hz, H_3-10), 1.30 (3H, s, H_3-12), 1.08 (3H, s, H_3-11), H-2b and 4b could not be assigned, due to overlapping signals; HR-ESI-TOF-MS (positive-ion mode) m/z: 411.1991 $[M+Na]^+$ (Calcd for $C_{19}H_{32}O_8Na$, 411.1989). **11c**: ^{1}H-NMR (CD_3OD, 400 MHz) δ: 5.40 (1H, d, J=6 Hz, H-8), 4.53 (1H, d, J=8 Hz, H-1′), 4.51 (1H, overlapped with HDO signal, H-9), 4.30 (1H, m, H-3), 3.80 (1H, dd, J=12, 2 Hz, H-6′a), 3.65 (1H, dd, J=12, 5 Hz, H-6′b), 3.12—3.36 (4H, m, H-2′, 3′, 4′, 5′), 2.41 (1H, m, H-4a), 1.83 (1H, m, H-2a), 1.40 (3H, s, H_3-13), 1.31 (3H, d, J=6 Hz, H_3-10), 1.29 (3H, s, H_3-12), 1.06 (3H, s, H_3-11), H-2b and 4b could not be assigned, due to overlapping signals; HR-ESI-TOF-MS (positive-ion mode) m/z: 411.1984 $[M+Na]^+$ (Calcd for $C_{19}H_{32}O_8Na$, 411.1989).

Enzymatic Hydrolysis of Crotalionoside C (3) Crotalionoside C (**3**) (4.5 mg) was hydrolyzed with emulsin (4.5 mg) and crude hesperidinase (4.0 mg) at 37 °C for 24 h. The reaction mixture was evaporated to dryness, and then the methanolic solution was adsorbed on silica gel and subjected to silica gel CC (25 g, Φ=22 mm, L=13 cm) with $CHCl_3$ (100 ml) and $CHCl_3$–MeOH (19 : 1, 100 ml, 9 : 1, 100 ml, 17 : 3, 100 ml, and 7 : 3, 300 ml), 5 ml fractions being collected. Crotalionol C (**3a**) (2.2 mg, 86%) and glucose (1.4 mg, 67%) were recovered in fractions 21—24 and fractions 49—55, repectively. Crotalionol C (**3a**): Amorphous powder; $[\alpha]_D^{24}$ −23.1° (c=0.15, MeOH); ^{1}H-NMR (CD_3OD, 400 MHz) δ: 5.74 (1H, d, J=16 Hz, H-7), 5.70 (1H, dd, J=16, 5 Hz, H-8), 4.32 (1H, br dd, J=6, 6 Hz, H-3), 4.29 (1H, qd, J=6, 5 Hz, H-9), 1.95 (1H, ddd, J=12, 6, 2 Hz, H-4a), 1.77 (1H, ddd, J=12, 6, 2 Hz, H-2a), 1.65 (1H, d, J=12 Hz, H-4b), 1.59 (1H, d, J=12 Hz, H-2b), 1.40 (3H, s, H_3-11), 1.24 (3H, d, J=6 Hz, H_3-10), 1.17 (3H, s, H_3-13), 0.86 (3H, s, H_3-12); ^{13}C-NMR (CD_3OD, 100 MHz) δ: 136.2 (C-8), 124.6 (C-7), 92.7 (C-6), 82.2 (C-5), 76.7 (C-3), 69.3 (C-9), 49.4 (C-2), 48.7 (C-4), 44.5

(C-1), 32.7 (C-12), 31.4 (C-13), 25.9 (C-11), 24.0 (C-10); HR-ESI-TOF-MS (positive-ion mode) *m/z*: 249.1463 $[M+Na]^+$ (Calcd for $C_{13}H_{22}O_3Na$, 249.1461).

Preparation of Crotalionol C (*R*)- and (*S*)-MTPA 9-Esters (3b, 3c) from 3a From **3a** (1.1 mg each), **3b** (1.3 mg, 60%) and **3c** (1.2 mg, 56%) were prepared with the respective amounts of (*R*)- and (*S*)-MTPA (26, 21 mg), EDC (15, 17 mg), and 4-DMAP (10, 10 mg). Crotalionol C 9-*O*-(*R*)-MTPA ester (**3b**): amorphous powder; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.51—7.54 (2H, m, aromatic protons), 7.37—7.39 (3H, m, aromatic protons), 5.88 (1H, d, *J*=16 Hz, H-7), 5.75 (1H, dd, *J*=16, 7 Hz, H-8), 5.61 (1H, dq, *J*=7, 6 Hz, H-9), 4.36 (1H, br dd, *J*=6, 6 Hz, H-3), 3.53 (3H, q, *J*=1 Hz, $-OCH_3$), 2.00 (1H, ddd, *J*=12, 6, 2 Hz, H-4a), 1.81 (1H, ddd, *J*=12, 6, 2 Hz, H-2a), 1.65 (1H, d, *J*=12 Hz, H-4b), 1.59 (1H, d, *J*=12 Hz, H-2b), 1.39 (3H, s, H_3-11), 1.38 (3H, d, *J*=6 Hz, H_3-10), 1.16 (3H, s, H_3-13), 0.85 (3H, s, H_3-12); HR-ESI-TOF-MS (positive-ion mode) *m/z*: 465.1840 $[M+Na]^+$ (Calcd for $C_{23}H_{29}O_5F_3Na$, 465.1859). Crotalionol C 9-*O*-(*S*)-MTPA esters (**3c**): amorphous powder; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.51—7.54 (2H, m, aromatic protons), 7.37—7.39 (3H, m, aromatic protons), 5.77 (1H, d, *J*=16 Hz, H-7), 5.68 (1H, dd, *J*=16, 7 Hz, H-8), 5.62 (1H, dq, *J*=7, 6 Hz, H-9), 4.34 (1H, br dd, *J*=6, 6 Hz, H-3), 3.55 (3H, q, *J*=1 Hz, $-OCH_3$), 1.99 (1H, ddd, *J*=12, 6, 2 Hz, H-4a), 1.79 (1H, ddd, *J*=12, 6, 2 Hz, H-2a), 1.64 (1H, d, *J*=12 Hz, H-4b), 1.58 (1H, d, *J*=12 Hz, H-2b), 1.43 (3H, d, *J*=6 Hz, H_3-10), 1.36 (3H, s, H_3-11), 1.14 (3H, s, H_3-13), 0.82 (3H, s, H_3-12); HR-ESI-TOF-MS (positive-ion mode) *m/z*: 465.1838 $[M+Na]^+$ (Calcd for $C_{23}H_{29}O_5F_3Na$, 465.1859).

Enzymatic Hydrolysis of 4 Compound **4** (21.5 mg) in 2 ml of H_2O was incubated with 9.4 mg of β-glucosidase at 37 °C for 24 h. The dried reaction mixture was subjected to silica gel (38 g) CC with a solvent system of $CHCl_3$ (200 ml) and $CHCl_3$–MeOH [(19 : 1, 200 ml) and (9 : 1, 200 ml)], 12 ml fractions being collected. An aglycone, melilotocarpan B (**4a**) (7.6 mg) was obtained in fractions 25—30, which was recrystallized from $CHCl_3$–MeOH to give 3.2 mg (23%) of colorless needles. Melilotocarpan B (**4a**): colorless needles ($CHCl_3$–MeOH), mp 111—113 °C, $[\alpha]_D$ −149.5° (*c*=0.21, dioxane); ^{1}H-NMR (acetone-d_6, 400 MHz) δ: 7.13 (1H, d, *J*=8 Hz, H-6′), 6.95 (1H, d, *J*=8 Hz, H-5), 6.71 (1H, d, *J*=8 Hz, H-6′), 6.37 (1H, dd, *J*=8, 2 Hz, H-5′), 6.28 (1H, d, *J*=2 Hz, H-3′), 5.53 (1H, d, *J*=6 Hz, H-4), 4.31 (1H, dd, *J*=10, 3 Hz, H-2b), 3.84 (3H, s, $-OCH_3$), 3.60 (2H, m, H-2a, 3); ^{13}C-NMR (acetone-d_6, 100 MHz) δ: 161.8 (C-2′), 159.7 (C-4′), 149.0 (C-7), 145.2 (C-9), 136.1 (C-8), 125.9 (C-6′), 121.5 (C-5), 119.1 (C-1′), 115.6 (C-10), 108.4 (C-5′), 106.9 (C-6), 98.6 (C-3′), 79.4 (C-4), 67.6 (C-2), 56.7 ($-OCH_3$), 40.6 (C-3); HR-ESI-TOF-MS (positive-ion mode) *m/z*: 309.0734 $[M+Na]^+$ (Calcd for $C_{16}H_{14}O_5Na$, 309.0733).

Sugar Analyses Glucoses derived on enzymatic hydrolyses of crotalionosides B (**2**) and C (**3**) were dissolved in 100 μl of H_2O, and an aliquot (10 ml) of each solution was analyzed by HPLC on a Shodex NH_2P-50 column (solvent: CH_3CN–H_2O, 3 : 1; flow rate: 1 ml/min) gave peak at 9.5 min exhibiting positive rotation (JASCO, OR-2090 polarimeter). The peaks were identified with authentic D-glucose. Compounds **4** and **5** (0.5 mg each) were hydrolyzed with 100 ml of 1 N HCl at 90 °C for 2 h. The hydrolyzates were partitioned with 100 ml of EtOAc and then 20 ml aliquot of the H_2O layers were analyzed by HPLC under the same conditions as above to give peaks at 9.5 min exhibiting positive rotation. The peaks were also identified with authetic D-glucose.

Known Compounds Isolated 3-Hydroxy-5,6-epoxy-β-ionol 9-*O*-β-D-glucopyranoside (**7**): $[\alpha]_D^{19}$ −50.7° (*c*=0.88, MeOH).[6] Kaempferol 3-*O*-robinoside (**8**): $[\alpha]_D^{25}$ −27.1°(*c*=0.75, MeOH).[7] Robinin (**9**): $[\alpha]_D^{25}$ −113.4° (*c*=0.47, pyridine).[8]

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