Contortisiliosides A – G: Isolation of Seven New Triterpene Bisdesmosides from *Enterolobium contortisiliquum* and Their Cytotoxic Activity

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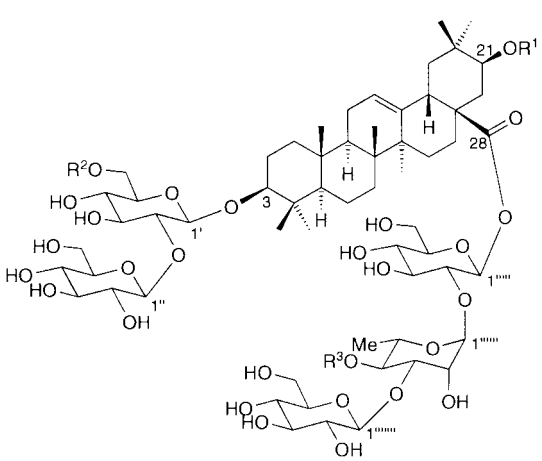
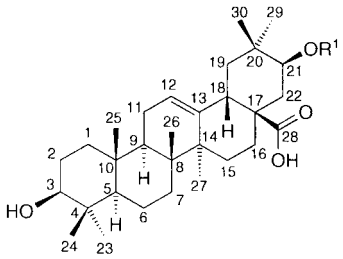
Seven new bisdesmosidic triterpene saponins, with up to eight monosaccharides, which were given the trivial names contortisiliosides A – G (**1** – **7**), were isolated from *Enterolobium contortisiliquum*. The structures of the new saponins were determined on the basis of extensive spectroscopic and chromatographic analyses of both intact and acid-hydrolyzed compounds. The isolated saponins were evaluated for their cytotoxic activities against BAC1.2F5 mouse macrophages, EL-4 mouse lymphoma cells, and L-929 mouse fibroblasts. Whereas contortisiliosides A (**1**) and C (**3**) were moderately cytotoxic to both BAC1.2F5 macrophages and EL-4 cells, and contortisiliosides D – G (**4** – **7**) did not show any apparent cytotoxic activities against the three cell lines, contortisilioside B (**2**) exhibited selective cytotoxic activity against BAC1.2F5 mouse macrophages, with an IC_{50} value of 3.4 μM. The macrophage death caused by **2** was shown to be neither necrotic nor apoptosis-inducing based on the unique morphological change of the killed cells, whose cytosols were transformed into large vacuoles, and according to the TUNEL assay.

Introduction. – *Enterolobium contortisiliquum* (VELL.) MORONG (Leguminosae) is a triterpene-saponin-rich herbal medicine used for the treatment of parasitism and gonorrhea in Brazil [1]. As part of our continuous studies on biologically active triterpene saponins from higher plants [2 – 7], a detailed phytochemical examination was carried out on the pericarps of *E. contortisiliquum*, with particular attention to the triterpene-saponin constituents, which resulted in the isolation of seven new bisdesmosidic triterpene saponins with up to a total of eight monosaccharide units. The new compounds were given the trivial names contortisilioside A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), F (**6**), and G (**7**), respectively. This paper reports the isolation and structural determination of the new saponins and their cytotoxic activities against BAC1.2F5 mouse macrophages, EL-4 mouse lymphoma cells, and L-929 mouse fibroblasts.

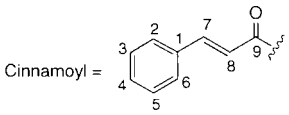
Results and Discussion. – *Isolation*. The H_2O extract of the dry pericarps of *E. contortisiliquum* was partitioned between BuOH and H_2O. The BuOH-soluble portion was separated by column chromatography (CC) over silica gel into seven subfractions (I – VII). Subfractions III – VI were repeatedly subjected to CC over silica gel and octadecylsilanized (ODS) silica gel to afford the contortisiliosides A – G (**1** – **7**).

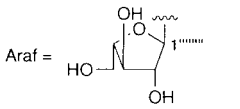


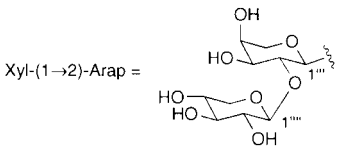
	R^1	R^2	R^3
1	cinnamoyl	Xyl-(1→2)-Arap	H
2	cinnamoyl	Xyl-(1→2)-Arap	Araf
3	cinnamoyl	Xyl-(1→2)-Gal	Araf
4	H	Xyl-(1→2)-Arap	H
5	H	Xyl-(1→2)-Arap	Araf
6	H	Xyl-(1→2)-Gal	Araf
7	H	H	Araf

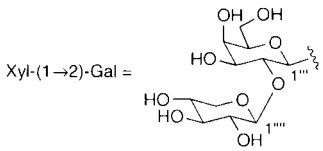


1a R^1 = cinnamoyl
4a R^1 = H









Structural Determination of **1**. Contortisilioside A (**1**) was obtained as an amorphous solid, and its molecular formula, $C_{79}H_{120}O_{37}$, was derived from a combination of negative-ion FAB-MS (*m/z* 1659 ($[M-H]^-$)), ^{13}C-NMR spectral (79 signals; see *Tables 2* and *3* in the *Exper. Part*), and elemental-analysis data.

The IR spectrum of **1** showed absorption bands at 3387 (OH) and 1755 (C=O) and 1686 cm^{-1} (C=O). The UV absorption at 277 nm (log $\varepsilon = 3.97$) was suggestive of a conjugated aromatic ring. The ^{1}H-NMR spectrum of **1** in (D_5)pyridine was typical for a triterpene saponin of the oleanolic acid type, showing signals for seven Me groups at quaternary C-atoms at δ_H 1.34, 1.29, 1.21, 1.17, 1.01, 1.00, and 0.97, and an olefinic group at δ_H 5.49 (*t*-like, $J = 3.2$), as well as signals for seven anomeric H-atoms at δ_H 6.36 (br. *s*), 6.16 (*d*, $J = 8.1$), 5.45 (*d*, $J = 7.8$), 5.36 (*d*, $J = 7.6$), 5.15 (*d*, $J = 5.1$), 4.99 (*d*, $J = 7.0$), and 4.89 (*d*, $J = 7.1$). The Me signals at δ_H 1.77 (*d*, $J = 6.0$) and δ_C 18.7 were indicative of **1** possessing one deoxyhexopyranosyl unit. In addition, the presence of an (*E*)-cinnamoyl group was evident from the signals at δ_H 7.92 (*d*, $J = 16.0$, 1 H), 6.71 (*d*, $J = 16.0$, 1 H), 7.65 (*dd*, $J =$ 7.6, 1.5, 2 H), and 7.37 (*m*, 3 H), and at δ_C 166.5 (C=O), 144.9 (CH), 135.0 (C), 130.6 (CH), 129.3 (2 CH), 128.6 (2 CH), and 119.2 (CH), respectively.

Acid hydrolysis of **1** with 0.7M HCl in dioxane/H_2O 1:1 produced a triterpene aglycon with a cinnamoyl group (**1a**), identified as 3β-hydroxy-21β-[(3-phenylprop-2-enoyl)oxy]olean-12-en-28-oic acid [8], and L-arabinose, D-glucose, D-xylose, and L-

rhamnose as the carbohydrate moieties. Thus, **1** was assumed to be a 21β-substituted olean-12-en-28-oic acid 3,28-bisdesmoside, whose sugar moieties were composed of a total of seven monosaccharides. The severe overlapping of the ^{1}H-NMR signals for the sugar moieties excluded the possibility of a complete assignment in a straightforward way by means of conventional 2D-NMR methods such as ^{1}H,^{1}H-COSY, 2D-TOCSY, and HSQC. However, analysis of the 1D-TOCSY spectra followed by interpretation of the ^{1}H,^{1}H-COSY, HSQC, HSQC/TOCSY, and HMBC spectra allowed us to solve the exact carbohydrate sequence and the linkage positions to the aglycon. The structure of **1** was established as β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl 21β-[((*E*)-3-phenylprop-2-enoyl)oxy]-3β-[(β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranosyl)oxy]-olean-12-en-28-oate.

The structures and absolute configurations of the monosaccharides were identified by direct HPLC analysis of the hydrolysate, which was performed on an aminopropyl-bonded silica-gel column, using MeCN/H_2O 17:3 as solvent system, with detection being carried out by means of a combination of refractive-index (RI) and optical-rotation (OR) detectors. The ^{13}C-NMR shifts of C(3) at δ_C 88.7 and C(28) at 174.9 implied that the sugar linkages were at both C(3) and C(28). The ^{1}H-NMR subspectra of individual monosaccharide units were obtained by selective irradiation of the easily identifiable anomeric H-atom signals, as well as by irradiation of the Me *d* of the rhamnosyl moiety in a series of 1D-TOCSY experiments [9–12]. Subsequent analysis of the ^{1}H,^{1}H-COSY spectrum resulted in the sequential assignments of all the H-atom resonances due to the seven monosaccharides, including identification of their multiplet patterns and coupling constants, as shown in *Table 2* (see *Exper. Part*). The HSQC and HSQC/TOCSY spectra helped us to correlate the H-atom resonances with those of the corresponding one-bond-coupled C-atoms, leading to unambiguous assignments of the C-atoms. Comparison of the C-atom chemical shifts thus assigned with those of the reference Me glycosides [13], taking into account the known effects of *O*-glycosylation, indicated that **1** contained *1*) a β-D-xylopyranosyl unit (Xyl C(1‴)/C(5‴)) and two β-D-glucopyranosyl units (Glc C(1″)/C(6″) and Glc C(1⁗)/C(6⁗)) as the terminal glycosyl moieties, and *2*) an α-L-arabinopyranosyl unit (Ara C(1‴)/C(5‴)), an α-L-rhamnopyranosyl unit (Rha C(1⁗)/C(6⁗)), and two β-D-glucopyranosyl units (Glc C(1′)/C(6′) and Glc C(1⁗)/C(6⁗)) as the substituted sugar moieties. The relatively large *J* values of the anomeric resonances of the arabinosyl, glucosyl, and xylosyl moieties indicated an α-anomeric orientation for the arabinosyl and β-orientations for the glucosyl and xylosyl anomeric positions. For the rhamnosyl moiety, the large ^1J(C,H) value of 171.3 Hz confirmed that the anomeric H-atom was equatorial (α-pyranoid anomeric form). In the HMBC spectrum, the anomeric H-atom signals at δ_H 5.45 (H–C(1⁗)), 6.36 (H–C(1⁗)), and 6.16 (H–C(1⁗)) showed long-range correlations with C(3⁗) of Rha at δ_C 83.9, C(2⁗) of Glc at 76.1, and C(28) of the aglycon at 174.9, respectively, indicating that the liner triglycoside Glc-(1 → 3)-Rha-(1 → 2)-Glc was attached at C(28) of the aglycon (*Fig. 1*). The anomeric H-atom at δ_H 4.99 (H–C(1⁗)) showed an HMBC correlation with C(2‴) of Ara at δ_C 80.7, whose anomeric resonance at δ_H 5.15 (H–C(1‴)), in turn, exhibited a long-range correlation with C(6′) of Glc at δ_C 69.3, whereas the anomeric H-atom at δ_H 5.36 (H–C(1″)) was correlated with C(2′) of Glc at δ_C 83.5. The anomeric H-atom of the 2,6-branched Glc at δ_H 4.89 (H–C(1′)) showed a ^3J(C,H) correlation with C(3) of the aglycon at δ_C 88.7 (*Fig. 1*). Thus, the branched tetraglycoside Xyl-(1 → 2)-Ara-(1 → 6)-[Glc-(1 → 2)]-Glc, which has not been reported as a sugar of either triterpene saponins or steroidal saponins, was revealed to be linked to C(3) of the aglycon.

Structural Determination of **2**. Contortisilioside B (**2**), obtained as an amorphous solid, exhibited a molecular formula of $C_{84}H_{128}O_{41}$ on the basis of the negative-ion FAB-MS (*m/z* 1791 ([*M* – H]$^-$)), ^{13}C-NMR spectral (84 signals; see *Tables 3* and *4* in the *Exper. Part*), and elemental-analysis data. The ^{1}H- and ^{13}C-NMR spectral data were very similar to those of **1**, except for the presence of the signals for eight anomeric centers. Acid hydrolysis of **2** afforded L-arabinose, D-glucose, L-rhamnose, and D-xylose, as well as the aglycon **1a**. Thus, **2** was shown to have one more pentose unit than **1**, identified as α-L-arabinofuranose, which was linked to C(4⁗) of the rhamnosyl

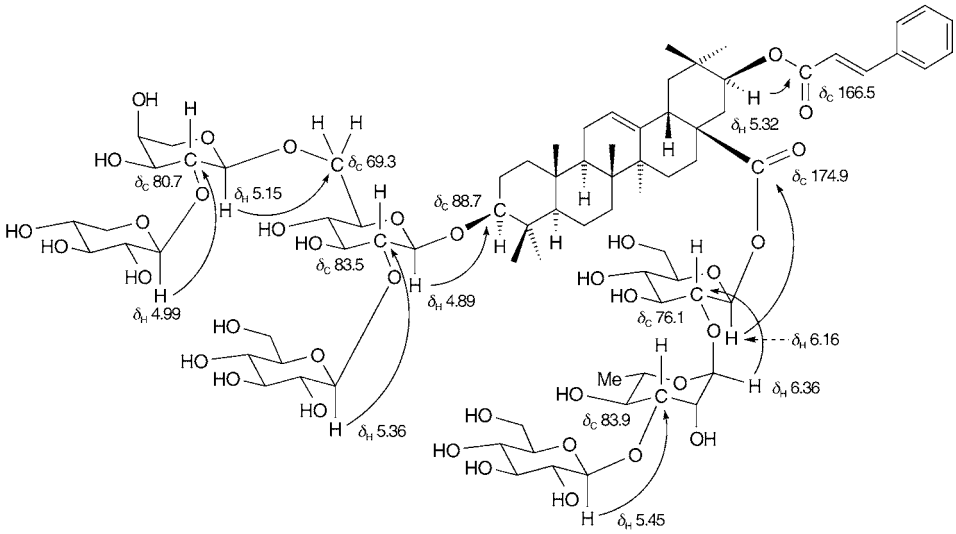


Fig. 1. *HMBC Correlations observed for the carbohydrate and cinnamoyl moieties of* **1**

residue. The structure of **2** was assigned as β-D-glucopyranosyl-(1 → 3)-[α-L-arabinofuranosyl-(1 → 4)]-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl 21β-[((*E*)-3-phenylprop-2-enoyl)oxy]-3β-[(β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-[β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranosyl)oxy]olean-12-en-28-oate.

Although the ^{1}H- and ^{13}C-NMR spectra of **2** allowed the identification of the aglycon as being the same as that of **1**, the molecular formula of **2** differed by $C_5H_8O_4$ from that of **1**, and the ^{1}H-NMR spectrum of **2** showed eight anomeric signals at δ_H 6.23 (*d*, *J* = 1.6), 6.12 (*d*, *J* = 7.5), 6.07 (br. *s*), 5.37 (*d*, *J* = 7.7), 5.34 (*d*, *J* = 8.0), 5.15 (*d*, *J* = 5.0), 4.99 (*d*, *J* = 7.0), and 4.87 (*d*, *J* = 6.8), suggesting that **2** structurally corresponds to **1** with one more pentose unit. The anomeric C-atom of the pentose appeared at δ_C 110.9, which was associated with the anomeric H-atom at δ_H 6.23 according to the HMQC spectrum. Analysis of the ^{1}H,^{1}H-COSY spectrum, starting from the anomeric resonance at δ_H 6.23, revealed the spin-coupling correlations and multiplet patterns of the pentose H-atoms. Since every H-atom coupled to their vicinal neighbors with relatively small coupling constant(s), the pentose was shown to be in the furanose form. The H-atoms assigned as H–C(1) to CH_2(5) were HMQC-correlated to the one-bond-coupled C-atoms, resulting in the assignments of C(1) to C(5) as δ_C 110.9 (CH), 84.8 (CH), 78.3 (CH), 84.7 (CH), and 62.4 (CH_2) in this order. Thus, the additional pentose was revealed to be α-L-arabinofuranose (Ara*f* C(1‴″)/C(5‴″)) [13]. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **2** with those of **1** revealed that the branched tetraglycoside group attached at C(3) of the aglycon was identical to that of **1**. However, significant differences were recognized in the signals from the Rha moiety linked to C(2″″) of Glc, which was attached at C(28) of the aglycon. In the HMBC spectrum, the anomeric H-atoms at δ_H 5.34 (H–C(1‴″)) and 6.23 (H–C(1‴″)) showed long-range correlations with C(3‴″) of Rha at δ_C 82.0 and C(4‴″) of Rha at 79.1, respectively. HMBC Correlations between the anomeric H-atom at δ_H 6.07 (H–C(1‴″)) and C(2‴″) of Glc at δ_C 76.7, and between the anomeric H-atom at δ_H 6.12 (H–C(1‴″)) and C(28) of the aglycon at δ_C 174.9 were also observed.

Structural Determination of **3**. Contortisilioside C (**3**) was shown to have the molecular formula $C_{85}H_{130}O_{42}$ on the basis of the negative-ion FAB-MS (*m*/*z* 1821 ([*M* – H]$^-$)), ^{13}C-NMR spectral (85 signals; see *Tables 2* and *3* in the *Exper. Part*), and

elemental-analysis data. The ^{1}H-NMR spectral features of **3** were analogous to those of **2**, showing signals for seven Me groups at quaternary C-atoms, an olefinic group, an (*E*)-cinnamoyl group, and eight anomeric H-atoms. However, acid hydrolysis of **3** gave D-galactose, along with L-arabinose, D-glucose, L-rhamnose, and D-xylose as the carbohydrate moieties, together with the aglycon **1a**. When the ^{13}C-NMR spectrum of **3** was compared with that of **2**, the signals arising from the aglycon, with a cinnamoyloxy group at C(21) and the branched tetraglycoside group linked to C(28), were superimposable on those of **2**, but the signals due to the tetraglycoside moiety attached at C(3) of the aglycon were not consistent with those of **2**, the major difference being the lack of the signals assignable to an α-L-arabinopyranosyl group in **3**. The above spectral and chemical information suggested that the α-L-arabinopyranosyl moiety in **2** was displaced by a β-D-galactopyranosyl group in **3**. The structure of **3** was formulated as β-D-glucopyranosyl-(1 → 3)-[α-L-arabinofuranosyl-(1 → 4)]-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl 21β-[((*E*)-3-phenylprop-2-enoyl)oxy]-3β-[(β-D-xylopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 6)-[β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranosyl)oxy]olean-12-en-28-oate.

In the 1D-TOCSY spectrum, irradiation of the anomeric H-atom at δ_H 5.12 (*d*, *J* = 7.7) gave a subspectrum, and the axial/axial relationships between H–C(1) and H–C(2), and between H–C(2) and H–C(3), and the axial/equatorial relationships between H–C(3) and H–C(4), and between H–C(4) and H–C(5), were revealed for the sugar protons with the help of ^{1}H,^{1}H-COSY data, indicative of a β-D-galactopyranosyl unit (Gal C(1‴)/C(6‴)). The ^{13}C-NMR shifts of C(1‴) to C(6‴) of the Gal moiety were identified by HSQC and HSQC/TOCSY experiments. In the HMBC spectrum, the anomeric H-atom at δ_H 5.06 (H–C(1⁗)) showed a ^3J(C,H) correlation with C(2‴) of Gal at δ_C 82.8, whose anomeric H-atom at δ_H 5.12 (H–C(1‴)), in turn, exhibited a long-range correlation with C(6′) of Glc at δ_C 69.9. HMBC Correlations from the anomeric H-atom at δ_H 5.40 (H–C(1″)) to C(2′) of Glc at δ_C 83.1, and from the anomeric H-atom at δ_H 4.89 (H–C(1′)) to C(3) of the aglycon at δ_C 88.1, were also observed.

Structural Determination of **4**–**6**. The molecular formulae of contortisiliosides D (**4**), E (**5**), and F (**6**) were determined by the negative-ion FAB-MS, ^{13}C-NMR spectral (*Tables 3* and *4*), and elemental-analysis data as $C_{70}H_{114}O_{36}$, $C_{75}H_{112}O_{40}$, and $C_{76}H_{124}O_{41}$, respectively. The ^{1}H-NMR spectrum of **4** showed signals for seven Me groups at quaternary C-atoms, an olefinic group, and seven anomeric H-atoms. Acid hydrolysis of **4** gave 3β,21β-dihydroxyolean-12-en-28-oic acid (**4a**) [14], L-arabinose, D-glucose, L-rhamnose, and D-xylose. The ^{13}C-NMR spectrum of **4** showed that the sugar units, linked to C(3) and C(28) of the aglycon, were identical to those of **1**. These data allowed us to confirm that **4** was the corresponding bisdesmosidic saponin of **1** without the cinnamoyl ester at C(21). Analysis of the ^{1}H- and ^{13}C-NMR spectra, and the results of acid hydrolysis of **5** and **6**, indicated that these two compounds were the corresponding cinnamoyl-free derivatives of **2** and **3**, respectively. Since neither acid hydrolysis of **1**–**3** (0.7M aqueous HCl, 95°, 90 min) nor alkaline treatment (3% aqueous KOH in EtOH, r.t., 3 h) cleaved the cinnamoyl ester linkages, **4**–**6** are believed to be natural products rather than artifacts produced during the extraction and isolation procedures.

Structural Determination of **7**. Contortisilioside G (**7**) analyzed for $C_{65}H_{106}O_{32}$ according to the negative-ion FAB-MS (*m*/*z* 1397 ([*M* – H]$^-$)), ^{13}C-NMR spectral (65 signals; see *Tables 3* and *4* in the *Exper. Part*), and elemental-analysis data. The ^{1}H-NMR spectrum displayed signals for six anomeric H-atoms at δ_H 6.21 (*d*, *J* = 1.6),

6.17 (br. *s*), 6.13 (*d*, *J* = 7.3), 5.36 (*d*, *J* = 7.7, 2 × 1 H), and 4.88 (*d*, *J* = 7.2), together with signals for seven Me groups at quaternary C-atoms, an olefinic resonance (H–C(12)), and an α-H–C(21) H-atom. Acid hydrolysis of **7** furnished **4a**, L-arabinose, D-glucose, and L-rhamnose. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **7** with those of **5** and **6** revealed that the aglycon and the tetraglycoside residue attached at C(28) were the same as those of **5** and **6**. Consequently, the sugar attached at C(3) of the aglycon was a diglycoside, identified as Glc-(1 → 2)-Glc by 2D-NMR data. The structure of **7** was, thus, assigned as β-D-glucopyranosyl-(1 → 3)-[α-L-arabinofuranosyl-(1 → 4)]-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl 3β-[(β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl)oxy]-21β-hydroxyolean-12-en-28-oate.

The anomeric H-atom at δ_H 4.88 was shown to be associated with the six signals at δ_C 105.0 (CH), 83.5 (CH), 78.1 (CH), 71.6 (CH), 78.0 (CH), and 62.8 (CH_2), which were indicative of a β-D-glucopyranosyl (Glc C(1′)/C(6′)) glycosylated at C(2′). The remaining six signals at δ_C 106.0 (CH), 77.1 (CH), 78.0 (CH), 71.8 (CH), 78.1 (CH), and 62.8 (CH_2) were attributed to a terminal β-D-glucopyranosyl unit (Glc C(1″)/C(6″)). Long-range correlations were observed from the anomeric H-atoms at δ_H 5.36 (H–C(1″)) to C(2′) of the inner Glc at δ_C 83.5, whose anomeric resonance at δ_H 4.88 (H–C(1′)) showed an HMBC correlation with C(3) of the aglycon at δ_C 89.1.

Biological Activity. Since several triterpene saponins with an acyl group have been reported to show selective cytotoxic activities against macrophages [5–7], the isolated saponins were incubated with BAC1.2F5 mouse macrophages, EL-4 mouse lymphoma cells, and L-929 mouse fibroblasts. Contortisilioside B (**2**) exhibited selective cytotoxic activity against BAC1.2F5 mouse macrophages, with an IC_{50} inhibitory concentration of 3.4 μM, whereas contortisiliosides A (**1**) and C (**3**) were only moderately cytotoxic to both BAC1.2F5 macrophages and EL-4 cells (*Table 1*). Contortisiliosides D–G (**4**–**7**) did not show any apparent cytotoxic activities against the three cell lines.

The above results imply that *1*) the cinnamoyl group at C(21) of the aglycon is essential for the cytotoxicities against macrophages and lymphoma cells, and *2*) that the selective cytotoxicity against macrophages is particular sensitive to the structures of the oligosaccharide moieties. It should be noted that the macrophage death caused by **2** was shown to be neither necrotic nor apoptosis-inducing according to the unique morphological change of the killed cells, whose cytosols were converted into large vacuolar structures (*Fig. 2*), as well as by the TUNEL assay [15], giving rise to no fluorescent emission (*Fig. 3*). Although the precise mechanism is unknown, it is possible that **2** caused the fusion of endosomal membranes to large vacuoles, after being internalized by macrophages. Further investigation into the detailed mechanism of action of **2** is now underway.

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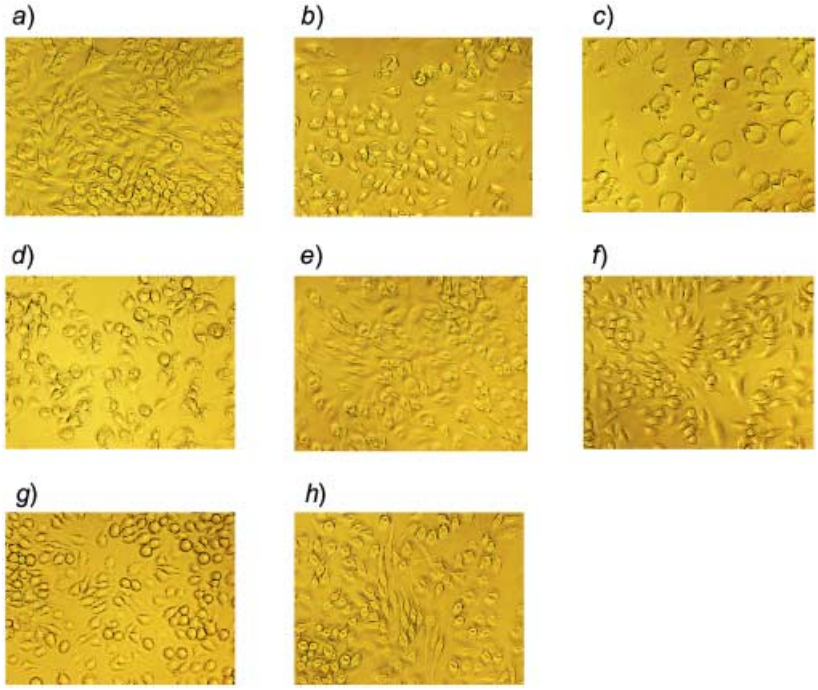

Fig. 2. *Photomicrographs of Cultured BAC1.2F5 Cells.* The cells were cultured for 72 h in the presence of 20% L-cell-conditioned medium, with 10 μg ml^{-1} each of *b*) **1**, *c*) **2**, *d*) **3**, *e*) **4**, *f*) **5**, *g*) **6**, and *h*) **7**. For vehicle control, BAC1.2F5 cells were cultured for 72 h in the presence of *a*) 20% L-cell-conditioned medium only.

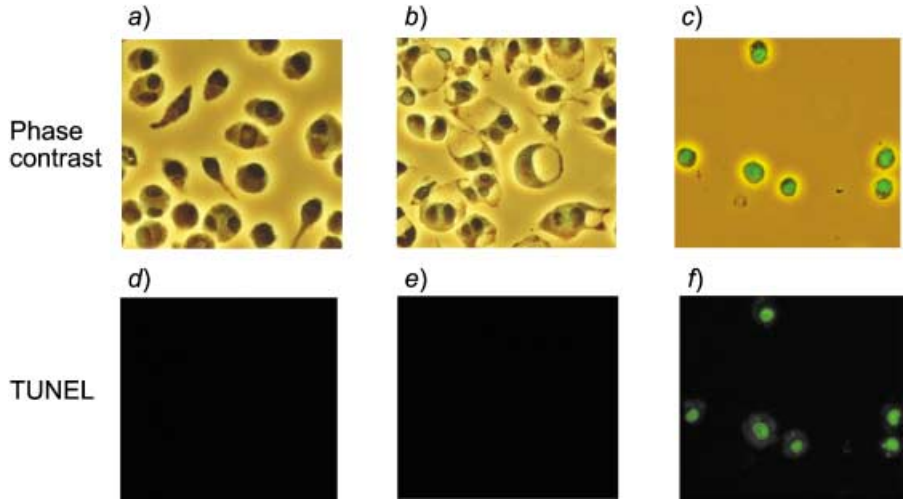

Fig. 3. *Analysis of the DNA Cleavage of Cultured BAC1.2F5 Cells.* The cells were cultured for 72 h either without (*a*, *d*) or with (*b*, *e*) contortisilioside B (**2**; 10 μg ml^{-1}) in the presence of 20% L-cell-conditioned medium. For positive controls (*c*, *f*), the cells were cultured for 24 h in 20% L-cell-conditioned medium containing 'cycloheximide' (=dihydro-4-[(2*R*)-2-((1*S*,3*S*,5*S*)-3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-pyridine-2,6(1*H*,3*H*)-dione; 1.0 μg ml^{-1}). The cells were stained by the TUNEL method, and observed by means of phase-contrast microscopy (*a*–*c*). DNA Breaks represented by fluorescein were detected by fluorescence microscopy (*d*–*f*).

Table 1. *Cytotoxic Activities of Contortisiliosides A–G* (**1**–**7**) *against BAC1.2F5, EL-4, and L-929 Cells.* Values for IC_{50} ('inhibitory concentration fifty') have been determined from three experiments, each performed in triplicate.

	IC_{50} [μM]		
Compound	BAC1.2F5	EL-4	L-929
1	23 ± 1.2	21 ± 0.9	> 50
2	3.4 ± 0.5	16 ± 1.1	> 50
3	21 ± 2.5	15 ± 0.8	> 50
4	> 50	> 50	> 50
5	> 50	> 50	> 50
6	> 50	> 50	> 50
7	> 50	> 50	> 50

Experimental Part

General. Column chromatography (CC): Silica gel (*Fuji-Silysia Chemical*, Aichi, Japan), and octadecylsilanized (ODS) silica gel (*Nacalai Tesque*, Kyoto, Japan). TLC: Precoated silica gel *60 F_{254}* (0.25 mm, *Merck*, Darmstadt, Germany) and *RP-18-F_{254}-S* (0.25 mm, *Merck*) plates; visualization by spraying with 10% aq. H_2SO_4 soln., followed by heating. HPLC: *CCPM* Pump (*Tosoh*, Tokyo, Japan), *CCP PX-8010* controller (*Tosoh*), *RI-8010* detector (*Tosoh*), *Shodex OR-2* detector (*Showa-Denko*, Tokyo, Japan), and *Rheodyne* injection port with a 20-μl sample loop. Anal. HPLC: *Capcell Pak-NH_2-UG80* column (4.6 mm × 250 mm, 5 μm, *Shiseido*, Tokyo, Japan) or *Capcell Pak-NH_2-SG80* column (4.6 mm × 250 mm, 5 μm, *Shiseido*); detection by refractive index (RI) and/or optical rotation (OR); t_R in min. Optical rotations: *Jasco DIP-360* automatic digital polarimeter. IR Spectra: *Jasco FT-IR 620* spectrophotometer. UV Spectra: *Jasco V-520* spectrophotometer. NMR Spectra: *Bruker DRX-500* spectrometer (500 and 125 MHz for ^{1}H- and ^{13}C-NMR, resp.) using standard *Bruker* pulse programs; chemical shifts δ in ppm rel. to $SiMe_4$ as internal standard. MS: *Finnigan MAT TSQ-700* mass spectrometer; in *m*/*z*.

Plant Material. The pericarps of *Enterolobium contortisiliquum* were collected in the fields of Igarapava City, São Paulo State, Brazil, in September 1998. The plant was identified by *M. H.*, and a specimen has been deposited at the Animal Health Center, Biological Institute, São Paulo State, Brazil (voucher No. 0202-EC).

Extraction and Isolation. The dried plant material (767 g) was extracted twice with hot H_2O. The aq. extract was concentrated under reduced pressure, the viscous concentrate was partitioned between BuOH and H_2O, and the extracts were evaporated. The BuOH-soluble residue (31.6 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH/H_2O 20:10:1, 7:4:1, 6:4:1, then MeOH): Fractions I–VII. Fraction III was submitted to repeated CC (1. SiO_2, $CHCl_3$/MeOH/H_2O 20:10:1, then 7:4:1; 2. ODS-SiO_2, MeOH/H_2O 13:5, then 1:2) to yield **1** (67.9 mg) and **2** (82.5 mg). Fraction IV was submitted to CC (1. SiO_2, $CHCl_3$/MeOH/H_2O 35:20:3, then 70:40:9; 2. ODS-SiO_2, MeCN/H_2O 1:1, then 2:5) to yield **3** (48.7 mg) and **6** (43.0 mg). Fraction V was submitted to CC (1. SiO_2, $CHCl_3$/MeOH/H_2O 35:20:4, then 70:40:9; 2. ODS-SiO_2, MeCN/H_2O 1:3) to yield **4** (84.4 mg) and **5** (78.7 mg). Fraction VI was submitted to CC (1. SiO_2, $CHCl_3$/MeOH/H_2O 6:4:1; 2. ODS-SiO_2, MeCN/H_2O 1:1, then 3:7) to yield **7** (34.9 mg).

Contortisilioside A (=*β-*D*-glucopyranosyl-(1 → 3)-α-*L*-rhamnopyranosyl-(1 → 2)-*β-D*-glucopyranosyl 21β-[((*E*)-3-phenylprop-2-enoyl)oxy]-3β-[(β-*D*-xylopyranosyl-(1 → 2)-α-*L*-arabinopyranosyl-(1 → 6)-[β-*D*-glucopyranosyl-(1 → 2)]-β-*D*-glucopyranosyl)oxy]olean-12-en-28-oate*; **1**). Amorphous solid. $[\alpha]_D^{27} = -22.0$ ($c = 0.10$, MeOH). UV (MeOH): 277 (3.97). IR (Film): 3387 (OH); 2938 (CH); 1755, 1686 (C=O); 1637, 1591, 1075, 1047. ^{1}H-NMR (500 MHz, (D_5)pyridine): 7.92 (*d*, *J* = 16.0, H–C(7) of Cin)[1]); 7.65 (2*dd*, *J* = 7.6, 1.5, H–C(2) and H–C(6) of Cin); 7.37 (3*m*, H–C(3), H–C(4), and H–C(5) of Cin); 6.71 (*d*, *J* = 16.0, H–C(8) of Cin); 5.49 (*t*-like, *J* = 3.2, H–C(12)); 5.32 (*dd*, *J* = 11.9, 4.7, H–C(21)); 3.48 (*dd*, *J* = 11.5, 4.3, H–C(3)); 3.22 (*dd*, *J* = 13.7, 4.2, H–C(18)); 1.34 (*s*, Me(23)); 1.29 (*s*, Me(27)); 1.21 (*s*, Me(24)); 1.17 (*s*, Me(26)); 1.01 (*s*, Me(30)); 1.00 (*s*, Me(29)); 0.97 (*s*, Me(25)). ^{13}C-NMR (125 MHz, (D_5)pyridine): see *Tables 2* and *3*. FAB-MS (neg.): 1659 ($[M-H]^-$). Anal. calc. for $C_{79}H_{120}O_{37} \cdot 6\,H_2O$ (1769.9053): C 53.61, H 7.52; found: C 53.49, H 7.32.

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[1]) Trivial atom numbering of the cinnamoyl (Cin) group; see chemical formulae.

Table 2. *^1H- (500 MHz) and ^{13}C-NMR (125 MHz) Data for the Glycosyl Moieties of* **1** *and* **3**. Solvent: (D_5)pyridine; δ in ppm, J in Hz. Abbreviations: Araf, arabinofuranosyl; Arap, arabinopyranosyl; Gal, galactopyranosyl; Glc, Glucopyranosyl; Rha, rhamnopyranosyl; Xyl, xylopyranosyl.

	1			**3**	
	^1H	^{13}C		^1H	^{13}C
Glc:			*Glc:*		
H–C(1′)	4.89 (*d*, *J* = 7.1)	104.9	H–C(1′)	4.89 (*d*, *J* = 6.9)	104.8
H–C(2′)	4.24 (*dd*, *J* = 8.5, 7.1)	83.5	H–C(2′)	4.25 (*dd*, *J* = 8.2, 6.9)	83.1
H–C(3′)	4.26 (*dd*, *J* = 8.5, 8.5)	78.0	H–C(3′)	4.24 (*dd*, *J* = 8.2, 8.2)	78.1
H–C(4′)	4.08 (*dd*, *J* = 8.5, 8.5)	71.9	H–C(4′)	4.05 (*dd*, *J* = 8.2, 8.2)	71.4
H–C(5′)	4.01 (*ddd*, *J* = 8.5, 2.8, 2.0)	76.0	H–C(5′)	4.04 (*m*)	76.7
CH_2(6′)	4.84 (*dd*, *J* = 11.7, 2.8, H_a)	69.3	CH_2(6′)	4.72 (br. *d*, *J* = 11.3, H_a)	69.9
	4.21 (*dd*, *J* = 11.7, 2.0, H_b)			4.32 (br. *d*, *J* = 11.3, H_b)	
Glc:			*Glc:*		
H–C(1″)	5.36 (*d*, *J* = 7.6)	106.1	H–C(1″)	5.40 (*d*, *J* = 7.6)	105.9
H–C(2″)	4.13 (*dd*, *J* = 9.0, 7.6)	77.2	H–C(2″)	4.13 (*dd*, *J* = 9.0, 7.6)	77.1
H–C(3″)	4.25 (*dd*, *J* = 9.0, 9.0)	77.9	H–C(3″)	4.24 (*dd*, *J* = 9.0, 9.0)	77.9
H–C(4″)	4.30 (*dd*, *J* = 9.0, 9.0)	71.8	H–C(4″)	4.30 (*dd*, *J* = 9.0, 9.0)	71.8
H–C(5″)	3.93 (*ddd*, *J* = 9.0, 4.3, 2.4)	78.1	H–C(5″)	3.91 (*m*)	78.1
CH_2(6″)	4.51 (*dd*, *J* = 11.2, 4.3, H_a)	62.8	CH_2(6″)	4.51 (br. *d*, *J* = 11.2, H_a)	62.7
	4.45 (*dd*, *J* = 11.2, 2.4, H_b)			4.44 (br. *d*, *J* = 11.2, H_b)	
Arap:			*Gal:*		
H–C(1‴)	5.15 (*d*, *J* = 5.1)	102.4	H–C(1‴)	5.12 (*d*, *J* = 7.7)	103.9
H–C(2‴)	4.51 (*dd*, *J* = 6.5, 5.1)	80.7	H–C(2‴)	4.51 (*dd*, *J* = 8.6, 7.7)	82.8
H–C(3‴)	4.39 (*dd*, *J* = 6.5, 3.1)	72.6	H–C(3‴)	4.22 (*dd*, *J* = 8.6, 4.0)	75.1
H–C(4‴)	4.40 (br. *s*)	67.5	H–C(4‴)	4.57 (br. *d*, *J* = 4.0)	69.5
CH_2(5‴)	4.31 (*dd*, *J* = 11.5, 2.5, H_a)	64.3	H–C(5‴)	4.02 (*m*)	76.8
	3.77 (*dd*, *J* = 11.5, 2.3, H_b)		CH_2(6‴)	4.38 (H_a)	62.0
				4.23 (H_b)	
Xyl:			*Xyl:*		
H–C(1⁗)	4.99 (*d*, *J* = 7.0)	106.4	H–C(1⁗)	5.06 (*d*, *J* = 6.7)	107.2
H–C(2⁗)	4.03 (*dd*, *J* = 8.5, 7.0)	75.5	H–C(2⁗)	4.08 (*dd*, *J* = 8.5, 6.7)	76.2
H–C(3⁗)	4.07 (*dd*, *J* = 8.5, 8.5)	77.9	H–C(3⁗)	4.05 (*dd*, *J* = 8.5, 8.5)	77.6
H–C(4⁗)	4.15 (*ddd*, *J* = 10.7, 8.5, 4.7)	70.9	H–C(4⁗)	4.13 (*ddd*, *J* = 10.7, 8.5, 4.3)	70.8
CH_2(5⁗)	4.46 (*dd*, *J* = 11.5, 4.7, H_a)	67.3	CH_2(5⁗)	4.43 (*dd*, *J* = 11.1, 4.3, H_a)	67.2
	3.61 (*dd*, *J* = 11.5, 10.7, H_b)			3.58 (*dd*, *J* = 11.1, 10.7, H_b)	
Glc:			*Glc:*		
H–C(1⁗′)	6.16 (*d*, *J* = 8.1)	95.3	H–C(1⁗′)	6.11 (*d*, *J* = 7.7)	95.4
H–C(2⁗′)	4.33 (*dd*, *J* = 9.1, 8.1)	76.1	H–C(2⁗′)	4.15 (*dd*, *J* = 8.3, 7.7)	76.8
H–C(3⁗′)	4.23 (*dd*, *J* = 9.1, 9.1)	79.1	H–C(3⁗′)	4.17 (*dd*, *J* = 8.3, 8.3)	78.3
H–C(4⁗′)	4.25 (*dd*, *J* = 9.1, 9.1)	71.3	H–C(4⁗′)	4.21 (*dd*, *J* = 8.3, 8.3)	71.2
H–C(5⁗′)	3.99 (*m*)	79.1	H–C(5⁗′)	3.96 (*m*)	79.0
CH_2(6⁗′)	4.39 (br. *d*, *J* = 11.0, H_a)	62.1	CH_2(6⁗′)	4.39 (H_a)	62.1
	4.28 (br. *d*, *J* = 11.0, H_b)			4.28 (H_b)	
Rha:			*Rha:*		
H–C(1⁗″)	6.36 (br. *s*)	102.0	H–C(1⁗″)	6.03 (br. *s*)	101.8
H–C(2⁗″)	5.08 (br. *d*, *J* = 2.9)	71.4	H–C(2⁗″)	5.18 (br. *d*, *J* = 2.8)	70.8
H–C(3⁗″)	4.70 (*dd*, *J* = 9.3, 2.9)	83.9	H–C(3⁗″)	4.89 (*dd*, *J* = 9.2, 2.8)	82.0
H–C(4⁗″)	4.53 (*dd*, *J* = 9.3, 9.3)	72.9	H–C(4⁗″)	4.49 (*dd*, *J* = 9.2, 9.2)	79.1
H–C(5⁗″)	4.63 (*dq*, *J* = 9.3, 6.0)	69.9	H–C(5⁗″)	4.53 (*dq*, *J* = 9.2, 5.9)	69.0
Me(6⁗″)	1.77 (*d*, *J* = 6.0)	18.7	Me(6⁗″)	1.82 (*d*, *J* = 5.9)	18.8

Table 2 (cont.)

	1			**3**	
	^{1}H	^{13}C		^{1}H	^{13}C
Glc:			*Glc:*		
H–C(1‴‴‴‴‴)	5.45 (*d*, *J* = 7.8)	107.0	H–C(1‴‴‴‴‴)	5.34 (*d*, *J* = 7.8)	105.7
H–C(2‴‴‴‴‴)	4.09 (*dd*, *J* = 9.2, 7.8)	76.0	H–C(2‴‴‴‴‴)	3.99 (*dd*, *J* = 9.0, 7.8)	75.4
H–C(3‴‴‴‴‴)	4.28 (*dd*, *J* = 9.2, 9.2)	78.3	H–C(3‴‴‴‴‴)	4.20 (*dd*, *J* = 9.0, 9.0)	78.4
H–C(4‴‴‴‴‴)	4.26 (*dd*, *J* = 9.2, 9.2)	71.3	H–C(4‴‴‴‴‴)	4.11 (*dd*, *J* = 9.0, 9.0)	71.8
H–C(5‴‴‴‴‴)	3.98 (*m*)	78.4	H–C(5‴‴‴‴‴)	3.97 (*m*)	78.1
CH_2(6‴‴‴‴‴)	4.44 (br. *d*, *J* = 10.8, H_a)	62.4	CH_2(6‴‴‴‴‴)	4.49 (br. *d*, *J* = 10.6, H_a)	62.8
	4.35 (br. *d*, *J* = 10.8, H_b)			4.23 (br. *d*, *J* = 10.6, H_b)	
			Araf:		
			H–C(1‴‴‴‴‴)	6.23 (*d*, *J* = 1.7)	110.9
			H–C(2‴‴‴‴‴)	4.95 (*dd*, *J* = 4.5, 1.7)	84.8
			H–C(3‴‴‴‴‴)	4.78 (*dd*, *J* = 7.0, 4.5)	78.3
			H–C(4‴‴‴‴‴)	4.72 (br. *d*, *J* = 7.0)	84.7
			CH_2(5‴‴‴‴‴)	4.29 (br. *d*, *J* = 12.4, H_a)	62.4
				4.16 (br. *d*, *J* = 12.4, H_b)	

Acid Hydrolysis of Contortisilioside A (**1**). A soln. of **1** (20.2 mg) in 0.7M HCl in dioxane/H_2O 1:1 (3 ml) was heated at 95° for 1.5 h under Ar atmosphere. The mixture was diluted with H_2O (2 ml) and extracted with AcOEt (3 × 3 ml). The org. extract was chromatographed (SiO_2; $CHCl_3$/MeOH 19:1) to yield 3.8 mg of **1a**. The aq. residue was neutralized by passage through an *Amberlite IRA-93ZU* (*Organo*, Tokyo, Japan) column, passed through a *Sep-Pak-C_{18}* cartridge (*Waters*, Milford, MA, USA; eluting with 30% aq. MeOH), and analyzed by anal. HPLC (MeCN/H_2O 17:3; 0.9 ml min^{-1} flow rate; refractive-index (RI) and optical-rotation (OR) detection): t_R 7.56 (L-rhamnose, neg. OR), 8.78 (L-arabinose, pos. OR), 9.27 (D-xylose, pos. OR), 14.04 (D-glucose, pos. OR).

Contortisilioside B (= *β-D-glucopyranosyl-(1 → 3)-[α-L-arabinofuranosyl-(1 → 4)]-α-L-rhamnopyranopyranosyl-(1 → 2)-β-D-glucopyranosyl 21β-[((*E*)-3-phenylprop-2-enoyl)oxy]-3β-[(β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-[β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranosyl)oxy]olean-12-en-28-oate*; **2**). Amorphous solid. $[\alpha]_D^{27} = -28.0$ ($c = 0.10$, MeOH). UV (MeOH): 278 (4.09). IR (Film): 3387 (OH); 2935 (CH); 1751, 1707 (C=O); 1638, 1591, 1076, 1045. ^{1}H-NMR (500 MHz, (D_5)pyridine): 7.92 (*d*, *J* = 16.0, H–C(7) of Cin)[1]); 7.66 (2*d*-like, *J* = 7.1, H–C(2) and H–C(6) of Cin); 7.38 (3*m*, H–C(3), H–C(4), and H–C(5) of Cin); 6.71 (*d*, *J* = 16.0, H–C(8) of Cin); 6.23 (*d*, *J* = 1.6, H–C(1‴‴‴‴‴)); 6.12 (*d*, *J* = 7.5, H–C(1‴)); 6.07 (br. *s*, H–C(1‴‴‴‴)); 5.48 (*t*-like, *J* = 3.5 Hz, H–C(12)); 5.37 (*d*, *J* = 7.7, H–C(1″)); 5.34 (*d*, *J* = 8.0, H–C(1‴‴‴‴‴)); 5.29 (*dd*, *J* = 11.7, 4.6, H–C(21)); 5.15 (*d*, *J* = 5.0, H–C(1‴)); 4.99 (*d*, *J* = 7.0, H–C(1‴)); 4.96 (*dd*, *J* = 4.9, 1.6, H–C(2‴‴‴‴‴)); 4.87 (*d*, *J* = 6.8, H–C(1′)); 4.79 (*dd*, *J* = 7.3, 4.9, H–C(3‴‴‴‴‴)); 4.73 (br. *d*, *J* = 7.3, H–C(4‴‴‴‴‴)); 4.30 (*dd*, *J* = 11.9, 3.5, H_a–C(5‴‴‴‴‴)); 4.16 (*dd*, *J* = 11.9, 4.2, H_b–C(5‴‴‴‴‴)); 3.47 (*dd*, *J* = 11.3, 4.3, H–C(3)); 3.23 (*dd*, *J* = 13.5, 3.6, H–C(18)); 1.83 (*d*, *J* = 5.9, Me(6‴‴)); 1.36 (*s*, Me(27)); 1.29 (*s*, Me(23)); 1.19 (*s*, Me(24)); 1.18 (*s*, Me(26)); 1.02 (*s*, Me(30)); 0.99 (*s*, Me(29)); 0.97 (*s*, Me(25)). ^{13}C-NMR (125 MHz, (D_5)-pyridine): see *Tables 3* and *4*. FAB-MS (neg.): 1791 ($[M-H]^-$). Anal. calc. for $C_{84}H_{128}O_{41} \cdot 5.5\ H_2O$ (1893.0147): C 53.30, H 7.40; found: C 53.26, H 7.56.

Acid Hydrolysis of Contortisilioside B (**2**). Compound **2** (20.0 mg) was subjected to acid hydrolysis, as described for **1**, to afford **1a** (3.3 mg) and a sugar fraction (aq. residue). HPLC Analysis of the latter (see above) showed the presence of L-rhamnose, L-arabinose, D-xylose, and D-glucose at t_R 7.54 (L-rhamnose, neg. OR), 8.92 (L-arabinose, pos. OR), 9.23 (D-xylose, pos. OR), and 13.86 (D-glucose; pos. OR).

Contortisilioside C (= *β-D-glucopyranosyl-(1 → 3)-[α-L-arabinofuranosyl-(1 → 4)]-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl 21β-[((*E*)-3-phenylprop-2-enoyl)oxy]-3β-[(β-D-xylopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 6)-[β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranosyl)oxy]olean-12-en-28-oate*; **3**). Amorphous solid. $[\alpha]_D^{27} = -18.0$ ($c = 0.10$, MeOH). UV (MeOH): 279 (4.15). IR (Film): 3363 (OH); 2928 (CH); 1750, 1702 (C=O); 1635, 1595, 1074, 1042. ^{1}H-NMR (500 MHz, (D_5)pyridine): 7.92 (*d*, *J* = 16.0, H–C(7) of Cin)[1]); 7.67 (2 br. *d*, *J* = 7.0, H–C(2) and H–C(6) of Cin); 7.38 (3*m*, H–C(3), H–C(4), and H–C(5) of Cin); 6.70 (*d*, *J* = 16.0,

Table 3. *^{13}C-NMR* (125 MHz) *Chemical Shifts for the Aglycon Parts of* **1**–**7**. Solvent: (D_5)pyridine; δ_C in ppm. 'Cinnamoyl' = 3-phenylprop-2-enoyl.

	1	**2**	**3**	**4**	**5**	**6**	**7**
Steroidal Part:							
CH_2(1)	38.9	38.9	38.9	38.9	38.9	38.9	38.9
CH_2(2)	26.9	26.8	26.9	26.8	26.8	26.9	26.6
CH(3)	88.7	88.7	88.1	88.7	88.7	88.2	89.1
C(4)	39.7	39.7	39.7	39.7	39.7	39.7	39.6
CH(5)	56.0	56.0	55.9	56.0	56.0	56.0	55.9
CH_2(6)	18.8	18.8	18.8	18.8	18.8	18.8	18.8
CH_2(7)	33.4	33.4	33.4	33.4	33.5	33.5	33.6
C(8)	39.9	39.9	40.0	40.0	40.0	40.0	39.9
CH(9)	48.1	48.0	48.0	48.1	48.1	48.1	48.1
C(10)	37.1	37.1	37.1	37.1	37.1	37.1	37.0
CH_2(11)	23.9	23.9	23.9	23.9	23.9	23.9	23.8
CH(12)	123.1	123.1	123.1	123.0	123.0	123.0	123.1
C(13)	142.9	142.9	142.9	143.0	143.0	144.0	143.5
C(14)	42.3	42.3	42.3	42.3	42.4	42.3	42.4
CH_2(15)	28.7	28.6	28.5	28.8	28.8	28.7	28.8
CH_2(16)	24.8	24.8	24.8	24.8	24.9	24.9	24.9
C(17)	48.7	48.7	48.7	48.9	48.9	48.9	48.9
CH(18)	41.4	41.3	41.2	41.7	41.6	41.6	41.7
CH_2(19)	46.6	46.6	46.6	47.0	47.1	47.0	47.2
C(20)	35.4	35.4	35.4	36.7	36.6	36.6	36.6
CH(21)	75.6	75.6	75.6	72.4	72.4	72.4	72.3
CH_2(22)	36.5	36.5	36.5	40.7	40.6	40.6	40.6
Me(23)	28.1	28.1	28.0	28.1	28.1	28.0	28.1
Me(24)	16.9	17.0	16.9	16.9	17.0	17.0	17.0
Me(25)	15.8	15.8	15.8	15.8	15.8	15.9	15.8
Me(26)	17.5	17.4	17.4	17.5	17.4	17.4	17.4
Me(27)	25.8	25.8	25.9	25.9	25.9	26.0	25.8
C(28)	174.9	174.9	174.9	176.0	175.0	175.0	175.4
Me(29)	28.8	28.8	28.7	29.7	29.6	29.6	29.6
Me(30)	18.5	18.5	18.5	17.8	17.7	17.7	17.7
Cinnamoyl Group[1]):							
C(1)	135.0	135.0	134.9				
CH(2,6)	128.6	128.6	128.6				
CH(3,5)	129.3	129.3	129.3				
CH(4)	130.6	130.6	130.6				
CH(7)	144.9	145.0	145.0				
CH(8)	119.2	119.1	119.1				
C(9)	166.5	166.5	166.5				

H–C(8) of Cin); 5.45 (*t*-like, $J = 3.3$, H–C(12)); 5.27 (*dd*, $J = 11.8$, 4.6, H–C(21)); 3.62 (*dd*, $J = 11.5$, 4.7, H–C(3)); 3.21 (*dd*, $J = 13.6$, 3.9, H–C(18)); 1.38 (*s*, Me(27)); 1.30 (*s*, Me(23)); 1.20 (*s*, Me(24)); 1.17 (*s*, Me(26)); 1.01 (*s*, Me(30)); 0.98 (*s*, Me(25)); 0.95 (*s*, Me(29)). ^{13}C-NMR (125 MHz, (D_5)pyridine): see *Tables 2* and *3*. FAB-MS (neg.): 1821 ($[M-H]^-$). Anal. calc. for $C_{85}H_{130}O_{42} \cdot 7\,H_2O$ (1950.0641): C 52.35, H 7.44; found: C 52.45, H 7.50.

Acid Hydrolysis of Contortisilioside C (**3**). Compound **3** (20.3 mg) was subjected to acid hydrolysis, as described for **1**, to give **1a** (2.8 mg) and a sugar fraction (aq. residue). HPLC Analysis of the latter (see above) showed the presence of L-rhamnose, L-arabinose, D-xylose, D-galactose, and D-glucose at t_R 9.38 (L-rhamnose, neg. OR), 11.24 (L-arabinose, pos. OR), 12.07 (D-xylose, pos. OR), 18.71 (D-galactose, pos. OR), and 21.07 (D-glucose, pos. OR).

Table 4. *^{13}C-NMR* (125 MHz) *Chemical Shifts for the Carbohydrate Parts of* **2** *and* **4**–**7**. Solvent: (D_5)pyridine; δ_C in ppm. Abbreviations: Araf, arabinofuranosyl; Arap, arabinopyranosyl; Gal, galactopyranosyl; Glc, Glucopyranosyl; Rha, rhamnopyranosyl; Xyl, xylopyranosyl.

	2	**4**	**5**	**6**	**7**
Glc:					
CH(1′)	104.9	104.9	104.9	104.8	105.0
CH(2′)	83.3	83.4	83.3	83.2	83.5
CH(3′)	78.0	78.0	78.0	78.1	78.1
CH(4′)	71.9	71.8	71.9	71.4	71.6
CH(5′)	76.0	75.9	75.9	76.7	78.0
CH_2(6′)	69.3	69.3	69.3	69.9	62.8
Glc:					
CH(1″)	106.0	106.1	106.0	105.9	106.0
CH(2″)	77.1	77.1	77.1	77.2	77.1
CH(3″)	77.9	77.9	77.9	77.9	78.0
CH(4″)	71.8	71.8	71.8	71.8	71.8
CH(5″)	78.1	78.1	78.1	78.1	78.1
CH_2(6″)	62.8	62.8	62.8	62.7	62.8
Arap:					
CH(1‴)	102.3	102.4	102.3		
CH(2‴)	80.7	80.7	80.7		
CH(3‴)	72.6	72.6	72.6		
CH(4‴)	67.4	67.5	67.4		
CH_2(5‴)	64.3	64.3	64.3		
Gal:					
CH(1‴)				103.9	
CH(2‴)				82.8	
CH(3‴)				75.2	
CH(4‴)				69.6	
CH(5‴)				76.7	
CH_2(6‴)				62.0	
Xyl:					
CH(1⁗)	106.4	106.4	106.4	107.2	
CH(2⁗)	75.5	75.5	75.5	76.0	
CH(3⁗)	77.9	77.9	77.9	77.6	
CH(4⁗)	70.8	70.9	70.8	70.8	
CH_2(5⁗)	67.3	67.3	67.3	67.2	
Glc:					
CH(1′′′′′)	95.4	94.9	95.2	95.3	95.2
CH(2′′′′′)	76.7	75.9	76.3	76.4	76.3
CH(3′′′′′)	78.4	79.3	78.6	78.6	78.6
CH(4′′′′′)	71.2	71.4	71.3	71.3	71.3
CH(5′′′′′)	79.0	79.0	79.0	79.0	79.0
CH_2(6′′′′′)	62.1	62.0	62.0	62.0	62.0
Rha:					
CH(1′′′′′)	101.8	101.5	101.5	101.5	101.5
CH(2′′′′′)	70.8	71.4	71.0	71.0	71.0
CH(3′′′′′)	82.0	83.2	81.9	81.9	81.9
CH(4′′′′′)	79.1	73.0	79.3	79.3	79.4
CH(5′′′′′)	68.9	69.7	68.7	68.8	68.7
Me(6′′′′′)	18.8	18.6	18.6	18.8	18.8

Table 4 (cont.)

	2	**4**	**5**	**6**	**7**
Glc:					
CH(1‴‴‴‴)	105.7	106.6	105.7	105.7	105.7
CH(2‴‴‴‴)	75.4	75.7	75.4	75.4	75.4
CH(3‴‴‴‴)	78.4	78.4	78.3	78.4	78.4
CH(4‴‴‴‴)	71.8	71.3	71.8	71.8	71.8
CH(5‴‴‴‴)	78.1	78.4	78.1	78.1	78.4
CH_2(6‴‴‴‴)	62.8	62.4	62.7	62.8	62.4
Araf:					
CH(1‴‴‴‴‴)	110.9	111.0	111.1	111.1	
CH(2‴‴‴‴‴)	84.8	84.8	84.8	84.9	
CH(3‴‴‴‴‴)	78.3	78.3	78.4	78.4	
CH(4‴‴‴‴‴)	84.7	84.8	84.8	84.8	
CH_2(5‴‴‴‴‴)	62.4	62.5	62.5	62.7	

Contortisilioside D (= *β*-D-*glucopyranosyl-(1 → 3)-α*-L-*rhamnopyranosyl-(1 → 2)-β*-D-*glucopyranosyl 21β-hydroxy-3β-[(β*-D-*xylopyranosyl-(1 → 2)-α*-L-*arabinopyranosyl-(1 → 6)-[β*-D-*glucopyranosyl-(1 → 2)]-β*-D-*glucopyranosyl)oxy]olean-12-en-28-oate*; **4**). Amorphous solid. $[\alpha]_D^{27} = -14.0$ ($c = 0.10$, MeOH). IR (Film): 3387 (OH), 2934 (CH), 1749 (C=O), 1074, 1046. ^{1}H-NMR (500 MHz, (D_5)pyridine): 6.43 (br. *s*, H−C(1‴‴‴‴)); 6.19 (*d*, *J* = 8.1, H−C(1‴‴‴)); 5.49 (*d*, *J* = 7.8, H−C(1‴‴‴‴‴)); 5.46 (*t*-like, *J* = 3.8, H−C(12)); 5.35 (*d*, *J* = 7.6, H−C(1″)); 5.14 (*d*, *J* = 5.0, H−C(1‴)); 4.98 (*d*, *J* = 7.0, H−C(1‴‴)); 4.88 (*d*, *J* = 6.8, H−C(1′)); 3.89 (*dd*, *J* = 12.0, 4.6, H−C(21)); 3.44 (*dd*, *J* = 11.4, 4.0, H−C(3)); 3.25 (*dd*, *J* = 13.5, 3.4, H−C(18)); 1.69 (*d*, *J* = 5.9, Me(6‴‴‴‴)); 1.32 (2*s*, Me(23), Me(27)); 1.21 (*s*, Me(29)); 1.19 (*s*, Me(24)); 1.16 (*s*, Me(26)); 1.10 (*s*, Me(30)); 0.96 (*s*, Me(25)). ^{13}C-NMR (125 MHz, (D_5)pyridine): see *Tables 3* and *4*. FAB-MS (neg.): 1529 ($[M-H]^-$). Anal. calc. for $C_{70}H_{114}O_{36} \cdot 7\,H_2O$ (1657.7732): C 50.72, H 7.75; found: C 50.58, H 7.63.

Acid Hydrolysis of Contortisilioside D (**4**). Compound **4** (19.9 mg) was subjected to acid hydrolysis, as described for **1**, to give **4a** (2.9 mg) and a sugar fraction (aq. residue). HPLC Analysis of the latter (see above) showed the presence of L-rhamnose, L-arabinose, D-xylose, and D-glucose at t_R 7.54 (L-rhamnose, neg. OR), 8.92 (L-arabinose, pos. OR), 9.23 (D-xylose, pos. OR), and 14.10 (D-glucose, pos. OR).

Contortisilioside E (= *β*-D-*glucopyranosyl-(1 → 3)-[α*-L-*arabinofuranosyl-(1 → 4)]-α*-L-*rhamnopyranosyl-(1 → 2)-β*-D-*glucopyranosyl 21β-hydroxy-3β-[(β*-D-*xylopyranosyl-(1 → 2)-α*-L-*arabinopyranosyl-(1 → 6)-[β*-D-*glucopyranosyl-(1 → 2)]-β*-D-*glucopyranosyl)oxy]olean-12-en-28-oate*; **5**). Amorphous solid. $[\alpha]_D^{27} = -22.0$ ($c = 0.10$, MeOH). IR (Film): 3387 (OH), 2939 (CH), 1750 (C=O), 1074, 1044. ^{1}H-NMR (500 MHz, (D_5)pyridine): 6.21 (*d*, *J* = 1.6, H−C(1‴‴‴‴‴)); 6.16 (br. *s*, H−C(1‴‴‴‴)); 6.13 (*d*, *J* = 7.5, H−C(1‴‴‴)); 5.48 (*t*-like, *J* = 3.5, H−C(12)); 5.36 (2*d*, *J* = 7.6, H−C(1″), H−C(1‴‴‴‴‴)); 5.14 (*d*, *J* = 4.8, H−C(1‴)); 4.98 (*d*, *J* = 7.0, H−C(1‴‴)); 4.86 (*d*, *J* = 6.9, H−C(1′)); 3.91 (*dd*, *J* = 12.6, 3.8, H−C(21)); 3.44 (*dd*, *J* = 11.5, 4.3, H−C(3)); 3.23 (*dd*, *J* = 13.6, 3.3, H−C(18)); 1.79 (*d*, *J* = 6.0, Me(6‴‴‴‴)); 1.39 (*s*, Me(27)); 1.28 (*s*, Me(23)); 1.19 (*s*, Me(29)); 1.18 (6*s*, Me(24), Me(26)); 1.07 (*s*, Me(30)); 0.97 (*s*, Me(25)). ^{13}C-NMR (125 MHz, (D_5)pyridine): see *Tables 3* and *4*. FAB-MS (neg.): 1661 ($[M-H]^-$). Anal. calc. for $C_{75}H_{122}O_{40} \cdot 6\,H_2O$ (1771.8749): C 50.84, H 7.62; found: C 50.87, H 7.56.

Acid Hydrolysis of Contortisilioside E (**5**). Compound **5** (20.1 mg) was subjected to acid hydrolysis, as described for **1**, to give **4a** (2.8 mg) and a sugar fraction (aq. residue). HPLC Analysis of the latter (see above) showed the presence of L-rhamnose, L-arabinose, D-xylose, and D-glucose at t_R 7.51 (L-rhamnose, neg. OR), 8.95 (L-arabinose, pos. OR), 9.24 (D-xylose, pos. OR), and 13.96 (D-glucose, pos. OR).

Contortisilioside F (= *β*-D-*glucopyranosyl-(1 → 3)-[α*-L-*arabinofuranosyl-(1 → 4)]-α*-L-*rhamnopyranosyl-(1 → 2)-β*-D-*glucopyranosyl 21β-hydroxy-3β-[(β*-D-*xylopyranosyl-(1 → 2)-β*-D-*galactopyranosyl-(1 → 6)-[β*-D-*glucopyranosyl-(1 → 2)]-β*-D-*glucopyranosyl)oxy]olean-12-en-28-oate*; **6**). Amorphous solid. $[\alpha]_D^{27} = -16.0$ ($c = 0.10$, MeOH). IR (Film): 3364 (OH), 2937 (CH), 1750 (C=O), 1073, 1042. ^{1}H-NMR (500 MHz, (D_5)pyridine): 6.21 (*d*, *J* = 1.6, H−C(1‴‴‴‴‴)); 6.14 (br. *s*, H−C(1‴‴‴‴)); 6.13 (*d*, *J* = 7.5, H−C(1‴‴‴)); 5.45 (*t*-like, *J* = 3.8, H−C(12)); 5.40 (*d*, *J* = 7.6, H−C(1″)); 5.37 (*d*, *J* = 7.7, H−C(1‴‴‴‴‴)); 5.13 (*d*, *J* = 7.7, H−C(1‴)); 5.07 (*d*, *J* = 7.0, H−C(1‴‴)); 4.89 (*d*, *J* = 7.2, H−C(1′)); 3.88 (overlapping, H−C(21)); 3.58 (*dd*, *J* = 11.1, 3.3,

H–C(3)); 3.22 (*dd*, *J* = 14.2, 3.8, H–C(18)); 1.79 (*d*, *J* = 6.1, Me(6‴‴‴)); 1.41 (*s*, Me(27)); 1.30 (*s*, Me(23)); 1.21 (*s*, Me(24)); 1.18 (*s*, Me(26)); 1.17 (*s*, Me(29)); 1.06 (*s*, Me(30)); 0.98 (*s*, Me(25)). ^{13}C-NMR (125 MHz, (D_5)pyridine): see *Tables 3* and *4*. FAB-MS (neg.): 1691 ($[M-H]^-$). Anal. calc. for $C_{76}H_{124}O_{41} \cdot 7\,H_2O$ (1819.9167): C 50.16, H 7.64; found: C 50.06, H 7.77.

Acid Hydrolysis of Contortisilioside F (**6**). Compound **6** (20.3 mg) was subjected to acid hydrolysis, as described for **1**, to give **4a** (2.7 mg) and a sugar fraction (aq. residue). HPLC Analysis of the latter (see above) showed the presence of L-rhamnose, L-arabinose, D-xylose, D-galactose, and D-glucose at t_R 9.32 (L-rhamnose, neg. OR), 11.19 (L-arabinose, pos. OR), 12.07 (D-xylose, pos. OR); 18.63 (D-galactose, pos. OR), and 20.00 (D-glucose, pos. OR).

Contortisilioside G (=*β*-D-*glucopyranosyl-(1* → *3)-[α*-L-*arabinofuranosyl-(1* → *4)]-α*-L-*rhamnopyranosyl-(1* → *2)-β*-D-*glucopyranosyl 3β-[(β*-D-*glucopyranosyl-(1* → *2)-β*-D-*glucopyranosyl)oxy]-21β-hydroxyolean-12-en-28-oate*; **7**). Amorphous solid. $[\alpha]_D^{27} = -22.0$ (*c* = 0.10, MeOH). IR (Film): 3387 (OH), 2940 (CH), 1751 (C=O), 1074, 1040. ^{1}H-NMR (500 MHz, (D_5)-pyridine): 6.21 (*d*, *J* = 1.6, H–C(1⁗⁗⁗)); 6.17 (br. *s*, H–C(1⁗⁗)); 6.13 (*d*, *J* = 7.3, H–C(1⁗⁗)); 5.52 (*t*-like, *J* = 3.7, H–C(12)); 5.36 (2*d*, *J* = 7.7, H–C(1″), H–C(1⁗⁗⁗)); 4.88 (*d*, *J* = 7.2, H–C(1′)); 3.93 (*dd*, *J* = 11.7, 4.7, H–C(21)); 3.28 (*dd*, *J* = 11.6, 4.3, H–C(3)); 3.26 (*dd*, *J* = 14.2, 4.0, H–C(18)); 1.79 (*d*, *J* = 6.0, Me(6⁗⁗⁗)); 1.37 (*s*, Me(27)); 1.27 (*s*, Me(23)); 1.22 (*s*, Me(29)); 1.18 (6*s*, Me(24), Me(26)); 1.07 (*s*, Me(30)); 0.93 (*s*, Me(25)). ^{13}C-NMR (125 MHz, (D_5)pyridine): see *Tables 3* and *4*. FAB-MS (neg.): 1397 ($[M-H]^-$). Anal. calc. for $C_{65}H_{106}O_{32} \cdot 5.5\,H_2O$ (1498.6331): C 52.10, H 7.87; found: C 52.00, H 7.94.

Acid Hydrolysis of Contortisilioside G (**7**). Compound **7** (20.1 mg) was subjected to acid hydrolysis, as described for **1**, to give **4a** (3.2 mg) and a sugar fraction (aq. residue). HPLC Analysis of the latter (see above) showed the presence of L-rhamnose, L-arabinose, and D-glucose at t_R 7.54 (L-rhamnose, neg. OR), 8.92 (L-arabinose, pos. OR), and 13.94 (D-glucose, pos. OR).

Cell-Culture Assay. Cells of BAC1.2F5 murine macrophages [16] were kindly provided by Dr. *E. Richard Stanley*, Einstein College of Medicine, NY, USA. The cells were maintained in RPMI-1640 medium (*Nissui-Seiyaku*, Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS; *Iwaki Glass*, Chiba, Japan), penicillin (100 U ml^{-1}), kanamycin (60 μg ml^{-1}), and 20% L-cell-conditioned medium as a source of CSF-1 [17]. For cytotoxicity assay, the cells were cultured in 96-well microplates (*Iwaki Glass*) at 10^4 cells/well with the samples to be tested, and simultaneously with 20% L-cell-conditioned medium for 72 h. EL-4 Mouse lymphoma cells and L-929 mouse fibroblasts were maintained in RPMI-1640 medium supplemented with 5% FBS. The cells were cultured in 96-well microplates at 10^4 cells/well with the samples to be tested for 72 h. Cell deaths of adherent BAC1.2F5 macrophages and L-929 fibroblasts were evaluated by crystal-violet staining. The cells in 96-well plates were stained with 0.2% crystal violet for 15 min, washed with PBS, and treated with sodium dodecyl sulfate (SDS; 100 μg ml^{-1}). The optical density at 595-nm wavelength was measured with a microplate reader (*Multiscan MS-UV*, *Labsystems*, Helsinki, Finland). Cell death of EL-4 cells was evaluated by an MTT assay [18]. The cells were added with 25 μl/well of MTT (5 mg ml^{-1}) and plates were incubated for an additional 3 h. Then, 150 μl of the supernatants was discarded, 100 μl of 0.04M HCl in i-PrOH was added to each well, and the optical density at 595 nm was measured.

DNA Breaks. DNA Breaks in the cultured BAC1.2F5 cells were detected *in situ* by terminal-deoxynucleotidyl-transferase-(TdT) mediated dUTP nick-end-labeling (TUNEL) [15]. The BAC1.2F5 cells (2.4×10^4 cells) were cultured in 8-well *Lab-Tek* slide chambers (*Nalge Nunc International*, Naperville, IL, USA) with the indicated concentrations of L-cell-conditioned medium and contortisilioside B (**2**) for 72 h. Once the damaged cells were detached from the culture substrate and removed by gentle washing with PBS (3 ×), the DNA breaks in the adherent cells were detected with the aid of *MEBSTEIN Apoptosis Kit Direct* (*Medical & Biological Laboratories*, Aichi, Japan). In the TUNEL reaction, free 3′-OH termini of the DNA strand breaks were enzymatically labeled with fluorescein-dUTP by means of TdT. The fluorescein incorporated into the cells was observed by fluorescence microscopy.

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