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Anti-Inflammatory Spirostanol and Furostanol Saponins from *Solanum macaonense*

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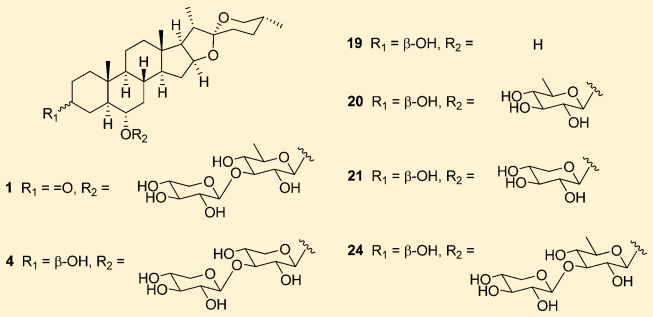
Supporting Information

ABSTRACT: Eight new spirostanol saponins, macaosides A–H (**1**–**8**), and 10 new furostanol saponins, macaosides I–R (**9**–**18**), together with six known spirostanol compounds (**19**–**24**) were isolated from *Solanum macaonense*. The structures of the new compounds were determined from their spectroscopic data, and the compounds were tested for in vitro antineutrophilic inflammatory activity. It was found that both immediate inflammation responses including superoxide anion generation and elastase release were significantly inhibited by treatment with compounds **20**, **21**, and **24** (superoxide anion generation: IC_{50} 7.0, 7.6, 4.0 μM; elastase release: IC_{50} 3.7, 4.4, 1.0 μM, respectively). However, compounds **1** and **4** exhibited effects on the inhibition of elastase release only, with IC_{50} values of 3.2 and 4.2 μM, respectively, while **19** was active against superoxide anion generation only, with an IC_{50} value of 6.1 μM. Accordingly, spirostanols may be promising lead compounds for further neutrophilic inflammatory disease studies.



The plant genus *Solanum* (Solanaceae) is distributed in tropical and subtropical regions of the world and has about 1200 species. Eighteen species are found in Taiwan, including *Solanum macaonense* Dunal, which grows in the southwestern plains.[1] As a part of an ongoing investigation on anti-inflammatory agents from Taiwanese *Solanum* species, it was found that the 95% MeOH-soluble and *n*-BuOH-soluble extracts of the aerial parts of *S. macaonense* showed activity against superoxide anion generation and elastase release induced by formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP)/cytochalasin B (CB) in human neutrophils. However, neither phytochemical nor biological studies of *S. macaonense* have been reported to date.

In the current study, chromatographic fractionation of the two above-mentioned extracts of *S. macaonense* led to the isolation of 24 compounds. Among these secondary metabolites, eight new spirostanol saponins, macaosides A–H (**1**–**8**), 10 new furostanol saponins, macaosides I–R (**9**–**18**), and six known spirostanol compounds, chlorogenin (**19**),[2] saponin Sc 4 (**20**),[2] saponin Sc 3 (**21**),[2] chlorogenin 6-*O*-β-D-glucopyranoside (**22**),[3] chlorogenin 6-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranoside (**23**),[4] and 6α-*O*-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl-(25*R*)-5α-spirostan-3β-ol (**24**),[2] were identified. Also, their antineutrophilic inflammatory effects and preliminary structure–activity relationship (SAR) properties were investigated.

RESULTS AND DISCUSSION

The MeOH extract of the aerial parts of *S. macaonense* was partitioned into *n*-hexane-, 95% MeOH-, *n*-BuOH-, and H_2O-soluble fractions, using an initial EtOAc and H_2O (1:1) extract. Fractionation of the active 95% MeOH- and *n*-BuOH-soluble

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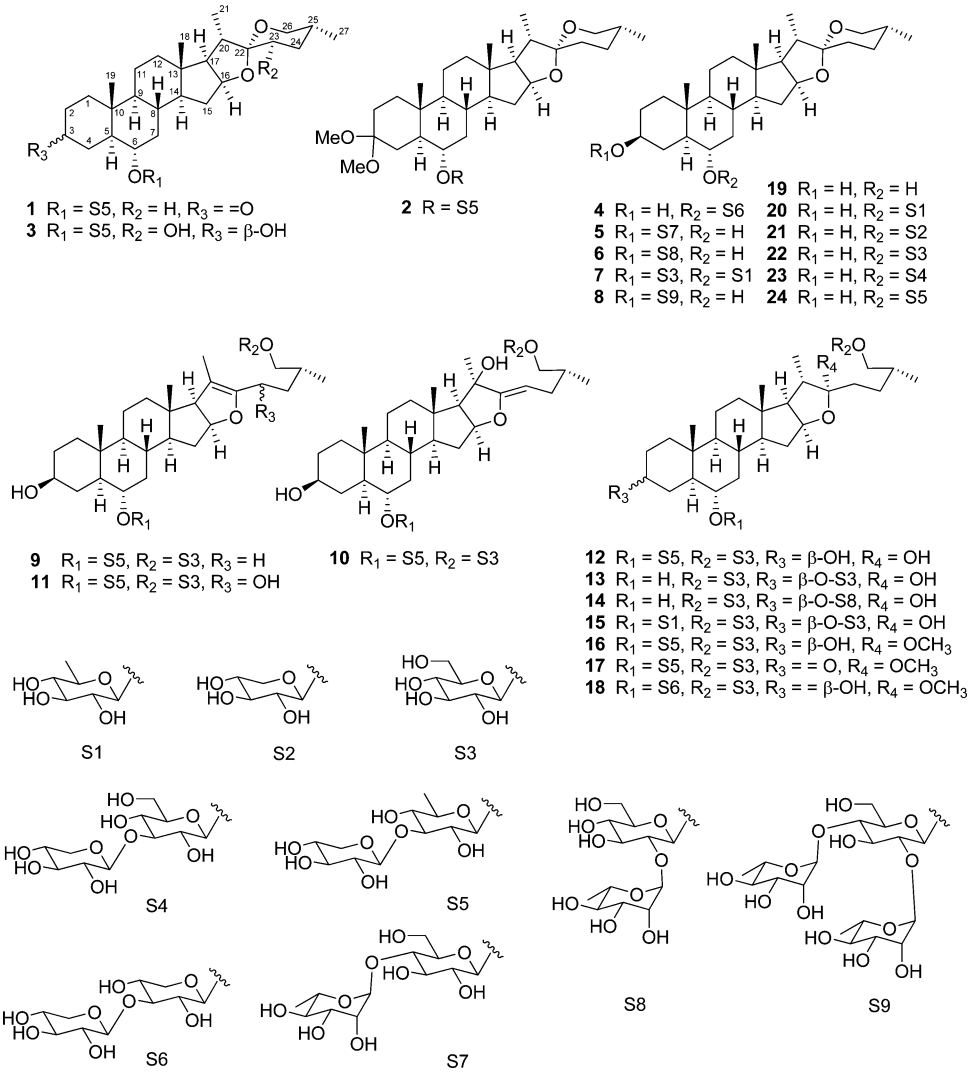
Chart 1



extracts afforded 18 new steroidal glycosides (**1**–**18**) and six known compounds (**19**–**24**).

HRESIMS of **1** showed a $[M + Na]^+$ ion at *m*/*z* 731.3982, indicating a molecular formula of $C_{38}H_{60}O_{12}Na$. The IR spectrum showed absorptions for hydroxy group (3401 cm^{-1}) and carbonyl (1705 cm^{-1}) functionalities. Thirty-eight carbon signals, including five methyls, 11 methylenes, 18 methines, and four quaternary carbons, were observed in the 1D NMR spectra of **1** (Tables 1 and 2). One quaternary carbon was identified as a carbonyl carbon on the basis of the chemical shift at δ_C 210.6. The 1D and HSQC NMR spectra displayed two anomeric [δ_H 4.75 (d, *J* = 7.7 Hz)/δ_C 105.4, δ_H 5.25 (d, *J* = 7.7 Hz)/δ_C 106.2] and two oxymethylene [δ_H 3.48 (t, *J* = 10.6 Hz), 3.57 o/δ_C 66.8, δ_H 3.69 o, 4.29 (dd, *J* = 11.5, 4.9 Hz)/δ_C 67.3] signals. The ^1H and ^{13}C NMR data of **1** were similar to those of **24**, a known spirostanol glycoside,[2] with differences apparent only in the chemical shifts at positions 2, 3, and 4 in the A ring [**1**: δ_H 2.31, 2.41 (H_2-2)/δ_C 38.1 (C-2), δ_C 210.6 (C-3), δ_H 2.46, 3.57 (H_2-4)/δ_C 39.8 (C-4); **24**: δ_H 1.74, 2.03 (H_2-2)/δ_C 32.1 (C-2), δ_H 3.77 (H-3)/δ_C 70.6 (C-3), δ_H 1.68, 3.21 (H_2-4)/δ_C 33.2 (C-4)]. The HMBC data (Figure 1) indicated that a carbonyl group (δ_C 210.6) was present at C-3 in **1** rather than the hydroxy group (δ_C 70.6) found in **24**. The 25*R* configuration of **1** was deduced from small differences in chemical shift values ($\Delta_{ab} = \delta_a - \delta_b$; 25*S*: Δ_{ab} > 0.35 ppm; 25*R*: Δ_{ab} < 0.2 ppm)[5] between the H_2-23 (Δ_{ab} = 0.05), H_2-24 (Δ_{ab} = 0), and H_2-26 (Δ_{ab} = 0.09) geminal protons, as well as the H_2-26 signal pattern [δ 3.48 (t, $J_{26eq,26ax}$ = 10.6 Hz, $J_{26eq,25ax}$ = 10.6 Hz) and δ 3.57, overlapped with H-4 and H-4′].[2,5−7] The absolute configurations of the sugar units, quinovose and xylose, were both determined to be D by GC analysis. Figure 1 shows key COSY and HMBC correlations observed for **1**. ROESY correlations (Figure 3) between H-5/H-9, H-6/Me-19, H-8, H-17/H-14, Me-21, and Me-18/H-8, H-20 as well as the absence of ROESY correlations between Me-19/H-5, H-9 or Me-18/H-14, H-16, H-17 indicated that the C-6 sugar group is α-oriented and the four A/B, B/C, C/D, and D/E ring junctions are *trans*, *trans*, *trans*, and *cis*, respectively. Macaoside A (**1**) was elucidated, therefore, as (25*R*)-6α-hydroxy-5α-spirostan-3-one 6-*O*-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranoside.

Compound **2** gave the molecular formula $C_{40}H_{66}O_{13}$, as established by HRESIMS (*m*/*z* 777.4399 $[M + Na]^+$). The 1D NMR data of **2** were also similar to those of **1** except that the chemical shift of one quaternary carbon was identified at δ_C 100.7 instead of 210.6 and two methoxy group signals appeared at δ_H 3.20 s/δ_C 47.3 and δ_H 3.24 s/δ_C 47.5, respectively. The HMBC ^3J correlations of H-1 (δ 1.21, 1.50) and OCH_3 (δ 3.20,

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Table 1. ^{1}H NMR Spectroscopic Data of Compounds 1−8 (500 MHz, C_5D_5N)[a]

position	1	2	3	4	5	6	7	8
1	1.20 (o)	1.21 (o)	0.96 (td, 13.6, 2.8)	0.96 (brt, 10.3)	0.91 (td, 10.2, 3.5)	0.91 (td, 10.7, 3.0)	0.83 (o)	0.92 (m)
	1.78 (o)	1.50 (o)	1.63 (o)	1.65 (o)	1.58 (o)	1.62 (o)	1.51 (o)	1.63 (o)
2	2.31 (brd, 13.8)	1.56 (o)	1.76 (o)	1.67 (o)	1.66 (o)	1.90 (m)	1.74 (m)	1.89 (m)
	2.41 (dd, 13.8, 7.8)	1.68 (o)	2.03 (brd, 10.6)	2.05 (o)	2.03 (m)	2.11 (o)	2.05 (m)	2.03 (m)
3			3.79 (o)	3.81 (m)	4.01 (o)	4.09 (m)	3.97 (m)	4.03 (m)
4	2.46 (m)	1.56 (o)	1.64 (o)	1.67 (o)	1.58 (o)	1.79 (o)	1.47 (o)	1.74 (m)
	3.57 (o)	1.97 (o)	3.20 (brd, 12.4)	3.18 (brd, 12.4)	3.13 (brd, 12.8)	3.19 (d, 12.7)	3.40 (brd, 11.5)	3.15 (brd, 11.5)
5	1.67 (o)	1.68 (o)	1.37 (t, 11.5)	1.39 (o)	1.20 (o)	1.20 (o)	1.24 (o)	1.23 (o)
6	3.69 (o)	3.64 (td, 10.8, 4.4)	3.65 (m)	3.71 (m)	3.60 (o)	3.60 (o)	3.60 (m)	3.59 (o)
7	1.18 (o)	1.23 (o)	1.23 (o)	1.23 (o)	1.20 (o)	1.15 (o)	1.19 (o)	1.21 (o)
	2.50 (dt, 12.8, 4.1)	2.52 (dt, 12.6, 4.2)	2.53 (dt, 12.5, 4.1)	2.45 (dt, 12.5, 3.9)	2.22 (dt, 12.6, 4.2)	2.22 (dt, 12.1, 4.0)	2.56 (dt, 13.0, 4.0)	2.21 (brd, 12.0)
8	1.69 (o)	1.66 (o)	1.63 (o)	1.68 (o)	1.64 (o)	2.11 (o)	1.60 (o)	1.63 (o)
9	0.62 (td, 11.4, 3.7)	0.63 (td, 10.6, 4.0)	0.61 (td, 11.0, 3.3)	0.61 (td, 9.6, 2.9)	0.66 (brt, 10.2)	0.66 (brt, 11.1)	0.55 (td, 10.0, 4.0)	0.66 (brt, 11.0)
11	1.26 (m)	1.25 (o)	1.23 (o)	1.26 (o)	1.22 (o)	1.26 (m)	1.19 (o)	1.27 (m)
	1.43 (o)	1.48 (o)	1.45 (brd, 13.2)	1.47 (m)	1.46 (o)	1.47 (o)	1.42 (o)	1.45 (o)
12	1.08 (o)	1.08 (o)	1.13 (o)	1.08 (o)	1.09 (td, 12.5, 3.7)	1.09 (m)	1.06 (m)	1.08 (m)
	1.69 (o)	1.68 (o)	1.76 (o)	1.70 (o)	1.70 (o)	1.68 (o)	1.64 (o)	1.69 (o)
14	1.08 (o)	1.11 (o)	1.17 (o)	1.10 (o)	1.17 (o)	1.15 (o)	1.07 (o)	1.21 (o)
15	1.47 (m)	1.43 (o)	1.52 (m)	1.41 (o)	1.45 (m)	1.44 (o)	1.45 (o)	1.45 (o)
	2.06 (m)	2.06 (m)	2.11 (o)	2.05 (o)	2.09 (m)	2.09 (o)	2.09 (m)	2.09 (m)
16	4.51 (q, 7.2)	4.48 (q, 7.4)	4.63 (q, 8.4)	4.50 (q, 7.7)	4.55 (q, 7.3)	4.55 (q, 7.3)	4.51 (q, 7.5)	4.54 (o)
17	1.78 (o)	1.79 (dd, 8.1, 8.1)	1.91 (dd, 8.6, 7.2)	1.79 (t, 7.3)	1.82 (dd, 7.8, 7.8)	1.83 (dd, 7.2, 7.2)	1.78 (dd, 8.5, 8.5)	1.83 (t, 8.0)
18	0.84 (s)	0.83 (s)	1.02 (s)	0.84 (s)	0.85 (s)	0.84 (s)	0.82 (s)	0.84 (s)
19	0.99 (s)	0.82 (s)	0.81 (s)	0.88 (s)	0.76 (s)	0.96 (s)	0.75 (s)	0.94 (s)
20	1.96 (quint, 6.8)	1.95 (o)	3.05 (quint, 7.0)	1.94 (quint, 6.8)	1.96 (quint, 7.1)	1.98 (quint, 6.8)	1.95 (quint, 7.0)	1.96 (quint, 6.5)
21	1.13 (d, 7.0)	1.13 (d, 6.9)	1.20 (d, 7.1)	1.13 (d, 6.9)	1.15 (d, 7.0)	1.15 (d, 7.0)	1.13 (d, 6.5)	1.14 (d, 6.5)
23	1.64 (o)	1.65 (2H, o)	3.86 (m)	1.65 (o)	1.58 (o)	1.58 (2H, o)	1.64 (2H, o)	1.66 (2H, o)
	1.69 (o)			1.74 (m)	1.66 (o)			
24	1.56 (2H, o)	1.49 (2H, o)	1.78 (o)	1.54 (2H, o)	1.66 (2H, o)	1.68 (2H, o)	1.57 (2H, o)	1.57 (2H, o)
			2.10 (o)					
25	1.56 (o)	1.56 (o)	1.83 (m)	1.54 (o)	1.58 (o)	1.62 (o)	1.57 (o)	1.57 (o)
26	3.48 (t, 10.6)	3.47 (t, 10.6)	3.47 (t, 10.9)	3.44 (t, 10.5)	3.50 (t, 10.6)	3.50 (t, 10.5)	3.48 (t, 10.5)	3.50 (o)
	3.57 (o)	3.57 (brd, 9.8)	3.54 (dd, 10.9, 3.3)	3.54 (dd, 11.0, 3.0)	3.59 (o)	3.59 (o)	3.57 (brd, 10.0)	3.57 (o)
27	0.68 (d, 5.4)	0.69 (d, 5.3)	0.73 (d, 6.2)	0.66 (d, 5.9)	0.70 (d, 5.4)	0.70 (d, 5.2)	0.69 (d, 4.5)	0.69 (d, 5.0)
OCH_3		3.20 (s)						
OCH_3		3.24 (s)						
	Qui	Qui	Qui	Xyl	Glc	Glc	Glc	Glc
1′	4.75 (d, 7.7)	4.79 (d, 7.4)	4.82 (d, 7.4)	4.80 (d, 7.3)	5.00 (o)	5.10 (d, 7.7)	5.14 (d, 8.0)	4.96 (o)
2′	4.03 (o)	4.02 (o)	4.05 (o)	4.04 (m)	3.99 (o)	4.28 (t, 8.6)	4.04 (t, 8.0)	4.20 (o)
3′	4.08 (t, 9.0)	4.09 (t, 8.9)	4.11 (m)	4.10 (o)	4.18 (t, 9.0)	4.21 (t, 8.8)	4.29 (o)	4.14 (m)
4′	3.57 (o)	3.58 (o)	3.62 (m)	4.10 (o)	4.46 (t, 9.3)	4.15 (o)	4.29 (o)	4.35 (o)
5′	3.73 (m)	3.72 (m)	3.74 (o)	3.63 (m)	3.64 (o)	3.79 (m)	3.86 (m)	3.50 (o)
				4.30 (m)				
6′	1.56 (d, 6.0)	1.56 (d, 6.0)	1.60 (d, 6.1)		4.13 (brd, 11.6)	4.36 (o)	4.35 (dd, 11.9, 5.3)	4.07 (m)
					4.27 (brd, 11.6)	4.50 (brd, 11.4)	4.43 (bd, 11.9, 2.3)	4.20 (o)
	Xyl	Xyl	Xyl	Xyl	Rha	Rha	Qui	Rha
1″	5.25 (d, 7.7)	5.27 (d, 7.7)	5.26 (d, 7.7)	5.27 (d, 7.6)	5.90 (s)	6.36 (s)	4.79 (d, 7.5)	6.36 (s)
2″	4.06 (o)	4.02 (o)	4.05 (o)	4.07 (m)	4.71 (brs)	4.76 (brs)	4.00 (t, 8.5)	4.78 (brs)
3″	4.14 (o)	4.14 (o)	4.18 (o)	4.19 (o)	4.59 (dd, 9.2, 3.1)	4.62 (dd, 9.2, 4.0)	4.13 (t, 8.5)	4.60 (brd, 9.0)
4″	4.14 (o)	4.15 (o)	4.20 (o)	4.19 (o)	4.35 (t, 9.3)	4.34 (o)	3.72 (t, 9.0)	4.33 (o)
5″	3.69 (o)	3.68 (m)	3.73 (o)	3.74 (m)	5.00 (o)	5.05 (m)	3.76 (m)	4.96 (o)

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Table 1. continued

position	1	2	3	4	5	6	7	8
	4.29 (dd, 11.5, 4.9)	4.29 (dd, 11.2, 5.0)	4.33 (dd, 11.3, 4.5)	4.34 (m)				
6″					1.74 (d, 6.2)	1.79 (d, 6.3)	1.63 (d, 6.0)	1.78 (d, 6.0)
								Rha
1‴								5.84 (s)
2‴								4.68 (brs)
3‴								4.54 (o)
4‴								4.33 (o)
5‴								4.90 (o)
6‴								1.63 (d, 6.0)

[a]o: overlapped with other signals; m: multiplet signals.

3.24) with C-3 (δ 100.7) as well as a long-range *W*-type COSY correlation of H-1 with OCH_3 suggested that the two methoxy groups are both at C-3. Key HMBC, COSY, and ROESY correlations are shown in Figures 1 and 3. Thus, compound **2** (macaoside B) was elucidated as (25*R*)-3,3-dimethoxy-5α-spirostan-6α-ol 6-*O*-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranoside, which is a ketal form of **1**. The dimethoxy group functionality may be produced from a carbonyl group under anhydrous conditions and acid catalysis.[3] However, compound **2** is not considered to be an extraction artifact from **1** since acidic or alkaline reagents were not applied during the extraction, partition, and isolation procedures.

The molecular formula of **3** was deduced as $C_{38}H_{62}O_{13}$ due to the appearance of a $[M + Na]^+$ ion at *m*/*z* 749.4086 in the HRESIMS. Furthermore, compound **3** showed similar spectroscopic data to five known 23-hydoxyspirostanols, namely, (22*R*,23*S*,25*R*)-3β,6α,23-trihydroxy-5α-spirostane 6-*O*-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranoside, (22*R*,23*S*,25*S*)-3β,6α,23-trihydroxy-5α-spirostane 6-*O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-quinovopyranoside, (22*R*,23*R*,25*S*)-3β,6α,23-trihydroxy-5α-spirostane 6-*O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-quinovopyranoside,[8] 6α-*O*-[β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl]-(25*S*)-5α-spirostan-3β,23β-ol, and 6α-*O*-[β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl]-(25*S*)-5α-spirostan-3β,23α-ol.[6] As shown in Figure 1, compound **3** has the same planar structure as the above-mentioned known glycosides. The configuration at C-22 in **3** was determined as *S* (22-α-*O*), since the signal of H-20 was shifted to δ 3.05 owing to the presence of an equatorial hydroxy group at C-23. Additionally, a 22*S* configuration was confirmed by the chemical shifts of H_3-21 (δ 1.20) and C-20 (δ 35.8) (22-α-*O*-23-hydoxyspirostanol: H_3-21 δ 1.07−1.26; C-20 δ 35.0−36.2; 22-β-*O*-23-hydoxyspirostanol: H_3-21 δ 1.53−1.54; C-20 δ 43.1−44.1).[7] ROESY correlations were observed between H-23 and H-25 (Figure 3), indicating H_3-27 to be in an equatorial position with a 25*R* configuration. The new compound **3** (macaoside C) was therefore identified as (22*S*,23*S*,25*R*)-3β,6α,23-trihydroxy-5α-spirostan 6-*O*-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranoside.

The HRESIMS of **4** showed a $[M + Na]^+$ ion at *m*/*z* 719.3987 ($C_{37}H_{60}O_{12}Na$), and hydroxy group (3242 cm^{-1}) absorption was observed in the IR spectrum. On the basis of its NMR data, compound **4** could be assigned as a having a C_{27} steroidal skeleton with two sugar moieties, resembling the spirostanol saponin **24**.[2] The 1D and HSQC NMR spectra displayed two anomeric [δ_H 4.80 (d, *J* = 7.3 Hz)/δ_C 106.0, δ_H 5.27 (d, *J* = 7.6 Hz)/δ_C 106.3] and three oxymethylene [δ_H 3.44 (t, *J* = 10.5 Hz), 3.54 (dd, *J* = 11.0, 3.0 Hz)/δ_C 66.8, δ_H 3.63 m, 4.30 m/δ_C 66.3, δ_H 3.74 m, 4.34 m/δ_C 67.4] signals. The key correlations shown in Figure 1 suggested that a hydroxy group substituent and a disaccharide unit were positioned at C-3 (δ 70.6) and C-6 (δ 78.9), respectively. In the HMBC spectrum, the anomeric protons H-1′ (δ 4.80, d, *J* = 7.3 Hz) and H-1″ (δ 5.27, d, *J* = 7.6 Hz) exhibited ^3J interactions with C-6 (δ 78.9) and C-3′ (δ 87.2), respectively. HMBC and COSY correlations (Figure 1) indicated that the glycosidic portion of **4** is a xylopyranosyl-(1→3)-xylopyranosyl unit, connected to C-6 of the aglycone moiety via an O-linkage. The absolute configuration of xylose was identified to be D by GC analysis. Key ROESY correlations are shown in Figure 3. Consequently, **4** (macaoside D) was characterized as (25*R*)-5α-spirostan-3β,6α-diol 6-*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranoside.

Compounds **5** and **6** gave the same molecular formula ($C_{39}H_{64}O_{13}Na$, *m*/*z* 763.4239 for **5**; $C_{39}H_{64}O_{13}Na$, 763.4235 for **6**) in the HRESIMS. The 1D NMR (Tables 1 and 2) and key COSY, HMBC, and ROESY correlations (Figures 1−3) supported the assignment of compounds **5** and **6** as chlorogenin 3β-*O*-[4-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside] and chlorogenin 3β-*O*-[2-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside], respectively. Both structures were synthesized by Song and associates in 2009 as H5N1 entry inhibitors.[9] Compounds **5** and **6**, which were isolated as new natural products, have been named macaosides E and F, respectively.

A $[M + Na]^+$ ion at *m*/*z* 763.4238 ($C_{39}H_{64}O_{13}$) was found in the HRESIMS of **7**. Compound **7** gave the same molecular formula and showed similar spectroscopic data. On the basis of the COSY and HMBC data of **7** (Figure 1), glucopyranosyl and quinovopyranosyl groups could be located at C-3 and C-6, in turn. Compound **7** (macaoside G) was elucidated as (25*R*)-5α-spirostan-3β,6α-diol 3-*O*-β-D-glucopyranosyl-6-*O*-β-D-quinovopyranoside.

Compound **8** (macaoside H) exhibited a $[M + Na]^+$ ion at *m*/*z* 909.4820 ($C_{45}H_{74}O_{17}Na$) in the HRESIMS. The 1D and HSQC NMR spectra displayed three anomeric [δ_H 4.96 o/δ_C 99.4, δ_H 6.36 s/δ_C 102.1, δ_H 5.84 s/δ_C 102.9], one oxymethylene [δ_H 3.50 o, 3.57 o/δ_C 66.9], and six methyl [δ_H 0.69 (d, *J* = 5.0 Hz)/δ_C 17.3, δ_H 0.84 s/δ_C 16.5, δ_H 0.94 s/δ_C 13.6, δ_H 1.14 (d, *J* = 6.5 Hz)/δ_C 15.0, δ_H 1.63 (d, *J* = 6.0 Hz)/δ_C 18.5, δ_H 1.78 (d, *J* = 6.0 Hz)/δ_C 18.6] signals. Using data shown in Figures 1 and 3, **8** was assigned as chlorogenin 3β-*O*-[2,4-di-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside], which was synthesized in 2005.[9,10] This is the first record of compound **8** from a natural source, and it was named macaoside H.

In their ^{13}C NMR spectra, compounds **9**−**18** showed a downfield-shifted signal at δ_C 74.9−75.5 in place of the spirostanol oxymethylene carbon signal (C-26) at δ_C 66.0−66.9

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Table 2. ^{13}C NMR Spectroscopic Data of Compounds 1−8 (125 MHz, C_5D_5N)

position	1	2	3	4	5	6	7	8
1	38.6	35.6	37.8	37.8	37.7	37.7	37.5	37.7
2	38.1	29.3	32.1	32.1	29.5	29.8	29.8	29.8
3	210.6	100.7	70.6	70.6	77.7	76.9	76.9	77.1
4	39.8	28.8	33.2	33.2	29.3	28.9	28.5	28.9
5	52.3	48.9	51.2	51.3	52.5	52.1	50.9	52.1
6	80.0	79.5	79.6	78.9	68.4	68.5	79.5	68.5
7	41.0	41.3	41.5	41.3	42.7	42.7	41.5	42.6
8	34.0	34.2	34.0	34.2	34.3	34.3	34.1	34.3
9	53.1	53.5	53.8	53.9	54.2	54.1	53.7	54.1
10	36.8	36.8	36.7	36.7	36.6	36.7	36.7	36.7
11	21.3	21.2	21.3	21.3	21.3	21.3	21.2	21.3
12	39.8	40.0	40.4	40.1	40.1	40.1	40.0	40.1
13	40.8	40.8	41.3	40.8	40.8	40.8	40.7	40.8
14	56.1	56.4	56.3	56.3	56.4	56.4	56.3	56.4
15	32.1	32.1	32.1	32.1	32.2	32.2	32.2	32.2
16	80.9	81.0	81.6	81.0	81.1	81.1	81.0	81.1
17	62.9	63.0	62.5	63.0	63.0	63.1	63.0	63.0
18	16.6	16.6	16.9	16.6	16.6	16.6	16.6	16.5
19	12.5	12.8	13.5	13.6	13.6	13.6	13.4	13.6
20	42.0	42.0	35.8	42.0	42.0	42.0	42.0	42.0
21	15.0	15.0	14.7	15.0	15.0	15.0	15.0	15.0
22	109.2	109.2	111.7	109.2	109.2	109.2	109.1	109.2
23	31.8	31.8	67.4	31.8	30.6	29.3	31.8	31.8
24	29.2	30.0	38.8	29.2	31.8	31.8	29.3	29.4
25	30.6	30.6	31.7	30.6	29.9	30.6	30.6	30.6
26	66.8	66.8	66.0	66.8	66.9	66.8	66.8	66.9
27	17.3	17.3	16.9	17.3	17.3	17.3	17.3	17.3
OCH_3		47.3						
OCH_3		47.5						
	Qui	Qui	Qui	Xyl	Glc	Glc	Glc	Glc
1′	105.4	105.4	105.3	106.0	102.0	99.6	101.6	99.4
2′	75.3	75.4	75.4	74.4	75.5	78.0	75.5	78.0
3′	87.3	87.6	87.6	87.2	76.7	79.6	78.7	77.9
4′	74.6	74.8	74.7	69.3	78.5	71.8	71.7	78.9
5′	72.3	72.2	72.3	66.3	77.0	78.2	78.1	76.8
6′	18.6	18.6	18.6		61.6	62.7	62.6	61.4
	Xyl	Xyl	Xyl	Xyl	Rha	Rha	Qui	Rha
1″	106.2	106.2	106.5	106.3	102.7	102.2	106.0	102.1
2″	74.6	74.6	74.8	75.5	72.7	72.5	75.9	72.4[a]
3″	78.2	78.2	78.3	78.2	72.8	72.8	78.1	72.7[b]
4″	70.9	70.9	70.9	71.0	74.0	74.3	76.8	73.9[c]
5″	67.3	67.4	67.4	67.4	70.5	69.5	72.6	69.4
6″					18.6	18.7	18.8	18.6
								Rha
1‴								102.9
2‴								72.5[a]
3‴								72.8[b]
4‴								74.2[c]
5‴								70.4
6‴								18.5

[a−c]Data with the same superscript letter are interchangeable.

found in **1**−**8**. On the basis of these data, compounds **9**−**18** were suggested as furostanol derivatives having an O-linkage with a glycopyranosyl group at C-26.[11,12]

With a $[M + H]^+$ ion at m/z 873.4842 in the HRESIMS, compound **9** showed the same molecular formula, $C_{44}H_{72}O_{17}$, as the furostanol glycoside torvoside P, 26-*O*-*β*-D-glucopyranosyl-25(*S*)-5*α*-furost-22(20)-ene-3*β*,6*α*,26-triol 6-*O*-[*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-quinovopyranoside].[13] Likewise, the two compounds were found to exhibit almost identical NMR spectroscopic data, with the only difference between **9** and torvoside P being their respective 25*R* and 25*S* configurations. For either a 25*R* or a 25*S* orientation of the Me-27 group in furostane-type steroidal saponins, a difference in chemical shift value between the H_2-26 geminal protons ($\Delta_{ab} = \delta_a - \delta_b$; 25*S*: $\Delta_{ab} > 0.57$ ppm; 25*R*: $\Delta_{ab} < 0.48$ ppm)[11] indicated a 25*R* configuration ($\Delta_{ab} = 0.33$) for **9**. As supported by the data

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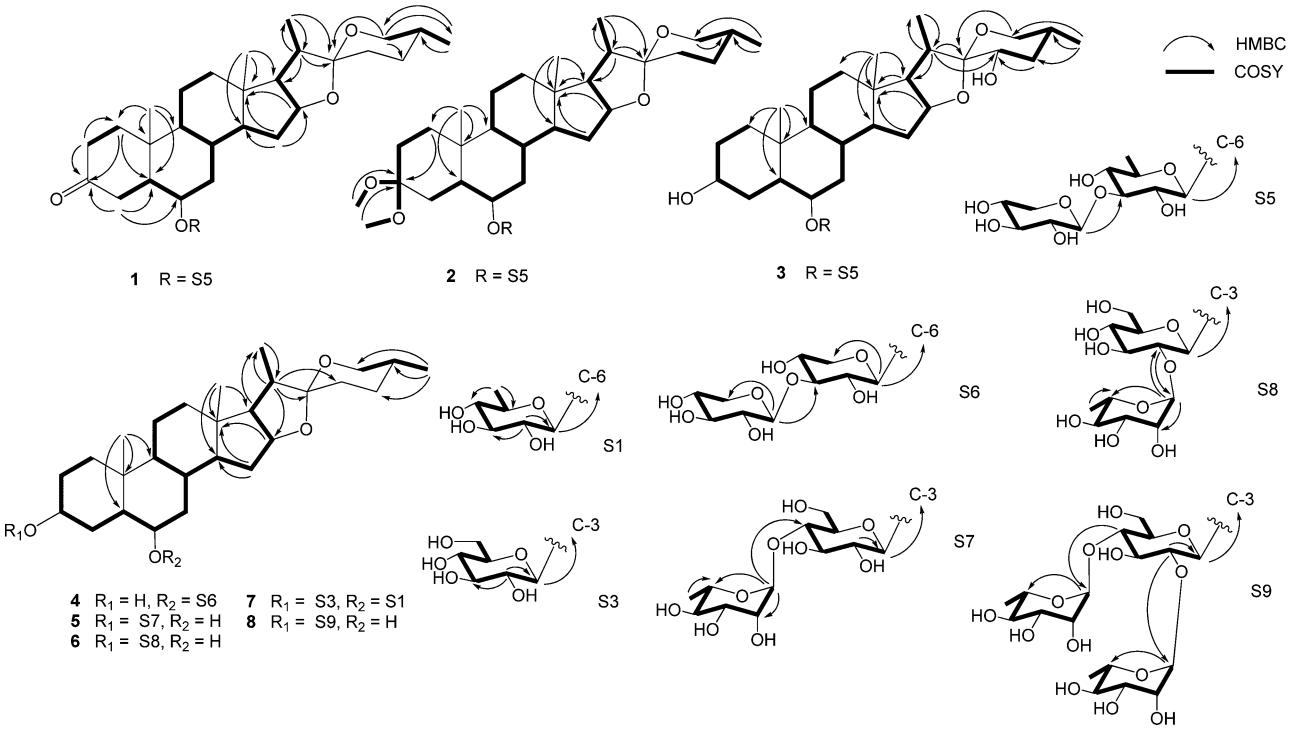

Figure 1. Key COSY and HMBC correlations of spirostanol glycosides **1**−**8**.

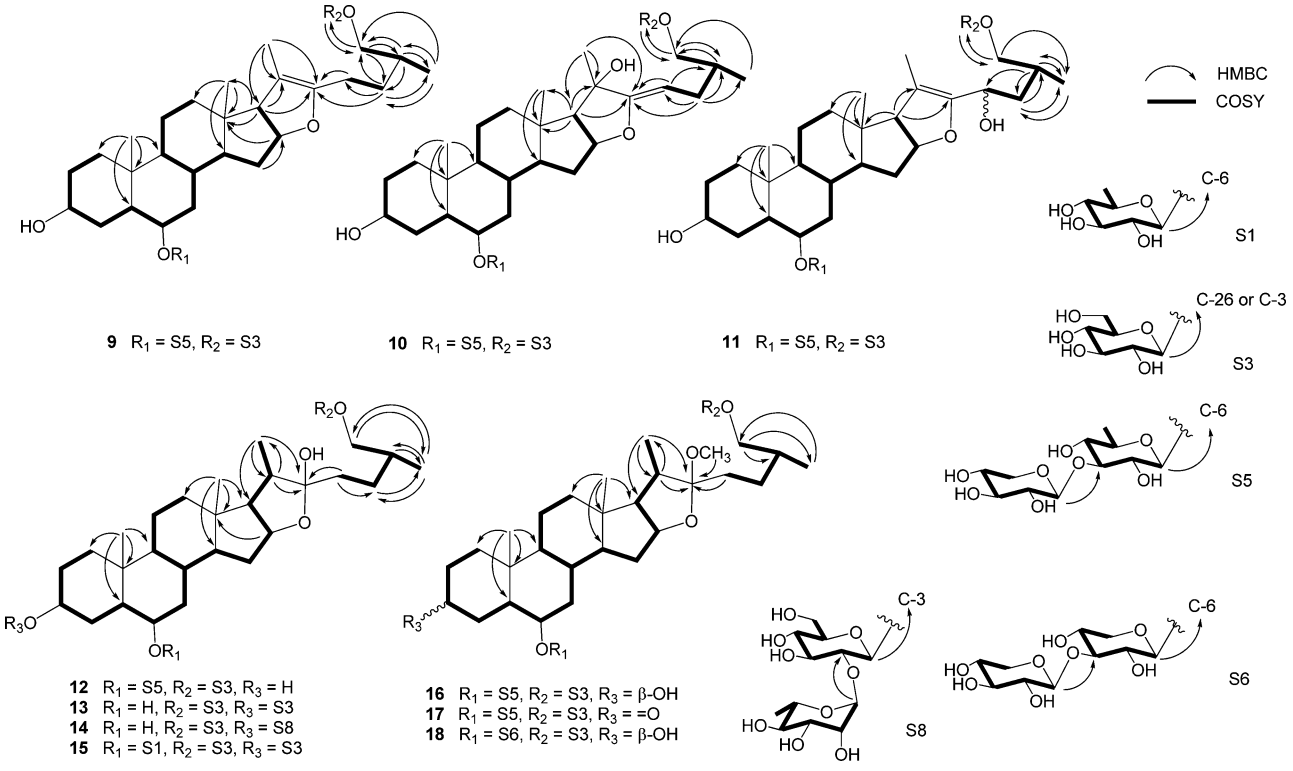

Figure 2. Key COSY and HMBC correlations of furostanol glycosides **9**−**18**.

shown in Figures 2 and 3, compound **9** (macaoside I) was elucidated as 26-*O*-*β*-D-glucopyranosyl-25(*R*)-5*α*-furost-22(20)-ene-3*β*,6*α*,26-triol 6-*O*-[*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-quinovopyranoside].

The molecular formula of **10** was shown to be $C_{44}H_{72}O_{18}$, from the HRESIMS peak $[M + Na]^+$ at *m*/*z* 911.4620, so this compound contains an additional oxygen atom in comparison with **9**. Its 1D and 2D NMR data (Table 3) showed that **10** is a furostanol glycoside like **9**. The main differences between **9** and **10** were the ^1H and ^{13}C NMR chemical shifts at H-18 and in the F ring (C-17, C-20, C-21) and the C-22 side chain (C-22, H-23/C-23, H-24/C-24, C-25). According to the HMBC correlations (Figure 2) (H-17/C-18, C-20, C-22, H-21/C-17, C-20, C-22, H-23/C-22, C-25, H-24/C-22, C-23, C-25, C-27,

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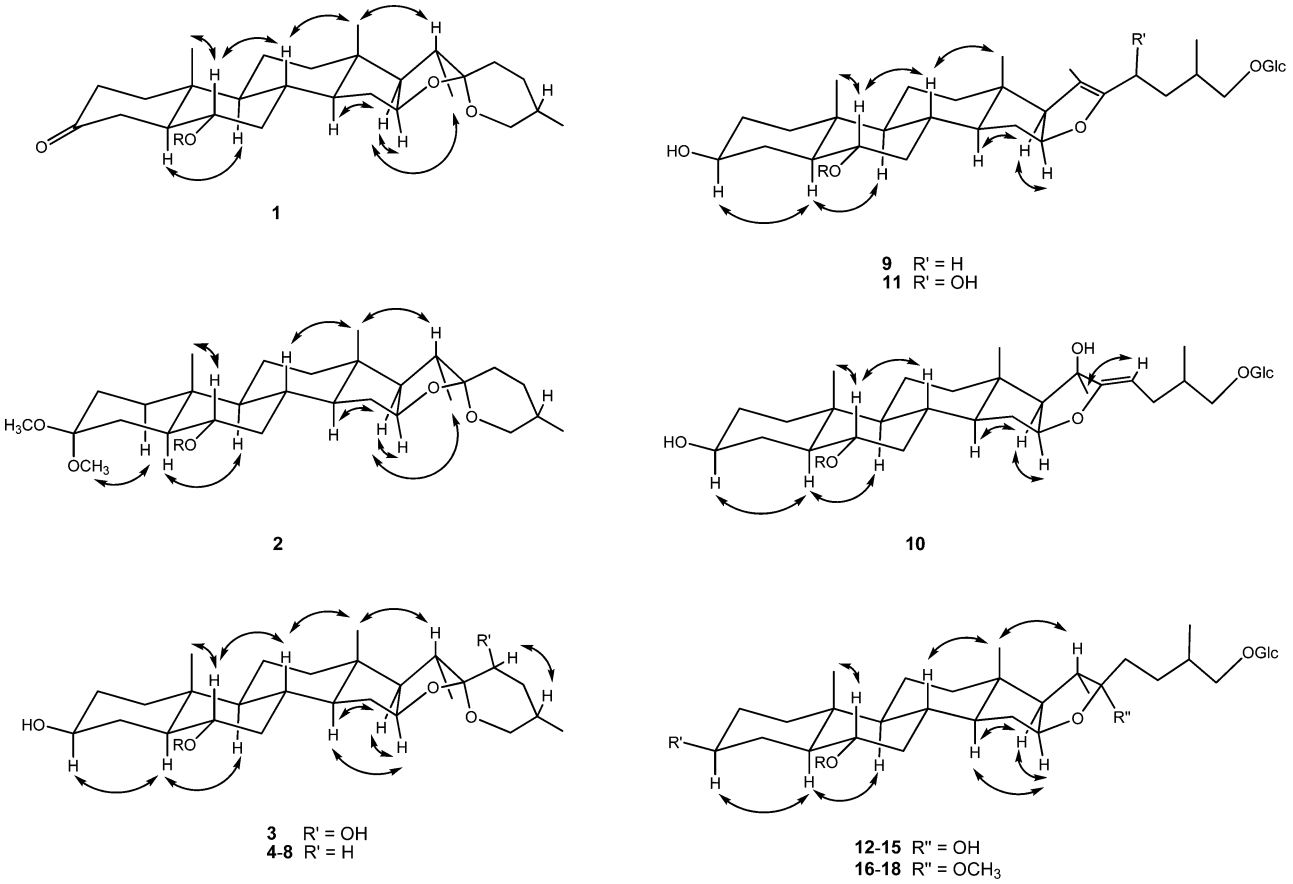

Figure 3. Key ROESY correlations of aglycone parts of **1**−**18**.

and H-25/C-26), the double bond could be located between C-22 and C-23 and the hydroxy group at C-20, respectively. The ROESY spectrum showed correlations between Me-21/H-23 (Figure 3), while no correlations were observed between Me-18/Me-21, and the small downfield shift of H_3-18 (**10**: δ_H 0.86, **9**: δ_H 0.69) indicated the double bond at C-22 and C-23 to be in the *Z* form and the hydroxy group at C-20 in the β position, respectively. Compound **10** (macaoside J) was assigned the structure 26-*O*-β-D-glucopyranosyl-25(*R*)-5α-furost-22(23)-ene-3β,6α,20β,26-tetraol 6-*O*-[β-D-xylopyranosyl-(1→3)-*O*-β-D-quinovopyranoside].

The molecular formula of **11** was determined to be $C_{44}H_{72}O_{18}$ on the basis of HRESIMS (*m*/*z* 911.4614 [M + Na]$^+$). As shown in Table 3, the chemical shifts of C-22, H-23/C-23, H-24/C-24, and H-25/C-25 of this compound showed differences from the analogous signal in **9**. The key COSY and HMBC correlations (Figure 2) indicated that a carbinol proton (δ_H 4.90/δ_C 63.7) occurs at C-23 in **11** instead of a methylene group (δ_H 2.20, 2.24/δ_C 23.7) in **9**. The ROESY data obtained are shown in Figure 3, and compound **11** (macaoside K) was assigned as 26-*O*-β-D-glucopyranosyl-25(*R*)-5α-furost-22(20)-ene-3β,6α,23ξ,26-tetraol 6-*O*-[β-D-xylopyranosyl-(1→3)-*O*-β-D-quinovopyranoside].

The HRESIMS of **12** showed a [M + Na]$^+$ ion at *m*/*z* 913.4773 ($C_{44}H_{74}O_{18}$Na). The 1D NMR data were very similar to those of torvoside B,[14] 25(*S*)-26-*O*-(β-D-glucopyranosyl)-22α-methoxy-5α-furostane-3β,6α,26-triol 6-*O*-[β-D-xylopyranosyl-(1→3)-*O*-β-D-quinovopyranoside], except for the loss of one methoxy unit at C-22. Additionally, the small difference in chemical shift values of the geminal protons H_2-26 indicated a 25*R* configuration[11] (Δ_{ab} = 0.32) for **12**. The key COSY, HMBC, and ROESY correlations observed for **12** are shown in Figures 2 and 3. However, the orientation of the C-22 hydroxy group could not be determined from the ROESY spectrum. Acid hydrolysis of **12** gave the spirostanol aglycone chlorogenin (**19**), so that it was considered that the C-22 hydroxy group in **12** is in an α-orientation. Compound **12** (macaoside L) was elucidated as 25(*R*)-26-*O*-(β-D-glucopyranosyl)-5α-furostane-3β,6α,22α,26-tetraol 6-*O*-[β-D-xylopyranosyl-(1→3)-*O*-β-D-quinovopyranoside].

HRESIMS of **13** gave a [M + Na]$^+$ ion at *m*/*z* 797.4295, indicating a molecular formula of $C_{39}H_{66}O_{15}$Na. The 1D and HSQC NMR spectra displayed two anomeric [δ_H 4.82 (d, *J* = 8.0 Hz)/δ_C 104.9, δ_H 5.09 (d, *J* = 7.5 Hz)/δ_C 102.2] and three oxymethylene [δ_H 3.63 m, 3.95 o/δ_C 75.2, δ_H 4.39 m, 4.56 (brd, *J* = 11.5 Hz)/δ_C 62.9, δ_H 4.39 m, 4.56 (brd, *J* = 11.5 Hz)/δ_C 62.8] signals. In the HMBC spectrum, the anomeric protons H-1′ (δ 5.09, d, *J* = 7.5 Hz) and H-1″ (δ 4.82, d, *J* = 8.0 Hz) exhibited ^3J interactions with C-3 (δ 77.6) and C-26 (δ 75.2), respectively. HMBC and COSY correlations (Figure 1) indicated that the glycosidic portion of **13** is constituted by two individual glucopyranosyl groups, connected, in turn, to C-3 and C-26 of the aglycone moiety via an O-linkage. The differences (Δ_{ab} = 0.32) of the chemical shift values between the H_2-26 geminal protons ($\Delta_{ab} = \delta_a - \delta_b$; 25*S*: Δ_{ab} > 0.57 ppm; 25*R*: Δ_{ab} < 0.48 ppm)[11] indicated a 25*R* configuration. Compound **13** (macaoside M) accordingly was elucidated as 25(*R*)-26-*O*-(β-D-glucopyranosyl)-5α-furostan-3β,6α,22α,26-tetraol 3-*O*-β-D-glucopyranoside.

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Table 3. ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds 9−11 (500 MHz; 125 MHz, C_5D_5N)[a]

	9		10		11	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	0.96 (t, 13.0)	37.8	0.96 (m)	37.7	0.95 (m)	37.8
	1.67 (o)		1.65 (o)		1.64 (m)	
2	1.76 (o)	32.1	1.74 (o)	32.1	1.77 (o)	32.1
	2.04 (brd, 11.0)		2.04 (o)		2.06 (m)	
3	3.79 (m)	70.6	3.78 (m)	70.6	3.81 (o)	70.6
4	1.70 (o)	33.2	1.68 (m)	33.2	1.69 (o)	33.2
	3.23 (brd, 11.5)		3.24 (brd, 12.5)		3.24 (brd, 12.0)	
5	1.38 (t, 12.0)	51.4	1.38 (m)	51.4	1.40 (m)	51.4
6	3.73 (o)	79.1	3.73 (o)	79.2	3.75 (o)	79.1
7	1.23 (m)	41.5	1.19 (m)	41.2	1.24 (o)	41.5
	2.49 (brd, 12.0)		2.46 (dt, 13.0, 4.5)		2.48 (o)	
8	1.57 (o)	33.9	1.55 (o)	33.5	1.56 (m)	33.8
9	0.60 (t, 10.5)	53.8	0.56 (td, 11.0, 3.5)	53.6	0.59 (brt, 11.0)	53.8
10		36.7		36.7		36.7
11	1.26 (m)	21.4	1.23 (m)	20.7	1.24 (o)	21.4
	1.48 (o)		1.44 (m)		1.44 (m)	
12	1.12 (t, 13.0)	39.8	1.11 (o)	39.4	1.11 (m)	39.8
	1.74 (o)		1.88 (brd, 11.5)		1.71 (o)	
13		43.7		40.6		43.8
14	0.88 (o)	54.6	1.00 (m)	56.6	0.86 (m)	54.6
15	1.46 (o)	34.3	1.48 (m)	33.4	1.41 (m)	34.3
	2.13 (m)		2.07 (o)		2.09 (m)	
16	4.73 (o)	84.3	5.17 (m)	84.1	4.75 (m)	84.5
17	2.43 (d, 10.0)	64.5	2.22 (d, 6.5)	67.8	2.45 (o)	64.8
18	0.69 (s)	14.4	0.86 (s)	13.5	0.68 (s)	14.5
19	0.88 (s)	13.6	0.88 (s)	13.7	0.88 (s)	13.6
20		103.5		76.7		104.9
21	1.63 (s)	11.8	1.74 (s)	21.8	1.75 (s)	11.6
22		152.4		163.6		154.4
23	2.20 (m)	23.7	4.51 (t, 7.5)	91.7	4.90 (o)	63.7
	2.24 (m)					
24	1.48 (o)	31.4	2.30 (m)	29.9	1.79 (o)	39.6
	1.83 (m)		2.40 (m)		2.45 (o)	
25	1.95 (m)	33.4	2.07 (o)	35.0	2.48 (o)	30.9
26	3.63 (m)	74.9	3.72 (o)	75.4	3.81 (o)	75.5
	3.96 (o)		4.01 (dd, 9.5, 7.0)		4.09 (o)	
27	1.07 (d, 6.5)	17.4	1.11 (d, 6.5)	17.7	1.20 (d, 6.0)	17.8
	Qui		Qui		Qui	
1′	4.87 (o)	105.1	4.87 (d, 7.0)	105.2	4.88 (o)	105.2
2′	4.06 (o)	75.3[b]	4.07 (o)	75.4	4.10 (o)	75.4[b]
3′	4.08 (o)	87.6	4.09 (o)	87.6	4.10 (o)	87.6
4′	3.62 (o)	74.9	3.62 (td, 9.0, 2.5)	74.9	3.62 (brt, 8.0)	74.7
5′	3.74 (o)	72.3	3.76 (o)	72.4	3.79 (o)	72.4
6′	1.54 (d, 6.0)	18.6	1.59 (d, 6.0)	18.7	1.58 (d, 5.5)	18.6
	Xyl		Xyl		Xyl	
1″	5.25 (d, 7.5)	106.4	5.26 (d, 8.0)	106.5	5.26 (d, 7.5)	106.5
2″	4.06 (o)	74.7[b]	4.07 (o)	74.7	4.07 (o)	74.9[b]
3″	4.24 (o)	78.2	4.21 (m)	78.2	4.19 (o)	78.3
4″	4.18 (o)	70.9	4.23 (m)	70.9	4.19 (o)	70.9
5″	3.70 (o)	67.4	3.73 (o)	67.4	3.70 (o)	67.4
	4.31 (brd, 11.5)		4.33 (dd, 11.0, 4.0)		4.33 (brd, 11.5)	
6″						
	Glc		Glc		Glc	
1‴	4.87 (o)	104.9	4.84 (d, 8.0)	104.9	4.88 (o)	105.0
2‴	4.06 (o)	75.2[b]	4.07 (o)	75.2	4.07 (o)	75.2[b]
3‴	4.18 (o)	78.6	4.25 (o)	78.6	4.24 (m)	78.7
4‴	4.24 (o)	71.7	4.25 (o)	71.7	4.22 (o)	71.8
5‴	3.96 (o)	78.5	3.95 (m)	78.5	3.97 (m)	78.6
6‴	4.40 (m)	62.8	4.41 (m)	62.8	4.31 (o)	62.9
	4.57 (brd, 11.0)		4.56 (brd, 12.0)		4.57 (brd, 10.5)	

[a] o: overlapped with other signals; m: multiplet signals. [b] Data with the same superscript letter are interchangeable.

Compounds **14** and **15** were found to be the 26-glycosylated furostanol derivatives of macaoside F (**6**) and macaoside G (**7**) and have been named macaosides N and O, respectively. The 1D and 2D NMR assignments obtained are detailed in Tables 4 and 5 and in Figures 2 and 3.

The HRESIMS of **16** showed an $[M + Na]^+$ ion at m/z 927.4927 ($C_{45}H_{76}O_{18}Na$). The 1D NMR data were very similar to those of a known furostanol, torvoside B.[14] The only difference was the *R* configuration (H_2-26: $\Delta_{ab} = \delta_a - \delta_b = 0.36$)[11] of C-25 in **16** rather than an *S* configuration at this position in torvoside B.[14] The key COSY, HMBC, and ROESY correlations are shown in Figures 2 and 3. In the ROESY spectrum, correlations between H-20/H-23, OCH_3-22/H-21, and OCH_3-22/H-16 were not observed. Also, the spirostanol aglycone chlorogenin (**19**) was obtained after acid hydrolysis of **16**. Therefore, the OCH_3 group at C-22 was proposed as being α-oriented. Compound **16** (macaoside P) was elucidated, therefore, as 25(*R*)-26-*O*-(β-D-glucopyranosyl)-5α-furostan-22α-methoxy-3β,6α,26-triol 6-*O*-[β-D-xylopyranosyl-(1→3)-*O*-β-D-quinovopyranoside].

Macaosides Q (**17**) and R (**18**) were found to be the 26-glycosylated furostanol derivatives of macaosides A (**1**) and D (**4**), respectively. The 1D and 2D NMR assignments are detailed in Tables 4 and 5 and in Figures 2 and 3.

Consequently, 24 steroidal saponins including 14 spirostanol compounds (**1**−**8** and **19**−**24**) and 10 furostanol glycosides (**9**−**18**) were isolated from *S. macaonense*. Chlorogenin (**19**) is the sapogenin of spirostanol saponins **1**−**8** and **20**−**24**. Among the 13 spirostanol glycosides, **1**−**6**, **23**, and **24** contain a xylopyranosyl-(1→3)-quinovopyranosyl, xylopyranosyl-(1→3)-xylopyranosyl, rhamnopyranosyl-(1→2)-glucopyranosyl, rhamnopyranosyl-(1→4)-glucopyranosyl, or xylopyranosyl-(1→3)-glucopyranosyl disaccharide unit, while **7** has two individual glucopyranosyl and quinovopyranosyl groups connected to different positions of the aglycone. The other four spirostanol glycosides, **8** and **20**−**22**, contain dirhamnopyranosyl-(1→2,1→4)-glucopyranosyl, quinovopyranosyl, xylopyranosyl, and glucopyranosyl units, respectively. These sugar moieties are usually attached at C-6 of the aglycone, with the exception of compounds **5**−**8** (C-3). Compounds **9**−**18** were found to be 26-glycosylated furostanol derivatives, and the sugar moieties

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Table 4. ^{1}H NMR Spectroscopic Data of Compounds 12−18 (500 MHz, C_5D_5N)[a]

position	12	13	14	15	16	17	18
1	0.98 (m)	0.88 (o)	0.92 (o)	0.85 (m)	0.94 (m)	1.21 (m)	0.98 (o)
	1.63 (o)	1.58 (o)	1.63 (o)	1.52 (m)	1.62 (o)	1.79 (o)	1.65 (o)
2	1.73 (o)	1.72 (o)	1.92 (o)	1.69 (o)	1.75 (o)	2.29 (brd, 14.0)	1.75 (o)
	2.02 (o)	2.08 (o)	2.08 (o)	2.04 (o)	2.00 (o)	2.43 (m)	2.04 (o)
3	3.78 (m)	4.05 (o)	4.10 (m)	3.96 (o)	3.77 (m)		3.79 (m)
4	1.65 (o)	1.54 (m)	1.79 (o)	1.45 (o)	1.66 (o)	2.48 (o)	1.67 (o)
	3.19 (brd, 11.0)	3.15 (brd, 12.5)	3.18 (d, 10.5)	3.40 (brd, 11.5)	3.19 (brd, 12.5)	3.57 (o)	3.16 (o)
5	1.36 (m)	1.21 (m)	1.19 (o)	1.19 (o)	1.35 (o)	1.65 (o)	1.36 (m)
6	3.70 (o)	3.56 (m)	3.61 (m)	3.61 (o)	3.69 (o)	3.73 (o)	3.70 (m)
7	1.21 (m)	1.17 (o)	1.15 (o)	1.15 (o)	1.20 (o)	1.55 (o)	1.23 (o)
	2.48 (brd, 10.5)	2.22 (m)	2.22 (m)	2.53 (m)	2.47 (m)	2.48 (o)	2.41 (dt, 12.5, 3.5)
8	1.67 (o)	1.62 (m)	1.61 (o)	1.62 (o)	1.61 (o)	1.65 (o)	1.63 (o)
9	0.59 (brt, 11.0)	0.64 (td, 12.0, 2.5)	0.64 (brt, 10.5)	0.56 (td, 10.0, 4.0)	0.58 (brt, 8.0)	0.61 (brt, 9.0)	0.58 (td, 11.5, 3.5)
11	1.25 (m)	1.26 (m)	1.27 (m)	1.15 (o)	1.22 (o)	1.28 (m)	1.23 (o)
	1.47 (m)	1.47 (o)	1.45 (o)	1.41 (m)	1.43 (m)	1.41 (m)	1.44 (m)
12	1.10 (o)	1.10 (m)	1.10 (o)	1.09 (o)	1.07 (o)	1.05 (o)	1.06 (o)
	1.73 (o)	1.75 (o)	1.75 (o)	1.69 (o)	1.67 (o)	1.67 (o)	1.67 (o)
14	1.08 (o)	1.15 (o)	1.14 (o)	1.09 (o)	1.07 (o)	1.05 (o)	1.06 (o)
15	1.44 (m)	1.47 (m)	1.45 (o)	1.41 (o)	1.38 (o)	1.39 (m)	1.33 (o)
	2.02 (o)	2.08 (o)	2.08 (o)	2.04 (o)	2.00 (o)	1.98 (o)	1.95 (o)
16	4.89 (q, 7.0)	4.94 (o)	4.95 (o)	4.87 (o)	4.40 (o)	4.40 (o)	4.39 (o)
17	1.91 (o)	1.95 (m)	1.97 (o)	1.90 (o)	1.73 (o)	1.71 (o)	1.75 (o)
18	0.86 (s)	0.90 (s)	0.89 (s)	0.87 (s)	0.77 (s)	0.80 (s)	0.79 (s)
19	0.87 (s)	0.76 (s)	0.95 (s)	0.76 (s)	0.85 (s)	0.98 (s)	0.86 (s)
20	2.23 (quint, 7.0)	2.23 (m)	2.24 (m)	2.23 (quint, 6.5)	2.19 (quint, 6.5)	2.19 (quint, 6.5)	2.19 (quint, 6.5)
21	1.31 (d, 6.0)	1.34 (d, 7.0)	1.34 (d, 6.5)	1.33 (d, 6.5)	1.16 (d, 7.0)	1.17 (d, 7.0)	1.16 (d, 7.0)
23	2.02 (2H, o)	2.04 (2H, o)	2.04 (2H, o)	2.04 (2H, o)	1.78 (o)	1.77 (o)	1.75 (o)
					2.00 (o)	1.98 (o)	1.95 (o)
24	1.67 (o)	1.69 (o)	1.70 (m)	1.69 (o)	1.35 (o)	1.35 (o)	1.33 (o)
	2.02 (o)	2.04 (o)	2.04 (o)	2.04 (o)	1.78 (o)	1.76 (o)	1.79 (m)
25	1.91 (o)	1.94 (m)	1.92 (o)	1.90 (o)	1.89 (m)	1.89 (m)	1.89 (o)
26	3.62 (m)	3.63 (m)	3.62 (m)	3.64 (m)	3.59 (o)	3.60 (o)	3.59 (dd, 9.5, 6.5)
	3.94 (o)	3.95 (o)	3.96 (o)	3.96 (o)	3.95 (o)	3.95 (o)	3.95 (o)
27	0.98 (d, 6.0)	0.99 (d, 7.0)	0.99 (d, 6.5)	1.00 (d, 6.5)	0.99 (d, 6.5)	0.99 (d, 8.5)	0.98 (d, 6.5)
22-OCH_3					3.22 (s)	3.24 (s)	3.20 (s)
	Qui	Glc	Glc	Glc	Qui	Qui	Xyl
1′	4.82 (d, 7.5)	5.09 (d, 7.5)	5.09 (d, 7.5)	5.14 (d, 7.5)	4.83 (d, 7.5)	4.76 (d, 7.5)	4.80 (d, 7.5)
2′	4.04 (o)	4.02 (o)	4.25 (o)	4.07 (o)	4.01 (o)	4.02 (o)	4.05 (o)
3′	4.09 (o)	4.28 (o)	4.18 (o)	4.28 (o)	4.06 (o)	4.07 (o)	4.12 (o)
4′	3.58 (o)	4.23 (o)	4.16 (o)	4.25 (o)	3.57 (o)	3.60 (o)	4.12 (o)
5′	3.70 (o)	3.89 (o)	3.79 (m)	3.87 (o)	3.73 (o)	3.73 (o)	3.68 (m)
							4.39 (m)
6′	1.54 (d, 5.0)	4.39 (m)	4.34 (o)	4.39 (dd, 12.0, 5.0)[b]	1.54 (d, 6.0)	1.55 (d, 6.0)	
		4.56 (brd, 11.5)	4.50 (brd, 11.0)	4.56 (dd, 12.0, 2.0)[c]			
	Xyl	Glc	Rha	Qui	Xyl	Xyl	Xyl
1″	5.23 (d, 7.5)	4.82 (d, 8.0)	6.35 (s)	4.80 (d, 7.5)	5.21 (d, 7.5)	5.22 (d, 7.5)	5.27 (d, 8.0)
2″	4.04 (o)	4.02 (o)	4.76 (brs)	4.03 (o)	4.01 (o)	4.02 (o)	4.05 (o)
3″	4.16 (o)	4.28 (o)	4.61 (brd, 9.0)	4.28 (o)	4.15 (o)	4.13 (o)	4.19 (o)
4″	4.16 (o)	4.23 (o)	4.32 (o)	3.72 (m)	4.15 (o)	4.13 (o)	4.19 (o)
5″	3.70 (o)	3.89 (o)	5.02 (o)	3.76 (m)	3.73 (o)	3.69 (o)	3.72 (m)
	4.30 (brd, 11.0)				4.29 (dd, 12.0, 4.5)	4.27 (o)	4.39 (m)
6″		4.39 (m)	1.79 (d, 5.5)	1.62 (d, 6.0)			
		4.56 (brd, 11.5)					
	Glc		Glc	Glc	Glc	Glc	Glc
1‴	4.80 (d, 7.5)		4.82 (d, 7.5)	4.82 (d, 8.0)	4.83 (d, 7.5)	4.83 (d, 7.5)	4.84 (d, 7.5)
2‴	4.04 (o)		4.04 (m)	4.03 (o)	4.01 (o)	4.02 (o)	4.05 (o)
3‴	4.22 (o)		4.25 (o)	4.28 (o)	4.21 (o)	4.21 (o)	4.24 (o)
4‴	4.22 (o)		4.25 (o)	4.25 (o)	4.18 (o)	4.21 (o)	4.24 (o)
5‴	3.92 (o)		3.96 (o)	3.96 (o)	3.93 (o)	3.93 (o)	3.95 (o)
6‴	4.37 (brd, 11.5)		4.39 (o)	4.33 (dd, 12.0, 5.5)[b]	4.36 (o)	4.37 (o)	4.39 (o)
	4.53 (brd, 11.5)		4.55 (brd, 11.5)	4.44 (dd, 12.0, 2.5)[c]	4.53 (dd, 12.0, 2.0)	4.54 (brd, 10.0)	4.56 (dd, 11.5, 2.0)

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Table 4. continued

[a]o: overlapped with other signals; m: multiplet signals. [b,c]Data with the same superscript letter are interchangeable.

Table 5. ^{13}C NMR Spectroscopic Data of Compounds 12−18 (125 MHz, C_5D_5N)

position	12	13	14	15	16	17	18
1	37.7	37.7	37.7	37.6	37.7	38.6	37.7
2	32.1	30.0[a]	29.8	29.9	32.0	38.0	32.1
3	70.6	77.6	77.0	76.9	70.6	210.8	70.5
4	33.1	29.5	28.9	28.5	33.0	39.8	33.1
5	51.3	52.2	52.1	51.0	51.2	52.3	51.3
6	79.2	68.5	68.5	79.4	79.2	79.8	78.9
7	41.4	42.8	42.7	41.5	41.3	40.9	41.3
8	34.1	34.3	34.3	34.3	34.1[a]	33.9	34.1
9	53.8	54.2	54.1	53.9	53.8	53.7	53.8
10	36.7	36.6	36.7	36.8	36.6	36.7	36.7
11	21.2	21.3	21.3	21.2	21.1	21.2	21.2
12	40.1	40.1	40.2	40.1	39.8	39.7	39.9
13	41.0	41.2	41.1	41.1	41.0	41.0	41.1
14	56.2	56.3	56.3	56.3	56.2	55.9	56.2
15	32.3	32.4	32.4	32.4	32.0	32.0	32.0
16	81.0	81.1	81.1	81.0	81.2	81.1	81.2
17	63.8	63.9	63.9	63.9	64.1	64.1	64.2
18	16.7	16.7	16.4	16.7	16.5	16.4	16.5
19	13.5	13.5	13.6	13.4	13.5	12.5	13.5
20	40.6	40.7	40.7	40.7	40.4	40.4	40.5
21	16.3	16.4	16.7	16.4	16.2	16.2	16.2
22	110.5	110.6	110.6	110.6	112.5	112.5	112.5
23	37.1	37.2	37.2	37.3	30.7	30.7	30.7
24	28.3	28.4[a]	28.4	28.4	28.1	28.2	28.2
25	34.2	34.3	34.3	34.1	34.0[a]	34.1	34.1
26	75.4	75.2	75.2	75.2	75.2	75.2	75.2
27	17.4	17.4	17.4	17.5	17.1	17.1	17.1
OCH_3					47.2	47.2	47.2
	Qui	Glc	Glc	Glc	Qui	Qui	Xyl
1′	105.1	102.2	99.6	101.6	105.1	105.2	105.9
2′	75.1	75.4[b]	78.1[a]	75.5[a]	75.1[b]	75.1[a]	74.4
3′	87.5	78.6	79.5	78.6[b]	87.5	87.3	87.2
4′	74.7	71.7	71.8	71.8[c]	74.6[c]	74.5	69.2
5′	72.4	78.3	78.5	78.5[d]	72.2	72.2	66.3
6′	18.6	62.9	62.7	62.9[e]	18.5	18.6	
	Xyl	Glc	Rha	Qui	Xyl	Xyl	Xyl
1″	106.3	104.9	102.1	105.9	106.3	106.1	106.3
2″	74.8	75.3[b]	72.5	76.0[a]	74.8[c]	74.6	75.2
3″	78.1	78.6	72.8	78.1[b]	78.1	78.1	78.3
4″	70.8	71.7	74.2	76.9	70.8	70.8	70.9
5″	67.3	78.5	69.4	72.6	67.3	67.3	67.4
6″		62.8	18.7	18.8			
	Glc		Glc	Glc	Glc	Glc	Glc
1‴	104.8		104.9	104.9	104.8	104.8	104.9
2‴	75.3		75.3	75.3[a]	75.2[b]	75.2[a]	75.4
3‴	78.4		78.6[a]	78.7[b]	78.5	78.5	78.6
4‴	71.6		71.7	71.8[c]	71.7	71.7	71.7
5‴	78.5		78.2	78.5[d]	78.4	78.4	78.5
6‴	62.7		62.8	62.7[e]	62.8	62.8	62.9

[a−e]Data with the same superscript letter are interchangeable.

and their positions of attachment on the aglycone were similar to those of the above-mentioned spirostanol derivatives. Most of the naturally occurring furostanol glycosides usually have a glucopyranosyl group at C-26 along with an OH/OMe group at C-22 (OH: **12**−**15**; OMe: **16**−**18**) or unsaturation between C-20 and C-22 (**9** and **11**). A rare unsaturation between C-22 and C-23 was found in this study in furostanol **10**. The OMe-22 furostanol saponins have been considered as genuine compounds by some researchers, but others have regarded them as artifacts from MeOH extraction.[11] The orientations of

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the 22-hydroxy and 22-methoxy groups present in certain compounds were not able to be determined using ROESY spectra in this investigation, but since the known aglycone chlorogenin was obtained on hydrolysis, the α-oriented configuration of OH-22 and OCH_3-22 was assigned for these furostanols (**9**–**18**). Both the spirostanol (**1**–**8** and **19**–**24**) and the furostanol saponins (**9**–**18**) isolated have 25*R* configurations, and their A/B, B/C, C/D, and D/E ring junctions were determined as *trans*, *trans*, *trans*, and *cis*, respectively, in this investigation.

Superoxide anion and elastase are produced from the activated neutrophils in response to FMLP/CB and are mediators of neutrophilic inflammation. Most of the isolates obtained were evaluated for antineutrophilic inflammatory effects (Table 6), although, the quantities of spirostanols **2**, **5**, **6**, and **23** isolated were insufficient for biological testing. The furostanol glycosides (**9**–**18**) did not show antineutrophilic inflammatory activity (Table 6). Among the 10 spirostanol compounds tested, **1** and **4** showed inhibition against elastase release with IC_{50} values of 3.2 and 4.2 μM, respectively, while **19** was active against superoxide anion generation with an IC_{50} value of 6.1 μM. In turn, compounds **20**, **21**, and **24** inhibited both of these inflammatory mediators (superoxide anion generation: IC_{50} 7.0, 7.6, 4.0 μM; elastase release: IC_{50} 3.7, 4.4, 1.0 μM, respectively). Compounds **1**, **4**, **20**, and **21** (up to 10 μM) did not affect neutrophilic cell viability, as measured by LDH release (Supporting Information). However, compounds **19** and **24** showed potential anti-inflammatory activity with toxicity toward neutrophils.

Table 6. Effects of Compounds 1–24 on Superoxide Anion Generation and Elastase Release in FMLP/CB-Induced Human Neutrophils[a]

	superoxide anion	elastase release
compound	IC_{50} (μM)[b]	IC_{50} (μM)[b]
1	>10	3.2 ± 0.2***
4	>10	4.2 ± 0.4***
19	6.1 ± 0.5***	>10
20	7.0 ± 0.8***	3.7 ± 0.3***
21	7.6 ± 0.4***	4.4 ± 0.6***
24	4.0 ± 0.1***	1.0 ± 0.2***
DPI[c]	0.8 ± 0.3	
sivelestat[c] (nM)		50.0 ± 0.4

[a]Results are presented as means ± SEM (n = 3 or 4) (***p < 0.001 compared with the control value). Compounds **2**, **5**, **6**, and **23** were not tested. Compounds **3**, **7**–**18**, and **22** were inactive in both assays (IC_{50} > 10 μM). [b]Concentration necessary for 50% inhibition (IC_{50}). [c]Diphenyleneiodonium (DPI) and sivelestat were used as positive controls for superoxide anion generation and elastase release, respectively.

Furthermore, to confirm whether the anti-inflammatory effect of **1** and **4** is reliant on the inhibition of degranulation, a β-hexosamindase degranulation assay was conducted to examine the antidegranulation effects on the basophils (RBL-2H3 cells). Compounds **1** and **4** did not exhibit significant cytotoxicity on cell viability as assessed by a MTT assay (Figure 4A). As shown in Figure 4B, RBL-2H3 cells treated with **1** at doses of up to 64 μM inhibited over 90% of DNP-BSA-stimulated degranulation in comparison with a control group. However, **4** showed only slight effects on antidegranulation even in the presence of the high dose used.

A preliminary SAR study conducted showed that the spirostanol compounds have greater potential antineutrophilic inflammatory activities than the furostanol glycosides. For the active spirostanols **1**, **4**, **19**, **20**, **21**, and **24**, the compositions of the sugar moieties do not seem to influence the resulting activity. Among the chlorogenin-type saponins, greater activity was observed for compounds in which the sugar units are attached to C-6 than at C-3. Compounds **1** (C-3: C═O, C-23: H), **3** (C-3: OH, C-23: OH), and **24** (C-3: OH, C-23: H) contain the same sugar moiety at C-6, but **3** was inactive, **1** was active only against elastase release, and **24** showed significant inhibition against both inflammatory mediators. In a previous study, neochlorogenin-type spirostanols (25*S*) isolated from *S. torvum* also showed significant activities against superoxide anion generation and elastase release induced by human neutrophils.[15] In order to establish a more meaningful SAR, a great diversity of spirostanols and spirostanosides and their antineutrophilic inflammatory effects should be further assembled and evaluated.

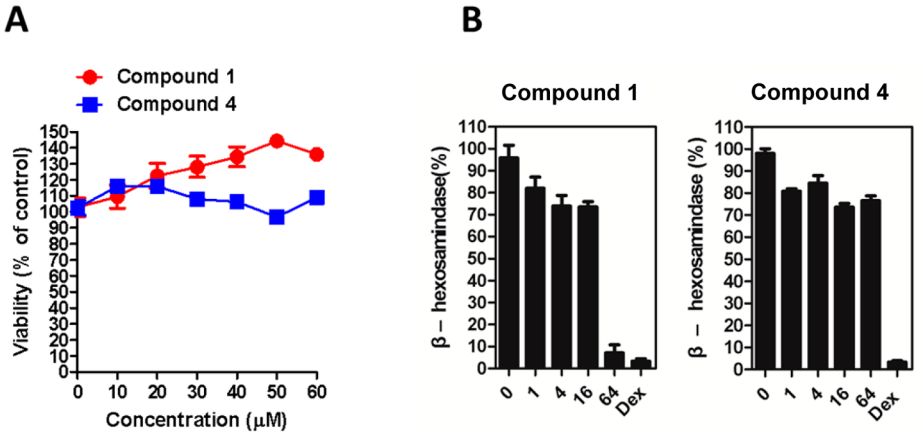

Figure 4. Effects of compounds **1** and **4** on the inhibition of immediate inflammation degranulation in RBL-2H3 cells. (A) The cytotoxicities of **1** and **4** were tested using an MTT viability assay. (B) The effects of antidegranulation were examined by a β-hexosaminidase release assay. Dexamethasone (Dex) was the positive control. The RBL-2H3 cells were treated with **1** and **4** at the indicated concentrations and with dexamethasone at a concentration of 100 nM.

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EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations and IR spectra were recorded on a JASCO-P-1020 polarimeter (cell length 10 mm) and a Shimadzu model IR Prestige-21 Fourier-transform infrared spectrophotometer, respectively. 1D and 2D NMR spectra were measured on a Bruker Ultrashield 500 Plus instrument. Chemical shift (δ) values are in ppm, and coupling constants (J) are in Hz with C_5D_5N used as the internal standard. Low- and high-resolution ESIMS were measured on a Bruker Daltonics Esquire HCT ultra-high-capacity trap mass spectrometer and an Orbitrap mass spectrometer (LTQ Orbitrap XL, Thermo Fisher Scientific), respectively. TLC was performed on Kieselgel 60 F_{254} (0.25 mm, Merck) and/or RP-18 $F_{254}S$ (0.25 mm, Merck) coated plates and then visualized by spraying with 10% H_2SO_4 and heating on a hot plate. Silica gel (Silicycle, 70−230 and 230−400 mesh), RP-18 (LiChroprep 40−63 μm, Merck), Sephadex LH-20 (GE Healthcare, Uppsala, Sweden), and Diaion HP-20 (Supelco) were used for column chromatography. A Shimadzu LC-20AT pump and a Shimadzu RID-10A refractive index detector (Shimadzu Inc., Kyoto, Japan) along with a Supelco Ascentis (250 × 10 mm i.d., 5 μm, C_{18}) column were used for HPLC. Sugar reagents, including D-xylose (Acros Organics, Fair Lawn, NJ, USA), L-xylose (Alfa Aesar, Heysham, England), D-rhamnose (Carbosynth Limited, Berkshire, UK), L-rhamnose (Acros Organics), D-glucose (MP Biomedicals, LLC, Illkirch, France), L-glucose (Alfa Aesar), and D-quinovose (Sigma, St. Louis, MO, USA) were used for GC/MS analysis.

Plant Material. Aerial parts of *S. macaonense* were collected in Kaohsiung, Taiwan, in March 2011 and identified by C.-L.K. A voucher specimen (SM201103) was deposited at the Natural Medicinal Products Research Center, CMUH, Taichung, Taiwan.

Extraction and Isolation. The aerial parts of *S. macaonense* (1.23 kg) were extracted four times with MeOH (8.0 L each) at room temperature to obtain a crude extract. The MeOH extract was partitioned between EtOAc and H_2O (1:1, v/v) to give an EtOAc-soluble fraction (25.09 g) and an aqueous phase (113.27 g), which were partitioned with *n*-hexane−95% MeOH and *n*-BuOH−H_2O (1:1), respectively, to give four fractions.

The 95% MeOH-soluble fraction (16.26 g) was subjected to open column chromatography on silica gel (70−230 mesh, column: 8 × 20 cm), using gradients of $CHCl_3$−MeOH−H_2O (100:0:0, 5.0 L; 50:1:0.1, 3.5 L; 30:1:0.1, 4.2 L; 20:1:0.1, 2.0 L; 10:1:0.1, 2.0 L; 8:1:0.1, 1.4 L; 0:1:0, 2.0 L), and gave 10 subfractions (SME1−SME10). Subfraction SME5 (2.04 g) was fractionated into seven fractions by Sephadex LH-20 (column: 5 × 57 cm; $CHCl_3$−MeOH, 1:1). Subfraction SME5-3 (304.0 mg) was subjected separation over to RP-18 (column: 3 × 28 cm; MeOH) and then purified by silica gel (230−400 mesh, column: 2 × 26 cm; $CHCl_3$−MeOH, 40:1) chromatography to give subfraction SME5-3-3-6 (20.0 mg). Subfraction SME5-3-3-6 was further purified using Sephadex LH-20 (column: 2 × 30 cm; $CHCl_3$−MeOH, 1:1) and preparative silica TLC ($CHCl_3$−MeOH, 45:1) to give **19** (13.0 mg). SME7 (1.40 g) was chromatographed over Sephadex LH-20 (column: 5 × 57 cm; $CHCl_3$−MeOH, 1:1), with subfraction SME7-3 (226.0 mg), then subjected to RP-18 column chromatography (column: 2.5 × 29 cm; MeOH−H_2O, 70:30 to 90:10), to give six subfractions. Subfraction SME7-3-4 (65.9 mg) was purified by passage over Sephadex LH-20 (column: 2 × 28 cm; MeOH) to furnish **1** (52.4 mg). Subfraction SME7-3-5 (13.9 mg) was purified using Sephadex LH-20 (column: 2 × 28 cm; MeOH) and RP-HPLC (MeOH−H_2O, 93:7; flow rate: 2.0 mL/min) to give **2** (2.6 mg, t_R = 30 min).

Subfraction SME8 (3.05 g) was subjected to separation over Sephadex LH-20 (column: 5 × 53 cm; $CHCl_3$−MeOH, 1:1) to obtain six subfractions. Subfraction SME8-3 (808.2 mg) was separated by silica gel chromatography (230−400 mesh, column: 3 × 23 cm; $CHCl_3$−MeOH, 15:1 to 10:1), and its subfraction SME8-3-3 (165.0 mg) was further subjected to RP-18 chromatography (column: 2 × 28 cm; MeOH−H_2O, 85:15, 90:10, 100:0), to give six additional subfractions (SME8-3-3-1−SME8-3-3-6). Of these, SME8-3-3-4 (35.5 mg) was subjected to RP-HPLC (MeOH−H_2O, 85:15; flow rate: 2.0 mL/min) to give **20** (17.0 mg, t_R = 56 min). SME8-3-4 (182.4 mg) was subjected to RP-18 chromatography (column: 2 × 28 cm; MeOH−H_2O, 90:10) to give seven subfractions (SME8-3-4-1−SME8-3-4-7), as well as pure compound **4** (20.2 mg). Subfraction SME8-3-4-3 (7.5 mg) was purified further with 75% MeOH by RP-HPLC (MeOH−H_2O, 73:27; flow rate: 2.0 mL/min) to give **3** (4.9 mg, t_R = 46 min). Subfraction SME8-3-4-5 (8.0 mg) was purified by RP-HPLC (MeOH−H_2O, 85:15; flow rate: 2.0 mL/min) to give **22** (3.5 mg, t_R = 29 min). In turn, subfraction SME8-3-5 (117.8 mg) was subjected to RP-18 chromatography (column: 2 × 28 cm; MeOH−H_2O, 90:10) to give **24** (42.6 mg). Subfraction SME8-4 (1.18 g) was chromatographed on a silica gel column (230−400 mesh, column: 3 × 23 cm), using $CHCl_3$−MeOH mixtures (10:1 to 8:1) for eluting. Subfraction SME8-4-3 (267.4 mg) was purified further using Sephadex LH-20 (column: 2 × 29 cm) with MeOH, along with RP-18 chromatography (column: 2.5 × 26 cm; MeOH−H_2O, 95:5) and RP-HPLC (MeOH−H_2O, 90:10; flow rate: 2.0 mL/min) to obtain **21** (6.0 mg, t_R = 24 min).

Subfraction SME9 (3.41 g) was subjected to separation over a Sephadex LH-20 (column: 5 × 58 cm; $CHCl_3$−MeOH, 1:1) and then silica gel chromatography (230−400 mesh, column: 3 × 28 cm; $CHCl_3$−MeOH, 10:1 to 6:1) to obtain six subfractions. SME9-3-3 (55.7 mg) was further separated into three subfractions by RP-18 chromatography (column: 2 × 27 cm; MeOH−H_2O, 80:20). Subfractions SME9-3-3-2 (4.0 mg) and SME9-3-3-3 (3.7 mg) were purified by RP-HPLC (MeOH−H_2O, 80:10 and 82:18; flow rate: 2.0 mL/min) to obtain **5** (2.2 mg, t_R = 43 min) and **23** (2.4 mg, t_R = 46 min), respectively. SME9-3-4 (38.9 mg) was subjected to RP-18 chromatography (column: 2 × 26 cm; MeOH−H_2O, 80:20) and purified by RP-HPLC (MeOH−H_2O, 80:20; flow rate: 2.0 mL/min) to obtain **6** (1.7 mg, t_R = 50 min). Subfraction SME9-3-5 (47.1 mg) was subjected to RP-18 chromatography (column: 2 × 26 cm; MeOH−H_2O, 80:20) to give **7** (6.4 mg).

The *n*-BuOH-soluble fraction (34.4 g) was chromatographed over a Diaion HP-20 column (7 × 14.5 cm; H_2O−MeOH−acetone, 100:0:0, 1.9 L; 80:20:0, 2.0 L; 60:40:0, 2.0 L; 40:60:0, 2.3 L; 20:80:0, 2.2 L; 0:100:0, 2.3 L; 0:0:100, 2.5 L), to give nine subfractions (SMB1−SMB9). Subfraction SMB4 (4.64 g) was purified over Sephadex LH-20 (column: 5 × 58 cm; MeOH) to produce seven subfractions. Subfraction SMB4-2 (2.92 g) was separated by silica gel chromatography (230−400 mesh, column: 4.5 × 30 cm; $CHCl_3$−MeOH, 5:1, 4:1, 3:1, 2:1, 1:1), and its subfraction SMB4-2-6 (217.0 mg) was further subjected to Sephadex LH-20 chromatography (column: 2 × 25 cm; MeOH−H_2O, 50:50) to give three additional subfractions (SMB4-2-6-1−SMB4-2-6-3). Subfraction SMB4-2-6-2 (192.0 mg) was purified by RP-18 chromatography (column: 2.5 × 24 cm; MeOH−H_2O, 70:30) and RP-HPLC (MeCN−H_2O, 30:70; flow rate: 2.0 mL/min) to give **10** (9.0 mg, t_R = 10 min), **11** (7.0 mg, t_R = 12 min), and **13** (5.0 mg, t_R = 16 min). Subfraction SMB4-2-7 (367.0 mg) was separated into four subfractions by column chromatography over Sephadex LH-20 (column: 2 × 25 cm; MeOH−H_2O, 50:50), and subfraction SMB4-2-7-1 (299.0 mg) was subjected to RP-18 chromatography (column: 2.5 × 24 cm; MeOH−H_2O, 80:20). Subfraction SMB4-2-7-1-2 (221.0 mg) was purified by RP-18 chromatography (column: 2.5 × 24 cm; MeOH−H_2O, 65:35) followed by HPLC (MeOH−H_2O, 60:40; flow rate: 2.0 mL/min) to obtain subfraction SMB4-2-7-1-2-2-2 (31.0 mg), which was further purified by RP-HPLC (MeCN−H_2O, 30:70; flow rate: 2.0 mL/min) to give **15** (2.0 mg, t_R = 16 min) and **14** (7.0 mg, t_R = 18 min).

Subfraction SMB5 (7.7 g) was separated over a Sephadex LH-20 column (5 × 58 cm; MeOH−H_2O, 100:0 to 80:20) to obtain six subfractions. Subfraction SMB5-2 (3.49 g) was purified by silica gel chromatography (230−400 mesh, column: 4.5 × 23 cm; $CHCl_3$−MeOH, 5:1), and its subfraction SMB5-2-3 (431.0 mg) was subjected to RP-18 chromatography (column: 2.5 × 24 cm; MeOH−H_2O, 70:30) and then HPLC (MeOH−H_2O, 70:30; flow rate: 2.0 mL/min) to give SMB5-2-3-2-4-1 (60.0 mg). Subfraction SMB5-2-3-2-4-1 was purified with RP-18 chromatography (column: 2.5 × 28 cm; MeCN−H_2O, 35:65) to give **17** (46.0 mg). Subfraction SMB5-2-4 (1.19 mg) was subjected to RP-18 chromatography (column: 2.5 × 24 cm; MeOH−H_2O, 80:20) to give SMB5-2-4-4 (661.0 mg), which was

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separated into four fractions by RP-18 chromatography (column: 2.5 × 24.5 cm; MeOH−H_2O, 80:20). SMB5-2-4-4-2 (510.0 mg) was purified by RP-HPLC (MeOH−H_2O, 80:20 and MeCN−H_2O, 35:65) and RP-18 chromatography (column: 2.5 × 28 cm; MeCN−H_2O, 35:65) to give **18** (21.0 mg). Subfraction SMB5-2-4-4-2-3 (315.0 mg) was subjected to RP-HPLC (MeCN−H_2O, 35:65) to give two major fractions, SMB-5-2-4-4-2-3-2 (80.0 mg) and SMB-5-2-4-4-2-3-3 (60.0 mg), which were purified by RP-18 chromatography (column: 2.5 × 28 cm; MeCN−H_2O, 30:70 and 35:65) to give **12** (37.0 mg) and **16** (47.0 mg), respectively. Subfraction SMB5-2-4-4-4 (37.0 mg) was subjected to RP-HPLC (MeOH−H_2O, 80:20) to give **9** (22.0 mg, t_R = 19 min).

Subfraction SMB7 (1.5 g) was subjected to Sephadex LH-20 (column: 5 × 48 cm; MeOH) and silica gel chromatography (230−400 mesh, column: 3 × 24 cm; $CHCl_3$−MeOH, 4:1) to obtain seven subfractions. SMB7-4-4 (90.0 mg) was further purified by RP-18 chromatography (column: 2 × 20.5 cm; MeOH−H_2O, 90:10) and RP-HPLC (MeOH−H_2O, 85:15; flow rate: 2.0 mL/min) to afford pure compound **8** (3.0 mg, t_R = 26 min).

*Macaoside A (**1**):* $[\alpha]_D^{26}$ −20.7 (*c* 0.15, MeOH); IR (neat) ν_{max} 3401 (OH), 2928 (CH), 1705 (C═O), 1452, 1379, 1240, 1169, 1074, 1049 (C−O−C), 982, 964, 918, 899, 866, 756 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 731.3982 $[M + Na]^+$ (calcd for $C_{38}H_{60}O_{12}Na$, 731.3977).

*Macaoside B (**2**):* $[\alpha]_D^{26}$ −26.7 (*c* 0.09, MeOH); IR (neat) ν_{max} 3364 (OH), 2949, 2928, 2872 (CH), 1454, 1375, 1240, 1175, 1096, 1047 (C−O−C), 982, 920, 899, 878, 866, 756 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 777.4399 $[M + Na]^+$ (calcd for $C_{40}H_{66}O_{13}Na$, 777.4396).

*Macaoside C (**3**):* $[\alpha]_D^{27}$ +17.1 (*c* 0.07, MeOH); IR (neat) ν_{max} 3389 (OH), 2951, 2930, 2874 (CH), 1452, 1379, 1244, 1169, 1070, 1049 (C−O−C), 1003, 961, 920, 897, 862, 756, 667 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 749.4086 $[M + Na]^+$ (calcd for $C_{38}H_{62}O_{13}Na$, 749.4083).

*Macaoside D (**4**):* $[\alpha]_D^{26}$ −25.5 (*c* 0.11, MeOH); IR (neat) ν_{max} 3242 (OH), 2930, 2857 (CH), 1242, 1215, 1173, 1076, 1053 (C−O−C), 982, 957, 920, 899, 866, 756, 667 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 719.3987 $[M + Na]^+$ (calcd for $C_{37}H_{60}O_{12}Na$, 719.3977).

*Macaoside E (**5**):* $[\alpha]_D^{28}$ +35.0 (*c* 0.08, MeOH); IR (neat) ν_{max} 3319 (OH), 2951, 2928, 2857 (CH), 1454, 1379, 1240, 1215, 1055 (C−O−C), 1038, 982, 920, 899, 866, 758, 665 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 763.4239 $[M + Na]^+$ (calcd for $C_{39}H_{64}O_{13}Na$, 763.4239).

*Macaoside F (**6**):* $[\alpha]_D^{27}$ +43.8 (*c* 0.08, MeOH); IR (neat) ν_{max} 3389 (OH), 2930, 2872 (CH), 1240, 1128, 1045 (C−O−C), 982, 955, 918, 897, 864, 814, 756 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 763.4235 $[M + Na]^+$ (calcd for $C_{39}H_{64}O_{13}Na$, 763.4239).

*Macaoside G (**7**):* $[\alpha]_D^{27}$ +48.8 (*c* 0.08, MeOH); IR (neat) ν_{max} 3381 (OH), 2924, 2850 (CH), 1449, 1379, 1360, 1242, 1175, 1155, 1076, 1047 (C−O−C), 1007, 984, 957, 922, 901, 864 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 763.4238 $[M + Na]^+$ (calcd for $C_{39}H_{64}O_{13}Na$, 763.4239).

*Macaoside H (**8**):* $[\alpha]_D^{28}$ −41.0 (*c* 0.10, MeOH); IR (neat) ν_{max} 3356 (OH), 2930 (CH), 1452, 1379, 1215, 1128, 1043 (C−O−C), 982, 955, 918, 899, 866, 837, 812, 756, 667 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 909.4820 $[M + Na]^+$ (calcd for $C_{45}H_{74}O_{17}Na$, 909.4818).

*Macaoside I (**9**):* $[\alpha]_D^{27}$ +27.0 (*c* 0.10, MeOH); IR (neat) ν_{max} 3360 (OH), 2914, 2874 (CH), 1692, 1643, 1449, 1379, 1304, 1215, 1167, 1074, 1045 (C−O−C), 939, 893, 856, 756, 665 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 3; HRESIMS *m*/*z* 873.4842 $[M + H]^+$ (calcd for $C_{44}H_{73}O_{17}$, 873.4842).

*Macaoside J (**10**):* $[\alpha]_D^{27}$ +39.0 (*c* 0.10, MeOH); IR (neat) ν_{max} 3360 (OH), 2932, 2872 (CH), 1643, 1452, 1379, 1169, 1074, 1047 (C−O−C), 1016, 959, 895, 756 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 3; HRESIMS *m*/*z* 911.4620 $[M + Na]^+$ (calcd for $C_{44}H_{72}O_{18}Na$, 911.4611).

*Macaoside K (**11**):* $[\alpha]_D^{27}$ −28.0 (*c* 0.10, MeOH); IR (neat) ν_{max} 3360 (OH), 2930, 2872 (CH), 1447, 1379, 1215, 1167, 1074, 1043 (C−O−C), 895, 758, 667 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 3; HRESIMS *m*/*z* 911.4614 $[M + Na]^+$ (calcd for $C_{44}H_{72}O_{18}Na$, 911.4611).

*Macaoside L (**12**):* $[\alpha]_D^{28}$ −82.5 (*c* 0.12, MeOH); IR (neat) ν_{max} 3360 (OH), 2930 (CH), 1454, 1379, 1337, 1310, 1248, 1167, 1072, 1045 (C−O−C), 893, 754 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 4 and 5; HRESIMS *m*/*z* 913.4773 $[M + Na]^+$ (calcd for $C_{44}H_{74}O_{18}Na$, 913.4767).

*Macaoside M (**13**):* $[\alpha]_D^{28}$ −83.8 (*c* 0.08, MeOH); IR (neat) ν_{max} 3381 (OH), 2932, 2849 (CH), 1643, 1454, 1381, 1165, 1076, 1040 (C−O−C), 1018, 955, 897, 868, 756 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 4 and 5; HRESIMS *m*/*z* 797.4295 $[M + Na]^+$ (calcd for $C_{39}H_{66}O_{15}Na$, 797.4294).

*Macaoside N (**14**):* $[\alpha]_D^{28}$ +22.0 (*c* 0.10, MeOH); IR (neat) ν_{max} 3401 (OH), 2934, 2847 (CH), 1643, 1452, 1383, 1265, 1134, 1101, 1080, 1043 (C−O−C), 953, 912, 837, 816, 779, 669 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 4 and 5; HRESIMS *m*/*z* 943.4879 $[M + Na]^+$ (calcd for $C_{45}H_{76}O_{19}Na$, 943.4873).

*Macaoside O (**15**):* $[\alpha]_D^{28}$ +25.0 (*c* 0.10, MeOH); IR (neat) ν_{max} 3381 (OH), 2932 (CH), 1643, 1452, 1379, 1260, 1171, 1074, 1045 (C−O−C), 953, 895, 629 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 4 and 5; HRESIMS *m*/*z* 943.4878 $[M + Na]^+$ (calcd for $C_{45}H_{76}O_{19}Na$, 943.4873).

*Macaoside P (**16**):* $[\alpha]_D^{28}$ −88.0 (*c* 0.10, MeOH); IR (neat) ν_{max} 3304 (OH), 2934 (CH), 1452, 1379, 1215, 1169, 1072, 1047 (C−O−C), 935, 895, 756 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 4 and 5; HRESIMS *m*/*z* 927.4927 $[M + Na]^+$ (calcd for $C_{45}H_{76}O_{18}Na$, 927.4924).

*Macaoside Q (**17**):* $[\alpha]_D^{28}$ +21.0 (*c* 0.10, MeOH); IR (neat) ν_{max} 3360 (OH), 2932 (CH), 1703 (C═O), 1452, 1379, 1167, 1072, 1045 (C−O−C), 893, 754 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 4 and 5; HRESIMS *m*/*z* 925.4770 $[M + Na]^+$ (calcd for $C_{45}H_{74}O_{18}Na$, 925.4767).

*Macaoside R (**18**):* $[\alpha]_D^{28}$ −62.2 (*c* 0.09, MeOH); IR (neat) ν_{max} 3379 (OH), 2930 (CH), 1643, 1452, 1379, 1248, 1169, 1076, 1045 (C−O−C), 897, 754 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 4 and 5; HRESIMS *m*/*z* 913.4770 $[M + Na]^+$ (calcd for $C_{44}H_{74}O_{18}Na$, 913.4767).

Acid Hydrolysis and GC/MS Analysis. Isolates (9.8 mg for **12**, 9.7 mg for **16**, 0.5−1 mg for the remaining compounds) were hydrolyzed in 1 M HCl−1,4-dioxane (1:1, 1.4 mL) at 90 °C for 3 h. Each was cooled and partitioned with $CHCl_3$−H_2O (1:1, 14 mL), with the $CHCl_3$ fraction providing an aglycone moiety. Each aqueous layer was neutralized with saturated aqueous Na_2CO_3 solution and then filtered. On drying, the filtrate was redissolved in pyridine (0.4 mL), and excess Ac_2O (0.1 mL) was added. The mixture was stirred overnight and then dried under vacuum before being extracted with $CHCl_3$ and H_2O (1:1, 14 mL). The acetylated $CHCl_3$ fraction was subjected to gas chromatography−mass spectrometry (DSQ II GC/MS, Thermo Scientific, Waltham, MA, USA) under the following conditions: DB-5MS capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness); injector, 250 °C; injection volume, 1 μL; column oven temperature, 155 °C for 60 min; column flow, 1.0 mL/min; ion source temperature, 250 °C; EI, 70 eV; mass range, *m*/*z* 50−800. Under these conditions, the retention times (t_R) for the acetate derivatives of standard sugars were D-xylose (13.51 and 14.76 min), L-xylose (13.50 and 14.78 min), D-rhamnose (14.09 and 14.31 min), L-rhamnose (14.06 and 14.32 min), D-glucose (49.27 and 49.95 min), L-glucose (49.15 and 49.83 min), and D-quinovose (13.13 and 13.30 min). Co-injection studies of the hydrolysates with the different standards were also conducted to confirm the presence of D-xylose, L-rhamnose, D-glucose, and D-quinovose in compounds **1**−**18**.

Isolation of Human Neutrophils. The study was approved by the Institutional Review Board of Chang Gung Memorial Hospital (IRB 102-1595A3). All healthy donors (20−30 years old) did not take any medication a week before blood sample collection. Human neutrophils were isolated from heparinized peripheral venous blood by dextran sedimentation and Ficoll-Hypaque centrifugation. After

hypotonic lysis of contaminating erythrocytes, neutrophils isolated were suspended in Ca^{2+}-free HBSS at pH 7.4 and maintained at 4 °C before use. Wright-Giemsa stain was used to confirm the purity of the neutrophil suspension, and greater than 98% viability was determined by trypan blue exclusion.

Measurement of Superoxide Anion Generation (refs 15 and 16). Neutrophils were equilibrated with 0.5 mg/mL ferricytochrome *c* and 1 mM Ca^{2+} at 37 °C for 2 min and then incubated with each test compound for 5 min. Cells were incubated with cytochalasin B (1 μg/mL) for 3 min, before activation by formyl-L-methionyl-L-leucyl-L-phenylalanine (100 nM) for 10 min. FMLP/CB was used as a stimulant to activate neutrophils to produce superoxide anion. The changes in absorbance with reduction of ferricytochrome *c* at 550 nm were continuously monitored in a double-beam, six-cell positioned spectrophotometer (Hitachi U-3010, Tokyo, Japan) with constant stirring. Calculations were based on differences in the reactions with and without superoxide dismutase (100 U/mL) divided by the extinction coefficient for the reduction of ferricytochrome *c* (ε = 21.1/mM/10 mm). The positive control was diphenyleneiodonium (DPI), an NADPH oxidase inhibitor.

Elastase Release Assay (refs 15 and 16). Neutrophils were equilibrated in an elastase substrate, MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide (100 μM), at 37 °C for 2 min and then incubated with test compounds for 5 min. Cells were activated by 100 nM FMLP and 0.5 μg/mL CB, and the changes in absorbance at 405 nm were monitored continuously to assay elastase release. The results were expressed as the percentage of elastase release in the FMLP/CB-activated, drug-free control system. Sivelestant, an inhibitor of human neutrophil elastase, was used as the positive control.

Lactate Dehydrogenase (LDH) Release. This assay was carried out according to an established protocol.[17] Release of LDH into the cell medium indicated cell membrane damage. Neutrophilic cytotoxicity was determined by LDH release in the cell-free medium as a percentage of the total LDH release.

Degranulation Assay. The cell viability was tested using an MTT assay.[18] Analysis of β-hexosaminidase release is a well-known assay for measuring the degree of degranulation in RBL-2H3 cells (rat basophilic leukemia cells).[19] In brief, RBL-2H3 cells were cultured in DMEM/F12 medium containing 10% FBS and then seeded into a 96-well plate at a concentration of 10^4 cells/well. The cells were treated with compounds **1** and **4** (0, 1, 4, 16, 64 μM) and the positive control dexamethasone (100 nM), respectively, for 48 h. The treated RBL-2H3 cells were incubated overnight with anti-DNP IgE (500 μg/mL) for sensitization. Cells were then washed with Tyrode's buffer and stimulated with 2,4-dinitrophenylated albumin from bovine serum (DNP-BSA, 0.1 μg/mL in Tyrode's buffer) for 1 h to induce the immediate-phase antigen-stimulated degranulation assay. The reaction of degranulation was stopped by cooling in an ice bath, and the supernatant was collected for β-hexosaminidase release measurement. The degree of β-hexosaminidase release was assessed by the enzymatic activity observed. The supernatant was incubated with substrate (0.1 M *p*-nitrophenyl-*N*-acetyl-D-glucosaminide in 0.1 M citrate buffer) for 1 h and was stopped by adding sodium bicarbonate stopping buffer. The activity of the test samples was measured by the absorbance observed at 405 nM. The ratio of β-hexosaminidase release was calculated by the following equation: the release % = (OD sample − OD background)/(OD total lysate − OD background) × 100.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The 1D and 2D NMR data of all new compounds are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on the Web on July 18, 2014, with an error in the name of compound **13** in the Results and Discussion section. The corrected version was reposted on August 12, 2014.