14,15-Secopregnane Derivatives from the Leaves of *Solenostemma argel*

Angela Perrone,[†] Alberto Plaza,[†] Simona Francesca Ercolino,[†] Arafa I. Hamed,[‡] Luca Parente,[†] Cosimo Pizza,[†] and Sonia Piacente*,[†]

Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, Via Ponte Don Melillo, 84084 Fisciano, Salerno, Italy, and Faculty of Science, South Valley University, Aswan 81528, Egypt

Received July 21, 2005

Five new 14,15-secopregnane derivatives, named argelosides K−O (**1**−**5**), have been isolated from *Solenostemma argel* leaves. Their structures were established by a detailed spectroscopic analysis. In particular, argeloside N (**1**) showed in the sugar portion an unusual 3-*O*-methyl-2,6-dideoxyhexopyranose unit characterized by the occurrence of a Δ^3 double bond, and argeloside O (**5**) displayed an unusual moiety linked to position 3 probably derived by the oxidation of a 3-*O*-methyl-2,6-dideoxyhexopyranose unit. The propensity of compounds **2**, **3**, and **5** and argeloside F (**6**) to inhibit TNF-α release by LPS-stimulated RAW 264.7 mouse cells was evaluated.

Solenostemma argel Hayne (Asclepiadaceae) is an Egyptian wild perennial erect shrub growing in the eastern desert and along the Nile in South Egypt.[1] The leaves are used widely in traditional medicine as a purgative, antipyretic, expectorant, and antispasmodic, and to treat bile congestion.[2] Previous investigations have reported the occurrence of monoterpenes,[3] pregnane glycosides,[4,5] and acylated phenolic glycosides in the leaves of this species.[6] In previous communications, we have reported the isolation of 15-keto-pregnane glycosides from the pericarps[7,8] and leaves,[8] and 14,-15-secopregnane glycosides from the pericarps[9,10] and hairy seeds.[10] The effect of these compounds on VEGF-induced Kaposi's sarcoma cell proliferation was also evaluated, and results indicated that 15-keto-pregnane glycosides and 14,15-secopregnane glycosides reduced the cell proliferation in a dose-dependent manner.[8,10]

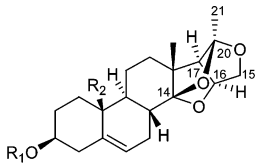
On the other hand, some pregnane compounds isolated from *Mandevilla illustris* and *Mandevilla velutina* (Apocynaceae) exerted anti-inflammatory activity by antagonizing bradykinin and related in vitro and in vivo kinin-mediated responses.[11] Among these compounds were velutinol A and illustrol, which are nor-secopregnanes. Furthermore, an illustrol glycoside produced a significant inhibition of carrageenan-induced paw edema while failing to affect bradykinin-induced paw edema.[12]

Here we report the occurrence of five new 14,15-secopregnane derivatives, namely argelosides K−O (**1**−**5**), along with the known compounds argelosides D, F (**6**), I, and J,[10] from the leaves of *S. argel*. Their structures were elucidated by extensive spectroscopic methods including 1D- (^{1}H and ^{13}C) and 2D-NMR (DQF-COSY, HSQC, HMBC, and HOHAHA) experiments as well as ESIMS analysis.

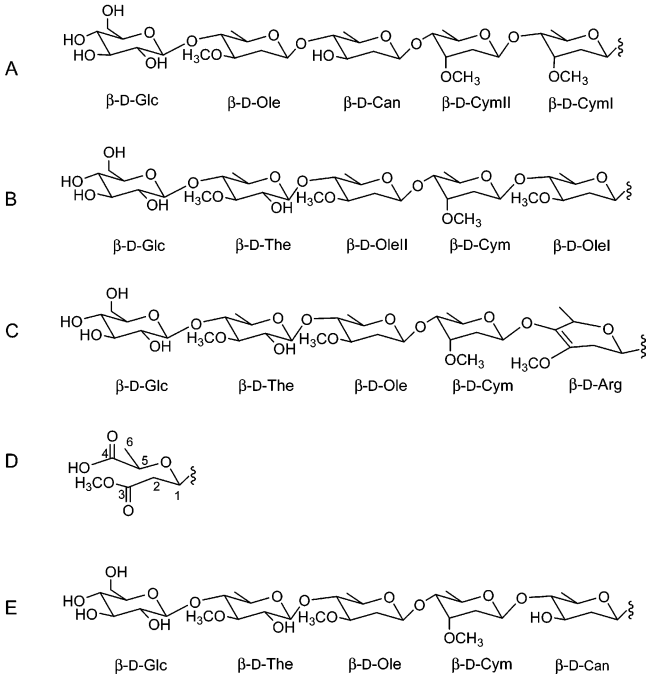
On the basis of the beneficial effect on acute inflammation exerted by nor-secopregnanes isolated from *M. illustris* and *M. velutina*, argelosides F (**6**), L (**2**), M (**3**), and O (**5**) were tested for inhibition of TNF-α release by LPS-stimulated RAW 264.7 mouse cells.

Results and Discussion

The leaves of *S. argel* were extracted with 70% EtOH and fractionated over Sephadex LH-20. The fractions containing pregnane glycosides were chromatographed by silica gel MPLC and reversed-phase HPLC to yield five new compounds, **1**−**5**, along with the known compounds argelosides D, F (**6**), I, and J[10] (see Experimental Section).



argeloside K	(**1**)	R_1 = A	R_2 = Me
argeloside L	(**2**)	R_1 = A	R_2 = CH_2OAc
argeloside M	(**3**)	R_1 = B	R_2 = CH_2OH
argeloside N	(**4**)	R_1 = C	R_2 = Me
argeloside O	(**5**)	R_1 = D	R_2 = Me
argeloside F	(**6**)	R_1 = E	R_2 = Me



The HRMALDIMS of **1** showed a major ion peak at *m*/*z* 1093.5551 $[M + Na]^+$, ascribable to a molecular formula of $C_{54}H_{86}O_{21}$ (calcd for $C_{54}H_{86}O_{21}Na$ 1093.5559). The ESIMS of **1** gave the highest mass ion peak at *m*/*z* 1093, which was assigned to the $[M + Na]^+$ ion. The MS/MS analysis of the ion at *m*/*z* 1093 showed the most intense ion at *m*/*z* 763 $[M + Na - 330]^+$

* To whom correspondence should be addressed. Tel: ++39089962616. Fax: ++39089962828. E-mail: piacente@unisa.it.
[†] Università degli Studi di Salerno.
[‡] South Valley University, Aswan.

10.1021/np050263c CCC: $33.50
Published on Web 12/10/2005

Table 1. ^{13}C and ^{1}H NMR Spectroscopic Data of the Sugar Portions of Compounds **1**−**5** (CD_3OD)

	1−**2** chain A		**3**, chain B		**4**, chain C		**5**, chain D	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
		β-D-CymI		β-D-OleI		β-D-Arg		3,4-secoargelose
1	96.9	4.90 dd (9.2, 2.0)	98.7	4.68 dd (9.6, 1.8)	97.6	4.86 dd (9.5, 2.0)	99.6	5.14 dd (7.9, 3.5)
2	36.8	2.08 m	37.9	2.23 ddd (13.0, 4.0, 1.8)	33.6	2.37 m	42.2	2.92 dd (14.9, 3.5)
		1.57 m		1.39 ddd (13.0, 9.6, 9.0)		2.37 m		2.63 dd (14.9, 7.9)
3	78.3	3.88 br m	80.1	3.38 ddd (9.5, 9.0, 4.0)	138.4		172.0	
4	83.7	3.25 dd (9.5, 6.1)	83.9	3.14 dd (9.5, 9.5)	138.9		180.7	
5	69.6	3.85 dq (9.5, 6.1)	72.0	3.36 dq (9.5, 6.1)	71.2	4.34 m	74.8	4.18 q (6.6)
6	18.3	1.22 d (6.1)	18.1	1.28 d (6.1)	18.7	1.28 d (6.1)	19.7	1.35 d (6.6)
OMe	58.1	3.46 s	57.3	3.45 s	56.7	3.61 s	51.7	3.69 s
		β-D-CymII		β-D-Cym		β-D-Cym		
1	100.9	4.83 dd (9.6, 2.0)	99.2	4.92 dd (9.2, 2.0)	101.2	5.00 dd (9.5, 2.0)		
2	36.2	2.17 m	36.2	2.16 m	35.8	2.34 m		
		1.62 m		1.57 m		1.63 m		
3	78.3	3.88 br m	78.2	3.87 br m	78.3	3.90 br m		
4	83.7	3.30 dd (9.5, 3.0)	83.6	3.30 dd (9.5, 3.0)	83.6	3.31 dd (9.5, 3.0)		
5	69.6	3.85 dq (9.5, 6.1)	70.0	3.85 dq (9.5, 6.1)	70.1	3.81 dq (9.5, 6.1)		
6	18.3	1.25 d (6.1)	18.1	1.26 d (6.1)	18.3	1.24 d (6.1)		
OMe	58.1	3.45 s	58.2	3.46 s	58.3	3.47 s		
		β-D-Can		β-D-OleII		β-D-Ole		
1	102.1	4.67 dd (9.6, 2.0)	102.4	4.62 dd (9.0, 2.0)	102.4	4.63 dd (9.6, 2.0)		
2	39.4	2.23 ddd (13.0, 4.0, 2.0)	37.5	2.34 ddd (13.0, 4.0, 2.0)	37.5	2.36 ddd (13.0, 4.0, 2.0)		
		1.52 ddd (13.0, 9.6, 9.0)		1.45 ddd (13.0, 9.0, 9.0)		1.47 ddd (13.0, 9.6, 9.0)		
3	70.5	3.63 ddd (9.5, 9.0, 4.0)	79.9	3.41 ddd (9.5, 9.0, 4.0)	80.2	3.41 ddd (9.5, 9.0, 4.0)		
4	88.5	3.04 dd (9.5, 9.5)	83.9	3.23 dd (9.5, 9.5)	84.2	3.25 dd (9.5, 9.5)		
5	71.5	3.39 dq (9.5, 6.1)	72.2	3.42 dq (9.5, 6.1)	72.4	3.42 dq (9.5, 6.1)		
6	17.9	1.32 d (6.1)	18.5	1.41 d (6.1)	18.4	1.41 d (6.1)		
OMe			57.3	3.45 s	57.5	3.45 s		
		β-D-Ole		β-D-The		β-D-The		
1	102.1	4.67 dd (9.6, 2.0)	104.4	4.47 d (7.5)	104.2	4.48 d (7.9)		
2	37.4	2.43 ddd (13.0, 4.0, 2.0)	75.1	3.27 dd (9.5, 7.5)	74.9	3.27 dd (9.5, 7.9)		
		1.49 ddd (13.0, 9.6, 9.0)						
3	79.7	3.49 ddd (9.5, 9.0, 4.0)	86.2	3.21 dd (9.5, 9.5)	86.3	3.22 m		
4	83.2	3.37 dd (9.5, 9.5)	82.8	3.38 dd (9.5, 9.5)	82.8	3.39 m		
5	72.7	3.55 dq (9.5, 6.1)	72.5	3.47 m	72.5	3.47 m		
6	18.1	1.43 d (6.1)	18.5	1.41 d (6.1)	18.4	1.41 d (6.1)		
OMe	58.0	3.51 s	61.1	3.66 s	61.1	3.67 s		
		β-D-Glc		β-D-Glc		β-D-Glc		
1	103.9	4.49 d (7.5)	104.4	4.45 d (7.5)	104.2	4.46 d (7.5)		
2	75.3	3.21 dd (9.0, 7.5)	75.5	3.20 dd (9.0, 7.5)	75.6	3.21 dd (9.0, 7.5)		
3	77.9	3.37 dd (9.0, 9.0)	78.1	3.38 dd (9.0, 9.0)	77.9	3.38 dd (9.0, 9.0)		
4	71.5	3.27 dd (9.0, 9.0)	71.6	3.24 dd (9.0, 9.0)	71.8	3.25 dd (9.0, 9.0)		
5	78.1	3.28 ddd (9.0, 4.5, 2.0)	78.2	3.28 ddd (9.0, 4.5, 2.0)	78.2	3.29 ddd (9.0, 4.5, 2.0)		
6	62.7	3.90 dd (12.0, 2.0)	62.8	3.88 dd (12.0, 2.0)	63.0	3.90 dd (12.0, 2.0)		
		3.67 dd (12.0, 4.5)		3.66 dd (12.0, 4.5)		3.67 dd (12.0, 4.5)		

ascribable to the loss of the aglycon moiety. Another intense peak was observed at *m/z* 603 $[M + Na - 330 - 160]^+$, corresponding to the loss of a methyldideoxyhexose unit. The ^{1}H and ^{13}C NMR chemical shifts of the aglycon moiety of **1** closely resembled those of argeloside C, previously isolated from the hairy seeds of *S. argel*[10] and characterized as (14*S*,16*S*,20*R*)-14,16−14,20−15,20-triepoxy-14,15-secopregn-5-en-3-ol.

The ^{1}H NMR spectrum of compound **1** for the sugar portion showed signals corresponding to four doublet methyls at δ 1.43 (3H, d, $J = 6.1$ Hz), 1.32 (3H, d, $J = 6.1$ Hz), 1.25 (3H, d, $J = 6.1$ Hz), and 1.22 (3H, d, $J = 6.1$ Hz), three methoxy groups at δ 3.51 (3H, s), 3.46 (3H, s), and 3.45 (3H, s), and signals for five anomeric protons at δ 4.90 (1H, dd, $J = 9.2$, 2.0 Hz), 4.83 (1H, dd, $J = 9.6$, 2.0 Hz), 4.67 (2H, dd, $J = 9.6$, 2.0 Hz), and 4.49 (1H, d, $J = 7.5$ Hz) (see Table 1). All of these data indicated that the sugar chain of compound **1** consists of five sugars, with four of them being 2,6-dideoxy sugars. The chemical shifts of all the individual protons of the five sugar units were ascertained from a combination of 2D-HOHAHA and DQF-COSY NMR spectroscopic analysis, and the ^{13}C NMR chemical shifts of their attached carbons could be assigned unambiguously from the HSQC spectrum (see Table 1). These data showed the presence of two β-cymaropyranosyl units (δ 4.90 and 4.83), one β-canaropyranosyl unit (δ 4.67), one β-oleandropyranosyl unit (δ 4.67), and one β-glucopyranosyl unit (δ 4.49). Glycosidation shifts were observed for C-4_{can} (δ 88.5), C-4_{cymI} (δ 83.7), C-4_{cymII} (δ 83.7), and C-4_{ole} (δ 83.2), suggesting that β-glucopyranosyl was a terminal unit. Finally, direct connectivity information was obtained from the HMBC spectrum, which showed the following key correlation peaks: δ_H 4.90 (H-1_{cymI}) and δ_C 78.9 (C-3), δ_H 4.83 (H-1_{cymII}) and δ_C 83.7 (C-4_{cymI}), δ_H 4.67 (H-1_{can}) and δ_C 83.7 (C-4_{cymII}), δ_H 4.67 (H-1_{ole}) and δ_C 88.5 (C-4_{can}), and δ_H 4.49 (H-1_{glc}) and δ_C 83.2 (C-4_{ole}). It is worthwhile noting that these results are in accordance with the fact that C-1 of β-cymarose characteristically resonates upfield (∼97.0 ppm) when linked at C-3 of the aglycon compared with a resonance at ∼101.0 ppm when it is linked to the hydroxyl group of a different sugar.[13] On the basis of all of this evidence, the sugar chain of compound **1** was deduced as 3-*O*-β-glucopyranosyl-(1→4)-β-oleandropyranosyl-(1→4)-β-canaropyranosyl-(1→4)-β-cymaropyranosyl-(1→4)-β-cymaropyranoside.

Due to the very small amount available for each isolated glycoside and the necessity to preserve it for biological assays, the absolute configurations of the sugar units were assigned after acid hydrolysis of the crude pregnane glycoside mixture and fractionation of the hydrolysate by silica gel column chromatography, which afforded canarose, cymarose, glucose, oleandrose, thevetose, and a sugar never reported in the literature that we called argelose. These sugars were established to be in the D-form on the basis of their optical rotation values.[14]

Thus compound **1** was identified as the new pregnane glycoside (14*S*,16*S*,20*R*)-14,16−14,20−15,20-triepoxy-14,15-secopregn-5-en-

3-ol-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-canaropyranosyl-(1→4)-β- D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside and has been named argeloside K.

The molecular formula of compound **2** was established unequivocally to be $C_{56}H_{88}O_{23}$ by HRMALDIMS (*m/z* 1151.5597 [M + Na]$^+$, calcd for $C_{56}H_{88}O_{23}Na$, 1151.5614). The ESIMS of **2** showed a major ion peak at *m/z* 1151 [M + Na]$^+$. Its MS/MS fragmentation showed an intense peak at *m/z* 1091 [M + Na − 60]$^+$ due to the loss of an acetate molecule. The MS^3 fragmentation of this ion showed two intense ions at *m/z* 763 [M + Na − 60 − 328]$^+$, corresponding to the loss of the aglycon moiety, and *m/z* 603 [M + Na − 60 − 328 − 160]$^+$, ascribable to the loss of a methyldideoxyhexose. The ^{1}H NMR spectrum of the aglycon portion of **2** in comparison to that of **1** showed the presence of a signal at 2.05 (3H, s) ascribable to an acetyl group, two signals at δ 4.62 (1H, dd, J = 11.8 Hz, H-19a) and 4.01 (1H, dd, J = 11.8 Hz, H-19b), and the absence of the methyl signal at δ 1.07 (3H, s, Me-19). Moreover, a detailed analysis of the ^{13}C NMR data and in particular of the HMBC spectrum, which showed correlations between the two proton signals at δ 4.62 and 4.01 and the carbon resonances at δ 34.8 (C-1), 46.4 (C-9), and 46.5 (C-10), suggested that compound **2** differed from **1** only by the presence of an acetoxy function at C-19 instead of the methyl group. Thus, compound **2** (argeloside L) was identified as the new substance (14*S*,16*S*,20*R*)-19-acetoxy-14,16−14,20−15,20-triepoxy-14,15-secopregn-5-en-3-ol-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-canaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

Compound **3** showed a major ion peak at *m/z* 1139 [M + Na]$^+$ and significant fragments at *m/z* 1109 [M + Na − 30]$^+$, *m/z* 793 [M + Na − 30 − 316]$^+$, and *m/z* 633 [M + Na − 30 − 316 − 160]$^+$ in the positive ESIMS. Its molecular formula was established unequivocally as $C_{55}H_{88}O_{23}$ by HRMALDIMS (*m/z* 1139.5603 [M + Na]$^+$, calcd for $C_{55}H_{88}O_{23}Na$, 1139.5614). The ^{1}H and ^{13}C NMR data of the aglycon moieties of **3** and argeloside D, previously isolated from the hairy seeds of *S. argel*,[10] were almost superimposable, suggesting these compounds have the same aglycon portion, namely, (14*S*,16*S*,20*R*)-14,16−14,20−15,20-triepoxy-14,-15-secopregn-5-en-3,19-diol. Additionally for **3**, resonances of anomeric protons were observed in the ^{1}H NMR spectrum at δ 4.92 (1H, dd, J = 9.2, 2.0 Hz), 4.68 (1H, dd, J = 9.6, 1.8 Hz), 4.62 (1H, dd, J = 9.0, 2.0 Hz), 4.47 (1H, d, J = 7.5 Hz), and 4.45 (1H, d, J = 7.5 Hz) (Table 1). A detailed analysis of the NMR data (^{1}H, ^{13}C, 2D-HOHAHA, DQF-COSY, HSQC) showed that **3** differed from argeloside D in the occurrence of an additional β-glucopyranosyl terminal unit (δ 4.45) linked at C-4 of the thevetose unit, as suggested by the downfield shift exhibited by this carbon (δ 82.8) (Table 1) when compared with the same carbon in argeloside D (δ 76.6).[10] On the basis of all of this evidence, the sugar chain of compound **3** was deduced as 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-oleandropyranoside. Thus, the structure (14*S*,16*S*,20*R*)-14,16−14,20−15,20-triepoxy-14,15-secopregn-5-en-3,19-diol-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-oleandropyranoside was assigned to argeloside M (**3**).

The molecular formulas of compounds **4** and **5** were established unequivocally to be $C_{55}H_{86}O_{22}$ and $C_{28}H_{40}O_9$, respectively, by HRMALDIMS (*m/z* 1121.5508 [M + Na]$^+$, calcd for $C_{55}H_{86}O_{22}$-Na, 1121.5508 for **4**; *m/z* 543.2642 [M + Na]$^+$, calcd for $C_{28}H_{40}O_9$-Na, 543.2570 for **5**). A detailed comparison of the aglycon portion of the NMR data (^{1}H, ^{13}C, 2D-HOHAHA, DQF-COSY, HSQC, HMBC) of compounds **1**, **4**, and **5** revealed that the aglycon was identical in all three compounds (see Experimental Section). The ^{1}H NMR spectrum of **4** for the sugar portion showed resonances for five anomeric protons at δ 5.00 (1H, dd, J = 9.5, 2.0 Hz), 4.86 (1H, dd, J = 9.5, 2.0 Hz), 4.63 (1H, dd, J = 9.6, 2.0 Hz), 4.48 (1H, d, J = 7.9 Hz), and 4.46 (1H, d, J = 7.5 Hz). Complete assignments of the ^{1}H and ^{13}C NMR signals of the sugar portion were accomplished by HSQC, HMBC, DQF-COSY, and 2D-HOHAHA experiments, which led to the identification of one β-cymaropyranosyl unit (δ 5.00), one β-oleandropyranosyl unit (δ 4.63), one β-thevetopyranosyl unit (δ 4.48), and one β-glucopyranosyl unit (δ 4.46). The sequence of the remaining sugar unit (δ 4.86) was deduced from the analysis of the DQF-COSY and HMBC spectra. In particular, the connectivities between δ 4.86 (H-1′) and 2.37 (H-2′) and between δ 4.34 (H-5′) and 1.28 (Me-6′) were confirmed by the COSY spectrum. Moreover, the following key HMBC correlations were observed: δ_H 4.86 (H-1′) and δ_C 33.6 (C-2′) and δ 78.9 (C-3); δ_H 2.37 (H-2′) and δ_C 97.6 (C-1′) and δ_C 138.4 (C-3′); and δ_H 1.28 (Me-6′) and δ_C 138.9 (C-4′) and δ_C 71.2 (C-5′). The placement of the methoxy group on C-3′ was deduced from the HMBC correlation of the signal at δ 3.61 with the carbon resonance at δ 138.4. All this evidence allowed us to deduce that this sugar unit is a 3-*O*-methyl-2,6-dideoxyhexopyranose characterized by a double bond between C-3′ and C-4′. This structure was confirmed by ESIMS analysis. The ESIMS of **4** showed a major ion peak at *m/z* 1121 [M + Na]$^+$ and a significant fragment at *m/z* 775 [M + Na − 346]$^+$ corresponding to the loss of the aglycon moiety. Another intense peak was observed at *m/z* 633 [M + Na − 346 − 142]$^+$, ascribable to the loss of a dehydromethyldideoxyhexose unit. On the basis of these results, this sugar unit was identified as the new 3,4-dehydro-3-*O*-methyl-2,6-dideoxyhexopyranose, named argelose. The β-configuration of this sugar was shown by the large (J = 9.5 Hz) coupling constant of the anomeric proton signal.[15] Once again, direct evidence of the sugar sequence and the linkage sites was derived from HSQC and HMBC experiments. The absence of any ^{13}C NMR glycosidation shift for the glucopyranose residue suggested that this sugar was the terminal unit. Finally, direct connectivity information was obtained from the HMBC spectrum, which showed the following key correlation peaks: δ_H 4. 86 (H-1$_{arg}$) and δ_C 78.9 (C-3), δ_H 5.00 (H-1$_{cym}$) and δ_C 138.9 (C-4$_{arg}$), δ_H 4.63 (H-1$_{ole}$) and δ_C 83.6 (C-4$_{cym}$), δ_H 4.48 (H-1$_{the}$) and δ_C 84.2 (C-4$_{ole}$), and δ_H 4.46 (H-1$_{glc}$) and δ_C 82.8 (C-4$_{the}$). On the basis of all this evidence, the sugar chain of compound **4** was established as 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-argelopyranoside, and compound **4** (argeloside N) as (14*S*,16*S*,20*R*)-14,16−14,20−15,20-triepoxy-14,15-secopregn-5-en-3-ol-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β- D-cymaropyranosyl-(1→4)-β-D-argelopyranoside.

The ^{1}H NMR spectrum of compound **5** showed, in addition to the signals corresponding to the aglycone, signals corresponding to one doublet methyl at δ 1.35 (3H, d, J = 6.6 Hz), one methoxy group at δ 3.69 (3H, s), and a signal for an acetal proton at δ 5.14 (1H, dd, J = 7.9, 3.5 Hz) (Table 1). The sequence of this unit was deduced from the analysis of the HMBC and DQF-COSY spectra. In particular, the following key HMBC correlations were observed: δ_H 5.14 (H-1′) and δ_C 42.2 (C-2′) and δ 78.9 (C-3); δ_H 4.18 (H-5′) and δ_C 180.7 (C-4′) and δ_C 19.7 (Me-6′); δ_H 3.69 (OMe) and δ_C 172.0 (C-3′); δ_H 2.92 (H-2′a) and δ_C 99.6 (C-1′) and δ_C 172.0 (C-3′); δ_H 2.63 (H-2′b) and δ_C 99.6 (C-1′) and δ_C 172.0 (C-3′); and δ_H 1.35 (Me-6′) and δ_C 180.7 (C-4′) and δ_C 74.8 (C-5′). Moreover, the connectivity between δ 5.14 (H-1′) and 2.92 (H-2′a) and 2.63 (H-2′b) and between δ 4.18 (H-5′) and 1.35 (Me-6′) was confirmed by the COSY NMR spectrum. Thus, the moiety linked to position 3 of the aglycon appeared to be derived from argelose by rupture of the linkage between C-3 and C-4 and oxidation of these carbons. This moiety was named 3,4-secoargelose, and compound **5** (argeloside O) was established therefore as (14*S*,-16*S*,20*R*)-14,16−14,20−15,20-triepoxy-14,15-secopregn-5-en-3-ol-3-*O*-3,4-secoargeloside.

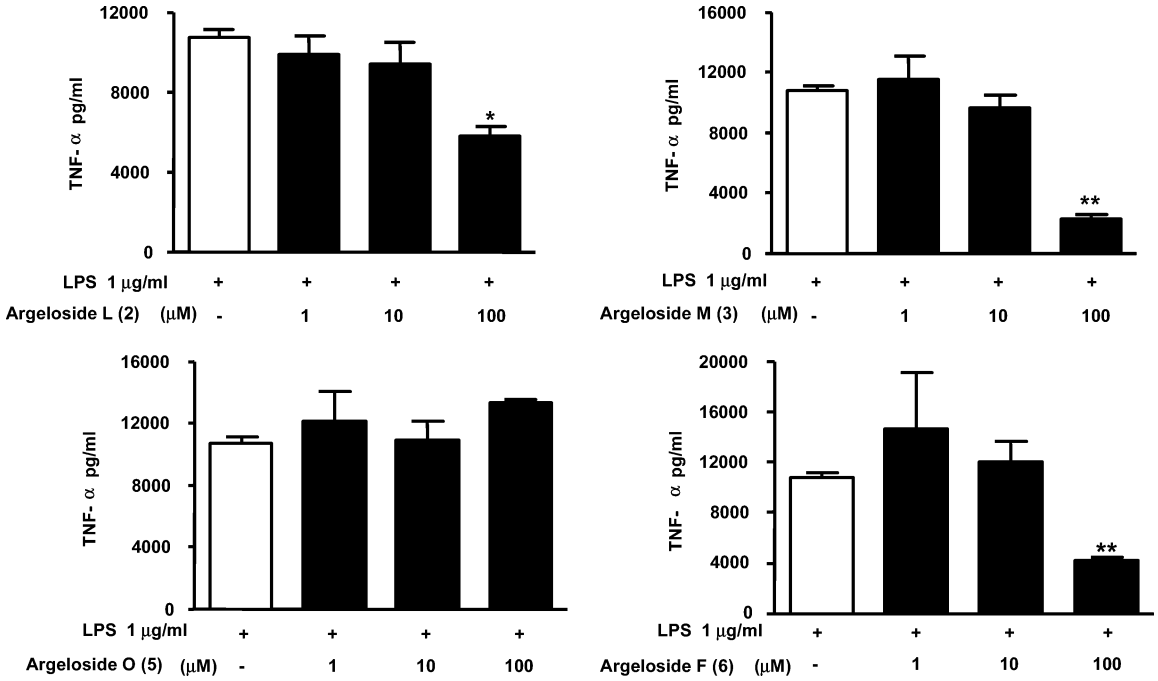


Figure 1. Effects of compounds **2**, **3**, **5**, and **6** on TNF-α release by RAW 264.7 mouse cells. Compounds were added to the cells for 30 min at the indicated doses. Cells were then stimulated with 1 μM LPS for 4 h. At the end of incubations TNF-α release in the supernatant was quantified by ELISA assay [($n = 3$); *$P < 0.01$; **$P < 0.001$, Student's t test].

On the basis of the beneficial effect on acute inflammation exerted by nor-secopregnanes isolated from *M. illustris* and *M. velutina*,[11,12] we tested the propensity of argelosides F (**6**), L (**2**), M (**3**), and O (**5**) to inhibit TNF-α release by LPS-stimulated RAW 264.7 mouse cells as an index of their potential anti-inflammatory activity. TNF-α is a potent pro-inflammatory cytokine able to induce inflammatory and immune-stimulating effects acting on specific receptors of cells involved in immune-inflammatory responses.[16]

The test compounds were chosen on the basis of their structural features. Argelosides F (**6**), L (**2**), and M (**3**) have at position C-3 sugar chains made up of five sugar units and aglycons differing in the occurrence of a methyl group, an acetoxy group, and a hydroxymethyl function at C-19, respectively. Argeloside O (**5**) is characterized by having a small substituent at position C-3. As shown in Figure 1, the compounds argeloside L (**2**), argeloside M (**3**), and argeloside F (**6**) significantly inhibited TNF-α release only at the highest concentration tested (100 μM), while argeloside O (**5**) had no inhibitory effect. Accordingly, these differences in activity could be related to the length of the substituent, attached to C-3.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco DIP 1000 polarimeter. IR measurements were obtained on a Bruker IFS-48 spectrometer. Exact masses were measured by a Voyager DE mass spectrometer. Samples were analyzed by matrix-assisted laser desorption ionization (MALDI) mass spectrometry. A mixture of analyte solution and α-ciano-4-hydroxycinnamic acid (Sigma) was applied to the metallic sample plate and dried. Mass calibration was performed with the ions from ACTH (fragment 18−39) at 2465.1989 Da and Angiotensin III at 931.5154 Da as internal standard. ESIMS analyses were performed using a ThermoFinnigan LCQ Deca XP Max ion trap mass spectrometer equipped with Xcalibur software. NMR experiments were performed on a Bruker DRX-600 spectrometer at 300 K. All the 2D-NMR spectra were acquired in CD_3-OD. The standard pulse sequence and phase cycling were used for DQF-COSY, 2D-TOCSY, HSQC, and HMBC spectra. Column chromatography was performed over Sephadex LH-20 (Pharmacia), and medium-pressure liquid chromatography (MPLC) was carried out on a Büchi 688 chromatography pump and Büchi B-685 borosilicate glass column (230 × 26 mm). Silica gel 60 (0.040−0.063 mm; Carlo Erba) was used as column material. HPLC separations were carried out on a Waters 590 system equipped with a Waters R401 refractive index detector, a Waters μ-Bondapak C_{18} column (300 × 7.8 mm), and a U6K injector. TLC was performed on silica gel F254 (Merck) plates, and reagent grade chemicals (Carlo Erba) were used throughout.

Plant Material. Fresh samples of *S. argel* leaves were collected at Allaqi (southeast of Aswan, Egypt) in May 2002 and identified by Dr. Mohamed G. Sheded. A voucher specimen (No. 10386) was deposited at the Botany Department Herbarium, Faculty of Science of Aswan, Egypt.

Extraction and Isolation. The leaves (100 g) were extracted with 70% EtOH, yielding 20 g of extract. Part of the extract (2.5 × 2 g) was fractionated on Sephadex LH-20 (100 × 5 cm) using MeOH as the mobile phase. Sixty-seven fractions (8 mL) were obtained. The fractions containing pregnane glycosides (fractions 13−17, 1.0176 g) were chromatographed by MPLC on silica gel with a gradient (flow rate 3.0 mL/min) of $CHCl_3$−MeOH (from 100:0 to 9:1, stepwise) as eluent to afford 1660 fractions (8 mL) monitored by TLC. Fractions 577−816 (F1, 24.4 mg) were chromatographed by semipreparative HPLC using MeOH−H_2O (78:22) as mobile phase (flow rate 2.5 mL/min) and loading 12 mg dissolved in MeOH (120 μL) each run to yield argeloside J (1.2 mg, $t_R = 10.1$ min) and argeloside I (1.0 mg, $t_R = 16.8$ min). Fractions 934−1068 (F2, 26.1 mg) were chromatographed by semipreparative HPLC using MeOH−H_2O (78:22) as mobile phase (flow rate 2.5 mL/min) and loading 13 mg dissolved in MeOH (130 μL) each run to yield compound **5** (1.1 mg, $t_R = 4.4$ min) and argeloside D (1.0 mg, $t_R = 8.0$ min). Fractions 1157−1191 (F3, 66.6 mg) were chromatographed by semipreparative HPLC using MeOH−H_2O (78:22) as mobile phase (flow rate 2.5 mL/min) and loading 10 mg dissolved in MeOH (100 μL) each run to yield compounds **2** (3.5 mg, $t_R = 11.2$ min) and **1** (3.3 mg, $t_R = 21.3$ min). Fractions 1219−1234 (F4, 33.6 mg) were chromatographed by semipreparative HPLC using MeOH−H_2O (73:27) as mobile phase (flow rate 2.5 mL/min) and loading 11 mg dissolved in MeOH (110 μL) each run to yield compound **4** (2.9 mg, $t_R = 34.5$ min). Fractions 1333−1393 (F5, 38.1 mg) were chromatographed by semipreparative HPLC using MeOH−H_2O (70:30) as mobile phase (flow rate 2.5 mL/min) and loading 10 mg dissolved in MeOH (100 μL) each run to yield argeloside F (**6**, 4.3 mg, $t_R = 32.4$ min). Fractions 1415−1451 (F6, 28.7 mg) were chromatographed by semipreparative HPLC using MeOH−H_2O (68:32) as mobile phase (flow rate 2.5 mL/min) and loading 10 mg

dissolved in MeOH (100 μL) each run to yield compound **3** (2.0 mg, t_R = 13.2 min).

Compound 1: white amorphous powder; $[\alpha]^{25}_D$ −16.7 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3470, 2992, 1509 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) (aglycon moiety) δ 5.44 (1H, dd, *J* = 3.3, 2.7 Hz, H-6), 4.57 (1H, br m, H-16), 3.87 (2H, m, H-15), 3.56 (1H, m, H-3), 2.53 (1H, d, *J* = 2.2 Hz, H-17), 1.95 (1H, m, H-1α), 1.93 (1H, m, H-8), 1.65 (3H, s, Me-21), 1.54 (1H, m, H-9), 1.20 (3H, s, Me-18), 1.16 (1H, m, H-1β), 1.07 (3H, s, Me-19); ^{13}C NMR (CD_3OD, 150 MHz) (aglycon moiety) δ 38.3 (C-1), 30.5 (C-2), 78.9 (C-3), 39.6 (C-4), 141.4 (C-5), 122.3 (C-6), 25.5 (C-7), 32.1 (C-8), 46.8 (C-9), 37.8 (C-10), 20.7 (C-11), 31.0 (C-12), 50.1 (C-13), 110.5 (C-14), 72.0 (C-15), 78.1 (C-16), 58.8 (C-17), 15.7 (C-18), 19.4 (C-19), 114.8 (C-20), 23.5 (C-21); ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) (sugar portion), see Table 1; ESIMS *m/z* 1093 [M + Na]$^+$; ESIMS/MS *m/z* 763 [M + Na − 330]$^+$, 603 [M + Na − 330 − 160]$^+$; HRMALDIMS *m/z* [M + Na]$^+$ calcd for $C_{54}H_{86}O_{21}Na$ 1093.5559, found 1093.5551.

Compound 2: white amorphous powder; $[\alpha]^{25}_D$ −14.4 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3463, 3005, 1724, 1517 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) (aglycon moiety) δ 5.68 (1H, dd, *J* = 3.3, 2.7 Hz, H-6), 4.62 (1H, dd, *J* = 11.8 Hz, H-19a), 4.57 (1H, br m, H-16), 4.01 (1H, dd, *J* = 11.8 Hz, H-19b), 3.86 (2H, m, H-15), 3.58 (1H, m, H-3), 2.53 (1H, d, *J* = 2.2 Hz, H-17), 2.26 (1H, m, H-8), 2.10 (1H, m, H-1β), 2.05 (3H, s, COC*H*$_3$), 1.65 (3H, s, Me-21), 1.57 (1H, m, H-9), 1.20 (3H, s, Me-18), 1.15 (1H, m, H-1α); ^{13}C NMR (CD_3OD, 150 MHz) (aglycon moiety) δ 34.8 (C-1), 30.6 (C-2), 78.8 (C-3), 39.7 (C-4), 136.0 (C-5), 126.6 (C-6), 25.3 (C-7), 33.3 (C-8), 46.4 (C-9), 46.5 (C-10), 21.1 (C-11), 30.8 (C-12), 49.7 (C-13), 110.0 (C-14), 71.8 (C-15), 78.0 (C-16), 58.5 (C-17), 15.5 (C-18), 65.1 (C-19), 114.6 (C-20), 23.3 (C-21), 172.0 (*C*OCH$_3$), 20.7 (COC*H*$_3$); ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) (sugar portion), see Table 1; ESIMS *m/z* 1151.5 [M + Na]$^+$, ESIMS/MS *m/z* 1091 [M + Na − 60]$^+$, 763 [M + Na − 60 − 328]$^+$, 603 [M + Na − 60 − 328 − 160]$^+$; HRMALDIMS *m/z* [M + Na]$^+$ calcd for $C_{56}H_{88}O_{23}Na$ 1151.5614, found 1151.5597.

Compound 3: white amorphous powder; $[\alpha]^{25}_D$ −11.9 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3461, 3010, 1507 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) (aglycon moiety) δ 5.69 (1H, dd, *J* = 3.3, 2.7 Hz, H-6), 4.57 (1H, br m, H-16), 3.90 (1H, dd, *J* = 11.5 Hz, H-19a), 3.87 (2H, m, H-15), 3.62 (1H, dd, *J* = 11.5 Hz, H-19b), 3.58 (1H, m, H-3), 2.52 (1H, d, *J* = 2.2 Hz, H-17), 2.43 (1H, m, H-8), 2.05 (1H, m, H-1β), 1.65 (3H, s, Me-21), 1.45 (1H, m, H-9), 1.23 (3H, s, Me-18), 1.05 (1H, m, H-1α); ^{13}C NMR (CD_3OD, 150 MHz) (aglycon moiety) δ 34.7 (C-1), 30.5 (C-2), 78.9 (C-3), 39.5 (C-4), 136.0 (C-5), 126.2 (C-6), 25.3 (C-7), 33.0 (C-8), 46.8 (C-9), 46.4 (C-10), 21.1 (C-11), 31.4 (C-12), 49.7 (C-13), 110.5 (C-14), 72.0 (C-15), 78.2 (C-16), 58.7 (C-17), 15.5 (C-18), 63.1 (C-19), 114.4 (C-20), 23.6 (C-21); ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) (sugar portion), see Table 1; ESIMS *m/z* 1139 [M + Na]$^+$; ESIMS/MS *m/z* 1109 [M + Na − 30]$^+$, 793 [M + Na − 30 − 316]$^+$, 633 [M + Na − 30 − 316 − 160]$^+$; HRMALDIMS *m/z* [M + Na]$^+$ calcd for $C_{55}H_{88}O_{23}Na$ 1139.5614 found 1139.5603.

Compound 4: white amorphous powder; $[\alpha]^{25}_D$ −2.8 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3454, 2989, 1520 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) (aglycon moiety), superimposable on those reported for compound **1**; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) (sugar portion), see Table 1; ESIMS *m/z* 1121 [M + Na]$^+$; ESIMS/MS *m/z* 775 [M + Na − 346]$^+$, 633 [M + Na − 346 − 142]$^+$; HRMALDIMS *m/z* [M + Na]$^+$ calcd for $C_{55}H_{86}O_{22}Na$ 1121.5508, found 1121.5508.

Compound 5: white amorphous powder; $[\alpha]^{25}_D$ +0.4 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3477, 3012, 1733, 1718, 1525 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) (aglycon moiety), superimposable on those reported for compound **1**; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) (sugar portion), see Table 1; ESIMS *m/z* 543 [M + Na]$^+$, ESIMS/MS *m/z* 453 [M + Na − 90]$^+$, 351 [M + Na − 90 − 102]$^+$; HRMALDIMS *m/z* [M + Na]$^+$ calcd for $C_{28}H_{40}O_9Na$ 543.2570, found 543.2642.

Acidic Hydrolysis of a Crude Pregnane Glycoside Mixture. The crude fraction containing pregnane glycosides (1 g) was heated at 60 °C with 1:1 0.5 N HCl−dioxane (100 mL) for 2 h, and the mixture was then evaporated in vacuo. The residue was partitioned with CH_2Cl_2−H_2O, and the H_2O layer was neutralized with Amberlite MB-3. The H_2O layer was then concentrated and passed through a silica gel column, using $CHCl_3$−MeOH−H_2O (7:1:1.2, lower layer) as eluting solvent to afford cymarose, oleandrose, thevetose, canarose, argelose, and glucose. The configuration of cymarose, oleandrose, thevetose, canarose, and glucose was established as D by comparison of their optical rotation values with those reported in the literature.[14] Optical rotation was determined after dissolving the sugars in H_2O and allowing them to stand for 24 h; D-cymarose: $[\alpha]^{25}_D$ +54.0 (*c* 1.0), D-oleandrose: $[\alpha]^{25}_D$ −12.4 (*c* 1.0), D-canarose: $[\alpha]^{25}_D$ −10.5 (*c* 0.1), D-thevetose: $[\alpha]^{25}_D$ +37.6 (*c* 0.1), D-glucose $[\alpha]^{25}_D$ +50.2 (*c* 0.5). In the case of argelose, we observed an optical rotation value $[\alpha]^{25}_D$ +15.6 (*c* 0.1); since this sugar is reported here for the first time, comparison with an optical rotation value reported in the literature was not possible; thus a D-configuration has been established on the basis of the consideration that this sugar derives from cymarose and oleandrose occurring in this plant in the D-form.

Biological Activity. Cell Culture. Raw 264.7 mouse cells (ATCC, LGC Promochem, Sesto S. Giovanni, Milan, Italy) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 4 mM L-glutamine, 10 000 units/mL penicillin, and 10 000 μg/mL streptomycine (all from Cambrex Bioscience, Verviers, Belgium) at 37 °C in an atmosphere of 95% O_2 and 5% CO_2. Compounds were dissolved in dimethyl sulfoxide (DMSO) to make a stock solution of 10^{-3} M, which was then diluted in culture medium to obtain the desired concentrations.

Measurement of TNF-α Production. RAW 264.7 cells were plated at 2.5×10^5 cells/mL in 24-well culture plates. Cells were allowed to adhere overnight and then rinsed twice and cultured for 16 h in serum-free DMEM. Vehicle (DMSO at the appropriate dilutions) and compounds (1−100 μM) were then added to the cells for 30 min before adding LPS (1 μg/mL) for 4 h. Supernatants were then removed, and TNF-α release was quantified by the Biotrak ELISA System (Amersham Biosciences, Cologno Monzese, Milan, Italy), according to the manufacturer's instructions.[17]

References and Notes

(1) Boules, L. *Flora of Egypt, Vol. 2*; Al Hadara: Cairo, 2000; p 212.
(2) Hocking, G. *A Dictionary of Terms in Pharmacognosy and Other Divisions of Economic Botany*; Charles C Thomas Publisher LTD: Springfield, IL, 1955; p 212.
(3) Kamel, M. S.; Ohtani, K.; Hasanain, M. H.; Mohamed, M. H.; Kasai, R.; Yamasaki, K. *Phytochemistry* **2000**, *53*, 937−940.
(4) Hassan, H.; Hamed, A. I.; El-Emary, N.; Springuel, I.; Mitome, H.; Miyaoka, H. *Phytochemistry* **2001**, *57*, 507−511.
(5) Hamed, A. I. *Fitoterapia* **2001**, *72*, 747−755.
(6) Kamel, M. *Phytochemistry* **2003**, *62*, 1247−1250.
(7) Plaza, A.; Piacente, S.; Perrone, A.; Hamed, A.; Pizza, C.; Bifulco, G. *Tetrahedron* **2004**, *60*, 12201−12209.
(8) Plaza, A.; Perrone, A.; Balestrieri, M. L.; Felice, F.; Balestrieri, C.; Hamed, A. I.; Pizza, C.; Piacente, S. *Steroids* **2005**, *70*, 594−603.
(9) Plaza, A.; Bifulco, G.; Hamed, A. I.; Pizza, C.; Piacente, S. *Tetrahedron Lett.* **2003**, *44*, 8553−8558.
(10) Plaza, A.; Perrone, A.; Balestrieri, C.; Felice, F.; Balestrieri, M. L.; Bifulco, G.; Carbone, V.; Hamed, A.; Pizza, C.; Piacente, S. *Tetrahedron* **2005**, *61*, 7470−7480.
(11) Bento, E. S.; Sant'Ana, A. E. G.; Hawkes, G. E.; Calixto, J. B.; Yunes, R. A. *Tetrahedron Lett.* **2003**, *44*, 8335−8337.
(12) Niero, R.; Vieira Alves, R.; Cechinel Filho, V.; Calixto, J. B.; Hawkes, J. E.; Sant'Ana, A. E. G.; Yunes, R. A. *Planta Med.* **2002**, *68*, 850−853.
(13) Aquino, R.; Peluso, G.; De Tommasi, N.; De Simone, F.; Pizza, C. *J. Nat. Prod.* **1996**, *59*, 555−564.
(14) Abe, F.; Mohri, Y.; Okanabe, H.; Yamauchi, T. *Chem. Pharm. Bull.* **1994**, *42*, 1777−1783.
(15) Agrawal, P. *Phytochemistry* **1992**, *31*, 3307−3330.
(16) Bazzoni, F.; Beutler, B. *N. Engl. J. Med.* **1996**, *334*, 1717−1725.
(17) Waage, A.; Bakke, O. *Immunology* **1988**, *63*, 299−302.

NP050263C