Stemucronatoside L, a Pregnane Glycoside from the Roots of *Stephanotis mucronata*, Inhibits Th1/Th2 Immune Responses *in vitro*

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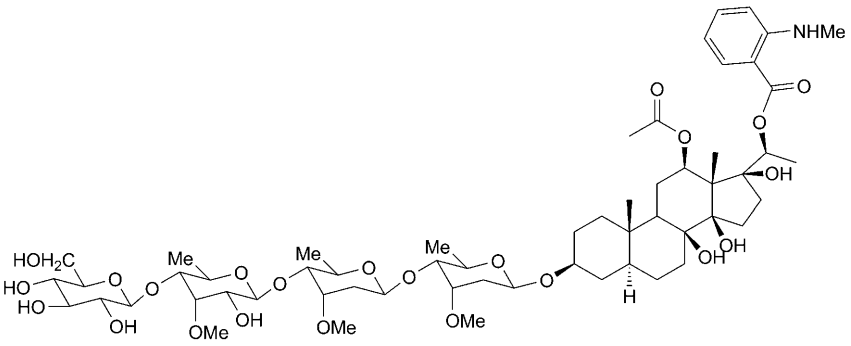
[illegible] *Stephanotis mucronata*, [illegible] *in vitro*. [illegible] Th1/Th2 [illegible] Th1 [illegible] -2 [illegible] -γ, Th2 [illegible] -4 [illegible] -10 [illegible] AT A-3 [illegible] C 4 [illegible] C 4 /C 8 [illegible] (AT A-3) [illegible] Th1/Th2 [illegible] Th1/Th2 [illegible] Th1/Th2 [illegible] Th1/Th2 [illegible] Th1/Th2 [illegible]

Introduction. *Stephanotis mucronata* (BLANCO) ERR. (A [illegible] C [illegible] 1. [illegible] *in vitro* 2 6. [illegible] (*Fig. 1*) [illegible] A (C A)- [illegible] *in vitro* 6. [illegible] Th1/Th2 [illegible] C 4 /C 8 [illegible] Th1- Th2- [illegible] (-γ, -2,



Formula: $C_{58}H_{91}NO_{23}$, M_r: 1192.5914

. 1. *Chemical structure of stemucronatoside L* ( )

Results. 1. *Effect of SML on Splenocytes CD4 /CD8 Subsets.* T b *Table 1* T C 4 T C 4 /C 8 b C A. , C 4 T C 4 /C 8 C A- 2 10 μ / ($P < 0.01$).

T b 1. *Effects of Stemucronatoside L* ( ) *on Splenocytes CD4 /CD8 Subsets.* b 0 10 μ / C A (3 μ /)

2. *Effect of SML on Cytokine Productions from ConA-Stimulated Splenocytes.* T b -2, -4, -10, -γ 0.9980. A *Table 2*, -2, -4, -10, -γ C A ($P \leq 0.05$, $P < 0.01$, $P \leq 0.001$), b T 1, T 2 C A-

T b 2. *Effects of Stemucronatoside L ( ) on Cytokine Production from Con A-Stimulated Mice Splenocytes.* b 0 10 μ / C A (3 μ /) 24 . T b -2, -4, -10, -γ A.T ± (*n* 3).

	C /			
	-2	-4	-10	-γ
C	20±10	2.33±0.33	20±1	936±197
C A	636±15	9.13±0.11	204±18	3366±265
C A± (0.08 μ /)	495±58)	6.35±0.57 b)	165±5)	1931±216 b)
C A± (0.4 μ /)	488±51 b)	5.92±0.66 b)	146±15)	1266±127)
C A± (2.0 μ /)	452±41 b)	4.83±0.88 b)	135±8 b)	1241±91)
C A± (10 μ /)	301±56)	2.60±0.28)	76±15)	1011±63)

C A) $P<0.05$, b) $P<0.01$,) $P<0.001$.

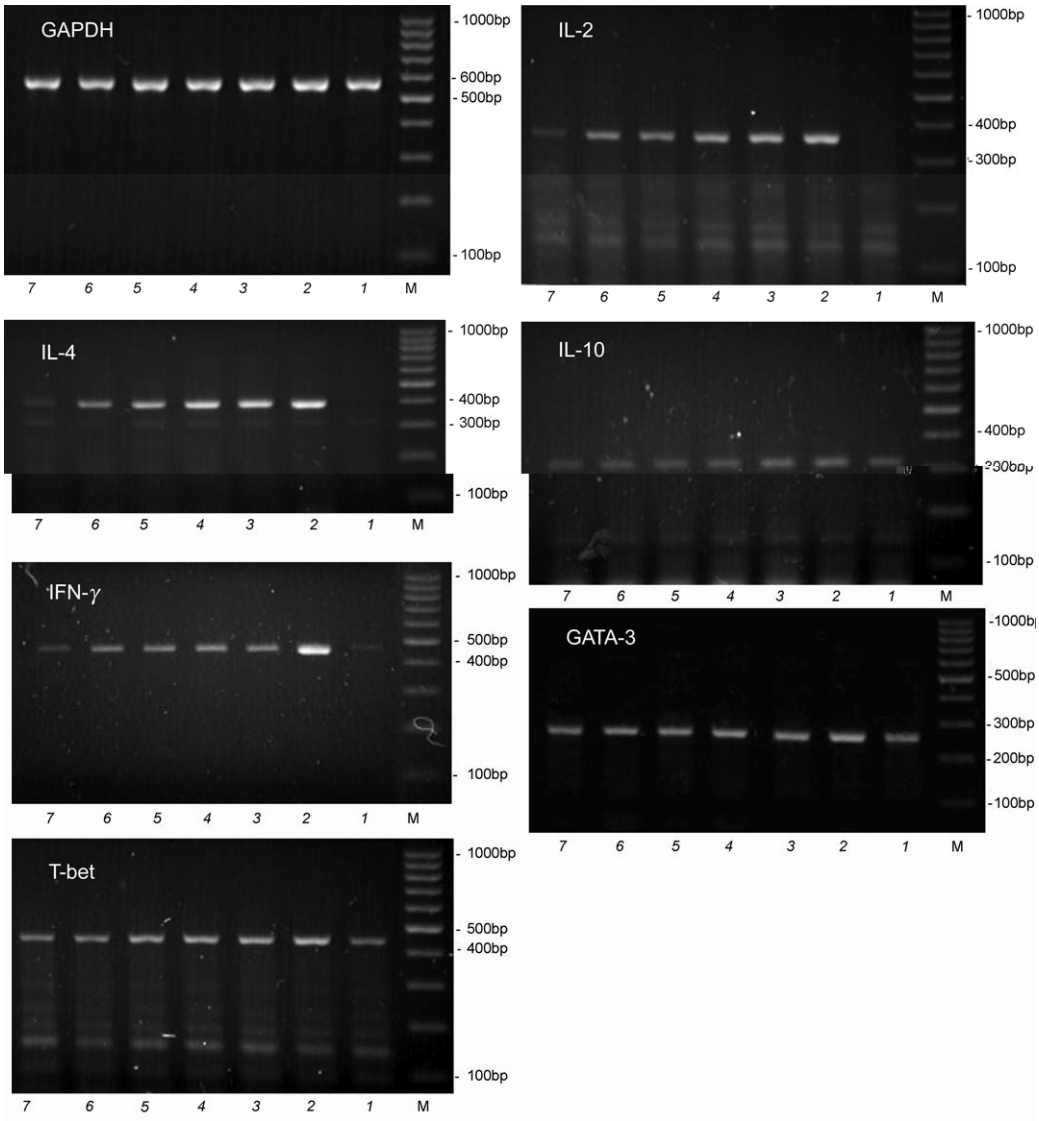
3. *Effect of SML on Expression of Cytokines and Transcription Factor mRNAs in ConA-Stimulated Splenocytes.* T A T 1/T 2 C A- *Fig. 2* *Table 3.* T 1 -2, -γ, T - b A ($P<0.05$, $P<0.01$, $P<0.001$), b T 2 -4 -10, AT A-3 A ($P<0.05$, $P<0.01$, $P<0.001$) C A- - . T T 1/T 2 b C A.

Discussion. A , , , -

T b 3. *The mRNA Expression Level of Cytokines and Transcription Factors in Mice Splenocytes Treated with Stemucronatoside L ( ) and Con A.* b ( ) 0 10 μ / C A (3 μ /) 16 . T b -2, -γ, -4, -10, T - b AT A-3 A b T - C b .T A . ± (*n* 3).

	C μ /					
	0	0.016	0.08	0.4	2	10
-2	0.49±0.01	0.42±0.02)	0.41±0.02 b)	0.30±0.03)	0.28±0.04)	0.09±0.01)
-γ	0.45±0.03	0.21±0.03)	0.19±0.03)	0.18±0.03)	0.15±0.03)	0.09±0.02)
T - b	0.39±0.03	0.33±0.01)	0.33±0.01)	0.33±0.01)	0.27±0.02 b)	0.22±0.02)
-4	0.52±0.04	0.42±0.04)	0.41±0.01)	0.30±0.01)	0.28±0.01)	0.05±0.01)
-10	0.39±0.02	0.34±0.02)	0.26±0.01)	0.22±0.01)	0.21±0.01)	0.13±0.01)
AT A-3	0.51±0.07	0.38±0.03)	0.37±0.01)	0.37±0.05)	0.37±0.02)	0.27±0.01 b)

0 μ /) $P<0.05$, b) $P<0.01$,) $P<0.001$.



. 2. *The mRNA expression level of GAPDH, cytokines and transcription factors in mice splenocytes treated with stemucronatoside J ( ) and ConA.* b
0 ,10 μ / C ; A (3 μ /) 16 .T A A ; -2, -γ, -10, -4,T- , AT A-3 b T- C .T A 1.5% (*w*/*v*) 7 b . *Lane M:* A , *Lane 1:* , *Lane 2:* C A, *Lane 3:* C A (0.016 μ /), *Lane 4:* C A (0.08 μ /), *Lane 5:* C A (0.4 μ /), *Lane 6:* C A (2 μ /), *Lane 7:* C A (10 μ /).

(AC) 7 12 , 13 b *S. mucronata* 18 , *in vitro* 2 6 . , b T 1/T 2 *in vitro.* T , B T . A , T , C 4 T , , 14 A b b T , C 4 T b 1 (T 1), T 2, T 0 15 , 16 . T 1 b T 2 17, T 1 -γ, -2, -12, T 1 - , T 2 , -4, -10, -5, -13, T 2 - 18 . -2 T T . -γ T 1 19 , -10, - T 1 T 2- , , B C 8 T 20 . b -4 T 2 7 1 21 . T b , , - , T , -γ -4 T 1 T 2 b T - b AT A-3 22 . T - b , b T - b , T 1- b , T 1 . - -γ, T AT -1- b . , T - b -γ , T - b b . , b b T . b b -γ/ -γ , b T - b . T - b T 2 , -γ- b - T 2 T 1 -γ - 17 . AT A-3, AT A b T 2 - , T 2- - b - -4. -4 b . T (T) T AT 6,

AT A-3. AT A-3 -4 b 22 .T b T - b b b AT A-3 ; AT A-3 b T - b T - b b AT A-3 17 . T - b AT A-3 b b T .

A *Tables 1* 2, C A C 4 T , C 4 /C 8 , -2, -γ, -10, -4 . C 4 T C 4 /C 8 , b -2, -γ, -4, -10 C A- - .T b T 1/T 2 T b -1 -2 , T - C A -2, -γ, -10, -4 C A- , T 1- T 2- T - b AT A-3 C A. T 1/T 2 A (*Fig. 2* *Table 3*). T , b *Stephanotis mucronata*, b T 1/T 2 b T - , b b T 1/T 2 . T b b *S. mucronata* .

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Experimental Part

General. C A, (C A) 3-(4,5- 7 -2-)-2,5- -2*H*- 7 b (TT) *Sigma Chemical Co.*, . , , A; *RPMI 1640* *Gibco BRL*, , A; (TC)- - C 4 (3T 4, 129.19) ( )- - C 8 (-2, 53-6.7) b *BD Biosciences Pharmingen*, CA, A; (-2, - , -4, -10) A *Wuhan Boster Biological Technology., Ltd.*, , b , . . C . *Trizol* *Invitrogen*, C b , CA, A; C C *Shanghai Sangon Biological Engineering Technology & Services Co., Ltd.*, , . . C . b (B) b *Hangzhou Sijiqing Corp.*, 1 , , . . C .

Extraction, Isolation, and Identification of SML. (; $C_{53}H_{91}O_{23}$; *M* : 1192.5914) *Stephanotis mucronata* b 6 , (, , ^{1} - ^{13}C- , ^{1} , ^{1} -C , C, BC). T b >99% b C .

T . , *RPMI-1640* ( ).T 0.1%

b .

Experimental Animals. C (, -) 18 22 *Zhejiang Experimental Animal Center* (C C 2003-0001, , C) 7 b b . *ad libitum*, : . 24±1°, 50±10%, 12- /12 . A . C b b b b , *Institute for Experimental Animals*, *Committee for Animal Experiments*.

Preparation of Splenocytes. b 23 , *Hank'* B (B ; *Sigma*), b , (0.8% (*w*/*v*)). A (1500×*g* 4° 10), B (*RPMI 1640* 12 M *HEPES* (7.1), 0.05 M 2- , 100 / , 100 µ / , 10% C). C b b T B C b 95%.

Flow Cytometry. 24- -b (*Costar*, C . . 3524) 5×10^6 / 1 , C A (3 µ /) *RPMI 1640* (0.016, 0.08, 0.4, 2, 10 µ /) 2 ( ). A 48 b b 5% C$_2$ 37°, B , T C- - C 4 - - C 8 b . A AG *CellQuest 3.0f* (*BD Biosciences Pharmingen*, CA, A) .

Measurement of Cytokines. b C A 24- -b b 5% C$_2$ 37°. A 24 , , 1400×*g* 5 , . -2, -4, -10, -γ A . B , b b b 37° 96- -b , b . 1.5 (-γ 2). b 37° 1 b -b (ABC). A b 30 T B (b) 37° 15 .T b b 100 µ .T b b A 450 .

RT-PCR for Cytokine and Transcription-Factor Gene Expression. 5×10^6 / 4 , C A (3 µ /) *RPMI 1640* (0.016, 0.08, 0.4, 2, 10 µ /) 8 . A 16 b b 5% C$_2$ 37°, B . 0.5 *Trizol* , A b 260 . A

M-MuLV Reverse Transcriptase (*Fermentas*, C . . 0441). A 1 µ A, 1 T A , 10 M T , 25 M C , 0.5 µ 20 µ C b (50 M C , 20 M *Tris* C , 8.4) , C 27 (A -γ), 29 (AT A-3), 31 (-2 T - b), 33 (-4 -10) *PTC-200 Thermal Cycler* (*MJ Research*, , A) 94° 2 , 55° (-2, AT A-3, T - b), 57° (A), 58° (, -4, -10, -γ) 50 , 72° 0.5 . A , 72° 10 .T : 5′-CCCACA T AAATT CAAC CAC-3′ 5′-CATT TT A , AACAC A-3′ A (570 b) 24 , 5′-CT CT ACA C AA CACA C-3′ 5′-CAT CT CCT CA AAA T C-

CACCA-3′ -2 (381 b), 5′-T AAC CT ACACACT CAT CTT -3′ 5′-C ACT CCTTTT CC CTT CCT A -3′ -γ (459 b) 25 , 5′-AT T CT CAACCCCCA C-T A T -3′ 5′- CT CTTT A CTTT CCA AA T C-3′ -4 (399 b) 26 , 5′-CCA TT-TT ACCT T A AA T AT -3′ 5′-T T CT A T CCT A T CCA CA ACT CAA-3′ -10 (324 b) 27 , 5′-AACCA T AT CCT TT CCCA C-3′ 5′-T T-C CCACT AA A-T A -3′ T - b (436 b) 28 , 5′- AA CAT CCA A CCC AAAC-3′ 5′-ACCCAT C T-ACCAT C-3′ AT A-3 (255 b) 11 . - . T - C A C .T C 1.5% , b *ImmageMaster VDS* (*Phamacia Biotech*, A) *GoldView*. T A (*Tiangen*, C).

Statistical Analysis. T ± ( ) A A - . *P* 0.05 b .

C

1 T B C B ', B C B ' (C), , , , 1975, . 270.
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