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

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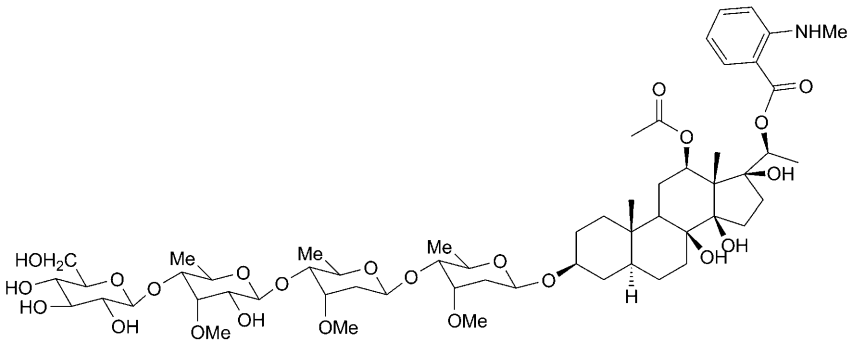
) , A ,T 182, 310013, . . C (: 86-(0)571-8821 5624; : 86-(0)571-8821 5624; - : 2005@163.)

) C A , , 268, 310029, . . C (: 86-(0)571-8697 1091; : 86-(0)571-8697 1091; - : @ . .)

( ), *Stephanotis mucronata*, T *in vitro*. , .T T1/T2 T - -2 -γ, T2 -4 -10 C A- - A , . , A T1/T2 T - AT A-3 T - C . C 4 T C 4 /C 8 , T1/T2 - . T A T1/T2 (T - AT A-3) .T T1/T2 T1/T2 .

Introduction. *Stephanotis mucronata* (BLANCO) ERR. (A) C 1 . *in vitro* 2 6 . T (; *Fig. 1*) , A (C A)- - *in vitro* 6 . , . T T1/T2 , , T - C 4 /C 8 ,T1- T2- (-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
C 4 /C 8 *Table 1.* T C 4 T
C 4 /C 8
C A. , C 4
T C 4 /C 8 C A-
2 10 μ / ($P < 0.01$).

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

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

T 2. *Effects of Stemucronatoside L ( ) on Cytokine Production from Con A-Stimulated Mice Splenocytes.* 0 10 μ / C A (3 μ /) 24 . T , -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)	165 ± 5)	1931 ± 216)
C A ± (0.4 μ /)	488 ± 51)	5.92 ± 0.66)	146 ± 15)	1266 ± 127)
C A ± (2.0 μ /)	452 ± 41)	4.83 ± 0.88)	135 ± 8)	1241 ± 91)
C A ± (10 μ /)	301 ± 56)	2.60 ± 0.28)	76 ± 15)	1011 ± 63)

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

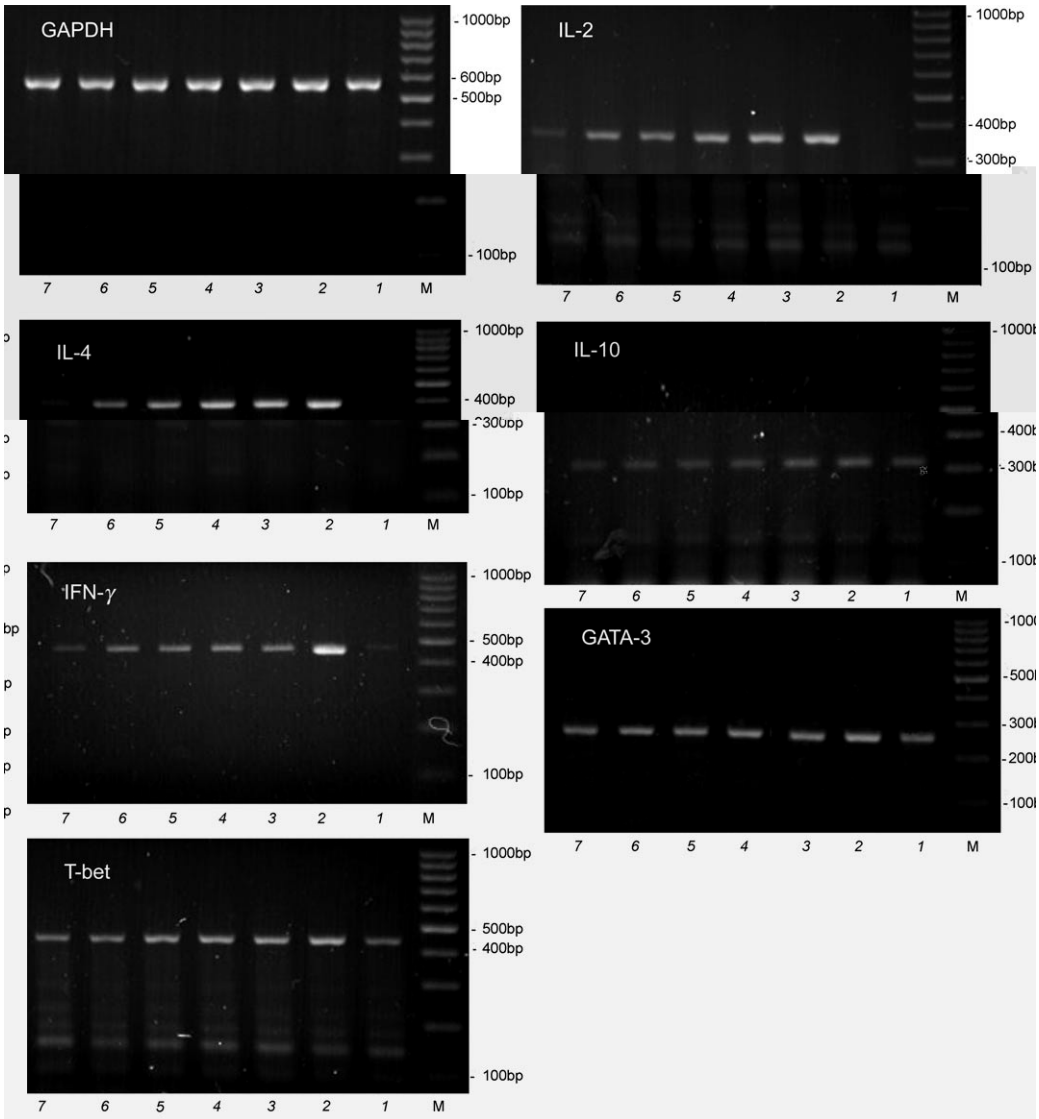
3. *Effect of SML on Expression of Cytokines and Transcription Factor mRNAs in ConA-Stimulated Splenocytes.* T A T 1/T 2 C A- , *Fig. 2* *Table 3.* T 1 -2, -γ, T - A ($P < 0.05$, $P < 0.01$, $P < 0.001$), T 2 -4 -10, AT A-3 A ($P < 0.05$, $P < 0.01$, $P < 0.001$) C A- - . T T 1/T 2 C A.

Discussion. A , , , -

T 3. *The mRNA Expression Level of Cytokines and Transcription Factors in Mice Splenocytes Treated with Stemucronatoside L ( ) and Con A.* ( ) 0 10 μ / C A (3 μ /) 16 . T -2, -γ, -4, -10, T - , AT A-3 A T - C . 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)	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 -	0.39 ± 0.03	0.33 ± 0.01)	0.33 ± 0.01)	0.33 ± 0.01)	0.27 ± 0.02)	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)

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



. 2. *The mRNA expression level of GAPDH, cytokines and transcription factors in mice splenocytes treated with stemucronatoside L ( ) and ConA.* 0 10 μ / C A (3 μ /) 16 . T A A , -2, -γ, -10, -4, T - , A T A-3 T - C . T A 1.5% (*w/v*) . *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 .

S. mucronata 18 *in vitro* 2 6 . , T 1/T 2 *in vitro.*

, , B T . A ,T , C 4 T , , , , , 14 . A , C 4 T T 1 (T 1),T 2, , , T 0 15 16 . , T 1 , 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 . -4 T 2 1 21 .

T T , , , , - , , , T . -γ -4 T 1 T 2 T - AT A-3 22 .T - , T - , T 1- T 1 . - -γ, T AT -1- . ,T - -γ , . , T - .T -γ/ -γ , T 2 , -γ- T - .T - - T 2 T 1 -γ - 17 . AT A-3, AT A , T 2- - T 2 - . - -4. -4 T (T) T AT 6,

AT A-3. AT A-3 -4 22 .T AT A-3 T - , T - AT A-3 17 . T - AT A-3 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 , -2, -γ, -4, -10 C A- - - .T T1/T2 .

T -1 -2 , T - C A -2, -γ, -10, -4 C A- . , T1- T2- T - AT A-3 C A. - T1/T2 A (*Fig. 2 Table 3*).

T *Stephanotis mucronata*, T - , T1/T2 T1/T2 . T *S. mucronata* .

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Experimental Part

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

Extraction, Isolation, and Identification of SML. (; C_{58} $_{91}$ $_{23}$, *M* : 1192.5914) *Stephanotis mucronata* 6 , (, , ^{1} - ^{13}C- , ^{1} , ^{1} -C , C, BC).T >99% C .

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

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

Preparation of Splenocytes. 23 . , *Hank'* B (B ; *Sigma*), , (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 T B . C 95%.

Flow Cytometry. 24- - (*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 5% C_2 37°, B , T C- - C 4 - - C 8 . A AC *CellQuest 3.0* (*BD Biosciences Pharmingen*, CA, A) .

Measurement of Cytokines. C A 24- - 5% C_2 37°. A 24 , 1400 × *g* 5 , . -2, -4, -10, -γ A . B , 96- - , 37° 1.5 (-γ 2). , . 37° 1 - - (ABC). A 30 , T B ( ) 37° 15 .T 100 μ .T 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 5% C_2 37°, B . 0.5 *Trizol* , A ' .T A 260 . A *M-MuLV Reverse Transcriptase* (*Fermentas*, C . . 0441).

A 1 μ A, 1 T A , 10 M T , 25 M C_2, 0.5 μ 20 μ C (50 M C , 20 M *Tris*, C , 8.4). C 27 (A -γ), 29 (AT A-3), 31 (-2 T -) , 33 (-4 -10) *PTC-200 Thermal Cycler* (*MJ Research*, , A) 94° 2 , 55° (-2, AT A-3, T -), 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) 24 , 5′-CT CT ACA C AA CACA C-3′ 5′-CAT CT CCT CA AAA T C-

CACCA-3′ -2 (381), 5′-T AAC CT ACACACT CAT CTT -3′ 5′-C ACT CCTTTT CC CTT CCT A -3′ -γ (459) 25 , 5′-AT T CT CAACCCCCA C-T A T -3′ 5′- CT CTTT A CTTT CCA AA T C-3′ -4 (399) 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) 27 , 5′-AACCA T AT CCT TT CCCA C-3′ 5′-T T C CCACT AA A-T A -3′ T - (436) 28 , 5′- AA CAT CCA A CCC AAAC-3′ 5′-ACCCAT C T-ACCAT C-3′ AT A-3 (255) 11 . - . T - C A

C .T C

1.5% , *ImmageMaster VDS* (*Phamacia Biotech*, A) *GoldView*. T A (*Tiangen*, C).

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

C

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