Supporting Information for

Physanolide A, a Novel Skeleton Steroid and Other Cytotoxic Principles from *Physalis angulata*

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Tzu-Hsuan Chen, and Kuo-Hsiung Lee

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Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. IR spectra were obtained with a Shimadzu FT-IR DR-8011 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on Bruker AVANCE-300 spectrometer. Chemical shifts are shown in δ values (ppm) with tetramethylsilane as an internal standard. Mass spectra were measured on a Jeol JMS-700 spectrometer with FAB ionization (positive-ion mode). Column chromatography was performed on silica gel (70-230 mesh, 230-400 mesh), and PTLC was executed on Merck precoated Si gel 60 F₂₅₄ plates, using UV light to visualize the spots.

Plant Material. The whole plants of *P. angulata* were collected in Tainan Hsien, Taiwan, Republic of China, in August 2002 and was authenticated by Prof. C. S. Kuoh, Department of Life Science, National Cheng Kung University. A voucher specimen (Wu 200200020) has been deposited in the Herbarium of National Cheng Kung University, Tainan, Taiwan.

Extraction and Isolation. Dried and powdered whole plants of *P. angulata* (6 kg) was refluxed with methanol (6 $\times$ 20 L) and filtered. The filtrate was concentrated to afford the crude extracts (600 g). The crude extracts were suspended in water and partitioned with chloroform and then resulted in concentrated chloroform extracts (190 g). Successively, the chloroform extracts were further partitioned between *n*-hexane and 80 % aqueous methanol to yield *n*-hexane solubles (45 g) and methanol extracts (145 g). Preliminary cytotoxicity examinations against the HONE-1 and NUGC cell lines displayed that the methanol extracts were active so that these syrups were subjected to open column chromatography over silica gel by eluting with the mixture of chloroform and methanol (30:1) and stepwise gradient of methanol to obtain 8 fractions. Fractions 1 and 2 were considered to be active fractions, since they showed cytotoxicity against the HONE-1 and NUGC cell lines with the inhibition percentage values of 93 % and 97 %, and 85 % and 96 %, respectively at 250 μM concentration. Fraction 1 on column chromatography over silica gel with the mixing eluents of benzene and acetone (9:1) and further purification by repeated column chromatography and thin-layer chromatography gave compounds 2 (6.0 mg), 3 (3.0 mg), physalin B (168.8 mg), F

(17.2 mg), J (16.2 mg), D (62.0 mg), and T (2.7 mg). Similarly, repeated column chromatography and preparative thin-layer chromatography of fraction 2 yielded **1** (5.0 mg), **2** (4.0 mg), physalin D (6.3 mg), G (3.9 mg), and I (8.9 mg).

Cytotoxicity Assay. Two human cancer cell lines, NUGC (gastric adenocarcinoma) and HONE-1 (nasopharyngeal carcinoma), were seeded in 96-well microliter plates at a density of 6000/well in 10 μ L culture medium (Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and nonessential amino acid) and maintained at 37 °C in a humidified incubator with 5 % CO₂. After an overnight adaptation period, test compounds (final concentration, 50 μ g/ml) in serum-free medium were added to individual wells. Cells were treated with test compounds for 3 days. Cell viability was determined by the 5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazolyl)-3-(4-sulfophenyl)-tetrazolium salt (MTS) reduction assay.¹² The 5 μ M (final concentration) of actinomycin D (showed 100% of inhibition at 10 μ M) and 0.3% (final concentration) of DMSO were used as positive and vehicle controls. Results were expressed as percent of DMSO control.

Physanolide A (1): Colorless needles (MeOH); mp 246—248 °C; $[\alpha]_D^{25} +114.3^\circ$ (c 0.01, CHCl_3); IR (KBr) ν_{max} 3449, 2944, 1750, 1736, 1436, 1375, 1246, 1182, 1142, 1105 cm^{-1} ; ^1H NMR (CDCl_3 , 300MHz), δ 0.87 (3H, s, CH_3 -18), 1.07 (3H, s, CH_3 -19), 1.19 (3H, d, $J = 10.7$ Hz, CH_3 -21), 1.20 (3H, s, CH_3 -28), 2.03 (3H, s, CH_3 -30), 2.69 (2H, m, H-20 & -24), 3.46 (1H, dd, $J = 10.1, 1.8$ Hz, H-22), 3.85 (1H, m, H-3), 4.12 (1H, dd, $J = 9.1, 6.9$ Hz, H-27), 4.44 (1H, dd, $J = 9.1, 6.9$ Hz, H-27), 5.05 (1H, br t, $J = 3.6$ Hz, H-1), 5.51 (1H, br d, $J = 4.8$ Hz, H-6); ^{13}C NMR (CDCl_3 , 75MHz), δ 10.6 (C-21), 13.6 (C-18), 19.5 (C-19), 20.2 (C-11), 21.1 (C-30), 22.0 (C-15), 23.9 (C-16), 26.5 (C-28), 28.9 (C-23), 31.1 (C-7), 31.4 (C-8), 34.5, 35.5 (C-2), 37.8 (C-24), 38.0 (C-20), 40.0 (C-10), 40.3 (C-12), 41.4 (C-9), 42.0 (C-4), 43.2 (C-13), 54.7 (C-17), 56.6 (C-14), 66.6 (C-3), 72.5 (C-27), 74.8 (C-22), 75.0 (C-1), 77.0 (C-25), 124.1 (C-6), 137.2 (C-5), 170.3 (C-29), 180.3 (C-26); FABMS m/z 501 ($[\text{M}+\text{H}]^+$, 1); HRFABMS m/z 501.3216 ($[\text{M}+\text{H}]^+$) (Calcd for $\text{C}_{30}\text{H}_{45}\text{O}_6$, 501.3216).

PAC28343C C0C13 2002-12-18 300MHz

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PAC28343C CDC13 2002-12-18 300MHz

PAC28343C COSY
C3C13 2002-12-18
300MHZ

PAC28343C NOESY
 CDC13 2002-12-12
 500MHz

PAC28343C HMBC
CDE13 2002-12-18
300MHZ

PAC28343C HMQC
 CDC13 2002-12-18
 300MHZ

PAC-015

Comment;
FTIR Measurement

Date/Time; 2004/02/18, U, 7:07:36:48
No. of Scans;
Resolution;
Apodization;
User; Tswul

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2	541.06	8.497	0.719	564.2	533.34	1.114
3	570.95	7.501	0.399	596.03	565.17	0.992
4	612.43	7.142	0.805	631.71	596.99	1.051
5	753.23	5.452	1.55	788.92	735.87	0.981
6	968.31	7.719	1.98	981.81	932.62	1.256
7	1022.32	12.112	2.167	1035.82	982.77	2.455
8	1051.25	12.886	3.286	1085.97	1036.78	2.259
9	1105.26	6.542	0.639	1128.48	1086.93	1.11
10	1141.91	6.479	1.054	1157.34	1127.44	0.805
11	1182.41	8.814	2.533	1208.46	1158.3	1.7
12	1246.07	13.768	7.612	1302.01	1209.42	3.985
13	1342.51	5.318	0.123	1345.41	1324.19	0.476
14	1375.3	10.411	5.537	1410.02	1346.37	2.097
15	1436.07	5.874	0.864	1447.84	1411.95	0.846
16	1454.39	5.502	0.033	1455.35	1448.6	0.163
17	1724.44	12.559	0.474	1726.36	1682.96	1.894
18	1736.01	13.049	0.538	1744.69	1729.26	0.919
19	1750.48	12.674	0.146	1754.33	1748.55	0.339
20	2901.06	8.733	0.96	2915.53	2534.57	3.68
21	2943.5	10.451	3.784	3046.7	2916.49	3.554
22	3420.9	10.964	0.146	3425.72	3403.54	1.1
23	3444.05	11.15	0.026	3445.01	3430.55	0.732
24	3448.87	11.177	0.089	3462.37	3445.98	0.833
25	3475.88	10.921	0.011	3477.8	3472.02	0.288
26	3481.66	10.841	0.099	3488.48	3478.77	0.383
27	3490.34	10.71	0.062	3492.27	3487.45	0.237
28	3499.02	10.817	0.275	3511.56	3493.24	0.896

[Mass Spectrum]
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 Sample: PAC-C15
 Note : NBA
 Inlet : Direct
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 RT : 0.32 min
 Scan# : 5
 BP : m/z 136.0000
 Int. : 356.25
 Output m/z range : 50.0000 to 570.0000
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 Cut Level : 0.00 %

[Elemental Composition]

Data : 20050311102

Sample: PAC-C15

Note : NBA

Inlet : Direct

RT : 2.67 min

Elements : C 30/0, H 45/0, O 6/0

Mass Tolerance : 5mmu

Unsaturation (U.S.) : 1.0 - 30.0

Date : 15-Mar-2005 10:58

Page: 1

Ion Mode : FAB+

Scan#: 17

Observed m/z	Int%	Err[ppm / mmu]	U.S. Composition
501.3216	3.0	-0.1 / -0.1	8.5 C 30 H 45 O 6

Physalin U (2): Colorless needles (MeOH); mp 281—283 °C; $[\alpha]_D^{25} -113.1^\circ$ (c 0.01, CHCl_3); IR (KBr) ν_{max} 3363, 2942, 1773, 1757, 1751, 1715, 1698, 1459, 1367, 1234, 1168, 1133, 1066 cm^{-1} ; ^1H NMR (CDCl_3 , 300MHz), δ 1.25 (6H, s, CH_3 -28 & -19), 1.90 (1H, dd, $J = 15.0, 11.0$ Hz, H-7 β), 1.99 (3H, s, CH_3 -21), 2.18 (1H, s, H-16), 2.45 (1H, d, $J = 4.6$ Hz, H-25), 2.55 (1H, ddd, $J = 12.1, 3.6, 2.5$ Hz, H-7 α), 2.67 (1H, d, $J = 17.2$ Hz, H-2), 2.84 (1H, dd, $J = 17.2, 3.8$ Hz, H-2), 3.25 (1H, d, $J = 2.5$ Hz, H-6), 3.28 (3H, s, OCH_3 -3), 3.67 (1H, d, $J = 13.0$ Hz, H-27), 3.70 (1H, m, H-3), 4.44 (1H, dd, $J = 13.0, 4.6$ Hz, H-27), 4.54 (1H, m, H-22); ^{13}C NMR (CDCl_3 , 75MHz), δ 15.1 (C-19), 21.5 (C-21), 23.3 (C-11), 24.9 (C-7), 25.6 (C-12), 26.5 (C-28), 31.1 (C-24), 33.0 (C-23), 33.5 (C-9), 36.6 (C-4), 36.7 (C-8), 42.3 (C-2), 50.8 (C-25), 53.8 (C-10), 56.1 (C-16), 56.2 (C-29), 60.7 (C-27), 63.0 (C-5), 63.4 (C-6), 73.4 (C-3), 76.8 (C-22), 79.3 (C-13), 80.0 (C-17), 81.0 (C-20), 107.0 (C-14), 166.6 (C-26), 172.2 (C-18), 207.6 (C-15), 213.3 (C-1); FABMS m/z 559 ($[\text{M}+\text{H}]^+$, 40), 541 (10); HRFABMS m/z 559.2180 ($[\text{M}+\text{H}]^+$) (Calcd for $\text{C}_{29}\text{H}_{35}\text{O}_{11}$, 559.2179).

PAC-C5 CDC13 2002-8-8 300MHZ

0.9500	1.5972	0.4147	1.3559	1.4063	1.6168	2.9137	0.7430	1.3245	0.4888	2.6686	0.7201
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Integral

PAC-C5 CDC13 2002-8-8 300MHZ

pac-c5 cosy
2002-9-6 300MHZ

pac-c5 NOESY
2002-9-6 300MHZ

pac-c5 HMBC
2002-9-6 300MHz

pac-c5 HMQC
2002-9-6 300MHz

Comment;
FTIR Measurement

Date/Time; 2004/02/18, U, 12:25:32
No. of Scans;
Resolution;
Apodization;
User; Tswul

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1	754.2	20.904	9.376	799.53	682.83	8.116	1.999
2	922.01	16.627	0.219	923.94	883.44	2.67	0.015
3	946.12	18.51	2.336	972.16	924.91	3.885	0.273
4	991.45	19.231	3.499	1029.07	973.13	4.58	0.404
5	1065.72	25.294	4.231	1079.22	1030.03	5.135	0.509
6	1085.01	23.151	0.855	1106.22	1080.18	2.74	0.052
7	1133.23	22.995	3.733	1153.48	1107.19	4.801	0.498
8	1167.95	23.021	4.477	1187.24	1154.45	3.347	0.448
9	1203.63	18.551	0.902	1216.17	1188.2	2.436	0.08
10	1233.53	19.51	2.151	1265.36	1217.14	4.185	0.237
11	1284.65	16.993	0.423	1294.29	1266.32	2.236	0.035
12	1299.11	16.611	0.431	1329.98	1295.26	2.493	0.064
13	1366.62	18.837	5.479	1409.06	1330.94	5.875	1.003
14	1459.21	17.897	4.898	1501.65	1410.02	6.499	0.935
15	1698.4	21.276	1.996	1714.79	1571.09	10.082	0.202
16	1742.76	26.79	0.55	1744.89	1715.76	3.339	0.042
17	1751.44	26.635	0.014	1752.4	1749.51	0.389	0
18	1757.23	26.899	0.097	1758.19	1754.33	0.522	0.001
19	1772.66	28.29	3.502	1857.53	1759.16	8.581	0.454
20	2889.49	15.576	0.38	2899.13	2842.23	3.931	0.039
21	2942.53	17.959	1.717	2965.68	2900.1	5.31	0.322
22	2981.11	17.253	1.103	3071.77	2966.65	7.382	0.143
23	3337.96	17.444	0.017	3338.92	3079.49	18.164	0.027
24	3363.03	17.575	0.058	3369.79	3338.92	2.582	0.005
25	3373.64	17.55	0.013	3376.54	3370.75	0.485	0
26	3385.22	17.565	0.055	3406.44	3377.5	2.421	0.005

[Mass Spectrum]
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 Cut Level : 0.00 %

[Elemental Composition]

Data : 20050311095

Sample: PAC-C4

Note : NBA

Inlet : Direct

RT : 1.00 min

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Mass Tolerance : 5mmu

Unsaturation (U.S.) : 1.0 - 30.0

Date : 14-Mar-2005 16:04

Ion Mode : FAB+

Scan#: 7

Page: 1

Observed m/z	Int%	Err [ppm / mmu]	U.S. Composition
559.2180	1.3	+0.1 / +0.1	12.5 C 29 H 35 O 11

Physalin V (3): Colorless needles (MeOH); mp 246—248 °C; $[\alpha]_D^{25} -58.7^\circ$ (c 0.01, CHCl_3); IR (KBr) ν_{max} 3502, 2924, 1775, 1755, 1734, 1455, 1369, 1277, 1168, 1064 cm^{-1} ; ^1H NMR (CDCl_3 , 300MHz), δ 1.28 (3H, s, CH_3 -28), 1.82 (3H, s, CH_3 -19), 2.00 (3H, s, CH_3 -21), 2.17 (3H, s, CH_3 -30), 2.18 (1H, s, H-16), 2.42 (1H, d, $J = 4.6$ Hz, H-25), 3.31 (2H, d, $J = 7.5$ Hz, H-4), 3.77 (1H, d, $J = 12.6$ Hz, H-27), 4.55 (1H, dd, $J = 12.6, 4.6$ Hz, H-27), 4.58 (1H, m, H-22), 5.05 (1H, m, H-1), 5.11 (1H, m, H-6), 5.48 (1H, td, $J = 8.0, 7.2$ Hz, H-2), 6.48 (1H, dd, $J = 8.0, 7.5$ Hz, H-3); ^{13}C NMR (CDCl_3 , 75MHz), δ 17.9 (C-19), 21.7 (C-21), 23.9 (C-11), 25.7 (C-7), 26.2 (C-12), 26.4 (C-28), 29.7 (C-24), 29.7 (C-30), 31.0 (C-23), 32.9 (C-9), 36.0 (C-4), 39.0 (C-8), 40.1 (C-10), 50.8 (C-25), 55.7 (C-16), 61.1 (C-27), 72.4 (C-1), 76.7 (C-22), 80.0 (C-13), 80.6 (C-17), 81.3 (C-20), 106.3 (C-14), 117.9 (C-3), 126.2 (C-6), 129.3 (C-2), 140.7 (C-5), 166.6 (C-26), 171.8 (C-29), 172.5 (C-18), 207.1 (C-15); FABMS m/z 555 ($[\text{M}+\text{H}]^+$, 1); HRFABMS m/z 555.2233 ($[\text{M}+\text{H}]^+$) (Calcd for $\text{C}_{30}\text{H}_{35}\text{O}_{10}$, 555.2230).

PAC 1-5-5-3-1T CDCL3 2002/07/25

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PPM

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207.082

C-10
noesy
2002-9-5

0206-07

No.	Peak	Height	Corr. He	Base (H)	Base (L)	Area	Corr. Ar
1	503.44	10.207	0.174	531.41	499.58	1.407	0.014
2	541.06	9.057	0.243	562.27	532.38	1.203	0.021
3	572.88	8.621	0.319	589.28	563.24	0.997	0.019
4	599.89	8.26	0.451	654.86	590.24	2.196	0.155
5	665.47	8.047	0.439	681.87	655.83	0.673	0.029
6	727.19	7.331	0.176	731.05	682.83	1.417	0.084
7	753.23	8.018	1.017	824.6	732.02	2.852	0.11
8	940.34	8.671	1.027	955.77	868	2.766	0.124
9	981.81	9.542	0.906	1005.92	956.73	2.031	0.106
10	1063.79	12.94	3.305	1103.33	1006.89	4.879	0.67
11	1144.8	11.631	0.367	1152.52	1104.29	2.383	0.027
12	1167.95	12.35	1.243	1255.71	1153.48	4.732	0.104
13	1276.93	9.411	1.248	1332.87	1256.68	2.924	0.258
14	1369.52	9.16	2.403	1417.74	1333.83	2.963	0.416
15	1455.35	7.897	1.857	1492	1418.71	2.278	0.292
16	1514.19	6.152	0.918	1566.27	1492.97	1.806	0.129
17	1734.08	14.081	9.997	1851.74	1567.23	11.109	5.802
18	2853.81	7.729	0.525	2869.24	2102.5	15.869	0.035
19	2924.21	9.906	2.906	3018.73	2870.2	5.421	0.809
20	3420.9	10.855	0.214	3430.55	3296.49	6.154	0.054
21	3449.84	11.16	0.277	3462.37	3431.51	1.564	0.023
22	3482.63	11.246	0.074	3488.41	3463.34	1.285	0.004
23	3501.92	11.481	0.245	3513.49	3489.38	1.282	0.014
24	3525.08	11.4	0.312	3544.35	3514.45	1.539	0.022
25	3560.75	11.059	0.42	3581	3545.32	1.78	0.04
26	3745.92	9.513	1.483	3780.64	3697.7	3.242	0.259

3

Date/Time; 2004/02/18, U, 01:43:56

No. of Scans;

Resolution;

Apodization;

User; Tswul

Comment;
FTIR Measurement

[Mass Spectrum]
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 Note : NBA
 Inlet : Direct
 Spectrum Type : Normal Ion [MF-Linear]
 Scan# : 7
 RT : 0.47 min
 BP : m/z 117.0000
 Int. : 289.91
 Output m/z range : 50.0000 to 600.0000
 Cut Level : 0.00 %

[Elemental Composition]

Data : 20050311103

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Sample: PAC-C7

Note : NBA

Inlet : Direct

Ion Mode : FAB+

RT : 6.34 min

Scan#: 39

Elements : C 30/0, H 45/0, O 10/0

Mass Tolerance : 5mmu

Unsaturation (U.S.) : 1.0 - 30.0

Observed m/z	Int%	Err [ppm / mmu]	U.S. Composition
555.2233	0.5	+0.5 / +0.3	13.5 C 30 H 35 O 10