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

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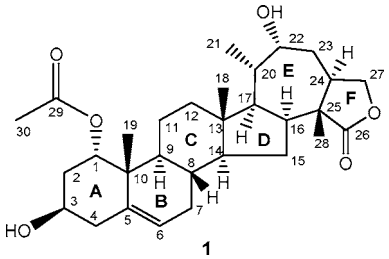
ABSTRACT



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A novel withasteroid, physanolide A (1), with an unprecedented skeleton containing a seven-membered ring, and two new physalins, physalins U (2) and V (3), were isolated from *Physalis angulata*. The structures were elucidated from spectroscopic analysis, and plausible biosynthetic pathways were postulated. Physalins B (4), D (5), and F (6) showed strong cytotoxicity against multiple tumor cell lines, including KB, A431, HCT-8, PC-3, and ZR751, with EC_{50} values less than 4 μg/mL.

Withasteroids comprise eight structural types of naturally occurring C_{28} steroidal lactones, containing an intact or modified ergostane skeleton. These compounds exhibit different biological activities and are produced mainly, but not exclusively, by genera in the Solanaceae family. Among these genera, *Physalis* species are highlighted since thus far, they contain the greatest variety of withasteroids known.[1]

Physalis angulata, known in Taiwan as "Kuzhi", is a branched annual shrub and is widely distributed throughout tropical and subtropical regions of the world. Extracts or infusions from this plant have been used in various countries in popular medicine as a treatment for different illnesses, such as malaria, asthma, hepatitis, dermatitis, diuretic, liver problems, and rheumatism, and as anticancer, antimycobacterial, antileukemic, antipyretic and immuno-modulatory

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(1) (a) Ray, A. B.; Gupta, M. *Prog. Chem. Org. Nat. Prod.* **1994**, *63*, 1−106. (b) Khan, P. M.; Ahmad, S.; Rubnawaz, H.; Malik, A. *Phytochemistry* **1999**, *51*, 669−671.

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agents.[2] Ergostane-type steroidal compounds, including physagulins A−G, withangulatin A, and physalins, some of which are novel, have been isolated from this plant.[3] In our integrated projects to discover new anticancer drugs, the cytotoxic assay guided fractionation of the methanol extract of *P. angulata* resulted in the isolation of a novel withanolide, physanolide A (**1**), with an unprecedented skeleton, and two new physalins, physalins U (**2**) and V (**3**), together with seven known ergostane-type steroidal compounds. Details of the isolation, structural elucidation, postulated biogenetic formation, and cytotoxicity of these compounds are presented below.

The whole plants of *P. angulata* were collected in Tainan Hsien, Taiwan, Republic of China. The air-dried and powdered whole plant of *P. angulata* (6 kg) was exhaustively extracted with MeOH, and the gum (600 g) obtained by concentrating the MeOH extract in vacuo was suspended in water and then partitioned with chloroform. The chloroform-soluble portion (190 g) was defatted with *n*-hexane to yield a brown residue (145 g), which was cytotoxic toward HONE-1 and NUGC-3 tumor cells with inhibition percentages of 88% and 98%, respectively. It was fractionated via silica gel column chromatography eluting with an increasing gradient of MeOH (0−100%) in chloroform to give eight fractions. The first and second fractions exhibited strong cytotoxicity toward HONE-1 and NUGC-3 tumor cells with inhibition percentages of 99% and 91% for the first fraction and 98% and 95% for the second fraction, respectively, and was further separated chromatographically to afford physanolide A (**1**) (5.0 mg), physalins U (**2**) (10.0 mg) and V (**3**) (3.0 mg), and seven known compounds, including physalins B (**4**), D (**5**), and F (**6**), which showed potent cytotoxicity as shown in Table 1.

Physanolide A (**1**) was obtained as a colorless solid, mp 246−248 °C and $[\alpha]^{25}_{D}$ +114.3. The molecular formula $C_{30}H_{44}O_6$ with nine degrees of unsaturation was established by the HREIMS, which had a molecular ion peak at *m*/*z* 501.3216. The absorption bands in the IR spectrum suggested the presence of hydroxyl (3449 cm^{-1}), γ-lactone (1750 cm^{-1}), acetyl (1736 cm^{-1}), and C−O bonds (1246, 1142, 1105 cm^{-1}). Analysis of ^{13}C NMR and HMQC spectral data revealed that **1** contained two carbonyl groups, four quaternary carbons including one olefinic carbon, eleven methines including one olefinic and three oxygenated carbons, eight methylenes, and five methyl groups. The ^1H NMR spectrum of **1** displayed two tertiary methyl signals at δ 0.87 (s) and 1.07 (s), an acetyl methyl at δ 2.03 (s), a downfield-shifted broad methine multiplet of the H-3 carbinol proton at δ 3.85, an oxygenated methine at δ 5.05 (m), and an olefinic proton at δ 5.51 (d, J = 4.8 Hz), indicating that **1** was an ergostane-type sterol with a 1α-acetyl-3β-hydroxyl-Δ^5 system. The ^1H−^1H COSY spectrum revealed the connectivities of C-1 to C-4, C-6 to C-12, and C-8−C-14 to C-17. Long-range HMBC correlations from CH_3-19 to C-1, C-5, and C-9; CH_3-18 to C-12, C-14, and C-17; and H-6 to C-10, constructed the ABCD rings of the ergostane skeleton. These NMR spectral data resembled those of steroids whose structures were identical with **1** in this region but showed significant differences mainly associated with signals arising from the side chain.[4] Typical methylene protons at δ 4.12 and 4.44, a methine at δ 2.69, and their HMBC correlations with a carbonyl resonance at δ 180.3 indicated the presence of a γ-lactone ring in the side chain. The ^1H NMR spectrum also displayed a tertiary methyl at δ 1.20, a secondary methyl at δ 1.19, and an oxygenated methine at δ 3.46 for the side chain. COSY cross-peaks indicated the sequences of C-22 to C-24−C-27 and C-17−C-20−C-21. The above moieties accounted for eight degrees of unsaturation, so the final one degree of unsaturation required the presence of an additional ring in **1**. Last, HMBC correlations from H-17 to C-21, and CH_3-28 to C-16, C-25, and C-26 showed that C-16 must be bonded to a quaternary carbon (C-25) to complete the final ring.

Table 1. EC_{50} Values of the Tested Compounds toward Different Tumor Cell Lines[a]

	EC_{50} (μg/mL)							
compd	KB	KB-VIN	A431	A549	HCT-8	PC-3	LNCAP	ZR751
2	11.9	20.0	13.4	16.3	11.1	18.1	15.9	8.2
4	3.0	1.3	1.8	5.9	1.5	0.9	5.3	2.6
5	1.2	7.0	1.4	1.6	1.2	1.6	1.3	0.4
6	0.9	1.9	1.1	1.3	1.0	1.1	1.0.	0.3
9	NA	NA	17.0	NA	NA	NA	NA	NA

[a] Compounds **1**, **7**, and **8** were not active at 20 μg/mL.

For the stereochemistry of **1**, NOESY cross-peaks for H-1 with CH_3-19 and H-3 with H-2α and H-4α showed that **1** had identical relative configurations at C-1, -3, and -10 to those of common steroids reported from *Physalis* species.[4] NOESY correlations of H-20/H-22, H-22/H-23, and H-23/H-24 and the coupling constants of H-20 and H-22 were also similar to those in certain withanolides having monooxygenation at C-22 supporting the same relative configurations at C-13, -20, -22, and -25 as a consequence of identical biogenesis.[4] Consequently, the structure of **1** was unambiguously established and named physanolide A.

(2) (a) Juang, J. K.; Huang, H. W.; Chen, C. M.; Liu, H. J. *Biochem. Biophys. Res. Commun.* **1989**, *159*, 1128−1134. (b) Di Stasi, L. C.; Santos, E. M. G.; Moreira dos Santos, C.; Hiruma, C. A. In *Plantas Medicinais da Amazonia*; Editora Unesp: Sao Paulo, 1989; pp 45−46. (c) Chiang, H. C.; Jaw, S. M.; Chen, P. M. *Anticancer Res.* **1992** *12*, 1155−1162. (d) Chiang, H. C.; Jaw, S. M.; Chen, C. F.; Kan, W. S. *Anticancer Res.* **1992** *12*, 837−843. (e) Lin, Y. S.; Chiang, H. C.; Kan, W. S.; Hone, E.; Shih, S. J.; Won, M. H. *Am. J. Chin. Med.* **1992**, *20*, 233−243. (f) Pietro, R. C.; Kashima, S.; Sato, D. N.; Januario, A. H.; Franca, S. C. *Phytomedicine* **2000**, *7*, 335−338. (g) Ismail, N.; Alam, M. *Fitoterapia* **2001**, *72*, 676−679. (h) Soares, M. B. P.; Bellintani, M. C.; Ribeiro, I. M.; Tomassini, T. C. B.; Santos, R. R. *Eur. J. Pharmacol.* **2003**, *459*, 107−112.

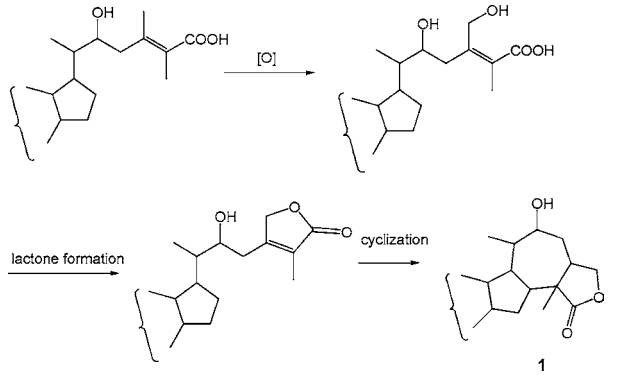
(3) (a) Shingu, K.; Marubayashi, N.; Ueda, I.; Yahara, S.; Nohara, T. *Chem. Pharm. Bull.* **1991**, *39*, 1591−1593. (b) Shingu, K.; Yahara, S.; Nohara, T.; Okabe, H. *Chem. Pharm. Bull.* **1992**, *40*, 2088−2091. (c) Shingu, K.; Yahara, H.; Okabe, H.; Nohara, T. *Chem. Pharm. Bull.* **1992**, *40*, 2448−2451. (d) Chen, C. M.; Chen, Z. T.; Hsieh, C. H.; Li, W. S.; Wen, S. Y. *Heterocycles* **1990**, *31*, 1371−1375. (e) Row, L. R.; Reddy, K. S.; Sarma, N. S.; Matsuura, T.; Nakashima, R. *Phytochemistry* **1980**, *19*, 1175−1181 and references therein.

(4) (a) Waiss, A. C., Jr.; Elliger, C. A.; Haddon, W. F.; Benson, M. *J. Nat. Prod.* **1993**, *56*, 1365−1372. (b) Elliger, C. A.; Haddon, W. F.; Harden, L.; Waiss, A. C., Jr.; Wong, R. Y. *J. Nat. Prod.* **1994**, *57*, 348−356. (c) Glotter, E. *Nat. Prod. Rep.* **1991**, *8*, 415−440.

(5) Makino, B.; Kawai, M.; Ogura, T.; Nakanishi, M.; Yamamura, H.; Butsugan, Y. *J. Nat. Prod.* **1995**, *58*, 1668−1674.

A plausible biogenetic pathway for physanolide A (**1**) was proposed as shown in Scheme 1. Physanolide A might be

Scheme 1. Possible Biosynthetic Pathway of **1**



derived by sequential cyclization via [C26−C27] lactonization and [C16−C25] bond formation from a sterol derivative of the *Physalis* species.[4]

Compound **2** was obtained as optically active colorless needles with mp 281−283 °C and $[\alpha]^{25}_{D}$ −113.1. The molecular formula $C_{29}H_{34}O_{11}$, established from its pseudo-molecular ion peak at *m*/*z* 559.2180 in the HRFABMS and characteristic hemiketal carbon signal at δ 107.0, indicated that compound **2** possessed a physalin skeleton.[5]

This postulate was further corroborated by the ^{13}C NMR spectrum, which showed signals for all 29 carbons of the molecule. The IR spectrum of **2** revealed the presence of hydroxyl (3363 cm^{-1}), γ-lactone (1773 cm^{-1}), five-membered-ring ketone (1757 cm^{-1}), δ-lactone (1751 cm^{-1}), and cyclohexanone functions (1715 cm^{-1}). The ^{1}H NMR spectrum of **2** displayed signals for three methyls at δ 1.25 (6H, s, CH_3-19 and -28) and 1.99 (3H, s, CH_3-21), and characteristic signals of a $-OCH_2CH-$ system at δ 2.45 (1H, d, J = 4.6 Hz, H-25), 3.67 (1H, d, J = 13.0 Hz, H-27), and 4.44 (1H, dd, J = 13.0, 4.6 Hz, H-27). These data suggested the physalin B-type basic skeleton for **2**. Furthermore, the presence of a doublet at δ 3.25 (1H, d, J = 2.5 Hz), and the signals at δ 1.90 (1H, dd, J = 15.0, 11.0 Hz) and 2.55 (1H, ddd, J = 12.1, 3.6, 2.5 Hz) attributable to H-6, H-7β, and H-7α, respectively, in the ^{1}H NMR spectrum, the corresponding carbons at δ 63.4, 24.9 and an oxygenated quaternary carbon at δ 63.0, indicated that the structure of **2** resembled closely that of 5β,6β-epoxyphysalin B.[6] However, disappearance of a characteristic α,β-unsaturated proton and the corresponding carbon signals and appearance of a CH_2 signal at δ 42.3, a OCH signal at δ 73.4, and a new methoxy signal at δ 56.2 suggest some structural changes in the vicinity of the C-1 center.

The HMBC correlations of methoxy protons (δ 3.28) with C-3 (δ 73.4) and H-2 (δ 2.84 and 2.67) with C-1 (δ 213.3) inferred that the C-2 and C-3 double bond was saturated and the methoxy substituent was present on C-3. All proton and carbon signals were confirmed by 2D NMR techniques and the structure of compound **2** was established as physalin U (Figure 1).

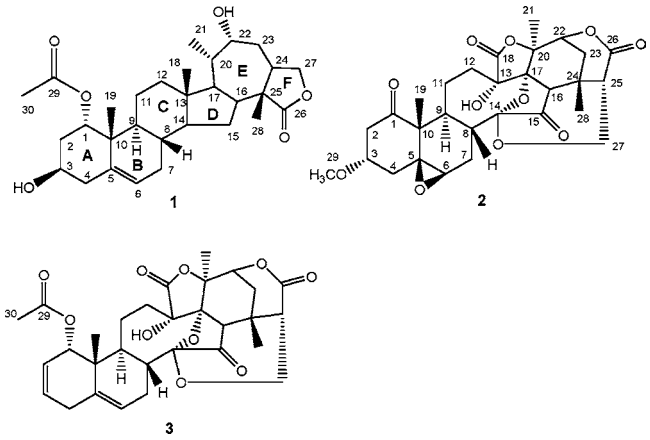


Figure 1. Structures of new compounds **1**, **2**, and **3**.

Compound **3**, obtained as optically active colorless needles with mp 246−248 °C and $[\alpha]^{25}_{D}$ −58.7, was shown to have the molecular formula $C_{30}H_{34}O_{10}$ from a pseudo-molecular ion peak at *m*/*z* 555.2233 in HRFABMS analysis. The IR spectrum displayed absorption bands at 3502, 1775, 1755, and 1734 cm^{-1}, which were compatible with the presence of hydroxyl, γ-lactone, five-membered-ring ketone, and δ-lactone functionalities, respectively. The ^{1}H NMR spectrum of **3** indicated the presence of three tertiary methyl groups at δ 1.28 (3H, s, CH_3-28), 1.82 (3H, s, CH_3-19), and 2.00 (3H, s, CH_3-21); a set of vinyl protons at δ 5.11 (1H, m, H-6), 5.48 (1H, td, J = 8.0, 7.2 Hz, H-2), and 6.48 (1H, dd, J = 8.0, 7.5 Hz, H-3); and an $-OCH_2CH-$ group at δ 2.42 (1H, d, J = 4.6 Hz, H-25), 3.77 (1H, d, J = 12.6 Hz, H-27), and 4.55 (1H, dd, J = 12.6, 4.6 Hz, H-27) commonly found in physalin B[7] and related physalins. When the 300 MHz ^{1}H NMR spectrum of **3** was analyzed, a comparison of the spectra of **3** and physalin B measured in $CDCl_3$ solution demonstrated that **3** differs from physalin B only in the A-ring. For the ring-A moiety, the coupling network from C-1 to C-4 was revealed by detailed ^{1}H−^{1}H COSY analysis. The easily assignable olefinic protons at δ 5.48 (H-2) and 6.48 (H-3) were coupled with an oxygenated methine proton at δ 5.05 (H-1) and methylene protons at δ 3.31 (H-4), respectively. Taken together, the above data indicated that compound **3** was structurally very similar to physalin B, with the exception of the reduction of the C-1 ketone group to the corresponding alcohol. This structural modification was completely confirmed by analysis of the ^{13}C NMR signals, in which an oxygenated methine carbon appeared at δ 72.4 rather than a ketone carbonyl signal for C-1. This assignment was also supported by the upfield shift of H-2 and H-3 signals in the ^{1}H NMR spectrum. Moreover, the presence

(6) Glotter, E.; Kirson, I.; Abraham, A.; Sethi, P. D.; Subramanian, S. S. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1370−1374.

(7) Matsuura, T.; Kawai, M.; Nakashima, R.; Butsugan, Y. *J. Chem. Soc. C* **1970**, 664−670.

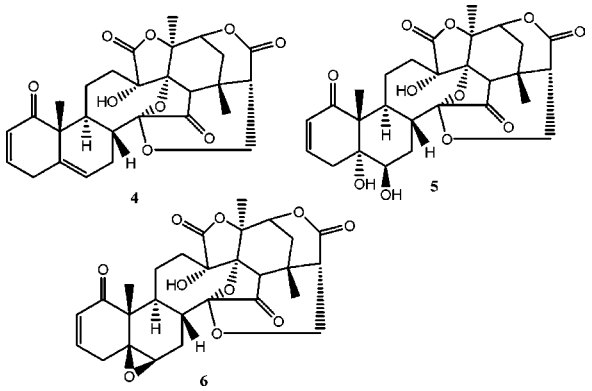


Figure 2. Structures of the most potent compounds **4**, **5**, and **6**.

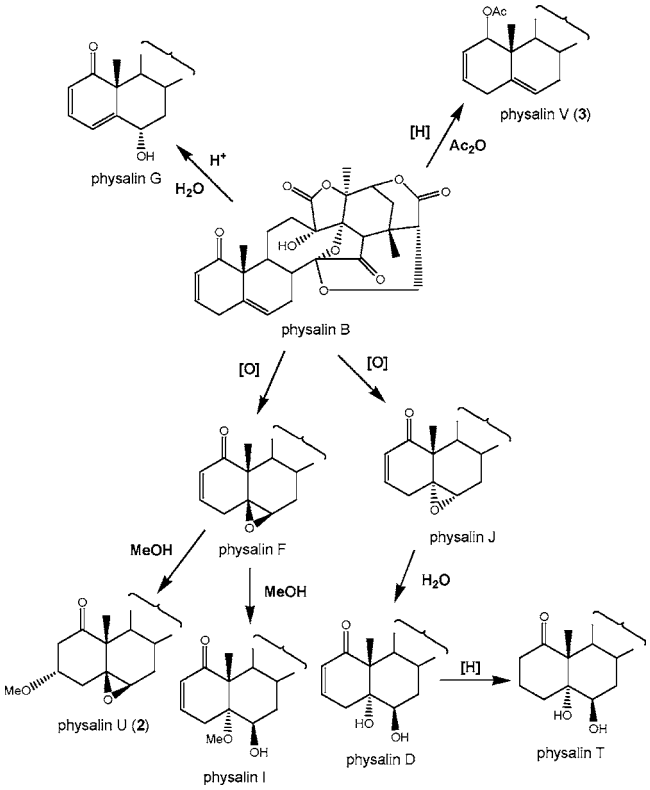
of an acetyl group in **3** was indicated by one methyl singlet at δ 2.17 (3H, s, CH_3-30) in the ^{1}H NMR spectrum together with the corresponding carbon signal at δ 29.7 (C-30) and an ester carbonyl signal at δ 171.8 (C-29) in the ^{13}C NMR spectrum. A HMBC correlation between H-1 (δ 5.05) and the ester carbonyl C-29 (δ 171.8) indicated that the acetyl group was attached to C-1. Thus the structure of **3** was elucidated as shown and named as physalin V.

In addition, the known isolates were identified as physalins B,[7] F,[6] J,[8] D,[9] G,[9] I,[9] and T[10] by comparison of their physical and spectral data with those reported in the literature.

Physalins B (**4**), D (**5**), F (**6**), G (**7**), I (**8**), and U (**2**) were screened for in vitro cytotoxicity against HONE-1 and NUGC-3 tumor cell lines.[11] Compounds **4**−**6** exhibited strong cytotoxicity against both HONE-1 and NUGC-3 cells. At 10 μM, the inhibition percentages were 97%, 95%, and 94%, respectively, against HONE-1 cells and 95%, 96%, and 83%, respectively, against NUGC cells. Physalin U (**2**) also displayed cytotoxicity toward both HONE-1 and NUGC-3 tumor cell lines at 50 μM with inhibition percentages of 92% and 86%, respectively.

Physanolide A (**1**) and physalins B (**4**), D (**5**), F (**6**), G (**7**), I (**8**), J (**9**), and U (**2**) were also examined for cytotoxicity against KB, KB-VIN (MDR-KB subline), A431, A549, HCT-8, PC-3, LNCAP, and ZR751 tumor cell lines, as described previously.[12] The EC_{50} values are shown in Table 1. Among the tested compounds, physalin F (**6**) showed strong cytotoxic activity against all tumor cell lines, with EC_{50} values ranging from 0.3 to 1.9 μg/mL. Physalins D (**5**) and B (**4**) also displayed strong cytotoxicity against most cell lines, but with decreased activity against KB-VIN and against LNCAP and A549 cells, respectively. Physalins J (**9**) and U (**2**) showed only weak cytotoxic activity against A431 and all cell lines, respectively.The co-occurrence of nine physalins, physalins B, D, F, G, I, J, T, U, and V, within the same plant *P. angulata* was highly interesting with respect to the biogenetic origin of the typical steroids of this genus and encourage speculation about their biogenetic relationship. It has been proposed that physalin B could be a biogenetic precursor for all of these physalins from *P. angulata*. Their plausible biogenetic interrelationship is presented in Scheme 2.

Scheme 2. Possible Biosynthetic Relationships of Isolated Physalins



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Supporting Information Available: Experimental procedure and full characterization of compounds **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Kawai, M.; Makino, B.; Taga, T.; Miwa, Y.; Yamamoto, T.; Furuta, T.; Yamamura, H.; Butsugan, Y.; Ogawa, K.; Hayashi, M. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 222−226.

(9) Row, L. R.; Reddy, K. S.; Sarma, N. S.; Matsuura, T.; Nakashima, R. *Phytochemistry* **1980**, *19*, 1175−1181.

(10) Kawai, M.; Yamamoto, T.; Makino, B.; Yamamura, H.; Araki, S.; Butsugan, Y.; Saito, K. *J. Asian Nat. Prod. Res.* **2001**, *3*, 199−205.

(11) Cheng, M. J.; Lee, S. J.; Chang, Y. Y.; Wu, S. H.; Tsai, I. L.; Jayaprakasam, B.; Chen, I. S. *Phytochemistry* **2003**, *63*, 603−608.

(12) Wang, X.; Bastow, K. F.; Sun, C. M.; Lin, Y. L.; Yu, H. J.; Don, M. J.; Wu, T. S.; Nakamura, S.; Lee, K. H. *J. Med. Chem.* **2004**, 47, 5816−5819.