Journal of Natural Products
Vol. 58, No. 11, pp. 1668-1674, November 1995

STRUCTURAL REVISION OF PHYSALIN H ISOLATED FROM *PHYSALIS ANGULATA*

BUNSHO MAKINO, MASAO KAWAI,* TOICHI OGURA, MASAKI NAKANISHI, HATSUO YAMAMURA, and YASUO BUTSUGAN

Department of Applied Chemistry, Nagoya Institute of Technology, Gokiso-cho, Showaku, Nagoya 466, Japan

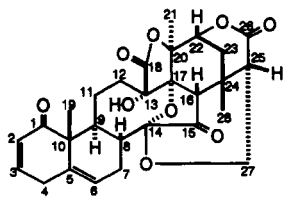
ABSTRACT.—The functionality of the A/B ring moiety of physalin H [**2**], a 16,24-cyclo-13,14-secosteroidal constituent of *Physalis angulata*, was revised as 5α-chloro-6β-hydroxy-2-en-1-one, after being erroneously assigned as 7β-hydroxy-2,5-dien-1-one.

Physalins are steroidal constituents of *Physalis* spp. and other closely related genera of the Solanaceae (1,2) and are characterized by their modified ergostane-type framework, being 16,24-cyclo-13,14-secosteroids. As a result of their polyoxyfunctional structures, physalins can be classified as the most advanced group in terms of the biogenetic oxidation level among the withanolide steroids (1,2). In the course of our study on the constituents of *P. alkekengi* L. var. *francheti* (Japanese name "Hôzuki"), physalins A–C and physalins L–Q were isolated and their structures were determined unambiguously (3–10). Row *et al.* reported the isolation of physalin B [**1**] and physalins D–K from *P. angulata* L. and *P. lancifolia* L. (11–13). Some of the physalins demonstrate cytotoxic activity against tumor cells in vitro and antineoplastic activity in vivo (6, 14–16), and recently Sunayama *et al.* reported the cell differentiation-inducing activity of physalin A (17).

In the course of our study on the chemistry and cytotoxic activity of the physalins and related compounds, physalin H [**2**] was obtained unexpectedly via the acid treatment of physalin F [**3**], a 5β,6β-epoxide of **1**. The reported functionality of **2** in the A/B ring moiety, namely, 7β-hydroxy-2,5-dien-1-one [**2***][1] (11), could not explain reasonably its formation from the 5β,6β-epoxy-2-en-1-one **3**. Spectroscopic study has revealed that the reported structure of physalin H should be revised. This paper describes the structural revision of physalin H and also the cytotoxic activity against HeLa cells of this and related compounds.

RESULTS AND DISCUSSION

Physalin F [**3**] was treated with HCl, which yielded a by-product, **2**, in addition to the desired glycol, physalin D [**4**] (Scheme 1) (13). The ^{1}H-nmr spectrum of **2** was found to be similar to the reported spectra of physalin H (11), and in fact **2** was indistinguishable from authentic physalin H provided by Row's group by Si gel tlc, reversed-phase



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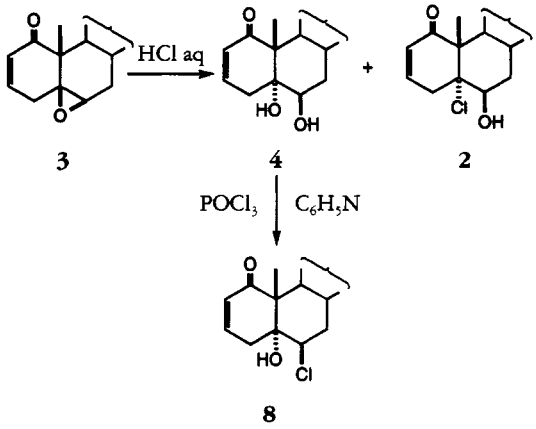
[1]Previously reported but now invalid structures are referred to by bold numerals with an asterisk.



3 4 2

8

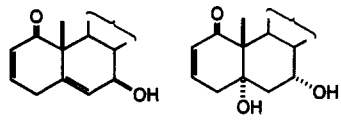
SCHEME 1

hplc, and ^{1}H-nmr spectral analysis. From these observations, we have concluded that physalin H is identical to compound **2** obtained from **3**.

Physalin H [**2**] is a minor constituent of *P. angulata*, the structure of which was elucidated as 7β-hydroxyphysalin B [**2***] by means of ^{1}H-nmr spectral analysis and chemical correlation with physalin E [**5***] (Figure 1) (11). It seemed difficult, however, to account for the reaction from the 5β,6β-epoxide **3** to the allyl alcohol, 5-en-7β-ol **2***, which prompted us to reinvestigate the structure of physalin H [**2**] by spectroscopic analysis.

According to Row *et al.*, **2** is isomeric with **3**, which has the molecular formula $C_{28}H_{30}O_{10}$. In the eims of **2**, however, a pair of molecular ion peaks containing one chlorine atom was observed at *m/z* 562 and 564 with intensity ratio 3.4:1, in addition to the peak at *m/z* 526, corresponding to $(C_{28}H_{30}O_{10})^+$. The molecular formula $C_{28}H_{31}O_{10}Cl$ was established by hreims analysis of the peak at *m/z* 562 and elemental analysis, indicating the addition of one molecule of HCl during the reaction from **3** to **2**.

When the 400 MHz ^{1}H-nmr spectrum of **2** was analyzed, a comparison of the spectra of **1** and **2** measured in DMSO-d_6 solution (as summarized in Table 1) demonstrated that **2** differs from **1** only at the A/B ring moiety. α- and β-Protons of the conjugated enone (δ 5.83 and 6.78) at the A ring were coupled with γ-methylene protons (δ 2.46 and 3.48), while the spin multiplicity of the γ-methylene protons indicated the absence of a proton at the δ-position, that is, at C-5. For the ring-B moiety, the coupling network from C-6 to C-9 was revealed by detailed ^{1}H-^{1}H COSY nmr analysis. The easily assignable methine proton at C-9 (δ 3.35) was coupled with the C-8 methine proton (δ 2.27) in a trans-diaxial relationship, which in turn was coupled with the C-7 methylene protons (δ 1.95 and 2.04). The methylene protons were correlated with the adjacent C-6 methine proton (δ 3.88) bearing a secondary hydroxyl group, to which the axial β-configuration was assigned based on the small coupling constants (both 3 Hz) with the C-7 protons.



2* 5*

FIGURE 1. Reported structures of physalins H [**2***] and E [**5***].

TABLE 1. 400 MHz ^{1}H-Nmr Spectral Data of Physalins B [**1**], H [**2**], and D [**4**] in DMSO-d_6 (chemical shift δ ppm, spin multiplicity, and coupling constant/Hz).[a]

Proton(s)	Compound		
	1[b]	**2**	**4**
H-2	5.80 dd ($J_{2,3}$=10) ($J_{2,4\beta}$=2)	5.83 dd ($J_{2,3}$=10) ($J_{2,4\beta}$=2.5)	5.70 dd ($J_{2,3}$=10) ($J_{2,4\beta}$=2)
H-3	6.89 ddd ($J_{3,2}$=10) ($J_{3,4\alpha}$=5) ($J_{3,4\beta}$=2)	6.78 ddd ($J_{3,2}$=10) ($J_{3,4\alpha}$=5) ($J_{3,4\beta}$=2)	6.62 ddd ($J_{3,2}$=10) ($J_{3,4\alpha}$=5) ($J_{3,4\beta}$=2)
H-4	2.89 dd (α) ($J_{4\alpha,4\beta}$=20) ($J_{4\alpha,3}$=5) 3.27 br d (β) ($J_{4\beta,4\alpha}$=20)	2.46 dd (α) ($J_{4\alpha,4\beta}$=21) ($J_{4\alpha,3}$=5) 3.48 ddd (β) ($J_{4\beta,4\alpha}$=21) ($J_{4\beta,2}$=2.5) ($J_{4\beta,3}$=2)	1.98 dd (α) ($J_{4\alpha,4\beta}$=20) ($J_{4\alpha,3}$=5) 3.11 br d (β) ($J_{4\beta,4\alpha}$=20)
H-5			4.25 s (OH)
H-6	5.59 br d ($J_{6,7\beta}$=6)	3.88 dt ($J_{6,OH}$=5) ($J_{6,7\alpha}$=3) ($J_{6,7\beta}$=3) 5.66 d (OH) ($J_{OH,6}$=5)	3.49 m ($W_{h/2}$=11) 4.90 d (OH) ($J_{OH,6}$=4)
H-7	1.97 m (α) 2.21 m (β)	1.95 m (α) 2.04 m (β)	—[c] —[c]
H-8	1.92 m	2.27 dt ($J_{8,9}$=11.5) ($J_{8,7\alpha}$=11.5) ($J_{8,7\beta}$=4)	2.20 dt ($J_{8,9}$=11) ($J_{8,7\alpha}$=11) ($J_{8,7\beta}$=5)
H-9	2.95 dd ($J_{9,8}$=11) ($J_{9,11\beta}$=9)	3.35 dd ($J_{9,8}$=11.5) ($J_{9,11\beta}$=8)	3.11 m
H-11	2.18 m (α) 1.10 m (β)	2.13 m (α) 0.97 m (β)	—[c] 0.94 m (β)
H-12	2.17 m (α) 1.45 m (β)	1.89 m (α) 1.44 br dd (β) ($J_{12\beta,12\alpha}$=16) ($J_{12\beta,11\beta}$=9)	2.11 m (α) 1.45 dd (β) ($J_{12\beta,12\alpha}$=16) ($J_{12\beta,11\beta}$=10)
H-13	6.28 s (OH)	6.06 s (OH)	5.76 s (OH)
H-16	2.86 s	2.82 s	2.77 s
H-19	1.09 s (Me)	1.24 s (Me)	1.11 s (Me)
H-21	1.78 s (Me)	1.81 s (Me)	1.81 s (Me)
H-22	4.56 dd ($J_{22,23R}$=3) ($J_{22,23S}$=2)	4.57 dd ($J_{22,23R}$=3) ($J_{22,23S}$=2)	4.56 dd ($J_{22,23R}$=3) ($J_{22,23S}$=2)
H-23	1.96 m (*S*) 2.14 m (*R*)	1.93 dd (*S*) ($J_{23S,23R}$=15) ($J_{23S,22}$=2) 2.10 dd (*R*) ($J_{23R,23S}$=15) ($J_{23R,22}$=3)	1.93 br d (*S*) ($J_{23S,23R}$=14) 2.10 dd (*R*) ($J_{23R,23S}$=14) ($J_{23R,22}$=4)
H-25	2.88 br d ($J_{25,27S}$=4)	2.89 dd ($J_{25,27S}$=4.5) ($J_{25,27R}$=1)	2.87 d ($J_{25,27S}$=4)

TABLE 1. Continued.

Proton(s)	Compound		
	1[b]	**2**	**4**
H-27	3.60 dd (*R*) ($J_{27R,27S}$=14) ($J_{27R,25}$=1) 4.26 dd (*S*) ($J_{27S,27R}$=14) ($J_{27S,25}$=4)	3.59 dd (*R*) ($J_{27R,27S}$=13) ($J_{27R,25}$=1) 4.26 dd (*S*) ($J_{25S,27R}$=13) ($J_{27S,25}$=4.5)	3.58 d (*R*) ($J_{27R,27S}$=13) 4.25 dd (*S*) ($J_{27S,27R}$=13) ($J_{27S,25}$=4)
H-28	1.16 s (Me)	1.17 s (Me)	1.17 s (Me)

[a]$W_{h/2}$ refers to half width (Hz).
[b]Data taken from Makino *et al.* (10).
[c]Not assigned.

The secondary hydroxyl proton signal (δ 5.66) at C-6 had been erroneously assigned as an alkenic proton signal leading to the incorrect structure **2***. The coupling network ended at the C-6 carbinol group and was not extended further, also indicating the absence of a proton at C-5, which was reasonably assumed to bear a chlorine atom on the basis of the established molecular formula. The stereochemistry at C-5 was determined to be α by the cd spectrum which showed a negative Cotton effect ([θ] −5700) at 334 nm indicating a trans-A/B ring junction of the steroids with a 2-en-1-one system (18,19). The resulting 5α-chloro-6β-hydroxy structure is quite reasonable considering the formation of **2** from **3** by trans-diaxial opening of the epoxy ring. The structure of physalin H [**2**] was thus established unequivocally as 5α-chloro-6β-hydroxy-2-en-1-one, i.e., 5α-chloro-6β-hydroxy-5,6-dihydrophysalin B.

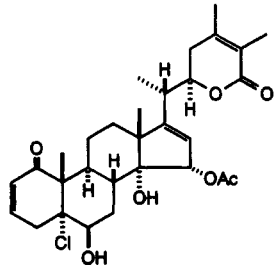
The ^{13}C-nmr spectral analysis of **2** was also performed with the aid of APT and the ^{13}C-^{1}H COSY nmr technique. As reported in Table 2 the spectral data of **1** and **2** in DMSO-d_6 were closely comparable, with the exception of the signals of the A/B rings. The signals of **2** assignable to the A/B ring moiety, on the other hand, corresponded well

TABLE 2. 100 MHz ^{13}C-Nmr Data of Physalins B [**1**] and H [**2**] and Physagulin B [**6**] (chemical shift δ ppm).[a]

Carbon	Compound			Carbon	Compound		
	1[a] (DMSO-d_6)	**2** (DMSO-d_6)	**6**[b] ($CDCl_3$)		**1**[a] (DMSO-d_6)	**2** (DMSO-d_6)	**6**[b] ($CDCl_3$)
C-1	202.4	200.3	201.0	C-15 ..	209.3	209.6	83.4
C-2	126.9	127.2	128.4	C-16 ..	54.1	53.9	120.6
C-3	146.1	142.8	141.7	C-17 ..	80.7	80.8	160.8
C-4	32.3	36.9	37.0	C-18 ..	171.8	171.5	16.7
C-5	135.5	82.3	81.1	C-19 ..	16.8	14.0	15.6
C-6	123.4	72.6	74.0	C-20 ..	80.3	80.4	35.4
C-7	24.4	26.7	26.6	C-21 ..	21.7	21.6	17.0
C-8	40.2	38.4	35.2	C-22 ..	76.3	76.3	78.3
C-9	33.1	30.9	36.9	C-23 ..	31.4	31.3	32.1
C-10 ...	52.0	54.3	52.8	C-24 ..	30.5	30.4	150.6
C-11 ...	24.1	24.3	22.8	C-25 ..	49.4	49.4	121.3
C-12 ...	25.6	25.8	38.5	C-26 ..	167.2	167.1	167.6
C-13 ...	78.2	78.4	52.1	C-27 ..	60.6	60.5	12.3
C-14 ...	106.3	106.4	82.1	C-28 ..	24.4	24.4	20.7

[a]Data taken from Makino *et al.* (10).
[b]Data taken from Shingu *et al.* (20).

to those of physagulin B [**6**] in $CDCl_3$, a withanolide isolated from *P. angulata* by Shingu *et al.* (20), which further supported the 5α-chloro-6β-hydroxy-2-en-1-one structure. Jaborosalactone E isolated from *Jaborosa integrifolia* is another example of a withanolide having the same A/B ring structure (18). It is interesting that these *trans*-5α,6β-chlorohydrins were isolated along with the corresponding 5β,6β-epoxy congeners, which can be considered their possible biogenetic precursors. The chlorohydrin **2** and another epoxy-opened product, *trans*-5α,6β-diol **4**, were actually isolated from *P. angulata* along with their precursor, epoxide **3**, suggesting that **2** and **4** are derived from the biogenetic precursor **3**, although the possibility that **2** and/or **4** are artifacts formed from **3** during the isolation procedure cannot be excluded.



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As described above, the structure of physalin H has been revised from **2*** to **2**. A compound possessing structure **2** was described previously by Row *et al.* as a reaction product of diol **4** treated with phosphoryl chloride in pyridine (13). Under the same reaction conditions, however, we obtained only the isomeric chlorohydrin, namely, 6β-chloro-5α-hydroxy-5,6-dihydrophysalin B [**8**] (Scheme 1). The spectral data of **8** were significantly different from those of **2**, but were in agreement with the reported data of the chlorohydrin (13).

The erroneous structural assignment of physalin H as the 7β-hydroxy-*2,5*-dien-*1*-one **2*** was based on chemical correlation with physalin E, namely, a 5α,7α-dihydroxy-2-en-1-one [**5***], which indicated that the proposed structure **5*** (11) is also doubtful. We have already reported that the proposed structure of so-called anhydrophysalin E as 7α-hydroxyphysalin B is inconsistent with the ^{1}H-nmr spectral data, which suggested its structure as 6β-acetoxy-5α-hydroxy-5,6-dihydrophysalin B, i.e., the 6-*O*-acetate of physalin D (8). The authentic samples of physalins D and E provided by Row's group were indistinguishable from each other on tlc and by reversed-phase hplc analysis. They also exhibited essentially the same 400 MHz ^{1}H nmr spectrum,[2] revealing that physalin E does not possess the structure **5*** but is identical to physalin D [**4**], namely, a 5α,6β-dihydroxy-2-en-1-one.[3] Synthetic **4**, derived from **3**, also afforded the same ^{1}H-nmr spectral data which are listed in Table 1.

It is already known that the presence of a conjugated cyclohexenone moiety at the

[2]The reported 100 MHz ^{1}H-nmr data of physalins D [**4**] (13) and E [**5***] (11) in DMSO-d_6 are very similar to one another except for the chemical shift values of the α-methine proton carrying a secondary hydroxyl group, i.e., H-6α in **4** (δ 3.85) and H-7β in **5*** (δ 3.52). We consider that the larger value of δ 3.85 for **4** is due to a typographical error.

[3]We erroneously reported the isolation of physalin E from *P. alkekengi* var. *francheti* (6) based on a comparison of the chemical shift value of the OH-carrying methine proton signal with that reported (see footnote 2) and tlc comparison with the authentic sample provided by Row's group.

A ring of the physalin structure is quite important for the exhibition of cytotoxicity against tumor cells (6,7). Physalin H [**2**] exhibited moderate in vitro cytotoxic activity against HeLa cells (ID_{50} 1.42 μg/ml), while physalin D [**4**] and its acetate were inactive. The regioisomeric chlorohydrin **8**, however, showed less potent activity (28.0 μg/ml) than **2**. The bromo analogue of **2** prepared by HBr treatment of **3**, namely, 5α-bromo-6β-hydroxy-5,6-dihydrophysalin B, demonstrated higher activity (0.71 μg/ml) than **2**. These results suggest that the functionalities at C-5 and C-6 also contribute to the observed cytotoxic activity. Further investigations on the structure-activity relationships are in progress.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Yanagimoto micro-melting point apparatus and are reported uncorrected. Cc and tlc were performed using SiO_2 (Fuji Silysia, FL60D) and precoated SiO_2 plates (Merck, Si gel $60F_{254}$), respectively. Analytical hplc was performed in the reversed-phase mode eluted with 65% aqueous MeOH (column; Tosoh, TSK Gel ODS-80 TM, 150×4.6 mm i.d.). Optical rotations were measured on a Jasco DIP-4 digital polarimeter and ir, uv, and cd spectra were recorded using Jasco A-102, Hitachi U-3500, and Jasco J-600 spectrometers, respectively. Mass spectra were measured on a Hitachi M-2000 spectrometer with electron-impact ionization and liquid-secondary-ion mass spectra (lsims) were taken in glycerol matrices. A Varian Unity 400 plus spectrometer was used for ^{1}H-nmr spectra at 400 MHz and ^{13}C-nmr spectra at 100 MHz and a Varian Gemini-200 spectrometer for 200 MHz ^{1}H-nmr spectra in DMSO-d_6 solution.

HYDROCHLORIC ACID-TREATMENT OF PHYSALIN F [**3**].—To a solution of **3** (1253 mg) in tetrahydrofuran (10 ml) was added 2 N HCl (10 ml). After stirring at room temperature for 80 min, the mixture was poured into H_2O and extracted with EtOAc. The extract was washed with 0.5 M $NaHCO_3$ and saturated NaCl solutions, and was dried over anhydrous Na_2SO_4. After the solvent was evaporated, the residue was subjected to Si gel cc using the solvent system $CHCl_3$/MeOH to afford **4** (644 mg, 50%) and **2** (497 mg, 37%).

Compound **4**.—Indistinguishable from authentic **4** by tlc, reversed-phase hplc, and ^{1}H-nmr data; colorless needles from Me_2CO; mp >300°; ir (KBr) ν max 3465, 1785, 1765, 1735, 1670, 1170, 1135, 1085, 1060, 980 cm^{-1}; ^{1}H-nmr data (400 MHz), see Table 1.

Compound **2**.—Indistinguishable from authentic physalin H; colorless needles from Me_2CO; mp 237–240°; $[\alpha]^{15}_D$ −92° (*c*=0.11, Me_2CO); ir (KBr) ν max 3440, 1780, 1765, 1735, 1670, 1170, 1135, 1080, 1060, 980, 930 cm^{-1}; uv λ max (MeOH) 226.5 nm (log ε 3.84); cd (MeOH) $[\theta]_{343}$ −5300, $[\theta]_{334}$ −5700, $[\theta]_{229}$ −13800; hreims *m/z* 562.1562 ($C_{28}H_{31}O_{10}{}^{35}Cl$; requires 562.1602, M^+); *anal.*, calcd for $C_{28}H_{31}O_{10}Cl{\cdot}CH_3COCH_3{\cdot}{}^1/_2H_2O$, C 59.09, H 6.08, Cl 5.63; found C 59.08, H 6.03, Cl 5.68; ^{1}H- (400 MHz) and ^{13}C- (100 MHz) nmr data, see Tables 1 and 2, respectively.

6β-CHLORO-5α-HYDROXY-5,6-DIHYDROPHYSALIN B [**8**] FROM PHYSALIN D [**4**].—To a solution of **4** (49 mg) in dry pyridine (2 ml) was added freshly distilled $POCl_3$ (70 μl, 8.4 equivalents); the mixture was stirred at room temperature for 80 min and at 90° for 25 min. After the usual workup, the product was purified by Si gel cc using $CHCl_3$/MeOH as solvent system, and crystallization from MeOH to afford **8** (40 mg, 79%).

Compound **8**.—Colorless needles from MeOH; mp 274–276°; ir (KBr) ν max 3510, 3440, 1780, 1760, 1745, 1735, 1675, 1170, 1135, 1065 cm^{-1}; lsims (glycerol matrix) *m/z* 565, 563 $[M+H]^+$; ^{1}H nmr (200 MHz) δ 0.95 (1H, m, H-11β), 1.13 (3H, s, Me-19), 1.16 (3H, s, Me-28), 1.44 (1H, br dd, *J*=16 and 9.5 Hz, H-12β), 1.80 (3H, s, Me-21), 1.92 (1H, dd, *J*=14 and 1.5 Hz, H-23*S*), 2.85 (1H, s, H-16), 2.90 (1H, d, *J*=4 Hz, H-25), 3.17 (1H, dm, *J*=19.5 Hz, H-4β), 3.20 (1H, m, H-9), 3.60 (1H, d, *J*=13.5 Hz, H-27*R*), 4.13 (1H, br s, $W_{h/2}$=3 Hz, H-6), 4.27 (1H, dd, *J*=13.5 and 4 Hz, H-27*S*), 4.58 (1H, br s, H-22), 4.98 (1H, s, OH-5α), 5.75 (1H, dd, *J*=10 and 2 Hz, H-2), 5.99 (1H, s, OH-13), 6.63 (1H, ddd, *J*=10, 4.5, and 1.5 Hz, H-3). Although **8** displayed a very similar tlc behavior to **2**, these were clearly distinguishable from one another by reversed-phase hplc.

5α-BROMO-6β-HYDROXY-5,6-DIHYDROPHYSALIN B FROM PHYSALIN F [**3**].—To a solution of **3** (69 mg) in tetrahydrofuran (2 ml) was added concentrated HBr (0.40 ml); the mixture was stirred at 0° for 30 min. After the usual work-up, the products were purified by Si gel cc using $CHCl_3$/MeOH as solvent system to afford the bromohydrin (72 mg, 90%) and **4** (6 mg, 9%).

5α-*Bromo-6β-hydroxy-5,6-dihydrophysalin B*.—Colorless amorphous powder from Me_2CO; mp 222–226°; ir (KBr) ν max 3450, 1780, 1765, 1735, 1670, 1170, 1135, 1080, 1055 cm^{-1}; lsims (glycerol matrix) *m/z* 609, 607 $[M+H]^+$; ^{1}H nmr (200 MHz) δ 0.96 (1H, m, H-11β), 1.16 (3H, s, Me-28), 1.24 (3H, s, Me-

19), 1.43 (1H, br dd, J=15 and 10 Hz, H-12β), 1.81 (3H, s, Me-21), 2.11 (1H, dd, J=14 and 3 Hz, H-23*R*), 2.64 (1H, dd, J=20.5 and 5 Hz, H-4α), 2.82 (1H, s, H-16), 2.89 (1H, d, J=4 Hz, H-25), 3.37 (1H, dd, J=10 and 9 Hz, H-9), 3.52 (1H, dm, J=20.5 Hz, H-4β), 3.58 (1H, d, J=13 Hz, H-27*R*), 4.08 (1H, m, $W_{h/2}$=7.5 Hz, H-6), 4.26 (1H, dd, J=13 and 4 Hz, H-27*S*), 4.57 (1H, br s, H-22), 5.69 (1H, d, J=4.5 Hz, OH-6), 5.85 (1H, dd, J=10 and 2 Hz, H-2), 6.04 (1H, s, OH-13), 6.82 (1H, ddd, J=10, 5, and 2 Hz, H-3).

ACKNOWLEDGMENTS

The authors are grateful to Professor Emeritus L. Ramachandra Row and Professor Teruo Matsuura for generous gifts of the samples of physalins, and also to Mr. Jun'ichi Ohya of Yokohama Research Center, Mitsubishi Chemical Corporation, for the cytotoxicity assays.

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Received 2 May 1995