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THE STRUCTURE OF PHYSALIN T FROM *PHYSALIS ALKEKENGI* VAR. *FRANCHETI*

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A new steroidal constituent named physalin T (**3**) was isolated from the aqueous extract of *Physalis alkekengi* var. *francheti*. Based on ^{1}H and ^{13}C NMR spectral studies the structure was assigned as 2,3-dihydrophysalin D, *i.e.*, $5\alpha,6\beta$-dihydroxy-2,3,5,6-tetrahydrophysalin B, which is the first example of a natural physalin possessing a saturated ring A moiety. The structure was confirmed by the chemical transformation from the known physalin D (**2**) to physalin T.

Keywords: Physalin; 16,24-cyclo-13,14-secosteroid; *Physalis alkekengi* var. *francheti*

Physalins are 16,24-cyclo-13,14-secosteroidal constituents of *Physalis* plants. The first members of this series, namely physalin A [1], physalin B (**1**) [2] and physalin C [3], were isolated as the bitter principles of *P. alkekengi* L. var. *francheti* Hort. (Japanese name; Hôzuki). Isolation of **1** and physalins D-K were reported from *P. angulata* and/or *P. lancifolia* [4–6]. However, we demonstrated that the reported physalin E [4] is identical with physalin D (**2**, $5\alpha,6\beta$-dihydroxy-5,6-dihydrophysalin B) [6] and that the reported structures

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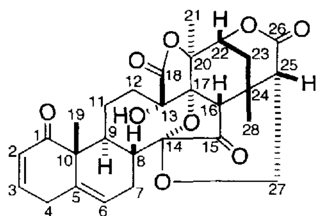
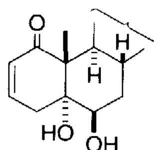
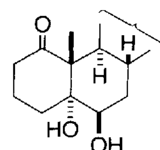
of physalin H [4] and physalin K [6] must be revised [7, 8]. We also reported the structures of physalins L-S isolated from *P. alkekengi* var. *francheti* [8–13]. Eight years after our report of physalin L [9], a constituent of *P. minima* was described by the name of 'physalin L' [14], but the proposed structure was completely different from our physalin L and was also inconsistent with the spectral data given [15]. Some of the physalins demonstrate cytotoxic activity against tumor cells *in vitro* and *in vivo* [7, 9, 16–18]. Judging from cell differentiation inducing activity, physalin A should be a new type of antitumor agent [19]. In the present study, further examination of the constituents of *P. alkekengi* var. *francheti* has led to the isolation of a new compound, named physalin T(**3**), lacking the C(2)—C(3) unsaturation which is known to be important for the cytotoxicity.



Physalin B (**1**)



Physalin D (**2**)



Physalin T (**3**)

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RESULTS AND DISCUSSION

Aqueous extract of the fresh epigeal parts of *P. alkekengi* var. *francheti* was transferred to $CHCl_3$ layer, and evaporation of the solvent afforded crude material, which was subjected to silica-gel chromatography. In addition to the known physalins A, B, D, L, M [10] and N [11], silica gel TLC analysis of the fractions indicated the presence of a new component whose R_f value was the same as that of physalin D (**2**) in $CHCl_3$-MeOH system but the R_f value was higher than that of **2** in benzene-AcOEt system. Repeated column chromatography followed by crystallization from MeOH-acetone afforded a new compound, physalin T (**3**), as colorless needles, mp 249–252°C. The molecular formula $C_{28}H_{34}O_{11}$ was established by high-resolution EI-MS and elemental analysis.

Except physalins R and S which contain an additional C—C bond at C(11)—C(15) and at C(3)—C(5), respectively [12], and physalin P with a rearranged 'neophysalin' skeleton [13], physalins previously reported

commonly possess the same skeletal structure. According to the presence/absence of C(14)—O—C(27) acetalic linkage, physalins are classified to types B/A, *i.e.*, while physalins A, C, L, M and O [11] and (25*S*)-25,27-dihydrophysalin C [16] belong to type A, the other physalins ever known are type B. The 400 MHz ^{1}H NMR spectra of **3**, taken in DMSO-d_6 solution, exhibited three methyl singlets (δ 1.14, 1.18 and 1.80) and the methylene signals at δ 3.56 (*d*, $J = 13$ Hz) and δ 4.23 (*dd*, $J = 13$ and 4 Hz) which are characteristic of the C(27)H_2—O—C(14) bridge indicating that **3** belongs to type B physalins. Among the three hydroxy proton signals, most deshielded one (δ 5.56, *s*) was assigned to the tertiary hydroxy group at C(13), while other two hydroxy groups (δ 4.06, *s* and δ 4.73, *d*, $J = 4$ Hz) were considered to be located at ring A and/or ring B. However, the most striking feature of the NMR spectra of **3** was the lack of alkenic proton signals, which was consistent with the evidence (no absorption maximum above 210 nm) of the UV spectrum. These spectral characteristics suggested the structure of **3** as dihydroxylated 2,3,5,6-tetrahydrophysalin B. Detailed ^{1}H-^{1}H and ^{1}H-^{13}C two dimensional NMR spectral analyses including DQF-COSY, HOHAHA, ROESY, HMQC and HMBC experiments enabled us to complete assignment of the ^{1}H and ^{13}C signals as summarized in Tables I and II, respectively, which indicated close similarity of **3** and physalin D (**2**) except the signals of the ring A moiety due to presence/absence of the C(2)—C(3) double bond in **2**/**3**. Therefore, the new physalin **3** was assumed to be 2,3-dihydrophysalin D.

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To confirm the structure of physalin T (**3**), chemical correlation between **2** and **3** was undertaken. Catalytic hydrogenation of **2** over palladium carbon yielded a hydrogenated product in 88% yield which was indistinguishable from **3** naturally obtained by TLC and ^{1}H NMR analyses. Thus, the structure of physalin T (**3**) was determined unambiguously as 2,3-dihydrophysalin D, namely, 5α,6β-dihydroxy-2,3,5,6-tetrahydrophysalin B.

Although the structure which corresponds to 2,3-dihydrophysalin D has apparently not been described, a dihydro derivative of physalin E was reported as 5α,7α-dihydroxy-2,3,5,6-tetrahydrophysalin B [4]. The identification work of physalin E and physalin D (**2**) conducted by the authors [7] supports the possibility that the 'dihydrophysalin E' can be identical with physalin T (**3**).

The new physalin **3** is unique since, unlike other physalins, it possesses a reduced ring A structure. All other physalins possess a double bond either at C(2)—C(3) or at C(3)—C(4), except physalin S which contains a cyclopropane ring instead of the unsaturation [13]. Physalins K and Q possess an endoperoxy function at C(2)—C(5) and C(3)—C(4) double bond

TABLE I 400 MHz ^{1}H NMR spectral data of physalins D (2) and T (3) in DMSO-d_6 solutions (chemical shift δ/ppm, spin multiplicity and coupling constant/Hz in parentheses). The signal of residual proton of the solvent (δ 2.49) was taken as the internal standard

	2	3
H-2	5.68 *dd* ($J_{2,3}=10$) ($J_{2,4\beta}=2$)	1.88 (α) m 2.64 (β) m
H-3	6.61 *ddd* ($J_{3,2}=10$) ($J_{3,4\alpha}=5$) ($J_{3,4\beta}=2$)	1.88 (α) m 1.77 (β) m
H-4	1.96 (α) *dd* ($J_{4\alpha,4\beta}=19$) ($J_{4\alpha,3}=5$) 3.10 (β) br *d* ($J_{4\beta,4\alpha}=19$)	1.20 (α) m 2.48 (β) m
H-5	4.21 (OH) s	4.06 (OH) s
H-6	3.47 m 4.87 (OH) *d* ($J_{OH,6}=4$)	3.44 m 4.73 (OH) *d* ($J_{OH,6}=4$)
H-7	1.78 (α) m 1.8 (β) m	1.8 (α) m 1.7 (β) m
H-8	2.19 *td* ($J_{8,7\alpha}=11$) ($J_{8,9}=11$) ($J_{8,7\beta}=5$)	2.13 *td* ($J_{8,7\alpha}=11$) ($J_{8,9}=11$) ($J_{8,7\beta}=3$)
H-9	3.10 m	3.05 *dd* ($J_{9,8}=11$) ($J_{9,11\beta}=8$)
H-11	1.75 (α) m 0.93 (β) m	1.38 (α) br *t* ($J_{11\alpha,11\beta}=14$) ($J_{11\alpha,12\alpha}=14$) 0.89 (β) m
H-12	2.08 (α) m 1.44 (β) *dd* ($J_{12\beta,12\alpha}=16$) ($J_{12\beta,11\beta}=10$)	1.97 (α) m 1.46 (β) m
H-13	5.70 (OH) s	5.56 s (OH)
H-16	2.77 s	2.77 s
H-19	1.10 (Me) s	1.18 (Me) s
H-21	1.80 (Me) s	1.80 (Me) s
H-22	4.55 m	4.56 m
H-23	2.08 (*R*) *dd* ($J_{23R,23S}=14$) ($J_{23R,22}=4$) 1.92 (*S*) br *d* ($J_{23S,23R}=14$)	2.08 (*R*) br *d* ($J_{23R,23S}-14$) 1.89 (*S*) m
H-25	2.86 *d* ($J_{25,27S}=4$)	2.87 *d* ($J_{25,27S}=4$)
H-27	3.57 (*R*) *d* ($J_{27R,27S}=13$) 4.23 (*S*) *dd* ($J_{27S,27R}=13$) ($J_{27S,25}=3.5$)	3.56 (*R*) *d* ($J_{27R,27S}=13$) 4.23 (*S*) *dd* ($J_{27S,27R}=13$) ($J_{27S,25}=4$)
H-28	1.15 (Me) s	1.14 (Me) s

TABLE II ^{13}C NMR Spectral chemical shifts (δ/ppm) of physalins D (2) and T (3) in DMSO-d_6 solutions. The most intense solvent peak (δ 39.5) was taken as the internal standard

C	2	3	C	2	3	C	2	3
1	204.4	215.4	11	24.7	25.0	21	21.6	21.4
2	127.2	36.5	12	25.8	25.7	22	76.3	76.3
3	142.9	20.5	13	78.7	79.0	23	31.3	31.3
4	35.2	29.7	14	106.9	107.0	24	30.5	30.5
5	76.4	77.6	15	209.8	209.7	25	49.4	49.4
6	72.5	73.4	16	54.0	54.2	26	167.3	167.2
7	26.6	27.0	17	80.7	80.5	27	60.5	60.5
8	38.3	38.0	18	171.8	171.8	28	24.5	24.4
9	29.9	30.0	19	13.3	14.6			
10	53.5	56.9	20	80.5	80.5			

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[8]. In the case of withanolides, which are biogenetically related to physalins, a few compounds with ring A saturation are known, and withametelin C isolated from *Datura* species [20] possesses the same ring A/B structure as that of **3**.

The presence of a double bond at C(2)—C(3) is known to be important for the cytotoxic activity of physalins against HeLa cells [9] and in fact, physalin T (**3**) did not exhibit any cytotoxicity. Physalin D (**2**) is also inactive, and 2,3-dihydrophysalin B showed weak activity, suggesting the additional contribution of the functionality at C(5)/C(6) to the cytotoxicity.

EXPERIMENTAL SECTION

General Experimental Procedures

Column chromatography was carried out on silica gel (Silica Gel 60, 230–400 mesh, Merck). Samples adsorbed on approximately equal amount of diatomaceous silica (Celite 545, John-Manville) were applied to the top of the silica-gel column and eluted with suitable solvent systems. Silica-gel TLC was performed using precoated plates (Silica Gel $60F_{254}$, Merck) and the spots were detected under UV light at 254 nm and also at 365 nm after spraying with 50% H_2SO_4 followed by heating. Mass spectra were measured with electron impact ionization. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded at 30°C with DMSO-d_6 solutions.

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Isolations of Physalins

Crude extracts (396 g) were obtained from fresh epigeal parts (240 kg) of *P. alkekengi* var. *francheti* harvested in Kyoto prefecture as described previously [9, 10]. Column chromatographic separation of the crude extract using $CHCl_3$-MeOH (100 : 0 – 100 : 8) as eluent afforded fractions #1(0.3 g), #2 (11.6 g), #3(9.1 g), #4 (11.0 g), #5 (10.9 g), #6 (2.4 g) and #7 (4.1 g). The fraction #2 was subjected to repeated chromatography ($CHCl_3$-MeOH) to give **1** (1.00 g) and physalin M [10] (1.21 g). Column chromatography of fraction #3 using $CHCl_3$-MeOH and C_6H_6-EtOAc systems followed by crystallization yielded physalin L (0.48 g) and the mixture of physalins A and N[11] (1.20 g). The fraction #4 was treated similarly as above to give physalin T (**3**, 0.075 g) and **2** (0.44 g). Further **2** (0.68 g) was obtained from the fraction #5. R_f values in TLC with the solvent systems $CHCl_3$-MeOH (9 : 1) and C_6H_6-EtOAc (3 : 7) were as follows: physalins A 0.49, 0.41; B (**1**)

0.67, 0.65; D (**2**) 0.38, 0.36; L 0.54, 0.50; M 0.62, 0.70; N 0.53, 0.41; T (**3**) 0.38, 0.40.

Physalin T (**3**). Colorless needles from MeOH-acetone; mp 249–252°C; $[\alpha]_D^{20}$ −64.3° (*c* 0.5, acetone); IR(KBr) ν_{max} 3430, 1780, 1760, 1735, 1685 cm^{-1}; ^1H NMR data, see Table I; ^{13}C NMR data, see Table II; CD(MeOH) $[\theta]_{221}$ −7100; EIMS *m/z* 546 $[M]^+$, 528 $[M\text{-}H_2O]^+$, 518 $[M\text{-}CO]^+$, 510 $[M\text{-}2H_2O]^+$; HR-EIMS *m/z* 546.2088 (M^+), calcd for $C_{28}H_{34}O_{11}$, 546.2098; anal. C 59.41%, H 6.24%, Calcd for $C_{28}H_{34}O_{11}\cdot H_2O$, C, 59.57%, H, 6.44%.

Conversion of Physalin D (2) to Physalin T (3)

A THF solution (20 ml) of **2** (113 mg) was hydrogenated with atmospheric H_2 over Pd-carbon (Pd content 5%, 150 mg) for 7 h at room temperature. The catalyst was filtered off and the filtrate was evaporated to dryness affording a white solid, which was subjected to column chromatography. Elution with C_6H_6-EtOAc (7 : 3) yielded the expected hydrogenated product (98 mg, yield 88%) which was identified as **3** by IR and ^1H NMR spectra and TLC analysis.

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