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# New Physalins Possessing An Additional Carbon-Carbon Bond from *Physalis alkekengi* var. *francheti*

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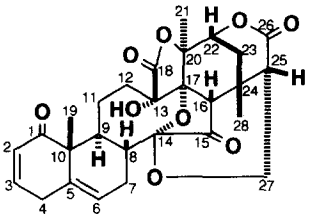
***Abstract***: Physalins R and S were isolated from *Physalis alkekengi* var. *francheti* and their structures were determined by spectroscopic studies as 15α-hydroxy-11β,15β-cyclo-15-deoxophysalin B and 3α,5α-cyclo-6β-hydroxy-2,3,5,6-tetrahydrophysalin B, respectively. The UV-irradiation of physalin B afforded physalin R, and this photo-induced C(11)–C(15) bond formation was shown to be sensitized intramolecularly by the conjugated enone group in the A ring.

Physalins are steroidal constituents of *Physalis* and of other closely related genera belonging to *Solanaceae*,[1,2] and are characterized by their modified ergostane-type framework, namely, 16,24-cyclo-13,14-secosteroid. From their polyoxyfunctional structures, physalins can be classified as part of the most advanced group in biogenetic oxidation level among the withanolide steroids.[1,2] In the course of our study on the constituents of *P. alkekengi* var. *francheti* (Japanese name; Hôzuki), physalins A–C[3-6] and physalins L–Q[7-11] were isolated and their structures were determined. Row *et al.* reported the isolation of physalin B (**1**) and physalins D-K from *P. angulata* and *P. lancifolia*.[12-14] Structural revisions of physalins H[15] and K[11] were also reported by us.[16] Due to their highly strained, polycyclic structure[17] unusually facile hydroxylation takes place at C(25) of physalins.[18] Acid-induced skeletal rearrangement of physalins yields neophysalins,[19] and physalin P actually possesses a neophysalin structure.[10] Some of the physalins demonstrate cytotoxic activity against tumor cells *in vitro* and *in vivo*,[7,15,20-22] and recently Sunayama *et al.* reported the cell differentiation inducing activity of physalin A as a new type of antitumor agent.[23] Further examination of the constituents of *P. alkekengi* var. *francheti* has led to the isolation of two new compounds, named physalins R (**2**) and S (**3**), possessing an additional carbon–carbon bond. Conversion of **1** to **2** has been attained by means of photo-induced isomerization. This paper describes the structural determinations of **2** and **3** and also the unique, self-sensitized photocyclization of physalins.



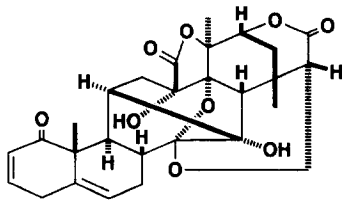
Physalin B (**1**)

## RESULTS AND DISCUSSION

From the aqueous extracts of the epigeal parts of *P. alkekengi* var. *francheti*, two new physalins, **1** and **2**, were isolated, along with the known constituents of this plant, *i.e.*, physalins A,[3] B (**1**),[4] F,[13] M,[8] N,[9] and O,[9] and (25*S*)-25,27-dihydrophysalin C.[20,24] The presence of the dihydrophysalin C in this plant has not been reported before.

*Structure of Physalin R*

Physalin R (**2**), $C_{28}H_{30}O_9$, is an isomer of **1**, but exhibits a lower $R_f$ value on silica gel TLC, suggesting its more polar nature than **1**. As summarized in Table 1, the 400 MHz $^1$H NMR spectra of **2**, measured in DMSO-$d_6$ solution, indicated the structural similarity of **2** and **1**, possessing the 2,5-dien-1-one structure at the A/B ring moiety (δ 5.83, *dd*, *J* = 10 and 2.5 Hz, H-2; δ 7.06, *ddd*, *J* = 10, 5.5, and 2 Hz, H-3; δ 5.57, *dd*, *J* = 3 and 1.5 Hz, H-6) and the C(14)–O–C(27) bridge structure. The chemical shifts and coupling constants of the methylene protons at C(27) of **2** (δ 4.05, *dd*, *J* = 11.5 and 4 Hz, H-27*S*; δ 4.71, *br d*, *J* = 11.5 Hz, H-27*R*), however, differed strikingly from those of **1** and related compounds (δ 4.22–4.36, *dd*, *J* = 13–14 and *ca.* 4 Hz, H-27*S*; δ 3.51–3.67, *br d*, *J* = 13–14 Hz, H-27*R*). In addition to the hydroxyl signal at C(13) (δ 6.13), another tertiary hydroxyl signal was observed at δ 5.66, which was consistent with the low $R_f$ value in the TLC. The $^{13}$C NMR spectral analysis of **2** in DMSO-$d_6$ solution using the APT (attached proton test) technique[25] revealed the absence of both the C(15) ketone carbonyl and C(11) methylene groups. Instead of those resonances the spectra of **2** exhibited a quaternary carbon signal at δ 75.6 and a methine signal at δ 46.9. Based on the detailed $^{13}$C and $^1$H NMR spectral analyses of **2** with the aid of 2D-correlational spectroscopy ($^1$H–$^1$H, $^{13}$C–$^1$H, and long range $^{13}$C–$^1$H COSY), the methine signal was assigned to C(11) and the quaternary carbon signal to C(15) which was assumed to carry the tertiary hydroxyl group. Considering the short distance between the C(11) methylene and the C(15) carbonyl groups in physalins demonstrated by X-ray crystallography,[17] the presence of an extra C–C bond between the C(11) methine and the C(15) carbinol groups was assumed in this new physalin. As shown in Table 2, large chemical shift differences between **1** and **2** were observed for the carbon atoms located in the vicinity of the C(11) or C(15), *e.g.*, C(9) and C(12) in **2** resonated at about 10 ppm lower field than those in **1**. The $^1$H NMR spectral data (Table 1) are also explained reasonably by the C(11)H–C(15)OH structure of **2**. The β-proton at C(12) and the *pro-R* proton at C(27), located in the anisotropic shielding region of the C(15) carbonyl group in **1**, are shifted to much lower field in **2** (H-12β: δ 1.45 to δ 2.27; H-27*R*: δ 3.60 to δ 4.71). The neighboring methine proton at C(16) of **2** (δ 1.90), on the other hand, resonated at much higher field than that of **1** (δ 2.86). Thus, the structure of physalin R (**2**) has been elucidated as 15α-hydroxy-11β,15β-cyclo-15-deoxophysalin B. Further confirmation of this structure is provided by the photochemical transformation of **1** to **2** as described below.




Physalin R (**2**)

*Photo-induced Conversion of Physalin B to Physalin R*

The structure of physalin R (**2**) suggested that **2** can be obtained from physalin B (**1**) by photo-induced

Table 1. 400 MHz $^1$H NMR Spectral Data of Physalins B (**1**), R (**2**), and S (**3**) in DMSO-$d_6$ Solutions (chemical shift δ/ppm, spin multiplicity, and coupling constant /Hz)[a)]

| Assignment | **1**[b)] | **2** | **3** |
|---|---|---|---|
| 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) | 2.84 *br dd* (α)[c)] ($J_{2\alpha,2\beta}$ = 17) ($J_{2\alpha,3}$ = 6) |
| | | | 1.92 *d* (β)[c)] ($J_{2\beta,2\alpha}$ = 17) |
| H-3 | 6.89 *ddd* ($J_{3,2}$ = 10) ($J_{3,4\alpha}$ = 5) ($J_{3,4\beta}$ = 2) | 7.06 *ddd* ($J_{3,2}$ = 10) ($J_{3,4\alpha}$ = 5.5) ($J_{3,4\beta}$ = 2) | 1.35 *m* |
| H-4 | 2.89 *dd* (α) ($J_{4\alpha,4\beta}$ = 20) ($J_{4\alpha,3}$ = 5) | 2.95 *dd* (α) ($J_{4\alpha,4\beta}$ = 21) ($J_{4\alpha,3}$ = 5.5) | 0.70 *ddd* (α) ($J_{4\alpha,4\beta}$ = 6) ($J_{4\alpha,3}$ = 6.5) ($J_{4\alpha,2\alpha}$ = 0.5) |
| | 3.27 *br d* (β) ($J_{4\beta,4\alpha}$ = 20) | 3.24 *dm* (β) ($J_{4\beta,4\alpha}$ = 21) | −0.21 *dd* (β) ($J_{4\beta,4\alpha}$ = 6) ($J_{4\beta,3}$ = 3.5) |
| H-6 | 5.59 *br d* ($J_{6,7\beta}$ = 6) | 5.57 *dd* ($J_{6,7\beta}$ = 3) ($J_{6,4\beta}$ = 1.5) | 3.18 *m* ($W_{1/2}$ = 6) |
| | | | 4.50 *d* (OH) ($J_{OH,6}$ = 3) |
| H-7 | 1.97 *m* (α) | 1.98 *m* (α) | 1.52 *ddd* (α) ($J_{7\alpha,7\beta}$ = 13) ($J_{7\alpha,8}$ = 10.5) ($J_{7\alpha,6}$ = 2.5) |
| | 2.21 *m* (β) | 1.98 *m* (β) | 2.17 *dm* (β) ($J_{7\beta,7\alpha}$ = 13) |
| H-8 | 1.92 *m* | 2.08 *ddd* ($J_{8,7\alpha}$ = 16) ($J_{8,9}$ = 10) ($J_{8,7\beta}$ = 5.5) | 2.26 *dt* ($J_{8,9}$ = $J_{8,7\alpha}$ = 10.5) ($J_{8,7\beta}$ = 1.5) |
| H-9 | 2.95 *dd* ($J_{9,8}$ = 11) ($J_{9,11\beta}$ = 9) | 2.27 *dd* ($J_{9,8}$ = 10) ($J_{9,11}$ = 5.5) | 2.58 *t* ($J_{9,8}$ = $J_{9,11\beta}$ = 10.5) |
| H-11 | 2.18 *m* (α) | 2.52 *m* | 1.67 *tm* (α) ($J_{11\alpha,11\beta}$ = $J_{11\alpha,12\alpha}$ = 14.5) |
| | 1.10 *m* (β) | | 0.95 *m* (β) |
| H-12 | 2.17 *m* (α) | 1.91 *m* (α) | 1.80 *m* (α) |
| | 1.45 *m* (β) | 2.27 *br d* (β) ($J_{12\beta,12\alpha}$ = 16) | 1.35 *m* (β) |
| H-13 | 6.28 *s* (OH) | 6.13 *s* (OH) | 6.63 *s* (OH) |
| H-15 | | 5.66 *s* (OH) | |
| H-16 | 2.86 *s* | 1.90 *s* | 2.85 *s* |
| H-19 | 1.09 *s* (Me) | 1.14 *s* (Me) | 0.83 *s* (Me) |
| H-21 | 1.78 *s* (Me) | 1.59 *s* (Me) | 1.78 *s* (Me) |
| H-22 | 4.56 *dd* ($J_{22,23R}$ = 3) ($J_{22,23S}$ = 2) | 4.41 *dd* ($J_{22,23R}$ = 3) ($J_{22,23S}$ = 1.5) | 4.57 *dd* ($J_{22,23R}$ = 3.5) ($J_{22,23S}$ = 2) |
| H-23 | 2.14 *m* (*R*) | 1.90 *dd* (*R*) ($J_{23R,23S}$ = 14.5) ($J_{23R,22}$ = 3) | 2.10 *dd* (*R*) ($J_{23R,23S}$ = 14.5) ($J_{23R,22}$ = 3.5) |
| | 1.96 *m* (*S*) | 1.80 *dd* (*S*) ($J_{23S,23R}$ = 14.5) ($J_{23S,22}$ = 1.5) | 1.90 *dd* (*S*) ($J_{23S,23R}$ = 14.5) ($J_{23S,22}$ = 2) |
| H-25 | 2.88 *br d* ($J_{25,27S}$ = 4) | 2.71 *br d* ($J_{25,27S}$ = 4) | 2.90 *d* ($J_{25,27S}$ = 4.5) |
| H-27 | 3.60 *dd* (*R*) ($J_{27R,27S}$ = 14) ($J_{27R,25}$ = 1) | 4.71 *br d* (*R*) ($J_{27R,27S}$ = 11.5) | 3.60 *d* (*R*) ($J_{27R,27S}$ = 13) |
| | 4.26 *dd* (*S*) ($J_{27S,27R}$ = 14) ($J_{27S,25}$ = 4) | 4.05 *dd* (*S*) ($J_{27S,27R}$ = 11.5) ($J_{27S,25}$ = 4) | 4.26 *dd* (*S*) ($J_{27S,27R}$ = 13) ($J_{27S,25}$ = 4.5) |
| H-28 | 1.16 *s* (Me) | 1.36 *s* (Me) | 1.15 *s* (Me) |

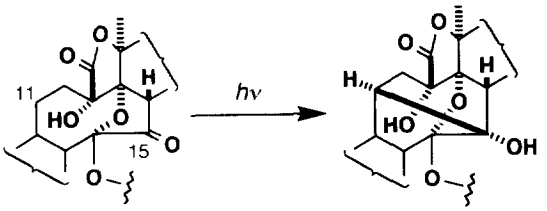
a) $W_{1/2}$ refers to half width (/Hz). b) Ref. 11. c) Ref. 26.

Table 2. 100 MHz $^{13}C$ NMR Spectral Data of Physalins B (**1**), R (**2**), and S (**3**) in DMSO-$d_6$ Solutions (chemical shift δ/ppm)

| Assignment | 1[a)] | 2 | 3 | Assignment | 1[a)] | 2 | 3 |
|---|---|---|---|---|---|---|---|
| C(1) | 202.4 | 202.7 | 217.0 | C(15) | 209.3 | 75.6 | 209.4 |
| C(2) | 126.9 | 127.1 | 39.5 | C(16) | 54.1 | 49.6 | 54.1 |
| C(3) | 146.1 | 148.6 | 14.5 | C(17) | 80.7 | 82.1 | 80.3 |
| C(4) | 32.3 | 32.1 | 17.0 | C(18) | 171.8 | 173.8 | 172.0 |
| C(5) | 135.5 | 137.7 | 34.4 | C(19) | 16.8 | 17.6 | 13.2 |
| C(6) | 123.4 | 124.4 | 70.2 | C(20) | 80.3 | 81.9 | 80.3 |
| C(7) | 24.4 | 25.4 | 31.7 | C(21) | 21.7 | 20.4 | 21.6 |
| C(8) | 40.2 | 43.2 | 36.2 | C(22) | 76.3 | 75.6 | 76.2 |
| C(9) | 33.1 | 44.7 | 36.7 | C(23) | 31.4 | 31.0 | 31.3 |
| C(10) | 52.0 | 51.4 | 52.9 | C(24) | 30.5 | 31.3 | 30.5 |
| C(11) | 24.1 | 46.9 | 20.6 | C(25) | 49.4 | 50.1 | 49.3 |
| C(12) | 25.6 | 34.7 | 24.6 | C(26) | 167.2 | 168.6 | 167.2 |
| C(13) | 78.2 | 85.9 | 78.1 | C(27) | 60.6 | 60.0 | 60.7 |
| C(14) | 106.3 | 112.0 | 106.1 | C(28) | 24.4 | 28.3 | 24.4 |

a) Ref. 11

reaction of the C(15) carbonyl group (Scheme 1). Therefore, an acetone solution of **1**, containing benzophenone as a sensitizer, was irradiated with a halogen-tungsten lamp under an argon atmosphere to give the expected **2** in 36% yield and also its epoxy derivative **4** (12%).[27] While no such product was formed under dark conditions, irradiation in the absence of benzophenone was found to afford a better yield of **2**, as given in Table 3. The reaction was therefore considered to proceed *via* the photo-excited carbonyl function at C(15), which is probably sensitized by the 2-en-1-one moiety at the A ring in the molecule. We propose the name "cyclophysalin" for the C(11)–C(15) bridged structure, *i.e.*, cyclophysalin X implies the structure in which the $CH_2$ and C=O groups at the 11- and 15-positions of physalin X are replaced by a CH–COH bridge. Therefore, **2** and **4** correspond to cyclophysalins B and F, respectively. Irradiation of physalin N (7α-hydroxyphysalin B) and physalin F (5β,6β-epoxy-5,6-dihydrophysalin B) in a similar manner also afforded cyclophysalins N and F (**4**), respectively, in 30–70% yields. In order to confirm the participation of the enone chromophore in




Scheme 1. Photo-induced cyclization of physalins

Table 3. Photo-induced Transformation of Physalins to Cyclophysalins

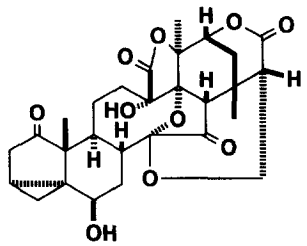
| substrate (/mM) | photosensitizer (/mM) | light source[a] | irradiation time /h | conversion /% | product | yield[b] /% |
|---|---|---|---|---|---|---|
| **1** (14) | $Ph_2CO$ (4) | W–$X_2$ | 20 | 100 | **2**<br>**4** | 36 (36)<br>12 (12) |
| **1** (2) | $Ph_2CO$ (1) | Hg | 6.3 | 100 | **2**<br>**4** | 33 (33)<br>trace |
| **1** (10) | none | W–$X_2$ | 44 | 100 | **2** | 49 (49) |
| physalin N (5) | $Ph_2CO$ (3) | W–$X_2$ | 12 | 100 | cyclophysalin N | 30 (30) |
| physalin N (11) | none | Hg | 5 | 66 | cyclophysalin N | 53 (80) |
| physalin F (16) | none | W–$X_2$ | 20.6 | 100 | **4** | 71 (71) |
| **5** (11) | none | W–$X_2$ | 40 | 3 | 2,3-dihydrophysalin F | 3 (97) |
| **5** (19) | $Ph_2CO$ (2) | W–$X_2$ | 40 | 32 | 2,3-dihydrophysalin F | 7 (22) |
| **5** (5) and physalin F (5)[c] | none | W–$X_2$ | 22 | 7<br>96 | 2,3-dihydrophysalin F<br>**4** | 6 (87)<br>88 (92) |
| isophysalin B (2) and **1** (0.1)[c] | none | W–$X_2$ | 44 | 16<br>100 | ----<br>**2** | --<br>33 (33) |
| physalin A (3) | none | Hg | 2.2 | 50 | cyclophysalin A<br>cyclophysalin N[e] | 23 (45)[d]<br>9 (18)[d] |
| physalin O (1) | $Ph_2CO$ (1) | W–$X_2$ | 11 | 69 | cyclophysalin O | 40 (58) |
| physalin O (1) | none | Hg | 4.5 | 100 | cyclophysalin O | 60 (60) |

a) Hg: high-pressure Hg lamp. W–$X_2$: halogen-tungsten lamp.
b) Isolated yield. Yield based on the consumed starting material in parentheses.
c) Binary mixture was subjected to the photo-irradiation.
d) Products were not isolated but yields were determined by $^1H$ NMR analysis.
e) This product is considered to be formed *via* physalin N or cyclophysalin A. See Ref. 28.

the A ring, 2,3-dihydrophysalin B (**5**),[5] lacking the conjugated enone moiety, was subjected to the reaction. Both in the absence and in the presence of benzophenone, **5** afforded no corresponding cyclophysalin but the epoxidation product, 2,3-dihydrophysalin F, was isolated in low yield. Isophysalin B[5] (3,4-didehydro-2,3-dihydrophysalin B), possessing a 3,5-dien-1-one system, also failed to give the cyclized product. In the case of an equimolar mixture of **5** and physalin F, most of **5** was recovered without any cyclophysalin formation, while physalin F was converted to cyclophysalin **4** in high yield. These results suggested that this photo-induced C(11)–C(15) bond formation reaction does not involve an intermolecular energy transfer process, but an intramolecular, self-sensitizing mechanism in which it can be assumed that the conjugated enone is an internal sensitizer. The reaction was found to proceed also in other types of physalins lacking the C(14)–O–C(27) bridge, *i.e.*, physalin A, possessing a C(25)=C(27) double bond, and physalin O, possessing a β-methyl group at C(25), also gave the corresponding cyclophysalins in high yields as summarized in Table 3. This newly found C(11)–C(15) bridge formation at the highly strained 14α,17-epoxy-13,14-seco C/D ring moiety constitutes an additional example of the characteristic reactions of physalins, which can also imply the possibility of the new physalin **2** being an artifact produced from the abundantly present precursor **1**.

*Structure of Physalin S*

Physalin S (**3**) possesses the molecular formula $C_{28}H_{32}O_{10}$, as established by high-resolution EI-MS. The 400 MHz $^1H$ NMR spectra of **3**, taken in DMSO-$d_6$ solution, were characterized by the absence of an olefinic signal and the presence of methylene protons which resonated at markedly higher field (δ −0.21, *dd*, *J* = 6 and 3.5 Hz and δ 0.70, *ddd*, *J* = 6, 6.5, and 0.5 Hz), indicating the presence of a cyclopropane ring in **3**. The characteristic methylene signals (δ 4.26, *dd*, *J* = 13 and 4.5 Hz and δ 3.60, *d*, *J* = 13 Hz) revealed the C(27)$H_2$–O–C(14) bridge commonly present in **1** and related physalins. Detailed $^1H$ and $^{13}C$ NMR spectral analyses using the 2D-correlational techniques were undertaken as summarized in Tables 1 and 2, which demonstrated that **3** differs from **1** only at the A/B ring moiety. The $^1H$–$^1H$ COSY revealed the proton coupling network from C(6) through C(12), in which the C(6) methine (δ 3.18, *m*) was assumed to carry a secondary hydroxyl group (δ 4.50, *d*, *J* = 3 Hz). An axial β-configuration was assigned to the secondary hydroxyl group, based on the coupling constant (2.5 Hz) between H-6 and H-7α. Another coupling network assignable to the A ring moiety was also revealed, *i.e.*, the highly shielded methylene protons (δ −0.21 and 0.70) mentioned above are coupled with a methine proton (δ 1.35), which in turn is further coupled with the protons of another methylene (δ 2.84 and 1.92) adjacent to a carbonyl group. Thus, the higher and lower field methylenes were reasonably assigned to C(4) and C(2), respectively, and the C(3) methine was assumed to form a cyclopropane ring with C(4) and C(5). The significantly shielded $^{13}C$ resonances of the C(3) methine (δ 14.5), C(4) methylene (δ 17.0), and the quaternary carbon atoms C(5) (δ 34.4) also support the cyclopropane structure. As for the stereochemistry of the cyclopropane ring, a β-configuration was assigned based on the chemical shift of the C(19) angular methyl protons (δ 0.83) observed at higher field than those of the known A/B-*trans* fused physalins (δ 1.05–1.34) considering the anisotropic shielding effect of the cyclopropane ring. The additional anisotropic shielding effect from the C(1) carbonyl group can reasonably account for the unusually higher field resonance (δ −0.21) of the H-4β proton of the cyclopropane ring. Accordingly, the structure of physalin S (**3**) has been established as 3α,5α-cyclo-6β-hydroxy-2,3,5,6-tetrahydrophysalin B, which is the first example of the cyclopropane-containing steroids isolated from *Physalis* plant.



Physalin S (**3**)

## EXPERIMENTAL

Column chromatography (CC) was carried out on $SiO_2$ (Fuji Silysia, FL60D). TLC were performed using precoated $SiO_2$ plates (Merck, Silica Gel $60F_{254}$) 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. HPLC were performed in the reversed-phase mode (Tosoh, TSK GEL ODS $80T_M$, 150 x 4.6 mm *i.d.*, elution with MeOH–$H_2O$ or $CH_3CN$–$H_2O$). Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-4 digital polarimeter and IR spectra were measured using a JASCO A-102 spectrophotometer with KBr discs. UV and CD spectra were recorded in MeOH using Hitachi U-3500 and JASCO J-600 spectrometers, respectively. Mass spectra were measured on a Hitachi M-2000 spectrometer with electron impact ionization. A Varian UNITY 400 plus spectrometer was used for $^1H$-NMR

spectra at 400 MHz and $^{13}C$ NMR spectra at 100 MHz, and a Varian Gemini-200 spectrometer was employed for 200 MHz $^{1}H$ NMR spectra in DMSO-$d_6$ solutions.

*Isolations of Physalins*

Fresh leaves (*ca.* 9 kg) and stems (*ca.* 2 kg) of *P. alkekengi* var. *francheti* harvested in Sobue, Aichi, Japan in August, were cut into small pieces and extracted with hot water for 10 min. The aqueous extracts were partitioned between 1-BuOH/EtOAc (1:5) and $H_2O$. The organic layer component (9.9 g) was fractionated by extractions with hexane, EtOAc, acetone, and MeOH, successively. The EtOAc soluble (3.6 g) and acetone soluble (0.8 g) fractions were combined and subjected to silica gel CC using the solvent systems $CHCl_3$–MeOH and $C_6H_6$–EtOAc repeatedly to afford the fractions containing new constituents, along with a mixture of physalins A, O, and N (401 mg), a mixture of physalin B (**1**) and isophysalin B[29] (973 mg), and a mixture of physalins F and M,[29] and (25*S*)-25,27-dihydrophysalin C (649 mg). Reversed-phase HPLC using 45% aqueous MeOH yielded physalin R (**2**; 3.1 mg) and purification with Sep-Pak $C_{18}$ cartridge (Waters) using 15–25% aqueous $CH_3CN$ yielded physalin S (**3**; 4.2 mg). $R_f$ values in TLC with the solvent systems $CHCl_3$–MeOH (9:1) and $C_6H_6$–EtOAc (3:7) were as follows: physalins A 0.42, 0.35; B (**1**) 0.68, 0.66; F 0.64, 0.60; M 0.58, 0.71; N 0.45, 0.35; O 0.42, 0.35; R (**2**) 0.44, 0.40; S (**3**) 0.46, 0.46; isophysalin B 0.68, 0.66; and (25*S*)-25,27-dihydrophysalin C 0.58, 0.71.

*Physalin R (2).* Colorless needles from MeOH; mp >300 °C; $[\alpha]_D^{15}$ −177° (*c* 0.13, acetone); IR $\nu_{max}$ 3545, 3460, 1765, 1730, 1660, 1645, 1365, 1285, 1240, 1210, 1185, 1145, 1110, 1060 $cm^{-1}$; UV $\lambda$max 222.5 nm (log ε 3.94); CD $[\theta]_{328}$ −3600, $[\theta]_{260}$ +6400, $[\theta]_{227}$ −20400, $[\theta]_{208}$ +1400; HRMS Found: m/z 510.1891. Calcd for $C_{28}H_{30}O_9$: $M^+$, 510.1888.

*Physalin S (3).* Colorless needles from MeOH; mp 287–289 °C; $[\alpha]_D^{15}$ −118° (*c* 0.08, acetone); IR $\nu_{max}$ 3430, 1755, 1725, 1625, 1165, 1125, 1095, 1060, 1030 $cm^{-1}$; UV: no apparent absorption maximum above 210 nm; CD $[\theta]_{304}$ −9400, $[\theta]_{296}$ −9400, $[\theta]_{224}$ −3900; HRMS Found: m/z 528.2008. Calcd for $C_{28}H_{32}O_{10}$: $M^+$, 528.1994.

*Photo-induced Transformation of Physalins to Cyclophysalins*

*Irradiation of Physalin B (1).* An acetone solution (8 ml) of **1** (58 mg) containing benzophenone (5 mg) in a Pyrex apparatus was irradiated by a 150 W halogen-tungsten lamp under argon-bubbling at ambient temperature for 20 h. The reaction was periodically monitored by TLC. After evaporation of the solvent, the residue was subjected to silica gel CC using the solvent system $CHCl_3$–MeOH and recrystallizations from MeOH to give **2** (14 mg) and **4** (7 mg).

Using a 100 W high-pressure Hg lamp, **1** (98 mg) and benzophenone (22 mg) in acetone (100 ml) in a Pyrex apparatus were irradiated at 15 °C for 6.3 h to afford **2** (33 mg) and a trace amount of **4** which was detected only by TLC.

Photo-irradiations of other physalins were performed as summarized in Table 3 in a similar manner to that described above to yield the following cyclophysalins:

*Cyclophysalin N.* Colorless needles from acetone–hexane, mp >300 °C; $[\alpha]_D^{15}$ −192° (*c* 0.13, acetone); IR $\nu_{max}$ 3410, 1780, 1720, 1655, 1635, 1360, 1185, 1055, 1030 $cm^{-1}$; UV λmax 222 nm (log ε 3.94); CD $[\theta]_{334}$ −2200, $[\theta]_{262}$ +2700, $[\theta]_{227}$ −28000, $[\theta]_{214}$ −17600; HRMS Found: m/z 526.1812. Calcd for $C_{28}H_{30}O_{10}$: $M^+$, 526.1837; $^1H$ NMR (200 MHz) δ 1.12 (*s*, $CH_3$-19), 1.36 (*s*, $CH_3$-28), 1.57 (*s*, $CH_3$-21), 2.02 (*s*, H-16), 2.09 (*dd*, $J_{8,9}$ = 12 and $J_{8,7}$ = 3 Hz, H-8), 2.26 (*br d*, $J_{12\beta,12\alpha}$ = 15 Hz, H-12β), 2.76 (*d*, $J_{25,27S}$ = 4 Hz, H-25), 2.96 (*dd*, $J_{4\alpha,4\beta}$ = 21 and $J_{4\alpha,3}$ = 5 Hz, H-4α), 3.27 (*br d*, $J_{4\beta,4\alpha}$ = 21 Hz, H-4β), 3.97 (*d*, $J_{OH,7}$ = 2.5 Hz, HO-7), 4.11 (*dd*, $J_{27S,27R}$ = 12 and $J_{27S,25}$ = 4 Hz, H-27*S*), 4.17 (*m*, H-7), 4.46 (*br s*, H-22), 4.72 (*d*, $J_{27R,27S}$ = 12 Hz, H-27*R*), 5.65 (*dd*, $J_{6,7}$ = 5 and $J_{6,4\beta}$ = 1 Hz, H-6), 5.80 (s, HO-15), 5.86 (*dd*, $J_{2,3}$ = 10 and $J_{2,4\beta}$ = 2 Hz, H-2), 6.09 (*s*, HO-13), 7.09 (*ddd*, $J_{3,2}$ = 10, $J_{3,4\alpha}$ = 5, and $J_{3,4\beta}$ = 2 Hz, H-3).

*Cyclophysalin F (4).* Colorless plates from acetone–MeOH, mp >300 °C; $[\alpha]_D^{15}$ −87° (*c* 0.13, acetone); IR $\nu_{max}$ 3430, 1770, 1725, 1650, 1370, 1250, 1210, 1185, 1145, 1130, 1060, 1045 $cm^{-1}$; UV λmax 221 nm (log ε 3.83); CD $[\theta]_{340}$ +8000, $[\theta]_{239}$ −11000, $[\theta]_{209}$ +8300; HRMS Found: m/z 526.1815. Calcd for $C_{28}H_{30}O_{10}$: $M^+$, 526.1837; $^1H$ NMR (200 MHz) δ 1.10 (*s*, $CH_3$-19), 1.32 (*s*, $CH_3$-28), 1.57 (*s*, $CH_3$-21), 1.99 (*s*, H-16), 2.70 (*d*, $J_{25,27S}$ = 4 Hz, H-25), 2.79 (*dm*, $J_{4\beta,4\alpha}$ = 18.5 Hz, H-4β), 3.21 (*br* s, $W_{1/2}$ = 3 Hz, H-6), 4.03 (*dd*, $J_{27S,27R}$ = 12 and $J_{27S,25}$ = 4 Hz, H-27*S*), 4.39 (*br s*, H-22), 4.66 (*br d*, $J_{27R,27S}$ = 12 Hz, H-27*R*), 5.69 (*s*, HO-15), 6.04 (*dd*, $J_{2,3}$ = 10 and $J_{2,4\beta}$ = 2.5 Hz, H-2), 6.24 (*s*, HO-13), 7.00 (*ddd*, $J_{3,2}$ = 10, $J_{3,4\alpha}$ = 7, and $J_{3,4\beta}$ = 2 Hz, H-3).

*2,3-Dihydrophysalin F.* White powder; IR $\nu_{max}$ 3420, 1760, 1700, 1165, 1135, 1060 $cm^{-1}$; HRMS Found: m/z 528.1976. Calcd for $C_{28}H_{32}O_{10}$: $M^+$, 528.1993; $^1H$ NMR (200 MHz) δ 0.81 (*s*, $CH_3$-19), 1.13 (*s*, $CH_3$-28), 1.77 (*s*, $CH_3$-21), 2.90 (*br s*, H-16), 2.90 (*br s*, H-25), 3.28 (*br s*, $W_{1/2}$ = 3 Hz, H-6), 3.58 (*d*, $J_{27R,27S}$ = 13.5 Hz, H-27*R*), 4.26 (*dd*, $J_{27S,27R}$ = 13.5 and $J_{27S,25}$ = 4 Hz, H-27*S*), 4.58 (*br s*, H-22), 6.64 (*s*, HO-13).

*Cyclophysalin A.* $^1H$ NMR (200 MHz) δ 1.08 (*s*, $CH_3$-19), 1.49 (*s*, $CH_3$-28), 1.60 (*s*, $CH_3$-21), 2.22 (*s*, H-16), 2.93 (*dd*, $J_{4\alpha,4\beta}$ = 21.5 and $J_{4\alpha,3}$ = 5 Hz, H-4α), 3.26 (*br d*, $J_{4\beta,4\alpha}$ = 21.5 Hz, H-4β), 4.37 (*m*, H-7), 4.47 (*br s*, H-22), 4.47 (*s*, HO-15), 4.56 (*d*, $J_{OH,7}$ = 3 Hz, HO-7), 5.64 (*m*, H-6), 5.73 (s, HO-13 or HO-14), 5.78 (*s*, H-27*E*), 5.86 (*dd*, $J_{2,3}$ = 10 and $J_{2,4\beta}$ = 2 Hz, H-2), 6.23 (*s*, H-27*Z*), 6.26 (*s*, HO-14 or HO-13), 7.08 (*ddd*, $J_{3,2}$ = 10, $J_{3,4\alpha}$ = 5, and $J_{3,4\beta}$ = 2 Hz, H-3).

*Cyclophysalin O.* White powder, $[\alpha]_D^{15}$ −151° (*c* 0.14, acetone); IR $\nu_{max}$ 3430, 1780, 1715, 1665, 1635, 1215, 1085, 1050 $cm^{-1}$; UV λmax 207 (log ε 3.90) and 224 nm (sh, log ε 3.80); CD $[\theta]_{333}$ −2700, $[\theta]_{257}$ +3300, $[\theta]_{227}$ −20400, $[\theta]_{216}$ −8700; HRMS Found: m/z 510.1917. Calcd for $C_{28}H_{30}O_9$: $M^+-H_2O$, 510.1888; $^1H$ NMR (200 MHz) δ 1.10 (*s*, $CH_3$-19), 1.14 (*d*, $J_{27,25}$ =7.5 Hz, $CH_3$-27), 1.16 (*s*, $CH_3$-28), 1.54 (*s*, $CH_3$-21), 2.01 (*s*, H-16), 2.95 (*dd*, $J_{4\alpha,4\beta}$ = 21 and $J_{4\alpha,3}$ = 5 Hz, H-4α), 3.26 (*br d*, $J_{4\beta,4\alpha}$ = 21 Hz, H-4β), 3.95 (*q*, $J_{25,27}$ = 7.5 Hz, H-25), 4.40 (*d*, $J_{22,23R}$ = 2.5 Hz, H-22), 4.47 (*m*, H-7), 4.65 (*d*, $J_{OH,7}$ = 3 Hz, HO-7), 4.97 (*s*, HO-15), 5.65 (*s*, HO-13 or HO-14), 5.65 (*d*, $J_{6,7}$ = 5 Hz, H-6), 5.86 (*dd*, $J_{2,3}$ = 10 and $J_{2,4\beta}$ = 2 Hz, H-2), 6.99 (*s*, HO-14 or HO-13), 7.08 (*ddd*, $J_{3,2}$ = 10, $J_{3,4\alpha}$ = 5, and $J_{3,4\beta}$ = 1.5 Hz, H-3).

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24. (25*S*)-25,27-Dihydrophysalin C (Ref. 20) was not isolated in the present study, but the isolation and characterization of this compound from other batches of this plant were already accomplished by us. (25*S*)-25,27-Dihydrophysalin C: White powder, $[\alpha]_D^{25}$ −129° (*c* 0.07, acetone); IR $\nu_{max}$ 3460, 1780, 1760, 1730, 1660, 1065 $cm^{-1}$ ; UV $\lambda_{max}$ 219.5 nm (log ε 3.98); CD $[\theta]_{344}$ −12100, $[\theta]_{261}$ +4600, $[\theta]_{236}$ −3400; HRMS Found: m/z 512.2043. Calcd for $C_{28}H_{32}O_9$: $M^+$, 512.2044; $^1H$ NMR (400 MHz) δ 1.06 (*s*, $CH_3$-19), 1.11 (*m*, H-11β), 1.13 (*d*, $J_{27,25}$ = 7 Hz, $CH_3$-27), 1.28 (*s*, $CH_3$-28), 1.65 (*br d*, $J_{23S,23R}$ = 15 Hz, H-23*S*), 1.74 (*s*, $CH_3$-21), 1.88 (*m*, H-8), 1.88 (*m*, H-12β), 1.99 (*m*, H-11α), 2.00 (*br d*, $J_{7\alpha,7\beta}$ = 16.5 Hz, H-7α), 2.07 (*dd*, $J_{23R,23S}$ = 15 and $J_{23R,22}$ = 4 Hz, H-23*R*), 2.25 (*m*, H-12α), 2.31 (*dm*, $J_{7\beta,7\alpha}$ = 16.5 Hz, H-7β), 2.70 (*q*, $J_{25,27}$ = 7 Hz, H-25), 2.80 (*s*, H-16), 2.81 (*dd*, $J_{9,8}$ = 11 and $J_{9,11\beta}$ = 7 Hz, H-9), 2.90 (*dd*, $J_{4\alpha,4\beta}$ = 22 and $J_{4\alpha,3}$ = 5 Hz, H-4α), 3.26 (*br d*, $J_{4\beta,4\alpha}$ = 22 Hz, H-4β), 4.47 (*dd*, $J_{22,23R}$ = 4 and $J_{22,23S}$ = 1.5Hz, H-22), 5.61 (*br d*, $J_{6,7\beta}$ = 6 Hz, H-6), 5.80 (*dd*, $J_{2,3}$ = 10 and $J_{2,4\beta}$ = 2 Hz, H-2), 6.14 (*s*, HO-13), 6.43 (*s*, HO-14), 6.92 (*ddd*, $J_{3,2}$ = 10, $J_{3,4\alpha}$ = 5, and $J_{3,4\beta}$ = 2.5 Hz, H-3); $^{13}C$ NMR (100 MHz) δ 16.4 (C-19), 16.6 (C-27), 20.9 (C-21), 23.8 (C-11), 25.5 (C-28), 25.8 (C-7 or C-23), 25.9 (C-23 or C-7), 29.1 (C-12), 32.2 (C-4), 34.4 (C-24), 34.4 (C-9), 40.8 (C-25), 42.3 (C-8), 52.5 (C-10), 54.0 (C-16), 76.4 (C-22), 78.7 (C-13), 81.9 (C-17), 82.4 (C-20), 101.1 (C-14), 124.1 (C-6), 126.9 (C-2), 135.9 (C-5), 146.5 (C-3), 171.7 (C-26 or C-18), 172.4 (C-18 or C-26), 202.5 (C-1), 216.0 (C-15)
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26. The configurational assignments of methylene protons at C(2) are based on the long range coupling ($^4J$ = 0.5 Hz) between H-2α (δ 2.84) and H-4α (δ 0.70) in the "M" arrangement (Wiberg, K. B.; Lowry, B. R.; Nist, B. J. *J. Am. Chem. Soc.* **1962**, 84, 1594–1597; Rassat, A.; Jefford, C. W.; Lehn, J. M.; Waegell, B. *Tetrahedron Lett.* **1964**, 233–243).
27. Although the photoreactions were performed under argon bubbling, residual oxygen is considered responsible for the photo-epoxidation (Shimizu, N.; Bartlett, P. D. *J. Am. Chem. Soc.* **1976**, 98, 4193–4200). Under the conditions with oxygen-bubbling, **2** and benzophenone in acetone were irradiated by a high-pressure Hg lamp to afford **4** in slightly improved yield. The photo-epoxidation of **2** and **5** proceeded even without benzophenone affording **4** and 2,3-dihydrophysalin F, respectively, reported in Table 3.
28. Slow interconversion between physalins A and N by the reversible Michael addition of the 14-OH group to the C(25)=C(27) double bond is known, and is commonly found for other physalins except those possessing a C(27) methyl structure. Details will be reported elsewhere.
29. We consider that isophysalin B is an artifact derived from **1**. It is also possible that physalin M, reported in Ref. 8, was produced from (25*S*)-25,27-dihydrophysalin C during the isolation procedures.

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