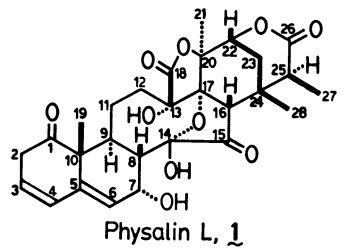
Structure of Physalin M Isolated from *Physalis alkekengi* var. *francheti*

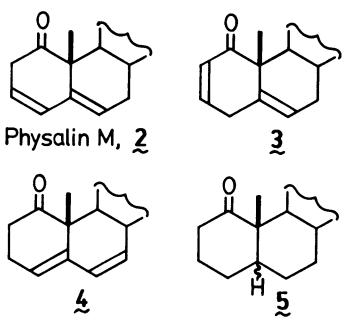
Masao KAWAI,* Toichi OGURA, Masaki NAKANISHI, Taketoshi MATSUURA,
Yasuo BUTSUGAN,* Yuji MORI,† Ken'ichi HARADA,†
and Makoto SUZUKI†
Department of Applied Chemistry, Nagoya Institute of Technology,
Showa-ku, Nagoya 466
†Faculty of Pharmacy, Meijo University, Tenpaku-ku, Nagoya 468
(Received March 15, 1988)

Synopsis. The structure of physalin M, isolated from *Physalis alkekengi* var. *francheti* was determined as 7-deoxyphysalin L by spectroscopic studies and chemical correlation with a known compound. Physalin M exhibited weak cytotoxicity against tumor cells.

Since the isolation of the first physalin, a novel 13,14-seco-16,24-cyclosteroid, from *Physalis alkekengi* var. *francheti* (Japanese name; Hôzuki),[1] more than ten physalins were isolated from *Physalis* plants grown in Japan[2-4] and in India.[5-11] Physalins were also isolated from *Witheringia coccoloboides* as cytotoxic constituents against tumor cells.[12] Very recently we reported the isolation and structure determination of a new physalin named physalin L (**1**) as a constituent of *P. alkekengi* var. *francheti*.[13] Unlike other physalins **1** possesses a conjugated diene structure in the A–B ring moiety instead of the conjugated cyclohexenone structure. In this note we will also report the isolation and structure determination of another new physalin which is structurally closely related to **1**.

From the extract of *P. alkekengi* var. *francheti*[13] a new compound, whose R_f value in SiO_2-TLC was the same as that of physalin B in benzene–ethyl acetate system but was lower than that of physalin B in chloroform–methanol system, was isolated and named physalin M (**2**). The new physalin **2** crystallized from 2-propanol as colorless prisims, mp 224—227 °C, and its molecular formula was established as $C_{28}H_{32}O_9$ by high-resolution mass spectroscopy and elemental analysis. Spectral data of **2** indicated that **2** possesses the similar structural characteristics to those of **1** as follows: i) Chemical shifts of olefinic protons (δ=5.67, 5.70, and 6.08) in the ^{1}H NMR spectrum (CD_3SOCD_3) and carbonyl stretching frequencies (1775, 1760, 1730, and 1695 cm^{-1}) in the IR spectrum indicated absence of the conjugated cyclohexenone moiety which is present in all known physalins except **1**. ii) Presence of a trisubstituted conjugated diene moiety was demonstrated by the UV absorption band at 228 nm (ε 11000) and by the three olefinic proton signals in the ^{1}H NMR spectrum. iii) Presence of a secondary methyl group (δ=1.14, d, J=7 Hz) in addition to three tertiary methyl groups (δ=1.18, 1.27, and 1.76) was also shown by the ^{1}H NMR spectrum. Assuming that **2** possesses the skeletal structure of physalins, the secondary methyl group was located at C(27) position as are the cases for **1** and also for the cytotoxic physalin from *Witheringia* species, namely 25-epi-25,27-dihydrophysalin C (**3**). Regarding the configuration of the secondary methyl group, a correlation between the stereochemistry at C(25) and the proton chemical shifts of C(27) and C(28) methyl groups was proposed;[12,13] i.e., in CD_3SOCD_3 solution C(27) methyl protons resonate at δ=1.07—1.10 and C(28) methyl protons at δ=1.42—1.48 for the compounds with the secondary methyl group in α-configuration, while C(27) and C(28) methyl protons resonate at δ=1.13—1.26 and at δ=1.14—1.30, respectively, for those with a β-methyl group. From the chemical shifts of C(27) and C(28) methyl groups of **2** (δ=1.14 and 1.27, respectively), β-configuration was assigned to the C(27) methyl group of **2**. The conjugated diene carrying three olefinic hydrogens could be placed only in the A–B ring moiety of the physalin skeleton. Thus two possible structures of **2** were obtained, i.e., one with the double bonds at C(3)–C(4) and C(5)–(6) positions and the other at C(4)–C(5) and C(6)–C(7). The former structure corresponds to the analog of **1** in which the secondary hydroxyl group is replaced by a hydrogen, namely 7-deoxyphysalin L, while the latter (**4**) is the known compound which was obtained by acid dehydration of tetrahydrophysalin A.[3] Spectral





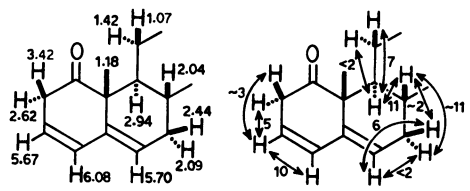


Fig. 1. Chemical shifts (δ) and vicinal coupling constants (Hz) of protons in the A–B ring moiety of physalin M (**2**) measured in CD_3SOCD_3 solution.

comparison clearly indicated that **2** is different from **4**, while the IR, UV, and ^1H and ^{13}C NMR spectral data of **2** resembled closely to those of **1**. The structure of physalin M (**2**) was thus reasonably assumed to be 7-deoxyphysalin L.

Detailed analysis of 400 MHz ^1H NMR spectrum of **2** was performed to ascertain the assumed structure. Homonuclear spin-spin decoupling experiment in combination with COSY analysis enabled the assignment of all of the 32 protons, and their chemical shifts and coupling constants were consistent with the 7-deoxyphysalin A structure. The results of ^1H NMR analysis in CD_3SOCD_3 solution concerning the A–B ring moiety of **2** is given in Fig. 1. Out of 28 carbons of the structure of **2**, 27 carbon resonances were observed in the ^1H noise-decoupled ^{13}C NMR spectrum recorded in CD_3SOCD_3 solution and remaining one carbon, which overlapped solvent signals, could be detected by DEPT (distortionless enhancement by polarization transfer) method. Chemical shifts of all the 4 methyl, 5 methylene, 8 methine, and 11 quaternary carbons were in consistent with the proposed structure as given in the experimental part.

In order to confirm the structure further, chemical tansformation of **2** to the known compound was attempted. Catalytic hydrogenation of **2** in methanol in the presence of palladium-charcoal was found to afford the expected tetrahydro derivative **5**, which was identical in all respects with the known 25-epi-7-deoxy-2,3,5,6,25,27-hexahydrophysalin A.[3,14] Thus, the structure of physalin M (**2**) has been established unequivocally as 7-deoxyphysalin A, namely 25-epi-3,4-dehydro-7-deoxy-2,3,25,27-tetrahydrophysalin A.

Physalins are of interest because of their antitumor activities.[12,13] The new physalin **2**, however, was found to show only very weak cytotoxicity against HeLa cells (IC_{50} 27.6 μg ml^{-1}). The low activity was not unexpected considering the lack of cytotoxicity of the 7-hydroxy analog **1**.[13] Taking into account the potential in vivo and in vitro activity of the double bond isomer (**3**) of **2**, having a double bond at C(2)–C(3) instead of C(3)–C(4),[12] it has been concluded that the conjugated cyclohexenone moiety at the A ring is quite important for the antitumor activity of physalins.

Experimental

Column chromatography and TLC were performed using SiO_2 (Merck, Silicagel 60, 7734) and precoated SiO_2 plates (Merck, Silica Gel 60 F_{254}), respectively. Melting points were determined with a Yanagimoto micro melting point apparatus and uncorrected. Optical rotation and UV spectrum were measured using a JASCO DIP-4 digital polarimeter and HITACHI 124 spectrophotometer, respectively. IR spectrum of **2** was recorded on a JASCO IRA-1 spectrometer with a KBr disk and FT-IR spectra of **5** were recorded on a JEOL JIR-100 spectrometer using a diffuse reflectance accessory. High-resolution MS was measured with a Hitachi M-80 mass spectrometer. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded on a JEOL JNM-GX 400 spectrometer, respectively. Elemental analysis was performed at Elemental Analysis Center of Kyoto University and cytotoxicity test was performed at Pharmaceuticals Laboratory, Life Science Research Sector, Research Center of Mitsubishi Chemical Industries Ltd.

Isolation of Physalin M (2). Water–$CHCl_3$ extract (10 g) of *P. alkekengi* var. *francheti*[13] was subjected to repeated column chromatography with the solvent systems $CHCl_3$–CH_3OH and C_6H_6–$CH_3CO_2C_2H_5$ to give a fraction (350 mg) which showed a single spot (R_f 0.75) on TLC with C_6H_6–$CH_3CO_2C_2H_5$ (3:7), but it showed two spots (R_f 0.70 and 0.58) when developed with $CHCl_3$–CH_3OH (9:1). Column chromatography of the fraction using $CHCl_3$–CH_3OH as eluent afforded physalin B (130 mg) and physalin M (**2**, 140 mg), and the latter was crystallized from 2-propanol to give colorless prisms of **2**: mp 224—227 °C; R_f 0.58 ($CHCl_3$:CH_3OH=9:1); $[\alpha]_D^{24}$ −106° (*c* 0.34, CH_3COCH_3); IR (KBr) 3480, 1775, 1760, 1730, and 1695 cm^{-1}; UV (CH_3OH) 228 nm (ε 11000); ^1H NMR (CD_3SOCD_3) δ=1.04 (6H, d, *J*=6 Hz, 2-propanol), 1.07 (1H, m, H-11β), 1.14 (3H, d, $J_{27,25}$=7 Hz, CH_3-27), 1.18 (3H, s, CH_3-19), 1.27 (3H, s, CH_3-28), 1.42 (1H, dd, $J_{11\alpha,11\beta}$=17 and $J_{11\alpha,12\alpha}$=9 Hz, H-11α), 1.64 (1H, d, $J_{23,23'}$=15 Hz, H-23), 1.76 (3H, s, CH_3-21), 1.86 (1H, dd, $J_{12\beta,12\alpha}$=16 and $J_{12\beta,11\beta}$=6 Hz, H-12β), 2.04 (1H, m, H-8), 2.08 (1H, m, H-23′), 2.09 (1H, m, H-7α), 2.18 (1H, ddd, $J_{12\alpha,12\beta}$=16, $J_{12\alpha,11\beta}$=12, and $J_{12\alpha,11\alpha}$=9 Hz, H-12α), 2.44 (1H, br dd, $J_{7\beta,7\alpha}$=15 and $J_{7\beta,6}$=6 Hz, H-7β), 2.62 (1H, dd, $J_{2\alpha,2\beta}$=20 and $J_{2\alpha,3}$=5 Hz, H-2α), 2.70 (1H, q, $J_{25,27}$=7 Hz, H-25), 2.78 (1H, s, H-16), 2.94 (1H, dd, $J_{9,8}$=11 and $J_{9,11\beta}$=7 Hz, H-9), 3.42 (1H, H-2β, partially overlapping H_2O signal), 3.77 (1H, m, 2-propanol), 4.35 (1H, d, *J*=4 Hz, 2-propanol), 4.48 (1H, d, $J_{22,23'}$=3 Hz, H-22), 5.67 (1H, m, H-3), 5.70 (1H, br d, $J_{6,7\beta}$=6 Hz, H-6), 6.08 (1H, br d, $J_{4,3}$=10 Hz, H-4), 6.20 (1H, s, OH), and 6.47 (1H, s, OH); ^{13}C NMR (CD_3SOCD_3) δ=16.6 and 18.0 (C-27 and C-28), 20.8 (C-19), 24.2 (C-11), 25.4 (2-propanol), 25.5 (C-21), 25.7, 26.1, and 29.0 (C-7, C-12, and C-23), 32.4 (C-9), 34.3 (C-24), 39.5 (C-2), 40.8 and 40.9 (C-8 and C-25), 54.0 (C-16), 55.1 (C-10), 62.0 (2-propanol), 76.4 (C-22), 78.8, 82.0, and 82.3 (C-13, C-17, and C-20), 101.2 (C-14), 122.5, 126.4, and 128.0 (C-3, C-4, and C-6), 140.4 (C-5), 171.7 and 172.2 (C-18 and C-26), 209.6 (C-1), and 215.8 (C-15). Found: C, 63.31; H, 6.84%; *m/z* 512.2031. Calcd for $C_{28}H_{32}O_9 \cdot C_3H_8O \cdot H_2O$: C, 63.04; H, 7.17%. Calcd for $C_{28}H_{32}O_9$: M, 512.2043.

Hydrogenation of Physalin M. Physalin M (9 mg) in CH_3OH (5 ml) was hydrogenated at room temperature and atmospheric pressure over platinum black (2 mg) for 18 h. TLC of the product showed two spots (R_f 0.65 and 0.55, $CHCl_3$:CH_3OH=9:1) and the compound having lower R_f value (3 mg) was isolated by column chromatography using $CHCl_3$ as eluent. Crystallization from CH_3COCH_3 gave colorless prisms: mp>300 °C. The FT-IR spectra of this product and authentic 25-epi-7-deoxy-2,3,5,6,25,27-hexahydrophysalin A (**5**)[3] prepared from physalin A were indistinguishable.

The authors are grateful to Mr. Jun'ichi Ohya of Pharmaceuticals Laboratory, Mitsubishi Chemical Industries Ltd. for the assay of cytotoxic activity and to FT-IR division of Instrument Center, Nagoya Institute of Technology for FT-IR measurement. Thanks are also to Professor Teruo Matsuura of Kyoto University and Professor Ruka Nakashima of Tottori University for helpful discussions.

References

1) T. Matsuura, M. Kawai, R. Nakashima, and Y. Butsugan, *Tetrahedron Lett.*, **1969**, 1083.

2) T. Matsuura and M. Kawai, *Tetrahedron Lett.*, **1969**, 1765.

3) T. Matsuura, M. Kawai, R. Nakashima, and Y. Butsugan, *J. Chem. Soc. C*, **1970**, 664.
4) M. Kawai and T. Matsuura, *Tetrahedron*, **26**, 1743 (1970).
5) S. S. Subramanian and P. D. Sethi, *Curr. Sci.*, **40**, 85 (1971).
6) E. Glotter, I. Kirson, A. Abraham, P. D. Sethi, and S. S. Subramanian, *J. Chem. Soc., Perkin Trans. 1*, **1975**, 1370.
7) R. R. Alluri, R. J. Miller, W. H. Shelver, and S. K. W. Khalil, *Lloydia*, **39**, 405 (1970).
8) L. R. Row, N. S. Sarma, T. Matsuura, and R. Nakashima, *Phytochemistry*, **17**, 1641 (1978).
9) L. R. Row, N. S. Sarma, K. S. Reddy, T. Matsuura, and R. Nakashima, *Phytochemistry*, **17**, 1647 (1978).
10) L. R. Row, K. S. Reddy, N. S. Sarma, T. Matsuura, and R. Nakashima, *Phytochemistry*, **19**, 1175 (1980).
11) K. K. Purushothaman and S. Vasanth, *Ind. J. Chem., Sect. B*, **20B**, 89 (1981).
12) M. D. Antoun, D. Abramson, R. L. Tyson, C. Chang, J. L. McLaughlin, G. Peck, and J. M. Cassady, *J. Nat. Prod.*, **44**, 579 (1981).
13) M. Kawai, T. Matsuura, S. Kyuno, H. Matsuki, M. Takenaka T. Katsuoka, Y. Butsugan, and K. Saito, *Phytochemistry*, **26**, 3313 (1987).
14) The stereochemistry at C(25) of **4** was not given in Ref. 3, but β-methyl configuration was assigned in Ref. 13 considering the chemical shifts of C(27) and C(28) methyl groups.