The Structure of Physalin P, a Neophysalin from *Physalis Alkekengi*

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Synopsis. The structure of physalin P, a constituent of *Physalis alkekengi* var. *francheti*, was determined to be 5α-hydroxy-6,7-didehydro-5,6-dihydroneophysalin B by spectroscopic study and chemical correlation with a known compound.

Physalins are the steroidal constituents of *Physalis* plants (*Solanaceae*) possessing a novel 13,14-seco-16, 24-cycloergostane skeleton. In the course of our study on the constituents of *P. Alkekengi* var. *francheti* (Japanese name: Hôzuki), physalin A,[1] physalin B,[1] physalin C,[2] physalin L,[3] physalin M,[4] physalin N,[5] and physalin O[5] have been isolated and characterized. Further attempts to isolate new physalins from this plant have led to the isolation of a new compound, named physalin P (**1**). In this note the structural determination of **1** will be described.

Repeated silica-gel column chromatography of the extract of the epigeal parts of *P. alkekengi* var. *francheti* yielded the new constituent, physalin P (**1**), mp 272—273°C. The mass spectrum of **1** exhibited a molecular ion peak at m/z 526 and a dehydrated ion peak at m/z 508, and the molecular formula $C_{28}H_{30}O_{10}$ was established by high-resolution mass spectroscopy and elemental analysis. The IR spectrum of **1** showed a hydroxyl band at 3520 cm^{-1} and carbonyl bands at 1770, 1715, and 1690 cm^{-1}. The ^{13}C NMR spectrum of **1** exhibited one ketone carbonyl (δ=202.6) and three lactone carbonyl groups (δ=173.0, 172.7, and 170.3), which demonstrated that **1** does not possess the usual physalin skeleton which commonly contains a tetrahydro-3-furanone, a 2-cyclohexen-1-one, and γ- and δ-lactone moieties. Physalins are known to undergo an acid-induced benzilic acid-type rearrangement reaction yielding products possessing a newly formed skeletal structure named "neophysalin".[6] The skeletal rearrangement from physalins to neophysalins involves a bond cleavage at C(15)–C(16) and a bond formation between C(14) and C(16), converting the tetrahydro-3-furanone *cyclo*[–C(14)–CO(15)–C(16)–C(17)–O–] to a γ-lactone *cyclo*[–C(14)–CO(15)–O–C(17)–C(16)–]. Therefore, the new constituent **1** could be assumed to possess a neophysalin structure (Chart 1). In fact, ^{13}C NMR spectra of **1** indicated the structural similarity between **1** and 4,7-didehydroneophysalin B (**2**), the latter of which had been obtained by refluxing the AcOH solution of physalin A in the presence of $AcONH_4$.[6] The detailed comparison of their ^{13}C NMR spectral data taken in DMSO-d_6 solutions as summarized in Table 1 demonstrated that compound **1** possesses a neophysalin structure which differs from the neophysalin **2** only at the A–B ring moiety.

The ^1H NMR spectra of **1** measured in DMSO-d_6 solution showed signals assignable to the α- and β-protons of the 2-cyclohexen-1-one (δ=5.75, br d, J=10 Hz and δ=6.58, ddd, J=10, 5, and 2 Hz) and another pair of mutually coupled protons on a cis-disubstituted double bond (δ=5.73, d, J=10 Hz and δ=5.96, dd, J=10 and 4.5 Hz). The cis-double bond was reasonably located at C(6)–C(7) in the B ring, and the presence of an OH group at the C(5) position was deduced from the molecular formula and the spin multiplicity of the C(6)

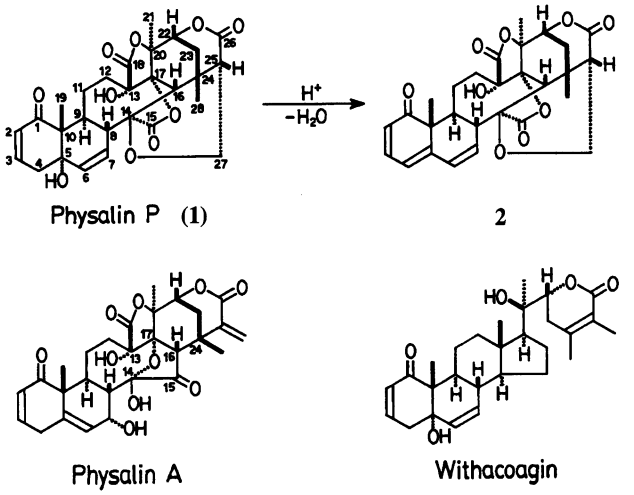

Chart 1.

Table 1. ^{13}C NMR Spectral Data (δ) of Physalin P (**1**) and 4,7-Didehydroneophysalin B (**2**)[a] in DMSO-d_6

	1	2		1	2		1	2
C-1	202.6	204.2	C-11	24.0	22.9	C-21	21.3	20.8
C-2	127.5	123.7	C-12	28.9	29.0	C-22	76.1	75.9
C-3	141.8	139.7	C-13	78.7	78.4	C-23	29.5	29.5
C-4	37.6	116.4	C-14	82.8	81.6	C-24	28.6	28.3
C-5	72.1	152.0	C-15	172.7	170.1	C-25	40.3	40.2
C-6	130.3	126.1	C-16	47.3	47.0	C-26	170.3	169.6
C-7	128.1	130.0	C-17	83.2	82.4	C-27	60.7	60.4
C-8	47.4	47.7	C-18	173.0	172.2	C-28	29.3	29.2
C-9	31.4	35.1	C-19	15.8	21.5			
C-10	53.4	50.8	C-20	81.8	81.5			

a) Ref. 6.

proton (δ=5.73). The stereochemistry of the OH group was determined by the CD spectrum of **1** which showed a negative Cotton effect ([θ] −6200) at 332 nm. The 5α- and 5β-steroids, respectively, with the 2-en-1-one system, are known to exhibit negative and positive Cotton effects due to the n–π^* transition reflecting negative and positive helicity of the transoid enone moiety.[7] Accordingly, the α-configuration was assigned to the OH group at the C(5) position. The close similarity of the ^{1}H NMR spectral data due to the A–B ring moiety of **1** to those of withacoagin (**3**), isolated from *Withania coagulans*,[8] also supported the 5α-hydroxy-2,6-dien-1-one structure.

Acid-induced dehydration of **1** afforded a conjugated trienone, which was characterized as the known 4,7-didehydroneophysalin B (**2**). Consequently, the structure of physalin P (**1**) has been established unequivocally as 5α-hydroxy-6,7-didehydro-5,6-dihydroneophysalin B. It is interesting that the compound possessing a neophysalin skeleton, which is known as an acid-induced rearrangement product of a physalin skeleton, has been found as a constituent of the plant.[9]

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 points apparatus and are uncorrected. IR and UV spectra were measured using JASCO A-102 and Hitachi 124 spectrophotometers, respectively. Optical rotation and CD spectra were recorded on a JASCO DIP-4 digital polarimeter and a J-600 spectropolarimeter, respectively. Mass spectra were measured on a Hitachi M-2000 spectrometer with electron impact ionization. NMR spectra were taken on a JEOL JNM GSX-400 spectrometer at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR.

Isolation of Physalin P (1). Epigeal parts of *P. alkekengi* var. *francheti* were boiled in hot water and the water layer was extracted with $CHCl_3$. The $CHCl_3$ extract was crystallized from acetone to yield physalin A and physalin B,[1] and the mother liquor was subjected to SiO_2 column chromatography using $CHCl_3$–MeOH and C_6H_6–AcOEt as eluents. Fractions containing the new compound **1** were collected and crystallized from MeOH and then from C_6H_6–AcOEt to give physalin P (**1**) as colorless fine needles, mp 272—273°C (yield, ca. 0.001% based on the fresh plant); R_f 0.39 ($CHCl_3$: MeOH=9 : 1), 0.40 (C_6H_6 : AcOEt=3 : 7), 0.48 (CH_2Cl_2 : Et_2O=3 : 1); $[\alpha]_D^{24}$ +54° (*c* 0.25, acetone); IR ν^{KBr} 3520, 1770, 1715, 1690, 1220, 1185, 1105, 1075, 1050, 1030 cm^{-1}; MS (EI) *m/z* 526 (M^+), 508 ($M-H_2O$); HRMS Found: *m/z* 508.1746. Calcd for $C_{28}H_{28}O_9$: $M-H_2O$, 508.1731. UV (MeOH) 225 nm (ε 6000); CD (MeOH) $[\theta]_{332}$ −6200, $[\theta]_{238}$ +12500; ^{1}H NMR (DMSO-d_6) δ=0.98 (3H, s, CH_3−19), 1.37 (3H, s, CH_3−28), 1.48 (1H, m, H-11α), 1.69 (3H, s, CH_3-21), 1.89 (1H, br d, *J*=15 Hz, H-23*S*), 2.04 (1H, dd, *J*=15 and 4.5 Hz, H-23*R*), 2.13 (1H, m, H-9), 2.26 (2H, m, CH_2-12), 2.37 (1H, dd, *J*=19.5 and 5 Hz, H-4α), 2.4 (1H, m, H-11β), 2.59 (1H, br d, *J*=19.5 Hz, H-4β), 2.82 (1H, m, H-8), 2.96 (1H, dd, *J*=12 and 2.5 Hz, H-25), 3.01 (1H, s, H-16), 4.06 (1H, dd, *J*=12 and 2.5 Hz, H-27*S*), 4.24 (1H, t, *J*=12 Hz, H-27*R*), 4.62 (1H, m, H-22), 5.73 (1H, d, *J*=10 Hz, H-6), 5.75 (1H, br d, *J*=10 Hz, H-2), 5.96 (1H, dd, *J*=10 and 4.5 Hz, H-7), 6.54 (1H, s, HO-13), 6.58 (1H, ddd, *J*=10, 5, and 2 Hz, H-3). Found: C, 58.99; H, 5.93%. Calcd for $C_{28}H_{30}O_{10}\cdot\frac{5}{2}H_2O$: C, 58.84; H, 6.17%.

Acid-Induced Dehydration of Physalin P (1) to 4, 7-Didehydroneophysalin B (2). A solution of **1** (8 mg) in AcOH (5 ml) was refluxed for 2.5 h. The AcOH was evaporated in vacuo and the yellow residue showed one spot on TLC (R_f 0.52, $CHCl_3$: MeOH=9 : 1). Without further purification the product was dissolved in DMSO-d_6 to measure the ^{1}H NMR spectrum. The product was indistinguishable from an authentic sample of **2** by the comparison of their TLC and the ^{1}H NMR spectra.

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9) The neophysalin **2** had been isolated from the root of *P. alkekengi* var. *francheti* as described at page 667 (as a footnote) in Ref. 1, where the structure of **2** had been erroneously assumed as an 8-epimer of 4,7-didehydrophysalin B.