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UPON 'PHYSALIN L' ISOLATED FROM *PHYSALIS MINIMA*

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Key Word Index—*Physalis minima*; Solanaceae; artefact; 16,24-cyclo-13,14-secosteroid; physalin.

Abstract—5α-Ethoxy-6β-hydroxy- and 6β-ethoxy-5α-hydroxy-5,6-dihydrophysalin B were derived from the corresponding 5β,6β- and 5α,6α-epoxides, respectively, by acid treatment in ethanol. The 5α-ethoxy derivative was isolated as an artefact from *Physalis alkekengi*, while other workers isolated the 6β-ethoxy compound as a constituent of *P. minima* and mistakenly named it as physalin L, which had already been assigned to (25*S*)-3,4-didehydro-2,3,25,27-tetrahydrophysalin A. The ^{1}H NMR spectrum of the 6β-ethoxy derivative prepared by us, however, did not agree with the reported data. Copyright © 1996 Elsevier Science Ltd

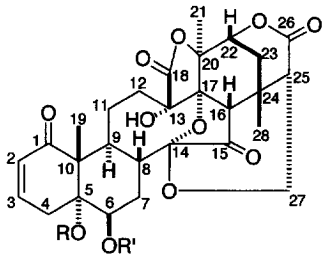
INTRODUCTION

Physalins are 16,24-cyclo-13,14-secosteroidal constituents of *Physalis* plants. In the course of our study on the constituents of *P. alkekengi* var. *francheti* (Japanese name: Hôzuki), physalins A–C and L–S were isolated and their structures determined unambiguously [1–3]. Row *et al.* [4, 5] described the isolation of physalins D–K from *P. angulata* and *P. lancifolia*, although some of the reported structures were found incorrect and had to be revised [6]. Recently, Sen and Pathak [7] reported the isolation from *P. minima* of a new compound named 'physalin L' possessing the structure 6β-ethoxy-5α-hydroxy-5,6-dihydrophysalin B (**1**). However, the name 'physalin L' had been assigned by us previously to (25*S*)-3,4-didehydro-2,3,25,27-tetrahydrophysalin A isolated from *P. alkekengi* var. *francheti* [2]. Therefore, we claim that compound **1** should not be called physalin L. We obtained a regioisomer of **1**, namely, 5α-ethoxy-6β-hydroxy-5,6-dihydrophysalin B (**2**), as an artefact during the isolation of physalins from *P. alkekengi* var. *francheti*. In this report we describe the compounds **1** and **2**.

RESULTS AND DISCUSSION

From the chloroform extracts of epigeal parts of *P. alkekengi* var. *francheti*, a new compound, **2** ($C_{30}H_{36}O_{11}$), was isolated in 0.014% yield along with the known components, physalins A–C, F, and N. The ^{1}H NMR spectrum of **2** taken in DMSO-d_6 solution indicated the presence of an ethoxyl group (δ 3.03 and 3.22 for OCH_2 and 0.90 for CH_3) in addition to three tertiary methyls (δ 1.15, 1.16 and 1.81), a conjugated enone (δ 5.72 and 5.64) and an OCH_2CH group (δ 3.59 and 4.27 for CH_2 and 2.88 for CH) commonly found in physalin B and the related physalins. The presence of an axially oriented secondary hydroxyl (δ 4.94, *d*, J = 3 Hz for OH and 3.82, *br s*, for CH), along with a tertiary hydroxyl group (δ 5.54) and absence of alkenic protons other than those of the enone group, suggested the 5α-ethoxy-6β-hydroxy-2-en-1-one structure as the AB-ring moiety of **2**. The 5α-configuration was based on the negative Cotton effect ($[\theta]_{333}$ −3000) indicating *trans*-juncture of the A/B-rings. In fact, the IR and ^{1}H NMR spectra of **2** resembled those of the corresponding 5α-methoxyl compound, namely, physalin I (**3**) [5]. Conversion of 5β,6β-epoxy-5,6-dihydrophysalin B (physalin F, **4**) [4] into **2** by HCl–ethanol treatment unambiguously established the structure of **2** as 5α-ethoxy-6β-hydroxy-5,6-dihydrophysalin B. Since chloroform, which we used as the solvent for extraction, contained ethanol (0.5%) as a stabilizer, it is reasonable to assume that **2** is an artefact formed from the epoxide **4** during extraction and/or isolation procedures. When acetone was used instead of chloroform to extract physalins, compound **2** was not detected in the extracts, but an acetone adduct of physalin C, namely 27-acetonyl-25,27-dihydrophysalin C, mp 191–193°, $[\alpha]_D^{25}$ −136° (*c* 0.1, acetone), was isolated in 0.007% yield.

Since the structure **1** described by Sen and Pathak [7] as 'physalin L' is a regioisomer of **2**, the ^{1}H NMR spectra of these compounds are expected to resemble each other. The reported chemical shifts of the CH_2 protons at the C-27 position (δ 3.75 and 3.99), however, differ significantly from those of **2**, physalin B, and other related physalins (δ 3.57–3.63 and 4.25–4.29). The corresponding methoxyl compound, 5α-hydroxy-6β-methoxy-5,6-dihydrophysalin B (**5**), prepared from the 5α,6α-epoxide (physalin J, **6**) [4], also



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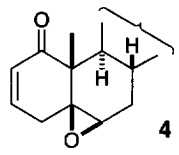
2 R = Et R' = H
3 R = Me R' = H
5 R = H R' = Me



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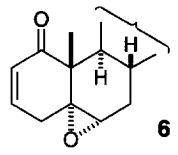

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exhibited reasonable spectra (δ 3.58 and 4.26, CH_2-27). The compound possessing structure **1** was then synthesized by H_2SO_4–ethanol treatment of **6**. As expected the ^1H NMR spectrum of synthetic **1** (δ 3.58 and 4.25, CH_2-27) was closely similar to that of **5** and therefore markedly different from the data reported by Sen and Pathak [7]. However, for the 2,3-dihydro derivative of **1**, the spectrum reported by them was comparable to that of our synthetic sample.

EXPERIMENTAL

Mps are uncorr. IR spectra were taken with KBr discs, UV and CD spectra were obtained in MeOH solns, NMR spectra were measured in DMSO-d_6 solns at 400 or 200 MHz for ^1H and 100 MHz for ^{13}C.

Isolation of 5α-ethoxy-6β-hydroxy-5,6-dihydrophysalin B (**2**). Air-dried epigeal parts (1.33 kg) of *P. alkekengi* var. *francheti* cultivated in Japan were defatted with hexane and kept in $CHCl_3$ under dark at room temp. for 1.5 months. The $CHCl_3$ extract was subjected to repeated silica gel CC using $CHCl_3$–MeOH and benzene–AcOEt as eluents. In addition to physalins B (1.42 g), C (28 mg) and F (**4**) (2.46 g) and a mixt. of physalins A, N and O, the new compound **2** (83 mg) was isolated as needles from MeOH, mp 224–226°; $[\alpha]_D^{25}$ −88° (Me_2CO; *c* 0.15). IR ν_{max} cm^{-1}: 3420, 1790, 1770, 1745, 1670, 1175, 1085, 1065. UV λ_{max}: 222 nm (log ε = 3.89). CD [θ]: −3000 (333 nm), −11500 (240 nm). ^1H NMR: δ 0.90 (*t*, *J* = 7 Hz, $C\underline{H}_3CH_2O$), 0.97 (*m*, H-11β), 1.15 (*s*, H_3-19), 1.16 (*s*, H_3-28), 1.48 (*dd*, *J* = 17, 10 Hz, H-12β), 1.66 (*td*, *J* = 12, 3 Hz, H-7α), 1.80 (*m*, H-11α), 1.81 (*s*, H_3-21), 1.83 (*m*, H-7β), 1.92 (*dd*, *J* = 14, 2 Hz, H-23*S*), 2.10 (*m*, H-12α), 2.10 (*dd*, *J* = 14, 4 Hz, H-23*R*), 2.18 (*td*, *J* = 12, 3 Hz, H-8), 2.34 (*dd*, *J* = 21, 5 Hz, H-4α), 2.78 (*s*, H-16), 2.88 (*d*, *J* = 4 Hz, H-25), 2.94 (*br d*, *J* = 21 Hz, H-4β), 3.03 (*quint*, *J* = 7 Hz, OC$\underline{H}$H'Me), 3.22 (*quint*, *J* = 7 Hz, OCH$\underline{H}$'Me), 3.27 (*m*, H-9), 3.59 (*d*, *J* = 13 Hz, H-27*R*), 3.82 (*br s*, H-6α), 4.27 (*dd*, *J* = 13, 4 Hz, H-27*S*), 4.56 (*dd*, *J* = 4, 2 Hz, H-22), 4.94 (*d*, *J* = 3 Hz, HO-6), 5.54 (*s*, HO-13), 5.72 (*dd*, *J* = 10, 3 Hz, H-2), 6.64 (*ddd*, *J* = 10, 5, 2 Hz, H-3). ^{13}C NMR: δ 13.5 (C-19), 15.5 ($C\underline{H}_3CH_2O$), 21.1 (C-21), 24.5 (C-28), 24.6 (C-11), 25.7 (C-12), 26.9 (C-7), 27.7 (C-4), 29.7 (C-9), 30.5 (C-24), 31.3 (C-23), 38.0 (C-8), 49.4 (C-25), 54.1 (C-16 or 10), 54.2 (C-10 or 16), 56.3 ($O\underline{C}H_2Me$), 60.5 (C-27), 66.7 (C-6), 76.3 (C-22), 78.8 (C-13), 80.4 (C-20 or 17), 80.5 (C-17 or 20), 81.3 (C-5), 106.8 (C-14), 127.6 (C-2), 141.7 (C-3), 167.3 (C-26), 171.8 (C-18), 204.5 (C-1), 209.8 (C-15). HR-EIMS *m/z*: 572.2299 $[M]^+$ ($C_{30}H_{36}O_{11}$ requires 572.2258).

Synthesis of **2** *from physalin F* (**4**). A mixt. of **4** (162 mg), conc. HCl (1 ml) and EtOH (25 ml) was stirred at room temp. for 40 min. Usual work-up and silica gel CC, using $CHCl_3$–MeOH, yielded **2** (81 mg, 46%), which was indistinguishable from that obtained from the plant as described above.

6β-Ethoxy-5α-hydroxy-5,6-dihydrophysalin B (**1**). This compound was prepd from **6** as described above for the synthesis of **2** from **4** using EtOH and H_2SO_4. Needles from EtOH, mp 171–175°; $[\alpha]_D^{20}$ −86° (Me_2CO; *c* 0.16). IR ν_{max} cm^{-1}: 3430, 1780, 1760, 1735, 1665, 1170, 1135, 1100, 1080, 1065. UV λ_{max}: 224.5 nm (log ε = 3.85). CD [θ]: −5700 (333), −7600 (237), +5300 (212 nm). ^1H NMR: δ *ca* 0.96 (*m*, 11β), 1.06 (*s*, H_3-19), 1.12 (*t*, *J* = 7 Hz, $C\underline{H}_3CH_2O$), 1.16 (*s*, H_3-28), 1.44 (*br dd*, *J* = 16, 9 Hz, H-12β), 1.81 (*s*, H_3-21), 2.80 (*s*, H-16), 2.87 (*d*, *J* = 4 Hz, H-25), 3.06 (*br d*, *J* = 20 Hz, H-4β), 3.15 (*dd*, *J* = 10.5, 7.5 Hz, H-9), 3.20 (*br s*, H-6α), 3.27 (*dq*, *J* = 9.5, 7 Hz, OC$\underline{H}$H'Me), 3.55 (*dq*, *J* = 9.5, 7 Hz, OCH$\underline{H}$'Me), 3.58 (*d*, *J* = 13.5 Hz, H-27*R*), 4.25 (*dd*, *J* = 13.5, 4 Hz, H-27*S*), 4.57 (*br s*, H-22), 4.43 (*s*, HO-5), 5.70 (*dd*, *J* = 10, 2 Hz, H-2), 5.83 (*s*, HO-13), 6.62 (*ddd*, *J* = 10, 4.5, 1.5 Hz, H-3). HR-EIMS *m/z*: 572.2246 $[M]^+$ ($C_{30}H_{36}O_{11}$ requires 572.2258).

6β-Ethoxy-5α-hydroxy-2,3,5,6-tetrahydrophysalin B. This compound was prepd in 92% yield by catalytic hydrogenation of **1** (50 mg) in THF (5 ml) with atmospheric H_2 over Pd–C (25 mg). Needles from MeOH–Me_2CO, mp 193–197°; $[\alpha]_D^{20}$ −70° (Me_2CO; *c* 0.14). IR ν_{max} cm^{-1}: 3420, 2980, 2945, 2880, 1780, 1750, 1685, 1170, 1135, 1090, 1065. ^1H NMR: δ 1.11 (*t*,

$J = 7$ Hz, $C\underline{H}_3CH_2O$), 1.14 (*s*, H_3-19), 1.14 (*s*, H_3-28), 1.80 (*s*, H_3-21), 2.10 (*dd*, $J = 14.5$, 3.5 Hz, H-23*R*), 2.81 (*s*, H-16), 2.88 (*d*, $J = 4$ Hz, H-25), 3.10 (*dd*, $J = 11$, 8 Hz, H-9), 3.17 (*br s*, H-6α), 3.27 (*dq*, $J = 9.5$, 7 Hz, OC$\underline{H}$H′Me), 3.58 (*dq*, $J = 9.5$, 7 Hz, OCH$\underline{H}$′Me), 3.58 (*d*, $J = 13.5$ Hz, H-27*R*), 4.24 (*dd*, $J = 13.5$, 4 Hz, H-27*S*), 4.28 (*s*, HO-5), 4.58 (*br s*, H-22), 5.65 (*s*, HO-13). HR-EIMS *m/z*: 574.2472 $[M]^+$ ($C_{30}H_{38}O_{11}$ requires 574.2414).

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