Pergamon

0031-9422(94)00736-5

Phytochemistry, Vol. 39, No. 5, pp. 1245–1246, 1995

0031-9422/95 $9.50 + 0.00

PHYSALIN L, A 13,14-SECO-16,24 CYCLOSTEROID FROM *PHYSALIS MINIMA*

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(*Received in revised form* 12 *August* 1994)

Key Word Index—*Physalis minima*; Solanaceae; 13,14-seco-16,24-cyclosteroid.

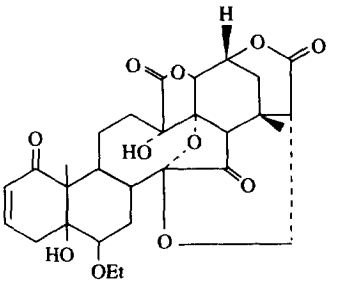
Abstract—A new 13,14-seco-16,24-cyclosteroid, physalin L, has been isolated, along with known compounds, from *Physalis minima*. The known compounds were physalin B, epoxyphysalin B and physalin D. The structure elucidation of physalin L showed it to be 6β-ethoxy-5α,13,20,22-tetrahydroxy-1,15-dioxo-16,24-cyclo-13,14-seco-ergost-2-ene 18,24-dioic acid, 18-20,27-22 dilactone.

INTRODUCTION

Physalis alkekengi varfranchatti has been reported to contain 13,14-seco-16,24-cyclosteroids [1]. *Physalis angulata* showed the presence of physalins B, E, F, H and I [2]. *Physalis minima* has been subjected to detailed chemical examination and reported to contain physalin B [1], 5,6β-epoxyphysalin B [2], withaphysalin A [3] and withaphysalin B [4, 5]. Physalin D [6] was isolated from this plant by Mulchandani *et al.* [3]. The plant has been used in the indigenous system of medicine and the abortifacient activity of physalins has been studied previously [6]. This paper presents the structural elucidation of a hitherto unreported physalin L (**1**) as a minor product from *Physalis minima*.

RESULTS AND DISCUSSION

The IR spectrum of physaslin L revealed the presence of hydroxyl 3440 cm^{-1}, γ-lactone (1780 cm^{-1}), five-membered ring ketone (1760 cm^{-1}), δ-lactone (1740 cm^{-1}) and cyclohexanone functions (1660 cm^{-1}). The compound showed the presence of an α,β-unsaturated ketone by its UV absorption at 226 nm. The NMR spectrum of the compound displayed the presence of three methyls at δ 1.12 (C-19, *s*, 3H), 1.13 (C-28, *s*, 3H) and 2.0 (C-21, *s*, 3H). Only two olefinic protons could be detected at δ5.8 (C-2, *d*, J = 11 Hz). The latter two signals disappeared in the dihydro derivatives. The signals for exo-methylene protons could not be detected. However, it gave signals at δ3.75 (*d*, J = 14 Hz) and 3.99 (*dd*, J = 14, 4 Hz) which are characteristics of the $-O-CH_2-CH-$ system in the molecule. Therefore, the structure of physalin L resembled



Physalin L (**I**)

physalin B rather than physalin A. The absence of an ABX pattern in the NMR spectrum of physalin L, such as that in the spectrum of withaferin A, ruled out the possibility of a hydroxyl at C-4.

The molecular formula of physalin L was $C_{30}H_{36}O_{11}$ (M_r 572) and could not be a withaphysalin. All known physalins (A–K) have M_rs ranging from 510 to 556. This particular compound showed that its molecular formula has (C_2H_4)- atoms more than physalin D. The mass spectral fragmentation pattern is similar to the physalin B and D type, since it showed prominent peaks at m/z 109 and 125. Hence the basic structural unit is of the physalin B or D type. The mass spectrum showed fragmentation for the loss of carbonyl which is typical for an α,β-unsaturated ketone. Hence an unsaturated carbonyl unit was present in the ring A of the physalin.

On hydrogenation with palladium over charcoal one molecule of hydrogen was added which is consistent with one double bond. Hence its structure was concluded to be more like physalin D and not like physalin A. Physalin A has an exocyclic methylene group at C-25 owing to which it takes up two molecules of hydrogen. The reaction with Jones reagent showed that it lost a molecule of ethanol readily, indicating that hydroxyl and ethoxy groups may be adjacent to each other.

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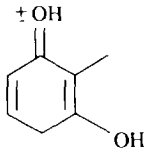

II

The signal for H-6 in the ^{1}H NMR spectrum was similar to that for physalin D and physalin I at δ 3.84 (*m*). Physalin D contained one secondary hydroxyl, since it gave a mono-acetyl derivative. Physalin L did not give any acetyl derivative indicating the absence of such a hydroxyl. Thus the hydroxyl group must be present at C-5 or C-7.

The mass spectrum resembled that physalin D. It gave a peak at *m/z* 125 owing to structural unit (**II**). This showed the presence of a hydroxyl at C-5. The position of the ethoxyl group at C-6 was ascertained through synthesis. α-Epoxy physalin B isolated from the same plant upon hydrolysis with methanol yielded a compound which possessed all the physical and chemical properties identical with those of physalin L. This conclusively proved the structure of physalin L to be 5α-hydroxy,6β-ethoxy-physalin B (**1**).

EXPERIMENTAL

Spectroscopy. IR spectra recorded as KBr pellets on a Perkin–Elmer model 137. NMR spectra were run in DMSO-d_6 with TMS as internal standard using a model A-60A spectrometer. MS analysis were carried out at the National Chemical Laboratory, Poona on model CEC 21-110B.

Chromatography. TLC was performed on silica gel G (Acme, India) using $CHCl_3$–Me_2CO (3:1) as solvent system for the separation of physalins and their derivatives. The spots were detected by spraying the plates with H_2SO_4 (10%) and heating at 100°.

Isolation of physalin B, 5,6β-epoxyphysalin B, physalin L and physalin D. Whole fresh plants of *P. minima* (5 kg) were macerated in a blender using water (3 l) and boiled over a heating mantle (24 hr). Three successive water extracts (7 l) were obtained, combined and concd (3.5 l) under red. pres. The aq. phase was then extracted with $CHCl_3$ (8 × 500 ml). The combined $CHCl_3$ extracts were dried (Na_2SO_4) and concd under red. pres. to yield a amorphous yellow solid (1.4 g). This was chromatographed over silica gel. Physalin B (220 mg) was first eluted, followed by 5,6β-epoxyphysalin B (60 mg) by using $CHCl_3$–Me_2CO (9:1) as solvent system. Physalin L (40 mg) was obtained using $CHCl_3$–Me_2CO (7:3) as solvent system. Lastly physalin D was eluted.

The identity of physalin B, 5,6β-epoxyphysalin B and physalin D was established by comparison of the physical and spectral properties reported in the literature.

Physalin L. Mp 265–266°, $[\alpha]_D^{25}$ − 43° (MeOH: *c* 1.0), $[M]^+$ at *m/z* 572. (Found: C, 63.0; H, 6.84; $C_{30}H_{36}O_{11}$ requires C, 62.93; H, 6.93%). UV λ_{max}^{MeOH} nm: 226 (log ε 3.66). IR ν_{max}^{KBr} cm^{-1}: 3440, 2920, 1780, 1760, 1740, 1660, 1380, 1060. ^{1}H NMR (DMSO-d_6): δ 1.12 (*s*, 3H), 1.3 (*s*, 3H), 1.7 (*s*, 3H), 3.75 (*d*, *J* = 14 Hz), 3.99 (*m*), 5.87 (*t*, *J* = 7 Hz), 6.97 (*q*, *J* = 7 Hz), 5.8 (*d*, *J* = 10 Hz), 6.7 (*dt*, *J* = 10, 3 Hz), 4.5 (*s*, OH).

Preparation of dihydrophysalin L. Physalin L (50 mg) was dissolved in EtOH and hydrogenated at room temp. under 20 lbs pressure using a PAAR hydrogenation apparatus and Pd over charcoal (50 mg) as catalyst. The crude compound obtained after evaporation of the solvent under red. pres. was purified by CC over silica gel. The pure compound was eluted with $CHCl_3$–Me_2CO (3:2) as solvent system. The compound (100 mg) was identified by mp 300° (crystallized from EtOAc); $[\alpha]_D^{25}$ − 45° (MeOH; *c* 1.0), $[M]^+$ at *m/z* 574, calculated for $C_{30}H_{38}O_{11}$. UV λ_{max}^{MeOH}: nil, IR ν_{max}^{KBr} cm^{-1}: 3480, 2920, 1780, 1760, 1740, 1680, 1380. ^{1}H NMR (DMSO-d_6): δ 1.2 (*s*,6H), 1.9 (3H), 3.58 (*d*, *J* = 14 Hz), 4.23(*dd*, *J* = 14, 4 Hz).

Preparation of dehydrophysalin L. Physalin L (50 mg) dissolved in pyridine (2 ml) with CrO_3 (10 mg) was left overnight at room temp. The excess reagent was destroyed by slow addition of crushed ice. The pptd compound was filtered off and purified by CC and crystallization. The compound (20 mg) showed mp 295–296°, $[\alpha]_D^{25}$ − 87.3° (MeOH; *c* 1.03), $[M]^+$ at *m/z* 526. UV λ_{max}^{MeOH} nm: 228 (log ε 3.4). IR ν_{max}^{KBr} cm^{-1}: 3506, 3218, 1779, 1748, 1689, 1250. ^{1}H NMR (DMSO-d_6): δ 1.21 (*s*, 3H), 1.25 (*s*, 3H), 1.31 (*s*, 3H), 3.56 (*d*, *J* = 14 Hz), 4.41 (*dd*, *J* = 14, 4 Hz), 5.85 (*dt*, *J* = 10, 3 Hz) and 6.85 (*d*, *J* = 10 Hz).

Hydrolysis of 5,6β-epoxyphysalin B. A soln of 5,6β-epoxyphysalin (10 mg) in EtOH was heated on a water bath overnight. The product was obtained by extracting the mixture with $CHCl_3$ (3 × 10 ml). The combined extract after drying over Na_2SO_4 and evapg *in vacuo*, yielded a solid. This was crystallized from EtOAc and was identical with physalin L in all respects.

Acknowledgements—Thanks are due to Dr L. P. Badheka and Dr S. A. Hassrajani, Bhabha Atomic Research Centre, Bombay for the reference samples of physalin A, B, β-epoxy and D.

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