

A NEW PHYSALIN FROM *PHYSALIS ALKEKENGI*: STRUCTURE OF PHYSALIN L

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(Received 24 February 1987)

Key Word Index—*Physalis alkekengi*; Solanaceae; structure determination; 13,14-*seco*-16,24-cyclosteroid; physalin L.

Abstract—A new physalin, physalin L, and the known physalins E and F were isolated from *Physalis alkekengi*. Spectroscopic studies and chemical transformation have established the structure of physalin L as 25-*epi*-3,4-dehydro-2,3,25,27-tetrahydrophysalin A.

INTRODUCTION

Physalis species are known to contain 13,14-*seco*-16,24-cyclosteroids called physalins. In 1969, Matsuura *et al.* reported the isolation and structural determination of physalin A (1) as a bitter principle of *Physalis alkekengi* var. *francheti* (Japanese name; Hôzuki) [1]. The structures of physalins B (2) and C (3), isolated from the same plant, were also determined [2, 3]. Row *et al.* isolated 2 and physalins D–K, possessing structures similar to that of 2, from *P. angulata* and *P. lancifolia* grown in India [4–6]. *P. minima* was shown to contain 1–3, dihydrophysalin B, 5 β ,6 β -epoxyphysalin B (physalin F), and 6,7-dihydro-6-hydroxydehydrophysalin B [7–10]. Physalin A (1) was also found in *P. peruviana* [7]. Antoun *et al.* isolated two cytotoxic substances against tumour cells from *Witheringia coccinloboides* (Solanaceae) and found them to be the known physalin B (2) and a new physalin, 25-*epi*-25,27-dihydrophysalin C (4) [11]. Physalin B also demonstrated *in vivo* activity against mouse leukaemia. The finding of physalins as potential antitumour agents prompted a further study upon the constituents of *P. alkekengi* var. *francheti*.

RESULTS AND DISCUSSION

Physalins E and F, known as constituents of Indian *Physalis* species, along with physalins A (1) and B (2) were isolated from a chloroform extract of *P. alkekengi* var. *francheti*. A new constituents having a slightly higher R_f value than 1 in silica gel TLC was isolated and was named physalin L (5).

Physalin L (5), $C_{28}H_{32}O_{10}$, showed a molecular ion peak at m/z 528. Its IR spectrum exhibited three bands (1770, 1740, and 1720 cm^{-1}) in the carbonyl region and a band at 1770 cm^{-1} was assigned to the γ -lactone function found in all known physalins. Out of 28 carbons in the molecular formula, 26 carbon resonances (4 methyls, 3 methylenes, 8 methines, and 11 quaternary carbons including 4 carbonyls) were observed in the ^{13}C NMR

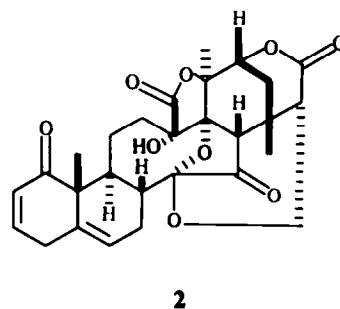
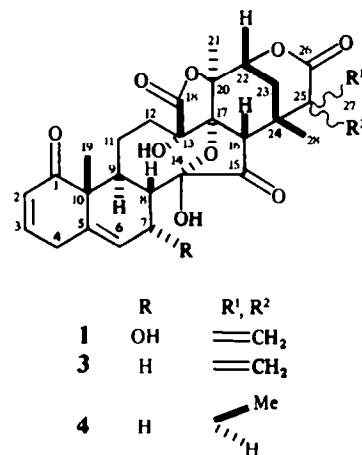


Fig. 1. Structures of physalins A (1), B (2), and C (3) and 25-*epi*dihydrophysalin C (4). Structures of physalins D–K differ from that of 2 at positions C-4, C-5, C-6, and/or C-7, having a double bond, an epoxide, a methoxy, and/or one or two hydroxyl function(s).

spectra of **5** and, as described later, 21 of them corresponded closely to those observed for the known physalin A (**1**), suggesting that **5** had a physalin type carbon skeleton. The 200 MHz ^1H NMR spectra of **5** taken in $\text{DMSO}-d_6$ solution indicated the presence of three *tert*-methyl groups (δ 1.16, 1.32, and 1.71), one *sec*-methyl group (δ 1.17, d , $J = 8$ Hz), two *tert*-hydroxyl groups (δ 5.52 and 6.85), one *sec*-hydroxyl group (δ 5.07, d , $J = 4$ Hz), three olefinic hydrogens (δ 5.76, d , $J = 5$ Hz; 5.88, br d , $J = 10$ Hz; and 6.14, d , $J = 10$ Hz), and two methine groups bearing an oxygen atom (δ 4.6, m , 2H). Assuming close correspondence between the structure of **5** and those of the known physalins (Fig. 1), the three tertiary methyl groups in **5** were assigned to C-19, C-28, and C-21. In physalins A (**1**) and C (**3**), C-27 carries an exocyclic double bond conjugated with the δ -lactone carbonyl and the terminal methylene protons resonate at $\delta \sim 5.6$ and ~ 6.4 as broad singlets [2, 3], while a pair of signals ($\delta \sim 3.6$ and ~ 4.3) exhibiting a geminal coupling ($J \approx 14$ Hz) are observed for physalin B (**2**) and other physalins having a C(27)-methylene connected to the oxygen atom on C-7 [2, 4–6]. From the absence of proton signals assignable to a terminal methylene or a $-\text{CH}_2\text{O}-$ group in the NMR spectrum of **5**, the secondary methyl group was assumed to be located at C-27 as is the case for the cytotoxic physalin (**4**) from *Witheringia* species [11].

The oxygen functions common to all the physalins are those of a γ - and a δ -lactone, two ketones at C-1 and C-15, a hydroxyl at C-13, an ether group C(14)–O–C(17), and an oxygen function at C-14, which is either a hydroxyl as in **1** and **3** (i.e. a hemiketal) or an ether oxygen connected to C-27 as in **2** and other physalins (i.e. a ketal). As **5** had a C-27 methyl group, the oxygen function at C-14 had to be a hydroxyl. This accounted for nine of the ten oxygen atoms. The remaining oxygen atom in **5** was accounted for by the presence of a secondary hydroxyl group indicated by the ^1H NMR spectrum. All the known physalins invariably possess a double bond at C(2)–C(3) constituting a conjugated enone system. The presence of such an α,β -unsaturated carbonyl group in **5**, however, was excluded by the chemical shifts of the olefinic proton signals (δ 5.76–6.14) in the NMR spectrum of **5** and also by the carbonyl absorption band (1720 cm^{-1} or higher) in the IR spectrum. The UV spectrum of **5** showed an absorption maximum at 233 nm ($\log \epsilon$ 3.99) indicating the presence of a conjugated system, which was not unreasonably assumed to be a conjugated diene composed of a *cis*-disubstituted double bond (δ 5.88 and 6.14, $J = 10$ Hz) and a trisubstituted double bond (δ 5.76, d , $J = 5$ Hz). Such a diene system could be located in the physalin skeleton either at C(3)H=C(4)H–C(5)=C(6)H or at C(4)H=C(5)–C(6)H=C(7)H. The doublet signal due to the olefinic proton at the trisubstituted double bond suggested the presence of an adjacent methine rather than a methylene group, which enabled us to locate the secondary hydroxyl group at the allylic position. These data showed that physalin L (**5**) had to have one of the two possible structures shown in Fig. 2.

Physalin A (**1**) was known to undergo dehydration on acid treatment to afford the conjugated trienone compounds, anhydrophysalin A (**6**) and dehydrophysalin B (**2**). It seemed reasonable to expect similar dehydration from either of the possible structures of **5** given in Fig. 2. A solution of **5** and *p*-toluenesulphonic acid in acetic acid was refluxed to give a light yellow product (**7**) ($\text{C}_{28}\text{H}_{28}\text{O}_9$, m/z 310 $[\text{M}]^+$), which was shown to possess the expected

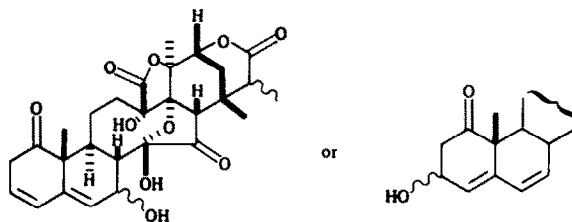
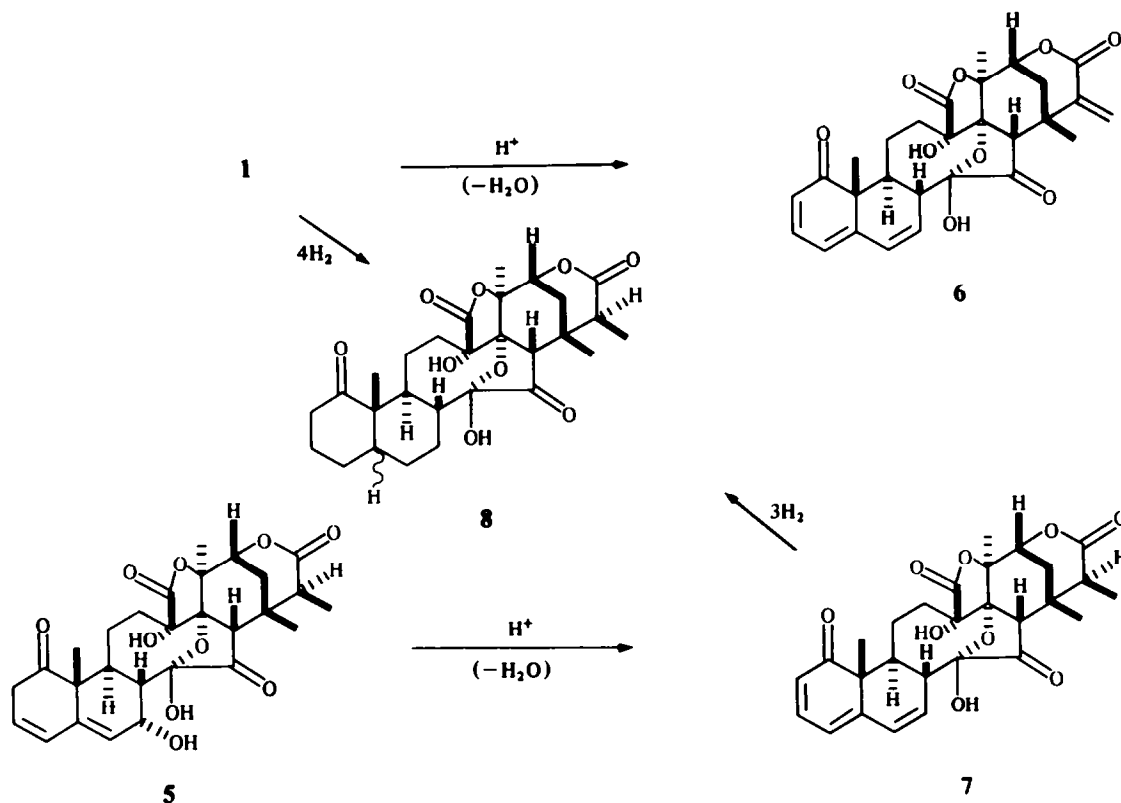


Fig. 2. Possible structures of physalin L (**5**).

trienone structure similar to that of **6** by UV (λ_{max} 347 nm, $\log \epsilon$ 3.81) and ^1H NMR spectra (δ $\text{DMSO}-d_6$ 5.87, d , $J = 10$ Hz; 7.13, dd , $J = 10$, 6 Hz; 6.09, d , $J = 6$ Hz). On catalytic hydrogenation the trienone (**7**) afforded a hexahydro derivative, which was identified as deoxyhexahydrophysalin A (**8**) known as a hydrogenation product of **1** [3]. Thus, chemical correlation of **5** to **1** has been established, as summarized in Scheme 1, which confirmed that **5** has the same skeletal structure as **1**. As for the stereochemistry at C-25 the stable β -configuration was assigned to the secondary methyl group in **5**, **7**, and **8**, taking into account the correlation between the proton chemical shift of the C(28)-methyl and the configuration at C-25 proposed by Antoun *et al.* [11]. As summarized in Table 1, the C(28)-methyl protons of the derivatives having a C(27)-methyl group in the β -configuration resonate at δ $\text{DMSO}-d_6$ 1.14–1.32, while those with the secondary methyl group in α -configuration resonate at δ $\text{DMSO}-d_6$ 1.42–1.48. It was also found out that the β -secondary methyl was more deshielded (δ $\text{DMSO}-d_6$ 1.13–1.26) than the α -methyl group (δ $\text{DMSO}-d_6$ 1.07–1.11), which validated further the assignment described above.

In order to determine the position and stereochemistry of the dienol function in the AB ring of **5** (Fig. 2), 400 MHz ^1H NMR analysis was carried out. Homonuclear spin decoupling experiment of **5** in $\text{DMSO}-d_6$ revealed vicinal coupling between the olefinic proton at δ 5.88 and a pair of methylene protons (δ 2.68 and 3.47), which agreed with a 7-hydroxy-3,5-dien-1-one structure but not with a 3-hydroxy-4,6-dien-1-one structure. Double quantum filtered correlated spectroscopy (DQF-COSY) and dipolar coupling NOE spectroscopy (NOESY) combined with usual spin decoupling study enabled the assignment of each proton signal as shown in Fig. 3. The small coupling constant between the C(7)- and C(8)-methine protons indicated the equatorial C(7)-hydrogen, i.e. α -axial orientation of hydroxyl group. Thus, the structure of physalin L (**5**) has been established unequivocally as 25-*epi*-3,4-dehydro-2,3,25,27-tetrahydrophysalin A as given in Scheme 1.

The ^{13}C NMR spectrum of physalin L (**5**) was compared with that of physalin A (**1**) as shown in Fig. 4. Reflecting the difference in the structures of **1** and **5**, chemical shifts of C-2, C-3, C-4, C-25, and C-27 of **1** were completely different from those of **5**. Resonances assignable to C-2 and C-25 could not be observed in the spectra of **5** and were assumed to overlap the solvent signals. C-1 and C-26 carbonyls of **5** resonated at considerably lower field than the corresponding signals of **1**, which was due to deconjugation of the carbonyl groups with the double bond in **5**. As for the remaining 21 carbons, differences in the chemical shifts of the corresponding signals of **1** and **5**



Scheme 1

Table 1. Chemical shifts of methyl (Me) protons of physalins and their derivatives having a secondary C(27)-Me group (δ , DMSO- d_6).

Functional groups in AB ring other than C(1)-carbonyl	Configuration of Me-27	Compound no. and/or reference	C-19	<i>tert</i> -Me C-21	C-28	<i>sec</i> -Me C-27
Δ^5	α	Ref. 3	1.07	1.74	1.42	1.10
Δ^5 , 7 α -OH	α	Ref. 2	1.09	1.67	1.46	1.11
Δ^5 , 7 α -OAc	α	Ref. 2	1.14	1.71	1.47	1.10
5 α -OAc, 6 β -Br, 7 α -OH	α	Ref. 2	1.31	1.71	1.48	1.10
5 α -OAc, 6 β -Br, 7 α -OAc	α	Ref. 2	1.33	1.75	1.47	1.07
none	β	8, Ref. 2	0.83	1.83	1.26	1.13
Δ^5 , 7 α -OH	β	Ref. 2*	1.12	1.69	1.29	1.15
Δ^5 , 7 α -OAc	β	Ref. 2	1.17	1.73	1.18	1.26
Δ^2 , Δ^5	β	Ref. 2	1.07	1.74	1.14	1.23
Δ^4 , Δ^6	β	4, Ref. 11	1.05	1.78	1.30	1.17
Δ^3 , Δ^5 , 7 α -OH	β	5 (physalin L)	1.16	1.71	1.32	1.17
Δ^2 , Δ^4 , Δ^6	β	7	1.11	1.65	1.23	1.14

*Chemical shifts of C(27)- and C(28)-Me were erroneously given in ref. [2].

were less than 3 ppm except for C-5 and one of C-12 and C-23, which showed slightly larger differences than the others.

Antitumour activities of physalins A, B, E, F, and L were determined using HeLa cells. Physalin A (1) exhibited moderate *in vitro* cytotoxicity (ID_{50} 3.4 μ g/ml) against HeLa cells. Physalin B (2) and physalin F, which were known to be cytotoxic against mouse tumour cells [11], showed higher activity (ID_{50} 0.32–0.35 μ g/ml) than 1, while physalin E and physalin L (5) were found to be inactive. Considering the activity of 4 which possesses a

similar structure to that of 5 with a β -C(27) methyl group [11], the inactivity of 5 seems to be attributable to the absence of the conjugated cyclohexenone moiety. Studies upon structure–cytotoxic activity relationships of physalins and their derivatives are in progress.

EXPERIMENTAL

Mps: uncorr. The purities of the compounds were checked by silica gel TLC with the solvent systems $CHCl_3$ –MeOH (9:1), $CHCl_3$ –Me₂CO (3:2), and C_6H_6 –EtOAc (3:7). The R_f values

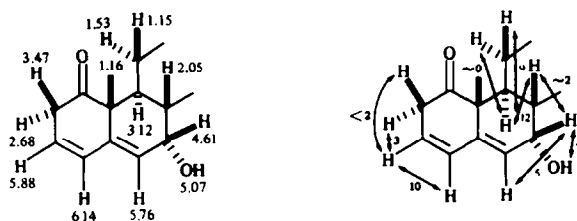


Fig. 3. Chemical shifts (δ DMSO- d_6) and vicinal coupling constants (Hz) of protons in the AB ring moiety of physalin L (5).

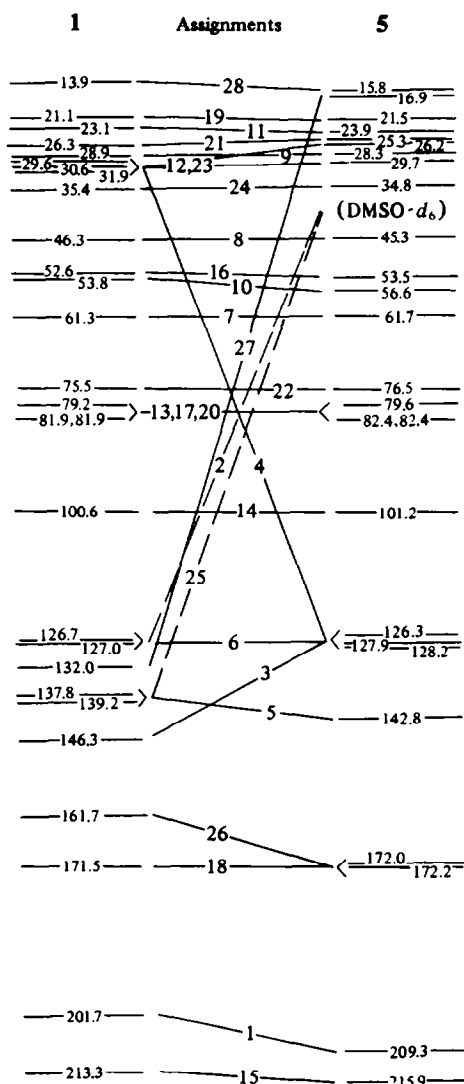


Fig. 4. Chemical shifts and signal assignments of ^{13}C NMR (50.31 MHz) spectra of physalin A (1) and physalin L (5) measured in DMSO- d_6 .

alkekengi var. *francheti* (10 kg), grown around Hamamatsu in Japan, were cut into pieces, boiled in hot H₂O (72 l), and filtered. Extraction of the filtrate with CHCl₃ (44 l) followed by evapn of the solvent afforded a dark green residual solid (18 g). A portion of the residue (5.0 g) was chromatographed over silica gel using CHCl₃-MeOH (100:1-10:1) as eluent. Physalins were eluted in the order: physalins B, F, L, A, E, but separation was incomplete and further purification by repeated silica gel CC and recrystallizations afforded pure physalins as follows: physalin A, *R_f* 0.5, 750 mg, mp 264-265° (Me₂CO) (lit. 265-266° [2]); physalin B; *R_f* 0.7, 700 mg, mp 243-245° (MeOH) (lit. 269-272° [2]); physalin E, *R_f* 0.4, CC with CHCl₃-Me₂CO and crystallization from MeOH, 145 mg, mp 252-254° (lit. 305-307° [4]); physalin F, *R_f* 0.6, CC with C₆H₆-EtOAc and crystallization from Me₂CO, 145 mg, mp 298-300° (lit. 295-296° [5]); *physalin L* (5), *R_f* 0.55, CC with CHCl₃-Me₂CO and with C₆H₆-EtOAc and crystallization from *i*-PrOH, 175 mg, mp 248-249° [α]_D²⁵ -118° (Me₂CO: c 0.3), IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3450, 1770, 1740, 1720; UV $\lambda_{\text{max}}^{\text{EtOH}}$ 233 nm (log ϵ 3.99); ¹H NMR (400 MHz, DMSO- d_6): δ 1.15 (m, H-11 β), 1.16 (s, Me-19), 1.17 (d, *J* = 8 Hz, Me-27), 1.32 (s, Me-28), 1.53 (dd, *J*_{11 α , 11 β} = 16 Hz, *J*_{11 α , 12 β} = 8 Hz, *J*_{11 α , 12 β} \approx 0 Hz, H-11 α), 1.71 (s, Me-21), 1.79 (br d, *J* = 15 Hz, H-23'), 1.93 (dd, *J*_{12 α , 12 β} = 16 Hz, *J*_{11 β , 12 β} = 5 Hz, *J*_{11 α , 12 β} \approx 0 Hz, 6-12 β), 2.05 (br d, *J* = 12 Hz, H-8), 2.11 (dd, *J*_{23, 23'} = 15 Hz, *J*_{22, 23} = 4 Hz, H-23), 2.16 (m, H-12 α), 2.62 (q, *J* = 8 Hz, H-25), 2.68 (dd, *J*_{2 α , 2 β} = 20 Hz, *J*_{2 α , 3} = 3 Hz, H-2 α), 2.97 (s, H-16), 3.12 (dd, *J*_{8, 9} = 12 Hz, *J*_{9, 11 β} = 9 Hz, *J*_{9, 11 α} \approx 0 Hz, H-9), 3.47 (br d, *J* = 20 Hz, H-2 β), 4.57 (m, H-22), 4.61 (m, H-7), 5.07 (d, *J* = 4 Hz, OH-7), 5.52 (s, OH-13), 5.76 (br d, *J* = 5 Hz, H-6), 5.88 (br d, *J* = 10 Hz, H-3), 6.14 (d, *J* = 10 Hz, H-4), 6.85 (s, OH-14) (Found: C, 61.9; H, 6.3. C₂₈H₃₂O₁₀ · H₂O requires: C, 61.5; H, 6.3%).

Anhydrophysalin L (7). A soln of 5 (40 mg) and *p*-toluenesulphonic acid (3 mg) in AcOH (5 ml) was refluxed for 1 hr. After evapn of the solvent, the residue was washed with aq. satd NaHCO₃ and H₂O to give a dehydration product as a yellow solid (35 mg). Purification by silica gel CC with CHCl₃-MeOH as eluent and crystallization from EtOAc-hexane afforded 7 as yellow crystals, mp 197-203°; UV $\lambda_{\text{max}}^{\text{EtOH}}$ 347 nm (log ϵ 3.81); ¹H NMR (200 MHz, DMSO- d_6): δ 1.11 (s, Me-19), 1.14 (d, *J* = 8 Hz, Me-27), 1.23 (s, Me-28), 1.65 (s, Me-21), 4.44 (m, H-22), 5.87 (d, *J* = 10 Hz, H-2), 6.09 (d, *J* = 6 Hz, H-4), 6.14 (s, OH), 6.49 (s, OH), 6.50 (m, H-6 and H-7), 7.13 (dd, *J*_{2, 3} = 10 Hz, *J*_{3, 4} = 6 Hz, H-3) (Found: C, 63.5; H, 5.8. C₂₈H₃₀O₉ · H₂O requires: C, 63.6; H, 6.1%).

Deoxyhexahydrophysalin A (8). A soln of 5 (20 mg) and *p*-toluenesulphonic acid (0.4 mg) in AcOH was refluxed for 1 hr. The resulting orange-yellow soln of 7 was hydrogenated over Pd black (10 mg) at room temp. and at atmos. pres. for 40 hr. The catalyst was filtered off, the filtrate evapd, and the residue crystallized from MeOH to give 4 mg of colourless crystals, which were identified as 8 by comparison of the IR and NMR spectra with those of an authentic sample [2].

Acknowledgement—The authors are grateful to emeritus Professor L. Ramachandran Row, Andhra University, for the samples of physalins E and F, to Professor Tatsuo Miyazawa, The University of Tokyo, for ¹H NMR measurements, and to Professor Teruo Matsuura, Kyoto University, for helpful discussions. Thanks are also due to Mr. Jun'ichi Ohya, Mitsubishi Chemical Industries, Ltd, for the performing cytotoxicity test.

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with the CHCl₃-MeOH system are given. Identifications of known physalins were based on comparisons of IR (KBr disks), EIMS and TLC properties with those of authentic samples.

Isolation of physalins. Leaves and stems of freshly harvested *P.*

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