Department of Applied Chemistry[1], Nagoya Institute of Technology, Nagoya, and Mitsubishi-Tokyo Pharmaceuticals, Inc.[2], Yokohama, Japan

Cytotoxic activity of physalins possessing modified skeletal structures against HeLa cells

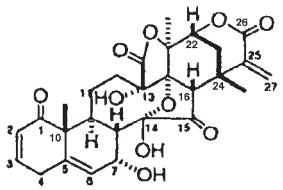
B. Makino[1], J. Ohya[2], H. Yamamura[1], S. Araki[1], Y. Butsugan[1], M. Kawai[1]

Received December 28, 2001
Accepted February 2, 2002

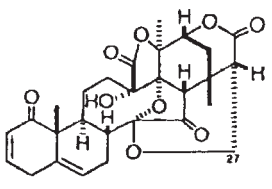
Professor Masao Kawai (Ph.D), Department of Applied Chemistry, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466–8555, Japan
kawai@ach.nitech.ac.jp

Pharmazie 58: 70–71 (2003)

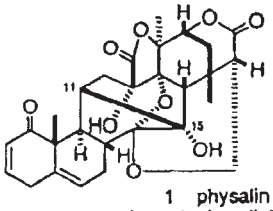
Physalins are steroidal constituents of *Physalis* plants and related species. Since the isolation of physalins A and B from *P. alkekengi* var. *francheti* (Japanese name; Hozuki) [1], more than 20 physalins were isolated [2]. Except physalins R and S containing an additional C–C linkage [3] and physalin P with a rearranged neophysalin skeleton [4], physalins commonly possess a 16,24-cyclo-13,14-seco-ergostane framework and are classified to types B and A according to the presence and absence of a C(14)–O–C(27) acetalic linkage, respectively [5]. Some physalins were known to demonstrate cytotoxic activity against tumor cells *in vitro* and *in vivo* [6, 7]. Very recently we reported the cytotoxic activity against HeLa cells of more than 70 physalins and their derivatives to investigate the structure-cytotoxic activity relationships, which revealed importance of the functionalities in the AB ring moiety for the cytotoxic activity [8]. In order to investigate the involvement of the skeletal structure itself for the cytotoxicity of physalins, we have examined anti-HeLa cells activity of physalin derivatives possessing modified skeletal structures.



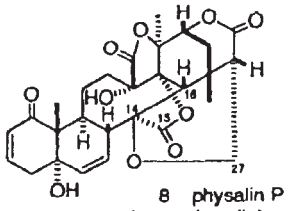
Physalin A



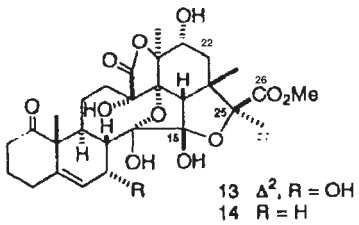
Physalin B



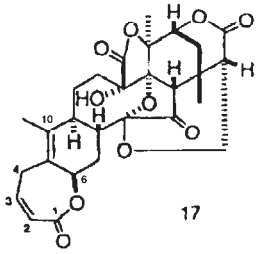
1 physalin R
(a cyclophysalin)



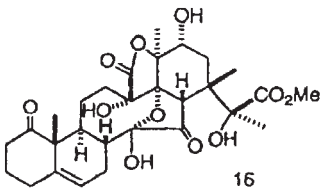
8 physalin P
(a neophysalin)



13 Δ^2, R = OH
14 R = H
15 R = OH



17



16

Upon UV-irradiation of physalins, unique C(11)–C(15)-bridged derivatives named cyclophysalins are formed by self-sensitized photocyclization [3]. Physalin R isolated from *P. alkekengi* var. *francheti* actually possesses a structure which corresponds to cyclophysalin B. Various cyclo-

Table: Cytotoxic activity of physalins with modified skeletal structures against HeLa cells

Compd.	Skeletal structure	AB ring (other than 1-oxo function)	C(25)–C(27)	IC_{50} (μg/ml)	Ref.
1[a]	Cyclophysalin	Δ^2, Δ^5	CH–CH_2–O–	11.7	[3] (physalin R)
2	Cyclophysalin	Δ^2, Δ^5, 7α-OH	CH–CH_2–O–	>100	[3]
3	Cyclophysalin	Δ^5	CH–CH_2–O–	>100	
4	Cyclophysalin	Δ^2, 5β,6β-epoxy	CH–CH_2–O–	>100	[3]
5	Cyclophysalin	Δ^2, Δ^5, 7α-OH	CH–CH_3 (*S*)	>100	[3]
6	Cyclophysalin	Δ^2, Δ^5, 7α-OH	C(OH)–CH_3 (*S*)	>100	
7	Neophysalin	Δ^2, Δ^4, Δ^6	CH–CH_2–O–	>100	[4]
8[a]	Neophysalin	Δ^2, Δ^6, 5α-OH	CH–CH_2–O–	>100	[4] (physalin P)
9	Neophysalin	Δ^2, Δ^6, 5α-OH	C(OH)–CH_2–O–	>100	
10	Neophysalin	Δ^6	CH–CH_2–O–	>100	
11	Neophysalin	Δ^2, Δ^4, Δ^6	CH–CH_3 (*S*)	>100	
12	Neophysalin	Δ^4, Δ^6	CH–CH_3 (*S*)	>100	[9]
13[a]	22,26-opened	Δ^2, Δ^5, 7α-OH	C(CH_3)–O– (*S*)	>100	
14[a]	22,26-opened	Δ^5	C(CH_3)–O– (*S*)	>100	
15[a]	22,26-opened	Δ^5, 7α-OH	C(CH_3)–O– (*S*)	68.1	
16[a]	22,26-opened	Δ^5	C(CH_3)–OH (*S*)	>100	
17[a]	Physalin	1,10-seco-1,6-lactone	CH–CH_2–O–	>100	
18	Physalin	Δ^2, Δ^5	CH(*S*)–CH_2-Acet[b)]	5.27	[12]
19	Physalin	Δ^2, Δ^5, 7α-OH	CH(*S*)–CH_2-THF[c)]	21.7	
20	Physalin	Δ^5, 7α-OH, 3-THF[c]	CH–CH_2–O–	>100	
21	Physalin	Δ^5, 7α-OH, 3-THF[c]	CH(*S*)–CH_2-THF[c)]	73.5	

a see structural formulas, b Acet = acetonyl, c THF = 2-tetrahydrofuranyl.

physalins were prepared and their *in vitro* cytotoxic activity against HeLa cells were examined. As given in the Table only physalin R showed cytotoxicity which was appreciably weaker than that of the parent physalin B [8]. Other cyclophysalins including cyclophysalins F, N and O (compounds **4**, **2** and **5**) were inactive although the corresponding parent physalins showed strong or moderate activity [8]. Thus, the cytotoxic activity of physalins was drastically impaired by the conversion to cyclophysalin structure.

Physalins are known to undergo acid-catalyzed benzilic acid rearrangement-type skeletal transformation at C(14)−C(15) position to yield derivatives named neophysalins [9]. Physalin P isolated from *P. alkekengi* var. *francheti* actually possesses the rearranged carbon skeleton [4]. Various neophysalins were also prepared and their anti-HeLa cells activity is given in the Table. All the neophysalins examined (**7**–**12**) were inactive suggesting that the neophysalin skeleton is incompatible with the cytotoxicity. However, further studies are necessary to ascertain that since corresponding normal physalins were either unavailable or also inactive [8].

In MeOH-containing solvent the activated charcoal-mediated C(25)-hydroxylation of type A physalins possessing C(27)-methyl group afforded the 25-hydroxy derivatives [10] and also the extensively skeletally-modified derivatives *via* methanolysis of the δ-lactone ring and hemiacetalization between C(25) and C(15). These derivatives were inactive or slightly active (**13**–**16**). The inactivity of the physalin O derivative **13** may be attributed to the lack of δ-lactone moiety or hemiacetalization which masked the C(15)-carbonyl function.

An A-ring cleaved derivative was obtained during the attempted photo-induced conversion of 6-epiphysalin G* to physalins K and Q [11] by photoisomerization. This ε-lactone structure-containing analog of physalin B was inactive being consistent with the loss of the conjugated cyclohexenone function (**17**).

During the isolation of physalins artifacts were formed by the reaction with solvent molecule(s). When acetone was used for the extraction from *P. alkekengi* var. *francheti* an acetone adduct of physalin C, namely 27-acetonyl-25,27-dihydrophysalin C, was isolated [12]. It is interesting that this compound showed almost the same activity as dihydrophysalin C (**18**). Solvent adducts were also formed upon the photosensitized reaction of physalins [3] when tetrahydrofuran was used as a solvent. 25-(2-Tetrahydrofuranyl)physalin O showed activity similar to that of physalin O (**19**). The addition occurred also at the pharmacologically essential 2-en-1-one moiety of the A ring [8] and the resulting C(3)-adducts showed low or no activity (**20**, **21**). In general the solvent adducts at C(25) position retained the activity suggesting that useful modification could be performed at this position.

In summary cytotoxic assay of skeletally modified physalin derivatives using HeLa cells indicated the importance of the whole skeletal structure of physalin for the cytotoxic activity in combination with the main pharmacophore, *i.e.* the functionalities in the AB ring moiety [8].

Experimental

1. Materials

Cyclophysalins were prepared by photo-induced isomerization of the corresponding physalins [3]. Physalin P was isolated from epigeal part of *P. alkekengi* var. *francheti* [4]. Other neophysalins were prepared by acid-induced rearrangement of physalins as reported [9].

2. Synthesis of the compounds

2.1. 1,10-Seco-1,6-lactone structure-containing physalin B analog

A solution of 6-epiphysalin G (662 mg) containing Rose Bengal (31 mg) in acetone (180 ml) was irradiated by a high-pressure mercury lamp (100 W) under O_2-bubbling for 5 h. Usual work up and silica gel column chromatography yielded the secolactone as a colorless powder (116 mg, 18% yield). HRMS(EI) Found: m/z 526.1895; Calcd for $C_{28}H_{30}O_{10}$: 526.1837 (M^+).

2.2. δ-Lactone ring-opened 25-methyl esters

Activated charcoal (Norit EXW, 1 g) was added to a solution of physalin O (190 mg) in MeOH-tetrahydrofuran (2 : 3 v/v, 25 ml) and the mixture was stirred at room temperature for 22 h and the charcoal was filtered off. Evaporation and silica gel column chromatography ($CHCl_3$-MeOH) of the residue afforded (25*S*)-25-hydroxyphysalin O (71 mg, 36%), (25*S*)-6β,25-dihydroxy-4,5-didehydro-5,6-dihydrophysalin O (14 mg, 7%), and the hemiacetal of δ-*lactone-opened* 25-hydroxy-26-methyl ester (34 mg, 16%), HRMS (EI) Found: m/z 558.2092; Calcd for $C_{29}H_{34}O_{11}$: 558.2099 $(M\text{-}H_2O)^+$. Other δ-lactone ring-opened analogs were obtained similarly as by-products of the 25-hydroxylation of 2,3,25,27-tetrahydrophysalin A and 2,3,25,27-tetrahydrophysalin C.

3. Cytotoxic assay

Cytotoxic assay was performed using HeLa cells as described in the previous communication [8].

* '6-Epiphysalin G' refers to 6β-hydroxy-4,5-didehydro-5,6-dihydrophysalin B [11] since the structure of physalin G isolated from *P. angulata* and *P. lancifolia* was described as 6α-hydroxy-4,5-didehydro-5,6-dihydrophysalin B [13]. However, the reported ^{1}H NMR data were inconsistent with those of the authentic 6α-hydroxy-2,4-dien-1-one prepared from physalin J but agreed with those of the epimeric 6β-hydroxy compound prepared from physalin F. Therefore, the natural product, physalin G, possesses the structure of 6-epiphysalin G.

References

1 Matsuura, T.; Kawai, M.; Nakashima, R.; Butsugan, Y.: J. Chem. Soc. (C), 664 (1970)
2 Kawai, M.; Yamamoto, T.; Makino, B.; Yamamura, H.; Araki, S.; Butsugan, Y.; Saito, K.: J. Asian Nat. Prod. Res. **3**, 199 (2001)
3 Makino, B.; Kawai, M.; Kito, K., Yamamura, H.; Butsugan, Y.: Tetrahedron **51**, 12529 (1995)
4 Kawai, M.; Matsumoto, A.; Makino, B.; Mori, H.; Ogura, T.; Butsugan, Y.; Ogawa, K.; Hayashi, M.: Bull. Chem. Soc. Jpn. **66**, 1299 (1993)
5 Makino, B.; Kawai, M.; Yamamura, H.; Araki, S.; Butsugan, Y.: Pharmazie **57**, 215 (2002)
6 Antoun, M. D.; Abramson, D.; Tyson, R. L.; Chang, C.-J.; McLaughlin, J. L.; Peck, G.; Cassady, J. M.: J. Nat. Prod. **44**, 579 (1981)
7 Makino, B.; Kawai, M.; Ogura, T.; Nakanishi, M.; Yamamura, H.; Butsugan, Y.: J. Nat. Prod. **58**, 1668 (1995)
8 Kawai, M.; Makino, B;.Yamamura, H.; Araki, S.; Butsugan, Y.; Ohya, J.: Pharmazie, **57**, 368 (2002)
9 Kawai, M.; Ogura, T.; Butsugan, Y.; Taga, T.; Hayashi, M.: Tetrahedron **47**, 2103 (1991)
10 Makino, B.; Kawai, M.; Yamamoto, T.; Yamamura, H.; Butsugan, Y.; Hayashi, M.; Ogawa, K.: J. Chem. Soc., Chem. Commun., 1430 (1992)
11 Makino, B.; Kawai, M.; Iwata, Y.; Yamamura, H.; Butsugan, Y.; Ogawa, K.; Hayashi, M.: Bull. Chem. Soc. Jpn. **68**, 219 (1995)
12 Kawai, M.; Makino, B.; Yamamura, H.; Butsugan, Y.: Phytochemistry **43**, 661 (1996)
13 Row, L. R.; Reddy, K. S.; Sarma, N. S.; Matsuura, T.; Nakashima, R.: Phytochemistry **19**, 1175 (1980)