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STRUCTURES OF TWO NEW HIGHLY OXYGENATED LABDANE-TYPE DITERPENOIDS AND A NEW CADINANE-TYPE SESQUITERPENOID POSSESSING A CYCLIC ETHER LINKAGE FROM THE LIVERWORT *PTYCHANTHUS STRIATUS*[§]

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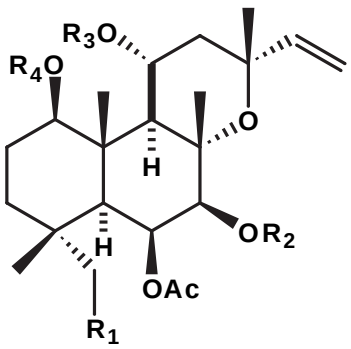
Abstract-As a part of our systematic investigation of biologically active substances of liverworts, we studied the chemical constituents of the liverwort *Ptychanthus striatus* belonging to the Lejeuneaceae family, and isolated two novel labdane-type diterpenoids, named ptychantins J and K, and a new cadinane-type sesquiterpene possessing a cyclic ether linkage**.** Their relative structures were determined by a combination of high resolution NMR spectrometry, X-Ray crystallographic analysis and chemical degradations.

1. INTRODUCTION

Liverworts contain both terpenoids and aromatic compounds which constitute the oil bodies. We have reported the distribution of a numer of novel terpenoids and aromatic compounds possessing biological activities such as antimicrobial, antifungul, antitumor and neuric sprouting activities in more than 500 species of the liverworts.[1-3] In the course of investigation of the biologically active substances from the liverworts, we already isolated nine novel labdane-type diterpenoids, ptychantins A-I [4, 5] (Figure 1), closely related to forskolin from the ether extract of the liverwort *Ptychanthus striatus* belonging to the

[§]Dedicated to the celebration of 75th birthday of Professor Yuichi Kanaoka.

Lejeuneaceae and three novel macrocyclic bis(bibenzyls), named ptychantols A-C [6] from the MeOH extract of *P. striatus*. Further fractionation of Et_2O and MeOH extracts of *P. striatus* resulted in the isolation of two new labdane-type diterpenoids, named ptychantins J (**1**) and K (**2**), and a new cadinane-type sesquiterpene (**3**) (Figure 1) possessing a cyclic ether linkage. We now report on the structure elucidation of three new compounds by two-dimension NMR spectra, X-Ray crystallographic analysis and chemical degradations.



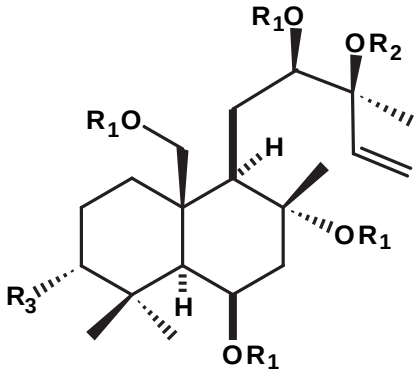
Ptychantin A: R_1= H, R_2= H, R_3= R4= Ac

Ptychantin B: R_1= H, R_2= R_3= R_4= Ac

Ptychantin C: R_1= R_2= R_3= H, R_4= Ac

Ptychantin D: R_1= R_2= R_4= H, R_3= Ac

Ptychantin E: R_1= OH, R_2= H, R_3= R_4= Ac

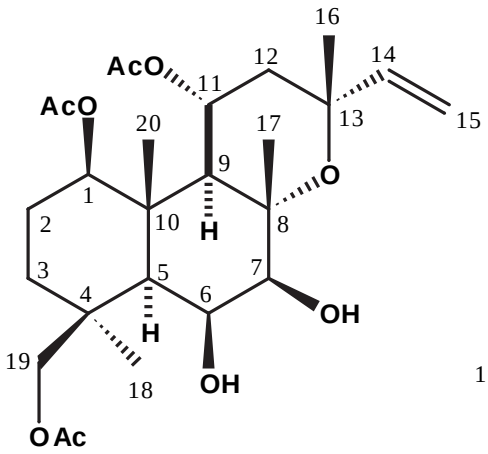


Ptychantin F: R_1= Ac, R_2= H, R_3= OAc

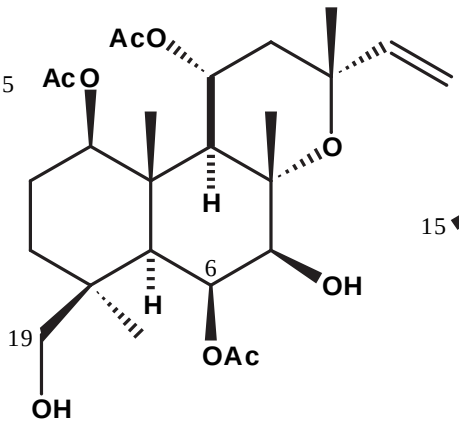
Ptychantin G: R_1= R_2= Ac, R_3= OAc

Ptychantin H: R_1= Ac, R_2= H, R_3= OH

Ptychantin I: R_1= Ac, R_2=R_3= H



Ptychantin J (1)



Ptychantin K (2)

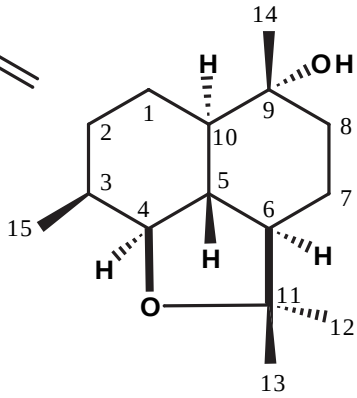


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Figure 1. Labdane type-diterpenoids and a cadinane-type sesquiterpene from *Ptychantus striatus*.

Isolation of two new labdane-type diterpenoids and a cadinane-type sesquiterpene

Ptychanthus striatus was extracted with Et_2O, and followed with MeOH. The MeOH extract was partitioned

between EtOAc and H_2O. The EtOAc and Et_2O extracts were combined and subjected to column chromatography on silica gel and Sephadex LH-20 to afford ptychantins J (**1**) and K (**2**), and a new cadinane-type sesquiterpene (**3**) with nine known labdane-type diterpenoids, ptychantins A-I.

Structural elucidation of ptychantins J and K Ptychantins J (**1**) {$[\alpha]_D$ -127.2° ($CHCl_3$)} was obtained as a colorless prisms (mp 213-214°), whose molecular formula, $C_{26}H_{40}O_9$ was established by high resolution chemical ionization mass spectroscopy (HR-CIMS)($[M]^+$ m/z 497.2748). Compound (**1**) had similar spectral data to those of ptychantins A~E. The IR spectrum of **1** indicated the presence of a hydroxyl (3491 cm^{-1}) and an acetoxyl (1731 cm^{-1}) groups. The ^1H (Table 1) and ^{13}C NMR (Table 2) spectra of **1** showed the presence of four tertiary methyl [δ_H 1.06, 1.20, 1.42, 1.56 (each 3H, *s*)] groups, three acetoxyl [δ_H 1.91, 1.92, 1.93 (each 3H, *s*); δ_C 20.9, 21.7, 21.9 (each *q*), 170.9, 171.1, 171.4 (each *s)*] groups, one vinyl [δ_H 4.93 (*dd*, J=10.7, 1.6 Hz), 5.16 (*dd*, J=17.0, 1.6 Hz), 5.81 (*dd*, J=17.0, 10.7 Hz); δ_C110.7 (t), 146.6 (d)] group, two secondary alcohol [δ_H 3.61 (*d*, J=3.8 Hz), 4.45 (*dd*, J=2.0, 3.8 Hz); δ_C 80.1 (*d*), 69.9 (*d*),], one methylene [δ_H 4.56, 4.59 (each *d*, J=11.8 Hz); δ_C 66.2 (t)] and two methine [δ_H 4.54 (*dd*, J=4.9, 11.0 Hz), 5.26 (*ddd*, J=1.1, 5.5, 7.1 Hz); δ_C 80.3 (d), 68.6 (d)] groups bearing acetoxyl groups, and an ether linkage [δ_C 72.3 (*s*), 76.1 (*s*)]. Acetylation of **1** with Ac_2O and pyridine afforded a tetraacetate (**4**) [^1H NMR: δ1.92, 1.93, 2.05, 2.18 (each 3H, *s*); IR (KBr) cm^{-1}: 3472 (OH), 1732 (COO)], and acetylation of an axial hydroxyl group at C-6β did not proceed even for a long time. Compound (**1**) showed the correlations between (i) H-19 / C-3, C-4, C-5, C-18 and OAc; (ii) H-18 / C-3, C-4, C-5 and C-19 in HMBC spectrum (Figure 2), and the NOEs between (i) H-18 / H-3α, H-5α, H-6 and H-19; (ii) H-19 / H-2β, H-18 and H-20 in the NOESY spectrum (Figure 2) indicating that an acetoxyl group was located at C-19. From the above spectral and chemical evifence, the relative structure of ptychantins J (**1**) was deduced and finally established by X-Ray crystallography of **1** as shown in Figure 3.

Ptychantin K (**2**) {$[\alpha]_D$ -81.9° ($CHCl_3$)} has the same molecular formula, $C_{26}H_{40}O_9$ (HR-CIMS; $[M]^+$ m/z 497.2748) as that of ptychantins J (**1**). The ^1H (Table 1) and ^{13}C (Table 2) NMR spectrum of **2** in $CDCl_3$ showed the presence of four tertiary methyl groups [δ_H 1.09, 1.20, 1.36, 1.50 (each 3H, *s*)], three acetoxyl groups [δ_H 1.92, 1.94, 2.12 (each 3H, *s*)] and were very similar to those (Tables 1 and 2) of **1** except for the chemical shifts of a methylene [δ_H 3.38, 3.89 (each *d*, J=11.3 Hz)] at C-19 and a methines [δ_H 5.85

(*dd*, J=2.2, 4.4 Hz)] at C-6. Acetylation of **2** afforded

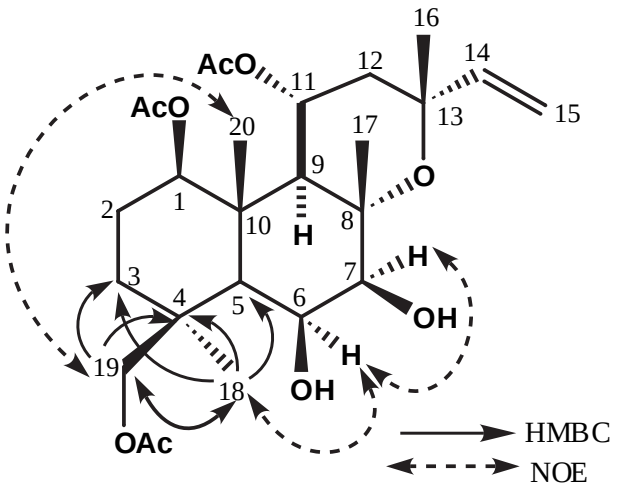


Figure 2. Important HMBC and NOESY correlations of **1**.

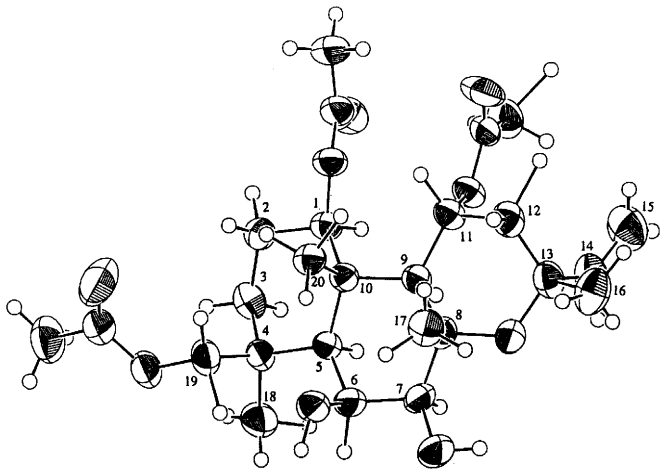


Figure 3. ORTEP drawing of **1**.

those (Tables 1 and 2) of **1** except for the chemical shifts of a methylene [δ_H 3.38, 3.89 (each *d*, J=11.3 Hz)] at C-19 and a methine [δ_H 5.85 (*dd*, J=2.2, 4.4 Hz)] at C-6. Acetylation of **2** afforded a petaacetate (**5**) [δ_H 1.92, 1.94, 2.02, 2.04, 2.16 (each 3H, *s*); IR (KBr)cm^{-1}: 1738 (COO)] indicating that compound (**2**) has two hydroxyl groups. The selective hydrolysis of **2** with KOH and MeOH afforded a diacetate (**6**) [δ_H 1.92, 1.93, (each 3H, *s*); $C_{24}H_{38}O_8$] in 97.7 % yield. Acetylation of **6** with Ac_2O and pyridine afforded a tetraacetate (**4**) [^{1}H NMR: δ1.92, 1.93, 2.05, 2.18 (each 3H, *s*)] whose mp, $[\alpha]_D$, CI-MS, IR, ^{1}H- and ^{13}C-NMR were identical to those of a tetraacetate (**4**) obtained by acetylation of ptychantins J (**1**). From the above experimental results and 2D-NMR (HMBC, NOESY etc.) data of **2**, the relative structure of ptychantin K (**2**) was determined as an epimer of ptychantin E at C-4. The absolute configurations of ptychantins J (**1**) and K (**2**) were elucidated by following experimental result described below (Figures 4 and 5). Protection of **6** with 2, 2-dimethoxypropane and *p*-TsOH afforded the major acetonide (**7**)(72.3 %) and the minor acetonide (**8**)(15.1 %). The structure of **7** was determined by HMBC spectrum as shown in Figure 4. Since the NMR of **7** showed that the hydroxyl group at C-7 was equatorial, we applied the modified Mosher's method [7] for MTPA ester of **7**. Compound **7** was esterified with (+)- and (-)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTPA chloride) and dimethylaminopyridene (DMAP) inCH_2Cl_2 to afford (+)-MTPA ester (**9**) and (-)-MTPA ester (**10**), respectively. The values of difference of each chemical shift [Δδ values; δ(-)-δ(+)] for **9** and **10** are shown in Figure 5. On the basis of the above

results, the configuration of **7** at C-7 is *S*., and the absolute configurations of ptychantins J and K were thus determined as **1** and **2**.

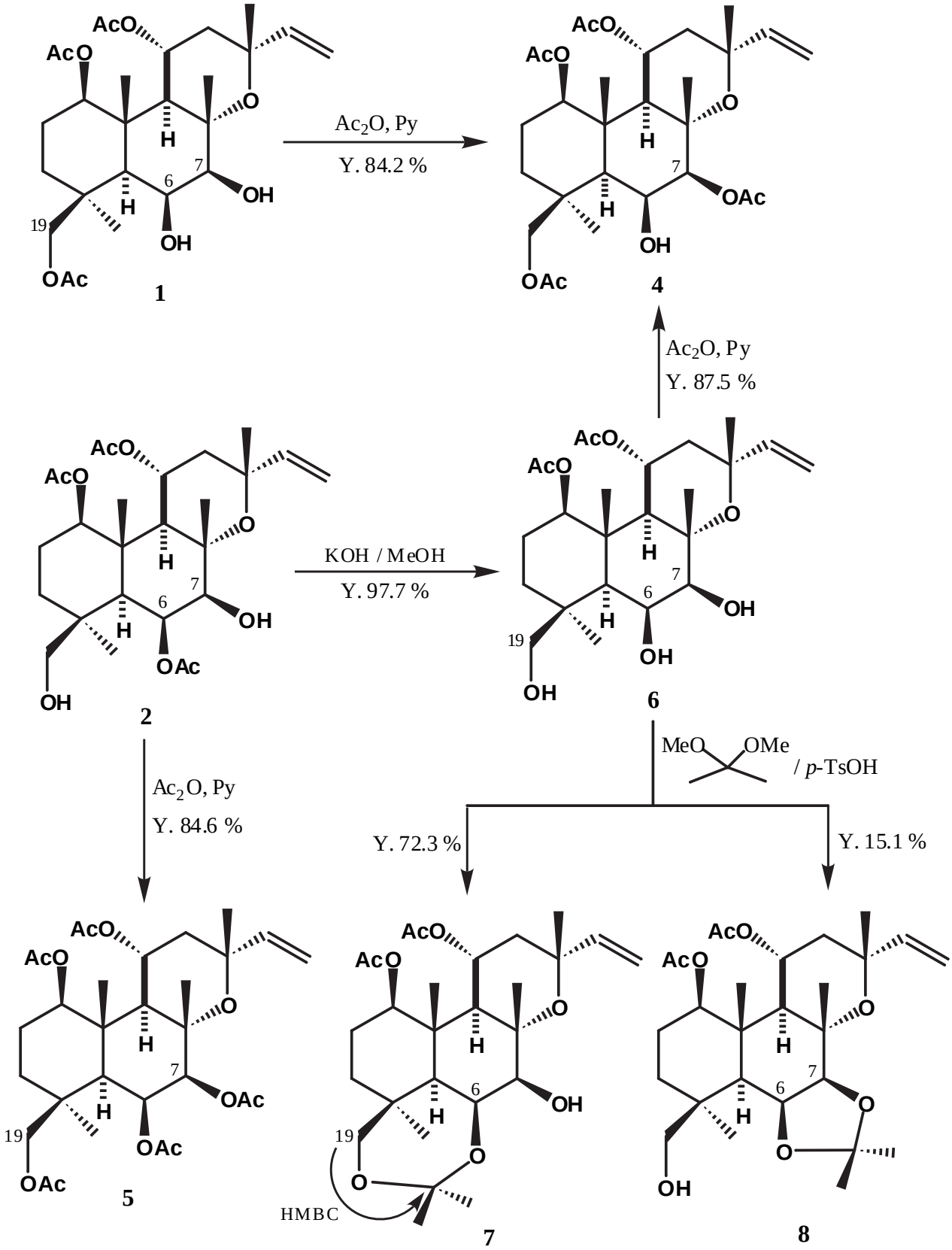


Figure 4. Chemical conversions of ptychantins J (**1**) and K (**2**).

Table 1. 600 MHz ^{1}H NMR spectral data of compounds (**1**, **2**, **4**, **5**, **6** and **7**) in $CDCl_3$

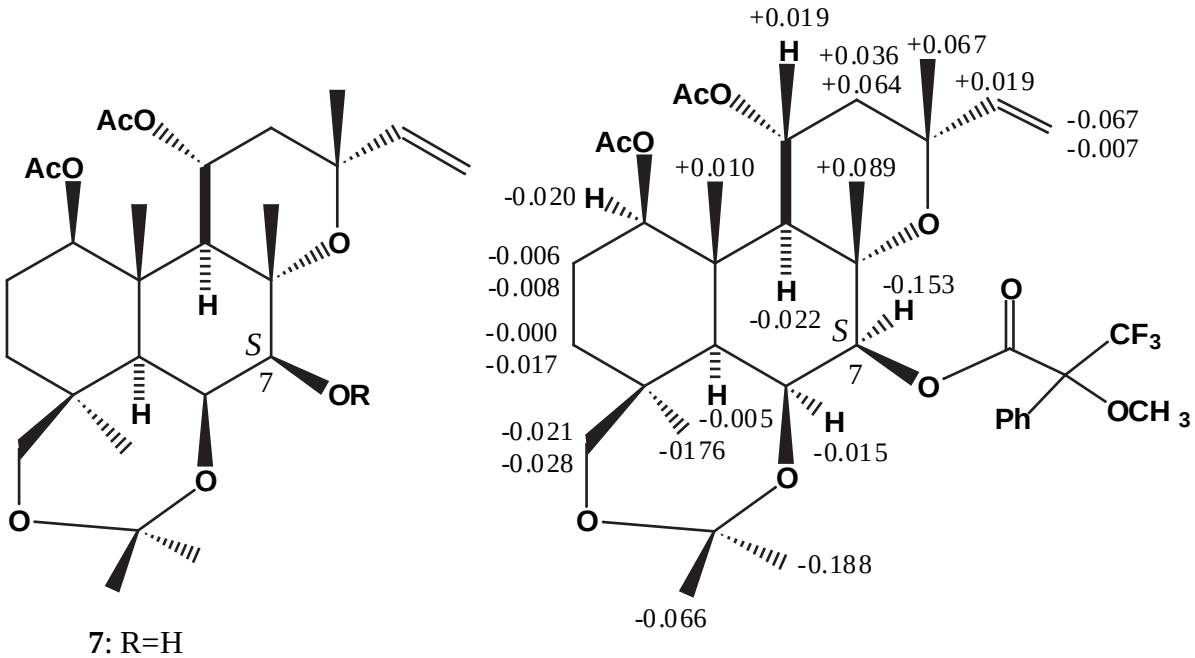
Position	**1**	**2**	**4**	**5**	**6**	**7**
1-H	4.54 *dd* [a]	4.57 *dd*	4.55 *dd*	4.58 *dd*	4.55 *dd*	4.75 *dd*
	(4.9, 11.0)	(4.7. 11.0)	(4.9, 11.0)	(4.9, 11.0)	(4.9, 10.4)	(3.0, 6.3)
2-H	1.58, 1.94 *m*	1.49, 1.93 *m*	1.55, 1.95 *m*	1.51, 1.98 *m*	1.44, 1.92 *m*	1.56, 1.90 *m*
3-H	1.11, 1.75 *m*	1.05, 1.96 *m*	1.13, 176 *m*	1.13, 178 *m*	1.37. 1.51 *m*	1.12. 1.50 *m*
5-H	1.18 *d*	1.42 *d*	1.27 *d*	1.48 *d*	1.23 *d*	1.02 *d*
	(2.0)	(2.2)	(2.0)	(2.7)	(2.2)	(2.2)
6-H	4.45 *dd*	5.85 *dd*	4.43 *dd*	5.80 *dd*	4.37 *dd*	4.35 *dd*
	(2.0, 3.8)	(2.2, 4.4)	(2.0, 3.8)	(2.7, 4.4)	(2.2, 4.2)	(2.2, 4.4)
7-H	3.61 *d*	3.76 *d*	4.99 *d*	5.01*d*	3.70 *d*	3.66 *d*
	(3.8)	(4.4)	(3.8)	(4.4)	(4.2)	(4.4)
9-H	1.92 *d*	1.98 *d*	2.04 *d*	1.92 *d*	1.95 *d*	1.96 *d*
	(7.1)	(7.1)	(7.1)	(6.3)	(7.1)	(6.3)
11-H	5.26 *ddd*	5.26 *ddd*	5.25 *ddd*	5.26 *ddd*	5.27 *ddd*	5.23 *ddd*
	(1.5, 5.5, 7.1)	(1.4, 5.2, 7.1)	(1.4, 5.2, 7.1)	(1.1, 5.2, 6.3)	(1.1, 5.2, 7.1)	(1.1, 5.5, 6.3)
12α-H	2.08 *dd*	2.10 *dd*	2.08 *dd*	2.10 *dd*	2.10 *dd*	1.94 *dd*
	(1.1, 16.5)	(1.4, 16.5)	(0.8, 16.4)	(1.0, 16.4)	(1.1, 16.5)	(1.1, 16.2)
12β-H	2.30 *dd*	2.29 *dd*	2.25 *dd*	2.24 *dd*	2.32 *dd*	2.33 *dd*
	(5.5, 16.5)	(5.2, 16.5)	(5.2, 16.4)	(5.2, 16.4)	(5.2, 16.5)	(5.5, 16.2)
14-H	5.81 *dd*	5.81 *dd*	5.73 *dd*	5.75 *dd*	5.81 *dd*	5.81 *dd*
	(10.7, 17.0)	(10.7, 17.3)	(10.4, 17.0)	(10.4, 17.0)	(10.7, 17.0)	(10.7, 17.0)
15-Ha	4.93 *dd*	4.94 *dd*	4.94 *dd*	4.93 *dd*	4.94 *dd*	4.95 *dd*
	(1.6, 10.7)	(1.6, 10.7)	(1.6, 10.4)	(1.6, 10.4)	(1.6, 10.7)	(1.6, 10.7)
15-Hb	5.16 *dd*	5.16 *dd*	5.16 *dd*	5.24 *dd*	5.17 *dd*	5.21 *dd*
	(1.6, 17.0)	(1.6, 17.3)	(1.6, 17.0)	(1.6, 17.0)	(1.6, 17.0)	(1.6, 17.0)
16-H	1.20 *s*	1.20 *s*	1.11 *s*	1.12 *s*	1.21 *s*	1.20 *s*
17-H	1.56 *s*	1.50 *s*	1.59 *s*	1.54 *s*	1.58 *s*	1.51 *s*
18-H	1.06 *s*	1.09 *s*	1.03 *s*	1.03 *s*	1.08 *s*	1.07 *s*
19-H	4.56, 4.59 *d*	3.38, 3.89 *d*	4.52, 4.57 *d*	3.81, 4.47 *d*	3.18, 4.24 *d*	3.00, 3.97 *d*
	(11.8)	(11.3)	(11.8)	(11.5)	(11.8)	(11.8)
20-H	1.42 *s*	1.36 *s*	1.44 *s*	1.42 *s*	1.45 *s*	1.46 *s*
OAc	1.91, 1.93,	1.92, 1.94,	1.92, 1.94,	1.92, 1.94,	1.92, 1.93 *s*	1.93, 1.95 *s*
	2.04 *s*	2.12 *s*	2.05, 2.18 *s*	2.02, 2.04, 2.16 *s*		
Others						1.39, 1.40 *s*

Chemical shifts are in δ values and assignments from ^{1}H-^{1}H COSY, NOESY and HMBC spectra. Coupling constants in Hz are in parenthesis.

Table 2 125 MHz ^{13}C NMR spectral data of compounds (**1**, **2**, **4**, **5**, **6** and **7**) in $CDCl_3$

position	**1**	**2**	**4**	**5**	**6**	**7**
C-1	80.3 (*d*) [a]	80.3 (*d*)	80.2 (*d*)	79.9 (*d*)	80.3 (*d*)	77.0 (*d*)
C-2	23.3 (*t*)	23.2 (*t*)	23.3 (*t*)	23.2 (*t*)	23.2 (*t*)	25.4 (*t*)
C-3	33.4 (*t*)	32.7 (*t*)	33.6 (*t*)	33.4 (*t*)	37.4 (*t*)	28.5 (*t*)
C-4	37.8 (*s*)	39.4 (*s*)	38.0 (*s*)	37.9 (*s*)	38.4 (*s*)	37.1 (*s*)
C-5	55.3 (*d*)	54.7 (*d*)	55.4 (*d*)	54.5 (*d*)	54.7 (*d*)	49.4 (*d*)
C-6	69.9 (*d*)	70.6 (*d*)	69.4 (*d*)	69.0 (*d*)	69.4 (*d*)	70.1 (*d*)
C-7	80.1 (*d*)	79.4 (*d*)	80.8 (*d*)	78.5 (*d*)	79.6 (*d*)	78.7 (*d*)
C-8	76.1 (*s*)	76.1 (*s*)	74.7 (*s*)	74.2 (*s*)	76.3 (*s*)	76.5 (*s*)
C-9	55.9 (*d*)	56.2 (*d*)	56.4 (*d*)	56.4 (*d*)	56.3 (*d*)	55.1 (*d*)
C-10	41.6 (*s*)	42.0 (*s*)	41.6 (*s*)	41.7 (*s*)	41.6 (*s*)	44.1 (*s*)
C-11	68.6 (*d*)	68.5 (*d*)	68.7 (*d*)	68.5 (*d*)	68.6 (*d*)	68.1 (*d*)
C-12	37.9 (*t*)	37.9 (*t*)	37.9 (*t*)	37.7 (*t*)	37.8 (*t*)	38.9 (*t*)
C-13	72.3 (*s*)	72.3 (*s*)	72.0 (*s*)	72.1 (*s*)	72.1 (*s*)	72.3 (*s*)
C-14	146.6 (*d*)	146.4 (*d*)	146.4 (*d*)	146.3 (*d*)	146.6 (*d*)	146.7(*d*)
C-15	110.7 (*t*)	110.8 (*t*)	111.2 (*t*)	111.3 (*t*)	110.6 (*t*)	110.9 (*t*)
C-16	31.2 (*q*)	31.1 (*q*)	31.7 (*q*)	31.6 (*q*)	31.0 (*q*)	31.4 (*q*)
C-17	23.3 (*q*)	22.5 (*q*)	23.9 (*q*)	23.3 (*q*)	22.9 (*q*)	22.7 (*q*)
C-18	26.6 (*q*)	26.2 (*q*)	26.5 (*q*)	26.3 (*q*)	26.3 (*q*)	27.0 (*q*)
C-19	66.2 (*t*)	63.8 (*t*)	66.3 (*t*)	65.1 (*t*)	66.7 (*t*)	69.2 (*t*)
C-20	15.5 (*q*)	15.2 (*q*)	15.5 (*q*)	15.3 (*q*)	14.5 (*q*)	14.7 (*q*)
OCO*CH*$_3$	20.9 (*q*)	21.5 (*q*)	20.9 (*q*)	20.8 (*q*)	21.6 (*q*)	21.6 (*q*)
OCO*CH*$_3$	21.7 (*q*)	21.7 (*q*)	21.2 (*q*)	20.9 (*q*)	21.8 (*q*)	21.8 (*q*
OCO*CH*$_3$	21.9 (*q*)	22.0 (*q*)	21.8 (*q*)	21.4 (*q*)		
OCO*CH*$_3$			22.0 (*q*)	21.8 (*q*)		
OCO*CH*$_3$				22.0 (*q*)		
O*CO*CH3	170.9 (*s*)	170.5 (*s*)	170.0 (*s*)	169.8 (*s*)	170.7 (*s*)	170.9 (*s*)
O*CO*CH3	171.1 (*s*)	170.7 (*s*)	170.1 (*s*)	170.1 (*s*)	171.3 (*s*)	171.5 (*s*)
O*CO*CH3	171.4 (*s*)	171.5 (*s*)	170.9 (*s*)	170.7 (*s*)		
O*CO*CH3			171.5 (*s*)	171.0 (*s*)		
O*CO*CH3				171.5 (*s*)		
Others						24.6 (*q*)
						24.7 (*q*)
						101.9 (*s*)

Chemical shifts from TMS and assignments from HMQC and HMBC spectra



7: R=H
9: R=(+)-MTPA
10: R=(-)-MTPA

Figure 5. Δδ values [$\delta_{(-)}$ - $\delta_{(+)}$] in ppm (600 MHz ^{1}H NMR) for (-)-MTPA ester (**10**) and (+)-MTPA ester (**9**).

Ptychantins A-E, J and K has the same skeleton, similar oxygen functionalities and the same absolute configuration as forskolin isolated from the Indian herb *Coleus forskohlii*, which shows a wide range of biological activities such as antihypertensive, positive inotoropic, bronchospasmolytic and antithrombotic activities. [8-10] Ptychantins A~E do not show any activities described above. This result suggests that C-11 carbonyl and C-9 hydroxyl groups in forskolin are essential for pharmacological activities. 1, 9-Dideoxyforskolin isolated from *C. forskohlii* has been found to selectively inhibit glucose transport in rats adipocytes. [11] 1,9-Dideoxyforskolin has been synthesized from ptychantins A and B by selective manipulations of four alkoxy groups in 8 steps in 32 % overall yield by our research groups.[12] New C-19 hydroxylated forskolin analogue will be synthesized from ptychantins J (**1**) and K (**2**) isolated from the liverwort *P. striatus* in this time.

Structural elucidation of a new cadinane-type sesquiterpene Compound (**3**) was colorless prisms (mp 213-214℃). The molecular formula $C_{15}H_{26}O_2$ was established by HR-EIMS ([M]$^+$ *m/z* 238.1928). The IR spectrum of **1** indicated the presence of a hydroxyl (3410 cm^{-1}) group. The ^{1}H and ^{13}C NMR spectra of **3** showed the presence of a secondary methyl [δ_H 0.90 (3H, *d*, J=7.1 Hz)], three tertiary methyl [δ_H 1.05, 1.19, 1.31 (each 3H, *s*)], a tertiary alcohol [δ_C 73.0 (*s*)] and a ether linkage [δ_H 3.45 (*dd*, J=4.9, 10.7 Hz); δ_C 81.5 (*d*), 81.6 (*s*)]. Compound **3** showed the correlations between (i) H-4 / C-5, C-6,

C10 and C-15; (ii) H-12 / C-6, C-11 andC-13; (iii) H-13 / C-6, C-11 and C-12; (iv) H-14 / C-8, C-9 and C-10 in HMBC spectrum (Figure 6) and the NOEs between H-4 / H-3, H-6 and H-12 in the NOESY spectrum (Figure 6). The relative structure of **3** could not be deduced completely, because ^{1}H NMR spectrum of **3** showed complicating features and some proton signals were overlaped. The relative structure of **3** was finally established by X-Ray crystallographic analysis as shown in Figure 7. Toyota *et al.* reported that a solution of (4*S*,7*R*)-germacra-1(10)*E*, 5*E*-dien-11-ol (**11**) isolated from the liverwort *Dumoritera hirsuta* in $CHCl_3$ or Et_2O was allow to stand at rt for a long time to afford compounds (**3**, **12** and **13**).[13] The possibility of natural product for compound (**3**) will be present, because compounds (**11**-**13**) were not isolated from the liverwort *P. striatus* and compound (**3**) was detected by GC-MS of the extract, which was obtained by the extraction of a fresh *P. striatus* with Et_2O for a short time. The absolute structure of compound (**3**) remains to be unidentified.

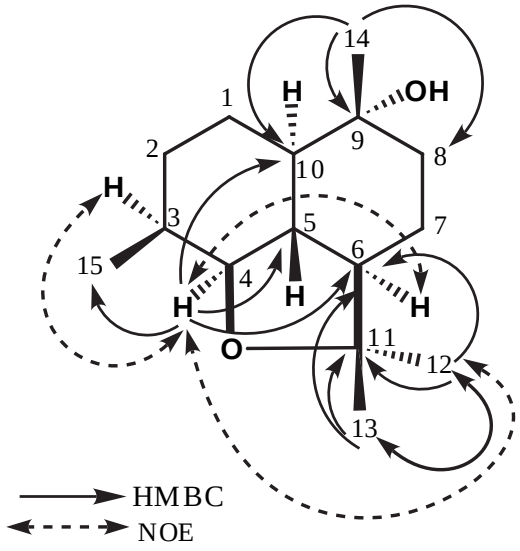


Figure 6. Important HMBC and NOESY correlations of **3**.

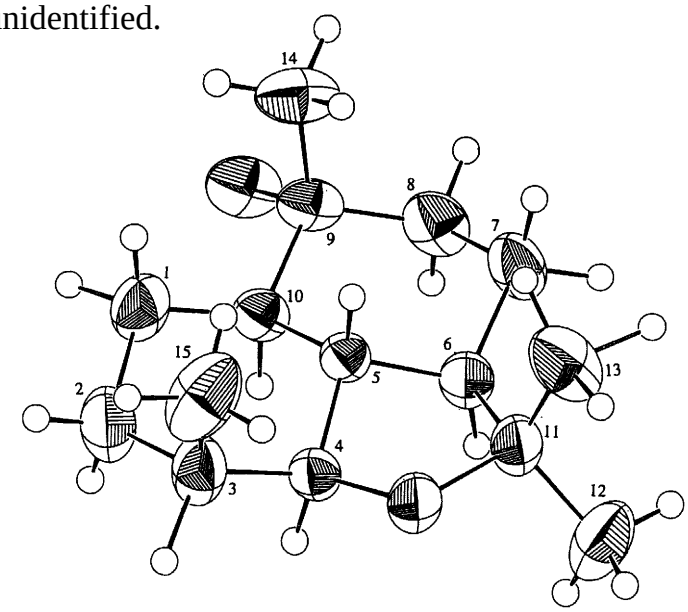


Figure 7. ORTEP drawing of **3**.

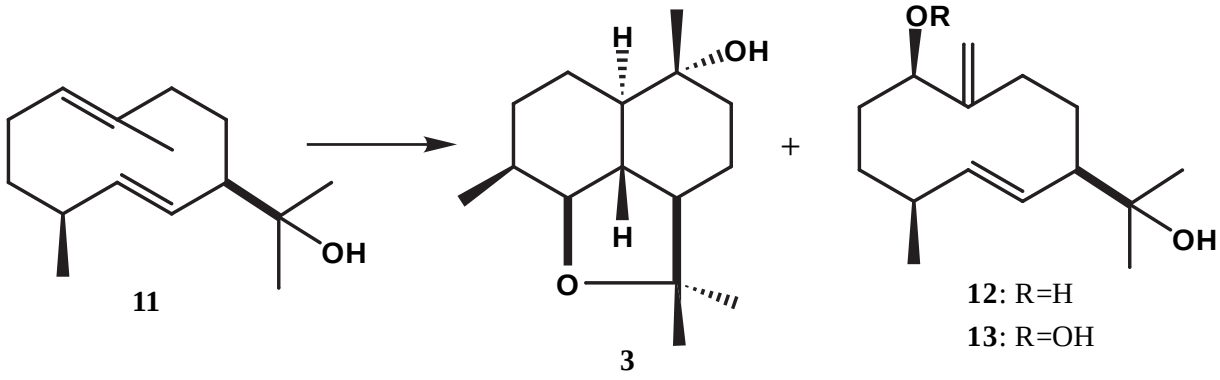


Figure 8. Chemical conversion of **11** into compounds (**3**, **12** and **13**).

EXPERIMENTAL

General Experimental Procedures. Melting points were uncorrected. TLC was carried out on silica gel precoated glass plates (Kieselgel 60 F_{254}, Merk) with *n*-hexane-EtOAc (2:1, 1:1) and $CHCl_3$-EtOAc (2:1, 1:1). Detection was carried with Godin reagent. For normal phase column chromatography (CC), Slica gel 60 (70-230 μm and 230-400 μm, Merk.) was used. IR spectrum was measured on a JASCO FT-IR 500 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Varian untiy 600 (^1H; 600 MHz, ^{13}C; 150 MHz) spectrometer. The solvent used for NMR spectra was $CDCl_3$. MS spectra were measured on a JEOL JMS HX-100 or a JEOL AX-500 spectrometer. The specific rotation was taken on a JASCO DIP-140 POLARIMETER.

Plant Material The liverwort *Ptychanthus striatus* was collected in May 2000 at Aioi, Tokushima, Japan and identified by Y. Asakawa and confirmed by Dr. M. Mizutani. A voucher specimen (H1203001) has been deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Isolation of two new diterpenoids and a new sesquiterpenoid. *P. striatus* was dried for 2 days and ground mechanically. The dry material (1.18 kg) was extracted with Et_2O (5 L) for 2 weeks at 20℃, followed by MeOH (5 L) for 1 month at rt. Each extract was filtered and the solvents were evaporated to give Et_2O extract (37.88 g) and MeOH extract (31.85 g) as a green oil. The latter extract was partitioned between EtOAc and H_2O. The EtOAc extract (17.66 g) was combined with the Et_2O extract. The total extracts were subjected to silica gel column chromatography (CC) with *n*-hexane-EtOAc gradient increasing the amount of 5% portions EtOAc stepwise to give 110 fractions (30 mL / fraction=fr.). A crude oil (2.605 g) from frs. 32~35 was chromatographed on silica gel with *n*-hexane-EtOAc gradient (20 mL / fr.) to give a cadinane-type sesquiterpene (**3**)(154.1 mg) from frs. 120-126. A crude oil (2.7810 g) from frs. 77-82 was chromatographed on Sephadex-LH-20 with $CHCl_3$-MeOH=1:1 (50 mL / fr.) to give a crude oil (1.913 g) from frs. 5-10, which was further subjected to silica gel CC with $CHCl_3$-EtOAc gradient to afford ptychantins J (**1**) (85 mg) and K (**2**) (1.224 g).

Ptychantin J (1) colorless prisms; mp 213-214℃ (EtOAc-Et_2O) ; $[\alpha]_D^{19}$ -127.2° (*c*1.6, $CHCl_3$); CIMS (iso-Bu): *m/z* 497 $[M+H]^+$, 481, 437, 421 (100%), 359, 343; HR-CIMS (iso-Bu): $[M+H]^+$ *m/z* 497.2748; $C_{26}H_{41}O_9$ requires 497.2750; FT-IR (KBr)cm^{-1}: 3491 (OH), 2972, 1731 (COO), 1371, 1255, 1030; ^1H-NMR (600 MHz) spectral data in $CDCl_3$ (Table 1); ^{13}C NMR (125 Hz) spectral data in $CDCl_3$ (Table 2).

Ptychantin K (2) colorless amorphous powder; $[\alpha]_D^{19}$ -81.9° (*c*1.1, $CHCl_3$); CIMS (iso-Bu): *m/z* 497 $[M+H]^+$, 481, 437, 421 (100%), 377, 317, 301; HR-CIMS (iso-Bu): $[M+H]^+$ *m/z* 497.2758; $C_{26}H_{41}O_9$ requires 497.2750; FT-IR (KBr)cm^{-1}: 3478 (OH), 2973, 1734 (COO), 1369, 1255, 1060; ^{1}H-NMR (600 MHz) spectral data in $CDCl_3$ (Table 1); ^{13}C NMR (125 Hz) spectral data in $CDCl_3$ (Table 2).

A new sesquiterpenoid (3) colorless prisms; mp 177-178°C (Et_2O); $[\alpha]_D^{18}$ +23.7° (*c*1.0, $CHCl_3$); EIMS : *m/z* 238 $[M]^+$, 233, 205, 162, 122 (100%), 95; HR-EIMS: $[M]^+$ *m/z* 238.1928; $C_{15}H_{26}O_2$ requires 238.1933; FT-IR (KBr)cm^{-1}: 3410 (OH), 2935, 1102, 1003, 838; ^{1}H NMR ($CDCl_3$): δ 0.90 (3H, *d*, J= 7.1 Hz, H-15), 1.05 (3H, *s*, H-12), 1.19 (3H, *s*, H-14), 1.20 (1H, *m*, H-1β), 1.23 (1H, *m*, H-7β), 1.23 (1H, *ddd*, J=10.7, 11.8, 12.1 Hz, H-5), 1.31 (3H, *s*, H-13), 1.33 (1H, *ddd*, J=3.8, 11.8, 11.8 Hz, H-10), 1.43 (1H, *ddd*, J=3.6, 12.1, 12.1 Hz, H-6), 1.47(1H, *m*, H-2α), 1.51 (1H, *dd*, J=3.3, 12.9 Hz, H-8α), 1.54 (1H, *m*, H-1α), 1.64 (1H, *m*, H-7), 1.67 (1H, *m*, H-2β), 1.82 (1H, *ddd*, J=3.3, 3.3, 12.9, H-8β), 2.10 (1H, *m*, H-3), 3.45 (1H, *dd*, J=4.9, 10.7 Hz, H-4); ^{13}C NMR ($CDCl_3$): δ 10.4 (*q*, C-15), 18.9 (*t*, C-1), 21.7 (*q*, C-14), 23.7 (*t*, C-7), 24.5 (*q*, C-12), 28.9 (*q*, C-13), 30.0 (*t*, C-2), 30.6 (*d*, C-3), 43.3 (*t*, C-8), 43.4 (*d*, C-5), 48.8 (*d*, C-10), 52.6 (*d*, C-6), 73.0 (*s*, C-9), 81.5 (*d*, C-4), 81.6 (*s*, C-11).

The crystal data for ptychantin J (1) Monoclinic, space group $P2_1$, a=6.133 (3) Å, b=20.422 (14) Å, c=10.809 (10) Å, V=1308 (2) $Å^3$, α=90.00°, β=104.88 (2)°, γ=90.00°, *Z* = 2, D_x= 1.266 Mg m^{-3}, μ (Mo Kα) =0.09 mm^{-1}, λ=0.71073, Final *R* was 0.060 for 1849 reflections. The structure was solved by direct method (Monte-Carlo Multan) and refined by full-matrix least-squares techniques. Diffraction data were obtained using a Mac Science MXC18 diffractiotometer at rt. All diagrams and calculations were performed using maXus (Mac Science, Japan).

Acetylation of ptychantin J (1) A solution of compound (**1**)(45.2 mg) in pyridine (2 mL) was treated with acetic anhydride (2 mL). The mixture was stirred at rt overnight. Ice water was added and the mixture was extracted with $CHCl_3$. The organic phase was washed with 1N HCl, 5% $NaHCO_3$ solution and brine, dried ($MgSO_4$), and evaporated to give a residue (45 mg). The residue was purified by a silica gel column chromatography with *n*-hexane-EtOAc gradient to afford a tetraacetate (**4**)(41.3 mg; 84.2 %) as colorless needles; mp 193-194℃ (EtOAc-Et_2O) ; $[\alpha]_D^{21}$ -70.4° (*c* 1.18, $CHCl_3$); CIMS (iso-Bu): *m/z* 539 $[M+H]^+$, 523, 479, 463 (100%), 403, 343; HR-CIMS (iso-Bu): $[M+H]^+$ *m/z* 539.2843; $C_{28}H_{43}O_{10}$ requires 539.2856; FT-IR (KBr)cm^{-1}: 3472 (OH), 2925, 1732 (COO), 1371, 1253, 1030; ^{1}H-NMR (600

MHz) spectral data in $CDCl_3$ (Table 1); ^{13}C NMR (125 Hz) spectral data in $CDCl_3$ (Table 2).

Acetylation of ptychantin K (2) A solution of compound (**2**)(71.4 mg) in pyridine (2 mL) was treated with acetic anhydride (2 mL). The mixture was stirred at rt overnight and the reaction mixture was treated in the same manner as described above to give the residue (87 mg), which was purified by a silica gel CC with *n*-hexane-EtOAc gradient to afford a pentaacetate (**5**)(70.6 mg; 84.6 %) as colorless needles; mp 183-186°C (EtOAc-Et_2O); $[\alpha]_D^{21}$-46.8° (*c* 0.93, $CHCl_3$); CIMS (iso-Bu): *m/z* 581 $[M+H]^+$, 565, 520, 505 (100%), 463, 343; HR-CIMS (iso-Bu): $M+H]^+$ *m/z* 581.2948; $C_{30}H_{46}O_{11}$ requires 581.2962; FT-IR (KBr)cm^{-1}: 2974, 1738 (COO), 1369, 1241, 1030; ^1H-NMR (600 MHz) spectral data in $CDCl_3$ (Table 1); ^{13}C NMR (125 Hz) spectral data in $CDCl_3$ (Table 2).

Hydrolysis of ptychantin K (2) To a solution of compound (**2**)(301.1 mg) in MeOH (15 mL) was added KOH powder (122 mg) and stirred at rt for 1 h. The reaction mixture was poured into ice water and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give the residue (300.3 mg) which was purified by a silica gel CC with $CHCl_3$-EtOAc gradient to afford a diacetate (**6**)(269.3 mg; 97.7 %) as colorless needles; mp 120-122°C (EtOAc-Et_2O) ; $[\alpha]_D^{19}$ -109.6° (*c*1.40, $CHCl_3$); CIMS (iso-Bu): *m/z* 455 $[M+H]^+$, 439, 424, 379 (100%), 301; HR-CIMS (iso-Bu): $[M+H]^+$ *m/z* 455.2646; $C_{24}H_{39}O_8$ requires 455.2645; FT-IR (KBr)cm^{-1}: 3330 (OH), 2957, 1732 (COO), 1370, 1258, 1028; ^1H-NMR (600 MHz) spectral data in $CDCl_3$ (Table 1); ^{13}C NMR (125 Hz) spectral data in $CDCl_3$ (Table 2).

Acetylation of compound (6) A solution of compound (**6**)(30.3 mg) in pyridine (3 mL) was treated with acetic anhydride (3 mL). The mixture was stirred at rt overnight and the reaction mixture was treated in the same manner as described above to give the residue (40.6 mg), which was purified by a silica gel CC with *n*-hexane-EtOAc gradient to afford a tetraacetate (**4**)(31.4 mg; 87.5 %) as colorless needles; mp 192-194°C (EtOAc-Et_2O); $[\alpha]_D^{21}$ -69.8° (*c* 1.52, $CHCl_3$). The physical and spectroscopic data (IR, ^1H and ^{13}C NMR spectra) of **4** was identical to those of a tetraacetate (**4**) obtained by acetylation of ptychantins J (**1**).

Reaction of compound (6) with 2,2-dimethoxypropane A solution of compound (**6**)(100.1 mg) in dry CH_2Cl_2 (10 mL) was treated with 2, 2-dimethoxypropane (5 mL) and *p*-TsOH (15 mg) and stirred at rt overnight. The reaction mixture was poured into ice water and extracted with $CHCl_3$. The organic layer

was dried over $MgSO_4$ and concentrated *in vacuo* to give the residue (300.3 mg) which was purified by a silica gel CC with *n*-hexane-EtOAc gradient to afford a major acetonide (**7**)(78.7 mg; 72.3 %) and a minor acetonide (**8**)(16.4 mg; 15.1 %).

A major acetonide (7); colorless needles; mp 200-202°C (Et_2O); $[\alpha]_D^{19}$ -87.1° (*c* 1.14, $CHCl_3$); CIMS (iso-Bu): *m/z* 495 $[M+H]^+$, 479, 434, 419 (100%), 377, 301; HR-CIMS (iso-Bu): $[M+H]^+$ *m/z* 495.2968; $C_{27}H_{43}O_8$ requires 495.2958; FT-IR (KBr)cm^{-1}: 3497 (OH), 2959, 1733 (COO), 1369, 1254, 1055; ^{1}H-NMR (600 MHz) spectral data in $CDCl_3$ (Table 1); ^{13}C NMR (125 Hz) spectral data in $CDCl_3$ (Table 2).

A minor acetonide (8); colorless needles; mp 241-243°C (Et_2O); $[\alpha]_D^{21}$ -69.7° (*c* 0.98, $CHCl_3$); CIMS (iso-Bu): *m/z* 495 $[M+H]^+$, 479, 437, 419 (100%), 359, 301; HR-CIMS (iso-Bu): $M+H]^+$ *m/z* 495.2978; $C_{27}H_{43}O_8$ requires 495.2958; FT-IR (KBr)cm^{-1}: 3493 (OH), 2928, 1732 (COO), 1369, 1251, 1029; ^{1}H NMR ($CDCl_3$): δ 1.09 (3H, *s*, H-18), 1.18 (3H, *s*, H-16), 1.30 (3H, *s*, H-20), 1.37 (1H, *m*, H-3α), 1.43, 1.44 (each 3H, s, $(CH_3)_2$C-O), 1.48 (1H, *m*, H-2β), 1.51 (1H, *d*, J= 4.1 Hz, H-5), 1.60 (3H, *s*, H-17), 1.62 (1H, *m*, H-3β), 1.93, 1.94 (each 3H, *s*, -OAc), 1.94 (1H, *d*, J=8.5 Hz, H-9), 1.99 (1H, *m*, H-2α), 2.03 (1H, *dd*, J=0.8, 16.5 Hz, H-12α), 2.31 (1H, *dd*, J= 5.5, 16.5 Hz, H-12β), 3.28, 4.17 (each 1H, J= 11.5 Hz, H-19), 4.28 (1H, *d*, J= 6.9 Hz, H-7), 4.61 (1H, *dd*, J=4.1, 6.9 Hz, H-6), 4.62 (1H, *dd*, J=4.7, 11.0 Hz, H-1), 4.95 (1H, *dd*, J=1.6, 10.7 Hz, H-15), 5.22 (1H, *dd*, J=1.6, 17.0 Hz, H-15), 5.26 (1H, *ddd*, J=0.8, 5.5, 8.5 Hz, H-11), 5.81 (1H, *dd*, J=10.7, 17.0 Hz, H-14).

Preparation of (+)-MTPA ester of compound (7) To a solution of compound (**7**)(20.1 mg) in pyridine (1.5 mL) was added (+)-MTPA chloride (100 mg) and DMAP (30 mg) and stirred at rt for 48 h. The reaction mixture was concentrated in vacuo and then the residue was partitioned between $CHCl_3$ and H_2O. The organic layer was washed with 1N HCl, 5% $NaHCO_3$, and sat. NaCl solution successively and dried over $MgSO_4$. Filtration and evaporation of solvent gave the crude oil (29.7 mg) which was purified by silica gel with *n*-hexane-EtOAc gradient to afford a recovered compound (**7**)(4.5 mg; 22.4 %) and (+)-MTPA ester (**9**)(21.3 mg; 73.7 %) as colorless oil; $[\alpha]_D^{21}$ -9.3° (*c* 2.13, $CHCl_3$); FAB(+)-MS: *m/z* 711 $[M+H]^+$, 189, 119, 55 (100%); HR-FAB(+)-MS: $[M+H]^+$ *m/z* 711.3362; $C_{37}H_{50}O_{10}F_3$ requires 711.3356; FT-IR (KBr)cm^{-1}: 2960, 1737 (COO), 1370, 1251, 1030; ^{1}H NMR ($CDCl_3$): δ 1.09 (3H, *s*, H-16), 1.09, 1.35 (each 3H, s, $(CH_3)_2$C-O), 1.12 (1H, *m*, H-3α), 1.15 (1H, *d*, J=1.4 Hz, H-5), 1.25 (3H, *s*, H-18), 1.52 (3H, *s*, H-20), 1.52 (1H, *m*, H-3β), 1.57 (1H, *m*, H-2β), 1.57 (3H, *s*, H-17), 1.90 (1H, *m*, H-2α), 1.94 (1H, *d*, J=16.2 Hz, H-12α), 1.95, 1.96 (each 3H, *s*, -OAc), 2.14 (1H, *d*, J=7.4 Hz, H-9), 2.27 (1H, *dd*, J= 5.5, 16.2 Hz, H-12β), 3.00, 3.98 (each 1H, J= 12.4 Hz, H-19), 4.44 (1H, *dd*, J=1.4, 4.4 Hz, H-6), 4.78 (1H, *dd*, J=2.7, 6.3 Hz, H-1), 5.00 (1H, *dd*, J=1.6, 10.7 Hz, H-15), 5.24 (1H, *dd*, J=5.5, 7.4 Hz, H-11), 5.32 (1H, *d*, J= 4.4 Hz, H-7), 5.41 (1H, *dd*, J=1.6, 17.0 Hz, H-15), 5.78 (1H, *dd*, J=10.7, 17.0 Hz, H-14).

Preparation of (-)-MTPA ester of compound (7) To a solution of compound (**7**)(20.4 mg) in pyridine (1.5 mL) was added (-)-MTPA chloride (100 mg) and DMAP (30 mg) and stirred at rt for 48 h. The reaction mixture was treated in the same manner as described above to give the residue (30.7mg), which was purified by a silica gel CC with hexane-EtOAc gradient to afford a recovered compound (**7**)(11.3 mg; 55.4 %) and (-)-MTPA ester (**10**)(10.3 mg; 35.1 %) as colorless oil; $[\alpha]_D^{21}$ -18.1° (*c* 2.22, $CHCl_3$); FAB(+)-MS: *m/z* 711 $[M+H]^+$, 189, 119, 55 (100%); HR-FAB(+)-MS: $[M+H]^+$ *m/z* 711.3359; $C_{37}H_{50}O_{10}F_3$ requires 711.3356; FT-IR (KBr)cm^{-1}: 2965, 1735 (COO), 1370, 1251, 1030; ^1H NMR ($CDCl_3$): δ 1.15 (3H, *s*, H-16), 1.09, 1.35 (each 3H, s, $(CH_3)_2C$-O), 1.12 (1H, *m*, H-3α), 1.15 (1H, *d*, J=1.4 Hz, H-5), 1.25 (3H, *s*, H-18), 1.52 (3H, *s*, H-20), 1.52 (1H, *m*, H-3β), 1.57 (1H, *m*, H-2β), 1.57 (3H, *s*, H-17), 1.90 (1H, *m*, H-2α), 1.94 (1H, *d*, J=16.2 Hz, H-12α), 1.95, 1.96 (each 3H, *s*, -OAc), 2.14 (1H, *d*, J=7.4 Hz, H-9), 2.27 (1H, *dd*, J= 5.5, 16.2 Hz, H-12β), 3.00, 3.98 (each 1H, J= 12.4 Hz, H-19), 4.44 (1H, *dd*, J=1.4, 4.4 Hz, H-6), 4.78 (1H, *dd*, J=2.7, 6.3 Hz, H-1), 5.00 (1H, *dd*, J=1.6, 10.7 Hz, H-15), 5.24 (1H, *dd*, J=5.5, 7.4 Hz, H-11), 5.32 (1H, *d*, J= 4.4 Hz, H-7), 5.41 (1H, *dd*, J=1.6, 17.0 Hz, H-15), 5.78 (1H, *dd*, J=10.7, 17.0 Hz, H-14).

The crystal data for compound (3) Orthorhombic, space group $P2_1P2_1P2_1$ a=8.390 (3) Å, b=11.306 (5) Å, c=14.988 (8) Å, V=1422 (11) $Å^3$, α=90.00°, β=90.00°, γ=90.00, Z = 2, D_x= 1114 Mg m^{-3}, μ (Mo Kα) =0.07 mm^{-1}, λ=0.71073, Final *R* was 0.071 for 1416 reflections. The structure was solved in the same method as described above.

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