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Structures of Five New Highly Oxygenated Labdane-Type Diterpenoids, Ptychantins A-E, Closely Related to Forskolin from the Liverwort *Ptychanthus striatus*

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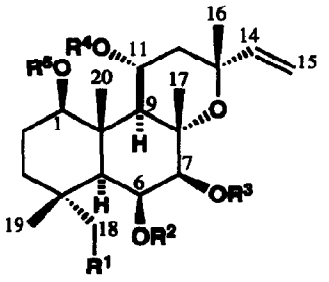
Abstract : Five novel labdane-type diterpenoids, named ptychantins A-E, closely related to forskolin have been isolated from the liverwort *Ptychanthus striatus*, and their absolute structures established by a combination of high resolution NMR and CD spectra, X-ray crystallographic analysis and chemical degradation.

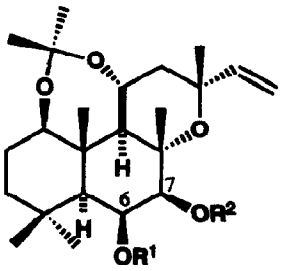
Liverworts contain both terpenoids and aromatic compounds which constitute the oil bodies. We have reported the distribution of a numer of new terpenoids and aromatic compounds in more than 100 species of the liverworts.[2,3] In the course of investigation of the biologically active substances from the liverworts, we isolated five novel labdane-type diterpenoids, ptychantins A-E (**1-5**), closely related to forskolin (**6**)[4] from the ether extract of the liverwort *Ptychanthus striatus* belonging to the Lejeuneaceae. Here we wish to report on the isolation and structure elucidation of **1-5**.

The ether extract (32.7 g) of dry material (1.02 kg) of *P. striatus* collected in Tokushima in 1992 was subjected repeatedly to column chromatography on Sephadex LH-20 ($CHCl_3$: MeOH = 1 : 1) and on silica gel (*n*-hexane-AcOEt, gradient) to afford ptychantins A (**1**)[5](4.54 g), B (**2**)[6](1.02 g), C (**3**)[7](0.32 g), D (**4**)[8] (0.73g) and E (**5**)[9](0.12g).

The IR spectrum of ptychantin A (**1**) ($C_{26}H_{40}O_8$) indicated the presence of a hydroxyl group (3387 cm^{-1}) and acetoxyl groups (1735 cm^{-1}). The ^1H NMR spectrum of **1** (Table 1) showed the presence of five tertiary methyl groups [δ 0.97, 1.02, 1.22, 1.41, 1.53 (each 3H, *s*)], three acetoxyl groups [δ 1.95, 1.96, 2.13 (each 3H, *s*)] and a vinyl group [δ 4.95 (*dd*, J=10.7, 1.5 Hz), 5.17 (*dd*, J=17.1, 1.5 Hz), 5.82 (*dd*, J=17.1, 10.7 Hz)], which was confirmed by the formation of a dihydro derivative (**7**) [δ 0.75 (3H, t, J=7.7 Hz)] on hydrogenation with 10% Pd-C. The stereostructure of **1** was deduced from careful analysis of NOE difference spectra, and finally established by X-ray crystallography[10] as shown in Fig. 1. The absolute configuration of **1** was elucidated by three experimental results described below. Reduction ($LiAlH_4/Et_2O$) of **1** afforded the tetrahydroxy derivative **8**, which was further converted to the monoacetonide **9** and the diacetonide **10** with 2, 2-dimethoxypropane and *p*-TsOH. Hydrolysis (KOH/MeOH, r.t., 5hr) of **1** gave the dihydroxy compound **11**. The CD spectra of **9** and **11** with a shift reagent $[Eu(fod)_3]$[11] showed the positive first extrema at 305 nm (in **9** and **11**), and the negative second extrema at 283 nm (in **9**) and 284 nm (in **11**). Compound **9** was treated with *p*-bromobenzoyl chloride and DMAP in pyridine to give only the mono-*p*-bromobenzoate **12**. The *p*-bromobenzoyl group at C-7 of **12** easily migrated to C-6 on treatment with base ($NaOH/MeCN\text{-}H_2O$) to give **13**. Further *p*-bromobenzoylation of **13** gave a dibenzoate **14**. The CD spectra of **14** showed the positive first

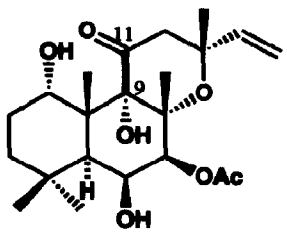
extremum at 251 nm and the negative second extremum at 233 nm. Compound **11** was esterified with (+)- and (-)-MTPA-Cl and DMAP in pyridine to afford the (+)-MTPA ester **15** and the (-)-MTPA ester **16**, respectively. The Δδ Values $[\delta_{(-)}-\delta_{(+)}]^{12}$ are shown in Fig. 2. The absolute configuration of ptychantin A was thus determined as **1**.





1 : R^1=R^3=H, R^2=R^4=R^5=Ac
2 : R^1=H, R^2=R^3=R^4=R^5=Ac
3 : R^1=R^3=R^5=H, R^2=R^4=Ac
4 : R^1=OH, R^3=H, R^2=R^4=R^5=Ac
5 : R^1=R^3=R^4=H, R^2=R^5=Ac
8 : R^1=R^2=R^3=R^4=R^5=H
11 : R^1=R^2=R^3=H, R^4=R^5=Ac
15 : R^1=R^2=H, R^3=(+)-MTPA, R^4=R^5=Ac
16 : R^1=R^2=H, R^3=(-)-MTPA, R^4=R^5=Ac
17 : R^1=OTs, R^3=H, R^2=R^4=R^5=Ac

9 : R^1=R^2=H
12 : R^1=H, R^2=*p*-Br-Bz
13 : R^1=*p*-Br-Bz, R^2=H
14 : R^1=R^2=*p*-Br-Bz



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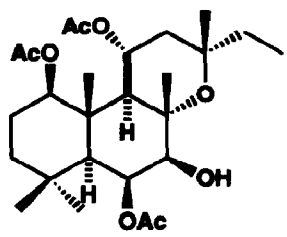

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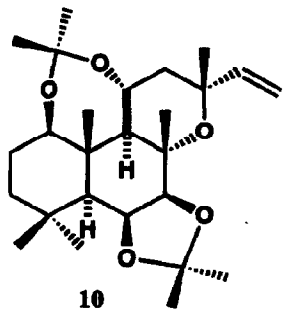

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Ptychantin B (**2**) ($C_{28}H_{42}O_9$) had very similar spectral data to those of **1**. The ^{1}H NMR spectrum of **2** (Table 1) showed the presence of four acetoxyl groups [δ 1.93, 1.94, 2.04, 2.11 (each 3H, *s*)]. As acetylation of **1** afforded **2**, the structure of **2** was determined as the C-7 acetylated compound of **1**.

Ptychantin C (**3**) and E (**5**) had the same molecular formula, $C_{24}H_{38}O_7$, and very similar spectral data to those of **1** and **2**. Acetylation of **3** and **5** afforded **2**. From the 2D NMR analysis and comparison of ^{1}H NMR spectra (Table1), the structures of **3** and **5** were determined as C-1 and C-11 deacetylated compound of **1**, respectively. Ptychantin D (**4**) ($C_{26}H_{40}O_9$) had also very similar spectral data to those of **1**, except for the presence of isolated one methylene signal bearing oxygen functions [δ 3.38, 3.89 (each 1H, *d*, J=11.2Hz)] in the ^{1}H NMR spectra (Table 1). Tosylation of **4** and reduction ($LiAlH_4$ /Et_2O) of the tosylate **17** afforded the tetrahydroxy compound **8**, whose spectral data (IR, ^{1}H and ^{13}C NMR) were identical with those of **8** derived

from **1** by reduction ($LiAlH_4$ /Et_2O). The position of the primary hydroxyl group was determined by the presence of the NOEs between H-19 and H-20, and H-18 and H-6.

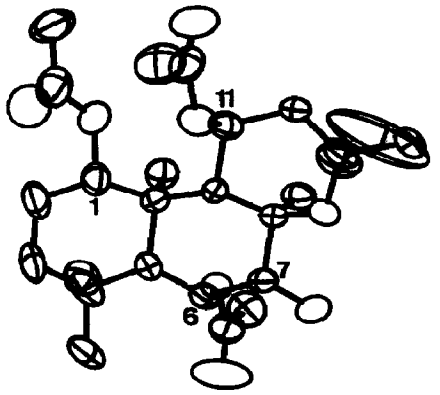

Fig.1 Perspective drawing of **1**.

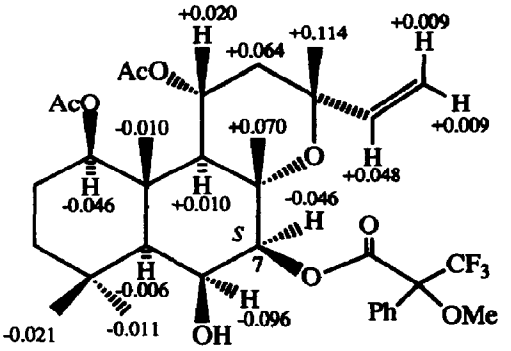

Fig. 2 $\Delta\delta$ values [$\delta_{(-)} - \delta_{(+)}$] were shown in ppm (600 MHz ^{1}H NMR).

Table 1. ^{1}H NMR spectral Data of **1-5**.

Position	1	2	3	4	5
1-H	4.52 *dd*	4.55 *dd*	3.40 *dd*	4.57 *dd*	4.50 *dd*
	(10.3, 5.1)	(10.3, 5.1)	(7.7, 1.5)	(10.8, 4.4)	(10.9, 4.8)
6-H	5.81 *dd*	5.76 *dd*	5.79 *dd*	5.83 *dd*	5.81 *dd*
	(4.2, 2.4)	(4.4, 2.4)	(4.2, 2.4)	(4.2, 2.4)	(4.2, 2.6)
7-H	3.73 *d* (4.2)	5.00 *d* (4.4)	3.75 *d* (4.2)	3.75 *d* (4.2)	3.74 *d* (4.2)
11-H	5.23 *m*	5.27 *m*	5.37 *m*	5.20 *m*	4.17 *m*
14-H	5.82 *dd*	5.74 *dd*	5.85 *dd*	5.81*dd*	5.96 *dd*
	(17.1, 10.7)	(16.8, 10.6)	(17.0, 10.6)	(17.2, 10.6)	(17.0, 10.6)
15-H	4.95 *dd*	4.92 *dd*	4.96 *dd*	4.94 *dd*	5.08 *dd*
	(10.7. 1.5)	(10.6. 1.6)	(10.6. 1.5)	(10.6. 1.5)	(10.6. 1.8)
	5.17 *dd*	5.24 *dd*	5.17 *dd*	5.16 *dd*	5.36 dd
	(17.1, 1.5)	(16.8, 1.6)	(17.0, 1.8)	(17.2, 1.5)	(17.0, 1.8)
16-H	1.20 *s*	1.12 *s*	1.19 *s*	1.20 *s*	1.20 *s*
17-H	1.52 *s*	1.55 *s*	1.47 *s*	1.50 *s*	1.48 *s*
18-H	1.01 *s*	0.97 *s*	1.00 *s*	3.38 *d* (11.2)	1.01*s*
				3.89 *d* (11.2)	
19-H	0.96 *s*	0.95 *s*	0.93 *s*	1.08 *s*	0.98 *s*
20-H	1.40 *s*	1.42 *s*	1.25 *s*	1.36 *s*	1.37 *s*
OAc	1.95 *s*	1.93 *s*	1.97 *s*	1.92 *s*	1.99 *s*
	1.96 *s*	1.94 *s*	2.12 s	1.94 s	2.13 s
	2.13 *s*	2.04 s		2.12 *s*	
		2.11 *s*			

^{1}H NMR Spectra were recorded at 400 MHz using $CDCl_3$ as solvents and TMS as internal standard. Chemical shifts are in δ values. Coupling constants in Hz are in parenthesis.

Ptychantins A-E (**1-5**) are the labdane-type diterpenoids possessing the same absolute configuration as forskolin (**6**) isolated from the Indian herb *Coleus forskohlii* which shows antihypertensive, positive inptoropic, bronchospasmolytic and antithrombotic activities.[13] Compounds **1** and **2** 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.

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References and notes

1. Present address: *Bioorganic Chemistry, Aburahi Laboratories, Shionogi Pharm. Co., Ltd., Koka, Shiga , 520-34 Japan*
2. Y. Asakawa, Chemical Constituents of Hepaticae in "*Progress in the Chemistry of Organic Natural Products*"(W. Herz, H. Grisebach, and W. G. Kirby eds.) Vol. **42**, P. 1, Springer, Wien (1982).
3. Y. Asakawa, Biologically Active Terpenoids and Aromatic Compounds from Liverworts and the Inedible Mushroom *Cryptoporus volvatus*. in "*Bioactive Natural Products: Detection, Isolation, and Structural Determination*" (S. M. Colegate and R. J. Molyneux eds.) P. 319, CRC Press, Florida (1993).
4. S. V. Bhat, B. S. Bajwa, H. Dornauer, N. J. de Souza, and H.-W. Fehlhaber, *Tetrahedron Lett.*, 1669 (1977).
5. mp 202-204°; $[\alpha]_D^{21}$=-75.3° (*c* 0.81, $CHCl_3$); EI-MS: *m/z* 465 (M^+-15), 405 (100), 365, 205; Anal. Calcd for $C_{25}H_{40}O_8$; C, 64.98; H, 8.39 ; Found : C, 64.79, H, 8.49; FT-IR (KBr) cm^{-1}: 3561 (OH), 1736(CO), 1367, 1255, 1103, 1044.
6. mp 222-223°; $[\alpha]_D^{25}$=-49.3° (*c* 0.51, $CHCl_3$); EI-MS: *m/z* 507 (M^+-15), 447 (100), 387, 285, 267; Anal. Calcd for $C_{28}H_{42}O_9$; C, 64.35; H, 8.10 ; Found : C, 64.33, H, 8.20; FT-IR (KBr) cm^{-1}: 1738 (CO), 1370, 1242, 1150, 1086, 1046.
7. mp 213-215°; $[\alpha]_D^{21}$=-54.0°(*c* 0.72, $CHCl_3$); HR-MS: m/z 438.2626, $C_{24}H_{38}O_7$ requires 438.2618; EI-MS: *m/z* 438 (M^+), 423, 378, 363 (100%), 285; 187, 177; FT-IR (KBr) cm^{-1}: 3497 (OH), 1739, 1715 (CO), 1366, 1242, 1148, 1105, 1040.
8. mp 131-132°; $[\alpha]_D^{20}$=-68.9° (*c* 0.57, $CHCl_3$); HR-MS: m/z 496.2678, $C_{26}H_{40}O_9$ requires 496.2673; EI-MS: *m/z* 496 (M^+), 438, 421 (100), 377, 299; 167 ;FT-IR (KBr) cm^{-1}: 3468(OH), 1738 (CO), 1371, 1258, 1078, 1040.
9. mp 192-193°; $[\alpha]_D^{21}$=-17.4° (*c* 0.47, $CHCl_3$); HR-MS: m/z 438.2595, $C_{24}H_{38}O_7$ requires 438.2618; EI-MS: *m/z* 438 (M^+), 423 (100%), 378, 363 , 285; 187, 177; FT-IR (KBr) cm^{-1}: 3449 (OH), 1715(CO), 1366, 1252, 1117, 1042.
10. The crystal data for **1** are as follows : monoclinic; space group $P2_1$ with a=12.863 (8), b=35.759 (34), c=6.199 (4)Å, β=115.37(5)°, V=2576(3)$Å^3$, Z=4. Final R value was 0.09 for 5503 reflections. The supplementary materials have been deposited at the Cambridge Crystallographic Data Centre.
11. J. J. Partridge, V. Toome, and M. R. Uskokovic, *J. Am. Chem. Soc.*, **98**, 3739 (1976).
12. T. Kusumi, T. Hamada, M. O.Ishitsuka, I. Ohtani, and H. Kakizawa, *J. Org. Chem.*, **57**, 1033 (1992).
13. S. V. Bhat, A. N. Dohadwalla, B. S. Bajwa, N. K. Dadkar, H. Dornauer, and N. J. de Souza, *J. Med. Chem.*, **26**, 486 (1983).

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