Lembehsterols A and B, Novel Sulfated Sterols Inhibiting Thymidine Phosphorylase, from the Marine Sponge Petrosia strongylata

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Lembehsterols A (1) and B (2), two novel sulfated sterols, were isolated from the marine sponge Petrosia strongylata. Both sterols showed inhibitory activity against thymidine phosphorylase, which is an enzyme related to angiogenesis in solid tumors. The structures of these sulfated sterols were established on the basis of chemical and physicochemical evidence.

Key words lembehsterol; thymidine phosphorylase; angiogenesis; sulfated sterol; sponge

In the growth and metastasis of solid tumors, angiogenesis has an important role. Thymidine phosphorylase (TP) is an enzyme catalyzing the reversible phosphorylization of thymidine and is identical with the platelet-derived endothelial cell growth factor (PD-ECGF), which has been shown to be an angiogenic factor.1,2) TP also catalyzes transport of deoxyribose from one deoxynucleoside to another nucleo-base to form a secondary deoxynucleoside.3–5) It was shown that TP was expressed at higher levels in a wide variety of solid tumors than in the adjacent nonneoplastic tissues6) and the increased expression of TP promotes angiogenesis, tumor growth, invasiveness and ability to metastasize.7,8) This evidence suggests that a compound which inhibits TP activity may delay abnormal angiogenesis and progression of various tumors. In fact, several inhibitors of TP showed suppression of angiogenesis and tumor growth.9)

In the course of our study of bioactive substances from marine organisms,10) we focused on a search for selective inhibitors of TP and isolated novel sulfated sterols, lembhsters A (1) and B (2), from the marine sponge Petrosia strongylata. This paper describes the elucidation of their absolute stereostructure.

Results and Discussion

A crude TP was obtained from the lysate of the cultured KB/TP cells,9) which was produced by transfection of PD-ECGF cDNA from human KB epidermoid carcinoma cells (KB 3-1). Using this crude TP, we have constructed the bioassay system by HPLC to search inhibitors of TP. The MeOH extract of the marine sponge Petrosia strongylata, collected at Bitung, Indonesia, showed inhibitory activity against TP and was subjected to bioassay-guided separation. The extract was partitioned into an AcOEt–water mixture to obtain an AcOEt soluble portion. The aqueous phase was further partitioned to give a secondary deoxynucleoside.3–5) It was shown that TP was expressed at higher levels in a wide variety of solid tumors than in the adjacent nonneoplastic tissues6) and the increased expression of TP promotes angiogenesis, tumor growth, invasiveness and ability to metastasize.7,8) This evidence suggests that a compound which inhibits TP activity may delay abnormal angiogenesis and progression of various tumors. In fact, several inhibitors of TP showed suppression of angiogenesis and tumor growth.9)

As shown in Fig. 2, the relative stereostructure of the ring part in 1 was elaborated on the basis of the nuclear Overhauser effect spectroscopy (NOESY) correlations and the $^{3}$JHH couplings. The small coupling constants (br s) for H-2 and H-3 suggested equatorial orientation of H-2 and H-3, respectively. Furthermore, the W-type coupling between H-1β and H-3 also indicated equatorial orientation of H-3. The β-axial orientation of H-6 was deduced from the large coupling constants between H-6 and H-5, H-7α, and the NOESY correlations between H-6 and H-8, H-19. The stereochemistry of C-17 and C-20 were determined by the NOESY correlations between H-17 and H-21, H-28; H-28 and C-8; C-15 to C-27) through C-12 and C-19 and H-8, H-19. The stereocchemistry of C-17 and C-20 were determined by the NOESY correlations between H-17 and H-21, H-28; H-28 and C-8; C-15 to C-27) through C-12 and C-19 and H-8, H-19. The stereocchemistry of C-17 and C-20 were determined by the NOESY correlations between H-17 and H-21, H-28;
and H-12α, H-12β and H-21; H-16β and H-20.\(^{13}\) Hydrolysis of 1 in dioxane and 10% aq. H\(_2\)SO\(_4\) mixture gave a tri-desulfated derivative 4, and then modified Mosher’s method\(^{14}\) was applied to the 6-hydroxyl group of 4. Thus, 4 was treated with S-(-)- or R-(-)-2-methoxy-2-phenyl-2-trifluoromethylacetic acid (MTPA), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), and \(N,N\)-dimethylaminopyridine (DMAP) to furnish the 6-O-R-(-)-MTPA ester 5a and the 6-O-S-(+)-MTPA ester 5b, respectively. The characteristic proton signals of 5a and 5b were assigned and the absolute configuration at C-6 of 4 was determined as S by the analysis of the \(\Delta\delta\) values (Fig. 3). Consequently, the total structure of lembehsterol A was established to be 5\(\alpha\)-cholest-9(11)-en-2\(\beta\),3\(\alpha\),6\(\alpha\)-triol-2,3,6-trisulfate 1 (Chart 1).

The molecular formula of lembehsterol B (2) was determined as C\(_{28}\)H\(_{44}\)O\(_8\)S\(_2\)Na\(_2\) by negative ion HR-FAB-MS. The IR spectrum of 2 exhibited an absorption band due to a sulfate group (1211 cm\(^{-1}\)). The \(^1\)H- and \(^{13}\)C-NMR spectra of 2 were closely similar to those of 1, except for the additional signals assignable to olefin [\(\delta\) 5.32 (d-like, \(J=4.9\) Hz), \(\delta\) 120.6 (d), 136.2 (s)] and disappearance of the oxygenated methine at C-6 of 1 (Table 1). On the basis of 2D-NMR analysis of 2, the planar structure of lembehsterol B was as...
shown in Chart 1. The relative stereochemistry of the ring part and C-20 in 2 were also elaborated on the basis of the NOESY correlations and the 3JHH couplings. Consequently, lembeshsterol B was elucidated to be cholest-5,9(11)-dien-2β,3α-diol-2,3-sulfate (2).

Lembeshsterols A (1) and B (2) showed inhibitory activity against TP at 41 and 45 μM concentration (IC50 value) in this assay system, respectively. Tri-desulfated derivative 4 of 1 did not show inhibition at 230 μM concentration, suggesting the importance of sulfate group for the inhibitory activity against TP. The IC50 value of TPI,71 which was a nucleoside analogue known as one of the most potent inhibitors, was 12 nm. Several sulfated sterols have been isolated from marine sponges, which show a variety of biological activities (e.g., HIV inhibitor).12,13 However, there is no report of sulfated sterols having inhibitory activity against TP. Most of the known TP inhibitors are nucleoside analogues, and lembeshsterols A and B are the first examples having a non-nucleoside skeleton. The action mechanism of these sulfated sterols is under investigation.

Experimental

Isolation The titled dried marine sponge (300 g), which was collected in July, 1999 at Lembeh Island, Bitung, Indonesia, was initially steeped in MeOH. The residue obtained by evaporation of the solvent under reduced pressure was partitioned into an AcOEt–water mixture (1:1) to obtain an AcOEt soluble portion (1.3 g). The aqueous phase was further partitioned with n-BuOH to obtain an n-BuOH soluble portion (3.7 g), and then the aqueous phase was evaporated under reduced pressure to give an H2O soluble portion (10 g). The H2O soluble portion (1 g) was separated by Diaion HP-20 column to give an active MeOH eluate, which was further separated by Sephadex LH-20 column (MeOH: H2O = 2:1) to afford an active fraction (120 mg) [30% inhibition at 25 μg/ml]. Then, the active fraction was purified by reversed-phase HPLC (YMC-Pack A-323 RP-18, MeOH: H2O = 8:2, 0.1 M NaClO4) to provide lembeshsterol A (41.4 mg, 4.1% yield from the H2O soluble portion) and bisteresterol sulfate B12 (4.3 mg, 0.2% yield). The n-BuOH soluble portion (1.42 g) was separated by SiO2 column (CHCl3: MeOH: H2O 8:2, 5 M NaClO4) to afford a crude fraction (200 mg) [33% inhibition at 25 μg/ml] and the active fraction was further purified by ODS column (MeOH:H2O–H2O) and reversed-phase HPLC (YMC-Pack A-323 RP-18, MeOH: H2O = 9:1, 0.1 M NaClO4) to afford lembeshsterol B (2, 65 mg, 4.6% yield from the n-BuOH soluble portion).

Lembeshsterol A (1): [α]25 +50.1° (c=0.41, MeOH). FAB-MS: m/z 715 (M+Na)+, m/z 613 (M+Na3SO4Na+), m/z 595 (M+Na3SO4Na2), m/z 493 (M+Na3SO4Na3), HR-FAB-MS m/z: C29H46O12Na3S4 (C29H45O12S3Na2: 715.1868; Found: 715.1849. IR (KBr): 3436, 1373, 1211 cm−1). The residue was purified by SiO2 column (CHCl3: MeOH: H2O 5:3:1) to furnish lembeshsterol A (2, 65 mg, 4.6% yield from the H2O soluble portion) and bisteresterol sulfate B12 (4.3 mg, 0.2% yield).

Lembeshsterol B (2): [α]25 +39.0° (c=0.32, MeOH). FAB-MS: m/z 595 (M+Na)+, m/z 493 (M+Na3SO4Na+), m/z 475 (M+Na3SO4Na2), HR-FAB-MS m/z: C30H45O12S4Na (C28H45O12S3Na2: 715.1868; Found: 715.1849. IR (KBr): 3436, 1373, 1211 cm−1). The residue was purified by SiO2 column (CHCl3: MeOH: H2O 5:3:1) to furnish lembeshsterol B (2, 65 mg, 4.6% yield from the H2O soluble portion) and bisteresterol sulfate B12 (4.3 mg, 0.2% yield).

Hydrolysis of Lembeshsterol A (1) A solution of 1 (1.0 mg) in dioxane (1.0 ml) and 10% acq. H2SO4 (1.0 ml) was refluxed at 140 °C for 3 h. The reaction mixture was poured into saturated acq. NaHCO3, then the whole was evaporated in vacuo. The residue was purified by Diaion HP-20 column (H2O–MeOH) to give a crude product. After evaporation of the solvent, the residue was purified by SiO2 column (CHCl3: MeOH = 3:1) to furnish a tri-desulfated derivative 4 (0.5 mg). FAB-MS: m/z 455 (M+Na)+, m/z 439 (M+Li)+. HR-FAB-MS m/z: C30H44O12Na3S5 (C28H43O12S4Na2: 692.2375; Found: 692.2391. IR (KBr): 3436, 1373, 1211 cm−1). The residue was purified by SiO2 column (CHCl3: MeOH = 3:1) to furnish a tri-desulfated derivative 4 (0.5 mg).