Pseudodistomin C, a New Piperidine Alkaloid with Unusual Absolute Configuration from the Okinawan Tunicate *Pseudodistoma kanoko*

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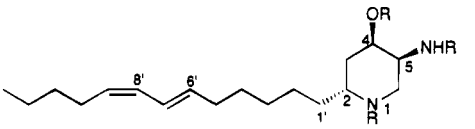
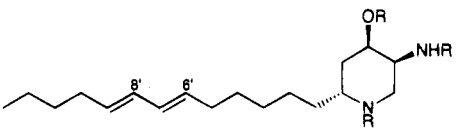
Pseudodistomin C, a new cytotoxic piperidine alkaloid, was isolated from the Okinawan tunicate *Pseudodistoma kanoko* and the structure elucidated by spectral data, chemical degradations, and synthesis. The absolute configurations of the C-4 and C-5 chiral centers of pseudodistomin C were revealed by chiral HPLC analysis to be 4*S* and 5*R*, which were opposite to those of pseudodistomins A and B, previously isolated from the same tunicate, having the same planar structure as to the piperidine nucleus.

During our studies on bioactive substances from marine organisms,[1] we previously investigated extracts of Okinawan tunicate *Pseudodistoma kanoko* and isoalted pseudodistomins A (**1**) and B (**2**),[2] the first piperidine alkaloids obtained from marine sources. The structure of their side-chain moiety was later revised,[3,4] while the structure of the piperidine nucleus was established by synthesis of tetrahydroacetyl derivatives of **1** and **2** achieved by three groups.[5] We recently further examined extracts of this tunicate and succeeded in isolating a new piperidine alkaloid, pseudodistomin C (**3**), which exhibited cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cells in vitro (IC_{50} values, 2.3 and 2.6 μg/mL, respectively). This paper describes the isolation and structure elucidation of **3**. Unexpectedly, pseudodistomin C (**3**) was revealed to have the opposite absolute configurations at the C-4 and C-5 positions from those found in **1** and **2**.

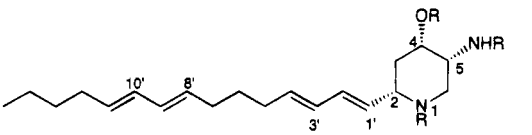
The tunicate *P. kanoko*, collected off Ie Island, Okinawa, was extracted with methanol/toluene (3:1), and the extract was partitioned between toluene and water. The toluene-soluble material was subjected to silica gel flash column chromatography ($CHCl_3$/*n*-BuOH/AcOH/H_2O, 1.5:6:1:1) followed by preparative TLC (silica gel, $CHCl_3$/MeOH/H_2O, 6:4:0.7) to afford pseudodistomin C (**3**, 0.03% yield, wet weight). A portion of the pseudodistomin-containing fraction from the silica gel column was acetylated (Ac_2O/pyridine), and the acetate mixture was purified by reversed-phase HPLC (ODS, MeOH/H_2O, 85:15) to give the acetate **6** of pseudodistomin C (**3**) together with acetates **4** and **5**[2] of pseudodistomins A (**1**) and B (**2**). The acetate **6** was used for characterizations and spectral studies.



1 R = H **4** R = Ac



2 R = H **5** R = Ac



3 R = H **6** R = Ac

Pseudodistomin C acetate (**6**), colorless oil, $[\alpha]^{22}_D$ +85° (*c* 0.98, $CHCl_3$), was shown to have a molecular formula of $C_{26}H_{40}N_2O_4$ by the HREIMS data (*m*/*z* 444.2925, M^+, Δ −4.3 mmu), implying that **6** contains two carbon and two hydrogen atoms (26 amu) more than the acetates of pseudodistomins A and B. The UV spectrum of **6** showed an absorption maximum at 235 nm with a molar absorption (ε 37000) being almost twice as large as those of **4** and **5** (ε 17000 and 18000, respectively),[2] which was suggestive of the presence of two conjugated diene chromophores for **6**.

The ^1H NMR spectra of **4** and **5** in $CDCl_3$ revealed signals of two conformers but all protons were assignable.[2,6] In contrast, the ^1H NMR spectrum of **6** in $CDCl_3$ showed such broad signals that no signals could be assigned. 2D NMR experiments (^1H–^1H COSY, HSQC, and HMBC) of **6** were therefore carried out in a CD_3OD solution, which showed relatively well resolved signals. Since the ^1H NMR spectrum of natural compound **3** in a C_5D_5N solution had better resolution, ^1H–^1H COSY and HSQC spectra of **3** were recorded in this solution. From these spectral data, pseudodistomin C (**3**) was suggested to consist of a piperidine moiety and an unsaturated side-chain; the piperidine ring has the same substituents (4-hydroxyl and 5-amino groups) as those of pseudodis-

[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1995.

(1) Takeuchi, S.; Kikuchi, T.; Tsukamoto, S.; Ishibashi, M.; Kobayashi, J. *Tetrahedron* **1995**, *51*, 5979–5986 and references cited therein.

(2) Ishibashi, M.; Ohizumi, Y.; Sasaki, T.; Nakamura, H.; Hirata, Y.; Kobayashi, J. *J. Org. Chem.* **1987**, *52*, 450–453.

(3) (a) Kiguchi, T.; Yuumoto, Y.; Ninomiya, I.; Naito, T.; Deki, K.; Ishibashi, M.; Kobayashi, J. *Tetrahedron Lett.* **1992**, *33*, 7389–7390. (b) Kiguchi, T.; Yuumoto, Y.; Ninomiya, I.; Naito, T. *Tennen Yuki Kagobutsu Toronkai Yoshishu* **1992**, *34*, 392–399.

(4) Pseudodistomin A (**1**) was revealed to possess 6′*E*,8′*Z*-diene in the side-chain: Ishibashi, M.; Deki, K.; Kobayashi, J. *J. Nat. Prod.* **1995**, *58*, 804–806.

(5) Racemate: (a) Naito, T.; Yuumoto, Y.; Ninomiya, I.; Kiguchi, T. *Tetrahedron Lett.* **1992**, *33*, 4033–4036. (b) Utsunomiya, I.; Ogawa, M.; Natsume, M. *Heterocycles* **1992**, *33*, 349–356. Optically active form: (c) Knapp, S.; Hale, J. J. *J. Org. Chem.* **1993**, *58*, 2650–2651. (d) Naito, T.; Ikai, M.; Shirakawa, M.; Fujimoto, K.; Ninomiya, I.; Kiguchi, T. *J. Chem. Soc. Perkin Trans. 1* **1994**, 773–775.

(6) The difference of the appearance in the ^1H NMR spectra of the acetate **6** from those of **4** and **5** might be ascribable to the difference of the relative stereochemistry of the piperidine nucleus.

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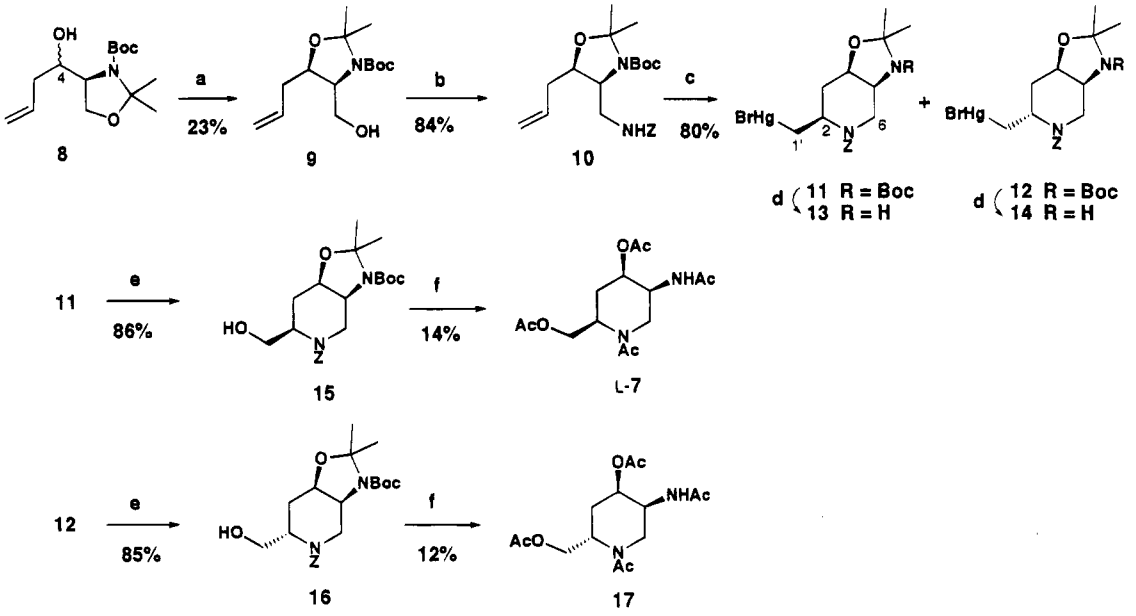
Scheme 1[a]

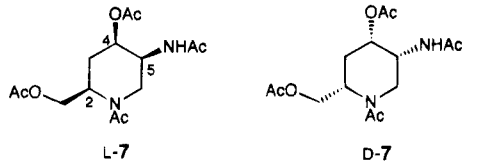


[a] (a) (1) *p*-TsOH, MeOH; (2) PivCl, pyridine; SiO_2 column, hexane/EtOAc (3:1); (3) DMP, BF_3·OEt; (4) 2.5 N KOH, MeOH; (b) (1) phthalimide, DIAD, PPh_3; (2) H_2NNH_2·H_2O, EtOH; (3) ZCl, 2 N NaOH; (c) (1) $Hg(OTFA)_2$, $CHCl_3$; (2) $NaHCO_3$; (3) NaBr; (d) TFA, CH_2Cl_2; (e) $NaBH_4$,O_2, DMF; (f) (1) TFA, CH_2Cl_2; (2) Ac_2O, pyridine; (3) H_2, Pd/C, EtOH; (4) Ac_2O, pyridine.

tomins A and B (**1** and **2**), and the side-chain attached to C-2 contains two dienes.

The $^1H-^1H$ COSY spectrum of **3** showed a clear cross-peak between a piperidine-ring proton on C-2 (δ_H 3.40) and an olefinic proton at C-1′ (δ_H 5.83). Thus, one of the two dienes was shown to be located at the C-1′–C-4′ position. To clarify the position of the second diene, compound **3** was treated with ozone followed by reduction with $NaBH_4$ and acetylation to give a crude product, from which the diacetate of 1,5-pentanediol was detected on the basis of reversed-phase TLC and HPLC analyses.[7] The second diene was therefore deduced to be at the C-8′–C-11′ position. The geometries of the double bonds were inferred to be all *E* from the coupling constants (**3**: $J_{1',2'} = J_{3',4'} = 15.4$ Hz) and the ^{13}C chemical shifts of the allylic methylenes (C-5′, C-7′, and C-12′: δ_C 31.7–32.4).[8]

To obtain an unambiguous evidence of the stereochemistry of the piperidine ring portion of pseudodistomin C (**3**), the tetraacetate **7**, which was obtained by HPLC



purification of the ozonolysis product derived from **3**, was prepared as an optically active form as follows (Scheme 1). Oxazolidine homoallyl alcohol **8**, prepared from L-serine by literature procedures,[9–11] was transformed via four steps into isomeric oxazolidine alcohol **9**. Mitsunobu reaction[12] of **9** followed by exchange of the protective group afforded a benzyl carbamate (**10**), which was subjected to amide mercuration[13] to give (2*R*)- and (2*S*)-piperidine derivatives (**11** and **12**) in a ratio of 54:46. The stereochemistries of C-2[14] of **11** and **12** were clearly assigned on the basis of NOESY spectra of **13** and **14**,[15] which were obtained by deprotection of the Boc group, respectively. The piperidine derivatives **11** and **12** were both oxidatively demercurated to give primary alcohols **15** and **16**, which were transformed via four steps into tetraacetates L-**7**[16] and **17**, respectively. The ^1H NMR spectrum of the tetraacetate **7** obtained from natural specimen of pseudodistomin C (**3**) was revealed to be identical with that of the former [(2*R*,4*R*,5*S*)-derivative, L-**7**]. Since the latter tetraacetate **17** possesses the same relative configurations at C-2, -4, and -5 positions on the

(7) Diacetates of 1,4-butanediol, 1,5-pentanediol, and 1,6-hexanediol were used for authentic samples for TLC analysis [ODS, 65% MeOH; spray reagent: 12 molybdo(IV)phosphoric acid *n*-hydrate (12 g) in 85% H_3PO_4 (7.5 mL), concd H_2SO_4 (25 mL), and H_2O (500 mL); R_f values: 0.56, 0.47, and 0.41, respectively], and the ozonolysis product showed a spot (R_f 0.47). HPLC analysis (Capcelpak C18; 55% MeOH; 4.6 × 250 mm; flow rate, 0.8 mL/min; RI detection) showed that the ozonolysis product gave a peak of the same retention time with diacetate of 1,5-pentanediol (t_R 9.2 min).

(8) The allylic carbons of *cis*- and *trans*-double bonds in the aliphatic chains are reported to resonate approximately at 27 and 32 ppm, respectively: Gunstone, F. D.; Pollard, M. R.; Scrimgeour, C. M.; Vedanayagam, H. S. *Chem. Phys. Lipids* **1977**, *18*, 115–129.

(9) (a) Garner, P.; Park, J. M. *J. Org. Chem.* **1987**, *52*, 2361–2364. (b) Garner, P.; Park, J. M. *Org. Synth.* **1991**, *70*, 18–28.

(10) The homoallyl alcohol **8** used in this study was a 1:1 mixture of diastereomers at C-4,[14] obtained by Grignard reaction using allylmagnesium bromide with the oxazolidine aldehyde.[9,11] After deprotection of the acetonide group of **8** and esterification of the primary alcohol with pivaloyl chloride, undesired 4,5-*threo* diastereomer was removed by silica gel column chromatography (see, Experimental Section).

(11) For studies on the stereoselective preparation of the homoallyl alcohol **8**, see: (a) Hafner, A.; Duthaler, R. O.; Marti, R.; Rihs, G.; Rothe-Streit, P.; Schwarzenbach, F. *J. Am. Chem. Soc.* **1992**, *114*, 2321–2336. (b) Roush, W. R.; Hunt, J. A. *J. Org. Chem.* **1995**, *60*, 798–806.

(12) Sisko, J.; Henry, J. R.; Weinreb, S. M. *J. Org. Chem.* **1993**, *58*, 4945–4951.

(13) Takahata, H.; Bandoh, H.; Momose, T. *J. Org. Chem.* **1992**, *57*, 4401–4404.

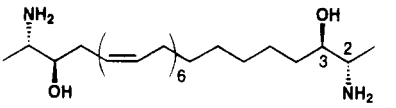
(14) The numberings described here correspond to that of pseudodistomin C (**3**).

(15) NOESY correlations clearly observed: **13**, H_2-1′/H-6β; **14**, H_2-1′/H-6α and H_2-1′/H-4.[14]

(16) For the synthetic compounds, those prepared from L-serine were prefixed by "L" (*e.g.*, L-**7**), while those prepared from D-serine were prefixed by "D" (*e.g.*, D-**7**).

piperidine ring as those of pseudodistomins A (**1**) and B (**2**),[17] relative configurations of **3** proved to be different from those of **1** and **2**. The sign of optical rotation of synthetic L-**7** ($[\alpha]_D$ −19°) was opposite to that of the tetraacetate **7** ($[\alpha]_D$ +16°) derived from the natural specimen of **3**. The absolute configuration of pseudodistomin C (**3**) was therefore revealed as 2*S*, 4*S*, and 5*R*. This result was, however, quite unexpected since the piperidine alkaloids isolated from the same tunicate possess different stereochemistries at C-4 and C-5 positions. Further unambiguous confirmation of this conclusion, therefore, may be required. Thus, we prepared the enantiomer D-**7**[16] from D-serine by the same procedures as above and subjected it to chiral HPLC analysis, which established that the tetraacetate **7**, derived from natural specimen **3**, showed the same retention time as the enantiomer D-**7** prepared from D-serine (see, Experimental Section), thus firmly corroborating the conclusion described above.

Structures of pseudodistomins are suggestive that they are biogenetically classified as sphingosine derivatives, and a precursor of pseudodistomins A (**1**) and B (**2**) may be reasonably assumed to be D-(+)-*erythro*-sphingosine; the absolute configurations of (4*R*)-hydroxyl and (5*S*)-amino groups of **1** and **2** are coincident with those of corresponding positions of D-(+)-*erythro*-sphingosine containing (3*R*)-hydroxyl and (2*S*)-amino groups. It was, however, quite surprising that pseudodistomin C (**3**), isolated from the same tunicate as **1** and **2**, possesses (4*S*,5*R*)-configurations. Pseudodistomin C (**3**), therefore, has to be derived from unusual L-(−)-*erythro*-spingosine (C_{20}-homolog), and the biosynthetic cyclization process of **1**−**3** is assumed to give commonly the same stereochemistry at C-2 of the piperidine ring since all these compounds **1**−**3** possess the same 2*S*-configuration.[18] Precedence for the existence of enantiomeric isomers within the same organisms have recently been reported. Leucetamol A (**18**) was isolated from a sponge *Leucetta microraphis*,[19] and this amino alcohol was described as a racemate; viz. (2*S*,3*R*)- and (2*R*,3*S*)-isomers were concurrently present in the sponge. From a sponge *Xestospongia* sp. an amino alcohol **19** was isolated[20] and was shown to have (2*R*,3*S*)-configurations by synthesis.[21] Crucigasterins (*e.g.*, **20**) were isolated from a tunicate *Pseudodistoma crucigaster* and were reported to possess (2*R*,3*S*)-configurations.[22] Thus, these amino alcohols (**19** and **20**) were proposed to be biosynthetically derived from unusual D-alanine. These facts may provide a sort of rationale of the coexistence of pseudodistomins A/B and C with opposite absolute stereochemistries at C-4 and C-5 positions in the extract of the tunicate *P. kanoko*.



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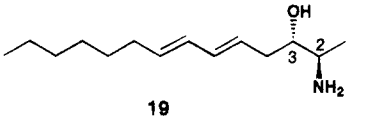

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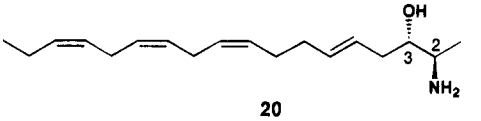

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Experimental Section

Collection, Extraction, and Isolation. The tunicate *P. kanoko*[23] was collected off Ie Islands, Okinawa, using SCUBA (−5 to −10 m) and kept frozen until used. The methanol/toluene (3:1, 1 L × 2) extract of this tunicate (0.4 kg) was partitioned between toluene (500 mL × 4) and 1 M NaCl (1 L). The toluene-soluble fraction was evaporated under reduced pressure to give a crude residue (1.68 g), which was partially (0.76 g) subjected to a silica gel column chromatography (2.4 × 36 cm) with $CHCl_3$/*n*-BuOH/AcOH/H_2O (1.5:6:1:1). The fraction (224 mg) eluting from 930 mL to 1100 mL was ninhydrin-positive and containing a mixture of piperidine alkaloids. A part of this fraction (11 mg) was separated by preparative TLC ($CHCl_3$/MeOH/H_2O, 6:4:0.7) to afford pseudodistomin C (**3**, 2.6 mg; 0.03% yield,[24] wet weight). Another part (65 mg) of the ninhydrin-positive fraction was treated with Ac_2O (0.6 mL) and pyridine (0.6 mL) at rt overnight, and the acetate mixture was purified by reversed-phase HPLC (Develosil ODS-5, 5 μm, 10 × 250 mm; eluent: MeOH/H_2O, 85:15; flow rate: 2.0 mL/min; detection: UV at 230 nm) to give pseudodistomin C acetate (**6**, 5.5 mg, t_R 25.1 min) together with acetates of pseudodistomins A and B (**4**, 6.2 mg, t_R 19.4 min; **5**, 8.6 mg, t_R 21.1 min).[2]

Pseudodistomin C acetate (6): colorless oil; $[\alpha]^{22}_D$ +85° (*c* 0.98, $CHCl_3$); IR (KBr) 3400, 1740, and 1640 cm^{-1}; UV (MeOH) λ_{max} 235 nm (ε 37000); ^1H NMR (CD_3OD) δ_H 0.95 (3H, t, *J* = 7.0 Hz, H$_3$-15′), 1.33 (4H, m; H$_2$-13′ and H$_2$-14′), 1.50 (2H, m; H$_2$-6′), 1.98 (1H, m; H-3a), 2.10 (4H, m; H$_2$-5′ and H$_2$-12′), 2.19 (2H, m; H$_2$-7′), 2.20 (9H, s; CH_3CO), 2.23 (1H, m; H-3b), 2.97 (1H, m; H-6a), 4.01 (1H, m; H-6b), 4.49 (1H, br s; H-5), 4.69 (1H, br s; H-2), 5.11 (1H, m; H-4), 5.59 (2H, m; H-1′ and H-4′), 5.70 (2H, m; H-8′ and H-11′), 6.00 (2H, m; H-2′ and H-3′), and 6.12 (2H, m; H-9′ and H-10′); ^{13}C NMR (CD_3OD) δ_C 14.2 (C-15′), 23.3 (C-14′), 30.2 (C-6′), 32.9, 33.0, 33.1, 33.3 (C-5′, 7′, 12′, and 13′), 37.2 (C-6), 42.4 (C-3), 49.2 (C-5), 70.2 (C-4), 130.8, 131.0, 131.1, 131.2, 131.8, 132.2, 132.4, 133.2 (C-1′−C-4′, and C-8′−C-11′), 172.0, 172.9, and 173.2 ($COCH_3$); EIMS *m*/*z* 444 (M^+), 384 (M^+ − AcOH), 181 (M^+ − AcOH − $C_{15}H_{23}$), 139 (M^+ − AcOH − $C_{15}H_{23}$ − CH_2CO), 122 (M^+ − AcOH − $C_{15}H_{23}$ − CH_3CONH_2), and 80 (M^+ − AcOH − $C_{15}H_{23}$ − CH_3CONH_2 − CH_2CO); HREIMS found *m*/*z* 444.2945 (M^+; calcd for $C_{26}H_{40}O_4N_2$, 444.2988).

Pseudodistomin C (3): ^1H NMR (C_5D_5N) δ_H 0.80 (3H, t, *J* = 7.0 Hz; H$_3$-15′), 1.4−1.3 (6H, br s; H$_2$-6′, 13′, and 14′), 2.01 (6H, m; H$_2$-5′, 7′, and 12′), 2.12 (2H, m; H$_2$-3), 3.11 (1H, br d, *J* = 13.0 Hz; H-6a), 3.40 (1H, br s; H-2), 3.93 (1H, br d, *J* = 13.0 Hz; H-6b), 4.03 (1H, br s; H-5), 4.28 (1H, m; H-4), 5.63 (3H, m; H-4′, 8′, and 11′), 5.83 (1H, dd, *J* = 15.4 and 6.8 Hz; H-1′), 6.00 (1H, dd, *J* = 15.4 and 10.3 Hz; H-3′), 6.11 (2H, m; H-9′ and H-10′), and 6.32 (1H, dd, *J* = 15.4 and 10.3 Hz; H-2′); ^{13}C NMR (C_5D_5N) δ_C 14.0 (C-15′), 22.2 (C-14′), 29.2, 29.9 (C-6′ and C-13′), 31.7, 32.3, 32.4 (C-5′, 7′, and 12′), 36.0 (C-3), 47.0

(17) It was reasonable that the coupling patterns of the ^1H NMR signals of **17** were similar to those of corresponding positions of piperidine ring protons of **4** and **5**. Particularly, the signals due to H-6α and H-6β[14] for **4**, **5**, and **17** appeared as broad doublets ($J_{6\alpha,6\beta}$ = *ca.* 14 Hz and $J_{5,6\alpha} = J_{5,6\beta}$ = 0−1.5 Hz),[2] implying that H-5 is equatorial, whereas the H-6α and H-6β signals of L-**7** were observed as a broad double doublet and a broad triplet, respectively ($J_{6\alpha,6\beta} = J_{5,6\beta}$ = *ca.* 12 Hz and $J_{5,6\alpha}$ = 3 Hz), suggesting that H-5 is axial.

(18) Two diastereomeric L-pipecolic acid derivatives ((4*R*,5*S*)- and (4*S*,5*S*)-dihydroxy-L-pipecolic acids) were coisolated from the same plant (leaves of *Derris elliptica*): Marlier, M.; Dardenne, G.; Casimir, J. *Phytochemistry* **1976**, *15*, 183−185.

(19) Kong, F.; Faulkner, D. J. *J. Org. Chem.* **1993**, *58*, 970−971.

(20) Gulavita, N. K.; Scheuer, P. J. *J. Org. Chem.* **1989**, *54*, 366−369.

(21) Mori, K.; Matsuda, H. *Liebigs Ann. Chem.* **1992**, 131−137.

(22) Jares-Erijman, E. A.; Bapat, C. P.; Lithgow-Bertelloni, A.; Rinehart, K. L.; Sakai, R. *J. Org. Chem.* **1993**, *58*, 5732−5737.

(23) The material *P. kanoko* was the same one as that used in the previous studies,[2,4] and was identified by Dr. T. Nishikawa, Graduate School of Human Informatics, Nagoya University.

(24) The % yield from wet weight of organism reported for the compound is the calculated yield, not the actual isolated yield.

(C-6), 51.5 (C-5), 57.4 (C-2), 67.3 (C-4), and 130.6, 131.0, 131.4, 132.0, 132.6, 132.6, 134.6, 135.7 (C-1′–C-4′, and C-8′–C-11′); EIMS m/z 318 (M^+), 115 ($M^+ - C_{15}H_{23}$), and 97 ($M^+ - C_{15}H_{23} - H_2O$).

Ozonolysis of Pseudodistomin C (3). A solution of pseudodistomin C (**3**, 14 mg) in MeOH (3.3 mL) was bubbled with O_3 at −78 °C for 4 min. After the removal of excess ozone by bubbling nitrogen, a solution of $NaBH_4$ (25 mg) in MeOH (1 mL) was added and the whole mixture was stirred for 45 min at 0 °C. After addition of 2 mL of 1 M potassium phosphate buffer (pH 7.0), the reaction mixture was partitioned between 1 N NaOH and *n*-BuOH. The organic phase was evaporated under reduced pressure. The residue was treated with acetic anhydride (1.5 mL) and pyridine (2.5 mL) for 1.5 h. After evaporation of the reagents, the residue was subjected to TLC and HPLC analysis for diacetate of 1,5-pentanediol.[7] This residue was, on the other hand, further separated by C_{18} HPLC [YMC-Pack AM323, S-5 μm 120A, 10 × 250 mm; flow rate, 1.5 mL/min; UV detection at 215 nm; eluent, 35% MeOH] to afford tetraacetate (**7**, 1.0 mg, t_R 17.6 min): amorphous powder; $[\alpha]^{23}_D$ +16° (*c* 0.10, MeOH); IR (neat) 3280, 1740, 1650, 1540, 1430, 1360, 1230, and 1040 cm^{-1}; ^1H NMR ($CDCl_3$, (*: signals for minor conformer)) δ 1.89 (1H, m; H-3), 2.00 (3H, s; Ac), 2.03 (3H, s; Ac), 2.15 (6H, s; Ac × 2), 2.73 (0.3H, br t, J = 12 Hz; H-6ax*), 3.24 (0.7H, dd, J = 12.3 and 12.6 Hz; H-6ax), 3.84 (0.7H, dd, J = 3.0 and 12.6 Hz; H-6eq), 4.03 (0.7H, m; H-5), 4.09 (0.3H, m; H-5*), 4.22 (0.3H, m; H-4*), 4.43 (0.7H, t, J = 10.0 Hz; H-1′a), 4.59 (0.3H, m; H-1′a*), 4.65 (0.3H, m; H-6eq*), 5.07 (0.7H, m; H-4), 5.10 (0.7H, br s; H-1′b), 5.13 (0.3H, br s; H-1′b*), 5.50 (0.3H, br s; NH-5*), and 5.75 (0.7H, br s; NH-5); EIMS m/z 315 ($M + H)^+$, 255 ($M - CH_3CONH_2)^+$, 212, 195, 181, 170, 152, 139, 122, 110, 93, and 80; HREIMS found m/z 315.1579 (M + H), calcd for $C_{14}H_{23}O_6N_2$ 315.1556.

***tert*-Butyl (4*S*,5*R*)-2,2-Dimethyl-4-(hydroxymethyl)-5-(2′-propenyl)oxazolidine-3-carboxylate (L-9).** To a solution of oxazolidine homoallyl alcohol[10] (L-**8**, 1:1 mixture of diastereomers, 10.0 g, 36.9 mmol) in MeOH (100 mL) was added *p*-toluenesulfonic acid monohydrate (30 mg), and the mixture was stirred at rt for 20 h and then heated at 45 °C for 3 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography (hexane/EtOAc, 3:2) to give 1,3-diol (1:1 mixture of diastereomers, 7.24 g, 31.4 mmol), part of which (6.9 g, 29.8 mmol) was then treated with pivaloyl chloride (3.85 mL, 29.9 mmol) and pyridine (100 mL) at rt for 3 h. The reaction mixture was, after evaporation of the solvent, separated by silica gel column chromatography (hexane/EtOAc, 97:3, repeated four times) to give the *erythro*-monopivaloate (3.8 g) together with the *threo*-isomer (3.6 g).[25] To a solution of the *erythro*-monopivaloate (3.1 g, 9.84 mmol) in CH_2Cl_2 (50 mL), 2,2-dimethoxypropane (15 mL) and BF_3·OEt_2 (1.0 mL) were added at 0 °C, and the mixture was stirred for 20 min. After addition of saturated $NaHCO_3$ aqueous solution (30 mL), extraction with CH_2Cl_2 (8.0 mL) followed by evaporation of the solvent afforded 1,2-acetonide (3.49 g, 9.8 mmol) without purification. The 1,2-acetonide (3.4 g, 9.57 mmol) was then treated with 2.5 N KOH (50 mL) in MeOH (50 mL) at 40 °C for 20 h. After removing MeOH under reduced pressure, extraction with EtOAc followed by purification with silica gel column chromatography (hexane/EtOAc, 3:1) afforded the alcohol (L-**9**, 2.2g, 23 % yield from L-**8**). L-**9**: colorless oil; $[\alpha]^{23}_D$ +16° (*c* 2.3, MeOH); IR (neat) 3420, 1680, 1380, 1250, 1170, 1150, and 900 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.81 (1H, m), 5.10 (2H, m), 4.09 (1H, m), 3.72 (3H, m), 2.40 (2H, m), 1.53 (6H, br s), and 1.42 (9H, s); EIMS m/z 256 ($M - CH_3)^+$, 240 ($M - CH_2OH)^+$, 216, 200, 184, 156, 140, and 59; HREIMS, found m/z 256.1527, calcd for $C_{13}H_{22}O_4N$ (M-CH_3) 256.1549.

***tert*-Butyl (4*S*,5*R*)-2,2-Dimethyl-4-[[(benzyloxycarbonyl)amino]methyl]-5-(2′-propenyl)oxazolidine-3-carboxylate (L-10).** To a solution of the alcohol (L-**9**, 2.1 g, 7.74 mmol) in THF (23 mL) were added phthalimide (1.44 g, 9.79 mmol), diisopropyl azodicarboxylate (DIAD, 2.16g, 10.9 mmol), and triphenylphosphine (2.6 g, 10.2 mmol), and the mixture was stirred at rt for 20 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography (hexane/EtOAc, 3:1) to give the phthalimide (3.0 g), which was then treated with hydrazine monohydrate (1.5 mL) in EtOH (30 mL) and stirred at rt for 15 h. After addition of 2 N NaOH (60 mL) and extraction with EtOAc (100 mL × 3), the organic phase was evaporated under reduced pressure to give a residue. The residue was dissolved in THF (15 mL), and to this solution 2 N NaOH (11 mL) and benzyl chloroformate (ZCl, 1.46 mL, 10.2 mmol) was added. After stirring at rt for 3 h, the reaction mixture was partitioned between brine and EtOAc (50 mL × 3). The organic solution was evaporated under reduced pressure to afford a residue, which was purified by silica gel column chromatography (hexane/EtOAc, 4:1) to give the benzyl carbamate (L-**10**, 2.56 g, 6.3 mmol, 84 % yeild from L-**9**). L-**10**: colorless oil; $[\alpha]^{23}_D$ +1.0° (*c* 1.5, MeOH); IR (neat) 3340, 1690, 1520, 1380, and 1240 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.50 (15H, br s) 2.38 (2H, m), 3.46 (2H, m), 5.80 (2H, m), 5.15 (4H, m), 4.02 (2H, m), and 7.33 (5H, br s); EIMS m/z 389 ($M - CH_3)^+$, 363, 349, 289, 273, 240, 197, 184, 139, and 59; HREIMS, found m/z 389.2100, calcd for $C_{21}H_{29}O_5N_2$ ($M - CH_3$) 389.2077.

Benzyl (3a*S*,6*R*,7a*R*)-6-[(Bromomercurio)methyl]-3-(*tert*-butoxycarbonyl)-2,2-dimethylperhydrooxazolo[4,5-*c*]pyridine-5-carboxylate (L-11) and Benzyl (3a*S*,6*S*,7a*R*)-6-[(Bromomercurio)methyl]-3-(*tert*-butoxycarbonyl)-2,2-dimethylperhydrooxazolo[4,5-*c*]pyridine-5-carboxylate (L-12). To a solution of compound L-**10** (285 mg, 0.70 mmol) in $CHCl_3$ (14 mL), mercuric trifluoroacetate (285 mg, 0.90 mmol) was added, and the mixture was stirred at rt for 20 h. After addition of saturated $NaHCO_3$ solution (10 mL) and saturated NaBr solution (10 mL), the mixture was stirred for 3 h at rt. Extraction with $CHCl_3$ (50 mL × 2) followed by evaporation of the solvent afforded a residue, which was purified by silica gel column chromatography (hexane/EtOAc, 4:1) to give two diastereomers of piperidine derivatives (L-**11**, 210 mg, 0.3 mmol, 43% and L-**12**, 177 mg, 0.26 mmol, 37 %). L-**11**: amorphous powder; IR (neat) 1690, 1390, 1250, 1150, and 1090 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.48 (15H, br s), 1.90 (1H, dt, J = 15.0 and 4.4 Hz), 2.28 (3H, m), 2.38 (2H, m), 2.86 (2H, dd, J = 13.5 and 10.6 Hz), 4.60–3.60 (4H, m), 5.30–5.05 (2H, m), and 7.36 (5H, br s); EIMS m/z 671 ($M - CH_3)^+$, 611, 569, 403, 347, 303, 202, 158, 91, and 57; HREIMS, found m/z 671.0869, calcd for $C_{21}H_{28}O_5N_2{}^{202}Hg^{81}Br$ ($M - CH_3$) 671.0868. L-**12**: amorphous powder; $[\alpha]^{23}_D$ +31° (*c* 0.36, MeOH); IR (neat) 1690, 1360, 1230, 1160, and 1090 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (15H, br s), 2.03 (3H, m), 2.93 (0.5H, dd, J = 15.0 and 3.3 Hz), 2.97 (0.5H, dd, J = 15.0 and 3.0 Hz), 3.80 (0.5H, m), 3.95 (0.5H, m), 4.98 (1H, dd, J = 12.9 and 4.1 Hz), 5.29 (1H, dd, J = 12.0 and 3.0 Hz), 7.33 (5H, br s); EIMS m/z 669 ($M - CH_3)^+$, 610, 568, 403, 347, 303, 202, 158, 91, and 57; HREIMS, found m/z 669.0927 calcd for $C_{21}H_{28}O_5N_2{}^{202}Hg^{79}Br$ ($M - CH_3$) 669.0888.

Benzyl (3a*S*,6*R*,7a*R*)-6-[(Bromomercurio)methyl]-2,2-dimethylperhydrooxazolo[4,5-*c*]pyridine-5-carboxylate (13) and Benzyl (3a*S*,6*S*,7a*R*)-6-[(Bromomercurio)-methyl]-2,2-dimethylperhydrooxazolo[4,5-*c*]pyridine-5-carboxylate (14). The (2*R*)-piperidine[14] derivative (L-**11**, 20 mg, 0.029 mmol) was dissolved in CH_2Cl_2 (1.5 mL), and to this solution trifluoroacetic acid (20 mL) was added. After stirring at rt for 40 min, saturated $NaHCO_3$ solution (3 mL) was added, and extraction with CH_2Cl_2 (15 mL × 2) and evaporation under reduced pressure afforded a residue, which was purified by silica gel column chromatography (hexane/EtOAc, 1:2) to give compound **13** (4.0 mg, 24 %): amorphous powder; $[\alpha]^{23}_D$ +4.7° (*c* 0.4, MeOH); IR (neat) 3400, 1680, 1360, 1500, 1420, 1360, 1240, and 1160 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (3H, s) 1.55 (3H, s), 1.88 (1H, ddd, J = 14.8, 3.4, and 1.8 Hz), 1.98 (1H, m), 2.21 (1H, m), 2.45 (1H, dd, J = 12.3 and 8.9 Hz), 3.12 (1H, t, J = 12.3 Hz), 3.62 (1H, br s), 4.11 (1H, m), 4.65 (1H, m), 4.85 (1H, br d, J = 8.5 Hz), 5.15 (2H, m), and 7.35 (5H, br s); EIMS m/z 584 and 582 (M^+), 569 and 567 ($M - CH_3)^+$, 526, 524, 303, 289, 202, 91, and 57; HREIMS, found m/z 584.0570, calcd for

(25) Structural assignment of the *erythro* and *threo* isomers was established by conversion of them into known 1,3-acetonides,[11a] respectively, in two steps [(i) 2.5 N KOH/MeOH, 45 °C, 10 h; (ii) 2,2-dimethoxypropane, CH_2Cl_2, 0 °C, 15 min].

$C_{17}H_{23}O_3N_2^{202}Hg^{79}Br$ (M) 584.0598. The (2*S*)-piperidine[14] derivative (L-**12**) was converted into compound **14** by the same procedures as above (24% yield). **14**: amorphous powder; $[\alpha]^{23}_D$ +25° (*c* 0.6, MeOH); IR (neat) 3400, 1680, 1360, 1500, 1420, 1360, 1240, and 1160 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (6H, s), 1.73 (1H, ddd, J = 13.4, 3.7, and 0.5 Hz), 1.91 (1H, ddd, J = 13.4, 7.1, and 1.1 Hz), 2.02 (1H, dd, J = 12.0 and 5.0 Hz), 2.26 (1H, t, J = 12.0 Hz), 3.22 (1H, br d, J = 13.4 Hz), 4.0 (1H, br s), 4.09 (1H, dt, J = 7.1 and 3.8 Hz), 4.18 (1H, dd, J = 13.4 and 1.5 Hz), 4.67 (1H, m), 4.86 (1H, br s), 5.13 (1H, d, J = 12.0 Hz), 5.23 (1H, d, J = 12.0 Hz), and 7.40 (5H, br s); EIMS *m*/*z* 584 and 582 (M^+), 569 and 567 $(M - CH_3)^+$, 526, 524, 303, 289, 202, 91, and 57; HREIMS, found *m*/*z* 584.0646, calcd for $C_{17}H_{23}O_3N_2^{202}Hg^{79}Br$ (M) 584.0598.

Benzyl (3a*S*,6*R*,7a*R*)-3-(*tert*-Butoxycarbonyl)-2,2-dimethyl-6-(hydroxymethyl)perhydrooxazolo[4,5-*c*]pyridine-5-carboxylate (L-15) and Benzyl (3a*S*,6*S*,7a*R*)-3-(*tert*-Butoxycarbonyl)-2,2-dimethyl-6-(hydroxymethyl)perhydrooxazolo[4,5-*c*]pyridine-5-carboxylate (16). Oxygen (O_2) was bubbled into a suspension of $NaBH_4$ (23 mg, 0.31 mmol) in DMF (7.5 mL) for 1 h, and to this was dropwise added a solution of L-**11** (170 mg, 0.218 mmol) in DMF (11 mL) over 2 h with continuous introduction of O_2. The bubbling of O_2 into the mixture was continued for 1 h. The reaction mixture was filtered through Celite, and the filtrate was evaporated under reduced pressure to give a residue, which was chromatographed (hexane/EtOAc, 2:1) to give L-**15** (92 mg, 0.128 mmol, 86 %): amorphous powder; $[\alpha]^{23}_D$ −11° (*c* 1.6, MeOH); IR (neat) 3450, 1690, 1390, 1250, 1170, and 1090 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.47 (15H, br s), 2.05 (2H, m), 2.90 (1H, br s), 3.83 (3H, m), 4.20 (1H, m), 4.43 (2H, m), 5.15 (2H, m), and 7.33 (5H, br s); EIMS *m*/*z* 421 $(M + H)^+$, 420 (M^+), 405 $(M - CH_3)^+$, 389, 345, 305, 289, 246, 91, and 57; HREIMS, found *m*/*z* 420.2274, calcd for $C_{22}H_{32}O_6N_2$ (M) 420.2260. L-**12** was converted into compound **16** by the same procedures as above (85% yield). **16**: amorphous powder; $[\alpha]^{23}_D$ +6.7° (*c* 2.5, MeOH); IR (neat) 3450, 1690, 1360, 1240, and 1090 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (15H, br s), 2.10 (1H, m), 2.41 (2H, br t), 3.06 (1H, m), 3.63 (3H, m), 4.25 (3H, m), 5.10 (2H, m), 7.33 (5H, br s); EIMS *m*/*z* 420 (M^+), 405 $(M - CH_3)^+$, 389, 345, 305, 289, 246, 91, and 57; HREIMS, found *m*/*z* 420.2264, calcd for $C_{22}H_{32}O_6N_2$ (M) 420.2260.

(2*R*,4*R*,5*S*)-1-Acetyl-4-acetoxy-5-(acetylamino)piperidine (L-7) and (2*S*,4*R*,5*S*)-1-Acetyl-4-acetoxy-5-(acetylamino)piperidine (17). To a solution of the alcohol (L-**15**, 20 mg, 0.047 mmol) in CH_2Cl_2 was added TFA (30 μL), and the mixture was stirred at rt for 20 h. After evaporation under reduced pressure, the residue was treated with acetic anhydride (0.3 mL) and pyridine (1.5 mL) at rt for 2 h. Evaporation of the reagent in vacuo afforded a residue, which was dissolved in EtOH (2.0 mL), and to this solution was added 5% Pd/C (5 mg). The mixture was stirred under H_2 atmosphere at rt for 24 h. Filtration off the catalyst and evaporation of the solvent afforded a residue, which was again treated with acetic anhydride (1.5 mL) and pyridine (2.5 mL) at rt for 1.5 h. After evaporation in vacuo, the residue was separated by silica gel column chromatography ($CHCl_3$/MeOH, 9:1) followed by further purification with C_{18} HPLC [YMC-Pack AM323, S-5 μm 120A, 10 × 250 mm; flow rate, 1.5 mL/min; UV detection at 215 nm; eluent, 35 % MeOH] to afford L-**7** (2.0 mg, 0.0064 mmol, 14 % yield, t_R 17.6 min): amorphous powder; $[\alpha]^{23}_D$ −19° (*c* 0.28, MeOH); IR, ^1H NMR, and EIMS data were identical with the ozonolysis product (**7**, vide supra) derived from **3**; HREIMS, found *m*/*z* 315.1585, calcd for $C_{14}H_{23}O_6N_2$ (M + H) 315.1556. Compound **16** was converted into the (2*S*)-isomer **17** by the same procedures as above (12% yield). **17**: amorphous powder; $[\alpha]^{23}_D$ +28° (*c* 0.10, MeOH); IR (neat) 3290, 1740, 1630, 1540, 1420, 1360, 1230, and 1040 cm^{-1}; ^1H NMR ($CDCl_3$, (*: signals for minor conformer)) δ 1.80 (1H, m; H-3′), 2.05 (12H, br s; Ac × 4), 2.20 (1H, m; H-3), 3.02 (0.3H, br d; H-6a*), 3.45 (0.7H, br d; H-6a), 4.03 (*ca.* 0.7H, br d; H-6b), 4.08 (*ca.* 0.7H, m; H-1′a), 4.45 (*ca.* 1.4H, m; H-5 and H-1′b), 4.5−4.7 (*ca.* 1.2H, br m; H-5*, 6b*, 1′a*, and 1′b*), 5.11 (2H, m; H-2 and H-4), and 5.60 (1H, br s; NH); EIMS *m*/*z* 315 $(M + H)^+$, 255 $(M - CH_3COOH)^+$, 212, 197, 181, 170, 154, 187, 139, 122, 110, 93, and 80; HREIMS, found *m*/*z* 315.1559, calcd for $C_{14}H_{23}O_6N_2$ (M + H) 315.1556.

Preparation of D-7. Starting with D-serine, the enantiomers (D-**9**, D-**10**, D-**11**, D-**15**, and D-**7**) were prepared by the same procedures as above.

D-**9**: colorless oil; $[\alpha]^{26}_D$ −22° (*c* 0.76, MeOH); HREIMS, found *m*/*z* 256.1566, calcd for $C_{13}H_{22}O_4N$ $(M - CH_3)$ 256.1549; 41% yield from D-**8**.

D-**10**: colorless oil; $[\alpha]^{26}_D$ −1.4° (*c* 0.90, MeOH); HREIMS, found *m*/*z* 389.2083, calcd for $C_{21}H_{29}O_5N_2$ $(M - CH_3)$ 389.2077; 88% yield from D-**9**.

D-**11**: amorphous powder; $[\alpha]^{26}_D$ 0° (*c* 1.0, MeOH); HREIMS, found *m*/*z* 671.0848, calcd for $C_{21}H_{28}O_5N_2^{202}Hg^{81}Br$ $(M - CH_3)$ 671.0868; 49% yield from D-**10**.

D-**15**: amorphous powder; $[\alpha]^{26}_D$ +19° (*c* 2.42, MeOH); HREIMS, found *m*/*z* 421.2316, calcd for $C_{22}H_{33}O_6N_2$ (M + H) 421.2339; 48% yield from D-**11**.

D-**7**: amorphous powder; $[\alpha]_D^{26}$ +20° (*c* 0.35 MeOH); HREIMS, found *m*/*z* 315.1577, calcd for $C_{14}H_{23}O_6N_2$ (M + H) 315.1556; 17% yield from D-**15**.

Chiral HPLC Analysis of the Tetraacetates L-7 and D-7. The enantiomers of synthetic tetraacetates L-**7** and D-**7** were revealed to be separable by chiral HPLC analysis using CHIRALPAK AD (Daicel Chemical Ind., Ltd.; 4.6 × 250 mm; flow rate: 0.5 mL/min; UV detection at 215 nm; eluent: hexane/2-propanol, 8:2) to show peaks at t_R 15.1 and 16.5 min, respectively. Under this condition, the tetraacetate **7** obtained by ozonolysis of **3** (vide supra) was also analyzed to show a peak at t_R 16.5 min, being identical with D-**7**. This fraction was collected and subjected to EIMS analysis, which showed the identical spectrum with that of **7**, confirming that this fraction veritably contained the tetraacetate **7**.

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Supporting Information Available: NMR spectra of compounds **3** and **6**, and chiral HPLC data (26 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; seen any current masthead page for ordering information.

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