Absolute Stereochemistry of Aplyronine A, a Potent Antitumor Substance of Marine Origin

Makoto Ojika, Hideo Kigoshi, Takeshi Ishigaki, Itaru Tsukada, Takaya Tsuboi, Takeshi Ogawa, and Kiyoyuki Yamada*

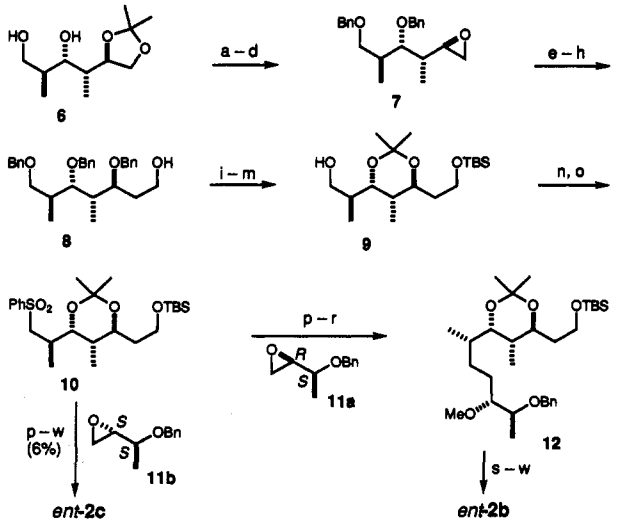
Department of Chemistry, Faculty of Science Nagoya University, Chikusa, Nagoya 464, Japan

Received March 17, 1994

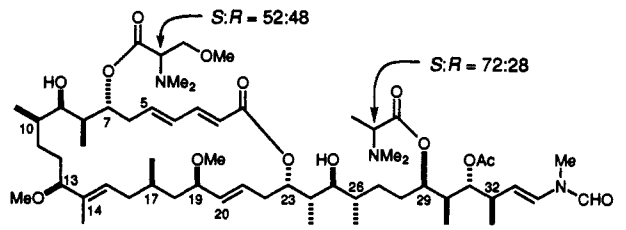
Recently, we reported the isolation of aplyronine A (**1**), a potent antitumor substance from the Japanese sea hare *Aplysia kurodai*, and the determination of its gross structure.[1] In the course of our stereochemical study on aplyronine A (**1**), the absolute stereochemistry of eight asymmetric centers (C23–C26 and C29–C32) of the right half of the molecule and the scalemic nature of the two amino acids[2] were clarified by spectroscopic analysis and the enantioselective synthesis of degradation products of **1**.[3] In this communication, we report determination of the complete absolute stereostructure of aplyronine A (**1**) on the basis of the enantioselective synthesis of other degradation products.

The absolute stereochemistry of seven asymmetric carbons (C7, C8, C9, C10, C13, C17, and C19) in **1** remained unknown, and these carbons are contained in two degradation products, the C5–C14 fragment **2** and the C15–C20 fragment **3**.[3a,4] We first examined the absolute stereochemistry of the C5–C14 fragment **2**, which contains five unassigned asymmetric carbons (C7, C8, C9, C10, and C13). The relative stereochemistry of the four contiguous asymmetric centers C7–C10 in **2** has been elucidated by comparison of ^{1}H NMR data between acetonide **4**[5] derived from **2** and the synthetic eight possible diastereomers including **5**:[3b] the spin–spin coupling constants[6] and the chemical shifts[6] of only the diastereomer **5** were found to be nearly identical to those of **4**, indicating that the relative stereochemistry of the four contiguous asymmetric centers C7–C10 in **2** is *anti-syn-anti*. Previously, we reported that the degradation of **1** afforded the C5–C14 fragments **2** and **2a**, the latter being the C14 diastereomer of the former.[3a,7] One of these natural fragments **2** and **2a** must be identical with one of the two stereoisomers **2b** and **2c**, the latter being the C13 diastereomer of the former. Thus, attempts were made to synthesize both stereoisomers **2b** and **2c** enantioselectively (Scheme 1).

Scheme 1[a]



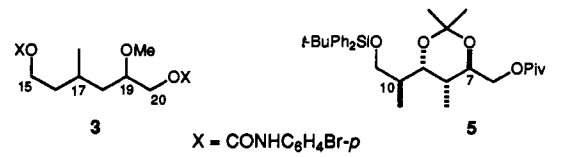
[a] (a) BnBr, NaH, THF–DMF, room temperature, 96%. (b) 2 N HCl, DME, reflux, 100%. (c) TsCl, pyridine, 0 °C. (d) K_2CO_3, MeOH, 0 °C, 80% (two steps). (e) 1,3-Dithiane, BuLi, THF, –23 °C, then **7**, room temperature, 100%. (f) BnBr, NaH, THF–DMF, room temperature, 88%. (g) CuO, $CuCl_2$, acetone–H_2O, reflux, 85%. (h) $NaBH_4$, EtOH, –29 to –20 °C, 97%. (i) *t*-$BuMe_2SiCl$ (TBSCl), imidazole, DMF, room temperature, 100%. (j) Li, liquid NH_3, *i*-PrOH, THF, –78 °C, 97%. (k) Pivaloyl chloride (PivCl), pyridine, 0 °C, 95%. (l) $Me_2C(OMe)_2$, CSA, acetone, room temperature, 98%. (m) $LiAlH_4$, Et_2O, 0 °C, 99%. (n) $(PhS)_2$, Bu_3P, DMF, room temperature, 96%. (o) *m*-CPBA, CH_2Cl_2, room temperature, 98%. (p) BuLi, THF, 0 °C, then **11a** (or **11b**), HMPA, room temperature, 34% (major isomer) and 11% (minor isomer). (q) 6% Na–Hg, Na_2HPO_4, MeOH, 0 °C, 90%. (r) MeI, NaH, THF, room temperature, 93%. (s) Bu_4NF, THF, room temperature, 93%. (t) Na, liquid NH_3, THF, –33 °C, 90%. (u) *p*-BrC_6H_4NCO, pyridine, room temperature, 100%. (v) AcOH, H_2O, room temperature. (w) Ac_2O, DMAP, pyridine, room temperature, 87% (two steps).



aplyronine A (**1**)

2 : R = Ac
2a : R = Ac (= C14-*epi*-**2**)
4 : R = acetonide

2b
2c (= C13-*epi*-**2b**)



3 X = $CONHC_6H_4Br$-*p*

5

Optically pure diol **6**,[3b] which possesses four contiguous asymmetric centers, was converted to epoxide **7** (Scheme 1). One-carbon homologation of **7** with the 1,3-dithianyl anion followed by *O*-benzylation, hydrolysis of the dithioacetal group,[8] and $NaBH_4$ reduction afforded alcohol **8**. By a series of protection–deprotection reactions, **8** was transformed into alcohol **9**, which

(1) Yamada, K.; Ojika, M.; Ishigaki, T.; Yoshida, Y.; Ekimoto, H.; Arakawa, M. *J. Am. Chem. Soc.* **1993**, *115*, 11020–11021.

(2) The ratios *S*/*R* varied with the animal samples employed, although the compounds with *S* configuration were always predominant. A sample of **1** that was used for the degradation study was found to contain trimethylserine and dimethylalanine in the ratios of *S*:*R* = 52:48 and 72:28, respectively. See refs 1 and 3a for details.

(3) (a) Ojika, M.; Kigoshi, H.; Ishigaki, T.; Yamada, K. *Tetrahedron Lett.* **1993**, *34*, 8501–8504. (b) Ojika, M.; Kigoshi, H.; Ishigaki, T.; Nisiwaki, M.; Tsukada, I.; Mizuta, K.; Yamada, K. *Tetrahedron Lett.* **1993**, *34*, 8505–8508.

(4) The numbering adopted in this paper corresponds to that of aplyronine A (**1**).

(5) Methanolysis (K_2CO_3, MeOH, room temperature) of **2** followed by acetalization ($Me_2C(OMe)_2$, camphorsulfonic acid, acetone, 0 °C) afforded **4** in 79% yield.

(6) For **5**: $J_{7,8}$ = 7.4 Hz, $J_{8,9}$ = 4.5 Hz, $J_{9,10}$ = 10.7 Hz, $\delta_{8\text{-Me}}$ = 0.92, and $\delta_{10\text{-Me}}$ = 0.92. For **4**: $J_{7,8}$ = 7.6 Hz, $J_{8,9}$ = 4.6 Hz, $J_{9,10}$ = 10.2 Hz, $\delta_{8\text{-Me}}$ = 0.87, and $\delta_{10\text{-Me}}$ = 0.82.

(7) These diastereomers were separable by reversed-phase HPLC, and the major isomer **2** has a longer retention time than the minor isomer **2a**. See ref 3a for details.

(8) Narasaka, K.; Sakashita, T.; Mukaiyama, T. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 3724.

(9) Nakagawa, I.; Hata, T. *Tetrahedron Lett.* **1975**, 1409–1412.

(10) Craig, D.; Smith, A. M. *Tetrahedron Lett.* **1990**, *31*, 2631–2632.

(11) Abushanab, E.; Vemishetti, P.; Leiby, R. W.; Singh, H. K.; Mikkilineni, A. B.; Wu, D. C.-J.; Sacha, R.; Panzika, R. D. *J. Org. Chem.* **1988**, *53*, 2598–2602.

(12) Abushanab, E.; Bessodes, M.; Antonakis, K. *Tetrahedron Lett.* **1984**, *25*, 3841–3844.

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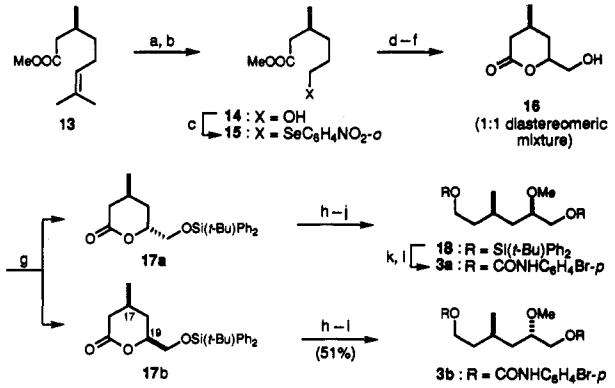
Scheme 2[a]



[a] (a) O_3, MeOH, −78 °C. (b) $NaBH_4$, MeOH, room temperature, 81% (two steps). (c) o-$NO_2C_6H_4SeCN$, Bu_3P, THF, room temperature, 100%. (d) 30% H_2O_2, THF, 0 °C. (e) OsO_4, pyridine, THF, room temperature. (f) TsOH, C_6H_6, room temperature then reflux, 55% (3 steps). (g) t-$BuPh_2SiCl$, imidazole, DMF, room temperature, 42% (**17a**) and 48% (**17b**). (h) $LiAlH_4$, Et_2O, room temperature, 99%. (i) t-$BuPh_2SiCl$, imidazole, DMF, 0 °C, 75%. (j) CH_2N_2, silica gel, hexane, 0 °C, 88%. (k) Bu_4NF, THF, room temperature, 90%. (l) p-BrC_6H_4NCO, pyridine, room temperature, 100%.

was further converted into sulfone **10** by a two-step sequence via a sulfide.[9] Addition[10] of the carbanion of **10** to (2*R*,3*S*)-3-(benzyloxy)-1,2-epoxybutane (**11a**)[11] afforded a separable 3:1 diastereomeric mixture of γ-hydroxy sulfones. The major isomer was subjected to reductive desulfurization followed by *O*-methylation to provide methyl ether **12**, which was further converted to urethane *ent*-**2b** in five steps. Urethane *ent*-**2c** was synthesized from sulfone **10** and (2*S*,3*S*)-3-(benzyloxy)-1,2-epoxybutane (**11b**)[12] by the same sequence of reactions as employed for the preparation of *ent*-**2b**. On comparison of the spectroscopic data and specific rotations, synthetic urethane *ent*-**2b** ($[\alpha]^{23}_D$ −24° (*c* 0.23, $CHCl_3$)) was found to be identical with the natural C5–C14 fragment **2** ($[\alpha]^{17}_D$ +26° (*c* 0.10, $CHCl_3$)) except for the sign of optical rotation, establishing the absolute stereochemistry at C7, C8, C9, C10, and C13.

We next turned our attention to the C15–C20 fragment **3**, which possesses two unassigned asymmetric centers (C17 and C19). To determine the absolute stereochemistry of these asymmetric centers, we synthesized two possible diastereomeric urethanes **3a** and **3b** (Scheme 2). Ozonolysis of methyl (−)-(*S*)-citronellate (**13**)[13] followed by $NaBH_4$ reduction produced alcohol **14**, which was converted to selenide **15** under the conditions of Grieco.[14] Oxidation[15] of **15** with H_2O_2 produced an olefin, which was subjected to osmylation followed by acid-induced cyclization to provide approximately a 1:1 diastereomeric mixture of lactone **16**.[16] After silylation, the two diastereomers were chromatographically separated to give lactones **17a** and **17b**. The relative stereochemistry of these two isomers was determined by ^{1}H NMR spectral analysis performed on **17b**: the axial orientation of H19 in **17b** was deduced from the spin–spin coupling constants, $J_{18,19}$ = 11.7 and 3.5 Hz, and on irradiation of H19, a 6% NOE was observed on the proton at C17, revealing the *cis* relationship of the two substituents at C17 and C19 in **17b**. Reduction of *trans*-lactone **17a** with $LiAlH_4$ provided a triol, which was subjected to selective silylation of two primary hydroxyl groups followed by *O*-methylation with diazomethane–silica gel[17] to afford the *syn* product **18**. By a two-step sequence, the *syn* product **18** was converted into *syn*-urethane **3a**. Transformation of *cis*-lactone **17b** to *anti*-urethane **3b** was executed by the same sequence of reactions as employed for the preparation of **3a** (Scheme 2). The ^{1}H NMR spectra of synthetic urethane **3a** and the natural C15–C20 fragment **3** were identical. The CD spectra of both **3a** and **3** showed a negative maximum at 251 nm (Δε values, −0.62 for **3a** and −1.17 for **3**),[18] disclosing the absolute stereochemistry at C17 and C19 in **3**.

With the absolute stereochemistry of the two degradation products **2** (=**2b**) and **3** (=**3a**) in hand, we can now define the complete stereostructure of aplyronine A as shown in the formula **1**.

Interestingly, the absolute stereochemistry of the right half of **1** is almost identical to that of the corresponding part of scytophycin C,[19] which was isolated from a blue-green alga. Aplyronine A (**1**) is presumed to be a metabolite of symbiotic algae in the sea hare *A. kurodai*.

Acknowledgment. This work was supported in part by Grants-in-Aid for Scientific Research (Nos. 04403009 and 03640472) from the Ministry of Education, Science, and Culture, Japan, the Fujisawa Foundation, the Shorai Foundation for Science and Technology, the Naito Foundation, the Ciba-Geigy Foundation (Japan) for the Promotion of Science, the Asahi Glass Foundation, and Ono Pharmaceutical Co. A fellowship of the Japan Society for the Promotion of Science for Japanese Junior Scientists to T.I. is gratefully acknowledged.

Supplementary Material Available: Characterization data for **4** and the compounds shown in Schemes 1 and 2; ^{1}H NMR data for all eight diastereomers of **5**; ^{1}H NMR spectra of **2**, **2a**, *ent*-**2b**, *ent*-**2c**, **3**, **3a**, and **3b** (13 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(13) **13** was prepared from optically impure (−)-(*S*)-citronellol, purchased from Aldrich Chemical Co., by pyridinium dichromate oxidation and methylation with diazomethane. **13**: $[\alpha]^{20}_D$ −4.5° (*c* 0.995, $CHCl_3$). Optically pure **13**: $[\alpha]^{22}_D$ −6.70° (*c* 2.28, $CHCl_3$). Asaoka, M.; Shima, K.; Tujii, N.; Takei, H. *Tetrahedron* **1988**, *44*, 4757–4766.

(14) Grieco, P. A.; Gilman, S.; Nishizawa, M. *J. Org. Chem.* **1976**, *41*, 1485–1486.

(15) Sharpless, K. B.; Young, M. W. *J. Org. Chem.* **1975**, *40*, 947–949.

(16) Another method for preparation of **16** was reported: Thurkauf, A.; Tius, M. A. *J. Chem. Soc., Chem. Commun.* **1989**, 1593–1594.

(17) Ohno, K.; Nishiyama, H.; Nagase, H. *Tetrahedron Lett.* **1979**, 4405–4406.

(18) Synthetic urethanes **3a** and **3b** are not optically pure for the reason described in ref 13.

(19) Ishibashi, M.; Moore, R. E.; Patterson, G. M. L.; Xu, C.; Clardy J. *J. Org. Chem.* **1986**, *51*, 5300–5306.