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

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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 acids² 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 ¹H NMR data between acetonide 4⁵ derived from 2 and the synthetic eight possible diastereomers including 5:3b the spin-spin coupling constants⁶ and the chemical shifts⁶ 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).

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(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.

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A (1).

(5) Methanolysis (K_2CO_3 , MeOH, room temperature) of 2 followed by acetalization (Me₂C(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, $\delta_{8.Me} = 0.92$, and $\delta_{10.Me} = 0.92$. For 4: $J_{7,8} = 7.6$ Hz, $J_{8,9} = 4.6$ Hz, $J_{9,10} = 10.2$ Hz, $\delta_{8.Me} =$ 0.87, and $\delta_{10-Me} = 0.82$.

(7) These diastercomers 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.

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^a (a) BnBr, NaH, THF-DMF, room temperature, 96%. (b) 2 N HCl, DMÈ, reflux, 100%. (c) TsCl, pyridine, 0 °C. (d) K₂CO₃, 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, CuCl₂, acetone-H₂O, reflux, 85%. (h) NaBH₄, EtOH, -29 to -20 °C, 97%. (i) t-BuMe₂SiCl (TBSCl), imidazole, DMF, room temperature, 100%. (j) Li, liquid NH₃, *i*-PrOH, THF, -78 °C, 97%. (k) Pivaloyl chloride (PivCl), pyridine, 0 °C, 95%. (1) Me₂C(OMe)₂, CSA, acetone, room temperature, 98%. (m) LiAlH4, Et2O, 0 °C, 99%. (n) (PhS)₂, Bu₃P, DMF, room temperature, 96%. (o) m-CPBA, CH₂Cl₂, 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, Na₂HPO₄, MeOH, 0 °C, 90%. (r) MeI, NaH, THF, room temperature, 93%. (s) Bu4NF, THF, room temperature, 93%. (t) Na, liquid NH₃, THF, -33 °C, 90%. (u) p-BrC₆H₄NCO, pyridine, room temperature, 100%. (v) AcOH, H₂O, room temperature. (w) Ac₂O, DMAP, pyridine, room temperature, 87% (two steps).



Optically pure diol 6,3b which possesses four contiguous asymmetric centers, was converted to epoxide 7 (Scheme 1). Onecarbon homologation of 7 with the 1,3-dithianyl anion followed by O-benzylation, hydrolysis of the dithioacetal group,⁸ and NaBH₄ reduction afforded alcohol 8. By a series of protectiondeprotection reactions, 8 was transformed into alcohol 9, which

 $X = CONHC_{6}H_{4}Br-p$

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^a (a) O₃, MeOH, -78 °C. (b) NaBH₄, MeOH, room temperature, 81% (two steps). (c) o-NO₂C₆H₄SeCN, Bu₃P, THF, room temperature, 100%. (d) 30% H₂O₂, THF, 0 °C. (e) OsO₄, pyridine, THF, room temperature. (f) TsOH, C₆H₆, room temperature then reflux, 55% (3 steps). (g) t-BuPh₂SiCl, imidazole, DMF, room temperature, 42% (17a) and 48% (17b). (h) LiAlH₄, Et₂O, room temperature, 99%. (i) t-BuPh2SiCl, imidazole, DMF, 0 °C, 75%. (j) CH2N2, silica gel, hexane, 0°C, 88%. (k) Bu₄NF, THF, room temperature, 90%. (l) p-BrC₆H₄NCO, pyridine, room temperature, 100%.

was further converted into sulfone 10 by a two-step sequence via a sulfide.⁹ Addition¹⁰ of the carbanion of **10** to (2R,3S)-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 Omethylation 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 (2S,3S)-3-(benzyloxy)-1,2epoxybutane $(11b)^{12}$ 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, CHCl₃)) was found to be identical with the natural C5–C14 fragment 2 ($[\alpha]^{17}_{D}$ +26° (c 0.10, CHCl₃)) 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 NaBH₄ reduction produced alcohol 14, which was converted to selenide 15 under the conditions of Grieco.¹⁴ Oxidation¹⁵ of 15 with H₂O₂ 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 silvlation, the two diastereomers were chromatographically separated to give lactones 17a and 17b. The relative stereochemistry of these two isomers was determined by ¹H 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 LiAlH₄ provided a triol, which was subjected to selective silvlation 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 cislactone 17b to anti-urethane 3b was executed by the same sequence of reactions as employed for the preparation of 3a (Scheme 2). The ¹H 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 ($\Delta\epsilon$ 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.

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Supplementary Material Available: Characterization data for 4 and the compounds shown in Schemes 1 and 2; ¹H NMR data for all eight diastereomers of 5; ¹H 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: $[\alpha]^{20}D^{-4.5^{\circ}}(c\,0.995, CHCl_3)$. Optically pure 13: [a]²²_D -6.70° (c 2.28, CHCl₃). Asaoka, M.; Shima, K.; Tujii, N.; Takei, H. Tetrahedron 1988, 44, 4757-4766.

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