Pergamon

Tetrahedron Letters, Vol. 37, No. 23, pp. 4023-4026, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)00752-6

Relative Stereochemistry of Pinnatoxin A, a Potent Shellfish Poison from *Pinna muricata*

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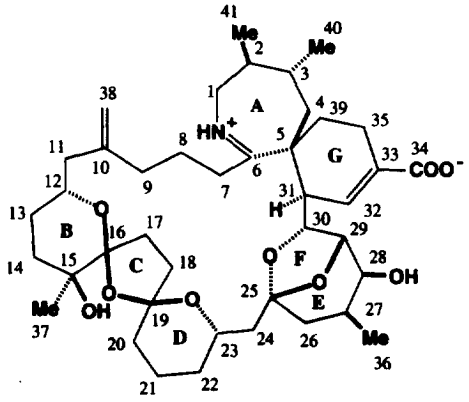
Abstract : Pinnatoxin A, the principal toxin isolated from *Pinna muricata*, is a novel amphoteric macrocyclic compound composed of a 6,7-spiro ring, a 5,6-bicyclo ring and a 6,5,6-trispiroketal ring involving 14 chiral centers. The relative stereochemistry was deduced based on detailed analysis of NOESY and ROESY data, and $^3J_{H\text{-}H}$ coupling constants. Copyright © 1996 Elsevier Science Ltd

Human intoxication resulting from ingestion of *Atrina pectinata* (*Pinna pectinata*), a commonly eaten shellfish, occurs frequently in coastal regions of Japan and China. In Japan, 6 outbreaks involving 2766 people were reported between 1975 and 1991.[1, 2] Fortunately, the mortality rate is very low. However, at least one death has been reported quite recently in Japan. Although toxic extracts from *Pinna attenuata*, referred to as pinnatoxin, have been reported,[3] identification of the specific toxin(s) and clarification of their physiological activity have proved to be challenging. We previously reported the isolation and planar structure of pinnatoxin A (**1**), one of the principal toxins.[4] We report here the relative stereochemistry of this rigid macrocyclic compound, which is composed of a 6,7-spiro ring, a 5,6-bicyclo ring and a 6,5,6-trispiroketal ring involving 14 chiral centers, based on a careful analysis of $^3J_{H\text{-}H}$ coupling constants and NOEs from NOESY and ROESY experiments.[5]



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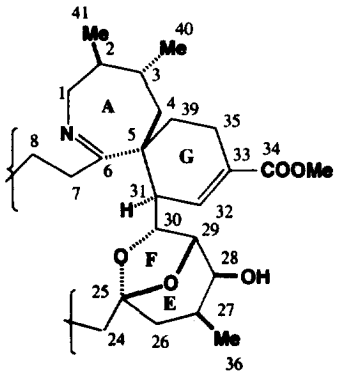

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First, the stereochemistry of the 5,6-bicyclo ring and 6,7-spiro ring portion (R segment) was assigned, as shown in Fig. 1. In the 5,6-bicyclo ring moiety (C25-C30), $^3J_{vic}$ values suggested the axial dispositions of protons at C26 (H26b) and C27, as well as equatorial protons at C28 and C29, which support a chair conformation for ring E ($^3J_{H26b\text{-}H27}$=10.0 Hz, $^3J_{H26a\text{-}H27}$=5.5 Hz, $^3J_{H27\text{-}H28}$=4.0 Hz, $^3J_{H28\text{-}H29}$=2.3 Hz).

NOESY crosspeaks, 36-Me/H26a, 36-Me/H26b, 36-Me/H28 and H27/H28, support the notion that 36-Me is equatorial and that the hydroxyl group on C28 is axial. Observation of a coupling constant of 4.7 Hz ($^3J_{H29\text{-}H30}$)[6] suggests that the proton at C30 is in an equatorial position. On the other hand, $^3J_{H31\text{-}H32}$ (0.5 Hz) and the NOESY crosspeak between H31 and H39a reveal a flattened-half chair conformation for cyclohexene ring G and an axial configuration for the protons at C31 and C39 (H39a). The large coupling constant ($^3J_{H30\text{-}H31}$=12.5 Hz) suggests an anti-relationship between H30 and H31. The configuration at C31 was readily deduced from the strong NOEs between H32/H30, H32/H29, H32/H28 and H31/H27. These NOE correlations, which are crucial for determining the stereochemistry at C31, C30 and C29, were further confirmed by observation not only in the ROESY spectrum of **1** but also in the NOESY spectrum[7] of methyl ester **2**, which was obtained by treatment of **1** with diazomethane. Since rings E and F compose a bicyclo system, the relative configuration at C25 is also confirmed. Furthermore, the configuration at C5 was assigned by the observation of NOESY crosspeaks: H30/H4b, H30/H3, H30/40-Me and H31/7-CH_2. The large coupling constant ($^3J_{H2\text{-}H3}$ =11.0 Hz) led us to locate H2 and H3 in axial positions, and $^3J_{H1b\text{-}H2}$, $^3J_{H3\text{-}H4b}$ values (<0.5 Hz) suggest that the dihedral angles between H1b/H2 and H4b/H3 are approximately 90°, respectively. NOESY crosspeaks H1a/41-Me, H1a/H2, H1b/H2; H4a/40-Me, H4b/H3 and H1b/H4a reveal that the 7-membered ring (ring A) that is torsionally strained by the imine double bond should be presented in a somewhat deformed chair conformation[8] (in CD_3OD) and that the relationship between 41-Me and 40-Me exhibits a trans configuration. Eventually, the relative stereochemistries at C2, C3, C5, C25, C27, C28, C29, C30 and C31 in the R segment were deduced.

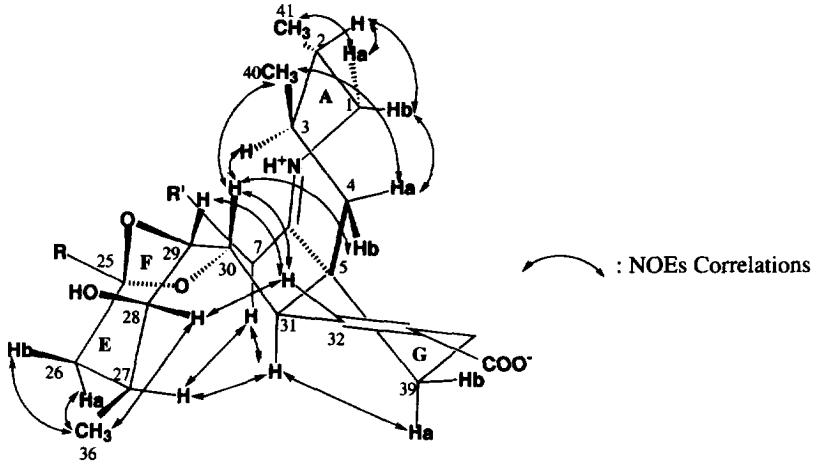

Fig. 1. Stereoview around the 5,6-bicyclo ring and the 6,7-spiro ring (R segment) of **1**.

Next, the stereochemistry of the L segment around the 6,5,6-trispiroketal ring was assigned, as shown in Fig. 2. Coupling constants related to H12 and H23 in two 6-membered ether rings (B and D rings) of the L segment could not be assigned from the ^{1}H-NMR or 2D DQF-COSY, due to their multiple couplings by AA'BB' and their similar proton chemical shifts. To resolve this problem, a triple resonance homodecoupling technique[9] was applied. When H11a and H11b or H24a and H24b were irradiated using this technique, H-12 (δ4.09) and H-23 (δ4.04) were observed as distinct double doublets, respectively ($^3J_{H12\text{-}H13a}$=11.0 Hz, $^3J_{H12\text{-}}$

$H13b$=1.6 Hz; and $^3J_{H23\text{-}H22a}$=12.0 Hz, $^3J_{H23\text{-}H22b}$<1.5 Hz) (Fig. 3). These two protons (H12 and H23) are characterized by large coupling on the order of 12.0 Hz, which unequivocally establishes an axial dispositions at C12 (H12) and C23 (H23). These findings are also supported by the NOEs observed between the axial protons H12/H14b and H23/H21b. Therefore, these two 6-membered ether rings (B and D rings) both possess a normal chair conformation. NOESY crosspeaks 37-Me/H14a and 37-Me/H13a reveal that 37-Me is axial and the hydroxyl group on C15 is equatorial. NOE correlations between the 37-Me group and the methylene protons at C17 (H17a and H17b) confirmed a thermodynamically stable spiro ketal with an axial ketal oxygen in the five-membered ring (ring C) at C16. In addition, the ketal oxygen in ring C was assigned an axial disposition at C19 based on the NOESY crosspeaks between H20a (axial proton) and H18a. These assignments are also supported by the fact that significant NOEs were not observed between H12 and 17-CH_2 or between H23 and 18-CH_2. Based on these results, the configurations at C12, C15, C16, C19 and C23 of the L segment were deduced.

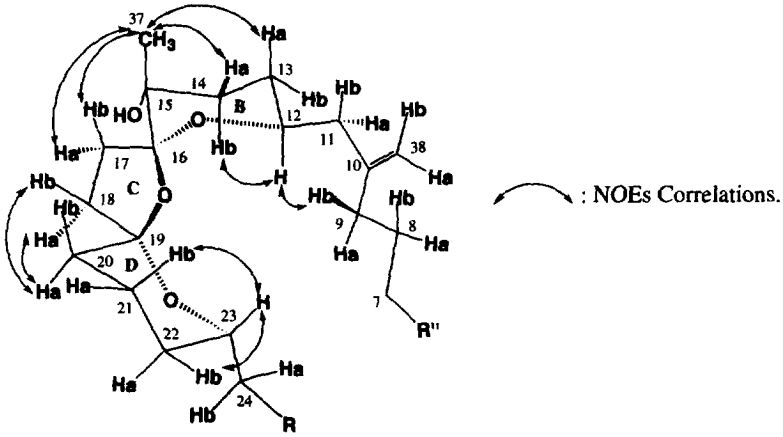

Fig. 2. Stereoview of the 6,5,6-trispiroketal ring (L segment) of **1**.

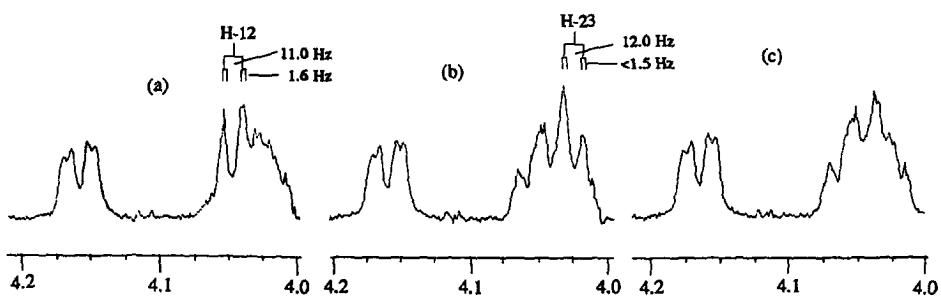

Fig. 3. Assignment of $^3J_{H\text{-}H}$ of H12 and H23 using the triple homodecoupling spectrum of **1**:
(a) H12, irradiated on H11a and H11b; (b) H23, irradiated on H24a and H24b; (c) not irradiated.

The relative configurations of the R and L segments were suggested as described above. However, the relationships between the R and L segments remain unclear. These two segments are connected to each other as

shown in Fig. 4 based on NOESY and ROESY crosspeaks H12/41-Me and H23/H3. The present findings confirmed the relative stereochemistry of the entire rigid macrocyclic compound pinnatoxin A (**1**). Studies on the absolute configuration of pinnatoxins are in progress.

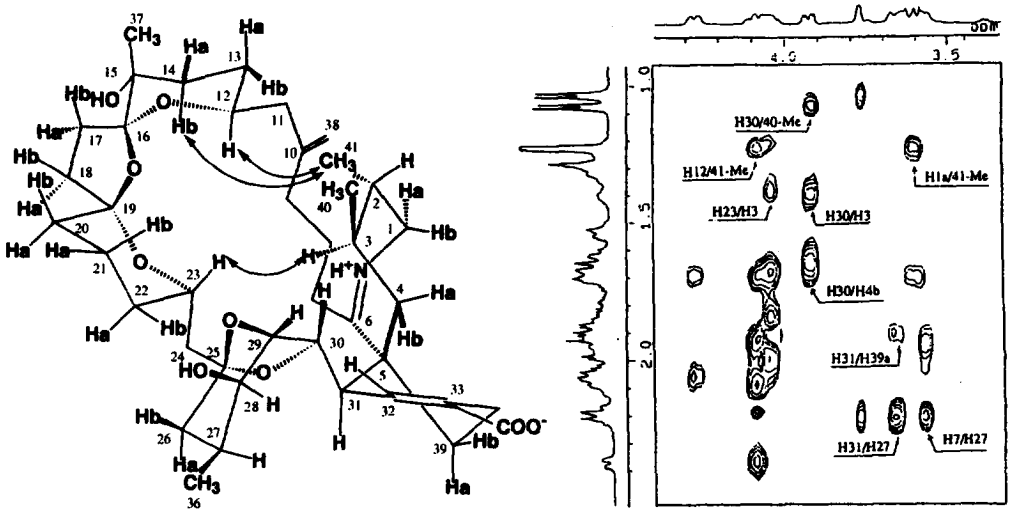

Fig. 4. The complete stereoview of pinnatoxin A (**1**) (and partial NOESY spectrum of pinnatoxin A in right).

Acknowledgments. This work was financially supported by Ono Pharmaceutical Company, the Shorai Foundation and by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

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5. NOESY spectra of **1** were acquired in CD_3OD with mixing times of 500 ms , while ROESY spectra of **1** were acquired in CD_3OD, with a mixing time of 250 ms .
   (5a) NOESY: Kumar, A.; Ernst, R. R.; Wuthrich, K., *Biochem. Biophys. Res. Commun.*, **1980**, 95, 1.
   (5b) ROESY: Bax, A.; Davis, D. G.; *J. Magn. Reson.*, **1985**, 63, 207.
6. $^3J_{H1\text{-}H6e}$ =4.6 Hz has been observed in the ^{1}H-NMR spectrum of camphor ($^3J_{H1\text{-}H6a}$ =0 Hz). In the case of norbornene, $^3J_{H1\text{-}H6e}$ =3.5 Hz and $^3J_{H1\text{-}H6a}$ =0 Hz were also reported: Brügel, W. *HandBook of NMR Spectral Parameters*; *Vol. 1*, Heyden & Son, Ltd.: London, Philadelphia, 1979; pp. 104-117.
7. NOESY spectra (400 MHz, Bruck) of **2** were acquired in C_6D_6 with mixing times of 400 ms. NOEs correlations were observed through the crosspeaks H32/H29, H32/H28, H32/H31, H31/H27, H31/H7, H30/H4b and H28/-OMe.
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   (b) Afanas'ev, V. A.; Bystrov, V. F.; *J. Magn. Reson.*, **1970**, 3, 357.

(*Received in Japan* 5 *March* 1996; *revised* 16 *April* 1996; *accepted* 17 *April* 1996)