Pergamon

Tetrahedron Letters, Vol. 35, No. 8, pp. 1223-1226, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $6.00+0.00

0040-4039(94)E0020-X

Absolute Configuration of C(1)-C(5) Fragment of AAL-toxin: Conformationally Rigid Acyclic Aminotriol Moiety.

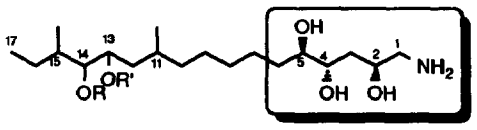
Hideaki Oikawa,*,[a] Isamu Matsuda[a], Akitami Ichihara,*,[a] and Keisuke Kohmoto[b]

[a]Department of Bioscience and Chemistry, Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan
[b]Laboratory of Plant Pathology, Faculty of Agriculture, Tottori University, Tottori 680, Japan

Key Words: AAL-toxin; host-specific toxin; *Alternaria alternata*; absolute configuration

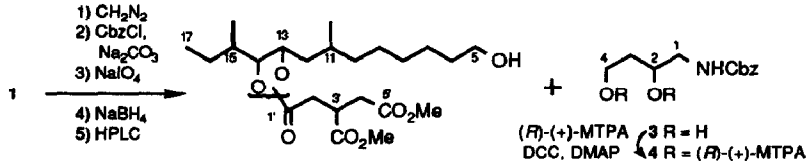
Abstract: Degradation of AAL-toxin **1** a host-specific phytotoxin and synthesis of model aminotriol **7a** - **7d** allowed us to determine the absolute configuration of C(1)-C(5) fragment as 2*S*, 4*S* and 5*R*. Unusually rigid conformation of this acyclic fragment was also discussed.

Host-specific toxins (HST) in plant diseases are interesting topics for studying host-parasite interaction.[1] AAL-toxin **1**,[2a,3] one of HST produced by *Alternaria alternata* f. sp. *lycopersici*, a causal fungus of tomato stem canker disease reproduces similar symptoms to that of the disease for susceptible genotype of tomato leaf in concentrations less than 10 ng/ml. From genetic analysis, Gilchrist *et al.* proposed that a single gene controls sensitivity to the toxin and susceptibility to the fungus.[4a] The same group also suggested that reduction of toxin activity by L-aspartate and orotate can be explained by inhibition of aspartate carbamoyl transferase,[4b] and that induction of necrosis in tomato caused by **1** requires *de novo* synthesis and external binding of ethylene.[4b] Although Bottini *et al.* determined the 2D-structure of **1** by the extensive analysis of MS, ^{1}H- and ^{13}C-NMR spectra,[2b,2c] relative and absolute stereochemistry of **1** remained to solve. For understanding the mechanism of the host-specificity of **1** in molecular level, the elucidation of 3D-structure and synthetic studies are essential. Here, we describe the absolute configuration and the local conformation of C(1)-C(5) fragment of **1**.

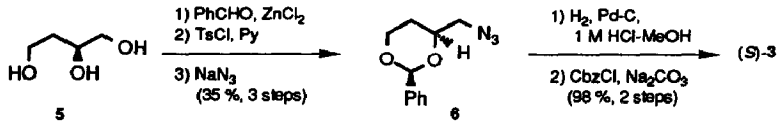


1 : R = $OCCH_2CH(CO_2H)CH_2CO_2H$ or H,
R' = H or $OCCH_2CH(CO_2H)CH_2CO_2H$
2 : R = R' = H

For degradative study, AAL-toxin **1** was isolated from the cultures of *Alternaria alternata* tomato pathotype (*A. alternata* f. sp. *lycopersici*) O-227.[3] Since large amount of **1** was not available in our hand, we at first concentrated our attention to the absolute stereochemistry at C-2. Degradation of **1** was carried out as shown in Scheme 1. After methylation, the amino group was protected by carbobenzyloxy (Cbz) group which also served as a chromophore in HPLC separation. Oxidative cleavage between C-4 and C-5, following reduction and HPLC separation gave diol **3**, which was further converted to bis-(*R*)-MTPA ester **4**. Starting from triol (2*S*)-**5**[5] derived from L-malic acid, authentic (2*S*)-**3** was prepared in five steps as shown in Scheme 2. Independently prepared (2*RS*)-**3**[6] and the (2*S*)-**3** were further converted to bis-(*R*)-MTPA esters (2*RS*)-**4** and (2*S*)-**4**, respectively. In their ^{1}H-NMR spectra, the signals of acyloxy methylene and methine protons were well resolved and were observed at 4.26 (CH) and 3.82 ppm (CH_2) in 2*R*-isomer, and at 4.13 (CH) and 3.99 ppm (CH_2) in 2*S*-isomer. Since the ^{1}H-NMR spectrum of the bis-(*R*)-MTPA ester **4** obtained from **1** was identical with that of synthetic (2*S*)-**4**, the absolute stereochemistry at C-2 of **1** was determined as *S*.

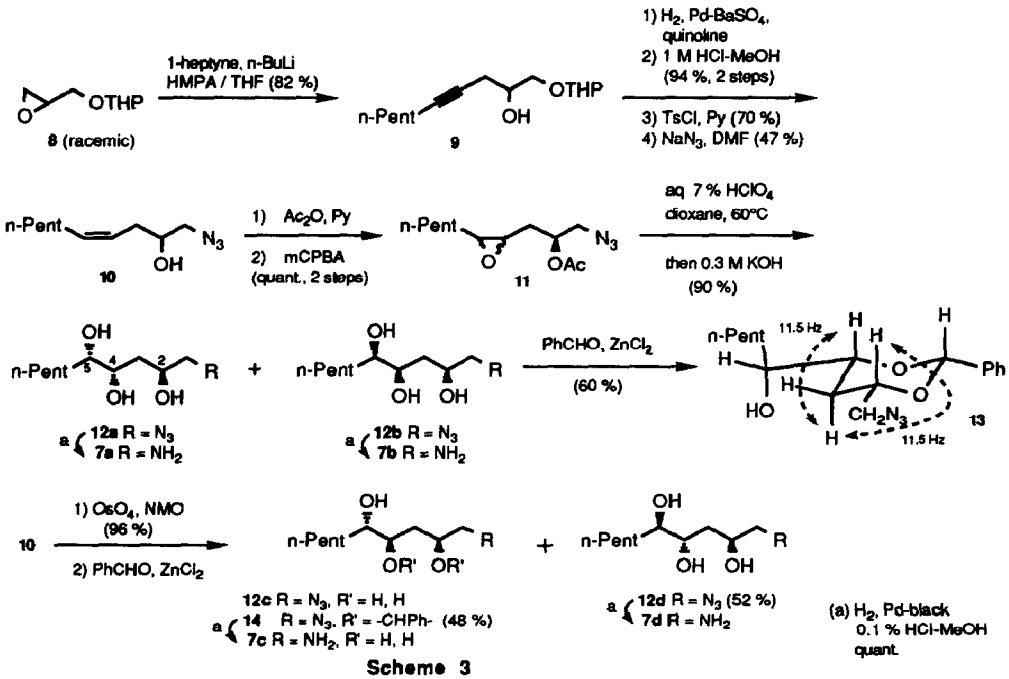


Scheme 1



Scheme 2

Structural difference of two components of AAL-toxin **1**, whose acyl group was substituted at either C-13 or C-14, did not affect the NMR resonances of right half of **1**.[2c] Therefore, we expected that aminotriols **7a** - **7d** (Scheme 3) are suitable model compounds for determining relative stereochemistries at C-2, C-4 and C-5 of **1**. The synthetic pathway of four possible isomers **7a** - **7d** were shown in Scheme 3. The required carbon skeleton of the model triols were constructed by condensation of epoxide **8** and 1-heptyne in high yield. The coupling product **9** was hydrogenated to corresponding *cis*-olefin which was further converted to azide **10** by deprotection, tosylation of primary alcohol and azidation. From this homoallyl alcohol **10**, 4,5-*syn*- and 4,5-*anti*-diols were prepared by the following transformations. After acetylation, treatment of *O*-acetyl-**10** with mCPBA gave diastereomeric epoxides **11**.[7] These were submitted to sequential hydrolysis with perchloric acid and aq. KOH to afford a 2:1 mixture of two 4, 5-*syn* triols **12a** and **12b**,[7] which were easily separated by SiO_2 chromatography. The major isomer **12b** was then converted to benzylidene acetal **13**, whose ^{1}H-NMR spectrum clearly shows 2, 4-*syn* relationship (Scheme 3). Whereas catalytic dihydroxylation with OsO_4 of azide **10** gave hardly separable 4, 5-*anti*-triols **12c** and **12d**. When this mixture was treated with benzaldehyde and $ZnCl_2$,



Scheme 3

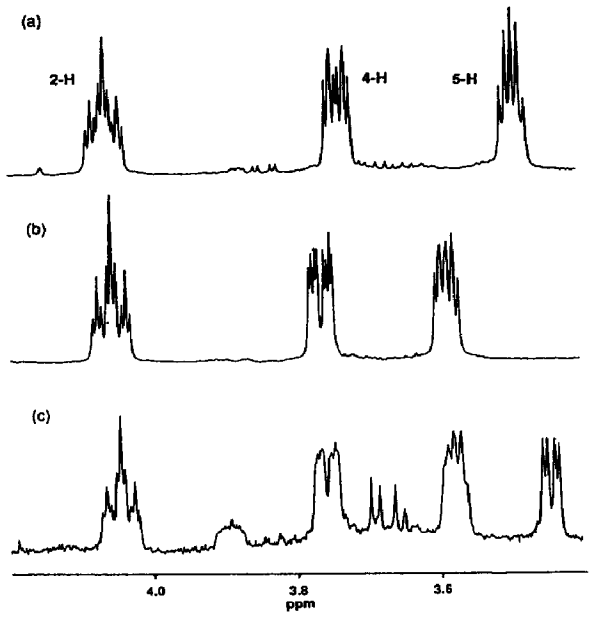


Fig. 1 ^{1}H-NMR spectra (500 MHz, D_2O) of: aminotriols (a) **12a**; (b) **12d**; (c) AAL-toxin **1**.

2, 4-*syn* isomer **12c** predominantly yielded acetal **14** while unreacted 2, 4-*anti*-isomer **12d** was recovered. The compounds **12a, 12b, 12d** and **14** were hydrogenated under acidic condition to afford the corresponding aminotriols **7a** - **7d**[8] as hydrochloride salts. The stereospecific transformations from **10** to the **7a** - **7d** and the NMR analysis of benzylidene acetals **13** and **14** enabled us to assign the relative stereocenters of aminotriols **7a** - **7d**.

Among the synthesized aminotriols, 2, 4-*syn* isomers **7b** and **7c** were easily excluded by the comparison of their ^{1}H-NMR spectral data[8] to that of aminopentol **2**.[2b,8] Although NMR spectra of the remaining 2, 4-*anti*-aminotriols[8,9] were very similar, inspection of the spectra (Fig.1) allowed us to determine that the relative stereochemistry of the right part of **1** is the same as that of **7d** (2, 4-*anti*-4, 5-*anti*). In addition, ^{13}C-NMR data supported this conclusion[9]; the resonances at 74.81 (C-5) and 35.88 ppm (C-3) in **7d** were nearly identical to that of **2**[2b,8] while those signals in **7a** were observed at 75.54 and 37.31 ppm. On the basis of *J*-values in the ^{1}H-NMR spectrum of **1**, Bottini *et al.* proposed the relative stereochemistries at C-2, C-4 and C-5 as all-*S* or all-*R* (2, 4-*anti*-4, 5-*syn*).[2b] Our data clearly indicate that 4, 5-*syn* **7a** and 4, 5-*anti* **7d** cannot be distinguished without comparing spectral data of the synthetic samples. Furthermore, the results of spectral analysis of the model aminotriol **7d** enabled us to conclude rigorously that the conformation of left part of **1** does not affect that of the right part in aqueous solution.

With the absolute configuration of fragment C(1)-C(5) of **1** established, the conformation of this part was examined next. From the calculated dihedral angles using Karplus equation and NOE studies for **7d**, the conformation of the right fragment of **1** was proposed. MM2 calculation of this conformation was undertaken and the energy-minimized conformation was shown in Fig. 2. This 3D-structure indicated the hydrogen bonds to be between NH→2-O and 4-OH→5-O even in aqueous solution. Thus, in order to avoid steric hindrance between two pseudo-cyclic systems, the right part of **1** predominates the conformation as shown in Fig. 2. Previous conformational studies of acyclic polyols[10] in D_2O showed that they exist as several energetically similar conformers. The conformation of aminotriol part of **1** is therefore a relatively unusual case in an acyclic system. The role of this conformer to biological activity is interesting.

In conclusion, we have determined the absolute stereochemistry of C(1)-C(5) fragment of **1**. The synthetic route described above can be applied to prepare a variety of analogues of **1**. Currently, we are undertaking to determine the remaining absolute structure of **1**.

We are grateful to Mr. K. Watanabe and Mrs. E. Fukushi in our department for analysis of MS spectra.

This work was supported by a Grant from the Ministry of Education, Science, and Culture of Japan.

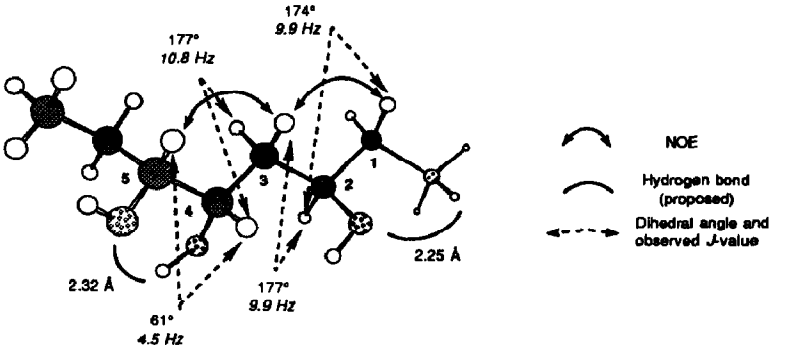


Fig. 2 The proposed conformation of fragment C(1)-C(5) of AAL-toxin **1**.

REFERENCES AND NOTES

1. Kohmoto, K.; Otani, H.; Nishimura, S. In *Phytotoxin and Plant Pathogenesis*; Graniti, A; Durbin, R. D.; Ballio, A. Eds; Springer-Verlag: Berlin, 1989; Series H, Vol. 27, pp 249-265; Kohmoto, K.; Otani, H.; Nishimura, S. In *Molecular Determinants of Plant Diseases*; Nishimura, S.; Vance, C. P.; Doke, N. Eds; Springer-Verlag: Berlin, 1987; pp 127-143; Kono, Y.; Knoche, H. W.; Daly, J. M. In *Toxins in Plant Disease*; Durbin, R. D. Ed; Academic Press: New York, 1981; pp 221-253.
2. (a) Siler, D. J.; Gilchrist, D. G. *Physiol. Plant Pathol.* **1983**, *23*, 263-274; (b) Bottini, A.T.; Gilchrist, D. G. *Tetrahedron Lett.* **1981**, *22*, 2719-2722; (c) Bottini, A.T.; Bowen, J. R.; Gilchrist, D. G. *Tetrahedron Lett.* **1981**, *22*, 2723-2726.
3. Identification based on chromatographic behavior: Kohmoto, K.; Verma, V. S.; Nishimura, S.; Takagi, M.; Scheffer, R. P. *J. Fac. Agric., Tottori Univ.* **1982**, *17*, 1-8; Identification using spectral data will be reported elsewhere.
4. (a) Gilchrist, D. G.; Harada, J. J. In *Phytotoxin and Plant Pathogenesis*; Graniti, A; Durbin, R. D.; Ballio, A. Eds; Springer-Verlag: Berlin, 1989; Series H, Vol. 27, pp 113-121; (b) Moussatos, V. V.; Gilchrist, D. G. *ibid.* pp 437.
5. Hanessian, S.; Ugolini, A.; Therien, M. *J. Org. Chem.* **1983**, *48*, 4427-4430.
6. The racemic **3** was prepared from 4-amino-3-hydroxybutanoic acid in three steps (i, CH_2N_2, ii, BH_3-Me_2S, iii, CbzCl, Na_2CO_3, 23 % overall).
7. For non protected epoxide *O*-deacetyl-**11**, the epoxide opening was attempted under acidic and basic conditions. In both cases, unidentified by-products were predominated. Since the epoxidation of *O*-acetyl-**10** gave a 1:1 diastereomer mixture, predominant formation of 2, 4-*syn*-diol **12b** could be explained by acyl group assisted epoxide opening in thermodynamically controlled manner.
8. ^{1}H-NMR data (500 MHz, D_2O): **7a**; δ 4.06 (1H, dddd, J = 3.3, 3.3, 9.6, 9.6 Hz, 2-H), 3.74 (1H, ddd, J = 3.3, 4.1, 9.6 Hz, 4-H), 3.49 (1H, ddd, J = 4.1, 4.1, 8.2 Hz, 5-H), 3.15 (1H, dd, J = 3.3, 13.1 Hz, 1-Ha), 2.93 (1H, dd, J = 9.6, 13.1 Hz, 1-Hb), 1.64 (1H, ddd, J = 3.3, 9.6, 14.5 Hz, 3-Ha), 1.59 (1H, ddd, J = 3.3, 9.6, 14.5 Hz, 3-Hb); **7b**; δ 4.07 (1H, dddd, J = 3.2, 6.4, 6.4, 9.6 Hz, 2-H), 3.70 (1H, ddd, J = 4.0, 6.4, 6.4 Hz, 4-H), 3.53 (1H, ddd, J = 4.0, 4.0, 8.5 Hz, 5-H), 3.19 (1H, dd, J = 3.2, 13.1 Hz, 1-Ha), 2.96 (1H, dd, J = 9.6, 13.1 Hz, 1-Hb), 1.77 (2H, t, J = 6.4 Hz, 3-H); **7c**; δ 4.09 (1H, dddd, J = 3.0, 6.4, 6.4, 9.6 Hz, 2-H), 3.69 (1H, ddd, J = 3.3, 4.6, 9.5 Hz, 4-H), 3.56 (1H, ddd, J = 3.4, 4.6, 9.1 Hz, 5-H), 3.19 (1H, dd, J = 3.0, 13.1 Hz, 1-Ha), 2.96 (1H, dd, J = 9.6, 13.1 Hz, 1-Hb), 1.81 (1H, ddd, J = 3.3, 5.4, 14.6 Hz, 3-Ha), 1.70 (1H, ddd, J = 6.4, 9.5, 14.6 Hz, 3-Hb); **7d**; δ 4.06 (1H, dddd, J = 2.9, 2.9, 9.9, 9.9 Hz, 2-H), 3.76 (1H, ddd, J = 2.0, 4.3, 10.8 Hz, 4-H), 3.59 (1H, ddd, J = 3.7, 4.3, 7.6 Hz, 5-H), 3.16 (1H, dd, J = 2.9, 13.1 Hz, 1-Ha), 2.93 (1H, dd, J = 9.9, 13.1 Hz, 1-Hb), 1.67 (1H, ddd, J = 2.0, 9.9, 14.5 Hz, 3-Ha), 1.54 (1H, ddd, J = 2.9, 10.8, 14.5 Hz, 3-Hb); 2^{2b}; δ 4.063 (1H, dddd, J = 3.0, 3.0, 9.9, 9.9 Hz, 2-H), 3.739 (1H, ddd, J = 2.0, 4.5, 10.8 Hz, 4-H), 3.556 (1H, ddd, J = 4.5, 6-8, 6-8 Hz, 5-H), 3.164 (1H, dd, J = 3.0, 13.1 Hz, 1-Ha), 2.938 (1H, dd, J = 9.9, 13.1 Hz, 1-Hb), 1.650 (1H, ddd, J = 2.0, 9.9, 14.8 Hz, 3-Ha), 1.533 (1H, ddd, J = 3.0, 10.8, 14.8 Hz, 3-Hb).
9. ^{13}C-NMR data (67.5 MHz, D_2O) **7a**; δ 75.54, 70.02, 65.02, 45.22, 37.31, 32.01, 31.20, 24.82, 22.11, 13.52; **7b**; δ 73.70, 71.00, 65.96, 44.48, 37.22, 32.10, 31.22, 24.93, 22.15, 13.54; **7c**; δ 74.60, 71.74, 66.24, 44.36, 36.18, 31.40, 31.20, 24.90, 22.11, 13.52; **7d**; δ 74.81, 70.48, 65.00, 45.24, 35.88, 31.51, 31.19, 24.93, 22.09, 13.52; 2^{2c}; δ 74.72 (C-5), 70.49 (C-4), 64.97 (C-2), 45.28 (C-1), 35.74 (C-3), 31.49 (C-6), 25.70 (C-7).
10. Hoffman, R. E.; Rutherford, T. J.; Mulloy, B.; Davies, D. B. *Magn. Reson. Chem.* **1990**, *26*, 458-464 and references are cited therein.

(*Received in Japan* 21 *June* 1993)