Design and synthesis of an antitumour cyclic hexapeptide *Rubia akane* analogue possessing an unnatural amide configuration[1]

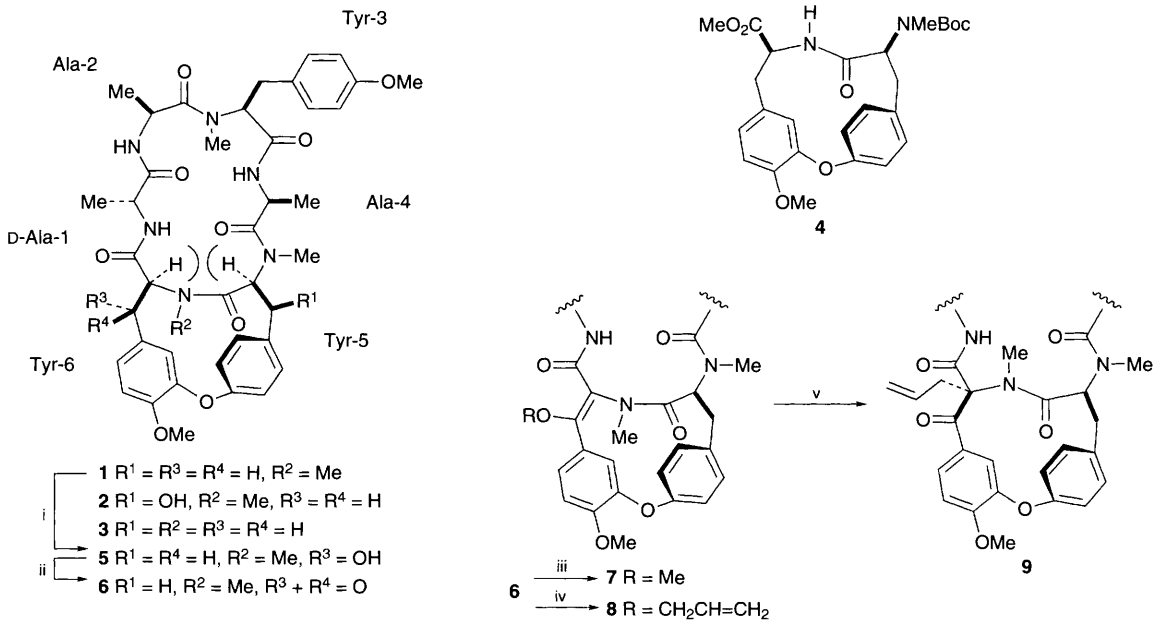
Yukio Hitotsuyanagi, Suguru Lee, Koichi Takeya and Hideji Itokawa*

Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan

An antitumour bicyclic hexapeptide analogue 9 possessing an unnatural *trans*-amide configuration between the Tyr-5 and Tyr-6 residues is designed and synthesised from RA-VII 1 (RA = *Rubia akane*).

RA-VII **1**[2] and bouvardin (NSC 259968) **2**[3] are a class of plant bicyclic hexapeptides possessing potent antitumour activity. Peptide **1** is currently undergoing clinical trials in Japan as an anticancer agent.[4] The mechanism of action of these peptides is considered to be the inhibition of protein synthesis through interaction with eukaryotic 80 S ribosomes.[5] It has been proposed that the 14-membered cycloisodityrosine constitutes the pharmacophore and the tetrapeptide, D-Ala-1-Ala-2-Tyr-3-Ala-4, constructing the 18-membered ring potentiates the biological properties and alters the conformation of cycloisodityrosine to that possessing the inherently disfavoured *cis*-amide bond within the 14-membered ring.[6] The cytotoxicity (B16, L1210) of N^{29}-desmethyl-RA-VII **3** is reported to be 50–60 times more potent than the cycloisodityrosine analogue **4** having a *trans*-peptide bond.[6b] However, it is unclear whether this potentiation is caused by the alteration of the cycloisodityrosine conformation or is due to the intrinsic effect of the tetrapeptide moiety. To obtain information about the influence of the amide geometry upon the activity, we proposed an analogue possessing a *trans*-amide bond between the Tyr-5 and the Tyr-6 residues and retaining the natural configuration at all the stereogenic centres. Prior to design of the analogue, a strategy is required that is capable of overriding this intrinsic bias that leads to the *cis*-amide bond. We deemed that substitution of the hydrogen atom at the α-position of either the Tyr-5 or the Tyr-6 residue by an alkyl group would force the geometry of the *cis*-amide to the *trans*-amide by steric repulsion. The successful realisation of this approach is outlined here.

We considered that RA-IV **5**,[7] a minor congener of **1**, would be a suitable precursor for the transformations since it has a hydroxy group at the β-position of the Tyr-6 residue which will serve as a foothold for the chemical manipulation. However, because of the quite small content (*ca.* 1×10^{-5}% of the dry roots of *Rubia cordifolia*) of **5** in the plant, its supply from the more available (*ca.* 1×10^{-2}%)† **1** had to be devised. After extensive experimentation, we found that oxidation of **1** with ceric ammonium nitrate (CAN) in MeCN–H_2O gave **5** in 69% yield (Scheme 1).‡ Swern[8] oxidation of **5** gave **6** (yield 83%).§ Initial attempts *via* direct alkylation using potassium carbonate and methyl iodide did not afford the *C*-alkyl product but yielded methyl enol ether **7** instead (yield 86%). Thus, **6** was converted into the allyl enol ether **8** (yield 94%) in the same manner. Claisen rearrangement of **8** proceeded when **8** was heated to 100 °C for 7 d and produced the *C*-allyl derivative **9** (yield 83%) as a single product. The stereochemistry of the allyl group was established by NOESYPH[9] experiments (Fig. 1).¶ Strong correlations were observed between the vinyl methine proton and Tyr-6-N*Me* and between the Tyr-5α proton and Tyr-6-N*Me*. These correlations are possible when the allyl group was introduced from the α-face of the molecule and the peptide bond has a *trans*-configuration. Another important correlation between Ala-2 Hα and Tyr-3 N*Me*, Tyr-3 N*Me* and Tyr-3 Hα, and Ala-4 *Me* and Tyr-5 N*Me* demonstrated that analogue **9** has the same *trans*-amide structure between the Ala-2 and Tyr-3,



Scheme 1 *Reagents and conditions*: i, CAN, MeCN–H_2O, room temp., 69%; ii, $(COCl)_2$, Me_2SO, CH_2Cl_2, −78 °C, Et_3N, 83%; iii, MeI, K_2CO_3, acetone, reflux, 86%; iv, allyl bromide, K_2CO_3, acetone, reflux, 94%; v, dioxane–heptane, sealed tube, 100 °C, 7 d, 83%

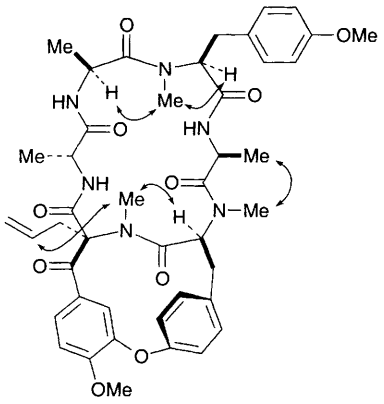


Fig. 1 Selected NOESYPH correlations for the main conformation of **9**

and between Ala-4 and Tyr-5 residues as the predominant conformation of **1**.[10] A preliminary biological evaluation using KB cells revealed that analogue **9** (IC_{50}: 0.014 μg cm^{-3}) is only 7 times less toxic than **1** (0.0020 μg cm^{-3}) and is slightly more toxic than the keto analogue **6** (0.021 μg cm^{-3}) possessing the same *cis*-amide bond between the Tyr-5 and Tyr-6 residues as peptide **1**. These results suggest that the *cis*-amide configuration between Tyr-5 and Tyr-6 residues is not essential for the 18-membered ring tetrapeptide potentiation of the 14-membered cycloisodityrosine subunit and that the potentiation is due to the intrinsic effect of the tetrapeptide (D-Ala-1-Ala-2-Tyr-3-Ala- 4) moiety.

Footnotes

† Although the plant contains some des-*O*-methyl congeners (*e.g.* RA-II[7] and RA-V[2b]), they are methylated to RA-VII **1** during the large-scale isolation process for reasons of convenience.

‡ It is interesting to note that the CAN oxidation of benzylic methylene/methyl groups generally affords carbonyl compounds.[11] The rigid cycloisodityrosine structure might be responsible for this alcohol formation which stereoelectronically hampers the oxidative abstraction of the pro-*R* hydrogen parallel to the aromatic ring.

§ We have previously oxidised RA-IV **5** using activated manganese dioxide, but the result was poor (yield 24%).[7,12]

¶ All proton and carbon resonances were assigned by H-H COSY, HMQC, HMBC and NOESYPH techniques.

References

1 Studies on *Rubia akane* (RA) derivatives. Part 9. For part 8 see: Y. Hitotsuyanagi, S. Lee, I. Ito, K. Kondo, K. Takeya, T. Yamagishi, T. Nagate and H. Itokawa, *J. Chem. Soc., Perkin Trans. 1*, 1996, 213.

2 (*a*) H. Itokawa, K. Takeya, K. Mihara, N. Mori, T. Hamanaka, T. Sonobe and Y. Iitaka, *Chem. Pharm. Bull.*, 1983, **31**, 1424; (*b*) H. Itokawa, K. Takeya, N. Mori, T. Hamnaka, T. Sonobe and K. Mihara, *Chem. Pharm. Bull.*, 1984, **32**, 284.

3 S. D. Jolad, J. J. Hoffmann, S. J. Torrance, R. M. Wiedhopf, J. R. Cole, S. K. Arota, R. B. Bates, R. L. Gargiulo and G. R. Kriek, *J. Am. Chem. Soc.*, 1977, **99**, 8040.

4 F. Yoshida, R. Asai, H. Majima, S. Tsukagoshi, H. Furue, M. Suminaga, K. Sakamoto, H. Niitani, A. Murata, M. Kurihara, T. Izumi, I. Nakao, Y. Ohashi and K. Ito, *Jpn. J. Cancer Chemother.*, 1994, **21**, 199.

5 M. Zalacaín, E. Zaera, D. Vázquez and A. Jiménez, *FEBS Lett.*, 1982, **148**, 95.

6 (*a*) D. L. Boger, D. Yohannes and J. B. Meyers Jr., *J. Org. Chem.*, 1992, **57**, 1319; (*b*) D. L. Boger, D. Yohannes, J. Zhou and M. A. Patane, *J. Am. Chem. Soc.*, 1993, **115**, 3420.

7 H. Itokawa, K. Takeya, N. Mori, T. Sonobe, S. Mihashi and T. Hamanaka, *Chem. Pharm. Bull.*, 1986, **34**, 3762.

8 A. J. Mancuso, S.-L. Huang and D. Swern, *J. Org. Chem.*, 1978, **43**, 2480.

9 G. Bodenhausen, H. Koger and R. R. Ernst, *J. Magn. Reson.*, 1984, **58**, 370.

10 (*a*) R. B. Bates, J. R. Cole, J. J. Hoffmann, G. R. Kriek, G. S. Linz and S. J. Torrance, *J. Am. Chem. Soc.*, 1983, **105**, 1343; (*b*) H. Morita, K. Kondo, Y. Hitotsuyanagi, K. Takeya, H. Itokawa, N. Tomioka, A. Itai and Y. Iitaka, *Tetrahedron*, 1991, **47**, 2757.

11 (*a*) L. Syper, *Tetrahedron Lett.*, 1966, 4493; (*b*) T.-L. Ho, *Synthesis*, 1973, 347.

12 N. Mori, PhD Thesis, Tokyo College of Pharmacy, 1985.

Received, 4th September 1995; Com. 5/05846E