Tetrahedron Letters, Vol. 34, No. 50, pp. 8119-8122, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

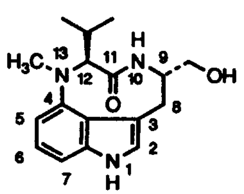
Designed Molecules Reproducing the Two Conformations of Teleocidins.

Michihiro Ohno, Yasuyuki Endo,* Masaaki Hirano, Akiko Itai and Koichi Shudo

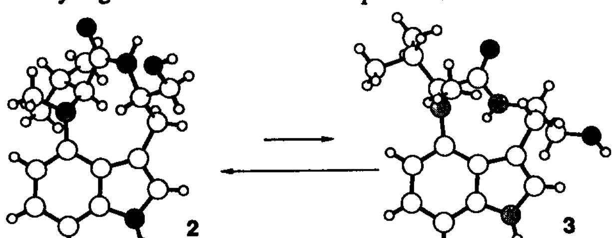
Faculty of Pharmaceutical Sciences, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113, Japan.

Abstract: Tumor-promoting teleocidins are known to exist in an equilibrium between two conformational states, the twist form and sofa form, in solution. Benzolactam-Vs, in which the indole ring of indolactams was replaced with a benzene ring, were designed in an attempt to reproduce the active conformation of teleocidins, and synthesized. The 8-membered lactam (benzolactam-V8-310) exists only in the twist form in solution and the 9-membered lactam (benzolactam-V9-310) exists only in the sofa form in solution. The stronger biological activities of the 8-membered lactam than those of indolactam-V and the lack of activity of the 9-membered lactam clearly indicated that active conformation for tumor-promoting activity of teleocidins is close to the twist form.

Teleocidins are tumor promoters as potent as 12-*O*-tetradecanoylphorbol-13-acetate (TPA).[1] Teleocidins and their active congeners (indolactam-V(1)s)[2] are known to exist in an equilibrium between at least two conformational states in solution, the twist (2) and sofa (3) form.[3] It is particularly important to determine the active ring conformation of teleocidins in order to explain the relationships between the structures and activities of several classes of TPA-type tumor promoters with various skeletal structures.[4] The conformational equilibrium is attributed to a *cis-trans* isomerization of the amide bond and the steric effects of substituents on the nine-membered lactams.[5] An importance of the twist form for the appearance of the activity has been suggested by the synthesis of indolactams having substituents on indole ring.[6] However, the low energy barrier between the two conformers (in case of **1**, the observed free energy of activation was $\Delta G^{\#} = 19.2$ kcal/mol at -10°C)[3] makes it difficult to identify the mode of interaction of these promoters with common macromolecular target(s). Synthesis of molecules in which the lactam ring is restricted to the twist or sofa form might allow us to solve the problem and to develop strategies for analyzing the mechanisms of tumor promotion.



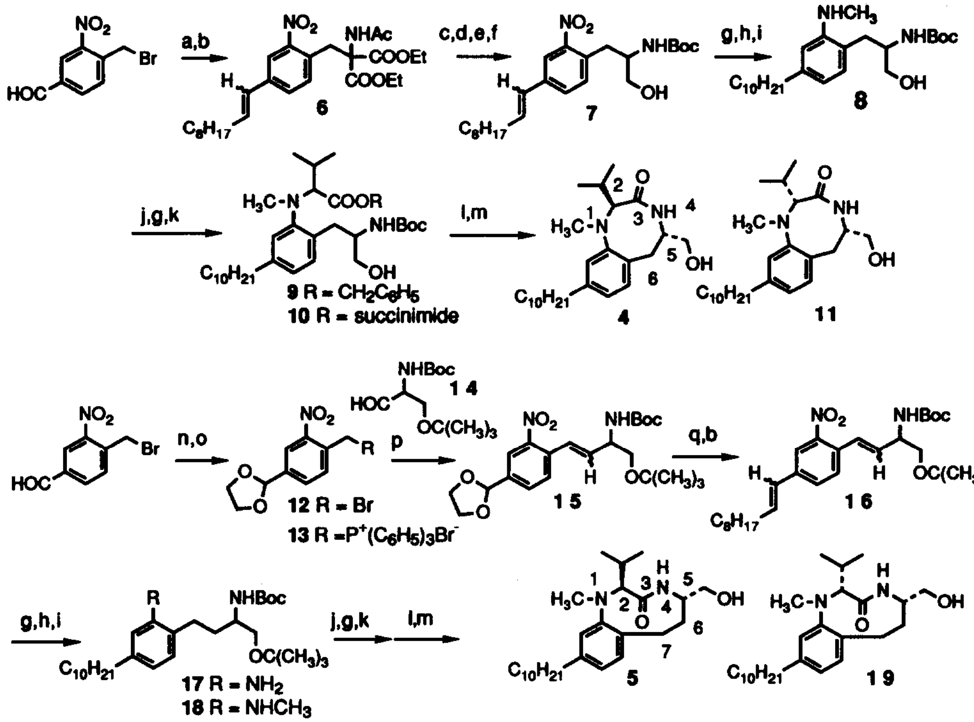
1



2 3

Figure 1. Conformation of indolactam-V in the twist (left) and sofa (right)

Conformational analyses of simple lactams have indicated that 5- to 8-membered lactams have the *cis* conformation and 10-membered and larger lactams have the *trans* conformation, while a 9-membered lactam, azacyclononanone, exists as an equilibrium mixture of *cis* and *trans* conformers.[7] Consequently, alteration of ring-size of the lactams seems to be effective for the restriction of conformation to the twist or sofa form. In connection with the design of suitable molecules for our purpose, two structure-activity results attracted our attention. 1) The presence of the hydrogen at N-1 of indolactam-V is not essential for the activity.[8] 2) The terpenoid side chain on teleocidins can be substituted by a simple alkyl group without loss of activity, as in 7-decylindolactam-V.[9] Based on the above considerations, benzolactam-V8-310 (4) was designed as a twist-restricted analogue and benzolactam-V9-310 (5) as a twist-sofa equilibrated analogue.



a) $CH_3CONHCH(COOC_2H_5)_2$, NaH/ DMF b) $C_9H_{19}P^+Ph_3\,Br^-$, *n*-BuLi/ THF c) HCl/ AcOH d) $SOCl_2$/ EtOH e) Boc_2O/ CH_2Cl_2 f) $LiBH_4$/ THF g) H_2, Pd-C/ EtOH h) HCOOH, AcOH i) BH_3/ THF j) triflate of benzyl DL-α-hydroxyisovalerate, 2,6-lutidine/ CH_2Cl_2 k) *N*-hydroxysuccinimide, DCC/ CH_3CN l) CF_3COOH/ CH_2Cl_2 m) $NaHCO_3$aq/ CH_3COOEt n) $HOCH_2CH_2OH$, TsOH/ toluene o) PPh_3/ toluene p) 14, K_2CO_3/ DMF q) pyridinium *p*-toluenesulfonate/ acetone, H_2O

The designed benzolactams were synthesized as follows. Reaction of 4-bromomethyl-3-nitrobenzaldehyde with diethyl acetamidomalonate without protection of the aldehyde group, followed by Wittig reaction employing nonyl phosphonium ylide gave **6** (55%). Deprotection and decarboxylation gave the amino acid, which was protected with ethyl ester for the carboxylic acid group and Boc for the amine group, and the ester group was reduced with $LiBH_4$ to afford **7** (59%). The nitroalcohol **7** was converted into methylamino alcohol (**8**, 75 %) by catalytic hydrogenation and formylation, followed by reduction with BH_3. Reaction of **8** with the triflate of benzyl DL-α-hydroxyisovalerate gave diastereomeric esters **9** (64 %). After hydrogenolysis of the benzyl ester, condensation with *N*-hydroxysuccinimide using DCC gave the activated esters **10** (96%). After removal of the Boc group using CF_3COOH, cyclization was carried out under dilute conditions to give **4** (48 %) and the epimer **11** (43 %), which were isolated on this stage.

The nine-membered lactam was also synthesized from 4-bromomethyl-3-nitrobenzaldehyde. After protection of the aldehyde group with acetal, the benzylbromide **12** was converted into the phosphonium salt **13**. The aldehyde **14**[10] derived from DL-serine was reacted with the ylide generated from **13** to give **15** (57 %, *cis:trans* 2:3). After deprotection of the acetal, the alkyl chain was introduced by the same procedure described above to give a mixture of four stereoisomers **16** (38 %). Catalytic hydrogenation of **16** gave a single amine **17**, which was converted to **18** (84 %), and **18** was further converted into **5** and the epimer **19** in the same manner as described for the synthesis of **4** in similar yields except for the cyclization step (20 % for **5** and 17 % for **19**).

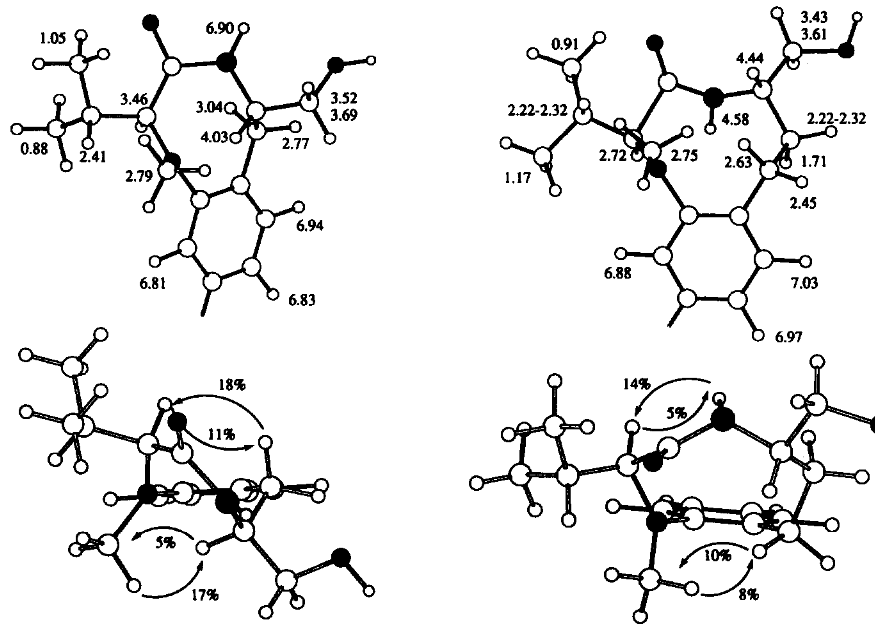


Figure 1. Conformations of 4 (left) and 5 (right); the alkyl side chain is omitted for the sake of clarity. Top: Views from the face of the benzene ring. The numerals are chemical shifts (ppm from TMS) in $CDCl_3$. Bottom: Views from the side of the benzene ring. The arrows indicated characteristic NOE enhancements.

Extensive conformational analysis of the twist and sofa forms of indolactam-V has been conduced[3] by the use of ^{1}H-NMR, and significant differences of chemical shifts between the two conformers have been observed. The conformational structures of **4** and **5** were deduced from ^{1}H-NMR spectral data and nuclear Overhauser effect (NOE) experiments.(Figure 1) The two benzolactams were each proved to exist in a single conformational state in solution, even at -40°C, despite the expectation that **5** might be in a twist-sofa equilibrium (see above). Although the chemical shifts of **4** could not directly indicate the conformation, in the NOE difference spectra, saturation of H-6α resulted in characteristic enhancement of the H-2 signal, as was found for the twist form of indolactam-V. No other conformation could explain the experimental results. Therefore, the 8-membered lactam **4** exists only in twist form in solution.

On the other hand, the spectral data of **5** were close to those of the sofa form of indolactam-V.[3] In particular, H-4 (NH) and H-2 were subject to high-field shielding by aromatic current anisotropy, while the large coupling constant between H-4 and H-5 indicated that the dihedral angle between these protons is near to 180°. These characteristic features are the same as those of the sofa form of indolactam-V. NOE enhancement of the H-2 signals by saturation of H-4 (NH) confirmed the sofa form of 5. Thus, the 9-membered lactam 5 exists only in the sofa form in solution.

The TPA-type tumor promoters induce growth inhibition, cell adhesion[11] and differentiation to monocytes of human promyelocytic leukemia cells (HL-60).[12] The twist-restricted benzolactam-V8-310 (4) caused growth inhibition and differentiation of HL-60 cells at the concentration of 10^{-8} M. On the contrary, the sofa-restricted benzolactam-V9-310 (5) proved to be inactive below the concentration of 10^{-6} M. The potency estimated from IC_{50} of the 8-membered lactam 4 was only one order weaker than that of teleocidin B-4 but 30 times stronger than that of indolactam-V. On the other hand, the 9-membered lactam 5 proved to be inactive. The benzolactams 4 and 5 were also evaluated in a standard [^{3}H]TPA competitive binding assay to protein kinase C (PKC) regulatory domain.[13] [^{3}H]TPA binding was inhibited by 45 % in the presence of 4 (1000 fold excess).[14] The binding was not inhibited in the presence of 5 (1000 fold excess).

It is difficult to rule out the possibility of conversion from the twist form to another putative conformers[4] (e.g. the fold form) through relatively low energy barrier (conversion being not due to *cis-trans* isomerization of amide bond) when the molecule interact with target. However, the above biological results indicate that the active conformation of teleocidins should not be the sofa form[15] but the twist form or another conformation converted from the twist form through low energy barrier.

References and Notes

1. Fujiki, H., Mori, M., Nakayasu, M., Terada, M., Sugimura, T., Moore, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 3872-3876.
2. Endo, Y., Shudo, K., Okamoto, T. *Chem. Pharm. Bull.* **1982**, *30*, 3457-3460. Endo,Y., Shudo, K., Furuhata, K., Ogura, H., Sakai, S., Aimi, N., Hitotsuyanagi, Y., Koyama, Y. *Chem. Pharm. Bull.* **1984**, *32*, 358-361. Irie, K., Hirota, M., Hagiwara, N., Koshimizu, K., Hayashi, H., Murao, S., Tokuda, H., Ito, Y. *Agric. Biol. Chem.*, **1984**, *48*, 1269-1274. deLaszlo, S. E., Ley, S. V., Porter, R. A. J. *J. Chem. Soc. Chem. Commun.* **1986**, 344-346. Kogan, T. P., Somers, T. C., Venuti, M. C. *Tetrahedron*, **1990**, *46*, 6623-6632.
3. Endo, Y., Shudo, K., Itai, A., Hasegawa, M., Sakai, S., *Tetrahedron*, **1986**, *42*, 5905-5924.
4. Kawai, T., Ichinose, T., Endo, Y., Shudo, K., Itai, A., *J. Med. Chem.* **1992**, *25*, 2248-2253. Kawai, T., Ichinose, T., Takeda, M., Tomioka, N., Endo, Y., Yamaguchi, K., Shudo, K., Itai, A., *J. Org. Chem.* **1992**, *57*, 6150-6155.
5. Jeffrey, A. M., Liskamp, R. M. J., *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 241-245. Wender, P. A., Koehler, K. F., Sharkey, N. A., Dell'Aquila, M. L., Blumberg, P. M., *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4214-4218. Itai, A., Kato, Y., Tomioka, N., Iitaka, Y., Endo, Y., Hasegawa, M., Shudo, K., Fujiki, H., Sakai, S., *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 3688-3692.
6. Irie, K., Hagiwara, N., Koshimizu, K., *Tetrahedron*, **1987**, *43*, 5251-5260.
7. Williamson, K. L., Roberts, J. D., *J. Am. Chem. Soc.*, **1976**, *98*, 5082-5086.
8. Fujiki, H., Suganuma, M., Hakii, H., Nakayasu, M., Endo, Y., Shudo, K., Irie, K., Koshimizu, K., Sugimura, T., *Proc. Jpn. Acad.* **1985**, *61*, 45-47.
9. Irie, K., Hayashi, H., Arai, M., Koshimizu, K. *Agric. Biol. Chem.* **1986**, *50*, 2679-2680. Endo, Y., Sato, Y., Shudo, K., *Tetrahedron*, **1987**, *43*, 2241-2247. Irie, K., Hagiwara, N., Tokuda, H., Koshimizu, K. *Carcinogenesis*, **1987**, *8*, 547-552.
10. Compound 14 was obtained from the O-t-Bu-N-Z-Ser methyl ester by exchange of the N-protective group from carbobenzoxy to Boc, followed by reduction using DIBAH (55 % in total).
11. Fujiki, H., Mori, M., Nakayasu, M., Terada, M., Sugimura, T., *Biochem. Biophys. Res. Commun.*, 1979, *90*, 976-983.
12. Huberman, E., Callaham, M. F., *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 1293-1297.
13. Jeng, A., Sharkey, N., Blumberg, P. *Cancer Res.*, **1986**, *46*, 1966-1972.
14. [^{3}H]TPA binding in the presence of indolactam-V was inhibited by below 20 %. Indolactam-V shows potent PKC activation but shows weak inhibition for TPA binding for PKC.
15. Recently, synthesis and lack of biological activity of sofa-restricted indolactam mimics were reported in Kozikowski, A. P., Ma, D., Pang, Y-P., Shum, P., Likic, V., Mishra, P., Macura, S., Basu, A., Lazo, J. S., Ball, R. G., *J. Am. Chem. Soc.*, **1993**, *115*, 3957-3965. We consider that a role of the sofa form can not be ruled out by only the results described in the above paper because the mimics are short of the hydrophobic alkyl groups which are important for the activity. We have found that an analogue of 4 lacking $C_{10}H_{21}$ group on benzene ring showed very weak activity by HL-60 assay and inhibition of TPA binding to PKC.

(Received in Japan 27 May 1993; accepted 14 July 1993)