Tetrahedron Letters,Vol.26,No.8,pp 1069-1072,1985 0040-4039/85 $3.00 + .00
Printed in Great Britain

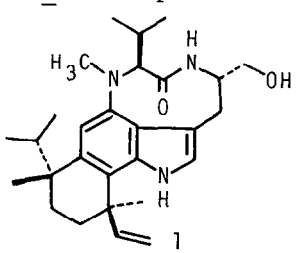
TUMOR PROMOTERS EXIST IN TWO CONFORMATIONAL STATES IN SOLUTION. STEREOCHEMISTRY OF (±)-INDOLACTAM-V.

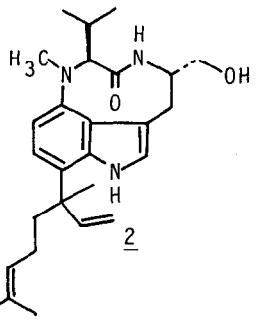
Yasuyuki Endo,[a] Masashi Hasegawa,[a] Akiko Itai,[a] Koichi Shudo,[a,*]
Motoo Tori,[b] Yoshinori Asakawa,[b] and Shin-ichiro Sakai[c]

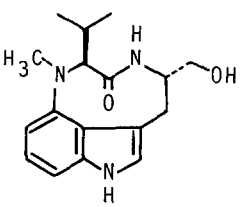
Faculty of Pharmaceutical Sciences, University of Tokyo,[a] Hongo, Bunkyo-ku, Tokyo,113, Japan, Faculty of Pharmaceutical Sciences, Tokushima Bunri University,[b] Yamashiro, Tokushima, 770, Japan, Faculty of Pharmaceutical Sciences, Chiba University,[c] Yayoi-cho, Chiba, 260, Japan.

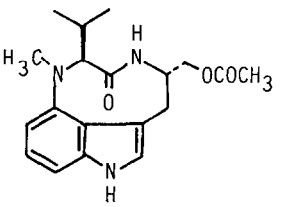
Abstract: ^{1}H-Nmr study and force field calculation of conformational Isomerism in (±)-indolactam-V which is a biologically active fragment of tumor promoter, teleocidin B, are described.

Teleocidin B (1) and lyngbyatoxin A (2) are produced by Streptomyces mediocidicus[1] and a blue-green alga, Lyngbya majuscula[2], respectively. They have been proved to be very potent skin tumor promoters.[3] They bind strongly to the recepter of another tumor promoter, tetradecanoylphorbol acetate (TPA), and manifest a variety of very important epigenetic effects in vitro.[4] Recently, we synthesized the structural fragment common to 1 and 2, i.e.,(-)-indolactam-V (3) and its stereoisomers.[5] (-)-Indolactam-V, but not the antipode or diastereoisomers, was found to exhibit several of the essential biochemical effects of tumor promoters.[6] More recently, the same compound and its acetate (4) were isolated from Streptoverticillium blastmyceticum together with 1 as compounds which induce Epstein-Barr virus early antigen.[7]


1


2


3


4

The ^{1}H-nmr spectrum of 3 suggested the existence of two stable conformers in solution.[5] Moore has reported the presence of extra signals in the nmr of 2, though the possible coexistence of an isomer could not be rule out.[2] Teleocidin B and olivoretins also show duplicate signals in the nmr.[8] In this paper we wish to report that these tumor promoters may exist in two stable conformational states in solution, on the basis of a thermodynamic analysis and nuclear magnetic resonance study of (±)-indolactam-V and its acetate (4).

The conformations of these compounds seem likely to play a critically important role in the appearance of the biological activity.[9]

The nmr spectra of 3 and 4 in CD_3OD and of 4 in $CDCl_3$ prepared at room temperature can be interpreted in terms of two sets of components (A and B), in a ratio that is almost independent of temperature between -30° and 60°, though it is dependent on solvent. Quite similar chemical shifts were assigned for the two sets of signals in the nmr spectra of 1, 2 and olivoretins.[8] However, when the spectrum of 4 at -30° immediately after dissolution of crystalline 4 in $CDCl_3$ precooled to -40° (fig. 1a), only a single component (A) was detectable. When the solution was warmed to 23° and then the spectrum was again recorded at -30° (Fig. 1b), an equilibrated spectrum was obtained, where the ratio of the two components (B/A) was 2.8. When the crystals recovered from the equilibrated solution were redissolved in precooled $CDCl_3$ and the nmr spectrum was again recorded at -30°, it showed only the signals of component A.

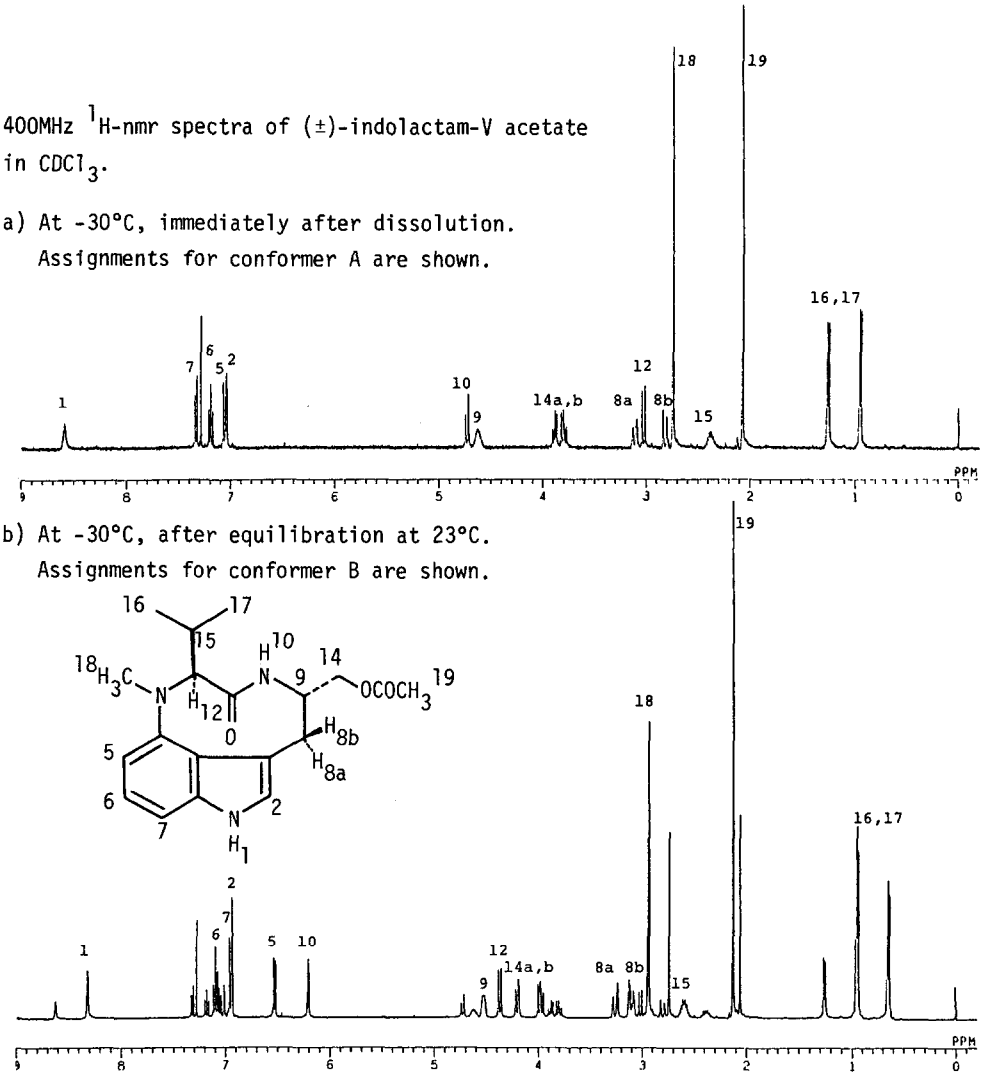


Figure 1. 400MHz ^{1}H-nmr spectra of (±)-indolactam-V acetate in $CDCl_3$.

a) At -30°C, immediately after dissolution. Assignments for conformer A are shown.

b) At -30°C, after equilibration at 23°C. Assignments for conformer B are shown.

In order to determine the thermodynamic parameters of the conversion between the two conformers, measurements of conversion rates were carried out by nmr. Good first-order kinetics were obtained for conversion of A to B. The equilibrium constant, B/A, at -10° was found to be 2.77, which corresponds to a free energy change of 0.53 kcal/mol. The rate constant of conversion of A to B was 1.03×10^{-3} s^{-1} (-10°C), and the activation free energy $\Delta G^{\#}$ was 18.9 kcal/mol. Table 1 also shows the values of $\Delta H^{\#}$ and $\Delta S^{\#}$. The 50%-equilibration time were calculated to be 14 min at -10° and 8 sec at 20°.[10]

Table 1. Conformational Conversion Parameters for A to B in $CDCl_3$ Solution.

temp. °C	rate constant s^{-1}	$\Delta G^{\#}$ kcal/mol	$\Delta H^{\#}$ kcal/mol	$\Delta S^{\#}$ cal/K·mol
-20	1.94×10^{-4}	19.0		
-10	1.03×10^{-3}	18.9	21.6± 0.1	+10.4± 0.4
0	4.92×10^{-3}	18.8		

The measurements for the determination of the rate constants were carried out using a nmr spectrometer and a variable temperature controller. Each sample was prepared as follows: 5 mg of (±)-4 was dissolved in 0.4 ml of $CDCl_3$ at -40°C, and spectrum was recorded at suitable intervals at each temperature using PG-200 auto stacking system. (interval 484 sec, accumulation 6 sec x 12 times at -20°C; interval 123.5 sec, accumulation 6 sec x 12 times at -10°C; interval 51.4 sec, accumulation 6 sec x 8 times at 0°C)

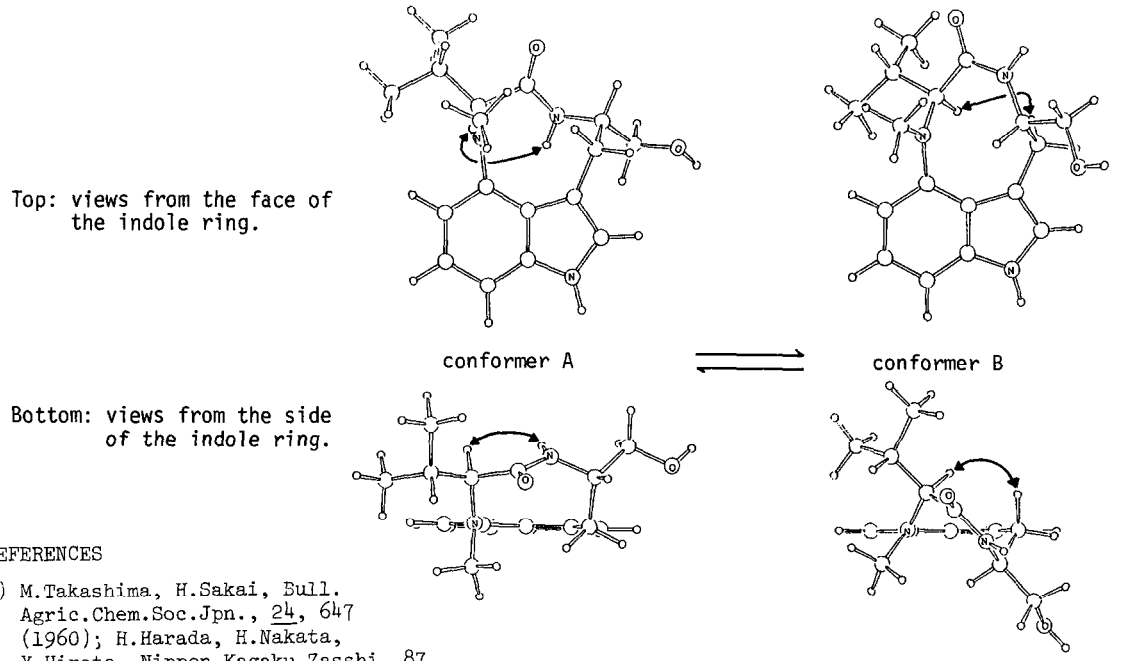
The structures of the two conformers were deduced from the 400 MHz nmr spectra of 3 and 4. It should be noted that some corresponding signals of the two conformers differ significantly in chemical shift (Fig. 1). Protons H-10 and H-12 of conformer A, and protons H-16 and H-17 of conformer B are subject to highfield shielding by the aromatic ring current anisotropy. The lowfield shielding of the aromatic protons (H-5 and H-7) of conformer A is due to the fixation of the molecule in a conformation which decreases the resonance between the lone pair electrons on the nitrogen atom and the aromatic electrons. The large coupling (J=11.5 cps) between H-10 (NH) and H-9 of conformer A indicates that the dihedral angle between these protons is near 180°.

Nuclear Overhauser effect (NOE) difference spectra of 3 and 4 in CD_3OD and 4 in $CDCl_3$ were taken. In conformer A, saturation of the H-10 proton (NH) caused a characteristic enhancement of the H-12 signal. In conformer B, saturation of H-12 resulted in a remarkable enhancement of the H-8a signal. Saturation of H-18 also produced an NOE at H-5.

On the basis of these observations and exhaustive examination of molecular models, conformer A and conformer B are presumed to be in SOFA conformation and in TWIST conformation, respectively. These molecular model structures were refined by empirical force field (EFF) calculation using Allinger's MM2 program.[11] The torsional barriers for the amide C-N bond and the N^{13}-C(aromatic) bond were estimated to be 24 and 8 kcal/mol, respectively. The optimized

structures for 3 are illustrated in Fig. 2.[12] The conversion of SOFA to TWIST could be accomplished mainly by the rotation of the amide C-N bond from trans to cis. The SOFA conformation is similar to the crystalline conformation of olivoretin B and the TWIST conformation is similar to that of teleocidin B.[8] It would be interesting to know which conformation, SOFA or TWIST, is of critical importance for the appearance of tumor promotion and other biological effects. We are planning to synthesize compounds whose conformations are fixed in one of these two conformations.

Figure 2. Conformation of Indolactam-V (3)
The arrows indicate NOE enhancements. (see text)



REFERENCES

1) M.Takashima, H.Sakai, Bull. Agric.Chem.Soc.Jpn., 24, 647 (1960); H.Harada, H.Nakata, Y.Hirata, Nippon Kagaku Zasshi, 87, 86 (1966); N.Sakabe, H.Harada, Y.Hirata, Y.Tomiie, I.Nitta, Tetrahedron Lett., 1966, 2523.
2) J.H.Cardellina II, F.J.Marner, R.E.Moore, Science, 204, 193 (1979).
3) H.Fujiki, M.Suganuma, N.Matsukura, T.Sugimura, S.Takayama, Carcinogenesis, 3, 895 (1983).
4) H.Fujiki, M.Mori, M.Nakayasu, M.Terada, T.Sugimura, Biochem.Biophys.Res.Commun., 90, 976 (1979); T.Hirakawa, T.Kakunaga, H.Fujiki, T.Sugimura, Science, 216, 527 (1982); H.Fujiki, M.Mori, M.Nakayasu, M.Terada, T.Sugimura, R.E.Moore, Pro.Nat.Acad.Sci.USA, 78, 3872 (1981); H.Fujiki, T.Sugimura, R.E.Moore, Environmental Health Perspectives, 50, 85 (1983).
5) Y.Endo, K.Shudo, K.Furuhata, H.Ogura, S.Skai, N.Aimi, Y.Hitotsuyanagi, Y.Koyama, Chem.Pharm. Bull., 32, 358 (1984); Y.Endo, K.Shudo, T.Okamoto, Chem.Pharm.Bull., 30, 3457 (1982).
6) H.Fujiki, M.Suganuma, M.Nakayasu, T.Tahira, Y.Endo, K.Shudo, T.Sugimura, Gann, 75, 866 (1984).
7) K.Irie, M.Hirota, N.Hasegawa, K.Koshimidzu, H.Hayashi, S.Murao, H.Tokuda, Y.Ito, Agric.Biol. Chem., 48, 1269 (1984).
8) S.Sakai, N.Aimi, K.Yamaguchi, Y.Hitotsuyanagi, C.Watanabe, K.Yokose, Y.Koyama, K.Shudo, A.Itai, Chem.Pharm.Bull., 32, 354 (1984).
9) D.H.Rich, P.K.Bhatnagar, R.D.Jasensky, J.A.Steele, T.F.Uchytil, R.D.Durbin, Bioorganic Chem., 7, 207 (1978).
10) cf., ref. 9, and K.Oda, T.Ohnuma, Y.Ban, K.Abe, J.Am.Chem.Soc., 106, 5378 (1984).
11) N.L.Allinger, J.Am.Chem.Soc., 99, 8127 (1977); J.Comput.Chem., 1, 30 (1980).
12) The calculated energy difference between the two structures is 0.53 kcal/mol.

(Received in Japan 27 November 1984)