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SYNTHESIS OF A BIOLOGICALLY ACTIVE FLUORESCENT INDOLACTAM DERIVATIVE; A METHOD OF PREPARING NEW PROBES FOR RECEPTOR ANALYSIS OF TUMOR PROMOTERS

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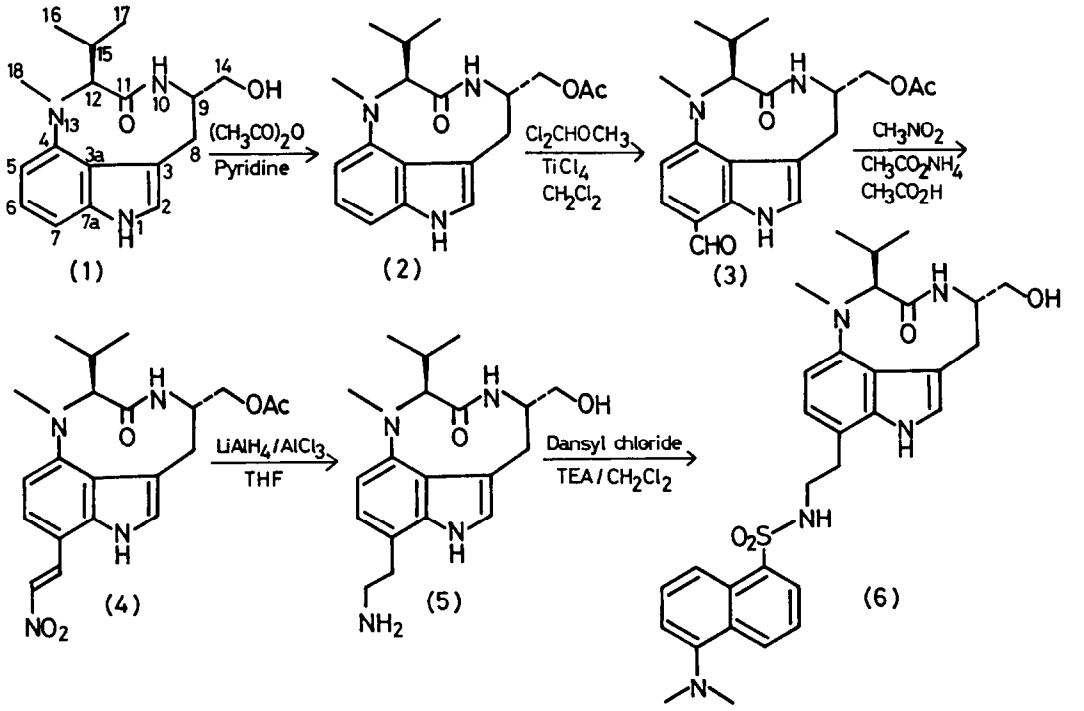
Abstract: A biologically active fluorescent compound, (-)-7-(2-N-dansylaminoethyl)indolactam V, was synthesized from (-)-indolactam V, which has the basic ring-structure of teleocidins. The effect of substituents on the two conformational states of (-)-indolactam V in solution was also discussed.

Recent studies have indicated that tumor promoters in the environment play a significant role in human cancer, and the mechanism of tumor promotion has been widely investigated.[1] Teleocidins are new potent tumor promoters[2] produced by Actinomycetes.[3] Simpler in structure and of higher stability than other potent tumor promoters, phorbol esters[4] and aplysiatoxins,[5] they are particularly convenient for investigating the mechanism of tumor promotion. The last few years have seen structure-activity studies on a wide range of teleocidin derivatives,[6] and these have helped to elucidate the structural requirements for tumor-promoting activity. In addition to this indirect approach, direct identification of the putative receptor sites of tumor promoters using photolabile and fluorescent derivatives is indispensable to reveal the mechanism of tumor promotion. In this communication, we report the synthesis of a biologically active fluorescent compound, (-)-7-(2-N-dansylaminoethyl)indolactam V, from (-)-indolactam V (1), which is readily obtainable from natural resources[7] and by organic synthesis,[8] and discuss the effect of substituents on the two conformational states of (-)-indolactam V (1) in solution.[9]

First, we tried to obtain a fluorescent indolactam derivative by acylation of the indole ring. As reported previously,[10] Friedel-Crafts acetylation on the indole ring of (-)-indolactam V (1) gave (-)-7-acetylindolactam V and a small amount of (-)-2,7-diacetylindolactam V, which was found to fluoresce strongly in methanol [λ(excitation): 400 nm, λ(emission): 515 nm]. However, unfortunately, this was completely inactive.[6c] Our recent study indicated that large substituents at positions 2 or 5 of the indole ring of 1 conspicuously lowered the activity, and that those at position 7 generally enhanced the activity.[6c] Furthermore, a low structural requirement at position 7 for the activity was also indicated.[6c] On the basis of these results, an attempt was made to introduce a fluorescent group onto position 7 of 1. The dansyl group was chosen because of its small molecular size and high stability.

(-)-Indolactam V (1) was converted to (-)-14-O-acetylindolactam V (2)[7,9] by the conventional method in 95 % yield. Treatment of 2 with titanium tetrachloride and dichloromethyl methyl ether in dry methylene chloride at room temperature for 24 hr afforded 3 [amorphous powder: MS m/z 371 (M^+); $[\alpha]_D^{21}$ -447° (c=2.15,EtOH); UV (EtOH) λ_{max} (ε): 371 (17,400), 261.5 (11,100); ^{1}H NMR δ($CDCl_3$) ppm 6.58 (1H,d,J=8.2Hz), 7.03 (1H,s), 7.51 (1H,d,J=8.2Hz), 9.85 (1H,s), 10.42 (1H,br.s)] in 25 % yield along with unreacted 2 (30 %). 2,7-Diformyl derivative was obtained as a minor product. These results are equivalent to those of Friedel-Crafts acylation using aluminum chloride and



acyl anhydride in nitrobenzene reported previously.[10] Condensation of 3 with nitromethane was achieved in ammonium acetate and acetic acid at 90 °C for 1.5 hr to give 4 [amorphous powder; MS m/z 414 (M^+); $[\alpha]_{650}^{23}$ -767.5° (c=0.31,EtOH); UV (EtOH) λ_{max} (ε): 472 (16,600), 282 (8800), 232 (16,800); ^{1}H NMR δ($CDCl_3$) ppm 6.58 (1H,d,J=8.4Hz), 7.04 (1H,s), 7.38 (1H,d,J=8.4Hz), 7.69 (1H,d, J=13.6Hz), 8.41 (1H,d,J=13.6Hz), 9.25 (1H,br.s)] in 75 % yield. In this reaction, the ratio of nitromethane and acetic acid was 10:1. This large excess of nitromethane was necessary to obtain sufficient yield.

Reduction of 4 with lithium aluminum hydride and aluminum chloride in tetrahydrofuran (refluxed for 1 hr) gave 5, which showed characteristic coloration with ninhydrin. Compound 5 was purified by extraction with water (pH 1), followed by partitioning between ethyl acetate and water (pH 14), and used in the next reaction without further purification. Treatment of 5 with dansyl chloride in triethylamine and methylene chloride gave 6[11] in 30 % yield from 4. The absorption and fluorescent spectra of 6 in methanol are shown in Figure 1. This compound has an absorption maximum at 335 nm and thus it can be selectively excited in the presence of protein and nucleic acids. The overall yield of 6 was ca. 5-10 %. The structures of all derivatives synthesized, except for 5, were confirmed by spectral measurements (UV, IR, ^{1}H NMR, EIMS and HR-EIMS). This synthetic method is also available for photolabile and other fluorescent indolactam derivatives by use of 5 as an intermediate.

It is known that teleocidins exist as two stable conformers in solution at room temperature: conformer A of the sofa type and B of the twist type.[9] Introduction of an electron-withdrawing group into position 7 will increase the resonance among the lone-pair electrons on N-13, aromatic electrons, and the substituent at position 7, and fix the molecule in conformer B, in which the lone-pair electrons on N-13 are more delocalizable onto the indole ring. As expected, 3 and 4 existed only as conformer B in chloroform-d at room temperature. This was determined by chemical shifts and coupling constants of the two conformers[7,9]: for example, in the ^{1}H NMR of 2 in

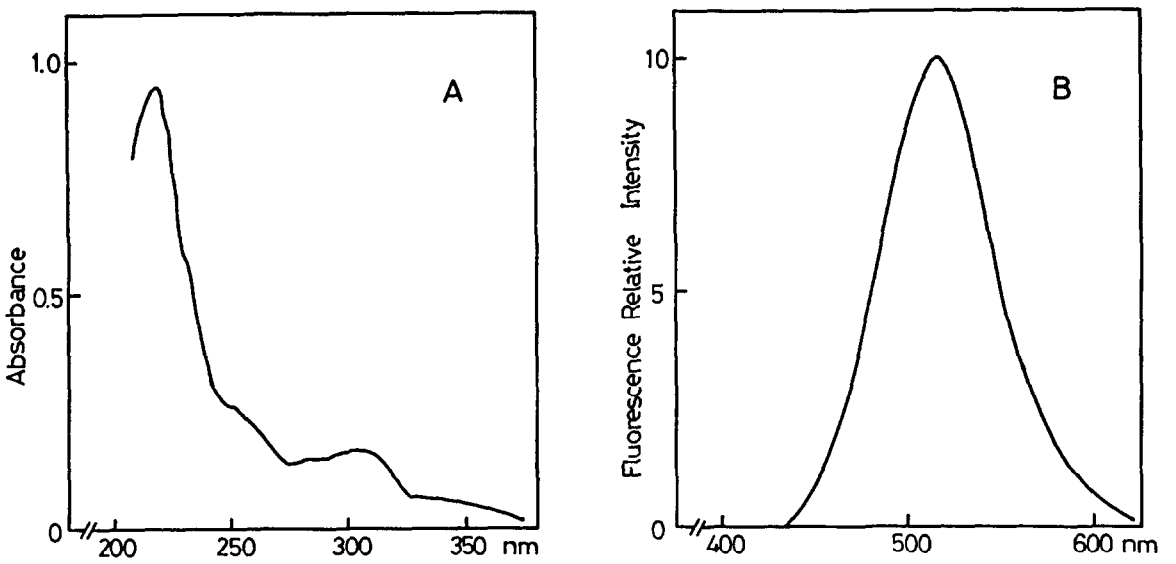


Figure 1. The absorption (A) and fluorescent (B) spectra of 6 in methanol; (A) 1.82 x 10^{-5} M, (B) 1.82 x 10^{-6} M, ex = 335 nm.

chloroform-d (conformer A:B = 1:2.6), the signals ascribable to protons 5, 10, 12, 16, 17 and 18 of conformer A were observed at δ7.04 (d,J=7.5Hz), 4.65 (d,J=12.0Hz), 2.98 (d,J=10.7Hz), 0.94 (d,J=6.1Hz), 1.24 (d,J=6.7Hz) and 2.75 (s), and those of conformer B at δ6.53 (d,J=7.6Hz), 6.01 (br.s), 4.36 (d,J=10.4Hz), 0.64 (d,J=6.7Hz), 0.93 (d,J=6.1Hz) and 2.93 (s). The chemical shifts and coupling constants of these protons of 3 were δ6.58 (d,J=8.2Hz), 6.11 (br.s), 4.54 (d,J=10.4 Hz), 0.59 (d,J=6.7Hz), 0.95 (d,J=6.1Hz) and 3.01 (s), and those of 4 were δ6.58 (d,J=8.4Hz), 6.26 (br.s), 4.49 (d,J=9.9Hz), 0.60 (d,J=7.0Hz), 0.96 (d,J=6.2Hz) and 2.99 (s). These data strongly suggest that the fixed conformer of 3 and 4 is conformer B. 14-O-Acetate of 6,[12] on the other hand, which has an electron-donating substituent at position 7, existed as the two conformers (conformer A:B = 1:2.6) in chloroform-d as expected.

The possible tumor-promoting activity of 6 was estimated by Epstein-Barr virus early antigen-inducing activity[13] and inhibition of specific binding of [^{3}H]12-O-tetradecanoylphorbol-13-acetate to a mouse epidermal particulate fraction.[14] The results suggested that 6 is about 10 times stronger a tumor promoter than (-)-indolactam V (1). Recently, cellular uptake and localization of fluorescent derivatives of phorbol ester-type tumor promoters have been reported.[15] Compound 6 might give a clue to the mechanism of tumor promotion in combination with these fluorescent phorbol esters. Cellular uptake and localization of 6 in several cells are under investigation.

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11) 6: Amorphous powder; HR-in-beam-EIMS m/z 577.2740 (M^+, calcd. for $C_{31}H_{39}N_5O_4S$, 577.2723); $[\alpha]_D^{22}$ -80° (c=0.26,MeOH); UV (MeOH) λ_{max} (ε): 335 (3900), 304.5 (9300), 287 (8300), 250 (14,600), 218.5 (51,800); IR (KBr) ν_{max} cm^{-1}: 1650, 1507, 1315, 1141; ^1H NMR δ($CDCl_3$) ppm (major): 0.61 (3H,d,J=6.4Hz), 0.92 (3H,d,J=6.4Hz), 2.58 (1H,m), 2.86 (3H,s), 2.88 (6H,s), 2.90-3.25 (6H,m), 3.54 (1H,m), 3.73 (1H,m), 4.24 (1H,br.s), 4.34 (1H,d,J=10.3Hz), 5.01 (1H,br.s), 6.36 (1H,d,J=8.1Hz), 6.68 (1H,d,J=8.1Hz), 6.92 (1H,s), 7.04 (1H,br.s), 7.18 (1H,d,J=7.7Hz), 7.51 (2H,m), 8.20 (1H,d,J=8.6Hz), 8.24 (1H,d,J=7.3Hz), 8.54 (1H,d,J=8.5Hz), 8.68 (1H,br.s).

12) 14-O-Acetate of 6: amorphous powder; HR-in-beam-EIMS m/z 619.2853 (M^+, calcd. for $C_{33}H_{41}N_5O_5S$, 619.2828); $[\alpha]_D^{22}$ -37° (c=0.20,MeOH); ^1H NMR δ($CDCl_3$) ppm (major): 2.09 (s).

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