Tetrahedron Letters, Vol.31, No.50, pp 7337-7340, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

ISOLATION AND BIOSYNTHESIS OF (-)-INDOLACTAM I, A NEW CONGENER OF INDOLE ALKALOID TUMOR PROMOTER TELEOCIDINS

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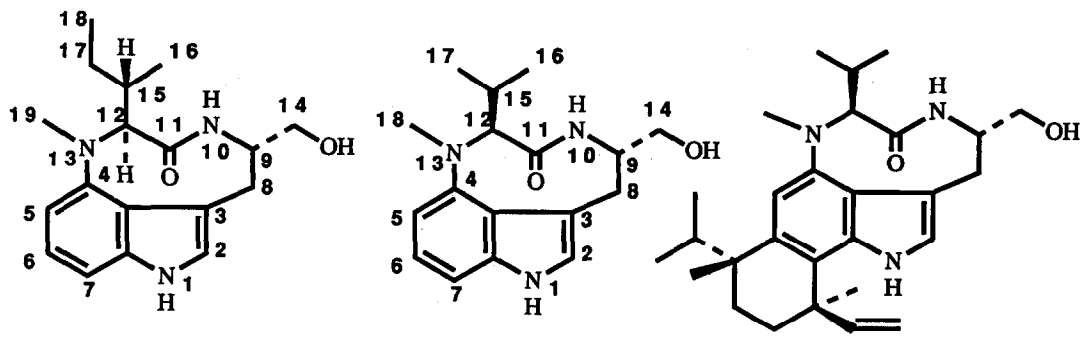
Abstract: (-)-Indolactam I, a new congener of indole alkaloid tumor promoter teleocidins, was isolated from *Streptoverticillium blastmyceticum*, and was shown to be biosynthesized from *N*-methyl-L-isoleucyl-L-tryptophanol.

(-)-Indolactam V,[1,2] an active fragment of indole alkaloid tumor promoter teleocidins,[3] has attracted much interest in the area of organic chemistry because of its peculiar structure involving a nine-membered lactam ring.[4] The name (-)-indolactam V is based on the logical biosynthetic precursors, L-tryptophan with an indole ring and L-valine, which provides the suffix V.[5] Endo *et al.*[5] have recently synthesized a series of indolactam analogues, (±)-indolactam G, A, L, F and t-L with DL-glycine, DL-alanine, DL-leucine, DL-phenylalanine and DL-t-leucine, respectively, instead of L-valine in (-)-indolactam V. Since (-)-indolactam V was shown to be biosynthesized from L-tryptophan, L-valine and L-methionine in *Streptoverticillium blastmyceticum*,[6] it is of interest to examine whether indolactam analogues with amino acids other than L-valine occur naturally.

We searched for new indolactam analogues in the culture broth of *S. blastmyceticum* NA34-17,[7] which has the characteristic feature of producing (-)-indolactam V in quantity, and thus found a new congener of teleocidins, (-)-indolactam I (**1**) with L-isoleucine in place of L-valine in (-)-indolactam V. This communication deals with the isolation, structure determination and biosynthesis of (-)-indolactam I (**1**).

Purification of **1** was guided by Ehrlich reagent,[8] with which (-)-indolactam V showed characteristic coloration (green) on TLC. *S. blastmyceticum* NA34-17 was cultured by deep aerated fermentation for 45hr, and the culture broth (100 liters) was extracted with dichloromethane. These extracts were chromatographed on silica gel using toluene and increasing volumes of acetone to give 40% acetone eluates (20g), which were further purified by repeated column chromatography and HPLC to give **1** (0.8mg) along with 3.5g of (-)-indolactam V.

Compound **1** was obtained as an amorphous powder. HR-EI-MS established its molecular fomula as $C_{18}H_{25}N_3O_2$ (obs. *m/z* 315.1963, calc. 315.1947). The presence of an indole chromophore was confirmed by UV spectrum [λ_{max} (MeOH) nm (ε): 228 (27,600), 289



(-)-Indolactam I (1) (-)-Indolactam V Teleocidin B-4

Fig. 1 Structure of teleocidin-related compounds.

Table I ^{1}H NMR (400MHz) chemical shifts of (-)-indolactam I (1) and (-)-indolactam V in chloroform-d[a]

No	(-)-indolactam I (1)	(-)-indolactam V
1	7.97 (1H, br.s)	7.98 (1H, br.s)
2	6.89 (1H, s)	6.90 (1H, s)
5	6.51 (1H, d, J=7.3Hz)	6.51 (1H, d, J=7.6Hz)
6	7.07 (1H, t, J=7.9Hz)	7.06 (1H, t, J=7.9Hz)
7	6.90 (1H, d, J=7.9Hz)	6.90 (1H, d, J=8.2Hz)
8	3.00 (1H, dd, J=17.4, 3.7Hz)	3.01 (1H, dd, J=17.4, 3.7Hz)
	3.21 (1H, br.d, J=17.4Hz)	3.20 (1H, br.d, J=17.4Hz)
9	4.28 (1H, m)	4.30 (1H, m)
10	6.62 (1H, br.s)	6.56 (1H, br.s)
12	4.51 (1H, d, J=10.1Hz)	4.39 (1H, d, J=10.4Hz)
14	3.54 (1H, m)	3.54 (1H, m)
	3.75 (1H, m)	3.75 (1H, m)
15	2.42 (1H, m)	2.61 (1H, m)
16	0.90 (3H, d, J=6.4Hz)	0.93 (3H, d, J=6.4Hz)
17	0.63 (1H, m)	0.63 (3H, d, J=6.7Hz)
	1.37 (1H, m)	
18	0.59 (3H, t, J=6.7Hz)	2.93 (3H, s)
19	2.92 (3H, s)	
OH	2.02 (1H, m)	1.95 (1H, m)

[a]Chemical shifts for twist conformer are expressed as ppm down field from TMS. twist : sofa = 2.5 : 1 in (-)-indolactam I (1); 3 : 1 in (-)-indolactam V at 0.004M, 27 °C.

(7500), 300 (7500)]. The EI-MS fragment pattern of **1** [*m/z* (%): 315 (M^+, 82), 297 (12), 268 (23), 258 (21), 215 (54), 171 (100)] was quite similar to that of (-)-indolactam V [*m/z* (%): 301 (M^+, 83), 283 (19), 268 (22), 258 (18), 215 (50), 171 (100)]. The fragment ion *m/z* 258 (M^+ −57) of **1** suggests the presence of a butyl moiety in **1** in place of an isopropyl group in (-)-indolactam V.

The ^1H NMR spectrum of **1** in chloroform-*d* (0.004M, 27 °C) revealed that **1** existed as two stable conformers[2] (twist : sofa = 2.5 : 1). The spectrum bore a striking resemblance to that of (-)-indolactam V, except for signals ascribable to the substituent at position 12. A comparison between the spectra of **1** and (-)-indolactam V (Table I) revealed the presence of a sec-butyl group [δ 0.59 (3H, t), 0.63 (1H, m), 0.90 (3H, d), 1.37 (1H, m) and 2.42 (1H, m) for twist conformer] at position 12 of **1**. The CD spectrum of **1** in methanol at 28 °C ($[\theta]_{320}$ 0, $[\theta]_{306}$ +9600, $[\theta]_{294}$ 0, $[\theta]_{255}$ −24,900, $[\theta]_{245}$ −19,900, $[\theta]_{226}$ −55,800, $[\theta]_{212}$ 0, *c*=0.00016M) was closely similar to that of (-)-indolactam V,[1] indicating that **1** and (-)-indolactam V had the same absolute configuration at positions 9 and 12. On the basis of these data, **1** was deduced to be (-)-indolactam I. The assignments of all proton signals established by ^1H-^1H COSY are summarized in Table I. This is the first example of the isolation of an indolactam analogue containing amino acid other than L-valine.

In our previous publication,[6] deuterium labeled *N*-methyl-L-valyl-L-tryptophanol, which was first isolated by Sakai *et al.*,[9] was shown to be efficiently incorporated into (-)-indolactam V. To confirm the structure of **1** and to examine its biosynthetic pathway, synthesis of **1** using *S. blastmyceticum* NA34-17 was tried: *N*-methyl-L-isoleucyl-L-tryptophanol[10] was added to the culture medium (4mg/100ml) of this micro-organism immediately prior to the start of (-)-indolactam V production as reported previously.[11] After 48hr of cultivation, *ca.* 15mg of (-)-indolactam I (*ca.* 40% yield) was obtained from 1000ml of the culture broth. The spectral data (UV, ^1H NMR, CD and EI-MS) of this "synthetic" (-)-indolactam I coincided with **1**, supporting the structure of **1** unambiguously.

The high rate of *N*-methyl-L-isoleucyl-L-tryptophanol biotransconversion into (-)-indolactam I indicates that (-)-indolactam I is biosynthesized through biosynthetic pathway analogous to (-)-indolactam V.[6] The very low levels of naturally occurring (-)-indolactam I (*ca.* 10μg/1000ml) compared with (-)-indolactam V (*ca.* 35mg/1000ml) can be interpreted by the hypothesis that the condensation enzyme for L-valine and L-tryptophan has a much higher substrate specificity to the valine moiety than the cyclization enzyme at position 4 of the indole ring. The above results also indicates that a variety of indolactam and teleocidin analogues for structure-activity studies is easily obtainable by this biotransconversion. In fact, (-)-indolactam L was similarly synthesized from *N*-methyl-L-leucyl-L-tryptophanol (not shown). Moreover, such indolactam precursors as *N*-methyl-L-isoleucyl-L-tryptophanol are especially useful for studying the cyclization enzyme because the cyclization products, for example (-)-indolactam I (**1**), rarely occur naturally, and the efficiency of this cyclization was very high. Studies on the isolation and substrate specificity of this cyclization enzyme are now in progress.

Acknowledgements

The authors thank Dr. K. Shudo of the Faculty of Pharmaceutical Sciences at Tokyo University for supplying (±)-indolactam A, F and L, Dr. J. Oda and Ms. K. Omine of the Institute for Chemical Research at Kyoto University, Mr. R. Imamura of the Faculty of Science at Kyoto University for NMR measurements, and Dr. K. Soda of the Institute for Chemical Research at Kyoto University for CD measurements. This work was supported in part by a Grant-in-Aid for Scientific Research from Ministry of Education, Science and Culture of Japan.

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10) *N*-methyl-L-isoleucyl-L-tryptophanol was synthesized from Boc-*N*-methyl-L-isoleucine and L-tryptophan methyl ester by the method reported previously.[6] *N*-methyl-L-isoleucyl-L-tryptophanol: $[\alpha]_D$ -38.2° (*c*=0.50, MeOH, 26°C). UV λ_{max} (MeOH) nm (ε): 222 (33,700), 275 (sh., 5400), 281 (5800), 290 (5000). ^{1}H NMR δ ($CDCl_3$) ppm: 0.75 (3H, t, *J*=7.3Hz), 0.78 (3H, d, *J*=7.0Hz), 0.92 (1H, m), 1.21 (1H, m), 1.66 (1H, m), 2.27 (3H, s), 2.79 (1H, d, *J*=4.6Hz), 2.99 (1H, dd, *J*=14.7, 7.9Hz), 3.06 (1H, ddd, *J*=14.7, 6.7, 0.6Hz), 3.67 (1H, dd, *J*=11.0, 6.1Hz), 3.77 (1H, dd, *J*=11.0, 3.5Hz), 4.26 (1H, m), 7.06 (1H, d, *J*=2.1Hz), 7.11 (1H, dt, *J*=7.9, 1.2Hz), 7.19 (1H, dt, *J*=8.2, 1.2Hz), 7.35 (1H, dd, *J*=7.9, 0.9Hz), 7.49 (1H, br.d, *J*=7.0Hz), 7.65 (1H, dd, *J*=8.2, 0.9Hz), 8.23 (1H, br.s). HR-EI-MS *m/z* : 317.2101 (M^+, calc. for $C_{18}H_{27}N_3O_2$, 317.2103).

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(Received in Japan 24 August 1990)