A New Actinomycin-type Chromopeptide from *Streptomyces* sp. HKI-0155

Sir:

The actinomycins form a family of more than 45 related heteromeric peptide antibiotics containing the same phenoxazinone chromophore but differing in the amino acid composition of the two peptidolactone side chains[1]. Binding to the DNA and intercalation into the polynucleic acid double strand has been shown as the main activity of actinomycin D containing identical peptidolactone moieties[2~4]. However, few information is available about DNA-binding activities of the other naturally occurring representatives of the actinomycin family[4].

In the course of our screening for new microbial metabolites we disclosed recently *Streptomyces* sp. HKI-0155 as the producer of an orange pigment which displayed moderate antibacterial activity against *Bacillus subtilis* ATCC 6633. Due to *m/z* 1295.3 ($[M+Na]^+$) in the ESI-MS spectrum and database searches suggesting the novelty of the metabolite we started an isolation program for this compound. The evaporated ethyl acetate extract of 10 liters of a 96 hours culture of *Streptomyces* sp. HKI-0155 was subjected to column chromatography on silica gel 60 (0.1~0.063 mm, elution by a) *n*-hexane, b) $CHCl_3$/MeOH 9:1 v/v). A yellowish fraction was further purified by preparative HPLC on silica gel RP_{18} (Spherisorb ODS-2, 25 mm×250 mm) using a gradient of water to 83% acetonitrile (2 minutes to 25 minutes) followed by an isocratic run with the latter eluent. 35 mg of **1** were thus obtained. The physico-chemical properties of **1** are shown in Table 1.

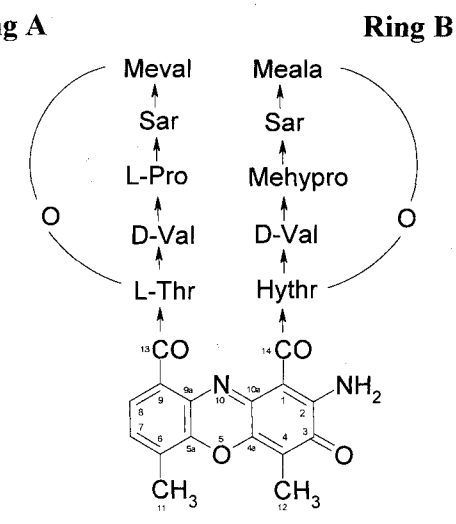
UV (λ_{max}) and IR spectroscopic data (Table 1) suggested the similarity of the chromophore of **1** with that of the actinomycins. The chemical formula $C_{61}H_{84}N_{12}O_{18}$ was readily inferred by HRESI-MS (*m/z* 1295.5894 ($[M+Na]^+$), calcd.: 1295.5924). FAB-MS displayed *m/z* 1273.8 ($[M+H]^+$) and diagnostic fragments such as *m/z* 762.8, 746.9, 451.9 and 435.8. The latter two diagnostic fragment ions were also observed in the daughter ion MS (CID-MS/MS) of *m/z* 1273 ($[M+H]^+$). Corroborative mass spectrometric data were furnished by positive ion ESI-MS^n experiments using an ion-trap mass analyzer. Starting with *m/z* 1295.0 ($[M+Na]^+$) the presence of *N*-methylvaline and sarcosine was shown by *m/z* 1182.5 ([M minus *N*-methylvaline]$^+$) and *m/z* 1093.7 ([M minus *N*-methylvaline, sarcosine and $H_2O]^+$) in the MS^2 and MS^3 experiments, respectively.

Conclusive evidence for the structure of **1** (Fig. 1) was furnished by 1D and 2D NMR spectroscopy (Table 1). The ^{13}C NMR spectrum of **1** displayed 12 amide carbonyls and an additional signal at 178.16 ppm attributable to C-3 of the phenoxazinone chromophore.

Table 1. Physico-chemical properties of **1**.

Appearance	Yellowish microcrystals
Chemical formula	$C_{61}H_{84}N_{12}O_{18}$
HRFAB-MS ($[M+Na]^+$):	*m/z* 1295.5894 (calcd.: 1295.5924)
$[\alpha]_D^{25}$ (in MeOH)	+226.0°
UV (λ_{max}; nm in MeOH (ε))	443 (24500)
IR ν_{max} (cm^{-1}, in KBr)	1022, 1090, 1118, 1153, 1194, 1265, 1315, 1344, 1440, 1466, 1510, 1580, 1654, 1738, 2965, 3310, 3420
Rf on TLC ($CHCl_3$/MeOH 9:1)	0.8
Rt (min) on analytical HPLC (RP_{18}; 0.25×250 cm; gradient 95% water/0.1% trifluoroacetic acid to 95% acetonitrile, 3~20 minutes)	12.2

Fig. 1. Structure of actinomycin HKI-0155 (**1**) from *Streptomyces* sp. HKI-0155.



Abbreviations: L-Pro: L-proline; Sar: sarcosine; Meval: *N*-methy-L-valine; Meala: *N*-methyl-L-alanine; Mehypro: 3-hydroxy-5-methyl-L-proline; Thr: L-threonine; Val: D-valine, Hythr: 4-hydroxy-L-threonine.

Table 2. Assignment of ^{1}H- and ^{13}C-NMR chemical shift data (in ppm) of **1** (in DMSO-d_6; coupling constants in Hz; concentration: 0.022 M).

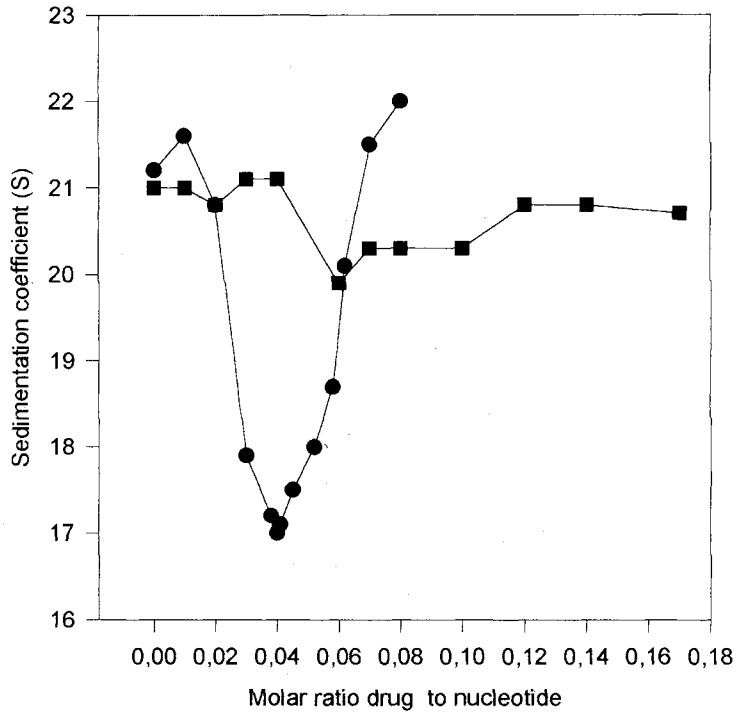
Moiety	Position	δ_C	δ_H	Moiety	Position	δ_C	δ_H
Phenoxazinone chromophore	C-1	97.5	—	Sar	C-1	167.1	—
	C-2	147.7	—		C-2	51.3	3.93 d, br; 17.5
	2-NH_2	—	4.6 br				4.79 d, br; 17.5
	C-3	178.2			N-CH_3	34.4	2.68 s
	C-4	112.5					
	C-4a	145.6	—	Meval	C-1	168.0	—
	C-5a	140.2	—		C-2	69.6	3.06 d; 6.1
	C-6	128.5	—		C-3	26.3	2.53 m
	C-7	129.7	7.44 d; 7.9		C-4	21.3	1.05 d; 7.0
	C-8	126.1	7.86 d; 7.9		C-5	18.9	0.75 d; 7.1
	C-9	127.8	—		N-CH_3	38.1	2.85 s
	C-9a	130.3	—				
	C-10a	145.9	—	Ring B			
	C-11	14.7	2.48 s	Hythr	C-1	168.9	—
	C-12	7.6	2.12 s		C-2	54.0	4.41 m
	C-13	165.0	—		C-3	68.4	4.14 m
	C-14	171.1	—		C-4	65.9	4.40 dd; 4.27 dd
					NH	—	7.31 d; 7.7
Ring A					OH	—	5.20 d; 5.4
L-Thr	C-1	169.3	—				
	C-2	56.0	4.92 dd; 9.0, 1.5	D-Val	C-1	169.9	—
	C-3	71.8	5.2 m		C-2	57.2	4.24 dd; 8.1, 7.2
	C-4	16.6	1.34 d; 6.5		C-3	27.9	1.95 m
	NH	—	8.85 d; 9.0		C-4	18.7	0.82 d; 7.1
					C-5	19.0	0.80 d; 7.2
D-Val	C-1	173.2	—		NH	—	7.58 d; 9.0
	C-2	57.8	3.30 dd; 8.3; 5.0				
	C-3	30.9	1.83 m	Mehypro	C-1	173.2	—
	C-4	18.95	0.67 d; 7.5		C-2	65.3	5.91 d, br
	C-5	18.9	0.87 d; 7.5		C-3	73.8	3.43 m, br
	NH	—	8.23 d; 8.3		C-4	39.9	1.70 m; 2.49 m
					C-5	51.2	4.08 m
L-Pro	C-1	171.6	—		5-CH_3	18.8	1.19 d; 6.1
	C-2	56.2	4.85 t, br		OH	—	5.19
	C-3	27.6	1.71 m; 2.15 m				
	C-4	24.1	1.83 m; 1.91 m	Sar	C-1	169.6	—
	C-5	46.9	3.49 m; 3.85 m		C-2	50.2	3.25 d, br; 4.38 d, br
					N-CH_3	37.9	3.22 s
				Meala	C-1	171.4	—
					C-2	52.3	5.07 q
					C-3	14.2	1.22 d; 7.1
					N-CH_3	30.9	2.94 s

Abbreviations: s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, br: broad; for the abbreviations of the amino acids see the legend of Fig. 1.

The ^{1}H- and ^{13}C-NMR signals of the chromophoric ring system were readily assigned on the basis of ^{1}H, ^{1}H-COSY, DEPT, HSQC and HMBC experiments whereby comparison of the measured ^{13}C data with carbon chemical shifts of other actinomycins was particularly helpful[1].

The assignment of the ^{1}H and ^{13}C carbon signals of the two different peptidolactone moieties A and B (Fig. 1) was based on the observable COSY, TOCSY, NOESY and C, H

Fig. 2. Effect of actinomycin D (-●-) and actinomycin HKI-0155 (-■-) on sedimentation coefficient of supercoiled plasmid DNA from pBR322[4,5].



long-range correlations. Thus the ^{1}H, ^{1}H couplings of the N-H protons with the neighboured C-H protons in the COSY spectrum and the C, H long-range and ^{2}J and ^{3}J couplings of the N-H and N-CH_3-protons, respectively, with the neighboured carbon atoms of the amino acids enabled the suggestion of carbonyl signals as part of a special amino acid (Table 2).

Acidic hydrolysis of **1**, derivatization of the amino acids by Marfey's reagent[5] and HPLC analysis of the amino acid derivatives suggested the presence of D-valine, L-threonine, *N*-methyl-L-alanine, L-proline and sarcosine. By this way the configuration of 4-hydroxy-L-threonine[8], 5-methyl-3-hydroxy-L-proline[9] and *N*-methyl-L-valine was not assignable but it could be expected to be the same as was shown for other actinomycins of the *Z*-type[8~10]. The physico-chemical data thus suggest compound **1** as a new representative of the actinomycin family of antibiotics showing different amino acid composition in each of the two peptidolactone rings (*aniso*-actinomycin).

1 displays only a moderate antimicrobial activity against some Gram-positive bacteria such as *Bacillus subtilis* ATCC 6633 (MIC≫200 μg/ml). In contrast to actinomycin D the new actinomycin HKI-0155 did not intercalate into the DNA. Fig. 2 displays that the sedimentation coefficient of supercoiled plasmid DNA undergoes a characteristic fall and rise with increasing actinomycin D concentration indicating the typical feature of an intercalation process[6,7]. In contrast, the compound **1** exhibited no similar change in the sedimentation profile. In accord with the missing DNA-intercalating properties, more than 200 times higher concentration of **1** were needed in comparison to actinomycin D as to cause comparable inhibition of bacterial gyrase[6]. Obviously the combination of an actinomycin-D-type peptidolactone (ring A) and a diminished variant containing two hydroxy amino acids (ring B) destabilizes the characteristic pseudo-C2-symmetrical overall conformation of[10] as a prerequisite for DNA intercalation. The additional hydroxyl groups, spatially adjacent to the chromophore[10], might also inhibit a correct intercalation by forming competing hydrogen bonds between 1 and the DNA chain.

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