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Jenamidines A to C: Unusual Alkaloids from *Streptomyces* sp. with Specific Antiproliferative Properties Obtained by Chemical Screening

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Three new naturally occurring bicyclic alkaloids, jenamidines A (**1**), B (**2**) and C (**3**), were discovered and isolated from the culture broth of *Streptomyces* sp. (strain HKI0297) *via* the chemical screening approach. Fermentation, isolation, structure and biological activities of these three new secondary metabolites are reported. The jenamidines have an unusual octahydro-pyrido[1,2-*a*]pyrimidine skeleton. Jenamidine A (**1**) shows antiproliferative effects against the chronic myeloid leukaemic cell line K-562. In addition, the new tricyclic sesquiterpenoid, africantriol (**4**) was isolated from the same strain.

Chemical screening is an efficient method for detecting secondary metabolites from microorganisms with different types of structural skeletons and biological activities[1]. In order to search for new secondary metabolites, the application of our chemical screening method to the culture broth of various Actinomycetes strains resulted in a number of new compounds[1]. In our continuing work[2~4] with different *Streptomyces* strains we have now investigated the secondary metabolites of *Streptomyces* sp. (strain HKI0297). A crude extract of this microorganism attracted our attention because thin-layer chromatograms (TLC) resulted in striking spots after staining with our routine staining reagents, especially a distinctive pink color after staining with Ehrlich's reagent (Table 1). Analysis of extracts using various solvent systems and staining reagents guided the isolation and led to the discovery of four new metabolites (**1** to **4**).

Material and Methods

General

For instrumentation and general methods see our preceding papers[2~4].

Cultivation of *Streptomyces* sp. (Strain HKI0297)

Pure cultures of strain HKI0297 were grown on a soybean mannitol culture medium (soybean meal 20 g/liter, mannitol 20 g/liter, H_2O 1 liter, pH=7.5 prior to sterilization). Storage of the strain was carried out in 50% glycerol at −20°C. The storage sample (2 ml) was used to inoculate a 300 ml Erlenmeyer flask containing 100 ml of culture medium. The strain was cultivated on a rotary shaker (180 rpm) at 28°C for 3 days for the primary TLC analysis of extracts. A 96-hour old culture, prepared in the same manner was used to inoculate a fermentor (20 liters working volume, inoculation volume 5%, 400 rpm, 28°C, aeration 5 liters/minute, 6 days). Foaming was decreased using PPG (polpropylenglycol). Details of the chemical screening method (cultivation, extract preparation, TLC analysis) have already been documented[5,6].

Isolation of Jenamidines A~C (**1~3**) and Africantriol (**4**)

The culture broth (18 liters) was filtered, and the culture filtrate was adsorbed on 3 liters of Amberlite XAD-16. The resin was washed with 6 liters of deionized water, and the metabolites were eluted with 5 liters of a mixture of MeOH/H_2O (25 : 75). The solution was reduced *in vaccuo*

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to an aqueous concentrate that was then lyophilized to yield 145 g of a dark brown crude material. The whole crude material was suspended in deionized water (1 liter), and extracted with EtOAc (3×1.5 liters) at room temperature. The combined organic layers were dried with anhydrous sodium sulfate (Na_2SO_4), and evaporated under reduced pressure to give a brown-red residue (*ca.* 18 g). This material was chromatographed on silica gel (column: 4.2×32 cm) with a $CHCl_3$-MeOH gradient (20 : 1~1 : 1) to yield 5 fractions: Fr. 3 ($CHCl_3$-MeOH 10 : 1~8 : 1, 800 mg) was again chromatographed on silica gel ($CHCl_3$-MeOH 9 : 1, column: 2.2×32 cm) and on RP-18 HPLC [GILSON, column: SP 250/21 Nucleosil 100-7 C18, Macherey-Nagel (2.2×21 cm), P: 120 bar, MeOH/H_2O (25 : 75), flow: 20 ml/minute], as well as on Sephadex LH-20 (acetone, column: 3.0×70 cm) resulting in jenamidine A (**1**, 1.75 mg/liter), jenamidine C (**3**, 0.30 mg/liter), and africantriol (**4**, 0.60 mg/liter). Jenamidine B (**2**, 0.55 mg/liter) was obtained from fr. 4 ($CHCl_3$-MeOH, 5 : 1, 150 mg) and purified by repetitive silica gel column chromatography (column: 2.2×32 cm, eluted with $CHCl_3$-MeOH, 8 : 1) and by gel permeation chromatography on Sephadex LH-20 (column: 3.0×76 cm) in MeOH. All yields are given as extrapolation of the isolated amounts in relation to culture volume.

Physico-chemical Properties

Jenamidine A (**1**): $C_{13}H_{18}N_2O_3$; ESI-MS (positive mode) *m/z*: 251 $[M+H]^+$, 273 $[M+Na]^+$, 501 $[2M+H]^+$, 523 $[2M+Na]^+$, 773 $[3M+Na]^+$; ESI-MS (negative mode) *m/z*: 249 $[M-H]^-$, 499 $[2M-H]^-$; EI-MS *m/z*: 250 $[M]^+$, 232 $[M-H_2O]^+$, 205, 177. Jenamidine B (**2**): $C_{13}H_{18}N_2O_4$; ESI-MS (positive mode) *m/z*: 267 $[M+H]^+$, 289 $[M+Na]^+$, 533 $[2M+H]^+$, 555 $[2M+Na]^+$, 799 $[3M+H]^+$, 821 $[3M+Na]^+$; ESI-MS (negative mode) *m/z*: 265 $[M-H]^-$, 531 $[2M-H]^-$; EI-MS *m/z*: 266 $[M]^+$, 248 $[M-H_2O]^+$, 233, 221, 193. Jenamidine C (**3**): $C_{13}H_{18}N_2O_4$; ESI-MS (positive mode) *m/z*: 267 $[M+H]^+$, 289 $[M+Na]^+$, 555 $[2M+Na]^+$, 821 $[3M+Na]^+$, 1087 $[4M+Na]^+$; ESI-MS (negative mode) *m/z*: 265 $[M-H]^-$, 531 $[2M-H]^-$; EI-MS *m/z*: 267 $[M+H]^+$, 221. Africantriol (**4**): $C_{15}H_{26}O_3$; ESI-MS (positive mode) *m/z*: 277 $[M+Na]^+$; ESI-MS (negative mode) *m/z*: 253 $[M-H]^-$; EI-MS *m/z*: 254 $[M]^+$, 236 $[M-H_2O]^+$, 221 $[M-H_2O-Me]^+$, 218 $[M-2H_2O]^+$, 203 $[M-2H_2O-Me]^+$, 179. Other physico-chemical properties of **1** to **4** are shown in Tables 1 to 4.

Antiproliferative and Cytotoxicity Assays

The target compounds were assayed against K-562 (DSM ACC 10) and L-929 (DSM ACC 2) for their antiproliferative effects, and against HeLa (DSM ACC 57) for their cytotoxic effects as described earlier[7].

Results and Discussion

For screening purposes, cultivation of the strain HKI0297 was carried out as 100 ml shaking cultures in soybean mannitol culture medium. In order to examine the secondary metabolite pattern of the strain, a standardized work-up procedure using solid phase extraction with Amberlite XAD-16, including a 1 : 50 concentration step, was applied to the culture filtrate[5]. To isolate the metabolites detected by the chemical screening method, *Streptomyces* sp. (strain HKI0297) was cultivated in a 20-liter fermentor using soybean mannitol medium at 28°C for 6 days. The detected metabolites were isolated from the culture filtrate after adsorption on Amberlite XAD-16 and subsequent elution with aq. MeOH (80%). Dilution of the concentrate in water, extraction with ethyl acetate and gradient chromatography on a silica gel column followed by gel permeation chromatography on Sephadex LH-20 yielded the pure metabolites.

Structure of Jenamidine A (**1**)

The molecular formula was determined by HR-EIMS to be $C_{13}H_{18}N_2O_3$ (*m/z*: 250.1309, M^+). The IR and UV data pointed to NH and hydroxyl groups, double bonds, a six-membered ring-ketone and an α,β-unsaturated ketone moiety (Table 1). The ^{13}C NMR spectrum (Table 2) showed thirteen carbon signals, indicating the presence of two carbonyl groups (δ 173.5, 204.7) and two double bonds [δ 93.8 (CH), 131.6 (C), 143.6 (CH) and 169.8 (C)]. Analysis of ^1H-^1H COSY NMR data showed the existence of three proton spin systems: $CH_3CH(OH)CH=C(CH_3)$– (2′-H, 3′-H, 4′-H, 5′-H); $-CH_2CH_2-$ (6-H, 7-H), and $-CH_2CH-$ (9-H, 9a-H). A long range allylic coupling between 2′-H and 5′-H caused a broadened doublet (δ 6.38) and a broad singlet (δ 1.91) for these protons. One bond proton-carbon connectivities were determined by a heteronuclear single quantum coherence (HSQC) NMR experiment. A heteronuclear multiple bond correlation (HMBC) NMR experiment indicated long range proton-carbon couplings (Fig. 1). Detailed analysis of the NMR data allowed the chemical shifts assignments for all carbons (Table 2) and protons (Table 3) in **1**. The stereochemistry of the double bond in the side chain was proved to be *E* by an unambiguous NOE effect (NOESY) between 3′-H (δ 4.65) and 5′-H (δ 1.91). The stereochemistry for the chiral

Table 1. Physico-chemical properties of jenamidines A (**1**), B (**2**), C (**3**) and africantriol (**4**).

	1	2	3	4
Appearance	light yellowish oil	light yellowish oil	light yellowish gum	colorless crystals ($CHCl_3$-MeOH)
Molecular formula	$C_{13}H_{18}N_2O_3$	$C_{13}H_{18}N_2O_4$	$C_{13}H_{18}N_2O_4$	$C_{15}H_{26}O_3$
HR-MS m/z (only for molecular ion)	found 250.1309[a] $C_{13}H_{18}N_2O_3$ $[M]^+$ requires 250.1313	267.1361[b] $C_{13}H_{19}N_2O_4$ $[M+H]^+$ requires 267.1340	267.1329[c] $C_{13}H_{19}N_2O_4$ $[M+H]^+$ requires 267.1340	found 236.1768[d] $C_{15}H_{24}O_2$ $[M-H_2O]^+$ requires 236.1770
MP (°C)				189~190
$[\alpha]_D^{25}$ (MeOH)	+6.8° (*c* 0.70)	+8.4° (*c* 0.60)	+ 1.8° (*c* 0.39)	+63.9° (*c* 0.44)
IR ν_{max} (film or KBr) cm^{-1}	3286 (br), 2972, 2929, 1699, 1644, 1558,1507, 1397, 1238, 1141, 1059	3334 (br), 2930, 1700, 1653, 1560, 1507, 1395, 1238, 1190, 1142, 1091	3350 (br), 2920, 2880, 1700, 1652, 1559, 1517, 1394, 1189, 1090	3382 (br), 3060, 2990, 2965, 2937, 2905, 1458, 1380, 1263, 1019
UV λ_{max} (MeOH) nm (log ε)	240 (4,01), 280 (3,11), 326 (3,32)	240 (4,12), 299 (3,08), 333 (3,26)	228 (4,23), 248 (3,53), 299 (3,13), 332 (3,48)	end absorption
R_f *(TLC silica gel) Solvent system*				
$CHCl_3$-MeOH (9:1)	0.34	0.40	0.22	0.36
n-BuOH/HAc/H_2O (4:1:5, upper phase)	0.53	0.69	0.60	0.86
Staining reagent[e]				
Ehrlich's reagent[f]	pink	pink	pink	blue-grey
Anisaldehyde/H_2SO_4	yellow	yellow	yellow	purple-grey
Orcinol reagent	green/violet	green-grey	green-grey	brown
Blue tetrazolium reagent	yellow	yellow	yellow	n.c.
2-Naphthol reagent	yellow-brown	yellow-brown	yellow-brown	purple

[a]HR-EIMS; [b]HR-FABMS; [c]HR-ESIMS; [d]HR-EIMS (254 $[M]^+$ found in EIMS was of insufficient intensity for HR-EIMS); [e]after spraying the plates were heated at 120°C for 5 min; [f]on a yellow background; n.c. no colorization.

centers at C-3′ and C-9a remained unassigned. Thus, jenamidine A (**1**) is 2-(3-hydroxy-1-methyl-but-1-enyl)-6,7,9,9a-tetrahydro-1*H*-pyrido[1,2-*a*]pyrimidine-4,8-dione.

Structure of Jenamidine B (**2**)

The molecular formula $C_{13}H_{18}N_2O_4$ was deduced by HR-FABMS (*m/z*: 267.1361, $[M+H]^+$) and supported by its ESIMS and the ^1H and ^{13}C NMR data. The IR and UV spectra (Table 1) as well as the ^1H (Table 3) and ^{13}C NMR (Table 2) spectra of **2** were similar to those of jenamidine A (**1**). ^1H-^1H COSY NMR data showed the presence of two proton spin systems. One is identical to a spin system in **1** [$CH_3CH(OH)CH=C(CH_3)$– (2′-H to 4′-H, 5′-H)], and the second is $-CH_2CH_2CH_2-$ (6-H to 8-H). These data suggested a translocation of the ketone carbon from C-8 (**1**: δ 204.7) to C-9 (**2**: δ 202.5). In addition, **2** differs from **1** by addition of a tertiary hydroxyl substituent in position C-9a as deduced from the ^{13}C NMR data, where a signal for a quaternary carbon bearing both an oxygen and a nitrogen atom is indicated (δ 97.1). This finding is also confirmed by the molecular formula. The structure elucidation of **2**

Fig. 1. Chemical structures and key $^{n}J_{C,H}$ HMBC correlations (H--->C) of jenamidines A (**1**), B (**2**) and C (**3**).

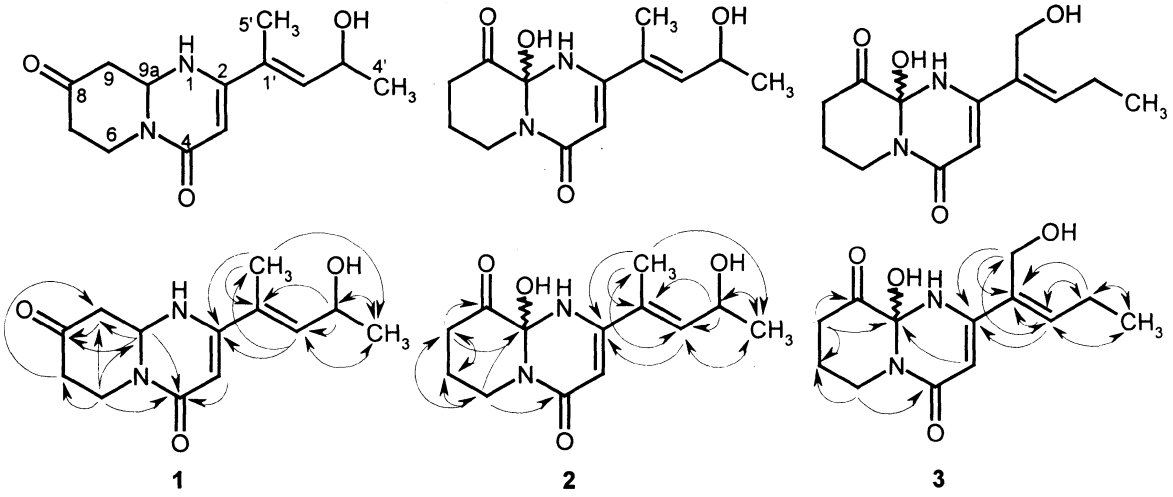

Table 2. ^{13}C NMR data (75 MHz, CD_3OD) for compounds **1**~**3** (δ in ppm, J in Hz).

Position*	**1** (DEPT)	**2** (DEPT)	**3** (DEPT)
2	169.8 (C)	170.2 (C)	168.4 (C)
3	93.8 (CH)	90.6 (CH)	90.4 (CH)
4	173.5 (C)	172.3 (C)	171.5 (C)
6	49.3 (CH_2)	49.2 (CH_2)	48.5 (CH_2)
7	28.8 (CH_2)	27.6 (CH_2)	27.8 (CH_2)
8	204.7 (C)	33.6 (CH_2)	33.6 (CH_2)
9	27.5 (CH_2)	202.5 (C)	202.4 (C)
9a	70.7 (CH)	97.1 (C)	97.0 (C)
1'	131.6 (C)	131.7 (C)	133.7 (C)
2'	143.6 (CH)	143.8 (CH)	146.9 (CH)
3'	65.3 (CH)	65.3 (CH)	22.4 (CH_2)
4'	22.7 (CH_3)	22.6 (CH_3)	13.6 (CH_3)
5'	12.9 (CH_3)	12.9 (CH_3)	57.0 (CH_2)

*Assignments made by 2D-NMR techniques (^1H-^1H COSY, HSQC, HMBC).

was independently proved by the HSQC and HMBC NMR data (Fig. 1). The configuration of the double bond in the side chain was found to be identical to that in **1** through a NOE effect (NOESY) between 3'-H (δ 4.62) and 5'-H (δ 1.90). Jenamidine B (**2**) is 9a-hydroxy-2-(3-hydroxy-1-methyl-but-1-enyl)-1,7,8,9a-tetrahydro-6*H*-pyrido[1,2-*a*]pyrimidine-4,9-dione.

Structure of Jenamidine C (**3**)

The EIMS spectrum showed a $(M+H)^+$ ion peak at *m/z* 267 and the molecular formula of **3** ($C_{13}H_{18}N_2O_4$)

Table 3. ^{1}H NMR data (300 MHz, CD_3OD) for compounds **1**~**3** (δ in ppm, J in Hz).

Position*	1	2	3
3	5.65 (1H, s)	5.63 (1H, s)	5.61 (1H, s)
6	3.20 (1H, m); 3.44 (1H, m)	3.22 (1H, m); 3.54 (1H, m)	3.15 (1H, m); 3.54 (1H, m)
7	2.15 (2H, m)	2.06 (1H, m); 2.35 (1H, m)	2.10 (1H, m); 2.35 (1H, m)
8		1.64 (1H, m); 1.95 (1H, m)	1.65 (1H, m); 1.91 (1H, m)
9	1.53 (1H, m); 2.20 (1H, m)		
9a	3.94 (1H, t, J = 7.8 Hz)		
2'	6.38 (1H, brd, J = 7.9 Hz)	6.35 (1H, brd, J = 7.8 Hz)	6.72 (1H, t, J = 7.6 Hz)
3'	4.65 (1H, dq, J = 6.5, 7.9 Hz)	4.62 (1H, dq, J = 6.4, 7.8 Hz)	2.31 (2H, dq, J = 7.6, 7.6 Hz)
4'	1.29 (3H, d, J = 6.5 Hz)	1.29 (3H, d, J = 6.4 Hz)	1.05 (3H, t, J = 7.6 Hz)
5'	1.91 (3H, brs)	1.90 (3H, brs)	4.45 (2H, brs)

*Assignments made by 2D-NMR techniques (^{1}H-^{1}H COSY, HSQC, HMBC).

being identical to that of **2** resulted from HR-ESIMS. ^{1}H-^{1}H COSY NMR data showed the differences of both metabolites in the proton spin system [CH_3CH_2CH=$C(CH_2OH)$– (2'-H to 4'-H, 5'-H)]. The terminal methyl group in the side chain showed a triplet at δ 1.05 (3H, t, J=7.6 Hz) which was different to the situation in **1** [δ 1.29 (3H, d, J=6.5 Hz)] and **2** [δ 1.29 (3H, d, J=6.4 Hz)]. Together with the finding of a missing allylic methyl group, **3** was determined to be a regioisomer of **2** with respect to the position of the hydroxyl group in the side chain (**2**: C-3', **3**: C-5'). This was confirmed by the low field shift of the vinyl methylene group as a broadened singlet at δ 4.45 (2H, brs, 5'-H) as assigned by 2D NMR experiments (^{1}H-^{1}H COSY, HSQC and HMBC; Fig. 1). The configuration of the double bond in the side chain is the same as in **1** and **2** (NOESY NMR data). Thus, jenamidine C (**3**) is 9a-hydroxy-2-(1-hydroxymethyl-but-1-enyl)-1,7,8,9a-tetrahydro-6*H*-pyrido[1,2-*a*]pyrimidine-4,9-dione.

The skeletons of jenamidines A (**1**), B (**2**) and C (**3**), represent an hitherto unknown type of alkaloid. The compounds show a certain relationship to piperidine alkaloids. In fact, they might be biosynthetically derived from a derivative of 3-piperidine carboxylic acid through condensation with the appropriate polyketide intermediate.

Structure of Africantriol (**4**)

The ESIMS spectra (positive and negative mode) indicated the molecular mass as M=254 g/mol. The molecular formula $C_{15}H_{26}O_3$ was deduced from a HR-EIMS of a characteristic fragment ion peak of *m/z* 236.1768 [$(M-H_2O)^+$, $C_{15}H_{24}O_2$] (Table 1). The ^{13}C NMR spectral data showed all fifteen carbon signals, corresponding with a sesquiterpenoid skeleton. The absorption bands in the IR spectrum (Table 1) indicated hydroxyl groups [3382 (br) cm^{-1}], as well as a cyclopropyl ring (3060, 1019 cm^{-1})[8,9]. In the ^{1}H NMR spectrum (CD_3OD) three tertiary methyl groups (δ 0.92, 1.00 and 1.16) and a secondary methyl group (δ 0.97, 3H, d, J=6.8 Hz) were observed. In the highfield portion of the ^{1}H NMR spectrum (CD_3OD) methylene protons (δ 0.36 and 0.56) and a methine proton (δ 0.85) of a double-substituted cyclopropyl ring were detected. Two other diastereotopic methylenes were detected as two doublets at δ 1.47 and 1.62 (J=14.6 Hz) and as a pair of multiplets (δ 1.64 and 2.21). Two methine protons neighbouring oxygen substituents were observed at δ 3.38 (1H, d, J=10.5 Hz) and 3.78 (1H, m). From the ^{1}H-^{1}H COSY NMR spectrum it was deduced that the carbinol proton at δ 3.38 was a neighbour of the cyclopropyl methine and the one at δ 3.78 was part of a $CH(CH_3)CH(OH)CH_2CH$– fragment. All of the above data revealed that metabolite **4** has an oxygenated tricyclic africanane skeleton[8] (Fig. 2). Therefore, in the ^{13}C NMR spectrum of **4** a quaternary carbon signal at δ 85.5 was assigned to C-8 bearing an additional hydroxyl group. A ^{1}H NMR spectrum in DMSO-d_6 showed exchangeable signals (D_2O) of three hydroxyl groups at δ 4.42 (1H, d, J=6.0 Hz, 10-OH), 4.23 (1H, d, J=5.2 Hz, 5-OH) and 3.34 (1H, s, 8-OH). Considering the ^{13}C-NMR chemical shifts of the methines C-5 and C-10, as well of the quaternary C-8, the

Fig. 2. Chemical structure and significant NOE correlations (NOESY) of africantriol (**4**, numbering of atoms according to literature[11,12]).

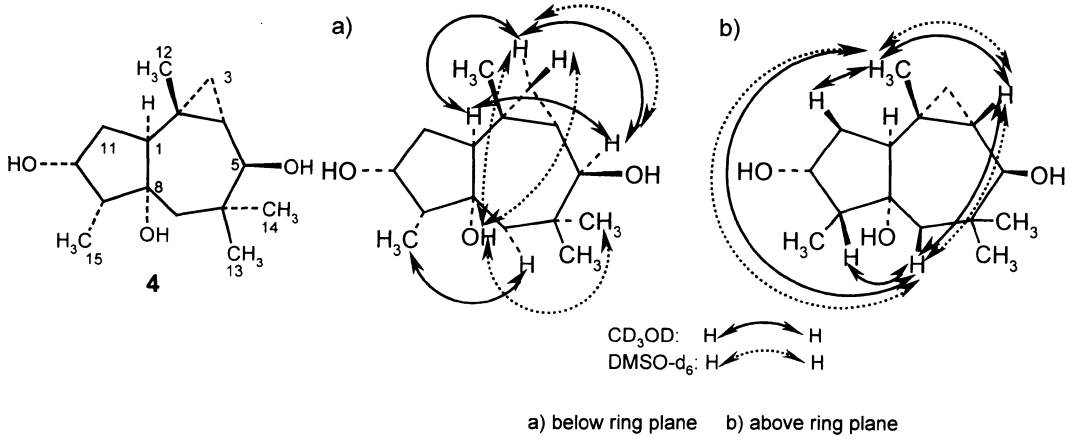

Table 4. ^{1}H and ^{13}C NMR data for compound **4** (δ in ppm).

Position*	$^{1}H^{a}$	$^{1}H^{b}$	Position	^{13}C (DEPT)[a]
1-Hβ	1.61 (1H, m)	1.45 (1H, m)	1	53.9 (CH)
3-Hβ	0.36 (1H, dd, J = 4.3, 4.6)	0.20 (1H, dd, J = 4.4, 4.5)	2	19.3 (C)
3-Hα	0.56 (1H, dd, J = 4.3, 8.1)	0.42 (1H, dd, J = 4.4, 8.1)	3	22.8 (CH_2)
4-Hα	0.85 (1H, m)	0.68 (1H, m)	4	28.5 (CH)
5-Hβ	3.38 (1H, d, J = 10.5)	3.20 (1H, dd, J = 5.2, 10.5)	5	79.0 (CH)
7-Hα	1.47 (1H, d, J = 14.6)	1.26 (1H, d, J = 14.2)	6	39.9 (C)
7-Hβ	1.62 (1H, d, J = 14.6)	1.44 (1H, d, J = 14.2)	7	47.7 (CH_2)
9-Hα	1.34 (1H, m)	1.11 (1H, m)	8	85.5 (C)
10-Hα	3.78 (1H, m)	3.62 (1H, m)	9	53.9 (CH)
11-Hα	1.64 (1H, m)	1.46 (1H, m)	10	77.9 (CH)
11-Hβ	2.21 (1H, m)	1.99 (1H, m)	11	34.1 (CH_2)
12-H_3	1.00 (3H, s)	0.89 (3H, s)	12	22.7 (CH_3)
13-H_3	0.92 (3H, s)	0.81 (3H, s)	13	24.7 (CH_3)
14-H_3	1.16 (3H, s)	1.05 (3H, s)	14	28.2 (CH_3)
15-H_3	0.97 (3H, d, J = 6.8)	0.83 (3H, d, J = 6.7)	15	10.3 (CH_3)
5-OH	n.d.	4.23 (1H, d, J = 5.2)		
8-OH	n.d.	3.34 (1H, s)		
10-OH	n.d.	4.42 (1H, d, J = 6.0)		

*Assignments were made by a combination of 1D- and 2D-NMR techniques (^{1}H-^{1}H COSY, HSQC, HMBC and NOESY); J values in Hz;
[a] ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR data measured in CD_3OD; n.d. = not detected;
[b] ^{1}H (500 MHz) NMR data measured in DMSO-d_6.

position of the hydroxyl groups was deduced from the ^2J long-range proton-carbon couplings in the HMBC NMR spectrum. The unambiguous assignments of the ^1H and ^{13}C NMR chemical shifts (Table 4) were confirmed by a combination of 1D and 2D NMR experiments (^1H-^1H COSY, HSQC, and HMBC).

The relative configuration of **4** was deduced on the basis of NOE correlations in NOESY spectra in CD_3OD and in DMSO-d_6 (Fig. 2). The NOE's between 8-OH and 3-CH_2, as well as between 1-H and 3-Hβ clearly indicated the *cis*-fusion of the 5- and 7-membered rings. The positioning of 1-H, cyclopropyl and 9-CH_3 on one side of the ring plane[10], and of 2-CH_3 and 5-OH on the other side was unambiguously deduced from the NOE correlations. The position of 10-H was proposed due to the absence of NOE's between 10-H and 1-H which were detected as strong signals in case of the structurally related 10α-Hydroxy-$\Delta^{9(15)}$-africanene.[12] There were also no NOE's found between 10-H and 8-OH, although both show otherwise strong effects. Thus, africantriol **4** was assigned to be of 1α5β8α9α10α configuration corresponding with the relative stereochemistry of isoafricanol.[11] The first congener of this structural class, africanol, was first detected in a marine invertebrate *Lemnalia africana* by TURSCH *et al.*[8] as an unusual tricyclic sesquiterpene in 1974. Since then, several africanane-type representatives were found in plants[9,10], fungi[11], and marine organisms[12]. Africantriol (**4**) is the first member of the africane family of metabolites to be isolated from *Streptomyces* (Actinomycetes).

Biological Activity of the Jenamidines

Jenamidine A (**1**), B (**2**) and C (**3**), as well as africantriol (**4**), were tested for antiproliferative and cytotoxic activities. Jenamidine A (**1**) inhibited proliferation of chronic myeloid leukaemic cell line K-562 was found with (GI_{50}=1.9 μg/ml). *In vitro* incubation for 72 hours with **1** led to cell enlargement of the K-562 cells as detected by a shift of the maximum of the cell volume distribution curve. An absence of antiproliferative activity against mouse fibroblasts L-929 and of cytotoxicity against HeLa-cells indicated a high specificity of the antiproliferative activity. Jenamidine B (**2**) and C (**3**), as well as africantriol (**4**) were not active in these assays. Furthermore, the jenamidines A to C (**1** to **3**) and africantriol (**4**) were inactive in a number of other biological tests (antibacterial, antifungal, antiviral and enzyme assays).

Table 5. Growth inhibition of chronic myeloid leukaemic cell line K-562 caused by jenamidine A (**1**).

Concentration (μg/ml)	Number of cells after treatment with **1***	Growth inhibition (GI, %)*
3.12	8.93×10^4	80
1.56	2.69×10^5	40
0.78	4.08×10^5	8

*The number of uninfluenced cells is 4.45×10^5.

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