Streptazones A, B_1, B_2, C, and D: New Piperidine Alkaloids from Streptomycetes

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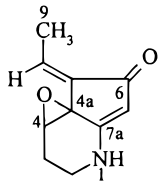
Five new piperidine alkaloids (**1**−**5**), named streptazones A, B_1, B_2, C, and D, were isolated from *Streptomyces* strains FORM5 and A1. Their structures were established on the basis of detailed MS and NMR analysis. Streptazone A (**1**) exhibited significant cytotoxic activity against selected human tumor cell lines.

Piperidine alkaloids are typical constituents of plants but have been rarely isolated from microbial sources until recently. Many of these metabolites have proved to be biologically active. Some of them, for example, the insecticide anabasine[1] or the antiarrhythmic drug ajmaline,[2] are of commercial interest.

A chemical screening approach[3] to detect piperidine alkaloids has been utilized to visualize such metabolites by their color reaction with Ehrlich's reagent. In this way we found four new octahydro-1-pyrindine compounds (**1**−**4**) and one new 4-pyridinone derivative (**5**) in the extracts of *Streptomyces* sp. strains FORM5 and A1. The current report describes the isolation and structure elucidation of these natural products.



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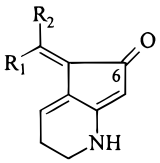

2: R_1 = H, R_2 = CH_3
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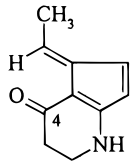

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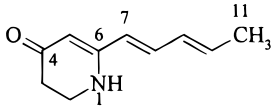

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Results and Discussion

The two strains were cultivated on an oatmeal medium and on a soybean meal/mannitol medium, respectively, for 4 days at 28 °C by using a stirred 10-L fermentor. TLC analysis (stationary phase: Si gel TLC plates; mobile phase: $CHCl_3$−MeOH, 9:1) of the culture filtrate extracts led to the observation that both strains produce some colorless compounds, which revealed violet spots after staining with Ehrlich's reagent. Purification of the extracts by several chromatographic steps furnished, in the case of strain A1, besides the known secondary metabolite streptazolin (**6**),[4,5] streptazones A (**1**) and B_1 (**2**). The extracts of strain FORM5 contained the streptazones B_1 (**2**), B_2 (**3**), C (**4**), and D (**5**).

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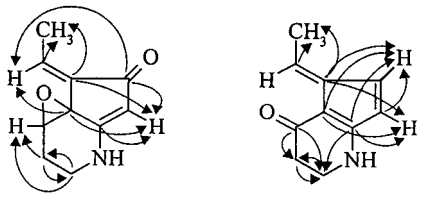

1 **4**

Figure 1. $^nJ_{C,H}$ long-range couplings observed in **1** and **4** by HMBC pulse sequences.

The molecular formula of **1** was shown to be $C_{10}H_{11}NO_2$ by HREIMS analysis. Its ^{1}H NMR spectrum displayed one exchangeable proton (δ 7.32), two olefinic protons (δ 5.69 and 5.18), and eight aliphatic protons (δ 3.95−2.06). The ^{13}C NMR spectrum of **1** indicated the presence of one C-methyl, two aliphatic methylenes, one O-substituted and two olefinic methines, one carbonyl, and three other quaternary carbons. On the basis of HMBC connectivities (Figure 1), biosynthetic considerations, and comparison to ^{13}C NMR data of **7**[6] and 3-(1-piperidyl)-2-cyclohexen-1-one,[7] structure **1** was assigned to streptazone A. Due to a NOE between δ_H 5.69 (H-8) and δ_H 3.95 (H-4), the configuration of the exocyclic methylene group at C-5 was unambiguously determined to be *Z*. The absolute configuration of the epoxide moiety is still unknown.

Streptazone B_1 (**2**) resembles **1**, but is more lipophilic. Comparison of the ^{13}C NMR data of these two compounds suggested that streptazone B_1 exhibits a double bond instead of the epoxide moiety. HREIMS of this compound revealed a molecular formula of $C_{10}H_{11}NO$. Information gleaned from ^{1}H−^{1}H COSY, HSQC, HMBC, and NOESY spectra resulted in assignment of structure **2**. It seems plausible that **2** is the biosynthetic precursor of **1**. Additionally, we isolated the (*E*)-isomer of **2**, named streptazone B_2 (**3**), as a minor component. In contrast to **2**, the NOESY spectrum of **3** shows a correlation between H-4 and H_3-9, which verifies the *E*-configuration of the exocyclic methylene group.

Compound **4** was isomeric with **2** and **3**. In contrast to **2**, the ^{1}H NMR spectrum of **4** shows, instead of signals of two single olefinic protons (H-4 and H-7), resonances of an olefinic AM-system at δ_H 7.20 and 6.28 (H-6 and H-7) with a ^3J coupling constant of J = 5.5 Hz typical of cis-positioned protons.[8] Remarkable is a lowfield shift of H-8 (δ_H 6.86), due to a neighboring carbonyl group. Detailed analysis of delay ^{1}H−^{1}H COSY, HSQC, and HMBC experiments (Figure 1) resulted in structure **4**. The configuration of the exocyclic double bond was assigned as *E* following the NOE that was observed between H-6 and H_3-9.

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Table 1. Cytotoxic Activity of **1** Against Different Tumor Cell Lines (GI_{50} and TGI Values in μmol/L)[18]

	HMO2	Kato III	HEP G2	MCF7
GI_{50}[a]	0.5	0.6	<0.1	15
TGI[b]	50	1.0	2.5	50

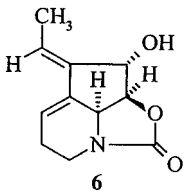
[a] GI_{50} = concentration resulting in a 50% inhibition of the cell growth. [b] TGI = concentration resulting in a complete inhibition of the cell growth.

The molecular formula of **5** was established as $C_{10}H_{13}NO$ on the basis of HREIMS and NMR data. The $^1H-^1H$ COSY spectrum made it possible to construct three fragments, an (*E*,*E*)-1,3-pentadienyl chain ($J_{H,H}$ = 15.0, 15.5 Hz), one ethylene group, and one olefinic proton, which appears as a singlet at δ_H 5.11. Complete analysis of the NMR spectra led to the conclusion that these three fragments are connected by a carbonyl group, a quaternary sp^2 carbon atom, and one NH group. The linkage of the fragments was achieved by interpretation of HMBC data and led to 6-[(1*E*,3*E*)-1,3-pentadienyl]-2,3-dihydro-4(1*H*)-pyridinone (= streptazone D, **5**).

Only a few octahydro-1-pyrindine derivatives are known from streptomycetes [e. g., dihydrolatumcidin (**7**),[6] pyrindicin,[9] 5-(2-butenylidene)-3-ethyl-1,2,3,4,5,7a-hexahydro-1-pyrindine-4,4a-diol,[10] kobutimycins A and B,[11] and epostatin[12]]. The streptazones A, B_1, B_2, and C (**1**−**4**) are not identical to any of them. Most of the mentioned metabolites are biologically active; for example, epostatin was found to be an inhibitor of dipeptidyl peptidase II.[13]

The 4-pyridinone derivative **5** is structurally related to the known secondary metabolites (*E*,*E*)-2-(1,3-pentadienyl)-piperidine, [2*S*(1*E*,3*E*),4*S*]-2-(1,3-pentadienyl)-4-piperidinol, and [2*S*(1*E*,3*E*),4*R*]-2-(1,3-pentadienyl)-4-piperidinol[14−16] and differs only from these three natural products in its level of oxidation.

Biosynthetic considerations led to the hypothesis that the streptazones are precursors or shunt products in the biosynthesis of streptazolin (**6**).[17] The isolation of **6** from both strains supported this assumption. In the case of the strain FORM5, the yields of **6** are dependent on the medium chosen. Cultivation of this strain for 4 d at 28 °C with a medium consisting of malt extract 1%, glucose 0.4%, and yeast extract 0.4% led to the isolation of 98 mg/L of **6**. In oatmeal medium, however, only traces of **6** were produced. We assume that the octahydro-1-pyrindine carbon skeleton of **1**−**4** is built from five acetate units via the polyketide pathway as it was described in the case of **7**.[6] In the course of the biosynthesis of **6**, an additional C_1 unit is incorporated.[17]



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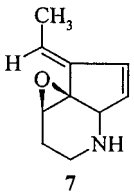

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Compounds **1**, **2**, **4**, **5**, and **6** were tested against the following tumor cell lines: HMO2 (stomach adenocarcinoma), HEP G2 (hepatocellular carcinoma), MCF 7 (breast adenocarcinoma), and Kato III (colon carcinoma).[18] However, only **1** showed significant cytotoxicity (Table 1), while the other metabolites gave no inhibition against the first three cell lines up to concentrations of 50 μmol/L. Due to the fact that **1** is the epoxide of **2**, this moiety seems to be responsible for the cytotoxicity of streptazone A (**1**) because it can easily be attacked by nucleophiles.

Experimental Section

General Experimental Procedures. Melting points were determined on a Reichert hot-stage microscope and are uncorrected. All homonuclear and heteronuclear 1D and 2D NMR spectra were recorded on a Bruker AMX 300 and a Varian Inova 500 instrument. Chemical shifts are expressed in δ values with solvents as internal standards. EIMS data were collected on a Finnigan MAT 95 mass spectrometer, at 70 eV (high resolution with perfluorkerosine as internal standard). IR spectra in pressed KBr disks were recorded on a Perkin-Elmer FT IR-1600 spectrometer, and the UV spectra, on a Varian Cary 3E spectrophotometer. Optical rotation values were measured with a Perkin-Elmer 241 polarimeter, and CD spectra, with a JASCO J 500 A spectrometer. TLC was carried out on Si gel 60 F_{254} plates (Merck, 0.25 mm), and column chromatography on Si gel (ICN, 32−63 μm) or Sephadex LH-20 (Pharmacia). Compounds were viewed under UV lamp at 254 nm and sprayed with Ehrlich's reagent[19] followed by heating.

Organisms and Fermentation. *Streptomyces* sp. strain FORM5 was isolated from a soil sample collected in Formentera (Spain) and is deposited at the Institute of Organic Chemistry in Göttingen. It was maintained as a stock culture on agar plates containing glucose 0.4%, malt extract 1%, yeast extract 0.4%, $CaCO_3$ 0.02%, and agar 2%. Precultures were carried out in 250-mL Erlenmeyer flasks with three baffles. Each flask was filled with 100 mL of medium (oatmeal 2% and trace element solution 0.25%,[20] adjusted to pH 7.2 before sterilization), sterilized 30 min at 121 °C, and then inoculated with a 1-cm^2 piece of agar from 5-day-old cultures that were grown at 28 °C. After 48 h on a rotary shaker (180 rpm, 28 °C) 800 mL of the precultures were used to inoculate a 10-L fermentor (Biostat E from Braun-Diessel) containing 9.2 L of sterilized medium (oat meal 3.0%, trace element solution 0.25%, and antifoam 0.09%). Fermentation was carried out at 28 °C for 4 days with aeration (10 L/min) under constant agitation (300 rpm). *Streptomyces* sp. strain A1 was maintained and cultivated as previously described and is deposited at the Sackler School of Medicine in Tel Aviv.[21] The precultures were grown on degreased soybean meal 2% and mannitol 2%. Additionally, the main culture contained antifoam 0.09%. With the exception of the medium, the fermentation of strain A1 in the Biostat E-fermentor was carried out under the same conditions as described for strain FORM5.

Extraction and Isolation. After filtration of the harvested culture broth of strain FORM5, the culture filtrate was passed through an Amberlite XAD-2 column (ca. 2 L of resin) and washed with H_2O (5 L). The bound compounds were eluted with MeOH (5 L) and evaporated to dryness in vacuo. The residue (10.0 g) was chromatographed twice on a Si gel column with CH_2Cl_2−MeOH (gradient from 19:1 to 9:1). The separation of the obtained main fraction by Si gel chromatography with EtOAc−cyclohexane−MeOH (10:5:1) afforded 46 mg of **2**, 14 mg of **4**, 162 mg of **5**, and 2 mg of **3**. The culture filtrate of strain A1 was analogously worked up as described for strain FORM5. The resulting crude product (5.4 g) was subjected to Si gel chromatography and eluted with CH_2Cl_2−MeOH (gradient from 14:1 to 6:1) into three fractions. The first fraction consisted of 202 mg of pure **6**. Purification of the second fraction by repeated gel filtration chromatography (Sephadex LH-20, MeOH, and acetone) led to 3 mg of **2**. The third fraction was also purified by gel filtration chromatography (Sephadex LH-20, MeOH) to furnish 12 mg of **1**. Under the mentioned conditions, strain A1 did not produce the rubromycins.[21]

Streptazone A (1): white solid, mp 133 °C; R_f 0.27 ($CHCl_3$-MeOH, 9:1), 0.28 (EtOAc−cyclohexane−MeOH, 10:5:1); color reaction with Ehrlich's reagent: pink; $[\alpha]^{22}_D$ +118° (*c* 0.34, MeOH); UV (MeOH) λ_{max} (log ϵ) 239 (4.19), 310 (4.37) nm; (MeOH + HCl) 220 (4.18), 315 (4.34) nm; CD (MeOH) λ ($\Delta\epsilon$) 240 (−1.50), 254 (+0.26), 290 (−5.50), 320 (+5.48) nm; IR (KBr) ν_{max} 1654, 1585 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 7.32 (1H, br s, H-1), 5.69 (1H, qd, J = 7.3, 0.7 Hz, H-8), 5.18 (1H, s, H-7), 3.95 (1H, d, J = 2.7 Hz, H-4), 3.35 (1H, ddd, J = 12.3,

12.3, 4.0 Hz, H-2β), 3.18 (1H, dddm, J = 12.3, 5.5, 5.5 Hz, H-2α), 2.28 (1H, dm, J = 15.0 Hz, H-3β), 2.15 (3H, d, J = 7.3 Hz, H_3-9), 2.13−2.06 (1H, m, H-3α); ^{13}C NMR (125.7 MHz, acetone-d_6) δ 191.7 (s, C-6), 166.6 (s, C-7a), 133.4 (s, C-5), 128.2 (d, C-8), 105.9 (d, C-7), 60.1 (s, C-4a), 59.6 (d, C-4), 37.6 (t, C-2), 26.2 (t, C-3), 13.6 (q, C-9); EIMS m/z 177 $[M]^+$ (100), 148 (52); HREIMS m/z 177.0789 (calcd for $C_{10}H_{11}NO_2$, 177.0789).

Streptazone B$_1$ (2): colorless oil, R_f 0.32 ($CHCl_3$−MeOH, 9:1), 0.36 (EtOAc−cyclohexane−MeOH, 10:5:1); color reaction with Ehrlich's reagent, violet; UV (MeOH) λ_{max} (log ϵ) 262 (4.14), 315 (3.99) nm; (MeOH + HCl) 262 (4.04), 279 (4.02), 327 (3.84) nm; IR (KBr) ν_{max} 1665, 1635, 1628, 1580 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 6.31 (1H, td, J = 4.5, 1.3 Hz, H-4), 6.25 (1H, q, J = 7.5 Hz, H-8), 5.17 (1H, s br, H-7), 3.36 (2H, t, J = 7.0 Hz, H_2-2), 2.47 (2H, td, J = 7.0, 4.5 Hz, H_2-3), 2.22 (3H, d, J = 7.5 Hz, H_3-9); ^{13}C NMR (125.7 MHz, CD_3OD) δ 195.1 (s, C-6), 165.7 (s, C-7a), 132.4 and 132.3 (s, C-4a, C-5), 126.7 (d, C-8), 119.2 (d, C-4), 102.4 (d, C-7), 39.9 (t, C-2), 24.0 (t, C-3), 13.6 (q, C-9); EIMS m/z 161 $[M]^+$ (100), 146 $[M - CH_3]^+$ (37), 132 (74); HREIMS m/z 161.0840 (calcd for $C_{10}H_{11}NO$, 161.0840).

Streptazone B$_2$ (3): colorless oil, R_f 0.26 ($CHCl_3$−MeOH, 9:1), 0.19 (EtOAc−cyclohexane−MeOH, 10:5:1); color reaction with Ehrlich's reagent, violet; UV (MeOH) λ_{max} (log ϵ) 257 (4.10), 319 (3.91) nm; (MeOH + HCl) 257 (4.02), 273 (3.99), 327 (3.78) nm; IR (KBr) ν_{max} 1663, 1636, 1576 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 6.58 (1H, tm, J = 4.5 Hz, H-4), 6.38 (1H, q, J = 7.5 Hz, H-8), 5.13 (1H, d, J = 1.0 Hz, H-7), 3.39 (2H, t, J = 7.5 Hz, H_2-2), 2.55 (2H, td, J = 7.5, 4.5 Hz, H_2-3), 2.03 (3H, d, J = 7.5 Hz, H_3-9); ^{13}C NMR (125.7 MHz, CD_3OD) δ 193.4 (s, C-6), 167.5 (s, C-7a), 133.7 (s, C-4a), 131.4 (s, C-5), 126.2 (d, C-4), 125.8 (d, C-8), 99.0 (d, C-7), 39.5 (t, C-2), 24.7 (t, C-3), 14.3 (q, C-9); EIMS m/z 161 $[M]^+$ (100), 132 (14); HREIMS m/z 161.0840 (calcd for $C_{10}H_{11}NO$, 161.0840).

Streptazone C (4): yellow oil, R_f 0.30 ($CHCl_3$−MeOH, 9:1), 0.29 (EtOAc−cyclohexane−MeOH, 10:5:1); color reaction with Ehrlich's reagent, gray-violet; UV (MeOH) λ_{max} (log ϵ) 286 (3.76), 368 (3.39) nm; (MeOH + HCl) 224 (3.58), 276 (3.71), 314 (3.57) nm; IR (KBr) ν_{max} 1613, 1571 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 7.20 (1H, dm, J = 5.5 Hz, H-6), 6.86 (1H, qm, J = 8.0 Hz, H-8), 6.28 (1H, dm, J = 5.5 Hz, H-7), 3.59 (2H, t, J = 8.0 Hz, H_2-2), 2.38 (2H, t, J = 8.0 Hz, H_2-3), 2.04 (3H, d, J = 8.0 Hz, H_3-9);^{13}C NMR (125.7 MHz, CD_3OD) δ 187.0 (s, C-4), 169.0 (s, C-7a), 141.0 (s, C-5), 136.2 (d, C-6), 131.0 (d, C-8), 124.2 (d, C-7), 103.8 (s, C-4a), 43.3 (t, C-2), 36.8 (t, C-3), 15.8 (q, C-9); EIMS m/z 161 $[M]^+$ (100), 133 (24); HREIMS m/z 161.0840 (calcd for $C_{10}H_{11}NO$, 161.0840).

Streptazone D (5): yellow solid, mp 105−109 °C; R_f 0.28 ($CHCl_3$−MeOH, 9:1) 0.24 (EtOAc−cyclohexane−MeOH, 10:5:1); color reaction with Ehrlich's reagent, violet; UV (MeOH) λ_{max} (log ϵ) 288 (4.34), 361 (4.11) nm; (MeOH + HCl) 215 (3.91), 314 (4.43) nm; IR (KBr) ν_{max} 1641, 1592, 1524 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 6.93 (1H, dd, J = 15.5, 10.3 Hz, H-8), 6.23 (1H, ddq, J = 15.0, 10.3, 1.0 Hz, H-9), 6.09 (1H, dq, J = 15.0, 7.0 Hz, H-10), 5.92 (1H, d, J = 15.5 Hz, H-7), 5.11 (1H, s, H-5), 3.54 (2H, t, J = 7.7 Hz, H_2-2), 2.37 (2H, t, J = 7.7 Hz, H_2-3), 1.84 (3H, dd, J = 7.0, 1.0 Hz, H_3-11); ^{13}C NMR (125.7 MHz, CD_3OD) δ 194.8 (s, C-4), 163.2 (s, C-6), 138.6 (d, C-8), 138.1 (d, C-10), 131.9 (d, C-9), 124.3 (d, C-7), 96.8 (d, C-5), 41.9 (t, C-2), 35.6 (t, C-3), 18.7 (q, C-11); EIMS m/z 163 $[M]^+$ (100), 134 (19), 120 (25), 106 (15); HREIMS m/z 163.0995 (calcd for $C_{10}H_{13}NO$, 163.0997).

Streptazolin (6): colorless oil; $[\alpha]^{22}_D$, ^{1}H NMR, ^{13}C NMR, EIMS, and HREIMS data were in agreement with literature values.[4,17,22]

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(19) 4-Dimethylaminobenzaldehyde (1 g) was dissolved in a mixture of concentrated HCl (25 mL) and MeOH (75 mL).
(20) In 1 L deionized water were dissolved 3 g $CaCl_2 \times 2\ H_2O$, 1 g Fe-III-citrate, 0.2 g $MnSO_4$, 0.1 g $ZnCl_2$, 0.025 g $CuSO_4 \times 5\ H_2O$, 0.02 g $Na_2B_4O_7 \times 10\ H_2O$, 0.004 g $CoCl_2$, and 0.01 g $NaMoO_4 \times 2\ H_2O$.
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