SECONDARY METABOLITES BY CHEMICAL SCREENING 4†

DETECTION, ISOLATION AND BIOLOGICAL ACTIVITIES OF CHIRAL SYNTHONS FROM *Streptomyces*

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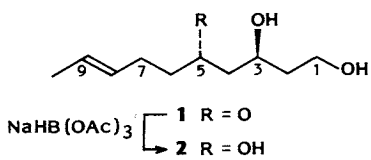
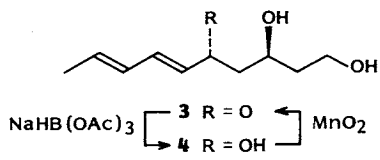
Chemical screening for secondary metabolites[2] in the culture broth of microorganisms has led to natural products with different types of structures and biological activities. This method allows the detection of compounds independent from their biological activity, which can lead to chiral synthons for synthesis. Therefore, this type of screening is a possibility for extending the chiral pool. For example, selective screening for secondary amines by using special staining reagents on TLC should result in interesting precursors for alkaloid synthesis.

The screening was performed by centrifugation of the culture-broth from different *Streptomyces* strains, adsorption of the compounds in the culture filtrate on Amberlite XAD-16, elution with methanol and concentration. Detection of the amines after TLC separation of the products was done using phenothiazine perbromide, an excellent reagent for visualization of secondary amines[3], which results in blue-colored spots on TLC. At room temperature primary amines were ruled out through a color change after treatment with a sodium hydroxide solution. *Streptomyces luteogriseus* (strain FH-S 1307), isolated from a soil sample collected in Kypcerissia, Greece, was found to be a potent producer of three alkaloids recognizable by striking blue-colored spots. Furthermore, four neutral metabolites were detected, which had shown no color reaction with phenothiazine perbromide but could be visualized with anisaldehyde.

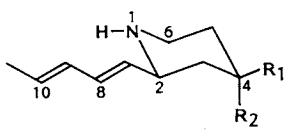
To receive more material for the identification and structure elucidation a fermentation was carried out for 120 hours at 30°C in a 10-liter Braun fermenter with a medium containing 2% soybean meal and 2% mannitol (pH 7). The isolation started with centrifugation of the culture broth. The filtrate was adjusted to pH 1 and extracted three times with 5-liter portions of ethyl acetate and was then adjusted to pH 10 followed by repeating this extraction procedure.

The first extract was concentrated and the residue was chromatographed on silica gel with ethyl acetate - hexane (3 : 1), which yielded mixtures of **1**, **3** and **2**, **4**. The compounds **1** and **3** were separated by silica gel chromatography with ethyl acetate - hexane - methanol (10 : 5 : 1). For the purification of

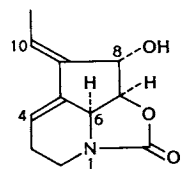




Relative stereochemistry



5 R_1=H R_2=OH
6 R_1=OH R_2=H
7 R_1=H R_2=H



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† See ref 1.

Table 1. Yields and properties of the isolated metabolites.

	1	2	3	4	5	6	7
Yield (mg/liter)	30	5	10	5	2.5	1.5	0.5
Rf[a]	0.49	0.37	0.48	0.36	0	0	0
Rf[b]	0.68	0.46	0.65	0.46	0.22	0.31	0.48
$[\alpha]_D^{20}$ (*c* 1, $CHCl_3$)	+26	+39	+62	+35	−15	−13	+27
Color reactions:							
Phenothiazine perbromide	—	—	—	—	Blue	Blue	Blue
Anisaldehyde	Light brown	Dark brown	Red brown	Dark brown	Blue	Blue	Blue
Ehrlich reagent	Green	—	Blue	—	—	—	—

[a] CH_2Cl_2-MeOH (9:1). [b] CH_2Cl_2-MeOH-conc NH_4OH (90:15:2).

2 and **4**, HPLC reversed phase chromatography (RP C-18) was used with the eluent methanol-water (4:6). We named the compounds **1**~**4** streptenols based on their structural features and their origin.

The structures were determined by means of 2D NMR spectroscopy. Streptenol A (**1**) was identified as (+)-(3*S*,8*E*)-1,3-dihydroxy-8-decen-5-one, a known compound first isolated from *Streptomyces fimbriatus*[4] and *Streptomyces cirratus*,[5] that exhibits an immunostimulating activity. Streptenol B (**2**) is the 5-dihydro derivative of **1**, whereas streptenol C (**3**) is the 6-dehydro derivative of **1**, a compound which was first isolated from *Streptomyces* sp. C57.[6] Streptenol D (**4**) turned out to be the 5-dihydro derivative of **3**. The assignments of the stereochemistry were performed by reduction with reagents where the stereoselectivity was predictable. For example, reaction of β-hydroxy ketones with $NaHB(OAc)_3$ as described by EVANS *et al.*[7] yields anti 1,3-diols preferentially. Application of this reduction to **1** resulted in streptenol B (**2**), which proved the (3*S*,5*S*,8*E*)-8-decen-1,3,5-triol structure of **2**. Furthermore, the anti position of the hydroxyl groups at C-3 and C-5 in **2** was ascertained by the synthesis of δ-lactones.[8] The (3*S*,5*R*,6*E*,8*E*)-6,8-decadiene-1,3,5-triol structure of **4** was determined by the analog reduction of **3**. Furthermore, streptenol C (**3**) was prepared from **4** by oxidation with MnO_2, which proved the (3*S*,6*E*,8*E*)-1,3-dihydroxy-6,8-decadien-5-one structure of **3**.

Drying and evaporation of the second organic layer yielded a dark syrup, which was chromatographed on silica gel with CH_2Cl_2-MeOH-conc NH_4OH (60:10:1) to yield the alkaloids **5**~**7**. Compounds **5** and **6** have the same molecular formula, $C_{10}H_{17}NO$ (EI-MS: *m/z* 167, M^+). The structures were determined by means of 2D NMR spectroscopy. Compound **5** was identified as [2α(1*E*,3*E*),4β]-2-(1,3-pentadienyl)-4-piperidinol,

Table 2. ^{13}C NMR chemical shifts of the streptenols, **1**~**3** in $CDCl_3$, **4** in CD_3OD (100 MHz, chemical shifts in ppm).

Position	1	2	3	4
1	60.7	61.9	61.3	60.1
2	37.9	38.3	37.8	41.1
3	67.4	69.8	68.2	69.9
4	49.3	42.6	46.3	45.7
5	211.2	69.2	200.9	66.6
6	43.3	37.0	127.6	134.9
7	26.5	29.0	141.6	130.9
8	129.2	130.7	144.3	137.3
9	126.1	125.6	130.1	129.7
10	17.3	17.9	18.8	18.2

which was first isolated from *Streptomyces* sp. S 20 846 and named SS 20 846A.[9] The piperidinol **5** has a restrictive action upon the digestive system.[9] Compound **6** is a C-4 epimer of **5** which was proved by the γ_{gauche} effect of 4-OH at C-2 in the ^{13}C NMR spectrum of **6** ($\Delta\delta_{5\sim6} = -4.5$ ppm). Therefore, the structure for **6** was deduced as [2α(1*E*,3*E*),4α]-2-(1,3-pentadienyl)-4-piperidinol. From this fact and the coupling pattern of 4-H in the ^1H NMR experiment the conformations of the piperidines were determined. The molecular formula of **7** ($C_{10}H_{17}N$, EI-MS: *m/z* 151, M^+) as well as the NMR spectra demonstrated that **7** is (*E*,*E*)-2-(1,3-pentadienyl)piperidine, a deoxy derivative of **5** and **6**. The absolute stereochemistry of **5**, **6** and **7** is presently under investigation.

The well known antibiotic streptazoline (**8**)[10] was isolated as a minor secondary metabolite (1 mg/liter) from the culture broth. A close examination of the different structures revealed that the piperidines **5** and **6** incorporate the structural elements of streptenol C (**3**), and streptazoline (**8**) possessed the partial structure of the piperidines **5** and **6**. Based on this comparison, we assume that **3** is the

Table 3. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data of alkaloids **5**, **6** and **7** in $CDCl_3$ (chemical shifts in ppm).

Position	5		6		7	
	δ (^1H)	δ (^{13}C)	δ (^1H)	δ (^{13}C)	δ (^1H)	δ (^{13}C)
2 ax	3.63 t (br)	52.8	3.16 dm	57.3	3.44 td	58.3
3 ax	1.59 td		1.24 q			
3 eq	1.82 dt	39.6	2.01 m	42.1	1.24	29.9
4 ax			3.67 tt		−2.02 m	
4 eq	4.20 t (br)	64.8		69.1	(6H)	22.7
5 ax	1.71 m		1.40 qd			
5 eq	1.72 m	32.8	1.97 m	35.4		22.9
6 ax	3.13 td		2.69 td		2.81 td	
6 eq	2.93 tdt	40.5	3.16 m	44.3	3.31 dt	44.9
7	5.54 dd	133.2	5.54 dd	132.3	5.61 dd	134.6
8	6.16 dd	130.5	6.13 dd	130.6	6.32 dd	130.5
9	6.04 dd	131.2	6.01 dd	131.0	6.01 dd	131.9
10	5.69 dq	129.2	5.69 dq	129.6	5.77 dq	127.0
11	1.78 d	18.0	1.74 d	18.5	1.73 d	18.1

biosynthetic precursor of the piperidines **5**~**7**, and **5** or **6** could be an intermediate in the biosynthesis of **8**. Due to the fact that **8** was only produced in low yield, it could be supposed that this particular strain lost the ability for the synthesis of more complex secondary metabolites. This would explain why this *S. luteogriseus* strain is an excellent source for the production of chiral building blocks.

The streptenols **1**~**4** as well as the alkaloids **5**~**7** should be useful synthons for the construction of more complex molecules. This was already demonstrated for **1** in the enantioselective synthesis of δ-lactones.[8] Investigation of the biological activity of the pure streptenols in different test systems have shown that **1**~**4** could be used as inhibitors of the cholesterol biosynthesis in HEP G_2-cells.[11] Compounds **1**~**4** exhibited an IC_{50} of 1×10^{-7} mol/liter in this test system. Further investigations concerning the mode of action are presently under investigation.

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