Biosynthetic Capacities of Actinomycetes. 3†

Naphthgeranine F, a Minor Congener of the Naphthgeranine Group Produced by *Streptomyces violaceus*

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The naphthgeranines A to E were isolated from *Streptomyces violaceus* (strain Tü 3556) in the course of a chemical screening program[2]. The naphthgeranines were detected in the extract of the culture filtrate by their coloured spots on TLC sheets. Naphthgeranines A and B show a weak antibacterial and antifungal activity in the agar disc diffusion assay with MIC values between 0.1 and 1 mg/ml, and a moderate cytocidal activity is described against various tumor cell lines *in vitro*[2].

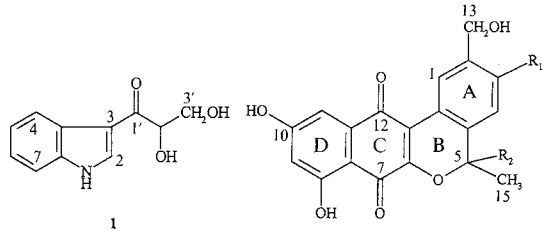
Strain Tü 3556 was included in our screening program for detection of new secondary metabolites, analyzing biosynthetically talented actinomycete strains by HPLC, diode array multiwavelength monitoring and a UV-visible absorbance spectral library database[3]. Besides phenazine-1-carboxylic acid (tubermycin B)[4] and methyl 1-phenazinecarboxylate[5], 1-(3-indolyl)-2,3-dihydroxypropan-1-one (**1**) was detected in the culture filtrate extract of strain Tü 3556. It has not previously been described as a natural product but only as a synthetic substance[6]. **1** was identified by its spectroscopic data†† in comparison with other natural indole derivatives[7]. In addition, a new compound was detected by this technique which showed a similar UV-visible spectrum to those of the naphthgeranines, especially to naphthgeranine E (**2**). This was confirmed by computer matching of the spectra. The retention time of 7.8 minutes in standardized gradient reversed-phase HPLC[3] differed from all other naphthgeranines, therefore, we had a good indication that this represented a new member of the naphthgeranine group of antibiotics, named naphthgeranine F (**3**). The structures of the compounds isolated from strain Tü 3556 are shown in Fig. 1.

Strain Tü 3556 was cultivated in 500-ml Erlenmeyer flasks on the rotory shaker at 120 rpm and 27°C containing 100 ml of medium consisting of mannitol 2% and soybean meal 2% in tap water (pH 7.5). After 48 hours incubation of the preculture, a 25-liter fermentor equipped with an intensor system (b20, Giovanola) containing 20 liters of the same medium was inoculated with 5% (v/v), and grown at 27°C, aeration of 0.5 v/v/m and agitation of 1200 rpm. Naphthgeranine F concentration was similar to naphthgeranine E and reached an amount of less than 1 mg/liter after a fermentation period of 120 hours. The main compound, naphthgeranine C, was produced in an amount of 9 mg/liter. No negative influence on the production of naphthgeranines was observed with variations of the carbon and nitrogen source, and of phosphate concentration. Therefore, no indication for C-, N- or P-repression of antibiotic production is evident.

Naphthgeranine F (**3**) was isolated from the culture filtrate by column chromatography using Amberlite XAD-16. The compound was desorbed from the resin by MeOH - H_2O (80 : 20), concentrated under *vacuo*, and extracted at pH 7 with ethyl acetate. The organic extract was concentrated to dryness, dissolved in CH_2Cl_2, and purified by chromatography using oxalic acid treated silica gel and elution with CH_2Cl_2 - MeOH (95 : 5). The naphthgeranine F containing fractions were concentrated to dryness and dissolved in a small volume of methanol. Purification of the raw product was carried out with Sephadex LH-20 column chromatography using methanol as eluent. Pure naphthgeranine F was obtained after preparative reversed-phase HPLC using Kromasil C-18 (particle size 10 μm, 16 mm i.d. × 250 mm stainless steel column) with H_2O - MeOH gradient elution and a flow rate of 20 ml/minute, yielding the antibiotic as a dark-red, amorphous substance. The physico-chemical properties are shown in Table 1.

The HREI-MS of naphthgeranine F (**3**) displayed a molecular peak at *m/z* 368 corresponding with the molecular formula $C_{20}H_{16}O_7$, which entails a high degree of unsaturation shown by 13 double bound equivalents. The ^1H and ^{13}C spectra (Table 2) revealed, besides signals

Fig. 1 Structures of 1-(3-indolyl)-2,3-dihydroxypropan-1-one (**1**), and naphthgeranines E (**2**) and F (**3**), produced by *Streptomyces violaceus* Tü 3556.



Naphthgeranine E (**2**) R_1 = OH, R_2 = CH_3
Naphthgeranine F (**3**) R_1 = H, R_2 = CH_2OH

† See ref 1.

†† Rf value 0.14 (TLC, silica gel, chloroform - methanol, 9 : 1), yellow spot with Ehrlich reagent, blue-violet spot with tetrazolium reagent; $[\alpha]_D^{20}$ −105° (*c* 0.005, MeOH); ^1H NMR (200 MHz, $CDCl_3$, CD_3OD) δ 3.80 (dd, J = 11.5 and 3.5 Hz, 3'-H_a), 3.95 (dd, J = 11.5 and 6.0 Hz, 3'-H_b), 4.90 (dd, J = 6.0 and 3.5 Hz, 2'-H), 7.24 (m, 5-H and 6-H), 7.45 (m, 7-H), 8.28 (m, 4-H), 8.30 (s, 2-H); ^{13}C NMR (50.3 MHz, CD_3OD) δ 66.9 (t, C-3'), 76.9 (d, C-2'), 112.8 (d, C-7), 115.3 (q, C-3), 122.8 (d, C-6), 123.3 (d, C-4), 124.4 (d, C-5), 127.1 (q, C-3a), 135.4 (d, C-2), 138.0 (q, C-7a), 196.4 (q, C-1').

of five aromatic hydrogen and 14 carbon atoms, the presence of a methyl group, two hydroxymethylene groups, a quarternary sp^3 carbon atom attached to oxygen, two enolic hydroxyl groups and two carbonyl groups. Detailed spectral analysis of **3**, aided by ^1H-^1H and ^1H-^{13}C shift correlations revealed the presence of a 5-hydroxy-1,4-naphthoquinone skeleton, in accordance with the UV spectra (Table 1). A comparison of the ^1H and ^{13}C signals with those of the isomeric naphthgeranine E (**2**) (Table 2) indicated a remarkable agreement. Thus, nearly identical chemical shifts were found for the naphthoquinone moiety. The main differences could be seen for the signals of ring D, especially for C-2 (δ_C 134.2), C-3 (δ_C 129.0, δ_H 7.43) and C-4 (δ_C 124.9, δ_H 7.21), and of C-14 (δ_C 67.7, δ_H 3.85/4.04). The missing phenolic hydroxy group at C-3 and an additional hydroxy group at C-14 resulting in a hydroxymethylene group at C-5 mark the structural variation. The obtained structure for naphthgeranine F (**3**), was finally confirmed by a $^nJ_{C,H}$ correlation experiment (COLOC) shown in Fig. 2.

Table 1. Physico-chemical properties of naphthgeranine F (**3**).

Appearence	dark-red powder
FAB-MS (positive ions)[a] $[M+H]^+$	369
HREI-MS (M^+)	found 368.0896; calcd. 368.0896
Molecular formula	$C_{20}H_{16}O_7$
MP	230°C (dec)
α_D^{20} (MeOH)	+207° (*c* 0.02)
UV λ_{max}^{MeOH} nm (ε)	217 (10,270), 259 (5,990), 299 (3,950), 317 (3,700), 343 (3,600), 438 (1,900)
UV $\lambda_{max}^{MeOH+NaOH}$ nm (ε)	212 (24,040), 257 (16,270), 310 (13,560), 377 (1,590), 546 (4,050)
IR (KBr) cm^{-1}	3386, 1622, 1583
Rf value (TLC, silica gel)[b]	0.26

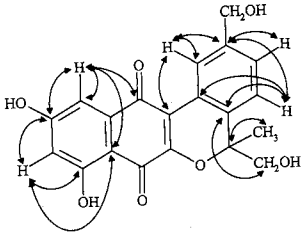
[a] Matrix nitrobenzyl alcohol (NBA).
[b] Chloroform - methanol (9 : 1).

Table 2. ^{13}C (50.3 MHz) and ^1H NMR (200 MHz) chemical shifts of naphthgeranine E (**2**) and F (**3**).

Atom No.	**2**		**3**[a]	
	δ_C[b]	δ_H[d]	δ_C[b]	δ_H[c]
1	129.4 d	8.43 s	126.7 d	8.51 d (1.5)
2	120.4 s		134.2 s	
3	152.7 d		129.0 d	7.43 dd (7.5, 1.5)
4	110.6 d	6.82 s	124.9 d	7.21 d (7.5)
4a	140.5 s		142.6 s	
5	81.9 t		85.1 s	
6a	158.7 s		156.2 s	
7	183.7 s		183.5 s	
7a	107.6 s		106.0 s	
8	167.7 s	12.18 (OH)	168.4 s	
9	108.9 d	6.57 d (2.0)	108.0 d	6.50 d (2.0)
10	165.5 s		165.8 s	
11	110.5 d	7.16 d (2.0)	116.6 d	7.14 d (2.0)
11a	136.1 s		135.5 s	
12	184.1 s		184.0 s	
12a	117.1 s		117.9 s	
12b	128.5 s		127.0 s	
13	60.9 t	4.82 s	65.1 t	4.69 s
14	26.0 q	1.69 s	67.7 t	3.85/4.04 d (12.0)
15	26.0 q	1.69 s	23.1 q	1.17 s

[a] Attached proton test (APT) experiment allowed distinction of carbon multiplicities; assignments based on COSY, HETCOR and COLOC data. δ values in ppm relative to internal TMS, *J* (Hz) in brackets. [b] CD_3OD; [c] $CD_3OD/CDCl_3$; [d] acetone-d_6.

Fig. 2. $^nJ_{C,H}$ long range couplings of **3** (COLOC).



The antimicrobial activity of **3** was tested by the agar plate diffusion assay and compared with the known naphthgeranines. Naphthgeranine F showed no activity against Gram-negative bacteria, yeasts and other fungi at concentrations of 1 mg/ml. A weak antibacterial activity against Gram-positive bacteria (*Bacillus subtilis* ATCC 6051, *Bacillus brevis* ATCC 9999, *Streptomyces viridochromogenes* Tü 57) was observed with similar MIC values as found for naphthgeranine C, which is the main compound of the fermentation product of strain Tü 3556.

The structural variations within the naphthgeranine family resulted from a high oxygenase activity directed towards the mevalonate derived part of the molecule[8]. The center of chirality at C-5 of **3** requires stereospecific acting enzymes. Thus, we assume that strain Tü 3556 may be suited for biotransformations of steroids.

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