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EXFOLIAMYCIN AND RELATED METABOLITES, NEW NAPHTHOQUINONE ANTIBIOTICS FROM *Streptomyces exfoliatus*

OLIVIER POTTERAT and HANS ZÄHNER*

Biologisches Institut, LB Mikrobiologie/ Antibiotika, Universität Tübingen D-7400 Tübingen, Germany

CORINNA VOLKMANN and AXEL ZEECK

Institut für Organische Chemie, Universität Göttingen, D-3400 Göttingen, Germany

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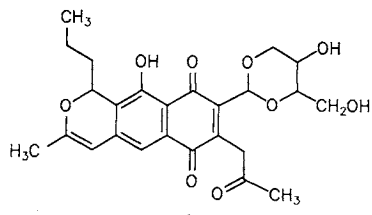
In the course of our chemical screening program, we observed that *Streptomyces exfoliatus* (strain Tü 1424) synthesized numerous quinoid pigments when rotary shaker cultures were supplemented with Amberlite XAD-1180. We report here on the isolation and structure elucidation of three antibiotics, which were shown to be new naphthoquinone C-glycosides (**2**~**4**).

After isolation from a soil sample collected in Turkey, the strain Tü 1424 was cultivated in 500 ml baffled Erlenmeyer flasks containing each 100 ml of a medium consisting of soybean meal 2% and mannitol 2% (pH 7.5). The fermentation was carried out on a rotary shaker for 4 days at 27°C. After 36 hours, each flask was supplemented with 30 g of Amberlite XAD-1180 suspended in 50 ml water. The mycelium and the resin, collected as a mixture from the harvested broth (6 liters) by filtration, were extracted with methanol - acetone (1 : 1, 5 × 2 liters). After concentration under reduced pressure, the aqueous residue was extracted with ethyl acetate (5 × 1 liter) and the evaporation residue of this extract (12 g) was fractionated by flash chromatography (silica gel, methylene chloride - methanol, 94 : 6→9 : 1). Further separations by low pressure liquid chromatography (LPLC) on Lobar columns (40~63 μm; i.d. 31 × 2.5 cm; Merck) provided compounds **1**~**4** as red to orange solids. Compounds **1** (76 mg), **2** (42 mg) and **3** (139 mg) were purified on RP-18 with methanol - water (7 : 3, 1 : 1 and 65 : 35, respectively). Compound **4** (120 mg) was isolated after two successive LPLC steps on RP-18 (methanol - water, 65 : 35) and Diol material (methylene chloride - methanol, 99 : 1).

Compound **1** ($C_{25}H_{28}O_9$; Rf = 0.55, silica gel/ methylene chloride - methanol, 9 : 1) was identified by its spectral data as naphthopyranomycin[2], an antibiotic recently isolated from *Streptomyces* sp. The physico-chemical properties and the ^{13}C and ^{1}H spectral data of the new compounds **2**~**4** are summarized in Tables 1 and 2. Anhydroexfoliamycin (**4**) showed UV data closely similar to those of **1**. In the FAB-MS a *quasi*-molecular ion was observed at *m/z* 417 ($[M+H]^+$) corresponding to the molecular formula $C_{22}H_{24}O_8$. A fragment due to the loss of a propyl chain was detected at *m/z* 373 ($[(M+H)-44)]^+$). NMR investigations, especially 2D-NMR experiments, led to the structure **4**. The long-range correlations revealed by a correlation spectroscopy *via* long range coupling (COLOC) experiment are depicted in the Fig. 1. The connectivity within the sugar moiety could not be



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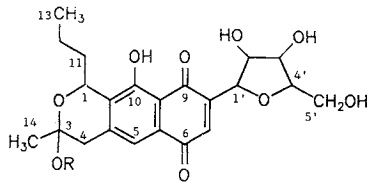
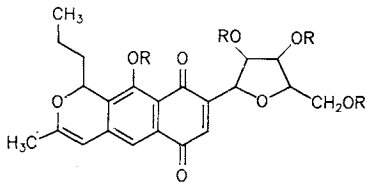


2 R = H
3 R = CH_3



4 R = H
5 R = Ac

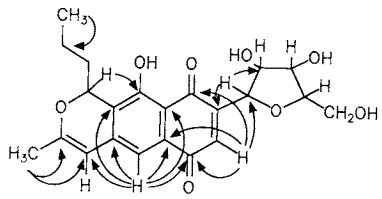
[†] See ref 1.

Table 1. Physico-chemical properties of compounds **2**~**4**.

	2	**3**	**4**
Appearance	Orange to brown solid	Orange to brown solid	Dark red solid
FAB-MS (positive ions)[a] $[M+H]^+$	435	449	417
Molecular formula	$C_{22}H_{26}O_9$	$C_{23}H_{28}O_9$	$C_{22}H_{24}O_8$
MP	90°C	129~131°C	167°C
$[\alpha]_D^{20}$ (MeOH)	+295° (*c* 0.16)	+478° (*c* 0.11)	+633° (*c* 0.14)
UV λ_{max}^{MeOH} nm (ε)	431 (4,130), 254 (10,840), 220 (31,600)	432 (3,970), 254 (10,950), 220 (28,970)	446 (4,570), 299 (18,430), 237 (15,560), 204 (22,870)
$\lambda_{max}^{0.01\,M\,HCl\text{-}MeOH}$ nm (ε)	437 (4,750), 299 (13,980), 230 (12,490), 213 (17,430), 207 (18,170)	455 (4,330), 299 (15,480), 237 (13,490), 206 (19,600)	445 (4,520), 300 (17,710), 237 (15,180), 204 (20,800)
$\lambda_{max}^{0.01\,M\,NaOH\text{-}MeOH}$ nm (ε)	558 (2,210), 444 (1,900), 283 (6,930), 229 (27,810)	568 (10,890), 229 (61,960)	553 (2,660), 293 (13,570), 263 (15,260), 223 (9,130)
IR (KBr) cm^{-1}	1640, 1605 (sh), 1595	1640, 1605 (sh), 1595, 1560	1640, 1605 (sh), 1595, 1560
Rf (TLC, silica gel)[b]	0.28	0.44	0.44

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

Fig. 1. Long-range heteronuclear correlations in **4** (COLOC).



completely deduced from 2D-NMR spectra because of signal overlapping. The furanosyl structure could be determined by the chemical shifts in the ^1H NMR spectrum (Table 2) of tetra-*O*-acetyl-anhydroexfoliamycin (**5**, orange needles from ethyl acetate-heptane, mp 177°C), which was isolated after treatment of **4** (15 mg) with acetic anhydride-pyridine followed by the usual work-up (95% yield). **5** has a molecular composition of $C_{30}H_{32}O_{12}$ determined by FAB-MS. In the ^1H NMR spectrum of **5**, 2'-H (+1.10 ppm), 3'-H (+1.12 ppm), 5'-H_a (+0.40 ppm) and 5'-H_b (+0.41 ppm) were shifted downfield whilst 1'-H and 4'-H remained little affected (see Table 2).

The FAB-MS of exfoliamycin (**2**) showed a *quasi*-molecular ion at *m/z* 435 ($[M+H]^+$) agreeing with the molecular formula $C_{22}H_{26}O_9$. Fragments were detected at *m/z* 417 ($[(M+H)-18]^+$) and 391 ($[(M+H)-44]^+$). In the ^{13}C NMR spectrum, C-4 was observed at δ 41.2, C-3 at δ 94.5 indicating the hydration of the 3, 4 double bond of **4**. Compared with **4**, 14-H_3 (δ 1.56) resonated at higher field. These data were consistent with the presence of a hemiacetal function located at C-3. Treatment of **2** with 0.1 M methanolic HCl readily afforded anhydroexfoliamycin (**4**) which definitively confirmed the structure of exfoliamycin.

3-*O*-Methylexfoliamycin (**3**) showed UV properties identical to those of **2**. The FAB-MS of **3** revealed a *quasi*-molecular ion at *m/z* 449 ($[M+H]^+$) in agreement with the molecular formula $C_{23}H_{28}O_9$. In the ^{13}C NMR spectrum, an additional signal due to a methoxy group was observed at δ 48.9. Compared with exfoliamycin, C-3 was shifted downfield while C-14 resonated at higher field. The C/H correlations detected in a COLOC experiment and in particular a long-range coupling between the methoxy protons and C-3 fully supported the structure of **3** to be 3-*O*-methylexfoliamycin. Final evidence was obtained by acidic degradation of **3**: Anhydroexfoliamycin (**4**) was readily produced in 0.1 M methanolic HCl while formation of exfoliamycin (**2**) was observed in aq 0.1% H_3PO_4. The elucidation of the stereochemistry of **2**~**4** is under investigation.

The naphthoquinones **1**~**4** possess similar antibacterial properties. In an agar plate diffusion assay, they were able to inhibit the growth of several Gram-positive microorganisms, including *Bacillus subtilis*, *B. brevis*, *Clostridium pasteurianum* and *Micrococcus luteus* but were completely ineffective against *Escherichia coli*, *Saccharomyces cerevisiae* and *Mucor hiemalis*. A comparision of the activity of **1**~**4** against *B. subtilis* is shown in Table 3.

Exfoliamycin and its derivatives are the first naphthoquinone C-glycosides containing a pentosyl

Table 2. NMR data of compounds **2**~**5**.

No.	**2**[a]		**3**[b]		**4**[c]		**5**[b]
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_H
1	69.8 d	5.08 (m)	69.8 d	4.90 (br s)	73.3 d	5.50 (dd, 3.5, 9.0)	5.30~5.45 (m)
3	94.5 s		97.9 s		157.1 s		
3-OMe			48.9 q	3.20 (s)			
4	41.2 t	2.93 (m)	40.7 t	2.90 (s)	100.3 d	5.61 (s)	5.64 (s)
4a	144.2 s		143.1 s		140.0 s		
5	119.8 d	7.28 (s)	120.1 d	7.30 (s)	114.7 d	7.10 (s)	7.41 (s)
5a	130.6 s		129.6 s		122.4 s		
6	184.8 s		184.3 s		185.2 s		
7	135.2 d	7.22 (d, 1.6)	134.6 d	7.05 (s)	134.4 d	7.02 (s)	7.01 (d, 1.5)
8	150.6 s		148.8 s		149.9 s		
9	191.3 s		190.7 s		188.7 s		
9a	113.9 s		113.0 s		113.8 s		
10	158.5 s	12.54 (OH)	158.1 s	12.20 (OH)	158.9 s		
10a	133.8 s		133.6 s		131.7 s		
11	36.6 t	1.90~2.00 (m)	35.9 t	1.80~2.10 (m)	35.1 t	1.40~1.60, 1.95 (m)	1.40~1.80 (m)
12	18.6 t	1.20~1.50 (m)	17.9 t	1.20~1.50 (m)	18.4 t	1.27~1.60 (m)	1.27~1.60 (m)
13	14.2 q	0.88 (t, 7.4)	14.0 q	0.90 (t, 7.0)	13.9 q	0.96 (t, 7.0)	0.89 (t, 7.2)
14	29.1 q	1.56 (s)	23.1 q	1.50 (s)	20.5 q	1.95 (s)	1.90 (s)
1′	80.5 d	5.03 (dd, 3.1, 1.6)	80.3 d	4.90 (br s)	80.6 d	5.02 (br s)	5.05 (dd, 4.0, 1.5)
2′	76.9 d	4.13 (dd, 3.1, 4.8)*	75.5 d	4.05 (br s)	76.0 d	4.13 (br s)	5.23 (m)
3′	71.3 d	4.03 (dd, 7.4, 4.8)*	71.6 d	4.05 (br s)	71.1 d	3.95~4.05 (m)	5.12 (m)
4′	84.1 d	3.96 (ddd, 7.4, 4.1, 2.8)*	83.8 d	4.05 (br s)	83.3 d	3.95~4.05 (m)	4.26 (m)
5′	62.0 t	3.72 (dd, 12.2, 4.1)*, 3.88 (dd, 12.2, 7.4)*	62.4 t	3.77 (br d, 12.2), 3.93 (br d, 12.2)	62.0 t	3.75 (br d, 12.0), 3.94 (br d, 12.0)	4.15 (dd, 12.1, 3.8), 4.35 (dd, 12.1, 2.7)
OAc							2.34, 2.08, 2.07, 2.01

Attached proton test (APT) and DEPT experiments allowed distinction of carbon multiplicities; attributions based on COSY, HETCOR and COLOC data. Spectra were recorded at 100.6/400 MHz (**2**); 50.3/200 MHz (**3** and **4**); 250 MHz (**5**).

[a] In acetone-d_6.

[b] In $CDCl_3$.

[c] In CD_3OD-$CDCl_3$.

* Multiplicities after D_2O exchange.

Table 3. Antimicrobial disc-diffusion assay of naphthoquinones **1**~**4** against *Bacillus subtilis*.

(mg/ml)	Compound			
	1	**2**	**3**	**4**
1.0	20	11	10	13
0.3	18	7	—	12
0.1	14	—	—	10
0.03	10	—	—	—

Inhibition diameters (mm).
15 µl of a solution were spotted onto filter discs (6 mm diameter).
—: No inhibition zone.

residue. Naphthopyranomycin (**1**), exfoliamycin (**2**) and small amounts of anhydroexfoliamycin (**4**) are detected in the crude methanol - acetone extract. On the other hand 3-*O*-methylexfoliamycin (**3**) as well as large amounts of anhydroexfoliamycin (**4**) appear to be produced from exfoliamycin during the further work-up procedure. Similar reactions were described in the case of fusarubins[3], a series of naphthoquinones isolated from *Fusarium solani*. At the moment we do not have any explanation, why Tü 1424 produces naphthoquinones in the presence of Amberlite XAD-1180 only. The so-called supplemented fermentation seems to be a promising method to affect the secondary metabolism of talented strains and to provide new metabolites. Further studies are presently in progress.

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