Notes

SECONDARY METABOLITES BY CHEMICAL SCREENING. 15†

STRUCTURE AND ABSOLUTE CONFIGURATION OF NAPHTHOMEVALIN, A NEW DIHYDRO-NAPHTHOQUINONE ANTIBIOTIC FROM *Streptomyces* sp.

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In the course of our chemical screening we detected a new naphthoquinone, naphthomevalin, in the culture broth of a *Streptomyces* sp. (strain Gö 28) besides a number of already known metabolites[2]. After isolating from a soil sample collected in Strathgordon, Australia, the strain Gö 28 was cultivated in 1-liter flasks at 28°C for 72 hours, using malt extract 1%, glucose 0.4% and yeast 0.4% as a culture medium. The culture broth could be separated by filtration, the mycelium and culture filtrate were subsequently extracted with acetone-ethyl acetate and ethyl acetate, respectively. The isolation of naphthomevalin from the resulting raw products has been successful by using silica gel flash chromatography (chloroform-methanol (98:2), ethyl acetate-hexane (2:1)) and gel chromatography on Sephadex LH-20 (MeOH), yielding naphthomevalin as a pale yellow oil (2.5 mg/liter culture broth). Naphthomevalin weakly absorbed UV light (254 nm) on Silica gel F_{254}, and turned dark brown with vanillin-sulfuric acid. The physicochemical properties of naphthomevalin are listed in Tables 1 and 2.

Extensive NMR-investigations, especially correlation spectroscopy *via* long range couplings (COLOC) experiments acquired with variable parameters (Fig. 1) in combination with the mass spectra, were the key tools for structure elucidation and unambiguously led to the structure of **1** for naphthomevalin. **1** shows very close correspondence to the recently described antibiotic SF2415B1 (**2**)[3] in which only an additional methyl group appears at C-7.

Treatment of **1** with sodium hydroxide in methanol resulted in the epoxide **3** (87% yield), probably by an S_N2-mechanism suggesting that the chlorine at C-2 and the hydroxyl group at C-3 in **1** are highly likely to be in *trans*-configuration. MYNDERSE *et al.* has recently described **3** as the natural product A80915G[4] but unfortunately reported neither NMR data nor the optical rotation. Thus, **1** is a new but closely related member of the dihydro-naphthoquinone metabolites, which

Table 1. Physico-chemical properties of **1** and **3**.

	1	**3**
Molecular formula	$C_{25}H_{31}O_5Cl$	$C_{25}H_{30}O_5$
FAB-MS (negative ions)[a]	448 (10%), 447 (38%), 446 (33%), 445 (100%), 273 (100%)	
HREI-MS (M^+)[b]	446.1860	410.2093
$[\alpha]_D^{20}$ (*c* 0.2, $CHCl_3$)	−5.0°	+98.7°
UV λ_{max}^{MeOH} nm (ε)	202 (5,700), 289 (sh, 4,100), 299 (1,800), 360 (2,700)	201 (10,200), 253 (8,100), 368 (sh, 4,200)
$\lambda_{max}^{MeOH+NaOH}$ nm (ε)	209 (14,500), 255 (5,600), 292 (6,800), 377 (4,500)	209 (12,300), 295 (sh, 7,100), 387 (6,000)
CD $\lambda_{extreme}$ nm ($[\theta]^{20}$)	244 (+5,150), 259 (+3,550), 281 (−860), 300 (+1,350), 335 (−7,500), 380 (0), 417 (−610)	223 (−4,100), 253 (+3,200), 270 (−650), 293 (+1,000), 323 (−1,100), 361 (+2,900)
IR (KBr) cm^{-1}	1705, 1620, 1585	1695, 1640, 1620, 1590
Rf value (TLC, silica gel)[c]	0.35	0.47

[a] Percentage of abundance.
[b] Found as calcd.
[c] *n*-Hexane-ethyl acetate (5:1).

† See ref 1.

Table 2. Selected ^1H and ^{13}C NMR signals of naphthomevalin (**1**) and its epoxide **3** in $CDCl_3$ (δ in ppm relative to internal TMS).

Proton	1[a]	3[a]	J in Hz
3-OH	4.15	—	s
5-H	7.03	7.04	d (2)
6-OH	7.16	6.28	br s
7-H	6.69	6.63	d (2)
8-OH	11.94	11.82	s

Carbon	1[b]	2[c]	m
C-1	195.6	191.2	s
C-2	82.3	67.3[d]	s
C-3	84.4	67.6[d]	s
C-4	196.6	195.2	s
C-4a	134.3	134.4	s
C-5	107.3	108.0	d
C-6	163.6	163.0	s
C-7	109.2	108.8	d
C-8	164.7	164.6	s
C-8a	110.5	109.3	s

All other signals of **1** and **3** are almost identical compared with **2** and SF 2415 B2[3], respectively.

[a] 200 MHz.

[b] 50.3 MHz.

[c] 125.7 MHz. [d]Interchangeable.

m: Multiplicity assignments by attached proton test (APT).

Fig. 1. $^3J_{C,H}$ Long range couplings observed in naphthomevalin (**1**) by COLOC pulse sequences.

The shown couplings are indicating the constitution. All other $J_{C,H}$ couplings are omitted for reason of clarity.

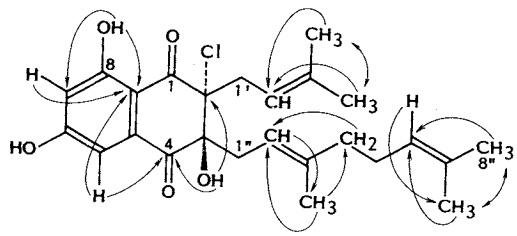
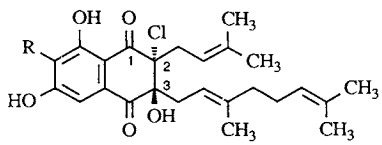


Table 3. Antimicrobial disc-diffusion assays of naphthomevalin (**1**) and **3**.

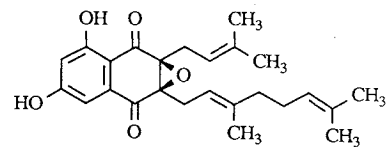
Test microorganism	Inhibition diameter (mm)					
	1 (mg/ml)			3 (mg/ml)		
	8.0	4.0	1.0	8.0	4.0	1.0
Bacillus subtilis	14	12	8	15	9	7
Escherichia coli	20	17	11	18	12	9
Mucor miehei	—	—	—	—	—	—

—: No inhibition zone.

Scheme 1.



1 R=H
2 R=CH_3



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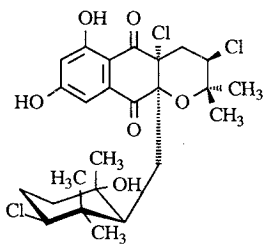

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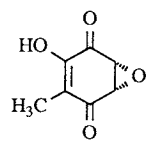

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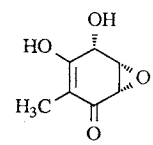

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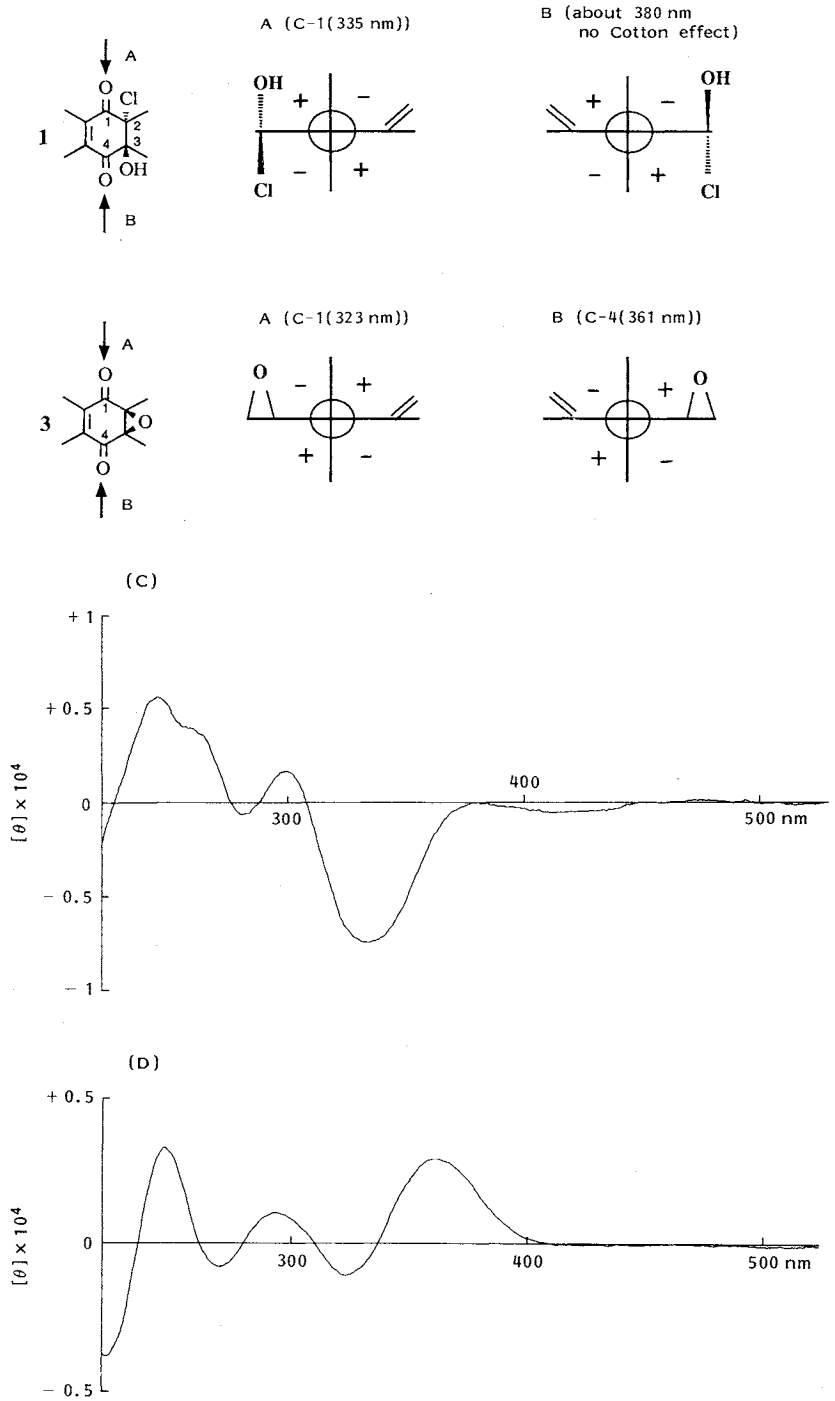
include a number of related A80915[4], SF2415[3] and napyradiomycin antibiotics[5]. All of these known compounds possess a very weak activity against Gram-positive bacteria which was also found for naphthomevalin (**1**) by disc-diffusion assays (Table 3). Despite an intensive search, none of these already known metabolites and no other analogous components could be detected in the culture broth of strain Gö 28.

Regarding the stereochemistry, only the absolute

Fig. 2. CD spectra and octant projections of **1** and **3**.

The view along both arrows A and B is symbolised by two octant projections, which connect the stereochemistry of C-2 and C-3 with the sign of Cotton effects. (C) CD curve of **1**, (D) CD curve of **3**.



configuration of napyradiomycins B2 and B4 (**4**)[5] has been determined by X-ray crystallography and this has led to the assumption that all other napyradiomycins have the same configuration. In contrast to this, the stereochemistry of the less cyclic dihydro-naphthoquinones, such as **2** and **3**, remained unsettled, obviously because most of these components have an oily consistency. Hence, the desired elucidation of the absolute configuration of **1** inevitably needed a second reliable method besides X-ray crystallography, namely the CD.

Naphthomevalin (**1**) and its epoxide **3** exhibit Cotton effects caused by their α,β-unsaturated ketone substructure, which can be interpreted by application of appropriate octant rules. The absolute configuration of the epoxide **3** could be ascertained by employing the inverse octant rule and by comparison of the CD spectrum of **3** with that of the benzoquinone epoxide (−)-terreic acid (**5**)[6~8], whose absolute stereochemistry was previously established by chemical correlation with (−)-terremutin (**6**)[9]. The CD spectrum of **3** (Fig. 2) exposes two Cotton effects for n-π^* transitions with opposite signs as found for **5** and is associated with the two individual carbonyl chromophores. The difference in their band positions was ascribed to internal hydrogen bonding to the C-1 carbonyl group[9] so that its n-π^* transition could be attributed to the Cotton effect with negative sign at 323 nm, whereas the transition of the C-4 carbonyl refers to the Cotton effect with the positive sign at 361 nm. With reference to the inverse octant rule, the resulting octant projections (Fig. 2) of both carbonyl groups undoubtly confirmed the absolute configuration of C-2 and C-3 of **3** to be (2*S*,3*R*). Additionally, compared to **3** the nearly mirror-imaged CD spectrum of **5**[9] manifests the inverse configuration of its epoxide substructure. Thus, with regard to the chemical relation between **3** and **1**, the stereochemistry of **1** at C-3 is ultimately indicated to be (3*S*).

The absolute stereochemistry of **1** at C-2 was assigned by using the α-haloketone rule[6,10]. The present α-halocycloketone chromophore, including the C-1 carbonyl group, is described to be dominant and controls the sign of the Cotton effect, which depends on the location of the chlorine inside the octant projection. The chlorine of **1** was highly likely to be in the axial position because of the voluminous C_5 chain at C-2. The expected intensive Cotton effect of the C-1 carbonyl appeared at 335 nm with a negative sign in the CD spectrum of **1** and unquestionably confirmed the (2*R*) configuration. The octant projection and the CD spectrum are shown in Fig. 2. Both independent assignments indicated the *trans*-configuration of the hetero substituents at C-2 and C-3 of **1** and were in agreement with its easy chemical conversion into the epoxide **3**.

In conclusion, we found the same stereochemistry in **1** as described for napyradiomycin B4 (**4**), which suggests **1** as a biosynthetic precursor of **4** and provides evidence to suggest a coincident pathway of their biosynthesis in the different *Streptomyces* strains.

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References

1) Hammann, P.; M. Katjär-Peredy, R. Klein, G. Kretzschmar, W. Raether, A. Kröger & F. Ditzel: Secondary matabolites by chemical screening. 14. Transformation of elaiophylin in subunits of naturally occurring acid ionophores: Synthesis, anticoccidio activity and studies concerning the ionophoric properties. Heterocycles, in preparation
2) Henkel, T.: Neue Naturstoffe aus einem modifizierten chemischen Screening und Strukturaufklärung der cytostatisch wirksamen Landomycine. Ph. D. Thesis, Univ. Göttingen, 1990
3) Gomi, S.; S. Ohuchi, T. Sasaki, J. Itoh & M. Sezaki: Studies on new antibiotics SF2415. II. The structural elucidation. J. Antibiotics 40: 740~749, 1987
4) Fukuda, D. S.; J. S. Mynderse, P. J. Baker, D. M. Berry, L. D. Boeck, R. C. Yao, F. P. Mertz, W. M. Nakatsukasa, J. Mabe, J. Ott, F. T. Counter, P. W. Ensminger, N. E. Allen, W. E. Alborn, Jr. & J. N. Hobbs, Jr.: A80915, a new antibiotic complex produced by *Streptomyces aculeolatus*. Discovery, taxonomy fermentation, isolation, characterization, and antibacterial evaluation. J. Antibiotics 43: 623~633, 1990
5) Shiomi, K.; H. Nakamura, H. Iinuma, H. Naganawa, T. Takeuchi, H. Umezawa & Y. Iitaka: New antibiotic napyradiomycins A2 and B4 and stereochemistry of napyradiomycins. J. Antibiotics 40: 1213~1219, 1987
6) Snatzke, G.: Circulardichroismus IX, Modifizierung der Oktantenregel für α,β-ungesättigte Ketone: Theorie. Tetrahedron 21: 421~438, 1965
7) Kis, Z.; A. Closse, H. Sigg, L. Hruban & G. Snatzke: Die Struktur von Panepoxydon und verwandten Pilzmetaboliten. Helv. Chim. Acta 53: 1577~1597, 1970
8) Shen, B.; Y. G. Whittle, S. J. Gould & D. A. Keszler: Structure and absolute stereochemistry of

the epoxyquinol LL-C10037α and related metabolites from *Streptomyces* LL-C10037. J. Org. Chem. 55: 4422~4426, 1990

9) MILLER, M.: The structure of terremutin. Tetrahedron 24: 4839~4851, 1968

10) DJERASSI, C. & W. KLYNE: Optical rotatory dispersion studies. X. Determination of absolute configuration of α-halocyclohexanones. J. Am. Chem. Soc. 79: 1506~1507, 1957