Paranolin: a New Xanthene-Based Metabolite from *Paraphaeosphaeria nolinae*

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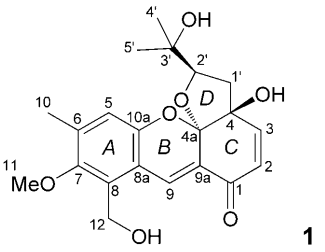
A new xanthene-based, polycyclic metabolite, paranolin (=(2*R*,3a*S*,12a*R*)-3,3a-dihydro-3a-hydroxy-8-(hydroxymethyl)-2-(1-hydroxy-1-methylethyl)-9-methoxy-10-methylfuro[3,2-*d*]xanthen-6(2*H*)-one; **1**) was isolated from a culture of *Paraphaeosphaeria nolinae* (IFB-E011), an endophytic fungus residing in the normal stem of the artemisinin-producing plant *Artemisia annua* (Asteraceae). The structure of **1** was elucidated by extensive spectroscopic analyses. Although not substantially active against the human colon (SW1116) and human cervical carcinoma (Hela) cell lines, this metabolite seems to be the first example of a *xanthene-derived* secondary metabolite. Its possible biosynthetic origin (*Scheme*) and its significance as a phyllogenetic marker are discussed in brief.

Introduction. – Endophytes, a rich source of bioactive products, are one of the hot topics of research owing to their excellent biosynthetic potential, presumably resulting from 'gene recombination' with the host [1][2]. In continuation of our characterization of chemically new and/or biologically active metabolites from the cultures of endophytic fungi harboring in *Artemisia* species [3–5], an unprecedented metabolite named paranolin (**1**) was isolated from the AcOEt extract of the culture broth of *Paraphaeosphaeria nolinae* (IFB-E011). *P. nolinae* is an endophytic fungus present in the stem of *Artemisia annua*, the producing plant of the antimalarial drug artemisinin. We, hereby, wish to describe the isolation and structure determination of this new fungal metabolite, as well as some biogenetic considerations



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Results and Discussion. – 1. *Structure Elucidation*. Paranolin (**1**) was isolated as an optically active, yellowish gum. Its molecular formula was determined as $C_{21}H_{24}O_7$ on the basis of its high-resolution ESI mass spectrum (*m/z* 389.1606 ($[M+H]^+$, $C_{21}H_{25}O_7^+$; calc. 389.1600)). The IR spectrum of **1** displayed absorption bands at 3384, 1667, and 1603 cm^{-1}, indicative of OH groups and an α,β-unsaturated keto function.

The ^{1}H-NMR spectrum of **1** (*Table*) revealed the presence of three OH groups (δ(H) 3.14 (br. *s*, 1 H), 4.63 (br. *t*, 1 H), 5.07 (br. *s*, 1 H)), three Me groups (δ(H) 1.09, 1.10, 2.30 (3*s*, 3×3 H)), one MeO function (δ(H) 3.76 (*s*, 3 H)), two CH_2 moieties (δ(H) 4.86 (*d*, 2 H), 2.35 (*dd*, 1 H), 2.74 (*t*, 1 H)), one CH group (δ(H) 3.77 (*dd*, 1 H)), and four aromatic/olefinic H-atoms (δ(H) 6.23 (*d*, 1 H), 6.92 (*d*, 1 H), 6.82 (*s*, 1 H), and 8.09 (*s*, 1 H)).

Table. *^{13}C- and ^{1}H-NMR Data for* **1**. At 125 (^{13}C) and 500 MHz (^{1}H), resp., in (D_6)acetone; δ in ppm, *J* in Hz. Arbitrary atom numbering.

Position	δ(C)	δ(H)	Position	δ(C)	δ(H)
1	185.0 (*s*)	–	9a	124.0 (*s*)	–
2	130.8 (*d*)	6.23 (*d*, *J* = 10.2)	4a	102.4 (*s*)	–
3	148.1 (*d*)	6.92 (*d*, *J* = 10.2)	10a	149.3 (*s*)	–
4	76.9 (*s*)	–	8a	133.2 (*s*)	–
5	119.2 (*d*)	6.82 (*s*)	1′	38.4 (*t*)	2.35 (*dd*, *J* = 6.0, 11.8, H_α), 2.74 (*t*, *J* = 11.8, H_β)
6	136.7 (*s*)	–	2′	85.1 (*d*)	3.77 (*dd*, *J* = 6.0, 11.8)
7	152.5 (*s*)	–	3′	71.2 (*s*)	–
8	118.2 (*s*)	–	4′	25.8 (*q*)	1.10 (*s*)
9	130.1 (*d*)	8.09 (*s*)	5′	25.5 (*q*)	1.09 (*s*)
10	16.6 (*q*)	2.30 (*s*)	3′-OH	–	3.14 (br. *s*)
11	62.2 (*q*)	3.76 (*s*)	4-OH	–	5.07 (br. *s*)
12	55.5 (*t*)	4.86 (*d*, *J* = 5.1)	12-OH	–	4.63 (br. *t*, *J* = 5.1)

All ^{1}H- and ^{13}C-NMR signals could be unequivocally assigned (*Table*) by a set of 2D-NMR correlation experiments including HMQC, HMBC (*Fig. 1*), COSY, and NOESY (*Fig. 2*) techniques. These experiments indicated the presence of the following four structural fragments: *1*) a trisubstituted vinyl group, *2*) a β-monosubstituted α,β-unsaturated ketone, *3*) an isoprenyl-derived five-carbon moiety with an OH group geminal to the two terminal Me groups, and *4*) a pentasubstituted phenyl nucleus (ring *A*) carrying a Me, a CH_2OH, and a MeO unit. Obviously, these fragments consumed seven degrees of unsaturation.

Owing to the absence of additional unsaturated bonds, the three remaining degrees of unsaturation had to be allotted to three more rings. Accordingly, rings *C* and *D* were proposed, supported by clear HMBC correlations between C(4)[1]) at δ(C) 76.9 and H–C(2′) at δ(H) 3.77, H–C(2) at 6.23, and 4-OH at 5.07, respectively, and between C(4a) at δ(C) 102.4 and H–C(1′) at δ(H) 2.35, H–C(2′) at 3.77, H–C(3) at 6.92, and H–C(9) at 8.09, respectively (*Fig. 1*). The deshielded phenyl resonance at δ(C) 149.3 (C(10a)) and an acetal-type quaternary C-atom resonance at δ(C) 102.4 (C(4a)) could only be

[1]) Arbitrary C-atom numbering. For systematic names, see *Exper. Part.*

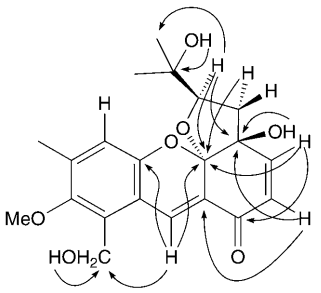


Fig. 1. *Key HMBC correlations for* **1**

rationalized by assuming the presence of ring *B* in **1**. This proposal was corroborated by the observed HMBC correlation between H–C(9) and both C(4a) and C(10a). It is noteworthy that the C=O group exerts a substantial paramagnetic effect on the neighboring H–C(9) H-atom, which resonates at δ(H) 8.09. In the NOESY spectrum of **1** (*Fig. 2*), H–C(9) showed an NOE correlation with the CH_2OH group at C(8).

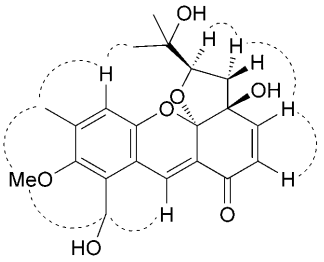


Fig. 2. *Key NOE correlations for* **1**

The proposed substitution pattern of the Ph ring was substantiated by NOE correlations between the 7-MeO group and both the CH_2OH and Me moieties at C(8) and C(6), respectively, the latter, in turn, correlating with H–C(5) at δ(H) 6.82. Therefore, the constitutional formula of **1** was concluded to correspond to 3,3a-dihydro-3a-hydroxy-8-(hydroxymethyl)-2-(1-hydroxy-1-methylethyl)-9-methoxy-10-methylfuro[3,2-*d*]xanthen-6(2*H*)-one.

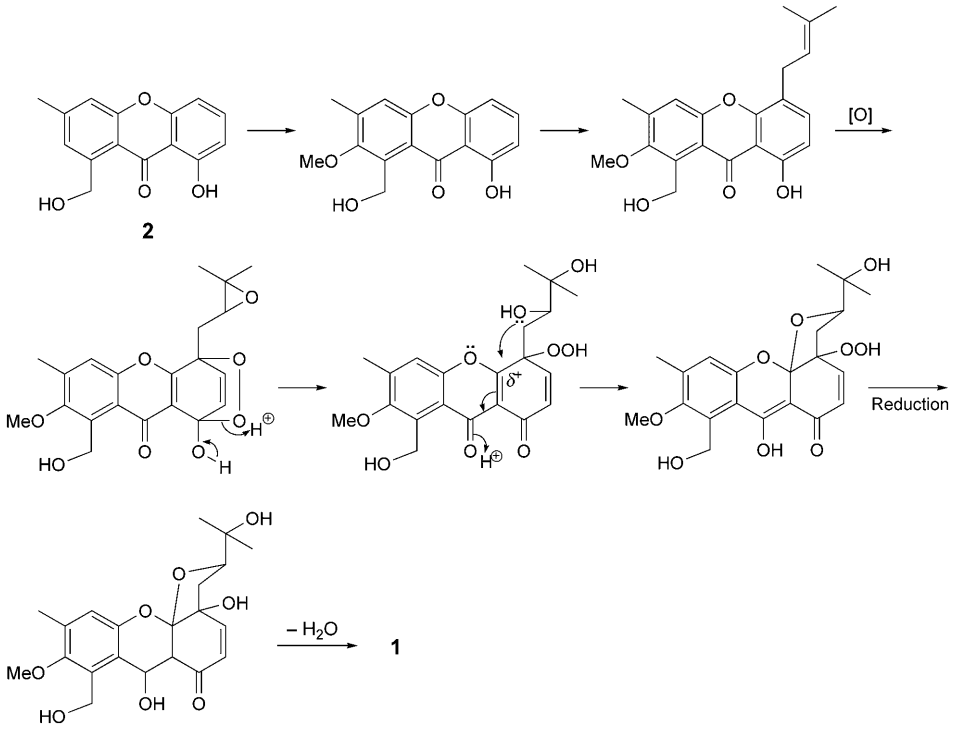
The absolute configurations at the three stereogenic centers of **1** were determined by circular-dichroism (CD) spectroscopy (in MeOH). The observed positive *Cotton* effect at 235 nm arising from the $\pi \rightarrow \pi^*$ transition of the α,β-unsaturated ketone connected to an exocyclic vinyl group (C(9)=C(9a)) was comparable to those reported for calycopterone and its demethoxy derivative [6]. Thus, C(4) and C(4a) were assigned the absolute (*S*)- and (*R*)-configurations, respectively. In the NOESY spectrum of **1**, clear correlations of H_α–C(1′) with both H–C(3) and H–C(2′) required the (*R*)-configuration at C(2′), as further substantiated by a NOESY cross-peak between H–C(5) and H–C(5′).

From the above data, compound **1** was, thus, identified as (2*R*,3a*S*,12a*R*)-3,3a-dihydro-3a-hydroxy-8-(hydroxymethyl)-2-(1-hydroxy-1-methylethyl)-9-methoxy-10-methylfuro[3,2-*d*]xanthen-6(2*H*)-one, and named paranolin. A cytotoxicity assay showed that this metabolite is not substantially active against the human colon (SW1116) and human cervical carcinoma (Hela) cell lines (IC_{50} values > 50 μg/ml).

2. *Biosynthetic Considerations.* Paranolin (**1**) could originate from the precursor 1-hydroxy-6-methyl-8-hydroxymethylxanthone (**2**), a fungal metabolite of *Cyathus intermedius* [7] *via* a set of sequential biotransformations (*Scheme*). Xanthones, not yet detected in any bacterial culture, have been characterized both as phytochemicals [8][9] and as fungal metabolites [10][11]. Thereby, a striking structural discrepancy between plant- and fungus-generated xanthones lies in their basic *vs.* methylated xanthane skeletons, respectively.

Interestingly, compound **1** has a dimethylated xanthane framework encountered before only in the secondary metabolites of *Ascodesmis sphaerospora* [12], *Cyathus intermedius* [13], and *Phomopsis* species [14]. Also, the xanthene-derived framework of paranolin (**1**) is unique in that it is presumably biosynthesized *via* reduction of the central C=O group of the basic xanthone skeleton, followed by elimination of the carbonyl O-atom to form the exocyclic C(9)=C(9a) bond. Thus, compound **1** could serve as a useful phyllogenetic marker.

Scheme. *Plausible Biosynthetic Pathway for Paranolin* (**1**)



Experimental Part

General. Silica gel (200–300 mesh) for column chromatography (CC) was purchased from *Qingdao Marine Chemical Factory*, China, and *Sephadex LH-20* gel was obtained from *Pharmacia Biotech*, Sweden. All chemicals used were of anal. grade. UV Spectra: *Hitachi U-3000* spectrophotometer; λ_{max} (log ε)

in nm. CD Spectra: *Jasco J-20C* automatic polarimeter; λ_{max} ($\Delta\varepsilon$) in nm. IR Spectra: *Nexus 870 FT-IR* spectrometer; in cm^{-1}. NMR Spectra: *Bruker DRX-500* spectrometer; δ in ppm rel. to Me_4Si, *J* in Hz. HR-ESI-MS: *Mariner 5304* mass spectrometer; in *m*/*z*.

Fungal Material. *Paraphaeosphaeria nolinae* (IFB-E011) was isolated from fresh stems of apparently healthy *Artemisia annua* collected in May 1997 in the suburb of Nanjing, China [15]. The seeds of a five-day-old fungal culture were cultivated for 14 d at 28° in a 200-l fermentor at 150 r.p.m. in *Charles Medium* containing (in g/l): sucrose (30.0), $NaNO_3$ (3.0), KCl (0.5), $MgSO_4 \cdot 7\,H_2O$ (0.5), $FeSO_4$ (0.01), K_2HPO_4 (1.0), and yeast extract (1.0).

Extraction and Isolation. The broth filtrate (total 80 l) was extracted with AcOEt. Evaporation of the solvent under reduced pressure gave a residue (9 g), which was purified by CC (SiO_2; column size: 50×4 cm; $CHCl_3$/MeOH 1:0 → 0:1): six fractions (*Fr. 1–6*) of 2.1, 0.5, 1.1, 0.9, 1.9, and 2.2 g, resp. *Fr. 3* was further purified by CC (SiO_2; column size 40×2.2 cm; petroleum ether (PE)/acetone 6:1, 3:1, 2:1 (500 ml each)): three subfractions (*Fr. 3.1–3.3*) of 0.5, 0.2, and 0.4 g, resp. Finally, gel filtration of *Fr. 3.2* (*Sephadex LH-20*; column size: 50×2.8 cm; MeOH) afforded compound **1** (30 mg).

Paranolin (= *(2*R*,3a*S*,12a*R*)-3,3a-Dihydro-3a-hydroxy-8-(hydroxymethyl)-2-(1-hydroxy-1-methylethyl)-9-methoxy-10-methylfuro[3,2-*d*]xanthen-6(2*H*)-one*; **1**). Yellowish gum. M.p. 114–116°. UV (MeOH): 315 (3.82), 370 (2.91). $[\alpha]_D^{20} = +77.3$ ($c = 0.065$, MeOH). CD ($c = 0.025$ mg/ml, MeOH): 241 (+0.113). IR (KBr): 3384, 2971, 2928, 2851, 1667, 1603, 1555, 1468, 1379, 1248. ^{1}H- and ^{13}C-NMR: see *Table*. ^{1}H- and ^{13}C-NMR data: see *Table*. HR-ESI-MS: 389.1606 ($[M+H]^+$; $C_{21}H_{25}O_7^+$; calc. 389.1600).

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