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A Biaryl Xanthone Derivative Having Axial Chirality from *Penicillium vinaceum*

Tomáš Řezanka,*,† Pavel Řezanka,‡ and Karel Sigler†

Institute of Microbiology, Vídeňská 1083, Prague 142 20, Czech Republic, and Department of Analytical Chemistry, Institute of Chemical Technology Prague, Technická 5, Prague 166 28, Czech Republic

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A new xanthone derivative having axial chirality was isolated from *Penicillium vinaceum*. Owing to the axial chirality, its structure, including absolute configuration, was determined by means of extensive spectroscopic data, such as UV, IR, MS, and 1D and 2D NMR spectra, and computational chiroptical methods. The new compound, (a*R*)-2′-methoxyvinaxanthone, has a structure containing two aromatic moieties with substituents hindering rotation about the biaryl axis. The compound gave positive results in a sea urchin egg test (*Paracentrotus lividus*) and a crown gall tumor on potato disks test (*Agrobacterium tumefaciens*).

The fungal kingdom includes many species with unique and unusual biochemical pathways. The products of these pathways include important pharmaceuticals or toxins. The most frequent producers of low molecular weight metabolites are the genera *Aspergillus* and *Penicillium*.[1–3]

Xanthones are secondary metabolites with interesting pharmacological properties.[4] Many are known not only as monomers but also as dimers, e.g., secalonic acids,[5,6] dicerandrols A, B, and C,[7] and phomoxanthones A and B.[8,9] Other isolated compounds include neosartorin,[10] rugulotrosins A and B,[11] and xanthonol.[12] Structurally very similar compounds were isolated from *Penicillum vinaceum*, which was found to contain a novel phospholipase C inhibitor, vinaxanthone, a polycyclic xanthone with polyacidic functional groups.[13]

Xanthofulvin and vinaxanthone were also isolated from a different strain of *Penicillium*.[14] A similar derivative, chaetocyclinone C, differing only in the substitution of aromatic rings was isolated from a culture of *Chaetomium* sp.[15]

This report is part of our investigation of compounds having axial chirality[16,17] within the framework of a comprehensive program on the chemistry and biotoxicity of natural compounds. We now report the identification of (a*R*)-2′-methoxyvinaxanthone, a dimeric xanthone having axial chirality.

Results and Discussion

P. vinaceum was cultivated at 25 °C in shaking flasks for 7 days, using a malt extract/glucose/yeast extract medium. The cultured broth was harvested and centrifuged. Both the cell cake and aqueous solution were independently extracted with acidic EtOAc, and both extracts were combined and evaporated to yield 2.41 g of a yellow, oily material. The oil was separated on Sephadex LH-20, and the fractions were further purified by RP-HPLC to give three compounds, **1** (13.6 mg), **2** (6.7 mg), and **3** (8.2 mg), as yellow powders. The compounds were identified by their IR, UV, CD, MS, and ^{1}H and ^{13}C NMR spectroscopic data.

The physicochemical properties of **1**, **2**, and **3** are summarized below. Similarity in these data suggested that they were structurally related. Compounds **2** and **3** were identified as the known compounds vinaxanthone[13,14] (or 411F)[18] and 411P,[18] respectively, by their UV, IR, MS, and ^{1}H and ^{13}C NMR data. The experiments supported the identity of **2** and **3** and were useful in determining the structure of **1**.

The molecular formula of **1** was established as $C_{29}H_{18}O_{15}$ by HRFAB-MS, differing from that of **2** and/or **3** by the addition of 30 amu (CH_2O) or 32 amu (CH_4O), respectively. The IR spectrum displays absorption bands characteristic for α,β-unsaturated ketone (1650 cm^{-1}) and carboxylic groups (1730 cm^{-1}), as in the case of **2** and **3**. Further, the UV spectrum and IR absorption bands (1620 and 1570 cm^{-1} for γ-pyrone) suggested the presence of a xanthone chromophore.

The ^{1}H NMR spectrum of **1** recorded in DMSO-d_6 exhibited six exchangeable, three aromatic, and three methyl hydrogen atoms, which were all observed as singlets. By contrast, a large number of carbon signals (29) were observed in the ^{13}C NMR spectrum of **1**.

Since additional spectroscopic analyses of **1** did not provide further structural information, 2D NMR experiments were carried out on permethylated derivative **1a**, which was prepared by the treatment of **1** with a large excess of diazomethane.

Six methoxy signals were observed in the ^{1}H NMR spectrum of **1a**. Four of these (δ 3.93, 4.03, 3.86, and 4.00) showed long-range CH couplings with aromatic carbons (δ 141.5, 153.6, 140.6, and 154.6, respectively), and two (δ 3.98 and 4.08) showed couplings with carbonyl carbons (δ 167.7 and 167.6). In addition, two methyl signals (δ 2.50 and 2.53) showed long-range CH couplings with ketone carbonyl carbons (δ 199.5 and 201.5). These results suggested the presence of four phenolic hydroxy, two carboxylic, and two acetyl groups in **1**.

The ^{1}H and ^{13}C NMR data of **1a** were very similar to those of permethylated vinaxanthone,[13] except that **1a** showed a signal for an additional *O*-methyl (δ_H 3.71, δ_C 55.6) in place of one of the aromatic methine protons observed in permethylated vinaxanthone. The analysis by 2D NMR, i.e., HMBC, determined the position of the *O*-methyl group at $C_{2'}$ and the structure was therefore established as **1a**; see also Figure 1.

Circular dichroism investigations were carried out to further confirm the absolute axial configuration of (a*R*)-2′-methoxyvinaxanthone (**1**). Because **1** constitutes the first 2,10-coupled chromone−xanthone dimer, an empirical comparison of the CD spectrum of **1** with that of configurationally known similar compounds was not possible. Dioxane was used as a solvent since the low concentration of the compound in MeOH did not provide a significant CD spectrum.

Two compounds may be regarded as models: the structurally similar chaetocyclinone C (**4**)[15] and the more distantly related rugulotrosin A.[19] The spatial arrangement of atoms has been determined in both compounds by X-ray analysis. As found for chaetocyclinone C, the two aromatic moieties are not in the same plane and the angle between them is $\theta = 117.1°$. However, further information on this compound states that "it exhibits no optical rotation and its CD spectrum is inconspicuous. Even if the rotation

* To whom correspondence should be addressed. Tel: +420 241 062 300. Fax: +420 241 062 347. E-mail: rezanka@biomed.cas.cz.
† Institute of Microbiology.
‡ Institute of Chemical Technology Prague.

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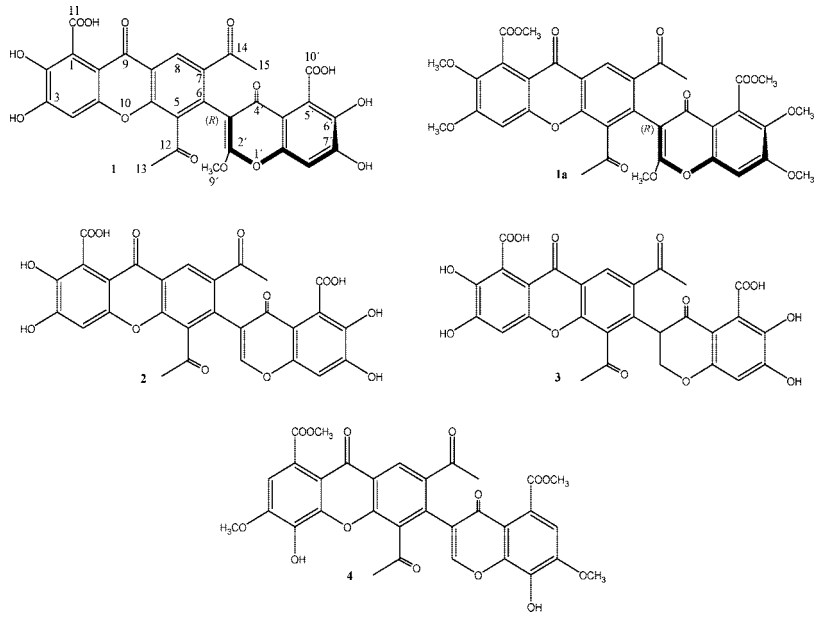

Figure 1. Structures of new (**1, 1a**) and known compounds (**2**–**4**).

between C-9 and C-11 were sterically hindered as in other similar compounds, no atropisomerism could be detected".[15]

The rotational barrier of biaryl derivatives depends on the nature, position, and number of substituents. The unsubstituted biphenyl has a rotational barrier of approximately 8.4 kJ/mol (2 kcal/mol). Increasing steric bulk of substituents in the *o*-positions causes increasing conformational stability as a result of steric interactions in the coplanar transition state. The majority of tetra-*o*-substituted biaryls have a rotational barrier sufficiently high to prevent racemization of the atropisomers at room temperature. The separation of stereoisomers at room temperature requires energy barriers of at least 92.1 kJ/mol (22 kcal/mol).[20] For this reason we computed (B3LYP functional and 6-31G* basis set, see below) the rotational barrier of our biaryl derivative (**1**) and compound **4** (chaetocyclinone C).[15] The rotational barrier for (a*R*)-2′-methoxyvinaxanthone (**1**) is 103.5 kJ/mol, and that for chaetocyclinone C (**4**) 82.1 kJ/mol. This clearly shows that at room temperature compound **1** cannot isomerize from *M* to *P* atropisomer and vice versa, whereas in compound **4** this isomerization is feasible. This explains why chaetocyclinone C was found to exhibit no optical rotation and its CD spectrum was inconspicuous.[15]

Rugulotrosin A has also been described to "lie on a 2-fold axis of symmetry, and the biaryl linkage is essentially orthogonal with a dihedral angle (C1−C2−C2′−C1′) of 87.4°. Although the molecule is chiral, the X-ray crystallographic analysis was unable to assign absolute configuration".[19]

Therefore, to elucidate the absolute configuration of **1**, a quantum mechanical calculation[21] of CD was carried out with Gaussian 03 software[22] using density functional theory with the B3LYP functional and 6-31G* basis set. The conformer used for CD calculation was the minimum-energy conformer. A comparison of theoretically predicted data with the corresponding experimental data has allowed us to elucidate the absolute configuration of **1**. Calculated and experimental spectra (Figure 2) are in good agreement. Hence the absolute configuration of **1** is *M* (i.e., *R*); the angle between the chromone−xanthone moieties is $\theta = 65.4°$, a value very similar to that measured in chaetocyclinone C (180° – 117.1° = 62.9°). A negative Cotton effect (Figure 2, negative exciton couplet caused by two substituted aryl chromophores) confirmed the structure of the *M*-isomer.[23–25]

In the standard agar plate disk diffusion assay of antibacterial and antifungal activity (*Bacillus subtilis*, *Staphyloccocus aureus*, *Escherichia coli*, *Saccharomyces cerevisiae*)[26–30] the compounds

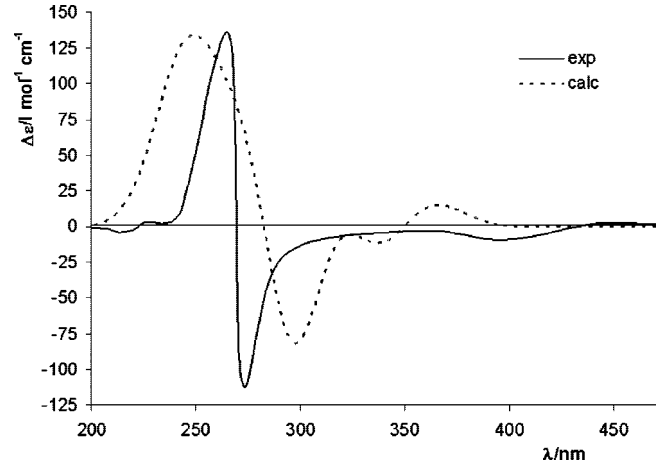

Figure 2. B3LYP/6-31G*-calculated CD spectrum for (a*R*)-**1** and experimental CD spectrum of **1**.

1–**3** showed neither antibacterial nor antifungal activity, but they gave positive results in a sea urchin egg test (*Paracentrotus lividus*) and a crown gall tumor potato disk test (*Agrobacterium tumefaciens*).

The antiproliferative, antimitotic, and cytotoxic effects of compounds **1**–**3** were evaluated by the sea urchin egg test using eggs and sperm from sea urchin (*P. lividus*) gonads. Egg fertilization and cleavage were determined according to Biyiti[31] as modified by Prokofeva.[32] The crown gall tumor bioassay used to test the compounds for antitumor activity was performed essentially according to Galsky.[33]

Compounds **1**–**3** showed significant inhibition of the growth of crown gall tumors on potato disks, suggestive of in vivo antitumor activity (Table 2). They also exhibited strong antitumor activity with *P. lividus*. As has been proposed,[34] xanthones, namely, 5,6-dimethylxanthenone-4-acetic acid, may have an allelopathic role, and their potent cytotoxicity may play a role in cancer therapy.

Experimental Section

General Experimental Procedures. UV–vis spectra were measured in MeOH within the range 220 to 550 nm in a Cary 118 (Varian) apparatus. A Perkin-Elmer (Perkin-Elmer, Norwalk, CT) model 1310 IR spectrophotometer was used for scanning IR spectroscopy as neat

Table 1. ^{1}H and ^{13}C NMR Data (measured in DMSO-d_6) of (a*R*)-2′-Methoxyvinaxanthone (**1**) and Its Permethylated Derivative (**1a**)

no.	^{1}H NMR of **1**	^{1}H NMR of **1a**	^{13}C NMR of **1**	^{13}C NMR of **1a**
1			119.9 s	119.9 s
2			140.6 s	140.6 s
2-OMe		3.86 (3H, s)		61.3 q
3			154.6 s	154.6 s
3-OMe		4.00 (3H, s)		56.1 q
4	6.93 (1H, s)	6.92 (1H, s)	102.1 d	102.1 d
5			133.7 s	133.7 s
6			131.7 s	131.7 s
7			135.8 s	135.8 s
8	8.64 (1H, s)	8.51 (1, Hs)	126.9 d	126.9 d
8a			120.4 s	120.4 s
9			172.6 s	172.6 s
9a			109.7 s	109.7 s
10a			152.7 s	152.7 s
11			167.6 s	167.6 s
11-COOMe		4.08 (3H, s)		51.5 q
12			201.5 s	201.5 s
13	2.53 (3H, s)	2.53 (3H, s)	32.6 q	32.6 q
14			199.5 s	199.5 s
15	2.50 (3H, s)	2.50 (3Hs)	29.7 q	29.7 q
2′			163.4 s	163.4 s
3′			117.7 s	117.7 s
4′			175.2 s	175.2 s
4a			150.0 s	150.0 s
4a′			111.9 s	111.9 s
5′			119.8 s	119.8 s
6′			141.5 s	141.5 s
6′-OMe		3.93 (3H, s)		61.3 q
7′			153.6 s	153.6 s
7′-OMe		4.03 (3H, s)		56.1 q
8′	6.96 (1H, s)	6.93 (1H, s)	102.5 d	102.5 d
8a′			150.7 s	150.7 s
9′	3.72 (3H, s)	3.71 (3H, s)	55.6 q	55.6 q
10′			167.7 s	167.7 s
10′-COOMe		3.98 (3H, s)		51.5 q

films. Optical rotations were recorded with a Perkin-Elmer 243 B polarimeter. Circular dichroism (CD) measurement was carried out under dry N_2 on a Jasco-500A spectropolarimeter at 24 °C. NMR spectra were recorded on a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 MHz (^{1}H) and 125.7 MHz (^{13}C). HRMS were recorded using a VG 7070E-HF spectrometer (70 eV). HRFABMS (positive ion mode) were obtained with a PEG-400 matrix.

Fermentation. *Penicillium vinaceum* from the Collection of Fungal Cultures (CFC), Department of Botany, Faculty of Science, Charles University, Prague, was grown on agar plates of medium (see below, 20 g/L agar was added for agar plates) at 25 °C for 7 days. A 1 cm^2 piece of agar from a 7-day-old culture was used to inoculate 100 mL of medium (malt extract 20 g/L, glucose 10 g/L, yeast extract 2 g/L, $(NH_4)_2HPO_4$ 0.5 mg/L, pH = 6.0 prior to sterilization) in 250 mL Erlenmeyer flasks with wall pits serving as baffles. These cultures were incubated on a rotary shaker (170 rpm, 25 °C) for 96 h.

Isolation. The cultured broth (4 L) was harvested on day 7 and centrifuged at 5000*g* for 10 min at 4 °C. The cell cake and also aqueous solution were independently extracted with 3 L of formic acid−EtOAc (1:99), and both acidic EtOAc extracts were combined and evaporated under reduced pressure to yield 2.41 g of oily material. The oil was further chromatographed by means of Sephadex LH-20 columns with $CHCl_3$−MeOH (1:)2 and then separated by RP-HPLC on a Discovery C18 column (Supelco), particle size 5 mm, length × i.d. (250 mm × 21.2 mm), using a linear gradient from 25% H_2O and 75% MeCN to 75% H_2O and 25% MeCN over 1 h, with a flow rate of 9.9 mL min^{-1}, and monitored by a variable-wavelength detector at 254 nm to give compound **1** (13.6 mg), compound **2** (6.7 mg), and compound **3** (8.2 mg) as yellow powders.

(a*R*)-2′-Methoxyvinaxanthone (1): pale yellow powder; $[\alpha]^{25}_D$ +210 (*c* 0.07, MeOH); IR (film) ν 3440, 2950, 1730, 1705, 1655, 1600 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 210 (4.35), 255 (4.25), 265 (4.10), 300 (3.85) nm; ^{1}H (500.1 MHz, DMSO-d_6) and ^{13}C (125.7 MHz, DMSO-d_6) NMR, see Table 1; HRFABMS (*m*/*z*) 629.0547 [M + Na]$^+$, calcd for $[C_{29}H_{18}O_{15}Na]^+$ 629.0543; CD (*c*, 0.08 dioxan) λ ($\Delta\epsilon$) 470 (1.8), 440 (1.7), 398 (−9.5), 355 (−3.1), 292 (−20.3), 272 (−108.5), 266 (133.7), 242 (8.7), 226 (3.3), 220 (−2.4), 213 (−4.6), 208 (−2.1), 200 (−1.4) nm.

Permethylated (a*R*)-2′-methoxyvinaxanthone (1a): pale yellow powder prepared from **1** and a large excess of diazomethane; $[\alpha]^{25}_D$ +238 (*c* 0.05, MeOH); IR (film) ν 3440, 2955, 1733, 1704, 1657, 1603 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 212 (4.30), 254 (4.22), 263 (4.07), 302 (3.83) nm; ^{1}H (500.1 MHz, DMSO-d_6) and ^{13}C (125.7 MHz, DMSO-d_6) NMR, see Table 1; HRFABMS (*m*/*z*) 713.1486 [M + Na]$^+$, calcd for $[C_{29}H_{18}O_{15}Na]^+$ 713.1482.

Known compounds **2** and **3** were identified by comparison of their spectroscopic data with literature data; see above.

Computations. The calculated CD intensity presented in Figure 2 was scaled up by a factor of 3. Gaussian band shapes and 20 nm half-width (at 1/e of peak height) were used to simulate the predicted spectrum.

Biological Activity Tests. For disk diffusion tests, bacterial suspensions of suitable OD obtained by 2–5 h incubation were diluted with H_2O and streaked evenly onto the surface of agar in Petri dishes with a cotton swab. After the inoculum had dried, disks containing 10, 50, 100, and 200 μg of compounds **1**–**3** were placed on the agar and the plates were incubated overnight.

With *Saccharomyces cerevisiae* a suitably diluted yeast inoculum suspension was used to inoculate likewise 90 mm diameter Petri dishes containing agar supplemented with 2% glucose. The plates were allowed to dry for 5–15 min, test disks containing again 10, 50, 100, and 200 μg of compounds **1**–**3** were placed on top of the agar, and the plates were incubated for 18–24 h at 28 °C. The diameters of zones around the disks were measured.

For the sea urchin egg test, the eggs were rinsed, filtered, and diluted by artificial seawater (ASW) to a concentration of 2000 eggs/mL. Sperm was collected "dry", and the semen was diluted (1:50) in ASW shortly before use. Fertilization was obtained by adding 10 μL of sperm to 1 mL of egg suspension at 20 °C. The eggs were then cultured at room temperature under gentle agitation, and the percentage of divided eggs was determined by light microscopy 30 min after fertilization. The compounds were used dissolved in DMSO at 50 μg/mL; as shown by compound-free DMSO-containing controls, the DMSO used in the experiments did not affect cell division.

Potato tubers were surface sterilized by immersing in 1% NaOCl for 20 min. Potato disks (5 mm thick) were made with cork borer and placed on 2% agar plates (10 disks per plate). A 0.5 mL volume of a 48 h culture of *Agrobacterium tumefaciens* mixed with 10 or 100 μg/mL of DMSO solution (0.1 mL) of the given compound was applied on the surface of each disk, and the Petri dishes were incubated at 28 °C for 21 days. The crown galls on the potato tuber disks were observed by the naked eye. Numbers of tumors per disk were counted, and percent inhibition for each concentration was determined as % inhibition = 100 – (average no. of tumors of sample/ditto for the control) × 100.

Table 2. Tumor Inhibition and Sea Urchin (*P. lividus*) Egg Test of Compounds **1**–**3**

compound	crown gall tumor 10 ppm (inhibition in %)[a]	crown gall tumor 100 ppm (inhibition in %)[a]	*Paracentrotus lividus*[b]
1	72 ± 6	100	0.05 ± 0.008
2	67 ± 5	100	0.12 ± 0.043
3	12 ± 3	88 ± 7	0.71 ± 0.14
DMSO	0	0	0

[a] Value ± SD, n = 10. [b] IC_{50} ± SD, n = 10; the compounds were used dissolved in DMSO at 50 μg/mL.

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