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## **Xanthones from a microfungus of the genus *Xylaria***

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## Abstract

Chemical investigations of a microfungus *Xylaria* sp. isolated from the Australian rainforest tree *Glochidion ferdinandi* have afforded two new natural products, 2-hydroxy-6-methyl-8-methoxy-9-oxo-9*H*-xanthene-1-carboxylic acid (**1**) and 2-hydroxy-6-hydroxymethyl-8-methoxy-9-oxo-9*H*-xanthene-1-carboxylic acid (**2**). Compound **1** has previously been synthesised but only partially characterised. Methylation of **1** using diazomethane afforded the crystalline compound 2,8-dimethoxy-6-methyl-9-oxo-9*H*-xanthene-1-carboxylic acid methyl ester (**3**), whose structure was determined by single crystal X-ray analysis. This paper reports the full spectroscopic characterisation of compounds **1-3** by NMR, UV, IR and MS data. All compounds were inactive in a brine shrimp lethality assay and several antimicrobial screens.

## Keywords

Microfungus; *Xylaria* sp.; Rainforest tree; *Glochidion ferdinandi*; Natural products; Secondary metabolites; Xanthone

## 1. Introduction

Xanthenes are a class of natural products that have been shown to display a wide range of pharmacological properties (Peres and Nagem, 1996; Peres *et al.*, 2000). Reported biological activities included anticancer (Ho *et al.*, 2002), antifungal (Rocha *et al.*, 1994), antioxidant (Minami *et al.*, 1994) antimicrobial (Malet-Cascon *et al.*, 2003), antiinflammatory (Lin *et al.*, 1996) and antiviral (Groveiss *et al.*, 2000). The majority of xanthenes reported in the literature have been isolated from higher plants, especially those belonging to the families Gentianaceae and Clusiaceae (Peres and Nagem, 1996; Peres *et al.*, 2000). However, fungi and lichens have also been sources of this class of secondary metabolite (Huneck, 2001; Schulz *et al.*, 2002). Microfungi belonging to the genus *Xylaria* have been previously investigated for their chemistry and have proven to be a good source of bioactive compounds. Examples include the chemokine receptor (CCR5) antagonist 19,20-epoxycytochalsin Q (Jayasuriya *et al.*, 2004), the antifungal metabolites multiplolides A and B (Boonphong *et al.*, 2001) and the NPY Y5 receptor antagonists xylarenals A and B (Smith *et al.*, 2002). We have recently embarked on a research program looking for new chemistry and bioactive metabolites from microfungi isolated from Australian endemic plants. Examination of a local rainforest tree, *Glochidion ferdinandi* (family Euphorbiaceae) afforded several microfungal strains, one of which was identified as *Xylaria* sp. This strain was fermented on solid media and chemical investigations of the resulting culture have resulted in the isolation of two new natural product xanthenes, 2-hydroxy-6-methyl-8-methoxy-9-oxo-9*H*-xanthene-1-

carboxylic acid (**1**) and 2-hydroxy-6-hydroxymethyl-8-methoxy-9-oxo-9*H*-xanthene-1-carboxylic acid (**2**).

## 2. Results and Discussion.

The fungus *Xylaria* sp. (FRR 5657) was grown on damp white rice under static conditions then extracted with EtOAc. This extract was separated by C18 flash column chromatography using H<sub>2</sub>O and increasing amounts of MeOH and yielded pure 2-hydroxy-6-methyl-8-methoxy-9-oxo-9*H*-xanthene-1-carboxylic acid (**1**, 37 mg) following precipitation from the 60% MeOH/40% H<sub>2</sub>O elution. An early eluting fraction from the flash column was subjected to C18 preparative HPLC (MeOH/H<sub>2</sub>O) followed by gel permeation chromatography using Sephadex LH-20 (MeOH) to afford pure 2-hydroxy-6-hydroxymethyl-8-methoxy-9-oxo-9*H*-xanthene-1-carboxylic acid (**2**, 4.3 mg).

The major metabolite, 2-hydroxy-6-methyl-8-methoxy-9-oxo-9*H*-xanthene-1-carboxylic acid (**1**) was isolated as a pale yellow amorphous solid. A pseudomolecular ion in the (-)-HRESIMS at *m/z* 299.0555 allowed a molecular formula of C<sub>16</sub>H<sub>12</sub>O<sub>6</sub> to be assigned to **1**. Broad IR absorptions at 3500-3000 and 1625 cm<sup>-1</sup> indicated the presence of hydroxyl and carbonyl groups, respectively. The presence of a phenol within compound **1** was established based on the UV spectrum, which underwent a bathochromic shift on addition of base. The <sup>1</sup>H NMR spectrum (Table 1) of **1** contained two exchangeable singlets [δ 12.49 (1H) and 9.96 (1H)], two mutually-coupled aromatic doublets [δ 7.43 (d, *J* = 9.0 Hz, 1H) and 7.29 (d, *J* = 9.0 Hz, 1H)], two aromatic singlets [δ 6.91 (1H) and 6.78 (1H)] a methoxyl singlet [δ 3.86 (3H)] and an aromatic methyl singlet [δ 2.41 (3H)]. The <sup>13</sup>C NMR spectrum displayed 16 signals of which 14

resonated between 107 and 174 ppm suggesting a polyaromatic system. DEPT analysis revealed resonances for four aromatic methines (107.1, 109.2, 118.4 and 123.2 ppm), one methoxyl (56.0 ppm) and a methyl signal (21.8 ppm). The HSQC spectra enabled all the proton signals to be assigned to their directly attached carbons. The aromatic methyl (6-CH<sub>3</sub>) at  $\delta$  2.41 was positioned *ortho* to both aromatic methine protons at  $\delta$  6.78 and 6.91 due to strong HMBC correlations to C-5 (109.2 ppm) and C-6 (107.1 ppm), respectively. ROESY correlations between 6-CH<sub>3</sub> and H-5 and H-7 further supported this assignment. The methoxyl group at  $\delta$  3.86 (8-OCH<sub>3</sub>) was positioned *ortho* to H-7 ( $\delta$  6.78) based on HMBC correlations from both sets of protons to C-8 (159.7 ppm) and a strong ROESY correlation between the proton singlets,  $\delta$  3.86 and 6.78. The remaining portion of ring B contained an oxygen substituted quaternary carbon at C-4b (156.9 ppm) and a carbonyl substituent at C-8a (109.0 ppm). Weak HMBC correlations ( $^4J_{CH}$ ) from both H-5 and H-7 to a carbonyl carbon at 173.5 ppm supported this substitution pattern. Ring A contained the pair of *ortho*-coupled ( $J = 9.0$  Hz) aromatic protons at  $\delta$  7.29 (H-3) and 7.43 (H-4). HMBC correlations from H-4 to carbons resonating at 147.9 ppm (C-4a) and 150.0 ppm (C-2) suggested two oxygenated quaternary carbons within ring B. The latter signal (C-2) was assigned to a phenol carbon based on a strong ROESY correlation between 2-OH ( $\delta$  9.96) and H-3 ( $\delta$  7.29). HMBC correlations from H-4 to C-9a (119.6 ppm) and C-9 (173.5 ppm) established a carbonyl *meta*-substitution of ring A relative to H-4. HMBC correlations from H-3 ( $\delta$  7.29) to C-1 (119.9 ppm) and 1-CO<sub>2</sub>H (167.8 ppm) established the substitution of a carboxylic acid moiety at position C-1 of ring A. With all the carbon and oxygen atoms of compound **1** accounted for the only remaining structural assignment was an ether bridge between C-4a and C-4b, which established two

linkages between rings A and B and hence structure **1** was assigned to 2-hydroxy-6-methyl-8-methoxy-9-oxo-9*H*-xanthene-1-carboxylic acid. Compound **1** is the 8-methyl ether analogue of the previously isolated fungal metabolite pinselic acid (**4**), which along with its methyl ester derivative pinselin (**5**) were both isolated from *Penicillium amarum* (Munekata, 1943, 1953). Pinselin has also been isolated from the plant *Cassia occidentalis* and is also known in the literature as cassiollin (**5**) (Ginde *et al.*, 1970; Moppett, 1971). Compound **1** has previously been synthesised as an intermediate in the total synthesis of pinselic acid (**4**) and pinselin (**5**), however **1** was only partially characterised with no <sup>13</sup>C NMR data reported (Law *et al.*, 1979).

Our attempts to obtain crystalline material of **1** suitable for single crystal X-ray analysis proved unsuccessful. However the methylation of **1** using CH<sub>2</sub>N<sub>2</sub>-Et<sub>2</sub>O in MeOH at 0 °C afforded pure 2,8-dimethoxy-6-methyl-9-oxo-9*H*-xanthene-1-carboxylic acid methyl ester (**3**, 8.0 mg, 98% yield), which yielded yellow needles (CHCl<sub>3</sub>) suitable for X-ray analysis. The structure of **3** was established as 2,8-dimethoxy-6-methyl-9-oxo-9*H*-xanthene-1-carboxylic acid methyl ester by X-ray crystallography. An ORTEP-3 (Farrugia, 1997) representation of the molecule is shown in figure 2. As for the previously reported crystal structure of 8-hydroxy-6-methyl-9-oxo-9*H*-xanthene-1-carboxylic acid methyl ester (Macias *et al.*, 2001) molecule **3** is planar except for the ester group which lies normal to the molecular plane with the C2-C1-C1a-O1b torsion angle of 81.4(5)°. Bond lengths and angles in the molecule are in accord with normal values (Allen *et al.*, 1987). Compound **3** is the dimethyl ether of pinselin and has been previously produced by *de novo* synthesis (Law *et al.*, 1979; Telange *et al.*, 1977) and via methylation of pinselin (**5**) (Munekata, 1943), however the crystal structure and full

NMR assignments for this metabolite have never been reported. NMR chemical shifts were assigned to structure **3** following analysis of  $^1\text{H}$ ,  $^{13}\text{C}$ , gCOSY, HSQC, gHMBC and ROESY NMR data.

The minor metabolite 2-hydroxy-6-hydroxymethyl-8-methoxy-9-oxo-9*H*-xanthene-1-carboxylic acid (**2**) was isolated as a stable yellow amorphous solid. An  $[\text{M}-\text{H}]^-$  ion in the (-)-HRESIMS at  $m/z$  315.0516 allowed a molecular formula of  $\text{C}_{16}\text{H}_{12}\text{O}_7$  to be assigned to **2**. The UV and IR data for **2** was essentially identical to that of **1** and the presence of a phenol substituent was confirmed by the bathochromic shift identified in the UV spectrum of **2** on addition of base. The  $^1\text{H}$  NMR spectral features of **2** were also similar to **1**, however **2** lacked the downfield exchangeable signals and the aromatic methyl resonance present in **1**. Compound **2** also contained two new mutually coupled proton signals at  $\delta$  4.59 (d,  $J = 5.5$  Hz, 2H) and  $\delta$  5.43 (t,  $J = 5.5$  Hz, 1H). HSQC analysis assigned the protons at  $\delta$  4.59 to a carbon at 62.4 ppm. This NMR data suggested the presence of a hydroxymethylene moiety (Pretsch *et al.*, 2000). Strong HMBC correlations from H-5 ( $\delta$  6.96) and H-7 ( $\delta$  6.84) of ring B to the carbon at 62.4 ppm indicated that **2** had the hydroxymethylene group positioned at C-6. Strong ROESY correlations between both H-5 and H-7 and the methylene protons of 6- $\text{CH}_2\text{OH}$  further supported this assignment. Hence the structure for **2** was assigned to 2-hydroxy-6-hydroxymethyl-8-methoxy-9-oxo-9*H*-xanthene-1-carboxylic acid. Compound **2** has the same oxygenation pattern and hydroxymethyl substitution to that of sydowinin B (**6**), which was isolated from the microfungus *Aspergillus sydowi* (Hamasaki *et al.*, 1975).

Compounds **1-3** were all tested for toxicity in a brine shrimp (*Artemia salina*) lethality assay (Solis *et al.*, 1993) and showed no activity at 20 or 200  $\mu\text{g/mL}$ .



Antimicrobial activities for compounds **1-3** were also evaluated using a modified Kirby-Bauer agar diffusion assay (Greenberg *et al.*, 1986) conducted at 12.5 and 6.25  $\mu\text{g}/\text{well}$  against *Escherichia coli* (NCCLS N25922), *Streptococcus pneumoniae* (NCCLS N49619), *Enterococcus faecalis* (NCCLS N27853), *Pseudomonas aeruginosa* (NCCLS N27853), *Staphylococcus aureus* (ATCC 49476) and *Candida albicans* (ATCC 14053). No antimicrobial activity was observed for any of the xanthenes **1-3**. Further biological investigations of compounds **1-3** are currently underway.

### **3. Experimental**

#### *3.1 General*

NMR spectra were recorded at 30 °C on a Varian 500 MHz Unity INOVA at 499.923 MHz for  $^1\text{H}$  and 124.981 MHz for  $^{13}\text{C}$ . The  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts were referenced to the solvent peak for DMSO- $d_6$  at  $\delta$  2.49 and 39.51 ppm, respectively. HRESIMS were recorded on a Bruker 4.7 T Apex III fourier-transform mass spectrometer. Melting points were determined using a Gallenkamp digital melting point apparatus and were uncorrected. FTIR and UV spectra were recorded on a Perkin-Elmer 1725X spectrophotometer and a GBC UV/vis 916 spectrophotometer, respectively. A Waters 600 pump equipped with a Waters 996 PDA detector and a Waters 717 autosampler were used for HPLC. Alltech Davisil 40-60  $\mu\text{m}$  60 Å C18 bonded silica packed into an open glass column (35 mm  $\times$  50 mm) was used for flash chromatography. An SPE cartridge (10 mm  $\times$  30 mm) packed with Alltech Davisil 40-60  $\mu\text{m}$  60 Å diol bonded silica was used for synthetic reaction purification. Sephadex LH-20 packed into

an open glass column (35 mm × 480 mm) was used for gel permeation chromatography. A Thermo Hypersil C18 BDS 5 µm 143 Å preparative column (21.2 mm × 150 mm) was used for HPLC separations. All solvents used for chromatography, UV and MS were Lab-Scan HPLC grade, and the H<sub>2</sub>O used was Millipore Milli-Q PF filtered. All synthetic reagents used were purchased from Sigma-Aldrich. All fungal culture media was purchased from Difco.

### 3.2 *Collection and identification*

*Xylaria* sp. was isolated from a surface sterilised (EtOH/flame) piece of outer bark from the rainforest tree *Glochidion ferdinandi* (Williams *et al.*, 1984) collected from Toohey Forest, Queensland, Australia during February of 2003. Fungal voucher specimens FRR 5657 and BRIP 39875 have been deposited at Food Science Australia, North Ryde NSW 1670 and the Department of Primary Industries and Fisheries, Indooroopilly, QLD 4068, respectively. *Xylaria* sp. did not sporulate under a variety of different growing conditions hence morphological taxonomic studies were not possible. Fungal identification was carried out by sequencing of the fungal ribosomal RNA genes (ITS1, 5.8s and ITS2 regions) and sequence comparisons were made using BLASTN searches through the NCBI website ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). The best scores for sequence matches were with fungi from the genus *Xylaria*.

### 3.3 *Fermentation, extraction and isolation*

The fungal isolate was initially grown in three culture tubes each containing malt extract broth (10 mL) at 30 °C for 5 days. These cultures were transferred to three conical flasks (500 mL) each containing damp white rice (50 g rice plus 100 mL H<sub>2</sub>O) and the fermentation was allowed to proceed under static conditions at 25 °C for 28 days. EtOAc extraction of the cultures followed by removal of the solvent in vacuo yielded a dark green gum (1.57 g). This extract was pre-absorbed to C18 silica overnight then loaded onto a C18 flash column and a 20% stepwise gradient was performed from 100% H<sub>2</sub>O to 100% MeOH. The 60% MeOH/40% H<sub>2</sub>O elution was allowed to slowly evaporate over 2 days and a fine amorphous precipitate formed. This solid was filtered and dried to yield pure 2-hydroxy-6-methyl-8-methoxy-9-oxo-9*H*-xanthene-1-carboxylic acid (**1**, 37 mg). The 20% MeOH/80% H<sub>2</sub>O elution was evaporated to dryness and the resulting material (136 mg) was subjected to C18 preparative chromatography using a linear gradient from 100% H<sub>2</sub>O to 50% MeOH/50% H<sub>2</sub>O in 50 min and a flowrate of 8 mL/min. Fraction 13 (15 mg, *t*<sub>R</sub> = 32-37 min) was further purified using a Sephadex LH-20 column using 100% MeOH as eluant at a flowrate of 4 mL/min. Analysis of the resulting fraction by (-)-LRESIMS and combining of the relevant test-tubes afforded pure 2-hydroxy-6-hydroxymethyl-8-methoxy-9-oxo-9*H*-xanthene-1-carboxylic acid (**2**, 4.3 mg).

### 3.3.1 2-Hydroxy-6-methyl-8-methoxy-9-oxo-9*H*-xanthene-1-carboxylic acid (**1**)

Stable pale yellow amorphous solid; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 245 (4.20), 257 (4.23), 288 (3.69), 312 sh (3.38), 371 nm (3.53); UV (MeOH+NaOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 207 (4.46), 259 (4.29), 412 nm (3.34); IR  $\nu_{\max}$  (NaCl) 3500-3000, 1625, 1569, 1471, 1392, 1314,

1227, 1209, 1107, 1016, 952, 897, 844, 814, 667  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data see Table 1; (-)-LRESIMS  $m/z$  (rel. int.) 255 (25), 299 (100); (-)-HRESIMS  $m/z$  299.0555 ( $\text{C}_{16}\text{H}_{11}\text{O}_6$   $[\text{M}-\text{H}]^-$  requires 299.0561).

### 3.3.2 2-Hydroxy-6-hydroxymethyl-8-methoxy-9-oxo-9H-xanthene-1-carboxylic acid (2)

Stable pale yellow amorphous solid; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 244 (4.01), 258 (4.01), 287 (3.51), 312 sh (3.18), 369 nm (3.20); UV (MeOH+NaOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 207 (4.69), 259 (4.10), 412 nm (3.26); IR  $\nu_{\text{max}}$  (NaCl) 3500-3035, 1651, 1567, 1557, 1539, 1506, 1471, 1455, 1367, 1271, 1205, 1093, 1059, 819  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data see Table 1; (-)-LRESIMS  $m/z$  (rel. int.) 271 (25), 315 (100); (-)-HRESIMS  $m/z$  315.0516 ( $\text{C}_{16}\text{H}_{11}\text{O}_7$   $[\text{M}-\text{H}]^-$  requires 315.0511).

### 3.3.3 2,8-Dimethoxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid methyl ester (3)

Xanthone **1** (7.4 mg, 0.0246 mmol) was dissolved in dry MeOH (1.5 mL) and Et<sub>2</sub>O (1.5 mL) then treated with excess CH<sub>2</sub>N<sub>2</sub>-Et<sub>2</sub>O at 0 °C for 1 h. The reaction was allowed to warm to rt overnight then the solvent were evaporated and the residue was purified using a diol SPE cartridge with a 20% stepwise elutions from 100% hexanes to 100% EtOAc. Compound **3** (8.0 mg, 98% yield) eluted with the 60% EtOAc/40% hexanes wash. Stable yellow needles (CHCl<sub>3</sub>); mp 219-221 °C (Ginde *et al.*, 1970); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 239 (4.25), 258 (4.29), 289 (3.72), 312 sh (3.41), 364 nm (3.57); IR  $\nu_{\text{max}}$  (NaCl) 1729, 1651, 1622, 1594, 1485, 1462, 1455, 1434, 1409, 1362, 1293, 1252,

1215, 1106, 1076, 1028, 968, 821, 730  $\text{cm}^{-1}$ ; (+)-LRESIMS  $m/z$  (rel. int.) 297 (40), 329 (40), 351 (100); (+)-HRESIMS  $m/z$  329.1030 ( $\text{C}_{18}\text{H}_{17}\text{O}_6$   $[\text{M}+\text{H}]^+$  requires 329.1020).

### 3.4 Crystallography

Diffraction data were collected on a crystal of **3** at 295 K on a Rigaku AFC7R rotating anode four circle diffractometer using monochromated  $\text{MoK}_\alpha$  radiation ( $\lambda = 0.71069 \text{ \AA}$ ). Found tetragonal, space group  $I4_1/a$ ,  $a = 14.660(2)$ ,  $c = 29.248(1) \text{ \AA}$ . The structure was solved by direct methods using the program SIR-97 (Altomare *et al.*, 1996) with atom positions and displacement parameters refined using SHELXL97 (Sheldrick, 1997) within the teXsan program package (Molecular Structure Corporation, 1997-2000). The final refinement to convergence was against  $F^2$  with 2770 unique reflections to give a final  $R$ -factor ( $I > 2\sigma(I)$ ) of 0.056 and  $wR$  (all data) = 0.199. The non-hydrogen atoms were refined anisotropically and the H-atoms placed in idealised geometries.

Crystallographic data for the structure reported in this paper have been deposited at the Cambridge Crystallographic Data Centre (CCDC No. 243683). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44-1223-336033; email: [deposit@ccdc.cam.ac.uk](mailto:deposit@ccdc.cam.ac.uk)).

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Table 1  
NMR data for xanthones 1-3.<sup>a,b</sup>

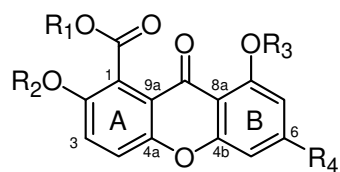
	Xanthone 1		Xanthone 2		Xanthone 3	
	<sup>13</sup> C	<sup>1</sup> H (mult., <i>J</i> , int.)	<sup>13</sup> C	<sup>1</sup> H (mult., <i>J</i> , int.)	<sup>13</sup> C	<sup>1</sup> H (mult., <i>J</i> , int.)
1	119.9		120.0		120.2	
2	150.0		155.3		151.9	
3	123.2	7.29 (d, 9.0, 1H)	122.4	7.07 (d, 9.0, 1H)	119.5	7.61 (d, 9.5, 1H)
4	118.4	7.43 (d, 9.0, 1H)	118.0	7.26 (d, 9.0, 1H)	119.5	7.65 (d, 9.5, 1H)
4a	147.9		147.4		148.2	
4b	156.9		156.4		157.0	
5	109.2	6.91 (s, 1H)	105.8	6.96 (s, 1H)	109.3	6.93 (s, 1H)
6	146.5		149.6		147.1	
7	107.1	6.78 (s, 1H)	103.3	6.84 (s, 1H)	107.3	6.81 (s, 1H)
8	159.7		158.9		159.8	
8a	109.0		111.7		108.8	
9	173.5		174.7		173.4	
9a	119.6		122.3		119.7	
1-CO <sub>2</sub> H	167.8	12.49 (br s, 1H)	168.1	<sup>c</sup>		
1-CO <sub>2</sub> CH <sub>3</sub>					166.8	
					52.1	3.83 (s, 3H)
2-OH		9.96 (br s, 1H)		<sup>c</sup>		
2-OCH <sub>3</sub>					56.7	3.84 (s, 3H)
6-CH <sub>3</sub>	21.8	2.41 (s, 3H)			21.8	2.42 (s, 3H)
6-CH <sub>2</sub> OH			62.4	4.59 (d, 5.5, 2H)		
				5.43 (t, 5.5, 1H)		
8-OCH <sub>3</sub>	56.0	3.86 (s, 3H)	55.9	3.86 (s, 3H)	56.1	3.87 (s, 3H)

<sup>a</sup> Assignments were determined by gCOSY, HSQC, gHMBC and ROESY data analysis.

<sup>b</sup> Spectra were recorded in DMSO-*d*<sub>6</sub> at 30 °C.

<sup>c</sup> Signals not observed.





<b>1</b>	R <sub>1</sub> =H	R <sub>2</sub> =H	R <sub>3</sub> =CH <sub>3</sub>	R <sub>4</sub> =CH <sub>3</sub>
<b>2</b>	R <sub>1</sub> =H	R <sub>2</sub> =H	R <sub>3</sub> =CH <sub>3</sub>	R <sub>4</sub> =CH <sub>2</sub> OH
<b>3</b>	R <sub>1</sub> =CH <sub>3</sub>	R <sub>2</sub> =CH <sub>3</sub>	R <sub>3</sub> =CH <sub>3</sub>	R <sub>4</sub> =CH <sub>3</sub>
<b>4</b>	R <sub>1</sub> =H	R <sub>2</sub> =H	R <sub>3</sub> =H	R <sub>4</sub> =CH <sub>3</sub>
<b>5</b>	R <sub>1</sub> =CH <sub>3</sub>	R <sub>2</sub> =H	R <sub>3</sub> =H	R <sub>4</sub> =CH <sub>3</sub>
<b>6</b>	R <sub>1</sub> =CH <sub>3</sub>	R <sub>2</sub> =H	R <sub>3</sub> =H	R <sub>4</sub> =CH <sub>2</sub> OH

Fig. 1. Structures for xanthenes **1-6**.

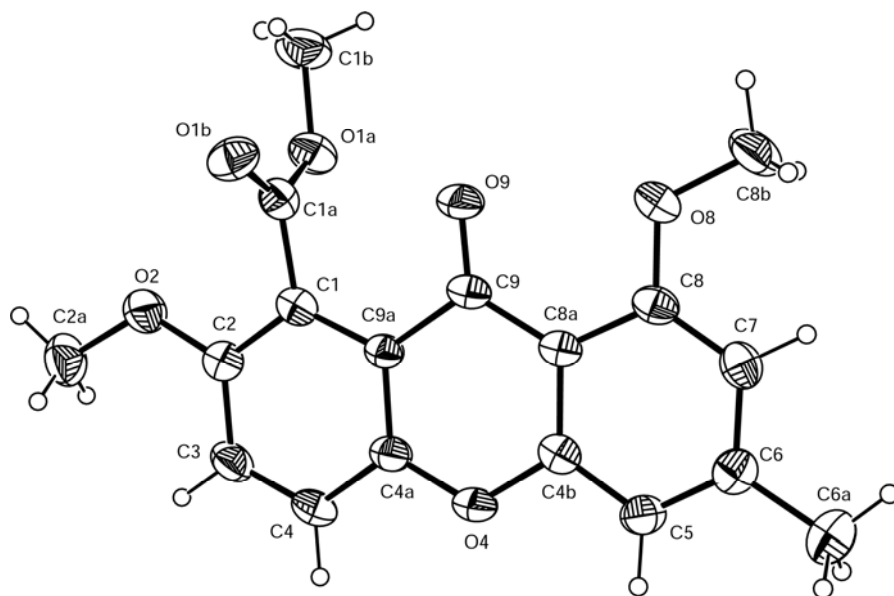


Fig. 2. ORTEP plot for xanthone **3**.