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NOTE

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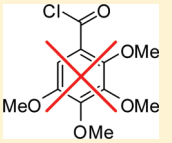
Is 2,3,4,5-Tetramethoxybenzoyl Chloride a Natural Product?

Kathryn A. Punch, Emilio L. Ghisalberti, and Matthew J. Piggott*

School of Biomedical, Biomolecular and Chemical Sciences, The University of Western Australia, Crawley, Western Australia, 6009

ABSTRACT: The title compound, which was reported to be a constituent of the fruiting body of the fungus *Antrodia camphorata*, has been synthesized. The reactivity and spectroscopic properties of the synthetic material do not match those of the natural product. There is currently insufficient information for a definitive structural reassignment.

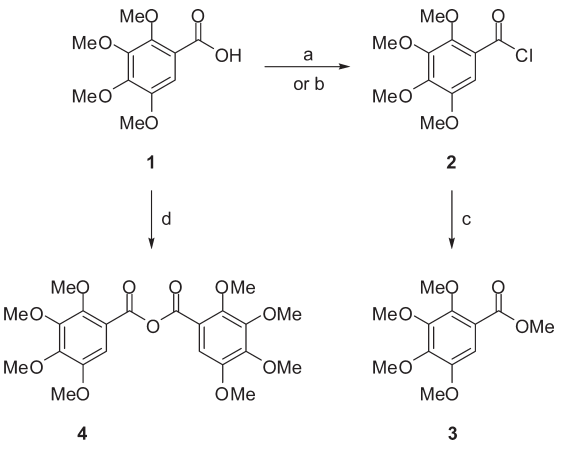


In 2007, 2,3,4,5-tetramethoxybenzoyl chloride (**2**) (Scheme 1) was purportedly isolated from the hexane extract of the fruiting body of *Antrodia camphorata*, a highly valued medicinal fungus from Taiwan.[1] This was surprising for two reasons. First, the unprecedented discovery of an acid chloride natural product had been published without comment. Second, the acid chloride not only was biosynthesised in an aqueous environment but was stable enough to survive the extraction and purification process, including multiple chromatographic separations using silica gel. This was an acid chloride unlike any other in our experience! Even the highly sterically hindered 2,4,6-tri-*tert*-butylbenzoyl chloride undergoes rapid hydrolysis.[2]

The structural elucidation of the natural product by Chen et al. was based upon detailed spectroscopic studies, including 2D NMR experiments, low- and high-resolution mass spectra, and the product of methanolysis. While the spectroscopic data could not be faulted at face value (in the absence of authentic material), close examination of the other evidence revealed several anomalies. Most immediately obvious was the absence of the ^{37}Cl isotope peaks from the low-resolution electron impact ionization mass spectral data. Furthermore, the molecular ion (containing ^{35}Cl) was the base peak, and there was no $[M - Cl]^+$ fragment at m/z 225. The $[M - Cl]^+$ fragment gives rise to the base peak in the mass spectra of *o*-, *m*-, and *p*-anisoyl chloride, 2,6-, 3,4-, and 3,5-dimethoxybenzoyl chloride, and all produce molecular ions of relatively low abundance (26% at most, but generally less than 10%).[3] Thus, on the basis of the mass spectrum alone, it seemed highly unlikely that the natural product was an acid chloride.

Treatment of the natural product with pyridine/MeOH resulted in substitution, affording a product that was assigned as the methyl ester **3**.[1] Again, at face value the characterization data for this compound seemed appropriate, but they were not compared with those already in the literature; methyl 2,3,4,5-tetramethoxybenzoate (**3**) has previously been isolated from *Relhania acerosa*.[4] The frequency of the carbonyl stretch absorption in the IR spectrum, the ^{1}H NMR data, and the EIMS mass spectra differ significantly between the publications, as detailed in Table 1. Thus, it appeared that the methanolysis product characterized by Chen et al. had been structurally misassigned and, ergo, so had the natural product.

Scheme 1[a]



[a] Reagents and conditions: (a) $(COCl)_2$, DMF, DCM; 2 × Kugelrohr dist. (38%); (b/c) 1. $SOCl_2$, 2. MeOH, pyridine (80% over two steps); (d) DCC, DCM.

The anomalies discussed above prompted us to synthesize **2**[5] and the corresponding methyl ester **3**. The acid chloride **2** was simply prepared from the known benzoic acid **1**[6] by treatment with oxalyl chloride and catalytic *N*,*N*′-dimethylformamide (Scheme 1). In contrast to the robust natural product described by Chen et al. (the UV spectrum was acquired in MeOH), we found **2** to be extremely moisture sensitive. Indeed, it was exceedingly difficult to obtain a clean ^{13}C NMR spectrum of **2** due to rapid hydrolysis resulting from adventitious water in $CDCl_3$. The hypersensitivity of **2** to hydrolysis presumably results from the electron-donating *o*/*p*-methoxy groups, which facilitate an S_N1 mechanism by stabilization of the intermediate acylium ion.[7]

The spectroscopic and MS data for synthetic **2** are presented in Table 2. As anticipated, the NMR data differ significantly from those reported for the natural product. As expected, the $[M - Cl]^+$ fragment gave rise to the base peak in the mass spectrum

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Table 1. Spectroscopic and MS Data for 3 from Various Sources

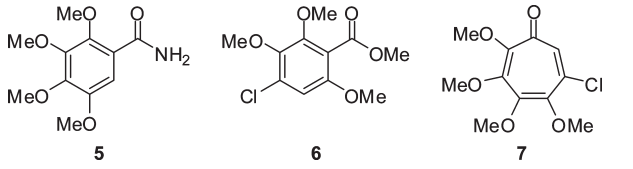
	Chen et al.[1]	Bohlmann et al.[4]	synthetic
IR (cm^{-1})	1714	1735	
^1H NMR (δ, ppm)	7.03[a]	7.12[b]	7.10[c]
	3.91	3.96	3.96
	3.91	3.91	3.91
	3.90	3.90	3.90
	3.90	3.88	3.88
	3.89	3.87	3.86
EIMS [m/z (%)][d]	256 (100)	256 (100)	256 (100)
	241 (54)		241 (45)
	225 (51)	225 (51)	225 (28)
	210 (57)		210 (4)
			198 (26)
		197 (65)	197 (1)

[a] Recorded at 400 MHz. [b] Recorded at 270 MHz. [c] Recorded at 500 MHz. [d] Not all reported data are shown.

Table 2. Spectroscopic and MS Data for 2 from Various Sources

	Chen et al.[1a]	synthetic
IR (C═O, cm^{-1})	1768	1775
^1H NMR (ppm)	7.13	7.30
	3.93	4.01
	3.92	3.91
	3.91	3.90
	3.89	3.89
^{13}C NMR (ppm)	168.1	163.5
	151.0	149.7
	148.2	149.2
	147.9	148.8
	146.5	147.3
	116.5	121.5
	109.7	110.7
	62.4	62.0
	61.2	61.5
	61.1	61.3
	56.3	56.4
EIMS [m/z (%)]		262 (0.5)
	260 (100)	260 (1.8)
	245 (64)	
	231 (16)	
	229 (54)	
		225 (100)
	227 (39)	
	217 (56)	217 (28)
	219 (19)	
	213 (32)	
		209 (19)
	202 (25)	
		195 (21)
	173 (21)	
	171 (54)	
		167 (18)

[a] Not all reported IR and MS data are shown.



5 6 7

Figure 1

of the synthetic material, and monoisotopic molecular ions were detected, albeit at very low abundance.

To help confirm the structure of the acid chloride **2** and allow comparison of the methyl ester **3** with literature reports, the former was subjected to the methanolysis conditions used by Chen et al. Thus, treatment of **2** with 1:1 pyridine/MeOH gave the corresponding ester **3**, which differed significantly from the methanolyzed natural product, but was virtually identical in all respects to that isolated by Bohlmann et al.[4] (Table 1). Clearly, 2,3,4,5-tetramethoxybenzoyl chloride (**2**) is not the natural product isolated by Chen et al. This evidence also indicates that the natural product is not a derivative of 2,3,4,5-teramethoxybenzoic acid **1**. Nevertheless, we considered two such derivatives with the aim of potentially identifying functional groups that match the chemical reactivity and at least some of the spectroscopic properties of the natural product.

Although an anhydride is likely also too reactive to be a natural product, we hypothesized that perhaps a benzoic acid was isolated and, in the presence of catalytic acid during the drying process, afforded the anhydride. Accordingly, anhydride **4** (Scheme 1) was prepared by heating **1** with DCC. Both the carbonyl carbons (153.5 ppm) and aromatic protons (6.47 ppm) of **4** resonate significantly upfield of the corresponding signals in the natural product, ruling out an anhydride as the reactive functional group in the latter.

Methanolysis of the primary benzamide **5** (Figure 1) is plausible, and it is also possible that the resonances due to the amide protons were overlooked in the ^1H NMR spectrum. However, **5** is known,[6] and while the carbonyl carbon resonates in the appropriate region (166.6 ppm), the aromatic proton (6.75 ppm) again resonates significantly upfield of the aryl signal in the ^1H NMR spectrum of the natural product. Furthermore, the amide carbonyl absorbs much lower energy IR radiation than the carbonyl group in the natural product.

We have been unable to propose an alternative structure that might be consistent with the reported physical and chemical properties. We initially considered a ring-chlorinated ester such as **6**, as the chlorine atom is much less readily lost in the mass spectra of chlorobenzenes compared to acid chlorides, and in principle, nucleophilic aromatic substitution with MeOH is possible (although unlikely). However, the natural product gives rise to four low-field resonances in the ^{13}C NMR spectrum, corresponding to four methoxy-substituted aromatic carbons, and one such resonance would be absent in **6** (or one of its regioisomers).

Another structure considered was the tropolone **7**, which should be susceptible to nucleophilic substitution with MeOH and might exhibit appropriate NMR chemical shifts. However, the carbonyl group of this highly conjugated ketone would be expected to absorb below 1700 cm^{-1} in the IR spectrum. Indeed DFT calculations predict a C═O stretch absorption at 1674 cm^{-1}, in contrast to the high-energy absorption of the natural product (1768 cm^{-1}).

It is apparent that more information is required for a structural reassignment. As a starting point, the presence of chlorine in the natural product needs to be confirmed by observation of the ^{37}Cl isotope peaks in the mass spectrum. Our attempts to derive a different molecular formula based on the accurate mass determination were fruitless.

In conclusion, we have presented irrefutable evidence that a natural product isolated from *Antrodia camphorata* has been misassigned as 2,3,4,5-tetramethoxybenzoyl chloride (**2**). The identity of the metabolite remains to be established.

EXPERIMENTAL SECTION

General experimental procedures have been published previously.[8]

Computational Modeling. Modeling was conducted using Spartan 08 at the density functional theory 6-31G* level.

2,3,4,5-Tetramethoxybenzoyl Chloride (2). Oxalyl chloride (61 mg; 0.47 mmol) was added to a stirred solution of **1** (110 mg, 0. 43 mmol) in 0.1% anhydrous DMF/dichloromethane (1.5 mL) at 0 °C under argon. The reaction mixture was stirred for 6 d, the volatiles were evaporated, and the residual brown oil was distilled twice (Kugelrohr, 100−150 °C, 45 mmHg), to give **2** as a pale pink oil, which solidified on cooling (45 mg; 38%). See Table 2 for spectroscopic data.

Methyl 2,3,4,5-tetramethoxybenzoate (3). Thionyl chloride (1 mL) was added to a stirred solution of **1** (59 mg; 0.25 mmol). After 30 min, a 1:1 pyridine/MeOH solution (1 mL) was added, and stirring was continued for 3 h. The solution was diluted with DCM (20 mL), washed with a saturated $CuSO_4$ solution (3 × 10 mL), dried, and evaporated to give **3** as a colorless oil (50 mg; 80%). The ^1H NMR spectrum (500 MHz, $CDCl_3$) matched that reported.[4]

2,3,4,5-Tetramethoxybenzoic Anhydride (4). DCC (10 mg, 0.050 mmol) was added to a solution of **1** (10 mg, 0.040 mmol) in anhydrous DCM, and the reaction mixture was heated under reflux for 36 h. Filtration of the reaction mixture through three plugs of neutral alumina and evaporation of the filtrate gave **4** as a white solid (11 mg, 60%): ^1H NMR (500 MHz, $CDCl_3$) δ 6.47 (s, 2H, ArH), 3.94 (s, 6H, CH_3O), 3.90 (s, 6H, CH_3O), 3.86 (s, 6H, CH_3O), 3.81 (s, 6H, CH_3O); ^{13}C NMR (125.8 MHz; $CDCl_3$) δ 153.6 (CO), 150.2 (ArO), 147.4 (ArO), 144.2 (ArO), 143.1 (ArO), 126.9 (*Ar*C), 104.1 (ArH), 62.6 (CH_3O), 61.3 (2 × CH_3O), 56.5 (CH_3O).

AUTHOR INFORMATION

Corresponding Author

*Tel: +61 8 6488 3170. Fax: +61 8 6488 1005. E-mail: piggott@cyllene.uwa.edu.au.

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