Journal of Chemical Crystallography, Vol. 28, No. 3, 1998

Enol tautomer of the acetate ester of 3-methoxy-4-hydroxyphenylpyruvic acid: Crystallographic and NMR spectroscopic evidence

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Received July 22, 1996

The *para* acetate ester azlactone of vanillin **2** was synthesized from vanillin **1** and hydrolyzed with sodium hydroxide. The yielded product **3** was investigated with X-ray crystallographic and nuclear magnetic resonance techniques. Compound **3** crystallized in the orthorhombic Pbca space group ($Z = 8$) and with cell dimensions $a = 14.732(2)$, $b = 12.756(3)$, $c = 12.747(6)$Å revealing the enolate tautomer and not the keto form of 3-methoxy-4-hydroxyphenylpyruvic acid as the acetate ester. The structure exhibited the pyruvic acid side chain in the *trans* extended conformation. A single proton on the benzylic carbon atom further suggested the existence of the enolate tautomer form of **3** in solution. The chemical shift values and peak integration in the NMR spectra add additional support to this finding.

KEY WORDS: x-ray; NMR; tautomerism; enolate; phenylpyruvic acid; vanillin.

Introduction

In a preceding paper[1] we reported the synthesis of 3-methoxy-4-hydroxy-5-chlorophenylpyruvic acid and its acetate ester as well as the X-ray and NMR studies of these structures. These studies revealed the existence of the phenylpyruvic acid in the enolate tautomer form and the crystallization of its acetate ester as three independent conformers. These results prompted the investigation of the structure of the unchlorinated phenylpyruvic acid molecule (3-methoxy-4-hydroxyphenylpyruvic acid). In continuation of our interest in exploring the synthesis of unnatural amino acids, we report in this study the synthesis of the intermediate **3** and its crystallographic and spectroscopic properties.

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Experimental

Synthesis

The synthesis of **3** involves the formation of the azlactone of vanillin **2** from vanillin **1**,[1,2] followed by hydrolysis. The basic hydrolysis of the azlactone somewhat surprisingly yielded the acetate ester of 3-methoxy-4-hydroxyphenylpyruvic acid **3** which was investigated with NMR spectroscopy and X-ray crystallography. The sodium hydroxide hydrolyses the phenolic acetyl ester as well as the azlactone during the final reaction. The fact that the enol ester was formed during this reaction may indicate that the enol hydroxy intermediate (formed from the azlactone hydrolysis) is sufficiently reactive to react with the released acetyl group. This phenomenon was also observed when the ring chlorinated phenylpyruvic acid was treated with acetic anhydride.[1] Efforts to use the same acid hydrolysis that was used for the ring chlorinated azlactone[1,2] were unsuccessful. The reaction mixtures went black, probably due to decomposition.

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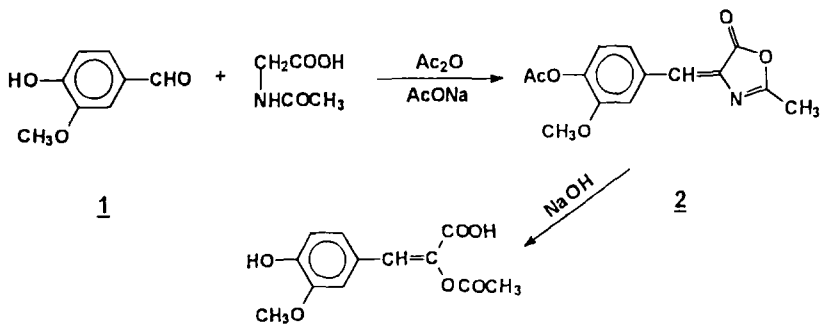

1 2 3

Synthetic Scheme

Synthesis of azlactone of vanillin **2**

A mixture of vanillin (15.2 g; 0.1 mol), N-acetylglycine (11.7 g; 0.1 mol), anhydrous sodium acetate (16.4 g; 0.2 mol) and acetic anhydride (30 ml) was heated at 105–110°C under stirring. After 2 h, a mixture (120 ml) of equal volumes of water and ethanol was added and stirred for 30 min. The crystals that formed were filtered off and dried at 45°C (17.6 g; 0.064 mol). Recrystallization from benzene gave yellow crystals of the azlactone **2** with a melting point 155–157°C.

Synthesis of acetate ester of 3-methoxy-4-hydroxyphenylpyruvic acid **3**

The azlactone of vanillin (2.0 g; 0.0073 mol) was heated at 80°C under stirring in a sodium hydroxide solution (20 ml; 20%). A dark wine-red solution formed. After 3 h, the mixture was cooled and acidified with hydrochloric acid (6 M) to a pH of 1.35. The mixture was stirred well and allowed to cool down to 4°C. The crystals that formed were filtered off and dried at 45°C (1.5 g; 0.006 mol). Recrystallization from ethanol gave pale yellow crystals of **3** with a melting point 214–218°C (decomp.).

X-ray analysis

Single crystals were selected and mounted on the tip of a glass fiber. Preliminary examination and data collection were performed with Cu $K\alpha$ radiation (λ = 1.54178 Å) on an Enraf-Nonius CAD4 computer controlled kappa axis diffractometer equipped with a graphite crystal incident beam monochromator. As a check on crystal quality, omega scans of several intense reflections were measured. The width at half-height was 1.50 with a take-off angle of 2.8°, indicating poor crystal quality. The cell parameters were, a = 12.747(6), b = 12.756(3) and c = 14.732(2)Å, V = 2395(4)Å^3 with extinctions: hk0 : k = 2n, 0kl : l = 2n, h0l : h = 2n, corresponding to the space group *Pcab*. We checked that no tetragonal space group satisfies these extinctions (hhl and hkl : no conditions). The parameters have then been permuted to satisfy the *Pbca* orthorhombic space group (N° 61), giving a = 14.732(2), b = 12.756(3) and c = 12.747(6)Å. The data were collected at room temperature using the ω-2θ scan technique with a scan width defined by $\Delta\omega$ = (1.5 + 0.15 tan θ) and a variable scan rate. A total of 2331 reflections were collected, of which 2161 were unique. Lorentz and polarization corrections were applied to the data. The linear absorption coefficient was μ = 9.3 cm^{-1}. No absorption correction was made. The structure was solved by direct methods using MOLEN.[3] The crystal data and a summary of the intensity data collection are listed in Table 1. Only the 1759 reflections having intensities greater than 3.0 times their standard deviations were used in the refinements. The final cycle of refinement included 211 variable parameters and converged with unweighted agreement factor of 0.080. The refined atomic coordinates are given in Table 2 while the molecular structure as well as the atomic numbering scheme adopted are shown in Fig. 1.

Spectroscopic analysis

NMR analysis of the acetate ester of 3-methoxy-4-hydroxyphenylpyruvic acid **3** was performed at 200

Table 1. Crystal data and summary of intensity data collection and structure refinement of **3**

Cmpd: $C_{12}H_{12}O_6$
Color/shape: Colorless prisms
For. wt., M = 252.23
Space group: *Pbca*
Temp., 23°C
Cell constants (25 reflections with $10° < \theta < 17°$)
a, Å = 14.732(2)
b, Å = 12.756(3)
c, Å = 12.747(6)
Cell volume, V(Å^3) = 2395(4)
Formula units/unit cell, Z = 8
D_{calc}, g cm^{-3} = 1.40
μ_{calc}, cm^{-1} = 9.3
Diffractometer/scan: ω-2θ
Range of relative transm. factors, %: absorption correction not applied
Radiation, graphite monochromator: Cu$K\alpha$, λ = 1.54178 Å
Max. crystal dimensions, mm: 0.22 × 0.17 × 0.10
Scan width: 1.5 + 0.15 tan θ
Standard reflections: 4 1 2, 3 2 −1
Decay of standards: −1%
Reflections measured: 2331
2θ range, deg: [4–130]
Range of h, k, l: 0/14, 0/14, 0/17
Reflections observed [$F_o \geq 3\sigma(F_o)$]: 1759
Corrections applied: Lorentz-polarization
Computer programs: MOLEN
Source of Structure factors used: *International Tables for X-ray Crystallography*
Structure solution: Direct methods
Treatment of Hydrogen Atoms: Theoret. positions or ΔF synthesis, refined isotropically
No. of parameters varied: 211
$R = \Sigma\,||F_o|-|F_c||/\Sigma|F_o| = 0.080$
$R_w = 0.121$
Largest feature final diff. map e.Å^{-3}: 0.00

Table 2. Positional parameters and equivalent isotropic thermal factors for nonhydrogen atoms of **3**; the standard deviations are given in parentheses[a]

Atom	x/a	y/b	z/c	B_{eq}(Å^2)
O10	0.0665 (3)	0.0740 (4)	1.0871 (3)	3.28 (9)
O11	0.0970 (3)	−0.0008 (4)	0.9321 (3)	3.64 (9)
O12	0.2303 (3)	0.1654 (3)	1.1027 (3)	2.94 (8)
O14	0.2820 (3)	0.0331 (3)	1.2060 (3)	2.60 (8)
O16	0.6509 (2)	0.1245 (3)	0.9046 (3)	2.27 (7)
O17	0.5674 (3)	0.2210 (3)	1.0589 (3)	2.71 (8)
C1	0.3694 (3)	0.0924 (4)	0.9314 (4)	1.64 (9)
C2	0.4167 (4)	0.0423 (4)	0.8489 (4)	2.00 (1)
C3	0.5096 (4)	0.0534 (4)	0.8384 (4)	1.90 (9)
C4	0.5589 (3)	0.1134 (4)	0.9092 (4)	1.71 (9)
C5	0.5128 (3)	0.1656 (4)	0.9915 (4)	1.71 (9)
C6	0.4208 (3)	0.1560 (4)	1.0022 (4)	1.95 (9)
C7	0.2739 (3)	0.0700 (4)	0.9420 (4)	1.75 (9)
C8	0.2126 (3)	0.0970 (4)	1.0152 (4)	1.69 (9)
C9	0.1205 (3)	0.0541 (4)	1.0105 (4)	2.10 (1)
C13	0.2612 (3)	0.1257 (4)	1.1946 (4)	1.82 (9)
C15	0.2701 (4)	0.2047 (5)	1.2825 (4)	3.40 (1)
C18	0.5255 (4)	0.2618 (6)	1.1523 (5)	3.80 (1)

[a] Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as: $4/3(a^2\beta_{1,1} + b^2\beta_{2,2} + c^2\beta_{3,3} + ab\cos\gamma\beta_{1,2} + ac\cos\beta\beta_{1,3} + bc\cos\alpha\beta_{2,3})$.

MHz (^{1}H) and 50 MHz (^{13}C) on a Bruker AC-200 spectrometer, with DMSO-D_6 as solvent and TMS as internal standard. The melting points (uncorrected) were determined on an Electrothermal melting point apparatus.

Results and discussion

X-ray analysis

The conformer of the enol acetate ester **3** crystallized as the enolate tautomer and not the keto form (Fig. 1). Bond length distances between the C(7) and C(8) and between C(8) and O(12) atoms clearly indicated the existence of the enolate form. The bond length value (1.346(7)Å) is in agreement with Csp^2 = Csp^2 bond length in enol esters[4] and similar to that we have recently reported for another phenylpyruvic acid derivative.[1] This was also evident for the Csp^2 -- O bond length (1.443(7)Å) in enol esters. The *trans* extended side chain conformation toward the acid moi-

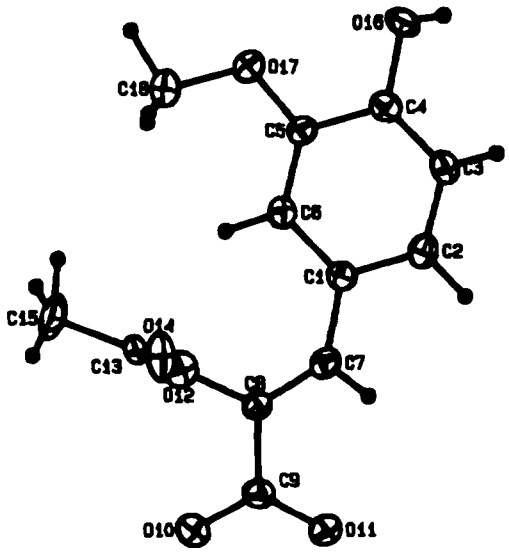

Fig. 1. ORTEP view of **3**.

Table 3. ^{1}H NMR data of **3**

Hydrogen atom	PPM
Ar; H - 2	7.32 (d, J_m = 1.6 Hz)[a]
Ar - C*H* = C	7.24 (s)
Ar; H - 6	7.11 (dd, J_o = 8.3 Hz, J_m = 1.6 Hz)
Ar; H - 5	6.82 (d, J_o = 8.3 Hz)
O*CH*$_3$	3.79 (s)
CH$_3$ - C = O	2.02 (s)

Table 4. ^{13}C NMR data of **3**

Carbon atom	PPM
CH$_3$ – *C* = O	169.2 (s)[a]
COOH	166.7 (s)
C - 3 (Ar)	148.2 (s)
C - 4 (Ar)	147.3 (s)
Ar - *C*H = C	132.7 (d)
Ar - CH = *C*	125.0 (s)
C - 6 (Ar)	124.3 (d)
C - 1 (Ar)	124.1 (s)
C - 5 (Ar)	115.5 (d)
C - 2 (Ar)	113.4 (d)
OCH$_3$	55.4 (q)
*C*H$_3$ – C = 0	22.5 (q)

[a] s = singlet; d = doublet; dd = double doublet; q = quartet.

ety (C(1)--C(7)--C(8)--C(9) = −174°) is similar to the observation for conformers I, II, and III in the *meta* chloro derivative of **3**.[1] Interestingly, the chloro derivative crystallized as three independent conformers compared to the single conformer observed in this study. The acetate ester portion of the molecule is orientated at −89° versus the side chain and aromatic moiety (C(7)--C(8)--O(12)--C(13)), which is significantly different from the ~115° that we observed for the three conformers of the *meta* chloro derivative of **3**. The orientation of the acid moiety, C(7)--C(8)--C(9)--O(10) = 174°, is similarly *trans* orientated as the conformers I and III of the *meta* chloro derivative of **3**. The carbonyl group of the ester is almost in the same plane as C(8), O(12), and C(13), i.e., the torsion angle C(8)--O(12)--C(13)--O(14) = 6°. The *meta* methoxy group is nearly coplanar with the aromatic ring with an angle C(6)--C(5)--O(17)--C(18) = 7°. The methoxy and acetate ester groups are further on the same side (*syn*) of the aromatic moiety as was previously observed.[1] The conformation of **3**, therefore, largely resembles conformer I of the *meta* chloro derivative of **3**.[1]

Spectroscopic analysis

The ^{1}H NMR data of **3** appear in Table 3. The integration of one proton at δ = 7.24 as well as the low field chemical shift value of this proton on benzylic carbon clearly support the sp^2 hybridization of the benzylic carbon and existence of the enol tautomer of the phenylpyruvic acid and not the keto form.[1] In support, no upfield peak was observed indicating the presence and integration of two benzylic protons. The integration of three protons at δ = 2.02 confirms the methyl protons of an enolic ester and not a phenolic ester. The methyl protons of a phenolic ester absorb further downfield, δ = ~ 2.27 (unpublished results). The ^{13}C NMR data of **3** appear in Table 4. The doublet signal for the benzylic carbon in the proton coupled ^{13}C spectrum (δ = 132.7) further support the existence of the enol tautomer. The quartet signal for the methyl carbon (δ = 22.5) and the signal at δ = 169.2 for carbonyl carbon also confirms the presence of the acetate function in the molecule.

This study along with the previous study clearly indicate that phenylpyruvic acid exists exclusively as the enol tautomer form in solid and in solution states.

Supplementary material. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-1003/5308. Copies of available material can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk.)

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