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Laboratory note

Synthesis and structure analysis of cyclodehydration product of piroxicam: A metabolite detected in dogs and monkeys

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ABSTRACT

We report here a novel synthesis of 6-methyl-6*H*-7-oxopyrido[1,2-*a*]pyrimido[5,4-*c*]-1,2-benzothiazine-5,5-dioxide or cyclodehydration product of piroxicam, a metabolite detected in dogs and monkeys that was synthesized in 6% yield earlier. The reaction of benzoyl chloride with piroxicam in the presence of triethylamine afforded the piroxicam metabolite in good yield. A comparison of spectral data of the synthesized compound with the reported values remained inconclusive. The structure of the compound was confirmed unambiguously by single-crystal X-ray analysis.

1. Introduction

Piroxicam (**1**, Fig. 1) or 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-1-(*N*-(2-pyridyl)carboxamide)-1,1-dioxide is a well-known non-steroidal anti-inflammatory drug (NSAID) used in the treatment of rheumatoid arthritis, osteoarthritis and other inflammatory disorders [1]. It is a non-selective inhibitor of cyclooxygenase (COX) possessing analgesic and antipyretic properties. Chemically, it belongs to the "oxicam" class of compounds that contain *N*-heterocyclic benzothiazine carboxamide moiety (Fig. 1). Pharmacokinetic and metabolism studies on piroxicam showed that it is readily absorbed after oral or rectal administration. Because of its long plasma half life of 35–60 h piroxicam is usually given in doses of 20 mg daily [2]. It is extensively metabolized by hepatic cytochrome P450 enzyme giving rise to several metabolites including the hydroxylated derivative as a major one. It was observed that hydroxylation predominantly took place at the C-5 position of the pyridyl ring followed by glucuronidation to generate 5-hydroxypiroxicam glucuronide. Among the several other metabolites reported a cyclodehydration product of piroxicam i.e. **3** (6-methyl-6*H*-7-oxopyrido[1,2-*a*]pyrimido[5,4-*c*]-1,2-benzothiazine-5,5-dioxide, Fig. 1) was detected especially in dogs and monkeys [3]. The synthesis of **3** has been reported earlier in 6% yield that involved the treatment of **1** with acetic anhydride in pyridine under refluxing condition. The high melting solid obtained after usual work-up followed by fractional crystallization from ethyl acetate was characterized as compound **3** based on NMR, IR, MS and HRMS along with the combustion analytical data.

2. Results and discussion

Due to our continuing interest in the chemical modifications of existing cyclooxygenase inhibitors [4,5] we have recently reported synthesis and pharmacological evaluation of a number of piroxicam derivatives [6]. In this study when piroxicam was treated with a number of aliphatic and aromatic acyl chlorides to afford the O-acylated products formation of an unidentified side product was observed. The formation of this unknown product (**3**) was found to be significant particularly when piroxicam (**1**) was reacted with benzoyl chloride (Scheme 1). Initially, the reaction was carried out for 6 h using benzoyl chloride and triethylamine, 1.0 equivalent each, when **1a** was isolated as a major product [6]. However, by increasing the reaction time from 6 h to 24 h and using 2.0 equivalent of each of these reagents, we were able to generate the other product **3** in significant quantity. After separating from the O-benzoylated compound **1a** the unexpected product **3** was characterized by various spectral data. The ^{1}H NMR data recorded for this compound in various solvents are listed in Table 1 (Entries 1–3) along with the reported values (Entry 4, Table 1). As evident from

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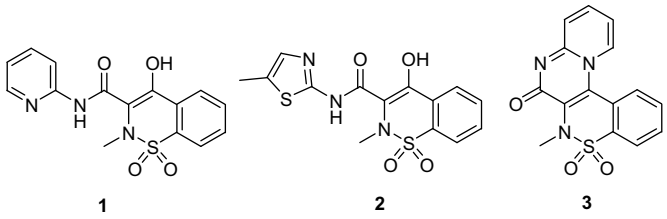
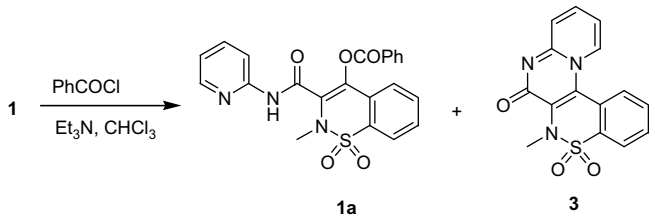


Fig. 1. Piroxicam (**1**), meloxicam (**2**) and a metabolite of piroxicam (**3**).



Scheme 1. Benzoylation of piroxicam.

Table 1
Comparison of spectral data of compound **3**.

Entry	Spectra	Solvent	δ values (ppm)
1.	^{1}H NMR (400 MHz)	CF_3CO_2H–DMSO-d_6	9.29 (d, J = 6.9 Hz, 1H), 8.43 (t, J = 7.3 Hz, 1H), 8.15 (d, J = 7.8 Hz, 2H), 8.06–7.9 (m, 2H), 7.80 (d, J = 8.7 Hz, 1H), 7.70 (t, J = 7.4 and 1.0 Hz, 1H), 3.40 (s, 3H)
2.	^{1}H NMR (200 MHz)	$CDCl_3$	8.30 (d, J = 7.3 Hz, 1H), 8.10–7.60 (m, 4H), 7.40 (d, J = 7.8 Hz, 1H), 6.80 (t, J = 6.9 Hz, 1H), 3.50 (s, 3H)
3.	^{1}H NMR (300 MHz)	DMSO-d_6	9.10 (d, J = 6.9 Hz, 1H), 8.30 (d, J = 7.9 Hz, 1H), 8.14 (t, J = 8.4 Hz, 2H), 8.10–7.90 (m, 2H), 7.90 (d, J = 8.7 Hz, 1H), 7.50 (t, J = 6.4 Hz, 1H), 3.20 (s, 3H)
4.	^{1}H NMR[a] (60 MHz)	CF_3COOD	9.3–7.6 (m, 8H), 3.47 (s, 3H) [doublets observed at 9.24 (d, J = 3.5 Hz) and 8.50 (d, J = 3.5 Hz)]

[a] Data collected from literature [as reported in ref [3]: ^{1}H NMR (60 MHz) τ 6.53 (s, 3H, CH_3), a multiplet at 2.4–0.7 (8H, aromatic protons) in which a pair of doublets at 0.76 and 1.50 (J = 3.5 Hz) could be distinguished] after converting the "τ" values into "δ" values.

Table 2
Crystal data and structure refinement parameters for $C_{15}H_{11}N_3O_3S\cdot 2H_2O$ (**3**).

Empirical formula	$C_{15}H_{11}N_3O_3S\cdot 2H_2O$
Formula weight	349.36
Temperature	298(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, $P2_1/c$
Unit cell dimensions	a = 8.3027(8) Å b = 27.641(3) Å c = 7.2861(7) Å β = 113.04(1)°
Volume	1538.7(3) Å^3
Z, Calculated density	4, 1.508 mg/m^3
Absorption coefficient	0.243 mm^{-1}
F(000)	728
Crystal size	0.22 × 0.28 × 0.38 mm
Theta range for data collection	2.67–25.97°
Reflections collected/unique	15,721/3006 [R(int) = 0.0259]
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	3006/5/233
Goodness-of-fit on F^2	1.017
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0449, wR_2 = 0.1205
R indices (all data)	R_1 = 0.0485, wR_2 = 0.1237
Largest diff. peak and hole	0.321 and −0.388 e A^{-3}

Table 3
Selected bond distances (Å) and bond angles (°) for $C_{15}H_{11}N_3O_3S\cdot 2H_2O$ (**3**).

S1–O1	1.423(2)	O1–S1–O2	119.1(1)
S1–O2	1.432(2)	O1–S1–N1	108.2(1)
S1–N1	1.637(2)	O2–S1–N1	107.6(1)
S1–C14	1.749(2)	N1–S1–C14	100.7(1)
N1–C1	1.407(2)	C1–N1–S1	114.2(1)
N1–C15	1.471(3)	C15–N1–S1	120.6(1)
N2–C3	1.328(2)	C1–N1–C15	121.0(2)
N2–C2	1.357(2)	C3–N2–C2	120.5(1)
N3–C3	1.384(2)	C3–N3–C7	119.9(1)
N3–C7	1.391(2)	C3–N3–C8	118.5(1)
N3–C8	1.409(2)	C7–N3–C8	121.4(1)
O3–C2	1.237(2)	C9–C14–S1	116.3(1)

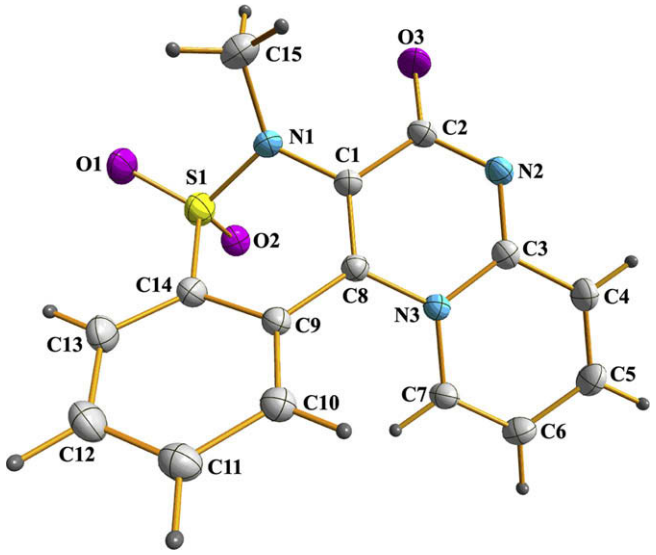


Fig. 2. ORTEP view of molecule in **3** with atom-labeling scheme. Displacement ellipsoids are drawn at the 50% probability level. Solvent water molecules are omitted for the sake of clarity.

Table 1 that comparison of the present data with the reported one remained inconclusive mainly due to the difference in MHz of the NMR instrument used and solvent for the preparation of NMR sample.

Analysis of other data for compound **3** [^{13}C NMR (DMSO-d_6) 157.3, 148.3, 141.9, 135.4, 134.2, 133.4, 132.8, 128.2, 127.8, 123.8, 123.9, 123.3, 118.6, 117.2, 34.6 (NCH_3); MS: 314; IR: 1701 (C=O)] did not provide enough information or evidence to confirm its structure. The structure of **3** was finally established from single-crystal X-ray analysis. Single crystals of **3** suitable for crystallographic study were obtained by slow crystallization from methanol. Relevant crystal data and structure refinement parameters are summarized in Table 2. Selected geometrical parameters of the molecule are listed in Table 3 [7].

Table 4
Selected hydrogen bonds for $C_{15}H_{15}N_3O_5S$ (**3**).

D–H···A	D–H (Å)	H···A (Å)	D···A (Å)	D–H···A (°)
O5–H5A···O2	0.97	2.15	3.029(3)	149
O5–H5B···O4	0.97	2.07	2.811(4)	131
C6–H6···O3^{i}	0.93	2.41	3.131(2)	134
O4–H4A···O3^{i}	0.97	1.89	2.855(3)	174
O4–H4B···N2^{ii}	0.97	1.95	2.920(2)	176
C15–H15B···O1^{iii}	0.96	2.49	3.365(3)	152

Symmetry codes: (i) $x+1$, $+y$, $+z+1$; (ii) $-x+1$, $-y$, $-z+1$, (iii) x, $-y^{+1/2}$, $z^{-1/2}$.

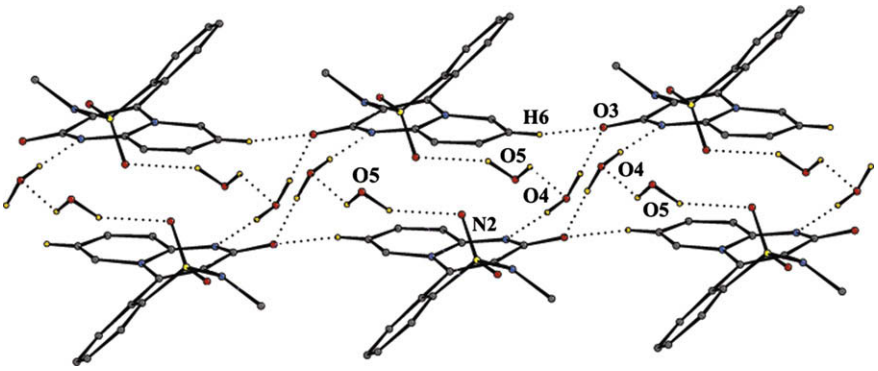
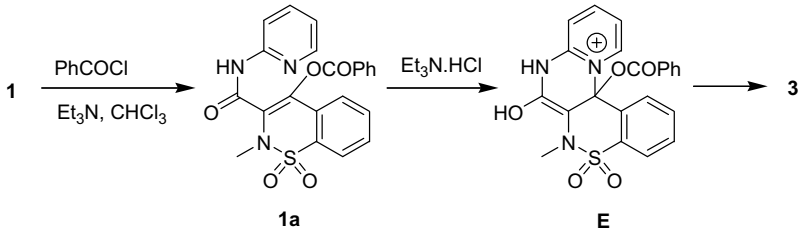


Fig. 3. Formation of polymeric chain in $C_{15}H_{15}N_3O_5S$ (**3**).



Scheme 2. Probable mechanism for the formation of **3**.

The molecular view of compound **3** is shown in Fig. 2. The compound **3** consists of 2-methyl-2*H*-benzo[*e*][1,2]thiazine-1,1-dioxide moiety and a pyrido[1,2-*a*]pyrimidin-2-one system fused across C1–C8. The ring-puckering parameters [9] of the thiazine ring (S1/N1/C1/C8–C9/C14), $Q = 0.585(2)$ Å, $\theta = 110.0(2)^\circ$ and $\varphi = 197.3(2)^\circ$, indicate a twist-boat conformation [10]. The dihedral angle between the planar benzene (C9–C14) and pyridine (N3, C3–C7) rings in **3** is 44.5(1)°. The S–O and S–N bond distances in **3** [Table 3] are similar to that reported for analogous structures [11–13]. The crystal packing in **3** is stabilized by intermolecular O–H···O, O–H···N and C–H···O hydrogen bonds (Table 4). Pairs of intermolecular O–H···O and O–H···N connect molecules of **3** forming $R_4^4(12)$ ring, in which the lattice water molecule (O4) acts as a double donor. Further linking of dimeric rings via C6–H6···O3 hydrogen bonds generates a one-dimensional polymeric chain (Fig. 3) along the [101] direction. Additional reinforcement between the molecules forming $R_4^4(12)$ rings and C(8) chains is provided by O–H···O and O–H···N hydrogen bonds in which the water molecule (O5) acts as a double donor.

Mechanistically, it was not clearly understood how the compound **3** formed during the reaction of piroxicam **1** with benzoyl chloride. Since the compound **3** was detected during the preparation of **1a** (Scheme 1) hence intermediacy of **1a** during the formation of **3** cannot be ruled out completely. Accordingly, formation of **3** was mediated by the reactivity of the pyridinium nitrogen towards the benzoyloxy bearing carbon of the benzothiazine ring and a possible mechanistic path leading to compound **3** via **1a** is shown in Scheme 2. Thus pyridine undergoes a Michael type of addition through its nitrogen with the olefinic moiety of the benzothiazine ring to form a six-membered ring intermediate **E** which on elimination of benzoyloxy group provided the compound **3**. Similarly, in a biological system such as dog or monkey, piroxicam is expected to undergo metabolism at the enolic hydroxyl group providing the corresponding *O*-sulphate or *O*-glucuronide derivative (Fig. 4) via a physiological process common to many well-known phenolic drugs [14–16]. This in turn can facilitate attack of the pyridinium nitrogen on the carbon bearing the sulfonated/glucuronated enolic hydroxyl group to provide the metabolite **3**.

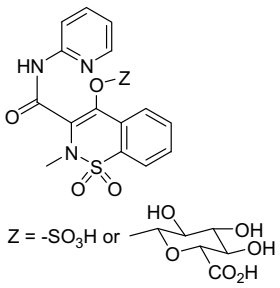


Fig. 4. Sulphate and glucuronide derivative of piroxicam.

3. Conclusion

In summary, the present work reports a novel synthetic root for preparing the cyclodehydration product of piroxicam, a metabolite detected in dogs and monkeys. The procedure involves the reaction of benzoyl chloride with piroxicam in the presence of triethylamine affording the piroxicam metabolite in good yield. As the spectroscopic characterization of the metabolite was ambiguous, the crystal structure of the compound has been established by single-crystal X-ray analysis. The cyclodehydration product of piroxicam consists of a 2-methyl-2*H*-benzo[*e*][1,2]thiazine-1,1-dioxide moiety and a pyrido[1,2-*a*]pyrimidin-2-one system fused across the C–C bond. In the solid state, the intermolecular hydrogen bonds connect the molecules to form $R_4^4(12)$ rings, in which one lattice water molecule acts as a double donor. A probable mechanism for formation of the compound under the present reaction condition as well as the physiological condition has been proposed. The present

study would undoubtedly help in better understanding of the chemistry of piroxicam and its metabolite.

4. Experimental

4.1. General methods

Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. ^{1}H NMR spectra were determined in a solvent as specified on 200 and 400 MHz spectrometers. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, $\delta = 0.00$) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet) and m (multiplet) as well as b (broad). Coupling constants (J) are given in hertz. A ^{13}C NMR spectrum was recorded in DMSO-d_6 at 50 MHz. Infrared spectra were recorded on an FT-IR spectrometer. Melting points were determined by using melting point apparatus and are uncorrected. MS spectra were obtained on a JMS-D 300 spectrometer.

4.2. Preparation of 6-methyl-6H-7-oxopyrido[1,2-a]pyrimido[5,4-c]-1,2-benzothiazine-5,5-dioxide (**3**)

To a solution of piroxicam (2 g, 0.006 mol) in chloroform (40 mL) was added benzoyl chloride (2.0 g, 0.014 mol) followed by triethylamine (2.0 mL, 0.014 mol) dropwise at room temperature. The mixture was then stirred at 30 °C for 24 h, poured into ice and extracted with chloroform (2× 25 mL). The organic layers were collected, washed with 5% NaOH (20 mL) solution followed by 5% HCl (20 mL) and then with water (2× 30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was then treated with methanol and the insoluble solid was isolated by filtration to afford the title compound **3** (1.0 g, 50% yield) as a light yellow powder, mp 282 °C (lit. [3] 286–287 °C).

4.3. Crystallographic study of 6-methyl-6H-7-oxopyrido[1,2-a]pyrimido[5,4-c]-1,2-benzothiazine-5,5-dioxide (**3**)

The compound **3** crystallizes in the monoclinic space group $P2_1/c$ with $Z = 4$. The intensity data were recorded using a Bruker SMART CCD area-detector diffractometer with graphite monochromated MoK_α radiation ($\lambda = 0.71073$ Å). The crystal structure was solved by direct methods, SHELXS97 [8] and refined on F^2 using SHELXL97 [8]. The hydrogen atoms associated with the carbon atoms were positioned geometrically and hydrogen atoms of the solvent water molecules were located from the difference Fourier map. Thermal parameters of all non-hydrogen atoms were refined anisotropically and hydrogen atoms were treated as riding (except the H-atoms of the water molecules) with fixed isotropic thermal parameters. After convergence of least-squares refinement based on 3006 unique reflections final R-values $R_1 = 0.045$ [for 2736 reflections with $I > 2\sigma(I)$] and $R_w = 0.121$ were obtained.

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Appendix. Supplementary material

Supplementary data associated with this article can be found in the online version, at doi: 10.1016/j.ejmech.2009.03.010.

References

[1] P. Schiantareli, D. Acerbi, G. Bovis, Arzneim.-Forsch.. (Drug Res.) 31 (1981) 92–97.
[2] Y. Michotte, H.P. Van Klaveren, M.R. Detaevernier, Ch.F. Gusdorf, L. Vanhaelst, Arzneim.-Forsch.. (Drug Res.) 41 (1991) 244–246.
[3] J.G. Lombardino, J. Med. Chem. 24 (1981) 39–42.
[4] S. Pericherla, J. Mareddy, D.P. Geetha Rani, P.V. Gollapudi, S. Pal, J. Braz. Chem Soc. 18 (2007) 384–390.
[5] S. Pal, P. Bindu, P.R. Venna, P.K. Dubey, Lett. Org. Chem. 4 (2007) 292–295.
[6] J. Jayaselli, J.M.S. Cheemala, D.P. Geetha Rani, S. Pal, J. Braz. Chem Soc. 19 (2008) 509–515.
[7] 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 695425. Copies of available material can be obtained, free of charge via www.ccdc.cam.ac.uk/data_request/cif, by e-mailing data_request@ccdc.cam.ac.uk, or contacting the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.
[8] G.M. Sheldrick, SHELXS97 and SHELXL97, University of Göttingen, Germany, 1997.
[9] D. Cremer, J.A. Pople, J. Am. Chem. Soc. 97 (1975) 1354–1358.
[10] D.G. Evans, J.C.A. Boeyens, Acta Crystallogr. Sect. B Struct. Crystallogr. Cryst. Chem. 45 (1989) 581–590.
[11] C. Glidewell, J.N. Low, J.M.S. Skakle, J.L. Wardell, Acta Crystallogr. Sect. C Cryst. Struct. Commun. 60 (2004) o350–o352.
[12] J. Bordner, J.A. Richards, P. Weeks, E.B. Whipple, Acta Crystallogr. Sect. C Cryst. Struct. Commun. 40 (1984) 989–990.
[13] L. Dupont, B. Pirotte, P.D. Tullio, J. Delarge, Acta Crystallogr. Sect. C Cryst. Struct. Commun. 52 (1996) 1741–1743.
[14] M.A. Tirmenstein, S.D. Nelson, J. Biol. Chem. 264 (1989) 9814–9819.
[15] H.J. Gonzalez, S. Kimura, Cancer Lett. 143 (1999) 199–204.
[16] C. Goldring, N. Kitteringham, R. Elsby, L. Randle, Y. Clement, Hepatology 39 (2004) 1267–1276.