Solvent-Mediated Effects in NMR Spectra of 2-Formyl Phenoxyacetic Acid-Chromatographic Evidence for Internal Cyclisation and Tautomerization

[1]AMJID IQBAL, [1]HAMID LATIF SIDDIQUI*, [2]CHAUDHRY MUHAMMAD ASHRAF,
[1]NAVEED AHMAD, [3]JAVED IQBAL QAZI AND [4]GEORGE WILLIAM WEAVER
[1]*Institute of Chemistry, University of the Punjab, Lahore- 54590, Pakistan.*
[2]*Department of Chemistry, Forman Christian College (A Chartered University) Lahore-54600, Pakistan.*
[3]*Department of Zoology, University of the Punjab, Lahore, Pakistan.*
[4]*Department of Chemistry, Loughborough University, Loughborough, LE 11 3TU, UK.*

(Received on 18th May 2009, accepted in revised form 13th October 2009)

Summary: The chromatographic analyses reveal that formylphenoxy acetic acid in methanol undergoes autocyclization to afford its cyclic hemiacetal, which slowly transforms into its enolic tautomer and develops equilibrium between them. The concentration of these time lapsed products significantly enhanced on adding conc. HCl. The ^{1}H- and ^{13}C-NMR data are used to establish their formation. Antimicrobial activity revealed that FPA showed good growth inhibition for Gram negative bacterium, and found to be ineffective for yeast tested, while the cyclised structures failed to exert any growth inhibitory effects on the micro-organisms tested.

Introduction

Salicyaldehyde and its derivatives have been widely used both in the industry, in synthetic and co-ordination chemistry, particularly to synthesize the salen family of ligands for catalytic applications [1]. In order to extend the scope of this reagent, it was decided to use 2-formyl phenoxyacetic acid, a derivative of salicyaldehyde. The action of aryloxyaliphatic acids on the permeability of blood vessels [2], and antimicrobial activity against the human pathogens [3] by *o*-substituted phenoxyacetic acid have been reported. The Schiff bases and their complexes derived from *o*-formylphenoxyacetic acid with aminothiazoles, a number of aminobenzene derivatives, some heterocyclic and aliphatic amines have shown biological significance such as anti-inflammatory agents [4], antimetabolites of pyridoxal phosphate [5], bacteriostatic activity [6], chorismate synthase inhibition [7], and antitumor activity [8]. Before using this compound as the starting ligand for extensive investigations, its purity was checked chromatographically. It was noticed that with time lapse, it started giving two spots and equilibrium was established in methanol or MeOH/$CHCl_3$ solvent system. The spot (A) corresponding to original FPA had R_f value 0.1 and the other product (B) is proposed to be its cyclised hemiacetal has R_f value 0.9 while using MeOH as mobile phase. However, when detailed chromatographic analysis of FPA was carried out, three spots were observed. These preliminary observations warrant further studies to identify and propose the transformation of (A) to (B) and (C) components, as mentioned in Scheme 1. This interesting behavior in FPA has not been studied before to the best of our knowledge and is reported herein.

Aldehydes are transformed into hemiacetal in the acidic alcoholic solution [9]. In the present case, FPA contains a carboxylic group, which provides the acidic medium to bring about its autocyclisation. Consequently, it was decided to record its ^{1}H- and ^{13}C-NMR spectra in polar solvents like methanol-d_4, acetone-d_6 and DMSO-d_6. Structural assignments of different protons and carbons were arrived at through two dimensional 400 MHz NMR spectra and the DEPT Method. The comparative chemical shifts of protons and carbons signals of FPA in CH_3OH-d_4, Me_2CO-d_6 and DMSO-d_6 solvents provide valuable information. It is worthwhile to infer the following observations from various chemical shifts collected in Table-1.

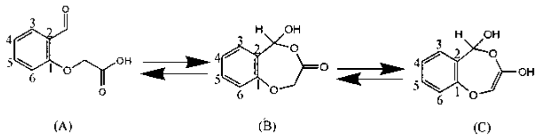
Results and Discussion

For Uncyclised FPA (A)

The carboxylic OH group of FPA recorded its absorption at δ 5.13 only in methanol-d_4, which

---

*To whom all correspondence should be addressed.



Scheme 1: Showing conversion of FPA (A) into Hemiacetal (B) and Tautomer (C).

Table-1(a): Comparative Chemical shifts of ^{1}H & ^{13}C-NMR signals in NMR spectra of FPA in three solvents.

(i) Methanol-d_4

δ	3CH	4CH	5CH	6CH	CH_2	-COOH	CHO	C-1	C-2
^{1}H	7.51	7.01	7.30	6.91	4.85	5.13	10.52	-	-
^{13}C	128.1	122.2	129.1	113.3	66.4	172.5	191.5	162.0	126.5

(ii) Acetone-d_6

Δ	3CH	4CH	5CH	6CH	CH_2	-COOH	-CHO	C-1	C-2
^{1}H	7.20	7.13	7.79	7.62	4.96	-	10.45	-	-
^{13}C	114.5	122.4	128.5	136.8	65.9	169.9	189.7	161.4	126.2

(ii) DMSO-d_6

Δ	3CH	4CH	5CH	6CH	CH_2	-COOH	-CHO	C-1	C-2
^{1}H	7.08	7.14	7.63	7.71	4.89		10.45	-	-
^{13}C	121.3	113.7	136.2	127.5	65.0	169.8	189.2	160.2	124.5

Table-1(b): Chemical shifts (δ) of resolved hemiacetal in Methanol-d_4.

Δ	CH_2	CHO-	Hemiacetal group	C-1	C-2
^{1}H	4.71	-	5,76	-	-
^{13}C	66.2	172.5	100.7	157.1	137.4

was not observed in the other two solvents (acetone-d_6 & DMSO-d_6). Moreover, recorded its chemical shift at δ 172.5, 169.9 and 169.8 for carboxylic carbon in methanol-d_4, acetone-d_6 and DMSO-d_6.

For Cyclised FPA (Hemiacetal) 5-hydroxy-2Hbenzo[e] [1,4] dioxepin-3(5H)-one

FPA was dissolved in distilled methanol and changes were monitored within one hour on silica gel TLC plate using mixture of distilled methanol and chloroform (1:9 respectively) as mobile phase system revealing the presence of two spots corresponding to original compound (R_f 0.1) and an other internally cyclised product (R_f 0.9) mentioned as (B) and (C) respectively in the text. The concentration of (B) was negligible.

The methanolic solution of FPA, which was allowed to stay for 5-days at room temperature, was used to run its ^{1}H- and ^{13}C–NMR spectra. Its TLC results indicated two spots corresponding to (A) and (B) as already mentioned. It has been observed that aldehydic form of FPA undergoes internal cyclisation only in methanol, while it failed to undergo similar cyclisation in acetone and DMSO. Hence, under our experimental conditions, no signal for the hemiacetal form would be observed in these more polar solvents. The cyclised (hemiacetal) and uncyclised (aldehydic) form of this ligand reflect pertinent additional variations. The preceding discussion about the behavior of FPA in methanol was however restricted only to its uncyclised aldehydic form.

However, the proposed hemiacetal (B) present in the methanolic solution manifests its own NMR signals for various protons and carbons as shown in Table-1. It is quite obvious that its proton signals for CH_2 and hemiacetalic-H record at δ 4.71 and 5.76, while ^{13}C signals for CH_2 (δ 66.2), hemiacetalic carbon (δ 100.7), C-1 (δ 157.1), C-2 (δ 137.4) and carbonyl carbon (δ 172.5) are noteworthy.

It may further be noted that in ^{1}H-NMR spectrum, aldehydic proton of (A) signal at δ 10.52 was very much reduced, while there was an intense and pronounced signal at δ 5.76 due to methine proton of the hemiacetalic group. The corresponding carbon signals were observed at δ 191.5 and 100.7 for the aldehydic and hemiacetal groups in DEPT spectrum in methanol-d_4. However, in the case under discussion, this ratio for aldehydic and hemiacetalic protons was 1:16 respectively, which was arrived at through the comparison of integration of aldehydic proton (0.62) and hemiacetal-H (10.06). The methanolic solution containing two components (A & B) when loaded on silica gel, gave three components, two of which had the same R_f values, while the third component (C) has changed R_f value of 0.85. At this stage, methanolic solution of FPA was kept for five days at room temperature was subjected to HPLC analysis, which confirmed the presence of three components, having 3.02, 3.31 and 5.04 min as retention time, while their relative ratio was 10.15:10.81:1. These observations confirmed the presence of solvent mediated transformation of FPA affording internally cyclised hemiacetal (B) and its enolic tautomer (C) mainly in methanolic solution. Their concentrations appeared to increase as the time lapse increases. One of the samples in methanolic solution was allowed to stay at room temperature for 90 days. During this long lapse of time, there was virtually only one spot corresponding to (C). The acidic medium significantly promoted these transformations even during much shorter time lapse. This behavior was, then utilized to isolate the three components in acetone and 400 MHz NMR spectra

Table-2(a): Chemical shifts (δ) of unresolved hemiacetal in Acetone-d_6

Δ	3CH	4CH	5CH	6CH	CH_2	CO-	Hemiacetal group	C-1	C-2
^1H	7.49	6.90	7.15	6.80	4.90	-	6.0	-	-
^{13}C	128.0	120.5	127.5	114.0	67.0	172.0	99.0	156.7	138.0

Table-2(b): Chemical shifts (δ) of tautomer (C) in Acetone-d_6

δ	3CH	4CH	5CH	6CH	Olefinic group	Tautomeric group	Hemi-acetal group	C-1	C-2
^1H	7.43	6.87	7.0	6.60	5.30	15.0	6.40	-	-
^{13}C	127.0	122.5	128.0	117.0	74.8	176.0	88.0	151.1	143.0

were run in acetone-d_6. The spectral results are shown for each component (Table-2).

The IR spectrum of the isolated component (A), superimposed on the similarly run IR spectrum of the original compound (FPA). The NMR spectral data of the chromatographically isolated components are also shown for proposed hemiacetal (B) and its tautomer (C) (Table-2). When the spectral data for the hemiacetal (B) formed in MeOH, which also contained uncyclised FPA, were compared with respective ^1H- and ^{13}C-NMR signals of the chromatographically isolated hemiacetal (B), there was very little difference between them.

Tautomer (C) (Z)-5H-benzo[e] [1,4] dioxepine-3,5 diol

The NMR spectral data, as shown in Table-2, for the chromatographically isolated tautomer (C) of cyclised hemiacetal (B) indicate olefinic proton absorptions at δ 5.30, the enolic OH at δ 15.0 and hemihedral proton at δ 6.40. Benzene ring protons at C-3, C-4, C-5, and C-6 correspond to δ 7.43, 7.00, 7.29 and 6.66. The carbon signals for olefinic, tautomeric and hemihedral forms indicate at δ 74.8, 176.0, and 95.0 respectively. Moreover, its benzene ring carbons-1,2,3,4,5 and 6 signal absorption at δ 151.1, 140.0, 127.0, 122.5, 128.0 and 117.0 respectively.

It may be pertinent to conclude that FPA undergoes internal cyclisation preferably in protic solvent like methanol. Under our experimental conditions, virtually no signals for hemiacetal or its tautomer could be traced in more polar aprotic solvents like acetone-d_6 and DMSO-d_6. Conspicuously, fluctuating behavior in the ^1H- and ^{13}C-NMR signals in these polar solvents cannot be fully rationalized. These complexities attributed partly to fluxional mark of FPA in these solvents which have quite diverging electronic environments and pose varying steric interactions in solution. This clearly demonstrates that FPA is capable of undergoing fluxional behavior.

This autocyclisation reaction is well known in carbohydrates [9]. The hemiacetal form is generally facilitated in the acidic medium [10]. In our case, the concentration of both cyclised hemiacetal (B) and its tautomer C initially increased many fold in the acidic medium within minutes. Hence, with the aid of spectral data and detailed chromatographic analysis, we can considerably rationalize the transformation of FPAA to its hemiacetal (B) which eventually transforms to tautomer (C).

Antimicrobial Activity

Compound (A), cyclic hemiacetal and its enolic form were screened for their antibacterial as well as antifungal activities against locally isolated *Escherichia coli* and *Saccharomyces cerevisiae* bacterial and yeast strains respectively by disc diffusion method. An overnight incubated cultures of these microbes were introduced onto the surface of sterile nutrient agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 10.15 mm in diameter were prepared from Whatman No. 1 filter paper and sterilized. The sterile discs were loaded with 10 μl of a standard solution of the test compound and were placed on a nutrient agar medium. The plates were inverted and incubated for one day at 37 °C. Solvent was used as a control. Solutions of the compounds A-C were prepared by dissolving 10 mg /ml in acetone for antimicrobial assays. From each of these equimolar solutions, 10 μl solution was loaded onto a sterile filter paper disc with the help of micropipette fitted with sterile tip. The inhibition zones were measured and compared with the controls. The bacterial and yeast growth inhibition zone values are summarized in Table-3. As the solutions of compounds **A-C** for antimicrobial assays were equimolar and moreover their equal amount 10 μl were loaded on to the filter paper discs, thus from the growth inhibition zone

Table-3: Zone of Growth Inhibition (mm).

Compound	*Escherichia coli*	*Saccharomyces cerevisiae*
	(ATTK-2006)	(SN-2006)
Control	0.00 ± 0.0	0.00 ± 0.0
FPA (A)	13.00 ± 0.5	0.00 ± 0.0
Hemicetal (B)	0.00 ± 0.0	0.00 ± 0.0
Tautomer (C)	0.00 ± 0.0	0.00 ± 0.0

Values are means of three replicates S.D

produced by the compound **A** for the bacterial species *E.coli,* it can be inferred that at the given dose the compound A is antibacterial as compared to compound B and C. The screening data obtained during this investigation revealed that the original compound showed good growth inhibition for the Gram negative bacterium, *E. coli.* It was found ineffective for the yeast tested. Regarding the evaluation of antimicrobial activities of the cyclic isomers (cyclic hemiacetal and its tautomer), it may be noticed that they failed to exert any growth inhibitory effects on the microorganisms tested. Thus, it can be safely concluded that cyclic forms of the compound cannot be used for antimicrobial activities and the target compound itself possesses valuable antibacterial activity. It might be considered for further investigations.

Acknowledgment

We greatly acknowledge the Higher Education Commission of Pakistan, as well as University of the Punjab, Lahore for financial support and Chemistry Department, Loughborough University U.K for spectral analysis.

References

1. P.G. Cozzi, *Chemical Society Reviews,* 410 (2004).
2. B. J Northover, J. Verghese, *Journal of Pharmacy and Pharmacology,* **14**, 615, (1962).
3. I. Zawadowska, *Acta Poloniae* Pharmaceutica, **20**, 25 (1963).
4. J.Nimala, P.Y. Sabnis, S.S. Bhide, *Bulletin of Haffkine,* **5**, 64 (1977).
5. T. L.Hullar, D.L. *J.* Failla, *Journal of Medicinal Chemistry,* **12**, 420 (1968).
6. B. C. Ma, X. M. Ma. L. R. Yan, D. Yang, *Xingyong-Huaxue,* **21**, 841 (2004).
7. M. G. Thomas, C. Lawson,. N. M. Allanson, B. W. Leslie, J. R. Bottomley, A. McBride, Olusanya, O.A. *Bioorganic and Medicinal Chemistry Letters,* **13**, 423 (2003).
8. X. Yang, P.Xu, Q. Gao, M. Tan, *Synthesis and reactivity in Inorganic and Metal-Organic Chemistry,* 59 (2002).
9. E. L. David, *Organic Chemistry A Modern Perspective,* Mcgraw-Hill College: USA, 422 (1996).
10. E. J. Grunwald, *Journal of the American Chemical Society,* **107**, 4715 (1985).