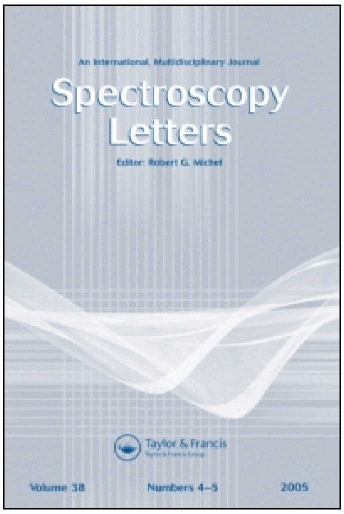


Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Spectral Determination of the Structure of 5-Hydroxymethylfurfurylidene Barbituric Acid

Marian N. Nikolov[a]; Mirjana V. Poneva[b]
[a] Department of Chemistry and Biochemistry, Medical Academy, Sofia, Bulgaria [b] Institute of Organic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria

To cite this Article Nikolov, Marian N. and Poneva, Mirjana V.(1987) 'Spectral Determination of the Structure of 5-Hydroxymethylfurfurylidene Barbituric Acid', Spectroscopy Letters, 20: 10, 821 — 834

To link to this Article: DOI: 10.1080/00387018708081590

URL: http://dx.doi.org/10.1080/00387018708081590

SPECTROSCOPY LETTERS, 20(10), 821-834 (1987)

SPECTRAL DETERMINATION OF THE STRUCTURE OF 5-HYDROXYMETHYLFURFURYLIDENE BARBITURIC ACID

KEY WORDS: 5-hydroxymethylfurfural, barbituric acid, 5-hydroxymethylfurfurylidene barbituric acid, hexose moiety of glycoproteins

Marian N.Nikolov[1] and Mirjana V.Poneva[2]
[1]-Department of Chemistry and Biochemistry, Medical Academy, 1431 Sofia, Bulgaria
[2]-Institute of Organic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria

ABSTRACT

5-hydroxymethylfurfural reacts with barbituric acid and forms a product with maximum absorbance at 395nm. Via UV-VIS and IR-spectra it becomes evident that in acidic conditions this compound has a conjugated system of bonds with a chynoide-like chromophore. In alkali conditions this chromophore breaks out and the solution is colorless. In this paper is reported a mechanism which is an attempt to explain the stages of this reaction.

1-Author to whom all correspondence should be sent.

INTRODUCTION

In so called colorimetric or chemical method the hexose moiety of glycoproteins[1] and glycohemoglobin[2] is converted to 5-hydroxymethylfurfural/HMF/by heating in acidic conditions.The liberated HMF is reacted with 2-thiobarbituric acid/TBA/and the resulting color is measured spectrophotometrically at 443 nm.In a short time appeard several methods based on this reaction[3-6]. All of the authors named the product obtained from the reaction between HMF and TBA yellow pigments or colored products and neglected the resulting structure.

In the present study we report a spectral determination of the structure of the yellow compound derived from the reaction between HMF and barbituric acid/BA/in the reaction mixture.We prefer BA than TBA because the first one absorbs the light to 340 nm while the second one absorbs to 400 nm.In addition,the absorbance of the product formed between HMF and BA /Fig.1,1/is more intensive than this one formed between HMF and TBA/Fig.1,2/derived in equal conditions.

EXPERIMENTAL

Chemicals: D/-/-fructose/Carlo Erba,Milano,Italy/,L/+/-arabinose/Koch-Light Labs Ltd,Colnbrook Bucks,England/, barbituric acid and 2-thiobarbituric acid/Reanal, Budapest,Hungary/,phenobarbital/5-phenyl-5-ethyl-barbituric acid/Loba Feinchemie,Vienna,Austria/and hydro-

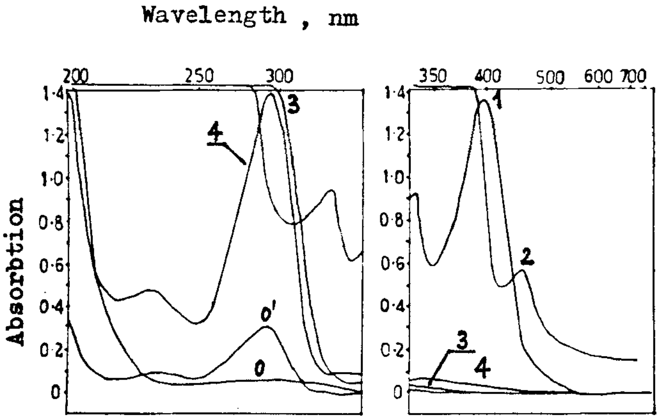


FIG.1 UV-VIS spectra of fructose, HMF, the condensation product of HMF and BA, the condensation product of HMF and TBA, HMF and equimolar quantity of phenobarbital, and diluted HMF.

chloric acid/Apolda,Berlin,GDR/-all analytycal grade were used in this study without further purification. Instruments: Spectrophotometer UV-VIS,Specord/Carl Zeiss, Jena,GDR/,spectrophotometer UV-VIS,Unicam SP 800,England, Furie-spectrometer Bruker IFS-113,FRG and mass-spectrometer JEOL 300-D,Japan were used in this study.

To determine the site of binding of HMF and BA we have made the following experiments:In two sealed tubes were placed 2 ml 0.005 mol/l fructose solution and 1 ml 0.1 mol/l HCl.They were heated in a boiling water bath. After 1 hour in the first tube was added 0.5 ml 0.01 mol/l BA and in the second tube 0.5 ml 0.01 mol/l phenobarbital.The reaction tubes were incubated 1h at

40^oC.The UV-VIS spectra revealed that BA and HMF produced a yellow product with a maximum absorbtion at 395nm while the phenobarbital and HMF did not produce a color solution.

The reaction between HMF and BA was studied via UV-VIS spectra/Fig.1/.On Fig.1: 0- 0.1 mol/l fructose solution; $0'$ - 0.1 mol/l fructose solution after 1h heating in a boiling water bath and after 10-fold dilution,maximum absorbance at 284nm/HMF/;1- the condensation product of HMF and BA after 10 min heating in a boiling water bath,maximum absorbance at 395nm;2- after addition of equimolar quantity of TBA and 10 min heating in a boiling water bath,maximum absorbance at 443nm;3- HMF and equimolar quantity of phenobarbital; and 4- HMF and 1:1.5 volume/volume distilled water.

The reaction solution of HMF and BA was vacuum dried.The solid residue was recrystallized 5 times in methanol.This yielded dark brown slab crystals with a m.p. 255-257°C.

Elemental analysis: C- 50.44%/calc.50.85%/,H-4.17% /calc.3.39%/and N- 8.81%/calc.11.86%/.Unfortunately, our attempts to achieve better results for nitrogen analysis failed.As a possible explanation of this fact may serve that the nitrogen is included in a pyrimidine ring.It is known that such compounds sometimes give lower results for N-analysis.

In the mass-spectrum obtained with electronic ionization there is a base peak at $M/_e$=205 which

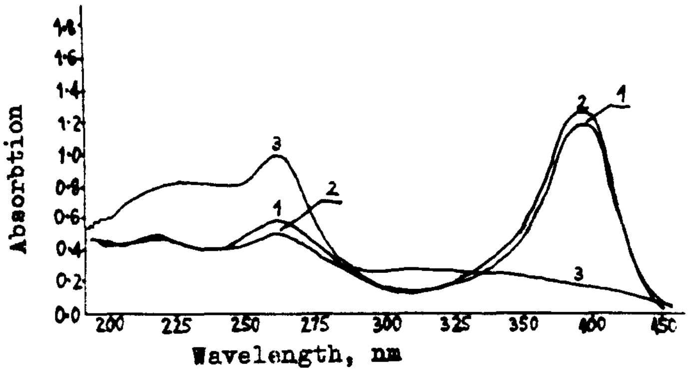


FIG.2 UV-VIS spectra of water solution of crystal product- 1,after introducingHCl to a pH about 1- 2,and after introducing NaOH to a pH about 13- 3.

corresponds to $(C_9H_5N_2O_4)^+$ i.e. an ion after a breaking away of the group HO-CH_2.In addition,there are two other peaks one for the molecule-ion,$M/_e$=236,$(C_{10}H_8N_2O_5)^+$ and one with $M/_e$=250.We suppose it is an ion formed after a recombination of the molecule ion with a CH_2 group- $(C_{11}H_{10}N_2O_5)^+$.

The water solution of these crystals absorbs at 395nm and has a pH=6/Fig.2,1/.After an addition of HCl to a pH about 1 the peak at 395nm slightly increases its intensity but this one at 260nm/aromatic structure/ slightly decreases/Fig.2,2/.After introducing a crystal of NaOH to a pH about 13 the absorbance at 395nm and the yellow color completely disappears,but the absorbance at 260nm shows a strong increase/Fig.2,3/.The reaction mixture of HMF and BA gives the same spectra when

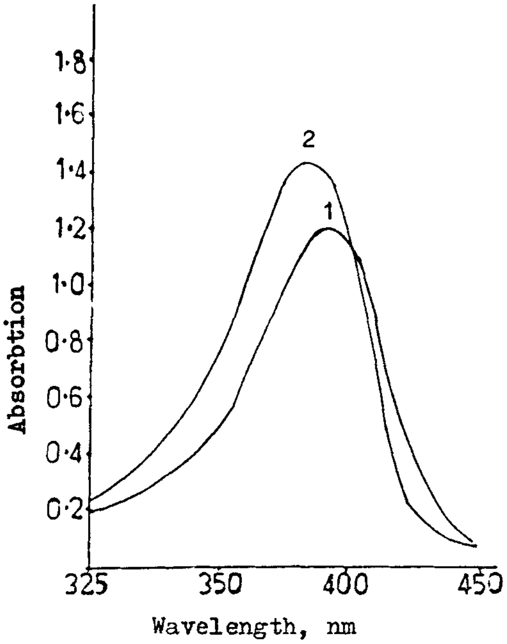


FIG.3 UV-VIS peak when crystals are dissolved in water-1; and in ethanol- 2

introducing HCl to a pH about 1 or NaOH to a pH about 13.

We have measured the absorbance of the crystal product when dissolved in different solvents.Fig.3: 1- in water and 2- in ethanol/more non-polar as compared with water/.

We have made IR-spectra of HMF/Fig.4/,BA/Fig.5/, the product of their reaction/Fig.6/and of the product after alkalization with NaOH in KBr tablets/Fig.7/.We have made also IR-spectrum of the crystal product in

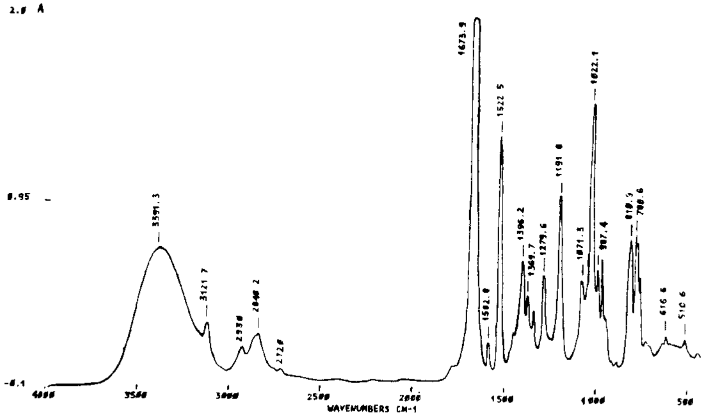


FIG.4 IR-spectrum of HMF

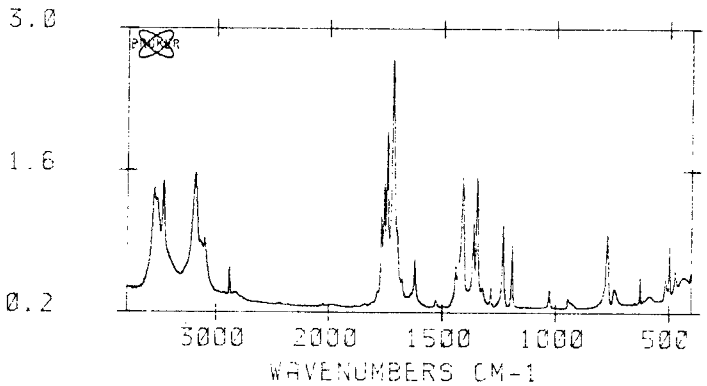


Fig FIG.5 IR-spectrum of BA

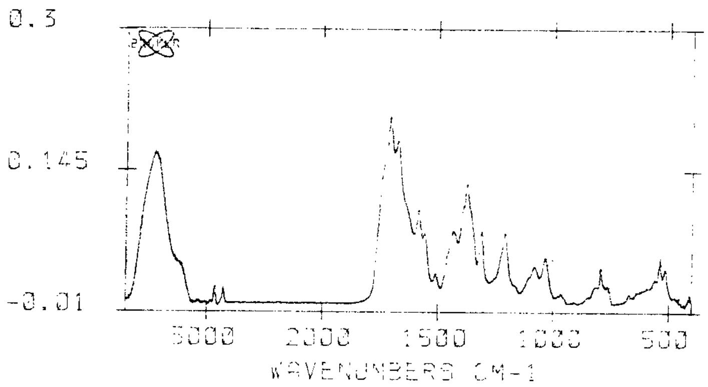


FIG.6 IR-spectrum of HMFBA

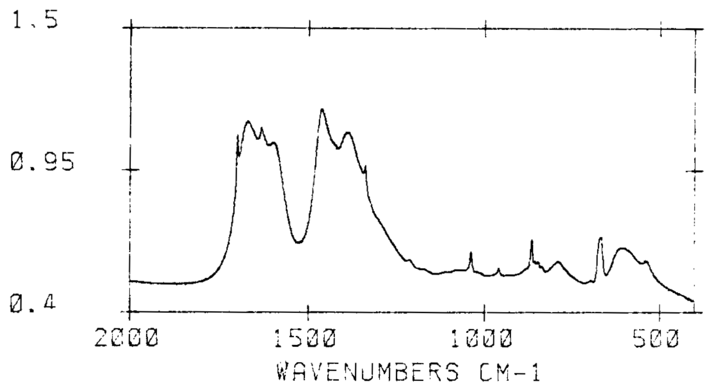
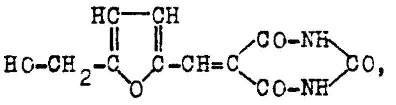


FIG.7 IR-spectrum of HMFBA after alkalization with NaOH/the sodium salt of the product after drying/

D_2O and after vacuum drying in the regions 4000-2800cm^{-1} and 1800-1250cm^{-1}/Fig.8/.The results are given in TABLE 1.

RESULTS AND DISCUSSION

The methylene group in pyrimidine ring of BA reacts with aldehyde group of HMF and leads to a product with the same structure as described in[7]:



named by us 5-hydroxymethylfurfurylidene-barbituric acid/HMFBA/.

Phenobarbital which has a blocked CH_2 group does not produce a color product.

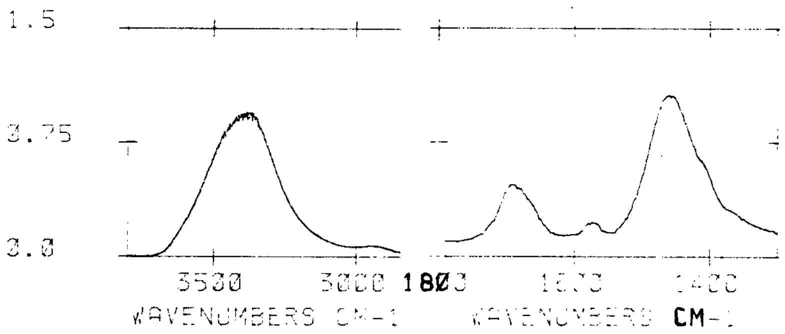


FIG.8 IR-spectrum of HMFBA after dissolution in D_2O and drying

From UV-VIS spectra it is evident that the product HMFBA has a maximum absorbance at 395nm and we suppose that it could be connected with a chynoide-like chrompphore after enolization of 4- and 6- CO groups in the BA ring/See Mechanism,4/.When introducing HCl in the HMFBA solution the 395nm peak increases and this one at 260nm decreases its intensity.It is in good agreement with the Mechanism.The Mechanism which we propose for this reaction,explains that in solution there is an equilibrium between 4b form of the compound and hydroxylic one/3a and 3b/.The additional acidifying of the HMFBA solution prevents the full enolization of 3a to 3b/Mechanism/and the peak at 260nm lowers.The additional alkalifying,on the opposite,breaks the chynoide-like structure/4b/and forms the product of full enolization/3b/.It is the reason of aromatization of compound in alkali conditions which leads to an

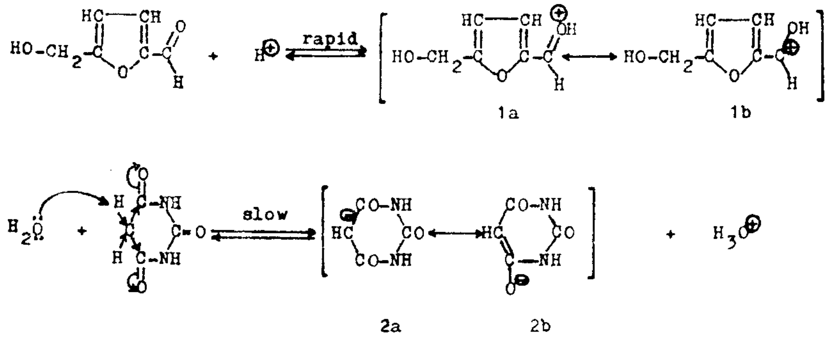
TABLE 1

Wave numbers, cm^{-1} of the functional groups of: HMF, BA, HMFBA, HMFBA as sodium salt.

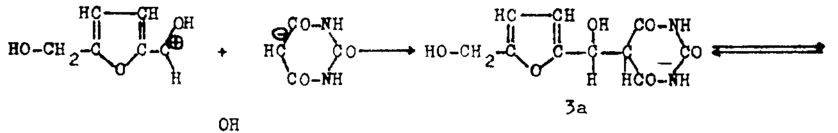
γ-valent vibrations and δ-deformation vibrations

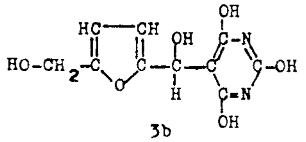
Compound	γC=O	γC=C	hidrogenium bond	γ =C–H	γC–H CH_2	γC–O–C	γC–OH	δC–H	γNH amide	γC=N
HMF see Fig.4	1674	1583 1523	3391	3121 2720	2930 2840	1192	1022	811 781		
BA see Fig.5	1768 1757 1744 1719				2925 2853			775	3246 3189 3101	
HMFBA see Fig.6	1708	1581 1559 1508	3453		2926 2853	1209	1078 1034	794		1673
HMFBA- sodium salt see Fig.7		1632 1598						666 865		1698 1670

MECHANISM:

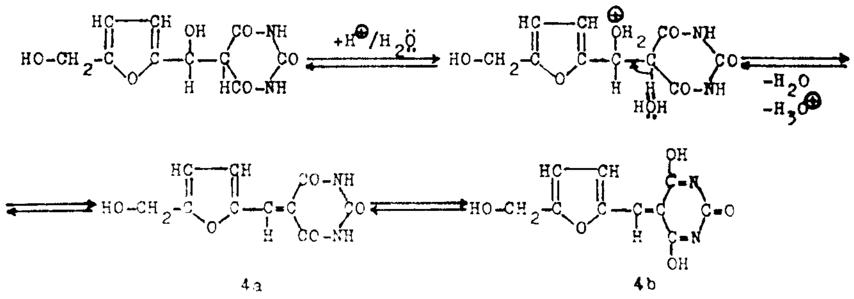


A nucleophylic addition of 2a to 1b:





The pyrimidine ring of 3b absorbs at 260nm



4b form of HMFBA has a chromophore/chynoide-like structure/which absorbs at 395nm.

increasement of the absorbance at 260nm.IR-spectrum of the dried residue from the alkaline solution/Fig.7/ confirms our assumption that in this situation HMFBA is fully enolized.There is no band at 1708cm^{-1}.The appearance in this spectrum of an intensive band consisting of four bands 1632, 1598, 1698 and 1670cm^{-1} connected with valent/ν/C=C vibrations, ν C=N vibrations and the bands 666 and 865cm^{-1} connected with C-H deformation/δ/vibrations near to a couple bond demonstrate the truth of the triple enolized structure of the sodium salt of HMFBA.

The red shift/to longer waves/of the absorbance at 395nm in more polar solvent/Fig.3/points out a π-conjugated system of bonds/π-π^* electron transitions, **Mechanism**,4b/.

IR-spectrum of HMFBA in D_2O after drying shows a band associated with connected OH groups and only one band for CO group which is in agreement with a chynoide-like chromophore of the compound in water solution.

In IR-spectrum of BA/Fig.5/there are four bands for νCO vibrations: 1768, 1757, 1744 and 1719cm^{-1} while in IR-spectrum of HMFBA/Fig.6/there are two bands: 1708 and 1673cm^{-1}.This fact gives us ground to think that the band at 1708cm^{-1}is associated with the CO group between the two N-atoms from the barbituric fragment and this at 1673cm^{-1}for C=N couple bonds and that HMFBA crystalized in double enolized form of the pyrimidine ring.The dark brown colored crystals of

HMFBA, their high melting point and hard burn out on a metal plate confirm the chynoide structure of the compound/Mechanism, 4b/. The presence in the spectrum of HMFBA of bands at 1708 and 1034cm^{-1} connected with simple C-OH bonds is in agreement with the above cited structure too.

We have made UV-VIS spectral observations of the reaction of furfural/F/and BA. Furfurylidene-barbituric acid/FBA/which is product of this reaction has two main absorbances: at 260nm and at 385nm. The first is associated with aromatic structure in the molecule and the second with the chromophore appearing from double enolized form of barbituric fragment. By acidifying to a pH about 1 the absorbance at 260 nm decreases slightly while this one at 385nm increases. That means an equilibrium shift to a chynoide-like form of FBA with a chromophore in the molecule. By alkalifying to pH about 13, on the opposite, the absorbance at 385nm completely disappeares while this at 260nm increases. It could be associated with a full enolization of the hydroxyl form of the compound/Mechanism, 3b/.

This paper devoted to spectral determination of the structure of HMFBA will serve as a starting point of a creation of a new chemical method for determination the extent of non-enzymatically glycated proteins and hemoglobin.

REFERENCES

1. M.Keeney and R.Bassette, J.Dairy Sci.,42,945(1959).
2. R.Fluckiger and K.H.Winterhalter, FEBS Lett.,71, 356(1976).
3. R.E.Pecoraro,R.J.Graf,J.B.Halter,H.Beiter and D. Porte,Jr, Diabetes,28,1120(1979).
4. K.H.Gabbay,J.M.Sosenko,G.A.Banuchi,M.J.Mininsohn and R.Fluckiger, Diabetes,28,337(1979).
5. C.V.Subramanian,B.Radhakrishnamurthy and G.S. Berenson, Clin.Chem., 26,1683(1980).
6. K.Michael Parker,J.D.England,J.Da Costa,R.L.Hess and D.E.Goldstein, Clin.Chem., 27,669(1981).
7. A.W.Dox and G.P.Plaisance, J.Am.Chem.Soc., 38, 2156(1916).

Date Received: 07/30/87
Date Accepted: 08/31/87