

SYNTHESIS OF PYRIDAZINONE DERIVATIVES OF CHELATING
BIOLOGICAL INTEREST

BY

N.A. Sharaf El-Din, M.El-Kersh and H.M. El-Fatraty
Department of Pharmaceutical Chemistry, Faculty of Pharmacy
University of Tanta, Egypt.

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ABSTRACT

Different 1-substituted-4,5-diacetyl-1,2,4,5-tetrahydropyridazin-3,6-dione were synthesised by the reaction of diacetyl L(+)-tartaric acid anhydride with the corresponding hydrazine derivatives. The structure of the produced compounds was confirmed by microanalytical and UV, IR and ^1H NMR spectral data. The obtained compounds showed chelating behaviour toward certain transition metals (Cu^{++} and Fe^{+++}). The chelating characteristics were studied using spectrophotometric method. These compounds have also biological activities.

INTRODUCTION

Compounds containing a diazine system have a wide spectrum of biological activities e.g. antimalarial [5], antifungal [10] antiinflammatory [8], and selective plant growth regulator [4]. Since the mode of action of some compounds was attributed to the formation of stable chelates with the metals in the cell, such process inhibits

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so many vital enzymatic reactions [3,12] and also metal chelates were suggested more toxic than the metal or the chelator itself [1,4,11].

It was of interest to prepare the compounds containing a diazine system having a chelating behaviour and to study their biological activities.

RESULTS AND DISCUSSION

In the present work we prepared anhydride diacetyl-L(+)- tartaric acid (I) according to the reported procedure [6]. The pyridazinone derivative (II) was synthesised by direct condensation between compound (I) and hydrazine at 90°C for 3 hr. The reaction between compound (I) and 2,4-dinitrophenylhydrazine in acetic acid at room temperature or by fusion gave compound (III).

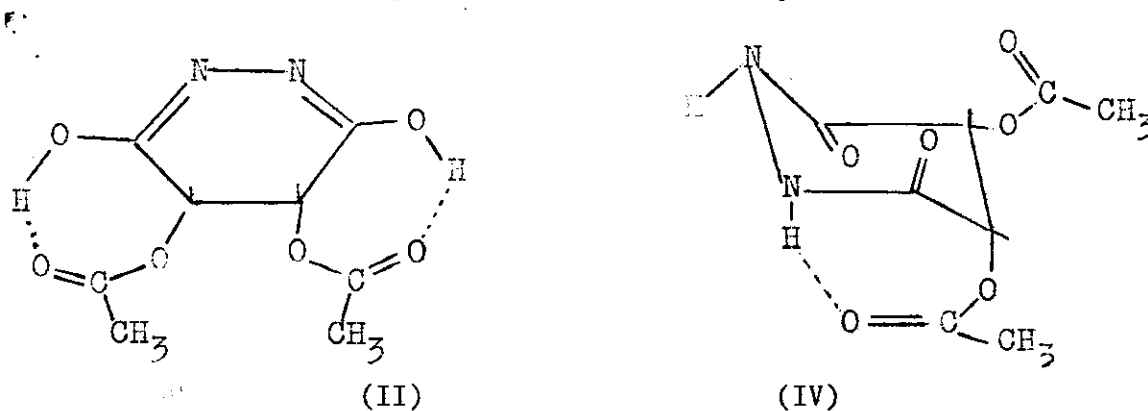
On the other hand, compound (IV) was obtained by condensation of compound (I) and phenylhydrazine at 120°C (see Scheme 1).

The structure of these compounds was established via microanalytical, UV, IR and ¹H-NMR spectral data.

The spectroscopic studies of the compounds II, III and IV led to the following results :-

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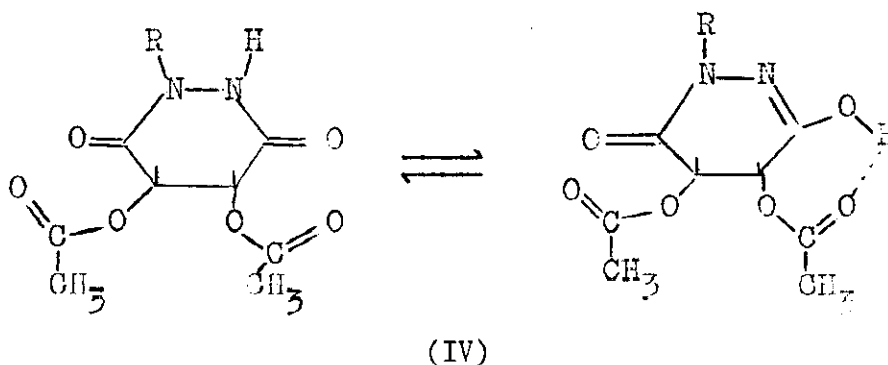
The presence of broad absorption bands in IR spectra at $3450 - 3300 \text{ cm}^{-1}$. This indicates that the compounds have associated hydrogen bond as the following :-



The electronic absorption spectra of the compound II (Fig. 1) show absorption on band at 245 nm at pH 9.0 while at pH 5.0 this band disappeared.

Also in $^1\text{H-NMR}$ spectra the N-H moiety appeared as two exchangeable separate signals (at 9.3, 9.7 and 9.7,10.3 for the compounds II and III respectively). These two signals are due to NH and/or OH associated hydrogen bonding. This gives an evidence that these compounds exhibit a tautomeric structure as shown below :

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The compounds II, III and IV reacted with Cu^{++} and Fe^{+++} producing coloured complexes. The chelation characters were investigated spectrophotometrically which was found to depend upon several factors as discussed below.

a- Chelation of copper with compounds (II) and (III):-

The reaction of the compound II with copper sulphate in aqueous media at pH 12 yielded bluish green coloured complex. The maximum absorbance of complex at $\lambda = 680 \text{ nm}$, $\epsilon = 6.5 \times 10^4 \text{ lit mol}^{-1} \text{ cm}^{-1}$. While the compounds III and IV gave deep green coloured complex, $\lambda 595 - 600 \text{ nm}$, $\epsilon = 12.5 \times 10^4 \text{ lit mol}^{-1}$ at pH 8.0.

b- Chelation of ferric ion with the compound (II):-

The compound II was reacted with FeCl_3 in aqueous solution at pH 12 to give a deep red coloured complex, $\lambda_{\text{max.}} = 455 \text{ nm}$, $\epsilon 9 \times 10^5 \text{ lit. mol}^{-1} \text{ cm}^{-1}$. (Fig. 2).

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c- Stoichiometry of metal compounds complexes :-

The stoichiometry of metal complexes with compounds (II) and (III) were investigated by continuous variation[9] method (Fig. 3). From the results we found that the maximum absorption occurs when the ratio of the compound II and metal ions are 1:1 and 3:2 for Cu^{++} and Fe^{++} respectively. While the maximum absorption of the compound III and Cu^{++} was produced at the ratio 2:1.

d- Effect of pH :-

The complex formation was affected by variation of pH of the solution (Fig. 4). The maximum absorbance was obtained at $\text{pH} > 8.0$.

e- Effect of time and temperature on the complexes formation:-

The colour of the complexes was produced immediately after the addition of the reagent. The maximum absorbances were obtained within 5 min. Also the absorbances of the complexes remained constant for long time (48 hr) at room temperature. The variation of temperature (25-80°C) did not affect the colour or absorbance of complexes.

f- Effect of interfering ions :-

The presence of other metals (5-30 ppm) did not interfere with the determination of Cu^{++} or Fe^{+++} . Also the copper- (II) complex colour was stable even in the presence of NH_3 or amines.

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The compounds were very sensitive to Cu^{++} and suitable for the determination of 1 ppm of copper specially the compound III.

On the other hand, the biological activities of the compounds and their copper II complexes were tested against bacteria, fungi and plant by agar diffusion method. The antimicrobial, antifungal results are summarized in Table 1.

From the test on the plant it can be noticed that the compound III has toxic action on the germination of seeds and inhibits the growth of roots. The action of the compound II was very interesting . At concentration 5.0 ppm the shoot length increased while at 100 ppm the root increased in length and in number.

EXPERIMENTAL

IR spectra were recorded on a Perkin Elmer 710 B. UV./vis. spectra were recorded on a Beckman Du 24 spectrophotometer.

The ^1H -NMR spectra were carried out using M-390-90 MHz NMR spectrometer. Both ^1H -NMR spectra and microanalysis were carried out at Microanalytical Unit, Cairo University.

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1- Synthesis of 4,5-diacetyl-1,2,4,5-tetrahydropyridazin-3,6-dione (II)

Equimolecular amounts of diacetyl-L(+)-tartaric acid anhydride and hydrazine hydrate were heated at 90°C for 3 hr. The crude product was triturated several times with acetone to a constant m.p. Yield 89% m.p. 170-172°C. The compound is insoluble in all ordinary solvents but soluble in the alkaline media.

Elemental analysis (C₈H₁₀N₂O₆)

	C %	H %	N %
found	41.2	4.5	12.5
Calcul.	41.7	4.3	12.2

IR spectrum : broad band 3600-3200 cm⁻¹ (OH and/or NH),
1710 cm⁻¹ (C=O), 1660 cm⁻¹ (amide), 1540 cm⁻¹
(C=N), 1250, 1080, 1140 cm⁻¹ (C-O-C).

¹H-NMR spectrum DMSO-D₆/pyrid. D₅
2.15 (s, 2CH₃), 5.1 and 5.3 (d, H-C-OAc), 9.3
and 9.7 (2s, NH and/ or OH).

2- Synthesis of 1-(2,4-dinitrophenyl)-4,5-diacetyl-1,2,4,5-tetra-hydropyridazin-3,6-dione (III) :-

Diacetyltartaric acid anhydride (0.1 mol) was added to the solution of 2,4-dinitrophenylhydrazine (0.1 mol) in

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acetic acid (20 ml). The mixture was left at room temperature for two days. The product was poured into cold 10% solution of Na_2CO_3 with stirring. The yellow crystalline product was obtained. The product was recrystallized from N,N-dimethylformamide and water. Mp. 173°C .

The same product was produced by direct fusion of the compound (I) with dinitrophenylhydrazine.

Elemental analysis ($\text{C}_{14}\text{H}_{12}\text{N}_4\text{O}_{10}$)

	C %	H %	N %
Found	42.5	3.1	14.5
calcul.	42.4	3.0	14.1

IR spectrum : 3450 cm^{-1} (OH), 3250 cm^{-1} (NH), 1720 cm^{-1} (ester), 1660 cm^{-1} (amide), 1560 cm^{-1} (C=N), $1250\text{--}1080\text{ cm}^{-1}$.

$^1\text{H-NMR}$ spectrum (DMSO-D_6) : 2.15 (s, 2CH_3), 7.2 and 7.4 (H-C-OAc) 8.2, 8.3 and 9.1 (d, $3\text{H}, \text{C}_6\text{H}_3(\text{NO}_2)_2$) 9.7 and 10.3 (s, NH and/or OH).

3- Synthesis of 1-phenyl-4,5-diacetyl-1,2,4,5-tetrahydro-pyridazin-3,6-dione (IV)

Equimolecular amounts of diacetyltartaric acid anhydride and phenylhydrazine were heated at 120°C for

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4hr. The product was poured into cold water with stirring. The yellow product was produced after recrystallization from N,N-dimethylformamide and water. Mp. 125°C.

Elemental analysis (C₁₄H₁₄N₂O₆)

	C %	H %	N %
found	54.3	4.8	9.8
calcul.	54.9	4.6	9.2

IR spectrum : 3450 cm⁻¹(OH), 3300 cm⁻¹(NH), 1715 cm⁻¹
(ester), 1660,1540 cm⁻¹(amide),1250,1080 cm⁻¹
(C-O, C-N).

Chelation with metal ions

- (0.025 mmol) Metals (CuSO₄.5H₂O and FeCl₃) solution in water were prepared and standardized by complexometric method [2].

- A stock solution of the compounds were prepared by dissolving a certain amount of each in appropriate volume of 0.1 N-NaOH.

General procedure :-

To a 1 ml of metals solution add a 2 ml (0.05 mmol)

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of the free ligand solution in 10 ml measuring flask and complete to mark with water. In all cases the pH of media must be adjusted at pH 12. The spectra were recorded within the wave length 200 - 800 nm.

Biological activities :-1- Antifungal, antibacterial activities :-

Procedure :-

Standards of 6 mm sterillized paper discs were impregnated with 20 mg/ml solution of the tested compound solution. The discs were placed on the top of agar plates seeded with the organisms. The seeded plates were incubated for 24 hr at 37°C.

The results were tabulated in Table 1.

Table 1 : Antifungal, antibacterial activities of the compounds and their Cu²⁺-complexes.

Organisms	Compounds			Cu ²⁺ only	Complex with Cu ²⁺		
	II	III	IV		II	III	IV
Fungi							
<u>Alternaria solani</u>	-	-	-	-	w	+	w
<u>aspergillus nigar</u>	w	+w	-	-	+	+	w
<u>Fusarium oxysporum</u>	+	+	-	- w	+	+	-
<u>S. cerevisia</u>	w	+		-	+	+	+
<u>Penicillium notatum</u>	-	-	-	w	w	w	w

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Bacteria

<u>Bacillus subtilis</u>	-	+	-	w	+	++	w
<u>Escherichia coli</u>	-		-	w	-	+	w

Whereas, - : wegative, + : active, ++ : more active,

2- Test on the plant :

Procedure :-

Agar solution 1.2% containing the required concentration (0.5, 5.0 and 100 ppm) of the compounds. This solution was poured while hot into 100 ml beaker and left to solidify. Each beaker was seeded with ten seeds (rice or barley) and left for 15 days.

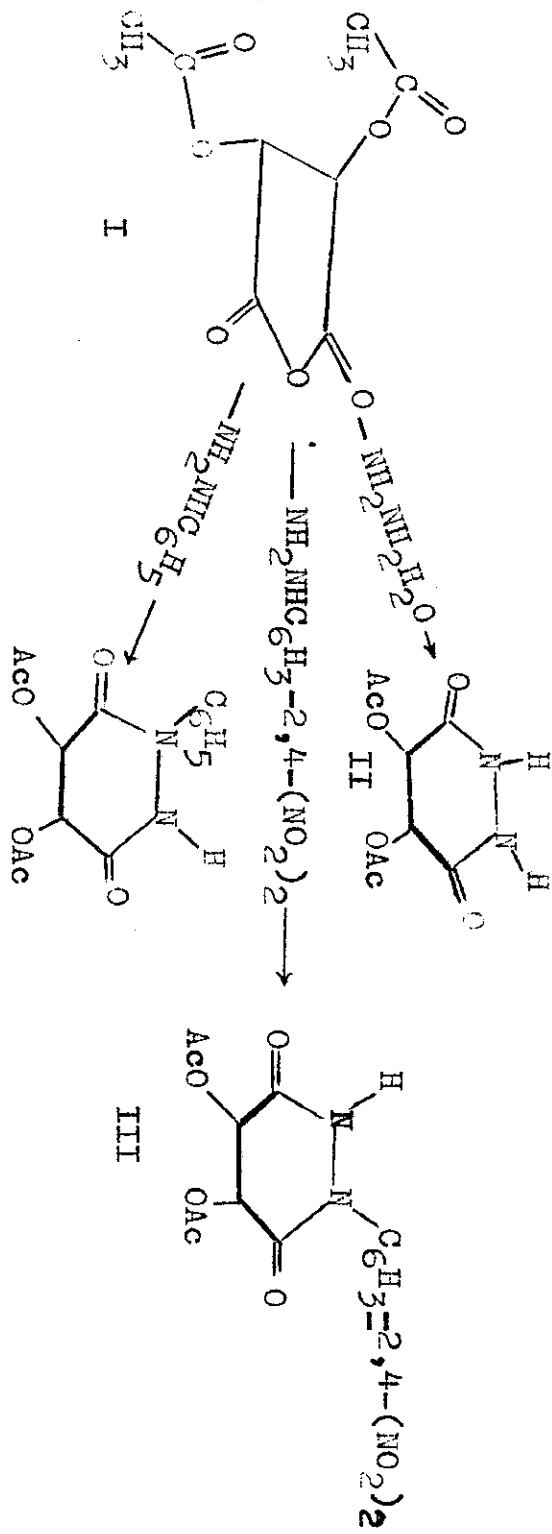
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Scheme 1

IV

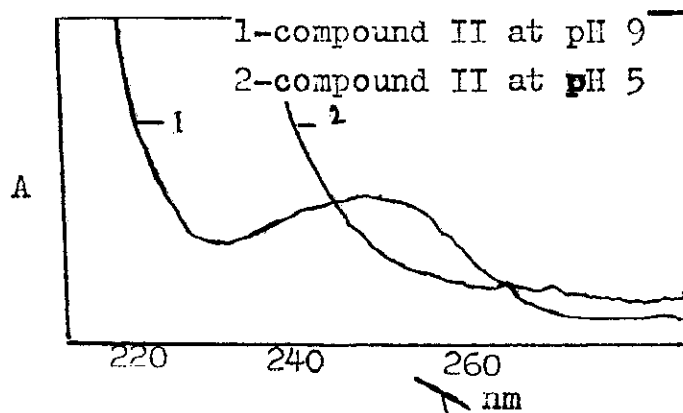


Fig. 1

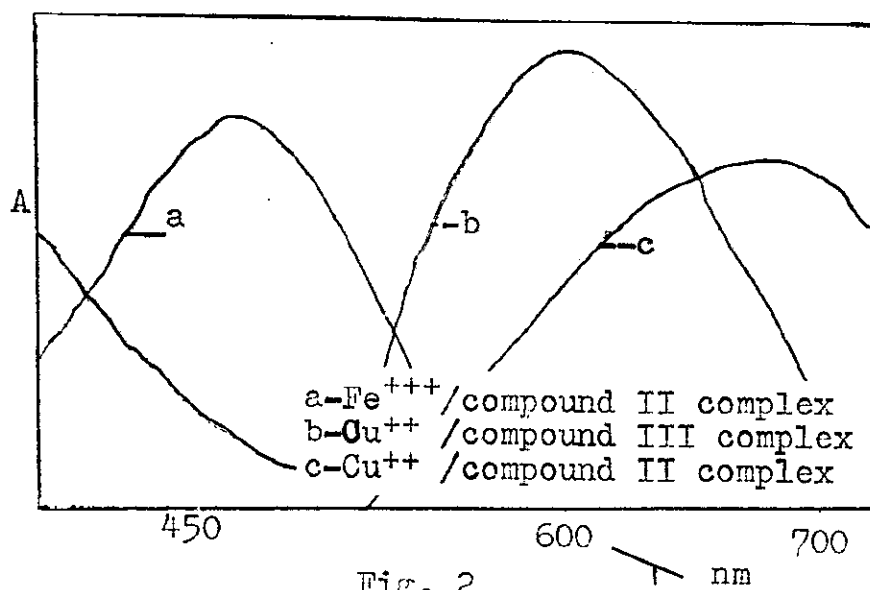


Fig. 2

where A = Absorbance
 λ_{nm} = wave length in nm

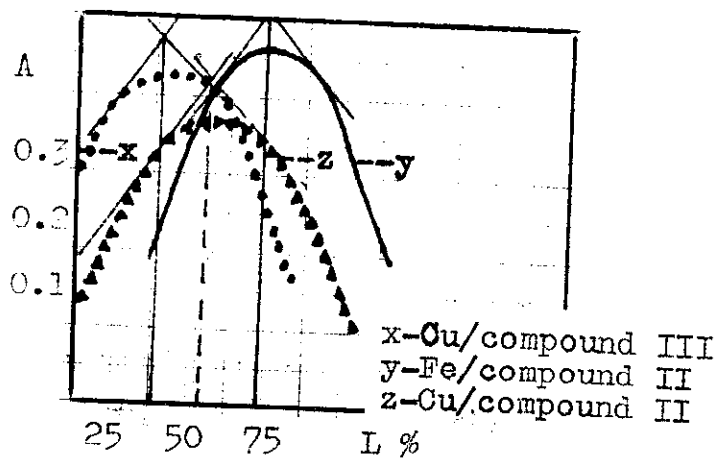


Fig. 3

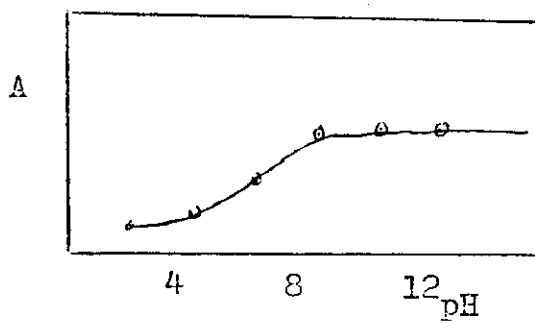


Fig. 4

تحضير مشتقات البيريدينون ذات الأهمية المخلاية والبيولوجية
نبوية شرف الدين - محمد القرش - حامد القطاطرى
قسم الكيمياء الصيدلانية - كلية الصيدلة - جامعة طنطا - مصر

تم تحضير ٥,٤ - ثنائي استيل - ١,٢,٤,٥ - رباعي
هيدروبيريدازين - ٣,٦ - ديون المستبدل فى الموقع ١ من تفاعل ثنائي استيل
أ تهيدريد الطرطريك مع مشتقات الهيدرازين المناظره .

وقد اثبتت التركيبات البنائية للمركبات بواسطة التحاليل الدقيقة
والقياسات الضوئية . وقد وجد أن لهذه المركبات خواص مخلاية تجاه بعض
العناصر . وتمت دراسته الخواص المخلاية بواسطة الطريقة الاسبكترو فوتومترية .
وايضا وجد أن لهذه المركبات أنشطة بيولوجية .

DETERMINATION OF THE SURFACE AREA OF CELLULOSE BY
NMR-SPECTROSCOPY AND PORE SIZE DISTRIBUTION

BY

A.A.Saafan and M.A.Sakran
Chemistry Department, Faculty of Science,
Tanta University, Egypt.

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ABSTRACT

Application of ^1H NMR spectroscopy, which has been shown to be an effective mean of measuring changes produced in the amount of bound water within cellulose in the wet state, has been extended to the study of alterations of the surface areas of cellulose produced by liquid ammonia treatments, under various conditions. These areas were compared with the surface areas as calculated from pore size distribution.

INTRODUCTION

Several workers [6,4,8] have shown that part of water within a moist cellulose polymer exhibits properties which are markedly different from those of the rest of the water. Filby and Maass [4] concluded from the high apparent density which could be calculated for cellulose when the volume was determined by the displacement of

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water that part of the water in the cellulose-water system had a higher density than that of free water. To estimate the quantity of the strongly sorbed water, Magne, Portas, and Wakeham [5] made use of the fact that chemically sorbed water will not freeze whereas capillary condensed or solvent water will freeze at a temperature lower than 0°C . Most of these investigations were carried out on cotton cellulose, and the experiments were repeated by many workers and yet there is a distinct lack of agreement upon the exact amount of bound water. Boesen has suggested that 0.12 g bound water per g cellulose was the best average value for cotton [1].

The application of NMR spectroscopy has also furthered the concept of bound water [2]. The water peak in the cellulose-water system is broader than that shown by free water, a fact which has been interpreted in terms of some of the water molecules being restricted in mobility owing to their proximity to the surface of the solid material. An attempt has been made to obtain more detailed information on determination of the amounts of bound water within cotton subjected to different kinds of liquid ammonia treatments. NMR technique was also used to determine the surface areas of moist cotton cellulose. These values have been compared with the pore surfaces calculated from the pore size distributions of the ammonia treated samples.

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EXPERIMENTAL

Materials

The starting material used was a woven cotton fabric (Printcloth 130 g/m²) warp 35 threads/cm, weft 322 threads/cm. crystallinity 79%.

Swelling treatments

The cotton samples were treated in a tensionless state with liquid ammonia for 5 minutes. The temperature of the ammonia swelling medium was maintained between -35 to -33°C. Ammonia was removed by either evaporation at room temperature or by immersing in boiling distilled water, and air dried. Some samples were then further used without intermediate drying.

NMR analysis

All samples were soaked in distilled water for two days. The samples were brought from a very wet state to the required moisture content by centrifugation. Each of the samples was packed as tightly as possible in the NMR tube. The tubes were kept stoppered until after the NMR analysis when an exact moisture content was determined by weighting the contents of the tubes before and after oven drying at 105°C for 20 hours. The instrument used was a Varian T60 NMR spectrometer. All experiments were carried out at standard measuring conditions (25°C and 65% R.H.).

Pore size distribution

The pore size distribution and surface areas were calculated from the pore volumes of the ammonia treated samples. The pore volumes were determined by sorption measurements with sugars and dextrans of graduated size as described in previous papers [2,7].

RESULTS AND DISCUSSION

In the following, consideration is given first of all to the method for calculating the amount of bound water from the values of the spin-spin relaxation time of the protons in the bound water. The mobility of a proton in NMR spectroscopy is correlated to its relaxation time (T_2), which can be calculated from the line width at half-height ($\nu_{1/2}$) according to the equation 1 :

$$T_2 = \frac{1}{\nu_{1/2}} \quad (1)$$

The amount of bound water can be calculated from the relaxation time (T_2) according to the equation 2 [3] :

$$T_2 = \frac{T_{21}}{[H_2O]_1} \times [H_2O]_T \quad (2)$$

Where T_{21} is the relaxation time of protons in the bound water and $[H_2O]_1$ is the concentration of bound water in

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the sample at a total moisture content of $[H_2O]_T$. According to equation 2, as long as $[H_2O]_1$ remains constant, a linear relationship should be found between T_2 and $[H_2O]_T$. From the slopes of the lines and by introducing $T_{21} = 3 \times 10^{-4}$ sec. for bound water, we obtained a value of the amount of bound water in the moist cotton samples. Table 1 lists the experimentally determined line with $\nu_{1/2}$ and the calculated relaxation time for the ammonia treated cottons at different moisture content $[H_2O]_T$. Figure 1 shows the linear relationships found between T_2 and $[H_2O]_T$ for the ammonia treated cottons. The obtained slopes of the lines $T_{21}/[H_2O]_1$, and the maximum values of the bound water as well as the fiber saturation values, which are the amounts of water in the fiber that are inaccessible for the macromolecule dextran 2000 [2] are given in Table 2. We notice a marked increase in the amounts of bound water has occurred as a result of liquid ammonia treatments, but to varying degrees depending on the method of removing the ammonia. Evaporative removal of ammonia caused an increase in the amount of bound water within cotton cellulose in the wet state of about 125%. Liquid ammonia treatment followed by removal of ammonia with boiling water caused an increase in the amount of bound water of about 129%. The strongest increase (156 %) being achieved by treatment with liquid ammonia and removal of ammonia with boiling water without intermediate drying.

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If it is assumed that the amount of bound water calculated by the NMR technique are attached to the cellulose surface in a monomolecular layer the required surface area for this can be calculated. Taking 3000 m^2 for the area of a gram of water spread out as a monomolecular layer [3] and multiplying the amounts of bound water by the factor 3000, we obtain values for the surface area of ammonia treated cottons (Table 2).

With the help of a determination of the pore size distribution of water swollen cotton cellulose by the exclusion method [9], it is possible to determine the surface area of moist cotton cellulose subjected to different kinds of liquid ammonia treatments. In Table 3 these areas are compared with the surface areas as calculated from the amounts of bound water. The results of this investigation show that the values of the pore surface available for monomolecular water sorption also increased after liquid ammonia treatments. The largest increase in the surface area values occurred in samples treated with liquid ammonia with removal of ammonia with boiling water without intermediate drying. The correlation between the surface area from NMR technique and those from the pore size distribution is shown in Figure 2. It can be seen that, as expected, the absolute values of surface area obtained by the two methods differ, the agreement is

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surprisingly close considering the wide difference in approach of the two methods in surface area determination of cotton in the wet state.

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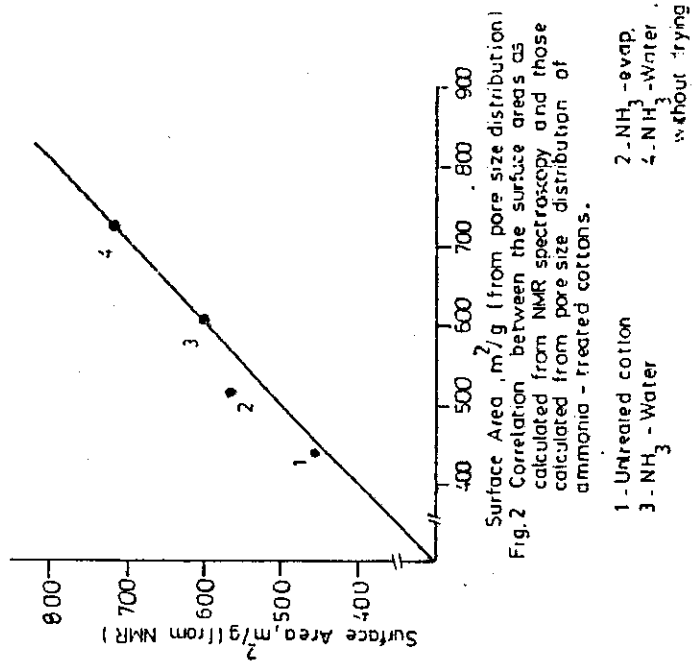
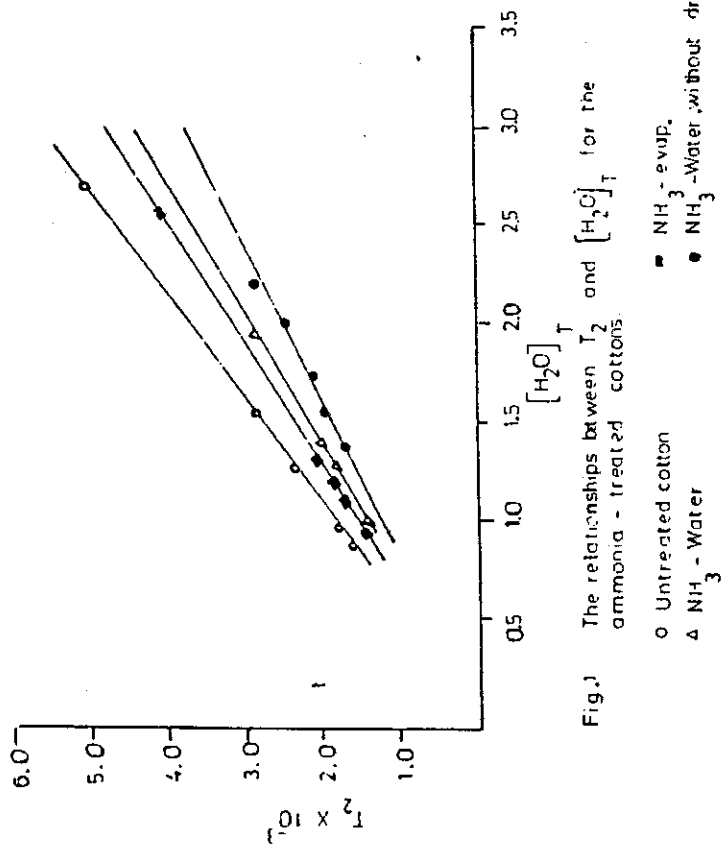


Table L. Line width at half-height ($\nu_{1/2}$) and the spin-spin relaxation time (T_2) at moist cottons at different moisture contents $[H_2O]_T$

Treatment	$\nu_{1/2}$	$T_2 \times 10^{-3}$	$[H_2O]_T$
Untreated cotton	63	5.055	2.72
	112	2.843	1.552
	174	1.83	1.145
	194	1.64	0.903
NH ₃ -evap.	79	4.03	2.53
	158	2.016	1.308
	176	1.809	1.18
	182	1.749	1.133
	212	1.502	0.977
NH ₃ -H ₂ O	109	2.94	1.92
	146	7.18	1.40
	170	1.87	1.31
	202	1.58	1.04
NH ₃ -H ₂ O without drying	115	2.769	2.203
	150	2.12	1.72
	162	1.965	1.59
	184	1.73	1.405

Table 2. Experimental Results on moist NH_3 - treated cottons

Treatment	SLOPE OF Fig.1 $T_{21}/[\text{H}_2\text{O}]_1$	Amount of bound water g/g ^{a)}	Fiber saturation value, g/g ^{b)}	surface area m^2/g
Untreated cotton	1.945×10^{-3}	0.154	0.49	462
NH_3 -evap.	1.562×10^{-3}	0.192	0.47	576
NH_3 - H_2O	1.5×10^{-3}	0.2	0.5	600
NH_3 - H_2O without drying	1.25×10^{-3}	0.24	0.63	720

a) Calculated from slope of Fig.1 using $T_{21} = 0.3 \times 10^{-3}$ sec.

b) The fiber saturation values of cottons were determined by the solute exclusion technique (c). Dextran 2000 was used.

Table 3. Surface areas for pores of various size in differently ammonia-treated cotton cellulose.

Specimen	Surface areas for the Pore diameter ranges					Total	
	$< 20 \text{ \AA}^\circ$	$= 20-50 \text{ \AA}^\circ$	$= 50-100 \text{ \AA}^\circ$	$= 100-150 \text{ \AA}^\circ$	$= 150-300 \text{ \AA}^\circ$		$> 300 \text{ \AA}^\circ$
Untreated cotton	340	66	17	7	4	2	436
NH ₃ -evap	410	7.1	19	6	2	-	508
NH ₃ -H ₂ O	0	74	18	4	2	-	598
NH ₃ -H ₂ O without drying	580	103	17	14	1	-	715

تعيين المساحة السطحية للسليولوز باستخدام طيف الرنين

المغناطيسى النووي وتوزيع المسامات

أحمد عبد الفتاح سعيان ، محمد عطية سكران

قسم الكيمياء - كلية العلوم - جامعة طنطا - مصر

تم تطبيق طيف الرنين المغناطيسى النووي لتعيين كمية الماء الرابطة كيميائيا على سطح السليولوز وبواسطة هذه الطريقة تم حساب المساحة السطحية للسليولوز ومقارنتها بالنتائج المحسوبة من توزيع المسامات داخل السليولوز ذو الحجم المختلفه والتي تم قياسها بواسطة محاليل مختلفة من الجلوكوز والرافينوز والديكسترانات.