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The Effect of Ring Size on the Biological Activity of Nitrogen Containing Compounds

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الخلاصة:

تمت دراسة العلاقة بين سرعة انفصال التريتيوم من الاسيتوفينون المعلَم اشعاعيا كمقياس للفاعلية البايولوجية واحد المؤشرات الفيزيوكميائية المهمة (معامل التوزيع) بين الاكتانول والماء.

الهدف من هذه الدراسة هو تحديد مختلف جوانب التركيب الكيميائي (حجم الحلقة وتاثير التعويضات فيها) للمركبات العضوية الحاوية على النايتروجين كجزء من مجموعة القلويات ذات التاثيرات البايولوجية المهمة واستعمال ثنائي مثيل السلفوكسيد كمذيب للمحافظة علي قلوية المواد.

تم قياس التحلل الاشعاعي بواسطة جهاز السنليشن و قياس معامل التوزيع بواسطة صفائح الكروماتوجرافيا الرقيقة TLC.

أظهرت النتائج وجود علاقة طردية بين المتغيرين عدا المورفولين حيث احتوائه على ذرة اوكسجين في تركيبه غير من طبيعته القاعدية، ولم تغير التعويضات (المثيل) في الحلقة او حتى عند ذرة النيتروجين على العلاقة الا اذا صاحبها عامل آخر مثل إمكانية دخول المزدوج الألكتروني على ذرة النتروجين برنين مع ذرة قريبة.

Abstract:

The biological activity of N-containing ring compounds was studied as a function of the distribution coefficient of these compounds in 1-octanol/water system. The reaction used to monitor activity is the detritiation of ^{3}[H]-acetophenone in dimethylsulfoxide affected by the nitrogen bases understudy at 25.0^{o}C

A strong correlation was found between the rate of detretiation of labeled acetophenone and the basicity of N-containing ring cyclic compounds. The correlation coefficient and regression constant were 1.0321 and R^2= 0.9415 respectively. This finding shows that similar factors were involved in both cases. Whenever these bases are part of the structure of a drug e.g., they may cause

deprotonation of some alkyl groups e.g an acyl hence altering its distribution and binding to the receptor.

Key Words: Detritiation, Partition Coefficient, TLC, DMSO

Introduction:

Recently amines have been used to prepare nanoparticle conjugates that can be encapsulated with drug for better loading and monitoring in multimodal imaging; positron emission tomography (PET), near infrared resonance florescence (NIRF) and magnetic resonance imaging (MRI) especially for early monitoring, at molecular level, of cancer [1]. In these studies the dipolar aprotic, dimethyl sulphoxide (DMSO) solvent with its unique salvation properties plays a major role in modifying solubility. In other cases DMSO is directly linked to the metal oxide of the nanoparticle [2].

The importance of amines in medicine comes from the fact that many neurotansmitters are mines. These include histamine, dopamine, serotonin, epinephrine and norepinephrine[3]. Therefore drugs are designed to interfere with the action of these neurotansmitters. Examples of these drugs include amphetamines, ephedrine, chlorpromazine and amoxapine. N-containing compound form a major part of an important group of biologically active compounds, the alkaloids. The activity of these natural products is attributed mainly to the basicity of the nitrogen atoms acting as lone pair donors. However, such a character is affected by neighboring groups or atoms. The solvation of the protonated amine as well as steric hindrance of groups attached to nitrogen affect the availability of electron, hence basicity. The latter has been shown to increase in the dipolar aprotic solvent dimethylsuphoxide, DMSO. The reaction rate of the reactions of *p*-nitrophenyl acetate with a series of alicyclic secondary amines in H_2O and in DMSO was studied. The solvent change from H_2O to DMSO resulted in rate enhancements. The effect of solvent on reactivity was found to be most significant for the reaction with piperazinium ion and least significant for the reaction with piperidine. The change in solvent from water to DMSO resulted in rate enhancements[4].

Frenna and Vivona studied the basicity of amines in benzene and water. They found that structural effects for secondary cyclic amines were higher in water than in benzene [5].

The relationship between structural changes and the physiochemical properties of which the partition constants play a central role in QASR studies and determines drug distribution in biological compartments was studied for some drugs[6]. This work concentrates on investigating structural changes of cyclic amines at 25.0^{o}C.

Material and Methods:

All chemicals used in this work were of analytical grade supplied by Sigma-Aldrich. DMSO was dried over 4A molecular sieves before being fractionally distilled under reduced pressure in the presence of nitrogen. Water content of DMSO < 0.1% w/w, was checked with GLC. The titration of acetophenone in DMSO was carried out by the solvent exchange method as has been described [4] ^{3}H-NMR was used to check for the correct positioning of the label. Determination of the observed rate constant values for the detritiation of tritiated acetopheneone was carried out as has been previously described [8] . The decrease in radioactivity of the substrate was followed using the initial-rate method. A small drop of dimethyl sulphoxide containing the tritiated substrate was added to a thermostated, at 25.0 ± 0.01^{o}C, an amine containing dimethyl sulphoxide. Fixed volumes, typically 2.0 ml of the reaction mixture were periodically injected into tubes containing 10.0 ml of scintillator, diphenyloxazole in sulfur free toluene, and 10.0 ml of demonized water. Radioactivity counting was carried using a Nuclear Enterprpises N. E. 250 Scintillator and the pseudo first-order rate constants, k_{obs}^T were determined using the initial rate constant technique.

The distribution coefficient or constant [9], as one of the main descriptors in QASR studies, is commonly determined by the classical shake and separate flask method. Other techniques including TLC and HPLC are also used [10]. In this work R_f values of amines were measured. A TLC plate is precoated with 1-octanol and allowed to dry. The plate is allowed to develop after applying the amine to the origin. The mobile phase is a mixture of 10% acetone-water [12]. UV lamp was used to visualize the spots. Measurements are made in duplicate and the average is taken. Partition coefficients were determined form the equation:

$$P = k/ (1/R_f -1)$$

Were k is a constant. In this work k it was determined from the slope of P against $(1-R_f/R_f)$ using data of similar compounds, aromatic amines determined previously.

This method was used because it works best for compounds of similar structures and properties. DMSO was chosen because it has high dielectric constant, 48.9, compared to organic solvents but lower than water 78 at 25^{o}C. It can penetrate organic layer and dissolve a wide range of substances, ionic and covalent. It mixes well with body fluids and non-harmful to heath [12].

Results and Discussion:

Studying proton transfer reactions using the detritiation reaction was recommended by a wide range of laboratories where facilities are available[13]. The study in DMSO was of particular importance as this solvent is unique in its

salvation properties hence use a carrier for some drugs as it has high penetration power in the lipid bilayer [14] .

The results and the corresponding graphs for the detritiation of tritiated acetophenone in DMSO catalyzed by unsubstituted and substituted nitrogen – containing compounds at 25.0±0.1^{o}C are shown in table-1 and Fig.1, 2.

The relationship is strong when similar structures, unsubstituted cyclic amines, are used with 1.0321 and R^2= 0.9415. Such a relationship falls apart with heavy substitution at the reaction center as in 1,2,2,6,6-petamethypyrrolidone (No.8) The situation becomes worse when such steric hinderance is accompanied by unavailability of the nitrogen lone pair due to conjugationas in 3,3,6,9,9-pentamethyl-2,10-diazobicyclo[4,4,0]-dec-1-ene (No.9). The introduction of an oxygen atom at the ring as in morpholine (No.12).

Ring size strain as well as the availability of the nitrogen lone pair is the main factors affecting such a relationship. Steric hinderance at the reactive centre, the nitrogen atom, has an important role in affecting the reactivity. Such a relationship still hold for similar ring compounds containing alkyl substituents but the presence of an oxygen atom in the ring changed the situation. The change in polarity in as in morpholine might have greater impact on the partition coefficient compared to reaction rate.

Other structural aspects of nitrogen containing compounds will be studied so that a clearer picture will be built about the structural aspects of the compound and their relation to activity. The acetyl containing compounds form a large group of biologically active compounds and proton transfer are important in drug binding as well as enzyme catalysed reactions.

No.	Name of Amine	Partition Coefficient	log Rate of Detritiation
1	Dimethylenimine	-0.41± 0.09	2.33± 0.03
2	Trimethyleneimine	-0.27±0.07	2.96±0.05
3	Pyrrolidone	0.21±0.07	3.34±0.05
4	Pipridine	0.57±0.19	3.78±0.07
5	Hexametheneimine	0.97±0.23	3.96±0.07
6	Heptamethyleneimine	1.38±0.21	4.4±0.08
7	N-methylpyrrolidone	0.55±0.19	4.62±0.08
8	1,2,2,6,6-petamethypyrrolidone	2.05±0.29	5.97±0.08
9	3,3,6,9,9-pentamethyl-2,10-diazobicyclo[4,4,0]-dec-1-ene	2.66±0.31	2.44±0.06
10	1,8-diazobicyclo-[5,4,0]undec-7-ene	0.42±0.14	2.47±0.04
11	1,4-diazobiyclo-[2,2,2]octane	-0.24±0.09	4.62±0.08
12	Morpholine	-0.77±0.18	5.4±0.08

Table -1: Results of Detritiation of Tritiated [^{3}H] Acetophenone by Cyclic Amines in DMSO at 25.0^{o}C

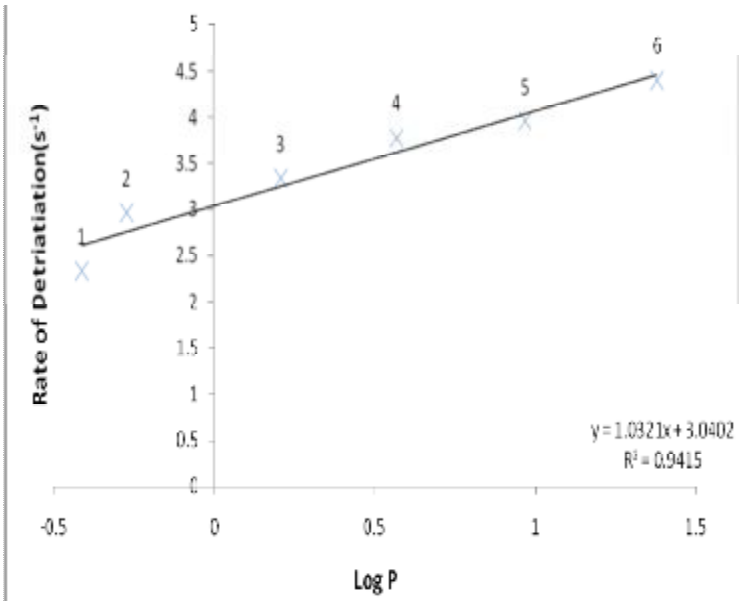

Fig. 1: Rate of Detritiation vs Distribution Coefficient for Some Unsubstituted Cyclic Amines.

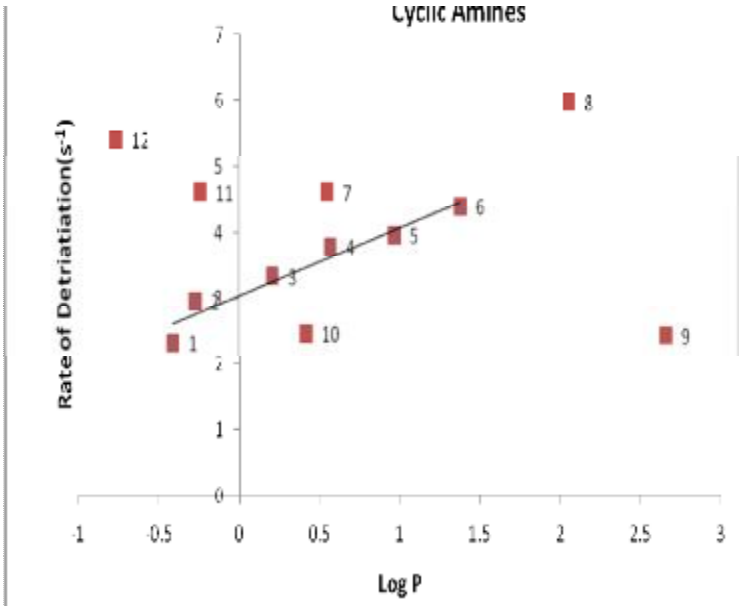

Fig. 2: Rate of Detritiation vs Distribution Coefficient for Some Cyclic Amines.

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