ON THE SUPPOSED "4-MORPHOLINYL-(THIOCARBONIC) ACID AMIDE"

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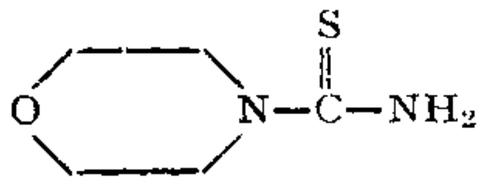
IN connection with our investigations of coordinating characteristics of morpholine and its derivatives, preparation of substituted thiourea compound of morpholine was attempted. It is well known that when secondary amines (RR'NH) are reacted with isocyanates and isothiocyanates in basic medium substituted ureas ($RR'NCONH_2$) and thioureas ($RR'NCSNH_2$) are formed respectively.[1] Morpholine is a typical secondary amine and so it is also expected to give similar type of compound, namely, 4-morpholinyl-(thiocarbonic) acid amide (MTCA) when it is treated with, say, potassium thiocyanate under alkaline conditions. Henry and Dehn[2] indeed reported the isolation of this compound,

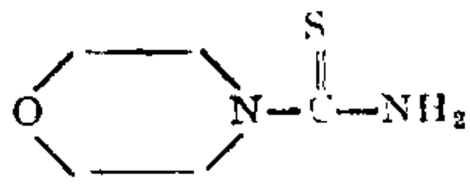


(m.p. 112·5–115·5° C.), under slightly different conditions by dissolution of morpholine hydrochloride and potassium thiocyanate in 1 : 1 mole ratio in water and evaporating the solution to dryness. The dry solid was extracted with anhydrous ethanol to separate the insoluble potassium chloride formed and the solution was concentrated to get the crystals of MTCA. We attempted the preparation of above compound exactly under similar conditions. The melting point of the compound prepared by us agreed with that of the reported value. The microanalytical estimation of carbon and hydrogen in the compound supported the formula $C_5H_{10}ON_2S$.

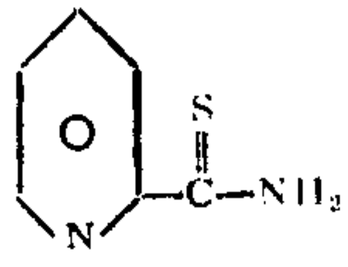
However, aqueous solution of this compound, even after its repeated recrystallisations gave an intense red colour with acidified ferric solution, a reaction which is characteristic of thiocyanate group and not of a thiocarbamide. The presence of morpholinium ion and thiocyanate ion in the aqueous solution of the compound was confirmed by ion exchange experiments. Passing 20·00 ml. of 0·1 M solution of the compound through a column of Dowex 50 W–X 8 cation exchanger (in the H^+ form) released 2 m. equivalents of hydrogen ions. In the same way passing 20·00 ml. of 0·1 M solution of the compound through a Dowex 2–X 4 anion exchanger (in the OH^- form) released 2 m. equivalents of OH^- ions. In the latter case thiocyanate ion was detected in the anion column by ferric test. In addition to simple chemical tests and ion exchange experiments the molar conductances of this compound were measured for 10^{-3} M solutions in water, anhydrous methanol and acetone at room temperature and they were found to be 139·3, 113·1 and 80·19 ohm^{-1} $cm.^2$ respectively. These values show the ionic nature of the compound in solution phase.

So, it appeared that the compound isolated by Henry and Dehn's method might have been only morpholinium thiocyanate. Or it could be that the compound existed as MTCA in the solid state but dissociated in polar solvents to give morpholinium and thiocyanate ions. This type of dissociation for various substituted thioureas has been studied by Shaw and Walker.[3] However, the rate of transformation reported by them for various thioureas is extremely small. To ascertain the state of the compound in the solid state infrared spectrum of this was taken using KBr pellet technique in a Perkin Elmer 337 instrument. The spectrum of the compound contained strong absorption bands around 2050 and 750 $cm.^{-1}$. These are characteristic of C–N and C–S stretching modes in ionic thiocyanates.[4] This strongly favours the formulation of the compound as

and not as



even in the solid state. It is to be pointed out that 2-amidothiopyridine



a thiocarbamide[5] with carbon attached to the ring system, as would have been also in MTCA,

does not exhibit any absorption bands around 2050 and 750 cm.$^{-1}$. This is a strong argument in favour of the ionic structure for the compound.

An alternate procedure for the preparation of the supposed MTCA was attempted starting from a mixture of morpholine and ammonium thiocyanate containing morpholine in slight excess than corresponding to 1 : 1 molar ratio. Ammonia, being more volatile than morpholine, was displaced by morpholine from ammonium thiocyanate by heating the mixture on the water-bath. The crystals obtained on cooling the concentrated solution were washed with acetone and recrystallised from anhydrous ethanol. The melting point, chemical behaviour and infrared spectrum of this compound were identical with those got for the compound which was prepared by adopting Henry and Dehn's procedure. Thus we conclude that both methods of preparation yield only morpholinium thiocyanate and not MTCA.

Keeping the morpholinium thiocyanate at the melting point for long periods and cooling it also did not bring about any conversion to thiocarbamide. Such conversions sometimes do occur as in the well-known case of ammonium thiocyanate to thiourea. It is strange that morpholine alone, among many of the secondary amines should behave in this manner to give morpholinium thiocyanate and not thiocarbamide.

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2. Henry, R. A. and Dehn, W. M., *J. Am. Chem. Soc.*, 1950, **72**, 2806.
3. Shaw, W. H. R. and Walker, D. G., *Ibid.*, 1957, **79**, 2681.
4. Burmeister, J. L., *Co-ordin. Chem. Rev.*, 1966, **1**, 205.
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CHROMOSOMAL ANALYSIS OF YOSHIDA ASCITES SARCOMA IN RATS

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INTRODUCTION

CHROMOSOMAL patterns of cancer cells have been studied extensively to elucidate correlation if any, between abnormalities of structure and number of chromosomes with the biological characteristics of the tumour.[1]

While the chromosome constitution of some tumours remain fairly stable during serial animal passages and different transfer generations, very often the derived sublines from the parent strain show considerable variation. This type of variations become more frequent with environmental changes like the strain of the animals used for maintenance of the tumours, the source material transplanted, whether ascites cells or cells obtained from solid forms of the same type of tumour, etc.

The present investigation was undertaken to study the chromosomal pattern of the Yoshida ascites sarcoma maintained in our laboratory and their stability or variability in different transfer generations during animal passage over the last few years.

MATERIALS AND METHODS

Yoshida ascites sarcoma (YAS) obtained from the Indian Cancer Research Centre, Bombay, who in turn had received the tumour from Professor Druckeray of Germany is being maintained in our laboratory since four years in inbred strain of wistar rats A/I.I.Sc. Intraperitoneal transplantation of 25 to 30 million cells every 5th day is the maintenance routine. The ascites developed with this dose becomes hæmorrhagic by 5th day and the average survival period of the animal is seven days.

At one stage, after about 80 transplant generations, the ascites form had to be regenerated from the solid tumour by homogenising the tumour mass and injecting the suspension intraperitoneally. Since then the ascites form is transplanted serially for over 208 generations.

Specimens for chromosomal analysis were prepared as follows: Colchicine in physiological saline (0·035 mg./kg.) was injected intraperitoneally into ascites tumour of five