SHORT COMMUNICATIONS

COPPER AND ZINC IN HUMAN SENILE CATARACT

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THERE are many metabolic reactions in normal physiological processes which are related to the presence of trace elements in eye lens[1]. With increased industrialization, environmental pollution by trace elements and its deleterious effects upon human health have led to the feasibility of trace elements as possible cataractogenic agent. Amongst a number of trace elements which are present in the lens, copper and zinc have recently attracted the attention as they are important constituents of various metalloenzymes and have possible relationship with senile cataract formation[2].

This study was carried out on 250 patients with matured senile cataracts. Cataractous lenses were extracted incapsularly and the classification of lenses was done on the basis of the method described by Pirie. Hypermature and mixed cataracts were eliminated from this study to allow better interpretation of the data and avoid complication due to their porosity and hence leakage of soluble constituents. The lenses were lyophilized and digested separately in 3 ml digestion mixture containing concentrated nitric acid and perchloric acid in the ratio of 5 : 1 (v/v) and was evaporated to dryness on a sand bath. The residue was dissolved in 10 mM nitric acid. Zinc and copper were determined using the double beam atomic absorption spectrophotometer, GBC-902.

Table 1 shows that the levels of copper and zinc were elevated significantly in all types of cataracts. The increase of copper in cataracts has been reported[3] to be more than ten times the normal. However, the level of zinc has increased to about two times the normal value and this has not been reported earlier. The presence of elevated levels of copper and zinc, in cataracts may lead to the damage of the lens through different pathways. Whereas excess copper in the lens may oxidize membrane sulphydryl groups to disulphide[4], excess level of zinc may induce formation of low molecular weight proteins[5] (metallothionein). It can thus be suggested that the combined effect of these trace metals may induce damage leading eventually to the formation of cataracts through the reduction or oxidation processes.

Table 1 *Levels of copper and zinc (μg/g lens weight)*

	Copper	Zinc
Normal lens	0.25 ± 0 06	12.8 ± 2 6
Cortical cataract	2.97 ± 0 8	25.83 ± 4 9
Nuclear cataract	3.75 ± 1.1	26.98 ± 6 6
Brunescent cataract	4.62 ± 1.4	28.20 ± 7.4

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A COMPARATIVE MASS SPECTRAL STUDY OF PYRIDINIUM ALDOXIMES

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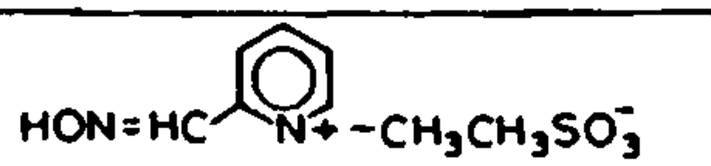
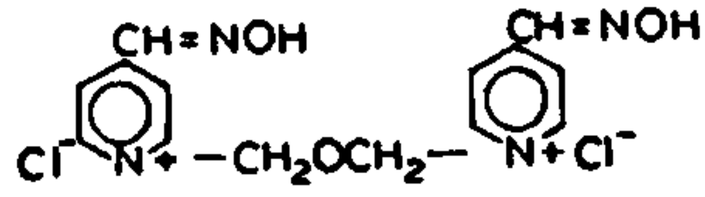
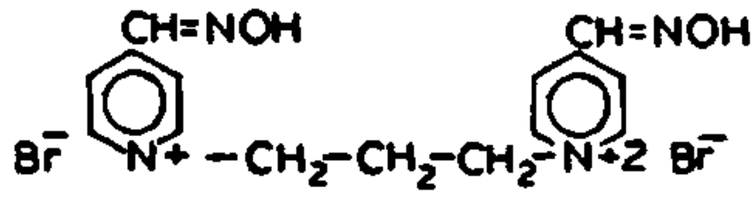
QUARTERNARY salts of pyridine aldoximes consist of an exclusive group of compounds having remarkable efficiency in regenerating phosphorylated cholinesterase enzymes. Since the time Wilson and coworkers[1] worked with pyridine-2-aldoxime methiodide, better known as 2-PAM, oximes have been steadily and successfully introduced in the therapy of poisioning by organophosphorus compounds.

Table 1 *Oximes reported*

Compound No.	Common nomenclature	M.P. (°C)	Molecular structure	Molecular weight
I	P_2S	155		232 (Mass spectral fragmentation is given in table-2)
II	Toxogonin	225		358
III	TMB-4	240		446

These aldoximes are salt-like in nature, having no sharp melting point. Indirect and often nonspecific TLC identification can be done by identifying the corresponding acids which would be formed on hydrolysis with water. Being nonvolatile, they cannot be identified by GLC. Mass spectrometry can play an important role in the identification and characterization of such a drug or antidote and its metabolites in biological system. However these oximes being nonvolatile and thermally labile, conventional electron impact (EI) or chemical ionisation cannot give any useful information in this regard. Field desorption (FD), fast atom bombardment (FAB) and secondary ion mass spectrometry have therefore been used[2,3] to characterize mono- and di-quarternary ammonium and phosphonium salts. In all the above cases, the salts have been desorbed and ionised either as di- or mono-cations. Our work[4] with oxime salts by FD could yield the mono-cation species, and the work on FD with rapidly heated activated emitter could yield the cluster ion peaks for all the oximes reported. Mass spectral informations obtained by using EI, FAB and rapidly heated FD are reported here for three of the oxime salts.

All mass spectra were obtained in a JEOL JMS DX–300 double focussing mass spectrometer combined with JMA 2000 on line computer. EI were recorded at 70 eV with the sample in direct inlet probe whose heating was programmed up to 300° at the rate of 16°C/min.

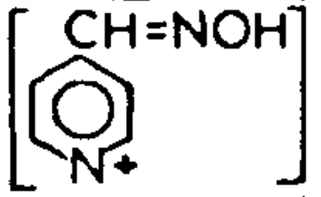
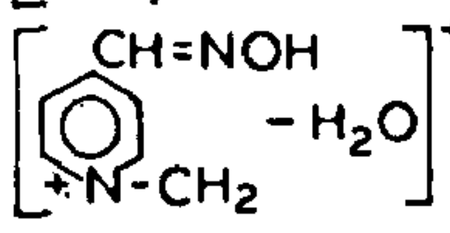
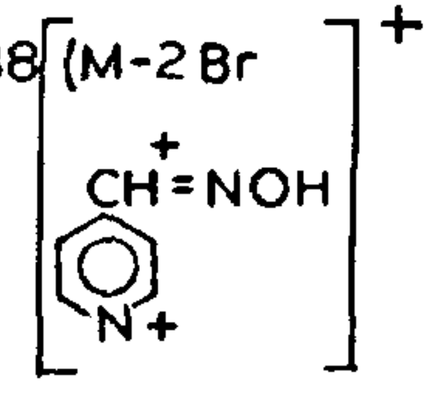
For FD spectra, rapid heating of emitter at the rate of 15–20 milli-amp./sec was employed and sampling at comparatively low conc. of 1–10 μg/ml was done conventionally as in FD techniques. Accelerating voltage was set at 2.3 kV. FD source was best focussed at a cathode voltage of 4.5 kV. FAB spectra were recorded at JEOL Application Centre, Tokyo, with $DMSO_4$ as solvent and glycerol as binding medium with FAB accessory attached to JMS DX–300

The three oxime salts and their molecular formulae are given in table 1. The EI, FAB and rapidly heated FD spectral data with peak intensities are given in table 2.

The EI spectra show the thermal decomposition of such oximes. As the molecule becomes complex with higher molecular weight, the basic moiety of pyridine and pyridinium oxime becomes less abundant due to thermal degradation. Loss of (H_2O) is characteristic of all such oximes.

The conventional FD spectra are characterized by the presence of mono-cation species and the absence of molecular ion and any fragmentation peaks. FAB spectra has high background because of the thermal energy generated by FAB. But it gives useful mass spectral information with $(M\text{-anion})^+$ and $(M\text{-acid})^+$ peaks with quite high intensity and a few fragmentation/degradation peaks as obtained in EI. FD with

Table 2 *Mass spectral data*

Compound No.	Fragmentation pattern in					
	EI	RA*	FAB	RA*	RAPIDLY HEATED FD	RA* (%)
I	$\frac{m}{e}$ 122 	High	$\frac{m}{e}$ 137(M-acid)$^+$	High	$\frac{m}{e}$ 272(M+K)$^+$	6
	104 $(122-H_2O)^+$		136(M-onion)$^+$	High	137(M-ocid)$^+$	25
	96 $(CH_3SO_3+H)^+$		119$(137-H_2O)^+$	High	136(M-anion)$^+$	62
	78 	High				
II	122	Bose	325$(M-Cl^{35})^+$	Low	380(M+Na)$^+$	30
	104	High	323$(M-Cl^{37})^+$	Low	357(M-H)$^+$	5
	78	High	170 $[(2CH=NOH+2Cl)+H]^+$	High	325 $(M-Cl^{35})^+$	14
			115 $(CH_2OCH_2+2Cl)^+$	Low	323 $(M-Cl^{37})^+$	44
III	293 [(M-(4 Cyane-N-methyl pyridinium-$2H_2O$-H) ion)]$^+$	Low	365(M-HBr)$^+$	High	468(M+Na)$^+$	25
	119 	Low	288 		366(M-anion)$^+$	23
					292 As in EI	36
	69 $[2CH=NOH-H_2O]^+$			Low	134(Diionic species)	17
	RA* : Relative obundance					

rapidly heated emitter is the only technique which could desorb the intact molecule along with potassium or sodium and thus gave cluster ion peaks at 272(P_2S-I), 380(Toxogonin-II) and 468(TMB-4-III). However these peaks are of comparatively low intensity (raw intensity of less than 2% in this spectrometer computer system) and thus could not give any isotope peaks.

The results suggest, the FD, FAB and rapidly heated FD emitter technique can be adopted as complimentary to each other for elucidating characteristic mass spectral peaks of such oximes.

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ANTIMICROBIAL ACTIVITY OF SULPHONYLCHALCONES AND SULPHONYLCYCLOHEXENONES

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BENZALACETOPHENONE (Chalcone) and its substituted derivatives have antibacterial[1-3], antifungal[4], antiparasitic[5], antitubercular[6], antiinflammatory[7] and insect repellent properties[8]. Studies on the structure-activity relationship of clavacin[9-12] and penicillic acid[13] indicate that such a structure as $-C=C-C=O$ that is found in chalcone, clavacin and penicillic acid is responsible for the development of antifungal and antibacterial activities. This structural feature is present in both the α-sulphonyl-chalcones (**1–10**) and sulphonylcyclohexenones (**11–20**), the synthesis of which would be published elsewhere. In the present investigation the antimicrobial activity of these compounds is assayed.

Ten mg of different α-sulphonylchalcones and sulphonylcyclohexenones which were insoluble in water were dissolved in the minimum required quantity of acetone. Twenty sterile Whatman filter paper No. 1 discs of 6 mm diameter were added to the solution and shaken thoroughly. The filter discs were allowed to dry and the amount of substance per paper disc was taken as 500 μg. Paper discs treated with acetone alone served as the control. The antibacterial activity was assayed[14] against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris* and *Candida albicans*. The filter paper discs treated as above were placed aseptically on nutrient agar plates seeded with test organisms and incubated at 37° for 24 h. The zone of inhibition of bacterial/yeast growth was measured.

Antifungal activity of the α-sulphonylchalcones and sulphonylcyclohexenones was assayed against two fungi—*Alternaria alternata* and *Curvularia lunata* following the procedure of Horsfall and Rich[15] with certain modifications. One mg of different test compounds was dissolved in 1 ml acetone (0.1%) and an aliquot (0.25 ml) was taken with a micropippette on demarcated area of microscopic slides. The solvent was allowed to evaporate leaving a deposit of the test compound on the top of the slide. Then the spore suspension (0.1 ml) was taken with a sterile pippette and deposited over the demarcated area of the test compound on slides. The areas were closed with cover slips. Suitable controls were kept using acetone dried area over slides. The spore suspension was adjusted to give 30–50 conidia per lower field (100×) of the microscope. The slides were incubated in a moist chamber at 20–25° for 24 h.

The percentage inhibition of conidial germination was calculated by the formula:

$$\text{Percentage of spore germination inhibition} = 100 - \frac{\text{Percentage of spore germination in the treatment}}{\text{Percentage of spore germination in the control}} \times 100.$$

The α-sulphonylchalcones were active against gram positive bacteria (*S. aureus* and *B. subtilis*) and inactive against the gram negative bacteria (*E. coli* and *P. vulgaris*) (table 1). The sulphonylcyclohexenones possessed no activity either against the gram positive or gram negative bacteria. This may be due to the phenylsulphonyl moiety being present at a position to the carbonyl group while in the α-sulphonylchalcones it is to the carbonyl group.