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ISOLATION OF A FLAVONE FROM POL YGALA STENOPETALA

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ABSTRACT:

A flavone has been isolated from the roots of *Polygala stenopetala.* The structure was established on the basis of UV, Mass spectrum, ^{1}HNMR, ^{13}CHMR and **NOE** difference experiments as 5,7,4 -trihydroxy-3 , 5 –dimethoxyflavone (tricin) This is for the first time that a flavone has been isolated from the species belonging to the family Polygalaceae.

INTRODUCTION

The genus *Polygala* comprises of approximately 500 species and distributed over temperate, subtropical and tropical region of all continents with the exception of Austriala (Melchor, H., 1964). Previous photochemical studies of the genus have shown the presence of a large spectrum of structurally and biogenetically diverse secondary metabolites such as saponins (Sakuma, S., 1981), lignans (Hokanson, J., 1978), coumarins (Hamburger, M., 1985), xanthones (Ghosal, S., 1981), sinapic acid esters (Bashir, A., 1985), flavonol glycosides (Ghosal S., 1974), chromonoeoumarins (Dipaolo, E.R., 1989) and isolfavones (Bashir, A., 1992) In continuation of our studies on African *Polygala* we undertook the investigation of *Polygala stenopetala*. There has been no reports on the secondary metabolites so far. Preliminary TLC and HPLC-UV analysis of the methanolic root extract suggested the presence of flavonoid.

RESULTS AND DISCUSSION

Fractionation of methanolic extract from the dried roots of Polygala stenopetala by MPLC on silica gel and RP-8 afforded a pure yellow colored compound. The UV spectrum showed absorption maxima at 269 nm (band II) and 351 run (band I) with a shoulder at 309 nm, respectively, suggesting a flavonoid nucleus. The presence of free hydroxyl groups at C-7 and C-4 was evidenced by bathochromic shift of band II (276 nm) and band I (391 nm) with NaOAc, respectively. A bathochromic shift observed upon addition of Al CI_3 not affected by the addition of HC1 indicated the presence of a chelated hydroxyl group at C-5. The EI mass spectrum exhibited a $[M]^+$ at m/z 330. The ions at m/z 315 $[M-15]^+$ resulted from successive elimination of two methyl radicals. The fragment ion at m/z 153 $[A_1]^+$, m/z 178 $[B_1]^+$ and 163 $[B_1-15]^+$ indicated the two hydroxyl groups in ring A and one hydroxyl and two methyl groups in the ring B.

In the ^{13}CHMR (50.3 MHz, DMSO-d $_{6)}$ spectrum signals at δ 103.65 is a typical of flavone nucleus whereas signal at 6 56.32 revealed the presence of two aromatic methoxyl groups which are not *ortho*- disubstituted ^{1}HNMR (200.6 MHz, DMSO-d_6) spectrum displayed signals of three

hydroxyl protons at δ 12.95 (IF], br., s, 110-5), 10.77 (110-7) and 9.27(110-4)'. The presence of two doublets at δ 6.21 (1=2.0 Hz, H-6) and 6.56 (1=2.0 Hz, H-8) indicated the substitution at C-5 and C-7. A six protons singlet at δ 3.89 indicated the presence of two equivalent aromatic methoxyl groups. The position of methoxyl groups and H-3 was assigned through a series of NOE difference experiments. Pre-saturation of the signal at δ 6.97 (H-3) gave enhancement of the two protons singlet at δ 7.33 (H-2′ and H-6′) The irradiation of the singlet at δ 7.33 (H-2′ and H-6′). The irradiation of the singlet at δ 7.33 (H-2 and H-6) resulted in the strong enhancement of signals at δ 3.89 (MeO-3 and MeO-5) and 6.97 (H-3). So the compound was identified as 5, 7, 4 -trihydroxy-3',5,-dimethoxyflavone (tricin), an antileukemic principle have already been isolated from *Wikstroemia indica*(lee, K.H., 1981) and *Artmisia ludoviciana* (Liu, Y.L., 1982).

There has been extremely few phytochemical studies on African *Polygala* species. Our investigation of methanolic root extract of *Polygala* stenopetala led to the isolation of flavone (tricin) which is a constituent very commonly found in species of Cypraceae family (Harbome 1.B., 1985). But here it is reported for the first time from the species belonging to Polygalaceae. The scant information about the flavonoid distribution within that family precludes at the moment the use of flavone as chemotexonomic marker for the Polygalaceae in particular of the genus *Polygala.*

EXPERIMENTAL

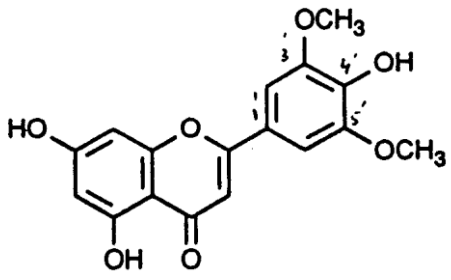
General: Melting point uncorrected. TLC was carried out on silica gel pre-coated Al sheets (Merck) and RP-8. The solvent system employed were $CHC1_3$-MeOH-H_2O (75: 25:3) (silica gel) and MeOH- H_2O(70:30) (RP-8). For medium pressure liquid chromatography (MPLC) silica gel (15-25 um, Merck) was used. UV spectra were recorded with the addition of usual shift reagents. Analytical HPLC was performed on a Hewlett-Packard 1090 series II instrument equipped with a photodiode array detector. Purity of the compound was checked on Nucleosil RP-8 column (7 urn, 250 x 4.6 mm, i.d., Macherey Nagel) at a flow rate of 1.5 ml/min.

MS was recorded on a Finnigan MAT. TSQ 700 triple stage quadruple instrument. D/CIMS: positive ion mode, NH_3 as reactant gas. ^{1}H and "CNMR spectra were measured at 200.6 MHz and 50.3 MHz, respectively. TMS was used as an internal standard.

Extraction and Isolation: Dried and ground roots of *Polygala stenopetala* (200 g) were successively extracted at room temperature with dichloromethane and methanol. The methanol extract (5.6 g) was fractionated by MPLC on slica gel into 12 fractions (1-12) using step gradient elution ($CHCI_3$MeOH-H_2O 75:25:3 65:35:5 60:40:10 + 0.05% TFA). Further separation of fraction 5 (180 g) by MPLC on RP-8 using MeOH – H_2O (37:63) yield a pure compound (6.0 mg).

Compound: Yellow needles; mp. 278°. UV λ_{max}^{MeOH}nm: 269, 309, 309 sh, 349; (NaOMe)276, 416; (NaOAc) 276, 319 sh, 391; (NaOAc+H_3BO_3) 270, 352; ($AICI_3$) 260 sh, 276, 307, 370 sh, 392; ($AICI_3$ + HCI) 256 sh, 277, 356 sh, 385sh. EIMS m/z: 330 [M]', 315 [M-151', 301 [M-29]', 287 [M-43]$^+$, D/CIMS m/z: 331 [M+I]$^+$. ^{1}HNMR (200.6 MHz , DMSO-d_6): δ 12.95 (1H,br. s, HO-5), 10.77 (1H, br. s, H0-7), 9.27 (1H, br., s, HO-4 ′) , 7.33 (2H, s, H-2 and H-6), 6.97 (1H,s, H-3), 6.56 (1 H, d, J= 2.0 Hz, H-8), 6.21 (1 H,d, J= 2.0 Hz, H-6), 3.89 (6H, s, MeO-3 and MeO-5). ^{13}CNMR (50.3 MHz, DMSO-d_6): δ 181.69 (C-4), 164.03 (C-2), 163.57^b (C-7), 161.31^b (C-5), 157.25 (C-9) ′ 148.13 (C-3 and C-5), 139.84 (C-4), 12034 (C-1); 104.41 = (C-2 and C-6), 103.65^a (C-3), 103.52^a (C-10), 98.74 (C-6), 94.09 (C-8), 56.32 (2 x MeO).

[a.b] Assignment interchangeable.



Tricin

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ROLE OF QUINONE MOIETY AS ANTITUMOUR AGENTS: A REVIEW

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ABSTRACT:
Quinone antitumour agents with wide spectrum of activity have been extensively used in different fonns of human cancers. Quinones have been isolated either from animals, microorganisms or plants. Extensive research on quinone compounds is going on to discover novel effective antitumour compound. We also discussed the mechanism of action, which have not been fully clarified. In this review we cover all the above mentioned aspects to emphasize the importance of quinone moiety as antitumour agents.

INTRODUCTION

Quinone containing compounds have been widely used for their antitumour and anticancer activity. Problems, such as, toxicity and drug resistance have served to stimulate an intense demand and all out research efforts for the discovery of new and novel anti tumour agents. In the last few decades significant progress has been made in the screening of quinone moiety for antitumour activity.

Nature is an important source of novel compounds useful directly as medicinal agents, as model compounds for synthetic or semisynthetic structure modifications and optimization, Compounds derived from natural sources serve as viable compounds for modem drug development.

QUINONE MOIETY IN NATURE

p-benzoquinone and very simple other alkyl derivatives are arthropod metabolites, which do not occur else where except in reduced form. A rare exception is the presence of *p*-toluquinone and the hydroquinone in the ascomycete *Nectria embescens.* Benzoquinone and its analogues are most widely used as chemical defensive agents by arthropods, millipedes, beetles, arachnids and termites. The actual compounds stored in the inner chamber of defensive glands are hydroquinones and hydrogen peroxide which then passed through another chamber where oxidase enzyme effect an explosive oxidation and as a result benzoquinone discharged as a hot spray, audibly in the case of bombardier beetles [Aneshansely, D.7., 1983].

Almost 350 natural naphthoquinones have been discovered and they constituents the largest

group. Now the co-existence of naphthoquinones and anthraquinones in plants is not rare and they have a common precursor. New types include isofuranonaphthaquinones and naphthoquinones linked to coumarine unit while dimers of various kind and benzisochromanquinones form substantial subgroups.

The Anthraquinones are as widely occurring as naphthoquinones. The greater number of anthraquinones has been found in marine animals and in insects and there is substantial group in digitalis plants. Carminic acid, which is an example of anthraquinone, is a coloring pigment of cochineal which contain the dried bodies of cochineal insect *Dactylopius coccus*. It is still used for coloring pork sausage, shrimps, jams and other food and drinks beside medicinal pills and cosmetic products [Lloyed, A.G., 1980].

In the 1950s Brockmann and Brown elucidated the structure of the red-orange pigments isolated from culture of different streptomyces species. The aglycon moieties of these compounds identified as antluacyclinones belonging to the type known as rhodomycinone, isorhodomycinone and pyrromycinone whose color was due to the presence of polyhydroxy-anthraquinone chromophore

Recently number of cyclofarensylated quinines have been isolated from Paluan ascidian and Aplidium longithora [Fu, X., 1997, Davis, R.A., 1999].

Naturally Occuring Quinone Having Antitumour Activity

Asterriquinone obtained from culture of a strain of *Aspergillus terreus* exhibits antitumour activity, other eleven di-indolylbenzoquinones were also isolated [Arai, K., 1981]. Lapachol obtained from heartwood and roots of Haplophragma adenophyllum and Frederieamycin A obtained from Culture of Streptomyces griseus have showed antitumour activity.

Carmine acid is a colouring pigment of cochineal, which contains the dried bodies of cochineal insect *Daclylopius coccus.* It is still used for coloring shrimps, jams and other food and drinks besides medicinal pills and cosmetic products [Lloyed, A.G., 1980]. In rats this pigment revealed antitumour activity and it is a potent feeding deterrent to ants which suggest that its natural function may be to act as a defensive agent against predation specially for males and newly born insects which are not protected by wax covering. Kidamycin and Hedamycinare are other examples of naturally obtained antitumour antibiotics belonging to this class [Kanda, N., 1972].

The Anthracyclines are the important class of antibiotics and great numbers of anthracyclines are being used for the treatment of a range of human cancers. Doxorubicin is the drug against which all other anthracyclines are compared experimentally and clinically, it is first isolated from the culture of Streptomyces strain. Aclacinomycin ‘‘A’’, which is isolated by oki etal. From a culture of *Streptomyces gelilacus* in 1975 is a clinically important antitumour agent which also belong to the anthracycline group [Oki, T., 1975].

The new nortriterpene methylene quinones were isolated from *Maytenus amazonica*, their structure were elucidated by spectroscopic methods and they showed low antitumour activity against four cancer cell lines [Chavez, H., 1999].

Synthetic Approaches Towards New Antitumour Derivatives

Taking Mitomycin C as a model, a number of p- benzoquinone have been synthesized [Khan, A.H., 1976], for example 3,6-diaziridinyl-2, 5-bis- (carbethoxy) amino-1, 4- benzoquinone

(Diazoquinone AZQ) and 2, 5, bis (l ,aziridinyl)-3 (-2-carbmoyloxy-1- methoxy ethyl-6- methyl-1, 4-benzoquinone (fig-2) have emerged as clinically useful derivatives as a result of the effort of two different teams.

Another derivative, 4-dialkylamino-5-methoxy-1,2 benzoquinone has also been synthesized by using a simple and efficient method [Viallen, L., 1995]. Lapachol is reported [Da cansolacao, M., 1975] to be available in Brazil as antitumour drug.

Clinical trials have showed undesirable side effects. Several synthetic analogues have been studied in which only geranyl compound (fig-3) and the dibromoallyl derivative (fig-4) showed activity. Fredericamycin A is a potent antitumour antibiotic [Misra, R., 1982] and its structure was elucidated by using x-ray technique [Misra, R., 1982]. For the signif 10-anthracenedione [Cheng, C.C., 1983].

The molecular modeling studies on the anthraquinone derivatives have been carried out to find out the effect of the different structural features present in the anthraquinone derivatives, i.e. the number of methylene groups (n) in the side chain, the steric bulk of the terminal amines and the presence of hydrophilic hydroxyl groups in the side chain, on their interaction with DNA [Agbandje, M., 1992]. The compound studied presented in fig-5.

Quite a few numbers of Anthracyclines are being used for the treatment of a range of human cancers. Determined efforts have been made to discover new chemotherapeutic agents having less side effects [Aubel-Sadron, G., 1984]. At present among all antineoplastic agents, doxorubicin exhibited the widest spectrum of antitumour activity and is the most utilized antitumour drug worldwide. Due to its cumulative dose-limiting cardiotoxicity, search for new analogues with reduced cardiotoxic potential and a more favorable therapeutic index was carried out. Hundreds of anthracyclines have been synthesized, utilizing new chemical reactions, most of them did not show significant activity as compared to that of doxorubicin [Weiss, G., 1986].

Analogues of daunorubicin possessing a fluorine atom at position C-8 of ring A have been synthesized with the aim of comparing their DNA-drug interaction and antitumour properties with those of the clinically useful anthracyclines doxorubicine and idambicin. The synthesis of (8s)-8-fluoro 4-demethoxydaunorubicin and molecular mechanism and NMR studies led to the synthesis of epimeric (8R)-8-fluoro-4-demethoxydaunorubicin The cytotoxic properties of the two 8-fluoro-anthracyclines analogues were markedly affected by the stereoselectivity of the fluorine substituent [Lomardi, p., 1995].

The fluoro derivatives were synthesized directly from daunorubicin and idarubicin following a non-deglycosidative approach [Pasqui, F., 1996]. In preclinical studies another halogenated anthracycline derivative, 4-deoxy-4-iododoxorubicin has demonstrated significantly reduced levels of cardiotoxicity compared to currently employed anthracyclines. The iodine atom at the 4-position of sugar ring reduces the basically and enhances the lipophilicity of this compound as compared to related anthracycline drugs. The iodine substituent does not alter the geometry of intercalation as compared to previously solved anthracyclines complexes, but appears to markedly

effect the solvent environment of the structures. This could have consequences for the interaction of this drug with DNA and DNA binding proteins in cells [Berger, I-, 1995].

The search for new antitumour agents having less toxicity, more cytotoxicity potency than their parent compound delivered the morpholino anthracycline. It possesses a morpholino ring incorporating the amino nitrogen of the daunosamine unit at the 3-position. It has been suggested that charged amine at the 3-position of the daunosamine sugar interacts with p-glycoprotein. From morpholinoanthracyclin Methoxy-morpholino-doxombicin and 3-deamino-3-morpholino-13-deoxo-l0-hydroxycarmomycin are under clinical trials [Rpamonti, M., 1992; Watanebe, W., 1988; Ogawa, M., 1989].

Recently a series of indolequinones bearing various functional groups have been synthesized which exhibited antitumour activity [Beall, H.D., 1998].

Mechanism of Action

There are number of mechanisms by which quinone containing compounds exerts their cytotoxic action, however it is not clear that which of these actions is the most important in inducing cell damage. The main target for their cytotoxic action is DNA.

The best known mechanism is the intercalation between two base pairs of DNA or RNA. The ring of the polycyclic chromophore plays a vital role in the intercalation by binding to DNA and RNA. By a strong electrostatic bond of the positively charged amino sugar portion of the anthracyclines to the sugar sugar-phosphate backbone of DNA the intercalated molecule is stabilized at intercellular pH. This way vital actions such as replication and transcription are blocked [Quigley, G.J., 1980; Wang, A.H., 1987].

Topoisomerase are essential for DNA metabolism by inserting transient breaks into single (topoisomerase 1) or double strand (topoisomerase II) DNA, thus solving mechanical problems caused by the double-helical structure of DNA [Pommier, Y., 1993]. The anthracycline-topoisomerase complex (cleavable complex) form stabilized DNA breaks, thus turning topoisomerase into cellular toxins. The nature of the substiutuent at 3'- position of the daunosamine moiety and of the C-14 position of the chromophore ring A are essential in the formation of cleavable complexes [Capranico, G., 1994; Bodley, A., 1989].

Several 5-alkyl-1, 3-dihydroxy benzene (5-alkylresorcinol) and 6-alkyl-1, 2,4 trihydroxy benzene derivatives were prepared and used to study the mechanism by which such compound effect Cu dependent DNA strand scission Te efficiency of DNA cleavage increased with increasing length of the alkyl substituent. DNA cleavage by the 5-alkylresorcinol appears to involve initial oxygenation of the benzene nucleus. Te resulting trihydroxylated benzene mediated DNA cleavage in a reaction dependent on the presence of both Cu^{2+} and O^{-2} The mechanism appears to involve reduction of Cu^{2+} by the trihydroxy benzene moiety with subsequent formation of reactive oxygen species [Sing, U.S., 1995].

Quinone containing compounds also exert cytotoxicity by radical formation through electron transport with a vital role for the quinone (C) ring. In the absence of oxygen they have the potency to generate aglycone free radicals (by splitting off the amino sugar of the semiquinone radical) that produce DNA and RNA single and double strand breaks. Free oxygen radicals can damage intra as

well as extracellular macromolecules (lipids and proteins). Their action cells and mitochondria inhibit swell, other organdies fragmentate, and essential intracellular enzymes.

Quinone moieties can also be cytotoxic by binding to membranes thus altering their functions. Cytotoxicity by membrane interaction in vitro where entrance of free doxombicin was prevented by coupling doxorubicine to an insoluble agarose support has been demonstrated.

Reaction of the anthracyclines, antitumour drugs adriamycin and daunorubicin with the self complementary DNA oligonucleotide $GCGCGCGC(GC)_4$ in the presence of reducing agent dithiothreitol, the oxidizing agent hydrogen peroxide, or the alkylating agent formaldehyde gives a similar mixture of DNA-drug adducts [Dylan, J., 1997].

Mitomycin is a bioreductive alkylating agent of DNA. About DNA-mitomycin ‘‘C’’ complex (fig-6) no specific information was available. However it has established that mitomycin binds to minor groove [Tomasz, M., 1987] not in major groove as believed earlier. Iye, and Szybalski proposed a mechanism [Iycr, V.N., 1964] and that is modified by Moore and Czemiak [Moore, KW, 1981] shown in Scheme A. Quinone moiety in mitomycin C is reduced by one electron to the semiquinone (1.2, R= electron) or by two electrons to hydmquinone (l.2, R= H) and this reduction of quinone converts the heterocyclic nitrogen from a non nucleophlic vinylogons amide nitrogen to an amine nitrogen which can eliminate the β–methoxide ion (1.2). The compound (1.4) is a set up for aziridine ring opening, which is produced by the tautom erization of the immonium ion (1.3) and thus the drug is activates by unmasking the electrophilic site at C-1 which alkylates the DNA (1.5). The cross linking of DNA is result (1.7) by the subsequent reaction of DNA at C-10 (1.6).

Reduction of the quinone is necessary for the covalent reaction of mitomycin C to DNA but there is controversy that whether the semiquinone (1.2, R= electron) or hydroquinonc (1.2, R= H) is the viable intermediate [Frank, R. W., 1990]. Chemical model studies on the mechanism of action of (1.1) indicate that the conversion of 1.1 to 1.7 can occur at the semiquinone stage and conversion of 1.6 to 1.7 occurs at the hydroquinone oxidation state [Kohn, H., 1990].

The bioreductive alkylation method based on quinone reduction to the corresponding hydroquinone was used in the prodrug design. Both mono (scheme B) [Antonini, I., 1982] and bisalkylating agents (scheme C) [Lin, A.J., 1975] were developed. Substituents, which are electron rich, lower the reduction potential of the quinone and make them more reactive. Both monoalkylated and bisalkylated DNA adducts have been identified and the extent of mono and bisalkylation increases as the guanine base composition of DNA increases [Borowy-Borowski, H., 1990]. The attachment site of these adducts is at N-2 of the guanine base [Tomasz, M, 1987),] thus DNA synthesis inhibited. In addition single strand breakage of DNA is caused by reduced mitomycin. In rodents mitomycin is tetratogenic and carcinogenic but the immunosupressive properties of mitomycin are relatively weak.

Scientists are working on quinone compounds because of its potential as effective anticancer agents. Quinones are abundant in nature so, with the help of all relevant structure and chemical information and by understanding how these molecules bind and interact on the molecular level, more new and safe anticancer drugs of the future will most certainly be found in very short time.

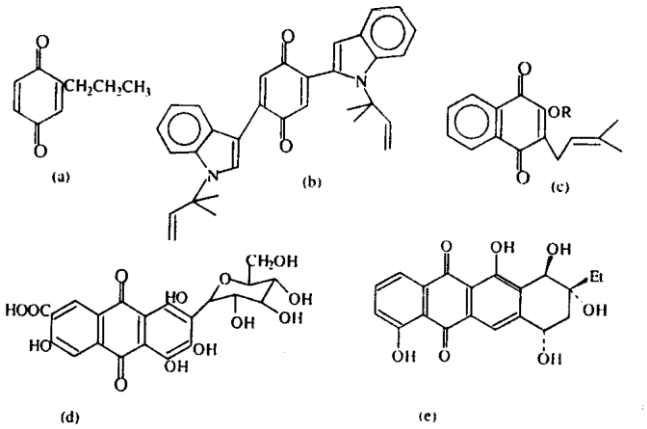
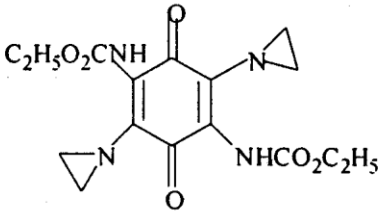
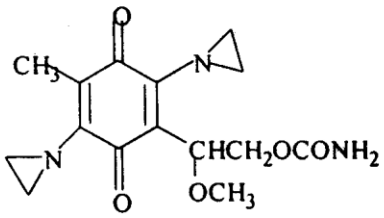


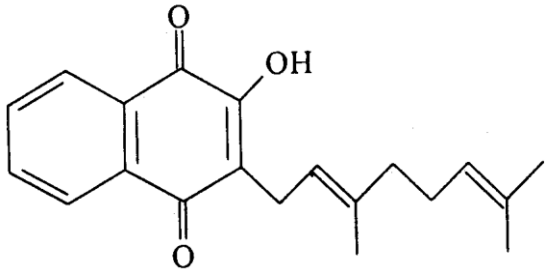
Fig. 1: Some naturally occuring quinone (a) n-propyl-1, 4 hen[oquinone which is adefensive secretion of Pedinini beetles (b) Asterriquinone from the culture f a strain of *Aspergillus terreus* this exhibits antitumour activity (c) Lapachol from the heartwood and roots of *Hoplophyragma* adenopltyllumm (d) Carminic acid from the insect Dactylopiusceylonicus, having antitumour activity. (e) a-citromycinone as a glycoside (cosmocarin) in culture of *St.* cosrnasus.

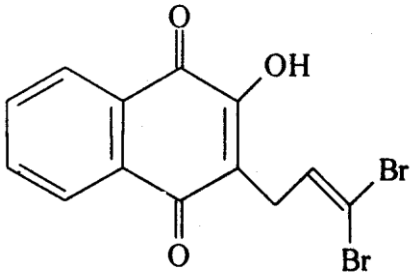


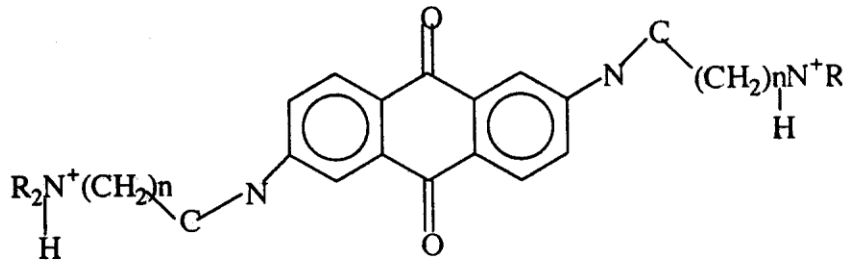
3,6-diaziridinyl-2,5-bis(carboxy)amino-1,4-benzoquinone



2,5bis(1-aziridinyl)-3-(2-carbamoyloxy)1-methoxy ethyl-6-methyl-1,4-benzoquinone







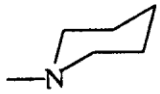
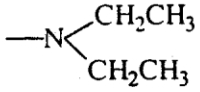
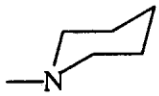
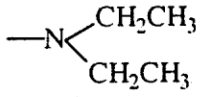
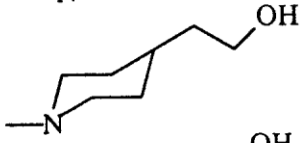
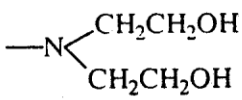
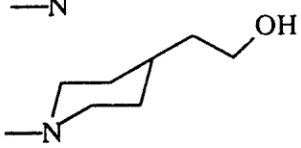
Compound	n	—NR_2	Compound	n	—NR_2
C_1	1		C_5	1	
C_2	2		C_6	2	
C_3	3		C_7	2	
C_4	4				

Fig. 5: The strcutural formulae of the compound used in the molecular modeling of the interaction of the interaction of the 2,6-bis(ω-aminoalkanamido)-9, 10 anthracenediones with DNA.

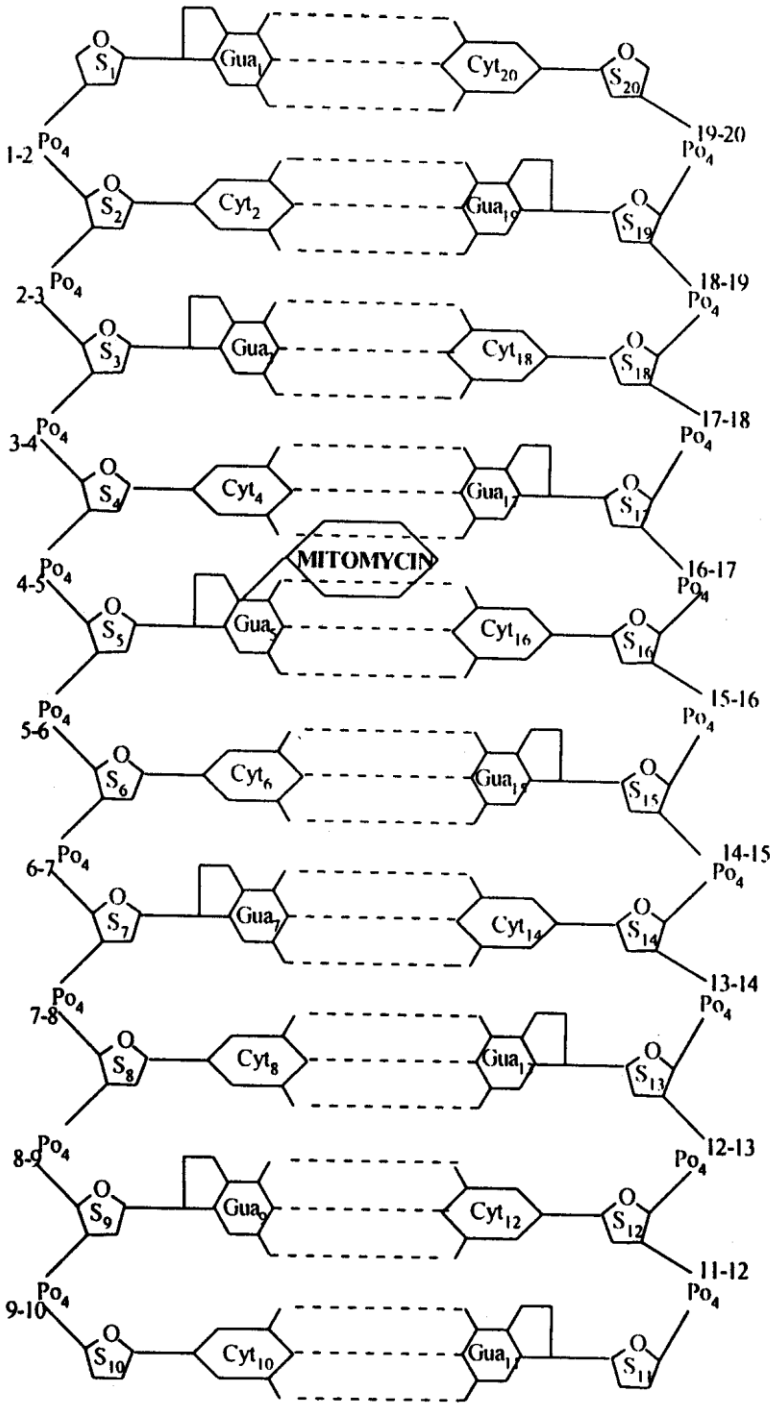
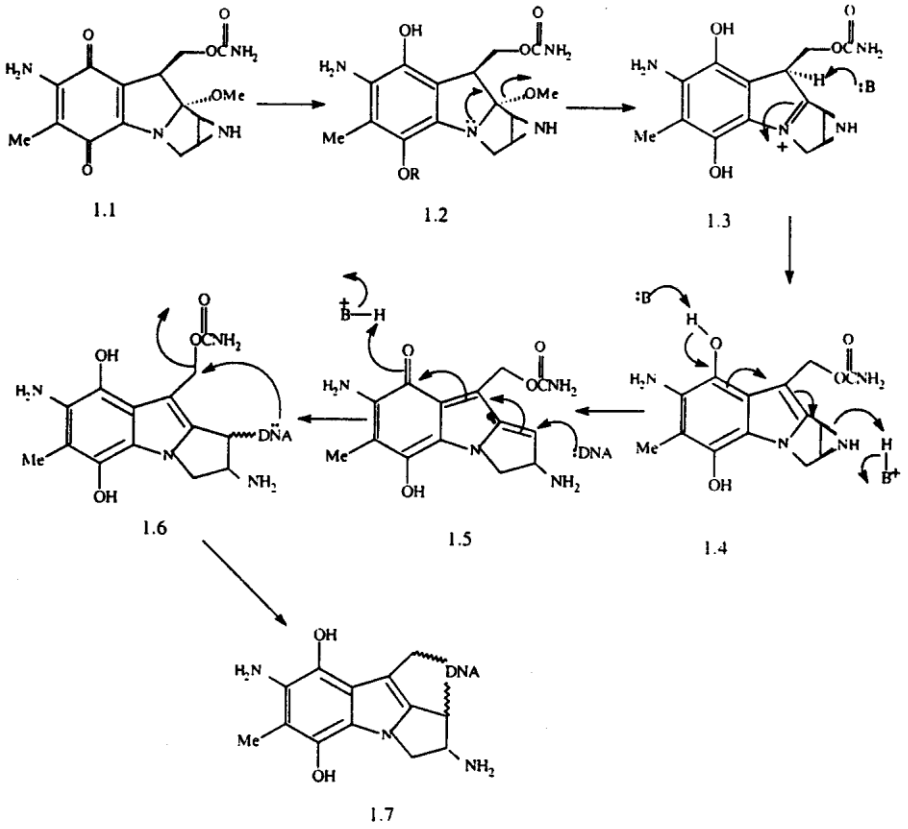
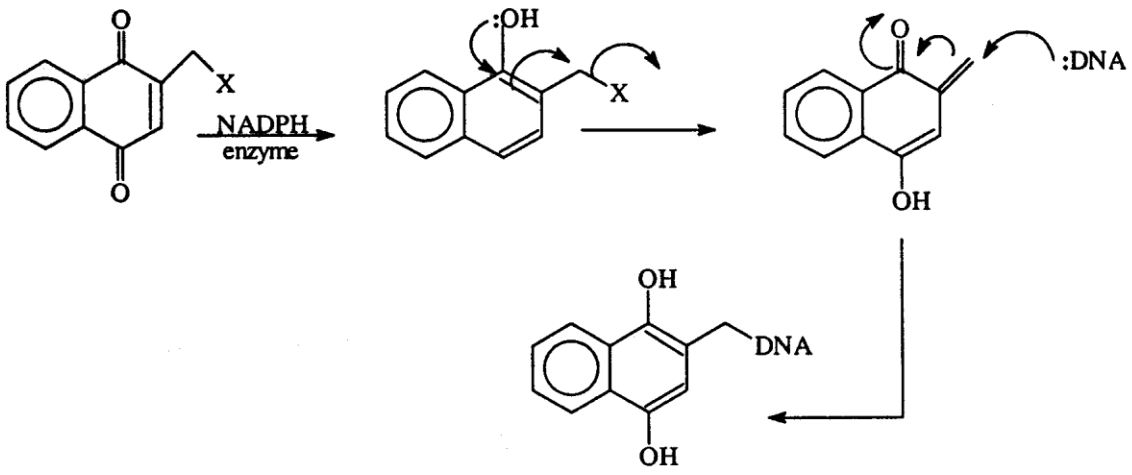


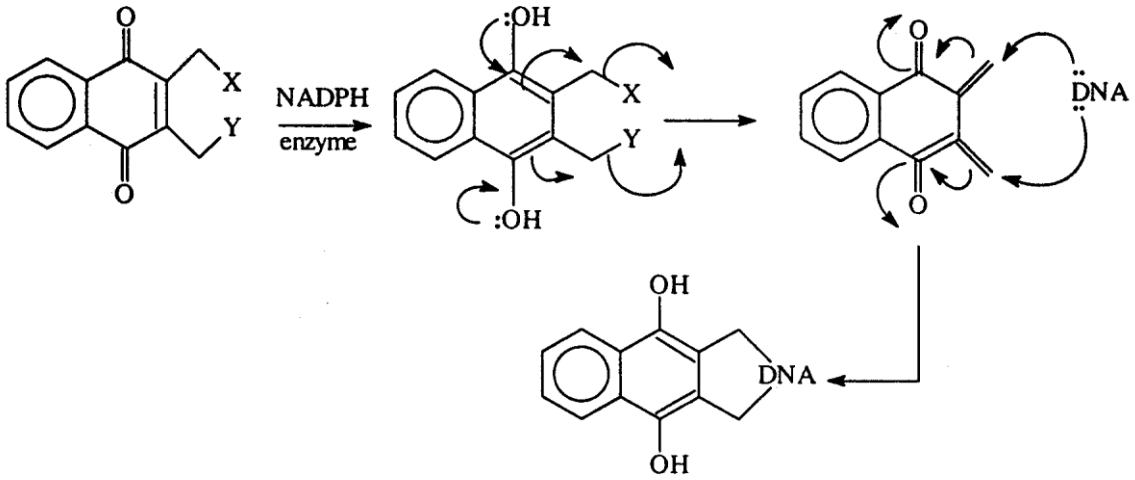
Fig. 6: Schematic representation of d (GCGCGCGCGC) $_2$ (GC $_{10}$) used in the studies on DNA-Mitomycin complex.



Scheme A:Bioactivation of Mitomhycin C.



Scheme B: Bioreductive monoalkylating agents.



Scheme C: Bioreductive bisalkylating agents.

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THE HYPOLIPIDAEMIC EFFECT OF GUM TRAGACANTH IN DIET INDUCED HYPERLIPIDAEMIA IN RATS

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ABSTRACT:

Previous research indicated that fibre in the diet of men lowers plasma lipid and LDL. Cholesterol concentration. To further study the lipid lowering effect of fibre, we conducted an animal study using rats with diet induced hyperlipidaemia. Rats were randomly assigned to one of the three experimental diets- Two of the diets contained cholesterol and choice acid to induce hyperlipidaemia, the fibre source in the hyperlipidaemic diet was gum tragacanth (5%). The rats consumed one of the three diets ad libitum for 4 weeks before they were killed. Plasma LDL cholesterol and total cholesterol concentrations were significantly higher in the hyperlipidaenuc group than in the non hyperlipidaemic control group. A marked improvement in the plasma LDL cholesterol and total cholesterol concentration was observed in the rats that were fed hyperlipidaemic diet containing gem tragacanth. No significant difference in the plasma triglyceride concentration was detected in the three groups. Plasma HDL. concentration was significantly higher in the non-hyperlipidaemic group than in the hyperlipidaemic group than. Addition of gum tragacanth to the hyperlipidaemic diet significantly improved the plasma HDL concentration in the hyperlipidacmic rats. These results suggest that fibre from gum tragacanth lowers plasma cholesterol and LDL in hyperlipidaemia. Gum tragacanth could be useful adjunct to the dietary management of hyperlipidaemia.

INTRODUCTION

Speculations on the evolution of human diet together with comparative studies with the diet of other primates suggest that the human gastrointestinal tract and metabolism are adapted to high fibre diets. Epidemiological studies support a negative association between dietary fibre intake and risk of coronary heart disease. Hypercholesterolaemia is one of the major risk factors for ischaemic heart disease and for the development of atherosclerotic plaques. Viscous fibre sources are likely to play a role since they reduce lipid risk factor for coronary heart disease including total and low density lipoproteins, cholesterol and apolipoproteins (Jenkins et al., 1998). Water soluble plant fibres have shown to exert a binding of intestinal cholesterol without being digested or metabolised (Hegele *et at.*, 1993). In addition, in linear regression analyses fibre intake has shown to be inversely associated with fasting glucose (Bakker *et al.*, 1998).

Results from animal and human studies suggest that fat digestion could be reduced or delayed in the presence of fibre rich fractions and that the intestinal absorption of fat and bile salts (Judd and Truswell, 1981) might be impaired. These changes could likely result in some alterations in the post prandial response to a meal as observed in humans (Dubois *et al.,* 995). Moreover, adults and children with familial hypercholesterolaemia are often resistant to standard dietary

management of their hyperchosterolaemia, and the management of hyperlipidaemia in children can involve special problems and consisderations (Kwiterovich, 1977). Pharmacological treatment is often necessary in addition to dietary treatment to significantly lower cholesterol and low density lipoprotein cholesterol (Zavarol *et al.*, 1983). It is therefore appealing to develop food products that contain safe, effective and hopefully, cost effective hypolipidaemic fibres that can be used long term in familial hypercholeterolaenuc children and adults as part of their every day diet.

MATERIALS AND METHODS

Female Wistar rats weighting 120 – 140 g were obtained from H.E.1. Research Institute of Chemistry, Karachi. The animals were housed in the animal house of the Department of Physiology, University of Karachi. The animals were grouped into three, according to the experimental diet: A: Control; B: Hyperlipidacmic; C: Hyperlipidaemic with added gum tragacanth (5%). A basal diet mixture (Table 1) was prepared from which the two experimental diets were derived. Hyperlipidaemic diet was prepared by adding 1% cholesterol and 0.1% cholic acid to the basal diet (Shinnick *et al.*, 1990). To test the effect of gum tragacanth, 5% gum tragacanth was added to the hyperlipidaemic diet. The animals had free access to the experimental diet and water for four weeks. The rats were weighted weekly during the experimental period

Blood and Tissue Collection

At the end of four weeks, the animals were sacrificed and the blood was collected. Plasma was separated from whole blood within 30 min of collection by centrifugation at 1200 x g for 20 minutes. Body organs including liver, small intestine and caecum were removed immediately, weighed and discarded.

Biochemical Analysis

Plasma VLDL, LDL and HDL concentration of total cholesterol and total triglyceride and total protein were determined by enzymatic analysis as previously reported (Ney et at., 1987).

Statistical Analysis

One-way ANOVA was computed using a general linear model with diet as the grouping factor to test against the null hypothesis for each of the response variables. Statistical analysis of body weights for which repeated measures were taken, were computed as two way ANOVA by diet and repeated measure over time.

RESULT

Body Weights and Organ Weights

Average body weights over 4 wk period differed significantly among the diet groups (Table 2) and over time as measured during wk 1,2, 3 and 4. (Fig 1). The body weights of animals fed hyperlipidaemic diet containing gum tragacanth showed a stable pattern of weight gain during the 4 week experimental period compared to the animals fed hyperlipidaemic diet only. The animals fed hyperlipidaemic diet containing showed significantly higher body weights ($P< 0.05$) compared to those fed on control (basal) and hyperlipidaemic diets. A significant difference in average body weights after four weeks was observed between all three the groups of animals fed on three different diets (Table 2).

Liver weights and small intestine weights (g wet weighu/100 g body weight) differed significantly among the groups (Table 2). Liver weights were significantly higher in animals fed hyperlipidaemic diet with and without fiber compared to the animals fed basal diet. The liver weights were significantly higher in animals fed hyperlipidaemic diet without fibre compared to those fed hyperlipidaemic diet with fibre. Small intestines and caecum from hyperlipidaemic animals weighed more than those from the control group and weighed slightly less than those from the hyperlipidaemic fibre group (Table 2).

Plasma Lipids and Lipoprotein Fractions

Plasma total cholesterol concentrations differed significantly among groups with concentration from the hyperlipidaemic group higher than in the control and hyperlipidacmic + 5% gum tragacanth group (Table 3). No significant difference could be observed in the plasma total triglyceride concentrations among the three diet groups (Table 3). The concentration of VLDL cholesterol was significantly higher in the hyperlipidaemic group than in both the control and hyperlipidaemic + 5% gum tragacanth group. However, the VLDL concentttion in the hyperlipidaemic + 5% gum tragacanth group was significantly higher ($P< 0.05$) than the control group. Concentrations of HDL cholesterol also differed significantly among the three diet groups, and was lower in the hyperlipidaemic group to the control and hyperlipidaemic + 5% gum tragacanth group (Table 3). Concentrations of LDL cholesterol also differed significantly among the three diet groups, with the higher concentration in the hyperlipidaemic group than in the control and 5% gum tragacanth group. The LDL cholesterol however, was significantly higher in the 5% gum tragacanth group than the control animals. The total protein concentration was significantly higher in the control diet group compared with the hyperlipidaemic and 5% gum tragacanth group. Among the three groups, the hyperlipidaemic group showed the lowest protein concentration.

DISCUSSION

Cardiovascular disease has a multiple aetiology, as a illustrated by the existence of numerous risk indicators, many of which can be influenced by dietary means. The deleterious effects of saturated animal fat and dietary cholesterol appear to be more important in the aetiology of ischaemic heart diseases (Mann *et al.,* 1997), yet the preventive effect of dietary fibre cannot be ignored. The present study investigated hypocholesterolaemic effects of gum tragacanth in rats with diet induced hyperlipidaemia.

Gum tragacanth is the plant exudate from ‘‘*Astragalus gummife*’’, native to Iran, Syria, Turkey and Asia Minor. It is a complex polysaccharide consisting of D-galactose, D-xylose and L-arabinose with cation association. It is probably made up of two major portions, 13assorin, the larger component comprises 60% of gum and tragacanthin (Klose and Glicksman, 1981).

Hypercholesterolaemia was induced in rats by feeding 1% cholesterol and 0.1% cholic acid in the diet. These amounts were chosen based on the report of Shinnick *et al.,* (1990), which demonstrated hypercholesterolaemia with minimal fatty infiltration. The hyperlipidemic animals had significatly higher body weights compared to the control animals and those fed on 5% gum tragacanth. Diets containing gum tragacanth (5%) did not appear to compromise food intake or growth in hyperlipidemic rats. However, higher small intestine and caecum weights were observed in the rats fed gum tragacanth compared with control and hyperlipidemic rats. Similar findings have been reported by Tinker et aL, (1994) and Judd and Truswell, (1985) from pectin

which is also a water soluble fibre. These result are likely due in part to the soluble fibre feeding.

Walter *et al*, (1986) reported higher concentration of microbes (indicative of increased fermentation) in rats fed a soluble source of fibre (gum arabic) compared with relatively insoluble source of fibre.

Plasma cholesterol and triglyceride concentrations were lower in gran tragacanth fed animals compared to the hyperlipidemic animals not fed gum tragacanth. These results are in agreement with those from other studies (Judd and Truswell, 1985; Tinker *et al*., 1994) in which another water soluble fibre pectin was fed to hypercholesterolemic animals. A lower HDL choleterol has shown to be associated with increased risk of cardiovascular disease in humans (Hegele *et al*., 1997). In rats, lower HDL concentrations are observed with cholesterol and cholic acid induced hyperlipidemia. Partial improvement in lipoprotein metabolism was seen abet feeding gum tragacanth to the hyperlipidaemic rats. An increase in dietary fibre has been associated with favourable alteration in lipid profiles (Benner et al., 1991). The present study shows a significant decrease in the cholesterol VLDL and LDL concentration after addition of gum tragacanth to the hyperlipidaemic diet. Feeding gum tragacanth to hyperlipidaemic rats shows hypcholesterolaemic activity, and probably accounts for the modest reduction in plasma cholesterol concentrations. In this hyperlipidaemic rat model, plasma cholesterol were lower in animals fed gum tragacanth compared with those fed on hyperlipidaemic diet only.

Viscous fibre sources have been shown to reduce lipid risk factors for coruary heart disease including total and low density lipoproteins, cholesterol and apolipoproteins by increasing fecal bile acid losses (Jenkins *et al.,* 1998). In addition soluble fibre may also reduce the rate of nutrient absorption so altering chylomicrons synthesis and reducing postprandial glucose and insulin levels and other risk factors for coronary heart disease (Cherbut *et al*, 1997; Jenkins *et al.,* 1998; Bakker *et al.,* 1998). Evidence is also now accumulating that a diet rich in fibre may protect against diseases associated with raised clotting factors (Neldman *et al.,* 1997).

Dietary intervention studies and specific dietary trials in the past have demonstrated the feasibility and efficacy of lowering serum cholesterol levels through dietary modification. The physiological effects of dietary fibres in experimental animals and humans are due to their physicochemical properties and these particular properties of gum tragacanth investigated in this study suggest that gum tragacanth would be a suitable ingredient in a healthy diet. Since the response of metabolic variables such as plasma lipoproteins and blood pressure to metabolic diets may be variable (Hegele *et al.,* 1997), the physiological effects of gum tragacanth in humans would require experiments in humans with particular emphasis on the dose related effects of this fibre.

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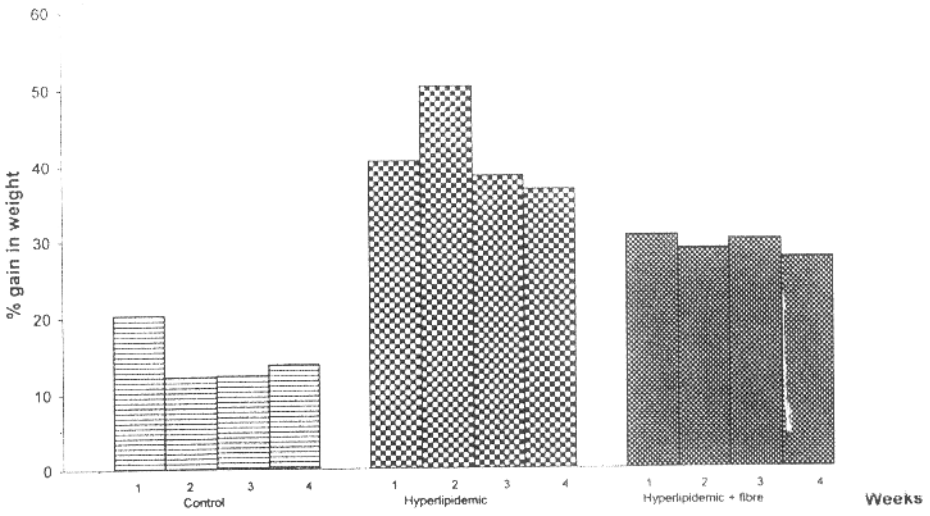

Fig. 1: Weekly weight changes in animals fed on basal, hyperlipidaemic and hyperlipidacmic + fibre diet.

Table1
Composition of three diets fed to different animal groups

Ingredients	Control (g)	Hyperlipidae-mic (g)	Hyperlipidaemic + fibre (g)
(A): Basal Composition Wheat flour	500	500	
Corn flour	250	250	
Gram flour	250	250	
Barley flour	250	250	
Wheat fine	250	250	
Corn oil	200 ml	200 ml	
(B): Experimental Hyperlipidaemic All the ingredients present in (A)			
Cholesterol		15 g (1.0%)	
Cholic acid		1.5g (0.1%)	
(C): Experimental Hyperlipidaemic +fibre All ingredients present in (A) & (B)			
Cholesterol			15 g (1%)
Cholic acid			1.5 g (0.1%)
Gum tragacanth			75g (5%)

Ingredients	Control (g)	Hyperlipidae-mic (g)	Hyperlipidaemic + fibre (g)
(A): Basal Composition Wheat flour	500	500	
Corn flour	250	250	
Gram flour	250	250	
Barley flour	250	250	
Wheat fine	250	250	
Corn oil	200 ml	200 ml	
(B): Experimental Hyperlipidaemic All the ingredients present in (A)			
Cholesterol		15 g (1.0%)	
Cholic acid		1.5g (0.1%)	
(C): Experimental Hyperlipidaemic +fibre All ingredients present in (A) & (B)			
Cholesterol			15 g (1%)
Cholic acid			1.5 g (0.1%)
Gum tragacanth			75g (5%)

Ingredients	Control (g)	Hyperlipidae-mic (g)	Hyperlipidaemic + fibre (g)
(A): Basal Composition Wheat flour	500	500	
Corn flour	250	250	
Gram flour	250	250	
Barley flour	250	250	
Wheat fine	250	250	
Corn oil	200 ml	200 ml	
(B): Experimental Hyperlipidaemic All the ingredients present in (A)			
Cholesterol		15 g (1.0%)	
Cholic acid		1.5g (0.1%)	
(C): Experimental Hyperlipidaemic +fibre All ingredients present in (A) & (B)			
Cholesterol			15 g (1%)
Cholic acid			1.5 g (0.1%)
Gum tragacanth			75g (5%)

Table 2
Body weights and organ weights in animals fed basal, hyperlipidaemic and hyperlipidaemic + fibre diet

	Control (n=10)	Ilyperlipidaemic (n = 10)	Ilyperlipidaemic +fibre (n= 12)
Body weight (%increase)	42.15***	56.31***	47.18
Liver (g wet weight1l00 g body weight)	3.23 _0,01***	4.52+ 0.04***	3.74+ 0.11
Small intestine (g wet weight/100 g body weight)	1.58+ 0.008*	1.79+ 0.02**	1.95+0.004
Caecum (g wet weight/100 g body weight)	0.75+ 11.001*	0.88+0.002**	1.07+0.001

Values are mean ! SEM
n = number of observations, *p<0.05, ** p<0.01, ***p< 0.005

Table 3
Profile of plasma lipids and proteins in rats fed basal, hyperlipidaemic and hyperlipidaemic + fibre diet

	Control	**Hyperlipidaemic**	**Ilyperlipidaemic +fibre**
1.Tolal Cholesterol (mmol/L)	2.27+ 0.12	4.02+0.14	2.84+ 0.081
2.Triglycerides (mmol/L)	0.59 + 0.11 NS	0.63 + 0.08 NS	0.71+ 0.107
3. V LDLcholesterol (mmol/L)	0.12+ 0,002***	2.47+ 0.05***	1.344 + 0.004
4. LDL cholestrol (rnmcl/L)	017+ 0.002***	0.76+0.003***	0,47+ 0.10
S.HDLcholesterol (mmol/L)	198+ 0.004***	0.79+ 0.002**	0.99+ 0.025
6. Protein (g/L)	1.33+ 0.013**	0.62+ 0.001*	0.80+ 0.015

Values are means + SEM
NS Not significant
* p<0.05, ** p<0.01, *** p<0.005

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KINETICS OF REACTION BETWEEN PHENOTHIAZINE DYE/METHYLENE GREEN AND PEROXYDISULPHATE ION IN PRESENCE OF BROMIDE ION

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ABSTRACT:

The rate of nuclear bromination was observed when the methylene green reacts with peroxydisulphate ion in presence of bromide ion. The reaction obeys zero order kinetics in the dye whereas first order kinetics in peroxodisulphate and bromide ion. The reaction was studied at various ionic strengths and temperatures to find out the effect of ionic strength on rate of nuclear bromination and energy of6

activation. The kinetics pertain to the rate determining stage of peroxydisulphate-bromide reaction. The study in different concentration of bromide and peroxydisulphate has shown that the rate constant increases by increasing the ionic strength and temperature of the system. The effect of ionic strength on the activation parameters was also studied.

INTRODUCTION

Phenothiazine-5-ium-3,7-bis(dimethylamdno)-4-nitrochloride (M.G.) does not react rapidly with peroxydisulphate, but the depolarization occurs very easily in presence of bromide ion. The reaction shows sharp absorption band at wave length 658 am. The bromide- pm oxydisulphatc reaction (F. Uddin 1980) in presence of mineral acid follows according to following schemc

$$Br + S_2O_8^{-2} \rightarrow BrS_2O_8^{-} \text{ (activatedcomplex)} \quad (1)$$

$$BrS_2O_8^{-3} \rightarrow BrSO_4^{-} + SO_4^{-2} \quad (2)$$

$$BrSO_4^{-} + Br^{-} \rightarrow Br + S_2O^{-2} \quad (3)$$

The kinetics of reaction in presence of dye follows the same mechanism. The tesults indicate that the reaction in presence of acid follows the zero order kinetics to dye and first order kinetics in $S_2O_8^{-2}$ and Br—each, both of these ion participates in the rate limiting step. The proposed mechanism is given as below:

$$S_2O_8^{-2} + Br^{-} \rightarrow BrSO_4 + HSO_4^{-} \quad (4)$$

$$BrSO_4^{-} + Br^{-} \rightarrow SO_4^{-2} + Br_2 \quad (5)$$

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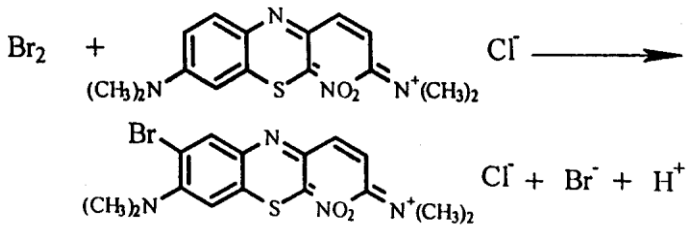

The experimental data for this reaction satisfies the following rate expression.

$$k_o = -d[M.G]/dt = k,[S_2O_8^{-2}][Br^-] \quad (7)$$

Where $k_\circ$ is specific rate constant of depolarization of dye and k, is the specific rate constant for the reaction between dye/methylene green and peroxydisulphate in the presence of bromide ion. The nuclear brominatlon, (P. V. Sub Rao, 1988) was observed when methyl orange interacts with persulphate in the presence of bromide ion. The effect of solvent on the reaction was also studied in various aqueous acetic acid mixtures. The effect of ionic strength and temperature on rate constant for a reactions such as bromoacetate-thiosulphate, (G. Corasaro, 1961) bromide-bromate, (F. Uddin, 1979) chloracetate-thiosulphate, (F. Uddin, 1985) dibromosuccinate-thiosulphate, (M. H., Bedford, 1934) and bromopropionate-thiosulphate, (F. Uddin, 1992, 1995) have been studied earlier.

In the present investigations the kinetics of reactions was studied in presence of thiazine dye. We considered it interesting to examine more thoroughly this reaction, to better clarify the mechanism and the effect of ionic strength on activation parameters.

EXPERIMENTAL

All the chemicals employed were of analytical reagent grade. Double distilled water was used throughout the investigation. The wave length maxima of the Methylene green (M G) was found to be 658 run. Molar absorption co-efficient was found to be 28000 (dm^3/mol.cm). Kinetics were investigated at constant pH, which was maintained with sodiumbisulphate. The change in ionic strength was brought about by the addition of calculated volumes of potassium bromide and peroxydisulphate. The concentration of Methylene green (MG) and mineral acid were kept constant. i.e. 2.3×10^{-5} moi.dm^{-3} and 1.0 mol.dm^{-3} respectively. The reaction was studied at various ionic strength i.e. 0.16-0.42 cool dm^{-3} and at temperatures 30^0, 35^0, 40^0, 45° and 50° C·

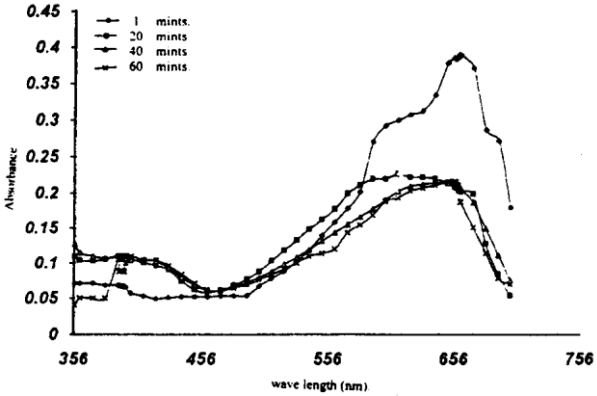

Fig. 1 :Spectra of reaction mixture at different time intervals.

Table-1
Rate measurements data at different time intervals
[M.G] =2.3x10^{-5} mol.dm^{-3} [Buffer]= 0.05 mol.dm^{-3} [KBr]= 0.15 mol.dm^{-3}
[K_2 Sr Os] = 0.017 mol.dnf ; µ-0 201 mol.dm$^{'3}$, Temperature - 40˚C 0.1 ° C

Time 1O^{4}5,	Abs. at X 658 tun_	Abs-- --_-__10"k,, at k 393.5 nm	(moldrn'
0.3	0.393	0.181	
0.6	0.375	0.188	
0.9	0.339	0.203	
1.2	0.299	0.216	
1.5	0.266	0.231	
1.8	0.251	i 0.250	
2.1	0.259	.. 0.279	
2.4	0.235	0.265	
2.7	0.230	0.275	
3.0	0.221	0.272	
3.30	0.216	0.279	
3.60	0.211	0.291	
3.90	0.211	0.308	
4.20	0.196	__ 0.303	
4.50	0.191	0.310	

Table -2
Influence of ionic strength on rate constant at 40^{0}C.
[M.G]=2.3x10^{-5} mol.dm^3

µ (mol. dm^{-3})	[Br $^-$] (mol. dm^3)	10^2[S_2 O_8 $^{-2}$] (mol.dm^{-3})	10^5 k_0 (mot dm^{-3} s $^{-1}$)	10^3 k_s dm^3 mol $^{-1}$.S $^{-1}$)
0.16	0.114	1.60	0.175	096
0.20	0.15	1.70	0.844	3.31
0.29	0.23	2.0	200	4.36
0.32	0.257	2.1	2.96	5.50
0.42	0.357	2.24	5.40	6.76

Table –3a
Rate measurements with respect to dye at various temperature and ionic strengths

Zero order rate constants 10^5 k_0 (mol.dm^{-3}s $^{-1}$) at temperatures					
µ (mol. dm^{-3})	30°C	35°C	40°C	45°C	50°C
0.16	0.113	0.161	0.175	0.366	0.450
0.20	0.56	0.644	0.844	0.925	1.13
0.29	1.21	1.56	2.00	2.20	3.11
0.32	1.67	2.05	2.96	3.17	4.18
0.42	2.95	3.41	5.40	6.06	7.81

Table - 3b
Rate measurement with respect to KBr and K_2S_2 Os at various temperature and ionic strengths

Rate constants 10^3 k_s (dm^3 mol^{-3} $*S^{-1}$)					
μ ($mokdm^{-3}$)	30°C	35°C	40^0C	45^0C	50^0C
0.16	0.621	0.885	0.963	2.00	2.47
0.20	2.20	2.51	3.31	3.63	4.46
0.29	2.63	3.39	4.36	4.79	6.76
0.32	3.10	3.80	5.50	5.88	7.76
0.42	3.70	4.27	6.76	7.58	9.77

Table - 4
Activation parameters at 25°C

μ ($mol.dm^{-3}$)	**Ea** $K.cal.mol^{-1}$	**$H^{\#}$** $K.cal.mol^{-1}$	**$S^{\#}$** $cal.mol^{-1}$	**$G^{\#}$** $K.cal.mol^{-1}$
0.20	10.40	9.81	-16.85	14.83
0.29	9.59	8.99	-17.25	14.13
0.32	9.15	8.55	-17.45	13.75
0.42	8.57	7.97	-17.65	13.23

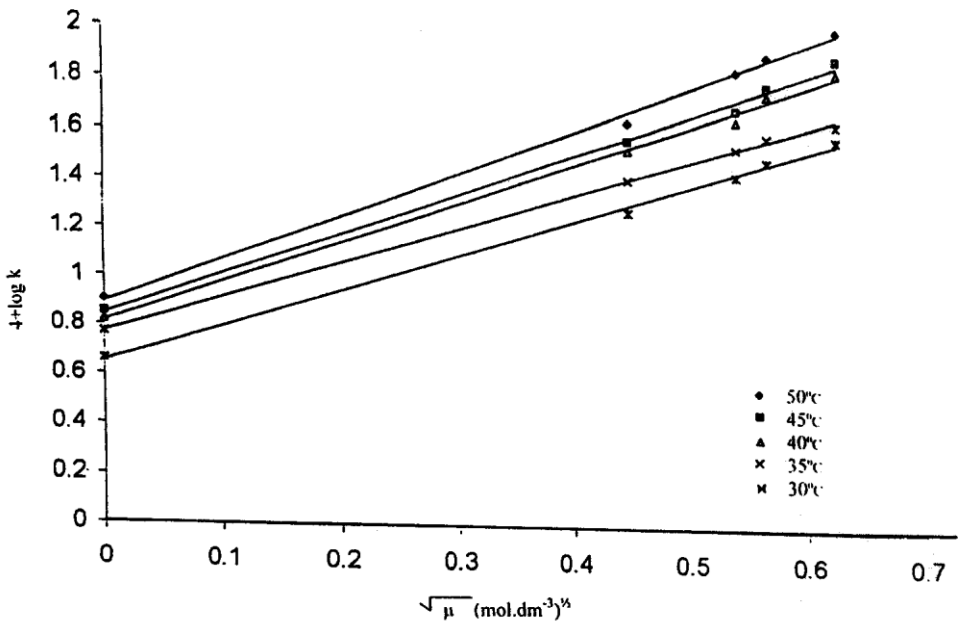

Fig. 2: log k vs √μ

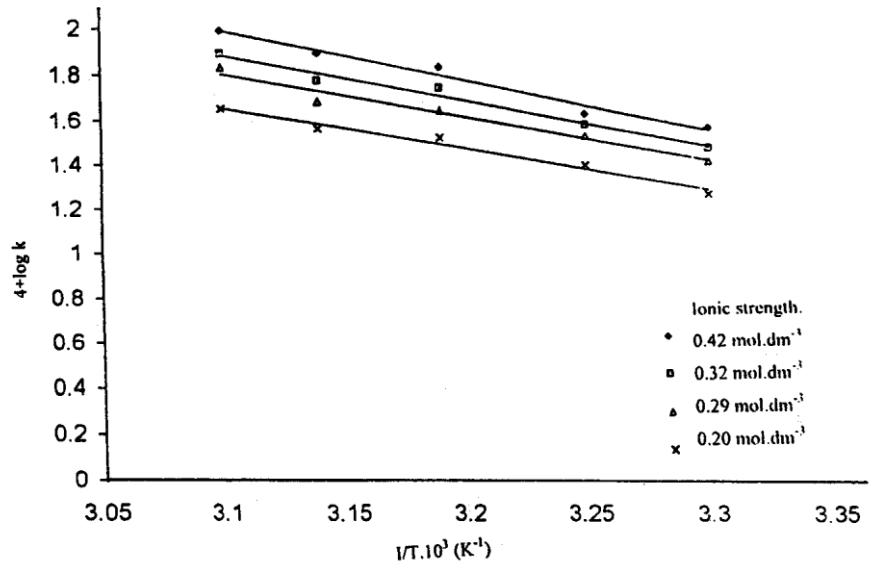

Fig. 3: log k vs T^{-1}

RESULTS AND DISCUSSION

Absorption spectra: The spectra of reaction mixture at different time interval, is shown in fig.l. The maximum absorbance of reaction mixture at different time intervals is also shown at the wave length 650 to 660 nm. Which is due to the unreacted methylene green and them is continuous decrease in the value of absorbance at different time, while a new peak arises at wave length 390 to 400 nm which is due to the formation of bromine during the reaction.

The ionic strength of the reaction mixture was varied by increasing the concentration of bromide and peroxydisphate ions. While the concentrations of methylene green and acid kept constant throughout the experiments. The values of absorbencies at ionic strength 0.201 $mol.dm^{-3}$ and 40°C are summarized in Table I. A staight line with a negative slope was obtained when absorbace at k658 nm was plotted against time, zero order kinetics was followed with respect to methylene green.

The values of rate constant of the reaction at various ionic strength are tabulated in Table 2. The influence of ionic strength and temperature on specific rate constant is shown by Table 3.

According to the (Table 3) there is a gradual increase in the value of rate constant at particular temperature and ionic strength. The behavior was not acceptable when the reaction was studied at lower ionic strength there was an inverse relationship between the ionic strength. The abnormal behavior was supported by the observations made by king *et al*. Results in Table (3.b) show that the increase in ionic strength and temperature also increase the rate constant. This is in accordance with the Debye – Huckel-Bronsted (S. Glasstone, 1941) theory. The plot of log k vs square root of ionic strength fig. 2 is a straight line with a positive slope. The average value of Z_ZA_Z calculated from the slope of the line was found to be 1.74. Where as the average value of Z_ZA_Z from the Kilpatrick relation was determined as 1.93. Which is more closer to the theoretical value.

The values of energy of activation were calculated from the slope of Arrhenius plot. The representative plots are shown in fig. 3. The results of activation parameters presented in Table. 4 were obtained from usual relationships. (S. Glasston, 1941, A. lndelli, 1960) Variation of activation parameters with ionic strength (greater than 0.16 mol.drn^{-1}) follows the same pattern described earlier. (F. Uddin, 1979, 1985, 1992, 1995, M. II., Bedford, 1934)

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