nificant amount of immunoglobulins is transmitted across the foetal membranes through the maternal circulation, the greater part is transmitted through colostrum. However, in the case of vaccinnia virus, Malkinson[10] has shown that the protection transferred across the foetal membrane is more effective. Since the protection obtained was only limited to ip challenge, one could speculate that blood-brain barrier restricts the diffusion of antibodies into the intracellular space.

Absence of alteration in the pattern of cross-reactivity despite five passages in the heterologous system indicates that there is no cross-protective passive immunity between these two viruses although antigenically they are known to exhibit cross-reactivity.

HI antibodies could be demonstrated in 2-ME treated and untreated sera of infants and their mothers when tested on various days after the delivery. In the studies with tritiated myeloma proteins, it was shown that only IgG molecules were transported across the epithelial barrier to blood while IgM and IgA were not absorbed in either monomeric or polymeric forms[11]. In the present study, therefore, HI titres in the infant sera could be due to IgG antibodies. Our inability to demonstrate HI antibodies in the stomach contents of the infants may be due to rapid absorption of antibodies across the gut[12]. It is of interest to study the persistence of these antibodies derived from maternal circulation and from colostrum separately so as to assess the effectiveness and duration of this protection.

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SYNTHESIS OF SOME FORMAZANS AND TETRAZOLIUM BROMIDES AS POTENTIAL ANTIVIRAL AGENTS

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ABSTRACT

1-Aryl-3-(3'-nitro-4'-methoxyphenyl)-5-phenyl formazans (II) were synthesized by the reaction of 3-nitro-4-methoxybenzaldehyde phenylhydrazone (I) with various aryl diazonium salts. Some of the formazans (II) on oxidation by H_2O_2/Fe^{++} were cyclized into their corresponding 3-aryl-5-(3'-nitro-4'-methoxyphenyl)-2-phenyl tetrazolium bromides (III). A majority of compounds II and III exhibited significant antiviral activity against ranikhet disease virus in a stationary culture of chorioallantoic membranes of chick embryos.

INTRODUCTION

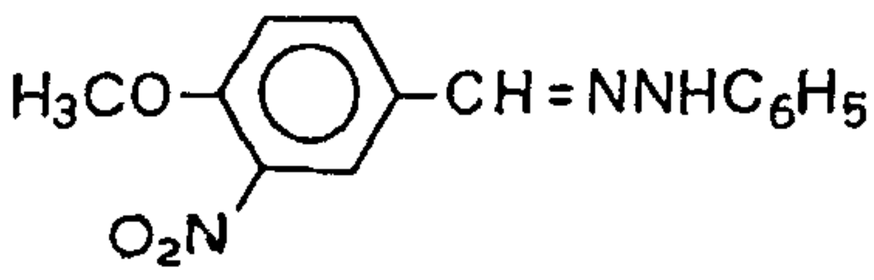
FORMAZANS and tetrazolium salts have since long been found to possess antiviral[1] and antibacterial[2,3] properties. Recently Misra *et al*[4] and Mukherjee *et al*[5-7] have synthesized various formazans and tetrazolium salts, some of which have significantly inhibited both animal as well as plant viruses. Formazans on oxidation are converted into their tetrazolium salts which because of their polar nature possess

potential antiviral activity[4]. In the present communication, the synthesis and antiviral activity of 1-aryl-3-(3'-nitro-4'-methoxyphenyl) -5-phenyl formazans (II) and 3-aryl-5-(3 -nitro-4'-methoxyphenyl) -2-phenyl tetrazolium bromides (III) against ranikhet disease virus are being reported.

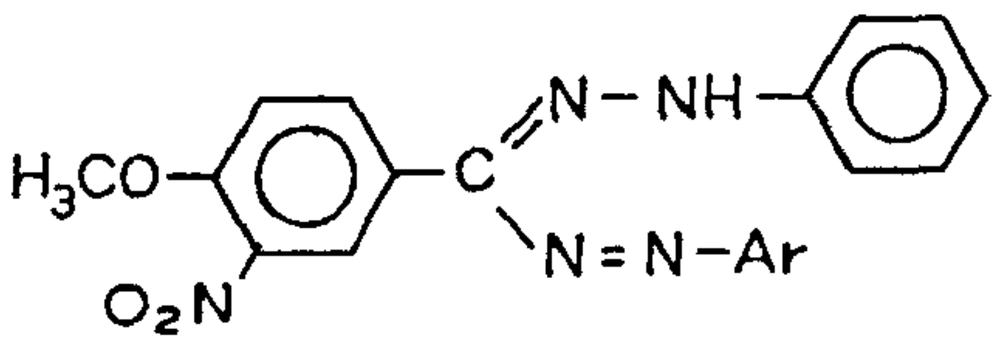
Condensation of 3-nitro-4-methoxybenzaldehyde with phenyl hydrazine afforded its phenylhydrazone (I). The reaction of 3-nitro-4-methoxybenzaldehyde phenylhydrazone (I) with aryl diazonium salts in pyridine yielded 1-aryl-3-(3'-nitro-4'-methoxyphenyl)-5-phenyl formazans (II) which were further oxidized into their corresponding tetrazolium bromides (III).

Experimental

Melting points were determined using sulphuric acid-bath in open capillary tubes and were uncorrected. IR spectra in KBr were recorded on a Perkin-Elmer 157 spectrophotometer (ν_{max} in cm^{-1}). 3-Nitro-4-methoxybenzaldehyde and 3-nitro-4-methoxybenzaldehyde phenylhydrazone (I) were prepared according to the earlier reported method[8].



I



II

III

1-Aryl-3-(3'-nitro-4'-methoxyphenyl)-5-phenyl formazans (II)

These were prepared by the reaction of 3-nitro-4-methoxybenzaldehyde phenylhydrazone (I) with an appropriate diazonium salt solution.

The diazonium salt solution of an appropriate aryl amine (0.015 mol) was added, dropwise with continuous stirring, to a solution of 3-nitro-4-methoxybenzaldehyde phenylhydrazone (I) (0.01 mol) in pyridine (20 ml), keeping the temperature below 12°. The reaction mixture was allowed to remain for about 4 hr and was then poured into 250 ml of ice-cooled water with continuous stirring. The dark-coloured solid, which separated out, was filtered, washed with cold water, followed by hot water, and finally with methanol and dried well in air. Formazans (II), thus synthesized, were recrystallised from ethanol or benzene and are recorded in table-I, yield 54-74%. Their IR spectra showed characteristic bands at 3450-3400 (NH), 2940-2910 (CH), 1690-1675 (CO), 1615-1605 (CN), 1520-1510 & 1345-1330 (NO_2).

3-Aryl-5-(3'-nitro-4'-methoxyphenyl)-2-phenyl tetrazolium bromides (III)

These were synthesized by the oxidation of the corresponding formazan (II), using hydrogen peroxide [in the presence of ferrous ions (H_2O_2/ Fe^{++})] as the oxidizing agent.

To an appropriate powdered fromazan (1 g) dissolved in the minimum amount of ethanol, was added 2N sulphuric acid (5 ml) containing a trace of ferrous sulphate. Thereafter, an excess of 20% hydrogen peroxide was added and the contents were heated on a water bath for 3 hr. After completion of the oxidation (disappearance of dark red colour), the excess of ethanol was distilled off under reduced pressure and the residual liquid was treated with an excess of sodium bromide to precipitate out the crude tetrazolium bromide, which was filtered, washed with a little water and dried well in air. It was finally crystallized by triturating it with petroleum ether (b.p. 40 60°). Five tetrazolium bromide (III), thus obtained are listed in table 2. Their IR spectra showed the characteristic bands at 2955-2920 (CH), 1690-1680 (CO), 1615-1610 (CN), 1525-1510 and 1340-1330 (NO_2).

Antiviral activity

All compounds II and III were tested for their antiviral activity against ranikhet disease virus (RDV) in a stationary culture of minced chorioallantoic membranes (CAM) of chick embryos according to an earlier procedure[9]. CAM of 10-day old chick embryos were used in all the experiments and the virus multiplication was measured by haemagglutination (HA) titre of the culture, collected after incubation. Inhibition in

TABLE 1

Physical and antiviral data of 1-aryl-3-(3'-nitro-4'-methoxyphenyl)-5-phenyl formazans (II)

Compound No.	Ar	m.p.°C	Molecular formula	Colour	Concentration of compound (mg/ml)		Percent inhibition
					Toxic to 50% CAM culture	Used for activity	
IIa	C_6H_5	98	$C_{20}H_{17}N_5O_3$	Dark purple	3	1.5	45
IIb	o-$CH_3.C_6H_4$	138	$C_{21}H_{19}N_5O_3$	Blackish brown	3	1.5	40
IIc	m-$CH_3.C_6H_4$	79-80	$C_{21}H_{19}N_5O_3$	Violet	3	1.5	50
IId	p-$CH_3.C_6H_4$	171	$C_{21}H_{19}N_5O_3$	Dark brown	3	1.5	10
IIe	o-$COOH.C_6H_4$	102	$C_{21}H_{17}N_5O_5$	Purple	2.5	1.3	50
IIf	m-$COOH.C_6H_4$	200	$C_{21}H_{17}N_5O_5$	Brown	3	1.5	60
IIg	p-$COOH.C_6H_4$	203-04	$C_{21}H_{17}N_5O_5$	Reddish purple	2	1.0	60
IIh	p-$COOC_2H_5.C_6H_4$	165	$C_{23}H_{21}N_5O_5$	Violet	2	1.0	45
IIi	p-$NHAc.C_6H_4$	115	$C_{22}H_{20}N_6O_4$	Reddish purple	2.5	1.3	25
IIj	p-$NO_2.C_6H_4$	118	$C_{20}H_{16}N_6O_5$	Violet	2.5	1.3	40
IIk	p-$OCH_3.C_6H_4$	105	$C_{21}H_{19}N_5O_5$	Brown	2.5	1.3	40
IIl	p-$OC_2H_5.C_6H_4$	125	$C_{22}H_{21}N_5O_4$	Dark violet	2.5	1.3	25
IIm	p-$Cl.C_6H_4$	172	$C_{21}H_{16}ClN_5O_3$	Purple	3.0	1.5	30
IIn	p-$Br.C_6H_4$	158	$C_{20}H_{16}BrN_5O_3$	Dark brown	3.0	1.5	40
IIo	p-$I.C_6H_4$	129	$C_{20}H_{16}IN_5O_3$	Dark purple	2.5	1.3	25

All the Compounds were analysed for C, H and N and the experimental values agreed with the calculated Values within the limits of experimental errors.

TABLE 2

Physical and antiviral data of 3-aryl-5-(3'-nitro-4'-methoxyphenyl)-2-phenyl tetrazolium bromides (III)

Compound No.	Ar	m.p.°C	Molecular formula	Concentration of Compound (mg/ml)		Percent inhibition
				Toxic to 50% CAM culture	Used for activity	
IIIa	m-$COOH.C_6H_4$	185	$C_{21}H_{16}BrN_5O_5$	2.5	1.3	30
IIIb	p-$COOH.C_6H_4$	190	$C_{21}H_{16}BrN_5O_5$	3.0	1.5	10
IIIc	p-$COOC_2H_5.C_6H_4$	122	$C_{23}H_{20}BrN_5O_5$	2.5	1.3	30
IIId	p-$NHAc.C_6H_4$	188	$C_{22}H_{19}BrN_6O_4$	3.0	1.5	50
IIIe	p-$Br.C_6H_4$	132	$C_{20}H_{15}Br_2N_5O_3$	3.0	1.5	20

All the Compounds were analysed for C, H and N and the experimental values agreed with the calculated Values within the limits of experimental errors.

virus multiplication was obtained by substracting this titre from that of control. The results of antiviral activity of compounds II are included in table 1 and those of compounds III in table 2.

The results of antiviral activity of compounds II indicate that all the compounds except IIi, IIl, IIo exhibited significant activity against RDV ranging from 30-60%, compound IIf and IIg showing significant inhibition (60%). However, among compounds III, only three compounds IIIa, IIIc and IIId significantly inhibited the multiplication of virus, compound IIId exhibiting maximum protection (50%).

These results of antiviral activity of compounds II reveal that when carboxylic group is introduced in the aryl moiety, the activity of the compound is increased, more so if the group is at *m* or *p*-position, while with the other substituents, the compounds are either less active or as active as the formazan with unsubstituted aryl ring. From the SAR point of view one can thus infer that the presence of a free ionic group in the molecule appears to enhance the antiviral activity of the formazans, since the compound with carboethoxy group (IIh) is as active as the parent compound (IIa).

The results of antiviral activity of compounds III indicate that among the tetrazolium bromides, only the compound (IIId), with an acetamide group at *p*-position in aryl exhibited a greater activity than the corresponding formazan (IIi). These results thus lead one to conclude that oxidation of formazans into the tetrazolium bromides, on the whole, renders them inactive against RDV.

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ANNOUNCEMENT

INTERNATIONAL SEMINAR ON 'IMPACT OF GENETICS ON BASIC PROBLEMS OF HUMAN BIOLOGY'

An International Seminar on "Impact of Genetics on Basic problems of Human Biology" is being organised at Bhopal (Madhya Pradesh) India under the joint auspices of Bhopal University, Bhopal and Society of Bionaturalists during 2-5 December 1983. The major sessions will include invited deliberations on Detection and Prevention of abnormal births, Child and Mother care, Twins and Twinning, Genetics of blood disorders, Chromosomes in cancer, Human ecological genetics: genetics of isolates, population migration, impact of urbanisation. The proceedings will be published in 'Bionature'. Abstracts of papers may be submitted before the end of October 1983. The registration fee of $50 may be sent along with the abstract. All payments may be made to Bionature.

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