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STUDIES ON DITHRANOL AND DITHRANOL-LIKE COMPOUNDS

I. *Binding to Nucleic Acids*

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Abstract. One of our most potent antipsoriatic agents, dithranol, forms molecular complexes with DNA. Systematic studies of dithranol and dithranol-like compounds have been undertaken in order to increase our understanding of the effect of dithranol on psoriasis. In the present paper the stereochemical criteria for the binding of anthracene and anthraquinone derivatives to nucleic acids have been studied. Most substitutions with OH-, NH_2- and OCH_3-groups are compatible with binding to native DNA. OH-substitutions in positions 2, 3, 6, and 7 make the compounds ionized at neutral pH, thus inhibiting binding to DNA. Chlorine substitutions give only a very weak binding to DNA. Substitutions with NO_2- and CH_3COO-groups give no binding. None of the substances studied except 2-amino-anthracene forms a complex with RNA or denatured DNA. Thus for most anthracene and anthraquinone derivatives studied a double helical structure of the nucleic acid seems to be necessary for complex formation.

Dithranol is one of our most potent antipsoriatic agents. Its mode of action on psoriasis is not fully known. In 1965 it was shown by Swanbeck & Thyresson (5) that dithranol interacts with DNA and it was assumed that this interaction was of importance for the therapeutic effect of dithranol on psoriasis. Later it was found that dithranol induces respiratory deficient mutants in yeast (1) and that dithranol inhibits thymidine incorporation in guinea pig epidermis (6). Krebs & Schaltegger (2) found that the regeneration of the *Xenopus laevis* larvae is inhibited by dithranol.

During the more than 50 years that dithranol has been used no compound of similar type with a higher antipsoriatic activity has appeared. Whether there is such a derivative, we do not know. It does not seem justifiable or possible to us to study different types of anthracene derivatives directly on psoriatic patients since some derivatives are highly carcinogenic. We have therefore chosen to study such properties of dithranol-like compounds that may be of importance for their antipsoriatic effect. In the present paper we give a report of our investigation on the interaction of dithranol-like compounds with nucleic acids.

MATERIAL AND METHODS

All substances studied except two were commercially available. Most of them were bought from Aldrich Chemical Company Inc., Milwaukee, Wisc. Emodin, chrysophanic acid, anthrarobin, dithranol and chrysarobin were obtained through the pharmacy of the University Hospital in Uppsala. 1,8-diacetoxy-anthraquinone and 1-methoxy-anthraquinone were kindly synthesized for us by Dr Gerd Bendz at the Department of Organic Chemistry, Uppsala University. The purity of the compounds was checked by thin layer chromatography on silicic acid and was found to be good except for chrysarobin and anthrarobin and also to a certain extent for dithranol. Impurities in the latter three compounds were detected in the chromatograms.

The solubility of most anthracene and anthraquinone derivatives in water or phosphate buffer is less than 1 mg/litre. To be spectroscopically analysable in the visible range these substances must be present at a concentration of several milligrams per litre. The substances were therefore first dissolved in pure ethanol and then added to a buffer and a solution of DNA in the same buffer. As a rule 60 mg of the substance studied was dissolved in 100 ml ethanol and 0.5 ml of this solution added to 100 ml of the buffer and the DNA solution giving a final concentration of 3 mg of the substance studied and 5 ml ethanol per litre solution. Two different buffers were used, one containing 0.05 M $Na_2HOP_4 + 0.05$ M NaH_2PO_4 giving pH 6.8 and the other containing 0.1 M NaH_2PO_4 giving pH 4.3. The DNA was grade A from calf thymus bought from Calbiochem, Los Angeles. The DNA concentration was 100 mg/l or 1000 mg/l. The spectra were recorded on a Beckman DB spectrophotometer with 4 cm cuvettes.

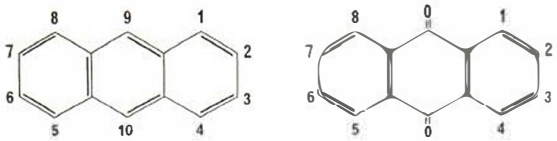

Fig. 1. Generally accepted numbering of the carbon atoms of anthracene and anthraquinone derivatives.

The criterion for binding of a substance to DNA was a red-shift of the absorption maximum in the visible range. If about the same red-shift was obtained at a concentration of 100 mg DNA per litre as for 1 000 mg DNA per litre the binding was defined as strong (*s*), which means a molar ratio of dye to DNA-phosphate in the complex of larger than 0.05. If the spectral shift increased by 25% to 3 times from 100 mg DNA per litre to 1 000 mg DNA per litre the binding was called weak (*w*) corresponding approximately to a molar ratio between 0.05 and 0.01, and if the spectral shift increased by more than 3 times, as very weak (*vw*), which corresponds to a molar ratio less than 0.01.

In the equilibrium dialysis experiments a DNA solution was dialysed against a solution of the substance in the buffer. The concentration of the substance after dialysis was estimated from the peak height of the absorption maximum.

Experiments with RNA were made in the same way as the DNA experiments. RNA was highly polymerized grade A from yeast.

RESULTS

Six anthracene and sixteen anthraquinone derivatives have been investigated with regard to binding to DNA. In order to facilitate further reading the generally accepted numbering of the carbon atoms in anthracene and anthraquinone derivatives is shown in Fig. 1.

Among the six anthracene derivatives studied, 1,9-dihydroxy-anthracene, 1,8,9-trihydroxyanthracene (dithranol), and the latter compound substituted with a methyl group at position 3 (chrysarobin), give a red-shift of the absorption maximum in the presence of DNA at neutral pH. Binding does not take place immediately as is the case for the anthraquinone derivatives. The red-shift is registered at the earliest 20 hours after mixing of the solution. Although chrysarobin is a rather impure product, it was included in this study because it is used in external dermatological therapy.

3,4,9-trihydroxy-anthracene does not interact with DNA at neutral pH, where it is evidently ionized, but is bound immediately to DNA at pH 4.3. 9-hydroxy-anthracene does not have an ab-

Table I. *Anthracene derivatives*

Each column represents one derivative and the type of substituted group is indicated in the row corresponding to the position of the substitution. (See text.) Binding to DNA at pH 6.8 and pH 4.3 are shown at the foot of the table.
s = strong binding; *w* = weak binding; *vw* = very weak binding.
– = no binding

	1	2	3	4	5	6
1		OH	OH		OH	
2						NH_2
3				OH	CH_3	
4				OH		
5						
6						
7						
8			OH		OH	
9	OH	OH	OH	OH	OH	
pH 6.8	?	*s*	*s*	–	*s*	–
pH 4.3	?			*s*		*s*

Table II. *Anthraquinone derivatives*

Each column represents one derivative and the type of substituted group is indicated in the row corresponding to the position of the substitution. (See text.) At the foot of the table, binding to DNA at pH 6.8 and pH 4.3.
s = strong binding; *w* = weak binding; *vw* = very weak binding. – = no binding.

	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	OH	OH	OH	OH	OH		OH	OH	OH	OH	OCH_3	NH_2	Cl	CH_3 COO	OH	OH
2		OH				OH	OH	OH								
3									CH_3	CH_3						
4			OH				OH								NH_2	NO_2
5				OH											NH_2	NO_2
6						OH				OH						
7								OH								
8					OH				OH	OH			Cl	CH_3 COO	OH	OH
pH 6.8	*w*	–	*s*	?	*s*	–	–	–	*s*	–	*w*	*w*	*vw*	–	*s*	–
pH 4.3		*s*		?		*w*	*s*	*w*		*s*				–		–

sorption maximum in the visible range, thus making it difficult to analyse by the present method. 2-amino-anthracene does not give a red-shift of the absorption maximum but both it and 9-hydroxy-anthracene are solubilised in appreciable amounts by DNA, as shown by equilibrium dialysis. At pH 4.3 2-amino-anthracene gives a red-shift of the absorption spectrum in the presence of both DNA and also RNA, contrary to all other anthracene and anthraquinone derivatives studied. Results for anthracene derivatives are summarized in Table I. Each substance is represented by a column and a substitution in position 9 by an OH-group is indicated by OH in row nine of that particular column. Thus for substance number 6 which is 2-amino-anthracene there is NH_2 in column 6, row 2. In row 10, binding at pH 6.8, and in row 11, binding at pH 4.3 is indicated.

The anthraquinones studied are listed in Table II in an analogous manner. At neutral pH OH-substituted anthraquinones are bound to DNA when there is one substitution at position 1, two substitutions at positions 1 and 8, or 1 and 4. Because of very low solubility in the buffer, 1,5-dihydroxy-anthraquinone could not be analysed. At neutral pH there seems, however, to be no binding to DNA when there is an OH-group in positions 2, 3, 6 or 7. These substances form a complex with DNA at pH 4.3. At this pH they are yellow and evidently not ionized which they seem to be at neutral pH where their colour is red or brown. An $-OCH_3$ (methoxy) or amino substitution at position 1 gives binding to DNA at neutral pH. This is also the case for methoxy substitution in positions 1 and 8. A chlorine substitution in positions 1 and 8 gives a very weak binding to DNA. Substitution in positions 1 and 8 by acetoxy groups gives no DNA binding. This compound hydrolyses within 24 hours giving 1,8-dihydroxy-anthraquinone which forms a complex with DNA. A simultaneous substitution of OH-groups in positions 1 and 8 and NH_2-groups in positions 4 and 5 gives binding to DNA, whereas a simultaneous substitution of OH in positions 1 and 8 and NO_2-groups in positions 4 and 5 gives no DNA binding. A methyl group in position 3, as in chrysophanic acid (1,8-dihydroxy-3-methyl-anthraquinone) does not seem to inhibit DNA binding.

None of the investigated anthracene or anthraquinone derivatives studied except 2-amino-anthracene gave a spectral shift in the presence of RNA. The RNA preparation was used in a control experiment with acridine orange which gave a spectral shift according to earlier findings. Thus in this respect the anthracene and anthraquinone derivatives differ from the acridines (4).

DISCUSSION

Several anthracene and anthraquinone derivatives have the property of forming complexes with DNA (7). Due to limitations of the spectroscopic technique used, anthracene and anthraquinone could not be studied but it is possible that they form complexes with DNA to a rather limited extent compared with their derivatives. Certain substitutions increase the affinity for DNA and others inhibit complex formation.

Substitution with small polar groups like hydroxy-, amino- and methoxy-groups increases the degree of binding to DNA. If the substitution makes the anthracene or anthraquinone derivative a negative ion at a particular pH, no binding to DNA occurs, probably because of repulsion between the derivative and the negatively charged phosphates of the DNA. This is the case for hydroxy substitutions at positions 2, 3, 6 or 7 at neutral pH. If the pH is lowered to 4.3 these derivatives become uncharged and form a complex with DNA. Substitution with large and bulky groups also inhibits binding to DNA. Examples of such groups are acetoxy- and nitro-groups. The diameter of the chlorine atom is somewhat larger than the thickness of the flat anthracene or anthraquinone ring and it is interesting to note that a chlorine substitution gives very poor binding to DNA. Unfortunately, other halogenated derivatives are difficult to obtain, but work is now in progress to synthesize fluorine derivatives.

Contrary to the acridines, no anthracene and anthraquinone derivatives studied, except 2-amino-anthracene, form complexes with RNA and do so very poorly with denatured DNA. Why 2-amino-anthracene is an exception, we do not know. The other derivatives seem to be dependent for binding on an helical structure of the nucleic acid.

Krebs & Schaltegger (3) investigated the antipsoriatic effect of different anthracene derivatives. There seems to be a marked parallelism between antipsoriatic activity as reported by these authors and their ability to form molecular complexes

with DNA as reported in the present paper. The anthraquinones chrysazin and chrysophanic acid do not have an antipsoriatic effect but readily form complexes with DNA.

We have not had the opportunity to study 1,8,9-triacetoxy-anthracene (dithranol-triacetate) but feel that all acetoxy substitutions inhibit binding to DNA because of the bulkiness of the substituting-group. In a water solution such groups are easily split off and a hydroxy derivative is formed. This might also occur in epidermal cells.

At present we believe that for dithranol-like compounds the ability to form complexes with DNA is necessary for an antipsoriatic effect. However, not all compounds which have this ability have antipsoriatic activity. Penetration, chemical alteration and binding to other substances in the cells are, of course, also of importance.

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