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The influence of dihydroxyanthracene derivatives on water and electrolyte movement in rat colon

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The purgative activities of 18 different dihydroxyanthracene derivatives, including free anthraquinones and anthrones, were investigated by determining their influence on the water, sodium and potassium absorption in the gastrointestinal tract by direct injection of the solutions in Tyrode to the rat colon *in situ*. The extent of the solubility of the compounds has also been assessed. The 1,8-dihydroxyanthracene structure seemed to be the best for hydragogue effect. Rhein-anthraquinone and -anthrone were the most active compounds tested.

Laxative medicinal plants like aloe, senna, frangula and rheum contain anthracene derivatives either as glycosides or aglycones in oxidized or reduced form. They are classified with the diphenylmethane derivatives, phenolphthalein and bisacodyl in the group of stimulant cathartics (Goodman & Gilman, 1965) which act mainly in the large intestine by altering the peristalsis and water absorption.

Forth, Rummel & Baldauf (1966), using the technique of the tied-off colon segments of rats *in vivo*, showed that bisacodyl and oxyphenisatin inhibited the absorption of water and electrolytes and increased their secretion into the intestinal lumen. This hydragogue effect is believed to be an essential part of the mechanism by which diphenols exert their laxative action.

The same technique of Forth & others (1966) has been used on the rat colon to extend information on the laxative substances present in senna fruits and senna leaves (Lemmens, 1974). Several derivatives of 1,8-dihydroxyanthracene-3-carboxylic acid (rhein) were injected into the colon on which sennosides showed no effect, but the aglycones rhein and rhein anthrone caused a significant inhibition of water and sodium absorption and an increased amount of potassium in the intestinal lumen.

A prerequisite for the hydragogue effect of the anthracene derivatives, as well as for the laxative action of diphenylmethane derivatives and bisacodyl, seems to be the presence of two phenolic functions (Forth & others, 1966). According to Schmid (1952, 1959) the orally administered senna glycosides should be hydrolysed and metabolized in the gastrointestinal tract by bacterial enzymes, and the free aglycones should cause the laxative effect. According to Fairbairn & Moss (1970), the sugars should have a transporter function enabling the aglycones to reach the site of action in the large intestine.

To assess the importance of the position of the phenolic hydroxyl groups and the degree of substitution and the oxidation on laxative properties, we have tested several dihydroxyanthraquinones and their corresponding anthrones on the rat colon.

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MATERIALS AND METHODS

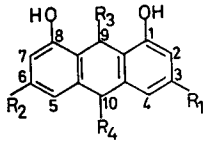
Anthraquinones

All the dihydroxyanthraquinones available commercially were recrystallized in boiling *N,N*-dimethylformamide. 1,4-dihydroxyanthraquinone (Quinizarine): Flüka AG; 1,5-dihydroxyanthraquinone (Anthrarufine): Flüka AG; 1,8-dihydroxyanthraquinone (Istizine): Merck AG; 2,6-dihydroxyanthraquinone: Aldrich; chrysophanol: Flüka AG. Aloeemodin was prepared from aloin by the method of Fairbairn & Simic (1963). Rhein was prepared from aloin by the method of Bellaart (1952) and emodin was extracted from Frangula bark according to Kinget (1966).

Anthrones

The anthrones were prepared by reduction of the corresponding anthraquinones with $SnCl_2$ in acid medium, according to Auterhoff & Scherff (1960). 2,6-Dihydroxyanthrone was prepared by reduction of 2,6-dihydroxyanthraquinone in sodium carbonate solution, with sodium dithionite as the reducing

agent. Istizine-10- and rhein-10-anthrone were prepared by reduction of the corresponding acetylated anthraquinones with $SnCl_2$ in acid medium according to Stoll, Becker & Helfenstein (1950). Aloe-emodin anthrone was prepared from aloin by the method of Kinget (1967).



R_1	R_2	R_3	R_4	
Me	H	O	O	Chrysophanol
CH_2OH	H	O	O	Aloe-emodin
COOH	H	O	O	Rhein
Me	OH	O	O	Emodin
H	H	O	H_2	1,8-Dihydroxy-9-anthrone
COOH	H	O	H_2	Rhein-9-anthrone
H	H	H_2	O_2	1,8-Dihydroxy-10-anthrone
COOH	H	H_2	O	Rhein-10-anthrone

Biological assay methods

The influence of the anthracene derivatives on the water and electrolyte absorption in the colon was tested by the method of Forth & others (1966).

Female Wistar rats, 200 g, received no food for 16 h before the experiment but had free access to water. Each rat was anaesthetized with ether and the large intestine, from the caecum to the rectum exposed and washed with a physiological salt solution. Then Tyrode solution, 2 ml containing 10^{-3} M of each product, was injected into the ligated colon (Tyrode solution, g litre^{-1}: NaCl, 8·0; KCl, 0·2; $CaCl_2.6H_2O$, 0·2; $MgCl_2.6H_2O$, 0·1; $NaHCO_3$, 1·0; NaH_2PO_4, 0·05; glucose, 2·7). Compounds that were slightly soluble were ground to a suspension in a mortar.

After 1 h the rat was killed under ether anaesthesia, the colon segment was excised and the residual fluid content of the segment was measured volumetrically.

The fluid was centrifuged 10 min at 3000 rev min^{-1}. The supernatant liquid was analysed for its sodium and potassium content using a flame photometer. The osomotic pressure was measured with a precision osmometer.

Amount of the compounds dissolved in Tyrode

To compare the activity of dihydroxyanthracene derivatives, 2 ml of 10^{-3} mol litre^{-1} of each preparation, in Tyrode solution, was injected into the rat colon. Some as a solution but most were in suspension.

To ascertain if a correlation of the amount of the compounds dissolved in Tyrode after 1 h, and the pharmacological effect after that time existed, we determined their solubility.

A suspension of each compound in 100 ml Tyrode was made by grinding in a mortar. The suspension was stirred for 1 h under N_2 and filtered through glass. 20 ml of the filtrate was transferred to a separating funnel and extracted with ethyl acetate. After drying the extract with sodium sulphate, the extinction of the ethyl acetate solution was determined at the corresponding wavelength.

RESULTS

The position of the phenolic groups

Table 1 shows the amounts of residual fluid volume and its Na and K content, 1 h after the injection of the compounds in 2 ml Tyrode solution into the ligated colon segments.

In the intestinal mucosa, transport of water and the soluble substances takes place in both directions, i.e. to and from intestinal lumen and blood. The difference between these two transports gives the *net flow* of water and soluble compounds.

All the products tested, except the 1,4- and 1,5-dihydroxyanthraquinones caused a decrease in water absorption i.e. there was more water in the lumen 1 h after the drug was administered in comparison with the control.

Na^+ absorption was also restricted and there was an apparent release of K^+ into the lumen.

Table 1 shows that the position and number of 'free' hydroxyl groups, present in each compound, affects its solubility in Tyrode solution. In the compounds having an α-hydroxyl group adjacent to a meso-carbonyl group in their molecule, a hydrogen bond is formed giving rise to a chelate structure. This will decrease the hydrophilic property of the hydroxyl group and consequently the solubility in Tyrode solution.

2,6-dihydroxy compounds showed the highest solubility possibly because of the absence of intramolecular hydrogen bonds.

There was no relation between the amount of the compounds dissolved and their activity after 1 h.

1,4- and 1,5-dihydroxyanthraquinones do not influence the water absorption in the colon. They have two hydroxyl groups, chelated with the adjacent carbonyl group. In their anthrone forms a free hydroxyl group appears and the compounds were active. 1,8-Dihydroxyanthraquinone and -9-anthrone were the most active products tested.

Increase in the number of free hydroxyl groups cannot be regarded strictly as a contributing factor to the increase in activity because the 2,6-dihydroxy-

Table 1. *Residual fluid, sodium and potassium concentration in colon segments of fasted rats 1 h after administration of the dihydroxyanthraquinones and -anthrones (10^{-3} M).* Mean of 5 rats ± s.e.* = $P < 0.01$ compared with the controls.

	Resid fluid (ml)	Na^+ concn (mM)	Na^+ (μmol)	K^+ concn (mM)	K^+ (μmol)	Sol† 10^{-7} mol litre^{-1}
Tyrode soln (2 ml)	0·78 ± 0·13	119 ± 4·3	93	18·1 ± 6·1	13·9	
1,4-diOHanthraquinone	0·98 ± 0·30	93 ± 12·6	94·5	14·8 ± 3	14·2	6·6
1,4-diOHanthrone	1·30* ± 0·17	136 ± 4·2	177	15·5 ± 3·8	20·3	51·3
1,5-diOHanthraquinone	1·08 ± 0·26	121 ± 6·9	131	17·3 ± 4·7	19·6	6·2
1,5-diOHanthrone	1·32* ± 0·10	135 ± 6·8	179	12·8 ± 3·9	16·5	83·6
1,8-diOHanthraquinone	1·76* ± 0·24	116 ± 6·6	206	15·5 ± 2·3	27·4	27·5
1,8-diOH-9-anthrone	1·76* ± 0·28	125 ± 5·5	222	15·1 ± 2·2	26·7	21·6
1,8-diOH-10-anthrone	1·60* ± 0·22	120 ± 5·0	192	18·7 ± 3·2	30·3	84·9
2,6-diOHanthraquinone	1·54* ± 0·17	126 ± 3·2	194	20·6 ± 3·6	31·7	310
2,6-diOHanthrone	1·62* ± 0·20	129 ± 2·1	209	16·4 ± 3·8	26·3	356
Tyrode solution	2·0	154	308	2·7	5·4	

† The amount of the dihydroxyanthracene derivatives, dissolved in Tyrode solution after 1 h at room temperature.

compounds were no more active than 1,8-dihydroxy-derivatives.

No significant difference in activity was observed between the anthraquinone and anthrone forms, nor between the 9- and 10-anthrone forms.

The importance of the substituent in 1,8-dihydroxy-anthracene derivatives

As the 1–8 position of the phenolic groups in the anthraquinone and anthrone molecule seems to be the optimum structure for a hydragogue effect (Table 2), we tested several natural 1,8-dihydroxy-anthracene derivatives of the medicinal plants, senna, frangula to see the effect of substitution in the molecule on laxative properties.

All the products tested inhibited water and electrolyte absorption in the colon. The rhein derivatives seem to be the most active.

There is a difference in solubility in Tyrode solution between the rhein compounds and the other 1,8-dihydroxyanthracene derivatives. This is due to the carboxylic group on the C_3 in the rhein compounds, through which water-soluble salts can easily be formed. The most soluble products were also the most active. All the 1,8-dihydroxy derivatives were active and the substituents did not change this activity, except the carboxylic group. There was no significant difference between the anthraquinone- and the anthrone form, or between the 9- and 10-anthrone forms.

Table 2. *Residual fluid, sodium and potassium concentration in colon segments of fasted rats 1 h after administration of the 1,8-dihydroxyanthraquinones and -anthrones (10^{-3} M).* Mean of 5 rats ± s.e.* = $P < 0.01$ as compared with the controls.

	Resid fluid (ml)	Na^+ concn (mM)	Na^+ (μmol)	K^+ concn (mM)	K^+ (μmol)	O.P. (m osm)	Sol† 10^{-5} mol litre^{-1}
Tyrode soln (2 ml)	0·62 ± 0·14	116 ± 15	74	12·5 ± 5·9	7·4	229	
Chrysophanol	1·18* ± 0·23	122 ± 4	145	11·7 ± 4·6	14·6	224	1·4
Chrysophanol-9-anthrone	1·24* ± 0·32	88 ± 11	110	5·6 ± 0·7	7·1	260	0·12
Aloe-emodin	1·10* ± 0·24	100 ± 6	110	6·3 ± 17	6·8	225	1·5
Aloe-emodin-9-anthrone	1·61* ± 0·07	110 ± 19	177	8·5 ± 3·6	13·8	250	11·3
Rhein	1·90* ± 0·20	124 ± 9	236	11·1 ± 1·2	21·1	247	196
Rhein-9-anthrone	2·07* ± 0·21	137 ± 7	284	10·4 ± 1·0	21·6	259	200
Rhein-10-anthrone	1·84* ± 0·22	130 ± 2	240	19·1 ± 3·4	35	—	191
Emodin	0·99* ± 0·10	127 ± 6	125	9·5 ± 1·3	9·4	230	10·8
Emodin-9-anthrone	105* ± 0·26	108 ± 18	112	6·3 ± 1·7	6·4	241	1·1
Tyrode soln	2·0	154	308	2·7	5·4	289	

† The amount of the 1,8-dihydroxyderivatives dissolved in Tyrode solution after 1 h at 37°C.

DISCUSSION

Forth & others (1966) classified the anthraquinone compounds in the same group as bisacodyl, oxyphenisatin and their derivatives, which are diphenols. Indeed, they assumed that the dihydroxyanthraquinones, like bisacodyl, when directly administered, prevented the absorption of water and Na^+ in the colon. This has been proved by Lemmens (1974, 1976) and also in the present investigation. Higher concentrations of these products in the ligated colon segment produced a net flow of water and Na^+ from the blood to the lumen.

A prerequisite for the hydragogue effect of the anthraquinone derivatives is the presence of free phenols preferably in the 1,8-position. The sennosides, diglucosides of rheindianthrone did not show any effect on the colon (Lemmens, 1976). However, the aglycones, rhein and rheinanthrone are active.

Generally there was no significant difference in activity on the colon between the anthraquinone- and anthrone forms, or between the 9- and 10-anthrone forms. Chemical analysis of the Tyrode solution after injection of rhein dianthrone or rhein into ligated colon segments did not show the formation of rhein anthrone, and we have found (unpublished) that after metabolism of Sennoside A and B in the caecum of the rat *in vivo*, no rhein anthrone, but rhein dianthrone and rhein were formed.

The anthraquinone form could be responsible for the hydragogue effect in the colon. These facts are in agreement with observations of Longo (personal communication) that after oral administration of the anthracene derivatives of frangula, no anthrones had been formed.

According to Ewe & Hölker (1974), the hydragogue effect of the diphenols can be explained by an increase in the permeability of the intestinal membrane towards the transfer of liquid and dissolved salts from the blood to the lumen. Another factor for this effect should be the hindrance of the Na^+ pump present in the mucosal cells. This inhibition of the Na^+ pump, should be the result of an inhibition of the microsomal ($Na^+ + K^+$) ATPase (Skou, 1965). Chignell (1968) showed that phenolphthalein, bisacodyl and 1,8-dihydroxyanthraquinone inhibited the microsomal ($Na^+ + K^+$) ATPase from rat small intestine brush border (epithelial) cells.

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