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Structure–Activity Relationships of Anthraquinones in the Decrease of Intestinal Motility

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Abstract

The effects of substituted anthraquinones on intestinal motility were evaluated in-vitro using rabbit small intestinal strips.

This structure–activity relationship study revealed the critical requirement of a hydroxy group at R_2 position. The intestinal motility was inhibited 50% (IC50) by emodine (8 μM), 2-hydroxy anthraquinone (20 μM), 2,6-dihydroxy anthraquinone (25 μM), 2,7-dihydroxy anthraquinone (10 μM), 1,2,4-trihydroxy anthraquinone (80 μM) and 1,2,5,8-tetra-hydroxy-anthraquinone (9 μM). The presence of other polar groups at R_2 position such as an amino, aldehyde and carboxylic acid group significantly reduced the activity (IC50 360–400 μM). The presence of a methyl group and esterification of the carboxylic acid at R_2 position was found to abolish the activity.

These data are useful for the future development of anthraquinones as laxative agents.

Anthraquinones have been widely used as laxative agents (Gaginella & Base 1978; de Wite & Lemli 1990). Emodin (7-methyl,2,4,5-trihydoroxyanthraquinone), the active principle of *Rheum palmatum* with antineoplastic and antibiotic activity (Wang 1993), has also been introduced as a vasorelaxant (Hung et al 1991). The anthraquinone moiety has an influence on the motility of intestinal smooth muscle (Jin et al 1994). The structural requirements of anthraquinones for the production of stimulation or inhibition and mixed stimulation/inhibition remain obscure although it is helpful for understanding those laxatives that may cause constipation (Bonnycastle 1965). We have reported that anthraquinone-2-carboxylic acid derivatives can inhibit intestinal motility (Kuo et al 1996). In this study, the structural features necessary for the inhibition, partial inhibition and stimulation of intestinal motility by anthraquinone derivatives are investigated.

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Materials and Methods

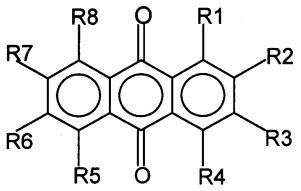
Materials

2-Hydroxy anthraquinone, 2,7-dihydroxy anthraquinone (John & Arthur 1923), 2-anthraquinone carbaldehyde (Jacob 1921), 2,6-dimethyl anthraquinone, 2,7-dimethyl anthraquinone (Morgan & Coulson 1929), 2,6-anthraquinone dicarboxylic acid, 2,7-anthraquinone dicarboxylic acid (Moshchinskaya et al 1980), 2,6-anthraquinone diethylcarboxylate and 2,7-anthraquinone diethylcarboxylate (Cohen et al 1968) were synthesized according to published methods. Other derivatives were purchased from Aldrich Chem. Co. (Wisconsin, USA). Solutions of **2**–**3**, **7** and **13**–**18** (Table 1) were prepared in a mixture of PEG400: alcohol: water (30 : 10 : 60); **1**, **4**–**6**, **8**–**12** and **19**–**20** were prepared in alkaline distilled water; and the others were dissolved in distilled water.

Bioassay

Male New Zealand albino rabbits, weighing 2·5–3·0 kg, were used. The small intestine prepared from these rabbits was cut into 0·5–0·6-cm strips and mounted in an organ bath (10 mL) containing

Table 1. Chemical structure of di-substituted anthraquinone derivatives and their effect on inhibiting motility of rabbit small intestine in-vitro.



Compound No.	R1	R2	R3	R4	R5	R6	R7	R8	IC50 (μM)[a]
1		OH		OH	OH		CH_3		8 ± 1
2		NH_2							380 ± 26
3		CHO							360 ± 34
4		COOH							400 ± 14
5		COOH				COOH			250 ± 14
6		COOH					COOH		100 ± 16
7		CH_2OH							300 ± 23
8		OH							20 ± 2
9		OH				OH			25 ± 2
10		OH					OH		10 ± 2
11	OH	OH		OH					80 ± 6
12	OH	OH			OH			OH	9 ± 1
13		CH_3							>1000[b]
14		CH_3				CH_3			>1000[b]
15		CH_3					CH_3		>1000[b]
16	OH	CH_3	CH_3	OH					>1000[b]
17		COOEt				COOEt			>1000[b]
18		COOEt					COOEt		>1000[b]
19	OH							OH	500 ± 34
20	OH		OH						550 ± 32
21	OH		CH_3					OH	650 ± 45

[a]Concentration (μM) for stimulation of small intestinal motility to produce 50% of maximal action. Values are presented as mean $\pm$ s.e.m. ($n = 6-8$). [b]Inhibitory response of >1000 was considered.

Tyrode-Ringer solution (pH 7·4) at $37 \pm 1^{\circ}$C under continuous aeration with a mixture of 5% CO_2 and 95% O_2. Tyrode-Ringer solution (pH 7·4) was prepared with NaCl (136·9 mM), KCl (2·68 mM), $CaCl_2$ (1·8 mM), $MgCl_2$ (1·05 mM), $NaHCO_3$ (11·9 mM), Na_2HPO_4 (0·42 mM) and glucose (5·55 mM). The spontaneous contraction was isometrically recorded on polygraphic paper (Physiography MKIII-S) through a mechanoelectrical transducer (NARCO, Bio-system). Incubation with noradrenaline (0·1 μM) abolished the contraction of smooth muscle. Recovery of motility was obtained by refreshing the Tyrode-Ringer solution twice. This treatment is used to recognize normality of the intestinal motility. When motility reached a stable plateau, anthraquinone derivatives were added into the medium cumulatively at doses of 1 μM–0·5 mM every 5 min. The lowered contractions induced by these compounds were expressed as percentage of spontaneous motility.

Data analysis

Data are expressed as means $\pm$ s.e.m. from a number ($n = 6-8$) of experiments. Statistical analysis was performed using paired comparison of Students *t*-test; $P \leq 0{\cdot}05$ was considered significant.

Results and Discussion

As a continuation of our interest in exploring new inhibitors of intestinal smooth muscle motility, we studied a series of mono-, di- and trisubstituted anthraquinone analogues and evaluated their pharmacological activity. For comparison, emodine (**1**) was first investigated and found to be a very potent inhibitor of intestinal smooth muscle motility (IC50 8 μM) using the protocol. This is similar to the effect in vascular smooth muscle (Hung et al 1991). Table 1 lists IC50 values of the studied anthraquinone analogues. All anthraquinone derivatives having amino- (**2**), carbaldehyde- (**3**), carboxylic- (**4**–**6**), hydroxymethylene- (**7**) and hydroxy-substituents (**1**, **8**–**12**) at the R_2 position showed inhibitory activity of intestinal motility; those with a methyl group (**13**–**16**) or carboxylic ester (**17**, **18**) at this position were inactive. It should also be pointed out that the anthraquinone moiety possesses a C_2 symmetry. When taking this factor into consideration, the environment occupied by the R_2

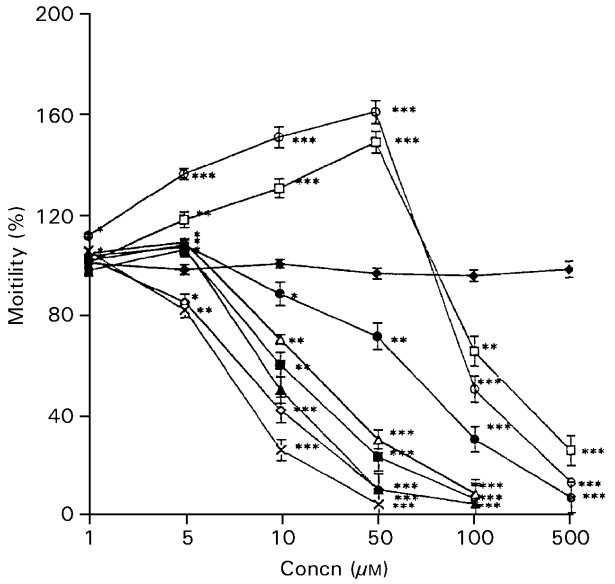


Figure 1. Concentration–response curves of anthraquinone derivatives on rabbit isolated small intestinal motility. Cumulative dose of compound **1** (×), **5** (□), **6** (○), **8** (■), **9** (△), **10** (▲), **11** (●), **12** (◇) or vehicle (◆). Data are mean ± s.e.m. (n = 6–8); $*P < 0.05$ $**P < 0.01$, $***P < 0.001$ compared with control.

substituent has a direct correlation with either the substituent at the R_3, R_6 or R_7 position respectively. Thus an anthraquinone having a substituent at any of the four positions, R_2, R_3, R_6 or R_7, will possess inhibitory activity.

In this study, we found the anthraquinones **8**–**12** which had a hydroxy substituent at the R_2 (or R_3, R_6 and R_7) position to be most effective and consistent inhibitors of intestinal motility with an efficacy comparable to that of emodine (**1**) (Figure 1). The presence of a hydroxy group at any other position in compounds **19**–**21** showed a very much reduced activity with an average IC50 of 500 μM. Our study suggested that the presence of a hydroxy substituent at the R_2 (or R_3, R_6 and R_7) position is an important factor in determining high inhibitory activity.

As the anthraquinone moiety possesses a C_2 symmetry whereby R_2, R_3, R_6 and R_7 are inter-related, the effect of having further substituents at any of these positions may have potentiating effects. We found that the disubstituted carboxylic acid at R_2, R_6 (**5**) and R_2, R_7 (**6**) were more potent that the monosubstituted counterpart (**4**). Surprisingly, potentiation was not observed in the case of the disubstituted hydroxy at R_2, R_6 (**9**) and R_2, R_7 (**10**). Furthermore, the R_2, R_7-disubstituted compounds (**6**, **10**) had a higher efficacy, illustrating the importance of the spatial requirement for optimal activity.

It was interesting to note that the dicarboxylic compounds **5** (R_2, R_6) and **6** (R_2, R_7) showed a pronounce mixed action of stimulation (< 50 μM)/inhibition (> 50 μM) for intestinal motility at different dosages. Such findings suggest that this biphasic property may be the main contributor to the side effect of laxative-induced constipation observed during overuse (Riecken et al 1990; Hogue 1996). The mechanism for this biphasic effect remains to be clarified.

Thus, this study strongly implies that future design of anthraquinone-based inhibitors of intestinal motility must ultimately incorporate a hydroxy group at the R_2 position. The nature and position of substituents on the anthraquinone ring also has an appreciable influence on the efficacy of the compound. A synergistic effect can sometimes be achieved by polysubstitution at the R_2, R_3, R_6 and R_7 positions, which are inter-related.

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