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The relative purgative activities of 1,8-dihydroxyanthracene derivatives

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The purgative activities of twelve different 1,8-dihydroxyanthracene derivatives including free anthraquinone, anthrone and dianthrone forms, anthraquinone *O*-glycosides and dianthrone *O*-glycosides were compared with senna pod powder using the production of wet faeces by mice as a criterion of purgation. The higher purgative activity of the dianthrone glycosides was confirmed for the compounds based on rhein. Sennidin (rhein dianthrone) was more active than had previously been reported. These highly active compounds had parallel dose response curves which were not parallel to those of the less active rhein anthrone, rhein, aloe-emodin and chrysophanol. Emodin and chrysazin were inactive in mice. The highly active compounds exerted a high activity during the initial 3 h after dosage while the less active compounds were virtually inactive during this period. Rhein anthrone appeared to act initially like the highly active primary sennosides, sennoside A and sennidin and later as the less active rhein. The results are discussed in relation to the mode of action of orally administered 1,8-dihydroxyanthracene derivatives.

Fairbairn (1949) showed that, in mice, orally administered anthracene derivatives* are highly active as anthrone glycosides, less active as free anthrones and much less active as free anthraquinones. Other workers have confirmed this; anthraquinone glycosides are more active than their corresponding aglycones (Ferguson, 1956); anthrone glycosides, from frangula, are more active than the anthraquinone glycosides (Jørgensen, 1950) and the glycosides glucofrangulin and frangulin are more active than their aglycone emodin (Longo, 1965; Cresseri, Peruto & Longo, 1966). It has been further suggested (Schmid, 1952, 1959; Fairbairn, 1965) that the anthrone form is the active substance *in situ* and that the sugar moiety prevents absorption of the aglycone from the intestine and subsequent detoxication in the liver, or protects it from breakdown in the intestine before it reaches its site of action in the colon and rectum.

To provide additional evidence for these ideas we investigated the relative purgative activities of a larger range of anthracene derivatives than have been studied before at one time.

EXPERIMENTAL

Anthraquinones (I–V)

Aloe-emodin, prepared by the method of Muhtadi (1969), m.p. 222–224° (lit. Fairbairn & Simic, 1963 give 225–226°); λ_{max} 226, 255, 287 and 430 nm (lit. Auterhoff & Scherf, 1960, give 220, 255 and 287 nm).

* The term "anthracene derivatives" refers to those compounds with a nucleus of 1,8-dihydroxyanthraquinone, 1,8-dihydroxyanthrone or dianthrones of the latter. Similarly the terms "anthraquinone", "anthrone" and "dianthrone" all refer to the 1,8-dihydroxy forms.

Rhein, prepared by the method of Oesterle (1902, 1903), m.p. 318–320° (lit. Nawa, Uchibayashi & Matsuoka, 1961, give 321°); λ_{max} 229, 259 and 430 nm (lit. Nawa & others, 1961, give 229 and 258 nm).

Chrysophanol, prepared by the method of Oesterle (1911), m.p. 193–195° (lit. Naylor & Gardner, 1931, give 196°); λ_{max} 229, 255, 289 and 430 nm (lit. Auterhoff & Scherf, 1960, give 222, 251 and 289 nm).

Emodin, obtained in a pure form from another member of the laboratory.

Chrysazin, Bayer Products Ltd.

Anthrones (VI)

Aloe-emodin anthrone, prepared by the method of Rosenthaler (1932), m.p. 196° (lit. Hauser, 1931, gives 195°); λ_{max} 223, 259, 292 and 378 nm (lit. Kinget, 1967, gives λ_{max} 220, 258 and 291 nm).

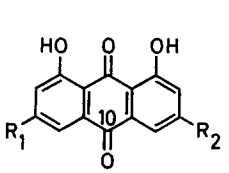
Rhein anthrone, prepared from an impure sample (containing 65% rhein anthrone) by preparative chromatography. Narrow bands of the impure rhein anthrone, dissolved in acetone, were applied to adsorbent layers (0·75 mm) of Kieselgel G (slurries prepared with citrate buffer at pH 6·3) and the plates developed in toluene–methanol (2:1). The band corresponding to the reference rhein anthrone was carefully scraped off and the rhein anthrone eluted from the Kieselgel G with 1% hydrochloric acid in acetone. The eluates from several plates were combined, evaporated to small volume and diluted with water. The precipitate of rhein anthrone was filtered, washed with water, and dried under vacuum at 90°. M.p. 270–300° (decomp.) (lit. Auterhoff & Scherf, 1960, decomp. at 288°); λ_{max} 207, 267, 296 and 372 nm (lit. Lemli, Dequeker & Cuveele, 1963, give λ_{max} 220, 264 and 295 nm).

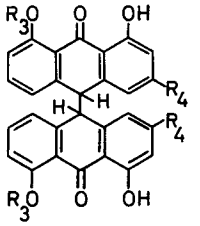
Dianthrones (VII–VIII)

Aloe-emodin dianthrone, prepared from aloe-emodin anthrone by a modification of the method of Kinget (1967). Aloe-emodin anthrone (100 mg) was dissolved in boiling ethanol (50 ml). To the boiling solution, kept in a dim light, a 1% ethanolic solution of hydrated ferric chloride (10·5 ml) was gradually added over 45 min. The green solution was diluted to 1 litre with water and left overnight at 4°. The precipitate was filtered, washed with water, and dried under vacuum at 90°. The crude aloe-emodin dianthrone was purified by dissolving 30 mg quantities in 5 ml of acetone and passing it through a column of Kieselgel H–Hyflo super cel (1:1) with 1% hydrochloric acid in acetone. The yellow band was eluated and evaporated to small volume and purified by preparative layer chromatography. Bands were applied to absorbent layers of Kieselgel G (0·75 mm) and the plates developed in toluene–cyclohexane–iso-octane-n-propanol (3:5:1:1). The band corresponding to aloe-emodin dianthrone was scraped off and the dianthrone eluted from the adsorbent with the acid acetone solvent. The eluates from several plates were combined, evaporated to small volume and diluted with water. The dianthrone was filtered, washed well with water and dried under vacuum at 90°. M.p. 250–255° (decomp.) (lit. Auterhoff & Scherf, 1960, decomp. 260°); λ_{max} 215, 271 and 370 nm (lit. Kinget, 1967, gives λ_{max} 220, 270 and 365 nm).

Sennidin (Rhein dianthrone) prepared by heating sennoside A (150 mg) in 3N sulphuric acid in a boiling water bath for 30 min. M.p. 330° (decomp.) (lit.

Auterhoff & Scherf, 1960, decomp. above 300°); λ_{max} 215, 270 and 385 nm (lit. Kinget, 1967, gives λ_{max} 220, 275 and 375 nm).





	R_1	R_2			R_3	R_4	
I	H	H	Chrysazin	VII	H	CH_2OH	Aloe-emodin
II	H	Me	Chrysophanol				dianthrone
III	H	CH_2OH	Aloe-emodin	VIII	H	COOH	Sennidin
IV	H	COOH	Rhein				(rhein dianthrone)
V	OH	Me	Emodin				
VI	The anthrones are formed by reduction of the = O on C (10) to H_2			IX	Glucose	COOH	Sennosides A and B

Anthraquinone glycoside

Aloe-emodin 1,8-*β*-D-*diglucoside*, prepared by the method of Muhtadi & Moss (1969), m.p. 224–226°; λ_{max} 222, 260 and 388 nm.

Dianthrone glycosides (IX)

Crude glycoside concentrate. As no primary sennosides were available an impure sample was prepared from senna pod by the method of Fairbairn, Friedmann & Ryan (1958) which gave a highly water soluble powder containing the calcium salts of the primary sennosides as well as other glycosides (28·1% rhein glycosides, calculated as sennosides A and B, 0·23% of aloe-emodin glycosides, calculated as aloe-emodin monoglucoside and 0·05% free anthraquinones, calculated as rhein).

Sennoside A. The crude glycoside concentrate (4 g) were dissolved in water (40 ml) and the pH of the solution lowered to 2·0–2·5 with a few drops of concentrated hydrochloric acid or the addition of ion-exchange resin [Zeocarb 225 (H^+)]. After standing (60 min) the dark brown precipitate was removed and discarded. The solution was left to stand for 48 h; during this time a yellow precipitate of sennoside A formed. This was filtered, washed with ether followed by water, and dried under vacuum at 90°. (Sennoside B did not precipitate from the solution until after the 48 h period). M.p. 200–220° (decomp.) (lit. Stoll, Becker & Kussmaul, 1949, decomp. 200–240°); λ_{max} 212, 270 and 380 nm (Ref. sennoside A gave λ_{max} 220, 270 and 375 nm).

Standard senna pod

One sample of Alexandrian senna pod in moderately fine powder was used as a standard in all the bioassays. This contained 4·79% rhein glycosides (as sennosides A and B), including primary sennosides, sennosides and rhein glucosides; 0·23% aloe-emodin glycosides (as aloe-emodin monoglucoside) and 0·17% free aglycones (as rhein) including dianthrones.

Biological assay methods

Relative purgative potencies. Dose-response curves and the relative purgative potencies were determined using a modification of the method described in Appendix

III of The Recommended Methods for the Evaluation of Drugs: The Chemical Assay of Senna Fruit and Senna Leaf (1965).

Three separate batches of male albino mice were used in three series of bioassays. 60 or 80 mice divided into groups of 10 were used in each assay. The mice within each group differed by only 1 to 2 g. The anthracene derivatives were orally administered in a dose volume of 1 ml in 2 or 3 dose levels to each group of 10 mice. The range of doses used is listed in Table 1. Senna pod powder was made up as an infusion in hot water. The soluble compounds were dissolved in water and the insoluble compounds were suspended in a 0·2–0·5% w/v solution of compound tragacanth powder. To standardize the conditions compound tragacanth powder was added to the infusion of senna pod powder and to the solutions in similar concentrations. The concentration of the anthracene derivatives in each dose was determined by chemically assaying 1 ml samples. Whenever possible a large number of results for each compound, at different dose levels, was obtained by making several bioassays with each compound. Senna pod powder, in two dose levels was given as a standard in each assay. As only one assay could be made each week the weight of the mice increased (from 20 g to about 50 g) during the series of assays and this was taken into account by expressing the doses as mol/kg of mouse.

Purgative activity was measured by counting the wet faeces produced by each pair of mice 3, 6, 9 and 24 h after administration of the anthracene derivatives. The total number of wet faeces produced by each group of 10 mice (each dose level) over the 24 h period were expressed as "the number of wet faeces/kg of mouse".

The dose levels of the anthracene derivatives were arranged such that the number of wet faeces produced by each group of 10 mice was never greater than 80 to 90 over 24 h. Doses producing this number or less ensured that the size of the wet faeces was constant. Higher doses produced very large wet faeces or such a number that individual ones were difficult to distinguish. When dosed mice were left in the cages for more than the 24 h period no more wet faeces were produced, indicating that the anthracene derivatives were only active during the 24 h period. The results obtained from each batch of mice, for each compound, were plotted as dose response curves. The correlation coefficient and regression coefficient for each compound were calculated if 5 or more results were obtained. If the correlation coefficient was greater than the tabulated value at the appropriate degrees of freedom ($P = 0{\cdot}95$) the regression line was drawn. The parallelity of the different regression lines was determined by comparison of the regression coefficients ($P = 0{\cdot}95$).

Rate of action. The rate of production of wet faeces by the mice after administration of each dose level of each anthracene derivative was determined by expressing the number of wet faeces produced by each group of 10 mice at 3, 6, 9 and 24 h as a percentage of the total number produced over 24 h. The mean of the results for each compound was then found. These ratios were only calculated if the total number of wet faeces produced by each group was greater than 20. The maximum number produced by a group of 10 mice was 90 while the mean was 42.

RESULTS

Chrysazin, emodin, chrysophanol, aloe-emodin, rhein, crude glycoside concentrate and senna pod were tested on the first batch of mice. Chrysazin and emodin produced no wet faeces. The results obtained from the other substances are plotted

Table 1. *Range of doses of anthracene derivatives orally administered to mice*

Anthracene derivative	mg/mouse	mg/kg of mouse	mol/kg of mouse ($\times 10^6$)
Senna pod standard .. .. (as sennosides A & B)	0·19– 1·53 (4–32 mg of powder)	4·4– 30·7	4·9– 35·6
Crude glycoside concentrate (as sennosides A & B)	0·28– 1·12 (1·00–2·00 mg of powder)	6·4– 25·0	7·4– 29·0
Sennoside A	0·31– 2·3	6·9– 51·0	8·0– 59·3
Anthraquinone glycoside ..	2·25– 9·0	45·0–180·0	76·0– 303·0
Dianthrones	0·39– 3·0	7·8– 71·9	14·5– 471·0
Anthrones	1·45– 5·7	29·7–124·0	105·0– 457·0
Anthraquinones	1·8 –11·6	40·0–300·0	115·0–1,111·0

All the anthracene derivatives were given in a dose volume of 1 ml per mouse.

as dose response curves in Fig. 1A. The crude glycoside concentrate was of equal activity to the senna pod standard (both calculated as sennosides A and B) while the anthraquinones were much less active and have parallel dose response curves which were not parallel to that of senna pod.

Sennoside A, sennidin, rhein anthrone, rhein and senna pod, were tested on the second batch of mice; the dose response curves are plotted in Fig. 1B. The dose

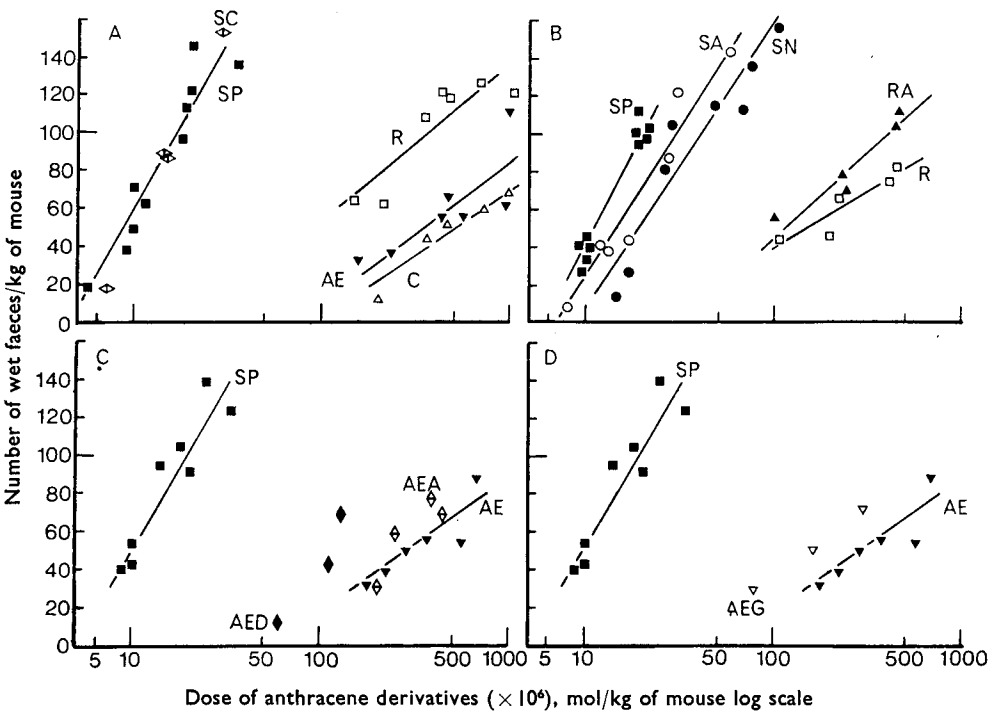


FIG. 1. Log dose response curves of anthracene derivatives tested on mice. A, first batch of mice; B, second batch of mice; C and D, third batch of mice with the same results from senna pod and aloe-emodin plotted in both for convenience. Regression lines are drawn in where there are five or more results. (AE ▼) aloe-emodin, (AEA ◈) aloe-emodin anthrone, (AED ◆) aloe-emodin dianthrone, (AEG ▽) aloe-emodin diglucoside, (C △) chrysophanol, (R □) rhein, (RA ▲) rhein anthrone, (SP ■) senna pod, as sennosides A & B, (SC ◈) crude glycoside concentrate, as sennosides A & B, (SA ○) sennoside A, (SN ●) sennidin.

response curves of sennoside A, sennidin and the senna pod standard (latter chemically assayed as sennosides A and B) were all parallel and sennoside A was 68% as active as senna pod and sennidin 53% as active. Rhein anthrone and rhein were much less active and had dose response curves which are neither parallel to the latter, more active compounds, nor to each other.

Aloe-emodin diglucoside, aloe-emodin dianthrone, aloe-emodin anthrone, aloe-emodin and senna pod, were tested on the third batch of mice. The results are plotted in Fig. 1C and D, with the same results of senna pod and aloe-emodin in each Figure. All these compounds based on aloe-emodin were far less active than the senna pod standard. Aloe-emodin dianthrone and diglucoside were only slightly more active than aloe-emodin. Unfortunately, not enough results were obtained to calculate regression lines for the former two compounds. Aloe-emodin anthrone had a similar activity to aloe-emodin but again no regression line could be calculated. However, the results for aloe-emodin anthrone, dianthrone and diglucoside all appear to lie on a slope more similar to that of aloe-emodin than senna pod.

Consistency of responses and relative potencies. The many experiments necessary involved using three successive batches of mice over two years. As already indicated the increase in weight of each mouse was compensated for by expressing the doses

Table 2. *Consistency of responses shown by the three different batches of mice after oral administration of senna pod*

Batch of mice adminstered with the standard senna pod	Correlation coefficient (r)	Tabulated r for appropriate ϕ ($P=0{\cdot}95$)	Regression coefficient†	*Dose to produce 70 wet faeces/kg mol/kg $\times 10^6$
1	0·94	0·58	178·2	11·5
2	0·96	0·67	191·3	13·5
3	0·93	0·71	169·5	14·4

* The doses are obtained from the regression lines plotted on the graphs in Fig. 2.
† The regression coefficients were shown to be not significantly different.
ϕ Degrees of freedom.

Table 3. *Relative potencies of anthracene derivatives compared to standard senna pod*

Anthracene derivative	Relative potency (in mol) (at dose * to produce 70 wet faeces/kg of mouse)	
Senna pod (as sennosides A & B)	100	
Crude glycoside concentrate (as sennosides A & B)	100	
Sennoside A	68	Parallel log dose/ response curves.
Sennidin (Rhein dianthrone)	53	
Rhein anthrone	7	
Rhein	5	
Aloe-emodin diglucoside	4	(approx.) Parallel log dose/
Aloe-emodin dianthrone	4	(approx.) response curves,
Aloe-emodin anthrone	2	(approx.) (not parallel to
Aloe-emodin	2	above).
Chrysophanol	2	
Chrysazin	Inactive	
Emodin	Inactive	

* Doses obtained from the regression lines wherever possible, plotted on the graphs illustrated in Fig. 1.

and responses as the weight, or number, per kg of mouse. Other variables were accounted for by using a standard sample of senna pod in each assay. The results in Table 2 show that there was a remarkable consistency in the responses of the different batches of mice to the standard senna pod. The regression coefficients were compared and found not to be significantly different, i.e., the regression lines of the dose response curves are parallel for all three batches of mice. The relative potencies of senna pod between the three batches was also similar. Rhein and aloe-emodin were the only other compounds tested on more than one batch of mice; the regression coefficients of these compounds were found to be similar in all three batches of mice, the relative potencies varied more than those found for senna pod. Thus the senna pod and two anthraquinones had a fairly consistent action on the different batches of mice. Although the dose response curves of the less active compounds were not parallel to those of the highly active sennoside A, sennidin and senna pod, the difference in potency of the two groups was such that the relative potencies of all the compounds tested could be compared at the dose level to produce 70 wet faeces/kg of mouse (the average of the number of wet faeces produced by the highest and lowest doses of senna pod) and related to senna pod as a standard. The results are shown in Table 3.

Comparison of the rates of production of wet faeces. The results are illustrated in Fig. 2. It is seen that they fall into two groups. Senna pod, senna pod concentrate, sennoside A, sennidin and rhein anthrone had their greatest activity, producing about 40% of the total number of wet faeces, in the initial 3 h after dosage while rhein, aloe-emodin and the other compounds based on aloe-emodin had only produced about 8% of the total number during this time. After 9 h the former group of compounds had produced about 90% of the total number of wet faeces while the latter group had produced only 67%.

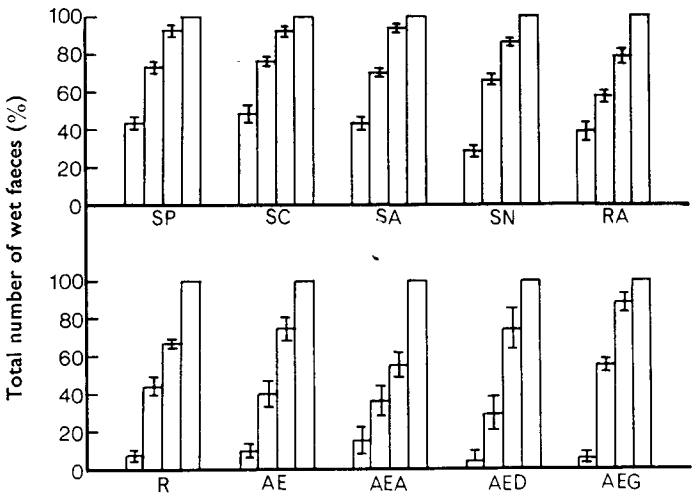


FIG. 2. Rate of production of wet faeces by mice after oral administration of anthracene derivatives. The columns represent the numbers of wet faeces, as a percentage of the total number produced over 24 h, produced during the 3, 6, 9 and 24 h periods after administration of the anthracene derivatives. The results are the mean of the results obtained from each dose level and the vertical lines represent the standard error of the mean. Key, as in Fig. 1.

DISCUSSION

Our results confirm the claims made by previous workers that glycosides are more active than aglycones and that anthrones are more active (only for rhein anthrone, not aloe-emodin anthrone) than anthraquinones as can be seen from Fig. 1 and Table 3. The present work respresents a more extensive and intensive study than has been done previously. The calculated correlation coefficients were greater than the tabulated values for those compounds from which five or more results were obtained showing that the logarithm of the dose was linearly related to the response for these compounds. This, together with the consistency of the results obtained from senna pod between the three batches of mice (Table 2) establishes that the biological assay method was reliable.

The dose-response curves of the active anthracene derivatives are parallel to each other but not to the low activity derivatives which are also parallel to each other (Fig. 1). A further difference is that the active group showed a high purgative activity during the first 3 h whereas the less active group showed little purgative activity initially but a relatively higher activity during the 9–24 h period (Fig. 2). This discrepancy in the parallelity of the dose response curves show that only the highly active compounds can be assayed using a 4-point assay method with senna pod as a standard. In this present series of assays an approximate estimate of the relative potencies between senna pod and the much less active aglycones was made by a comparison of the distances between the log-dose response curves at the dose levels to produce 70 wet faeces/kg. Although this estimate is only valid at this dose level it does illustrate the great difference in purgative activity between the two groups of compounds.

Sennoside A was only 68% as active as senna pod (chemically assayed as sennosides A and B), a result close to that given by Fairbairn (1965) who found that the sennoside content of the pod accounted only for 60% of the total activity. This was attributed to the presence of highly active primary glycosides of the sennosides and since we found that the pod and crude glycoside concentrate were equipotent and their log-dose response curves were parallel to that of sennoside A and sennidin (rhein dianthrone) it seems that the primary sennosides act similarly to the "secondary" sennosides and sennidin. A possible explanation of the differences in potency is suggested earlier (p. 584); that the sugar moiety prevents absorption of the glycosides from the alimentary canal; the more soluble the glycosides, the more will be retained in the lumen. Furthermore the sugars "protect" the active anthrone moiety from oxidation in the relatively alkaline conditions of the small intestine.

The present work has revealed some exceptions to the above suggestions. The aglycone sennidin (rhein dianthrone) was significantly more active than had previously been thought (Fairbairn, 1949). Table 3 shows that it has an activity of about 80% of sennoside A with a parallel dose-response curve. In contrast, rhein anthrone had only about 10% of the activity of sennoside A and the dose response curves are not parallel. This suggests that the dianthrone structure itself is an important factor in protecting the anthrone form from oxidation *en route*. Rhein anthrone, although of relatively low activity (probably because it is readily oxidized in the slightly alkaline conditions of the small intestine), shows high initial activity like that of the active glycosides (Fig. 2) and its dose response curve is not parallel

to either the highly active or less active groups (such as its oxidized form rhein) which indicates that some of it may reach the site of action in the large intestine in the reduced form in the first few hours after dosage. The other aglycones however have a low initial activity and a higher activity during the 9–24 h period, which may suggest that they take longer to reach the large intestine than the more soluble active compounds, or that the presence of larger amounts in the large intestine is necessary for purgation. Thus, these various suggestions may be reasons for the difference in parallelity of the dose response curves. The highly active compounds act in the anthrone form, while the less active compounds act, in a different manner, in the quinone form. The results obtained from the derivatives based on aloe-emodin (Fig. 1 and Table 3) also suggest some modification of the above generalizations. Although formation of the dianthrone and diglucoside leads to an increase in activity, the amount of increase is markedly less than that with the corresponding rhein series and aloe-emodin anthrone has the same activity as aloe-emodin. The slopes of the dose-response curves also appear to differ for the dianthrone derivatives of rhein. Moreover the relative potencies in the aloe-emodin series are much less than in the rhein series, and they have a lower initial activity. The increased activity of the rhein series may be due to the presence of the carboxylic group in rhein, acting directly because of its acidity or by increasing the solubility of the aglycone.

For high purgative activity we therefore suggest the following factors:

(i) Prevention of absorption from the alimentary canal *en route*. This is effected by the presence of sugars increasing water solubility. High molecular weight may also be a factor, since the active substances have molecular weights varying from about 560 to 1200 whereas the less active are about 270; this would explain why rhein dianthrone, though not very water soluble, is of high activity.

(ii) Prevention of oxidation of the active anthrone to the inactive quinone form. This again is effected by the presence of sugars or by a dianthrone structure.

(iii) Water solubility of the *aglycone*, so that it is in solution at its probable site of action in the large intestine.

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