Synthetic Laxative Drugs*

By N. M. FERGUSON

Data are presented on the laxative effects produced in mice by the administration of several synthetic polyhydroxyanthraquinone glycosides and their aglycones. These compounds are compared in laxative activity to standard senna powder using the fecal staining technique. Through the addition of a mixture of potassium hydroxide and magnesium hydroxide, the laxative activity of some of the synthetic glycosides is greatly increased.

LAXATIVE DRUGS and their constituents, particularly those of the polyhydroxyanthraquinone type, have been under investigation for many years. The work of Léger (1–3), Rosenthaler (4, 5), Beal and his co-workers (6, 7), Fairbairn (8, 9), Stoll (10, 11), and many others centered around the isolation and identification of the polyhydroxyanthraquinones and their corresponding glycosides from aloe, cascara, frangula, rhubarb, and senna. As a result of these investigations, the aglycones present in the the above drugs were isolated and identified. Their glycosides, however, could not be purified and identified due to the ease of hydrolysis and the difficulties involved in separation. Because of these facts, no plant substance of this type which produces laxation in man or animals has been isolated, and its structure established.

The difficulties involved in the identification of plant glycosides led Gardner and his co-workers (12–17), to the synthesis of several polyhydroxyanthraquinone glycosides in an attempt to duplicate the chemical properties of the natural glycosides and, thereby, effect an identification of these natural products.

The purpose of this research is to study the laxative effect in mice produced by the administration of certain of these synthetic polyhydroxyanthraquinone glycosides.

EXPERIMENTAL

The glycosides used in the present study were synthesized using the method of Takahashi (18). This method consists of reacting the appropriate aglycone with the desired bromo-acetylated sugar in the presence of quinoline and dry silver oxide. The acetylated glycoside is then separated and purified and the pure glycoside obtained from it by deacetylation. The preparation of some of the glycosides used in this study is described elsewhere (19), the remainder will be described in a later paper.

The laxative testing technique used was the mouse fecal staining method described by Hazelton (20), and by Lou (21), except that free access to food and water was provided at all times during the test. Female albino mice, weighing 20 to 25 grams which had been acclimated to the laboratory for two weeks prior to the test, were placed in small cages protected from drafts and provided with food[1] and water and observed for fecal stains for thirty minutes before dosing. The tendency to stain was determined by examining the lower surface of Whatman #1 filter paper placed beneath each cage. Mice producing stools which were moist enough to produce a fecal stain through the filter paper were discarded.

The remaining mice were then dosed by stomach tube with a single dose of the laxative preparation dissolved or suspended in 0.5 cc. of the dosing solution. The stomach tube consisted of a blunted spinal puncture needle attached to a syringe. The nature of the dosing solution and the results obtained are shown in Table I. Readings were taken every two hours for twelve hours and mice which produced a stain at any time during this period were recorded as positive. It was found that the mice could be re-employed in the test after a rest period of one week.

Since mice are very susceptible to air currents, and changes in room temperatures, they often become stainers if the room temperature drops below 25°. In order to control these two factors, individual treatment cages made by fitting a hinged lid to wire test tube baskets 12.5 cm. × 10 cm. × 10 cm. and fitted with water drinking tubes were used. Each set of 10 cages was covered with a cardboard carton equipped with an electric light bulb and small ventilator holes. In this way, it was possible to maintain the proper minimum temperature and at the same time keep the laboratory drafts from chilling the mice.

The dosing solutions used were water, 0.4% sodium carbonate solution, and a suspension of magnesium hydroxide in a dilute solution of potassium hydroxide. The sodium carbonate solution was used in order to solubilize the drugs being tested. The magnesium hydroxide–potassium hydroxide solution was chosen first in order to solubilize the drugs used and second because of the fact that ash determinations revealed the presence of magnesium oxide in senna leaves, as well as in frangula bark, and cascara bark.

In order to determine what effect a change in the concentration of magnesium hydroxide and potassium hydroxide in the dosing solution would have

* Received April 13, 1956, from the Pharmacognosy Research Laboratory, University of Houston, College of Pharmacy, Houston, Texas.
Presented to the Scientific Section, A. PH. A., Detroit meeting, April, 1956.
The author wishes to thank Dr. Chen and his associates at Eli Lilly and Company for running certain of the assays as well as for many helpful suggestions.

[1] Ralston Purina Checker Tabs.

TABLE I.—THE LAXATIVE EFFECT IN MICE PRODUCED BY SEVERAL ANTHRAQUINONE DERIVATIVES AND THEIR CORRESPONDING GLYCOSIDES COMPARED WITH THAT PRODUCED BY ALOE AND SENNA

Drug	Drug Dose, mg.	Dosing Solution (per 0.5 ml. Dose): Distilled Water — Number Treated	Distilled Water — Per Cent Positive	Na_2CO_3 Solution 1.88×10^{-5} Moles — Number Treated	Na_2CO_3 Solution 1.88×10^{-5} Moles — Per Cent Positive	$Mg(OH)_2$ Solution 14.2×10^{-7} Moles KOH Solution 23.3×10^{-6} Moles — Number Treated	$Mg(OH)_2$ Solution 14.2×10^{-7} Moles KOH Solution 23.3×10^{-6} Moles — Per Cent Positive
Senna-Like Stools							
Dosing solution	...	10	0	10	0	20	0
Sodium chloride	0.016	10	0	..	...	10	0
Standard senna powder	6.25	40	50	..	...	..	...
Chrysazin	20.0	20	0	..	...	20	0
Chrysazin	5.0	20	0	..	...	..	...
Chrysazin	0.128	20	0	..	...	..	...
Chrysazin glucoside	5.0	10	50	10	50	..	...
Chrysazin glucoside	2.5	10	10	20	50	..	...
K chrysazin glucoside	2.56	20	75	..	...	10	100
K chrysazin glucoside	1.28	20	40	..	...	10	100
K chrysazin glucoside	0.64	10	10	..	...	10	100
K chrysazin glucoside	0.128	..	...	..	...	10	100
K chrysazin glucoside	0.064	..	...	..	...	20	65
Rhein	0.128	10	0	..	...	10	0
Rhein glucoside	0.141	10	0	..	...	..	...
K rhein glucoside	0.141	10	10	..	...	20	60
K rhein glucoside	0.070	10	0	..	...	10	60
Chrysophanic acid	5.0	10	20	..	...	10	20
Chrysophanic acid glucoside	4.6	10	20	30	43	..	...
Chrysophanic acid glucoside	2.3	10	0	10	20	..	...
Chrysophanic acid acetyl glucoside	2.3	10	0	10	10	..	...
Chrysophanic acid maltoside	6.5	10	30	10	100	..	...
Chrysophanic acid maltoside	5.0	10	20	..	...	..	...
Chrysophanic acid acetyl maltoside	6.5	10	0	10	0	..	...
K chrysophanic acid anthranol-9-glucoside	2.47	10	0	..	...	..	...
K chrysophanic acid anthranol-9-glucoside	0.128	10	0	..	...	10	60
Aloe-Like Stools							
Powdered extract aloes N. F. VII	40.0	25	77	..	...	..	...
Powdered extract aloes N. F. VII[a]	30.0	50	58	..	...	..	...
Powdered extract aloes N. F. VII[a]	20.0	93	54	..	...	..	...
Powdered extract aloes N. F. VII[a]	15.0	29	45	..	...	..	...
Aloin U. S. P. XIV	20.0	10	40	..	...	..	...
Aloin U. S. P. XIV	7.5	10	10	10	20	..	...
Aloin U. S. P. XIV	5.0	10	10	10	20	..	...
Aloe emodin	10.0	10	10	10	40	..	...
Aloe emodin	5.0	10	0	10	0	..	...
Aloe emodin	3.15	10	0	10	0	..	...
Aloe emodin glucoside	7.5	10	70	10	100	..	...
Aloe emodin glucoside	5.0	..	...	10	80	..	...
Aloe emodin glucoside	2.0	..	...	10	10	..	...
Aloe emodin maltoside	5.0	10	80	10	100	..	...
K aloe emodin maltoside	1.0	8	0	10	10	..	...
Frangula emodin	10.0	10	0	10	10	..	...
Frangula emodin	5.0	10	0	10	0	..	...

Aloe-Like Stools

Drug	Drug Dose, mg.	Dosing Solution (per 0.5 ml. Dose) — Distilled Water — Number Treated	Distilled Water — Per Cent Positive	Na_2CO_3 Solution 1.88×10^{-5} Moles — Number Treated	Na_2CO_3 Solution 1.88×10^{-5} Moles — Per Cent Positive	$Mg(OH)_2$ Solution 14.2×10^{-7} Moles KOH Solution 23.3×10^{-6} Moles — Number Treated	$Mg(OH)_2$ Solution 14.2×10^{-7} Moles KOH Solution 23.3×10^{-6} Moles — Per Cent Positive
Frangula emodin glucoside	5.0	15	66	..	...	..	...
K frangula emodin glucoside	0.75	8	0	..	...	..	...
Frangula emodin maltoside	6.5	10	50	10	70	..	...
Frangula emodin maltoside	4.0	12	30	..	...	..	...
Frangula emodin maltoside	2.0	10	10	..	...	..	...
K frangula emodin maltoside	1.0	10	100	..	...	..	...
Alizarin-2-glucoside	8.4	10	20	10	40	..	...
Alizarin-2-glucoside	4.2	10	10	10	20	..	...
K alizarin-2-glucoside	2.4	10	50	..	...	..	...
Hydroxy-chrysazin-2-glucoside	5.0	..	...	10	70	..	...
K hydroxy-chrysazin-2-glucoside	0.128	..	...	..	...	10	20

[a] See reference (20).

on the laxative action of potassium chrysazin glucoside several tests were run in which the concentration of these substances was varied. The results of these tests are shown in Table II. Additional tests in which calcium hydroxide is substituted mole-for-mole for the magnesium hydroxide in the above dosing solution and another in which aluminum hydroxide in equimolar concentration is substituted for the magnesium hydroxide are shown in Table III.

In order to rule out the salt effect of the compounds tested a group of 10 mice were each dosed with 0.016 mg. of sodium chloride in water and again in the magnesium hydroxide–potassium hydroxide dosing solution. Here the amount of sodium chloride used is equivalent in number of particles to 0.128 mg. of potassium chrysazin glucoside.

TABLE II.—THE EFFECT OF CHANGING THE CONCENTRATION OF POTASSIUM HYDROXIDE AND MAGNESIUM HYDROXIDE ON THE LAXATIVE ACTION OF POTASSIUM CHRYSAZIN GLUCOSIDE 0.128 MG. (1.45×10^{-7} MOLES) PER DOSE OF 0.5 ML. IN MICE

Senna-Like Stools

Dosing Solution Concentration: Moles KOH $\times 10^{-6}$	Moles $Mg(OH)_2$ $\times 10^{-7}$	Number of Animals Treated	Per Cent Positive
23.30	...	20	15
23.30	1.42	20	10
23.30	2.84	10	10
23.30	5.64	10	30
23.30	8.46	10	40
23.30	14.20	10	100
23.30	71.00	10	100
...	14.20	20	25
4.66	14.20	10	20
14.10	14.20	10	40
23.30	14.20	10	100
18.60	71.00	10	50

RESULTS AND DISCUSSION

In observing the laxative effects in mice of senna and aloe preparations, as well as those of the synthetic aglycones and glycosides reported here, it was apparent from the beginning that two distinct types of laxative activity were possible. The first which we will refer to as the senna type, is produced by senna and its preparations as well as by all of the glycosides investigated having the sugar component attached in the 1 or 8 position in such anthraquinone derivatives that have no hydroxy groups in positions 2, 3, 6, or 7. The presence of a carboxy or methyl group in position number 3 such as is the case in rhein or chrysophanic acid does not change the senna-like properties of the glycosides prepared from these aglycones. The administration of senna-like drugs causes the mice to produce stools which are soft and watery and show distinct stains when collected on filter paper. Such stools are sufficiently moist to enable the stains to be seen from the underneath side of Whatman #1 filter paper. Aside from the number of stains observed, the extent of staining was also found to be important since reduced dosage of drug does not always cause a decrease in the number of mice producing stains but only in the intensity of staining.

The second type of laxative activity is that displayed by aloes and aloin as well as by aglycones and

TABLE III.—A COMPARISON OF THE LAXATIVE EFFECTS PRODUCED BY POTASSIUM CHRYSAZIN GLUCOSIDE IN POTASSIUM HYDROXIDE SOLUTION IN THE PRESENCE OF MAGNESIUM HYDROXIDE, CALCIUM HYDROXIDE, AND ALUMINUM HYDROXIDE IN MICE

Dosing Solution Concentration: Moles KOH $\times 10^{-6}$		Number of Animals Treated	Per cent Positive
	Moles $Ca(OH)_2$ $\times 10^{-7}$		
23.3	14.2	19	35
..	Moles $Al(OH)_3$ $\times 10^{-7}$	..	...
23.3	14.2	20	30
..	Moles $Mg(OH)_2$ $\times 10^{-7}$	..	...
23.3	14.2	10	100

The second type of laxative activity is that displayed by aloes and aloin as well as by aglycones and

their glycosides having hydroxy groups in positions 2, 3, 6, or 7. The aloe-like action results in larger, shinier, more pasty stools which will adhere to the filter paper but will not produce distinct stains. Because of this fact, it is difficult in low concentrations to differentiate between the stools obtained from normal as against those obtained from treated animals. Anthraquinone derivative aglycones such as aloe emodin, frangula emodin, alizarin and hydroxychrysazin, as well as preparations of cascara, frangula, and rhubarb, produce an aloe-like laxative action in mice.

It can be seen from the data reported in Table I, that standard senna powder is 50% active in doses of 6.25 mg. whereas powdered extract of aloe must be given in doses of 20 mg. to produce the same activity. It is further seen, that aloin in doses of 20 mg. produces laxation in only 40% of the mice treated. The aglycones such as chrysazin, rhein, chrysophanic acid, aloe emodin, and frangula emodin, are either inactive or only slightly active under the same conditions.

The glycosides prepared from the aglycones mentioned above show increased activity over the corresponding aglycones when given in water. When suspended in sodium carbonate solution, these glycosides show a marked increase in activity due probably to increased solubility and uniformity of dosage.

The use of the magnesium hydroxide–potassium hydroxide dosing solution had no effect on the activity of the aglycones such as chrysazin, rhein, or chrysophanic acid over that produced when these compounds were administered in water. This was not the case with the glycosides, however. It can be seen from Table I, that potassium chrysazin glucoside given in doses of 2.56 mg. in aqueous solution gave a positive response of 75%. The same effect, however, was produced by only 0.064 mg. of this glucosidal salt when administered in the magnesium hydroxide–potassium hydroxide solution showing that this solution produced a definite increase in activity. Similarly, potassium rhein glucoside in doses of 0.070 mg. is inactive when administered in aqueous solution but produces 60% positive responses in the magnesium hydroxide–potassium hydroxide medium.

Potassium chrysophanic acid anthranol-9-glucoside was also prepared and tested since it has been suggested that the active constituents of aloe as well as those of senna might be anthranol glycosides. It is seen that this compound is moderately active in the magnesium hydroxide–potassium hydroxide dosing solution.

The effect of changing the potassium hydroxide as well as the magnesium hydroxide concentrations are shown in Table II. Here it is seen that the optimum concentration for potassium hydroxide is of the order of 23.30×10^{-6} moles per dose of 0.128 mg. (1.45×10^{-7} moles) of potassium chrysazin glucoside. The optimum concentration for magnesium hydroxide under the same conditions is 14.20×10^{-7} moles.

Substituting an equal amount of calcium hydroxide or aluminum hydroxide for magnesium hydroxide resulted in a decrease in the activity of potassium chrysazin glucoside. These data are presented in Table III.

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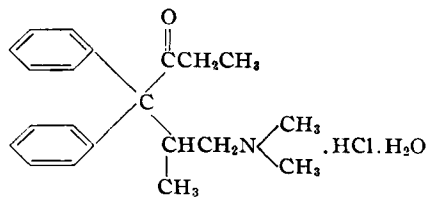
A Note on the Crystallography of *l*-Isomethadone Hydrochloride Hydrate*

By HARRY A. ROSE and ANN VAN CAMP

l-ISOMETHADONE is referred to in the literature as a narcotic and analgetic (1). Data are presented here which permit the identification of this compound by crystallographic methods.

The structural formula of *l*-isomethadone hydrochloride hydrate is as follows:



* Received April 14, 1956, from the Lilly Research Laboratories, Eli Lilly and Co., Indianapolis 6, Ind.