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Constituents in Cascara Sagrada Extract

IV. Aloe-Emodin, Chrysophanic Acid and Emodin*

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A number of publications have dealt with the isolation and biological assay of individual compounds that occur in, or can be derived directly from, extracts of the dried bark from *Rhamnus Purshiana* trees (cascara sagrada). The isolated compounds to which cathartic activity has been attributed are all hydroxy-methylanthraquinones of the emodin type. A large part of the anthraquinone material is apparently linked in glycosidic form to rhamnose and glucose, the two sugars being present in approximately equal quantities. In no case, however, has an isolated compound been found to exert a physiological activity that was comparable with the activity of the standard U. S. P. crude extract (on the basis of the extract's content of known compounds).

Emodin was identified in the drug by the early studies of Schwabe (1) and Jowett (2). The latter also identified isoemodin, rhamnol, syringic acid, pyrocatechuic acid and two of the fatty acids. Sipple, King and Beal (3) isolated frangulin, the rhamnoside of emodin, and Green, King and Beal (4) reported the identification of isoemodin and methylhydrocotoin. Daels (5) and Beal and Tumminkatti (6) showed that the emodin-type constituents were characteristically present in both the free form and as glycosides. Proof of the structure of emodin as 1,6,8-trihydroxy-3-methylanthraquinone was established by the studies of Eder and Widmer (7) and Jacobson and Adams (8). Gunton and Beal (9) also synthesized the natural rhamnoside of emodin, frangulin.

The present study was directed toward (*a*) further isolation of active compounds and also toward (*b*) checking the activity of known mixtures of pure compounds to test their synergistic effects in comparison with single compounds and the crude extract. We believe that the latter part of the study has provided an essentially new experimental basis for interpreting the activity of cascara sagrada and perhaps of many other crude extracts of a similar nature.

EXPERIMENTAL

A preliminary concentration of the most active material in cascara sagrada was accomplished as follows. The standard U. S. P. fluidextract of cascara sagrada (100 ml.) was dialyzed in cellophane tubes against 10 volumes of distilled, deaerated water for 48 hrs. Carbon dioxide was passed in during this time to exclude oxygen, pro-

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mote stirring and prevent mold growth. Nearly pure emodin occasionally separated from the dialysate, apparently subsequent to hydrolysis of a glycoside or other soluble conjugate. The dialysate was concentrated to 50 ml. *in vacuo* and then hydrolyzed by refluxing in the presence of 250 ml. of chloroform at *p*H 1.0 for $2^1/_2$ hrs. After cooling and separation of the two layers, it was found that practically all of the activity was in the aqueous portion. This layer was brought to the *p*H of the original extract (4 to 5) by treatment with sodium bicarbonate, and then treated with 7 volumes of acetone or absolute methyl alcohol to remove inorganic and tarry organic material. The results with acetone were more variable, so methyl alcohol was used in the later work. The insoluble material was centrifuged and discarded as it was found to be almost inactive. The dilute methyl alcohol solution was then evaporated to dryness *in vacuo* at approximately 40° C., using a capillary flow of carbon dioxide. The spongy, dried material was extracted by stirring with 25 ml. and then 15 ml. of absolute methyl alcohol. About 50 ml. of water was then added to the solution, and the methyl alcohol and part of the water were removed *in vacuo*. This water-soluble extract contained approximately 7 Gm. of solid material and had from two to three times the biological activity of the fluidextract on a total solids basis. The solids were relatively insoluble in ethyl acetate, chloroform and butanol. At room temperature in aqueous or methanol solutions, there was a fairly marked loss in activity after storage for only a few days.

Isolation of Aloe-Emodin.—An aqueous solution of 10 Gm. of the methyl alcohol-soluble material was treated with ferric chloride solution according to the method of Cahn and Simonsen (10). Because extraction of the resulting tar with toluene or other organic solvents gave only a small yield of anthraquinones, it was found necessary to dissolve the tarry material in 5% sodium hydroxide solution. The solution was then acidified and stirred with several volumes of ether. After centrifuging to separate the ether and water layers, the ether solution was decanted. The solid material in the aqueous layer was again dissolved in dilute sodium hydroxide solution, and the acidification and ether extraction were repeated. From four to seven such treatments were necessary for the removal of the anthraquinones. The ether solution was then extracted with small portions of 5% sodium hydroxide solution until the extracts were almost colorless. The aqueous solution was in turn acidified and extracted with ether until the ether extracts were almost colorless. The entire ether solution was then extracted five times with 50-ml. portions of 5% sodium bicarbonate solution. This treatment removed a large amount of the color, but left the anthraquinone content practically unchanged. The ether solution was then extracted twice with 50-ml. portions of 5% sodium carbonate to remove small amounts of β-hydroxyanthraquinones (with an intervening water wash), washed several times with water and then evaporated to dryness. The residue was extracted with small amounts of boiling toluene until almost completely dissolved. On cooling, aloe-emodin crystallized from solution. After recrystallization from toluene and glacial acetic acid, it formed orange-red shiny platelets or needles which melted at 218–219° C. Further purification by sublimation *in vacuo* brought the melting point to 222–223° C. Yield, 4% to 8%. Analysis: Calcd.: C, 66.64%; H, 3.73%. Found: C, 66.62% and 66.42%; H, 3.81% and 3.86%. The crystals were very soluble in pyridine, easily soluble in glacial acetic acid, soluble in acetone, slightly soluble in ethyl alcohol, methyl alcohol, ether, chloroform, benzene and toluene, and insoluble in water and petroleum ether.

Upon treating aloe-emodin with acetic anhydride and sodium acetate, the triacetate was obtained as golden needles (from ethyl alcohol) which melted at 175° C. Heating the aloe-emodin in a sealed Pyrex tube for 2 hrs. in a boiling water bath with benzoyl chloride and pyridine, followed by triturating with water and 5% sodium hydroxide solution, drying the residue and crystallizing from benzene, gave the benzoate as pale green needles which melted at 234–235° C. Refluxing the aloe-emodin for $1^1/_2$ hrs. with a 1:1 mixture of hydriodic acid (sp. gr. 1.7) and glacial acetic acid, or reduction with tin and hydrochloric acid, formed an anthrone which crystallized from ethyl alcohol in pale orange platelets, melting point 203–204° C. The anthrone yielded an acetyl derivative which melted at 238–239° C. with darkening. All of the above derivatives that were prepared from aloe-emodin corresponded in properties with the derivatives recorded in the literature.

Isolation of Chrysophanic Acid.—The toluene filtrate, after separation of the aloe-emodin, was evaporated and treated with 25 ml. of chloroform. The mixture was allowed to stand over night, and any precipitate that formed was removed by centrifuging. The solution was then extracted a number of times with 0.5% potassium hydroxide solution to remove any remaining aloe-emodin and with 5% sodium hydroxide solution to dissolve the chrysophanic acid. Carbon dioxide was passed into the solution until a heavy brown precipitate was formed. The precipitate was washed several times with 5% sodium carbonate solution and water, dried by evaporation at room temperature and recrystallized from acetic acid, petroleum ether and ethyl alcohol, giving orange-yellow needles melting at 190–191° C. No depression of melting point resulted when the crystals were mixed with chrysophanic acid (m. p. 191–192° C.) prepared from chrysarobin by the method of Gardner (11). Yield, 0.5% to 1.5% on a dry solids basis.

Chrysophanic acid was nearly insoluble in dilute sodium carbonate solution but dissolved slowly with an intense red coloration in dilute sodium hydroxide. It was somewhat more soluble than aloe-

emodin in toluene and much more soluble in chloroform. By use of the methods referred to for aloe-emodin, the material formed an acetyl derivative, pale yellow in color, melting at 205–206° C. The benzoyl derivative was prepared and crystallized from acetic acid diluted with hot ethyl alcohol, as pale yellow prisms melting at 202–204° C. Reduction with hydriodic acid or by the method of Gardner, using tin and hydrochloric acid, gave an anthrone melting at 202–203° C. The acetyl derivative of the anthrone melted at 237–238° C. Refluxing with constant-boiling (48%) hydrobromic acid and acetic acid, or treatment with 80% sulfuric acid at 160° C. for 20 min., produced no change in the melting point, showing that the compound was not a methyl ether.

The melting point of the product corresponded with that of the chrysophanic acid obtained from rhubarb by Tutin and Clewer (12), and from frangula by Gunton and Beal (9), but this is lower than the value given for chrysophanic acid that was prepared synthetically (193–194° C.). The color of the crystalline material was more orange than that of the synthetic material, or of that prepared from chrysarobin. However, all of the derivatives that were prepared gave the correct melting points.

Isolation of Syringic Acid.—The soluble material in the standard aqueous solution (equivalent to 200 ml. of fluidextract) was treated with 50 ml. of concentrated hydrochloric acid and then hydrolyzed in a Pyrex bottle for 6 hrs. at 125° C. in the autoclave. Both the acid solution and the tar were extracted several times with 30-ml. portions of ether. The solvent was allowed to evaporate and the brown, semi-crystalline material was dissolved in acetone, treated with Norit and the acetone allowed to evaporate. After recrystallization from glacial acetic acid and then from water, the product formed long colorless needles melting at 205–206° C. yield, 100–200 mg.

Acetylation with acetic anhydride and sodium acetate, followed by crystallization from dilute acetic acid or ethyl alcohol, gave an acetate which melted at 190–191° C. Syringic acid was also obtained by hydrolysis of the standard extract in 0.5 *N* sodium hydroxide solution.

Repeated attempts (over a wide *p*H range and with many different reducing agents) to concentrate and then purify the anthraquinone types of material in the fluidextract by the procedure of reduction to form products with a higher solubility in water, followed by aeration or chemical oxidation to re-form the less soluble quinones, did not give encouraging results. The precipitations secured by oxidation were slight, and the products could not be purified with as good success as that achieved by the procedures described above.[1]

Bioassays.—Although the anthraquinones obtained from cascara by degradation processes do not in all cases possess the same structure as when present in the fluidextract, particularly with regard to glycosidic linkage, it was thought desirable to assay them in order to relate structure to activity and to test the synergistic activity of known mixtures in comparison with single components. An earlier study had shown that hydrolysis of the native glycosides exerted little effect upon their cathartic activity, although oxidation may cause fairly steady losses in potency (4). The emodin and aloe-emodin were obtained from cascara. The chrysophanic acid was prepared from chrysarobin by the method of Gardner, since the low yields that could be obtained from cascara made that source less satisfactory. The solid products were fed in capsules; the capsules were "washed down" with 1 to 2 ml. of water or dilute sucrose solution.

When fed individually in 25-mg. dosage, the three compounds showed negligible or only slight cathartic activity. Mixtures of 10 mg. of emodin with 10 mg. of chrysophanic acid, 10 mg. of aloe-emodin with 10 mg. of emodin, and 10 mg. of aloe-emodin with 5 mg. of chrysophanic acid were found to be only slightly to moderately active. These results were in striking contrast, however, with the subsequent tests in which mixtures of all three compounds were fed. A 25-mg. (total) dosage of the tripartite mixture was highly active. Even one-half of this amount of the mixture (12.5 mg.) showed moderate activity. For example, a mixture of 10 mg. of aloe-emodin, 10 mg. of emodin and 5 mg. of chrysophanic acid possessed a very strong cathartic activity; and one-half of this dosage showed greater activity than 25 mg. of any one of the individual substances.

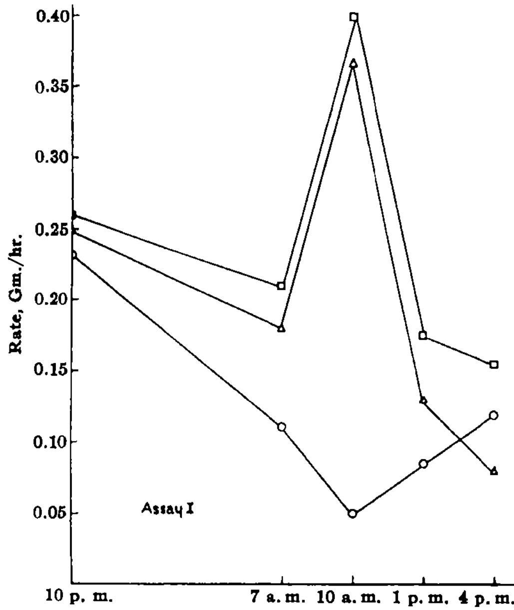
Chrysophanic acid anthrone was inactive in 25-mg. dosage, aloin showed only slight activity in 25-mg. dosage, and chrysarobin in the same dosage showed only slight or no activity.

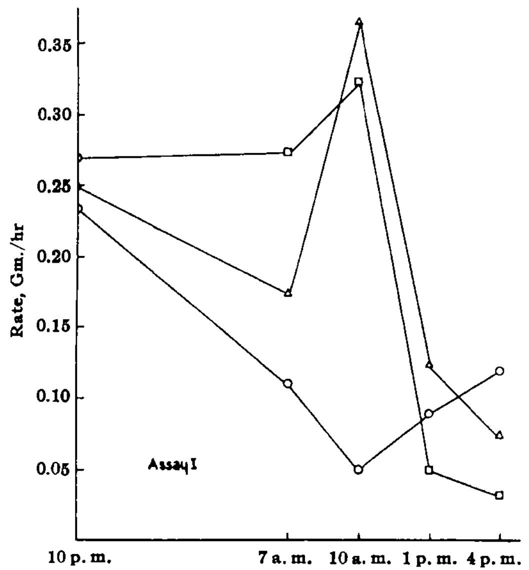
After the anthraquinones were fed, they could be detected in the feces by treatment with dilute alkali solutions. The urine from treated animals was also reddened when made alkaline with sodium or potassium hydroxide solution; the coloration effect was particularly strong with emodin, less evident with aloe-emodin and weak with chrysophanic acid.

Groups of 15 to 20 animals (preferably with an average weight of approximately 300 Gm.) for each test level gave reasonably consistent results. A preliminary 6-hr. record of the fecal output rate served both to eliminate animals with abnormal records and to match the satisfactory animals, so that their group averages were comparable at the beginning of the assay. A reasonably convenient schedule was obtained by feeding the test preparation at 10 p. m., followed by collections for weighing at 7 a. m., 10 a. m., 1 p. m. and 4 p. m.

The activity of fluidextracts obtained from different sources varied considerably, although the total solids value was about 0.25 Gm. per ml. in each case. Fluidextracts prepared in the laboratory according to the directions given in the U. S. P.

[1] The authors are indebted to Mr. Charles Spiegl for his assistance in carrying out a number of chemical studies and for making a recheck of the assay values reported in the present paper.





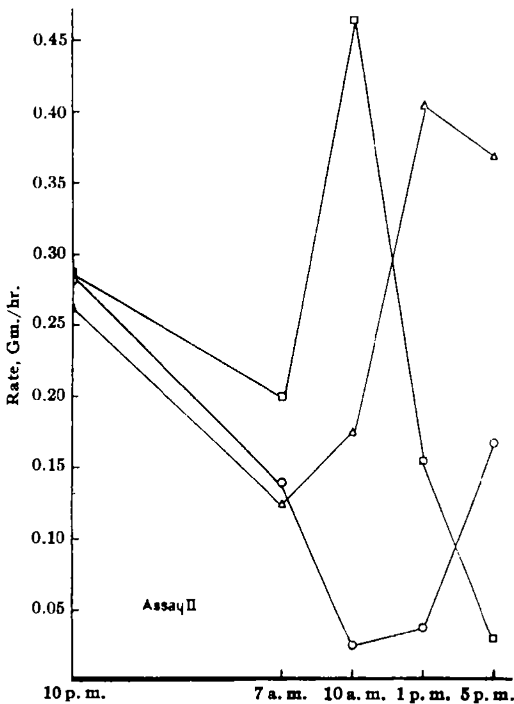


Fig. 1—Assay of mixture containing emodin (10 mg.), aloe-emodin (10 mg.) and chrysophanic acid (5 mg.). Twenty guinea pigs in each test group. The 1.5 ml. of cascara sagrada extract contained approximately 250 mg. total solids.

□ Aloe-emodin (10 mg.)
Emodin (10 mg.)
Chrysophanic acid (5 mg.)
Δ Cascara (1.5 ml.)
O Negative controls

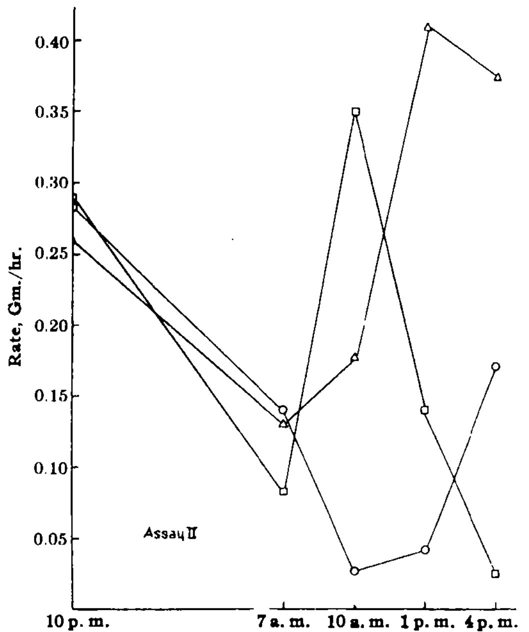


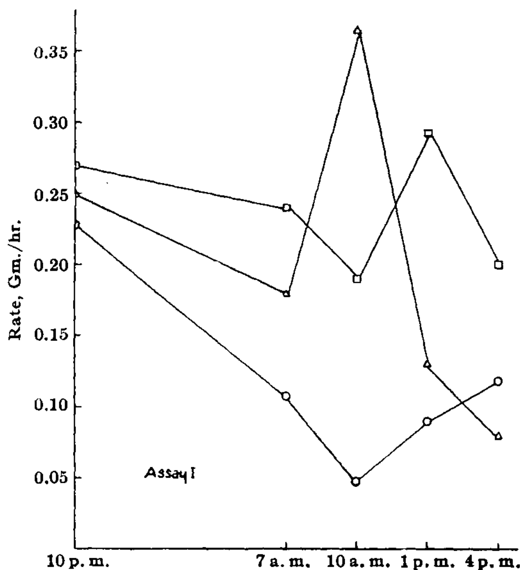
Fig. 2—Assay of mixture containing emodin (5 mg.), aloe-emodin (5 mg.) and chrysophanic acid (2.5 mg.). Twenty guinea pigs in each test group.

□ Aloe-emodin (5 mg.)
Emodin (5 mg.)
Chrysophanic acid (2.5 mg.)
Δ Cascara extract (1.5 ml.)
O Negative controls.

XI were found to be uniformly active in 1-ml. dosages, but 1.5-ml. portions of the four commercial samples tested were required for a comparable effect.

DISCUSSION

In all, considerable information has been gained concerning the active ingredient of cascara sagrada. The fact that the active materials are water-soluble and will dialyze rapidly through a viscose membrane indicates that the most important compounds are of relatively low molecular weight. However, the products are not very stable; they tend to be oxi-



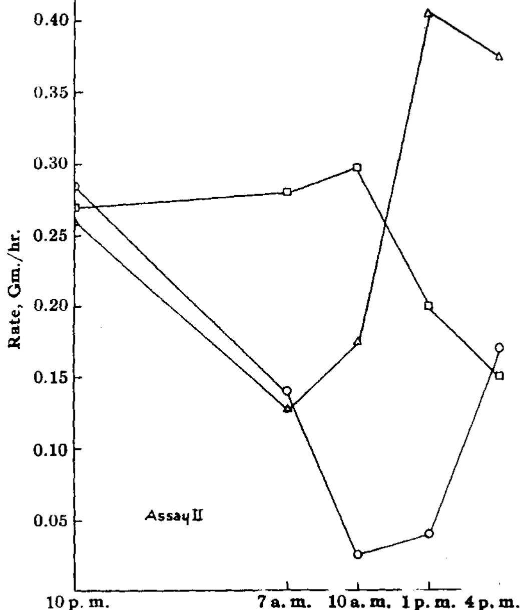


Fig. 3.—Assay of mixture containing aloe-emodin (15 mg.) and chrysophanic acid (10 mg.). Eighteen guinea pigs used in each test group.

□ Aloe-emodin (15 mg.) Chrysophanic acid (10 mg.)
Δ Cascara extract (1.5 ml.)
O Negative controls.

dized in parallel with condensation or polymerization reactions during further treatment, making the isolation of a high percentage of the initial individual compounds present in crude extracts exceedingly difficult. Some substances in the active fraction are apparently closely related to aloin, since they (*a*) give (initially), in borax solution, a strong fluorescence with ultraviolet light, (*b*) are difficult to hydrolyze and (*c*) yield hydroxy-anthraquinones after treatment with ferric chloride.

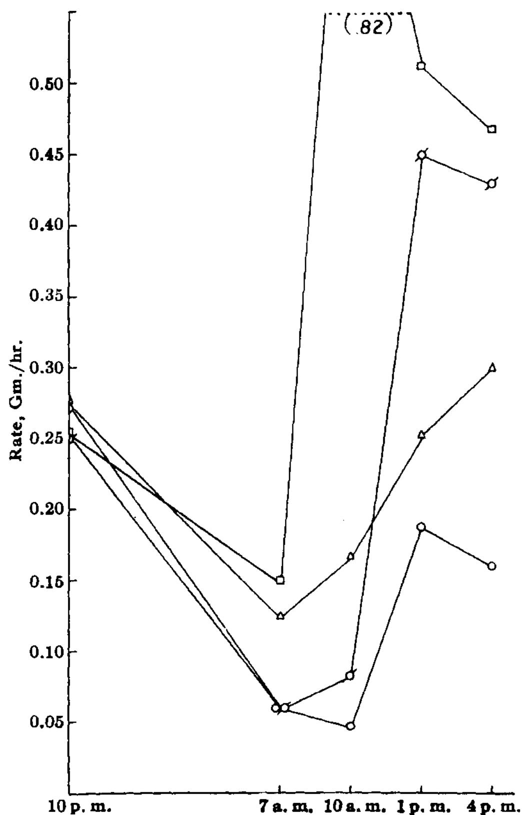


Fig. 4.—Assay of aloe-emodin and mixture of compounds obtained from cascara sagrada extract. Twelve guinea pigs in each test group. The mixture had the same composition as that referred to in Figs. 1 and 2.

Δ Aloe-emodin (25 mg.)
□ Mixture (25 mg.)
ø Mixture (12.5 mg.)
O Negative controls.

The fact that the Bornträger reaction for anthraquinones was strongly positive in the urine and feces after administration of the compounds assayed gives an indication that these materials were in large part excreted unchanged; at least they did not undergo simple reduction to the anthrone series of products, since they gave no borax fluorescence test in ultraviolet light. Further destruction of the compounds that undergo reduction to the anthrone stage appears probable, since after feeding chrysophanic acid anthrone, no test for either the anthrone or the corresponding anthraquinone could be obtained in the urine or feces.

The method of bioassay with guinea pigs gave occasional erratic results, but repeated tests with animals that were comparable in size and age were in reasonably good agreement, even when carried out by two independent investigators at different times of the year. The results did not fully conform with those recorded for humans, however. In the latter case, very few definite records are available. Thus it has been reported that 25 mg. of aloin is a cathartic dose for most human

subjects, but that quantity produced little or no cathartic effect with guinea pigs; and, though 50 mg. of aloe-emodin was quite active for guinea pigs, Tutin and Clewer (12) reported that this material was only slightly active with human subjects in 100-mg. dosage. It is of some interest to note that although the guinea pig serves as a satisfactory test animal, the dosage per unit body weight, compared to the human dosage, is about 250:1.

The marked supplementary or synergistic effect observed when a mixture of three products was fed is of special interest because it provides evidence to account for the high activity of the crude extracts in comparison with the relatively low activity of any of the isolated or highly purified fractions contained in the conventional types of extracts.

SUMMARY

Aloe-emodin and chrysophanic acid have been isolated from cascara sagrada extract, subsequent to dialysis, acid hydrolysis and treatment with ferric chloride. Syringic acid and emodin were also isolated a number of times, confirming earlier identifications.

The cathartic activity of weighed single dosages of three of the substituted anthraquinones that have been isolated from cascara sagrada extract was assayed by the technique previously described, using guinea pigs as test animals. Known, weighed mixtures of the three ingredients were also assayed and found to be markedly more active than similar quantities of any one of the compounds given alone. A mixture of 10 mg. each of aloe-emodin and emodin plus 5 mg. of chrysophanic acid was distinctly more active than 25 mg. of any one of the three compounds when tested separately.

Chrysophanic acid anthrone, aloin and chrysarobin were practically inactive in 25-mg. doses when assayed individually. The supplementary or synergistic effect observed for mixtures of the compounds appears to provide an explanation of the marked activity of crude extracts compared to the relatively poor activity of any of the purified fractions thus far studied.

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A Study of Wild and Cultivated Stramonium in Puerto Rico*

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Since stramonium grows profusely in Puerto Rico and since the leaves are widely used in the island for the relief of asthma and other ailments, a study of the content of the active medicinal principles of Puerto Rican stramonium was deemed desirable. A comparison of the wild and cultivated forms was made, together with the effects of fertilizer.

The three species of the genus Datura in Puerto Rico according to Britton (1) are *Datura Stramonium* L., *Datura Metel* L. and *Datura fastuosa* L. Their morphology has been studied by de Grosourdy (2), Stahl

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