upon the administered dose of drug. A threefold increase in dose caused a twofold increase in urinary S^{35} output (Table II). An eightfold increase in the dose of compound III caused only a slight increase in the urinary S^{35} excretion of this compound.

The difference in structure between compounds II and III other than the salt form is in the substitution in the 2 position in the phenothiazine nucleus; compound II contains a chlorine atom, while compound III contains the trifluoromethyl grouping. This was the only case in which substitution in the 2 position of the nucleus appeared to have an effect upon the excretion pattern.

Although the dosages of compounds IV and V were increased two- and fourfold, respectively, the urinary S^{35} excretion of these compounds was increased only slightly (Table II). The increase in urinary S^{35} excretion following the increase in dose of compound II was similar to that obtained with promazine-S^{35}, 10-(3-dimethylaminopropyl) phenothiazine hydrochloride. Fyodorov (3), following the oral administration of promazine-S^{35} to rats at dosage levels of 12–20 mg./Kg., reported that the urine contained 40% of the administered S^{35} activity. At dosage levels of 50 mg./Kg. of promazine-S^{35} to rats intragastrically by stomach tube, Walkenstein and Seifter (8) reported that approximately 65% of the S^{35} dose was excreted in the urine. Compound I (chlorpromazine-S^{35}) was administered at only one dosage level due to a limited supply of this compound.

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Relation of Cathartic Activity to Structural Modifications of Ricinoleic Acid of Castor Oil

By M. S. MASRI, L. A. GOLDBLATT, F. DeEDS, and G. O. KOHLER

Castor oil and certain of its derivatives were tested in rats to elucidate the specific structural configuration of castor oil responsible for its cathartic action. The results indicate that the hydroxyl function on carbon-12 and the double bond between carbons 9 and 10 of the ricinoleic acid moiety are essential for the cathartic action, as evidenced by the loss of activity upon either masking of the hydroxyl group or hydrogenation of the double bond. On the other hand, the naturally occurring *cis* configuration of the double bond is not essential for this action, as evidenced by the retention of the activity upon elaidinization to the *trans* configuration.

CASTOR OIL, which is obtained from the seeds of *Ricinus communis*, consists mainly of the triglycerides of an unsaturated hydroxy fatty acid, ricinoleic acid, having the formula $C_{17}H_{32}$-(OH)COOH. Castor oil acts as a cathartic for man although considerable amounts may be absorbed and utilized when fed as part of the diet of many animals. Paul and McCay (1) reported 92.1% utilization by rabbits and 99% utilization by sheep when castor oil was fed to the extent of 6%. Stewart and Sinclair (2) fed adult rats a diet containing 48.4% castor oil and found that the oil was readily metabolized with no evidence of catharsis. Perkins, *et al.* (3), recently reported that weanling rats fed 10% triricinolein "gained as much weight as those fed corn oil (10%) diets" and found (private communication) no indication of catharsis.

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Since ricinoleic acid differs from oleic acid, generally considered to be the most plentiful as well as the most widely distributed of all fatty acids in natural fats, only in having a hydroxyl group at carbon-12, it was of interest to investigate the effect of chemical modifications of the functional groups of ricinoleic acid upon the cathartic activity. The modifications planned included esterification of the hydroxyl group, hydrogenation of the double bond, and elaidinization, that is, conversion of the naturally occurring *cis* to the *trans* isomer. Materials tested included castor oil and the hydrogenated, elaidinized and acetylated oil. Castor oil, although it is predominantly the triglyceride of ricinoleic acid, is not a single chemical compound. Therefore, the investigation was extended to include highly purified methyl ricinoleate and its elaidinized, hydrogenated, and acetylated derivatives. The structural formulas for these four compounds are given in Fig. 1.

EXPERIMENTAL

Material and methods.—Castor oil grade AA,

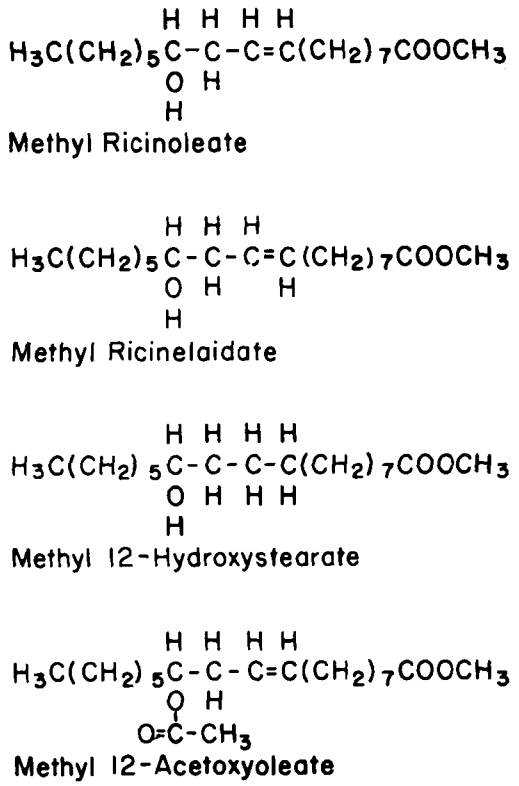

Fig. 1.—Formulas of methyl esters tested.

hydroxyl value 165, a commerical product,[1] was employed.

Hydrogenated Castor Oil.—Fifty grams of castor oil in 250 ml. ethanol was hydrogenated in the presence of 0.3 Gm. of platinum dioxide at a pressure of 55–39 p.s.i. gauge in a Parr hydrogenator. After 5 hours the theoretical amount of hydrogen had been taken up and absorption of hydrogen ceased. Hydrogenation was begun at room temperature of 24° and the temperature rose spontaneously to 29°. However, as the hydrogenation progressed the saturated product began to crystallize from the solution and it was necessary to raise the temperature by external heating to above 50° to keep the product in solution. After hydrogenation was discontinued the catalyst was filtered off and the solvent removed with a rotary evaporator at reduced pressure at a temperature below 70°. The hydrogenated oil, obtained in practically quantitative yield as an almost colorless white solid, melted at 83.5° to 85.2°.

Elaidinized Castor Oil.—Castor oil was elaidinized with a relatively small quantity of nitrite-nitric acid solution as the isomerization agent essentially according to the procedure of McCutchon, *et al.* (4), for the elaidinization of methyl ricinoleate. *To 150 Gm. castor oil maintained at 60°* was added with rapid stirring (magnetic stirrer) 10 ml. of 2 *M* sodium nitrite and 6.7 ml. of 6 *M* nitric acid. The heating and stirring was continued for 4 hours, after which the mixture was poured into 500 ml. of hot distilled water contained in a separator. The water layer was drawn off and the elaidinized oil washed again with 500 ml. hot distilled water. The oil, which was orange-brown in color and solidified below 50°, was then extracted with 500 ml. of a mixture of ethyl ether and Skellysolve F (1:1). The solution was dried with sodium sulfate and decolorized by percolating through a 200-Gm. column of alumina previously wetted with ether-Skellysolve F. The colorless eluate was evaporated to dryness at reduced pressure. The waxy, nearly colorless product melted at 48.9° to 51.0° and showed characteristic absorption in the infrared region at 10.3 μ indicating about 80% conversion of the *cis* olefinic unsaturation to the *trans* (elaidinized) configuration.

Methyl Ricinoleate.—This ester was obtained by alcoholysis of castor oil, followed by fractional distillation of the crude esters according to the procedure of Swern and Jordan (5) and purified further by low temperature crystallization. A heart cut of the distillate which was already better than 98% pure, α_D^{26} + 4.68°,[2] was further purified by fractional crystallization from Skellysolve F-acetone. One hundred and fifty grams of methyl ricinoleate was dissolved in a mixture of 250 ml. Skellysolve F and 50 ml. acetone and cooled to −30°. An impure first crop, α_D^{26} + 4.57°, amounting to 12 Gm. was thus obtained. The filtrate was then cooled to −36° whereby a second crop amounting to 83 Gm. was obtained, α_D^{26} + 4.70°. A third crop obtained at −50° was again somewhat impure, α_D^{26} + 4.65°. Recrystallization of a portion of the second crop did not increase its optical activity and this material, liquid at room temperature, was used in the biological tests.

Ricinoleic Acid.—Ricinoleic acid was prepared by saponification of purified methyl ricinoleate, α_D^{26} + 4.68°, essentially according to the procedure of McCutchon, *et al.* (4).

Anal. Calcd. for $C_{18}H_{34}O_3$: Acid value and hydroxyl value 187.9. Found: acid value 183.2; hydroxyl value 181.3.

Methyl Ricinelaidate.—Methyl ricinoleate was elaidinized with a small quantity of nitrite-nitric acid solution as the isomerization agent and purified by crystallization from Skellysolve F-acetone followed by decolorization with carbon and recrystallization from acetone-water (4). The product thus obtained melted at 29.2° to 30.8°. The magnitude of the characteristic absorption in the infrared region at 10.3 μ indicated quantitative conversion of the *cis* to the *trans* configuration in the purified product.

Methyl 12-Hydroxystearate.—Methyl ricinoleate was hydrogenated in the same manner previously described for the hydrogenation of castor oil except that no external heating was required since the product remained in solution at all times. Using 50 Gm. methyl ricinoleate, 250 ml. 95% ethanol, and 0.2 Gm. platinum oxide at a pressure of 50–30 p.s.i. the calculated amount of hydrogen was absorbed in about 2 hours at 30° but shaking under hydrogen was continued for another hour. The catalyst was then filtered off and the product allowed to crystallize at −2° overnight. The precipitate was collected and recrystallized from

[1] We are indebted to the Baker Castor Oil Co. for a generous supply of castor oil.

[2] Rotations reported are those observed in a 10-cm. tube.

methanol, 5 ml. per Gm. at 2°. The product thus obtained was a white crystalline solid, m.p. 56.4° to 57.6°.

Methyl 12-Acetoxyoleate.—Methyl ricinoleate was acetylated by treating 62.4 Gm. (0.2 mole) of the ester with 320 ml. of an acetic anhydride-pyridine mixture (1:3) in a 1-L. round-bottom flask which was fitted with a reflux condenser and thermometer. The mixture was stirred and heated to 100° and maintained at this temperature for 2 hours. After cooling to 75°, 300 ml. of water was added, and the mixture was stirred rapidly at this temperature for 15 minutes. It was then cooled to room temperature, transferred to a separator, and extracted with 400 ml. of ether. The ether extract was washed four times with 500-ml. portions of 3 *M* hydrochloric acid, after which it was dried over anhydrous sodium sulfate. The recovered product was fractionally distilled at reduced pressure. The main fraction, 40 Gm. distilling at 154° at 0.23 mm., had $\alpha_D^{26} + 23.01°$. This liquid distillate was used for the biological tests.

Acetylated Castor Oil.—Castor oil (hydroxyl value 165) was acetylated in the same manner as methyl ricinoleate. The crude reaction product was extracted with ether and washed and dried but was not distilled. The 3.3 hydroxyl value of the product indicated that 98% of the hydroxyl groups originally present in the oil had been acetylated.

Biological Tests.—Young adult male albino rats from a colony maintained in our laboratory since 1931 were used. The rats were fed a diet previously described (6). For the determination of cathartic activity, the rats were fasted overnight but allowed free access to water. In the morning they were lightly anesthetized with ether and stomach-tubed with the test substances. Samples which are solid at room temperature were warmed and given in the melted state with or without warm corn oil as a diluent to overcome their tendency to crystallize at body temperature. The relatively high melting point of hydrogenated castor oil, in particular, necessitated the use of a relatively large volume of corn oil. Two pellets weighing about 15 Gm. of a commercial ration for rats and a water bottle were supplied immediately after stomach-tubing. The rats were housed individually in hanging-wire cages with soft tissue paper spread underneath. The bottom of each cage was 9.5″ × 8″ made from 2-mesh, 16-gauge galvanized wire cloth which allowed the feces to drop freely on the paper. The number of wet feces produced in 24 hours was taken as an index of catharsis (7). Feces were scored wet when they were so fluid as to be unformed and produced distinct brown stains on the tissue paper surrounding them. Also, wet feces, when allowed to dry, could not be brushed off without tearing the paper. When catharsis was evident almost all the wet feces were usually produced within a few hours of stomach-tubing and occasionally it was necessary to replace the tissue papers with fresh ones in order to facilitate counting of the wet feces. In one experiment in which castor oil constituted 10% of the diet, weanling instead of adult rats were used. They were observed for growth and catharsis over a period of 5 weeks. For this experiment the diet with the following percentage composition was employed: corn meal 53, sucrose 10, casein 17, salt mixture U.S.P. XIV 4, corn oil 4, adequate vitamin mixture triturated in dextrose 2, and castor oil 10.

Table I.—Cathartic Action of Test Substances in Rats

Material Stomach-Tubed	No. of Rats	Body wt., Gm. Mean ± S.E.	Wet Feces/Rat Mean	Wet Feces/Rat S.D.
Castor oil 0.5 ml.	32	152 ± 3	6.8	4.6
Castor oil[a] 0.5 ml.	20	173 ± 3	7.2	3.6
Elaidinized castor oil[a] 0.5 ml.	10	164 ± 2	7.7	3.2
Hydrogenated castor oil[b] 0.5 ml.	10	186 ± 8	2.1	1.8
Acetylated castor oil 0.6 ml.	20	165 ± 4	0.8	1.3
Ricinoleic acid 0.5 ml.	10	153 ± 2	3.6	4.7
Ricinoleic acid[a] 0.5 ml.	10	156 ± 5	4.7	4.9
Methyl ricinoleate 0.5 ml.	10	178 ± 8	6.5	3.5
Methyl ricinoleate[a] 0.5 ml.	10	174 ± 7	6.6	5.0
Methyl ricinelaidate 0.5 ml.	9	152 ± 4	5.5	3.3
Methyl ricinelaidate[a] 0.5 ml.	10	198 ± 7	6.1	3.6
Methyl 12-hydroxystearate[a] 0.5 ml.	10	168 ± 9	0.2	0.4
Methyl 12-acetoxyoleate 0.5 ml.	10	150 ± 6	0.9	1.1
Corn oil (control) 0.5 ml.	20	166 ± 3	0.1	0.4
Corn oil (control) 2.0 ml.	10	202 ± 4	1.3	2.2
Corn oil (control) 3.5 ml.	8	216 ± 9	3.3	2.8

[a] With 1.5 ml. corn oil. [b] With 3.0 ml. corn oil.

RESULTS AND DISCUSSION

The results obtained with stomach-tubed samples are shown in Table I. It is evident that castor oil, elaidinized castor oil, methyl ricinoleate, methyl ricinelaidate, and ricinoleic acid all produced catharsis under our conditions. On the other hand, hydrogenated castor oil, acetylated castor oil, methyl 12-hydroxystearate, and methyl 12-acetoxyoleate were without cathartic action. The apparent slight cathartic activity of the sample of acetylated castor oil tested may be due to its slight content of incompletely acetylated oil. It should be emphasized that the number of wet feces given in Table I represent only a rough but measurable index of the degree of catharsis. Although the individual wet feces differed with respect to size and degree of wetness, each was assigned the same numerical value of one. The subjective appraisal is therefore also important, and a strict statistical comparison of the numbers of wet feces is not intended. However, it should be stated that the subjective appraisal of the individual tests paralleled the conclusions to be drawn from the numerical values.

In the feeding experiment in which 10 four-week-old male rats were fed *ad libitum* the diet containing 10% castor oil for approximately 5 weeks, no catharsis was observed and growth was comparable to that obtained with a similar control group in which the castor oil was replaced by corn oil. The mean weight and its standard error of the rats receiving castor oil was 42.2 ± 0.6 Gm. at the start and 155.6 ± 4.6 Gm. at the end of the experiment.

The corresponding means for the control group were 42.2 ± 0.5 Gm. and 160.5 ± 4.1 Gm., respectively.

Gross examination at autopsy showed no abnormalites. Histopathological examination of the perirenal fat also showed no abnormalities.

The results indicate that either saturation of the double bond or masking of the hydroxyl group of the hydroxy-double bond system of ricinoleic acid nullifies the cathartic action. On the other hand, neither isomerization of the double bond from the *cis* to the *trans* configuration, nor conversion of the neutral triglyceride to the methyl ester or to the free acid, appears to affect the cathartic action to an appreciable extent. The fact that the highly purified (distilled and crystallized) methyl ricinoleate is very effective militates against the possibility that the cathartic action of castor oil may be due to a trace contaminant.

The quite different results obtained when castor oil was given to fasted rats by stomach tube or as a part of the diet is striking. In the latter case no catharsis was observed, although the daily food intake toward the end of the feeding experiment provided well over 1 Gm. of castor oil per rat. This is in agreement with the observations of others, *e.g.*, Stewart and Sinclair (2) found that no catharsis occurred even when castor oil was included in the diets of rats at levels as high as 48.4%. There is no ready explanation for this difference. The reported use of castor oil as an article of the diet in China (8), presumably without untoward effects, may be related to this phenomenon. It is noteworthy that when used as a cathartic, castor oil is usually taken on an empty stomach (9).

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Technical Articles

Water Vapor Sorption and Diffusion through Hard Gelatin Capsules

By W. A. STRICKLAND, Jr., and MARTHA MOSS

Gelatin contains appreciable amounts of water under normal environmental conditions. This water may be transferred to a hygroscopic powder contained in a gelatin capsule. This report describes a study of this water transfer and of the diffusion of water vapor through gelatin capsules.

THE TWO-PIECE hard gelatin capsule is a convenient single dose medicine container that prevents ill taste on swallowing and promptly dissolves on reaching the stomach. The development of high speed filling equipment and the availability of well made, low cost capsules has prompted renewed interest in capsules as a dosage form. Capsules are made from refined animal gelatin and may contain very small amounts of other substances such as dyes or preservatives; however, the physical properties of hard gelatin capsules are essentially the properties of the gelatin from which they were made. Gelatin normally contains 9 to 12% water, but the water content can vary from about 4 to about 16%, depending on the environment. It is conceivable that the water content of normal capsules may exercise a deleterious effect on the capsule content provided it can be transferred. In addition, atmospheric water vapor may diffuse through capsules if the content is hygroscopic. Gelatin cannot be completely dehydrated by normal desiccating procedures and, if low water content is achieved, the capsules become brittle and fracture easily; conversely, greater than normal water content causes

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