then treated as just described. After two hours halogen could just barely be detected in the sample and finally when no more bubbles could be raised in the resin no appreciable Beilstein test could be obtained.

The physical characteristics of the resin are similar to those described by Cherbuliez. It showed no tendency to crystallize. The resin is very sparingly soluble in water, slightly soluble in petroleum ether and miscible in all proportions with alcohol, benzene, ethyl acetate and chloroform. It contains no nitrogen. *Anal.* Found: C, 68.6, 68.3; H, 8.69, 8.84; mol. wt., 600;[10] Hanus iodine no. 52.5, 53.6; OCH$_3$, 1.13, 1.23, 1.19;[11] sap. no. 254.5, 233.2.

Preparation of Croton Oil.—Fifty-one grams of ground croton beans was extracted in a Soxhlet with petroleum ether (max. b. p. 85°) for about nine hours. The petroleum ether was evaporated from the extract and the oil dried to constant weight at 105°; yield 12.9 g. (25.3%). A further extraction with the same solvent for about six hours removed only 0.07 g. of oil of sap. no. 265.8.

Preparation of Alcohol-Soluble Portion of Croton Oil.—One hundred grams of croton oil (obtained by petroleum ether extraction) was shaken in the cold with 150 ml. of alcohol (95%). The alcoholic layer was withdrawn and the oil was then extracted with a further 100-ml. portion. The combined alcoholic extracts were filtered, centrifuged to remove suspended particles of oil and finally the alcohol was evaporated; yield 31 g. (31%).

Summary

1. Croton resin has been separated into fractions which have different compositions and possess different toxicities to goldfish.

2. Complete survival time–concentration curves, using goldfish as the test organism, have been determined for croton oil, the alcohol-soluble portion of croton oil and croton resin.

3. Croton resin has been shown to be more toxic to goldfish than rotenone.

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(10) (a) Rast, *Ber.*, **54**, 1979 (1921); (b) Spies, THIS JOURNAL, **55**, 250 (1933).

(11) Clark, *J. Assoc. Off. Agr. Chem.*, **15**, 136 (1932).

[CONTRIBUTION FROM THE UNIVERSITY OF MARYLAND STATION OF THE INSECTICIDE DIVISION, BUREAU OF CHEMISTRY AND SOILS]

Croton Resin. II. The Toxic and Vesicant Action of Certain of its Derivatives[1]

BY JOSEPH R. SPIES

The work of previous investigators has indicated that the croton bean contains a physiologically active principle which owes its toxic and vesicant properties to a condition of unsaturation. A more extensive study, using goldfish as the test organism, has demonstrated, however, that the physiological action of the active constituent is more intimately related to the presence of free hydroxyl groups.

Croton oil was first hydrogenated by Paal and Roth,[2] who used a palladium catalyst and found the irritating property of the oil to be proportional to the iodine number. This property disappeared entirely when saturation was complete. These authors also hydrogenated Boehm's[3] resin, causing a drop in its iodine number from 77 to 12.5. The product was no longer toxic to frogs or rabbits. Catalytic hydrogenation of our croton resin,[4] with both nickel and platinum, caused a reduction of the iodine number from 53 to 38 but no apparent decrease in toxic or vesicant action at the concentration used for the tests. The product, a harder resin, had lost its transparency and assumed a turbid or milky appearance.

Cherbuliez *et al.*[5] brominated the resin and found the product to be without physiological activity, as shown by tasting. Bromination of our resin produced a marked decrease in toxic and vesicant properties but did not destroy them completely.

The important relation of the free hydroxyl groups to the physiological activity of croton resin was not observed by earlier investigators. Boehm[3] reported the absence of free hydroxyl groups in his resin, while Cherbuliez *et al.*[5] found that their resin contained approximately 3.4% hydroxyl on the basis of the saponification number before and after acetylation. The latter authors noted a decrease in activity of the acetylated product but did not attribute this to esterification of the hydroxyl groups. Acetylation of our resin, which, however, resulted in only partial esterification of the hydroxyl groups shown

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(1) From a thesis submitted by Joseph R. Spies to the Graduate School of the University of Maryland in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

(2) Paal and Roth, *Ber.*, **42**, 1544 (1909).

(3) Boehm, *Arch. Path. Pharmakol.*, **79**, 138 (1915). This resin was obtained by a process which altered its nature. Despite this fact its composition, physical characteristics and toxic action are similar to the resin used in this study. At a concentration of 1:10^8 Boehm's material killed tadpoles in three to four hours.

(4) Isolated as described in the first article of this series. THIS JOURNAL, **57**, 180 (1935).

(5) Cherbuliez, Ehninger and Bernhard, *Helv. Chim. Acta*, **15**, 658 (1932).

to be present by methylation by Purdie's reagents, produced some decrease in physiological action. Complete methylation to the extent of 11.7% methoxyl, however, yielded a resin which is without either toxic or vesicant action. The average methoxyl content of the original resin is 1.2%. Detailed results of these studies are collected in Tables I and II.

TABLE I

THE TOXICITY OF CERTAIN DERIVATIVES OF CROTON RESIN TO GOLDFISH

Temperature 27 ± 0.3°; concn. 2.0 mg./liter[a]

Substance		OCH_3, %	Sap. no.	Iodine no.	Survival time, min.[b]
Original resin		1.2	244	53.1	20
Hydrogenated resin		..	...	39.2	18
Acetylated resin	(1)[d]	..	358	..	35
Acetylated resin	(2)	..	336	..	30
Methylated resin		11.7	181	..	no deaths
Methylated acetylated resin	(3)	..	183	..	in 1740[c]
Bromo resin	(1)	..	...	..	62
Bromo resin	(2)	..	...	..	71

[a] This relatively high concentration was used to reduce the survival times to convenient limits. [b] Average of four fishes. See this series, article I, *loc. cit.* [c] A few fishes showed slight irritation of the gills. [d] These numbers refer to the number of the preparation in the experimental section.

TABLE II

VESICANT ACTION OF CROTON RESIN AND CERTAIN OF ITS DERIVATIVES

Substance		Concn. of test soln.	Mg./sq. in. of skin[a]	Solvent used	Individuals tested	Results
Original resin		1:1000	0.04	Alcohol	1	Moderate burn
Original resin		1:100	.4	Alcohol	1	Serious burn
Hydrogenated resin	[b]	1:100	.4	Alcohol	1	Serious burn
Methylated resin		1:100	.4	Acetone	2	No burn
Acetylated resin	(1)[c]	1:100	.4	Acetone	2	Moderate burn
Bromo resin	(1)	1:100	.4	Acetone	2	Very slight burn

[a] Approximate. [b] Iodine number 39.2. [c] These numbers refer to the number of the preparation in the experimental section.

Croton resin forms no water-soluble salt when its ethereal solution is agitated with cold dilute aqueous potassium hydroxide and its alcoholic solution gives no color with ferric chloride. It is probable nevertheless that the free hydroxyl groups are phenolic or enolic in nature but are masked by substituents which repress their normal reactions. This conclusion is based on the fact that the average saponification number of the methylated resin is 181, while that of the original resin is 244, indicating that part of the alkali was neutralized by the free hydroxyls after saponification. The saponification number would otherwise be lowered only in the ratio of the molecular weights of the original and methylated resin, *i. e.*, in the ratio 600:628.

Experimental

Hydrogenation.—In a preliminary experiment 16.4 g. of resin was dissolved in 200 ml. of alcohol and the solution was shaken for five hours with hydrogen and 0.2 g. platinum oxide[6] at 50–60 lbs. per sq. in. This process was repeated twice using fresh portions of catalyst at 45–55° for one and three hours, respectively. The hydrogenated product, which was still toxic[7] and vesicant, was harder than the starting resin and possessed a turbid or milky appearance, iodine number 39.2.[8]

To 18.5 g. of resin in 110 ml. of 90% methanol, 3 g. of nickel catalyst (Raney)[9] was added. This solution was shaken for twenty-four hours with hydrogen at 50–60 lb. per sq. in. at 40–50° and the process was repeated three times with fresh portions of nickel. The resin was recovered and dried as usual, iodine no. (Hanus) 38.8, 36.2. In an attempt to lower further the iodine number this sample was subjected to further hydrogenation in alcoholic solution to which 0.5 g of platinum oxide was added. The solution was shaken for five hours with hydrogen at 55–60 lb. at 48–58°. The product, however, showed no decrease in iodine number.

Bromination. 1.—To 1 g. of resin dissolved in 15 ml. of *cold* glacial acetic acid bromine was added drop by drop until an excess was present. Liberation of hydrobromic acid showed that some substitution occurred. After half an hour the solution was poured into dilute sodium bisulfite. The bromide, which precipitated in white flocs, was filtered off and again stirred up with fresh bisulfite solution, filtered, and washed with water on the filter until the washings were neutral. The precipitate was pressed on a porous plate and dried in a vacuum desiccator over phosphorus pentoxide. The material was amorphous and possessed a light yellow or orange color.

Anal. Found: Br, 35.67, 35.14;[10] C, 45.56, 45.50; H, 5.69, 5.55.

2.—One gram of resin was dissolved, with *gentle warming* to hasten solution, in glacial acetic acid, the solution was cooled and treated with bromine as before. More substitution seemed to occur and the product was much darker in appearance. This material was less toxic than the preceding preparation.

Acetylation. 1.—One gram of resin was dissolved in 25 ml. of acetic anhydride, 2 g. of anhydrous sodium acetate was added and the solution was refluxed for one hour, after which the excess acetic anhydride was removed by distillation under diminished pressure on the steam-bath. The residue was dissolved in ether and the solution washed with water until neutral. The solution was dried over anhydrous sodium sulfate, and the resin recovered by

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(6) Adams, Voorhees and Shriner, "Organic Syntheses," Coll., Vol. I, 1932, p. 452.

(7) Samples of non-crystalline derivatives were prepared for analysis and toxicity tests as described in the first article of this series, THIS JOURNAL, **57**, 180 (1935).

(8) Gattermann and Wieland, "Laboratory Methods of Organic Chemistry," 1932, p. 142.

(9) Covert and Adkins, THIS JOURNAL, **54**, 4116 (1932).

(10) The author is indebted to Mr. S. A. Shrader of the University of Maryland for the micro bromine analyses.

evaporation of the ether. The product was darker in appearance than the starting material, sap. no. 355.3, 360.9.[11]

2.—Four grams of resin was dissolved in 10 ml. of dry pyridine, the solution was cooled and 10 ml. of distilled acetic anhydride was added slowly. After thirty-six hours the solution was poured into water and allowed to stand for one-half hour to ensure hydrolysis of the excess acetic anhydride. The aqueous suspension was extracted with ether and the ether solution washed with dilute hydrochloric acid and then with water until neutral. It was dried over anhydrous sodium sulfate and the resin recovered as before, sap. no. 341.6, 331.1.

3.—Six grams of methylated resin (OCH_3 11.7%) was acetylated with acetic anhydride in pyridine as described under 2, sap. no. 176.9, 189.4.

Methylation.—Ten grams of resin was dried by solution in benzene followed by distillation of the solvent. Fifty ml. of methyl iodide and 7 g. of dry silver oxide were added and the flask was connected to a reflux condenser closed with a calcium chloride tube. The mixture was refluxed for twenty-one hours over a small flame so that vigorous boiling and consequent thorough agitation of the silver oxide occurred. Five ml. of the solution was withdrawn to obtain a sample for analysis. This solution was boiled up twice with acetone to remove all trace of methyl iodide and the resin was recovered and dried as before. *Anal.* OCH_3, 10.41, 10.61.[12]

The methyl iodide was distilled from the remainder of the methylated product, which was then dried with benzene as before. The methylation process was repeated with twenty hours of refluxing. *Anal.* OCH_3, 11.45, 11.85.

This is believed to represent the maximum amount of methylation attainable by this method because, when another sample with a methoxyl content of 10.6% was refluxed as before for two hundred and fifty-eight hours, the methoxyl content increased only to 11.3%. The product gave no evidence of having been decomposed as a result of this prolonged treatment.

Vesicant Tests.—The tests for vesicant action were carried out in the following manner: 3 drops from a pipet (calibrated 75 drops/ml.) containing the test solution were placed on the skin and allowed to spread over an area of about 6.5 sq. cm. Each drop was allowed to dry before the following one was applied. The treated area was left exposed to the air and in cases of positive reaction redness, swelling and vesication resulted in from eight to ten hours, probably reaching a maximum within twenty-four hours. The pustulation caused by slight burns disappeared within a few days but sometimes persisted for several weeks in more severe cases, as was observed in some minor accidents that occurred during the course of extraction of the resin.

Summary

A study of the toxicity to goldfish and the vesicant action of croton resin and certain of its derivatives has shown that the free hydroxyl groups, probably enolic or phenolic, are more intimately related to maximum physiological activity than is the condition of unsaturation.

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[11] Titration of the unused alkali was carried out in a volume of about 500 ml.: this large volume was necessary in order to distinguish the end-point, as the saponification products produced a characteristic dark discoloration which tended to obscure the end-point.

[12] Clark, *J. Assoc. Off. Agr. Chem.*, **15**, 136 (1932).

[CONTRIBUTION FROM THE UNIVERSITY OF MARYLAND STATION OF THE INSECTICIDE DIVISION, BUREAU OF CHEMISTRY AND SOILS]

Croton Resin. III. The Combined Acids[1]

BY NATHAN L. DRAKE AND JOSEPH R. SPIES

Saponification of the complex mixture known as "croton resin" has been the subject of several investigations. Dunstan and Boole[2] noted the loss of vesicant action and the formation of acids resulting from treatment of the resin with boiling potassium hydroxide. Later Boehm[3] studied the action of 30% aqueous potassium hydroxide at room temperature on a "croton resin," and found that a dark brown solution resulted. Acidification produced a mixture of acids in a yield of 35%, among which formic and acetic acids were identified qualitatively, and isobutyric and tiglic acids by actual isolation.[4] Cherbuliez *et al.*[5] also recognized that the resin contained esters, but did not study the products of their hydrolysis.

The present paper is a report of a study of the saponification products of the resin, and in particular of the acids liberated. The presence of tiglic, caprylic, capric, lauric, myristic, palmitic, oleic and linoleic acids has been demonstrated conclusively, as well as the absence of any ap-

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[1] From the Ph.D. dissertation of Joseph R. Spies. Presented at the 88th meeting of the American Chemical Society held at Cleveland, Ohio, September, 1934. Parts I and II, THIS JOURNAL, **57**, 180, 182 (1935).

[2] Dunstan and Boole, *Proc. Roy. Soc.* (London), **58**, 238 (1895).

[3] Boehm, *Arch. Path. Pharmakol.*, **79**, 138 (1915).

[4] It should be noted that the resin used by Boehm in his early work was not prepared in the same manner as the material employed here. Our "croton resin" was prepared by the method described in the first article of this series, Spies, THIS JOURNAL, **57**, 180 (1935).

[5] Cherbuliez, Ehninger and Bernhard, *Helv. Chim. Acta*, **15**, 658 (1932).