Compound III.—Colorless crystals, resembling II; m. p. 139–140°. Soluble in benzene and ethyl alcohol. It does not add bromine in carbon tetrachloride, and is similar to VII and II in this respect. *Anal.* Calcd. for $C_{23}H_{18}O_2NBr$: C, 65.73; H, 4.28; N, 3.33. Found: C, 65.58; H, 4.41; N, 3.23.

1,3-Diphenyl-5-(*p*-bromophenyl)-5-ethoxypyrrolone-2 (II) and 1,3-Diphenyl-5-(*p*-bromophenyl)-5-methoxypyrrolone-2 (III) from IV.—Two grams of IV, 12 cc. of absolute alcohol (ethyl alcohol for II, methyl for III), and 34 cc. of absolute ether were mixed and saturated at room temperature with dry hydrogen chloride. After standing at room temperature overnight, the solution was evaporated at steam-bath temperature to crystallization. The product was then recrystallized from the appropriate alcohol, yields 80% approx. The products were identified by melting points and mixed melting points with products described above.

1,3-Diphenyl-5-(*p*-bromophenyl)-5-ethoxypyrrolone-2 (II) from III, and 1,3-Diphenyl-5-(*p*-bromophenyl)-5-methoxypyrrolone-2 (III) from II.—The procedure described immediately above was carried out, substituting first III, and then II, for IV. When III was thus treated with absolute ethyl alcohol–ether saturated with dry hydrogen chloride, compound II was obtained. Similarly, when II was treated with absolute methyl alcohol and ether and dry hydrogen chloride, III was the product. Products were identified by melting point methods.

1,3-Diphenyl-5-(*p*-bromophenyl)-pyrrolone-2 (V) from 1,3-Diphenyl-5-(*p*-bromophenyl)-5-ethoxypyrrolone-2 (II).—A mixture of 100 cc. of glacial acetic acid, 10 g. of powdered zinc, and 7 g. of II (or of III) was heated at a gentle boil for four hours, during which time 30 g. more of zinc was added in small portions at intervals of fifteen minutes. The supernatant liquid was decanted while hot and the residue extracted several times with hot glacial acetic acid. The combined solutions were poured into water and allowed to stand overnight. The solid which precipitated was recrystallized from ethyl alcohol, yield 85%, white plates of m. p. 151.5–152.5°. Compound V is soluble in carbon tetrachloride, benzene, pyridine and toluene.

Anal. Calcd. for $C_{22}H_{16}ONBr$: C, 67.70; H, 4.10; N, 3.59; mol. wt., 389.9; active hydrogen atoms, 0.0. Found: C, 67.92; H, 4.00, 3.82; N, 3.43; mol. wt., 361; active hydrogen atoms, 0.1, 0.2, 0.2.

1,3,5-Triphenylpyrrolone-2 (VIII) from 1,3,5-Triphenyl-5-ethoxypyrrolone-2 (VII).—Compound VIII was prepared from VII by the same method as used above for the preparation of V from II. VIII was recrystallized from glacial acetic acid, m. p. 197–198°.

Summary

Acetophenone condenses with benzoylformanilide to give an aldol-like compound which will form an hydroxypyrrolone ring upon treatment with dry hydrogen chloride. Alkoxypyrrolones result if alcoholic hydrogen chloride is employed. These alkoxypyrrolones yield 1,3,5-triphenylpyrrolone-2 as their common reduction product.

NEW YORK, N. Y. RECEIVED OCTOBER 26, 1934

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

Croton Resin. I. Toxicity Studies Using Goldfish[1]

BY JOSEPH R. SPIES

The oil of the croton bean[2] has been the subject of numerous investigations during the last century. Its purgative action as well as its vesicant and toxic properties were noted by earlier investigators, and various attempts have been made to isolate the active principle. Recently Cherbuliez[3] and his co-workers have isolated an extremely active, non-homogeneous, resin from both the oil and beans which is undoubtedly responsible for their vesicant and toxic properties.[4]

In a search for new natural insecticides, in which a number of plant materials were examined,[5] it became apparent that the croton bean[6] contains a substance which surpasses rotenone in its toxicity to goldfish. The process of Cherbuliez was modified to obtain the resin for this study, and it was shown by tests on goldfish that no appreciable quantity of toxic material was lost in the process.

In the hope that some resolution of components

(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) *Croton tiglium* (Linné) is a species of the *Croton* genus of the *Euphorbiaceae* family. The seed or bean is sometimes used as a fish poison.

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

(4) Boehm and Flaschenträger, *Arch. Path. Pharmakol.*, **157**, 115 (1930), claim to have isolated the pure toxic principle from croton oil by purely physical means which they did not describe.

(5) (a) Drake and Spies, *J. Econ. Entomol.*, **25**, 129 (1932); (b) Spies, *ibid.*, **26**, 285 (1933).

(6) Dr. G. P. Jung of the Bureau of Entomology of China kindly furnished the major supply of beans for this study. According to Jung a "croton emulsion" made from the beans is used as an insecticide in China; other sources from which the beans were obtained: (a) Schimmel & Co., A. G., Miltitz bei Leipzig (through Fritzsche Bros., N. Y.), shelled beans, yield of resin 0.6%; (b) Anandji Virgi & Co., Box 153, Bombay, India, shelled beans, yield of resin 0.94%; (c) H. C. Neibert, Milbuk, P. I. *Cf.* Jung, *Lingnan Sci., J.*, [3] **13**, 557 (1934).

leading to crystalline material might be effected, a solution of 50 g. of resin in 300 ml. of 90% methanol was extracted continuously with redistilled petroleum ether (b. p. 55–70°). The color of successive fractions deepened materially; the first extracted material was light yellow and the resin soft and sticky, that obtained toward the end of the experiment was brown and yielded a hard and brittle resin. About 81% of the resin, in which portion the more toxic substances were concentrated, was extracted during the first forty-eight hours. The carbon and hydrogen content of the fractions decreased from 69.9 to 66.4% and 9.2 to 8.2% from first to last, respectively, showing that a constituent which contains more oxygen than the most toxic substances concentrated in the methanol. The extraction was continued for one hundred and sixty-eight hours and the residue which remained in the methanol was not toxic to goldfish. No crystalline products were obtained from any extract.

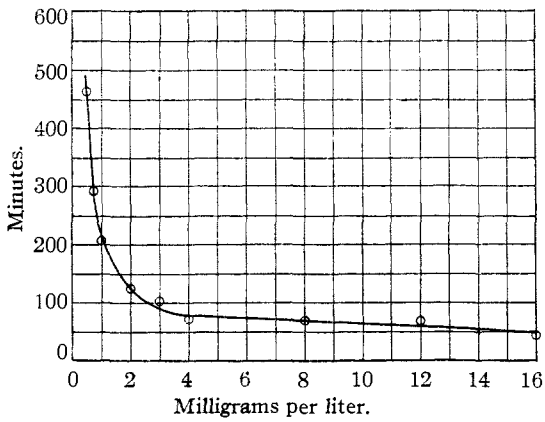

Fig. 1.—Curve showing toxicity of croton oil to goldfish.

The method of Gersdorff[7] using goldfish[8] (*Carassius auratus*) was employed to study the relative toxicity of croton oil, the alcohol-soluble portion of the oil, and croton resin, which constitute, respectively, about 25, 8 and 0.5% of the unshelled croton bean. The results obtained are illustrated graphically in Figs. 1, 2 and 3, where average survival time is plotted as ordinates against concentration as abscissas. The survival time–concentration curve for rotenone published by Gersdorff[7] has been included in Figs. 2 and 3 for the sake of comparison. Since the tests were not made on the same lot of fish, exact comparison is not possible, but it is evident that croton resin is appreciably more toxic than rotenone.

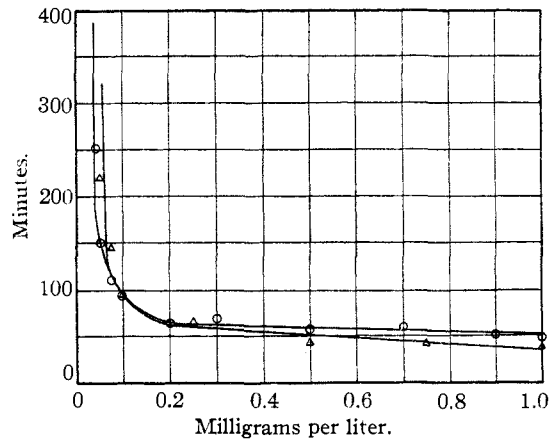

Fig. 2.—Curves showing toxicity of alcohol-soluble fraction of croton oil and rotenone: △, alcohol soluble fraction of croton oil; ○, rotenone.

Experimental

Isolation of Croton Resin.—The extraction with methanol of the ground unshelled beans was carried out on 5-kg. lots in a large capacity Soxhlet.[9] The resin was isolated from the extract by a modification of the process of Cherbuliez.[3] The details of this procedure can be obtained from the author's dissertation. The resin was stored in a desiccator out of contact with air and protected from light.

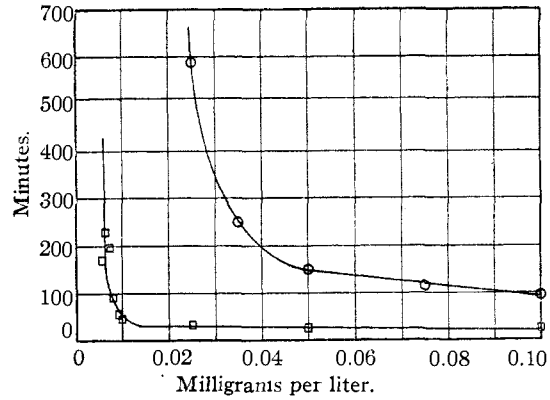

Fig. 3.—Curves showing toxicity of croton resin and rotenone: □, croton resin; ⊙, rotenone.

For removal of solvent from the resin before analysis or toxicity studies, the following procedure was adopted. The sample was placed in a platinum boat in an Abderhalden drier heated by boiling toluene. Air was alternately removed and admitted until no more bubbles formed in the resin when the pressure was reduced (water pump). To test the efficiency of this method the following experiment was performed. About 2 g. of resin was dissolved in 150 ml. of pure distilled chloroform and most of the solvent was removed on the steam-bath. The sample was

(7) Gersdorff, THIS JOURNAL, **52**, 3440 (1930).

(8) The average weight of each fish was about 2 g. Four fish were used to determine the average survival times on the straight portions of the curves while eight to ten fish were used on the curved portions.

(9) Drake and Spies, *Ind. Eng. Chem., Anal. Ed.*, **5**, 284 (1933).

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.

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

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

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.

COLLEGE PARK, MD. RECEIVED OCTOBER 26, 1934

[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 entire y 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

(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 $1:10^3$ 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).