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 of the solution was withdrawn to obtain a sample for analysis.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).

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[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).

preciable quantity of unsaturated acids containing three or more double bonds. Furthermore, absence of any large quantity of stearic or other higher saturated acid has been shown by means of the lead salt–ether method applied to the residue after removal of the more volatile fractions from the mixture of methyl esters of all the acids.

## Experimental

**Saponification of Croton Resin.**—30.4 g. of resin was heated under reflux for one and one-half hours with 125 ml. of 1.6 *N* alcoholic potassium hydroxide in an atmosphere of nitrogen.[6] After removal of the alcohol (nitrogen atmosphere) under diminished pressure, a dark-colored residue remained which dissolved completely in 200 ml. of distilled water. Evaporation of a dried ether extract of this alkaline solution yielded 0.5 g. of brown resinous material. This substance gave no Liebermann–Burchard test for sterols.

The alkaline solution was made acid to congo red with dilute hydrochloric acid, causing the separation of a dark-colored material with a pronounced fatty acid odor. Thorough extraction of the acidified mixture with petroleum ether (b. p. 50–60°) removed a part of the substances thrown out by the acid, but left a large lump of dark-brown gum. The latter was separated mechanically, washed several times with petroleum ether and finally with water. It was then dissolved in methyl alcohol, the solution dried over sodium sulfate and filtered, and the alcohol evaporated, leaving a residue which was heated under nitrogen on the steam-bath to constant weight (11.6 g.).

The combined petroleum ether extracts were washed once with water, dried, separated from drying agent and evaporated until all solvent was removed. There remained 9.6 g. of a brown liquid mixture of acids.

The aqueous solution remaining from the petroleum ether extraction process was a golden yellow. It gave a very persistent deep purple color with ferric chloride solution. The solution was made slightly alkaline to litmus and the water removed by distillation under reduced pressure in a current of nitrogen. As the solution became more concentrated a small quantity of dark gummy matter separated. Such material was removed on two occasions during the evaporation. When all the water had been removed, there remained a golden-yellow residue consisting of the water-soluble saponification products and some potassium chloride. To remove the major part of the inorganic salts the residue was treated with absolute alcohol, filtered and the alcoholic solution evaporated to dryness under reduced pressure in a current of nitrogen. The light-brown amorphous residue so obtained was readily soluble in water or alcohol but not in ether (8.7 g.). Warming an acid (HCl) aqueous solution of this substance caused some change in the material which gives the color test with ferric chloride, for attempts to isolate the organic material from its alkali salt by acidification (litmus) and evaporation of the acid solution resulted in a product which was much darker in color and would no longer give the ferric chloride color test. This water-soluble hydrolysis product gave a negative test for glycerol. Further work on the identification of this material is in progress.

**Lead Salt–Ether Separation of the Liquid and Solid Acids.**—The method described by Jamieson[7] was used. The results are given in the summary:

| | | |
|---|---|---|
| Mixed acids, g. | 3.3 | 3.4 |
| KOH to neut., milli-equiv. | 17.1 | 17.5 |
| Solid acids, g. | 0.94 | 0.92 |
| Liquid acids, g. | 1.53 | 1.74 |

The presence of a considerable quantity of saturated acids of low molecular weight gives these figures little quantitative significance. The lead salts obtained, however, were completely soluble in ether at room temperature (30–33°), thus indicating the absence of any appreciable quantity of the higher saturated fatty acids.

**The Bromides of the Unsaturated Acids.**—To 5.3 g. of unsaturated fatty acids, obtained by a lead salt–ether separation and dissolved in 80 ml. of ether containing 4 ml. of glacial acetic acid, bromine was added at 0–5°, drop by drop, until an excess was present. The solution was allowed to stand for three hours at this temperature. Lack of any precipitate indicated the absence of acids containing three or more double bonds. The ethereal solution was washed with dilute sodium bisulfite to remove any excess bromine and then with saturated sodium chloride solution (to prevent emulsification) until neutral to litmus. The dried ethereal solution was evaporated, the residue taken up in 20 ml. of petroleum ether (b. p. 50–70°), and filtered to remove a small quantity of insoluble tarry material. Concentration to about 10 ml. and cooling caused separation of the tetrabromide of linoleic acid. Owing to the presence of the dibromide of oleic acid and some saturated fatty acids, separation of the tetrabromide was slow. After careful purification by recrystallization from petroleum ether, the tetrabromide melted from 113.5–114.5°.[8] In another experiment 3.64 g. of mixed acids yielded 4.66 g. of bromides, from which 1.55 g. of crude tetrabromide was obtained.

*Anal.* Calcd. for $C_{18}H_{32}O_2Br_4$: C, 36.00; H, 5.38; Br, 53.29. Found: C, 36.11, 36.30; H, 5.59, 5.33; Br, 53.36.[9]

The absence of Matthes and Boltze's[10] so-called liquid tetrabromide of linoleic acid, which is soluble in petroleum ether, was demonstrated in the experiment described above by dissolving the residue after removal of the petroleum ether in a small quantity of methanol and allowing this solution to stand for two and one-half days in the ice box. No crystals were formed.

**Permanganate Oxidation of the Unsaturated Acids.**—The method of Lapworth and Mottram[11] was used to show the presence of oleic acid and to confirm the presence of linoleic acid; 5.1 g. of the fatty acid mixture was used in the experiment, and 2.4 g. of crude polyhydroxystearic

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[6] In alkaline solution a characteristic brown color is produced which appears to be equally intense whether air or nitrogen forms the atmosphere above the solution.

[7] Jamieson, *J. Assoc. Off. Agr. Chem.*, **11**, 303 (1928).

[8] All melting points were taken with standardized Anschütz thermometers.

[9] We wish to thank R. P. Jacobsen for the bromine determination, and S. A. Shrader for the carbon and hydrogen analysis.

[10] Matthes and Boltze, *Arch. Pharm.*, **250**, 225 (1912).

[11] Lapworth and Mottram, *J. Chem. Soc.*, **127**, 1629 (1925).

acids was obtained. This material was treated with 100–150 ml. of petroleum ether (50–70°) to remove the saturated acids and any unoxidized acids; 0.8 g. of product remained undissolved, and 1.6 g. dissolved. The dried mixture of di- and tetrahydroxystearic acids (0.80 g.) was shaken with 200 ml. of ether, and the solution was decanted from the residue. Concentration of the ethereal solution resulted in the separation of crystals which melted at 98°. Two further recrystallizations from ether yielded a product which shrunk at 117° and melted sharply at 123.5°. Three more recrystallizations from ethyl acetate resulted in a product which shrunk at 119–120° and melted sharply at 123.5–124°.[12] Analysis showed this substance to be a dihydroxystearic acid.

*Anal.* Calcd. for $C_{18}H_{36}O_4$: C, 68.29; H, 11.44. Found: C, 68.50, 68.25; H, 11.88, 11.69.

The ether-insoluble residue from the experiment described above was recrystallized from water and dried. It softened at 143° and melted at 155–156°. After a lengthy purification by crystallization[13] from alcohol, water and again from alcohol, the melting point of the acid was raised to 167–168° with previous softening at 156–157°. Further crystallization from alcohol failed to raise the melting point. Analysis demonstrated this compound to be a tetrahydroxystearic acid.

*Anal.* Calcd. for $C_{18}H_{36}O_6$: C, 62.02; H, 10.42. Found: C, 62.03, 61.80; H, 10.65, 10.56.

**Preparation of the Methyl Esters of the Mixed Acids.**—33.7 g. of the mixed acids above described was dissolved in 337 ml. of absolute methanol, and the solution was saturated with dry hydrogen chloride at room temperature. After saturation the mixture was allowed to stand for one hour and then poured into 1685 ml. (5 vol.) of cold water. The esters were removed by ether extraction and isolated in the usual way, yield, 30.6 g.

**Fractional Distillation of the Methyl Esters.**—An apparatus similar to that described by Podbielniak[14] was used. The rate of distillation at no time exceeded 0.1 ml./min., and was much less than this between pure fractions; Table I gives the details. The temperatures recorded at the top of the fractionating column were considerably lower than the recorded boiling points at 15 mm. for the corresponding esters. The very slow rate of distillation is responsible.

TABLE I

DISTILLATION DATA AND CHARACTERISTICS OF THE ESTERS

Vol. of esters, 34.1 ml. Pressure (start), 15.1 mm. Pressure (finish), 13.1 mm. Time, 6 hrs. Total distillate, 19.8 ml.

| Fraction | Vol., ml. | $n^{20}_D$ | $d^{20}$ g./cc. | Mol. wt. of ester from saponification equiv. | | | M. p. of *p*-toluidide of acid, °C. | | Acid in *p*-toluidide |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Found | | Calcd. | Found | Recorded[a] | |
| I | 1.6 | 1.4351 | 0.9482 | 116.9 | | 114 | 70–71.5 | .. | Tiglic |
| Ia[b] | 0.3 | .... | ... | ... | | ... | ....... | .. | ...... |
| II | 0.9 | 1.4253 | .8946 | 155.9 | 155.8 | 158 | 67.5–68.8 | 70 | Caprylic |
| IIa | 0.4 | .... | ... | ... | | ... | ....... | .. | ...... |
| III | 6.0 | 1.4269 | .8762 | 186.3 | 185.1 | 186 | 76.5–77.2 | 78 | Capric |
| IIIa | 0.9 | .... | ... | ... | | ... | ....... | .. | ...... |
| IV | 2.9 | 1.4338 | .8767 | 214.7 | 215.2 | 214 | 82.5–83.2 | 87 | Lauric |
| IVa | 0.9 | .... | ... | ... | | ... | ....... | .. | ...... |
| V | 2.6 | 1.4387 | .8741 | 234.4 | 239.7 | 242 | 89–90.2 | 93 | Myristic |
| Va | 0.8 | 1.4512 | .8804 | 278.3 | | 270 | 95–96 | 98 | Palmitic |
| VI[c] | 1.5 | 1.4581 | .8862 | ... | | ... | ....... | .. | ...... |

[a] The melting points recorded in the literature vary widely. The values given are those of Robertson, *J. Chem. Soc.*, **115**, 1211 (1919). [b] The fractions labeled a are mixtures between purer fractions. [c] The distillation was not carried farther because the boiling points of the methyl esters of stearic, oleic and linoleic acids are so close that separation is impossible.

**Isolation of Tiglic Acid.**—0.3 g. of Fraction 1 was saponified with alcoholic potassium hydroxide. After acidification the organic acid was extracted with ether and isolated by evaporation of the ether. The crude tiglic acid was crystallized from petroleum ether and finally sublimed at 20 mm. The purified product melted at 63.5°.[15]

*Anal.* Calcd. for $C_5H_8O_2$: C, 60.00; H, 8.00. Found: C, 59.76; H, 7.90.

**Preparation of the *p*-Toluidides.**—*p*-Toluidides were prepared directly from the methyl esters by the method of Koelsch and Tenenbaum.[16] All the products were crystallized from aqueous alcohol and finally from methanol to constant melting point before analysis. Table II lists the analyses of the various *p*-toluidides; the related acids are tiglic, caprylic, capric, lauric, myristic and palmitic, respectively.

**Search for Other Saturated Fatty Acids.**—Stearic acid or any higher homolog would have escaped detection in the experiments so far described; 23 ml. of mixed methyl esters were therefore subjected to fractional distillation in the Podbielniak apparatus, and the high boiling residue, which should contain any methyl stearate present origi-

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[12] Because of the danger of lactone formation, this compound was dried in a vacuum at room temperature. Melting point capillaries were introduced into the bath only when the latter was near the melting point of the substance. A large number of isomeric dihydroxystearic acids are possible; *cf.* Lewkowitsch, "Chemical Technology and Analysis of Oils, Fats and Waxes," 6th ed., Vol. I, pp. 233–235.

[13] Rollett, *Z. physiol. Chem.*, **62**, 420 (1909), has discussed the purification of the tetrahydroxystearic acid resulting from linoleic acid on oxidation. His method was used in this instance.

[14] Podbielniak, *Ind. Eng. Chem., Anal. Ed.*, **5**, 135 (1933).

[15] The value 64.5° is given in Beilstein, 3d ed., Vol. I, p. 513.

[16] Koelsch and Tenenbaum, THIS JOURNAL, **55**, 3049 (1933).

TABLE II

THE ANALYSIS OF THE *p*-TOLUIDIDES

| No. | Formula | Carbon, % Calcd. | Carbon, % Found | Hydrogen, % Calcd. | Hydrogen, % Found |
|---|---|---|---|---|---|
| I | $C_{12}H_{15}ON$ | 76.19 | 76.03 | 7.94 | 8.09 |
| | | | 76.16 | | 8.13 |
| II | $C_{15}H_{23}ON$ | 77.19 | 76.86 | 9.94 | 9.98 |
| III | $C_{17}H_{27}ON$ | 78.10 | 78.04 | 10.42 | 10.53 |
| IV | $C_{19}H_{31}ON$ | 78.81 | 79.09 | 10.80 | 10.92 |
| V | $C_{21}H_{35}ON$ | 79.42 | 79.10 | 11.12 | 11.19 |
| VI | $C_{23}H_{39}ON$ | 79.93 | 79.45 | 11.38 | 11.36 |

nally, was saponified with alcoholic potassium hydroxide. The lead salt–ether method was then employed to separate any stearic acid from the liquid unsaturated acids. Only a small quantity of insoluble lead salt was obtained, from which 0.5 g. of free acid was isolated. This free acid was practically completely soluble in *cold* methanol; the higher saturated fatty acids are insoluble in methanol. It is unlikely, therefore, that any considerable quantity of stearic acid or higher homolog is combined in the original resin.

**The Saponification Equivalents of the Methyl Esters.**—The method of Chargaff[17] was used. Since no *n*-propyl alcohol was available, isobutyl alcohol was used instead; 25 to 50 mg. samples were used for each determination. The results are given in Table I.

## Summary

1. The saponification of croton resin has been studied.

2. The petroleum ether–soluble fatty acids have been shown to comprise approximately 32% of the saponification products.

3. Tiglic, caprylic, capric, lauric, myristic, palmitic, oleic and linoleic acids have been shown to be present in the mixed acids obtained by saponification.

[17] Chargaff, *Z. physiol. Chem.*, **199**, 221 (1931).

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[CONTRIBUTION FROM THE RESEARCH LABORATORY OF ORGANIC CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, No. 115]

# The Reactivity of Atoms and Groups in Organic Compounds. XV. The Relative Reactivities of the Hydrogen of the Hydroxyl Group in Benzoic Acid and Certain of its Derivatives[1]

BY JAMES F. NORRIS AND WILLIAM H. STRAIN

The determination of the influence of the structure of alcohols on the relative reactivities of the hydroxyl-hydrogen in these compounds[2] led to simple and definite conclusions in regard to the effect of the position of a substituent on the reactivity. The results were used in developing a new synthetic method for the preparation of ethers which contain two different alkyl radicals.[3]

As it seemed probable that a similar study of the acidic hydrogen atoms in acids would give information of value, the work was undertaken.

The results of such an investigation would show whether or not there exists a relationship between the relative ionization of acids in water and the relative reactivities of the acidic hydrogen atoms of these same acids when measured under conditions that apparently do not lead to ionization. If any such relationship were found to exist, the large number of ionization constants which have been determined would prove of great value to the organic chemist. A knowledge of the effect of the structure of the radical joined to the carboxyl group in an acid on the reactivity of the acidic hydrogen atom would be very helpful in elucidating the mechanism of esterification and other types of reactions.

The acids selected for study were benzoic acid and certain of its substitution products, because these acids have widely different ionization constants and form esters at rates that differ greatly. The reaction selected for study was that between the acids and di-*p*-tolyldiazomethane. The solvent used was toluene except with the acids which were so slightly soluble in the hydrocarbon that solutions of sufficient concentration could not be prepared with which to make accurate measurements. In the case of these acids ethyl acetate was the solvent. The particular diazo compound selected was used because it reacted with all the acids at such rates that they could be measured conveniently. Staudinger and Gaule[4] in their study of aliphatic diazo compounds made some semi-quantitative measurements of the rates at which these compounds reacted with several aliphatic acids.

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[1] From the thesis of William H. Strain submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy, 1931.

[2] Norris and Cortese, THIS JOURNAL, **49**, 2640 (1927).

[3] Norris and Rigby, *ibid.*, **54**, 2088 (1932).

[4] Staudinger and Gaule, *Ber.*, **49**, 1897 (1916).