molecules of one-half and one-third of the normal molecular weight are present together with the normal.

UPSALA, SWEDEN

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF THE COLLEGE OF THE CITY OF NEW YORK]

THE FATTY ACIDS ASSOCIATED WITH RICE STARCH

BY LEO LEHRMAN

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It has been shown that in several naturally occurring starches fatty acids are combined with carbohydrate.[1] In the case of corn and rice the fatty acids constitute part of the molecule of one of the components, namely, the α-amylose.[2] The fatty acids liberated by the hydrolysis of corn starch free from extraneous material have been identified and their amounts determined.[1b,3] From a review of the "fat by hydrolysis,"[1b] acid number and iodine number of the fatty acids liberated from corn and rice starch free from extraneous material,[1b] a difference in amounts and possibly in kind is apparent. Therefore, it is important that these fatty acids which are in the rice starch molecule[1] (α-amylose portion) should be determined both qualitatively and quantitatively. This would enable a comparison of these two starches and furnish additional evidence in the understanding of the different behavior of the starches.

In the course of the investigation the probable absence of sterols or other substances that might occur in the starch and be present in the liberated fatty acids was shown. As the fatty acids occurring in the germ are present as glycerides, the aqueous filtrate from the hydrolysis was examined for glycerol; a negative result was obtained. This is added evidence that the fatty acids identified were not present extraneously in the starch.

Experimental Part

Mixed Fatty Acids from Rice Starch.—The rice starch[4] which was used as the starting material had a negligible amount of extractable extraneous material, using petroleum ether as the solvent. In the course of the work a total of twenty pounds was used. The hydrolysis[1b] was carried out using hydrochloric acid and a much more concentrated suspension of the rice starch. Five pounds of the starch yielded a suspension of 3.5 liters; the acid solution was made up by diluting 525 cc. of concentrated hydrochloric acid to 1.5 liters. The filtrate from the hydrolysis, which had a dark brown color, was reserved for the examination for glycerol. The fat by hydrolysis, obtained by

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[1] (a) Sostegni, *Gazz. chim. ital.*, **15**, 376 (1885); (b) Taylor and Nelson, THIS JOURNAL, **42**, 1726 (1920); (c) Aoi, *J. Chem. Soc., Japan*, **44**, 755 (1923).

[2] Taylor and Iddles, *Ind. Eng. Chem.*, **18**, 713 (1926).

[3] Taylor and Lehrman, THIS JOURNAL, **48**, 1739 (1926).

[4] The author wishes to thank Stein, Hall and Co., Inc., New York City, for their kindness in supplying this material.

extracting the dry residue with petroleum ether and then allowing the solvent to evaporate spontaneously, was a light yellow colored solid; five pounds of the original rice starch gave 14.75 g. of mixed fatty acids free from traces of the solvent.

Examination of Filtrate for Glycerol.—In order to concentrate the glycerol if it was present, a distillation of some of the filtrate from the hydrolysis was attempted but there was so much frothing that the process had to be stopped. Two liters was put in a large beaker and evaporated to about 300 cc., a dark brown precipitate being obtained. This was filtered off and the filtrate extracted with chloroform. After the chloroform was distilled off, a black tarry material was left. This was boiled up with a small amount of water, filtered, treated with potassium permanganate in acid solution and tested for formaldehyde.[5] The result was negative.

In order to be sure that the acid present from the hydrolysis had no effect in the subsequent procedure, one liter of the filtrate from the hydrolysis was neutralized with calcium carbonate, extracted with chloroform and the chloroform distilled off. The small amount of tarry material left gave negative results for glycerol.[5]

Isolation and Identification of Saturated Fatty Acid.—The mixed fatty acids were dissolved in alcohol, the solution chilled in an ice–salt mixture until nearly solid, filtered quickly by suction and washed with several portions of cold alcohol. The solid white residue was recrystallized several times from alcohol, when it gave a melting point of 62°.

The phenylhydrazide, made by heating with an excess of phenylhydrazine,[6] was a white crystalline solid, m. p. 110°,[7] which corresponded with that made similarly from pure palmitic acid.

The molecular weight of the acid was determined by dissolving a weighed amount in neutral alcohol and titrating with standard alkali to phenolphthalein, running a blank at the same time.

Anal. Subs., 0.0784, 0.1245: 7.50, 11.86 cc. of $N/25$ NaOH. Calcd. for palmitic acid, $C_{16}H_{32}O_2$: mol. wt. 256.3. Mol. wt. found: (monobasic acid) 261.3, 262.4.

Anal. Subs., 0.1252, 0.1163: CO_2, 0.3446, 0.3206; H_2O, 0.1387, 0.1295. Calcd. for palmitic acid, $C_{16}H_{32}O_2$: C, 74.91; H, 12.59. Found: C, 75.06, 75.18; H, 12.40, 12.46.

From the above data the presence of palmitic acid in the mixed fatty acids is shown.

Identification of Unsaturated Fatty Acids

A. Oxidation.—The mixture of unsaturated fatty acids separated from the saturated fatty acid by means of the magnesium soap alcohol method[3] was a light brown oil.

A portion of this mixture of unsaturated fatty acids was oxidized with potassium permanganate in alkaline solution.[8] It was found that about half of the oil could be recovered unoxidized, so the oxidation was repeated on this and the product combined with that from the first oxidation. After the hydroxy acids were filtered off, they were washed with water and allowed to dry on the filter paper. In the course of this investigation it was found that the lower hydroxy acid fraction (ether-soluble part) always contained a small amount of the higher hydroxy acid which was difficult to eliminate. This was due to the slight solubility of the higher hydroxy acid in ether, a fact not mentioned previously. In order to make a sharper separation a different solvent was sought and it was found that chloroform on prolonged extraction dissolves the lower hydroxy acid and none of the higher.

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[5] Gettler, *J. Biol. Chem.*, **42**, 311 (1920).

[6] Brauns, THIS JOURNAL, **42**, 1480 (1920).

[7] J. van Alphen, *Rec. trav. chim.*, **44**, 1064 (1925).

[8] Lapworth and Mottram, *J. Chem. Soc.*, **127**, 1628 (1925).

After several recrystallizations from chloroform and a final one from ether, a white crystalline solid was obtained, m. p. 124–126°. Equal parts of this substance were mixed with dihydroxystearic acid, m. p. 127–128° (obtained from corn starch),[3] ground to a fine powder and the melting point determined (123–124°).

Anal. Subs., 0.0619, 0.1072; CO_2, 0.1549, 0.2691; H_2O, 0.0608, 0.1074. Calcd. for dihydroxystearic acid, $C_{18}H_{36}O_4$: C, 68.29, H, 11.47. Found: C, 68.25, 68.44; H, 10.99, 11.21.

The silver salt was prepared[9] and the molecular weight determined by igniting a weighed amount in a porcelain crucible and weighing the residue of metallic silver.

Anal. Subs., 0.0365: Ag, 0.0090. Calcd. for dihydroxystearic acid, $C_{18}H_{36}O_4$: mol. wt., 316.3. Found: 330.7.

The above results indicate the presence of oleic acid in the mixture of unsaturated fatty acids.

The residue of the chloroform extraction of the solid hydroxy acids was extracted with hot water and the solution allowed to cool slowly, silky white crystals coming out. These crystals were filtered off, recrystallized from water and dried, giving a melting point of 154–155°.

Anal. Subs., 0.1354, 0.1421: CO_2, 0.3084, 0.3245; H_2O, 0.1239, 0.1314. Calcd. for tetrahydroxystearic acid, $C_{18}H_{36}O_6$: C, 62.02; H, 10.42. Found: C, 62.12, 62.28; H, 10.24, 10.35.

These data indicate the presence of linolic acid in the mixture of unsaturated fatty acids.

The filtrate from the filtration of the insoluble hydroxy acids was now examined for higher hydroxy acids according to the method of Lewkowitsch[10] but the results were negative, thus indicating the absence of acids more unsaturated than linolic.

B. Bromination.—A sample of the unsaturated fatty acid mixture was brominated[11] and gave no ether-insoluble bromides, indicating the absence of acids more unsaturated than linolic, which substantiates the results as obtained by oxidation. After evaporation of the ether, the residue was recrystallized several times from petroleum ether, yielding white crystals, m. p. 113°.

Anal. (Carius). Subs., 0.1587, 0.1637; AgBr, 0.1984, 0.2050. Calcd. for tetrabromostearic acid, $C_{18}H_{32}O_2Br_4$: Br, 53.33. Found: Br, 53.18, 53.27.

These data are additional indication of the presence of linolic acid in the mixture of unsaturated fatty acids.

Examination for Other Substances.—The result of a sodium fusion on the mixed fatty acids showed the absence of nitrogen, sulfur and halogens. A fusion of another sample of the mixed fatty acids with a mixture of equal parts of sodium carbonate and sodium nitrate showed the absence of phosphorus.

During the alcoholic saponification of the mixed fatty acids necessary in the preparation of the magnesium soaps[3] no unsaponifiable matter was noticed. The Liebermann–Burchard[12] test for phytosterol was also

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[9] Clarke, "A Handbook of Organic Analysis," 2nd ed., p. 216.

[10] Lewkowitsch, "Chemical Technology and Analysis of Oils, Fats and Waxes," 5th ed., Vol. I, p. 564.

[11] Ref. 10, p. 573.

[12] Ref. 10, p. 270.

negative. The modified Kerr and Sorber[13] method for detecting phytosterol was applied but again the results were negative.

Thus the results given above show the presence of three fatty acids, that is, palmitic, oleic and linolic, and the probable absence of other substances in the fatty acid mixture obtained by extracting the solid material resulting from the hydrolysis of rice starch.

Determination of Iodine Numbers and Amounts of the Fatty Acids.—Having the qualitative data, the amounts of the fatty acids were estimated[3] from the determinations of the iodine numbers[3] of the mixed and unsaturated fatty acids portions.

TABLE I

RESULTS OF DETERMINATIONS

Fatty acid sample	Mixed	Mixed	Unsaturated	Unsaturated
Weight, g.	0.1277	0.2558	0.1979	0.1664
Iodine number	85.68	83.73	133.2	130.5

TABLE II

RESULTS OF DETERMINATIONS

Fatty acid	Palmitic	Oleic	Linolic
Percentage	36	35	29

Summary

The fatty acids (saturated and unsaturated) liberated by the hydrolysis of rice starch free from extraneous material have been identified and their amounts determined.

NEW YORK CITY

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

THE ACETATE OF THE FREE ALDEHYDE FORM OF GLUCOSE

BY M. L. WOLFROM[1]

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The formula of glucose is at present considered to be best represented by the closed chain or lactal type of structure. In the study of the reactivity of the sugars, there has frequently arisen a need for characterizing these compounds as open-chain hydroxy-aldehydes or ketones, capable of showing keto–enol tautomerism. This is particularly true in the study of their behavior in alkaline media, as exemplified by the work of Nef and his students, and by the work being conducted in this Laboratory by Evans and co-workers. Accordingly, we have attempted to isolate a crystalline derivative of the open-chain, free aldehyde form of glucose. We have succeeded in obtaining this as the acetate. This compound may be char-

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[13] Hertwig, Jamieson, Baughman and Bailey, *J. Assocn. Off. Agr. Chem.*, **8**, 439 (1925).

[1] National Research Fellow in Chemistry.