on standing by more or less complete reduction of these compounds in the more concentrated acid solutions.

3. The absorption curves of fuchsone, aurin and triphenyl carbinol in concd. sulfuric acid have been determined.

4. The absorption curves of fuchsone and aurin in alcoholic solution containing various amounts of potassium hydroxide, and of the latter compound in concentrated aqueous potassium hydroxide have been obtained.

5. A table showing the frequency numbers of the peaks of the absorption bands of the various solutions studied has been prepared.

6. A chart, showing graphically the results of our studies on di- and triphenylmethane and some of their derivatives, and on fuchsone, benzaurin and aurin, has been prepared.

ITHACA, NEW YORK

[CONTRIBUTION FROM THE BUREAU OF PLANT INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE]

IDENTIFICATION OF SOME OF THE PRODUCTS FORMED BY BACTERIUM PRUNI IN MILK

BY S. L. JODIDI

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Dr. Erwin F. Smith, who was the first to study and to name *Bacterium pruni*, the microbe that causes a disease of plum and peach trees,[1] has made the observation that when the organism is grown in milk, crystals are formed in the culture medium. Because of their characteristic form and interest, Dr. Smith requested me to try to ascertain their chemical nature.

Available for identification were four flasks which contained pure cultures of *Bacterium pruni* grown in skim milk for about four months at room temperature. The crystals formed were separated from the wine-red, slimy fluid by filtration, after which they were spread on porous, unglazed porcelain. A preliminary macroscopic examination of the dry substance showed it to contain chiefly white, star-like needles mixed with some globular aggregates. Under the microscope one could see fine, transparent, prismatic needles partly arranged in bunches. The needles gave the Millon, Mörner and xanthoprotein reactions, indicating the presence of tyrosine. A melting point of the repeatedly purified crystals taken simultaneously with the melting point of tyrosine, a Kahlbaum preparation, showed them to decompose, respectively, at 294–299° and 295–300°, with evolution of gas.

[1] Smith, *Science*, [N. S.] **17**, 456 (1903); **30**, 224 (1909). "Bacteria in Relation to Plant Diseases," *Carnegie Inst. Wash. Publ.*, **1911**, vol. II, pp. 57–60. See also Rorer, *Mycologia*, **1**, 23 (1909). Rolfs, *Cornell Agr. Exptl. Sta. Memoir* No. 8, **1915**. Roberts, *U. S. Dept. Agr. Bull.*, 543, **1917**.

As mentioned already, the needles representing tyrosine were mixed with some globular crystals which had the appearance of impure leucine. Therefore, it was decided to effect their separation by means of boiling glacial acetic acid and 95% alcohol[2] in which tyrosine is almost insoluble. The insoluble residue obtained thereby, on being repeatedly recrystallized from hot water, yielded crystals containing 7.66% of nitrogen; calcd. for $C_9H_{11}NO_3$, 7.74%. Thus, the nitrogen estimation fully corroborates the identity of the substance as tyrosine.

The filtrate from tyrosine was first distilled and then evaporated to dryness. The remainder, which was found to be free from cystine, was purified by recrystallization from 95% alcohol. The purified crystals obtained showed, under the microscope, white, round, refractive balls and radially streaked crystals characteristic of leucine. When a portion of the crystals was transferred to a test-tube and heated carefully on a Bunsen burner, a white, wool-like sublimate formed which could be driven before the flame. When heated rapidly in a closed capillary tube, the substance melted at 292–293°, under complete decomposition and evolution of gas. Under the same conditions Fischer[3] found the melting point of leucine at 293–295°. The data secured appear to establish the identity of the material as leucine beyond any reasonable doubt.

When the fraction containing leucine was heated with water, it was noticed that on the surface of the liquid large, brown drops formed which solidified on cooling. The solidified substance was insoluble in cold and hot water, difficultly soluble in cold alcohol, but readily soluble in hot alcohol, ether and chloroform. The material, recrystallized from chloroform and alcohol, melted at 63°. It made a fatty spot on paper. The light density of the substance (it floats on water), its solubilities as given above, as well as its property of making a fatty spot on paper, indicate it to be a fatty acid or acids which *Bacterium pruni* undoubtedly produced from the butter fat of milk. This fat is known to consist chiefly of the glycerides of palmitic (m. p., 62.6°), myristic (m. p., 53.8°), oleic and stearic (m. p., 69.3°) acids. This, coupled with the circumstance that the substance melts at 63°, points to the fact that it is made up of a mixture of palmitic, myristic and stearic acids.

When the fatty material was extracted with chloroform, there remained an insoluble residue. Ignited on a platinum spatula, it burned with a bright flame, leaving a white ash. This proved to be lime, as shown by its flame and precipitation reactions. It was quite natural to assume, as we did, that the chloroform-insoluble substance might be the calcium salt of the fatty acids referred to above. If such be the case, its distillation

[2] Habermann and Ehrenfeld, *Z. physiol. Chem.*, **37**, 27 (1902–1903).

[3] Hoppe-Seyler, "Handbuch der Physiologisch-Pathologisch Chemischen Analyse," A. Hirschwald, Berlin, **1909**, p. **235**.

should give the corresponding ketones. Actually, its distillation has led to a solid product which was insoluble in water but soluble in alcohol. Under the microscope it was seen to consist of leaflets. The substance (not quite pure) melted at 74°. The pure ketones, stearone, palmitone and myristone, have the melting points of 87.8, 82.8 and 75°, respectively. However, taking into consideration the mode of preparation of the substance, and the inability to purify it for lack of material and, furthermore, that a mixture of compounds not infrequently melts below the constituent having the lowest melting point, it seems reasonable to regard it as a mixture of the ketones mentioned.

Summary

Of the solid products formed by *Bacterium pruni* in skim milk, tyrosine, leucine and the higher fatty acids (a mixture of myristic, palmitic and stearic) have been identified. The latter have been shown to be present partly as such, and partly in the form of a calcium salt.

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HYDROBENZAMIDE AND BENZYLIDENE IMINE AS AMMONO ALDEHYDES

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Working upon the hypothesis that ammonia is analogous to water, Franklin[1] has shown that it is possible to prepare a number of acids, bases and salts which are related to ammonia as the familiar oxygen acids, bases and salts are related to water. Most of the compounds discussed by Franklin were inorganic substances. In a recent article,[2] however, he has indicated that organic substances containing trivalent negative nitrogen should be considered as ammonia derivatives and he has briefly shown the relation of some of these ammono compounds to the corresponding aquo compounds.

Expatiating the analogies outlined above, one would expect a number of compounds which are related to ammonia as the alcohols, ethers, aldehydes, acetals, ketones, carboxylic acids, etc., to be related to water. As a matter of fact, many such substances have been prepared. Their relationship to the aquo compounds, which is quite evident after a little study, is slightly complicated because of the trivalence of nitrogen which makes possible a greater number of combinations than is possible in the case of oxygen. Thus, corresponding to the alcohols one finds the primary

[1] (a) Franklin, *Am. Chem. J.*, **47**, 285 (1912); (b) *Proc. Eighth Int. Cong. Appl. Chem.*, **6**, 119 (1912).

[2] Franklin, THIS JOURNAL, **46**, 2137 (1924).