Crystalline Ficin

By A. Walti

The latex of certain fig trees has been used as an anthelmintic by the natives of Central America for a long time. It also has been known that latex of certain species of *Ficus* contains a proteolytic enzyme. Robbins[1] has shown that such a latex which was proteolytically potent also possessed the ability to digest ascarids in vitro.

We have been able to obtain the active principle in crystalline form.[2] This substance readily can be recrystallized. A convenient manner for obtaining this crystalline substance is the following: The clarified active latex was brought to *p*H 5 by the addition of a normal sodium hydroxide solution while stirring, and the clear solution was allowed to remain at approximately 5° for several weeks. Crystals formed which could be recognized readily by the golden sheen they imparted to the brown solution. Under the microscope at a magnification of 100 to 200 diameters the crystals represent beautiful hexagonal thin plates. The crystals were centrifuged off preferably at a lower temperature. They had a yellowish color. By dissolving the crystals in a dilute hydrochloric acid solution of approximately 0.02 normality, filtering through filter cell if necessary, and neutralizing to *p*H 5 with a sodium hydroxide solution, the material could be recrystallized readily. This procedure may be repeated. The white recrystallized substance possessed similar anthelmintic properties to the original latex when tested on living ascarids and hydrolyzed gelatine and benzoylglycylamide without the addition of an activator. The substance gave a positive Millon and biuret test and also its nitrogen, carbon, hydrogen and sulfur content were in agreement with the chemical nature of a protein. The enzyme could be obtained practically free from ash. On treatment of this enzyme with phenylhydrazine, inactivation occurred toward gelatine and benzoylglycylamide. On the addition of cysteine reactivation occurred. The enzyme was also inactivated by iodine and hydrogen peroxide. Ficin is a papainase and is the first proteolytic enzyme to be obtained from plant sources.[3]

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[1] B. H. Robbins, *J. Biol. Chem.*, **87**, 251 (1930).

[2] Presented before the Biochemical Section of the American Chemical Society meeting in Pittsburgh, September 7, 1936.

[3] Recently A. K. Balls, H. Lineweaver and R. R. Thompson have obtained crystals with the properties of papain, *Science*, October, 22, 1937, p. 379.

COMMUNICATIONS TO THE EDITOR

A NEW KETONE FROM THE URINE OF PREGNANT MARES

Sir:

Recently Marker, Kamm, Crooks, Oakwood, Lawson and Wittle [THIS JOURNAL, **59,** 2297 (1937)] have reported the preparation of pregnanedione and *allo*-pregnanedione and the isolation of pregnanediol from the non-phenolic extract of mares' pregnancy urine. This material has been under examination in these laboratories during the past year and has yielded pregnanetriol [in confirmation of the earlier work of Haslewood, Marrian and Smith, *Biochem. J.*, **28,** 1316 (1934)], a water soluble semicarbazone (m. p. 253–254° (decomp.)] which has not been investigated to date and small amounts of an undescribed saturated ketone. A preliminary statement concerning the latter is considered advisable at this time although its identity has not yet been established.

Combustion figures indicate the empirical formula $C_{19}H_{26}O_3 \pm C \pm 2H$ for the new ketone. It gives a golden yellow color with the Liebermann-Burchardt reagents and a yellow to orange solution with a green fluorescence on warming with concentrated sulfuric acid. Esterifiable hydroxyl groups are absent; no product was obtained with acetic anhydride at 100°. Quantitative hydrolysis of the semicarbazone and its composition clearly establish the fact that only one oxygen atom is present in a reactive carbonyl group. The nature of combination of the remaining two oxygen atoms has not been determined.

The apparent inertness suggests a possible relationship to the steroids of the adrenal cortex [Mason, Myers and Kendall, *J. Biol. Chem.*, **116,** 267 (1936); Reichstein, *Helv. Chim. Acta,* **19,** 1107 (1936)] but so far no additional evidence in support of this attractive hypothesis has been obtained.

The substance was isolated from the neutral total ketonic fraction as the semicarbazone which is strikingly characterized by its practically complete insolubility in boiling organic solvents and water. For analysis the semicarbazone was freed from as many impurities as possible by repeated extractions with hot water, alcohol and acetone. The melting point varied from 300 to 315° (decomp.) with the rate of heating. On hydrolysis with dilute alcoholic sulfuric acid, 103.6 mg. gave 86.0 mg. of the crude ketone; the theoretical yield from a monosemicarbazone of $C_{19}H_{26}O_3$ is 87.1 mg. The product was readily soluble in cold acetone, benzene and chloroform, moderately so in ether and alcohol. The analytical sample was recrystallized four times from alcohol and twice from aqueous acetone. It separated in long white needles melting at 252° (uncorrected) with preliminary softening 3° below this. Nitrogen, halogen and sulfur were absent. *Anal.* (Schoeller) Semicarbazone: calcd. for $C_{20}H_{29}O_3N_3$: C, 66.85; H, 8.08; N, 11.69. Found: C, 67.05, 67.02; H, 7.75, 7.82; N, 10.85, 10.74. Free ketone: calcd. for $C_{19}H_{26}O_3$: C, 75.50; H, 8.61. Found: C, 75.47, 75.50; H, 8.52, 8.45.

The investigation is being continued. Complete experimental details will be published at a later date.

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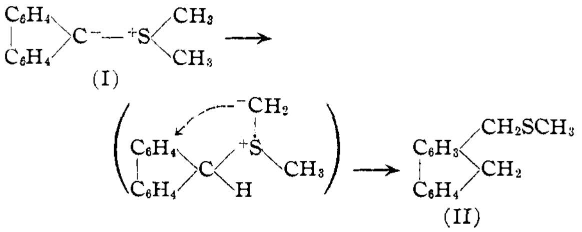
R. D. H. HEARD

RECEIVED JANUARY 18, 1938

REARRANGEMENT OF FLUORYLIDENE DIMETHYL SULFIDE TO FLUORENE-1-DIMETHYL SULFIDE

Sir:

Recent developments [Sommelet, *Compt. rend.*, **205,** 56 (1937); Krollpfeiffer and Schneider, *Ann.*, **530,** 38 (1937)] along lines closely related to those on which we are working have prompted us to record observations dealing with fluorylidene dimethyl sulfide. Our experiments have revealed that fluorylidene dimethyl sulfide (I), which was discovered by Ingold and Jessop [*J. Chem. Soc.*, 713 (1930); see also Hughes and Kuriyan, *ibid.*, 1609 (1935)], rearranges in an alkaline medium such as alcoholic sodium hydroxide or liquid ammonia to fluorene-1-dimethyl sulfide (II).



Evidence favoring the structure assigned to (II) was secured by graded oxidation. By means of hydrogen peroxide (II) is converted to fluorene-1-dimethyl sulfone, whereas with a stronger oxidizing agent such as sodium dichromate in acetic acid, fluorenone-1-dimethyl sulfone and fluorenone-1-carboxylic acid are formed. The last mentioned compound was characterized by comparison with fluorenone-1-carboxylic acid prepared by the oxidation of fluoranthene. As is to be expected, (II) is converted by methyl alcoholic hydrochloric acid to fluorene-1-dimethyl ether and by a solution of hydrogen bromide in acetic acid to fluorene-1-methyl bromide, which with zinc and acetic acid is reduced to 1-methylfluorene.

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GUIDO E. HILBERT
LOUIS A. PINCK

RECEIVED NOVEMBER 17, 1937

INTERNAL FREE ROTATION IN HYDROCARBONS

Sir:

In a recent note Kassel[1] puts forward certain arguments which imply that free internal rotation occurs in saturated hydrocarbon molecules at room temperatures. Since we cannot agree with several of the points which he has put forward, we should like to discuss them here and at the same time present a very brief summary of the arguments on the other side of this vexatious question.

Kassel bases his reasoning on a comparison for the reaction *n*-butane ⟷ iso-butane of the entropy change obtained from equilibrium and combustion data (−2.1 e. u.) with that from Third Law measurements by Parks, Shomate, Kennedy and Crawford[2] (−5.8 e. u.). He argues that

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[1] Kassel, THIS JOURNAL, **59,** 2745 (1937).

[2] Parks, Shomate, Kennedy and Crawford, *J. Chem. Phys.*, **5,** 359 (1937).