

### 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<sup>1</sup> 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.<sup>2</sup> This substance readily can be recrystallized. A convenient manner for obtaining this crystalline substance is the following: The clarified active latex was brought to pH 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

(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.

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 pH 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 benzoylglucylamide 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 benzoylglucylamide. 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.<sup>3</sup>

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(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.

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## COMMUNICATIONS TO THE EDITOR

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### 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 satu-

rated 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  $C_{19}H_{26}O_3 = C = 2H$  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.