

[CONTRIBUTION FROM THE WILLIAM G. KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES OF THE CALIFORNIA INSTITUTE OF TECHNOLOGY]

Chemical Studies of Pineapple (*Ananas sativus* Lindl). II. Isolation and Identification of a Sulfur-Containing Ester in Pineapple¹

BY A. J. HAAGEN-SMIT, JUSTUS G. KIRCHNER, CLARA L. DEASY² AND ARTHUR N. PRATER³

In a study of the volatile constituents of the smooth Cayenne variety of pineapple, *Ananas sativus* Lindl, it was found that a sulfur-containing compound was present in the higher-boiling fractions.⁴

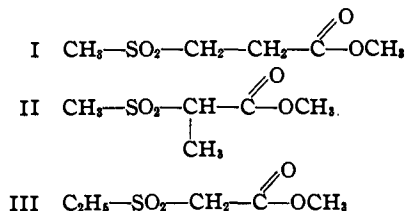
The amount of the compound present in pineapple is small, as was shown by sulfur analyses of all the fractions obtained. A total of 0.250 g. of sulfur was found in 995 kg. of the winter fruit pulp; a slightly smaller amount, 0.1125 g. of sulfur, was present in 740 kg. of the summer fruit pulp. The peel from the summer fruit contained negligible amounts.

Qualitative tests on the impure fractions indicated that the compound was neither a thio-ketone nor a mercaptan⁵; the absence of nitrogen eliminated the possibility of an isothiocyanate.

Since it was impossible to purify the compound completely by distillation, a solid derivative was prepared. This was obtained by oxidation of the purest fraction (Fraction 16 of Table V⁴) with an excess of a 50-50 mixture of 30% hydrogen peroxide and glacial acetic acid. The same product was obtained when a trace of ammonium molybdate, which catalyzes the oxidation of sulfides to sulfones,⁶ was added to the oxidizing mixture.

The oxidized compound was found by micro-analysis to have the empirical formula C₆H₁₀SO₄. A Rast micro-molecular weight determination confirmed the simple formula. A qualitative test indicated that two of the oxygens were present in an ester linkage.⁷ This was confirmed by the determination of the saponification equivalent of the compound.

Since the amount of the solid derivative available was extremely small, and since there were only nine possible structures for the compound,



(1) This investigation was supported by a grant from the Pineapple Research Institute of Hawaii.

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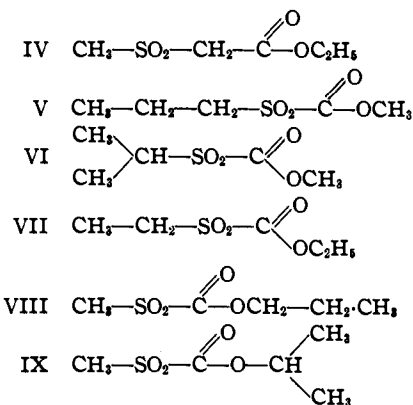
(3) Present address: C. B. Gentry Co., Los Angeles, California.

(4) Fractions S-16 through S-21 of Table VI and Fractions 9 through 20 of Tables IV and V of Part I (THIS JOURNAL, **67**, 1646 (1945)).

(5) Feigl, "Qualitative Analysis by Spot Tests" (translated by J. W. Matthews), Nordemann Publishing Co., New York, N. Y., 1939, p. 291

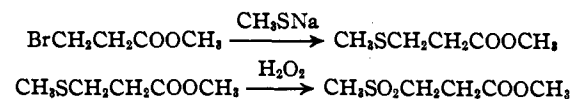
(6) Toennies and Kolb, *J. Biol. Chem.*, **140**, 131 (1941).

(7) Feigl, ref. 5, p. 295.



it was decided to attack the problem of the structural determination by synthesis.

The methyl ester of β -methylsulfonylpropionic acid (I) was prepared by the series of reactions



The compound so prepared was found by analysis and by mixed melting point determination to be identical with the oxidized sulfur compound from pineapple.

The identification of the oxidized sulfur compound as methyl β -methylsulfonylpropionate proves that the original sulfide occurring in pineapple is methyl β -methylthiolpropionate, CH₃SCH₂CH₂COOCH₃. From the analytical data for sulfur, it can be calculated that the sulfide is present in the winter and summer fruit pulp in approximately equal quantities of 1 g. per 1000 kg. of fruit pulp.

The similarity of the formula of this sulfide to that of cysteine, HSCH₂CH(NH₂)COOH, indicates the possibility that the compound is formed from this amino acid in the fruit.

Experimental

Isolation from Pineapple.—The method of isolation of the sulfur-containing compound from pineapple already has been described.⁴

As the possibility existed that the sulfur compound might be present in larger quantities in the peel, the peel from the summer fruit (182 kg.) was run through the Waring Blendor and then subjected to steam distillation and fractionation. Sulfur determinations were run on the peel fractions whose boiling points were similar to those of the sulfur-containing fractions from the pulp. The peel was found, however, to contain very little of the sulfur compound.

Oxidation of the Sulfur Compound.—Two hundred and eight mg. of the purest fraction obtained (Fraction 16 of Table V⁴), containing 17.89% sulfur, was dissolved in excess of a 50-50 mixture of 30% hydrogen peroxide and

glacial acetic acid. The reaction was exothermic. The mixture was allowed to stand at room temperature for five days and then the excess peroxide was decomposed with platinum black. After filtration, the reaction mixture was distilled to dryness in a vacuum still below 50°. Sixty-seven mg. of crude solid material was obtained, which was recrystallized once from carbon tetrachloride and five times from alcohol to yield a white crystalline compound, m. p. 93.6–94° (cor.).

*Anal.*³ Calcd. for C₈H₁₀SO₄: C, 36.15; H, 6.07; S, 19.26. Found: C, 36.27; H, 5.93; S, 18.75.

The same compound was obtained when a trace of ammonium molybdate was used as catalyst in the oxidation.

A determination of the molecular weight by the Rast micro method gave 170; the calculated value for C₈H₁₀SO₄ is 166.

The saponification equivalent of the compound was determined using an ethylene glycol solution of potassium hydroxide.⁹

Anal. Calcd. for C₈H₁₀SO₄: sapon. equiv., 166. Found: sapon. equiv., 178.3, 178.5.

Preparation of Methyl β-Methylthiolpropionate.—A solution of sodium methylate, prepared by dissolving 2.85 g. sodium (0.124 atom) in 30 cc. of absolute methyl alcohol, was placed in an ice-cooled three-necked flask, equipped with reflux condenser, mercury-sealed stirrer and dropping funnel, and a cooled solution of 5.95 g. (0.124 mole) of methyl mercaptan in 25 cc. of absolute methyl alcohol

was added gradually. The mixture was then gradually heated to boiling, a solution of 20.7 g. (0.124 mole) of methyl β-bromopropionate in absolute methyl alcohol slowly added, and the solution was refluxed for an hour. After dilution with water, the solution was extracted with ether. The ether solution was washed with water, dried over sodium sulfate, and the ether was removed by distillation on the water-bath. A yield of 9.1 g. (54.7% yield) of methyl β-methylthiolpropionate boiling at 69° at 11 mm. was thus obtained.

Oxidation of Methyl β-Methylthiolpropionate.—One and a half grams of methyl β-methylthiolpropionate was oxidized by the method used for the pineapple compound, except that approximately 5 mg. of ammonium molybdate was used as catalyst, and the mixture was allowed to stand for only three hours. After three recrystallizations from ethyl alcohol-water, white needles melting at 94–94.6° were obtained. A mixed m. p. with the oxidized pineapple compound showed no depression.

Anal. Calcd. for C₈H₁₀SO₄: C, 36.15; H, 6.07; S, 19.26. Found: C, 36.24; H, 6.10; S, 18.73, 18.83.

Summary

A sulfur-containing ester, CH₃SCH₂CH₂COO-CH₃, has been isolated from the higher-boiling volatile material from pineapple fruit pulp. This compound has been converted by oxidation to the sulfone, m.p. 93.6–94°, which was identified by synthesis.

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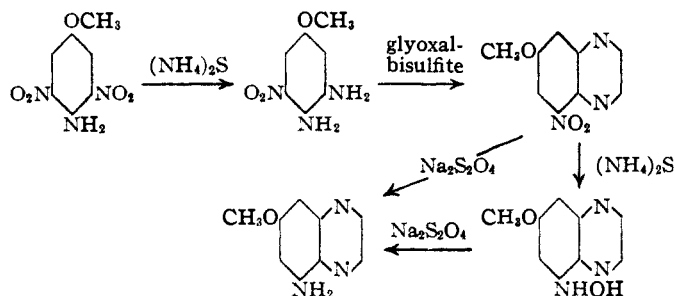
RECEIVED JULY 11, 1945

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE POLYTECHNIC INSTITUTE OF BROOKLYN]

Syntheses in the Quinoxaline Series. Preparation of 7-Methoxy-5-aminoquinoxaline and 7-Methoxy-5-hydroxylaminoquinoxaline

BY R. H. MIZZONI AND PAUL E. SPOERRI

The literature of quinoxaline derivatives is surprisingly meager in view of the potential usefulness of some of them as medicinals. It was thought that certain compounds which were structurally analogous to the plasmochin type might possess useful properties. This investigation necessitated the preparation and characterization of 7-methoxy-5-amino-quinoxaline, along with several hitherto unknown intermediates.



Gawron and Spoerri¹ have prepared 7-methoxy-5-aminoquinoxaline through condensation of 3,4,5-triaminoanisole with glyoxal-bisulfite. In the

(1) Gawron and Spoerri, *THIS JOURNAL*, **67**, 514 (1945).

present investigation the synthesis was accomplished through the condensation of 3,4-diamino-5-nitroanisole with glyoxal-bisulfite and subsequent reduction of the resulting 7-methoxy-5-nitroquinoxaline. The accompanying flow chart, starting with 3,5-dinitro-4-aminoanisole, shows the reactions involved in this synthesis.

3,5-Dinitro-4-aminoanisole was prepared according to the method of Reverdin² by nitration of *m*-nitrobenzenesulfonyl-*p*-aminoanisole. *m*-Nitrobenzenesulfonyl chloride was prepared by known methods.³

Reduction of 3,5-dinitro-4-aminoanisole with ammonium sulfide gave the desired 3,4-diamino-5-nitroanisole, while with sodium sulfide only resin formation resulted. The compound is weakly basic, its hydrochloride reverting to the free base at the melting point of the base. The hydrochloride is readily hydrolyzed in warm acetone and in warm ethyl methyl ketone. The amine could not be acetylated by any of the usual means, nor did it form a picrate.

(2) Reverdin, *Ber.*, **42**, 1525 (1909); *ibid.*, **45**, 349 (1912).

(3) German Patent 89,997 (1896).