

[CONTRIBUTION FROM THE FOOD RESEARCH DIVISION, BUREAU OF CHEMISTRY AND SOILS, AND THE OFFICE OF HORTICULTURAL CROPS AND DISEASES, BUREAU OF PLANT INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE]

SOME ORGANIC ACIDS OF WHEAT PLANTS¹

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Many of the constituents of wheat plants have been repeatedly and thoroughly investigated. In Wehmer's book, "Die Pflanzenstoffe," second edition, 1929, mention is found of a considerable number of organic and inorganic constituents. However, the non-volatile organic acids are not mentioned.

In the course of a study on the buffer system of the wheat plant,⁴ Hurd-Karrer found that the form of the titration curve between P_{H} 2.0 and 6.0 suggested the presence of malic acid in the juice, which observation directed attention to the fact that the nature of the organic acids of wheat had not been reported. The present work was accordingly undertaken, in coöperation with the office of Cereal Investigations of the United States Department of Agriculture, to determine the identity and relative amounts of the acids in the tissues of the wheat plant.

The material used for this study was winter wheat grown on the Arlington Experimental Farm at Rosslyn, Virginia, during the season of 1927-1928. Samples were collected from May 15 until May 28, 1928. The plants at this time were in a vigorous state, free from rust and other diseases. When cutting was begun, no heads had appeared, but toward the end of the cutting, heads had begun to show. The plants were cut about four inches or more above the ground so that none of the basal, yellowing leaves were included in the samples.

The material was immediately taken into the laboratory, cut into short pieces in a meat slicer, weighed and thoroughly extracted with boiling water. The total weight of the green plants was 76,054 g., 14,527 g. of which was dry matter.

The liquid was strained through a closely woven bag to which the solid residue was finally transferred. The remaining liquor in the residue was pressed out in a tincture press, and was added to the strained portion.

The liquid portion was treated with successive portions of a saturated solution of basic lead acetate until no precipitate was formed by further addition of lead acetate. The precipitates were collected on Büchner funnels and dried at room temperature. Combined, they amounted to 1715 g.

¹ Food Research Division Contribution No. 86.

² Bureau of Chemistry and Soils.

³ Bureau of Plant Industry.

⁴ Hurd-Karrer, "Titration Curves of Etiolated and of Green Wheat Seedlings Reproduced with Buffer Mixtures," *Plant Physiol.*, 3, 307-328 (1930).

For the separation of the non-volatile acids by the ester distillation method, 685 g. of the lead precipitate was decomposed by sulfuric acid, and filtered from lead sulfate. The excess of sulfuric acid in the filtrate was removed by its exact equivalent of barium hydroxide. The barium sulfate was removed by filtration, and the solution of organic acids was concentrated to small volume, treated with a liberal quantity of absolute alcohol, and filtered from precipitated proteids and extraneous materials.

The alcoholic solution was evaporated to dryness and esterified with alcoholic hydrochloric acid (one liter of absolute alcohol containing 25 g. of dry hydrochloric acid). The excess alcohol was evaporated and the esterification repeated. The alcohol was again evaporated and the residue dissolved in ether. Considerable insoluble material was left. The ethereal solution was filtered, shaken with a solution of sodium hydroxide to remove free acids and evaporated. Fifty-two grams of the crude mixed esters were obtained.

The esters were submitted to fractional distillation at 10 mm., and the distillates were re-fractionated at the same pressure.

The results of the fractionation are as follows

	G.
1 90-100° (redistilled at 85-90°)	2.2
2 100-120°	0.1
3 120-125°	.1
4 125-135° ($\alpha_D - 10.7^\circ$)	21.0
5 135-145°	1.8
6 145-155°	1.5
7 155-165°	0.6
8 165-170°	13.2
Residue (oily)	6.8
	47.3

The hydrazides were prepared from these fractions.

Fraction 1 yielded a hydrazide which came down at once in needles. These were in very small quantity and when recrystallized melted at 235°. They agreed in optical properties⁶ with oxalic hydrazide.

The filtrate from this hydrazide, on longer standing, yielded another hydrazide, crystallizing in plates, melting after recrystallization at 149-151°. Optical examination confirmed its identity with malonic hydrazide.

The filtrate from malonic hydrazide, on evaporation, yielded a small quantity of an alcohol-soluble hydrazide, melting at 68-75°, which indicated levulinic hydrazide. As levulinic acid may have been formed by the action of alcoholic hydrochloric acid on carbohydrates, it is not considered as originally present in the plant.

Fractions 2 and 3, being small in quantity, were disregarded.

⁶ Optical crystallographic examinations were made by G. L. Keenan of the Food, Drug and Insecticide Administration.

Fraction 4 yielded a copious precipitate of a hydrazide having the appearance of malic hydrazide. It melted at 178–179°. Admixture with *l*-malic hydrazide caused no depression in the melting point. The optical rotation of this fraction also indicates that it is the ester of *l*-malic acid.

Fractions 5 and 6 also yielded impure malic hydrazide. These fractions were saponified and tested for tartaric acid by the tentative method of the A. O. A. C., with negative results.

Fraction 7 yielded an impure hydrazide which appeared to contain malic and citric hydrazide.

Fraction 8 also yielded a pasty hydrazide which was partly crystalline. As its behavior indicated that it was aconitic acid, a small quantity of the fraction was saponified, acidified and extracted with ether. Aconitic acid was identified in the residue from the ether. Therefore fraction 8 was refractionated. Six grams boiling at 170° at 10 mm. was obtained which afforded a hydrazide melting at 104–105° and agreeing with citric hydrazide in optical properties.

For the separation of aconitic acid, the mixed acids, liberated from 500 g. of the lead precipitate, were dissolved in water and extracted with ether in an apparatus designed for the continuous extraction of liquids with ether until negligible quantities of ether-soluble acids were left in the aqueous liquid. The extracted acids were partly crystalline. They were dissolved in ether, and the ether solution was shaken out with five 5-cc. portions of water. The water solution was diluted and shaken out four times with an equal volume of ether, which was then united with the main ether solution.

The ether was evaporated in a tared beaker, and the residue was dried on a steam-bath at reduced pressure until free from water and volatile acids. It was a solid, crystalline acid, weighing 9.0 g. On recrystallizing from water the acid melted at 183–185°. It decolorized permanganate solutions, gave the acetic anhydride test for aconitic acid, and agreed with that acid in its optical crystallographic properties.

The citric and malic acids were further determined in a portion of the lead precipitate by the tentative methods given in "Methods of Analysis," A. O. A. C., second edition.⁶ Citric acid was determined as pentabromoacetone and malic acid by the polariscope method. The figures were somewhat higher than those obtained by the ester distillation method.

Oxalic acid, left in the extracted plants as an insoluble salt, was determined by the Arbenz⁷ method.

The percentages of non-volatile acids, calculated on the basis of green wheat plants, were found to be as follows

Malonic acid (by fractionation of esters)	0.005%
Aconitic acid (by ether extraction of acids)	.040%

⁶ These analyses were made by H. H. Mottern of the Food Research Division.

⁷ Arbenz, *Mitt. Lebensm. Hyg.*, 8, 98 (1917).

Malic acid (by fractionation of esters)	0.056%
Malic acid (by polarization)	.064%
Citric acid (by fractionation of esters)	.016%
Citric acid (by pentabromoacetone)	.019%
Oxalic acid	Trace
Oxalic acid in residue, dry basis	0.11%
Oxalic acid in residue, fresh basis	.02%

Aconitic acid has been identified previously in two species of the *Gramineae* (sugar cane and sorghum).

Malonic acid has not been found in any of the investigations on the organic acids of fruits, but was reported present in alfalfa by Turner and Hartman.⁸

Summary

In this investigation of the organic acids in growing wheat plants the water extract was found to contain aconitic, citric, malic and malonic acids with a trace of oxalic acid. The residue after water extraction was found to contain oxalic acid.

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[A COMMUNICATION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

THE RELATION OF THE STRUCTURE OF KETONES TO THEIR REACTIVITY AND AFFINITY IN ACETAL FORMATION. II

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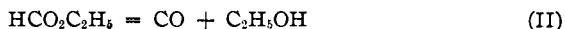
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The reaction of orthoformic ester and ketones to form acetals and ethyl formate (Equation I) is a reversible reaction suited to the study of the relationship of the structure of compounds to the strength of oxygen to carbon linkages.



Recently the extent of acetal formation for eight ketones was reported.¹ In extending the study of this reaction it became apparent that the previous evaluation of the analytical data was in error in the case of one ketone, so the method of analysis was subjected to a more thorough study.

The analytical method used by Carswell for the determination of the extent of the acetal reaction was based upon the observation of Geuther that ethyl formate in the presence of sodium ethoxide decomposed into carbon monoxide and alcohol. Geuther formulated the reaction as in Equation II.



⁸ Turner and Hartman, *THIS JOURNAL*, **47**, 2044 (1925).

¹ Carswell and Adkins, *ibid.*, **50**, 235 (1928).