

with the numbers required by theory as tested by actually writing all the possible structural formulas.

4. The usefulness of the formulas advanced is not limited to the calculation of the number of structurally isomeric alcohols. Since the latter are typical mono-substituted derivatives of the paraffins, the figures recorded in Table I more generally represent the number of structurally isomeric mono-substitution products of the saturated hydrocarbons inclusive of the eicosanes.

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## SOME ORGANIC ACIDS IN BARLEY, MAIZE, OATS AND RYE PLANTS

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In continuation of the work on the organic acids of cereal plants, which began with a study of the acids of wheat plants,<sup>1</sup> an investigation has been made of the non-volatile acids in the green parts of the growing plants of barley, maize, oats and rye. The maize was raised in the field. The barley, oats and rye plants were grown in the greenhouse. All plants were beginning to head when cut. As in the case of wheat plants the investigation was confined to the non-volatile acids, extracted by boiling water from the dried, ground plants. These have not been previously reported in the literature.

Aconitic acid, previously reported as present in sugar cane<sup>2</sup> and in sorghum<sup>3</sup> and found in the wheat plant,<sup>1</sup> is present also in these plants of the grass family. Other acids in some of these plants which have not been found in numerous investigations of the acids of fruits are tricarballylic acid and malonic acid.

**The Acids of Barley Plants.**—The acids were separated from one hundred pounds of barley plants and converted into the ethyl esters, yielding 84 g. of crude esters. Fractionation at 10 mm. yielded: 1, 80–95°, 0.4 g.; 2, 95–105°, 0.6 g.; 3, 105–115°, 1.5 g.; 4, 115–125°, 5.2 g.; 5, 125–135°, 42.2 g.; 6, 135–150°, 1.1 g.; 7, 150–165°, 2.8 g.; 8, 165–173°, 21.9 g.; residue, 8 g.

Fraction 1 yielded oxalic dihydrazide, melting at 235° and further identified by optical crystallographic data.<sup>4</sup> The filtrate, seeded with malonic hydrazide, yielded a second crop of crystals, melting at 148–149° and further identified as malonic hydrazide by optical data.

<sup>1</sup> Nelson and Hasselbring, *THIS JOURNAL*, 53, 1040–1043 (1931).

<sup>2</sup> Behr, *Ber.*, 10, 35 (1877).

<sup>3</sup> Parsons, *Jahresb.*, 1444 (1882).

<sup>4</sup> Optical crystallographic examinations were made by G. L. Keenan of the Food and Drug Administration.

Fractions 4 and 5 yielded malic hydrazide melting at 179°.

Fraction 7 was seeded with tricarballic hydrazide, but the result was negative. Malic hydrazide was obtained instead, and was identified as such by its melting point.

Fraction 8 yielded a small quantity of hydrazide melting at 186°. When this was mixed with tricarballic trihydrazide, the melting point was between that of tricarballic trihydrazide and 186°. Optical crystallographic data showed it to be slightly impure tricarballic trihydrazide.

The filtrate from tricarballic hydrazide was seeded with citric hydrazide. On standing, citric trihydrazide separated. It melted at 108°, and was identified by the optical method. The yield of tricarballic hydrazide was small as compared with that of the citric hydrazide, showing the fraction to consist mainly of ethyl citrate.

Aconitic acid was obtained from the ether extraction of the acids previous to esterification. The ether extraction afforded about 2 g. of crystals contaminated with an oily material. On purification the crystals melted at 180–181° and were further identified as aconitic acid by optical crystallographic examination.

Oxalic acid was determined by the method of Arbenz<sup>5</sup> and 0.019% was found on the basis of the green plants.

**The Acids of Maize Plants.**—The acids, separated as lead salts from ninety-six pounds of fresh maize plants in the roasting-ear stage of maturity, were converted into the ethyl esters, yielding 83 g. of crude esters.

Fractionated four times at 10 mm. the following fractions were obtained: 1, 85–95°, 2.1 g.; 2, 95–105°, 3.6 g.; 3, 105–115°, 2.8 g.; 4, 115–125°, 3.5 g.; 5, 125–135°, 20.2 g.;  $\alpha_D$  -10.5°; 6, 135–145°, 1.8 g.; 7, 145–155°, 3.3 g.; 8, 155–165°, 11.1 g.; 9, 165–179°, 6.2 g.; residue, 28.0 g.

The hydrazides were prepared from these fractions. Fraction 1 yielded an immediate precipitate of oxalic dihydrazide, melting at 235° and further identified by optical crystallographic examination. The filtrate from oxalic dihydrazide was seeded with malonic dihydrazide, but with a negative result.

Fraction 2 yielded a mixture of oxalic and malic hydrazides; fraction 3, impure malic hydrazide; and fractions 4 and 5, pure malic hydrazide melting at 179°. From fraction 6 a few crystals of malic hydrazide were obtained. Fraction 8 afforded an oily hydrazide. The acid, isolated from the ester after saponification, melted at 180–181°, and was unsaturated. It was identified as aconitic acid by optical crystallographic data.

Fraction 9 gave an immediate precipitate of a crystalline hydrazide which after recrystallization from 75% alcohol melted at 182°, and which by optical crystallographic data was further identified as tricarballic trihydrazide.

Citric acid was identified in this fraction by the Stahre<sup>6</sup> reaction, in which pentabromoacetone was formed. Oxalic acid, which was determined by the Arbenz method, amounted to 0.029% on the fresh basis.

**The Acids of Oat Plants.**—The acids, isolated as lead salts from one hundred pounds of oat plants, were brought into a rather concentrated water solution and extracted with ether in a continuous extraction outfit. The ether solution was washed three times with 5-cc. portions of water, and the washings were added to the aqueous solution of acids.

On evaporation, 9.42 g. of yellow, crystalline acids was obtained. This was recrystallized from ethyl acetate. It was mainly aconitic acid, as shown by optical crystallographic data and by the color reaction given by aconitic acid on heating with acetic anhydride.

The acids left after ether extraction were esterified, affording 13.5 g. of crude esters,

<sup>5</sup> Arbenz, *Mitt. Lebensm. Hyg.*, 8, 98 (1917).

<sup>6</sup> Stahre, *Nord. Farm. Tidskrift.*, 2, 41 (1895).

which, on fractionating at 10 mm., yielded the following fractions: 1, 95–125°, 2.0 g.; 2, 125–130°, 8.7 g.; 3, 130–150°, 0.6 g.; 4, 150–160°, 0.5 g.

Fraction 1, on refractionating, gave fraction 1a, 90–110°; 1b, 110–120°; 1c, 120–125°. Hydrazides were prepared from these fractions.

From fraction 1a, a small quantity of malonic hydrazide was separated. There was not enough for a melting point determination, but it was identified by optical crystallographic data.

Fractions 1b, 1c and 2 yielded malic hydrazide. Fraction 3 yielded a mixture of malic and an oily hydrazide, and from fraction 4 a few crystals, identified by the usual methods as citric hydrazide, were obtained. These were associated with the oily hydrazide resulting, no doubt, from aconitic acid which had remained unextracted by ether. The content of oxalic acid, determined by the Arbenz method, was found to be 0.04% on the basis of the fresh material.

**The Acids of Rye Plants.**—The acids from one hundred pounds of rye plants, separated as lead salts and esterified, yielded 43 g. of crude esters.

Fractionation at 10 mm. afforded the following fractions: 1, 90–100°, 1.4 g.; 2, 125–135°, 14.9 g.,  $\alpha_D$   $-9.75^\circ$ ; 3, 135–145°, 2.5 g.; 4, 145–155°, 0.9 g.; 5, 155–165°, 8.8 g.; 6, 165–175°, 4.0 g.; residue, 3.7 g.

The hydrazides were prepared from these fractions.

Fraction 1 afforded an immediate precipitate of a hydrazide, crystallizing in needles and melting at 235°, which corresponds to the hydrazide of oxalic acid. The filtrate from oxalic hydrazide was examined for malonic hydrazide, but none was found.

Fractions 2, 3 and 4 yielded malic hydrazide, melting at 178–179°.

Fraction 5 did not yield a pure crystalline hydrazide. It was, therefore, saponified and the acid separated. The acid was not completely soluble in water. Aconitic acid was identified in the water-soluble portion by optical crystallographic data and the color reaction with hot acetic anhydride. The water-insoluble fraction, a sirupy acid, was not identified.

Fraction 6 afforded an impure hydrazide. This fraction was then redistilled, and the hydrazide obtained from the redistilled fraction, melting at 104–105°, was further shown to be citric trihydrazide by optical crystallographic data. A determination of oxalic acid gave 0.048% on the basis of the fresh material.

### Summary

Barley plants were found to contain aconitic, malic, citric, malonic, tricarballic and oxalic acids. A determination of oxalic acid showed that it contained 0.019% on the basis of the fresh material.

Maize plants were found to contain aconitic, malic, citric, tricarballic and oxalic acids. The percentage of oxalic acid in the fresh material was found to be 0.029.

Oat plants were found to contain aconitic, malic, citric, malonic and oxalic acids. 0.04% of oxalic acid was found in the fresh plants.

Aconitic, malic, citric and oxalic acids were found in rye plants. The undried plants contained 0.048% of oxalic acid.

The occurrence of aconitic acid in these members of the grass family, as well as in sugar cane and sorghum, is noteworthy.

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