tion was apparent, the flask was heated to 45° for four hours. Methanol was added to destroy excess chromic acid, then 200 mg. of zinc dust, and the mixture heated on the steam-bath for one hour. The solution was concentrated in a vacuum. After addition of water, it was extracted with ether. The ether extract was washed with 1 *N* potassium hydroxide and with water, dried over sodium sulfate, and evaporated to dryness. The residue weighed 45 mg. It was refluxed with semicarbazide hydrochloride and potassium acetate in ethanol for an hour, water was added, the precipitate filtered, washed with water and ether, and recrystallized several times from chloroform–ethanol. Seventeen milligrams of pure 3-acetoxydehydroandrosterone semicarbazone was obtained, m. p. 275–278°. A mixed m. p. with an authentic specimen showed no depression.

*Anal.* Calcd. for $C_{22}H_{33}O_3N_3$: C, 68.18; H, 8.59. Found: C, 67.90; H, 8.88.

**$\Delta^5$-3-Acetoxypregnenol-17-one-20 Oxime from (IV).**—The oxime was prepared from the ketone in the usual manner. It was crystallized from benzene–petroleum ether, m. p. 253–256°; mixed m. p. with the 3-acetoxy oxime prepared from the $\Delta^5$-pregnenediol-3,17-one-20 isolated directly from the products of the anil hydrolysis showed no depression.

*Anal.* Calcd. for $C_{23}H_{35}O_4N$: C, 70.92; H, 9.08; N, 3.60. Found: C, 70.71; H, 9.21; N, 3.79.

**Rearrangement of $\Delta^5$-3-Acetoxypregnenol-17-one-20 (IV) to $\Delta^5$-3,17-Dihydroxy-18-keto-chrysopregnene (V).**—Fifty milligrams of $\Delta^5$-3-acetoxypregnenol-17-one-20 was refluxed with 3% methanolic potassium hydroxide for two hours. The alkali was neutralized with carbon dioxide, the solution concentrated, and water added. The precipitate was filtered, washed with water, and crystallized in beautiful hexagonal prisms from acetone, m. p. 278–280°; $[\alpha]^{22}D$ −104° (9.8 mg. in 2.0 cc. dioxane, 1 dm. tube, $\alpha^{22}$ −0.51). A mixed m. p. with $\Delta^5$-3,17-dihydroxy-18-keto-chrysopregnene prepared by the direct hydration of $\Delta^5$-17-ethynyl-androstenediol-3,17[2] showed no depression. The specific rotations of the two substances were the same.

## Summary

$\Delta^5$-17-Ethynyl-androstenediol-3,17 and aniline have been condensed in the presence of ether–boron fluoride and mercuric oxide catalysts to form $\Delta^5$-pregnenediol-3,17-one-20-anil.

The anil is partially hydrolyzed by contact with water to form $\Delta^5$-pregnenediol-3,17-one-20. The acetate of this substance has been oxidized to 3-acetoxydehydroandrosterone, thus proving its pregnane structure.

By saponification of $\Delta^5$-3-acetoxypregnenol-17-one-20 with methyl alcoholic potassium hydroxide a rearranged product is obtained for which the name $\Delta^5$-3,17-dihydroxy-18-keto-chrysopregnene is proposed. The substance is identical with the ketone obtained by the direct hydration of $\Delta^5$-17-ethynylandrostenediol-3,17.

NEW BRUNSWICK, N. J. RECEIVED DECEMBER 13, 1939

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[CONTRIBUTION FROM THE BIOCHEMISTRY DEPARTMENT OF THE UNIVERSITY OF OKLAHOMA MEDICAL SCHOOL]

# The Isolation of Keturonic Acids. II[1,2]

BY L. T. CREWS, J. P. HART AND M. R. EVERETT

The authors are reporting the isolation of crystalline brucine salts of keturonic acids prepared by oxidation of *l*-xylose, *l*-arabinose and *d*-glucosamine, together with a new type of oxidation product, an anhydride of a dicarbonyl sugar, from oxidized levoglucosan. Brucine keturonates also have been prepared from oxidized $\alpha$-*d*-glucoheptose and *l*-fucose but to date these salts have not been separated completely from accompanying non-reducing substances. Ultimate analysis and melting point determinations of the reported preparations are given in Table I and other properties of these substances in Table II.

## Experimental

Oxidation of the 1% carbohydrate solutions, preparation of the mixed barium salts, and isolation of the crystalline brucine salts were conducted according to the general methods of Hart and Everett.[2] Yields of mixed barium salts from 4-g. quantities of the carbohydrates were as follows: *l*-xylose, 4.1 g.; *l*-arabinose, 3.0 g.; and *d*-glucosamine hydrochloride, 1.7 g. Reducing barium salts could not be obtained from oxidized levoglucosan solutions but during concentration of the barium salt solution *in vacuo* the substance designated as ketolevoglucosan crystallized directly. Non-reducing barium salts could be precipitated from the mother liquor by the addition of acetone.

**Brucine-*l*-xyloketuronate.**—A solution of this salt was prepared from 3.75 g. of the mixed barium salts obtained from oxidized *l*-xylose. The reducing brucine salt began to crystallize during concentration of the solution *in vacuo*. After two hours in the refrigerator it was filtered off, washed with alcohol and ether and dried in a desiccator. The yield was 2.5 g. The salt was recrystallized from water.

**Brucine-*l*-araboketuronate.**—A solution of this salt was prepared from 2.9 g. of the mixed barium salts obtained

(1) Aided by a grant from the Research Appropriation of the University of Oklahoma Medical School.

(2) For previous paper in this series see THIS JOURNAL, **61**, 1822 (1939).

TABLE I

| Brucine- | Source | M. p., °C.[a] | Formula | Ultimate analysis, % Found C | Found H | Calcd. C | Calcd. H |
|---|---|---|---|---|---|---|---|
| *l*-Xyloketuronate | *l*-Xylose | 147–148 (dec.) | $C_{23}H_{26}N_2O_4 \cdot C_5H_8O_6 \cdot H_2O$ | 58.11 | 6.12 | 58.32 | 6.30 |
| *l*-Araboketuronate | *l*-Arabinose | 160–161[b] | $C_{23}H_{26}N_2O_4 \cdot C_5H_8O_6 \cdot 2H_2O$ | 56.79 | 6.61 | 56.54 | 6.44 |
| *d*-Chitoketuronate[c] | *d*-Glucosamine·HCl | 177–178 | $C_{23}H_{26}N_2O_4 \cdot C_6H_8O_6 \cdot 1^1/_2H_2O$ | 58.53 | 6.08 | 58.26 | 5.91 |
| Ketolevoglucosan | Levoglucosan | 181–182 (dec.) | $C_6H_8O_5 \cdot {}^1/_2H_2O$ | 42.62 | 5.37 | 42.77 | 5.36 |

[a] All melting points uncorrected with short stem thermometers. [b] Mixed m. p. with brucine *d*-xyloketuronate was 162°. [c] % N: found by ultimate analysis, 4.73; calcd., 4.69.

TABLE II

OPTICAL ROTATIONS, REDUCING EQUIVALENTS AND COLOR REACTIONS[d]

| Brucine- | Color reactions: Naphtho-resorcinol | Bial | Molisch | $[\alpha]^{25}D$[a] | *d*-Glucose equivalent by Sumner's method[b]: Salt | Calcd. for free acid |
|---|---|---|---|---|---|---|
| *l*-Xyloketuronate | Pink | Negative | Brown | −29.5° | 0.23 | 0.81 |
| *l*-Araboketuronate | Pink | Negative | Brown | −13° | .20 | .71 |
| *d*-Chitoketuronate | Orange | Negative | Red purple | −50.5° | .34 | 1.15 |
| Ketolevoglucosan | Red purple | Green[d] | Negative | −62° | [e] | |

[a] For 1% aqueous solutions. [b] For 2 mg. per ml. concentrations of the salts. [c] For crystalline ketolevoglucosan, 0.52; calcd. for the anhydrous substance, 0.55; Folin–Wu equivalent for crystalline ketolevoglucosan, 0.26. [d] Color developed slowly. [e] M. R. Everett and Fay Sheppard, *Proc. Soc. Exp. Biol. Med.*, **34**, 7 (1936).

from oxidized *l*-arabinose. Crystals appeared during concentration *in vacuo*. The mixture was then placed in the refrigerator for twenty-four hours and filtered. A second crystalline fraction obtained by concentrating the mother liquor *in vacuo* proved to be non-reducing. The first fraction was recrystallized several times by dissolving in hot water and adding five volumes of alcohol. The yield of recrystallized salt was 0.78 g. This keturonate was difficult to free from a small contamination of non-reducing salt. With repeated recrystallization, properties of this substance approached those of the brucine *d*-xyloketuronate isolated by Hart and Everett.[3] Ultimate analysis indicated the presence of an extra mole of water of crystallization in the *l*-arabinose derivative and the melting point of this brucine salt was 8° lower than that of brucine-*d*-xyloketuronate. The mixed melting point was intermediate (Table I). If future experimentation confirms these indications of similarity, *d*-xyloketuronic acid will be shown to have the structure postulated by Everett and Sheppard,[3] namely, *l*-xyloketo-5-uronic acid according to the classification of Hart and Everett.[4]

**Brucine-*d*-chitoketuronate.**—A solution of this salt was prepared from 1.95 g. of the mixed barium salts obtained from oxidized *d*-glucosamine hydrochloride. Concentration *in vacuo* gave a crystalline fraction of reducing salt that was filtered off after twenty-four hours in the refrigerator. The salt was recrystallized by dissolving in hot water, adding four volumes of alcohol and placing in the refrigerator for two hours. After filtering, washing with alcohol and ether and drying in the desiccator the yield was 0.52 g. This salt lost no weight upon heating to 175° for several hours *in vacuo* over phosphorus pentoxide. Ultimate analysis indicated a brucine salt of a nitrogen-free anhydroketuronic acid. The authors have therefore provisionally designated the acid as *d*-chitoketuronic acid, with the position of the reducing carbonyl group undetermined.

**Ketolevoglucosan.**—A well-crystallized barium-free substance separated during concentration of the barium salt solution prepared from oxidized levoglucosan. After placing in the refrigerator for several hours it was filtered off, washed twice with cold water and recrystallized from water. The yield was 0.65 g. of recrystallized substance from 4.0 g. of levoglucosan. The substance reduced Sumner's reagent at room temperature, contained no titratable acid group and was very sparingly soluble in water at room temperature (1% aqueous solution could be obtained by warming). It lost no weight upon heating to 156° *in vacuo* over phosphorus pentoxide. Hydrolysis with 0.6 *N* sulfuric acid for 2.5 hours on a boiling water-bath caused a gradual diminution in specific rotation from −62 to −34° together with a 33% decrease in reducing power (Sumner's method). The Sumner/Folin–Wu ratio of reducing equivalents also changed from 1.99 to 1.45, indicating hydrolysis of the anhydride linkage.[3,5] Subsequent oxidation of the hydrolyzate with bromine for forty-eight hours at 25° caused a 50% loss of reduction, which indicated the appearance of an aldose radical during hydrolysis. The original reducing substance was not affected by similar bromine oxidation. The derivation and properties of this product of levoglucosan oxidation indicate that it is the anhydride of a dicarbonyl sugar.[6]

(3) M. R. Everett and Fay Sheppard, University of Oklahoma Medical School Monograph, "Oxidation of Carbohydrates in Acid Solution," 1936.

(4) J. P. Hart and M. R. Everett, "A Suggested Nomenclature for Keturonic Acids," Abst. of Papers, 96th Meeting of Am. Chem. Soc.

(5) M. R. Everett and Fay Sheppard, THIS JOURNAL, **60**, 1792 (1938).

(6) In view of the present ambiguity in dicarbonyl sugar nomenclature and the possible need for naming several new dicarbonyl sugars whose preparation we are attempting, the following suggestions are made:

1. That the group name for sugars with two potential aldehyde radicals be *alduroses* and that their configurations be referred to the parent polyhydric alcohols. Example: *d-sorbitoaldurose* for the identical structures, 6-aldehydo-*d*-glucose and 6-aldehydo-*d*-gulose.

2. That the group name for sugars with one potential aldehyde

Determination of the position of the reducing carbonyl radical necessitates the preparation of larger quantities of ketolevoglucosan.

**Molecular Weights of the Anhydro Derivatives.**—The ordinary cryoscopic method for determination of molecular weight could not be employed due to the slight solubility of ketolevoglucosan in ordinary solvents and the limited quantities of *d*-chitoketuronic acid available. Barger's method as modified by Rast[7] gave satisfactory results in control determinations of the molecular weight of glucose but it proved entirely inapplicable to the anhydro derivatives under investigation. A series of dilutions lost water to an intermediate glucose standard, probably because of gradual association or polymerization of the anhydro derivatives. Rast's camphor method[8] gave the following molecular weight values: 330 for ketolevoglucosan, 1100 for brucine *d*-chitoketuronate and 350 for pure levoglucosan. These values are approximately dimolecular but levoglucosan has been shown by the ordinary cryoscopic method to be monomolecular. A common interpretation would imply that the anhydro derivatives reported here are also monomolecular.

and one potential ketone radical be *keturoses* and that their configurations be referred to the parent ketoses, as in the suggested nomenclature for keturonic acids.[4] Examples: *l-sorbo-6-urose* for the identical structures, *d*-gluconose and *l*-idonose; and *d-fructo-1-urose* for *d*-glucosone.

This terminology avoids multiple naming, is generally applicable and suggests functional relations of these sugars to their uronic acid oxidation products. Present indications are that the dicarbonyl sugar anhydride isolated by the authors is 1,6-anhydro-*l*-sorbo-6-urose (1,6-anhydro-gluconose) but the temporary designation as ketolevoglucosan is preferred until the position of the second carbonyl radical is proven.

(7) Abderhalden, "Handbuch der biologischen Arbeitsmethoden," 1928, Abt. III, Teil AI, p. 751.

## Summary

1. *l*-Xyloketuronic acid and *d*-chitoketuronic acid have been isolated as crystalline brucine salts.

2. The keturonic acid similarly isolated from oxidized *l*-arabinose solutions resembles *d*-xyloketuronic acid closely but complete identity of these acids has not been proven.

3. An anhydride of a dicarbonyl sugar has been isolated from oxidized levoglucosan solutions.

OKLAHOMA CITY, OKLA. RECEIVED NOVEMBER 23, 1939

(8) Kamm, "Qualitative Analysis," John Wiley and Sons, New York, N. Y., 1932, p. 131.

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[CONTRIBUTION FROM THE PEARSON MEMORIAL LABORATORY OF TUFTS COLLEGE]

# The Action of Aromatic Amines on 3-Nitro-6-iodonitrostyrene

BY DAVID E. WORRALL AND FREDERICK BENINGTON

Whereas nitration of 2-chloro- and 2-bromonitrostyrene does not entirely inhibit its addition capacity for certain aromatic amines,[1] the halogenated derivatives are not as reactive as nitrostyrene itself. We now find that the analogous iodo derivative closely parallels nitrostyrene in all its reactions with nitrogen bases.

## Experimental

**α-Nitro-β-(2-iodophenyl)-ethylene.**—Iodobenzaldehyde prepared through the diazo reaction[2] from *o*-aminobenzaldehyde[3] was condensed with nitromethane in the presence of triethylamine; yield, after steam distillation, 65–70%. It separated from alcohol as pale yellow needles, m. p. 113–114°. On oxidation with potassium permanganate it gave 6-iodo-3-nitrobenzoic acid[4] which recrystallized from alcohol in long yellow needles, m. p. approx. 190°.

*Anal.* Calcd. for $C_8H_6INO_2$: C, 34.8; H, 2.2. Found: C, 35.2; H, 2.5.

**α-Nitro-β-(6-iodo-3-nitrophenyl)-ethylene.**—Prepared by nitration of the above compound using fuming acid, it was obtained from alcohol as tiny pale yellow needles, m. p. 145–146°.

*Anal.* Calcd. for $C_8H_5IN_2O_4$: C, 30.0; H, 1.5. Found: C, 30.2; H, 1.3.

The product from bromination of the iodo compound separated as an oil on treatment with a warm alcoholic solution of potassium acetate. On mixing with fuming nitric acid, it solidified and was recrystallized from alcohol in yellow prismatic needles, m. p. 136–137°.

*Anal.* Calcd. for $C_8H_4BrIN_2O_4$: C, 24.0; H, 1.0. Found: C, 24.4; H, 1.2.

It was observed to form an addition compound with *p*-toluidine, but was not further investigated.

The same procedure previously developed[1] was used for the preparation of the addition compounds. The products, yellow or orange-yellow and deeper in color than the parent nitrostyrene, were recrystallized from alcohol, separating as narrow plates or prismatic needles. The melting points frequently were not sharp and were always accompanied by decomposition so that they varied with the rate of heating. Difficulty was experienced in preparing the *o*-toluidino compound as it did not always separate even on long standing. The anilino derivative formed only after several days. The *o*- and *m*-anisidine derivatives formed more readily than the corresponding toluidine. The hydroxylamino and semicarbazido products were colorless.

(1) Worrall, THIS JOURNAL, **60**, 2845 (1938).
(2) Patterson, *J. Chem. Soc.*, **69**, 1006 (1896).
(3) Bamberger, *Ber.*, **60**, 319 (1927).
(4) Goldstein and Grampoloff, *Helv. Chim. Acta*, **13**, 310 (1930).