Δ^{16}-Allopregnene-3α,15β-diol-20-one Diacetate (XVI).—The side chain degradation of 4.38 g. of the diol XV was carried out exactly as described above for digitogenin and after recrystallization from methanol led to 1.79 g. of the unsaturated ketone XVI, m.p. 142–143°, $[\alpha]_{\mathrm{D}}$ −152°, $\lambda^{\mathrm{EtOH}}_{\mathrm{max}}$ 231 mμ, log ε 4.00.

Anal. Calcd. for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found: C, 71.74; H, 8.70.

Allopregnane-3α,15β-diol-20-one (XVIIb).—The above unsaturated ketone XVI (265 mg.) was hydrogenated as described for XI and yielded the saturated diacetoxyketone XVIIa as a colorless glass (no high selective ultraviolet absorption) which resisted all attempts at crystallization. Consequently, the material was directly saponified with 2% methanolic potassium hydroxide[47] to furnish a colorless solid (223 mg.) which was recrystallized from chloroform–hexane; m.p. 239–241°, $[\alpha]_{\mathrm{D}}$ +59° ($CHCl_3$), +84° (pyridine).

Anal. Calcd. for $C_{21}H_{34}O_3$: C, 75.40; H, 10.25. Found: C, 75.37; H, 10.30.

Allopregnane-3,15,20-trione (XVIII).[57]—A solution of 26 mg. of allopregnane-3α,15β-diol-20-one (XVIIb) in 2.5 cc. of glacial acetic acid was treated dropwise over a period of 15 minutes with a 0.5% solution of chromium trioxide in 95% acetic acid until a definite excess of the reagent was noted (15.32 mg., theory 10.4 mg.). The excess reagent was destroyed with ethanol and the product was isolated by means of chloroform and crystallized; m.p. 222–223°, $[\alpha]_{\mathrm{D}}$ +137°. The mutarotation (final value +55°) was determined by dissolving 6 mg. of the substance in 1.2 cc. of methanol, determining the rotation and then adding 0.02 cc. of 1.35 *N* methanolic potassium hydroxide solution and measuring the rotation at intervals until equilibrium was reached (*ca.* 15–20 hours). The substance proved to be identical by mixture melting point, infrared comparison, rotation and rate of mutarotation with samples prepared at the Squibb Institute[42,43] by two different routes from 15β-hydroxyprogesterone (XXI).

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.72; H, 8.97.

22a,25a,5α-Spirostane-2β,3β-diol (IX).[26]—Gitogenin (IV) was converted into the 2,3-dimesylate with mesyl chloride in pyridine solution and recrystallized from methanol; m.p. 238–240°, $[\alpha]_{\mathrm{D}}$ −79°.

One gram of the dimesylate was heated in a sealed tube at 110° for 24 hours with 1.5 g. of sodium iodide and 20 cc. of dry acetone, water was added and the product (0.67 g.) was isolated by extraction with chloroform and recrystallized from acetone. The resulting **Δ^2-22a,25a,5α-spirosten (VIII)**, m.p. 182–184°, was shown to be identical by mixture melting point and infrared comparison with a specimen prepared[20] from tigogenin.

To a stirred solution of 400 mg. of the olefin VIII in 75 cc. of glacial acetic acid was added 270 mg. of freshly prepared silver oxide and the mixture was refluxed until clear. A trace (6.5 mg.) of acetic anhydride was added (to remove excess water formed during the above reaction), the solution was cooled to 20° and 255 mg. of iodine dissolved in acetic acid was added with stirring and heating was continued at 90–95° for 3 hours. The coagulated silver iodide was filtered, the filtrate evaporated to dryness *in vacuo*, taken up in hot methanol, boiled with a small amount of potassium bromide and again filtered. Saponification of the intermediate acetate was completed by adjusting the *p*H of the solution to 10 with methanolic potassium hydroxide solution and leaving overnight at room temperature. Dilution with water, extraction with chloroform, washing, drying and evaporation furnished 390 mg. of crude glycol, m.p. 218–230°. The analytical sample was purified by chromatography through a short alumina column and recrystallization from hexane–acetone and from methanol; m.p. 223–224° (flat plates from hexane–acetone) or 235–237° (needles from methanol) (Kofler), $[\alpha]_{\mathrm{D}}$ −49°, rate of lead tetraacetate oxidation[58] in acetic acid solution,[19] $k = 31.9 \times 10^{-3}$ l.-mole^{-1} sec.$^{-1}$.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.61; H, 10.29.

Oxidation of 40 mg. of the diol IX with chromium trioxide in acetic acid led to 20 mg. of gitogenic acid (III), m.p. and mixture m.p. 243–245°.

The acetonide was prepared by the acetone–*p*-toluenesulfonic acid method[20] and eluted from alumina with benzene; m.p. 239–241° (after recrystallization from acetone), depressed to 205–230° when mixed with the starting glycol IX, $[\alpha]_{\mathrm{D}}$ −31°.

Anal. Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 76.41; H, 10.36.

DETROIT, MICHIGAN

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[57] This experiment was carried out by Dr. Josef Fried and Miss Emily F. Sabo of the Squibb Institute for Medical Research (*cf.* ref. 7).

[58] The value of 3.69×10^{-3} reported earlier (ref. 19) was obtained with a product which proved to be chiefly gitogenin (see footnote in ref. 6).

[RESEARCH AND DEVELOPMENT DEPARTMENT, U. S. NAVAL POWDER FACTORY]

Decarboxylation *via* the Acid Chloride of Penta-*O*-acetyl-D-gluconic Acid[1a,1b]

BY F. A. H. RICE

RECEIVED JANUARY 23, 1956

It has been found that the acid chloride of penta-*O*-acetyl-D-gluconic acid reacts with silver oxide and bromine in carbon tetrachloride solution to yield carbon dioxide and *aldehydo*-1-bromo-D-arabinose penta-*O*-acetate. The reaction in terms of carbon dioxide evolution is essentially quantitative. Although the reaction yields the same product that is obtained when a silver salt is treated with bromine, the use of the acid chloride eliminates the difficulties experienced in drying the silver salts.

In previous communications[2] it was shown that the silver salts of either alginic acid or penta-*O*-acetyl-D-gluconic acid would, when treated with bromine, react in the same manner as aromatic or aliphatic acids.[3] That is, the carboxyl group, which is lost as carbon dioxide, is substituted by bromine. The reaction is very sensitive to traces of water, the presence of which leads to the isolation of the unreacted free acid. The silver salts are also quite sensitive to heat and thus are difficult to dry thoroughly. In attempts to use acetic anhydride as a drying agent, it was observed that a carbon tetrachloride solution of the anhydride reacted with dry silver oxide and bromine to produce two moles of carbon dioxide per mole of acetic anhydride. When a carbon tetrachloride solution of acetyl chloride was treated with dry silver oxide and bromine, one

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[1] (a) Published with the permission of The Bureau of Ordnance Navy Department. The opinions and conclusions are those of the author. (b) Presented in part at the 128th Meeting of the American Chemical Society, Minneapolis, Minn., September, 1955.

[2] F. A. H. Rice, Abstracts of the 127th Meeting of the Am. Chem. Soc., Cincinnati, O., **11E**, (1955); F. A. H. Rice and A. H. Johnson, THIS JOURNAL, **78**, 428 (1956).

[3] J. Kleinberg, *Chem. Revs.*, **40**, 381 (1947).

mole of carbon dioxide per mole of acetyl chloride was evolved.

It seemed that this reaction might provide a method of degrading the carbon chain of a sugar in non-aqueous media, which would not involve the use of the sensitive silver salts. Unlike the silver salts the anhydrous acid chlorides should be prepared with little difficulty.

Accordingly the acid chloride of penta-*O*-acetyl-D-gluconic acid was prepared by allowing penta-*O*-acetyl-D-gluconic acid to react with thionyl chloride. The acid chloride, which was a crystalline compound of m.p. 65° and $[\alpha]^{20}\mathrm{D}$ −2.6° (CCl_4, *c* 5.6), was dissolved in carbon tetrachloride which had been dried over phosphorus pentoxide and freshly distilled. The solution was then added to a stirred suspension of dried silver oxide in carbon tetrachloride–bromine. Carbon dioxide was evolved when the solution was heated to reflux temperature. The carbon dioxide was washed out of the reaction flask with nitrogen and was then freed of bromine vapor by passing the gases through two Dry-Ice traps; the carbon dioxide was absorbed in a 0.2 *N* barium hydroxide solution. The amount of carbon dioxide liberated was determined by titration with 0.1 *N* hydrochloric acid.[4] Table I shows the results obtained on a series of 1-g. to 13-g. samples. We believe that the results indicate that essentially a quantitative decarboxylation takes place under the above conditions.

TABLE I

AMOUNT OF CARBON DIOXIDE LIBERATED AND YIELD OF *aldehydo*-1-BROMO-PENTA-*O*-ACETYL-D-ARABINOSE FROM THE REACTION OF THE ACID CHLORIDE OF PENTA-*O*-ACETYL-D-GLUCONIC ACID WITH SILVER OXIDE AND BROMINE

Wt. of sample, g.	CO_2 equivalent, ml. 0.1 *N* HCl Calcd.	Found	Yield,[a] g.
1.88	92.5	93	1.8
2.94	145	140	2.7
14.27	705	548	13.5
3.41	164	157	3.2
5.30	249	240	5.0
2.31	114	125	2.1

[a] Yields obtained after the crude *aldehydo*-1-bromopenta-*O*-acetyl-D-arabinose was dissolved in 10 ml. of benzene and precipitated at 0° by the addition of 100 to 200 ml. of petroleum ether (b.p. 30–60°).

The carbon tetrachloride solution, after filtration and concentration, yielded a sirup which analysis indicated was *aldehydo*-1-bromo-D-arabinose penta-*O*-acetate and which on reaction with silver acetate gave *aldehydo*-D-arabinose hexa-*O*-acetate.

The optical rotation of the *aldehydo*-1-bromopenta-*O*-acetyl-D-arabinose is low compared to the corresponding crystalline compound prepared from L-arabinose.[5]

It was suggested[2] that the low optical rotation of of the product obtained by the decarboxylation of the silver salt of penta-*O*-acetyl-D-gluconate with bromine, could be accounted for either by assuming the reaction to proceed *via* the ionization of the carboxyl carbon, followed by the formation of the C–Br bond or by a free radical intermediate[6] Either reaction would yield a mixture of α- and β-forms. It is also possible that although only one form is produced by the reaction this form is racemized by the silver bromide present.[7]

The simplest picture of decarboxylation *via* the acid chloride is that the acid chloride first reacts with the silver oxide. The reaction then proceeds in the same manner as it would with the silver salt of the acid.

The problem is under investigation.

Experimental

Preparation of the Acid Chloride of Penta-*O*-acetyl-D-gluconic Acid.—Penta-*O*-acetyl-D-gluconamide (m.p. 183°, $[\alpha]^{22}\mathrm{D}$ +23°) was prepared after the manner of Robbins and Upson.[8] The free acid was obtained by treating the amide with nitrosyl chloride[9] in alcohol-free chloroform. The penta-*O*-acetyl-D-gluconic acid was crystallized from toluene[10] to yield the anhydrous acid, m.p. 110°, $[\alpha]^{20}\mathrm{D}$ +11° (alcohol-free $CHCl_3$, *c* 2) in agreement with the values reported in the literature.

Anal. Calcd. for $C_{16}H_{22}O_{12}$: C, 47.43; H, 5.46. Found: C, 47.45; H, 5.44.

Ten grams of penta-*O*-acetyl-D-gluconic acid was dissolved in 25 ml. of thionyl chloride and heated at the boiling point for 30 min. The excess thionyl chloride was removed at 30–40° under reduced pressure. The sirup which resulted, crystallized when it was dried under high vacuum in a desiccator over solid potassium hydroxide. The compound had m.p. 65°, $[\alpha]^{20}\mathrm{D}$ −2.6° (CCl_4, *c* 5.6), after recrystallization from a mixture of ether and petroleum ether (b.p. 30–60°).

Anal. Calcd. for $C_{16}H_{21}O_{11}Cl$: C, 45.23; H, 4.95; Cl, 8.35. Found: C, 45.50; H, 4.96; Cl, 8.30.

Decarboxylation *via* the Acid Chloride of Penta-*O*-acetyl-D-gluconic Acid.—The acid chloride of penta-*O*-acetyl-D-gluconic acid (2.81 g.) was dissolved in 25 ml. of carbon tetrachloride which had been freshly distilled from phosphorus pentoxide. The solution was then added through a dropping funnel to a stirred suspension of 5 g. of silver oxide (which had been dried for several days under high vacuum at the temperature of boiling water) in 30 ml. of carbon tetrachloride. One milliliter of bromine[11] in 10 ml. of carbon tetrachloride was then added and the stirred solution heated to reflux temperature. The carbon dioxide liberated was washed out of the reaction flask by a stream of nitrogen (which had been freed of traces of carbon dioxide and water, respectively, by passing through a saturated aqueous solution of sodium hydroxide and *concd.* sulfuric acid) and after passing through two Dry Ice traps to remove any bromine vapor, the carbon dioxide was absorbed in a 0.2 *N* barium hydroxide solution. After 4 hours the evolution of carbon dioxide had ceased. The amount of carbon dioxide evolved from the reaction was determined by titration with 0.1 *N* hydrochloric acid; calcd. 0.1 *N* hydrochloric acid equivalent to the carbon dioxide liberated from 2.81 g. of the acid chloride 139 ml., found 140 ml.

In order to maintain an excess of bromine (determined visually) additional quantities of bromine dissolved in carbon tetrachloride were added from time to time. At the end of 4 hours the solution was filtered and concentrated to dryness at 30–40° under reduced pressure; yield 2.9 g. of

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(4) R. B. Schmitt and J. B. Niederl, *Mikrochem.*, **24**, 59 (1938); F. Pregl and J. Grant, "Quantitative Organic Microanalysis," the Blakiston Company, Philadelphia, Penna., 1946, p. 61.

(5) G. E. Felton and W. Freudenberg, THIS JOURNAL, **57**, 1637 (1935); M. L. Wolfrom and M. Konigsberg, *ibid.*, **60**, 288 (1938).

(6) C. C. Price, "Mechanisms of Reactions at Carbon-Carbon Double Bonds," Interscience Publishers Inc., New York, N. Y., (1946), p. 55; M. Rottenberg, *Experientia*, **7**, 432 (1951).

(7) C. L. Arcus, A. Campbell and T. Kenyon, *J. Chem. Soc.*, 1510 (1949).

(8) G. B. Robbins and F. W. Upson, THIS JOURNAL, **60**, 1788 (1938).

(9) M. L. Wolfrom, M. Konigsberg and D. I. Weisblat, *ibid.*, **61**, 574 (1939).

(10) R. T. Major and E. M. Cook, *ibid.*, **58**, 2474 (1936).

(11) Merck Reagent Grade, which was freshly distilled from phosphorus pentoxide and dissolved in the ratio of 1:10 by volume in carbon tetrachloride (distilled from phosphorus pentoxide). The carbon tetrachloride solution was stored over a few lumps of solid barium oxide.

a thick sirup. The sirup was redissolved in 100 ml. of alcohol-free chloroform and shaken, first, with an ice-cold half-saturated aqueous sodium bicarbonate solution (50 ml. × 2) and then with ice-water (50 ml. × 2) and dried over anhydrous sodium sulfate. Concentration of the chloroform solution at 30–40° under reduced pressure yielded a solid colorless glassy substance $[\alpha]^{21}_{\mathrm{D}}$ +11.3° (alcohol-free $CHCl_3$, *c* 6).

Anal. Calcd. for $C_{15}H_{21}O_{10}Br$: C, 40.84; H, 4.80; Br, 18.12. Found: C, 40.90; H, 4.75; Br, 18.1.

Preparation of *aldehydo*-D-Arabinose Hexa-*O*-acetate.—The sirup (1 g.) obtained by the reaction of the acid chloride described above was dissolved in 10 ml. of toluene and added to a suspension of silver acetate (1 g.) in boiling toluene.[12] The mixture was immediately allowed to cool to room temperature and after standing at room temperature overnight filtered under suction through a fritted glass filter. Petroleum ether (b.p. 30–60°) was added to the filtrate and the gummy precipitate which resulted removed by decantation. After washing with ice-cold half-saturated aqueous sodium bicarbonate solution, the gum was crystallized from ethanol. Constants found were: m.p. 89–90°, $[\alpha]^{21}_{\mathrm{D}}$ +30° ($CHCl_3$, *c* 2) in accord with the values reported for *aldehydo*-D-arabinose hexa-*O*-acetate.[13,14] The compound showed no depression in melting point when mixed with an authentic sample of *aldehydo*-D-arabinose hexa-*O*-acetate prepared from D-arabinose after the manner of Wolfrom.[14]

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[12] R. S. Tipson, *J. Biol. Chem.*, **130**, 55 (1939).

[13] E. M. Montgomery, R. M. Hann and C. S. Hudson, This Journal, **59**, 1124 (1937).

[14] M. L. Wolfrom, *ibid.*, **57**, 2498 (1935).

INDIAN HEAD, MD.

[CONTRIBUTION FROM THE KERCKHOFF LABORATORIES OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

Preparation of N-Substituted 1-Amino-1-deoxy-D-arabino-hexuloses[1] of Arginine, Histidine and Lysine.[2] II

BY PETER H. LOWY AND HENRY BORSOOK

RECEIVED JANUARY 9, 1956

The preparation of N-substituted 1-amino-1-deoxy-D-arabino-hexuloses of L-arginine, L-histidine and L-lysine is described. The parent sugar was D-glucose. Like the derivatives of the neutral and acidic amino acids, they reduce ferricyanide in 0.1 *N* alkali rapidly at room temperature, give low values for sugar with the anthrone reagent, nearly equivalent values for sugar in boiling alkaline solution with ferricyanide and stimulate incorporation *in vitro* of labeled amino acids into the proteins of rabbit reticulocytes.

We have reported previously the isolation from hog liver of compounds of the N-substituted 1-amino-1-deoxy-2-ketohexose type, their ability to stimulate incorporation *in vitro* of amino acids into the proteins of rabbit reticulocytes[3] and the synthesis of such compounds by condensation of hexoses with L-alanine, L-aspartic acid, L-glutamic acid, glycine, L-leucine, L-serine, L-threonine and L-valine.[4]

These compounds may be viewed as the Amadori rearrangement products of the N-glycosylamino acids. The amino acid-deoxy-fructoses of the neutral and acidic amino acids had been separated from the parent sugar and amino acid by adsorption on Dowex-50 (H^+ form) resin, removal of the sugar by elution with water and of the amino acid-deoxy-fructose with trichloroacetic acid whose concentration was varied from 0.05 to 0.50 molar depending on the constituent amino acid.[4] This chromatographic procedure was unsatisfactory for isolation of the amino acid-deoxy-fructoses of arginine, histidine and lysine from the respective reaction mixtures. These compounds are adsorbed so strongly on Dowex-50 (H^+ form) resin that, to elute them, 4 *N* hydrochloric acid was necessary. This also eluted the parent amino acid without sufficient separation from the amino acid-deoxy-fructose. The following procedure was successful. The reaction mixture was passed through a column of Amberlite IRC-50 (H^+ form); the sugar passed through and was completely removed with water, the amino acid-deoxy-fructose and the free basic amino acid were retained on the column from which both together were eluted with acetic acid, 1 *N* for the arginine and lysine, 0.1 *N* for the histidine preparations. The separation of the amino acid-deoxy-fructose from the corresponding free amino acid was achieved by adsorption on a cellulose column and elution with pyridine–water (3:1).

After drying by lyophilization, the amino acid-deoxy-fructoses were dissolved in absolute methanol from which they were precipitated by dry ether. All three compounds were thus obtained as white powders. L-Arginino-deoxy-fructose and L-histidino-deoxy-fructose can be dried to constant weight at 80° *in vacuo*. L-Lysino-deoxy-fructose begins to brown after 45 minutes at 80° *in vacuo*. We have not determined which nitrogen atom of the dibasic amino acids is linked to the carbohydrate; furthermore, the anomeric configuration and the ring structure of the sugar moiety have not been ascertained.

Most of the amino acid-deoxy-fructoses begin to brown on the melting stage at about 120° when they give off a pleasant and characteristic odor, which is different from caramel and reminiscent of freshly baked cereal foods. On further heating all the compounds darken and decompose.

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[1] These compounds have been called N-substituted 1-amino-1-deoxy-2-ketohexoses and for convenience fructose amino acids, *e.g.*, fructose alanine. To comply with the Rules of Carbohydrate Nomenclature [*Chem. Eng. News*, **31**, 1776 (1953)], we now use the generic name N-substituted 1-amino-1-deoxy-D-arabino-hexulose. We designate the compound with a specific amino acid, *e.g.*, 1-(L-alanino)-1-deoxy-D-fructose. As a shorter generic name we use the term amino acid-deoxy-fructose and as a short specific name, *e.g.*, alanino-deoxy-fructose.

[2] This work was aided by a contract between the Atomic Energy Commission and the California Institute of Technology. It was also supported by a research grant from the National Institutes of Health, United States Public Health Service, by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council and by a grant from Eli Lilly and Co.

[3] H. Borsook, A. Abrams and P. H. Lowy, *J. Biol. Chem.*, **215**, 111 (1955).

[4] A. Abrams, P. H. Lowy and H. Borsook, This Journal, **77**, 4794 (1955).