copper(II) complexes, are similar in gross detail to the corresponding hydroxyanthraquinone curves.

Experimental

The experimental procedure for absorption spectra measurements[12] was the same as that described in our earlier publication.[10] The concentrations of the *n*-butanol solutions ranged from 0.00003 to 0.0002 *M*. Values of the extinction coefficients were calculated by Beer's law.

Catalytic Behavior of the Inner Complex[10]

It was found that certain of the inner complexes prepared could catalyze the chemiluminescent oxidation of luminol (3-amino-phthalhydrazide) by hydrogen peroxide. The cobalt(II) and copper(II) complexes of 1-hydroxyanthraquinone and the copper(II) complexes of 2-acetylalizarin and 3-nitroalizarin all exhibit decreasing degrees of catalytic activity, in that order. The free quinones are inactive.

The 1-hydroxyanthraquinone-cobalt(II) complex produces a bluish violet-white luminescence, lasting over three hours, but not as intense as the light produced with the cobalt(II) complex of phthiocol. The copper(II) complex produces a blue-white light of three-hour duration, but of lower intensity than either the 1-hydroxyanthraquinone cobalt(II) complex or the phthiocol copper(II) complex. Solubility and stability of the complex appear to be the governing factors in the catalysis of chemiluminescent oxidation.

(12) With H. M. Haendler.

Summary

1. The preparation and properties of the cobalt(II), copper(II), magnesium, manganese(II), nickel(II), diaquonickel(II), and uranyl inner complexes of 1-hydroxyanthraquinone, as well as the copper(II) inner complexes of 2-acetylalizarin and 3-nitroalizarin have been described.

2. The absorption spectra of these inner complexes in *n*-butanol solution have been determined in the visual region.

3. The color of the complex is due to the chelation of the quinone with the metal.

4. The cobalt(II) and copper(II) inner complexes of 1-hydroxyanthraquinone and the copper(II) inner complexes of 2-acetylalizarin and 3-nitroalizarin catalyze the chemiluminescent oxidation of luminol by hydrogen peroxide.

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[CONTRIBUTION FROM THE IOWA AGRICULTURAL EXPERIMENT STATION]

The Molecular Weights of the Schardinger Alpha and Beta Dextrins[1]

BY DEXTER FRENCH AND R. E. RUNDLE

The work of Freudenberg[2] and others[3] indicates that the Schardinger dextrins are composed solely of glucose residues bonded by α-1,4-glucosidic linkages as in starch. Though these dextrins are of much lower molecular weight than starch, they are completely non-reducing. The only structure which appears to be consistent with these chemical properties is a cyclic structure.[4]

Several molecular species with the chemical properties given above are known to occur in the dextrin mixture obtained by the *B. macerans* enzymolysis of starch. The main components of the mixture have been separated and characterized by Freudenberg and Jacobi.[5] The molecular weights which these authors suggest for these species were determined by cryoscopic methods, but since these dextrins are of comparatively high molecular weight and are very difficult to free from low molecular weight impurities (solvent of crystallization and inorganic ash), the cryoscopic molecular weights can be expected to be but rough approximations.

A method better adapted to the determination of the molecular weights of high molecular weight crystalline compounds is X-ray diffraction combined with crystal density measurement. In this method low molecular weight impurities are of minor importance, and their contribution to the crystal density can be determined and corrected for with satisfactory accuracy.

By this method we have found Schardinger's

(1) Journal Paper No. J-979 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 639. Supported in part by a grant from the Corn Industries Research Foundation.

(2) K. Freudenberg, G. Blomquist, L. Ewald and K. Soff, *Ber.*, **69**, 1258 (1936).

(3) J. C. Irvine, H. Pringsheim and J. MacDonald, *J. Chem. Soc.*, **125**, 942 (1924).

(4) K. Freudenberg, *Ann. Rev. Biochem.*, **8**, 81 (1939).

(5) K. Freudenberg and R. Jacobi, *Ann.*, **518**, 102 (1935).

α-dextrin to contain six glucose residues per molecule and the β-dextrin to contain seven glucose residues per molecule, whereas Freudenberg and Jacobi[5] report five and six, respectively.[6] That the cryoscopic values are too low is not unexpected.

Naming of the Compounds.—The chemical properties of the α- and β-dextrins together with the molecular weights reported here leave no doubt as to the gross structure of these compounds. Their present names have no structural significance, nor have the names been standardized in usage.[7] The term amylose has been generally applied to those compounds possessing the α-1,4-glucosidic linkage of starch. The prefix *cyclo* adequately describes the distinguishing structural characteristics of this class of compounds, and the usual Greek terms can be used to identify the members of the class through the number of glucose residues in the ring. We therefore propose that Schardinger's α-dextrin be named cyclohexaamylose, and the β-dextrin be named cycloheptaamylose.

Experimental

Preparation of the Cycloamyloses.—The cycloamyloses were prepared by enzymolysis of potato starch as described by Schoch,[8] and were separated and purified by the method of Freudenberg and Jacobi.[5] The authors are indebted to Drs. T. J. Schoch and R. W. Kerr of Corn Products Refining Company for part of the material used in this investigation. Crystals of cyclohexaamylose were prepared by adding 95% ethanol to a hot, 30% aqueous solution of the cyclohexaamylose until the concentration of alcohol reached 60–80%. On cooling, crystals deposited in large, glass-clear, orthorhombic prisms. For anhydrous cyclohexaamylose $[\alpha]_D$ is 151.4 ± 0.5°. Crystals of cycloheptaamylose were prepared by evaporation of a saturated, aqueous solution at room temperature. For anhydrous cycloheptaamylose $[\alpha]_D$ is 161.9 ± 0.5°. The identity of these compounds with the α- and β-dextrins was confirmed by the formation of characteristic iodine addition products; blue-black hexagonal prisms and greenish needles from the cyclohexaamylose, and red-brown prisms from the cycloheptaamylose.

X-Ray Diffraction Patterns.—Twenty degree oscillation patterns were taken about all three axes of both crystals reported here. They were obtained using Ni filtered Cu Kα radiation and a cylindrical camera of 5-cm. radius. Reciprocal lattice goniometer patterns[9] were also made with Cu Kα radiation and a sample to film distance of $2\sqrt{3}$ cm., with the axis of rotation inclined 60° to the X-ray beam.

Cyclohexaamylose.—The density, measured by flotation in a mixture of chloroform and toluene, was 1.436 ± 0.004. The amount of volatile matter (water and/or alcohol of crystallization) amounted to 12.2 ± 0.1% of the weight of the crystals, and an ash determination showed the presence of 1.63 ± 0.02% inorganic impurity. The average value for the carbohydrate density is then 1.237 ± 0.005.

The primitive translations of the lattice, as obtained by layer line separations on oscillation patterns, are a_0 = 15.49 Å.; b_0 = 24.06 Å.; c_0 = 13.93 Å. The volume of the orthorhombic unit is 5195 cu. Å. Since the molecular weight of a glucose residue is 162.1, the number of glucose residues per unit cell is

$$\frac{5195 \times 1.237 \times 6.06 \times 10^{-1}}{162.1} = 24.02 \cong 24$$

The crystal has orthorhombic symmetry as shown by oscillation and goniometer diffraction patterns. Since the molecules are optically active only those space groups isomorphous with the point group V-222 are allowed. Many reflections from all types of planes eliminate all space groups based on any but the primitive orthorhombic lattice. The possible space groups are then V^1-$P222$, V^2-$P222_1$, V^3-$P2_12_12$, and V^4-$P2_12_12_1$. Examination of the ($h00$), ($0k0$), and ($00l$) reflections on intense oscillation and goniometer patterns reveals that all odd orders of these reflections are missing. It was possible to observe 16 orders of ($h00$), 26 orders of ($0k0$), and 14 orders of ($00l$), so that the space group is doubtless V^4-$P2_12_12_1$. This space group requires a multiple of four molecules per unit cell regardless of molecular symmetry. The number of glucose residues per molecule is then 6, 3, or 2. The latter two possibilities may be excluded on the basis of the chemical behavior of this compound and the fact that a ring of three or two glucose residues is sterically impossible. Moreover, it is quite unusual for a unit cell to contain a number of molecules greater than that required by the crystal symmetry.[10]

Cycloheptaamylose.—Crystals were obtained having a density of 1.444 ± 0.004 and a water content of 14.18 ± 0.02%. The carbohydrate density is therefore 1.240 ± 0.004. The crystals exhibit monoclinic symmetry as shown by oscillation and reciprocal lattice patterns. The unit translations determined by layer line separations are a_0 = 15.27 Å.; b_0 = 10.24 Å.; c_0 = 20.93 Å. The monoclinic angle β = 68.0° ($\sin\beta$ = 0.9272) was determined by direct measurement on reciprocal lattice patterns and confirmed by Laue patterns. The volume of the unit cell is

(6) O. Kratky and B. Schneidmesser [*Ber.*, **71**, 1413 (1938)], claim to have found five glucose residues per molecule of the alpha-dextrin, but their work contains a discrepancy. The possible space groups which they find for their crystal are V^3 — $P2_12_12$ and V^4 — $P2_12_12_1$. V^4 requires four asymmetric molecules per cell, and V^3 requires four asymmetric molecules or two molecules possessing a two-fold axis. They find for their unit cell two molecules of five glucose residues each, molecules which cannot possess the required symmetry.

(7) Schardinger called these compounds "crystallized amylose" and "crystallized amylopectin" [*Zentr. Bakt. Parasitenk. II*, **22**, 98 (1908)]. Pringsheim used the name "polyamylose," and referred to the individual compounds as α-tetraamylose, β-hexaamylose, etc. [*Ber.*, **47**, 2565 (1914)]. Freudenberg and Jacobi (ref. 5) refer to these compounds as α-dextrin, β-dextrin, etc., and later as pentaosan, hexaosan, etc. [K. Freudenberg and H. Boppel, *Ber.*, **73**, 609 (1940)].

(8) T. J. Schoch, Report to the Corn Industries Research Foundation (1940). This method is an adaptation of the method of E. B. Tilden and C. S. Hudson, THIS JOURNAL, **61**, 2900 (1939).

(9) The instrument used was the Clark–Gross modification of the reciprocal lattice X-ray goniometer described by W. F. deJong and J. Bouman, *Z. Krist.*, **98**, 456 (1938); **99**, 326 (1938).

(10) A. E. H. Tutton, "Crystalline Form and Chemical Constitution," The Macmillan Co., London, 1926, p. 31.

3032 cu. Å. The number of glucose residues per unit cell is then

$$\frac{3032 \times 1.240 \times 6.06 \times 10^{-1}}{162.1} = 14.05 \cong 14$$

The monoclinic space groups allowed for an optically active molecule are C_2^1-$P2$, C_2^2-$P2_1$, and C_2^3-$C2$. Many reflections of the form (hkl) with $(h + k)$ odd are present so that C_2^3-$C2$ is eliminated. Intense reciprocal lattice goniometer patterns were made rotating the crystal about the zone [100]. All reflections of the form $(0kl)$ were observed to $(\sin\theta)/\lambda = 0.56$, except the odd orders of $(0k0)$, through (090), which were absent. The space group can then be taken as C_2^2-$P2_1$. This space group requires an even number of molecules per unit cell so we must have two molecules of seven glucose residues each.

Summary

1. The molecular weights of the Schardinger α- and β-dextrins have been accurately determined by X-ray diffraction and crystal density measurements.

2. The α-dextrin contains six glucose residues per molecule and has been renamed cyclohexaamylose; the β-dextrin contains seven glucose residues and has been renamed cycloheptaamylose.

AMES, IOWA RECEIVED FEBRUARY 17, 1942

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

Sterols. CXLVII. Sapogenins. LXI. The Bio-reduction of Steroids

BY RUSSELL E. MARKER, R. B. WAGNER AND PAUL R. ULSHAFER

It has been postulated that Δ^5-3-hydroxysteroids arise as reduction products of Δ^4-3-ketosteroids under biological conditions.[1] Using Δ^4-dehydrotigogenone as a model substance for the bio-reduction process, we[3] have recently shown that this when administered to a dog on a biscuit diet gave diosgenin (I), smilagenin (iso-sarsasapogenin) (II) and *epi*-smilagenin (*epi*-iso-sarsasapogenin) (III). These results not only support our hypothesis, but also support the conception that cholestenone is an intermediate in the formation of coprosterol in the organism[1,5] since the products, (I), (II) and (III) correspond in nuclear structure to cholesterol, coprosterol and *epi*-coprosterol. The sapogenin derivatives having the characteristic side-chain act as effective indicators and are not subject to the suggestions of Fieser and Wolfe.[2]

We have now extended this work along the line of that of Rosenheim and Webster[4] who showed that β-sitosterol administered together with brain-powder to rats was converted into an isomeride of sitostanol which they named *copro*-sitostanol. The latter agrees in composition and properties with 24-ethylcoprostanol-3(β). Since a coprostane derivative has not been obtained directly from a Δ^5-3-hydroxysteroid by chemical action Rosenheim and Webster's experiment indicates a Δ^4-3-ketosteroid as a very probable intermediate for the formation of the *copro*-sitostanol.

Accordingly, diosgenin (I) was administered to a dog fed on a meat diet containing small portions of pig brain. The non-saponifiable fraction of the feces gave smilagenin (II) and *epi*-smilagenin (III) products which correspond to coprosterol and its epimer. This and the previous conversion support the hypothesis of Schoenheimer[5] that there is a reversible biological reaction of the type cholestenone $\rightleftarrows$ cholesterol. Δ^4-Dehydrotigogenone (IV) may be reduced by one enzyme system to smilagenin (II) and *epi*-smilagenin (III) or by another enzyme system converted to diosgenin (I). The present work indicates the reversible reaction involving the following oxidation–reduction mechanism; diosgenin (I) $\underset{[R]}{\overset{[O]}{\rightleftarrows}}$ Δ^4-dehydrotigogenone (IV) $\xrightarrow{[R]}$ smilagenin (II) and *epi*-smilagenin (III). These reactions are summarized in the accompanying chart.

Another observation in the present work is that the bio-reduction of keto-sapogenins gives hydroxy compounds of both alpha and beta configuration. This is contrary to the earlier statements[1] that reduction *in vivo* of 3-ketosteroids appears to give only alpha compounds. Tigogenone and sarsasapogenone having the cholestane and the coprostane configuration, respectively, were administered to a dog fed on a meat diet. In the case of the tigogenone the feces contained both

(1) Marker, THIS JOURNAL, **60**, 1725 (1938).
(2) Fieser and Wolfe, *ibid.*, **63**, 1485 (1941).
(3) Marker, Wittbecker, Wagner and Turner, *ibid.*, **64**, 818 (1942).
(4) Rosenheim and Webster, *J. Soc. Chem. Ind.*, **XL**, 486 (1941)
(5) Schoenheimer, Rittenberg and Graff, *J. Biol. Chem.*, **111**, 185 (1935).