Infrared Absorption Studies.—Chloroform solutions of each of the substances listed in Table I were made up at a concentration of 0.025 *M*. These solutions were in turn placed in a 0.105-mm. sodium chloride cell, and the infrared spectrum of each was scanned in the region 5.0–6.2 μ, using a Perkin–Elmer model 21 double-beam infrared spectrometer. Chloroform solvent was used in a 0.107-mm. cell in the I_0 beam, and a slit-width of 57 μ was employed. The integrated absorption intensity under the region 5.72 μ carbonyl band for each sample was calculated by relationship 1

$$A = k \log (T_0/T)\nu_{max} \times \Delta\nu^{a}1/2 \qquad (1)$$

where T_0 represents the transmittance of the chloroform blank and T that of the sample, and where $\Delta\nu^{a}1/2$ is the apparent half-band width. Equation 1 represents a simplification of the "Method of Direct Integration" of Jones and co-workers.[15] The constant k in (1) cancels when taking the pertinent ratios in Table I, all instrumental and other factors being constant in the successive determinations, and was therefore not evaluated. Thus the "Integrated Absorption Intensity" column in Table I is given in arbitrarily dimensionless numbers which are significant only when converted to the ratios in Table I. It is interesting to note that when the apparent molecular extinction coefficients of the five carbonyl absorption intensities in question were calculated in the usual way, the theoretical ratios of 5/3 and 4/3 for nos. 1, 2 and 3, 4, respectively, in Table I were only very poorly approximated, thus emphasizing the validity of Jones and co-workers' contention[15] of the superiority of integrated absorption intensities over molecular extinction coefficients for such empirical comparisons.

STANFORD, CALIFORNIA

[CONTRIBUTION FROM THE LABORATORY OF CHEMICAL PHARMACOLOGY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Synthetic Polysaccharides. III. Polyglucose Sulfates[1]

BY JOHN W. WOOD AND PETER T. MORA

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From synthetic polyglucoses of different molecular weight and degree of branching, polyglucose sulfates were prepared with different degrees of sulfation, preparatory to correlating certain biological activities (such as anticoagulant activity) with macromolecular properties.

The acid-catalyzed polycondensation of D-glucose in vacuum,[2] and the influence of polymerization conditions and of fractionation on the molecular weight, branching and certain other molecular properties of the polyglucoses obtained[3] were described in the previous communications in this series. We now report the preparation of polyglucose sulfates differing in molecular weight, branching and degree of sulfation.

Sulfuric acid esters of certain polymers, especially polysaccharides, are known to have blood anticoagulant activity similar to that of heparin[4] (for example, sulfuric acid esters of polyvinyl alcohol,[5] cellulose,[6] xylan,[7] chitin,[6,7] chondroitin sulfuric acid[6,7] and dextran).[8] Most of these products also exhibited toxicity, ascribed chiefly to precipitation of fibrinogen and agglutination of platelets. It appears from the literature that the common molecular features associating with these activities are macromolecular structure (preferably that of a polysaccharide) and strong electronegative charges (such as are imparted by the sulfuric acid ester groups). In the case of dextran sulfate, the influence of molecular weight and degree of substitution on these biological activities has been studied[9,10]; a relatively low molecular weight product, with two to three sulfate groups per anhydro-glucose unit, was developed in England for clinical use as an anticoagulant.

The sulfuric acid esters prepared from synthetic polyglucoses also were observed[11,12] to have anticoagulant activity similar to heparin; this activity can be reversed by protamine.[11] Moreover, it was observed recently that these preparations possess several biological activities which may be useful in the study of some aspects of blood coagulation, and lipemia clearing.[13] Sodium polyglucose sulfates, and the free acids obtained from them after eliminating the sodium by ion exchange treatment, interact with basic proteins and enzymes, often producing insoluble precipitates.[14] The synthetic polysaccharides offer a suitable model system for correlation of these biological activities with systematic changes in the molecular features, including the degree of branching.

We selected two pairs of polyglucose preparations, obtained by two different polymerization methods[2] which result in certain differences in the degree of branching,[15] and in molecular weight (see Table I). Each pair consisted of a lower and a higher molecular weight sample (preparations A, G and E, F, respectively), with the higher molecular weight sample having about twice as high a molecular weight as the other sample prepared by the same polymerization method. The molecular weights ranged from 9,300 to 28,400. From each of these polyglucoses we prepared a sulfuric acid ester by the chlorosulfonic acid–pyridine method, introducing 2.6 to 3 acid sulfate groups per an-

(1) Presented in part before the Polymer Division of the American Chemical Society at the 132nd National Meeting in New York, N. Y., September 8, 1957.

(2) P. T. Mora and J. W. Wood, THIS JOURNAL, **80**, 685 (1958).

(3) P. T. Mora, J. W. Wood, P. Maury and B. G. Young, *ibid.*, **80**, 693 (1958).

(4) For mechanism of heparin activity *cf.*, for example, A. S. Douglas, *J. Clin. Invest.*, **35**, 533 (1956).

(5) E. Chargaff, F. W. Bancroft and M. Stanley-Brown, *J. Biol. Chem.*, **115**, 155 (1936); P. Karrer, E. Usteri and B. Camerino, *Helv. Chim. Acta*, **27**, 1422 (1944).

(6) P. Karrer, H. Koenig and E. Usteri, *ibid.*, **26**, 1296 (1943).

(7) K. H. Meyer, R. P. Pirue and M. E. Odier, *ibid.*, **35**, 574 (1952).

(8) A. Grönwall, B. Ingelman and H. Mosiman, *Upsala Läkareförен. Förh.*, **50**, 397 (1945).

(9) C. R. Ricketts and K. W. Walton, *Chemistry & Industry*, 809 (1952).

(10) C. R. Ricketts and K. W. Walton, *Brit. J. Pharmacol.*, **8**, 476 (1953).

(11) P. T. Mora, unpublished results with collaborators at the Experimental Station and Stine Laboratory, du Pont Co., Wilmington, Del., 1953.

(12) E. London, R. S. Theobald and G. D. Twigg, *Chemistry & Industry*, 1060 (1955).

(13) Personal communication from Drs. S. Farber, E. Klein and I. Djerassi, Children's Cancer Research Foundation, Boston, Mass.

(14) P. T. Mora and B. G. Young, *Nature*, in press.

(15) See reference 3 for discussion of differences in branching as indicated by periodate titrations.

TABLE I

PREPARATION AND ANALYSES OF SODIUM POLYGLUCOSE SULFATES

Prepn.	Polyglucose intermediate[d] Method of prepn.[a]	[η],[b] dl./g.	Mol. wt.[c]	$ClSO_3H$ amount used, ml.	$ClSO_3H$ A.G.U.,[e] mole/mole	Yield,[f] g.	Analyses,[g] % Ash[h]	Sodium Found[i]	Sodium Calcd.[j]	Sulfur[k]	SO_4 groups per D-glucose unit
A	Soln.-melt	0.03	17,750	10.0	4.94	12.3[bb]	48.6	15.8	14.2	19.8	2.7
B	Soln.-melt	.03	17,750	6.0	2.98	10.1	42.3	13.7	14.3	19.9	2.7
C	Soln.-melt	.03	17,750	3.5	1.74	7.8	33.4	10.6	9.5	13.3	1.2
D	Soln.-melt	.03	17,750	2.5	1.23	3.6	24.0	7.6	6.2	8.6	0.6
E	Soln.-melt	.06	28,400	10.0	4.94	13.3	47.8	15.6	13.9	19.4	2.6
F	Infra-red	.05	18,400	10.0	4.94	12.9	46.9	15.3	14.6	20.4	2.9
G	Infra-red	.04	9,350	10.0	4.94	14.0	47.0	15.4	14.6	20.4	2.9
H	Two-stage	.05	19,300	25.0	6.49	26.3	50.6	16.5	14.9	20.8	3.1

[a] For a description of the methods see Experimental section and ref. 2. [b] [η] = intrinsic viscosity, as determined in ref. 2; [bb] in 0.5% NaCl, 0.04. [c] Number-average molecular weights determined by the modified Scales end-group reducing method.[2] [d] Used 5 g. each of the various polyglucoses in preparations A through G, and 9.5 g. of the polyglucose in preparation H; the following amounts of dry pyridine were used: in prepn. D, 38 ml.; in prepn. H, 100 ml.; and in all of the other prepns. 40 ml. [e] Anhydro-glucose unit. [f] The moisture content of the purified freeze-dried products varied from 12.1 to 13.7%. [g] The analyses are reported on a moisture free basis. All of the products except H gave a negative Beilstein test for chlorine. All of the products contained 0.06%, or less, of nitrogen. [h] Determined by treating the samples with concentrated sulfuric acid in a platinum crucible and heating to a dull red stage. [i] On the basis of the ash determinations. [j] On the basis of the sulfur determination. [k] Sulfur content was determined by sodium peroxide fusion method using a micro Parr bomb.

hydro-glucose unit (A.G.U.), which is near to the maximum possible substitution of the three free hydroxyls.[16] When the amount of chlorosulfonic acid was three moles/A.G.U. (B) the substitution was already at the maximum, and it was not necessary to increase further the mole percentage of the reagent under the reaction conditions employed (compare with A and H). We also prepared sulfates with progressively less substitution from one of the polyglucose samples, by decreasing the mole percentage of chlorosulfonic acid in the same amount of pyridine, thereby diluting the reaction mixture. The molar ratio of $ClSO_3H$/A.G.U. was 1.74 in preparation C and 1.2 hydroxyls were substituted per A.G.U., while in preparation D the average substitution was only 0.6 hydroxyl/A.G.U. obtained with 1.23 moles of chlorosulfonic acid. Besides the above preparations, we describe a somewhat larger scale preparation (H) where an improved method of isolation was employed by eliminating the bulk of the pyridine and sodium sulfate before dialysis. The final dialyzed polyglucose sulfate sodium salts were free from sodium sulfate, as indicated by the agreement of found sodium with the calculated values based on sulfur analysis, assuming a sodium to sulfur ratio of one.

Below we report the synthetic procedures for the polyglucose sulfates, certain data on molecular properties and qualitative indication of metachromatic and anticoagulant activity. Detailed biological evaluation will be presented elsewhere.

Experimental

Polyglucose Samples.—For sulfation experiments A, B, C, D and E (see Table I) the polyglucose employed was prepared by the "solution-melt" polymerization method in the presence of the inert solvent tetramethylene sulfone. This was described in experiment 3 of reference 2, except that the polymerization temperature was lower: 140° for 5 hr., and 120° for an additional 12 hr. The polyglucose was fractionated as described in reference 3. The low molecular weight fraction, precipitated with a large excess of alcohol (over 75%), was used for sulfation experiments A, B, C and D, while the higher molecular weight polyglucose used for experiment E was recovered between 49–75% alcohol and refractionated between 60–75% alcohol limits. For sulfation experiments F and G the polyglucose was prepared by the 140° "infrared" polymerization, as reported in reference 2, polymerization experiment 1. Fraction II (58–62% ethanol), obtained as reported in Table I, reference 3, was used for sulfation experiment F, while for experiment G the lower molecular weight fraction VI (75–80% ethanol) was employed. Finally, for the somewhat larger scale sulfation H, the polyglucose was prepared by 170° "two-stage" polymerization method (polymerization experiment 7, reference 2), and the 66–70% alcohol fraction (Table I, reference 3) was used. The intrinsic viscosities of these polyglucose fractions and the number average molecular weights determined by reducing end-group method[17] are reported in Table I. These starting polyglucose preparations were freeze-dried white powders, and they were further dried at 60° in vacuum (0.1 mm.) for approximately 16 hr. before sulfation.

Reagents.—Pyridine (Fisher, reagent grade) was dried with solid potassium hydroxide and then distilled over barium oxide. The chlorosulfonic acid (Eastman, practical grade) was used without redistillation.

Sulfation.—The method was similar to that used for preparation of dextran sulfate,[8,18,19] but incorporated certain alterations. The conditions employed are given below and in Table I.

In a reaction vessel, under anhydrous conditions, 40 ml. of pyridine was chilled under vigorous stirring to −15 to −20°, in a Dry Ice–methyl Cellosolve-bath. Chlorosulfonic acid (the different amounts are shown in Table I) was added dropwise. The pasty product was then heated to 45–55°, when it partially liquified. Five g. of polyglucose powder was added as rapidly as possible to the well-stirred sulfation mixture; heating and stirring was maintained at 60–70° for 4–6 hr. After this the reaction was allowed to cool, when the product separated as a viscous, dark amber-colored mass. The entire reaction mixture was poured onto 150–300 g. of cracked ice. The resulting clear solution was neutralized with 40% sodium hydroxide to *p*H 7–7.2, whereupon the mixture separated into two layers. (It is preferable at this point to discard the upper layer, which was done in experinent H, since a great deal of pyridine and sodium sulfate can be eliminated at this point. We were reluctant to do this in the earlier experiments A through G because of the possibility of losing some polyglucose sulfates of lower molecular weight.) The entire solution (lower layer) was chilled with ice and precipitated with the addition of three volumes of absolute alcohol, and

(16) The number of free hydroxyl groups in polyglucoses approaches three per A.G.U. with increasing molecular weight. This is independent of the degree of branching, since each branch which eliminates one free hydroxyl leads to one new end-group with one additional free hydroxyl.

(17) *Cf.* ref. 2 for the modified Scales method employed, and also ref. 3 for limitations.

(18) C. R. Ricketts, *Biochem. J.*, **51**, 129 (1952).

(19) C. R. Ricketts, K. W. Walton and S. M. Saddington, *ibid.*, **58**, 532 (1954).

the precipitate was centrifuged off. (In the case of sample H, the precipitate was centrifuged off, dissolved in a minimum amount of water and reprecipitated with alcohol.) The precipitate was dissolved in a convenient amount of water and the solution was dialyzed in a rotated Visking cellophane casing for 3 hr. (for 8.5 hr. in the case of preparation H) against running distilled water. The dialyzed solution, which had become slightly acidic, usually was adjusted to neutrality with dilute sodium hydroxide solution. The neutral solution was precipitated by the addition of three volumes of absolute alcohol, and the sirupy precipitate was separated by centrifuging. Alcohol precipitation was repeated from an aqueous solution. The sodium polyglucose sulfate was then dissolved in water and freeze-dried to give a light, stable and almost colorless powder. The properties of the resulting polyglucose sulfate sodium salts are reported in Table I.

The polyglucose sulfate can be obtained in the free acid form by passing the aqueous solution of the salt through IR-120-H Amberlite ion exchange resin. The solution (*p*H 1) can be freeze-dried to a brown sirup.

Anticoagulant Activity.—The U.S.P. assay for heparin activity was used on sheep plasma.[20] Polyglucose sulfates gave approximately the same anticoagulant activity by the assay as clinical heparin preparations, which is about one-third of the international heparin standard.

Metachromatic Activity.—Interaction with the basic thiazine dye toluidine blue was measured spectrophotometrically. Metachromatic activity was in the same order of magnitude as dextran sulfates.[21]

Acknowledgment.—We are indebted to Dr. W. C Alford for analytical determinations, and to Miss Vivian S. Williams for viscosity measurements.

(20) L. M. Tocatins, "The Coagulation of Blood," Grune and Stratton, New York, N. Y., 1955, Chapter XIII, p. 214.

(21) K. W. Walton and C. R. Ricketts, *Brit. J. Exp. Pathol.*, **35**, 227 (1954).

BETHESDA 14, MD.

[CONTRIBUTION FROM THE CHEMICAL RESEARCH AND DEVELOPMENT DIVISION OF THE SCHERING CORP.]

The Dienol–Benzene Rearrangement.[1] Some Chemistry of 1,4-Androstadiene-3,17-dione

BY MARGARET JEVNIK GENTLES, JANE B. MOSS, HERSHEL L. HERZOG AND E. B. HERSHBERG

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1,4-Androstadiene-3ξ,17β-diol, upon treatment with weak acid or activated magnesium silicate, rearranges with loss of water to 4-methyl-1,3,5(10)-estratrien-17β-ol. The structure of the product was proved by dehydrogenation of the corresponding 17-ketone over Pd-on-carbon to the known 1-ethyl-2,8-dimethylphenanthrene.

Subsequent to the renaissance of interest in steroid 1,4-diene-3-ketones[2] we had occasion to study the chemistry of 1,4-androstadiene-3,17-dione (I) looking toward the introduction of various substituent groups at C-17. It had been shown previously that 4-androstene-3,17-dione reacts with one mole of ethylene glycol at either C-3 or C-17 depending upon the ratio of catalyst (*p*-toluenesulfonic acid) to steroid employed.[3] Under conditions most favorable for the formation of a 3-ethylene ketal it was now found that I forms only the 17-ethylene ketal II. By increasing the amount of ethylene glycol employed, an 87% yield of II was achieved. It was not possible to demonstrate the presence of either the 3-ethylene ketal of I[3a] or the 3,17-bisethylene ketal.

Since it was impossible to achieve selective protection at C-3 in this way an attempt was made to reduce the 3-carbonyl group of II. Following reaction of II with a large excess of lithium aluminum hydride[3b] in ethereal solution the excess reagent was destroyed by the addition of acetone and then a limited amount of water was added in order to afford a filterable precipitate of hydrated alumina. The reaction products were then isolated by *chromatography over Florisil* and a *ca.* 65% yield of a benzenoid, steroidal product III resulted.

The structure of III was established in the following fashion: The benzenoid nature of III was apparent from the ultraviolet [bands at 262 mμ (ε 259) and 269 mμ (ε 197)] as was the disappearance of the dienone group originally present in II. The infrared spectrum of III displayed aromatic absorption at 6.30 and 6.38 μ and lacked the bands customarily assigned to 1,4-diene-3-ketones. The loss of an atom of oxygen was clear from the carbon and hydrogen analyses of III and its transformation products. Hydrolysis of the 17-ethylene ketal group of III in aqueous acetic acid yielded IV, which had ultraviolet and infrared spectra consistent with the assigned structure. Reduction of IV with sodium borohydride or of I with lithium aluminum hydride (*with chromatography*) afforded V. Oxidation of IV or V with chromic acid in acetic acid gave a diketone VI, which contained a six-membered ring carbonyl group conjugated to the benzene ring [ultraviolet bands at 253 mμ (ε 9,000) and 299 mμ (ε 2,000)].

Recapitulation of the evidence to this point led to the logical surmise that a dienone–phenol type rearrangement had occurred during the lithium aluminum hydride step. Isolation of the lithium

(1) The name for this rearrangement was first suggested to us by Dr. J. Meinwald of Cornell University. Since that time a preliminary communication of H. Plieninger and G. Keilich, *Angew. Chem.*, **68**, 618 (1956), has appeared in which the rearrangement of 4-trichloromethyl-4-methylcyclohexadienol to *o*-trichloromethyltoluene by the action of mineral acid is described. These authors propose that the rearrangement be designated in the same way. A related aromatization, without rearrangement, was postulated by U. Weiss, *et al.*, *Science*, **119**, 774 (1954), to account for the conversion of prephenic acid to phenylpyruvic acid.

(2) H. L. Herzog, A. Nobile, S. Tolksdorf, W. Charney, E. B. Hershberg, P. L. Perlman and M. M. Pechet, *ibid.*, **121**, 176 (1955).

(3) H. L. Herzog, M. A. Jevnik, M. E. Tully and E. B. Hershberg, THIS JOURNAL, **75**, 4425 (1953).

(3a) *Cf.* J. A. Hogg, *et al.*, *ibid.*, **77**, 4438 (1955), who claim to have prepared the 3-cycloethylene ketal of methyl-3,11-diketo-1,4,17(20)-[*cis*]-pregnatrien-21-oate. Neither detailed procedure nor constants of the ketal are reported.

(3b) *Cf.* F. Sondheimer, *et al.*, *Chemistry & Industry*, 1482 (1954), who noted that reduction of 1,4-androstadiene-3,17-dione with sodium borohydride affords a mixture of diols containing products in which the double bond at 1- has been reduced.