[CONTRIBUTION FROM THE STARCH AND DEXTROSE SECTION, NORTHERN UTILIZATION RESEARCH BRANCH[1]]

Evaluation of the Periodate Oxidation Method for Structural Analysis of Dextrans

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Specific structural information was required on hundreds of dextrans within a relatively short time. The only sufficiently rapid method available was that of Jeanes and Wilham based on oxidation with sodium metaperiodate. Although the dextrans were structurally diverse, uniform conditions of periodate oxidation were employed for all. The reliability of the results has been established through experimentation with the oxidation and analytical procedures and through correlation with data from other methods of structural analysis. Formic acid measurements, from which the percentage of 1,6-linked anhydroglucopyranose units is calculated, were obtained with 99% accuracy for our most studied dextran, NRRL B-512. For this dextran, the accuracy of periodate reduced, upon which calculation of 1,4-like and 1,3-like linked units is based, was about 95% by the Fleury–Lange method, but about 97–98% when the iodometric titration in this method was made at 4°. This difference appeared to result mainly from reduction of iodine by the oxidized dextran during titration at 25° for periodate reduced. These accuracies are believed to apply to other dextrans excepting certain ones with high contents of 1,4-like links which over-oxidize. The utility of the periodate oxidation method for structural analysis was extended by applying it to bacterial levans and by establishing a simplified procedure for analysis of water-insoluble dextrans.

A previously reported periodate oxidation method for determining the types and proportions of glucosidic linkages in dextrans was established using six dextran preparations from four different strains of *Leuconostoc mesenteroides*.[2] We now have applied this method to dextrans from over a hundred strains comprising five genera of microörganisms.[3] These data were needed for characterizing the dextrans, and for selecting typical ones for further investigations and for correlating the data therefrom.

The six original dextrans reacted with periodate as if the non-1,6-linkages were 1,4-, and thus the results agreed with published methylation data on other dextrans.[2] However, many of the new dextrans reacted as if 1,3-linkages were present in addition to 1,6- and 1,4-. Since the reactions of 1,4- and 1,3-linked units with periodate are not specific, but merely are indicative of two or of three hydroxyl groups, respectively, in contiguous positions in the anhydroglucopyranose units, a variety of previously unencountered structures were possible in the new dextrans.

We have established the validity of our periodate oxidation data on these dextrans of diverse and often unprecedented structural nature through critical examination of the method as well as of the results and their interpretation. The significance of these observations is augmented by the extensive use of the periodate oxidation measurements for correlating chemical,[4] physical chemical,[5] fractionation[6] and serological[7] data on the dextrans. New information reported here on periodate oxidation procedures and on the analytical methods has fundamental significance as well as general applicability in structural analysis of other polysaccharides by this method.

Experimental

Materials.—All the native bacterial polysaccharides[3] and the dextran fractions[6] used in this investigation were prepared and characterized as described in the references cited. They are designated by the NURB Culture Collection strain number of the organism which produced them. The suffix, E, indicates an enzymatically synthesized dextran. Other suffixes indicate fractions of native dextrans. In a few cases, data are reported for several dextran preparations from a given strain. The polysaccharides usually contained no more than about 0.05% ash, 0.01% nitrogen, 0.001% phosphorus and, unless stated otherwise, less than 0.2% fructose.

Dialdehyde dextrans were produced by periodate oxidation, isolated and purified as already reported elsewhere.[4c]

Ethylene glycol was distilled *in vacuo* over sodium hydroxide.

Sodium metaperiodate was a commercial preparation used without further purification.

Analytical Methods.—The moisture[3] and fructose[8] contents of the polysaccharides were determined as described in the references cited.

Formic acid determinations were made on 10-ml. aliquots as described previously[2] except that 0.01 N barium hydroxide was used and was standardized to ±0.00005 N with potassium acid phthalate weighed on a microbalance. To conserve time, end-points were found by phenolphthalein indicator rather than potentiometrically.

Periodate was measured on aliquots of duplicate oxidation mixtures by the method of Fleury and Lange.[9] A 5-ml. aliquot of the oxidation mixture was added to 10 ml. of saturated sodium bicarbonate solution and immediately thereafter 5 ml. of 0.1 N sodium arsenite solution and 1 ml. of 20% potassium iodide were added. After standing at least 15 minutes, the solution was titrated rapidly with 0.1 N iodine solution to a starch end-point, using a microburet.

In addition to these titrations for periodate carried out at room temperature (25°), in many cases, the solutions were cooled to 4° before and during titration with iodine.

For comparison with results of the Fleury–Lange method, some measurements of periodate reduced were made by the method of Malaprade.[10] To a 5-ml. aliquot was added rapidly a solution containing 40 ml. of water, 2 ml. of 20% potassium iodide solution and 3 ml. of 0.5 N sulfuric acid, and the mixture was titrated immediately with 0.1 N thiosulfate solution. Duplicate analyses were made at 25° as well as on test solutions which were prepared from ice-cold reagents, and held at 4° during titration.

(1) One of the Branches of the Agricultural Research Service, U. S. Department of Agriculture. Article not copyrighted.

(2) Allene Jeanes and C. A. Wilham, THIS JOURNAL, **72**, 2655 (1950).

(3) Allene Jeanes, W. C. Haynes, C. A. Wilham, J. C. Rankin, C. E. Rist, *et al.*, forthcoming publication.

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(7) (a) E. J. Hehre, J. Y. Sugg and J. M. Neill, *Ann. N. Y. Acad. Sci.*, **55** (3), 467 (1952); (b) E. A. Kabat and D. Berg, *J. Immunol.*, **70**, 514 (1953); (c) P. H. Maurer, *Proc. Soc. Exptl. Biol. Med.*, **83**, 879 (1953).

(8) C. W. Wise, R. J. Dimler, H. A. Davis and C. E. Rist, Abstracts Papers Am. Chem. Soc., **124**, 2D (1953).

(9) P. P. Fleury and J. Lange, *J. pharm. chim.*, **17**, 107 (1933); E. L. Jackson, "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 361.

(10) L. Malaprade, *Compt. rend.*, **186**, 382 (1928).

TABLE I

COMPARISON OF METHODS FOR MEASURING PERIODATE REDUCED BY DEXTRANS AND OTHER CARBOHYDRATES

Sample	Run no.[a]	Titration condition[b]	Mole/mole AGU: IO_4^- reduced, Reaction time, hr. 72	IO_4^- reduced, 96	HCOOH produced, 72	HCOOH produced, 96	AGU linked, %[c] 1,6-	1,4-like	1,3-like
B-512 dextran	1	A	1.94	1.96	0.945	0.948	95	5	0
	2	A	1.97	1.96	.946	.956	95	8	−3
	2	A-4	1.92	1.93			95	3	2
	3	A	1.93	1.94	.951	.950	95	3	2
	3	A-4	1.90	1.89			95	0	5
	3	S	1.95	1.90			95	5	0
	3	S-4	1.97	1.91			95	7	−2
B-512-E dextran	1	A	1.99	2.00	.943	.961	94	10	−4
	1	A-4	1.93	1.94			94	4	2
B-742 (L) dextran, fraction L-R	1	A	1.83	1.86	.792	.802	79	25	−4
	1	A-4	1.78	1.82			79	20	1
	1	S	1.83	1.81			79	25	−4
	1	S-4	1.82	1.80			79	24	−3
B-1146 dextran	1	A	1.98	2.00	.958	.966	96	6	−2
	1	A-4	1.92	1.93			96	0	4
	2	A	1.92	1.98	.955	.965	96	1	4
	2	A-4	1.89	1.91			96	−2	6
	2	S	1.94	1.91			96	3	1
	2	S-4	1.92	1.91			96	1	4
B-1254 dextran, fraction C-3	..[d]	A	1.71	1.72	.679	.690	68	35	−3
	..[d]	A-4	1.68	1.71			68	32	0
	4	A	1.76	1.75	.686	.696	69	39	−8
	4	S	1.74	1.69			69	37	−6
	4	S-4	1.70	1.70			69	33	−4
B-1299 dextran fraction S-R	1	A	1.52	1.56	.502	.528	50	52	−2
	1	A-4	1.50	1.54			50	50	0
	2	A	1.52	1.58	.503	.527	50	51	−1
	2	S	1.50	1.53			50	49	1
	2	S-4	1.49	1.54			50	48	2
B-1355 dextran fraction B-3	1	A	1.61	1.62	.757	.763	76	10	14
	1	A-4	1.55	1.57			76	4	20
	1	S	1.56	1.57			76	5	19
	1	S-4	1.55	1.57			76	4	20
Waxy corn starch	1	A	0.88	0.93	.047	.047			
	1	A-4	0.88	0.93					
Levan	1	A	1.00	1.00	.007	.015			
	1	A-4	1.00	1.00					
Levoglucosan	1	A	2.00	2.01	.995	1.002			
	1	A-4	1.985	2.01					
	1	S	1.985	1.96					
	1	S-4	1.985	1.99					

[a] A given number indicates a specific periodate-oxidation run for the sample, but all numbers 1 were not necessarily run simultaneously. [b] "A" designates the bicarbonate-arsenite procedure, "S" the acid-thiosulfate procedure, "4" indicates titration at 4°. [c] Calculated from 72-hour values as follows: % 1,6- = FA × 100; % 1,4- = (PR − 2FA) × 100; % 1,3- = remainder from 100. [d] Average of three closely agreeing runs.

Periodate Oxidation Procedure. (a) **Water-Soluble Dextrans.**—All water-soluble dextrans were oxidized under standard conditions which were the same as those previously reported[2] except that the sample weight was increased to 0.1000 g. (dry basis). This change insured sufficient acid for titration from dextrans having high proportions of non-1,6-linked units. Duplicate samples of the dextrans were dissolved in 50 ml. of boiled distilled water. Autoclaving (15 lb./sq. in. for 30 minutes) was required to dissolve some dextrans; the remainder dissolved at room temperature. Then three moles of sodium metaperiodate per mole of anhydroglucopyranose unit (AGU) were added (50 ml. of 0.0374 *M* solution). The initial *p*H of blanks was 5.0–5.2; the final value of the oxidation solutions was 3.1 to 3.3. Blanks and a dextran control were included in each run.

(b) **Water-Insoluble Dextrans.**—The previously used procedure of dissolving water-insoluble dextrans in alkali and neutralizing with acid in preparation for oxidation[2] presented the difficulty of obtaining solutions which were exactly neutralized and free of buffering action. Reliable results now have been obtained from oxidation of such dextrans under heterogeneous conditions if duplicate oxidation mixtures were set up for each time of analysis. Two methods for preparation of the heterogeneous suspensions have been used: (1) The dry sample merely was suspended in carbon dioxide-free water and (2) this suspension was autoclaved for 30 minutes at 15 lb./sq. in. to increase hydration of particles or, in some cases, to produce partial solubilization. At suitable times during oxidation, the heterogeneous mixtures were shaken gently. Aliquots free of suspended particles were taken for analysis.

Results

Measurement of Formic Acid.—Titrations for formic acid carried out potentiometrically at 4°

gave results identical with those obtained at 25° on formic acid controls as well as on periodate oxidized dextrans B-512 and B-742. The titration curves for these periodate oxidized dextrans and for the formic acid controls were superimposable between *p*H 6.0 and 8.5. This indicated that free acid alone was being measured in these dextran oxidation mixtures and not, in addition, weakly acidic groups of unknown type. For these oxidized dextran solutions and for the controls as well, acid values obtained using phenolphthalein indicator were approximately 1% higher than those obtained by potentiometric titration.

Occasionally solutions of the dextrans themselves were titrated with barium hydroxide, especially when the ash content was higher than usual. No evidence of acidity or alkali-binding power ever was observed.

Formic acid and sodium metaperiodate did not interact during five weeks of observation in mixtures simulating exactly (except that polysaccharide was absent) those of typical dextrans after oxidation under standard conditions.

The excellent reproducibility of formic acid measurements in different runs is shown for the dextrans in Table I.

Measurement of Periodate Reduced.—Doubt concerning the reliability of the Fleury–Lange procedure for determination of periodate reduced by carbohydrates has been raised by several investigators.[11–14] Although difficulties have been reported usually for early stages of oxidation of reducing sugars, we examined all aspects of the method as applied in our work to the determination of periodate reduced by dextrans when the reaction was complete or nearly so.

Contrary to the observations of others reported in the references cited, we established that for our measurements of periodate reduced by the Fleury–Lange procedure, the order of addition of components to the test solution did not change the result[11]; the periodate remaining in dextran oxidation solutions was reduced instantaneously by arsenite–bicarbonate–iodide[11–13]; and increasing the concentration of iodide fivefold did not affect the results,[12] although higher values were obtained if the iodide was omitted. Further oxidation of the carbohydrate will occur after addition of the test aliquot to bicarbonate unless the arsenite and iodide are added immediately.

The first permanent end-point for the iodine titration at 25° in the measurement of periodate reduced by dextrans faded badly. The extent of fading differed with the dextran. It appeared most pronounced for dextrans having highest proportions of 1,6-linked units (such as B-512 and B-1146) and less pronounced for dextrans having high proportions of 1,3-like linked units (such as B-1355 S-R and B-1149). After the end-point was reached for dextrans such as B-512 and B-1146, the color faded completely within 10 minutes. Under identical conditions, the end-point of a test solution of oxidized waxy corn starch persisted 10 minutes and that of a levan sample lasted an hour. The color of dextran end-points was more stable at 4°.

Keeping the test solution for determination of periodate cold (4°) during back-titration with iodine decreased fading of the end-point and permitted more accurate titration as well as better duplication between different runs. Titrations at 4° almost invariably indicated a lower reduction of periodate by dextrans than did titrations at 25° as shown by typical data in Table I. Titration of levoglucosan oxidation solutions at 4° always gave slightly lower values than titrations at 25° when reaction was not entirely complete as indicated by formic acid measurements (72 hours reaction time). The 4° values were in better agreement with the formic acid than were the 25° values. However, at 96 hours, when formic acid values indicated complete reaction, periodate values obtained at 4° and 25° were identical (Table I).

Also shown in Table I are results for periodate reduced by dextrans when the Malaprade method of analysis was employed. No consistent difference between titrations at 4 and 25° was found, and usually the differences were not significant.

Solutions of isolated dialdehyde products from periodate oxidation of five typical dextrans were titrated with iodine under conditions simulating exactly those used for measurement of periodate reduced by dextrans (condition C), and also in solutions containing bicarbonate but not arsenite and iodide (condition D). The results show that the dialdehydes reduced iodine at 25° but not at 4° (Table II). The amount reduced was greater under condition D than under C.

TABLE II

TITRATION OF DIALDEHYDE DEXTRANS WITH IODINE AT 25°

Dialdehyde dextran	Iodine, reduced, ml.[a,b] (C) HCO_3^-, AsO_3^{---}, I^- [c]	Iodine, reduced, ml.[a,b] (D) HCO_3^-	AGU linked in dextran, %[d] 1,6-	1,4-like	1,3-like
B-512	0.12	0.33	95	5	0
B-523	.13	.42	85	11	4
B-742, S–R	.05	.32	58	18	24
B-1064	.12	.29	95	5	0
B-1355, S–R	.05	.56	57	8	35
Blank		.04			

[a] In excess of that required to reach the first end-point. Over a period of 30 minutes, 0.1 *N* iodine solution was added to bring the starch–iodine color back after each time it disappeared. The test solution contained the inorganic substances indicated plus dextran dialdehyde, each in concentration simulating a normal periodate oxidation test solution. [b] Usually 0.01 ml. of iodine is equivalent to 0.01–0.015 mole IO_4^-/mole AGU. [c] At 4°, all values in this column were 0.00. [d] Obtained by the standard periodate procedure.

Influence of Conditions on Oxidation of Soluble Dextrans.—No practical advantage was observed when five typical dextrans were oxidized at 4°, all other conditions being standard. At 17 days, the formic acid produced and periodate reduced were essentially the same as obtained at 96 hours by oxidation at 25°. All test solutions for measurement of periodate reduced were prepared at 4°; one set of duplicates was allowed to warm to 25° for titration with iodine, the second set was

(11) G. Hughes and T. P. Nevell, *Trans. Faraday Soc.*, **44**, 941 (1948).

(12) P. P. Fleury, J. E. Courtois and A. Bieder, *Bull. soc. chim. France*, 118 (1952).

(13) J. E. Taylor, THIS JOURNAL, **75**, 3912 (1953).

(14) J. C. Speck, Jr., private communication.

maintained at 4° until after titration with iodine was completed. Differences between the 4 and 25° titrations were of the same order as those obtained when these same dextrans were oxidized at 25°.

When standard conditions of periodate oxidation were modified by using 2 instead of 3 moles periodate per mole AGU, 96-hour values were comparable in all respects with those usually obtained at 72 hours for dextrans such as B-512 and B-1254 C-3, thus confirming previous observations.[2] For dextran B-1299 S-R this modification seemed to give a slight advantage, resulting in percentages of linkages as follows: (25°) 50, 50, 0; (4°) 50, 47, 3.

A tenfold increase or decrease in the dextran concentration in the oxidation mixture in which the ratio of 3 moles periodate per mole AGU was retained, resulted in proportionate changes in the rate and extent of reaction for a given reaction interval.

The *p*H range of about 5 to 3 in which our oxidations were carried out has been shown by other investigators to be most satisfactory for oxidation of their dextran preparation.[15]

After 72 or 96 hours of oxidation under standard conditions, the majority of water-soluble dextrans gave values of periodate reduced and formic acid produced which did not show any further significant change. Data for some representative dextrans, B-512, B-1254, B-1308 and B-1394, are shown in Table III. Three of the dextrans (B-512, B-742 (C), B-1254) showed no significant change even at the greatly extended reaction times of 192 and 240 hours. Other dextrans, such as B-742 (L) L-R, B-1399 and B-1422 had not reached constant formic acid values at 120 hours. The observation that for most dextrans reaction was essentially complete at 72 hours, and the necessity for uniformity in obtaining and reporting results on many dextrans, are the reasons for our use of 72-hour values in reporting all periodate oxidation results on dextrans. When more intensive investigation is conducted on individual dextrans, it would be advisable to adjust oxidation conditions in accordance with the properties of the specific dextran.

TABLE III

EFFECT OF LENGTH OF OXIDATION TIME ON ANALYTICAL VALUES

	Mole/mole AGU					
	Periodate reduced (25°)			Formic acid produced		
	Reaction time, hr.					
Carbohydrate	72	96	120	72	96	120
Dextran B-						
512-E		2.00	1.97[a]	0.947	0.952	0.952[b]
742 (C)		1.58	1.58[a]	.671	.681	.696[c]
742 (L) L-R	1.82	1.84	1.87	.793	.806	.813
1254		1.90	1.90[a]	.879	.891	.892[d]
1308	1.94	1.98	1.96	.949	.951	.952
1394	1.89	1.90	1.94	.923	.924	.924
1399	1.65	1.71	1.71	.651	.674	.682
1442	1.96	1.99	1.98	.937	.941	.952
Levan B-[e]						
512-E	0.99	1.00		0.008	0.006	
1072	1.32	1.33		.327	.338	
1386	1.00	1.02		.025	.029	
Levoglucosan	2.00	2.01	2.02	0.996	1.000	1.001

[a] At 192 hr.; value same at 240 hr. [b] At 192 hr.; value 0.954 at 240 hr. [c] At 192 hr.; value 0.699 at 240 hr. [d] At 192 hr.; value 0.894 at 240 hr. [e] The fructose contents (%) of these substances, measured by the resorcinol procedure,[8] were as follows: B-512-E, 96; B-1072, 60; B-1386, 95. By the anthrone procedure,[8] B-1072 contained 64%.

The periodate reduced values shown in Table III were measured at 25°. They did not always parallel increases in formic acid liberated, as is shown for dextrans B-742 (C) and B-1399. For other dextrans (B-512, B-742 (L) L-R, B-1308 and B-1422) the values are higher than is theoretically possible for anhydroglucopyranose units having only 1,6-, 1,4-like and 1,3-like linkages. For most of these dextrans, titration at 4° has been found to give more reasonable results.

Oxidation of Levans and Levoglucosan.—The oxidation of three levans appeared to be essentially complete at 72 or 96 hours (Table III). Two of these, from B-512-E and B-1072, respectively, were by-products from dextran preparations.[6] The other, from B-1386, was the major product from the bacterial culture. The fructose units appear to reduce only one mole of periodate; the formic acid apparently is from the glucose residues present. The fructose contents calculated from the periodate values show excellent agreement with the measured fructose contents given in Table III. Thus for B-512-E, the calculated percentage of fructose units is $[0.99-2(0.008)] \times 100 = 97$. These results permit the interpretation that fructofuranose units are present and linked through the C_2- and C_1- or/and C_6-positions.

Theoretical formic acid was obtained from our control, levoglucosan, at 96 hours reaction time, and no significant increase was observed at 120 hours.

Oxidation of "Water-insoluble" Dextrans.—Some dextran samples which were insoluble in water by normal treatment were obtained in water solution by very slow addition of cold water. This permitted comparison of results from oxidation of dextrans under heterogeneous and homogeneous conditions, as is shown for dextrans B-1144 and B-1384 in Table IV. The values obtained at 72 hours for the homogeneous solutions were in good agreement with those obtained at about 192 hours under heterogeneous conditions. The fact that for dextran B-1144 only the homogeneous solution had been autoclaved, did not appear to influence the agreement in results.

The water-insoluble dextrans differed in the extent to which liberation of formic acid leveled off, although in all cases the apparent reduction of periodate had practically ceased. This difference appears to be related to the particular dextran, since different preparations from the same strain were consistent in leveling off well (B-1139) or poorly (B-1299). The continued liberation of acid from dextrans such as B-1355, B-1299 and B-1433 might result from slow hydrolysis of sensitive linkages in the acidic solution and further oxidation of the products during the long period of observation. The water-soluble component of B-1299 dextran behaved similarly to the insoluble component (Table I).

Discussion

Interpretation of Results.—The dextrans used in this investigation had molecular weights of many

(15) G. Neumüller and E. Vasseur, *Arkiv Kemi*, **5**, 235 (1953).

TABLE IV

PERIODATE OXIDATION OF "WATER-INSOLUBLE" DEXTRANS

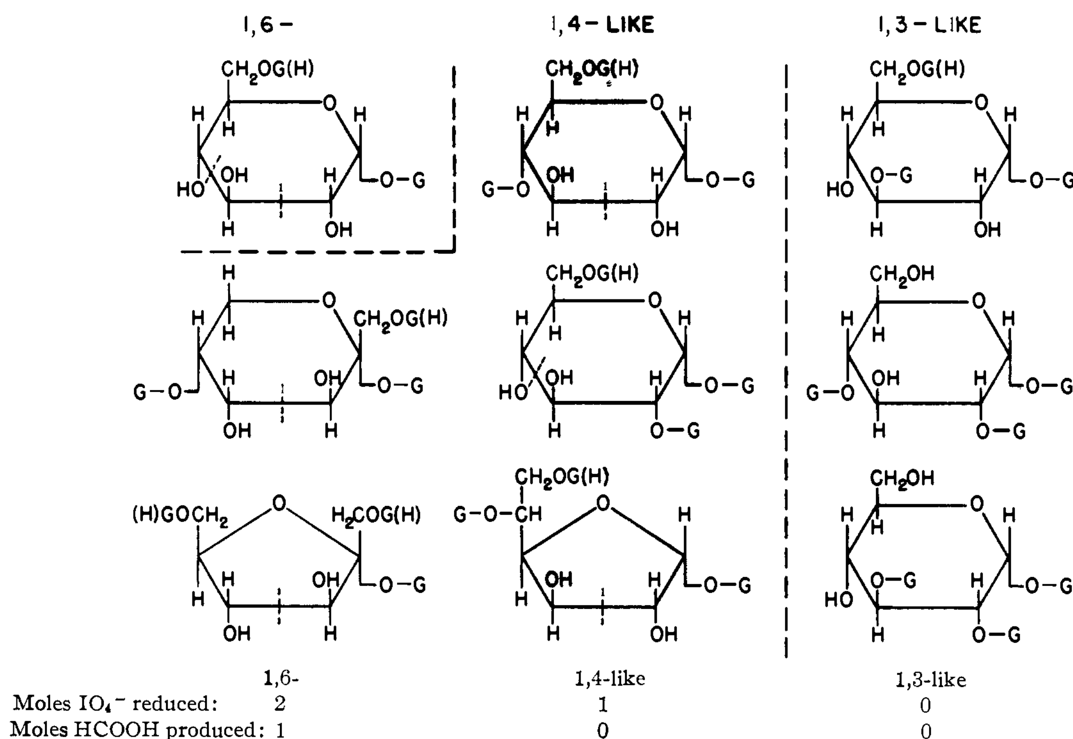
Dextran, B-	Oxidation system	Mole IO_4^-/mole AGU,[a] reaction time, hr. 72	144	192	240	Mole HCOOH/mole AGU, reaction time, hr. 72	144	192	240
1144	Homo.[b]	1.87	1.87			0.873	0.879	0.882	0.884
	Hetero.		1.79	1.83	1.83		.877	.881	.877
1384	Homo.	1.73		1.74	1.75	.828		.838	.840
	Hetero.	1.73		1.75	1.75	.811	.824	.838	.843
1118	Hetero.[b]			1.54	1.57			.756	.764
1121	Hetero.[b]			1.32	1.34			.652	.659
1139[c]	Hetero.		1.65	1.68	1.70		0.801	.810	.810
1139[c]	Hetero.		1.63	1.67	1.69		.796	.812	.812
1299, L-R	Hetero.[b]		1.49	1.53	1.56		.559	.584	.610
1299, A-2	Hetero.[b]			1.48	1.50			.538	.562
1355, A-5	Hetero.[b]			1.53	1.58			.687	.700
1433	Hetero.[b]			1.56	1.61			.630	.651

[a] Titrations were made at 25°. [b] The aqueous solution or mixture had been autoclaved before oxidation. [c] Different protein supplements were used in the culture medium for these two preparations.

millions. They were of high but not of absolute purity. Structural determination of these dextrans by the procedure of periodate oxidation reaction analysis employed in this work is made upon the whole sample and gives average results based upon the total composition. Alternative methods of analysis employing the techniques of methylation or of periodate oxidation product isolation,[4c,16] and with which our results are compared, have the apparent advantages of effecting some purification of the sample before the critical analyses are made. However, possible preferential losses counterbalance this advantage.

The data reported here give no information about the position of the various types of units within the polysaccharide molecule and, therefore, provide no measure or proof of branching. It merely can be inferred that units carrying non-1,6-linkages might constitute branch points because this has been found to be true for dextrans for which reliable methylation data are available.[17,18]

The action of sodium metaperiodate on dextrans differentiates only among three types of anhydrohexose units. Some structures representative of these types are shown in Fig. 1. Units glycosidically linked only through C_1 and/or C_6 can be determined specifically; those linked in other ways can be determined only in certain categories. Steric hindrance may give the same result as a C_3-bond, but it seems unlikely here.[16] In keeping with the limits of our present information, we assume the



	1,6-	1,4-like	1,3-like
Moles IO_4^- reduced:	2	1	0
Moles HCOOH produced:	1	0	0

Fig. 1.—Structures representative of the three anhydrohexose types differentiated by periodate oxidation: G = AGU

(16) M. Abdel-Akher, J. K. Hamilton, R. Montgomery and F. Smith, THIS JOURNAL, 74, 4970 (1952).

(17) Key references have been cited elsewhere.[2,3]

(18) J. W. Van Cleve, W. C. Schaefer and C. E. Rist, Abstracts Papers Am. Chem. Soc., 125, 8D (1954).

presence of only anhydroglucopyranose units, but they cannot be differentiated in all cases from fructofuranose units by periodate oxidation (Fig. 1). For polymers as large as native dextrans, the effect of the reducing end group can be neglected.

The non-reducing end groups are not differentiated from units linked through both C_1 and C_6, and the sum of these two types of units constitutes our so-called 1,6-linked units.

Tests have not been made for formaldehyde, which would be expected only from original or partially oxidized units having free hydroxyl groups at both C_5- and C_6-positions.

Expression of Results.—Usually the most suitable way to report the data on dextrans is in terms of the percentage of each type of unit present, as is shown in Tables I and II. At times, it has been desirable to express the results in terms of the ratio (R) of 1,6- to non-1,6-linked units as calculated from formic acid measurements.[2,5,7b,c] However, the relationship is not linear, and the sensitivity of R as an expression of the measured formic acid increases deceptively as R increases. This is shown by some typical values of moles of formic acid per mole AGU and the corresponding values of R, as follows: 0.500, 1.0; 0.600, 1.5; 0.700, 2.3; 0.800, 4.0; 0.810, 4.3; 0.900, 9; 0.910, 10; 0.950, 19; 0.955, 21; 0.960, 24; 0.970, 32. Values of R less than 7.0 are expressed to the first decimal place.

Calculated percentages of less than 2 for non-1,6-linkages are reported as "0," but were added to the percentage of the other type of non-1,6-linkage, which usually was a significant value.

Reliability of Results.—Recently the reliability of measurement of formic acid produced and periodate reduced during periodate oxidation of sugars and various polysaccharides has been questioned frequently. Formic acid values have been reported to be low,[11,19,20] and to include the effect of unidentified groups, presumably aldehydic, unless special techniques were used.[21] Periodate values have been reported to be high[11,14,20] and to vary with the method of analysis.[11,12,14] Active hydrogen atoms appear to reduce periodate[20] or iodine during analysis for periodate.[22] Some investigators have reported the Fleury–Lange method of periodate analysis unreliable,[11–14] others have preferred to use the Malaprade,[11–13] the Rappaport, *et al.*,[15,23] or the spectrophotometric[14,24] methods.

However, we have achieved accuracies within 1% in measurement of formic acid and periodate reduced (Fleury–Lange procedure) for our standard levoglucosan, and for isomaltose and isomaltotriose.[25] For dextrans, our formic acid values are precise to 0.004 mole per mole AGU, and our accuracy is within 1% on B-512 dextran as compared with results of methylation[18] and of periodate oxidation product isolation techniques.[4c,16]

For dextrans, the precision of our measurements for periodate reduced by the Fleury–Lange titration at 25° is ±0.02 mole per mole AGU. However, by typical analyses for B-512 dextran (1A,2A, Table I), the periodate reduced value is believed to be high by 5% since tentative data from methylation analysis on this dextran preparation indicate it has 5% 1,3-linked units but no 1,4-.[18]

This inaccuracy might be interpreted to indicate excessive reduction of periodate; however, production of excess formic acid did not occur. It did not result from interaction between dextran and periodate in the presence of arsenite–bicarbonate–iodide during preparation of the test solutions for periodate analysis. Our data indicate that this inaccuracy resulted in part from reaction of iodine with the periodate oxidized dextrans in the bicarbonate-buffered solution during analysis for periodate. This conclusion is supported by the fact that the apparent excessive reduction of periodate was not eliminated by carrying out the oxidation in the cold, or by combining in the cold the components of the test solution for periodate analysis. However, keeping the test solution at 4° during titration with iodine gave more stable end-points and almost invariably resulted in lower values of periodate reduced (Table I). The only exceptions were a few cases where titrations at 4° and 25° were identical. These observations cannot be explained by periodate being held unreactive in a complex as has been proposed for glucose oxidation mixtures.[11,14] Furthermore, when analysis for periodate was carried out in acid solution by use of thiosulfate, temperature had no consistent effect on the results and usually the results were in closer agreement with the 4° Fleury–Lange values than with the 25° values. This is shown for dextrans B-512, B-742, B-1146 and B-1355 in Table I. However, by the acid thiosulfate procedure, which is well known for its low accuracy,[13] we achieved lower precision and no greater accuracy than by the Fleury–Lange titration at 4°.

Further evidence that periodate oxidized dextrans react with iodine at 25° in bicarbonate-buffered solutions during back titration with iodine is provided by the fact that dialdehyde dextrans reduced iodine under the same conditions. In agreement with our observations during titration for periodate, the rate of reduction of iodine by the dextran dialdehydes appeared to be related directly to the percentage of 1,6- and inversely to the 1,3-like linked units in the dextrans. The rate of reduction was slower in the presence of arsenate and iodide. This sensitivity of 1,6-linked units to iodine probably has a common structural basis with the extensive over-oxidation produced in B-512 dextran by alkaline hypoiodite.[26] Additional indication that structure is a significant factor in the behavior of dextran periodate oxidation products toward iodine is provided by the lesser reactivity of the corresponding products from waxy corn starch, and especially of those from the levans studied.

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(20) M. L. Wolfrom, A. Thompson, A. N. O'Neill and T. T. Galkowski, THIS JOURNAL, **74**, 1062 (1952).

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(22) W. A. Bonner and R. W. Drisko, *ibid.*, **73**, 3699 (1951).

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(24) F. S. H. Head and H. A. Standing, *J. Chem. Soc.*, 1457 (1952).

(25) Allene Jeanes, C. A. Wilham, R. W. Jones, H. M. Tsuchiya and C. E. Rist, THIS JOURNAL, **75**, 5911 (1953).

(26) Allene Jeanes and C. A. Wilham, unpublished results.

Still further evidence for the validity of periodate oxidation measurements by the Fleury–Lange procedure at 4° is provided by infrared analysis, which shows "Type II" absorption[27] by dextrans proportionate to their content of 1,3-like linked units.[3] Infrared analysis indicates the presence of approximately 5% and 3% 1,3-like linked units in dextrans B-512 and B-1146, respectively, none in the dextran fractions B-742 (L) L-R and B-1254 C-3, and approximately 9% in B-1299 S-R. Thus, there is agreement between the results of infrared analysis and periodate oxidation measurements at 4° for all these representative dextran products except B-1299 S-R, which appears to be unusually susceptible to over-oxidation. Results comparable with these for B-1299 have been obtained for other dextrans (B-1298, B-1399, B-1402, B-1424) which have less than 75%, 1,6-linked units and no more than about 10% 1,3-like linked units.[3] The unusually sensitive structure in these dextrans might be 1,2-linked units.

(27) S. C. Burket and E. H. Melvin, *Science*, **115**, 516 (1952).

By comparison with preliminary methylation data for B-512 dextran, our results for non-1,6-links by 4° titration may still be off by 2–3% (runs 2, A-4 and 3, A-4, Table I). This appears to be the limiting error of the method and is believed to be due partly to over-reduction of periodate by B-512 as well as by many other dextrans, and partly to accumulation of errors in calculating by difference the content of 1,3-like linked units. The over-reduction of periodate can be diminished somewhat by decreasing the amount of excess periodate used in oxidations, especially for dextrans having low proportions of units linked 1,6.

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[CONTRIBUTION FROM THE CHEMICAL RESEARCH LABORATORIES OF SCHERING CORPORATION]

Simple Analogs of the Antiarthritic Steroids[1]

BY DOMENICK PAPA, HELEN F. GINSBERG AND FRANK J. VILLANI

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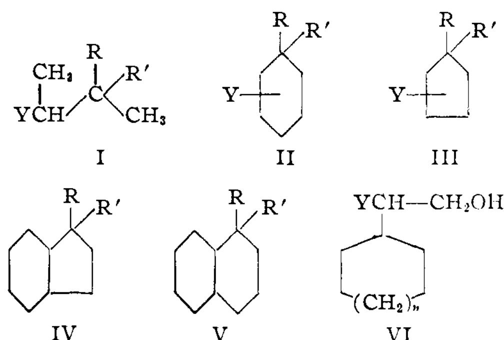
Five series of compounds representing fragments of the steroids proposed for and/or in clinical use as antiarthritic agents have been prepared. These simple analogs are for the most part aliphatic (comparable to the steroid carbons 11-17 inclusive) and alicyclic (rings C and D and ring CD) substances with substituents characteristic of the C_{17} moieties of the steroids.

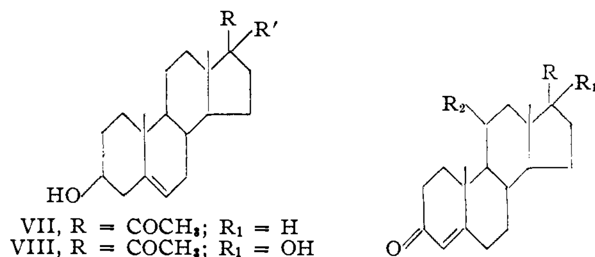
Shortly after the initial clinical successes with compound E and the investigations of related steroids as antiarthritic agents, we undertook the synthesis and the pharmacological evaluation of simple analogs of these compounds. The object of this investigation was to determine whether the dissection of the cyclopentanophenanthrene nucleus with the retention of the C-17 substituents of these steroids, as shown in formulas I–VI would yield relatively simple compounds retaining even to a small degree the pharmacodynamic action of the parent steroids. This approach in the case of other complex natural medicinal agents has indeed been fruitful in recent years.

At the time this study was initiated, the steroids which were proposed or in clinical study as antiarthritic agents were pregnenolone (VII), 17α-hydroxypregnenolone (VIII), desoxycorticosterone (IX), compound E (X) and the polyhydric adrenal cortical hormones of types XI and XII. The structures I–V denote the D and CD rings of these steroids as well as fragments thereof and the next higher cyclic homologs of these rings, the quantities R and R_1 representing the C-17 substituents of the steroids. Along with these five types, several representative glycols of formula VI were prepared in view of their relationship to XI and XII.

In the course of the preparation of compounds of type I–V, several syntheses were explored and in a



Y = H or lower alkyl, n = 0,1



VII, R = $COCH_3$; R_1 = H
VIII, R = $COCH_3$; R_1 = OH
IX, R = $COCH_2OH$; R_1 and R_2 = H
X, R = $COCH_2OH$; R_1 = OH; R_2 = O
XI, R = $CHOHCH_2OH$; R_1 = OH; R_2 = O
XII, R = $CHOHCH_3$; R_1 = OH; R_2 = O

number of instances the intermediates in these syntheses, on pharmacological examination, were shown to possess sedative-hypnotic and anticon-

(1) Presented in abstract before The Division of Medicinal Chemistry, American Chemical Society Meeting, Atlantic City, N. J., September 15, 1952.