[CONTRIBUTION FROM THE WOOD CONVERSION LABORATORY OF THE UNIVERSITY OF IDAHO]

A Study of Polysaccharide Hydroxylation Using *p*-Toluenesulfonyl Chloride and Triphenylchloromethane[1a]

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The study of structural configuration in the complex polysaccharides has been hindered seriously by a lack of suitable reactions and analytical methods. Practically the only accepted technique available even at the present time lies in the methylation procedure, developed and refined by Haworth and co-workers and others, followed by partial or complete hydrolysis of the methylated compound and the tedious analytical separation and identification of the components of the glycosidic sirup.

In many cases, particularly in the preliminary stages of an investigation, it is of distinct advantage and importance to learn the elementary details of the substance under consideration. This applies not only to solubility, reducing power, furfural yield and the like, but also to the character, number and type of the reactive hydroxyl groups present in the "basic" or "repeating unit" of the polymer.

At least two methods for differentiating between primary and secondary hydroxyl have been known for some time. Both have been subjected to justifiable criticism and the non-specificity of each is recognized. One method employs *p*-toluenesulfonyl chloride[2,3] which reacts rapidly with primary hydroxyl in pyridine solution; more slowly with the secondary type. Curiously enough, treatment of the tosyl derivative with sodium iodide in acetone results in replacement of tosyl by iodine only in the primary position.[3] The disadvantage of the method lies in obtaining complete reaction of the polysaccharide without dehydration or other degradation of the reaction product. A second method, wherein the carbohydrate is treated with triphenylchloromethane[4] in pyridine solution, has been shown non-specific for primary hydroxyl in a number of instances.[5,6,7] According to Hockett and co-workers,[7] the "preference" or "selectivity" of trityl chloride is a function of the relative reaction rates of the two hydroxyl types and that of the primary group is considerably greater than for the corresponding secondary.

In addition to the above objections, both methods usually yield amorphous reaction products whose purification is always a matter of some uncertainty.

Despite the obvious disadvantages, both reactions are of importance and, as will be shown, are remarkably reliable when carefully applied. In the present instance each method accurately confirms one part of the tentative structure proposed for arabo-galactan[8] as established through the methylation-hydrolysis technique.

The polysaccharide, a water-soluble gum separated from larch wood, *Larix occidentalis*, was treated in pyridine medium with *p*-toluenesulfonyl chloride under various conditions of time, temperature and moles of reactants. The reaction proceeds as a competition between primary and secondary hydroxyls for reagent, the more reactive primary group being rapidly substituted after which reaction rate decreases. The course of the reaction was followed by change in sulfur content of the derivative and the results of a number of experiments are given in Table I. The partially tosylated products were then treated individually with sodium iodide in acetone solution and the resulting compounds analyzed for iodine and sulfur. Using the arabogalactan repeating unit as basis, these analyses now permit calculation of primary hydroxyl content in the original compound expressed either as moles tosyl replaced or as moles iodine substituted. The results of a series of experiments are given in Table II and it is to be noted that, regardless of degree of esterification, tosyl replacement and iodine substitution remain constant within experimental error. This, of course, would not be true for tosyl derivatives of low sulfur content separated from the reaction before primary tosylation was complete. The average value of three moles iodine substituted confirms the previously reported structure for arabogalactan showing three primary hydroxyl groups at the terminal arabinosan and galactosan units of the repeating unit.

These experiments are again supported by the reaction of arabogalactan with triphenylchloromethane. The polysaccharide was treated with the reagent in pyridine medium and the reaction product separated as an insoluble precipitate by addition of excess acetone. The degree of trityl substitution was determined by change in carbon content of the product which was then acetylated in pyridine solution with acetic anhydride. In this manner the remaining hydroxyl groups of the trityl ether were esterified and the resulting compound was analyzed for acetyl content. Again using the arabogalactan repeating unit as a basis,

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(2) Freudenberg and Raschig, *Ber.*, **60**, 1633 (1927).

(3) Oldham and Rutherford, THIS JOURNAL, **54**, 366 (1932).

(4) Helferich, *Angew. Chem.*, **41**, 871 (1928).

(5) Hockett and Hudson, THIS JOURNAL, **56**, 945 (1934).

(6) Jackson, Hockett and Hudson, *ibid.*, **56**, 947 (1934).

(7) Hockett, Fletcher and Ames, *ibid.*, **63**, 2516 (1941).

(8) White, *ibid.*, **64**, 2838 (1942).

calculation of the molar ratio[9] trityl to acetyl gives 3:17.2, closely approximating the theoretical value 3:17.0. The ratio is of course dependent upon the increased reaction rate of the primary hydroxyl with trityl chloride as compared with that of the secondary type and it is significant to note in this connection that trityl substitution was always slightly lower than the theoretical value.

In view of these experiments it would seem reasonable to believe that both *p*-toluenesulfonyl chloride and triphenylchloromethane can be applied more generally to the study of complex polysaccharides. Complete esterification by the acid chloride is not a prerequisite for primary hydroxyl evaluation since the more reactive primary tosyl is replaced by iodine in subsequent reaction. Experimental results can be expressed either upon a repeating unit basis or in arbitrary units. On the other hand, the reaction of trityl chloride with the polysaccharide followed by acetylation necessitates no consideration of molecular weight basis since results are expressed as a molar ratio of more reactive to less reactive hydroxyl groupings.

Experimental

Isolation and Purification of Arabo-galactan.—Larch sawdust, *Larix occidentalis*, was extracted with the minimum quantity of water and the extract purified, after filtering through norite and Super-Cel, by fractional precipitation with ethyl alcohol according to a previously described method.[10] The white amorphous powder so obtained possessed the usual characteristics of solubility, furfural yield, etc., and was stored in a glass-stoppered bottle. Prior to reaction the polysaccharide was dried under reduced pressure at 50°.

TABLE I

REACTION OF ARABO-GALACTAN WITH *p*-TOLUENESULFONYL CHLORIDE

Temp., °C.	Time, hours	Tosyl chloride[a]	Yield, g.[b]	S, %	Tosyl sub., moles
55	5	28.94	2.3	11.42	8.8
55	10	28.94	2.4	12.06	10.0
55	18	28.94	2.5	12.78	11.5
55	28	28.94	2.5	13.18	12.5
55	38	28.94	2.7	13.42	13.2
70	1	28.94	1.7	8.20	4.7
70	5	28.94	2.3	11.19	8.4
70	10	28.94	2.5	12.18	10.2
70	16	28.94	2.5	12.72	11.4
70	37	28.94	2.8	12.32	10.5
90	1	28.94	1.8	9.34	5.8
90	5	28.94	2.2	11.80	9.5
90	10	28.94	2.3	12.10	10.0
90	14	28.94	2.4	12.40	10.7
90	38	28.94	2.6	11.92	9.7
55	21	28.94	...	13.05	12.1
55	21	60.00	...	13.29	12.7
55	21	99.85	...	13.43	13.1

[a] Moles tosyl chloride per mole arabo-galactan. [b] All products gave negative tests for chlorine and nitrogen.

Reaction of Arabo-galactan with *p*-Toluenesulfonyl Chloride.—Arabo-galactan was treated with pure *p*-toluenesulfonyl chloride under a variety of controlled conditions as indicated in Table I. In a typical experiment 1 g. of the polysaccharide was placed in a glass reaction tube and 20 cc. of anhydrous pyridine containing 5 g. of tosyl chloride added. The tube was sealed, shaken to mix the components and warmed in a rocking heater at 55° for twenty-eight hours. The reacting solution was then cooled and slowly precipitated into rapidly stirred ice water. Unreacted tosyl chloride thereby hydrolyzed to the water-soluble acid and the product was obtained as a flocculent precipitate. The latter was filtered, washed free from chloride ion and dried; yield, 2.5 g. (Found: S, 13.18. Calcd. tosyl substitution, 12.5 moles).

Iodination of Tosylated Arabo-galactan.—The above tosyl derivative was iodinated by treatment of 5 g. of ester (13.18 % S) dissolved in 50 cc. of acetone with 10 g. of sodium iodide. The reaction mixture was heated in a sealed glass tube for six hours at 100° and, after cooling, was filtered from the crystallized sodium *p*-toluenesulfonate. The solution was then dropped slowly into rapidly stirred ice water and the insoluble iodo derivative filtered, washed free from iodide ion and dried; yield, 4.8 g. (Found: I, 13.51; S, 10.23. Calcd. iodine substitution 3.08 moles; tosyl replacement 3.21 moles.)

The results of a series of experiments performed on tosyl derivatives prepared at 55° reaction temperature are given in Table II.

TABLE II

IODINATION OF TOSYLATED ARABO-GALACTAN

Tosyl derivative S, %	Iodinated tosyl derivative S, %	Iodinated tosyl derivative I, %	Tosyl replaced, moles	Iodine sub., moles
11.42	7.56	15.98	3.33	2.93
12.06	8.84	14.94	3.08	2.96
12.78	9.78	14.30	3.12	3.09
13.18	10.23	13.51	3.21	3.08
13.42	10.67	13.72	3.16	3.22

Reaction of Arabo-galactan with Triphenylchloromethane.—A variety of reaction conditions were investigated, the most satisfactory results being obtained when 3 g. of arabo-galactan was placed in a glass tube containing 20 cc. of anhydrous pyridine. Twelve grams of trityl chloride[11] dissolved in 40 cc. of pyridine was added and the tube sealed, shaken to give an opalescent colloidal solution and then warmed at 50° for twelve hours. The cooled reaction was dropped slowly into an excess of dry acetone and the resulting precipitate, settled in a centrifuge, washed free from chloride and carbinol with fresh acetone, was dissolved directly in pyridine. The latter solution was then precipitated into rapidly stirred ice water, washed to remove chloride ion and dried; yield, 5 g. (Found: C, 62.76; H, 6.59. Calcd. trityl substitution, 2.80 moles.) No explanation can be offered for the slightly lower than theoretical carbon content of the trityl ether. The value given is constant over rather widely varying experimental conditions and substitution therefore assumed complete.

Acetylation of Tritylated Arabo-galactan.—The above trityl derivative was found somewhat resistant to the action of acetic anhydride in pyridine solution and samples analyzed after twenty-four hours of treatment at room temperature gave low acetyl values. Such resistance was overcome by warming 2 g. of the ether dissolved in 5 cc. of pyridine with 5 cc. of acetic anhydride on a steam-bath for four hours. The reaction was then cooled and added dropwise to rapidly stirred ice water. Unreacted anhydride was thus hydrolyzed and removed from the insoluble product by filtering and washing. After drying, the crude acetate was dissolved in pyridine, precipitated as above, washed and dried; yield, 2.5 g. (Found: $COCH_3$, 27.2. Calcd. acetyl substitution, 15.6 moles.) Reacetylation under the same conditions and similar experiments per-

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[9] Calculation of molar ratio of course involves evaluation of trityl substitution in the trityl ether ester as calculated from the trityl ether analysis.

[10] White, THIS JOURNAL, **63**, 2871 (1941).

[11] Evans and Reynolds, *ibid.*, **60**, 2559 (1938).

formed in boiling pyridine-acetic anhydride, 1:1, gave products of the same acetyl content.

Summary

1. Two independent methods, one employing the use of *p*-toluenesulfonyl chloride and a second using triphenylchloromethane, have been employed successfully to confirm primary hydroxylation in the previously reported structure for arabogalactan.

2. It is suggested that both *p*-toluenesulfonyl chloride and triphenylchloromethane can be more generally applied in the study of polysaccharide hydroxylation.

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An Unexpected Rearrangement in the Application of the Skraup Reaction to 3-Nitro-4-aminoveratrole

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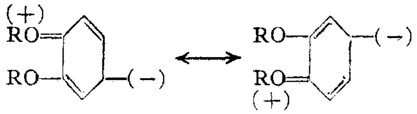
In the course of one of our antimalarial research projects, it became necessary to prepare 8-nitro-6,7-dimethoxyquinoline, and the synthesis which quite naturally suggested itself was the application of the Skraup reaction to 3-nitro-4-aminoveratrole, just as 8-nitro-6-methoxyquinoline is obtained from 3-nitro-4-aminoanisole in the manufacture of Plasmochin.

The preparation of the requisite 3-nitro-4-aminoveratrole from vanillin has been described by Pisovschi,[1b] and we had no difficulty in duplicating his results.

No complications were expected in subjecting this amine to the Skraup reaction, for this reaction has failed very rarely with primary aromatic amines. It is true, that, in the case of some nitroanilines, even when arsenic pentoxide is used as oxidizing agent, as recommended by Knueppel,[2] the yields are not infrequently quite unsatisfactory. In the manufacture of Plasmochin, the patents are significantly silent as to the yields in the Skraup step. Strukov,[3] who carried this out under special conditions, appears to have secured the best result (78%). So far as our own examination of the literature has shown, no molecular rearrangements have been observed in the application of this reaction.

In the veratrole series, 6,7-dimethoxyquinoline is readily synthesized from 4-aminoveratrole by this method.[4] The directive influence of the two methoxyl groups is shown by the formation of the 6,7- rather than 5,6-dimethoxyquinoline. The synthesis of this same quinoline from 6-aminoveratric acid by Goldschmidt,[5] involving simultaneous decarboxylation, is further evidence of this strong orienting property.

Arnold and Bordwell,[6] as a theoretical explanation of this directive influence, have postulated the existence of the following resonance forms for the veratrole molecule



In the experiments recorded beyond, when we subjected 3-nitro-4-aminoveratrole to standard Skraup reactions, using either nitrobenzene or arsenic pentoxide as oxidizing agent, strong or dilute sulfuric acid, and varying the temperature and length of heating, with the free amine or its acetyl derivative, no quinoline compound could be isolated.

When phosphoric (85%) was substituted for the sulfuric acid in the reaction mixture, an orange product was isolated which, crystallized from alcohol, melted at 172–174°, and on analysis gave figures for carbon, hydrogen and nitrogen, agreeing with those calculated for the initial nitro amine.

That these orange crystals were not identical with the brilliant red 3-nitro-4-aminoveratrole (m. p. 74°) was obvious from the m. p.'s of the two and of a number of their derivatives, and that the product actually separated (m. p. 172–174°) was the 5-nitro-4-aminoveratrole, and mixtures of this product with authentic samples of 5-nitro-4-aminoveratrole showed no change in the melting point.

To ascertain what conditions were requisite for this rearrangement, the arsenic pentoxide and the glycerol were omitted successively, and it was found that it could not be accomplished by heat alone (in tetralin solution), but that the best yield (30%) was obtained by digesting the 3-nitro isomer in a mixture of phosphoric (85%) and glacial acetic acids for one and one-half hours at 140–160°. There was always a great deal of carbonization in all of these experiments.

Superficially, this rearrangement can be explained by the migration either of the nitro group or of one of the methoxyls, but the mechanism of the change is obscure.

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[1b] Pisovschi, *Ber.*, **43**, 2137 (1910).

[2] Knueppel, *ibid.*, **29**, 703 (1896).

[3] Strukov, *Org. Chem. Ind.* (U. S. S. R.), **4**, 523 (1937).

[4] Frisch and Bogert, *J. Org. Chem.*, **8**, 331 (1943).

[5] Goldschmidt, *Monatsh.*, **8**, 343 (1887).

[6] Arnold and Bordwell, THIS JOURNAL, **64**, 2983 (1942).