was added dropwise to the reaction flask, and the mixture was refluxed seven hours. Continuous stirring was maintained. The reaction mixture was decomposed with ice and a little hydrochloric acid. The carbon disulfide layer was separated and washed with water. The aqueous layer was extracted with ether, and the extract was washed with water. The ether and carbon disulfide solutions were combined and dried over sodium sulfate. Removal of the solvent by distillation gave a dark red oil. High-boiling ligroin was added, and the mixture was cooled. A brown crystalline solid was obtained. The filtrate was diluted with ether, extracted with 10% sodium hydroxide solution and dried over sodium sulfate. The ether was removed, and the residue was distilled at atmospheric pressure. A small amount of propionitrile (b. p. 95–102°) and 10 g. of mesitylene (b. p. 160–164°) were obtained.

The brown solid was dissolved in 10% sodium hydroxide solution and the solution was extracted with ether, the aqueous solution was acidified and the solid was collected. This was crystallized by dissolving it in hot methyl alcohol, adding a little water and cooling. Further purification was effected by crystallization from benzene diluted with a little ligroin: yield 11.6 g.; m. p. 127–128°.

Anal. Calcd. for $C_{13}H_{15}ON$: C, 77.67; H, 7.5; N, 6.96. Found: C, 77.73, 77.35; H, 7.73, 7.68; N, 6.93, 7.15.

α-Methyl-β-methoxy-β-mesitylacrylonitrile (VIII).—Ten grams of α-cyanopropiomesitylene was dissolved in a solution of 16.6 g. of potassium hydroxide in 150 cc. of water. The solution was heated to boiling and 33.5 g. of methyl sulfate was added dropwise. The reaction mixture was boiled twenty minutes after the methyl sulfate was added. The mixture was cooled, a small amount of 10% potassium hydroxide was added and the mixture was extracted with ether. The ether solution was dried over calcium chloride and evaporated. The residue was crystallized from ligroin. Four grams of a solid melting at 78–81° was obtained. Recrystallization raised the melting point to 83–84°. The aqueous filtrate was acidified, and 2.3 g. of starting material was recovered.

Anal. Calcd. for $C_{14}H_{17}ON$: C, 78.10; H, 7.95; N, 6.51; OCH_3, 14.42. Found: C, 78.73, 78.26; H, 7.9, 7.97; N, 6.69; OCH_3, 14.8.

Summary

β-Methoxy-β-mesitylacrylonitrile (VI) and α-methyl-β-methoxy-β-mesitylacrylonitrile (VIII) have been prepared by methylation, respectively, of α-cyanoacetomesitylene (IV) and α-cyanopropiomesitylene (V). In each instance only O-methylation occurred.

Two forms—presumably *cis* and *trans* modifications—were obtained of VI; they form a solid solution when mixed in equal proportions.

URBANA, ILLINOIS RECEIVED FEBRUARY 26, 1938

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

The Oxidation of Sugar Alcohols by *Acetobacter suboxydans*[1]

BY RAYMOND M. HANN, EVELYN B. TILDEN AND C. S. HUDSON

Through the coöperation of Messrs. H. T. Herrick and P. A. Wells, of the Industrial Farm Products Research Division of the Bureau of Chemistry and Soils, U. S. Department of Agriculture, we have been enabled to test the oxidizing action of *Acetobacter suboxydans*, in submerged growth, upon a series of rare sugar alcohols and some related derivatives.

The optimum cultural conditions for oxidation of perseitol were first determined, and were then applied to the other oxidations. The substance to be tested was made up in a 2% aqueous solution containing 0.5% of Difco yeast extract, 0.3% potassium acid phosphate, and 0.05% glucose as nutrients, and then sterilized in 500-cc. Jena glass gas-washing bottles. A bacterial suspension of *A. suboxydans* was added, and sterile air passed through the culture at a rate of 200 cc. per minute, the temperature being maintained at 30°. Copper reduction values were determined on 2-cc. sub-samples by the Shaffer–Hartmann method after six and twelve days. The results are summarized in Table I.

The oxidation of *d*-α-mannoheptitol (perseitol) to perseulose, first investigated by Bertrand[2] using *A. xylinum* and obtained in 45% yields, has been shown by copper reduction studies, using crystalline perseulose as a standard, to be quantitative with *A. suboxydans*. It was possible to isolate the ketose in crystalline condition in a yield of 95%, and studies detailing the production of perseulose under various bacteriological conditions and confirming its structure as *l*-gala-heptulose have been completed.

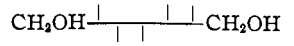
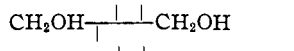
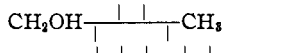
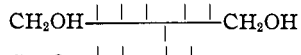
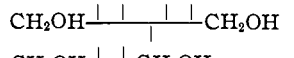
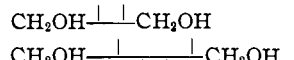
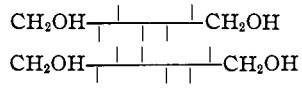
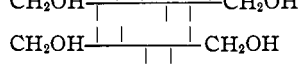
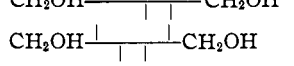
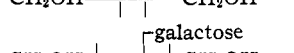
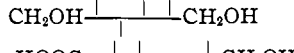
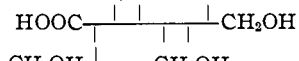
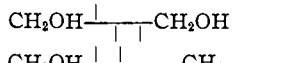
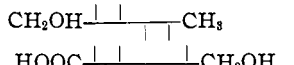
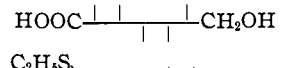
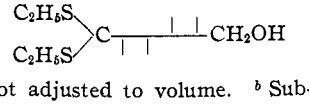
Oxidized solutions of *d*-arabitol, following removal of the bacteria and a lead acetate purification, gave a specific rotation of −30° in good agreement with the rotation of *d*-xylulose, quoted by Schmidt and Treiber[3] as −33.2°. Upon treatment with phenylhydrazine the oxidized

(1) Publication authorized by the Surgeon General, U. S. Public Health Service.

(2) Bertrand, *Compt. rend.*, **126**, 762 (1908).

(3) Schmidt and Treiber, *Ber.*, **66**, 1765 (1933).

TABLE I

Substance	Configuration of hydroxyl groups	Copper reduction values in mg. on 2-cc. sub-samples 6 days[a]	12 days[b]
d-α-Mannoheptitol (perseitol)		114	114
d-Arabitol		63	78
l-Fucitol		70	90
d-α,β-Glucooctitol		74	82
d-α-Glucoheptitol		62	72
meso-Erythritol		58	54
d-β-Galaheptitol		9	19
d-α,α-Galaoctitol		10	17
d-α-Galaheptitol		9	
Dulcitol			2
d-Lactositol			2
d-α,α-Galaoctonic acid			0.65
l-Arabitol			.65
l-Rhamnitol		No reduction	
d-α-Galaheptonic acid		No reduction	
d-Mannose diethyl mercaptal		No reduction	

[a] Sub-sample from oxidized solution not adjusted to volume. [b] Sub-sample from oxidized solution readjusted to volume of 200 cc.

solution yielded *d*-xylose phenylosazone. The physiological importance of this ketose, which is the enantiomorph of the sugar obtained in the urine in cases of pentosuria[4] has been cited by Larson and his co-workers.[5] The biochemical method of synthesis would seem to have many advantages over the preparation from *d*-xylose.[6]

The solution resulting from the oxidation of *l*-fucitol, after removal of the bacteria but without a lead acetate clarification, showed a specific rotation of −7°. This result was unexpected, since the configuration of fucitol is similar to that of dulcitol, which is reported to be unattacked by *A. suboxydans*.[7] Moreover, fucitol does not possess a configuration considered favorable for biochemical oxidation, if the generalization expressed by Bertrand for *A. xylinum* may be extended to include *A. suboxydans*.

Under the prescribed conditions *d*-α-glucoheptitol apparently is converted to *l*-glucoheptulose, the bacteria-free oxidized solution showing a specific rotation of −54°. Bertrand and Nitzberg[8] have oxidized this heptitol with *A. xylinum* and obtained crystalline *l*-glucoheptulose rotating −67.1°. This value corresponds with Austin's[9] recorded rotation of +67.5° for *d*-glucoheptulose prepared by the Lobry de Bruyn rearrangement of *d*-α-glucoheptose.

The oxidation of *d*-α,β-glucooctitol gave a solution whose specific rotation was −57°, indicating considerable conversion to a new substance, probably a keto-octose.

No effort was made to investigate the products of oxidation giving the lower copper reduction values, since the present study was preliminary in scope; however, in the oxidation of *d*-α-mannoheptitol certain changes in environment resulted in marked increase in oxidizing power, and it is hoped that further investigation will yield results with compounds which up to the present time have resisted biochemical oxidation. It appears that the optimal cultural conditions need to be determined for each sugar alcohol.

An interesting fact may be developed by con-

(4) Levene and LaForge, *J. Biol. Chem.*, **18**, 319 (1914).

(5) Larson, Blaterwick, Bradshaw, Ewing and Sawyer, *ibid.*, **117**, 719 (1937).

(6) Levene and Tipson, *ibid.*, **115**, 731 (1936).

(7) F. Visser't Hooft, Dissertation, Delft, 1925.

(8) Bertrand and Nitzberg, *Compt. rend.*, **186**, 925 (1928).

(9) Austin, THIS JOURNAL, **52**, 2106 (1930).

sideration of the configurations of some of the compounds studied. According to the generalization of Bertrand the favorable configuration for oxidation by *A. xylinum* is a *cis* arrangement of two secondary hydroxyl groups adjacent to a primary alcohol grouping. In Table I there are two pairs of *d*- and *l*-forms, namely, *d*- and *l*-arabitol and *d*- and *l*-α-mannoheptitol (since *d*-α-galaheptitol is the enantiomorph of *d*-α-mannoheptitol). In each of these pairs, only the *d*-form is oxidized readily.

For the four and higher carbon sugar alcohols the configuration which is oxidized readily is OH OH / C——C——CH_2OH / H H and its mirror image is either not oxidized at all or only at a slow rate. It seems possible that *A. suboxydans* is more specific than *A. xylinum* because, while both organisms require *cis* arrangement of the two hydroxyl groups indicated above, *A. xylinum* acts when the *cis* pair is above or below (*e. g.*, sorbitol (above) and *l*-arabitol (below)), whereas *A. suboxydans* does not act if the *cis* hydroxyls are below (*e. g.*, *l*-arabitol (no action) and *d*-arabitol (oxidized)). The behavior of *l*-fucitol must be studied further before deciding what generalization may apply to the alcohols derived from the methylose sugars.

We are indebted to Dr. W. D. Maclay for preparing many of the rare sugar alcohols used in the present investigation. Further investigations of the biochemical oxidations are under way in the Division of Chemistry of the National Institute of Health.

Summary

The oxidizing action of *Acetobacter suboxydans* on a considerable number of sugar alcohols and related carbohydrate derivatives has been tested in a preliminary survey. The present results indicate a specific relationship between configuration of the substrate and oxidizing attack. Perseitol is oxidized to perseulose, which is obtained as the crystalline sugar in nearly quantitative yield. *d*-Arabitol is oxidized to *d*-xylulose, but *l*-arabitol is not attacked. *l*-Rhamnitol is not oxidized, but *l*-fucitol gives a reducing substance in large amount, presumably a new ketose. *d*-α-Glucoheptitol readily yields what is presumably *l*-glucoheptulose. The study is being continued.

WASHINGTON, D. C. RECEIVED MARCH 24, 1938

[CONTRIBUTION FROM THE CELLULOSE DEPARTMENT, CHEMICAL FOUNDATION, BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.]

The Dimolar Tosylation of β-Methylcellobioside: α-Cellobiomethylose

BY JACK COMPTON

In a previous communication[1] it has been shown that the unimolar tosylation of α- and β-methylglucosides in pyridine solution with tosyl chloride, followed by acetylation, results in the formation of 6-tosyl-triacetyl-α-methylglucoside in 36% yield and 6-tosyl-triacetyl-β-methylglucoside in 41% yield, respectively. Extension of this reaction to other alkylglycosides may serve as a convenient method for obtaining the ω-desoxy sugars, since the tosyl group in the primary position may be replaced by an iodo group[2] which is reduced easily.

The present investigation is concerned with the relative reactivity of the primary hydroxyl groups in β-methylcellobioside as shown by treatment in pyridine solution with tosyl chloride. Also, in applying the general procedure suggested above for the production of ω-desoxy sugars, a new disaccharide (cellobiomethylose) has been obtained in crystalline form.

The dimolar tosylation of β-methylcellobioside in pyridine solution with tosyl chloride, followed by acetylation, resulted in formation of 6,6′-ditosylpentaacetyl-β-methylcellobioside in 67% yield. The structure of this compound may be considered proved since it is identical with that obtained by Helferich, Bohn, and Winkler,[3] upon tosylating 2,3,4,2′,3′-pentaacetyl-β-methylcellobioside which in turn was prepared from 6,6′-ditrityl-pentaacetyl-β-methylcellobioside. In comparison with α- and β-methylglucosides, under comparable conditions, it may be stated that the primary hydroxyl groups in β-methylcellobioside, are considerably more reactive.

Reduction of 6,6′-diiodopentaacetyl-β-methylcellobioside, obtained by treating 6,6′-ditosylpentaacetyl-β-methylcellobioside with sodium io-

(1) J. Compton, THIS JOURNAL, **60**, 395 (1938).

(2) J. W. H. Oldham and J. K. Rutherford, *ibid.*, **54**, 366 (1932).

(3) B. Helferich, E. Bohn, and S. Winkler, *Ber.*, **63**, 989 (1930).