have as the second substituent a hydrogen atom. The basis for this statement, as well as the basis for the structure assigned to the free radical formed in step 3, will be presented in our next publication dealing with this interesting reaction.

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RECEIVED SEPTEMBER 14, 1945

CHROMATOGRAPHY OF CARBOHYDRATES AND SOME RELATED COMPOUNDS

Sir:

We wish to report that we have developed a method for the chromatography of the carbohydrates and related polyhydroxy compounds. Columns of adsorptive clays were used as adsorbents; an example is a Florida clay sold by the Floridin Co. of Warren, Pennsylvania, under the name Florex XXX. As developer we have used various low molecular weight hydrophilic solvents such as the alcohols, hydroxylic ethers, ether, dioxane, ketones, acids, pyridine, and water, either singly or in admixture.

Streak reagents that have been used on the extruded columns to detect the zones are alkaline permanganate, 2,6-dichlorophenolindophenol and acid-base indicators. Water was the eluting agent.

By the use of this method we have arranged a large number of the members of this class of compounds in an adsorption series. This chromatographic sequence shows the position each compound occupies on the adsorption column in relation to the other members. We have included in this series three pentoses, nine hexoses, two heptoses, six disaccharides, two trisaccharides, one tetrasaccharide, two other oligosaccharides, four glycosides, twenty polyhydric alcohols, seven polyhydric alcohol inner ethers, and ten acids, lactones or salts. Several positions in the sequence are occupied by more than one compound with the degree of resolution currently in use.

As examples of the applicability of this method the following are given. On chromatographing a mixture containing equal amounts of sorbitol and D-glucose, the separated D-glucose zone showed the presence of 100% of the added sugar by a reducing sugar determination, and the sorbitol zone showed 99.7% of the sorbitol by periodic acid oxidation. The sorbitol zone gave a negative reaction toward Fehling solution. A mixture containing 98% sorbitol and 2% D-mannitol was chromatographed to exhibit the presence of two zones. A mixture of equal amounts of D-mannitol and dulcitol was chromatographed and each component eluted and crystallized. The first crop of D-mannitol gave a recovery of 90% with m. p. 166–167°; a second crop gave a further 6% with the same melting point. The accepted value for the m. p. of D-mannitol is 166°. The first crop of dulcitol gave 95% recovery with m. p. 187–188° (accepted value 188°). A mixture, in disproportionate amounts, of 1,4:3,6-dianhydrosorbitol, 1,4:3,6-dianhydro-D-mannitol, and 1,4:3,6-dianhydro-L-iditol was chromatographed to show three zones. The rate of lactonization and delactonization of D-gluconic acid was followed qualitatively since the acid and the lactones occupied different positions on the column. Lemon juice was chromatographed and the ascorbic acid content was concentrated into a zone detectable by 2,6-dichlorophenolindophenol.

Work is being continued on this method and details will be communicated at a later date.

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THE CONFIGURATION OF THE PYRANOSE RINGS IN POLYSACCHARIDES

Sir:

If one accepts the assumption that the C–C and C–O bond angles and distances occurring in polysaccharides are those found in simple molecules, then two *trans* configurations are possible for a pyranose ring. The important difference between these two *trans* configurations is the angle which the C_1- and C_4-to-glycosidic-oxygen bond makes with the "plane" of the pyranose ring.

In the present discussion we are concerned only with those polysaccharides in which the glycosidic oxygens are *trans* to one another. This includes polymers of β-glucose (cellulose), β-mannuronic acid (alginic acid) and α-galacturonic acid (pectic acid). With this restriction it follows, then, that in one *trans* configuration of the pyranose ring (I) the two carbon-glycosidic bonds make an angle of about 20° with the "plane" of the pyranose ring. In the other *trans* configuration (II) this angle is about 90°. This difference in angle results in a difference in the fiber identity period when units of the same configuration are joined together to make a long chain. A chain made with I has a projection per pyranose unit in the direction of the fiber axis equal to 5.15 Å., while the projection for a chain made from II is 4.37 Å.

It is now generally accepted that the pyranose rings in cellulose are I.[1] Recent X-ray studies on fibers of sodium pectate[2] and alginic acid[3]

(1) Astbury and Davies, *Nature*, **154**, 84 (1944); Cox, *ibid.*, **154**, 84 (1944).

(2) Palmer and Lotzkar, THIS JOURNAL, **67**, 883 (1945); Palmer and Hartzog, *ibid.*, in press.

(3) Astbury, *Nature*, **155**, 667 (1945). We wish to express our thanks to Dr. Astbury for kindly sending us a copy of this paper before publication.

indicate that in these two compounds the pyranose rings probably have the configuration II. Astbury[3] suggests that there is reason to believe that the pyranose rings can change from type I to II and *vice versa*, and suggests that such may occur in certain derivatives of cellulose. In this connection some recent results obtained in this Laboratory are of interest.

We have been making a rather extensive X-ray study of pectin and its derivatives, as well as other uronic acid-containing compounds including sodium alginate. Of interest to this discussion is the fiber identity period of 15.0 Å. obtained for sodium alginate from oriented fibers. Although fiber photographs of pectic acid are more difficult to interpret, it can be established that the fiber identity period is very close to 13.0 Å. The conclusions to be deduced from these values as well as the value 13.1 Å. for sodium pectate[2] and 8.7 Å. for alginic acid[3] are summarized in Table I.

TABLE I

	Fiber period	Symmetry of chain	Projection per unit along fiber axis	Type of pyranose ring
Alginic acid	8.7	2-fold	4.37	II
Sodium alginate	15.0	3-fold	5.0	I
Pectic acid	13.0	3-fold	4.3	II
Sodium pectate	13.1	3-fold	4.37	II
Cellulose	10.3	2-fold	5.15	I
Soda cellulose-II[4]	15.4	3-fold	5.13	I

Column 4 of Table I shows that the identity period per unit falls into two classes, the value of which agrees with the calculated value for the two *trans* configurations of the pyranose ring. In addition, two other points are of particular interest: (1) the galacturonide chain would appear less flexible than the others, since in both pectic acid and sodium pectate the screw symmetry remains three-fold; and (2) alginic acid and sodium alginate not only have chains of different screw symmetry but the pyranose rings apparently have different configurations.

(4) Gundermann, *Z. physik. Chem.*, **37B**, 387 (1937).

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RECEIVED SEPTEMBER 4, 1945

STREPTOMYCES ANTIBIOTICS. II. CRYSTALLINE STREPTOMYCIN TRIHYDROCHLORIDE–CALCIUM CHLORIDE DOUBLE SALT

Sir:

A crystalline double salt of streptomycin trihydrochloride and calcium chloride has been obtained. Streptomycin appears to have the composition $C_{21}H_{37}N_7O_{12}$.

Observations made during chromatographic purification of crude streptomycin concentrates led to experiments which yielded a crystalline double salt of streptomycin trihydrochloride and calcium chloride. This salt has certain advantages over the previously described non-crystalline streptomycin hydrochloride.[1] Since it can be crystallized, the double salt is obtained as a product with constant biological, chemical, and physical properties. In this respect it is more satisfactory than the hydrochloride, which is obtained by precipitation. The double salt can be prepared from streptomycin hydrochloride or from the crystalline streptomycin helianthate.[1] The latter salt is preferred for preparative work and a typical experiment follows.

Seven grams of calcium chloride in 50 ml. of methanol was acidified with 3 drops of concd. hydrochloric acid and added to 30 g. of streptomycin helianthate suspended in 900 ml. of methanol. The insoluble calcium helianthate was removed by filtration. The filtrate, concentrated *in vacuo* to 75 ml., deposited colorless crystals overnight. The crystals (9.2 g.) were dried at 100° *in vacuo;* activity, about 750 units/mg.; $[\alpha]^{25}D$ −76° (*c*, 1% in water). They decomposed between 200–230° on the microblock.

Anal. Calcd. for $(C_{21}H_{37}N_7O_{12}\cdot 3HCl)_2\cdot CaCl_2$: C, 33.88; H, 5.42; N, 13.17; Cl, 19.05; Ca, 2.69. Found: C, 33.73; H, 5.78; N, 13.18; Cl, 18.65; Ca, 2.76.

A second crop (2.3 g.) of crystals with identical properties was obtained. Recrystallization of another sample with identical properties from methanol–ethanol gave crystals showing unchanged activity and rotation.

Anal. Found: C, 33.67; H, 5.79; N, 13.13; Cl, 19.48; Ca, 2.87.

Analytical data on streptomycin hydrochloride and helianthate,[1] which are also in agreement with the above formulation, follow:

Anal. Calcd. for $C_{21}H_{37}N_7O_{12}\cdot 3HCl$: C, 36.61; H, 5.85; N, 14.23; Cl, 15.44. Found: C, 36.80; H, 6.09; N, 14.39; Cl, 15.59.

Anal. Calcd. for $C_{21}H_{37}N_7O_{12}(C_{14}H_{15}N_3O_3S)_3$: C, 50.59; H, 5.53; N, 14.99. Found: C, 50.53; H, 5.83; N, 14.81.

At present, the formula for streptomycin appears to be $C_{21}H_{37}N_7O_{12}$. However, it is possible though improbable that the number of hydrogen atoms is 39 instead of 37. The tentative formula $(C_{10}H_{19}N_3O_{7-8})_n$, suggested by Fried and Wintersteiner,[2] is not in agreement with our present data. A cryoscopic molecular weight determination on streptomycin trihydrochloride in water gave about 800 for the free base (calcd., 580). Necessary corrections for the chloride ion and the non-ideal cryoscopic behavior of the trivalent streptomycin ion were made. The uncertainties seem sufficient to account for the observed disparity.

The extension of this work to other inorganic salts and to streptothricin will be described later

(1) Kuehl, Peck, Walti and Folkers, *Science*, **102**, 34–35 (1945)
(2) Fried and Wintersteiner, *ibid.*, **101**, 613–615 (1945)