POLYCONDENSATION OF D-GLUCOSE AND OTHER SIMPLE SUGARS IN PRESENCE OF ACIDS

Sir:

The acid-catalyzed hydrolysis of polysaccharides to simple sugars is a widely known process of both scientific and industrial importance. The reverse action produced by acids on monosaccharides has been studied by a number of investigators, since Emil Fischer in 1890 isolated the osazone of "isomaltose" from a 25% solution of D-glucose in concentrated hydrochloric acid. The products of such "reversion" or condensation reaction in solution are mostly disaccharides, the reaction representing a pseudo-unimolecular change similar to the hydrolysis of disaccharides (Harrison, 1914; Moelwyn-Hughes, 1928). However, formation of "polyoses" with fairly high degree of polymerization (D.P.) should be possible, provided that water could be completely, or nearly so, eliminated from the sugar–water–acid system, and provided that excessive degradation caused by the acid would be avoided.

In this preliminary communication we wish to report that we have succeeded in establishing optimal conditions for the polycondensation of the simple mono- and disaccharides such as D-glucose, D-mannose, D-fructose, L-arabinose, maltose and cellobiose, and mixtures of these sugars. Essentially, our procedure consists in concentration by rapid evaporation under greatly diminished pressure at 0 to 45° of a concentrated (about 50%) sugar solution in about 5% hydrochloric acid to a dry and brittle, glassy product, in which part of the hydrogen chloride remains entrapped.

In the case of D-glucose, subsequent dialysis in a cellophane bag for six days against running tap water of an aqueous solution of "D-glucopolyose" resulted, after concentration and precipitation with methanol, in a colorless powder; yield 15 to 20%. The product had $[\alpha]^{20}D$ 108.0° (in water), and showed an absorption maximum, log I_0/I = 0.471, 1 cm. cell) at 280 mμ in a 2.17% solution. On heating in a capillary tube, it decomposed between 230 and 250° to a brown voluminous mass. Its osmotic molecular weight corresponded to D.P. 42 (by extrapolation to infinite dilution of the osmotic pressures measured at various concentrations). In cuprammonium solution it had an intrinsic viscosity, $[\eta]$ = 0.14, corresponding to D.P. 37 (using Kramer's constant, Km = 260). The product barely reduced hot Fehling solution, but both salivary enzyme and "hemicellulase" (Rohm and Haas Company, Philadelphia) showed definite activity. The rate constant for acid hydrolysis to D-glucose (osazone isolated) at 100° in 0.1 N hydrochloric acid was $k = 2.4 \times 10^{-3}$ (log, min.) calculated from polarimetric measurements and from changes in reducing power. Acetylation by the pyridine method gave rise to a colorless, amorphous material. Methylation yielded a light colored solid which, after methanolysis and hydrolysis, contained 2,3,6-trimethyl- and 2,3,4,6-tetramethyl-D-glucose (identified by paper chromatography). Since an authentic sample of 2,3,4-trimethylglucose did not satisfactorily develop in the chromatogram, the presence of this substance in the acid hydrolysate is not excluded.

By means of our procedure a large number of synthetic polysaccharides has become available for chemical, enzymatic, immunological and industrial investigation. Detailed description of our experiments will be published after completion of the current structural examination.

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THE ISOLATION OF NEOMYCIN B

Sir:

Paper chromatograms have indicated that the neomycin isolated in our laboratory from elaboration products of *Streptomyces fradiae*,[1] contains at least two biologically active components. The predominant antibiotic, isolated as the crystalline helianthate salt, has been shown to be different from the neomycin A hydrochloride reported by Peck, *et al.*,[2] and has been designated neomycin B. Neomycin B sulfate, regenerated from the crystalline helianthate by means of triethylamine sulfate, is a homogeneous single compound and assays about 215 neomycin units per milligram against a strain of *B. subtilis* and 260 neomycin units per milligram against a strain of *K. pneumoniae* using the turbidimetric streptomycin procedure and a standard supplied by Dr. Waksman.

The activity of neomycin A hydrochloride, described by Peck, *et al.*,[2] is 1700 neomycin units per milligram against a strain of *B. subtilis*. In view of the differences in biological activities of neomycins A and B, the *p*-(*p'*-hydroxyphenylazo)-benzenesulfonate salt of neomycin B was prepared from our neomycin complex by the method described by Peck, *et al.*[2] As in the case of the helianthate, a high proportion of neomycin B was obtained as the homogeneous dye salt. Repeated recrystallizations from 50% methanol showed little variation in analysis. After three recrystallizations, the dye salt was dried *in vacuo* at room temperature; $[\alpha]^{25}D$ +30° (*c*, 0.5 in methanol).

Anal. Found: C, 46.20, 46.19; H, 5.33, 5.37; N (Dumas), 10.12, 10.14; S (Carius), 8.33, 8.38.

By paper chromatography, it was shown that the neomycin B sulfate, $[\alpha]^{25}D$ +58° (*c*, 0.5 in water), regenerated from recrystallized *p*-(*p'*-hydroxyphenylazo)-benzenesulfonate salt by means of triethylamine sulfate in methanol, was a single active substance. It gave 255 neomycin units per milligram against *K. pneumoniae* and 220 neomycin units per milligram against *B. subtilis*.

(1) Waksman and Lechevalier, *Science*, **109**, 305 (1949).

(2) Peck, Hoffhine, Gale and Folkers, THIS JOURNAL, **71**, 2590 (1949).

Anal. Found: C, 29.35, 29.45; H, 6.86, 6.92; N (Dumas), 9.21; $SO_4^=$ (as barium sulfate), 28.26, 28.44.

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STEROIDS. II.[1] A METHOD FOR THE CONVERSION OF *ALLO*-STEROIDS INTO Δ^4-3-KETOSTEROIDS

Sir:

The current interest in corticosteroids as possible therapeutic agents in arthritis has made the availability of starting materials extremely important. Since the majority of the *abundant* steroidal plant sapogenins, representing a potentially unlimited source for 20-keto-pregnanes, either belong to the *allo* series or possess a Δ^5-3-hydroxy grouping, which in turn is convertible in nearly quantitative yield into the 3-keto*allo*steroid system (I), it has become an urgent matter to develop a general procedure for the transformation of I into the essential Δ^4-3-keto moiety.

We have observed that while 3-keto-4-bromosteroids of the *normal* series (rings A/B *cis*) do not react with sodium iodide in acetone solution, 2-bromo-3-keto*allo*steroids (rings A/B *trans*) readily react to yield the corresponding iodo derivatives, which on treatment with zinc dust in ethanol, chromous chloride in acetone, or even short *boiling with collidine* regenerate the saturated 3-keto*allo*steroids. When applied to 2,4-dibromo-3-keto*allo*steroids (II), obtainable in high yield from I, short boiling with sodium iodide affords a 2-iodo-4-bromo-3-keto*allo*steroid (*e. g.*, 2-iodo-4-bromoandrostan-17-ol-3-one 17-hexahydrobenzoate, m. p. 146–149°. Calcd. for $C_{26}H_{38}O_3BrI$: C, 51.58; H, 6.33. Found: C, 51.71; H, 6.35), which on refluxing with collidine suffers simultaneous dehydrobromination and deiodination to lead directly to the required Δ^4-3-ketosteroid. Even more strikingly, if II is refluxed with sodium iodide in acetone solution for twenty hours, there is obtained in good yield a 2-iodo-Δ^4-3-ketosteroid (III), which is smoothly transformed to the Δ^4-3-ketosteroid. The generality of this method has already been demonstrated in five diverse instances in this Laboratory and will be exemplified here by the preparation of the important adrenal hormone 17α-hydroxyprogesterone.

N-Bromoacetamide oxidation of *allo*pregnane-3β,17α-diol-20-one[2] gave a high yield of *allo*pregnan-17α-ol-3,20-dione (IV) (m. p. 251–253°, $[\alpha]^{20}D$ + 24°), which on dibromination in acetic acid led to the 2,4-dibromo derivative (V) (m. p. 183–185° $[\alpha]^{20}D$ 0°. Calcd. for $C_{21}H_{30}O_3Br_2$: C, 51.44; H, 6.17. Found: C, 51.64; H, 5.88). Twenty hours of refluxing with sodium iodide in acetone yielded 2-iodo-17α-hydroxyprogesterone (m. p. 112–115°, $[\alpha]^{20}D$ + 71°, maximum 244 mμ (log E 4.15). Calcd. for $C_{21}H_{29}O_3I$: I, 27.81. Found: I, 28.32), which without isolation on deiodination afforded 17α-hydroxyprogesterone (VI) (m. p. 220–222°, $[\alpha]^{20}D$ + 103° (acetone), maximum 241 mμ (log E 4.30)). The present method, in addition to ready availability of starting materials and good yields, has the marked advantage over the corresponding *normal* ketones in that from each saturated 3-keto*allo*steroid (*e. g.*, IV), three unsaturated ketones of interest for clinical trial can be prepared: in addition to VI, V on collidine treatment afforded the interesting $\Delta^{1,4}$-pregnadien-17α-ol-3,20-dione (m. p. 232–234°, $[\alpha]^{20}D$ + 38.5°, maximum 244 mμ (log E 4.14). Calcd. for $C_{21}H_{28}O_3$. C, 76.79; H, 8.59. Found: C, 76.96; H, 8.21), while the monobromination product of IV on dehydrobromination yielded the Δ^1-isomer of VI, Δ^1-allopregnen-17α-ol-3,20-dione (m. p. 254–257°, $[\alpha]^{20}D$ + 71°, maximum 230 mμ (log E 4.05). Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.49; H, 9.33).

Details, applications and extension of this method to other cortical hormones and analogs will be reported shortly.

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RECEIVED DECEMBER 5, 1949

NITROGEN FIXATION IN AN ULTRASONIC FIELD

Sir:

Our observations on the oxidative fixation of molecular nitrogen in ultrasonic field have led to results which are of special interest in the connection of biological nitrogen fixation.

The experiments were carried out in water solution at ordinary pressure; radiating surface of the vessel was 42 mm. in diameter; radiation intensity in the radiation point was ~10 W/sq. cm., frequency 300 kc./sec. The hydrogen and nitrogen gases were led to the other side of the solution at the rate of about 1 l./min., carbon monoxide gas 0.4 l./min. Thus, oxygen was present in the solution in each experiment.

The nitrogen fixation in ultrasonic field does not, at least essentially, depend on the hydrogen ion concentration of the solution as far as the total amount of fixed nitrogen, nitrite *plus* nitrate N (other N-compounds have not been found), is considered. On the other hand, the mutual relation of nitrite and nitrate N is decided by the *p*H of the solution (Figs. 1 and 2). These results explain the observation of Loiseleur[1] on the rapid

(1) Paper I, THIS JOURNAL, **71**, 3689 (1949).

(2) Kritchevsky and Gallagher, *J. Biol. Chem.*, **179**, 507 (1949).

(1) Loiseleur, *Compt. rend.*, **218**, 876 (1944).