series of monochloro-, bromo-, and iodonitrobenzenes using both reducing monosaccharides and disaccharides in alkaline medium.

Before carrying out reduction studies on halogenated nitrobenzenes, some preliminary reductions were performed on nitrobenzene with the reducing sugars galactose, fructose, lactose, and maltose, in order to compare the effectiveness of these reducing agents. Azoxybenzene was obtained in fair yield using galactose and fructose and no aniline was isolated. With the disaccharides a lower yield of azoxybenzene was obtained, together with some aniline. The results are given in Table I.

In this study, the monosaccharides were employed as mild reducing agents for the preparation of dihalogenated azoxybenzenes. It was expected that the reducing monosaccharides would be equally effective as reducing agents because of their similar reducing powers. The ketose, fructose, should have similar reducing power to that of the aldoses. The disaccharides, lactose and maltose, having less reducing power than the monosaccharides were expected to be milder reducing agents. The results obtained from the reduction of chloro-, bromo-, and iodonitrobenzenes with various sugars are shown in Tables II and III.

CONCLUSIONS

Comparison of the effectiveness of the reducing sugars with regard to the preparation of dihalogenated azoxybenzenes from monohalogenated nitrobenzenes leads to some interesting conclusions. Preliminary experiments on the reduction of nitrobenzene showed that monosaccharides were stronger reducing agents for the preparation of azoxybenzene than the disaccharides.

For the first time, galactose, fructose, and mannose were used as reducing agents for halogenated nitrobenzenes. The main product of these reductions was the dihalogenated azoxybenzene and in some cases the amine was formed. Monosaccharides were found to be the best reducing agents for the preparation of dichloroazoxybenzenes. 3,3′-dichloroazoxybenzene was obtained in higher yield than 4,4′-dichloroazoxybenzene regardless of the sugar employed. Similar results were obtained with the reductions of the bromonitrobenzenes. Reductions of 4-chloro- and 3-bromonitrobenzene with the disaccharides led to substantial yields of the halogenated anilines.

Reduction of 2-iodonitrobenzene to the azoxybenzene was not easy—only glucose and maltose gave any of this substance. For the preparation of 3,3′-diiodoazoxybenzene, lactose and maltose were good reducing agents; on the other hand, 4,4′-diiodoazoxybenzene was obtained in highest yield when using glucose and galactose.

The 2-halogenated nitrobenzenes were the most difficult to reduce. The nitro group in 2-bromo- and 2-iodonitrobenzene is sterically hindered and these compounds are not very stable in the reducing medium, thus reduction is rendered difficult as shown by the low yields of the azoxybenzenes, the lower recovery of unreacted starting material, and the increased appearance of tars.

Monosaccharides, especially glucose and galactose, are good reducing agents for the preparation of dichloro- and dibromoazoxybenzenes, whereas the milder reducing disaccharides are more suitable for the preparation of diiodoazoxybenzenes.

EXPERIMENTAL[22]

Materials. The chloro-, bromo-, and iodonitrobenzenes used in this work were commercially available reagent grade samples.

Reduction of monohalogenated nitrobenzenes. As a similar procedure was employed for all compounds, only one reduction will be described as an example of the method employed.

Reduction of 4-chloronitrobenzene with galactose. 4-chloronitrobenzene (26.7 g., 0.17 mole) and sodium hydroxide (30.0 g., 0.75 mole) in water (260 ml.) were heated to 60° whereupon galactose (23.0 g., 0.13 mole) as a paste in water (25 ml.) was added in small portions. The mixture was heated at 70–72°, for 40 min. with stirring. Steam distillation produced a distillate, which on being acidified with hydrochloric acid and standing overnight gave recovered 4-chloronitrobenzene, m.p. 84–85°, (lit. m.p. 83.5°),[23] yield 3.5 g. (13.1%). The acid filtrate was made alkaline with sodium hydroxide and allowed to stand overnight. 4-chloroaniline separated out, yield 0.1 g., (0.5%) and was recrystallized from aqueous ethanol, m.p. 70–72°, (lit. m.p. 70–71°).[23] Acetylation gave 4-chloroacetanilide (from aqueous ethanol), m.p. 178–179°, (lit. m.p. 178.4°).[23]

The granular orange-yellow residue left in the reaction flask after steam distillation was 4,4′-dichloroazoxybenzene, m.p. 149–151°, yield, 16.0 g. (70.8%), which recrystallized from ethanol as pale yellow needles, m.p. 154.5–155.5°, alone or with an authentic sample (lit. m.p. 154–155°).[12]

In reductions of bromo- and iodonitrobenzenes, the azoxybenzenes were extracted from the reaction residues with ethanol and the extracts given several charcoal treatments, before evaporation and dilution with water to isolate the products.

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(22) All melting points are uncorrected.

(23) *Handbook of Chemistry & Physics*, 42nd ed., Chemical Rubber Co., Hodgman, Weast, and Selby, Cleveland, Ohio (1960–1961).

Synthesis of Aldosterone-1α,2α-H[3][1]

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The need for radioactive aldosterone of high specific activity for use as an indicator in analysis

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of peripheral blood prompted us to undertake this study. Aldosterone-7α-H^3 of high specific activity had already been prepared; however, the radioactivity had been introduced at an early stage, and the biosynthetic process seemed altogether too involved to be used as a routine method.

Therefore, it was decided to investigate the recently published method of Osinki *et al.*,[2] which consists in the selective reduction of the less substituted 1,2-double bond of a 1,4-dien-3-one system. Thus 1-dehydroaldosterone 21-acetate in dioxane was reduced catalytically with one mole of tritium over 5% palladium-on-charcoal. The catalyst was filtered off and the residue equilibrated with methanol. The mixture was then chromatographed on a Celite partition column. The first eluates contained the saturated 3-ketone (no ultraviolet absorption from 220–260 mμ) and were not further investigated. The following fractions contained the Δ^1-5α-H^3 fractions (λ_{max} 231 mμ (ϵ, 10,000) no fluorescence[3] after spraying with a sodium hydroxide solution) and were put aside. The most polar fractions contained the desired aldosterone acetate-1α,2α-H^3 [4,5] which was isolated in a yield of 10%, calculated on the tritium.

The acetate was hydrolysed with lipase and from the mixture aldosterone-1α,2α-H^3 with identical specific activity was isolated after chromatography in a yield of 50%.

In another experiment the acetate was hydrolysed with sodium bicarbonate following the directions of Simpson *et al.*,[7] whereby aldosterone-1α,2α-H^3 was isolated, again with identical specific activity. This is noteworthy, since it demonstrates that weak bases like sodium bicarbonate solution (*p*H 8.85) are not able to enolize the conjugated 3-ketone in fifteen hours at room temperature. The stability in biological systems remains to be shown.

EXPERIMENTAL[8]

Hydrogenation of Δ^1-aldosterone acetate. Into a 25-ml. three-neck flask (one neck having a break seal, one connected to the vacuum line, and a third serving for introduction of the starting material and the reagent) was put a solution of 70 mg. of Δ^1-aldosterone 21-acetate,[9,10] m.p. 192–194°, in 8 ml. of dioxane and 30 mg. of 5% palladium-on-charcoal. Then one neck was closed off, the flask connected to the vacuum line, degassed, evacuated, and 6 ml. of carrier-free tritium ($\simeq$ 15 c) transferred by means of a Toepler pump. Then the flask was sealed off, removed from the line and shaken at 25° for 18 hr. After connecting the flask to the line through the break seal, the break seal was broken, traces of tritium toeplered out, and the solvent pumped off. The residue was dissolved in 50 ml. of methanol, the catalyst filtered off and washed with methanol, and the filtrate concentrated, whereby the distilled solvent was replaced with a fresh one. Finally the solution was reduced to a small volume and the radioactivity measured on an aliquot. There was a total activity of 10 $\pm$0.5 c in the mixture.

The above mixture was divided into three portions. A typical run using one portion of the material is described below. One portion was taken down to dryness and transferred to 1 g. of Celite 545 with a small quantity of methane. The Celite was dried with a gentle stream of nitrogen, 0.5 ml. of the stationary phase added, and the mixture packed on top of a 60 cm.-long column. The column contained 28 g. Celite 545 and 14 ml. of the stationary phase. The mobile phase was benzene–Skellysolve C (1:1 v./v.) and stationary phase methanol-water (4:1 v./v.). The mobile phase was run through the column and 5 ml. fractions collected with an automatic fraction collector. From every fraction 5 λ was taken out and added to 10 ml. of a specially prepared quenched scintillation liquid in a glass vial. The quenched scintillation liquid contained one part of the usual scintillation liquid (4 g. diphenyl oxazole and 100 mg. 1,4-bis-2-(5-phenyl-oxazoyl) benzene, per l. of toluene) and 10 parts of absolute ethanol. The vials were counted in a Tricarb liquid scintillation counter at a high voltage setting giving an efficiency of 16.4% for tritium. In this quenched solution 1 μc of tritium gave about 1000 c.p.m. A solution of 20 μc progesterone-7-H^3 in the quenched scintillation liquid was used as standard.

The first few fractions from the solvent front did not show any ultraviolet absorbance at 240 mμ, characteristic of Δ^4-3-ketones, and were considered to contain the saturated 3-ketones of aldosterone-21-acetate-1,2-H^3 (R aldosterone-21-monoacetate 0.15). Aliquots from the second peak fractions did not give any alkali fluorescence and contain Δ^1-5α-H^3-aldosterone-21-acetate (R aldosterone-21-monoacetate 0.6). The first and second peak fractions were not further investigated. The third peak comprising fractions 19, 20, 21, and 22 were pooled and identified as aldosterone-21-acetate-1,2-H^3 by paper chromatographic comparison with an authentic sample in a Bush B_2 system [mobile phase toluene–Skellysolve C (2:1 v./v.); stationary phase methanol–water (4:1 v./v.)]. It gave a color with the blue tetrazolium reagent and alkali fluorescence on heating. The radiochemical purity of the substance was tested by running about 1 μc of the radioactive material alone with carrier aldosterone-21-monoacetate in the Bush B_2 system. Carrier aldosterone-21-acetate was added and scanned in a Vanguard automatic windowless flow chromatogram scanner (Model 800 autoscanner, efficiency about 3%). It showed a single sharp peak corresponding to the marked spot due to aldosterone-21-monoacetate and thus found to be pure.

In order to clean the column before reuse, 500 ml. of the mobile phase was allowed to run through and 1 g. of Celite from the top removed. The second batch of the aldosterone

(2) P. Osinki and H. Vanderhaeghe, *Rec. trav. chim.*, **79**, 216 (1960).

(3) I. E. Bush, *Biochem. J.* **50**, 370 (1952).

(4) Only followed by nonradioactive starting material.

(5) The assignment of the α-configuration to the tritium at C-1 and C-2 is an assumption based on analogy to other reactions,[6] for which proof was furnished that the attacking species approached the steroid molecule from the less hindered α-side.

(6) L. F. Fieser and M. Fieser, *Experientia*, **4**, 285 (1948).

(7) S. A. S. Simpson, J. F. Tait, A. Wettstein, R. Neher, J. v. Euw, O. Schindler, and T. Reichstein, *Helv. Chim. Acta*, **37**, 1163 (1954).

(8) Purification of the solvents and washing of the Celite were carried out as described by C. Flood, D. S. Layne, S. Ramcharan, E. Rossipal, J. F. Tait, and S. A. S. Tait, *Acta Endocrinol.* **36**, 265 (1961). In all chromatographic separations only the peak fractions were combined and counted.

(9) E. Vischer, J. Schmidlin, and A. Wettstein, *Exp.*, 1?, 50 (1956).

(10) Thanks are due to Dr. Maurice Pechet who supplied us with the starting material.

21-monoacetate-1,2-H^3 was put on the column as described above. From every three runs, four peak fractions containing the 1,2-H^3-aldosterone-21-monoacetate were pooled, and the total activity was about 1500 mc. The specific activity of the substance was determined and found to be 100 $\mu c/\mu g$.

Hydrolysis of the aldosterone-21-monoacetate-1,2-H^3. (a) *Enzymatic hydrolysis.* The solution of 10 ml. of 0.05*M* monohydrogen sodium orthophosphate was prepared and the *p*H adjusted to 7.0 with a few drops of acetic acid. Then 40 mg. of wheat germ lipase (Worthington Co., N. J.) was dissolved in that solution and warmed to 37° in a constant temperature oven. The solution of 200 mc of aldosterone-21-monoacetate-1,2-H^3 in 0.4 ml. propylene glycol was added to the enzyme solution in four portions with approximate 0.5 hr. intervals between additions. Incubation was continued for a total period of 4 hr. The incubation mixture was extracted with 4 × 15 ml. methylene dichloride. The combined solvent extract was washed with 2 ml. of water and taken down to dryness. It was further purified using a partition column made up with 28 g. Celite 545 in 14 ml. of the stationary phase.

The mobile phase (benzene) of the solvent system was equilibrated against the stationary phase [methanol-water (1:1 v./v.)]. Fractions of 5 ml. were collected and assayed for tritium using 5 λ from every fraction dissolved in the quenched scintillation liquid. The peak fractions 9, 10, and 11 containing aldosterone-1,2-H^3 were pooled and identity with standard aldosterone confirmed by paper chromatography, blue tetrazolium reaction, and soda fluorescence. The radiochemical purity of free compound and diacetate, prepared by acetylation with acetic anhydride and pyridine was determined as follows. Chromatography of a mixture of 1 μc of the 1,2-H^3-aldosterone and inert aldosterone (2 μg) in a Bush B_5 system gave a spot due to inert aldosterone, which was marked by pencil as observed in the ultraviolet light, and a strip (1.25 in. wide) of the chromatogram was scanned in a Vanguard automatic chromatogram scanner. It showed only one peak corresponding to the inert aldosterone; yield 100 mc. The specific activity of the aldosterone-1,2-H^3 was found to be 100 $\mu c/\mu g$.

A mixture of about 1 μc of the aldosterone-1,2-H^3 and 3 μg. inert aldosterone was acetylated with 0.15 ml. acetic anhydride and 0.3 ml. pyridine. The diacetate was run in the Bush B_3 system and scanned in a chromatogram scanner. Again only one sharp peak corresponding to the aldosterone diacete was observed thereby establishing the radiochemical purity of the aldosterone-1,2-H^3.

(b) *Alkaline hydrolysis.* Aldosterone 21-monoacetate-1,2-H^3 (220mc) was hydrolyzed according to the method of Simpson *et al.*[4] in a sealed tube. The reaction mixture was extracted with 4 × 15 ml. methylene dichloride; the total extract with 2 ml. of water and evaporated in dryness. It was purified by column chromatography and the fractions containing aldosterone-1,2-H^3 were identified. The radiochemical purity[11] of the product was established as described above in the case of aldosterone-1,2-H^3 obtained from the enzymatic hydrolysis; yield 66 mc. The specific activity was found to be 98 $\mu c/\mu g$.

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(11) It should be noted that no 17-isoaldosterone-1,2-H^3 could be detected (chromatography of a mixture of authentic aldosterone and its 17-isomer showed feasibility of its separation with a Bush B_5 system on paper, as well as on a Celite column).

β-Condensation Reactions of Piperidine with Aldehydes

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Saturated amines commonly exhibit nitrogen reactivity only. We wish to report a reaction in which piperidine is attacked on the carbon skeleton without loss of the nitrogen function. Skraup and Böhm in 1926 isolated from the reaction of benzaldehyde and piperidine a compound of unknown structure, m.p. 88–89°, to which they assigned the formula $C_{20}H_{19}N$.[4] In 1958 Parker and Furst reported that *p*-dimethyl- and diethylaminobenzaldehyde react with piperidine to give compounds $C_{23}H_{27}N_3$ and $C_{27}H_{35}N_3$, respectively.[5]

We have reinvestigated the reaction of benzaldehyde with piperidine and have shown the product to be 3,5-dibenzylpyridine (Ia). The NMR spectrum of the product exhibits a high field singlet (CH_2; $\tau = 6.17$), a broad, poorly resolved multiplet in the region for phenyl protons ($\tau = 2.90$) and a doublet (J = 1.7 c.p.s.) at such low field ($\tau = 1.82$) as to be attributable only to the 2,6-protons of a pyridine ring. The methiodide prepared from the free base was not sufficiently soluble in water for NMR analysis, and was converted by treatment with silver chloride to a hygroscopic methochloride (II). The 4-proton resonance, obscured by the phenyl multiplet in the spectrum of the free base, is shifted downfield by quaternization ($\delta = -3.32$ relative to DHO in deuterium oxide) and the intensity is half that of the 2,6-proton resonance ($\delta = -3.60$; the doublet is not resolved). Methylene ($\delta = 0.67$), *N*-methyl ($\delta = 0.57$) and phenyl ($\delta = -2.53$) protons have relative intensities in agreement with II. The doublet in the spectrum of the free base arises from spin coupling of the 2- and 4-protons of the pyridine nucleus. The coupling constant of 1.7 c.p.s. is close to the value of 1.9 c.p.s. calculated by Pople, Schneider, and Bernstein for the 2- and 4-protons of pyridine.[6] For 2,6-dibenzylpyridine, a mechanistically attractive alternative for Ia, coupling of the 3- and 4- protons should give splitting of about 8 c.p.s. by analogy

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(4) S. Skraup and K. Böhm, *Ber.*, 59, 1015 (1926).

(5) E. D. Parker and A. Furst, *J. Org. Chem.*, 23, 201 (1958).

(6) J. A. Pople, W. G. Schneider, and H. J. Bernstein, *High-resolution Nuclear Magnetic Resonance*, McGraw-Hill, New York, 1959, p. 266.