naphthylazo)-benzenesulfonate salt,[4] by ion-exchange techniques,[5] or by other known methods of effecting partial purification of the antibiotic. Hydrogenation, followed by the addition of sodium sulfate and sodium iodide, precipitates the complex salt, which is readily recrystallized from water.

Dihydrostreptomycin iodide sulfate has a bacterial spectrum, resistance pattern, acute and chronic toxicity comparable with crystalline dihydrostreptomycin sulfate. The synergistic action of combined streptomycin and potassium iodide therapy has been reported[6]; accordingly a similar investigation with this complex salt is being carried out.

Experimental

Among the simple dihydrostreptomycin salts utilized in this investigation were the hydroiodide, hydrobromide, hydrochloride, nitrate and sulfate. When these were dissolved in water at a concentration of about 650 mg. per ml. and treated with one or more equivalents of the salt of a dissimilar anion, crystallization occurred in a number of instances.

Dihydrostreptomycin Bromide Sulfate.—Crystalline dihydrostreptomycin sulfate[3] (14.6 g., 0.02 mole) was dissolved in 14 ml. of water. Sodium bromide (4.0 g., 0.035 mole) dissolved in 5 ml. of water was added and the solution was allowed to stand for several hours. The crystals of dihydrostreptomycin bromide sulfate were filtered, washed with cold water and dried; weight 10.1 g. This salt was dissolved in 36 ml. of water at 80°, filtered and then concentrated under vacuum until a heavy slurry of crystals was obtained. The resulting crystals were filtered, washed with cold water and dried *in vacuo* at room temperature and then at 78° for 48 hours; weight 6.9 g., $[\alpha]^{25}D$ −88° (*c* 1, water), potency 795 γ/mg.

Anal. Calcd. for $C_{21}H_{41}N_7O_{12}\cdot HBr\cdot H_2SO_4$: C, 33.06; H, 5.82; N, 12.86; $SO_4^=$, 12.60; Br, 10.48. Found: C, 33.18; H, 5.99; N, 12.75; $SO_4^=$, 12.62; Br, 10.66.

Concentration of the combined wash and mother liquor yielded additional dihydrostreptomycin bromide sulfate.

The characterizations of similar salts are summarized in Table II.

Isolation of Dihydrostreptomycin Iodide Sulfate from Solutions of Crude Streptomycin.—An aqueous solution, containing about 400 mg. per ml. of crude streptomycin hydrochloride, such as is obtained from the streptomycin salt of *p*-(2-hydroxy-1-naphthylazo)-benzenesulfonate,[4b,c] was reduced with Raney nickel catalyst under a hydrogen pressure of 1000 p.s.i. at 75°[7] until the streptomycin content was less than 1%. A portion of this solution (100 ml.) containing microbiological activity[8] equivalent to 33.5 g. of dihydrostreptomycin iodide sulfate was treated with 13 g. of anhydrous sodium sulfate. A non-crystalline precipitate was filtered, 14 g. of sodium iodide was added to the filtrate and the mixture was allowed to crystallize with stirring for about 48 hours. The dihydrostreptomycin iodide sulfate was filtered, washed with a small amount of ice-water and dried; weight 25.7 g., potency 655 γ/mg. Concentration of the wash liquor afforded an additional 6.1 g. assaying 405 γ/mg. (56% pure); the principal contaminants of this second crop product were inorganic salts. The first crop of dihydrostreptomycin iodide sulfate was purified by dissolving it in warm water and then concentrating under vacuum to give a product which, after drying under vacuum, assayed 745 γ/mg. (theoretical potency 725 γ/mg.), $[\alpha]^{25}D$ −78.5° (*c* 1, water).

Anal. Calcd. for $C_{21}H_{41}N_7O_{12}$: C, 31.14; H, 5.48; N, 12.11; $SO_4^=$, 11.87; I, 15.67. Found: C, 31.08; H, 5.56; N, 12.23; $SO_4^=$, 11.98; I, 15.54.

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[FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

Differential Reduction of Steroid Ketones[1]

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The selective reduction at C-3 of steroid ketones by means of sodium borohydride has been described. This carbonyl was preferentially reduced in 3,20-diketones, 3,11,20-triketones and in 17α,21-dihydroxy-3,11,20-triketones of both the normal and allo series. The preparation of 11β,17α-dihydroxy-3α,21-diacetoxypregnane-20-one is described.

The very useful selective reduction of a carbonyl group in steroids of the normal and allo series reported from this Laboratory[3] has been extended to more complicated compounds containing multiple hydroxyl and carbonyl functions. It was noted in our earlier report that 20-ketosteroids were reduced with difficulty to the corresponding alcohols by sodium borohydride and the first group of substances tested were 3,20-diketones of the pregnane and allopregnane series. The conditions of the reaction were altered somewhat from those previously used in order to establish standardized conditions for the reaction and thus aid in the reproducibility of the procedure. The important variations were careful standardization of a relatively stable pyridine solution of the reducing agent and the addition of a small amount of alkali to stabilize the sodium borohydride in methanol.[4] The yield of 3α- and 3β-hydroxy-20-ketosteroids obtained was comparable to that found on reduction of other 3-ketones.[3,5,6]

(1) This investigation was supported by grants from the Anna Fuller Fund, the Lillia Babbit Hyde Foundation, and the National Cancer Institute, United States Public Health Service.

(2) Post-doctorate Fellow of the National Cancer Institute, United States Public Health Service.

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TABLE I[a]

Steroid	Concn. $\times 10^{-2}$, mmoles of $NaBH_4$/ml.	Concn. $\times 10^{-2}$, mmoles of steroid/ml.	Ratio of moles of H_2 avail./moles of steroid	Time of reaction in min.	Compounds isolated[b]	Yield[c] of products, %
Allopregnane-3,20-dione	0.85	1.62	2.1	10	3β-Hydroxyallopregnane-20-one	62
					3α-Hydroxyallopregnane-20-one	10
					Allopregnane-3,20-dione	10
Pregnane-3,20-dione	1.13	2.30	2.0	10	3α-Hydroxypregnane-20-one	70
					3β-Hydroxypregnane-20-one	14
					Pregnane-3,20-dione	4
Allopregnane-3,11,20-trione	1.08	2.01	2.1	10	3β-Hydroxyallopregnane-11,20-dione	49
					3α-Hydroxyallopregnane-11,20-dione[d]	7
					Allopregnane-3,11,20-trione	15
Pregnane-3,11,20-trione	0.35	1.12	1.3	10	3α-Hydroxypregnane-11,20-dione	70
					3β-Hydroxypregnane-11,20-dione	10
					Pregnane-3,11,20-trione	8
11β,17α-Dihydroxy-21-acetoxypregnane-3,20-dione	0.82	2.52	1.3	10	11β,17α-Dihydroxy-3α,21-diacetoxypregnane-20-one[e]	30
					11β,17α-Dihydroxy-21-acetoxypregnane-3,20-dione	12
17α-Hydroxy-21-acetoxy-pregnane-3,11,20-trione	1.54	2.22	2.8	5	17α-Hydroxy-3α,21-diacetoxypregnane-11,20-dione	10
					17α-Hydroxy-3α,20,21-triacetoxypregnane-11-one[d]	2
	0.78	2.27	1.4	10	17α-Hydroxy-3α,21-diacetoxypregnane-11,20-trione	26
					17α-Hydroxy-21-acetoxypregnane-3,11,20-trione	22
17α-Hydroxy-21-acetoxy-allopregnane-3,11,20-trione	0.79	1.82	1.7	10	17α-Hydroxy-3β,21-diacetoxyallopregnane-11,20-dione	15
					17α-Hydroxy-21-acetoxyallopregnane-3,11,20-trione	11

[a] Solvent was 80% methanol and 20% pyridine with the exception of pregnane-3,11,20-trione (90% methanol–10% pyridine). [b] Products were determined by melting points and infrared spectra unless otherwise specified. [c] Yield refers to pure products isolated. [d] Determined by infrared spectra only. [e] Characterized by infrared spectrum, melting point and rotation determinations.

In contrast to the C_{19}-steroids, however, no dihydroxy compounds were obtained from the reaction although there was a small and somewhat variable amount of unreacted 3,20-diketone. In agreement with previous results, ketones of the normal series were reduced to 3α-hydroxy steroids in somewhat higher yield than 3-ketones of the allopregnane series were reduced to 3β-hydroxy steroids. This result implies that the 3-ketones of the allo series are more sterically hindered than the corresponding compounds of the normal series since electronic factors appear to be the same in both types of compound. Completely analogous results were obtained with 3,11,20-triketones of the normal and allo series. In the meantime, it was reported[7] that pregnane-3,11,20-trione could be reduced selectively at the C-3 position by sodium borohydride.

In view of the favorable results obtained with 3,20-diketones and 3,11,20-triketones (Table I) it was of interest to study the partial reduction of steroids having a dihydroxyacetone side chain. The expected products were of particular interest to us because this Laboratory has for some time been concerned with the partial synthesis of steroid hormone metabolites that have been isolated from urine. The first studies were carried out in pyridine solution under a nitrogen atmosphere at room temperature, but this proved to be unsatisfactory since the reaction was very sluggish and with increase in time or concentration of the reagent, the expected compounds were not obtained. Better results were obtained using the methanol–pyridine mixture as described above for the case of simple 3,20-diketones. Steroids with a dihydroxyacetone side chain are easily altered in alkaline medium and, probably for this reason, the reduction of the 3-ketone was achieved with the formation of more side products than was the case with less sensitive materials. It may be that alkaline rearrangement of the ketol, a reaction well known in the sugar series, the formation of an alkyl borate difficult to hydrolyze[8] or transformation to a D-homosteroid was responsible for the lower yield of product. Nevertheless it was possible to carry out the selective reduction of a 3-ketone in compounds of this type and to obtain the desired products in acceptable yield.

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Experimental

Reagents.—Small pieces of powdered sodium borohydride were extracted continuously with pyridine in a soxhlet type apparatus until an approximately saturated solution was obtained. This procedure was necessary with the samples of borohydride we had available but there appeared to be differences between several lots of the material since other investigators have informed us that in their experience the reagent readily dissolved in pyridine, either at room temperature or after gentle warming. Sodium borohydride is quite stable in pyridine solution but after long standing an amorphous precipitate settled gradually from the solution. The solution was standardized by measuring the hydrogen evolved from 1 ml. of solution upon addition to an excess of dilute hydrochloric acid. In general, 1 ml. of solution contained between 0.2 and 0.3 millimole of reagent.

Conditions for a Typical Reduction.—A stream of nitrogen was passed through a solution of 0.34 millimole of compound dissolved in 9 ml. of methanol. To this solution, a mixture of 3 ml. of methanol, 0.1 ml. of 2.5 *N* sodium hydroxide and 2.8 ml. of a 0.18 molar solution of sodium borohydride in pyridine was added. After 10 minutes an ex-

(8) S. N. Chaikin and W. G. Brown, *ibid.*, **71**, 122 (1949).

cess of hydrochloric acid was added and the solution was extracted with ether. The ether was washed with dilute sodium hydroxide and with water until neutral. After drying over sodium sulfate, the solvent was removed. The reduction products were isolated by chromatography upon alumina except in the case of compounds having the dihydroxyacetone side chain. These latter products were acetylated with acetic anhydride and pyridine at room temperature. The acetates were chromatographed on a partition column using silica gel containing 40 ml. of ethanol per 100 g. of silica gel as the stationary phase and developing the column with a 1% solution of ethanol in methylene chloride. A good separation of product and starting material was obtained in all cases. In some instances, a good separation between the two C-3 isomers was achieved with the partition column; this was much more difficult with very polar substances than was the separation of the simple monohydroxy ketosteroids.

This report describes the first partial synthesis of 11β,17α-dihydroxy-3α,21-diacetoxypregnan-20-one. As with the natural material isolated from urine,[9] the melting point was not a criterion of purity. An analytical sample had a m.p. 212–222° but the rotation was constant through several recrystallizations, $[\alpha]^{24}D$ +90.8° (chloroform), $[\alpha]^{24}D$ +85.0° (acetone). The product was identical with the urinary metabolite, as was the infrared spectrum of the two products.

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Separation of the Air-oxidation Products of Cholesterol by Column Partition Chromatography

BY ERWIN H. MOSBACH,[1] MARTHA NIERENBERG AND FORREST E. KENDALL

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The oxygenated sterols resulting from the air-oxidation of colloidal solutions of cholesterol have been separated by column partition chromatography. Two solvent systems proved effective: aqueous methanol–cyclohexane and propylene glycol–petroleum ether. The stationary phase in each case consisted of Celite 545, partially saturated with the alcohol. It was shown that the isomeric 7-hydroxycholesterols were formed in nearly equal amounts during the air-oxidation of cholesterol sols. Cholestane-3β,5α,6β-triol which had not been identified previously as a reaction product was shown to be a minor component of air-oxidized cholesterol.

Introduction

A number of investigators have shown that the cholesterol obtained from the non-saponifiable fractions of animal tissues is accompanied by relatively minute amounts of structurally related sterols. Thus 7-ketocholesterol (or its dehydration product $\Delta^{3,5}$-cholestadiene-7-one), 7α-hydroxycholesterol, 7β-hydroxycholesterol and cholestane-3β,5α,6β-triol have been isolated from a variety of different organs such as aortas, liver, testes, spleen and blood.[2–5] Schwenk has shown recently that cholestane-3β,5α,6β-triol may be present in most samples of cholesterol prepared from animal sources.[6] It has been pointed out repeatedly, however, that with the possible exception of 7-ketocholesterol these substances may be artefacts derived from cholesterol during the isolation procedures.[2,3,7] This point of view received strong support from the work of Bergström and Wintersteiner[7,8,9] who showed that at *p*H 8 and temperatures near 85° about 65% of colloidally dispersed cholesterol was converted to 7-ketocholesterol and the isomeric 7-hydroxycholesterols within a few hours. It was found by the same authors that the oxidation took place under physiological conditions of *p*H and temperature although at a slower rate. They suggested that a conversion of cholesterol to the 7-oxygenated sterols might occur *in vivo*, and that an attack on the sensitive 7-position of the cholesterol molecule might be involved in the biological degradation of this sterol.[7]

The present study on the separation of the air-oxidation products of cholesterol had as its objective the development of procedures for an investigation of the biological fate of these sterols by radioactive tracer techniques.[10] Although the separation of weakly polar sterols has been achieved by paper chromatography,[11] it was decided to investigate the use of columns so that somewhat larger quantities of sterols, required for C^{14}-tracer studies, could be handled.

Two solvent systems proved useful for partition-chromatographic separations: 95% aqueous methanol–cyclohexane, and propylene glycol–petroleum ether (b.p. 60 to 68°). The alcoholic component, supported on Celite 545, was used as the stationary phase in both cases. It was found that the major components of air-oxidized cholesterol sols could be

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