imine is stable or labile. In the symmetrical dimethyl-diaminodurene, the diimine is rather stable in 80% methanol; in tetramethyldiiminodurene it is extremely labile, yet, in both cases, no radical is formed at all.

Of the many details presented in this table one item is worth special attention. No. 18 is considerably more labile than 17; correspondingly no. 26 is more labile than 25, but no. 26 is much more stable than 18. While in unsymmetrical dimethyldiaminobenzene the introduction of one CH₃ group in the ring ortho to the methylated amino group has a strong destabilizing effect, this effect is smaller for a corresponding substitution in tetramethyldiamino-benzene. In the latter case, the introduction of two methyl groups in the ring is necessary to exert a really strong destabilizing effect. Compare also the difference of this effect according to the position of the two methyl groups (nos. 27 and 28).

4. Absorption Spectra.—Ten cc. of a 0.01 M solution of the dihydrochloride in the same solvent as indicated in the tabulation, was mixed with 1 cc. of 0.001 N aqueous solution of bromine. The concentration of the radical was calculated according to the amount of bromine added, neglecting the overlapping of the two steps, which causes no appreciable error if the excess of unoxidized diamine is as large as in these experiments. A König–Marten's spectrophotometer was used for the readings.

Summary

The radicals derived from aromatic p-diamines as modified by substitutions at the amino groups and at the benzene ring, by univalent oxidation, are compared with each other with regard to stability and color. The stability is measured approximately in terms of the lifetime in a properly chosen solvent. A distinction has to be made as to whether the disintegration is due to the direct breakdown of the radical ensuing from interaction with the solvent, or is caused indirectly by the lability of the diimine with which it always is in equilibrium. The indirect lability is most obvious in compounds not methylated at the amino groups. It may make the radicals appear to be much more labile than they really are. The direct spontaneous lability of the radicals is increased whenever there is a steric hindrance preventing the coplanar arrangement of the molecule necessary for resonance. This is the case when one or two methyl groups substituted in the benzene ring are in ortho position to a methylated amino group. Direct observations of changes in color are supported by potentiometric oxidative titrations of the diamines. The absorption spectra are recorded. It is especially noteworthy that diamino durene forms a very stable radical, but that any methylation of its amino groups entirely prevents the formation of a radical, due to the steric hindrance preventing coplanar arrangement of the molecule.

Studies on the Wolff–Kishner Reduction of Steroid Ketones[1]

BY JAMES D. DUTCHER AND O. WINTERSTEINER

In connection with a problem related to the steroid compounds of the adrenal cortex we had occasion, in a practice experiment, to reduce the semicarbazone of cholestanone by the method of Wolff and Kishner. However, the usual agent, sodium ethylate, as well as the benzylate modification described by Ruzicka and Goldberg,[2] yielded not the expected hydrocarbon, cholestane, but α- and β-cholestanol as the sole reaction products. This unusual result prompted us to study the behavior of other ketonic steroids in the Wolff–Kishner reduction, in order to learn whether constitutional or experimental factors were primarily involved.

A search of the literature revealed only two cases in which a similar atypical course of the Wolff–Kishner reduction had been observed. According to Eisenlohr and Polenske,[3] β-decalone semicarbazone yielded, besides the hydrocarbon, 33% of β-decalol. When the experiment was repeated under strictly anhydrous conditions, the yield of alcohol was but little lower, 25%. A complete failure to obtain the normal product had been reported by Reindel and Niederländer,[4] who after treating the semicarbazone of acetyl-nor-lithocholyl methyl ketone with sodium ethylate at 200° were able to isolate only the corresponding carbinol. The formation of secondary alcohols from cyclic steroid ketones

(1) This report is from a dissertation submitted by James D. Dutcher in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.

(2) Ruzicka and Goldberg, *Helv. Chim. Acta*, **18**, 668 (1935).

(3) Eisenlohr and Polenske, *Ber.*, **57**, 1639 (1924).

(4) Reindel and Niederländer, *Ann.*, **522**, 218 (1936).

has not, to our knowledge, previously been observed.[5]

Methods.—The usual method of heating the semicarbazones with sodium ethylate in a bomb tube was employed in preference to the benzylate procedure of Ruzicka and Goldberg,[2] since in the latter some benzoic acid is always formed, and this interfered with the isolation of the reduction products in the experiments with bile acids.

In order to obtain quantitative information as to the ratio of carbinols and non-alcoholic products formed, the reaction products were treated with an excess of succinic anhydride in pyridine. The acid succinates of the alcohols can be extracted readily from ether with dilute sodium carbonate solution, a property not possessed by the acid phthalates with which we at first experimented. Both types of acid esters are easily crystallizable and have served in some instances for the identification of the alcohols formed. In the experiments with bile acids the reduced acids had to be converted into the ethyl esters prior to the esterification with succinic acid in order to retain the non-alcoholic products in the ether phase on extraction with sodium carbonate.

Table I summarizes the results obtained with seven ketonic sterols and bile acids. It is immediately apparent from the data that compounds with a semicarbazone group in position 3 are reduced preponderantly to the corresponding C_3-carbinols; semicarbazone groups at positions 7 and 12, on the other hand, do not exhibit this abnormal behavior. Dehydrocholic acid trisemicarbazone and dehydrodesoxycholic acid disemicarbazone consequently yield a high percentage of lithocholic acid (3-hydroxycholanic acid). It is also evident that the observed anomaly is not dependent upon the configuration of carbon atom 5, since it is exhibited by members of both the cholestane and coprostane series.

As was to be expected, the C_3-carbinols produced in the reaction were epimeric mixtures. In accordance with the observations of Windaus[6] on the epimerization of sterols with alkali at high temperature, the epimer with the hydroxyl group

in *trans* position to the hydrogen on C_5 is the main product. Thus cholestanone yields chiefly β-cholestanol (dihydrocholesterol) besides some α-cholestanol (*epi*-dihydrocholesterol); coprostanone, on the other hand, gives preponderantly the *epi* compound, α-coprosterol. A small amount of the β-epimer undoubtedly was present but could not be isolated. The ketonic bile acids, which correspond sterically to coprostanone, are reduced to mixtures of α- and β-lithocholic acids, in which the α-epimer likewise preponderates.

The proportion of C_3-carbinol to the normal C_3-methylenic reduction product depends largely on the nature of the ketone reduced and less so on experimental factors. Under standard conditions the saturated C_3-monoketones (cholestanone, coprostanone, dehydrolithocholic acid) give the highest yields of carbinols (70–80%). With the di- and triketo acids the yields of normal reduction product (cholanic acid) are increased, but always fall short of that of the C_3-carbinol (lithocholic acid). No evidence was obtained, in the latter cases, for the presence of polyhydroxy acids which might be formed by an abnormal reduction of the C_7 or C_{12} keto groups. This point was further checked by experiments with 3-hydroxy-12-ketocholanic acid semicarbazone which were thought to provide a better chance for isolating a dihydroxy acid. No compound of this description could be isolated, but some of the original 3-hydroxy-12-ketocholanic acid was recovered besides the normal reduction product, lithocholic acid. The same acid, 3-hydroxy-12-ketocholanic acid, was also invariably found to be present, to the extent of 8–10%, among the reduction products of dehydrodesoxycholic acid disemicarbazone. Since the nitrogen content of the two semicarbazones in question was close to the theoretical value, the possibility that they contained material in which the 12-keto group was left free appears remote. It seems rather that under the conditions of the reaction the semicarbazone group in position 12 undergoes partial hydrolysis, with regeneration of the free ketone.

The reduction of the semicarbazone of the α,β-unsaturated ketone, cholestenone, follows a more complex course. Besides the normal reduction product, Δ_4-cholestene (pseudocholestene), a large proportion of alcohols is formed. One of the steps in the abnormal reaction is probably the formation of the epimeric α,β-unsaturated alcohols, the *allo*-cholesterols. The presence of these com-

(5) After this work had been completed Marker and Lawson [THIS JOURNAL, **61**, 852 (1939)] reported that pregnanol-20(α)-one-3-semicarbazone on reduction by the Wolff–Kishner method yielded almost exclusively pregnanediol-3(α), 20(α), and Marker and Rohrmann [*ibid.*, **61**, 1284 (1939)] reported that the reduction of the semicarbazone of sarsasapogenone, a C_3 ketone (see Marker and Rohrmann, [*ibid.*, **61**, 943 (1939)]), yielded chiefly the carbinol, sarsasapogenin. These results are entirely in harmony with our observations on the reduction of saturated C_3-monoketones.

(6) Windaus, *Ber.*, **49**, 1724 (1916).

TABLE I

WOLFF–KISHNER REDUCTION OF STEROID KETONES

Reaction time eight hours, temperature 200° unless indicated otherwise in Column 1. The melting points in Column 4 are those of the products for which the yield is given; the figures in brackets are the melting points of the pure compounds. When the yield given pertains to a crude fraction, reference is made in Column 4.

Compound reacted	Reaction products	Yield, % of theory	Remarks
Cholestanone semicarbazone (at 180°)	α-Cholestanol β-Cholestanol	90	Weighed as crude carbinol fraction. Both epimers isolated in pure form. Hydrocarbon fraction negligble
Cholestanone semicarbazone (at 200°)	α-Cholestanol β-Cholestanol	75	Weighed as crude succinates. Both epimers isolated in pure form.
	Cholestane	13	M. p. 80° (81°).
Cholestanone semicarbazone + hydrazine	Cholestane	75	M. p. 80–81° (81°).
Cholestanone hydrazone	(α + β)-Cholestanols	82	Weighed as crude succinates.
	Cholestane	15	M. p. 78–80° (81°).
Cholestanone hydrazone + hydrazine	Cholestane	93	M. p. 81° (81°).
Cholestanone ketazine	(α + β)-Cholestanols	75	Weighed as crude succinates.
	Cholestane	18	Crude hydrocarbon fraction.
Cholestanone ketazine + hydrazine	Cholestane	98	M. p. 81° (81°).
Coprostanone semicarbazone (at 180°)	α-Coprostanol	64	M. p. 116–118° (118°).
	β-Coprostanol	Small amount	Indicated by digitonin precipitate. Hydrocarbon fraction negligible.
Cholestenone semicarbazone (at 210°)	α-Coprostanol β-Cholestanol Cholesterol	35	Weighed as crude succinates. Saturated alcohols isolated in pure form; cholesterol indicated by color reaction.
	allo-Cholesterols	Small amount	Isolated as Δ₃,₅-cholestadiene.
	Δ₄-Cholestene	40	Weighed as crude hydrocarbon fraction. Pure compound (m. p. 79°) isolated.
Cholestenone ketazine + hydrazine	Δ₄-Cholestene	64	M. p. 77° (79°).
Dehydrocholic acid trisemicarbazone	α-Lithocholic acid	38	M. p. 188° (188°).
	β-Lithocholic acid	Small amount	Not isolated.
	Cholanic acid	22	M. p. 161° (164°); part isolated as ethyl ester, m. p. 93° (93°).
Dehydrodesoxycholic acid disemicarbazone	α-Lithocholic acid β-Lithocholic acid	38	M. p. 188° (188°). M. p. 179° (179°).
	Cholanic acid	31	M. p. 164° (164°).
	(α + β)-3-Hydroxy-12-ketocholanic acid	9	M. p. 157°; pure α-epimer (m. p. 164°) and derivatives prepared.
Dehydrolithocholic acid semicarbazone	(α + β)-Lithocholic acid	73	Isolated as crude ethyl lithocholate acid sucinnate, m. p. 140–145° (147°).
	Cholanic acid	7.7	Isolated as ethyl ester, m. p. 93° (93°).
3(α)-Hydroxy-12-ketocholanic acid semicarbazone	(α + β)-Lithocholic acid	70	M. p. 172–176°.
	(α + β)-3-Hydroxy-12-ketocholanic acid	10	M. p. 157° (9 parts α, 1 part β).

pounds, indicated by a strong Rosenheim reaction, was proved by their conversion on acid treatment to the doubly unsaturated hydrocarbon melting at 79° of Schoenheimer and Evans,[7] later shown to be Δ₃,₅-cholestadiene by Stavely and Bergmann.[8] The allo-cholesterols, under the

conditions of the Wolff–Kishner reduction, may be partially transformed into the corresponding epimeric β,γ-unsaturated alcohols. Cholesterol could not be isolated as such, but its presence in the digitonin precipitable portion of the carbinol fraction was indicated by a strong Liebermann–Burchard reaction. Reduction of the double bond, as evidenced by the isolation of α-coprosterol and

(7) Schoenheimer and Evans, J. Biol. Chem., 114, 567 (1936).
(8) Stavely and Bergmann, J. Org. Chem., 1, 567 (1937).

β-cholestanol, also takes place. Since no saturated hydrocarbons could be found in the hydrocarbon fraction, it appears that double bond reduction does not occur once the keto group has been eliminated.

These results confirm and amplify those of Lettré,[9] who found pseudo-cholestene, cholesterol and a saturated alcohol which he believed to be β-cholestanol, among the reduction products of cholestenone semicarbazone. They also accord with those of Stange,[10] who reduced the disemicarbazone of 3,6-diketocholestene with sodium ethylate and obtained a small amount of Δ_4-cholestene, presumably together with other reduction products. When the 3-monosemicarbazone was subjected to the reaction, cholestanediol-3,6 was obtained.

Influence of Experimental Conditions.—The results discussed so far were obtained under standard conditions. The influence of the reaction time was studied in the case of dehydrodesoxycholic acid. The results recorded in Table II indicate that the proportion of normal and abnormal reduction products does not vary greatly when the period of heating is extended from 4.5 to 8, 10 and 22 hours. No trend is discernible in the figures; the fluctuations observed are probably fortuitous.

TABLE II

REDUCTION OF DEHYDRODESOXYCHOLIC ACID DISEMI-
CARBAZONE; REACTION TIME VARIED

1.0 g. of disemicarbazone yielded the products listed in the percentage given.

Reaction time, hrs.	Cholanic acid		Lithocholic acid		3-Hydroxy-12-keto-cholanic acid	
	Mg.	%	Mg.	%	Mg.	%
4.5	274	38	263	35	71	9
8	238	33	293	39	62	8
10	285	40	224	30	72	9
22	265	36	248	33	64	8

Since the practicable temperature range for the reaction is comparatively narrow (180–210°), the influence of this factor was not systematically investigated. From the first two experiments with cholestanone semicarbazone recorded in Table I it would appear that raising the temperature from 180 to 200° favors slightly the production of the hydrocarbons.

That the addition of 10% of water to the reaction mixture or the exclusion of oxygen do not perceptibly alter the proportion of normal and abnormal reaction products was ascertained in

separate experiments with cholestanone semicarbazone.

A profound effect, however, is exerted by the presence of an added excess of hydrazine hydrate. The abnormal reaction is entirely suppressed and a practically quantitative yield of hydrocarbon is obtained. The hydrazone and ketazine of cholestanone, which under standard conditions react in the same way as the semicarbazone to give high yields of carbinol, are also reduced in the normal manner when hydrazine is present.

Among C_3-ketonic steroids of known structure, only the two unsaturated ketones studied by Lettré and by Stange, and a few saturated bile acids previously have been subjected to the reaction. The reduction of the trisemicarbazone of dehydrocholic acid to cholanic acid has been described by Borsche and Hallwass,[11] but no yield was reported, and other products may well have been present. An instance of complete reduction in the presence of hydrazine is found in the paper of Wieland and Kapitel,[12] who obtained good yields of 7-hydroxycholanic acid from the disemicarbazone of 3,12-diketo-7-acetoxycholanic acid. The experiment of Wieland and Jacobi,[13] on the other hand, in which a mixture of the mono- and disemicarbazones of 3,7-diketocholanic acid was reduced to lithocholic acid, can, in the light of our experience, be interpreted as an instance of reduction of the semicarbazone grouping at C_3 to carbinol and that at C_7 to methylene. Fieser,[14] commenting on the anomaly of this result, suggests that the lithocholic acid was formed from the 7-monosemicarbazone by elimination of the C_7-keto group and reduction of the free C_3-keto group to carbinol. In all probability both reactions occurred simultaneously.

In all other instances recorded in the literature, reduction of keto groups other than on C_3 are involved. It is evident from these reports that the reaction proceeds in the normal manner on keto groups at C_6,[15] C_7,[16] C_{12}[16,17] and C_{17}.[18]

(9) Lettré, Z. physiol. Chem., **221**, 82 (1933).

(10) Stange, ibid., **223**, 245 (1934).

(11) Borsche and Hallwass, Ber., **55**, 3324 (1922).

(12) Wieland and Kapitel, Z. physiol. Chem., **212**, 269 (1932).

(13) Wieland and Jacobi, ibid., **148**, 232 (1925).

(14) Fieser, "The Chemistry of Natural Products Related to Phenanthrene," Reinhold Publishing Corp., New York, N. Y., 1937, p. 139.

(15) Sugiyama, J. Biochem. (Japan), **25**, 157 (1937); Wieland, Dane and Martius, Z. physiol. Chem., **215**, 15 (1932); Wieland and Dane, ibid., **212**, 41 (1932).

(16) Wieland and Dane, ibid., **210**, 268 (1932); Kawai, ibid., **214**, 71 (1932).

(17) Wieland, Dane and Scholz, ibid., **211**, 261 (1932).

(18) Butenandt, Stoermer and Westphal, ibid., **208**, 149 (1932); Danielli, Marrian and Haslewood, Biochem. J., **27**, 311 (1933).

No satisfactory explanation can be offered at present as to why the C_3-keto group displays this unique resistance to complete reduction. It may be classed with other known differences between the chemical reactivity of C_3-hydroxyl or keto groups and that of similar groups in other positions of the steroid molecule. The C_3-keto groups can be reduced selectively to carbinol catalytically or with sodium and alcohol, and to methylene by the Clemmensen method. Acylation as well as ester saponification proceeds faster at C_3 than at C_7, C_{12}, C_{17} and C_{20}.

For the abnormal reaction the following mechanism is suggested: Wolff[19] believed that the course of the normal reaction from the semicarbazone to the methylene compound proceeded with the intermediate formation of the hydrazone, followed by its decomposition to the methylene compound as shown by Reaction 1. This view is supported by the fact that hydrazones are readily reduced by this method.

Reaction 1

$$>C=N-NH-CO-NH_2 \xrightarrow{H_2O}$$
(I)

$$>C=N-NH_2 + NH_3 + CO_2 \xrightarrow{NaOEt} >CH_2 + N_2$$
(II)   (III)
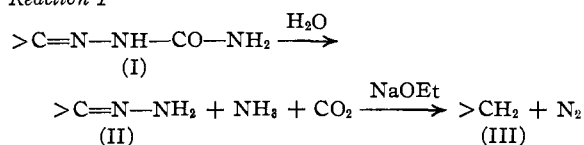
The first step in the abnormal reduction is probably the same as I to II in Reaction 1. The subsequent step, involving the production of a hydroxyl group, can best be explained by the assumption that the hydrazone is hydrolyzed to the free ketone and hydrazine base (II to IV). The regenerated keto group reacts then by oxidation-reduction with sodium ethylate, giving the secondary alcohol and acetaldehyde (IV to V). This reaction can be demonstrated by treating cholestanone itself with sodium ethylate at 180°. The epimeric cholestanols are obtained in good yield. The acetaldehyde which should be formed presumably undergoes further changes such as condensation or oxidation.

Reaction 2

$$>C=N-NH_2 + H_2O \longrightarrow >C=O + H_2N-NH_2$$
(II)   (IV)

$$>C=O + C_2H_5OH \longrightarrow >CH-OH + CH_3CHO$$
(IV)   (V)
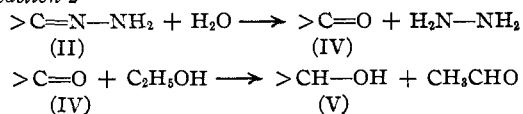
If the postulated equilibrium between free ketone and hydrazine on the one hand and hydrazone on the other actually exists, then the introduction of an excess of hydrazine should keep the

(19) Wolff, *Ann.*, **394**, 86 (1912).

concentration of free ketone at a low level and thereby promote the normal reaction (Reaction 1). This is indeed the case, since in the presence of hydrazine the reduction to the hydrocarbon is quantitative. Splitting of the semicarbazone occurs to a measurable extent only at comparatively high temperatures since refluxing with sodium ethylate at 90° does not regenerate the ketone or any other ether-soluble material.

Experimental

The conditions were, in the majority of runs, those customarily employed. The amount of sodium dissolved in absolute alcohol was equal to the weight of the semicarbazone; the temperature was 200° and the reaction time usually eight hours, except in experiments designed to test the influence of the last two variables. Deviations from the standard procedure, such as the addition of hydrazine hydrate, will be referred to in the text.

In the ensuing account, all melting points are uncorrected.

Semicarbazones of Cholestanone and Coprostanone.— These were obtained in quantitative yield as colorless, amorphous powders by refluxing an alcoholic solution of the ketone for two hours with twice its equivalent of semicarbazide acetate. Cholestanone semicarbazone decomposed at 238° after sintering at 227°; coprostanone semicarbazone decomposed at 192° after sintering at 178°.[20]

Anal. Calcd. for $C_{28}H_{49}ON_3$: N, 9.5. Found: N, 9.4, 9.4.

β-Cholestanol Acid Phthalate.—This was prepared by boiling a pyridine solution containing the alcohol and a large excess of phthalic anhydride (10 mols) for one hour. It could neither be extracted with sodium carbonate solution, nor adsorbed on solid sodium carbonate, from its solution in ether or petroleum ether; needles from alcohol, m. p. 160°.

Anal. Calcd. for $C_{35}H_{52}O_4$: C, 78.4; H, 9.7; mol. wt., 537. Found: C, 78.4; H, 9.7; neut. equiv., 535, 536.

α-Coprostanol Acid Phthalate.—This was isolated from the reduction products of coprostanone after treatment with phthalic anhydride in pyridine. It likewise did not possess the desired solubility properties; needles from alcohol, m. p. 218–220°.

Anal. Calcd. for $C_{35}H_{52}O_4$: C, 78.4; H, 9.7; mol. wt., 537. Found: C, 78.3; H, 9.5; neut. equiv., 537.

β-Cholestanol Acid Succinate.—The treatment of β-cholestanol with succinic anhydride in the ratio of 1 to 5 mols in pyridine yielded a mixture of acid ester and di-ester. Only when a ratio of 1 to 10 was employed was the acid ester quantitatively formed; plates from dilute alcohol or ether–methanol, m. p. 171°.

Anal. Calcd. for $C_{31}H_{52}O_4$: C, 76.2; H, 10.7. Found: C, 76.4; H, 10.8.

Reduction of Cholestanone and Coprostanone Semicarbazones.—A mixture of 400 mg. of cholestanone semicarbazone with a solution of 400 mg. of sodium in 15 cc. of absolute alcohol was heated in a bomb tube at 180° for

(20) Dorée and Gardner, *J. Chem. Soc.*, **93**, 1630 (1908).

eight hours. The contents of the tube were poured into water, neutralized with hydrochloric acid and extracted with ether. The ethereal residue (361 mg.) was refluxed with 1.4 g. of succinic anhydride and 5 cc. of pyridine for one hour, poured into water and extracted with ether. The ether layer was then washed several times with dilute sulfuric acid and finally with dilute sodium carbonate solution. The ether, which retained the neutral fraction (hydrocarbon), was washed with water, dried and evaporated, leaving 22 mg. of oily, non-crystallizable material. The sodium carbonate solution was acidified with hydrochloric acid and extracted with ether. This ether solution, containing the acid esters of the alcohols, was washed, dried and evaporated (413 mg.).

The succinates were saponified with methyl alcoholic potassium hydroxide at room temperature, yielding 316 mg. of alcohols; 150 mg. of the latter was treated with digitonin in 80% alcohol, and precipitate and filtrate worked up in the usual manner: 102 mg. of β-cholestanol, m. p. 140°, $[\alpha]D$ +29° (ethanol); and 27.2 mg. of α-cholestanol, m. p. 181°, $[\alpha]D$ +34° (ethanol), were thus obtained and identified by specific rotations and mixed melting point determinations with authentic samples. β-Cholestanol[21] has m. p. 141°, $[\alpha]D$ +28.8° (ether). α-Cholestanol[22] has m. p. 181°, $[\alpha]D$ +33.95° (alcohol).

Four hundred mg. of coprostanone semicarbazone, reduced under the same conditions, yielded 17.4 mg. of non-crystalline material in the hydrocarbon fraction, and 269 mg. of alcohols. The latter yielded on treatment only a small amount of digitonide which was not worked up. From the digitonin filtrate 225 mg. of α-coprostanol was recovered, m. p. 116–118°, unchanged when mixed with an authentic sample; $[\alpha]D$ +30° (alcohol); Dorée and Gardner[20] report $[\alpha]D$ +31.3°.

In another experiment 1.2 g. of the semicarbazone was treated with sodium ethylate (1 g. of sodium in 15 cc. of alcohol) at 200° for ten hours, and the ether-soluble reaction products (1.034 g.) separated with succinic anhydride. The weight of the ester fraction (1.04 g.) indicated that 75% of the sterol portion of the semicarbazone had been converted to alcohols. One hundred mg. of the recrystallized succinate (m. p. 155–160°) was saponified. The precipitate obtained with digitonin yielded 40 mg. of β-cholestanol, m. p. 142°, and the digitonin filtrate 24 mg. of α-cholestanol, m. p. 181–182°.

The hydrocarbon fraction (204 mg.) was recrystallized from alcohol–ether, giving 136 mg. of plates melting at 80°, $[\alpha]D$ +25° (chloroform); cholestane,[23] m. p. 80°; $[\alpha]D$ +24.4°.

The lack of effect of water and of oxygen was shown by the following experiment. Each of three bomb tubes (a, b, c) was charged with 300 mg. of the semicarbazone, and with ethylate prepared from 300 mg. of sodium and 5 cc. of absolute alcohol. Tube a served as control; to tube b, 0.5 cc. of water was added; in tube c the air was replaced by nitrogen. After six hours at 200° the contents of the tubes were subjected to the usual fractionation procedure. The weights of the hydrocarbon fractions were (a) 15.7 mg., (b) 14 mg., (c) 10 mg.; those of the

carbinol fractions (calculated from the weights of the ester fractions) (a) 252 mg., (b) 252 mg., (c) 241 mg.

Hydrazine hydrate had a marked effect: 434 mg. of the semicarbazone was reduced with sodium ethylate at 200° in the presence of 0.5 cc. of 100% hydrazine hydrate. The crystalline material (330 mg.) obtained by ether extraction of the reaction products yielded on recrystallization 273 mg. (75%) of cholestane, m. p. and mixed m. p. 80–81°.

Reduction of Cholestanone Hydrazone.—The hydrazone was prepared by refluxing 1 g. of the ketone with 1.0 cc. of hydrazine hydrate in 50 cc. of absolute alcohol. Water was added to the hot solution to incipient cloudiness. After standing overnight the hydrazone had precipitated out quantitatively; light yellow needles, m. p. 248° (dec.) after softening at 230°.

Anal. Calcd. for $C_{27}H_{48}N_2$: N, 7.0. Found: N, 7.1.

The reduction of 300 mg. of this compound under standard conditions (300 mg. sodium, 5 cc. alcohol, 200°, eight hours) yielded 280 mg. of ether-soluble material, which was separated into alcoholic (300 mg. of acid succinate) and hydrocarbon (42 mg.) fractions. The crude ester after one recrystallization from dilute alcohol melted at 155–160°, showing that it consisted of a mixture of α- and β-cholestanol acid succinates. The hydrocarbon fraction gave on recrystallization cholestane melting at 78–80°, mixed m. p. with cholestane (m. p. 81°) 80°; yield of alcohols, computed from weight of crude succinates, 80%; of hydrocarbon, 15%.

The same experiment, repeated with the addition of 0.5 cc. of hydrazine hydrate, yielded 260 mg. (93%) of cholestane, m. p. 81°, $[\alpha]D$ +24.6° (chloroform).

Reduction of Cholestanone Ketazine.—When acetic acid was used to precipitate the cholestanone hydrazone from the above alcoholic solution, the ketazine, an amorphous white powder decomposing at about 200°, was obtained. *Anal.* Calcd. for $C_{54}H_{92}N_2$: N, 3.7. Found: N, 3.8. The reduction of 500 mg. of this compound, carried out under standard conditions, yielded 87 mg. (18%) of hydrocarbon and 477 mg. of acid succinate, corresponding to a 75% yield of alcohols.

When the reduction was carried out in the presence of 0.5 cc. of hydrazine hydrate, 500 mg. of the ketazine yielded 475 mg. (98%) of cholestane, m. p. 82°.

Reduction of Cholestenone Semicarbazone.—The semicarbazone of cholestenone was prepared by the pyridine–alcohol method to be described in the section on bile acids. The amorphous material which formed within an hour after mixing crystallized in clumps of needles after standing at room temperature for twenty-four hours, m. p. 215–235° (dec.).[24] The yield was quantitative.

Anal. Calcd. for $C_{28}H_{47}ON_3$: N, 9.5. Found: N, 9.5.

One and one-half grams of semicarbazone was treated with a solution of 1.5 g. of sodium in 25 cc. of absolute alcohol in a bomb tube at 210° for eight hours. The contents of the tube were poured into water, and the resulting alkaline solution extracted with ether. (Some material remained in the alkaline solution from which it was later extracted with ether after neutralization.) The crude residue (1.08 g.) from the ether was treated with 1.0 g. of

(21) Willstätter and Mayer, *Ber.*, **41**, 2199 (1908).

(22) Windaus and Uibrig, *ibid.*, **47**, 2384 (1914).

(23) Mauthner, *Monatsh.*, **30**, 635 (1909).

(24) Heilbron, Morton and Sexton, *J. Chem. Soc.*, 50 (1928).

succinic anhydride in 5 cc. of pyridine at room temperature for twenty-four hours.[25] The hydrocarbon fraction (500 mg.) after two recrystallizations from acetone–water and one from ether–methanol, yielded Δ_4-cholestene, m. p. and mixed m. p. 79°; $[\alpha]_D$ +65.0° (chloroform).

The virtual absence of saturated hydrocarbons was established in the following way. The material from the mother liquor was dissolved in ether and treated with a solution of bromine in acetic acid, and as much of the dibromide (m. p. 117°) as possible was removed by crystallization. The residue of the mother liquor was dissolved in carbon tetrachloride and treated with acetic anhydride and sulfuric acid according to Anderson and Nabenhauer.[26] The Δ_4-cholestene dibromide was decomposed completely by these reagents. The carbon tetrachloride on evaporation left only 3 mg. of an oily residue, which still gave a positive Liebermann–Burchard reaction.

The acid ester fraction (580 mg.) was saponified with a 20% solution of potassium hydroxide in 50% methanol. On cooling, a crystalline mass formed which was filtered off, washed with dilute methyl alcohol and water and dried *in vacuo*. This crude alcoholic fraction (450 mg.) gave a strongly positive Liebermann–Burchard reaction (presence of cholesterol) and a negative Rosenheim reaction (absence of Δ_4-alcohols). Treatment with 1.0 g. of digitonin in 85% alcohol gave 1.2 g. of digitonide. This, when decomposed with pyridine and ether, yielded 290 mg. of material which on repeated crystallization from 95% ethanol and from aqueous methanol gave a fraction (100 mg.), m. p. 141°. This material gave a negative Liebermann–Burchard reaction and showed no depression of its melting point when mixed with β-cholestanol. The material which remained in the mother liquors still gave an intense Liebermann–Burchard reaction but could not be separated into pure compounds. It probably consisted of cholesterol mixed with some β-coprostanol.

The digitonin filtrate was evaporated to dryness and extracted with ether. The extract after several recrystallizations from aqueous acetone melted at 117–118° and was identified as α-coprostanol. No α-cholestanol could be isolated from the mother liquors.

The acidic fraction (137 mg.), obtained on neutralization of the alkaline solution of the original reaction mixture, showed an intense Rosenheim reaction and contained 4.3% of nitrogen. After recrystallization from boiling 95% ethanol it formed long needles (50 mg.). The Rosenheim reaction was negative. *Anal.* Calcd. for $C_{28}H_{48}ON_3$: C, 76.9; H, 9.9; N, 9.6. Found: C, 77.2; H, 10.0; N, 9.6. This compound is probably identical with a substance of similar properties and composition (C, 77.07; H, 10.19; N, 9.66) which Stange[10] obtained as a by-product from the Wolff–Kishner reduction of Δ_4-cholestenedione-3,6 monosemicarbazone.

Since the residue from the mother liquors (70 mg.) still gave a very strong Rosenheim reaction, indicative of the presence of *allo*-cholesterols, it was dissolved in 50 cc. of alcohol containing a few drops of concentrated sulfuric acid, and the solution boiled for four hours. The solution was then poured into water and extracted with ether.

The ether after repeated washings with dilute sodium carbonate solution, yielded 13 mg. of a crystalline residue. On recrystallization from dilute alcohol long needles melting at 78° and exhibiting a specific rotation in benzene, $[\alpha]_D$ −113°, were obtained. $\Delta_{3,5}$-Cholestadiene[7] has m. p. 78–79°, $[\alpha]_D$ −112.5° (benzene).

Reduction of Cholestenone Ketazine in Presence of Hydrazine.—The ketazine was prepared in the same manner as the corresponding compound from cholestanone. It is an amorphous, yellow powder decomposing above 190°. It was dried *in vacuo* at 110° for analysis. *Anal.* Calcd. for $C_{54}H_{88}N_2$: N, 3.7. Found: N, 4.0.

The reduction of 500 mg. of the ketazine (500 mg. of sodium, 10 cc. of alcohol, 0.5 cc. of hydrazine hydrate, 200° for eight hours) yielded 490 mg. of ether-soluble material from which no alcohols could be isolated by the succinic anhydride procedure. On recrystallization from aqueous acetone 310 mg. of needles, m. p. 77°, was obtained. Several recrystallizations from alcohol gave Δ_4-cholestene, m. p. and mixed m. p. 78–79°.

Reduction of Dehydrodesoxycholic Acid Disemicarbazone.—The customary method of preparing semicarbazones by refluxing the ketonic compound with an alcoholic solution of semicarbazide acetate proved to be unsatisfactory in the case of the polyketonic bile acids. Although Borsche and Hallwass[11] had used this method for preparing the trisemicarbazone of dehydrocholic acid, we were unable to obtain a preparation with the required nitrogen content, no matter whether their procedure was employed in its original form or whether the conditions were varied in respect to the time of heating and the amounts of reagent used. In the preparation of the semicarbazone of 3-hydroxy-12-ketocholanic acid by this method the yield was very low, and the product was pigmented. Better results were obtained by the use of butyl alcohol as solvent. However, the method of Haller and LaForge,[27] employing semicarbazide acetate in a pyridine–alcohol solution, gave very satisfactory results, especially when the mixture was allowed to stand at room temperature for several days instead of being refluxed for a shorter period.

Five grams of commercial dehydrodesoxycholic acid, m. p. 186°, was dissolved in a mixture of 50 cc. of absolute alcohol and 50 cc. of dry pyridine. To this was added a solution of semicarbazide acetate, prepared by dissolving 5.0 g. of semicarbazide hydrochloride in 15 cc. of water and 5.0 g. of potassium acetate in 50 cc. of absolute alcohol, mixing, and filtering off the precipitated potassium chloride. After the addition of 10 cc. more water the solution was allowed to stand at room temperature for several days. The gelatinous mass which had formed was broken up, poured into a large volume of cold water, filtered and washed as thoroughly as possible to remove pyridine. After drying *in vacuo* at 110° a nearly colorless amorphous product was obtained in theoretical yield; decomposition point 215° after discoloration beginning at 190°. *Anal.* Calcd. for $C_{26}H_{42}O_4N_6$: N, 16.7. Found: N, 16.7.

Five grams of disemicarbazone was heated with a solution of 2.0 g. of sodium dissolved in 25 cc. of absolute alcohol at 200° for ten hours. The reaction mixture was

(25) Considerable amounts of pigment and resinous material were formed at 130°.

(26) Anderson and Nabenhauer, THIS JOURNAL, **46**, 1957 (1924).

(27) Haller and LaForge, *J. Org. Chem.*, **1**, 38 (1936).

diluted with water, acidified and extracted with ether. The ether residue was esterified with absolute alcohol and sulfuric acid and fractionated with succinic anhydride as previously described: succinic half-esters, 2.40 g.; cholanic acid ester, 1.58 g.

The neutral fraction (1.58 g.) was crystallized from absolute alcohol, yielding 1.0 g. of ethyl cholanate, colorless needles, m. p. and mixed m. p. 93°. The mother liquors, saponified with potassium hydroxide in methyl alcohol, yielded 200 mg. of crystalline cholanic acid, m. p. 164°. The over-all yield of cholanic acid from the disemicarbazone was 31%.

A small amount of 3-hydroxy-12-ketocholanic acid was isolated from the acid succinate fraction by the following procedure. The esters (2.4 g.) were saponified with alcoholic potassium hydroxide at room temperature for twelve hours. The solution was poured into water, acidified with hydrochloric acid and extracted with ether. The ether solution was washed with water and repeatedly extracted with 0.067 M disodium phosphate solution, which was acidified and again extracted with ether. The two ether fractions were again washed, dried and evaporated.

The material remaining in the first ether solution (1.43 g.) was crystallized from aqueous ethyl alcohol. Colorless plates, m. p. 175–177°, were obtained; 100 mg. of this fraction was treated with digitonin in 90% ethyl alcohol; 73 mg. of digitonide was obtained, indicating that the epimeric mixture contained 17.5% of the β-compound. Decomposition of the digitonide yielded material which on crystallization from aqueous ethanol gave colorless plates, m. p. 179°; [α]D +25.1° (alcohol). β-Lithocholic acid[28] has m. p. 180° and [α]D +25° (alcohol).

The filtrate from the digitonin precipitation yielded α-lithocholic acid which crystallized from ether–petroleum ether in colorless plates, m. p. 188°. *Anal.* Calcd. for $C_{24}H_{40}O_3$: C, 76.6; H, 10.6. Found: C, 76.7; H, 10.4. [α]D +33.6° (95% alcohol). α-Lithocholic acid[11] has [α]D +32.7°. The ethyl ester melted at 92°. Ethyl α-lithocholate[29] has m. p. 92–93°. *Anal.* Calcd. for C_{26}-$H_{44}O_3$: C, 77.3; H, 10.9. Found: C, 77.4; H, 10.6. The acetyl derivative melted at 169°. Acetyl α-lithocholic acid[29] has m. p. 169°.

The product recovered from the phosphate solution (0.36 g.), after crystallization from aqueous acetone, melted at 157°. It was shown to be a mixture of α- and β-3-hydroxy-12-ketocholanic acids similar to that obtained by Kyogoku[30] by the biological reduction of dehydrodesoxycholic acid. *Anal.* Calcd. for $C_{24}H_{38}O_4$: C, 74.0; H, 9.8. Found: C, 74.1, 74.1; H, 9.8, 9.9. The acetyl derivative formed needles from aqueous acetic acid; the melting point, 198°, is that reported by Kyogoku, and was not depressed by admixture with authentic α-3-acetoxy-12-ketocholanic acid. *Anal.* Calcd. for C_{26}-$H_{40}O_5$: C, 72.2; H, 9.3. Found: C, 72.2; H, 9.2. The acid succinate of the ethyl ester formed colorless prisms or needles from aqueous alcohol, m. p. 170°; [α]D +96.3° (ethanol); neutralization equivalent found, 517, 529. The melting point was unchanged on admixture with the acid succinate ([α]D +95.7° in alcohol) prepared from an authentic sample of ethyl α-3-hydroxy-12-ketocholanate.

Saponification of the acetyl derivative yielded free α-3-hydroxy-12-ketocholanic acid, m. p. 164°, mixed m. p. 165°.[30] The ethyl ester of this had m. p. and mixed m. p. 132–133°.

The yields of crystalline products from 5.0 g. of semicarbazone were: cholanic acid, 1.13 g., 31%; ($\alpha + \beta$)-lithocholic acids, 1.43 g., 38% of the theory; 3-hydroxy-12-ketocholanic acids (90% α-, 10% β-), 0.36 g., 9.2%.

To determine whether the time of reduction influenced the nature or proportion of the products, the following experiment was carried out. Each of four tubes was charged with 1.0 g. of disemicarbazone and 0.75 g. of sodium dissolved in 20 cc. of absolute alcohol. The tubes were placed in the oven at 210° and removed at intervals of 4.5, 8.0, 10.0 and 22 hours. The contents of the tubes were poured into water, acidified and extracted with petroleum ether. The petroleum ether was repeatedly extracted with 0.067 M disodium phosphate solution to remove the 3-hydroxy-12-ketocholanic acid present. The residue obtained on evaporating the petroleum ether was then subjected to the succinic anhydride procedure. The distribution of the material in the three fractions is recorded in Table II.

Reduction of 3-Hydroxy-12-ketocholanic Acid.—The acid was prepared by the method of Kaziro and Shimada,[31] m. p. 161° with sintering at 113–115°; [α]D +110° (alcohol); acetyl derivative, m. p. 198–199°; ethyl ester, m. p. 133°. The semicarbazone, which was obtained in quantitative yield by the pyridine–alcohol procedure, melted at 240° with decomposition.

Two grams of the semicarbazone was reduced under standard conditions. The reaction mixture was diluted with water, acidified and extracted with ether. A small amount of unreacted semicarbazone (80 mg.) collected at the interphase and was removed by filtration. The ether phase was washed with water and extracted with 0.067 M disodium phosphate solution. From the material contained in the phosphate solution (229 mg.) 3-hydroxy-12-ketocholanic acid, m. p. 157°, [α]D +84° (alcohol), was recovered. These properties are those of a mixture of 9 parts of α- and 1 part of β-acid.[30] The acid succinate of the ethyl ester was prepared (m. p. 169°) and identified by mixed melting point determination with the corresponding compound from the pure α-epimer (m. p. 170°); yield of crude acid, 10%.

The residue of the ether solution (1.215 g.) yielded on crystallization from dilute alcohol, lithocholic acid, m. p. 172–176°. Treatment of a small sample with digitonin showed the presence of 18% of β-compound in the epimeric mixture; yield of crude acid, 70%.

Reduction of Dehydrolithocholic Acid Semicarbazone.—Dehydrolithocholic acid (m. p. 140°) was obtained by oxidation of lithocholic acid with chromic acid by the method of Reindel and Niederländer.[28] The semicarbazone was prepared in the usual manner with semicarbazide acetate in alcohol. The colorless, semicrystalline product melted at 230° with decomposition.

Anal. Calcd. for $C_{25}H_{41}O_3N_3$: N, 9.7. Found: N, 9.8.

Five hundred mg. of semicarbazone was treated with 500 mg. of sodium dissolved in 10 cc. of absolute alcohol in a bomb tube at 200° for seven and one-half hours. The

(28) Reindel and Niederländer, *Ber.*, **68**, 1245 (1935).

(29) Reindel and Niederländer, *ibid.*, **68**, 1969 (1935).

(30) Kyogoku, *Z. physiol. Chem.*, **246**, 99 (1937).

(31) Kaziro and Shimada, *Z. physiol. Chem.*, **249**, 220 (1937).

reaction mixture was worked up in the usual manner. The acid succinate fraction weighed 425 mg., and the cholanic ester fraction 35 mg. This represents a lithocholic acid yield of 73% and of cholanic acid 7.7%. The succinic acid ester melted at 140–145°. For comparison the acid succinate of ethyl α-lithocholate was prepared from an authentic sample of ethyl α-lithocholate. It crystallized from ether–petroleum ether solution in large, hexagonal plates, m. p. 147°; neutralization equivalent calcd. 504; found, 505. On saponification of the succinic ester fraction, m. p. 140–145°, with alcoholic potassium hydroxide, a mixture of α- and β-lithocholic acids was obtained, m. p. 175–177°. Precipitation of a small sample with digitonin showed that approximately 15% of the β-epimer was present.

The neutral fraction on crystallization from absolute alcohol yielded needles of ethyl cholanate, m. p. and mixed m. p. 93°.

Reduction of Dehydrocholic Acid.—Recrystallized dehydrocholic acid, m. p. 236°, was used. The trisemicarbazone was obtained in theoretical yield by the alcohol-pyridine method. The nearly colorless, amorphous product melted above 290° with decomposition. The material was dried *in vacuo* at 110° for analysis.

Anal. Calcd. for $C_{27}H_{48}O_5N_9$: N, 22.0. Found: N, 21.9.

One gram of the semicarbazone was treated with a solution of 1.0 g. of sodium in 15 cc. of absolute alcohol at 200° for eight hours. The highly pigmented contents of the tube were poured into water, acidified with hydrochloric acid and extracted with ether. The residue from the ether solution (525 mg.) was dissolved in 90% acetic acid; 50 mg. of crystalline material, melting at 161°, deposited after prolonged standing. Recrystallization from acetone gave cholanic acid, m. p. and mixed m. p. 164°. The remaining oily material was esterified and the ester fraction (463 mg.) subjected to the succinic anhydride procedure in the usual way. The neutral fraction yielded 100 mg. of ethyl cholanate which after two recrystallizations from absolute alcohol showed m. p. and mixed m. p. 93°. From the sodium carbonate solution 397 mg. of succinic acid esters was obtained. Crystallization from aqueous methanol and aqueous acetone yielded plates melting at 140–145°. This material was therefore similar to the mixture of α- and β-lithocholic ester acid succinates obtained from the reduction of dehydrodesoxycholic acid disemicarbazone. Saponification at room temperature with alcoholic potassium hydroxide yielded, after two recrystallizations from aqueous acetone, colorless plates (250 mg.) of α-lithocholic acid, m. p. and mixed m. p. 188°; [α]D +33.6° (alcohol). The acetate melted at 169°. α-Lithocholic acid[29] has m. p. 188°; [α]D +33.5° (al-

cohol); acetate, m. p. 169°. The over-all yields from dehydrocholic acid were: cholanic acid, 142 mg., 22%; α-lithocholic acid, 250 mg., 38%.

The majority of the microanalyses reported in this paper were carried out by Mr. William Saschek.

Summary

The products found in the Wolff–Kishner reduction of the semicarbazone of cholestanone, coprostanone, cholestenone, dehydrocholic acid, dehydrodesoxycholic acid, dehydrolithocholic acid and 3-hydroxy-12-ketocholanic acid have been investigated. When the usual conditions are employed, the semicarbazone group at carbon atom 3 yields mainly the corresponding C_3-epimeric carbinols besides smaller amounts of the normal C_3-methylenic reduction products. Semicarbazone groups at C_7 and C_{12} are reduced in the normal manner to methylene. In this way lithocholic acid, in addition to cholanic acid, may be obtained in good yield from the ketonic bile acids. The occurrence of the abnormal type of reduction is not dependent on the configuration of carbon atom 5 in the C_3-ketone used.

The hydrazone and ketazine of cholestanone behave in the same manner as the semicarbazone, yielding on reduction mainly the epimeric cholestanols.

The abnormal reduction to carbinol is completely suppressed by the addition of an excess of hydrazine hydrate to the reaction mixture. Under these conditions a quantitative yield of cholestane was obtained from the semicarbazone, hydrazone and ketazine of cholestanone.

The reduction of cholestenone semicarbazone yields chiefly α-coprostanol, β-cholestanol, and Δ4-cholestene. Evidence for the formation of *allo*-cholesterol and cholesterol has been obtained. No saturated hydrocarbons are produced in the reaction.

A possible mechanism for the abnormal type of reduction is suggested.