the above unsaturated acid was converted to the acetate. The excess acetic anhydride was evaporated *in vacuo* and the residue was dissolved in 50 cc. of acetic acid. To this solution was added a solution of 4 g. of chromic anhydride in 50 cc. of 80% acetic acid. The mixture was allowed to stand at 50–55° for four hours. The reaction mixture was cooled and poured into water. The mixture was extracted with ether and the ether extract was washed with water and 10% sodium hydroxide. The ether was evaporated and the residue was warmed with 5% alcoholic potassium hydroxide solution. The mixture was cooled, poured into water and extracted with ether. The ether was evaporated and the residue was dissolved in 50 cc. of 90% ethanol. To the hot alcoholic solution was added 2 g. of semicarbazide hydrochloride and 3 g. of sodium acetate. After refluxing for one hour, water was added and the precipitated solid was filtered. This material was crystallized from ethanol, m. p. 241–243° dec., yield 1 g. This gave no depression when mixed with an authentic sample of the semicarbazone of *etio*-cholan-3(α)-ol-17-one.

Anal. Calcd. for $C_{20}H_{33}N_3O_2$: C, 69.1; H, 9.6. Found: C, 69.3; H, 9.6.

Ozonolysis of 3(β)-Hydroxy-Δ^{17-20}-pregnenoic Acid-21.—The product obtained from the acetylation of 3.5 g. of 3(β)-hydroxy-Δ^{17-20}-pregnenoic acid-21 was dissolved in 300 cc. of dry chloroform. Oxygen containing 7% ozone was bubbled through this solution at the rate of 30 liters per hour for ten minutes. At the end of this time no more ozone was absorbed. The reaction mixture was poured into water and stirred for thirty minutes. It was then heated on the steam-bath with stirring until the chloroform had all distilled off. After cooling it was extracted with ether. The ether was evaporated and the residue was warmed with 3.5 g. of potassium hydroxide dissolved in 200 cc. of ethanol for one hour. The mixture was then treated as described above isolating the product as the semicarbazone. This latter was crystallized from ethanol, m. p. 253° dec., yield 2.0 g. It gave no depression in melting point when mixed with an authentic sample of the semicarbazone of *etio*-cholan-3(β)-ol-17-one.

Anal. Calcd. for $C_{20}H_{33}N_3O_2$: C, 69.1; H, 9.6. Found: C, 69.4; H, 9.5.

Hydrolysis of the Semicarbazone of *etio*-Cholan-3(β)-ol-17-one.—A solution of 1.3 g. of the semicarbazone of *etio*-cholan-3(β)-ol-17-one in 75 cc. of ethanol containing 7 cc. of concentrated sulfuric acid and 15 cc. of water was refluxed for one hour and then poured into water. The precipitated solid was extracted with ether. The ether extract was washed with water and the ether was evaporated. The crystalline residue was crystallized from ether–pentane to give long needles, m. p. 150–152°, which gave no depression in melting point when mixed with an authentic sample of *etio*-cholan-3(β)-ol-17-one.

Anal. Calcd. for $C_{19}H_{30}O_2$: C, 78.6; H, 10.4. Found: C, 78.4; H, 10.3.

Summary

17,21-Dibromo-pregnan-3(β)-ol-20-one acetate has been converted to 3(β)-hydroxy-Δ^{17-20}-pregnenoic acid-21 and the latter reduced to 3(β)-hydroxy-pregnanoic acid-21.

3(β)-Hydroxy-Δ^{17-20}-pregnenoic acid-21 has been oxidized to *etio*-cholan-3(β)-ol-17-one.

STATE COLLEGE, PENNA. RECEIVED JUNE 25, 1941

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

Sterols. CXXXIX. Sapogenins. LIX. The Bio-reduction of 4-Dehydrotigogenone[1,1a]

BY RUSSELL E. MARKER, EMERSON L. WITTBECKER, R. B. WAGNER AND D. L. TURNER

Since coprosterol has not been obtained directly from cholesterol by chemical action, Schoenheimer believed that cholestenone was an intermediate in the formation of coprosterol in the animal intestine. He therefore fed cholestenone to a dog and examined the sterol content of the feces.[2] When the dog was on a basic meat diet an increased coprosterol excretion was observed, but with a diet of dog biscuits there was an increased cholesterol excretion. It seemed reasonable to conclude that the formation of cholestenone from cholesterol is a biologically reversible process. Additional evidence to this effect was secured by Diels[3] who found an increase in cholesterol content of the livers of guinea pigs after the administration of cholestenone. However, these experiments are subject to the objection noted by Anchel and Schoenheimer[4] that the cholesterol may not arise from the administered cholestenone but by a secondary effect of the latter on sterol metabolism, especially since it is known that the feeding of squalene and various other substances increases the excretion of cholesterol.[5] In order to make the experiments more exact, Schoenheimer and his co-workers introduced deuterium as an indicator. However, the feeding of deuterocholestenone to mice did not give information concerning the formation of cholesterol from cholestenone. Cholesterol with insignificant deuterium content was isolated from the feces but

(1) *Cf.* Marker, *et al.*, THIS JOURNAL, **63**, 1769 (1941).

(1a) Original manuscript received June 23, 1941.

(2) Schoenheimer, Rittenberg and Graff, *J. Biol. Chem.*, **111**, 183 (1935).

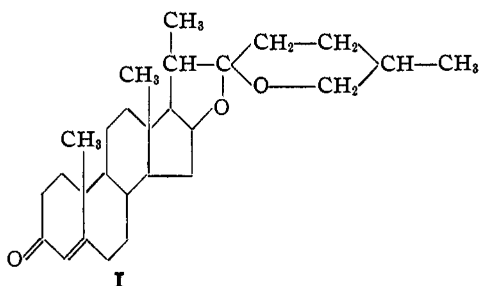
(3) Diels, *Z. ges. exp. Med.*, **100**, 527 (1937).

(4) Anchel and Schoenheimer, *J. Biol. Chem.*, **125**, 23 (1938).

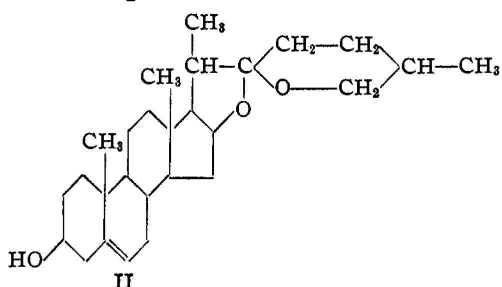
(5) Channon and Tristram, *Biochem. J.*, **31**, 738 (1937).

it was pointed out[4] that the deuterium may have been lost during the conversion process.

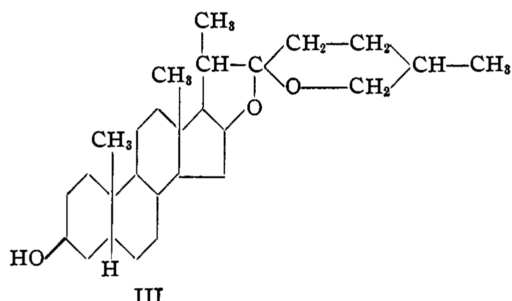
We have administered 4-dehydrotigogenone (I) to a dog fed on a diet of dog biscuit. While this substance has the nuclear structure of cholestenone, it has the characteristic side-chain of the sapogenins, which constitutes an effective indicator. Since it is entirely foreign to the animal metabolism, it can be the only source of sapogenin derivatives found in the feces. The non-saponifiable fraction of the feces gave diosgenin (II), smilagenin (III) (iso-sarsasapogenin) and *epi*-smilagenin (IV) (*epi*-iso-sarsasapogenin). These products correspond in structure to cholesterol, coprosterol and *epi*-coprosterol, respectively.



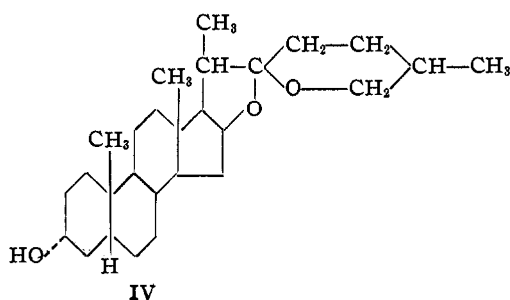
I



II



III



IV

The reduction products of 4-dehydrotigogenone thus correspond to those obtained from cholestenone by Schoenheimer[2] except that he did not isolate *epi*-coprosterol. We found no bio-reduction products of 4-dehydrotigogenone with the *allo*-configuration. However, the defects of the experimental technique prevent a completely quantitative analysis. Cholestanol accompanies coprosterol in small quantities in feces.[6] There is evidence that it is secreted into the intestine and accumulates because it cannot be resorbed.[7] On the other hand, the coprosterol from the feces is probably a product of bacterial reduction since administered cholesterol causes an increase in the coprosterol content of the feces of animals on a meat diet[2,8] but is excreted unchanged by animals fed diets which do not contain meat.[9,10] The origin of coprosterol by bacterial reduction is confirmed by the experiments of Dam[11] and Bischoff.[12,13]

The isolation of *epi*-smilagenin from the feces of the dog to which 4-dehydrotigogenone was administered led us to expect the presence of *epi*-coprosterol in normal feces. It seemed probable that the failure of previous workers to isolate *epi*-coprosterol from feces was dependent on the usual practice of isolating the sterols as digitonides and neglecting the non-precipitable fraction.

Accordingly, we examined the feces of another dog which had been fed a normal diet. A considerable amount of *epi*-coprosterol was isolated. This experiment lends additional support to Schoenheimer's theory[4] that cholestenone is an intermediate in the formation of coprosterol in the organism. It is interesting that bacterial reduction of the C-3 carbonyl group in steroids seems to favor the formation of the $3(\alpha)$ carbinol. Thus dehydro-chenodesoxycholic acid was reduced by fermentation with *B. coli* to chenodesoxycholic acid[14] and testosterone has been converted to *etio*-cholane-$3(\alpha),17(\alpha)$-diol[15] by bacterial reduction.[16]

The conversion of a Δ^4-3-keto steroid to a Δ^5-3-

(6) Windaus and Uibrig, *Ber.*, **48**, 857 (1915).
(7) Schoenheimer and Hrdina, *Z. physiol. Chem.*, **212**, 161 (1932).
(8) Bondzynski and v. Huminicki, *ibid.*, **22**, 396 (1896).
(9) Muller, *ibid.*, **29**, 129 (1900).
(10) Doree and Gardner, *Proc. Roy. Soc.* (London), **80B**, 227 (1908).
(11) Dam, *Biochem. J.*, **28**, 820 (1934).
(12) Bischoff, *Biochem. Z.*, **222**, 211 (1930).
(13) Bischoff, *ibid.*, **227**, 230 (1930).
(14) Sihn, *J. Biochem.* (Japan), **28**, 165 (1938).
(15) Ercoli, *Ber.*, **71**, 650 (1938).
(16) Schramm and Mamoli, *ibid.*, **71**, 1322 (1938).

hydroxy-steroid demonstrated in this paper supports the hypothesis of Marker[17] that there is a reversible reaction of the type cholestenone ⇆ cholesterol which can occur under biological conditions. The cholestenone is reduced by one enzyme system to coprosterol and *epi*-coprosterol but there is probably another enzyme system capable of reconverting it to cholesterol, which may in turn be reoxidized to cholestenone. Although neither cholestenone nor coprostanone has been isolated from the organism, the possibility of bio-oxidation is indicated by the isolation of androsterone, *etio*-cholan-3(α)-ol-17-one[18,19] and isoandrosterone[20] from the urine of men to whom testosterone propionate was administered.

The presence or absence of Δ^5-3-hydroxy steroids in excreta depending on the character of the diet possibly indicates that the reduction of the Δ^4-3-keto steroid may be more complete in some cases and lead to the exclusive formation of saturated steroids, while in others the Δ^5-3-hydroxy steroid can be formed. In our experiments a large excess of Δ^4-3-keto steroid was deliberately given to the dog so that there would be an opportunity for incomplete reduction. Fieser[21] has emphasized the failure of Callow[18] to isolate dehydro-isoandrosterone from the urine of a patient given testosterone propionate. However, a single negative experiment does not prove the impossibility of the biological conversion of androstenedione to dehydro-isoandrosterone. A constituent of urine may be missed because of the defects inherent in the isolation technique. A striking example was observed in this Laboratory[22] when we failed to isolate even a trace of pregnane-3(α),20(α)-diol from a large quantity of human pregnancy urine collected from many different patients and treated by a standard technique which ordinarily leads to the successful isolation of this substance.[23,24]

The various biochemical experiments have been of value in showing what conversions are possible in biological systems, but the demonstration of one type of bio-reaction does not eliminate other possibilities. Thus the assumption of Fieser that the conversion of a Δ^4-3-keto steroid to a Δ^5-3-hydroxy steroid was impossible because of Callow's and Mamoli's[25] experiments is at least questionable.

The theory of Marker[17] was not intended to be construed in an absolutely rigid sense. Thus it does not imply as Callow appears to think[16] that androstenedione is the *only* possible precursor of testosterone. Adrenal cortical steroids also have been considered.[17,26,27]

The bio-reduction of 4-dehydrotigogenone to smilagenin and *epi*-smilagenin is another proof that diosgenin and smilagenin have the same side-chain configuration.[28] *epi*-Smilagenin has not been made previously. In order to prove its identity we have prepared it by the reduction of smilagenone with sodium and ethanol or with hydrogen using Adams catalyst in ethanol. Both methods gave the same product. It did not precipitate with digitonin. Oxidation of the *epi*-smilagenin gave smilagenone and energetic reduction in acetic acid with hydrogen in the presence of Adams catalyst gave dihydro-*epi*-sarsasapogenin.[29] This is in agreement with the previous observation[30] that smilagenin and sarsasapogenin gave the same dihydrosapogenin.

We wish to thank Parke, Davis and Company for their assistance.

Experimental

A 20-kg. male dog was fed daily a mixture of 300 g. of dog biscuits and 30 g. of lard containing 3 g. of 4-dehydrotigogenone, for three consecutive days. In addition a solution of 1 g. of 4-dehydrotigogenone in 20 cc. of peanut oil was injected subcutaneously daily for the three days he was maintained on the above diet. The 4-dehydrotigogenone used in this experiment was carefully purified and gave no precipitation with digitonin. After these feedings and injections, the dog was maintained for three additional days on a diet of dog biscuits. His feces were collected during the entire period and immediately ground up in acetone and thoroughly extracted. The residue was finally extracted with ether. The solvent was removed from the total extract and the residue was hydrolyzed by refluxing for one hour with a 5% solution of alcoholic potassium hydroxide. The non-saponifiable fraction was extracted with ether and the solvent was removed. This fraction weighed 9.5 g. It was dissolved in a small amount of hot alcohol and to it was added a solution of 10 g. of digitonin in 1 liter of 95% alcohol. After standing overnight the precipitate was filtered and dried. It weighed 9.5 g.

(17) Marker, THIS JOURNAL, **60**, 1725 (1938).
(18) Callow, *Biochem. J.*, **33**, 559 (1939).
(19) Dorfman, Cook and Hamilton, *J. Biol. Chem.*, **130**, 285 (1939).
(20) Dorfman and Fish, *ibid.*, **135**, 349 (1940).
(21) Fieser, THIS JOURNAL, **63**, 1485 (1941).
(22) Unpublished experiments.
(23) Marrian, *Biochem. J.*, **23**, 1090 (1929).
(24) Butenandt, *Ber.*, **63**, 659 (1930).
(25) Mamoli, *et al.*, *Ber.*, **71**, 156, 650, 2083, 2698 (1938).
(26) Marker and Lawson, THIS JOURNAL, **60**, 2928 (1938).
(27) Marker and Rohrmann, *ibid.*, **61**, 3476 (1939).
(28) Marker, Tsukamoto and Turner, *ibid.*, **62**, 2525 (1940).
(29) Marker and Rohrmann, *ibid.*, **61**, 943 (1939).
(30) Marker and Rohrmann, *ibid.*, **61**, 846 (1939).

The digitonide was decomposed in the usual manner with pyridine. The digitonin precipitable sterol fraction, 2.0 g., was dissolved in 1 liter of ether. When the solution was concentrated to about 15 cc., a considerable amount of precipitate formed. The ethereal solution was cooled and filtered. The precipitate was boiled with 25 cc. of ether. A portion of this did not dissolve. It was filtered hot, and the precipitate which was insoluble in ether was recrystallized from a small amount of methanol–acetone mixture. It was very insoluble in ether and acetone, but dissolved readily in a small amount of methanol. It melted at 300–305° and weighed 0.1 g. This fraction was not investigated further. The mother liquors from this fraction were crystallized from methanol and from acetone; m. p. 207–209°; yield 0.2 g.; there was no depression when the product was mixed with an authentic sample of diosgenin.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 78.0; H, 10.1.

When refluxed with acetic anhydride, the above product gave an acetate which was crystallized from acetic anhydride and from methanol; m. p. 199–200°; it gave no depression when mixed with an authentic sample of diosgenin acetate.

Anal. Calcd. for $C_{29}H_{44}O_4$: C, 76.3; H, 9.7. Found: C, 76.4; H, 9.5.

The ether-soluble digitonin precipitable sterol fraction was crystallized several times from methanol and from acetone. An intermediate fraction which appeared to be a mixture of products was discarded. A fraction of 0.1 g. was obtained which melted at 178–180°; this gave no depression when mixed with an authentic sample of smilagenin.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.6. Found: C, 77.5; H, 10.6.

The above product was converted to the acetate by boiling with acetic anhydride. It was crystallized from methanol, m. p. 152°; there was no depression when it was mixed with an authentic sample of smilagenin acetate.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 76.0; H, 10.0.

The non-digitonin precipitable fraction, 7.2 g. was crystallized from acetone. This yielded 3.2 g. of recovered 4-dehydrotigogenone. The mother liquors of this product were treated with succinic anhydride to remove the carbinol fraction from the non-carbinol fraction. The non-carbinol fraction was crystallized from acetone and yielded an additional 1 g. of 4-dehydrotigogenone, m. p. 186–188°.

The succinic ester of the carbinol fraction was hydrolyzed with alcoholic potassium hydroxide and the carbinols were extracted with ether. The solvent was removed and the residue was crystallized from acetone and from methanol, m. p. 215–217°; yield 0.4 g.; there was no depression when it was mixed with an authentic sample of *epi*-smilagenin (*epi*-isosarsasapogenin).

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.6. Found: C, 77.6; H, 10.4.

When refluxed with acetic anhydride it gave an acetate which melted at 158–160°; there was no depression when it was mixed with an authentic sample of *epi*-smilagenin acetate.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 75.9; H, 10.0.

***epi*-Smilagenin.**—(a) A mixture of 3.5 g. of smilagenone (isosarsasapogenone), 1 g. of platinum oxide catalyst and 300 cc. of absolute ethyl alcohol was shaken with hydrogen at room temperature and three atm. pressure for seventy-five minutes. The mixture was filtered, and the filtrate was evaporated *in vacuo*. The residue was crystallized from acetone and from methanol, m. p. 217–220°.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.6. Found: C. 77.9; H, 10.5.

(b) A solution of 2 g. of smilagenone in 300 cc. of absolute alcohol was treated with 20 g. of sodium in small pieces. After the sodium had all dissolved, water was added and the mixture was extracted with ether. The ether layer was washed with water and then evaporated. The product was crystallized from methanol and then from acetone, m. p. 217–220°; it gave no depression in melting point when mixed with the above product.

When refluxed with acetic anhydride, this compound gave an acetate which was crystallized from acetic anhydride and from methanol; m. p. 158–159°.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 75.8; H, 10.3.

Oxidation of *epi*-Smilagenin.—A solution of 0.5 g. of *epi*-smilagenin in 100 cc. of acetic acid was oxidized at 25° with 250 mg. of chromic anhydride in 20 cc. of 90% acetic acid. The mixture was allowed to stand for forty-five minutes. Water was added and the mixture was extracted with ether. The ether layer was washed with dilute sodium carbonate solution, water, and then evaporated. The residue crystallized from acetone; m. p. 185–187°; yield 0.4 g.; it gave no depression in melting point when mixed with an authentic sample of smilagenone.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 78.3; H, 10.0.

***epi*-Dihydrosarsasapogenin.**—A mixture of 0.5 g. of *epi*-smilagenin, 1.0 g. of platinum oxide catalyst and 500 cc. of acetic acid was shaken with hydrogen at 70–75° and three atmospheres pressure for ten hours. The mixture was filtered and the filtrate was evaporated *in vacuo*. The residue crystallized from ether–pentane, m. p. 134–136°; mixed with dihydro-*epi*-sarsasapogenin it gave no depression in melting point. When crystallized from acetone a polymorphic form was obtained which melted at 180–182°.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.4; H, 11.1. Found: C, 77.1; H, 10.9.

Isolation of *epi*-Coprosterol from Dog Feces.—The feces used in this experiment were collected from a male dog which was maintained on a normal well-balanced dog food diet. This dog had not previously been used in any feeding experiments. The feces were extracted with acetone and with ether. The solvent was removed and the residue was hydrolyzed with alcoholic potassium hydroxide. The non-saponifiable fraction was extracted with ether. A solution of 3 g. of this product in alcohol was treated with an excess of digitonin in 90% alcohol. This digitonin precipitable fraction which gave 3.5 g. of digitonides was

not investigated since a careful study of this fraction has already been made by other workers. The non-digitonin precipitable fraction was treated with succinic anhydride in pyridine to separate the carbinols from the non-carbinol fraction. This yielded 0.8 g. of carbinols upon hydrolysis. These were sublimed at about 10^{-3} mm.; a fraction distilling at 110–140° was collected. This gave no depression in melting point when mixed with an authentic sample of *epi*-coprosterol prepared by the reduction of coprostanone; yield 0.1 g. It gave no precipitation with digitonin in alcohol.

Anal. Calcd. for $C_{27}H_{48}O$: C, 83.4; H, 12.5. Found: C, 83.4; H, 12.4.

Summary

1. *epi*-Smilagenin has been prepared from smilagenone.
2. The administration of 4-dehydrotigogenone to a dog led to an excretion of diosgenin, smilagenin and *epi*-smilagenin in the feces.
3. *epi*-Coprosterol is excreted normally in the feces of the dog.
4. The significance of these facts has been discussed.

STATE COLLEGE, PENNA. RECEIVED JANUARY 8, 1942

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

Sterols. CXL. 17-Bromo-*allo*-pregnanone-20 and 17,21-Dibromo-*allo*-pregnanone-20*

BY RUSSELL E. MARKER, HARRY M. CROOKS, JR., R. B. WAGNER, ANTHONY C. SHABICA, ELDON M. JONES AND EMERSON L. WITTBECKER

For a study of the reactions of the 20-keto-pregnane series, *allo*-pregnanone-20 is a model compound since it is easily obtained from *allo*-pregnanedione-3,20[1] and there are no other complicating reactive groups in the molecule. We have, therefore, used it as a test compound for many of the reactions of the 17-bromo- and 17,21-dibromo-20-keto-pregnane series.[2] Some of these reactions are now presented for the *allo*-pregnanone-20 series.

allo-Pregnanone-20 was prepared by partial Clemmensen reduction of *allo*-pregnanedione-3,20 as previously reported.[1] Treatment of *allo*-pregnanone-20 with 1 mol of bromine in acetic solution in the presence of hydrogen bromide led to formation in good yield of a crystalline 17-monobromide. Reduction of the monobromide with zinc or iron and acetic acid or with hydrogen–palladium in the presence of pyridine gave *allo*-pregnanone-20. Refluxing of a pyridine solution of the monobromide for four hours gave a good yield of Δ^{16}-*allo*-pregnanone-20. The latter compound was reconvertible to *allo*-pregnanone-20 by hydrogen–palladium reduction.

The direct treatment of *allo*-pregnanone-20 with 2 mols of bromine in acetic acid solution or treatment of the monobromide with 1 mol of bromide gave good yields of 17,21-dibromo-*allo*-pregnanone-20. The dibromide was reconvertible to the parent ketone by reduction with zinc or iron in acetic acid or by potassium formate–formic acid (bomb reaction, ten hours at 130°).

Refluxing of the 17,21-dibromide with alcoholic potassium hydroxide yielded Δ^{17-20}-*allo*-pregnenoic acid-21 which on ozonolysis gave androstanone-17 isolated as the semicarbazone and characterized as the free ketone.

The reactions are summarized in the accompanying chart.

We thank Parke, Davis and Company for their assistance.

Experimental

17-Bromo-*allo*-pregnanone-20.—To a solution of 10 g. of *allo*-pregnanone-20 in 200 cc. of glacial acetic acid were added 10 drops of concentrated hydrobromic acid and 33.2 cc. of 1 *M* bromine solution in acetic acid. After standing for fifteen minutes, the solution was poured into water, extracted with ether and the extract washed free of acetic acid with water and dilute sodium carbonate solution. Evaporation of the ether gave a residue which was crystallized from ether–methanol to give 8 g., m. p. 113–121°. Recrystallization from acetone gave crystals, m. p. 127–9° containing halogen (Beilstein test) which depressed the melting point of *allo*-pregnanone-20, m. p. 132°, twenty degrees.

Anal. Calcd. for $C_{21}H_{33}OBr$: C, 66.2; H, 8.7. Found: C, 65.6; H, 8.8.

Reduction of 17-Bromo-*allo*-pregnanone-20. (a) **Zinc and Acetic Acid.**—A solution of 500 mg. of the 17-monobromide in 20 cc. of glacial acetic acid was warmed on the steam-bath one hour with 500 mg. of powdered zinc. The solution was decanted into water from the excess zinc, extracted with ether and the ether washed well with water.

* Original manuscript received July 5, 1941.

(1) Marker and Lawson, THIS JOURNAL, **61**, 852 (1939).

(2) Marker and co-workers, *ibid.*, **64**, 210, 213, 817 (1942).