By combination of a determination of total MG with a 1-MG determination before isomerization, the 2-MG content of fatty mixtures may be obtained by difference.

Perchloric Acid Recovery Following Isomerization.—The perchloric acid enters into the isomerization reaction only as a catalyst since perchloric acid recovery by precipitation as nitron perchlorate following isomerization was found to be 99.0% as compared with 98.6% in a blank sample.

Equilibrium Composition of Monoglycerides.—The primary purpose of this work has been to develop a method to determine total monoglyceride concentrations in fats in which significant quantities of 2-MG may be present. In addition to achieving the above purpose the study has led to the conclusion that 1- and 2-MG co-exist in an equilibrium composition which is considerably higher in 2-MG concentration than commonly recognized. The summarizing data of Table III lead to the conclusion that equilibrium between 1- and 2-MGs exists in the composition range of 90–92% 1-MG and 10–8% 2-MG.

Equilibrium is most certainly achieved in the diglyceride (DG) system by action of perchloric acid on chloroform solutions of either 1,2- or 1,3-DG as evidenced by melting point changes with isomerization, but in this work no attempt was made to ascertain the equilibrium composition of the DG system.

TABLE III

COMPOSITION OF FAT RECOVERED FROM PERCHLORIC ACID ISOMERIZATION OF MONOGLYCERIDES

Criteria for fat composition	Indicated composition of fat
Periodic acid detn. of 1-MG after isom. and recovery of fat from soln.	88.5% 1-MG
Detn. of glycerol freed during isom.	2–4% diglyceride
Decrement in hydroxyl val. resulting from isom.	4% diglyceride
Craig separator anal. and periodic acid anal. of isomerized MG	9% 2-MG

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CINCINNATI 31, OHIO

[CONTRIBUTION FROM THE CHEMICAL RESEARCH LABORATORIES OF SCHERING CORPORATION]

11-Oxygenated Steroids. VII. The Acylation of 11β-Hydroxy Steroids: The Synthesis of Compound F 11-Acetate and Related Compounds[1]

BY EUGENE P. OLIVETO, CORINNE GEROLD, LOIS WEBER, H. E. JORGENSEN, RICHARD RAUSSER AND E. B. HERSHBERG

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A general method is described for the acylation of 11β-hydroxy steroids containing no activating groups at C-9 or C-12. Pregnan-3α,11β,17α-ol-20-one 11-acetate has been prepared and converted to Kendall compound F 11-acetate.

Several successful syntheses of Kendall compound F have been described, starting with cortisone[2] or with intermediates from its synthesis.[3] Microbiological processes[4] starting with Reichstein compound S also have been reported. It was our hope that a relatively simple synthesis of compound F could be developed which would be exactly analogous to the Gallagher[5] synthesis of cortisone, except that the starting material would be pregnan-3α,11β,17α-triol-20-one[6] instead of the corresponding 11-ketone. The sequence involved bromination at C-21, replacement of the halogen with acetate, and oxidation (*via* the Oppenauer reaction) at C-3 to yield 4,5-dihydro compound F acetate. This latter compound had been transformed to compound F previously.[3b] In our hands, the predominant reaction upon adding bromine to pregnan-3α,11β,17α-triol-20-one in chloroform was not bromination at C-21, but oxidation at C-11,[7] and consequently this reaction scheme was dropped.[8]

The obvious way to avoid oxidation at C-11 during the bromination is to protect the C-11 hydroxyl group in some manner, such as by ester formation. The literature, however, records no instance of the acetylation of an 11β-hydroxyl group, when there are no activating groups at C-9 or at C-12.[9] Indeed, the failure of an 11-hydroxyl group to esterify

(1) (a) Paper VI, E. P. Oliveto, H. L. Herzog, M. A. Jevnik, H. E. Jorgensen and E. B. Hershberg, THIS JOURNAL, **75**, 3651 (1953). (b) A preliminary report of this work has appeared in *Arch. Biochem. Biophys.*, **43**, 234 (1953).

(2) (a) R. Antonucci, S. Bernstein, M. Heller, R. Lenhard, R. Littell and J. Williams, *J. Org. Chem.*, **18**, 70 (1953); (b) N. Wendler, Huang-Minlon and M. Tishler, THIS JOURNAL, **73**, 3818 (1951).

(3) (a) R. Levin, B. Magerlein, A. McIntosh, A. Hanze, G. Fonken, J. Thompson, A. Searcy, M. Scheri and E. Gutsell, *ibid.*, **75**, 502 (1953); (b) N. Wendler, R. Graber, R. Jones and M. Tishler, *ibid.*, **72**, 5793 (1950).

(4) H. C. Murray and D. H. Peterson, U. S. Patent 2,602,769; D. Colingsworth, M. Brunner and W. Haines, THIS JOURNAL, **74**, 2381 (1952).

(5) T. H. Kritchevsky, D. C. Garmaise and T. F. Gallagher, *ibid.*, **74**, 483 (1952).

(6) E. P. Oliveto, T. Clayton and E. B. Hershberg, *ibid.*, **75**, 486 (1953).

(7) Other workers, however, have reported successful bromination at C-21 in the presence of an 11β-hydroxyl group, but no details are as yet available (ref. 3a and 8).

(8) Exactly the same sequence of reactions for the synthesis of compound F had been discussed by Dr. P. L. Julian at the Laurentian Hormone Conference, Sept., 1950 ("Recent Progress in Hormone Research," Academic Press, Inc., New York, N. Y., 1951, p. 206). However, no further reports have been released from his laboratory.

(9) The presence of a 3,9-epoxide apparently facilitates acetylation of an 11β-hydroxyl group: H. Heymann and L. F. Fieser, THIS JOURNAL, **73**, 5252 (1951); V. R. Mattox, R. B. Turner, B. F. McKenzie, L. L. Engel and E. C. Kendall, *J. Biol. Chem.*, **173**, 283 (1948). Similarly, a 12-keto group facilitates the alkaline hydrolysis of an 11β-acetate: W. P. Long and T. F. Gallagher, *ibid.*, **162**, 511 (1946).

at room temperature using acetic anhydride and pyridine invariably was taken as excellent evidence that the hydroxyl at C-11 was in the β-configuration.[10] More drastic treatment (refluxing acetic anhydride in pyridine) usually tended to destroy the hydroxyl group.

We have found, however, that acetylation of 11β-hydroxy groups is easily accomplished, in good yield, by treatment of the steroid with an acylating agent in the presence of a strong acid catalyst at room temperature. Thus treatment of pregnan-3α,11β,17α-triol-20-one[6] (I) with (a) acetic acid, acetic anhydride and *p*-toluenesulfonic acid[11]; or (b) acetic acid, acetic anhydride and perchloric acid; or (c) isopropenyl acetate and *p*-toluenesulfonic acid, all at room temperature overnight, gave a new compound, m.p. 209–210°, whose infrared spectrum showed no hydroxyl peak. Integration of the acetate carbonyl frequency area gave a value in agreement with that expected for three acetate groups, as did the carbon and hydrogen analyses. Since the first set of conditions is known to acetylate the 17α-hydroxyl group without causing D-homo rearrangement,[11] the new compound must be pregnan-3α,11β,17α-triol-20-one triacetate (II).

The triacetate II could be partially hydrolyzed by acid to the 11,17-diacetate, or by alkali (either sodium carbonate or sodium hydroxide) to the 11-monoacetate IV. This latter compound could also be prepared by another sequence starting with pregnan-3α,11β-diol-20-one.[6] Treatment with acetic anhydride and *p*-toluenesulfonic acid overnight produced the 3,11-diacetate XV. This was not isolated, but was converted to the 17α-hydroxy-20-ketone IV *via* enol acetate formation, perbenzoic acid oxidation and alkaline hydrolysis.[12]

Although the yield of IV obtained by this method was satisfactory, its quality did not compare favorably with that obtained by the sequence I → II → IV. This may be due to the prolonged heating the diacetate XV undergoes during the enol acetylation reaction.

The 11-monoacetate IV could be reconverted to the triacetate II by treatment with acetic acid, acetic anhydride and *p*-toluenesulfonic acid, thus indicating that no rearrangement or dehydration had occurred during the hydrolysis.

The conversion of IV to Δ^4-pregnen-11β,17α-diol-3,20-dione-11-acetate (VII) and to compound F 11,21-diacetate XII was accomplished by procedures analogous to the Gallagher synthesis[5] of cortisone acetate. The reactions are outlined in the accompanying flow sheet. Only in the conversion of IV to the 21-acetate IX *via* the bromide VIII was any difficulty encountered, and this was believed to be due to the presence of varying amounts of the $\Delta^{9(11)}$-compound which could have formed during the acetylation reaction. When the temperature of the acetylation was not allowed to rise above room temperature at any time, the yields from the bromination and acetoxylation reactions rose to about 80%, and no longer fluctuated.[13]

Compound F 11,21-diacetate XII was hydrolyzed to the 11-monoacetate XIII by acid hydrolysis, using hydrochloric acid.

The hypsochromic effect on 3-keto-Δ^4- systems produced by acetylation of an 11α-hydroxyl group has already been noted.[2a,14] Acetylation of an 11β-hydroxyl group (compounds VII and XII) also produces the same hypsochromic shift of 2 mμ.

Experimental[15]

Pregnan-3α,11β,17α-triol-20-one Triacetate (II).—To a solution of 4.0 g. of pregnan-3α,11β,17α-triol-20-one[6] in 40 ml. of acetic acid and 8 ml. of acetic anhydride was added 0.4 g. of *p*-toluenesulfonic acid. A temperature rise always occurred after an induction period of 1–2 hr., and the best results were obtained by maintaining the temperature of the solution at room temperature (*ca.* 25°). After standing overnight, the solution was poured into water and the resulting solid collected by filtration, washed with water and dried; wt. 5.22 g. (98.4%), m.p. 196–200°. The analytical sample, crystallized twice from acetone–hexane, melted at 209.0–210.0°, $[\alpha]_D$ +54.3° (chl.). The infrared spectrum showed no hydroxyl peak, and integration of the acetate carbonyl frequencies gave a value in agreement with that expected for three acetate groups.

Anal. Calcd. for $C_{27}H_{40}O_7$: C, 68.04; H, 8.46. Found: C, 68.28; H, 8.39.

Similar results were obtained with a mixture of acetic acid, acetic anhydride and perchloric acid, or of isopropenyl acetate and *p*-toluenesulfonic acid.

Pregnan-3α,11β,17α-triol-20-one 11,17-Diacetate (III).—A solution of 1.0 g. of II in 10 ml. of chloroform and 35 ml. of methanol containing 2.1 ml. of concd. hydrochloric acid and 3.5 ml. of water was allowed to stand at 20° for 24 hr. An additional 120 ml. of water was then added, and the resulting mixture placed in an ice-chest for another 24 hr. Water was then added and the mixture extracted with chloroform. The organic extracts were washed neutral with water, dried over sodium sulfate and concentrated to a residue to yield crude III, m.p. 212–222°. The analytical sample was crystallized from ether and had a m.p. of 233.5–235.0°, $[\alpha]_D$ +28.0° (chl.). The infrared spectrum confirmed the loss of one acetate group, which had not been adjacent to a ketone carbonyl.

Anal. Calcd. for $C_{25}H_{38}O_6$: C, 69.09; H, 8.81. Found: C, 68.85; H, 9.12.

Pregnan-3α,11β,17α-triol-20-one 11-Acetate (IV).—A solution of 5.0 g. of II in 100 ml. of methanol and 30 ml. of water containing 2.2 g. of sodium carbonate was refluxed for 24 hr. Excess base was neutralized by the addition of acetic acid. The methanol was removed under reduced pressure, water was added and crude IV was removed by filtration: wt. 4.0 g. (97%), m.p. 107–115° dec. Recrystallization from ether gave m.p. 103.0–105.0° dec., $[\alpha]_D$ +35.6° (chl.). The infrared spectrum indicated the presence of one acetate group, not adjacent to a ketone carbonyl. Reacetylation gave the original triacetate, indicating that no rearrangement or dehydration had occurred during the hydrolysis.

(10) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publishing Corp., New York, N. Y., 1949, pp. 654–658.

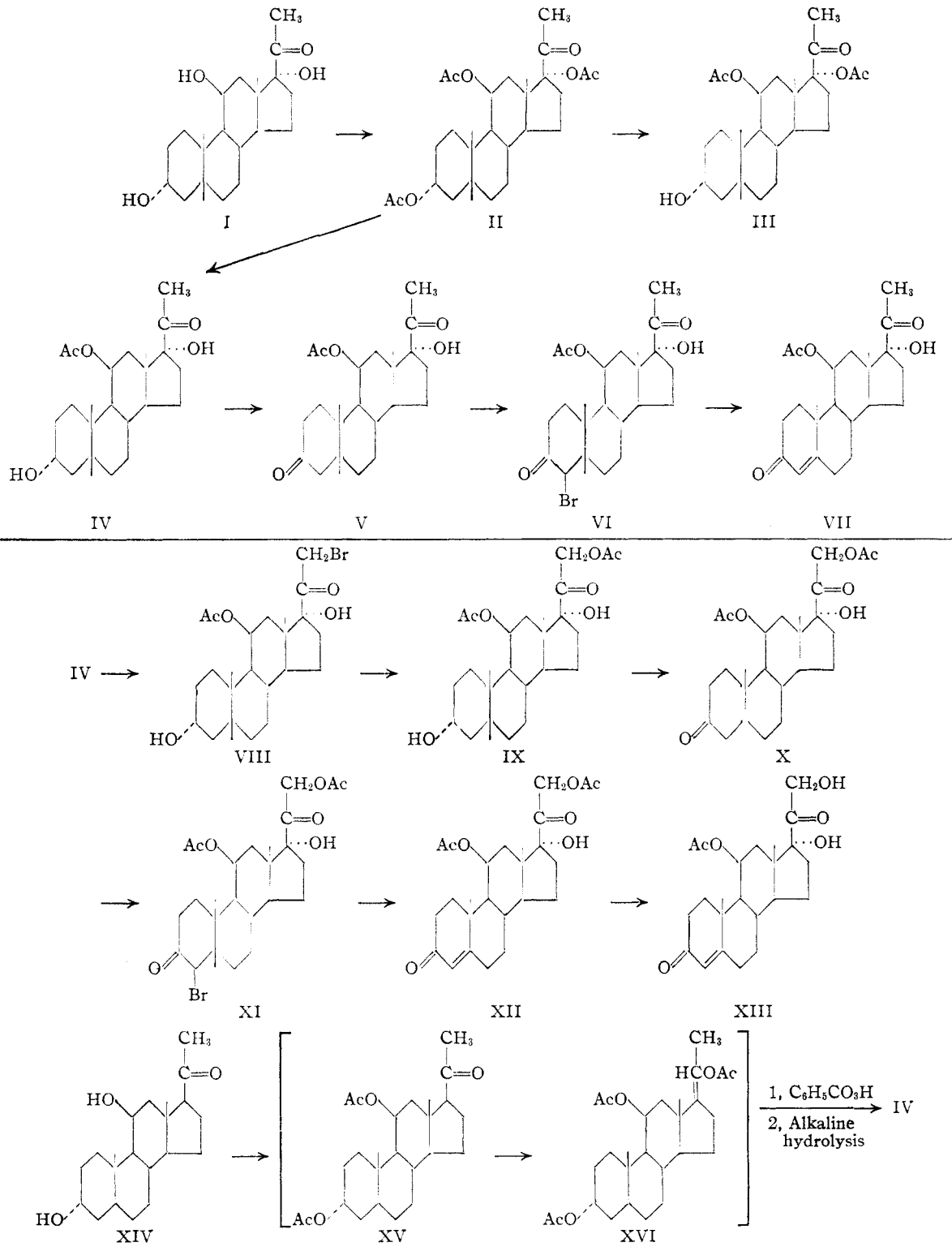
(11) During the period of our investigations two other laboratories reported on the acetylation of 17α-hydroxy-20-keto steroids using similar conditions [R. B. Turner, THIS JOURNAL, **74**, 4220 (1952); Huang-Minlon, E. Wilson, N. L. Wendler and M. Tishler, *ibid.*, **74**, 5394 (1952)].

(12) T. H. Kritchevsky and T. F. Gallagher, THIS JOURNAL, **73**, 184 (1951).

(13) A compound believed to be $\Delta^{9(11)}$-pregnen-3α,17α-diol-20-one diacetate has been isolated from some acetylation reactions. Evidence for its structure rests solely on its infrared spectrum at the moment, and unequivocal chemical evidence is being sought.

(14) *Cf.* D. H. Peterson, S. H. Eppstein, P. D. Meister, B. J. Magerlein, H. C. Murray, H. M. Leigh, A. Weintraub and L. M. Reineke, THIS JOURNAL, **75**, 412 (1953); P. D. Meister, D. H. Peterson, H. C. Murray, G. B. Spero, S. H. Eppstein, A. Weintraub, L. M. Reinke and H. M. Leigh, *ibid.*, **75**, 416 (1953). The same effect apparently also holds for 3-keto-$\Delta^{4,6}$ systems: D. H. Peterson, A. H. Nathan, P. D. Meister, S. H. Eppstein, H. C. Murray, A. Weintraub, L. M. Reineke and H. Marian Leigh, *ibid.*, **75**, 419 (1953).

(15) All melting points are corrected. All rotations were taken in a one decimeter tube at 25° and at a concentration of about 1%. Analyses and optical data were obtained by the Microanalytical and Physical Chemistry Departments of these laboratories.



Anal. Calcd. for $C_{23}H_{36}O_5$: C, 70.37; H, 9.25. Found: C, 70.21; H, 9.58.

Essentially the same results were obtained by the use of sodium hydroxide, either overnight at room temperature or 1.5 hr. at reflux.

Pregnan-11β,17α-diol-3,20-dione 11-Acetate (V).—A solution of 11.0 g. of IV in 110 ml. of methylene chloride and 110 ml. of *t*-butyl alcohol was cooled to 3–5° in an ice-bath, and 13.2 g. of N-bromoacetamide was added. The mixture was kept in an ice-chest overnight, and the excess of oxidizing agent was then destroyed by the addition of sodium sulfite solution. Water was added and the organic material was extracted by means of methylene chloride. After drying and removal of the solvent, the residue was crystallized from ether–hexane to yield 7.64 g. (69.5%) of V, m.p. 188.0–189.0°, $[\alpha]_D$ +43.6° (chl.). The melting point was not changed by further crystallization.

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.37; H, 8.92.

Δ^4-Pregnen-11β,17α-diol-3,20-dione 11-Acetate (VII).—A solution of 7.35 g. of V in 75 ml. of acetic acid containing 20 drops of 10% hydrogen bromide in acetic acid was bromi-

nated, at 16–17°, with a solution of 3.10 g. of bromine in 50 ml. of acetic acid containing 1.55 g. of sodium acetate. Excess water was then added, and crude VI was removed by filtration; wt. 8.0 g., m.p. 182–183° dec. Recrystallization from aqueous acetone gave m.p. 181–183°. This bromide was split in the usual fashion[16] using the semicarbazone; 3.86 g. of VI gave 2.20 g. (69%) of crude VII, m.p. 110–115°. The analytical sample crystallized from aqueous methanol as the monohydrate, m.p. 116-120°, $[\alpha]_D$ +141.2° (chl.), ϵ 15,100 at 240 mμ (methanol).

Anal. Calcd. for $C_{23}H_{32}O_5 \cdot H_2O$: C, 67.95; H, 8.43. Found: C, 67.65, 67.50; H, 8.66, 8.73.

Δ^4-Pregnen-11β,17α-diol-3,20-dione (prepared *via* bromination and dehydrobromination of pregnan-11β,17α-diol-3,20-dione[8]) had m.p. 223.0–224.5°, $[\alpha]_D$ +112.3° (chl.), ϵ 14,200 at 242 mμ (methanol).

Pregnan-3α,11β,17α,21-tetrol-11,21-Diacetate (IX).—A solution of 5.0 g. of IV in 50 ml. of C.P. chloroform was brominated at room temperature over a period of 20 min. with a solution of 2.20 g. of bromine in 22 ml. of C.P. chloroform. The chloroform was removed under reduced pressure, 10 g. of potassium acetate and 100 ml. of acetone were added and the mixture refluxed for 5 hr. Steam was then introduced to remove the acetone, and the organic residue was extracted with methylene chloride. Removal of the organic solvent left an oil which crystallized easily from ether to yield 4.0 g. of IX, m.p. 189–191°. The analytical sample, crystallized once more from ether, had a m.p. of 195.0–196.0°, $[\alpha]_D$ +88.4° (chl.).

Anal. Calcd. for $C_{25}H_{38}O_7$: C, 66.64; H, 8.50. Found: C, 66.45; H, 8.56.

Pregnan-11β,17α,21-triol-3,20-dione 11,21-Diacetate (X).—At ice-bath temperature, 51.0 g. of N-bromosuccinimide was added to a solution of 42.5 g. of IX in 760 ml. of acetone and 190 ml. of water. A temperature of about 3–5° was maintained for 2.5 hr., and the excess oxidizing agent was then destroyed by the addition of sodium sulfite solution. Water was then added to precipitate 39.8 g. (93.7%) of X, m.p. 199–201°. The analytical sample, crystallized from aqueous methanol, had a m.p. of 206.0–207.5°, $[\alpha]_D$ +92.8° (chl.).

Anal. Calcd. for $C_{25}H_{36}O_7$: C, 66.94; H, 8.09. Found: C, 67.00; H, 8.09.

4-Bromopregnan-11β,17α,21-triol-3,20-dione 11,21-Diacetate (XI).—A solution of 50.0 g. of X in 340 ml. of acetic acid and 160 ml. of methylene chloride, and containing 10 ml. of 10% hydrogen bromide in acetic acid, was cooled to *ca.* 3–5° and then brominated at this temperature over a period of 20 min. with a solution of 18.0 g. of bromine and 9.0 g. of sodium acetate in 250 ml. of acetic acid. The methylene chloride was removed under reduced pressure, and water was then added to precipitate 57 g. (96.5%) of crude XI, m.p. 161–164° dec. The pure 4-bromide was obtained by first sludging the crude with ether, and then recrystallizing the residue from aqueous acetone: m.p. 185–187° dec., $[\alpha]_D$ +95.5° (chl.).

Anal. Calcd. for $C_{25}H_{35}O_7Br$: Br, 15.15. Found: Br, 15.05.

Δ^4-Pregnen-11β,17α,21-triol-3,20-dione 11,21-Diacetate (Compound F 11,21-Diacetate) (XII).—A solution of 14.75 g. of XI in 300 ml. of acetic acid was dehydrobrominated in the usual way[16] using 3.82 g. of semicarbazide hydrochloride. After splitting the semicarbazone with pyruvic acid, the crude XII was extracted into methylene chloride. Pure compound F 11,21-diacetate (7.50 g., 76.3%) was obtained by crystallization from aqueous methanol, m.p. 191.0–191.8°, $[\alpha]_D$ +167.1° (chl.), ϵ 17,200 at 240 mμ (95% ethanol).

Anal. Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.68. Found: C, 67.19; H, 7.47.

Δ^4-Pregnen-11β,17α,21-triol-3,20-dione 11-Acetate (Compound F 11-Acetate) (XIII).—A solution of 4.5 g. of XII in 40 ml. of C.P. chloroform, 140 ml. of C.P. methanol, 8.5 ml. of concd. hydrochloric acid and 14 ml. of water was allowed to react at 25° for 48 hr. Water was added, and the mixture was extracted with methylene chloride. Removal of the organic solvent under reduced pressure gave 4.2 g. of a white resin which crystallized on trituration with ether, m.p. 110–112° dec. Two further crystallizations gave 2.7 g. of compound F 11-acetate as the monohydrate, m.p. 113–118°, $[\alpha]_D$ +163.2° (chl.), ϵ 16,250 at 240 mμ (95% ethanol). The infrared spectrum confirmed the loss of one acetate group which had been adjacent to a ketone carbonyl.

Anal. Calcd. for $C_{23}H_{32}O_6 \cdot H_2O$: C, 65.38; H, 8.11. Found: C, 65.77, 65.32; H, 8.27, 8.27.

Compound F or its 21-acetate has λ_{max} at 242 mμ.[2a,8b]

BLOOMFIELD, N. J.

(16) W. F. McGuckin and E. C. Kendall, THIS JOURNAL, **74**, 5811 (1952); V. R. Mattox and E. C. Kendall, *J. Biol. Chem.*, **188**, 287 (1951); B. Koechlin, T. Kritchevsky and T. F. Gallagher, *ibid.*, **184**, 393 (1950); E. B. Hershberg, *J. Org. Chem.*, **13**, 542 (1948).

[CONTRIBUTION FROM THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

The Action of *t*-Butyl Hypochlorite on Organic Compounds. IV. Cholesterol[1]

BY DAVID GINSBURG[2]

RECEIVED JULY 13, 1953

t-Butyl hypochlorite adds to the double bond of cholesterol and oxidizes the secondary alcoholic function to a carbonyl group. The primary reaction product is 6β-chloro-Δ^4-cholesten-3-one. Dehydrochlorination of this compound gives Δ-4,6-cholestadien-3-one which is isolated as its 2,4-dinitrophenylhydrazone. The mechanism of the reaction is discussed.

In previous communications in this series[3] it has been indicated that in a molecule containing an olefinic double bond, *t*-butyl hypochlorite (TBH) introduces a chlorine atom by addition to the double bond rather than through substitutive chlorination in the allylic position, *i.e.*, through formulation A rather than B

$$\text{A, } RCH_2CH{=}CHR' \xrightarrow{TBH} RCH_2\overset{+}{C}HCHClR' \xrightarrow{-H^+} RCH{=}CHCHClR'$$

$$\text{B, } RCH_2CH{=}CHR' \xrightarrow{TBH} RCHClCH{=}CHR'$$

This point is of interest, as if it can be shown to hold in many cases, TBH could become as versatile a halogenating agent as N-bromosuccinimide. While the latter is an allylic halogenating agent in the majority of cases,[4] the former could perform halogenation concurrent with a *shift* of the double bond. In a system such as 1-phenylcyclohexene[5] it is not possible to distinguish between formulations A and B because of the inherent symmetry of the resulting chlorination product. Of several unsymmetrical systems which could be employed to investigate this problem, cholesterol

(1) A preliminary communication on this subject has appeared: D. Ginsburg, *Bull. Res. Council Israel*, **2**, 269 (1952).

(2) U. S. Public Health Service Fellow, Harvard University, 1952–1953. On leave of absence from the Weizmann Institute, Rehovoth, Israel.

(3) D. Ginsburg, THIS JOURNAL, **73**, 2723 (1951), and additional references given therein.

(4) C. Djerassi, *Chem. Revs.*, **43**, 271 (1948).

(5) D. Ginsburg and R. Pappo, *J. Chem. Soc.*, 516 (1951).