TABLE II

PREPARATIONS OF UNSATURATED 2-MONOGLYCERIDES BY BORIC ACID CLEAVAGE OF 1,3-BENZYLIDENE-2-ACYLGLYCEROLS

Compound	Mole ratio, H_3BO_3/1,3-benzylidene 2-acyl glycerol	Yield, %	M.p., °C.[a] (uncor.)	Analytical data: Hydroxyl val.[b] Found	Hydroxyl val.[b] Calcd.	Iodine val.[c] Found	Iodine val.[c] Calcd.	Acid val.	1-MG Content, % Without isom.[d]	1-MG Content, % With isom.[e]
2-Monoölein	1.8	65	23.2	313	315	70.7	71.2	0.8	4.13	85.0
2-Monoölein	1.8	58	23.5	317	315	...	...	0.6	2.0	..
2-Monoelaidin	1.1	27	53.7	...	...	69.2	71.2	...	1.4	84.6
2-Monoelaidin	1.4	86	..	...	...	...	...	...	2.1	
2-Monoelaidin	(Composite of (3 prepns.)		54.2	314	315	...	...	0.2	1,2	..
2-Monolinolein	1.8	30	8.9	...	...	141.0	143.2	...	1.8	83.1

[a] Values obtained on crystals from petroleum ether except for 2-monolinolein which was melted and chilled before melting point measurement. [b] Determination of hydrogen evolved by reaction with lithium aluminum hydride. [c] Wijs method. [d] According to Pohle and Mehlenbacher, ref. 7, with reduction in concn. of reagents to permit determinations on 3–9 mg. quantities of 1-MG. [e] Isomerization of 2-MG to 1-MG by treatment with 56% aqueous perchloric acid, followed by analysis for 1-MG content, according to a preliminary report in a paper by F. H. Mattson, *et al.*, *J. Nutrition*, **48**, 335 (1952), and a forthcoming paper by the author; the isomerization technique applied to pure 1- or 2-MG results in a 1-MG content of 85 ± 3%.

Boric oxide was practically inactive while metaboric acid was essentially identical in splitting activity to the more readily available orthoboric acid.

The effects of variations in reaction temperature and time as well as in the molar ratio of the reactants are indicated by the data in Table I.

The data illustrate the importance of keeping the reaction temperature and time as low as possible to minimize the formation of the 1-MG impurity.

The boric acid cleavage of the benzylidene group from the glyceride has not been found to follow a simple stoichiometric behavior, but this may be the result of several factors, namely: (1) the polyfunctionality of boric acid, (2) the reaction as used is not carried to completion, and (3) boric acid interacting with triethyl borate may yield a mixture of ethylboric acids of variable composition, which are the active agents in the reaction. Molar quantities in the range of 0.5 to 2.0 moles of boric acid per mole of 1,3-benzylidene-2-acylglycerol give good yields of 2-MG.

Unsaturated 2-Monoglycerides from Boric Acid Cleavage of 1,3-Benzylidene-2-acylglycerols.—The properties of 2-monoölein, 2-monoelaidin and 2-monolinolein prepared by this method are shown in Table II.

Acknowledgment.—The assistance of Messrs. R. G. Folzenlogen and R. A. Volpenhein in this work is gratefully acknowledged.

CINCINNATI 31, OHIO

[CONTRIBUTION FROM THE CHEMICAL DIVISION OF THE PROCTER & GAMBLE CO.]

The Equilibrium between Symmetrical and Unsymmetrical Monoglycerides and Determination of Total Monoglycerides

BY JAMES B. MARTIN

RECEIVED JULY 6, 1953

Perchloric acid as a catalyst isomerizes 1- and 2-monoglycerides in chloroform solution to an equilibrium mixture containing about 90% 1-monoglyceride. 2-Monoglycerides do not react with periodic acid commonly used for the determination of monoglycerides; however, by application of the periodate analysis before and after perchloric acid isomerization, it is possible to determine 1-monoglycerides and total monoglycerides present in a mixture from which the original 2-monoglyceride content is readily obtained by difference. After isomerization, the total monoglyceride content of the fat is calculated by multiplying the per cent. 1-monoglyceride from periodic acid oxidation by a factor of 1.15 to convert from the equilibrium composition and correct for the slight effect of side reactions. The method is applicable to both saturated and unsaturated compounds; no interference from other fatty substances has been observed.

Acid and alkaline media as well as fusion are known to promote the isomerization of 2-monoglycerides (2-MG) to 1-monoglycerides (1-MG).[1] Monoglycerides dissolved in purified chloroform containing 0.5% ethanol are isomerized rapidly and reproducibly at room temperature with an aqueous 56% perchloric acid solution as a catalyst to yield an equilibrium mixture of the fatty MG isomers containing 90–92% 1-MG and 10–8% 2-MG. Evidence for the existence of an equilibrium in this concentration range has not been reported previously, but Verkade[2] has indicated that aromatic monoglycerides exist in equilibrium at a composition of 88% 1-MG and 12% 2-MG.

The perchloric acid-catalyzed isomerization is applicable as a step in the determination of total MG in fats containing 2-MG. The 2-MG content of a fat is the difference between total MG calculated after isomerization and the 1-MG content determined before isomerization. Equilibrium formation by isomerization makes about 85% of the total MG present in the reaction mixture accessible to periodic acid oxidation, and the total MG value is obtained by using a factor of 1.15 to correct for the equilibrium composition and the slight interference from side reactions. The monoglycerides can be saturated or unsaturated and other fatty materials have not been found to interfere with the isomerization.

(1) B. F. Daubert and C. G. King, THIS JOURNAL, **60**, 3003 (1938).

(2) (a) P. E. Verkade, private communication; see also, *Chim. Ind.*, **69**, 239 (1953). (b) P. E. Verkade and O. E. van Lohuizen, *Proc. Roy. Dutch Acad. Sci.* (to be publ.).

Experimental

Materials. Perchloric Acid.—An aqueous 56–57% perchloric acid is prepared by dilution of a C.P. acid of 60 or 70% concentration.

Chloroform.—Technical or C.P. chloroform must be purified by the following steps in sequence or incomplete isomerization may result: (1) the chloroform is washed with 1/4 its volume of concentrated sulfuric acid; (2) it is water-washed until clear; (3) it is subjected to distillation, discarding the first and last 10% portions; (4) the distillate is dried by shaking with approximately 1/20 its weight of anhydrous sodium sulfate; (5) after drying, it is filtered into an amber glass bottle and 0.5% by volume of ethanol is added. The satisfactory performance of the chloroform solvent is established by isomerization of a known 2-MG sample.

Methods. Perchloric Acid Isomerization Procedure.—The sample is dissolved in purified chloroform in a small glass-stoppered flask to obtain a solution containing 0.1 to 2.0% total MG. From a pipet, 0.003 ml. of 56% perchloric acid is added per ml. of the chloroform solution. The flask is stoppered and shaken for one minute, then allowed to stand for 10 minutes from the time of the perchloric acid addition. A volume of water approximately equal to the volume of the chloroform solution is added and the mixture is shaken for five seconds. It is transferred to a separatory funnel and the chloroform solution is washed a total of five times with approximately equal volumes of water. The water washes are combined and extracted at least twice with small volumes of chloroform to recover entrained sample. In the meantime the washed chloroform solution is filtered into a volumetric flask which will permit taking a suitable aliquot for MG analysis (3–9 mg. of 1-MG in 5–20 ml. of solution). The chloroform extracts of the water washes are used to wash all glassware and the filter paper with which the chloroform solution of the sample has been in contact. The chloroform solution and washings are diluted to volume and analyzed for 1-MG by periodic acid oxidation. Ethers inhibit the acid-catalyzed isomerization of 2-MG and it is recommended that samples be kept free of ethers before isomerization.

Periodic Acid Analysis for 1-MG.—Pohle and Mehlenbacher's method[3] was modified by Dr. J. H. Benedict of these laboratories for the determination of micro quantities of 1-MG.

Reagents are as described by the authors[3] with the following exceptions.

Periodic Acid Solution.—A quantity of 1.67 g. of periodic acid (H_5IO_6) is dissolved in 50 ml. of water and diluted to 1 liter with glacial acetic acid. The periodic acid is tested by running blanks and the concentration of the solution is adjusted to give a titration of 48–50 ml. of the standard sodium thiosulfate solution.

Sodium Thiosulfate Solution (0.006 *N*).—An aqueous solution of 1.49 g. of sodium thiosulfate is prepared, made up to 1 liter and standardized.

Procedure.—A suitable aliquot of the monoglyceride chloroform solution (3–9 mg. of 1-MG) is pipetted into a 125-ml. glass-stoppered erlenmeyer flask, sufficient acetic acid is added to the sample to give a solvent mixture of 2 parts chloroform–1 part acetic acid and to obtain a solution volume in the range of 10 to 30 ml. Five ml. of the periodic acid reagent is added by pipet and mixed by swirling. The flask is stoppered and allowed to stand 15 minutes. Two ml. of potassium iodide solution is added, the sample is shaken and allowed to stand 1 minute. The stopper is rinsed with water and while stirring vigorously (a magnetic stirrer is desirable) the solution is titrated with sodium thiosulfate until the color nearly disappears. One ml. of starch solution is added and the titration is continued to the starch-iodine end-point. Blanks are run in the same manner as the sample.

Calculations.—Per cent. 1-MG is calculated by the equation of Pohle and Mehlenbacher.[3] Per cent. total MG is obtained by multiplying % 1-MG after perchloric acid isomerization by 1.15.

Results and Discussion

Preparation of Solvent for Isomerization.—It should be stressed that the purification of the chloroform according to the directions is essential. The most critical features are (1) the need to avoid drying prior to distillation and (2) the necessity for the addition of 0.5% ethanol. Using solvent prepared as directed, it has been possible to get identical results for isomerization of MGs at concentrations within the range 0.1 to 2% MG in the solution.

Optimum Concentration and Quantity of $HClO_4$ for Isomerization of MG.—Aqueous 56% perchloric acid is most satisfactory as an isomerization catalyst for MG isomers in that the reaction is rapid and the effects of side reactions are minimized. A 70% perchloric acid, although about 10 times more rapid in its isomerizing action, induces considerable rearrangement of monoglyceride to diglyceride and glycerol.

The quantity of 56% perchloric acid required for isomerization of MG in chloroform solution is a function of the volume of the solution rather than of the MG content; see Fig. 1. The method is not sensitive to moderate variations in the quantity of 56% perchloric acid used, but it is desirable to maintain the quantity to within ±50% of the recommended amount (*i.e.*, 0.003 ml. of 56% perchloric acid per ml. of chloroform solution).

Rate of Isomerization of 2-MG.—The rate of isomerization of 2-monopalmitin by 56% perchloric acid is shown in Fig. 2. It is evident that a time interval in the range of 10 to 30 min. is satisfactory for the isomerization. Again the isomerization reaction is independent of MG concentration in the solvent (see also Fig. 1) since concentrations of 0.1 and 1.0% of 2-MG isomerize at identical rates.

Action of Perchloric Acid on Partial Glycerides.—The action of 56% perchloric acid on partial glycerides including various mono- and diglyceride isomers has been investigated and analytical data for the glycerides before and after isomerization are given in Table I.

TABLE I

ACTION OF PERCHLORIC ACID ON A VARIETY OF PARTIAL GLYCERIDES

Glyceride investigated	Treatment	Fat recovery,[a] %	1-MG content of fat,[b] % CH-Cl₃ soln.	1-MG content of fat,[b] % Recovered fat	M. p.,[c] °C.	Hydroxyl val.	Acid val.
2-Monopalmitin	Unisom.			3	68.0	335	0.5
	Isom.	98.8	84.2	88.4	73.8	323	1.1
2-Monostearin	Unisom.			10	73.7	316	0.8
	Isom.	94.8	84.1	87.0	75.1	308	0.7
2-Monoölein[4]	Unisom.			7	21.0		1.3
	Isom.	92.7	85.6	88.5	34.8		1.7
1-Monopalmitin	Unisom.			95.2	76.1	337	0.2
	Isom.	97.1	87.4	89.4	74.0	328	0.9
1,2-Dipalmitin	Unisom.			0.1	63.4	108	0
	Isom.	98.9	0.8	0.7	70.4	109	0.7
1,3-Dipalmitin	Unisom.			0.6	72.1	104	0
	Isom.	97.8	1.2	1.2	69.6	107	0.3

[a] Fat recovery is the per cent. of the fat recovered by evaporation of the solvent following isomerization; average recovery = 96.7%. [b] 1-MG analyses were made on both the soln. of the fat after isomerization and the fat recovered from solution by evaporation of the solvent at temperatures not above 40°. Analyses on the fat without isomerization are included under recovered fats. [c] Melting points are the complete melting points determined on the original glyceride crystallized from solvent or on the isomerized glyceride recovered by evaporation of the solvent under vacuum.

(3) W. D. Pohle and V. C. Mehlenbacher, *J. Am. Oil Chem. Soc.*, **27**, 54 (1950).

(4) J. B. Martin, THIS JOURNAL, **75**, 5482 (1953).

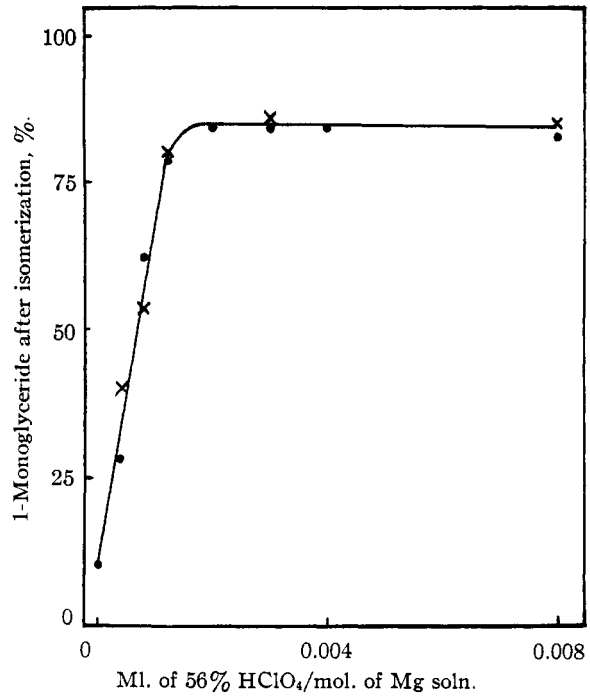

Fig. 1.—Isomerization of 2-monopalmitin with various quantities of 56% $HClO_4$ using a 10-min. isomerization period: ●, 1.0%, and ×, 0.0%, solutions of MG in $CHCl_3$.

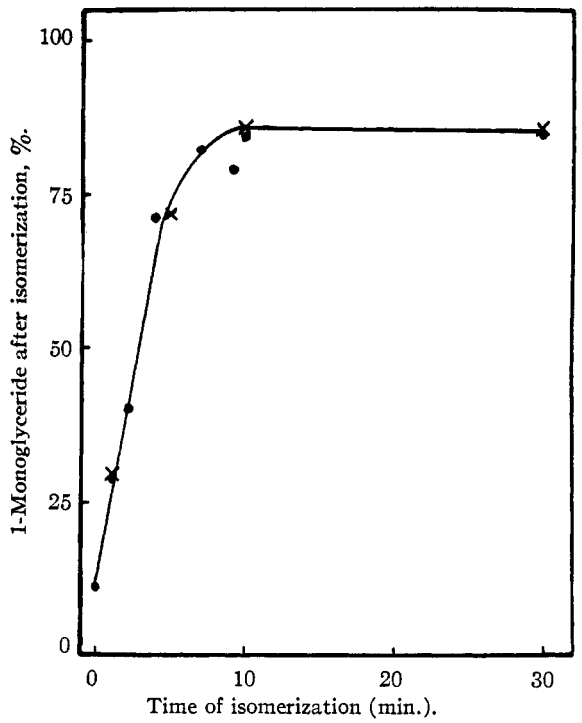

Fig. 2.—Isomerization of 2-monopalmitin as a function of time using 0.003 ml. of 56% $HClO_4$ solution per ml. of MG solution: ●, 1.0%, and ×, 0.1%, solutions of MG in $CHCl_3$.

In addition to the data in Table I, the following observations should be noted as an aid in interpreting the nature of the isomerization reaction.

(A) Glycerol contents of the water washes from perchloric acid-isomerized partial glycerides fall in the range of from 0.2 to 0.45% for mono- and 0.08% for diglycerides (based on fat content of the sample). The maximum quantity of glycerol observed in the water washes would account for the hydrolysis of 1.7% of the MG or rearrangement of 3.5% of the MG to diglyceride and glycerol.

(B) Craig separator analyses on samples of crude 2-monopalmitin before and after isomerization with 70% perchloric acid revealed that the unisomerized "monoglyceride" consisted of 98% MG and 2% di- and triglyceride and after isomerization (1 min.) 92% MG and 8% di- and triglyceride. Periodic acid analysis for 1-MG showed 28 and 83%, respectively, before and after the isomerization treatment. The difference between the periodic acid analysis and the Craig separator analysis of 9% in MG content on the isomerized sample is particularly significant in indicating the presence of 2-MG in the sample after isomerization.

Isomerization of MGs in Fat Mixtures.—To establish the applicability of this isomerization technique to the problem of total monoglyceride determination in fatty mixtures,[5] the method has been evaluated for mixtures of 2-monopalmitin with other fatty compounds; see Table II.

The value of perchloric acid isomerization as a step in the determination of total monoglycerides in fatty mixtures is readily apparent from inspection of the data in Table II. The method for total monoglyceride based on perchloric acid isomerization and periodic acid oxidation is not as precise as would be desired but this difficulty arises from the following known factors: (1) loss of fat during isomerization, (2) slight rearrangement of mono- to diglyceride, and (3) establishment of an equilibrium composition.

(5) F. H. Mattson, J. H. Benedict, J. B. Martin and L. W. Beck, *J. Nutrition*, **48**, 335 (1952).

TABLE II

DETERMINATION OF TOTAL MONOGLYCERIDE IN FATTY MIXTURES

A Composition of mixture 2-MP,[a] %	Other fatty material	B 1-MG found after isom.,[b] %	C Total MG added/ 1-MG after isom.	D Total MG found (Bxl.15),
50	Palmitic acid	41.2	1.21	47.4
25	Palmitic acid	21.2	1.18	24.4
50	1-MP	87.9	1.14	101.3
50	1-MP	85.2	1.16	99.3
50	1,3-Dipalmitin	41.5	1.21	47.8
25	1,3-Dipalmitin	21.5	1.16	24.8
50	1,2-Dipalmitin	43.0	1.16	49.5
25	1,2-Dipalmitin	22.4	1.11	25.8
25	Fat A[c]	21.4	1.17	24.6
33.3	Fat B	29.6	1.13	34.1
33.3	Fat B	28.9	1.15	33.3
19.2	Fat C	16.8	1.14	19.4
5.7	Fat C	4.9	1.17	5.6
1.5	Fat C	1.3	1.11	1.56
20	Fat D	17.8	1.12	20.5
20	Fat D	17.8	1.12	20.5

[a] MP = Monopalmitin. [b] By periodate determination, ref. 3. [c] Fats have compositions as follows: A, 33.3% palmitic acid, 33.3% 1,3-dipalmitin and 33.3% 1,2-dipalmitin; B, 50% 1,3-dipalmitin and 50% 1,2-dipalmitin; C, 31% palmitic acid, 14% 1,3-dipalmitin and 55% tripalmitin; D, 32% palmitic acid, 12% 1,3-dipalmitin and 56% cottonseed oil.

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

Acknowledgment.—The assistance of Mr. C. B. Stewart and Dr. E. S. Lutton in performing the Craig Separator Analyses reported herein is gratefully acknowledged.

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).