high-speed mixing. Indeed, an examination of Fig. 3 reveals that when a DNA sample (8LD) with an average sedimentation coefficient of 29 *S* was homogenized, those molecules with sedimentation coefficients above 26 *S* were almost quantitatively degraded. This decrease in sedimentation coefficients was accompanied by a drop in the intrinsic viscosity from 103 to 15 in units of 100 cc./g. This treatment did not however lead to a lowering of the alkali hyperchromic shift (Table I).[36]

The effect on DNA of high-speed mixing is reminiscent of the effects of sonic treatment; in that case there also occurs a scission of the twin-helical chain which is not accompanied by a separation of the twin strand (denaturation).[8-11]

It should be borne in mind that these studies were carried out using a small volume of DNA solution in a small homogenization cup. It may be that the extent of the alteration of the size of the DNA is dependent upon the design of the apparatus.

The question can be asked whether the scission of the DNA fibers was perhaps accomplished by the cutting action of the blades. It would seem that the effect of high-speed mixing is similar to that of sonication, hence the major factor leading to the degradation might be cavitation. This method of degradation lends itself well to the preparation from DNA specimens with very high sedimentation coefficients of specimens of smaller size with sedimentation coefficients below 26 *S*. An advantage of this method of degradation is the extremely short period of time necessary to bring about these alterations. In view of the experiments reported here, it should be possible to prepare a graded series of DNA preparations from sedimentation coefficients in excess of 30 *S* down to 26 *S* in less than a minute. Under these circumstances minimal denaturation is to be expected.

The data suggest that the widely-used process of high-speed mixing in the course of the isolation of DNA might give a product which is appreciably smaller than that obtained when such a step is not included in the isolation procedure. However, high-speed mixing has frequently been used to speed up the dissolution of fibers of DNA and the present data indicate that such a procedure leads to degradation.

High-speed mixing could be used in the preparation of DNA samples with high values of sedimentation coefficients and a narrower distribution of sedimentation coefficients than untreated samples. Such DNA samples might prove to be useful specimens for physical studies such as the effects of a decreased M_w/M_n ratio on the molecular weight obtained by light-scattering (see discussion in ref.[28]).

Acknowledgments.—The authors are very grateful to Dr. G. B. Brown for his interest and advice in the course of this study and to Dr. G. di Mayorca for a gift of the DNA samples used in this study as well as for helpful suggestions.

(36) When these sedimentation and viscosity data are substituted in the relationship of Doty, *et al.*,[11] or in the equation of Mandelkern and Flory (*J. Chem. Phys.*, **20**, 209 (1952)) values of *ca.* 16 and 3 × 10^5 are obtained for the molecular weights of the control and homogenized samples, respectively.

[CONTRIBUTION FROM THE COLLIP MEDICAL RESEARCH LABORATORY, UNIVERSITY OF WESTERN ONTARIO, AND FROM THE DEPARTMENT OF CHEMISTRY, LAVAL UNIVERSITY]

Steroids and Related Products. XII.[1] The Synthesis of 17α-Bromo-11-dehydrocorticosterone[2]

BY R. DEGHENGHI AND CH. R. ENGEL[3]

RECEIVED NOVEMBER 2, 1959

The synthesis of 17α-bromo-11-dehydrocorticosterone, the first 17-halogenated derivative of a glucocorticoid, from 3α-acetoxy-11,20-dioxopregnane, is described.

A comparison of the glucocorticoid activity of 11-dehydro-17α-methylcorticosterone, 11-dehydrocorticosterone and cortisone shows an apparent parallelism of this activity with the electronegativity of the 17α-substituent.[4] It seemed attractive to investigate whether or not this parallelism was merely coincidental, and therefore to extend the series by synthesizing glucocorticoid derivatives with 17α-substituents more electronegative than a hydroxy group. The preparation of 17α-halogenated products appeared to merit particular attention because in a number of cases halogen substitution in positions vicinal to carbonyl and carbinol groupings results in a marked increase in biological activity.[5] We decided to include in our study parent compounds of the related progesterone and mineralocorticoid series; recently we reported the synthesis and high progestational activity of 17α-bromoprogesterone.[6a] Now we wish to record the synthesis of the first 17-halogenated glucocorticoid, 17α-bromo-11-dehydrocorticosterone (XVII).

Since 17α-bromo-21-methyl-20-ketones and the corresponding 17,21-dibromo and 17-bromo-21-iodo derivatives are relatively easy to prepare, it would seem attractive to convert such compounds directly to 21-hydroxy- or 21-acyloxy-17α-bromo-20-ketones. In a subsequent paper[6b] we show that this approach is not practical in the 17α-bromo

(1) Paper XI of this series: Ch. R. Engel and W. W. Huculak, *Can. J. Chem.*, **37**, 2031 (1959).

(2) The main results reported in this paper were included in a communication presented before the 4th International Congress of Biochemistry in Vienna, September, 1958.

(3) Correspondence should be addressed to this author, at the Faculty of Science, Laval University, Quebec.

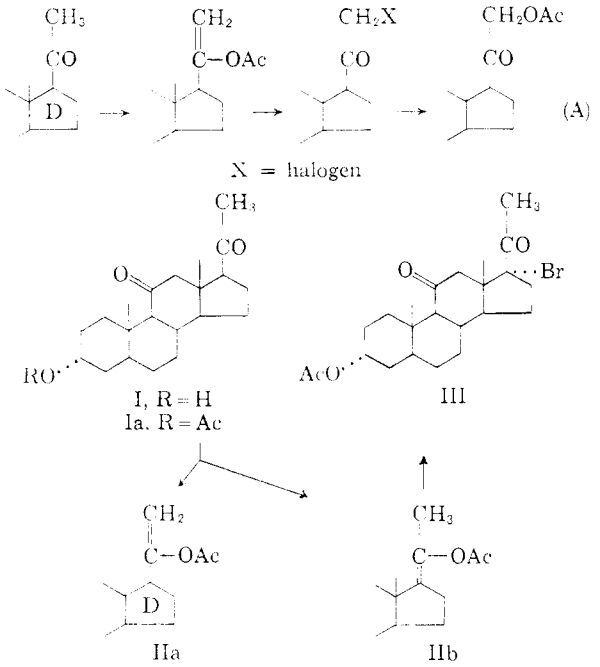
(4) Ch. R. Engel, THIS JOURNAL, **78**, 4727 (1956).

(5) Compare, for instance, ref. 8, 11, 12, 13, 14, 18, 19, 34 and 35 of the article quoted under footnote 6a.

(6)(a) Ch. R. Engel and H. Jahnke, *Can. J. Biochem. Physiol.*, **35**, 1047 (1957). (b) Ch. R. Engel, R.-M. Hoegerle and R. Deghenghi, *Can. J. Chem.*, **38**, in press (1960).

series. The 21-hydroxy-20-keto moiety has to be developed prior to the introduction of the bromine substituent in position 17.

We chose the readily available 3α-acetoxy-11,20-dioxopregnane (Ia) as starting material for our experiments, and first intended to introduce the 21-hydroxy function by transforming the 20-ketone to its 20,21-enol acetate, by subsequently halogenating the enol acetate and by replacing the halogen atom of the resulting 21-halo-20-ketone in the usual fashion with an acetoxy substituent, according to reaction sequence A. This method, developed by Moffett and Weisblat,[7] had become particularly attractive since Djerassi and Lenk[8] showed that 20,21-enol acetates could be transformed directly with N-iodosuccinimide into 21-iodo-20-ketones. However, upon treatment of the 11,20-diketone Ia with isopropenyl acetate, under various reaction conditions, we could obtain



only, either the pure 17,20-enol acetate IIb or a mixture of the enol acetates IIa and IIb, from which the desired isomer IIa could not be isolated. When the crude mixture of IIa and IIb was treated with one equivalent of bromine, only the 17α-bromide III,[4,9] derived from the 17,20-enol acetate IIb, was obtained. One must assume that the 20,21-enol acetate of the acetoxy diketone Ia rearranges with particular ease to the 17,20-enol acetate.

Therefore we took recourse to Ruschig's method[10] for the elaboration of the ketol side chain. Condensation of the acetoxy diketone Ia with dimethyl oxalate in the presence of sodium methylate gave the 3α-hydroxy-21-oxalyl derivative IV, the 3-acetate being saponified under the reaction conditions. The product was iodinated (IVa) in the usual fashion and the oxalyl substituent removed with alkali, preferably with potassium acetate.[10c] The resulting iodide V was isolated but not purified and subjected directly to the action of potassium acetate in acetone, thus giving 3α-hydroxy-21-acetoxy-11,20-dioxopregnane (VI)[11] in an over-all yield of 39% (from Ia). The product was further characterized by its acetate VIa,[12] and readily transformed with chromic acid in acetic acid to 21-acetoxy-3,11,20-trioxopregnane (IX).[13]

It seemed tempting to try to transform this acetoxy triketone IX *via* the trienol tetraacetate XII and the 4,17-dibromide XV to the 17-bromo-3-semicarbazone XVI and hence to the acetate XVIIa of the desired 17α-bromo-11-dehydrocorticosterone, using the elegant method developed by Lyttle and Levin[14] in the 21-unsubstituted series. However, the formation of a 17,20-enol acetate in the presence of a 21-acetoxy group proved even more difficult than expected.[15] When the triketone IX was treated with acetic anhydride in the presence of *p*-toluenesulfonic acid at boiling temperature for a prolonged period of time, an amorphous mixture was obtained; this gave upon bromination with N-bromosuccinimide a crystalline (but not completely pure) monobromide to which we assigned the structure of the 4-monobromide XIV, in accordance with its physical and spectral constants and its transformation through the 3-semicarbazone to 11-dehydrocorticosterone acetate (XIII) by the use of McGuckin and Kendall's method.[16,17]

Treatment of the triketone IX with isopropenyl acetate and *p*-toluenesulfonic acid afforded the crystalline 3-monoenol diacetate X. This gave

(7) R. B. Moffett and D. I. Weisblat, THIS JOURNAL, **74**, 2183 (1952).

(8) C. Djerassi and C. T. Lenk, *ibid.*, **75**, 3493 (1953); **76**, 1722 (1954).

(9) (a) H. V. Anderson, E. R. Garrett, F. H. Lincoln, Jr., A. H. Nathan and J. A. Hogg, *ibid.*, **76**, 743 (1954); (b) R. U. Schock and W. J. Karpel, U. S. Patent 2,684,963 (1954); (c) Ch. R. Engel, THIS JOURNAL, **77**, 1064 (1955).

(10) (a) H. Ruschig, *Angew. Chem.*, **60A**, 247 (1948); *Chem. Ber.*, **88**, 878 (1955). Compare also: (b) G. I. Poos, R. M. Lukes, G. E. Arth and L. H. Sarett, THIS JOURNAL, **76**, 5031 (1954); and (c) J. Schmidlin, G. Anner, J.-R. Billeter, K. Heusler, H. Ueberwasser, P. Wieland and A. Wettstein, *Helv. Chim. Acta*, **40**, 2291 (1957).

(11) E. von Euw, A. Lardon and T. Reichstein, *ibid.*, **27**, 1287 (1944).

(12) L. H. Sarett, THIS JOURNAL, **70**, 1454 (1948).

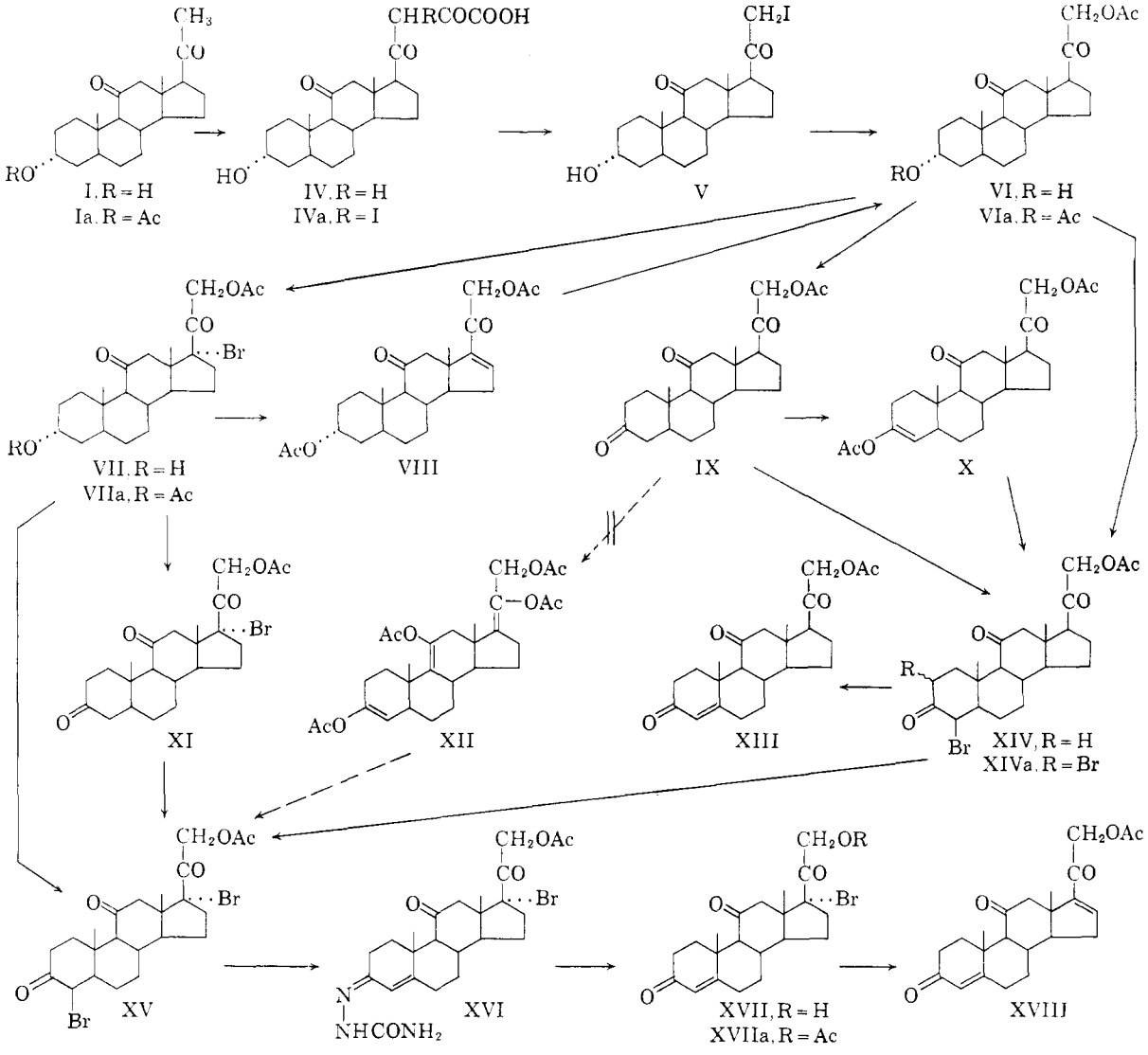
(13) A. Lardon and T. Reichstein, *Helv. Chim. Acta*, **26**, 747 (1943).

(14) D. A. Lyttle and R. H. Levin, U. S. Patent 2,705,720 (1955), compare also B. J. Magerlein, D. A. Lyttle and R. H. Levin, *J. Org. Chem.*, **20**, 1709 (1955).

(15) Apart from a steric effect of the 21-acetoxy group, one also has to consider the electrical situation. There is good spectral evidence [compare R. N. Jones, P. Humphries, F. Herling and K. Dobriner, THIS JOURNAL, **74**, 2820 (1952), R. N. Jones and C. Sandorfy, in "Technique of Organic Chemistry," Vol. IX, Interscience Publishers, Inc., New York, N. Y., 1956, pp. 480-481; L. J. Bellamy and R. J. Williams, *J. Chem. Soc.*, 861 (1957)] for the fact that the carbonyl function of the 21-acetate and the 20-keto group affect each other, each diminishing the other's polarization, probably by an interaction of their respective dipole fields. The reduction of the polarization of the 20-keto group would, of course, result in a reduced ability to undergo enolization. Very recently Levine and Wall [THIS JOURNAL, **81**, 2829 (1959)] reported that a 17α-bromo-16β-acetoxy-21-methyl-20-ketone could not be brominated under the usual conditions in position 21. We consider that this might also be due to the interaction of the dipoles of the two carbonyl functions (as evidenced by a hypsochromic shift of their absorption bands in the infrared), which in turn should impede the enolization of the 20-keto group and hence its bromination.

(16) W. F. McGuckin and E. C. Kendall, *ibid.*, **74**, 5811 (1952).

(17) The mother liquors of the bromide XIV obtained in this sequence of reactions gave, upon treatment with collidine, an amorphous product which contained, according to its ultraviolet and infrared spectra, some $\Delta^{4,16}$-21-acetoxy-3,11,20-trioxopregnadiene (XVIII). This indicates that to a small extent bromination in position 17, and therefore enolization in 17–20, had occurred.



with two equivalents of molecular bromine a mixture from which we were able to isolate in the impure state a dibromide to which we assign tentatively the structure of a 2,4-dibromide (XIVa) since it yielded with pyridine a product with the spectral characteristics of a $\Delta^{1,4}$-3-ketone.

The pure 4-monobromide XIV was readily obtained either by direct bromination of the triketo acetate IX with one molecular equivalent of bromine in acetic acid or by treatment of 3α-hydroxy-21-acetoxy-11,20-dioxopregnane (VI) with four equivalents of N-bromosuccinimide.[18] When the monobromide XIV was subjected to the action of N-bromosuccinimide under the conditions generally used for 17-bromination of a 20-ketone, at elevated temperature and under illumination,[9b] a crystalline mixture of bromides was obtained, which contained, according to its infrared spectrum, appreciable amounts of authentic (*vide infra*) 4β,17α-dibromo-21-acetoxy-3,11,20-trioxopregnane (XV). It was however not possible to isolate, by crystallization, the pure dibromide XV in reasonable yields. Introduction of the Δ^4-double bond *via* the 3-semicarbazone[16] gave an amorphous mixture of Δ^4-3-keto-steroids which was separated by repeated chromatography into authentic (see below), but not entirely pure, 17α-bromo-11-dehydrocorticosterone acetate (XVIIa), 11-dehydrocorticosterone acetate (XIII) and small amounts of a third product, the constitution of which was not further investigated. We thus established that the mixture obtained upon treatment of the monobromide XIV with N-bromosuccinimide contained, apart from the desired dibromide XV, unreacted monobromide XIV and other bromination products.

Because this route seemed to hold little promise from a practical point of view, we decided to introduce the 17-bromine substituent by direct bromination with molecular bromine. In a preliminary series of experiments, we treated 3α,21-diacetoxy-11,20-dioxopregnane (VIa) with one equivalent of bromine in acetic acid, holding the temperature at 40–45°, since no reaction took place at room temperature. The resulting crude monobromide VIIa crystallized badly and proved very difficult

(18) Compare E. B. Hershberg, C. Gerrold and E. P. Oliveto, THIS JOURNAL, **74**, 3849 (1952).

to purify; however, when treated with pyridine it gave a product which represented, according to its infrared and ultraviolet spectra, crude Δ^{16}-3α,21-diacetoxy-11,20-dioxopregnene (VIII),[19] which was reconverted smoothly to 3α,21-diacetoxy-11,20-dioxopregnane (VIa) by hydrogenation over palladium-on-charcoal. This sequence of reactions clearly establishes the location of the bromine atom in position 17. We assign the α-configuration to the 17-bromine substituent, in agreement with the established course of 17-brominations of 20-ketones.[20,21]

We now applied our method of 17-bromination to the free 3α-hydroxy adduct VI which was obtained directly during the elaboration of the ketol acetate side chain as described above. When the bromination was carried out quickly, the desired 17-monobromide VII was obtained in 55–65% yield. As a side product, the 3-acetate VIIa was isolated; furthermore, small amounts of products, the bromine content of which was higher than that of a monobromide, were formed during the reaction. When the bromination was carried out in chloroform at room temperature over a prolonged period of time, the desired 17-bromide VII was obtained in low yield only.[22] The structure of bromide VII, which crystallizes readily and analyzes well, was proved by its conversion with acetic anhydride in pyridine to the acetate VIIa and by the transformation of its derivative XVIIa to the Δ^{16}- adduct XVIII (*vide infra*).

Oxidation of the hydroxy monobromide VII with chromic acid in acetic acid gave, in approximately 60% yield, the triketo bromide XI, which was readily brominated in acetic acid to the 4,17-dibromide XV. The same product was obtained directly from the hydroxy bromide VII by treatment with six equivalents of N-bromosuccinimide.[18] The location of the second bromine substituent in position 4 is proved by the following steps. Treatment of the dibromide XV with semicarbazide base[16] gave indeed the 3-semicarbazone XVI, which confirms the findings made by Lyttle and Levin in the 21-unsubstituted series.[14] Hydrolysis of the semicarbazone with aqueous acetic acid and pyruvic acid gave a mixture of Δ^4-17α-bromo-21-acetoxy-3,11,20-trioxopregnene (XVIIa) and the free alcohol XVII; reacetylation of this mixture, under nitrogen, gave pure acetate XVIIa in a yield of over 70% (from XV).[23] Hydrolysis with perchloric acid in methanol[6a,24] gave the crystalline, but unstable 17α-bromo-11-dehydrocorticosterone (XVII), which could be reacetylated to the acetate XVIIa.[23] The transformation of the latter with pyridine to the known $\Delta^{4,16}$-21-acetoxy-3,11,20-trioxopregnadiene (XVIII)[19,25,26] constitutes a further proof of the structure of the new 17-brominated hormone derivative.

Throughout the series of experiments described, we observed the typical levorotatory shift due to bromination of a 20-ketone in position 17.[4,6a,27] On the other hand, it is of interest to note that, whereas 17-bromination of 21-unsubstituted 20-ketones does not result in any marked shift of the carbonyl absorption band in the infrared,[28] the introduction of a bromine substituent in position 17 of a 21-acetoxy-20-ketone seems to produce an easily detectible shift of the 20-carbonyl absorption maximum toward lower frequencies.[29]

According to preliminary biological tests, for which we are indebted to Drs. S. Tolksdorf and P. L. Perlman, 17α - bromo - 11 - dehydrocorticosterone (XVII) and its acetate XVIIa exhibit eosinophil activity, but to a lesser degree than cortisone and its acetate. These findings, borne out by other results to be published at a later date, seem to indicate that the electronegativity of the 17-substituent is at least not a major activating factor of glucocorticoid activity.[30]

Acknowledgments.—We wish to express our most sincere thanks to Mrs. J. Capitaine for her expert and devoted assistance in the described experiments; to Drs. S. Tolksdorf and P. L. Perlman, Schering Corporation, Bloomfield, N. J., for the biological tests; and to Mr. R. W. White, Science Service Laboratory, London, Ontario, for his unfailing help with a considerable part of the infrared analyses. We are greatly indebted to the Federal–Provincial Departments of National Health and Welfare, Ottawa and Toronto; The National Research Council of Canada; the Schering Corporation, Bloomfield, N. J.; The Schering Corporation Ltd., Montreal; and the Canadian Life Insurance Officers Association, for generous financial support. The constant encouragement of Dean J. B. Collip and the kind co-operation of the authorities of Laval University were highly appreciated.

(19) H. L. Slates and N. L. Wendler, *J. Org. Chem.*, **22**, 498 (1957).

(20) N. L. Wendler, R. P. Graber and G. G. Hazen, *Tetrahedron*, **3**, 144 (1958).

(21) Previously, Plattner, Heusser and Angliker [*Helv. Chim. Acta*, **29**, 468 (1946)] described in the 11-unoxygenated 5-allo-pregnane series the 17-bromination of 20-ketones at elevated temperatures.

(22) Under these conditions, oxidation in position 3 seems to occur to a considerable degree. One of the isolated reaction products was 4β-bromo-21-acetoxy-3,11,20-trioxopregnane (XIV), which must have been formed by an initial oxidative attack of the 3-hydroxy function of VI and by subsequent bromination in position 4. Furthermore, we were able to isolate from the combined mother liquors of bromination experiments in chloroform and in acetic acid a pure dibromide to which we tentatively assign the structure of a 17,21-dibromide, in accordance with its infrared spectrum and the improbability of brominations in other sites under the reaction conditions used. When the bromination was carried out in acetic acid, even with relatively large amounts of material, it was never possible to isolate this dibromide.

(23) For complete acetylation the reaction time had to be extended beyond the usual period.

(24) J. Fried and F. Sabo, THIS JOURNAL, **74**, 3849 (1952).

(25) W. S. Allen and S. Bernstein, *ibid.*, **77**, 1028 (1952).

(26) We wish to thank sincerely Drs. S. Bernstein and W. F. McGuckin for providing us with authentic samples of this compound.

(27) H. Heusser, Ch. R. Engel, P. Th. Herzig and Pl. A. Plattner, *Helv. Chim. Acta*, **33**, 2229 (1950).

(28) Compare the first article cited in footnote 15. Jones, Ramsey, Herling and Dobriner [THIS JOURNAL, **74**, 2828 (1952)] report, however, a shift of the 20-keto band of a 5-allo-pregnane derivative toward lower frequencies upon bromination in position 17.

(29) In most cases, contrary to 17-unbrominated 21-acetoxy-11,20-diketones, which show in potassium bromide or chloroform distinct 11- and 20-carbonyl absorption bands, the corresponding 17-brominated products show the 20-ketone absorption only as a shoulder in a combined 11,20-dicarbonyl band. We intend to publish, in collaboration with Dr. N. R. Jones of the National Research Council in Ottawa, a more extensive study on this subject.

(30) We wish to thank Drs. H. Herzog, M. J. Gentles and E. B. Hershberg for kindly informing us of their work on 17-chlorinated and 17-fluorinated corticoids. It was agreed that their results would be published simultaneously with our parallel work on 17-chlorinated steroids (compare footnote 6b).

Experimental[31-33]

Attempted Preparation of $\Delta^{20,21}$-3α,20-Diacetoxy-11-oxopregnene (IIa). (a).—To a solution of 1.5 g. of 3α-acetoxy-11,20-dioxopregnane (Ia) in 5 cc. of freshly-distilled isopropenyl acetate a drop of concentrated sulfuric acid was added and the mixture was refluxed for one hour. A small quantity of solvent was removed under reduced pressure and the solution was refluxed for another hour. The cooled mixture was extracted with ether and the ethereal solution was washed repeatedly with cold sodium bicarbonate solution and with water and was dried over sodium sulfate. Upon removal of the solvent there was obtained 1.5 g. of a light brown oil which resisted crystallization and was chromatographed on 45 g. of aluminum oxide (*p*H 6.5). Petroleum ether–benzene mixtures eluted 700 mg. (37% yield) of **Δ^{17}-3α,20-diacetoxy-11-oxopregnene (IIb)**, m.p. 113–124°. The product was recrystallized four times from acetone–hexane for analysis; silky needles, m.p. 127–129° dec., $[\alpha]^{22}D$ +55° (*c* 0.94 in $CHCl_3$). The product gave a positive tetranitromethane reaction.

Anal. Calcd. for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found: C, 71.99; H, 8.68.

Elutions with benzene gave 700 mg. of starting material Ia, m.p. 124–126°, not depressed upon admixture of an authentic sample; the identity of the product with Ia was also confirmed by infrared analysis. A sample was recrystallized from ether–hexane for analysis; m.p. 128.5–130.5° dec., $[\alpha]^{22}D$ +132° (*c* 0.90 in $CHCl_3$), negative tetranitromethane reaction.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.82; H, 9.07.

Considering the recovery of starting material, the yield of IIb was 79%.

(b).—A mixture of 2 g. of the acetoxy diketone Ia and 300 mg. of *p*-toluenesulfonic acid was dissolved in 40 cc. of isopropenyl acetate. The latter was distilled slowly through a Vigreux column over a period of 10 hours, the volume of the solution being kept above 20 cc. by occasional additions of isopropenyl acetate. Subsequently the mixture was cooled and worked up as described under (a). The crude product (2.1 g.) resisted crystallization and was chromatographed on 50 g. of silica gel. Benzene–ethyl acetate (94:6) eluted 1.8 g. of amorphous fractions, the infrared spectra of which showed a band at 1650 $cm.^{-1}$, typical of the double bond of a terminal methylene group, but gave at the same time a positive tetranitromethane reaction; these fractions were thus assumed to represent a mixture of the enol acetates IIa and IIb. They were rechromatographed on 45 g. of neutral aluminum oxide (*p*H 7.0). Petroleum ether–benzene (1:1) eluted 1.6 g. of crude crystalline enol acetate IIb, m.p. 107–108°. The identity of the product was ascertained by its infrared spectrum, a positive tetranitromethane reaction and a mixed melting point with authentic material. Elutions with benzene afforded 300 mg. of starting material Ia; the yield of IIb was 85%, considering this recovery of starting material.

(c).—A mixture of 2 g. of Ia and 300 mg. of sulfosalicylic acid was dissolved in 25 cc. of isopropenyl acetate. The solution was refluxed for 2 hours and the solvent removed *in vacuo* almost completely. The dark residue was dissolved in chloroform and the organic solution was washed with cold sodium bicarbonate solution and with water and dried over sodium sulfate. The residue was taken up in benzene and decolorized by filtration through a short column of aluminum oxide. There was obtained 1.8 g. (80% yield) of crystalline IIb.

(d).—A quantity of 2 g. of Ia was treated exactly as described by Moffett and Weisblat[7] with isopropenyl acetate and *p*-toluenesulfonic acid to give 2.638 g. of an amorphous product which resisted crystallization, gave a positive tetranitromethane test and showed the infrared absorption band of a terminal methylene group at 1650 $cm.^{-1}$.

Bromination.—To a solution of the above-mentioned crude enolization product in 30 cc. of dichloromethane was added at −10°, dropwise and with stirring, 20 cc. of an 0.45 *N* solution of bromine in dichloromethane. The solvent was removed and the residue chromatographed on 100 g. of silica gel. Benzene–ethyl acetate (98:2 and 96:4) eluted 2.2 g. of a crude crystalline bromide, m.p. 131–135°. Recrystallization from ether afforded 1.8 g. (74%) of 17α-bromo-3α-acetoxy-11,20-dioxopregnane (III), m.p. 156° dec., $[\alpha]^{24}D$ +10.6° (*c* 0.94 in $CHCl_3$). The product gave no depression of melting point upon admixture of an authentic sample and its infrared spectrum was identical with that of genuine III.

Benzene containing a higher percentage of ethyl acetate and pure ethyl acetate eluted small quantities of unidentified oils.

3α-Hydroxy-11,20-dioxopregnane-21-oxalyl Acid (IV).—To a solution of 3.453 g. of methyl oxalate and 1.5 g. of sodium methoxide in 30 cc. of absolute benzene, 3.745 g. of 3α-acetoxy-11,20-dioxopregnane (Ia) was added. The solution, which slowly turned solid upon standing, was kept overnight at room temperature. The gelatinous mass was poured into a mixture of 20 cc. of 2 *M* phosphoric acid which was overlayed with ether. Subsequently, further quantities of ether were added and the mixture was extracted with this solvent. The acid was removed as its potassium salt from the organic layer by treatment with 120 cc. of 1 *N* potassium hydroxide solution. The remaining ethereal solution was worked up in the usual way and gave 200 mg. of 3α-hydroxy-11,20-dioxopregnane (I), m.p. 164–166°, not depressed upon admixture of authentic I. The alkaline extract was poured slowly into ice-cold 2 *M* phosphoric acid and the resulting precipitate was filtered, washed and dried. There was obtained 4.175 g. (93% yield) of the oxalic acid derivative IV, m.p. 200° dec. Considering the recovery of I, the yield was quantitative. A sample of the product was recrystallized from tetrahydrofuran–ether for analysis; m.p. 208–210° dec., $[\alpha]^{24}D$ +105° (*c* 0.98 in tetrahydrofuran).

Anal. Calcd. for $C_{25}H_{42}O_6$: C, 68.29; H, 7.97. Found: C, 68.15; H, 7.99.

3α-Hydroxy-21-acetoxy-11,20-dioxopregnane (VI).—To a mixture of 48 g. of the oxalic acid derivative IV, m.p. 200° dec., and 58 g. of dry potassium acetate in 900 cc. of absolute methanol was added within 15 minutes, dropwise and with stirring, at 0° and under nitrogen, 30.8 g. of iodine in 300 cc. of absolute methanol. The stirring was continued for another 4 hours at 0° and the reaction product was poured into ice-water. After one hour, the crystalline precipitate was filtered at 0° and washed with 50% aqueous methanol and with water, and dried in a vacuum desiccator over sulfuric acid for 12 hours. The dry product (39.75 g.), representing crude **21-iodo-3α-hydroxy-11,20-dioxopregnane (V)**, melted at 90°. The filtrate was extracted with dichloromethane, the organic solution was washed with ice-water and was dried over sodium sulfate. Removal of the solvent afforded 1.804 g. of an amorphous product, which was combined with the solid iodide V and dissolved in 600 cc. of dry acetone. To this solution was added potassium acetate, freshly prepared from 190 g. of potassium bicarbonate and 110 cc. of acetic acid. The mixture was refluxed for one hour under nitrogen and concentrated *in vacuo*. The residue was poured into cold water, the precipitate was extracted with ether and the ethereal solution was washed with cold sodium bicarbonate solution and with water and was dried over sodium sulfate. Removal of the solvent gave 31.21 g. of an amorphous material which upon chromatographic purification with aluminum oxide, afforded 17.52 g. (38.8% yield from IV or I) of crystalline **3α-hydroxy-21-acetoxy-11,20-dioxopregnane (VI)**, m.p. 129–132°. A sample was recrystallized twice from ether for analysis; colorless prisms, m.p. 137–138°, $[\alpha]^{20}D$ +109° (*c* 1.0 in $CHCl_3$); ν_{max}^{KBr} 1240 (acetate), 1708 (11-ketone), 1725 and 1755 (21-acetoxy-20-ketone doublet), 3550 $cm.^{-1}$ (3-hydroxy).

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.83; H, 8.69.

Diacetate VIa.—A quantity of 5.86 g. of the monoacetate VI was acetylated in the usual fashion with acetic anhydride in pyridine at room temperature. The amorphous reaction product (6.27 g.) was chromatographed on 200 g. of aluminum oxide (Woelm, activity II). Petroleum ether–benzene

(31) All melting points were taken in evacuated capillaries and the temperatures were corrected.

(32) The microanalyses were carried out by Mr. J. F. Alicino, Dr. O. Schwarzkopf and Mr. W. Manser, to whom we express our sincere appreciation.

(33) For chromatography there was employed either Merck Reagent aluminum oxide, treated as described in footnote 31 of the first article of this series [Ch. R. Engel and G. Just, THIS JOURNAL, **76**, 5909 (1954)], or Woelm's standardized, non-alkaline aluminum oxide, activity II–III. We wish to express sincere thanks to Merck and Co., Montreal, for kindly providing us with their aluminum oxide. The silica gel used for chromatography was Davison silica gel no. 923.

(1:1 and 1:4) and benzene eluted 3.7 g. of solvated crystals of 3α,21-diacetoxy-11,20-dioxopregnane (VIa), m.p. 70–100°. A sample was recrystallized twice from ether for analysis; colorless needles, m.p. 106–108°, ν_{max}^{KBr} 1235 and 1250 (diacetate); 1704 (11-ketone); 1725 and 1750 (21-acetoxy-20-ketone doublet); 1720 cm.$^{-1}$ (carbonyl of 3-acetate).

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39; acetyl, 19.90. Found: C, 69.65; H, 8.41; acetyl, 20.37.

21-Acetoxy-3,11,20-trioxopregnane (IX).—To a solution of 4.6 g. of the hydroxy acetate VI, m.p. 130–132°, in 50 cc. of acetic acid, was added 1.42 g. of chromic acid in 27.5 cc. of 90% acetic acid at 10°. After 18 hours, a few cc. of methanol was added and the product stirred into water. The crystalline precipitate was filtered, washed until neutral and dried. There was obtained 5.375 g. (95% yield) of IX, m.p. 134–144°. A sample was recrystallized three times from ether for analysis; colorless prisms, m.p. 148–150°, $[\alpha]^{23}D$ +117° (*c* 1.2 in $CHCl_3$); ν_{max}^{KBr} 1230 (acetate); 1708 (11-ketone); 1710 (3-ketone); 1725 and 1758 cm.$^{-1}$ (21-acetoxy-20-ketone doublet).

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 71.14; H, 8.34.

Attempted Trienol Triacetylation of 21-Acetoxy-3,11,20-trioxopregnane (IX). (a).—A solution of 800 mg. of 21-acetoxy-3,11,20-trioxopregnane (IX), m.p. 146–148°, and of 250 mg. of *p*-toluenesulfonic acid in 35 cc. of acetic anhydride was heated to boiling temperature and the solvent allowed to distil slowly. In the course of 3 hours, 27 cc. of distillate was collected and at the same time 15 cc. of acetic anhydride was replaced. The solvent was removed *in vacuo*, and the residue was dried by repeated distillation with toluene and subsequently dissolved in benzene. The extract was washed with cold sodium bicarbonate solution and with water, dried over sodium sulfate, and partly decolorized by filtration over 1 g. of silica gel. Removal of the solvent afforded 820 g. of a yellow oil.

Bromination.—The above-obtained product was treated in the dark at room temperature in 105 cc. of *t*-butyl alcohol with 600 mg. of N-bromosuccinimide and 3.5 cc. of 1 *N* sulfuric acid for a period of 2 hours. The usual working-up afforded 1.0 g. of crude **4β-bromo-21-acetoxy-3,11,20-trioxopregnane (XIV)**, which melted after one recrystallization from methylene chloride–ether at 169–170° dec., $[\alpha]^{23}D$ +95° (*c* 1.24 in $CHCl_3$). *Anal.* Found: Br, 16.24.

A quantity (0.298 g.) of this bromide was transformed according to McGuckin and Kendall's method[16] to **Δ^4-21-acetoxy-3,11,20-trioxopregnene (XIII)** in 40% yield. The identity of the product was confirmed by melting point and infrared analysis; λ_{max}^{EtOH} 238 mμ (log ε 4.3).

(b).—A solution of 1.942 g. of acetoxy triketone IX and 2.853 g. of *p*-toluenesulfonic acid in 300 cc. of acetic anhydride and 100 cc. of isopropenyl acetate was slowly distilled in a carbon dioxide atmosphere for a period of 8 hours. The remaining solution was taken to dryness *in vacuo* and further dried by distillation with toluene. The resulting black gum was taken up in ether, the ethereal solution was washed with iced sodium bicarbonate solution and water and dried over sodium sulfate. The solvent was removed and the 2.2 g. of dark oil obtained was chromatographed on 80 g. of aluminum oxide (Woelm, activity III). Petroleum ether–benzene (4:1) eluted 1.75 g. of a slightly yellow oil which was rechromatographed on 40 g. of aluminum oxide. A sample of homogeneous petroleum ether–benzene fractions (600 mg.) was analyzed for its acetyl content. *Anal.* Calcd. for 2 acetyl groups: 19.9. Found: 21.58.

Benzene–ether mixtures eluted 50 mg. of a crystalline material, m.p. 165–170°, which was recrystallized from methylene chloride–hexane for analysis; long needles, m.p. 173–175°. *Anal.* Found: C, 70.97; H, 7.36; acetyl, 13.01. The compound differed from both starting material IX and Δ^3-3,21-diacetoxy-11,20-dioxopregnene (X) (*vide infra*).

Δ^3-3,21-Diacetoxy-11,20-dioxopregnene (X).—A solution of 3 g. of the 21-acetoxy triketone IX in 50 cc. of isopropenyl acetate and two drops of concd. sulfuric acid was refluxed for 17 hours. The usual working-up gave 3.9 g. of a brown oil which was chromatographed on 100 g. of aluminum oxide (Woelm, activity III). Petroleum ether–benzene mixtures eluted 2.122 g. (64% yield) of crystalline monoenol diacetate X, m.p. 108–118°. A sample was recrystallized twice from dichloromethane–hexane for analysis; small needles, m.p. 127–129°, $[\alpha]^{26}D$ +112° (*c* 0.97 in $CHCl_3$); ν_{max}^{KBr} 1220 (acetate), 1696 (11-ketone), 1712 (20-ketone), 1740 (3-enol acetate), 1755 cm.$^{-1}$ (shoulder) (21-acetate).

Anal. Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96; acetyl, 19.99. Found: C, 69.64; H, 7.95; acetyl, 19.97.

Bromination of Δ^3-3,21-Diacetoxy-11,20-dioxopregnene (X) with Two Moles of Bromine.—To a solution of 1.871 g. of the monoenol diacetate X in 30 cc. of acetic acid, was added dropwise and with stirring at room temperature, in the course of 25 minutes, 14.5 cc. of an 0.6 *M* bromine solution in acetic acid. The almost colorless reaction mixture was poured into water and the precipitate was extracted with dichloromethane. The usual working-up gave 2.48 g. of a yellow oil which was chromatographed on 100 g. of silica gel. Benzene–ethyl acetate (84:16) eluted 1.0 g. of a crystalline bromide, m.p. 110–125° dec., $[\alpha]^{24}D$ +17° (*c* 0.84 in $CHCl_3$). A sample was recrystallized three times from ether–hexane for analysis; small needles, m.p. 153–154° dec. (beginning of darkening at 144°).

Anal. Calcd. for $C_{23}H_{30}O_5Br_2$: Br, 29.26. Found: Br, 34.15.

A sample of this product (430 mg.) was refluxed for 8 hours with 10 cc. of pyridine and the reaction product was reacetylated in the usual fashion with acetic anhydride in pyridine. The amorphous reaction product was chromatographed on 8 g. of aluminum oxide. Petroleum ether–benzene (1:4) and benzene eluted 100 mg. of crystals, m.p. 70–80°, λ_{max}^{EtOH} 243 mμ; ν_{max}^{KBr} 1590, 1612 and 1660 (Δ^{1,4}-3-ketone triplet), 1698 (11-ketone), 1718 and 1742 cm.$^{-1}$ (21-acetoxy-20-ketone doublet).

4β-Bromo-21-acetoxy-3,11,20-trioxopregnane (XIV). (a) **From the Acetoxy Triketone IX.**—To a solution of 1.71 g. of 21-acetoxy-3,11,20-trioxopregnane (IX), m.p. 157–159°, in 23 cc. of acetic acid, there were added 20 drops of a hydrogen bromide solution in acetic acid and subsequently, dropwise and with stirring, 16.67 cc. of a 0.5 *N* solution of bromine in acetic acid. The usual working-up afforded 1.913 g. of a white solid which gave, upon crystallization from methylene chloride–ether, 1.345 g. of colorless crystals, m.p. 171° dec. (65.4% yield). A sample was recrystallized four times from dichloromethane–ether for analysis; m.p. 176–178° dec., $[\alpha]^{26}D$ +102.5° (*c* 1.0 in $CHCl_3$). The infrared spectrum of the product was found to be identical with that of the product obtained by bromination of the mixture formed during the attempted trienol triacetylation of IX (experiment a).

Anal. Calcd. for $C_{23}H_{31}O_5Br$: C, 59.10; H, 6.69; Br, 17.10. Found: C, 59.03; H, 6.73; Br, 17.31.

(b) **From the Hydroxy Diketo Acetate VI.**—To a solution of 1.486 g. of 3α-hydroxy-21-acetoxy-11,20-dioxopregnane (VI) in 30 cc. of dry *t*-butyl alcohol was added a solution of 2.73 g. of N-bromosuccinimide in 70 cc. of dichloromethane and 40 cc. of *t*-butyl alcohol. The mixture was kept in the dark at room temperature for 12 hours and was subsequently refluxed for 6 minutes under illumination with a photoflood lamp. After cooling, the colorless solution was washed with cold 5% sodium bisulfite solution and with water, and was dried over sodium sulfate. Removal of the solvent *in vacuo* gave 1.79 g. of a white solid, m.p. 135–145° dec. Upon one recrystallization from methylene chloride–methanol, purified bromide XIV, m.p. 160–161° dec., was obtained. The infrared spectrum of the product was identical with that of an authentic sample.

Dehydrobromination.—A sample of the bromide XIV (1.05 g.) was refluxed for 8 hours with 20 cc. of pyridine and the crude reaction product was reacetylated in the usual way with acetic anhydride in pyridine. The reaction product was chromatographed on 30 g. of aluminum oxide to yield 150 mg. of crude crystalline **11-dehydrocorticosterone acetate (XIII)**, m.p. 155–165°, λ_{max}^{EtOH} 238 mμ, (log ε 4.1). The melting point, raised by recrystallization from methanol to 168–170°, was not depressed upon admixture of an authentic sample and the product showed the correct infrared spectrum.

Bromination of 4β-Bromo-21-acetoxy-3,11,20-trioxopregnane (XIV) with N-Bromosuccinimide.—To a solution of 1.06 g. of the monobromide XIV, m.p. 171° dec., in 20 cc. of methylene chloride and 200 cc. of carbon tetrachloride, 520 mg. of N-bromosuccinimide was added. The mixture was refluxed for 10 minutes over a photoflood lamp. After cooling, the mixture was washed with iced dilute sodium bisulfite solution, cold sodium bicarbonate solution and water and was dried over sodium sulfate. Upon removal of the sol-

vent *in vacuo* a yellowish solid (1.28 g.) was obtained which was recrystallized from methylene chloride–hexane. This afforded 120 mg. of crystals, m.p. 160° dec., and a second crop of the same product, m.p. 158° dec., $[\alpha]^{26}D$ +45° (*c* 1.0 in $CHCl_3$); in spite of the high value of the optical rotation the infrared spectrum of the product corresponded in all salient points to that of authentic 4β,17α-dibromo-21-acetoxy-3,11,20-trioxopregnane (XV) (*vide infra*). By further crystallizations of the mother liquors 240 mg. of crystals, m.p. 143–148° dec., and 200 mg. of another crop, m.p. 142–144° dec., were obtained. A sample of the highest melting crystals was recrystallized for analysis.

Anal. Calcd. for $C_{23}H_{30}O_5Br_2$: Br, 29.26. Found: Br, 28.86.[34]

Dehydrobromination.—A quantity of 439 mg. of the bromide mixture obtained upon bromination of XIV with N-bromosuccinimide was dissolved in 30 cc. of methylene chloride and 50 cc. of *t*-butyl alcohol. The air was displaced with carbon dioxide, 120 mg. of semicarbazide base was added, the flask was flushed with carbon dioxide and sealed. The usual color changes[16] were observed. After two hours the colorless solution was taken to dryness *in vacuo*, the residue was dissolved in 20 cc. of 70% aqueous ethanol and precipitated in 1 l. of water. The dried precipiatate (440 mg.) was dissolved in 28 cc. of acetic acid and 12 cc. of water. After addition of 1 cc. of a 1.6 *N* solution of pyruvic acid the air was displaced with carbon dioxide and the flask stored for 20 hours at room temperature. The usual working-up afforded 345 mg. of a slightly yellowish foam, the infrared spectrum of which indicated a mixture. The product was reacetylated in the usual fashion and the resulting amorphous material (340 mg.) filtered through a column of silica gel. The first two fractions (323 mg.), eluted by benzene–ethyl acetate (87:13) were chromatographed on 15 g. of silica gel. Benzene–ethyl acetate (90:10) eluted a bromine-containing product, m.p. 163–167° dec., absorbing in the ultraviolet at 238 mμ, which probably represented a mixture and the structure of which was not further investigated. Benzene–ethyl acetate (80:20) eluted 60 mg. of crystalline and authentic **11-dehydrocorticosterone acetate (XIII)**, identified by mixed melting point and infrared analysis.

A third fraction of the first filtration through silica gel (*vide supra*), (50 mg.), was chromatographed on 5 g. of silica gel. Benzene–ethyl acetate (93:7) eluted 30 mg. of authentic (see below), but not entirely pure **17α-bromo-11-dehydrocorticosterone acetate (XVIIa)**.

17α-Bromo-3α,21-diacetoxy-11,20-dioxopregnane (VIIa).—To a solution of 925 mg. of 3α,21-diacetoxy-11,20-dioxopregnane (VIa) in 20 cc. of acetic acid were added six drops of a 15% hydrogen bromide solution in acetic acid and subsequently, with stirring and at room temperature, 335 mg. of bromine in 13.5 cc. of acetic acid in the course of 30 minutes. The stirring was continued for 2 hours, but no appreciable discoloration occurred. The temperature was raised to 45°, whereupon the color disappeared within 4 minutes. The mixture was extracted with dichloromethane, the organic solution was washed with iced sodium bicarbonate solution and with water and was dried over sodium sulfate. Upon removal of the solvent *in vacuo*, 1.16 g. of a white foam was obtained. Part of the product (580 mg.) was chromatographed on 15 g. of aluminum oxide (Woelm, activity II–III). Petroleum ether–benzene mixtures eluted 400 mg. of crystals, m.p. 105–122° dec. The product crystallized very badly, and was most difficult to recrystallize. A sample was recrystallized three times from ether–methanol for analysis; needles, m.p. 146–148° dec.

Anal. Calcd. for $C_{25}H_{35}O_6Br$: Br, 15.63. Found: Br, 16.60 and 14.44.

Dehydrobromination and Reduction.—A sample (520 mg.) of the above-described bromide VIIa was refluxed for 1 hour under nitrogen with 15 cc. of γ-collidine. Crystalline collidine hydrobromide was formed during the reaction. To the cooled reaction mixture, 0.5 cc. of acetic anhydride was added. After 18 hours, the product was worked up in the usual fashion and gave 450 mg. of a yellow oil, λ_{max}^{EtOH} 237 mμ. Chromatography on 15 g. of aluminum oxide afforded 215 mg. of crude **Δ[16]-3α,21-diacetoxy-11,20-dioxopregnene (VIII)**,[19] λ_{max}^{EtOH} 237 mμ (log ε 3.7); $\nu_{max}^{CHCl_3}$ 1250 (acetate), 1590 (Δ[16]), 1705 (11-ketone), 1718 (Δ[16]-21-acetoxy-20-ketone), 1737 cm.$^{-1}$ (double band of 3- and 21-acetates).

A quantity (22 mg.) of the crude 16-unsaturated diketo diacetate VIII, λ_{max}^{EtOH} 237 mμ (log ε 3.7), was dissolved in 50 cc. of 95% ethanol and hydrogenated at room temperature and atmospheric pressure during 4 hours in the presence of 35 mg. of a 5% palladium-on-charcoal catalyst. The usual working-up gave 20 mg. of the saturated 3α,21-diacetoxy-11,20-dioxopregnane (VIa), identified by mixed melting point and infrared spectrum. The product did not show the ultraviolet absorption typical of an α,β-unsaturated ketone.

17α-Bromo-3α-hydroxy-21-acetoxy-11,20-dioxopregnane (VII). (a).—A solution of 6.004 g. of 3α-hydroxy-21-acetoxy-11,20-dioxopregnane (VI), m.p. 135–137°, in 130 cc. of acetic acid was warmed to 40–45°. Two drops of hydrogen bromide in acetic acid was added, and subsequently, with stirring, within 6 minutes, 2.457 g. of bromine in 27.5 cc. of acetic acid. A further six drops of hydrogen bromide in acetic acid was added to the orange-colored solution and the mixture was stirred for another two minutes. The almost-colorless solution was poured into 1.5 l. of ice-water and the precipitate was filtered, washed to neutral and dried in a vacuum desiccator over phosphorus pentoxide. There was obtained 6.4 g. of a colorless solid which, upon crystallization from a small quantity of ethyl acetate, gave 1.114 g. of colorless crystalline VII, m.p. 130° dec. The mother liquors gave upon recrystallization from acetone–ether another 478 mg. of the same product with the same melting point. The remaining mother liquors were chromatographed on 500 g. of silica gel. Benzene–ethyl acetate (89:11, 87:13 and 85:15) eluted 1.59 g. of VII, m.p. 122–124° dec., and 920 mg. of the same, but less pure, crystalline product (total yield of crystalline VII, 57%). Benzene–ethyl acetate (85:15) eluted 235 mg. (5%) of impure and only partly crystalline VII. A sample of the crystalline product was recrystallized twice from ethyl acetate for analysis; colorless needles, m.p. 138–139.5° dec., $[\alpha]^{22}D$ +4.3° (*c* 0.934 in $CHCl_3$); ν_{max}^{KBr} 1240 (acetate), 1698 (11-ketone), 1715 and 1740 (21-acetoxy-20-ketone doublet), 3530 cm.$^{-1}$ (3-hydroxy).

Anal. Calcd. for $C_{23}H_{33}O_5Br$: C, 58.85; H, 7.09; Br, 17.02. Found: C, 58.71; H, 7.08; Br, 16.98.

The benzene–ethyl acetate (95:5 and 93:7) fractions of the above-described chromatogram (1.02 g.) consisted of the diacetate VIIa in the amorphous state.[35]

(b).—To a solution of 1.0 g. of VI, m.p. 135–137°, in 100 cc. of chloroform, was added dropwise and with stirring, at room temperature, within 3.5 hours, a solution of 409 mg. of bromine in 30 cc. of carbon tetrachloride. The colorless solution was washed with iced sodium bicarbonate solution and with water and was dried over sodium sulfate. Removal of the solvent *in vacuo* afforded 1.3 g. of a foamy material which upon crystallization with very small quantities of ethyl acetate gave 665 mg. of a crystalline mixture which was chromatographed on 40 g. of silica gel. Benzene–ethyl acetate (94:6) eluted 75 mg. of a crude crystalline bromide, m.p. 130–133° dec., identified by infrared analysis as impure **4β-bromo-21-acetoxy-3,11,20-trioxopregnane (XIV)**. Benzene–ethyl acetate (91:9, 89:11 and 87:13) gave 400 mg. of a crystalline product melting between 105 and 115° dec., which afforded upon recrystallization from ethyl acetate 95 mg. of **17α-bromo-3α-hydroxy-21-acetoxy-11,20-dioxopregnane (VII)**, m.p. 120° dec.; benzene–ethyl acetate (80:20, 75:25 and 50:50) eluted 180 mg. of the unbrominated starting material VI, m.p. 131–135°.

The mother liquors of the crystallization of the fractions containing VII were rechromatographed on 50 g. of silica gel. Benzene–ethyl acetate (97:3, 95:5 and 93:7) eluted further quantities of the 3-keto bromide XIV. The total amount of 3-keto fractions separated was 225 mg.

(34) This analysis was carried out by Dr. A. Bernhardt, Mühlheim, Germany.

(35) In a similar experiment carried out on 12.119 g. of VI, and with a reaction time of 15 minutes, the quantities of VII obtained were smaller, those of VIIa appreciably larger. Furthermore, it was possible to isolate, by repeated chromatography, small quantities of amorphous bromides, eluted with benzene–ethyl acetate (95:5); the bromine content of these fractions was higher than that of a monobromide. The best fractions (*Anal.* Found: Br, 24.66) may represent, according to the infrared spectrum [ν_{max}^{KBr} 1198 (21-bromo-21-acetate), 1250 (3-acetate), 1710 (11-ketone), 1737 (double band of 21-acetoxy-20-ketone and 3-acetate), 1780 cm.$^{-1}$ (21-bromo-20-keto-21-acetate)], 17α,21-dibromo-3α,21-diacetoxy-11,20-dioxopregnane in the impure state.

(c).—The combined mother liquors (2.613 g.) of bromination experiments in acetic acid and chloroform (1.0 g. from an acetic acid experiment, 1.163 g. from a chloroform experiment) were chromatographed on silica gel. Benzene–ethyl acetate (87:13, 85:15 and 83:17) eluted 860 mg. of crude VII which gave upon recrystallization from ethyl acetate–ether 178 mg. of pure VII, m.p. 134–135° dec. The preceding chromatogram fractions (benzene–ethyl acetate, 89:11 and 87:13) eluted 630 mg. of a crystalline product, m.p. 122–126° dec., which gave upon recrystallization from ethyl acetate–ether 93 mg. of colorless crystals, m.p. 167–168° dec., $[\alpha]^{23}D$ +67.2° (*c* 1.02 in $CHCl_3$); $\nu_{max}^{CHCl_3}$ 1200 (21-bromo-21-acetate), 1711 (11-ketone), 1730 (21-bromo-21-acetoxy-20-ketone), 1775 (21-bromo-21-acetate), 3600 cm.$^{-1}$ (3-hydroxy); ν_{max}^{KBr} 1205, 1698, 1720, 1760, 3400 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{30}O_5Br_2$: Br, 29.10. Found: Br, 28.78.

The structure of **17α,21-dibromo-3α-hydroxy-21-acetoxy-11,20-dioxopregnane** is tentatively assigned to this product.

Acetylation of the Hydroxy Bromide VII.—A solution of 240 mg. of the hydroxy monobromide VII in 7 cc. of pyridine was treated in the usual way with 4.5 cc. of acetic anhydride at room temperature in a nitrogen atmosphere for 17.5 hours. The infrared spectrum of the reaction product (293 mg.) was identical with that of authentic diacetate VIIa.

17α-Bromo-21-acetoxy-3,11,20-trioxopregnane (XI).—To a solution of 2.389 g. of the 3-hydroxy bromide VII, m.p. 133.5–136° dec., in 127 cc. of acetic acid, 567 mg. of chromic acid in 5.7 cc. of 90% acetic acid was added at 6°. The solution was allowed to warm to room temperature and was kept for 13.5 hours. After addition of 20 cc. of methanol, the mixture was stirred into ice-water and the precipitate was filtered, washed to neutral and dried. The white solid (1.845 g.) was chromatographed on 200 g. of silica gel. Benzene–ethyl acetate (91:9 and 89:11) eluted 1.413 g. of the bromo triketo acetate XI, m.p. 140–146°, and 41 mg. of slightly less pure crystals (62% yield). A sample was recrystallized twice from ether–hexane–acetone for analysis; colorless needles, m.p. 149–150° dec., $[\alpha]^{23}D$ (*c* 0.45 in $CHCl_3$); ν_{max}^{KBr} 1238 (acetate), 1698 and 1705 (double band with shoulder of 3- and 11-ketones), 1717 (marked shoulder of 20-ketone), 1746 cm.$^{-1}$ (21-acetate).

Anal. Calcd. for $C_{23}H_{31}O_5Br$: C, 59.10; H, 6.69; Br, 17.1. Found: C, 59.03; H, 6.80; Br, 16.97.

In another run, 1.467 g. of hydroxy bromide VII of a slightly lesser purity, m.p. 126–128° dec., was oxidized to 1.079 g. of crude crystalline bromo triketo acetate XI (74% yield).

4β,17α-Dibromo-21-acetoxy-3,11,20-trioxopregnane (XV).

(a). From the Triketo Acetoxy Bromide XI.—To a solution of 946 mg. of 17α-bromo-21-acetoxy-3,11,20-trioxopregnane (XI), m.p. 138–139° dec., in 20 cc. of acetic acid, were added two drops of a 15% hydrogen bromide solution in acetic acid and subsequently, dropwise and with stirring, 6.4 cc. of a 0.63 *N* solution of bromine in acetic acid. During the addition, a colorless crystalline precipitate formed. The mixture was stirred for another 15 minutes; the crystals were filtered, washed to neutral and dried. Thus there was obtained 608 mg. of dibromide XV, m.p. 164° dec. Ice and water were added to the filtrate, and the resulting precipitate was filtered and dried. Recrystallization of this product (370 mg., m.p. 151–152° dec.) from methylene chloride–methanol gave another 254 mg. of dibromide XV, m.p. 159–160° dec. (yield of pure XV, 77%.) A sample was recrystallized from methylene chloride–methanol for analysis; small and fine needles, m.p. 169–170.5° dec., $[\alpha]^{22}D$ +26.9° (*c* 0.866 in $CHCl_3$), ν_{max}^{KBr} 1240 (acetate), 1701 and 1704 (double band with small shoulder of 3- and 11-ketones), 1718 (20-ketone), 1748 cm.$^{-1}$ (21-acetate).

Anal. Calcd. for $C_{23}H_{30}O_5Br_2$: C, 50.56; H, 5.54; Br, 29.26. Found: C, 50.28; H, 5.71; Br, 29.09.

(b) From the Bromo Hydroxy Diketo Acetate VII.—To a solution of 900 mg. of 17α-bromo-3α-hydroxy-21-acetoxy-11,20-dioxopregnane (VII), m.p. 140° dec., in 10 cc. of methylene chloride and 30 cc. of *t*-butyl alcohol, 1.365 g. of N-bromosuccinimide in 50 cc. of dichloromethane and 30 cc. of *t*-butyl alcohol was added at room temperature. The mixture was kept in the dark for 12 hours. When it was subsequently exposed to daylight, it developed a bromine color which was removed by refluxing for 10 minutes under illumination with a photoflood lamp. The solution was cooled, washed with cold dilute sodium bisulfite solution and with water and was dried over sodium sulfate. Removal of the solvent *in vacuo* gave 1.293 g. of a foam which crystallized from ethyl acetate (m.p. 137–143° dec.). Upon one recrystallization from methylene chloride–ethyl acetate, the product, representing crude dibromide XV, melted at 144–146° dec. The infrared spectrum of the product was identical with that of authentic dibromide XV. It was transformed to the 4-unsaturated triketone XVIIa in the same manner as the pure bromide, obtained by bromination of the triketone XI.

Δ⁴-17α-Bromo-21-acetoxy-3,11,20-trioxopregnene (XVIIa).—A solution of 1.623 g. of 4β,17α-dibromo-21-acetoxy-3,-11,20-trioxopregnane (XV), m.p. 166–171.5° dec., in 59 cc. of absolute, alcohol-free chloroform and 73 cc. of dry *t*-butyl alcohol was flushed with carbon dioxide. Semicarbazide base (459 mg.) was added and the air was again displaced by carbon dioxide. One-half minute after the addition of the semicarbazide, the solution turned yellow, reaching the maximum intensity of color after 3.5 minutes. The mixture was shaken repeatedly for 135 minutes, whereupon the almost colorless solution was separated from the very small quantities of remaining crystals by filtration. The solvent was evaporated *in vacuo* at a temperature not exceeding 45° and the white solid residue was dissolved almost completely in 815 cc. of ethanol and 50 cc. of water at 40°. The solvent was reduced to 220 cc. *in vacuo* and the mixture, which now contained an appreciable amount of precipitate, was poured into 1.7 l. of cold water; the mixture was kept for 4 hours at −3° and filtered. There was obtained 1.337 g. of **semicarbazone XVI**, λ_{max}^{EtOH} 270 mμ (log ε 4.4). Extraction of the mother liquors afforded 120 mg. of the same product, of a slightly lesser degree of purity (total yield 96.5%).

A solution of 1.377 g. of the above-described crystalline semicarbazone XVI in 42.5 cc. of acetic acid and 15 cc. of water was flushed with carbon dioxide and 3.3 cc. of a 1.58 *N* aqueous pyruvic acid solution was added. The air was displaced by carbon dioxide and the flask was sealed. After 18.5 hours, the product was stirred into ice-water and the precipitate was filtered and dissolved in dichloromethane. The organic solution was washed with water, iced dilute hydrochloric acid, cold sodium bicarbonate solution and with water, dried over sodium sulfate and taken to dryness *in vacuo*. Thus, 1.073 g. of a colorless foam was obtained. The filtrate was extracted with dichloromethane and worked up as described above. This gave 153 mg. of a slightly yellowish oil. The infrared spectra of both fractions indicated that hydrolysis in 21 had occurred to some extent. The material was reacetylated for a prolonged period, with excess acetic anhydride in pyridine, and purified by crystallizations from acetone–ether–hexane and chromatography. The mother liquors were reacetylated and chromatographed. There was obtained 907 mg. (74% yield) of crystalline **17α-bromo-11-dehydrocorticosterone acetate (XVIIa)**, which melted upon one recrystallization at 113–117° dec. There was also obtained 136 mg. (11%) of the same product in a less pure amorphous state. A sample of the crystals was recrystallized twice for analysis; transparent needles, m.p. 116–118° dec., $[\alpha]^{23}D$ +93.7° (*c* 0.546 in $CHCl_3$), λ_{max}^{EtOH} 238 mμ (log ε 4.2); ν_{max}^{KBr} 1230 (acetate), 1610 and 1665 (Δ⁴-3-ketone doublet), 1705 (11-ketone), 1718 (shoulder) and 1750 cm.$^{-1}$ (17α-bromo-21-acetoxy-20-ketone); $\nu_{max}^{CHCl_3}$ 1240 (acetate), 1615 and 1665 (Δ⁴-3-ketone doublet), 1708 (11-ketone), 1718 (shoulder) and 1750 cm.$^{-1}$ (20-keto-21-acetate).

Anal. Calcd. for $C_{23}H_{29}O_5Br$: C, 59.35; H, 6.28; Br, 17.17. Found: C, 59.56; H, 6.40; Br, 17.04.

Δ⁴-17α-Bromo-21-hydroxy-3,11,20-trioxopregnene (17α-Bromo-11-dehydrocorticosterone) (XVII).—A solution of 133 mg. of 17α-bromo-11-dehydrocorticosterone acetate (XVIIa), m.p. 115–117° dec., in 5.7 cc. of a 39:1 mixture of methanol and 70% perchloric acid was left at room temperature in a carbon dioxide atmosphere for 21 hours. The solution was poured into 160 cc. of ice-water and the resulting mixture was extracted with dichloromethane. The organic solution was washed with water, iced sodium bicarbonate solution and water, and was dried over sodium sulfate. Removal of the solvent afforded 126 mg. of a colorless foam which crystallized from acetone–hexane. Recrystallization from the same mixture afforded 55 mg. of colorless crystals, m.p. 85° dec. The product decomposed very readily in solution. A sample was recrystallized once at reduced pressure from acetone–hexane for analysis; colorless needles,

m.p. 85° dec., $[\alpha]^{22}D$ +85° (*c* 0.807 in acetone), λ_{max}^{EtOH} 238 mμ (log ε 4.2); ν_{max}^{KBr} 1612 and 1665 (Δ⁴-3-ketone), 1705 (double band of 11- and 20-ketones), 3400 cm.⁻¹ (21-hydroxy).

Anal. Calcd. for $C_{21}H_{27}O_4Br$: C, 59.57; H, 6.43; Br, 18.88. Found: C, 59.35; H, 6.95; Br, 18.40.

Reacetylation.—A solution of 99 mg. of XVII in 5 cc. of pyridine was treated with 2 cc. of acetic anhydride and kept for 20 hours at room temperature in the dark in an argon atmosphere. The usual working-up gave 60 mg. of a foamy material, the infrared spectrum of which was identical with that of authentic acetate XVIIa. The product was dissolved in benzene–ethyl acetate (85:15) and filtered through 1 g. of silica gel. Thus there was obtained crystalline XVIIa, m.p. 108–112° dec., not depressed upon admixture of authentic XVIIa.

Δ⁴,¹⁶-21-Acetoxy-3,11,20-trioxopregnadiene (XVIII).—A solution of 315 mg. of 17α-bromo-11-dehydrocorticosterone acetate (XVIIa) in 10 cc. of pyridine was refluxed for 40 minutes in a nitrogen atmosphere. The product was extracted with ether and worked up in the usual way. The resulting crude amorphous product (275 mg.) showed the infrared spectrum of XVIII. Chromatography on aluminum oxide gave 165 mg. of a clear oil which was rechromatographed on silica gel. Benzene–ethyl acetate (89:11, 85:15 and 80:20) eluted 48 mg. of crystalline Δ⁴,¹⁶-21-acetoxy-3,11,20-trioxopregnadiene (XVIII), m.p. 175–182°, λ_{max}^{EtOH} 237 mμ (log ε 4.3); the melting point was not depressed upon admixture of an authentic sample[26] and the infrared spectra of both products were identical.

LONDON, ONTARIO, AND
QUÉBEC, QUÉBEC, CANADA

[CONTRIBUTION FROM THE DANIEL SIEFF RESEARCH INSTITUTE, THE WEIZMANN INSTITUTE OF SCIENCE]

Syntheses in the Cardiac Aglycone Field. III.[1] The Conversion of a 14α- to a 14β-Hydroxy Group in the Androstane Series. The Ultraviolet Spectra of Δ¹⁵-Androsten-17-ones

BY FRANZ SONDHEIMER, SUMNER BURSTEIN AND RAPHAEL MECHOULAM

RECEIVED NOVEMBER 10, 1959

A method for converting the readily available androstane-3β,14α-diol-17-one 3-monoacetate (I) to the corresponding 14β-hydroxy compound Va is described, which involves successive dehydration of I to the Δ¹⁴-17-one II, peracid oxidation to the 14β,15β-oxide III, rearrangement to the 14β-hydroxy-Δ¹⁵-17-one IV and hydrogenation. The chemical evidence for the structures assigned to the 14β-hydroxy compounds IV and Va is supplemented by the optical rotatory dispersion data. Δ¹⁵-Androsten-3β-ol-17-one (XV) and the corresponding 14-iso compound XVI have also been prepared. The anomalous ultraviolet spectra of the various Δ¹⁵-androsten-17-ones are discussed.

Nearly all of the naturally occurring cardiac-active steroidal lactones possess a 14β-hydroxy substituent[2] and the introduction of this grouping into the steroid nucleus is therefore a prerequisite for the successful synthesis of this important group of compounds. Until now only one method for bringing about 14β-hydroxylation has been described, which uses steroidal Δ¹⁴,¹⁶-dienes as intermediates.[3]

As opposed to the 14β-hydroxy group, the 14α-hydroxy group can be introduced directly in one step into simple steroids by microbiological means[4] and also, in the androstane series, by chemical means.[5] Our objective was to effect the conversion of the resulting 14α-hydroxy steroids to the corresponding 14β-hydroxy compounds. In this paper we describe the realization of this type of transformation in the androstane series by a four-step route which proceeds in high yield at each step.[6]

The starting material for our work was androstane-3β,14α-diol-17-one 3-monoacetate (I), readily prepared from dehydroisoandrosterone acetate dibromide by chromic acid oxidation, zinc debromination and catalytic hydrogenation.[5,7] The compound I was dehydrated by means of potassium bisulfate in acetic anhydride. The resulting Δ¹⁴-androsten-3β-ol-17-one acetate (II),[5,8] m.p. 156°, was obtained in 70% yield when the reaction was carried out in the refluxing anhydride, but only little dehydration occurred under the recommended conditions (95–100° for 15 minutes).[5]

Treatment of the unsaturated acetoxy-ketone II with perbenzoic acid gave 70% of one pure oxide, m.p. 160°, and no other isomer could be isolated. Unlike Δ¹⁴-steroids containing a β-orientated side-chain at C-17, which are hydrogenated from the α-side[9], Δ¹⁴-androsten-17-ones have been found to

(1) For Part II, see F. Sondheimer and S. Burstein, *Proc. Chem. Soc.*, 228 (1959).

(2) For a review, see L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, Chapter 20.

(3) P. A. Plattner, L. Ruzicka, H. Heusser, *et al.*, *Helv. Chim. Acta*, **29**, 942 (1946); **30**, 385, 395, 1342 (1947).

(4) S. H. Eppstein, P. D. Meister, D. H. Peterson, H. C. Murray, H. M. Leigh Osborn, A. Weintraub, L. M. Reineke and R. C. Meeks, Abstracts of Papers, 123rd Meeting of the American Chemical Society, Los Angeles, Calif., March, 1953, p. 5C; THIS JOURNAL, **80**, 3382 (1958); J. Fried, R. W. Thoma, D. Perlman, J. E. Herz and A. Borman, *Recent Progr. Hormone Research*, **11**, 157 (1955); E. J. Agnello, B. L. Bloom and G. D. Laubach, THIS JOURNAL, **77**, 4684 (1955); A. Schubert, D. Onken, R. Siebert and K. Heller, *Ber.*, **91**, 2549 (1958).

(5) A. F. St. André, H. B. MacPhillamy, J. A. Nelson, A. C. Shabica and C. R. Scholz, THIS JOURNAL, **74**, 5506 (1952). The orientation of the 14-hydroxyl group was not specified definitely by these authors, but was later shown to be alpha by Eppstein *et al.*[4]

(6) For a preliminary communication, see footnote 1.

(7) Although compound I itself has not been obtained microbiologically, Eppstein, *et al.*,[4] have converted testosterone by incubation with *M. griseo-cyanus* to 14α-hydroxytestosterone, which was oxidized to Δ⁴-androsten-14α-ol-3,17-dione. The latter could undoubtedly be transformed to I, *e.g.*, through successive chemical reduction of the double bond, preferential reduction of the 3-ketone with sodium borohydride and acetylation.

(8) This substance has also been prepared, though only in very low yield, by the dehydrobromination of 16α-bromo-isoandrosterone acetate (R. Pappo, B. M. Bloom and W. S. Johnson, THIS JOURNAL, **78**, 6347 (1956)).

(9) *Inter al.*, F. Schenck, K. Buchholz and O. Wiese, *Ber.*, **69**, 2696 (1936); M. Steiger and T. Reichstein, *Helv. Chim. Acta*, **21**, 828 (1938); F. Hunziker and T. Reichstein, *ibid.*, **28**, 1472 (1945); A. Lardon and T. Reichstein, *Pharm. Acta Helv.*, **27**, 287 (1952).