(17$\alpha$-ethinyltestosterone) on oral administration.[11] As an ovulation inhibitor in the rabbit, II was 10-20 times more active parenterally than progesterone. When administered parenterally to pregnant rats castrated on the 8th day of pregnancy, II was 25-100 times progesterone in maintaining pregnancy to term. These results, which indicate 6$\alpha$-methyl-17$\alpha$-hydroxyprogesterone 17-acetate to be a progestational agent of exceptional potency, will be described in greater detail shortly.[12]

(11) In the same assays, the corresponding non-methylated steroid, 17$\alpha$-hydroxyprogesterone 17-acetate,[7b] was *ca.* 6 times progesterone (parenterally) and 2–4 times ethisterone (orally).

(12) The chemical portion of this work will be submitted to THIS JOURNAL (Babcock, Gutsell, Herr and Hogg); the endocrine studies will be reported elsewhere (Stucki, Barnes and Dulin).

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RECEIVED APRIL 24, 1958

## DIRECT INTRODUCTION OF A NITROGEN FUNCTION AT C-18 IN A STEROID[1]

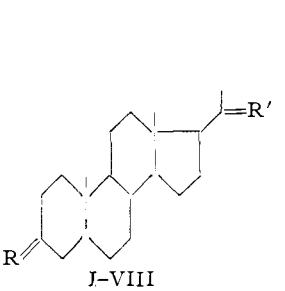
*Sir:*

Current interest in aldosterone,[1a] has prompted several investigations on the synthesis of C-18 oxygenated steroids, total[2] as well as partial synthesis using steroids lacking substitution at C-18 as starting material.[3] In the latter case opening of ring D has been used for modification of C-18.

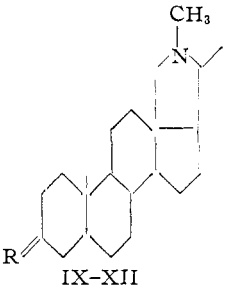
We have developed a third way of approaching the problem, the direct introduction of a substituent into the *intact* tetracyclic steroid molecule by the Loeffler-Freytag reaction.[4,5]

3$\beta$-Acetoxy-20-keto-5$\alpha$-pregnane (I)[6] was converted via the oxime (II) (m.p. 198°, $[\alpha]^{20}D$ +17° ($CHCl_3$), *Anal.* found: C, 73.49: H, 9.94) to 3$\beta$-acetoxy-20$\alpha$-amino-5$\alpha$-pregnane (III)[7] (m.p. 171°; $pK$*[8] 9.30) (Pt–$H_2$), thence with ethyl formate to the N-formyl derivative (IV) (m.p. 185–186°, $[\alpha]^{19}D$ −3° ($CHCl_3$), *Anal.* found: C, 74.01; H, 10.13; N, 3.52) and further with $LiAlH_4$ to 3$\beta$-hydroxy-20$\alpha$-N-methylamino-5$\alpha$-pregnane (V) (m.p. 211°, $[\alpha]^{20}D$ +27° ($CHCl_3$), $pK$* 9.17, *Anal.* found: C, 79.20; H, 11.79; N, 4.16). Oxidation of (V) ($CrO_3$–HOAc) gave the 3-keto-derivative (VI) ($pK$* 9.20; N-acetate: m.p. 216-217°, $[\alpha]^{19}D$ +15° ($CHCl_3$), *Anal.* found: C, 77.06; H, 10.69) which was reduced by the Huang-Minlon procedure to 20$\alpha$-N-methylamino-5$\alpha$-pregnane (VII) (m.p. 96°, $[\alpha]^{20}D$ +32° ($CHCl_3$), $pK$* 9.32, *Anal.* found: C, 83.22; H, 12.33; N, 4.31). The N-chloro derivative (VIII), made from (VII) according to the procedure of Ruschig, *et al.*,[9] furnished by treatment with a mixture of concd. sulfuric and acetic acid[10] a tertiary base, m.p. 107°, formulated as the hitherto unknown conanine (IX),[11,12] $[\alpha]^{19}D$ +61° ($CHCl_3$), ($pK$* 8.28 *Anal.* found: C, 83.66; H, 12.08; N, 4.43).

The constitution of (IX) was proved as follows: dihydro-isoconessimine (X)[13] was converted via the N-chloro derivative (XI) to 3-keto-conanine (XII) (m.p. 146°, $[\alpha]^{20}D$ +80° ($CHCl_3$), $pK$* 8.26, *Anal.* found: C, 80.15; H, 10.64; N, 4.13 ($NaOCH_3$ followed by $H_3O^+$). Removal of the 3-keto group in the latter afforded conanine (IX) (m.p. 107°, $[\alpha]^{20}D$ +63° ($CHCl_3$), $pK$* 8.28, *Anal.* found: C, 83.85; H, 11.79, N, 4.39), identical in all respects with the tertiary base from the cyclization reaction. Assuming that C-20 is not involved in the latter reaction the precursors of (IX) must therefore be formulated as 20$\alpha$-compounds.[12]



I–VIII



IX–XII

| | | |
|---|---|---|
| I | R = {—H, —OAc} | R′ = O |
| II | R = same | R′ = NOH |
| III | R = same | R′ = {—H, —$NH_2$} |
| IV | R = same | R′ = {—H, —NHCHO} |
| V | R = {—H, —OH} | R′ = {—H, —$NHCH_3$} |
| VI | R = O | R′ = same |
| VII | R = $H_2$ | R′ = same |
| VIII | R = same | R′ = {—H, —$NClCH_3$} |
| IX | R = $H_2$ | |
| X | R = {—H, —$NHCH_3$} | |
| XI | R = {—H, —$NClCH_3$} | |
| XII | R = O | |

(1) This paper is part of a joint effort of CIBA AG,[2a] Basle, Organische Anstalt der Universitat,[2b] Basel, N.V. Organon,[2c] Oss, and organisch-chemisches Laboratorium der Eidg. Technischen Hochschule, Zurich, on synthesis of C-18 oxygenated steroids.

(1a) S. Â. Simpson, J. F. Tait, A. Wettstein, R. Neher, J. von Euw, O. Schindler and T. Reichstein, *Helv. Chim. Acta*, **37**, 1163, 1200 (1954).

(2) (a) P. Wieland, K. Heusler, H. Ueberwasser and A. Wettstein, *Helv. Chim. Acta*, **41**, 416 (1958) and earlier papers; (b) A. Lardon, O. Schindler and T. Reichstein, *ibid.*, **40**, 666, 1034 (1957); (c) W. J. van der Burg, D. A. van Dorp, O. Schindler, C. M. Siegmann and S. A. Szpilfogel, *Rev. Trav. Chim.*, **77**, 171 (1958), and previous papers.

(3) D. H. R. Barton, A. da S. Campos-Neves and A. I. Scott, *J. Chem. Soc.*, 2698 (1957).

(4) (a) A. W. Hofmann, *Ber.*, **18**, 5, 109 (1885); (b) K. Loeffler and C. Freytag, *Ber.*, **42**, 3427 (1909); K. Loeffler, *ibid.*, **43**, 2035 (1910).

(5) For references *cf.* R. Lukes and M. Ferles, *Coll. Czech.*, **20**, 1227 (1955).

(6) A. Butenandt, U. Westphal and W. Hohlweg, *Z. physiol. Chem.*, **227**, 84 (1934).

(7) The stereochemistry of C-20 in III and related compounds is discussed below.

(8) Measured in methylcellosolve according to W. Simon, E. Kovats, L. H. Chopard-dit-Jean and E. Heilbronner, *Helv. Chim. Acta*, **37**, 1872 (1954).

(9) H. Ruschig, W. Fritsch, J. Schmidt-Thome and W. Haede, *Ber.*, **88**, 883 (1955).

(10) According to a private communication of Prof. E. J. Corey the cyclization reaction of aliphatic N-chloro-amines is promoted by traces of ferrous sulfate. We thank Prof. Corey for this information. A similar effect we have now noted in the cyclization of compound (VIII).

(11) Nomenclature as proposed by R. D. Haworth and M. Michael, *J. Chem. Soc.*, 4973 (1957).

(12) For the proof of configuration of C-20 *cf.* V. Cerny, L. Labler and F. Sorm, *Coll. Czech.*, **22**, 76 (1957).

(13) R. D. Haworth, J. McKenna and G. H. Whitfield, *J. Chem. Soc.*, 1102 (1953).

Full details of this and further work will be published in *Helvetica Chimica Acta*.[14]

(14) Drs. E. J. Corey and W. R. Hertler also have been concerned with this problem and our results are published simultaneously with theirs by friendly agreement.

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RECEIVED MAY 1, 1958

## ACYL AMIDES AS EPIMERIZATION REAGENTS[1,2]

*Sir:*

We wish to report the novel epimerizing action of acyl amides on certain tosylates.

$\beta$-Cholestanyl tosylate in a 2.5% solution in N,N-dimethylformamide[3] (DMF) heated at 78°, reacted completely in 23 hours to form a product which when chromatographed on Florisil[4] was cleanly separated into 75% of $\alpha$-cholestanyl formate (m.p. 114.5–116.0°, $[\alpha]_D$ +30.3° chf, $\lambda_{max}^{CS_2}$ 5.82; 8.40, 8.45, 8.65$\mu$.[5] *Anal.* Found: C, 81.00; H, 11.80), identical with the ester prepared from epicholestanol[1] and formic acid, and 22% of 2-cholestene.[6] However, chromatography on alumina[7] instead of Florisil resulted in hydrolysis of the formate, permitting a facile quantitative separation[8] into $\alpha$-cholestanol and olefin.

In DMF purified[9] by treatment with barium oxide and distillation, the reaction, although slower (60 hr.), gave essentially the same yields of products. At reflux temperature, with untreated DMF, the product contained 35% formate and was predominantly olefin.

Methyl 3$\alpha$-tosyloxycholanate with untreated DMF at 78° gave when chromatographed on Florisil 78% methyl 3$\beta$-formoxycholanate (m.p. 104–107.5°, $[\alpha]_D$ +13.4° chf., $\lambda_{max}^{CS_2}$ 5.77, 5.82; 8.45, 8.68$\mu$. *Anal.* Found: C, 74.90; H, 10.27) identical with the product of esterification of methyl 3$\beta$-hydroxycholanate[10] with formic acid, and 20% methyl 3-cholenate. Chromatography on alumina similarly yielded methyl 3$\beta$-hydroxycholanate and olefin quantitatively.

Obtained at 78° from DMF and the corresponding epimeric tosylates were: $\beta$-cholestanyl formate,[11] 45 hr., 36%, m.p. 85–86.0°, $[\alpha]_D$ + 14.4° chf, $\lambda_{max}^{CS_2}$ 5.82; 8.47$\mu$ (*Anal.* Found: C, 80.91; H, 11.85); androsterone formate, 45 hr., 73%, m.p. 181–181.5°, $[\alpha]_D$ +94.2° chf, $\lambda_{max}^{CS_2}$ 5.76, 5.82; 8.44, 8.65$\mu$. (*Anal.* found: C, 75.25; H, 9.60). Cholesteryl tosylate under these conditions in 40 hr. gave 54% of cholesteryl formate,[12] m.p. 97.5–98.0°, $[\alpha]_D$ −49.1° chf, $\lambda_{max}^{CS_2}$ 5.81; 8.45, 8.52$\mu$.

With N,N-dimethylacetamide,[3] $\beta$-cholestanyl tosylate required 92 hr. for complete reaction (disappearance of strong tosylate infrared bands), yielding 21% of $\alpha$-cholestanyl acetate,[13] and much ether-insoluble product.

Formamide did not react with $\beta$-cholestanyl tosylate because of the extremely low solubility, but when heated for 65 hr. at 78° with methyl 3$\alpha$-tosyloxycholanate, formed 53% of methyl 3$\beta$-hydroxycholanate and 32% of methyl 3-cholenate.

Observed facts pertinent to a study of the mechanisms of the reactions reported are: the alcoholic products are stereochemically nearly homogeneous; the reaction in formamide yields inverted alcohol, not formate; purified DMF gives the same yields as untreated DMF, albeit more slowly; an equimolar amount of dimethylammonium *p*-toluenesulfonate was recovered (from DMF reaction); DMF with added *p*-toluenesulfonic acid does not formylate epicholestanol to any appreciable extent under the conditions of the inversion; formamide does not hydrolyze methyl 3$\beta$-formoxycholanate under like conditions; cholesterol is formylated without rearrangement.[14]

Full details and further work on this reaction will be reported subsequently.

(1) Paper VII in Seroflocculating Steroids series. Previous paper VI, *Chem. & Ind.*, in press (1958).

(2) This work is supported by grants CS-9053, C-2249 and C-3407 from the National Cancer Institute, of the National Institutes of Health, Public Health Service.

(3) Eastman Kodak Co. product, "Eastman" grade, used as purchased.

(4) Floridin Co. product, 60–100 mesh.

(5) The very strong carbonyl band at 5.82 $\mu$ permits estimation of the formate concentration. Such estimates were found to check actual isolations by chromatography to within 1%. Furthermore, the C—O stretching bands near 8.5 $\mu$ appear to be characteristic of the 3-formates (L. J. Bellamy, "The Infra-Red Spectra of Complex Molecules," Methuen & Co. Ltd., London, 1956, p. 161).

(6) Elsevier's "Encyclopaedia of Organic Chemistry," Vol. III, 14 Supplement, 1422S.

(7) Fisher Scientific Co. product A-540.

(8) In another experiment in which $\beta$-cholestanyl tosylate was heated for 29 hr. at 78° as a 5% solution in DMF, some unreacted tosylate remained, and 3.5% of $\beta$-cholestanol was obtained as the final fraction from the chromatography on alumina.

(9) *Cf.* G. R. Leader and J. F. Gorley, THIS JOURNAL, **73**, 5731 (1951). S. R. Ross and M. M. Labes (*ibid.*, **79**, 4155 (1957)), report that DMF purified similarly contains 0.09–0.13% water, more than the equimolar proportions needed for reaction.

(10) R. T. Blickenstaff and F. C. Chang, *ibid.*, **80**, 2726 (1958).

(11) Elsevier's "Encyclopaedia of Organic Chemistry," Vol. III, 14, p. 58.

(12) Elsevier's "Encyclopaedia of Organic Chemistry," Vol. III, 14, p. 1630S.

(13) C. W. Shoppee, *J. Chem. Soc.*, 1138 (1946).

(14) *Cf.* S. Winstein and R. Adams, THIS JOURNAL, **70**, 838 (1948).

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RECEIVED MARCH 28, 1958

## THE REACTION OF SODIUM BOROHYDRIDE WITH MUSCLE PHOSPHORYLASE[1]

*Sir:*

The presence of a firmly bound, non-protein constituent in skeletal muscle phosphorylase has been investigated independently in two different laboratories.[2,3,4] Identification of this material as pyridoxal 5′-phosphate (PLP) was first reported by Baranowski, *et al.*[2] Cori and Illingworth[3] found

(1) Supported by the Institutional Grant to the University of Washington by the American Cancer Society, the Initiative 171 Fund of the State of Washington, and the United States Public Health Service (Grant No. A-850).

(2) T. Baranowski, B. Illingworth, D. H. Brown and C. F. Cori, *Biochim. et Biophys. Acta*, **25**, 16 (1957).

(3) C. F. Cori and B. Illingworth, *Proc. Nat. Acad. Sci.*, **43**, 517 (1957).

(4) A. B. Kent, E. G. Krebs and E. H. Fischer, *J. Biol. Chem.*, in press.