scribed for the preparation of IIb. Crystals (0.68 g.), m.p. 140–145°, were collected and crystallized five times from acetone–petroleum ether to furnish 0.33 g. (48%), m.p. 169–171°. A portion (0.2 g.) was further crystallized twice from methanol to yield 0.15 g. of VIb, m.p. 184.5–187°; λ_{max} 225.5 mμ (ε 11,600) and 262 mμ (ε 570); ν_{max} 1595, 1340, 1170, 1094 and 815 cm.$^{-1}$; $[\alpha]^{25}D$ −12° (17 mg., αD −0.10°), [M]D −69.

Anal. Calcd. for $C_{32}H_{44}O_7S$ (572.67): C, 67.11; H, 7.74; S, 5.58. Found: C, 67.36; H, 7.80; S, 5.86.

Treatment of VIb with 5% alcoholic potassium hydroxide as in the preparation of IIIc yielded only material with m.p. 184–187°. Its infrared absorption spectrum was identical to that of the starting material VIb.

Δ⁴-Pregnene-3,11,20-trione-17α,21-oxide 20-Ethylene Ketal (VIIa). A.—A solution of the 11-one-oxide-bis-ethylene ketal IIId (0.15 g.) in methanol (20 ml.) was treated with 8.5% (v./v.) sulfuric acid (1.5 ml.) in the fashion described for IVb. Solid (0.103 g., 77%) was collected, m.p. 248–251°. Two crystallizations from acetone–petroleum ether afforded 0.095 g. of VIIa, m.p. 247–250°, λ_{max} 238 mμ (ε 16,600); ν_{max} 1709, 1680, 1621, 1055 and 1022 cm.$^{-1}$; $[\alpha]^{24}D$ +198° (9.9 mg., αD +0.98°), [M]D +765.

Anal. Calcd. for $C_{23}H_{30}O_5$ (386.47): C, 71.48; H, 7.82. Found: C, 71.75; H, 7.90.

B.—A solution of 0.1 g. of IIId in 4 ml. of 75% acetic acid was heated 20 minutes on a steam-bath, cooled and added slowly to a saturated solution of sodium bicarbonate. Water was added, and the solid was filtered and washed well with water. Four crystallizations from acetone–petroleum ether gave 0.055 g. of VIIa, m.p. 247–250°. There was no depression of mixed melting point with the sample above, and the infrared absorption spectra were identical.

Δ⁴-Pregnene-3,11,20-trione-17α,21-oxide 3-(2,4-Dinitrophenylhydrazone) 20-Ethylene Ketal (VIIb).—A solution of 75 mg. of the 20-ketal VIIa in 6 ml. of acetic acid was treated with 100 mg. of 2,4-dinitrophenylhydrazine, and was heated for 10 minutes on the steam-bath. The mixture was cooled, and crystallization was induced by scratching. The solid was collected and was washed with cold methanol to give 63 mg., m.p. 290–291°. This material was adsorbed on an alumina column with benzene–petroleum ether (1:1), and was eluted with benzene. Recrystallization with chloroform–methanol furnished 39 mg. of VIIb, m.p. 293–294°; $\lambda_{max}^{1\%\ CHCl_3\ in\ abs.\ EtOH}$ 256–257 mμ (ε 19,200) and 384–386 mμ (ε 31,400); ν_{max} 3330, 1707, 1625, 1595, 1116 and 1056 cm.$^{-1}$.

Anal. Calcd. for $C_{29}H_{36}O_8N_4$ (568.61): N, 9.85. Found: N, 9.23.

Δ⁴-Pregnene-3,11,20-trione-17α,21-oxide 3-Semicarbazone 20-Ethylene Ketal (VIIc).—To a solution of 50 mg. of the 20-ketal VIIa in 10 ml. of warm methanol, water was added to the point of turbidity. Semicarbazide hydrochloride (40 mg.) and sodium acetate (60 mg.) were added and the mixture was heated on the steam-bath for one-half hour. The mixture was cooled and filtered to afford 37 mg. of crystals, m.p. 159–160°. Crystallization from dilute methanol and from acetone–chloroform–petroleum ether yielded 10 mg. of VIIc, m.p. 269–271°.

Anal. Calcd. for $C_{24}H_{33}O_5N_3$ (443.53): N, 9.47. Found: N, 9.52.

PEARL RIVER, NEW YORK

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, DUKE UNIVERSITY]

The Synthesis of Some Steroidal Amines

BY ROBERT A. MICHELI[1,2] AND CHARLES K. BRADSHER

RECEIVED DECEMBER 20, 1954

A variety of new steroidal 21-tertiary aminoalcohols and aminoketones have been prepared for biological experiments. The method of incorporating the nitrogen function involved the reaction of a haloketone or haloalcohol with an amine. The steroidal ring systems included the 5-pregnene, 5,16-pregnadiene and allopregnane-17-α-ol. In addition, a 21-amino derivative of desoxycorticosterone has been prepared.

The partial synthesis of steroidal alkaloids from non-nitrogenous naturally occurring steroids has been reported in several cases.[3,4] Some related steroidal amines[5,6,a,b] have been synthesized for testing as hypotensive agents, and a number of mono- and diamino derivatives of cholesterol and cholestanol have been reported to exhibit high antibacterial activity.[7–9] The amebacidal activity of conessine[10] has prompted the synthesis of amino derivatives of androstane,[11] allopregnane[9,12] and trihydroxynorcholane.[11]

It was considered of interest to make available certain 21-aminosteroids for biological testing. A simple displacement type reaction appeared to be most suitable for the initial introduction of the nitrogen function.

$$R'COCH_2X + R_2NH \longrightarrow R'COCH_2NR_2 + HX$$
$$+ R_3N \longrightarrow R'COCH_2N^+R_3$$

The two iodoketones, 21-iodo-5-pregnene-3β-ol-20-one acetate (Ia)[13] and 21-iodo-5,16-pregnadiene-3β-ol-20-one acetate (IIa),[14] were used for the preparation of the majority of the amino compounds. With dimethylamine these afforded 3β-acetoxy-21-dimethylamino-5-pregnene-20-one (Ib) and the corresponding 5,16-pregnadiene compound (IIb).[15] Similarly, the new quaternary salts (Ic, IIc) were obtained directly from gaseous trimethylamine or indirectly *via* the corresponding amine hydrochlorides. Reaction of morpholine with the two iodoketones (Ia, IIa) to give the 21-morpholino series (Ie, IId) was also quite successful. Hydrogenation of the pregnadiene amines to the pregnene analogs interrelated the two series.

During the synthesis of the methiodides (Ic, IIc,

(1) Taken in part from the thesis submitted by Robert A. Micheli in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Duke University, 1954.

(2) Monsanto Chemical Co. Fellow, 1953–1954.

(3) F. C. Uhle and W. A. Jacobs, *J. Biol. Chem.*, **160**, 243 (1945).

(4) F. C. Uhle, THIS JOURNAL, **75**, 2280 (1953); **76**, 4245 (1954).

(5) F. C. Uhle, *ibid.*, **73**, 883 (1951).

(6a) L. F. Fieser and W. Huang, *ibid.*, **75**, 6306 (1953).

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(10) T. A. Henry, "The Plant Alkaloids," 4th ed., The Blakiston Co., Philadelphia, Pa., 1949. p. 742.

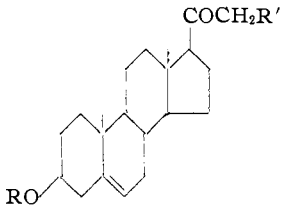
(11) D. P. Dodgson and R. D. Haworth, *J. Chem. Soc.*, 67 (1952).

(12) J. Joska, V. Cerny and F. Sorm, *Coll. Czech. Chem. Communs.*, **19**, 551 (1954).

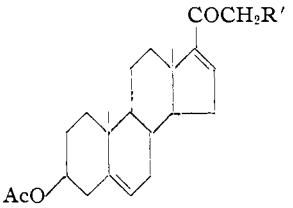
(13) C. Djerassi and C. T. Lenk, THIS JOURNAL, **75**, 3493 (1953).

(14) C. Djerassi and C. T. Lenk, *ibid.*, **76**, 1722 (1954).

(15) The iodoketone Ia afforded a nice crystalline phthalimido derivative Id. Attempts to convert Id to the free base were unsuccessful.



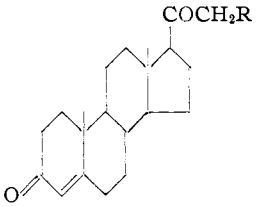
I R = Ac
a, R′ = I
b, R′ = $N(CH_3)_2 \cdot HCl$
c, R′ = $\overset{+}{N}(CH_3)_3I^-$
d, R′ = $N(CO)_2C_6H_4$
e, R′ = $N(CH_2—CH_2)_2O \cdot HCl$
f, R′ = $\overset{+}{N}(CH_2—CH_2)_2OI^-$ (CH_3)
R = H
g, R′ = $N(CH_2—CH_2)_2O$



IIa, R = I
b, R = $N(CH_3)_2 \cdot HCl$
c, R = $\overset{+}{N}(CH_3)_3I^-$
d, R = $N(CH_2—CH_2)_2O \cdot HCl$
e, R = H

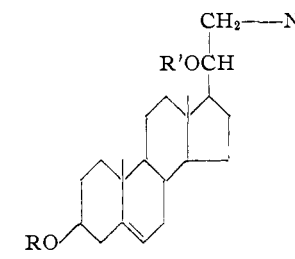
If and IIIb) a large difference in rates of formation was observed. The quaternary salts from the dimethylamino derivatives precipitated within a few hours as compared to the four days or more required for the morpholine derivatives.[16]

A desired 21-amino analog of desoxycorticosterone, 21-morpholino-4-pregnene-3,20-dione (IIIa), was obtained from the alcohol (Ig) by means of the Oppenauer reaction



IIIa, R = $N(CH_2CH_2)_2O$
b, R = $\overset{+}{N}(CH_2CH_2)_2OI^-$ (CH_3)

Hydrogenation of 3β-acetoxy-21-morpholino-5,16-pregnadiene-20-one hydrochloride followed by sodium borohydride reduction and basic saponification (IId → IVa) or direct treatment of the hydrochloride of 21-morpholino-5-pregnene-3β-ol-20-one acetate with a large excess of the hydride effected simultaneous reduction of the 20-keto and hydrolysis of the 3-acetate groups (Ie → IVa), respectively, giving 21-morpholino-5-pregnene-3β,20β-diol (IVa). Short treatment of Ie with only a slight excess of the borohydride resulted in the reduction of the 20-keto function to the alcohol IVb with retention of the ester group at position three.



IVa, R = R′ = H
b, R = Ac; R′ = H
c, R = R′ = Ac

The hydride reduction products were obtained in good yields as nicely crystalline material with sharp melting points, indicating that only one of the two possible C-20 epimers had been isolated. It is not generally true that only one isomer is isolated from the sodium borohydride reduction of a ketone, when a new asymmetric center is formed. However, this stereospecificity has been observed in several cases involving the 20-carbonyl group. For example predominant products from the hydride reduction of pregnane-3β-ol-11,20-dione and pregnane-3α,17α-diol-11,20-dione[17] as well as pregnane-3,11,20-trione[18] are reported to have the 20β-hydroxy configuration. During the course of investigating the 17α-hydroxy series (see below), the sodium borohydride reduction of allopregnane-3β,17α-diol-20-one 3-acetate (Reichstein's Substance L acetate) (Va) followed by acetylation resulted in the isolation of only the known allopregnane-3β,17α,20β-triol 3,20-diacetate (Reichstein's Substance J diacetate)[19,20] (Vc). Furthermore, the change in molecular rotations of the alcohols and their corresponding acetates are characteristic of the 20 β-series.[21] Therefore, by analogy the 20β-configuration has been assigned to the amino-alcohols (and the bromohydrin Vd) obtained in this work.

For the preparation of 17α-hydroxy amino compounds, Reichstein's Substance L acetate[22-25] (Va), was selected as a suitable intermediate and was found to be obtained conveniently from 5,16-pregnadiene-3β-ol-20-one acetate (IIe). Epoxidation and reacetylation[26] to 16α,17α-oxido-5-pregnene-3β-ol-20-one acetate, followed by catalytic hydrogenation of the 5,6-double bond over a palladium–charcoal catalyst afforded the known 16α,17α-oxidoallopregnane-3β-ol-20-one acetate.[27,28] The latter was converted by means of hydrogen bromide in acetic acid to the bromohydrin, followed by hydrogenolysis over palladium–calcium carbonate catalyst (the Julian[26,29] and Kendall[30] reactions (*cf.* ref. 18)) to the desired allopregnane-3β,17α-diol-20-one 3-acetate (Va). The conversion (Va) to 21-bromo-allopregnane-3β,17α-diol-20-one 3-acetate (Vb) has been described previously.[24,25]

Treatment of the bromoketone Vb with morpholine produced, in good yield, the desired 21-morpholinoallopregnane-3β,17α-diol-20-one 3-acetate, isolated as the hydrochloride Ve. An extended reaction period with a large excess of hydride on Ve

(16) At higher temperatures (refluxing benzene) inferior products were obtained.

(17) E. P. Oliveto and E. B. Hershberg, THIS JOURNAL, **75**, 4888 (1953).

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(21) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd ed., Reinhold Publishing Corp., New York, N. Y., 1949, pp. 434, 506.

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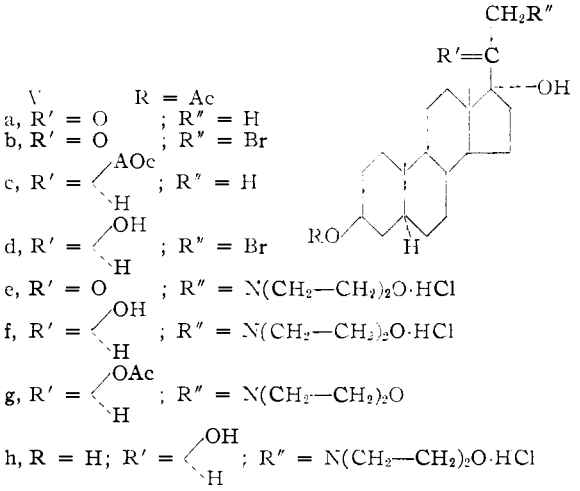
(28) P. A. Plattner, L. Ruzicka, H. Heusser and E. Angeliker, *Helv. Chim. Acta*, **30**, 385 (1947).

(29) P. L. Julian, E. W. Meyer, W. J. Karpel and I. Ryden, THIS JOURNAL, **71**, 3574 (1949).

(30) F. B. Colton, W. R. Nes, D. A. vanDorp, H. L. Mason and E. C. Kendall, *J. Biol. Chem.*, **194**, 235 (1952).

followed by treatment with a base proceeded smoothly to give the uniform product 21-morpholinoallopregnane-3β,17α,20β-triol, isolated as the hydrochloride Vh or free base. Acetylation in the usual manner afforded the 3,20-diacetate Vg.

In the synthesis of the trihydroxyamino compound Vh, it was considered of interest to find a route that would allow the retention of the 3-ester function. It was found that the bromoketone Vb could be reduced under suitable conditions with sodium borohydride to give in 50% yield 21-bromoallopregnane-3β,17α,20β-triol 3-acetate (Vd).



When this bromohydrin Vd was refluxed overnight in benzene, in the presence of morpholine, the amino compound, isolated as the hydrochloride Vf, was formed in good yield. Hydrolysis afforded the free base of Vh.

Antimicrobial screening[31] of four steroidal amine derivatives (Ic, IIb, IId and Ve) revealed that none completely inhibited the growth of *E. coli* at 250 p.p.m. At the same concentration, IIb and Ic were the only compounds in the group which completely inhibited the growth of *B. subtilis*, Ic affording complete inhibition even at 100 p.p.m.[32] The two compounds screened against *A. niger*[33] showed significant fungistatic activity, IId causing 96% and IIb 100% inhibition of growth at 250 p.p.m.

We are indebted to Dr. J. Berlin and Syntex, S. A., Mexico City, D.F., for a sample of Reichstein's Substance L acetate and for a generous gift of 5,16-pregnadiene-3β-ol-20-one acetate. We also wish to express our appreciation to Professor C. Djerassi of Wayne University for an authentic sample of 5-pregnene-3β,21-diol-20-one diacetate and to Parke, Davis and Co. for some 5,16-pregnadiene-3β-ol-20-one.

Experimental

Melting Points.—All melting points are corrected. Those obtained on the Kofler micro hot-stage are indicated by (Kof.); all others have been carried out on the Fisher–Johns block. All compounds were dried *in vacuo* at 68°, unless otherwise specified. The analytical samples were dried *in vacuo* over phosphorus pentoxide at the temperature given.

Absorption Spectra.—The ultraviolet absorption spectra were measured in 1-cm. quartz cells in 95% ethanol solution using a Beckman model DU quartz spectrophotometer. Infrared absorption spectra were obtained with a Perkin–Elmer model 21 double beam spectrophotometer using a Nujol mull between sodium chloride plates.

3β-Acetoxy-21-dimethylamino-5,16-pregnadiene-20-one Hydrochloride (IIb).—A solution of 1.20 g. (2.49 mmoles) of 21-iodo-5,16-pregnadiene-3β-ol-20-one-acetate (IIa) in 75 ml. of ether was added in one portion to 100 ml. of ether previously saturated at room temperature with anhydrous dimethylamine. A white solid rapidly precipitated. After standing for 12 hours the mixture was treated with dilute potassium hydroxide solution and extracted with ether. The ethereal extract was washed with water and brine until neutral, dried over anhydrous sodium sulfate and filtered. Treatment with anhydrous hydrogen chloride produced the amine salt IIb as a white solid. One crystallization gave 0.70 g. (64.5%) as tiny needles from absolute ethanol–ether. A further recrystallization from absolute ethanol produced the analytical sample (dried 110°). The melting point varies somewhat with rate of heating and type of apparatus and should not be used as a criterion of purity. Thus, in a capillary tube the compound exhibited a melting point of 238–240° dec., while on the Kofler block it was 222–225° dec. Repeated crystallization did not alter the melting point, $[\alpha]^{26}D$ −32.9° (methanol); λ_{max} 2.98, 3.75, 5.78, 5.99, 6.32, 8.01 and 8.12 μ; λ_{max} 246 mμ (ε 8860). The solution was treated with dilute ethanolic potassium hydroxide to yield the free base, λ_{max} 240.5 mμ (ε 6800).

Anal. Calcd. for $C_{25}H_{38}ClNO_3$ (436.02): C, 68.86; H, 8.78; Cl, 8.13; N, 3.21. Found: C, 68.54; H, 8.83; Cl, 8.28; N, 3.65.

3β-Acetoxy-21-dimethylamino-5,16-pregnadiene-20-one Methiodide (IIc). (a) **From the Amino Ketone.**—A suspension of 56 mg. (0.128 mmole) of 3β-acetoxy-21-dimethylamino-5,16-pregnadiene-20-one hydrochloride (IIb) in dilute potassium hydroxide was extracted with ether, the ethereal solution after being washed and dried (sodium sulfate) was treated with 0.25 ml. of methanol and 3 ml. of methyl iodide. The total volume was 15 ml. After several minutes, white needles began to form; the solution was allowed to remain overnight at room temperature.

The product was collected, washed with ether and dried, yielding 56.5 mg. (81%), m.p. 231–233° dec. The analytical sample was obtained by recrystallization from methanol–ether and methanol and drying at 110°, m.p. 241.5–242° dec. (Kof.), $[\alpha]^{27}D$ −25.9° (methanol), λ_{max} 243 μ (ε 8050); λ_{max} 5.78, 5.95, 6.31 and 8.07 μ.

Anal. Calcd. for $C_{26}H_{40}INO_3$ (541.51): C, 57.66; H, 7.45. Found: C, 57.82; H, 7.44.

(b).—Neutralization of 58 mg. of IIb was carried out as above. The ether was displaced and the oily residue taken up in a mixture of 2 ml. of dry benzene and 1 ml. of methyl iodide. After refluxing for 3 hours (a second portion of methyl iodide was added after 1.5 hours) the yellow, amorphous solid was collected and crystallized from methanol–ether, m.p. 218° dec., with previous browning. Further purification was not possible.

(c) **From Iodoketone IIa.**—One hundred milligrams (0.207 mmole) of 21-iodo-5,16-pregnadiene-3β-ol-20-one acetate (IIa) in 20 ml. of methanol–ether (1:1) was treated with gaseous trimethylamine and allowed to remain at room temperature for 48 hours. The precipitate of short, stout, tan needles weighed 30 mg. (27%), m.p. 233–235° dec. One recrystallization from the same solvent pair gave colorless needles, m.p. 242–243° dec., λ_{max} 243 μ (7700). Identity with the above product (a) was established by mixture melting determination and comparison of infrared spectra.

3β-Acetoxy-21-dimethylamino-5-pregnene-20-one Hydrochloride (Ib).—A mixture of dimethylamine (10 ml.) and 195 mg. (0.404 mmole) of 21-iodo-5-pregnene-3β-ol-20-one acetate (Ia) in 25 ml. of ether was allowed to react at room temperature for three days. The work-up was identical with that for the unsaturated analog IIb; yield 120 mg. (68%) of crude hydrochloride Ib. The compound appeared somewhat hygroscopic and was employed without further purification.

(31) We are indebted to Mrs. Dorcas G. Clarke for carrying out all of these tests.

(32) Complete inhibition is reported only when there was no visible growth on the inoculated agar plates after 72 hours. The test organisms were *E. coli*, ATCC strain 4157 and *B. subtilis* ATCC strain 9945.

(33) T. C. 215-4247 Steinberg. The testing procedure was essentially that of J. M. Leonard and V. L. Blackford, *J. Bact.*, **57**, 339 (1949).

3β-Acetoxy-21-dimethylamino-5-pregnene-20-one Methiodide (Ic). (a) **From the Corresponding Amine Hydrochloride** Ib.—A 70-mg. (0.160 mmole) portion of the hydrochloride of 21-dimethylamino-5-pregnene-3β-ol-20-one acetate (Ib) was converted into the methiodide (*cf.* substance IIc method a) by reaction at 25° for 12 hours; yield 67.6 mg. (78%), m.p. 232–234° dec. One crystallization from methanol–ether gave tiny, pale yellow needles (dried at room temperature), m.p. 239.5–241.5° dec. (Kof.).

Anal. Calcd. for $C_{26}H_{42}INO_3 \cdot {}^1/_2H_2O$ (552.54): C, 56.51; H, 7.84; N, 2.53. Found: C, 56.88; H, 7.59; N, 2.16.

Drying at 110° gave the unsolvated product with m.p. 241.5–242.5° dec. (Kof.).

(b) **By Hydrogenation of the Unsaturated Base** IIb.—3β-Acetoxy-5,16-pregnadiene-20-one hydrochloride (IIb) (150 mg., 0.344 mmole) was partitioned between dilute ammonia and ether. The ethereal portion was washed, dried (sodium sulfate) and evaporated *in vacuo*. The solid residue was taken up in 10 ml. of absolute ethanol and hydrogenated in the presence of 50 mg. of 5% palladium-on-barium sulfate. Hydrogen uptake ceased after 15 minutes; the catalyst was filtered off (Super-cel), and the residue, after evaporation, was converted into the methiodide by the action of 5 ml. of methyl iodide in 10 ml. of ether at room temperature overnight. The product weighed 143 mg. (77%), m.p. 239–240° dec. Recrystallization from methanol–ether raised the melting point of the pale yellow crystals to 241.5–242.5° dec. (Kof.).

(c) **From the Iodoketone** Ia.—A solution of 100 mg. (0.207 mmole) of 21-iodo-5-pregnene-3β-ol-20-one acetate (Ia) in 25 ml. of ether and 3 ml. of methanol was saturated at 25° with anhydrous trimethylamine. A precipitate was noticeable after a few hours, but the total reaction time was 12 hours. The yield of ether-washed product (Ic) was 87 mg. (78%), m.p. 230–233° dec. Recrystallization from methanol–ether and drying at 110° afforded the analytical sample (73 mg.), m.p. 241.5–242.5° dec. (Kof.), $[\alpha]^{27}D$ +10.0° (methanol); λ_{max} 5.77, 5.81 and 8.07 μ.

Anal. Calcd. for $C_{26}H_{42}INO_3$ (543.53): C, 57.45; H, 7.79; I, 23.35. Found: C, 57.34; H, 7.86; I, 23.68.

Identity was established with the products from experiments a, b and c by mixed melting point determination and superposition of the infrared curves.

3β-Acetoxy-21-morpholino-5,16-pregnadiene-20-one Hydrochloride (IId).—A solution of 3.0 g. (6.2 mmoles) of 21-iodo-5,16-pregnadiene-3β-ol-20-one acetate (IIa), m.p. 150–152°, in 30 ml. of benzene gave an immediate precipitate of white crystalline material when treated with 10 ml. of morpholine. The reaction was allowed to continue at 25° for 16 hours. The hydrochloride IId was isolated as described for compound IIb; yield 2.75 g. (92%) of pale tan crystals, approximate m.p. 210–218° dec. and sintering. The analytical sample was obtained by two recrystallizations from absolute alcohol–ether as long, colorless needles (dried 80°) and melted at approximately 215–222° dec. (varied with rate of heating and initial block temperature), $[\alpha]^{28}D$ −16.6° (methanol); λ_{max} 3.82, 3.98, 4.13, 5.77, 5.99, 6.32, 7.94, 8.08 and 8.89 μ; λ_{max} 245.5 mμ (ε 8600), λ_{max} 240.5 mμ (ε 6540) (free base, obtained by addition of dilute potassium hydroxide to cells).

Anal. Calcd. for $C_{27}H_{40}ClNO_4$ (478.06): C, 67.83; H, 8.43. Found: C, 67.67; H, 8.62.

3β-Acetoxy-21-morpholino-5-pregnene-20-one Hydrochloride (Ie).—A mixture of 21-iodo-5-pregnene-3β-ol-20-one acetate (Ia) (195 mg., 0.404 mmole), benzene (10 ml.) and morpholine (5 ml.), treated as described for IId, yield 174 mg. (85%) of (Ie) with approximate m.p. 184–188° dec. (Kof.). Small colorless needles were formed from absolute ethanol and ether (dried 80°), m.p. 189–192° dec. (Kof.) varying somewhat with initial block temperature and rate of heating; $[\alpha]^{23}D$ +17.5° (methanol); λ_{max} 2.97, 4.07, 5.78, 5.80 (shoulder), 8.01 and 8.92 μ.

Anal. Calcd. for $C_{27}H_{42}ClNO_4 \cdot 1{}^1/_2H_2O$ (507.11): C, 63.94; H, 8.94. Found: C, 63.97; H, 9.13.

3β-Acetoxy-21-morpholino-5-pregnene-20-one Methiodide (If).—Conversion of 64.5 mg. (0.127 mmole) of the hydrochloride of 21-morpholino-5-pregnene-3β-ol-20-one acetate (Ie) into an ethereal solution (25 ml.) of the free base was performed as described for IIc, part a. Methyl iodide (3 ml. in methanol (1 ml.)) was added and the quaternary salt slowly crystallized out as small, white needles during a period of 7 days at room temperature; yield 62 mg. (82%), m.p. 210–211° dec., after drying at room temperature; $[\alpha]^{29}D$ +10° (methanol); λ_{max} 5.78, 5.82, 8.03, 8.13 and 8.93.

Anal. Calcd. for $C_{28}H_{44}INO_4 \cdot {}^1/_2H_2O$ (594.57): C, 56.56; H, 7.63; N, 2.36. Found: C, 56.88; H, 7.49; N, 1.98.

3β-Acetoxy-21-phthalimido-5-pregnene-20-one (Id).—A mixture of 410 mg. (0.848 mmole) of 21-iodo-5-pregnene-3β-ol-20-one acetate (Ia) and 175 mg. (0.946 mmole) of potassium phthalimide in 20 ml. of dimethylformamide was maintained at 80–85° for 45 minutes. The yellow solution was diluted with chloroform, washed (0.2 *N* sodium hydroxide, water and brine), dried and evaporated (*vacuo*). The oily residue which crystallized readily when triturated with cold aqueous methanol was filtered, yielding 236 mg. (55%) of the phthalimido derivative Id, m.p. 193–197° (sintering). The analytical sample was obtained from methanol as needles, m.p. 198.5–200° (Kof.).

Anal. Calcd. for $C_{31}H_{37}NO_5$ (503.62): N, 2.78. Found: N, 2.83.

Experiments designed to yield the corresponding primary amine or aminoalcohol were not successful.

21-Morpholino-5-pregnene-3β-ol-20-one Acetate. (a) **From the Corresponding Hydrochloride** (Ie).—Neutralization of a solution of 50 mg. (0.104 mmole) of 3β-acetoxy-21-morpholino-5-pregnene-20-one hydrochloride (Ie) in 5 ml. of 50% methanol with dilute ammonia followed by cooling gave 44 mg. (95%) of the free base as needles, m.p. 122–124.5°; dried at 80°. Recrystallization from aqueous methanol raised the melting point to 129.5–131° (Kof.), $[\alpha]^{28}D$ +16.6° (methanol); λ_{max} 5.77, 5.83, 5.88, 8.05 and 9.00 μ.

Anal. Calcd. for $C_{27}H_{42}NO_4$ (443.61): C, 73.10; H, 9.32. Found: C, 72.79; H, 9.49.

(b) **By Hydrogenation of the Amine IId.**—A sample (1.38 g., 2.89 mmoles) of the hydrochloride of 21-morpholino-5,16-pregnadiene-3β-ol-20-one acetate (IId) was neutralized, taken up in 30 ml. of 95% ethanol and hydrogenated over 400 mg. of 5% palladium-on-barium sulfate (*cf.* compound Ic method b). The product crystallized out of dilute ethanol as long, colorless needles, m.p. 129–131°, yield 1.05 g. One recrystallization from aqueous ethanol afforded material with a constant melting point of 129.5–131.5°. Admixture with above sample (part a) exhibited no melting point depression.

21-Morpholino-5-pregnene-3β-ol-20-one (Ig).—Hydrolysis of the hydrochloride of 21-morpholino-5-pregnene-3β-ol-20-one acetate (Ie) was carried out by refluxing for one hour 802 mg. (1.67 mmoles) in 40 ml. of methanol containing 800 mg. of potassium carbonate in 10 ml. of water. The solution was diluted and extracted with ether. Evaporation of the washed and dried ethereal portion followed by crystallization from aqueous methanol yielded 541 mg. (78%), m.p. 153–154°. Recrystallization from aqueous methanol or benzene–petroleum ether (30–60°) gave long, colorless needles, m.p. 158.5–160° (dried 80°), $[\alpha]^{28}D$ +28.0° (methanol); λ_{max} 3.00, 5.81 and 8.93 μ.

Anal. Calcd. for $C_{25}H_{39}NO_2 \cdot {}^1/_2CH_3OH$ (417.59): C, 73.34; H, 9.90. Found: C, 73.63; H, 9.79.

The above alcohol, after drying at 110° for 24 hours, melted at 161–163° (Kof.).

21-Morpholino-4-pregnene-3,20-dione (**21-Morpholinoprogesterone**) (IIIa).—A solution of 1300 mg. (3.23 mmoles) of 21-morpholino-5-pregnene-3β-ol-20-one (Ig) in 125 ml. of toluene and 13 ml. of cyclohexanone was concentrated to 85 ml. To the hot solution was added (10 minutes) 1.3 g. of aluminum isopropoxide in 40 ml. of toluene and refluxing was continued for 20 minutes. The cooled, yellow solution was washed (dilute potassium hydroxide and water), dried and the solvent evaporated (*vacuo*). The oily residue was taken up in ether and treated with gaseous hydrochloric acid. The amorphous solid which resulted was dissolved in dilute methanol and neutralized with ammonia. Upon cooling, the free base IIIa crystallized out; yield 656 mg. (51%), m.p. 137–141°

The pale yellow material in benzene–petroleum ether (1:1) was chromatographed on 16 g. of alumina. Benzene and ether eluted the colorless base which, after evaporation of the solvent and on crystallization from aqueous methanol, weighed 455 mg. (colorless needles), m.p. 142–144°. A sample was recrystallized from benzene–petroleum ether

(30–60°) as colorless plates, m.p. 142.5–143.5 (Kof.) (dried 80°), $[\alpha]^{29}D$ +130° (acetone), λ_{max} 241 mμ (ε 15,951); λ_{max} 5.86, 5.96, 6.19 and 8.99 μ.

Anal. Calcd. for $C_{25}H_{37}NO_3$ (399.55): C, 75.15; H, 9.33. Found: C, 75.25; H, 9.70.

In one experiment the amorphous hydrochloride was obtained from methanol as short, stout, tan crystals, m.p. 210° dec. (sintering), λ_{max} 240.5 mμ (ε 14,150). Recrystallization failed to remove the colored impurity.

A 50-mg. sample of IIIa in 6 ml. of methanol–ether (1:5) and 1 ml. of methyl iodide was allowed to remain at room temperature for several days during which time white plates crystallized out; yield 61.5 mg., m.p. 169–172° dec. This compound, presumably the methiodide IIIb, could not be recrystallized satisfactorily.

21-Morpholino-5-pregnene-3β,20β-diol (IVa). (a) **From the Amine Salt (Ie).**—Reduction and hydrolysis was carried out simultaneously on a solution of 127 mg. (0.264 mmole) of 3β-acetoxy-21-morpholino-5-pregnene-20-one hydrochloride (Ie) in 30 ml. of methanol by treatment with a large excess of sodium borohydride (200 mg.) in 5 ml. of methanol. After remaining for 20 hours at 25°, the reaction mixture was warmed and diluted and 92 mg. (86%) of white needles collected, m.p. 194–194.5° (turbid, cleared by 200°). Recrystallization did not improve the melting point. The analytical sample was obtained by sublimation in high vacuum at 200–220°; m.p. 199.5–200° (Kof.), $[\alpha]^{27}D$ −11.7° (methanol); λ_{max} 2.93 and 8.89 μ.

Anal. Calcd. for $C_{25}H_{41}NO_3$ (403.59): C, 74.39; H, 10.24; N, 3.47. Found: C, 74.57; H, 10.44; N, 3.65.

(b) **By Hydrogenation Followed by Reduction of the Unsaturated Salt (IId).**—A 710-mg. (1.49 mmoles) sample of 3β-acetoxy-21-morpholino-5,16-pregnadiene-20-one hydrochloride (IId) was neutralized, hydrogenated (*cf.* Ic, part b) and the product, in 20 ml. of 95% ethanol, was allowed to react at room temperature for one hour with 300 mg. of sodium borohydride in 3 ml. of water. Subsequently, 400 mg. of sodium hydroxide in 3 ml. of water was added and the mixture allowed to remain for 18 hours at 25°. The dialcohol was collected after warming and diluting the solution; yield 523 mg. (87%), m.p. 193–195° (turbid). A sample was obtained from aqueous methanol (needles) with m.p. 196.5–198° (turbid melt, clear by 200°). Mixture melting point with the sample from Ie gave no depression.

21-Morpholino-5-pregnene-3β,20β-diol 3-Monoacetate (IVb).—The reduction of 110 mg. (0.229 mmole) of the hydrochloride[34] of 21-morpholino-5-pregnene-3β-ol-20-one acetate (Ie) in 20 ml. of methanol by 60 mg. of sodium borohydride in 2 ml. of the same solvent proceeded with vigorous evolution of gas for the first few minutes. After one hour, the solution was warmed, treated with charcoal, filtered and diluted to give 96 mg. (94%) of colorless needles with m.p. 190–195°. The analytical sample was obtained by recrystallization from aqueous methanol; m.p. 198–199° (Kof.) (admixture with dialcohol IVa caused a marked depression of melting point), followed by sublimation in high vacuum (200–220°), $[\alpha]^{27}D$ −7.4° (methanol); λ_{max} 2.90, 5.78, 8.02 and 8.97 μ.

Anal. Calcd. for $C_{27}H_{43}NO_4$ (445.63): C, 72.77; H, 9.72. Found: C, 72.43; H, 10.08.

21-Morpholino-5-pregnene-3β,20β-diol Diacetate (IVc).—A sample of the 3-monoacetate IVb, 44 mg. (0.099 mmole), was treated at room temperature with 0.2 ml. of acetic anhydride in 0.4 ml. of pyridine, overnight. The product weighed 42 mg. (87%), m.p. 176–178.5° (mixture melting point with starting material was depressed about 30°). Two recrystallizations from methanol gave colorless rectangular plates, m.p. 185–186° (dried 110°), $[\alpha]^{27}D$ +6.1° (chloroform); λ_{max} 5.78, 8.03, 8.11 and 8.99 μ.

Anal. Calcd. for $C_{29}H_{45}NO_5$ (487.66): N, 2.87. Found: 2.82.

16α,17α-Oxidoallopregnane-3β-ol-20-one Acetate.—Hydrogenation of 5.16 g. (0.014 mole) of 16α,17α-oxido-5-pregnene-3β-ol-20-one acetate[35] in 240 ml. of 95% ethanol was carried out at atmospheric pressure and room temperature over 0.75 g. of a 10% palladium–charcoal catalyst. Hydrogenation uptake ceased in about 6 hours. The product crystallized from methanol as plates; yield 2.85 g. (54%), m.p. 180–185°. The yield varied in several runs from 50–66%. Two recrystallizations from methanol, and drying at 80°, furnished a sample with m.p. 188–189° (Kof.) (reported[27,28] 185–186°, 186–187°), $[\alpha]^{29}D$ +50.5° (chloroform), reported[28] $[\alpha]^{30}D$ +51.6° (chloroform).

Allopregnane-3β,17α-diol-20-one 3-Acetate (Reichstein's Substance L Acetate) (Va).—To a solution of 2.57 g. (6.86 mmoles) of 16α,17α-oxidoallopregnane-3β-ol-20-one acetate in 30 ml. of glacial acetic acid was added 1.8 ml. of acetic acid saturated with hydrogen bromide. After 30 minutes at room temperature, the solution was poured into ice-water, filtered and thoroughly washed. The damp bromohydrin was hydrogenated in 120 ml. of 95% ethanol for 3 hours in the presence of 7 g. of 1.5% palladium–calcium carbonate catalyst. Dilution, extraction with ether and evaporation gave a white solid residue which crystallized from acetone–petroleum ether; yield 1.79 g. (69%) of Substance L acetate as white plates or needles (the two forms, depending on rate of crystallization, also were reported by v. Euw and Reichstein[22]) with m.p. 185–188°. A crystallized sample was obtained as needles, m.p. 188–190° (a slight change in the appearance of the material was observed at about 184° when the sample was placed on the block at 175°; this material did not depress an authentic sample, m.p. 187–190°, kindly supplied by Dr. J. Berlin, Syntex, S.A.), reported m.p. 190–191°,[22,24] 188–190°,[24] 187–189°[25]; $[\alpha]^{29}D$ +16.5 (acetone), reported $[\alpha]^{18}D$ +14.7° (acetone),[22,23] $[\alpha]^{29}D$ +16° (acetone),[24] $[\alpha]^{20}D$ +18° (acetone).[25]

Hydrochloride of 21-Morpholinoallopregnane-3β,17α-diol-20-one 3-Acetate (Ve).—A mixture of 350 mg. (0.768 mmole) of 21-bromoallopregnane-3β,17α-diol-20-one 3-acetate[36] (Vb) and 5 ml. of morpholine in 10 ml. of benzene was allowed to react overnight at room temperature (a precipitate of colorless needles was observed after 10 minutes). The usual work up (*cf.* IIb) yielded 265 mg. (69%)[37] of the hydrochloride Ve, m.p. 189–193° dec. Recrystallization (absolute ethanol–ether and absolute ethanol) gave the analytical sample as rectangular plates, m.p. 194–196° dec. (the m.p. showed fair reproducibility provided the block temperature was raised at a very slow rate), dried at 80°; $[\alpha]^{28}D$ +41.3° (methanol); λ_{max} 3.03, 3.75–3.80, 5.76, 5.84, 8.05 and 8.83 μ.

Anal. Calcd. for $C_{27}H_{44}ClNO_5$ (498.10): N, 2.81. Found: N, 2.87.

21-Morpholinoallopregnane-3β,17α,20β-triol Hydrochloride (Vh).—Reduction of the hydrochloride, 21-morpholinoallopregnane-3β,17α-diol-20-one 3-acetate (Ve), was realized by the dropwise addition of sodium borohydride (140 mg.), in water (2 ml.), to a cooled solution (138 mg. 0.277 mmole) in methanol (8 ml.). After 10 hours[38] at room temperature the mixture was treated with 100 mg. of sodium hydroxide in 2 ml. of water and allowed to remain at 25° for 12 hours more. (Some precipitate was noticed within the first hour.) It was found most expedient to add sufficient acetic acid to bring about solution of the precipitate and to follow with the addition of ether and dilute sodium hydroxide (the aqueous phase must be made decidedly basic before extracting). The ethereal phase was washed and dried, and the amine was precipitated as a white, semi-solid material by addition of hydrogen chloride. Decantation followed by crystallization from absolute ethanol–ether afforded 104 mg. (78%) of hydrated Vh. Two recrystallizations from absolute ethanol gave the analytical sample as plates (dried 80°), m.p. of all samples was 255–265° with decomposition and sintering, $[\alpha]^{26}D$ +17.0° (methanol); λ_{max} (shoulders at 2.88–2.93 and 3.07), 3.02, 3.77, 3.88, 4.08, 6.05 and 8.86 μ.

Anal. Calcd. for $C_{25}H_{44}ClNO_4 \cdot 1.5H_2O$ (485.10): C, 61.89; H, 9.77. Found: C, 61.77; H, 9.82.

(34) A poorer yield was obtained with the free base.

(35) This material was prepared as described by Julian, *et al.*[26] It was found more convenient to extract with ether the flocculent precipitate formed upon dilution of the reaction mixture. After washing and drying the solution, the ether was distilled *in vacuo*. The acetylation was carried out for one hour on the steam-bath.

(36) Prepared as described by Rosenkranz, *et al.*[25] (see also, ref. 24), crystallized once from aqueous acetone, m.p. 165–170°, and used as such for most subsequent experiments. Recrystallization from ethyl acetate–hexane and ethyl acetate raised the melting point to 190–193° (Kof.).

(37) Only a slightly better yield (77%) was obtained with the bromo ketone melting at 190–193°.

(38) It was found that reduction was incomplete after an hour.

21-Morpholinoallopregnane-3β,17α,20β-triol. (a) From the Amine Hydrochloride (Vh).—Neutralization of 97 mg. (0.200 mmole) of the hydrochloride of 21-morpholinoallopregnane-3β,17α,20β-triol (Vh) in 10 ml. of warm 50% methanol with ammonia followed by further dilution and cooling at 5°, overnight, gave 85 mg. (95%) of the free base, m.p. 184–185°. Recrystallization from dilute methanol gave long, colorless, solvated needles, m.p. 186–187° (Kof.) (dried at 68°). These needles became opaque when dried for analysis at 80° with m.p. 187–187.5° (Kof.). The loss of solvent was quite obvious when the initial block temperature was high, but the change over was not discernible when the initial temperature was below 100°; $[\alpha]^{26}D$ +8.6° (methanol); λ_{max} 2.93 (no band in the 6 μ region) and 8.99 μ.

Anal. Calcd. for $C_{25}H_{43}NO_4 \cdot 1.5H_2O$ (448.63); C, 66.93; H, 10.33. Found: C, 67.29; H, 10.31.

(b) **From the Ketone Ve without Isolation of the Intermediates.**—After 10 hours at room temperature a mixture of 1.25 g. (2.51 mmoles) of the hydrochloride 21-morpholinoallopregnane-3β,17α-diol-20-one 3-monoacetate (Ve) in 80 ml. of methanol and 1.3 g. of sodium borohydride in 10 ml. of water was treated with sodium hydroxide (900 mg.) in water (8 ml.) and allowed to remain overnight. The solution along with precipitated material was worked up as described for Vh. Crystallization from aqueous methanol furnished the amino-alcohol as long needles, yield 710 mg., m.p. 175–180°. Recrystallization (Norite) raised the m.p. to 182–183.5° (no depression with material from part a) and gave 645 mg. of long, white needles.

(c) **From the Bromohydrin Vd.**—21-Bromoallopregnane-3β,17α,20β-triol 3-monoacetate (Vd) (43 mg., 0.094 mmole), with m.p. 199–201.5°, was dissolved in 5 ml. of dry benzene and refluxed with 0.8 ml. of morpholine for 16 hours (colorless needles were noticed precipitating after a few hours).[39] The usual treatment (*cf.* IIb) yielded 35 mg. of presumably the hydrochloride of 21-morpholinoallopregnane-3β,17α,-20β-triol 3-monoacetate (Vf), as a white crystalline product (in one experiment a sample was recrystallized from absolute ethanol–ether as platelets but had a poor m.p. of approximately 260° dec. with previous sintering).

The 35-mg. sample of Vf was dissolved in 5 ml. of hot methanol and refluxed one hour with 70 mg. of potassium carbonate in 1 ml. of water. Dilution and cooling gave 25 mg. of opaque needles, m.p. 184.5–187.5°. One recrystallization from aqueous methanol gave the aminoalcohol with m.p. and mixture melting point (with sample from a) 186–187.5°.

21-Morpholinoallopregnane-3β,17α,20β-triol 3,20-Diacetate (Vg).—21-Morpholinoallopregnane-3β,17α,20β-triol with m.p. 184–185° (92 mg., 0.20 mmole) after being allowed to remain in 2 ml. of pyridine and 1 ml. of acetic anhydride for 18 hours was poured on ice, methanol being used to complete the transfer. The product was collected and washed with water; wt. 85 mg. (82%), m.p. 165.5–167.5°. The analytical sample was obtained as tufted needles following two recrystallizations from ether–petroleum ether (30–60°), m.p. 170.5–171°, $[\alpha]^{29}D$ +36° (acetone); λ_{max} 2.88, 5.78, 5.87, 8.09 and 8.98 μ.

Anal. Calcd. for $C_{29}H_{47}NO_8$ (505.68): C, 68.88; H, 9,37. Found: C, 68.94; H, 9.34.

21-Bromoallopregnane-3β,17α,20β-triol 3-Monoacetate (Vd).—Conversion of 402 mg. (0.881 mmole) of 21-bromoallopregnane-3β,17α-diol-20-one 3-monoacetate (Vb), in 30 ml. of methanol, to the bromohydrin Vd was brought about by the action of 200 mg. of sodium borohydride in methanol (10 ml.) for one hour at room temperature. Decomposition of excess hydride by the addition of a few ml. of acetic acid followed by dilution of the warmed solution precipitated the crude product; yield 350 mg., m.p. 135–145° (sintering). One crystallization from a very small amount of methanol (1–2 ml.) gave 202 mg. (50%)[40] of tiny crystals, m.p. 195–201°. Further recrystallization from ether–petroleum ether (30–60°) afforded the analytical sample as colorless needles, m.p. 204–205° (dried 80°), $[\alpha]^{29}D$ + 7.5° (acetone); λ_{max} 2.84, 2.92, 5.79, 7.90 and 7.96 μ.

Anal. Calcd. for $C_{23}H_{27}BrO_4$ (457.45): C, 60.38; H, 8.15. Found: C, 60.23; H, 7.68.

Allopregnane-3β,17α,20β-triol 3,20-Diacetate (Reichstein's Substance J Diacetate) (Vc).—To 40 mg. (0.106 mmole) of Substance L acetate (Va) in 6 ml. of methanol was added 25 mg. of sodium borohydride in 2 ml. of methanol. After one hour at room temperature (35°) the excess hydride was decomposed with a few drops of acetic acid, warmed, diluted and the white solid product collected and dried. Acetylation was carried out at room temperature for 18 hours with a mixture of acetic anhydride (0.25 ml.) and pyridine (0.5 ml.). Fractional crystallization of the product from aqueous ethanol (following treatment with Norite) gave 11 mg. (25%) of the diacetate Vc, m.p. 155–158°, as small, colorless needles. A second crop of material melting lower, with sintering, was obtained but was not investigated further. One recrystallization from the same solvent pair gave material melting 157.5–159° (reported 161–162°[19] from ether–pentane, 154–154.5°[20] from aqueous ethanol); $[\alpha]^{29}D$ +26° (acetone) (reported $[\alpha]^{19}D$ +24.6 ± 1° (acetone),[19] $[\alpha]^{21}D$ +29.8° (acetone[20]).

In another experiment 175 mg. of the acetate Va in 25 ml. of 95% ethanol was allowed to react overnight at room temperature with 190 mg. of the borohydride in 3 ml. of water. Crystallization of the acetylated product from aqueous ethanol furnished 100 mg. (m.p. 142–146°) and 19 mg. (138–141°), total 61%, of product. Combination and recrystallization from ether–petroleum ether gave 61 mg. of white crystalline material, m.p. 148–151° (sintering). The m.p. could not be sharpened or raised by recrystallization.

DURHAM, NORTH CAROLINA

(39) At room temperature a reaction time of four days was necessary before a precipitate was discernible.

(40) Variation of reaction time from 30 to 90 minutes gave comparable yields of pure material; a longer period of 6 or 12 hours, on the other hand, gave poor results.