with hydrogen and after about 30 min. shaking at room temperature the pressure had dropped to 47 lb./in.^{2}. The hydrogenation was stopped and analysis of the mixture revealed that 25.0% of the 1,3-dichloropropene had been converted and the remaining 1,3-dichloropropene was 73.0% *cis* and 27.0% *trans*. This corresponds to 1.9 ± 0.4% isomerization of *cis* to *trans* isomer in the unchanged 1,3-dichloropropene.

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The Microbial Hydroxylation of Tomatidine[1a,b]

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The steroidal alkaloid tomatidine has been hydroxylated by the fungus *Helicostylum piriforme* to yield 7α,11α-dihydroxytomatidine, 7α-hydroxytomatidine, and 9α-hydroxytomatidine. The proofs for their structures are discussed.

With the successful microbial hydroxylation of the steroidal alkaloid solasodine,[3a] it became of interest to study the effect of the fungus *Helicostylum piriforme* on tomatidine (I), the 5,6-dihydro C-25 epimer of solasodine. We previously had observed[3b] that the reduction of the double bond in the steroidal sapogenin, diosgenin, to the 5,6-dihydro derivative, tigogenin, had hindered its hydroxylation entirely.

With tomatidine, however, the hydroxylation proceeded smoothly to give the triol, 7α,11α-dihydroxytomatidine (IIa), in fairly good yields (25–30%). A lesser amount (*ca.* 5%) of the monohydroxylated compound, 7α-hydroxytomatidine (IIB), and a very small amount (*ca.* 0.5%) of 9α-hydroxytomatidine (IIC) also were isolated.

The assignment of a 3β,7α,11α-triol formulation to IIA is based on the degradation of the triol IIA to the known allopregnane derivatives, allopregnane-3β,7α,-11α-triol-20-one triacetate (Va) and allopregnane-3,7,11,20-tetrone (Vc). For the removal of the side chain,[4] the amorphous tetraacetate (IIa) was isomerized with boiling glacial acetic acid to a resinous pseudo-derivative (IIIa) which could not be resolved by alumina chromatography. The reaction product, without further efforts at purification, was oxidized with chromic acid and the acyloxy side chain removed by treatment with boiling acetic acid.[5] Although the products crystallized at this stage, chromatography over alumina failed to resolve them into homogeneous components. The mixture, therefore, was hydrogenated in ethyl acetate over palladium–barium sulfate, whereupon an absorption of about 1.6 moles of hydrogen occurred, and the product became resolvable through alumina chromatography. The purified component agreed in properties with an authentic specimen of 3β,7α,11α-trihydroxyallopregnan-20-one triacetate[6] (Va). For further confirmation, the triol Vb also was oxidized to the known allopregnane-3,7,11,20-tetrone[6] (Vc). In the course of the separation of Va through chromatography, paper chromatographic spot tests of the various fractions indicated the presence of a second component concentrated mainly in the mother liquors after removal of Va. Accordingly the mother liquors were combined, hydrolyzed with base, and chromatographed over Florisil. The crystalline dihydroxyallopregnane (Vd) thus obtained was then oxidized to the known allopregnane-3,11,20-trione[6] (Ve). Apparently, the 7α-hydroxyl moiety in the triol IIA had partially dehydrated during the course of the degradation.

The structure of the diol IIB also was determined by its degradative conversion into the known 3β-acetoxyallopregnane-7,20-dione[7] (Vf) and into 3β,7α-dihydroxyallopregnane-20-one (Vg). For the conversion into Vf, the crude acetate IIb (acetic anhydride–pyridine, 15 hr. at room temperature) was isomerized as usual with glacial acetic acid and the pseudoproduct chromatographed over alumina to yield O,N-diacetylhydroxypseudo- (IIIb) and O,O,N-triacetylpseudotomatidine (IIIc). The 7α-hydroxyl is not completely acetylated under these conditions of acetylation.[8] Barton and Laws[9] have observed the same phenomenon in regard to the acetylation of ergost-22-ene-3β,7α-diol. The subsequent oxidation and acid hydrolysis (acetic acid) of IIIb and the reduction of the resultant 16-dehydro derivative (IVa) affords the known 3β-acetoxyallopregnane-7,20-dione[7] (Vf). For conversion of IIB into 3β,7α-dihydroxyallopregnan-20-one (Vg), the acetylation is conducted under more vigorous conditions (acetic anhydride–pyridine, 1 hr. at steam-bath temperature) to the O,O,N-triacetyl derivative (IIb) and submitted to the usual degradative procedure (IIb → IIIc → IVb → Vh).

The resistance of IIB toward acetylation, its relationship to the triol IIA, and its molecular rotation data[10] (ΔM_D −39) all support our assignment of a 7α configuration.

The structure of compound IIC was unequivocally established as 9α-hydroxytomatidine by its degradative conversion in the usual manner (IIc → IIId →

(1) (a) In remembrance of the late Dr. Erich Mosettig of this Institute; (b) a preliminary account of portions of this work was published in *J. Org. Chem.*, **26**, 4181 (1961).

(2) Visiting Scientist (1960–1962), National Institutes of Health.

(3) (a) Y. Sata and S. Hayakawa, *J. Org. Chem.*, **28**, 2739 (1963); (b) S. Hayakawa and Y. Sato, *ibid.*, **28**, 2742 (1963).

(4) Y. Sato, N. Ikekawa and E. Mosettig, *ibid.*, **24**, 893 (1959).

(5) A. F. B. Cameron, K. M. Evans, J. C. Hamlet, J. S. Hunt, P. C. Jones, and A. G. Long, *J. Chem. Soc.*, 2807 (1955).

(6) C. Djerassi, O. Mancera, J. Romo, and G. Rosenkranz, *J. Am. Chem. Soc.*, **75**, 3505 (1953). We thank Dr. P. G. Holton of Syntex, S.A., Mexico, for providing us with an authentic specimen of the triacetate.

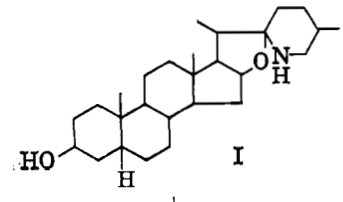
(7) W. Klyne, *J. Chem. Soc.*, 3449 (1951).

(8) L. F. Fieser, J. E. Herz, M. W. Klohs, M. A. Romero, and T. Utne, *J. Am. Chem. Soc.*, **74**, 3309 (1952).

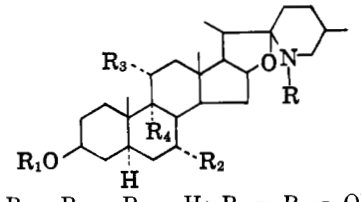
(9) D. H. R. Barton and G. F. Laws, *J. Chem. Soc.*, 52 (1954).

(10) ΔM_D = M_D (7β-hydroxytomatidine) − M_D (tomatidine) = −39. Cited values for 7α-OH, −59, and for the 7β-OH, +110. From L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p. 179.

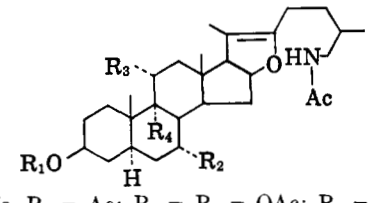
IVc) to 3β,9α-dihydroxyallopregnan-20-one acetate (Vj), the authentic specimen of which was obtained from the catalytic reduction of 5-pregnene-3β,9α-dihydroxy-20-one acetate.[11]



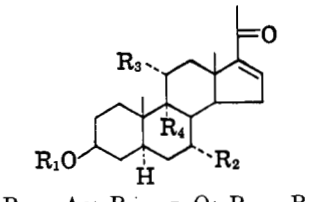
I



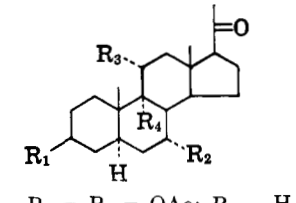
IIA, R = R_1 = R_4 = H; R_2 = R_3 = OH
B, R = R_1 = R_3 = R_4 = H; R_2 = OH
C, R = R_1 = R_2 = R_3 = H; R_4 = OH
a, R = R_1 = Ac; R_2 = R_3 = OAc; R_4 = H
b, R = R_1 = Ac; R_2 = OAc; R_3 = R_4 = H
c, R = R_1 = Ac; R_4 = OH; R_2 = R_3 = H



IIIa, R_1 = Ac; R_2 = R_3 = OAc; R_4 = H
b, R_1 = Ac; R_2 = OH; R_3 = R_4 = H
c, R_1 = Ac; R_2 = OAc; R_3 = R_4 = H
d, R_1 = Ac; R_4 = OH; R_2 = R_3 = H



IVa, R_1 = Ac; R_2 = =O; R_3 = R_4 = H
b, R_1 = H; R_2 = OH; R_3 = R_4 = H
c, R_1 = Ac; R_4 = OH; R_2 = R_3 = H
d, R_1 = Ac; R_2 = R_3 = OAc; R_4 = H
e, R_1 = H; R_4 = OH; R_2 = R_3 = H



Va, R_1 = R_2 = R_3 = OAc; R_4 = H
b, R_1 = R_2 = R_3 = OH; R_4 = H
c, R_1 = R_2 = R_3 = =O; R_4 = H
d, R_1 = R_3 = OH; R_2 = R_4 = H
e, R_1 = R_3 = =O; R_2 = R_4 = H
f, R_1 = OAc; R_2 = =O; R_3 = R_4 = H
g, R_1 = R_2 = OH; R_3 = R_4 = H
h, R_1 = H; R_2 = OH; R_3 = R_4 = H
i, R_1 = R_3 = OAc; R_2 = R_4 = H
j, R_1 = OAc; R_4 = OH; R_2 = R_3 = H

(11) Obtained from the degradation of 9α-hydroxysolasodine; see ref. 3a.

It is of some interest to note that 7α-hydroxylation of steroids in the C-5 allo series is an unique occurrence previously unencountered in this field.

The steroidal alkaloids in recent years have achieved some significance as starting material for the production of biologically active steroid hormones. The hydroxylation of these alkaloids enhances their utility.

Experimental[12]

Fermentation.—The medium was prepared by mixing 20 g. of peptone, 3 g. of corn steep liquor, 50 g. of technical dextrose, and 1000 ml. of tap water. Two hundred-milliliter batches of the sterile medium (pH 4.5) were innoculated with *Helicostylum piriforme* (A.T.C.C. 8992) and agitated for 2 days at 28° on a platform shaker. Forty milligrams of tomatidine in 2 ml. of ethanol was then introduced into each flask and shaking continued for 24 hr.

Isolation of 7α,11α-Dihydroxytomatidine (IIA), 7α-Hydroxytomatidine (IIB), and 9α-Hydroxytomatidine (IIC).—The contents of the flasks were filtered through a bed of Celite to remove the mycelium; the filtrate, after being made basic with alkali, was extracted with chloroform. The extract from a ten-flask run yielded about 420 mg. of crude residue. It was triturated with acetone to afford a crude batch of IIA (yield, 25–30%) which was repeatedly and alternately recrystallized from methanol and ethanol to give rods of 7α,11α-dihydroxytomatidine, m.p. 266–270° dec., $[\alpha]^{20}D$ +23.3° (C_2H_5OH); λ_{max}^{Nujol} 2.94 and 3.33 μ (OH and NH).

Anal. Calcd. for $C_{27}H_{45}O_4N$: C, 72.44; H, 10.13. Found: C, 72.42; H, 10.43.

The mother liquors, after removal of IIA, were combined, evaporated to dryness, and chromatographed over alumina (Woelm neutral, activity I) with the following solvent mixtures: 0.1, 0.2, 0.3, 0.4, 0.5, 1, and 5% methanol in ether. Each fraction was tested by paper chromatography using 30% propylene glycol as the stationary phase and a mixture of toluene–dioxane (7:3 v./v.) as the mobile phase. The compounds were detected by spraying the paper first with an 1% ethanolic cinnamic aldehyde solution, followed by a saturated solution of antimony trichloride in nitrobenzene, and warming over a hot plate. Fractions which gave the same coloration (reddish gray) and having approximately the same R_f values were collected (0.2 ~ 0.3% methanol in ether eluates). The compound, 9α-hydroxytomatidine (0.5%), crystallized as plates, m.p. 188–191°, from acetone. Recrystallization from methanol raised the melting point to 192–195°, $[\alpha]^{20}D$ +2.3° ($CHCl_3$); $\lambda_{max}^{CHCl_3}$ 2.78 and 2.93 μ (OH and NH).

Anal. Calcd. for $C_{27}H_{45}O_3N$: C, 75.13; H, 10.51. Found: C, 74.49; H, 10.44.

Fractions eluted with 0.5 ~1% methanol in ether in the aforementioned chromatogram afforded rods of 7α-hydroxytomatidine (IIB) from methanol. A recrystallized sample from methanol melted at 243–247° dec., $[\alpha]^{20}D$ −3.5° ($CHCl_3$); $\lambda_{max}^{CHCl_3}$ 2.78 and 2.93 μ (OH and NH).

Anal. Calcd. for $C_{27}H_{45}O_3N$: C, 75.13; H, 10.51. Found: C, 75.22; H, 10.68.

O,O,O,N-Tetraacetate of 7α,11α-Dihydroxytomatidine (IIa).—A mixture of 455 mg. of 7α,11α-dihydroxytomatidine, 5 ml. of pyridine, and 2.5 ml. of acetic anhydride was warmed on the oil bath at 70–80° for 20 min. until the solution became clear. It was then allowed to stand at room temperature overnight. After the addition of ice–water, the precipitate was filtered, washed with water, and dried. The resulting amorphous powder (626 mg.) was then chromatographed over alumina (Woelm neutral, activity I) but could not be induced to crystallize. The infrared spectrum of the acetate failed to show any hydroxyl absorption. The amorphous acetate possessed a rotation of +28° in chloroform.

Anal. Calcd. for $C_{35}H_{53}O_8N$: C, 68.26; H, 8.68. Found: C, 68.09; H, 8.91.

Isomerization of IIa to IIIa.—The aforementioned amorphous acetate (459 mg.) was added in small portions to 10 ml. of boiling

(12) Melting points were taken on the Kofler block and are uncorrected. Microanalyses were performed by the Institute's Analytical Service Laboratory under the direction of Mr. Harold G. McCann. The infrared spectra were taken on the Model 21 Perkin-Elmer infrared spectrometer by Mr. H. K. Miller and Mrs. Anne H. Wright of this laboratory.

acetic acid and refluxed for 15 min. The resinous residue, after removal of the solvent, was submitted to alumina chromatography but failed to crystallize.

16-Allopregnene-3β,7α,11α-triol-20-one Acetate (IVd).—A solution of chromic anhydride (210 mg.) in 10 ml. of 80% acetic acid was added dropwise over a period of 15 min. to a stirred solution of IIIa (534 mg.) in 30 ml. of acetic acid with attendant cooling (15°) of the reaction mixture. After the solution had been stirred for 1 hr. at room temperature, water (*ca.* 100 ml.), a small amount of sodium sulfite, and excess salt were added and the saturated mixture extracted with a solution of chloroform–ether (1:9). The residue from the extraction was dissolved in 50 ml. of acetic acid and refluxed for 2 hr. After removal of the acetic acid, the resulting semicrystalline residue crystallized from ether to give 169 mg. of plates, m.p. 180–225°, $[\alpha]^{20}D$ +43.8° ($CHCl_3$); $\lambda_{max}^{C_2H_5OH}$ 236 mμ (log ε 3.98); $\lambda_{max}^{CHCl_3}$ 5.77 (OAc), 5.99 and 6.25 μ (Δ^{16}-20-one). Several attempts at purification proved unsuccessful.

Allopregnane-3β,7α,11α-triol-20-one (Vb) and Its Acetate (Va), Allopregnane-3β,11α-diol-20-one (Vd) and Its Acetate (Vi), and Allopregnane-3,11,20-trione (Ve).—Compound IVd (97.4 mg.) was dissolved in 30 ml. of ethyl acetate with 200 mg. of 10% palladium on barium sulfate and hydrogenated at atmospheric pressure and room temperature. The hydrogen uptake came to about 1.6 moles before it ceased. The product (57.5 mg.) crystallized from ether–pentane to give prisms of m.p. 165–176°. Chromatography over alumina (Woelm neutral, activity I) afforded prisms of m.p. 177–180°, $[\alpha]^{20}D$ +53.4° ($CHCl_3$); $\lambda_{max}^{CS_2}$ 5.75 (OAc), 5.84 (20-ketone). The compound agreed in properties with an authentic specimen of Va.[6]

Anal. Calcd. for $C_{27}H_{40}O_7$: C, 68.04; H, 8.46. Found: C, 67.77; H, 8.50.

The alcohol Vb was prepared from the acetate Va by treatment with 1% methanolic potassium hydroxide. It crystallized as prisms from methanol–ether, m.p. 261–263°, $[\alpha]^{20}D$ +88.5° (C_2H_5OH); λ_{max}^{Nujol} 2.76 and 3.01 (OH), 5.72 μ (20-ketone).

Anal. Calcd. for $C_{21}H_{34}O_4$: C, 71.96; H, 9.78. Found: C, 71.73; H, 10.11.

The mother liquors, after removal of Va, were combined, hydrolyzed with 1% methanolic potassium hydroxide, and extracted with chloroform. The residue from the extraction was submitted to Florisil chromatography and the fractions eluted with 10% acetone in chloroform crystallized from ether. It yielded rods (2% based on IVd) of Vd, m.p. 186–191°; $\lambda_{max}^{CHCl_3}$ 2.76 and 2.89 (OH), 5.85 μ (20-ketone).

The allopregnanolone (Vd, 16.4 mg.) was acetylated with acetic anhydride–pyridine (1 hr. on steam bath). The diacetate Vi crystallized from acetone–pentane to give 6.4 mg. of crystals of m.p. 164–168°. The compound was identical in properties with an authentic specimen of Vi.[13,14]

A small sample of crude Vd was oxidized with chromic anhydride in acetic acid; the trione Ve crystallized from acetone and then from methanol as plates, m.p. 209–213°. The infrared spectrum of the trione was indistinguishable from an authentic compound of Ve.[6]

Allopregnane-3,7,11,20-tetrone (Vc).—Vb (40 mg.) in 1.5 ml. of acetic acid was oxidized with a solution of 30 mg. of chromium trioxide in 0.3 ml. of 80% acetic acid. Crystallization of the reaction product from acetone–pentane gave 26.9 mg. of plates, m.p. 243–246°, $[\alpha]^{20}D$ +52.5° ($CHCl_3$); $\lambda_{max}^{CHCl_3}$ 5.83 μ (ketone). The tetrone agreed in properties (melting point, mixture melting point, and infrared spectrum) with an authentic sample.[6]

3β-Acetoxyallopregnane-7,20-dione (Vf).—For conversion of IIB into Vf, the acetylation of IIB was conducted at room temperature for 15 hr. and the product without separation submitted to isomerization. At this stage, alumina chromatography (Woelm neutral, activity I) readily separated the products into O,O,N-triacetylpseudotomatidine (IIIc) and hydroxy-O,N-diacetylpseudotomatidine (IIIb). The former was eluted with 0.5% methanol in ether and the latter with 1% methanol in ether. Thus from the acetylation and isomerization of 191 mg. of IIB, 40 mg. of the triacetylpseudoderivative (IIIc) and 110 mg. of the hydroxy diacetylpseudoderivative (IIIb) were obtained. The latter (IIIb) crystallized as rods from aqueous methanol and melted at 88–91°.

Anal. Calcd. for $C_{31}H_{49}O_5N$: C, 72.19; H, 9.58. Found: C, 72.15; H, 9.83.

The pseudoderivative IIIb was oxidized and hydrolyzed in acetic acid in the manner described for IIIa → IVd. The 16-dehydroallopregnan-3β-ol-7,20-dione acetate (IVa) crystallized as plates from aqueous methanol, m.p. 203–208.5°, $[\alpha]^{20}D$ −56.9° ($CHCl_3$).

Anal. Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.35; H, 8.67.

The 16-dehydro derivative (IVa, 20 mg.) was reduced over 42 mg. of 10% palladium–barium sulfate. The product crystallized as needles from ether–hexane, m.p. 168–170°. It agreed in melting point, mixture melting point, and infrared spectrum with an authentic sample of Vf.[7]

O,O,N-Triacetate of 7α-Hydroxytomatidine (IIb).—7α-Hydroxytomatidine (500 mg.) was dissolved in a mixture of 10 ml. of pyridine and 5 ml. of acetic anhydride and warmed for 1 hr. on the steam bath. The crude resinous acetate (579 mg.) was chromatographed on alumina (Woelm neutral, activity III) and the fractions eluted with hexane and 10% benzene in hexane. IIb was crystallized from methanol–water and then from acetone–pentane to yield plates (194 mg.) of m.p. 186–188°, $[\alpha]^{20}D$ −29.8° ($CHCl_3$); $\lambda_{max}^{CHCl_3}$ 5.80 (OAc), 6.10 μ (NAc). A second crop (97 mg.) of crystals, m.p. 183–187°, also was obtained.

Anal. Calcd. for $C_{33}H_{51}O_6N$: C, 71.06; H, 9.22. Found: C, 70.77; H, 9.47.

16-Allopregnane-3β,7α-diol-20-one (IVb).—The aforementioned acetate (IIb, 184 mg.) was isomerized in 7 ml. of glacial acetic acid in the manner described previously for the preparation of IIIa. It crystallized from acetone–pentane to afford 139 mg. of needles of the so-called O,O,N-triacetylpseudotomatidine (IIIc) of m.p. 169–171°, $[\alpha]^{20}D$ −48.7° ($CHCl_3$); $\lambda_{max}^{CHCl_3}$ 2.89 (NH), 5.79 (OAc), 5.99 and 6.60 μ (HNAc).

Anal. Calcd. for $C_{33}H_{51}O_6N$: C, 71.06; H, 9.22. Found: C, 71.25; H, 9.51.

The isomerized product (IIIc, 125 mg.) was dissolved in 7 ml. of acetic acid and oxidized with 50 mg. of chromic anhydride in 1 ml. of 80% acetic acid. The oxidation product (see IVd for experimental details) was refluxed for 1.5 hr. in a mixture of 4.5 ml. of *t*-butyl alcohol, 0.5 g. of potassium hydroxide, and 0.5 ml. of water. The resinous reaction product (71.3 mg.) was submitted to Florisil chromatography. The 5% acetone in dichloromethane eluants afforded 18.3 mg. of rods of IVb from acetone which melted at 245–251°, $[\alpha]^{20}D$ +25.7° (C_2H_5OH); λ_{max}^{Nujol} 2.91 (OH), 6.04 and 6.31 μ (Δ^{16}-20-one).

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 76.05; H, 9.93.

Allopregnane-3β,7α-diol-20-one (Vg).—The aforementioned crude 16-dehydro derivative (57 mg.) was dissolved in 20 ml. of ethyl acetate and reduced in the presence of 21 mg. of 5% palladium on carbon. The crude hydrogenated product was chromatographed over Florisil and the fraction eluted with 5% acetone in chloroform crystallized from acetone as prisms, m.p. 208–211°, $[\alpha]^{20}D$ +83.6° (C_2H_5OH).

Anal. Calcd. for $C_{21}H_{34}O_3$: C, 75.40; H, 10.25. Found: C, 75.47; H, 10.55.

9α-Hydroxytomatidine O,N-Diacetate (IIc).—IIC (545 mg.) was dissolved in a mixture of 10 ml. of pyridine and 5 ml. of acetic anhydride and allowed to stand overnight at room temperature. The crude acetate (635 mg.) was chromatographed over alumina (Woelm neutral, activity I), and the 40% ether in benzene and 0.2% methanol in ether eluates worked up to yield 385 mg. of plates, m.p. 188–201°. Thin layer chromatography (silica gel G, cyclohexane–ethyl acetate, 1:1 v./v.) indicated the presence of a contaminant less polar than the main product. Repeated crystallizations from methanol–ether and then from methanol raised the melting point to 205–209°; the impurity could not be completely eliminated. Compound IIc showed a specific rotation of +4.2° ($CHCl_3$) and possessed infrared absorption bands in carbon disulfide at 2.77 (OH), 5.75 (OAc), and 6.01 μ (N-Ac).

Anal. Calcd. for $C_{31}H_{49}O_5N$: C, 72.19; H, 9.58. Found: C, 72.29; H, 9.78.

16-Allopregnene-3β,9α-diol-20-one (IVc).—IIc (221 mg.) in 5 ml. of acetic acid was isomerized (IIId) and oxidized in 12 ml. of acetic acid with 88 mg. of chromic anhydride in 4.5 ml. of 80% acetic acid in the manner described previously for IVd. The oxidized product (216 mg.) was then hydrolyzed for 2 hr. with a mixture of 9 ml. of *t*-butyl alcohol, 1 g. of potassium hydroxide,

(13) C. Djerassi, E. Batres, J. Romo, and G. Rosenkranz, *J. Am. Chem. Soc.*, **74**, 3634 (1952). We thank Dr. O. Halpern of Syntex, S.A., Mexico, for providing us with 5,16-pregnadiene-3β-11α-diol-20-one diacetate[14] from which Vi was prepared according to their directions.

(14) O. Halpern and C. Djerassi, *ibid.*, **81**, 439 (1959).

and 1 ml. of water. The resulting crude product (98 mg.) was submitted to chromatography over Florisil; the fractions eluted by 5% acetone in chloroform were crystallized from acetone–pentane and then from methanol to yield 33 mg. of prisms, m.p. 206–209°, $[\alpha]^{20}D$ +31.6° ($CHCl_3$); $\lambda_{max}^{CHCl_3}$ 2.79 and 2.91 (OH), 6.02 and 6.29 μ (Δ^{16}-20-one).

The **acetate IVc** of IVe, which was prepared in the usual manner, crystallized as needles from benzene–pentane, m.p. 181–184°, $[\alpha]^{20}D$ −19.5° ($CHCl_3$); λ_{max}^{Nujol} 2.86 (OH), 5.79 (OAc), 6.01 and 6.29 μ(Δ^{16}-20-one).

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.57; H, 9.33.

Allopregnane-3β,9α-diol-20-one Acetate (Vj).—The aforementioned 16-dehydro derivative (IVc, 36 mg.) was dissolved in 4 ml. of ethyl acetate with 32 mg. of 10% palladium–barium sulfate and hydrogenated at atmospheric pressure. Chromatography of the reduction product over alumina (Woelm neutral, activity II) and elution with benzene and 10% ether in benzene afforded a compound (29 mg.) which crystallized as plates, m.p. 187–191°, $[\alpha]^{20}D$ +58.2° ($CHCl_3$); $\lambda_{max}^{CS_2}$ 2.78 and 2.83 (OH), 5.76 (OAc), 5.85 μ (20-ketone).

The substance was identical in properties (melting point, mixture melting point, and infrared spectrum) with an authentic specimen of Vj prepared from 9α-hydroxysolasodine.[3a]

Reactions of Mercaptoamines. II. With Chloroformates and Chlorothiolformates[1]

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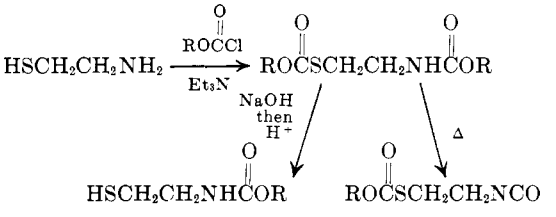
2-Mercaptoethylamine reacted with alkyl chloroformates and chlorothiolformates to give the N,S-disubstituted products, which on basic hydrolysis gave 2-(mercaptoethyl)carbamates in the first case and regenerated 2-mercaptoethylamine in the second. On pyrolysis or treatment with silver nitrate, the S-alkyl N-[2-(alkyldithiolcarbonato)ethyl]thiolcarbamates gave the 2-(alkyldithiolcarbonato)ethyl isocyanates. These reacted with amines to give the corresponding ureas, hydrolyzable to 2-mercaptoethyl ureas.

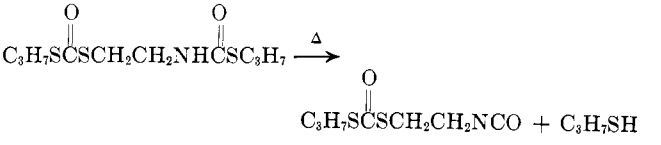
In continuation of studies of the reaction of mercaptoamines with compounds potentially capable of reacting at both the mercapto and amino functions,[2] the reaction of 2-mercaptoethylamine (MEA) with chloroformates and chlorothiolformates has been examined.

In the presence of triethylamine as an acid acceptor, solutions of 2-mercaptoethylamine in dry acetonitrile reacted with ethyl, *n*-propyl, *n*-butyl, and *n*-hexyl chloroformates to give good yields of the N,S-disubstituted products, the O-alkyl N-[2-(alkylthiolcarbonato)ethyl]carbamates. The ethyl and *n*-propyl



compounds could be distilled at reduced pressure, but the higher molecular weight compounds underwent partial pyrolysis when distillation was attempted, giving products with isocyanate absorption in their infrared spectra. The critical temperature for the onset of pyrolysis appeared to be about 150°. All the N,S-disubstituted compounds were hydrolyzed readily at the thiolcarbonato function by sodium hydroxide in aqueous alcohol to give the 2-(mercaptoethyl)carbamates in good yield. The properties of the N,S-disubstituted compounds are reported in Table I and those of the 2-(mercaptoethyl)carbamates in Table II.

Alkyl chlorothiolformates reacted with 2-mercaptoethylamine in the same manner as the chloroformates to give N,S-disubstituted products (Table I). The compounds from methyl and ethyl chlorothiolformate were solids which could be purified by recrystallization, but the product from *n*-propyl chlorothiolformate was a liquid. Like the higher molecular weight chloroformate products, it underwent partial pyrolysis when distillation was attempted. Several repetitions of the distillation completed the pyrolysis, and eventually pure 2-(*n*-propyldithiolcarbonato)ethyl isocyanate was obtained in 30% yield. A number of attempts were



made to hydrolyze the S-alkyl N-[2-(alkyldithiolcarbonato)ethyl]thiolcarbamates to the corresponding 2-(mercaptoethyl)thiolcarbamates, but hydrolysis occurred as readily at the thiolcarbamate function as at the thiolcarbonato function, and it proved impossible to isolate the half-hydrolyzed products.

It is readily apparent that a convenient intermediate for the preparation of 2-mercaptoethylureas would be an S-substituted 2-mercaptoethyl isocyanate wherein the mercapto function was covered by a group easily removed after urea formation. As noted before, pyrolysis of the S-alkyl N-[2-(alkyldithiolcarbonato)-ethyl]thiolcarbamates provides one route to such an intermediate. A more convenient route is offered by the metal ion-assisted mercaptan elimination reaction.[3] Treatment of S-methyl N-[2-(methyldithiolcarbonato)-ethyl]thiolcarbamate with a solution of silver nitrate in acetonitrile gave a solution of 2-(methyldithiolcarbonato)ethyl isocyanate which reacted with water to give 1,3-bis[2-(methyldithiolcarbonato)ethyl]urea and with amines to give 2-(methyldithiolcarbonato)-ethylureas (Table III). Successful reaction was obtained with aniline, *t*-butylamine, *n*-octylamine, morpholine, and diethyl iminodiacetate. All the products from primary amines, including 1,3-bis[2-(methyldithiolcarbonato)ethyl]urea, underwent basis hydrolysis to give the corresponding 2-mercaptoethylureas. The secondary amine products were completely de-

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(2) Previous paper in this series: A. F. Ferris and B. A. Schutz, *J. Org. Chem.*, **28**, 3140 (1963).

(3) A. F. Ferris and B. A. Schutz, *ibid.*, **28**, 71 (1963)