The **perchlorate** crystallized from methanol as orange needles, m.p. 291–292° dec. (sealed tube).

*Anal.* Calcd. for $C_{20}H_{18}ClNO_8$: C, 54.00; H, 4.30; N, 3.15; $OCH_3$, 22.36. Found: C, 53.75; H, 4.64; N, 3.33; $OCH_3$, 21.83.

**10-Hydroxy-2,3,9-trimethoxydibenzo[*a*,*g*]quinolizidine (I).**—A suspension of 0.2 g. of 10-hydroxy-2,3,9-trimethoxybenz[*a*]acridizinium bromide (XIII) in 100 ml. of ethanol was hydrogenated in the presence of platinum oxide (0.03 g.) for 72 hr. The colorless solution was filtered and concentrated under reduced pressure. The residue was treated with a dilute solution of ammonium hydroxide and extracted with chloroform. The residue obtained by evaporation of the chloroform was crystallized from methanol in colorless prisms, m.p. 187.5–188.5°.

*Anal.* Calcd. for $C_{20}H_{23}NO_4$: C, 70.38; H, 6.74; N, 4.10. Found: C, 70.58; H, 6.51; N, 4.15.

This compound (I) gave evidence of complete homogeneity when chromatographed on alumina plates with benzene–methanol, ether–methanol, and methanol. The melting point was unchanged by vacuum sublimation.

**2,3,9,10-Tetramethoxydibenzo[*a*,*g*]quinolizidine (II, Tetrahydropalmatine).**—A solution of 10-hydroxy-2,3,9-trimethoxybenz[*a*,*g*]quinolizidine (0.2 g.) in 10 ml. of dry methanol was mixed with an excess of diazomethane in dry ether (100 ml.) and the mixture was allowed to remain at refrigerator temperature for 7 days and then was allowed to evaporate at room temperature. The residue crystallized from methanol as colorless needles, m.p. 148° (lit.[11] m.p. 147°), and was shown to be identical with an authentic sample (no mixture melting point depression).

(11) R. D. Haworth, J. B. Koepfli, and W. H. Perkin, Jr., *J. Chem. Soc.*, 548 (1927).

# Structure of Tomatillidine

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Tomatillidine (I) subjected to Wolff–Kishner reduction gave deoxotomatillidine (VII), which afforded dihydrodeoxotomatillidine (VIII) by catalytic hydrogenation. VIII was converted into O,N-diacetyl-$\Delta^{22}$-dihydrodeoxotomatillidine (X) by treatment with acetic anhydride, then into 3$\beta$-acetoxy-26-acetylaminocholestan-22-one (XII), by acid hydrolysis. The latter (XII) yielded 3$\beta$-acetoxybisnorallocholanic acid (XIV) by chromic acid oxidation. Selenium dehydrogenation of deoxotomatillidine (VII) afforded a 2,5-disubstituted pyridine derivative which aided in the elucidation of the structure of the basic moiety of the molecule. Mass spectral patterns of hexahydrotomatillidine (III), tetrahydrodeoxotomatillidine (XV), deoxotomatillidine (VII), tomatillidine (I), its O-acetyl derivative (Ia), and dihydrotomatillidine (II) and spectral data (ultraviolet, infrared, and n.m.r.) of O,N-diacetyltomatillidine (VI) showed the location of the carbonyl group.

Solasodine and two new alkaloids, tomatillidine (I)[2] and dihydrotomatillidine (II), have been isolated from *Solanum tomatillo* (Philippi) of Chilean botanical origin. When the crude extract of leaves from old plants of *S. tomatillo* (Philippi) collected in March–April (beginning of fall in the southern hemisphere) was chromatographed, a small amount (0.02%) of crude tomatillidine followed by the major component solasodine in yields of 1.3%, was obtained. Further purification of the crude fraction of tomatillidine by gradient elution chromatography and fractional recrystallization afforded dihydrotomatillidine (*ca.* 15–20%, based on crude tomatillidine). From leaves of young plants collected in December (beginning of summer), the yields of dihydrotomatillidine were much higher (*ca.* 80%, based on crude tomatillidine), although the total alkaloidal content was somewhat lower.

From consideration of its empirical formula, $C_{27}H_{41}NO_2$, and the fact that it accompanied solasodine,[3] a well-known steroidal alkaloid, it was suspected that tomatillidine (I) was steroidal in nature. Of the two oxygen functions in tomatillidine, one was shown to be alcoholic by its conversion to O-acetyltomatillidine (Ia) in the usual manner (acetic anhydride–pyridine). Alkaline hydrolysis of Ia readily regenerated the parent compound. A double bond, whose presence was revealed in the n.m.r. spectrum of tomatillidine (olefinic proton, 5.35 p.p.m.), was found to be located in the proximity of the alcoholic function by Oppenauer oxidation which converted I into an $\alpha,\beta$-unsaturated oxo derivative V, $\lambda_{max}^{C_2H_5OH}$ 240 m$\mu$ (log $\epsilon$ 4.24). The same compound V was also obtained by isomerizing the product IV of the Kiliani[4] oxidation of tomatillidine with methanolic alkali.[5] The ketonic nature of the second oxygen atom in tomatillidine was indicated by its characteristic low ultraviolet absorption at about 285 m$\mu$ and verified by the formation of a semicarbazone.[6a] The rotatory dispersion curve of tomatillidine displayed a positive Cotton effect but the shift of the peak from the normal[6b] toward the shorter wave length pointed to the presence of an external disturbing factor in its environment. The failure of tomatillidine to form the N-acetyl derivative under normal acetylating conditions, along with the infrared absorption band in tomatillidine at 6.18 $\mu$ of medium intensity, indicated that the nitrogen atom in all probability was present as a part of a C=N group.

In order to explore the compound more fully without the ketonic function, tomatillidine was submitted to Wolff–Kishner reduction (Huang-Minlon modification).[7] The resulting deoxotomatillidine (VII, mol. wt. 397, mass spectrum) possessed an infrared absorption band at 6.04 $\mu$ ascribable to a >C=N– moiety

(1) (a) Appointment supported by International Cooperation Administration under the visiting research scientist program administered by the National Academy of Sciences of the United States of America. Guest investigator at Stanford University, 1962–1963, and at the National Institutes of Health, 1963–1964. (b) To whom inquiries should be made at College of Pharmacy, University of Arizona, Tucson, Ariz. (c) National Institute of Arthritis and Metabolic Diseases.

(2) E. Bianchi, F. Diaz, and J. A. Garbarino, *Gazz. chim. ital.*, **90**, 894 (1960).

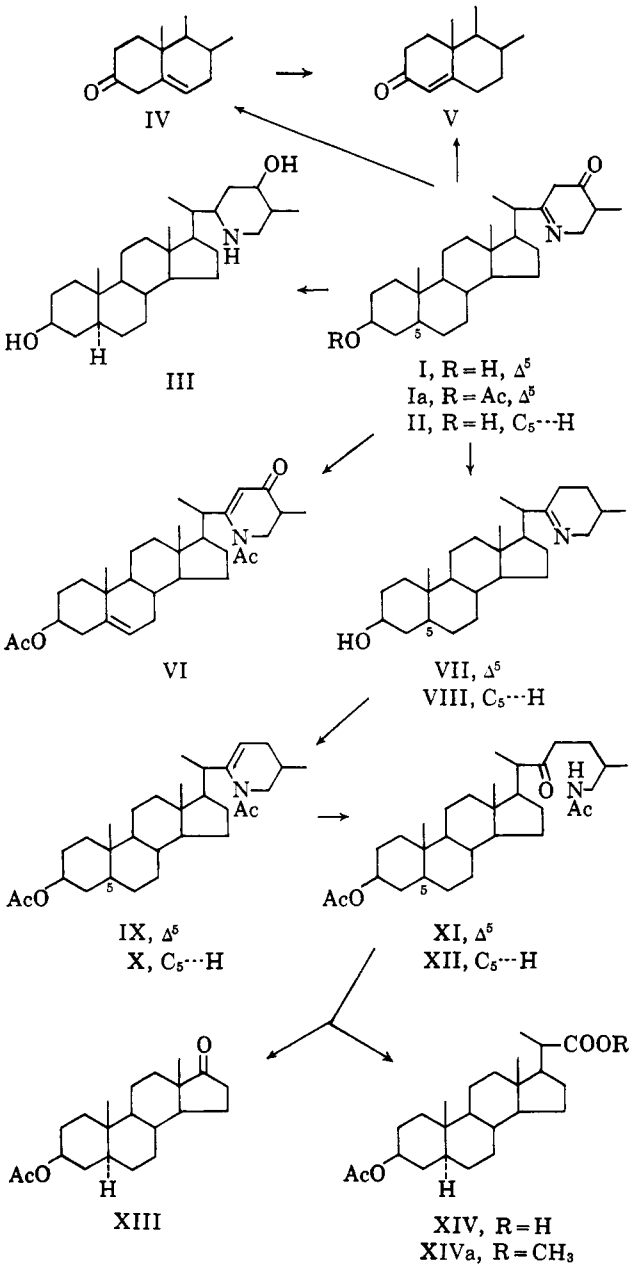
(3) L. Briggs, W. E. Harvey, R. H. Locker, W. A. McGillivray, and R. N. Seelye, *J. Chem. Soc.*, 3013 (1950).

(4) H. Kiliani, *Ber.*, **46**, 676 (1913).

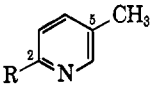
(5) C. Djerassi, R. R. Engle, and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956).

(6) (a) W. Dirscherl and H. Nahm, *Ber.*, **76**, 710 (1943); (b) C. Djerassi, "Optical Rotary Dispersion," McGraw-Hill Book Co., Inc., New York, N. Y., 1960, Chapter 4.

(7) Huang-Minlon, *J. Am. Chem. Soc.*, **71**, 3301 (1949).

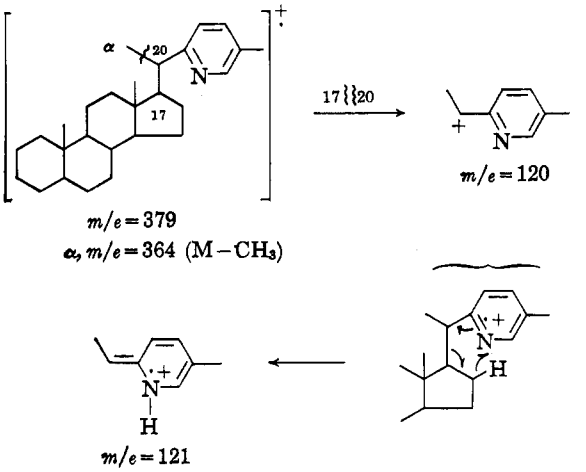



whose rotatory dispersion curve also agreed with the rotary dispersion curve of a >C=N- chromophore.[8] Hydrogenation of the deoxo derivative VII with a palladium–charcoal catalyst in acetic acid readily afforded dihydrodeoxoto natillidine (VIII) with the absorption of 1 mole of hydrogen. Under these conditions only the $\Delta^5$-bond was saturated, since the n.m.r. spectrum of VIII was devoid of any signal for an olefinic proton. As was evident from the infrared and rotatory dispersion measurements, the C=N chromophore remained intact. Additional information on the nature of the side chain was observed from the selenium dehydrogenation of deoxotomatillidine (VII). The neutral fraction, unfortunately, yielded no identifiable product but the basic fraction afforded a crystalline compound which possessed ultraviolet absorption bands at 268 mμ (log ε 3.66) and 277 mμ (log ε 3.50) in good agreement with the absorption bands of 2-methyl-5-ethylpyridine or 2,5-dimethylpyridine.[9] Comparison between n.m.r. spectra of the two 2,5-substituted pyridine and the isolated base show identical chemical shifts and splitting patterns for the protons on the aromatic ring. Furthermore, the signal of the methyl group at C-5 (2.3 p.p.m.) was in exact agreement with the methyl signal in 2,5-dimethylpyridine. From these data, we can assume that the basic portion of the product of selenium dehydrogenation bears the following structure.

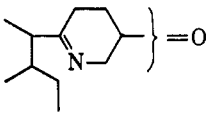


R = steroidal moiety

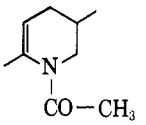
This observation was confirmed by the information obtained from the patterns of the mass spectrum of the base which showed peaks at $m/e$ = 379, 364, 120, and 121 corresponding to the following parent ion and fragments.




Further proof for the side-chain structure of tomatillidine (I) was afforded by the conversion of deoxoto-



matillidine into the O,N-diacetyldeoxo derivative IX by treatment with acetic anhydride. The compound IX showed an ultraviolet absorption band at 235 mμ (log ε 3.90) and infrared bands at 5.78 (3-OAc), 5.99, and 6.09 μ (C=C–N–Ac) in very good agreement for an α,β-unsaturated aminoacetyl system.[10] The n.m.r.



spectrum revealed two olefinic protons at 5.35 and 5.05 p.p.m., the former ascribed to the C-6 olefinic proton and the latter to the C-23 olefinic proton. The parent compound, deoxotomatillidine (VII), has only a single C-6 olefinic proton at 5.35 p.p.m.

(8) We are indebted to Professor W. Klyne, Department of Chemistry, Westfield College, London, who confirmed the presence of the >C=N- by the optical rotatory dispersion studies of this compound.

(9) J. P. Phillips and F. C. Nachod, "Organic Electronic Spectra Data," Vol. IV, Interscience Publishers, Inc., New York, N. Y., 1958–1959.

(10) Y. Sato, H. G. Lathan, and N. Ikekawa, *J. Org. Chem.*, **25**, 1962 (1960).

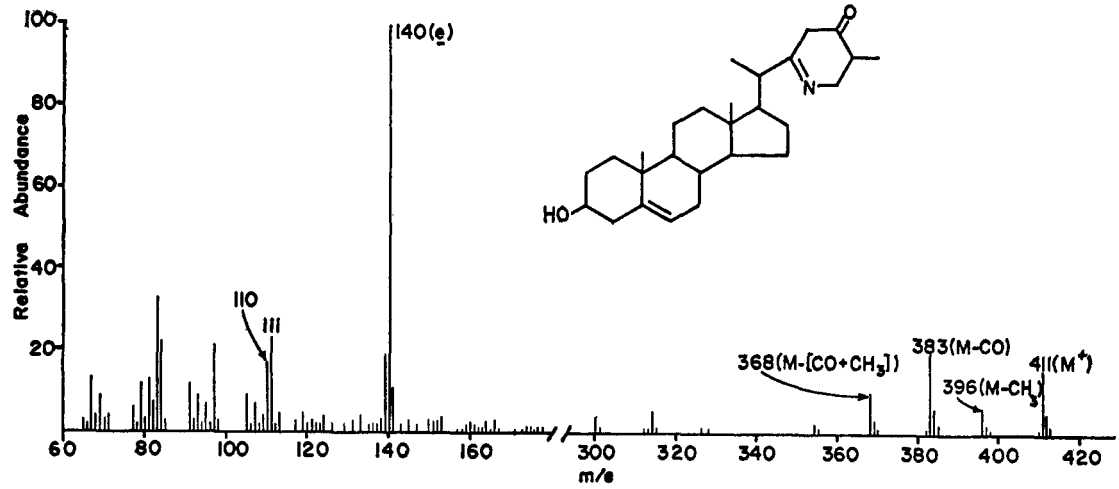
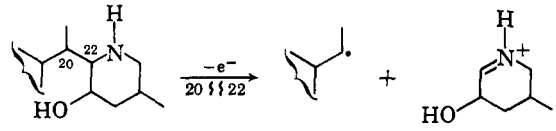


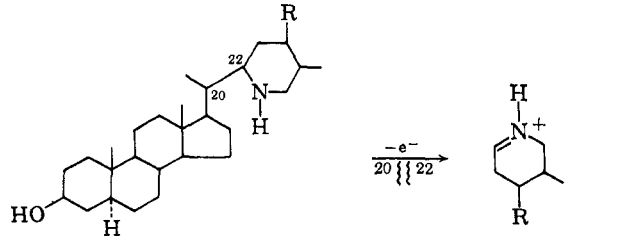

Figure 1.—The mass spectrum of tomatillidine (I).

That the carbonyl chromophore is located in the side chain and in the γ-position with respect to the nitrogen atom was inferred from mass spectral patterns of tetrahydrodeoxotomatillidine (XV), hexahydrotomatillidine (III), deoxotomatillidine (VII), tomatillidine (I), its O-acetyl derivative (Ia), and dihydrotomatillidine (II), and from the ultraviolet absorption and n.m.r. spectra of O,N-diacetyltomatillidine (VI), prepared by treatment of tomatillidine with acetic anhydride. It has been shown[11] that steroidal alkaloids characterized by the presence of a piperidine ring linked to C-20 of the steroidal side chain (*e.g.*, veratramine or isojervine) form their main cleavage ion by rupture of the 20,22-bond.




*m/e* = 114

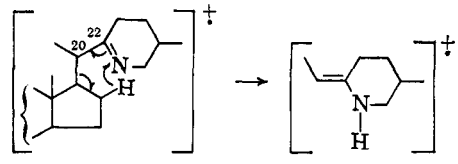
Tetrahydrodeoxotomatillidine (XV) shows in the molecular region of its mass spectrum both an M − 1 and an M − $CH_3$ species in addition to the molecular ion (*m/e* = 401). By far the most abundant ion, however, occurs at *m/e* = 98 (**a**), formed in the expected manner by α-cleavage of the 20,22-bond. For hexahydrotomatillidine (III), in which the oxo function of tomatillidine (I) has been reduced to a hydroxyl group, the main fragment occurs at *m/e* = 114 (**b**), the increment of 16 mass units over **a** corresponding to an additional hydroxyl group. This finding confirms that the second oxygen function of tomatillidine (I) must be located in the piperidine ring.




III, R = OH
XV, R = H

**a**, R = H; *m/e* = 98
**b**, R = OH; *m/e* = 114

The presence of the C-22,N double bond, as in deoxotomatillidine (VII), makes rupture of the 20,22-bond an energetically unfavorable process (cleavage of a bond next to a double bond) and the principal fragmentation takes place as expected in a typical McLafferty rearrangement, *i.e.*, β-cleavage accompanied by γ-hydrogen transfer to yield **c** (*m/e* = 125).




VII

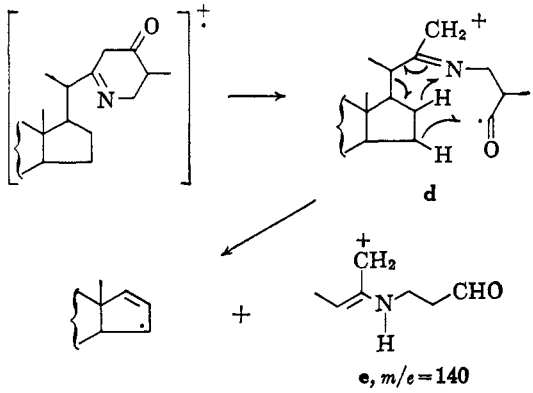
**c**, *m/e* = 125

The mass spectrum of tomatillidine (I) (Figure 1) itself exhibits an anomalous feature, since the most abundant cleavage ion does not occur at *m/e* = 139 (**c** with an additional oxo group), but rather at *m/e* = 140. A more detailed discussion of this compound is therefore warranted.[12] The spectra of tomatillidine (I), its dihydro product (II), and the O-acetyl derivative (Ia) exhibit fragments in the molecular-ion region which are due to the loss of a methyl group and carbon monoxide (as established by high resolution measurements), as well as 43 mass units ($CH_3$ + CO). In all cases the principal fragment shows a mass of *m/e* = 140 and high resolution data establish its composition as $C_8H_{14}NO$, clearly indicating that it comprises the piperidone moiety. In addition to the transfer of one hydrogen atom in the McLafferty rearrangement (see VII → **c**) during the formation of the *m/e* = 140 ion, a second hydrogen radical must migrate to the charged species. A possible explanation for this behavior is the assumption that the first fragmentation step is breakage of the 23,24-bond, giving an allylic ion and a carbonyl radical **d** which, either prior to or during the rearrangement step, stabilizes itself by abstraction of a hydrogen radical from the steroid nucleus. The process may be tentatively depicted as in-

(11) (a) H. Budzikiewicz, *Tetrahedron*, **20**, 2267 (1964); (b) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, Inc., San Francisco, Calif., 1964, Chapter 1.

(12) It has been noticed also with other alkaloids that introduction of a keto group close to the centers of fragmentation can cause deviation from the normal breakdown routes (*cf.* ref. 11b, Vol. I, pp. 87, 109, 123, 124, and 136).

dicated below, but it should be kept in mind that labeling experiments would be necessary to establish rigorously the migrating hydrogen atoms. Minor peaks at $m/e$ = 110 and 111 in the spectrum of I (Figure 1) could be shown to be doublets: $m/e$ = 110 being $C_7H_{12}N$ (principally) and $C_6H_8NO$ (formed probably by the loss of $CH_2O$ and $C_2H_6$ from **e**, respectively) and $C_7H_{13}N$ (principally) and $C_6H_9NO$ (loss of a formyl and an ethyl radical from **e**, respectively).

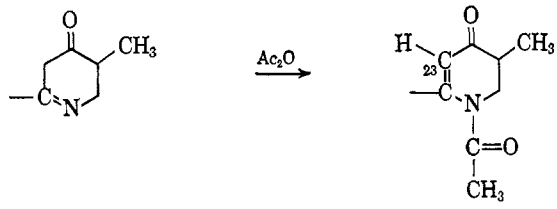


**d**

**e**, $m/e$ = 140

Tomatillidine (I) and its derivatives constitute additional examples for the strong fragmentation-directing effect of the nitrogen atom in steroidal alkaloids[11] which leads to predominant fragmentation in the neighborhood of this hetero atom, irrespective of substitutional alterations in the steroid skeleton. Mass spectrometry is thus demonstrated to be a rather powerful tool for the recognition of structural types in this class.

In addition the ultraviolet absorption spectrum, $\lambda_{max}$ 222 mμ (log ε 3.50) and 275 mμ (log ε 3.35), of VI indicated that the following change had taken place. Observance of two olefinic protons in the n.m.r. spectrum (C-6 proton, 5.40 p.p.m.; C-23 proton, 6.05 p.p.m.) supports the above observation.



Finally, an unambiguous proof of the structure of deoxotomatillidine (VII) was obtained through its degradative conversion into 3β-acetoxybisnorallocholanic acid (XIV). For this purpose deoxotomatillidine (VII) was catalytically reduced to the 5,6-dihydrodeoxo derivative VIII, and thence converted into O,N-diacetyldihydro-$\Delta^{22}$-deoxotomatillidine (X) by refluxing with acetic anhydride. Submission of compound X to hydrolysis in an acetic–hydrochloric acid mixture resulted in the cleavage of the nitrogenous ring[13] to form 3β-acetoxy-26-acetylamino-5α-cholestan-22-one (XII). After several attempts at cleavage of the molecule, it was found that oxidation with chromic acid at 63–65° for 4 hr. resulted in the cleavage of the nitrogen-bearing side chain to afford the known 3β-acetoxybisnorallocholanic acid (XIV).[14] In addition, 3β-acetoxyandrostan-17-one (XIII),[15] was isolated from the neutral portion of the oxidation mixture. The isolation of XIII is not unexpected in an oxidation of this type. The formation of the acetoxyacetylaminocholestanone (XII) by hydrolysis of X and its subsequent degradation to XIV clearly establishes the structure of deoxotomatillidine as VII and I as the structure of tomatillidine. In the course of these investigations, tomatillidine (I) was subjected to hydrogenation under varying conditions. Efforts to obtain a preferential reduction of one chromophore (C=O or C=N) over the other were unsuccessful and only 5,6-dihydrotomatillidine (II) and the completely hydrogenated hexahydro derivative III were obtained. The 5,6-dihydro derivative II obtained by the catalytic reduction (palladium–charcoal, acetic acid) of tomatillidine proved to be identical with dihydrotomatillidine of natural origin isolated from the plant by gradient elution chromatography.

(13) Y. Sato and N. Ikekawa, *J. Org. Chem.*, **25**, 786 (1960).

(14) E. Fernholz, *Ann.*, **507**, 128 (1933).

## Experimental[16]

**Separation of Tomatillidine (I) from 5,6-Dihydrotomatillidine (II).**—Crude tomatillidine (3 g.) was dissolved in hot dichloromethane and then hexane was added until the ratio 2:1 (dichloromethane–hexane) was reached. The crystalline precipitate, obtained after standing overnight in the icebox, was recrystallized in the above manner three more times and finally from acetone to afford 1.69 g. of I, m.p. 219–222°. When the compound was checked on thin layer chromatography (benzene–ethyl acetate, 4:1), it showed a violet-brown spot when the plate was sprayed with 2% cerium sulfate solution in sulfuric acid (2 *N*). The mother liquor yielded a second crop of crystals (1.17 g.) which showed by t.l.c. a partially violet-brown and yellow spot. This material was subjected to gradient elution chromatography on a column of 115 g. of alumina (grade III) with the following eluents: hexane, followed progressively and continuously with mixtures of dichloromethane–hexane, 1:4 and 1:2. The dichloromethane–hexane eluate (1:2) afforded 173 mg. of II, m.p. 179–181°, when recrystallized from acetone; t.l.c., yellow spot. Then 238 mg. of material richer in II, followed by 447 mg. of material richer in I, were obtained. The latter, after four crystallizations from dichloromethane–hexane, yielded an additional amount of 220 mg. of I. The material obtained by evaporation of this mother liquor (220 mg.) was joined with the 238 mg. of material richer in II and subjected to a second gradient elution chromatography. This afforded 97 mg. more of II. Over-all, from 3.0 g. of crude tomatillidine, 1.89 g. of I and 270 mg. of II were obtained. On this basis the content of 5,6-dihydrotomatillidine (II) from crude tomatillidine from old plants of *S. tomatillo* amounts to about 15%. In the material obtained from young plants the quantities are reversed, *i.e.*, 15% of tomatillidine (I).

**Tomatillidine** (I) had the following properties: m.p. 219–222°; $[\alpha]D$ −18.1° ($CHCl_3$); $\lambda_{max}^{KBr}$ 5.98 μ (C=N and CO); n.m.r. *t*-$CH_3$, 0.64 p.p.m.; *t*- and *sec*-$CH_3$, 0.96 p.p.m.; *sec*-$CH_3$, 1.08 p.p.m.; one olefinic proton, 5.35 p.p.m.; (pyridine addition) two *t*-$CH_3$, 0.75 and 0.99 p.p.m.; two *sec*-$CH_3$, 0.90 ($J$ = 5 c.p.s.) and 1.12 p.p.m. ($J$ = 6 c.p.s.); and one olefinic proton, 5.35 p.p.m.; O.R.D. in methanol (*c* 0.12) $[\alpha]_{290}$ +366°

(15) A. Butenandt, *Z. Physiol. Chem.*, **234**, 223 (1935).

(16) Melting points were taken on a Kofler block and are uncorrected. All column chromatograms were carried out with Woelm alumina. For thin layer chromatography, silica gel G, Merck, was used. Microanalyses were performed by the Microanalytical Laboratory of the National Institutes of Health under the direction of Dr. W. C. Alford and by Mr. E. Meier of Stanford University. The infrared spectra were determined with a Perkin-Elmer Model 21 spectrophotometer by Mr. H. K. Miller and Mrs. A. H. Wright of National Institutes of Health. The n.m.r. spectra were determined on the Model A-60 Varian Associates spectrophotometer by Mrs. J. A. Goodwin of National Institutes of Health and Dr. L. J. Durham (Stanford University). The spectra were determined in deuteriochloroform with tetramethylsilane as internal standard. The mass spectra in these experiments have been measured at Stanford partly with a CEC-21-103C mass spectrometer and partly with an Atlas CH-4 instrument both of which were equipped with direct inlet systems; the high resolution spectra, with an AEI MS-9 instrument.

and $[\alpha]_{240}$ −666°; in dioxane (*c* 0.129), $[\alpha]_{405}$ −48°, $[\alpha]_{392}$ −15°, $[\alpha]_{375}$ −48°, $[\alpha]_{360}$ −3°, $[\alpha]_{369}$ −18°, $[\alpha]_{352}$ +42°, $[\alpha]_{348}$ +33°, and $[\alpha]_{298}$ +446°; and mass spectrum, mol. wt., 411.

*Anal.* Calcd. for $C_{27}H_{41}NO_2$: C, 78.78; H, 10.04. Found: C, 78.89; H, 10.03.

**5,6-Dihydromatillidine (II)** had t.l.c. (benzene–ethyl acetate, 4:1), yellow spot; m.p. 179–181°; $[\alpha]^{20}D$ +21.4° ($CHCl_3$); $\lambda_{max}^{KBr}$ 5.95 μ (C=N and CO); n.m.r. two *t*-$CH_3$, 0.62 and 0.75 p.p.m.; two *sec*-$CH_3$, 0.96 and 1.08 p.p.m.; and no olefinic protons; O.R.D., in dioxane (*c* 0.159), $[\alpha]_{589}$ +124°, $[\alpha]_{410}$ +430°, $[\alpha]_{400}$ +400°, $[\alpha]_{385}$ +550°, $[\alpha]_{380}$ +525°, $[\alpha]_{362}$ +880°, $[\alpha]_{355}$ +750°, $[\alpha]_{298}$ +2900°, and $[\alpha]_{255}$ −828°; and mass spectrum, mol. wt., 413.

*Anal.* Calcd. for $C_{27}H_{43}NO_2$: C, 78.40; H, 10.48. Found: C, 78.70; H, 10.73.

**II by Partial Hydrogenation of Tomatillidine (I).**—Tomatillidine (I, 100 mg.) was dissolved in 4 ml. of glacial acetic acid and hydrogenated over 100 mg. of palladium–charcoal (10%) catalyst under atmospheric pressure. After about 8 hr., the absorption of gas ceased with the uptake of 1.8 mole equiv. of hydrogen. The filtrate, after removal of the catalyst, was poured into ice–water, basified with a saturated solution of sodium carbonate, and saturated with sodium chloride. The mixture was extracted with dichloromethane. After partial evaporation of the solvent, the residue was absorbed onto 1.0 g. of deactivated alumina (with methanol) and placed on 10 g. of alumina (grade III) for chromatography. From the benzene eluate, 15 mg. of 5,6-dihydrotomatillidine was obtained which, when crystallized from methanol–acetone melted at 179–181°. It was identical (mixture melting point and infrared spectrum) with a specimen of II from natural sources. From the dichloromethane eluate a few milligrams of an unidentified product (m.p. 160–169°) was obtained. Finally, from the chloroform eluate, 16 mg. of hexahydrotomatillidine (III), m.p. 237–239°, was obtained. The melting point and the infrared spectrum (KBr) coincided with a specimen obtained from the platinum oxide hydrogenation of I.

**O-Acetyltomatillidine (Ia).**—Acetic anhydride (1 ml.) was added to a solution of 64 mg. of tomatillidine (I) in 2 ml. of anhydrous pyridine (refluxed on barium oxide) and allowed to stand at room temperature for 5 hr. The reaction mixture, worked up in the conventional way, yielded 50 mg. of O-acetyltomatillidine (Ia): m.p. 141–144° (from acetone); t.l.c., benzene–ethyl acetate (9:1); $[\alpha]^{20}D$ +9.6° ($CHCl_3$); $\lambda_{max}^{CHCl_3}$ 5.95 μ (C=N and CO).

*Anal.* Calcd. for $C_{29}H_{43}NO_3$: C, 76.80; H, 9.47. Found: C, 76.71; H, 9.76.

**I by Hydrolysis of Ia.**—To a solution of Ia in methanol, a few drops of an aqueous methanolic solution of potassium carbonate was added, and the mixture was allowed to stand. After 2 hr. at room temperature the mixture was worked up in the conventional way, and afforded I.

**Tomatillidine Semicarbazone.**[6]—A solution of 20 mg. of tomatillidine (I) in 0.2 ml. of dry pyridine was added to 15 mg. of semicarbazide hydrochloride dissolved in 1 drop of water. After warming on the steam bath until complete dissolution, the mixture was allowed to stand at room temperature for 30 min. It was then poured into water, saturated with sodium chloride, and allowed to stand overnight. The filtered product was crystallized from acetone–methanol and afforded 10 mg. of the crystalline tomatillidine semicarbazone: m.p. 239–240°; t.l.c., ethyl acetate–acetone (1:1).

*Anal.* Calcd. for $C_{28}H_{44}N_4O_2$: C, 71.75; H, 9.46; N, 11.96. Found: C, 71.79; H, 9.63; N, 12.09.

**3-Ketotomatillidine (IV).**—To a stirred cold solution (8–9°) of 266 mg. of tomatillidine (I) in 40 ml. of acetone (distilled over potassium permanganate) was added dropwise, a solution of Kiliani's reagent[17] until the mixture turned slightly orange. After 5 min., a few drops of isopropyl alcohol, some sodium hydrogen carbonate, and anhydrous magnesium sulfate were added. The liquid was then filtered through a sintered-glass filter and evaporated at reduced pressure. The residue, dissolved in benzene and chromatographed on alumina (grade IV; eluent, benzene), yielded 102 mg. of 3-ketotomatillidine (IV): m.p. 169–172°; t.l.c., benzene–ethyl acetate (9:1); $[\alpha]^{20}D$ +17.4° ($CHCl_3$); $\lambda_{max}^{KBr}$ 5.85 (CO at C-3) and 5.95 μ (C=N and CO).

(17) Standard chromium trioxide reagent: a solution of 26.72 g. of chromium trioxide in 23 ml. of concentrated sulfuric acid diluted with water to a volume of 100 ml.

*Anal.* Calcd. for $C_{27}H_{39}NO_2$: C, 79.37; H, 9.53. Found: C, 79.27; H, 9.68.

**3-Keto-$\Delta^4$-tomatillidine (V).**[5] **Method A.**—3-Ketotomatillidine (IV, 40 mg.) was suspended in 2.5 ml. of hot methanol and 1 drop of 10% methanolic potassium hydroxide solution, and heated on the steam bath for 5 min. Then it was poured into water, neutralized with dilute acetic acid, and extracted with ether. After evaporation of the solvent, the crude residue was purified by chromatography on alumina (grade II; eluent, benzene) and yielded 9 mg. of 3-keto-$\Delta^4$-tomatillidine (V): m.p. 142–144.5°; t.l.c., benzene–ethyl acetate (9:1); $[\alpha]^{20}D$ +99.03° ($CHCl_3$); $\lambda_{max}^{KBr}$ 5.95 (C=N and CO at C-24) and 6.02 μ (conjugated CO at C-3); and $\lambda_{max}^{C_2H_5OH}$ 240 mμ (log ε 4.25).

*Anal.* Calcd. for $C_{27}H_{39}NO_2$: C, 79.37; H, 9.53. Found: C, 79.22; H, 9.77.

**Method B. By Oppenauer Oxidation of Tomatillidine (I).**—Tomatillidine (I, 200 mg.) was dissolved in a mixture of toluene (9 ml.) and cyclohexanone (1.8 ml.) and the solution was heated to boiling. A solution of 100 mg. of aluminum isopropoxide in 0.9 ml. of toluene was added. The mixture was refluxed for 60 min. Then it was poured into water and basified with dilute sodium hydroxide. The organic layer was separated and the aqueous liquid was extracted with ether. The combined organic extracts, after evaporation, yielded a residue. This residue was purified by chromatography (grade II; eluent, benzene). It was crystallized from methanol and afforded 80 mg. of 3-keto-$\Delta^4$-tomatillidine (V), m.p. 143–144°. A mixture melting point and the infrared (KBr) and ultraviolet spectra were compatible with the product obtained by isomerization of 3-ketotomatillidine (IV).

**O,N-Diacetyl-$\Delta^{22}$-tomatillidine (VI).**—A solution of 100 mg. of tomatillidine (I) in 5–6 ml. of acetic anhydride was refluxed for 75 min. in a nitrogen atmosphere. The reaction mixture was poured into excess ice–water, basified with ammonium hydroxide (15%), and extracted with ether. After evaporation of the solvent, the residue crystallized from methanol. It yielded 80 mg. of O,N-diacetyl-$\Delta^{22}$-tomatillidine (VI): m.p. 155.5–157°; t.l.c., chloroform–ethyl acetate (7:3); $[\alpha]^{20}D$ −88.5° ($CHCl_3$); $\lambda_{max}^{CHCl_3}$ 5.80, (O-Ac), 5.90 (conjugated CO), and 6.02 μ (N-Ac); $\lambda_{max}^{C_2H_5OH}$ 222 mμ (log ε 3.68) and 275 mμ (log ε 3.55); and n.m.r., olefinic protons, 5.45 and 6.05 p.p.m.

*Anal.* Calcd. for $C_{31}H_{45}NO_4$: C, 75.11; H, 9.15. Found: C, 75.22; H, 9.27.

**Hexahydrotomatillidine (III).**—Tomatillidine (I, 100 mg.) was dissolved in 4 ml. of glacial acetic acid and hydrogenated over 100 mg. of platinum oxide catalyst under atmospheric pressure. In about 15 min., the uptake of 3 mole equiv. of hydrogen was accomplished. The catalyst was removed by filtration and washed three times with hot methanol. The combined filtrate was poured into ice–water, basified with ammonium hydroxide (15%), and saturated with sodium chloride. The precipitate was filtered on the following day. The crude material, crystallized from methanol, afforded 15 mg. of hexahydrotomatillidine (III), m.p. 239–241°. The mother liquor, diluted with a mixture of dichloromethane and methanol, was absorbed onto 1 g. of deactivated alumina (with methanol). After complete removal of the solvent, the alumina–compound mixture was placed on top of 10 g. of alumina (grade III) and chromatographed with the following eluents: benzene–dichloromethane (1:1) and then chloroform. An additional 16 mg. of crystalline III, m.p. 229–231°, was obtained: t.l.c., ethyl acetate–acetone–methanol (3:3:1); $[\alpha]^{20}D$ −2.5° ($CH_3OH$); and infrared, no C=N or CO band.

*Anal.* Calcd. for $C_{27}H_{47}NO_2$: C, 77.64; H, 11.34. Found: C, 77.73; H, 11.32.

**Deoxotomatillidine (VII).**[7]—Tomatillidine (I, 1.0 g.) was refluxed in an atmosphere of nitrogen with 40 ml. of ethanol, 40 ml. of β,β′-dihydroxyethyl ether, and 7.3 ml. of 85% hydrazine hydrate for 24 min. Following the addition of 4.0 g. of potassium hydroxide and refluxing for 35 min., the condenser was removed, the temperature of the mixture was allowed to rise to 190°, and the refluxing was continued for another 2.5 hr. After cooling the reaction mixture, it was poured into water, saturated with sodium chloride, and let stand overnight. The precipitate was filtered, washed thoroughly with water, and dried at low temperature (50–60°). The crude material, after crystallization from acetone, yielded 700 mg. of deoxotomatillidine (VII): m.p. 139–141°; t.l.c., chloroform–ethyl acetate–acetone (1:1:1); $[\alpha]^{20}D$ +4.19° ($CH_3OH$); $\lambda_{max}^{KBr}$ 6.05

$\mu$ (C=N); and O.R.D. in methanol (*c* 0.178), $[\alpha]_{249}$ +405° and $[\alpha]_{219}$ −175°.

*Anal.* Calcd. for $C_{27}H_{43}NO$: C, 81.50; H, 10.80. Found: C, 81.46; H, 11.10.

**Pyridine Derivative from Selenium Dehydrogenation of Deoxotomatillidine (VII).**—Deoxotomatillideine (VII, 300 mg.) was ground thoroughly with 900 mg. of selenium dust. The mixture was poured into a Carius tube, sealed under vacuum, and placed in a salt bath (10 parts of potassium nitrate and 7 parts of sodium nitrite), and kept at 305–315° for 14 hr. Afterwards, the tube was removed from the bath, allowed to cool, and opened (hood). The reaction mixture was removed from the tube with ether and hydrochloric acid (1:2). The ethereal layer containing the neutral part (aromatic and partially aromatic hydrocarbons) was extracted four times with hydrochloric acid (1:2). Attempts to isolate some of the hydrocarbons failed, and the neutral part was set aside for later studies. The aqueous acid phase was extracted five times with ether, then it was basified with dilute sodium hydroxide, and extracted several times again with ether. The combined extracts, dried and evaporated, left a residue (155 mg.) smelling of pyridine derivatives. It was checked by t.l.c. [petroleum ether (b.p. 30–60°)–ether, 2:1] giving two spots (iodine solution in chloroform, 0.5%). The material was separated with preparative t.l.c. (petroleum ether–ether, 1:1). The substance with the lower $R_f$ (12 mg.) was crystallized from ethanol to afford 2 mg. of crystalline material. Checked by t.l.c. (benzene–methanol, 9:1), it gave a spot with only a slight contamination. The substance with the higher $R_f$ (20 mg.) was crystallized from ethanol and yielded 3.7 mg. of crystalline material. When checked by t.l.c. (petroleum ether–ether, 2:1), it showed a single spot. From the alcoholic mother liquor 7.2 mg. more of the latter crystalline material was obtained.

The substance with lower $R_f$ had $\lambda_{max}^{C_2H_5OH}$ 268 m$\mu$ (log $\epsilon$ 3.90) and 277 m$\mu$ (log $\epsilon$ 3.70); and n.m.r. H-6, 8.35 p.p.m., $J_{4,6}$ = 3 c.p.s.; H-4, 7.38 p.p.m., $J_{3,4}$ = 8 c.p.s., $J_{4,6}$ = 3 c.p.s.; H-3, 7.01 p.p.m., $J_{3,4}$ = 8 c.p.s.; methyl group at C-5, 2.30 p.p.m.

The substance with higher $R_f$ had m.p. 176–180° with previous sintering at about 153°; $\lambda_{max}^{C_2H_5OH}$ 268 m$\mu$ (log $\epsilon$ 3.66) and 277 m$\mu$ (log $\epsilon$ 3.50); n.m.r. H-6, 8.35 p.p.m., $J_{4,6}$ = 3 c.p.s.; H-4, 7.38 p.p.m., $J_{3,4}$ = 8 c.p.s., $J_{4,6}$ = 3 c.p.s.; H-3, 7.01 p.p.m., $J_{3,4}$ = 8 c.p.s.; methyl group at C-5, 2.28 p.p.m.; and mass spectrum, *m/e* = 379, 364, 121, and 120.

**5,6-Dihydrodeoxotomatillidine (VIII).**—The deoxotomatillidine (VII, 100 mg.) was dissolved in 4 ml. of glacial acetic acid and hydrogenated over 100 mg. of palladium–charcoal (10%) catalyst under atmospheric pressure. The absorption of gas ceased with the uptake of 1 mole equiv. of hydrogen. After removal of the catalyst, the filtrate was poured into water and made alkaline with a solution of sodium carbonate. The mixture was extracted with dichloromethane; the organic layer was washed with water and evaporated to dryness. The residue was crystallized from acetone (25 mg., m.p. 163–166°): t.l.c., ethyl acetate–acetone–methanol (1:1:1); $[\alpha]^{20}D$ +25.5 ($CHCl_3$); $\lambda_{max}^{CHCl_3}$ 6.05 $\mu$ (C=N); n.m.r., no olefinic proton; and O.R.D. in methanol (*c* 0.20), $[\alpha]_{249}$ +570°.

*Anal.* Calcd. for $C_{27}H_{45}NO$: C, 81.14; H, 11.35. Found: C, 81.92; H, 11.64.

**Tetrahydrodeoxotomatillidine (XV).**—Deoxotomatillidine (VII, 76 mg.) was dissolved in 4 ml. of glacial acetic acid and hydrogenated over 76 mg. of platinum oxide catalyst under atmospheric pressure. When the uptake of 2 mole equiv. of hydrogen had ceased, the reaction mixture was worked up in the same manner described for the preparation of III. The crude material (50 mg.) recrystallized three times from methanol afforded tetrahydrodeoxotomatillidine (XV): m.p. 239–241°; t.l.c., chloroform–acetone–methanol (2:2:1); $[\alpha]^{20}D$ 0° ($CHCl_3$); infrared no C=N band; and mass spectrum, mol. wt., 401 for $C_{27}H_{47}NO$.

**VIII from 5,6-Dihydrotomatillidine (II).**—Natural dihydrotomatillidine (100 mg.) was subject to Huang-Minlon reduction in the same manner described for the preparation of VII. The crude material crystallized from acetone to yield 60 mg. of dihydrodeoxotomatillidine, m.p. 166–168°. The compound was identical (mixture melting point and infrared spectrum) with a specimen obtained from the hydrogenation of deoxotomatillidine.

**O,N-Diacetyl-5,6-dihydro-$\Delta^{22}$-deoxotomatillidine (X).**[10,18]—A solution of 190 mg. of 5,6-dihydrodeoxotomatillidine (VIII) in 7 ml. of acetic anhydride was refluxed for 30 min. in a nitrogen atmosphere. The reaction mixture was poured into ice–water, basified with ammonium hydroxide (15%), and extracted with ether. The substance, after evaporation of the ether and crystallization from methanol, afforded 77 mg. of crystalline (plates) O,N-diacetyl-5,6-dihydro-$\Delta^{22}$-deoxotomatillidine (X): t.l.c., chloroform; m.p. 153–156°; $[\alpha]^{20}D$ +28.5° ($CHCl_3$); $\lambda_{max}^{CHCl_3}$ 5.78 (O-Ac), 5.99, and 6.10 $\mu$ (C=C—N—Ac); $\lambda_{max}^{C_2H_5OH}$ 235 m$\mu$ (log $\epsilon$ 3.86); and n.m.r., olefinic proton, 5.20 p.p.m.

*Anal.* Calcd. for $C_{31}H_{49}NO_3$: C, 76.97; H, 10.21. Found: C, 77.16; H, 10.26.

**O,N-Diacetyl-$\Delta^{22}$-deoxotomatillidine (IX).**—Deoxotomatillidine (VII, 200 mg.), acetylated in the same manner described for the preparation of X, yielded O,N-diacetyl-$\Delta^{22}$-deoxotomatillidine (77 mg.): t.l.c., chloroform; m.p. 160–161°; $[\alpha]^{29}D$ +3.0° ($CHCl_3$); $\lambda_{max}^{CHCl_3}$ 5.78 (O-Ac), 5.99, and 6.09 $\mu$ (C=C–N-Ac); $\lambda_{max}^{C_2H_5OH}$ 235 m$\mu$ (log $\epsilon$ 3.90); and n.m.r., olefinic protons, 5.05 and 5.35 p.p.m.

*Anal.* Calcd. for $C_{31}H_{47}NO_3 \cdot 0.5CH_3OH$: C, 76.00; H, 9.86. Found: C, 76.12; H, 9.86.

**3$\beta$-Acetoxy-26-acetylaminocholestan-22-one (XII).**[13]—A solution of 110 mg. of O,N-diacetyl-5,6-dihydrodeoxotomatillidine (X), 2.4 ml. of acetic acid, and 0.52 ml. of 4 *N* hydrochloric acid was allowed to stand at room temperature for 45 min. After the addition of water and neutralization with sodium hydrogen carbonate solution, it was extracted with dichloromethane. The organic layer was washed with water, dried over anhydrous magnesium sulfate, and evaporated at reduced pressure. The residue was subjected to preparative t.l.c. (1 mm. thick, chloroform–ethyl acetate, 1:1). The material containing XII was removed from the plate and, after elution with acetone, yielded 90 mg. of amorphous 3$\beta$-acetoxy-26-acetylaminocholestan-22-one (XII). All attempts to crystallize it failed. The amorphous substance possessed the following physical constants: $[\alpha]^{20}D$ −2.5° ($CHCl_3$); $\lambda_{max}^{CHCl_3}$ 2.93 (N–H), 5.80 (O–Ac and CO), 5.99, and 6.61 $\mu$ (HN–Ac); $\lambda_{max}^{CS_2}$ 5.75 (O–Ac), 5.82 (CO), and 5.91 $\mu$ (N–Ac); and $\lambda_{max}^{C_2H_5OH}$ 280 m$\mu$ (log $\epsilon$ 2.03).

*Anal.* Calcd. for $C_{31}H_{51}NO_4$: C, 74.21; H, 10.25. Found: C, 74.40; H, 10.07.

**3$\beta$-Acetoxy-26-acetylaminocholest-5-en-22-one (XI).**—O,N-diacetyldeoxotomatillidine (IX), hydrolyzed and worked up in the same manner described for the preparation of XII, yielded an amorphous 3$\beta$-acetoxy-26-acetylaminocholest-5-en-22-one (XI): $[\alpha]^{20}D$ −30.4° ($CHCl_3$); $\lambda_{max}^{CHCl_3}$ 2.90 (N–H), 5.82 (O–Ac CO), 6.00, and 6.62 $\mu$ (HN–Ac); $\lambda_{max}^{CS_2}$ 5.78 (O–Ac), 5.82 (CO), and 5.95 $\mu$ (N–Ac); and $\lambda_{max}^{C_2H_5OH}$ 285 m$\mu$ (log $\epsilon$ 2.4).

**Oxidation of XII to 3$\beta$-Acetoxybisnorallocholanic Acid (XIV) and 3$\beta$-Acetoxyandrostan-17-one (XIII).**—A solution of chromic acid (356 mg. of chromium trioxide in 1.9 ml. of 80% acetic acid) was added to a solution of 214 mg. of XII in 5.2 ml. of acetic acid and heated at 63–66°. The reaction mixture was kept at this temperature for 4 hr. After cooling, water and a small amount of sodium sulfite were added and the mixture was extracted with ether.

**A. Acidic Part. Insoluble Sodium Salt.**—The ether extract was washed with water and was then shaken vigorously with 3 ml. of an aqueous solution of sodium hydroxide (2 *N*). A solid white precipitate (insoluble sodium salt) was produced at the interphase. After removal of the suspension, this operation was repeated once more with 2 ml. of 2 *N* NaOH. The combined suspensions were centrifuged. The liquid layer was removed with a capillary pipet. Sodium hydroxide (2 ml., 2 *N*) and 4–5 ml. of ether were added and shaken in the centrifuge tube itself, then centrifuged. The etheral layer was removed and the suspension was shaken with ether and centrifuged twice more. Finally after the removal of the aqueous and ethereal layers, sulfuric acid (33%) and ether were added, and the mixture was transferred to a separatory funnel. The free carboxylic acid dissolved in the organic layer and was washed with water until neutral. The solution was dried on magnesium sulfate and the solvent was evaporated. The residue, recrystallized from acetone, afforded 13.5 mg. of 3$\beta$-acetoxybisnorallocholanic

(18) NOTE ADDED IN PROOF.—Our compound X has been identified with (25*R*)-3$\beta$-acetoxy-22,26-acetimino-5$\alpha$-cholest-22-en (XIV) prepared by K. Schreiber and G. Adam [*Tetrahedron*, **20**, 1707 (1964)]. The discrepancy in the reported melting point and optical rotation value of our compound VIII, m.p. 163–166°, $[\alpha]^{20}D$ +25.5° ($CHCl_3$), and those of Schreiber's (25*R*)-22,26-imino-5$\alpha$-cholest-22(N)-en-3$\beta$-ol (XIII), m.p. 170–174°, $[\alpha]^{20}D$ +43.1° ($CHCl_3$), respectively, is probably due to partial racemization at C-25 during the Huang-Minlon reduction.

acid (XIV). The analytical sample was purified by four recrystallizations from acetone: m.p. 192–194° [14]; t.l.c., ethyl acetate–acetone–methanol (3:3:1). Mixture melting point and infrared (KBr) were coincident with an authentic specimen.

*Anal.* Calcd. for $C_{24}H_{38}O_4$: C, 73.80; H, 9.81. Found: C, 73.67; H, 9.98.

**Soluble Sodium Salt.**—The alkaline aqueous phase containing the soluble sodium salts was not studied.

**B. Neutral Part.**—The ether layer, after removal of the acidic material and the insoluble sodium salt, was washed with water, dried, and evaporated. The residue was checked by t.l.c. (chloroform), after spraying with a 2% solution of cerium sulfate in 2 *N* sulfuric acid, and showed an intensely blue colored spot. All of the neutral crude material (*ca.* 50 mg.) was subjected to preparative thin layer chromatography (chloroform) and the material corresponding to the blue spot was eluted with acetone. It afforded 6 mg. of 3β-acetoxyandrostan-17-one (XIII), m.p. 89–92°,[15] melting point and infrared spectrum coincident with an authentic specimen.

**Methyl 3β-Acetoxybisnorallocholanate (XIVa).**—An ether solution of diazomethane was added to an ice-cold suspension of 3β-acetoxybisnorallocholanic acid (XIV, 21 mg.) and allowed to stand at room temperature for 45 min. After removal of the solvent at reduced pressure, the residue was dissolved in methanol, shaken with Norit, and filtered. After partial evaporation of the solvent, 100 mg. of crystalline (plates) methyl 3β-acetoxybisnorallocholanate was obtained, m.p. 128–130°[14]; the t.l.c. (benzene–ethyl acetate, 9:1), melting point, and infrared spectrum were coincident with an authentic specimen.[14]

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# Transformation of Progesterone and Related Steroids by *Aspergillus tamarii*

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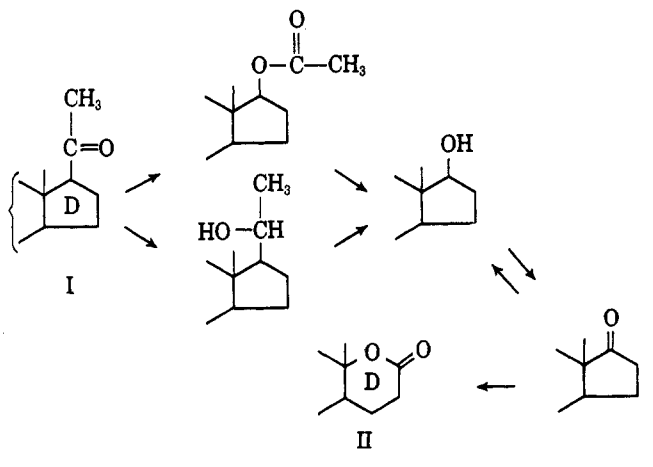
Progesterone is readily converted into testololactone and 11β-hydroxytestosterone by the mold *Aspergillus tamarii*. 11β-Hydroxytestosterone is an end product and is not converted into testololactone. 11β- and 11α-hydroxyprogesterone are transformed into 11β- and 11α-hydroxytestosterone, respectively, but not into the corresponding testololactone. Δ⁴-Androsten-11β-ol-3,17-dione was unaffected by *A. tamarii*, but Δ⁴-androstene-3,17-dione and testosterone were transformed into testololactone. Unlike related microbiological transformations, hydroxylation at C-11 inhibits oxidative cleavage of ring D by *A. tamarii*. Furthermore, this is the first report that an *Aspergillus* sp. of any kind produces 11β-hydroxylation of steroids.

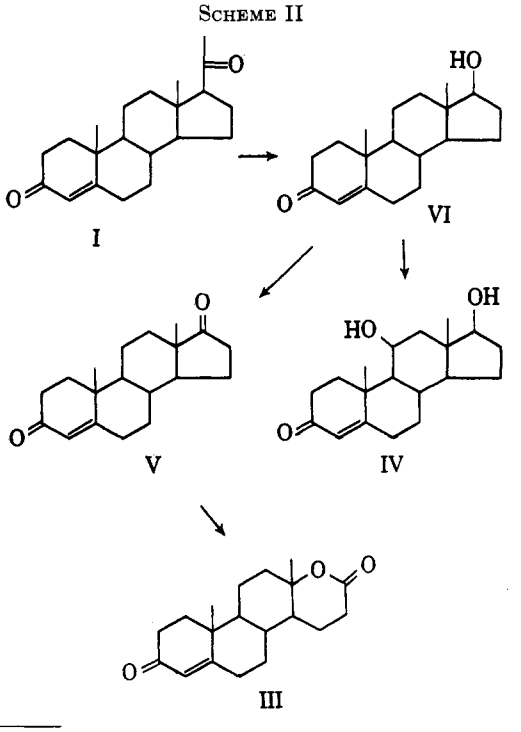
A number of microorganisms are known[1–4] to stereospecifically degrade the C-17 β-acetyl side chain of progesterone (I) and related compounds to give D-ring lactones (II), and the present evidence supports the pathway[4–7] outlined in Scheme I. Another well-known microbiological transformation is C-11 hydroxylation, undoubtedly the most important of the chemical changes carried out by microorganisms.[8]

SCHEME I




We have found that *Aspergillus tamarii* transforms progesterone by two independent paths. The major pathway leads to the end product testololactone (III), which is produced at an early stage *via* the intermediates testosterone (VI) and Δ⁴-androstene-3,17-dione (V) as previously postulated (see Scheme II). The second path leads to the end product 11β-hydroxytestosterone (IV). These findings differ from a previous report[9]

SCHEME II

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