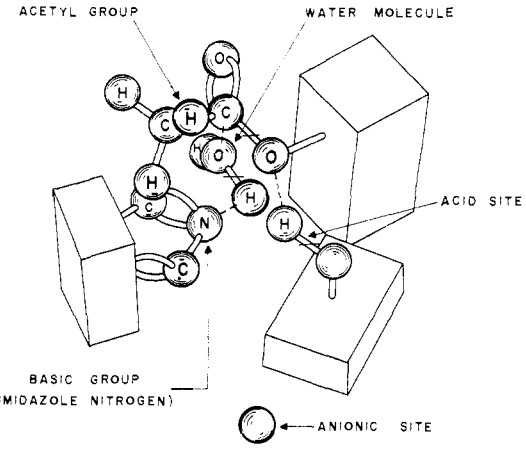


Fig. 3.—Proposed structure for the acetyl enzyme, showing the water molecule that is involved in the deacetylation reaction.

These results may be explained if, for steric reasons, the proton does not have access to the imidazole nitrogen atom.

The nature of the acetylation process is easily envisaged in the light of Fig. 2. New bonds are formed at the dotted lines, and bonds are broken as indicated on the diagram. It is to be noted that there is simultaneous rupture of three bonds and making of three new bonds. Such a process will be highly efficient provided that, as is the case, the atoms are initially held in the appropriate positions.

The Acetyl Enzyme, and the Deacetylation Process.—Figure 3 shows the proposed structure of the acetyl enzyme. Included in the figure is a water molecule arranged in the correct position for deacetylation to occur. The structure of the acetyl-enzyme is such that it is easy to see how inhibitors such as *cis*-2-dimethylaminocyclohexanol can become attached to it as strongly as to the free enzyme. Inhibitors of the second type, however, are too large to become attached other than at the anionic site.

The *p*H results[7] showed that both the acidic and basic groups (in their protonated and unprotonated forms, respectively) are involved in the deacetylation process; that the acidic group is involved is confirmed by the inhibition studies.[8] The role of the imidazole nitrogen atom is presumably to attract a proton from the water molecule, leaving the hydroxide ion available for attack on the carbonyl carbon atom. At the same time the hydrogen atom of the acid site may become transferred to the serine oxygen atom. The various bond breaking and bond making processes are assumed to occur simultaneously, and the process may again be expected to be a very efficient one.

Acknowledgment.—The authors are indebted to the Defence Research Board of Canada for their support of this work under grant no. 9510-06.

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, HARVARD MEDICAL SCHOOL, BOSTON 15, MASS.]

The Synthesis of Azaoxaspirane Steroid Alkaloids[1]

BY FREDERICK C. UHLE

RECEIVED MARCH 15, 1960

The transformations of kryptogenin and of diosgenin to solasodine, of neotigogenin to tomatidine and of sarsasapogenin to 5β-tomatidine, as well as to N-methyl-5β-tomatidine, are described. The conversion of pregnenolone to piperidine derivatives related in skeletal structure to the hydrogenolysis products of the alkaloids is reported.

Structural investigations in several laboratories culminated in provisional formulation of the natural products solasodine[2] and tomatidine[3] as ring F nitrogenous counterparts of the spiroketal sapogenins. The alkaloids merit close study both as members of the steroid family and as representatives of a mode of juncture not encountered elsewhere.

In a first synthetic approach, methods were sought for selective replacement of the 27-hydroxyl of kryptogenin by an amino group. Since no reagent capable of differentiating the primary alcoholic group of the side chain from the unusually reactive 3β-homoallylic secondary function of ring A was known, judicious choice of conditions, followed by fractionation of the expected mixture of products was considered most promising. After persistent experimentation, direct preparation of kryptogenin 27-*p*-toluenesulfonate (II) was achieved in 40% yield through 15 hours treatment with two equivalents of *p*-toluenesulfonyl chloride in anhydrous pyridine at 0°. Use of less, or of more, sulfonyl chloride diminished the yield.

A second, superior preparation of II was developed, however, through selective solvolysis[4] of the 3β,27-di-*p*-toluenesulfonate I, readily available in 90% yield. When a 0.04 molar aqueous acetone solution of I was heated under reflux for 2 hours, the 27-*p*-toluenesulfonate II remained in yields as high as 80%, corresponding to greater than 70% over-all transformation from the sapogenin.

Methanolysis of the 27-*p*-toluenesulfonate in the

(1) Announcements of introductory phases of this work were made in Communications to the Editor of *J. Am. Chem. Soc.*, **73**, 883 (1951); **75**, 2280 (1953); **76**, 4245 (1954); with J. A. Moore, *ibid.*, **76**, 6412 (1954). The expression "azaoxaspirane" has been recommended as a generic term to denote compounds in which appositely placed amino, hydroxyl and keto functions have entered into ketal-like spirane formation: F. C. Uhle and F. Sallmann, *J. Am. Chem. Soc.*, **82**, 1190 (1960).

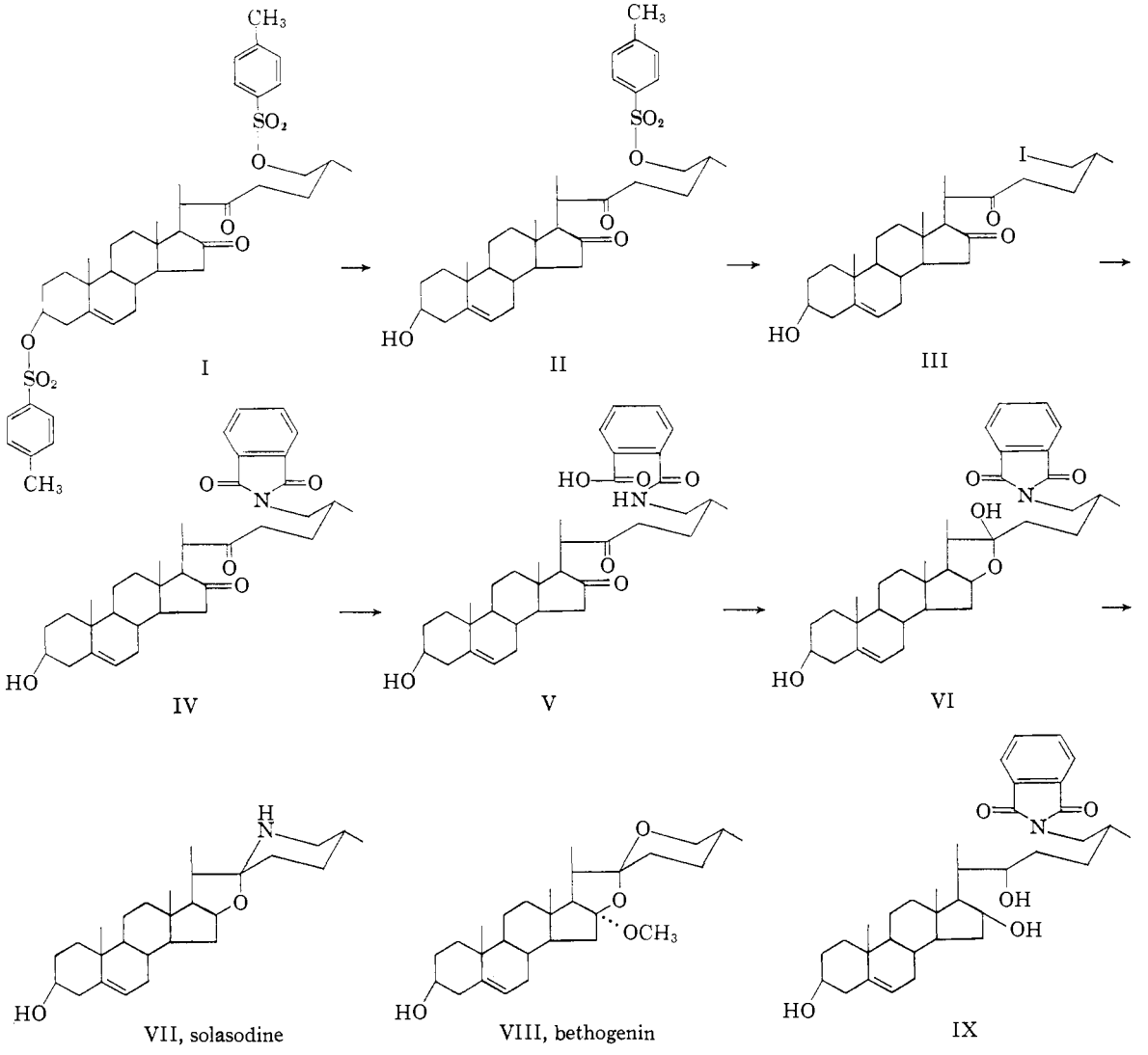
(2) L. H. Briggs, W. E. Harvey, R. H. Locker, W. A. McGillivray and R. N. Seelye, *J. Chem. Soc.*, 3013 (1950).

(3) (a) T. D. Fontaine, J. S. Ard and R. M. Ma, *J. Am. Chem. Soc.*, **73**, 878 (1951); (b) Y. Sato, A. Katz and E. Mosettig, *ibid.*, **73**, 880 (1951); (c) R. Kuhnand I. Löw, *Chem. Ber.*, **85**, 416 (1952); R. Kuhn, I. Löw and H. Trischmann, *ibid.*, **86**, 372 (1953).

(4) *Cf.* the observed[5] and rationalized[6] rapid solvolysis of cholesteryl 3β-*p*-toluenesulfonate.

(5) W. Stoll, *Z. physiol. Chem.*, **207**, 47 (1932).

(6) S. Winstein and R. Adams, *J. Am. Chem. Soc.*, **70**, 838 (1948); R. M. Dodson and B. Riegel, *J. Org. Chem.*, **13**, 424 (1948).



presence, as well as in the absence, of potassium acetate gave bethogenin[7] (VIII); treatment of the 3β,27-di-*p*-toluenesulfonate with refluxing methanol led to bethogenin 3β-methyl ether. With sodium iodide in butanone at 25°, the 27-*p*-toluenesulfonate furnished 80% of kryptogenin 27-iodide (III), which, with potassium phthalimide in dimethylformamide[8] at 25°, afforded the 27-phthalimido derivative IV in 85% yield.

For the next step, a preferential reduction of the 16-keto function, sodium borohydride in isopropyl alcohol[9] was employed. Since the imide grouping, as well, unexpectedly was found to undergo a reductive change,[10] the phthalimido derivative IV was opened to the less vulnerable phthalamidic acid V with potassium bicarbonate in boiling aqueous methanol. After 15 hours treatment of the potassium phthalamidate in isopropyl alcohol with 16 equivalents of sodium borohydride, the phthalimide ring was reconstituted at 0° with N-ethyl - N′ - [2 - (4 - morpholinyl) - ethyl] - carbodiimide metho-*p*-toluenesulfonate.[11]

Chromatography on aluminum oxide gave two crystalline substances, the desired hemiketal 3β,22-dihydroxy-27-phthalimido-25α-5-furostene (VI) and the triol IX arising from attack at both carbonyl groups. Kryptogenin itself, with sodium borohydride, afforded a mixture of diosgenin (48%) and of the tetrol 3β,16,22,27-tetrahydroxy-25α-5-cholestene.[12] Treatment of the phthalimido

(7) S. Lieberman, F. C. Chang, M. R. Barusch and C. R. Noller, *J. Am. Chem. Soc.*, **64**, 2581 (1942); C. R. Noller and M. R. Barusch, *ibid.*, **65**, 1435, 1786 (1943); R. E. Marker, R. B. Wagner, P. Ulshafer, E. L. Wittbecker, D. P. J. Goldsmith and C. H. Ruof, *ibid.*, **69**, 2210 (1947).

(8) J. C. Sheehan and W. A. Bolhofer, *ibid.*, **72**, 2786 (1950).

(9) Isopropyl alcohol was favored despite its relatively low solvent power for the reagent because of the stability of the solution (no evolution of hydrogen is observed at ordinary temperature over protracted periods) and because of the rapid rate of carbonyl reduction in the medium; *cf.* H. C. Brown, E. J. Mead and B. C. Subba Rao, *ibid.*, **77**, 6209 (1955).

(10) In a model study prompted by this finding, simpler N-substituted phthalimides gave, in isopropyl alcohol alone, 3-hydroxy- and 3-isopropoxy-1-isoindolinones; in aqueous isopropyl alcohol, reduction proceeded further to *o*-hydroxymethylbenzamide derivatives: F. C. Uhle, in press.

(11) Although dicyclohexylcarbodiimide effected phthalimide ring closure equally well, use of a water-soluble carbodiimide was particularly advantageous since dicyclohexylurea collected in the same chromatographic fraction as the hemiketal VI and was difficult to remove by crystallization even when present in trace amounts.

(12) The tetrol appeared unexpectedly acid-labile in isopropyl alcohol under the processing conditions. When hydrochloric acid had

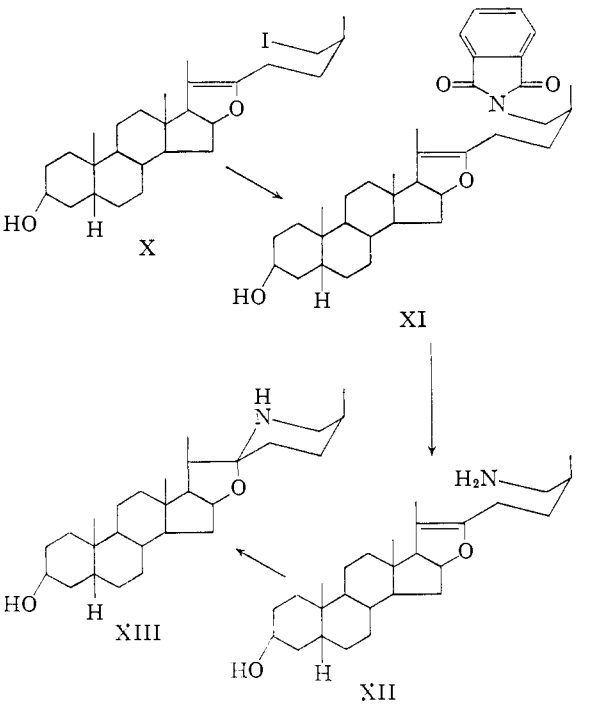
furostene VI with hydrazine[13] in dichloromethane–methanol at 25°, followed by 1 *N* hydrochloric acid in refluxing aqueous ethanol, gave solasodine (VII),[14] identical with the natural product.

Concurrently with the kryptogenin approach, an effort to employ pseudosapogenins as starting compounds in a more general synthesis of aza-oxaspirane alkaloids had been initiated. Unpropitious trials with the intractable pseudodiosgenin led to work with pseudosarsasapogenin, a stable, nicely crystalline substance prepared according to an improved isomerization procedure with acetic anhydride in the presence of pyridine hydrochloride.[15]

The crude product from esterification of pseudosarsasapogenin with two equivalents of *p*-toluenesulfonyl chloride in pyridine at 0° was allowed to react with sodium iodide in butanone. Chromatography on alumina gave two major fractions, the second of which afforded, from methanol, pseudosarsasapogenin 27-iodide (X) in 46% over-all yield. Treatment of the iodide with potassium phthalimide in dimethylformamide gave 80% of the 27-phthalimidofurostene XI.

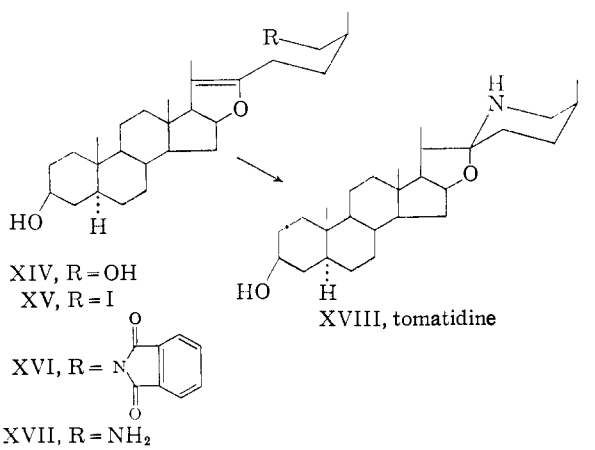
Rejection of the phthalimido moiety with hydrazine provided the 27-amino compound XII, an alkanolamine which gave 27-acetyl and 3β,-27-diacetyl derivatives. The pentacyclic aminofurostene was found to be fully stable to 20% acetic acid, as well as to one equivalent of hydrochloric acid, and to require relatively vigorous treatment with mineral acid for cyclization to the aza-oxaspirane XIII. Addition of the 27-amino group to the 20(22)-double bond of the cyclic vinyl ether thus proceeds much less readily than does addition of the 27-hydroxyl group of the pseudosapogenins. Consequently, the 27-amino furostenes appeat unlikely precursors for preparation of nitrogenous analogs of the metastable cyclopseudosapogenins.

Attention was next devoted to tomatidine, the tomato alkaloid, for which a skeletal kinship to solasodine had been proposed.[3] An especially informative sequence had given rise to demissidine, a 5,6-dihydride of solanidine.[3c] Solanidine, in turn, already had been interrelated with sarsasapogenin.[16] Hence, the 25-methyl group of tomatidine could be assigned the configuration of that of the new synthetic azaoxaspirane XIII, epimeric with that of the 25-substituent of solasodine.



Moreover, the remaining break-down products, tigogenin lactone and 3β-hydroxy-5α-16-pregnen-20-one, reaffirmed the *trans* lock of rings A and B as opposed to *cis* fusion in the case of XIII. Opportunely, the sapogenin satisfying these spatial qualifications at positions 5 and 25, neotigogenin, had been rediscovered as a subordinate companion of hecogenin in East African *Agave sisalana*.[17]

Transformation to pseudoneotigogenin (XIV) and thence to intermediates analogous to those of the pseudosarsasapogenin route gave pseudoneotigogenin 27-iodide (XV) (40%), 3β-hydroxy-27-phthalimido-5α-25β-20(22)-furostene (XVI) (80%) and 3β-hydroxy-27-amino-5α-25β-20(22)-furostene (XVII) (70%), an alkanolamine characterized as the 27-acetyl and 3β,27-diacetyl derivatives. Cyclization of the aminofurostene with hydrochloric acid gave tomatidine (XVIII), establishing its



been used to complete cyclization of the presumed intermediate precursor of diosgenin, the hemiketal triol 3β,22,27-trihydroxy-25α-5-furostene [the "16-dihydrokryptogenin" of St. Kaufmann and G. Rosenkranz, *J. Am. Chem. Soc.*, **70**, 3502 (1948), and of R. S. Miner and E. S. Wallis, *J. Org. Chem.*, **21**, 715 (1956)], the tetrol was found in only minimal amounts. On the other hand, when introduction of strong acid had been omitted in an unsuccessful endeavor to isolate the hemiketal triol in crystalline form, 35% of the tetrol was obtained readily, together with 10% of diosgenin. Apparently the triol accompanied the tetrol in the final chromatographic fraction since acidification of the filtrate from crystallization of the tetrol gave an additional amount of diosgenin. The fate of the tetrol in the presence of acid was not ascertained.

(13) H. R. Ing and R. H. F. Manske, *J. Chem. Soc.*, 2350 (1926).

(14) In the absence of contrary evidence, the alkaloids have been accorded the conformational representations accepted for their sapogenin precursors; rest on analogy alone, however, is by no means wholly justified.

(15) W. G. Dauben and G. J. Fonken, *J. Am. Chem. Soc.*, **76**, 4618 (1954).

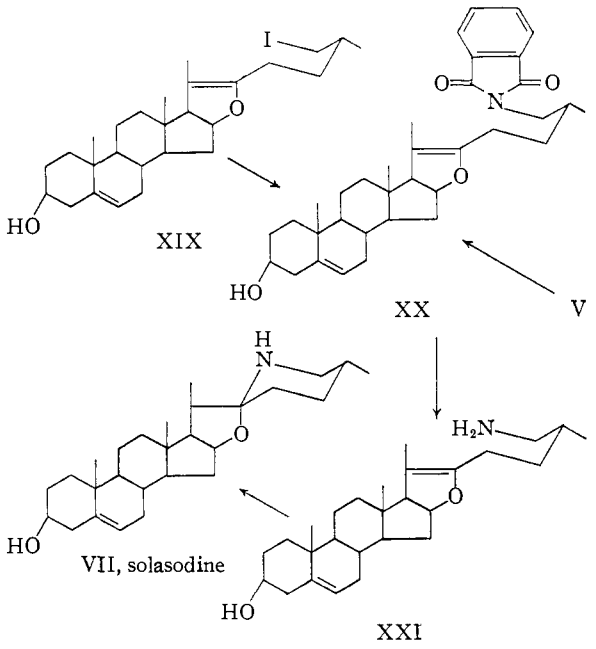
(16) F. C. Uhle and W. A. Jacobs, *J. Biol. Chem.*, **160**, 243 (1945).

(17) R. K. Callow and V. H. T. James, *Chemistry & Industry*, 691 (1954); *J. Chem. Soc.*, 1671 (1955). The sapogenin had first been isolated from *Chlorogalum pomeridianum* Kunth by L. H. Goodson and C. R. Noller, *J. Am. Chem. Soc.*, **61**, 2420 (1939).

structure and stereochemistry. The closely related XIII from sarsasapogenin subsequently was referred to most simply as 5β-tomatidine.

With the experience gained with sarsasapogenin and with neotigogenin as a guide, the possibility of preparing solasodine from diosgenin was re-examined. Treatment of pseudodiosgenin with two equivalents of *p*-toluenesulfonyl chloride in pyridine at 0°, followed by reaction with sodium iodide in butanone, afforded a very large, early chromatographic fraction, assumed to represent material in which attack had occurred at both 3β- and 27-positions. Fractional crystallization, after tedious rechromatography, provided two crystalline substances, the 3β,27-diiodide of pseudodiosgenin and the elimination product 27-iodo-25α-3,5,20(22)-furostatriene.[18] The second major eluate of the original column separation gave, from methanol, a 16% yield of pseudodiosgenin 27-iodide (XIX).

Since these results signified a much greater involvement at the 3β-position during esterification than was the case with the ring B saturated sapogenins, the selective solvolysis which had proved so advantageous with kryptogenin 3β,27-di-*p*-toluenesulfonate was invoked. When a 0.01 molar aqueous acetone solution of the crude pseudodiosgenin *p*-toluenesulfonate was heated under reflux for 1.5 hours and the product allowed to react with sodium iodide in butanone, 53% of pseudodiosgenin 27-iodide (XIX) was isolated without chromatography.[19]



Treatment of XIX with potassium phthalimide in dimethylformamide gave 75% of 3β-hydroxy-27 - phthalimido - 25α - 5,20(22) - furostadiene (XX). A second preparation of XX was accomplished from kryptogenin, linking the two series, when, after sodium borohydride reduction of the phthalamidic acid V, the phthalimide ring had been reconstructed in the presence of two equivalents of N-ethyl-N′-[2-(4-morpholinyl)-ethyl]-carbodiimide metho-*p*-toluenesulfonate at 25°, rather than at 0°. Under these conditions, imide cyclization was attended by hemiketal dehydration, affording the 20(22)-unsaturated derivative XX in lieu of the 22-hydroxy compound VI.

Hydrazinolysis of XX provided 3β-hydroxy-27-amino-25α-5,20(22)-furostadiene (XXI). Acetylation with acetic anhydride in pyridine gave the ester amide which was hydrolyzed with dilute alkali to the 27-acetyl derivative. Cyclization of the amino furostadiene with mineral acid afforded solasodine (VII).

Finally, consideration was given to the preparation of N-methyl tertiary homologs of the alkaloids,[20] substances of potential interest in conformational arguments. Since in early experiments the pseudosapogenin 27-iodides had not reacted with ammonia, an indirect procedure modeled on the Gabriel phthalimide method, was developed for the introduction of a substituted N-methyl function. Treatment of pseudosarsasapogenin 27-iodide (X) with potassium N-methyl-*p*-toluenesulfonamide in dimethylformamide at 25° gave the 27-N-methyl-*p*-toluenesulfonamide XXII.[21]

Hydrogen bromide in acetic acid in the presence of phenol[22] failed to remove the *p*-toluenesulfonyl residue of XXII, however, and Hofmann alkylation of X with methylamine itself was attempted. When pseudosarsasapogenin 27-iodide (X) was allowed to react with 20% methanolic methylamine in a sealed tube at 90°, 3β-hydroxy-27-methylamino-5β-25β-20(22)-furostene (XXIII) was formed in 80% yield.[23] Treatment of the methylaminofurostene with *p*-toluenesulfonyl chloable hydrolysis to pseudodiosgenin. In the presence of pseudodiosgenin, separation of amino derivatives was seldom clear-cut, seriously compromising final purification, since the neutral pseudosapogenin unfailingly tends to form opalescent dispersions in 10–20% acetic acid which defy coagulation.

(18) A 3β-hydroxy elimination product, 27-iodo-5α-25β-2(3),20,-(22)-furostadiene, had been isolated from the chromatographic fraction carrying less polar constituents in the pseudoneotigogenin–tosylation–iodination sequence. Treatment of the doubly unsaturated iodide with potassium phthalimide in dimethylformamide gave the 27-phthalimido derivative which, with hydrazine, followed by hydrochloric acid, afforded $\Delta^{2(3)}$-dehydrotomatidine.

(19) The 27-iodo derivatives from pseudodiosgenin, pseudosarsasapogenin and pseudoneotigogenin closely resemble one another in crystallizing habit and in melting behavior. Pseudodiosgenin 27-iodide appears more sensitive to basic reagents, however, since unusual precautions for anhydrous conditions were required to avert appreci-

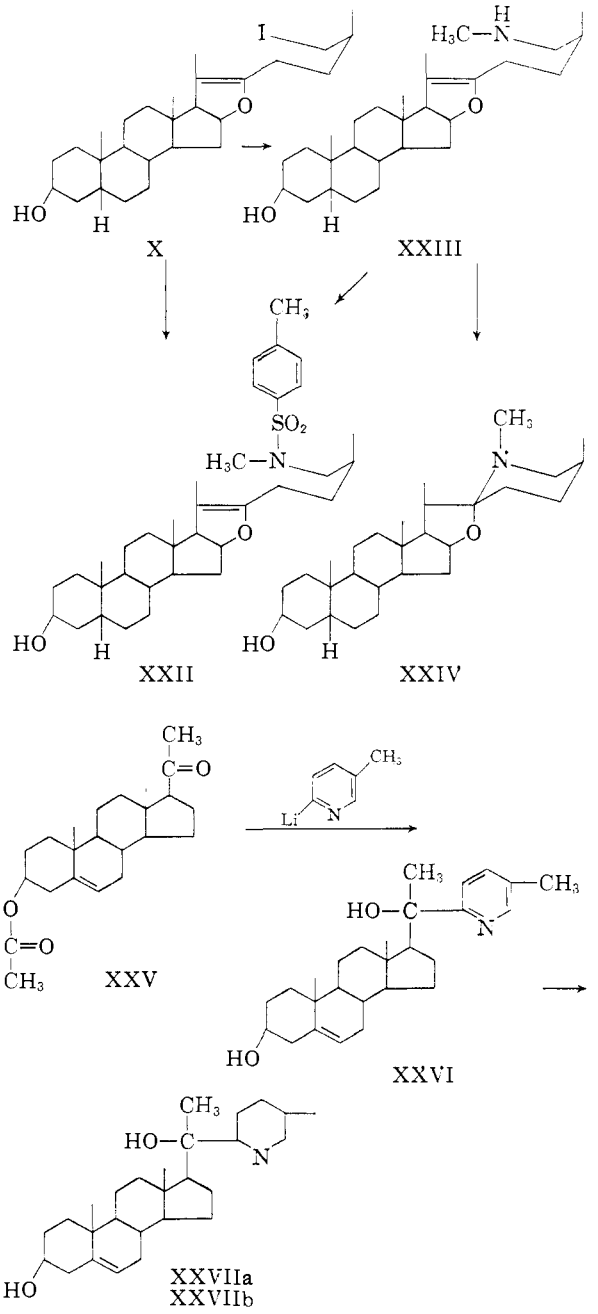
(20) Attempts to methylate solasodine with methyl iodide or by the Clarke–Eschweiler procedure have failed: ref. 2; Y. Sato, H. G. Latham, Jr., and E. Mosettig, *J. Org. Chem.*, **22**, 1496 (1957).

(21) The practicability of the procedure was established with benzyl chloride which gave 95% of N-benzyl-N-methyl-*p*-toluenesulfonamide. The method appears to have been little exploited, although W. Markwald and O. Frobenius [*Ber.*, **34**, 3547 (1901)] had allowed sodium N-methyl-*p*-toluenesulfonamide to react with various β-haloethyl compounds. Dimethylformamide aids by promoting homogeneous conditions.

(22) D. I. Weisblat, B. J. Magerlein and D. R. Myers, *J. Am. Chem. Soc.*, **75**, 3630 (1953); H. R. Snyder and R. E. Heckert, *ibid.*, **74**, 2006 (1952); H. R. Snyder and H. C. Geller, *ibid.*, **74**, 4864 (1952). Difficulty in the present instance doubtless is to be ascribed to acid-sensitivity of the cyclic vinyl ether. It had been hoped that prompt cyclization, after rapid cleavage, would obviate extensive decomposition. Apparently, no authentic example of alkaline hydrolysis of a substituted sulfonamide has been recorded; *cf.* C. M. Suter, "The Organic Chemistry of Sulfur," John Wiley and Sons, Inc., New York, N. Y., 1944, p. 581.

(23) Pseudodiosgenin 27-iodide, with methanolic methylamine under the same circumstances, gave a lower conversion to 3β-hydroxy-27-methylamino-25α-5,20(22)-furostadiene; *cf.* footnote 19.

ride under Schotten–Baumann conditions gave the amide XXII obtained directly from the iodide XV and potassium N-methyl-*p*-toluenesulfonamide. Although the open-chain methylamino compound appeared to cyclize less readily[24] than did the primary homolog XII, N-methyl-5β-tomatidine (XXIV) was secured in a conversion of 50% after treatment with dilute mineral acid.



(24) This assertion rests principally on the observation that, with 3 *N* HCl, conversion (10%) of XXIII to N-methyl-5β-tomatidine (XXIV) was much below that of XII to 5β-tomatidine. With 0.5 *N* HCl, the yield rose to 50%, suggesting that with high acid concentrations, vinyl ether fission took precedence over cyclization. Starting XXIII could not be retrieved from mother liquors of reactions which gave minimal quantities of XXIV. The phthalimido furostene XI failed to survive 15 hours treatment with 1% methanolic sulfuric acid at 25°.

Of the reactions typical of solasodine and tomatidine, hydrogenolysis at the spirane junction with lithium aluminum hydride or with catalytically activated hydrogen to give pentacyclic 2′-piperidyl-20-pregnene derivatives has received particularly careful study.[25] In the synthesis of compounds of this framework, 5-methyl-2-pyridyllithium was prepared from 2-bromo-5-methylpyridine by halogen–metal interchange with *n*-butyllithium and was allowed to react at very low temperature with pregnenolone acetate (XXV) to afford 3β,20β-dihydroxy-20α-(5′-methyl-2′-pyridyl)-5-pregnene (XXVI). Reduction of the pyridine nucleus with sodium and *n*-propyl alcohol at 100° gave rise to two stereoisomeric secondary amines XXVIIa and XXVIIb. Separation of the piperidine isomers, whose properties were closely allied, was achieved by fractional crystallization of their picrates.

Experimental

The melting points were observed on a calibrated micro hot-stage. Compounds in these series, as seen under the microscope, frequently melt without sharpness. Samples of analytical purity not uncommonly display melting ranges of 3–5°, and in a few cases, even of 8–10°.

The microanalyses were performed by Dr. S. M. Nagy and his associates of the Massachusetts Institute of Technology, Cambridge, Mass. A number of the rotations were measured by the Schwarzkopf Microanalytical Laboratories, Woodside 77, N. Y. The infrared spectra, in potassium bromide, were recorded with Perkin–Elmer spectrophotometers, models 21 and 137. Only those maxima of significance in interpretation are mentioned. Bands not of full intensity are designated as medium (m) or weak (w).

Since steroid amino derivatives in general form sparingly water-soluble salts with the common mineral acids, these reagents were excluded from all manipulative operations. Separation of neutral and basic products was accomplished by trituration of solid residues with 10–20% aqueous acetic acid. Woelm non-alkaline aluminum oxide was used for the chromatographic separations.

In order to conserve space in the experimental descriptions, the following abbreviated conventions have been adopted: "at 25°" = at ordinary temperature; "concentrated" = concentrated under diminished pressure with a Rinco rotating evaporator; "dried" = dried in a vacuum desiccator, usually over phosphorus pentoxide; "stirred" = stirred by means of a magnetic apparatus; "pyridine" = anhydrous pyridine; "dimethylformamide" = anhydrous dimethylformamide; "$[\alpha]_D$" = a rotation in chloroform at a concentration of approximately 1% at 25 ± 2° unless otherwise stated; "petroleum ether" = petroleum ether (b.p. 30–60°).

Kryptogenin Series

3β,27-Di-*p*-toluenesulfonyloxy-25α-5-cholesten-16,22-dione (Kryptogenin 3β,27-Di-*p*-toluenesulfonate) (I).—To a solution of 1.07 g. (0.0025 mole) of kryptogenin in 10 ml. of pyridine at 0° was added 1.9 g. (0.01 mole) of *p*-toluenesulfonyl chloride. After 25 hours at 0°, the mixture was added to 250 ml. of water. The precipitate was collected by filtration, washed with water and dried. Recrystallization from a mixture of dichloromethane and anhydrous ether gave 1.66 g. (90%) of needles, m.p. 119–121°; $[\alpha]_D$ −165°; infrared spectrum: 5.82 (16-CO), 5.88 (22-CO), 7.40, 8.45, 8.55 μ (OSO_2).

Anal. Calcd. for $C_{41}H_{54}S_2O_8$ (738.97): C, 66.63; H, 7.37; S, 8.68. Found: C, 66.49; H, 7.17; S, 8.62.

3β-Hydroxy-27-*p*-toluenesulfonyloxy-25α-5-cholesten-16,22-dione (Kryptogenin 27-*p*-Toluenesulfonate) (II). **(A) From Kryptogenin.**—To a solution of 8.60 g. (0.02 mole) of kryptogenin in 50 ml. of pyridine at 0° was added, in 6 portions over a period of 4 hours, 7.60 g. (0.04 mole) of

(25) L. H. Briggs and R. H. Locker, *J. Chem. Soc.*, 3020 (1950); Y. Sato and H. G. Latham, Jr., *J. Am. Chem. Soc.*, **78**, 3146 (1956); ref. 3b and 3c.

p-toluenesulfonyl chloride. After 15 hours at 0°, the mixture was added to 1000 ml. of water. The clear supernatant liquor was decanted from the viscous deposit which was washed repeatedly by swirling with water, followed by decantation. The dried material was triturated with 100 ml. of anhydrous ether. After 18 hours at 25°, the precipitate was collected by filtration and was washed with ether. Recrystallization from a mixture of dichloromethane and anhydrous ether gave 4.67 g. (40%) of heavy needles, m.p. 155–165°. The analytical sample, from the same solvent pair, melted at 165–166°, $[\alpha]_D$ − 144°; infrared spectrum: 3.05 (OH), 5.82 (16-CO), 5.88 (22-CO), 7.40, 8.45, 8.55 μ (OSO_2).

Anal. Calcd. for $C_{34}H_{48}SO_6$ (584.58): C, 69.82; H, 8.27; S, 5.48. Found: C, 69.82; H, 8.20; S, 5.28.

(B) From **Kryptogenin 3β,27-Di-*p*-toluenesulfonate (I).**—A solution of 2.22 g. (0.003 mole) of kryptogenin 3β,27-di-*p*-toluenesulfonate (I) and 15 ml. of water in 60 ml. of acetone was maintained at reflux temperature for 2 hours. The solution was diluted with 300 ml. of water and was concentrated. After 15 hours at 0°, the precipitate was collected by filtration, washed with water and dried. The anhydrous material (1.67 g.) was recrystallized from a mixture of dichloromethane and anhydrous ether to afford 1.4 g. (80.5 %), m.p. 155–165°.

Evaporation of the aqueous filtrate gave 640 mg., m.p. 90–105°, which, with *p*-toluidine, afforded 800 mg. (90%) of the *p*-toluidine salt of *p*-toluenesulfonic acid, m.p. 194–198°.[26]

Bethogenin (VIII).—A solution of 117 mg. (0.0002 mole) of kryptogenin 27-*p*-toluenesulfonate (II) and 100 mg. (0.001 mole) of anhydrous potassium acetate in 10 ml. of methanol was heated under reflux for 7 hours. After the solution had been diluted with water and concentrated, the precipitate was collected by filtration, washed with water and dried. Two recrystallizations from methanol containing 2% potassium hydroxide gave 35 mg. (40%), m.p. 186–192° with a minor portion softening at 150–165°. The infrared spectrum was identical with that given by a sample prepared from kryptogenin with methanol and concentrated hydrochloric acid[7]: absence of carbonyl absorption complex fingerprint region with prominent bands at 10.25, 11.10 and 11.35 μ attributed to the spirane junction.

Methanolysis in the absence of potassium acetate gave a product with broader melting range but with identical infrared spectrum.[27]

Bethogenin 3β-Methyl Ether.—A solution of 158 mg. (0.0002 mole) of kryptogenin 3β,27-di-*p*-toluenesulfonate (I) in 100 ml. of methanol was kept at reflux temperature for 6 hours. After the mixture had been diluted with 100 ml. of water and concentrated, the precipitate was collected by filtration, washed with water and dried. The anhydrous material (90 mg.) was recrystallized from methanol to give 50 mg. (54%). The analytical sample was recrystallized 6 times from a mixture of dichloromethane and methanol to afford rectangular needles, m.p. 167–182°; infrared spectrum: absence of carbonyl and hydroxyl absorption, complex fingerprint region with prominent bands at 10.25, 11.15 and 11.35 μ attributed to the spirane junction.

Anal. Calcd. for $C_{29}H_{46}O_4$ (458.66): C, 75.94; H, 10.11. Found: C, 75.73; H, 10.15.

3β,27-Diiodo-25α-5-cholesten-16,22-dione (Kryptogenin 3β,27-Diiodide).—A solution of 148 mg. (0.0002 mole) of kryptogenin 3β,27-di-*p*-toluenesulfonate (I) and 150 mg. (0.001 mole) of sodium iodide in 1 ml. of 2-butanone was stirred for 115 hours at 25°. After the mixture had been diluted with 10 ml. of water, the precipitate was collected by filtration, washed with water and dried. Recrystallization from a mixture of dichloromethane and methanol gave 80 mg. (62%) of prismatic needles, m.p. 157–158°.

Anal. Calcd. for $C_{27}H_{40}O_2I_2$ (650.41): C, 49.86; H, 6.20. Found: C, 50.25; H, 6.22.

3β-Hydroxy-27-iodo-25α-5-cholesten-16,22-dione (Kryptogenin 27-Iodide (III).—A solution of 584 mg. (0.001 mole) of kryptogenin 27-*p*-toluenesulfonate (II) and 300 mg. (0.002 mole) of sodium iodide in 3 ml. of 2-butanone was stirred for 60 hours at 25°. After the mixture had been diluted with 25 ml. of water, the precipitate was collected by filtration, washed with water and dried. Recrystallization from methanol afforded 460 mg. (85%) of white lustrous plates, m.p. 141–142°, $[\alpha]_D$ −161°; infrared spectrum: 5.82 (16-CO), 5.88 μ (22-CO).

Anal. Calcd. for $C_{27}H_{41}O_3I$ (540.52): C, 59.99; H, 7.65; I, 23.48. Found: C, 60.20; H, 7.76; I, 23.20.

3β-Hydroxy-27-phthalimido-25α-5-cholesten-16,22-dione (IV).—A mixture of 540 mg. (0.001 mole) of kryptogenin 27-iodide (III), 194 mg. (0.0011 mole) of potassium phthalimide and 2 ml. of dimethylformamide was stirred for 70 hours at 25°. After the turbid solution had been diluted with 20 ml. of 10% aqueous potassium chloride, the precipitate was collected by filtration, washed with water and dried. Recrystallization from a mixture of dichloromethane and methanol afforded felt-like needles; yield 450 mg. (80%), m.p. 212–214°, $[\alpha]_D$ −147°; infrared spectrum: 5.82 (16-CO), 5.88 (22-CO), 5.65, 5.90, 13.8, 14.1 μ (phthalimido).

Anal. Calcd. for $C_{35}H_{45}NO_5$ (559.72): C, 75.10; H, 8.10; N, 2.50. Found: C, 74.91; H, 8.01; N, 2.60.

3β-Acetoxy-27-phthalimido-25α-5-cholesten-16,22-dione.—A solution of 56 mg. (0.0001 mole) of 3β-hydroxy-27-phthalimido-25α-5-cholesten-16,22-dione (IV) in 1 ml. of acetic anhydride was heated under reflux for 10 minutes. After the mixture had been diluted with 20 ml. of water, the precipitate was collected by filtration, washed with water and dried. From methanol the compound deposited in needles as a solvate, m.p. 100–105°. Recrystallization from isopropyl alcohol gave 50 mg. (81%) of prisms, m.p. 172–174°.

Anal. Calcd. for $C_{37}H_{47}NO_6$ (601.76): C, 73.84; H, 7.87; N, 2.33. Found: C, 73.74; H, 7.88; N, 2.40.

3β,22-Dihydroxy-27-phthalimido-25α-5-furostene (VI.).—A mixture of 560 mg. (0.001 mole) of 3β-hydroxy-27-phthalimido-25α-5-cholesten-16,22-dione (IV), 600 mg. of potassium bicarbonate, 20 ml. of methanol and 10 ml. of water was heated under reflux for 2 hours. The initially insoluble phthalimido derivative rapidly entered into solution. The opalescent residue from concentration of the methanol was diluted with 50 ml. of water and was acidified with 1 ml. of 6 *N* aqueous hydrochloric acid. The highly hydrated, gelatinous precipitate was collected by filtration, repeatedly washed with water and dried to give 580 mg., m.p. *ca.* 105–110°.

To a solution of this phthalamidic acid derivative (V) in 100 ml. of isopropyl alcohol containing 56 mg. (0.001 mole) of potassium hydroxide was added 156 mg. (0.004 mole, 16 equivalents for one carbonyl group) of sodium borohydride. The mixture was stirred for 16 hours at 25°. After 1 ml. of 6 *N* aqueous hydrochloric acid had been added, the solution was concentrated to give a residue which was diluted with water. An ether extract of the precipitate was washed with water and was concentrated. To a solution of the dried residue (580 mg.) in 4 ml. of methanol was added, at 0°, 800 mg. (0.002 mole) of N-ethyl-N′-[2-(4-morpholinyl)-ethyl]-carbodiimide metho-*p*-toluenesulfonate.[28] After 15 hours at 0° (a precipitate had separated from the medium), the mixture was diluted with 25 ml. of water. An ether extract of the precipitate was washed with dilute aqueous ammonium hydroxide and with water and was concentrated. (No alkali-soluble material was evident upon subsequent acidification of the ammoniacal extract). A benzene solution of the dried remainder (570 mg.) was chromatographed over 17.0 g. of aluminum oxide to give the following fractions: (1) benzene, 5 mg.; (2) ether, 5 mg.; (3) ether–dichloro-methane (9:1), 210 mg.; (4) ether–dichloromethane (1:1), 20 mg.; (5) dichloromethane, 10 mg.; (6) dichloromethane–methanol (95:5), 230 mg.

Fraction 3 afforded, from methanol, 155 mg. (28%) of needles, m.p. 166–171°, $[\alpha]_D$ −54°; infrared spectrum: 5.65, 5.90, 13.8, 14.0 μ (phthalimido).

Anal. Calcd. for $C_{35}H_{47}NO_5$ (561.73): C, 74.83; H, 8.43; N, 2.49. Found: C, 74.99; H, 8.45; N, 2.54.

(26) L. F. Fieser, "Experiments in Organic Chemistry," third edition, D. C. Heath and Co., Boston, Mass., 1957, p. 145.

(27) Bethogenin exhibits a peculiarly erratic melting behavior even after recrystallization from alkaline solvent. Nevertheless, very low and imprecisely melting samples, such as a specimen which had been stored for 2 years (beginning m.p. now in the neighborhood of 120°) give infrared spectra devoid of carbonyl absorption and virtually identical with those of new and more sharply melting preparations.

(28) J. C. Sheehan and J. J. Hlavka, *J. Am. Chem. Soc.*, **79**, 4528 (1957).

3β,16,22-Trihydroxy-27-phthalimido-25α-5-cholestene (IX).—Fraction 6 of the chromatographic separation of the sodium borohydride reduction product of 3β-hydroxy-27-phthalimido-25α-5-cholesten-16,22-dione (IV) described immediately above was recrystallized from methanol to give 150 mg. (27%), m.p. 105–110°, $[\alpha]_D$ −45°; infrared spectrum; 5.65, 5.90, 13.8, 14.1 μ (phthalimido).

Anal. Calcd. for $C_{35}H_{49}NO_5$ (563.75): C, 74.56; H, 8.76; N, 2.48. Found: C, 73.93; H, 8.84; N, 2.62.

3β,16,22-Triacetoxy-27-phthalimido-25α-5-cholestene.—A solution of 113 mg. (0.0002 mole) of 3β,16,22-trihydroxy-27-phthalimido-25α-5-cholestene (IX) in 2 ml. of acetic anhydride was heated under reflux for 30 minutes. After the solution had been concentrated, the crystalline residue was recrystallized from methanol to give 120 mg. (80%) of plates, m.p. 200–203°, $[\alpha]_D$ +7°; infrared spectrum: 5.80 (acetoxy), 5.65, 5.90, 13.8, 14.1 μ (phthalimido).

Anal. Calcd. for $C_{41}H_{55}NO_8$ (689.86): C, 71.38; H, 8.04; N, 2.03. Found: C, 71.18; H, 7.86; N, 2.41.

Solasodine [3β-Hydroxy-22(27)-imino-25α-5-furostene] (VII).—A solution of 140 mg. (0.00025 mole) of 3β,22-dihydroxy-27-phthalimido-25α,5-furostene (VI), 64 mg. (0.002 mole) of hydrazine and 1 ml. of dichloromethane in 1 ml. of methanol was allowed to remain at 25° for 15 hours. A felt-like deposit, presumably the hydrazine salt of phthalhydrazide[29] had begun to separate after 2 hours. The mixture was diluted with 60 ml. of ether and with 20 ml. of water. The organic phase was washed with dilute aqueous ammonium hydroxide and with water and was concentrated. A solution of the residue and 1 ml. of 6 *N* aqueous hydrochloric acid in 5 ml. of ethanol was kept at reflux temperature for 1 hour. After 2.5 ml. of 3 *N* aqueous potassium hydroxide had been added, the mixture was concentrated. An ether extract of the precipitate was washed with water and was concentrated. A benzene solution of the dried residue (110 mg.) was chromatographed over 3.3 g. of aluminum oxide. The material which eluted with ether (80 mg.) was recrystallized from methanol to give lustrous white plates; yield 35 mg. (35%), m.p. 198–201° (on the micro hot-stage the plates slowly rearranged to long slender needles as the melting point was approached), mixed m.p. with a specimen of naturally occurring solasodine (m.p. 199–202°) 198–202°, $[\alpha]_D$ −102° (methanol); infrared spectrum identical with that given by a sample of naturally occurring solasodine: 10.3, 10.4, 11.2, 11.5 μ (azaoxaspirane bands).

Anal. Calcd. for $C_{27}H_{43}NO_2$ (413.62): C, 78.40; H, 10.48; N, 3.39. Found: C, 78.24; H, 10.54; N, 3.53.

The picrate was prepared in ethanol and was recrystallized from methanol; m.p. 141–142°.

Anal. Calcd. for $C_{33}H_{46}N_4O_9$ (642.74); C, 61.66; H, 7.21; N, 8.72. Found: C, 62.00; H, 7.30; N, 8.95.

Diosgenin and 3β,16,22,27-Tetrahydroxy-25α,5-cholestene from Kryptogenin.—To a solution of 645 mg. (0.0015 mole) of kryptogenin in 150 ml. of isopropyl alcohol was added 234 mg. (0.006 mole) of sodium borohydride. After the mixture had been stirred for 19 hours at 25°, it was acidified cautiously with 5 ml. of 6 *N* aqueous hydrochloric acid and was concentrated. The residue was diluted with 50 ml. of water and was extracted with ether. The organic phase was washed with dilute aqueous ammonium hydroxide and with water and was concentrated. A solution of the dried residue in a mixture of ether and ethyl acetate (1:1) was chromatographed over 19.5 g. of aluminum oxide to give the following fractions: (1) ether–ethyl acetate (1:1), 350 mg.; (2) ether–methanol (95:5), 300 mg.

Fraction 1 was recrystallized from a mixture of dichloromethane and methanol to give 300 mg. (48%) of diosgenin, m.p. 198–205°, $[\alpha]_D$ −116°; infrared spectrum identical with that given by a specimen of naturally occurring diosgenin. A solution of 200 mg. of this product in 5 ml. of acetic anhydride was heated under reflux for 15 minutes. The residue from concentration of the solution was recrystallized from a mixture of dichloromethane and methanol to afford diosgenin acetate, m.p. 195–197°; infrared spectrum identical with that provided by a sample prepared from naturally occurring diosgenin.

Anal. Calcd. for $C_{29}H_{44}O_4$ (456.64): C, 76.27; H, 9.71. Found: C, 76.23; H, 9.73.

Fraction 2 was recrystallized from methanol to give 72 mg. (11%) of 3β,16,22,27-tetrahydroxy-25α-5-cholestene, m.p. 205–210°.[30] The analytical sample was recrystallized five times from methanol, m.p. 210–213°, $[\alpha]_D$ −25° (chloroform–ethanol, 1:1); infrared spectrum: absence of functional bands other than hydroxyl.

Anal. Calcd. for $C_{27}H_{46}O_4$ (434.64): C, 74.61; H, 10.67. Found: C, 73.91; H, 10.62.

A solution of 80 mg. of the tetrol in 2 ml. of acetic anhydride was heated under reflux for 1 hour. The residue from concentration of the solution was recrystallized from petroleum ether to give 85 mg. (76%) of dense prisms of 3β,16,22,27-tetraacetoxy-25α-5-cholestene,[30] m.p. 110–113°; the analytical sample was recrystallized 6 times from petroleum ether; m.p. 113–115°, $[\alpha]_D$ +3°; infrared spectrum: 5.80 μ (acetoxy).

Anal. Calcd. for $C_{35}H_{54}O_8$ (602.78); C, 69.74; H, 9.03. Found: C, 69.25; H, 8.72.

A second sodium borohydride reduction of kryptogenin was carried out exactly as described above with the exception that treatment with hydrochloric acid was omitted in an endeavor to isolate "16-dihydro-kryptogenin."[12] A solution of the product in a mixture of ether and ethyl acetate (1:1) was chromatographed over 19.5 g. of aluminum oxide to give the following fractions: (1) ether–ethyl acetate (1:1), 120 mg.; (2) ethyl acetate, 40 mg.; (3) ether–methanol (95:5), 500 mg.

Fraction 1 was recrystallized from a mixture of dichloromethane and methanol to give 62 mg. (10%) of diosgenin, m.p. 195–200°. Fraction 3 was recrystallized from a mixture of dichloromethane and methanol to afford 230 mg. (35%) of 3β,16,22,27-tetrahydroxy-25α-5-cholestene, m.p. 205–210°. The mother liquors from recrystallization of the tetrol, following treatment with dilute aqueous hydrochloric acid, gave 42 mg. (7%) of diosgenin, m.p. 195–200°, suggesting the presence in fraction 3 of the hemiketal "16-dihydrokryptogenin."

In a third experiment (actually the first carried out) in which the isopropyl alcohol solution had been kept at reflux temperature for 15 minutes following acidification with hydrochloric acid, diosgenin (48%) was the sole product isolated. The ether–methanol (95:5) eluate (250 mg.) failed to yield a crystalline product.

Pseudosarsasapogenin Series

3β-Hydroxy-27-iodo-5β-25β-20(22)-furostene (Pseudosarsasapogenin 27-Iodide) (X).—To a solution of 8.33 g. (0.02 mole) of pseudosarsasapogenin,[15] m.p. 169–171°,[31] in 50 ml. of pyridine, at 0°, was added, in 12 portions over a period of 6 hours, 7.63 g. (0.04 mole) of *p*-toluenesulfonyl chloride. After 15 hours at 0°, 1200 ml. of water was added. The major fraction of the product deposited as a viscous oil. After the supernatant liquor had been removed by decantation, the deposit was washed by swirling, followed by decantation, with successive amounts of cold water. The first supernatant fluid and the washings were filtered by gravity to retrieve particles in suspension. The pink, difficultly mobile resinous product was dried. A solution of the anhydrous substance and 6.0 g. (0.04 mole) of sodium iodide in 150 ml. of 2-butanone was stirred for 100 hours at 25°. A crystalline precipitate of sodium *p*-toluenesulfonate had begun to separate after 6 hours. After the mixture had been diluted with 1800 ml. of water and kept at 0° for 15 hours, the precipitate was collected by filtration, washed with water and dried. A benzene solution of the anhydrous product (10.5 g.) was chromatographed over 315 g. of aluminum oxide to give the following fractions: (1) benzene, followed by ether, 2.5 g. of colorless oil; (2) ethyl acetate, 7.5 g. Fraction 2 was crystallized from 70 ml. of methanol to give, after 15 hours at 0°, 4.83 g. (46.5%) of white, felt-like needles. The substance melted initially rather unsharply in the neighborhood of 60°; as the temperature of the hot stage was elevated slowly, the melt virtually completely resolidified to characteristic sheaves of needles which, in turn, melted at 110–115°, $[\alpha]_D$ +15°; infrared spectrum: 5.92 (m) μ (vinyl ether).

Anal. Calcd. for $C_{27}H_{43}O_2I$ (526.53): C, 61.59; H, 8.23; I, 24.12. Found: C, 61.38; H, 8.42; I, 24.25.

(29) R. G. Jones, *J. Am. Chem. Soc.*, **78**, 159 (1956).

(30) *Cf.* the preparation of the tetrol by lithium aluminum hydride reduction of kryptogenin: A. Sandoval, J. Romo, G. Rosenkranz, St. Kaufmann and C. Djerassi, *J. Am. Chem. Soc.*, **73**, 3820 (1951).

(31) R. E. Marker and E. Rohrmann, *ibid.*, **62**, 518 (1940).

3β-Hydroxy-27-phthalimido-5β-25β-20(22)-furostene (XI).—A mixture of 2.63 g. (0.005 mole) of 3β-hydroxy-27-iodo-5β-25β-20(22)-furostene (X), 1.11 g. (0.006 mole) of potassium phthalimide and 25 ml. of dimethylformamide was stirred for 115 hours at 25°. After the solution had been diluted with 100 ml. of water and stored for 5 hours at 0°, the precipitate was collected by filtration, washed with water and dried. Recrystallization from a mixture of dichloromethane and methanol gave 2.05 g. (75%) of needles, m.p. 170–185°. The analytical sample was recrystallized five times from a mixture of dichloromethane and methanol, m.p. 185–187°, $[\alpha]_D$ +5°; infrared spectrum: 5.92 (m) (shoulder) (vinyl ether), 5.70, 5.90, 13.85, 14.0 μ (phthalimido).

Anal. Calcd. for $C_{35}H_{47}NO_4$ (545.74): C, 77.02; H, 8.68; N, 2.57. Found: C, 76.71; H, 8.64; N, 2.81.

3β-Acetoxy-27-phthalimido-5β-25β-20(22)-furostene.—A solution of 55 mg. (0.0001 mole) of 3β-hydroxy-27-phthalimido-5β-25β-20(22)-furostene (XI) in 1 ml. of acetic anhydride was heated under reflux for 10 minutes. After the mixture had been diluted with 25 ml. of water and stored at 0° for 30 minutes, the precipitate was collected by filtration, washed with water and dried. Recrystallization from methanol afforded 50 mg. (86%), m.p. 132–134°.

Anal. Calcd. for $C_{37}H_{49}NO_5$ (587.77): C, 75.60; H, 8.40; N, 2.38. Found: C, 75.74; H, 8.40; N, 2.40.

3β-Hydroxy-27-amino-5β-25β-20(22)-furostene (XII).—A solution of 140 mg. (0.00025 mole) of 3β-hydroxy-27-phthalimido-5β,25β-20(22)-furostene (XI), 64 mg. (0.002 mole) of hydrazine and 1 ml. of absolute ethanol in 1 ml. of dichloromethane was kept at 25° for 20 hours. A heavy precipitate of the hydrazine salt of phthalhydrazide pervaded the medium. After the mixture had been diluted with 75 ml. of ether and 15 ml. of water, the organic phase was washed with dilute aqueous ammonium hydroxide and with water and was concentrated. The residue was triturated with 10 ml. of 10% aqueous acetic acid. After clarification by gravity filtration, the solution was basified with dilute aqueous ammonium hydroxide. An ether extract of the suspended mass was washed with water and was concentrated. Recrystallization of the dried remainder from ethyl acetate gave 65 mg. (65%) of tiny needles, m.p. 142–147°, $[\alpha]_D$ +11°; infrared spectrum: 5.92(m) (vinyl ether), 3.0–3.2 (multiple), 6.25(w) μ (NH_2).

Anal. Calcd. for $C_{27}H_{45}NO_2$ (415.64): C, 78.02; H, 10.91; N, 3.37. Found: C, 77.82; H, 10.85; N, 3.26.

3β-Hydroxy-27-acetamino-5β-25β-20(22)-furostene.—To a solution of the total hydrazinolysis product from 140 mg. (0.00025 mole) of 3β-hydroxy-27-phthalimido-5β,25β-20(22)-furostene (XI) in 2 ml. of pyridine was added 150 mg. of acetic anhydride. After 20 hours at 0°, the mixture was diluted with 40 ml. of 10% aqueous potassium chloride to give a precipitate which was collected by filtration, washed with water and dissolved in 5 ml. of ethanol containing 0.3 ml. of 3 *N* aqueous potassium hydroxide. After 15 minutes at reflux temperature, the solution was concentrated and diluted with water to give a precipitate which was collected by filtration, washed with water and dried. Recrystallization from ethyl acetate afforded 80 mg. (70%) of broad needles, m.p. 154–157°; infrared spectrum: 5.92(m) (vinyl ether), 6.10, 6.50 μ ($NHCOCH_3$).

Anal. Calcd. for $C_{29}H_{47}NO_3$ (457.67): C, 76.10; H, 10.35; N, 3.06. Found: C, 75.77; H, 10.32; N, 3.12.

3β-Acetoxy-27-acetamino-5β-25β-20(22)-furostene.—A solution of the total hydrazinolysis product from 140 mg. (0.00025 mole) of 3β-hydroxy-27-phthalimido-5β-25β-20-(22)-furostene (XI) and 1 ml. of acetic anhydride in 2 ml. of pyridine was kept at 25° for 45 hours. After the mixture had been diluted with 15 ml. of saturated aqueous potassium chloride, the resinous deposit was collected by filtration, washed with water and dried. A benzene solution of the anhydrous material was chromatographed over 3.0 g. of aluminum oxide. After 20 mg. of oil which eluted with benzene had been discarded, the product (80 mg.) appeared with ether. Recrystallization from a mixture of dichloromethane and petroleum ether gave 50 mg. (40%), m.p. 148–152°, $[\alpha]_D$ +13°; infrared spectrum: 3.05 (NH), 5.80 (acetoxy), 5.92(m) (vinyl ether), 6.10, 6.50 μ ($NHCOCH_3$).

Anal. Calcd. for $C_{31}H_{49}NO_4$ (499.71): C, 74.51; H, 9.85; N, 2.80. Found: C, 74.23; H, 9.52; N, 2.80.

5β-Tomatidine [3β-Hydroxy-22(27)-imino-5β-25β-furostane] (XIII).—A solution of the total hydrazinolysis product from 140 mg. (0.00025 mole) of 3β-hydroxy-27-phthalimido-5β-25β-20(22)-furostene (XI) and 3 ml. of 6 *N* aqueous hydrochloric acid in 3 ml. of ethanol was heated under reflux for 2 hours. The solution was diluted with 10 ml. of ethanol and was basified with 8 ml. of 3 *N* aqueous potassium hydroxide. After 3 minutes at reflux temperature, the solution was concentrated to give a residue which was diluted with water. An ether extract of the precipitate was washed with water and was concentrated. A benzene solution of the dried residue (100 mg.) was chromatographed over 3.0 g. of aluminum oxide. After negligible traces had been removed with benzene, the product appeared in the ether eluate. Recrystallization from ethyl acetate gave 55 mg. (55%), m.p. 212–217°. The analytical sample was recrystallized from ethyl acetate; m.p. 221–223°, $[\alpha]_D$ −17° (methanol); infrared spectrum: complex fingerprint region with prominent azaoxaspirane bands at 10.25, 11.20, 11.50, 11.80 μ.

Anal. Calcd. for $C_{27}H_{45}NO_2$ (415.64): C, 78.02; H, 10.91; N, 3.37. Found: C, 78.23; H, 10.85; N, 3.25.

5β-Tomatidine Hydrochloride.—A solution of 52 mg. (0.000125 mole) of 5β-tomatidine (XIII) and 1.25 ml. of 0.1 *N* aqueous hydrochloric acid in 10 ml. of ethanol was concentrated. The dried remainder was recrystallized from a mixture of methanol and ethyl acetate; infrared spectrum: 3.25, 4.00(m), 4.20(w) μ (ammonium bands[32]); absorption in 10–12 μ region considerably weaker than in the case of the free base.

Anal. Calcd. for $C_{27}H_{46}NO_2Cl$ (452.10): N, 3.10. Found: N, 3.28.

3β,N-Diacetyl-5β-tomatidine.—A solution of 104 mg. (0.00025 mole) of 5β-tomatidine (XIII) and 1 ml. of acetic anhydride in 2 ml. of pyridine was kept at 0° for 4 days. After the mixture had been diluted with 10 ml. of saturated aqueous potassium chloride, the precipitate was collected by filtration, washed with water and dried. Recrystallization from methanol gave 80 mg. (64%) of needles, m.p. 182–187° when the substance was placed on the hot-stage at *ca.* 170°, $[\alpha]_D$ +24°; infrared spectrum: 5.80 (acetoxy), 6.10 μ (tertiary amide).

Anal. Calcd. for $C_{31}H_{49}NO_4$ (499.71): C, 74.51; H, 9.85; N, 2.80. Found: C, 74.24: H, 9.93; N, 2.82.

The combined mother liquors from preparation of the analytical sample were concentrated to give a residue which was dissolved in glacial acetic acid. After 15 minutes at reflux temperature, the solution was diluted with water to give a precipitate which was collected by filtration, washed with water and dried. Recrystallization from a mixture of dichloromethane and petroleum ether gave a product with properties and infrared spectrum identical with those of 3β-acetoxy-27-acetamino-5β-25β-20(22)-furostene.

3β-Hydroxy-27-methylamino-5β-25β-20(22)-furostene (XXIII).—A solution of 270 mg. (0.0005 mole) of 3β-hydroxy-27-iodo-5β-25β-20(22)-furostene (X) in 10 ml. of 20% methylamine in methanol was sealed in a Pyrex tube and kept at 90° for 20 hours. The colorless solution was concentrated to give a residue which was dissolved in 3 ml. of ethanol containing 0.5 ml. of 3 *N* aqueous potassium hydroxide. After 5 minutes at reflux temperature, the solution was diluted with water to give a precipitate which was extracted with ether. The organic phase was washed with water and was concentrated. Recrystallization of the dried residue from ethyl acetate afforded 172 mg. (80%), m.p. 139–142°, $[\alpha]_D$ +13°; infrared spectrum: 3.00–3.20 (multiple) (NH and OH), 5.92(m) μ (vinyl ether).

Anal. Calcd. for $C_{28}H_{47}NO_2$ (429.66): C, 78.27; H, 11.03; N, 3.26. Found: C, 78.30; H, 10.93; N, 3.33.

N-Methyl-N-benzyl-*p*-toluenesulfonamide.—To a solution of 1.85 g. (0.01 mole) of N-methyl-*p*-toluenesulfonamide and 1.18 g. (0.03 mole) of potassium hydroxide in 10 ml. of water was added a solution of 5.6 g. (0.10 mole) of potassium hydroxide in 10 ml. of water. After 3 hours at 0°, the white lustrous plates were collected by filtration on a sintered glass funnel, washed with acetone and dried to give 1.95 g. of potassium N-methyl-*p*-toluenesulfonamide.

(32) For a general discussion of infrared spectra of amine salts see R. A. Heacock and L. Marion, *Can. J. Chem.*, **34**, 1782 (1956).

A solution of 334 mg. (0.0015 mole) of the potassium derivative and 127 mg. (0.001 mole) of benzyl chloride in 2 ml. of dimethylformamide was stirred for 20 hours at 25°. After dilution with ether, the solution was washed with water, with 3 *N* aqueous potassium hydroxide and with water and was concentrated. The dried residue was recrystallized from a mixture of dichloromethane and petroleum ether to give 260 mg. (95%), m.p. 93–96°. The analytical sample was recrystallized three times from methanol; m.p. 98–99°[33]; infrared spectrum: 7.50, 8.60 μ (SO_2N).

Anal. Calcd. for $C_{15}H_{17}NSO_2$ (275.37): C, 65.42; H, 6.22; N, 5.09. Found: C, 65.61; H, 6.28; N, 5.10.

3β-Hydroxy-27-N-methyl-*p*-toluenesulfonamido-5β-25β-20(22)-furostene (XXII). (A).—A solution of 263 mg. (0.0005 mole) of 3β-hydroxy-27-iodo-5β-25β-20(22)-furostene (X) and 170 mg. (0.00075 mole) of potassium N-methyl-*p*-toluenesulfonamide (prepared as described immediately above) in 1 ml. of dimethylformamide was stirred for 20 hours at 25°. After dilution with ether, the solution was washed twice with 1 *N* aqueous potassium hydroxide and three times with water and was concentrated. A benzene solution of the dried residue (300 mg.) was chromatographed over 9.0 g. of aluminum oxide. The fraction (260 mg.) which eluted with benzene–ether (1:1) was recrystallized from a mixture of dichloromethane and petroleum ether to give 75 mg. (25%). (This value does not represent the actual conversion since additional product can be secured by careful processing of the mother liquors. The substance crystallizes very slowly and tends to separate as an oil if solutions are not properly dilute. An appropriate single solvent was not found.) M.p. 110–115°; infrared spectrum: 7.50, 8.60 (SO_2N), 5.92(m) μ (vinyl ether).

Anal. Calcd. for $C_{35}H_{53}SO_4N$ (583.85): C, 72.00; H, 9.15; N, 2.40; S, 5.49. Found: C, 71.69; H, 9.11; N, 2.41; S, 5.61.

(B).—A mixture of 43 mg. (0.0001 mole) of 3β-hydroxy-27-methylamino-5β-25β-20(22)-furostene (XXIII), 95 mg. (0.0005 mole) of *p*-toluenesulfonyl chloride, 1 ml. of benzene and 1 ml. of 3 *N* aqueous potassium hydroxide was stirred vigorously for 3 hours at 25°. After dilution with ether, the organic phase was washed with water and was concentrated. Recrystallization of the dried residue from a mixture of dichloromethane and petroleum ether gave 50 mg. (86%), m.p. 110–115°; infrared spectrum indistinguishable from that given by the product prepared from 3β-hydroxy-27-iodo-5β-25β-20(22)-furostene (X) and potassium N-methyl-*p*-toluenesulfonamide.

N-Methyl-5β-tomatidine [3β-Hydroxy-22(27)-methyl-imino-5β-25β-furostane] (XXIV).—A solution of 86 mg. (0.0002 mole) of 3β-hydroxy-27-methylamino-5β-25β-20-(22)-furostene (XXIII) and 2 ml. of 1 *N* aqueous hydrochloric acid in 2 ml. of methanol was heated under reflux for 2 hours. The solution was diluted with 3 ml. of methanol and was basified with 1 ml. of 3 *N* aqueous potassium hydroxide. After 5 minutes at reflux temperature the mixture was diluted with water to give a precipitate which was extracted with ether. The organic phase was washed with water and was concentrated. The dried residue was recrystallized from ethyl acetate to give 43 mg. (50%), m.p. 232–235°; infrared spectrum: rich fingerprint region with prominent azaoxaspirane bands at 10.2, 11.0, 11.2, 11.4, 11.6 μ.

Anal. Calcd. for $C_{28}H_{47}NO_2$ (429.66): C, 78.27; H, 11.03. Found: C, 78.25; H, 11.07.

N-Methyl-5β-tomatidine Hydrochloride.—A solution of 43 mg. (0.0001 mole) of N-methyl-5β-tomatidine (XXIV) and 0.1 ml. of 1 *N* aqueous hydrochloric acid in 3 ml. of methanol was concentrated. The dried remainder was recrystallized from a mixture of methanol and ethyl acetate; infrared spectrum: 4.00(w), 4.20, 4.35(m) μ (ammonium bands[32]).

Anal. Calcd. for $C_{28}H_{48}NO_2Cl$(466.13): N, 3.00. Found: N, 3.06.

3β-Acetyl-N-methyl-5β-tomatidine.—A solution of 43 mg. (0.0001 mole) of N-methyl-5β-tomatidine (XXIV) and 0.5 ml. of acetic anhydride in 1 ml. of pyridine was kept at 0° for 15 hours. The mixture was diluted with 10 ml. of saturated aqueous potassium chloride to give a precipitate which was collected by filtration, washed with water and dried. Recrystallization from methanol gave 35 mg. (74%) of needles which were freely soluble in 20% aqueous acetic acid; m.p. 215–217°; infrared spectrum: 5.80 (acetoxy), 10.2, 11.0, 11.2, 11.4, 11.6 μ (azaoxaspirane).

Anal. Calcd. for $C_{30}H_{49}NO_3$ (471.70): C, 76.38; H, 10.46; N, 2.97. Found: C, 76.33; H, 10.43; N, 3.07.

Pseudoneotigogenin Series

3β-Hydroxy-27-iodo-5α-25β-20(22)-furostene (Pseudoneotigogenin 27-Iodide) (XV).—A solution of 1.5 g. (0.0033 mole) of neotigogenin acetate, m.p. 178–181°, and 0.50 g. of pyridine hydrochloride in 7.5 ml. of acetic anhydride was heated under reflux for 5 hours. The solution was diluted with 100 ml. of water and was stored at 0° for 15 hours to give a viscous deposit which was isolated and dissolved in 50 ml. of methanol containing 1.0 g. of potassium hydroxide and 5 ml. of water. After 1 hour at reflux temperature the solution was concentrated and was diluted with water. An ether extract of the precipitate was washed with water and was concentrated. The dried residue was recrystallized from acetone to give 1.09 g. (80%) of pseudoneotigogenin (XIV), m.p. 165–175°[34]; infrared spectrum: 5.92(m) μ (vinyl ether).

To a solution of 0.99 g. (0.0024 mole) of this product in 6 ml. of pyridine at 0° was added, in 6 portions over 3 hours, 0.76 g. (0.004 mole) of *p*-toluenesulfonyl chloride. After 15 hours at 0°, the mixture was diluted with 100 ml. of water to give a viscous deposit which was isolated as was the corresponding product from pseudosarsasapogenin. A solution of the dried material and 750 mg. (0.005 mole) of sodium iodide in 20 ml. of 2-butanone was stirred for 60 hours at 25°. After the mixture had been diluted with 100 ml. of water and kept at 0° for 5 hours, the deposit was collected, washed with water and dried. A benzene solution of the resinous solid (1.2 g.) was chromatographed over 36 g. of aluminum oxide to give the following fractions: (1) benzene, 280 mg.; (2) ether–ethyl acetate (1:1), 1 g. Fraction 2 was recrystallized from methanol to afford 500 mg. (40%) of white lustrous plates, m.p. 127–129°; when the melt had cooled to *ca.* 110°, it solidified to characteristic sheaves of needles, $[\alpha]_D$ +9°; infrared spectrum: 5.92(m) μ (vinyl ether).

Anal. Calcd. for $C_{27}H_{43}O_2I$ (526.53): C, 61.59; H, 8.23; I, 24.12. Found: C, 61.86; H, 8.42; I, 23.92.

3β-Hydroxy-27-phthalimido-5α-25β-20(22)-furostene (XVI).—A solution of 131 mg. (0.00025 mole) of 3β-hydroxy-27-iodo-5α-25β-20(22)-furostene (XV) and 74 mg. (0.0004 mole) of potassium phthalimide in 1 ml. of dimethylformamide was stirred for 65 hours at 25°. After the mixture had been diluted with 10 ml. of water, the precipitate was collected by filtration, washed with water and dried. Recrystallization from methanol gave 110 mg. (81%) of plates, initial m.p. 120–122°; as the temperature was raised, the molten substance crystallized throughout the entire field to characteristic sheaves and rosettes of needles which re-melted at 170–175°, $[\alpha]_D$ +3°; infrared spectrum: 5.92(m) (vinyl ether), 5.70(m), 5.90, 13.9, 14.1 μ (phthalimido).

Anal. Calcd. for $C_{35}H_{47}NO_4$ (545.74): C, 77.02; H, 8.68; N, 2.57. Found: C, 76.90; H, 9.02; N, 2.58.

3β-Hydroxy-27-amino-5α-25β-20(22)-furostene (XVII).—A solution of 140 mg. (0.00025 mole) of 3β-hydroxy-27-phthalimido-5α-25β-20(22)-furostene (XVI), 64 mg. (0.002 mole) of hydrazine and 1 ml. of dichloromethane in 1 ml. of methanol was kept at 25° for 24 hours. After dilution with 60 ml. of ether, the solution was washed with dilute aqueous ammonium hydroxide and with water and was concentrated to give a residue which was triturated with 10 ml. of 10% aqueous acetic acid. After clarification by filtration, the solution was basified with dilute aqueous ammonium hydroxide. An ether extract of the precipitate was washed with water and was concentrated. Recrystallization of the dried residue from ethyl acetate (in which the amine is rather difficultly soluble) afforded 70 mg. (70%) of fine needles, m.p. 165–170°, $[\alpha]_D$ +10°; infrared spec-

(33) E. L. Holmes and C. K. Ingold, *J. Chem. Soc.*, 1813 (1925), report 95°.

(34) *Cf.* R. K. Callow and V. H. T. James, *J. Chem. Soc.*, 1674 (1955), for preparation with refluxing octanoic acid containing a small quantity of acetic anhydride.

trum: 3.0–3.2 (multiple), 6.25(w) (NH_2), 5.92(m) μ (vinyl ether).

Anal. Calcd. for $C_{27}H_{45}NO_2$ (415.64): C, 78.02; H, 10.91; N, 3.37. Found: C, 77.93; H, 10.86; N, 3.21.

3β-Acetoxy-27-acetamino-5α-25β-20(22)-furostene.—To a solution of 104 mg. (0.00025 mole) of crude 3β-hydroxy-27-amino-5α-25β-20(22)-furostene (XVII) (recovered from mother liquors) in 1 ml. of pyridine was added 1 ml. of acetic anhydride. After 45 hours at 0°, the solution was diluted with 40 ml. of water to give a precipitate which was collected, washed with water and dried. A benzene solution of the anhydrous product was chromatographed over 3.0 g. of aluminum oxide. The material (40 mg.) which eluted with benzene was recrystallized from a mixture of dichloromethane and petroleum ether; m.p. 129–132°, $[\alpha]_D$ +4°; infrared spectrum: 3.00 (NH), 5.80 (acetoxy), 5.92(m) (shoulder) (vinyl ether), 6.10, 6.45 μ ($NHCOCH_3$).[35]

3β-Hydroxy-27-acetamino-5α-25β-20(22)-furostene.—A solution of the total hydrazinolysis product from 140 mg. (0.00025 mole) of 3β-hydroxy-27-phthalimido-5α-25β-20(22)-furostene (XVI) in 1 ml. of pyridine was treated at 0° with 0.20 ml. of acetic anhydride. After 15 hours at 0°, the solution was diluted with 25 ml. of water to give a deposit which was collected, washed with water and dissolved in 10 ml. of methanol containing 0.25 ml. (0.00075 mole) of 3 *N* aqueous potassium hydroxide. After 15 hours at 0°, the solution was concentrated to give a residue which was extracted with ether. The organic phase was washed with water and was concentrated. Recrystallization of the dried residue from a mixture of methanol and ethyl acetate gave 80 mg. (70%) of small plates (very slightly soluble in ethyl acetate alone). Six recrystallizations furnished the analytical sample, m.p. 183–190°, $[\alpha]_D$ +12°; infrared spectrum: 5.92(m) (vinyl ether), 6.05, 6.50 μ ($NHCOCH_3$).[36]

Anal. Calcd. for $C_{29}H_{47}NO_3$ (457.67): C, 76.10; H, 10.35; N, 3.06. Found: C, 76.01; H, 10.10; N, 2.87.

Tomatidine [3β-Hydroxy-22(27)-imino-5α-25β-furostane] (XVIII).—A solution of the total hydrazinolysis product from 140 mg. (0.00025 mole) of 3β-hydroxy-27-phthalimido-5α-25β-20(22)-furostene (XVI) in 3 ml. of ethanol and 3 ml. of 6 *N* hydrochloric acid was heated under reflux for 1 hour. The solution was basified with 3 *N* aqueous potassium hydroxide and was concentrated to give a precipitate which was extracted with ether. The extract was washed with water and was concentrated to give a residue which was triturated with 10 ml. of 10% aqueous acetic acid. After clarification, the filtrate was basified with dilute aqueous ammonium hydroxide to give a precipitate which was extracted with ether. The organic phase was washed with water and was concentrated. A benzene solution of the dried residue was chromatographed over 3.0 g. of aluminum oxide. The material (80 mg.) which eluted with benzene was recrystallized from ethyl acetate to afford 50 mg. (50%) of glistening plates. Three additional recrystallizations gave the analytical sample, m.p. 202–206°, mixed m.p. with a specimen of naturally occurring tomatidine (m.p. 203–208°) 202–208°, $[\alpha]$ +8°; infrared spectrum indistinguishable from that of the natural product: 10.1, 10.25, 10.4, 11.1, 11.5 μ (azaoxaspirane bands).

Anal. Calcd. for $C_{27}H_{45}NO_2$ (415.64): C, 78.02; H, 10.91; N, 3.37. Found: C, 77.69; H, 10.83; N, 3.34.

Tomatidine Hydrochloride.—A solution of 21 mg. of synthetic tomatidine and 0.5 ml. of 0.1 *N* aqueous hydrochloric acid in 2 ml. of ethanol was concentrated. The dried residue was recrystallized from absolute ethanol; m.p. 265–270°, $[\alpha]_D$ −5° (methanol); infrared spectrum: 3.85, 4.10(m), 4.30(w) μ (ammonium bands[32]).

Anal. Calcd. for $C_{27}H_{46}NO_2Cl$ (452.11): C, 71.73; H, 10.26; N, 3.10. Found: C, 71.35; H, 10.02; N, 3.11.

3β,N-Diacetyltomatidine.—A solution of 45 mg. of synthetic tomatidine and 1 ml. of acetic anhydride in 2 ml. of pyridine was kept at 0° for 18 hours. After the solution had been diluted with 20 ml. of water, the precipitate was collected, washed with water and dried. Recrystallization from methanol gave 30 mg. of broad needles, m.p. 181–190°; mixed m.p. with a sample of the diacetyl derivative (m.p. 183–191°) prepared from the natural product, 181–191°; infrared spectrum indistinguishable from that of the derivative prepared from the natural product: 5.80 (acetoxy), 6.05 μ (tertiary amide).

Anal. Calcd. for $C_{31}H_{49}NO_4$ (499.71): C, 74.51; H, 9.88; N, 2.80. Found: C, 74.54; H, 9.86; N, 2.72.

27-Iodo-5α-25β-2(3),20(22)-furostadiene.—Fraction 1 from the chromatographic separation of 3β-hydroxy-27-iodo-5α-25β-20(22)-furostene (XV) (described in the first paragraph of the pseudoneotigogenin section) was dissolved in a mixture of petroleum ether and benzene (4:1) and rechromatographed over 8.4 g. of aluminum oxide. The colorless oil which eluted with a mixture of petroleum ether and benzene (4:1) was recrystallized from a mixture of dichloromethane and methanol to give long, slender needles (very slightly soluble in methanol alone), m.p. 103–105°; infrared spectrum: 5.92(m) μ (vinyl ether).

Anal. Calcd. for $C_{27}H_{41}OI$ (508.52): C, 63.76; H, 8.13. Found: C, 64.05; H, 8.21.

27-Phthalimido-5α-25β-2(3),20(22)-furostadiene.—A solution of 152 mg. (0.003 mole) of 27-iodo-5α-25β-2(3),-20(22)-furostadiene and 111 mg. (0.0006 mole) of potassium phthalimide in 2 ml. of dimethylformamide was stirred for 96 hours at 25°. After the mixture had been diluted with water, the precipitate was collected, washed with water and dried. Recrystallization from a mixture of dichloromethane and methanol gave 110 mg. (70%), m.p. 110–115°. Five recrystallizations furnished the analytical sample as large plates, m.p. 116–122°; infrared spectrum: 5.92(m) (shoulder) (vinyl ether), 5.70(m), 5.90, 13.9, 14.1 μ (phthalimido).

Anal. Calcd. for $C_{35}H_{41}NO_3$ (527.72): C, 79.65; H, 8.60; N, 2.65. Found: C, 79.36; H, 8.47; N, 2.71.

Cleavage of the phthalimido moiety with hydrazine in dichloromethane and methanol, followed by treatment with 3 *N* aqueous ethanolic hydrochloric acid at reflux temperature for 2 hours, gave, from ethyl acetate, plates, m.p. 162–172°, of **22(27)-imino-5α-25β-2(3)-furostene** ($\Delta^{2(3)}$-**dehydro-tomatidine**). Insufficient material remained for complete characterization; infrared spectrum: 10.25, 11.1, 11.2, 11.5 μ (azaoxaspirane bands).

Pseudodiosgenin Series

Pseudodiosgenin 3β,27-Dibutyrate.—A solution of 1.0 g. of diosgenin in 10 ml. of butyric anhydride was heated under reflux for 15 hours. The solution was concentrated to give a residue which was recrystallized from methanol; m.p. 79–80°, $[\alpha]_D$ −17° (methanol); infrared spectrum: 5.80 (ester), 5.92(m) (shoulder) (vinyl ether), 8.45 μ (butyrate).

Anal. Calcd. for $C_{35}H_{54}O_5$ (554.78): C, 75.77; H, 9.81. Found: C, 75.60; H, 9.61.

Pseudodiosgenin 3β-Acetate 27-Butyrate.—A solution of 1.0 g. of diosgenin 3β-acetate in 10 ml. of butyric anhydride was heated under reflux for 15 hours. The mixture was concentrated to give a residue which was recrystallized from methanol; m.p. 119–120°; infrared spectrum: 5.80 (ester), 5.92(m) (shoulder) (vinyl ether), 8.00 (acetyl) 8.45 μ (butyryl).

Anal. Calcd. for $C_{33}H_{50}O_5$ (526.73): C, 75.24; H, 9.57. Found: C, 75.49; H, 9.66.

Pseudodiosgenin.[37]—A mixture of 40 g. of crude commercial diosgenin acetate, m.p. 175–195°, 13.0 g. of pyridine hydrochloride and 200 ml. of acetic anhydride was heated under reflux for 5 hours. After the solution had been diluted with 800 ml. of water and stored at 0° for 15 hours, the precipitate was collected by filtration, washed with water and dried. Four recrystallizations from meth-

(35) *Cf.* the production from naturally occurring tomatidine through acetylation followed by refluxing acetic acid: Y. Sato, N. Ikakawa and E. Mosettig, *J. Org. Chem.*, **24**, 893 (1959).

(36) *Cf.* the preparation from naturally occurring tomatidine with refluxing acetic anhydride, followed by dilute alkali: Y. Sato and H. G. Latham, Jr., *J. Am. Chem. Soc.*, **78**, 3150 (1956).

(37) Because of the technical difficulties associated with the preparation of pseudodiosgenin, the full details are given here. Since no satisfactory, reproducible method of recystallization was found, the procedure features hydrolysis of the scrupulously purified diacetate under mild conditions which give directly a crystalline solid suitable for use. For comments concerning disparities in literature reports concerning the compound, see A. F. B. Cameron, R. M. Evans, J. C. Hamlet, J. S. Hart, P. G. Jones and A. G. Long, *J. Chem. Soc.*, 2807 (1955).

anol, each employing 200 ml. of solvent,[38] left 14.0 g. of lustrous white plates of pseudodiosgenin diacetate, m.p. 101–104°.

To a solution of the four-times recrystallized diacetate (14.0 g., 0.028 mole) in 500 ml. of methanol at 25° was added a solution of 15.6 g. (0.28 mole, 5 equivalents) of potassium hydroxide in 25 ml. of water. Within 15 hours at 25°, the solution had become completely pervaded with a white, felt-like deposit. After 10 days at 0°, the crystalline precipitate was collected by filtration, pressed well with a spatula, washed with 2 liters of cold water and dried. The dull-white product had a texture reminiscent of that of asbestos; yield 8.5 g. (73%), m.p. 163–168°, $[\alpha]_D$ −36°; infrared spectrum: 5.92(m) μ (vinyl ether).

3β-Hydroxy-27-iodo-25α-5,20(22)-furostadiene (Pseudodiosgenin 27-Iodide) (XIX). (A).—To a solution of 8.28 g. (0.02 mole) of pseudodiosgenin in 50 ml. of pyridine at 0° was added, in 12 equal portions over a period of 6 hours, a total of 7.63 g. (0.04 mole) of *p*-toluenesulfonyl chloride. After 15 hours at 0°, the mixture was added to 500 ml. of water. The precipitate was collected by filtration, washed with water and dried. A solution of the anhydrous product (13.1 g.) and 6.0 g. (0.04 mole) of sodium iodide in 120 ml. of 2-butanone was stirred for 75 hours at 25°. (A precipitate of sodium *p*-toluenesulfonate had begun to separate after 6 hours.) After the mixture had been diluted with 1 liter of water and stored at 0° for 6 hours, the precipitate was collected by filtration, washed with water and dried. (The aqueous filtrate was found to contain 0.024 mole of iodide ion.) A benzene solution of the anhydrous product (12.93 g.) was chromatographed over 390 g. of aluminum oxide to give the fractions: (1) benzene followed by ether; 3.3 g.[39]; (2) ether–ethyl acetate (3:1), 5.5 g.

Fraction 2 was crystallized from a mixture of 10 ml. of dichloromethane and 50 ml. of methanol to give 870 mg., m.p. 70–72°. The residue from concentration of the mother liquors was dissolved in 2 ml. of dichloromethane and was treated with 22 ml. of methanol to afford, after 50 hours at 0°, 1.6 g. of a resinous solid. Recrystallization from a mixture of 1 ml. of dichloromethane and 10 ml. of methanol gave 820 mg., m.p. 70–72°, which was combined with the first crop to provide a total of 1.69 g. (16%). The analytical sample, after five recrystallizations, melted at 72–75°, followed by resolidification, as the temperature of the hot-stage slowly was elevated, to characteristic sheaves of needles which re-melted at 120–123°, $[\alpha]_D$ −32°; infrared spectrum: 5.92(m) μ (vinyl ether).

Anal. Calcd. for $C_{27}H_{41}O_2I$ (524.51): C, 61.82; H, 7.88; I, 24.20. Found: C, 61.68; H, 8.16; I, 24.26.

(B).—To a solution of 2.07 g. (0.005 mole) of pseudodiosgenin in 12 ml. of pyridine was added, in 4 portions at hourly intervals, a total of 1.90 g. (0.01 mole) of *p*-toluenesulfonyl chloride. After 15 hours at 0°, the solution was diluted with 200 ml. of water to give a precipitate which was collected and washed with water. A solution of the crude product and 100 ml. of water in 400 ml. of acetone was kept at reflux temperature for 90 minutes. After the solution had been diluted with 1200 ml. of water and stored at 0° for 15 hours, the precipitate was collected, washed with water and dried. A solution of the anhydrous material (2.35 g.) and 3.0 g. (0.02 mole) of sodium iodide in 30 ml. of 2-butanone was stirred for 75 hours at 25°. After the mixture had been diluted with 500 ml. of water and kept at 0° for 15 hours, the precipitate was collected, washed with water and dried. (The filtrate was found to contain 0.0156 mole of iodide ion.) Recrystallization from a mixture of 2 ml. of dichloromethane and 10 ml. of methanol gave 1.33 g. (53%), m.p. 70–72°, followed by resolidification to characteristic rosettes of needles, re-melting at 120–123°.

27-Iodo-25α-3,5,20(22)-furostatriene.—The fluid resin (3.3 g.) from fraction 1 of the chromatographic separation of 3β-hydroxy-27-iodo-25α-5,20(22)-furostadiene (XIX) described immediately above (procedure A) was extracted with 200 ml. of petroleum ether to discard 1.8 g. of insoluble residue. The extract was chromatographed over 45 g. of aluminum oxide The material which eluted with petroleum ether–benzene (9:1) was recrystallized from a mixture of dichloromethane and methanol to give 110 mg., m.p. 75–90°. The analytical sample, after 5 recrystallizations from methanol melted at 90–95°; infrared spectrum: 5.92-(m) μ (vinyl ether).

Anal. Calcd. for $C_{27}H_{39}OI$ (506.49): C, 64.02; H, 7.76; I, 25.06. Found: C, 64.19; H, 7.82; I, 24.76.

3β,27-Diiodo-25α-5,20(22)-furostadiene (Pseudodiosgenin 3β,27-Diiodide).—To a solution of 414 mg. (0.001 mole) of pseudodiosgenin in 4 ml. of pyridine at 0° was added in 3 portions, over 3 hours, 762 mg. (0.004 mole) of *p*-toluenesulfonyl chloride. After 20 hours at 0°, the mixture was diluted with 125 ml. of water to give a precipitate which was collected, washed with water and dried. A solution of the anhydrous product, 2.4 g. (0.016 mole) of sodium iodide and 0.24 ml. of water in 24 ml. of 2-butanone was stirred for 100 hours at 25°. After the mixture had been diluted with 250 ml. of water and stored at 0° for 15 hours the precipitate was collected, washed with water, and dried. A solution of the anhydrous material (620 mg.) in a mixture of petroleum ether and benzene (10:3) was chromatographed over 18 g. of aluminum oxide. The fraction which eluted with petroleum ether–benzene (1:1) was recrystallized from a mixture of dichloromethane and methanol (very slightly soluble in methanol alone) to give 140 mg. (22%) of needles. The analytical sample, after 6 recrystallizations, melted at 129–131°, $[\alpha]_D$ −11°; infrared spectrum: 5.92(m) μ (vinyl ether).

Anal. Calcd. for $C_{27}H_{40}OI_2$ (634.41): C, 51.11; H, 6.36; I, 40.01. Found: C, 52.90; H, 6.45; I, 38.14.

Since analytical determinations with several highly purified samples of the well crystalline substance checked closely, the possibility cannot be excluded that the crystals represent a definite molecular complex with the elimination product 27-iodo-25α-3,5,20(22)-furostatriene. Calcd. for a complex with a ratio of 3β,27-diiodide to elimination product of 7:1: C, 52.73; H, 6.53; I, 38.14.

3β-Iodo-25α-5-spirostene (Diosgenin 3β-Iodide).—A solution of 228 mg. (0.0004 mole) of diosgenin 3β-*p*-toluenesulfonate[40] and 120 mg. (0.0008 mole) of sodium iodide in 2 ml. of 2-butanone was heated under reflux for 45 minutes. After the mixture had been diluted with 25 ml. of water, the precipitate was collected, washed with water and dried. Recrystallization from a mixture of dichloromethane and methanol gave 150 mg. of flat needles, m.p. 155–165°. The analytical sample melted at 164–166°.

Anal. Calcd. for $C_{27}H_{41}O_2I$ (524.51): C, 61.82; H, 7.88; I, 24.20. Found: C, 61.95; H, 8.02; I, 24.36.

3β-Hydroxy-27-methylamino-25α-5,20(22)-furostadiene.—A solution of 270 mg. (0.0005 mole) of 3β-hydroxy-27-iodo-25α-5,20(22)-furostadiene (XIX) and 2.25 g. (0.072 mole) of methylamine in 8 g. of methanol was sealed in a Pyrex tube containing a magnetic stirring bar and kept at 90° with stirring for 23 hours. After the solution had been concentrated, the residue was dissolved in 5 ml. of methanol and was basified with 3 *N* aqueous potassium hydroxide. After dilution with 100 ml. of ether, the organic phase was washed with water and was concentrated. The residue was triturated with 20 ml. of 10% aqueous acetic to give an opalescent solution which was filtered several times with little apparent clarification. The filtrate was basified with dilute aqueous potassium hydroxide to give a voluminous gel which was extracted with a mixture of ether and dichloromethane (3:1). The organic phase was washed with water and was concentrated. Recrystallization of the dried remainder from ethyl acetate gave 45 mg. (20%) of plates, m.p. 150–161°; infrared spectrum: 3.0–3.2 (multiple) (NH_2), 5.92(m) μ (vinyl ether).

(38) Recrystallization from rather large volumes of solvent was found to be advantageous, giving a granular solid in acceptable recovery. From concentrated solution, the ester invariably separates as an intractable gum, permitting little purification despite a number of repetitions. The properties of the 3β-acetate 27-butyrate are somewhat superior to those of the diacetate.

(39) Fraction 1 was found to consist principally of a mixture of 27-iodo-25α-3,5,20(22)-furostatriene and 3β-27-diiodo-25α-5,20(22)-furostadiene which was very difficult to separate, even with rechromatography. In sodium iodide experiments carried out under strictly anhydrous conditions the former appeared to predominate, while in the presence of small amounts of water, the diiodide was the sole compound isolated in crystalline form from the first fractions.

(40) W. J. Peal, *J. Chem. Soc.*, 3081 (1957); M. E. Wall and S. Serota, *J. Am. Chem. Soc.*, **78**, 1747 (1956).

Anal. Calcd. for $C_{28}H_{45}NO_2$ (427.65): C, 78.63; H, 10.61; N, 3.28. Found: C, 77.84; H, 10.36; N, 2.60.

3β-Hydroxy-27-phthalimido-25α-5,20(22)-furostadiene (XX). (A).—A mixture of 524 mg. (0.001 mole) of 3β-hydroxy-27-iodo-25α-5,20(22)-furostadiene (XIX) and 222 mg. (0.0012 mole) of potassium phthalimide in 5 ml. of dimethylformamide was stirred for 45 hours at 25°. After the solution had been diluted with 50 ml. of water, the precipitate was collected, washed with water and dried. A benzene solution of the anhydrous material was chromatographed over 16 g. of aluminum oxide. Essentially the entire product (530 mg.) appeared with benzene–ether (9:1). The oil was treated with 30 ml. of methanol. After 15 hours at 0°, the supernatant liquor was removed to discard a small resinous deposit and was concentrated to a volume of 4 ml. After 15 hours at 0°, the precipitate was collected by filtration to give 410 mg. (75%). The analytical sample, after 5 recrystallizations from methanol, melted initially at 70–72°; as temperature elevation was continued, long slender needles began to appear in the melt at *ca.* 105°. If the temperature now was allowed to rise only very slowly, virtually the entire molten field solidified and re-melted in the range 135–148°, $[\alpha]_D$ −18°; infrared spectrum: 5.92(m) (shoulder) (vinyl ether), 5.70(m), 5.90, 13.8, 14.0 μ (phthalimido).

Anal. Calcd. for $C_{35}H_{45}NO_4$ (543.72): C, 77.31; H, 8.34; N, 2.58. Found: C, 77.03; H, 8.20; N, 2.60.

(B).—A mixture of 1.12 g. (0.002 mole) of 3β-hydroxy-27-phthalimido-25α-5-cholesten-16,22-dione (IV), 1.2 g. of potassium bicarbonate, 50 ml. of methanol and 15 ml. of water was kept at reflux temperature for 3 hours. The experiment was continued exactly as was the sodium borohydride reduction of the phthalamidic acid V described in the kryptogenin section with the exception that the imide ring closure with 1.6 g. (0.004 mole) of N-ethyl-N′-[2-(4-morpholinyl)-ethyl]-carbodiimide metho-*p*-toluenesulfonate[28] was allowed to proceed for 60 hours at 25°. After the solution had been diluted with 100 ml. of ether, the organic phase was washed with dilute aqueous ammonium hydroxide and with water and was concentrated. A benzene solution of the dried residue was chromatographed over 33.6 g. of aluminum oxide to give the fractions: (1) ether–dichloromethane (3:1), 650 mg.; (2) ether–methanol (95:5), 430 mg.

When the absence in fraction 1 of the hemiketal VI had been ascertained, the entire fraction was rechromatographed over 20 g. of aluminum oxide. The product which appeared with benzene–ether (9:1) was recrystallized from isopropyl alcohol to give 325 mg. (30%), m.p. 70–72° with prompt resolidification, followed by re-melting at 135–148°; mixed m.p. with the product prepared according to procedure A: 70–72°/135–148°; infrared spectrum identical with that of compound prepared from pseudodiosgenin 27-iodide according to procedure A. Isopropyl alcohol appeared preferable to methanol as a crystallizing solvent. In any event, the product prepared according to procedure B crystallized in a superior fashion and was virtually pure after the first crystallization.

3β-Hydroxy-27-amino-25α-5,20(22)-furostadiene (XXI).—A solution of 270 mg. (0.0005 mole) of 3β-hydroxy-27-phthalimido-25α-5,20(22)-furostadiene (XX), 128 mg. of hydrazine (0.004 mole) and 2 ml. of methanol in 2 ml. of dichloromethane was kept at 25° for 19 hours. A gelatinous precipitate had begun to fill the medium after 3 hours. After the solution had been diluted with 80 ml. of ether, the organic phase was washed with dilute aqueous ammonium hydroxide and with water and was concentrated. Recrystallization of the dried residue from methanol gave 125 mg. (60%) of a hydrate, m.p. 142–150°, $[\alpha]_D$ −19°; infrared spectrum: 3.0–3.2 (multiple), 6.25(w) (NH_2), 5.92(m) μ (vinyl ether).

Anal. Calcd. for $C_{27}H_{43}NO_2H_2O$ (431.64): C, 75.13; H, 10.51; N, 3.25. Found: C, 74.98; H, 10.00; N, 3.16.

3β-Hydroxy-27-acetamino-25α-5,20(22)-furostadiene.—A solution of the total hydrazinolysis product from 140 mg. (0.00025 mole) of 3β-hydroxy-27-phthalimido-25α-5,20(22)-furostadiene (XX) and of 1 ml. of acetic anhydride in 2 ml. of pyridine was kept at 0° for 18 hours. After the solution had been diluted with saturated aqueous potassium chloride, the precipitate was collected, washed with water and dissolved in 20 ml. of methanol containing 0.5 ml. of 3 *N* aqueous potassium hydroxide. After 1 hour at reflux temperature, the solution was concentrated to give a residue which was diluted with water. An ether extract of the precipitate was washed with water and was concentrated. A benzene solution of the dried residue was chromatographed over 3.3 g. of aluminum oxide. The material which eluted with benzene–ether (1:1) was recrystallized from ethyl acetate to give 55 mg. (40%), m.p. 170–175°; infrared spectrum: 3.0 (OH and NH), 5.92(m) (vinyl ether), 6.05, 6.45 μ ($NHCOCH_3$).

Anal. Calcd. for $C_{29}H_{45}NO_3$ (455.66): C, 76.44; H, 9.95; N, 3.07. Found: C, 76.44; H, 10.10; N, 2.93.

3β-Acetoxy-27-acetamino-25α-5,20(22)-furostadiene.—A solution of 30 mg. of 3β-hydroxy-27-amino-25α-5,20(22)-furostadiene (XXI) and 0.5 ml. of acetic anhydride in 1 ml. of pyridine was kept at 25° for 18 hours. After the solution had been diluted with 10 ml. of saturated aqueous potassium chloride, the precipitate was collected by filtration, washed with water and dried. Recrystallization from a mixture of dichloromethane and petroleum ether afforded small plates, m.p. 123–133°; infrared spectrum: 3.0 (NH), 5.80 (acetoxy), 5.92(m) (shoulder) (vinyl ether), 6.05, 6.50 μ ($NHCOCH_3$).[41]

Solasodine [3β-Hydroxy-22(27)-imino-5-furostene] (VII).—A solution of the total hydrazinolysis product from 272 mg. (0.0005 mole) of 3β-hydroxy-27-phthalimido-25α-5,20(22)-furostadiene (XX) and 1 ml. of 6 *N* aqueous hydrochloric acid in 5 ml. of methanol was kept at reflux temperature for 1 hour. After basification with 3 *N* aqueous potassium hydroxide, the solution was concentrated to give a residue which was extracted with ether. The organic phase was washed with water and was concentrated. A benzene solution of the dried residue was chromatographed over 6 g. of aluminum oxide. The material (140 mg.) which eluted with dichloromethane was recrystallized from methanol to afford 60 mg. (30%) of plates. The analytical sample, from methanol, melted at 198–201°, mixed m.p. with a specimen of naturally occurring solasodine (m.p. 199–203°) 198–203°, $[\alpha]_D$ −118°[42]; infrared spectrum indistinguishable from that provided by a sample of the natural product: 10.3, 10.4, 11.2, 11.5 μ (azaoxaspirane).

Anal. Calcd. for $C_{27}H_{43}NO_2$ (413.62): C, 78.40; H, 10.48; N, 3.39. Found: C, 78.50; H, 10.35; N, 3.41.

The hydrochloride, from absolute ethanol, melted at 290–300°.

Anal. Calcd. for $C_{27}H_{44}NO_2Cl$ (450.09): C, 72.05; H, 9.85; N, 3.11. Found: C, 72.24; H, 9.92; N, 3.05.

Pregnenolone Series

3β-Acetoxy-20β-hydroxy-20α-(5′-methyl-2′-pyridyl)-5-pregnene.—To a solution of 6.88 g. (0.04 mole) of 2-bromo-5-methylpyridine[43] in 40 ml. of anhydrous ether at −70° was added 80 ml. of an ethereal solution containing 0.04 mole of *n*-butyllithium.[44] The dark red mixture then was added to a solution of 3.6 g. (0.01 mole) of 3β-acetoxy-5-pregnen-20-one (XXV) in 150 ml. of anhydrous ether at −15°. A cream colored precipitate separated at once. After 16 hours at 25°, the solution was concentrated to give a solid residue which was triturated with 150 ml. of 25% aqueous acetic acid. After the insoluble neutral material (1.65 g.) had been collected, the filtrate was basified with dilute aqueous ammonium hydroxide to give a precipitate which was extracted with ether. The organic phase was washed with water and was concentrated. A solution of the dried remainder in 5 ml. of pyridine was treated with 1 ml. of acetic anhydride. After 15 hours at 0°, the mixture was diluted with 50 ml. of water to give a precipitate which was collected, washed with water and dried. Recrystallization from absolute ethanol gave 1.5 g. of white lustrous plates. Two additional recrystallizations afforded 1.0 g. (22%), m.p. 224–226°, $[\alpha]_D$ −80°.

Anal. Calcd. for $C_{29}H_{41}NO_3$ (451.63): C, 77.12; H, 9.15; N, 3.10. Found: C, 77.07; H, 9.11: N, 3.21.

3β, 20β-Dihydroxy-20α-(5′-methyl-2′-pyridyl)-5-pregnene (XXVI).—To a solution of 1.25 g. of 3β-acetoxy-20β-

(41) *Cf.* the production from naturally occurring solasodine through acetylation followed by refluxing acetic acid.[35]

(42) *Cf.* L. H. Briggs and T. O'Shea, *J. Chem. Soc.*, 1654 (1952).

(43) F. H. Case, *J. Am. Chem. Soc.*, **68**, 2574 (1946).

(44) H. Gilman, J. A. Bell, C. H. Brennen, N. W. Bullock, G. E. Dunn and L. S. Miller, *ibid.*, **71**, 1499 (1949).

hydroxy-20α-(5′-methyl-2′-pyridyl)-5-pregnene in 150 ml. of ethanol was added a solution of 5.0 g. of potassium hydroxide in 10 ml. of water. After 5 hours at reflux temperature, the solution was concentrated to give a residue which was diluted with water. The precipitate was collected by filtration, washed with water and dried. Recrystallization from absolute ethanol afforded 1.1 g., m.p. 281–282°, $[\alpha]_D$ −77°.

Anal. Calcd. for $C_{27}H_{39}NO_2$ (409.59): C, 79.17; H, 9.60; N, 3.42. Found: C, 79.39; H, 9.61; N, 3.42.

3β,20β-Dihydroxy-20α-(5′-methyl-2′-piperidyl)-5-pregnene (XXVIIa).—To a solution of 1.02 g. (0.0025 mole) of 3β,20β-dihydroxy-20α-(5′-methyl-2′-pyridyl)-pregnene (XXVI) in 150 ml. of *n*-propyl alcohol was added, in 4 portions over 0.5 hour, a total of 11.5 g. of sodium. After the metal had dissolved entirely, the solution was concentrated. The residue was diluted with water to give a precipitate which was extracted with ether. The organic phase was washed with water and was concentrated to give a remainder which was treated with 12 ml. of a 5% solution of picric acid in absolute ethanol. After 15 hours at 0°, the yellow precipitate of the picrate of **XXVIIa** was collected by filtration to give 750 mg. (46%), m.p. 240–250°. Three recrystallizations from absolute ethanol afforded the analytical sample as golden yellow needles, m.p. 248–250°.

Anal. Calcd. for $C_{33}H_{48}N_4O_9$ (644.75): C, 61.47; H, 7.50; N, 8.69. Found: C, 61.38; H, 7.60; N, 8.82.

A suspension of the picrate in water was treated with dilute aqueous lithium hydroxide and was extracted repeatedly with ether. The organic phase was washed with water and was concentrated. Recrystallization of the dried residue from methanol gave 100 mg., m.p. 207–208°, $[\alpha]_D$ −55°.

Anal. Calcd. for $C_{27}H_{45}NO_2$ (415.64): C, 78.02; H, 10.91; N, 3.37. Found: C, 78.15; H, 10.92; N, 3.64.

The hydrochloride of **XXVIIa** was recrystallized from absolute ethanol; m.p. 294–296°.

Anal. Calcd. for $C_{27}H_{46}NO_2Cl$(452.11): C, 71.72; H, 10.26; N, 3.10. Found: C, 71.28; H, 10.20; N, 3.04.

Treatment of a 10% aqueous acetic acid solution of XXVIIa with 10% aqueous sodium nitrite gave the N-nitroso derivative of **XXVIIa** which was recrystallized from methanol to give hexagonal plates, m.p. 210–218°.

Acetylation of XXVIIa with acetic anhydride in pyridine, followed by treatment with dilute alkali, afforded the N-acetyl derivative of **XXVIIa** which was recrystallized from methanol to give small plates, m.p. 246–250°.

3β,20β-Dihydroxy-20α-(5′-methyl-2′-piperidyl)-5-pregnene (XXVIIb).—The mother liquor from the picrate of XXVIIa was concentrated to a small volume to give, after 15 hours at 0°, a deposit which was collected and was recrystallized from a mixture of methanol and benzene to afford orange-yellow needles of the picrate of **XXVIIb**, m.p. 238–243°.

Anal. Calcd. for $C_{33}H_{48}N_4O_9$ (644.75): C, 61.47; H, 7.50; N, 8.69. Found: C, 61.74; H, 7.31; N, 8.75.

A solution of the picrate in ethanol was treated with dilute aqueous lithium hydroxide and was diluted with water to give a precipitate which was extracted with ether. The organic phase was washed with aqueous lithium hydroxide and with water and was concentrated. The dried residue was recrystallized from methanol to give needles, m.p. 202–205°, $[\alpha]_D$ −62°.

Anal. Calcd. for $C_{27}H_{45}NO_2$ (415.64): C, 78.02; H, 10.91; N, 3.37. Found: C, 77.89; H, 10.92; N, 3.48.

An intimately ground mixture of XXVIIa and XXVIIb melted at 197–208°. The infrared spectra of the isomers, which displayed quite uneventful fingerprint regions, were difficult to distinguish.

The hydrochloride of **XXVIIb**, from absolute ethanol, melted at 320–330°.

Treatment of a 10% aqueous acetic acid solution of XXVIIb with dilute aqueous sodium nitrite, gave, after recrystallization from a mixture of dichloromethane and methanol, short, thin needles of the N-nitroso derivative of **XXVIIb**, m.p. 252–258°.

Acetylation of XXVIIb with acetic anhydride in pyridine, followed by treatment with dilute alkali, gave, after recrystallization from methanol, the N-acetyl derivative of **XXVIIb**, m.p. 185–190°.

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The Relation between the Minor Components of Whole Normal Human Adult Hemoglobin as Isolated by Chromatography and Starch Block Electrophoresis

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Three hemoglobin components may be separated from hemolysates of normal adult red blood cells by starch block electrophoresis: the main component, A_1, moves at a position intermediate between the most slowly moving component, A_2, and the most rapidly moving component, A_3. By chromatography, eight hemoglobin components may be detected: five (A_{1a}, A_{1b}, A_{1c}, A_{1d} and A_{1e}) move down the column more rapidly than the main component (A_{1I}) and two move more slowly (A_{1IIa} and A_{1IIb}). Non-heme containing proteins are also present. The present investigation has determined the correspondence between the components as isolated by the two methods: A_1 contains A_{1c}, A_{1d} and A_{1e}, and A_{1I}; A_2 contains A_{IIb} and non-heme protein(s); A_3 contains A_{1a}, A_{1b} and non-heme protein(s).

Introduction

Inasmuch as the pertinent literature relating to the heterogeneity of whole normal human hemoglobin was reviewed in some detail by Clegg and Schroeder,[1] it is necessary here to mention only data that were not discussed by them. To the

(1) M. D. Clegg and W. A. Schroeder, THIS JOURNAL, **81**, 6065 (1959).