[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, HARVARD MEDICAL SCHOOL]

# The Synthesis and Transformations of a Steroid Pyrroline Derivative

BY FREDERICK C. UHLE AND F. SALLMANN

RECEIVED JUNE 9, 1959

The preparation of the steroid pyrroline derivative I by zinc–acetic acid reduction of kryptogenin diacetate 2,4-dinitrophenylhydrazone is described. A discussion of the chemical behavior of I, and of its transformation products with acetic anhydride, is presented. The synthesis of 22,25-isosolanidine is reported.

The naturally occurring azaoxaspirane[1] alkaloids solasodine[2] and tomatidine[3] may be considered nitrogen analogs of the steroid sapogenins in which the ether oxygen of the terminal tetrahydropyran ring has been replaced by a secondary amino function. Isomeric substances in which the hetero atom of the tetrahydofuran nucleus, on the other hand, appears in the guise of a basic grouping have not been described.

In a synthetic approach to such compounds we set out to transform the C.16 carbonyl group of kryptogenin to a primary amino function by a procedure assuring the preservation of the oxidation status at position 22 undisturbed. Initial exploratory efforts to secure a C.16 monoxime proved without issue. Even with less than molar amounts of hydroxylamine, the sole product, other than unchanged diketone, was found to be the known 16,22-dioxime.[4] Phenylhydrazine led to a dihydropyridazine derivative as a consequence of cyclization following phenylhydrazone formation.

1-Methyl-1-phenylhydrazine, with which pyridazine condensation is excluded, gave a mobile oil when allowed to react with the sapogenin diacetate.[5] Reduction of this product with zinc in refluxing glacial acetic acid furnished a large basic fraction which was separated into two major components by chromatography on aluminum oxide. Zinc–acetic acid reduction of the previously reported kryptogenin diacetate 2,4-dinitrophenylhydrazone[6] afforded comparable yields of the same steroid amines.

The predominant product, the pyrroline I, m.p. 148–150°, $pK$ 5.45, isolated in amounts of the order of 20%, gave analytical figures for a composition of $C_{27}H_{43}NO_2$ and provided an infrared spectrum notable for the presence of a band of medium intensity at 6.13 $\mu$. With methyl iodide the base readily formed a crystalline quaternary salt.

Sodium borohydride in neutral, as well as in acid solution, produced a dihydro derivative, m.p. 175–177°, $pK$ 9.03, which proved to be identical with the subordinate product (10%) of the hydrazone reduction. This secondary amine was formulated as the pyrrolidine II and was characterized by the preparation of mono- and triacetyl derivatives. Sodium borohydride reduction of the methiodide of I afforded the N-methyl derivative of II, a tertiary amine obtained, as well, by Clarke–Eschweiler methylation of the pyrrolidine.

Catalytic hydrogenation of I with platinum in acetic acid resulted in the uptake of two moles of hydrogen to supply the 5,6-dihydride of II. The structure of the tetrahydro compound was confirmed by a second preparation through hydrogenation of kryptogenin dioxime.[7]

(1) The expression "azaoxaspirane," consistent with Ring Index precedent, is introduced here as a convenient generic term.

(2) L. H. Briggs, W. E. Harvey, R. H. Locker, W. A. McGillivray and R. N. Seeyle, *J. Chem. Soc.*, 3013 (1950); F. C. Uhle, THIS JOURNAL, **75**, 2280 (1953); **76**, 4245 (1954).

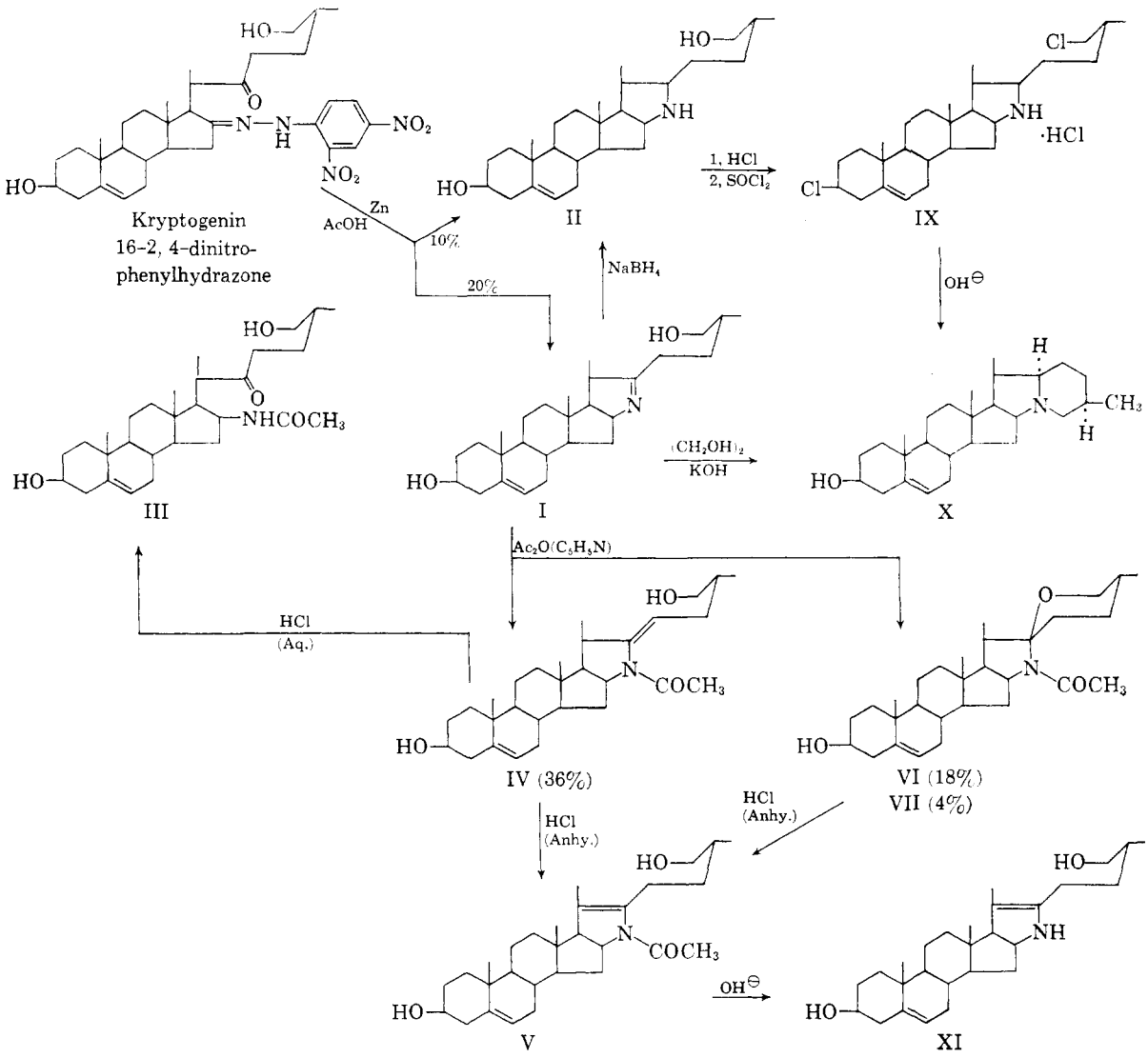
(3) T. D. Fontaine, J. S. Ard and R. M. Ma, *ibid.*, **73**, 878 (1951); Y. Sato, A. Katz and E. Mosettig, *ibid.*, **73**, 880 (1951); R. Kuhn, I. Löw and H. Trischmann, *Chem. Ber.*, **85**, 416 (1952); F. C. Uhle and J. A. Moore, THIS JOURNAL, **76**, 6412 (1954).

(4) R. E. Marker, R. B. Wagner, C. H. Ruof, P. R. Ulshafer and D. P. J. Goldsmith, *ibid.*, **65**, 1658 (1943).

(5) Hydrolysis of the oil with aqueous methanolic potassium bicarbonate gave a crystalline derivative, m.p. 154–155°, identical with the product of treatment of the free 3$\beta$,27-diol with 1-methyl-1-phenylhydrazine. Since the infrared spectrum of the compound displayed no absorption in the carbonyl region, the C.27 hydroxyl is presumed to have entered into hemiketal formation with the C.22 keto group. This conclusion was reached also by R. S. Miner and E. S. Wallis, *J. Org. Chem.*, **21**, 715 (1956), in the case of a substance formulated as 3$\beta$,27-dihydroxy-16$\alpha$-chloro-22-keto-5-cholestene. However, the infrared spectra of 3$\beta$,27-dihydroxy-22-cholestanone (I. Scheer, M. J. Thompson and E. Mosettig, THIS JOURNAL, **79**, 3218 (1957)), of 3$\beta$,27-dihydroxy-16$\beta$-acetamino-22-keto-5-cholestene (III) and of the related 3$\beta$,27-dihydroxy-16$\beta$-benzoylamino-22-keto-5-cholestene of the present work exhibit the full intensity of the free C.22 carbonyl function. It is not immediately apparent why the C.27 hydroxyl and C.22 carbonyl groups should participate in hemiketal formation in the case of certain 16-substituted compounds and not in others. In fact, it is perhaps surprising that a cyclic form is not the favored arrangement in the case of kryptogenin itself. Nevertheless, the infrared spectrum of the sapogenin, both in solution and in the solid state, gives unmistakable evidence of the integrity of the 16, 22 and 27 functions.

(6) G. Rosenkranz, St. Kaufmann, A. Landa, J. J. Corona and A. Olalde, *ibid.*, **70**, 3518 (1948). These authors tacitly assumed the hydrazone to be the C.16 derivative. It is true that, with a single recorded exception, the carbonyl group at position 16 of kryptogenin proves more accessible to reaction than that at position 22. Hydrogen in the presence of Raney nickel (St. Kaufmann and G. Rosenkranz, *ibid.*, **70**, 3503 (1948)), sodium borohydride (unpublished work from this Laboratory), and 1,2-ethanedithiol (I. Scheer, M. J. Thompson and E. Mosettig, *ibid.*, **79**, 3218 (1957)), give products which have resulted from primary attack at the group in the 5-membered ring. Only in the case of the Clemmensen reduction does the C.22 function appear to be preferentially involved for the end result of the change comprises a 1:1 mixture of 3$\beta$,27-dihydroxy-5-cholestene and 3$\beta$,27-dihydroxy-16-keto-5-cholestene (I. Scheer, M. J. Thompson and E. Mosettig, *ibid.*, **78**, 4733 (1956)).

Support for the C.16 formulation of the 2,4-dinitrophenylhydrazone may be gleaned from examination of the infrared spectra of the sapogenin and of its derivatives. According to our measurements, the carbonyl groups of kryptogenin itself are clearly defined at 1735 (C.16) and at 1700 cm.$^{-1}$ (C.22). Again, the carbonyl band of 3$\beta$,27-dihydroxy-16$\beta$-acetamino-22-keto-5-cholestene (III) (and of the corresponding 16$\beta$-benzoylamino derivative) is located precisely at 1700 cm.$^{-1}$. However, in the case of 3$\beta$-hydroxy-16,22-diketo-27-tosyloxy-5-cholestene and of 3$\beta$-hydroxy-16,22-diketo-27-iodo-5-cholestene, intermediates in the synthesis of solasodine (to be reported in detail in a forthcoming publication), the two bands are found at 1735 and at 1710 cm.$^{-1}$. Moreover, in the case of kryptogenin diacetate, the C.22 absorption is seen only as an inflection point at 1715 cm.$^{-1}$, superimposed on the deep ester–C.16 carbonyl band at 1730–1735 cm.$^{-1}$. It thus appears that in free 27-hydroxyl compounds, a certain interaction with the C.22 carbonyl group intervenes, betrayed in a lowering of the frequency of the C.22 keto band by approximately 10 cm.$^{-1}$. In the case of the 2,4-dinitrophenylhydrazone of kryptogenin (obtained by potassium bicarbonate hydrolysis of the 3$\beta$,27-diacetate), the carbonyl band is again found at 1710 cm.$^{-1}$. Perhaps the spatial demands of the 2,4-dinitrophenyl residue are of such dimensions as to effectively forestall propinquity of the hydroxyl and keto functions




$$\text{I} \xrightarrow{Ac_2O(H_2O)} \text{III}\ (60\%) + \text{VI}\ (26\%)$$

$$\text{I} \xrightarrow[2.\ p\text{-}CH_3C_6H_4SO_3H(C_6H_6)]{1.\ Ac_2O\ (C_5H_5N)} \text{V}\ (85\%)$$

$$\left.\begin{array}{l} \text{VI} \xrightarrow{AcOH} \text{IV} \xrightarrow{HCl\ (aq.)} \\ \text{VII} \xrightarrow{AcOH} \text{IV} \xrightarrow{HCl\ (aq.)} \end{array}\right\} \begin{array}{c} \text{III} \\ + \\ \text{V} \end{array}$$

With acetic anhydride in aqueous isopropyl alcohol, compound I gave a high melting substance whose infrared spectrum (5.88, 6.10, 6.46 $\mu$) characterized it as an acetyl amino ketone[8] arising from hydrolytic acetylation, a reaction typical of 1-pyrrolines.[9] Location of the carbonyl band at the wave length (5.88 $\mu$) identified with the C.22 carbonyl group of kryptogenin was considered to warrant confidence in assignment of structure III to the ring fission product.[10] Benzoyl chloride, under Schotten–Baumann conditions, afforded a related benzoylamino ketone with infrared absorption again at 5.88 $\mu$. As a consequence of these findings, the pyrroline representation I, with placement of the double bond at the site provisionally adopted on the basis of the mode of synthesis, was adjudged substantiated.[11]

with the consequence that the C.22 carbonyl band of the hydrazone appears at the frequency characteristic of those derivatives devoid of a C.27 primary alcoholic group.

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

(8) As noted in the sequel, the acetylamino ketone (60%) was accompanied by 26% of VI.

(9) *Cf. inter alia*, P. G. Haines, A. Eisner and C. F. Woodward, THIS JOURNAL, **67**, 1258 (1945); G. G. Evans, *ibid.*, **73**, 5230 (1957), M. C. Kloetzel, J. L. Pinkus and R. N. Washburn, *ibid.*, **79**, 4222 (1957); B. Belleau, *Can. J. Chem.*, **35**, 651 (1957); V. Cerný and F. Šorm, *Chemistry & Industry*, 516 (1959).

(10) Hydrolysis with 40% ethanolic potassium hydroxide afforded a basic fraction which gave an infrared spectrum identical with that of I. However, difficulty was encountered in purification to a product of correct melting point. Apparently, the free keto group suffers under the harsh concentration of alkali required for amide cleavage, giving rise to contaminating by-products. Acid hydrolysis is precluded by the sensitivity to dehydration of the homoallylic alcohol function of ring A.

(11) Nitrous acid, in dilute aqueous acetic acid solution, gave a complex mixture of products. In general, 1-pyrrolines may be expected to react with this reagent, as with acylating agents, in the manner of the amino ketone available through hydrolytic equilibrium; *e.g.*, a pyrroline derived from conessine has given 18-hydroxyprogesterone: F. Buzzetti, W. Wicki, J. Kalvoda and O. Jeger, *Helv. Chim. Acta*, **42**, 388 (1959). In the past, alicyclic primary amines have been found to afford virtually quantitative yields of the corre-

With acylating agents in the presence of anhydrous tertiary bases, compound I underwent a much more complex change. Treatment with acetic anhydride in pyridine, followed by ester hydrolysis with dilute aqueous potassium bicarbonate, gave rise to a mixture of three isomeric tertiary amides which was fractionated on a column of alumina.

The major product (36%), that most firmly bound to the adsorbent, has been considered to be IV. Spectroscopic support for the assignment was found in the infrared spectrum with an amide band at 6.16 $\mu$ and in the ultraviolet spectrum with absorption at 244 m$\mu$ (log $\epsilon$ 3.90), attributed to coupling of the ethylenic and carbonyl unsaturation through the unshared electron pair of the nitrogen atom.[12]

Treatment of IV with 40% fixed alkali in refluxing ethyl alcohol led to scission of the amide linkage and to reconstitution of I. In acid media the substance was found to display an especially pronounced sensitivity.[13] Exposure to anhydrous hydrogen chloride in benzene solution led to abrupt transformation to a new tertiary amide, V, a compound pictured as a nitrogenous counterpart of pseudodiosgenin. With dilute aqueous mineral acid, the amide V was accompanied by a certain amount of the acetylamino ketone III.[14]

The infrared spectrum of V was distinguished chiefly by the presence of a sharp band at 5.98 $\mu$ reminiscent of that associated with the 20,22 center of unsaturation of the pseudosapogenins. Ultraviolet absorption at 251 m$\mu$ (log $\epsilon$ 4.16), as compared with that at 244 m$\mu$ (log $\epsilon$ 3.90) in the case of IV, was considered in accord with tetrasubstitution of the 20,22-double bond of V.[15] Moreover, shift of the unsaturated linkage into ring E in the presence of acid was recognized as consistent with recently enunciated generalizations concerning the greater relative stability of endocyclic, as opposed to exocyclic, double bonds.[16] Once formed, V resisted further change in the presence of dilute aqueous mineral acid.

The endocyclic isomer was most advantageously prepared directly from I by treatment of the total acetic anhydride–pyridine reaction product with anhydrous *p*-toluenesulfonic acid in benzene solution. Following this expedient, V was obtained as its 3$\beta$,27-diacetate in 85% yield.

The exocyclic isomer IV failed to give a solid acetic ester, repeated efforts to induce its crystallization notwithstanding. 3,5-Dinitrobenzoyl chloride, with IV in refluxing pyridine, afforded the 3$\beta$,-27-di-(3,5-dinitrobenzoyl) ester of V.[17]

Amide hydrolysis of V with concentrated alcoholic potassium hydroxide at 78° gave a new basic substance[18] (XI) in which the double bond appears to have remained in the 20,22-position.[19] The free vinylamine was found to be wholly indifferent to brief exposure to 1 *N* hydrochloric acid in boiling methanol.

Although the expression IV implies the possibility of "*cis–trans*" isomers, convincing evidence for the existence of a second exocyclic olefin was not secured. Uncertainty persisted for some time at this stage, inasmuch as IV deposited from ethyl acetate in two distinct crystalline forms, one of which invariably took precedence when the compound had been dissolved in glacial acetic acid. Although the infrared spectra of the forms in potassium bromide were not coincident at all points, curves obtained with solutions were indistinguishable. The rotations of the two in pyridine, as well as in chloroform, were identical. Again, the melting points were in the same range and no appreciable depression of melting point was observed on admixture. Consequently, there appeared little cause to defer the conclusion that the crystalline variants merely represent lattice modifications.

Of somewhat greater concern was the high positive rotation of IV, as opposed to negative values for all other compounds in the group, particularly in view of the recent disclosure of very little difference in the rotations of pseudotigogenin diacetate and of an isomeric 22,23-exocyclic vinyl ether, the

sponding alcohol with complete retention of configuration provided the amino group is equatorially situated, while axial amines give a mixture of the isomeric alcohol and of the end products of elimination: W. G. Dauben, R. C. Tweit and C. Mannerskantz, THIS JOURNAL, **76**, 4420 (1954). Since the concept of equatorial–axial relationships has no meaning in reference to position 16 of the steroid nucleus, it is not possible, on the basis of this simple generalization, to predict the outcome of nitrous acid treatment in the present case. However, in terms of recent theoretical interpretations of the reaction (C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p. 395), it is doubtless to be anticipated that the deamination product embodying retention of configuration (diosgenin) would represent only a small percentage of the total, if indeed it were formed at all. Search of the ether eluate of a chromatographic separation of the products failed to reveal the presence of diosgenin. This fraction, which accounted for less than 20% of the whole, gave only a substance melting at 225–235° which has not as yet been identified.

(12) *Cf.* G. Rosenkranz, O. Mancera, F. Sondheimer and C. Djerassi, *J. Org. Chem.*, **21**, 520 (1956); R. S. Montgomery and G. Dougherty, *ibid.*, **17**, 823 (1952); R. H. Mazur, THIS JOURNAL, **81**, 1454 (1959); R. Griot and T. Wagner-Jauregg, *Helv. Chim. Acta*, **42**, 121 (1959).

(13) While it would be desirable to bring forth oxidative confirmation of the position of the center of unsaturation, the lability of the substance in acid media thus far has discouraged initiation of the task. We must, of course, contend also with the presence of the 5,6-double bond.

(14) In the first "test-tube" experiments in which the behavior of the acetyl derivatives IV, VI and VII with mineral acid was examined, the impression had been gained that in aqueous media the predominant, if not the exclusive product, was the acetylamino ketone III. However, later repetition on a preparative scale, *i.e.*, with amounts of the order of 100 mg., followed by chromatography, disclosed the presence of a mixture of III and V in a ratio which did not appear to be constant from run to run. The factors responsible for influencing the direction of the change have not as yet been unravelled.

(15) R. B. Woodward, THIS JOURNAL, **63**, 1123 (1941); **64**, 76 (1942).

(16) R. B. Turner and R. H. Garner, *ibid.*, **80**, 1424 (1958).

(17) Reaction in the cold was incomplete, furnishing no homogeneous product.

(18) The compound melted nearly fifty degrees higher than did the pyrroline I and provided an infrared spectrum with a band of medium intensity at 6.15 $\mu$ as well as an ultraviolet spectrum with absorption at 224 m$\mu$ (log $\epsilon$ 3.64). *Cf.* the ultraviolet spectrum of 11-azabicyclo-[4.4.1]-1-undecene: 226 m$\mu$ (log $\epsilon$ 3.71); A. C. Cope, R. J. Cotten and S. G. Roller, THIS JOURNAL, **77**, 3590 (1955). For general statements concerning the ultraviolet absorption spectra of unsaturated amines see K. Bowden, E. A. Braude, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.*, 48 (1946); N. J. Leonard and D. M. Locker, THIS JOURNAL, **76**, 5230 (1954).

(19) The authenticity of a number of 2,3-unsaturated pyrroles described in the older literature has been disputed in the claim that there are probably no genuine secondary $\Delta^2$-pyrrolines and that all substances of this class are more correctly formulated as $\Delta^1$-derivatives: B. Witkop, *ibid.*, **76**, 5507 (1954). If our assignment to the hydrolysis product of V withstands the scrutiny of further experimental study, and at the moment we see no reason to fear that it will not, the generalization must be called into question.

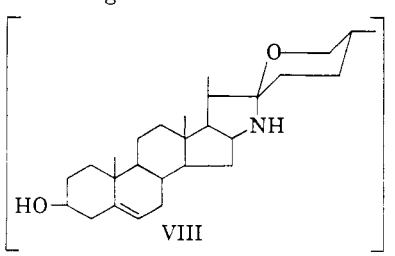
first sapogenin derivative of its class to be reported.[20] Although it is not possible to perceive a uniform correlation of the rotations of nitrogen-bearing steroid relatives with those of their oxygen analogs, or to fashion "rules" in these series, it is worthy of remark that in no other case has the substitution of $NHCOCH_3$ for O led to so striking a disparity.

The elution of IV from the chromatographic column had been preceded by the appearance of two isomeric substances, VI and VII, whose infrared spectra classified them, likewise, as tertiary amides.[21] The isomer obtained in greater amount, VI (18%), was encountered once again as a by-product in the preparation of the acetylamino ketone III from I and acetic anhydride in aqueous isopropyl alcohol. The companion substance VII was present in the pyridine–acylation mixture in relatively insignificant amount (4%) and was retrieved readily only by rechromatography of the first eluate and careful selection of the introductory fractions.

The early abstraction of VI and VII from the aluminum oxide, in itself, suggested that the compounds were characterized by the possession of only a single hydroxyl group and, indeed, a crystalline monoacetyl derivative of each isomer was acquired. As opposed to the vulnerability to alkali of IV, both amides VI and VII were recovered unchanged after 20 hours with 40% caustic potash in refluxing ethanol, pointing to the sequestration of the acetamino function in a comparatively inaccessible environment. Again in contradistinction to IV, alcoholic solutions of the compounds were transparent in the ultraviolet above 200 mμ, demonstrating the absence of unsaturation in proximity to the amide group.

In their behavior in strongly acid media, however, VI and VII closely resembled IV. In fact, with glacial acetic acid both substances were converted promptly to IV. With mineral acid they furnished the endocyclic unsaturated amide V, accompanied by the acetylamino ketone III when the reaction was carried out in aqueous solution.

In the perspective of the experimental evidence assembled at this juncture there appears little alternative to attribution of hexacyclic structures to VI and VII. In this sense, the compounds have been regarded as amide derivatives of a parent spirane VIII resulting from addition of the terminal hydroxyl group of the side chain of I to the unsaturated center of ring E.[22]



VIII

It must be admitted that the inflexible nature of I had been somewhat unexpected in the early phases of the study. In projection of the work, the pyrroline had been envisaged simply as a rather labile, possibly not isolable, intermediate, obtention of which would be tantamount to successful completion of the synthesis of an azaoxaspirane derivative isomeric with solasodine since no impediment to final ring closure had been foreseen. However, the pentacyclic integrity of I appeared to be maintained over a comprehensive compass of experimental conditions. Despite assiduous effort to bring about transformation to the hexacyclic secondary base, no evidence for its presence, even at low temperatures, could be detected.[23]

Failure of normal addition of the C.27 hydroxyl to the azomethine linkage has been ascribed to a destabilization stemming from van der Waals interaction of the amino hydrogen with neighboring hydrogen atoms in the most probable conformation VIII of the prospective azaoxaspirane. Measurements with Dreiding stereomodels[24] indicate that the amino hydrogen, in one position, and the axial hydrogen at C.24, for example, are separated by a distance of approximately 2.0 Å., a value which rather seriously oversteps the critical approach of 2.4 Å. defined in consideration of a van der Waals radius of 1.2 Å. for hydrogen.[25] Since attenuation of these purely repulsive hydrogen–hydrogen interactions could readily be achieved by development of a trigonal carbon atom at C.22, it is perhaps not surprising that the pyrroline I proves to be the only stable arrangement under ordinary circumstances.

The hexacyclic amides VI and VII, of course, are themselves molecules severely sterically compressed. Once formed, however, they obviously cannot split apart in the direct fashion of the hypothetical free secondary amine VIII. Nevertheless, their survival in neutral or in alkaline solution, even at fairly high temperatures, is promptly cut short by the introduction of acid reagents.

Since the problem of the mode of formation, and

(20) H. Hirschmann and F. B. Hirschmann, *Tetrahedron*, **3**, 243 (1958).

(21) It was possible to separate IV and VI fairly cleanly through fractional crystallization alone by virtue of the differential solubility of the compounds in methanol and ethyl acetate.

(22) The known azaoxaspirane alkaloids solasodine, tomatidine and 5β-tomatidine, as opposed to allied open chain compounds, give infrared spectra especially rich in the finger-print region with at least 4 prominent bands between 10 and 12 μ considered diagnostic. However, the intensity of these bands is very appreciably subdued in the case of the N-acetyl derivatives of the bases, rendering imputation of hexacyclic structure to a new compound on the basis of infrared evidence alone rather hazardous. Certainly, the problem is far less straight-forward than in the related situation of the spiroketal sapogenins and their pseudo isomers, for in the oxygen series comparison of infrared spectra provides an unambiguous answer as to whether or not spirane ring closure has occurred. The amides VI and VII do, in fact, give curves more eventful than the rather "flat" tracings provided by the pentacyclic compounds of the series. For an unknown reason, the 6-12 μ region of the spectrum of VII is somewhat more complex than that of VI, to a degree which, on casual inspection alone, might justify suspicion of spirane fusion. It may be mentioned as a curious, but not explained, fact that while the melting point of VII is characteristically precise, that of VI, and of its 3β-acetate, extends over a range of at least ten degrees and is not brought into sharper focus after repeated recrystallization or after rechromatography.

(23) The experiments included exposure of the pyrroline to acidic and basic reagents of a range of power, under aqueous as well as under anhydrous condition, at low and at elevated temperatures. Attempts also were made to influence the course of the reaction with acetic anhydride in pyridine at very low temperature. Conversion of the methiodide of I to the quaternary hydroxide, followed by various manipulations, gave rise to no definitive product.

(24) A. S. Dreiding, *Helv. Chim. Acta*, **42**, 1339 (1959).

(25) L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1948, p. 189.

of configuration, of the cyclic amides presents a challenge of some subtlety, we elect to reserve a discussion of possible mechanisms until more revealing information is at hand. Thus far we have not found the opportunity of ascertaining the relative stability of the isomers, which presumably differ in orientation at C.20, or C.22, or both.

Treatment of I with trifluoroacetic anhydride in pyridine gave only a single amide which readily reverted to the starting pyrroline with dilute potassium hydroxide at 0°. The rotation and ultraviolet spectrum of this substance confirmed its kinship to the exocyclic olefin IV. With a mixture of anhydrous formic acid and acetic anhydride in pyridine, on the other hand, compound I provided a mixture of amides in which the hexacyclic fraction appeared to predominate,[26] as witnessed by end absorption only in the ultraviolet spectrum. The decreasing output of hexacyclic amide in proceeding through the series N-formyl, N-acetyl, N-trifluoroacetyl may be viewed as consistent with the relative spatial demands of the acyl groups and suggests a controlling influence of this factor in the formation of the substances, although electrical effects pertaining to the individual acid residues, to be sure, cannot be discounted altogether.

With thionyl chloride, the hydrochloride of the pyrrolidine II gave the 3$\beta$,27-dichloride hydrochloride IX. Cautious neutralization of the salt, followed by treatment with refluxing absolute ethanol, led to internal alkylation to afford a tertiary amine. Potassium acetate in acetic acid transformed the 3$\beta$-chloro product to the 3$\beta$-acetate which was hydrolyzed to the free alkanolamine X, an octahydropyrrocoline derivative characterized by the solanidane skeleton.

Inasmuch as solanidine itself has been related to sarsasapogenin,[7] a representative of the normal (25$\beta$-methyl)[27] sapogenin series, as opposed to the iso (25$\alpha$-methyl) configuration of diosgenin (and of kryptogenin), the C.25 methyl group of the new hexacyclic amine X must occupy the position epimeric with that of the corresponding substituent in the case of the well known alkaloid from *Solanum tuberosum*. Moreover, since the pyrrolidine II was obtained from the pyrroline I by sodium borohydride reduction, and since the hydrogenation products of I and II are identical, the C.22 hydrogen of II, and hence of X, is almost certainly directed in the $\beta$-configuration[27] as a consequence of ingress of the saturating reagents to the less hindered surface of the molecule. The C.22 hydrogen of solanidine, on the other hand, has been regarded,[28] principally on the ground of interrelationship with one of the dihydro derivatives of tomatidine, as most probably oriented in the $\alpha$-configuration.[29] The synthetic (X) and natural compounds therefore appear to be epimeric at both centers 22 and 25 and the new octahydropyrrocoline base, accordingly, has been designated 22,25-isosolanidine.[30]

In a somewhat surprising transformation, X was obtained directly from I in 65% yield following a three-hour reaction period with refluxing ethylene glycol containing potassium hydroxide. This conversion was recognized first during an attempt to cleave the amide rest of VI at elevated temperature. Apparently ring F was severed under the rigors of these conditions with ensuing hydrolysis to the pentacyclic pyrroline. The actual course of formation of the tertiary amine in this contingency has not been clarified, although abundant precedent for the reducing capacity of alkaline ethylene glycol is available in the literature.[31] The pyrrolidine II under identical conditions gave only neligible amounts of 22,25-isosolanidine. In still another preparation, brief pyrolysis of the hydrochloride of I in a sealed tube at 200° afforded approximately 10% of the tertiary base.

On a later occasion we hope to present an account of additional facets of the chemistry of the pyrroline I and to marshall further corroborative support for those assignments as yet not rigorously established.

## Experimental

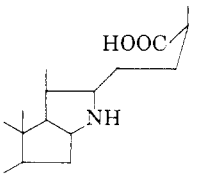
The melting points were observed on a calibrated micro hot-stage. The microanalyses were performed by Dr. S. M. Nagy and associates of the Massachusetts Institute of Technology, Cambridge, Mass. A number of the rotations were measured (at 25 ± 3°) by the Schwarzkopf Microanalytical Laboratories, Woodside 77, N. Y. The infrared

(26) The mixture proved exceedingly difficult to separate with the result that an accurate quantitative accounting of its composition is not yet possible. The total reaction product was a well crystalline mass, m.p. 100–225°, with which attempts at fractional crystallization were entirely unavailing. Only a tedious chromatographic process served to subdivide the whole into a "hexacyclic fraction" and an apparently homogeneous olefin amide. The former was composed of at least two substances, one of which melted considerably higher than the other. While it cannot be said with complete certainty that the cyclic fraction was the major one, there is little doubt that the amount of N-formylspirane amide exceeded that of the corresponding N-acetyl derivative produced under identical conditions.

(27) For terminology see L. F. Fieser and M. Fieser, "Steroids," Reinhold Publ. Corp., New York, N. Y., 1959, p. 337.

(28) Y. Sato and H. G. Latham, Jr., THIS JOURNAL, **78**, 3146 (1956).

(29) If the evidence on the basis of which the assignment of configuration at C.22 in the case of solanidine is accepted as satisfactory, it must be assumed that the hydrogen atom at this center in the intermediate amino acid which led to the synthesis[7] of 5$\alpha$-solanidan-3$\beta$-ol




was fixed in the same orientation. However, the amino acid was derived from sarsasapogenoic acid dioxime by a hydrogenation procedure closely allied to that which, with kryptogenin dioxime, gave the secondary amine identical with the 5,6-dihydride of II. These findings can be reconciled readily only on the assumption that the respective pyrrolidine derivatives which crystallized fortuitously were those of opposite configuration at position 22. To be sure, both substances were isolated in low yields: 10% in the case of the reduction of sarsasapogenoic acid dioxime and 33% in the case of the hydrogenation of kryptogenin dioxime. Moreover, the actual course of the dioxime reduction and subsequent cyclization is probably not a simple process, leading to a single isomer.

(30) Catalytic hydrogenation, followed by chromic acid oxidation, gave a ketone, m.p. 160–162°, which provided an infrared spectrum with intensive absorption at 5.82 $\mu$ (carbonyl group in six-membered ring). Although this substance remains to be fully characterized, it appears not to be identical with the 3-keto compound, m.p. 147–148°, derived from dihydrosolasodine by chromic acid oxidation, followed by hydrogenation in the presence of palladium: Y. Sato, H. G. Latham, Jr., and E. Mosettig, *J. Org. Chem.*, **22**, 1496 (1957).

(31) *Cf., e.g.*, J. U. Nef, *Ann.*, **335**, 310 (1904); H. S. Fry and P. E. Bowman, THIS JOURNAL, **52**, 1531 (1930); H. Gilman, D. L. Esmay and R. K. Ingham, *ibid.*, **73**, 470 (1951); W. Tadros, M. S. Ishak and E. Bassili, *J. Chem. Soc.*, 2351 (1954); 627 (1959).

spectra were determined in potassium bromide with Perkin–Elmer infrared spectrophotometers, models 21 and 137. The ultraviolet absorption spectra, in ethanol, were determined with a Cary ultraviolet recording spectrophotometer, model 11 MS. Woelm non-alkaline aluminum oxide was employed for chromatographic separations.

**Zinc–Acetic Acid Reduction of Kryptogenin Diacetate 2,4-Dinitrophenylhydrazone.**—To a solution of 20 g. (0.029 mole) of kryptogenin diacetate 2,4-dinitrophenylhydrazone in 200 ml. of glacial acetic acid at reflux temperature was added, in portions over a period of 1 hour, 160 g. of zinc dust (Merck Reagent). The zinc was removed by filtration and washed with glacial acetic acid. The filtrate was concentrated under reduced pressure. The residue was diluted with water and basified with dilute aqueous ammonium hydroxide. The precipitate was extracted with a mixture of ether and chloroform (3:1). The organic extract was washed with water and concentrated under diminished pressure. The residue was dissolved in a mixture of 900 ml. of methanol and 400 ml. of 5% aqueous potassium bicarbonate. After 4 hours at reflux temperature the methanol was distilled under reduced pressure. The residue was shaken with a mixture of ether and chloroform (3:1). The organic solution was washed with water and concentrated *in vacuo*. The remainder (15 g.) was extracted by trituration in the cold with 5 successive portions of 10% aqueous acetic acid. The acetic acid solution was basified with dilute ammonium hydroxide. The precipitate was extracted with a mixture of ether and chloroform (3:1). The organic phase was washed with water and dried over anhydrous $Na_2SO_4$. The residue (7.1 g.) from vacuum distillation of the solvents was chromatographed over 213 g. of aluminum oxide to give the fractions: (1) ether–ethyl acetate (1:1) 2.7 g.; (2) ethyl acetate–methanol (99:1) 1.0 g.

Fraction 1 gave, from ethyl acetate, 2.1 g. (17.5%) of lustrous white plates of **3β,27-dihydroxy-16β,22-imino-5,22(N)-cholestadiene (I)**, m.p. 143–145°. The analytical sample was recrystallized 5 times from ethyl acetate; m.p. 148–150°, $[\alpha]_D$ −101° ($CHCl_3$), $pK$ 5.45;[32] infrared spectrum: 6.13(m) μ (C=N); ultraviolet spectrum: end absorption.

*Anal.* Calcd. for $C_{27}H_{43}NO_2$ (413.62): C, 78.40; H, 10.48; N, 3.39. Found: C, 78.32; H, 10.51; N, 3.45.

**The hydrochloride** was recrystallized from absolute ethanol; m.p. 230–250°; infrared spectrum: 6.03(m) μ (protonated C=N).

*Anal.* Calcd. for $C_{27}H_{44}NO_2Cl$ (449.08): C, 72.05; H, 9.85; N, 3.11. Found: C, 71.81; H, 10.12; N, 3.05.

Fraction 2 gave, from ethyl acetate, 1.1 g. (9.2%) of needles of **3β,27-dihydroxy-16β,22-imino-5-cholestene (II)**, m.p. 176–178°, $[\alpha]_D$ −37° ($CHCl_3$), $pK$ 9.03.[32]

*Anal.* Calcd. for $C_{27}H_{45}NO_2$ (415.63): C, 78.02; H, 10.91; N, 3.37. Found: C, 77.89; H, 11.16; N, 3.16.

A solution of 165 mg. (0.0004 mole) of II and 0.4 ml. of acetic anhydride in 2 ml. of anhydrous pyridine was kept at ordinary temperature for 15 hours. The mixture was diluted with water. The precipitate was extracted with ether. The organic phase was washed with water and dried over anhydrous $Na_2SO_4$. The remainder from vacuum distillation of the solvent was recrystallized twice from a mixture of ether and petroleum ether (b.p. 30–60°) to give 130 mg. (60%) of plates of **3β,27-diacetoxy-16β,22-acetylimino-5-cholestene**, m.p. 95–96°, $[\alpha]_D$ −37° ($CHCl_3$).

*Anal.* Calcd. for $C_{33}H_{51}NO_5$ (541.75): C, 73.16; H, 9.49; N, 2.59. Found: C, 73.36; H, 9.47; N, 2.60.

A solution of 70 mg. of the triacetyl derivative and 600 mg. of potassium bicarbonate in a mixture of 20 ml. of methanol and 10 ml. of water was maintained at reflux temperature for 4 hours. The solution was concentrated under reduced pressure. The residue was diluted with water and extracted with a mixture of ether and chloroform (3:1). The organic phase was washed with water and dried over anhydrous $Na_2SO_4$. The remainder from vacuum distillation of the solvents was recrystallized from ethyl acetate to give 60 mg. of prisms of **3β,27-dihydroxy-16β,22-acetylimino-5-cholestene**, m.p. 169–172°, $[\alpha]_D$ −25° ($CHCl_3$).

*Anal.* Calcd. for $C_{29}H_{47}NO_3$ (457.67): C, 76.10; H, 10.35; N, 3.06. Found: C, 75.85; H, 10.19; N, 3.11.

(32) We are indebted to Dr. W. Simon of the Eidgenössische Technische Hochschule, Zurich, Switzerland, for this measurement.

**Sodium Borohydride Reduction of I. A.**—To a solution of 40 mg. of I in a mixture of 2 ml. of 3 *N* aqueous hydrochloric acid and 2 ml. of methanol at 0° was added, in portions, 40 mg. of sodium borohydride. After 10 minutes the solution was basified with 3 *N* aqueous sodium hydroxide and extracted with a mixture of ether and chloroform (3:1). The organic phase was washed with water and dried over anhydrous $Na_2SO_4$. The residue from vacuum distillation of the solvents was recrystallized twice from ethyl acetate to give 27 mg. of needles, m.p. 175–177°; mixed m.p. with II, 175–177°.

B.—A solution of 45 mg. of I and 45 mg. of sodium borohydride in 10 ml. of isopropyl alcohol was stirred by means of a magnetic device at ordinary temperature for 1 hour. The mixture was diluted with water. The precipitate was collected by filtration, washed with water and dried; 45 mg. m.p. 160–170°. Recrystallization from ethyl acetate gave needles, m.p. 175–177°; mixed m.p. with II, 175–177°.

**Catalytic Hydrogenation of I.**—A solution of 100 mg. (0.00024 mole) of I in 4 ml. of glacial acetic acid was stirred by means of a magnetic device in an atmosphere of hydrogen at 23°, in the presence of platinum prepared by hydrogenation of 100 mg. of platinum oxide. After 11.2 ml. of hydrogen had been absorbed during a period of 3 hours (theory for 2 moles, 11.6 ml.), the catalyst was removed by filtration. The filtrate was concentrated under reduced pressure and diluted with water. The solution was basified with dilute aqueous ammonium hydroxide and extracted with a mixture of ether and chloroform (3:1). The organic phase was washed with water and dried over anhydrous $Na_2SO_4$. The residue from vacuum distillation of the solvents was recrystallized from ethyl acetate to afford 70 mg. (70%) of plates of **3β,27-dihydroxy-16β,22-iminocholestane**, m.p. 174–177°,[7] $[\alpha]_D$ +18° ($CHCl_3$).

*Anal.* Calcd. for $C_{27}H_{47}NO_2$ (417.66): C, 77.64; H, 11.34; N, 3.35. Found: C, 77.90; H, 11.52; N, 3.41.

**Catalytic Hydrogenation of II.**—A solution of 100 mg. (0.00024 mole) of II in glacial acetic acid was stirred by means of a magnetic device in a hydrogen atmosphere at 25°, in the presence of platinum prepared by hydrogenation of 100 mg. of platinum oxide in 4 ml. of acetic acid. After 1 hour at ordinary temperature, 6.3 ml. of hydrogen had been absorbed (theory for 1 mole, 5.9 ml.). The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was diluted with water and basified with dilute aqueous ammonium hydroxide. The precipitate was extracted with a mixture of ether and chloroform (3:1). The organic phase was washed with water and dried over anhydrous $Na_2SO_4$. The residue from vacuum distillation of the solvents was recrystallized from ethyl acetate to give 71 mg. (71%) of plates of **3β,27-dihydroxy-16β,22-iminocholestane**, m.p. 175–177°.

**Catalytic Hydrogenation of Kryptogenin Dioxime.**—A solution of 200 mg. (0.00043 mole) of kryptogenin dioxime in 5 ml. of glacial acetic acid was stirred by means of a magnetic device in an atmosphere of hydrogen at 23°, in the presence of platinum prepared by reduction of 100 mg. of platinum oxide in 5 ml. of glacial acetic acid. After 60 ml. of hydrogen had been absorbed during 15 hours (theory for 5 moles, 53 ml.) the platinum was removed by filtration. The solution was concentrated under reduced pressure, diluted with water and treated with dilute aqueous ammonium hydroxide. The precipitate was extracted with a mixture of ether and chloroform (3:1). The organic phase was washed with water and dried over anhydrous $Na_2SO_4$. The residue from distillation of the ether was chromatographed over 6.0 g. of aluminum oxide. The material, which was eluted with chloroform–methanol (95:5), was recrystallized from a mixture of ethanol and water to give 61 mg. (33%) of plates of **3β,27-dihydroxy-16β,22-iminocholestane**, m.p. 174–177°.

A solution of 75 mg. of this substance and 0.2 ml. of acetic anhydride in 2 ml. of anhydrous pyridine was kept at ordinary temperature for 15 hours. The pyridine was distilled under reduced pressure. The residue was diluted with water and extracted with ether. The organic phase was washed with 2 *N* aqueous hydrochloric acid, 2 *N* aqueous sodium carbonate and water. The residue from the dried ($NaSO_4$) ether solution was recrystallized from a mixture of ether and petroleum ether (b.p. 30–60°) to give 76 mg. of flat needles of **3β,27-diacetoxy-16β,22-acetyliminocholestane**, m.p. 140–143°, $[\alpha]_D$ −10° ($CHCl_3$).

*Anal.* Calcd. for $C_{33}H_{53}NO_5$ ((543.76): C, 72.89; H, 9.82; N, 2.58. Found: C, 73.15; H, 9.99; N, 2.68.

**Lithium Aluminum Hydride Reduction of I.**—A solution of 60 mg. of I in 50 ml. of anhydrous tetrahydrofuran was added slowly to a suspension of 110 mg. of lithium aluminum hydride in 20 ml. of tetrahydrofuran. After 10 hours at reflux temperature, water was added cautiously to the cooled mixture. The tetrahydrofuran was distilled under reduced pressure. The residue was acidified with 6 *N* hydrochloric acid. The mixture was concentrated under diminished pressure. The precipitate of amine hydrochlorides was collected by filtration and dissolved in ethanol. The solution was basified with dilute aqueous ammonium hydroxide. The ethanol was distilled under reduced pressure. The precipitate was extracted with ether. The ether extract was washed with water and dried over anhydrous sodium sulfate. The residue (50 mg.) from distillation of the ether was chromatographed over 1.5 g. of aluminum oxide. Fractions 1–6 (with ether–chloroform 1:1) gave, from a mixture of ethanol and water, 15 mg. of unchanged I, m.p. 140–144°. Fractions 8–9 (with chloroform–methanol 95:5) gave, from a mixture of ethanol and water, 12 mg. of II, m.p. 169–174°.

**3β,27-Dihydroxy-16β,22-methylimino-5-cholestene. A.**—To 4 ml. of methyl iodide was added 104 mg. (0.00025 mole) of I. A deposit began to separate almost at once. After 20 hours at 0°, the precipitate was collected by filtration and washed with benzene; yield 120 mg. (86%) of the **methiodide of I**, m.p. 225–228°; infrared spectrum: 6.02(m) μ. This material was added to a solution of 125 mg. of sodium borohydride in 50 ml. of isopropyl alcohol. After 3 hours at ordinary temperature, the isopropyl alcohol was distilled under reduced pressure. The residue was treated with water. The precipitate was collected by filtration, washed with water and dried. Recrystallization from ethyl acetate gave plates, yield 85 mg. (92%), m.p. 198–206°. Two additional recrystallizations from ethyl acetate, followed by two recrystallizations from methanol, gave the analytical sample, m.p. 202–206°.

*Anal.* Calcd. for $C_{28}H_{47}NO_2$ (429.66): C, 78.27; H, 11.03; N, 3.26. Found: C, 78.44; H, 10.98; N, 3.20.

B.—A solution of 208 mg. (0.0005 mole) of 3β,27-dihydroxy-16β,22-imino-5-cholestene (II) in a mixture of 1 ml. of 98% formic acid and 1 ml. of 37% aqueous formaldehyde was maintained at reflux temperature for 4 hours. The solvents were distilled under reduced pressure. The residue was diluted with water and treated with dilute aqueous ammonium hydroxide. The precipitate was collected by filtration and washed with water. A solution of the material in a mixture of 4 ml. of ethanol and 0.5 ml. of 3 *N* aqueous potassium hydroxide was kept at reflux temperature for 0.5 hour. The ethanol was distilled under diminished pressure. The residue was extracted with a mixture of ether and chloroform (3:1). The extract was washed with water and dried over anhydrous sodium sulfate. The remainder (175 mg.) from vacuum distillation of the solvents was recrystallized from ethyl acetate to give 40 mg. (18%), m.p. 165–198°. Two additional recrystallizations from ethyl acetate brought the m.p. to 200–206°; mixed m.p. with the substance prepared according to procedure A, 200–206°; infrared spectrum identical with that given by the compound prepared according to procedure A.

**Reaction of I-Methiodide with Potassium Hydroxide.**—A solution of 200 mg. of the methiodide of I in 2 ml. of methanol was treated with a dilute aqueous solution of potassium hydroxide. The mixture was diluted with water. The precipitate was collected by filtration, washed with water and dried in a vacuum desiccator over phosphorus pentoxide. The precipitate, which was completely soluble in anhydrous benzene and which did not give a Beilstein test for halogen, was strongly alkaline. A solution of this material in anhydrous benzene was allowed to remain at ordinary temperature for 4 days. At this stage an orange deposit had separated from the medium. The solid substance was insoluble in benzene, ether and ethyl acetate but dissolved readily in dichloromethane and in methanol. A solution of the compound (150 mg.) in dichloromethane was chromatographed over 4.5 g. of aluminum oxide to give the fractions: (1) dichloromethane, 40 mg.; (2) ethyl acetate, 20 mg.; (3) ethyl acetate–methanol (99:1), 10 mg. No one of these fractions gave a crystalline compound.

**Treatment of I with Nitrous Acid.**—To a solution of 208 mg. (0.0005 mole) of I in 4 ml. of 20% aqueous acetic acid was added a solution of 20 mg. of sodium nitrite in 4 ml. of water. A resinous precipitate separated at once, accompanied by the evolution of gas. After 1 hour at 0°, the precipitate was collected by filtration and washed with water. A solution of the material in 2 ml. of ethanol containing 0.1 ml. of 6 *N* hydrochloric acid was kept at reflux temperature for 5 minutes. The mixture was diluted with water and extracted with ether. The organic phase was washed with dilute aqueous ammonium hydroxide and with water. The ether was distilled under reduced pressure. The residue was dried in a vacuum desiccator over phosphorus pentoxide. A solution of this material in ether was chromatographed over 6.0 g. of aluminum oxide to give the fractions: (1) ether, 45 mg.; (2) ether–methanol (95:5), 165 mg. Fraction 1, after two recrystallizations from methanol, gave 10 mg. of plates, m.p. 225–235°; infrared spectrum: no bands in functional region, complex finger-print region.

**Reaction of I with Acetic Anhydride in Isopropyl Alcohol.**—To a solution of 104 mg. (0.00025 mole) of I in a mixture of 4 ml. of isopropyl alcohol and 1 ml. of water at 0° was added 1 ml. of acetic anhydride. After 4 hours at 0° a crystalline precipitate had separated from the medium. The mixture was diluted with 5 ml. of water. After 15 hours at 0°, the precipitate was collected by filtration, washed with water and dried; 120 mg., m.p. 190–240°. This material was chromatographed in dichloromethane over 3.6 g. of aluminum oxide to give the fractions: (1) dichloromethane, 40 mg.; (2) dichloromethane–methanol (99:1), 80 mg.

Fraction 1 was recrystallized 3 times from methanol to give 30 mg. of **VI** (26%), m.p. 235–245°; infrared spectrum: 6.20 μ (tertiary amide).

Fraction 2 was recrystallized from a mixture of methanol and ethyl acetate to give 70 mg. (60%) of heavy rhombs of **3β,27-dihydroxy-16β-acetylamino-22-keto-5-cholestene (III)**, m.p. 265–273°, $[\alpha]_D$ −1° (MeOH); infrared spectrum: 5.88 ($C_{22}$-carbonyl), 6.10 (secondary amide carbonyl), 6.45 μ (secondary amide).

*Anal.* Calcd. for $C_{29}H_{47}NO_4$ (473.67): C, 73.53; H, 10.00; N, 2.96. Found: C, 73.56; H, 10.04; N, 2.95.

**Reaction of I with Benzoyl Chloride.**—To a mixture of 50 mg. (0.00083 mole) of I and 2 ml. of 3 *N* aqueous sodium hydroxide was added 0.1 ml. of benzoyl chloride. After the suspension had been stirred by means of a magnetic device for 1 hour at ordinary temperature the precipitate was collected by filtration and washed with water. A solution of this material in 2 ml. of methanol containing 0.2 ml. of 3 *N* aqueous potassium hydroxide was maintained at reflux temperature for 2 hours. The mixture was concentrated under reduced pressure and extracted with a mixture of ether and chloroform (3:1). The organic phase was washed with water and dried over anhydrous sodium sulfate. The residue from distillation of the solvent was recrystallized from ethyl acetate to give plates of **3β,27-dihydroxy-16β-benzoylamino-22-keto-5-cholestene**; yield 26 mg. (58%), m.p. 216–217°; infrared spectrum: 5.88 (C.22 carbonyl), 6.10 (secondary amide carbonyl), 6.45 μ (secondary amide).

*Anal.* Calcd. for $C_{34}H_{49}NO_4$ (535.74): C, 76.22; H, 9.22; N, 2.61. Found: C, 76.00; H, 9.15; N, 2.59.

**Reaction of I with Acetic Anhydride in Pyridine.**—A solution of 2.0 g. (0.0048 mole) of I and 8 ml. of acetic anhydride in 30 ml. of anhydrous pyridine was kept at ordinary temperature for 16 hours. The mixture was added to ice and extracted with ether. The organic phase was washed with water and concentrated under reduced pressure. The residue was dissolved in a mixture of 300 ml. of methanol and 200 ml. of water containing 10.0 g. of potassium bicarbonate. After 4 hours at reflux temperature the methanol was distilled under reduced pressure. The remainder was extracted with a mixture of ether and chloroform (3:1). The organic phase was washed with water and dried over anhydrous sodium sulfate. The residue (2.2 g.) from distillation of the solvents was chromatographed in benzene over 66.0 g. of aluminum oxide to give the fractions: (1) ether–ethyl acetate (3:1), 122 mg.; (2) ether–ethyl acetate (1:1), 559 mg.; (3) ethyl acetate, 1242 mg.

Fraction 1, from ethyl acetate, gave 90 mg. (4%) of **VII**, m.p. 200–201°, $[\alpha]_D$ −59° ($CHCl_3$); infrared spectrum: 6.10 μ (tertiary amide); ultraviolet spectrum: end absorption only.

*Anal.* Calcd. for $C_{29}H_{45}NO_3$ (455.66): C, 76.43; H, 10.00; N, 3.07. Found: C, 76.25; H, 10.00, N, 3.47.

Fraction 2 gave, from methanol, 400 mg. (18%) of plates of **VI**, m.p. 226–235°; $[\alpha]_D$ −43° ($CHCl_3$); infrared spectrum: 6.20 μ (tertiary amide); ultraviolet spectrum: end absorption only.

*Anal.* Calcd. for $C_{29}H_{45}NO_3$ (455.66): C, 76.43; H, 10.00; N, 3.07. Found: C, 76.87; H, 10.02; N, 3.00.

Fraction 3 gave, from ethyl acetate, 810 mg. (36%) of plates of **3β,27-dihydroxy-16β,22-acetylimino-5,22(23)-cholestadiene (IV)**, m.p. 195–197°, $[\alpha]_D$ +122° ($CHCl_3$), $[\alpha]_D$ +102° ($C_5H_5N$); infrared spectrum: 6.00(w), 6.16 μ (tertiary amide); ultraviolet spectrum in ethanol: 197.5 mμ (log ε 4.08), 244 mμ (log ε 3.90).[33]

*Anal.* Calcd. for $C_{29}H_{45}NO_3$ (455.66); C, 76.43; H, 10.00; N, 3.07. Found: C, 76.12; H, 10.30; N, 2.97.

**Reaction of VII with Acetic Anhydride in Pyridine.**—A solution of 50 mg. of VII and 0.2 ml. of acetic anhydride in 1.5 ml. of anhydrous pyridine was kept at ordinary temperature for 15 hours. The mixture was diluted with ice-water. The precipitate was collected by filtration, washed with water and dried. Recrystallization from methanol gave prismatic needles of the **3-acetate of VII**, m.p. 184–186°, $[\alpha]_D$ −71° ($CHCl_3$); infrared spectrum: 5.80 (ester carbonyl), 6.10 μ (tertiary amide).

*Anal.* Calcd. for $C_{31}H_{47}NO_4$ (497.69): C, 74.81; H, 9.52; N, 2.81. Found: C, 74.77; H, 9.70; N, 2.83.

**Reaction of VI with Acetic Anhydride in Pyridine.**—A solution of 40 mg. of VI and 0.2 ml. of acetic anhydride in 1.5 ml. of anhydrous pyridine was kept at ordinary temperature for 15 hours. The mixture was diluted with ice-water. The precipitate was collected by filtration, washed with water and dried. Two recrystallizations from methanol gave 25 mg. of the **3-acetate of VI**, m.p. 168–175°, $[\alpha]_D$ −50° ($CHCl_3$); infrared spectrum: 5.80 (ester carbonyl), 6.15 μ (tertiary amide).

*Anal.* Calcd. for $C_{31}H_{47}NO_4$ (497.69): C, 74.81; H, 9.52; N, 2.81. Found: C, 74.98; H, 9.46; N, 2.84.

**Reaction of IV with Potassium Hydroxide in Ethanol.**—A solution of 40 mg. of IV in 2 ml. of 40% ethanolic potassium hydroxide was maintained at reflux temperature for 16 hours. The solution was concentrated under reduced pressure and diluted with water. The precipitate was collected by filtration, washed with water and dried. Recrystallization from ethyl acetate gave 28 mg. of plates, m.p. 142–145°; mixed m.p. with I, 142–145°; infrared spectrum identical with that of I.

**Reaction of IV with Aqueous Hydrochloric Acid.**—To a solution of 20 mg. of IV in 1 ml. of methanol was added 1 drop of 6 *N* hydrochloric acid. After 0.5 hour at ordinary temperature the mixture was diluted with 3 ml. of water. The precipitate was collected by filtration, washed with water and dried. Recrystallization from a mixture of methanol and ethyl acetate gave heavy rhombs, m.p. 265–273°; infrared spectrum: identical with that of 3β,27-dihydroxy-16β-acetylamino-22-keto-5-cholestene (III).

**Reaction of IV with Anhydrous Hydrogen Chloride.**—A slow stream of dry hydrogen chloride gas was introduced during 2 minutes to a solution of 800 mg. (0.0017 mole) of IV in a mixture of 65 ml. of anhydrous ether and 65 ml. of anhydrous benzene. After 4 hours at 0°, the mixture was concentrated to dryness under reduced pressure. A solution of the residue in benzene was chromatographed over 24.0 g. of aluminum oxide. The material (650 mg.) which eluted with ether–ethyl acetate (1:1) was recrystallized from a mixture of ethyl acetate and methanol to give 480 mg. (60%) of needles of **3β,27-dihydroxy-16β,22-acetylimino-5,20(22)-cholestadiene, (V)**, m.p. 181–192°. Recrystallization was accomplished from ethyl acetate; m.p. 194–197°, $[\alpha]_D$ −71° ($CHCl_3$); infrared spectrum: 5.98 (20.22 C=C), 6.15 μ (tertiary amide); ultraviolet spectrum in ethanol: 196.5 mμ (log ε 4.02), 251.5 mμ (log ε 4.16.)[33]

*Anal.* Calcd. for $C_{29}H_{45}NO_3$ (455.66): C, 76.44; H, 10.00; N, 3.07. Found: C, 75.37; H, 9.99; N, 3.29.

A solution of 30 mg. of V and 120 mg. of 3,5-dinitrobenzoyl chloride in 1 ml. of anhydrous pyridine was maintained at reflux temperature for 15 minutes. The mixture was diluted with water. The crystalline precipitate was collected by filtration, washed with water and dried; 46 mg. Recrystallization from a mixture of dichloromethane and methanol gave 31 mg. of the **3β,27-di-(3,5-dinitrobenzoate) of V**, m.p. 205–208°; infrared spectrum: 5.80 (ester carbonyl), 5.98 (20, 22 C=C), 6.15 μ (tertiary amide).

*Anal.* Calcd. for $C_{43}H_{49}N_5O_{13}$ (843.86): C, 61.20; H, 5.85; N, 8.30. Found: C, 61.34; H, 5.97; N, 8.15.

**3β,27-Diacetoxy-16β,22-acetylimino-5,20(22)-cholestadiene. A.**—A solution of 60 ml. of V and 0.15 ml. of acetic anhydride in 1 ml. of pyridine was kept at ordinary temperature for 15 hours. The mixture was diluted with ice-water. The crystalline precipitate was collected by filtration, washed with water and dried; 65 mg. Recrystallization from methanol gave 50 mg. of needles, m.p. 115–120°. The analytical sample was recrystallized twice from methanol; m.p. 119–121°, $[\alpha]_D$ −70° ($CHCl_3$); infrared spectrum: 5.80 (ester carbonyl), 5.98 (20.22 C=C), 6.08 μ (tertiary amide).

*Anal.* Calcd. for $C_{33}H_{49}NO_5$ (539.73): C, 73.43; H, 9.15; N, 2.59. Found: C, 73.18; H, 8.55; N, 2.93.

**B.**—A solution of 207 mg. of 3β,27-dihydroxy-16β,22-imino-5,22(N)-cholestadiene (I) and 0.8 ml. of acetic anhydride in 3 ml. of anhydrous pyridine was kept at 0° for 15 hours. The mixture was diluted with water and extracted with ether. The ether extract was washed with water and concentrated under reduced pressure. The residue was dried in a vacuum desiccator over phosphorus pentoxide. To this material was added 10 ml. of a benzene solution of *p*-toluenesulfonic acid prepared as follows: a solution of 1.0 g. of *p*-toluenesulfonic acid in 1 ml. of acetic annydride was kept at ordinary temperature for 15 minutes; the mixture was diluted with 9 ml. of anhydrous benzene and kept at ordinary temperature for 15 minutes.

After 15 hours at ordinary temperature, the solution was diluted with 50 ml. of ether. The organic phase was extracted with 3 successive quantities of 50 ml. of water and finally with a 5% aqueous solution of potassium bicarbonate. The organic solvents were distilled under reduced pressure. The residue was dried in a vacuum desiccator over phosphorus pentoxide. Recrystallization from methanol gave 230 mg. (85%), m.p. 119–121°.

**3β,27-Dihydroxy-16β,22-imino-5,20(22)-cholestadiene (XI).**—A solution of 225 mg. (0.00042 mole) of 3β,27-diacetoxy-16β,22-acetylimino-5,20(22)-cholestadiene (m.p. 115–118°), 800 mg. of potassium hydroxide and 0.2 ml. of water in 2 ml. of absolute ethanol was maintained at reflux temperature for 23 hours. The solution was diluted with water and extracted with ether. The ether phase was washed with water and concentrated under reduced pressure. The residue was dried in a vacuum desiccator over phosphorus pentoxide. Recrystallization from ethyl acetate gave 140 mg. (74%), m.p. 175–195°. The analytical sample was recrystallized 5 times from methanol (in which the substance is very slightly soluble); m.p. 184–197°; infrared spectrum: 6.15 μ (vinylamine); ultraviolet spectrum: 224 mμ (log ε 3.64).

*Anal.* Calcd. for $C_{27}H_{43}NO_2 \cdot 2H_2O$ (449.65): C, 72.12; H, 10.54; N, 3.12. Found: C, 71.75; H, 9.92; N, 3.22.

**Reaction of VI with Glacial Acetic Acid.**—A solution of 35 mg. of VI in 2 ml. of glacial acetic acid was kept at ordinary temperature for 2 hours. The solution was diluted with water and extracted with a mixture of ether and chloroform (3:1). The organic phase was washed with 2 *N* aqueous sodium carbonate and water. The solution was dried over anhydrous sodium sulfate. The residue from distillation of the solvents was chromatographed over 1.0 g. of aluminum oxide. The material (27 mg.) which eluted with ether–chloroform(1:1) gave, from ethyl acetate, needles of **3β,27-dihydroxy-16β,22-acetylimino-5,22(23)-cholestadiene** (IV), m.p. 195–197°; mixed m.p. with IV (plates, 194–195°; $[\alpha]_D$ +123° ($CHCl_3$), $[\alpha]_D$ +104° ($C_5H_5N$); infrared spectrum: 6.00(w), 6.20 μ (tertiary amide).

*Anal.* Calcd. for $C_{29}H_{45}NO_3$ (455.66): C, 76.43; H, 10.00; N, 3.07. Found: C, 76.47; H, 10.08; N, 3.20.

**Reaction of VI with Aqueous Hydrochloric Acid.**—To a solution of 30 mg. of VI in 8 ml. of methanol was added 8 drops of 6 *N* aqueous hydrochloric acid. After 1 hour at ordinary temperature the solution was diluted with 12 ml. of water. The precipitate was collected by filtration, washed with water and dried. Recrystallization from a mixture of methanol and ethyl acetate gave 15 mg., m.p.

(33) We are indebted to Dr. G. Rotzler of the Organische Anstalt of the University of Basel, Basel, Switzerland, for this measurement.

250–265°; infrared spectrum identical with that of 3β,27-dihydroxy-16β-acetamino-22-keto-5-cholestene (III).

**Reaction of VII with Aqueous Hydrochloric Acid.**—To a solution of 15 mg. of VII in 0.5 ml. of methanol was added 2 drops of 6 *N* hydrochloric acid (aqueous). After 30 minutes at ordinary temperature the mixture was diluted with water. The crystalline precipitate was collected by filtration, washed with water and dried; m.p. 245–255°; infrared spectrum identical with that of 3β,27-dihydroxy-16β-acetamino-22-keto-5-cholestene (III).

**Reaction of IV with 3,5-Dinitrobenzoyl Chloride in Pyridine.**—A solution of 50 mg. of 3β,27-dihydroxy-16β,22-acetylimino-5,22(23)-cholestadiene (IV) and 150 mg. of 3,5-dinitrobenzoyl chloride in 1 ml. of anhydrous pyridine was maintained at reflux temperature for 0.5 hour. The cooled mixture was diluted with water. The precipitate was collected by filtration, washed with water and dried. Recrystallization from a mixture of dichloromethane and methanol gave 60 mg., m.p. 194–204°. Two additional recrystallizations from the same solvent pair afforded 48 mg., m.p. 205–208°; infrared spectrum identical with that of the 3β,27-di-(3,5-dinitrobenzoate) of V.

**Reaction of VI with Anhydrous Hydrogen Chloride.**—A slow stream of dry hydrogen chloride gas was introduced during 2 minutes to a solution of 111 mg. (0.00024 mole) of VI in a mixture of 15 ml. of anhydrous benzene and 15 ml. of anhydrous ether. After 4 hours at 0°, the crystalline precipitate was collected by filtration, washed with water and dried. The product was recrystallized twice from a mixture of ethanol and water and twice from ethyl acetate; yield 85 mg. (76%), m.p. 193–195°; mixed m.p. with V, 193–195°; infrared spectrum identical with that of 3β,27-dihydroxy-16β,22-acetylimino-5,20(22)-cholestadiene (V).

**Reaction of I with Trifluoroacetic Anhydride in Pyridine.**—To a solution of 207 mg. (0.0005 mole) of I in 2 ml. of anhydrous pyridine at 0° (ice–isopropyl alcohol-bath) was added slowly 75 drops (*ca.* 600 mg.) of trifluoroacetic anhydride. After 4 hours at 0° the mixture was diluted with ether and extracted 4 times with water. The dry residue from distillation of the ether was dissolved in 40 ml. of methanol. The solution was maintained at reflux temperature for 1 hour to effect hydrolysis of the ester linkage. (In an earlier experiment in which the methanol solution had been allowed to remain at 0° for 15 hours, the product, m.p. 155–185°, was found by infrared analysis to contain esterified material.) The methanol was distilled under reduced pressure. The remainder was dissolved in ether and washed with water. The dry residue from evaporation of the ether was chromatographed in benzene over 6.0 g. of aluminum oxide. The material (230 mg.) which eluted with ether–dichloromethane (1:1) was recrystallized from ethyl acetate to give 170 mg., m.p. 216–218°, $[\alpha]_D$ +98° ($CHCl_3$); infrared spectrum: 6.10 μ (tertiary amide); ultraviolet spectrum: 255 mμ (log ε 3.72).

*Anal.* Calcd. for $C_{29}H_{42}NO_3F_3$ (509.64): C, 68.34; H, 8.31. Found: C, 66.83; H, 8.30.

**Reaction of I with a Mixture of Anhydrous Formic Acid and Acetic Anhydride in Pyridine.**—A solution of 4 ml. of 98% formic acid and 1.6 ml. of acetic anhydride was kept at ordinary temperature for 4 hours.[34] The solution was cooled to 0° and added dropwise in 6 portions to a solution at 0° of 207 mg. (0.0005 mole) of I in 8 ml. of anhydrous pyridine. After 15 hours at 0°, the mixture was diluted with 50 ml. of 5% aqueous potassium chloride solution. The precipitate was collected by filtration and washed with water. A solution of this material in 3 ml. of 10% ethanolic potassium hydroxide was maintained at reflux temperature for 2.5 hours. The ethanol was distilled under reduced pressure. The residual solution was extracted with ether. The organic phase was washed with water. The ether was distilled under diminished pressure. The residue was extracted in the cold with 50 ml. of 10% aqueous acetic acid. The precipitate was collected by filtration through cotton and washed with water. The filtrate was basified with dilute aqueous ammonium hydroxide. No precipitate appeared, indicating that amide hydrolysis had not occurred. The acetic acid-insoluble material was dried in a vacuum desiccator over phosphorus pentoxide. A solution of the substance in benzene was chromatographed over 6.0 g. of aluminum oxide. About 70 mg. eluted very slowly with ether. Another 30 mg. was obtained with ether–dichloromethane (9:1). These fractions were combined and crystallized from methanol to give 60 mg., m.p. 195–240°. The crystalline product obviously consisted of a mixture of at least two substances, one (plates) melting considerably higher than the other; $[\alpha]_D$ +17° ($CHCl_3$); infrared spectrum: 6.10 μ (tertiary amide); ultraviolet spectrum: end absorption only.

With ether–methanol (95:5) 90 mg. was removed from the alumina column. Recrystallization from methanol gave 55 mg. The analytical sample was recrystallized from methanol, m.p. 212–215°, $[\alpha]_D$ +91° ($CHCl_3$); infrared spectrum: 6.10 μ (tertiary amide); ultraviolet spectrum: 255 mμ (log ε 3.72).

*Anal.* Calcd. for $C_{28}H_{43}NO_3$ (441.63): N, 3.17. Found: N, 3.35.

**3β,27-Dichloro-16β,22-imino-5-cholestene Hydrochloride (IX).**—A solution of 600 mg. (0.0013 mole) of the hydrochloride of II, m.p. 240–250°, in 6 ml. of freshly distilled thionyl chloride was kept at ordinary temperature for 15 hours. The thionyl chloride was removed under reduced pressure. The residue was recrystallized from a mixture of methanol and ether to give 490 mg. (80%) of plates, m.p. 230–236°. The analytical sample was recrystallized from a mixture of methanol and ether; m.p. 232–236°.

*Anal.* Calcd. for $C_{27}H_{44}NCl_3$ (489.00): C, 66.31; H, 9.07; N, 2.86. Found: C, 66.01; H, 8.89; N, 2.70.

**22,25-Isosolanidine 3β-Acetate.**—To a solution of 350 mg. (0.00072 mole) of 3β,27-dichloro-16β,22-imino-5-cholestene hydrochloride in 4 ml. of absolute ethanol was added a solution of 16.5 mg. (0.00072 mole) of sodium in 2 ml. of absolute ethanol. The sodium chloride which precipitated was removed by filtration. The filtrate was maintained at reflux temperature for 4 hours. The ethanol was distilled under reduced pressure. The residue was dissolved in 20 ml. of glacial acetic acid containing 600 mg. of fused potassium acetate. The mixture was maintained at reflux temperature under nitrogen for 4 hours. The solution was concentrated under diminished pressure. The residue was diluted with water and treated with dilute aqueous ammonium hydroxide. The precipitate was collected by filtration, washed with water and dried. Recrystallization from methanol gave 150 mg. (48%) of flat needles, m.p. 153–157°.

*Anal.* Calcd. for $C_{29}H_{45}NO_2$ (493.66): C, 79.22; H, 10.32; N, 3.19. Found: C, 79.02; H, 10.32; N, 3.16.

**22,25-Isosolanidine (X).**—A solution of 50 mg. of 22,25-isosolanidine 3β-acetate and 1.0 g. of potassium bicarbonate in a mixture of 40 ml. of methanol and 10 ml. of water was maintained at reflux temperature for 4 hours. The methanol was distilled under reduced pressure. The residue was diluted with water. The precipitate was collected by filtration, washed with water and dried. Recrystallization from a mixture of dichloromethane and methanol gave 42 mg. of long needles, m.p. 205–210°. The analytical sample was recrystallized 3 times from a mixture of dichloromethane and methanol; m.p. 209–212°, $[\alpha]_D$ −11° ($CHCl_3$).

*Anal.* Calcd. for $C_{27}H_{48}NO$ (397.62): C, 81.55; H, 10.90; N, 3.52. Found: C, 81.22; H, 10.93; N, 3.44.

A solution of 35 mg. of 22,25-isosolanidine and 80 mg. of 3,5-dinitrobenzoyl chloride in 1 ml. of anhydrous pyridine was kept at ordinary temperature for 15 hours. The mixture was 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 long slender needles, m.p. 220–223°, of the **3β,27-di-(3,5-dinitrobenzoyl)-derivative** of 22,25-isosolanidine.

*Anal.* Calcd. for $C_{34}H_{45}N_3O_6$ (591.72): C, 69.01; H, 7.67; N, 7.10. Found: C, 69.34; H, 7.65; N, 7.43.

**Reaction of I with Ethylene Glycol and Potassium Hydroxide.**—A solution of 680 mg. (0.0016 mole) of I and 2.0 g. of potassium hydroxide in 20 ml. of ethylene glycol was maintained at reflux temperature for 3 hours. A heavy crystalline mass began to separate from the red solution after 1 hour. The mixture was diluted with water. The precipitate was collected by filtration, washed with water and dried. Recrystallization from a mixture of dichloromethane and methanol gave 405 mg. (65%) of long needles, m.p. 205–209°, $[\alpha]_D$ −17° ($CHCl_3$); infrared spectrum

(34) F. Reber, A. Lardon and T. Reichstein, *Helv. Chim. Acta*, **27**, 54 (1954).

identical with that of 22,25-isosolanidine; mixed m.p. with 22,25-isosolanidine 205–209°.

*Anal.* Calcd. for $C_{27}H_{43}NO$ (397.62): C, 81.55; H, 10.90; N, 3.52. Found: C, 81.44; H, 11.05; N, 3.63.

**Pyrolysis of the Hydrochloride of I.**—An evacuated sealed tube containing 400 mg. (0.0009 mole) of the hydrochloride of I was immersed in a Dowtherm-bath at 260° for 2 minutes. The contents of the cooled tube were dissolved in ethanol and treated with dilute aqueous ammonium hydroxide. The ethanol was distilled under reduced pressure. The residue was extracted with ether. The ether solution was washed with water and dried over anhydrous $Na_2SO_4$. The remainder from vacuum distillation of the solvent was chromatographed over 10 g. of aluminum oxide. The material (80 mg.) which eluted with ether–chloroform (9:1) was recrystallized from a mixture of dichloromethane and methanol to give 35 mg. (10%) of long needles, m.p. 208–210°; mixed m.p. with 22,25-isosolanidine, 208–210°; infrared spectrum identical with that of 22,25-isosolanidine.

**Reaction of VI with Potassium Hydroxide in Refluxing Ethylene Glycol.**—A solution of 90 mg. of VI and 300 mg. of potassium hydroxide in 2 ml. of ethylene glycol was maintained at reflux temperature for 15 hours. The mixture was diluted with water. The precipitate was collected by filtration and stirred with 10% aqueous acetic acid. The acetic acid solution was clarified by filtration and basified with dilute aqueous ammonium hydroxide. The precipitate was extracted with ether. The organic phase was washed with water and dried over anhydrous $Na_2SO_4$. The residue (50 mg.) was recrystallized from a mixture of dichloromethane and methanol to give needles, m.p. 207–209°; mixed m.p. with 22,25-isosolanidine, 207–210°; infrared spectrum identical with that of 22,25-isosolanidine.

**Catalytic Hydrogenation of 22,25-Isosolanidine.**—A solution of 90 mg. (0.00023 mole) of 22,25-isosolanidine in 5 ml. of glacial acetic acid was added to a suspension of platinum prepared by hydrogenation of 50 mg. of platinum oxide in 5 ml. of glacial acetic acid. After 5.4 ml. of hydrogen had been absorbed during a period of 15 hours (theory for one mole, 5.6 ml.) the catalyst was removed by filtration. The filtrate was concentrated under reduced pressure and diluted with water. The solution was treated with dilute aqueous ammonium hydroxide. The precipitate was collected by filtration, washed with water and dried. Recrystallization from methanol gave needles, m.p. 185–197°, yield 71 mg. (79%). The analytical sample was recrystallized from methanol; m.p. 193–197°, $[\alpha]_D$ +34° ($CHCl_3$).

*Anal.* Calcd. for $C_{27}H_{45}NO$ (399.64): C, 81.14; H, 11.35. Found: C, 80.94; H, 11.51.

A solution of 48 mg. of this substance in a mixture of 7 ml. of acetone and 0.5 ml. of glacial acetic acid (bichromate test) was treated with 0.2 ml. of Kiliani reagent.[35] After 2 hours at ordinary temperature 5 drops of methanol was added. After 0.5 hour the solution was concentrated under reduced pressure to a volume of 2 ml. The mixture was diluted with water and basified with dilute aqueous ammonium hydroxide. The precipitate was extracted with ether. The organic phase was washed with 2 *N* aqueous sodium carbonate solution and with water. The solution was dried over anhydrous sodium sulfate. The remainder from vacuum evaporation of the solvent was chromatographed over 1.5 g. of aluminum oxide. The material (12 mg.) which eluted with ether was recrystallized from ethyl acetate to give 4 mg. of flat needles, m.p. 160–162°; infrared spectrum: 5.82 $\mu$ (keto group in six-membered ring).

**Reaction of Kryptogenin Diacetate with Phenylhydrazine.**—To a solution of 206 mg. (0.0004 mole) of kryptogenin diacetate and 216 mg. (0.002 mole) of phenylhydrazine in 10 ml. of ethanol was added 200 mg. (0.0002 mole) of potassium acetate. The potassium chloride was removed by filtration. After 2 hours at reflux temperature, the ethanol was distilled under reduced pressure. The residue was diluted with water and extracted with ether. The organic extract was washed with water. The remainder from vacuum distillation of the ether was recrystallized from a mixture of chloroform and methanol; yield 110 mg. (49%), m.p. 178–180°; infrared spectrum: 5.78 $\mu$ (ester), 6.06(w), 6.25 $\mu$.

*Anal.* Calcd. for $C_{37}H_{50}N_2O_4$ (586.79): C, 75.73; H, 8.59; N, 4.77. Found: C, 75.31; H, 8.37; N, 4.79.

**Reaction of Kryptogenin with 1-Methyl-1-phenylhydrazine. A.**—A solution of 200 mg. (0.00046 mole) of kryptogenin, 0.3 ml. of 1-methyl-1-phenylhydrazine and 3 drops of acetic acid in 3 ml. of ethanol was maintained at reflux temperature for 18 hours. The ethanol was distilled under reduced pressure. The residue was diluted with water and basified with dilute aqueous ammonium hydroxide. The precipitate was extracted with ether. The organic extract was washed with water and dried over anhydrous $Na_2SO_4$. The ether was distilled *in vacuo*. The residue was chromatographed over 6.6 g. of aluminum oxide. The fraction which eluted with benzene–ether (95:5) gave, from a mixture of ether and petroleum ether (b.p. 30–60°), 104 mg. (42%) of plates, m.p. 154–155°; infrared spectrum: 6.25(m) $\mu$.

*Anal.* Calcd. for $C_{34}H_{50}N_2O_3$ (534.76): C, 76.36; H, 9.43. Found: C, 76.62; H, 9.42.

B.—A solution of 1.28 g. (0.0025 mole) of kryptogenin diacetate, 2.5 ml. of 1-methyl-1-phenylhydrazine and 0.5 ml. of acetic acid in 25 ml. of ethanol was maintained at reflux temperature for 15 hours. The ethanol was distilled under reduced pressure. The residue was diluted with water and basified with dilute aqueous ammonium hydroxide. The precipitate was extracted with ether. The organic extract was washed with water and dried over anhydrous $Na_2SO_4$. A solution of the residue and 3.0 g. of potassium bicarbonate in a mixture of 75 ml. of methanol and 25 ml. of water was maintained at reflux temperature for 5 hours. The methanol was distilled under diminished pressure. The precipitate was collected by filtration, washed with water and dried. Recrystallization from methanol gave 1.04 g. (78%), m.p. 153–155°; mixed m.p. with the compound prepared according to procedure A, 153–155°; infrared spectrum identical with that given by the substance prepared according to procedure A.

**Acknowledgments.**—It is a pleasure to acknowledge the assistance of Grace Swanson in the later phases of the work. The investigation was supported by grants from the National Heart Institute of the National Institutes of Health (USPHS H-2205) and from the Eugene Higgins Trust.

BOSTON 15, MASS.

(35) A solution of 53 g. of chromium trioxide and 80 g. of sulfuric acid in 400 g. of water.