do not support any view that the activity of NMI is promoted by the anionic site. The initial rates of reactivation by NMI and NH appear to be about equal at their optimum *p*H but slightly greater for NH if both are compared at *p*H 8.0. The rate of reactivation of DFP inhibited cholinesterase by NH at 3 hours is only about 30% of TEPP reactivation which can be readily understood in terms of shielding of the phosphorus atom by the more extensive isopropyl groups or in terms of small "chemical" differences. At any rate, the change is quite characteristic of chemical reactions involving isopropyl and ethyl derivatives.[11]

If we accept the comparative data as being essentially correct, is it plausible to conclude that the anionic site is functional toward an N-methylpyridine structure when the esteratic site bears a diethylphosphoryl group, but practically non-functioning when the esteratic site bears a diisopropyl phosphoryl group? Such a conclusion would not seem too difficult if we bear in mind that in the diethylphosphoryl enzyme the affinity of the anionic site toward methylated ammonium ions has been diminished some sixty-fold as compared to the active enzyme and toward the larger pyridinium ions the diminution might be even greater. Evidently, this effect arises from the geometrical closeness of the esteratic and anionic sites whereby the ethyl groups in some configurations physically interfere with the close approach of the substituted ammonium ions and it should not, therefore, be unrealistic to assume that the larger isopropyl groups could further diminish the effectiveness of the anionic site. However, because of the uncertainty in the experimental interpretation of the data, as mentioned before, it is not clear whether there may not be some small promoting effect.

(11) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., New York, N. Y., 1949.

Acknowledgments.—The authors wish to acknowledge the assistance of Miss Carole Quan and to thank Dr. David Nachmansohn for his continuous interest.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE UNIVERSITY]

Constitution and Stereochemistry of Samogenin, Markogenin and Mexogenin[1]

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Employing both degradative and synthetic evidence, samogenin has been shown to be 22a,25a-spirostane-2β,3β-diol (Ia). In view of earlier interrelations, markogenin is now established as the 25b-epimer of samogenin while mexogenin is best defined as 12-ketosamogenin. These three sapogenins are the first examples in the steroid series of naturally occurring *cis*-glycols and mention is made of the consequences of this observation on the current thoughts on the biogenesis of steroidal sapogenins. Several 3,4-dihydroxysapogenins and both 3,4-*seco*-acids of the 5α- and 5β-series have been synthesized and attention is called to the observation that in the 5β-series, attack by peracid or osmium tetroxide on ring A olefins (Δ^2 or Δ^3) proceeds from the front side (β) while rearward approach always is observed with 5α-isomers. A modification of the current sapogenin nomenclature is suggested which defines the configuration at C-25.

The large majority of the known steroidal sapogenins[2] either possess a double bond in the 5,6-position or belong to the 5α(*allo*) series. In fact, only two sapogenins, sarsasapogenin and smilagenin have been shown unequivocally to belong to the 5β(*normal*) series by degradation[3] to pregnane derivatives of established stereochemistry. In 1947, Marker and collaborators[4] recorded the isolation of three new sapogenins, samogenin, texogenin (markogenin[5]) and mexogenin and assigned them to the 5β-series on insufficient evidence.[6] The present paper is concerned with the elucidation of the structure and stereochemistry of these three sapogenins.

Samogenin, first isolated[4] from *Samuela carnerosana* Trel., is the most abundant one and since markogenin (a side chain epimer of samogenin) and mexogenin (x-ketosamogenin) have been interrelated with it,[4,5] most of the structural arguments given below can be applied *ipso facto* to the other two sapogenins. The fallacies in the earlier[4] structure assignment already have been reviewed[1,6] and in particular it has been pointed out that no unequivocal correlation between samogenin and a sapogenin of known structure has been accomplished. A supply of samogenin[7] from the original Marker collection has enabled us to settle most of the outstanding points in the chemistry of these three sapogenins.

Samogenin (Ia) has been shown[4] earlier to be a vicinal dihydroxy sapogenin by oxidation to a dibasic acid V. Conversion of the sapogenin to the dimesylate Ic and treatment with sodium iodide in acetone solution[8] now has furnished an olefin (subsequently proved to possess structure II) which

(1) A preliminary note covering part of this material already has been published (C. Djerassi, J. Fishman and J. A. Moore, *Chemistry & Industry*, 1320 (1954)).

(2) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publ. Corp., New York, N. Y., 1949, 3rd Edit., Chapter VIII.

(3) *Inter al.*, R. E. Marker, THIS JOURNAL, **62**, 3350 (1940).

(4) R. E. Marker, R. B. Wagner, P. R. Ulshafer, E. L. Wittbecker, D. P. J. Goldsmith and C. H. Ruof, *ibid.*, **69**, 2167 (1947).

(5) M. E. Wall, C. R. Eddy, S. Serota and R. F. Mininger (*ibid.*, **75**, 4437 (1953)) isolated a dihydroxysapogenin with presumably the same structure as "texogenin" but with completely different physical constants. The substance was renamed "markogenin" and this term is being used throughout the present paper.

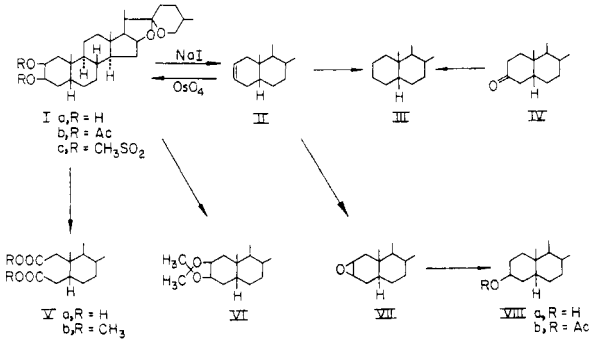
(6) *Cf.* C. Djerassi and R. Ehrlich, *J. Org. Chem.*, **19**, 1351 (1954).

(7) We are greatly indebted to Dr. J. A. Moore of Parke Davis and Co., and to Dr. R. B. Wagner, formerly of the Pennsylvania State College, for donating this material.

(8) This procedure first was introduced into sapogenin chemistry by N. L. Wendler, H. L. Slates and M. Tishler (THIS JOURNAL, **74**, 4894 (1952)) in the case of manogenin.

upon catalytic hydrogenation furnished 22a-spirostan (III), identical with a sample obtained by Huang–Minlon reduction[9] of 22a-spirostan-3-one (smilagenone) (IV). This constitutes the first correlation of samogenin with a known sapogenin and it also establishes the stereochemistry of all asymmetric centers (with the exception of the vicinal hydroxyl groups) since this particular sample of smilagenone (IV) had been synthesized[10] from diosgenin[11] by Oppenauer oxidation followed by catalytic hydrogenation. Samogenin, therefore, can be defined as x,y-dihydroxy-22a-spirostan and is correctly[4] assigned to the 5β-series.

The presence of a *cis*-glycol moiety in samogenin (Ia) is demonstrated by the ready formation of an acetonide VI under conditions where gitogenin (22a,5α-spirostane-2α,3β-diol) is recovered unchanged.[12] Confirmation was provided by the osmium tetroxide hydroxylation of the olefin II, derived from samogenin dimesylate (Ic), which regenerated samogenin (Ia). In order to obtain some information concerning the stereochemistry of the *cis*-glycol system, the olefin II was oxidized with monoperphthalic acid to an epoxide (subsequently shown to be VII) which led to smilagenin (22a-spirostan-3β-ol) (VIIIa)[13] after reduction with lithium aluminum hydride. This reaction sequence (I → II → VII → VIII) not only represents a second correlation of samogenin with a known sapogenin (smilagenin) but also serves to support two additional arguments: (a) Since the hydroxyl group in smilagenin (VIII) is β-oriented,[2] the epoxide VII from which it has been prepared must be a β-epoxide. On the reasonable assumption that attack of both peracid and osmium tetroxide proceeds from the same side of the molecule, the glycol system in samogenin can be assigned the β-configuration. (b) The correlation of samogenin (I) with smilagenin (VIII) fixes the position of one of the hydroxyl groups at position 3, thus leaving only two structural possibilities, 22a-spirostan-2β,3β-diol (Ia) or 22a-spirostan-3β,4β-diol (XXa) open for consideration.



Since oxidation[4] of samogenin yields a *seco*-acid which can now only be the 2,3- or 3,4-isomer of the 5β-series, an attempt was made to differentiate between these two possibilities by partial synthesis. This approach was initiated at a time when a 3,4-dihydroxy-5α-structure[6] for samogenin could not yet be ruled out and the preparation of the third alternative, the 5α-3,4-*seco* acid XVII also was attempted. Lardelli and Jeger[14] have demonstrated in the case of Δ^4-cholesten-3-one that standard Wolff–Kishner reduction conditions result in bond migration with formation of Δ^3-cholesten. Since this appeared to offer a path to at least one of the desired *seco*-acids, we have undertaken a study of the Wolff–Kishner reduction of Δ^4-22a-spirosten-3-one (Δ^4-diosgenone) (IX).[15]

Careful chromatography of the Wolff–Kishner reduction mixture of Δ^4-22a-spirosten-3-one (IX) resulted in the separation of three isomeric olefins ($C_{27}H_{42}O_2$); subsequent work showed that qualitatively the same results were obtained with the Huang-Minlon modification[9] of the Wolff–Kishner reduction. The most dextrorotatory isomer was readily shown to be the unrearranged reduction product, Δ^4-22a-spirosten (X), since it also was obtained by desulfurization[16] of the cycloethylene mercaptal XI of the unsaturated ketone.

The isomer eluted last was the expected Δ^3-22a,5α-spirosten (XII) as could be demonstrated by a series of reactions. Catalytic hydrogenation afforded the known[12,17a] 22a,5α-spirostan (XIII) while perbenzoic acid oxidation led to 3α,4α-oxido-22a,5α-spirostan (XIV),[17b] the structure of which was established by lithium aluminum hydride reduction to 22a,5α-spirostan-3α-ol (epitigogenin) (XVa).[12,18] Acetolysis of the epoxide XIV furnished a separable mixture[17b] of the 4-monoacetate XVIc and the 3,4-diacetate XVIb of 22a,5α-spirostane-3α,4β-diol and the free glycol XVIa, obtained by saponification of the acetates, was oxidized to the desired 3,4-*seco*-acid XVII, which proved to be different from samogenic acid (Va) derived from samogenin (Ia). For comparison purposes, Δ^3-22a,5α-spirosten (XII) also was hydroxylated with osmium tetroxide to the *cis*-glycol XVIIIa, assigned the α-configuration by analogy to the course of the epoxidation reaction (XII → XIV), and oxidation of this isomer yielded the same 3,4-*seco*-acid XVII mentioned above. A quantitative comparison of the rate of cleavage of these two glycols (XIVa, XVIIIa) with lead tetraacetate already has been recorded.[6]

The third olefin isomer produced in the Wolff–Kishner reduction of IX and eluted first in the chromatogram is assigned the Δ^3-22a-spirosten (XIX) structure since catalytic hydrogenation yielded 22a-spirostan (III), a result compatible with a Δ^2- or Δ^3-22a-spirosten formulation. The

(9) Huang-Minlon, THIS JOURNAL, **71**, 3301 (1949).

(10) C. Djerassi, R. Yashin and G. Rosenkranz, *ibid.*, **74**, 422 (1952).

(11) V. H. T. James, *Chemistry & Industry*, 1388 (1953), has related the configuration of carbon atom 25 in diosgenin with D-glyceraldehyde.

(12) J. Pataki, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **73**, 5375 (1951).

(13) We are grateful to Dr. M. E. Wall (United States Department of Agriculture) and Dr. J. A. Moore (Parke, Davis and Co.) for gifts of authentic smilagenin.

(14) G. Lardelli and O. Jeger, *Helv. Chim. Acta*, **32**, 1817 (1949).

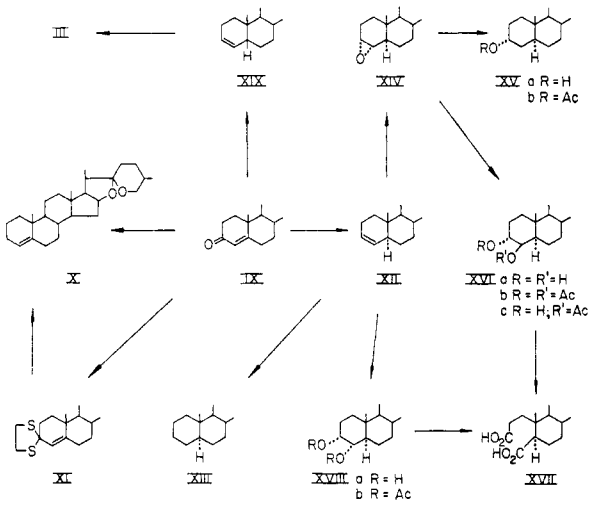
(15) R. E. Marker, T. Tsukamoto and D. L. Turner, THIS JOURNAL, **62**, 2525 (1940).

(16) H. Hauptmann, *ibid.*, **69**, 562 (1947), has shown that no rearrangement of the double bond is involved in this type of desulfurization.

(17) (a) R. E. Marker and D. L. Turner, *ibid.*, **63**, 767 (1941); J. Romo, M. Romero, C. Djerassi and G. Rosenkranz, *ibid.*, **73**, 1528 (1951); (b) A. Fürst and R. Scotoni, *Helv. Chim. Acta*, **36**, 1332 (1953), made a similar observation in the cholestane series.

(18) R. E. Marker, THIS JOURNAL, **62**, 2621 (1940).

occurrence of shift of the 4,5-double bond during Wolff–Kishner reduction to the 3,4-position already has been established[14] (*cf.* IX → XII) and it appeared reasonable that both possible 3,4-isomers (5α- and 5β-configuration) should be pro-



duced even though only one (5α) had been identified earlier.[14] A double migration of the 4,5-double bond to the 2,3-position seemed quite unlikely and could be excluded rigorously by employing a procedure first developed by Fieser and Ettorre[19] in the bile acid field.

Monobromination of 22a-spirostan-3-one (IV) yielded a nearly inseparable mixture of the 4-monobromo XXIV and side-chain brominated products, but the presence of the former could be established by infrared means and by dehydrobromination with 2,4-dinitrophenylhydrazine to the dinitrophenylhydrazone XXV of Δ^4-diosgenone (IX). The crude bromination product was reduced with sodium borohydride and the reduction product was treated with base and subjected to careful chromatography. The first substance eluted proved to be an oxide, to which is assigned the 3α,4α-oxido structure XXVII and which arises from an intermediate 3α-hydroxy-4β-bromo derivative XXVI. The second product eluted from the column was 22a-spirostan-3-one (IV), formed by dehydrobromination of the isomeric 3β,4β-bromohydrin, and these results are in complete accord with the earlier observations[19] in the bile acid series. Lithium aluminum hydride reduction of the 3α,4α-oxide XXVII furnished a new alcohol,[20] 22a-spirostan-4α-ol (XXVIIIa), while acetolysis of the oxide followed by direct saponification furnished 22a-spirostane-3β,4α-diol (XXIII). The same diol XXIII was obtained from the Wolff–Kishner product Δ^3-22a-spirosten (XIX) by perbenzoic acid oxidation to an isomeric epoxide, which, therefore, must be 3β,4β-oxido-22a-spirostan (XXII), and subsequent acetolysis and saponification.

The above reaction sequence proves the 3,4-location of the double bond in the olefin XIX as well as the 5β-configuration and since this olefin was not identical with the olefin IV produced in the sodium iodide reaction of samogenin dimesylate (Ic), the latter must be the 2,3-isomer by exclusion. The non-identity of the two olefins, the physical constants of which were very similar, was confirmed by the course of the osmium tetroxide oxidation of Δ^3-22a-spirosten (XIX) which produced a *cis*-glycol, 22a-spirostan-3β,4β-diol (XXa),[21] and upon oxidation the derived 3,4-*seco*-acid XXIa of the 5β-series. Both substances (XXa and XXIa) proved to be different from samogenin (Ia) and samogenic acid (Va) and this also applied to the corresponding derivatives (XXb, XXIb). Since one of the hydroxyl groups in samogenin must be at C-3 because of its conversion to smilagenin (VIIIa) (*vide supra*), samogenin must be 22a-spirostan-2β,3β-diol (Ia).

It is instructive to note that in contrast to the 5α-series, where attack on either the 2,3 or 3,4-double bonds proceeds from the rear (α-side), the converse applies to the 5β-series. An inspection of models further illustrates this point since in the 5β-series, the hindering effect of the C-19 angular methyl group to frontal attack becomes negligible in view of the fact that ring A is nearly perpendicular to the plane formed by rings B, C and D.[22]

In addition to the above exclusion evidence, it has been possible to provide support for the structure assignments by partial synthesis. 22a-Spirostan-3α-ol (epismilagenin) (XXIXa), derived from diosgenin,[11] was converted into the tosylate XXIXb and refluxed with γ-collidine. In one experiment, the olefin mixture (consisting chiefly of the Δ^3-olefin XIX accompanied by some Δ^2-isomer II) was hydroxylated with osmium tetroxide and the crude hydroxylation product was oxidized and methylated. Chromatography of the methyl esters furnished pure methyl samogenate (Vb) as the minor component, the major product being the isomeric 3,4-derivative XXIb. In a second experiment, the glycol mixture was chromatographed directly and a small amount of pure samogenin (Ia), further characterized as the diacetate Ib, could be separated from the predominating 3,4-isomer XXa.

With the establishment of the constitution and stereochemistry of samogenin, it is now possible to consider the structures of markogenin and mexogenin. According to Wall, *et al.*,[5] markogenin forms a furosten derivative *different* from that derived from samogenin; vigorous acid treatment of markogenin, however, transforms it into samogenin. It is clear, therefore, that samogenin and markogenin differ only in the nature of the side chain[23] and that they bear the same relationship to each other as does smilagenin to sarsasapogenin. According to recent work[24] the side chain of

(19) L. F. Fieser and R. Ettorre, THIS JOURNAL, **75**, 1700 (1953).

(20) This represents further evidence for the α-configuration since the epimeric oxide would yield smilagenin.

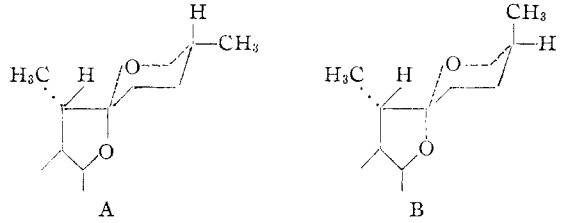
(21) The β-configuration is assigned on the usual basis, namely, that attack by osmium tetroxide most likely occurs from the same side as does perbenzoic acid.

(22) *Cf.* D. H. R. Barton, *Experientia*, **6**, 316 (1950).

(23) The configuration of the hydroxyl groups in ring A is the same as demonstrated by quantitative lead tetraacetate oxidation studies (ref. 6).

(24) *Cf.* D. A. H. Taylor, *Chemistry & Industry*, 1066 (1954), and I. Scheer, R. B. Costic and E. Mosettig, THIS JOURNAL, **77**, 641 (1955), for leading references.

smilagenin (and hence samogenin) is best depicted by A and that of sarsasapogenin (and hence markogenin) by B.



A B

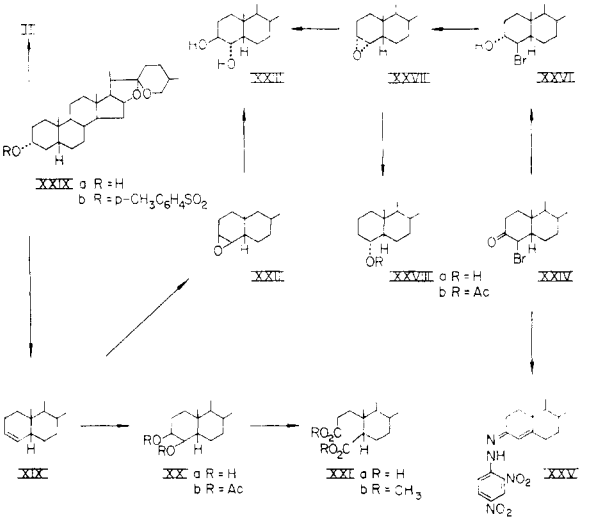
This implies that the only inversion produced is that at C-25 although some authors[25] claim that inversion at C-22 also is involved. Until general agreement is reached, presumably when additional evidence is forthcoming, nothing is gained by changing the generally accepted sapogenin nomenclature[26] or structural representation of the spiroketal side chain. However, one change already can be introduced and that refers to the configuration at C-25 which is not defined in the earlier nomenclature.[26] It is now suggested that "22a" and "22b" be still retained in the original sense[26] until definite agreement is reached concerning the configuration at C-22 but that the configuration at C-25 be defined[27] by introducing "25b" for the sarsasapogenin side chain (related to L-glyceraldehyde)[11] and "25a" for the smilagenin (diosgenin) side chain (related to D-glyceraldehyde).[11] Samogenin (I) therefore is described at the present time as 22a-25a-spirostane-2β,3β-diol and markogenin as 22b,25b-spirostane-2β,3β-diol.

Mexogenin was isolated[4] together with samogenin and Marker, *et al.*, suggested that it was 12-ketosamogenin since it could be converted by Wolff–Kishner reduction to samogenin and because the reactivity of the keto group in mexogenin closely resembled that of other 12-ketosapogenins but not that of 6- or 7-ketosapogenins. We were able to confirm the conversion of mexogenin to samogenin, using the Huang-Minlon procedure,[9] and since the infrared carbonyl band of mexogenin is typical of 6-membered ketones (thus excluding C-15), mexogenin almost certainly can be referred to as 22a,25a-spirostane-2β,3β-diol-12-one.

It is noteworthy that samogenin, markogenin and mexogenin are the first examples in the steroidal sapogenin series in which the presence of a *cis*-glycol system has been demonstrated. Gitogenin (22a,25a,5α-spirostane-2α,3β-diol) has been assigned[12,28,29] the *trans*-2α,3β-configuration and this should apply also to all the other sapogenins (yuccagenin,[29] lilagenin, kammogenin and manogenin) related by Marker[4] with gitogenin.

Marker and collaborators[30] in a biogenetic scheme of the steroidal sapogenins proposed that yuccagenin (Δ^5-22a,25a-spirosten-2α,3β-diol) is the precursor in the plant for both gitogenin (22a,25a,5α-spirostane-2α,3β-diol) and samogenin (22a,25a-spirostane-2β,3β-diol) (Ia), biological reduction of the 5,6-bond yielding in one instance the 5α- and in the other the 5β-dihydro derivative. The demonstration in the present work that gitogenin and samogenin differ in configuration *both* at C-2 and C-5 indicates that the biogenetic pattern appears to be involved considerably more than originally suggested[30] or that, in fact, it may proceed by a completely different sequence.

Recently,[31] Nawa reported the isolation of a new dihydroxy sapogenin from *Rhodea japonica* Roth. and suggested that it was 22b-spirostane-2β,3α-diol on the basis of various chemical reactions, none of which led to a connection with a known steroid, and the fact that the substance was not identical with any known sapogenin. Through the kindness of Dr. Nawa we have been able to secure a small sample of iso-rhodeasapogenin, the acid isomerization product of rhodeasapogenin, and which should now have the 22a-configuration in the side chain. If the suggested[31] structure were correct, the previously unknown iso-rhodeasapogenic acid should be identical with samogenic acid (V). We have prepared iso-rhodeasapogenic acid and have found it to be different from all four possible 2,3- and 3,4-*seco* acids with the 22a,25a-side chain and with either the 5α- or 5β-configuration. Rhodeasapogenin can, therefore, not have the suggested[31] structure and the problem remains an unsolved one.



Experimental[32]

Samogenin Acetonide (VI).—A sample of samogenin (0.25 g.) was extracted continuously for 16 hours in a soxhlet extractor with 50 cc. of acetone containing 50 mg. of *p*-toluenesulfonic acid. Sodium carbonate was added, the volume was reduced to *ca.* 20 cc. and the product was extracted with ether. After washing with water, the solvent

(25) M. E. Wall and S. Serota, THIS JOURNAL, **76**, 2850 (1954); J. B. Ziegler, W. E. Rosen and A. C. Shabica, *ibid.*, **76**, 3865 (1954).

(26) G. Rosenkranz and C. Djerassi, *Nature*, **166**, 104 (1950). Ciba Conference on Steroid Nomenclature, *Chemistry & Industry*, June 23 (pp. SN 1–SN 11) (1951).

(27) This suggestion has now also been made by Scheer, Costic and Mosettig (ref. 24).

(28) *Cf.* J. Herran, G. Rosenkranz and F. Sondheimer, THIS JOURNAL, **76**, 5531 (1954).

(29) C. Djerassi, L. B. High, T. T. Grossnickle, R. Ehrlich, J. A. Moore and B. R. Scott, *Chemistry & Industry*, 474 (1955).

(30) Reference 4, p. 2216.

(31) H. Nawa, *Proc. Japan Acad.*, **29**, 214 (1953); *J. Pharmaceut. Soc. Japan*, **73**, 1192, 1195, 1197 (1953).

(32) Melting points are uncorrected. Unless noted otherwise, rotations and infrared spectra (Baird double beam recording spectrophotometer using 0.1-mm. cells) were measured in chloroform solution. The microanalyses were carried out by Geller Laboratories, Hackensack, N. J.

was evaporated and the residue recrystallized once from ethanol yielding 0.14 g., m.p. 163–168°. Further recrystallization raised the m.p. to 167–170°, $[\alpha]^{29}D$ −72°.

Anal. Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 76.02; H, 10.07.

Samogenin Dimesylate (Ic).—A solution of 0.98 g. of samogenin (Ia) in 3 cc. of pyridine and 2 cc. of methanesulfonyl chloride was left at room temperature for 36 hours and then partitioned between chloroform and dilute hydrochloric acid. Thorough washing of the chloroform solution followed by drying and evaporation furnished a solid residue which was recrystallized once from benzene–hexane; yield 1.05 g., m.p. 195–198°. The analytical sample exhibited m.p. 201–202°, $[\alpha]^{28}D$ −63°.

Anal. Calcd. for $C_{29}H_{48}O_8S_2$: C, 59.15; H, 8.22. Found: C, 59.55; H, 7.98.

Δ^2-22a,25a-Spirosten (II).—The above dimesylate (0.25 g.) was heated in a sealed tube at 100° for 24 hours with 0.68 g. of sodium iodide and 8 cc. of acetone. The dark colored solution was concentrated, chloroform was added and the extract was washed with sodium thiosulfate, water, dried and evaporated. Recrystallization from methanol–chloroform led to 0.13 g., m.p. 147–150°, raised on further recrystallization to m.p. 149–150°, $[\alpha]^{26}D$ −84°.

Anal. Calcd. for $C_{27}H_{42}O_2$: C, 81.35; H, 10.62. Found: C, 81.20; H, 10.77.

22a,25a-Spirostan (III). (a) By Hydrogenation of Δ^2-22a,25a-spirosten or Δ^3-22a,25a-Spirosten (XIX).—The olefin II (50 mg.) was hydrogenated with platinum oxide catalyst in absolute ethanol solution for 20 hours. Filtration of the catalyst, evaporation to dryness and crystallization from ethanol yielded 40 mg. of 22a,25a-spirostan (III), m.p. 139–140°, $[\alpha]^{25}D$ −72°, which was shown by mixture melting point and infrared comparison to be identical with a specimen prepared according to b.

The same results were observed in the catalytic hydrogenation of the Δ^3-isomer XIX; the product, m.p. 138–139°, $[\alpha]^{25}D$ −74°, was identical in all respects with the material obtained from the Δ^2-derivative II.

In one hydrogenation, carried out by hydrogenation of the olefin II with palladium black in glacial acetic acid (20 min.), the product crystallized as needles, m.p. 119–120°. Recrystallization from ethanol and seeding with the higher melting form (blades) transformed it into the latter, indicating that there exist at least two polymorphic forms of the compound.

(b) By Huang-Minlon[9] Reduction of 22a,25a-Spirostan-3-one (IV).—The reduction of the ketone IV (0.4 g.)[10] was carried out exactly as described below for the Huang-Minlon reduction of Δ^4-diosgenone (IX) using diethylene glycol as the solvent. The product (0.35 g.) after recrystallization exhibited m.p. 138–140°, $[\alpha]^{25}D$ −75°, no carbonyl absorption in the infrared.

Anal. Calcd. for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07. Found: C, 80.97; H, 11.18.

Synthesis of Samogenin (Ia). (a) From Δ^2-22a,25a-Spirosten (II).—A mixture of 100 mg. of the olefin II (formed from samogenin dimesylate), 100 mg. of osmium tetroxide, 3 cc. of pyridine and 5 cc. of benzene was left at room temperature for 72 hours and then evaporated to dryness. The residue was refluxed for 4 hours with 1 g. of potassium hydroxide, 1 g. of mannitol, 10 cc. of ethanol, 5 cc. of benzene and 3 cc. of water and the product was extracted with more benzene. After the usual processing and repeated recrystallization from acetone there was isolated 30 mg. of samogenin (m.p. 205–206°, $[\alpha]^{29}D$ −88°) which proved to be identical by mixture melting point and infrared comparison with samogenin[7] (m.p. 205–207°, $[\alpha]^{29}D$ −86°) isolated from *S. carnerosana*. Similarly, the diacetate Ib (m.p. 196–198°, $[\alpha]^{25}D$ −75°) proved to be identical with the derivative prepared from the natural sapogenin.

(b) From 22a,25a-Spirostan-3α-ol (XXIXa).—22a,25a-Spirostan-3-one (IV)[10] (10 g.) was reduced with sodium borohydride (rather than lithium aluminum hydride as described earlier[10]) and the resulting 3α-alcohol **XXIXa** was transformed into the corresponding tosylate by treatment with *p*-toluenesulfonyl chloride in pyridine solution for 36 hours at room temperature. A sample of the **tosylate XXIXb** was recrystallized from acetone; m.p. 168–170°, $[\alpha]^{29}D$ −38°.

Anal. Calcd. for $C_{34}H_{50}O_5S$: C, 71.55; H, 8.83; S, 5.61. Found: C, 71.55; H, 9.09; S, 5.62.

The crude tosylate was refluxed for 6 hours with 200 cc. of γ-collidine and after processing in the usual manner, the crude product was passed through unwashed alumina to furnish 5.2 g. of mixed olefins (II and XIX). Hydroxylation with 3.5 g. of osmium tetroxide and cleavage with mannitol and alkali was performed as described under (a) and the residue (4.4 g.) was chromatographed on 135 g. of alumina which had been deactivated by shaking for 30 minutes with 4.5 cc. of 10% acetic acid. Since the separation of the two glycols I and XX is very inefficient, approximately 70 fractions were eluted with chloroform. The samogenin concentrated in the last eluates, as shown by infrared spectroscopy, and the last ten fractions were rechromatographed and again eluted with chloroform. Recrystallization from acetone gave 20 mg. of pure samogenin (Ia), m.p. 205–207°, and, upon acetylation, the diacetate Ib, m.p. 196–198°. The infrared spectra were identical with those of authentic specimens and no melting point depression was observed on admixture. The bulk (*ca.* 2 g.) of the glycol fraction proved to be the expected 22a,25a-spirostane-3β,-4β-diol (XXa) described below.

Samogenic Acid (Va). (a) From Samogenin (Ia).—A solution of 100 mg. of samogenin (Ia) in 15 cc. of acetic acid was oxidized with 150 mg. of chromium trioxide in 2.5 cc. of 80% acetic acid for 35 minutes at room temperature. The crude acid fraction, isolated in the usual manner, was recrystallized from acetone yielding 70 mg., m.p. 270–273°, $[\alpha]^{25}D$ −37° (chloroform), −39° (pyridine); lit.[4] m.p. 270–271°, no rotation recorded. The melting point was depressed on admixture with the 3,4-*seco*-acids of both the 5α (XVII) and 5β (XXIa) series.

The methyl ester (Vb), prepared with diazomethane, was recrystallized from methanol; m.p. 144–145°, $[\alpha]^{25}D$ −32°. Marker, *et al.*,[4] report m.p. 159–160° (no rotation given) but we have been unable to raise the melting point above 145°; this also applied to the specimen obtained by partial synthesis (below) and it is conceivable that this is a further example of polymorphism, so predominant in the sapogenin series.

Anal. Calcd. for $C_{29}H_{46}O_6$: C, 70.98; H, 9.45. Found: C, 70.75; H, 9.35.

(b) From 22a,25a-Spirostan-3α-ol (XXIXa).—In view of the poor separation of the epimeric glycols I and XX in the above partial synthesis of samogenin, a better indication of the relative proportions of the two olefin isomers II and XIX can be obtained from the following experiment.

22a,25a-Spirostan-3α-ol tosylate (3.0 g., m.p. 164–165°) was refluxed for 6 hours with 50 cc. of γ-collidine and the reaction product was passed through 50 g. of unwashed alumina to furnish 1.5 g. of colorless crystals (m.p. 140–147°) representing a mixture of olefins (II, XIX). This mixture was hydroxylated with 1.5 g. of osmium tetroxide in the usual manner and slow crystallization of the resulting glycol mixture from acetone furnished 0.7 g. of 22a,25a-spirostane-3β,4β-diol (XXa), m.p. 191–194°, further characterized as the diacetate XXb (m.p. 209–211°). The mother liquors were evaporated to dryness and the residue oxidized with chromium trioxide. The acid fraction (0.34 g.) was methylated and the mixed methyl esters were chromatographed on 15 g. of neutral alumina. The hexane–benzene (9:1) eluates furnished 87 mg. of the dimethyl ester of the 3,4-*seco*-acid XXIb, m.p. 195–197°, while from the hexane–benzene (1:1) eluates, there was isolated 80 mg. of dimethyl samogenate (Vb), m.p. 143–145°, $[\alpha]^{25}D$ −34°, identical with the product obtained in a.

2β,3β-Oxido-22a,25a-spirostan (VII).—Δ^2-22a,25a-Spirosten (II) (0.6 g.) was oxidized in ether solution with excess monoperphthalic acid for 72 hours. Chromatographic purification of the crude oxidation product followed by recrystallization from acetone furnished 0.31 g. of epoxide with m.p. 229–231°, $[\alpha]^{25}D$ −73°.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21; Found: C, 77.85; H, 10.08.

Lithium Aluminum Hydride Reduction of 2β,3β-Oxido-22a,25a-spirostan (VII).—The above epoxide VII (65 mg.) was refluxed with an excess of lithium aluminum hydride in ether solution for 4 hours. Two recrystallizations of the crude product, obtained in quantitative yield, from acetone led to smilagenin (VIIIa), m.p. 187–189°, acetate VIIIb, m.p. 149–151°. Identity with authentic samples[13] was established by mixture melting point determination and infrared spectra.

Wolff-Kishner Reduction of Δ^4-22a,25a-Spirosten-3-one (Δ^4-Diosgenone) (IX).—A mixture of 5.0 g. of Δ^4-diosgenone (IX) (m.p. 181–183°[15]) in 200 cc. of absolute ethanol and 5 cc. of anhydrous hydrazine[33] was refluxed for 30 minutes, a solution of 5 g. of sodium in 100 cc. of absolute ethanol was added and heating was continued in an autoclave for 16 hours at 200–210°. The combined reaction product (18.6 g., no infrared carbonyl band) from four identical runs was chromatographed on 900 g. of unwashed alumina using hexane and hexane–benzene (9:1) as eluting agents. Ninety-five fractions were collected and combined in four groups according to melting points: A, 2.69 g., m.p. 134–147°; B, 7.23 g., m.p. 104–166°; C, 4.8 g., m.p. 138–163°; D, 1.79 g., m.p. 160–172°. Groups B and C were combined and rechromatographed in the same manner on 700 g. of alumina collecting 40 fractions which were divided as follows: A′, 2.2 g., m.p. 141–148°; B′, 5.02 g., m.p. 114–145°; C′, 3.57 g., m.p. 125–150°; D′, 1.33 g., m.p. 150–168°.

Fractions A and A′ after two recrystallizations from methanol–chloroform yielded 4.1 g. of **Δ^3-22a,25a-spirosten (XIX)**, m.p. 143–146°; the analytical sample had m.p. 142.5–144° (Kofler), $[\alpha]_D^{25}$ −86°, pale yellow color with tetranitromethane. A mixture melting point with the Δ^2-isomer was depressed to 130–140°.

Anal. Calcd. for $C_{27}H_{42}O_2$: C, 81.35; H, 10.62. Found: C, 81.14; H, 10.74.

Three slow crystallizations of fraction B′ from ethanol furnished 1.67 g. of Δ^4-22a,25a-spirosten (X) as two, interconvertible polymorphic forms, m.p. 134–135° and 143–145°, $[\alpha]_D^{23}$ −30°.

Anal. Calcd. for $C_{27}H_{42}O_2$: C, 81.35; H, 10.62. Found: C, 81.25; H, 10.75.

For further characterization, 0.5 g. of the olefin was oxidized with perbenzoic acid to give, after recrystallization from ethanol, 0.3 g. of 4ξ,5ξ-oxido-22a,25a-spirostan, m.p. 195–198°, $[\alpha]_D^{25}$ −26°.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 77.93; H, 10.03.

No pure material could be obtained from C′, but recrystallization of combined D and D′ from ethanol led to 2.06 g. of Δ^3-22a,25a,5α-spirosten (XII), m.p. 172–174° (Kofler), $[\alpha]_D^{25}$ −34°.

Anal. Calcd. for $C_{27}H_{42}O_2$: C, 81.35; H, 10.62. Found: C, 81.49; H, 10.55.

Catalytic hydrogenation as described above (II → III) produced in 80% yield 22a,25a,5α-spirostan (desoxytigogenin) (XIII), m.p. 172–174°, $[\alpha]_D$ −76°, identified by direct comparison with an authentic specimen.[12,17]

Huang-Minlon Reduction[9] of Δ^4-22a,25a-Spirosten-3-one (IX).—It seemed of interest to determine the composition of the reduction mixture when Huang-Minlon's conditions[9] are employed and 1.0 g. of the unsaturated ketone IX was, therefore, refluxed with 30 cc. of ethanol, 30 cc. of diethylene glycol and 4 cc. of commercial 85% hydrazine hydrate for 35 minutes, followed by the addition of 3 g. of potassium hydroxide and refluxing for 25 minutes. The condenser was removed, the temperature of the solution was permitted to rise to 190° (15 cc. of diethylene glycol having been added to redissolve some precipitated material) and refluxing was then continued for 2.5 hours. The crude reduction product was processed exactly as described above for the standard Wolff–Kishner reduction and yielded 80 mg. of Δ^3-22a,25a-spirosten (XIX), 50 mg. of Δ^4-22a,25a-spirosten (X) and 200 mg. of Δ^3-22a,25a,5α-spirosten (XII). The reversal in the proportion of XIX and XII as compared to the preceding experiment is noteworthy.

Desulfurization of Δ^4-22a,25a-Spirosten-3-one Cycloethylene Mercaptal (XI) to Δ^4-22a,25a-Spirosten (X).—A mixture of 2.0 g. of the unsaturated ketone IX, 2.0 cc. of ethanedithiol, 10 g. of anhydrous sodium sulfate, 10 g. of freshly fused zinc chloride and 20 cc. of dioxane was permitted to stand at room temperature overnight and then was poured into excess dilute ammonium hydroxide. The product was collected and recrystallized from benzene–ethanol; yield 1.8 g., m.p. 265–267°, $[\alpha]_D^{25}$ +30°. Molecular rotation difference calculations[34] confirm that no double bond migration had occurred.

Anal. Calcd. for $C_{29}H_{44}O_2S_2$: C, 71.26; H, 9.07; S, 13.12. Found: C, 71.08; H, 9.15; S, 13.17.

The mercaptal (300 mg.) in 100 cc. of dioxane was refluxed for 3 hours with 5 g. of W-4 Raney nickel and filtered. The filtrate was diluted with an equal volume of water and the milky solution was left overnight in the refrigerator. Collection of the precipitate (200 mg.) and slow recrystallization from ethanol furnished 100 mg. of Δ^4 22a,25a-spirosten (X),[16] m.p. 142–144°, $[\alpha]_D^{22}$ −32°, identical (including infrared spectrum) with the product isolated in the Wolff–Kishner reduction.

3α,4α-Oxido-22a,25a,5α-Spirostan (XIV).—Perbenzoic acid oxidation of 1.0 g. of the Δ^3-5α-olefin XII with excess perbenzoic acid in ether solution for 72 hours yielded 0.75 g. of the epoxide after recrystallization from acetone; m.p. 195–198°, $[\alpha]_D^{22}$ −60°.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 77.93; H, 10.42.

Lithium aluminum hydride reduction (ether solution, 2 hours refluxing) of 0.3 g. of the epoxide XIV led to 0.25 g. of **22a,25a,5α-spirostan-3α-ol (XVa)**, m.p. 243–245°, **acetate (XVb)**, m.p. 199–203°. Identity with authentic material[12] was established by direct comparison (infrared spectrum and mixture melting point).

22a,25a,5α-Spirostane-3α,4β-diol (XVIa).—One-half gram of the above 3α,4α-epoxide XIV was refluxed for 3.5 hours with 50 cc. of glacial acetic acid and diluted with water. Extraction with ether, washing with bicarbonate solution and water, drying, evaporation and slow crystallization from acetone produced 0.28 g. of crystals, m.p. 250–265°, representing a mixture[18] of mono- (XVIc) and diacetate (XVIb). Saponification with 10% methanolic potassium hydroxide followed by recrystallization from acetone furnished 0.21 g. of the 3α,4β-diol XVIa, m.p. 263–264°, $[\alpha]_D^{25}$ −70°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 75.11; H, 10.36.

In a second experiment starting with 1.0 g. of epoxide XIV, the mixture of acetates was chromatographed on 30 g. of neutral alumina. Elution with petroleum ether resulted in the recovery of 0.18 g. of starting material XIV while elution with benzene and recrystallization from chloroform–acetone gave 0.19 g. of **22a,25a,5α-spirostane-3α,4β-diol diacetate (XVIb)**, m.p. 274–276°, $[\alpha]_D^{25}$ −87°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 72.02; H, 9.14.

Further elution with ether and recrystallization from acetone gave the monoacetate XVIc, m.p. 216–218°, $[\alpha]_D^{25}$ −63°.

Anal. Calcd. for $C_{29}H_{46}O_5$: C, 73.38; H, 9.77. Found: C, 73.30; H, 10.07.

22a,25a,5α-Spirostane-3α,4α-diol (XVIIIa).—Δ^3-22a,25a,-5α-Spirosten (XII) (0.78 g.) was hydroxylated in the usual manner (48 hours) with 0.5 g. of osmium tetroxide in 15 cc. of pyridine and 20 cc. of benzene. Cleavage of the osmate ester was accomplished by refluxing the residue for 4 hours with a mixture of 3.5 g. of mannitol, 3.5 g. of potassium hydroxide, 35 cc. of ethanol, 20 cc. of benzene and 10 cc. of water. Recrystallization from methanol–ether led to 0.41 g. of colorless crystals, m.p. 248–251°, raised on further recrystallization to m.p. 254–256°, $[\alpha]_D^{25}$ −83°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.95, H, 10.25. Found: C, 75.02; H, 10.29.

The diacetate XVIIIb was recrystallized from methanol–ether; m.p. 237–238°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 72.34; H, 9.47.

3,4-*seco*-22a,25a,5α-Spirostane-3,4-dioic Acid (XVII).—Chromium trioxide oxidation of either the 3α,4β-diol XVIa or the 3α,4α-isomer XVIIIa in the manner described above (I → V) and recrystallization from dilute methanol yielded the desired *seco*-acid, m.p. 273–275°, $[\alpha]_D^{27}$ −69° (pyridine).

Anal. Calcd. for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15; neut. equiv., 231. Found: C, 69.99; H, 9.23; neut. equiv., 238.

22a,25a-Spirostane-3β,4β-diol (XXa).—Hydroxylation of 0.8 g. of Δ^3-22a,25a-spirosten (XIX) with osmium tetroxide furnished after recrystallization from acetone 0.35 g. of diol, m.p. 192–195°, $[\alpha]_D^{29}$ −82°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.69; H, 10.11.

(33) L. I. Smith and K. L. Howard, *Org. Syntheses*, **24**, 53 (1944).
(34) C. Djerassi and M. Gorman, THIS JOURNAL, **75**, 3704 (1953).

The diacetate XXb, recrystallized from methanol–ether, exhibited m.p. 210–212°, $[\alpha]^{29}D$ −46°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 71.92; H, 9.40.

Chromium trioxide oxidation of 0.1 g. of the diol XXa and recrystallization of the acid fraction from acetone produced 0.08 g. of **3,4-*seco*-22a,25a-spirostane-3,4-dioic acid (XXIa)**, m.p. 264–266°, $[\alpha]^{25}D$ −19° (pyridine).

Anal. Calcd. for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15. Found: C, 69.90; H, 8.92.

The dimethyl ester XXIb was recrystallized from methanol; m.p. 195–197°, $[\alpha]^{25}D$ −47°.

Anal. Calcd. for $C_{29}H_{46}O_6$: C, 70.98; H, 9.45. Found: C, 70.80; H, 9.31.

3α,4α-Oxido-22a,25a-spirostan (XXVII).—A solution of 4.14 g. of 22a,25a-spirostan-3-one (IV)[10] in 250 cc. of anhydrous ether was treated dropwise over a period of 24 hours with a solution of 1.6 g. of bromine in 50 cc. of chloroform at 5°. Removal of the solvent left a residue, which by analysis (Calcd. for $C_{27}H_{41}BrO_3$: C, 65.86; H, 8.39. Found: C, 66.27; H, 8.18) represented chiefly monobrominated material but which appeared to be approximately a 60–40 mixture of the 4-bromoketone XXIV ($\lambda_{max.}^{CHCl_3}$ 5.80 μ) and 23-bromoketone ($\lambda_{max.}^{CHCl_3}$ 5.87 μ). The presence of the 4-bromo-3-ketone XXIV was established by dehydrobromination with dinitrophenylhydrazine in glacial acetic acid[35] which yielded the **2,4-dinitrophenylhydrazone XXV**, m.p. 272–274° (after recrystallization from ethanol–chloroform), identical with a sample prepared from Δ^4-diosgenone (IX).

Anal. Calcd. for $C_{33}H_{44}N_4O_6$: C, 66.87; H, 7.48. Found: C, 66.36; H, 7.29.

The bromination product (4.8 g.) was suspended in 300 cc. of ethanol and stirred for 24 hours with 2.5 g. of sodium borohydride. Dilution with water and collection of the precipitate gave 4.85 g. of product which did not exhibit any carbonyl absorption in the infrared (presumably mixture of epimeric bromohydrins and some side-chain brominated epismilagenin). The crude material (4.3 g.) was refluxed for 2.5 hours with 20 g. of potassium hydroxide in 500 cc. of ethanol and the product was chromatographed on 150 g. of neutral alumina. Elution with hexane–benzene (9:1) and recrystallization from acetone gave 0.96 g. of the desired **epoxide XXVII**, m.p. 177–179°, $[\alpha]^{25}D$ −103°; admixture with the epimeric epoxide XXII depressed the melting point by 15°.

(35) C. Djerassi, THIS JOURNAL, **71**, 1003 (1949).

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.03; H, 10.07.

The hexane–benzene (1:1) eluates yielded 0.4 g. of smilagenone (IV), arising from a 3β,4β-bromohydrin (epimer of XXVI), while subsequently eluted (benzene and ether mixtures) material (m.p. 205–225°, 0.65 g.) contained bromine, presumably in the side chain.

22a,25a-Spirostan-4α-ol (XXVIIIa).—A sample (150 mg.) of the epoxide XXVII was reduced with lithium aluminum hydride and the product recrystallized from acetone; yield 100 mg., m.p. 170–171°, depressed to 140° upon admixture with the starting material, $[\alpha]^{25}D$ −70°.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.66; H, 10.58.

The acetate XXVIIIb crystallized as needles from ethanol; m.p. 166–168°, $\lambda_{max.}^{CHCl}$ 5.78 μ and type B band at 8.0 μ.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.84; H, 10.28.

22a,25a-Spirostan-3β,4α-diol (XXIII).—Acetolysis of either isomeric epoxide (XXII or XXVII) and direct saponification of the reaction product in the manner described above (XIV → XVIa) produced in *ca.* 50% yield the identical diol XXIII which was recrystallized from acetone; m.p. 181–184°, $[\alpha]^{25}D$ −74°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 75.08; H, 10.23.

Conversion of Mexogenin to Samogenin.—Mexogenin (100 mg., m.p. 237–238°, $[\alpha]^{25}D$ −6°, $\lambda_{max.}^{CHCl_3}$ 5.88 μ) was reduced by the Huang-Minlon procedure exactly as described above (IV → III) to yield 85 mg. of samogenin (Ia) (m.p. 206–208°), identified with authentic material by mixture melting point determination and infrared comparison.

Oxidation of Isorhodeasapogenin.—Chromium trioxide oxidation of a sample of the sapogenin (m.p. 245–248°) kindly furnished by Dr. H. Nawa[31] led to colorless needles (from acetone) of the derived *seco*-acid, m.p. 246–248°; mixture melting point determinations showed depressions (indicated after each acid) with the following acids: gitogenic acid (225–235°), samogenic acid (235–245°), 3,4-*seco*-acid XVII (230–240°). The dimethyl ester after two recrystallizations from methanol exhibited m.p. 155–157°, depressed to 138–145° upon admixture with the dimethyl ester XXIb.

Anal. Calcd. for $C_{29}H_{46}O_6$: C, 70.98; H, 9.45. Found: C, 70.86; H, 9.49.

DETROIT, MICHIGAN

[CONTRIBUTION FROM THE INSTITUTE OF ORGANIC CHEMISTRY, TECHNICAL UNIVERSITY OF BUDAPEST]

Structural Proof of Sugar Phenylhydrazones

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The controversial question as to whether sugar phenylhydrazones are cyclic or acyclic can be settled by the formazan reaction in which only open-chain (*aldehydo*-) structures participate. The structures of several sugar phenylhydrazones, including the three D-glucose phenylhydrazones, have been established by this reaction. In addition, the proportion of open-chain material in solution of a mixture of sugar phenylhydrazones can be determined, making it possible to follow and interpret structurally the optical changes in a mutarotating phenylhydrazone solution. The formazan reaction is also applicable to acetylated *aldehydo*-phenylhydrazones.

When Emil Fischer,[1] almost 70 years ago, prepared sugar phenylhydrazones for the first time, he represented them as open-chain compounds. The conception of ring structure[2–5] arose later to account for mutarotational effects in solution. The question is still moot, although many workers have attempted to clarify the point. Frèrejacque[6] found that definitive evidence could not be obtained from a study of hydrolysis rates and rotational values of the hydrolyzates. Later, Stempel[7] on the basis of similar experiments came to the same conclusion. In earlier work, attempts[2] have been made to elucidate the structure of the sugar phenylhydrazones on the basis of their mutarotations, but the later work of Butler and Cretcher[8] and of Stempel[7] revealed that while mutarotational studies permitted many interesting observations,

(1) E. Fischer, *Ber.*, **20**, 821 (1887).
(2) H. Jacobi, *Ann.*, **272**, 170 (1893).
(3) R. Behrend, *ibid.*, **353**, 106 (1907).
(4) R. Behrend and F. Lohr, *ibid.*, **362**, 78 (1908).
(5) A. Hofmann, *ibid.*, **366**, 277 (1909).
(6) M. Frérejacque, *Compt. rend.*, **180**, 1210 (1925).
(7) G. H. Stempel, THIS JOURNAL, **56**, 1351 (1934).
(8) C. L. Butler and L. H. Cretcher, *ibid.*, **53**, 4356 (1931).