[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S.A.]

Steroidal Sapogenins. XII.[1] The Configuration of the Hydroxyl Groups in Kammogenin, Yuccagenin, Lilagenin and Gitogenin

BY J. PATAKI, G. ROSENKRANZ AND CARL DJERASSI

Δ^2-22-Isoallospirosten (V) underwent hydroxylation with osmium tetroxide to yield 22-isoallospirostan-2α,3α-diol (VIa), which readily formed an acetonide (VIc). Since 22-isoallospirostan-2,3-diol (III) (gitogenin) is not identical with VIa and does not form an acetonide, it is assumed to be a *trans*-glycol. Perbenzoic acid oxidation of V led to 2α,3α-oxido-22-isoallospirostan (VIII), as proved by lithium aluminum hydride reduction to 22-isoallospirostan-3α-ol (IXa), and thence by acetolysis and saponification to 22-isoallospirostan-2β,3α-diol (Xa). Since this *trans*-glycol proved to be different from gitogenin (III), the latter as well as kammogenin (I), yuccagenin (IIa) and lilagenin (IIb) are believed to have the 2α,3β-dihydroxy configuration. 22-Isoallospirostan-2β,3α-diol 2-acetate 3-mesylate (Xd) on alkaline treatment afforded the 2β,3β-oxido-22-isoallospirostan (XI), which formed 22-isoallospirostan-2β-ol (XIIa) upon reduction with lithium aluminum hydride.

The hydroxyl group in steroidal sapogenins with only one alcoholic function in ring A exhibits the β-configuration.[2] On the other hand, the orientation of the hydroxyl groups in the ring A-disubstituted sapogenins is unknown. Since Δ^5-22-isospirostene-2,3-diol-12-one[3] (I) (kammogenin), Δ^5-22-isospirostene-2,3-diol (IIa) (yuccagenin) and its 22-normal analog, lilagenin (IIb), have all been correlated[2] with 22-isoallospirostan-2,3-diol (III) (gitogenin), a solution of the stereochemical problem of this latter sapogenin will automatically apply to the other three. The results to be described below strongly suggest that all four sapogenins possess the 2α,3β-dihydroxy configuration.

22-Isoallospirostan-3β-ol tosylate (IV) (tigogenin tosylate) on treatment with collidine led to Δ^2-22-isoallospirosten (V), which was hydroxylated with osmium tetroxide to yield 22-isoallospirostan-2α,3α-diol (VIa), further characterized by the formation of a diacetate (VIb) and acetonide (VIc). The position of the hydroxyl groups was proved by oxidation to 22-isoallo-2//3-spirostan-2,3-dioic acid (VII) (gitogenoic acid)[4] while the α-configuration follows from the fact that perbenzoic acid oxidation of V yields exclusively the α-oxide VIII, and both reagents can be expected to attack the double bond from the less hindered α-side. The formation of the α-oxide was to be anticipated on the basis of similar results in the cholesterol series[5] and the configuration was established rigorously by lithium aluminum hydride reduction to 22-isoallospirostan-3α-ol (IXa) (epitigogenin).[6]

22-Isoallospirostan-2,3-diol (III) (gitogenin) was not identical with the 2α,3α-dihydroxy isomer VIa nor did it form an acetonide derivative. Although we were unable to prepare the other *cis*-isomer (2β,3β-diol) and thus prove the point conclusively,[7] it seems fairly certain that 22-isoallospirostan-2,3-diol (III) is a *trans*-glycol.

Acetolysis of the α-oxide VIII led to a *trans*-diol monoacetate, which most likely possesses the 22-isoallospirostan-2β,3α-diol 2-monoacetate (Xc) structure.[8] Saponification produced the free 2β,3α-diol Xa and acetylation the diacetate Xb. Both substances proved to be different from the corresponding 22-isoallospirostan-2,3-diol (III) (gitogenin) derivatives and it can thus be assumed with reasonable certainty that "kammogenin (I)," "yuccagenin (IIa)," "lilagenin (IIb)" and "gitogenin (III)" are spirostan-2α,3β-diols.

In complete concordance with earlier results in the cholesterol series,[5,9] inversion of the α-oxide to the β-isomer could be accomplished by alkaline treatment of 22-isoallospirostan-2β,3α-diol 2-acetate 3-mesylate (Xd). As was to be expected, the resulting β-oxide XI upon reduction with lithium aluminum hydride afforded a new alcohol, not identical with the C-3 epimeric 22-isoallospirostan-3-ols, which is best formulated as 22-isoallospirostan-2β-ol (XIIa).

Experimental[10]

22-Isoallospirostan-2α,3β-diol (III) (Gitogenin).—The free sapogenin exhibited m.p. 272–273°, $[\alpha]^{20}D$ −63°, while the diacetate showed m.p. 242–244°, $[\alpha]^{20}D$ −90°. The specimens obtained in our Laboratory from *Agave schottii* proved to be identical with samples isolated from *Chlorogalum pomeridianum*.[11] When a few crystals of the sapogenin are covered with concentrated sulfuric acid, a deep purple color develops. This color test appears to be specific for the 2α,3β-dihydroxy moiety, since I, IIa and III produced the same color, whereas the *cis*-glycol VIa or the epimeric *trans*-glycol Xa showed only a light yellow color under those conditions.

22-Isoallospirostan-3β-ol Tosylate (IV).—An ice-cold solution of 5.0 g. of 22-isoallospirostan-3β-ol (tigogenin) (m.p. 204–206°, $[\alpha]$ −72°) isolated from *Agave pariflora* in 80 cc. of pyridine was treated with stirring in portions with 5.0 g. of *p*-toluenesulfonyl chloride and then permitted to stand at room temperature for 18 hours. The product was precipitated by dilution with water, the crystals collected, washed well with dilute hydrochloric acid and dried; yield 5.8 g. (85%), m.p. 162–166°. Several recrystallizations

(1) Paper XI. C. Djerassi, H. Martínez and G. Rosenkranz, *J. Org. Chem.*, **16**, 1278 (1951).

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

(3) For nomenclature of steroidal sapogenins, see G. Rosenkranz and C. Djerassi, *Nature*, **166**, 104 (1950).

(4) *Cf.* R. Tschesche, *Ber.*, **68**, 1090 (1935). We are grateful to Prof. Tschesche, University of Hamburg, for an authentic sample.

(5) A. Fürst and Pl. A. Plattner, *Helv. Chim. Acta*, **32**, 275 (1949).

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

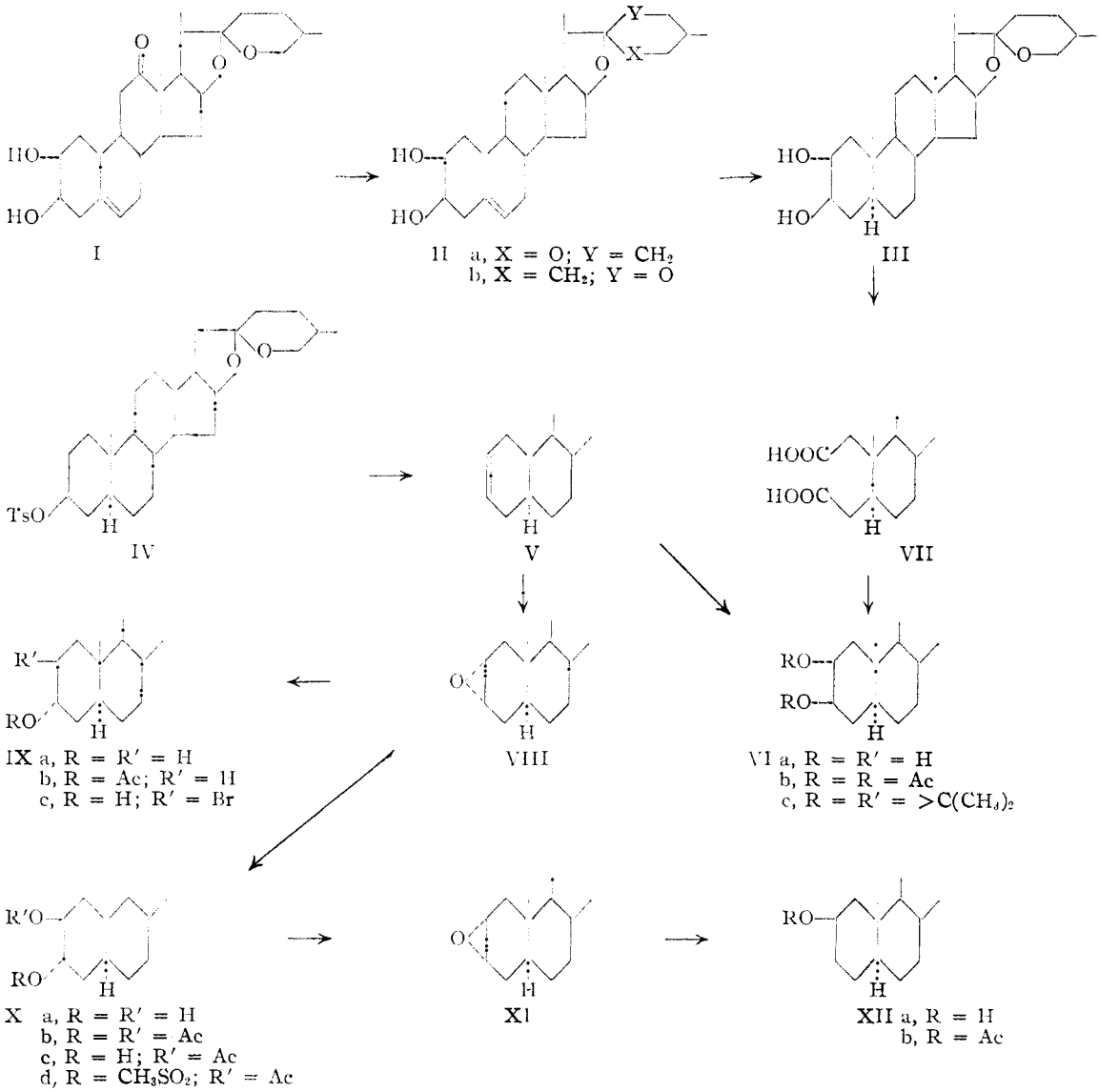
(7) On the basis of models, acetonide formation seems quite feasible with a 2β,3β-glycol.

(8) A similar structure has been advanced for the analogous reaction in the cholesterol series (ref. 5). The product in both instances must be a *trans*-glycol and it seems very probable that attack occurs at the less hindered 2β-position and thus leads to X rather than the isomeric 2α,3β-diol.

(9) A. Fürst and F. Koller, *Helv. Chim. Acta*, **30**, 1454 (1947).

(10) Melting points are uncorrected. Unless noted otherwise, rotations were determined in chloroform and infrared spectra in carbon disulfide solution. We are indebted to the Srtas. Amparo Barba and Rachel Cervera for the microanalyses and to Srta. Paquita Revaque for the rotations and spectral determinations. Thanks are also due to the Srtas. Lucia Llamosas Gutiérrez and Maria Luisa Franco for technical assistance.

(11) We are grateful to Prof. C. R. Noller, Stanford University, for samples isolated in his laboratory (C. R. Noller, L. H. Goodson and M. Synerholm, THIS JOURNAL, **61**, 1707 (1939)).



from acetone afforded the analytical sample with m.p. 175–176°, $[\alpha]^{20}D$ −55° (acetone).

Anal. Calcd. for $C_{34}H_{50}O_5S$: C, 71.54; H, 8.83; S, 5.62. Found: C, 71.64; H, 8.99; S, 5.35.

Δ^2-22-Isoallospirosten (V).—A solution of 5 g. of the tosylate IV was refluxed with 50 cc. of γ-collidine for 6 hours and then poured into ice-cold dilute sulfuric acid. Extraction with ether, washing with dilute acid, carbonate and water, evaporating and chromatographing on 100 g. of alumina afforded 2.1 g. (60%) of colorless crystals which after recrystallization from acetone showed m.p. 182–184°,[12] $[\alpha]^{20}D$ −22°. The product gave a yellow color with tetranitromethane.

Anal. Calcd. for $C_{27}H_{42}O_2$: C, 81.35; H, 10.62. Found: C, 81.28; H, 10.36.

Hydrogenation in ether solution with platinum oxide catalyst afforded in 85% yield 22-isoallospirostan with m.p. 173–175°, $[\alpha]^{20}D$ −69°, which gave no depression in melting point upon admixture with an authentic specimen.[13]

22-Isoallospirostan-2α,3α-diol (VIa).—A mixture of 2.2 g. of Δ^2-22-isoallospirosten (V), 1.4 g. of osmium tetroxide, 30 cc. of pyridine and 50 cc. of benzene was allowed to stand at room temperature for 48 hours. The black solution was evaporated to dryness and the residue was refluxed for 4 hours with 10 g. of potassium hydroxide, 10 g. of mannitol, 100 cc. of ethanol, 50 cc. of benzene and 25 cc. of water. Extraction with benzene, washing with water, drying, evaporating and recrystallizing twice from methanol yielded 1.2 g. (51%) of colorless crystals with m.p. 263–266°, $[\alpha]^{20}D$ −54°, which gave a light yellow color with concd. sulfuric acid.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 75.02; H, 9.92.

Oxidation of a small sample in chloroform–acetic acid with chromium trioxide and recrystallization of the acid fraction from dilute acetic acid afforded crystals with m.p. 241–244°, which gave no depression on admixture with an authentic sample[4] (m.p. 242–244°) of 22-isoallo-2//3-spirostan-2,3-dioic acid (VII) (gitogenoic acid). The infrared spectra (chloroform) of the two specimens proved to be identical.

The diacetate VIb crystallized from ether–hexane as small blades with m.p. 265–267°, $[\alpha]^{20}D$ −42.5°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 72.47; H, 9.46.

The acetonide VIc was prepared by placing 0.3 g. of the diol VIa into a thimble of a soxhlet extractor and extracting the material with 50 cc. of acetone containing 30–50 mg. of *p*-toluenesulfonic acid for six hours. After addition of carbonate solution and concentrating to 30 cc., the solution was extracted with ether, washed until neutral, dried, evapo-

(12) R. E. Marker and D. L. Turner, THIS JOURNAL, **63**, 767 (1941), obtained presumably the same compound by another procedure, but reported m.p. 163–166°.

(13) J. Romo, M. Romero, C. Djerassi and G. Rosenkranz, *ibid.*, **73**, 1531 (1951).

rated and recrystallized from methanol; yield 0.16 g., m.p. 197–198.5°, $[\alpha]^{20}D$ −16°, no hydroxyl band in the infrared. The zinc chloride procedure gave somewhat poorer results.

Anal. Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 76.05; H, 10.56.

Under the above conditions or employing zinc chloride, 22-isoallospirostan-2α,3β-diol (III) (gitogenin) was recovered in 80–92% yield.

2α,3α-Oxido-22-isoallospirostan (VIII).—A solution of 10 g. of Δ^2-22-isoallospirosten (V) in 50 cc. of chloroform was treated for 24 hours at room temperature with 200 cc. of a chloroform solution of perbenzoic acid (0.26 g./cc.). After washing with 5% sodium iodide solution, sodium thiosulfate solution and sodium bicarbonate, the chloroform was evaporated and the residue recrystallized from acetone; yield 7.4 g. (71%), m.p. 171–173°, $[\alpha]^{20}D$ −49°.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.51; H, 10.47.

22-Isoallospirostan-3α-ol (IXa) (Epitigogenin).—To a mixture of 1.5 g. of lithium aluminum hydride in 75 cc. of ether was added a solution of 3.0 g. of the above oxide VIII in 100 cc. of the same solvent. After refluxing for two hours and decomposing the excess reagent with ethyl acetate, the layers were separated, the ether solution was washed with dilute acid and water, dried and evaporated. Two recrystallizations of the residue from acetone afforded 2.1 g. (70%) of colorless crystals with m.p. 242–245°, $[\alpha]^{20}D$ −58°; lit.[6] m.p. 242–245°. Chromium trioxide oxidation produced 22-isoallospirostan-3-one, m.p. 208–210°, identical with an authentic sample.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.84; H, 10.59.

The acetate IXb possessed m.p. 201–203°, $[\alpha]^{20}D$ −41°; reported[6] 199–202°.

2β-Bromo-22-isoallospirostan-3α-ol (IXc).—The oxide ring was opened by refluxing 2.0 g. of the α-oxide VIII in 200 cc. of ethanol with 2.4 g. of pyridine hydrobromide[14] for one hour. Dilution with water, filtration of the colorless solid and recrystallization from ether–hexane gave 1.45 g. of the bromohydrin IXc with m.p. 203–205°, $[\alpha]^{20}D$ −35°.

Anal. Calcd. for $C_{27}H_{43}O_3Br$: C, 65.44; H, 8.75. Found: C, 64.97; H, 8.83.

22-Isoallospirostan-2β,3α-diol 2-Monoacetate (Xc).—The acetolysis was carried out exactly as described for 2α,3α-oxidocholestane[5] by refluxing 4.0 g. of the oxide VIII with 400 cc. of glacial acetic acid for 3 hours. The solution was concentrated under reduced pressure to a volume of *ca.* 100 cc., diluted with water and the product was extracted with ether. Recrystallization of the ether residue from acetone yielded 2.4 g. (52%) of the monoacetate with m.p. 219–222°, $[\alpha]^{20}D$ −41°.

Anal. Calcd. for $C_{29}H_{46}O_5$: C, 73.38; H, 9.77. Found: C, 73.19; H, 9.70.

Saponification with methanolic potassium hydroxide followed by recrystallization from acetone afforded in 87% yield **22-isoallospirostan-2β,3α-diol (Xa)** with m.p. 240–242°, $[\alpha]^{20}D$ −52°. The substance gave a yellowish color with concentrated sulfuric acid.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 75.23; H, 10.48.

The diacetate Xb was recrystallized from ether–acetone, whereupon it showed m.p. 246–248°, $[\alpha]^{20}D$ −26°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 72.36; H, 9.52.

22-Isoallospirostan-2β,3α-diol 2-acetate 3-mesylate (Xd) was prepared in 82% yield from the monoacetate Xc and methanesulfonyl chloride in pyridine solution as described above for the tosylate IV. Recrystallization from acetone led to the analytical sample with m.p. 184–186° (dec., Kofler block), $[\alpha]^{20}D$ −18°.

Anal. Calcd. for $C_{30}H_{48}O_7S$: C, 65.15; H, 8.75. Found: C, 65.06; H, 8.58.

2β,3β-Oxido-22-isoallospirostan (XI).—A solution of 0.35 g. of the mesylate Xd and 0.21 g. of potassium hydroxide in 80 cc. of methanol was refluxed for 2 hours, concentrated under diminished pressure, diluted with water and extracted with ether. After washing and drying, the ether was removed and the residue was recrystallized from acetone; yield 0.21 g. (80%), m.p. 198–201°, $[\alpha]^{20}D$ −33°.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.68; H, 10.10.

22-Isoallospirostan-2β-ol (XIIa).—The lithium aluminum hydride reduction of 0.3 g. of the β-oxide XI was carried out exactly as described for the α-epimer and after recrystallization from acetone afforded 0.23 g. of alcohol with m.p. 197–199°, $[\alpha]^{20}D$ −50°. The substance exhibited a free hydroxy band in the infrared.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.70; H, 10.82.

The acetate XIIb had m.p. 187–189°.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 76.12; H, 10.34.

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(14) *Cf.* reaction of cholesteryl oxide with pyridine hydrochloride (P. N. Chakravorty and R. H. Levin, THIS JOURNAL, **64**, 2317 (1942)).

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF DUKE UNIVERSITY]

Rhodanine Derivatives in Reactions of the Michael Type[1]

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By a suitable choice of conditions, rhodanine can be made to react with either acetaldehyde or 5-ethylidenerhodanine to yield 1,1-bis-(2-thio-4-ketotetrahydro-5-thiazolyl)-ethane. The structure of this product was demonstrated by hydrolysis and hydrogenolysis to yield β-methylglutaric acid. Products analogous to that obtained with acetaldehyde were obtained by the reaction of rhodanine (two moles) with six other aliphatic aldehydes.

While much study has been devoted to the condensation of rhodanine with aromatic aldehydes,[2] condensation with saturated aliphatic aldehydes appear to have received less attention. The reaction of rhodanine with acetaldehyde,[2a,3,4] isobutyraldehyde,[4b] or 3-methylbutyraldehyde[4b] has been shown to yield 5-alkylidenerhodanines (II).

As a continuation of an earlier[5] investigation of the reaction of rhodanine with carbonyl compounds, we restudied the sodium acetate-catalyzed reaction with paraldehyde.[3,4] It was found that in addition to the expected 5-ethylidenerhodanine (II, R = CH_3), an unidentified high-melting product (m.p. 246.5–248.5°) was often produced. Better yields of the high-melting compound were obtained when pure acetaldehyde was allowed to react with *two*

(1) This research was sponsored in part by the Biological Division, Chemical Corps, Camp Detrick, Frederick, Maryland under Contract no. DA-18-064-CML-120 with Duke University.

(2) *E.g.* (a) M. Nencki, *Ber.*, **17**, 2277 (1884); (b) N. Campbell and J. McKail, *J. Chem. Soc.*, 1251 (1948); and (c) P. Julian and B. Sturgis, THIS JOURNAL, **57**, 1126 (1935).

(3) C. Granacher, M. Gero, A. Ofner, A. Klopfenstein and E. Schlatter, *Helv. Chim. Acta*, **6**, 458 (1923).

(4) (a) R. Andreasch, *Monatsh.*, **39**, 419 (1918); (b) R. Andreasch, *ibid.*, **49**, 122 (1928).

(5) F. Brown, C. K. Bradsher, S. G. McCallum and M. Potter, *J. Org. Chem.*, **15**, 174 (1950).