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

STEROIDAL SAPOGENINS. VII.[1] EXPERIMENTS IN THE HECOGENIN SERIES (PART 1)

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Received December 6, 1950

In spite of the fact that the potential usefulness of C-12 oxygenated steroidal sapogenins as starting materials for the partial synthesis of cortisone and related cortical hormones has received considerable publicity (1), no experiments have as yet been reported[2] in which such substances were employed for synthetic purposes. In our hands, 22-isoallospirostan-3β-ol-12-one (IIIa)[3] (hecogenin) has proved to be the most abundant, naturally occuring 12-keto sapogenin[4] and the present paper deals with certain exploratory experiments in that series, notably the preparation and transformation of certain brominated 22-isoallospirostan-3β-ol-12-one derivatives.

According to Marker and co-workers, spirostan-3β-ol (sarsasapogenin) (I) (2), 22-isoallospirostan-3β-ol (IIa) (tigogenin) (3), and 22-isoallospirostan-3β-ol-12-one (hecogenin) (III) (4) can be *mono*brominated in the side chain to afford the corresponding 23-bromo derivatives; as emphasized in the case of spirostan-3β-ol *further bromination appears to be impossible.* Furthermore, these investigators showed that the 23-bromine atom is resistant towards base, but can be reductively removed (zinc) to regenerate the parent substance. If it should prove to be a fact that 22-isoallospirostan-3β-ol-12-one could only be *mono*brominated, then it would considerably reduce the usefulness of C-12 oxygenated steroidal sapogenins for cortical hormone syntheses, since one of the most useful methods for the shifting of an oxygen function from C-12 to C-11—Gallagher's procedure (5) employing an 11-bromo-12-ketone—would be impossible. A reinvestigation of the behavior of steroidal sapogenins towards bromine, therefore, was clearly indicated.

The first compound to be reinvestigated[5] was spirostan-3β-ol (sarsasapogenin) (I). It was immediately found that if its acetate was treated with two moles of bromine in acetic acid, 23,23-*di*bromospirostan-3β-ol 3-acetate could be isolated in nearly quantitative yield. Since this dibromo compound is rather insoluble and can be reconverted to spirostan-3β-ol (I) on reduction with zinc, it has proved to be quite useful in this laboratory for the separation of pure spirostan-

[1] Paper VI, Rosenkranz, Romo, Batres and Djerassi, *J. Org. Chem.*, preceding paper.

[2] The conversion of Δ^5-22-isospirosten-3β-ol-12-one (botogenin) to Δ^5-pregnen-3β,17α-diol-12,20-dione has been reported by Marker (*J. Am. Chem. Soc.*, **71**, 4149 (1949). This claim has since been shown to be incorrect [Fukushima and Gallagher, *J. Am. Chem. Soc.*, **72**, 2306 (1950)].

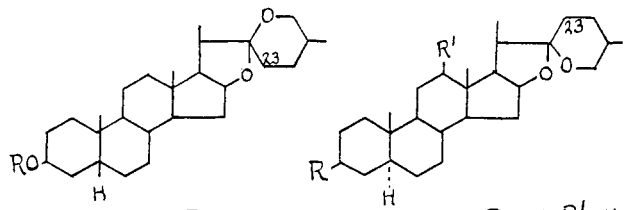
[3] For nomenclature of steroidal sapogenins see Rosenkranz and Djerassi, *Nature*, **166**, 104 (1950).

[4] The position of the keto group at C-12 was established by Wagner, Moore and Forker, *J. Am. Chem. Soc.*, **72**, 1856 (1950).

[5] We are indebted to J. Berlin and E. Batres for assistance in these experiments.

3β-ol (I) from plant extracts containing also 22-isospirostan-3β-ol (smilagenin) and 22-isoallospirostan-3β-ol (tigogenin) (IIa). Since this first observation already was in complete contrast to Marker's report (2), 22-isoallospirostan-3β-ol (IIa), 22-isoallospirostan (desoxytigogenin) (IIb) (6) and 22-isoallospirostane-3β,12-diol 3,12-diacetate (IIc) (rockogenin) were similarly brominated, but in this instance only a 23-monobromo derivative was formed. From these observations it appears that *the steroidal sapogenin side chain can be dibrominated in the normal (neo) configuration, but only monobrominated when it possesses the 22-iso configuration.*

With this information at hand, we turned to an investigation of the action of bromine upon 22-isoallospirostan-3β-ol-12-one (III), which according to Marker, *et al.* (4) affords a 23-monobromo derivative. Treatment of the acetate IIIb with 2.0, 2.5, and 3.0 moles of bromine in acetic acid at 35° led to solid derivatives on dilution with water, which essentially appeared to be 11,23-dibromo-22-isoallospirostan-3β-ol-12-one (IV)[6] on the basis of the following transformations. By analogy to Gallagher's work (5) in the desoxycholic acid series, the



Ia R = H
Ib R = Ac

IIa R = OH, R' = H
IIb R = R' = H
IIc R = R' = OAc

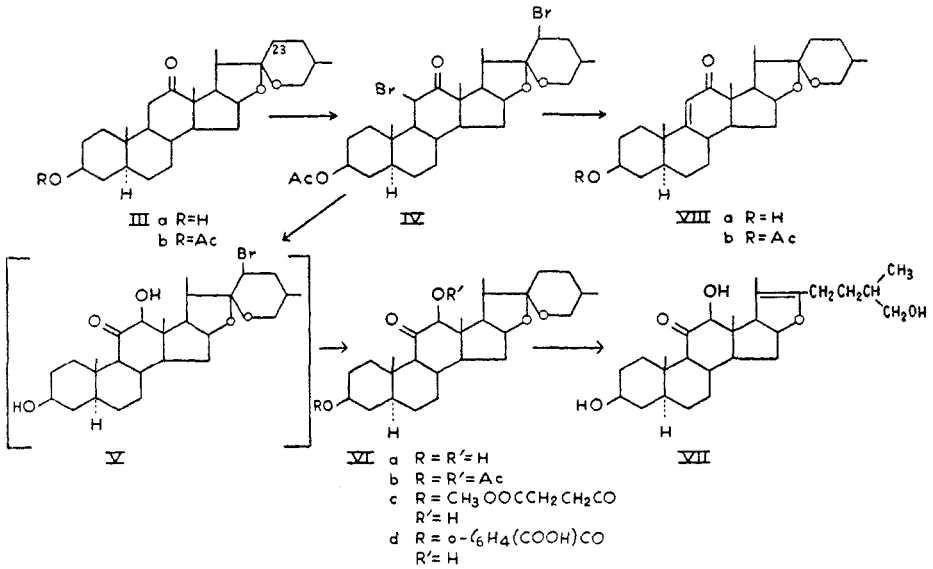
dibromo derivative IV was refluxed with strong alkali in order to hydrolyze both possible 11-bromo isomers to the corresponding ketol and simultaneously convert the latter to the stable 11-keto-12-hydroxy configuration. This intermediate, most likely formulated as V, was directly debrominated with zinc in acetic acid or ethanol to afford in 60% over-all yield (based on IIIb) the desired 22-isoallospirostan-3β,12-diol-11-one (VIa), *the first steroidal sapogenin with an oxygen function at C-11.* The correctness of the structure assignment follows from analogy to the proved case in the bile acid series (5), the infrared spectrum[7] which showed a carbonyl band at 1706 cm^{-1} identical with that exhibited by the "Marker-Lawson" acid methyl ester (5), and the formation of a *di*acetate (VIb) as well as a 3-monohemisuccinate methyl ester (VIc) and 3-monohemiphthalate

[6] The bromine content of the crude mixture in various experiments ranged from 23–27%; the correct analysis (calc'd, 25.35) was obtained several times (e.g. 24.99; 25.31). The product, which crystallized poorly, most likely represents a mixture of 11-bromo stereoisomers as has been observed in the desoxycholic acid series [Longwell and Wintersteiner, *J. Am. Chem. Soc.*, **62**, 200 (1940); Seebeck and Reichstein, *Helv. Chim. Acta*, **62**, 536 (1943); Gallagher and Long, *J. Biol. Chem.*, **162**, 521 (1946)].

[7] We are grateful to Dr. K. Dobriner and Mrs. P. Humphries, Sloan-Kettering Institute for Cancer Research, for measuring the infrared spectrum.

(VId). This key substance VI, further transformations of which will be reported in a later paper of this series, on heating with acetic anhydride at 200° was readily converted to the crystalline $\Delta^{20(22)}$-allofurostene-3β,12,26-triol-11-one (VII), important because it can be degraded to the corresponding 20-ketopregnane derivative. Further confirmation for the presence of a C-11 bromine atom in 11,23-dibromo-22-isoallospirostan-3β-ol-12-one (IV) was afforded by collidine treatment, followed by removal of the bromine atom in the side chain with zinc, which led to an α,β-unsaturated ketone, $\Delta^{9(11)}$-22-isoallospirosten-3β-ol-12-one (VIII) [9(11)-dehydrohecogenin] with a characteristic ultraviolet absorption maximum at 238 mμ.

As will become apparent from work to be reported later, this α,β-unsaturated ketone VIII represents a very useful intermediate, since it opens a route to $\Delta^{9(11)}$-pregnenes which are interesting substrates for enzymatic and perfusion studies.



EXPERIMENTAL[8]

23,23-Dibromospirostan-3β-ol (dibromosarsasapogenin). A solution of 4.58 g. of spirostan-3β-ol 3-acetate (Ib) in 250 cc. of glacial acetic acid containing two drops of 4 *N* hydrogen bromide in acetic acid was treated at 25° with a solution of 2.0 moles of bromine in the same solvent. Decolorization proceeded rapidly and crystals started to appear soon after the last drops of bromine solution were added. After standing at room temperature for 2.5 hours, the precipitate (4.66 g., m.p. 235–237°) was washed with acetic acid and water. The analytical sample of *23,23-dibromospirostan-3β-ol 3-acetate* crystallized from ethanol-chloroform as colorless needles with m.p. 242–244°, $[\alpha]_D^{20}$ −73.1°.

Anal. Calc'd for $C_{29}H_{44}Br_2O_4$: C, 56.49; H, 7.19; Br, 25.93.
Found: C, 56.50; H, 6.87; Br, 25.72.

Debromination of the dibromo acetate by refluxing with zinc dust and ethanol (four

[8] Melting points are uncorrected unless noted otherwise. Rotations were carried out in chloroform and ultraviolet absorption spectra in 95% ethanol solution. We are grateful to Srta. Paquita Revaque for these measurements and to Srta. Amparo Barba of our Microanalytical Department for the analyses.

hours) or acetic acid (one hour) yielded spirostan-3β-ol 3-acetate (Ib), m.p. 143–145° in over 80% yield. "Sarsasapogenin" thus can be readily purified through its dibromo derivative. Chromous chloride in acetone solution did not affect the bromo compound.

23-Bromo-22-isoallospirostan (23-bromodesoxytigogenin). A solution of 1.0 g. of 22-isoallospirostan (IIb) (6) in 60 cc. of warm acetic acid was treated as above with either one or two moles of bromine. In the latter case, decolorization of the solution was not complete and in either instance nearly 90% of 23-bromo-22-isoallospirostan was isolated. The analytical sample had m.p. 206–210° (dec.), $[\alpha]_D^{20}$ −94.3°, after recrystallization from ethanol-chloroform.

Anal. Calc'd for $C_{27}H_{43}BrO_2$: C, 67.64; H, 9.04.

Found: C, 67.56; H, 9.16.

23-Bromo-22-isoallospirostane-3β,12-diol 3,12-diacetate (23-bromorockogenin diacetate). 22-Isoallospirostane-3β,12-diol 3,12-diacetate (IIc) (670 mg., m.p. 198–202°) was treated with two moles of bromine exactly as described for Ib. Decolorization was not complete and after pouring into water, the precipitated solid (770 mg.) was purified by chromatography and recrystallization from aqueous methanol; yield, 430 mg., m.p. 98–103° (Kofler block), $[\alpha]_D^{20}$ −52.5°.

Anal. Calc'd for $C_{31}H_{47}BrO_6$: Br, 13.42. Found: 12.99.

11,23-Dibromo-22-isoallospirostan-3β-ol-12-one 3-acetate (IV). A solution of 20 g. of 22-isoallospirostan-3β-ol-12-one 3-acetate (IIIb) (hecogenin acetate) (m.p. 244–246°, $[\alpha]_D^{20}$ −2°) in 600 cc. of acetic acid containing 2 drops of hydrogen bromide (in acetic acid) was warmed to 35° and treated dropwise with a solution of 2.5–3.0 moles of bromine in 200 cc. of acetic acid. Decolorization was *not* complete[9] and after 1.5 hours at room temperature, the solution was diluted with a large volume of water and filtered. The well-washed precipitate was dried at 90° under vacuum; yield, 25.1 g., m.p. *ca.* 140–150° (dec.). In a number of experiments, the rotation ranged from $[\alpha]_D^{20}$ −14° to −21°. The bromine content varied from 23–27% (Calc'd for $C_{29}H_{42}Br_2O_5$: Br, 25.35) and the product obviously represented a mixture.[6] Refluxing with zinc-acetic acid (for conditions, see below) regenerated 22-isoallospirostan-3β-ol-12-one 3-acetate (IIIb). In view of the relative lability and poor crystallizability, the material was used as such for the subsequent transformations. A detailed study of the composition of this bromination mixture is now under way.

22-Isoallospirostane-3β,12-diol-11-one (VI). The bromination product from 10.0 g. of 22-isoallospirostan-3β-ol-12-one 3-acetate (IIIb) was refluxed for two hours with 400 cc. of methanol and 77 g. of potassium hydroxide, poured into water, filtered, and washed well with water. The nearly colorless solid (V) (m.p. *ca.* 135–145° in various runs) was debrominated by refluxing for 2.5 hours, with stirring, with 420 cc. of acetic acid and 75 g. of zinc dust. Zinc in ethanol was also satisfactory, but required a longer time (see below in the preparation of VIII). The product (negative Beilstein test) obtained on filtering the zinc dust and diluting the acetic acid filtrate with water, was refluxed once more with 730 cc. of methanol and 7.3 g. of potassium hydroxide for one hour and then poured into water. Filtration and washing with water afforded consistently between 6.2–6.5 g. of 22-isoallospirostane-3β,12-diol-11-one (VIa) with m.p. 202–207°. Further recrystallization from methanol raised the m.p. to 215–217° (corr.) $[\alpha]_D^{20}$ −13°, u.v. maximum at 288 mμ (log ε 1.74), infrared band[7] at 1706 cm^{-1} (carbon disulfide).

Anal. Calc'd for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48.

Found: C, 72.90; H, 9.38.

The *diacetate* (VIb), prepared by boiling with acetic anhydride and pyridine, crystallized from methanol-chloroform as felt-like needles with m.p. 225–225.5°, $[\alpha]_D^{20}$ −67°. The strongly negative rotation is noteworthy.

Anal. Calc'd for $C_{31}H_{46}O_7$: C, 70.16; H, 8.74.

Found: C, 70.14; H, 8.91.

The *methyl ester of the 3-hemisuccinate* (VIc) was prepared as follows: 0.7 g. of the diolone

[9] Based on empirical observations, a better yield of subsequent transformation products (VI and VIII) was obtained when the bromination was carried out with an excess of bromine.

VIa and 1.5 g. of succinic anhydride was heated on the steam bath in 20 cc. of pyridine for *ca.* forty minutes and then left at room temperature overnight. The product (0.77 g., m.p. 100–104°) isolated on pouring the reaction mixture into ice cold, dilute hydrochloric acid, was methylated by treatment with ethereal diazomethane for five minutes. Several recrystallizations from hexane-ethyl acetate afforded the ester VIc as rosettes of nearly colorless prisms with m.p. 166–170°, $[\alpha]_D^{20}$ −24.6°. The melting point range could not be sharpened on further recrystallization.

Anal. Calc'd for $C_{32}H_{48}O_8$: C, 68.54; H, 8.63; methoxyl, 5.53.

Found:[10] C, 68.64; H, 8.89; methoxyl, 5.17.

Treatment with phthalic anhydride in the same fashion (without methylation) followed by recrystallization from hexane-acetone led to the *hemiphthalate* VId which exhibited m.p. 169–172° (Kofler), $[\alpha]_D^{20}$ −19°. The substance is quite soluble in aqueous sodium carbonate solution.

Anal. Calc'd for $C_{35}H_{46}O_8$: C, 70.68; H, 7.80.

Found: C, 70.26; H, 7.52.

$\Delta^{20\,(22)}$-*Allofurostene-3β,12,26-triol-11-one* (VII). A solution of 1.0 g. of 22-isoallospirostane-3β,12-diol-11-one (VIa) in 4 cc. of acetic anhydride was heated in a sealed tube for eight hours at 196° and then poured into water. The oily acetate was extracted with ether and saponified by refluxing for one hour with 2.0 g. of potassium hydroxide and 20 cc. of ethanol. Dilution with water and filtration yielded 0.89 g. of a brownish precipitate, which was nearly decolorized by trituration with ether and filtration. Two recrystallizations from acetone-hexane afforded the analytical sample of the triolone VII with m.p. 188–191°, $[\alpha]_D^{20}$ +71° (chloroform), $[\alpha]_D^{20}$ +68.3° (dioxane). The large dextrorotatory shift is typical for the conversion of a spirostan derivative to the corresponding furosten and has been observed in numerous instances in this laboratory. The degradation of this substance to the corresponding pregnene analog will be published at a later date.

Anal. Calc'd for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.76; H, 9.47.

$\Delta^{9(11)}$-*22-Isoallospirosten-3β-ol-12-one* [*9(11)-dehydrohecogenin*] (VIII). A solution of 2.64 g. of dibromo derivative (IV) was refluxed for one hour with 30 cc. of γ-collidine, which produced 0.81 g. of collidine hydrobromide (completely water-soluble), corresponding to 0.95 mole of hydrogen bromide. The usual work-up (ether extraction, washing with acid, carbonate and water) gave a tan colored, crystalline residue[11] with a u.v. maximum at 238 mμ (log ε 4.05), which was directly debrominated by refluxing for eight hours with 15 g. of zinc dust and 100 cc. of ethanol. Filtration of the zinc dust, dilution of the filtrate with water, extraction with ether, evaporation and crystallization from methanol produced 1.1 g. (56%) of colorless crystals with m.p. 212–215°, negative Beilstein test. Further recrystallization from chloroform-methanol afforded the analytical sample of $\Delta^{9(11)}$-*22-isoallospirosten-3β-ol-12-one 3-acetate* (VIIIb) with m.p. 218–220° (corr.), $[\alpha]_D^{20}$ −8.7°, u.v. maxima at 238 mμ (log ε 4.17) and 322 mμ (log ε 1.80).

Anal. Calc'd for $C_{29}H_{42}O_5$: C, 74.01; H, 9.00.

Found: C, 73.75; H, 8.85.

Saponification with methanolic potassium hydroxide followed by recrystallization from chloroform-methanol led to $\Delta^{9(11)}$-*22-isoallospirosten-3β-ol-12-one* (VIIIa) with m.p. 223–225° (Kofler), $[\alpha]_D^{20}$ −3°, u.v. maxima at 240 mμ (log ε 4.13) and 322 mμ (log ε 1.85).

Anal. Calc'd for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41.

Found: C, 75.53; H, 9.56.

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[10] This analysis was carried out by Mr. Joseph F. Alicino, Metuchen, N. J.

[11] In one experiment, this intermediate $\Delta^{9(11)}$-23-bromo-22-isoallospirosten-3β-ol-12-one 3-acetate was recrystallized from methanol-chloroform; m.p. 209–213° (dec.), U.V. maximum at 238 mμ (Log ε 4.12).

Anal. Calc'd. for $C_{29}H_{41}BrO_5$: Br, 14.54. Found: Br, 14.29.

SUMMARY

A reinvestigation of the bromination of steroidal sapogenins has resulted in a correction and expansion of some of Marker's earlier observations that only mono-bromo derivatives are formed.

22-Isoallospirostan-3β-ol-12-one (hecogenin) (III) yields an 11,23-dibromo derivative (IV), which on alkali treatment and removal of the bromine in the side chain leads to 22-isoallospirostan-3β,12-diol-11-one (VI), the first steroidal sapogenin with an oxygen function at C-11. Conversion to $\Delta^{20(22)}$-allofurostene-3β,12,26-triol-11-one (VII) is accomplished readily by heating VI with acetic anhydride. Collidine dehydrobromination of the 11,23-dibromo derivative IV leads to $\Delta^{9(11)}$-22-isoallospirosten-3β-ol-12-one (VIII) [9(11)-dehydrohecogenin].

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