crystallization from methanol–water, then from methanol using Darco, and finally from methanol, gave material of m.p. 216.5–218°.

Anal. Calcd. for $C_{25}H_{32}O_7$: C, 67.55; H, 7.26. Found: C, 66.98; H, 7.06.

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[CONTRIBUTION NO. 131 FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF TENNESSEE]

The Isomeric Mono- and Dibromohecogenin Acetates

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Bromination of hecogenin acetate gives rise to two isomeric monobromides in which the bromine atoms are located on the side chain. In the absence of direct evidence for the exact position and configuration of the halogen they are tentatively designated as 23a and 23b isomers. Further bromination of each isomer yields a mixture of dibromides which can be dehydrohalogenated to the isomeric 9,11-dehydro-23-bromo derivatives. In the 23a series this mixture was resolved, yielding the 11α,23a- and 11β,23a-dibromides.

Although dibromination of hecogenin acetate has been used in several laboratories[2-5] as a convenient route to the ring-C ketols, 11-ketosteroids and 9,11-dehydro derivatives, we have felt that a more detailed study of the bromides themselves was indicated by the complex nature of mixtures which are always obtained. We have previously commented on this situation and have reported the isolation of 11α- and 11β,23ξ-dibromohecogenin acetates from the mixture of dibromides.[5]

It now appears that there are two isomeric monobromohecogenin acetates; we have isolated these and derived the foregoing dibromides from one of them. The second pair of dibromides has defied separation but has yielded a second 9,11-dehydro-23ξ-bromohecogenin (IX) convertible to 9,11-dehydrohecogenin acetate, thus linking the isomerism to a bromine atom in the side chain.

Introduction of two bromine atoms at C-23 in sarsasapogenin acetate[2] suggested that either of these hydrogens might also be replaceable in the isosapogenins. Substantiation of this possibility appears in the same publication where a preparation of 9,11-dehydro-23-bromohecogenin acetate showed a lower melting point, 209–213°. We have found this melting range characteristic of a mixture of equal parts of 9,11-dehydro-23a-bromohecogenin acetate and the 23b isomer. Finally, the isolation earlier[5] of two bromoketols, each yielding 3β,12β-dihydroxy-5α,22a-spirostan-11-one on debromination, pointed toward this type of isomerism.

In the absence of any direct evidence for the position or configuration of the halogen in the side chain, the parent compounds of the two series have been arbitrarily designated as 23a- and 23b-bromides, II and III. The usual placement of bromine at C-23 is based on analogy with Marker's proof that this is the position occupied in bromosarsasapogenin acetate, a 22b-spirostane.[6]

We attempted a similar oxidation of 23a-bromohecogenin acetate (II) but had no success in isolating a twenty-two carbon diketo acid. We also tried making a C-20 bromide by adding bromine to pseudohecogenin in the cold, followed by gentle warming with acid. No product was isolated which corresponded to either of the bromohecogenins. The investigation of these structures is being continued.

Crystallization techniques appear particularly advantageous for the separation of these isomers, since mixtures of II and III as well as VIII and IX were not separable by chromatography. Due to the relative insolubility of the 23a series, VI and VII could be crystallized from the mixture obtained by dibromination of hecogenin acetate (I).[5] The melting point of VI and rotation of VII differ slightly from the dibromides previously isolated; these isomers were prepared by bromination of pure 23a-bromohecogenin acetate. Separations were in all cases followed by rotations as well as melting points, and the crystal forms, though similar, are distinctive. Differentiation of VI and VII was made through dehydrohalogenation, VI being stable in boiling pyridine; however, both compounds yield VIII when refluxed with collidine.[7]

The mixed dibromides in the 23b series were lower melting and resisted separation. Dehydrohalogenation with pyridine and chromatography were used in the hope of isolating 11α,23b-dibromohecogenin acetate and 9,11-dehydro-23b-bromohecogenin acetate. Only the latter, IX, was obtained in these experiments.

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Experimental

23a-Bromohecogenin Acetate (II) and 23b-Bromohecogenin Acetate (III).—Hecogenin acetate, 2.0 g., in 100 ml. of glacial acetic acid was stirred at 20° and treated successively with one drop of hydrogen bromide saturated acetic acid and one equivalent of purified bromine in 25 ml. of acetic acid during ten minutes. Decolorization was complete in this time and the mixture was stirred into 1.5 l. of water. The flocculent white precipitate was washed thoroughly and dried at 55°, yielding 2.26 g. (97%) of product, m.p. 220–223° dec., $[\alpha]^{26}_{D}$ −21.1° (dioxane). This mixture was not resolved by the various chromatographic procedures

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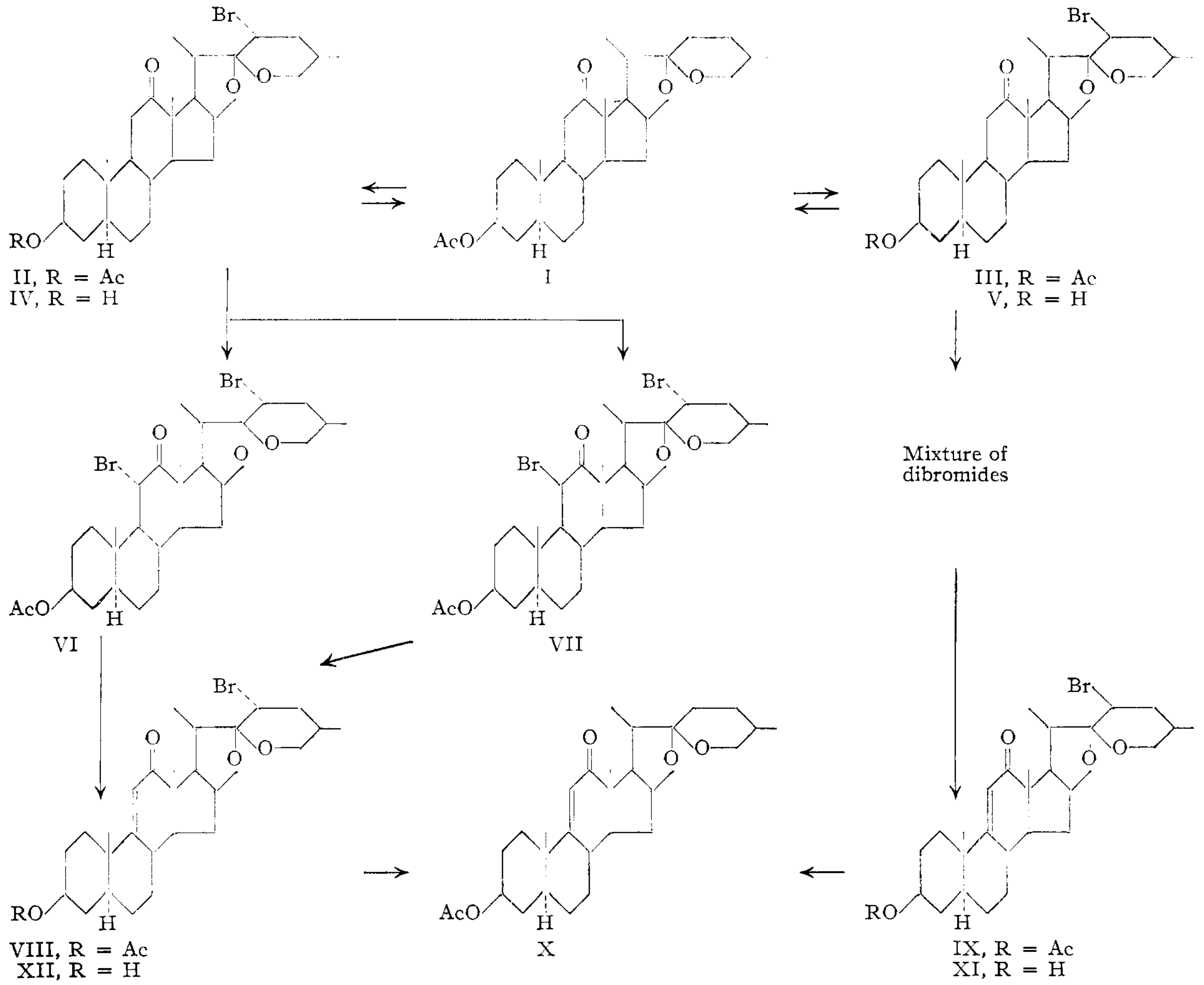

tried. A long series of slow crystallizations from ether or ether–petroleum ether solutions, accompanied by manual separation of crystal forms, afforded the most satisfactory initial separation after which the two isomers were further purified by recrystallization. In this manner 146 mg. of transparent plates of pure 23a-bromohecogenin acetate (II), m.p. 230–232.5° dec. (sinters and darkens at 227°),[8] $[\alpha]^{26}\text{D}$ −9.5° (dioxane), was obtained. *Anal.* Calcd. for $C_{29}H_{43}O_5Br$: C, 63.14; H, 7.86; Br, 14.48. Found: C, 62.99; H, 8.01; Br, 14.39. The other isomer, 23b-bromohecogenin acetate (III), was similarly obtained with a yield of 163 mg. as flat, transparent needles, m.p. 221–222° dec. (darkens at 210°), $[\alpha]^{26}\text{D}$ −36.6° (dioxane). *Anal.* Found: C, 63.08; H, 7.72; Br, 14.71. A mixture of the pure isomers softened at 212°, melting at 226–227.5° dec.

Each isomer was heated at 150° for four hours in a collidine–xylene mixture but showed no change in melting point, rotation or ultraviolet absorption on subsequent isolation and purification. The absorption spectrum revealed the complete absence of any α,β-unsaturated carbonyl grouping.

Debromination by heating 67 mg. of II at 95° with 1.0 g. of zinc dust and 25 ml. of acetic acid followed by precipitation in water and recrystallization from ether afforded 32 mg. of hecogenin acetate, m.p. 248–250°, $[\alpha]^{32}\text{D}$ −2.3° (dioxane). Similar treatment of 65 mg. of III yielded 30 mg. of hecogenin acetate, m.p. 247–250°, $[\alpha]^{32}\text{D}$ −1.9° (dioxane).

23a-Bromohecogenin (IV).—A solution obtained by heating 50 mg. of II with 15 ml. of alcohol was treated with 1.4 g. of potassium hydroxide in 10 ml. of alcohol at room temperature. After standing 28 hours, the mixture was poured into 400 ml. of water, extracted with ether and the ethereal solution washed, dried and concentrated to a small volume. This was treated with methanol and water, whereupon fine needles separated from the turbid solution. Recrystallization of these twice from methanol gave 30.5 mg. of square, transparent plates, m.p. 220–222° dec. (softening at 217°), $[\alpha]^{30}\text{D}$ −2.7° (dioxane). *Anal.* Calcd. for $C_{27}H_{41}O_4Br$: C, 63.65; H, 8.11. Found: C, 63.46; H, 8.19.[3] Acetylation of this product with boiling acetic anhydride yielded the original acetate.

23b-Bromohecogenin (V).—Hydrolysis of 50 mg. of III as described above yielded clusters of fine needles, m.p. 242.5° dec. (sintering at 235°), $[\alpha]^{30}\text{D}$ −30.0° (dioxane). *Anal.* Found: C, 63.87; H, 8.04. Reacetylation here gave a product crystallizing on slow cooling as long needles. These dissolved on shaking with concomitant crystallization in the form of the starting material first observed.

11α,23a-Dibromohecogenin Acetate (VI) and 11β,23a-Dibromohecogenin Acetate (VII).—One gram of 23a-bromohecogenin acetate (II) was dissolved by warming in 50 ml. of glacial acetic acid. This solution was warmed to 50°, treated with a trace of hydrogen bromide and then with 1.1 equivalents of purified bromine in 12.5 ml. of glacial acetic acid over a period of 25 minutes. Stirring was continued for three hours, and the product was isolated by precipitation in water. The yield of dry product was quantitative. After treating with decolorizing charcoal, fractional crystallization by a process of concentration and cooling of the ether solution gave 330 mg. of relatively pure less soluble material from which further crystallization gave 175 mg. of pure 11α,23a-dibromohecogenin acetate (VI) as long, flat, transparent needles, m.p. 180–183° dec., $[\alpha]^{24}\text{D}$ −39.3° (chloroform). *Anal.* Calcd. for $C_{29}H_{42}O_5Br$: C, 55.24; H, 6.72;

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[8] All melting points were observed at fifty magnifications with the Kofler apparatus and are corrected. The reported melting points were determined by slowly heating the samples from room temperature until decomposition occurred. Decomposition points vary as much as 20° in some cases depending on the temperature at which heating is begun.

Br, 25.34. Found: C, 55.59; H, 6.79; Br, 25.19. The more soluble reaction product crystallized slowly as small cubes; from 148 mg. of essentially pure product, 78 mg. of pure 11β,23a-dibromohecogenin acetate (VII), was obtained by ether recrystallization as small, flat, transparent needles, m.p. 175–177° dec., $[\alpha]^{24}_{\mathrm{D}}$ $-34.8°$ (chloroform). *Anal.* Found: C, 55.23; H, 6.63; Br, 25.23. Ultraviolet absorption spectrum showed the complete absence of an α,β-unsaturated carbonyl function.

Dehydrobromination of 11α,23a-Dibromohecogenin Acetate (VI).—A solution of 100 mg. of the dibromide, VI, in 25 ml. of dry pyridine was refluxed two hours, and the pyridine was distilled *in vacuo* over steam. The residue was dissolved in ether, concentrated to about 15 ml. and cooled to effect crystallization. The product was identical in all respects with the starting material and showed no maximum at 238 mμ.

Similarly, 50 mg. of the dibromide was refluxed for three hours in a mixture of 25 ml. of γ-collidine and 25 ml. of xylene. Collidine and xylene were distilled *in vacuo,* and the residue was dissolved in ether, washed repeatedly with dilute hydrochloric acid and then water. The dried ethereal solution was treated with decolorizing charcoal and concentrated to about 5 ml. Crystallization yielded 22 mg. (44%) of 9,11-dehydro-23a-bromohecogenin acetate (VIII) as long, flat, transparent needles, m.p. 220–223° dec., sweating beginning at 200°, $[\alpha]^{22}_{\mathrm{D}}$ $-21.3°$ (dioxane), $\lambda^{\text{alc}}_{\max}$ 238 mμ (log ϵ 4.02). The mixed melting point with an analytically pure sample of VIII, which was obtained by the procedure given below, was not depressed.

Dehydrobromination of 11β,23a-Dibromohecogenin Acetate (VII).—Treatment of 76 mg. of VII in 25 ml. of dry pyridine as described above yielded 28 mg. (43%) of compound VIII, m.p. 220–222° dec. (sinter at 216°), $[\alpha]^{22}_{\mathrm{D}}$ $-21.9°$ (dioxane), $\lambda^{\text{alc}}_{\max}$ 238 mμ (log ϵ 4.06), identical in crystalline form, melting point and mixed melting point with the previous sample.

Bromination of 23b-Bromohecogenin Acetate (III).—A solution of 895 mg. of III in 50 ml. of glacial acetic acid was treated with 1.2 equivalents of purified bromine under the conditions outlined above. The solution was colorless but changed to a green color with the addition of a slight excess of bromine. The products were more soluble than the 23a series and precipitated as oils from petroleum ether as well as various mixtures of ether–petroleum ether. Attempts to crystallize the product from benzene, ethyl acetate and hexane or combinations of these failed. An amorphous white solid, m.p. 120–125° followed by decomposition to a green colored melt at 130–135°, was obtained from 95% alcohol. The absence of an α,β-unsaturated carbonyl function was shown by the ultraviolet absorption spectrum. The mixture in general was colored as though by decomposition products, and defied separation by crystallization, fractional precipitation and chromatography.

9,11-Dehydro-23a-bromohecogenin Acetate (VIII) **and 9,11-Dehydro-23b-bromohecogenin Acetate** (IX).—A solution of 2.0 g. of the 11,23-dibromohecogenin acetate mixture obtained by direct bromination of hecogenin acetate was refluxed two hours in 40 ml. of γ-collidine and 40 ml. of xylene and processed as described earlier. An ethereal solution of the product was concentrated to incipient crystallization, and the first crop of crystals, m.p. 219–222° dec., $[\alpha]^{21}_{\mathrm{D}}$ $-29°$ (chloroform), was removed. The filtrate was concentrated and diluted with petroleum ether; the rosettes of very fine needles obtained at this point were recrystallized from ether, and the mother liquor therefrom was seeded with the needles and allowed to stand in the refrigerator. Filtration yielded 430 mg. of needles, which, after five recrystallizations from ether–petroleum ether, afforded 121 mg. of pure 9,11-dehydro-23b-bromohecogenin acetate (IX) as flat, transparent needles, m.p. 220–221° dec. (sinters and darkens at 215–218°), $[\alpha]^{22}_{\mathrm{D}}$ $-47.2°$ (dioxane), $\lambda^{\text{alc}}_{\max}$ 238 mμ (log ϵ 4.14). *Anal.* Calcd. for $C_{29}H_{41}O_5Br$: C, 63.37; H, 7.52; Br, 14.54. Found: C, 63.13; H, 7.45; Br, 14.70.

The first crop of crystals removed was recrystallized three times from ether, giving 109 mg. of 9,11-dehydro-23a-bromohecogenin acetate (VIII) as flat, transparent needles, m.p. 220–220.5° dec. (sinters and darkens at 215–218°), $[\alpha]^{23}_{\mathrm{D}}$ $-21.4°$ (dioxane), $\lambda^{\text{alc}}_{\max}$ 238 mμ (log ϵ 4.13).[9] *Anal.* Found: C, 63.24; H, 7.58; Br, 14.42.

A mixture of the 23a and 23b isomers melts at 212–214°, the melt decomposing at higher temperatures.[10]

A portion of the inseparable mixture, 330 mg., obtained by bromination of III was dissolved in 25 ml. of dry pyridine, refluxed three hours and processed as described earlier. An ethereal solution of the product upon seeding yielded 30 mg. of 9,11-dehydro-23b-bromohecogenin acetate, m.p. 219–220° dec., $[\alpha]^{21}_{\mathrm{D}}$ $-45°$ (dioxane) and 230 mg. of yellow oily residue which was not separated into crystalline material by chromatography.

Elution of the mixture of dibromides from an acid-washed alumina column caused dehydrobromination, giving IX as the only isolable crystalline compound, m.p. 220–222° dec., $\lambda^{\text{alc}}_{\max}$ 238 mμ (log ϵ 4.08), identical in all respects with the sample above.

Reduction of the 9,11-Dehydro-23-bromohecogenin Acetates to 9,11-Dehydrohecogenin Acetate (X).—Zinc dust, 1.2 g., was added in three portions at 30-minute intervals to a hot acetic acid solution of 100 mg. of 9,11-dehydro-23a-bromohecogenin acetate. After stirring three hours on the steam-bath, the solution was filtered into 300 ml. of water, and the product was isolated by extraction with ether in the usual way. The crystalline crude product weighing 87 mg. was decolorized with charcoal and recrystallized from 3 ml. of ether–petroleum ether. Long, flat needles, 35 mg., of 9,11-dehydrohecogenin acetate (XII) were obtained, m.p. 219–221°, $[\alpha]^{21}_{\mathrm{D}}$ $-5.5°$ (dioxane).[2,4,5,11]

Similar treatment of 88 mg. of 9,11-dehydro-23b-bromohecogenin acetate afforded 23 mg. of 9,11-dehydrohecogenin acetate, m.p. 218–221°, $[\alpha]^{22}_{\mathrm{D}}$ $-3.7°$, not depressing the melting point of the sample above.

9,11-Dehydro-23b-bromohecogenin (XI).—The acetate IX, 42 mg., was dissolved by warming with 15 ml. of alcohol, and treated with 1.4 g. of potassium hydroxide in 10 ml. of alcohol at room temperature. After 19 hours the product was precipitated in water, collected, washed, dried and recrystallized from 5 ml. of ether. Recrystallization from methanol gave 18 mg. of the pure product crystallizing as thin, transparent plates, m.p. 226–227.5° dec., $[\alpha]^{28}_{\mathrm{D}}$ $-52.7°$ (dioxane), $\lambda^{\text{alc}}_{\max}$ 239 mμ (log ϵ 4.09). *Anal.* Calcd. for $C_{27}H_{39}O_4Br$: C, 63.90; H, 7.75. Found: C, 63.81; H, 7.75.

9,11-Dehydro-23a-bromohecogenin (XII).—Hydrolysis of 50 mg. of VIII by the foregoing procedure yielded 13 mg. of 9,11-dehydro-23b-bromohecogenin as transparent plates, m.p. 211–212.5° dec., $[\alpha]^{32}_{\mathrm{D}}$ $-25.2°$ (dioxane), $\lambda^{\text{alc}}_{\max}$ 239 mμ (log ϵ 4.08). *Anal.* Found: C, 63.77; H, 7.68.

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[9] 9,11-Dehydro-23-bromohecogenin acetate was first reported to have the constants: m.p. 228–230° dec., $[\alpha]^{26}_{\mathrm{D}}$ $-24.6°$ (chloroform) (*cf.* ref. 3). On the basis of rotation this corresponds to the isomer isolated here.

[10] The physical constants reported, ref. 2, m.p. 209–213° dec., $\lambda^{\text{alc}}_{\max}$ 238 mμ (log ϵ 4.12) correspond to this mixture of isomers.

[11] *Cf.* R. B. Wagner, R. F. Forker and P. F. Spitzer, THIS JOURNAL, **73**, 2494 (1951).