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

STEROIDAL SAPOGENINS. V. $\Delta^{5,7}$-22-ISOSPIROSTADIEN-3β-OL[1]

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During the past lustrum, numerous investigations on 7-dehydrocholesterol and related compounds have been published (2–8) making use of the Wohl-Ziegler reaction of N-bromosuccinimide (9) with Δ^5-cholestene derivatives, followed by dehydrobromination of the resulting 7-bromo compounds.

It seemed interesting to extend these studies to the steroidal sapogenin series for two reasons: 1. It was thought possible that the doubly unsaturated sapogenins might exhibit properties similar to those of 7-dehydrocholesterol; that is, that on irradiation with ultraviolet light they would yield products with vitamin D_3 activity. Though the vitamin D activity of a steroid is linked with the structure of its side chain, it is known that at least two types of side chain, the ergosterol and the cholesterol type, are concomitant with vitamin D activity. However, the irradiation products which the Wisconsin Alumni Research Foundation prepared from the presently described 7-dehydrosapogenins exhibited no antirachitic activity.[2] 2. The 7-dehydrosapogenins, possessing two double bonds in ring B, are interesting starting materials for transformations leading eventually to the introduction of functional groups into ring C of the cyclopentanophenanthrene nucleus and, after degradation of the sapogenin side chain, to valuable intermediates in the synthesis of cortical hormone analogs, now so important in the treatment of rheumatoid arthritis. Bergmann (10) has carried out experiments along this line in the ergosterol series. The greater facility of side chain degradation in the spirostan series was an important factor of the present investigation. The present paper deals only with the preparation of the spirostadiene derivatives: the results of further transformation studies will be dealt with separately.

As starting materials the acetate and the benzoate of Δ^5-22-isospirosten-3β-ol (diosgenin, I) were used. Among the different conditions for bromination and dehydrobromination described in the literature, those described by Bernstein and co-workers (5) were found to be the most suitable for this type of compound. Whereas it had been found that the 23-position of the side chain is susceptible to reaction with N-bromosuccinimide[3], it was fortunate that under these conditions

[1] In the preceding paper of this series (Ref. 1) Rosenkranz and Djerassi proposed a new nomenclature for steroidal sapogenins. For the convenience of the reader, we give in brackets the conventional names of the known compounds which were used as starting material in the present investigation.

[2] The assay report of the Wisconsin Alumni Research Foundation states that our sample of $\Delta^{5,7}$-22-isospirostadien-3β-ol "under the conditions of the irradiation technique as described . . . showed no evidence of vitamin D potency at a level of 1,000 U.S.P. units of vitamin D per gram. On the other hand, the irradiated ergosterol used as a positive control revealed a potency of approximately 5,000,000 U.S.P. units of vitamin D per gram."

[3] Unpublished results from this laboratory.

a selective bromination in the 7-position could be accomplished without affecting the side chain. By reacting the isospirostenol esters with N-bromosuccinimide under artificial illumination, the corresponding 7-bromo derivatives (II) were obtained. In accordance with the postulates of Bide, Henbest and co-workers (4), we assign to the bromine substituent at C_7 the "β"-configuration. These compounds were obtained as crystalline substances characterized by a strong levo rotation. The molecular rotation differences calculated according to Barton's method (11) are in good agreement with those reported for the cholesterol series (Table I).

While the 7-Br-Δ^5-22-isospirosten-3β-ol benzoate is rather stable, the acetate decomposes slowly, liberating acetic acid, which is readily detected by its odor. The rotation of the acetate, measured in chloroform solution immediately after preparation is $[\alpha]_D$ $-303°$, this strong levorotation dropping to about $-200°$ if the solution is allowed to stand for 24 hours.

TABLE I

MOLECULAR ROTATIONS OF 7-BROMO-Δ^5-STENOLS

COMPOUND	M_D(CHCl$_3$)		M_D (Br-deriv.) $-M_D$(Stenol)	REFERENCE
	Δ^5-Stenol	7-Br-Δ^5-stenol		
Δ^5-22-Isospirosten-3β-ol acetate	−580	−1622	−1042	
Cholesteryl acetate	−201	−1245	−1044	4
Δ^5-22-Isospirosten-3β-ol benzoate	−430	−1345	−915	
Cholesteryl benzoate	−67	−940	−873	5
		−980	−913	4
Δ^5-Norcholesten-3β-ol benzoate	−85	−920	−835	6

On treatment with collidine, dehydrobromination occurred with formation of a second double bond. When this latter reaction was carried out in xylene-collidine solution, the desired $\Delta^{5,7}$-22-isospirostadien-3β-ol esters (III) were obtained as the main products, accompanied by smaller amounts of the $\Delta^{4,6}$-isomers (IV). Similar observations have been reported by the workers in the cholesterol field (3–7). While the corresponding reaction in the cholesterol series leads to mixtures of isomers which are difficult to separate, there exists in the spirostan series a marked difference in the solubility of the $\Delta^{5,7}$- and $\Delta^{4,6}$-compounds, which permits easy separation of these two isomers. A further by-product, which we suspect is $\Delta^{3,5,7}$-22-isospirostatriene, was isolated from the dehydrobromination mixture, and is now being investigated.

When the dehydrobromination was carried out in undiluted collidine, the $\Delta^{4,6}$-isomer was obtained as the main product. The dehydrobromination method of Lowenbein (12), using calcium oxide as dehydrobrominating agent was also tried, but we failed to isolate doubly unsaturated compounds from the reaction mixture.

The formation of IV can be explained on the basis of Hughes' and Ingold's work (13), if it is assumed that the dehydrobromination proceeds as a unimolecular elimination reaction (E_1), which would include the formation of a carbo-

nium ion followed by a shift of the double bond from the 5,6- to the 6,7-position.

The assignment of structures to the isomers was based primarily on u.v. absorption spectra measurements. These results are contained in Table II.

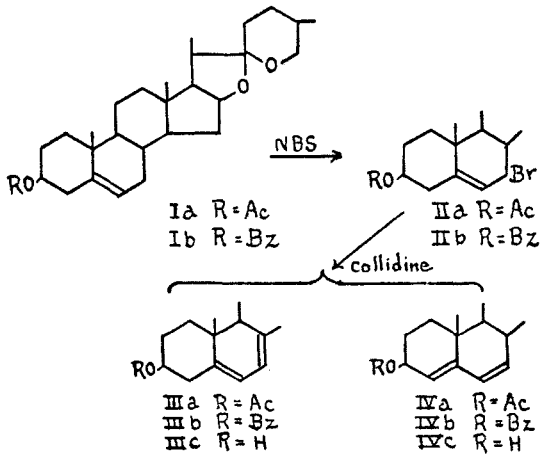

TABLE II

PHYSICAL CONSTANTS OF THE $\Delta^{5,7}$-22-ISOSPIROSTADIEN-3β-OL AND ITS ESTERS

COMPOUND	M.P., °C	$[\alpha]_D$ ($CHCl_3$)	PRINCIPAL U.V. ABSORPTION MAXIMA WITH LOGARITHMS OF MOLECULAR EXTINCTION COEFFICIENTS				
$\Delta^{5,7}$-Isospirostadien-3β-ol	167–170 (188.5–190)[3a]	−173.9°	λ max		270	282	292
			log ε		4.16	4.18	3.96
$\Delta^{5,7}$-Isospirostadien-3β-ol acetate	190–192 (202–205)[3a]	−127°	λ max		270	280	292
			log ε		4.19	4.20	3.99
$\Delta^{5,7}$-Isospirostadien-3β-ol benzoate	206–209	−91°	λ max	228	270	282	292
			log ε	4.17	4.14	4.14	3.89
$\Delta^{5,7}$-Isospirostadien-3β-ol *p*-nitrobenzoate	221–223	−93.5°	λ max		270	282	
			log ε		4.50	4.44	
$\Delta^{5,7}$-Isospirostadien-3β-ol 2,4-dinitrobenzoate	223–225	−89.6°	λ max	260	270	280	292
			log ε	4.18	4.18	4.13	3.91

[3a] The melting points of IIIa and IIIc were consistently found higher when taken in a sulfuric acid bath, 202–205° and 188.5–190° respectively.

The maxima at wave lengths 270 and 280 mμ are those recorded in Dannenberg's monograph (14) for the conjugated ethylenic linkage 5,6; 7,8 in the ergostane, cholestane and androstane series. In addition, a third, somewhat less pronounced, maximum at 292 mμ was found, in agreement with the findings of Bernstein (5) and Huber (15) (see Fig. 1). It may be mentioned here that Rochelmeyer and Böttcher (16) prepared 7-dehydrosolanidine and found its u.v. spectrum to be identical with that of ergosterol (maxima at 270 and 280 mμ).

The maxima at wave lengths 232, 238–240 and 248 mμ are the same that Alberti and co-workers (6) report for the $\Delta^{4,6}$-dienes in the cholestane series (see Fig. 2). Obviously, the spectrum of the benzoate (and of the *p*-nitrobenzoate) is not suitable for identification purposes in this instance, since the benzoyl group itself has a strong absorption in the region characteristic for the 4,6-double bond, as was observed by Huber (15).

This difference in the absorption spectra has been widely used for a quantitative estimation of the isomers in the crude dehydrobromination product (of the acetates). Furthermore, the determination of the molecular extinction coefficient afforded a useful tool for determining the completeness of separation of the two

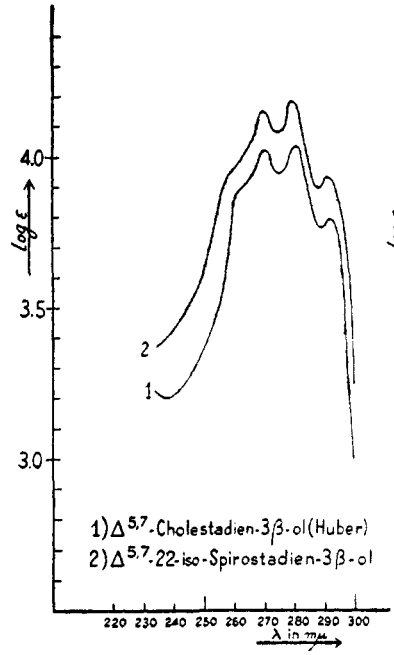

Fig. 1

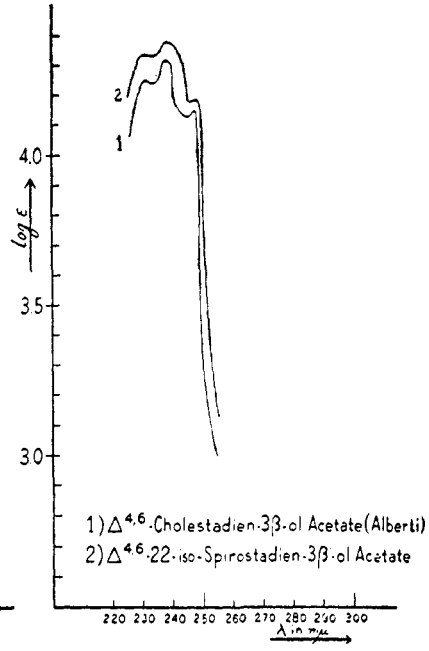

Fig. 2

isomers, absence of the maxima corresponding to the contaminating isomer being the criterion of complete separation.

For further characterization of the 22-iso*allo*spirostanol derivatives the method of molecular rotation differences was applied and a comparison with the corresponding values in the cholesterol series was made (Table III). It was found that the differences in the behaviour of the two series are negligible as long as only positions 3 (esterification) and 5,6 (double bond) are involved; on the other hand the introduction of a second double bond has a markedly different influence in the two series; though, according to generally accepted considerations, they supposedly differ only in the structure of the side chain. As a possible explanation it is suggested that the spiro-ketal side chain, with its obviously restrained mobility, exercises some sort of "vicinal effect" on ring B, resulting possibly

from a distortion of the valency angles, propagated from the tetrahydrofuran ring to ring B, *via* rings D and C (18).

However, such an explanation does not account for the surprising agreement between the rotations of IIIc (−173.9°) and IVc (−159°). On the basis of the work of Barton and Cox (19), who recorded considerable differences in the rotation of heteroannular ($\Delta^{4,6}$) and homoannular ($\Delta^{5,7}$) dienic systems, it could be predicted that IIIc would be much more levorotatory than IVc. Methods for an unambiguous preparation of steroidal $\Delta^{4,6}$-diene-3-ols are now being investigated and will be reported at a later date.

TABLE III

MOLECULAR ROTATION DIFFERENCES FOR DERIVATIVES OF 22-ISOALLOSPIROSTANOL AND OF CHOLESTANOL

TYPE OF DERIVATIVE	DERIVATIVE OF		ΔM_D	ΔM_D (DERIVATIVES) $-\Delta M_D$ (PARENT COMPOUNDS)	REFERENCE
	22-Isoallospirostanol (Tigogenin)[a] M_D	Cholestanol M_D			
Parent compound	−304	+93	+397	—	17
Δ^5-Stenol (diosgenin and cholesterol)	−534	−151	+383	−16	17
Δ^5-Stenol acetate	−578	−201	+377	−20	17a
Δ^5-Stenol benzoate	−472	−69	+403	+6	17a
$\Delta^{5,7}$-Stadienol	−716	−460	+256	−141	5
$\Delta^{5,7}$-Stadienol acetate	−576	−328	+248	−149	5
$\Delta^{5,7}$-Stadienol benzoate	−464	−259	+205	−192	5
$\Delta^{5,7}$-Stadienol *p*-nitrobenzoate	−522	−288	+234	−163	5

[a] The $[\alpha]_D$ of 22-isoallospirostanol (tigogenin) (mol. wt. 416) found in the literature (−49°) is not comparable, since it was measured in pyridine solution. It is replaced by the value of −73° (chloroform), determined in this laboratory $\left(\frac{-73 \times 416}{100} = -304\right)$.

EXPERIMENTAL[4]

7-Bromo-Δ^5-22-isospirosten-3β-ol acetate (IIa). Δ^5-22-Isospirosten-3β-ol acetate (diosgenin acetate) (13.65 g.) was dissolved in 105 cc. of carbon tetrachloride and 30 cc. was distilled to remove traces of moisture. Then 6.3 g. (1.2 mole) of N-bromosuccinimide was added and the mixture was heated with two photospot lamps (General Electric Co. No. RSP2) and refluxed for five minutes. The succinimide was filtered and washed with ether. The combined filtrates were washed with water, dried with sodium sulfate, decolorized with charcoal, filtered and evaporated to dryness under reduced pressure at about 30° using a vertical tube evaporator. The residue was crystallized from ether to yield 8.5 g. of crystals, m.p. 165–167° (dec.), $[\alpha]_D^{20}$ −303°.

Anal. Calc'd for $C_{29}H_{43}O_4Br$: C, 65.02; H, 8.09; Br, 14.93.
Found: C, 65.31; H, 8.03; Br, 14.35.

$\Delta^{5,7}$-22-Isospirostadien-3β-ol acetate (IIIa). Five grams of IIa was dissolved in 20 cc.

[4] All melting points were determined on the Kofler block (see however footnote 3[a]). Rotations were carried out on 60–100 mg. of substance in 10 cc. of chloroform in a 2 dm. tube, while all spectra were taken in 95% ethanol solution. We are indebted to Srta. Paquita Revaque for these determinations and to Srta. Amparo Barba of our Microanalytical Department for the microanalyses.

of collidine and 200 cc. of xylene and refluxed for forty minutes. After cooling, the precipitate of collidine hydrobromide was filtered and washed with ether, wt. 1.75 g. (calc'd wt. 1.87 g.). The combined filtrates were washed several times with dilute sulfuric acid to remove the collidine and then with water to neutrality. The solution was steam-distilled to remove all organic solvents, the residue was taken up in ethyl acetate, the solution dried with sodium sulphate, and evaporated to dryness, yielding 4.1 g. (calc'd wt. 4.2 g.) of a light brown powder. The absorption spectrum of this crude reaction product, by comparison with the corresponding spectra of the pure $\Delta^{5,7}$ and $\Delta^{4,6}$ acetates (see Figs. 1 and 2), indicates that the isomers are formed in about 60:40 proportion. A less pronounced maximum at 306 mμ probably is indicative of the presence of some triene (presumably $\Delta^{3,5,7}$-22-isospirostatriene), now under investigation.

The crude dehydrobromination product was twice stirred with small portions of methanol, then dissolved in pentane. After treatment with charcoal, 2.0 g. of white needles was obtained; m.p. 190–192°[3a] $[\alpha]_D^{20}$ −127°; absorption maxima at 270, 280 and 292 mμ; log ε 4.19, 4.20 and 3.99 respectively (see Fig. 1).

Anal. Calc'd for $C_{29}H_{42}O_4$: C, 76.61; H, 9.31.
Found: C, 76.86; H, 9.41.

$\Delta^{4,6}$-*22-Isospirostadien-3β-ol acetate* (IVa). The mother liquors of IIIa were evaporated to dryness and then carefully chromatographed on alumina (Merck Reagent Aluminum Oxide). Four different crystalline fractions were obtained: (a) with hexane-benzene 19:1 a substance, which after recrystallization from ethyl acetate melted at 186–188°; (b) with hexane-benzene 9:1 followed by recrystallization from ethyl acetate, white crystals, m.p. 160–163°; $[\alpha]_D^{20}$ −126°; absorption maxima at 232, 238 and 248 mμ with log ε 4.34, 4.38 and 4.18 respectively (see Fig. 2).

Anal. Calc'd for $C_{29}H_{42}O_4$: C, 76.61; H, 9.31.
Found: C, 76.63; H, 9.24.

(c) With hexane-benzene 1:1, an additional crop of IIIa was eluated; (d) with benzene, a substance was eluated, which, after recrystallization from ethyl acetate melted at 240–243° and which is now being investigated.

$\Delta^{5,7}$-*22-Isospirostadien-3β-ol* (IIIc). Five grams of IIIa was refluxed for one hour with 100 cc. of a 1% ethanolic potassium hydroxide solution under nitrogen atmosphere. The reaction mixture was poured into water and extracted with ether. The ether solution was washed with water until neutral, dried with sodium sulfate and evaporated to dryness leaving a residue of 4.3 g. After several recrystallizations from ethyl acetate, the product melted at 167–170°[3a]; $[\alpha]_D^{20}$ −173.9°; absorption maxima at 270, 282 and 292 mμ; log ε 4.16, 4.18 and 3.96 respectively.

Anal. Calc'd for $C_{27}H_{40}O_3$: C, 78.59; H, 9.77.
Found, C, 78.75; H, 9.99.

$\Delta^{5,7}$-*22-Isospirostadien-3β-ol benzoate* (IIIb). From 1 gr. of IIIc, on benzoylation in pyridine, 1 g. of white needles was obtained, m.p. 203–204°. Further recrystallization from ethyl acetate-methanol raised the m.p. to 206–209°; $[\alpha]_D^{20}$ −91°; absorption maxima at 228, 270, 282 and 292 mμ; log ε 4.17, 4.14, 4.14 and 3.89 respectively.

Anal. Calc'd for $C_{34}H_{44}O_4$: C, 79.02; H, 8.58.
Found: C, 79.00; H, 8.72.

$\Delta^{5,7}$-*22-Isospirostadien-3β-ol p-nitrobenzoate.* One gram of IIIc treated with *p*-nitrobenzoyl chloride in pyridine yielded 1.2 g. of yellow needles. One recrystallization from chloroform-methanol raised the m.p. to 223°; $[\alpha]_D^{20}$ −93.5°; absorption maximum at 270, 282 mμ; log ε 4.50 and 4.44 respectively.

Anal. Calc'd for $C_{34}H_{43}O_6N$: C, 72.69; H, 7.71.
Found: C, 72.49; H, 7.51.

$\Delta^{5,7}$-*22-Isospirostadien-3β-ol 2,4-dinitrobenzoate.* One gram of IIIc treated with 2,4-dinitrobenzoyl chloride in pyridine yielded 1 g. of yellow needles, m.p. 223–225°; $[\alpha]_D^{20}$ −89.6°; absorption maxima at 260, 270, 280 and 292 mμ; log ε 4.18, 4.18, 4.13 and 3.91 respectively.

Anal. Calc'd for $C_{34}H_{42}O_8N_2$: C, 67.30; H, 6.97; N, 4.61.
Found: C, 67.45; H, 7.21; N, 4.66.

$\Delta^{4,6}$-22-Isospirostadien-3β-ol (IVc). Five grams of IVa was refluxed for ninety minutes with 800 cc. of a 0.5% ethanolic potassium hydroxide solution, under nitrogen atmosphere. The reaction mixture was poured into water, the precipitate was filtered, washed with water until neutral and recrystallized from ethanol, yielding 3.8 g. of white needles, m.p. 148–150°. Repeated recrystallizations from ethanol raised the m.p. to 160–161°; $[\alpha]_D^{20}$ −159°; absorption maxima at 232, 240 and 248 mμ with log ε 4.38, 4.41 and 4.22 respectively.

Anal. Calc'd for $C_{27}H_{40}O_2$: C, 78.59; H, 9.77.

Found: C, 78.29; H, 9.78.

$\Delta^{4,6}$-22-Isospirostadien-3β-ol benzoate (IVb). Four grams of IVc treated in the usual way yielded 3.2 g. of needles, m.p. 188–190°; $[\alpha]_D^{20}$ −115°, absorption maximum at 240 mμ, log ε 4.63.

Anal. Calc'd for $C_{34}H_{44}O_4$: C, 79.02; H, 8.58.

Found: C, 79.14; H, 8.74.

$\Delta^{4,6}$-22-Isospirostadien-3β-ol p-nitrobenzoate. Three grams of IVc treated in the usual way yielded 2.7 g. of pale yellow needles, m.p. 200–202°. Recrystallization from chloroform-methanol raised the m.p. to 204–206°; $[\alpha]_D^{20}$ −114°; absorption maximum at 250 mμ, log ε 4.47.

Anal. Calc'd for $C_{34}H_{43}O_6N$: C, 72.69; H, 7.71.

Found: C, 72.54; H, 7.66.

7-Bromo-Δ^5-22-isospirosten-3β-ol benzoate (IIb). Eight grams of Δ^5-22-isospirosten-3β-ol benzoate (diosgenin benzoate, m.p. 233°, $[\alpha]_D^{20}$ −83°) was dissolved in 320 cc. of carbon tetrachloride and 60 cc. was distilled to remove traces of moisture. Then 3.3 g. (1.2 mole) of N-bromosuccinimide was added and the mixture was heated as described above for the acetate. In several runs, no difference in the yield and purity of the reaction product was found, regardless of whether the illumination time was five, ten or fifteen minutes. The reaction mixture was worked up as described above, yielding 4.5 g. Recrystallization from methylene dichloride-hexane gave the pure product, m.p. 160° (dec.); $[\alpha]_D^{20}$ −226°.

Anal. Calc'd for $C_{34}H_{45}O_4Br$: C, 68.31; H, 7.58; Br. 13.38.

Found: C, 67.95; H, 7.43; Br, 13.05.

$\Delta^{5,7}$-Isospirostadien-3β-ol benzoate (IIIb). Nine grams of IIb was dissolved in 45 cc. of collidine and 450 cc. of xylene and refluxed for ninety minutes. After cooling, the precipitate of collidine hydrobromide which had formed during the reaction was filtered and washed with ether, wt. 2.8 g. (calc'd wt. 3.0 g.). The combined filtrates were treated as described above for the acetate. The crude reaction product had absorption maxima at 232, 270, 280 and 292 mμ; log ε 4.41, 3.82, 3.80 and 3.60 respectively. The high extinction at 232 mμ is indicative of the presence of some $\Delta^{4,6}$-compound. No attempt was made to isolate this compound from the crude reaction product; recrystallization from ethyl acetate-methanol gave 2 g. of the pure $\Delta^{5,7}$-22-isospirostadien-3β-ol benzoate (IIIb), m.p. 205–208°, identical in every respect with the product obtained by benzoylation of IIIc (see above).

SUMMARY

Bromination and subsequent dehydrobromination of Δ^5-22-isospirosten-3β-ol (diosgenin) leads to $\Delta^{5,7}$-22-isospirostadien-3β-ol, a sapogenin analog of provitamin D.

The main by-product formed on dehydrobromination of the intermediate 7-Br-Δ^5-22-isospirosten-3β-ol is the $\Delta^{4,6}$-22-isospirostadien-3β-ol.

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