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Nature of the Hydrogen Chloride Induced Isomers of α-Spinasterol

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Abstract — It was established that α-spinasterol (**I**) was isomerized by hydrogen chloride to a mixture of two isomers. These isomers were separated into each of components and identified as $\Delta^{8(14),22}$-stigmastadienol (**III**) and $\Delta^{14,22}$-stigmastadienol (**IV**), respectively. The mass spectra of these compounds were found to be particularly suitable to identify the position of nuclear double bond of sterols possessing 22 : 23-double bond in side chain.

α-Spinasteryl-D-glucoside was first isolated by Obata, *et al.*,[1] in 1955 from *Beta vulgaris*, and has since been identified in several plant tissues; the genera *Spinacia*[2,3], *Pitecellobium*[4,5], *Platycodon*,[6] *Medicago*,[7] *Samania*,[8] and *Phytolacca*[9,10]. In the course of the study on structure elucidation of steryl glucoside from *Phytolacca americana*, we found that the melting points of aglucones obtained by acid hydrolysis under drastic conditions were lower than those under milder conditions. This phenomenon was already observed by Ito, *et al.*[6] Although positive identification was not made, they suggested that on the basis of glc analysis α-spinasterol might be converted to $\Delta^{8(14),22}$-stigmastadienol during acid hydrolysis.

It is well known that when α-spinasterol is shaken in the presence of hydrogen with platinum catalyst in acetic acid[11] or with palladium catalyst in ether,[12] the 22, 23-double bond is saturated, but the 7, 8-double bond migrates to give $\Delta^{8(14)}$-stigmastenol which is isomerized by hydrogen chloride to Δ^{14}-stenol, in about 80% yield.[11]

On the other hand, it has been established that 5-dihydroergosterol is isomerized by hydrogen chloride in chloroform,[13] but the product is suggested by consideration of optical rotation evidence to be an inseparable mixture of about equal parts of $\Delta^{8(14),22}$-and $\Delta^{14,22}$-ergostadienol.[14]

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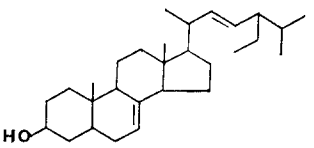
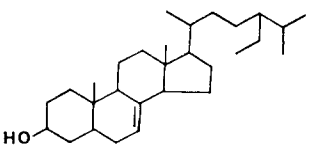
It has, however, been reported that a double bond at 8, 14-position of $\Delta^{8(14)}$-dehydroisosapogenin acetate and 20-keto-$\Delta^{8(14)}$-5α-pregnen-3β-ol are not rearranged to the 14, 15-position by hydrogen chloride.[15]



I



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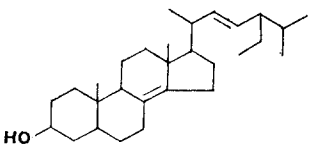
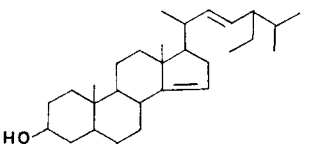


III



IV

Since in none of the publications were the hydrogen chloride induced isomers of α-spinasterol completely identified, it is desirable to clear up the nature of them. We now wish to report the separation of these isomers and their structure elucidation.

α-Spinasteryl-D-glucoside was hydrolyzed under the various conditions which were used by various authors for hydrolysis of steryl glucosides. Aglucones formed were crystallized once from $CHCl_3$-MeOH (1:1) for comparison purpose and their mps are shown in Table I. As mentioned before, acid hydrolysis under drastic conditions gave compounds having lower melting points.

Table I — Hydrolysis of glucoside under various conditions.

No.	Catalyst (v/v%)	Reflux time (hr)	Mp of product	Authors used by
1	AmOH·EtOH(10:3)-3% HCl	2	137–141	Power & Salway[16,17]
2	MeOH-5% HCl	70*	139–143	Tunmann[18]
3	EtOH-2/3% H_2SO_4	12	140–142	Thornton, *et al.*[7,19]
4	MeOH-7.5% HCl	5	149–150	Ito[6]
5	EtOH-1% H_2SO_4	24	149–151	Obata[1]
6	EtOH-0.28% H_2SO_4	2	162–164	This Expt.

* at 30°.

The change of color developed in the Liebermann-Burchard reaction for sterols obtained in Expt. Nos. 2 and 6 is shown in Fig. 1, in which the absorption at 620 nm is plotted

against the period of reaction. The curve (a in Fig. 1) for sterol (No. 6) is quite similar to that (b in Fig. 1) for α-spinasteryl-D-glucoside, and the maximal molar L value is in excellent agreement with the value for α-spinasterol,[20] while the curve (c in Fig. 1) for sterol (No. 2) closely resembles that for a $\Delta^{8(14)}$-stenol or Δ^{14}-stenol.[21-23] These results suggest that during acid hydrolysis a part of Δ^{7}-sterol is converted into a compound which is intermediate acting in color test.

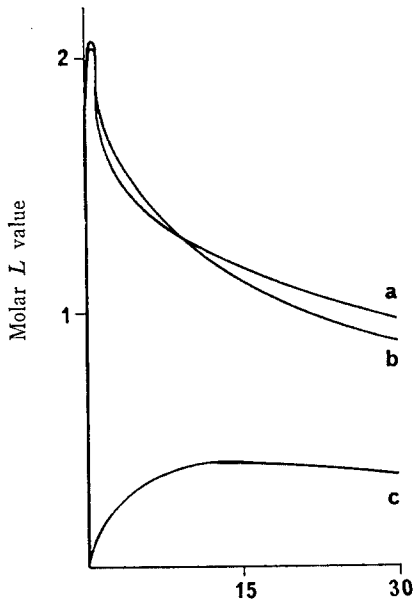


Fig. 1 — Reactivity of aglucones with Liebermann-Burchard reagent. a, aglucone obtained under the conditions of Expt. No. 6 in Table I; b, α-spinasteryl-D-glucoside; c, aglucone of Expt. No. 2.

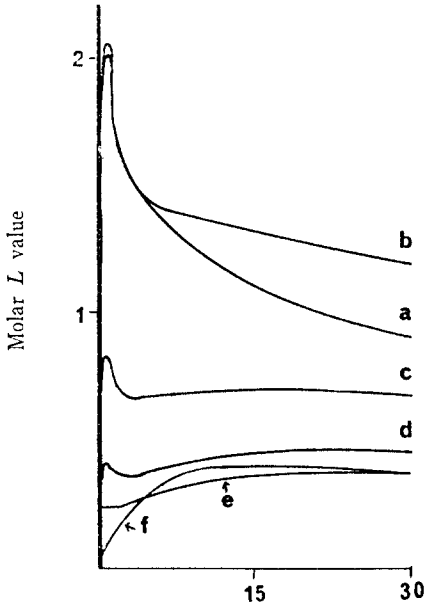


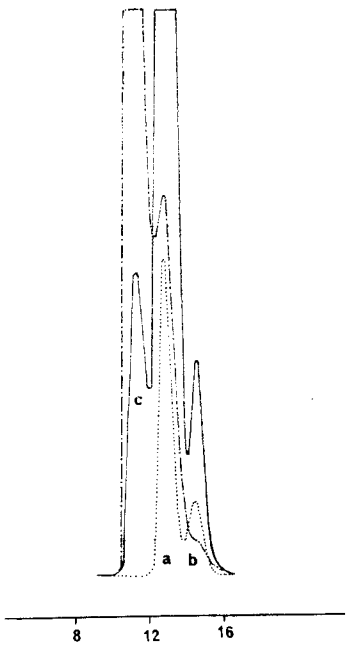
Fig. 2 — Change of reactivity of α-spinasterol with LB reagent due to acid treatment. a, control; b, 0.28% EtOH-H_2SO_4, 1hr; c, 2hr; d, 3hr; e, 4hr; f, 5% MeOH-HCl, 5hr.

This view is also supported by a study of the product formed by acid treatment of α-spinasterol itself. Fig. 2 shows that α-spinasterol is affected by acid treatment even under milder conditions. Reaction rates are decreased in proportion to increment of acidity and period of treatment.

Glc analysis shows that the sterol (No. 6) is a mixture of α-spinasterol (peak a in Fig. 3), Δ^{7}-stigmastenol* (peak b in Fig. 3) and unknown sterol (peak c in Fig. 3), which is

* See Experimental.

an artefact as mentioned below. However, chromatogram for the sterol (No. 2) is quite different from that of the sterol (No. 6), notwithstanding they were derived from the same origin.



Time in min.

Fig. 3 — Glc of aglucones. —— aglucone obtained under the conditions of Expt. No. 6 in Table I; —·—·— aglucone of Expt. No. 2; ··· standard sterols : a, α-spinasterol; b, Δ^7-stigmastenol.

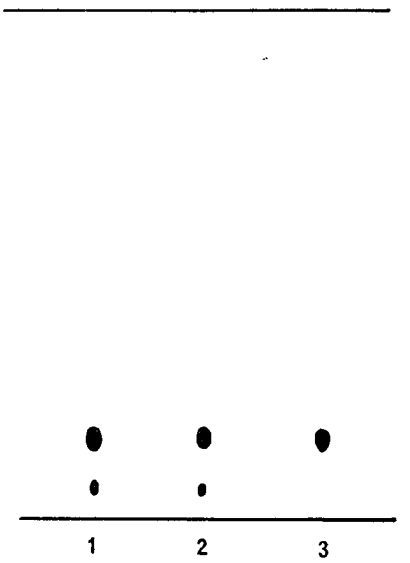


Fig. 4 — Tlc on 15% $AgNO_3$-SiO_2 of aglucones obtained under the conditions of Expt. No. 6 in Table I; (1), product fromed under the same conditions (2), and α-spinasterol as control (3). Developer, benzene.

All aglucones derived by acid hydrolysis of the glucoside and all products formed by acid treatments of α-spinasterol under various conditions could be separated by tlc on $AgNO_3$ impregnated SiO_2 plates into 2 portions, respectively. Typical chromatograms are shown in Fig. 4. It is worth mentioning that we have observed no significant differences between the chromatograms of fast acting sterols and intermediate acting sterols.

After separation by preparative tlc on 15% $AgNO_3$-SiO_2 plates, reactivity of each fraction with the Liebermann-Burchard reagent was investigated. All of lower zones were interme-

diate acting sterols (curved in Fig. 5), but the curves for upper zones were different according to acidity and period of treatment. For example, upper zone of sterol derived on hydrolysis of the glucoside with 0.28% EtOH-H_2SO_4 for 2 hr was fast acting (curve a in Fig. 5), whereas that for 12hr was intermediate acting (curve e in Fig. 5).

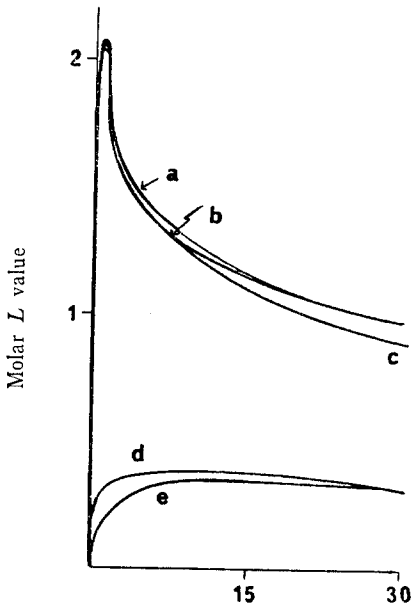


Fig. 5 — Reactivity with LB reagent of the components separated from the products formed on treatment of α-spinasterol and its glucoside with 0.2% EtOH-H_2SO_4. a, upper zone from glucoside for 2 hr; b, upper zone from sterol for 1hr; c, α-spinasterol as control; d, lower zone from sterol or glucoside for 12hr; e, upper zone from sterol or glucoside for 12hr.

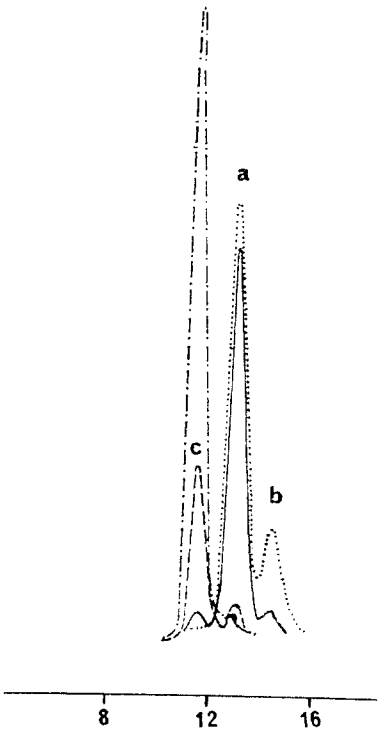


Fig. 6 — Glc of the components separated from the products formed on acid treatment of α-spinasterol. —— upper zone by 0.28% EtOH-H_2SO_4 for 2hr; —·—·— upper zone and — — — lower zone by 5% MeOH-HCl for 5 hr; ····· standard sterols : a, α-spinasterol; b, Δ^7-stigmastenol.

Glc analysis of the products formed from α-spinasterol on treatment with acid gave the results presented in Fig. 6. The upper zone of the product on treatment with 0.28 % EtOH-H_2SO_4 for 2 hr consisted of three components, α-spinasterol, Δ^7-stigmastenol, and unknown sterol (peak c in Fig. 6); the content of the latter compound was decreased as compared to that of nonpurified aglucone derived from the glucoside under the same consitions (peak c in Fig. 2). The observed mass spectrum (Fig. 7, A) of this fraction

was in good agreement with that of α-spinasterol published elsewhese [20] and molecular peak of Δ^7-stigmastenol can be seen at m/e 414. Retention times of both fractions, upper zone, and lower zone, from the products under drastic conditions are identical (peak c in Fig. 6). These data suggest that under drastic conditions α-spinasterol is almost completely isomerized to two sterols, isomer A (upper zone) and B (lower zone), which could be separated by tlc but not on the glc system used. Therefore, peak c substance in Fig. 6 for the upper zone of the product obtained under milder conditions is isomer A. Small peak a substances for these fractions seem not to be α-spinasterol

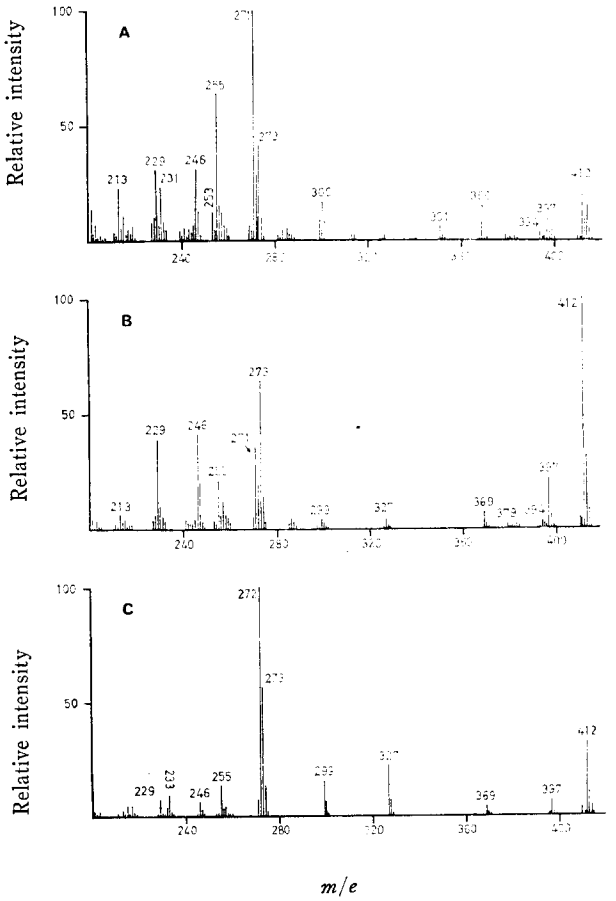


Fig. 7 — Mass spectra of purified aglucone of Exptl. No. 6 in Table I (A), and $\Delta^{8(14),22}$-stigmastadienol (B) and $\Delta^{14,22}$-stigmastadienol (C) which were separated from the products formed by acid treatment of α-spinasterol under drastic conditions.

unchanged but might be isomers derived from Δ^7-stigmastenol, respectively.

It was also found that α-spinasterol was completely isomerized by treatment of HCl in $CHCl_3$ for 2hr to a mixture of isomer A and B (25:75), whereas the ratio was 60:40 by treatment of HCl in MeOH for 5 hr.

Isomer A——Mp 159-160°, (M^+ 412), $[\alpha]_D^{22} = \pm 0°$, λ_{max}^{EtOH} 204 nm(logε, 4.14), acetate, mp 138-139°, $[\alpha]_D^{22} = -12.4°$, benzoate, mp 117-118°, $[\alpha]_D^{22} = -10.8°$. It gave a positive Tortelli-Jaffe test, characteristic of sterols with a tetrasubstituted nuclear double bond.[24] The infrared spectrum showed a hydroxyl band at 3340 cm^{-1} and the presence of a *trans*-disubstituted double bond by a strong band at 970 cm^{-1} [25,26], but no peaks in the 800-840cm^{-1} region, which corresponded to a trisubstituted double bond.[27,28]

The presence of 22 : 23-unsaturation was also provided by the nmr spectrum which showed a vinylic multiplet centered at δ 5.17, integrating for two protons and the 21-methyl doublet at relatively low field 58.8 and 65.8Hz (J=7.0 Hz).[29,30] The proton magnetic frequencies for C-18 and C-19 methyl signals (52.3 and 42.0 Hz, respectively) agreed well with the calculated values for $\Delta^{8(14),22}$-sterol (50.5 and 42.0 Hz, respectively).[31] By these data, 5α-stigmast-8(14),22-dien-3β-ol is assigned to this isomer. As a matter of fact, frequencies for C-18 and C-19 methyl signals of 5α-stigmast-8(14),22-dien-3β-ol isolated from *Gymnocladus dioica* have been reported to be 52.0 and 41.0 Hz, respectively.[32]

In addition, the shift in molecular rotation observed on acetylation and benzoylation (-56.3° and -55.7°, respectively) are identical, within the limits of experimental error, with those recorded in the literature for 5α-stigmast-8(14),22-diene-3β-ol isolated from *Aplopappus heterophyllus* (-48° and -67°, respectively).[33]

Hydrogenation of acetate at atmospheric pressure using palladium in ether afforded $\Delta^{8(14)}$-stigmastenol acetate which was identical with authentic sample.

The mass spectum of this compound (Fig. 7) shows that the cracking pattern is similar to that of α-spinasterol. It has a parent peak at *m/e* 412 and fragmentation peaks at *m/e* 397, 394, 383, 379, 369, 273, 271, 255, 253, 246, 229 and 213, which are all observed in mass spectrum of α-spinasterol. But the intensity of the high mass peaks in the compound is much higher than those in α-spinasterol. Most notable is appearance of peaks at *m/e* 327 and 299 (unknown origin) and the peaks at *m/e* 300 and 231 disappear in striking contrast to the high intensity of these peaks in the spectrum of α-spinasterol (Fig. 7A).

The intensity of the peak at *m/e* 271 due to the loss of side chain together with two

hydrogen atoms from molecular ion is lower than that of the peak at *m/e* 273.

This fragmentation appears to be characteristic of $\Delta^{8(14),22}$-sterols and to be distinguished from $\Delta^{5,22}$ -and $\Delta^{7,22}$ -sterols.

Isomer B — Mp 128-129°, (M^+ 412), $[\alpha]_D^{26} = -4.61°$, λ_{max}^{EtOH} 201 nm (log ε, 3.74), acetate, mp 61—66°, $[\alpha]_D^{25.5} = -12°$, benzoate, mp 78-82°, $[\alpha]_D^{24} = +3.4°$.

It gave a negative test with the selenious acid reagent of Fieser [34] and did not develop a green color (Tortelli-Jaffe reaction) when bromination experiments were attempted.[24]

It showed a peak at 970 cm^{-1} in ir and a doublet centered at δ 1.01 (J = 7.0 Hz) in nmr, indicating the presence of a *trans*-disubstituted double bond in side chain. [25,26,29,30]

The frequencies for C-18 and C-19 methyl signals (55.2 and 49.8 Hz, respectively) are in excellent agreement with the calculated values for $\Delta^{14,22}$-sterol (55.0 and 49.5 Hz, respectively).[31]

The molecular rotation differences on acetylation and benzoylation (−35.5° and +36.5°, respectively) are in good agreement with values obtained in Δ^{14}-stenol series.[35] Hydrogenation of benzoate using palladium catalyst gave stigmastanol benzoate which was identified with authentic sample. From a study of its properties and reactions isomer was identified as 5α-stigmasta-14,22-dien-3β-ol.

The mass spectrum of $\Delta^{14,22}$ -sterol(Fig. 7 C) is quite different from those of $\Delta^{7,22}$ -and $\Delta^{8(14),22}$ -sterols. It has a base peak at *m/e* 272 probably due to the loss of the side chain plus one hydrogen atom. The peaks at 327, 299, and 233 are very prominent which are not the case in spectrum of α-spinasterol. The complete absence of peaks at *m/e* 231 and 213 due to the cleavage of ring D is not surprising, since this cleavage is blocked effectively by Δ^{14} -double bond. While we were preparing this paper Schiller[36] published a report of the fragmentation of the side chain together with one H-atom in $\Delta^{14,22}$ -sterols.

EXPERIMENTAL*

Sterol and Steryl Glucoside—α-Spinasterol (I) and its glucoside used in this study were

* The melting points were taken on a Mitamura-Riken apparatus and are uncorrected. The ir spectra were determined in KBr pellets on a JASCO Model IR-S spectrophotometer. The nmr spectra were recorded in $CDCl_3$ with a JEOL Model JEM-3H-60 instrument. The uv spectra were obtained on a Shimadzu Model MPS-50L recording spectrometer. Glc was carried out with a Shimadzu Model GC-4B gas chromatograph equipped with a FID. The chromatographic column was 150 cm×4 mm glass tube contained chromosorb W (60-80 mesh) coated with 2% OV-17. Column temp., 265°; injector temp., 240°; detector temp., 320°; carrier gas, N_2 (40ml/min).

isolated from the roots of *Phytolacca americana*. Although they contained small amounts of Δ^7-stigmastenol (**II**) and its glucoside, respectively, this contamination did not disturb our study.

The Liebermann-Burchard Reaction——This reaction was carried out as described previously.[20]

Isomerization of α-Spinasterol (I) by HCl in MeOH—A sample (20mg) of **I** was dissolved in 43 ml of MeOH and refluxed with 7 ml of *c*-HCl for 5 hr. After cooling the reaction mixture, the substance was taken up in Et_2O and separated into upper zone and lower zone by preparative tlc on silica gel with $AgNO_3$ (15%) using benzene as developer. Both zones were dissolved in 1 ml of $CHCl_3$, respectively, and subjected to glc for determination of amounts using cholesterol as internal standard. **I** was completely isomerized to mixture of $\Delta^{8(14),22}$-stigmastadienol (**III**) and $\Delta^{14,22}$-stigmastadienol (**IV**) (60:40).

Isomerization by dry HCl in $CHCl_3$——The dry hydrogen chloride gas was passed for 2 hr into the solution of **I**-acetate (30mg) in $CHCl_3$ (50ml). A part of acetate was hydrolyzed in the course of treatment. After the reaction mixture was evaporated under reduced pressure, the remainder was completely hydrolyzed with 3% MeOH-NaOH. The products were separated and analysed by glc as above. The ratio of **III** and **IV** was 25:75.

$\Delta^{8(14),22}$-Stigmastadienol (III)——Upper zones of the products by acid treatment under the drastic conditions were combined and crystallized from MeOH to give **III**, mp 159-160°, $[\alpha]_D^{22} = \pm 0°$ (c=0.4 in $CHCl_3$), λ_{max}^{EtOH} 204nm (log ε, 4.14), ir 3340cm^{-1} (OH) and 970 cm^{-1} (*trans*-disubstituted double bond), nmr δ 0.70 (s, 19-CH_3), 0.87 (s, 18-CH_3), 1.04 (d, J=7Hz, 21-CH_3), 3.50 (b,**H**-C-OH), and 5.0-5.2 (C**H**=C**H**-).

$\Delta^{8(14),22}$-Stigmastadienol Acetate——A sample (30mg) of **III** was heated with Ac_2O (1ml) and pyridine (3ml) for 2 hr. Pouring onto ice gave solids which were crystallized from MeOH to give **III**-acetate, mp 138-139°, $[\alpha]_D^{22} = -12.4°$ (c=0.14 in $CHCl_3$), ir 1730 and 1245 cm^{-1} (acetate).

$\Delta^{8(14),22}$-Stigmastadienol Benzoate-A sample (30mg) of **III** was heated with benzoyl chloride (0.2ml) and pyridine (3ml) for 90 min. Working up in the usual way aflorded a **III**-benzoate, which was crystallized from MeOH, mp 117-118°, $[\alpha]_D^{22} = -10.8°$ (c=0.32 in $CHCl_3$), ir 1715 and 1279 cm^{-1} (benzoate).

Hydrogenation of $\Delta^{8(14),22}$-Stigmastadienol Acetate—**III**-Acetate (50mg) was dissolved in Et_2O and palladium catalyst was added to the solution. The mixture was shaken in an atmosphere of hydrogen for 7 hr. After removal of solvent and recrystallization from

MeOH, the product, mp 116—118° was obtained, which was identified as $\Delta^{8(14)}$-stigmastenol acetate by mixed mp and ir with an authentic specimen prepared from α-spinasterol acetate under the same conditions.

$\Delta^{14,22}$-Stigmastadienol and Its Derivatives —Crystallization of the lower zones from MeOH gave IV, mp 128—129°, $[\alpha]_D^{26} = -4.61°$ (c=0.15 in $CHCl_3$), λ_{max}^{EtOH}201 nm (logε, 3.74), ir 3280(OH), 970 (*trans*-disubstituted double bond), and 823, 805, 797 (trisubstituted double bond), nmr δ 0.83 (s, 19-CH_3), 0.92 (s, 18-CH_3), 1.02 (d, J = 7 Hz, 21-CH_3), 3.5 (b, **H**-C-OH), 5.1—5.2 (m,=C=C**H**- and -CH=C**H**-). Acetylation and benzoylation of **IV** as above yielded **IV**-acetate, mp 61—66° $[\alpha]_D^{26} = -12°$ (c=0.23 in $CHCl_3$), ir 1732 and 1242cm^{-1} and **IV**-benzoate, mp 78—82°, $[\alpha]_D^{26} = +3.4°$ (c=0.2 in $CHCl_3$) ir 1785 and 1284 cm^{-1}, which by catalystic hydrogenation as above gave stigmastanol benzoate, mp 137°, identified by mixed mp and ir with an authentic sample.

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