THE SYNTHESIS AND THE MASS AND NUCLEAR MAGNETIC RESONANCE SPECTRA OF SIDE CHAIN ISOMERS OF CHOLESTA-5,22-DIEN-3β-OL AND CHOLESTA-5,22,24-TRIEN-3β-OL

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ABSTRACT

The previously unknown 22-cis-cholesta-5,22-dien-3β-ol, 22-cis and 22-trans isomers of cholesta-5,22,24-trien-3β-ol, 20-iso-22-trans-cholesta-5,22,24-trien-3β-ol, 22,24-trans,trans-26-homocholesta-5,22,24-trien-3β-ol, and the known 22-trans-dehydrocholesterol were synthesized. Their infrared, mass and nuclear magnetic resonance spectra are presented. These compounds undergo fragmentation processes that are governed primarily by the site of the unsaturation and not by their stereochemistry. However, the C-methyl resonances in the nuclear magnetic resonance spectra are influenced by their chemical environment.

As part of our continuing study of the metabolism and mechanism of dealkylation of sterols in insects, it became necessary for us to prepare the 22-cis and trans isomers of cholesta-5,22-dien-3β-ol and the 22-trans isomers of cholesta-5,22,24-trien-3β-ol. This paper reports on the synthesis of these compounds, their infrared, mass and nuclear magnetic resonance spectra and their chromatographic behavior by gas-liquid chromatography (GLC) and thin-layer chromatography (TLC).

The 22-trans isomer of cholesta-5,22-dien-3β-ol had been prepared by Bergmann and Dusza (1) by means of the Wittig reaction of 3β-acetoxydinorchol-5-en-22-al and the appropriate generated ylid. Our approach was quite similar; however, we protected the Δ^5-double bond

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of stigmasterol by formation of i-sterol ether (2). Thus, the ozonolysis of i-stigmasterol methyl ether (II) by the method of Slomp and Johnson (3) gave the aldehyde (III) in 70% yield. A Corey-modified Wittig reaction (4) of III with the ylid generated from 3-methylbutyltriphenylphosphonium bromide gave the 22-cis isomer (IVa) almost exclusively. The rearrangement of IVa with zinc acetate in boiling acetic acid followed by the saponification of the acetate (Va) yielded the 22-cis isomer of cholesta-5,22-dien-3β-ol (VIa) in an overall 53% yield. The unusually high proportion of the cis-olefin obtained by this method has been reported by others (5-7) and mechanistically explained by House et al. (8). The infrared spectrum of VIa (Fig. I) and of its acetate (Va) (Fig. 2) exhibited a medium band at 725 cm^{-1} that indicated a cis-oriented disubstituted double bond (9). The acetate (Va) showed only one spot when subjected to TLC on silver nitrate-impregnated silica gel H plates (10). Interestingly, the 22-cis and trans isomers were not distinguishable from one another by GLC on two different chromatographic systems (SE-30 and QF-1) (11).

A mixture of the 22-cis and trans isomers IVa and IVb was obtained when the aldehyde (III) was refluxed in hexane with the ylid generated from 3-methylbutyltriphenylphosphonium bromide with n-butyl lithium (12). The mixture of acetates (Va and Vb) obtained by rearrangement of IVa and IVb with zinc acetate in boiling acetic acid (13) was separated on a silver nitrate-impregnated Unisil (14) column.

Saponification of Va and Vb gave the respective cis and trans isomers VIa and VIb. Compound VIb had the typical strong peak at 965 cm^{-1} that indicated a trans-disubstituted double bond (Fig. 1), and agreed both in melting point and optical rotation with the 22-dehydrocholesterol of Bergmann and Dusza (1).

A Corey-modified method of the Wittig reaction of the aldehyde (III) with the ylid generated from 3-methylbut-2-enyltriphenylphosphonium bromide gave predominantly the 22-trans-$\Delta^{22,24}$-isomer (VIIb) accompanied by other by-products. Evidently the double bond stabilized the ylid and altered the reaction to favor the formation of the trans-compound (15). After the rearrangement of the i-methyl ether mixture (VII), the predominant $\Delta^{5,22,24}$-acetoxy compound (VIIIb, λ max. 240 mμ, ϵ 30,500 shoulders at 234, 247 mμ) was isolated by column chromatography on Unisil impregnated with silver nitrate. The infrared spectrum of this 22-trans isomer (VIIIb) also exhibited very strong absorption at 965 cm^{-1}. Repetitive column chromatography in this manner separated some 22-cis-$\Delta^{5,22,24}$-compound (VIIIa) and a third isomer. The amount of the third isomer varied but the largest proportions were obtained when a large excess of ylid was used. We have assigned the C-20 iso-configuration to the third isomer (VIIIc) on the basis of its more positive specific rotation of -4°, in contrast to the rotation of -62° for the 22-trans-cholesta-5,22,24-trien-3β-acetate (VIIIb), and on the basis of its infrared spectrum that exhibited both the Δ^{5}- and the trans Δ^{22}-double bond absorptions.

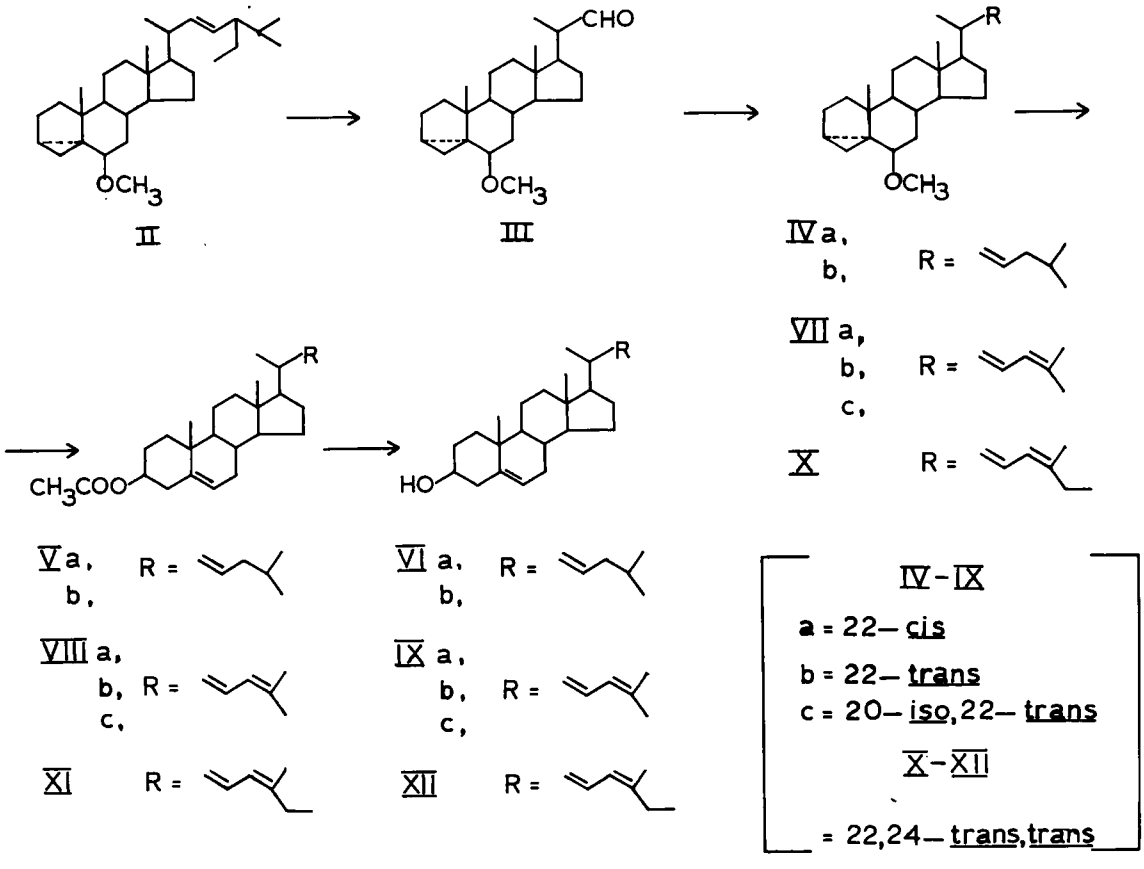
OCH3
II
CHO
OCH3
III
R
OCH3
IV a,
b, R =
VII a,
b, R =
c,
X R =
R
CH3COO
V a,
b, R =
VIII a,
b, R =
c,
XI R =
R
HO
VI a,
b, R =
IX a,
b, R =
c,
XII R =
IV-IX
a = 22-cis
b = 22-trans
c = 20-iso,22-trans
X-XII
= 22,24-trans,trans

Figure 1

Moreover, ultraviolet spectrum of the third isomer supports the conjugated diene structure of the side chain. Saponification of VIIIa, b, and c gave the free sterols IXa, b, and c, respectively. Their infrared (Fig. 1) and ultraviolet absorption spectra, specific rotations, and chromatographic behavior on GLC and TLC supported the assigned structures.

The Corey-modified Wittig reaction of the aldehyde (III) with 3-methylpent-2-enyl phosphorane produced a total of four compounds. Since the best separation of these compounds was obtained as their acetates, the i-methyl ether mixture (X) was rearranged to a mixture of Δ^5-3β-acetoxy compounds. In this case, even the acetates were difficult to separate, and we were only able to obtain the predominant (> 50% by GLC analysis) component, namely the 22,24-trans, trans isomer (XI) in 9% overall purified yield. Saponification of XI gave the 22,24-trans, trans-26-homocholesta-5,22,24-trien-3β-ol (XII). We have assigned the trans, trans orientation of the double bonds in the side chain for XI and XII for the following reasons: (i) The trans-3-methylpent-2-enyl bromide was used to prepare the ylid, and it is unlikely that inversion occurred during reaction. (ii) The trans isomer was the major isomer in the preparation of VIII; by analogy, the 22-trans isomer was also expected to be the major component in the preparation of XI. (iii) The trans isomers are faster moving than cis isomers on silver nitrate-impregnated Unisil columns or TLC plates because of a lesser tendency to complex with the silver ion.

Figure 2

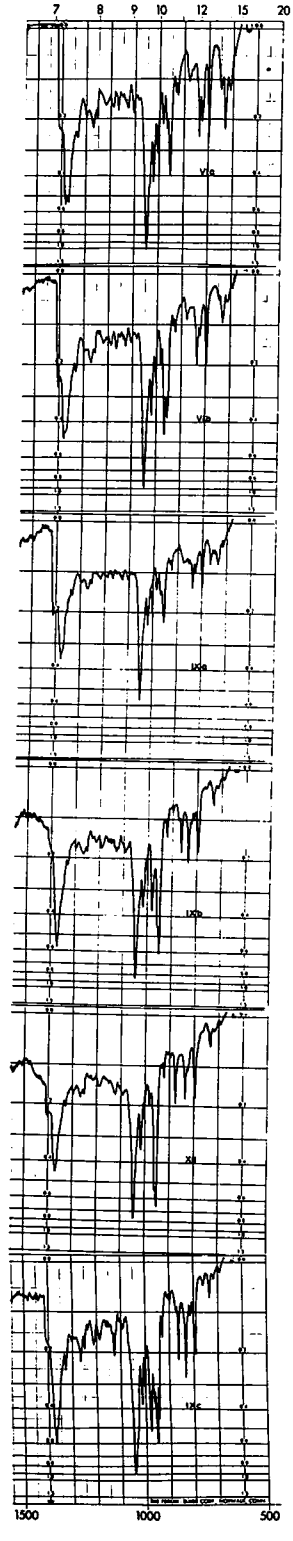

Fig.1 The Infrared Spectra of the Free Sterols

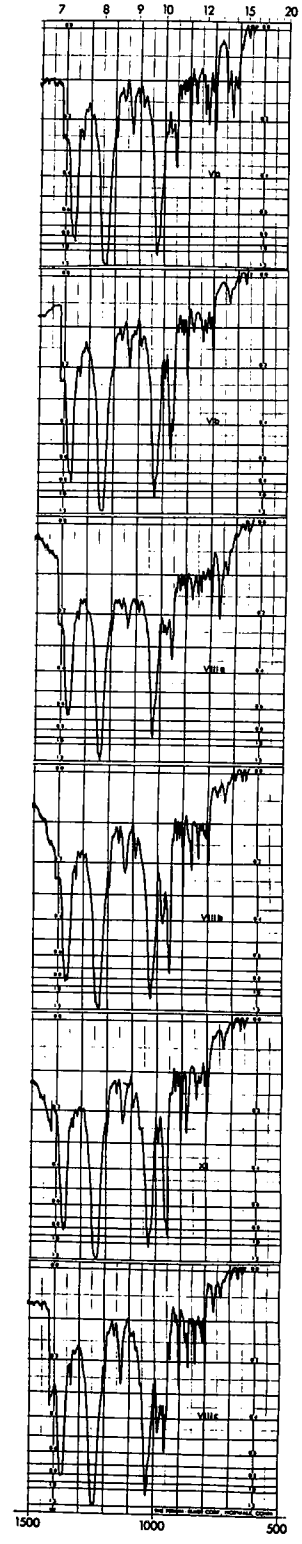

Fig.2. The Infrared Spectra of the Steral Acetates

(iv) By GLC analyses, the trans isomers with a conjugated diene system are slower moving than the corresponding cis components on SE-30 and QF-1 columns. (v) The infrared spectrum for XI shows the strong peak for a trans disubstituted double bond at 965 cm^{-1} (see Fig. 2).

On the basis of their chromatographic behaviour, the other isomers, though present in small amounts are most likely the 20-iso-22,24-trans, trans-26-homo compound and 22,24-cis, cis-26-homo compound and another that could be a 22,24-cis, trans or trans, cis-26-homo compound or a mixture of both.

The modified Wittig reaction of Corey is a convenient method and yielded the following previously unreported compounds in modest amounts after a sequence of reactions: 22-cis-cholesta-5,22-dien-3β-ol (VIa), 22-trans-cholesta-5,22,24-trien-3β-ol (IXb), and 22,24-trans, trans-26-homocholesta-5,22,24-trien-3β-ol (XII). Although all three of these compounds were unknowns as far as we knew, we recently isolated and identified 22-trans-cholesta-5,22,24-trien-3β-ol (IXb) from an insect source (16) as an intermediate in the conversion of stigmasterol to cholesterol. Additional studies with insects are being conducted with these compounds to obtain further insight into the mechanism of the conversion of the phytosterols to cholesterol.

The 22-trans-cholesta-5,22-dien-3β-ol (XIb) has been found in the German cockroach, Blatella germanica (L.) (17, 18) the Alaskan king crab, Paralithodes camtschatica (19) and in marine algae, Rhodophyta (20-23). Since compounds VIa, VIb, IXb, and XII could

be present in other invertebrates or vertebrates, we have included a brief discussion of the nuclear magnetic resonance (NMR) and mass spectral data of these compounds that should aid in future identification and structural elucidation of compounds containing similar side chains.

Parent Ion. All the sterols showed a respectable parent ion (Fig. 1-6). Indeed, under the conditions used, the parent ion for cholesterol was the base peak and was almost equal to the base peak for the trans-$\Delta^{5,22}$-sterol. However, introduction of a second double bond into the side chain at C-24 reduced the parent ion to 10-20% of base peak. Correspondingly, the M-15, M-18, and M-15-18 peaks were of relative abundance in both the cholesterol and $\Delta^{5,22}$-sterol spectra; these peaks were very small ($<1\%$) in the $\Delta^{5,22}$-sterol spectra.

Interestingly, the $\Delta^{5,22,24}$-sterol acetates exhibited parent ions that were approximately 5% of the base peak while the Δ^{5}- and $\Delta^{5,22}$-sterol acetate parent ions were almost non-existent.

Base Peak. As expected, the base peak for both the cholesterol acetate and the cholesta-5,22-dien-3β-acetates occurred at M-60. Unexpectedly, the cholesta-5,22,24-trien-3β-acetates exhibited a M-60 peak that was only 10% of the base peak that appeared at m/e 109, and at m/e 123 for the 26-homocholesta-5,22,24-trien-3β-acetate. These peaks at m/e 109 and m/e 123 were also base peaks for the $\Delta^{5,22,24}$-sterols; the 14-unit difference for the homosterol immediately defined them as the appropriate side chain fragments.

Figure 3

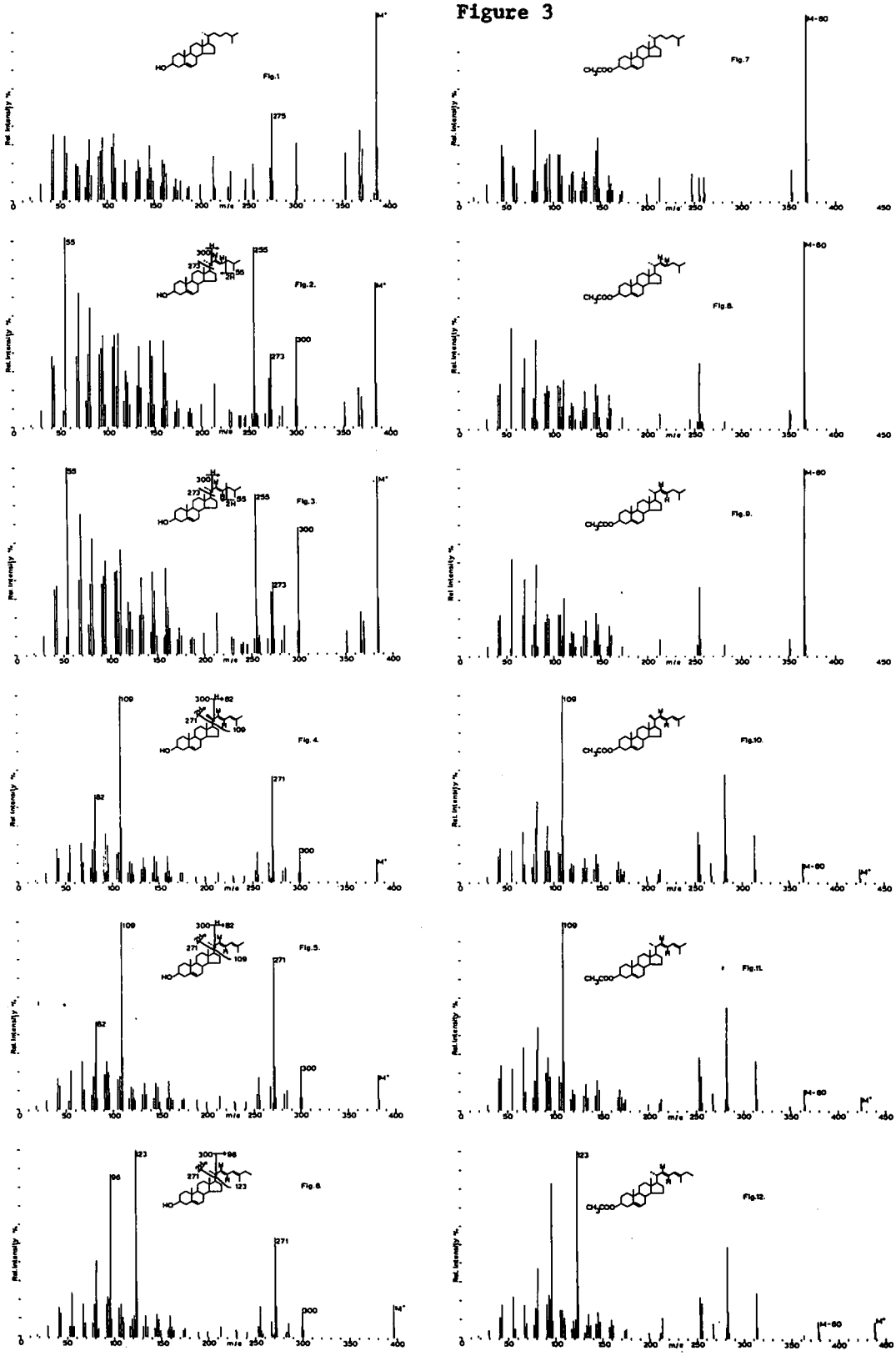
Fig.1
Fig.2.
Fig.3.
Fig.4.
Fig.5.
Fig.6.
Fig.7
Fig.8.
Fig.9.
Fig.10.
Fig.11.
Fig.12.
Rel. Intensity %
m/e
M+
M-60
M-82
M-96

The conjugated double bond system stabilized the side chain moiety as is obvious from the small peak at m/e 273, the remaining fragment. Both the cis- and trans-$\Delta^{5,22}$-sterols had their base peak at m/e 55. The large m/e 300 peak (48 and 68% of base peak for the cis and trans-sterol, respectively) was derived by fragmentation of the 20-22 bond accompanied by a transfer of hydrogen. Wyllie and Djerassi (24) observed in their mass spectra study of Δ^{22}-cholestene a strong peak at m/e 286 from which they projected an m/e 300 peak for a $\Delta^{5,22}$-sterol, and this projection was confirmed by our study. The m/e 55 peak may arise from a similar fragmentation on the other side of the Δ^{22}-bond, though without labelling studies, this suggestion can only be speculative. Cleavage of the 20-22 bond accompanied by hydrogen transfer also occurs in the $\Delta^{5,22,24}$-sterols and in homo-$\Delta^{5,22,24}$-sterol as attested by the large peaks at m/e 82 and m/e 96, respectively.

Peaks m/e 271 and m/e 273. As already stated, the $\Delta^{5,22,24}$-sterols and the homoanalog had a very small m/e 273 peak. At first, we attributed this to minor allylic cleavage of the 17-20 bond, but the base peaks at m/e 109 and m/e 123 for the respective side chain indicate that this is indeed the major point of cleavage. In these $\Delta^{5,22,24}$-sterols there appears to be a second competing type of fragmentation, also of the 17-20 bond, that is accompanied by a transfer of two hydrogens from the sterol nucleus, as exemplified by the peak at m/e 271 that was 81 and 52% of the base peak for the trans-and iso-isomers, respectively. Both types of cleavage

were also observed in the $\Delta^{5,22}$-sterols; however, the corresponding m/e 271 and m/e 273 ions were not as prominent as those observed in the $\Delta^{5,22,24}$-sterol spectra. While these peaks have been suggested for diagnostic purposes in the $\Delta^{5,22}$-sterols (24), this can certainly be extended with great reliability to the $\Delta^{5,22,24}$-sterols.

The C-methyl Signals in the NMR Spectra. The position of the C-18 and C-19 methyl groups has been shown to be highly sensitive to the stereochemistry of the steroid nucleus and to the nature and orientation of the functional groups. The compounds we examined differed only in the side chain, and, the major variations in the spectra occurred, as expected, with the C-21, and C-26 and C-27 methyl protons. Surprisingly though, the C-18 methyl protons were greatly influenced by the stereochemistry of the double bond at the C-22 position. In the cis-Δ^{22}-compound, the C-18 methyl appeared further downfield at 0.733 ppm than the trans-Δ^{22}-C-18 methyl (0.700 ppm), a discernable difference of only 2 cps. Nevertheless, a sample of the cis- compound that appeared to be pure by all other physical methods was in fact shown to contain about 5% of the trans-isomer by NMR. A prepared 1:1 mixture of the cis- and the trans-isomers showed a doublet indicating a similar concentration of the isomers. This difference of 2 cps for the C-18 methyl group appeared in both the free sterol and the acetate of the Δ^{22}-compounds. The cis- and trans-isomers of the $\Delta^{22,24}$-compounds also showed a difference of 2 cps for the C-18 methyl protons and the cis-C-18 methyl protons again appeared further downfield at 0.74 ppm. Oviously the

position of the C-18 methyl resonance can be used as a diagnostic tool to distinguish between the cis- and the trans-isomer at the C-22 position. However, the more important use is for the detection of small quantities of one isomer that are contaminating the other. Although the chemical shift of the C-18 methyl will not distinguish the cis-Δ^{22}- from the cis-$\Delta^{22,24}$-compound or the trans-Δ^{22}- from the trans-$\Delta^{22,24}$-compound, the far downfield shift for the C-26 and C-27 methyl signal at 1.74 ppm will readily indicate a double bond at the C-24 position. The position of the C-26 and C-27 methyl resonance was identical for all of the $\Delta^{5,22,24}$-isomers, including the C-27 methyl resonance for the homo compound. Although we, and others, have observed a doublet in the NMR spectra of the $\Delta^{5,24}$-cholestadienol (25, 26), our trans-$\Delta^{5,22,24}$-sterols gave only a single peak for the isopropylidene methyls but the cis-$\Delta^{5,22,24}$-sterol had an extra small peak at 1.81 ppm on the downfield side of the main peak at 1.74 ppm.

The 20-iso-$\Delta^{5,22,24}$-compound exhibited the typical singlet for the C-26 and C-27 methyl resonance at 1.74 ppm as the normal trans-$\Delta^{5,22,24}$-compound. The C-21 methyl resonance for this 20-iso-compound appeared at higher field (0.86 ppm), although both peaks of the expected doublet for the C-21 methyl signal could not be observed The chemical shifts of the C-18 and the C-19 methyls of the iso-compound were similar to those of cholesterol.

EXPERIMENTAL

All melting points were determined on a Kofler block. The optical rotations were determined in approximately 1% solutions in chloroform at 23° C. Infrared spectra were obtained in CS_2 with a Perkin-Elmer model 221 prism-grating double beam spectrophotometer, and the ultraviolet spectra of the acetates were taken in cyclohexane and that of the free sterols in methanol with a Bausch and Lomb spectrophotometer 505. Gas-liquid chromatographic analyses were made on Barber-Colman models 10 and 15. A radium sulfate ionization

source was used in the detector cell, and argon was the carrier gas. The inert support was prepared and coated according to the method of Horning et al. (27), and the phases used were SE-30 and QF-1. The compounds were shown to be of the correct molecular weight by mass spectrometry. The mass spectra were recorded on an LKB Model 9000 gas chromatograph-mass spectrometer (LKB Produkter AB, Stockholm). The compounds were introduced directly into the ion chamber; the ionization energy was 70 ev. NMR spectra were recorded at 60 Mc with a Varian A-60A NMR spectrometer by using deuterated chloroform as the solvent and TMS as an internal NMR standard.

Trans 3-methylpent-2-enyl bromide. To 7.0 g of 48% aqueous hydrogen bromide (2 M equiv.) stirred in an ice bath was added dropwise 2.0g of 3-hydroxy-2-methylpent-1-ene(28). After the addition was completed, stirring was continued at room temperature for 10 minutes. The solution was poured into a mixture of 10% aqueous sodium carbonate and cracked ice and extracted into ether. The ethereal solution was washed with water, dried over anhydrous sodium sulfate, and the ether was removed in vacuo to give the desired bromide.

3-Methylpent-2-enyltriphenylphosphonium Bromide, 3-Methylbut-2-enyltriphenylphosphonium Bromide and 3-Methylbutyltriphenylphosphonium Bromide. To 5.26 g of triphenylphosphine dissolved in 25 ml of dry benzene was added 3.0 g of trans 3-methylpent-2-enyl bromide. The reaction mixture was left overnight at room temperature. However, within one hour crystallization had commenced. The precipitate was collected, washed with ether, and then dried at 65° in vacuo overnight to give 5 g of 3-methylpent-2-enyltriphenylphosphonium bromide, m.p. 200-201°. A similar reaction of triphenylphosphine with 3-methylbut-3-enyl bromide yielded its triphenylphosphonium bromide m.p. 236-237°. By refluxing triphenylphosphine and 3-methylbutyl bromide in benzene overnight the 3-methylbutyltriphenylphosphonium bromide was obtained in 50% yield, m.p. 158-159°.

In each case, the phosphonium bromide was stored in a desiccator and used without any further recrystallization.

i-Stigmasterol methyl ether (II). i-Stigmasterol methyl ether was prepared according to the procedure of Nes and Steele (2).

i-Dinorcholan-22-al methyl ether (III). In a typical run, ozone prepared from predried oxygen was bubbled through a solution of 2.0 g of i-stigmasterol methyl ether in 75 ml of dry methylene chloride containing 1% pyridine, at -70° (3). The reaction was followed by the disappearance of the i-stigmasterol methyl ether peak and the emergence of a new peak for the aldehyde on an SE-30 column. (On a QF-1 column, the aldehyde and i-stigmasterol methyl ether have similar retention times.) At the end of the reaction, 3.0 g of zinc dust and 5 ml of glacial acetic acid were added, and the stirred mixture was quickly brought to room temperature. Stirring was continued for one hour and the mixture was filtered through a coarse sintered glass funnel. The filtrate was concentrated to small volume, diluted with water, and extracted with

hexane. The hexane solution was washed with saturated sodium bicarbonate solution, and dried over anhydrous sodium sulfate. Removal of solvent in vacuo gave 1.14 g of the oily aldehyde (III); νmax sharp bands at 3012, 3060; shoulder 3030 cm^{-1} (cyclopropane ring); medium band at 2690 and strong band at 1723 cm^{-1} (aldehyde).

22-cis-Cholesta-5,22-dien-3β-acetate (Va). Approximately 250 mg of sodium hydride (52% dispersion in mineral oil) was washed three times, by decantation, with dry ether and blown dry with nitrogen. To this was added 5 ml of freshly distilled dry dimethyl sulfoxide (DMSO). Then the mixture was heated with stirring under nitrogen at 75-80° (4). After 40-45 min, the evolution of hydrogen had ceased and the solution had become light green. This solution was chilled and 2.0 g of 3-methylbutyltriphenylphosphonium bromide in 10 ml of warm DMSO was added rapidly to produce a deep red solution. After 10 min at room temperature, 1.13 g of aldehyde (III) dissolved in 10 ml of dry tetrahydrofuran and 10 ml of dry DMSO was added. Stirring was continued overnight at room temperature under nitrogen. The solution was poured into 25 ml of water, extracted with hexane, and the hexane solution was dried over sodium sulfate and concentrated to dryness in vacuo. The residue was dissolved in a minimal amount of hexane and filtered through 20 g of activity grade II neutral alumina (Woelm). The 22-cis-i-methyl ether (IVa) was eluted in the first two 25 ml fractions of hexane to give 0.81 g of IVa, νmax sharp bands at 3012, 3060 shoulder 3030 cm^{-1} (cyclopropane ring), no carbonyl absorption. Its GLC showed a single component.

A mixture of 0.8 g of IVa, 1.5 g of freshly fused zinc acetate in 20 ml of acetic acid was refluxed with magnetic stirring for 3 hrs. The solution was cooled, diluted with water and extracted with hexane. The hexane solution was washed with saturated sodium bicarbonate solution, water, dried over anhydrous sodium sulfate, and concentrated to dryness in vacuo to give 1.01 g of crystalline residue. Recrystallization from ether-methanol gave the 22-cis-cholesta-5,22-dien-3β-acetate (Va), m.p. 115-116°, αD -68°; νmax 1730 cm^{-1} (acetate), three pronounced peaks between 710 and 750 cm^{-1} (expected region for a cis-oriented double bond). (See Fig. 2.), NMR, δ 0.73(18-H), 1.03(19-H), 0.95(21-H), 0.85, 0.91(26-and 27-H), 2.03($OCOCH_3$)

22-cis-Cholesta-5,22-dien-3β-ol (VIa). Saponification of 0.8 g of Va with 4% methanolic potassium hydroxide gave VIa. Crystallization from dilute acetone yielded 0.68 g of VIa, m.p. 137-139°, αD -65°, NMR, δ 0.73(18-H), 1.02(19-H), 0.95, 1.07(21-H), 0.85, 0.91(26- and 27-H)

22-trans-Cholesta-5,22-dien-3β-acetate (Vb). To a mechanically stirred suspension of 12 g of 3-methylbutyltriphenylphosphonium bromide in 150 ml dry hexane under nitrogen was added 25 ml of n-butyl lithium in hexane (22% dispersion). The mixture was brought to reflux, and a solution of 5.0 g of aldehyde (III) in 50 ml dry hexane was added and refluxed for one hr. After stirring under nitrogen for 18 hr, the solution was poured into ice water and worked up as in the preparation of Va to give 4.0 g of a mixture of i-methyl ether trans-isomer IVb (30%) and the cis-isomer IVa.

The mixture of i-methyl ethers was rearranged to the acetates Va and Vb by refluxing with 10 g of freshly fused zinc acetate and 50 ml of glacial acetic acid for 2 hr. The crude mixture of the cis and trans isomers (3.9 g) was chromatographed over 100 g of hexane-washed 20% silver nitrate-impregnated Unisil and the following fractions were collected: 1-10, 50 ml each of benzene-hexane (15:85); 11-26, 15 ml each of benzene-hexane (15:85) and then the column was stripped with 500 ml of benzene. Fractions 1-7 were discarded, fractions 8-26 contained mostly trans-isomer, and the 27th fraction contained mainly cis isomer.

Fractions 8-26 were combined and rechromatographed on a similar column and eluted with benzene-hexane (13:87). Fractions of 50 ml each were collected and monitored by TLC. Fractions 1-29 were discarded, fractions 30-71 contained mainly saturated side chain sterol impurities originally present in the stigmasterol, and fractions 72-104 contained the desired trans isomer (Vb). Fractions 105-126, which contained a mixture of trans and cis isomers, were combined with the benzene fraction from the first chromatography. A similar rechromatography of this predominately cis fraction gave fractions 1-10, discarded; 11-20, mainly impurities; 21-25, the trans isomer, 26-34, a mixture of trans and cis isomers; 35-39 (a benzene column stripping), the cis isomer.

Fractions 72-104 from the second chromatography and fractions 21-25 from the third chromatography were combined and recrystallized from ether-methanol to give 0.61 g of 22-trans-cholesta-5,22-dien-3β-acetate (Vb), m.p. 125-128°, αD -61° NMR, δ 0.70(18-H), 1.03(19-H), 0.95, 1.06(21-H), 0.81, 0.91(26- and 27-H), 2.03($OCOCH_3$) (Lit. (1), m.p. 126°; αD -63.2°).

Fractions 35-39 from the third chromatography on recrystallization from ether-methanol gave 1.44 g of 22-cis-cholesta-5,22-dien-3β-acetate (Va), m.p. 115-116°.

22-trans-Cholesta-5,22-dien-3β-ol (VIb). Saponification of 0.39 g of Vb by refluxing with 2% potassium carbonate in 10% aqueous methanol for 0.5 hr gave VIb. Crystallization from aqueous acetone yielded 0.27 g of VI b, m.p. 133-135°, αD -60° (Lit. (1), m.p. 133.5-134°; αD -57.3°). NMR, δ 0.70(18-H), 1.01(19-H), 0.95, 1.07 (21-H), 0.81, 0.91(26- and 27-H).

The Cholesta-5,22,24-trien-3β-acetates (VIIIa), (VIIIb) and (VIIIc). The reaction of 2.0 g of the aldehyde (III) with the ylid generated from 4.0 g of 3-methylbut-2-enyltriphenylphosphonium bromide as in the preparation of Va yielded 1.2 g of an oily mixture of i-methyl ethers (VII). The mixture VII was rearranged with zince acetate and acetic acid in the usual manner to give a mixture of the acetates (VIII). The compounds were effectively separated by a single column chromatography using the silver nitrate impregnated Unisil adsorbent as in the separation and purification of Vb. The eluant used was benzene-hexane (3:7), and fractions of 15 ml each were collected and monitored by GLC (SE-30). Fractions 1-50 contained the satu-

rated side chain impurities; 55-81 were predominantly VIIIc; 82-86 were a mixture of VIIIc and VIIIb; 87-115 were mainly VIIIb; 116-end (benzene stripping of the column) were a mixture of VIIIb and VIIIa.

Fractions 55-81 were combined and crystallized from ether-methanol to give 0.154 g of 20-iso-22-trans-cholesta-5,22,24-trien-3β-acetate (VIIIc), m.p. 124-127°, αD -4°, λ max 235 sh, 242, 248 sh mμ, ε26,500,29,000, 21,500, NMR, δ 0.67(18-H), 1.00(19-H), 0.88(21-H), 1.74(26- and 27-H), 2.03($OCOCH_3$).

Fractions 87-115 on crystallization from ether-methanol gave 0.21 g of 22-trans-cholesta-5,22,24-trien-3β-acetate (VIIIb), m.p. 133-135°, αD -62°, λ max 234 sh, 240, 247 sh mμ, ε28,000, 30,500, 22,500, NMR, δ 0.71(18-H), 1.03(19-H), 0.995, 1.10(21-H), 1.74 (26- and 27-H), 2.03($OCOCH_3$).

The 22-cis-cholesta-5,22,24-trien-3β-acetate (VIIIa) was obtained from fractions 116-end by preparative GLC. A recrystallization of the 25 mg sample from ether-methanol gave 10 mg of VIIIa of 87% purity m.p. 93-95°, λ max 235 sh, 241, 248 sh mμ, ε21,000, 22,000, 16,000. NMR, δ 0.74(18-H), 1.03(19-H), 0.995, 1.10(21-H), 1.74, 1.81(26- and 27-H), 2.03($OCOCH_3$).

The acetates VIIIc, VIIIb, and VIIIa were converted to the free sterols IXc, IXb, and IXa, respectively, by refluxing with 2% potassium carbonate in 10% aqueous methanol for 30 min.

20-iso-22-trans-Cholesta-5,22,24-trien-3β-ol (IXc). Crystallization from aqueous acetone gave fine needles, m.p. 145-146°, αD -4°, λ max 235 sh, 241, 248 sh mμ, ε25,000, 28,000,21,000, NMR, δ 0.67 (18-H), 0.99(19-H), 0.86(21-H), 1.74(26- and 27-H).

22-trans-Cholesta-5,22,24-trien-3β-ol (IXb) was obtained as thin plates from methanol m.p. 133-135°, αD -56°, λ max 234 sh, 240, 247 sh mμ, ε30,500, 32,500, 25,000. NMR, δ 0.71(18-H), 1.01(19-H), 1.10(21-H), 1.74(26- and 27-H).

22-cis-Cholesta-5,22,24-trien-3β-ol (IXa) was obtained as needles from dilute acetone, m.p. 115-118°. By GLC, this material was still contaminated by 10% of IXb.

22,24-trans,trans-26-Homocholesta-5,22,24-trien-3β-acetate (XI). The i-methyl ether isomers (X) were obtained when the Corey-modified Wittig procedure was used with 2.0 g of 3-methylpent-2-enyltriphyenylphosphonium bromide and 1.0 g of the aldehyde (III). The 0.74 g of product obtained after filtering through 20 g alumina was rearranged to the acetates. Analysis by GLC indicated a complex mixture. Repetitive chromatography on the same type of column as used previously separated 0.16 g of the major component. This material was crystallized from ether-methanol to give 0.13 g of 22,24-trans, trans-26-homocholesta-5,22,24-trien-3β-acetate (XI), m.p. 132-134°, αD -61°, λ max 235 sh, 242, 248 sh mμ, ε27,500, 30,000, 22,500, NMR, δ 0.71(18-H), 1.03(19-H), 0.995, 1.10(21.H), 1.74(26- and 27-H), 0.89(28-H), 2.03($OCOCH_3$).

The three other components representing about 40% were not satisfactorily separated.

22,24-trans,trans-26-Homocholesta-5,22,24-trien-3β-ol (XII). Saponification of 0.13 g of XI by refluxing with 2% potassium carbonate in 10% aqueous methanol for 30 min gave XII. Crystallization from aqueous acetone yielded 0.10 g of XII, m.p. 120-122°, αD -55°, λ max 235 sh, 241, 248 sh mμ, ε 30,000, 32,500, 24,500, NMR, δ 0.71(18-H), 1.01(19-H), 1.10(21-H), 1.74(26- and 27-H), 0.88(28-H).

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