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Shidasterone, an Insect Metamorphosing Substance from *Blechnum niponicum*: Structure[1]

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The structure of shidasterone, the phytoecdysone isolated from *Blechnum niponicum* (Blechnaceae) has been studied. In connection with this, stereoisomers of the cholestane-20,22-diol derivatives in regard to C-20 and C-22 have been synthesized and their chemical and spectral properties have been examined. Chemical and physico-chemical data and, in particular, the ^{13}C NMR spectrum of shidasterone have revealed that shidasterone is identified as 22,25-oxido-5β-cholest-7-en-6-one-2β,3β,14α,20-tetraol (**1**).

During the course of our systematic survey of Japanese ferns for phytoecdysones by means of bioassay, we found that the methanol extract of the whole plant of *Blechnum niponicum* Makino (Blechnaceae) shows intense insect moulting hormone activity.[3] Chromatography of the polar fraction of the extract revealed the plant to contain, besides ecdysterone and ponasterone A, a new active principle which was designated as shidasterone.[4] The present paper deals with the structure elucidation of the phytoecdysone.[5]

Shidasterone gives a positive color reaction for steroids. The analytical data for carbon and hydrogen approximated the figures for a composition $C_{27}H_{44}O_7$. In consistent with this finding, the mass spectrum exhibited a very weak peak at *m/e* 480 which was first erroneously considered to be corresponding to the molecular ion (M^+). From these observations, the molecular formula of shidasterone was once deduced to be $C_{27}H_{44}O_7$. The infrared (IR) spectrum are quite similar to the spectra of the common phytoecdysones such as ecdysterone (**3**), a strong band at 3430 cm^{-1} attributed to hydroxyls and a fairly strong band at 1643 cm^{-1} characteristic of a cyclohexenone being visible. The latter band together with an ultraviolet (UV) spectrum maximum at 244 nm and an ^1H nuclear magnetic resonance (NMR) signal (1H) at 6.21 ppm associated with an α-hydrogen on an enone system are indicative of the presence of a β,β-disubstituted α,β-unsaturated carbonyl moiety in the molecule. When shidasterone was heated in ethanol in the presence of hydrochloric acid, the resultant product showed UV maxima at 298 and 243 nm.[6] The combined evidence points to the 7-en-6-one system having a 14-hydroxyl group in a steroid skeleton. The ^1H NMR spectrum of shidasterone shows that it has five carbon terminals all of which are methyl groups (Table I). Although the splitting patterns of the five methyls which are all singlets are in accord with those in ecdysterone (**3**), their chemical shifts are not consistent with those of a series of the

1) This paper constitutes Part XXIV in the Tohoku University series on Steroids. Part XXIII: H. Jin, H. Hikino, and T. Takemoto, *Yakugaku Zasshi*, **95**, 596 (1975).
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3) H. Hikino, T. Okuyama, H. Jin, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **21**, 2292 (1973).
4) T. Takemoto, T. Okuyama, H. Jin, T. Arai, M. Kawahara, C. Konno, S. Nabetani, S. Arihara, Y. Hikino, and H. Hikino, *Chem. Pharm. Bull.* (Tokyo), **21**, 2336 (1973).
5) Part of the material contained herein has been reported in a preliminary communication: T. Takemoto, Y. Hikino, T. Okuyama, S. Arihara, and H. Hikino, *Tetrahedron Letters*, **1968**, 6095.
6) P. Karlson, H. Hoffmeister, H. Hummel, P. Hocks, and G. Spitellar, *Chem. Ber.*, **98**, 2394 (1965).

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Table I. ^{1}H NMR Signals of the Ecdysones (in C_5D_5N, ppm from TMS)

Substance	2-H	3-H	5-H	7-H	9-H	18-H	19-H	21-H	22-H	26-H 27-H
Shidasteone	~4.10	4.19	2.95	6.29	3.53	1.06	1.06	1.39	~4.06	1.22
	m	ddd	dd	d	ddd	s	s	s	dd	s
Ecdysterone	4.12	4.19	2.95	6.23	3.54	1.20	1.07	1.57	3.83	1.37
	m	ddd	dd	d	ddd	s	s	s	dd	s

known phytoecdysones. The spectrum also exhibits three carbinyl hydrogen signals. The mass spectrum of shidasterone discloses the fragmentation pattern similar to that of ecdysterone (**3**). In particular, the peaks at *m/e* 363, 345, and 327 are attributable to the nucleus fragments and those at *m/e* 99 (base peak) and 81 are ascribable to the side-chain fragments generated by the cleavage of the C-20: C-22 bond and the following dehydration without rearrangement which is predicted in a case where oxygen functions are attached to C-20 and C-22. This finding suggests that the nucleus and the side-chain of shidasterone resemble those of ecdysterone (**3**). Although the properties of shidasterone and ecdysterone are very similar to each other as described above, the significant difference between both the substances in their chemical properties is that shidasterone afforded the 2,3-diacetate (**2**) on treatment with acetic anhydride in pyridine at 5°, while ecdysterone formed the 2,3,22-triacetate under the same conditions. The following facts demonstrate the 2β,3β-dihydroxy-5β(*H*)-stereochemistry for shidasterone: 1) the chemical shifts and splitting patterns of two signals originating from two hydrogens on acetoxyl-carrying carbons of shidasterone diacetate (**2**) are in good agreement with those of the C-2 and C-3 carbinyl hydrogens of ecdysterone triacetate (Table II), 2) the line positions of the C-19 methyl hydrogen signals of shidasterone and its diacetate (**2**) are consistent with those of ecdysterone and its triacetate, respectively (Table I and II),

Table II. ^{1}H NMR Signals of the Ecdysone Acetates (in $CDCl_3$, ppm from TMS)

Substance	2-H	3-H	7-H	9-H	18-H	19-H	21-H	22-H	26-H	27-H
Shidasterone	5.05	5.32	5.86	3.10	0.80	1.00	1.23	3.86	1.16	1.21
2,3-diacetate	ddd	ddd	d	ddd	s	s	s	dd	s	s
Ecdysterone	5.04	5.31	5.85	3.10	0.85	1.02	1.24	4.79	1.18	1.21
2,3,22-triacetate	ddd	ddd	d	ddd	s	s	s	dd	s	s

and 3) the optical rotatory dispersion (ORD) and circular dichroism (CD) curves of shidasterone showing a positive Cotton effect centered at 337 nm (a +77, [θ] +3.2×10^3) for the n-π^* transition are almost overlapping on the curves of the A/B *cis* companions, *e.g.*, ecdysterone (**3**). On the other hand, the C-22 oxygen function, whose presence has been assumed from the mass spectral data, remained unchanged on the acetylation. In fact, the C-22 carbinyl hydrogen signal in the ^{1}H NMR spectrum of the diacetate (**2**) occurs at 3.87 ppm. The splitting pattern of the C-22 hydrogen signal, a doublet of doublets, indicates the adjacent (C-23) carbon to be a methylene. One more oxygen atom is involved in the side-chain portion, and it is deduced to be present at C-25 from the observation that in the ^{1}H NMR spectra the C-26 and C-27 methyl hydrogens appear as singlets at deshielded regions, 1.22 and 1.22 ppm for shidasterone and 1.16 and 1.21 ppm for the diacetate (**2**). All the carbon and hydrogen atoms in the side-chain have already been accommodated by the above spectral data except for a missing methylene group which consequently must connect the (C-23) methylene and the (C-25) oxygenated carbon bearing two tertiary methyls. From the accumulated data, it was once concluded that shidasterone might be a stereoisomer of ecdysterone in regard to C-20 and/or C-22 (**1'**).[5] Assuming it to be the case, the C-22 hydroxyl was concluded to be free but to show resistance to acetylation. Periodate oxidation and acetonide formation

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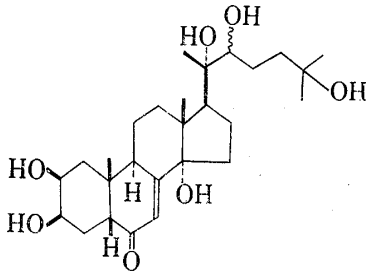
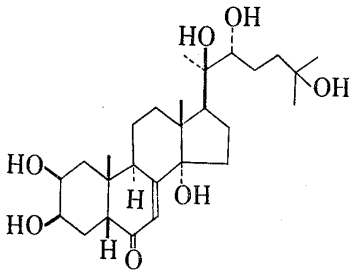
of the diacetate (**2**) were further attempted to recover the starting diacetate. Based on the above results, it was considered that the C-22 hydroxyl is sterically hindered for these reactions. The possibility that shidasterone is the 22-epimer of ecdysterone was excluded by the direct comparison with the synthetic 22-*epi*-ecdysterone (**4**), revealing both the substances to be different.



1′



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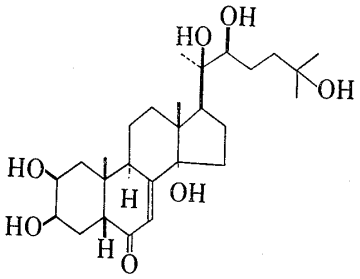

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Although the above physico-chemical data suggested that shidasterone might be one of the (20*S*)-5β-cholest-7-en-6-one-2β,3β,14α,20,22,25-hexaols, isomeric at C-20, or C-20 and C-22 with ecdysterone (**3**), further unambiguous evidence was required. The most direct proof must have been obtained by the synthesis of the two isomers. However, the problem now was whether there is any one among the stereoisomers of 20,22-dihydroxy steroids in which the C-22 hydroxyl group is hindered for acetylation and in which the 20,22-dihydroxyl group is resistant to periodate oxidation and acetonide formation. This prompted us first to carry out the synthesis of the model compounds, four all the possible isomers of 5α-cholestane-3β,20,22,25-tetraol, which were considered to be mach easier to be prepared.

Synthesis of ecdysterone has been independently achieved by three groups.[7-9] Therefore, one of the model compounds (20*R*,22*R*)-5α-cholestane-3β,20,22,25-tetraol and possibly its 22-epimer were expected to yield by application of the above mentioned synthesis. The procedure adopted here was that reported by Mori and Shibata,[9] because it is the most stereospecific. Thus, 5α-pregnan-20-on-3β-ol (**5**) was treated with vinylmagnesium bromide to afford (20*S*)-20-vinyl-20-hydroxy derivative (**6**) accompanied by a small amount of the (20*R*)-isomer (**7**). The introduction of a vinyl group into the product (**6**) was substantiated by the vinyl hydrogen signals appearing as an ABX pattern in the ^{1}H NMR spectrum. The addition reaction of 20-oxo-steroids (metal hydride reduction,[10] cyanohydrin formation,[11] or Grignard reaction[12]) is known to be highly stereospecific and the configuration of the introduced hydroxyl group has been proven to be β_F from chemical evidence, which is now integrated as Cram's rule. The present assignment of the configuration at C-20 was performed based on the high stereospecificity of the reaction in the light of Cram's rule. Ozonolysis of the main product (**6**) in tetrahydrofuran containing a trace amount of pyridine[13] afforded the hydroxyaldehyde (**8**) with a small amount of the further oxidized 20-oxo derivative (**5**) whose generation was indicated by means of thin-layer chromatography (TLC) and ^{1}H NMR spectroscopy. The structure of the aldehyde (**8**) was confirmed by the IR spectrum exhibiting bands at 2750 and 1700 cm^{-1} and by

7) G. Hüppi and J.B. Siddall, *J. Am. Chem. Soc.*, **89**, 6790 (1967).
8) U. Kerb, R. Wiechert, A. Furlenmeier, and A. Fürst, *Tetrahedron Letters*, **1968**, 4277.
9) H. Mori and K. Shibata, *Chem. Pharm. Bull.* (Tokyo), **17**, 1970 (1969).
10) *a*) N.G. Gaylord, "Reduction with Complex Metal Hydride," Interscience Publ. Inc., New York, 1956, p. 268; *b*) K. Heusler, P. Wieland, and C. Meystre, "Org. Synth." Vol. 45, 1965, p. 57.
11) N.K. Chaudhuri, R. Nickolson, H. Kimball, and M. Gut, *Steroids*, **15**, 525 (1970).
12) *a*) A. Mijares, D.I. Cargill, J.A. Glasel, and S. Lieberman, *J. Org. Chem.*, **32**, 810 (1967); *b*) N.K. Chaudhuri, J.G. Williams, R. Nickolson, and M. Gut, *J. Org. Chem.*, **34**, 3759 (1969).
13) G. Slomp, Jr. and J.L. Johnson, *J. Am. Chem. Soc.*, **80**, 915 (1958).

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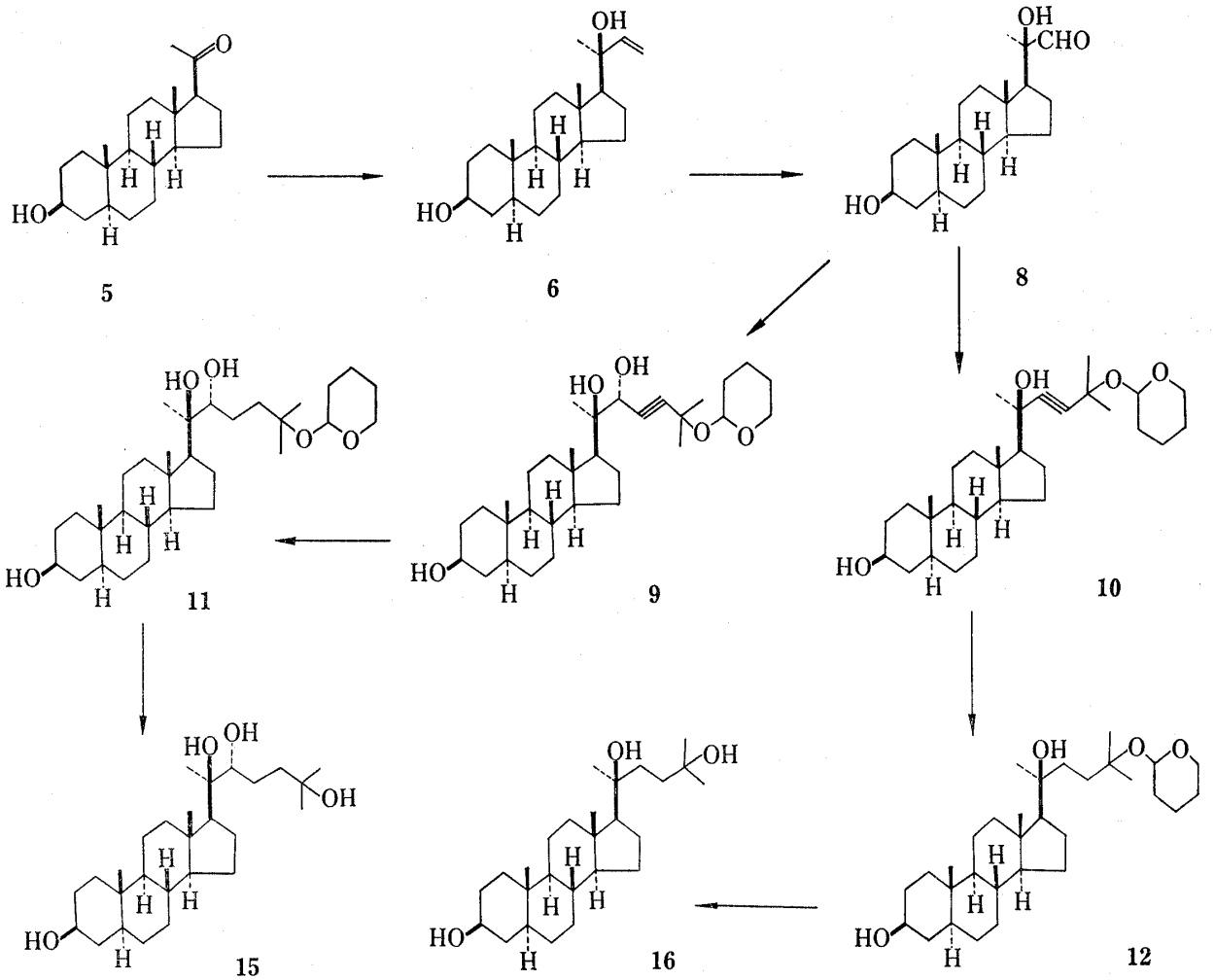
the ^1H NMR spectrum showing the aldehyde hydrogen signal at 9.63 ppm as a singlet. The aldehyde (**8**) was considerably unstable especially under basic condition to convert back to the 20-ketone (**5**). Grignard reaction of the aldehyde (**8**) with 3-methyl-3-(tetrahydropyran-2-yloxy)butynylmagnesium bromide led to a mixture of the acetylenic alcohols (**9** and **10**) which was also obtained by reaction of the aldehyde (**8**) with the corresponding lithium compound in an improved yield. Without purification, a mixture of the crude acetylenes (**9** and **10**) was hydrogenated over palladized carbon to give the hydrogenation product which on preparative TLC gave the crystalline tetraol ether (**11**) as the main product together with the oily triol ether (**12**) as the minor product. The ethers (**11** and **12**) were characterized as the 3,22-diacetate (**13**) and the 3-acetate (**14**), respectively. Removal of the tetrahydropyranyl group with hydrochloric acid[14] from the ethers (**11** and **12**) gave the tetraol (**15**) and the triol (**16**), respectively, which were further characterized as the corresponding 3,22-diacetate (**17**) and 3-acetate (**18**). That the tetraol (**15**) is identified as cholestane-3β,20,22,25-tetraol was



corroborated by the following spectral and chemical evidence. In the mass spectrum, the peaks at *m/e* 319, 301, 117, 99, and 81 are attributed to the fragments generated by the fission of the C-20: C-22 bond without rearrangement which is expected to a vicinal diol, and therefore are consistent with the presence of the 20,22-dihydroxyl group. When the tetraol (**15**) was made to react with acetone and acetone-d_6 in the presence of *p*-toluenesulfonic acid giving the 20,22-acetonide and the 20,22-acetonide-d_6 which were characterized as their 3-acetates

14) J.B. Siddall, A.D. Cross, and J.H. Fried, *J. Am. Chem. Soc.*, **88**, 862 (1966).

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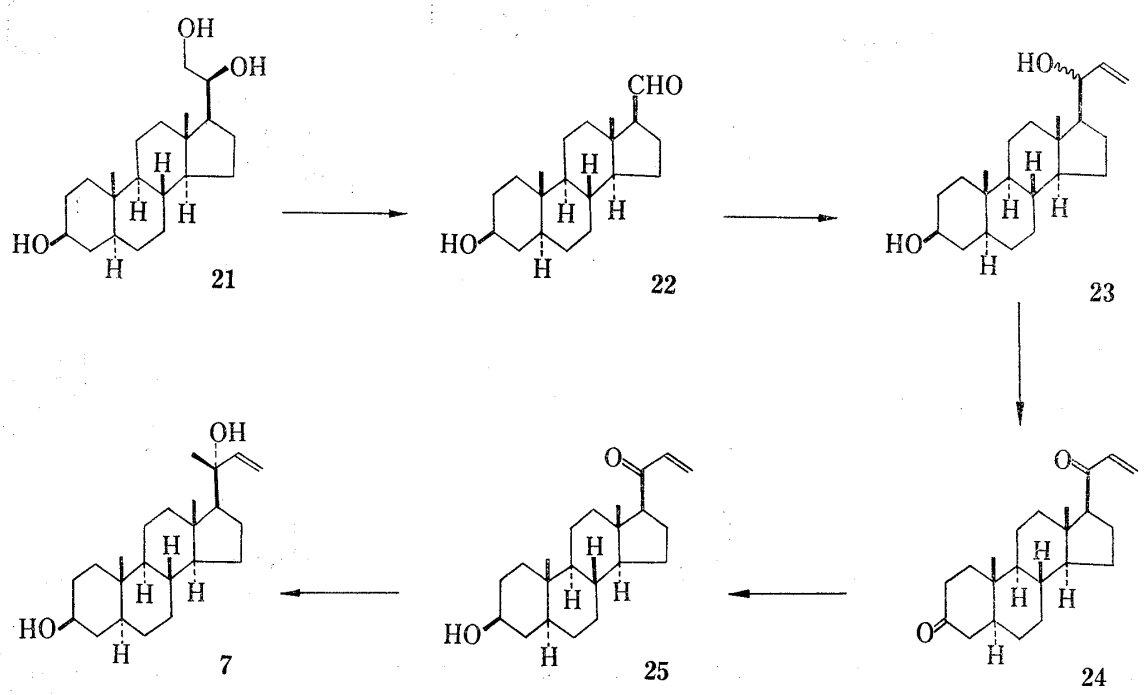
(**19** and **20**), respectively, while the tetraol (**15**) was treated with periodate to afford the 20-oxo compound (**5**). The structure of the minor product (**16**) was deduced from the results of elemental analysis, mass spectral analysis, ^{1}H NMR spectral analysis, acetylation yielding the monoacetate (**18**), and the synthesis of the triol ether (**12**) from pregnanolone (**5**) by a Grignard reaction followed by catalytic hydrogenation. The formation of the by-product (**10**) in the previous Grignard reaction may be rationalized by the reaction of the Grignard reagent with pregnanolone (**5**) which was spontaneously generated from the starting material, the hydroxy-aldehyde (**8**). Thus, the high stereospecificity of the Grignard reaction of the aldehyde (**8**) failed to give a pair of C-22 epimers but only one, contrary to expectation. The absolute configurations at C-20 and, in particular, at C-22 as *R*, *R* will be discussed later in relation to those of the deoxy analogs.

Synthesis of the (20*S*)-5α-cholestane-3β,20,22,25-tetraols might be completed by a similar procedure using the 20-epimeric diol (**7**) as the intermediate. This diol (**7**), however, was the minor product in the previous Grignard reaction and, consequently, was not obtained in a sufficient quantity for further reactions. Therefore, a better synthetic route for the diol (**7**) was sought. Oxidative cleavage of (20*R*)-5α-pregnane-3β,20,21-triol (**21**) with periodate yielded the aldehyde (**22**).[15] Grignard reaction of the aldehyde (**22**) with vinylmagnesium bromide gave a 1:1 mixture of the vinyl alcohols (**23**) epimeric with respect to C-20. It should be noted that the Grignard reaction of the aldehyde (**22**) was not so stereospecific in contrast to that of 20-oxopregnane derivatives. Since all attempts to convert the diols (**23**) into the ketol (**25**) by selective oxidation with manganese dioxide[16] and with dichlorodicyanoquinone,[17] and by mild Oppenauer oxidation[18] failed, the diols (**23**) were first converted by oxidation with Jones reagent into the dione (**24**) which then was partially reduced by sodium borohydride[19] to the ketol (**25**). Grignard reaction of the ketol (**25**) with methyl-



15) *a*) K. Miescher, F. Hunziker, and A. Wettstein, *Helv. Chim. Acta*, **23**, 400 (1940); *b*) *Idem*, *ibid.*, **23**, 1367 (1940).
16) F. Sondheimer, C. Amendolla, and G. Rosenkranz, *J. Am. Chem. Soc.*, **75**, 5930 (1953).
17) S.H. Burstein and H.J. Ringold, *J. Am. Chem. Soc.*, **86**, 4952 (1964).
18) K. Heusler, J. Kalvoda, P. Wieland, and A. Wettstein, *Helv. Chim. Acta*, **44**, 179 (1951).
19) H. Mori, K. Shibata, K. Tsuneda, and M. Sawai, *Chem. Pharm. Bull.* (Tokyo), **16**, 2416 (1968).

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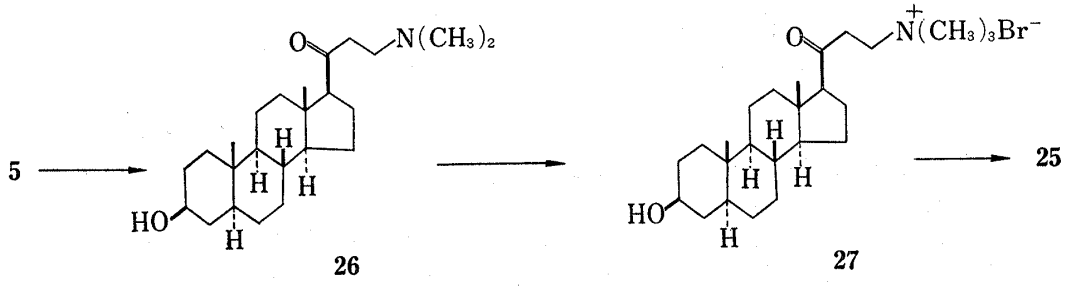
magnesium bromide furnished the desired intermediate (**7**) but in a poor yield probably on account of the 1,4-addition of the reagent to the α,β-unsaturated function. It was later found that the reaction of the ketone (**25**) with methyllithium proceeded in a good yield. Since the overall yield from the starting material (**21**) was not necessarily satisfactory, another synthetic procedure for the vinyl ketone (**25**), which is simpler and gives a better yield, was searched for and, as a result, a route involving a Mannich reaction was devised. Thus, pregnanolone (**5**) was transformed to the Mannich base (**26**) by the action of dimethylamine and paraformaldehyde in 1,2-dimethoxyethane[20] in an excellent yield, while only unsatisfactory results were obtained when diethylene glycol dimethyl ether (diglyme) or alcoholic solvent was used as the reaction solvent. Treatment of the dimethylamine (**26**) with methyl bromide in methanol gave the quaternary ammonium bromide (**27**) which on treatment with sodium bicarbonate[21] in water at room temperature afforded the desired ketone (**25**). The overall yield from the starting material (**5**) was much improved. Although the key intermediate, the methyl vinyl carbinol (**7**), was obtained from the ketone (**25**) by Grignard reaction with



methylmagnesium bromide, the yield was poor. However, the yield was found to be satisfactory when reacted with methyllithium. The configuration at C-20 of the vinyl alcohol (**7**) was deduced to be *R* according to Cram's rule and, in fact, the diol (**7**) thus obtained is the same as the (20*R*)-diol (**7**) but not identical with the (20*S*)-diol (**6**) prepared previously. Subsequent procedure essentially followed the route as in the synthesis of the corresponding tetraol (**15**) of the (20*R*)-series. Thus, ozonolysis of the vinyl derivative (**7**) gave the aldehyde (**28**), a characteristic singlet due to the isolated aldehyde hydrogen appearing at 9.58 ppm in the ^{1}H NMR spectrum. Grignard reaction of the aldehyde (**28**) with 3-methyl-3-(tetrahydropyran-2-yloxy)-2-butynylmagnesium bromide at room temperature afforded no desired compound but only pregnanolone (**5**). Formation of the 20-ketone (**5**) was partly observed in the previous Grignard reaction on the 20-isomeric aldehyde (**8**), suggesting that the conformation of the hydroxyaldehyde (**28**) at C-20 makes the aldehyde group more hindered than in the isomer (**8**). Reaction of the aldehyde (**28**) with 3-methyl-3-(tetrahydropyran-2-yloxy)-butynylmagnesium bromide at reflux temperature or with the corresponding lithium compound at room temperature afforded a mixture of the addition products (**29** and **10**). Catalytic hydrogenation of the mixture (**29** and **10**) over palladized charcoal yielded the saturated tetrahydropyranyl ether (**30**) along with the same by-product (**12**) obtained in the corresponding reaction in the (20*R*)-series. On acid hydrolysis, the ether (**30**) gave an isomer of the (20*S*)-5α-cholestane-3β,20,22,25-tetraols (**32**). The tetrahydropyranyl ether (**30**) and tetraol (**32**) were characterized as the 3,22-diacetates (**31** and **33**). The mass spectrum of the (20*S*)-tetraol (**32**) was found to be very similar to that of the (20*R*)-tetraol (**15**) as expected. Treatment of the tetraol (**32**) with acetone and acetone-d_6 in the presence of acid furnished the 20,22-acetonide and the 20,22-acetonide-d_6 which were characterized as the 3-acetates (**34** and **35**) and periodate oxidation of the tetraol (**32**) afforded pregnanolone (**5**). Discussion about the

20) S. Hirai, R.G. Harvey, and E.V. Jensen, *Tetrahedron Letters*, **1963**, 1123.
21) R.G. Berg, S.K. Frigdor, and G.D. Laubach, U.S. Patent 3033855 (1962).

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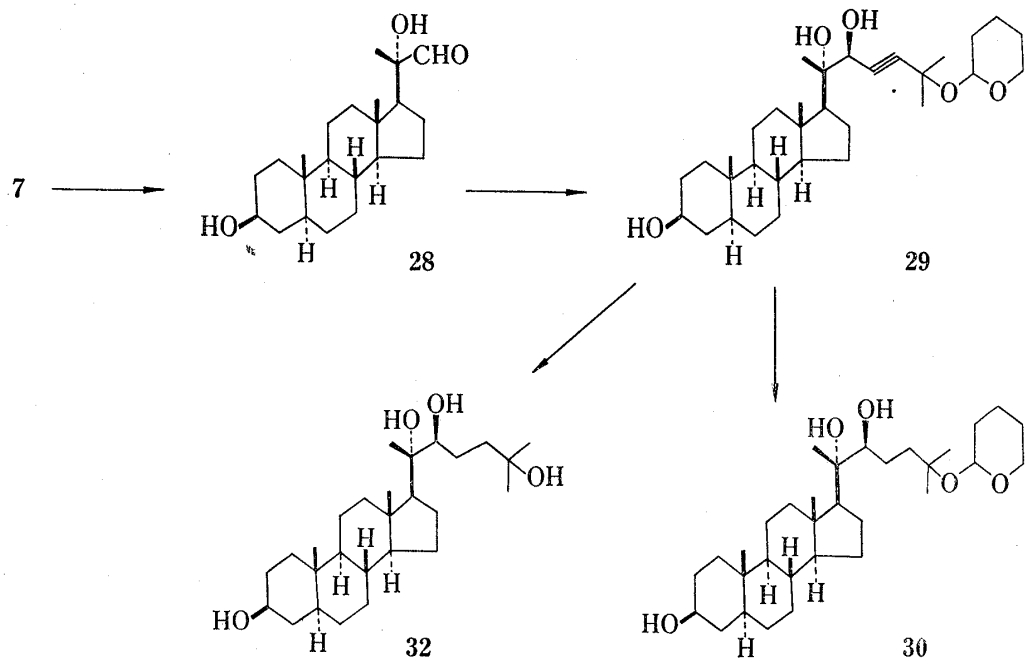

absolute configurations at C-20 and, in particular, at C-22 as *S*, *S* will be made below in connection with the deoxy congeners.

As descrived above, the addition reactions of the 20-formyl derivatives (**8** and **28**) proceed stereospecifically so that all the four required ((20*R*, 22*S*)-, (20*R*, 22*S*)-, (20*S*, 22*R*)-, and (20*S*, 22*S*)-) stereoisomers of cholestane-3β,20,22,25-tetraol could not be prepared but only two of them. Hence, the next endeavor was directed towards the synthesis of the stereoisomers with respect to C-20 and C-22 of cholestane-3β,20,22-triol having no hydroxyl at C-25, since this alteration might give the possibility to prepare all the stereoisomers.

Thus, the vinyl alcohol (**6**) after acetylation to the 3-acetate (**36**) was ozonized to yield the aldehyde (**37**) which on Grignard reaction with isoamylmagnesium bromide afforded a mixture of the (20*R*)-triols epimeric in regard to C-22. Chromatography of the mixture led to the isolation of the two triols (**38** and **39**) in the ratio 9:1. The structures of the triols (**38** and **39**) were substantiated by elemental analysis and analysis of the mass spectra (see experimental), the IR spectra (strong hydroxyl bands), and the ^{1}H and ^{13}C NMR spectra (two secondary methyls, three tertiary methyls, eleven methylenes, eight methines, three quaternary carbons, two secondary hydroxyls, and one tertiary hydroxyl (Table III and VI)), acetylation

Table III. ^{1}H NMR Singals of the Tetraols and the Triols (in C_5D_5N, ppm from TMS)

Substance	3-H	18-H	19-H	21-H	22-H	26-H 27-H
Cholestane-3β,20*R*,22*R*,25-tetraol (**15**)	3.80[a]	1.16	0.83	1.55	3.85[b]	1.47
Cholestane-3β,20*S*,22*S*,25-tetraol (**32**)	3.80[a]	1.12	0.82	1.38	4.12[b]	1.48
Cholestane-3β,20*R*,22*R*-triol (**38**)	3.80[a]	1.17	0.83	1.51	3.78[b]	0.94[c]
Cholestane-3β,20*R*,22*S*-triol (**39**)	3.80[a]	1.16	0.83	1.60	3.70[b]	0.90[c]
Cholestane-3β,20*S*,22*S*-triol (**46**)	3.80[a]	1.10	0.82	1.32	4.02[b]	0.96[c]
Cholestane-3β,20*S*,22*R*-triol (**47**)	3.80[a]	1.12	0.81	1.44	3.84[b]	0.89[b]

a) multiplet, *b*) doublet of doublets (*J* 4 and 9 Hz), *c*) doublet (*J* 6 Hz)

forming the 3,22-diacetates (**40** and **41**), formation of the 20,22-acetonides characterized as the 3-acetates (**42** and **43**), and periodate oxidation giving pregnanolone (**5**). The stereochemistry at C-22 will be discussed later.

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TABLE IV. ^{1}H NMR Signals of the Tetraol Diacetates and the Triol Diacetates (in $CDCl_3$, ppm from TMS)

Substance	3-H	18-H	19-H	21-H	22-H	26-H 27-H
3β,20*R*,22*R*,25-Tetraol 3,22-diacetate (**17**)	4.70[a]	0.84	0.81	1.25	4.80[b]	1.21
3β,20*S*,22*S*,25-Tetraol 3,22-diacetate (**33**)	4.70[a]	0.90	0.80	1.07	4.80[b]	1.20
3β,20*R*,22*R*-Triol 3,22-diacetate (**40**)	4.65[a]	0.85	0.82	1.23	4.77[b]	0.87[c]
3β,20*R*,22*S*-Triol 3,22-diacetate (**41**)	4.70[a]	0.82	0.82	1.27	4.78[b]	0.87[c]
3β,20*S*,22*S*-Triol 3,22-diacetate (**48**)	4.70[a]	0.91	0.82	1.06	5.17[b]	0.88[c]
3β,20*S*,22*R*-Triol 3,22-diacetate (**49**)	4.70[a]	0.85	0.82	1.17	4.71[b]	0.97[c]

a) multiplet, *b*) doublet of doublets (*J* 4 and 9 Hz), *c*) doublet (*J* 6 Hz)

TABLE V. ^{1}H NMR Signals of the Acetonides and the Hexadeuterioacetonides of the Tetraols and the Triols (in $CDCl_3$ and C_6D_6, ppm from TMS)

Subst. No.	$CDCl_3$							C_6D_6						
	3-H	18-H	19-H	21-H	22-H	26-H 27-H	$-O-$ $-O-$ >C(CH$_3$)$_2$	3-H	18-H	19-H	21-H	22-H	26-H 27-H	$-O-$ $-O-$ >C(CH$_3$)$_2$
19	4.65[a]	0.80	0.84	1.18	3.65[b]	1.25	1.30, 1.44	4.85[a]	0.69	0.98	1.26	3.78[b]	1.18	1.38, 1.50
34	4.65[a]	0.84	0.82	1.10	3.86[b]	1.24	1.34, 1.42	4.85[a]	0.72	0.90	1.16	3.90[b]	1.20	1.40, 1.47
42	4.65[a]	0.77	0.81	1.12	3.64[b]	0.88[c]	1.27, 1.38	4.85[a]	0.68	0.98	1.22	3.74[b]	0.88[c]	1.36, 1.45
43	4.60[a]	0·78	0.81	1.34	3.65[b]	0.90[c]	1.35, 1.45	4.82[a]	0.70	0.96	1.38	3.67[b]	0.90[c]	1.36, 1.51
50	4.65[a]	0.84	0.82	1.08	3.80[b]	0.90[c]	1.31, 1.39	4.80[a]	0.70	0.92	1.15	3.59[b]	0.90[c]	1.41, 1.50
51	4.65[a]	0.92	0.84	1.32	3.57[b]	0.86	1.32, 1.43	4.80[a]	0.70	0.93	1.32	3.61[b]	0.92[c]	1.36, 1.52
20	4.65[a]	0.79	0.81	1.15	3.64[b]	1.23		4.80[a]	0.70	0.96	1.25	3.75[b]	1.18	
35	4.65[a]	0.83	0.81	1.09	3.85[b]	1.24		4.80[a]	0.72	0.90	1.16	3.90[d]	1.19	
52	4.65[a]	0.78	0.82	1.11	3.64[d]	0.88[c]		4.85[a]	0.67	0.98	1.22	3.72[d]	0.92[c]	
53	4.65[a]	0.79	0.82	1.37	3.65[d]	0.90[c]		4.80[a]	0.69	0.99	1.40	3.70[d]	0.92[c]	
54	4.65[a]	0.85	0.82	1.08	3.80[d]	0.90[c]		4.80[a]	0.69	0.91	1.11	3.64[d]	0.95[c]	
55	4.65[a]	0.90	0.84	1.32	3.52[d]	0.86[c]		4.80	0.70	0.96	1.34	3.60[d]	0.95[c]	

a) multiplet, *b*) doublet of doublets (*J* 4 and 9 Hz), *c*) doublet (*J* 6 Hz), *d*) doublet of doublets (*J* 3 and 9 Hz)

Synthesis of the triols in the (20*S*)-series was performed in a similar manner as above. Thus, the epimeric vinyl alcohol (**7**) was first converted into the 3-acetate (**44**) which then on ozonolysis furnished the epimeric aldehyde (**45**). Grignard reaction of the aldehyde (**45**) with isoamylmagnesium bromide gave a mixture of the (20*S*)-triols epimeric with respect to C-22 which was subjected to repeated chromatography to separate the two triols (**46** and **47**) in the ratio 12: 1. Confirmation of the structures were carried out by the same spectral criteria as in their congeners (**38** and **39**). Further, acetylation gave the 3-acetates (**48** and **49**), treatment with acetone in the presence of acid afforded the 20,22-acetonides which were characterized as the 3-acetates (**50** and **51**), and periodate oxidation yielded pregnanolone (**5**). It may be worthy to note that the mass spectra of the (20*R*)-triols (**38** and **39**) show peaks at *m/e* 419 for M^+-1 but not those for M^+, while those of the (20*S*)-triols (**46** and **47**) exhibit peaks at *m/e* 420 for M^+.

As evident from the aforementioned results, the stereospecificity in the cases of the Grignard reactions of the aldehydes (**37** and **45**) with isoamylmagnesium bromide is not so high as compared with that with 3-methyl-3-(tetrahydropyran-2-yloxy)butynylmagnesium bromide, because the former reactions furnished a pair of 22-epimers in both (20*R*)- and (20*S*)-series, though the yields were still unbalanced.

After the completion of the synthesis of all the four stereoisomers of 5α-cholestane-3β,20,22-triol, the absolute configurations at C-20 and C-22 and the chemical and physico-chemical properties of these isomers were examined in connection with the relationship between the

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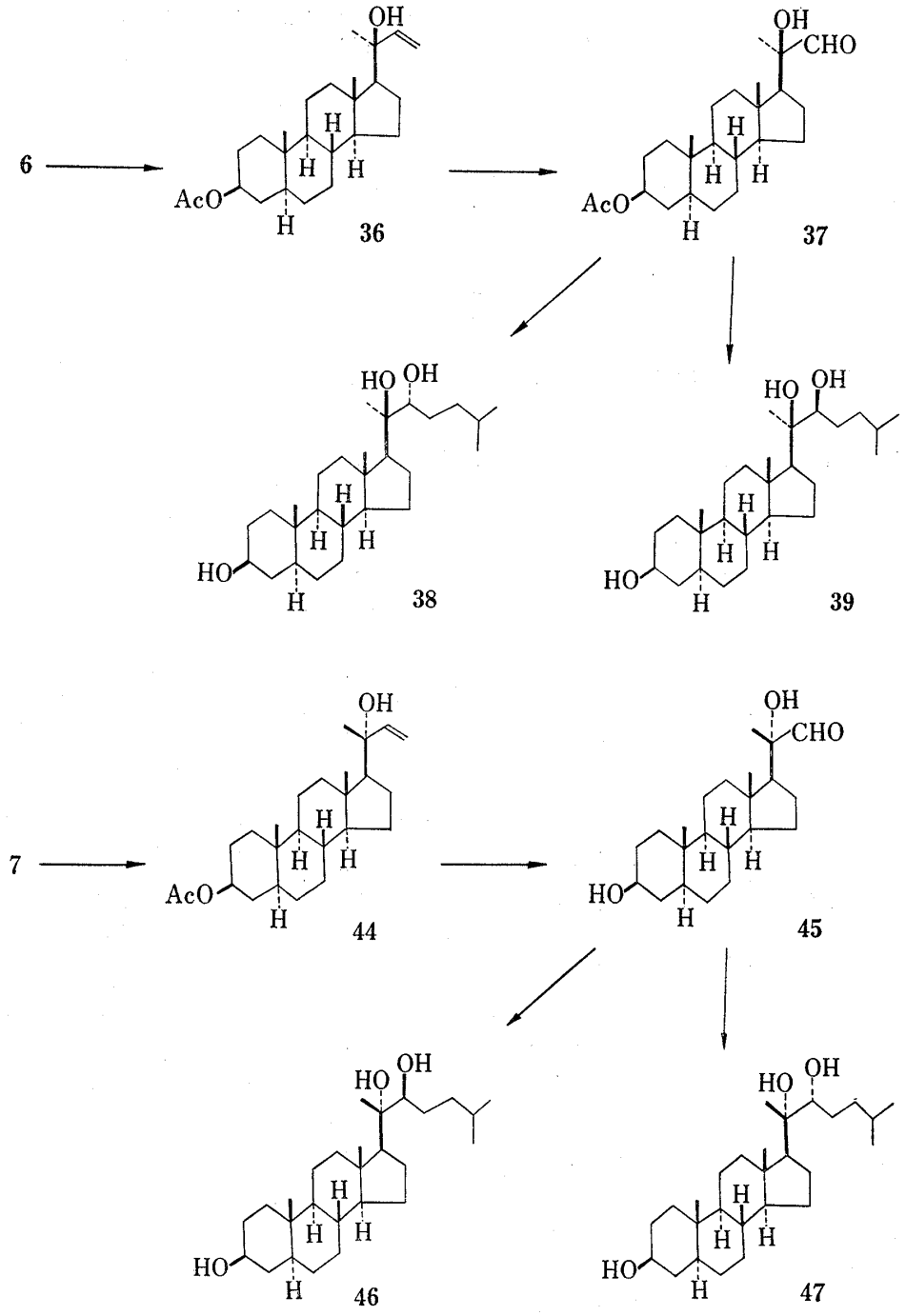

absolute configurations and the properties of the stereoisomers of ecdysterone. First the ^{1}H NMR spectra of the 20,22-acetonides were subjected to examination if there is the W-type long range coupling or the intramolecular nuclear Overhauser effect (NOE) between the C-21 methyl hydrogens and the C-22 hydrogen in each isomer.[22] Since the C-3 and C-22 hydrogen signals are somewhat overlapping in the spectra of the acetonides. the double resonance experiments were first tried on the spectra of the acetonide 3-acetates (**42**, **43**, **50**, and **51**) where the C-3 hydrogen signals show acetylation (lower-field) shifts to insulate the C-22 hydrogen signals in question. However, it was further found that a C-21 methyl hydrogen signal appears closely to two signals due to the methyls of the acetonide linkage origin. To simplify the situation, the triols were converted into the corresponding hexadeuterioacetonide 3-acetates

22) K. Nakanishi and J. Dillon *J. Am. Chem. Soc.*, 93, 4058 (1971).

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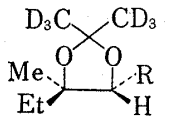
(**52**, **53**, **54**, and **55**). Saturation of the C-21 hydrogen signals of the two deuterioacetonides (**53** and **55**) caused significant increases (21 and 33%) in the integrated areas of the C-22 hydrogen signals, indicating a *cis*-relationship between the C-20 methyl and the C-22 hydrogen in each substance. Apparent enhancement (10%) in the areas of the C-22 hydrogen signals on double irradiation at the C-21 hydrogen signals in the spectra of the acetonides (**52** and **54**) was observed which must be due to the proximate occurrence of the signals arising from some other hydrogens located close to this hydrogen. Then, disappearance of the small 4σ couplings between the C-21 hydrogens and the C-22 hydrogens have to be found on double irradiation in the spectra of the two acetonides (**52** and **54**). However, this was not the case when repeated experiments were performed in chloroform-*d* and further in benzene-d_6 (Table V), though the reasons for this have been unknown. Since the 20*R*- and 20*S*-configurations have been previously deduced from Cram's rule for the triols (**38** and **39**) and the triols (**46** and **47**), respectively, the 22*R*- and 22*S*-configurations are consequently allotted to the triols (**38** and **47**) and the triols (**39** and **46**), respectively, the absolute configurations at C-20 and C-22 of the triols (**38**, **39**, **46**, and **47**) being assigned to *R*,*R*, *R*,*S*, *S*,*S*, and *S*,*R*, respectively. These assignments were further corroborated by the following evidence. 1) First, the triols were submitted to apply the method for determination of the absolute configurations of α-glycols by measuring the Cotton effect for the n-π^* region induced by the addition of tris(dipivaloylmethanato)praseodymium (Pr(dpm)$_3$).[23] As a result, it was found that the two *threo* triols (**38** and **46**) exhibited Cotton effects in the presence of Pr(dpm)$_3$ ($\Delta\varepsilon_{307}$—2.1 and $\Delta\varepsilon_{303}$+3.4, respectively) in the CD curves, corroborating the above conclusion. On the other hand, the two *erythro* triols (**39** and **47**) displayed no Cotton effects with Pr(dpm)$_3$, a fact which demonstrates that the formation of the Pr complexes is restricted in the latter cases probably due to some stereochemical factors such as steric hindrance and/or relative location of the hydroxyls. Information about the conformations (the relative situations of the two hydroxyls) at C-20 and C-22 in these triols has been obtained also from the reaction rates in periodate oxidation and the ^{13}C NMR spectroscopic properties of these triols (*vide infra*). 2) Nakanishi, *et al.*[24] found that in certain acetonides (such as **56**), the ^{1}H NMR signals of methyl groups located *cis* to the adjacent alkyl groups (*threo*) appear 0.10—0.15 ppm higher as compared to those located *trans* (*erythro*). Similar displacements were also observed in the present cases, the C-21 methyl hydrogen signals of the *threo* isomers (**52** and **54**) occurring at higher field by 0.26 and 0.24 ppm relative to those of the corresponding *erythro* isomers (**53** and **55**), respectively (Table V). 3) In extention of an NMR study by Mijares, *et al.*,[25] Middleton, *et al.*[26] reported that in either methanol-d_4 or pyridine-d_5, ^{1}H NMR signals for the C-21 methyl hydrogens of $20\alpha_F$-hydroxy sterols are at lower field than those of the corresponding $20\beta_F$-hydroxy epimers, $\Delta\delta$ being 0.07—0.14 ppm. The present comparison of the C-21 methyl hydrogen signals of two pairs of the epimeric 20,22-glycols (**38** and **47**, and **39** and **46**) revealed that although the values showed remarkable variation ($\Delta\delta$ 0.07 and 0.28 ppm in pyridine-d_5, respectively), analogous chemical shift differences were observable (Table III).



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After the establishment of the absolute configurations of the four triols (**38**, **39**, **46**, and **47**), their chemical properties were next examined. The first examined was the acetylation rates whose determination was one of the main purposes of the preparation of these triols. Each triol was made to react with acetic anhydride in pyridine-d_5 under the identical conditions, the reaction rate being determined by measuring the integrated areas of the ^{1}H NMR signals

23) J. Dillon and K. Nakanishi, *J. Am Chem. Soc.*, **96**, 4059 (1974).
24) K. Nakanishi, D.A. Schooley, M. Koreeda, and J. Dillon, *Chem. Commun.*, **1971**, 1235.
25) A. Mijares, D.I. Cargill, J.A. Glasel, and S. Lieberman, *J. Org. Chem.*, **32**, 810 (1967).
26) E.J. Middleton, D.H.S. Horn, and M.N. Galbraith, *Aust. J. Chem.*, **25**, 1245 (1972).

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attributed to the C-3 and C-22 hydrogens which changed in the spectrum of the reaction mixture as the acetylation proceeded. As a result, it was found that all the 3β-hydroxyls were acetylated naturally in the same rate and faster than any 22-hydroxyl. The 22-hydroxyls of the *threo* isomers (**38** and **46**) were acetylated approximately in the same rate and faster than those of the *erythro* isomers (**39** and **47**) (Fig. 1). The rate determination of periodate oxidation of

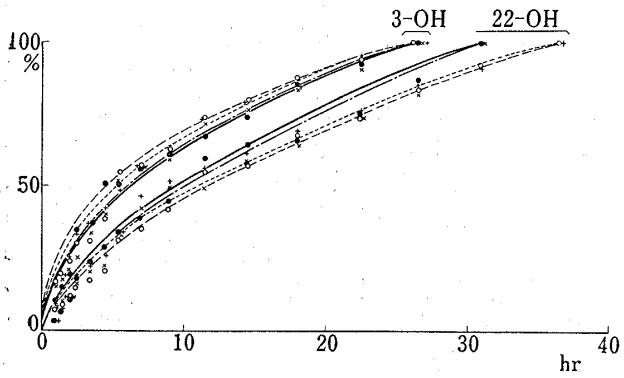
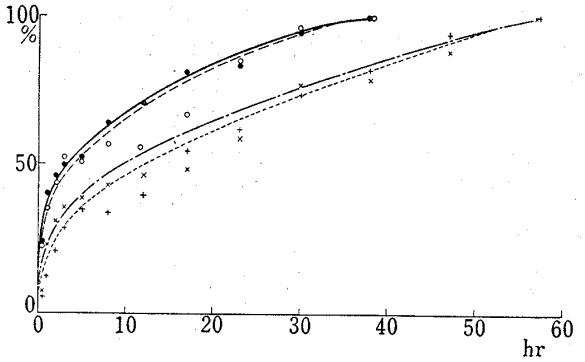


Fig. 1. Reaction Rate of Acrtylation of the Triols

Each triol (10 mg) treated with Ac_2O (0.03 ml) in C_5D_5N (0.3 ml) at 26°.
—●—: triol (**38**) —×—: triol (**46**)
—○—: triol (**39**) —+—: triol (**47**)



Fgi. 2. Reaction Rate of Periodate Oxidation of the Triols

Each triol (5 mg) treated with $NaIO_4$ (0.2 mg) in CD_3OD (0.3 ml), C_5D_5N (0.05 ml), and D_2O (0.02 ml) at 24°.
—●—: triol (**38**) —×—: triol (**46**)
—○—: triol (**39**) —+—: triol (**47**)

these four triols was the next objective. Each triol was treated with sodium periodate in a mixture of methanol-d_4, pyridine-d_5, and water-d_2, and the rate was determined by measuring the area of the ^{1}H NMR signal originating from the C-21 methyl hydrogens of pregnanolone (**5**) generated as the oxidation proceeded. The results demonstrate the C-20: C-22 bonds of the *threo* isomers (**38** and **46**) to be cleaved faster than those of the *erythro* isomers (**39** and **47**) (Fig. 2). This finding indicates that the periodate molecule is more restricted to join with the hydroxyls at C-20 and C-22 of the latter (**39** and **47**) in comparison with those of the former (**38** and **46**) due to some stereochemical factors such as steric hindrance and/or the dihedral angles of the hydroxyls, which is compatible with the previous observations in the CD study on Cotton effects with $Pr(dpm)_3$.

Inspection of the ^{1}H NMR spectra in pyridine-d_5 of the triols (Table III) revealed that the C-18 methyl hydrogen signals show little alteration while the C-21, C-26, and C-27 methyl hydrogen signals in the *threo* isomers (**38** and **46**) appear at higher-field, lower-field, and lower-field, respectively, than the corresponding signals in the *erythro* isomers (**39** and **47**). The ^{1}H NMR spectra in chloroform-*d* of the 3,22-diacetates (Table IV) exhibit the properties different from those of the free triols. This, the C-18 and C-21 methyl hydrogen signals in the *threo* isomers (**40** and **48**) occur in lower-field and higher-field regions, respectively, as compared with the corresponding signals in the *erythro* isomers (**41** and **49**) while the C-26 and C-27 methyl hydrogen signals show essentially no change. The C-22 carbinyl hydrogen signals disclose the characteristic variation. Accumulated data point to that all the four stereoisomers of the triol can be characterized by the combined spectral properties of the free triols and their diacetates, which will further be discussed later.

The ^{13}C NMR spectra of the triols (**38**, **39**, **46**, and **47**) were next examined. The ^{13}C spectrum of the (20*R*, 22*R*)-triol (**38**) has already been analyzed previously.[27] The present assignments of the spectra of the other triols were performed by the results of noise decoupling and off-resonance decoupling experiments coupled with the chemical shift considerations (Table VI). The results for the four triols clearly show the identity ($\Delta\delta \pm 0.3$ ppm) for the

27) H. Hikino, T. Okuyama, C. Konno, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **23**, 125 (1975).

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TABLE VI. Carbon-13 Shieldings in Shidasterone and Related Substances (in C_5D_5N, ppm from TMS)

Substance No.	58	38[27]	39	15	59	46	47	32	3[27]	1
C-1	37.3	37.5	37.5	37.5	37.1	37.6	37.5	37.5	37.9	37.7
C-2	28.3	32.3	32.4	32.3	28.1	32.4	32.4	32.4	68.0	67.9
C-3	74.1	70.5	70.5	70.5	73.9	70.6	70.6	70.6	68.0	67.9
C-4	38.5	39.2	39.2	39.2	38.2	39.3	39.2	39.3	32.2	32.2
C-5	140.0	45.2	45.2	45.2	139.8	45.3	45.2	45.2	51.2	51.2
C-6	122.9	29.1	29.3	29.1	122.5	29.3	29.2	29.3	203.4	203.5
C-7	32.2	32.2	32.4	32.3	31.9	32.4	32.4	32.4	121.5	121.5
C-8	31.6	35.1	35.1	35.1	31.4	35.3	35.2	35.1	166.0	166.0
C-9	50.3	54.6	54.7	54.6	50.1	54.7	54.8	54.5	34.3	34.2
C-10	36.8	35.7	35.8	35.7	36.6	35.8	35.8	35.8	38.5	38.5
C-11	22.5	21.5	21.6	21.5	22.2	21.6	21.5	21.5	20.4[a]	21.0
C-12	40.6	40.9	41.0	40.9	39.9	41.0	40.8	40.9	31.6	31.5
C-13	43.0	43.7	43.3	43.6	43.0	43.5	43.8	43.4	48.0	47.5
C-14	57.2	56.8	57.0	56.7	56.9	57.1	56.9	57.0	84.1	84.0
C-15	24.3	24.4	24.4	24.3	24.0	24.2	24.5	24.2	31.6	31.5
C-16	28.3	22.5	22.5	22.5	28.1	22.6	22.6	22.6	21.3[a]	21.5
C-17	58.7	55.6	55.1	55.5	58.7	55.8	55.0	55.8	50.5	51.2
C-18	14.0	14.1	14.1	14.1	13.7	14.3	14.5	14.3	17.6	17.8
C-19	19.4	12.5	12.5	12.5	19.2	12.6	12.5	12.5	24.3	24.3
C-20	74.1	76.5	76.5	76.6	74.5	77.5	77.3	77.6	76.8	80.3
C-21	21.2	21.1	21.9	21.2	21.0	23.8	24.2	23.7	21.0	21.0
C-22	45.2	76.5	78.3	77.1	43.3	76.2	77.9	76.3	77.4	84.8
C-23	22.8	30.1	29.3	30.5	23.1	30.5	29.9	30.2	29.8	27.6
C-24	40.2	37.1	37.5	42.5	39.9	37.0	37.1	42.4	42.4	38.8
C-25	26.9	28.2	28.6	69.5	27.4	28.8	28.5	69.6	69.6	75.4
C-26	22.9	22.5	22.8	27.1	22.6	23.0	22.6	27.8	27.3	28.2
C-27	22.9	23.2	23.1	29.7	22.6	23.0	23.2	29.9	29.8	28.7

a) The assignments of the asterisked signals are ambiguous and might have to be reversed.

shieldings of C-1—C-12, C-14—C-16, C-19, and C-27. Resonances which undergo significant chemical shift changes as a result of alteration in the configurations at C-20 and C-22 are those for C-20—C-23, among which the C-21 resonance is most sensitive to the stereochemical changes. As has been noticed before,[27] a comparison of the results for the triol (**38**) and ponasterone A (**57**) gives an excellent match for the signals of the side-chain carbons (C-20—C-27). These data further corroborated the (20*R*,22*R*)-configuration for the triol (**38**) because if it were not the case the ^{13}C chemical shifts of C-20—C-23 in the spectrum of the triol would be expected to deviate from ponasterone A (**57**) whose (20*R*, 22*R*)-feature has been established.

We have previously found that in the ^{13}C NMR spectra of 1,2-glycols the observed chemical shifts of the carbinyl carbons are in good agreement with the predicted values, calculated using the shieldings of a carbinyl carbon in a monool as the reference and the additive substituent parameter for an additional hydroxyl, when the two hydroxyls are situated in a far (antiparallel) relationship, while the observed values are deviated upfield from the predicted values when the two hydroxyls are located in a near (*gauche*) relationship.[28] This rule is now applied to the C-20 and C-22 NMR signals of the triols (**38**, **39**, **46**, and **47**) if some valuable information about the conformations at C-20 and C-22 of the triols will be obtained. The C-22 resonances of (20*S*)- and (20*R*)-20-hydroxycholesterol acetates (**58** and **59**),[25,29] reference substances for the (20*R*)- and (20*S*)-triols (**38** and **39**, and **46** and **47**), appear at 45.2 and 43.3 ppm, respectively. Introduction of a hydroxyl at C-22 causes a downfield shift of the C-22

28) C. Konno and H. Hikino, to be published.
29) N.K. Chaudhuri, J.G. Williams, R. Nikolson, and M. Gut, *J. Org. Chem.*, **34**, 3759 (1969).

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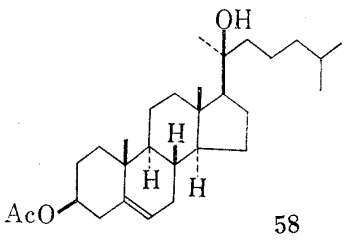

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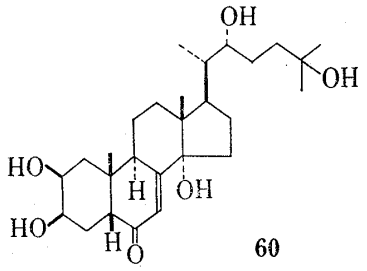

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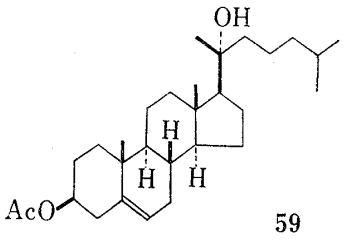

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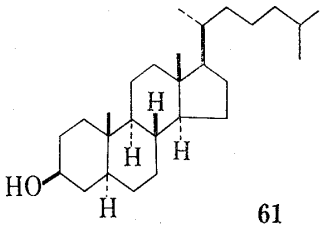

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resonance. In fact, the C-22 resonances of the (20*R*)-triols (**38** and **39**) and of the (20*S*)-triols (**46** and **47**) occur at 76.5 and 78.3, and 76.2 and 77.9 ppm, respectively, those of the *threo* isomers (**38** and **46**) being present at higher field than those of the corresponding *erythro* isomers (**39** and **47**). This finding indicates that the C-22 resonances of the *threo* isomers (**38** and **46**) are more deviated towards upper-field than those of the *erythro* isomers (**39** and **47**) from the hypothetical values for the C-22 where the two hydroxyls are not interacted, which, in the light of the previously found rule,[28] shows the two hydroxyls at C-20 and C-22 in the formes (**38** and **46**) to be located in closer relations than those in the latters (**39** and **47**). This conclusion is considered as being consistent with the previous observations in the CD study on the Cotton effects with $Pr(dpm)_3$ and in the periodate oxidation of the triols. Although the C-20 resonances of each pair of the triols (**38** and **39**, or **46** and **47**) appear at the same position (76.5 and 76.5, or 77.5 and 77.3 ppm, respectively), no conclusion cannot be drawn from these data since the reference substrances, the stereoisomers of cholestan-22-ol in regard to C-20 and C-22, are not available. It may be worthy to note that there is a trend to smaller substituent effects upon increasing substitution at a carbinyl carbon in an alcohol[30] and an ether,[28] which is confirmed also in the present case. Thus, the signal for C-20 of ecdysone (**60**) appears at 42.3 ppm[27] which on addition of the substituent effect of the C-20 hydroxyl derived from the difference (37.0 ppm) between the C-20 resonances of (20*S*)-20-hydroxycholesterol acetate (**58**) and cholestan-3β-ol (**61**) (74.1 (Table VI) and 36.1 ppm,[27] respectively) affords a calculated value of 80.8 ppm. In fact, the resonance for C-20 of ecdysterone (**3**) is observed at 76.8 ppm which shows a deviation of 3.5 ppm from the above calculated value. While, the C-22 resonance of (20*S*)-20-hydroxycholesterol acetate (**58**) occurs at 45.2 ppm which on addition of the substituent effect of the C-22 hydroxyl deduced from the difference (37.3 ppm) between the C-22 resonances of ecdysone (**60**) and cholestanol (**61**) (73.8 and 36.5 ppm,[27] respectively) gives a calculated value of 82.5 ppm. In reality, C-22 of ecdysterone (**3**) resonates at 77.4 ppm, showing a deviation of 5.1 ppm. Since the above calculation has been done on the same glycol, ecdysterone (**3**), the difference between both the deviation values (3.5 ppm for the quaternary C-20 and 5.1 ppm for the tertiary C-22) demonstrates that substitution at a carbinyl carbon reduces the deviation.

Assignments of the absolute configurations at C-20 and, in particular, at C-22 of the tetroals (**15** and **32**), which have remained unsettled, must be mentioned here. The absolute configurations for C-20 in the tetraols have been above deduced on the basis of Cram's rule.

30) J.D. Roberts, F.J. Weigert, J.I. Kroschwitz, and H.J. Reich, *J. Am. Chem. Soc.*, **92**, 1338 (1970).

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When taken into account the facts that a Grignard reaction of the aldehyde (**8** or **28**) afforded only one of the C-22 epimers (**15** or **32**) and a Grignard reaction of the aldehyde (**37** or **45**) gave a pair of the C-22 epimers (**38** and **39**, or **46** and **47**) in a ratio of 9:1 or 12:1, the tetraols (**15** and **32**) are suggested to have the same 20*R*,22*R*- and 20*S*,22*S*-configurations as the main products obtained in the latter reactions. In support of this supposition, the ^{1}H NMR parameters of the tetraols (**15** and **32**), their 3,22-diacetates (**17** and **33**), their 20,22-acetonide 3-acetates (**19** and **34**), and their 20,22-hexadeuterioacetonide 3-acetates (**20** and **35**) are in accord with the corresponding ones of the triols (**38** and **46**) and their derivatives (**40** and **48**, **42** and **50**, and **52** and **54**), respectively, except for the C-22 hydrogen signals of the diacetates, the acetonide acetates, and the hexadeuterio acetonide acetates, the reasons for these discrepancies, however, being unknown (Table III, IV, and V). The ^{13}C NMR spectra of the tetraols (**15** and **32**) were also analyzed comparing with those of the triols (**38**, **39**, **46**, and **47**) and considering the resonance changes on passing from ponasterone A (**57**) to ecdysterone (**3**) (Table VI). The remarkable coincidence of the resonances for the side-chain carbons C-20 C-22 in the spectra of the tetraol (**15**)and ecdysterone (**3**) demonsterates the identity of the absolute configurations (*R*, *R*) at C-20 and C-22 in both the substances. The C-21 and C-22-resonances of the tetraols (**15** and **32**) were found to be in accord with those of the triols (**38** and **46**), respectively, also indicating the two pairs of polyols possess the same absolute configurations at C-20 and C-22, respectively. Combined evidence has established the absolute configurations at C-20 and C-22 of the tetraols (**15** and **32**) as *R*,*R* and *S*,*S*, respectively. Here, it was considered that the method for determination of the absolute configurations of 1,2-glycols by measuring the Cotton effects of the glycols with $Pr(dpm)_3$[23] above applied would be useful also in these cases. The results obtained for the tetraols (**15** and **32**) ($\Delta\varepsilon_{314}$+1.1 and $\Delta\varepsilon_{314}$−1.3, respectively), however, are not consistent with those expected and opposite to those obtained with the corresponding deoxy companions (**38** and **46**). The reversal in signs suggests the formation of complexes involving the 1,4-glycol systems preferentially to those of the 1,2-glycols presumably because the 20,22-glycol moiety is more sterically hindered and the two hydroxyls at C-22 and C-25 may be allowed to approach closer by rotation of the side-chain.

As has been clarified from the above observations, in all the four stereoisomers of cholestane-3β,20,22-triol with respect to C-20 and C-22, the 22-hydroxyl groups are acetylated, and the 20,22-dihydroxyl systems form the acetonide linkages and are slit by periodate oxidation, though the reaction rates vary depending upon the configurations at C-20 and C-22. These chemical properties are clearly different from those previously presumed for shidasterone. At this point, the possibility that shidasterone may be a 22-epimer of (20*S*)-5β-cholest-7-en-6-one-2β,3β,14α,20,22,25-hexaol is excluded, and another structure has to be allotted to the phytoecdysone.

Previously we performed assignments of the resonances in the ^{13}C NMR spectra of some phytoecdysones and indicated that this technique will provide useful information in the structural analysis of potential congeners.[27] Thus, the ^{13}C NMR spectrum of shidasterone is now submitted to analysis as compared with that of a representative of the ecdysones, ecdysterone (**3**). Comparison of the ^{13}C chemical shifts of shidasterone and ecdysterone (Table VI) shows that the resonances for the nucleus carbons in shidasterone are invariant from those in ecdysterone, while those for the side-chain carbons in the former undergo various changes on going to the latter. Among them, those most significantly shifted are C-22 and C-25 which move downfield 7.4 and 5.8 ppm, respectively, from ecdysterone. These shifts are those expected as the two hydroxyls on the two carbons in question in ecdysterone are dehydrated to form an ether linkage.[31] If this were the case, *i.e.*, shidasterone were 22,25-oxido-5β-cholest-7-en-6-one-2β,3β,20-tetraol, its molecular formula must be revised to $C_{27}H_{42}O_6$ from $C_{27}H_{44}O_7$ as previously postulated.[5] Then shidasterone was subjected to careful re-

31) *cf.*, ref. 28.

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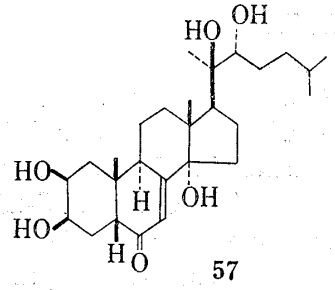
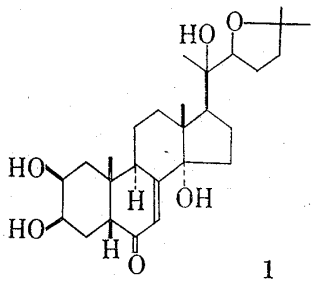
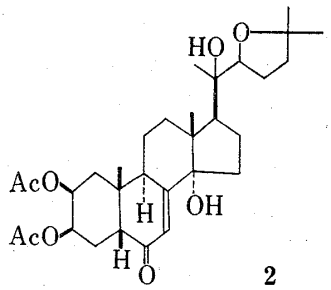
examination, revealing that the mass spectrum exhibited no peak at *m/e* 480 but a M^+ peak at *m/e* 462 (0.2% relative to the base peak at *m/e* 99), though even after prolonged drying of the sample at high temperatures *in vacuo* the analytical data for carbon and hydrogen remained the figures for the composition $C_{27}H_{44}O_7$ which must now be construed as $C_{27}H_{42}O_6 \cdot H_2O$. The previons obseruation of the peak at *m/e* 480 in the mass speetrum is judged to be a technical mistake. Now, shidasterone may possibly be identical with stachysterone D later isolated from *Stachyurus praecox* SIEBOLD et ZUCCARINI (Stachyuraceae)[32] or one of its stereoisomers. Direct comparison of both the phytoecdysones confirmed the identity. Hence, shidasterone has been identified as 22,25-oxido-5β-cholest-7-en-6-one-2β,3β,14α,20-tetraol (**1**).



57



1



2

The reasons of the previous misunderstanding of the structure are that 1) there was the aforementioned mistake in the mass spectral measurements. Further, crystals of shidasterone firmly retain one molecule of water of crystallization. These led us to the erroneous conclusion about its molecular formula. 2) In the behaviors on TLC shidasterone is more polar than ponasterone A (**57**), which convinced us of a trihydroxy feature of the side-chain. 3) Shidasterone shows the moulting hormone activity in the *Sarcophaga* test, which has reminded us of the free 20,22-dihydroxy moiety in shidasterone, and in fact stachysterone D exhibits an extremely weak or occasionally non-observable activity in the *Chilo* test.[32]

In 1966—1967, several zooecdysones and phytoecdysones possessing similar properties were isolated from various sources but given different names, *i.e.*, β-ecdysone, crustecdysone, 20-hydroxyecdysone, ecdysterone, the same structure being proposed for the last three.[33] Although comparison of β-ecdysone, 20-hydroxyecdysone, ecdysterone, and crustecdysone of plant origin proved their identity, no persuasive evidence for the identity of crustecdysone of animal origin was presented.[33] The main evidence for the identification of the crustecdysone of arthropod origin with the other congeners was originating from its ^1H NMR spectrum which is in agreement with those of the other 20-hydroxy-ecdysones. To be exact, however, the possibility that the crustecdysone of animal origin may possibly be one of the stereoisomers of ecdysterone (**3**) cannot be eliminated until the spectral properties of the stereoisomers have been fully clarified. In relation to the structure study of shidasterone, all the four stereoisomers of a 20,22-dihydroxy steroid have been prepared above and their ^1H NMR spectra give support to the identity of the crustecdysone in question with the other 20-hydroxyecdysones.

Experimental[34]

Isolation of Shidasterone——The dried whole plants of *Blechnum niponicum* MAKINO (Blechnaceae) (67 kg) were extracted 3 times with refluxing MeOH (25 liters. each) for 7 hr (each extraction). The combined

32) S. Imai, E. Murata, S. Fujioka, and T. Matsuoka, *Chem. Commun.*, **1970**, 352.

33) *cf.*, H. Hikino and Y. Hikino, "Fortschritte d. Chem. Org. Naturst," Vol. 28, ed. by W. Herz, H. Grisebach, and A.I. Scott, Springer-Verlag, Wien, 1970, pp. 256—312.

34) Mps are uncorrected. The ^1H NMR spectra were determined in $CDCl_3$ at 60 MHz unless otherwise specified. The ^{13}C NMR spectra were recorded in C_5D_5N at 25 MHz as previously described.[27] Chemical shifts are given in ppm downfield from internal TMS and coupling constants (J) expressed in Hz. Abbreviations: s=singlet, d=doublet, m=multiplet, and dd=doublet of doublets.

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MeOH solution was concentrated to yield an extract (8.0 kg) which on extraction with AcOEt and evaporation gave a residue (0.98 kg). The residue (0.98 kg) was chromatographed over neutral alumina (1.2 kg), and the fraction eluted with AcOEt: MeOH (10: 1) was further subjected to chromatography on silica gel twice. Fractions containing a substance less polar than ecdysterone but more polar than ponasterone A were combined and crystallized from MeOH–AcOEt to furnish shidasterone (**1**) as colorless leaflets (25 mg). mp 257—258°. *Anal.* Calcd. for $C_{27}H_{42}O_6 \cdot H_2O$: C, 67.47; H, 9.23. Found: C, 67.85; H, 9.21. ORD (*c* 0.118, dioxane): $[\phi]_{358}^{peak}$ +3670, $[\phi]_{307}^{trough}$ −4060; CD (*c* 0.118, dioxane): $[\theta]_{337}$ $+3.2\times10^3$. Mass Spectrum *m/e*: 462 (M^+), 363 (M^+-99), 345 ($M^+-99-18$), 99 (M^+-363, base), 81 ($M^+-363-18$). UV λ_{max}^{EtOH} nm (ε): 244 (9110); IR ν_{max}^{KBr} cm^{-1}: 3430 (hydroxyl), 1643 (cyclohexenone); ^1H NMR (C_5D_5N, 100 MHz): shown in Table I; ^{13}C NMR: shown in Table VI. Liebermann-Burchard reaction: positive (pale blue).

Acid Treatment of Shidasterone——Shidasterone (**1**) (0.26 mg) in 1% ethanolic HCl (2 ml) was heated under reflux for 1 hr to give the mixture of the 8,14-dien-6-one and the 7,14-dien-6-one. UV $\lambda_{max}^{EtOH(HCl)}$ nm; 243, 298.

Acetylation of Shidasterone——Shidasterone (**1**) (20 mg) was treated with Ac_2O (0.5 ml) in pyridine (1 ml) at 5° for 2 days. After working up in the customary manner, the product was crystallized from MeOH–AcOEt to give shidasterone 2,3-diacetate (**2**) as colorless needles (19 mg). mp 188—189°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (hydroxyl), 1745, 1235 (acetoxyl), 1665 (cyclohexenone); ^1H NMR (100 MHz): shown in Table II.

Attempted Periodate Oxidation of Shidasterone Diacetate——Shidasterone diacetate (**2**) (7.7 mg) in MeOH (0.5 ml) was treated with $NaIO_4$ (10 mg) in water (0.5 ml) under stirring at 25° overnight and the mixture was heated under reflux for 1 hr. The mixture was worked up in the customary way to give the product. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (hydroxyl), 1745, 1235 (acetoxyl), 1665 (cyclohexenone). The identity with the starting material (**2**) was confirmed by comparisoin of TLC and IR spectra.

Attempted Acetonide Formation of Shidasterone Diacetate——Shidasterone diacetate (**2**) (5 mg) in acetone (4 ml) containing *p*-TsOH (5 mg) was stirred at room temperature overnight. The mixture was worked up as usual to yield the product. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (hydroxyl), 1745, 1235 (acetoxyl), 1665 (cyclohexenone). Identification with the starting material (**2**) was carried out by comparison of TLC and IR spectra.

Grignard Reaction of Pregnanolone with Vinylmagnesium Bromide——Pregnanolone (**5**) (20 g) in tetrahydrofuran (400 ml) was added with stirring under cooling with ice under N_2 during the period of 1.5 hr to a Grignard reagent which was made from 1,2-dibromoethane (450 g), KOH (360 g), Mg powder (22 g), and tetrahydrofuran (150 ml). The mixture was stirred for a further 1 day. After cooling with dry ice-acetone, the excess Grignard reagent was decomposed with 10% NH_4Cl solution. Extraction with *n*-BuOH–ether, working up in the usual manner, and crystallization from acetone gave (20*S*)-20-vinyl-5α-pregnane-3β,20-diol (**6**) as colorless needles (15 g). mp 148—150°. *Anal.* Calcd. for $C_{23}H_{38}O_2$: C, 79.71; H, 11.05. Found: C, 79.65; H, 11.04. IR ν_{max}^{KBr} cm^{-1}: 3340 (hydroxyl), 1632, 995, 918 (vinyl); ^1H NMR: 3H s at 0.81 ($C_{(18)}\underline{H}_3$), 3H s at 0.81 ($C_{(19)}\underline{H}_3$), 3H s at 1.32 ($C_{(21)}\underline{H}_3$), 1H m at 3.65 ($C_{(3)}\underline{H}$), 1H dd at 4.92 (*J* 2, 10, $C_{(20)}$-CH=C$\underline{H}_2$), 1H dd at 5.09 (*J* 2, 18, $C_{(20)}$-CH=C$\underline{H}_2$), 1H dd at 6.00 (*J* 10, 18, $C_{(20)}$-C$\underline{H}$=CH_2); ^1H NMR (C_5D_5N): 3H s at 0.84 ($C_{(19)}\underline{H}_3$), 3H s at 1.06 ($C_{(18)}\underline{H}_3$), 3H s at 1.54 ($C_{(21)}\underline{H}_3$), 1H m at 3.85 ($C_{(3)}\underline{H}$), 1H dd at 5.09 (*J* 2, 10, $C_{(20)}$-C$\underline{H}$=CH_2), 1H, dd, at 5.48 (*J* 2, 18, $C_{(20)}$-CH=C$\underline{H}_2$), 1H, dd, at 6.27 (*J* 10, 18, $C_{(20)}$-C$\underline{H}$=CH_2).

The mother liquor was evaporated (4 g) and treated with Ac_2O (6 ml) in pyridine (12 ml) at room temperature overnight. After the mixture was worked up in the customary way, the product was chromatographed over silica gel (250 g). Elution with benzene–AcOEt (15: 1) and crystallization from acetone gave (20*R*)-20-vinyl-5α-pregnane-3β,20-diol 3-acetate (**44**) as colorless leaflets (1.8 g). mp 233—235.5°. IR ν_{max}^{KBr} cm^{-1}: 3450 (hydroxyl), 1722, 1274 (acetoxyl), 1625, 999, 922 (vinyl). The vinylpregnanediol acetate (**44**) (1.5 g) and $LiAlH_4$ (1.5 g) in tetrahydrofuran (120 ml) were heated under reflux for 2 hr. Working up in the customary manner and crystallization from acetone yielded (20*R*)-20-vinylpregnane-3β,20-diol (**7**) as colorless needles (1.2 g). mp 181—183°. IR ν_{max}^{KBr} cm^{-1}: 3450 (hydroxyl), 1628, 1003, 922 (vinyl). Identification with the (20*R*)-vinylpregnanediol (**7**) obtained from pregnanolone (**5**) through Mannich reaction (*vide infra*) was carried out by mixed mp and comparision of IR spectra.

Ozonolysis of (20*S*)-20-Vinyl-5α-pregnane-3β,20-diol——Ozonized O_2 was passed through the (20*S*)-vinylpregnanediol (**6**) (2 g) in tetrahydrofuran (200 ml) and pyridine (0.1 ml) at −70° until the starting material was not detectable by TLC. After stirring with Zn powder (4.8 g) and AcOH (6 ml) at room temperature under N_2 for 2 hr and filtration, the filtrate was extracted with AcOEt. Working up in the customary manner afforded a crystalline mass (1.9 g) which on crystallization from acetone yielded (20*R*)-20-formyl-5α-pregnane-3β,20-diol (**8**) as colorless prisms. mp 144—146°. IR ν_{max}^{KBr} cm^{-1}: 3400 (hydroxyl), 2720, 1725 (formyl); ^1H NMR: 3H s at 0.76 ($C_{(18)}\underline{H}_3$), 3H s at 0.80 ($C_{(19)}\underline{H}_3$), 3H s at 1.32 ($C_{(21)}\underline{H}_3$), 1H m at 3.65 ($C_{(3)}\underline{H}$), 1H s at 9.63 ($C_{(20)}$-C$\underline{H}$O).

Grignard Reaction of (20*R*)-20-Formyl-5α-pregnane-3β,20-diol with 3-Methyl-3-(tetrahydropyran-2-yloxy)butynylmagnesium Bromide——To a Grignard reagent prepared from Mg (1.78 g) and ethynyl bromide (7.98 g) in tetrahydrofuran (100 ml), the aldehyde (**8**) (0.5 g) in tetrahydrofuran (30 ml) was added under cooling with ice under N_2 during the period of 20 min. The mixture was further stirred at room temperature overnight. After addition of 10% NH_4Cl (0.2 liter.), extraction with *n*-BuOH–ether gave a mixture of

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(20*R*, 22*R*)-25-(tetrahydropyran-2-yloxy)-5α-cholest-23-yne-3β,20,22-triol (**9**) and (20*R*)-24,24-dimethyl-24-(tetrahydropyran-2-yloxy)-5α-chol-22-yne-3β,20-diol (**10**).

Reaction of (20*R*)-20-Formyl-5α-pregnane-3β,20-diol with 3-Methyl-3-(tetrahydropyran-2-yloxy)-butynyllithium——To CH_3Li prepared from Li (2.32 g) and CH_3I (11.40 g) in ether (100 ml), 3-methyl-3-(tetrahydropyran-2-yloxy)-butyne (7.04 g) in tetrahydrofuran (60 ml) was added under N_2 at room temperature during the period of 20 min. Stirring was continued for a further 4 hr. The aldehyde (**8**) (1.0 g) in tetrahydrofuran (120 ml) was added to the mixture at room temperature under N_2 during the period of 40 min. The reaction mixture was further stirred at room temperature overnight. Working up as above yielded a mixture of (20*R*,22*R*)-25-(tetrahydropyran-2-yloxy)-5α-cholest-23-yne-3β,20,22-triol (**9**) and (20*R*)-24,24-dimethyl-24-(tetrahydropyran-2-yloxy)-5α-chol-22-yne-3β,20-diol (**10**).

Catalytic Hydrogenation of the Acetylenes over Palladium-Charcoal——The mixture of the acetylenes (**9** and **10**) (2.0 g) in EtOH (100 ml) was hydrogenated over 10% Pd-C (1.5 g) in the presence of piperidine (0.1 ml) at room temperature overnight. After filtration and evaporation, the product was chromatographed over silica gel (150 g).

Elution with benzene–AcOEt (1: 1) and crystallization from MeOH–AcOEt afforded (20*R*,22*R*)-25-(tetrahydropyran-2-yloxy)-5α-cholestane-3β,20,22-triol (**11**) as colorless needles (0.75 g). mp 129—130.5°. $[\alpha]_D^{28}$ +1.43° (*c* 5.19, EtOH). Mass Spectrum *m/e*: 502 (M^+-18), 484 ($M^+-18\times2$), 469 ($M^+-18\times2-15$), 466 ($M^+-18\times3$), 444 ($M^+-58-18$), 426 ($M^+-58-18\times2$), 419 (M^+-101), 418 ($M^+-84-18$), 401 ($M^+-101-18$), 400 ($M^+-84-18\times2$), 385 ($M^+-84-18\times2-15$), 383 ($M^+-101-18\times2$), 319 (M^+-201), 301 ($M^+-201-18$), 283 ($M^+-201-18\times2$), 275 (M^+-245), 273 (M^+-247), 257 ($M^+-245-18$), 255 ($M^+-247-18$), 201 (M^+-319), 184 ($M^+-319-17$), 161 ($M^+-275-84$), 143 ($M^+-275-84-18$), 125 ($M^+-275-84-18\times2$), 117 ($M^+-319-84$), 107 ($M^+-275-84-18\times3$), 99 ($M^+-319-84-18$), 85 (M^+-435), 81 ($M^+-319-84-18\times2$). IR ν_{max}^{KBr} cm^{-1}: 3400 (hydroxyl); ^1H NMR (C_5D_5N, 100 MHz): 3H s at 0.81 ($C_{(19)}\underline{H}_3$), 3H s at 1.12 ($C_{(18)}\underline{H}_3$), two 3H s's at 1.29, 1.32 ($C_{(26)}\underline{H}_3$, $C_{(27)}\underline{H}_3$), two 1H m's at 3.50, 3.90 (–O–C$\underline{H}_2$–), 1H m at 3.80 ($C_{(3)}\underline{H}$), 1H dd at 3.84 (*J* 4, 9, $C_{(22)}\underline{H}$), 1H dd at 4.98 (*J* 6, 12, –O–C$\underline{H}$–O–).

Elution with benzene–AcOEt (3: 1) and crystallization from acetone gave (20*S*)-24,24-dimethyl-24-(tetrahydropyran-2-yloxy)-5α-cholane-3β,20-diol (**12**) as colorless needles (0.2 g). mp 65—67.5°. IR ν_{max}^{KBr} cm^{-1}: 3380 (hydroxyl). Identification with the triol tetrahydropyranyl ether (**12**) obtained from the pregnanolone (**5**) (*vide infra*) was performed by mixed mp and comparison of IR spectra.

Acetylation of (20*R*,22*R*)-5α-Cholestane-3β,20,22,25-tetraol Tetrahydropyranyl Ether——The tetraol tetrahydropyranyl ether (**11**) (0.05 g) in pyridine (2 ml) was made to react with Ac_2O (1 ml) at room temperature overnight. Working up in the customary manner and crystallization from MeOH–AcOEt gave (20*R*,22*R*)-25-(tetrahydropyran-2-yloxy)-5α-cholestane-3β,20,22-triol 3,22-diacetate (**13**) as colorless needles (0.04 g). mp 160—161.5°. IR ν_{max}^{KBr} cm^{-1}: 3480 (hydroxyl), 1740, 1242 (acetoxyl); ^1H NMR: 3H s at 0.81 ($C_{(18)}\underline{H}_3$), 3H s at 0.81 ($C_{(19)}\underline{H}_3$), 3H s 1.14 ($C_{(21)}\underline{H}_3$), 6H s at 1.20 ($C_{(26)}\underline{H}_3$, $C_{(27)}\underline{H}_3$), two 1H m's at 3.55, 3.85 (–O–C$\underline{H}_2$), 1H m at 4.65 ($C_{(3)}\underline{H}$), 1H dd at 4.70 (*J* 6, 12, –O–C$\underline{H}$–O–), 1H dd at 4.70 (*J* 4, 9, $C_{(22)}\underline{H}$).

Acetylation of (20*S*)-24,24-Dimethyl-5α-cholane-3β,20,25-triol Tetrahydropyranyl Ether——The triol tetrahydropyranyl ether (**12**) (0.05 g) and Ac_2O (0.5 ml) in pyridine (1 ml) were left standing at room temperature overnight. Working up in the usual manner and crystallization from AcOEt afforded (20*S*)-24,24-dimethyl-24-(tetrahydropyran-2-yloxyl)-5α-cholane-3β,20-diol 3-acetate (**14**) as colorless needles (0.042 g). mp 127—128.5°. IR ν_{max}^{KBr} cm^{-1}: 3500 (hydroxyl), 1725, 1250 (acetoxyl); ^1H NMR: 3H s at 0.81 ($C_{(18)}\underline{H}_3$), 3H s at 0.81 ($C_{(19)}\underline{H}_3$), 3H s at 1.15 ($C_{(21)}\underline{H}_3$), 6H s at 1.18 ($C_{(24)}(C\underline{H}_3)_2$), two 1H m's at 3.55, 3.85 (–O–C$\underline{H}_2$–), 1H m at 4.65 ($C_{(3)}\underline{H}$), 1H m at 4.70 (–C$\underline{H}$–O–).

Acid Hydrolysis of (20*R*,22*R*)-5α-Cholestane-3β,20,22,25-tetraol Tetrahydropyranyl Ether——The tetraol tetrahydropyranyl ether (**11**) (0.15 g) was treated with 0.05N HCl in 10% aqueous tetrahydrofuran (10 ml) at room temperature for 2 hr. Dilution with water, extraction with *n*-BuOH–ether, and crystallization from MeOH–AcOEt afforded (20*R*,22*R*)-5α-cholestane-3β,20,22,25-tetraol (**15**) as colorless needles (0.12 g). mp 216—217.5°. $[\alpha]_D$ +1.6 (*c* 4.26, EtOH); CD (5×10^{-4} mole with $Pr(dpm)_3$ 1.6×10^{-4} mole, $CHCl_3$): $\Delta\varepsilon_{314}$ +1.1. *Anal.* Calcd. for $C_{27}H_{48}O_4$: C, 74.26; H, 11.08. Found: C, 73.93; H, 10.81. Mass Spectrum *m/e*: 435 (M^+-1), 418 (M^+-18), 403 ($M^+-18-15$), 400 ($M^+-18-15$), 387 ($M^+-18\times2-15$), 367 ($M^+-18\times3-15$), 319 (M^+-117), 301 ($M^+-117-18$), 283 ($M^+-117-18\times2$), 275 (M^+-161), 273 (M^+-163), 257 ($M^+-161-18$), 161 (M^+-275), 143 ($M^+-275-18$), 125 ($M^+-275-18\times2$), 117 (M^+-319), 107 ($M^+-275-18\times3$), 99 ($M^+-319-18$), 81 ($M^+-319-18\times2$). IR ν_{max}^{KBr} cm^{-1}: 3370 (hydroxyl); ^1H NMR (C_5D_5N, 100 MHz): shown in Table III; ^{13}C NMR: shown in Table VI.

Acid Hydrolysis of (20*S*)-24,24-Dimethyl-5α-cholane-3β,20,24-triol Tetrahydropyranyl Ether——The triol tetrahydropyranyl ether (**12**) (0.2 g) was treated with 0.05N HCl in 10% aqueous tetrahydrofuran (14 ml) at room temperature for 2 hr. The mixture was worked up in the customary way and the product was crystallized from MeOH–AcOEt to yield (20*S*)-24,24-dimethyl-5α-cholane-3β,20,24-triol (**16**) as colorless needles (0.15 g). mp 190—191.5°. *Anal.* Calcd. for $C_{26}H_{46}O_3$: C, 76.79; H, 11.40. Found: C, 76.76; H, 11.39. Mass Spectrum *m/e*: 406 (M^+), 405 (M^+-1), 388 (M^+-18), 373 ($M^+-18-15$), 370 ($M^+-18\times2$), 355 $M^+-18\times2-15$), 319 (M^+-87), 301 ($M^+-87-18$), 283 ($M^+-87-18\times2$), 273 (M^+-133), 257 ($M^+-131-18$), 255 (M^+-133), 131 (M^+-275), 113 ($M^+-275-18$), 95 ($M^+-275-18\times2$). IR ν_{max}^{KBr} cm^{-1}: 3320 (hydroxyl);

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^{1}H NMR (C_5D_5N, 100 MHz): 3H s at 0.85 ($C_{(19)}\underline{H}_3$), 3H s at 1.13 ($C_{(18)}\underline{H}_3$), 6H s at 1.46 (($C_{(24)}\underline{H}_3)_2$), 3H s at 1.54 ($C_{(21)}\underline{H}_3$).

Acetylation of (20*R*,22*R*)-5α-Cholestane-3β,20,22,25-tetraol——The tetraol (**15**) (0.03 g) and Ac_2O (0.5 ml) in pyridine (1 ml) were set aside at room temperature overnight. The mixture was worked up as usual to give the product which was crystallized from MeOH–AcOEt to yield (20*R*,22*R*)-5α-cholestane-3β,20,22,25-tetraol 3,22-diacetate (**17**) as colorless needles (0.025 g). mp 171—172.5°. IR ν_{max}^{KBr} cm^{-1}: 3550, 3480 (hydroxyl), 1724, 1710, 1245 (acetoxyl); ^{1}H NMR: 3H s at 0.81 ($C_{(19)}\underline{H}_3$), 3H s at 0.84 ($C_{(18)}\underline{H}_3$), 6H s at 1.21 ($C_{(26)}\underline{H}_3$, $C_{(27)}\underline{H}_3$), 3H s at 1.25 ($C_{(21)}\underline{H}_3$), 1H m at 4.70 ($C_{(3)}\underline{H}$), 1H dd at 4.80 (*J* 4, 9, $C_{(22)}\underline{H}$).

Acetylation of (20*S*)-24,24-Dimethyl-5α-cholane-3β,20,24-triol——The triol (**16**) (0.03 g) in pyridine (1 ml) was treated with Ac_2O (0.5 ml) at room temperature overnight. After working up in the usual way, the product was crystallized from AcOEt to give (20*S*)-24,24-dimethyl-5α-cholane-3β,20,24-triol 3-acetate (**18**) as colorless needles (0.023 g). mp 200.5—202°. IR ν_{max}^{KBr} cm^{-1}: 3450 (hydroxyl), 1708, 1245 (acetoxyl); ^{1}H NMR (100 MHz): 3H s at 0.82 ($C_{(19)}\underline{H}_3$), 3H s at 0.84 ($C_{(18)}\underline{H}_3$), 6H s at 1.20 (($C_{(24)}\underline{H}_3)_2$), 3H s at 1.25 ($C_{(21)}\underline{H}_3$), 1H m at 4.65 ($C_{(3)}\underline{H}$).

Grignard Reaction with 3-Methyl-3-(tetrahydropyran-2-yloxy)-butynylmagnesium Bromide followed by Catalytic Hydrogenation of Pregnanolone——To 3-methyl-3-(tetrahydropyran-2-yloxy)-butynylmagnesium bromide in tetrahydrofuran prepared as above, pregnanolone (**5**) (0.5 g) in tetrahydrofuran (150 ml) was added under N_2 at room temperature for 30 min. The mixture was stirred overnight and worked up in the usual way to give the crude acetylene (**10**) (*ca.* 0.6 g). The crude acetylene (**10**) (*ca.* 0.6 g) in EtOH (30 ml) was hydrogenated over Raney Ni (0.4 g) in the presence of piperidine (0.1 ml) at room temperature overnight. The product was crystallized from acetone to furnish (20*S*)-24,24-dimethyl-24-(tetrahydropyran-2-yloxy)-5α-cholane-3β,20-diol (**12**) as colorless needles (0.25 g). mp 67—68°. Mass Spectrum *m/e*: 490 (M^+), 489 (M^+-1), 472 (M^+-18), 457 ($M^+-18-15$), 454 ($M^+-18\times2$), 439 ($M^+-18\times2-15$), 432 (M^+-58), 414 ($M^+-58-18$), 388 ($M^+-84-18$), 371 ($M^+-101-18$), 370 ($M^+-84-18\times2$), 355 ($M^+-84-18\times2-15$), 353 ($M^+-101-18\times2$), 319 (M^+-171), 301 ($M^+-171-18$), 283 ($M^+-171-18\times2$), 273 (M^+-217), 257 ($M^+-215-18$), 255 ($M^+-217-18$), 215 (M^+-275), 157 ($M^+-275-58$), 131 ($M^+-275-84$), 113 ($M^+-275-84-18$), 95 ($M^+-275-84-18\times2$), 85 (M^+-405). IR ν_{max}^{KBr} cm^{-1}: 3380 (hydroxyl); ^{1}H NMR (C_5D_5N, 100 MHz): 3H s at 0.82 ($C_{(19)}\underline{H}_3$), 3H s at 1.10 ($C_{(18)}\underline{H}_3$), two 3H s's at 1.30, 1.32 ($C_{(24)}(C\underline{H}_3)_2$), 3H s at 1.49 ($C_{(21)}\underline{H}_3$), two 1H m's at 3.50, 3.90 (–O–C$\underline{H}_2$–), 1H m at 3.80 ($C_{(3)}\underline{H}$), 1H dd at 4.92 (*J* 6, 12, –O–C$\underline{H}$–O–).

Periodate Oxidation of (20*R*)-Pregnane-3β,20,21-triol——To pregnanetriol (**21**) (2.0 g) in tetrahydrofuran (100 ml), $NaIO_4$ (1.50 g) in water (10 ml) was added. The mixture was set aside at room temperature for 30 min and worked up in the customary way to give the product (1.7 g) which was crystallized from acetone yielding 17β-formyl-5α-androstan-3β-ol (**22**) as colorless leaflets. mp 144—146°. IR ν_{max}^{KBr} cm^{-1}: 3350 (hydroxyl), 2700, 1705 (formyl); ^{1}H NMR: 3H s at 0.75 ($C_{(18)}\underline{H}_3$), 3H s at 0.82 ($C_{(19)}\underline{H}_3$), 1H m at 3.65 ($C_{(3)}\underline{H}$), 1H d at 9.68 (*J* 2, $C_{(17)}$–C$\underline{H}$O).

Grignard Reaction of 17β-Formyl-5α-androstan-3β-ol with Vinylmagnesium Bromide——To tetrahydrofuran solution (100 ml) of vinylmagnesium bromide prepared from Mg (1.2 g) and vinyl bromide, which was made from ethylene dibromide (9.2 g) and KOH (40 g) in EtOH (15 ml), the aldehyde (**22**) (1.5 g) in tetrahydrofuran (150 ml) was added under N_2 during the period of 30 min. The sitrring was continued for a further 6 hr. After working up in the usual way, the product was chromatographed over silica gel (120 g). Elution with hexane–AcOEt (12: 1) and crystallization from acetone furnished a mixture of (1′*R*)- and (1′*S*)-17β-(1′-hydroxy-2′-propenyl)-5α-androstane-3β,20-diols (**23**) as colorless needles (0.8 g). mp 135—145.5°. IR ν_{max}^{KBr} cm^{-1}: 3400 (hydroxyl), 1645, 960, 925 (vinyl); ^{1}H NMR: 3H s at 0.80 ($C_{(19)}\underline{H}_3$), two 1.5H′ s at 0.82, 0.84 ($C_{(18)}\underline{H}_3$), 1H m at 4.05 ($C_{(1')}\underline{H}$), 3H m in the region 5—6 ($C_{(2')}\underline{H}$, $C_{(3')}\underline{H}_2$).

Chromic Acid Oxidation of the Mixture of (1′*R*)- and (1′*S*)-17β-(1′-Hydroxy-2′-propenyl)-5α-androstan-3β-ols——To the mixture of the diols (**23**) (1.0 g) in acetone (90 ml), a chromic acid solution (50 ml), prepared from CrO_3 (26.72 g) and conc. H_2SO_4 (23 ml) in water (100 ml), was added at 0—5°. After stirring for 15 min, the reaction mixture was poured into ice. Ether extraction and crystallization from acetone-hexane gave 17β-(1′-oxo-2′-propenyl)-5α-androstan-3-one (**24**) as colorless needles (0.4 g). mp 164—164.5°. *Anal.* Calcd. for $C_{22}H_{32}O_2$: C, 80.44; H, 9.83. Found: C, 80.40; H, 9.72. IR ν_{max}^{KBr} cm^{-1}: 1710, 1662 (ketone), 1618, 982, 914 (vinyl); ^{1}H NMR: 3H s at 0.61 ($C_{(18)}\underline{H}_3$), 3H s at 1.01 ($C_{(19)}\underline{H}_3$), 1H dd at 5.58 (*J* 3, 8, $C_{(3')}\underline{H}$), 1H dd at 6.05 (*J* 3, 16, $C_{(3')}\underline{H}$), 1H dd at 6.44 (*J* 8, 16, $C_{(2')}\underline{H}$).

Partial Reduction of 17β-(1′-Oxo-2′-propenyl)-5α-androstan-3-one with Sodium Borohydride——To the dione (**24**) (0.35 g) in CH_2Cl_2 (15 ml) and EtOH (15 ml), $NaBH_4$ (0.04 g) was added under stirring at 5—7° during the period of 15 min. Dilution with water, extraction with ether, and crystallization from acetone gave 17β-(1′-oxo-2′-propenyl)-5α-androstan-3β-ol (**25**) as colorless needles (0.32 g). mp 151.5—153°. *Anal.* Calcd. for $C_{22}H_{34}O_2$: C, 79.95; H, 10.37. Found: C, 79.69; H, 10.32. IR ν_{max}^{KBr} cm^{-1}: 3450 (hydroxyl), 1685, 1659 (ketone), 1619, 990, 921 (vinyl); ^{1}H NMR: 3H s at 0.58 ($C_{(18)}\underline{H}_3$), 3H s at 0.80 ($C_{(19)}\underline{H}_3$), 1H m at 3.65 ($C_{(3)}\underline{H}$), 1H dd at 5.60 (*J* 3, 8, $C_{(3')}\underline{H}$), 1H dd at 6.08 (*J* 3, 16, $C_{(3')}\underline{H}$), 1H dd at 6.45 (*J* 8, 16, $C_{(2')}\underline{H}$).

Mannich Reaction of Pregnanolone with Formaldehyde and Dimethylamine——To pregnanolone (**5**) (11.0 g) in dimethoxyethane (4 liters.), paraformaldehyde (1.92 g) and $(CH_3)_2NH\cdot HCl$ (4.94 g) were added and the mixture was heated under reflux for 2 days. After filtration, the filtrate was treated with 40% NaOH

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(100 ml). $CHCl_3$ extraction and crystallization from acetone gave 17β-(3′-dimethylamino-1′-oxopropyl)-5α-androstan-3β-ol (**26**) as colorless needles (9.5 g). mp 87—88. *Anal.* Calcd. for $C_{24}H_{41}O_2N$: C, 76.75; H, 11.00; N, 4.01. Found: C, 76.58; H, 11.06; N, 4.11. IR ν_{max}^{KBr} cm^{-1}: 3550, 3400 (hydroxyl), 2810, 1760 (N-methyl), 1702, 1684, 1640 (carbonyl); ^1H NMR: 3H s at 0.60 ($C_{(18)}\underline{H}_3$), 3H s at 0.81 ($C_{(19)}\underline{H}_3$), 6H s at 2.25 ($-N(C\underline{H}_3)_2$), 4H s at 2.58 ($C_{(2')}\underline{H}_2$, $C_{(3')}\underline{H}_2$), 1H m at 3.65 ($C_{(3)}\underline{H}$).

Reaction of 17β-(3′-Dimethylamino-1′-oxopropyl)-5α-androstan-3β-ol with Methyl Bromide——To the dimethylamine (**26**) (1.0 g) in MeOH (70 ml), CH_3Br (20 ml) was added under cooling with ice. After left standing at room temperature, the solvent was evaporated to yield the residue which was crystallized from MeOH to give the quaternary ammonium bromide (**27**) as colorless prisms (9.5 g). mp 232—232.5°. *Anal.* Calcd. for $C_{25}H_{44}O_2NBr$: C, 63.82; H, 9.43; N, 2.98; Br, 16.98. Found: C, 64.00; H, 9.69; N, 2.80; Br, 16.88. IR ν_{max}^{KBr} cm^{-1}: 3300 (hydroxyl), 1708 (carbonyl).

Alkaline Treatment of the Quaternary Ammonium Bromide——To the ammonium salt (**27**) (20.0 g) in MeOH (1.2 liters.), $NaHCO_3$ (10.2 g) in water (250 ml) was added at 0°. The mixture was stirred at room temperature for 2 hr and poured into ice. Neutralization with 1% H_2SO_4, evaporation of the solvent, extraction with AcOEt, and crystallization from acetone furnished the enone (**25**) as colorless needles (8.0 g). mp 152—153.5°. IR ν_{max}^{KBr} cm^{-1}: 3450 (hydroxyl), 1682, 1652 (carbonyl), 1617, 985, 918 (vinyl). Identification with the unsaturated ketol (**25**) obtained from the unsaturated diketone (**25**) by partial reduction was carried out by mixed mp and comparison of IR spectra.

Reaction of the Enone with Methyllithium——The enone (**25**) (6.0 g) in tetrahydrofuran (50 ml) was added under N_2 to CH_3Li solution, prepared from Li (1.16 g), CH_3I (25.3 g), and ether (50 ml). The mixture was stirred for a further 2 hr. The mixture was worked up in the customary way to afford the oily product (6.5 g) which was acetylated with Ac_2O (10 ml) in pyridine (15 ml) in the usual way. The product was chromatographed over silica gel (350 g). Elution with benzene–AcOEt (15: 1) and crystallization from acetone–hexane gave (20*R*)-20-vinyl-5α-pregnane-3β,20-diol 3-acetate (**44**) as colorless leaflets (4.2 g). mp 234—235°. IR ν_{max}^{KBr} cm^{-1}: 3470 (hydroxyl), 1718, 1270 (acetoxyl), 1625, 998, 918 (vinyl); ^1H NMR (100 MHz): 3H s at 0.74 ($C_{(18)}\underline{H}_3$), 3H s at 0.80 ($C_{(19)}\underline{H}_3$), 3H s at 1.20 ($C_{(21)}\underline{H}_3$), 1H m at 4.65 ($C_{(3)}\underline{H}$), 1H dd at 4.99 (J 2, 10, $C_{(20)}$–CH=C$\underline{H}_2$), 1H dd at 5.20 (J 2, 18. $C_{(20)}$–CH=C$\underline{H}_2$), 1H dd at 6.05 (J 10, 18, $C_{(20)}$–C$\underline{H}$=CH_2). The acetate (**44**) (3.0 g) and $LiAlH_4$ (2.5 g) in tetrahydrofuran (250 ml) were heated under reflux for 2 hr. Working up in the customary manner gave the product which was crystallized from acetone to yield (20*R*)-20-vinyl-5α-pregnane-3β,20-diol (**7**) as colorless needles (2.4 g). mp 182—183.5°. *Anal.* Calcd. for $C_{23}H_{38}O_2$: C, 79.71; H, 11.05. Found: C, 79.42; H, 11.01. IR ν_{max}^{KBr} cm^{-1}: 3350 (hydroxyl), 1625, 1002, 922 (vinyl); ^1H NMR (100 MHz): 3H s at 0.78 ($C_{(18)}\underline{H}_3$), 3H s at 0.82 ($C_{(19)}\underline{H}_3$), 3H s at 1.24 ($C_{(21)}\underline{H}_3$), 1H m at 3.65 ($C_{(3)}\underline{H}$), 1H dd at 5.60 (J 3, 8, $C_{(20)}$–CH=C$\underline{H}_2$), 1H dd at 6.05 (J 3, 16, $C_{(20)}$–CH=C$\underline{H}_2$), 1H dd at 6.40 (J 8, 16, $C_{(20)}$–C$\underline{H}$=CH_2).

Ozonolysis of (20*R*)-20-Vinyl-5α-pregnane-3β,20-diol——The vinylpregnanediol (**7**) (1.2 g) in tetrahydrofuran (180 ml) was ozonized at −70° until the starting material was not detectable by TLC. The mixture was stirred with Zn powder (3.5 g) and AcOH (5.2 ml) under N_2 for 2 hr and filtered. Extraction of the filtrate with AcOEt and crystallization from acetone furnished (20*S*)-20-formyl-5α-pregnane-3β,20-diol (**28**) as colorless needles (0.8 g). mp 175—177.5°. IR ν_{max}^{KBr} cm^{-1}: 3400 (hydroxyl), 2720, 1726 (formyl); ^1H NMR: 3H s at 0.68 ($C_{(18)}\underline{H}_3$), 3H s at 0.79 ($C_{(19)}\underline{H}_3$), 3H s at 1.24 ($C_{21()}\underline{H}_3$), 1H m at 3.65 ($C_{(3)}\underline{H}$), 1H s at 9.58 ($C_{(20)}$–C$\underline{H}$O).

Reaction of (20*S*)-20-Formyl-5α-pregnane-3β,20-diol with 3-Methyl-3-(tetrahydropyran-2-yloxy)butynyllithium——To 3-methyl-3-(tetrahydropyran-2-yloxy)butynyllithium in ether and tetrahydrofuran, prepared as described previously, the aldehyde (**28**) (1.0 g) in tetrahydrofuran (120 ml) was added under N_2 at −40 ∼ −60°. The mixture was stirred at room temperature for 1 hr and worked up in the usual way to give a mixture of the crude acetylenes (**29** and **10**) (*ca.* 1 g).

Hydrogenation of the crude Acetylene over Palladium-Carbon——The mixture of the crude acetylenes (**29** and **10**) (1.5 g) in EtOH (80 ml) was hydrogenated over 10% Pd-C (1.2 g) in the presence of piperidine (0.1 ml) at room temperature overnight. Working up in the customary manner and crystallization from MeOH–AcOEt to yield (20*S*,22*S*)-25-(tetrahydropyran-2-yloxy)-5α-cholestane-3β,20,22-triol (**30**) as colorless needles (0.6 g). mp 234—235°. Mass Spectrum *m/e*: 484 ($M^+-18\times2$), 469 ($M^+-18\times2-15$), 466 ($M^+-18\times3$), 444 ($M^+-58-18$), 436 (M^+-84), 426 ($M^+-58-18\times2$), 419 (M^+-101), 418 ($M^+-84-18$), 400 ($M^+-84-18\times2$), 385 ($M^+-84-18\times2-15$), 319 (M^+-201), 301 ($M^+-201-18$), 283 ($M^+-201-18\times3$), 275 (M^+-245), 273 (M^+-247), 257 ($M^+-245-18$), 255 ($M^+-247-18$), 201 (M^+-319), 184 ($M^+-319-19$), 161 ($M^+-275-84$), 143 ($M^+-275-84-18$), 125 ($M^+-275-84-18\times2$), 117 ($M^+-319-84$), 107 ($M^+-275-84-18\times3$), 99 ($M^+-319-84-18$), 85 (M^+-435), 81 ($M^+-319-84-18\times2$). IR ν_{max}^{KBr} cm^{-1}: 3350 (hydroxyl); ^1H NMR (C_5D_5N): 3H s at 0.88 ($C_{(18)}\underline{H}_3$), 3H s at 0.88 ($C_{(19)}\underline{H}_3$), 6H s at 1.20 ($C_{(26)}\underline{H}_3$, $C_{(27)}\underline{H}_3$), 3H s at 1.44 ($C_{(21)}\underline{H}_3$).

The mother liquor (0.9 g) was chromatographed over silica gel (120 g). Elution with benzene–AcOEt (3: 1) and crystallization from acetone afforded (20*S*)-24,24-dimethyl-24-(tetrahydropyran-2-yloxy)-5α-cholane-3β,20-diol (**12**) as colorless needles (0.15 g). mp 67—69°. IR ν_{max}^{KBr} cm^{-1}: 3375 (hydroxyl). Identification with the triol tetrahydropyranyl ether (**12**) obtained from the pregnanolone (**5**) (*vide supra*) was

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carried out by mixed mp and comparision of IR spectra.

Acetylation of (20*S*,22*S*)-5α-Cholestane-3β,20,22,25-tetraol Tetrahydropyranyl Ether——The tetraol tetrahydropyranyl ether (**30**) (0.03 g) and Ac_2O (0.5 ml) in pyridine (1 ml) were set aside at room temperature overnight. After isolation in the usual way, the product was crystallized from AcOEt to afford (20*S*,22*S*)-25-(tetrahydropyran-2-yloxy)-5α-cholestane-3β,20,22-triol 3,22-diacetate (**31**) as colorless needles (0.025 g). mp 176.5—177.5°. IR ν_{max}^{KBr} cm^{-1}: 3500 (hydroxyl), 1723, 1245 (acetoxyl); ^1H NMR: 3H s at 0.84 ($C_{(19)}\underline{H}_3$), 3H s at 0.86 ($C_{(18)}\underline{H}_3$), 6H s at 1.24 ($C_{(26)}\underline{H}_3$, $C_{(27)}\underline{H}_3$), 3H s at 1.28 ($C_{(21)}\underline{H}_3$), 2H m at 3.55, 3.95 (–O–$C\underline{H}_2$–), 1H m at 4.65 ($C_{(3)}\underline{H}$), 1H m at 4.70 (–O–C<u>H</u>–O–), 1H dd at 4.70 (*J* 4, 9, $C_{(22)}\underline{H}$).

Acid Hydrolysis of (20*S*,22*S*)-5α-Cholestane-3β,20,22,25-tetraol Tetrahydropyranyl Ether——The tetrahydropyranyl ether (**30**) (0.3 g) was treated with 0.05 N HCl in 10% aqueous tetrahydrofuran (25 ml) at room temperature for 30 min. The mixture was worked up in the customary way to give a crystalline mass which was crystallized from MeOH–AcOEt to yield (20*S*,22*S*)-5α-cholestane-3β,20,22,25-tetraol (**32**) as colorless needles (0.12 g). mp 182—183.5°. CD (5×10^{-4} mole with $Pr(dpm)_3$ 1.6×10^{-4} mole, $CHCl_3$): $\Delta\varepsilon_{314}$ −1.3. Mass Spectrum *m/e*: 436 (M^+), 435 (M^+-1), 403 ($M^+-18-15$), 400 ($M^+-18\times2$), 385 ($M^+-18\times2-15$), 367 ($M^+-18\times3-15$), 319 (M^+-117), 301 ($M^+-117-18$), 283 ($M^+-117-18\times2$), 273 (M^+-163), 257 ($M^+-161-18$), 161 (M^+-275), 143 ($M^+-275-18$), 125 ($M^+-275-18\times2$), 107 ($M^+-275-18\times3$), 99 ($M^+-319-18$), 81 ($M^+-319-18\times2$). IR ν_{max}^{KBr} cm^{-1}: 3400 (hydroxyl); ^1H NMR (C_5D_5N, 100 MHz): shown in Table III; ^{13}C NMR: shown in Table VI.

Acetylation of (20*S*,22*S*)-5α-Cholestane-3β,20,22,25-tetraol——The tetraol (**32**) (0.03 g) was treated with Ac_2O (0.5 ml) in pyridine (1 ml) to give the product which on crystallization from AcOEt furnished (20*S*,22*S*)-5α-cholestane-3β,20,22,25-tetraol 3,22-diacetate (**33**) as colorless needles (0.025 g). mp 198—202°. IR ν_{max}^{KBr} cm^{-1}: 3450 (hydroxyl), 1730, 1245 (acetoxyl); ^1H NMR: shown in Table IV.

Acetylation of (20*S*)-20-Vinyl-5α-pregnane-3β,20-diol——The (20*S*)-vinylpregnanediol (**6**) (1.0 g) and Ac_2O (1.6 ml) in pyridine (0.8 ml) were left standing at room temperature overnight. Working up in the usual manner and crystallization from AcOEt-hexane afforded (20*S*)-20-vinyl-5α-pregnane-3β,20-diol 3-acetate (**36**) as colorless leaflets (0.92 g). mp 206—207°. IR ν_{max}^{KBr} cm^{-1}: 3470 (hydroxyl), 1724, 1278 (acetoxyl), 1632, 1010, 898 (vinyl); ^1H NMR: 3H s at 0.81 ($C_{(18)}\underline{H}_3$), 3H s at 0.81 ($C_{(19)}\underline{H}_3$), 3H s at 1.31 ($C_{(21)}\underline{H}_3$), 1H m at 4.70 ($C_{(3)}$H), 1H dd at 4.95 (*J* 2, 10, $C_{(20)}$–CH=C<u>H</u>$_2$), 1H dd at 5.12 (*J* 2, 18, $C_{(20)}$–CH=C<u>H</u>$_2$), 1H dd at 6.02 (*J* 10, 18, $C_{(20)}$–CH=CH_2).

Ozonolysis of (20*S*)-20-Vinyl-5α-pregnane-3β,20-diol 3-Acetate——The vinylpregnanediol acetate (**36**) (1.0 g) was ozonized in CH_2Cl_2 (150 ml) and AcOEt (150 ml) in the presence of pyridine (0.1 ml) at −70° until starting material was not detectable by TLC. Isolation in the customary manner and crystallization from acetone gave (20*R*)-20-formyl-5α-pregnane-3β,20-diol-3-acetate (**37**) as colorless needles. mp 132.5—133°. IR ν_{max}^{KBr} cm^{-1}: 3490 (hydroxyl), 2700, 1718 (formyl), 1726, 1262 (acetoxyl); ^1H NMR: 3H s at 0.83 ($C_{(18)}\underline{H}_3$), 3H s at 0.83 ($C_{(19)}\underline{H}_3$), 3H s at 1.33 ($C_{(21)}\underline{H}_3$), 1H m at 4.65 ($C_{(3)}\underline{H}$), 1H s at 9.61 ($C_{(20)}$–C<u>H</u>O).

Grignard Reaction of (20*R*)-20-Formyl-5α-pregnane-3β,20-diol 3-Acetate——The (20*R*)-formypregnanediol acetate (**37**) (0.9 g) in tetrahydrofuran (100 ml) was made to react with isoamylmagnesium bromide, prepared from Mg (1.23 g) and isoamyl bromide (6.35 g) in tetrahydrofuran (100 ml), as described previously. After isolation in the usualy way, the product was subjected to preparative TLC (silica gel, $CHCl_3$–acetone (12: 1)).

Extraction of the more polar portion with MeOH and crystallization from MeOH–AcOEt afforded (20*R*,22*R*)-5α-cholestane-3β,20,22-triol (**38**) as colorless needles (0.56 g). mp 96—96.5°. $[\alpha]_D$ −1.3 (*c* 2.29, EtOH). CD (5×10^{-4} mole with $Pr(dpm)_3$ 1.14×10^{-4} mole, CCl_4): $\Delta\varepsilon_{307}$ −2.1. *Anal.* Calcd. for $C_{27}H_{48}O_3$: C, 77.09; H, 11.50. Found: C, 77.06; H, 11.60. Mass Spectrum *m/e*: 419 (M^+-1), 402 (M^+-18), 387 ($M^+-18-15$), 384 ($M^+-18\times2$), 369 ($M^+-18\times2-15$), 319 (M^+-101), 301 ($M^+-101-18$), 283 ($M^+-101-18\times2$), 275 (M^+-145), 273 (M^+-147), 257 ($M^+-145-18$), 255 ($M^+-147-18$), 145 (M^+-275), 127 ($M^+-275-18$), 109 ($M^+-275-18\times2$), 107 ($M^+-275-18\times2-2$), 71 ($M^+-275-74$), 57 ($M^+-275-88$), 55 ($M^+-275-90$). IR ν_{max}^{KBr} cm^{-1}: 3350 (hydroxyl); ^1H NMR (C_5D_5N, 100 MHz): shown in Table III; ^{13}C NMR: shown in Table VI.

Extraction of the less polar portion with MeOH and crystallization from MeOH–AcOEt gave (20*R*,22*S*)-5α-cholestane-3β,20,22-triol (**39**) as colorless needles (0.07 g). mp 183—184°. $[\alpha]_D$ −0.48 (*c* 2.06, EtOH). CD (5×10^{-4} mole with $Pr(dpm)_3$ 1.14×10^{-4} mole, CCl_4): no maximum. *Anal.* Calcd. for $C_{27}H_{48}O_3$: C, 77.09, H, 11.50. Found: C, 77.16; H, 11.58. Mass Spectrum *m/e*: 419 (M^+-1), 402 (M^+-18), 387 ($M^+-18-15$), 384 ($M^+-18\times2$), 369 ($M^+-18\times2-15$), 319 (M^+-101), 301 ($M^+-101-18$), 283 ($M^+-101-18\times2$), 275 (M^+-145), 273 (M^+-147), 257 ($M^+-145-18$), 255 ($M^+-147-18$), 145 (M^+-275), 127 ($M^+-275-18$), 109 ($M^+-275-18\times2$), 107 ($M^+-275-18\times2-2$), 71 ($M^+-275-74$), 57 ($M^+-275-88$), 55 ($M^+-275-90$). IR ν_{max}^{KBr} cm^{-1}: 3400 (hydroxyl); ^1H NMR (C_5D_5N, 100 MHz): shown in Table III; ^{13}C NMR: shown in Table VI.

Ozonolysis of the (20*R*)-20-Vinyl-5α-pregnane-3β,20-diol 3-Acetate——The (20*R*)-vinylpregnanediol acetate (**44**) (1.0 g) was ozonized in CH_2Cl_2 (150 ml) and AcOEt (150 ml) in the presence of pyridine (0.1 ml) at −70° until starting material was not detectable by TLC. After isolation as usual, the product was crystallized from acetone to give (20*S*)-20-formyl-5α-pregnane-3β,20-diol 3-acetate (**45**) as colorless needles.

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mp 182—183.5°. IR ν_{max}^{KBr} cm^{-1}: 3450 (hydroxyl), 2700, 1722 (formyl), 1718, 1270 (acetoxyl); ^{1}H NMR: 3H s at 0.80 ($C_{(18)}\underline{H}_3$), 3H s at 0.82 ($C_{(19)}\underline{H}_3$), 3H s at 1.25 ($C_{(21)}\underline{H}_3$), 1H m at 4.65 ($C_{(3)}\underline{H}$), 1H m at 9.58 ($C_{(22)}\underline{H}$).

Grignard Reaction of the (20*S*)-20-Formyl-5α-pregnane-3β,20-diol Acetate——The (20*S*)-formylpregnanediol acetate (**45**) (0.9 g) was treated with isoamylmagnesium bromide prepared as above. After working up in the customary manner, the product was submitted to preparative TLC (silica gel, $CHCl_3$–acetone (12: 1)).

Extraction of the more polar portion and crystallization from MeOH–AcOEt furnished (20*S*,22*S*)-5α-cholestane-3β,20,22-triol (**46**) as colorless needles (0.6 g). mp 171—172.5°. $[\alpha]_D$ −0.40 (*c* 2.48, EtOH). CD (5×10^{-4} mole with $Pr(dpm)_3$ 1.14×10^{-4} mole, CCl_4): $\Delta\varepsilon_{322}$ −1.3, $\Delta\varepsilon_{303}$ +3.4. *Anal.* Calcd. for $C_{27}H_{48}O_3$: C, 77.09; H, 11.50. Found: C, 76.97; H, 11.52. Mass Spectrum *m/e*: 420 (M^+), 419 (M^+-1), 402 (M^+-18), 387 ($M^+-18-15$), 384 ($M^+-18\times2$), 369 ($M^+-18\times2-15$), 319 (M^+-101), 301 ($M^+-101-18$), 283 ($M^+-101-18\times2$), 275 (M^+-145), 273 (M^+-147), 257 ($M^+-145-18$), 255 ($M^+-147-18$), 145 (M^+-275), 127 ($M^+-275-18$), 109 ($M^+-275-18\times2$), 107 ($M^+-275-18\times2-2$), 71 ($M^+-275-74$), 57 ($M^+-275-88$), 55 ($M^+-275-90$). IR ν_{max}^{KBr} cm^{-1}: 3400 (hydroxyl); ^{1}H NMR (C_5D_5N, 100 MHz): shown in Table III; ^{13}C NMR: shown in Table VI.

Extraction of the less polar portion and crystallization from MeOH–AcOEt yielded (20*S*,22*R*)-5α-cholestane-3β,20,22-triol (**47**) as colorless needles (0.05 g). mp 145—147°. $[\alpha]_D$ +3.1 (*c* 3.04, EtOH); CD (5×10^{-4} mole with $Pr(dpm)_3$ 1.14×10^{-4} mole, CCl_4): no maximum. Mass Spectrum *m/e*: 420 (M^+), 419 (M^+-1), 402 (M^+-18), 387 ($M^+-18-15$), 384 ($M^+-18\times2$), 369 ($M^+-18\times2-15$), 319 (M^+-101), 301 ($M^+-101-18$), 283 ($M^+-101-18\times2$), 275 (M^+-145), 273 (M^+-147), 257 ($M^+-145-18$), 255 ($M^+-147-18$), 145 (M^+-275), 127 ($M^+-275-18$), 109 ($M^+-275-18\times2$), 107 ($M^+-275-18\times2-2$), 71 ($M^+-275-74$), 57 ($M^+-275-88$), 55 ($M^+-275-90$). IR ν_{max}^{KBr} cm^{-1}: 3400 (hydroxyl); ^{1}H NMR (C_5D_5N, 100 MHz): shown in Table III; ^{13}C NMR: shown in Table VI.

Acetylation of the Cholestane-3β,20,22-triols——a) Each cholestane-3β,20,22-triol (**38**, **39**, **46**, or **47**) (0.03 g) was treated with Ac_2O (0.5 ml) in pyridine (1 ml) at room temperature overnight. The mixture was worked up in the usual way and each product was crystallized from MeOH–AcOEt to give the respective diacetates.

(20*R*,22*R*)-5α-cholestane-3β,20,22-triol 3,22-diacetate (**40**) as colorless needles (from AcOH–MeOH). mp 163—163.5°. IR ν_{max}^{KBr} cm^{-1}: 3580, 3450 (hydroxyl), 1722, 1234 (acetoxyl); ^{1}H NMR (100 MHz): shown in Table IV. (20*R*,22*S*)-5α-cholestane-3β,20,22-triol 3,22-diacetate (**41**) as colorless needles (from AcOEt–MeOH). mp 122—123.5°. IR ν_{max}^{KBr} cm^{-1}: 3500 (hydroxyl), 1721, 1228 (acetoxyl); ^{1}H NMR (100 MHz): shown in Table IV. (20*S*,22*S*)-5α-cholestane-3β,20,22-triol 3,22-diacetate (**48**) as colorless needles (from MeOH). mp 125—126.5°. IR ν_{max}^{KBr} cm^{-1}: 3500 (hydroxyl), 1724, 1234 (acetoxyl); ^{1}H NMR (100 MHz): shown in Table IV. (20*S*,22*R*)-5α-cholestane-3β,20,22-triol 3,22-diacetate (**49**) as colorless prisms (from AcOEt–MeOH). mp 115—117°. IR ν_{max}^{KBr} cm^{-1}: 3450 (hydroxyl), 1715, 1234 (acetoxyl); ^{1}H NMR (100 MHz): shown in Table IV.

b) Each triol (**38**, **39**, **46**, or **47**) (10 mg) in C_5D_5N (0.3 ml) was made to react with Ac_2O (0.003 ml) at 26°. The acetylation rate was determined by measuring the ^{1}H NMR spectra of the reaction mixture periodically (Fig. 1).

Acetonide Formation followed by Acetylation of the Cholestane-3β,20,22,25-tetraols and the Cholestane-3β,20,22-triols——a) Each tetraol (**15** or **32**) or each triol (**38**, **39**, **46**, or **47**) (0.03 g) in acetone (25 ml) containing *p*-TsOH (0.03 g) was stirred at room temperature for 2 hr. The mixture was diluted with water and extracted with AcOEt. Each product was chromatographed over silica gel (8 g). Elution with benzene–AcOEt (40: 1) gave the corresponding 20,22-acetonide as a crystalline solid. The acetonide from the tetraol (**15**) was crystallized from MeOH as colorless needles. mp 235—236.5°. IR ν_{max}^{KBr} cm^{-1}: 3450 (hydroxyl); NMR (100 MHz): 3H s at 0.78 ($C_{(18)}\underline{H}_3$), 3H s at 0.80 ($C_{(19)}\underline{H}_3$), 3H s at 1.15 ($C_{(21)}\underline{H}_3$), 6H s at 1.22 ($C_{(26)}\underline{H}_3$, $C_{(27)}\underline{H}_3$), 6H s at 1.29, 1.40 (–O–C($C\underline{H}_3$)$_2$–O–), 1H m at 3.50 ($C_{(3)}\underline{H}$), 1H dd The at 3.63 (*J* 3, 9, $C_{(22)}\underline{H}$). acetonide from the triol (**46**) was crystallized from MeOH as colorless needles. mp 113—115°. IR ν_{max}^{KBr} cm^{-1}: 3450 (hydroxyl); NMR (100 MHz): 3H s at 0.80 ($C_{(18)}\underline{H}_3$), 3H s at 0.80 ($C_{(19)}\underline{H}_3$), 6H d at 0.91 (*J* 6, $C_{(26)}\underline{H}_3$, $C_{(27)}\underline{H}_3$), 3H s at 1.37 ($C_{(21)}\underline{H}_3$), 6H s at 1.37, 1.48 (–O–C($C\underline{H}_3$)$_2$–O–), 1H m at 3.55 ($C_{(3)}\underline{H}$), 1H dd at 3.65 (*J* 3, 9, $C_{(22)}\underline{H}$).

Each monoacetonide (0.03 g) and Ac_2O (0.4 ml) in pyridine (0.8 ml) were set aside at room temperature overnight. After working up in the usual manner, each product was chromatographed over silica gel (1 g). Elution with benzene–AcOEt (80: 1) gave the respective 20,22-acetonide 3-acetate.

(20*R*,22*R*)-5α-Cholestane-3β,20,22,25-tetraol 20,22-acetonide 3-acetate (**19**) as colorless leaflets (from hexane-acetone). mp 160—163°. IR ν_{max}^{KBr} cm^{-1}: 1726, 1708, 1239 (acetoxyl); ^{1}H NMR (100 MHz): shown in Table V. (20*S*,22*S*)-5α-Cholestane-3β,20,22,25-tetraol 20,22-acetonide 3-acetate (**34**) as colorless amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1727, 1242 (acetoxyl); ^{1}H NMR (100 MHz): shown in Table V. (20*R*,22*R*)-5α-Cholestane-3β,20,22-triol 20,22-acetonide 3-acetate (**42**) as colorless needles (from $CHCl_3$–MeOH) mp 155—156.5°. IR ν_{max}^{KBr} cm^{-1}: 1728, 1235 (acetoxyl); ^{1}H NMR (100 MHz): shown in Table V. (20*R*,22*S*)-5α-cholestane-3β,20,22-triol 20,22-acetonide 3-acetate (**43**) as colorless needles (from MeOH). mp 71—72°. IR ν_{max}^{KBr} cm^{-1}: 1729, 1232 (acetoxyl); ^{1}H NMR (100 MHz): shown in Table V. (20*S*,22*S*)-5α-Cholestane-3β,20,22-triol 20,22-acetonide 3-acetate (**50**) as colorless amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1723, 1238 (acetoxyl); ^{1}H NMR (100

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MHz): shown in Table V. (20*S*,22*R*)-5α-Cholestane-3β,20,22-triol 20,22-acetonide 3-acetate (**51**) as colorless needles (from hexane–acetone). mp 178—179.5°. IR ν_{max}^{KBr} cm^{-1}: 1728, 1231 (acetoxyl); ^{1}H NMR (100 MHz): shown in Table V.

b) The tetraols (**15** and **32**) and the triols (**38**, **39**, **46**, and **47**) were converted into the corresponding acetonide-d_6 3-acetates (**20**, **35**, **52**, **53**, **54**, and **55**, respectively) by similar procedures described above but instead using acetone-d_6. ^{1}H NMR (100 MHz): shown in Table V.

Periodate Oxidation of the Cholestane-3β,20,22-triols——a) Each cholestanetriol (**38**, **39**, **46**, or **47**) (5 mg) in MeOH (3 ml) was treated with $NaIO_4$ (10 mg) in water (3 ml) under stirring at room temperature for 30 min. Isolation as usual and crystallization from MeOH–acetone afforded pregnan-20-on-3β-ol (**5**) as colorless needles. The idendity was confirmed in the usual criteria (TLC, IR, and ^{1}H NMR).

b) Each triol (**38**, **39**, **46**, or **47**) (5 mg) in CD_3OD (0.3 ml) containing C_5D_5N (0.05 ml) was made to react with $NaIO_4$ (0.2 mg) in D_2O (0.02 ml) at 24°. The oxidation rate was dertermined by measuring the ^{1}H NMR spectra of the reaction mixture periodically (Fig. 2).

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