SYNTHESIS OF STEROLS WITH MODIFIED SIDE CHAINS

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ABSTRACT

Synthesis of sterols with side chain containing from four to nine carbons are described.

Earlier gc - ms examination in our laboratory of a number of extracts from marine sources indicated the presence of trace amounts of unusual sterols containing 23, 24 and 25 carbons in addition to the more common C_{26} and C_{27} sterols. The availability of very small amounts of these compounds as well as the difficulties associated with the isolation and differentiation of various isomers by gc - ms clearly emphasize the need for the preparation of authentic samples. We now describe the synthesis, physical characteristics and chromatographic behavior of sterols 1 - 13 which either are naturally occurring or might be encountered in the future in marine sources.

22-Trans-24-norcholesta-5,22-dien-3β-ol (8) was first isolated by Idler, et al.[1], from the scallop Placopecten magellanicus, and since then its wide distribution in a number of marine animals has been recorded by others.[2] More recently, the isolation of 22-trans-27-nor-(24S)-24-methylcholesta-5,22-dien-3β-ol (12) - an isomer of cholesterol - and 22-trans-27-nor-(24S)-24-methylcholesta-7,22-dien-3β-ol[3] (14) has been described. The former (12) could conceivably serve as a biogenetic precursor for the C-26 sterol 8.

As a general preparative procedure solvolysis of stigmasterol tosylate[4] (17) in methanol furnished the i-methyl ether 18 which on sub-

sequent ozonization in dichloromethane-pyridine gave the aldehyde 19. Wittig reaction with the appropriate phosphorane in ether furnished a cis-trans mixture of Δ^{22}-steroidal i-Me ethers 20 which on refluxing with fused zinc acetate in acetic acid yielded the sterol acetates 1a - 13a. Separation of cis and trans isomers was performed by column chromatography over silica gel followed by repeated preparative thin layer chromatography over 15% silver nitrate impregnated silica gel using benzene-hexane (2-8 multiple developments) as irrigant.

Physical characteristics and retention times relative to cholesterol are given in Table 1. Pairs of cis and trans isomers of sterols depicted essentially identical mass spectra and in some cases could only be distinguished by silver nitrate impregnated silica gel thin layer chromatography (e.g., 2 and 3, where no discernible differences on gc relative retention times could be observed). Similarly sterols 6, 7, 8, 9 and 10, 11, 13 furnished similar mass spectra (see Table II).

The ions corresponding to cleavage of the C-22,23 bond with transfer of one hydrogen (m/e 300) and to loss of side chain (m/e 273) with transfer of two hydrogens (m/e 271) had comparable intensities in compounds 6, 7, 8, 9 and 10, 11 and 13 (see Table II).

Pairs of cis and trans isomers depicted marked differences in their nmr spectra. Thus, the C-18 methyl signal invariably appeared at 0.69-0.70 δ in the trans isomers and at 0.72-0.73 δ in their cis counterparts. Although it was not possible at 100 MHz to identify the multiplicity of the C-22,23 olefinic proton signals, the cis isomers generally furnished better resolved structure at 5.0 - 5.3 δ, while these signals were complex and shifted to 5.20 - 5.46 δ in the corresponding

trans compound. This should be helpful in future assignment of geometry of the Δ^{22} double bond in 22-unsaturated steroids.

In conclusion, it is apparent that for conclusive identification of marine sterols, gc-retention times and mass spectra alone are not sufficient.

EXPERIMENTAL

Melting points were determined with a Kofler hot stage apparatus and are uncorrected. Infrared spectra were obtained for a solution in chloroform or as potassium bromide pellets with a Perkin-Elmer 421 spectrometer. ^{1}H nmr spectra were obtained in deuteriochloroform as solvent and tetramethyl silane as internal reference on a Varian XL-100 spectrometer. Optical rotations were recorded with a Perkin-Elmer model 141 spectropolarimeter for solution in chloroform. Low resolution mass spectra were obtained by Messrs. R. G. Ross and R. Conover with A.E.I. MS-9 and Atlas CH-4 spectrometers. GLC-MS were obtained by Miss Annemarie Wegmann using a Varian Mat 711 spectrometer. Gas liquid chromatography was carried out with a Hewlett Packard hp 402 instrument. High resolution measurements were obtained with an MS-9 instrument by peak matching using perfluoro kerosene as a standard or with a Mat 711 instrument on line to the ACME Computer facility of the Stanford University Medical Center.

General Synthetic Procedure

The following is a modification of the procedure described in ref. 6. To a suspension of isoamyl triphenyl phosphonium bromide (4.80 g) in ether (50 ml) was added n-butyl lithium [2.1 N, 4.5 ml]. The mixture was stirred at room temperature for 1 hr and a solution of aldehyde prepared by ozonization of stigmasterol-i-methyl ether (2 g) in ether (20 ml) was added and the mixture stirred at room temperature for 48 hr and then poured into water and the aqueous phase extracted with hexane. The hexane-ether layer was washed with water, dried over sodium sulfate and evaporated to a gum. This gum was dissolved in acetic acid (30 ml) and heated under reflux with zinc acetate (4.0 g) for 5 hr. The mixture was poured into water and extracted with ether. The ether layer was washed with water dried over sodium sulfate and evaporated to a white solid. The separation of cis and trans isomers was effected by preparative thin layer chromatography over 15% silver nitrate impregnated silica gel. The physical constants and the spectral characteristics are given in Table I. The yields of cis and trans isomers were of the order of 30-50 and 50-70% respectively with the exception of 13 (>70%).

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Table I. Physical and Chromatographic Characteristics of Synthetic Sterols

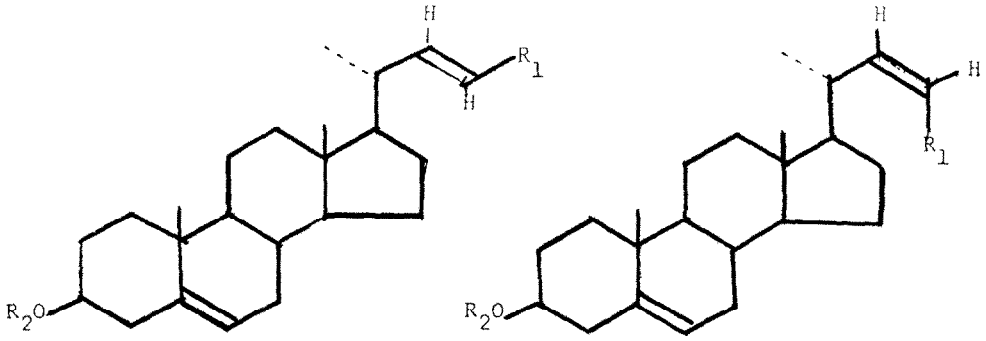
Compound	mp	$[\alpha]_D^{CHCl_3}$	C-22,23 H $[\frac{4.75\text{-}5.0}{5.50\text{-}6.0}]_\delta$	RRT+	Rf*
1	-	-		-	-
1a	102-105° (poor crystal)	-	-	0.49	0.15
2	121°	-62.7	5.25-5.43	0.44	-
2a	126-8°	-61.9	5.25-5.45	0.64	0.42
3	144°	-63.6	5.15-5.27	0.41	-
3a	149-50°	-71.8	5.15-5.30	0.64	0.32
4	120°	-59.1	5.24-5.45	0.55	-
4a	118-9°	-61.9	5.23-5.47	0.81	0.46
5	123-4°	-62.3	5.10-5.30	0.55	-
5a	121-3°	-71.4	5.10-5.30	0.80	0.35
6	122-3°	-	5.20-5.40	0.76	-
6a	166°	-62.0	5.20-5.36	1.08	0.49
7	121-2°	-	5.10-5.15	0.73	-
7a	111°	-63.5	5.10-5.15	1.06	0.41
8	143-4°[5a]	-64.0	5.10-5.30	0.65	-
8a	142-3°	-	4.95-5.10	-	0.32
9	161-63°[5b]	-6.5±2	-	-	-
9a	-	-	-	-	-
10	131-33°[6]	-60.6	5.21-5.31	0.95	-
10a	120-22°	-61.0	-	-	-
11	139-139.5°[5]	-63.0	5.12-5.27	0.89	-
11a	117-19°	-69.0	-	-	-
12	128-29°[2]	-43±2	5.08-5.20	-	-
12a	138-141°	-	-	-	-
13	147-48°[7]	-57.4	4.80-5.25	0.88	-
13a	132-33°	-	4.80-5.30	1.18	0.52
15	147-48°[8a]	-66	5.10-5.25	1.05	-
15a	-	-	5.10-5.25	-	-
16	134-36°[8b]	-	5.20-5.30	1.11	-
16a	138-39°	-	-	-	-

+ Gas Chromatographic retention times are relative to cholesterol on 3% OV3 column. Oven temperature 200°; Detector 300°; Injector 300°.

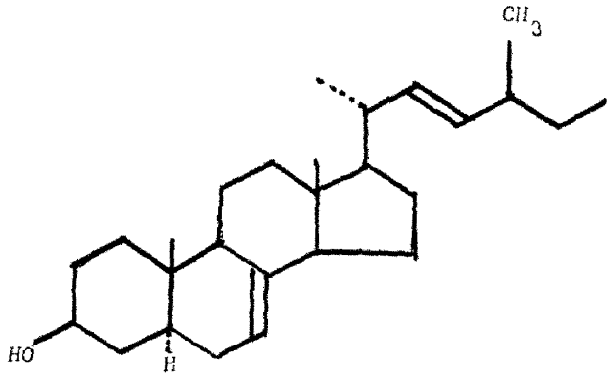
* Rf values are for 15% silver nitrate impregnated silica gel using benzene-hexane (1-1) as the irrigant (one development).

Table II. Partial Mass Spectra of Sterols.

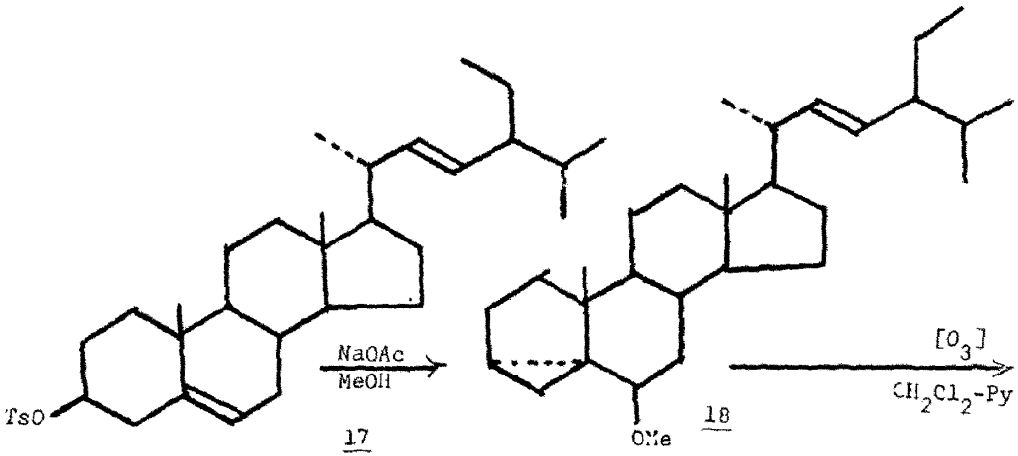
Compound	M^+ (%)	m/e 300	m/e 273	m/e 271	m/e 213
6	26.0	18.0	10.0	12.0	10.0
7	16	6.0	4.0	10.0	8.0
8	65	26	20	20	13.0
9	67	49	20	18	25
10	38	18	22	12	14
11	38	21	21	13	14
13	38	20	21	16	15



1 R_1=R_2=H
1a R_1=H; R_2=CH_3CO
2 R_1=CH_3; R_2=H
2a R_1=CH_3; R_2=CH_3CO
4 R_1=C_2H_5; R_2=H
4a R_1=C_2H_5; R_2=CH_3CO
6 R_1=n-C_3H_7; R_2=H
6a R_1=n-C_3H_7; R_2=CH_3CO
8 R_1=$(CH_3)_2CH$; R_2=H
8a R_1=$(CH_3)_2CH$; R_2=CH_3CO
10 R_1=$(CH_3)_2CH\ CH_2$; R_2=H
10a R_1=$(CH_3)_2CH\ CH_2$; R_2=CH_3CO
12 R_1=$CH_3CH_2CH(CH_3)$-; R_2=H; 24S
12a R_1=$CH_3CH_2CH(CH_3)$-; R_2=CH_3CO; 24S
15 R_1=$(CH_3)_2CH\ CH(CH_3)$-; R_2=H; 24R
3 R_1=CH_3; R_2=H
3a R_1=CH_3; R_2=CH_3CO
5 R_1=C_2H_5; R_2=H
5a R_1=C_2H_5; R_2=CH_3CO
7 R_1=n-C_3H_7; R_2=H
7a R_1=n-C_3H_7; R_2=CH_3CO
9 R_1=$(CH_3)_2CH$; R_2=H
9a R_1=$(CH_3)_2CH$; R_2=CH_3CO
11 R_1=$(CH_3)_2CH\ CH_2$; R_2=H
11a R_1=$(CH_3)_2CH\ CH_2$; R_2=CH_3CO
13 R_1=$CH_3CH_2CH(CH_3)$-; R_2=H; 24S
13a R_1=$CH_3CH_2CH(CH_3)$-; R_2=CH_3CO; 24S
16 R_1=$(CH_3)CH\ CH(CH_3)$-; R_2=H; 24S


CH3
HO
H

14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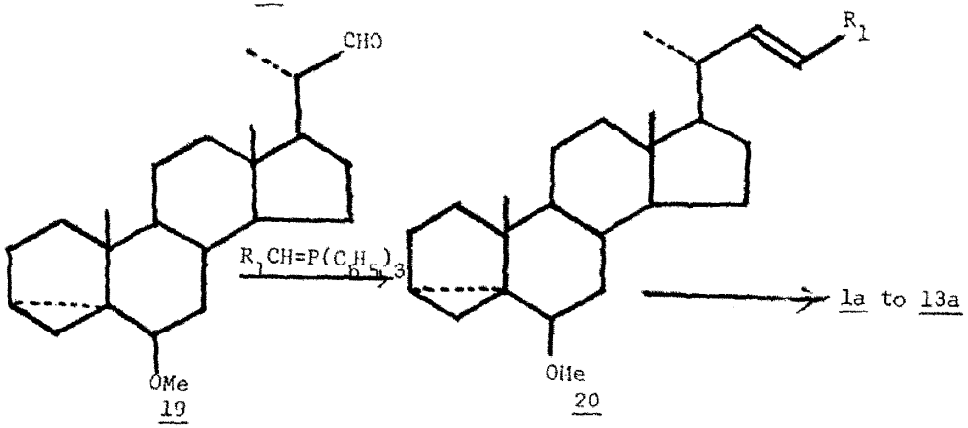
17
OMe
18
[O3]
CH2Cl2-Py


CHO
R1CH=P(C6H5)3
OMe
19
R1
OMe
20
1a to 13a

REFERENCES

1. Idler, D. R., Wiseman, P. M. and Safe, L. M., STEROIDS, 16, 451 (1971).

2. Kobayashi, M. and Mitsuhashi, H., STEROIDS, 24, 399 (1974).

3. Kobayashi, M. and Mitsuhashi, H., TETRAHEDRON, 30, 2147 (1974).

4. Winstein, S. and Schlesinger, A. H., J. AMER. CHEM. SOC., 70, 3528 (1948).

5. a. Fryberg, M., Oehlschlager, A. C. and Unrau, A. M., J.C.S. CHEM. COMM., 1194 (1971).
   b. Metayer, A. and Barbier, M., J. CHEM. SOC., 424 (1973).

6. Huchins, R. F. H., Thomson, M. J. and Svoboda, J. A., STEROIDS, 15, 113 (1970).

7. We are thankful to Professor H. S. Mosher for a sample of 2-S-2-methyl-1-butanol which after conversion to its bromide (Wiley, G. A., Hershkowitz, R. L., Rein, B. M. and Chunge, B. D., J. AMER. CHEM. SOC., 86, 964 (1964)), was reacted with triphenylphosphine in benzene to furnish the corresponding phosphonium salt.

8. a. Sheikh, Y. M., Leclercq, J. and Djerassi, C., J. CHEM. SOC. Perkin 1, 909 (1974).
   b. Thomson, M. J., Cohen, C. F. and Lancaster, S. M., STEROIDS, 5, 745 (1965).
   c. Teshima, Shin-ichi, Kanazawa, A. and Ando, T., BIOCHEM. PHYSIOL, 47b, 507 (1974).