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ISOLATION OF 22-METHYLENECHOLESTEROL. FURTHER EVIDENCE FOR DIRECT BIOALKYLATION OF 22-DEHYDROCHOLESTEROL

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Abstract: The first isolation of a 22-monoalkylated analog of cholesterol, i.e., 22-methylenecholesterol is reported and the biosynthetic implications of this observation discussed.

Our recent isolation[2] of 22(S),23(S)-methylenecholesterol (4) constitutes the first evidence for direct bioalkylation of 22-dehydrocholesterol (1) without the intermediacy of a 24-methyl substituent. Presumably,[3] the first biosynthetic step is biomethylation by S-adenosylmethionine (SAM) to provide 2 or 3, which loses a C-28 proton to produce the cyclopropane 4. Since no 22-monoalkyl sterol has so far been encountered in nature, whereas 22-dehydro-23-methylcholesterol (5) has been detected recently,[4] one might be inclined towards the cation 3 as the preferred biosynthetic intermediate to 4. However caution has to be exercised, because the 23-methylated sterol 5 need not necessarily arise by direct SAM alkylation of 22-dehydrocholesterol (1) via 3, but may also arise in nature by dealkylation of the C-24 substituent in the naturally occurring[5] 23,24-dimethyl-22-dehydrocholesterol (6). We now report the first isolation[6] of a 22-monoalkylated cholesterol derivative - 22-methylenecholesterol (8) - whose eventual detection we had predicted several years ago[7] and which considerably narrows the plausible biosynthetic options. In the absence of actual tracer experiments, it is theoretically feasible that 22-methylenecholesterol (8) does not actually arise from C-22 SAM bioalkylation but rather by initial C-23 attachment (via 3 and 4). However, it seems much more likely that its biosynthetic origin mimics the well established[3] biosynthesis of 24-methylenecholesterol (12) from desmosterol (11) in that initial SAM attack of 1 leads to the 22-methyl cation 2, which then undergoes a 1,2-hydrogen shift to the more stable cation 7, followed by loss of a C-28 proton.

The complicated sterol mixture of the Black Sea (Bulgarian coast) sponge Halichondria panicea was first subjected to separation by silica gel tlc and digitonin precipitation[8] followed by HPLC using a reverse phase Whatman Partisil M9 10/50 ODS-2 column with absolute methanol as solvent. The peak with relative retention time (rrt in HPLC) 0.719 (cholesterol = 1.00) proved to be homogeneous by GLC (rrt 1.13 on a 3% OV17 column at 260°C) and was shown by chromatographic

criteria, 360 MHz PMR spectroscopy (C-6 H, 5.351; C-18 methyl, 0.704; C-19 methyl, 1.016; C-21 methyl, 1.039 (d, J = 6.77 Hz); C-26,27 methyl (6 H), 0.902 (d, J = 6.59 Hz), C-28 methylene, 4.612 and 4.695) and characteristic mass spectrometric fragmentation (notably the two intense peaks at m/z 342 and 272 associated with the two alternative McLafferty rearrangement processes involving migration of the C-25 or C-16 protons respectively) to be completely identical with a synthetic specimen of 22-methylenecholesterol (8) prepared as follows.

6β-Methoxy-3α,5α-cyclocholestan-22-one (13)[9] was subjected to Wittig condensation (benzene, 40 h reflux, 80% yield) and the i-methyl ether protecting group of the oily product 14 cleaved in 70% yield by heating under reflux with pTSA in aqu. dioxane to give 22-methylenecholesterol (8) mp 114-116°C, $[\alpha]_D$ -71° ($CHCl_3$), high resolution mass spectrum: m/z 398.3547,(27% ($C_{28}H_{46}O,M^+$); 342.2912, 100% ($C_{24}H_{38}O$); 324.2808, 15.83% ($C_{24}H_{36}$); 313.2872, 2.84% ($C_{23}H_{37}$); 287.2738, 6.17% ($C_{21}H_{35}$); 273.2187, 29.36% ($C_{19}H_{29}O$); 272.2132, 74% ($C_{19}H_{28}O$); 271.2058, 10% ($C_{19}H_{27}O$); 255.2109, 34.73% ($C_{19}H_{27}$); 213.1658, 14.44% ($C_{16}H_{21}$). The PMR spectrum and chromatographic mobility data were identical with those of the natural product. From the mother liquors of 8, there was isolated by HPLC the 21-isomer 15, m.p. 150-151°C, $[\alpha]_D$ -24° ($CHCl_3$), whose mass spectrum was indistinguishable from that of its "natural" isomer 8, but which displayed characteristic PMR ($CDCl_3$) shifts (C-18 methyl, 0.665; C-19 methyl, 0.988; C-21 methyl, 0.944 (d, J = 6.88 Hz); C-26,27 methyl, (6 H), 0.909 (d, J = 6.59 Hz); C-28 (2 H), 4.638 and 4.781).

As outlined in the introduction, the most plausible biosynthetic route to 22-methylenecholesterol (8) is loss of the C-28 proton of the precursor 7. However, alternative proton loss from the other two neighboring carbon atoms is also conceivable and in order to learn something about the properties and relative stability of the resulting double bond isomers 9 and 10, we subjected 22-methylenecholesterol (8) to isomerization with N-lithioethylenediamine[10] at 115°C. After 2 h, aside from 6% of starting material (8), there was obtained 77% of the 22E (9a) and 6% of the 22Z (9b) isomers,[11] together with 8% of the Δ^4-isomer of 9a. Increasing the reflux time to 24 h increased slightly the proportion of the last two compounds at the expense of 9a, but did not produce any of the tetra-substituted olefin 10. Essentially the same equilibrium mixture was obtained when the 22E isomer 9a rather than the 22-methylene analog 8 was subjected to the isomerization conditions.

The isolation of 22-methylenecholesterol (8) from a natural source greatly increases the likelihood that other 22-alkyl sterols will eventually be found. By analogy[3] to 24-methylenecholesterol (12), it is likely that 8 could be the biosynthetic precursor to saturated 22-methylcholesterols and by further biomethylation to 23-ethylidene or 23-ethyl homologs. We are synthesizing such sterols by stereoselective means in order to facilitate their eventual recognition in nature.

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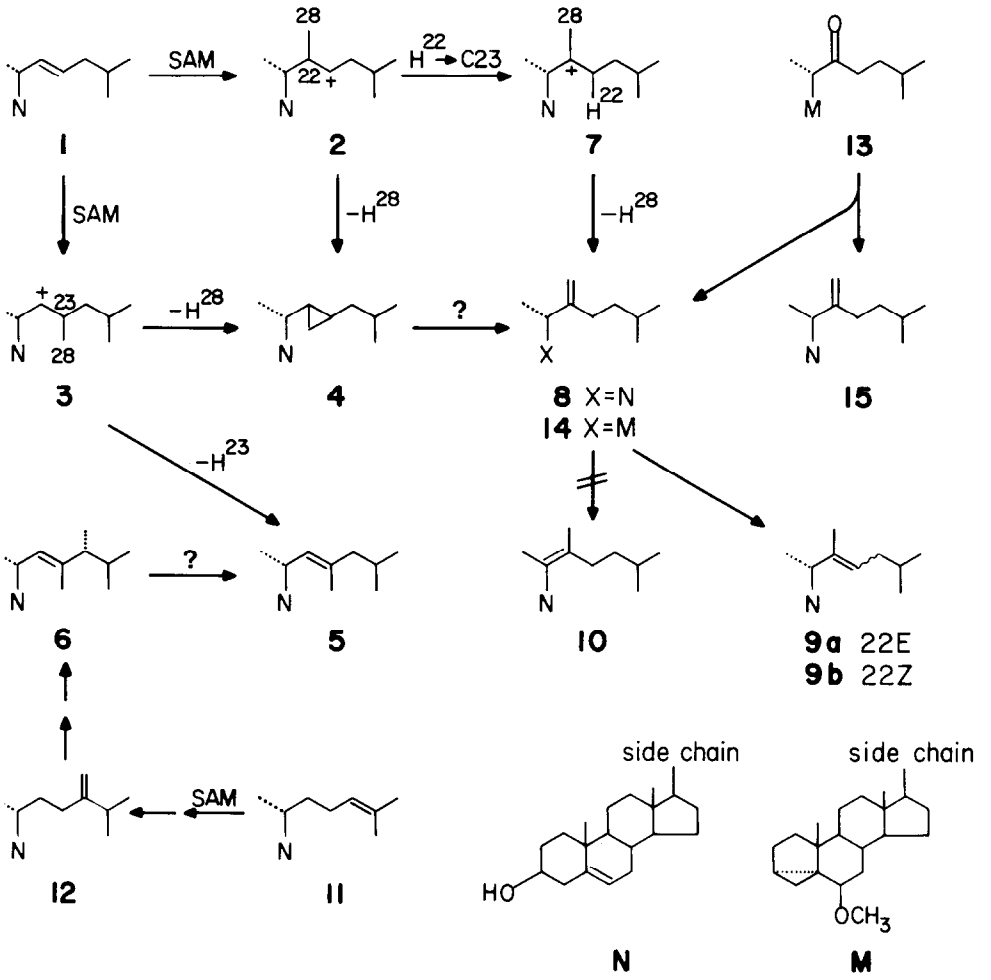
SAM
28
22
H → C23
1
2
7
13
SAM
−H28
−H28
23
−H28
?
3
4
8 X=N
14 X=M
15
−H23
?
6
5
10
9a 22E
9b 22Z
SAM
12
11
side chain
side chain
HO
OCH3
N
M

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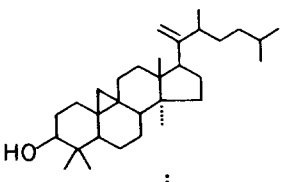

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11. The two isomers are readily separable by HPLC and distinguishable by PMR spectroscopy as will be reported in the detailed paper.

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