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AN EFFICIENT SYNTHESIS OF 25-HYDROXYCHOLESTEROL FROM STIGMASTEROL

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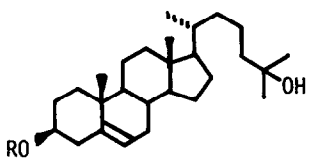
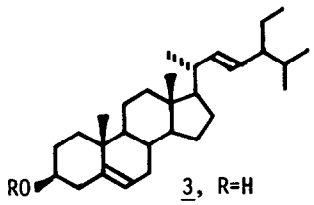
A synthesis of 25-hydroxycholesterol (1) from stigmasterol (3) in an overall yield of 30 percent in seven steps has been published recently.[1] We report here a particularly facile process which proceeds from stigmasterol, also in seven steps, to either the 3-alcohol (1) or 3-acetate (2) with an overall yield of greater than 50 percent. Although seven discrete chemical steps were employed, only three isolations by crystallization were necessary thus streamlining the entire sequence.

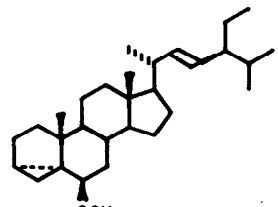
The first three steps of the sequences are common, namely the protection of the 3β-hydroxy-Δ^5-system as the 3α,5α-cyclo-6β-methoxy system. Accordingly, stigmasterol was converted with pyridine and tosyl chloride to the tosylate (4).[2] The yield was essentially quantitative.



1, R=H
2, R=CH_3CO



3, R=H
4, R=Ts
5, R=CH_3



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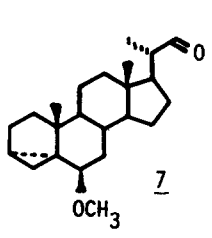
The tosylate (4) was then transformed into the stigmasteryl-i-ether (6) by boiling in methanol in presence of potassium acetate.[2] At the end of the reaction (*ca.* 20 hours) the reaction was cooled, water was added, and the stigmasteryl-i-ether extracted into hexane. After drying, the extracts were evaporated to yield a semi-solid residue. Although a small amount of stigmasteryl methyl ether (5) contaminates the product, it is unnecessary, and indeed inefficient to remove it. The weight of the residual material corresponded to an almost quantitative conversion.

Ozone was now passed through a solution of this crude stigmasteryl-i-ether in a mixture of methylene chloride and methanol (ratio 3:1) at -70° until the end of the reaction was indicated by a faint blue coloration. At this stage, tlc examination of the reaction still showed a small amount of material with the same R_f as starting material; this was undoubtedly sitosteryl-i-ether since sitosterol (22,23-dihydrostigmasterol) is a usual contaminant in commercial stigmasterol.[3] The peroxidic products of the reaction were reduced by trimethylphosphite[4], and the

reaction mixture washed with sodium sulfite solution (to ensure complete removal of peroxides) and then water. Evaporation of the solution yielded an oil which still contained some trimethyl phosphite, removed by redissolution of the oil in toluene and re-evaporation *in vacuo* at 65°. Although tlc of the residual oil indicated minor impurities, the nmr spectrum showed that the aldehyde (7)[5] was by far the major component R_f (5% ethyl acetate/hexane) - 0.25. Nmr ($CDCl_3$): δ 0.3-0.63m; 0.77s(3H); 1.03s(3H); 1.10d, J=ca. 6Hz(3H); 2.76m(1H); 3.33s(3H); 9.58d, J=3Hz(1H). Because of the comparative ease with which aldehyde (7) underwent epimerization at C-20 on prolonged standing (a few weeks at room temperature), it proved to be advisable to prepare it, and to carry on to the side chain elaboration as soon as possible.



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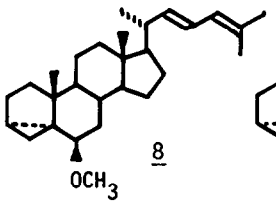

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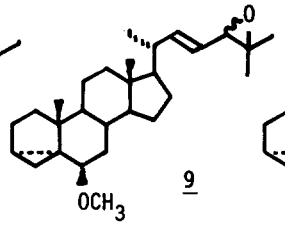

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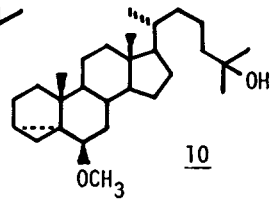

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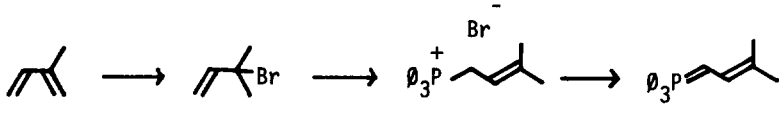
In order to introduce the side chain, we chose to make use of the ylide (13), readily and cheaply available from isoprene according to the scheme:



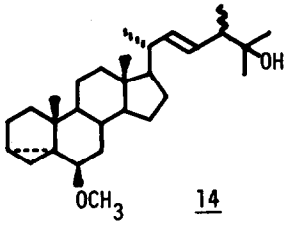
11 12 13

Thus, a solution of isoprene in methylene chloride is treated with one mole of hydrogen bromide at -10 to +5°. The resulting bromide (11) is then allowed to react with triphenylphosphine at room temperature for 2 hours. Addition of hexane then precipitates the salt (12) in 96% yield, based on the phosphine.

The ylide (13) was prepared from a suspension of the salt (12) in hexane by addition of n-butyl lithium, and was then condensed with the aldehyde (7) at 25-30°. Reaction was immediate and, at the end of the addition of the aldehyde to the ylide, the mixture was cooled to 0° before removal of the precipitated triphenyl phosphine oxide by filtration. The hexane solution was washed twice with methanol containing 10% water, dried, and evaporated to yield the diene (8)[6] as an oil R_f (10% ethyl acetate/hexane) - 0.5. Nmr ($CDCl_3$): δ 0.30-0.63m; 0.75s(3H); 1.03s(3H); 1.05d, J=6Hz(3H); 1.73s(6H); 2.77m(1H); 3.32s(3H); 5.18-6.38m(3H). It was expected that Δ^{22} would possess E-configuration.[6,7] The upfield region of the proton nmr spectrum of the diene does indeed suggest strongly that one geometrical isomer predominates (> 90%), but the vinyl region is too complex for simple interpretation. Nevertheless, solid information concerning the configuration of the Δ^{22}-double bond was forthcoming from the product of the next step of the sequence.

The carbon atoms of the cholestane side chain now in place, the oxygen function at C-25 was to be introduced next: a transformation which was the key step of the synthesis. This was

achieved by the selective epoxidation of the Δ^{24}-double bond. It had been anticipated that epoxidation of the diene (8) would be regioselective, first because of less steric hindrance to attack at Δ^{24}, and secondly, the trisubstituted Δ^{24} would be expected to undergo electrophilic attack more readily than the disubstituted Δ^{22}. Such proved to be the case. The diene (8) was allowed to react with peracetic acid, buffered with sodium acetate[8], in the presence of sodium carbonate in methylene chloride[9] for 30 minutes[10] at room temperature. The solution was now filtered and the filtrates washed sequentially with sodium sulfite solution, sodium bicarbonate solution, and then water. After filtration through a little sodium sulfate the solution was used directly for the next step. Evaporation of the solution yielded the epoxide (9) as an oil R_f (10% ethyl acetate/hexane) - 0.2. Nmr ($CDCl_3$): δ 0.30-0.63m; 0.75s(3H); 1.03s(3H); 1.05d, J=6Hz(3H); 1.27s(3H); 1.33s(3H); 2.77m(1H); 3.12d, J=7Hz(1H); 3.32s(3H); 5.53 octet, split AB system (2H). This nmr confirmed the regioselectivity of the epoxidation. In addition, the perturbed AB system in the vinyl region revealed the coupling J_{22-23}=16 Hz, confirming the E-configuration of Δ^{22}. Information concerning the stereoselectivity at C-24 was not obtainable from this nmr. Nevertheless, reaction of the epoxide with methyl magnesium chloride gave an approximately equal mixture of 24-R- and 24-S-methyl-25-hydroxy compounds (14) the 24-R-diastereoisomer being ultimately converted[11] to 25-hydroxy Vitamin D_2 (15).[12]



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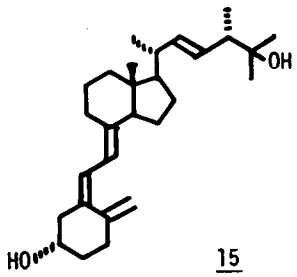

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Concomitant reduction of the Δ^{22}-double bond and the 24,-25-oxide in the epoxy-olefin (9) was accomplished by hydrogenation of the methylene chloride solution obtained from the previous step, under an atmosphere of hydrogen (15 psi) over 5% platinum on carbon catalyst at room temperature for 2 hours. After removal of the catalyst the solution was concentrated (to *ca.* 2.5 ml. per gram of tosylate (4) used originally) and then acetonitrile (5 ml. per gram of tosylate input) was added. Although tlc of this solution showed a number of impurities accumulated during the sequence, the desired product crystallized beautifully from this solvent mixture to give 3α,5α-cyclo-6β-methoxy-25-hydroxycholestane (10)[1,13] in 56% yield (based on stigmasteryl tosylate).

The reversal of the i-ether to the 3-acetate (2) is trivially straightforward, by heating at boiling point in acetic acid for 3 minutes, or at 70° for 3 hours. After conventional work-up and crystallization from acetone, the yield of 25-hydroxycholesteryl acetate is 94%. Alternatively, the i-ether may be converted to the 3,25-diol (1) by heating in aqueous dioxane in presence of tosic acid in greater than 90% yield.[1]

The overall yield of the diol (1) and the 3-acetate (2), from the raw material stigmasterol, is thus in each case greater than 50 percent.

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10. Prolonged reaction times were necessary before diepoxidation took place.
11. Unpublished work by J. A. Campbell and D. M. Squires.
12. T. Suda, H. F. DeLuca, H. K. Schnoes and J. W. Blunt, *Biochemistry*, 8, 3515 (1969).
13. For other syntheses of this compound evaluated by us in connection with our efforts in the Vitamin D metabolite area, see:
W. G. Salmond and K. D. Maisto, *Tetrahedron Letters*, in press, and W. G. Salmond, M. C. Sobala and K. D. Maisto, *Tetrahedron Letters*, in press.