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SYNTHESIS OF 26,27-DIFLUORO-25-HYDROXY- AND *(25R,S)*-27-FLUORO-25,26-DIHYDROXY-CHOLESTEROL DERIVATIVES FROM METHYL 3β-HYDROXY 5-CHOLENOATE

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SUMMARY

26,27-Difluoro-6β-methoxy-3α,5-cyclo-5α-cholestan-25-ol **1a** *and (25R,S)-27-fluoro-6β-methoxy-3α,5-cyclo-5α-cholestan-25,26-diol* **1b** *were obtained from methyl 3β-hydroxy-5-cholenoate* **2** *via opening of the oxirane ring of (25R,S)-25,26-epoxy-27-fluoro-6β-methoxy-3α,5-cyclo-5α-cholestan* **4a** *with tetrabutylammonium fluoride.*

INTRODUCTION

Recently it has been found that vitamins D_2 and D_3 inhibit proliferation of certain tumour cells [1]. In the search for vitamin D analogues possessing anti-leukemic activity it has been proposed to introduce a fluorine atom(s) at the metabolically active sites of the molecule, particularly at the side-chain [1a,2]. During the past decade many synthetic strategies have been elaborated to obtain vitamin D analogues with a fluorine atom at C-23, C-24, C-25, C-26, and C-27 positions [3]. Several of such fluorinated compounds inhibit growth of malignant cells; most of them are, however, also involved in calcium and phosphorus metabolism. One of these F-analogues, 26,27-F_6-1α-OH-vitamin D_3, greatly affects cell differentiation, whereas its hypercalcemic activity is low [4].

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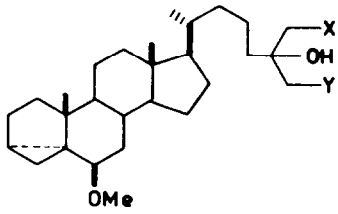
In this connection, at our laboratory it was attempted to synthesize cholesterol-type precursors of vitamin D_3 with a fluorine atom(s) located at the metabolically active sites of the side-chain. In a previous paper [5] we have reported preparation of *(25R,S)*-26-fluoro-5-cholesten-3ß,25-diol 3-acetate and its 27-alkyl C_1-C_3 homologues from methyl 3ß-hydroxy-5-cholenoate. Fluorine was introduced by our procedure [6] involving synthesis of ∝-fluoromethyl ketones *via* allene oxides. In continuation, we present the synthesis of 26,27-difluoro-25-hydroxy- and *(25R,S)*-27-fluoro-25,26-dihydroxy cholesterol derivatives 1a and 1b (scheme) from methyl 3ß-hydroxy-5-cholenoate 2. Compounds 1a and 1b may be considered as cholesterol precursors of 26,27-difluoro-1∝,25-dihydroxy- and *(25R,S)*-27-fluoro-1∝,25,26-trihydroxy- vitamin D_3, respectively. These two fluorine containing vitamin D_3 analogues may be expected to have anti-leukemic properties.

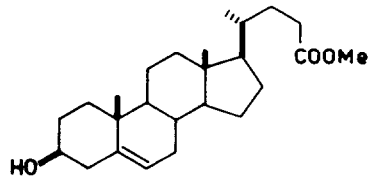
RESULTS

Fluoroketone 3, the key compound in our synthesis of *(25R,S)*-26-fluoro-5-cholesten-3ß,25-diol 3-acetate and its 27-alkyl homologues, has been obtained [5] from methyl 3ß-hydroxy-5-cholenoate 2 in eight steps with the overall yield of 40%. At present, we report application of 3 for the synthesis of 26,27-difluoro-25-hydroxy- and 27-fluoro-25,26-dihydroxy-cholesterol derivatives 1a and 1b. The strategy of the synthesis required transformation of the carbonyl group of fluoroketone 3 into the epoxide group, followed by its cleavage with fluoride anions.

Treatment of 26-fluoro-6ß-methoxy-3∝,5-cyclo-27-nor-5∝-cholestan-25-one 3 with an ylide - generated from trimethylsulphoxonium iodide $[(CH_3)_3S(O)I]$ and sodium hydride (NaH) in dimethylsulphoxide (DMSO) - afforded 27-fluoro-25,26-epoxide 4a in 83% yield. Compound 4a was obtained as a mixture of epimers (1:1) at C-25. The presence of epimers in epoxide 4a was confirmed by the ^{19}F NMR (470 MHz) spectrum in which signals of C-27 fluorine appeared as two doublets of triplets $J_1(HF)$=48 Hz, $J_2(HF)$=4 Hz at δ -228.447 and -228.450 ppm, respectively. Tetrabutylammonium fluoride trihydrate ($TBAF \cdot 3H_2O$), readily



1 a. X=Y=F
b. X=F, Y=OH
c. X=Y=OH



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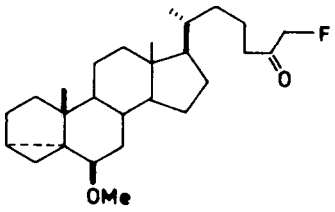

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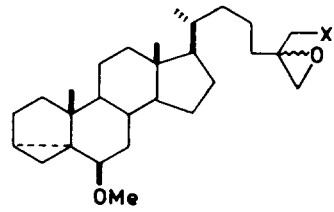

4 a. X=F
b. X=OH

Scheme.

soluble in tetrahydrofuran (THF), a source of nucleophilic fluoride, was chosen for opening of the oxirane ring of 4a. Thus, the reaction of 4a (1 mmol) with TBAF·$3H_2O$ (1.2 mmol) in boiling THF over 8 hours led to a mixture of four products and a small amount of starting material (5%). These compounds were separated by column chromatography on silica gel and their structures were assigned by spectroscopic methods.

The major, less polar product (43%) exhibited the structure of expected 26,27-difluoro-6ß-methoxy-3∝,5-cyclo-5∝-cholestan-25-ol 1a. In its ^{19}F NMR spectrum, the fluorines of C-26 and C-27 appeared at δ -231.756 and -231.780 ppm (two triplets, J(HF)=48Hz). The structure of the second product which was obtained in 36% yield, was shown to be *(25R,S)*-27-fluoro-6ß-methoxy-3∝,5-cyclo-5∝-cholestan-25,26-diol 1b. In the ^1H NMR

(500 MHz) spectrum of 1b, protons of C-27 were present as two doublets (one proton each) at δ 4.375 and 4.377 ppm with geminal H-F coupling J=47.49 Hz. The remaining two products, isolated in minute amounts (5% each), contained no fluorine. Their structures were assigned as *(25R,S)*-6ß-methoxy-25,26-epoxy-3α,5-cyclo-5α-cholestan-27-ol 4b and 6ß-methoxy-3α,5-cyclo-5α-cholestan-25,26,27-triol 1c.

For optimization of conditions for the synthesis of 1a (and possibly 1b), it was necessary to elucidate the role of TBAF $3H_2O$ in the cleavage of the oxirane ring in 4a. Thus, treatment of 1a (1 mmol) with TBAF·$3H_2O$ (1.2 mmol) in THF for 8 hours afforded a mixture of the following five compounds: 1a (54%), 1b (29%), 1c (11%), 4a (6%), and 4b (4%). Likewise, refluxing of hydroxy-epoxide 4b (1 mmol) and TBAF·$3H_2O$ (1.2 mmol) in THF yielded compounds: 1a (6%), 1b (8%), 1c (34%), 4a (32%), and 4b (13%). In the above reactions, prolongation of refluxing time gradually increased the yield of 1c, to finally lead to exclusive formation of 1c.

The above experiments exemplified the dual behaviour of fluoride ions: as a nucleophile (in cleavage of the oxirane ring 4a → 1a) and as a base (permitting formation of epoxides (1a → 4a and 1a → 4b). The method used for opening the oxirane ring of 4a with TBAF·$3H_2O$ gave neither 1a or 1b as single products (because of competition between F^- and H_2O. However, if TBAF $3H_2O$ was replaced by anhydrous TBAF [7], compound 1a was obtained in high yield (83%).

Summing up, two new fluorine analogues of 25-hydroxy-cholesterol, 26,27-difluoro-6ß-methoxy-3α,5-cyclo-5α-cholestan-25-ol 1a and *(25R,S)*-27-fluoro-6ß-methoxy-3α,5-cyclo-5α-cholestan-25,26-diol 1b were synthesized from methyl 3β-hydroxy-5-cholenoate 2 with the aim of serving as precursors of F-analogues of 25-hydroxy vitamin D_3.

EXPERIMENTAL

Melting points were recorded on a Kofler hot-stage apparatus and are uncorrected. The spectra were recorded using the following equipment: IR spectra - Beckman 4240 or Unicam SP 200, ^1H NMR spectra - Bruker AM 500 (500 MHz, in $CDCl_3$ solution),

^{19}F NMR spectra - Bruker AM 500 (470 MHz, in $CDCl_3$ solution), mass spectra (high resolution at 70 eV ionisation potential) - Finnigan MAT 8200. Chemical shifts were recorded in δ units (ppm), downfield shift from Me_4Si (^1H) and $CFCl_3$ (^{19}F). Column chromatography was performed on Kieselgel 60 (70-230 mesh), Merck, and TLC - on aluminium sheets, Kieselgel 60, Merck. Organic solutions were dried over anhydrous $MgSO_4$ and solvents were evaporated under reduced pressure on a rotary evaporator. Yields refer to homogeneous products (TLC).

(25R,S)-27-Fluoro-25,26-epoxy-6ß-methoxy-3∝,5-cyclo-5∝-cholestan 4a

To an ylide, which was prepared in DMSO (2 ml) at room temperature and under argon from $(CH_3)_3S(O)I$ (660 mg, 3 mmol) and NaH (145 mg, 50% dispersion in oil, 3 mmol), ketone 3 (418 mg, 1 mmol) was added in DMSO (3 ml). The reaction mixture was stirred at room temperature for 2 hours, whereupon water was added and the product was extracted with Et_2O. The ether layer was washed 3 times with water and dried. After evaporation of solvent the residue was chromatographed on a silica gel column (10 g) with hexane-Et_2O (98:2) as eluent, to give epoxide 4a (359 mg, 83%), m.p.=69-72 °C (hexane-Et_2O).
^1H NMR, δ: 0.430 (1H, dd, J_1=5.05 Hz, J_2=7.95 Hz, cyclopropyl-H) 0.645 (1H, dd, J_1=3.98 Hz, J_2=4.98 Hz, cyclopropyl-H), 0.713 (3H, s, 18-H), 0.919 (3H, d, J=6.60 Hz, 21-H), 1.021 (3H, s, 19-H), 2.72 (1H, dd, J_1=4.35 Hz, J_2=8.29 Hz, 26-H of one epimer) 2.75 (1H, d, J=1.95 Hz, 26-H of second epimer), 2.76 (1H, t, J= 2.96 Hz, 6-H), 3.323 (3H, s, OMe), 4.351 and 4.362 (1H, 2dd, J(HH)=10.24 Hz, J(HF)=47.38 Hz, 27-H), 4.467 and 4.777 [1H, 2dd, J(HH)=10.24 Hz, J(HF)=47.63 Hz].
^{19}F NMR, δ: -228.447 and -228.450 [2dt, J(HF)=48Hz, J(HF)=4 Hz].
m/e: 432 (M^+, 38%), 417 (M-15, 31%), 400 (M-32, 44%), 377 (49%), 57 (100%);
High resolution: for $C_{28}H_{45}O_2F$ calculated - 432.3404
found - 432.3404
Elemental analysis: for $C_{28}H_{45}O_2F$ calculated C-77.73%, H-10.48%
found C-77.86%, H-10.29%

Reaction of 4a with TBAF·$3H_2O$

A solution of epoxide 4a (432 mg, 1 mmol) and TBAF·$3H_2O$ (379 mg, 1.2 mmol) in THF (5 ml) was refluxed for 8 hours, whereupon water was added and the products were extracted with Et_2O. The ether layer was washed 3 times with water and dried. Subsequently, the solvent was evaporated *in vacuo* and the residue was chromatographed on a silica gel column with the following eluates:

1) hexane-Et_2O (98:2) to give 4a (22 mg, 5%);

2) hexane-Et_2O (98:2) to give 26,27-difluoro-6ß-methoxy-3∝,5-cyclo-5∝-cholestan-25-ol 1a (194 mg, 43%),
m.p.=168-172 °C (hexane-Et_2O);
IR ($CHCl_3$): 3600 (OH) cm^{-1};
^1H NMR, δ: 0.430 (1H, dd, J_1=5.05 Hz, J_2=7.96 Hz, cyclopropyl-H) 0.645 (1H, dd, J_1=3.98 Hz, J_2=4.98 Hz, cyclopropyl-H), 0.715 (3H, s, 18-H), 0.926 (3H, d, J=6.58 Hz, 21-H), 1.021 (3H, s, 19-H), 2.77 (1H, t, J=2.80 Hz, 6-H), 3.324 (3H, s, OMe), 4.341 [2H, tdd, J(HF)=47.48 Hz, J_1(HH)=9.31 Hz, J_2(HH)=1.43 Hz, 26-H and 27-H], 4.377 [2H, ddd, J(HF)=47.06 Hz, J_1(HH)=9.43 Hz, J_2(HH)=1.85 Hz, 26-H and 27-H];
^{19}F NMR, δ: -231.756 and -231.780 [2t, J(HF)=48 Hz, 26-F and 27-F];
m/e: 452 (M^+, 23%), 437 (M-15, 12%), 420 (M-32, 18%), 397 (M-55, 33%), 215 (41%), 156 (30%), 125 (35%), 111 (48%), 97 (59%), 85 (60%), 83 (60%), 71 (77%), 57 (100%);
High resolution: for $C_{28}H_{46}O_2F_2$ calculated - 452.3466
found - 452.3466
Elemental analysis: for $C_{28}H_{46}O_2F_2$ calculated C-74.30%, H-10.24%
found C-74.41%, H-10.46%

3) hexane-Et_2O (95:5) to give (25R,S)-25,26-epoxy-6ß-methoxy-3∝,5-cyclo-5∝-cholestan-27-ol 4b (17 mg, 4%), oil;
IR ($CHCl_3$): 3620 (OH) cm^{-1};
^1H NMR, δ: 0.429 (1H, dd, J_1=4.92 Hz, J_2=7.95 Hz, cyclopropyl-H) 0.648 (1H, t, J=4.13 Hz, cyclopropyl-H), 0.710 (3H, s, 18-H), 0.913 (3H, d, J=6.55 Hz, 21-H), 1.020 (3H, s, 19-H), 2.657 (1H, t, J=4.23 Hz, 26-H), 2.769 (1H, t, J=2.66 Hz, 6-H), 2.892 (1H, d, J=4.78 Hz, 26-H), 3.323 (3H, s, OMe), 3.648 and 3.781 [2H, q(AB), J(AB)=12.32 Hz, 27-H];

m/e: 430 (M^+,54%), 415 (M-15, 52%), 398 (M-32, 70%), 375 (M-55, 85%), 255 (32%), 107 (69%), 95 (98%), 81 (92%), 71 (82%), 55 (100%);

High resolution: for $C_{28}H_{46}O_3$ calculated - 340.3447
found - 430.3493

Elemental analysis: for $C_{28}H_{46}O_3$ calculated C-78.09%, H-10.77%
found C-78.22%, H-10.65%

4) hexane-Et_2O (95:5) to give *(25R,S)*-6ß-methoxy-27-fluoro-3∝,5-cyclo-5∝-cholestan-25,26-diol 1b (162 mg, 36%), m.p.=151-154 °C (hexane-Et_2O);

IR (KBr): 3450 (OH) cm^{-1};

^1H NMR, δ: 0.430 (1H, dd, J_1=5.06 Hz, J_2=7.98 Hz, cyclopropyl-H) 0.647 (1H, dd, J_1=3.97 Hz, J_2=4.89 Hz, cyclopropyl-H), 0.713 (3H, s, 18-H), 0.921 (3H, d, J=6.58 Hz, 21-H), 1.021 (3H, s, 19-H), 2.77 (1H, t, J=2.78 Hz, 6-H), 3.323 (3H, s, OMe), 3.540 and 3.639 [2H, q(AB), J(AB)=11.20 Hz, J(HH)=3.14 Hz - coupling constant of part B of quartet, 26-H], 4.375 and 4.377 [2H, 2d, each with J(HF)=47.49 Hz, 27-H];

^{19}F NMR, δ: -231.816 [t, J(HF)=48 Hz, 27-F];

m/e: 450 (M^+, 80%), 435 (M-15, 62%), 418 (M-32, 100%), 395 (M-55, 94%);

High resolution: for $C_{28}H_{47}O_3F$ calculated - 450.3509
found - 450.3509

Elemental analysis: for $C_{28}H_{47}O_3F$ calculated C-74.62%, H-10.51%
found C-74.73%, H-10.81%

5) hexane-Et_2O (1:1) to give 6ß-methoxy-3∝,5-cyclo-5∝-cholestan-25,26,27-triol 1c (22 mg, 5%), oil;

IR (film): 3450 (OH) cm^{-1};

^1H NMR, δ: 0.430 (1H, dd, J_1=5.05 Hz, J_2=7.98 Hz, cyclopropyl-H) 0.647 (1H, dd, J_1=3.98 Hz, J_2=4.90 Hz, cyclopropyl-H), 0.710 (3H, s, 18-H), 0.914 (3H, d, J=6.59 Hz, 21-H), 1.020 (3H, s, 19-H), 2.77 (1H, t, J=2.56 Hz, 6-H), 3.322 (3H, s, OMe), 3.579 and 3.672 [4H, q(AB), J(AB)=11.17 Hz, J(HH)=4.0 Hz - coupling constant of part at δ 3.579 ppm, 26-H and 27-H];

m/e: 448 (M^+, 100%), 433 (M-15, 58%), 416 (M-32, 97%), 393 (M-55 96%), 385 (87%), 255 (40%), 95 (54%);

High resolution: for $C_{28}H_{48}O_4$ calculated - 448.3553
found - 448.3553

Elemental analysis: for $C_{28}H_{48}O_4$ calculated C-74.96%, H-10.78%
found C-74.79%, H-10.85%

Reaction of 4a with anhydrous TBAF

A solution of epoxide 4a (216 mg, 0.5 mmol) and TBAF (785 mg, 1.5 mmol) in THF (4 ml) was refluxed. The reaction mixture was monitored by TLC. After 4 hours, when the whole of epoxide 4a has reacted, the product was extracted with Et_2O. After chromatography on silica gel (hexane-Et_2O, 98:2), difluoro compound 1a was isolated (179 mg, 83%).

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REFERENCES

1 a) V. K. Ostrem and H. F. DeLuca, Steroids, 49 (1987) 73;
b) T. Suda, C. Miura, E. Abe and T. Kuroki, in W.A. Peck (ed.) 'Bone and Mineral Research', 4, Elsevier Amsterdam, 1986, p. 1.

2. a) G. Jones, D. Vriezen, D. Lohnes, and N. S. Edwards, Steroids, 49 (1987) 29.
b) N. Ikekawa and Y. Fujimoto, Yuki Kagaku Kyokaishi, 46 (1988) 455.

3 a) Y. Kobayashi and T. Taguchi, in R. Filler and Y. Kobayashi (eds.) Biomedicinal Aspects of Fluorine Chemistry, Kodansha Tokyo, and Elsevier Biomedical Press, Amsterdam, New York, Oxford, 1982, p. 33;
b) Y. Kobayashi, T. Taguchi, S. Mitsuhashi, T. Eguchi, E. Oshima, and N. Ikekawa, Chem. Pharm. Bull., 30 (1982) 4297;
c) N. Ikekawa, T. Eguchi, N. Hara, S. Takatsuto, A. Honda, Y. Mori, and S. Otoma, Chem. Pharm. Bull., 35 (1987) 4362;
d) S-J. Shiuey, J. J. Partridge, and M. R. Uskokovič, J, Org. Chem., 53 (1988) 1040;
e) Y. Kobayashi, M. Nakajima, M. Nakazawa, T. Taguchi, N. Ikekawa, H. Sai, Y. Tanaka, and H. F. DeLuca, Chem. Pharm. Bull., 36 (1988) 4144;

f) Y. Kobayashi and T. Taguchi, in A.W. Norman, K. Schaefer, H.-G. Grigoleit and D. v. Herrath (eds.) 'Vitamin D, Molecular, Cellular and Clinical Endocrinology', Walter de Gruyter, Berlin, New York, 1988, p. 3;

g) J. S. Gill, J. M. Londowski, R. A. Corradino, A. R. Zinsmeister, and R. Kumar, J. Med. Chem., 33 (1990) 480.

4 M. Inaba, K. Yukioka, Y. Nishizawa, S. Okuno, S. Otani, S. Morisawa, H.F. DeLuca and H. Morii, in D.V. Cohn (ed.) 'Calcium and Bone Metabolism. Basic and Clinical Aspects', 9, Elsevier, Amsterdam, 1987, p. 523.

5 M. M. Kabat, J. Fluorine Chem., 46 (1990) 123.

6 M. M. Kabat, J. Fluorine Chem., 42 (1989) 435.

7 a) R. K. Sharma and J. L. Fry, J. Org. Chem., 48 (1983) 2112

b) D. P. Cox, J. Terpiński, and W. Lawrynowicz., J. Org. Chem., 49 (1984) 3216.