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RUTHENIUM TETROXIDE OXIDATION OF GRUNDMANN'S KETONE DERIVED FROM VITAMIN D_3

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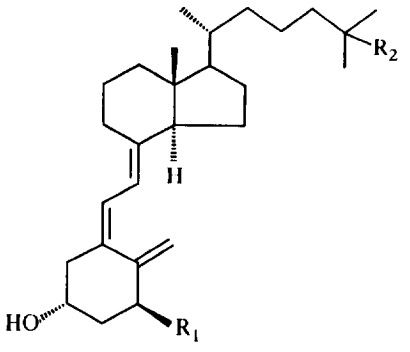
Abstract: The reaction of Grundmann's ketone **2a** with ruthenium tetroxide, generated *in situ* from ruthenium trichloride and sodium metaperiodate, has been investigated. In addition to the main 25-hydroxylated compound **2b** the minor oxidation products have been isolated and identified. The examined catalytic process results in the oxidation of methylene groups and hydroxylation at tertiary positions occurring with retention of configuration.

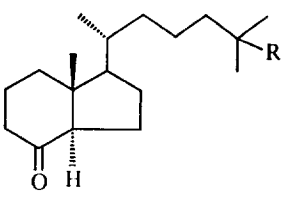
The discovery of the metabolic pathway of vitamin D_3 (**1a**) and an isolation of its two main active forms, 25-OH-D_3 (**1b**)[1] and 1,25-$(OH)_2D_3$ (**1c**),[2] has stimulated great interest in the synthesis of vitamin D metabolites and analogs. One of the most important synthetic strategies, pioneered by the Lythgoe group,[3] involves attachment of the cyclohexane A-ring fragment to the hydrindane CD unit. Grundmann's ketone **2a**, readily available from the ozonolysis of **1a**,[4] usually serves the role of the CD fragment during the synthesis of vitamin D analogs possessing saturated cholestane side chain[4b,5] whereas **2b** (usually with protected tertiary hydroxyl group) is valuable intermediate for the preparation of 25-hydroxylated vitamin D_3 analogs.[5b,6,7] Since oxidative cleavage of 25-OH-D_3 (**1b**) has to be considered as an extremely expensive way of preparing hydroxylated hydrindane derivative **2a**, other synthetic approaches have been developed.[6,8]

As part of an ongoing project on the modified vitamin D compounds we searched for a method suitable for the synthesis of larger quantities of **2b**. Our attention has been focused on the direct 25-hydroxylation[9] of **2a** with $NaIO_4/RuCl_3$ system recently reported by Uskokovic group.[8b] We investigated the catalytic oxidation of Grundmann's ketone **2a** with ruthenium tetroxide generated *in situ*, carrying out the reactions[10] at room temperature and using 0.15 mol equivalent of ruthenium chloride and 3.6 mol equivalent of sodium periodate as the cooxidant in a solvent system containing CCl_4-CH_3CN-H_2O.[11] We performed several experiments changing the reaction time and separated the unreacted substrate from the desired 25-hydroxylated product **2b** by flash chromatography. It has been established that the optimal length of the ruthenium tetroxide oxidation process is close to 3 days. Elongation of the reaction time up to 5 and more days did not improve yield of **2b** but resulted in diminished amounts of recovered starting material **2a**. During chromatographical separation of the reaction mixtures we obtained always fractions containing several compounds of higher polarity than the substrate. Since the ruthenium tetroxide oxidation of non-acivated C-H bonds is recognized as an important process and the derivatization of Grundmann's ketone seems to be also of considerable importance, it was therefore of interest to isolate and identify the minor products formed in this reaction.

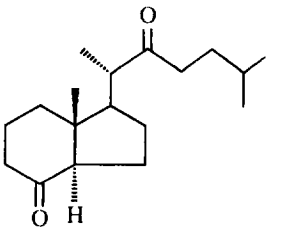
Separation of the oxidation mixture (reaction time 72 hours) was done by flash chromatography on silica followed by careful HPLC (Zorbax Sil column) of the corresponding fractions. In addition to the unreacted substrate **2a** (32.2%), the following oxidation products were isolated (in order of increasing polarity; yields based on recovered starting material): **3** (2.3%), **4** (4.8%), **5** (5.4%) , **6** (1.7%), **7** (3.3%), **8** (1.2%), **9** (1.0%) and 25-hydroxy Grundmann's ketone **2b** (43.3%). The structural assignments of the isolated compounds have been based on the ^1H NMR and mass spectroscopy.[12] The distinct molecular peaks have been observed in the



1a. R_1=H, R_2=H
1b. R_1=H, R_2=OH
1c. R_1=OH, R_2=OH



2a. R=H
2b. R=OH



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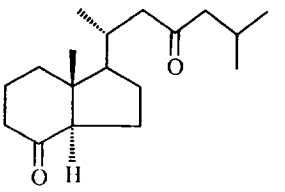

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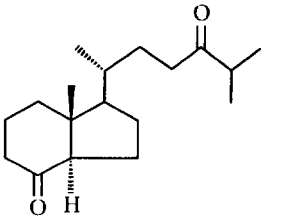

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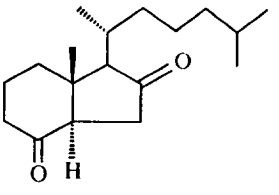

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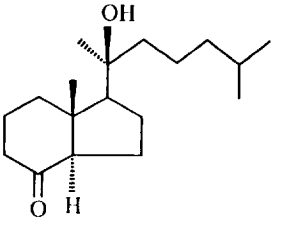

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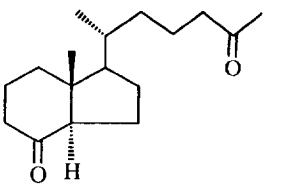

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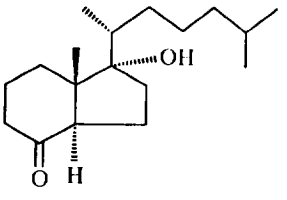

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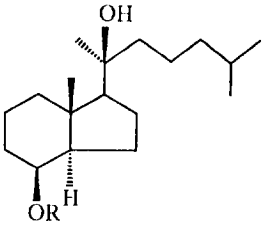
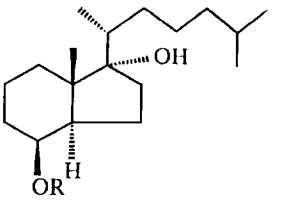


10a. R=H
10b. R=Ac



11a. R=H
11b. R=Ac

mass spectra of all products. Moreover, characteristic fragmentation patterns have been found for the closely related ketones **3**, **4** and **5** which undergo preferential cleavages of the bonds α to their side-chain ketonic groups. Similarly, the peaks resulting from scission of the C-C bonds α to the hydroxyl-substituted carbon in the mass spectra of the tertiary alcohols **7** and **9** confirmed the positions of the newly introduced oxygen functions. The chemical shifts of the methyl groups observed in the ^{1}H NMR spectra of products provided the important contribution to their structural investigation. We have computed the substituent increments from the relevant literature ^{1}H NMR data and used these values for the calculation of the chemical shifts of methyl groups for the corresponding compounds derived from the parent Grundmann's ketone **2a**. The calculated chemical shifts (Table) of methyl groups resonances were found to be in a very good agreement with the observed values.

Table. ^{1}H Chemical Shifts† of Methyl Groups for Grundmann's Ketone **2a** and Its Oxidation Products

	18-H_3		21-H_3¶		26- and 27-H_3¶	
Compound	obs.	calc.§	obs.	calc.§	obs.	calc.§
2a	0.64		0.95		0.87	
2b	0.64		0.97		1.22	
3	0.66	0.67[a]; 0.68[b]	1.13	1.13[a]; 1.15[b]	0.90	0.90[a]; 0.91[b]
4	0.68	0.70[b]	0.95	0.95[b]	0.91	0.93[b]
5	0.64	0.64[b]	0.95	0.95[b]	1.10	1.10[b]
6	0.79	0.81[b]	0.99	1.02[b]	0.87	0.88[b]
7	0.82	0.81[c]; 0.83[a]	1.30	1.31[a]	0.88	0.88[c]
8	0.64	0.64[a]	0.97	0.97[a]	2.14	2.14[a]
9	0.72	0.75[c]	0.94		0.87	0.87[c]

†400 MHZ, $CDCl_3$ solutions, δ values given in ppm downfield from internal Me_4Si; ¶δ values given for the mid points of signals (singlets, doublets or double doublets); §chemical shifts computed using the δ values for **2a** and substituent increments calculated from the following model structures: [a] cholesterol and its 22-ketone (ref. 13), 20S-hydroxy (ref. 14) and 27-nor-25-ketone (authentic specimen, see ref. 15) derivatives; [b] cholestane-3β,5α,6β-triyl triacetate and its 16-, 22-, 23- and 24-ketone analogs (ref. 16); [c] des-AB-cholestan-8β-yl acetate and its 17α- and 20S-hydroxylated analogs (ref. 8a).

Although the above data seemed to be sufficient for structure establishments we have decided to support the configurational assignments given for the tertiary alcohols **7** and **9** by converting them into the derivatives described in the literature. 8-Keto group in these compounds was reduced with $NaBH_4$ and the resulted diols **10a** and **11a** were selectively acetylated (Ac_2O, pyr, rt) to the known hydroxy acetates **10b** and **11b**, respectively. Their ^{1}H NMR spectra were found to be in close agreement with those reported by Mazur et al.[8a]

Studies on ruthenium tetroxide oxidation of branched saturated hydrocarbons have showed that the reaction is sensitive to steric hindrance and the order of reactivity is as follows: $CH>CH_2>>CH_3$.[17] These findings are in agreement with our observations. Thus, we isolated all possible tertiary alcohols with the exception of the 14-hydroxy compound, and the main oxidation product resulted from the hydroxylation of the least hindered tertiary atom C-25. We also isolated all ketones derived from the oxidation of the secondary positions of the side chain fragment. Interestingly, only two ring positions (C-16 and C-17) underwent oxidation. Another important finding was the stereoselectivity of the hydroxylation process and the absence of products resulting from Wagner-Meerwein type rearrangements. All these experimental facts do not support the mechanism of RuO_4 oxidation proceeding with the intermediate carbocation[17] but they seem to be in agreement with the mechanistic pathway postulated by Tenaglia et al.[18] These authors explained the ruthenium tetroxide oxidation of non-activated carbon-hydrogen bonds suggesting a concerted mechanism where polarization of C-H

bond by approaching RuO_4 facilitates the insertion of the oxoruthenium group in the cyclic transition state. Since the crucial step of this mechanistic pathway involves the activation of C-H bond and developing of a partial positive charge on the carbon, such mechanism also offers an explanation of the observed resistance of the ring positions neighbouring to the 8-keto group toward RuO_4 oxidation.

References and Notes

1. Blunt, J. W.; DeLuca, H. F.; Schnoes, H. K. *Biochemistry* **1968**, *7*, 3317-3322.
2. Suda, T.; DeLuca, H. F.; Schnoes, H. K.; Blunt, J. W. *Biochemistry* **1969**, *8*, 3515-3530.
3. Lythgoe, B. *Chem. Soc. Rev.*, **1981**, 449-475.
4. (a) Inhoffen, H. H.; Quinkert, G.; Shütz, S.; Kampe, D.; Domagk, G. F. *Chem. Ber.*, **1957**, *90*, 664-673; (b) Peterson, P. E. ; Breedlove Leffew, R. L.; Jensen, B. J. *J. Org. Chem.*, **1986**, *51*, 1948-1954; (c) Maestro, M. A.; Sardina, F. J. ; Castedo, L. ; Mourino, A. *J. Org. Chem.*, **1991**, *56*, 3582-3587.
5. (a) Lythgoe, B.; Moran, T. A.; Nambudiry, M. E. N.; Tideswell, J.; Wright, P. W. *J. Chem. Soc., Perkin Trans. 1*, **1978**, 590-595; (b) Trost, B. M.; Dumas, J.; Villa, M. *J. Am. Chem. Soc.*, **1992**, *114*, 9836-9845.
6. (a) Baggiolini, E. G.; Iacobelli, J. A.; Hennessy, B. M.; Uskokovic, M. R. *J. Am. Chem. Soc.*, **1982**, *104*, 2945-2948; (b) Baggiolini, E. G.; Iacobelli, J. A.; Hennessy, B. M.; Batcho, A. D.; Sereno, J. F.; Uskokovic, M. R.*J. Org. Chem.*, **1986**, *51*, 3098-3108; (c) Hatakeyama, S.; Sugawara, K.; Numata, H.; Takano, S. *J. Org. Chem.*, **1991**, *56*, 461-463.
7. (a) Shiuey, S.-J.; Kulesha, I.; Baggiolini, E. G.; Uskokovic, M. R. *J. Org. Chem.*, **1990**, *55*, 243-247; (b) Kabat, M.; Kiegiel, J.; Cohen, N.; Toth, K.; Wovkulich, P. M.; Uskokovic, M. R. *Tetrahedron Lett.*, **1991**, *32*, 2343-2346; (c) Perlman, K. L.; Swenson, R. E.; Paaren, H. E.; Schnoes, H. K.; DeLuca, H. F. *Tetrahedron Lett.*, **1991**, *32*, 7663-7666; (d) Posner, G. H.; Nelson, T. D.; Guyton, K. Z. ; Keusler, T. W. *J. Med. Chem.*, **1992**, *35*, 3280-3287; (e) Posner, G. H.; Dai, H. Biochem. Chem. Lett. **1993**, *3*,1829-1834.
8. (a) Cohen, Z.; Berman, E.; Mazur, Y. *J. Org. Chem.*, **1979**, *44*, 3077-3080; (b) Kiegiel, J.; Wovkulich, P. M.; Uskokovic, M. R. *Tetrahedron Lett.*, **1991**, *32*, 6057-6060; (c) Bovicelli, P.; Lupattelli, P.; Mincione, E.; Prencipe, T.; Curi, R. *J. Org. Chem.*, **1992**, *57*, 5052-5054.
9. Steroidal numbering is used throughout this paper.
10. General oxidation procedure: to the magnetically stirred mixture of $RuCl_3$ (31 mg, 0.15 mmol) and $NaIO_4$ (770 mg, 3.6 mmol) in water (3 mL) a solution of Grundmann's ketone **2a** (264 mg, 1 mmol) in CCl_4-CH_3CN (1:1, 4.5 mL) was added. Vigorous stirring was continued for 60 to 120 h at room temperature, then the reaction was stopped by the addition of a few drops of 2-propanol. The heterogenous mixtured was poured into water and extracted with CCl_4-$CHCl_3$ (1:1) solvent system. The combined extracts were washed with water, dried (Na_2SO_4) and evaporated to give an oily residue which was initially separated by silica gel (Merck 230-400 mesh) flash chromatography. Elution with ethyl acetate-hexane (2:8) gave the unreacted substrate **2a** followed by fraction L containing keto compounds **3**, **4**, **5**, **6** and more polar fraction M consisting of products **7**, **8**, **9**; pure 25-hydroxy Grundmann's ketone **2b** was eluted with ethyl acetate-hexane (4:6). Less polar fraction L was then subjected to HPLC (9.4 mm x 25 cm Zorbax Sil column, 20% ethyl acetate in hexane) to give pure compound **6** (R_V 38 mL) in addition to unseparated mixture of **3**, **4** and **5** (R_V ~ 35 mL). Rechromatography of this mixture with ethyl acetate-hexane (1:9) solvent system afforded pure ketones: **3** (R_V 75 mL), **4** (R_V 77 mL) and **5** (R_V 80 mL). Fraction M, in turn, was succesfully separated by HPLC using ethyl acetate-hexane (2:8) solvent system; compounds **7** (R_V 53 mL), **8** (R_V 57 mL) and **9** (R_V 61 mL) were gradually eluted.
11. Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.*, **1981**, *46*, 3936-3938.
12. Spectral data of selected compounds: ^1H NMR (400 MHz, $CDCl_3$; the methyl signals given in the Table are not listed), MS (EI, 70 eV, relative intensity). **3**: ^1H NMR δ 2.38 and 2.46 (1H and 1H, each m, 23-H_2), 2.53 (1H, dq, J = 10.6, 7.0 Hz, 20-H); MS m/z 278 (M^+, 50), 207 (M^+ - isopentyl, 16), 179 (88), 99 ($C_6H_{11}O^+$, 100). **4**: ^1H NMR δ 2.17 (1H, m, one of 22-H_2), 2.25 (2H, d, J = 7.1 Hz, 24-H_2), 2.43 (1H, m, one of 22-H_2); MS m/z 278 (M^+, 20), 221 (M^+ - isobutyl, 10), 179 (100), 85 ($C_5H_9O^+$, 67). **5**: ^1H NMR δ 2.40 and 2.46 (1H and 1H, each m, 23-H_2), 2.61 (1H, sept, J = 6.7 Hz, 25-H); MS m/z 278 (M^+, 59), 235 (M^+ - isopropyl, 56), 193 (100), 71 ($C_4H_7O^+$, 66). **6**: ^1H NMR δ 2.81 (1H, dd, J = 12.1, 7.6 Hz, 14α-H); MS m/z 278 (M^+, 47), 263 (96), 151 (100). **7**: ^1H NMR δ 2.44 (1H, dd, J = 10.8, 7.2 Hz, 14α-H); MS m/z 280 (M^+, 6), 265 (10), 195 (M^+ - isohexyl, 59), 152 (59), 135 (56), 129 (54), 111 (100). **8**: MS m/z 264 (M^+, 64), 249 (11), 152 (52), 125 (61), 111 (100). **9**: ^1H NMR δ 3.14 (1H, dd, J = 10.2, 7.8 Hz, 14α-H); MS m/z 280 (M^+, 32), 265 (18), 167 (M^+ - side chain, 22), 156 (34), 125 (36), 111 (100).
13. Burrows, E. P.; Hornby, G. M.; Caspi, E. *J. Org. Chem.*, **1969**, *34*, 103-107.
14. Nes, W. R.; Varkey, T. E.; Crump, D. R.; Gut, M. *J. Org. Chem.*, **1976**, *41*, 3429-3433.
15. Lam, H.-Y.; Schnoes, H. K.; DeLuca, H. F. *Steroids* **1975**, *25*, 247-256.
16. Barton, D. H. R.; Göktürk, A. K.; Jankowski, K. *J. Chem. Soc., Perkin. Trans. 1*, **1985**, 2109-2117.
17. (a) Bakke, J. M.; Lundquist, M. *Acta Chem. Scand.*, **1986**, *B40*, 430-433; (b) Carlsen, P. H. J. *Synth. Commun.*, **1987**, *17*, 19-23.
18. (a) Tenaglia, A.; Terranova, E.; Waegell, B. *Tetrahedron Lett.*, **1989**, *30*, 5271-5274; (b) Tenaglia, A.; Terranova, E.; Waegell, B. *J. Org. Chem.*, **1992**, *57*, 5523-5528.

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