Conformational Equilibria in Vitamin D. Synthesis of 1β-Hydroxyvitamin D_3[1]

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Received September 28, 1976

1 β-Hydroxyvitamin D_3 was prepared from 1α-hydroxyvitamin D_3 by oxidation to 1-ketoprevitamin D_3, followed by sodium borohydride reduction and subsequent thermal isomerization. The conformational equilibria in 1β-hydroxyvitamin D_3 were established using ^1H NMR technique. These data indicate that in nonpolar solvent this compound assumes mainly the conformation in which hydroxy groups are both axial, while in H-bonding solvent mainly the conformation where these groups are both diequatorial.

1α,25-Dihydroxyvitamin D_3, **1** (C_{25}-OH), is a natural hormone,[2] inducing the formation of calcium binding proteins, responsible for the calcium transport and its mobilization in the body. A number of other vitamin D_3 analogs, like 1α-hydroxyvitamin D_3 (**1**),[3] dihydrotachysterol$_3$ (**2**), 5,6-transvitamin D_3 (**3**), 1α-hydroxy-3-deoxyvitamin D_3 (**4a**), its 3α-methyl analog (**4b**)[3] and their respective 25-hydroxy derivatives[2] exert in various degrees similar biological activity in vivo. One of the common features in all these compounds is the presence of a hydroxy function at C_1 having an α configuration.[4]

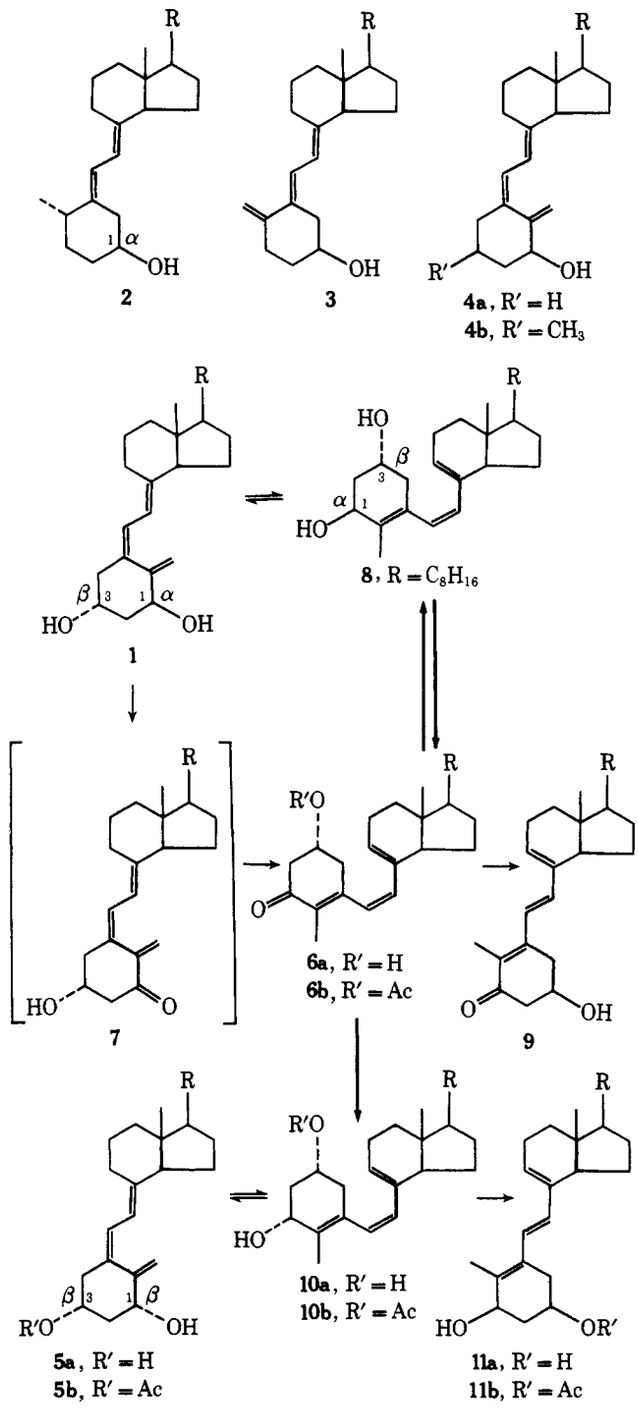
In order to establish whether this 1α-hydroxy substituent is essential for the hormonal activity of the vitamin D_3 analogs, we have synthesized 1β-hydroxyvitamin D_3 (**5a**) the C_1 epimer of 1α-hydroxyvitamin D_3 (**1**) and evaluated its biological activity.[1]

The starting material, 1α-hydroxyvitamin D_3 (**1**), was oxidized with freshly prepared active manganese dioxide in ether resulting in the ketone **6a**. The spectral data indicated that this ketone possessed the previtamin D and not the vitamin D skeleton. Thus its NMR spectrum showed three vinylic protons due to the endocyclic 5(10),6,8-triene system (AB quartet of the two protons at C_6 and C_7 and a broad singlet of the proton at C_9) instead of the four protons of 1-ketovitamin D_3 (**7**) exocyclic 5,7,10(19)-triene system. The UV spectrum of **6a** exhibited two bands at λ_{max} 236 and 287 nm (ε 9500, 10 000) the latter indicating an extension of conjugation of the previtamin chromophore by 28 nm (1α-hydroxyprevitamin D_3 (**8**) absorbs at λ_{max} 259 nm; ε 10 000) in accord with the assigned structure.[5] Furthermore, on exposing **6a** to sunlight in the presence of iodine the UV spectrum changed; the two bands were replaced by one appearing at higher wavelength with enhanced intensity (λ_{max} 320 nm; ε 22 000). This UV change was indicative of a C_6–C_7 double bond $Z \rightleftarrows E$ isomerization, with a formation of tachysterol$_3$ derivative, **9**, possessing an extended planar conjugated 1-keto-triene chromophore.[6]

Oxidation of 1α-hydroxyprevitamin D_3 (**8**)[7] with an active manganese dioxide resulted also in 1-ketoprevitamin D_3 (**6a**). This oxidation, however, proceeded at a faster rate than the corresponding oxidation of 1α-hydroxyvitamin D_3 (**1**) and gave the ketone in higher yield.

The formation of 1-ketoprevitamin (**6a**) instead of 1-ketovitamin **7** from **1** implied that the thermal equilibrium **6a** $\rightleftarrows$ **7** is totally on the side of the 1-ketoprevitamin D_3 (**6a**) differing thus from the equilibrium vitamin $D_3 \rightleftarrows$ previtamin D_3 which is predominant on the side of the vitamin. This shift in the position of the equilibrium is consistent with the increased stability due to the linearly conjugated carbonyl system present in the ketone **6a**.

Reduction of ketone **6a** with sodium borohydride in methanol resulted in a single product 1β-hydroxyprevitamin D_3 (**10a**) which had a UV spectrum identical with 1α-hydroxyprevitamin D_3 (**8**) and isomerized with iodine and light to a tachysterol derivative **11a** (λ_{max} 272, 282, 292 nm; ε 22 000, 25 000, 21 000). The NMR of **10a** differed from that of its C_1

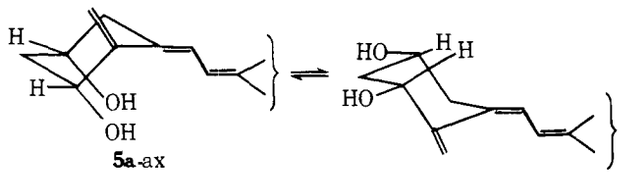


epimer 8 only by the chemical shift of the protons at C_1 and C_3.

Reduction of the ketone **6a**, with lithium aluminum hydride proceeded differently from that with sodium borohydride, resulting in a mixture of both C_1 epimers **10a** and **8** in a ca. 2.8:1 ratio. The formation of the 1α-hydroxyprevitamin D_3 (**8**) in the lithium aluminum hydride reduction may be explained by the coordination of the aluminum atom to the hydroxy function which allows an attack of the hydride from the sterically hindered α side of the molecule.

Heating 1β-hydroxyprevitamin D_3 (**10a**) at 70 °C for 3.5 h gave a mixture containing the starting material and 1β-hydroxyvitamin D_3 (**5a**) (λ_{max} 264 nm; ϵ 18 000) in a 1:4 ratio (as established by the NMR spectrum of the total product mixture). The NMR spectrum of **5a** was similar to that of its C_1 epimer, the 1α-hydroxyvitamin D_3 (**1**), but for the signals of the protons at C_1 and C_3, while the mass spectra of both compounds were practically identical. 1β-Hydroxyvitamin D_3 (**5a**) isomerized with visible light in the presence of iodine to 1β-hydroxy-5,6-*trans*-vitamin D_3 (λ_{max} 272 nm; ϵ 22 000).

1β-Hydroxyvitamin D_3 (**5a**) exists as other vitamin D derivatives as a mixture of two chair conformers, which in solution are in dynamic equilibrium.[8] This equilibration can be deduced from its NMR spectrum in a nonpolar solvent (carbon tetrachloride), where the protons at C_1 and C_3 appear at 4.22 and 3.96 ppm as two triplets with J = 3.6 Hz and with half-height width, $w_{1/2}$ = 9.5 Hz. These triplets are due to two vicinal interactions, one axial:equatorial ($J_{ax:eq} = J_{eq:ax}$ = ca. 3.6 Hz) and the other representing an average between axial:axial and equatorial:equatorial coupling (J = 3.6 Hz). Using $J_{ax:ax}$ = 11 Hz, $J_{eq:eq}$ = 3 Hz,[3] and the experimental value J = 3.6 Hz we have calculated the ratio of the two conformers **5a-ax** and **5a-eq** to be 9:1. The strong preference for



5a-ax **5a**-eq

a conformation having the hydroxyl group in diaxial orientation is due to an intramolecular hydrogen bonding between these groups. This internal H bonding breaks down in H-bonding solvents, as the following NMR data show. Upon addition of acetone-d_6 to the carbon tetrachloride solution of **5a** both signals shifted to a higher field appearing at 3.79 and 4.02 ppm, the former as a heptet (J = 8.4 Hz and ca. 4.0 Hz) and the latter as a broad multiplet ($w_{1/2}$ = 17 Hz). These NMR data indicated that the intermolecular hydrogen bonding becomes predominant and the compound assumes mainly the conformation in which both hydroxyl groups are equatorial, the ratio of **5a-ax:5a-eq** being 3:7.

Additional information about the hydrogen bonding of the two hydroxyl groups at C_1 and C_3 can be gained from the NMR spectrum of 1β-hydroxyvitamin D_3 3-acetate (**5b**).

This compound was synthesized from **6a** by acylation with 4-(dimethylamino)pyridine and acetic anhydride resulting in the ketoacetate **6b** (λ_{max} 287, 236 nm; ϵ 10 000, 9500). Reduction with sodium borohydride in methanol yielded 1β-hydroxyprevitamin D_3 3-acetate (**10b**) (λ_{max} 259; ϵ, 10 000) which on hydrolysis gave 1β-hydroxyprevitamin D_3 (**10a**). The acetate (**10b**) isomerized to tachysterol derivative **11b** with iodine and sunlight (λ_{max} 272, 282, 292 nm; ϵ 22 000, 25 000, 21 000).

Heating **10b** at 70 °C for 3.5 h gave 1β-hydroxyvitamin D_3 3-acetate (**5b**) (λ_{max} 264; ϵ 18 000). The NMR spectrum in carbon tetrachloride was similar to that of **10a** except for the signals of the protons at C_1 and C_3 which appeared at 4.2 ppm (quartet, J = 9 Hz and ca. 4.0 Hz) and 4.88 ppm (heptet, J = 9 Hz and ca. 4.0 Hz), respectively, indicating that **5b** exists mainly in the diequatorial conformation, the ratio of **5b-eq: 5b-ax** being 8:2.

Preliminary biological assays in chicks indicated that 1β-hydroxyvitamin D_3 (**5a**) as well as its 3-acetate **5b** are devoid of any activity in inducing calcium transport and mobilization in the body.[1] Thus it appears that the high physiological activity of C_1-hydroxylated vitamin D_3 derivatives is limited to compounds possessing this function in the α-configuration.

Experimental Section

Nuclear magnetic resonance spectra were recorded on a Bruker 270 MHz using carbon tetrachloride as a solvent and cyclohexane-d_{12} as an internal lock. All chemical shifts are reported in δ values relative to tetramethylsilane standard. The ultraviolet spectra were taken on a Cary 118 spectrophotometer, using ether as a solvent. Mass spectra were recorded on Varian MAT 731 high resolution mass spectrometer.

1α-Hydroxyprevitamin D_3 (8). A solution of 400 mg of 1α,3β-dihydroxycholesta-5,7-diene in 250 mL of dry ether was irradiated at 0 °C under a nitrogen atmosphere with 3000-Å light (Rayonet) using 0.4% solution of sodium nitrate as a filter. The solvent was evaporated and the residue was chromatographed on Sephadex LH-20. Elution with a mixture of chloroform–hexane (6.5:3.5) gave 100 mg of 1α-hydroxyprevitamin D_3 (**8**). UV λ_{max} 259 nm (ϵ 10 000). NMR δ 0.71 (3H, s, 18-H), 1.70 (3H, s, 19-H), 5.50 (1H, m, 9-H), 5.68 and 5.86 (2H, ABq, J = 12 Hz, 6-H and 7-H), 4.02 (1H, m, $w_{1/2}$ = 9.4 Hz, 1-H), 3.89 (1H, m, $w_{1/2}$ = 17.2 Hz, 3-H). *m/e* (M^+) 400.3297 (calcd 400.3330, 382 ($M^+ - H_2O$), 364 ($M^+ - 2H_2O$), 152 ($M^+ - C_{18}H_{32}$) and 134 ($M^+ - C_{18}H_{32} - H_2O$).[9]

1-Ketoprevitamin D_3 (6a).[1] A solution of 100 mg of 1α-hydroxyvitamin D_3 (**1**) in 10 mL of dry ether was treated at room temperature with 350 mg of freshly prepared manganese dioxide for 6 h. The reaction mixture was filtered through a celite column and the filtrate was evaporated to dryness at room temperature. Chromatography on silica gel using an ethyl acetate–chloroform mixture (3:7) resulted in 35 mg of 1-ketoprevitamin D_3 (**6a**). UV λ_{max} 287, 236 nm (ϵ 10 000, 9500). NMR δ 0.74 (3H, s, 18-H) 1.69 (3H, s, 19-H), 5.99 and 6.13 (2H, ABq, J = 11 Hz, 6-H and 7-H), 5.51 (1H, m, 9-H) and 4.02 (1H, heptet, J = 8.6 and 4.3 Hz, 3-H). *m/e* (M^+) 398.6349 (calcd 398.3174), 380 ($M^+ - H_2O$), 157 ($M^+ - C_{16}H_{31} - H_2O$).[9]

A solution of 100 mg of 1α-hydroxyprevitamin D_3 (**8**) in 10 mL of ether was treated with 650 mg of freshly prepared manganese dioxide for 6 h. Isolation as above resulted in 85 mg of 1-ketoprevitamin D_3 (**6a**).

1β-Hydroxyprevitamin D_3 (10a).[1] A solution of 50 mg of 1-ketoprevitamin D_3 (**6a**) in 20 mL of methanol was treated with 85 mg of sodium borohydride at 0 °C for 30 min, extracted with ether, and washed with brine. The ether extract was dried over magnesium sulfate and evaporated at 0 °C to dryness. The residue was chromatographed on silica gel. Elution with a mixture of ethyl acetate–chloroform (3:7) gave 35 mg of 1β-hydroxyprevitamin D_3 (**10a**). UV λ_{max} 259 nm (ϵ 10 000), and on addition of iodine and exposure to sunlight λ_{max} 272, 282, 292 nm (ϵ 22 000, 25 000, 21 000). NMR δ 0.70 (3H, s, 18-H), 1.70 (3H, s, 19-H), 5.56 (1H, s, 9-H), 5.78 and 5.94 (2H, AB q, J = 11.5 Hz, 6-H and 7-H), 3.93 (1H broad s, $w_{1/2}$ = 11Hz, 3-H), and 4.22 (1H, m, $w_{1/2}$ = 10.5 Hz 1-H). *m/e* (M^+) 400.3333 (calcd 400, 3333), 382 ($M^+ - H_2O$), 364 ($M^+ - 2H_2O$), 152 ($M^+ - C_{18}H_{32}$), and 134 ($M^+ - C_{18}H_{32} - H_2O$).

A solution of 100 mg of 1-ketoprevitamin D_3 (**6a**) in 10 mL of anhydrous ether was treated with 20 mg of lithium aluminum hydride at 0 °C for 3 h with stirring. The reaction mixture was then triturated with saturated sodium sulfate solution followed by filtration and the residue was evaporated to dryness. Chromatography on silica gel and elution with a mixture of ethyl acetate chloroform (3:7) gave 1β-hydroxyprevitamin D_3 (**10a**) and 1α-hydroxyprevitamin D_3 (**8**) in a 2.8:1 ratio (60% yield).

1β-Hydroxyvitamin D_3 (5a).[1] A solution of 30 mg of 1β-hydroxyprevitamin D_3 (**10a**) in 10 mL isooctane was heated under nitrogen atmosphere at 70 °C for 3.5 h. The solvent was evaporated to dryness and the residue was chromatographed on silica gel. Elution with ether gave 25 mg of 1β-hydroxyvitamin D_3 (**5a**). UV λ_{max} 264 nm (ϵ 18 000) and on addition of iodine and exposure to sunlight λ_{max} 272 nm (ϵ 22 000). NMR δ 0.54 (3H, s, 18-H), 5.19 (1H, m, 19E-H), 4.88 (1H, m, 19Z-H), 5.92 and 6.29 (2H, ABq, J = 11.5 Hz, 6-H and 7-H), 3.96 (1H,

m, $w_{1/2}$ = 9.5 Hz, 3-H), and 4.22 (1H, t, J = 3.5 Hz, 3-H). *m/e* (M^+) 400.3335 (calcd 400.3331), 382 ($M^+ - H_2O$), 364 ($M^+ - H_2O$), 152 ($M^+ - C_{18}H_{32}$), and 134 ($M^+ - C_{18}H_{32} - H_2O$).[9]

1-Ketoprevitamin D_3 3-Acetate (6b). A solution of 50 mg of 1-ketoprevitamin D_3 (**6a**) in 4 mL of methylene chloride was treated with 10 mg of 4-(dimethylamino)pyridine and 15 mg of acetic anhydride at room temperature for 2 h. The reaction mixture was evaporated and the residue was chromatographed on silica gel. Elution with ether gave 45 mg of 1-ketoprevitamin D_3 3-acetate (**6b**). UV λ_{max} 287, 236 nm (ϵ 10 000, 9500) and on addition of iodine and exposure to sunlight λ_{max} 320 nm (ϵ 22 000). NMR δ 0.69 (3H, s, 18-H), 1.74 (3H, s, 19-H), 1.99 (3H, s, acetate methyl), 5.45 (1H, m, 9-H), 5.90 and 6.08 (2H, ABq, J = 12 Hz, 6-H and 7-H), 5.04 (1H, heptet, J = 8.5 Hz and 4.0 Hz, 3-H). *m/e* (M^+) 440.3277 (calcd 440.3279), 396 ($M^+ - C_2H_4O$), 380 ($M^+ - C_2H_4O_2$) 220, 202.[9]

Reduction and Hydrolysis of 1-Ketoprevitamin D_3 3-Acetate (6b). A solution of 40 mg of 1-ketoprevitamin D_3 3-acetate (**6b**) in 3 mL of methanol was treated with 20 mg sodium borohydride at 0 °C for 30 min, extracted with ether, and washed with brine.

The ether extract was dried over magnesium sulfate and evaporated at 0 °C to dryness. The residue was chromatographed on silica gel. Elution with a mixture of ether–hexane (4:6) gave 25 mg of 1β-hydroxyprevitamin D_3 3-acetate (**10b**). UV λ_{max} 259 nm (ϵ 10 000) and on addition of iodine and exposure to sunlight: λ_{max} 272, 282, and 292 nm (ϵ 22 000, 25 000, 21 000). A solution of 20 mg (**10b**) in methanol was treated at 0 °C with a solution of 40 mg of potassium hydroxide in 1 mL of methanol for 4 h. The reaction mixture was extracted with ether and water and washed with brine. The ether extract was dried over magnesium sulfate and evaporated at 0 °C to dryness. The residue was chromatographed on silica gel. Elution with ether gave 15 mg of material which was identical with 1β-hydroxyprevitamin D_3 (**10a**).

1β-Hydroxyvitamin D_3 3-Acetate (5b). A solution of 10 mg of 1β-hydroxyprevitamin D_3 3-acetate (**10b**) in 2 mL of isooctane was heated under nitrogen atmosphere at 70 °C for 3.5 h. The solvent was evaporated to dryness and the residue was chromatographed on silica gel. Elution with a mixture of ether–hexane (4:6) gave 7 mg of 1β-hydroxyvitamin D_3 3-acetate (**5b**). UV λ_{max} 264 nm (ϵ 18 000) and on addition of iodine and exposure to sunlight λ_{max} 272 nm (ϵ 22 000). NMR δ 0.54 (3H, s, 18-H), 1.98 (3H, s, methyl acetate), 4.90 (1H, m, 19Z-H), 5.29 (1H, m, 19E-H), 5.87 and 6.15 (2H, ABq, J = 11.5 Hz, 6-H and 7-H), 3.97 (1H, quartet J = 9Hz and J = 4 Hz, 1-H), 4.82 (1H, heptet J = 9 Hz and J = 4 Hz 3-H).

1-Ketotachysterol$_3$ (9). A solution of 50 mg of 1-ketoprevitamin D_3 (**6a**) in 10 mL of ether was treated with 0.1 mL of 5% iodine solution in ether and exposed to visible light for 30 min. The ether solution was washed with water and evaporated under vacuum to give 35 mg of 1-ketotachysterol$_3$ (**9**). UV λ_{max} 320 nm (ϵ 22 000). NMR δ 0.70 (3H, s, 18-H), 1.82 (3H, s, 19-H), 4.1 (1H, m, 1-H), 5.77 (3H, m, 6-H, 7-H and 9-H).

Acknowledgment. We are indebted to Dr. Z. V. I. Zaretskii for the mass spectra determinations and to Mr. E. Berman for the NMR spectra. We thank United States–Israel Binational Science Foundation, Jerusalem, for financial support.

Registry No.—2, 41294-56-8; **5a,** 63181-13-5; **5b,** 63181-14-6; **6a,** 63181-15-7; **6b,** 63181-16-8; **8,** 41461-13-6; **9,** 63181-17-9; **10a,** 63181-18-0; **10b,** 63181-19-1; 1α,3β-dihydroxycholesta-5,7-diene, 43217-89-6.

References and Notes

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(5) In the alternative structure **7** the carbonyl chromophore is not expected to shift the UV band of vitamin system (λ_{max} 264 nm) considerably.
(6) The comparatively short wavelength absorption in **6a**, as well as in the other previtamin D_3 derivatives, indicates that the triene system in these compounds is not planar as in their respective tachysterol derivatives. The ring A of previtamin is probably tilted considerably in one or both directions; G. M. Sanders, J. Pot, and E. Havinga, *Fortschr. Chem. Org. Naturst.*, **27,** 131 (1969).
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(9) Full mass spectral data will be published elsewhere by Dr. Z. V. I. Zaretskii.

Reduction of Sterigmatocystin and Versicolorin A Hemiacetals with Sodium Borohydride[1]

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Received April 11, 1977

Reduction of sterigmatocysin or versicolorin A hemiacetals with a limited amount of sodium borohydride yielded two major products in each case. The hemiacetal derived from sterigmatocystin gave a new diol as the complete reduction product and a new hemiacetal as a partial reduction product, and the structure of this new hemiacetal was established by ^{13}C NMR spectroscopy and by chemical conversions. The hemiacetal derived from versicolorin A behaved similarly. The bearing of this work on the structure of versiconal acetate, isolated from *Aspergillus flavus*, is discussed.

The aflatoxins and the related sterigmatocystins are a group of toxic and carcinogenic metabolites of certain strains of the fungi *Aspergillus flavus, Aspergillus parasiticus,* and *Aspergillus versicolor,* and have aroused considerable interest because of their widespread occurrence in human and animal foodstuffs.[4,5] Previous theoretical proposals and experimental studies on the biosynthesis of these compounds have indicated that the most probable biosynthetic pathway lies from acetate through the anthraquinones averufin (**1**) and versicolorin A (**3**) to sterigmatocystin (**4**) and thence to aflatoxin B_1 (**5**) (Scheme I).[6-13] The conversion of averufin to versicolorin A is of considerable interest, since the latter contains the same