

Synthesis of Viridiofungin A Trimethyl Ester and Determination of the Absolute Structure of Viridiofungin A¹

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Abstract: Four diastereoisomeric trimethyl esters of viridiofungin A, a member of novel family of aminoacyl alkyl citrate compounds, were synthesized in a highly stereoselective manner and the absolute configuration of natural viridiofungin A was determined to be 3S, 4S, 2'S. © 1998 Elsevier Science Ltd. All rights reserved.

Viridiofungin A (1) was isolated from a strain of *Trichoderma viride* Pers. (Fungi, Hyphomycetes) together with viridiofungin B and C by researchers at Merck² after screening for substances that exhibit inhibitory activity against squalene synthase.³ Although viridiofungins were found to be about four orders of magnitude less active than zaragozic acids in inhibition of squalene synthase, they have newly attracted much attention as novel potent inhibitors of sphingolipid synthesis.⁴ These viridiofungins have unique structures consisting of a common alkylated citric acid moiety having a C-16 long chain and an aromatic amino acid residue such as tyrosine, phenylalanine, and tryptophan. The flat structures of these natural products were determined by spectroscopic analysis² but either absolute or relative structure has not been determined yet. We, therefore, need to develop a reliable synthetic route leading to their all diastereoisomers for the confirmation of the absolute structures. We describe herein the first synthesis of this novel family of aminoacyl alkyl citrate compounds, viridiofungin A trimethyl ester (2) and its three diastereoisomers, thereby establishing the absolute structure of viridiofungin A as depicted in 1.



p-Methoxybenzylation of 3-butyn-1-ol followed by reaction with paraformaldehyde in the presence of *n*-butyllithium gave propargylic alcohol 3.5 Successive treatment of 3 with Red-Al[®] and iodine⁶ allowed stereoand regioselective formation of (Z)-iodoalkene which was directly protected as its tetrahydropyranyl ether to give 4. After treatment of 4 with *tert*-butyllithium, the resulting alkenyllithium was successively reacted with carbon dioxide and methyl iodide to give ester 5 almost quantitatively.⁷ Upon sequential DIBAH reduction, *p*- methoxybenzylation, and acidic methanolysis, the ester 5 gave trisubstituted allylic alcohol 6. Katsuki-Sharpless catalytic asymmetric epoxidation⁸ of 6 afforded epoxide 7 with enantiomeric purity⁹ of 88% ee, $[\alpha]^{20}_{D} - 19.8^{\circ}$ (c 1.17, CHCl₃). Exposure of 7 to vinylmagnesium bromide in the presence of CuI caused regio- and stereoselective nucleophilic opening of the epoxide¹⁰ to give diol 8, $[\alpha]^{21}_{D} + 15.6^{\circ}$ (c 0.93, CHCl₃), exclusively. After protection of 8 as its acetonide, the alkenic double bond was cleaved by a sequence involving osmylation and NaIO₄ oxidation to yield (2*R*,3*S*)-aldehyde 9.



Scheme 1. (a) p-(MeO)C₆H₄CH₂Cl, NaH, n-Bu₄NI, THF; (b) n-BuLi, (CH₂O)_n, THF; (c) Red-Al[®], Et₂O, 0 to 25 °C, then I₂, -50 to 25 °C; (d) PPTS, DHP, CH₂Cl₂; (e) t-BuLi, CO₂, Et₂O, -78 °C, then MeI, DMF; (f) DIBAH, CH₂Cl₂, -78 °C; (g) p-(MeO)C₆H₄CH₂Cl, NaH, n-Bu₄NI, THF, reflux; (h) PPTS, MeOH, reflux; (i) diisopropyl L-tartrate (0.09 equiv), Ti(O-i-Pr)₄ (0.07 equiv), t-BuOOH (2 equiv), 4 Å molecular sieves, CH₂Cl₂, -30 °C; (j) CH₂=CHMgBr, CuI, THF, -25 °C; (k) (MeO)₂CMe₂, PPTS, benzene, reflux; (l) OsO₄ (0.1 equiv), NMO, THF-H₂O (3 : 1), then NaIO₄.

Wittig reaction of 9 with the ylide, generated from 10 by the action of *n*-butyllithium, was conducted in THF at 0 °C to give (Z)-alkene 11, $[\alpha]^{16}D$ +20.1° (c 1.51, CHCl₃), stereoselectively.¹¹ Although Birch reduction of 11 gave the diol 12 quantitatively, all attempts at direct oxidation of its two hydroxymethyl groups to the carboxylic acids failed. However, their oxidations were eventually achieved by the following stepwise transformations. Crude diol 12 thus obtained was directly silvlated with tert-butyldiphenylsilvl chloride. It is important to note that the silulation took place with complete siteselectivity to give mono-silul ether 13, $[\alpha]^{26}$ – 0.6° (c 1.31, CHCl₂), exclusively and the other mono-silvl ether was not obtained in this particular case. Successive Swern oxidation, NaClO, oxidation, and esterification allowed quantitative conversion of 13 to ester 14, $[\alpha]^{23}_{D}$ +17.6° (c 0.99, CHCl₃). A four-step sequence involving acid treatment to form the keto-lactone, HF-promoted desilylation, Jones oxidation, and esterification led 14 to lactone 15, $[\alpha]^{19}_D$ +21.4° (c 0.87, CHCl₃) along with 16, $[\alpha]^{28}$ _D -29.9° (c 1.00, CHCl₃).¹² Photo-isomerisation of the alkenic double bond was carried out at this stage and the desired E-isomer 17, $[\alpha]^{19}_D - 17.1^\circ$ (c 0.39, CHCl₃), was obtained in a pure form¹¹ by separation of the resulting 82:18 E/Z-mixture using SiO₂ preparative TLC. Upon sequential saponification, esterification, and Jones oxidation, 17 gave carboxylic acid 18 which was directly subjected to condensation with L-tyrosine methyl ester. After considerable experimentation, it was found that the condensation was best accomplished using a water soluble carbodiimide as a dehydrating agent in the presence of 1-hydroxybenzotriazole and N-methylmorpholine¹³ in DMF to furnish (35,45,2'S)-viridiofungin A trimethyl ester 2, $[\alpha]^{26}$ –19.1° (c 0.43, MeOH). Similarly, (3S,4S,2'R)-viridiofungin A trimethyl ester 19, $[\alpha]^{26}$ – 15.5° (c 0.43, MeOH), was synthesized by condensation of 18 with D-tyrosine methyl ester.



Scheme 2. (a) *n*-BuLi, THF, 0 °C; (b) Li, THF-liq. NH₃, -33 °C; (c) *t*-BuPh₂SiCl, Et₃N, CH₂Cb; (d) (COCl)₂, DMSO, Et₃N, CH₂Cl₂, -60 to 25 °C; (e) NaClO₂, NaH₂PO₄, 2-methyl-2-butene, *t*-BuOH-H₂O (4:1); (f) CH₂N₂, Et₂O; (g) conc. HCl, *t*-BuOH; (h) 46% HF-MeCN (1:7); (i) H₂CrO₄, aq. acetone, -10 °C; (j) CH₂N₂, Et₂O; (k) hv, PhSSPh, *c*-hexane; (l) NaOH, MeOH; (m) CH₂N₂, Et₂O; (n) H₂CrO₄, aq. acetone, -10 °C; (o) Me₂N(CH₂)₃N=C=NEt·HCl, *N*-methylmorpholine, 1-hydroxybenzotriazole, DMF.

Furthermore, (3S,4R,2'S)-viridiofungin A trimethyl ester 22, $[\alpha]^{27}_D$ +33.3° (c 0.47, MeOH), and (3S,4R,2'R)-viridiofungin A trimethyl ester 23, $[\alpha]^{27}_D$ +31.6° (c 0.47, MeOH), were also synthesized from (2S,3S)-aldehyde 21 which was obtained from the diol 8 via 20, $[\alpha]^{23}_D$ +5.0° (c 0.91, MeOH), as summarized in Scheme 3.



Scheme 3. (a) *t*-BuCOCl, Et₃N, CH₂Cl₂; (b) OsO₄ (0.1 equiv), NMO, THF-H₂O (3 : 1), then NaIO₄; (c) NaBH₄, MeOH; (d) (MeO)₂CMe₂, PPTS, CH₂Cl₂; (e) NaOH, MeOH; (f) (COCl)₂, DMSO, Et₃N, CH₂Cl₂, -60 to 25 °C; (g) as in Scheme 2.

Comparison (${}^{1}H{}^{14}$ and ${}^{13}C$ NMR, $[\alpha]_{D}$) of these four diastereomers 2, 19, 22, and 23 with viridiofungin A trimethyl ester, $[\alpha]^{25}D - 23.0^{\circ}$ (*c* 0.47, MeOH), derived from natural viridiofungin A allowed us to conclude that viridiofungin A has 3S, 4S, and 2'S configurations.

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References and Notes:

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2: δ 0.90 (t, 3H, J = 7.5 Hz), 1.29 (m, 14H), 1.53 (m, 4H), 1.98 (m, 2H), 2.43 (t, 4H, J = 7.5 Hz), 2.60 (d, 1H, J = 16.0 Hz), 2.87 (dd, 1H, J = 8.5, 14.5 Hz), 2.92 (d, 1H, J = 16.0 Hz), 3.07 (dd, 1H, J = 5.0, 14.5 Hz), 3.19 (d, 1H, J = 8.5 Hz), 3.64 (s, 3H), 3.70 (s, 3H), 3.71 (s, 3H), 4.61 (dd, 1H, J = 5.0, 8.5 Hz), 5.51 (m, 2H), 6.67 (d, 2H, J = 8.5 Hz), 6.98 (d, 2H, J = 8.5 Hz).

19: δ 0.90 (t, 3H, J = 7.0 Hz), 1.29 (m, 14H), 1.53 (quint, 4H, J = 6.5 Hz), 1.99 (q, 2H, J = 7.0 Hz), 2.44 (t, 4H, J = 8.0 Hz), 2.58 (d, 1H, J = 16.0 Hz), 2.80 (d, 1H, J = 16.0 Hz), 2.87 (dd, 1H, J = 9.0, 14.0 Hz), 3.09 (dd, 1H, J = 5.0, 14.0 Hz), 3.14 (d, 1H, J = 9.5 Hz), 3.62 (s, 3H), 3.69 (s, 3H), 3.70 (s, 3H), 4.61 (dd, 1H, J = 5.0, 9.0 Hz), 5.45 (dd, 1H, J = 9.0, 15.0 Hz), 5.56 (dt, 1H, J = 7.0, 15.0 Hz), 6.70 (d, 2H, J = 8.5 Hz), 7.00 (d, 2H, J = 8.5 Hz).

22: δ 0.90 (t, 3H, J = 7.5 Hz), 1.29 (m, 14H), 1.54 (m, 4H), 2.08 (q, 2H, J = 7.0 Hz), 2.43 (t, 2H, J = 7.5 Hz), 2.44 (t, 2H, J = 7.5 Hz), 2.65 (d, 1H, J = 15.5 Hz), 2.72 (d, 1H, J = 15.5 Hz), 2.87 (dd, 1H, J = 8.5, 14.0 Hz), 3.01 (dd, 1H, J = 5.5, 14.0 Hz), 3.30 (d, 1H, J = 9.5 Hz), 3.61 (s, 3H), 3.62 (s, 3H), 3.67 (s, 3H), 4.52 (dd, 1H, J = 6.0, 8.5 Hz), 5.49 (ddt, 1H, J = 1.5, 9.5, 15.0 Hz), 5.72 (dt, 1H, J = 7.0, 15.0 Hz), 6.69 (d, 2H, J = 8.5 Hz), 6.98 (d, 2H, J = 8.5 Hz).

23: δ 0.89 (t, 3H, J = 7.0 Hz), 1.29 (m, 14H), 1.53 (quint, 4H, J = 5.5 Hz), 2.05 (q, 2H, J = 6.5 Hz), 2.43 (t, 2H, J = 7.5 Hz), 2.44 (t, 2H, J = 7.5 Hz), 2.68 (d, 1H, J = 15.0 Hz), 2.73 (d, 1H, J = 15.0 Hz), 2.85 (dd, 1H, J = 8.5, 14.0 Hz), 3.00 (dd, 1H, J = 5.5, 14.0 Hz), 3.34 (d, 1H, J = 9.5 Hz), 3.62 (s, 3H), 3.68 (s, 3H), 3.72 (s, 3H), 4.56 (dd, 1H, J = 5.5, 8.0 Hz), 5.45 (ddt, 1H, J = 1.5, 9.5, 15.0 Hz), 5.66 (dt, 1H, J = 6.5, 15.0 Hz), 6.67 (d, 2H, J = 8.5 Hz), 6.95 (d, 2H, J = 8.5 Hz).