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Synthesis of *dl*-7-Methyldemethylvariotin†

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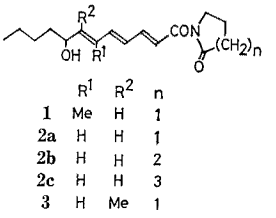
The synthesis of *dl*-7-methyldemethylvariotin with a less antibiotic potency than variotin is described.

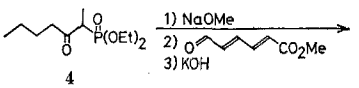
Variotin **1** is an antifungal antibiotic isolated from the culture fluid of *Paecilomyces varioti Bainier* var. *antibioticus* and the structure was established by Takeuchi and Yonehara.[1] We previously published some papers[2] on the syntheses of *dl*-variotin **1** and some analogues of this antibiotic, *e.g.* **2a**, **2b**, and **2c**. Interestingly all of these analogues, lacking a methyl group at the 6-position of the acid moiety, showed no bioactivity on the same microorganisms as used for the bioassay of variotin. Based on this fact, it was conceivable that a methyl group of the original antibiotic might be necessary for the bioactivity on the microorganisms. This idea prompted us to synthesize some variotin analogues possessing a methyl group or methyl groups in the acid moiety, to assay them, and to clarify the relationship between molecular structure and bioactivity. Here, we report the synthesis of the titled compound **3**, one of the structural isomers of the original antibiotic.

Our starting material, a keto phosphonate **4**, was prepared by acylation of diethyl lithioethylphosphonate with methyl valerate in THF at −70°C in 61% yield. The keto phosphonate was treated with NaOMe in DMF, followed by addition of methyl 6-oxo-2,4-(E,E)-hexadienate, prepared from monomethyl muconate (see ref. 2d), on an ice bath. The mixture was then stirred at room temperature for 16 hr to give a ketotrienic ester **5**, presumably an E,E,E-isomer, as a single product in 54% yield, which was converted to a ketotrienic acid **6** by hydrolysis in 82% yield. The acid **6** was treated with oxalyl chloride in THF at room temperature for 16 hr, and then after the addition of N-trimethylsilyl 2-pyrrolidone[3] the mixture was stirred at reflux temperature for 1.5 hr to provide 7-methyldemethylketovariotin **7** in 79% yield. Compound **7** was reduced with $LiAl(t\text{-}BuO)_3H$ in THF on an ice-salt bath for 30 min and worked up in a usual manner to afford *dl*-7-methyldemethylvariotin **3** in 82% yield. Satisfactory spectroscopic data were obtained for this substance.

Compounds **7** and **3** were less active on some microorganisms than variotin.



	R^1	R^2	n
1	Me	H	1
2a	H	H	1
2b	H	H	2
2c	H	H	3
3	H	Me	1



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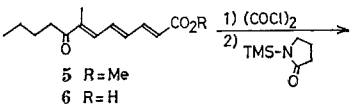
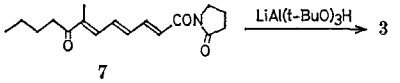


5 R=Me
6 R=H



7

† Synthetic Studies on Variotins VI. See Ref. 3).

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EXPERIMENTAL

Melting points are not corrected. IR spectra were measured in nujol mulls for solid substances and in liquid film for a liquid substance. NMR spectra were measured at 60 or 100 MHz in $CDCl_3$ or CCl_4 with TMS as an internal standard. MS spectra were measured at 70 eV.

Diethyl ethylphosphonate

A mixture of 78 g (0.5 mole) of ethyl iodide and 83 g (0.5 mole) of triethylphosphite was boiled at reflux temperature for 11 hr. The mixture was fractionated to afford 80.5 g (96% yield) of a colorless oil, bp 92~93.5°C/22 mmHg.

Diethyl 1-methyl-2-oxohexylphosphonate **4**

To a stirred dry THF (50 ml) at −70°C under N_2 atmosphere, 1.2 N *n*-BuLi solution (100 ml; 0.12 mole) in hexane and then diethyl ethylphosphonate (20.0 g; 0.12 mole) were added. After 1 hr stirring, the resulting orange solution was added dropwise to the solution of methyl valerate (10.0 g; 0.086 mole) in dry THF (100 ml) at −70°C under N_2 atmosphere. After 1 hr stirring, the reaction mixture was poured into ice water and extracted with two portions of ether. The ethereal extracts were combined, washed with two portions of brine, dried over anhyd. $MgSO_4$ and evaporated. The obtained residue was fractionated to afford 13.4 g of a pale yellow oil, bp 156.5~159°C/16.5 mmHg. Redistillation of the obtained oil provided 13.0 g (61% yield) of the product, bp 150°/22 mmHg; n_D^{23} 1.4359. IR ν_{max}^{neat} cm^{-1}: 1720, 1265, 1050, 1030. NMR ($CDCl_3$; 60 MHz) δ: 0.80~1.65 (m, 16H), 2.65 (t, J=5 Hz, 2H), 3.25 (dq, J=8 and 24 Hz, 1H), 4.10 (q, 7 Hz, 2H), 4.20 (q, J=7 Hz, 2H). *Anal.* Found: C, 52.68; H, 9.36. Calcd. for $C_{11}H_{23}O_4P$: C, 52.80; H, 9.20.

Methyl 7-methyl-8-oxo-2,4,6-(E,E,E)-dodecatrienate **5**

A solution of the ketophosphonate **4** (6.35 g; 0.025 mole) in dry DMF (10 ml) was added to the stirred solution of NaOMe, prepared from Na (0.5 g; 0.022 mole) and MeOH, in dry DMF (15 ml). After 40 min stirring, the solution of methyl 6-oxo-2,4-(E,E)-hexadienate (1.59 g; 0.011 mole) in dry DMF (4 ml) was added dropwise to the stirred mixture, cooled on an ice bath. The reaction mixture was kept at room temperature overnight, poured into ice water (100 ml) and extracted with ether (50 ml×3). The combined ethereal extracts were washed with water (×2) and brine (×1), dried over anhyd. $MgSO_4$ and evaporated. The obtained residue was filtered with suction and washed with a little amount of ether to provide 1160 mg of pale yellow crystals. The combined filtrate and washings gave pale yellow crystals by silica gel column chromatography. The total amount of the crystals was 1412 mg (54% yield). Recrystallization from AcOEt-pet. ether (1:1) gave pale yellow prisms, mp 102~103°C. IR ν_{max}^{nujol} cm^{-1}: 1710, 1679, 1619, 1588. NMR ($CDCl_3$; 60 MHz) δ: 0.91 (t, J=7 Hz, 3H), 1.1~1.8 (m, 4H), 1.95 (s, 3H), 2.70 (t, J=7 Hz, 2H), 3.80 (s, 3H), 6.00 (d, J=15 Hz, 1H), 6.4~7.7 (m, 4H). UV λ_{max}^{MeOH} nm (ε): 315 (54,200). *Anal.* Found: C, 71.25; H, 8.44. Calcd. for $C_{14}H_{20}O_3$: C, 71.19; H, 8.47.

7-Methyl-8-oxo-2,4,6-(E,E,E)-dodecatrienic acid **6**

A solution of KOH (484 mg; 8.64 mmoles) in MeOH (20 ml) and water (1 ml) was added to a solution of the methyl ester **5** (1360 mg; 5.8 mmoles) in MeOH (80 ml), and the mixture was boiled for 2.5 hr, quenched into ice water, acidified with dil. HCl and extracted with two portions of ether. The ethereal extracts were combined and extracted with satd. aq. Na_2CO_3 twice. The alkaline extracts were combined, acidified with dil. HCl, and extracted with two portions of ether. The combined ethereal extracts were washed with brine four times, dried over anhyd. Na_2SO_4 and evaporated. The obtained crystalline residue was filtered with suction and washed with a little amount of ether to give 576 mg (45% yield) of pale yellow crystals. Recrystallization from $CHCl_3$ afforded pale yellow needles, mp 137~138°C. The neutral ethereal fraction provided 610 mg of the ester; the yield of the acid based on the consumed ester was 82%. IR ν_{max}^{nujol} cm^{-1}: 3100~2400, 1695, 1665, 1623, 1580. NMR ($CDCl_3$; 60 MHz) δ: 0.93 (t, J=7 Hz, 3H), 1.1~1.8 (m, 4H), 1.99 (s, 3H), 2.74 (t, J=7 Hz, 2H), 6.06 (d, J=15 Hz, 1H), 6.4~7.9 (m, 4H), 11.87 (s, 1H). UV λ_{max}^{MeOH} nm(ε): 315 (42,500). *Anal.* Found: C, 70.13; H, 7.96. Calcd. for $C_{13}H_{18}O_3$: C, 70.27; H, 8.11. MS (*m/e*): 222 (M^+), 165 (M^+-C_4H_9).

7-Methyldemethylketovariotin **7**

A mixture of the keto acid **6** (386 mg; 1.74 mmoles), oxalyl chloride (228 mg; 1.8 mmoles), and dry THF (3 ml) was stirred at room temperature for 16 hr. To the stirred mixture, N-trimethylsilyl 2-pyrrolidone (728 mg; 4.0 mmoles) was added. After boiling for 1.5 hr, the mixture was evaporated. The obtained residue was dissolved into $CHCl_3$, and the $CHCl_3$ solution was washed with brine (×1), aq. $NaHCO_3$ (×1), and brine (×2), dried over anhyd. Na_2SO_4, and evaporated. Recrystallization of the obtained residue from ether in a refrigerator afforded 332 mg (66% yield; 79% yield based on the consumed acid) of yellow crystals. Recrystallization from $CHCl_3$-ether gave yellow crystals, mp 106~107°C. IR ν_{max}^{nujol} cm^{-1}: 1735, 1665, 1615, 1593, 1567. NMR ($CDCl_3$; 60 MHz) δ: 0.93 (t, J=7 Hz, 3H), 1.1~1.8 (m, 4H), 1.97 (s, 3H), 2.07 (quintet J=7 Hz, 2H), 2.65 (t, J=7Hz, 2H), 2.72 (t, J=7Hz, 2H), 3.90 (t, J=7Hz, 2H), 6.4~7.9(m, 5H). UV λ_{max}^{MeOH} nm(ε): 330 (48,000). *Anal.* Found: C, 70.34; H, 7.89;

N, 4.75. Calcd. for $C_{17}H_{23}O_3N$: C, 70.59; H, 7.96; N, 4.84. MS (*m/e*): 289 (M^+), 232 (weak, $M^+-C_4H_9$), 205 ($M^+-C_4H_6ON$).

dl-7-Methyldemethylvariotin **3**

Dry *t*-BuOH (333 mg; 4.5 mmoles) was added dropwise to a stirred suspension of $LiAlH_4$ (57 mg; 1.5 mmoles) in dry THF (10 ml) on an ice bath. After 10 min, to the stirred suspension on an ice bath, the ketone **7** (289 mg; 1.0 mmole) was added. After additional stirring for 30 min, the reaction mixture was poured into ice water and the mixture was extracted with ether (×3). The combined ethereal extracts were washed with brine, dried over anhyd. Na_2SO_4, and evaporated at low temperature under reduced pressure to provide a pale yellow syrup (251 mg), which was purified by silica gel column chromatography eluted with $CHCl_3/Et_2O$ (4/1) to afford a pale yellow syrup (238 mg; 82% yield). IR ν_{max}^{neat} cm^{-1}: 3440, 1740, 1665, 1595, 1580. NMR (CCl_4; 100 MHz) δ: 0.89 (t, J=8 Hz, 3H), 1.1~1.6 (m, 6H), 1.78 (s, 3H), 2.00 (quintet, J=8 Hz, 2H), 2.53 (t, J=8 Hz, 2H), 2.93 (broad, 1H), 3.78 (t, J=8 Hz, 2H), 3.92 (d, J=7 Hz, 1H), 6.05~7.53 (m, 5H). MS (*m/e*): 291 (weak, M^+), 273.

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