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SYNTHESIS OF OPTICALLY ACTIVE FORMS OF A-FACTOR, THE INDUCER OF STREPTOMYCIN BIOSYNTHESIS IN INACTIVE MUTANTS OF *STREPTOMYCES GRISEUS*†

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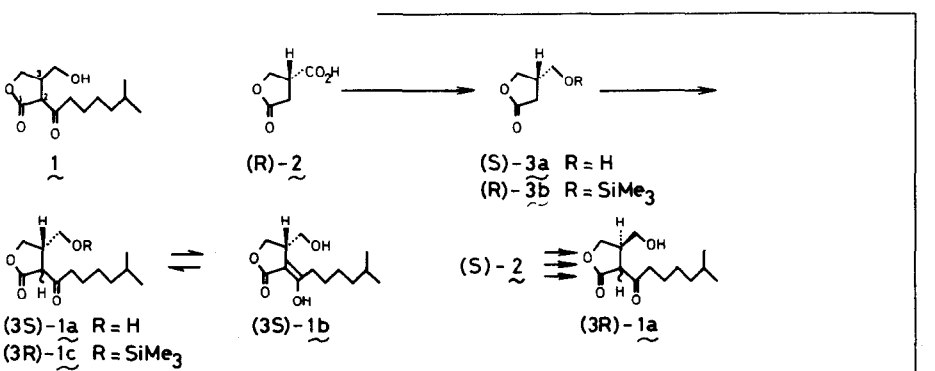
Abstract—The absolute configuration at C-3 of A-factor, 2-(6-methylheptanoyl)-3-hydroxymethyl - 4 - butanolide **1a**, was determined to be S by synthesizing both enantiomers of it from paraconic acid. (3S)-A-factor was 2.5 times more active than the (3R)-isomer as the inducer of streptomycin biosynthesis in inactive mutants of *Streptomyces griseus*.

Khokhlov *et al.* discovered A-factor as the inducer of the biosynthesis of streptomycin in inactive mutants of *Streptomyces griseus*.[1] It also induces the formation of spores in asporophological modifications of *S. griseus*.[1] In 1976, Russian workers proposed the gross structure of A-factor to be **1** by chemical and spectroscopic studies,[2] which was later confirmed by a synthesis of (±)-**1**.[3] They also deduced the absolute stereochemistry of A-factor to be 2S, 3S as shown in **1a**.[2,4]‡ This was done on the basis of the application of the Klyne lactone sector rule[5] to this system **1**. Since A-factor can exist either as a keto-form **1a** or as an enol-form **1b**, the applicability of the lactone sector rule is not unambiguous. We therefore decided to synthesize both enantiomers of A-factor from an optically active compound with known absolute stereochemistry in order to establish the configuration at C-3 unambiguously.[6] For this purpose paraconic acid **2** seemed to be the most appropriate starting material, because the absolute configuration of (−)-**2** was known to be R by its conversion to (S)-(−)-methylsuccinic acid.[7]

(±)-Paraconic acid **2** was resolved carefully with (R)-(+)-α-phenethylamine to give a pure crystalline salt. This was dissolved in MeOH–H_2O and filtered through Amberlite IR-120 (H^+-form) to give (R)-(−)-**2**, m.p. 57–58°, $[\alpha]_D^{23}$ −59.6° (MeOH), (lit[7] m.p. 48°, $[\alpha]_D^{30}$ +49° (MeOH) for (S)-(+)-**2**), after two recrystallizations from $CHCl_3$. Reduction of (R)-(−)-paraconic acid **2** with $BH_3 \cdot Me_2S$ in THF afforded (S)-(−)-3-hydroxymethyl-4-butanolide **3a**. Unfortunately attempts to determine the optical purity of (S)-(−)-**3a** by analyzing it or its (S)-(−)-MTPA ester (α-methoxy-α-trifluoromethylphenylacetate) were unsuccessful. The hydroxy lactone **3a** as well as A-factor **1a** itself was highly susceptible to racemization in the presence of a base or an acid due to intramolecular transesterification. Careless work-up of **3a** or **1a** led to partial or complete racemization. To avoid any racemization, the OH group of (S)-**3a** was protected as Me_3Si ether (R)-(−)-**3b** by treatment with $(Me_3Si)_2NH$ and Me_3SiCl in C_5H_5N. The lactone enolate generated by treating (R)-(−)-**3b** with 2.5 eq of $LiNPr^i_2$ in THF at −78° was acylated with 6-methylheptanoyl chloride at



−78° to give (3R)-**1c**. This was dissolved in EtOH–H_2O and the soln was heated under reflux to remove the Me_3Si protective group. Subsequent work-up followed by chromatographic purification afforded (3S)-(−)-A-factor **1a** as a waxy solid, $[\alpha]_D^{23}$ −13.1° ($CHCl_3$); CD(MeOH) 283.5 nm ($\Delta\epsilon$ +0.699), 221 nm ($\Delta\epsilon$ +0.420).§ The natural A-factor was reported to show the following CD spectrum: CD(MeOH) 285 nm (ϵ +0.349), 225 nm (ϵ +0.215).[2] The comparison of these CD data proved the

†Synthetic microbial chemistry—II. Part I, K. Mori and K. Sato, *Tetrahedron* **38**, 1221 (1982). The experimental part on the synthesis of (3R)-A-factor was taken from the B.Sc. thesis of K. Yamane (March 1982).

‡In their preliminary communication, Khokhlov *et al.* reported a 2S, 3R-stereochemistry for A-factor, while their formula in that paper showed a 3S-configuration.[2] They later revised this to the correct 2S, 3S-stereochemistry.[4]

§In our preliminary communication[6] we erroneously wrote (3S)-(+)-A-factor which should read (3S)-(−)-A-factor.

stereochemical identity of (3S)-(−)-A-factor **1a** with the natural product. This established beyond doubt the 3S-configuration of A-factor. Interestingly the CD data also suggested the higher optical purity of our synthetic A-factor than that of the natural one. Since the C-2 of (3S)-(−)-**1a** is readily enolizable via keto-enol tautomerization, the configuration at that position is uncertain. As suggested by Khokhlov, (2S, 3S)-**1a** seems to be thermodynamically more stable than (2R, 3S)-**1a**.[2,4]

So as to synthesize the unnatural enantiomer (3R)-(+)-**1a** of A-factor, (±)-paraconic acid **2** was resolved with (S)-(−)-α-phenethylamine to give (S)-(+)-**2**, m.p. 60–63°, $[\alpha]_D^{21.5}$ +61.7° (MeOH). In a similar fashion to that described above for (3S)-(−)-**1a**, (S)-(+)-paraconic acid **2** yielded (3R)-(+)-A-factor **1a**, $[\alpha]_D^{22}$ +12.7° ($CHCl_3$). Its CD spectrum was antipodal to that of (3S)-(−)-**1a**.

Bioassay of the enantiomers of A-factor was kindly carried out by Prof. T. Beppu and Mr. O. Hara of this Department. The least concentration effective as the inducer of streptomycin biosynthesis in inactive mutants of *Streptomyces griseus* was 2 ng/disc for (3S)-(−)-A-factor and 5 ng/disc for the (3R)-(+)-isomer. This means that the unnatural enantiomer also possesses weak biological activity (40% of the natural product).

EXPERIMENTAL

All b.ps were uncorrected. IR spectra were determined as film or as Nujol mull on a Jasco A-102 spectrometer. NMR spectra were recorded at 60 MHz with TMS as an internal standard on a Hitachi R-24A spectrometer unless otherwise stated. Optical rotations were measured on a Jasco DIP-140 automatic polarimeter.

Paraconic acid **2**

(*a*) *Racemate.* To a stirred and heated soln of ethyl (±)-paraconate[7] (81 g) in 95% EtOH (170 ml) was added dropwise a soln of KOH (60 g) in H_2O (590 ml) during 1 h keeping the soln at reflux temp. After the addition the mixture was stirred and heated under reflux for 15 min. After cooling, the soln was slowly filtered through a column of Amberlite IR-120 (H^+-form, 2 l.). The resin was washed with H_2O. The eluant (5 l.) was concentrated continuously with a flash evaporator. The residue was distilled to give a fraction with b.p. 170–180°/1.2 mm. This was mixed with $CHCl_3$ and left to stand in a refrigerator to give 47 g (70.6%) of crude (±)-**2**, m.p. 47–54°. Recrystallization of the crude product from $CHCl_3$ yielded prisms, m.p. 58–61.5° (lit[7] m.p. 60–63°), ν_{max} ~3200 (m), ~2600 (m), ~1735 (br. s), 1270 (m), 1230 (s), 1190 (s), 1100 (w), 1030 (s), 1000 (m), 860 (m), 840 (m) cm^{-1}; δ($CDCl_3$) 2.79 (1H, d, J = 2 Hz), 2.92 (1H, s), 3.30–3.80 (1H, m), 4.55 (2H, d, J = 6 Hz), 10.52 (1H, s). This acid is hygroscopic. (Found: C, 45.74; H, 4.54. Calc. for $C_5H_6O_4$: C, 46.16; H, 4.65%.)

(*b*) (*R*)-(−)-*Isomer.* (R)-(+)-α-Phenethylamine (44 g) was added to a soln of (±)-**2** (47 g) in MeOH (150 ml). Ether (50 ml) was added to the soln and the mixture was left to stand at room temp. The separated crystalline salt (52 g) was collected on a filter and recrystallized from MeOH-ether to give 29 g of the salt, $[\alpha]_D^{21}$ −13.4° (c = 1.12, MeOH). This was recrystallized from MeOH–ether to give 18 g of the salt, $[\alpha]_D^{21}$ −17.3° (c = 1.19, MeOH). Further recrystallization from MeOH–ether gave 11 g of crystals, $[\alpha]_D^{21}$ −20.6° (c = 1.06, MeOH). This was again recrystallized from MeOH–ether to give 7 g (15.4%) of the (R)-acid-(R)-amine salt as needles, m.p. 150–152°, $[\alpha]_D^{21}$ −25.0° (c = 0.80, MeOH); ν_{max} ~2650 (m), 2520 (w), 2200 (w), 1775 (s), 1625 (m), 1455 (m), 1395 (m), 1370 (m) 1170 (m), 1160 (m), 1020 (m), 770 (m), 700 (m) cm^{-1}. (Found: C, 61.98; H, 6.64; N, 5.57. Calc. for $C_{13}H_{17}O_4N$: C, 62.14; H, 6.82; N, 5.57%.) This salt (7 g) was dissolved in MeOH (20 ml)–H_2O (50 ml). The soln was filtered through Amberlite IR-120 (H^+-form, 100 g). The resin was washed with H_2O (500 ml). The combined filtrate and washings were concentrated *in vacuo* with a flash evaporator. The residue was dissolved in THF-C_6H_6 and concentrated *in vacuo*. This was repeated several times to remove H_2O. The residue was dissolved in THF–$CHCl_3$. The soln was filtered to remove solid impurities and the filtrate was concentrated *in vacuo*. The residue was recrystallized from $CHCl_3$ to give 2.5 g (69%) of (R)-**2**. Further recrystallization from $CHCl_3$ yielded 1.39 g (39% from the salt) of (R)-**2** as hygroscopic prisms, m.p. 57–58°, $[\alpha]_D^{23}$ −59.6° (c = 0.614, MeOH). Because of its hygroscopic nature (R)-**2** did not give correct analytical data. (Found: C, 45.03; H, 4.73. Calc. for $C_5H_6O_4$: C, 46.16; H, 4.65%.) The IR and NMR spectra were identical to those of the racemate.

(*c*) (*S*)-(+)-*Isomer.* (S)-(−)-α-Phenethylamine (59 g) was added to a soln of (±)-**2** (62.7 g) in MeOH (170 ml). Ether was added to the soln and the mixture was left to stand overnight at room temp. The precipitated salt was recrystallized six times from *i*-PrOH to give 7 g (10.8%) of the (S)-acid-(S)-amine salt, m.p. 138–141°, $[\alpha]_D^{23}$ +24.0° (c = 1.032, MeOH). The IR spectrum was identical to that of the (R)-acid-(R)-amine salt. (Found: C, 62.36; H, 6.91; N, 5.52. Calc. for $C_{13}H_{17}O_4N$: C, 62.14; H, 6.82; N, 5.57%.) This salt (13.11 g; $[\alpha]_d^{23}$ +23.8° (c = 1.016, MeOH)) was dissolved in MeOH (47 ml)–H_2O (50 ml) and filtered through Amberlite IR-120 (H^+-form, 200 g). The resin was washed with H_2O (1 l.). The combined filtrate and washings were concentrated *in vacuo* with a flash evaporator. The residue was worked up as described above for (R)-**2** to give 3.55 g (52.3% from the salt) of (S)-**2**, m.p. 60–63°, $[\alpha]_D^{21.5}$ +61.7° (c = 0.625, MeOH), as hygroscopic prisms. (Found: C, 45.56; H, 4.82. Calc. for $C_5H_6O_4$: C, 46.16; H, 4.65%.) The IR and NMR spectra were identical with those of the racemate.

3-*Hydroxymethyl-4-butanolide* **3a**

(*a*) *Racemate.* $BH_3 \cdot Me_2S$ (1 ml) was added dropwise to a stirred and ice-cooled soln of (±)-**2** (1.09 g) in dry THF (7.5 ml) under Ar at 0–5. The mixture was stirred for 3 h at 0–5°. Then MeOH (10 ml) was added dropwise with cooling to destroy BH_3. The soln was concentrated *in vacuo*. The residue was dissolved in MeOH (10 ml) and concentrated *in vacuo*. This was repeated once more. The residue was distilled to give 897 mg (92%) of (±)-**3a**, b.p. 160°/8 mm, n_D^{21} 1.4608; ν_{max} 3400 (br. s), 2930 (s), 2860 (s), 1760 (vs), 1475 (m), 1415 (m), 1380 (m), 1340 (m), 1290 (m), 1260 (m), 1180 (s), 1045 (s), 1020 (s), 970 (m), 940 (m), 895 (w), 835 (w) cm^{-1}; δ ($CDCl_3$) 2.3–2.9 (3H, m), 3.65 (2H, d, J = 6 Hz), 3.90 (1H, s), 4.1–4.6 (2H, m). (Found: C, 51.64; H, 7.36. Calc. for $C_5H_8O_3$: C, 51.72; H, 6.94%).

(*b*) (*S*)-(−)-*Isomer.* $BH_3 \cdot Me_2S$ (1.5 ml) was added dropwise to a stirred and ice-cooled soln of (R)-(−)-**2** (1.3 g, $[\alpha]_D^{23}$ −59.6° (MeOH)) in dry THF (10 ml) under Ar at 0–5°. The mixture was stirred at 0–5° for 1 h and quenched with MeOH (5 ml). The soln was concentrated *in vacuo* below 30°. The residue was dissolved in MeOH (10 ml) and concentrated *in vacuo*. This was repeated again. The residue was chromatographed over SiO_2 (Mallinckrodt Silicar CC-7, 3 g) in *n*-hexane. Elution with $CHCl_3$ yielded 995 mg (89%) of (S)-**3a**, n_D^{22} 1.4661; $[\alpha]_D^{22}$ −46.3° (c = 1.224, $CHCl_3$). The IR and nmr spectra were identical with those of the racemate. Higher work-up temp or distillation of the product caused racemization.

(*c*) (R)-(+)-*Isomer.* In the same manner as above (S)-(+)-**2** (1.12 g, $[\alpha]_D^{21.5}$ +59.7° (MeOH)) yielded 800 mg (80%) of (R)-(+)-**3a**, $n_D^{21.5}$ 1.4671; $[\alpha]_D^{21.5}$ +44.8° (c = 1.553, $CHCl_3$). This showed the same spectral properties as those for (±)-**3a**.

3-*Trimethylsilyloxymethyl-4-butanolide* **3b**

(*a*) (*R*)-(−)-*Isomer.* $(Me_3Si)_2NH$ (1 ml) and Me_3SiCl (1 ml) were added to a stirred and ice-cooled soln of (S)-(−)-**3a** (990 mg, $[\alpha]_D^{22}$ −46.3° ($CHCl_3$)) in dry C_5H_5N (1 ml) and the mixture was kept at 0–5° for 2 h. After dilution with C_6H_6-n-hexane, the mixture was filtered through Celite to remove $C_5H_5N \cdot HCl$. The filtrate was concentrated *in vacuo*. The residue was distilled to give 830 mg (52%) of (R)-**3b**, b.p. 140–144°/26 mm, n_D^{23} 1.4368; $[\alpha]_D^{23}$ −34.8° (c = 0.736, ether); ν_{max} 2950 (m), 2900 (m), 2860 (m), 1775 (s), 1470 (w), 1415 (m), 1370 (m), 1335 (w), 1250 (s), 1170 (s), 1090 (s), 1020 (m), 1000 (m), 870 (s), 840 (s), 745 (m), 680 (w) cm^{-1}; δ (CCl_4) 0.20 (9H, s), 2.25–3.05

(3H, m) 3.66 (2H, d, *J*-6 Hz), 4.10–4.70 (2H, m). (Found: C, 50.71; H, 8.35. Calc. for $C_8H_{16}O_3Si$: C, 51.03; H, 8.57%).

(*b*) (*S*)-(+)-*Isomer.* In the same manner as above (R)-(+)-**3a** (800 mg, $[\alpha]_D^{21.5}$ + 44.8° ($CHCl_3$)) yielded 687 mg (52%) of (S)-**3b**, b.p. 140°/12 mm, $n_D^{21.5}$ 1.4671; $[\alpha]_D^{21.5}$ + 36.0° (c = 0.86, ether). The IR and NMR spectra were identical with those of (R)-(−)-**3b**.

2-(6-*Methylheptanoyl*)-3-*hydroxymethyl*-4-*butanolide* (*A-factor*) **1***a*

(*a*) (3*S*)-(−)-*Isomer* (= *natural A-factor*). A soln of $LiNPr^i_2$ was prepared by the addition of n-BuLi (1.53 N in n-hexane, 7.3 ml) to a stirred and cooled soln of Pr^i_2NH (1.1 g) in dry THF at −78° under Ar. To this was added with stirring and cooling at −78° a soln of (R)-**3b** (830 mg) in dry THF (5 ml) during 5 min. The soln was stirred at −78° for 10 min. Then 6-methylheptanoyl chloride (0.8 g) in dry THF (2 ml) was added dropwise during 5 min at −78°. After stirring for 1 h at −78°, the mixture was poured into ice–AcOH–H_2O and extracted with ether. The ether soln was washed with H_2O, $NaHCO_3$ soln and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give (3R)-**1c** as an oil with ν_{max} 1770 (s), 1720 (s), 865 (s), 840 (s) cm^{-1}. This was dissolved in 95% EtOH–H_2O (4 : 1, 15 ml) and the soln was stirred and heated under reflux for 10 min. After the removal of EtOH *in vacuo*, the product was extracted with ether. The ether soln was washed with H_2O and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed over SiO_2 (Mallinckrodt Silicar CC-7, 6 g) in n-hexane. Impurities were first eluted with n-hexane-ether (4 : 1) and then (3S)-**1a** was eluted with n-hexane–ether (1 : 1) as 318 mg (30%) of a waxy solid, $[\alpha]_D^{23}$ − 13.1° (c = 1.18, $CHCl_3$); ν_{max} 3460 (m), 2960 (s), 2930 (s), 2860 (m), 1765 (s), 1720 (s), 1640 (w), 1470 (m), 1380 (m), 1365 (m), 1215 (m), 1170 (s), 1130 (m), 1095 (m), 1025 (s), 960 (w), 945 (w), 900 (w), 870 (w), 835 (w), 735 (w), 680 (w) cm^{-1}; δ ($CDCl_3$) 0.82 (6H, d, *J* = 6 Hz), 1.0–2.0 (9H, br), 2.5–3.3 (3H, m), 3.5–3.8 (2H, m), 3.9–4.2 (2H, m); ^{13}C NMR (25 MHz, $CDCl_3$) 22.260, 23.167, 26.443, 27.466, 38.348, 39.225, 42.297, 54.758, 61.310, 69.120, 172.728 (C=O), 203.5 (C=O); MS: *m/z* 242.1604 ($M^+ = C_{13}H_{22}O_4$, 3%), 211.1356 ($C_{12}H_{19}O_3$, 34%), 158.0531 ($C_7H_{10}O_4$, 30%), 153.0539 ($C_8H_9O_3$, 25%), 143.0461 ($C_{10}H_7O$, 20%), 141.0781 ($C_{11}H_9$, 28%), 127.1118 ($C_8H_{15}O$, 30%), 127.0380 ($C_6H_7O_3$, 32%), 116.0492 ($C_5H_8O_3$, 33%), 109.1039 (C_8H_{13}, 100%, base peak), 102.0319 ($C_4H_6O_3$, 30%), 85.0278 ($C_4H_5O_2$, 77%), 83.0853 (C_6H_{11}, 35%), 82.0782 (C_6H_{10}, 46%), 69.0709 (C_5H_9, 20%), 67.0543 (C_5H_7, 28%), 57.0716 (C_4H_9, 69%), 55.0556 (C_4H_7, 33%), 55.0191 (C_3H_3O, 29%), 43.0573 (C_3H_7, 51%), 41.0420 (C_3H_5, 40%); CD (c = 0.062, MeOH): 283.5 nm ($\Delta\epsilon$ + 0.699, peak), 221 nm ($\Delta\epsilon$ + 0.420, peak).

(*b*) (3*R*)-(+)-*Isomer.* In the same manner as above (S)-**3b** (687 mg) yielded 256 mg (29%) of (3R)-(+)-**1a**, $[\alpha]_D^{22}$ + 12.7° ($CHCl_3$); CD (c = 0.109, MeOH): 283.5 nm ($\Delta\epsilon$ − 0.593, trough), 222 nm ($\Delta\epsilon$ − 0.402, trough). This showed the same IR, ^1H NMR, ^{13}C NMR and mass spectra as those of (3S)-(−)-**1a**.

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