July, 1975] BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 48 (7), 2081—2083 (1975) 2081

Synthesis of (2*R*, 3*S*, 4*S*)-4-Amino-3-hydroxy-2-methyl-5-(3-pyridyl)-pentanoic Acid Present in Antibiotic Pyridomycin

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(Received April 12, 1975)

Natural (2*R*,3*S*,4*S*)-4-amino-3-hydroxy-2-methyl-5-(3-pyridyl)pentanoic acid (**1**) present in antibiotic pyridomycin was synthesized. The 5,6-anhydro-1,2-*O*-isopropylidene-3-*C*-methyl-α-D-allofuranose (**4**) was prepared from (the known) 3-deoxy-1,2;5,6-di-*O*-isopropylidene-3-*C*-methyl-α-D-allofuranose (**2**). Regiospecific introduction of 3-pyridyl group to the C-6 of **4** was effected by 3-pyridyllithium. The product (**5**) was converted to the 5-azido derivative (**7**) by the S_N2 substitution reaction of the 5-mesylate (**6**) of **5**. Hydrolysis of **7** followed by two-stage oxidation with periodate-bromine in aqueous acetic acid afforded (2*R*,3*S*,4*S*)-4-azido-3-formyloxy-2-methyl-5-(3-pyridyl)pentanoic acid (**9**), which was transformed to the dihydrochloride of **1**.

A novel amino acid, 4-amino-3-hydroxy-2-methyl-5-(3-pyridyl)pentanoic acid (**1**) present in antimycobacterial antibiotic pyridomycin[1,2] was isolated as a degradation product of pyridomycin by Ogawara, *et al.*[3] Its absolute configuration was established as "2*R*,3*S*,4*S*" (structure **1**) based on the complete structure of the parent antibiotic determined by X-ray crystallographic analysis.[4] This paper presents a synthesis of **1** with the natural configuration from an appropriate sugar derivative through regiospecific and stereospecific routes as shown in the Chart.

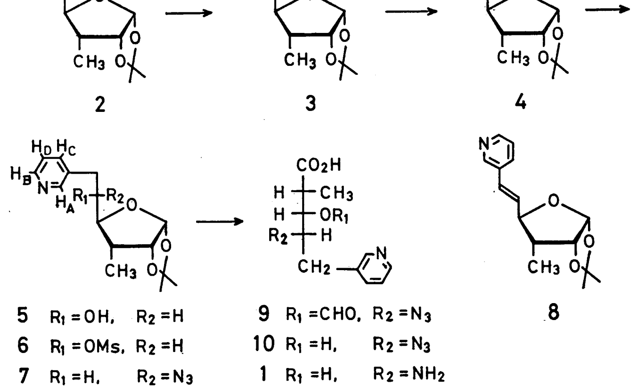

Chart.

Removal of 5,6-*O*-isopropylidene group of (the known) 3-deoxy-1,2; 5,6-di-*O*-isopropylidene-3-*C*-methyl-α-D-allofuranose[5] (**2**) with a warm 91% aqueous acetic acid followed by selective *O*-tosylation in the usual way afforded the 6-tosylate (**3**) in a 65% yield. Treatment of **3** with sodium methoxide in chloroform gave 5,6-anhydro-3-deoxy-1,2-*O*-isopropylidene-3-*C*-methyl-α-D-allofuranose (**4**) in a 76% yield. The epoxide ring of **4** was regiospecifically opened with 3-pyridyllithium[6] in ether at −10 °C to afford 3,6-dideoxy-1,2-*O*-isopropylidene-3-*C*-methyl-6-*C*-(3-pyridyl)-α-D-allofuranose (**5**) in an 88% yield, whose structure was confirmed by PMR spectroscopy. Mesylation of **5** with mesyl chloride in pyridine gave crystalline 5-mesylate (**6**) in a 75% yield. Substitution reaction of **6** with sodium azide in dimethylsulfoxide at 85 °C afforded two kinds of products, which were separated from each other by silica gel column chromatography in homogeneous states. Structural elucidations of the reaction products isolated on the basis of elemental and spectroscopic analyses revealed that the reaction produced one substitution reaction product and one elimination reaction product in 70 and 23% yield, respectively, none of the rearrangement reaction product being formed. Since this substitution reaction seemed to be very characteristic of the S_N2 reaction which should theoretically have only one alternation (*E*2 reaction),[7] the major product above-obtained was considered the expected S_N2 substitution reaction product, 5-azido-3,5,6-trideoxy-1,2-*O*-isopropylidene-3-*C*-methyl-6-*C*-(3-pyridyl)-β-L-talofuranose (**7**), the C-5 configuration of which was opposite to that of **6**. The structure of the minor product assigned to the elimination reaction product was determined as 3,5,6-trideoxy-1,2-*O*-isopropylidene-3-*C*-methyl-6-*C*-(3-pyridyl)-α-D-*ribohex-trans*-5-enofuranose (**8**) by PMR analysis. When the mesylate (**6**) was kept without the nucleophile (azide ion) in dimethyl sulfoxide at ~85 °C, the formation of **8** was never observed. This fact indicated that the compound (**8**) should be formed in the *E*2 reaction of **6** under S_N2 condition. Moreover, the fact that in the case of **6**, such an *E*2 reaction practically occured to some extent, may demonstrate that the 3-pyridylmethyl hydrogens at C-6 of **6** are so active that they are removed by the weakly basic azide ion.

Hydrolysis of **7** in a boiling 20% aqueous acetic acid, followed by successive oxidations of the resultant free sugar with sodium periodate and with bromine in the same aqueous acetic acid at room temperature afforded (2*R*,3*S*,4*S*)-4-azido-3-formyloxy-2-methyl-5-(3-pyridyl)pentanoic acid (**9**) as needles in a 59% yield. It should be noted that the two-stage oxidation of the free sugar must be carried out in the acidic condition at pH≤2, in order to prevent the complicated side reactions including the β-elimination of the formyloxy group which may probably be catalysed by the free pyridyl residue present in its own molecule at heigher pH values than 2.

De-*O*-formylation of **9** with dilute hydrogen chloride in aqueous dioxane at room temperature gave the corresponding azido hydroxy acid (**10**) as a crystalline hydrochloride in an 81% yield. The hydrochloride of **10** was hydrogenolyzed on palladium black in methanol to afford the title compound (**1**) as a monohydrochloride in a 97% yield, from which the dihydrochloride of **1** was obtained as a colorless hygroscopic powder with a negative rotation ($[\alpha]_D -2°$ in 5 M hydrochloric acid). The synthetic dihydrochloride of **1** proved to be identical with the natural amino acid dihydrochloride derived from pyridomycin according to Ogawara, *et al.*[3)] in all respects.

Experimental

Melting points were determined on a Mitamura Riken micro hot-stage and are uncorrected. IR spectra were taken on a Hitachi 225 spectrophotometer, PMR spectra were recorded on a Varian A-60D spectrometer using TMS as an internal standard. Specific rotations were determined with a Zeiss Photoelectric Precision Polarimeter. Tlc was performed on WAKOGEL B-5 and silica gel column chromatography on WAKOGEL C-200 which was activated at 110 °C for 1 hr. Paper chromatography was conducted on Toyoroshi No. 525 with butanol–acetic acid–water (3: 1: 1). Paper electrophoresis was carried out with a Savant 1V-5000A in formic acid–acetic acid–water (1: 3: 36). pK_a values were measured with a Metrohm Herisau Potentiograph E436. In general, all concentration were carried out under reduced pressure below 40 °C.

3-Deoxy-1,2-O-isopropylidene-3-C-methyl-6-O-tosyl-α-D-allofuranose (3). Treatment of **2** (7.00 g) prepared according to Rosenthal, *et al.*[5)] with a 91% aqueous acetic acid (38.8 ml) at 60 °C for 30 min followed by evaporation afforded a syrup, which was chromatographed through silica gel (140 g) with benzene–ethyl acetate (1: 1) to give a practically pure sample of the de-5,6-*O*-isopyridenation product (5.36 g, 90.5% yield). The product (4.15 g, 18.9 mmol) was tosylated with tosyl chloride (3.99 g, 20.9 mmol) in pyridine by the usual way to afford **3** (5.06 g, 72%) as a colorless syrup: $[\alpha]_D^{28}$ +23° (*c* 1.15, chloroform); $\nu_{max}^{CCl_4}$ 3550 (OH) and 1175 cm^{-1} (sulfonate); $\delta(CDCl_3)$, 1.15 (d, 3-CH_3, J_{3,CH_3}=6.8 Hz), 1.32 and 1.48 [each s, $(CH_3)_2C$], 3.33—4.08 (m, OH and H-3), 4.13 (s, Ar–CH_3), 3.62—4.33 (m, H-4, H-5, and H-6), 4.57 (dd, H-2, $J_{2,3}$=4.9 Hz), 5.77 (d, H-1, $J_{1,2}$=3.9 Hz), and 7.33—7.97 (m, Ar).

Found: C, 55.09; H, 6.53; S, 8.30%. Calcd for $C_{17}H_{24}O_7S$: C, 54.83; H, 6.50; S, 8.60%.

5,6-Anhydro-3-deoxy-1,2-O-isopropylidene-3-C-methyl-α-D-allofuranose (4). A 2.12M solution of sodium methoxide in methanol (6.5 ml) was added dropwise with stirring to a solution of **3** (5.06 g, 13.6 mmol) in dry chloroform (20 ml) under ice-cooling. After the cooled mixture was vigorously stirred, it was allowed to stand at room temperature for 2 hr. The resultant solution was poured into cold water (20 ml) and the mixture was extracted with chloroform. The chloroform layer was washed with satd. aq. NaCl, dried, and evaporated. The syrupy residue (2.69 g) was chromatographed through silica gel (150 g) with benzene–ethyl acetate (5: 1) to afford **4** (2.06 g, 76%) as a colorless syrup: $[\alpha]_D^{25}$ +12° (*c* 0.85, chloroform); $\nu_{max}^{CCl_4}$ 1250 and 870 cm^{-1} (epoxide); $\delta(CDCl_3)$, 1.18 (d, 3-CH_3, J_{3,CH_3}=6.8 Hz), 1.35 and 1.52 [each s, $(CH_3)_2C$], 1.75—2.42 (m, H-3), 2.58—3.17 (m, H-5 and H-6), 3.58 (dd, H-4, $J_{3,4}$=10.0 Hz and $J_{4,5}$=5.9 Hz), 4.63 (dd, H-2, $J_{2,3}$=4.9 Hz), and 5.88 (d, H-1, $J_{1,2}$=3.9 Hz).

Found: C, 59.84; H, 7.88%. Calcd for $C_{10}H_{16}O_4$: C, 59.98; H, 8.05%.

3,6-Dideoxy-1,2-O-isopropylidene-3-C-methyl-6-C-(3-pyridyl)-α-D-allofuranose (5). The reaction was carried out under nitrogen. An ethereal solution (16.6 ml) of butyllithium (8.61 mmol) was cooled to −35 °C in a dry ice-methanol bath and there was added dropwise with stirring, a solution of 3-bromopyridine (1.82 g, 11.5 mmol) in ether (8 ml) during a period of 30 min. After 15 min, to the bright yellow colored suspension was added dropwise with stirring, a solution of **4** (573 mg, 2.87 mmol) in ether (7 ml), the mixture being maintained at −10 °C for 1.5 hr. The resultant brown mixture was then poured into cold water and extracted with ether twelve times. The ether extracts were washed with satd. aq. NaCl, dried and evaporated to afford a brown syrup (1.24 g). The syrup was chromatographed through silica gel (80 g) with benzene–acetone (1: 1) to give pale yellow crystals of **5** (700 mg, 88%). Recrystallization from ethyl acetate–petroleum ether afforded an analytical sample as a colorless needle: mp 114.0—115.0 °C; $[\alpha]_D^{14}$ +39° (*c* 0.74, chloroform); ν_{max}^{KBr} 3460 (OH), 1585, 1430, 795 and 710 cm^{-1} (pyridine); $\delta(CDCl_3)$, 1.23 (d, 3-CH_3, J_{3,CH_3}=6.8 Hz), 1.38 and 1.55 [each s, $(CH_3)_2C$], 1.83—2.50 (m, H-3), 2.75—3.01 (m, H-6 and OH), 3.75—4.17 (m, H-4 and H-5), 4.65 (dd, H-2, $J_{2,3}$=4.9 Hz), 5.87 (d, H-1, $J_{1,2}$=3.9 Hz), 7.30 (m, H_D, $J_{A,D}$=1.0 Hz, $J_{B,D}$=5.0 Hz and $J_{D,C}$=8.3 Hz), 7.73 (m, H_C, $J_{A,C}$=2.6 Hz and $J_{B,C}$=2.0 Hz), 8.40 (m, H_B, $J_{A,B}$=0.5 Hz), and 8.55 (m, H_A).

Found: C, 64.73; H, 7.59; N, 4.91%. Calcd for $C_{15}H_{21}O_4N$: C, 64.49; H, 7.58; N, 5.01%.

3,5,6-Trideoxy-1,2-O-isopropylidene-5-O-mesyl-3-C-methyl-6-C-(3-pyridyl)-α-D-allofuranose (6). Mesylation of **5** (1.20 mmol) with mesyl chloride (1.44 mmol) in dry pyridine (3.4 ml) at room temperature followed by work up in the usual manner afforded a crystalline solid of **6**(540 mg), which was recrystallized from ethyl acetate–petroleum ether to give a pure sample of **6** (333 mg, 75%): mp 111.5—112.0 °C; $[\alpha]_D^{18}$ +31° (*c* 0.89, chloroform); ν_{max}^{KBr} 1360 and 1175 cm^{-1} (sulfonate); $\delta(CDCl_3)$, 1.23 (d, 3-CH_3, J_{3,CH_3}=6.8 Hz), 1.37 and 1.51 [each s, $(CH_3)_2C$], 4.47 (s, –SO_3CH_3), 1.83—2.33 (m, H-3), 3.17 (d, H-6, $J_{5,6}$=6.0 Hz), 3.98 (dd, H-4, $J_{3,4}$=10.0 Hz and $J_{4,5}$=4. 5 Hz), 4.65 (dd, H-2, $J_{2,3}$=4.8 Hz), 5.01 (dt, H-5), 5.88 (d, H-1, $J_{1,2}$=3.8 Hz), 7.3—7.9 and 8.5—8.9 (each m, 3-pyridyl).

Found: C, 53.67; H, 6.47; N, 3.77; S, 9.02%. Calcd for $C_{16}H_{23}O_6NS$: C, 53.77; H, 6.49; N, 3.92; S, 8.96%.

*5-Azido-3,5,6-trideoxy-1,2-O-isopropylidene-3-C-methyl-6-C-(3-pyridyl)-β-L-talofuranose (7) and 3,5,6-Trideoxy-1,2-O-isopropylidene-3-C-methyl-6-C-(3-pyridyl)-α-D-*ribo*hex*-trans-*5-enofuranose (8).* A solution of **6** (331 mg, 0.925 mmol) in dimethylsulfoxide (1.7 ml) was heated with sodium azide (181 mg, 2.78 mmol) at 85 °C for 2 hr: tlc [benzene–acetone (3: 1)] revealed the absence of **6** (R_f 0.37) and the presences of the two products (R_f 0.60 and 0.52). The cooled solution was diluted with water (2 ml) and extracted with ethyl acetate seven times. The extracts were washed with satd. aq. NaCl, dried and evaporated to afford a yellow syrup (295 mg). Column chromatography [silica gel 45 g, benzene–acetone (10: 1)] of the syrup afforded the substitution product (**7**) [R_f 0.60, colorless syrup, 196 mg (70%)] and the elimination product (**8**) [R_f 0.52, colorless needle, 54 mg (23%)].

7: $[\alpha]_D^{15}$ +11 ° (*c* 1.40, chloroform); $\nu_{max}^{CCl_4}$ 2105 cm^{-1} (N_3); $\delta(CDCl_3)$, 1.02 (d, 3-CH_3, J_{3,CH_3}=6.8 Hz), 1.36 and 1.46 [each s, $(CH_3)_2C$], 2.0—2.6 (m, H-3), 3.0—3.5 (m, H-5 and H-6), 3.80 (dd, H-4, $J_{3,4}$=10.2 Hz and $J_{4,5}$=1.3 Hz), 4.66 (dd, H-2, $J_{2,3}$=4.0 Hz), 5.93 (d, H-1, $J_{1,2}$=3.6 Hz), 7.2—7.9 and 8.5—8.8 (each m, 3-pyridyl).

Found: C, 59.21; H, 6.67; N, 18.55%. Calcd for $C_{15}H_{20}O_3N_4$: C, 59.19; H, 6.62; N, 18.41%.

8: mp 80.0—81.5 °C (two recrystallizations from petroleum ether); $[\alpha]_D^{20}$ +13 ° (*c* 0.75, chloroform); $\nu_{max4}^{CCl_4}$ 965 cm^{-1} (disubstituted *trans*-olefine); δ $(CDCl_3)$, 1.12 (d, 3-CH_3, J_{3,CH_3}=6.8 Hz), 1.38 and 1.59 [each s, $(CH_3)_2C$], 1.7—2.1 (m, H-3), 4.13 (dd, H-4, $J_{3,4}$=10.0 Hz and $J_{4,5}$=7.0 Hz), 4.78 (t, H-2, $J_{2,3}$=3.9 Hz), 6.07 (d, H-1, $J_{1,2}$=3.9 Hz), 6.37 (dd, H-5, $J_{5,6}$=16.3 Hz), 6.91 (d, H-6), 7.3—8.1 and 8.6—9.1 (each m, 3-pyridyl).

Found: C, 68.80; H, 7.33; N, 5.31%. Calcd for $C_{15}H_{19}O_3N$: C, 68.94, H, 7.33; N, 5.36%.

*(2R,3S,4S)-4-Azido-3-formyloxy-2-methyl-5-(3-pyridyl)-pentanoic Acid (**9**).* A solution of **7** (506 mg, 0.172 mmol) in a 20% aqueous acetic acid (25 ml) was refluxed for 5 hr. After evaporation, the residual syrup (free sugar) was dissolved in a 33% aqueous acetic acid (15 ml) and then cooled at 5 °C, there was added dropwise under stirring a solution of $NaIO_4$ (716 mg) in water (7.2 ml). After 20 min, bromine (0.45 ml) was added to the reaction mixture (pH 2) and allowed to stand at room temperature overnight. The resultant solution was aerated to remove an excess of bromine and evaporated. The residual solid mass was extracted with ethanol (*ca.* 5 ml) and the extract was evaporated to give a yellow syrup (958 mg). Column chromatography [silica gel 50 g, chloroform–methanol–acetic acid (80:5:1)] of the syrup afforded a yellow solid, which was recrystallized twice from acetone to give a pure sample of **9** (275 mg, 59%) as colorless needles: mp 127.5—129.5 °C (decomp.); $[\alpha]_D^{26}$ -114 ° (*c* 0.57, methanol); ν_{max}^{KBr} 2100 (N_3) and 1725 cm^{-1} (OCHO and COOH); $\delta(CDCl_3)$, 1.34 (d, 2-CH_3, $J_{2,CH_3}=7.1$ Hz), 2.73 (dd, H-5, $J_{4,5}=11.0$ Hz, $J_{5,5'}=14.5$ Hz), 3.16 (dq, H-2, $J_{2,3}=4.5$ Hz), 3.54 (dd, H-5′, $J_{4,5'}=3.6$ Hz), 4.13 (dq, H-4, $J_{3,4}=8.0$ Hz), 5.53 (dd, H-3), 7.4—8.2 and 8.7—9.1 (each m, 3-pyridyl), 8.47 (s, OCHO), and 12.0—12.3 (broad s, COOH).

Found: C, 52.00; H, 5.06; N, 19.90%. Calcd for $C_{12}H_{14}O_4N_4$: C, 51.79; H, 5.07; N, 20.14%.

*(2R, 3S, 4S)-4-Azido-3-hydroxy-2-methyl-5-(3-pyridyl)pentanoic Acid (**10**) Hydrochloride.* Treatment of **9** (21.7 mg) with 2 ml of a 2% HCl (in 50% (v/v) aq. dioxane) at room temperature for 1 hr followed by evaporation afforded a crystalline solid, which was recrystallized twice from methanol–ether to give a pure sample of **10** hydrochloride (19.2 mg, 81%) as colorless needles: mp 127.0—132.0 °C (decomp.); $[\alpha]_D^{19}$ $-40°$ (c 0.63, methanol); ν_{max}^{KBr} 3340 (OH), 2090 (N_3), and 1720 cm^{-1} (COOH).

Found: C, 45.97; H, 5.40; N, 19.75; Cl, 12.46%. Calcd for $C_{11}H_{14}O_3N_4 \cdot HCl$: C, 46.08; H, 5.27; N, 19.54; Cl, 12.37%.

*(2R,3S,4S)-4-Amino-3-hydroxy-2-methyl-5-(3-pyridyl)pentanoic Acid (**1**).* A solution of the hydrochloride of **10** (18.2 mg) in methanol (2 ml) was stirred with palladium black for 1 hr in a hydrogen atmosphere to afford a monohydrochloride of **1** (17.1 mg, 97%) as a hygroscopic monohydrate: mp 140—143 °C (decomp.); $[\alpha]_D^{24}$ -12 ° (*c* 0.35, methanol).

Found: C, 46.86; H, 6.91; N, 9.62; Cl, 13.08%. Calcd for $C_{11}H_{16}O_3N_2 \cdot HCl \cdot H_2O$: C, 47.40; H, 6.87; N, 10.05; Cl, 12.72 %. The dihydrochloride of **1** was obtained as a monohydrate in a quantitave yield by treatment of aqueous solution of the monohydrochloride with 0.1M hydrochloric acid: colorless hygroscopic powder, mp 175—180 °C (decomp.) [lit,[3] mp 177—180 °C (decomp.)]; $[\alpha]_D$ $-2°$, $[\alpha]_{546}$ $-4°$, $[\alpha]_{436}$ $-8°$, $[\alpha]_{405}$ $-10°$ and $[\alpha]_{365}$ $-11°$ (*c* 0.53, 5M HCl at 24 °C) [A natural specimen of the dihydrochloride monohydrate isolated from pyridomycin by authors gave the following rotations: $[\alpha]_D$ $-2°$, $[\alpha]_{546}$ $-4°$, $[\alpha]_{436}$ $-8°$, $[\alpha]_{405}$ $-10°$ and $[\alpha]_{365}$ -11 ° (c 0.37, 5M HCl at 27 °C)]; pK_a, 3.20, 4.70, and 8.70 (titration equivalent 305) [lit,[3] pK_a, 3.25, 4.75, and 8.80 (300)].

Found: C, 41.74; H, 6.58; N, 9.06; Cl, 22.61%. Calcd for $C_{11}H_{16}O_3N_2 \cdot 2HCl \cdot H_2O$: C, 41.91; H, 6.40; N, 8.89; Cl, 22.50%. The IR (KBr) and PMR (D_2O) spectra of the synthetic specimen of **1** were identical with those[8] of authentic sample of natural amino acid. R_f-values on paper chromatography and mobilities of paper electrophoresis of the synthetic product and the natural one (above described) were all identical.

The authors wish to express their sincere thanks to Dr. Kenji Maeda, National Institute of Health of Japan, for his helpful advice and kind supply of pyridomycin and to Mr. Saburo Nakada, Keio University, for the elemental analyses. We also wish to thank gratefully Dr. Hamao Umezawa, Institute of Microbial Chemistry and Prof. Sumio Umezawa, Keio University, for their encouragements.

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8) The IR and PMR spectral charts of the authentic sample were generously supplied by Dr. Hiroshi Ogawara, the National Institute of Health of Japan, to whom the authors' thanks are due.