ANTIMETABOLITES PRODUCED BY MICROORGANISMS. XV

SYNTHESIS OF 2-METHYL-L-ARGININE, 2-METHYL-L-ORNITHINE AND THEIR ENANTIOMERS

H. MAEHR, L. YARMCHUK and M. LEACH

Chemical Research Department, Hoffmann-La Roche Inc.
Nutley, New Jersey 07110, U.S.A.

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5-(3-Azidopropyl)-5-methyl-2,4-imidazolidinedione was prepared either from 5-(3-chloropropyl)-5-methyl-2,4-imidazolidinedione or 5-methyl-5-{3-[(4-methylphenyl)sulfonyloxy]-propyl}-2,4-imidazolidinedione and hydrogenolyzed to the corresponding amine which, after carbamimidoylation, afforded 2-methyl-DL-arginine upon acid hydrolysis. Racemic 2-methylarginine was converted enzymically to a mixture of 2-methyl-D-arginine and 2-methyl-L-ornithine. 2-Methyl-L-arginine was reconstructed from 2-methyl-L-ornithine *via* its Cu(II) chelate with *O*-methyl-isourea and 2-methyl-D-ornithine was obtained by alkaline hydrolysis of 2-methyl-D-arginine.

The most recently discovered member of the group of arginine antagonists is 2-methyl-L-arginine (L-**9**) which was isolated from cultures of a new *Streptomyces* species and described in a preceding paper.[1]

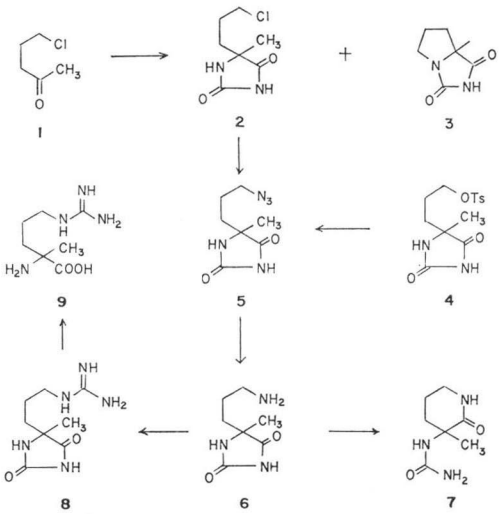
To permit extended biological evaluation, a chemical synthesis was desired leading to 2-methyl-L-arginine (L-**9**) as well as 2-methyl-L-ornithine (L-**10**), the latter heretofore only available in racemic form but possessing some biochemical interest.[2,3]

Two general approaches can be considered for the synthesis of these optically active 2-methylamino acids. Among the dissymmetric methods, the STRECKER-type synthesis with [4S, 5S]-(+)-5-amino-2,2-dimethyl-4-phenyl-1,3-dioxane, or its enantiomer, although highly enantioselective does not readily allow prediction of chirality of the resulting aminonitrile due to the functionalized alkyl methylketone required.[4] Another conceivable route to chiral 2-methylamino acids consists of the alkylation of amino-protected (4S,5S)-4,5-dihydro-4-(methoxymethyl)-α-methyl-5-phenyloxazole-2-methylamine, or its enantiomer, *via* the lithio salt, similar to the recently described synthesis of methylalkanoic acids.[5]

Alternative approaches leading to racemic products and requiring subsequent resolution could involve alkylation of the metalated 2-isocyanopropionic ester and hydrolysis,[6] methylation and alkylation of N-[bis(methylthio)methylene]- or N-(1,3-dithiolan-2-ylidene)glycine ethyl esters followed by oxidative-hydrolytic desulfurization,[7] methylation of metalated 3-[(4-nitrophenylmethylene)amino]piperidin-2-one leading to 2-methylornithine,[3] or, among others, a normal STRECKER-type condensation with a suitable methyl ketone[2,3] which we chose.

Chemical resolutions of racemic **9** and **10** were not attractive due to the limited tendency of these compounds to form good crystals. Although enzymic resolutions of amino acids are well established, the extension to the 2-methyl analogs is somewhat equivocal.[8] The action of arginase, however, known for high stereoselective substrate specificity and heretofore untested with 2-methylarginine as substrate offered a route to both isomers of **9** and **10** when employed

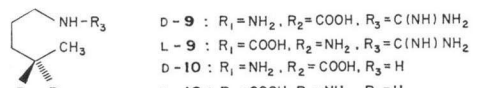
in conjunction with hydrolysis of D-9 and N-carbamimidoylation of L-10.



Subjecting 5-chloro-2-pentanone (**1**) to the BUCHERER-HENZE reaction afforded **2** in modest yield, readily separated from coproduced **3**, a precursor of 2-methylproline.[2] Nucleophilic displacement with sodium azide effectively converted **2** to **5**. Alternatively, azido compound **5** was prepared from **4**, accessible *via* the known BUCHERER-HENZE product of 5-hydroxy-2-pentanone.[2] Hydrogenolysis of **5** was preferably carried out in the presence of one equivalent of mineral acid to eliminate the conversion of **6** to **7**, both compounds being precursors of 2-methylornithine. Thus, **6** was obtained as the hemisulfate which was reacted with S-methylisothiourea hemisulfate and barium hydroxide to afford **8**. Although the carbamimidoylation of **6** could be achieved consistently in nearly quantitative yield on a millimolar scale, the efficiency of the same process diminished upon scale-up. Similar to **6**, compound **8** is presumably present in zwitterionic form.[2] Hydrolysis of **8** with 6 N hydrochloric acid gave **9** which was isolated most efficiently as the 2-nitroindanedione salt.

Racemic **9** showed the same antibacterial spectrum as the naturally occurring L-form[1] and, in a specific test with *B. subtilis*, exhibited 50 % of the activity of the L-form.

Arginase-catalyzed hydrolysis of racemic **9** afforded a mixture of unchanged 2-methyl-D-arginine (D-**9**) with an optical rotation of essentially equal magnitude but opposite sign of that reported for the naturally occurring L-form[1] and 2-methyl-L-ornithine (L-**10**) readily separated by chromatography on sulfonated polystyrene resin and isolated as the crystalline 2-nitroindanedione salt and hydrochloride, respectively. 2-Methyl-L-ornithine was converted to the Cu(II) chelate and reacted with *O*-methylisourea according to a known procedure[9] to yield crystalline L-**9** 2-nitroindanedione salt, identical with the 2-nitroindanedione salt prepared from natural L-**9**.[1] Precluding racemization of optically active **9** and **10** due to the absence of α-hydrogen atoms 2-methyl-D-ornithine (D-**10**) was accessible *via* alkaline hydrolysis of D-**9**.



Derivatives

5-Chloro-2-pentanone was redistilled before use. Arginase (bovine liver) was purchased from Worthington Biochemical Corporation, Freehold, New Jersey, U.S.A. 07728. Tlc was performed with silica gel G plates (E. Merck, Darmstadt) using systems

A. (chloroform, methanol, conc. ammonium hydroxide, 2:2:1, v/v);
B. (chloroform, methanol, conc. ammonium hydroxide, water, 1:4:2:1, v/v);
C. (ethanol, water, conc. ammonium hydroxide, 49:49:2, v/v);
D. (1-butanol, acetic acid, water, 4:1:1, v/v) and
E. (chloroform, 2-propanol, 9:1, v/v).

Spots were detected with iodine vapors, ninhydrin- or SAKAGUCHI sprays. All evaporations were performed under reduced pressure, melting points (uncorrected) were observed on a Reichert Thermopan hot stage, elemental analyses were within ± 0.4 % of calculated values and ^{1}H-nmr spectra were recorded on a Varian HA-100 spectrometer.

5-(3-Chloropropyl)-5-methyl-2,4-imidazolidinedione (**2**)

To a stirred solution of 5-chloro-2-pentanone (87 g, 0.72 mol) in ethanol (750 ml) contained in a 3-liter round-bottom flask equipped with reflux condenser and mechanical stirrer was added a solution of ammonium carbonate (214 g) in water (525 ml), followed by a solution of potassium cyanide (50 g) in water (265 ml). The mixture was stirred at 50~55°C for 2.5 hours, cooled to 5~10°C and excess carbonate and cyanide were removed by the slow addition of 6 N hydrochloric acid (approx. 650 ml) to pH 4 under stirring. The resulting mixture was concentrated to a volume of approximately 1 liter and refrigerated overnight. The crystalline deposit of **2** was filtered off, dried and recrystallized from chloroform-hexane (25.1 g, 0.13 mol, 18 %), mp 119~121°C (reported 127~129°C)[2]; Rf 0.50 (E). *Anal.* $C_7H_{11}ClN_2O_2$ (190.63), C, H, Cl, N.

The aqueous filtrate was extracted with five 600-ml portions of chloroform. The chloroform extracts and the mother liquor resulting from the recrystallization of **2** were combined, concentrated and a chloroform solution of the concentrate was applied to a column of silicic acid (470 g) packed and developed with chloroform. The column was developed with chloroform (1,150 ml) followed by chloroform containing 1.5 % 2-propanol (2,000 ml) eluting traces of **1** followed by **3** (5.4 g, 0.035 mol, 5 %, after solvent removal and crystallization from chloroform-hexane); mp 134~136°C; Rf 0.63 (E). *Anal.* $C_7H_{10}N_2O_2$ (154.17), C, H, N. Development with chloroform containing 3 % 2-propanol (2,500 ml) eluted **2** (9.6 g, 0.05 mol, 7 %, after solvent removal and crystallization from chloroform-hexane).

5-Methyl-5-{3-[(4-methylphenyl)sulfonyloxy]-propyl}-2,4-imidazolinedione (**4**)

p-Toluenesulfonyl chloride (22.5 g, 0.118 mol) was added to a stirred solution of 5-(3-hydroxypropyl)-5-methyl-2,4-imidazolidinedione[2] (20.0 g, 0.116 mol) in pyridine (250 ml) at 5°C. The mixture was stirred for 1 hour, refrigerated overnight and diluted with water (275 ml). The resulting solution was extracted three times with chloroform (375, 190 and 190 ml), the combined extracts were washed with 5 N sulfuric acid (4×125 ml), water (2×150 ml) and saturated sodium bicarbonate solution (2×125 ml). Concentration of the dried chloroform phase yielded **4** (22.2 g, 0.068 mol, 58 %) upon addition of ether and hexane, mp 126~127°C, Rf 0.56 (E). *Anal.* $C_{14}H_{18}N_2O_5S$ (326.37), C, H, N; δ_{TMS} in $CDCl_3$/DMSO-d_6 (20:1) 1.35 (s, CH_3-C), 1.46~1.86 (m, $(CH_2$-$CH_2)$, 2.45 (s, CH_3-C⩽), 3.98 (m, O-CH_2), 7.13 (s, NH), 7.32, 7.73 (AA′, BB′, 4, J_0=8 Hz, arom.) and 9.77 (s, CO-NH-CO).

5-(3-Azidopropyl)-5-methyl-2,4-imidazolidinedione (**6**)

A solution of sodium azide (13 g, 0.2 mol) in water (100 ml) was added to a solution of **2** (19.06 g, 0.1 mol) in dimethylformamide (900 ml) and the mixture was kept at 75°C for 21 hours. Iodometric determinations of azide ions,[10] conducted in a separate experiment, indicated the consumption of the expected quantities of azide at that time. The mixture was concentrated to a syrup which was redissolved in chloroform (200 ml), filtered and diluted with chloroform (500 ml) and water (100 ml). The chloroform phase was collected after equilibration and combined with four additional chloroform extracts which were dried ($MgSO_4$) and concentrated. Crystalline **6** was obtained from chloroform-hexane (17.3 g, 88 %), mp 104°C after one recrystallization from the same solvent; Rf 0.54 (E). *Anal.* $C_7H_{11}N_5O_2$ (197.20), C, H, N; $\delta_{TMS}^{CDCl_3}$ 1.45 (s, CH_3-C), 1.49~1.97 (m, CH_2-CH_2), 3.28 (m, CH_2-N), 6.83 (s, NH) and 9.10 (broad, CO-NH-CO); ν_{max}^{KBr} 3200 broad (NH), 2175 weak and 2105 strong (azido group), 1770 and 1710 cm^{-1} (carboximide).

5-(3-Aminopropyl)-5-methyl-2,4-imidazolidinedione hemisulfate (**6**)

A suspension of platinum oxide (200 mg) in 2-propanol (50 ml) and 1 N sulfuric acid (20 ml) was briefly hydrogenated (3.4 atm) and **5** (3.94 g, 0.020 mol) was added. Hydrogenation was

continued for 7 hours at 3.4 atm. The catalyst was removed by filtration and the filtrate concentrated to a small volume, addition of 2-propanol afforded **6** hemisulfate hemihydrate as prisms (4.40 g, 0.019 mol, 95 %); mp 183~185°C after one crystallization from aqueous 2-propanol; Rf 0.43 (A). *Anal.* $C_7H_{13}N_3O_2 \cdot \frac{1}{2}H_2SO_4 \cdot \frac{1}{2}H_2O$ (229.25), C, H, N; $\delta^{D_2O}_{ext.TMS}$ 1.93 (s, CH_3-C), 1.99~2.42 (m, CH_2-CH_2), 3.50 (m, CH_2-N).

5-{3-[(Aminoiminomethyl)amino]propyl}-5-methyl-2,4-imidazolidinedione (**8**)

Amine **6** hemisulfate hemihydrate (229 mg, 1 mmol) was dissolved in 0.418 N barium hydroxide (3.5 ml) and S-methylisothiourea hemisulfate (153.1 mg, 1.1 mol), was added. The suspension was shaken at room temperature for $6\frac{1}{4}$ hours and 0.418 N barium hydroxide (1 ml) was added, followed by another 0.5 ml of barium hydroxide solution 18 hours later. After 4 hours, S-methylisothiourea (70 mg, 0.5 mmol) was added and shaking of the suspension was continued for 66 hours. At that time only traces of **6** (A) could be detected. Barium sulfate was filtered off and washed with water, filtrate and washings were applied to a column containing 5 ml of Dowex 50W-X4 (H^+) and the column was washed consecutively with water (50 ml), 10 % aqueous pyridine (25 ml) and 2.5 M ammonium hydroxide solution (25 ml). Concentration of the ammoniacal effluent gave a colorless syrup from which crystalline **8** was deposited on standing (207 mg, 0.97 mmol, 97 %). Recrystallization from water afforded fine needles, mp 294~295°C (dec.); Rf 0.17 (A). *Anal.* $C_8H_{15}N_5O_2$ (213.24), C, H, N; δ^{TFA}_{TMS} 1.62 (s, CH_3-C), *ca.* 1.68~2.20 (m, CH_2-CH_2) and 3.33 (m, CH_2-N).

2-Methyl-DL-arginine (**9**)

A solution of **8** (1.39 g, 6.52 mmol) in 6 N hydrochloric acid (21 ml), contained in a sealed glass-tube, was heated at 125°C for 24 hours. The hydrolyzate was evaporated to dryness, redissolved in water and applied to a column (15×245 mm) containing Dowex 50W-X4 (H^+, 100~200 mesh). The column was washed with water until the effluent was neutral and eluted with 1 N ammonium hydroxide. Some impurities preceded those fractions containing ninhydrin- and SAKAGUCHI-positive solutes; the latter were combined, concentrated, filtered and taken to dryness to yield **9** as amorphous, hygroscopic powder (1.37 g), R_f 0.16 (A), 0.33 (B), 0.09 (C) and 0.10 (D). This material was dissolved in water (10 ml) and an aqueous solution of 2-nitroindanedione was added until a pH of 5.1 was reached. The crystal suspension was concentrated to a small volume and cooled to 0°C for several hours prior to filtration. The resulting **9** 2-nitroindanedione salt dihydrate (2.60 g, 6.26 mmol, 96 % with respect to the conversion of **8**) was recrystallized from hot water to give yellow prisms, mp 168~170°C (dec), *Anal.* $C_7H_{16}N_4O_2 \cdot C_9H_5NO_4 \cdot 2H_2O$ (415.41), C, H, N, H_2O, after drying at room temperature under high vacuum for 3 hours.

Similarly, amorphous **9** base was converted to the hydrochloride by pH-adjustment to 5.1 with 1 M hydrochloric acid. Although crystalline **9** hydrochloride could be obtained by the addition of 2-propanol to the methanolic solution of **9** hydrochloride, the yields of crystalline product were only 30%, mp 243~246°C. *Anal.* $C_7H_{16}N_4O_2 \cdot HCl$ (224.69), C, H, N.

2-Methyl-D-arginine (D-**9**) and 2-methyl-L-ornithine (L-**10**)

A solution of 2-methyl-DL-arginine 2-nitroindanedione salt dihydrate (1.02 g, 2.46 mmol) in water (100 ml) was charged to a 15-ml column of Dowex 50-X4 (H^+, 50~100 mesh). 2-Nitroindanedione was eluted with water and 2-methylarginine with 5 N ammonium hydroxide solution. Concentration of the ammoniacal effluent gave a colorless residue (550 mg) which was dissolved in water (15 ml), adjusted to pH 9.5 (HCl) and digested at 37°C while stirring for 4 days with a pre-incubated (37°C, 4 hours) solution prepared by dissolving arginase (10 mg) in 20 ml of 0.05 M manganese maleate buffer (dissolve 845 mg $MnSO_4$ in 30 ml water; dissolve 580.4 mg maleic acid in 50 ml water and adjust pH to 8.1 with NaOH; combine the solutions and make a final volume of 100 ml with water).[11] The reaction was quenched by pH-adjustment to 2.5 (H_2SO_4), the resulting solution was stirred for 10 minutes with charcoal (Norit A, 200 mg) before filtering and the filtrate was applied to a column of Dowex 50-X4 (H^+, 200~400 mesh, 10×310 mm). Washing the column with water removed maleic and sulfuric acids and subsequent development with 1 N ammonium hydroxide solution first eluted L-**10** followed by D-**9** as deter-

mined by tlc. The appropriate fractions were pooled and concentrated to dryness to yield L-**10**, 227 mg, Rf 0.23 (A), 0.59 (B), 0.14 (C), 0.07 (D), and D-**9**, 281 mg, Rf 0.16 (A), 0.33 (B), 0.09 (C), 0.10 (D), as colorless syrups.

An aqueous solution of L-**10** (180 mg, 1.23 mmol) was adjusted to pH 5 with hydrochloric acid, concentrated and diluted with ethanol to yield L-**10** hydrochloride as prisms (165 mg, 0.90 mmol, 73 %), mp 222~224°C. *Anal.* $C_6H_{14}N_2O_2 \cdot HCl$ (182.65), C, H, N; $[\alpha]_D$+10.5° (*c* 0.76, 5 N HCl), $\delta^{D_2O}_{ext.TMS}$ 1.90~2.54 (m, $(CH_2)_2$), 2.03 (s, CH_3-C) and 3.55 (t, CH_2-N, $J_{4,5}$=7.2 Hz).

The fractions containing D-**9** showed Rf values identical with those of natural L-**9**[1] and the racemic form and were concentrated and recrystallized as D-**9** 2-nitroindanedione salt, as described previously, to give yellow prisms (290 mg, 0.71 mmol, 58 %), mp 165~170°C (dec). *Anal.* $C_7H_{16}N_4O_2 \cdot C_9H_5NO_4 \cdot 1.5\ H_2O$ (406.40), C, H, N, H_2O, after drying at room temperature under high vacuum overnight; $[\alpha]_D$−7.9° (*c* 0.33, water).

2-Methyl-L-arginine (L-**9**)

A solution of L-**10** hydrochloride (143 mg, 0.78 mmol) in water (2 ml) was heated on the steam bath for 5 minutes with basic cupric carbonate and filtered. Filtrate and washings were cooled to 5°C, mixed with *O*-methylisourea hydrogen sulfate (148 mg, 0.86 mmol) and 1.0 N sodium hydroxide solution (1.72 ml) and allowed to stand at room temperature for 6 days. The solution was acidified to pH 1.8 with 2 N hydrochloric acid, saturated with hydrogen sulfide and filtered through charcoal. Filtrate and washings were applied to a column of Dowex 50-X4 (H^+, 200~400 mesh, 12.7×240 mm) which was washed with water (150 ml) and developed with 1 N ammonium hydroxide solution eluting unreacted L-**10** followed by pure L-**9** as indicated by the systems A~D and isolated as 2-nitroindanedione salt as described previously (80 mg, 0.19 mmol), *Anal.* $C_7H_{16}N_4O_2 \cdot C_9H_5NO_4 \cdot 2.5\ H_2O$ (424.41), C, H, N, H_2O; $[\alpha]_D$+9.2° (*c* 0.3, water) after drying for 3 hours at room temperature under high vacuum.

2-Methyl-D-ornithine (D-**10**)

An aqueous solution of D-**9** 2-nitroindanedione salt sesquihydrate (100 mg, 0.25 mmol) was converted to the free amino acid as described previously; the ammoniacal column effluent was concentrated to dryness, redissolved in 2.5 N sodium hydroxide solution (5 ml) and kept on the steam bath for 24 hours. The alkaline solution was neutralized by successive additions of Dowex 50W-X8 (H^+, 100~200 mesh) and the suspension was applied to the top of a resin column containing 5 ml of the same resin as used above. The column was washed with water and then with 1 N ammonium hydroxide first eluting D-**10** with Rf values identical with those exhibited by L-**10**, followed by traces of unreacted D-**9**. Fractions containing D-**10** were pooled and concentrated to give a colorless residue which was redissolved in water, adjusted to pH 4.7 with 1 N hydrochloric acid, concentrated and diluted with ethanol to afford D-**10** hydrochloride having properties identical with those reported for the L-isomer but opposite sign of rotation. Alternatively, D-**10** was converted to the dipicrate by the addition of 2 equivalents of picric acid in ethanol to an aqueous solution of the base. The solution was concentrated with the product crystallizing from water and recrystallized from ethanol-ether to give yellow prisms of the dipicrate (80 mg, 0.13 mmol), mp 196~197°C (dec) *Anal.* $C_6H_{14}N_2O_2 \cdot (C_6H_3N_3O_7)_2$ (604.40), C, H, N; $[\alpha]_D$−4.2° (*c* 0.31, 5 N HCl).

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