ANTIMETABOLITES PRODUCED BY MICROORGANISMS XVI[1]

SYNTHESIS OF N^5-HYDROXY-2-METHYLARGININE AND N^5-HYDROXY-2-METHYLORNITHINE

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N^5-Hydroxy-2-methylornithine and N^5-hydroxy-2-methylarginine were synthesized. 2-Amino-5-hydroxy-2-methylpentanoic acid was prepared from 5-hydroxy-2-pentanone and converted to N-(tetrahydro-3-methyl-2-oxo-2H-pyran-3-yl) acetamide which was treated with hydrogen bromide affording 2-(acetylamino)-5-bromo-2-methylpentanoic acid. This acid was esterified with methanol and used to alkylate *anti*-benzaldoxime yielding methyl 2-(acetylamino)-2-methyl-5-[(phenylmethylene)amino]-pentanoate N^5-oxide which, upon hydrolysis, yielded N^5-hydroxy-2-methylornithine, and, upon aminolysis and short acid-treatment, gave N^2-acetyl-N^5-hydroxy-2-methylornithinamide. Carbamimidoylation and hydrolysis of the latter compound furnished N^5-hydroxy-2-methylarginine.

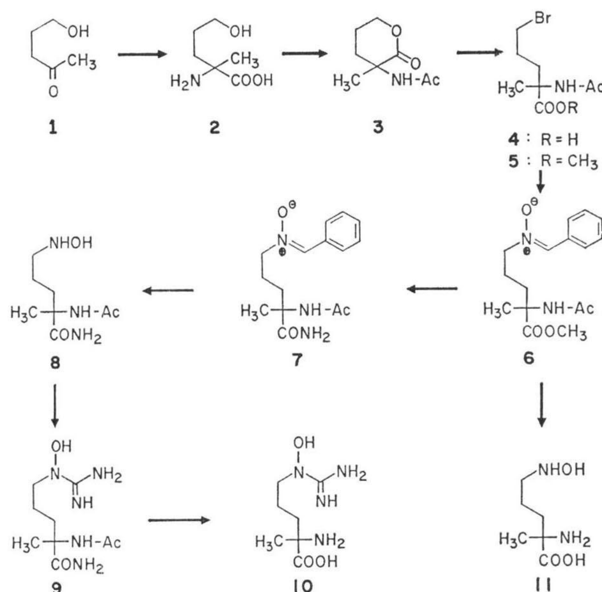
N^5-Hydroxy-L-arginine[2,3] and 2-methyl-L-arginine[4] are microbial products whose antibiotic activities are reversed by L-arginine, L-citrulline, L-ornithine and N^2-acetyl-L-ornithine but not by L-glutamic acid and N^2-acetyl-L-glutamic acid. Introduction of either an N^5-hydroxy function or a 2-methyl group into the arginine molecule apparently creates arginine antagonists with very similar or identical modes of antibacterial actions. For these reasons it was obviously of interest to prepare synthetically the arginine derivative **10** containing both of those structural anomalies in one molecule and to investigate its biological properties.

Similar considerations prompted us to synthesize 2-methyl-N^5-hydroxyornithine (**11**). 2-Methylornithine is a potent ornithine-decarboxylase inhibitor[5] and N^5-hydroxyornithine is not only an important building block of many naturally occurring hydroxamic acids[6] but exhibits weak antibiotic activities of its own[7]. The presence of both an N^5-hydroxy- and a 2-methyl-group in the ornithine molecule, therefore, was also expected to be an interesting combination.

A STRECKER reaction with 5-hydroxy-2-pentanone (**1**) led to 2-amino-5-hydroxy-2-methylpentanoic acid (**2**) which was converted to lactone **3** with acetic acid and acetic anhydride without prior purification (Scheme 1). Crude **3** was treated with hydrogen bromide in acetic acid[8] to afford 2-(acetylamino)-5-bromo-2-methylpentanoic acid (**4**), esterification of **4** with methanolic hydrogen chloride yielding crystalline methyl 2-(acetylamino)-5-bromo-2-methylpentanoate (**5**) after chromatography on silica gel.

N-Alkylation of *anti*-benzaldoxime[7] by **5** gave nitrone **6** which was converted to the carboxamide **7** to prevent the expected formation of 3-amino-1-hydroxy-3-methyl-2-piperidone after generation of the hydroxylamine function. Thus, **7** was treated briefly with hydrochloric acid and the resulting 2-(acetylamino)-5-(hydroxyamino)-2-methylpentanamide (**8**) was reacted with S-methylisothiourea to give **9**, yielding the desired N^5-hydroxy-2-methyl-D,L-arginine (**10**) after acid hydrolysis and chromatographic separation from the coproduced 2-methyl-D,L-ornithine. The conversion of **6** to **10** could be accomplished without intermittent product isolation.

Scheme 1.



N^5-Hydroxy-2-methyl-D,L-ornithine (**11**) was accessible directly by acid hydrolysis of **6** and was obtained as the crystalline picrate.

No significant *in vitro* antimicrobial activity was observed for **10** or **11** when each was tested at 200 mcg/ml against *Serratia* sp. 101, *Bacillus subtilis* NRRL-558, *Escherichia coli* B, and *Candida albicans* NRRL-477 under conditions described in reference 3.* Thus, the introduction of both a 2-methyl- and an N^5-hydroxy-group into arginine or ornithine results in a loss of the activity observed when these substituents are present individually.

Interestingly, however, **11** picrate was as active an ornithine-decarboxylase inhibitor as 2-methyl-D,L-ornithine hydrochloride,[5] but N^5-hydroxy-D,L-ornithine[7], comparable in activity to sarkomycin, was twice as active as 2-methyl-D,L-ornithine hydrochloride. Since 2-methyl-D-ornithine dipicrate was either inactive or at least twenty times less active than the racemic mixture of the hydrochlorides, it appears that 2-methyl-L-ornithine, N^5-hydroxy-D,L-ornithine and N^5-hydroxy-2-methyl-D,L-ornithine (**11**) are about equipotent ornithine-decarboxylase inhibitors when compared as the free bases. Assuming inactivity of the D-isomers would then suggest N^5-hydroxy-L-ornithine and N^5-hydroxy-2-methyl-L-ornithine to be about twice as active as 2-methyl-L-ornithine**. Neither N^5-hydroxy-D,L-ornithine nor N^5-hydroxy-2-methyl-D,L-ornithine, however, exhibited significant antitumor activity in mice.***

N^5-Hydroxy-D,L-arginine hydrochloride, N^5-hydroxy-2-methyl-D,L-arginine hydrochloride, and 2-methyl-L-arginine 2-nitroindanedione salt were either inactive or at least twenty times less active than N^5-hydroxy-2-methyl-D,L-ornithine picrate or 2-methyl-D,L-ornithine hydrochloride when tested for ornithine-decarboxylase activity.**

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Experimental Section

Melting points were observed on a Reichert Thermopan hot stage and are uncorrected; ^1H nmr spectra were recorded on a Varian XL-100 spectrometer with TMS as internal reference. Precoated silica gel F-254 layers (E. Merck, Darmstadt) were used for tlc in connection with systems A (chloroform - 2-propanol, 9: 1, v/v), B (chloroform - methanol, 4: 1, v/v), C (chloroform - ethyl acetate - methanol, 5: 5: 1, v/v), and D (1-butanol - acetic acid - water, 4: 1: 1, v/v). Adsorption chromatography was performed with silicic acid, 100 mesh (Mallinckrodt); the columns were prepared by slurrying silicic acid in chloroform. All concentrations were carried out under reduced pressure. Reported elemental analyses were within $\pm$ 0.4% of the theoretical values.

Methyl 2-(acetylamino)-5-bromo-2-methylpentanoate (**5**)

A solution of ammonium chloride (55.6 g) in water (150 ml) was added to an ice-cooled solution of potassium cyanide (61.3 g) in water (115 ml) contained in a 1-liter round-bottom flask, followed by conc. ammonium hydroxide (62.3 ml) and a solution of 5-hydroxy-2-pentanone (**1**) (100 g, 97.9 mol) in methanol (150 ml). The flask was securely stoppered, placed into a constant temperature bath (55~60°C) and stirred magnetically for 5 hours. The mixture was concentrated to a volume of approximately 350 ml to remove methanol, diluted with water (*ca.* 150 ml), cooled in an ice-bath and treated cautiously (HCN!) with conc. HCl (500 ml). The resulting solution was refluxed on a steam bath for 5 hours, treated with charcoal, filtered through Celite, concentrated to a paste and extracted with hot methanol (3×300 ml). The combined extracts were concentrated and extracted again with hot methanol (2×200 ml). Methanol-removal from the resulting extracts yielded crude **2** which was dissolved in hot acetic acid (750 ml), diluted with acetic anhydride (375 ml) and refluxed for 1 hour. The resulting solution was concentrated to a syrup, repeatedly redissolved in xylene and evaporated to remove acetic acid and acetic anhydride. After the residue was kept under reduced pressure over sodium hydroxide for 24 hours, it was dissolved in acetic acid containing 30% hydrobromic acid (500 g) and kept at room temperature for $6\frac{1}{2}$ days in the dark. The dark brown solution was concentrated to a syrup and most residual acid was removed by several co-distillations with toluene. The resulting oily residue was dissolved in dry methanol (500 ml) and added to methanolic hydrogen chloride prepared by the slow addition of acetyl chloride (780 ml) to stirred methanol (4 liters) at ice-bath temperature. The solution was concentrated to a syrup after remaining at room temperature for 24 hours. Most of the residual hydrogen chloride was removed by several co-distillations with toluene. The dark-brown oil was taken up in chloroform (1.5 liters), washed successively with water, satd. sodium hydrogencarbonate solution and water, dried with sodium sulfate, concentrated and charged to a column of silicic acid (1.2 kg). The column was developed with chloroform (750 ml) and chloroform containing 1% 2-propanol. Elution of pure **5** started after 4 liters of solvent mixture had passed through the column. After continued development with the same mixture (1 liter), elution of **5** was completed with chloroform containing 2% 2-propanol (2 liters). The effluent was monitored by tlc and the fractions containing pure **5** were pooled, evaporated to dryness and crystallized from chloroform-petroleum ether (61 g, 0.23 mol, 23% based on **1**), mp 84~86°C, Rf 0.57 (A). *Anal.* $C_9H_{16}BrNO_3$ (266.14), C,H,N; $\delta_{TMS}^{CD_3OD}$ 1.41 (s, CH_3C), 1.73~2.03 (m, CH_2-CH_2), 1.90 (s, CH_3CO), 3.41 (t, CH_2-Br, $J_{4,5}$=6 Hz), and 3.66 (s, CH_3O).

Methyl 2-(acetylamino)-2-methyl-5-[(phenylmethylene)amino]pentanoate, N^5-oxide (**6**)

A solution of **5** (21.7 g, 81.5 mmol) and *anti*-benzaldoxime[7] (9.9 g, 81.7 mmol) in dry methanol (200 ml) was diluted with methanolic sodium methoxide (4.085 N, 20.0 ml), allowed to stand at room temperature 24 hours and at 5°C for an additional 2 days and was concentrated to dryness. The residue was extracted exhaustively with chloroform. The combined extracts were filtered, concentrated and chromatographed on a column of silicic acid (320 g) with chloroform containing 1.25% 2-propanol (1 liter) as mobile phase eluting by-products with high Rf-values. Development with chloroform containing 2.5% 2-propanol (2 liters) eluted pure **6**, the elution was completed with chloroform - 2-propanol, 4: 1, v/v (1 liter). The fractions containing pure **6** (tlc) were pooled and evaporated. The residue was allowed to crystallize from ethyl acetate - diethyl ether (14.3 g, 46.7mmol, 57% based on **5**), mp 134~135°C, Rf 0.79 (B) and 0.30 (C). *Anal.* $C_{16}H_{22}N_2O_4$ (306.36), C,H,N; $\delta_{TMS}^{CDCl_3}$ 1.55 (s, CH_3-C), 1.8~2.3

(m, CH_2–CH_2), 1.97 (s, CH_3CO), 3.71 (s, OCH_3), 3.91 (t, CH_2–N, $J_{4,5}$=6.5 Hz), 6.94 (s, broad, NH), 7.41 (s, –CH=, superimposed H–3′, H–4′, and H–5′), and 8.22 (m, H–2′ and H–6′).

2-Acetylamino-2-methyl-5-[(phenylmethylene)amino]pentanamide, N^5-oxide (**7**)

A solution of **6** (485 mg, 1.58 mmol) in liquid ammonia (15 ml) was kept in a sealed glass tube at 60°C for 7 days. Ammonia was allowed to evaporate and the resulting residue was dissolved in chloroform and chromatographed on a column of silicic acid (20 g). The column was developed with chloroform (60 ml) and system C (360 ml) eluting a compound with Rf 0.30 (tlc, system C) followed by **7**. Elution of **7** was completed with system B (150 ml) as mobile phase. The fractions containing **7** were pooled, evaporated to dryness and crystallized from methanol - chloroform (390 mg, 1.19 mmol, 75%). For analysis, a sample was recrystallized from acetone - chloroform, mp 103~104.5°C, Rf 0.53 (B) and 0.05 (C). *Anal.* $C_{15}H_{21}N_3O_3 \cdot H_2O$ (309.37), C,H,N; $\delta_{TMS}^{CDCl_3+DMSO-D_6}$ 1.56 (s, CH_3–C), 1.8~2.3 (m, CH_2–CH_2), 1.98 (s, CH_3CO), 3.93 (t, CH_2–N, $J_{4,5}$=6 Hz), 6.23 (s, broad, NH), 7.04 (s, broad, NH) 7.40 (m, H–3′, H–4′, and H–5′, superimposed NH), 7.51 (s, –CH=), and 8.24 (m, H–2′ and H–6′).

N^5-Hydroxy-2-methyl-D,L-arginine (**10**)

A solution of **6** (10.6 g, 34.6 mmol) in dry methanol (250 ml) was saturated with ammonia at 0°C and kept at room temperature for 42 days. The clear solution containing primarily **7** was evaporated to dryness, the residue redissolved in 100 ml of conc. hydrochloric acid and the solution heated on the steam bath for 10 minutes to hydrolyze the nitrone **7**. After evaporation to dryness and drying over sodium hydroxide under reduced pressure overnight, the resulting crude **8** was dissolved in water (65 ml) and combined with S-methylisothiourea hemisulfate (10 g). The pH of the solution was brought to 7.2 with 50% sodium hydroxide and was readjusted daily to the same value. After 9 days, the solution was diluted with an equal volume of conc. hydrochloric acid and was heated on the steam bath for 11 hours to hydrolyze both carboxamide and N-acetyl group of **9**. The solution was evaporated to dryness and the resulting crude **10** was purified by chromatography on a column of Dowex 50W–X8 (200~400 mesh, 33×680 mm). The resin was previously converted to the sodium form and equilibrated with a buffer prepared by combining 0.1 M citric acid and 0.2 M disodium hydrogen phosphate to pH 6.1. A solution of crude **10** in water (50 ml) was adjusted to pH 3 and applied to the column. Development was initiated with the buffer described above (800 ml) to which sodium chloride (5.8 g/liter) had been added and was continued with the same buffer containing 17.4 g of sodium chloride per liter (3 liters). The effluent volume 1,220~1,800 ml contained 2-methyl-D,L-ornithine whereas **10** emerged in the effluent volume 2,420~3,100 ml. To desalt **10**, the effluent was passed through a column of Dowex 50W–X4 (H^+, 50~100 mesh, 500 ml), the column was washed with water until the effluent was neutral and **10** was eluted with 1 M ammonium hydroxide. The ninhydrin-positive fractions were evaporated to dryness, the residue redissolved in water, the solution adjusted to pH 5.1 with hydrochloric acid and concentrated to dryness. The residue was crystallized from aqueous ethanol to yield pure **10** hydrochloride as small prisms (2.57 g, 12.6 mmol, 36% based on **6**), mp 248~249°C, Rf 0.11 (System D). *Anal.* $C_7H_{16}N_4O_3 \cdot HCl$ (240.69), C,H,N; $\delta_{TMS}^{DMSO-D_6+D_2O}$ 1.40 (s, CH_3–C), 1.4~2.1 (m, CH_2–CH_2), and 3.51 (s, broad, CH_2–N).

N^5-Hydroxy-2-methyl-D,L-ornithine (**11**)

A solution of **6** (410 mg, 1. 34 mmol) in 6 N hydrochloric acid (8 ml) was heated on the steam bath for 4.5 hours. The solution was concentrated to dryness and stored under reduced pressure over potassium hydroxide for 20 hours to yield a very thick, syrupy residue (315 mg, 1.34 mmol) assumed to be the dihydrochloride salt of **11** and homogeneous on tlc, Rf 0.09 (D).

This residue was dissolved in water (5 ml) and a 0.23 M picric acid solution in ethanol (11.7 ml) was added. The solution was adjusted to pH 4.1 with 1 N aqueous sodium hydroxide and concentrated to a volume of *ca.* 4 ml. Upon standing some picric acid deposited which was filtered off and washed quickly with a small amount water. The filtrate deposited brown prisms of the monopicrate of **11** (165 mg, 0.42 mmol, 31%).

Additional **11** picrate was recovered upon concentration of the mother liquor, mp 175~178°C (dec). *Anal.* $C_6H_{14}N_2O_3 \cdot C_6H_3N_3O_7$ (391.30), C,H,N; $\delta_{TMS}^{DMSO-D_6}$ 1.37 (s, CH_3–C), 1.2~2.0 (m, CH_2–CH_2), 3.03 (s, broad, CH_2–N), and 8.59 (s, H–C$\leqslant$).

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