N-[2-(Dimethylamino)ethyl]-N-ethyl-2-(2-thienyl)-quinolin-4-amine (46): obtained from N,N-dimethyl-N'-ethylethylenediamine and 13; yield 95%; an oil; ^{1}H NMR δ 1.20 (t, J = 7 Hz, 3 H), 2.26 (s, 6 H), 2.54 (t, J = 7 Hz, 2 H), 3.46 (m, 4 H), 7.15 (m, 1 H), 7.31 (s, 1 H), 7.40 (t, J = 8 Hz, 1 H), 7.44 (d, J = 5 Hz, 1 H), 7.61 (t, J = 8 Hz, 1 H), 7.69 (d, J = 4 Hz, 1 H), 8.02 (m, 2 H); MS m/e 325 (7, M$^+$), 267 (52), 58 (100). 47·2HBr·2H$_2$O: mp 263-264 °C. Anal. ($C_{19}H_{23}N_3S$·2HBr·2H$_2$O) C, H, N.

4-(4-Methylpiperazino)-2-(2-thienyl)quinoline (47): obtained from N-methylpiperazine and 13; yield 91%; an oil; ^{1}H NMR δ 2.44 (s, 3 H), 2.74 (m, 4 H), 3.32 (m, 4 H), 7.15 (m, 1 H), 7.26 (s, 1 H), 7.42 (t, J = 8 Hz, 1 H), 7.45 (d, J = 5 Hz, 1 H), 7.63 (t, J = 8 Hz, 1 H), 7.70 (d, J = 4 Hz, 1 H), 7.97 (d, J = 8 Hz, 1 H), 8.04 (d, J = 8 Hz, 1 H); MS m/e 309 (51, M$^+$), 294 (12), 238 (15), 70 (100). 48·2HBr·H$_2$O: mp 330-334 °C. Anal. ($C_{18}H_{19}N_3S$·2HBr·H$_2$O) C, H, N.

General Procedure C. Preparation of Ketimines 49-52. The procedure for condensation of 2-aminobenzonitrile (48) with methyl ketones has been published.[5] Yields, melting points, and ^{1}H NMR spectra of 2-[(1-phenylethylidene)amino]benzonitrile (49), 2-[[1-(2-pyridyl)ethylidene]amino]benzonitrile (50), and 2-[[1-(2-thienyl)ethylidene]amino]benzonitrile (52) have also been presented previously.[5]

2-[[1-(3-Pyridyl)ethylidene]amino]benzonitrile (51): obtained from 3-acetylpyridine and 48; yield 87%; mp 45-46 °C; ^{1}H NMR (60 MHz) δ 2.30 (s, 3 H), 6.75-7.73 (m, 4 H), 8.30 (m, 1 H), 8.68 (m, 1 H), 9.17 (m, 1 H). Anal. ($C_{14}H_{11}N_2$) C, H, N.

General Procedure D. Preparation of Quinolin-4-amines 53-56. The procedure for LDA-mediated cyclization of ketimines 49-52 has been published.[5] Yields, melting points, and ^{1}H NMR spectra for 2-phenylquinolin-4-amine (53), 2-(2-pyridyl)-quinolin-4-amine (54), and 2-(2-thienyl)quinolin-4-amine (56) have also been presented previously.[5]

2-(3-Pyridyl)quinolin-4-amine (55): obtained from 51; yield 52%; mp 185-188 °C; ^{1}H NMR (60 MHz, DMSO-d_6) δ 7.03 (br s, 2 H), 7.27 (s, 1 H), 7.42-8.80 (m, 7 H), 9.38 (d, J = 2 Hz, 1 H); MS m/e 221 (100, M$^+$), 195 (16). Anal. ($C_{14}H_{11}N_3$) C, H, N.

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Communications to the Editor

Synthesis and Bioactivity of N^{ω}-Hydroxyarginine: A Possible Intermediate in the Biosynthesis of Nitric Oxide from Arginine

Nitric oxide (NO) has recently been found to be an endogenous molecule of extreme biological importance in mammalian cells. It has been demonstrated to play a vital role in a variety of physiological responses[1] including smooth muscle relaxation.[2] Though a significant amount of research has been conducted in determining the physiological role of NO, little work has been performed in elucidating the mechanistic pathway of NO formation. Early studies have demonstrated that NO generation is the result of the enzymatic oxidation of a terminal guanidinium nitrogen on arginine with citrulline being the other product. To date, two mechanisms for the generation of NO have been put forth (Figure 1) with little supporting evidence. One pathway (A-B-C-D, Figure 1), proposed by Marletta et al.,[3] involves initial N^{ω}-hydroxylation of arginine to give N^{ω}-hydroxyl-L-arginine (NOHA) followed by a series of reactions to generate NO. The other pathway (A-E-F, Figure 1), proposed by DeMaster et al.,[4] also

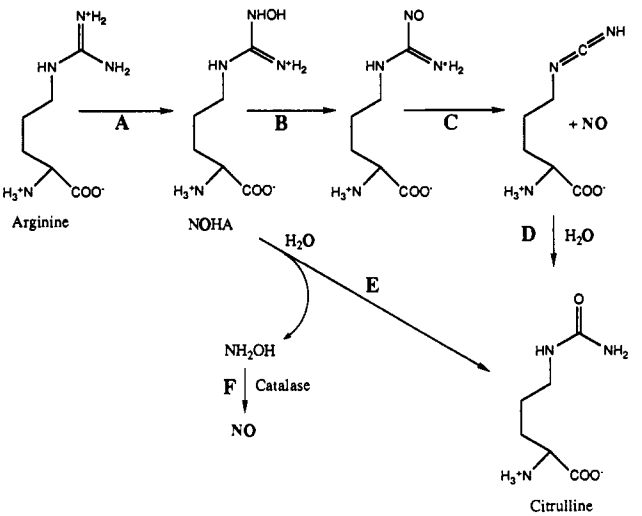

Figure 1. Proposed pathways for the biosynthesis of NO from arginine.

requires an initial N-hydroxylation of arginine to generate NOHA. This pathway differs from the first in that free hydroxylamine is generated and subsequently oxidized by another enzyme (possibly catalase) to give NO. Both pathways have as their first step the formation of NOHA. In the macrophage, it is unlikely that either mechanism is entirely correct since oxygen isotope studies have indicated that the oxygen in citrulline originates from molecular oxygen and not water.[5] Although NOHA is proposed to be a critical biosynthetic intermediate, no reports on its synthesis or bioactivity have appeared in the literature. Herein, we describe the synthesis of NOHA and report our preliminary findings regarding its biological activity.

NOHA was synthesized according to the scheme outlined in Figure 2 and is described below. NMR spectra

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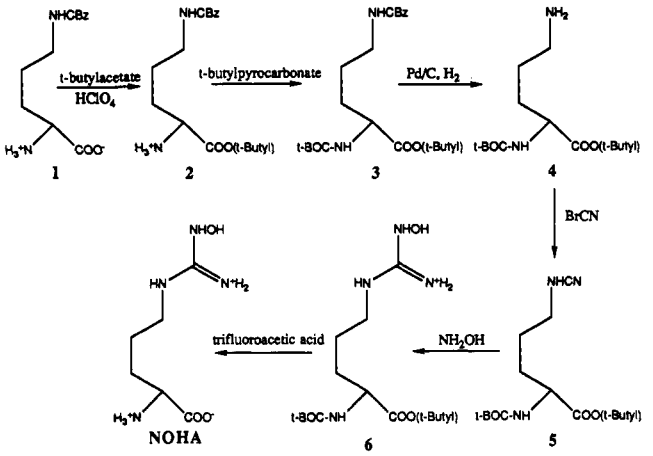

Figure 2. Synthetic scheme for the synthesis of NOHA. For experimental conditions, see the text.

were obtained on a Bruker AM360, and the mass spectrum was obtained on a ZAB model spectrometer (VG Analytical Inc., Manchester, U.K.).

N^{δ}-(Benzyloxycarbonyl)-L-ornithine *tert*-Butyl Ester (2). The *tert*-butyl ester of N^{δ}-(benzyloxycarbonyl)-L-ornithine (1) (Sigma, St. Louis, MO) was synthesized from 13.0 g (48.8 mmol) of 1 according to the method of Bodanszky[6a] with the following modifications. The reaction was stirred for 2 days. Product isolation was accomplished by addition of water to the reaction mixture, treatment with NaOH to basic, and extraction with ethyl acetate. Rotary evaporation of the organic phase afforded 11.25 g (34.9 mmol) of an oil (71.5%). NMR (deuteriochloroform): δ 1.44 (s, 9 H), 1.5–1.9 (m, 4 H), 3.23 (t, 2 H), 3.31 (t, 1 H), 5.09 (s, 2 H and s, 1 H broad), 7.35 (s, 5 H).

N^{α}-(*tert*-Butyloxycarbonyl)-N^{δ}-(benzyloxycarbonyl)-L-ornithine *tert*-Butyl Ester (3). N^{δ}-(Benzyloxycarbonyl)-L-ornithine *tert*-butyl ester (11.25 g, 34.9 mmol) was dissolved in 40 mL of methylene chloride and cooled (ice bath). A solution of 8.79 g (40.3 mmol) of *tert*-butyl pyrocarbonate (Aldrich, Milwaukee, WI) in 15 mL of methylene chloride was slowly added. The reaction mixture was then stirred for 1 h at 0 °C and 3 h at room temperature, the solvent removed by rotary evaporation, and the product isolated by flash chromatography (silica) using hexane/ethyl acetate (3:1). Fractions from the column were analyzed for 3 by TLC (R_f = 0.33) using the eluting solvent. Fractions containing pure product were pooled, and the solvent was removed by rotary evaporation. This procedure afforded 13.81 g (32.7 mmol) of a product oil (93.7%). NMR (deuteriochloroform): δ 1.44 (s, 9 H), 1.46 (s, 9 H), 1.5–1.9 (m, 4 H), 3.23 (t, 2 H), 4.17 (t, 1 H), 4.88 (s, 1 H broad), 5.09 (s, 2 H and s, 1 H broad), 7.35 (s, 5 H).

N^{α}-(*tert*-Butyloxycarbonyl)-L-ornithine *tert*-Butyl Ester (4). Removal of the benzyloxycarbonyl protecting group from 3 (13.81 g, 32.7 mmol) was performed by the method described by Bodanszky.[6b] This procedure yielded 9.05 g (31.4 mmol) of a product oil (96%) which solidified on freezing. NMR (deuteriochloroform): δ 1.44 (s, 9 H), 1.46 (s, 9 H), 1.5–1.9 (m, 4 H), 2.73 (t, 2 H), 4.17 (t, 1 H), 5.20 (s, 1 H broad).

N^{α}-(*tert*-Butyloxycarbonyl)-N^{δ}-cyano-L-ornithine *tert*-Butyl Ester (5). Cyanamide formation was performed on 9.05 g (31.4 mmol) of 4 by the method of Bailey[7] with the following modifications. After completion of the reaction, solvent was removed by rotary evaporation, and the residue was taken up in water and extracted three times with ether. The organic extracts were combined and dried, and the solvent was removed by rotary evaporation. The product was purified by flash chromatography (silica) using hexane/ethyl acetate (2:1). Fractions were analyzed by TLC using the eluting solvent (R_f = 0.6). Fractions containing pure product were pooled, and the solvent was removed by rotary evaporation. This procedure afforded 8.16 g (26.0 mmol) of a product oil (83%). NMR (deuteriochloroform): δ 1.44 (s, 9 H), 1.46 (s, 9 H), 1.5–1.9 (m, 4 H), 3.17 (t, 2 H), 4.17 (t, 2 H and s, 1 H broad), 5.19 (s, 1 H broad).

N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-hydroxy-L-arginine *tert*-Butyl Ester (6). The procedure of Bailey[7] was used to make the hydroxylamine derivative from 8.16 g (26.0 mmol) with the following modifications. Dry dioxane (Na/benzophenone) was used as solvent. The reaction mixture was refluxed for 1 h and filtered, and the solvent was removed by rotary evaporation. The residue was dissolved in methylene chloride and extracted three times with water acidified to pH 5.5 (acetic acid). The aqueous extracts were washed twice with methylene chloride, and the water was removed by rotary evaporation. This resulted in 6.31 g of a crude material which contained 70% of the desired material, 6, as determined by NMR. NMR (deuteriochloroform): δ 1.44 (s, 9 H), 1.46 (s, 9 H), 1.5–1.9 (m, 4 H), 3.17 (t, 2 H), 4.19 (t, 2 H), 4.29 (s, 1 H broad), 5.19 (s, 1 H broad). The residue was used directly in the following step.

NOHA. Removal of the protecting groups from 6.31 g of the crude material from above was performed according to the general method of Bodanszky[6c] with the following modifications. The residue remaining after rotary evaporation of trifluoroacetic acid was dissolved in water and purified with use of a cationic exchange resin (Dowex-50W, 4% cross-linked, dry mesh 200–400) and 0.05 M ammonium hydroxide as eluting solvent. The fractions were monitored by NMR for product after solvent removal. The desired solid was isolated in 53% yield for the last two steps. The entire procedure gave 2.37 g (12.5 mmol) in overall 28% yield.

The exact mass measurement from the high-resolution mass spectrum (EI, 20 eV) gave a molecular formula of $C_6H_{14}N_4O_3$ (190.106 590 5). The principle fragments were m/z = 190 (M^+), 173, 172, 128, 69, 56. The NMR spectra (D_2O) showed the following peaks: δ 4.06 (t, 1 H), 3.25 (t, 2 H), 1.50–2.05 (m, 4 H).

The biological activity of NOHA was tested in isolated rings of bovine pulmonary artery by the method described earlier.[8] Preliminary results indicate that NOHA is capable of causing vasorelaxation in a concentration-dependent manner similar to that of arginine itself (Figure 3). Also, the NOHA-dependent vasorelaxation is reversible by the addition of known inhibitors of NO biosynthesis such as N^{ω}-methyl (Figure 3) or N^{ω}-nitroarginine (data not shown). These results are consistent with the role of NOHA as an intermediate in the biosynthetic pathway. However, whether NOHA is actually a metabolic intermediate or is simply capable of generating NO by an independent pathway cannot be discerned at this time. Studies are in progress to further evaluate these possibilities.

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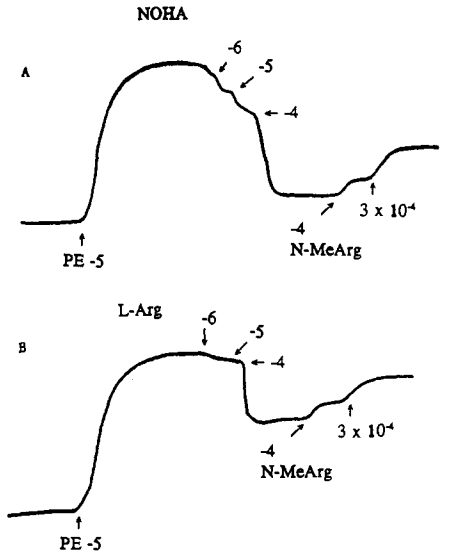

Figure 3. NOHA-elicited vasorelaxation in arginine-depleted bovine pulmonary artery. Isolated rings of endothelium intact bovine pulmonary artery were precontracted with phenylephrine (PE) and challenged with acetylcholine (ACh) added cumulatively (not shown). The tissues were then allowed to stand in oxygenated Krebs solution for 24 h to deplete endogenous arginine levels. To these depleted tissues were then added PE, NOHA (A), and L-arginine (B) as shown. Inhibitor was added after NOHA and arginine addition was completed as indicated. Concentrations are expressed as the base power of 10 and represent final bath concentrations.

NOHA is a relatively stable molecule, now accessible by a simple synthetic route, that can cause vasorelaxation in bovine pulmonary arteries. To our knowledge, it is the only simple N-substituted arginine analogue found to have biological activity similar to that of arginine itself (L-homoarginine can also elicit vasorelaxation[8,9]). All other arginine analogues tested (i.e., N^{ω}-methyl-, N^{ω}-amino-, N^{ω}-nitroarginine)[10,11] in a variety of cells and tissues are inhibitors of NO biosynthesis.

Registry No. 1, 3304-51-6; 2, 53054-01-6; 3, 53054-02-7; 4, 53054-03-8; 5, 133374-42-2; 6, 133374-43-3; NOHA, 53054-07-2; NO, 10102-43-9; H-Arg-OH, 74-79-3.

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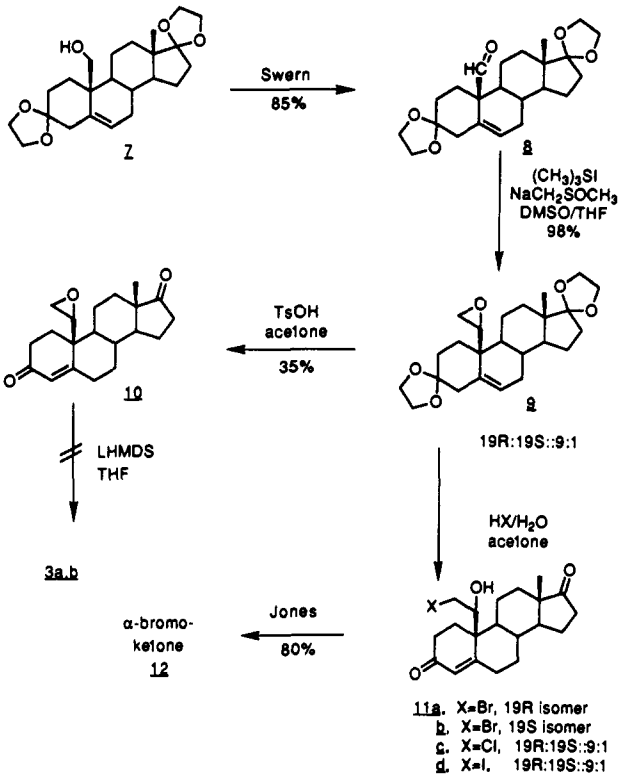
Novel Time-Dependent Inhibitors of Human Placental Aromatase

Aromatase is a cytochrome P_{450} enzyme system that converts androgens to estrogens by oxidative dealkylation of the angular C-19 methyl group and aromatization of the steroidal A ring. This transformation requires 3 equiv of oxygen and NADPH.[1] The initial hydroxylations produce

Scheme I



the 19-hydroxy and 19-oxo androgen derivatives,[2] whereas the site of the third hydroxylation has not yet been determined.[3] A 2β-hydroxylation has been proposed,[4] i.e., to produce 1 from 19-oxoandrostenedione, which would allow the intermediacy of lactol **2**, although reports on the aromatization of 1[5,6] and recent labeling experiments and studies on peroxide models for aromatase[7-9] suggest an alternate process. The C-10, C-2 hydroxyethyl bridged species **3a** and **3b** could be viewed as stable, carbon analogues of lactol **2**.[10] This communication describes the

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