Synthesis and Biological Activity of the Novel Nitric Oxide Synthase Inhibitor $N^{\omega'}$-Hydroxy-N^{ω}-methyl-L-arginine

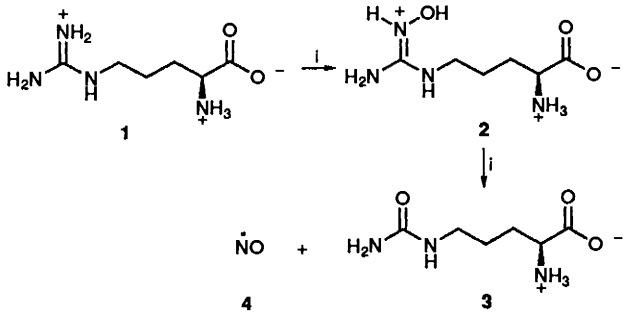
Humphrey A. Moynihan,[a] Stanley M. Roberts,[a] Hazel Weldon,[a] Graham H. Allcock,[b] Erik E. Änggård[b] and Timothy D. Warner[b]

[a] *Department of Chemistry, Stocker Road, Exeter EX4 4QD, UK*

[b] *The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ, UK*

$N^{\omega'}$-Hydroxy-N^{ω}-methyl-L-arginine has been synthesised in eight steps from N^{δ}-(benzyloxycarbonyl)-L-ornithine and has been found to inhibit the biosynthesis of nitric oxide.

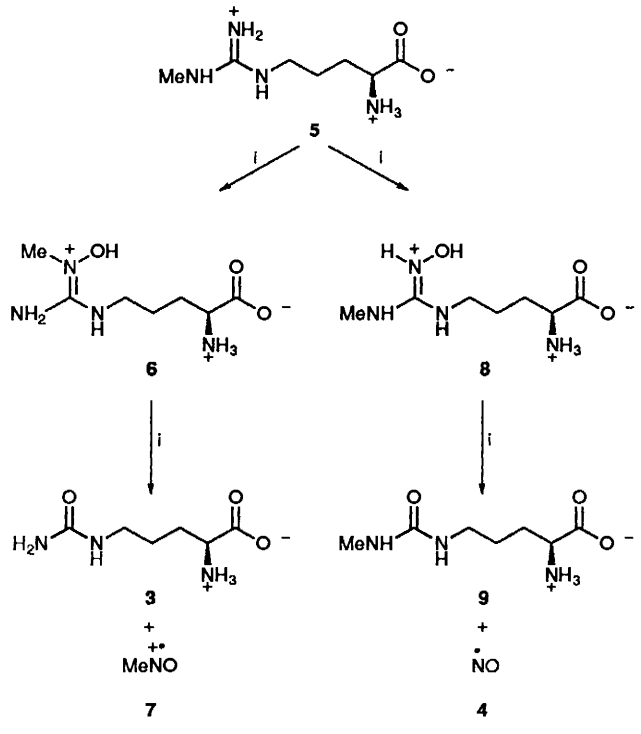
Following the discovery, in 1987, that the unidentified vasodilator termed the endothelium derived relaxation factor (EDRF) was nitric oxide[1,2] (NO) there has been much interest as to its biological action. NO released from the endothelium stimulates smooth muscle relaxation[3] and thus is important in the regulation of blood pressure. Other roles include inhibition of platelet aggregation, cytotoxic effects and its action as a messenger in the central nervous system.[4] There has been significant attention directed towards elucidation of the mechanism of the biosynthesis of NO. It has been shown[5] that, in an NADPH-dependent hydroxylation, nitric oxide synthase enzymes (NOS) convert L-arginine **1** into $N^{\omega'}$-hydroxy-L-arginine **2**. This compound is further oxidised to give L-citrulline **3** and NO **4** in an NADPH-dependent, three-electron oxidation (Scheme 1).



Scheme 1 i, NOS, NADPH

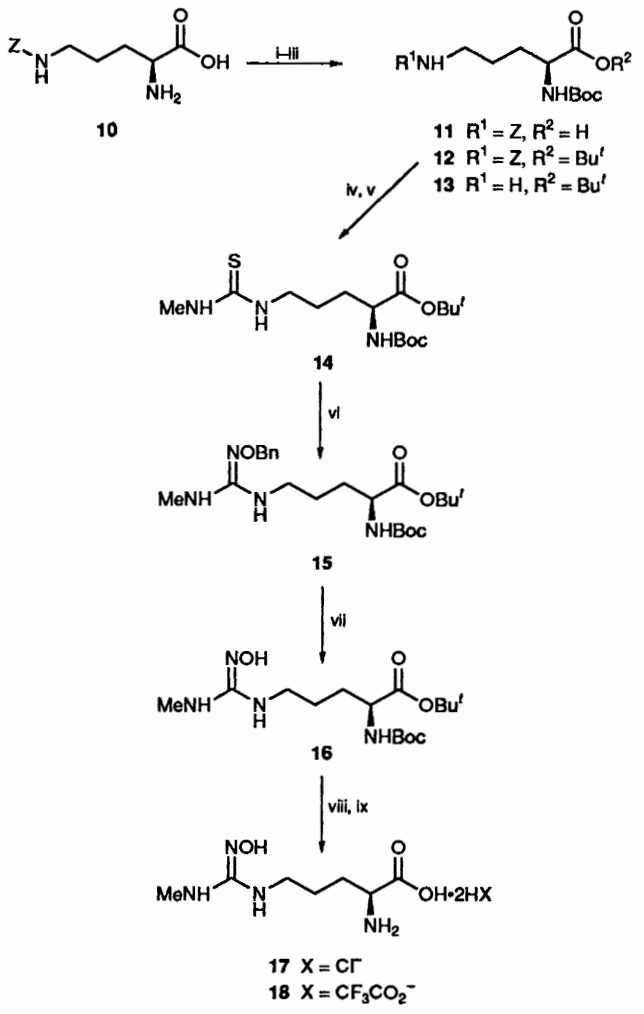
N^{ω}-Methyl-L-arginine (NMA) **5** has been found to irreversibly inactivate NOS.[6] As this inactivation was found to be NADPH-dependent it was proposed[7] that NMA **5** would undergo an initial hydroxylation, following a pathway similar to that of arginine, on either of the two terminal guanidino nitrogens to give $N^{\omega'}$-hydroxy-$N^{\omega'}$-methyl-L-arginine **6** or $N^{\omega'}$-hydroxy-N^{ω}-methyl-L-arginine **8** (Scheme 2). Further oxidation of compound **6** would give citrulline **3** and the nitrosomethane radical cation **7**. However, oxidation of compound **8** would lead to N^{ω}-methyl-L-citrulline **9** and NO **4**. Feldman *et al.*[7] synthesised an authentic sample of $N^{\omega'}$-hydroxy-$N^{\omega'}$-methyl-L-arginine **6** and confirmed that NMA **5** was metabolised by the first of the two proposed pathways to give compound **6** as the inactivating species. It was thus proposed that $N^{\omega'}$-hydroxy-N^{ω}-methyl-L-arginine **8** would not act as an inactivating species and would, in fact, release NO.

We report herein the synthesis of $N^{\omega'}$-hydroxy-N^{ω}-methyl-L-arginine (Scheme 3) and its action as an inhibitor of NOS. The synthesis of compound **8** was planned following adaptation of the Feldman synthesis of $N^{\omega'}$-hydroxy-L-arginine **2**.[8] Protection[9] of the α-amino group of N^{δ}-(benzyloxycarbonyl)-L-ornithine **10** gave the *tert*-butoxycarbonyl (Boc) derivative **11** in



Scheme 2 i, NOS, NADPH

quantitative yield. Treatment of compound **11** with *N*,*N*-dimethylformamide di-*tert*-butyl acetal[10] afforded the ester **12** in 81% yield. The benzyloxycarbonyl (Z) group was removed by hydrogenation using 10% Pd–C as catalyst to afford the δ-amine **13** (95% yield). Nucleophilic displacement of imidazole from 1,1'-thiocarbonyldiimidazole by the free δ-amine gave an intermediate which was not isolated. A second nucleophilic displacement of imidazole with methylamine introduced the *N*-methylthiourea functionality into compound **14** in 77% yield. The benzyloxyguanidine group was constructed following activation of the thiocarbonyl group by mercury(II) oxide and subsequent displacement with *O*-benzylhydroxylamine hydrochloride[11] to give **15** (42% yield). It was found that optimum yields were obtained on addition of an excess of triethylamine to the reaction flask prior to the addition of diethyl ether and, in fact, if the order of addition of the triethylamine and the diethyl ether was reversed no reaction occurred. Hydrogenation of the benzyl group using 20% $Pd(OH)_2$–C as the catalyst at 0 °C gave the hydroxyarginine derivative **16** in 16% yield together with 60% recovered starting material which was recycled. It was found that if the reaction was allowed to reach completion



Scheme 3 *Reagents and conditions:* i, $(Boc)_2O$, dioxane, $NaHCO_3$, H_2O, room temp, 3 days, 99%; ii, dimethylformamide di-*tert*-butyl acetal, toluene, 111 °C, 2 h, 81%; iii, 10% Pd–C, H_2, EtOH, room temp, 4 h, 95%; iv, 1,1'-thiocarbonyldiimidazole, CH_2Cl_2, 0 °C, 2.5 h; v, $MeNH_2$, MeOH, 0 °C, 2 h, 77% from **13**; vi, *O*-benzylhydroxylamine hydrochloride, Et_3N, HgO, Et_2O, room temp, 3 days, 42%; vii, 20% $Pd(OH)_2$–C, H_2, MeOH, 0 °C, 10 min, 16% and 60% recovered starting material; viii, HCl (4 mol dm^{-3}) in dioxane, room temp, 18 h, 82%; ix, prep. HPLC using 90% MeCN–H_2O [0.1% trifluoroacetic acid (TFA)] as eluent, 65%

the product was obtained in yields varying from 18–25% with the rest of the material decomposing to an unidentified by-product. Hydrolysis of the Boc group and the *tert*-butyl ester using HCl (4 mol dm^{-3}) in dioxane afforded the desired product **17** as the dihydrochloride salt in 82% yield as a white precipitate. Purification was carried out by C18 reverse-phase HPLC (Spherisorb ODS2 preparatory reverse-phase column) using 90% MeCN–H_2O (0.1% TFA) (10 cm^3 min^{-1}) as the eluent. The product bis-trifluoroacetate salt **18** was obtained as a hygroscopic oil in 65% yield* after lyophilizing the frozen aqueous solution.

* Data for $N^{\omega'}$-hydroxy-N^{ω}-methyl-L-arginine bis-trifluoroacetate: $[\alpha]_D^{24}$ +9.3 10^{-1} deg cm^2 g^{-1} (*c* 1.0, $CHCl_3$); δ_H(300 MHz; [$^2H_6$]dimethyl sulfoxide; 60 °C) 7.96 (1 H, br s, MeN*H*), 7.81 (2 H, br s, NH_2), 3.82 (1 H, br, t, *J* 5.5, α-H), 3.25 (2 H, br t, *J* 6, 2 × δ-H), 2.79 (3 H, d, *J* 4, Me), 1.87 (2 H, br m, 2 × β-H) and 1.70 (2 H, br m, 2 × γ-H); δ_C(75.5 MHz; [$^2H_4$]MeOH), 166–162 (br, C=O), 159.17 (C=N), 158.56 and 158.18 (CF_3CO_2), 117.76 and 114.00 (CF_3CO_2), 49.81 (CH), 41.28 (CH_2), 28.46 (CH_2), 27.76 (CH_3) and 25.57 (CH_2) [Found: *m/z* (FAB) $(M + H)^+$, 205.1299. $C_7H_{16}N_4O_3$ requires $(M + H)^+$, 205.1302].

Biological Results and Discussion

Injection of boluses containing 10, 30 and 100 μmol kg^{-1} of $N^{\omega'}$-hydroxy-N^{ω}-methyl-L-arginine bis(trifluoroacetate) **18** to anaesthetised rats caused dose-dependent increases in mean arterial pressure of approximately 7, 12 and 15 mmHg, respectively. These effects were sustained for *ca.* 10–15 min. In organ bath experiments $N^{\omega'}$-hydroxy-N^{ω}-methyl-L-arginine bis-trifluoroacetate at a concentration of 100 μmol dm^{-3} caused an inhibition of endothelium-dependent relaxations induced by acetylcholine. This effect was not obtained at the two lower concentrations used (10 and 30 μmol dm^{-3}). These results indicate that $N^{\omega'}$-hydroxy-N^{ω}-methyl-L-arginine is an inhibitor of NOS both *in vivo* and *in vitro*. However, it is not as strong an inhibitor as N^{ω}-methyl-L-arginine **5** for similar effects are observed *in vivo* and *in vitro* with three to ten-fold smaller concentrations of this compound.[12,13] Further studies are currently aimed at elucidating if this difference in activity can be explained by the additional ability of $N^{\omega'}$-hydroxy-N^{ω}-methyl-L-arginine to stabilise and potentiate the effects of NO.[14] Alternatively, the possibility that compound **18** is being metabolised back to N^{ω}-methyl-L-arginine **5** and it is the latter compound that is causing the inhibition is also being investigated.

Experimental

$N^{\omega'}$-*Benzyloxy*-N^{α}-tert-*butoxycarbonyl*-N^{ω}-*methyl*-L-*arginine* tert-*Butyl Ester* **15**.—Triethylamine (5 cm^3) was added to a stirred mixture of N^{α}-*tert*-butoxycarbonyl-δ-(*N*-methylthioureido)-L-norvaline *tert*-butyl ester **14** (0.50 g, 1.39 mmol), mercury(II) oxide (0.30 g, 1.39 mmol) and *O*-benzylhydroxylamine hydrochloride (0.22 g, 1.39 mmol) under a nitrogen atmosphere. Diethyl ether (15 cm^3) was added to the mixture which after being vigorously stirred for 3 days was filtered through Celite and evaporated. The residue was subjected to column chromatography with ethyl acetate–light petroleum (b.p. 40–60 °C) (9:1) as eluent to give the title compound **15**, a mixture of geometric isomers, as a yellow oil (0.26 g, 42%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3442 (NH), 3009 and 2985 (CH), 1710 (C=O), 1632 (Ar), 1501, 1370, 1249, 1155, 1058, 843 and 695; δ_H(300 MHz; [$^2H_6$]dimethyl sulfoxide) 7.41–7.22 (5 H, m, Ar), 7.06 (1 H, d, *J* 8.0, α-NH), 5.25 and 4.92 (1 H, 2 × q, *J* 5.0 MeN*H*), 5.16 and 4.80 (1 H, 2 × t, *J* 6.0, δ-NH), 4.71 and 4.69 (2 H, 2 × s, PhCH_2), 3.78 (1 H, br, α-H), 2.92 and 2.83 (2 H, 2 × q, *J* 6.0, 2 × δ-H), 2.59 and 2.43 (3 H, 2 × d, *J* 5.0, Me), 1.71–1.48 (4 H, m, 2 × β-H and 2 × γ-H) and 1.38 (18 H, s, 2 × Bu^t) [Found: *m/z* (CI) $(M + H)^+$, 451.2920. $C_{23}H_{38}N_4O_5$ requires $(M + H)^+$, 451.2922].

Acknowledgements

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