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Hydroxamic acids are nitric oxide donors. Facile formation of ruthenium(II)-nitrosyls and NO-mediated activation of guanylate cyclase by hydroxamic acids

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Hydroxamic acids are shown for the first time to be effective NO donors by their ability to readily form ruthenium(II)-nitrosyls, and to cause vascular relaxation of rat aorta by NO-mediated activation of the iron-containing guanylate cyclase.

Hydroxamic acids, a group of weak organic acids of general formula RC(O)N(R′)OH, fulfil a variety of roles in biology and medicine, for example, as siderophores for iron(III),[1] as potent and selective inhibitors of enzymes such as peroxidases,[2] ureases,[3] and matrix metalloproteinases,[4] and as hypotensive,[5] anti-cancer, anti-tuberculous and antifungal agents.[6] While some of these roles are undoubtedly due to the chelating ability of the hydroxamate group, others, such as the hypotensive effects (a known nitric oxide property), may be due to their ability to release nitric oxide, a view strengthened by the now established importance of NO in many physiological processes.[7] We report herein the first evidence that hydroxamic acids can indeed act as NO donors, as shown by the fact that they readily transfer NO to ruthenium(III) and cause vascular relaxation in rat aorta by activation of the iron-containing guanylate cyclase enzyme.

Reaction of K[Ru(Hedta)Cl]·2H_2O (200 mg, 0.40 mmol), with benzohydroxamic acid (177 mg, 1.29 mmol) in aqueous solution (30 cm^3) at room temperature resulted in an immediate colour change from straw-yellow to red. A brown product was obtained (*ca.* 55% yield without attempted optimisation) following purification on a Sephadex LH 20 column and removal of solvent. The unambiguous formation of a ruthenium nitrosyl complex was confirmed by microanalysis,† IR, mass and ^{1}H NMR spectra, which were consistent with the formulation K_2[Ru(edta)(NO)Cl], containing a linear, diamagnetic Ru^{2+}–NO^+ group[8] (IR: distinctive ν_{NO} at 1880 cm^{-1}, broad, strong ν_{CO} at 1660 cm^{-1}, no absorption at 1730 cm^{-1}, confirming fully depronated edta[9] and ν_{Ru-Cl} at 300 cm^{-1}; ESI-MS: mass peaks at 420 and 390 amu, corresponding to $[Ru(edta)(NO)]^-$ and $[Ru(edta)]^-$, respectively, with correct isotopic abundances; ^{1}H NMR: δ 3.6 due to ethylenic protons, 3.8, 4.1, 4.3 and 4.4 due to CH_2COO^- protons, in D_2O solution). This complex is similar to the previously reported six-coordinate Ru(H_2edta)(NO)Cl·2H_2O,[9] but contains fully deprotonated tetradentate edta with two pendant carboxylate groups. A related complex, $[Ru(edta)NO]^-$, is formed in solution by reaction of NO with K[Ru(Hedta)Cl].[10] Similar reactions of K[Ru(Hedta)Cl]·2H_2O with acetohydroxamic acid and salicylhydroxamic acid also gave the same product in high yields. The products of the denitrosylation reactions were shown to be the corresponding carboxylic acids by TLC, UV and ^{1}H NMR analysis.‡§ Reaction of $RuCl_3$·xH_2O with aceto-, benzo-, salicyl- and anthranilic hydroxamic acids in ethanol followed by purification on Sephadex LH 20 columns also gave ruthenium(II) nitrosyl complexes, all of which have very distinctive ν_{NO} bands at *ca.* 1885 cm^{-1}.¶

The ability of hydroxamic acids to release nitric oxide in simple biological systems was shown by vascular relaxation of endothelium-denuded rings of rat aorta.[11] These were set up in organ baths for isometric tension recording. Rings were contracted with the α_1-adrenoreceptor agonist phenylephrine (1 μM), and the ability of increasing concentrations of hydroxamic acid derivatives to produce relaxation was examined; the results are shown in Fig. 1. Of the hydroxamic acids investigated, benzohydroxamic acid proved most effective, causing approximately 45% relaxation of rat aorta at a concentration of 300 μM. Although this value is higher than that quoted for the well known NO donor 3-morpholinosydnonimine, SIN-1 (1 μM), it compares favourably with that of another NO donor 4-(3-butoxy-4-methoxybenzo)-2-imidazolidilone, Ro-20-1724 (100 μM).[12] Relaxation occurred by activation of the enzyme guanylate cyclase (a definitive receptor for NO),[13] as shown by the fact that methylene blue (10 μM), a known inhibitor of this enzyme,[14] prevented the relaxation (*e.g.* relaxation to 300 μM acetohydroxamic acid: vehicle (distilled water) treated, 31.4±10.9% relaxation; methylene blue treated, 6.4±3.1% relaxation, $n = 4$, $P < 0.05$).

In this work, we have shown conclusively that hydroxamic acids can act as effective NO donors by demonstrating that they can readily transfer NO to ruthenium(III), the first reported metal nitrosyl complexes formed from hydroxamic acids. Furthermore, hydroxamic acids can cause vascular relaxation in rat aorta by NO-mediated activation of the iron-containing enzyme guanylate cyclase, thus confirming the biological relevance of our novel results.

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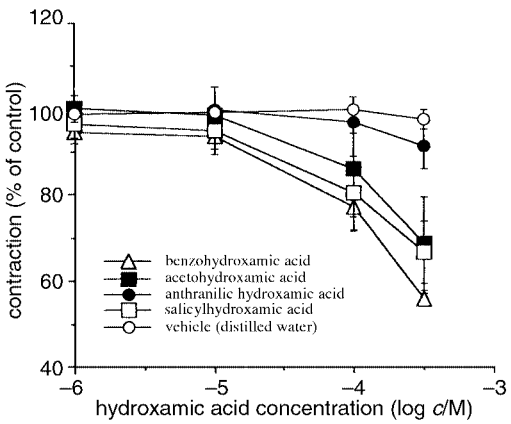

Fig. 1 The effects of hydroxamic acids on relaxation of rat aorta.

Notes and references

† Microanalysis: found: C, 21.03; H, 2.78; N, 7.51; Cl, 9.11. K_2[Ru(edta)-(NO)Cl]·0.5HCl·H_2O ($C_{10}H_{14.5}N_3O_{10}Cl_{1.5}K_2Ru$) requires C, 21.10; H, 2.57; N, 7.39; Cl, 9.34%.

‡ An aqueous mixture of benzohydroxamic acid and an excess of [Ru(Hedta)Cl]$^-$, after completion of the reaction, was analysed by TLC [silica plates and a toluene–ether–acetic acid–methanol (120:60:18:1) or ethanol–water–ammonia (8:1:1) solvent mixture] which confirmed the presence of benzoic acid and the absence of benzohydroxamic acid. The UV spectrum in H_2O has a band at 224 nm due to benzoic acid which is not present in the spectrum of the nitrosyl complex. The ^{1}H NMR spectrum of a D_2O solution containing [Ru(Hedta)Cl]$^-$ and a twofold excess of benzohydroxamic acid, to ensure complete removal of the paramagnetic ruthenium(III) complex, showed a signal at δ 8.01 which is characteristic of benzoic acid, but not of benzohydroxamic acid. The acid-catalysed hydrolysis of hydroxamic acids to carboxylic acids (half-life *ca*. 1 h at 88 °C) has previously been reported.[15]

§ A possible mechanism for the denitrosylation of the hydroxamic acid (RC(O)NHOH) involves aquation of [Ru(Hedta)Cl]$^-$, followed by nucleophilic attack by the hydroxo conjugate base ligand on the hydroxamic acid carbonyl group, giving an intermediate from which hydroxylamine is eliminated. Abstraction of NO from this causes displacement of the carboxylate ligand ($RCOO^-$) and this, together with displacement of coordinated edta carboxylate by chloride in the work up procedure, gives [Ru(edta)(NO)Cl]$^{2-}$.

¶ IR(Nujol mull): strong ν_{NO} at 1885 cm^{-1} (acetohydroxamate complex), 1885 cm^{-1} (benzohydroxamate), 1883 cm^{-1} (salicylhydroxamate) and 1885 cm^{-1} (anthranilic hydroxamate).

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