Short Communications

The generality of the nitroso-glyoxylate reaction: Conversion of *p*-nitronitrosobenzene to the hydroxamic acid[1]

M.D. Corbett and B.R. Corbett

Pesticide Research Laboratory, Food Science and Human Nutrition Department, University of Florida, Gainesville (Florida 32611, USA), 18 March 1982

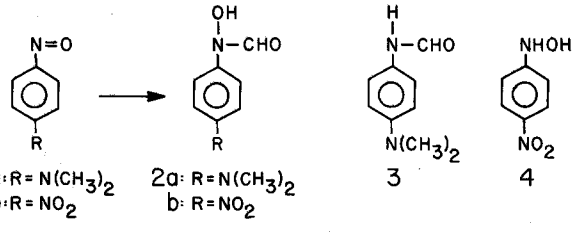
Summary. The reaction of glyoxylic acid with *p*-nitronitrosobenzene (**1b**) in dilute aqueous solution gave the hydroxamic acid (**2b**) as the major detectable product. The significance of this observation with respect to the title reaction is discussed.

In 1980 a new and unusual chemical conversion was reported for aromatic C-nitroso compounds (general structure **1**), in which their reaction with glyoxylic acid in aqueous media resulted in the production of the corresponding N-formyl derived hydroxamic acids (general structure **2**) in nearly quantitative yields[2]. This reaction was found to be general for *meta* and *para* substituted nitrosobenzenes with substituents ranging from electron withdrawing halogens and the carbethoxy group to electron donating alkyl and alkoxy groupings[2]. However, in the case of *p*-dimethylaminonitrosobenzene (**1a**) further reduction of the hydroxamic acid **2a** occurred to give the formanilide **3**[3]. This discovery that a strongly electron donating substituent resulted in a deviation from the general nitroso-glyoxylate reaction led us to consider the possibility that substituents with extreme opposite properties (i.e. strongly electron withdrawing functional groups) might also cause a deviation from this general reaction. This possibility, coupled with our more recent observations on the extreme ease of chemical reduction of *p*-nitronitrosobenzene (**1b**)[4], prompted an investigation of the reaction of **1b** with glyoxylic acid.

Experimental. The synthesis of *p*-nitronitrosobenzene (**1b**) was achieved by oxidation of *p*-nitroaniline (Sigma Chemical Co.) with neutralized Caro's acid[5]. Reduction of **1b** with ascorbic acid[6] gave *p*-nitrophenylhydroxylamine (**4**), which was converted to N-(*p*-nitrophenyl)formohydroxamic acid (**2b**) by a previously published method[2]. The desired hydroxamic acid (**2b**) was also prepared by reaction of **1b** with glyoxylic acid according to our literature precedence[2]. **2b** was obtained as fine tan crystals (m.p. 135–136 °C, decomposition); λmax (EtOH) 320 nm (ε 12,350). Elemental analysis and NMR-data were consistent with the structure **2b**; mass spectral analysis indicated the presence of the parent ion m/e 182 and a base ion at m/e 166. The M-16 mass spectral fragmentation is characteristic of hydroxamic acids[2]. The table gives the retention times and relative peak height ratios at dual wavelengths for these compounds in the high performance liquid chromatography (HPLC) systems employed in this study.

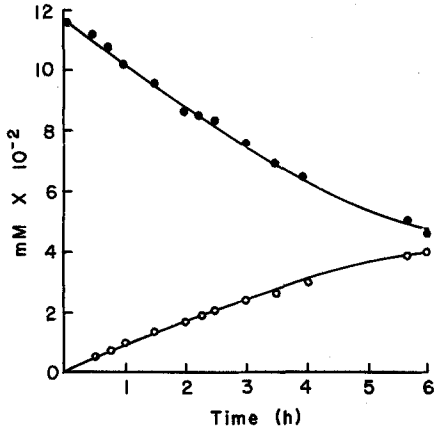
The time course of the reaction of 0.12 mM substrate **1b** with 5.0 mM glyoxylate in pH 6.0, 0.05 M, KH_2PO_4 buffer at 25 °C was followed by direct HPLC analysis of 10 µl aliquots taken from the reaction at timed intervals. The HPLC system consisted of 0.01% desferal mesylate[7] in 40% MeOH buffered to pH 3.5 with 0.01 M KH_2PO_4 as eluent at a flow rate of 1.5 ml/min through a Waters Associates µBondapak C_{18} (3.9 mm × 30 cm). Peak detection and quantitation was achieved by the use of a Waters Model 440 dual wavelength detector with combinations of 254, 280 and 313 nm windows. A 2nd HPLC eluent consisting of 60% MeOH was also employed.

Results and discussions. Our success in synthesizing **2b** by the reaction of **1b** with glyoxylic acid was the 1st proof that the title reaction does occur even for this highly deactivated substrate, *p*-nitronitrosobenzene (**1b**). Nevertheless, it was necessary to employ a highly sensitive HPLC analytical procedure to determine if an alternate reaction pathway might be operative in this particular case. We were interested in the possibility that an initial redox reaction might occur to produce the hydroxylamine **4** as a by-product or as a necessary intermediate in the eventual production of the hydroxamic acid **2b**. The possibility that other reaction intermediates might be detected by HPLC was also of great interest in our attempt to prove the mechanism of the title reaction, which had been previously proposed[2]. The rela-



HPLC retention times and dual wavelength ratios of synthetic standards. Solvent flow rate was 1.5 ml/min through a µBondapak C_{18} column. See experimental section for details on solvent composition

Compound	Solvent	Retention time (min)	A_{313}/A_{254}	A_{313}/A_{280}
1b	40% MeOH	10.8	1.46	-
1b	60% MeOH	4.04	-	0.46
2b	40% MeOH	5.0	5.55	-
2b	60% MeOH	2.61	-	2.1
4	40% MeOH	4.13	1.66	-



The reaction of *p*-nitronitrosobenzene (**1b**) with glyoxylic acid as a function of time. The reaction was initiated by the addition of sufficient **1b** as a concentrated solution in ethanol to give an initial concentration of 0.117 mM in a 5.0 mM solution of glyoxylic acid in pH 6.0, 0.05 M, KH_2PO_4 buffer at 25 °C. The concentrations of reactant **1b** (●) and product **2b** (○) were determined by direct HPLC analysis at the indicated sampling times.

tively slow rate of reaction of **1b** with glyoxylate was predicted from our earlier results on substituent effects upon the rate of the reaction[2]; and it was on this basis that we thought it might be possible to detect certain discrete intermediates during the reaction of **1b** with glyoxylate. However, as was the case for other nitrosobenzenes the only significant product observed at any time in the present reaction was the hydroxamic acid **2b**. This single product of the reaction was readily observed in HPLC chromatograms as a peak with identical retention times and peak height ratios to those observed for authentic **2b** (table). The figure illustrates the time-dependent conversion of **1b** to **2b** for a 6-h period, during which time period a close material balance between the starting material (**1b**) and the hydroxamic acid product (**2b**) was observed. A very slight deviation from linearity in the time-dependent formation of **2b** and disappearance of **1b** was accounted for by the tendency of **1b** to volatilize from aqueous solutions, as is typical of many aromatic nitroso compounds[2]. We conclude that *p*-nitronitrosobenzene (**1b**) behaves like other nitroso aromatics towards glyoxylate, and that the nitroso-glyoxylate reaction is a general chemical reaction for aromatic C-nitroso compounds.

1 This study was supported by grant No.CA 32395 from the National Cancer Institute, and by Research Career Development Award ES 00120 from the National Institute of Environmental Health Sciences, DHHS.
2 Corbett, M.D., and Corbett, B.R., J. org. Chem. *45* (1980) 2834.
3 Corbett, M.D., and Corbett, B.R., J. org. Chem. *46* (1981) 466.
4 Corbett, M.D., and Corbett, B.R., unpublished results.
5 McIntyre, J., and Simpson, J.C.E., J. chem. Soc., Lond. *1952* 2606.
6 Kuhn, R., and Weygand, F., Ber. dt. chem. Ges. *69* (1936) 1969.
7 Corbett, M.D., and Chipko, B.R., Analyt. Biochem. *98* (1979) 169.

Microbiological reduction of steroidal ketones using the thermophilic bacterium *Caldariella acidophila*

G. Sodano, A. Trabucco, M. De Rosa and A. Gambacorta

Istituto per la Chimica di Molecole di Interesse Biologico del C.N.R., Via Toiano, 2, Arco Felice, Napoli (Italy), 2 March 1982

Summary. Twelve steroidal ketones have been subjected to reduction with *C. acidophila* resting cells, regio- and stereospecific reduction of the 3-keto groups being observed, as well as reduction of the Δ^4-double bond. The presence of oxo groups at C-11 or C-12 and the presence of hydrophobic side chains on the steroidal molecules inhibit the reduction.

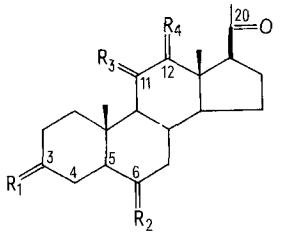
In a previous communication[1] we have reported that the main microbiological transformation induced by MT-4 strain of *Caldariella acidophila* on progesterone (**1**) consists in hydroxylation-oxidation at the allylic carbon (C-6), some reduction products also being isolated.

From these preliminary results it could be predicted that the reduction processes would predominate when a steroidal molecule lacking an available allylic position is used as a substrate. In this report we describe the observed modifications of steroid substrates which have been chosen with the purpose of verifying the practicability of reductions of steroidal ketones with *C. acidophila*.

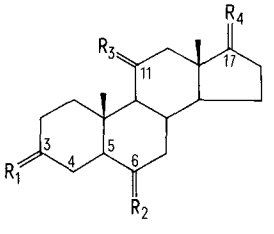
The substrates were incubated with resting cells of *C. acidophila* at 85 °C without agitation for 30 h, as previously described[1], and the metabolites were recovered by extraction of the incubation mixture with chloroform. The extracts were chromatographed on silica gel columns (benzene and increasing amounts of diethyl ether or chloroform and increasing amounts of methaol) and the single products further purified by preparative TLC. The identity of the metabolites was established mainly by the chemical shifts of the angular methyl groups and the chemical shifts and coupling constants of the C*H*OH protons in the ^{1}H NMR-spectra[2,3], by mass spectroscopy[4] and by comparison with authentic samples, when possible. The results are reported in the table and can be summarized as follows.

When carbonyl reduction occurs, mainly 3β-alcohols are formed (substrates **2**, **4**, **7**), indicating the presence in the bacterium of a constitutive oxo-steroid reductase activity which allows a regio- and stereo-specific reduction of steroidal polyketones.

Compounds containing carbonyl groups at C-11 or C-12



1, $R_1=0$; $R_2=R_3=R_4=H_2$; Δ^4;
2, $R_1=R_2=0$; $R_3=R_4=H_2$;
3, $R_1=H$, βOH; $R_2=0$; $R_3=R_4=H_2$;
7, $R_1=R_3=0$; $R_2=R_4=H_2$;
8, $R_1=H$, βOH; $R_2=R_4=H_2$; $R_3=0$;
9, $R_1=R_4=0$; $R_2=R_3=H_2$; 5βH;
12, $R_1=0$; $R_2=H$, βOH; $R_3=R_4=H_2$; Δ^4;
13, $R_1=R_2=0$; $R_3=R_4=H_2$; Δ^4;
16, $R_1=H$, βOH; $R_2=0$; $R_3=R_4=H_2$; Δ^4;



4, $R_1=R_4=0$; $R_2=R_3=H_2$;
5, $R_1=H$, βOH; $R_2=R_3=H_2$; $R_4=0$;
6, $R_1=R_4=H$, βOH; $R_2=R_3=H_2$;
10, $R_1=R_3=R_4=0$; $R_2=H_2$; Δ^4;
11, $R_1=R_3=R_4=0$; $R_2=H_2$; Δ^1; Δ^4;
14, $R_1=R_4=0$; $R_2=H$, βOH; $R_3=H_2$; Δ^4;
15, $R_1=R_2=R_4=0$; $R_3=H_2$;
17, $R_1=R_2=R_4=0$; $R_3=H_2$; Δ^4;