COMMUNICATION

The first electrochemical study of epidithiopiperazine-2,5-diones, a special class of α,α′-disulfide bridged cyclic dipeptides

Christina L. L. Chai,*[a] Graham A. Heath,[b] Paul B. Huleatt[a] and Gavin A. O'Shea[a]

[a] *Department of Chemistry, The Faculties, Australian National University, ACT 0200*
[b] *Research School of Chemistry, Institute of Advanced Studies, Australian National University, ACT 0200*

Received (in Cambridge) 26th November 1998, Accepted 28th January 1999

Polarographic studies of 3,6-epidithiopiperazine-2,5-diones (ETP) including naturally occurring gliotoxin and simple synthetic analogues are pertinent to understanding their biological action; coulometric measurements on 1,4-diethyl ETP in acetonitrile establish the existence of a ready *one-electron* reduction overall, in sharp contrast to the familiar two-electron cleavage of acyclic disulfides.

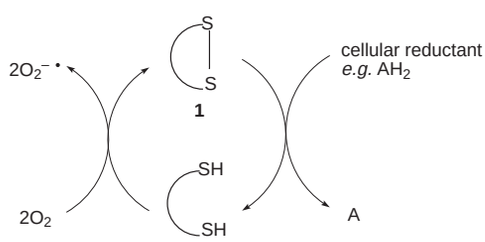
Epidithiopiperazine-2,5-diones (abbreviated as ETP) **1** are an important class of biologically active compounds.[1] The fungal metabolite gliotoxin **2** displays antitumor, antiviral and immunosuppressive properties whereas sporidesmin **3** is the causative agent of facial eczema in ruminants. Comparable patterns of activity persist in certain simple synthetic analogues such as 1,4-dimethyl and 1,4-diethyl ETP.[2,3] One proposal[4] to account for this behaviour envisages redox cycling of the disulfide to the dithiol piperazine-2,5-dione, in a 2-electron/2H$^+$ process coupled with a cellular reductant such as glutathione (see Scheme 1). However little is known directly of the redox properties of this class of compounds so even an empirical correlation with biological activity remains to be established. As part of a broad investigation of the mode of action of epidithiopiperazine-2,5-diones,[3] we now report the voltammetric behaviour of gliotoxin (**2**), selected synthetic analogues (**4**–**7**) and a structural variant (**8**). Epidithiopiperazine-2,5-diones **4**–**7** were prepared by modifications of known procedures[2,5] and 1,4-diacetylcystine anhydride (**8**) by acetylating cystine anhydride.[6] These studies suggest that the bridgehead disulfide moiety in ETP compounds is exceptionally easy to reduce, and that one-electron reduction is a special property of ETP compounds. To our knowledge, this is the first reported electrochemical study of this particular class of disulfides.

4 $R^1 = R^2 = Et$
5 $R^1 = Bn$, $R^2 = Me$
6 $R^1 = R^2 = Bn$
7 $R^1 = R^2 = p$-MeOBn

Table 1 Redox properties of cyclic disulfides at the dropping mercury electrode[a,b]

Compound	Peak 1/*V*	Peak 2/*V*
Ferrocene (internal reference)	+0.55	
1,4-Diethyl ETP (**4**)	−0.38	−0.60
1-Benzyl-4-methyl ETP (**5**)	−0.39	−0.53
1,4-Dibenzyl ETP (**6**)	−0.43	−0.53
1,4-Di(*p*-methoxybenzyl) ETP (**7**)	−0.50	−0.58
Gliotoxin (**2**)[c]	−0.45	−0.52 (shoulder)
1,4-Diacetylcystine anhydride (**8**)	not observed	not observed

[a] $E_{1/2}$ values *vs.* Ag/AgCl/Cl$^-$ in acetonitrile, recorded as ac peak potentials. [b] Polarographic conditions: scan rate 10 mV s^{-1}, drop time 0.5 s, ac frequency 205 Hz. [c] Gliotoxin has dc $E_{1/2}$ values of −0.37 and −0.48 V, with a strong broad maximum at −0.9 V.



Scheme 1

Reduction of the synthetic ETP compounds **4**–**7** in acetonitrile takes place readily on mercury, between *ca.* −0.4 to −0.6 V, although a complex wave with two components is observed in each case. Typical ac and dc polarograms are shown in Fig. 1. Rather than the two components representing successive one-electron steps, detailed examination established one-electron reduction of the ETP compound *overall* (see below). The shape of the first feature suggests specific adsorption of the ETP compounds **4**–**7** on the negatively polarised electrode. For gliotoxin (**2**), the first two cathodic processes are similar to those of the synthetic ETP compounds, though the ac peaks are not fully resolved. Gliotoxin also displays a strong broad polarographic maximum at *ca.* −0.9 V, apparently unrelated to its primary reduction. In contrast to all the ETP compounds, no reduction of 1,4-diacetylcystine anhydride (**8**) is observed within the scan limit (−2.0 V) under these conditions.

The choice of acetonitrile enabled us to compare a range of simple organosoluble derivatives and to explore the partici-

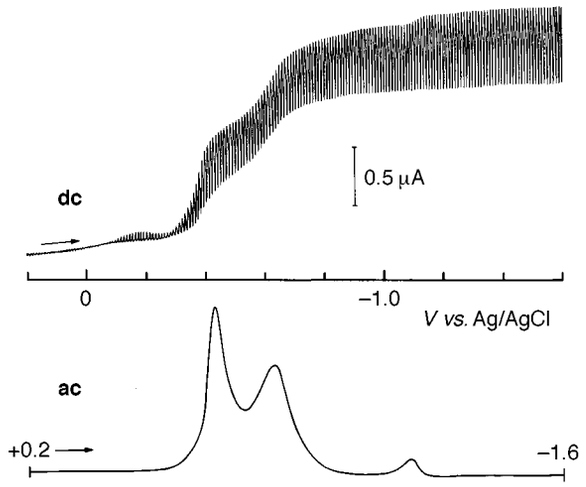


Fig. 1 Ac and dc polarograms of 3,6-epidithio-1,4-diethylpiperazine-2,5-dione (**4**).

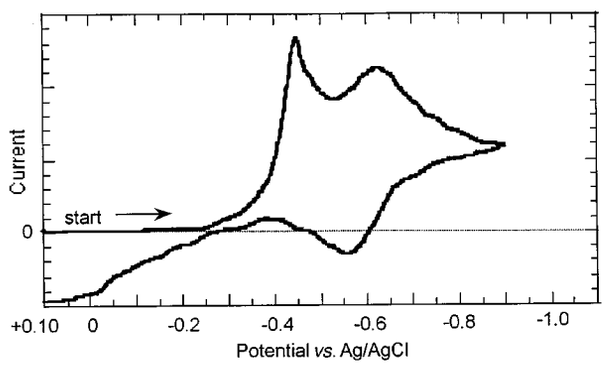


Fig. 2 CV of 3,6-epidithio-1,4-diethylpiperazine-2,5-dione (**4**) (scan rate of 500 mV s^{-1}).

pation of water. These conditions may mimic the biological environment of ETP compounds as there is much evidence that ETP compounds are readily taken up by cells.[7] Progressive addition of water (up to 2% by volume, *i.e. ca.* 100 × the concentration of ETP) did not greatly affect the observations or undermine the pattern emerging in Table 1. Further work should be directed to the comparison of *i–E* curves of **4**–**7** in aqueous media and to developing water soluble analogues.

The reduction of 1,4-diethyl ETP (**4**) on the dropping mercury electrode was examined in greater detail. The impression that wave 1 is due to adsorption was reinforced by concentration-dependence studies and by the observation that low levels of added Li^+ completely suppress the first component in both ac and dc scans, leaving component 2 unchanged. Cyclic voltammetric experiments on ETP **4** at a stationary mercury electrode (hanging mercury drop electrode, HMDE) confirm that component 1 is tensammetric in nature, while component 2 represents a reversible Faradaic reduction (Fig. 2). This pattern of behaviour (including inhibition by cations) is in accord with specific adsorption of the ETP compound on the negatively polarised electrode surface (in the −0.3 to −0.5 V domain).[8] This, along with observations on the shape of the curves,† suggests that the first wave is characteristic of an adsorption phenomenon, whereas the second wave is that resulting from a 'true' reduction process. In accord with this, attempted coulometry at the Hg pool polarised at −0.5 V leads to complete current decay within seconds with very obvious simultaneous development of a dark, apparently insulating patina on the mercury surface. The adsorption of disulfides and diselenides onto mercury electrodes is widely documented, though there is no consensus on the chemical formulation of the adsorbed species.[9]

Coulometry of **4** at a Hg-pool polarised at −1.1 V, *i.e.* well beyond the second step in the overall reduction, consumes only one Faraday per mole of ETP compound. This unexpected outcome is consistent with initial formation of a radical anion disulfide species (as opposed to the more obvious RSSR/2 × RSH couple). Although no definitive evidence was obtained to support this formulation, a persistent orange–red colour of the homogeneous electrolysed solution was observed. This sensitive solution was immediately decolourised on exposure to air. Pulse radiolysis studies on a number of dialkyl disulfides have shown that $[RSSR]^{-\bullet}$ species can be generated and characterised spectrophotometrically by broad absorption maxima at *ca.* 410 nm.[10] In particular, the cyclic disulfide radical anion of lipoic acid has a lifetime of the order of 0.1 millisecond;[11] the present ETP structure is much more conducive to achieving the >100-fold increase in lifetime implicit in our reversible ac polarography and CV results. Polarographic analysis after bulk electrolysis revealed the disappearance of the original ETP cathodic processes, and the emergence of a new anodic wave of comparable height near +0.1 V. The oxidation of *cis*-1,4-diethyl-3,6-dithiolpiperazine-2,5-dione (the dithiol related to ETP **4**) was measured separately and was found to occur at +0.65 V at the dropping mercury electrode.

Accordingly, the ETP compounds described here are reduced more readily than simple dialkyl disulfides which generally display a two-electron polarographic process well beyond −1.0 V.[12] This may reflect the atypical conformation and more strained S–S bond in ETP compounds[13] compared to acyclic analogues (S–S bond length 2.08 Å *vs.* 2.04 Å; CSSC dihedral angle of *ca.* 10° *vs.* 90°). The cyclic disulfide system of **8**, by contrast, is relatively normal in conformation (for the parent cystine anhydride, S–S bond length 2.00 Å; CSSC dihedral angle 90°).[14] It appears that the inherent constraint on the ETP disulfide bridge has two (exquisitely balanced) consequences *i.e.* to weaken the bond by ~0.04 Å such that the σ* level is lowered in energy, making formation of the hemi-bonded disulfide radical more accessible, and to protect the (albeit weak) hemi bond from heterolytic cleavage, or further reduction, by the enforced apposition of the neighbouring atoms. This suggests that the original $2e/2H^+$ redox cycling scheme (Scheme 1) deserves further consideration, with possible participation at a microscopic level of a one electron process.

These results illustrate the practical utility of the Hg electrode in eliciting an electro-analytical response for ETP derivatives, despite the attendant complications which are often encountered in the polarography of organo-thio compounds.[9,15] The present studies indicate that the reduction processes for these five ETP compounds are broadly similar to one another, notwithstanding the more marked biological activity of gliotoxin itself.[3] There is room to believe that redox cycling is fundamental to their common mode of action, while attendant molecular features of **2** *vs.* **4**–**7** lend selectivity to gliotoxin's role in the physiological cycle.

Experimental

Polarographic and coulometric experiments were performed using a PAR 170 Electrochemistry System coupled to Metrohm 505 and 663 electrode stands using a Pt-wire counter electrode, a Ag/AgCl reference electrode, and tetrabutylammonium tetrafluoroborate (recrystallized *ex* methanol) as the supporting electrolyte in acetonitrile. The purified solvent was distilled from calcium hydride under nitrogen, immediately before use. Typical experiments utilised 2×10^{-3} M solution of the ETP derivative in 10 ml of CH_3CN–Bu_4NBF_4 (0.1 M) electrolyte, stringently purged of oxygen. Polarograms were recorded in the range 0.0 V to −2.0 V (*vs.* Ag/AgCl) and also calibrated with ferrocene. Multiple coulometric measurements on 25 mg of ETP **4** yielded concordant values of 1.0 Faraday per mole.

Acknowledgements

The authors thank Dr P. Waring for the gift of gliotoxin and the Australian Research Council for financial support of this project.

Notes and references

† We thank a referee for encouraging us to explore this issue further.

1 A. Taylor, in *Microbial Toxins*, ed. S. Kadis, A. Ciegler and S. Ajl, Academic Press, New York, 1971, Part VII, p. 337–371; T. W. Jordan and S. J. Cordiner, *Trends Pharmacol. Sci.*, 1987, **8**, 144; P. G. Sammes, *Fortschr. Chem. Org. Naturst.*, 1975, **32**, 51; P. Waring, R. D. Eichner and A. Müllbacher, *Med. Res. Rev.*, 1988, **8**, 499; P. Waring and J. Beaver, *Gen. Pharmacol.*, 1996, **27**, 1311.

2 P. W. Trown, *Biochem. Biophys. Res. Commun.*, 1968, **33**, 402.

3 A. M. Hurne, J. Simpson, P. Waring and C. L. L. Chai, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2645.

4 R. Munday, *J. Appl. Toxicol.*, 1987, **7**, 17.

5 H. Poisel and U. Schmidt, *Angew. Chem., Int. Ed. Engl.*, 1971, **10**, 130.

6 B. Kamber, *Helv. Chim. Acta*, 1971, **54**, 927.

7 P. Waring, N. Newcombe, M. Edel, Q. H. Lin, H. Jiang, A. Sjaarda, T. Piva and A. Müllbacher, *Toxicon.*, 1994, **32**, 491.

8 B. Breyer and H. H. Bauer, in *Chemical Analysis*, New York, eds. P. J. Elving and I. M. Kolthoff, Interscience Publishers, 1963, vol. 8.

9 B. Nygård, *Ark. Kemi*, 1967, **28**, 75; B. Nygård, *Ark. Kemi*, 1967, **28**, 89; B. Nygård and L. Schotte, *Acta Chem. Scand.*, 1956, **10**, 469; J. Ludvík and B. Nygård, *J. Electroanal. Chem.*, 1997, **423**, 1.

10 R. L. Wilson, *J. Chem. Soc., Chem. Commun.*, 1970, 1425.

11 K.-D. Asmus, in *Methods in Enzymology*, Academic Press, New York, eds. L. Packer and A. N. Glazer, 1990, vol. 186, p. 168; M. Z. Hoffman and E. Hayon, *J. Am. Chem. Soc.*, 1972, **94**, 7950.

12 See for example M. E. Hall, *Anal. Chem.*, 1953, **25**, 556.

13 J. Fridrichsons and A. McL. Mathieson, *Acta Crystallogr.*, 1965, **18**, 1043; J. Fridrichsons and A. McL. Mathieson, *Acta Crystallogr.*, 1967, **23**, 439.

14 H. Mez, *Cryst. Struct. Commun.*, 1974, **3**, 657.

15 For early examples, see I. M. Kolthoff and C. Barnum, *J. Am. Chem. Soc.*, 1941, **63**, 520; D. G. Davis and E. Bianco, *J. Electroanal. Chem.*, 1966, **12**, 254.

Communication 8/09266D