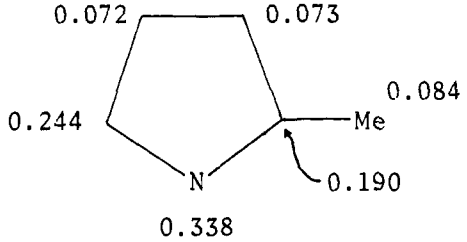
1,2-dimethylpyrrole cation radical with the nucleophile is considered, there are two initial possibilities. Firstly, the anodically generated cation radical **1** is attacked by the cyanide ion to produce the radical **2,** followed by further anodic oxidation and successive proton release, thus leading to the aromatic cyanation product. Alternatively, **1** could undergo deprotonation to afford an analogue of a benzylic radical intermediate **3,** which would subsequently undergo anodic oxidation to give a cation **4,** followed by nucleophilic attack by cyanide ion to give 5-methylene-1-methyl-3-pyrroline-2-carbonitrile (**5,** Scheme I) which should be eventually aromatized in protic solvents. Indeed, the compound of the type of **5** would be important as a reaction intermediate. 5-Methylene-2,5-dihydro-2-furonitrile is a primary product of abnormal product formation in the reaction of 2-(chloromethyl)furan with aqueous cyanide solution.[4] The reaction of 2-dimethylaminomethyl-1-methylpyrrole methiodide with sodium cyanide in water[5] gave rise to 1,2-dimethylpyrrole-5-carbonitrile (**6**) as well as 1-methylpyrrole-2-acetonitrile (1:6). To distinguish these two possibilities, anodic oxidation of 1,2-dimethylpyrrole was examined in the CH_3OD–NaCN system. Incorporation of deuterium in the 2-methyl group of compound **6,** 1,2-dimethylpyrrole-5-carbonitrile-*2-d,* was not observed (mass and NMR spectroscopies). Trace amounts of 1-methylpyrrole-2-acetonitrile were detected. Therefore, the latter mechanism is not so important for this reaction ($k_a \gg k_e$).

The current efficiency for these reactions was 60% or so and the remainder of the current would consumed with side reactions. Anodically generated cationic species chemically oxidize cyanide ion to regenerate the parent neutral substrate or the radical **2** and produce cyano radical, which might attack the coexisting cyanide ion to form cyanogen anion radical or dimerize to cyanogen.

In order to evaluate qualitatively the distribution of the positive charge in the 1,2-dimethylpyrrole cation radical, a MO calculation by the ω technique was carried out.[6] These data showed a relatively high charge density at position 5, thus supporting the observed reactivity at this position.



Acknowledgment. This work was supported by a grant from the Ministry of Education.

References and Notes

(1) (a) H. R. Snyder and E. L. Eliel, *J. Am. Chem. Soc.*, **70,** 1857 (1948); (b) W. Herz and J. L. Rogers, *ibid.*, **73,** 4921 (1951); (c) H. J. Anderson, *Can. J. Chem.*, **37,** 2053 (1959); (d) P. Fournari, *Bull. Soc. Chim. Fr.*, 488 (1963); (e) T. F. Spande, A. Fontana, and B. Witkop, *J. Am. Chem. Soc.*, **91,** 6199 (1969); (f) M. Artico, R. Giuliano, G. C. Porretta, and M. Scalzo, *Farmaco, Ed. Sci.*, **27,** 60 (1972); (g) J. K. Chakrabarti and T. M. Hotten, *J. Chem. Soc., Chem. Commun.*, 1226 (1972); (h) E. E. Ryskiewicz and R. T. Silverstein, *J. Am. Chem. Soc.*, **76,** 5802 (1954).
(2) (a) K. Yoshida and T. Fueno, *Bull. Chem. Soc. Jpn.*, **42,** 2411 (1969); *J. Org. Chem.*, **36,** 1523 (1971); (b) K. Yoshida, T. Saeki, and T. Fueno, *ibid.*, **36,** 3673 (1971); (c) K. Yoshida, *J. Am. Chem. Soc.*, **98,** 254 (1976).
(3) (a) H. R. Snyder and E. L. Eliel, *J. Am. Chem. Soc.*, **70,** 1703 (1948); (b) A. H. Jackson and A. E. Smith, *J. Chem. Soc.*, 5510 (1964); (c) S. Clementi, P. Linda, and G. Marino, *J. Chem. Soc., Chem. Commun.*, 427 (1972).
(4) S. Divald, M. C. Chun, and M. M. Joullié, *J. Org. Chem.*, **41,** 2835 (1976).
(5) W. Herz and J. L. Rogers, *J. Am. Chem. Soc.*, **73,** 4921 (1951).
(6) The parameters used were as follows: $h = 3.0$ and $k = 0.8$ for methyl group, $h = 1.5$ and $k = 1.0$ for pyrrole nitrogen (same for N–H, N–CH_3), $\delta = 0.1$, $\omega = 1.4$ (heteroatom model).

Kunihisa Yoshida

Department of Chemistry, Faculty of Engineering Science
Osaka University, Toyonaka, Osaka 560, Japan

Received April 18, 1977

Novel Phosphonothioate Substrates for Phosphodiesterases

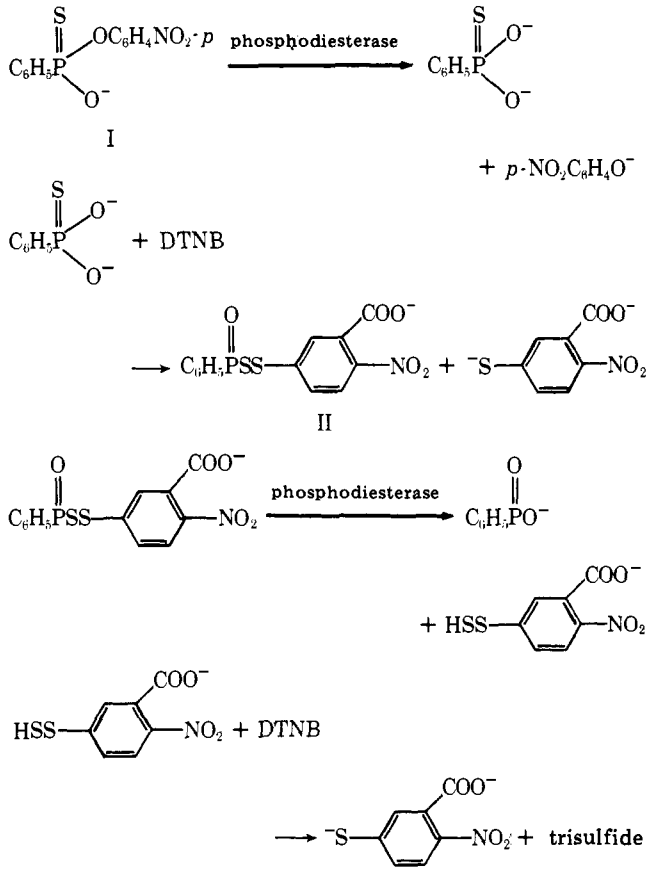
Sir:

Phosphodiesterases of the 5′-nucleotidase type[1] have been found to hydrolyze *O-p*-nitrophenyl phenylphosphonothioate (I). In addition they hydrolyze very rapidly 3-carboxy-4-nitrobenzene phenylphosphonyl disulfide (II), bis(phenylphosphonyl) disulfide (III), and probably 3-carboxy-4-nitrobenzene phenylphosphonyl trisulfide.

Compound I was made by hydrolysis of bis(*O-p*-nitrophenyl) phenylphosphonothioate[2] (10 g) in 1 M KOH (82.4 mL) with CH_3CN (150 mL) with vigorous shaking for 50 min at 25 °C. After removal of CH_3CN the aqueous residue was adjusted to pH 5 with Dowex 50W/H^+, extracted with ether (12 × 25 mL), and then evaporated. Addition of excess aqueous 1 M cyclohexylammonium chloride to a concentrated aqueous solution of I (K^+ salt) yielded the cyclohexylammonium salt of I as an oil which crystallized on cooling. Recrystallization from CH_3CN yielded white needles;[3] the ultraviolet spectrum of this salt had $\lambda_{max}^{H_2O}$ at 292 (ϵ 9560). ^{31}P NMR of the K^+ salt (D_2O) showed one peak (δ −68.4); ^1H NMR of the same solution showed the presence of one phenyl group (multiplet, δ 7.54) per nitrophenyl ester group (quartet, δ 7.0–8.1). Hydrolysis of I to phenylphosphonothioate and *p*-nitrophenolate was monitored either spectrophotometrically or by pH stat autotitration with KOH, at pH 8.8. Venom phosphodiesterase from *Crotalus adamanteus* (type II, Sigma Chemical Co.) catalyzed the hydrolysis of I until a measured 49.5% of the anticipated *p*-nitrophenol had been released, after which no further hydrolysis occurred, indicating the high stereospecificity of this enzyme toward the chiral phosphorus center. The 5′-nucleotide phosphodiesterase from bovine gut[1,4] behaved in the same way.

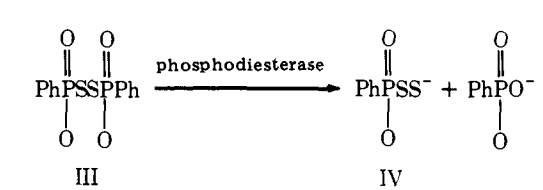
Attempts to oxidize dilute solutions of I and its hydrolysis product, phenylphosphonothioate,[5] with mild oxidants (iodate,

Scheme I



potassium ferricyanide, and 2,2′-dinitro-5,5′-dithiobis(benzoic acid) (DTNB)) showed that only the unesterified phenylphosphonothioate would react. Thus the hydrolysis of I in the presence of DTNB resulted in the release of 3-carboxy-4-nitrothiophenolate which, together with the *p*-nitrophenolate released, was observed at 412 nm. When excess DTNB was added to assays containing I and snake venom phosphodiesterase it was observed that 1 mol of *p*-nitrophenol and 2 mol of 3-carboxy-4-nitrothiophenol were being produced at the same rate. This result suggested that the disulfide II which was produced by the reaction of phenylphosphonothioate and DTNB was being hydrolyzed to phenyl phosphonate and 3-carboxy-4-nitrobenzene hydrogen disulfide, catalyzed by the phosphodiesterase. 3-Carboxy-4-nitrobenzene hydrogen disulfide would then react with DTNB to produce bis(3-carboxy-4-nitrobenzene) trisulfide and a second molecule of 3-carboxy-4-nitrothiophenolate. This idea, illustrated in Scheme I, was tested in two ways. Firstly, snake venom phosphodiesterase and I were incubated in the presence of Mg^{2+} (which is necessary for the enzymatic activity) in Tris buffer, pH 8.8, until hydrolysis had ceased. Sufficient EDTA was then added to chelate all Mg^{2+} and to inhibit the enzyme completely. Following this, excess DTNB was added, and the release of 1.02 mol of 3-carboxy-4-nitrothiophenolate/mol of *p*-nitrophenolate previously released was observed. However, if DTNB was added to the hydrolyzed ester plus enzyme in the absence of EDTA, a very rapid release of 2.00 mol of 3-carboxy-4-nitrothiophenolate/mol of *p*-nitrophenolate occurred. This result demonstrated stepwise the hydrolysis of I followed by the very much faster hydrolysis of II. Secondly, venom phosphodiesterase was added to a mixture already containing phenylphosphonothioate, DTNB, and Mg^{2+}. The formation of compound II which occurred prior to the addition of the enzyme was signaled by the production of 3-carboxy-4-nitrothiophenolate; the hydrolysis of II after addition of enzyme resulted in the further release of 3-carboxy-4-nitrothiophenolate inhibitable by EDTA.

Compound III was prepared by dropping methanolic iodine into a suspension of disodium phenylphosphonothioate[6] (1.09 g in 2 mL of MeOH) at 0 °C until the iodine was no longer decolorized. The white crystalline precipitate was filtered off, washed with MeOH and CCl_4, and recrystallized by adding cold acetone to a cold saturated solution of disulfide in MeOH until the appearance of turbidity and heating to yield silky white needles.[3] The ultraviolet spectrum (H_2O) rose continuously from 290 down, with shoulders at λ 283 (ε 132), 276 (191), and 219 (488). ^{31}P NMR showed one peak (δ −33.69) and ^1H NMR showed multiplets at δ 7.26 (8 H) and 7.47 (2 H). Hydrolysis of III at pH 8.8, catalyzed by snake venom phosphodiesterase with Mg^{2+}, was observed using the pH stat autotitrator; ~1.7 protons were released per molecule of III hydrolyzed. Spectrophotometric assay of this reaction in the presence of DTNB showed that 2.0 mol of 3-carboxy-4-nitrothiophenolate were released/mol of III; this reaction was fully inhibited by EDTA. Reactions in the spectrophotometric assay were envisaged to occur commencing with the hydrolysis of III as shown, followed by formation of the mixed 3-carboxy-4-nitrobenzene phenylphosphonyl trisulfide from IV upon



III IV

reacting with DTNB; this trisulfide apparently is also subject to hydrolysis by phosphodiesterase. An alternative scheme in which the enzyme rapidly hydrolyzes IV is not favored, as this would require the release of at least two protons per molecule of III, assayed at the pH stat. Values of K_m and V_{max} for I, III, and bis(*p*-nitrophenyl) phosphate for one batch of snake venom phosphodiesterase are listed in Table I.

Table I

Substrate	Assay[a]	K_m (mM)	V_{max}[b] (units/mg enzyme)
I	Spectrophotometric	0.775[c]	0.405
III	pH stat	0.592	20.2
Bis(*p*-nitrophenyl) phosphate	Spectrophotometric	1.20	0.889

[a] At pH 8.8, 30 mM Tris (except for pH stat), 30 mM $MgCl_2$, 25 °C. [b] Units are μmol/min. [c] K_m for the reactive enantiomer.

Competition experiments with the venom enzyme at the pH stat showed that bis(*p*-nitrophenyl)phosphate behaved as a competitive inhibitor of hydrolysis of III, with $K_i = 7.95 \times 10^{-4}$ M, very similar to K_m for hydrolysis of the diester. Thus it seems most likely that the phosphodiesterase in the venom preparation which catalyzes the hydrolysis of bis(*p*-nitrophenyl) phosphate is also catalyzing hydrolysis of III. This view is strengthened by the behavior of the bovine gut 5′-nucleotide phosphodiesterase. This enzyme, ~10% pure, catalyzed hydrolysis of I, II, and III, at rates whose ratios were similar to those of the snake venom enzyme. Neither enzyme appeared to catalyze hydrolysis of tetrathionate, a structural analogue of III.

An enzyme present in crude wheat germ acid phosphatase (Sigma, type I) which was shown by inhibition and inactivation studies not to be the acid phosphatase itself, slowly hydrolyzed I at pH 5 (acetate buffer).[7] Enzymes which did not hydrolyze I were bovine spleen phosphodiesterase (Sigma, type I) and bovine heart cyclic phosphodiesterase (Sigma). The hydrolysis of I is therefore catalyzed by the same pattern of enzymes which catalyze hydrolysis of *p*-nitrophenyl phenylphosphonate, enzymes which have been labeled the 5′-nucleotide phosphodiesterases.[1]

These observations expand the range of compounds known to be substrates for phosphodiesterases and establish an enzymatic method for desulfurizing phosphonothioates under gentle conditions; presumably phosphorothioate esters would undergo a parallel desulfurization. Further, the stereochemical course of hydrolysis of optically active I can now be followed by reesterifying the product phenylphosphonothioate (labeled by hydrolysis in [^{18}O]) and resolving the resulting ester using the phosphodiesterase. The ease with which phenylphosphonothioate is oxidized should make this step of value in protecting sulfur during reesterification. We are proceeding with these studies at present.

Acknowledgment. This research was supported by a grant from the National Institutes of Health (GM 13306).

References and Notes

(1) S. J. Kelly, D. E. Dardinger, and L. G. Butler, *Biochemistry*, **14**, 4983 (1975).
(2) Bis(*O*-*p*-nitrophenyl) phenylphosphonothioate was prepared by refluxing phenylphosphonothioic dichloride (9.9 g) with rigorously dried sodium *p*-nitrophenoxide (20 g) in 70 mL of dry ether for 39 h, after which the mixture was filtered, and the precipitate washed with ether (4 × 12 mL). Combination of the washings with the filtrate resulted in crystallization of essentially all the diester (12.97 g). This material, 97.7% pure by alkaline hydrolysis, was used to prepare I. The diester, recrystallized from EtOH, gave chunky needles,[3] creamy white, mp 93–94 °C; the ultraviolet spectrum (H_2O containing 6% EtOH by volume) had λ_{max} 277.6 (ε 21 900); the diester yielded 100.6% expected *p*-nitrophenol on alkaline hydrolysis.
(3) Bis(*O*-*p*-nitrophenyl) phenylphosphonothioate and compounds I and III each gave satisfactory elemental analyses.
(4) Kindly provided by Dr. L. G. Butler, Purdue University, Lafayette, Ind.
(5) Phenylphosphonothioate (Na_2^+ salt) was prepared by the method of D. C. Gray and N. K. Hamer, *J. Chem Soc. B*, 1123 (1970), and crystallized by the

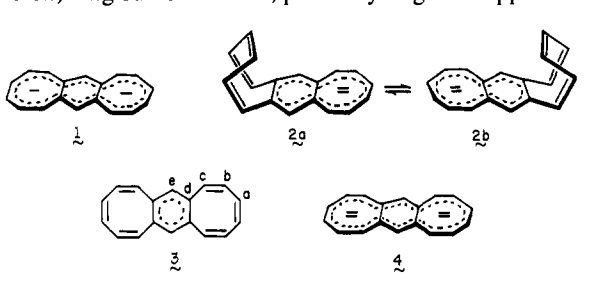
method used for compound III.
(6) Compound III was prepared by a modification of the method of G. Peters, *J. Org. Chem.*, **27**, 2198 (1962).
(7) Purified wheat germ acid phosphatase catalyzes the hydrolysis of the corresponding phosphonate esters as reported by M. E. Hickey, P. P. Waymack, and R. L. Van Etten, *Arch. Biochem. Biophys.*, **172**, 439 (1976).

N. P. Dudman, S. J. Benkovic*
Department of Chemistry
The Pennsylvania State University
University Park, Pennsylvania 16802
Received May 9, 1977

Dicyclooctatetraeno[1,2:4,5]benzene Dianion and Tetraanion. Experimental Assessment of Extended Paratropic vs. Restricted Diatropic π-Electron Delocalization

Sir:

Although our perception of aromatic stabilization and destabilization has been greatly extended in recent years, it is not yet possible to predict on an a priori basis the ground-state electronic character of molecules capable of adopting either a diatropic or paratropic ring current. Systems having two fused $4n$ π rings and therefore $[4n + 2]$ π electrons have commanded the greatest experimental and theoretical scrutiny.[1] In general, such compounds appear not to be strongly stabilized, in keeping with the antiaromatic character of their monocyclic components. For several reasons, one would anticipate that diamagnetic π-electron delocalization should dominate in those molecules where the simpler structural unit is $[4n + 2]$ and the larger $4n$. However, this need not be so if the species is charged, and particularly multiply charged, since repulsive electron-electron interactions must now also be considered. Very little is known about the extent to which these counterproductive energetic factors might be offset.[2] In this communication, we report the preparation and direct observation (by NMR spectroscopy) of the dicyclooctatetraeno-[1,2:4,5]benzene dianion and present a detailed analysis of its structure. Were this system to adopt a paramagnetic (20 π electron) ring current as in **1**, planarity might be approached

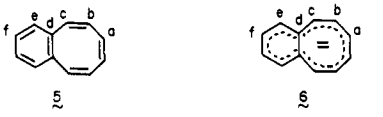


(both eight-membered rings would thereby become equivalent) and negative charge would become distributed over the entire framework at one time. This would suggest that electrostatic repulsion between the pair of electrons is adequate to overcome both the compressional strain energy associated with flattening the medium-sized rings and the "antiaromatic" component of the ring current. On the other hand, the existence of this dianion in two interconverting equivalent nonplanar diamagnetic (14 π electron) forms (**2a** ⇌ **2b**) would indicate that dispersal of negative charge is less important than resonance stabilization.

For completeness, we have also examined the 4-electron reduction of neutral parent hydrocarbon **3**[3] to tetraanion **4**, the most highly charged analogue of anthracene presently known.

Initially, the electrochemical behavior of **3** in anhydrous HMPA (vacuum line conditions; oxygen and moisture excluded) was discovered to differ meaningfully in several ways from that of closely related benzocyclooctatetraenes (Table I). Thus, not only is **3** uniquely reduced with greater facility than the parent COT (by 135 mV), but it is the only system which exhibits full chemical reversibility (cyclic voltammetry experiments) at 100-mV/s scan rates (I_{pa}/I_{pc} = 100%). Secondly, because the diffusion current constant (I_p) exhibited by **3** is nearly twice that of COT, rarely precedented[2,5,6] synchronous 2ϵ uptake or closely spaced transfer of two single electrons is occurring.[7] As expected, additional reduction of the dianionic species to the tetraanion was not seen prior to onset of solvent breakdown. Like COT itself, electron transfer to **3** is non-Nernstian as indicated by the scan rate dependency of the peak current constant.[7,8] These characteristics serve to securely classify **3** as a molecule particularly susceptible to dianion formation.[9]

Generation of $\mathbf{3}^{2-}$ either by electrochemical methods or by exposure to Na-K alloy in anhydrous THF-d_8 produced stable dark emerald green solutions displaying ^{1}H NMR signals at δ 6.67 (s, 2), 5.57–5.34 (m, 4), and 4.93 (narrow m, 8).[10] Comparison of this spectrum with that of neutral **3** in THF-d_8 (6.49 (s, H_e), 6.41 (d, J = 12 Hz, H_c), 5.92 (dd, J = 12 and 2 Hz, H_b), and 5.80 (d, J = 2 Hz, H_a)) reveals the benzenoid protons to be downfield shifted ($\Delta\delta$ = −0.18 ppm) but H_a–H_c to be substantially shielded ($\Delta\delta$ = +0.87, 0.99, and 0.84–1.07 ppm, respectively). These characteristics differ from those observed for benzo-COT[11] (**5**, $\delta_{TMS}^{THF\text{-}d_8}$ 7.13 (dd, 2, H_f),



6.90 (dd, 2, H_e), 6.51 (d, J = 12 Hz, H_c), 5.94 (dd, J = 12 and 2 Hz, H_b), and 5.82 (d, J = 2 Hz, H_a)) and its well-delocalized 14 π electron dianion[11,12] (**6**, $\delta_{TMS}^{THF\text{-}d_8}$ 7.74 (dd, H_e), 6.70 (d, H_c), and 6.41–5.84 (m, H_a, H_b, and H_f)). Here the realization of a diamagnetic ring current more than cancels the "charge effect"[13] and causes all protons except H_f (now additionally shielded owing to the increase in π-electron density

Table I. Cyclic Voltammetry of Several Benzocyclooctatetraenes at a Platinum Electrode in Anhydrous Hexamethylphosphoramide (0.1 M in $(n\text{-Bu})_4N^+ClO_4^-$)[a]

Compd	$E_{1/2}$[b]	$E_{1/2}^1 - E_{1/2}^1$ (COT), mV	$E_{C_1} - E_{A_1}$, mV	$E_{C_2} - E_{A_2}$, mV	I_P	n_{app}
COT	−1.606		118[c,d]	60	0.42	1
	−1.921				0.40[e,f]	1
Benzo-COT (**5**)	−1.724	−118	64[c,d]	54[e]	0.50	1
	−1.948				0.30[g]	1
sym-Dibenzo-COT	−1.896	−290	58[d,e]	64[e]	0.45	1
	−2.016		—		0.43[g]	1
3	−1.471	+135	60[c,d]		0.73	2

[a] Reference 4. [b] In volts vs. SCE. [c] At 100-mV scan rate. [d] Scan rate dependent. [e] Scan rate independent. [f] I_p measured as I_{pA_2}, but second cathodic wave is scan independent. [g] Measured as second anodic wave at 100 mV s^{-1}.