very broad resonance located approximately 30 c.p.s. downfield from the water resonance. The intensity of this resonance suggests that about 60% of these protons have undergone exchange during the time required by sample preparation and recording of the spectrum.

The two α-methylene hydrogens of the glycine ring exhibit a triplet at 62 c.p.s. upfield from the water resonance, which has side bands of approximately one-third the intensity one normally expects. This splitting is due to those protons on the adjacent nitrogen atom. In the alanine complex, $[Co(en)_2(alanine)](NO_3)_2$, the single α-hydrogen exhibits a poorly defined quartet centered at the same location, and the methyl resonance is split into a doublet centered at 180 c.p.s. upfield with a splitting of 7 c.p.s. Second-order splitting is evident as the components of the doublet exhibit some additional splitting. The poor definition of the quartet at 62 c.p.s. (split by the three methyl hydrogen atoms) probably results from the same cause and also because of interaction with the protons of the nitrogen.

The addition of a drop of 2 *N* sodium hydroxide in D_2O, making the solution barely alkaline to litmus, causes the hydrogens on the nitrogen atom to undergo exchange but, of particular interest, brings about rapid exchange of the α-hydrogens. The weak triplet in the glycine complex and the quartet in the alanine, both at 62 c.p.s. upfield, disappear as do the resonances ascribed to the protons attached to the nitrogen atoms, at 30 c.p.s. downfield. In the alanine complex the methyl doublet collapses to a singlet, but in both cases the en ring hydrogens remain unexchanged. These observations serve as dramatic evidence of the electron-withdrawing action of the central metal ion upon the chelate ring to enhance the lability, as well as the acidity, of the α-methylene hydrogens. Observations of a similar but more subtle nature have been made on Co(EDTA)$^-$.

Weakleim and Hoard[4] have determined the structure of the Co(EDTA)$^-$ ion in two of its salts, and the conformations of the chelate rings have been considered earlier.[5] The glycine-like chelate rings in octahedral EDTA complexes exist as two distinct pairs of rings. These are most readily identified as mutually planar with the ethylenediamine chelate ring, the *in-plane* pair, and as not in that plane, the *out-of-plane* pair of rings.[6] Day and Reilley[7] correctly identified AB patterns with the methylene hydrogen atoms of each of these kinds of glycine-like chelate rings and we have independently achieved complete assignment of the p.m.r. spectrum of CoEDTA$^-$.[8] The two AB patterns are centered at δ_{av} = 39 and 49 c.p.s. *vs.* H_2O at 22°. The AB pattern occurring at lower fields is assigned on the basis of its relative position in the spectrum and because of the environmental distinction between the two hydrogen atoms of the *out-of-plane* chelate rings. This is required by the fact that the *out-of-plane* rings are virtually strain free while the *in-plane* rings suffer severe valence angle distortions and substantial elongation of the Co–O bonds.

When solutions of Co(EDTA)$^-$ in D_2O are carefully made basic and heated gently, the downfield AB pattern (*out-of-plane* rings) slowly decreases in intensity. After relatively long periods of time, the second AB pattern (*in-plane* rings) also shows a measurable diminution in intensity. Confirmation of the nature of the process has been provided by observing the appearance of the AB patterns when ethylenediaminetetraperdeuterioacetatocobaltate(III) is allowed to undergo exchange with H_2O.

Although present data are semiquantitative at best, it can be concluded that the rates of exchange of the hydrogen atoms on the two kinds of glycine-like chelate rings are quite different. Since all the acetato functions of the free ligand are equivalent, it is seen that the metal ion that coordinates to EDTA in an octahedral manner may serve both to activate and to bring a sharp chemical distinction between pairs of these groups. The effect is at once stereochemical and electronic. It again serves to illustrate the decisive, very easily understood, yet kaleidoscopically varied effects that metal ions can exert on the properties and reactivities of molecules and ions that may serve as ligands.

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Phenyldiimide

Sir:

Phenyldiimide (**1**) has been postulated as an intermediate in the oxidation of phenylhydrazine,[1] the hydrolysis of benzoylphenyldiimide, and the decarboxylation of phenylazoformic acid.[2] The sodium salt of **1** has been claimed as the product of the reaction of sodium amide and phenylazoformic acid amide,[3] but no further report on this work has appeared.

We have now detected **1** as a somewhat unstable intermediate in the decarboxylation of phenylazoformic acid anion (**2**) according to eq. 1.

$$\underset{\mathbf{2}}{C_6H_5N{=}{=}NCOO^-} + H^+ \longrightarrow CO_2 + \underset{\mathbf{1}}{C_6H_5N{=}{=}NH}\ (cis \text{ or } trans) \quad (1)$$

The anion **2** decomposes at a rate linear in hydrogen ion over the pH range 10.5–12.0 in a first-order process. The rate constant is independent of the concentration of **2** and only somewhat sensitive to ionic strength (see Figure 1). The rates are followed at 2830 Å. (the maximum for **2**) with samples taken from *oxygen-free* solutions, quenched in 1.2 *N* sodium hydroxide, and extracted with *n*-heptane to remove strongly absorbing products.

A degassed solution of **2** in 0.6 *M* sodium hydroxide ($0.9–1.6 \times 10^{-2}$ *M*, 50 μl.) is mixed with 5.0 ml. of degassed phosphate buffer, pH 7.10, in an all-glass system fitted with a quartz cell. Recording of spectra

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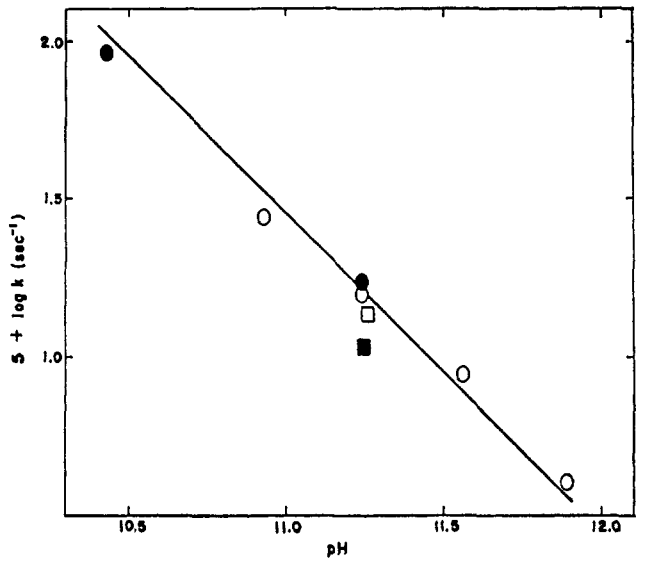

Figure 1. Plot of log k *vs.* pH. Straight line was drawn so that d log k/dpH = −1. ○, phosphate buffer, ionic strength 0.18–0.20; $C_6H_5N{=}NCOO^-$, 2.2–2.4 × 10^{-4} *M*; ●, carbonate buffer, ionic strength 0.18–0.20; $C_6H_5N{=}NCOO^-$, 2.2–2.4 × 10^{-4} *M*; □, phosphate buffer, ionic strength 0.45; $C_6H_5N{=}NCOO^-$, 2.4 × 10^{-4} *M*; ■, phosphate buffer, ionic strength 4.91; $C_6H_5N{=}NCOO^-$, 2.6 × 10^{-4} and 2.1 × 10^{-2} *M*.

(at 25° with a Cary Model 14 spectrophotometer) is begun within 30–70 sec. after mixing.

The half-life of **2** at the pH of the reaction solution (7.34) can be estimated from the figure as about 0.5 sec. Three new maxima are observed: 2140 (ϵ ~10,000), 2700 (ϵ ~7000), and 4000 Å. (ϵ ~160). The latter two correspond quite well to the expected $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions for a molecule like **1**.[4] The new absorptions decrease with a half-life of approximately 80,000 sec. The same phenomena are observed with more difficulty at pH 9.10 since the intermediate disappears at a much greater rate, $t_{1/2}$ *ca.* 1000 sec. Base-catalyzed decomposition of phenyldiimide is thus implied (eq. 2), as suggested by Cram and Bradshaw[5] for alkyldiimides, and in accord with the formation of 2-bromophenyl anion through the action of ethoxide ion on ethyl 2-bromophenylazoformate in ethanol.[6] In fact, **1** is completely destroyed within 100 sec. on raising the pH from 7.34 to 13.7.

$$C_6H_5N{=}NH + OH^- \longrightarrow HOH + [C_6H_5N{=}N^-] \longrightarrow \text{products} \quad (2)$$

The chief products of the decomposition of **1** are benzene, azobenzene, and hydrazobenzene. Oxygen reacts rapidly with **1**. In the presence of oxygen, **1** is not observed to form from **2**.

Many questions of interest may now be studied directly with solutions of phenyldiimide. The preparation and reactions of other aryldiimides and possibly alkyldiimides are being actively pursued. Phenyldiimide has been implicated as the compound responsible for the loss of glutathione in red blood cells treated with phenylhydrazine, acetylphenylhydrazine, and methyl phenylazoformate, a finding which throws light on the mechanism of drug-induced hemolysis and which might be useful in the design of antimalarial drugs.[7]

(4) Benzeneazomethane, $C_6H_5N{=}NCH_3$, has been reported to have λ_{max} 2605 Å. (ϵ 7800, ethanol); λ_{max} 4035 Å. (ϵ 87, hexane) [A. Burawoy, *J. Chem. Soc.*, 1177 (1939)].

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A Synthesis of Cyclic Peptides Utilizing High Molecular Weight Carriers

Sir:

Cyclic peptides are usually prepared from linear peptides by intramolecular cyclization. The carboxylic end of the peptide is as a rule activated by the formation of active esters, anhydrides, azides, or chlorides, and the free terminal amino end allowed to react with the active terminal carbonyl group at high dilution.[1-4] Because of intermolecular condensation occurring even under these conditions, linear oligopeptides are formed in addition to the desired cyclic peptide. The techniques available so far thus lead to reaction mixtures from which cyclic peptides are usually isolated only in relatively low yields. In the following we report on the development of a new method for the synthesis of cyclic peptides in which high molecular weight peptide active esters of type II (see Figure 1), in which the peptide is bound to a high molecular weight polyalcohol carrier, are used as intermediates. When insoluble esters of this type are employed condensation between the activated peptide moieties is suppressed, and internal aminolysis leads to the formation of the desired cyclic peptide (IV) which is released from the insoluble polyhydroxy carrier (III). Intermolecular condensation might be expected to be markedly reduced even when soluble high molecular weight peptide esters of type II are used.

Two high molecular weight poly(nitrophenol) derivatives have been used in the preparation of the peptide active esters: cross-linked poly-4-hydroxy-3-nitrostyrene (IIIa) and a branched copolymer of DL-lysine and 3-nitro-L-tyrosine (IIIb) in which free amino groups have been blocked by acetylation. The former has been prepared according to the literature[5] and is insoluble in the usual organic solvents. The latter has been prepared by total acetylation, with acetic anhydride, of a branched copolymer of DL-lysine and L-tyrosine (molar residue ratio 3:1),[6] removal of the O-acetyl groups in alkali, and nitration in concentrated nitric acid at 0°. IIIb is insoluble in dioxane, ether, and acetone, but is soluble in dimethylformamide (DMF),

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