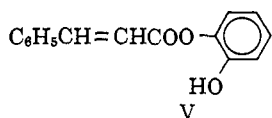


$$v = k'\alpha[\text{phenol}] = \frac{k'K_N}{K}[\text{phenolate}^-]$$

where K is the dissociation constant of the phenol, α is the fraction of the nucleophile in the anionic form, and K_N is the dissociation constant of the nucleophile, *viz.*

$$K_N = [\text{H}^+][\text{N}^-]/[\text{HN}] \cong [\text{H}^+][\text{N}^-]/[\text{N}]_{\text{total}}$$

Experiments are now in progress to distinguish between these two possible rate enhancement mechanisms. Hydroxyl groups, quite remote from a susceptible bond, when properly oriented can cooperate with a vicinal peptide group to very markedly enhance the reactivity. In some enzymes, such as pepsin⁹ and carboxypeptidase,¹⁰ where tyrosine groups are involved in the activity, the role of the phenolic group may be related to one or the other of the above-mentioned mechanisms. It should follow that the esters as well as imides of the *o*-OH phenyl derivatives should exhibit enhanced reactivity. The hydrolysis of monocinnamoyl catechol (V) was followed spectrophotometric-



ally utilizing the change in optical density at 3000 Å. accompanying the hydrolytic reaction. The second-order specific rate of OH⁻ catalyzed hydrolysis is $2 \times 10^4 M^{-1} \text{min.}^{-1}$ near neutral pH. This is 300 times greater than the specific rate of phenyl cinnamate hydrolysis; another manifestation of the marked effect of an adjacent hydroxyl on reactivity. Similar results have been recently reported for monoacetylcatechol.¹¹

A detailed report of these kinetic results, and of their potential pertinence to specific examples of enzymic catalysis, will be presented shortly.

Acknowledgment.—This work was supported by grants from the National Science Foundation and the Public Health Service.

(9) R. Herriott, *J. Gen. Physiol.*, **19**, 283 (1935).

(10) R. T. Simpson, J. F. Riordan, and B. L. Vallee, *Biochemistry*, **2**, 616 (1963).

(11) B. Hansen, *Acta Chem. Scand.*, **17**, 1375 (1963).

(12) On leave of absence from the Department of Biophysics, The Weizmann Institute of Science, Rehovot, Israel.

INSTITUTE OF MOLECULAR BIOLOGY AND DEPARTMENT OF CHEMISTRY
UNIVERSITY OF OREGON
EUGENE, OREGON

YECHIEL SHALITIN¹²
SIDNEY A. BERNHARD

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Neighboring Group Effects on Ester Hydrolysis. II. Neighboring Carbonyl Groups

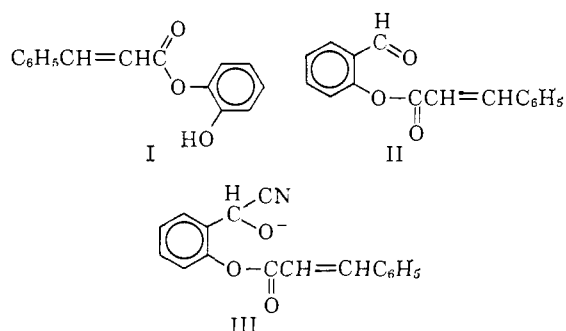
Sir:

Previously,¹ and in an accompanying communication,² large rate enhancements in ester hydrolysis have been demonstrated in compounds containing a neighboring hydroxyl group. These results may be of pertinence to the mechanism of enzymic hydrolysis of acyl derivatives, wherever a serine hydroxyl residue is a constituent of the catalytically active site. Other neighboring functional groups may possibly exert rate

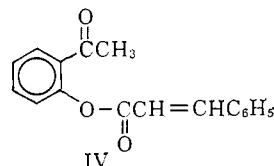
(1) S. A. Bernhard, A. Berger, J. H. Carter, E. Katchalski, M. Sela, and Y. Shalitin, *J. Am. Chem. Soc.*, **84**, 2421 (1962).

(2) Y. Shalitin and S. A. Bernhard, *ibid.*, **86**, 2291 (1964).

enhancement effects as well. Carbonyl groups have been shown to increase the rate of hydrolysis of a neighboring ester.^{3,4} Likewise, we have found that the aldehyde analog of I, cinnamoylsalicylaldehyde (II), undergoes very rapid hydrolysis. The rate is first order in OH⁻ concentration. The second-order specific rate is approximately $7 \times 10^5 M^{-1} \text{min.}^{-1}$. The activity is presumably due to the hydrated aldehyde or its conjugate base. This is indicated by the fact that in 0.002 *M* KCN solution, where the reactive species is probably the cyanohydrin (III), the rate of hydrolysis is increased tenfold. The catalysis may proceed similarly to either Scheme A or B of ref. 2, or *via* a nucleophilic attack on the carbonyl ester as suggested for the hydrolysis of *o*-formyl benzoate esters.⁴



A related ester containing a neighboring ketone, the cinnamoyl ester of *o*-hydroxyacetophenone (IV), is hydrolyzed with a specific rate of $2.6 \times 10^3 M^{-1} \text{min.}^{-1}$; very much slower than the hydrolysis of III,



but nevertheless 40-fold faster than the hydrolysis of phenyl cinnamate. Rate enhancement by CN⁻ is similar to that found with II.

Carbonyl groups of aldehydes and ketones, especially the former, can have a great influence on the rate of hydrolysis of neighboring ester groups. The available evidence indicates, however, that the rate enhancement is, in reality, exerted by the hydroxylic adduct or its conjugate base.

Acknowledgment.—This work was supported by grants from the National Science Foundation and the Public Health Service.

(3) M. S. Newman and S. Hishida, *ibid.*, **84**, 3582 (1962).

(4) M. L. Bender and M. S. Silver, *ibid.*, **84**, 4589 (1962).

(5) On leave of absence from the Department of Biophysics, The Weizmann Institute of Science, Rehovot, Israel.

INSTITUTE OF MOLECULAR BIOLOGY AND DEPARTMENT OF CHEMISTRY
UNIVERSITY OF OREGON
EUGENE, OREGON

YECHIEL SHALITIN⁵
SIDNEY A. BERNHARD

RECEIVED APRIL 27, 1964

Search For Accidental Degeneracy in Purines

Sir:

The strong adenine absorption at 260 mμ (38.5 kk.) appears to be a single electronic transition. Mason¹

(1) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).

using substitutional perturbations has suggested that there might actually be two transitions superimposed—accidental degeneracy. Recently, Clark and Tinoco² have examined a most appropriate perturbation series: purine and the 6-chloro, 6-methoxy, and 6-amino derivatives. Their work seems to corroborate Mason's suggestion, though they find that one band in the 260- $m\mu$ region for 6-aminopurine (adenine) may be considerably weaker than the other. Stewart and Davidson³ have measured the pleochroism of a very thin single crystal of 9-methyladenine, and find a change in dichroic ratio toward the short wave length side of the 260- $m\mu$ absorption band which they have attributed to a second transition having an extinction about one-tenth that of the stronger band.

It cannot be absolutely guaranteed that correlation of electronic transitions between members of a perturbational series will go smoothly; moreover, granted that the correlations are correct, quantitative interpretations are not inherent to the method. Absorption measurements on molecular crystals of the weak and intermediate coupling type can be quantitative; however, constancy of dichroic ratio is not to be expected with more than one molecule per unit cell and with intermediate coupling,⁴ so that departures from constancy of dichroic ratio cannot always be used to show the existence of more than one molecular electronic transition.

Polarized fluorescence of dilute (rigid) solutions has been most useful as a way of detecting two transitions, oppositely polarized and nearly superimposed.⁵ Here briefly is the idea: if one uses polarized light to excite to a particular upper electronic state, which excitation then disappears by fluorescence, one finds that the emitted light is strongly polarized in the same direction as the polarization of the exciting light. This is understandable because, for molecules which are rigidly held, only those having their transition moments lined up along the polarization direction of the exciting light will be (maximally) excited. In the case of two perpendicular transitions, if the higher energy state is the one excited, it internally converts to the lower state prior to fluorescence, which is from the lower excited state. Again in this case the molecules which are excited are those whose transition moments are lined up with the polarization direction of the exciting light. However, the emitted light comes from a transition which is polarized perpendicularly to the absorbing transition. Fluorescence from the same fixed molecules is then polarized oppositely to that of the exciting radiation. The polarization ratio is defined as $(I_{||} - I_{\perp}) / (I_{||} + I_{\perp})$, where, e.g., $I_{||}$ is the fluorescence intensity taken with a polarizer parallel to the polarization of the exciting light. This ratio theoretically is +0.5 when the absorbing and emitting levels are the same, or polarized the same, and -0.33 when the absorbing and emitting levels have perpendicular transition moment directions.⁶

We have measured the polarized fluorescence of guanine and 9-ethylguanine in the region from 230 to 300 $m\mu$ with the results shown in Fig. 1 and 2.⁷

(2) L. B. Clark and I. Tinoco, to be published.

(3) R. F. Stewart and N. Davidson, *J. Chem. Phys.*, **39**, 255 (1963).

(4) Apparent weak coupling cases may show intermediate coupling intensity behavior (R. L. Fulton and M. Gouterman, *J. Chem. Phys.*, in press).

(5) F. C. Adam and W. T. Simpson, *J. Mol. Spectry.*, **3**, 363 (1959).

(6) F. Dörr and M. Held, *Angew. Chem.*, **72**, 287 (1960).

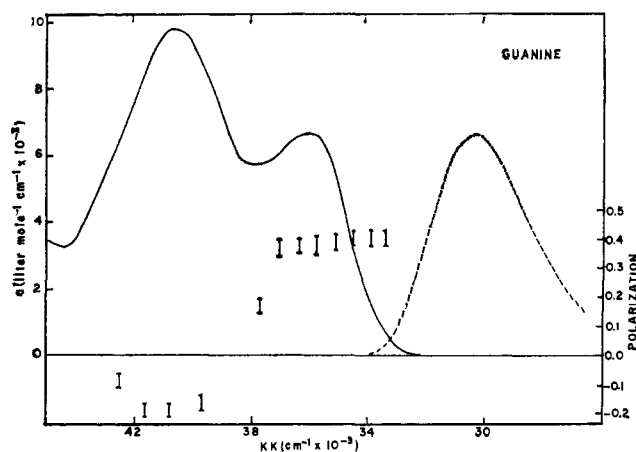


Fig. 1.—Absorption (solid line), fluorescence (dashed line), and polarization ratio of guanine.

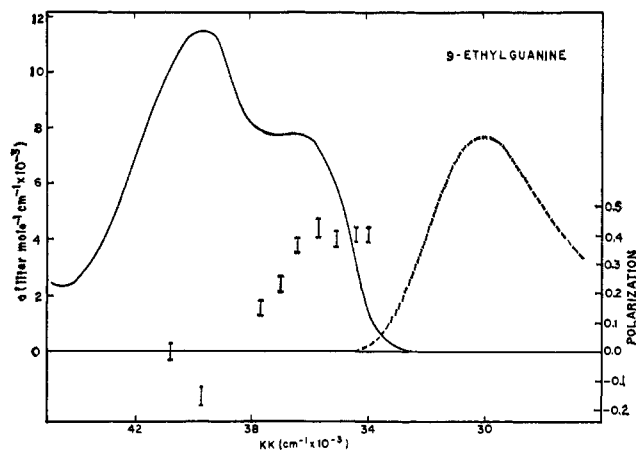


Fig. 2.—Absorption (solid line), fluorescence (dashed line), and polarization ratio of 9-ethylguanine.

Note that the polarization ratio goes negative in the middle of the second band; this shows that there are two approximately perpendicular transition moments. Note also that the fluorescence is approximately the mirror image of the first absorption band. The polarization ratios are around +0.4 and -0.2, which according to experience is about as close to the theoretical as one might expect. We have included 9-ethylguanine because here especially the bands are close together, and considering 9-ethylguanine by itself one might conceivably think that he is looking at a single electronic transition.

We have also studied adenine (Fig. 3) and one sees right away that there are not two electronic transitions in the sense that there are in guanine. The polarization ratio has the full positive value typical for a single transition over most of the absorption band.

A close study of Fig. 3⁷ will show, however, that some weak perpendicular absorption on the blue side of the 260- $m\mu$ band can possibly be inferred because the polarization does not stay up at the normal high value all the way across the band. The molecule *p*-dimethoxybenzene⁸ is not expected to have a weak transition underlying the first band, but has an oppositely polarized transition nearby though not overlapping. The dichroic ratio determined for the (001) crystal

(7) Experiments were conducted using dilute (10^{-4} M) ethylene glycol-water (7:3) glasses at -78° . In all cases the fluorescence appeared independent of exciting frequency.

(8) A. C. Albrecht and W. T. Simpson, *J. Chem. Phys.*, **23**, 1480 (1955).

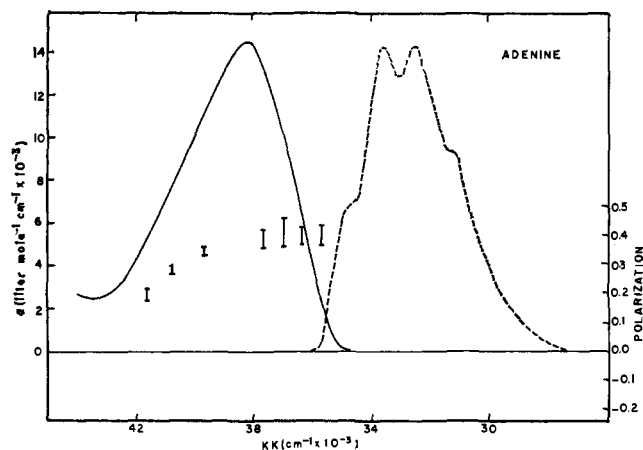


Fig. 3.—Absorption (solid line), fluorescence (dashed line), and polarization ratio of adenine.

face changes toward the high frequency side of the first band, which may indicate intermediate coupling. An additional possibility is that high vibronic transitions in the first band can be perpendicularly polarized owing to the nearness of a second band. We have measured the polarized fluorescence here as a sort of blank and find that the polarization ratio stays up at around +0.4 considerably farther across the band than in the case of adenine. Apparently selective borrowing for dimethoxybenzene is absent; perhaps also then for adenine. The ratio could be changed by the overlapping tail of the next strong band, but quantitative consideration from this point of view requires postulating a tail having an implausible shape. Thus there appears to be a weak second transition. According to our quantitative consideration of the depolarization, the second transition would have a maximum extinction coefficient about one-tenth that of the first transition. All this harmonizes with, and, we believe, tangibly reinforces the conclusions of Mason, Stewart and Davidson, and Clark and Tinoco.

Acknowledgment.—We wish to acknowledge helpful discussions especially with Drs. Stewart and Clark, and wish to thank Drs. Clark and Tinoco for showing us their paper prior to publication.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WASHINGTON
SEATTLE 5, WASHINGTON

PATRIK R. CALLIS
EUGENE J. ROSA
WILLIAM T. SIMPSON

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The Tetrameric Structure of Anhydrous, Crystalline Cobalt(II) Acetylacetonate

Sir:

Structural investigations of β -ketoenolate complexes of various transition metals have sometimes revealed interesting and unsuspected complications.^{1,2} We report here the rather novel and complicated structure of anhydrous, crystalline bis(2,4-pentanedione)cobalt(II). It was recently reported that this compound in solution in noncoordinating solvents exists in polymeric equilibrium, with oligomers at least as large as $[\text{Co}(\text{AA})_2]_4$. The monomer was considered to be tetrahedral but the oligomers were believed to have the metal atoms in distorted octahedral environments.³

(1) G. J. Bullen, R. Mason, and P. Pauling, *Nature*, **189**, 291 (1961).

(2) A. G. Swallow and M. R. Truter, *Proc. Roy. Soc. (London)*, **A254**, 205 (1960); **A254**, 218 (1960).

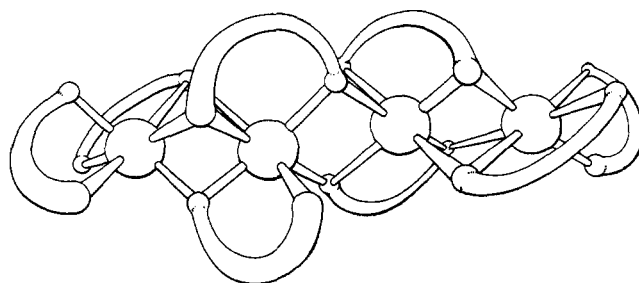


Fig. 1.—A schematic diagram of the structure of $[\text{Co}(\text{AA})_2]_4$. The O-C-C-O rings are indicated by curved lines.

The anhydrous crystals are prepared only with difficulty by sublimation⁴ (~ 2 mm. at $\sim 100^\circ$) and we have yet to discover a crystal which is not twinned and/or cracked.⁵ From one crystal which was twinned but not seriously cracked, all data were obtained.

The triclinic unit cell has the dimensions: $a = 8.61$, $b = 10.38$, $c = 13.72$ Å.; $\alpha = 93^\circ 50'$, $\beta = 90^\circ 25'$, $\gamma = 98^\circ 35'$; there are four $\text{Co}(\text{AA})_2$ groups in the unit cell. The intensities of 880 reflections were visually estimated from precession photographs on which the interpenetrating reciprocal lattices could be separately indexed. The accuracy of these data is not as good as would normally be expected because of the poor quality of the crystal. A Patterson function showed the positions of the cobalt atoms and indicated that the space group is the centric one. A first Fourier map phased on the cobalt atoms revealed the oxygen atoms, and a second Fourier map phased on both cobalt and oxygen atoms provided trial positions for all of the carbon atoms. In the course of five cycles of least-squares refinement, the residual dropped steadily from 37 to 25%.

The structure is that of a tetramer (Fig. 1), which is centrosymmetric. There are three distinct types of rings: those with both oxygen atoms bonded to only one terminal cobalt atom, those with one oxygen atom serving as a bridge between two cobalt atoms, and those with both oxygen atoms serving as bridges. In a general way, though *not* in detail, the structure can be thought of as consisting of two fragments of the $[\text{Ni}(\text{AA})_2]_3$ structure¹ with a common octahedral edge. This structure is consistent with the previously reported³ study of solutions. The apparent leveling off of the polymerization number at a value of 2 at high concentrations at CCl_4 at 77° may represent a genuine stability of the dimeric unit $[\text{Co}(\text{AA})_2]_2$, or it may show that the presence of traces of coordinating impurities (*e.g.*, alcohols, ether, H_2O) tends to split the tetramers into dimers in which each metal atom achieves a coordination number of six.

On the basis of the structure reported here for $[\text{Co}(\text{AA})_2]_4$ and that previously found for $[\text{Ni}(\text{AA})_2]_3$, the structures of the $[\text{M}(\text{AA})_2]_2\text{py}$ ($\text{M} = \text{Co}, \text{Ni}$) compounds prepared by Fackler⁶ might very well correspond, in general if not in detail, to one-half of the $[\text{Co}(\text{AA})_2]_4$ molecule, with one of the nonbridging coordination sites filled by the pyridine molecule.

(3) F. A. Cotton and R. H. Soderberg, *Inorg. Chem.*, **3**, 1 (1964).

(4) It is extremely difficult, if not impossible without extraordinary precautions, to dry solvents sufficiently to obtain completely anhydrous material by crystallization from solution.

(5) Over fifty were examined on the precession camera; they are exceedingly fragile, cracking under the slightest stress.

(6) J. P. Fackler, Jr., *J. Am. Chem. Soc.*, **84**, 24 (1962); *Inorg. Chem.*, **2**, 266 (1963).