ergetically impossible for it to add to the double bond.

As final examples, results of some of our studies of atomic carbon, both hot and thermal, are cited.[30] We showed that with hydrocarbons reaction proceeds mainly by insertion of C atoms into existing bonds, to form an intermediate adduct which subsequently decomposes (e.g., eq 14). The polyvalent character of

$$^{11}C + C_2H_6 \longrightarrow H^{11}CCH_2CH_3 \longrightarrow$$
$$H^{11}C \equiv CH + (CH_3 + H) \quad (14)$$
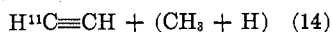
atomic carbon allows it to be bound to alkanes. Because of the increased number of C–C bonds, the complex in its ground state will be stable by several electron volts with respect to both reactants and products. ϵ^* will therefore be large, in marked contrast to hydrogen atom–alkane systems, and criterion A may thus be satisfied.

Reactions analogous to (14) might be expected to be possible for perfluorinated systems as well, since in this case also a strongly bound intermediate should be capable of existing. Very recent studies[31] have, however, uncovered no evidence of this. The only reaction tentatively identified, F abstraction to form CF, probably proceeds directly. Apparently there is a barrier to insertion of C into C–F bonds. This again indicates the importance of our second criterion (B). The fact that a strongly bound complex is capable of existing does not guarantee that it will be formed.

In conclusion we must emphasize again that systematic study of the competition between direct reaction and persistent intermediate complex formation is still at an early stage. Yet it appears that the factors underlying this competition are becoming clear and are starting to provide a basis for making at least tentative predictions.

(30) C. MacKay and R. Wolfgang, *Science*, **148**, 899 (1965).

(31) D. Blaxell, C. MacKay, and R. Wolfgang, *J. Am. Chem. Soc.*, in press.

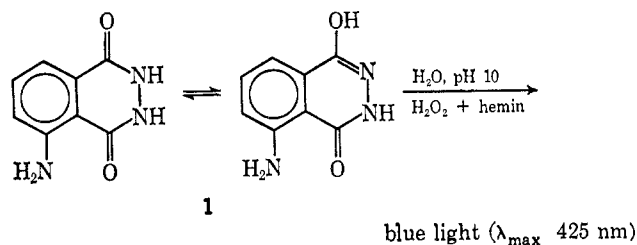# The Chemiluminescence of Organic Hydrazides

Emil H. White

The Johns Hopkins University, Baltimore, Maryland 21212

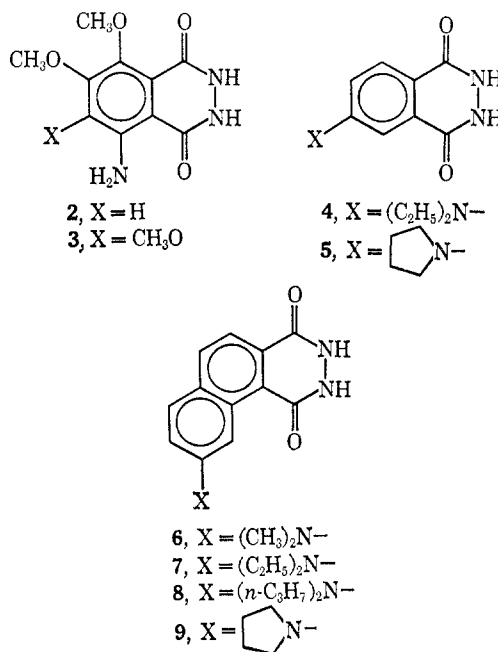David F. Roswell

Loyola College, Baltimore, Maryland 21210

Chemiluminescence is the production of light in excess of black-body irradiation by a chemical reaction. The definition effectively includes any reaction that yields visible light at room temperature, since emission by "black bodies" at this temperature is negligible in the visible region of the spectrum. A wide variety of compounds are known to be chemiluminescent, but this paper will be concerned only with acyl derivatives of hydrazine. Since several reviews of chemiluminescence have appeared recently,[1] our treatment of the historical aspects of the problem will not be exhaustive. Probably the best known example of chemilumines-

2, X = H
3, X = CH₃O

4, X = (C₂H₅)₂N–
5, X = ⟨N⟩–

blue light (λ_max 425 nm)

6, X = (CH₃)₂N–
7, X = (C₂H₅)₂N–
8, X = (n-C₃H₇)₂N–
9, X = ⟨⟩N–

(1) (a) K. D. Gundermann, "Chemilumineszenz Organischer Verbindungen," 1st ed, Springer-Verlag, New York, N. Y., 1968; (b) K. D. Gundermann, *Angew. Chem. Intern. Ed. Engl.*, **4**, 566 (1965); (c) F. McCapra, *Quart. Rev.* (London), **20**, 485 (1966); (d) J. W. Haas, *J. Chem. Educ.*, **44**, 396 (1967); (e) E. H. White in "Life and Life," 1st ed, W. D. McElroy and B. Glass, Ed., Johns Hopkins Press, Baltimore, Md., 1961, p 183.

cence is the oxidation of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione, 1) to give an intense blue light, first reported by Albrecht in 1928.[2] A large number of related hydrazides have since been tested, but only about eight analogs[3] distributed among three groups (examples 2–9) are more efficient in light production than luminol. Both cyclic and acyclic hydrazides are chemiluminescent; however it is convenient to discuss the two classes separately.

Cyclic Hydrazides

Compounds of this type (1–9) are generally prepared by the reaction of a dicarboxylic acid anhydride or imide with hydrazine. The isomeric N-amino imide 12 is an intermediate in the reaction (eq 1).[4]
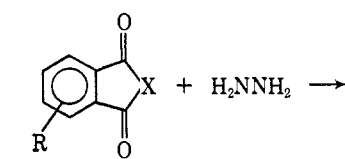

10, X = O
11, X = NH, NCH$_3$

12   13
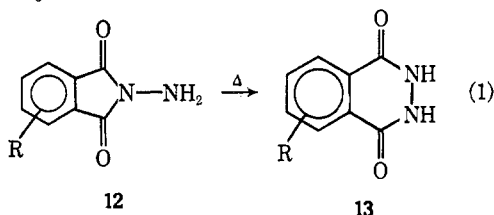
Analogs with substituents on the nonheterocyclic ring are readily prepared (i.e., as in compounds 1–9), but any substitution on the heterocyclic ring (e.g., as in compounds 14 and 15, R = 5-NH$_2$) renders the compounds nonchemiluminescent.[5] Furthermore, related compounds isomeric with 1, e.g., compounds 12 and 16, are not chemiluminescent.[6] Thus, 13 represents the type of structure required for chemiluminescence of the cyclic hydrazides; as we shall see, an unsubstituted heterocyclic ring is required to permit facile

14   15   16
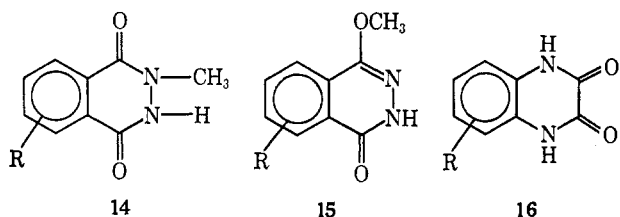
oxidation to the corresponding carboxylic acid and nitrogen.

Both protic and aprotic solvents may be used in the chemiluminescence,[1e,7] although the chemical reactions occurring are different in the two media. Water is the most common protic solvent; a similar chemistry is observed, however, in the lower alcohols and in mixtures of water and organic solvents. In these systems, a base, hydrogen peroxide, and an oxidizing agent are required; ferricyanide or hypochlorite are normally used, although the system derived from hydrogen peroxide and a chelated transition metal such as iron or copper can be substituted. Free radicals appear to be implicated, and the reaction path given by eq 2 has been proposed.[1e] A similar mechanism

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + \cdot OH$$

17   18
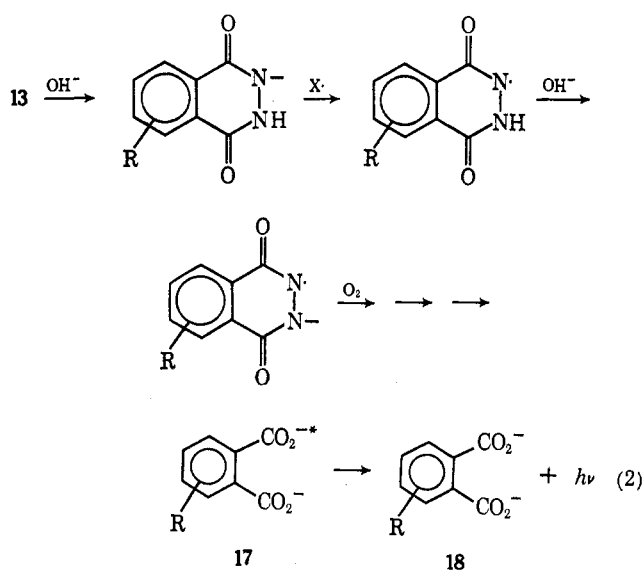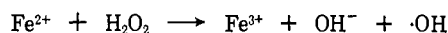
involving free radicals has been proposed for the electrochemical chemiluminescence of luminol.[8] The reaction conditions are rather vigorous in the protic systems, and degradation of the products occurs. However, using copper(II)–ammonia solutions, hydrogen peroxide, and luminol (1) at 0°, it was possible to show with the aid of ultraviolet absorption, fluorescence emission, and paper chromatography that 3-aminophthalate (17, R = 3-NH$_2$) was formed in the reaction as the only fluorescent product.[9]

Simpler and milder systems can be devised in aprotic solvents. Dimethylformamide, hexamethylphosphoric triamide, and dimethyl sulfoxide are suitable solvents, although the latter has been the most useful to date.[1e] In aprotic solvents, the cyclic hydrazides require only a strong base and oxygen for chemiluminescence, and the corresponding phthalate ions and nitrogen are the principal products.[10] Alkali metal hydroxides

(2) H. O. Albrecht, Z. Physik. Chem., 136, 321 (1928).
(3) (a) E. H. White and M. M. Bursey, J. Org. Chem., 31, 1912 (1966); (b) K. D. Gundermann, W. Horstmann, and G. Bergmann, Ann., 684, 127 (1965).
(4) In some cases, the N-amino imide is stable (H. D. K. Drew and F. H. Pearman, J. Chem. Soc., 26 (1937)).
(5) H. D. K. Drew and R. F. Garwood, ibid., 836 (1939).
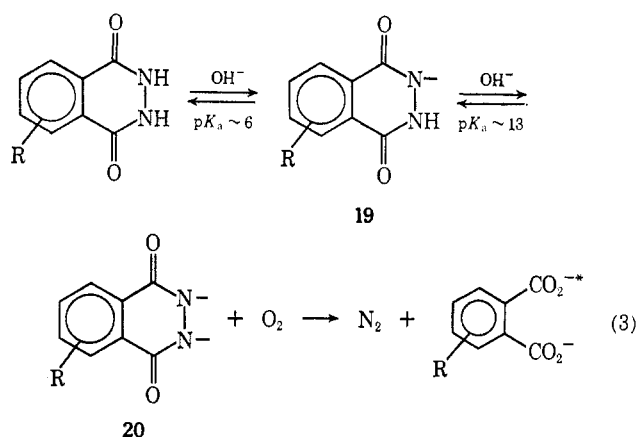(6) E. H. Huntress and J. V. K. Gladding, J. Amer. Chem. Soc., 64 2644 (1942).

(7) E. H. White, J. Chem. Educ., 34, 275 (1957).
(8) B. Epstein and T. Kuwana, Photochem. Photobiol., 4, 1157 (1965).
(9) Dr. C. Kulier, unpublished results.
(10) E. H. White, O. C. Zafiriou, H. M. Kagi, and J. H. M. Hill, J. Amer. Chem. Soc., 86, 940 (1964).

$$ 19 + O_2 \longrightarrow N_2 + \quad (3) $$

20

and salts of *t*-butyl alcohol, other than the lithium derivatives, are suitable bases. In the chemiluminescence of luminol, the aminophthalate formed has been converted into both dimethyl 3-aminophthalate and 3-acetamidophthalic anhydride in about 90% yields.[10,11] Nitrogen is evolved and oxygen consumed in very nearly molar amounts.[10]

The monosodium salts of the cyclic hydrazides, of luminol, for example, are quite stable to oxygen.[10] The addition of more base leads to some light evolution, the brightness increasing as the ratio of base to salt reaches 1.[9] The dinegative ion **20** is undoubtedly the critical intermediate formed here, and not an ion involving the NH_2 group, since the same dependence of chemiluminescence on the base ratio was also found for phthalic hydrazide. In fact, solutions of phthalic hydrazide and sodium dimethyl sulfoxylide (1:2) on evaporation *in vacuo* yield a yellow solid (**20**, R = H) which glows on exposure to air in either the solid state or when suspended in dimethyl sulfoxide. In the absence of oxygen, the salts **19** and **20** are stable indefinitely. The chemiluminescence step must therefore involve simply the reaction of oxygen with the dinegative ion (**20**) (see last step of eq 3).

To confirm this sequence of events for luminol and also to show that the two oxygen atoms introduced into the aminophthalic acid framework came from the oxygen gas and not from hydroxide ion or from adventitious water (*e.g.*, in "anhydrous" dimethyl sulfoxide), the chemiluminescence was carried out with ^{18}O-enriched oxygen gas in a system of 70 mol % dimethyl sulfoxide, 30 mol % water, and an excess of sodium hydroxide. It was found that over 85% of the label ended up in aminophthalate ion (the 15% loss of ^{18}O was shown by suitable blanks to result from exchange reactions occurring during the analysis).[10]

The kinetics of the chemiluminescence has also been measured in this system by following the light emission.[10] The second ionization constant for luminol is estimated[12] to be about 10^{-13}, and presumably only a low concentration of the dinegative ion **20** is present in the reaction mixture. Substitution of amino or methoxy groups on the phthalic hydrazide skeleton

increases the rate of the reaction, indicating that the oxidation is the slow step in the chemiluminescence. The reaction, therefore, involves an equilibrium with base leading to the dinegative ion, followed by a slow oxidation step (eq 4). Using the steady-state approximation, and with the logical assumption that k_{-1} (H_2O) $\gg k_2(O_2)$, the expression for the rate of photon emission becomes $(Z(k_1k_2/k_{-1})[H_2O][\mathbf{19}][OH^-][O_2] = k[\mathbf{19}][OH^-][O_2]$. Experimentally, in the presence of an excess of base and with oxygen saturation at 35° ([**19**] $\simeq$ concentration of luminol = 4.0×10^{-4} M, [OH^-] = 6.0×10^{-2} M, [O_2] = 1.6×10^{-3} M), the reaction was strictly pseudo first order, $k' = 2.5 \times 10^{-3}$ sec^{-1}. Changing the concentration of luminol 10-fold, the concentration of base 40-fold, and the concentration of oxygen 10-fold (independently) showed that the reaction was first order in each of these reactants.

$$ \mathbf{19} + OH^- \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathbf{20} + H_2O $$
$$ \xrightarrow[k_2]{+O_2} \mathbf{17} + N_2 \quad (4) $$
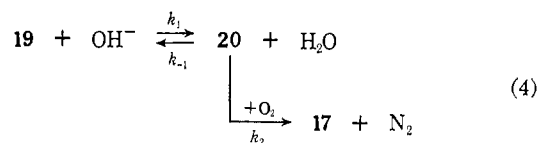
In the chemiluminescence of liquid solutions, the conversion of chemical energy into light energy involves the formation of a reactant, intermediate, or product molecule in an excited electronic state (directly or by energy transfer), and the emission of a photon by that species (Chart I). Since excited electronic states are also reached by the absorption of light, it should be possible to correlate the wavelength distribution in chemiluminescence with that in the phosphorescence of the product if a triplet state were involved, or with the distribution in the fluorescence of the product if a singlet state were involved. A number of such wavelength matches have been made,[13-19] and in all of the cases it has been established that the singlet state of the emitter is involved. This is not too surprising in view of the known efficient quenching of triplet states, especially by oxygen.[20]

In the chemiluminescence of luminol in dimethyl sulfoxide and also in water, a good match has been found for the wavelength distribution in chemiluminescence, the fluorescence of the total reaction product, and the fluorescence of sodium aminophthalate.[13] All three emissions are found at 485 nm in DMSO and at

(11) Dr. J. H. M. Hill, unpublished results.
(12) F. H. Stross and G. E. K. Branch, *J. Org. Chem.*, **3**, 385 (1938).
(13) E. H. White and M. M. Bursey, *J. Amer. Chem. Soc.*, **86**, 941 (1964).
(14) H. H. Seliger in ref 1e, p 200.
(15) J. Lee and H. H. Seliger, *Photochem. Photobiol.*, **4**, 1015 (1965).
(16) A similar effect operates in the chemiluminescence of the lophines: (a) E. H. White and M. J. C. Harding, *J. Amer. Chem. Soc.*, **86**, 5686 (1964); (b) E. H. White and M. J. C. Harding, *Photochem. Photobiol.*, **4**, 1129 (1965).
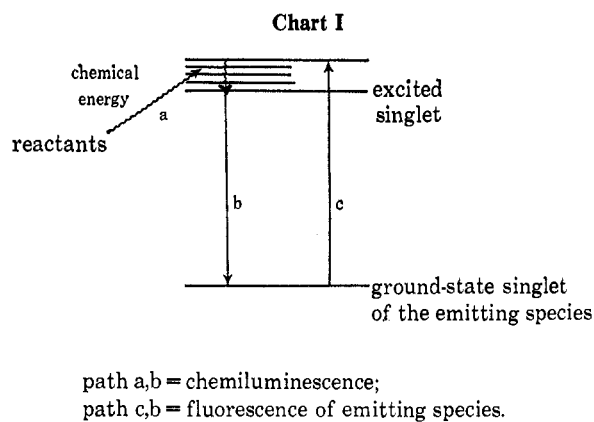(17) J. R. Totter, *ibid.*, **3**, 231 (1964).
(18) (a) T. A. Hopkins, H. H. Seliger, E. H. White, and M. W. Cass, *J. Amer. Chem. Soc.*, **89**, 7148 (1967); (b) E. H. White, E. Rapaport, T. A. Hopkins, and H. H. Seliger, *ibid.*, **91**, 2178 (1969); (c) F. McCapra, Y. C. Chang, and V. P. Francois, *Chem. Commun.*, 22 (1968).
(19) F. McCapra and Y. C. Chang, *ibid.*, 1011 (1967).
(20) J. G. Calvert and J. N. Pitts, Jr., "Photochemistry," 1st ed, John Wiley & Sons, Inc., New York, N. Y., 1966, p 598.

Chart I

path a,b = chemiluminescence;
path c,b = fluorescence of emitting species.

424 nm in water.[21] In mixtures of dimethyl sulfoxide and water (or other protic solvents), furthermore, a double emission occurs in *both* chemiluminescence and fluorescence, with peak positions unchanged from the values in the pure solvents (*i.e.*, 424 and 485 nm).[13] Similar shifts were also found for compounds **2** and **3** and for the 5-OH compound (**13**, R = 5-OH).[22] The occurrence of two emission peaks is attributed to the formation of two different kinds of excited-state molecules that differ by a proton. Either the 485-nm emitter in the chemiluminescence of luminol is **17** (R = 3-NH₂) and the 424-nm emitter is species **17** hydrogen bonded to water or, more probably, **17** emits at 424 nm and the 485-nm emitter stems from a molecule formed by proton loss to the medium (**21**) or a proton transfer to the carbon group (**22**).[13] In any event, we
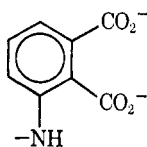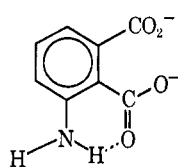
have found that only hydrazides with an ionizable group in the 5 position (**13**) produce light in the DMSO system and only phthalic acids bearing such ionizable groups in the 3 position are fluorescent in DMSO solutions. The coplanarity and rigidity of **22** are attractive in this context. Interesting modifications of the above rule are found in the section on energy transfer.

As we have indicated, the addition of water to DMSO solutions shifts both the chemiluminescence wavelength of luminol and the fluorescence wavelength of aminophthalate from 485 nm to 424 nm. The rate at which the wavelengths shift, however, differs, the chemiluminescence peak at 424 nm developing at a slower rate than the fluorescence peak at 424 nm. This effect is not understood at present; it may be the result of emission of the aminophthalate ion from slightly different solvent cages in the two excitation pathways (Chart I), possibly as the result of the involvement of

nitrogen in only the chemiluminescence pathway.[13]

Wavelength matches have also been found for a number of other hydrazides where the chemiluminescence emission has been compared with the fluorescence emission of the corresponding phthalate ion.[3,13,14,23,24] Thus, despite the fact that the emissions are usually broad, featureless bands,[25] we feel confident that the product phthalate ions are the light emitters in the chemiluminescence. Measurements of the fluorescence emissions of luminol itself, aminophthalic anhydride, and the aminobenzoic acids eliminate these compounds from consideration as light emitters in the luminol chemiluminescence.

Although demonstrations of the chemiluminescence of luminol (milligram quantities) can be quite startlingly bright, the over-all efficiency of the reaction is only about 1% (quantum yield = number of photons/number of molecules of **1** that react = 0.01).[15] The observed efficiency would appear to be a product of three factors: (1) the fraction of hydrazide molecules that react chemically by the correct "light" path, it being presumed that side reactions are occurring, (2) the fraction of intermediate or product molecules that cross over onto the energy surface for the excited singlet state of the light emitter,[26] and (3) the fraction of the excited molecules that emit a photon (=the apparent fluorescence quantum yield). Information concerning the first two factors is lacking at the moment, but data exist to show that factor 3 is an important one.

It has long been known that the substituent R in **13** has a profound effect on the over-all chemiluminescence efficiency of the cyclic hydrazides.[16] Thus, luminol (**1**), bearing a 5-amino group, is one of the most efficient of the hydrazides, whereas the parent hydrazide (**13**, R = H) and those bearing methyl, chloro, and nitro substituents are very inefficient in light production.[27] The expected light emitters in the latter cases, the corresponding phthalates, are essentially nonfluorescent (see section on phthalic hydrazide), and, of course, one would not expect efficient chemiluminescence under those circumstances (eq 2). In general, electron-releasing substituents (NH₂, OH, etc.) lead to highly fluorescent acids and to efficient hydrazides, whereas electron-attracting substituents (Cl, NO₂, etc.) lead to nonfluorescent acids and poorly efficient hydrazides. The dependence of these efficiencies on the σ constants of the R groups is reasonably regular for monosubstituted hydrazides, but the effect of the group is not necessarily additive in polysubstituted hydrazides. That is, compounds **2** and **3** are more efficient than luminol (in the DMSO system), but compound **23** is less efficient than luminol in both the water and

(21) These values are in contrast to those published by Y. Omote, T. Miyake, S. Ohmori, and N. Sugiyama, *Bull. Chem. Soc. Jap.*, **40**, 899 (1967).

(22) M. M. Bursey, Ph.D. Thesis, The Johns Hopkins University, Baltimore, Md., 1963.

(23) O. C. Zafiriou, Ph.D. Thesis, The Johns Hopkins University, Baltimore, Md., 1966.

(24) E. H. White, E. G. Nash, D. R. Roberts, and O. C. Zafiriou, *J. Amer. Chem. Soc.*, **90**, 5932 (1968).

(25) Vibrational fine structure has been seen in certain cases, *e.g.*, in compound **32** (see also the sections on energy transfer and mono-acyl hydrazides).

(26) M. G. Evans, H. Eyring, and J. F. Kincaid, *J. Chem. Phys.*, **6**, 349 (1938).

(27) H. D. K. Drew and R. F. Garwood, *J. Chem. Soc.*, 791 (1938).

DMSO systems.[28a] The environment of the groups

23   **24**
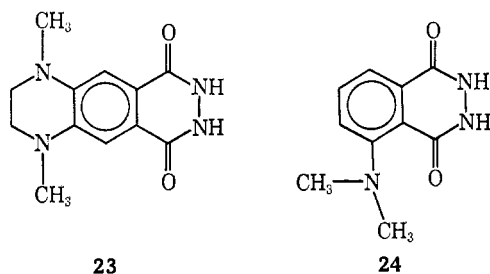
must also be considered. For example, compound **24** is far less efficient than luminol. This result probably stems from steric inhibition of resonance of the dimethylamino group caused by nonbonded interactions with the carbonyl group. Compound **24** is, in fact, about as efficient as phthalic hydrazide itself in DMSO and not much more efficient in water.[28b]

Mechanism of the Formation of Excited States. A sizable fraction of all chemiluminescent reactions in liquid solution appear to involve a four-membered peroxide (an oxaoxetane, e.g., **25**) as the critical reaction intermediate, the decomposition of which leads to two carbonyl groups.[1c,16] Most of these cases involve the prior formation of a hydroperoxide, which in turn is generated from either oxygen or hydrogen peroxide, depending on the oxidation state of the organic component.

At first, it appeared that the special virtue of the four-membered peroxides that enabled them to form excited electronic states resided in the fact that their geometry approaches that of the excited states of the carbonyl group; the excited singlet and triplet states of formaldehyde have been shown to be bent, with


$$2 \overset{}{>}C{=}O + h\nu \quad (5)$$

25

excited state
of $>$C$=$O?
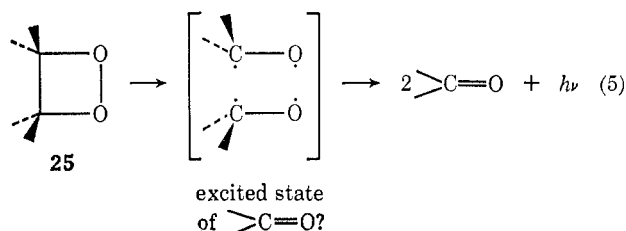
the oxygen being about 20° out of the plane of the carbon and its two substituents.[29] The exothermic nature of the cleavage of the peroxides and the generation of two carbonyl groups, which could extend conjugation, are additional attractive features of the decomposition.[16b] The success of the Woodward–Hoffmann rules of orbital symmetry in interpreting a wide variety of organic reactions[30] leads to another possibility, however. The formation of cyclobutane from two ground-state ethylenes is forbidden, but the formation from one molecule of ethylene in an excited

state and one ground-state ethylene is an allowed process. This suggests that, in the reverse process, the synchronous cleavage of **25**, one of the carbonyl compounds will be formed in an excited state.[31]

It has been difficult to establish the role of four-membered peroxides in the hydrazide chemiluminescence because of the difficulty of trapping and detecting intermediates. The chemiluminescence of luminol in aprotic solvents proceeds at $\sim-60°$, and esr signals have not been detected from the reaction.

The critical reaction of the dinegative ion of the hydrazides with oxygen (eq 6) could, a priori, follow several courses (eq 6). Pthalazinediones (azaquinones,
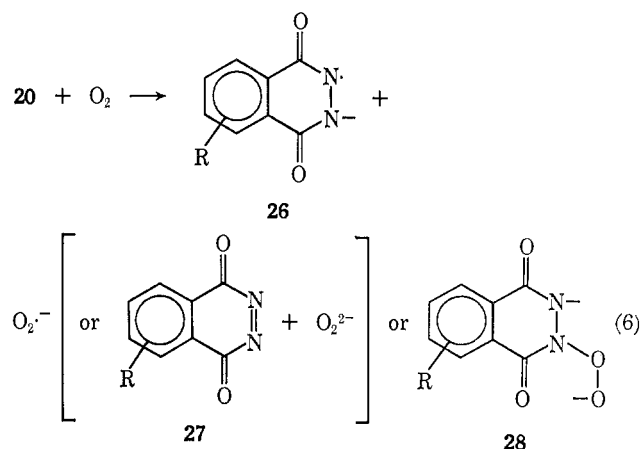
27) were proposed as intermediates in the first paper published on the chemiluminescence of luminol.[2] Their role in chemiluminescence has not been established until recently, however, since the azaquinones are generally very reactive and difficult to obtain in a pure form. As an exception to this rule, we have found that azaquinone **30**, corresponding to 2,3-naphthalic hydrazide, is relatively stable and as a result has been obtained analytically pure.[24] This azaquinone is chemiluminescent in the aqueous system (glyme added for solubility) with hydrogen peroxide as the only

added requirement. 2,3-Naphthalate ion (**31**) is formed in the chemiluminescence of **30**, as it is in the
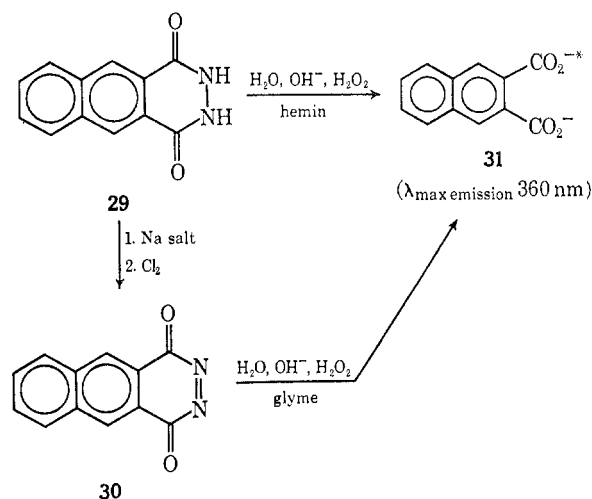
(28) (a) K. D. Gundermann and M. Drawert, *Chem. Ber.*, **95**, 2018 (1962); this compound was prepared independently in our laboratories by Dr. J. H. M. Hill; (b) E. H. White and K. Matsuo, *J. Org. Chem.*, **32**, 1921 (1967).
(29) (a) G. W. Robinson and V. E. DiGiorgio, *Can. J. Chem.*, **36**, 31 (1958); (b) W. T. Raynes, *J. Chem. Phys.*, **44**, 2755 (1966).
(30) R. Hoffmann and R. B. Woodward, *Accounts Chem. Res.*, **1**, 17 (1968).

(31) See also F. McCapra, *Chem. Commun.*, 155 (1968).

chemiluminescence of **29**, and the wavelength maximum of the chemiluminescence is the same in the two cases. As expected, this wavelength maximum matches that of the fluorescence of **31**.[24] Kinetic data also suggest that the azaquinones are in the main stream of the chemiluminescence. The rate of the chemiluminescence of luminol in the water–hydrogen peroxide system with persulfate as the oxidizing agent shows a first-order dependence on luminol and a first-order dependence on persulfate.[32] Since free radicals were not detected in the reaction, it was proposed that a two-electron oxidation of luminol had occurred, presumably to generate the azaquinone. Future work concerning the mechanism of the chemiluminescence of the cyclic hydrazides will presumably follow leads uncovered in the study of, for example, the luciferins,[18] the lophines,[16] and the acridines.[33]

The general picture of the chemiluminescence of cyclic hydrazides—that light emission occurs from the corresponding phthalate ion in an excited state (eq 2)—appears to be correct for all the efficient compounds that have been tested. The parent compound, phthalic hydrazide itself (and also **13**, R = CH₃), does not fall into this category, however.[34] The reaction with base and oxygen in DMSO yields a yellow light of only moderate intensity, whereas little if any light is obtained when the water system is used.[35] The wavelength maximum of emission is anomalous, as a comparison with the emissions of compounds **29** and **32** shows (358 and 411, 428 nm, respectively). An extrapolation of the λ_{max} as a function of ring number would have led to an expected emission in the low 300-nm range for **13**, R = H, whereas the value found was 525 nm.[34] Further, phthalate ion is nonfluorescent in both water and DMSO, and chemiluminescence by


32   **33**

34
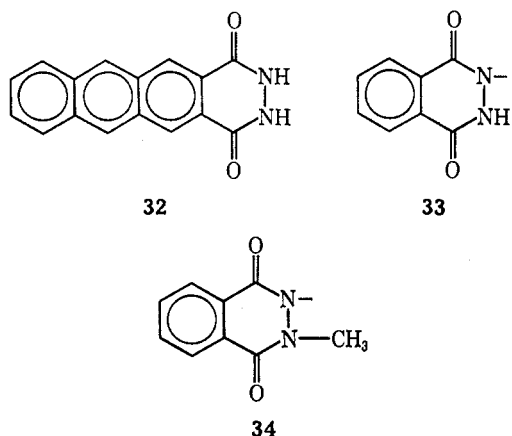
the normal mechanism is not to be expected. The observation that the mononegative ion of phthalic hydrazide (**33**) fluoresces at 525 nm, as does the salt

of N-methylphthalic hydrazide (**34**), suggested that in this case, because of the nonfluorescence of phthalate ion, light emission was a result of energy transfer back to a reactant molecule, followed by fluorescence of this species (**33**) (eq 7). The quantum efficiency is low, high concentrations are required, and under these conditions the energy transfer step is presumably favored.

(7)
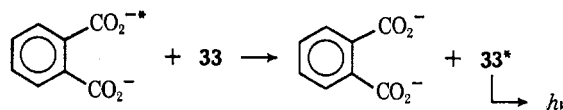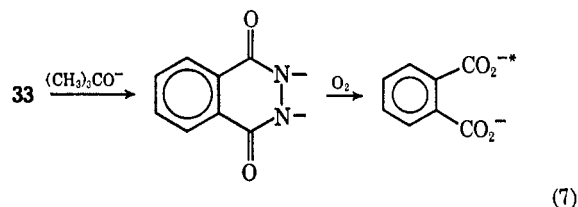
As a further complication, however, light yields were not markedly enhanced by added **34**. It has been proposed, to account for this observation, that a hydrogen bond must be formed between the phthalate ion and the mononegative ion of phthalic hydrazide (**33**) (eq 7) for energy transfer to occur efficiently enough to compete with quenching of the phthalate ion.[34,36] Energy transfer to other fluorescent compounds has not been observed to date; however, no fluorescent compound with the proper acidity (p$K_a \sim$ 13) has been tested.

Intramolecular Energy Transfer. Chemiluminescent compounds have a dual function: they must generate electronically excited states and they must generate products that can emit light efficiently. These functions can be incompatible within a single molecule. An efficient energy generator may lead to nonfluorescent intermediates and products, or a molecule designed to yield a highly fluorescent product may be unstable to the reaction conditions. Therefore, it would appear to be reasonable to allocate these functions to different molecular systems. This is the case, in fact, for several types of chemiluminescence involving intermolecular energy transfer. A highly fluorescent molecule, A,

$$B + C \longrightarrow D^*$$
$$D^* + A \longrightarrow D + A^*$$
$$ \longrightarrow A + h\nu \qquad (8)$$
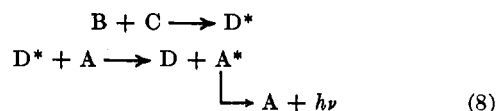
in the presence of various reacting systems such as oxalyl chloride plus hydrogen peroxide,[37] or chlorine, base, and hydrogen peroxide,[38] leads to light emission from A (eq 8).

(32) M. M. Rauhut, A. M. Semsel, and B. G. Roberts, *J. Org. Chem.*, **31**, 2431 (1966).

(33) F. McCapra, D. G. Richardson, and Y. C. Chang, *Photochem. Photobiol.*, **4**, 957 (1965).

(34) E. H. White, D. F. Roswell, and O. C. Zafiriou, *J. Org. Chem.*, **34**, 2462 (1969).

(35) J. Stauff and G. Hartmann, *Ber. Bunsenges. Phys. Chem.*, **69**, 145 (1965).

(36) Several cases of energy transfer in fluorescence involving hydrogen bonds have been found recently by Professor M. Kasha, Florida State University (private communication).

(37) (a) E. A. Chandross, *Tetrahedron Letters*, **761** (1963); (b) M. M. Rauhut, B. G. Roberts, and A. M. Semsel, *J. Amer. Chem. Soc.*, **88**, 3604 (1966).

(38) A. Khan and M. Kasha, *J. Chem. Phys.*, **39**, 2105 (1963).

Intermolecular energy transfer can be inefficient at low concentrations of reagents. We have, therefore, constructed bifunctional compounds composed of a hydrazide energy-generating end (C) and a fluorescent light-emitting end (A), in which the excitation energy is transferred intramolecularly (eq 9).[39]

$$A-C \xrightarrow[\text{oxidation}]{} A-D^* \longrightarrow A^*-D \longrightarrow h\nu + A-D \qquad (9)$$
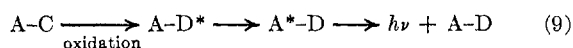
In the first compound of this type prepared (35), diphenylanthracene was selected as the ultimate emitter (moiety A) since its fluorescence quantum yield is high (almost 1.0).[40] 2,3-Naphthalic hydrazide was chosen as part C and the A and C parts were joined via a methylene group.

Compound 35 was shown to have a quantum yield of chemiluminescence of 2.6 × 10⁻³, whereas the 2,3-naphthalic hydrazide itself is only about one-fourth
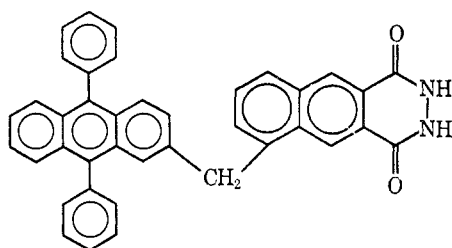
**35**

as efficient. Further, the chemiluminescence spectrum of 35 in the aqueous system showed that light emission occurred from both the diphenylanthracene and the 2,3-naphthalate portions of the molecule. Some of the primary excited states undergo radiative decay leading to emission from the naphthalate portion of the molecule whereas others transfer their energy to the diphenylanthracene moiety, which in turn emits. The ratio of the two emissions shows that the majority of the excitation is transferred rather than emitted directly. The transfer was shown to be intramolecular.[39,41] In compound 41, the fluorescence of 2,3-naphthalate ion overlaps well the first absorption band of diphenylanthracene, leading to efficient singlet-singlet transfer, presumably of the dipole-dipole type proposed by Forster.

In the DMSO system, compound 35 is chemiluminescent, but all the light comes from the diphenylanthracene part of the molecule. This fact is not surprising, since naphthalate ion is not fluorescent in DMSO. (Note in this connection that naphthalic hydrazide (29) is not chemiluminescent in the DMSO system.)

The second compound in this series, 36a, was prepared since phthalic hydrazide itself (13, R = H) is not chemiluminescent in the aqueous system. Com-

pound 36a, on testing in the aqueous system, proved to be moderately efficient (quantum yield = ∼8 × 10⁻⁴). This light emission also shows that energy transfer can be important in chemiluminescence. To determine the generality of energy transfer, three other compounds (37a–39a) in which phthalic hydrazide is linked to different acceptors were studied. The relative chemiluminescence quantum yields of the four hydrazides and the fluorescence quantum yields of the four acids are given in Table I. From these data it can be seen that there is a marked decrease in chemiluminescence efficiency in going from 36a to 39a. This decrease can only be partially explained by the differences in fluorescence quantum yields of the emitters. This anomaly argues against the singlet-singlet resonance transfer mechanism for the chemiluminescence of 36a–39a. Another argument against this
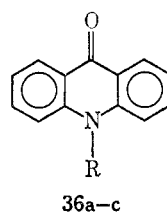
36a–c
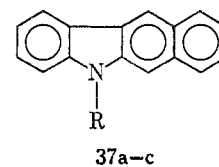
37a–c
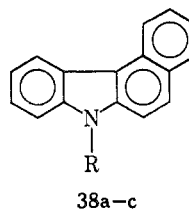
38a–c
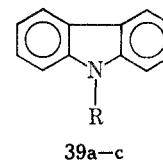
39a–c
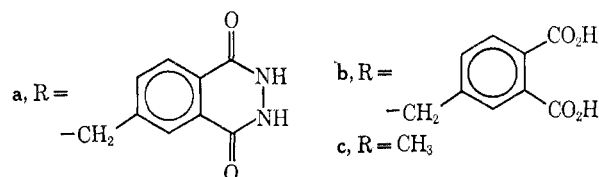
type of transfer is that phthalate ion is completely nonfluorescent. The maximum of the $s_1 \rightarrow s_0$ singlet emission distribution would be expected at about 305 nm[41] and consequently should overlap well with the absorption bands of all four acceptors. In fact, because of the relative extinction coefficients, compound 38a should have the greatest chemiluminescence quantum yield since it absorbs most strongly in the region. Phthalate ion shows an intense phosphorescence centered at 410 nm which overlaps to different extents the absorption bands of the four acceptors (36c–39c)

(39) E. H. White and D. F. Roswell, *J. Amer. Chem. Soc.*, **89**, 3944 (1967). For references about analogs in which only fluorescence is studied, see the literature citation above and also O. Schnepp and M. Levy, *ibid.*, **84**, 172 (1962).

(40) I. B. Berlman, "Handbook of Fluorescence Spectra of Aromatic Molecules," 1st ed, Academic Press, New York, N. Y., 1965, p 130.

(41) E. H. White, D. R. Roberts, and D. F. Roswell in "Molecular Luminescence," 1st ed, E. C. Lim, Ed., W. A. Benjamin, Inc., New York, N. Y., 1969, p 479.

Table I

Chemiluminescence and Fluorescence Quantum Yields

Φ(chemiluminescence)ᵃ	36a, 100.0	37a, 50.0	38a, 5.8	39a, 0.3
Φ(fluorescence)ᵇ	36b, 0.89	37b, 0.45	38b, 0.21	39b, 0.02

ᵃ Relative chemiluminescence quantum yield. ᵇ Fluorescence quantum yield determined by comparison with quinine bisulfate [Φ(fluorescence) 0.55]: W. H. Meluish, *J. Phys. Chem.*, **65**, 229 (1961).

in these compounds. For this reason triplet–singlet energy transfer has been proposed to explain the chemiluminescence of **36a–39a**.[41] If this triplet–singlet energy transfer were of the dipole–dipole type proposed by Forster,[42] the chemiluminescence efficiency would be directly related to the overlap integrals between the phosphorescence of phthalate and the absorption bands of the acceptors. These overlap integrals have been obtained, and although they do not completely confirm a dipole–dipole mechanism they suggest that the mechanism is triplet–singlet transfer of a mixed dipole–dipole and exchange type.[43]

Noncyclic Hydrazides

Monoacyl Hydrazides. Various compounds of this type have been reported to be chemiluminescent (**40–42**).[44] The oxidizing systems used are similar to
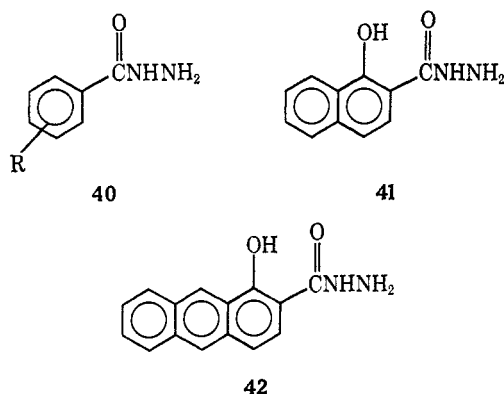
those used for cyclic hydrazides, and both water and DMSO have been used as solvents.

The effect of substituents is exerted in part at the carboxylate ion stage since electron-releasing sub-

$$RCNHNH_2 \xrightarrow[\text{base}]{\text{``oxidn''}} RCO_2^{-*} \longrightarrow RCO_2^- + h\nu \qquad (10)$$
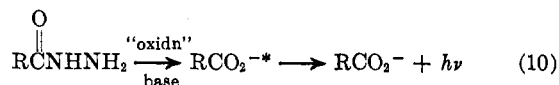
stituents increase both the chemiluminescence efficiency of the hydrazides and the fluorescence efficiency of the products. In the benzenoid compounds (**40**, R = NH$_2$), the *ortho* and *meta* isomers are chemiluminescent and the *para* isomer is not. The same pattern of luminescence is seen in the fluorescence of the corresponding aminobenzoic acids.[44d]

The most efficient monoacyl hydrazides we have prepared are the hydrazides of dehydroluciferin (**43**) and acridine-9-carboxylic acid (**45**). The structure of firefly luciferin (**44**)[45] is given for comparison. Both hydrazides **43** and **45** are about 0.3% efficient in light production; in comparison, luminol is ~1% efficient.[14] The chemiluminescence of compounds **41**, **42**, and **43**
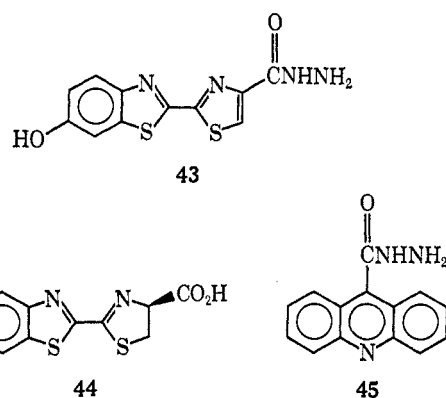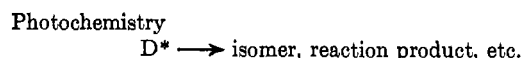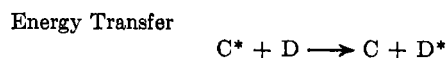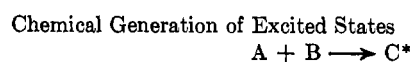
involves light emission from the corresponding carboxylic acids, and presumably the mechanism is related to that of the cyclic hydrazides. The light path for compound **45** is uncertain at present. It either involves the usual pathway to the excited carboxylate ion or an oxidative decarboxylation leading to acridone in an excited state.[46]

Photochemistry without Light

Chemiluminescent reactions are, in effect, systems for generating electronically excited states. We have shown in a previous section that the energy of these chemically produced excited states can be transferred to other molecules, which in the cases cited were fluorescent and capable of light emission. It should be possible to utilize this excitation energy to do work, as in ordinary photochemistry.

Chemical Generation of Excited States

$$A + B \longrightarrow C^*$$

Energy Transfer

$$C^* + D \longrightarrow C + D^*$$

Photochemistry

$$D^* \longrightarrow \text{isomer, reaction product, etc.}$$

Such systems would constitute "photochemistry without light."

We have been able to achieve this result within a complex molecule C* and also *via* intermolecular energy transfer. In the former category, compound **46** was oxidized in the water system and the resulting acids were converted into the methyl esters. Analysis by glpc showed that a few per cent of the *cis* isomer was formed in the reaction as a result of the chemical production of excited states.[47]
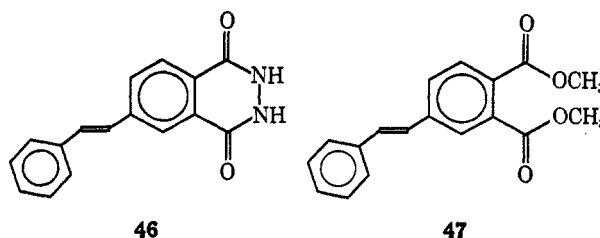
A more useful method involves intermolecular energy transfer. The synthesis, decomposition, and product

(42) Th. Forster, *Discuss. Faraday Soc.*, **27**, 7 (1959).
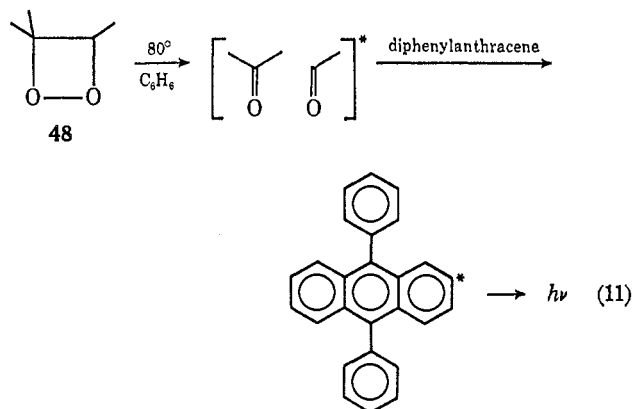
(43) Dr. D. R. Roberts, manuscript in preparation.

(44) (a) A. A. M. Witte, *Rec. Trav. Chim.*, **64**, 471 (1935); (b) J. S. Wassermann and G. P. Mikluchin, *Zh. Obshch. Chim.*, **9**, 606 (1939); (c) H. Ojima, *Naturwissenschaften*, **48**, 600 (1961); (d) E. H. White, M. M. Bursey, D. F. Roswell, and J. H. M. Hill, *J. Org. Chem.*, **32**, 1198 (1967); (e) J. Kroh and J. Luszczewski, *Roczniki Chem.*, **30**, 647 (1956).

(45) E. H. White, F. McCapra, and G. F. Field, *J. Amer. Chem. Soc.*, **85**, 337 (1963).

(46) E. Rapaport, unpublished results.

(47) Dr. D. F. Roswell, unpublished results.

analysis (eq 11) of an oxaoxetane (48) has been reported recently[48] (eq 11). We have found that the

decomposition carried out in the presence of *trans*-stilbene leads to about 10% isomerization of the stilbene into the *cis* form.[49] Other "photochemical" reactions have been effected,[49] and now that the principle has been established, efforts will be made to make this a practical, general approach to photochemistry. Two advantages of this method of generating excited states are: (1) the method of excitation ensures that all the energy resides initially in one molecular species (in the donor part of a molecule) and (2) only simple apparatus is needed, beakers, not monochromators.

The research described here and carried out in the laboratories of the Department of Chemistry of The Johns Hopkins University was supported by the National Institutes of Health. We wish to thank the students and postdoctoral fellows, whose names appear in the bibliography, for their contributions to the work summarized in this paper.

(48) K. R. Kopecky, J. H. VanDeSande, and C. Mumford, *Can. J. Chem.*, **46, 25** (1968); K. R. Kopecky and C. Mumford, Abstracts, 51st Annual Conference of the Chemical Institute of Canada, Vancouver, Canada, June 1968, p 41; K. R. Kopecky and C. Mumford, *Can. J. Chem.*, **47,** 709 (1969). Dr. Kopecky kindly furnished us with a preprint of the latter article.

(49) E. H. White, J. Wiecko, and D. F. Roswell, *J. Amer. Chem. Soc.*, **91,** 5194 (1969).

Protein–Solvent Interactions and Protein Conformation

SERGE N. TIMASHEFF

Graduate Department of Biochemistry, Brandeis University,[1] Waltham, Massachusetts, and Pioneering Research Laboratory for Physical Biochemistry, United States Department of Agriculture, Eastern Utilization Research and Development Division, Waltham, Massachusetts 02154

Proteins are natural high polymers with molecular weights in the range between 5000 and several million. They are polycondensates of amino acids, with the general formula $(-NHCHRCO-)_n$, where R can be any of some 22 different amino acid side chains.

At any given set of conditions at which the protein is not undergoing a conformational transition, all the polymeric chains of a given protein are folded in essentially identical fashion, resulting frequently in compact globular structures. Thus, within the limits of thermal fluctuations of the polypeptide chain fold, all the molecules have identical secondary and tertiary structures.[2]

A feature of particular importance is the fact that, in globular proteins which are soluble under physiological conditions, when the molecules are native, *i.e.*, in the conformation in which they occur in their natural surroundings, most polar (and in particular ionizable) residues are located on the surface of the molecule in contact with the aqueous solvent, while the interior (or core) is made up to a large extent of nonpolar amino acid side chains.[3] When the aqueous medium is perturbed by the addition of some organic molecules, such

as urea or alcohol, or some salts, such as guanidinium chloride, at a given concentration of the perturbant the protein becomes denatured, *i.e.*, its compact structure opens up and the chain largely unfolds, exposing much of the core to contact with solvent. This pattern of folding and unfolding of the polypeptide chain raises the questions: what are the forces that stabilize the native structure of globular proteins, and in what manner is this structure disrupted by the denaturing agent? It is the purpose of this paper to consider some aspects of the second question.

Protein Structure Stabilizing Forces

It is quite evident that the folding of a long-chain polymer into a compact structure results in loss of freedom of motion of the chain and, thus, in a decrease of the configurational entropy. Thus, for a typical protein of 200 amino acid residues, folding into the native structure may result in the loss of 200–250 kcal/mol of protein of structure stabilization free energy.[4,5] This must, evidently, be overcome by opposite effects of equal magnitude. Let us examine what these may be.

The forces which stabilize the protein structures are predominantly of a noncovalent nature.[6,7] The prin-

(1) Publication No. 685.

(2) The secondary structure of a protein refers to the manner in which the chain is folded, *i.e.*, to the relative three-dimensional space coordinates of consecutive amino acid residues; the tertiary structure refers to the manner in which folded chain segments are mutually arranged with respect to each other and to the mutual ordering of side chains.

(3) See, for example, J. C. Kendrew, *Sci. Am.*, **205** (6), 96 (1961), or D. C. Phillips, *ibid.*, **215** (5), 78 (1966).

(4) W. Kauzman in "The Mechanism of Enzyme Action," W. D. McElroy and B. Glass, Ed., Johns Hopkins University Press, Baltimore, Md., 1954, p 10.

(5) C. Tanford, *J. Am. Chem. Soc.*, **84,** 4240 (1962).

(6) C. Tanford, *Advan. Protein Chem.*, **23,** 121 (1968).

(7) R. Lumry and R. Biltonen in "Structure and Stability of