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CHEMILUMINESCENCE IN ORGANIC COMPOUNDS

/Following is a translation of an article by Dr. Heinz Stork, in the German-language periodical Chemiker-Zeitung Chemiscue Apparatur, (Chemists' Journal Chemical Apparatus), Vol 85, No 13, 5 July 1961, pages 467-473.7

The manifold group of luminescence phenomena may be divided into six types, according to their respective differences in origin $\frac{17}{\text{numbers}}$ in brackets refer to similarly numbered items in Bibliography at end?:

- 1. Electroluminescence, which may be observed under the influence of electrical fields with changing polarity (e. g. on ZnS);
- Photoluminescence, which is incited by absorption of light (fluorescence, phosphorescence);
- 3. Triboluminescence, which may be traced to mechanical causes (e.g. pulverization of sugar crystals);
- Crystalloluminescence, occurring during crystallization of certain substances (e.g. As₂0₃);
- 5. Sonoluminescence, observable in the ultrasonic field (e.g. in electrolytic solutions containing argon);
- 6. Chemiluminescence

The energy of the radiation emitted in chemiluminescence originates from a chemical reaction; it may be interpreted as the reversal of a photochemical reaction: in the same manner as the radiation absorbed in a photochemical process will entail a chemical reaction, the analogous is true in reverse in the case of chemiluminescence where radiation is a consequence of a chemical reaction. In both cases the

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following important limitation will apply: the ensuing process must not be caused by an increase in temperature resulting from the primary (source of) energy. In a true photochemical process, for example, the chemical reaction will not occur because of a temperature increase (caused by incidence of radiation) may have stimulated a certain percentrge of the molecules (according to Maxwell's distribution theorem) to an energy level permitting the subsequent reaction; each reacting molecule must receive its stimulation in one single stage of absorption. In analogous mannor, the emission of light during chemiluminescence must not occur as a consequence of a temperature increase but the energy emitted by a particle must have been absorbed by the latter from one single impulse. These impulses are given by molecules generated during the particular reaction whose energy has not yet been depleted by collisions with other particles incapable of radiation. The cause of chemiluminescence, in other words, is the transference of reaction energy to atoms or molecules capable of radiation; shown in a schematic formulation:

 $\begin{array}{c} \mathbf{A^*} + \mathbf{B} & \cdots & \mathbf{B^*} + \mathbf{A} \\ \mathbf{B^*} & \cdots & \mathbf{B} + \mathbf{hr}. \end{array}$

The particle with a higher energy level (A^*) , originating from the chemical reaction, will transfer its energy to the particle B which, under emission of light (h_{γ}) will return to the low energy level state. The following substances may act as agent B:

- 1. One of the reaction partners (e.g. the D-line of the sodium spectrum is emitted in the reaction of sodium vapor with chlorine under reduced pressure /1, 2, 3/);
- 2. An intermediate substance occurring during the course of the reaction (e.g. in the oxidation of phosphorus and P_2O_3 to P_2O_5 the spectrum of P_2O_3 is emitted /1, 4, 5/);
- 3. A product of reaction (e.g. only CO₂ bands-i.e., spectral lines - occur in the carbon monoxide-oxygen flame $\sqrt{17}$;
- 4. A foreign admixture (e.g. if oxidisilin is oxidized with permanganate in the presence of the dying agent Rhodanite B, strong red luminescence will be observed; luminescence will be weak if the coloring agent is not present in the reaction $/\Gamma$, 6, 77).

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One of the first authors having performed extensive research work in the field of chemiluminescence of organic compounds is B. RADZISZEWSKI /8, 9, 107. Among other phenomena he observed luminescence upon entrance of oxygen into alkaline solutions of sebacic acids, aldehydes, amides and alcohols; particularly bright luminescence occurs during the alkaline oxidation of Lophine and Amarine. M. TRAUTZ /117 published a comprehensive historical survey of chemi-Tuminescence phenomena and has himself observed luminescence during oxidation of numerous aldehydes, alcohols, phenols and sebacic acids; luminescence is especially intensive in the joint oxidation of aldehydes and phenols. He verifies luminescence of Amarine and Lophine in alkaline $H_2 \hat{v}_2$ solution; the same phenomenon is observed in Chrysenë," Anthracene and Carbazole. TRAUTZ has tested the influence of the oxidation agent on the chemiluminosity in a great number of experiments; bromine water proved to be a particularly effective reagent, showing its greatest effect on heterocyclic compounds containing nitrogen (e.g. quinoline and its derivatives); bright luminescence was generated in the latter. A number of publications $\sqrt{12}$, 13, 147 deals with the chemiluminescence of Grignard type compounds, occurring during their reaction with air, oxygen and other oxidizing agents. The most noteworthy contributions are those published by W. V. EVANS, R. T. DUFFORD, D. NIGHTINGALE and their colleagues /15, 16, 17, 187. Chemiluminescence in approximately 200 Grignard compounds is compared by these authors. Nearly all magnesium compounds tested showed luminescence, with the exception of such compounds where magnesium is bonded to a short alkyl remainder. Capacity for luminescence increases in proportion to molecular weight; it is generally greater in the bromides than in the chlorides which, in turn, surpass the iodides. In the phenyl-magnesium compounds, m-substitution was found to have a favorable effect, while an osubstituent will have an even better and a p-substitution the best result. The compound with maximal intensity of luminescence proved to be p-chloride-phenyl-magnesium bromide. No luminescence was shown by organic zinc and mercury Attention is also devoted by these authors to the halides. relationship between chemiluminescence and fluorescence. According to their statements fluorescence was observed in the oxidation products of numerous Grignard compounds (in the section of the long ultraviolet rays), even such substances which had shown no luminescence. Spectral distribution of fluorescence differs from that found for luminescence.

Another phenomenon of chemiluminescence which had been treated repeatedly /19, 207 is the bright green light occurring during alkaline oxidation of N, N'-dimethyl-diacridylic

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salt. H. KAUTSKY and H. H. KAISER /217 proved that the spectrum of luminescence is identical with the spectrum of fluorescence of the resulting oxidation product, i.e., N-methyl-acridone.

All luminescence phenomena listed thus far are surpassed by far in regard to intensity of luminescence by the cyclic 3-amino-phthalic acid hydrazide, called Luminol (I). This luminescence was discovered by W. LOMMEL in the Bayer factory laboratories at Leverkusen; H. O. ALBRECHT, upon LOMMEL's recommendation, conducted a thorough investigation of this phenomenon /227. He found that (1) will show inten-sive blue finerescence in diluted acid solution: sour solution, however, will not show luminescence when adding NaClO solution. Conversely, (I) will show no fluorescence in alkaline solution, but will emit intensive blue light upon addition of NaClO solution and still stronger intensity will be obtained when adding H_2O_2 and NaClO solutions. Luminescence will still occur after dilution of the solution to $1/10^7$ the original value; its spectrum is not identical with the spectrum of fluorescence. ALBRECHT ascribes the latter fact to changes occurring in the 3-amino group during oxidation. **E**quilibrium between the two tautomeric forms of (I) will be moved toward the left in alkaline and toward the right in acid solution.



This indicates that fluorescence is attributable to the cyclic hydrazide (I), while luminescence is effected by the dihydroxy compound (Ia). Consequently, ALBRECHT bases his formulation of the reaction process causing luminescence on (Ia), where the first step is shown to be oxidation to form the azodiacyclic compound (II):



(II) quickly hydroyses in alkaline solution, forming 3-aminophthalic acid (III) and diimine:



Difmine then reacts with one molecule of (II), which causes re-formation of (I):



This Luminol molecule is rich in energy and is converted to a less energized stage under emission of light. An important experimental foundation for this reaction path is given by the experiments conducted by H. KAUTSKY and K.H. KAISER $\sqrt{237}$. These authors agitated (I) with Ca(OC1), under acetone, and obtained a violet-red solution displaying the characteristics of an azodizcyclic compound; it emits bright light upon addition of a watery, diluted alkaline solution. A solution of (I) in acetone, however, does not show luminescence when alkaline solution is added; an additional oxidizing agent is required. Luminescence, therefore, is obviously preceded by formation of the azodiacyclic compound and its hydrolyhydrolysis. In accordance with equation (3), re-formation of (I) then takes place which, upon addition of more oxidation agent, again permits luminescence until, finally, all of (I) will have been converted to 3-amino-phthalic hydrazide.

An attempt to classify the chemiluminescence of (I) in the above schematization (in accordance with the reaction mechanism stated by ALBRECHT) will show that it occupies a

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special position in this respect. In this case we will not find a reaction product (A*) which is rich in energy, transferring a portion thereof (by impulse) onto a particle B which thus becomes capable of emission; the particle A* is here identical with B*. ALBRECT interprets luminescence in strongly diluted solutions as a verification of his mechanism, since under those circumstances a transfer of energy by means of impulse is practically impossible.

GLEU and PFANNSTIEL /247 have worked on the luminescence of Luminol derivatives. While LOMMEL had used 3-aminophthalic imide in reaction with watery hydrazide for the production of Luminol, the latter authors' synthesis is based on benzisoxazolon-4-carboxylic acid-methyl ester (IV) which they had been the first to produce:



The ester (IV) is boiled shortly with an excess of hydrazine which will cause its transformation to the hydrazide (V) which, in turn, isomerizes to N-amino-3-hydroxylaminophthalic amide (VI). By means of reduction with an excess of hydrazine the N,3-diamino-phthalic imide (VII) is formed which is denoted as "yellow hydrazide", according to its (Note: GLEU and PFANNSTIEL have pointed out that, color. according to a private note received from). BAYER this "yellow hydrazide" had already been produced by Dr. RAST in the BAYER factory laboratories at Leverkusen: Dr. RAST permitted watery hydrazine to act upon 3-amino-phthalic imide, at temperatures below 75° Celsius.) This substance shows neither fluorescence nor chemiluminescence. Boiling with concentrated hydrochloric acid will transform the yellow product into the isomeric, stable "white hydrazide", i.e., Luminol:

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If (IV) is boiled for an extended period with watery hydrazine, (I) will result directly, which by this method may be produced easily, permitting a high yield and offering a high degree of purity. If manufacture is based on the Nmethyl derivative of the ester (IV), the end product will be 3-methylamino-phthalic acid hydracide (VIII). This substance will show fluorescence in alkaline but not in acid solution (compare I!); chemiluminescence is equally strong as that shown by (I) while the color of the emitted light, however, is no longer a pure blue. If dimethyl-sulphate is permitted to act upon (I) in lye solution, a dimetnylized product will result, showing (according to the authors' statements) uniform melting point; it was assigned the formula (IX). It shows fluorescence in glacial acetic acid and in alkaline solution but does not show the slightest trace of light emission during alkaline oxidation:



The authors have stated, concerning this fact, that presence of the hydrazine rest without substitutions having been made in it obviously forms the prerequisite for occurrence of chemiluminescence. R. WEGLER /257, however, does not agree with this conclusion: he produces N-benzyl-phthalic acid hydrazide (X) from phthalic acid anhydride and benzylic hydrazine and then manufactures a mixture of the two isomeric N-benzyl-3-amino-phthalic acid hydrazides (XI) by means of reaction between 3-nitro-phthalic acid and benzylic hydrazine, -ith subsequent reduction. Both substances will show emission of light in H₂O₂ solution containing an addition of hemin. Since, however, the high degree of purity required in these substances cannot be guaranteed and thus formation of phthalic acid hydrazide or Luminol, respectively, through the easy separability of the benzyl rest, WEGLER does not even consider this

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luminescence as proof of the theory. For this reason he let 3-nitro-phthalic acid react with phenylic hydrazine and, after subsequent reduction, obtained a mixture of the isomeric N-phenyl-3-amino-phthalic acid hydrazides (XII). These compounds also display a clearly distinguishable emission of 14 with U_20_2 addition; despite a strong decrease in intensity of luminescence, noted after the substance had been purified repeatedly, the author considers this luminescence an indication that phthalic acid hydrazides with one substitution having been made at a nitrogen atom will still show emission of light.



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In addition to the above quoted compounds, showing substitution in the hydrazine residue, WEGLER produced the dicarboxylic acid hydrazides listed in Table 1: he conducts a comparative study of their luminescent capacity in H2O2 alkaline solution, in the presence of hemin.

Brightest luminescence in all these substances is shown by (I); the isomeric 4-amino compound shows equal intensity. XIV, without hemin, shows an intensity equal to that of I with hemin, but no increase in intensity is obtained in XIV after addition of hemin. In a similar manner it is impossible to achieve a catalytic increase in the luminous intensity of XVIII, in spite of the fact that this substance emits a

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Table 1

Chemiluminescent cyclic dicarboxylic hydrazides, according to R. WEGLER /257

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XXVII Diamine, romellithic acid hydrazide	XXVI	Succinic ~cid hydrazide
	XXVII	Diamine, romellithic acid hydrazide

brighter light with H_2O_2 alone, than I under identical conditions. XV shows more intensive luminescence than the unsubstituted compound XX, but less intensive than I; XVI and XVII will not even reach the intensity of the mono-hydroxy compound XV. XX and XXI show equal intensity, both of them surpass XXII. No comment is made concerning the luminescence intensity of the monocyclic hydrazides XXIV, XXV and XXVI; luminescence of the dihydrazide XXVII is stated as being hardly perceptible.

E. S. VASSERMANN and G. P. MIKHLUKHIN $\sqrt{267}$ report on another group of luminescent hydrazides:

Table 2

Chemiluminescent cyclic dicarboxylic acid hydrazides according to E. S. VASSERMANN and G. P. MIKHULKIN /267

XXVIII	Naphthalic acid hydrazide
XXIX	N-amino-phthalimide
XXX	4-nitro-phthalic acid bydrazide
XXXI	Diphenyl-2-2'-dicarboxylic acid hydrazide
XXXII	m-diphenyl-2,2'-dicarboxylic acid hydrazide
XXXIII	cis-delta 3,5-dihydro-phthalic acid hydrazide
XXXIV	delta 2,6-dihydro-phthalic acid hydrazide

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XXXIII and XXXIV are the compounds displaying most intensive luminosity.

C. N. ZELLNER and G. DOUGHERTY /277 are not content with a qualitative comparison of luminescence intensities, based only on visual perception. They have measured the relative luminescence intensity of 10 phthalic acid hydrazides by means of a Weston photo element connected to a mir_or galvanometer with an internal resistance of 896 Ohms. Deflection of the pointer as shown on the galvanometer scale constitutes a measure of light intensity

Table 3

Luminescence of phthalic acid hydrazide derivatives according to C. N. ZELLNER and G. DOUGHERTY /277

(Measurements were made at two concentrations in 0.25% sodium lye; 0.2 ccm n-NaCl0 solution were used in each case as oxidation agent. Distance between scale and galvanometer was 1 meter.)

Phthalic	acid hydrazide	(a)	(b)
XV	3-hydroxy-(not clean in an-		
	alysis!)	38.0	65,0
XXXV	3-acetylamino-	28.0	31.5
XXXVI	Acety1-3-acety1amino	11.5	26.5
XXXVII	3-chloro-(not pure in analy-		
	sis!)	4.0	3.5
XXXVIII	Diacety1-3-benzoylamino-	0.8	1.1
XXXIX	alpha-N-methy1-3-acetylamino-	0.0	0.4
XL	4-acetylamino-	9.0	11.0
XI I	Acety1-4-acety1amino-	8.5	10.0
XLII	Diacetyl-4-benzoylamino-	0.2	0.2
XLIII	alpha-N-methyl-4-acetylamino-	0.0	0.2

/Legend:7 a) Deflection (cm) (c=0.001 mol/1); b) Deflection (cm) (c=0.004 mol/1).

Measuring of the luminescence of Luminol with the apparatus described above was possible only after installing of an additional resistor of 700,000 Ohms in front of the galvanometer: pointer deflection at c=0.004 mol/l was 22 cm. This examination also does not permit a final decision as to

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the cheuiluminescence of phthalic acid hydrazides with substitution(s) in the hydrazine remainder. XXXVI is formed from XXXV by acetylation; XLI is formed by the same process from 4-amino-phthalic acid hydrazide. There is a possibility that in both compounds the amino group exists in the diacety-In XXXVIII and XLII which are formed from the Inter Corr. percentive beaughamino compounds by acetylation, at least one or the acetyl groups must be bonded to one of the N-atoms of the hydrazine rest. Luminescence in these compounds is, however, of such extreme weakness that it may be attributable to a lack in purity. The same is true of the compounds XXXIX and XLIII which are obtained from the respective acetylaminophthalic acid anhydrides and methyl hydrazine, together with the isomeric beta-compounds (e.g. XXXIXa) (Positioning of the N-methyl group within the alpha and beta derivatives was not ascertained). Isomeric compounds, when contained in a mixture, may be separated by means of fractionized crystalliza-Luminescence of alpha-N-phenyl-4-acetylamino-phthalic tion. acid hydrazide (manufactured as number XLIV) could not even be measured with the apparatus used in these tests.

Through oxidation of the hydrazides with NaClO solution and measuring of the nitrogen quantities generated within certain time intervals the so-called oxidation rate may be determined; a certain relationship was found to exist between the latter and the luminescence intensity. All substances with low luminescence displayed a (more or less marked) deceleration in their nitrogen generation; an extreme case was presented by the dimethyl compound XLV which neither showed luminescence not nitrogen generation. On the other hand, a high oxidation rate does not constitute a criterion for occurrence of luminescence; e.g. no light emission is shown by the nitro-phthalic acid hydrazides, despite the fact that their oxidation progresses as rapidly as that of Luminol.

ZELLNER and DOUGHERTY included in their investigation the amino-benzoyl hydrazides and showed that open hydrazides may also display luminescence; o-amino-benzoyl hydrazide shows well visible light emission, while that of the m-derivatives is hardly perceptible and the p-derivative emits no light at all. Further examples of open hydrazine derivatives showing luminescence are given by isonicotinic acid hydrazide, N,N'-di-isonicotinic acid hydrazine and the N-isonicotinic acid-N'-nicotinic acid hydrazine; these compounds show rather intensive luminescence, according to J. KROH and J. LUSCZEW-SKI $\sqrt{287}$, in the presence of hemin.

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K. H. DREW and his colleagues have reported, in a number of publications, on the manufacture and testing of numerous phthalic acid hydrazides containing substitutions /29, 30, 31, 32, 33, 347. They investigated the influence of the substituents in the benzol ring as well as in the hydrazine rest and have developed a new theory on the mechanism of the luminescence reaction, supported by their experiments. Table 4 shows the results of a comparison of 19 derivatives of phthalic acid hydraide containing substitutions in their benzol rings.

Table 4

Luminescence of phthalic acid hydrazide derivatives, according to K. H. DREW and colleagues /327

(Luminescence intensities were compared in a Karrerporitsky photometer with the optimal conditions for Luminol (0.04 mol/l hydrazide, 12 x molar quantity NaOH, 4 drops of 6% H_2O_2 solution and 6 drops of 1% solution of hemoglobine in 0.1% nsodium lye). First measurement was made at 18° Celsius with hemoglobine addition; the compounds have been arranged in order of decreasing light intensity (column 3). A second test measurement at 100°C without catalyst yielded the results represented in column 4.)

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Phthalic acid hydrazide		Color of light emitted	with without hemoglobin	
			18 C	100 C
ĩ	3-amino	blue	1	4
VIII	3-methylamino	green-blue	2	2
XLVI	3.6-di-acetyl-amino	greenish	ं 3	1
XV	3.hvdroxy	blue-violet	4	3
XVIII	3-hydrazino	blue	5	7
XLVII	Pyromellithic acid dihydrazide	yellow	6	5
XXXV	3-acetylamino	green-blue	7	6
XIII	4-amino	blue	8	8
XLVIII	3.6-dichloro	blue-violet	9	9
XXXVII	3-chloro	blue -viole t	10	11
XL	4-acetylamino	blue	11	13
XLIX	3.5-diamino(*)	white-blue	12	12
L	3-benzoylamino	blue	13	14
LI	3-(beta-sulfo)-hy- drazino (sodium			
	salt)	white-blue	14	18
XX	Phthalic acid hydra-			
	zide	white-blue	15	10
LII	4.5-diamino(*)	blue-violet	16	16
LIII	3-nitro(*)	white-yellow	17	15 -
LIV	4.5-dichloror	blue-violet	18	17
XXX	4-nitro (*)	white-yellow	19	19

(*) Solutions of these substances are strongly colored; light intensity values noted in these cases are too low, due to autoabsorption.

The most important results from these comparisons are as follows:

a) o,p directing groups $(-NH_2, -NHCH_3, -OH, -NHNH_2,$ and also-somewhat weaker-Cl) will increase luminescence intensity; m-directing groups will have a contrary effect $(-NO_2, \text{ and } -COCH_3, -COC_6H_5, -SO_3Na \text{ in the side chains});$

b) Double substitution (disubst.) in 3,6-position will show a better effect than single substitution in only one of these positions. (The fact that XLIX forms an exception is due to autoabsorption in the red solution);

c) Substitution in the 3 and 6 positions shows a greater effect than in the 4 and 5 positions.

d) Luminescence is strongly increased by a second hydrazide ring in the molecule.

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e) Order of luminescence intensity is temperature dependent.

In addition to the comparison of luminescence values the authors have paid particular attention to the methods of synthesis (production); their reports contain a great number of exact preparative instructions showing the most feasible approach for the manufacture of many of the phthalic acid hydrazide derivatives. All syntheses are based on imides and anhydrides with respective substitutions. During reaction of hydrazine and phthalic imide as well as phthalic acid anhydride, N-amino-phthalic imide (XXIX) will form as an interim stage (via LV or LVI, respectively); after prolonged boiling it will be converted to the stable isomeric form (XX). Reversal of this procedure can only be effected through a carbonyl group; in this manner XXIX and XX will be converted (with p-anisaldehyde) into hydrazone (LVII) /297. Frequently, only one of the isomeric forms imide in reaction with hydrazine, for example, will only yield the hydrazide 3,6-dichloro phthalic imide will produce only N-amino-XV; 3,6-dichloro-phthalic imide. 3-chloro-phthalic imide with 1 mol hydrazine will form N-amino-3-chloro-phthalic imide, but with excessive hydrazine will produce the hydrazide XXXVII; the same reaction is shown by 3-amino-phthalic imide. 3-methylamino-phthalic imide, -3-hydrazino-phthalic imide and by the sodium salt of the 3-(beta-sulfo)-hydrazino-phthalic imide. Substituted phthalic acid anhydrides show a strong tendency toward formation of the six-numbered heterocyclic ring. While only N-amino-3,6-dichloro-phthalic imide will be formed during reaction between 3,6-dichloro-phthalic acid anhydride and 1 mol of hydrazine, it will be possible to obtain the hydrazide XLVIII by increasing the hydrazine quantity to more than 2 mols. Tetrachloric phthalic acid and its anhydrides, on the other hand, will produce only N-amino-tetrachloro-phthalic imide, regardless of the hydrazine quantity This indicates that some substituents (-Cl, -NH₂) will nsed. impede formation of the six-numbered ring if they are placed in the o-position during ring linkage. No such effect is observed in the m-position; XII and LIV are formed from 4-amino-phthalic imide or 4,5-dichloro-phthalic imide, respectively, with only 1 mol of hydrazine.

The best approach for obtaining phthalic acid hydrazides with substitutions in the hydrazine rest is presented by the reaction of phthalic acid anhydride with hydrazine derivatives $\sqrt{317}$; N,N'-dimethyl-hydrazine, e.g. will produce LVIII and N,N'-dimethyl hydrazine LIX. In the reaction of methyl hydrazine with 3-nitro- or 4-nitro-phthalic acid anhydride, respectively, separation of the isomeric mixture

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through fractional crystallization and subsequent reduction the isomeric pair LX and LXI will be obtained. Positioning of the methyl group could be determined via the copper salts of the diazo compounds generated from LX and LXa by means of diazotization and coupling with beta-naphthol /33/.

Like all the other compounds produced (containing the five-numbered heterocyclic ring) LIX showed no luminescence, mither did the isomeric dimethyl compound LVIII (N-aminophthalic imides also show no fluorescence, even when derived from the fluorescent phthalic imides. All phthalic imides with an amino group in the "3" position show fluorescence, the same group in "4" position will cause absence of fluorescence. Lack of fluorescence in N-amino-phthalic imides is not attributable to the losing of the imide hydrogen but is a characteristic effect of the N-amino group; N-methyl- and N-phenyl-phthalic imide will show fluorescence).



The four nitro compounds corresponding to the monomethyl compounds LX, LXa, LXI and LXIa also show no luminescence, while LXI was the only amino compound for which nonluminescence was clearly established. The other three amino compounds showed weak but distinctly discernible luminescence. According to the authors' view this luminescence may be

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traced to impurities caused by matter not containing methyl which, in turn, may have resulted from traces of unsbustituted hydrazine in the methyl hydrazine. For the purpose of verification of this theory LX was converted into its acetyl form, re-crystallized 8 times while in this form and thereafter the acetyl group removed by hydrolysis; the product when thoroughly purified in this manner showed no further luminescence. The obvious prerequisite for occurrence of luminescence thus seems to be the fact that both nitrogen atoms in the hydrazine rest be free from substitutions.

In the luminescence mechanism theory established by H. D. DREW and R. F. GARWOOD /347 a peroxyde is substituted in place of the azoidacyl shown by ALBRECHT. The existence of such peroxyds derivatives of cyclic hydrazide is proven by the isolation of LXII and LXIII in relative pure from. A watery solution of LXII (after addition of hemoglobins) will show bright luminescence, which will still be increased after addition of alkaline matter. Evidently, an equilibrium is reached between the peroxyde and its components.

The first ste) in the reaction would thus be the conversion of the ion LXIV into the peroxyde LXV. The latter (by oxidation of the imide hydrogen) will conver to the stable intermediate product LXVI which will either re-form LXIV

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(where oxygen will be separated) or (under generation of nitrogen) will be converted into the anion of phthalic acid LXVII:



This shows that the luminescent hydrazide is not oxidized itself but only serves as the carrier of oxidation of H_2O_2 caused by the latter itself or by other oxidation agents.

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This also explains the observation that benzophenon-o.o;-dicarboxylic acid is formed during oxidation of phthali. acid hydrazide with NaClO solution, while only phthalic acid will be produced if the same oxidation is effected by H_2O_2 .

The great superiority of the luminescence intensity of Luminol compared to that of any of the other hydrazides is shown impressively in the test results compiled by A. A. PON-OMARENKO et alii /357.

Dependence of the Luminol luminescence on the pH value has been repeatedly investigated. R. WEGLER /257 already pointed out that a strongly alkaline Luminol solution containing hemin will emit much brighter light than in weak alkaline concentration. Measurements performed by C. N. ZELL-NER and G. DOUGHERTY /277 indicate that a maximum of luminescence intensity is reached at a sodium lye concentration of 0.015 n; higher alkaline concentrations will cause the intensity to decrease again. According to A. BERNANOSE /367, nearly linear dependence exists on either side of this maximum located at a pH value of 10.5 between the logarithm of the total light emission and the pH value. A. A. PONOMARENKO et alii /377 are even using the luminescence beginning at a pH value of 8 to 8.5 as an indicator in acidimetric titration.

Table 5

Luminescence of phthalic acid hydrazide derivatives, according to A. A. PONOMARENKO et al. /357

(Comparison of luminescence values was made with a colorimeter, where the intensity shown by substance I was set equal to 100. Column 2 shows the pH value above which luminescence was observed.)

Phthalic acid hydrazide		Minimal pH value	Relative in- tensity 100	
I 3-amino-		8.0 8.5		
XIII	4-am1no-	7.7 7.8	4.5	
LIII	3-nitro-	8.4 8.5	12.0	
XXX	4-aitro-	8.4 8.5	1.5	
XXXVII	3-chloro-	8.8 9.2	0.22	
LXVIII	4-chloro-		0.08	
LIV	4.5-chloro-	8.5 8.7	0.20	
LXIX	Tetrachloro-	9.0	0.53	

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Intensive study was devoted to the influence of numerous catalysts on luminescence intensity. H. O. ALBRECHT /22/ had already observed that luminescence will be intensified by simultaneous action of H_2O_2 and NaClO or of H_2O_2 and X_{\odot} [Fe(CN)6_7; he also reported on the intensification causcd by blood. K. GLEU and K. PFANNSTIEL /247 noted that crystalline hemin surpasses blood by far in effectiveness and causes a brighter and more prolonged emission; R. WEGLER $\sqrt{257}$ reports the same results for radish and horse radish juice. Many other ferruginous complex compounds besides hemin have been shown to have a favorable effect on luminescence, e.g. hemoglobin $\sqrt{387}$, Ferritin and methemoglobin $\sqrt{397}$, Iron-chlorophyll derivatives /407, iron-phthalic cyanine /387, the inner iron complex salts of phthiocol /417 and the azomethines resulting from substituted slicylic aldehydes and ethylene diamine /427. TOKUICHI TSUMAKI et al. /437, therefore, recommend utilization of the positive influence on Luminol luminescence as a constitutional criterion for inner complex salts ... Other compounds examined, e.g. copper-ammonia compounds /447, ruthenium chloride and vanadium sulphate /457, Fe⁺⁺-, Mn⁺⁺-, Ce⁺⁺- and Co⁺⁺⁺-ions /467 displayed a (more or less) smaller catalytic effectiveness compared to the inner complex salts.

Despite the fact that many of the luminescence phenomena intensified by catlysts have been described as extremely bright and K. PFANNSTIEL /477 has even been able to take photographs in luminescent light, and the actual light quantity yielded during emission is relatively small, as shown by measurements made by L. HARRIS and A. S. PARKER /487. The authors created a very small zone of reaction (in a very precisely described apparatus), collected the light emitted by this nearly point shaped source by means of a quartz lense and projected this light beam onto the surface of a highly sensitive micro-thermo element which was connected to a galvancmeter. The galvanometer-thermo element system was calibrated by means of standard light sources. Maximal yield in light quants measured with this method amounted to app.ox1mately 0.3%. Thorough investigation of agreement between the spectra of luminescence and fluorescence will yet have to be carried out. Success of this research project will probably depend to a large extent on the choice of the correct catalyst. It should be pointed out in this connection that not only the intensity but also the duration of luminescence is increased by some catalysts which fact may provide a temporal margin sufficient for the measuring of spectral distributions. A catalyst of particular suitability might be the inner cobalt-(II)-complex salt of phthiocol: it causes intensive luminescence for a duration of 3 hours /417. Luminescence will continue even for several days under the influence of radish and

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horse radish juices $\sqrt{257}$.

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