OH⁻ attached to the triangle borons (Nos. 1 and 4). This structure explains very nicely the fibrous nature of the excellent cleavages (100) and (001), since the bonds holding neighboring chains together are much weaker than the bonds in the chains.

The oplyanion in kernite can be formed from that of borax as indicated by the equation (*3*):

$$n[B_4O_5(OH)_4]^{2-} = nH_2O + [B_4O_6(OH)_2]_n^{2n-}$$

This transformation is not a simple dehydration of the sort that occurs, for example, in the inyoite-colemanite transformation since a boron-oxygen bond must also be broken (*5*). The breaking of the bond would require considerably more energy than simple dehydration and explains the difficulty in forming kernite from borax.

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5. I thank C. L. Christ and Joan R. Clark for comments regarding the transformation.

31 October 1966

Ozone-Induced Chemiluminescence of Organic Compounds

Abstract. *Chemiluminescence was observed when organic compounds in a dry state, as well as in organic solvents, were exposed to ozone in oxygen. The peak intensity of the light emission is related quantitatively to the amount of compound. Many compounds can be identified by this method, and some are listed. Sensitivity of detection approximates that of fluorescence methods and extends to less-than-nanogram quantities for some compounds. Ozone-induced chemiluminescence of compounds in acetone solution was less sensitive than it was in the dry analysis.*

Although there have been reports (*1, 2*) of luminescence induced with ozone, this phenomenon has not been studied extensively. We have been impressed by the brilliant chemiluminescence evident in solutions of certain organic compounds into which ozone (*3*) had been bubbled. For instance, the dye safranin (10 mg/ml in acetone) emitted enough light to read by when exposed to ozone. It occurred to us that this chemiluminescence might be used to detect and measure small amounts of chemicals, and we now report on the examination of some organic compounds in apparatus of our own design.

In order to find out if ozone would induce visible chemiluminescence a large number of organic compounds were examined by adding 50 mg of each compound to about 5 ml of acetone and observing whether light was produced as ozone was bubbled through the solution, the experiment being conducted in a darkened room (Table 1). We also observed the effect of the test substance in acetone solutions containing potassium hydroxide pellets, which have been reported to promote chemiluminescence on heating (*2*, pp. 18 to 21), and the effect of other additives and solvents as well (Table 1). In the cases of anthracene, 4-methylumbelliferone, and kynurenic acid, KOH altered the results from a "negative" to a "bright" reaction with ozone. Generally, where a positive result was obtained, the chemiluminescence persisted in solution as much as a minute after ozone bubbling was stopped.

Next, it was desirable to devise a method whereby the amount of light could be quantitatively related to the amount of material present. We found that it was not necessary to have the compounds in solution in order to observe the chemiluminescence; solutes al-

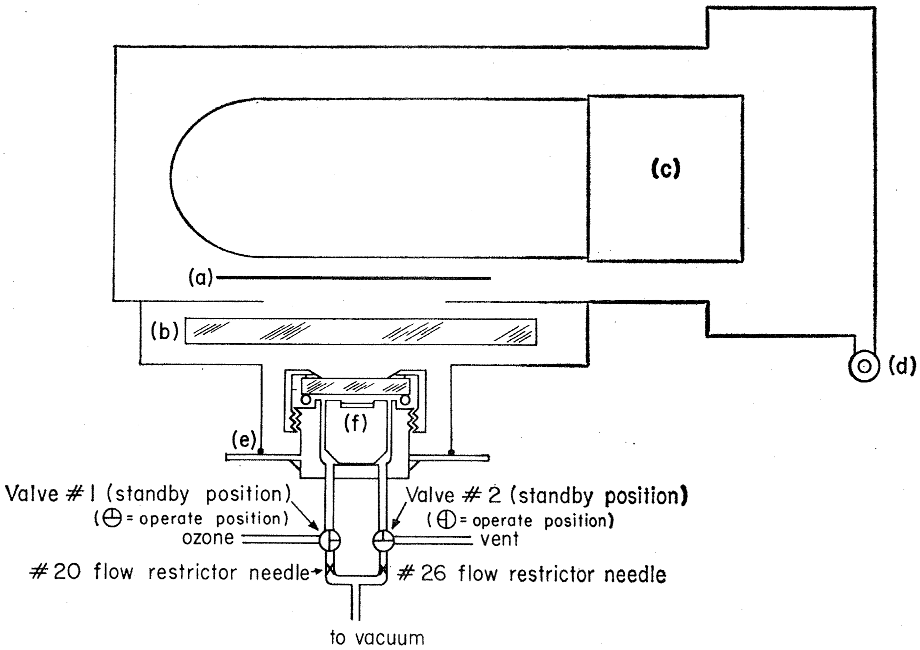

Fig. 1. Arrangement of optical parts for light measurement during exposure of samples to ozone. *a*, Shutter; *b*, optical filter if required; *c*, photomultiplier (RCA 1P28); *d*, hinge, permitting raising and lowering of the phototube housing assembly; *e*, soft rubber light seal; *f*, stainless steel holder has substrate in recess. Ozone is admitted to sample chamber when the valves are in the "operate" position.

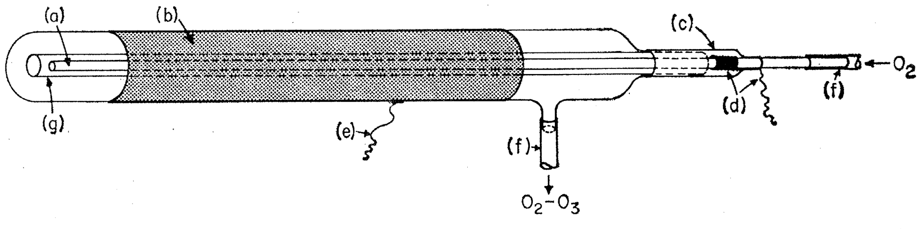

Fig. 2. Ozonizer. *a*, Stainless steel tube 1.5 mm in diameter (oxygen inlet and high-voltage electrode); *b*, aluminum foil (ground electrode) on glass tube (1 × 10 cm); *c*, shrinkable Teflon glass-to-metal seal; *d* and *e*, leads to Tesla coil and ground; *f*, 2.25-mm Teflon tubing; *g*, glass-tube centering (*a*).

lowed to dry on various solid media emitted light when exposed to a stream of ozone.

This chemiluminescence was measured with the apparatus (Fig. 1). The sample, in 1 μl of acetone solution, was allowed to dry on a disc (6.3 mm diameter) of silica-gel film (*4*) in the sample chamber. The chamber was made gas-tight by a sealed quartz window. The multiplier phototube housing was hinged to permit access to the sample chamber and was shielded from ambient light by a soft rubber seal. Ozone was produced by flowing oxygen passed through the electrical discharge of a Tesla coil (*5*) in an ozonizer (see Fig. 2).

When the valves were turned to the "operate" positions, a stream of ozone in oxygen flowed through the sample chamber at 2.5 ml/sec at 1.25 atm abs. Used gas was drawn off by laboratory vacuum through a constriction in the line. The light produced was detected by a type 1P28 phototube operated at 900 volts, amplified (*6*), and recorded (*7*) as a function of time. Under these conditions, the chemiluminescence reached a peak in less than a second, then decreased exponentially, apparently owing to exhaustion of the sample by reaction with ozone. The peak height of the phototube signal was related to the amount of compound present when plotted on a log-log scale (Fig. 3). (It would probably be more correct to integrate the curve of phototube response plotted against time, but our method with the peak heights was satisfactory since the response curves were quite similar for the chemiluminescent substances tested.) The results (Fig. 3) suggested immediately that ozone-induced luminescence could be used as a way of determining substances in nanogram amounts. The sensitivity of the method is limited by the signal-to-noise ratio, the noise arising primarily from light emitted by the silica-gel substrate.

If we arbitrarily define the lowest detectable quantity of compound as that amount which results in a signal-to-noise ratio of 1 (where the blank represents an anode current of 0.06 na), the sensitivity of the method can be quantitatively described (Table 2) for a number of compounds. The detection limit is in the nanogram and subnanogram range, and therefore the method is approximately as sensitive as many fluorescence assays (*8*).

Table 1. Brightness of ozone-induced chemiluminescent solutions. The mixture of O_3 and O_2 was bubbled through solutions containing approximately 10 mg/ml of compound. Chemiluminescence intensity was estimated by subjectively comparing the luminescence with the glow of the ozonizer due to internal electric discharge. The glow of the discharge was such that it could be discerned in the dark after a minute or two of accommodation, approximately 0.005 millilambert (*10*). The intensity was measured relatively, as follows: VBrt, brighter than ozonizer; Brt, comparable to ozonizer; RBrt, rather bright; less bright than ozonizer; WK, positive chemiluminescence; VWK, near detectable limit; Neg, not visible. The code for the source of the compounds accompanies Table 2. The abbreviation KOH(s) indicates solid potassium hydroxide. ZnO—Green Seal 8, New Jersey Zinc Co.

Compound	Source (code)	In acetone	Other conditions; remarks
Aesculin	J	Brt	RBrt in acetonitrile—slow response
Anthracene	G	Neg	RBrt in acetone + KOH(s)
Anthranilic acid	D	VWK	Brt in acetone+KOH(s)
3-Hydroxyanthranilic acid	D	Neg	RBrt in acetone +KOH(s)
5-Aminonaphthalene-1-sulfonic acid (dissolved in 4 drops H_2O)	G	Neg	RBrt in acetone +KOH(s)
Benzoin	K	Brt	Neg in pyridine
Benzoin acetate	G	Neg	Neg in water
9,10-Benzophenanthrene	K	WK	
Biphenyl	G	Neg	Neg in pyridine
Bovine serum albumin	G	Neg	Neg in ethanol
Calcofluor-Y & RWP	B	RBrt	RBrt in water
Calcofluor-5-BT & LD	B	WK	
Catechol	G	Neg	WK in pyridine +KOH(s)
O-Chlorophenol	J	WK	
Chrysene	G	Brt	
Coriphosphine	E	WK	
Creatinine-ZnCl	O	Neg	RBrt in pyridine + ZnO
5-Dimethylaminonaphthalene-1-sulfonyl chloride	D	Neg	WK in pyridine +KOH(s)
Dinitrophenol	J	Brt	
Dopamine	D	Neg	WK in acetic acid + ZnO
Epinephrine	J	WK	
Epinephrine bitartrate	P	Neg	RBrt in acetic acid + ZnO
Hematoxylin	N	Brt	
Hydroxybenzaldehyde	G	WK	
Hydroxybenzoic acid	K	RBrt	
2-Hydroxyquinoline-4-carbolic acid	K	WK	
Isokynurenic acid	D	RBrt	Test made in 10 drops solution KOH
Kynurenine sulfate	D	Neg	WK in ethanol
Kynurine (4-hydroxyquinoline)	D	RBrt	VBrt in acetone + KOH(s)
Methylene blue	L	VBrt	
4-Methylumbelliferone	G	Neg	VBrt in acetic acid + ZnO
Morin (dissolved in 3 drops alum solution)	E	Neg	Brt in acetic acid + ZnO
Naphthalene	L	VWK	WK in pyridine
Neutral red	N	WK	
Niacin	M	Neg	WK in pyridine + ZnO
Nitrophenol	J	Neg	RBrt in pyridine + ZnO
Phloridzin	O	Neg	WK in pyridine
Pontacyl BY-8G	H	VBrt	
Pontacyl BP-B	H	Brt	Rapid fade
Pyrogallol	G	Neg	RBrt in pyridine + KOH(s)
Pyronin-B	E	RBrt	
Quinine	D	WK	
Rose Bengal	N	VBrt	
Rhodamine B, 3GO & 6GO	E	RBrt	VBrt in pyridine or acetic acid
Riboflavine (dissolved in 10 drops KOH solution)	O	Neg	Brt in pyridine + ZnO
Serotonin	D	Neg	Brt in acetic acid + ZnO
Sanguinarine nitrate	D	WK	
Thioflavine-S	E	Brt	
Thioflavine-T	E	Neg	RBrt in acetic acid
Trypaflavine	N	RBrt	
Tryptophan (dissolved in 10 drops H_2O)	D	WK	
Tyrosine	O	Neg	WK in pyridine

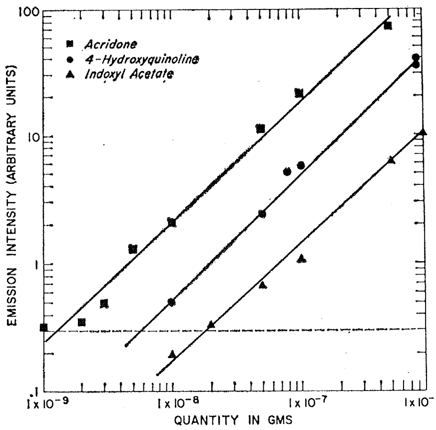

Fig. 3. Chemiluminescent emission on arbitrary units as a function of quantity of compound. 1 unit = 0.2 na at the multiplier anode with 900 volts at the photocathode. Dotted line indicates system noise level. Each point indicates the mean of three determinations.

Table 2. Quantitative estimation (nanograms) of compounds in acetone, or on silica-gel films prepared by drying of acetone solutions; signal-to-noise ratio was one.

Compound	Source (code)*	Amount detected (ng)	
		Dry	Liquid
Acridine orange	E	2	20
Acridone	G	2	10
2-Aminoanthracene	Q	5	1000
3-Aminophthalic acid hydrochloride	G	20	100
8-Aminonaphthalene sulfonic acid	G	5	50
Dimethylamino-benzaldehyde	J	10	500
3,5-Dinitrophenol	Q	6	500
Eosin-Y	F	0.4	100
Fluorescein	G	4	1000
4-Hydroxyquinoline	D	5	100
Iodeosin	G	1.0	50
Indole	J	30	100
Indoxyl acetate	N	20	50
Janus green-B	N	1.0	10
Luminol	G	0.8	50
3-Methyl indole	P	700	1000
Phenosafranin	N	0.001	0.02
Primuline	E	3	50
Quercetin	D	3	100
Rhodamine-B	N	0.1	5
Rubrene	A	3	5000
Safranin bluish (methylene violet)	N	0.001	0.05
Safranin-O	N	0.02	0.1
Salicylic acid	J	300	500
Thionin	E	2	1000

* Commercial sources of compounds conform to the following code: A, Aldrich Chemical Co.; B, American Cyanamid Co.; C, Armour & Co.; D, California Corp. for Biochem. Res.; E, Chroma-Gesellschaft, Stüttgart; F, CIBA; G, Eastman Organic Chemicals; H, E. I. duPont de Nemours & Co.; J, Fisher Scientific Co.; K, K & K Laboratories, Inc.; L, Matheson Coleman & Bell; M, Merck & Co.; N, National Aniline; O, Nutritional Biochemicals Corp.; P, Sigma Chemical Co.; Q, Synthesized.

To confirm the greater sensitivity of the "dry" method, we put the same quantity of sample (1 μl) in 0.2 ml of acetone in the sample holder without the silica gel. The ozone entering the sample chamber was conducted via a piece of stainless-steel hypodermic tubing under the surface of the acetone solution so that the luminescence of the solution was measured by the phototube. The total sample required to match the luminescence of the dry sample was estimated to the nearest order of magnitude and is listed in the last column of Table 2.

While fluorescence techniques are extremely useful for analysis of a large number of compounds, ozone-induced chemiluminescence may be useful in special cases where the fluorescence is rather weak and the background interference from scattered light is a problem, as in chromatogram scans. In the chemiluminescence assay system the phototube can be placed very close to the sample to receive a large percentage of the emitted photons; whereas in fluorescence assay one must consider separating the detector from the exciting light by filters, special placement of parts, and other means.

Substances other than silica-gel film have been tested for suitability as substrates; among them were various papers, plastics, metal foils, and glasses, but these were less satisfactory because of a high blank or a low output of light from the sample. Apparently the substrate medium must be porous enough to have a large surface area onto which the sample can be dispersed and made accessible to the ozone.

The color of the ozone-induced light emission generally is similar to that of the fluorescence excited with ultraviolet light. We are examining this point more closely by obtaining spectra of the solution chemiluminescence with a spectrophotofluorometer (Aminco-Bowman equipped with a liquid-nitrogen-cooled photomultiplier). So far results show that the spectrum of the emission from several compounds is indeed similar to the fluorescence spectrum, but some compounds show changes as ozone is bubbled through and as the compound is transformed to other chemiluminescent materials before exhaustion of the effect.

The chemiluminescence studied was produced with ozone in a stream of pure oxygen. It has been almost axiomatic that oxygen quenches luminescence (*9*), and it is perhaps for this reason that not much study has been directed toward ozone-induced chemiluminescence.

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3. "Ozone" as used here implies the few percent of ozone produced in oxygen exposed to an electrical discharge.
4. Eastman Chromagram Sheet, Type K301R2.
5. Lepel High Frequency Laboratories, Inc., Type E7896.
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10. Metrastar exposure meter, Metrawatt A. G. Nurnberg (U.S. Distribution: Leitz Wetzlar).
11. B. Bush of Washington Sanitarium and Hospital on a fellowship supported by American Instrument Company made most of the preparations and measurements. We thank all concerned.

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Suggested Revision for West Mexican Archeological Sequences

Abstract. *A review of the radiocarbon dates and published and unpublished archeological data from the West Mexican states of Sinaloa, Nayarit, Jalisco, and Colima has resulted in a revised tentative chronology for West Mexico.*

West Mexico, while definitely peripheral to cultural developments in the Valley of Mexico, is an area of considerable importance to any understanding of Mesoamerican and U.S. Southwestern culture history. During the Early Classic Period of the Valley of Mexico, Indians in the states of Colima, Jalisco, and Nayarit developed an elaborate burial complex of shaft-tombs which contained multiple burials and hollow clay figurines. This was a development generally unrelated to that in the Valley of Mexico—in fact it resembled developments in Central and Northern South America. During the Postclassic Period, West Mexico appears to have