For analysis by the present procedure a substance must have a hydroxyl group on the carbon atom adjacent to the primary carbinol group, and must yield no product precipitating with the dimedon reagent except formaldehyde. (The latter point may be checked conveniently by the sharp and characteristic melting point of the formaldehyde–dimedon compound.) Unsatisfactory for one or both of the above reasons are monoacetone glucose and α-methyl glucoside. The low value obtained with fructose (86% theory) remains to be explained.

TABLE I

FORMALDEHYDE FROM CARBOHYDRATES BY OXIDATION WITH PERIODATE

Substance	Mg. taken	Mg. ppt.	M. p. ppt., °C.	% theor. CH_2O
d-Glucose	18.0	29.6	189–90	101.3
		29.3	....	100.0
		29.0	....	99.3
		29.4	....	100.6
d-Galactose	18.0	28.8	189–90	98.8
		29.1	....	99.7
d-Xylose	15.0	28.8	188–90	98.8
		29.1	....	99.7
Mannitol	9.1	29.5	189–90	101.0
		29.5	....	101.0
2,3-Dimethyl glucose	20.8	29.2	189–90	100.0
		28.6		98.0
2,3,4,6-Tetramethylglucose	23.6	0.0	....	...
		0.0	....	...
Fructose	9.0	25.1	189–90	86.0
		25.1	....	86.0
Monoacetone glucose	22.0	30.2	187–90	(103.3)
		33.6	....	(115.0)
α-Methyl glucoside	19.4	1.9	200–210	...
		2.7	....	...

Reagents and Procedure

Periodic acid solution, approximately 0.3 molar, is prepared by dissolving 7.0 g. of $HIO_4 \cdot 2H_2O$ in water and making up to a volume of 100 cc. After standing overnight the solution is decanted from a small yellow precipitate.

Sodium arsenite solution, 1.2 *N*, is prepared by dissolving 20.4 g. of pure Na_2HAsO_3 in water and making up to 100 cc. This solution should completely reduce an equal volume of the periodic acid reagent in the presence of 0.1–0.2 *N* hydrochloric acid.

The dimedon solution contained 80 mg. of 5,5-dimethyldihydroresorcinol per cc. of 95% alcohol. We used *N* sodium bicarbonate solution, *M* sodium acetate solution and *N* hydrochloric acid solution.

The following directions provide sufficient reagent to oxidize 0.1 mmole (18 mg.) of hexose and to precipitate 0.1 mmole (3 mg.) of formaldehyde. The substance to be analyzed may be dissolved in water or dilute acid, but not more than 0.6 mequiv. of free acid or buffer should be introduced with the sample. The sample dissolved in 2.0 cc. of solution is placed in a test-tube and treated with 2.0 cc. of *N* sodium bicarbonate and 2.0 cc. of periodic acid reagent. The solutions are mixed and allowed to stand at room temperature for one hour (with glucose the reaction is nearly complete in ten minutes). Then are added in turn with thorough mixing 3.0 cc. of *N* hydrochloric acid and 2.0 cc. of sodium arsenite solution. When the precipitate and yellow color have completely disappeared 2.0 cc. of sodium acetate solution and 1 cc. of dimedon reagent are added. The test-tube is then placed in a boiling water-bath for ten minutes, after which it is allowed to stand at room temperature for at least one hour (or it may stand at room temperature overnight without heating). The precipitate is then filtered on a weighed sintered glass filter stick, washed thoroughly with water, and dried in a current of dry air at 85–95° for twenty minutes. (If this temperature is exceeded there will be loss due to sublimation.) The crystalline precipitate should melt sharply at 189–190° cor. The weight of formaldehyde is equal to 0.10274 times the weight of the precipitate.[4,5]

(4) D. Vorländer, *Z. anal. Chem.*, **77**, 241 (1929).

(5) M. V. Ionescu and C. Bodea, *Bull. Soc. Chim.*, [4] **47**, 1408 (1930).

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Chemiluminescence of Luminol Catalyzed by Iron Complex Salts of Chlorophyll Derivatives

BY ERICH SCHNEIDER

Cook[1] demonstrated that the chemiluminescence of luminol (3-aminophthalhydrazide) which takes place when the peroxide of luminol formed during oxidation is decomposed,[2] occurs with iron phthalocyanines as catalysts as well as with catalysts like hemin, hemoglobin, and catalases (iron porphyrin complex salts) as reported by earlier investigators. Other metal phthalocyanines have a weaker or no catalytic action upon the reaction.

I wish to report that iron chlorophyll derivatives have the same catalytic effect, and have tested a number of these and related compounds as to their ability to catalyze the chemiluminescence of luminol. Iron chlorin e_6, iron pheophorbide a and iron bacteriochlorin e_6 showed strong luminescence; copper chlorin e_6, copper deuteroporphyrin and sulfonated copper phthalocyanine a weak luminescence; chlorophyllin a still weaker or no luminescence; pheophorbide, chlorin e_6, deuteroporphyrin and coproporphyrin showed no luminescence.

(1) A. H. Cook, *J. Chem. Soc.*, 1845 (1938).

(2) H. D. K. Drew and R. F. Garwood, *ibid.*, 791 (1938).

The compounds were added to the test solution (0.1 g. of luminol dissolved in 100 ml. of 5% sodium carbonate solution, to which was then added 20 ml. of 3% hydrogen peroxide) in the form of aqueous solutions of their sodium salts. The iron complex salts thus naturally were present in the *alkaline* solution as "hematins," the halogen atom having been replaced by hydroxyl.

Iron bacteriochlorin e_6 trimethyl ester (bacteriochlorin e_6 trimethyl ester-hemin) which is not reported in the literature so far, was prepared by the following method: bacteriochlorin e_6 trimethyl ester derived from bacteriochlorophyll[3] was treated in the usual way (under nitrogen, for half an hour on a steam-bath) with a freshly prepared ferrous acetate–sodium chloride solution in glacial acetic acid.[4] The reaction mixture was poured into chloroform and the chloroform solution was washed free of acetic acid and excess iron. It was then evaporated to dryness, the residue was dissolved in hot glacial acetic acid containing a little sodium chloride, and the solution was filtered. On standing the bacteriochlorin e_6 trimethyl ester-hemin crystallized.

Absorption Spectra.—In pyridine–ether or in chloroform: three maxima at 695, about 596, 543.5 $\mu\mu$ and an end absorption at the blue end of the spectrum. The first maximum of absorption is very intense, the second one very weak (in pyridine–ether almost invisible). Sequence of intensities: I; III, II. In glacial acetic acid–ether (1 + 1): 686, 623 $\mu\mu$. II, I. The solutions do not fluoresce.

A solution of a sodium salt of bacteriochlorin e_6-hemin, as used in the chemiluminescence test, was obtained by shaking the ester at room temperature with 1 *N* sodium hydroxide in methanol. This brought enough sodium salt (mono-, di-, or tri-sodium salt ?) of bacteriochlorin e_6-hemin in solution. The solution was prepared freshly each time for the luminescence test, as side reactions (dehydrogenation ?) occur on standing. It is not possible to saponify the ester by refluxing it in alcoholic sodium hydroxide, as the complexly bound iron is partly eliminated and other side reactions take place.

Chlorin e_6-hemin was prepared from chlorin e_6 with ferrous acetate–sodium chloride in glacial acetic acid and worked up over chloroform as described above. (The yield of crystalline material is very poor.) The absorption spectrum is identical with that of the chlorin e_6 trimethyl ester-hemin given by Fischer and Wunderer[5] the main absorption maximum being at 619 $\mu\mu$ (chloroform solution).

From what was known it was to be expected that those substances free of complexly bound metal (pheophorbide, chlorin e_6, deuteroporphyrin, coproporphyrin) would have no catalytic effect. Furthermore, in view of the fact that Cook[1] found a weak catalytic action of a number of metal phthalocyanines other than iron phthalocyanine, it is not surprising that sulfonated copper phthalocyanine and copper deuteroporphyrin also exhibit such weak catalytic properties. However, a catalytic effect as strong as that of hemin could not have been postulated from iron complexes of such chlorophyll derivatives which are not porphyrins but have the phorbine structure[6] (dihydroporphin structure with cyclopentanone ring) as iron pheophorbide, or have the chlorin structure (dihydroporphin structure) as iron chlorin e_6, or even have the dihydrochlorin structure (tetrahydroporphin structure) as iron bacteriochlorin e_6. With these results in mind, the weak catalytic effects of chlorophyllin (magnesium complex) and copper chlorin e_6 only complete the picture.

The iron complexes of chlorophyll derivatives which were found to have a strong catalytic action have the coördinative association of the iron atoms with four pyrrole nitrogen atoms as found in hemin or iron phthalocyanine, which configuration obviously is sufficient to make the substance potentially catalytically active, other structural factors (side chains, cyclopentanone ring, degree of saturation, di- and tetrahydroporphin structure) having no influence.

Chemiluminescence also has been observed by Helberger and Hevér[7] and by Rothemund[8] when metal complexes of porphyrins were heated with organic solvents containing peroxides. However, whereas these authors report no or a very weak effect of iron and copper complexes of porphyrins and a strong catalytic action of magnesium (and zinc) complexes, the chemiluminescence (peroxide decomposition) of luminol is very strongly catalyzed by iron complexes and only very little by the magnesium complex chloroprophyllin. Furthermore, whereas the luminescence observed by Helberger and Hevér and by Rothemund goes on simultaneously with a rapid decomposition of the catalysts, the iron chlorophyll derivatives catalyzing the luminescence of luminol are destroyed so slowly that it is hard to observe their decomposition.

(3) H. Fischer, R. Lambrecht and H. Mittenzwei, *Z. physiol. Chem.*, **253**, 38 (1938).
(4) H. Fischer and H. Orth, "Die Chemie des Pyrrols," Vol. 2, II, **1940**, p. 332.
(5) H. Fischer and A. Wunderer, *Ann.*, **533**, 241 (1938).
(6) H. Fischer and H. Orth, ref. 4, Vol. 2, II, 1940, p. 41.
(7) J. H. Helberger and D. B. Hevér, *Ber.*, **72B**, 11 (1939).
(8) P. Rothemund, THIS JOURNAL, **60**, 2005 (1938).

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Xylyl Methyl Carbinols

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Levene[1] proved the structures of secondary alcohols, obtained from the reaction of phenyl-

(1) Levene and Walti, *J. Biol. Chem.*, **90**, 86 (1931).