of these sugars and their lactones are principally due to their common element of structure which is within the lactonic ring, and that the configuration, weight, etc., of the groups which they do not possess in common have only a minor influence on the rotatory powers. Since such is the case it is hardly possible to agree with Tollens that the configuration of any of the groups below the ring can be decided from the rotations of the sugars. The only way at present known by which the configuration of the doubtful $CH_3.CHOH$ group of fucose and rhodeose can be determined is from a knowledge of the sign of the rotation of the methyl tetronic lactones which should be yielded by these sugars. This method has been used in determining the configuration of the similar group in the related methyl pentose, rhamnose,[1] but the methyl tetronic lactones from fucose and rhodeose have not yet been prepared, and the difficulty of obtaining these rare sugars in quantity prevents me from attempting the preparation of these substances.

To summarize, the structure of the antipodal sugars fucose and rhodeose has been determined by three independent methods. In each of these the fact that fucose yields *d*-trihydroxyglutaric acid on oxidation, and rhodeose, *l*-trihydroxyglutaric, is used to limit the possible structures to forms I and II. A choice between these forms is then made, according to the first method (Votocek's) from the fact that Bertrand's sorbose bacillus does not attack rhodeitol, according to the second (Tollens' and Votocek's) from the fact that the oxidation of fucohexonic and rhodeohexonic acids does not yield crystallin mucic acid, and according to the third method, from the fact that rhodeonic and rhodeohexonic lactones are of strong negative rotations. The three methods give the same conclusion, namely, that rhodeose has the structure I and fucose the antipodal configuration. The configuration of the first asymmetric group of these sugars $CH_3.CHOH$ remains entirely undetermined, but could doubtless be decided from a knowledge of the rotatory powers of the methyl tetronic lactones from fucose and rhodeose, which however have not yet been prepared on account of the difficulty of obtaining the sugars.

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LUCIFERESCEINE,[2] THE FLUORESCENT MATERIAL PRESENT IN CERTAIN LUMINOUS INSECTS.

BY F. ALEX. MCDERMOTT.

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In 1909, Dr. W. W. Coblentz,[3] of the U. S. Bureau of Standards, an-

[1] THIS JOURNAL, 31, 348.

[2] In my own notes I have adopted this name as representing the fluorescent

nounced that during the course of his work with Dr. Ives on the luminous efficiency of the firefly, *Photinus pyralis*,[4] he had discovered that this insect contains a substance whose solutions possess a bright blue fluorescence, and that the spectrum of the fluorescent light of such solutions is complementary to that of the emitted light of the insect. At this time I was engaged with Professor Joseph H. Kastle, of the University of Virginia (then Chief of the Division of Chemistry of the Hygienic Laboratory), in a study of the effects of various chemical reagents on the luminous tissue of the firefly,[5] and after some correspondence with Dr. Coblentz, took up a study of the chemical behavior of this fluorescent substance, with the view of determining its nature. As yet the work is by no means complete, owing partly to the lack of sufficient material, and hence the following paper will present only certain qualitative results which have been obtained on the small amount so far available; it is hoped to secure a larger quantity of the fluorescent material during the coming summer, and extend the inquiry.

Since its original discovery by Dr. Coblentz in *Photinus pyralis*, it has been found by him in *Photinus corruscus* and in *Photuris pennsylvanica*, all species of Lampyridae. I have been unable to verify the presence of this substance in *Photuris pennsylvanica*, but have, in addition, found it in *Photinus scintillans*. All of these insects possess a strong, rather rank, characteristic odor, and the Photinini exude, when irritated, a sticky, milky fluid, which hardens in the air; in this secretion the fluorescent material is largely present. These insects, and especially their larvae, are carnivorous, feeding on snails, earth-worms, etc.

In addition to the Lampyridae above noted, Dubois[6] records that there is present in the juices of the cucuyo, the large Cuban elaterid firefly, *Pyrophorus noctiluca*, a substance whose solutions show a blue fluorescence. Dubois considered this substance to be essential to the photo-

substance derived from the firefly, the first three syllables agreeing with Dubois' "Luciferine" and "Luciferase" and the last three being merely the termination of "fluorescein" with "*e*" added to make the termination representing an alkaloid. Whether the formation of the name is justified by the constitution of the substance remains to be seen; I have no intention of encumbering the nomenclature of organic chemistry with a new variation.

Right here I might say that there is an unfortunate confusion in the minds of many people between "fluorescent" and "phosphorescent" materials, especially when mentioned in connection with the firefly, and that the substance which forms the basis of this paper is not the substance which produces the light of the Lampyridae and other photogenic insects.

[3] Coblentz, *Physik. Z.*, 10, 955–6 (1909). See also Ives and Coblentz, 2.

[4] Ives and Coblentz, *Bulletin of the Bureau of Standards*, Washington, **6**, 321–36 (1910).

[5] Kastle and McDermott, *Am. J. Physiol.*, **27**, 122–51 (1910).

[6] Dubois, *Bull. soc. entomologique de France*, **11**, 1–275 (1886).

genic process of the insect, and ascribed to the actinic power of the blue rays in its fluorescent light, the actinic power of the emitted light of the insect. The substance itself he considered as analogous to esculin, indeed his work might be taken to indicate that he considered it to be esculin.[1] Dubois observed that the substance he extracted from the cucuyo acquired its maximum intensity of fluorescence at wave length 0.391μ in the ultraviolet. Without developing this line further, it will suffice to say that the fluorescent solutions derived from the material present in the Lampyridae, fail to give any of the reactions for esculin given in Watts' Dictionary, or any that I have found elsewhere. The cucuyo belongs to a group of beetles entirely unlike the Lampyridae, and known as the Elateridae; it is herbivorous, living largely on sugar cane. In view of these differences there may be some reasonable doubt as to whether the substances found in the two groups of insects are identical; I have had no opportunity to test this point. It is nevertheless remarkable that two insects as widely separated in classification as *Pyrophorus noctiluca* and *Photinus pyralis*, and having nothing else in common except the possession of the photogenic function, should both yield substances whose solutions show a blue fluorescence.

I have extracted the margined soldier beetle (*Chauliognathus marginatus*), a near relative of the Lampyridae and formerly classed with them, but which is diurnal and non-luminous, and obtained an extract containing a yellow dye, and non-fluorescent. Extracts of the local species of cantharid (*Epicauta pennsylvanica*) also yielded yellow dyes, but no fluorescence; and a single species of carabid (*Chlaenius sericeus*), which has a strong, characteristic odor, failed to give either a dye or fluorescence. In all these cases, alcohol was used as the extractant. In a private communication, Mr. T. Davidson Arndt, of Trinidad, B. W. I., states that he has observed independently "the production by most insects of substances which under the X-rays are strongly fluorescent."

Fluorescent substances are by no means uncommon, and in spite of the great amount of work that has been done upon it, it is pretty difficult to ascribe to any special group or arrangement the production of fluorescence. Fluorescent substances of various natures are not infrequent among lower animals (see von Fürth),[2] and the fluorescent pigments known as lipochromes occur in many Coleoptera. Luciferesceine, however, appears to offer objections to including it in any of the classes of fluorescent materials so far found in animal organisms. Of the insects named as containing luciferesceine, all are luminous and nocturnal in

[1] Esculin (aesculin) is a glucoside found in the horse chestnut, which on hydrolysis yields glucose and esculetin (dihydroxycoumarin). In alkaline alcoholic and aqueous solutions it shows a strong blue fluorescence.

[2] von Fürth, *Vergleichende chemische Physiologie der niederen Tiere*, Jena, 1903, 551–60.

habit, except *Photinus corruscus*; this species, although a true lampyrid, has become diurnal in habit, and has lost its luminous function, except, it is said, while in the larval stage. Lampyrids as a class are rejected as inedible by most insectivorous creatures; toads and frogs present perhaps the only exception to this rule, and in view of the well-known wide scope of the gastronomic attainments of these animals, this can scarcely be taken as a fair criterion of the edibility of Lampyridae for most other insectivorous creatures. Children in this locality (Washington, D. C.) seem to quite generally regard the fireflies as poisonous, but this view appears to be by no means universal. In some localities, the larvae of the cucuyo are said to be used as food.

To obtain this substance for study, Dr. Coblentz extracted the insects with ether, added water to the extract, precipitated the proteins with lead acetate, and concentrated by boiling. The material I have used has for the most part been obtained in either of two ways: The first of these was to extract the entire insects (in the early stages of the work the luminous segments had been removed for other work) with absolute alcohol in a Soxhlet extractor; the extract is yellow, and becomes brownish orange on concentration. It is impossible to bring this extract to dryness on the water bath or in a vacuum desiccator. A thick, dark syrup is left, which decomposes if heated to a temperature materially higher than 100°. For the purpose of removing as much of the foreign matters as possible, the alcoholic extract was treated with lead acetate solution and allowed to stand 24 hours, filtered, and the precipitate washed with absolute alcohol, crystallin ammonium sulfate added and the solution allowed to stand for another 24 hours, and then decanted through a filter. The resulting solution is yellow, with a strong blue fluorescence, and on evaporation yields a brownish syrup from which the excess of ammonium sulfate crystallizes out. On taking up again with absolute alcohol the excess of sulfate remains, and the fluorescent substance goes into fairly pure solution. Some other organic compounds are, however, undoubtedly present. The second method of obtaining the luciferesceine was to wash out the tubes, bottles, etc., in which the insects had been collected, with absolute alcohol, preferably allowing it to stand for several hours in them. In these tubes the sticky secretion before referred to may be seen hardened in specks all over their inside surfaces, and from this secretion the alcohol dissolves out the luciferesceine. Such solutions, after filtration, are clear and colorless, and show a strong blue fluorescence. The solution may be evaporated to dryness, and leaves a white or slightly yellow, solid, non-crystallin material, apparently the fluorescent material in the purest form in which it has yet been obtained. So far all attempts to secure the luciferesceine in crystallin condition have ailed, but that obtained by the evaporation of the alcoholic extract of

the deposited secretion of the insects acts in such a way as to lead me to believe that it is very nearly a single substance, even if not quite pure. Heat, up to the boiling point of water, does not appear to affect the stability of luciferesceine; at higher temperaures it melts and boils, giving off an unpleasant, fishy odor, chars with the evolution of an inflammable gas, and finally leaves a very slight ash. Under ordinary conditions of temperature and humidity, the luciferesceine seems to be very stable. Insects collected in Massachusetts over thirteen years ago, and preserved dry, pinned in a cabinet ever since, yielded fluorescent extracts with alcohol.

In the alcoholic extract of the whole insects, after purification, chloroplatinic acid produces a yellow precipitate which appears to be a mixture of the chloroplatinates of potassium and lecithin. The filtrate from these chloroplatinates, containing an excess of platinum, may be rendered alkaline, the platinum precipitated by hydrogen sulfide in large excess, and the platinum sulfide filtered off, and a clear, slightly yellow, fluorescent solution obtained, which still contains other organic matters. As long as hydrochloric acid is present, the solution does not show its fluorescence. In this extract of the whole insect, silver nitrate solution produces a white precipitate, which rapidly turns brown; mercuric chloride produces a white precipitate; ferric chloride gives a brown precipitate. Examined in a layer 15 mm. thick, the moderately concentrated solution shows a shortening of the violet end of the solar spectrum. The extract leaves a greasy feeling on evaporation on the fingers. On standing exposed to the light, the fluorescence gradually weakens, and a light brown precipitate is formed. The precipitate dissolves in aqueous alkali, forming a brownish, non-fluorescent solution.

With the extract derived from the deposited secretion, and which I regard as being fairly pure, chloroplatinic acid produces no precipitate. Lead acetate produces no immediate precipitate, but after boiling and standing, a very slight, white precipitate is formed (this may, of course, be a basic lead compound); the supernatant fluid is fluorescent. Mercuric chloride produces a slight cloudiness at first; after boiling and standing, a slight yellow precipitate is formed; supernatant fluid not fluorescent. Silver nitrate produces no immediate precipitate; after boiling and standing, a slight brown precipitate is formed. The supernatant fluid is not fluorescent. Magnesia mixture produces a white, flaky precipitate, but the supernatant fluid retains its fluorescence. Ammonium molybdate produces a faint opalescence, but does not destroy the fluorescence. Uranium nitrate produces no precipitate, but its yellow color masks the fluorescence; the same is true of ferric chloride, potassium dichromate, picric acid, and iodine. One per cent. osmic acid causes a faint opalescence, which darkens on heating. Ammonium sulfate produces no pre-

cipitate. Potassium ferrocyanide produces in some solutions a dense, cream-colored precipitate, and the supernatant fluid is no longer fluorescent. I have not as yet had an opportunity to study this precipitate. Three per cent. phosphotungstic acid produces only a slight opalescence, but 3 per cent. phosphomolybdic acid produces in some solutions a cream-colored precipitate, and what appear to be drops of oil adhere to the sides of the test tube; these, however, fail to dissolve in ether. Alkaline cupric sulfate solution produces a white precipitate.

The luciferesceine remaining on the evaporation of the extract from the secretion dissolves best, and with the production of fluorescent solutions, in water, ethyl alcohol, amyl alcohol, and glycol; it is less easily soluble in ether, and the solution shows a weaker fluorescence. It does not appear to be soluble in carbon tetrachloride, benzene, acetone, or chloroform; it dissolves to some extent in pyridine, and the resulting solution is slightly fluorescent. With glycerol it appears to react slowly, forming a yellow solution, and with concentrated sulfuric acid it reacts energetically, with charring, and reddening of the acid. It does not give the murexide test.

The injection into mice of weak solutions in physiologic salt solution produced no noticeable immediate results, although after twelve hours the animal was evidently somewhat sleepy, and showed slow respiration. In larger doses it acts first as an irritant; later the animal shows drowsiness, and sometimes slow respiration. Dr. W. H. Schultz, who was good enough to make these injections for me, suggests that these effects might be due to a trace of cantharidine, which is present in many Coleoptera.

The urates and phosphates of sodium, potassium, ammonium and calcium have been found in the tissue and ash of the luminous organs of the Lampyridae and Elateridae, and the inner layer of the photogenic organs of these insects is said to consist of guanine, and sodium or ammonium urate.

Most of the substances yielding fluorescent solutions, which have been found in the tissue of lower organisms, have been lipochromes or other pigments. The fact that luciferesceine is not a colored substance and that it does not dissolve in the usual fat solvents—benzene and carbon tetrachloride—are sufficient to render the view that it might be a lipochrome untenable. Its conduct with various precipitants, its solution with a reddish color in sulfuric acid, and its chemical conduct in general have led me to regard it as alkaloidal in nature. That it is not a necessary factor in biophotogenesis is evidenced by its existence in non-luminous species. It cannot be a fluorescent chlorophyll derivative since it is found in carnivorous insects.

By the researches of Tappeiner and others, it has been shown that

fluorescent materials in the organism affect the activity of diastase and other enzymes. In the instance of luciferesceine, we have a strongly fluorescent material normally present in considerable amounts in an organism; it can here evidently exert no deleterious action upon the organism containing it. Most, but not all of the organisms containing it, however, are nocturnal in habit, and their activities depressed by exposure to light. It is of interest to note in this connection, that according to the theory of fluorescence as a "step-down" process of radiation, and the law of Stokes expressing this theory, luciferesceine should fail to show fluorescence in the emitted light of the insect, since all of the light emitted by the insect is less refrangible than the longest wave length in the fluorescent light of luciferesceine; however, Nichols and Merritt[1] have shown that Stokes' law fails to hold good for many common fluorescent substances, and my own experiments indicate that any light within the visible spectrum of shorter wave length than the yellow-orange, will excite a visible fluorescence in solutions of luciferesceine. On the whole it seems that this substance is contained in a defensive secretion of the insect, and that its fluorescence is a property dependent on its chemical constitution and having no reference to the life processes of the organism or possibly to its defensive function either.[2]

In conclusion I wish to express my indebtedness to Dr. Wm. W. Coblentz, of the Bureau of Standards, the original discoverer of luciferesceine in the Lampyridae, to Dr. Carl L. Alsberg, of the Bureau of Plant Industry, and to Dr. W. H. Schultz and other workers in the Hygienic Laboratory, for assistance and advice in this work, and to Dr. E. A. Schwarz and Mr. H. S. Barber, of the U. S. National Museum, for entomologic information.

THE PREPARATION AND PROPERTIES OF AN OXIDASE OCCURRING IN FRUITS.

By H. P. Bassett and Firman Thompson.

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Within recent years there have been numerous reports on the existence of a class of enzymes which are capable of promoting various oxidizing processes, and which appear to be very widely distributed in both plants and animals. To this class of enzymes the generic name of oxidases has been given.

[1] Nichols and Merritt, *Physic. Rev.*, 16, 18–36 (1904).

[2] Jordan (*Botanical Gaz.*, Chicago, 27 (1899)) arrived at a somewhat similar conclusion in regard to the fluorescent pigment of *Bacillus fluorescens liquefaciens*. Although this pigment is yellow, with a green fluorescence, it shows some analogies to luciferesceine; it is soluble in water, but not in carbon tetrachloride, chloroform, ethyl alcohol or ether, and does not appear to be precipitated by lead acetate or ammonium sulfate.