Fluorescence Analysis as Applied to Some Alkaloids and Crude Drugs*

By A. Slesser† and C. B. Jordan‡

PART I—ALKALOIDS

In the course of routine analysis it is sometimes advantageous to apply rapid tests for identity or purity of materials. For example, a rapid method of identification of the components present in a mixture of alkaloids would be quite desirable.

Analysis by fluorescence methods has, during the past few years, been successfully applied for rapid qualitative and quantitative analysis of chemicals and drugs. The method is comparatively simple; the only apparatus required is a source of ultraviolet rays and a screen to filter out the visible rays so that any fluorescence shown by the substance under examination can be easily discerned when the experiment is performed in a dark room.

Where the fluorescence produced is characteristic of the substance irradiated, it may be used as a means of analysis. Ultraviolet rays are desirable for fluorescence analysis because (1) they stimulate fluorescence in many substances, and (2) they are, in themselves, invisible and do not mask any fluorescence produced.

The source of the ultraviolet rays used in the work was a quartz mercury-vapor arc and a control unit so devised that a constant operating voltage, and thus a constant output of ultraviolet rays, could be maintained. A light-proof hood to encase the arc was an essential part of the apparatus. To an aperture in the bottom of the hood was fitted a special glass filter to remove the visible, and yet allow the passage of ultraviolet rays, chiefly those having a wavelength of 3650 Å.

Table Showing Results of Fluorescence Analysis

				Precipitate with—						
				A	B	C	D	E	F	G
Number	Alkaloid	Sulfate	Hydrochloride	Picrolonic Acid	Mayer's Reagent	Wagner's Reagent	Picric Acid	Phosphotungstic Acid	Phosphomolybdic Acid	Tannic Acid
1	Quinine	Blue	Weak	Neg.	Neg.	Neg.	Neg.	—	Neg.	Neg.
2	Strychnine	Weak	Weak	Neg.	Neg.	Neg.	Neg.	—	Neg.	Neg
3	Brucine	Weak	Weak	Neg.	Neg.	Neg.	Neg.	—	Neg.	Neg.
4	Hydrastine	—	—	GY	Neg.	—	Neg.	—	Neg.	—
5	Hydrastinine	—	WB	Neg.	Neg.	—	Neg.	IW	Neg.	—
6	Emetine	—	Blue	Neg.	Neg.	—	Neg.	—	—	—

WB = whitish blue, GY = golden yellow, IW = intense white.

EXPERIMENTAL

Initial experimental attempts were devoted to examination of the fluorescence of several alkaloids before and after purification. The following alkaloids were studied: quinine, strychnine, hydrastine, hydrastinine and emetine. The purpose of this part of the work was (1) to note if differences either in color or intensity of fluorescence were shown after recrystallization, and (2) to prepare comparatively pure forms of the alkaloids for subsequent salt formation.

The results indicated that three recrystallizations did not alter to any great extent the color or intensity of the fluorescence. The presence of moisture in the sample, however, did lessen the intensity, as evidenced by the increase in intensity after drying.

The second portion of the experimental work was performed with the object of ascertaining whether or not corresponding salts of similar alkaloids showed the same type of fluorescence; if the fluorescence differed, two similar alkaloids, *e. g.*, strychnine and brucine, could be detected in the presence of each other by adding the proper reagent and examining under the lamp. The salts formed were those yielded by a solution of the alkaloid upon treatment with the common alkaloidal precipitating reagents: Wagner's Reagent, Mayer's Reagent, picric acid

* An abstract of a thesis presented to Purdue University in partial fulfilment of the requirements for the Master of Science degree by A. Slesser, 1937.

† Eli Lilly & Company Fellow, Purdue University.

‡ Dean, Purdue University School of Pharmacy.

(saturated aqueous solution), tannic acid (10% aqueous solution), phosphotungstic acid, phosphomolybdic acid and picrolonic acid (1% alcoholic solution).

The results of examination under the lamp are shown in the preceding table.

The alkaloidal precipitants themselves showed no fluorescence in ultraviolet light.

A dash in the table indicates either that no precipitate was formed or else the amount formed was too slight to handle.

Each of the precipitates was next examined in three different acids, namely, acetic, sulfuric and hydrochloric.

SUMMARY AND CONCLUSIONS

(1) The nature of fluorescence of several alkaloids before and after purification has been noted.

(2) The presence of moisture in the powder was found in certain cases to influence the color and intensity of the fluorescence.

(3) Precipitates of the alkaloids with common alkaloidal precipitating reagents have been examined under quartz light and were found with a few exceptions to show no visible fluorescence, thus making impossible identification or differentiation by this method.

(4) The appearance in ultraviolet light of the alkaloidal precipitates in different solvents has been carried out.

PART II—CRUDE DRUGS

Nearly all crude drug extracts display luminescence in ultraviolet light, the intensity of color usually increasing on dilution of the solution up to a certain point. The color depends on the fluorescently active substances present in the extract, provided, of course, that the solvent is itself non-fluorescent. Capillary analysis applied to the extracts will show a number of colored zones on the filter paper, but the color visible to the eye under the ultraviolet rays may be due to only one substance, which exhibits a more intense fluorescence than any of the others present.

Alcoholic extracts of the drug ipecac on dilution show an intense bluish white luminescence in reflected, and a greenish color in transmitted ultraviolet light. There is no record in the literature that the causative agent for the luminescence has been sought. It was of scientific interest to the writer to attempt to isolate and, if possible, identify the fluorescently active principle.

EXPERIMENTAL

An alcoholic extract of coarsely powdered Brazilian ipecac was prepared by the percolation method, the alcohol removed by heating on a steam-bath, extraneous matter removed by the usual method of precipitation with lead acetate solution and the lead removed with hydrogen sulfide gas. The resulting filtrate displayed the characteristic colors under the lamp. A portion of the liquid was treated with Mayer's Reagent and the precipitate filtered off. The resulting filtrate displayed no luminescence. This seemed to indicate that alkaloidal material was responsible for the fluorescence.

The alkaloids occurring in ipecac are emetine, cephalline, psychotrine, *o*-methyl psychotrine and emetamine. Of these the first two are present in much more abundant quantities than any of the others.

The removal of emetine and cephalline from the other alkaloids, which was accomplished by making use of the ready solubility of these two alkaloids in ether and the insolubility of the others in this solvent, did not alter in any way the color or intensity of fluorescence of the filtrate. This seemed to be a pretty good indication that the luminescence was not due to emetine or cephalline.

The deëmetinized filtrate was evaporated to low volume under diminished pressure at a temperature not exceeding 50° C. Tests for the presence of glycoside, tannin and flavones performed on the solution were negative. The test for alkaloid, however, was positive.

The evaporation was continued as before, small amounts of alcohol being added from time to time in order to remove all traces of water, and anhydrous ether, to remove the alcohol.

The dark, viscid residue was then extracted successively with the following solvents: petrolatum benzin, anhydrous ether and chloroform. Before each extraction the preceding solvent was removed by evaporation. Of these extracts, only the chloroformic fraction showed the characteristic blue-green color under the lamp.

After several attempts at recrystallization, two crystalline hydrochlorides were obtained from the residue of the chloroform fraction. The microscope showed that prismatic and needle-shaped crystals were present. The latter were removed by solution in absolute alcohol.

The prismatic crystals gave a distinct test for alkaloids, while the needle-shaped ones did not. Neither salt showed fluorescence under the lamp in the dry state, but both gave the intense bluish white color on the addition of a few drops of water.

SUMMARY AND CONCLUSIONS

(1) The substance or substances causing the characteristic fluorescence of alcoholic extracts of ipecac in ultraviolet light is alkaloidal in nature as proved by the disappearance of the fluorescence after alkaloidal precipitation has been effected.

(2) The alkaloid responsible is not emetine or cephalline, because removal of these from the extract in no way alters the color or intensity of fluorescence.

(3) The substance responsible for the fluorescence is not a decomposition product produced by heating at steam-bath temperature because non-heat treated extracts show the same color as the others.

(4) The alkaloid may be psychotrine, *o*-methyl psychotrine or emetamine. Of these, the first two are the more likely suspects because emetamine and solutions of its salts are non-fluorescent in daylight; this argues against the likelihood of their showing such an intense fluorescence as displayed by the extracts of ipecac under quartz light.

(5) Solutions of the unknown substance darken on standing, and deposit a brown substance. Psychotrine solutions are said to act in this manner.

(6) Ether extracts of ipecac do not display the same type of fluorescence that alcoholic extracts show. Psychotrine is insoluble in ether and soluble in alcohol.

(7) Chloroform will extract the fluorescently active material from ipecac. Psychotrine is soluble in chloroform.

(8) Psychotrine or *o*-methyl psychotrine or both are apparently responsible for the fluorescence of the alcoholic extracts of ipecac.

Incompatibilities in Prescriptions

IV. The Use of Inert Powders in Capsules to Prevent Liquefaction Due to Deliquescence*,†

By William J. Husa‡ and Charles H. Becker

In a previous paper (1) a study was made of the effectiveness of various inert powders in capsules in which the contents liquefy due to formation of a eutectic mixture. In the present paper a report is made on the relative efficiency of various inert powders in preventing liquefaction of the contents of capsules due to deliquescence.

EXPERIMENTAL

Prescription No. 1

℞ Salol	℈ ii
Sodii Nitrite	grs. xx
Sodii Bromid	℈ iv

M. ft. caps. no. xxiv.
Sig.: Take one after meals.

On the above prescription as obtained from the file of a pharmacy, there appeared a notation indicating that the dispenser had added 40 grs. of lactose.

Upon mixing the ingredients of this prescription as written, a dry powder was obtained and single doses were put in No. 2 capsules. After four days the capsules in a beaker liquefied and acquired a rusty-red color. After a week, the capsules were entirely colored and adhered to the bottom of the container. On longer standing, the capsules hardened, and upon observing the interior, the ingredients were found dry and white in color except for a thin colored layer where the powder was in contact with the capsule. The capsules stored in a tightly stoppered jar had undergone no changes after three weeks. Similar results were obtained when 40 grs. of lactose were added for 24 capsules. The results show that lactose did not prevent liquefaction of the capsules when placed in an open container, whereas, in a tightly stoppered container, lactose was not necessary since the capsules had undergone no change.

The prescription was also filled using various amounts of inert powders. In all cases of compounding, the sodium bromide and sodium nitrite were first triturated together to obtain a fine powder. The inert powder and salol were then added in the order named. The temperature during the time of compounding was between 72° and 73° F. When using from 1 to 5 grs. of each absorbent per individual dose, the size of the capsule ranged from No. 2 to No. 00.

The results on capsules stored in open containers were as follows:

Light Magnesium Oxide and Magnesium Carbonate.—When using as much as 5 grs. of the absorbents per individual dose, the capsules developed a rusty-red color and became soggy and sticky after standing two weeks; the contents were dry.

* Read before the Section on Practical Pharmacy and Dispensing, A. PH. A., Atlanta meeting, 1939.

† This paper is based on part of a thesis presented to the Graduate Council of the University of Florida by Charles H. Becker, in partial fulfilment of the requirements for the degree of Master of Science in Pharmacy.

‡ Head Professor of Pharmacy, University of Florida.