Jelinek,[2] and appears to be evidence of the fact that the reaction $3Au_2O = Au_2O_3 + 4Au$ takes place. The constancy of the potential over a considerable period of time is considered significant, as well as the fact that the color of the blue-black precipitate had during this time changed to that of metallic gold. Dr. Gerke believes that the change in potential with the time can be accounted for by assuming that the electrode reaction is Au (colloidal) = Au (cryst.).

It is certain that the black precipitate does undergo a change with the time, suggesting considerable instability. Further work on this problem is planned to study other possible methods of preparation in an endeavor to establish conclusively whether or not aurous oxide is capable of existence, and to measure accurately the value of its electrode potential.

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FLUORESCEIN AND SOME OF ITS DERIVATIVES

BY W. R. ORNDORFF AND A. J. HEMMER[1]

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Fluorescein was first prepared by Baeyer[2] in 1871 by fusing a mixture of phthalic anhydride and resorcinol. Baeyer[3] gave the formula $C_{20}H_{12}O_5 + H_2O$, to the *yellow* product precipitated from alkaline solution by acids, while for the product crystallized from ethanol as a dark *red* powder, he gave the formula $C_{20}H_{12}O_5$. In 1882, Baeyer[4] assigned a lactoid structure to fluorescein (see Formula III below) and fluorescein dichloride. Bernthsen[5] retained the lactoid structure for fluorescein, but assigned a quinoid structure to its salts and esters. Meyer[6] proved that both hydroxyl groups in fluorescein were in the *para* positions to the methane carbon atom. Kehrmann and Dengler[7] assigned an *ortho-quinoid* structure to fluorescein, holding that as it is colored it cannot be a dihydroxyfluoran, but must have a quinoid structure. Kropp and Decker[8]

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[2] Jirsa and Jelinek, *Z. Elektrochem.*, **30**, 286, 534 (1924).

[1] From a dissertation presented by A. J. Hemmer to the Faculty of the Graduate School, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] Baeyer, *Ber.*, **4**, 555 (1871).

[3] Baeyer, *Ann.*, **183**, 1 (1876); see also Fischer, *Ber.*, **7**, 1211 (1874).

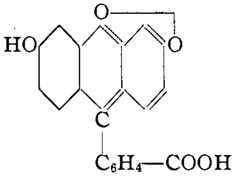
[4] Baeyer, *Ann.*, **212**, 351 (1882).

[5] Bernthsen, *Chem.-Ztg.*, **16**, 1956 (1892); *Ber.*, **26** [4], 376 (1893).

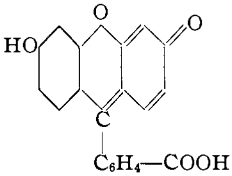
[6] Meyer, *Ber.*, **28**, 428 (1895).

[7] Kehrmann and Dengler, *Ber.*, **41**, 3440 (1908).

[8] Kropp and Decker, *Ber.*, **42**, 578 (1909).



I. Fluorescein (*o*-quinoid)



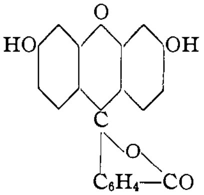
II. Fluorescein (*p*-quinoid)

and also Pope and Howard[9] took exception to this formula and gave reasons for believing that it has a *p*-quinoid structure (II).

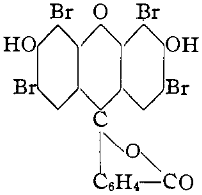
Von Liebig[10] described *five* different forms of *yellow* fluorescein, all of which, however, gave the same diacetate. Schultz[11] made the statement, "If one wishes to recrystallize fluorescein, one dissolves it in dil. sodium hydroxide solution, precipitates with sulfuric acid and extracts with ether, which dissolves the hydrated fluorescein ($C_{20}H_{12}O_5 + H_2O$). The ethereal solution is treated with absolute alcohol and the ether distilled off, whereupon the fluorescein separates out as dark red, crystalline grains or crusts of the composition $C_{20}H_{12}O_5$." In a number of textbooks on organic chemistry, the formula of fluorescein is given as $C_{20}H_{12}O_5 + H_2O$.

Theoretically, resorcinol, like orcinol,[12] should give three phthaleins. For a number of years one of us has been endeavoring to prepare the *ortho-para* and the di-*ortho* isomers of fluorescein and in the course of this investigation it became necessary to prepare pure fluorescein, free from any possible isomers, to investigate its properties and to prepare some of its derivatives, using the pure product. It was also deemed advisable to determine whether fluorescein existed in more than one form and to isolate these forms in view of the work of von Liebig.[10]

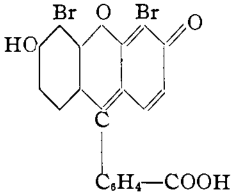
Our investigations show that fluorescein exists in two forms, *red* and a *yellow*, the two forms being isomeric and having the formula, $C_{20}H_{12}O_5$. The yellow form is obtained by precipitating an alkaline solution of fluorescein with acetic acid at room temperature. Baeyer's statement that this product is a hydrate is incorrect. The red form is obtained by precipitating a boiling, alkaline solution of fluorescein with acetic, formic or hydro-



III. Yellow fluorescein (lactoid)



IV. Eosin (lactoid)



V. Dibromofluorescein

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[9] Pope and Howard, *J. Chem. Soc.*, **97**, 1023 (1910); **99**, 545 (1911).

[10] Von Liebig, *J. prakt. Chem.*, [II] **85**, 97, 258 (1912).

[11] Schultz, "Farbstofftabellen," **1923**, 6th ed., vol. 1, p. 196.

[12] Orndorff and Allen, This Journal, **37**, 1201 (1915).

chloric acid. We believe that the yellow form of fluorescein, despite its color, has a lactoid structure (III) and that the red form of fluorescein has the *p*-quinoid structure (II).

Dry, *yellow* fluorescein does not form an ammonium salt with dry ammonia gas, whereas *red* fluorescein does so very readily. Dry, yellow fluorescein forms a yellow hydrochloride with dry hydrogen chloride, which loses its hydrogen chloride and forms yellow fluorescein by heating at 150° or by standing with cold water; but upon boiling with water, the hydrochloric acid is lost and red fluorescein results. This is in agreement with the conversion of yellow fluorescein into red fluorescein by boiling with water containing mineral acid. Red fluorescein forms a yellow hydrochloride with dry hydrogen chloride, which by heating at 150° or by standing with cold water loses its hydrochloric acid and gives red fluorescein. Yellow fluorescein is converted into crystals of red fluorescein, without loss in weight, by heating at 250–260°.

Some objection may be made to assigning a lactoid structure to yellow fluorescein because of its color. If, however, this structure is correct, we should expect by the introduction of halogens into the molecule to obtain lighter colored or colorless compounds. This was found to be the case with tetrachlorofluorescein,[13] the lactoid form of which is pale yellow, while tetrachloro-eosin is colorless. In further corroboration of this viewpoint, we have obtained eosin in its colorless lactoid form (IV). The observation of Fischer and Hoffmann[14] that fluorescein forms *colorless* addition products with pyridine or quinoline, and that these colorless products lose their pyridine and quinoline with the formation of yellow fluorescein, is in accord with a lactoid structure for yellow fluorescein.

We have found no indication of the existence of yellow fluorescein in more than one form, as von Liebig claims. Recrystallization of fluorescein (either the red or the yellow form) from a large number of solvents gives either the red or the yellow form and in some cases the red or yellow form with solvent of crystallization. Repetition of von Liebig's directions for the preparation of his β-fluorescein II, $C_{20}H_{12}O_5 + C_4H_{10}O$, gives a product which has been found to contain methoxyl; von Liebig's β-fluorescein II is, therefore, an impure compound containing a methyl ester or ethers of fluorescein. Both red and yellow fluoresceins give the same derivatives, such as the bisphenyl carbamate, hydrazide diacetate, methyl ester, etc. This is explained by assuming that in solution an equilibrium exists between the lactoid and quinoid forms.

Fluorescein reacts very readily with the calculated amount of bromine to form a dibromofluorescein, which has been obtained as a red powder by acidification of the alkaline solution.

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[13] Orndorff and Hitch, THIS JOURNAL, **36**, 680 (1914).

[14] Fischer and Hoffmann, *Ber.*, **47**, 1578 (1914).

Tetrabromofluorescein (eosin) was obtained pure only by saponification of the purified diacetate. The colorless, lactoid form was obtained by crystallization from glacial acetic acid, and a red hydrate, $C_{20}H_8Br_4O_5 + 2.5H_2O$, by acidification of the cold alkaline solution with acetic acid. At 150°, this red hydrate loses one and a half moles of water and forms the rose-colored hydrate, $C_{20}H_8Br_4O_5 + H_2O$.

Experimental Part

All of the fluorescein used in this work was purified by converting it into the diacetate, crystallizing this until it was colorless and melted at 200°, and then saponifying the diacetate with alcoholic sodium hydroxide solution. The alcohol was removed *completely* by distillation, water was added and the fluorescein precipitated with acid. When acetic acid was used at ordinary room temperature, yellow fluorescein resulted. When hydrochloric, acetic or formic acid was added to the boiling solutions, crystals of red fluorescein were obtained.

Fluorescein Diacetate.—Crude fluorescein[15] was prepared according to Gattermann[16] with the exception that the temperature of the fused mixture was held at 180° throughout, giving a much purer product than when the temperature was increased to 210°. For the preparation of the diacetate, the crude fluorescein was heated to boiling for two hours with five parts by weight of acetic anhydride. The crude diacetate obtained by allowing the acetic anhydride solution to cool was recrystallized repeatedly from hot acetic anhydride, and finally from ethanol. The pure diacetate forms colorless needles from ethanol, and melts at 200°. The melting point is unchanged by crystallization from other solvents; von Liebig's melting point of 205–206° must be wrong.[3]

Yellow Fluorescein.—The air-dried, yellow fluorescein was heated to 200° in a current of dry air, in a tube heated electrically.[17] It did not change color and the loss in weight was very small. In no case were results obtained anywhere approaching the loss of a molecule of water from the compound, $C_{20}H_{12}O_5 + H_2O$.

Anal.[18] Subs. (dry), 0.2570, 0.2395: CO_2, 0.6799, 0.6359; H_2O, 0.0836, 0.0783. Calcd. for $C_{20}H_{12}O_5$: C, 72.27; H, 3.64. Found: C, 72.15, 72.41; H, 3.64, 3.66.

Yellow fluorescein, therefore, has the formula $C_{20}H_{12}O_5$ and not $C_{20}H_{12}O_5 + H_2O$. When heated slowly in a sealed melting-point tube, yellow fluorescein turns slightly orange and then red at about 260°, and melts at 314–316°, with decomposition.[19] Dr. C. W. Mason, Instructor in Chemical Microscopy in this Department, kindly furnished the following report on the conduct of yellow fluorescein when heated and observed under the microscope. "Yellow fluorescein is very finely granular, no crystal form being recognizable, but shows strong double refraction. On heating, it darkens to orange at about 100°, with no apparent change in form. Further heating results in the liberation of a pink liquid as a condensate at about 180°. At this temperature a

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[15] We are indebted to the Heller and Merz Company for the donation of five pounds (2.3 kg.) of fluorescein.

[16] Gattermann, "The Practical Methods of Organic Chemistry," MacMillan Co., New York, **1916**, p. 357.

[17] Orndorff and Nichols, *Am. Chem. J.*, **48**, 477 (1912).

[18] International atomic weights for 1925 were used in the calculations in this paper.

[19] The melting point of commercial fluorescein is given as 312–318°.

yellow to orange liquid is formed in the vicinity of the solid. Numerous yellow-orange rosets, spherulites and single, lath-shaped crystals are formed either from this yellow liquid or from the unaltered yellow fluorescein by the growth of the original fine crystals. The yellow crystals are strongly doubly refractive and show parallel extinction, and are probably rhombic. At still higher temperatures (250–300°) the crystal aggregates and rosets melt and, after being held for some time at this temperature, red prisms, apparently rhombic, grow in the deep orange melt. On cooling, none of the above changes reverse."

Yellow fluorescein dried at 200° is completely converted into *crystals* of red fluorescein at 250–260° without loss of weight. Yellow fluorescein does not undergo any change when boiled with dil. acetic acid, but is converted into *red*, crystalline fluorescein when boiled with dil. hydrochloric acid. Yellow fluorescein is insoluble in petroleum ether, slightly soluble in ethyl ether, chloroform, benzene and xylene, difficultly soluble in ethanol, glacial acetic acid, nitrobenzene and *iso*-amyl alcohol, soluble in methanol and acetone and very soluble in 85% formic acid. It is slightly soluble in hot water, but less soluble in cold water, the solubility increasing upon the addition of acids. The solutions in methanol, ethanol, glacial acetic acid, acetone and *iso*-amyl alcohol have an orange color and the solutions in the alcohols have a green fluorescence. The nitrobenzene and formic acid solutions are deep red with no fluorescence. Freshly precipitated, yellow fluorescein is much more soluble in the ordinary solvents than the air-dried product, being very soluble in ether. Yellow fluorescein dissolves slowly in concd. sulfuric acid with a yellow color and slight green fluorescence, which gradually change upon standing for a few days to an orange color and strong green fluorescence. The colors, compared with a Mulliken Color Chart, are Y-normal tone and OR-normal tone. The color change is probably due to the formation of a sulfate.

Action of Boiling Water on Yellow Fluorescein. Red Fluorescein.—When yellow fluorescein is boiled with a large quantity of water, it gradually changes in the course of five or ten minutes to a dark red crystalline powder, with a green surface color. The change is apparently catalyzed by the presence of mineral acids or adhering solvents. The air-dried crystals grind to a red powder, exactly like the red fluorescein obtained by precipitating a boiling alkaline solution with acids. The loss in weight on heating the air-dried crystals at 130° is very small, and at 195–215° they do not lose any further weight.

Anal. Subs. (dry), 0.2445: CO_2, 0.6453; H_2O, 0.0835. Calcd. for $C_{20}H_{12}O_5$: C, 72.27; H, 3.64. Found: C, 71.98; H, 3.82.

The yellow and red fluorescein, therefore, are isomers. They both give the same diacetate and one may be very readily converted into the other.

Preparation of Red Fluorescein.—The alkaline solution of fluorescein obtained by saponification of pure fluorescein diacetate with alcoholic sodium hydroxide solution, after the *complete* removal of all of the alcohol and addition of water, was heated to boiling with steam, and precipitated boiling with a slight excess of hydrochloric acid. The fluorescein separated as a bright red, crystalline powder. If the acidification takes place before the solution has reached the boiling point the fluorescein comes down at first as a yellow, flocculent precipitate, which changes upon boiling to a dark red, crystalline powder. This is in agreement with the action of boiling water containing a mineral acid on yellow fluorescein. The loss in weight of the air-dried crystals at 145° was very small.

Anal. Subs. (dry), 0.1589, 0.1893: CO_2, 0.4206, 0.5000; H_2O, 0.0535, 0.0628. Calcd. for $C_{20}H_{12}O_5$: C, 72.27; H, 3.64. Found: C, 72.19, 72.04; H, 3.77, 3.71.

This red crystallized fluorescein is, therefore, identical with the red crystals obtained by boiling the yellow fluorescein with water, as shown by the analyses and an examination under the microscope. The red fluorescein is very readily converted into its isomer, yellow fluorescein, by dissolving it in solutions of the alkalies and precipitating the fluorescein in the cold with *acetic* acid. Red fluorescein melts at 314–316°, with slight decomposition, in a sealed melting-point tube. It dissolves in concd. sulfuric acid with an orange color and green fluorescence. Upon standing, the color changes slowly to orange-red with strong green fluorescence. It is insoluble in petroleum ether and only very slightly soluble in diethyl ether. It is easily soluble in hot 85% formic acid, 80% acetic acid and in hot, freshly-distilled aniline. It is soluble in hot acetone, difficulty soluble in methanol and ethanol, and slightly soluble in glacial acetic acid. It is slightly soluble in hot water, the solubility increasing upon the addition of acids. Red fluorescein is less soluble in the common organic solvents than yellow fluorescein.

Several experiments were then carried out in an attempt to separate fluorescein into other components than the yellow and red forms. For this purpose, fluorescein was crystallized from several solvents of widely different nature and the pure product obtained was subjected to analysis. The results are embodied in Table I. In three experiments the fluorescein crystallized with solvent of crystallization, and except in these the fluorescein was usually dried at 150–160° before analysis. It will be seen that in

TABLE I

PRODUCTS AND ANALYSES OF SUBSTANCES FORMED BY CRYSTALLIZATION OF YELLOW AND RED FLUORESCEIN FROM VARIOUS SOLVENTS

RF, red fluorescein; YF, yellow fluorescein

Starting product	Solvent	Product	Analyses					
			Calcd.		Found			
			C, %	H, %	C, %	H, %	Calcd., %	Found, %
yellow	CH_3OH	$F+2CH_3OH$[a]	69.21	4.43	69.61	4.09	CH_3OH	CH_3OH
		RF	72.27	3.64	71.99	3.58	8.80	8.67–8.93
yellow or red	C_2H_5OH	RF[b]	72.27	3.64	72.04	3.69		
					72.19	3.60		
yellow	*iso*-Amyl alc.	RF[c]	72.27	3.64	72.17	3.91		
yellow or red	HCOOH	YF[d]	72.27	3.64	71.99	3.83		
yellow	80%	$F+2CH_3CO$	F		66.96	4.06	CH_3CO	CH_3CO
	CH_3COOH	$1CH_3CO$	67.33	4.11	66.95	4.28	15.31	13.00
					66.93	4.05		
red	Ether	Red	72.27	3.64	72.12	3.83		
red	Ether, reduced press.	Yellow	72.27	3.64	71.94	4.06		
yellow	Ether-benzene	YF[e]	72.27	3.64	72.49	3.75		
yellow	Ethyl acetate	YF	72.27	3.64	72.41	4.04		
yellow	Acetone	RF	72.27	3.64	72.07	3.91		
yellow	Nitrobenzene	$F+2C_6H_5NO_2$	72.27	3.64	71.95	4.04	$C_6H_5NO_2$	$C_6H_5NO_2$
		F					27.03	26.43
								26.45

[a] These yellow crystals lost the methyl alcohol of crystallization on being heated to 150–160° and were red fluorescein.

[b] An attempt was made to obtain yellow γ fluorescein of Liebig,[10] by dissolving fluorescein in cold ethanol and allowing it to stand at room temperature. During the course of two months some *red* fluorescein was obtained, but no yellow product was formed.

[c] No yellow form was obtained.

[d] No fluorescein with a mole of formic acid of crystallization was obtained.

[e] Four parts of benzene to 3 parts of ether was used to extract *yellow* fluorescein.

several cases a rearrangement in form occurs but in no cases were other forms of fluorescein found as reported by Liebig.[10]

Table II contains the experimental data from several experiments concerning the action of various reagents on both forms of fluorescein. The fact that yellow fluorescein does not take up dry ammonia gas is evidence for a lactoid structure of *yellow* fluorescein.

TABLE II

PROPERTIES AND ANALYSES OF SUBSTANCES FORMED FROM YELLOW AND RED FLUORESCEIN

Reagent	Form of fluorescein	Product	M. p., °C.	Analyses Calcd., %	 Found, %
Dry HCl	yellow	yellow-HCl		HCl, 9.89	HCl, 9.62 10.25 9.57 9.89
Dry HCl	red	red-HCl		9.89	HCl, 9.93 10.39
Dry NH$_3$[a]	yellow	no action[a]			
Dry NH$_3$	red	red 2$^1/_2$ NH$_3$		*NH$_3$*, 11.36	*NH$_3$*, 11.99 11.48
NH$_3$-alcohol	red or yellow	F.NH$_3$.$^1/_2$-C$_2$H$_5$OH		N, 3.76	N, 3.73 3.83
Phenyl isocyanate	red or yellow	bisphenyl carbamate	197–198[b]	N, 4.91	N, 4.94 5.01
CH$_3$OH 3% HCl[c]	red or yellow	methyl ester	269–270	C, H, CH$_3$O, 72.81 4.08 8.96	C, H, CH$_3$O, 72.76 4.16 9.16 8.76
Dry HCl	methyl ester	methyl ester hydrochloride		HCl, 9.56	HCl, 9.54 9.36
(CH$_3$CO)$_2$O	methyl ester	methyl ester acetate	210–211	CH$_3$CO, 11.08	CH$_3$CO, 10.89 10.98
Hydrazine hydrate	yellow or red	hydrazide H$_2$O	Decomposes 280	N, 7.69	N, 7.56 7.52
NaAc (CH$_3$CO)$_2$O	hydrazide H$_2$O	hydrazide tetraacetate	233.5–234.5	C, H, CH$_3$CO, N, 65.35 4.31 33.47 5.48	C, H, CH$_3$CO, N, 65.53 4.77 33.28 5.41 32.87 33.57
Br$_2$ in glac. acetic acid	yellow	dibromofluorescein	Decomposes 280–290	Br, *32.62*	Br, *32.71 32.72*
C$_6$H$_5$COCl	dibromo	dibromo dibenzoate	239–240	Br, 22.90	Br, 23.00 23.15
Dry HCl	dibromo	dibromo 2HCl		HCl, 12.96	HCl, 12.92 13.38
Expose to KOH	dibromo 2HCl	dibromo HCl		HCl, 7.09	HCl, 6.80
Dry NH$_3$	dibromo	dibromo ca. 2NH$_3$		NH$_3$, 6.50	NH$_3$, 7.15 7.15

[a] Yellow fluorescein absorbs a small amount of ammonia.

[b] Haller and Guyot [*Compt. rend.*, **116**, 479 (1893)] obtained a melting point of 195° (with decomposition), on a product consisting of yellow grains, but gave no analyses. This carbonate was obtained in feathery needles and was saponified in the cold by sodium hydroxide solution or concd. sulfuric acid.

[c] Sulfuric acid also may be used as a catalyst.

Bromination of Fluorescein. Colorless Eosin.—Fluorescein was brominated by suspension of the phthalein in glacial acetic acid and subsequent addition of a glacial acetic acid solution of bromine, to obtain the tetrabromo derivative. Variation of conditions, such as the rate of addition of the bromine, the use of an excess of bromine, heating after bromination, etc., was tried, but in no case was a pure product obtained, bromination being incomplete. The best results were obtained by the use of an excess of bromine, the addition of all of the bromine solution at once and heating after the addition of the bromine. A suspension of 10 g. of *yellow* or *red* fluorescein in 40 g. of glacial acetic acid, was rapidly mixed with a solution of 28 g. of bromine in 110 g. of glacial acetic acid. All of the fluorescein dissolved and then crystals separated from the solution. After heating for one hour on a boiling water-bath, the mixture was poured into a large volume of water, the crude eosin filtered off, dissolved in dil. sodium hydroxide solution and precipitated with an excess of acetic acid. This eosin was converted into the diacetate, which was purified, shown to be *pure* by analyses, and then saponified with alcoholic sodium hydroxide solution.

The alcoholic sodium hydroxide solution, obtained from the pure diacetate, was distilled to remove part of the alcohol. It was then acidified with acetic acid, boiled, and a large volume of hot water was added. Upon the addition of some concd. hydrochloric acid, a turbid solution was obtained which, upon boiling, deposited flesh-colored crystals. These crystals were dissolved in hot glacial acetic acid and, upon cooling, colorless crystals of pure eosin were obtained. In the air they turn to a cream color with a trace of pink.

Anal. Subs., 0.1476, 0.1744: AgBr, 0.1704, 0.2018. Calcd. for $C_{20}H_8Br_4O_5$: Br, 49.34. Found: Br, 49.13, 49.24.

Colorless eosin turns pink at about 200° and then melts at 295–296.5° to a dark red liquid. It is insoluble in petroleum ether, slightly soluble in water and diethyl ether, sparingly soluble in ethyl acetate, benzene, chloroform, glacial acetic acid and acetone, these latter solutions being orange with no fluorescence. It is soluble in 95% ethanol with a red color. The solution in concd. sulfuric acid is orange with no fluorescence, the color changing, upon standing, to a dark red. The solution in 10% aqueous sodium hydroxide is dark red, with no fluorescence, the color changing to red, orange and pink upon dilution, all with green fluorescence. Colorless eosin takes up but a very small amount of hydrochloric acid, when exposed to the dry gas, eosin being more acidic than fluorescein or dibromofluorescein.[3,20] Table III contains data concerning experiments on colorless eosin.

TABLE III

PROPERTIES AND ANALYSES OF DERIVATIVES FORMED FROM COLORLESS EOSIN

Reagent	Starting product	Product	Analyses			
			Calcd., %		Found, %	
			H_2O,	Br,	H_2O,	Br,
$H_2O + CH_3CO_2H$	Eosin	Eosin[a] 2.5H_2O:1.5H_2O	3.90	48.02	3.91, 3.82, 4.17	48.00, 48.24, 47.79
Dry NH_3	Colorless eosin	Eosin 4NH_3	NH_3, 9.52		NH_3, 9.11 8.77	
Expose to concd. H_2SO_4	Eosin 4NH_3	Eosin 2NH_3	NH_3, 5.00		NH_3, 5.15 5.05	
Dry NH_3	Eosin hydrate	Eosin H_2O.4NH_3	NH_3, 9.28		NH_3, 9.60 9.05	
Expose to concd. H_2SO_4	Eosin H_2O.4NH_3	Eosin H_2O.2NH_3	NH_3, 4.87		NH_3, 4.90	

[a] Decomposes at 276–281°.

Summary

The results of this investigation may be briefly stated as follows.

1. Pure fluorescein has been prepared and proved to exist in two isomeric forms, *red* and *yellow*, having the formula, $C_{20}H_{12}O_5$. The *yellow*

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[20] For previous work on eosin, see Hofmann, *Ber.*, **8**, 62 (1875). von Liebig, *Ber.*, **46**, 2743 (1913).

form is obtained by precipitation of a cold alkaline solution with acetic acid and is believed to have a lactoid structure. The *red* form is obtained by precipitation of a boiling alkaline solution with acids and is believed to have a *p*-quinoid structure.

2. No other forms of fluorescein were obtained than the yellow lactoid form and the red quinoid form; von Liebig's claims to the existence of five different yellow forms must be in error.
3. The yellow lactoid fluorescein and the red quinoid fluorescein give different yellow hydrochlorides.
4. The colorless diacetate, bisphenyl carbamate and hydrazide of fluorescein have been prepared. These are derivatives of lactoid fluorescein.
5. The red mono-ammonium salt and the red methyl ester and its orange acetate have been prepared. These are derivatives of quinoid fluorescein.
6. Dibromofluorescein, its hydrochloride and diammonium salt have been prepared. These colored compounds are derivatives of quinoid fluorescein.
7. Dibromofluorescein dibenzoate has been prepared. This is colorless and a derivative of lactoid fluorescein.
8. Colorless eosin has been prepared. The introduction of bromine has made the lactoid form stable and colorless.
9. Two colored hydrates of eosin have been prepared.

ITHACA, NEW YORK

[CONTRIBUTION FROM THE MARTIN MALONEY CHEMICAL LABORATORY OF THE CATHOLIC UNIVERSITY OF AMERICA]

THE VAPOR-PRESSURE CURVE OF BENZOIC ACID

BY SIMON KLOSKY, LEO P. L. WOO AND ROBERT J. FLANIGAN

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Introduction

The determination of the vapor pressure of benzoic acid from 100 to 180° was suggested by Zanetti.[1] The authors did not limit themselves to this range, but have determined values from 50 to 250°. As two different methods were used, one for each phase, this paper is divided into two parts for convenience.

I.[2] The Vapor Pressure of Benzoic Acid (Liquid) from 251 to 128.6°

The vapor pressure of liquid benzoic acid has already been determined by Kahlbaum.[3] He used a static method and his results are very accurate

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[1] Zanetti, *Ind. Eng. Chem.*, **16**, 304 (1924).

[2] Extract from a thesis submitted in partial fulfilment of the requirements for the degree of Master of Arts, by Robert J. Flanigan.

[3] Kahlbaum, *Z. physik. Chem.*, **26**, 603 (1898).