TABLE I

PHYSICAL PROPERTIES AND ANALYSES OF N-ETHANOL AMIDES

No. C atoms in acid	M. p., °C.	n^{25}_D	B. p., °C.	Mm.	Yield, %	% Nitrogen Calcd.	Found
1	Glass	1.4785	150–155	2[a]	65	15.73	15.61
2	40	1.4710	155–160	2–3[a]	87	13.60	13.40
3	Glass	1.4681	160–168	1–2[a]	89	11.98	11.93
4	Glass	1.4672	155–162	1–1.5	78	10.69	10.60
5	32.0	1.4642	192	6	84	9.65	9.63
6	46.0				91	8.80	8.83
7	53.6				87	8.08	8.00
8	63.2				85	7.51	7.46
9	71.6				88	6.97	6.93
10	77.1				82	6.51	6.50
11	84.8				86	6.10	6.06
12	78.2				90	5.77	5.79
13	91.8				87	5.45	5.41
14	87.4				80	5.17	5.13
15	97.0				81	4.91	4.86
16	94.4				84	4.67	4.70
17	99.2				84	4.47	4.44
18	96.1				86	4.28	4.20

[a] Ref. (5) gives for no. 1, b. p. 191–193° (10 mm.), d^{25}_{25} 1.180; for no. 2 b. p. 195–196° (10 mm.), d^{25}_{25} 1.115; for no. 3 b. p. 201–203° (10 mm.), d^{25}_{25} 1.071. Ref 3 gives m. p. for no. 2 of 63–65° while we found 40° from a heating and cooling curve.

TABLE II

MELTING POINTS AND ANALYSES

No. C atoms in acid	Diethanol amides M. p., °C.	Nitrogen, % Calcd.	Found	Isopropanol amides M. p., °C.	Nitrogen, % Calcd.	Found
9	Glass	. .	. .	53.8	6.52	6.50
10	Glass	. .	. .	58.1	6.10	6.00
11	34.9	5.13	5.08	63.1	5.76	5.70
12	38.7	4.88	4.81	66.6	5.45	5.43
13	45.3	4.66	4.64	71.0	5.17	5.15
14	47.9	4.44	4.40	74.2	4.91	4.87
15	50.9	4.25	4.20	75.1	4.75	4.69
16	65.1	4.08	4.00	78.2	4.53	4.54
17	67.9	3.93	3.88	82.0	4.34	4.30
18	69.7	3.79	3.75	86.1	4.15	4.13

invert at no. 14. The melting points of the N-diethanol amides appear to rise in groups of three. Pharmacological results have been published.[9] For the ninth members of the four series of N-substituted amides the activities are in the order: $RCONHCH_3 > RCONHCH_2CH_2OH > RCON(CH_2CH_2OH)_2 > RCONHCH_2CH(OH)CH_3$.

Summary

The N-ethanol amides of the normal acids from formic to stearic have been prepared and characterized. The corresponding N-diethanol and N-isopropanol amides have been made of the higher acids. Comparative pharmacological effects are also reported.

BALTIMORE, MARYLAND RECEIVED NOVEMBER 14, 1936

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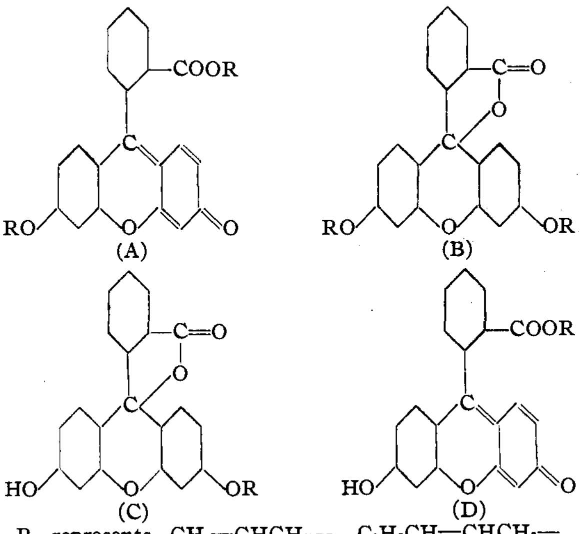
[9] Macht and D'Alelio, *Am. J. Pharmacol.*, **116**, 104 (1936).

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

Alkenyl Derivatives of Fluorescein

BY CHARLES D. HURD AND LOUIS SCHMERLING[1]

Surprisingly few O-alkylated derivatives of fluorescein have been prepared. Only the methyl and ethyl derivatives are reported in the literature, but they have been investigated by many workers[2] chiefly because of the confusion arising from difficulties involved in their purification. In the present study, the allyl, pentenyl and hexenyl derivatives were synthesized. The various types involved are the "ether ester" (A), the "diether" (B), the "monoether" (C) and the "monoester" (D).



R represents $CH_2{=}CHCH_2{-}$, $C_2H_5CH{=}CHCH_2{-}$, $n\text{-}C_3H_7CH{=}CHCH_2{-}$.

Two general methods of synthesis were used. In the first, fluorescein was reacted with the

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[1] Parke, Davis and Company Fellow, 1933–1935.

[2] Fischer and Hepp, *Ber.*, **27**, 2790 (1894); **28**, 396 (1895); **46**, 1951 (1913); Nietzki and Schroeter, *ibid.*, **28**, 44 (1895); Herzig and Meyer, *ibid.*, **28**, 3258 (1895); Kehrmann and Dingler, *ibid.*, **42**, 810 (1909); **46**, 3028 (1913); **47**, 84 (1914); von Liebig, *J. prakt. Chem.*, **85**, 97, 241 (1912); **86**, 472 (1912); **88**, 26 (1913); *Ber.*, **46** 3593 (1913).

TABLE I

O-ALKENYL DERIVATIVES OF FLUORESCEIN

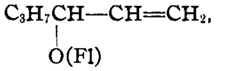
Symbol	Descriptive name of compound	Systematic name
I	Allyl ether ester	Allyl 6-alloxy-9-phenylfluorone-11-carboxylate
II	Allyl diether	3,6-Dialloxyfluoran
III	Allyl ether	3-Alloxy-6-hydroxyfluoran
IV	Acetate of III	3-Alloxy-6-acetoxyfluoran
V	Allyl ester	Allyl resorcinolbenzein-11-carboxylate
VI	Pentenyl ether ester	γ-Ethylallyl 6-(γ-ethylalloxy)-9-phenylfluorone-11-carboxylate
VII	Pentenyl diether	3,6-Di-(γ-ethylalloxy)-fluoran
VIII	Pentenyl ether	3-γ-Ethylalloxy-6-hydroxyfluoran
IX	Acetate of VIII	3-γ-Ethylalloxy-6-acetoxyfluoran
X	Hexenyl ether ester	γ-*n*-Propylallyl 6-(γ-*n*-propylalloxy)-9-phenylfluorone-11-carboxylate
XI	Hexenyl diether	3,6-Di-(γ-*n*-propylalloxy)-fluoran
XII	Hexenyl ether	3-γ-*n*-Propylalloxy-6-hydroxyfluoran
XIII	Acetate of XII	3-γ-*n*-Propylalloxy-6-acetoxyfluoran

alkenyl bromide in acetone solution in the presence of potassium carbonate. All four type compounds were obtained in the reaction mixture. The first step in separating them was based on the fact that A and B are alkali-insoluble, whereas C, D and unreacted fluorescein are alkali-soluble. Separation of orange colored A from colorless B was accomplished by virtue of the fact that the latter is more soluble in carbon tetrachloride and less soluble in ethyl alcohol. The monoether and the monoester were separated from the fluorescein by precipitation from the alkaline solution with carbon dioxide. The amount of material so obtained was usually very small. The dark-red monoallyl ester was found to be less soluble in cold alcohol than the nearly colorless monoether and was separated from it by use of this property.

The second method, which has the advantage of being much more rapid than the first, was applied to phenols as well as to fluorescein types. It involves the reaction of the alkenyl bromide with the sodium phenolate in an aqueous acetone solution. The proportions of water and acetone were so adjusted as to give a clear solution. With this procedure, there were higher yields of C, lower yields of B, and approximately the same yields of A, as in the first method. Since little unreacted fluorescein remained, the monoether (C) could be obtained in a pure state by acidifying the alkaline solution and recrystallizing the precipitate from ethyl alcohol. It was not found possible to isolate any of the monoester by this second method.

It was found as expected that the various allyl and alkenyl ethers (types A, B and C) rearranged on heating to form C-substituted fluoresceins. When, for example, the allyl diether was heated to 210°, an exothermic reaction occurred, and most of the material became alkali-soluble. The product consisted chiefly of 2,7-diallylfluorescein with some 2-allylfluorescein and fluorescein. The presence of the latter compounds is analogous to the formation of phenol[3] from phenyl allyl ether. In the present study, fluorescein and the C-alkylated rearrangement products were found to be too similar in physical and chemical properties to permit the complete separation of the latter from the former.

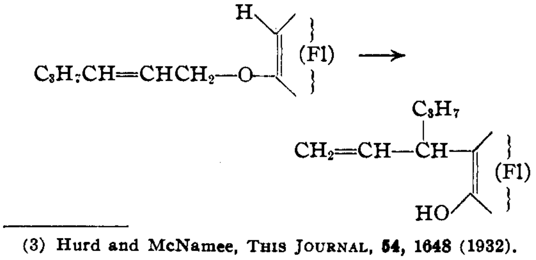
The structure of the hexenyl portion of fluorescein hexenyl diether was determined by ozonolysis. It was found that the volatile acid obtained from the ozonide was a mixture of formic and butyric acids, with butyric considerably in excess. The butyric acid would come from $\mathrm{C_3H_7CH{=}CHCH_2{-}O{-}(Fl)}$ and the formic from



$$\mathrm{C_3H_7\underset{\underset{O(Fl)}{|}}{C}H{-}CH{=}CH_2},$$

wherein (Fl) represents the fluorescein residue. Such a result indicates that the hexenyl bromide used in the synthesis of the ether was an equilibrium mixture of 1-bromo-2-hexene and 3-bromo-1-hexene.

Ozonolysis of the rearrangement product of the diether gave evidence which supported the idea



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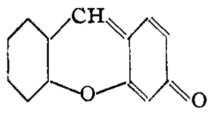
[3] Hurd and McNamee, THIS JOURNAL, **54**, 1648 (1932).

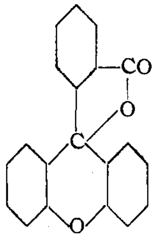
that the "wandering" group undergoes inversion of an allylic type. Formic and butyric acids were indicated as before, but this time it was the formic which was in excess.

The saponification of the diallyl ether ester led to some very interesting results. It was found that the solvent used during the reaction was a determining factor. Thus, it was shown that either fluorescein, or its monomethyl, ethyl or allyl ethers could be obtained by using, respectively, water, methanol, ethanol or allyl alcohol (or aqueous acetone) as the solvent. A similar observation was made by von Liebig[4] who obtained the monoethyl ether of fluorescein in an attempt to prepare the monomethyl ether by saponifying the dimethyl ether ester with ethyl alcoholic potash.

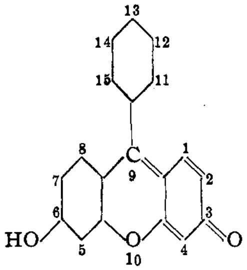
The O-alkenyl compounds studied are listed in Table I. The systematic names are derived from the following nuclei: fluorone (E), fluoran (F) and resorcinolbenzein (G).



(E)



(F)



(G)

The allyl ether ester (I) and pentenyl ether ester (VI) were tested bactericidally in the Parke, Davis and Company Laboratories, but were found to be ineffective (phenol coefficients less than 50 toward *Staph. aureus*). Their extreme insolubility may have been a contributing factor.

Two new reactions of fluorescein are also recorded. One is the production of diacetylfluorescein by addition of ketene, and the other is the formation of 3,6-dichlorofluoran by reaction with thionyl chloride.

Experimental Part

Diacetylfluorescein.—Ketene, generated at the rate of 0.1 mole per hour, by passing acetone vapors over a hot platinum filament,[5] was passed through a suspension of 1.66 g. of red fluorescein in 50 cc. of acetone. The calculated time to obtain the diacetate was six minutes. In five minutes, it was noticed that the fluorescein was beginning to dissolve. In seven minutes, solution was complete and the flow of ketene was stopped. The acetone was evaporated. The residual oil solidified on the addition of ethanol The first crystallization from 100 cc. of hot alcohol gave crystals which melted at 204° (the same as that observed with material prepared from fluorescein and acetic anhydride). A second recrystallization raised the m. p.[6] to 205°; yield, 1.9 g., or 91%.

3,6-Dichlorofluoran.—Ten cc. (16.5 g.) of thionyl chloride was added to 5 g. of red fluorescein. A yellow color was imparted to the mass. It turned red and dissolved after an hour of refluxing. The heating was continued for another hour and the excess of thionyl chloride was distilled off. The red-brown residue was digested with water, then treated with dilute sodium hydroxide, and finally crystallized from hot toluene. White crystals of 3,6-dichlorofluoran, m. p. 262°, uncorr., were obtained; yield 5.4 g., or 90%. The m. p. was unchanged by recrystallization from acetic anhydride or from alcohol. When dissolved in hot toluene and precipitated therefrom by one to two volumes of alcohol, the m. p. changed to 256°.

3,6-Dichlorofluoran, prepared from fluorescein and phosphorus pentachloride according to Baeyer's direction,[7] melted at 255°. Baeyer reported 252°. When reduced with zinc dust and alcohol,[8] both products yielded fluorescein dichloride, m. p. 229°.

Preparation of Ethers

Allyl *p*-Cresyl Ether.—This ether was prepared by a new method. An aqueous solution of sodium *p*-cresolate was prepared from 5.4 g. of *p*-cresol, 2.0 g. of sodium hydroxide and 30 cc. of water. To this were added 6.1 g. of allyl bromide and sufficient acetone (30 cc.) to give a single clear solution. The solution was allowed to stand at room temperature for four hours during which there separated an oily supernatant layer. The oil was extracted with petroleum ether, washed with water, dried over potassium carbonate, and distilled. Allyl *p*-cresyl ether was obtained as a colorless oil boiling at 96–98° (15 mm.); $n^{24}\text{D}$ 1.5157. These constants agree with those in the literature.[9] The yield was 5.3 g., which is 72%.

Hexenyl *p*-Cresyl Ether.—A solution was made of 4.4 g. of *p*-cresol and 1.6 g. of sodium hydroxide in 50 cc. of water. Then 6.5 g. of 1-bromo-2-hexene in 100 cc. of acetone was added. In four hours the mixture had separated in two layers which were separated by extraction with petroleum ether. After washing, drying and

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[4] Von Liebig, *J. prakt. Chem.*, **88**, 26 (1912).

[5] Hurd and Williams, THIS JOURNAL, **58**, 965 (1936).

[6] The m. p. is reported variously in the literature. Orndorff and Hemmer, THIS JOURNAL, **49**, 1275 (1927), listed 200°; von Liebig, *J. prakt. Chem.*, **85**, 241 (1912) reported 205–206°; Sah and Yen, *Chem. Zentr.*, **104**, I, 3560 (1933), found 206–207°.

[7] Baeyer, *Ann.*, **183**, 18 (1876).

[8] Meyer, *Ber.*, **25**, 1387 (1892).

[9] Claisen and Eisleb, *Ann.*, **401**, 44 (1913).

distilling, the colorless ether was obtained in 66% yield (5 g.); b. p. 142–146° (14 mm.), n^{26}_{D} 1.5071.

Anal. Calcd. for $C_{13}H_{18}O$: C, 82.1; H, 9.46. Found: C, 81.7; H, 9.39.

Preparation of O-Alkenyl Derivatives of Fluorescein

The properties and analyses of the various compounds are summarized in Table II.

TABLE II

PROPERTIES OF COMPOUNDS

Symbol	Color	M. p., °C.	Analyses,[a] % Calcd. C	Calcd. H	Found C	Found H
I	Orange	155	75.69	4.89	75.16	5.02
II	Pale yellow	124	75.69	4.89	75.37	4.61
III	Pale yellow	205	74.17	4.32	73.97	4.61
IV	Colorless	143	72.46	4.35	72.01	4.43
V	Dark red	233	74.17	4.32	73.76	4.53
VI	Orange	118	76.91	6.00	76.97	5.89
VII	Pale yellow	131	76.91	6.00	76.89	5.95
VIII	Yellow	220	75.00	5.00	74.66	5.22
IX	Yellow-white	108	73.30	4.97	73.12	4.85
X	Orange	109	77.42	6.45	76.69	6.24
XI	Pale cream	103	77.42	6.45	77.30	6.50
XII	Pale yellow	187	75.36	5.31	74.95	5.37
XIII	Yellow-white	154	73.68	5.26	73.68	5.35

[a] All analyses except XI were performed by Mr. M. A. Pollack.

The Allyl Ether Ester of Fluorescein (I)

First Method.—Thirty-three grams (0.1 mole) of fluorescein was mixed with 28 g. (0.2 mole) of potassium carbonate. A solution of 24 g. (0.2 mole) of allyl bromide in 300 cc. of acetone was added. After refluxing with stirring for twelve hours, the mixture was poured into 500 cc. of cold water. The allyl ether ester and the allyl diether of fluorescein precipitated, while the unreacted fluorescein as well as a small amount of the monoallyl ether and the monallyl ester dissolved, giving rise to a deep red solution which exhibited a green fluorescence. The orange precipitate was filtered off, washed with water, and dried in a desiccator. On recrystallizing the 30 g. of crude material from 350 cc. of carbon tetrachloride, there was obtained 16 g. of the orange-colored ether ester, m. p. 155°. Concentration of the mother liquor to 75 cc. yielded an additional 1.2 g. of the compound, bringing the total yield to 17.2 g. or 42%. On complete evaporation of the solvent, there was left a brown gummy residue from which the allyl diether could be obtained.

Second Method.—Thirty-eight grams (0.1 mole) of the disodium salt of fluorescein was dissolved in 250 cc. of water and a solution of 24 g. (0.2 mole) of allyl bromide in 150 cc. of acetone was added, giving a single clear solution. (The sodium salt was prepared by adding an excess of fluorescein to a solution of sodium hydroxide, filtering, and evaporating the filtrate to dryness.) The aqueous acetone solution was refluxed for two hours during which time the allyl ether ester and the monoallyl ether separated as a dark red oil. On pouring the supernatant solution into water, an orange, colloidal solution was obtained. Dilute alkali was added to dissolve the monoallyl ether and cause the precipitation of the ether ester as a flocculent orange precipitate. The dark red oil was dissolved in 50 cc. of acetone, poured into water, and the resulting colloidal solution treated similarly with alkali. After filtering and washing the precipitates, the combined yield of crude alkali-insoluble material was 25 g. On recrystallizing from carbon tetrachloride there was obtained 18 g. of (I), m. p. 155°. This is 43.5% of the theoretical.

The second method is not only more rapid than the first, but it is also to be preferred since a 30% yield of the monoallyl ether rather than unreacted fluorescein was obtained from the alkaline filtrate. It was found that the yield of ether ester could be made practically quantitative by using a 25% excess of allyl bromide and by adding sufficient acetone during the refluxing to maintain a clear solution.

The Allyl Diether of Fluorescein (II).—The brown, gummy residue from the final mother liquor from the recrystallization of (I), prepared by the first method, was dissolved in 30 cc. of hot 95% ethanol. Dark brown crystals began to separate on cooling. These were filtered and washed with alcohol to remove most of the brown impurity. On recrystallizing from 20 cc. of hot alcohol there was obtained 3.6 g. of pale yellow crystals which melted sharply at 124°.

The Allyl Ether of Fluorescein (III).—The alkaline filtrates from the synthesis of (I) by the second method were combined and acidified with hydrochloric acid. There was obtained a yellow precipitate which coagulated to form a tan gum on warming. (This serves to distinguish the monoether from unreacted fluorescein, the yellow form of which coagulates as red granules on warming in hydrochloric acid solution.) The coagulated precipitate was washed by decantation and then dissolved in 75 cc. of hot alcohol. On standing overnight 9.2 g. of the pale yellow allyl ether of fluorescein, m. p. 205°, separated. An additional 2.1 g. of the compound was obtained by evaporating the mother liquor and treating the resulting tar with a solution of 0.5 cc. of acetone in 10 cc. of alcohol which removed the impurities, leaving the ether undissolved. The total yield of (III) was 30% of the theoretical.

Acetate of (III).—This derivative was prepared by dissolving 0.5 g. of (III) in 2 cc. of acetic anhydride and heating for five minutes. After several recrystallizations from ethanol, the acetate was obtained colorless, m. p. 143°.

The Allyl Ester of Fluorescein (V).—The alkaline filtrate from the synthesis of (I) by the first method was acidified with hydrochloric acid and the yellow precipitate filtered and washed. This gave 10.8 g. of material which was dissolved in a solution of 4 g. of sodium carbonate in 200 cc. of water. Carbon dioxide was bubbled through the solution for several hours, at the end of which time 2.4 g. of orange-red solid had separated. This was a mixture of the allyl ether and the allyl ester. The former was extracted by washing the mixture with several 10-cc. portions of warm ethanol. The residue was dissolved in 4 cc. of hot ethanol. The solution was intensely fluorescent. On cooling, (V) slowly crystallized in small clusters of dark red crystals, m. p. 233°.

The pentenyl ether ester (VI) and the pentenyl diether of fluorescein (VII) were obtained in essentially the same

TABLE III

Substance heated	Compound formed		M.p., °C.	Analyses			
				Calcd.		Found	
	Symbol	Name		C	H	C	H
III	XIV	2-Allylfluorescein	168–176	74.17	4.32	73.57	4.46
II	XV	2,7-Diallylfluorescein	158–161	75.69	4.89	74.99	4.78
I	XVI	Allyl 2-allylresorcinolbenzein-11-carboxylate (Allyl ester of 2-allylfluorescein)	137–143	75.69	4.89	74.40	4.84
VIII	XVII	2-Pentenylfluorescein	156–160	75.00	5.00	74.25	4.86
XI	XVIII	2,7-Dihexenylfluorescein	135–140	77.42	6.45	75.28	6.52

way as the corresponding allyl derivatives, (I) and (II), using the first method. The mixture of 1-bromo-2-pentene[10] (0.1 mole), fluorescein (0.05 mole) and potassium carbonate (0.1 mole) in acetone (50 cc.) was refluxed for twelve hours over a period of two days. On pouring into water, an oily layer separated. The oil solidified after four hours in the ice box. It was filtered, washed with a 1% solution of sodium hydroxide, then water and finally dried. In this case, it was found that a better separation of the two isomers could be obtained by dissolving the ether ester in cold ethanol, which procedure left the diether undissolved. Recrystallized from hot ethanol, the pentenyl diether of fluorescein was obtained as pale yellow needles, m. p. 131°.

The pentenyl ether ester was obtained as orange-colored flakes, m. p. 118°, by precipitation from the alcoholic solution with water and recrystallization from carbon tetrachloride. The yields of diether and ether ester, respectively, were 23 and 17%.

The pentenyl ether of fluorescein (VIII) was obtained in 32% yield by the same method as that used to prepare the allyl ether (III). Its melting point, 220°, was found to depend somewhat on the rate of heating, for rearrangement takes place on melting. **Its acetyl derivative** (**IX**), prepared analogously to IV, melted at 108°. The crystallization from alcohol required several hours.

The hexenyl ether ester (**X**) **and the hexenyl diether of fluorescein** (**XI**) were prepared in the same manner as the pentenyl compounds. It was necessary to reflux with stirring the mixture of 1-bromo-2-hexene[11] (0.2 mole) fluorescein (0.1 mole), and potassium carbonate (0.2 mole) in acetone (120 cc.) for sixty hours over a period of eight days. The first sign of reaction (*i. e.*, the formation of alkali-insoluble material) was not observed until the fifth day, after thirty-six hours of refluxing. The yields of diether and of ether ester, respectively, were 22 and 12%.

The Hexenyl Ether of Fluorescein (**XII**).—This compound and its acetyl derivative (XIII) were obtained by the same methods as for the allyl and pentenyl compounds. Like the other monoethers, the impure yellow crystals of the monohexenyl ether tended to turn orange when exposed to air and alcohol. It was only after three crystallizations from ethanol that it was obtained as pale yellow crystals, m. p. 187°; yield 12%.

Pyrolysis of the Ethers

General Procedure.—The compound (2–5 g.) was heated in a test-tube immersed in an oil-bath. Inner and outer temperatures were recorded. In all cases an exothermic reaction occurred at 210–220°, at which time the melt became viscous and darkened in color. The temperature was maintained at 210–220° for about one hour. The material was cooled, and the friable, dark red glass so obtained was crushed and extracted with a hot 5% solution of sodium bicarbonate. (In the case of the product from (I) it was necessary to use a 0.5% solution of sodium hydroxide.) The alkaline solutions were strongly fluorescent. The filtered alkaline solution was acidified, thereby precipitating the substituted fluorescein as a yellow-orange precipitate (yields, 50%) which formed a dark red glass on drying in an oven at 115°. The compounds were readily soluble in alcohol, acetone, ethyl acetate, glacial acetic acid or dioxane but insoluble in petroleum ether. In no case was it found possible to recrystallize the product from an organic solvent or combination of solvents.

The compounds treated in this manner were the allyl ether (III), the allyl ether ester (I), the allyl diether (II), the pentenyl ether (VIII), and the hexenyl diether (XI). Data regarding the substances formed are collected in Table III. Since purification was only by bicarbonate extraction and subsequent acidification, high purity for the compounds could not be expected. Analyses were fairly satisfactory in most cases, however. Small amounts of fluorescein or other by-products of the pyrolysis were undoubtedly present.

The material insoluble in sodium bicarbonate was washed with 10% sodium hydroxide, dissolved in alcohol and precipitated therefrom by water. About 0.3–0.6 g. of orange-yellow solid was obtained in each case. These were probably the cumarane analogs of XIV–XVIII.

Ozonolysis of Fluorescein Hexenyl Diether.—One gram (0.002 mole) of **XI** in 60 cc. of dry carbon tetrachloride was ozonized, the ozonide hydrolyzed with warm water and the aldehydes liberated were oxidized to acids by warming for four hours with a mixture of sodium hydroxide (1 g.) and silver oxide (from 3 g. of silver nitrate). After filtration, acidification with dilute sulfuric acid and refiltration, the filtrate was distilled to obtain the volatile acids. Both formic and butyric acids were identified, the former by permanganate titration and the latter (via the sodium salt and *p*-bromophenacyl bromide) as *p*-bromophenacyl butyrate, m. p. 61–63°. On the assumption that these were the only two volatile acids, they were analyzed by the Duclaux method and found to be in the ratio of 30:70 (formic:butyric). This assumption may not be wholly justified but the answer is valuable at least qualitatively.

Ozonolysis of 2,7-Dihexenylfluorescein.—Ozonolysis of XVIII (0.5 g.) and subsequent treatment as before yielded a mixture of volatile acids which was high in formic acid as revealed by permanganate titration.

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[10] Hurd and McNamee, THIS JOURNAL, **59**, 104 (1937).

[11] Hurd and Parrish, *ibid.*, **57**, 1732 (1935).

Duclaux analysis seemed to show 85% formic and 15% butyric acids, but here again the possible presence of other volatile acids limits the reliability of this quantitative figure. It does serve to demonstrate the preponderance of formic acid in the mixture, however.

Hydrolysis and Alcoholysis of (I)

In Water.—Three grams of the allyl ether ester was refluxed with a solution of 2 g. of potassium hydroxide in 100 cc. of water. The compound slowly dissolved, giving an intensely fluorescent solution. Saponification was complete after thirty minutes. Acetic acid was added to the clear solution until incipient precipitation. Filtration yielded 0.35 g. of the allyl ether (III); m. p. 204° after crystallization from ethyl alcohol. Complete acidification of the filtrate yielded 1.4 g. of material which was shown to be fluorescein; m. p. of the diacetate, 203–204°.

In Allyl Alcohol.—Two grams of the allyl ether ester was dissolved in 20 cc. of dry allyl alcohol. One gram of crushed sodium hydroxide was added, and the mixture was refluxed for one hour. The completeness of the reaction was determined by pouring a test portion into water; a clear solution indicated that the saponification was complete. Part of the alcohol was then distilled off and the residue poured into 100 cc. of water, filtered to remove traces of insoluble impurities, and acidified with hydrochloric acid. There was obtained 1.8 g. of (III), m. p. 205° (recrystallized from ethyl alcohol); a mixed m. p. with an authentic sample showed no depression.

In Aqueous Acetone.—One gram of the allyl ether ester was dissolved in 50 cc. of acetone and 1.5 g. of sodium hydroxide dissolved in 10 cc. of water was added. Two layers were formed. Refluxing for two hours resulted in very little reaction. When, however, sufficient water to give a single, clear solution was added, the solution almost immediately showed a green fluorescence and after ten minutes saponification was complete. The solution was poured into 100 cc. of water and acidified. The tan precipitate thus obtained melted at 197–200°. One recrystallization from alcohol yielded 0.5 g. of the pure allyl ether, m. p. 205°.

This procedure was found to be quite satisfactory for obtaining the allyl monoether (III), using the crude ether ester prepared by the second method described above. The yield of (III) was 55–65%, with fluorescein formed as a by-product.

In Methanol.—The procedure and quantity of materials were the same as in the case of allyl alcohol. The product formed on acidifying the alkaline solution melted at 262–265°; yield 1.3 g. On recrystallization from 95% ethyl alcohol, very pale yellow crystals, m. p. 266°, were obtained. The melting point given in the literature for fluorescein methyl monoether[12] is 262, 265 or 266°. The identity of the monoether was confirmed by converting it to the dimethyl ether ester by refluxing 0.8 g. of the compound and 2 cc. of concentrated sulfuric acid in 20 cc. of methanol for eight hours. The solution was poured into dilute alkali and the orange precipitate filtered off. Recrystallized from ethyl acetate, it melted at 208°; yield 0.6 g.

3-Methoxy-6-acetoxyfluoran (Acetate of Fluorescein Methyl Ether).—This new ester was obtained by heating 0.5 g. of fluorescein methyl monoether in 2 cc. of acetic anhydride and working up the product in the usual manner: m. p. 141° (recrystallized from ethyl alcohol).

Anal. (By Mr. M. A. Pollack) Calcd. for $C_{23}H_{16}O_6$: C, 71.13; H, 4.12. Found: C, 70.95; H, 4.10.

In Ethanol.—The method and results were similar to those in the experiments with the other two alcohols. Acidification of the alkaline solution yielded 1.35 g. of tan material. It was purified from 95% ethyl alcohol from which it crystallized in nearly colorlesss crystals, m. p. 250°. This is correct for fluorescein ethyl monoether.[13] Its identity was confirmed by preparing the monoacetyl derivative, m. p. 222°.

Summary

β,γ-Unsaturated ethers of fluorescein have been synthesized. These include the allyl, pentenyl and hexenyl monoethers, diethers and ether esters. The monoallyl ester also has been prepared.

The behavior of these ethers toward heat is analogous to that of the other allyl aryl ethers. Rearrangement gives rise to C-alkenylated fluoresceins with attachment to the nucleus taking place at the gamma-carbon atom of the allyl chain.

The hydrolysis or alcoholysis of the diallyl ether ester of fluorescein has been shown to yield fluorescein, the monomethyl ether, the monoethyl ether, or the monoallyl ether when water, methanol, ethanol or allyl alcohol (or aqueous acetone), respectively, are used as solvent. The acetate of fluorescein methyl ether was prepared.

It was shown that diacetylfluorescein may be made very readily from fluorescein and ketene. 3,6-Dichlorofluoran was suitably prepared from fluorescein and thionyl chloride.

A rapid, new method of synthesis of alkenyl aryl ethers was presented.

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[12] Von Liebig, *J. prakt. Chem.*, **88**, 39 (1913).

[13] Nietzki and Schroeter, *Ber.*, **28**, 56 (1895).