Summary

1. 11-Hydroxyhexadecanoic acid and 11-hydroxypentadecanoic acid have been synthesized by the condensation of methyl 10-aldehydodecanoate with *n*-amyl magnesium bromide and *n*-butyl magnesium bromide, respectively, and subsequent saponification of the esters obtained.

2. Jalapinolic acid has been shown to be the *d*-form of 11-hydroxyhexadecanoic acid. This has been accomplished through oxidation of both substances to the same keto acid, and also by the reduction of the keto acid derived from jalapinolic acid to a substance identical with the synthetic 11-hydroxyhexadecanoic acid.

3. Convolvulinolic acid has been shown to have a structure different from that of 11-hydroxypentadecanoic acid. The procedure used in investigating this substance was identical with that used in the study of jalapinolic acid.

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[CONTRIBUTION FROM THE ROCKEFELLER PHYSICAL AND THE BAKER CHEMICAL LABORATORIES OF CORNELL UNIVERSITY]

THE ABSORPTION SPECTRA OF SULFONEFLUORESCEIN AND SOME OF ITS DERIVATIVES[1]

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Sulfonefluorescein[3] is a sulfonic acid derivative of resorcinolbenzein and is analogous in many of its chemical properties to fluorescein. The absorption spectra of resorcinolbenzein[4a] and fluorescein[4b] have already been investigated in this Laboratory. All of the substances used in this study were analytically pure samples prepared by Dr. Vose.[3]

Fig. 1 gives the absorption spectra of neutral alcoholic solutions of sulfonefluorescein, dibromosulfonefluorescein and 2,4-dihydroxybenzoylbenzene-*o*-sulfonic acid, the intermediate product in the formation of sulfonefluorescein from resorcinol and *o*-sulfobenzoic acid anhydride. The absorption of 2,4-dihydroxybenzoylbenzene-*o*-sulfonic acid, Curve C, is characterized by three absorption bands and is entirely similar to the absorption of 2,4-dihydroxybenzoyl-*o*-benzoic acid,[4b] the bands for the former being shifted slightly toward higher frequency numbers. The structure of these intermediate acids has been the subject of considerable

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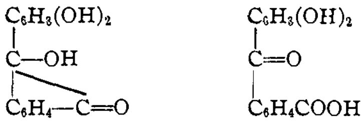
[3] Orndorff and Vose, THIS JOURNAL, **46,** 1896 (1924).

[4] Orndorff, Gibbs and Shapiro, *ibid.*, (a) **48,** 1327 (1926); (b) **50,** 819 (1928).

debate, Graebe, Kohn and Huguenin[5] assigning to them a lactone form, I, while Heller[6] proposed the ketone structure, II.



I II

2,4-Dihydroxybenzoyl-*o*-benzoic acid

A comparison of the position of the bands in the absorption spectra of these acids with those of benzoic aldehyde and acetophenone,[7] Table I, as well as the general similarity in the shape of the curves, shows conclusively that the intermediate acids, in the free state, are to be regarded as ketone derivatives. If they were present in solution in the lactone form, it is to be expected that they would have the same type of absorption spectra as diphenylphthalide and phenolphthalein,[8] but there is no indication of such a relation. As was pointed out above, the tautomeric nature of these acids was early recognized from their chemical behavior but no decisive criterion, such as the present study of absorption spectra, has been applied to determine which form is present in the free state.

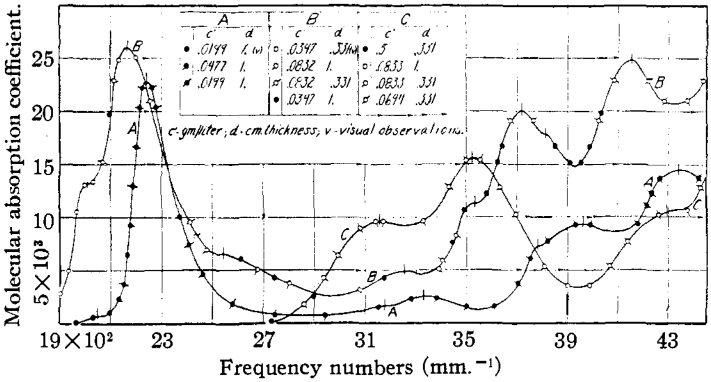


Fig. 1.—Neutral alcoholic solutions. *A*, sulfonefluorescein; *B*, dibromosulfonefluorescein; *C*, 2,4-dihydroxybenzoylbenzene-*o*-sulfonic acid.

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[5] Graebe, Kohn and Huguenin, *Arch. sci. phys. nat.*, **30**, 91 (1893).

[6] Heller, *Ber.*, **28**, 315 (1895). See also Orndorff and collaborators, THIS JOURNAL, **40**, 1239 (1918); **44**, 1518 (1922); **46**, 2276 (1924).

[7] Bielecki and Henri, *Ber.*, **47**, 1690 (1914). The absorption spectrum of benzophenone, the parent substance of the intermediate acids, has the same general outline according to data presented by Scheibe, *Ber.*, **59**, 2626 (1926), although Castille, *Bull. Acad. Roy. Belg.*, **12**, 498 (1926), has shown that the first band at about frequency number 2900 can be resolved into five narrow bands. This effect is to be correlated with the presence of two unsubstituted phenyl groups in the benzophenone molecule, in contrast with the intermediate acids, in which both phenyl groups are substituted.

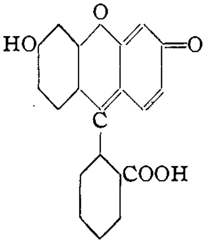
[8] Orndorff, Gibbs and McNulty, THIS JOURNAL, **48**, 1994 (1926).

TABLE I

FREQUENCY NUMBERS OF BANDS IN ALCOHOLIC SOLUTIONS OF:

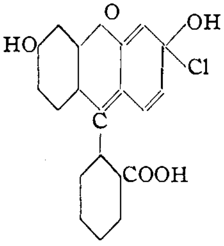
Benzoic aldehyde	Aceto-phenone	2,4-Dihydroxy-benzoyl-*o*-benzoic acid	2,4-Dihydroxy-benzoylbenzene-*o*-sulfonic acid
3050	3140	3144	3156
3563	3590	3515	3522
4097	4140	4208	4294

Sulfonefluorescein, Curve A, and dibromosulfonefluorescein, Curve B, exhibit fairly similar though displaced spectra. The absorption of these two compounds, however, shows no evident relation to that of either fluorescein or resorcinolbenzein. As was noted in the case of fluorescein,[4b] its resemblance to resorcinolbenzein does not extend beyond the first four bands. In the present case, the two visible bands of sulfonefluorescein are only slightly reminiscent of the two in neutral fluorescein. If we compare, however, the absorption of an alcoholic solution of fluorescein containing hydrogen chloride with that of a *neutral* solution of sulfonefluorescein, the identity of the absorption bands is striking: fluorescein (1 mole: 960 moles HCl), 2258, 3240, 3349, 3790, 4018; sulfonefluorescein (neutral solution), (2050), 2241, 3177, 3328, 3781, 3962, (4340). It is well known that the sulfonic acid group is much more highly ionized in solution than the carboxylic group. Further, sulfonefluorescein does not form a hydrochloride, nor does the addition of hydrogen chloride to a neutral alcoholic solution affect the absorption in the slightest degree, even when the ratio of acid to sulfonefluorescein is as high as 10,000 molecules to one. Sulfonefluorescein must, therefore, be regarded as an inner carbonium salt, III, similar in structure to fluorescein hydrochloride, II, while fluorescein probably exists as the free acid, I.



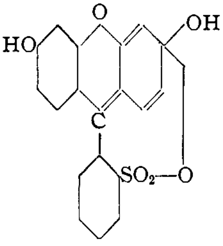
I

Fluorescein



II

Fluorescein hydrochloride



III

Sulfone-fluorescein

Dibromosulfonefluorescein is likewise assigned an inner salt structure on the basis of its resemblance to sulfonefluorescein. This assignment appears to be the most satisfactory way to account by means of structural formulas for the similarity of both chemical and optical properties of these compounds.

Orndorff and Vose[3] prepared colored diacetates of both sulfonefluorescein and its dibromo derivative, which dissolve in absolute alcohol to give yellow solutions, but which were, nevertheless, assigned lactoid structures. A study of the absorption spectra of these diacetates, Fig. 2, Curves A and B, not only shows them to be entirely different from the absorption of the corresponding diacetate of fluorescein,[4b] but also brings out a degree

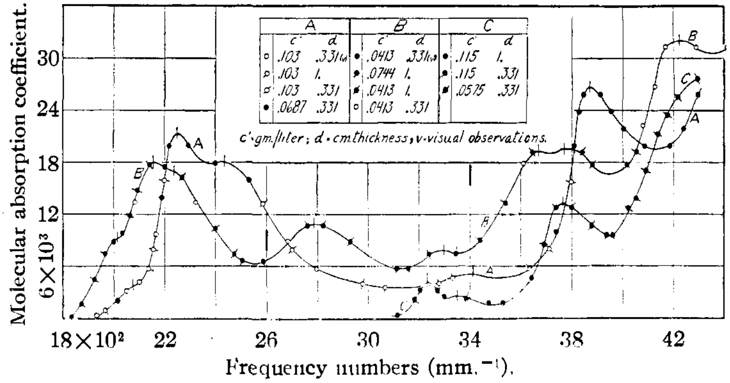


Fig. 2.—Neutral alcoholic solutions. *A*, sulfonefluorescein diacetate; *B*, dibromosulfonefluorescein diacetate; *C*, 3,3′-dichlorosulfonefluoran.

of similarity with neutral sulfonefluorescein. We are inclined, therefore, to regard the diacetates as possessing a quinoid structure which is derived directly from the inner carbonium salt structure of sulfonefluorescein. This would account for the high color of the compounds and for the relative

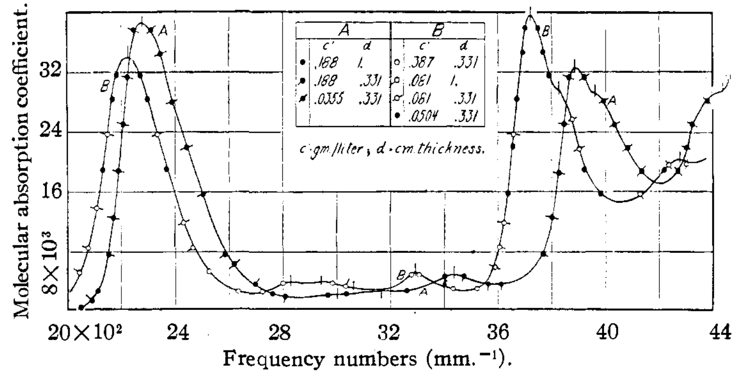


Fig. 3.—Concentrated sulfuric acid solutions. *A*, sulfonefluorescein; *B*, dibromosulfonefluorescein.

complexity of the absorption spectra. On the other hand, sulfonefluorescein dichloride or, more correctly, 3,3′-dichlorosulfonefluoran, which can only be assigned a lactoid structure, is a colorless compound and exhibits an absorption, Curve C, more nearly resembling that of the lactoid derivatives of fluorescein.

Sulfonefluorescein and dibromosulfonefluorescein dissolve in concentrated sulfuric acid to give yellow solutions having a pronounced green fluorescence. The absorption of these solutions, Fig. 3, Curves A and B, is typical of all compounds structurally related to fluorescein thus far examined in concentrated sulfuric acid. The absorption spectrum always contains a prominent band in the blue or violet, a series of weak bands in the middle ultraviolet and a prominent double or complex band in the further ultraviolet. These are followed in the present case by a band in the extreme ultraviolet region, but if such a band were present in resorcinolbenzein or fluorescein, calculation shows that it would lie just beyond the range of our spectrograph. A band similar to this last one has also been located in sulfuric acid solutions of dibromo- and tetrabromofluorescein.[9] The remarkable resemblance of the absorption curves for these various compounds in sulfuric acid solution and the consistent confirmation of Hartley's rule, that increase in molecular weight shifts the band toward longer wave lengths, is well brought out in Part A, Fig. 4.

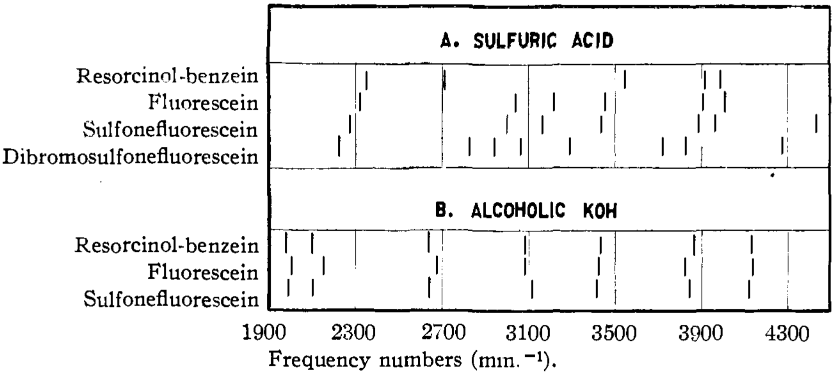


Fig. 4.—Relative positions of absorption bands in acid and alkaline solutions of some fluoresceins.

Fig. 5 shows the absorption of alcoholic solutions of sulfonefluorescein containing varying amounts of potassium hydroxide. Curve A, A′, for one molecule of potassium hydroxide to one of sulfonefluorescein shows bands characteristic of both the neutral and alkaline states. The two bands of Curve A are entirely comparable with the first two bands in a neutral solution of fluorescein. There is, apparently, sufficient alkali present to break the inner carbonium salt bond of the neutral sulfonefluorescein and set free the acid, but not enough to bring out the prominent band at 1990 which is characteristic of the alkali salt. Increasing the ratio of alkali to 4 and 25 molecules, Curves B, B′ and C, C′, respectively,

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[9] Unpublished data.

brings out the distinctive absorption of the alkali salt and, as can be seen from B, Fig. 4, this absorption is practically identical with that of the alkali salts of resorcinolbenzein and fluorescein. It is significant in this connection that the acid and basic salts of the various derivatives of resorcinolbenzein considered thus far give uniform types of absorption, whereas the neutral solutions show decided variations. While these variations are primarily due to the specific chemical properties of the added substituent groups, such as carboxyl and sulfoxyl and hence are less susceptible of expression in a rigid structural formula, the spectroscopic evidence in this case justifies the use of different structures for fluorescein and sulfonefluorescein. On the other hand, the presence of external forces, such as strong acid or basic solutions, can overcome these individual differences and produce a uniformity of structure, as is made evident by the striking similarity in the absorption spectra of these salts.

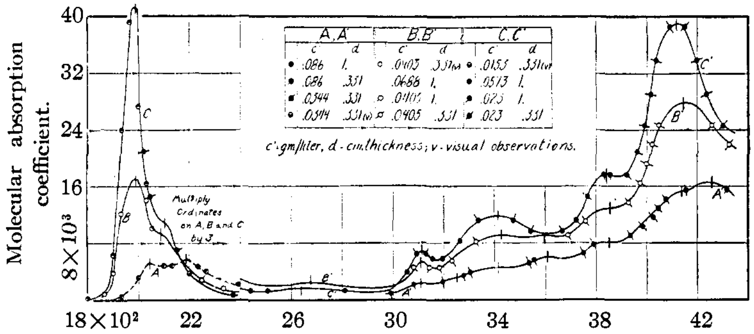

Fig. 5.—Sulfonefluorescein in alcoholic KOH. *A*, 1 molecule: 1 molecule KOH; *B*, 1 molecule: 4 molecules KOH; *C*, 1 molecule: 25 molecules KOH.

When dissolved in 33% aqueous potassium hydroxide, sulfonefluorescein yields an orange-colored solution with an intense green fluorescence. On standing for several days, the color gradually changes to purple with an evident decrease in intensity. As in the case of fluorescein,[4b] the production of the purple color can be hastened, either by heating the alkaline solution or by dissolving the solid potassium hydroxide and sulfonefluorescein in water simultaneously. The absorption of solutions prepared in both ways is shown in Fig. 6. Curve A, A′ for a freshly prepared cold solution, Curve B, B′ for the same solution two weeks later and Curve C, C′ for a freshly prepared hot solution, examined after twelve hours to allow for cooling, all show the same general type of absorption. This again emphasizes the point that was made in discussing the behavior of fluorescein under like conditions, namely, that the rupture of the pyrone ring, to which Baeyer ascribed the change in color from orange to purple, has no radical effect on the absorption of the solution, except for the growth of an existent band at frequency number 1740. However, the breaking of the pyrone ring with the accompanying formation of a tetrabasic salt is followed by a gradual disruption of the molecule, as indicated by the decrease in intensity of the bands at 1740 and 1997. After eight months the solution had faded to a pale pink, and the absorption in the ultraviolet region, Curve D′, had changed completely in character. This slow fading of the solution is not due to carbinol formation entirely, for the original color of the fresh solution cannot be restored by dilution with water, after the process has gone on for some time, nor can the fluorescein or sulfonefluorescein be reprecipitated by acidification, as is possible with faded solutions of the phthaleins. It appears, rather, that after the pyrone ring is broken the molecule is disrupted, with the formation of decomposition products whose identity has not yet been established.

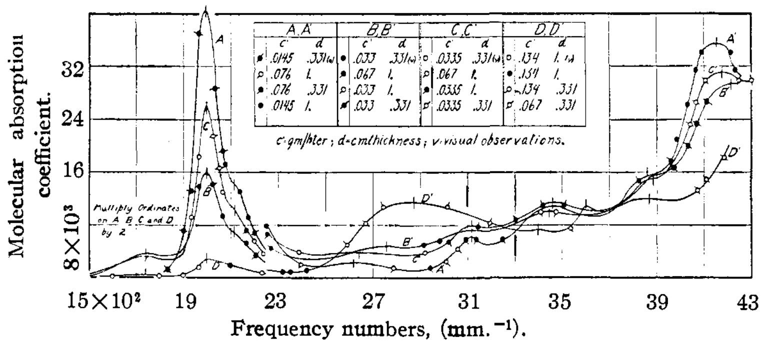

Fig. 6.—Sulfonefluorescein in 33% aqueous KOH: *A*, freshly prepared, cold solution; *B*, solution "A," after two weeks; *C*, hot solution, after twelve hours; *D*, solution "C," after 8 months.

Table II summarizes the positions of the absorption bands in the various solutions of sulfonefluorescein and its derivatives.

TABLE II

FREQUENCY NUMBERS OF ABSORPTION BANDS

ABSOLUTE ALCOHOL SOLUTION

2,4-Dihydroxybenzoylbenzene-*o*-sulfonic acid				3156		3522		4294
Sulfonefluorescein	2050	2241		3177	3328	3781	3962	4340
Dibromosulfonefluorescein	2010	2165	2543	3253	3535	3720	3813	4154
Sulfonefluorescein diacetate	2075	2250	2436		3411		3868	
Dibromosulfonefluorescein diacetate	2013	2155	2236	2786	3297	3671	3770	4223
Sulfonefluorescein dichloride					3233	3348	3765	

CONCENTRATED SULFURIC ACID SOLUTION

Sulfonefluorescein	2279		3001	3165	3436	3885	3961	4435
Dibromosulfonefluorescein	2224	2828	2942	3062	3291	3721	3826	4278

Table II (*Concluded*)

Alcoholic Potassium Hydroxide Solution										
Sulfonefluorescein (1 mole):1 mole of potassium hydroxide	2045	2188	2665	3110	3252	3414	3609	3853	4139	4260
Sulfonefluorescein (1 mole):4 moles of potassium hydroxide			1990	2088	2676	3109	3427	3598	3856	4145
Sulfonefluorescein (1 mole):25 moles of potassium hydroxide			1990	2104	2639	3113	3415		3841	4120
33% Aqueous Potassium Hydroxide Solution										
Sulfonefluorescein, freshly prepared cold solution			1740	1997	2114	2618	3118	3460	3861	4155
Solution above after standing for two weeks			1740	1997	2114	2757	3118	3445	3885	4215
Freshly prepared hot solution after twelve hours			1740	1997	2117	2642	3118	3460	3885	4175
Solution above after standing for eight months			1740	1997		2873		3398	3601	3869

Summary

1. It has been shown from a comparative study of their absorption spectra that 2,4-dihydroxybenzoyl-*o*-benzoic acid and 2,4-dihydroxybenzoylbenzene-*o*-sulfonic acid exist in the free state as ketone derivatives.

2. A comparison of the absorption spectrum of sulfonefluorescein in neutral alcoholic solution with that of fluorescein in neutral and acid solution has led to the suggestion that sulfonefluorescein has an inner salt structure.

3. The absorption spectra of sulfonefluorescein in concentrated sulfuric acid solution and in alcoholic alkaline solution bear a close relationship to the spectra of resorcinolbenzein and fluorescein in the same solvents.

4. The absorption of sulfonefluorescein in strong, aqueous potassium hydroxide changes progressively, indicating two successive chemical changes: (1) the breaking of the pyrone ring and (2) the probable disruption of the molecule.

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