—36.64° as is the case in the oils from various parts of the two grains studied, namely, wheat and corn.

Summary

An effort has been made to prepare sitosterol in as pure a condition as possible and to study the physical properties of the purified preparations.

The results indicate that sitosterol as ordinarily prepared is contaminated with small amounts of dihydro-sitosterol. The greater part of this impurity can be removed by fractional precipitation of the dibromo compound.

The sitosterol regenerated from the dibromo compound possesses a higher melting point and a higher levorotation than preparations purified only by ordinary recrystallization.

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THE INFLUENCE OF CONSTITUTIONAL VARIATION IN DYES UPON THEIR RELATIVE ABSORPTION IN AQUEOUS AND ALCOHOLIC SOLUTIONS[1]

BY WALTER C. HOLMES

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It was recognized by the earliest investigators in spectroscopy that colorless solvents affect the absorption spectra of colored solutes. The rule was formulated by Kundt[2] that the position of the absorption band of the solute depends on the refractive index of the solvent, being displaced toward the red end of the spectrum as the refractive index increases.

Subsequent investigations bearing on the question[3] have not, for the greater part, confirmed the validity of Kundt's rule. It has been established that if any fundamental relation exists between the refractive index of the solvent and the absorption of the solute its influence is greatly obscured by the predominant effect of other factors. In numerous instances, as in the recent investigation of the absorption of alizarin-cyanine in organic solvents by Meek,[4] it has been made apparent that the chemical nature of the solvent has a decisive influence on the phenomenon.

The method of procedure employed in these investigations was a study of the absorption of a comparatively small number of dyes in as compre-

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[1] Presented before the Dye Section of the American Chemical Society at its meeting, April 21–26, 1924.

[2] Kundt, *Pogg. Ann. Jubelband*, **1874**, 615–624. *Sitzb. Bayer. Akad.*, **7**, 234 (1877). *Wied. Ann.*, **4**, 34 (1878).

[3] Most of these have been reviewed and discussed by Kayser, "Handbuch der Spectroscopie," S. Hirzel, Leipzig, **1905**, III, pp. 80–89.

[4] Meek, *J. Chem. Soc.*, **111**, 969 (1917).

hensive a selection of solvents as possible. The alternate procedure of a study of as comprehensive a selection of dyes as possible in a limited number of solvents does not appear to have been employed.

In the course of spectrophotometric examinations of dilute aqueous and alcoholic solutions of several hundred dyes, the writer observed a definite correlation between the relative absorption of the dyes in the solvents and their constitutional variation. Many data substantiating the observed relationships were found in the literature, although their significance did not appear to have been noted. The text and tables of Formánek and Grandmougin's "Untersuchung und Nachweis organischer Farbstoffe auf spektroskopischem Wege" (1908, 1911 and 1913), in particular, supply very extensive data on both pure and technical products, by reference to which the generalizations to be outlined may be tested, and make it unnecessary to record further general lists of measurements.

For convenience the term *alcopositive* will be applied to dyes of which the absorption band is displaced in the direction of longer wave length (toward the red) in passing from aqueous to alcoholic solution. Dyes exhibiting the opposite phenomenon will be referred to as *alconegative*, while dyes which give no appreciable displacement of absorption with the change in solvent will be termed *alconeutral.*

In accordance with Kundt's rule all dyes should prove alcopositive in a definite and invariable degree. In point of fact alcopositive dyes exhibit a wide range in alcopositivity, while a very large proportion of dyes are alconegative.

Azo Dyes

It is probable that most of the azo dyes are alcopositive. The data in Formánek and Grandmougin's tables indicate that the proportion of alconegative dyes increases with the complexity of the dye molecule and that alconegative dyes predominate in the trisazo class.

The constitutional variation in azo dyes is so extreme and so few data are, as yet, available on compounds of closely related structure that it does not appear advisable to undertake further generalization at this time.

Triphenylmethane Dyes. Diamino Derivatives

Döbner's violet, the parent substance of the group, is strongly alcopositive, whereas the basic dyes derived by alkyl substitution at the amino groups are only moderately alcopositive, from which it is evident that this type of substitution exerts an alconegative influence.

Döbner's Violet: aq. max., 560$\mu\mu$; alc. max., 575 $\mu\mu$; diff. = +15
Victoria Green: aq. max., 616$\mu\mu$; alc. max., 620 $\mu\mu$; diff. = +4

Most of the acid dyes of the group, including all amido-oxy derivatives, are alconegative. It would appear that sulfonation in the amino substituents is without decided influence but that sulfonation in the non-aminated nucleus has a considerable alconegative effect.

Guinea green B (Schultz 502): aq. max., 619 μμ; alc. max., 627 μμ; diff. = +8
Light green SF yellowish (Schultz 505): aq. max., 633μμ; alc. max., 633μμ; diff. = 0
Xylene blue AS (Schultz 508): aq. max., 638μμ; alc. max., 630μμ; diff. = −8

The diamino derivatives of diphenylnaphthylmethane are alconegative.

Triphenylmethane Dyes. Triamino Derivatives

Pararosaniline and its homologs are moderately alcopositive (pararosaniline: aq. max., 539μμ; alc. max., 546μμ; diff. = +7), while the basic derivatives obtained by alkyl substitution at the amino groups are substantially alconeutral. The effect of these substitutions is analogous to that observed with diamino derivatives.

The character of the acid dyes of the group appears to depend primarily upon the nature of the amino substitutions. Dyes in which little or no amino substitution occurs (Schultz No. 524, 526) are moderately alcopositive; dyes with alkyl substitution predominant at the amino groups (Schultz No. 527 to 534) are somewhat alconegative; while dyes with aryl substitution predominant (Schultz No. 535 to 539) are decidedly alcopositive. It seems evident that the substitution of phenyl groups in the amino positions exerts a considerable alcopositive effect. The influence of sulfonation does not appear to be very decided.

Rhodamines

Rhodamine chloride, the parent substance of the group, is moderately alcopositive. With the basic derivatives alkyl substitution at the amino groups has the alconegative effect noted with triphenylmethane derivatives, while esterification exerts an alcopositive influence. These effects are evident in technical products but may be illustrated to better advantage by means of data obtained by Formânek and Grandmougin on pure derivatives.

	Aq. max. μμ	Alc. max. μμ	Diff. μμ
Rhodamine chloride	494.0	500.0	+6.0
sym-Diethyl derivative	519.3	516.3	−3.0
sym-Diethyl derivative (ethyl ester)	521.9	524.7	+2.8
sym-Tetra-ethyl derivative	552.6	545.5	−7.1
sym-Tetra-ethyl derivative (ethyl ester)	555.5	551.9	−3.6

The acid dyes of the group appear to afford evidence of an alcopositive influence of aryl substitution at the amino groups.

Phthaleins

The data supplied by Formânek and Grandmougin for the absorption of fluorescein and its halogenated derivatives in neutral solvents cannot be accepted, in all instances, as applying to the normal dye salts. Their measurements in alkaline solvents on technical products, however, as well as those of E. Vogel[5] on pure derivatives may be utilized.

The halogenated dyes derived by substitution within the resorcin residues are distinctly less alcopositive than those derived by substitution within the phthalic anhydride residue or substitution within both the resorcinol and phthalic anhydride residues. Esterification exerts an appreciable alcopositive effect, as was the case with the rhodamines.

	Schultz No.	Aq. max. μμ	Alc. max. μμ	Diff. μμ
Eosin	587	516	523	+ 7
Eosin S	589	525	537	+12
Phloxin	596	537.5	549	+11.5

Quinonimide Dyes

The great majority of technical thiazine and oxazine dyes are alconegative, the principal exceptions occurring within the class of aminophenonaphthazoxonium derivatives. With technical azine dyes the proportion of alcopositive dyes is greater than with the thiazines and oxazines.

The influence of amino substitution with these classes of dyes, in general, is analogous to that already noted with triphenylmethane and rhodamine derivatives. It is strikingly evident in the following table of the absorption maxima of a number of pure parent substances and their derivatives, which has been compiled from data obtained by Formánek and Grandmougin.

Table I

Influence of Amino Substitutions with Thiazine, Oxazine and Azine Dyes

Dye	Absorption maximum in water μμ	Absorption maximum in alcohol μμ	Difference μμ
Thiazine			
Diamino-phenazthionium chloride	602.5	605.3	+ 2.8
sym-dimethyl derivative[a]	620.1	617.8	− 2.3
sym-diethyl[a]	621.6	619.2	− 2.4
tetramethyl	667.5	657.4	−10.1
tetra-ethyl	673.0	661.1	−11.9
Amino-oxy-phenazthionium chloride	592.2	595.3	+ 3.1
dimethyl derivative	622.3	601.6	−20.7
diethyl	625.5	607.2	−18.3
Diamino-pheno-tolazthionium chloride	604.4	607.2	+ 2.8
trimethyl derivative	654.5	643.1	−11.4
tri-ethyl	658.9	647.2	−11.7
oxazine			
Diamino-phenazoxonium chloride	586.8	593.0	+ 6.2
tetramethyl derivative	648.9	643.3	− 5.6
tetra-ethyl	654.6	646.9	− 7.7

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[5] Vogel, *Wied. Ann.*, **43,** 449 (1891).

TABLE I (*Concluded*)

Dye	Absorption maximum in water mμ	Absorption maximum in alcohol mμ	Difference mμ
Resorufamine	577.7	578.7	+ 1.0
dimethyl derivative	591.7	580.0	−11.7
Diamino-pheno-tolazoxonium chloride	...	...	....
asym-diethyl derivative	628.2	625.7	− 2.5
tetramethyl	660.0	653.7	− 6.3
dimethyl-phenyl	656.3	658.1	+ 1.8
azine	...	...	...
Phenosafranine chloride	525.0	534.4	+ 9.4
asym-dimethyl derivative	555.0	551.9	− 3.1
asym-diethyl	561.8	556.1	− 5.7
tetramethyl	589.0	578.7	−10.3
sym-diphenyl	570.7	580.7	+10.0

[a] The unsymmetrical dialkyl derivatives are decidedly more alconegative than the symmetrical derivatives.

Alkyl substitution at the amino groups has a decided alconegative influence, whereas the effect of the substitution of phenyl groups is alcopositive. In general the influence of ethyl groups appears somewhat more alconegative than that of methyl groups.

Indigos

Indigotin disulfonate is approximately alconeutral, the trisulfonate is slightly alcopositive and the tetrasulfonate is decidedly alcopositive.[6] In this instance sulfonation exerts an opposite effect to that noted with diamino-triphenylmethane derivatives.[7]

Most of the investigators who have accepted, in whole or in part, the validity of Kundt's rule have found therein support either for the hypothesis that the phenomenon is indicative of solvation, or the opposed hypothesis that it precludes the possibility of solvation and is purely physical. Others have attributed the differences of absorption of dyes in water and in alcohol to phenomena of electrolytic dissociation or of molecular aggregation of solute.

The evidence cited in the present paper clearly indicates that the absorption of dyes in the solvents employed is not primarily dependent upon the refractive indices or other physical constants of the solvents. Further evidence has been obtained which indicates that the presence of the solvent employed does not *necessarily* influence the absorption of a dye in any appreciable degree. The absorption bands of crystal violet and methylene

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[6] THIS JOURNAL, **46**, 210 (1924).

[7] An instance of opposite effect has also been noted with carboxyl substitution. Victoria Green is somewhat alcopositive, whereas Chrome Green Powder (By.) is alconeutral. On the other hand, rhodamine chloride is more strongly alcopositive than is rosamine chloride.

blue in relatively concentrated aqueous solutions, for example, are practically identical with those of the dry dyes, while the aqueous band of eosin at all dilutions is essentially the same as that given by the dye in the absence of solvents.

On the other hand, sufficient evidence has been cited to demonstrate that the relative absorption of the dyes in aqueous and alcoholic solvents is primarily dependent upon constitutional factors. The influence of alkyl and aryl substitution at the amino groups, in particular, is plainly defined with dyes of a considerable variety of constitutional types. The effects of other types of substitution are also clearly apparent. The influence of substituent groups depends, in some measure, upon the structure of the parent substance and upon the position in which substitution occurs.

In a previous paper by the writer it was shown that the absorption of aqueous solutions of a considerable number of dyes underwent radical modification with suitable change in concentration.[8] In the instance of this phenomenon, also, the influence of constitutional factors was apparent. The conclusion was reached that many dyes exist in aqueous solution in a state of tautomerism between two forms in which the distribution of affinity, if not the actual atomic arrangement, is different.

It now appears to the writer that the only satisfactory explanation of the phenomena observed, both with change in concentration and with change in solvent, is found in the hypothesis that the absorption of dyes is primarily dependent upon the distribution of residual affinity in the dye molecule, and that with solutions of dyes the interplay of the residual affinities of solvent and solute may bring about affinity rearrangements in the dye molecule. In the light of this hypothesis, the dependence of the relative absorptions of dyes in aqueous and alcoholic solutions upon constitutional factors, and the further dependence of the absorptions of many dyes upon the concentration of their aqueous solutions may be readily comprehended.

It will be recognized that the hypothesis advanced is not novel. It is essentially merely a re-statement of certain fundamental assumptions which were made by Baly on the basis of other evidence and deductions, and which were utilized by him in developing his theory of chemical reactivity.[9]

Summary

The relative absorption of dyes in aqueous and alcoholic solutions does not conform to Kundt's rule but is primarily dependent upon constitutional factors.

The observed results in this investigation, as well as those obtained in a previous study of concentration effects, are accounted for by the hy-

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[8] *Ind. Eng. Chem.*, **16**, 35 (1924).

[9] Baly, THIS JOURNAL, **37**, 979 (1915).

pothesis that the absorption of dyes is dependent upon the distribution of residual affinity in the molecule, and that with solutions of dyes the interplay of the residual affinities of the solvent and solute may bring about rearrangement in the distribution of affinity of the dye.

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EVIDENCE CONCERNING THE CONSTITUTION OF GUINEA GREEN B

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Although many articles have appeared on the use of the spectrophotometer for identifying dyes, little has been said as to its quantitative application.

From Beer's law the concentration of any solution of a dye can be determined if we know the amount of absorption of light at any given wave length for some definite concentration of this dye.[1] The method of establishing the "standard" absorption of a dye is comparatively simple. The absorption spectra (extinction coefficient-wave length) are plotted for three or four samples of the same dye from different batches, all of which have been carefully analyzed and the color content determined by chemical means. From these figures the absorption curve for a 100% sample is calculated. For example, if a 0.002% solution of a sample containing 85% of dye shows an extinction coefficient at some definite wave length of 1.02, the extinction coefficient for a 0.002% solution of the pure dye would be 1.20. By determining the extinction coefficients for several samples and averaging their 100% curves, errors due to observation are practically eliminated from the standard. This method has been used to obtain the absorption constants for the eleven permitted food colors. Analyses based on these figures have proved entirely satisfactory in every way for all except three dyes, Light Green S F Yellowish, Guinea Green B and Indigotin. The last named dye offers entirely different difficulties from the greens and will be dealt with at another time.

Three methods are in general use for determining the exact color content of the permitted food dyes: color by difference, sulfated ash, and titration with titanium trichloride.

The color content can be found by determining all the possible impurities, such as inorganic salts and uncombined intermediates, and subtracting their total from 100%. In the cases of the permitted greens the "color

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[1] The variations in Beer's law are negligible over the concentrations which can be used in the standard 1-cm. cell.