tions with stirring. The temperature was held at 15° during the addition of the aluminum chloride, and was then lowered to 10°, while stirring was continued for three hours. After decomposing the reaction mixture with ice-hydrochloric acid, the product was worked up by the same procedure used with the reaction product from succinic anhydride and iodobenzene. For purification, the crude acid fraction was esterified with ethyl alcohol and distilled under reduced pressure. A fraction, boiling at 188–206° (1.5 mm.), was obtained in a yield of 26.8 g. From this, after digestion with 10% sodium bisulfite solution, and crystallization from alcohol, a yield of 23 g. (32%) of pure ethyl δ-(p-iodobenzoyl)-n-valerate, m. p. 66–67°, was obtained.

Anal. Calcd. for $C_{14}H_{17}O_3I$: I, 35.2. Found: I, 35.2.

δ-(p-**Iodobenzoyl**)-n-**valeric Acid.**—Hydrolysis of ethyl δ-(p-iodobenzoyl)-n-valerate with sodium hydroxide in 50% alcohol gave a nearly quantitative yield of δ-(p-iodobenzoyl)-n-valeric acid; m. p. 154–156°.

Anal. Calcd. for $C_{12}H_{13}O_3I$: neut. equiv., 332. Found: neut. equiv., 332.

By oxidation of δ-(p-iodobenzoyl)-n-valeric acid with aqueous alkaline permanganate, a 67% yield of p-iodobenzoic acid was obtained.

Ethyl ε-(p-**Iodophenyl**)-n-**caproate.**—By Clemmensen reduction, δ-(p-iodobenzoyl)-n-valeric acid was converted to ε-(p-iodophenyl)-n-caproic acid. The product was isolated and purified as the ethyl ester which was obtained in 14% yield; b. p. 205–210° (10 mm.).

Anal. Calcd. for $C_{14}H_{19}O_2I$: I, 36.7. Found: I, 37.0.

ε-(p-**Iodophenyl**)-n-**caproic Acid.**—Hydrolysis of the ethyl ester with alcoholic sodium hydroxide gave ε-(p-iodophenyl)-n-caproic acid. The pure product, m. p. 66–67°, was obtained by crystallization first from petroleum ether (b. p. 40–60°) and finally from 60% acetic acid.

Anal. Calcd. for $C_{12}H_{15}O_2I$: neut. equiv., 318. Found: neut. equiv., 320.

Summary

With the object of developing an absorbable liquid contrast medium for radiographic diagnoses, a number of esters of iodinated aromatic derivatives of glycolic acid, of iodinated phenoxy fatty acids, and of iodinated phenyl fatty acids have been prepared. Of these, ethyl iodophenylundecylate, a mixture of isomers obtained by the addition of iodobenzene to ethyl undecylenate in the presence of aluminum chloride, appears to satisfy most of the clinical requirements.

Studies on the Carotenoids. III. Distribution of Pure Pigments between Immiscible Solvents[1,2]

BY JONATHAN W. WHITE, JR.,[3] AND F. P. ZSCHEILE

Most methods for the determination of carotene depend upon a partition between two immiscible solvents for its separation from other pigments. Peterson[4] has discussed such methods from a historical and critical viewpoint. Many methods in use today employ 85 to 90% methanol or ethanol and a hydrocarbon as the two phases. Following the work of Clausen and McCoord[5] who used diacetone alcohol in the determination of carotene in blood, Hegsted, Porter, and Peterson,[6] Zimmerman, Tressler and Maynard,[7] and Beadle and Zscheile[8] have employed aqueous diacetone alcohol as the hypophasic solvent.

Despite the wide use of such partition methods in the carotene determination, very little quantitative work has been done with pure pigments. Clausen and McCoord[5] determined the distribution coefficients for "carotene" and "xanthophyll" between hexane and aqueous solutions of methanol, ethanol, and diacetone alcohol. The method developed from these studies was based upon a single distribution between hexane and a 100:14 solution of diacetone alcohol and water.

The determination of the carotenoids in corn grain is complicated by the presence of relatively large amounts of cryptoxanthol. Efforts to include this pigment in an analytical method have been based on the degree of adsorption of the pigment by calcium carbonate[9] or magnesium carbonate.[10] However, no attempt is usually made

(1) Based upon a portion of a thesis to be submitted by Jonathan W. White, Jr., to the faculty of Purdue University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, May, 1942.

(2) Journal Paper Number 10 of the Purdue University Agricultural Experiment Station.

(3) Present Address: Eastern Regional Research Laboratory, Wyndmoor, Pennsylvania.

(4) W. J. Peterson, *Ind. Eng. Chem., Anal. Ed.*, **13**, 212 (1941).

(5) S. W. Clausen and A. B. McCoord, *J. Biol. Chem.*, **113**, 89 (1936).

(6) D. M. Hegsted, J. W. Porter and W. H. Peterson, *Ind. Eng. Chem., Anal. Ed.*, **11**, 256 (1939).

(7) W. I. Zimmerman, D. K. Tressler and L. A. Maynard, *Food Res.*, **5**, 93 (1940).

(8) B. W. Beadle and F. P. Zscheile, *J. Biol. Chem.*, **144**, 21 (1942).

(9) L. O. Buxton, *Ind. Eng. Chem., Anal. Ed.*, **11**, 128 (1939).

(10) G. S. Fraps and A. R. Kemmerer, *ibid.*, **13**, 806 (1941).

to separate the cryptoxanthol from the final "carotene" solution. Since cryptoxanthol is intermediate in structure between β-carotene and zeaxanthol, it should have solubility properties intermediate between the two. It has adsorption properties between those of β-carotene and zeaxanthol.[11]

The results of a quantitative study of the distribution of three representative carotenoid pigments between solvent pairs are presented here for three different pairs of solvents. The pigments β-carotene, cryptoxanthol, and zeaxanthol are representatives of three types of carotenoids, those with hydrocarbon, monohydric alcohol, and dihydric alcohol structures, respectively. All have the same system of double bonds. The solvent pairs studied were hexane and aqueous solutions of each of three alcohols: methanol, diacetone alcohol, and 2-methyl-2,4-pentanediol.

Experimental

Materials.—The pigments were from preparations which have been described previously.[12] They were isolated by chromatography and were recrystallized until constant absorption coefficients were obtained following successive crystallizations. Specific absorption coefficients in liters/g. cm. were as follows: β-carotene 258 at 4500 Å. and cryptoxanthol 246 at 4515 Å. in hexane solution and zeaxanthol 248 at 4520 Å. in ethanol solution. The absorption spectra reported there were used as standards for photoelectric spectrophotometric analyses of all solutions. Diacetone alcohol was purified by distillation under reduced pressure; commercial 2-methyl-2,4-pentanediol was used without further purification. Other solvents were purified as previously described.[12]

Procedure.—For β-carotene and cryptoxanthol the following procedure was used. A sample of approximately 1.5 mg. of the pigment was dissolved in 1 liter of hexane and a 25-ml. aliquot was added to 25 ml. of each hypophasic solvent in a separatory funnel. The mixtures were shaken several times and allowed to stand in the dark at room temperature for ten minutes before the phases were separated. The volume of each phase was noted. The epiphase was washed three times with water, made to 25 ml., and the pigment concentration determined.

For zeaxanthol a different method was used. An unweighed sample of the pigment was dissolved in ethyl ether and aliquots were evaporated to dryness at room temperature under reduced pressure. The pigment residues were dissolved in 25 ml. of the particular alcohol–water solution to be studied, added to 25 ml. of hexane and equilibrated as above. The more highly colored phase was selected and its pigment was transferred through ether to ethanol for determination.

The concentration of the pigment in the phase not measured was taken as the difference between the measured

(11) L. Zechmeister, "Carotinoide," Berlin, 1934.

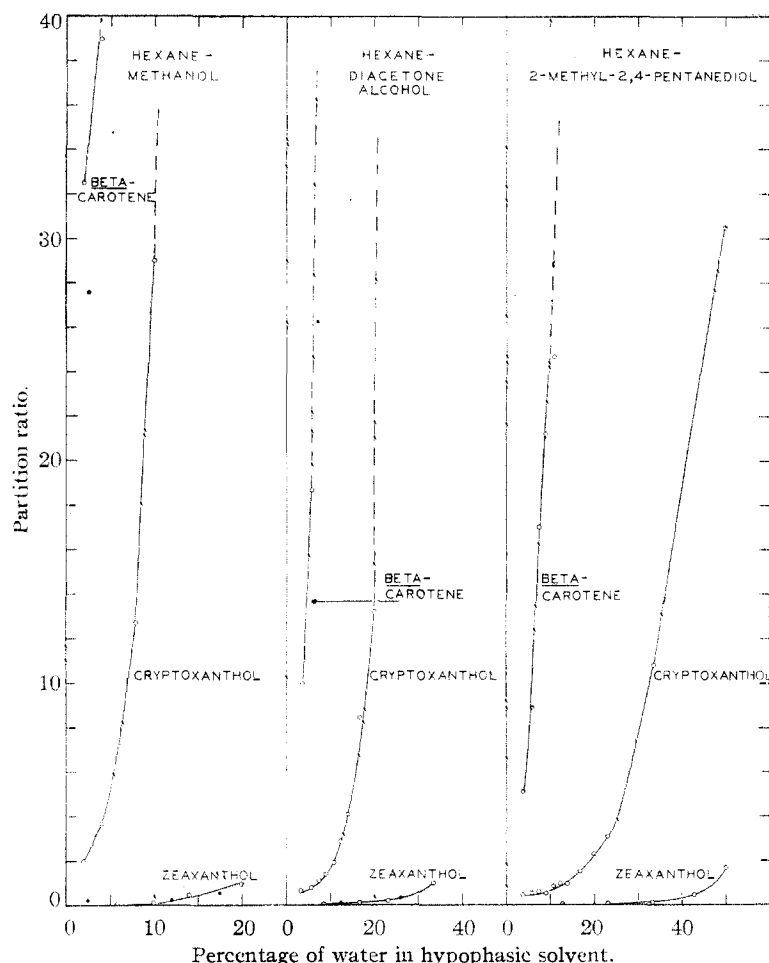(12) F. P. Zscheile, J. W. White, Jr., B. W. Beadle and J. R. Roach, *Plant Physiol.*, in press.

concentrations in the original solution and in the measured phase. Concentrations in each phase were then corrected for volume changes in the partition.

The methanol, diacetone alcohol and 2-methyl-2,4-pentanediol solutions were made up by the addition of water to the alcohol and are reported in per cent. by volume. The methylpentanediol was studied because it is so closely related structurally to diacetone alcohol. It was hoped that it might give results superior to those obtained with diacetone alcohol, which on standing develops a yellow color that interferes with the determination of carotenol concentration.

Results

Figure 1 shows the effect of the water content of the alcoholic solvent upon the partition ratios (ratio of concentration in epiphase to concentration in hypophase) of the three carotenoids for each of the three solvent pairs. The dotted lines represent continuation of the curves to experimental points beyond the limit of the graph.

TABLE I

PARTITION COEFFICIENTS

	Solvent pair		
Pigment	Hexane–90% methanol	Hexane–78.5% diacetone alcohol	Hexane–70% methyl-pentanediol
β-Carotene	54	>100	>325
Cryptoxanthol	29	40	8
Zeaxanthol	0.12	0.15	0.04

The values in Table I which were selected from Fig. 1 give the optimum water content of the hypophasic solvent for the best separation of zeaxanthol from a solution containing carotene, cryptoxanthol, and zeaxanthol.

TABLE II

PARTITION COEFFICIENTS

	Solvent pair		
Pigment	Hexane–98.0% methanol	Hexane–92.5% diacetone alcohol	Hexane–92.0% methyl-pentanediol
β-Carotene	32.5	54	19
Cryptoxanthol	1.98	1.1	0.54

The data in Table II were selected to indicate the optimum water content of the alcoholic solvent for the separation of carotene from cryptoxanthol in the absence of dihydroxycarotenes.

As an application of the solubility data, the carotenoids of three samples of corn grain were subjected to a separation based on their differential solubilities in hexane and 78.5% diacetone alcohol (see Table I). This resulted in two solutions—one containing carotene plus cryptoxanthol in hexane and the other the dihydroxy carotenols in diacetone alcohol. The former, after washing with water, was extracted by 92.0% 2-methyl-2,4-pentanediol, which removed most of the cryp-

Fig. 1.—Distribution of carotenoids between hexane and aqueous solutions of three alcohols. The data of Clausen and McCoord[5] are plotted as solid circles.

Discussion

Agreement with the distribution values of Clausen and McCoord[5] is good.

It appears that diacetone alcohol and methanol are of equal practical value in the separation of the carotene plus monohydroxycarotene from the dihydroxycarotene. The figure shows that the diacetone alcohol values change less rapidly than the methanol values with a change in the water concentration. This is of importance in application of these data to cases in which the water content of the system is not known accurately.

In the absence of cryptoxanthol, there is little practical difference between the solvents for the separation. When this pigment is present, strict control of the water content of the system is necessary if methanol is used. 2-Methyl-2,4-pentanediol is not satisfactory for this separation since the partition coefficient for cryptoxanthol does not increase rapidly enough with respect to that for zeaxanthol to permit the selection of an advantageous concentration.

The separation of carotene from crytoxanthol by solvent partition was not as complete as was the separation previously discussed, since the partition coefficients are not so advantageous. Since the partition coefficient of cryptoxanthol is less than unity in the case of 92% methylpentanediol, this solvent is preferred.

Peterson[4] has pointed out that efforts to separate carotene from cryptoxanthol by the extraction of petroleum ether extracts of yellow corn with diacetone alcohol have been unsuccessful. The use of adsorbents to separate the two pigments is not easily adapted to a routine procedure because it requires testing and standardization of each new lot of adsorbent. The possibility of loss of pigment (which Peterson has reported to average 16%)[4] also discourages the use of chromatographic methods in a routine analytical procedure. Fraps and Kemmerer[10] have recently

toxanthol (see Table II). The solutions were then examined chromatographically to determine their qualitative composition. Quantitative determinations on the eluted pigment from each fraction of the adsorption column were made spectrophotometrically. No carotene or cryptoxanthol was found in the dihydroxycarotene fraction, no dihydroxycarotene in the carotene fraction, and only traces of dihydroxycarotene in the cryptoxanthol fraction. The degree of separation of carotene from cryptoxanthol in this second extraction is presented in Table III.

TABLE III

DEGREE OF SEPARATION OF CAROTENE FROM CRYPTOXANTHOL

Sample	% of total carotene in carotene fraction	% of total cryptoxanthol in cryptoxanthol fraction
1	88	90
2	82	82
3	80	74

described a chromatographic method for the separation of carotene from cryptoxanthol. Their losses averaged 3%; in 10% of their columns it was 10–18%.

The degree of the separation as shown in Table III is of the order that would be expected from the relative values of the distribution coefficients. Error in quantitative analysis caused by the incomplete separation would be nearly compensated when the two pigments are present in corn in approximately equal proportions.

Summary

1. A quantitative study was made of the distribution of β-carotene, cryptoxanthol, and zeaxanthol between hexane and various aqueous solutions of three alcohols—methanol, diacetone alcohol, and 2-methyl-2,4-pentanediol.

2. In the absence of monohydroxycarotene, partition of carotene–dihydroxycarotene mixtures between hexane and diacetone alcohol solutions (94.5 to 77.0% diacetone alcohol by volume) should be satisfactory for the separation of these two classes of pigment.

3. In the presence of cryptoxanthol, extraction by 78.5% diacetone alcohol gives satisfactory separation of the carotene plus cryptoxanthol from the dihydroxycarotenes.

4. Extraction of a hexane solution by a 92.0% solution of 2-methyl-2,4-pentanediol in water gives fair separation of cryptoxanthol from carotene, as shown by analysis of corn grain pigments.

The Temperature–Composition Relations of the Binary System Manganous Nitrate–Water

BY WARREN W. EWING AND HERBERT E. RASMUSSEN

This article and the succeeding one[1] are continuations of the series of studies that have been made in this Laboratory on the thermodynamic properties of concentrated solutions. The nitrates of certain bivalent metals have been chosen for these studies because very concentrated solutions can be made from them, particularly so since the supersaturated solutions in the concentrated range are quite stable. Supersaturated solutions of the present system, manganous nitrate–water, up to 24 m, can be prepared and worked with at room temperature without too much difficulty.

It was necessary to establish the temperature–composition equilibria in order to facilitate the vapor pressure and calorimetric studies.[1] Various hydrates have been reported in the literature. Mellor[2] mentions a hexahydrate, a tetrahydrate, a trihydrate, a hemipentahydrate, and a monohydrate. The solubility data of Funk[3] seem to be erroneous especially in the more concentrated range. Ewing and Glick[4] found the hexahydrate, tetrahydrate, dihydrate, sesquihydrate, monohydrate, and hemihydrate existing in the ternary

system, manganous nitrate–nitric acid–water, at 20°. They also prepared the anhydride.

It seemed probable that all these forms did not exist in the manganous nitrate–water system. This present investigation indicates that only the hexahydrate, the tetrahydrate, the dihydrate, and the monohydrate are in stable equilibrium with water in the temperature range ·10 to 75°.

Experimental

The preparation and analysis of solutions of manganous nitrate have been described elsewhere.[4]

The freezing point method employed in previous work[5] was used for concentrations up to 75%. It was found that for concentrations greater than this, the extreme viscosity and the slow attainment of equilibrium made the freezing point method unfeasible. Consequently, a solubility method was used on the more viscous solutions.

In the solubility method crystals of the desired concentration were melted and then cooled to just below the freezing temperature in a tube that was placed in a constant temperature bath ($\pm 0.02°$). A seed crystal was added and the mixture was stirred until equilibrium was attained between the crystals and the solution. Due precautions were

(1) Ewing, Glick and Rasmussen, THIS JOURNAL, **64**, 1445 (1942).

(2) Mellor, "Comprehensive Treatise on Inorganic and Theoretical Chemistry," Vol. XII, Longmans, Green and Co., London, 1932, p. 440.

(3) "International Critical Tables," Vol. IV, 1930, p. 224.

(4) Ewing and Glick, THIS JOURNAL, **62**, 2174 (1940).

(5) Ewing, Krey, Law and Lang, ibid., **49**, 1958 (1927).