Chemical Composition of the Red Eye Pigment of *Drosophila melanogaster*[1]

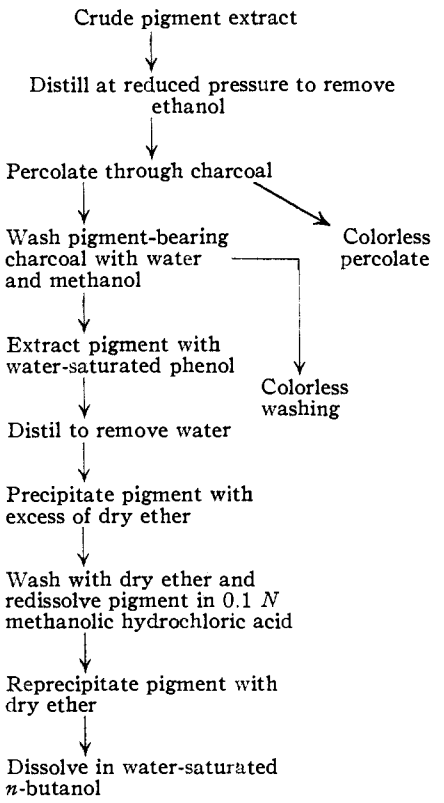
By Frank L. Chan, Hans Heymann and Clarence W. Clancy

Recently we described the application of partition chromatography to the fractionation of red eye pigments of the mutant, *vermilion*.[2] The method has enabled us to secure enough material for elementary analysis; and although our preparations were not crystalline, we believe that the details of the purification procedure and the analytical results merit publication at this time, particularly since our investigations have been interrupted for an indefinite period of time.

The source of pigment, as before,[2] was an inbred stock of the eye color mutant, *vermilion*, raised at 25 ± 1° on standard cornmeal-molasses-agar medium,[3] fortified with dried brewer's yeast. Five to seven days after eclosion the flies were etherized, checked for phenotype, and their number estimated by volume displacement. They were macerated in a Waring blendor with a small amount of 30% ethanol containing HCl to a *p*H of 2.0, and the brei dialyzed through a sausage casing ("No-Jax," 27/37, Visking Casing Company) against 200–300 cc. of the same solvent at 18–20° for 24–48 hours. The dialysis was repeated three or four times with fresh solvent, and the dialysate pooled and stored at 4°. After several months about eleven liters of crude extract derived from about one million flies was on hand for the isolation work.

Chart 1

Outline of procedures used in the concentration and relative purification of crude extracts of the red pigment of *Drosophila melanogaster*



The extract was processed in three batches by adsorption with charcoal and elution with phenol essentially as described before.[2] The procedure is summarized in Chart 1. By comparison of the optical densities at 485 mμ of the original solution and of the final concentrate, the recovery of colored material was found to be approximately 42%.

Partition chromatography in water–butanol solution was carried out on three large columns as described previously,[2] and no new phenomena were encountered. However, elution of the colored bands was more advantageously accomplished with phenol than with the solvents previously used. Only the central portion of the carrot-red band, IIb, which contains most of the tinctorial power,[2] was eluted separately; the remaining nine colored constituents were eluted and purified conjointly.

The pigment-bearing silica was placed in a 200-cc. sintered glass filter funnel, and water-saturated phenol added in small portions. When most of the pigment had been eluted and appeared in the liquid accumulating at the bottom of the silica cake, the eluate was removed by suction. Elution was continued until the silica appeared white. Suspended silica (traces) was removed by centrifugation, and the pigment precipitated by addition of methanol and ether. The latter process required about five hours.

The pigment was collected, washed with ether, redissolved in 0.1 *N* methanolic HCl, and reprecipitated with ether. The pigment was then collected, dried in air, and finally in a vacuum desiccator over 98% sulfuric acid. Table I records the amounts of purified fractions thus obtained.

Table I

Summary of Data Preliminary to Microanalysis

Initial volume of crude extract, ml.	Dry weight of pigment before solution in *n*-butanol, g.	% of original as calculated from relative optical densities	Amount of purified pigment after partition chromatography, mg. Carrot-red band	Nine-band composite pigment
3930	0.538	39.8	6.5	32.6
2973	1.145	45.1	10.9	28.2
3860	0.750	39.9	4.5	38.2
		Total	21.9	99.0

Table II

Analytical Results

	C, %	H, %	N, %	Cl, %	Ash, %
Pigment, Band II b (Carrot-red)	39.46	5.32	18.32	12.51	0.42
	39.59	5.19	18.11	12.64	0.47
Average (ash-free basis)	39.70	5.27	18.30	12.63	
Combined residual pigments	41.68	5.15	18.54	10.48	no ash
	41.42	5.09	18.48	10.29	no ash
Lederer's data	42	5.5	19	...	....
$C_9H_{14}N_4O_4Cl$	38.92	5.08	20.18	12.77	
$C_{10}H_{16}N_4O_5Cl$	39.02	5.24	18.21	11.52	
$C_{11}H_{17}N_4O_5Cl$	41.19	5.34	17.47	11.05	

Analytical results are recorded in Table II.[4] As pointed out previously,[2] elements other than those analyzed for were not detected. The analyses are compared with Lederer's analysis,[5] of a preparation of *wild-type* eye pigment, and with data calculated for a possible empirical formula. The similarity between Lederer's data and those of the present work is apparent. The molecular

(1) Work executed under a contract between the University of Oregon and the Office of Naval Research.

(2) H. Heymann, F. L. Chan and C. W. Clancy, This Journal, **72**, 1112 (1950).

(3) C. B. Bridges and H. H. Darby, *Am. Naturalist*, **67**, 437 (1933).

(4) Microanalyses by Dr. Carl Tiedcke, Laboratory of Microchemistry, Teaneck, N. J.

(5) E. Lederer, *Biol. Rev. Cambridge Phil. Soc.*, **15**, 273 (1940).

weight reported by Maas[6] requires a content of nine to eleven carbon atoms, and Lederer's suggestion that the eye pigments are pterine derivatives still appears to be a plausible one.[7]

(6) W. Maas, *Genetics*, **33**, 177 (1948).

(7) We are indebted to Mrs. Evelyn Shirck McConnaughey for technical assistance in the culture of flies and in the preparation of the crude extracts.

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Densities in the Methanol–Water System at 25.00°

BY GERALDINE CLIFFORD AND J. A. CAMPBELL

Much work has been done on the methanol–water system but none of the vapor pressure data are sufficiently good to give a consistent check with the Duhem equation. The most convenient way to analyze solutions in this system is by means of density determinations. Since we find no data at 25.00° available (a convenient temperature for our purposes), we have determined the density–mole fraction relations for the system at 25.00° in order to undertake a complete study of the vapor–liquid equilibrium.

Experimental

The water used was distilled from acid dichromate solution and shown to be of conductivity grade. Reagent grade methanol was diluted with an equal volume of water and distilled from a ten-plate fractionating column to give a practically odorless methanol of density d^{25}_4 0.78687. "International Critical Tables" lists 0.78683.

Solutions of known composition were made by adding water to weighed ground glass stoppered bottles, weighing, adding methanol and weighing again. Each mole fraction should be accurate to within ±0.00002 unit.

Density determinations were made at each composition with two pycnometers, one of about 8 ml. volume, the other about 10 ml. calibrated with water as a standard. Every determined value of the density at each composition agreed with the values tabulated below to within ±0.00004. Deviations were independent of the pycnometer used.

Weights standardized against a Bureau of Standards calibrated set were used throughout and all values were corrected for the buoyancy of air.

The thermometer was standardized against a thermometer newly calibrated at the Bureau of Standards.

Results

The density of methanol–water solutions at 25.00° as a function of the mole fraction of methanol is given in Table I. No simple equation (third power or less) fits the data because of the high curvature at the ends of the composition range.

Mole fraction methanol (±0.00002)	d^{25}_4 (±0.00004)
0.00000	0.99707
.04998	.98225
.13479	.96202
.23820	.93912
.37528	.90852
.51984	.87733
.58901	.86306
.78902	.82386
.86674	.80968
1.00000	.78687

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Ultraviolet Absorption Spectra of N-Nitrocarbamates, N-Nitroamines and Salts of N-Nitroamines

BY HOWARD M. CURRY[1] WITH J. PHILIP MASON

Shortly after this study was begun, the ultraviolet absorption spectra of some compounds containing N-nitro and N-nitroso groups were reported by Carmack and Leavitt.[2] Since our program included the ultraviolet absorption spectra of N-nitrocarbamates and amine salts of N-nitroamines the investigation was continued. Recently, a comprehensive study of the ultraviolet absorption spectra of N-nitroso and N-nitro compounds has been reported by Jones and Thorn.[3] They did not include any data on N-nitrocarbamates or amine salts of N-nitroamines.

Seven N-nitrocarbamates have been investigated and found to exhibit absorption maxima in the range 235–240 mμ whereas the nitroamines and nitroamine salts have maxima in the range 232–235 mμ. The results for the nitroamines are consistent with those of Carmack and Leavitt[2] who observed maxima for their compounds in the region 228–236 mμ. These investigators used aqueous solutions.

TABLE I

Compound	$\lambda_{max.}$, mμ	$\epsilon_{max.}$
Ethyl N-nitro-N-isopropyl carbamate	240	4900
Ethyl N-nitro-N-*n*-propylcarbamate	239	6190
Methyl N-nitro-N-ethylcarbamate	235	6030
Ethyl N-nitro-N-ethylcarbamate	237	5630
n-Butyl N-nitro-N-*n*-butylcarbamate	238	6710
Ethyl N-nitro-N-*t*-butylcarbamate	239	2690
N,N′-Dinitro-N,N′-dicarbethoxy-1,6-diaminohexane	238	12070
Isopropyl-N-nitroamine	232	6810
Potassium salt of isopropyl N-nitroamine	234	8210
Isopropylamine salt of isopropyl-N-nitroamine	233	8200
Isobutylamine salt of cyclohexyl-N-nitroamine	235	8080

The results indicate that there is a slight shift toward the visible in the spectra of the N-nitrocarbamates although the shift is not of sufficient magnitude to serve as a means of distinguishing N-nitrocarbamates from N-nitroamines.

The intensities of absorption of the amine salts of N-nitroamines and of the one potassium salt which was measured were found to be consistently greater than the intensities of N-nitrocarbamates and N-nitroamines.

An appreciable decrease in intensity is noted in the N-nitrocarbamate series when the alkyl group attached to the nitrogen atom exhibits branching at the carbon atom adjacent to the nitrogen atom. This is exemplified in Table I for the two isomeric compounds ethyl N-nitro-N-*n*-propylcarbamate and ethyl N-nitro-N-isopropylcarbamate. Although the absorption maxima for these compounds fall in approximately the same position, the

(1) Abstracted from a portion of the dissertation submitted by Howard M. Curry in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) M. Carmack and J. J. Leavitt, THIS JOURNAL, **71**, 1221 (1949).

(3) R. N. Jones and G. D. Thorn, *Can. J. Research*, **27B**, 828 (1949).