yield 0.7 g. of white needles which underwent decomposition at 305°. Prior to analysis, the material was dried *in vacuo* at 100°, during which process the needles collapsed to a white powder.

Anal. Calcd. for $C_4H_5ClON_4$: N, 34.9; Cl, 22.1. Found: N, 34.6; Cl, 21.9.

The **picrate** decomposed slowly above 250°.

Anal. Calcd. for $C_{10}H_8ClN_7O_8$: N, 25.2. Found: N, 24.9.

2,4-Diamino-5,6-dichloropyrimidine (I).—2,4-Diamino-6-chloropyrimidine (1.5 g.) was dissolved in 25 ml. of dilute hydrochloric acid and treated with a rapid stream of chlorine for seven minutes (prolonged chlorination resulted in the complete loss of product). The solution was neutralized and filtered leaving 0.5 g. of material melting at 218–219°. After recrystallization once from alcohol and once from water, 0.2 g. of material melting at 218–220° was obtained.

Anal. Calcd. for $C_4H_4Cl_2N_4$: N, 31.3; Cl, 39.6. Found: N, 31.3; Cl, 39.6.

2-Amino-4,5,6-trichloropyrimidine.—Six grams of 2-amino-4,6-dihydroxypyrimidine was refluxed overnight with 45 g. of phosphorus pentachloride and 25 g. of phosphorus oxychloride. After pouring onto crushed ice, a solid formed which was removed and suspended in 50 cc. of water. It was then observed to liquefy and to resolidify. After filtration and sublimation, 1.5 g. of material melting at 234° was obtained.[3] Four recrystallizations from benzene yielded a product of constant melting point, 236–237°.

Anal. Calcd. for $C_4H_2Cl_3N_3$: N, 21.2; Cl, 53.6. Found: N, 21.2; Cl, 53.5.

Neutralization of the original acidic filtrate from this reaction resulted in the separation of 2-amino-4,6-dichloropyrimidine, which after sublimation weighed 1.0 g. and melted at 220°.[4]

Dechlorination of 2,4,6-Triamino-5-chloropyrimidine.—One hundred and ten milligrams of 2,4,6-triamino-5-chloropyrimidine in 20 ml. of methanol containing excess sodium acetate was treated with hydrogen at 75° and 850 p. s. i. in the presence of palladium–charcoal catalyst containing a trace of Adams catalyst.[1] The solution was filtered, made distinctly basic with sodium carbonate, evaporated to dryness, and the residue extracted with 20 ml. of absolute ethanol. The residue from evaporation of the latter was sublimed *in vacuo* to give 30 mg. of triaminopyrimidine melting at 246° (dec.), alone and when intimately mixed with an authentic sample.[5]

(3) This product had a nitrogen content of 21.7% and was probably contaminated with 2-amino-4,6-dichloropyrimidine.

(4) Büttner, *Ber.*, **36**, 2227 (1903).

(5) Traube, *ibid.*, **37**, 4544 (1904).

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RECEIVED FEBRUARY 25, 1950

A New Method for Ascertaining the Coördination Number in Choleic Acids

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It is well known that desoxycholic acid forms molecular compounds, the so-called choleic acids, with a wide variety of organic derivatives. The method used for ascertaining the molar ratio of these compounds depends upon the nature of the acholic component.[1] A new method which may prove to be useful in many cases is reported in this paper. It consists in determining the ultraviolet or visible light absorption of alcoholic solutions of the choleic acid and of the pure acholic component. Provided both solutions have the same spectrum, from their concentrations and extinctions at a definite wave length, the percentage of the acholic component in the choleic acid—and thus the coördination number—can readily be ascertained.

Desoxycholic acid itself does not interfere with the method. Preliminary investigations have shown that it absorbs to some extent ultraviolet light of only very short wave length indicating that, working with a diluted (about 200 mg. per liter) solution of choleic acid, its absorption would be completely negligible above 230 mμ, when using a 1-cm. cell.

It is interesting to note that all investigations hitherto reported on the stability of choleic acids in alcoholic solutions indicate that the complex is practically or completely dissociated.[2–5] Actually one may expect that, independently of the extent of its dissociation, a solution of a choleic acid should show the same spectrum of the acholic component.

We decided to assay the method with the well-investigated choleic acid of naphthalene[6–9] and phenanthrene,[4,9,10] which can easily be prepared without being contaminated by the solvent (ethanol) choleic acid.

It has been found that naphthalene choleic acid shows the same ultraviolet spectrum as the pure hydrocarbon. Therefore we carried out the usual calculations and deduced, in agreement with earlier investigations[6–8] that naphthalene combines with two molecules of desoxycholic acid.

Phenanthrene choleic acid showed practically the same spectrum as the pure hydrocarbon below 300 mμ. However, above 320 mμ discrepancies were observed. The reason for those discrepancies is rather doubtful, though they may have been produced by some impurity. In spite of this fact, when the percentage of phenanthrene was com-

TABLE I

ABSORPTION DATA FOR DESOXYCHOLIC ACID IN ETHANOL

(c 14 g./liter; d 1.002 cm.)

λ, mμ	$\log_{10}(I_0/I)$	λ, mμ	$\log_{10}(I_0/I)$
230	0.116	270	0.013
240	.065	280	.011
250	.037	290	.008
260	.017		

(1) Harry Sobotka, "The Chemistry of the Sterids," The Williams and Wilkins Company, Baltimore, Md., 1938, pp. 118–120.

(2) H. Sobotka and J. Kahn, *Biochem. J.*, **26**, 898 (1932).

(3) H. Sobotka and J. Kahn, *Ber.*, **65**, 227 (1932).

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(5) E. Lorenz (quoted by L. F. Fieser and M. S. Newman), THIS JOURNAL, **57**, 1602 (1937).

(6) H. Wieland and H. Sorge, *Z. physiol. Chem.*, **97**, 24 (1916).

(7) E. Flume, Dissertation, Bonn, 1929, p. 26.

(8) P. Braun, Dissertation, Bonn, 1931, p. 34.

(9) P. Senise, *Bol. faculdade filosofia ciênc. letras. São Paulo Univ. XIV, Quimica No.* **1**, 35 (1942).

(10) L. F. Fieser and M. S. Newman, THIS JOURNAL, **57**, 1602 (1937).

TABLE II

ANALYSES OF CHOLEIC ACIDS BY ULTRAVIOLET OR VISIBLE LIGHT ABSORPTION

	Choleic acid soln. Concn., mg./l.	Choleic acid soln. $\log_{10} I_0/I$	λ, mμ	Pure acholic soln. Concn., mg./l.	Pure acholic soln. $\log_{10} I_0/I$	% Acholic component in choleic acid Calcd.	% Acholic component in choleic acid Found	Co-ordin. No.
	141.0	0.851	275[a]	20.5	0.900		13.7	
Naphthalene	110.4	.669	275	16.3	.712	14.0	13.9	2
	108.5	.658	275	16.3	.712		13.9	
	52.8	1.415	240	3.58	0.744		12.9	
Phenanthrene	24.0	1.159	250[a]	3.92	1.453	13.1	13.0	3
	24.1	1.162	250	3.92	1.453		13.0	
p-Aminoazobenzene	100.5	1.342	385[a]	10.1	1.298		10.4[b]	
	80.4	1.050	385	10.1	1.298	11.1	10.2[b]	4

[a] Approximately, wave length of the absorption maximum. [b] Calcd. for $C_{12}H_{11}N_3 + 5C_{24}H_{40}O_4$: 9.1. Calcd. for $C_{12}H_{11}N_3 + 6C_{24}H_{40}O_4$: 7.7.

puted from the extinction of the main band (around 250 mμ), the expected value for a coördination number of three[4,10] was obtained.

Next, we applied the method to the choleic acids of *p*-aminoazobenzene and its N-dimethyl derivative, which were prepared in connection with a research in azo-carcinogenesis. From the binary "thaw–melting point diagram" of these azo compounds with desoxycholic acid,[11] it was impossible to decide in both cases whether the coördination number in their choleic acids was four or six—five being *a priori* ruled out by symmetry considerations.[12]

It has been found that both choleic acids give the spectrum of the pure azo compound and that *p*-aminoazobenzene combines with four molecules of the biliary acid. However, no consistent results were obtained with *p*-dimethylaminoazobenzenecholeic acid. In this case different samples showed a different content of the azo compound which, however, was consistent within each sample. This choleic acid is still under investigation.

The author is indebted to Prof. H. Rheinboldt for helpful suggestions.

Experimental

Naphthalene and phenanthrene were several times recrystallized from absolute ethyl alcohol. Naphthalene was dried in Abderhalden drying apparatus at 60°, m. p. 80.2–80.5°. Phenanthrene was dried in a vacuum desiccator over calcium chloride; m. p. 99.4–100.4°. *p*-Aminoazobenzene was a sample of a product prepared by Dr. Lucy L. Nazario; m. p. 123.7–124.8°. Desoxycholic acid was purified according to Sobotka and Goldberg,[13] m. p. 171–173°. Absolute ethyl alcohol was used in both preparative and spectrophotometric work.

Choleic acids were prepared by pouring a hot alcoholic solution of the acholic component (0.3–0.4 g. in 5 ml.) into a filtered hot alcoholic solution of desoxycholic acid (3 g. in 13 ml.). The choleic acid crystallized upon cooling. Naphthalenecholeic acid was dried in an Abderhalden drying apparatus at 60°, m. p. 180–183°, reported m. p. 181–182°,[8] 182°,[6,7] 181.5–182.5°.[9] Phenanthrenecholeic acid was dried in a vacuum desiccator over calcium chloride; m. p. 183.8–185°, reported m. p. 184–185°,[10] 184–186°,[9] 186–187°.[4] *p*-Aminoazobenzenecholeic acid was dried in the Abderhalden apparatus at 94°, yellow crystals melting at 180–183°.

The extinction curves were measured by means of a D. U. Beckman quartz spectrophotometer, using 1.002-cm. silica cells. Absorption data for a solution of desoxycholic acid having a concentration of 14 g./liter are shown in Table I. In Table II are summarized the analyses of choleic acids by light absorption.

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RECEIVED MAY 15, 1950

(11) G. Cilento, unpublished data.

(12) H. Rheinboldt, *Ann.*, **451**, 256 (1927).

(13) H. Sobotka and A. Goldberg, *Biochem. J.*, **26**, 555 (1932).

The Separation of Adenosine Polyphosphates by Ion Exchange and Paper Chromatography[1]

BY WALDO E. COHN AND C. E. CARTER

The purity of preparations of ADP[2] and ATP[2] is usually established by the ratio of acid-labile to total phosphate[3] and enzymatic assay,[4] while the preparation of either in pure form, from a mixture of the two, is achieved by differential solubility of their salts.[5] Application of ion exchange and paper chromatography to the resolution and quantitation of a series of adenine compounds encountered in preparations of ATP and ADP is reported in this paper. The principles of these methods have been previously described.[6,7] The chromatographic separation of adenine nucleotides has also been studied by Chargaff, *et al.*,[8] Crammer,[9] and Hummel and Lindberg,[10] although the systems employed by them did not permit the present application. The results reported below indicate that both techniques have certain advantages in the analysis of mixtures of AMP[1], ADP, and ATP and that ion exchange can, in addition, be used as an exact preparative method

(1) Work performed under Contract Number W-7405-Eng-26 for the Atomic Energy Commission.

(2) ATP, Adenosine triphosphate; ADP, Adenosine diphosphate; AMP, Adenosine monophosphate.

(3) A. L. Dounce, *et al.*, *J. Biol. Chem.*, **174**, 361 (1948).

(4) (a) H. M. Kalckar, *ibid.*, **167**, 445 (1947); (b) G. A. LePage, and V. R. Potter, *ibid.*, **179**, 1229 (1949).

(5) H. M. Kalckar, *ibid.*, **148**, 127 (1943).

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(7) C. E. Carter, *ibid.*, **72**, 1466 (1950); Oak Ridge National Laboratory Report ORNL-313 (unclassified).

(8) E. Chargaff, *et al.*, *ibid.*, **71**, 1513 (1949).

(9) J. L. Crammer, *Nature*, **161**, 349 (1948).

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