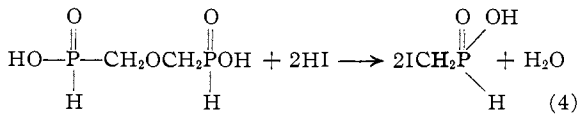
fication of this compound was made first through separation by paper chromatography and then by a gradient elution ion exchange method.

A paper chromatographic solvent consisting of 80 ml. of acetone, 25 ml. of water and 5 ml. of ammonium hydroxide was found effectively to resolve chloromethyl-, hydroxymethyl- and methylphosphinic acid from each other, as well as from all the phosphonic acids, phosphorous acid, hypophosphorous acid and the ether compound. The ether compound was found to be a trivalent phosphorus compound that titrated as a monobasic acid. By infrared analysis, and by the acetic anhydride method, it was shown that it did not contain any alcoholic OH group. A sample of the ether was isolated by gradient elution through a Dowex anion exchange resin column by means of 1 *M* KCl solution. The eluted fractions were checked by paper chromatograms. The fraction containing this compound had the same R_f value as that assigned to this fraction in the original paper chromatogram of the reaction mixture. It was evaporated, weighed, and the KCl content determined by flame photometry for potassium and silver nitrate titration for chloride. The phosphorus and phosphinate values were determined by the colorimetric vanadate and $HgCl_2$ procedures. Using these values and accounting for the presence of KCl, the respective equivalent weights of this compound were 88 and 89. The theoretical equivalent weight as shown by the formula is 87.

Another sample of this compound was isolated by ion exchange separation, and an ether determination was made by the standard reaction with HI in accordance with eq. 4.



(4)

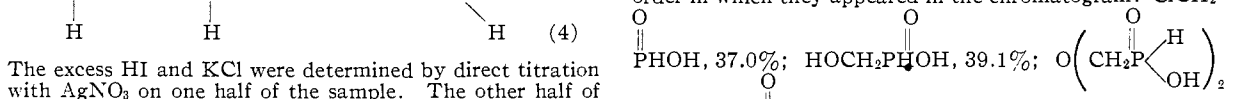
The excess HI and KCl were determined by direct titration with $AgNO_3$ on one half of the sample. The other half of the sample was boiled with NaOH to liberate the iodine atom from iodomethylphosphinate as iodide ion. The total iodide was then titrated with $AgNO_3$. The difference in the $AgNO_3$ titration gave an equivalent weight of 91 for the compound.

3. Hydrolysis with Calcium Hydroxide.—To 57 g. (0.5 mole) of chloromethylphosphinic acid in 200 cc. water was added portionwise 41 g. (0.55 mole) of $Ca(OH)_2$. The mixture was heated to 100°. After 3 hours, 80% of the chlorine had become ionic. After a total of 5 hours, 97% of the chlorine atoms in the original chloromethylphosphinic acid had been ionized. If appreciable dibasic acid, such as methylphosphonic acid, has been formed, there would not have been sufficient $Ca(OH)_2$ for the hydrolysis reaction to go to completion.

The reaction mixture was worked up by the addition of one mole of sodium hydroxide in 200 cc. of water at room temperature to convert the calcium salts to $Ca(OH)_2$. The solution was filtered to remove the precipitated $Ca(OH)_2$, and that $Ca(OH)_2$ remaining in solution was removed by bubbling CO_2 through the filtrate and filtering the $CaCO_3$ formed. The sodium salts were then converted to the free acid with concd. HCl and worked up as described in the NaOH hydrolysis.

The product thus obtained weighed 44 g. It contained 33.3% P and negligible amounts of Cl. Upon separation by a gradient elution ion exchange method, it was shown to contain 39% hydroxymethylphosphinic acid and 53.0% of α,α'-bis-(phosphinyl)-dimethyl ether, the remainder being a phosphonic acid which was assumed to be methylphosphonic acid.

4. Hydrolysis with $Mg(OH)_2$.—To 22.8 g. (0.2 mole) of chloromethylphosphinic acid dissolved in 200 cc. of H_2O was added 17.5 g. (0.3 mole) of magnesium hydroxide. The mixture was heated to reflux for 62 hours. A sample of the mixture was oxidized with $HgCl_2$ and results indicated that 96% of the phosphorus present was still in the tripositive state. The reaction mixture was worked up by adding 0.4 mole of NaOH dissolved in 100 cc. of H_2O to convert the magnesium salts to $Mg(OH)_2$, which was removed by filtration. The resulting solution was then worked up as described for the hydrolysis in $Ca(OH)_2$ solution. The product thus obtained weighed 16.8 g. Elemental analysis showed 29.3% P and 10.6% Cl. Paper chromatographic analysis showed that the following compounds were present. These compounds are given in the order in which they appeared in the chromatogram: $ClCH_2$-



16.0%; $CH_3P(OH)_2$ and unidentified cmpd., 8.0%.

Acknowledgment.—The authors wish to express their appreciation to Jacob Kleinberg, Robert Parry, Edward Walsh, T. M. Beck and L. E. Netherton for their valuable discussions and suggestions. We are also especially grateful to Donald Bernhart and William Chess for the analytical results, the paper chromatograms, and the characterization of α,α'-bis-(phosphinyl)-dimethyl ether.

[CONTRIBUTION FROM TUFTS UNIVERSITY SCHOOL OF MEDICINE, BOSTON, MASS.]

The Incorporation of the Four Nitrogen Atoms of Purines into the Pyrimidine and Pyrazine Rings of Riboflavin[1]

BY WALTER S. MCNUTT

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Experiments involving the use of N^{15}-adenine and xanthine labeled with N^{15} and C^{14} indicate that all the atoms of the purine ring except the carbon atom in position 8 are incorporated as a unit into riboflavin by the yeast, *Eremothecium ashbyii*.

Earlier studies suggested that naturally occurring purines may serve as donors of the pyrimidine ring in the biosynthesis of riboflavin and pteridines by *Eremothecium ashbyii*.[2-5] This direct biosynthetic relationship of purines to the pyrimidine rings of other molecules contrasts with the lack of a precursor relationship between the purines and pyrimidines of polynucleotides. As previous studies of this conversion dealt with purines labeled in the carbon atoms only, it was not evident whether any nitrogen in the imidazole ring of the purine was incorporated into riboflavin, and suggestions that a diaminopyrimidine derivative might arise biologically from the purine and serve as a precursor of riboflavin[2,6,7] were thus mere speculations. Although enzyme preparations from microorganisms

(1) This work was supported by U. S. Public Health Service Grant A-3675.

(2) W. S. McNutt, *J. Biol. Chem.*, **219**, 365 (1955).

(3) W. S. McNutt and H. S. Forrest, *J. Am. Chem. Soc.*, **80**, 951 (1958).

(4) H. S. Forrest and W. S. McNutt, *ibid.*, **80**, 739 (1958).

(5) W. S. McNutt, *ibid.*, **82**, 217 (1960).

(6) T. Masuda, *Pharm. Bull. (Japan)*, **5**, 136 (1957).

(7) T. W. Goodwin and D. H. Treble, *Biochem. J.*, **70**, 14P (1958).

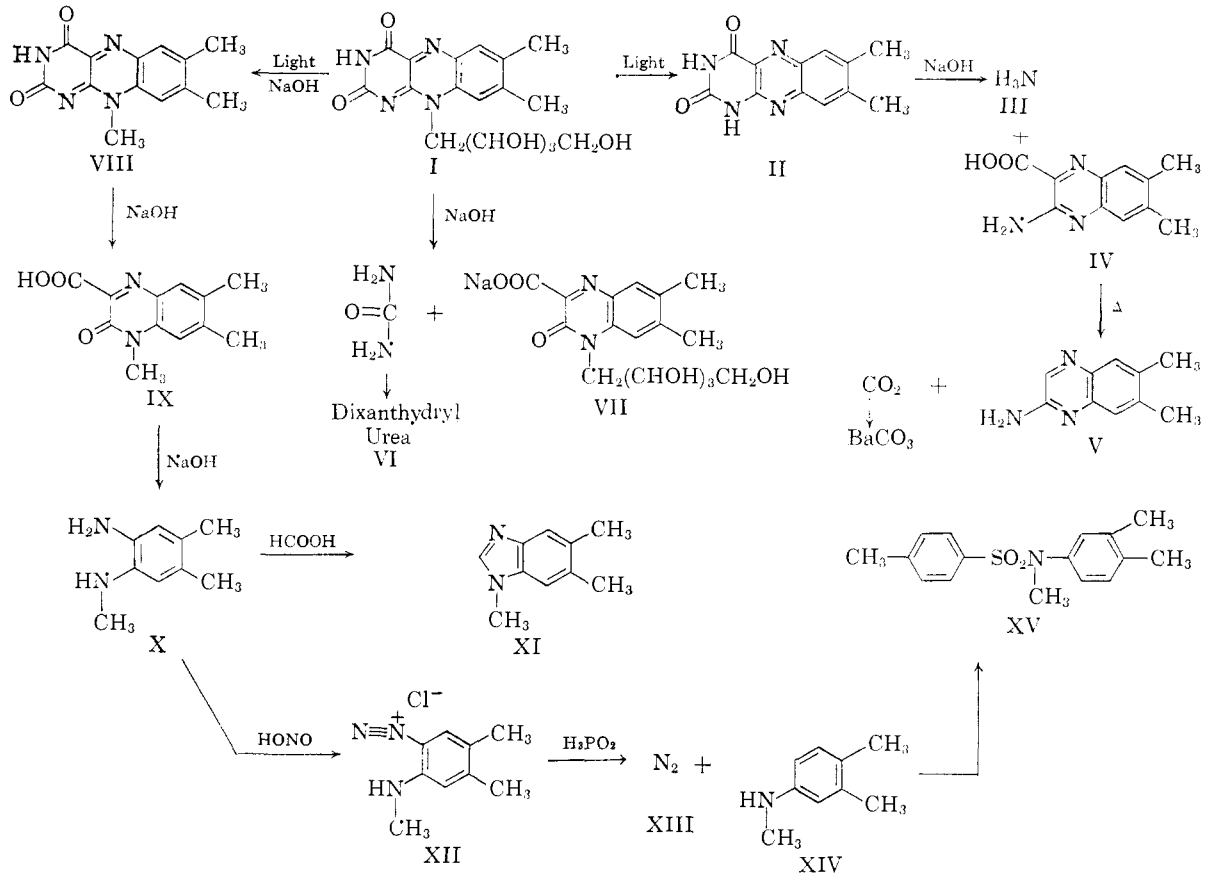

Fig. 1.—The chemical degradation of riboflavin.

catalyze reactions between diaminopyrimidines and diacetyl thus forming pteridines,[8] the lack of substrate specificity in these reactions leaves doubt in regard to their biological meaning. The nature of the conversion of purines into riboflavin has been studied further by means of two experiments, one involving the use of N^{15}-uniformly labeled adenine and the other xanthine labeled with both C^{14} and N^{15}.

The former was carried out as follows: uniformly labeled N^{15}-adenine was fed to a culture of the yeast grown in the presence of an excess of unlabeled asparagine. In this way it was hoped that the N^{15} in the amino group of adenine and any N atom lost from the ring would be so diluted by the unlabeled "ammonia pool" as to leave little doubt which N atoms of riboflavin arose directly from the purine ring and which arose from the "ammonia pool." The riboflavin thus obtained, after dilution with carrier, was degraded chemically, following in part the scheme represented in Fig. 1, and the products were combusted and analyzed for N^{15}. The results (Table I) show that the riboflavin had an equal distribution of N^{15} between the pyrimidine and pyrazine rings (VI and VII) as well as equal amounts of isotope in the two N atoms of the pyrimidine ring; that is, III, IV and VI are of approximately equal specific isotopic activity. These results like those previously reported[9] suggested that the two N atoms in the pyrazine ring were also each similarly labeled and that all four N atoms of the purine ring of adenine were incorporated as a unit into riboflavin.

TABLE I

THE DISTRIBUTION OF N^{15} IN THE RIBOFLAVIN PRODUCED BY THE CULTURE CONTAINING U-N^{15}-ADENINE

The atoms % excess N^{15} shown are the results of single analyses.

Degradation product of riboflavin	Atoms % excess N^{15}
Lumichrome (II)	0.251
Dixanthydryl urea (VI)	.252
Sodium 1,2-dihydro-1-D-ribityl-2-keto-6,7-dimethyl-3-quinoxaline carboxylate monohydrate (VII)	.266
2-Amino-6,7-dimethyl-3-quinoxaline carboxylic acid (IV)	.255
Ammonia (III)	.219

The latter experiment was carried out to gain further evidence on this point. Xanthine uniformly labeled with respect to N^{15} and uniformly labeled with respect to C^{14} was fed to the yeast in a medium containing unlabeled asparagine and un-

(8) H. Katagiri, I. Takeda and K. Imai, *J. Vitaminology (Japan)*, **5**, 81 (1959).

(9) W. S. McNutt, *Federation Proc.*, **19**, 241 (1960).

TABLE II

THE RADIOACTIVITY AND THE ATOMS % EXCESS N^{15} IN THE DOUBLY LABELED XANTHINE AND THE RESULTING RIBOFLAVIN

The atoms % excess N^{15} shown are the averages of duplicate analyses. The specific radioactivity was computed from the average count of samples plated on glass in groups of 6.

Compound	Specific radioactivity c.p.m. per 10^{-6} mole	Theory[a]	Atom % excess N^{15}	Ratio c.p.m. per 10^{-6} mole / Atom % excess N^{14}	Theoretical ratio[b]
Administered xanthine	26,700				
Xanthine after dilution	182		0.035	5200	
Riboflavin after dilution (I)	323	326	.074	4360	4400
Lumichrome (II)	328	326	.071	4620	4400
Ammonia (III)			.071		
2-Amino-6,7-dimethyl-3-quinoxaline carboxylic acid (IV)	226	245	.072	3140	3300
Dixanthydryl urea (VI)	81	81.5	.082	988	1100
Na 1,2-dihydro-1-D-ribityl-2-keto-6,7-dimethyl-3-quinoxaline carboxylate·H_2O (VII)	244	245	.075	3250	3300
$BaCO_3$	62	81.5			
2-Amino-6,7-dimethyl quinoxaline (V)	162	163			
N-Methyl-1,2-diamino-4,5-dimethyl benzene 2HCl (X)	0.06	0.00			
1,5,6-Trimethyl benzimidazole (XI)	0.07	0.00			
N_2 (XIII) from the diazonium salt of N-methyl-1,2-diamino-4,5-dimethyl benzene (XII)			.040[c]		
p-Toluene sulfon (N-methyl-3,4-dimethyl) anilide (XV)			.078		

[a] Calculated upon the basis that each carbon atom in the pyrimidine ring of riboflavin has a specific radioactivity of 81.5 c.p.m. per 10^{-6} gram atoms of C. [b] The ratio calculated upon the basis that each carbon atom in the pyrimidine ring of riboflavin has a specific radioactivity of 81.5 c.p.m. per 10^{-6} gram atoms of C and each N atom in the molecule has an atoms % excess of 0.074. [c] As 1/2 the N_2 came from the HONO used in the deamination reaction, the actual specific isotopic activity is twice the value shown.

labeled glucose. A portion of this doubly labeled xanthine after dilution with carrier and recrystallization was used to determine the ratio of C^{14} to N^{15} in the administered xanthine. The result is shown in Table II. The riboflavin produced by the culture was diluted with carrier, and the ratio was similarly determined (Table II). Assuming the loss of C atom No. 8 in the incorporation of xanthine into riboflavin the ratio of C^{14} to N^{15} in the riboflavin should be $5,200 \times {}^4/_5$ or 4,160. The value found, 4,360, shows that the C^{14} and N^{15} of the purine ring were incorporated into riboflavin with roughly comparable efficiencies, as the interpretation requires. The riboflavin was degraded according to the scheme shown in Fig. 1. The atoms % excess N^{15} in the four N atoms in the molecule was determined for each atom individually, and all were labeled to a similar extent (Table II, column 4). Also, the C^{14} was limited to the pyrimidine ring of riboflavin with the individual C atoms within this ring showing equal specific radioactivities (columns 2 and 3). Thus, the ratios of C^{14} to N^{15} in the products of chemical degradation of riboflavin agree with expectation (columns 5 and 6). It is concluded, therefore, that all the N atoms and the four C atoms of the pyrimidine ring of purines are incorporated into the formally related positions of riboflavin.

This mechanism for the biosynthesis of riboflavin differs from that proposed by Woolley[10] for other microörganisms in which 1,2-diamino-4,5-dimethylbenzene is considered to be an intermediate in the formation of the pyrazine and dimethylbenzene rings of the molecule. Although one likes to think that a substance so essential to life as riboflavin is made in much the same way in those organisms which have the capacity to make it, this is not known to be the case.

As adenine, guanine and xanthine all act similarly in the biosynthesis of riboflavin by this yeast, it seems likely that all of these naturally occurring purines are first converted to a common intermediate which serves as a precursor of riboflavin and certain other pteridines; although it is not proven that in the normal course of riboflavin biosynthesis by this yeast, or other organisms, in the absence of added purines, a similar biochemical course is followed.

The biological intermediates between the purine ring system and the pteridine ring system are not yet known. Attempts have been made to find in cultures of *Eremothecium ashbyii* substances other than riboflavin which are capable of supporting the growth of riboflavin-requiring mutants[8] of *Neurospora* and *Aspergillus*, thus far without success; nor does Masuda's compound G,[6] 6,7-dimethyl - 8(*ribo* - 2,3,4,5 - tetrahydroxypentyl)-2,4(3*H*,8*H*)-pteridinedione, support significant growth of any of the five mutants at concentrations as high as 5 μg. per ml. of medium. Efforts are being made to bring about the accumulation of an intermediate by means of enzyme preparations and by use of 1,2-dichloro-4,5-diaminobenzene, an inhibitor of riboflavin biosynthesis,[11] in growing cultures of this yeast.

The result of the biological experiments reported in this article is somewhat analogous to the findings of Pfleiderer[12] and Albert[13] that certain purines may be converted to pteridines by chemical means. The demonstration of Goodwin and Treble[7] that

(10) D. W. Woolley, "The Strategy of Chemotherapy," Cambridge University Press, London, Great Britain, 1958, p. 158.

(11) D. W. Woolley, *Proc. Soc. Exp. Biol., N. Y.*, **75**, 745 (1950).

(12) W. Pfleiderer, Ciba Foundation Symposium, "The Chemistry and Biology of Purines," Little, Brown and Co., Boston, Mass., 1957, p. 77.

(13) A. Albert, *ibid.*, p. 97.

C^{14}-acetoin is incorporated specifically into the dimethylbenzene ring of riboflavin is not inconsistent with Masuda's view that riboflavin arises biologically through building up a dimethylbenzene ring on to a preexisting pteridine derivative.[6] Further work is required to determine whether in nature purines serve as precursors of pteridines in general or whether this precursor relationship is restricted to the 2,4-dioxypteridines present in *Eremothecium ashbyii* and certain other microorganisms.

Experimental

The Analysis of N^{15}.—Samples of N_2 (0.5 mg.) were prepared and analyzed for N^{15} according to standard methods[14] making use of Nier's[15] value of 0.365 atoms % N^{15} as the natural abundance of the isotope. I am indebted to Dr. Gerald O. Dudek of Harvard University for instruction in the use of the mass spectrometer.

Adenine-U-N^{15}.—Uniformly labeled ribonucleic acid (60 mg.) containing 70 atoms % excess N^{15} (Schwarz Laboratories) was hydrolyzed with 3 ml. of 1 *N* HCl at 100° for 1 hr., and the solution was chromatographed on paper in 2-propanol–HCl–water.[16] The adenine, R_f, 0.33, was eluted from the paper and rechromatographed along 160 cm. of Whatman No. 3 MM filter paper in 1-butanol–NH_3–H_2O.[17] The adenine band, R_f, 0.26, was cut into small pieces (12.5 g. of paper), transferred to a 3 l. culture flask and autoclaved.

Culture of the Organism in the Presence of N^{15}-Adenine.—150 ml. of a $2^1/_2$ day old culture of the yeast, grown in a medium[3] containing excess asparagine (2 g. of L-asparagine per l.) was transferred to the flask containing the N^{15}-adenine and paper. The mixture was protected from light and cultured at 26° for an additional 7 days with continual shaking. Glacial acetic acid, 10 ml., was added and the mixture was filtered. To the filtrate 1.7 g. of carrier riboflavin were added, and the recrystallized riboflavin was purified by treatment with a little charcoal and two additional crystallizations from 10% acetic acid solution, m.p. 293–295°, dec.

Doubly Labeled Xanthine.—The guanine obtained by hydrolysis of 80 mg. of N^{15}-ribonucleic acid, as described above, was eluted from the first paper chromatogram, R_f, 0.25, and converted to xanthine with $NaNO_2$ and HCl. It was combined with the xanthine similarly prepared from 20 μc. of guanine-U-C^{14} (Schwarz Laboratories) 1.86 μc per mg. The xanthine was taken up in dil. NaOH, diluted with carrier, precipitated with HCl and dried to give 63 mg. of doubly labeled xanthine. As this preparation was found to be impure by paper chromatography, the entire amount of material was chromatographed along 46 sheets of Whatman No. 3 MM paper in a 1-butanol–water–formic acid system.[17] Xanthine, R_f, 0.24; guanine, R_f, 0.13. The xanthine bands were cut from the sheets. A portion of this purified material equivalent to 1.3 mg. of sodium xanthine was eluted from the paper. Its specific radioactivity was determined (Table II). 1.27 mg. of this doubly labeled xanthine was diluted with approximately 200 mg. of pure disodium xanthine and twice crystallized from water. The molar specific radioactivity and the atoms % excess N^{15} of this diluted disodium xanthine·H_2O are presented in Table II.

The remainder of the doubly labeled xanthine was used in the fermentation below.

Culture of the Organism in the Presence of Doubly Labeled Xanthine.—The areas of paper containing approximately 60 mg. of doubly labeled xanthine were cut into small pieces and placed in four 2 liter culture flasks. The contents and flasks were sterilized by autoclaving and inoculated with a total of 1.1 liters of a $3^1/_2$ day old culture of the organism grown in the medium described above. After incubating the stationary cultures for 16 days at about 20° the mixture was filtered. To the filtrate 2.5 g. of carrier riboflavin were added, and the diluted riboflavin was purified by crystallizing twice from hot 12% acetic acid solution.

Sodium 1,2-Dihydro-1-D-ribityl-6,7-dimethyl-3-quinoxaline Carboxylate Monohydrate.—Riboflavin, 160 mg., was degraded as described by Surrey and Nachod.[18] The product (VII) was taken up in hot water and crystallized twice more from water–ethanol, m.p. 244–246°. Surrey and Nachod report 242–243°.

Dixanthydryl Urea.—The mother liquor from (VII) was reduced in volume, and treated with excess xanthydrol in glacial acetic acid.[19] The product (VI) was purified by dissolving it in a large volume of glacial acetic acid at room temperature and evaporating the solution in vacuum. The substance is unstable in boiling acetic acid, m.p. 284–286° dec.

Degradation of Lumichrome to Ammonia and 2-Amino-6,7-dimethyl-3-quinoxaline Carboxylic Acid.—Lumichrome, prepared as described earlier,[20] 40 mg., was heated with 15 ml. of 1 *N* NaOH at 85° for 20 hr. in a stainless steel tube. Ammonia-free air was bubbled through the solution, and the liberated NH_3 (III) was trapped in 20 ml. of 0.1 *N* H_2SO_4. The crystalline sodium salt of (IV) which separated upon cooling was filtered off, washed with cold 1 *N* NaOH and converted to the free acid (IV) which was crystallized from acetic acid solution,[2] m.p. 224–229°, dec. Wolf, *et al.*,[21] report m.p. 215–220°.

Decarboxylation of 2-Amino-6,7-dimethyl-3-quinoxaline Carboxylic Acid.—40 mg. of (IV) were melted in an evacuated tube at 240°. The CO_2 was collected in $Ba(OH)_2$ solution, and the $BaCO_3$ was washed with CO_2-free water and dried. 32 mg. were obtained. From the charred mass in the bottom of the tube (V) was obtained by sublimation and crystallization as described elsewhere,[2] m.p. 276–279° dec. Wolf, *et al.*,[21] report m.p. 275–278°.

1,2-Dihydro-2-keto-1,6,7-trimethyl-3-quinoxaline Carboxylic Acid.—Riboflavin, 50 mg., in 100 ml. of 0.5 *N* NaOH at 4° was exposed to incandescent light for two days. The solution was acidified and extracted with $CHCl_3$. From the residue of the $CHCl_3$ extract lumiflavin (VIII) contaminated with some lumichrome was obtained by crystallization from 12% acetic acid solution. The impure specimens of (VIII), 100 mg., were heated with 100 ml. of 0.1 *N* NaOH at 100° for 1 hr. After acidification the acid (IX) was taken up in $CHCl_3$ and chromatographed along 5.5 meters of Whatman No. 3 MM, in ethanol, 100; water, 24; concd. NH_4OH, 12 ml. The band of ammonium 1,2-dihydro-2-keto-1,6,7-trimethyl-3-quinoxaline carboxylate, R_f, 0.56 (thus separated from lumichrome, R_f, 0.35), was eluted. The solution was acidified and extracted with $CHCl_3$. From the residue of the $CHCl_3$ extract, (IX) was obtained, and it was purified by crystallization from 12% acetic acid solution.[22]

The Distribution of N^{15} Between the Two N Atoms in the Pyrazine Ring of Riboflavin.—140 mg. of (IX) were degraded by heating at 150° for 13 hr. with 64 ml. of 5 *N* NaOH.[23] The crystals of (X) were taken up in ether and purified by extracting into dil. HCl solution and back into ether again. Evaporation of the ether gave 67 mg. of a dark crystalline product. It was taken up in 0.2 ml. of conc. HCl and 0.2 ml. of water. Upon cooling to 0° long needle-like crystals of the dihydrochloride of (X) separated, and they were redissolved by adding 0.4 ml. of water and warming. The solution was cooled to 0° and 0.033 mg. of $NaNO_2$ (1 molecular equivalent)[24] in 0.2 ml. of H_2O were added. For analysis, 0.5 ml. of this solution was used. It was transferred to one arm of the reaction vessel which contained 0.2 ml. of 50% hypophosphorus acid in the other arm. After freezing, degassing and thawing, the two solutions were mixed. The N_2 thus evolved was derived from the N atoms of the primary amino group of the diamino-compound which had been diluted with an equal amount of unlabeled N_2 from the $NaNO_2$. As the corrected value for the atoms

(14) A. San Pietro, in "Methods in Enzymology," Vol. IV, Editors S. P. Colowick and N. O. Kaplan, Academic Press, Inc., New York, N. Y., 1957, p. 485.

(15) Alfred O. Nier, *Phys. Rev.*, **77**, 789 (1950).

(16) G. R. Wyatt, *Biochem. J.*, **48**, 584 (1951).

(17) R. Markham and J. D. Smith, *ibid.*, **45**, 294 (1949).

(18) A. R. Surrey and F. C. Nachod, *J. Am. Chem. Soc.*, **73**, 2336 (1951).

(19) R. E. Phillips and B. M. Pitt, *ibid.*, **65**, 1355 (1943).

(20) W. S. McNutt, *J. Biol. Chem.*, **210**, 511 (1954).

(21) F. J. Wolf, R. H. Beutel and J. R. Stevens, *J. Am. Chem. Soc.*, **70**, 2572 (1948).

(22) O. Warburg and W. Christian, *Biochem. Z.*, **266**, 377 (1933).

(23) G. W. E. Plaut, *J. Biol. Chem.*, **211**, 111 (1954).

(24) O. N. Witt and H. Truttevin, *Ber.*, **47**, 2794 (1914).

% excess N^{15} in this N atom of the pyrazine ring equals that of the sum of the two N atoms of the pyrazine ring, it is apparent that the other atom is similarly labeled.

It was determined directly as follows: the solution from the above reaction containing N-methyl-3,4-dimethyl aniline derived from 66 mg. of (X) was extracted well with ether, and the acidic aqueous phase was collected. The solution was made alkaline and again extracted with ether. Evaporation of the ether gave a brown oil which was suspended in dil. NaOH and steam distilled. The steam distillate containing (XIV) was treated with *p*-toluenesulfonyl chloride and NaOH solution. The crude *p*-toluenesulfonamide of (XIV) separated, and it was washed well with dil. NaOH. The low-melting solid (40–60°) containing (XV) was combusted and analyzed for N^{15} without further purification.

1,5,6-Trimethylbenzimidazole.—15 mg. of (X) were refluxed with 5 ml. of 1 *N* HCl and 5 drops of HCOOH for 3 hrs.[25] The *p*H was adjusted to 8, and (XI) was taken up into $CHCl_3$. The residue was sublimed at 120°; 10 mg. were obtained, m.p. 139° (in a sealed tube). The m.p. reported for the authentic substance is 143–144°.[2]

(25) N. G. Brink and K. Folkers, *J. Am. Chem. Soc.*, **72**, 4442 (1950).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF STANFORD UNIVERSITY, STANFORD, CALIF., AND THE INSTITUTE OF CHEMISTRY, UNIVERSITY OF UPPSALA, SWEDEN]

Optical Rotatory Dispersion Studies. XLVIII.[1] The Nitroso Chromophore[2]

BY CARL DJERASSI, E. LUND, E. BUNNENBERG[3] AND B. SJÖBERG

RECEIVED JANUARY 20, 1961

Optically active nitroso compounds exhibit Cotton effects which can be related in terms of wave length to their $\eta \rightarrow \pi^*$ absorption transition. The rather rare C-nitroso chromophore shows a Cotton effect in the visible centered around 675 mμ, while that of a N-nitrosodialkylamine occurs between 350–400 mμ. These single Cotton effects are in marked contrast to the multiple Cotton effect curves exhibited by N-nitrosoamides which are found in the region 450–300 mμ. The rotatory dispersion curves of a number of N-nitroso derivatives of N-acetyl- or N-benzoyl-α-amino acids have been investigated and their Cotton effects can be used for stereochemical assignments. N-Nitroso-N-methylamides of optically active carboxylic acids also show multiple Cotton effects, but these cannot always be employed for determining the absolute configuration of the α-asymmetric center.

Initially, our studies on the role of optical rotatory dispersion measurements in organic chemistry[4] centered on the carbonyl chromophore. Once the utility of this physical method had been demonstrated for structural and especially stereochemical problems, we turned to an investigation[5] of "chromophoric" derivatives which afford Cotton effect curves for otherwise "nonchromophoric" functional groups (*e.g.*, hydroxyl, carboxyl, amino, etc.). Concurrent with this work, we have also undertaken a screening program of various chromophores—notably those with low intensity extinction coefficients—whose spectral properties would suggest that they might exhibit anomalous optical rotatory dispersion. As pointed out elsewhere,[1,4] it is this area of optical rotatory dispersion measurements which has by far the greatest scope in terms of applications to organic chemical problems. In this connection, we completed recently a survey of optically active disulfides and diselenides,[6] and we should now like to record our experience with the nitroso chromophore.

The recorded[7] maxima for the $\eta \rightarrow \pi^*$ transition of the nitroso chromophore occur approximately at 680 mμ for the C-nitroso function, 370 mμ for N-nitrosoamines and nitrites and at 415 mμ for N-nitrosoamides; if these bands should be optically active, then Cotton effects would be expected in these spectral regions. In fact, the only two earlier studies[8,9] show that this is so. Organic alkyl nitroso compounds are rather unavailable and this applies particularly to optically active ones. It is not surprising, therefore, that no rotatory dispersion studies have been recorded for the isolated C-nitroso chromophore, but Mitchell's measurements[8] of the circular dichroism of caryophyllene and bornylene nitrosites (nitroso nitrites) indicate that the 680 mμ transition is optically active. Through the kind coöperation of Prof. G. Büchi and Dr. F. W. Bachelor, we have been able to secure a specimen of the aconitine derivative I,[10] which represents a non-enolizable C-nitroso compound. Its single absorption band above 300 mμ occurs at 679 mμ (ε 14) and, as can be seen from Fig. 1, this gives rise to a negative Cotton effect.

While this result is principally of theoretical interest because of the inaccessability of optically active tertiary C-nitroso compounds and is thus unlikely to be of any stereochemical utility, this is not necessarily the case with N-nitrosoamines. The rotatory dispersion behavior of such compounds has hitherto not been examined, but their comparative ease of preparation makes them of potential interest as "chromophoric" derivatives of optically active amines, since their low-intensity

(1) Paper XLVII, C. Djerassi, *Tetrahedron*, in press (1961).

(2) Supported by Grant No. CRTY-5061 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

(3) National Institutes of Health Postdoctorate Research Fellow.

(4) C. Djerassi, "Optical Rotatory Dispersion: Applications to Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1960

(5) (a) B. Sjöberg, A. Fredga and C. Djerassi, *J. Am. Chem. Soc.*, **81**, 5002 (1959); (b) C. Djerassi and K. Undheim, *ibid.*, **82**, 5755 (1960); (c) E. Bunnenberg and C. Djerassi, *ibid.*, **82**, 5953 (1960); (d) C. Djerassi, K. Undheim, R. C. Sheppard, W. G. Terry and B. Sjöberg, *Acta Chem. Scand.*, in press (1961).

(6) C. Djerassi, A. Fredga and B Sjöberg, *ibid.*, in press (1961).

(7) See J. W. Sidman, *Chem. Revs.*, **58**, 689 (1958); J. Mason, *J. Chem. Soc.*, 3904 (1957); R. N. Haszeldine and B. J. H. Mattinson, *ibid.*, 4172 (1955); R. N. Haszeldine and J. Jander, *ibid.*, 691 (1954); R. L. Peck and K. Folkers in "Chemistry of Penicillin" (ed. by H. T. Clarke, J. R. Johnson and R. Robinson), Princeton University Press, Princeton, N. J., 1949, pp. 144–206.

(8) S. Mitchell, *J. Chem. Soc.*, 3258 (1928); S. Mitchell and S. B. Carmack, *ibid.*, 415 (1932).

(9) W. Kuhn and H. L. Lehmann, *Z. physik. Chem.*, **B18**, 32 (1932); W. Kuhn and H. Biller, *ibid.*, **B29**, 1 (1935); H. B. Elkins and W. Kuhn, *J. Am. Chem. Soc.*, **57**, 296 (1935).

(10) F. W. Bachelor, R. F. C. Brown and G. Büchi, *Tetrahedron Letters*, No. 10, 1 (1960).