for a period of at least six days. The solubility analysis was carried out in a thermostatically controlled constant temperature bath at 35 ± 0.1°. The procedure used was essentially that of Dalton and Schmidt.[9]

Titration of Terramycin Hydrochloride.—Since aqueous solutions of terramycin tend to precipitate terramycin base in the range *p*H 5 to 7.5, the potentiometric titrations were carried out at less than 0.002 molar concentration. The solution was titrated at 28 ± 0.2° under nitrogen using 0.2 *N* sodium hydroxide. pK'_a values were calculated from the titration data plotted in Fig. 1 in the regions of half equivalents using the equation[10]

$$pK_{a'} = P_{aH} - \log \frac{(Na^+) - (OH^-)}{(acid) - (Na^+) + (OH^-)}$$

where (Na^+) and (OH^-) are the molar concentrations of the respective ions and (acid) is the initial molar concentration of terramycin hydrochloride being titrated. pK'_a values of 3.49, 7.55 and 9.24 were obtained for terramycin hydrochloride. The precision of the measurements was of the order of ±0.05 *pK* unit. Values obtained from titration curves for methanol–water mixtures were found to be in good agreement with those obtained from titration of aqueous solutions. The titration data for the terramycin–calcium chloride solutions were obtained similarly.

(9) J. B. Dalton and C. L. A. Schmidt, *J. Biol. Chem.*, **103**, 549 (1933).

(10) P. L. Kirk and C. L. A. Schmidt, *ibid.*, **81**, 237 (1929).

Acknowledgments.—The authors are indebted to Dr. B. A. Sobin for the early work on the isolation and the preliminary characterization of terramycin. We wish to express our appreciation to Dr. J. A. Means for the microanalyses and to Mr. R. C. Kersey for the biological assays contained in this paper. We are also indebted to Mr. G. B. Hess for the infrared and ultraviolet absorption measurements.

BROOKLYN, N. Y. RECEIVED MARCH 15, 1951

[CONTRIBUTION FROM DEPARTMENT OF CHEMISTRY AND SCHOOL OF MEDICINE, STANFORD UNIVERSITY]

The Isolation in Crystalline Form and Characterization of the Two Isomeric Cytidylic Acids Derived from Yeast Nucleic Acid[1]

BY HUBERT S. LORING AND NYDIA G. LUTHY

The problem of the variation in rotation shown by different samples of cytidylic acid, $[\alpha]_D$ +23° to +49°, has been resolved by the isolation of a new isomer with an optical rotation of $[\alpha]_D$ +20.7° as well as of that formerly obtained with $[\alpha]_D$ +49°. The latter was prepared by a new procedure involving fractionation of dibrucine uridylate and dibrucine cytidylate with pyridine. Crystals of the two isomers differ in melting point behavior, in solubility, in crystalline habit and in indices of refraction. The $[\alpha]_D$ +49° compound could be readily deaminated with the formation of a product which gave a highly insoluble dibrucine salt with similar solubility properties and optical activity as the dibrucine uridylate usually isolated from ribonucleic acid hydrolysates. On deamination and treatment with brucine, the new isomer gave a relatively soluble dibrucine salt, which was not definitely characterized.

In the course of a search for an isomeric dibrucine uridylate different from that usually isolated from yeast nucleic acid, the specific rotations of the fractionated dibrucine salts of the pyrimidine nucleotides were determined in pyridine. It was found that the dibrucine salts corresponding, in general, to the cytidylate fraction, in contrast to dibrucine uridylate, failed to dissolve in this solvent. Examination of the properties of the pyridine-insoluble residues proved them to consist of free cytidylic acid as well as of the brucine salt. Since a sample of dibrucine cytidylate prepared from crystalline cytidylic acid[2] behaved similarly, it became evident that dibrucine cytidylate could be largely decomposed by pyridine with the formation of free cytidylic acid.

The above mentioned method was applied on a preparative scale to the dibrucine salts of the pyrimidine nucleotide fraction present after acid hydrolysis of yeast ribonucleic acid. After prolonged extraction of the mixture of dibrucine salts with pyridine, the residue, recrystallized from aqueous alcohol, showed the typical behavior of free cytidylic acid and gave a specific rotation of $[\alpha]_D$ +50.3°. Recrystallization did not significantly affect either the optical activity or decomposition point. The cytidylic acid fractions obtained from the mother liquors of the $[\alpha]_D$ +50° gave specific rotations between $[\alpha]_D$ +31° and +49°, and evidently were comparable to those prepared by alkaline hydrolysis,[2] $[\alpha]_D$ +39° to +42° or to those prepared by acid or alkaline hydrolysis,[3] $[\alpha]_D$ +37°. Although an optical rotation of $[\alpha]_D$ +49° has been reported previously for cytidylic acid,[4] a value of $[\alpha]_D$ +23.4° was also found on one occasion by Thannhauser and Dorfmuller.[5] No explanation has been offered, heretofore, for the seeming discrepancies in optical activity. It occurred to us, in view of the optical rotation of $[\alpha]_D$ +21.4° for the reported synthetic cytidine-2-phosphate,[6] that the above results might be explained by the presence of varying amounts of an isomeric product with a lower rotation than $[\alpha]_D$ +49°. Recrystallization of the samples of lower specific rotation from aqueous alcohol resulted in some increases in the rotation of the products, but in general, did not lead readily to material either of $[\alpha]_D$ +49° or of less than $[\alpha]_D$ +31°.

Attention was next turned to the possibility of fractionating the cytidylic acid mixture as the relatively insoluble crystalline phosphotungstate.[2] The latter was isolated from the pyrimidine nucleotide fraction and was recrystallized from 1 *N* sulfuric acid. The cytidylic acid phosphotungstate which first separated was converted to free cytidylic acid, and in contrast to the sample of highest specific rotation mentioned above, gave $[\alpha]_D$ +20.7°. Repeated recrystallization of this material from aque-

(1) Aided by a grant from the Rockefeller Foundation.

(2) Loring, Roll and Pierce, *J. Biol. Chem.*, **174**, 729 (1948).

(3) Barker, Gulland, Smith and Thomas, *J. Chem. Soc.*, 904 (1949).

(4) Levene, *J. Biol. Chem.*, **41**, 484 (1920); Bredereck and Richter, *Ber.*, **71**, 718 (1938).

(5) Thannhauser and Dorfmuller, *Z. physiol. Chem.*, **104**, 65 (1919).

(6) Gulland and Smith, *J. Chem. Soc.*, 1527 (1948).

ous alcohol did not result in any significant change in optical activity. A careful examination of the properties of the new cytidylic acid and the $[\alpha]_D$ +49° isomer showed them to differ in several respects as summarized in Table I.

TABLE I

PROPERTIES OF ISOMERIC CYTIDYLIC ACIDS

$[\alpha]^{20}D$	+20.7°, *c*, 1.0 in H_2O[a]	+49.4°, *c*, 1.0 in H_2O
Solubility in water	Difficultly	Moderately
M.p. (in bath at 230°, rate of heating 3° per minute)	238–240° (dec. with striking swelling and evolution of gas)	232–234° (dec. in similar manner)
Indices of refrac.[b]	*n*, 1.600, 1.495	*n*, 1.585, 1.512
Extinction angle	Approx. 9°	Approx. 4.5°

[a] In order to prepare a 1% solution, the suspension must be heated at 100° for 2–3 hours. [b] For crystals obtained from water or aqueous alcohol.

Further concentration of the cytidylic acid phosphotungstate mother liquors gave additional crystalline fractions which after conversion to free cytidylic acid proved to have specific rotations varying from $[\alpha]_D$ +30° to $[\alpha]_D$ +40°. These, like the intermediate fractions found by the pyridine extraction procedure, apparently consisted of various mixtures of the two isomers which again could not be readily fractionated by recrystallization from aqueous alcohol.

As the two natural cytidylic acids fail to reduce periodate,[7] they are evidently phosphorylated in either the 2′- or 3′-positions. A comparison with the synthetic cytidylic acids previously thought to be 2′-phosphates[6,8] is unwarranted because of the recent work of Brown, Haynes and Todd[9] which shows that these preparations are in reality 5′-phosphates. It was of interest to determine if any relationship could be shown between the natural cytidylic acids and the uridylic acid usually isolated from hydrolysates of yeast nucleic acid. Cytidylic acid, $[\alpha]_D$ +49° on treatment with sodium nitrite and glacial acetic acid[10] dissolved readily, and the product was easily converted in 87% yield to the highly insoluble dibrucine salt with $[\alpha]_D$ −58.9° in pyridine characteristic of dibrucine uridylate.[11,12] The isomer of lower specific rotation in the presence of sodium nitrite and acetic acid dissolved very slowly and the deaminated product formed a highly soluble brucine salt which could not be isolated in the pure form.

The great difference in the solubilities of the dibrucine uridylates prepared from the two cytidylic acids provides an explanation for the usual recovery of dibrucine uridylate with $[\alpha]_D$ −59° ($[\alpha]_D$ +21° for diammonium salt) from nucleic acid hydrolysates even though as indicated by the work of Cohn[13] both isomers may be present. This solubility difference similarly explains the recovery of disodium uridylate, $[\alpha]_D$ +21°, by Barker, Gulland, Smith and Thomas[3] after deamination of cytidylic acid, $[\alpha]_D$ +36.7°, and purification of the uridylic acid by recrystallization of the dibrucine salt. The same explanation is suggested for the recovery of this compound only in uridylic acid syntheses where both uridine-2′-phosphate and uridine-3′-phosphate should have been formed.[8,14] As mentioned by Brown, Haynes and Todd,[9] the structure of the uridylic acid forming the highly insoluble dibrucine salt is usually considered to be uridine-3′-phosphate. From the above considerations, it appears that either the 2′- or 3′-phosphate structure may be equally well assigned to this compound and correspondingly to cytidylic acid, $[\alpha]_D$ +49°.

As already presented briefly,[15] a more efficient separation of the two cytidylic acids and isolation in crystalline form can be effected by ion exchange on Dowex-1(formate) by procedures similar to those described by Cohn for the fractionation of nucleotides.[16] In a report[13] which appeared simultaneously with our own, the latter author has also presented evidence in certain ion exchange elution diagrams for heterogeneity in a sample of cytidylic acid with $[\alpha]_D$ +39°. It is evident from the optical density ratios published[13,15] that the cytidylic acid isomer with $[\alpha]_D$ +21° is identical with that called cytidylic acid "a" by Cohn and that the $[\alpha]_D$ +49° isomer is identical with cytidylic acid "b." The experiments on the isolation of the two isomers by ion exchange procedures as well as further studies on the properties and chemistry of the two compounds, including the data on periodate titration, will be presented in a forthcoming publication.

Experimental

Preparation of Pyrimidine Nucleotide Fractions.—Two hundred grams of yeast nucleic acid was refluxed for one hour in 2 l. of 1 *N* sulfuric acid. The purines were precipitated as silver salts by the addition of 112 g. of dry silver sulfate. The solution failed to give any additional precipitate with more silver sulfate and was filtered through Hyflo-Super Cel, which was washed with several portions of 1 *N* sulfuric acid. The combined filtrates and washings were warmed on a steam-bath and 125 ml. of 1 *N* hydrochloric acid was added in small portions to remove excess silver. After removal of silver chloride, the clear brown solution was divided into two equal parts for fractionation of the pyrimidine nucleotides.

Fractionation of Dibrucine Salts of Pyrimidine Nucleotides Into Cytidylic and Uridylic Acid Components.—To one portion of the solution was added a hot saturated solution of barium hydroxide until alternate titration of aliquots with clear barium hydroxide solution and 1 *N* sulfuric acid showed only a slight excess of sulfate ions (just blue to congo red paper). The barium sulfate was filtered from the hot solution on Hyflo-Super Cel and the filtrate was reduced to 250 ml. *in vacuo*. Brucine (95 g. in 250 ml. alcohol) was added slowly and the mixture from which some brucine salts had begun to precipitate allowed to stand 24 hours at 5°. The dense mass, consisting chiefly of the brucine salts of the pyrimidine nucleotides, was filtered on a Buchner funnel, washed with alcohol and dried on a clay plate under an infrared lamp. Three crops of crystals were collected totaling 185 g. The combined dibrucine salts were extracted three times with about 400-ml. portions of pyridine at 50° and the suspension allowed to stand at room temperature for 12 hours each time. The insoluble residue was filtered, washed with small portions of pyridine and finally dried on

(7) Loring and Bortner, unpublished data.
(8) Michelson and Todd, *J. Chem. Soc.*, 2476 (1949).
(9) Brown, Haynes and Todd, *ibid.*, 408 (1950); *ibid.*, 2299 (1950).
(10) Brederick, *Z. physiol. Chem.*, **224**, 79 (1934).
(11) Levene and Tipson, *J. Biol. Chem.*, **106**, 113 (1934).
(12) Schwerdt and Loring, *ibid.*, **167**, 593 (1947).
(13) Cohn, THIS JOURNAL, **72**, 2811 (1950).
(14) Gulland and Hobday, *J. Chem. Soc.*, **746** (1940).
(15) Loring, Luthy, Bortner and Levy, THIS JOURNAL, **72**, 2811 (1950).
(16) Cohn, *ibid.*, **71**, 2275 (1949); **72**, 1471 (1950).

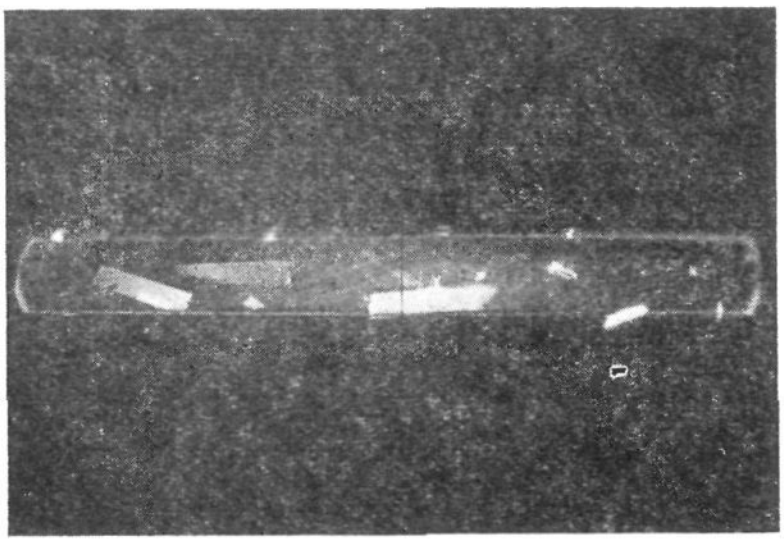

Fig. 1.—Cytidylic acid $[\alpha]_D$ +20.7° (from water) 450×. The large crystal although lying parallel to the cross-hairs of a polarizing microscope, does not show extinction. Oblique extinction and biaxial interference figure indicate the monoclinic system.

a clay plate (infrared lamp); yield 30.3 g. A crystal of the material with a drop of nitric acid gave the bright-red color characteristic of a positive brucine test, indicating the presence of some undecomposed brucine salts. The pyridine mother liquors were used as described later for the preparation of dibrucine uridylate.

Isolation of Cytidylic Acid, $[\alpha]_D$ +49°.—In an exploratory experiment 1.5 g. of the insoluble residue (30.3 g.) was treated with another 50 ml. of pyridine and the suspension allowed to stand for 24 hours in the cold. After the pyridine had been filtered, the solid was recrystallized from 35% alcohol. The first crop of crystals weighed 0.18 g. and gave a m.p. of 178–180° in agreement with dibrucine cytidylate prepared from previously isolated cytidylic acid.[2] Upon concentration of the mother liquor, a second crop was obtained, 0.26 g., m.p. 223–225° (with decomposition and evolution of gas characteristic of free cytidylic acid) and $[\alpha]_D$ +30.8°, *c* 1.0 in H_2O.

In a second experiment 12.7 g. of the apparent mixture of brucine cytidylate and cytidylic acid was dissolved in 300 ml. 35% alcohol. The solution was filtered from a flocculent brown insoluble material (1.85 g.) and three crops of crystals were collected with the following properties: 5.57 g., m.p. 180–182°; 0.42 g. and 0.0365 g. both m.p. 193–195°, all giving a positive brucine test and representing 47.4% of the original sample. The mother liquor was concentrated under reduced pressure to 75 ml. and a fourth crop was obtained weighing 2.65 g., m.p. 210–215° (dec.). The latter was dissolved in 60 ml. hot water, treated twice with Norite, and finally 95% alcohol was added to the solution until cloudiness persisted. After standing in the cold, 1.1 g. of white crystals separated, m.p. 220–230° (dec.), $[\alpha]_D$ +44.7°. The ultraviolet absorption spectrum in 0.01 *N* hydrochloric acid showed a maximum at 278 mμ and a minimum at 240 mμ, in agreement with cytidylic acid.[17] It was evident from the above results that a large portion of the brucine salts still remained in the insoluble residue.

Prolonged extraction with pyridine in a Soxhlet apparatus was next attempted as a means of increasing the yield of free cytidylic acid. The material used in one experiment was the first crystalline fraction 5.57 g., m.p. 180–182°, obtained from 12.7 g. of pyridine-extracted residue by recrystallization from aqueous alcohol. After extraction for 16 hours and recrystallization from 35% alcohol, 1.0 g. of cytidylic acid was recovered m.p. 233–234° (in bath at 220°) $[\alpha]_D$ +50.3°, *c*, 1.0 in water. Recrystallization from aqueous alcohol gave 0.49 g. of product, m.p. 233–234°, $[\alpha]_D$ +48.6° (sample dried to constant weight *in vacuo* over P_2O_5). Ultraviolet absorption in 0.01 *N* hydrochloric acid: max. 278 mμ, min. 240 mμ; $E_{max.}$ 13,000.

Anal.[18] Calcd. for $C_9H_{14}O_8N_3P$: N, 13.0; P, 9.6. Found: N, 13.06; P, 9.36.

(17) Ploeser and Loring, *J. Biol. Chem.*, **178**, 431 (1949).

(18) Microchemical Specialties Co., Berkeley, California.

In other experiments various dibrucine cytidylate fractions, m.p. 178–210°, were subjected to pyridine extraction in a Soxhlet apparatus for 40 hours. Recrystallization of the residues, which in every case still contained some brucine, gave further yields of free cytidylic acid, m.p. 225–230° dec.

Recovery of Dibrucine Uridylate and Brucine.—The original pyridine extracts (1.2 l.) were concentrated under reduced pressure to 500 ml. and allowed to stand in the cold room for 16 hours. A first crop was obtained of a white crystalline material weighing 29 g. On further concentration and standing in the cold, second and third crops of crystals were collected weighing 3.9 g. and 0.12 g., respectively. Concentrating the filtrate to dryness gave a sirupy mass which dissolved almost entirely in chloroform, and evidently consisted of free brucine. A sample recrystallization of the combined brucine uridylate fraction gave 70.6% of crystals, m.p. 180–183° and $[\alpha]_D$ −59°, *c*, 1.0 in pyridine. These properties are in agreement with those usually given for dibrucine uridylate prepared by other methods.[11,12]

Fractionation of Pyrimidine Nucleotides with Phosphotungstic Acid. Isolation of Cytidylic Acid, $[\alpha]_D$ +20.7°.—The second half of the purine-free filtrate from the acid hydrolysis of yeast nucleic acid (described above) was concentrated to 300 ml. Sixty grams of phospho-12-tungstic acid ($H_3PW_{12}O_{40}\cdot 14H_2O$) dissolved in 50 ml. of hot water was added and a precipitate formed at once. This dissolved on warming to 80° and the clear solution was allowed to stand at room temperature. A crystalline precipitate formed and the solution gave a solid mass on standing in the cold room.[19] The crystalline cytidylic acid phosphotungstates were filtered (after standing for five months in a cold room) and recrystallized from 500 ml. of 1 *N* sulfuric acid. The crystals formed at room temperature were filtered and washed with small amounts of cold water; yield 31 g.

The resulting product was decomposed with ammonium sulfate as previously described,[2] and the resulting solution of free cytidylic acid concentrated to 20 ml. and decolorized with Norite. An equal volume of alcohol was added and the main solution was separated from a small amount of oil by decantation. The solution was further concentrated to incipient crystallization and placed overnight in the cold. The crystals were filtered and upon recrystallization from water a small amount of oil formed once more from which the supernatant was removed. On cooling, the latter gave a flocculent precipitate which was recrystallized again. The

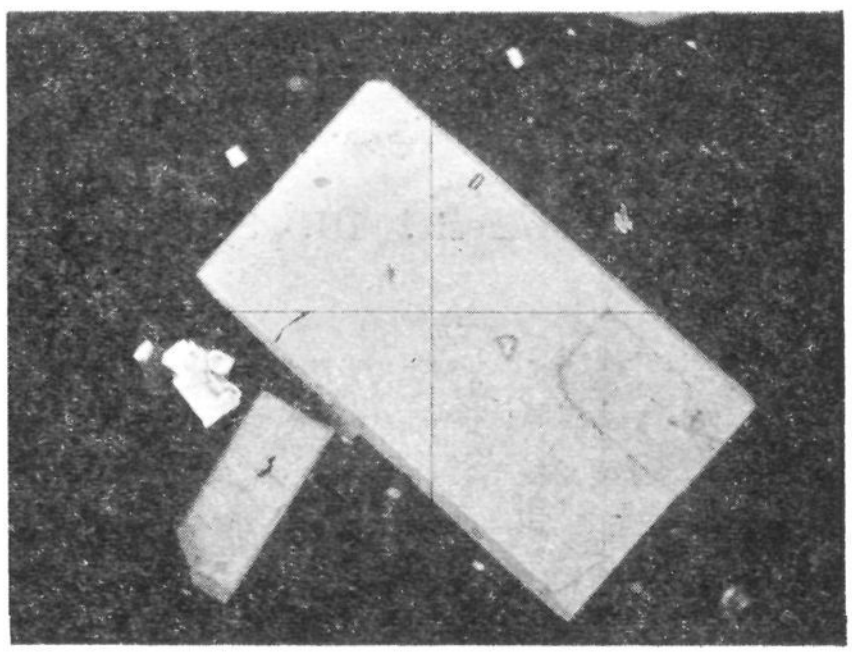

Fig. 2.—Cytidylic acid $[\alpha]_D$ +49° (from aqueous alcohol) 60×. The large crystal (oblique extinction) lying between crossed nicols of a polarizing microscope is in its brightest position. In a position parallel to the cross-hairs, these crystals are so nearly completely extinguished that a satisfactory photograph cannot be taken.

(19) Cytidylic acid phosphotungstate tends to form a gelatinous mass if cooled suddenly in the cold. The salt decomposes when exposed to an infrared lamp and is best dried at room temperature.

crystals so obtained showed a m.p. 238–240° dec. (in bath 230°) $[\alpha]_D$ +20.7°, *c*, 1.0 in water; yield approximately 200 mg.

Anal. Calcd. for $C_9H_{14}O_8N_3P$: N, 13.0; P, 9.6. Found: N, 12.9; P, 9.75.

The combined mother liquors on standing gave a second crop of crystals of $[\alpha]$ +40.5° consisting evidently of a mixture of the cytidylic acid isomers. The small amount of oil from the recrystallization procedures (soluble in water, insoluble in alcohol) finally solidified to yield a white powdery material which darkened in the m.p. at 245° but did not show the characteristic decomposition melting point of cytidylic acid.

The original mother liquor containing cytidylic acid phosphotungstates, on standing in the cold, produced a second crystalline fraction which was treated as above to obtain free cytidylic acid. When the acid was recrystallized, there was collected a first crop of crystals, 0.23 g., $[\alpha]_D$ +38.2°, and a second crop, $[\alpha]_D$ +29°. The latter upon recrystallization gave an additional yield of 81 mg. of the $[\alpha]_D$ +20.7° isomer.

Optical Properties.—A sample of the cytidylic acid $[\alpha]_D$ +20.7° was compared with cytidylic acid $[\alpha]_D$ +49.4° between crossed nicols of a polarizing microscope. Both sets of crystals were anisotropic showing oblique extinction for the views most often encountered (see Table I and Figs. 1 and 2). Determination of the indices of refraction by the Becke-line immersion method[20] gave for cytidylic acid $[\alpha]_D$ +20.7°, *n*, 1.600, 1.495; cytidylic acid $[\alpha]_D$ +49.6, *n*, 1.585, 1.512.

Deamination of Cytidylic Acid $[\alpha]_D$ +48°.—The high rotating isomer (0.1 g.) was suspended in 1.5 ml. of water to which 0.41 g. of sodium nitrite had been added.[10] Upon the gradual addition of 0.41 ml. of glacial acetic acid, there was considerable evolution of gas from the surface of the crystals. As the cytidylic acid was deaminated to form the highly water-soluble uridylic acid, the mixture became clear, indicating completion of the reaction. Although the solution had cleared in about two hours, it was permitted to stand 15 hours at 5°, after which it was taken to dryness twice at reduced pressure to remove excess acetic acid. Two moles brucine (0.245 g.) in 4 ml. of warm alcohol were added. A dense white crystalline precipitate formed on standing in ice. After filtering, washing with several small portions of 95% alcohol, and drying, the crude dibrucine uridylate, 0.32 g., 87% theory, was recrystallized and its optical rotation was found to be $[\alpha]_D$ −58.9°, *c*, 1.0 in pyridine. The rotation of dibrucine uridylate isolated from hydrolysates of yeast nucleic acid has ranged from $[\alpha]_D$ −55.0° to −57.7°.[11,12]

Deamination of Cytidylic Acid $[\alpha]_D$ +20.7°.—The same procedure was followed as in the preceding experiment; 50 mg. of this isomer was treated with 0.205 g. of sodium nitrite and 0.2 ml. of glacial acetic acid. The reaction proceeded more slowly than in the previous experiment and a clear solution was effected only after four hours at room temperature. After standing 12 hours at 5°, the solution was taken to dryness twice in a vacuum desiccator, and two moles brucine (0.123 g.) in 2 ml. of warm alcohol were added. This time, however, a precipitate did not form immediately, and after 48 hours in refrigeration, only a small amount of material was isolated. From its solubility in chloroform and crystalline behavior it was evidently unreacted brucine. In two other experiments in which 100-mg. and 180-mg. samples and sodium nitrite and glacial acetic or hydrochloric acid were used as deaminating agents, only small amounts of products ranging in rotation from $[\alpha]_D$ −59° to −37.6° could be isolated. These could not be definitely characterized.

(20) Chamot and Mason, "Handbook of Chemical Microscopy," Vol. I, 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1939, p. 362.

STANFORD, CALIF. RECEIVED DECEMBER 11, 1950

[CONTRIBUTION FROM THE LABORATORY OF PLANT PHYSIOLOGICAL CHEMISTRY, DEPARTMENT OF BOTANY, COLUMBIA UNIVERSITY]

Alkaloid Biogenesis. III. Specificity of the Nicotine–Nornicotine Conversion

BY R. F. DAWSON

The specificity of the biological conversion of nicotine to nornicotine has been examined by feeding homologs and analogs of nicotine to excised leaves of *Nicotiana glutinosa* rendered initially alkaloid free by grafting to tomato roots. Nornicotine was produced from *l*-nicotine, *d,l*-nicotine and *d,l*-N′-ethylnornicotine. Anabasine was produced from *d,l*-N′-methylanabasine and *d,l*-N′-ethylanabasine. The specificity of the process is thus so low that the formation of nornicotine in the green tobacco leaf can be considered as a specific case of a more general N-dealkylating activity of the leaf tissues.

It has been shown[1,2] that nornicotine, a principal Nicotiana alkaloid, is derived from nicotine by N′-demethylation in the plant leaf. The overall chemical nature of the transformation, its inherited character and restriction to leaf tissues suggest the possibility of a coupling with some non-alkaloidal phase of plant metabolism. Since alkaloid biosynthetic pathways and their metabolic couplings in plants are at present almost wholly unknown, the nicotine–nornicotine conversion has been subjected to closer examination in this Laboratory. It has been found that excised leaves of *Nicotiana glutinosa* can remove N-methyl and N-ethyl groups from both pyrrolidine and piperidine ring moieties of the Nicotiana alkaloids. The process may also lack stereochemical specificity. Thus, the demethylation of nicotine in certain strains of cigarette tobaccos and in many wild species of Nicotiana emerges as a single instance, albeit the naturally occurring one, of a more general N-dealkylating activity of the leaf tissues of these plants.

Completion of this phase of the inquiry depended upon the availability of two simple but highly propitious botanical techniques. One of these, the grafting of tobacco scions to tomato rootstocks,[3] permitted the production of virtually alkaloid-free leaves for use in the feeding experiments. Secondly, by the use of ice-cooled culture solutions, bacterial degradation of the test substances as well as clogging of the vascular tissues of the leaf petioles were avoided. Absorption of large volumes of the culture solutions by the leaf blades was thus facilitated. By these devices it was possible to feed substantial amounts of the various homologs and analogs of nicotine and to determine their metabolic fate without encountering the exceedingly difficult problem of separating such substances from pre-existing quantities of nicotine and nornicotine.

(1) R. F. Dawson, THIS JOURNAL, **67**, 503 (1945); *Am. J. Bot.*, **32**, 416 (1945).

(2) G. S. Il'in, *Biokhimya*, **13**, 193 (1948).

(3) R. F. Dawson, *Am. J. Bot.*, **29**, 66 (1942).