this point. Similar observations have been made elsewhere for nonadditivity of 19-H frequency shifts due to substituents on six-membered rings where steric strain or interactions leads to ring conformational changes.[6,24a,e,35]

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Biosynthesis of the Nicotiana Alkaloids. X. The Incorporation of Glycerol-2-C^{14} into the Pyridine Ring of Anabasine[1]

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RECEIVED OCTOBER 15, 1963

The administration of glycerol-2-C^{14} to *Nicotiana glauca* plants leads to the formation of radioactive anabasine which has 38% of its activity located in the pyridine ring. A systematic degradation is described whereby the distribution of activity around the pyridine ring can be determined. The anabasine derived from glycerol-2-C^{14} was found to have substantial activity at C-2, C-3, and C-5, with only low activity at C-4 and C-6. These results are consistent with the hypothesis that nicotinic acid, the precursor of the pyridine ring of anabasine, is formed in *Nicotiana* species from glycerol and succinic acid or closely related metabolites.

In our previous publication on the biosynthesis of anabasine[4] we reported that the administration of sodium acetate-2-C^{14} to *Nicotiana glauca* led to the formation of radioactive anabasine which had 37% of its activity located in the pyridine ring. It was shown that all this activity was located at C-2 and C-3 and was divided approximately equally between these positions. It was suggested that the acetate-2-C^{14} enters the Krebs cycle leading to the formation of succinic acid-2,3-C^{14} which is then incorporated into nicotinic acid, the established precursor of the pyridine ring of anabasine.[5] Ricinine, and the pyridine ring of nicotine, are also derived from nicotinic acid, and recent investigations on their biosynthesis[6-8] are consistent with this hypothesis. We considered that carbons 4,5, and 6 of the pyridine ring of nicotinic acid were derived from glycerol or a closely related three carbon metabolite. When glycerol-2-C^{14} was fed to *N. glauca* plants, radioactive anabasine was indeed obtained. Oxidation of the anabasine yielded nicotinic acid which was decarboxylated affording pyridine having 38% of the total activity of the alkaloid. We have now developed a systematic degradation of nicotinic acid (I) which enables us to determine the distribution of activity around the pyridine ring. Initial steps in this degradation have been previously described[4] and result in the formation of 1,3-dimethyl-2-phenylpiperidine (II). Oxidation of this piperidine derivative with chromic acid afforded a mixture of benzoic acid (representing the activity at C-2) and acetic acid which was further degraded to methylamine (C-7) and carbon dioxide (C-3) by the Schmidt reaction. The piperidine II was converted to its methiodide III which on treatment with sodium in liquid ammonia afforded 1-dimethylamino-4-methyl-5-phenylpentane (IV). Vapor phase chromatography of the crude reaction product from the Emde reduction indicated that only one of the two possible isomers was obtained. The n.m.r. spectrum of the product was consistent with structure IV. A doublet at 7.43 τ was assigned to the benzylic hydrogens at C-5 (the benzylic hydrogens of ethylbenzene absorb at 7.38 τ[9]). The hydrogen at C-1 in the isomeric structure VI would be expected to produce a doublet at about 6.68 τ (the position at which the benzylic hydrogens of N,N-dimethylbenzylamine absorb[9]). No absorption was present in this region of the spectrum. A doublet was present at 7.78 τ which was assigned to the hydrogens at C-1 (the hydrogens on the α-carbons of triethylamine absorb at 7.58 τ[9]). Furthermore, subsequent steps in the degradation confirmed the structure IV. A Hofmann elimination reaction on the methiodide of IV yielded 4-methyl-5-phenyl-1-pentene (V) which had an absorption in the ultraviolet similar to toluene. A similar series of reactions on the amine VI would have yielded a styrene derivative having quite a different ultraviolet spectrum. The alkene V was cleaved with sodium metaperiodate in the presence of a catalytic amount of osmium tetroxide yielding formaldehyde, collected as its dimedone derivative (C-6), and 3-methyl-4-phenylbutanal (VIII). A solution of this aldehyde in acetone was oxidized with a calculated amount of chromic acid in sulfuric acid to 3-methyl-4-phenylbutanoic acid (VII).[10] On treatment of this acid with sodium azide in sulfuric acid, carbon dioxide (C-5) was obtained in 33% yield. A neutral by-product which was assigned the structure IX was also obtained in this reaction. It presumably arises by cyclization of the butanoic acid to 3-methyltetralone (X) followed by ring enlargement with hydrazoic acid. The lactam IX was also obtained by a Beckmann rearrangement on the oxime of 3-methyltetralone. Lack of material prevented us from proceeding further to determine the activity at C-4 directly. In the present work we have calculated the activity at this position by difference.

The activities of the degradation products obtained from the radioactive anabasine isolated from the plant which had been fed glycerol-2-C^{14} are recorded in Table I. The distribution of activity in the pyridine ring was: C-2, 11; C-3, 11; C-4, 2.4; C-5, 12; C-6, 1.6%. Low activity at C-4 and C-6 with high activity at C-5 is consistent with the direct participation of the three carbons of glycerol-2-C^{14} in the biosynthesis of this part of the pyridine ring. A similar pattern of labeling was found at C-4, C-5, and C-6 of ricinine obtained from *Ricinus communis* which had been fed glycerol-2-C^{14}.[11]

The appreciable activity which was found at C-2 and C-3 may be rationalized by postulating that the glyc-

(1) An account of this work was presented at the 145th National Meeting of the American Chemical Society, Sept. 8–13, 1963, New York, N. Y. This investigation was supported by a research grant MH-02662 from the National Institutes of Health, U. S. Public Health Service.

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TABLE I

ACTIVITIES OF ANABASINE DERIVED FROM GLYCEROL-2-C^{14} AND ITS DEGRADATION PRODUCTS

	Activity, d.p.m./mmole × 10^{-5}	Labeled atoms	Distribution of activity, %
Anabasine diperchlorate	7.9	All	100
Nicotinic acid	4.1		
Nicotinic acid hydrochloride	4.0	2, 3, 4, 5, 6, 7	50
3-Methyl-2-phenylpyridine methiodide	3.8		
1-Dimethylamino-4-methyl-5-phenylpentane methiodide	3.9		
Barium carbonate[a]	0.94	7	12
N-Methylbenzamide[b]	0.91		
(by difference)		Pyridine ring	38
Benzoic acid[c]	0.84	2	11
Barium carbonate[d]	.86	3	11
Barium carbonate[e]	.97	5	12
Formaldehyde dimedone	.13	6	1.6
(by difference)		4	2.4

[a] Obtained from the carbon dioxide produced by the decarboxylation of the nicotinic acid. [b] Obtained from the methylamine resulting from the Schmidt reaction on the acetic acid produced by the Kuhn–Roth oxidation of 1,3-dimethyl-2-phenylpiperidine. [c] Obtained by the oxidation of 1,3-dimethyl-2-phenylpiperidine with chromic acid and sulfuric acid. [d] Obtained from the Schmidt reaction on the acetic acid from the Kuhn–Roth oxidation. [e] Obtained by the Schmidt reaction on 3-methyl-4-phenylbutanoic acid.

erol is metabolized by well-established routes to phosphoenolpyruvate. An enzyme is known which catalyzes the carboxylation of this compound to oxaloacetate.[12] If the glycerol-2-C^{14} fed to the *N. glauca* plants were metabolized by this sequence of reactions, oxaloacetate labeled on the carbonyl group would result. Redistribution of activity between the two central carbons of oxaloacetate could be accomplished by reduction (known to occur in some anaerobic bacteria) to malic acid, and then dehydradation to fumaric acid. A more likely route for the randomization of activity is *via* citric and succinic acids by operation of the Krebs cycle. Pyruvate derived from glycerol-2-C^{14} would be labeled at C–2 and would yield acetate-1-C^{14}. Participation of this acetate in the Krebs cycle would result in labeling of the carboxyl groups of succinic acid. The succinic acid derived from glycerol-2-C^{14} would thus have activity on all its carbons and its incorporation into nicotinic acid would lead to labeling at C–2, C–3, and C–7. However the carboxyl group (C–7) of nicotinic acid is not incorporated into anabasine.[5] Glycerol has also been shown to be an effective precursor of nicotinic acid in *Escherichia coli*[13] and *Mycobacterium tuberculosis*.[14] However the distribution of activity was not determined. Glycerol and succinic acid are such active metabolites that it seems unlikely that the immediate precursors and the actual mechanism of the formation of nicotinic acid will be determined until the enzyme systems responsible for its synthesis are isolated from higher plants.

In the present work the anabasine derived from glycerol-2-C^{14} was found to have considerable activity in the piperidine ring (62%), with about one-fifth of the activity present on the carbon attached to the pyridine ring (C-2′). An almost identical pattern of

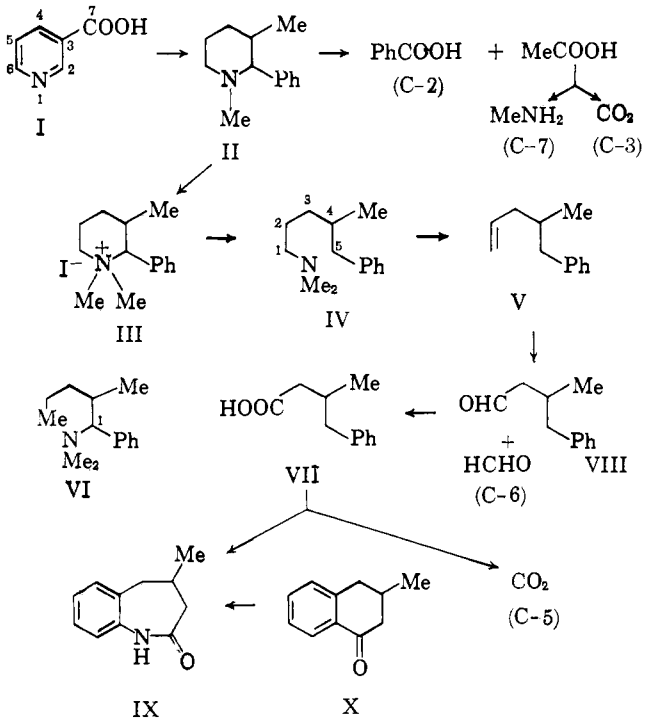

Fig. 1.—Systematic degradation of nicotinic acid.

labeling was found in this ring when acetate-2-C^{14} was fed to *N. glauca*.[4] At that time we suggested that the acetate-2-C^{14} was converted to uniformly labeled lysine which is the established precursor of the piperidine ring of anabasine. Our present results are also consistent with the formation of uniformly labeled lysine from metabolites of glycerol-2-C^{14}.

Experimental[15]

Administration of Glycerol-2-C^{14} to *N. glauca* and Isolation of the Anabasine.—Glycerol-2-C^{14} (10.7 mg., 0.5 mc.)[16] was administered to ten 4-month old *N. glauca* plants[17] growing in soil by means of a cotton wick inserted into the stems. Extraction of the fresh plants (1519 g.) as previously described[4] yielded anabasine diperchlorate (1.82 g.) having a specific activity of 7.9 × 10^5 d.p.m./mmole. (0.36% incorporation).

Degradation of the Anabasine.—In the previous paper in this series[4] the conversion of anabasine to nicotinic acid, 3-methylpyridine, 3-methyl-2-phenylpyridine, and 1,3-dimethyl-2-phenylpiperidine methiodide was described.

1-Dimethylamino-4-methyl-5-phenylpentane Methiodide.—1,3-Dimethyl-2-phenylpiperidine methiodide (1.43 g.) was suspended in liquid ammonia (70 ml.) and sodium (0.75 g.) added. When all the ammonia had evaporated, more (70 ml.) was added followed by more sodium (0.30 g.). The residue obtained after allowing the ammonia to evaporate a second time was dissolved in water (30 ml.) and extracted with ether. The residue obtained on evaporation of the dried ether extract was distilled (110°, 0.2 mm.) affording 1-dimethylamino-4-methyl-5-phenylpentane as a colorless oil (0.83 g., 95%). Vapor phase chromatography of this oil on a silicone grease–firebrick column at 185° with a helium flow rate of 30 ml./min. showed only one peak with an emergence time of 43 min. The tertiary amine was dissolved in methanol (2 ml.) and methyl iodide (2 ml.) added. After standing for 17 hr. at room temperature the excess methyl iodide was removed and ether added when the methiodide separated (0.80 g.). Crystallization from a mixture of methanol and ether afforded colorless needles, m.p. 119–120°.

Anal. Calcd. for $C_{16}H_{26}NI$: C, 51.87; H, 7.51; N, 4.03. Found: C, 52.07; H, 7.55; N, 4.25.

4-Methyl-5-phenyl-1-pentene.—1-Dimethylamino-4-methyl-5-phenylpentane methiodide (0.761 g.) was dissolved in water (10 ml.) and shaken with freshly prepared silver hydroxide (from

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(15) Melting points are corrected. We thank Mrs. Olga Hamerston and her assistants at the University of Minnesota for the analyses. Radioactive compounds were assayed in a Nuclear Chicago Model 724 liquid scintillation spectrometer using the solvents and scintillators previously described.[4]

(16) Purchased from New England Nuclear Corp., Boston, Mass.

(17) We thank Robert C. McLeester of the Botany Department of the University of Minnesota for the cultivation of the plants.

0.39 g. of silver nitrate) for 15 min. The filtered solution was evaporated to dryness *in vacuo* and the residue distilled (150°, 0.2 mm.) yielding 4-methyl-5-phenyl-1-pentene (0.238 g., 68%); λ_{max} mμ (log ε) in methanol: 242 (2.21), 248 (2.22), 253 (2.22), 259 (2.22), 262 (2.21), 265 (2.11), and 269 (2.11).

Oxidation of the 4-Methyl-5-phenyl-1-pentene.—The pentene derivative (160 mg.) was dissolved in ether (5 ml.), and water (5 ml.) added. After the addition of a small crystal of osmium tetroxide (20 mg.) a solution of sodium metaperiodate (580 mg.) in water (5 ml.) was added during 25 min. to the rapidly stirred solution cooled to 0°. The reaction mixture was then allowed to warm to room temperature and stirred for an additional 3 hr. Sodium iodate was removed by filtration and the filtrate extracted with ether. The aqueous solution was distilled into a saturated aqueous solution of dimedone (125 ml.), which on standing overnight deposited crystals of the formaldehyde dimedone derivative (132 mg., 45%). Evaporation of the dried ether extract afforded 3-methyl-4-phenylbutanal (48.6 mg., 30%).

3-Methyl-4-phenylbutanoic Acid.—3-Methyl-4-phenylbutanal (48.6 mg., 0.30 mmole) was dissolved in acetone (5 ml.) and a solution of chromic acid in sulfuric acid (0.075 ml., 0.20 mmole, made by dissolving 26.7 g. of chromium trioxide in 23 ml. of concentrated sulfuric acid and diluting with water to 100 ml.) added rapidly with stirring. After 3 min., water (20 ml.) was added, the solution saturated with sodium chloride, and then extracted with ether (4 × 20 ml.). The combined ether extract was extracted with aqueous 5% sodium bicarbonate which was then acidified with sulfuric acid and extracted with ether. Evaporation of the dried ether extract afforded 3-methyl-4-phenylbutanoic acid (25.5 mg., 50%). This acid was dissolved in 50% ethanol and titrated with 0.1 *N* sodium hydroxide to a pH of 8. Evaporation of the solution afforded the sodium salt.

Schmidt Reaction on 3-Methyl-4-phenylbutanoic Acid.—The sodium salt of 3-methyl-4-phenylbutanoic acid (15 mg.) was dissolved in concentrated sulfuric acid (0.1 ml.) and cooled to −10°. Sodium azide (20 mg.) was added and the mixture warmed to 40–43° in a stream of carbon dioxide-free nitrogen for 1 hr. The evolved carbon dioxide was absorbed in barium hydroxide solution affording barium carbonate (4.9 mg. 33%). The acidic residue in the flask was diluted with water (5 ml.) and extracted with ether. The ether was washed with 5% sodium bicarbonate and then dried over magnesium sulfate. The residue obtained on evaporation of the ether was sublimed (90°, 10^{-3} mm.) yielding a colorless solid. Recrystallization from petroleum ether (b.p. 60–70°) afforded benzo[*f*]-4-methyl-2,3,4,5-tetrahydroazepin-2-one (IX) as colorless needles, m.p. 116–117° (2.9 mg., 22%). This material was identical (mixture m.p., infrared spectrum) with the product obtained by the Beckmann rearrangement on 3-methyl-α-tetralone oxime.

Benzo[*f*]-4-methyl-2,3,4,5-tetrahydroazepin-2-one.—3-Methyl-α-tetralone oxime[18] (0.267 g.) was stirred with polyphosphoric acid (7.0 g.) at 110° for 10 min. The reaction mixture was poured onto ice and extracted with ether. The dried ether extract was evaporated and the residue crystallized from petroleum ether yielding colorless needles of the lactam (0.147 g., 55%), m.p. 116–117°.

Anal. Calcd. for $C_{11}H_{13}NO$: C, 75.40; H, 7.48; N, 7.99. Found: C, 75.70; H, 7.10; N, 7.99.

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[CONTRIBUTION FROM THE ATLANTIC REGIONAL LABORATORY, NATIONAL RESEARCH COUNCIL, HALIFAX, CANADA]

The Ultraviolet Spectra of Native and Denatured Deoxyribonucleic Acid[1]

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RECEIVED JUNE 10, 1963

The ultraviolet absorption spectra of native and denatured calf-thymus deoxyribonucleic acid were studied from 3500 to 1830 Å. The spectral changes which are brought about by heating deoxyribonucleic acid in H_2O or D_2O solution, or by drying in solid films, are similar. The absorption maxima near 1850 and 2600 Å. shift by about 50 and 14 Å., respectively, to longer wave lengths upon denaturation. The integrated intensity of the 2600-Å. band increases upon denaturation by a factor of about 1.43, while the integrated intensity of the 1850-Å. band (of which only the part above 1830 Å. was measured) certainly does not increase by a comparable factor and may possibly decrease slightly.

Introduction

The denaturation of deoxyribonucleic acid (DNA) is accompanied by the well known increase in the absorbance of the ultraviolet band centered at 2600 Å.[2] While many studies of the changes of absorbance of DNA upon denaturation have been reported, none of them extend below about 2100 Å., in spite of considerable theoretical interest in measuring the spectrum as far as possible toward short wave lengths.[3-7] In the present work the spectra of DNA in the native and denatured state were examined from 3500 to 1830 Å., with special care in the short wave-length region. The effect of denaturation on the spectrum was studied both in aqueous solution and in solid films. The study made use of the recent observation that solid DNA undergoes, upon drying, structural changes which are analogous to denaturation.[8]

Experimental

Two samples of the sodium salt of calf-thymus DNA were used. One was purchased from the Nutritional Biochemicals Corp., Cleveland, Ohio, and the other was kindly donated by Dr. Goeffrey Brown, Biophysics Department, King's College, London. The purity and integrity of these samples were checked by means of their infrared and ultraviolet spectra, orientability and dichroism, melting temperature, and extent of hypochromism at 2600 Å. A negative silver nitrate test showed the absence of chloride ions, and a negative orcinol test showed that the samples contained little or no ribonucleic acid. X-Ray diffraction patterns confirmed the absence of salt, protein, and ribonucleic acid.[9]

The spectra were recorded on a Beckman DK-2 "far ultraviolet" spectrophotometer.[10] The instrument was purged at the rate of about 40 l. per min. with "purified grade" nitrogen (which contains less than 15 parts per million of oxygen[11]), supplied by the Canadian Liquid Air Co. This purging rate was found to be sufficient to eliminate practically all absorption due to oxygen and water vapor. The stray light at short wave lengths[12] was determined by inserting in the sample beam a plate of Vycor 2 mm. in thickness (absorbing 100% below 2200 Å.) or a 1 cm. silica cell containing a 0.01 *M* solution of sodium chloride in water (absorbing 100% below 1940 Å.). The values of stray light of wave length above 1940 Å. and above 2200 Å., determined in the above manner, were the same. The part of each spectrum in

(1) Issued as N.R.C. No. 7833. A part of this work was carried out at the Spectroscopy Laboratory, Massachusetts Institute of Technology, and was supported by Grant No. A-2262 (C3) from the National Institutes of Health.

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