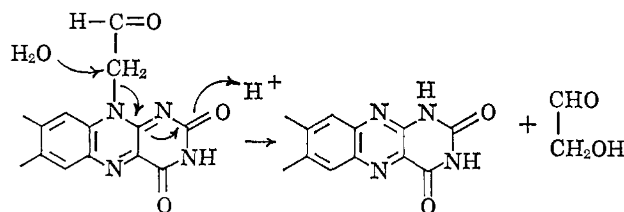
This alkaline cleavage reaction is quite analogous to the alkaline hydrolysis of β-diketones reported by Pearson and co-workers.[10,11]

The second and quantitatively less important reaction is the production of lumichrome according to eq. 2:



In a preliminary work, we found formic acid as a product in the photolysis of FMF at pH 7.4 and 8.0. However, it remains to be established to what extent the alkaline hydrolysis contributes to the rate of photolysis of both RF and FMF, which is an intermediate in the photolysis of the former. No triplet-state species (thermochromic) of isoalloxazine ring was involved in the alkaline hydrolysis of FMF, as revealed by unaffected rate in the presence of 1.2×10^{-4} M KI.

In conclusion, it is significant to note that the nonphotochemical formation of LF in the alkaline photolysis of RF has been established for the reaction path in which FMF is the confirmed intermediate, as studied previously in our laboratory. Studies on the mechanism of the photochemical formation of LF from FMF are to be published elsewhere.

(10) R. G. Pearson and E. A. Mayer, *J. Am. Chem. Soc.*, **73**, 926 (1951).
(11) R. G. Pearson and A. C. Sandy, *ibid.*, **73**, 931 (1951).

Appendix

If we assume reaction 2 is a reasonable approximation to the mechanism of LC formation, equation (3) can be written as

$$\frac{-\mathrm{d(FMF)}}{\mathrm{d}t} = \frac{k_1K_w}{(\mathrm{H}^+)}(\mathrm{FMF}) + \frac{\mathrm{d(LC)}}{\mathrm{d}t} = \left[\frac{k_1}{(\mathrm{H}^+)} + \frac{k_2}{(\mathrm{OH}^-)}\right]K_w(\mathrm{FMF}) \simeq k\ (\mathrm{FMF}) \quad (3)$$

Integrating the last expression, we get

$$(\mathrm{FMF}) = (\mathrm{FMF})_0e^{-kt}$$

Thus, eq. 1 can be expressed as

$$\frac{\mathrm{d(LF)}}{\mathrm{d}t} = \frac{k_1K_w}{(\mathrm{H}^+)}(\mathrm{FMF})_0e^{-kt}$$

with the integrated relation

$$(\mathrm{LF}) = (\mathrm{LF})_0 + \frac{\frac{k_1K_w}{(\mathrm{H}^+)}(\mathrm{FMF})_0}{k}(1 - e^{-kt})$$

Similarly, for eq. 2

$$(\mathrm{LC}) = (\mathrm{LC})_0 + \frac{\frac{k_2K_w}{(\mathrm{OH}^-)}(\mathrm{FMF})_0}{k}(1 - e^{-kt})$$

Plots of (LF) and (LC) against $(1 - e^{-kt})$ would then yield k_1 and k_2 if accurate kinetic measurements of LF and LC were possible.

Studies on Nicotine Biosynthesis[1]

K. S. Yang, R. K. Gholson, and G. R. Waller

Contribution from the Department of Biochemistry, Agricultural Experiment Station, Oklahoma State University, Stillwater, Oklahoma. *Received March 19, 1965*

Quinolinic acid was found to be an efficient precursor of nicotine in whole tobacco plants (Nicotiana tabacum L.). A method for the stepwise degradation of the pyridine ring of nicotine has been developed which involves the oxidation of nicotine to nicotinic acid, the biological conversion of nicotinic acid to ricinine, and the chemical degradation of ricinine. Using this degradation method it has been shown that glycerol is incorporated without randomization into carbons 4, 5, and 6 of the pyridine ring of nicotine. This degradation method has also been used to confirm the mechanism of fusion of the pyridine ring of nicotinic acid with the pyrrolidine ring during nicotine biosynthesis, previously proposed by Dawson, et al.[2a]

Nicotine and several other pyridine alkaloids have been utilized in studies of the biosynthetic pathway leading to nicotinic acid. Most of the available evidence[2b-8] supports the view that in higher plants and bacteria nicotinic acid is formed by the condensation of glycerol (or a closely related compound) and a four-carbon dicarboxylic acid. This suggests that pyridine-2,3-dicarboxylic acid, quinolinic acid, may be the first aromatization product resulting from this condensation. In fact it has been found that quinolinic acid is converted into nicotinic acid in *E. coli*[9] and plants.[10]

(1) A preliminary report of a portion of this work has been presented: R. K. Gholson, J. L. R. Chandler, K. S. Yang, and G. R. Waller, *Federation Proc.*, **23**, 528 (1964).
(2) (a) R. F. Dawson, D. R. Christman, A. F. D'Adamo, M. L. Solt, and A. P. Wolf, *J. Am. Chem. Soc.*, **82**, 2629 (1960); (b) M. V. Ortega and G. M. Brown, *J. Biol. Chem.*, **235**, 2939 (1960).
(3) D. Gross, H. R. Schütte, G. Hubner, and K. Mothes, *Tetrahedron Letters*, **No. 9**, 541 (1963).
(4) T. Griffith, K. P. Hellman, and R. U. Byerrum, *Biochemistry*, **1**, 336 (1962).
(5) G. R. Waller and L. M. Henderson, *Biochem. Biophys. Res. Commun.*, **5**, 5 (1961).
(6) P. F. Juby and L. Marion, *ibid.*, **5**, 461 (1916).
(7) P. F. Juby and L. Marion, *Can. J. Chem.*, **41**, 117 (1963).
(8) J. M. Essery, P. F. Juby, L. Marion, and E. Trumbell, *ibid.*, **41**, 1142 (1962).
(9) A. J. Andreoli, M. Ikeda, Y. Nishizuka, and O. Hayaishi, *Biochem. Biophys. Res. Commun.*, **12**, 92 (1963).

However, it has been reported that quinolinic acid is not a precursor of niacin in *Mycobacterium tuberculosis*[11] or of nicotine in sterile root cultures of the tobacco plant, *Nicotiana tabacum* L.[12]

The results of the present study show that quinolinic acid is an efficient precursor of nicotine in the tobacco plant.

A method for the degradation of the pyridine ring of nicotine involving its biological conversion to ricinine has been developed. Using this technique, it has been found that glycerol is incorporated into positions 4, 5, and 6 of the pyridine ring of nicotine, thus confirming similar results previously obtained with ricinine.[7,8,13] This degradation procedure has also been employed to determine the mechanism for the fusion of the carbon skeletons of the pyridine and pyrrolidine rings of nicotine.

Experimental Section

Preparation of Labeled Compounds. Quinolinic acid-2,3,7,8-^{14}C was prepared by condensing aniline-U-^{14}C[14] with glycerol[15,16] and oxidizing the resulting quinoline with H_2O_2 in the presence of Cu^{2+}. The quinolinate was treated with H_2S to regenerate quinolinic acid and purified by repeated recrystallization from hot water to constant specific activity (m.p. 190°, uncorrected). Nicotinic acid-2,3,7-^{14}C was prepared by pyrrolysis of quinolinic acid at its melting point and purified by sublimation at about 150° under vacuum. The specific activity of nicotinic acid obtained agreed with the value calculated directly from quinolinic acid by subtracting 25% of its activity which is lost from carbon 7. Both quinolinic and nicotinic acids thus prepared were checked for radiochemical purity by paper chromatography prior to administration to tobacco plants.

Glycerol-1-^{14}C and glycerol-2-^{14}C were purchased from Nuclear Chicago Corp. Quinolinic acid-^{3}H was a gift of Dr. R. F. Dawson of Columbia University.

Biosynthesis of Nicotine. Quinolinic acid-2,3,7,8-^{14}C with a specific activity of 4.0×10^8 d.p.m./mmole was administered to young *Nicotiana tabacum* L. plants (10–15 cm. long) through a newly regenerated root system, as described by Henderson, *et al.*[17] The isotope was completely taken up in 3–4 hr. After 1 week nicotine was isolated by the method of Brown and Byerrum[18] and converted to nicotine dipicrate, which was recrystallized from hot water to constant specific activity. The purity of the dipicrate was checked by paper chromatography in five different solvent systems.[19] In order to compare the efficiency of isotope incorporation, nicotinic acid-2,3,7-^{14}C was fed to another group of tobacco plants under similar conditions. The biosynthesis of nicotine from glycerol-^{14}C and quinolinic acid-^{3}H was carried out in the manner described above.

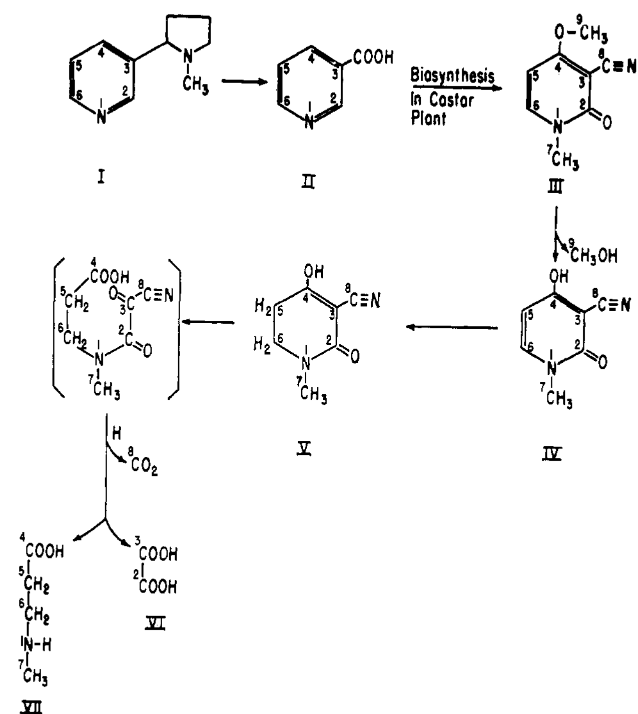


Figure 1. Degradation of the pyridine ring of nicotine *via* biosynthesis of ricinine.

Isotope Analyses. The specific activity of nicotine dipicrate was determined by the wet combustion procedure of Van Slyke, *et al.*,[20] followed by counting the $^{14}CO_2$ activity with the vibrating reed electrometer. In most cases, liquid scintillation counting[21] was employed for those compounds appreciably soluble in the scintillation solvent which was composed of 58.75% toluene, 39.25% ethanol, and 2.0% water. The phosphor was 0.5% 2,5-diphenyloxazole and 0.02% *p*-bis-2(5-phenyloxazolyl)benzene. Internal standards were used to correct for quenching. All counts were corrected for counting efficiency to yield absolute values (d.p.m.).

Degradation of the Pyridine Ring of Nicotine. The method of degradation developed is based on the previous observation[22] that nicotinic acid is converted to ricinine in high yield in the castor plant with retention of the complete carbon skeleton, and on the availability of a method for the stepwise degradation of the ricinine molecule.[23] The scheme of degradation is outlined in Figure 1.

About 40 mg. of nicotine (I), isolated from 20 tobacco plants to which carbon-14 labeled compound had been administered, were oxidized to nicotinic acid (II), as described by Lamberts and Byerrum.[24] This

(10) L. A. Hadwiger, S. E. Badiei, G. R. Waller, and R. K. Gholson, *Biochem. Biophys. Res. Commun.*, 13, 466 (1963).
(11) A. G. Moat and J. N. Albertson, *Federation Proc.*, 23, 528 (1964).
(12) D. R. Christman and R. F. Dawson, *Biochemistry*, 2, 182 (1963).
(13) K. S. Yang and G. R. Waller, in press.
(14) Purchased from New England Nuclear Corp.
(15) H. T. Clarke and A. W. Davis, *Org. Syn.*, 2, 79 (1922).
(16) R. K. Gholson, I. Ueda, N. Ogasawara, and L. M. Henderson, *J. Biol. Chem.*, 239, 1208 (1964).
(17) L. M. Henderson, J. F. Someroski, D. R. Rao, P. H. L. Wu, T. Griffith, and R. U. Byerrum, *ibid.*, 234, 93 (1959).
(18) S. A. Brown and R. U. Byerrum, *J. Am. Chem. Soc.*, 74, 1523 (1952).
(19) Ethanol (95%), 1 *N* ammonium acetate (7:3); 85% 2-propanol; 1-butanol–acetic acid–water (4:1:1); 1-butanol, saturated with 15% NH_4OH; 60% 1-propanol.
(20) D. D. Van Slyke, J. Plazin, and J. R. Weisiger, *J. Biol. Chem.*, **191**, 299 (1951).
(21) Tricarb, Model 314, Packard Instrument Co., LaGrange, Ill.
(22) G. R. Waller and L. M. Henderson, *J. Biol. Chem.*, 236, 1186 (1961).
(23) U. Schiedt and G. Boeckh-Behrens, *Z. Physiol. Chem.*, 330, 58 (1962).
(24) B. L. Lamberts and R. U. Byerrum, *J. Biol. Chem.*, 233, 939 (1958).

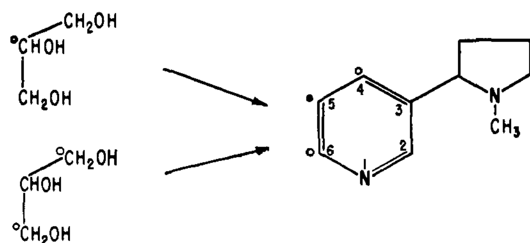


Figure 2. Labeling patterns of nicotine formed from glycerol-1-^{14}C and glycerol-2-^{14}C.

nicotinic acid was purified by repeated sublimation (m.p. 232°, uncorrected) and was converted to ricinine-^{14}C in approximately 10% radiochemical yield as follows: castor seedlings 10–15 cm. high were each injected with 100 μg. of nicotinic acid. Four days later ricinine was isolated from the whole plants as previously described.[20] This ricinine (III, m.p. 201°, uncorrected) was converted to ricininic acid (IV, m.p. 299°, uncorrected) by refluxing with 1 *N* NaOH. Ricininic acid was hydrogenated to form dihydroricininic acid (V, m.p. 240°, uncorrected) with 5% sodium amalgam in alcoholic NaOH. This compound (V) was oxidized with 1 *N* $KMnO_4$ in alkaline solution followed by acid hydrolysis to yield oxalic acid (VI) and N-methyl-β-alanine (VII). The carbon dioxide evolved during the hydrolysis step originated from the nitrile carbon (C-8). Oxalic acid, derived from carbons 2 and 3, was separated from N-methyl-β-alanine by ion exchange on a Dowex-50 column and was precipitated as calcium oxalate. This procedure is modified from the method of Schiedt and Boeckh-Behrens[23] chiefly by the careful control of the $KMnO_4$ oxidation of V to avoid the further oxidation of carbons 2 and 3 to carbon dioxide.

N-Methyl-β-alanine (VII) was methylated and pyrolyzed to yield carbon 7 as the tetramethylammonium salt and carbons 4, 5, and 6 as acrylic acid. This acrylic acid was hydrogenated at atmospheric pressure over Adams catalyst to produce propionic acid, which was purified by chromatography on a Celite column. Propionic acid was degraded by the Schmidt procedure to isolate carbons 4, 5, and 6 of the pyridine ring of ricinine.

Results and Discussion

A comparison of the incorporation of nicotinic and quinolinic acids into nicotine by tobacco plants (*Nicotiana tabacum* L.) is shown in Table I.

Nicotine was converted to nicotinic acid in order to determine whether the pyridine ring of quinolinic acid is incorporated directly into the pyridine ring of nicotine. Nicotine (40 mg., 495 mμcuries/mmole), isolated from 20 tobacco plants to which quinolinic acid-2,3,7,8-^{14}C had been administered, was oxidized to nicotinic acid as described by Lamberts and Byerrum.[24] This nicotinic acid was purified by repeated sublimation (m.p. 232°). Its specific activity was 463 mμcuries/mmole, demonstrating that almost all the radioactivity of nicotine formed from quinolinic acid-2,3,7,8-^{14}C is localized in the pyridine ring. The per cent of ^{14}C incorporation (radiochemical yield) of nicotinic acid in these experiments with whole plants is about the same

Table I. Incorporation of Quinolinic Acid-2,3,7,8-^{14}C, Quinolinic Acid-^{3}H, and Nicotinic Acid-2,3,7-^{14}C into Nicotine

	Q.A.-2,3,-7,8-^{14}C	N.A.-2,3,-7,-^{14}C	Q.A.-^{3}H
Specific activity d.p.m./mmole × 10^{-8}	4.00	2.98	0.585
Total activity used, d.p.m. × 10^{-5}	9.10	12.10	1.493
Specific activity of nicotine dipicrate, d.p.m./mmole × 10^{-6}	1.02	3.31	0.248[b]
Total activity isolated, d.p.m. × 10^{-4}	2.55	8.03	0.680
% incorporation (corrected)[a]	5.6	10.0	4.6

[a] Values reported here have been corrected for the loss of activity residing originally in the carboxyl groups. This is based on the fact that when nicotinic acid is incorporated into nicotine its carboxyl group is lost while the pyridine ring carbon skeleton remains unchanged.[25,26] It is expected that both carboxyl groups of quinolinic acid would be lost during its incorporation into nicotine. [b] Radioactivity determined by Dr. D. R. Christman, Brookhaven National Laboratory.

as that previously reported in tobacco root cultures by Dawson, *et al.*[25]

Since the radiochemical yields of nicotine from quinolinic acid-^{3}H andquinolinic acid ^{14}C are quite similar, negligible incorporation of quinolinic acid-^{3}H into nicotine previously reported[12] is probably not due to loss of tritium during isolation of nicotine as the dipicrate. The incorporation of quinolinic acid labeled with either tritium or carbon-14 into nicotine in root cultures (see Appendix) is in fact much lower than that observed in whole plants. This phenomenon may be due to a more efficient absorption of quinolinic acid in whole plants than in root cultures.

The finding that quinolinic acid is an efficient precursor of nicotine indirectly supports the concept that a three-carbon glycerol derivative and a four-carbon dicarboxylic acid take part in the pyridine ring formation with retention of both carboxyl groups before condensing with the pyrrolidine ring to form nicotine. The same conclusions with respect to pyridine ring formation have been reached on the basis of studies of ricinine biosynthesis in the castor plant in this laboratory and of anabasine biosynthesis in Leete's laboratory.[27]

Incorporation of Glycerol into the Pyridine Ring of Nicotine. Direct evidence for the incorporation of glycerol into positions 4, 5, and 6 of the pyridine ring of nicotine is provided by the data shown in Table II.

These results were obtained by feeding glycerol-1-^{14}C and glycerol-2-^{14}C to tobacco plants and degrading the nicotine formed as outlined in Figure 1. The labeling patterns obtained (Figure 2) are very similar to those found for the incorporation of glycerol into ricinine by the castor plant[28] and into anabasine by *Nicotiana glauca.*[27] Glycerol-^{14}C is incorporated into carbons 4, 5, and 6 of the pyridine ring of nicotine without randomization. Incorporation of glycerol-^{14}C into carbons 2 and 3 of the pyridine ring probably results

(25) R. F. Dawson, D. Christman, and R. C. Anderson, *J. Am. Chem. Soc.*, 75, 5114 (1953).
(26) R. F. Dawson, D. R. Christman, R. C. Anderson, M. L. Solt, A. F. D'Adamo, and U. Weiss, *ibid.*, 78, 2645 (1956).
(27) E. Leete, *ibid.*, 26, 1224 (1964).
(28) G. R. Waller and K. S. Yang, *Federation Proc.*, 22, 356 (1963).

Table II. Distribution of Radioactivity in Nicotine Biosynthetically Formed from Glycerol-2-^{14}C and Glycerol-1-^{14}C

Compd.	Nicotine Carbon	Glycerol-2-^{14}C d.p.m./mmole	%	Glycerol-1-^{14}C d.p.m./mmole	%
Ricinine	2–7	34,900	100	102,000	100
CO_2 (–C–N)	7	5,110	14.6	4,620	4.5
Calcium oxalate	2,3	9,550	27.4	33,500	32.9
CO_2 (propionate C-1)	4	0	0	35,400	34.7
CO_2 (propionate C-2)	5	22,200	63.5	Trace	0
CO_2 (propionate C-3)	6	0	0	30,600	30.0

from its conversion to a 2-carbon unit which is then incorporated into a dicarboxylic acid which is the precursor of carbons 2, 3, and 7 of the pyridine ring.[18] These findings further support the concept that the pyridine ring is formed in plants by a condensation of glycerol (or a glycerol derivative) and a four-carbon dicarboxylic acid.

Mechanism of Condensation of the Pyridine and Pyrrolidine Rings of Nicotine. A symmetrical relationship between carbon atoms 2′,5′ and 3′,4′ of the pyrrolidine ring of nicotine has been demonstrated by Leete[29] and confirmed by other workers[24,30] in studies on nicotine biosynthesis. The mesomeric intermediate of the pyrrolidine moiety formed from glutamic acid,[24] or a structurally related compound,[29] undergoes condensation with the pyridine ring moiety with an equal frequency on positions 2′ and 5′ to form the nicotine molecule.

Several mechanisms are theoretically possible for the decarboxylation of nicotinic acid and its condensation with the pyrrolidine ring in the biosynthesis of nicotine: (1) decarboxylation of nicotinic acid to form free pyridine followed by condensation with the pyrrolidine ring; (2) attachment of the pyrrolidine ring on carbon 4 (or carbons 2 or 6) of nicotinic acid with subsequent decarboxylation and intramolecular rearrangement; (3) attachment of the pyrrolidine ring on carbon 5 of nicotinic acid with subsequent decarboxylation on carbon 3; (4) bond breakage and bond formation on the same position, carbon 3.

The results of Dawson, *et al.*,[2a] obtained using nicotinic acid substituted on different carbons with tritium or deuterium, strongly support mechanism 4. These workers found that the hydrogens on positions 2, 4, and 5 of nicotinic acid are retained in the corresponding positions in nicotine. The availability of nicotinic acid-2,3,7-^{14}C makes it possible to check these results by an independent method, since nicotine having the labeling patterns shown in Figure 3 would be formed from this precursor.

Nicotinic acid-2,3,7-^{14}C was administered to young *Nicotiana tabacum* L. plants, and nicotine was isolated as described previously. This nicotine-^{14}C was oxidized to nicotinic acid with retention of all its radioactivity in the pyridine ring. This nicotinic acid was then converted biosynthetically to ricinine. Ninety-six hours after injecting the nicotinic acid ricinine was isolated[5] and degraded as previously described. The results of this degradation are shown in Table III. Essentially all of the activity of the pyridine ring was

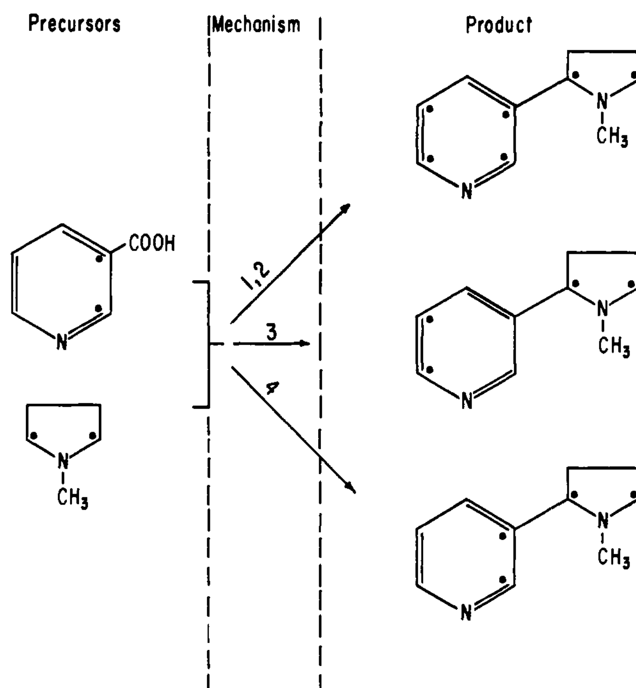


Figure 3. Hypothetical mechanisms for the condensation of the pyridine and pyrrolidine rings of nicotine.

present in carbons 2 and 3. This labeling pattern is consistent with mechanism 4 and indicates that during the biosynthesis of nicotine the pyrrolidine ring is attached to the pyridine ring at the position (carbon 3) where the decarboxylation takes place. This finding confirms the conclusions reached by Dawson, *et al.*,[2a] on the basis of the retention of hydrogens on the pyridine ring of nicotine.

Table III. Distribution of Radioactivities in Ricinine Derived from Nicotine Using Nicotinic Acid-2,3,7-^{14}C as Precursor

Compound	Specific activity, d.p.m./mmole $\times 10^{-4}$
Nicotine dipicrate	1190
Ricinine	1.49
O-Me, N-Me, and –CN (carbons 7, 8, 9)	0
Calcium oxalate (carbon 2, carbon 3)	1.30
Propionate (carbons 4, 5, 6)	0

Acknowledgment. This research was supported in part by Grant GM-08624-03 from the United States Public Health Service and in part by Grant GB-1659 from the National Science Foundation.

Appendix[31]

Quinolinic acid labeled with tritium by the Wilzbach procedure (specific activity 472,850 d.p.m./mg.) was supplied to two samples of 50 sterile cultures each of excised Turkish tobacco roots. The concentration of the acid in the culture medium was 0.033 mg./ml. After 4 weeks of incubation, the nicotine picrates obtained from

(29) E. Leete, *J. Am. Chem. Soc.*, 80, 2162 (1958).

(30) W. L. Alworth, A. A. Liebman, and H. Rapoport, *ibid.*, 86, 3375 (1964).

(31) The research described was performed under the auspices of the U. S. Atomic Energy Commission at Brookhaven National Laboratory and under Contract No. AT(30-1)-1778 at Columbia University.

these cultures assayed 329 and 326 d.p.m./mg., respectively. Radiochemical yields were 0.86 and 0.84%, and the ratio of specific activities was 0.37%. These figures were substantially lower than those obtained from the use of nicotinic acid and also of certain aliphatic intermediates (*e.g.*, alanine and β-alanine) with respect to label incorporated into the pyridine ring of nicotine. Thus we concluded[12] that quinolinic acid is probably not an important precursor of the pyridine ring of nicotine.

The results reported in the preceding paper with carbon-14 ring-labeled quinolinic acid necessitate a reappraisal of our conclusions. Using quinolinic acid-2,3,7,8-^{14}C,[32] we have obtained the following results.

Treatment of a portion of the carbon-14 labeled material with charcoal in hot water and recrystallization yielded quinolinic acid-2,3,7,8-^{14}C with a specific activity of 1006 mμcuries/mg. of carbon. The recrystallized and original quinolinic acid-2,3,7,8-^{14}C samples were supplied at about 0.01 mg./ml. to tobacco root culture fluid. After 7 days of incubation, the nicotine picrates obtained were found to assay 0.929 and 0.659 mμcurie/mg. of carbon, respectively. The respective radiochemical yields were 0.78 and 0.83%.

The latter figures can be corrected for predictable losses (*e.g.*, carbon atoms in positions 7 and 8) by doubling. Thus, the corrected radiochemical yields would be 1.56 and 1.66%, respectively. Similar corrections may be made for the incorporation of the tritium-labeled acid if we assume that the hydrogen atom on position 6 is lost during the formation of nicotine.[2a] In this case, radiochemical yield figures are multiplied by three-halves, assuming that the distribution of label on the ring of quinolinic acid was uniform. The resulting values are 1.17 and 1.24%, respectively. (For justification of the latter procedure, see Dawson, *et al.*[2a]

(32) Supplied by Dr. R. K. Gholson, Oklahoma State University.

Communications to the Editor

Studies on the Helix–Coil Transition by Polarization of Fluorescence Measurements[1]

Sir:

Polarization of fluorescence measurements are ideally suited to the study of the rigidity of macromolecules[2–4] and its changes during the helix–coil transition. It is more sensitive to changes in rigidity than the usual hydrodynamic measurements[5] and can be applied to molecules of all sizes. The rotational relaxation time and the degree of polarization of fluorescence at a given temperature reflect the over-all rigidity of the molecule. The transition temperature T_T, *i.e.*, the temperature at which $(1/p + {}^1/_3) = (1/p_0 + {}^1/_3)\cdot(1 + RT\tau/V\eta)$ deviates from a linear dependence upon T/η to follow the exponential equation $(1/p + {}^1/_3) = Ke^{+aT/\eta}$, measures the stability of the internal structure.[5–7] T_T is a function of the kinetic energy of the molecule and not of the viscosity of the medium up to 9 cp.[6] The rotational relaxation time ρ_h[5] and the equivalent volume of the rotating segment V_e[5] were calculated from the straight line portion of the curve (at 5°). The values of these two parameters for a rigid sphere were calculated from the molar volumes of the polypeptides.

The helix–coil transitions in poly Glu^{97}Lys^{3}, polyLys (No. 2), and poly Glu^{63}Lys^{37} (No. 3)[8] were studied in order to investigate the rigidity and stability of the glutamic acid helix, the lysine helix, and the helix in a copolymer containing both glutamic acid and lysine. Poly Glu^{97}Lys^{3} was used as a model for the glutamic acid helix, because a few lysine residues were necessary to introduce the fluorescein isothiocyanate or 1-dimethylaminonaphthalene-5-sulfonyl chloride (DNS) dye. The polarization of fluorescence was studied as a function of pH and temperature in a modified Brice-Phoenix light-scattering apparatus as previously described[5]; all studies were performed in 0.2 *M* NaCl and 0.1 *M* buffer (citrate, phosphate, or carbonate). As the polymer undergoes a transition from the rather flexible coil at neutral pH to the more rigid helical conformation at pH 4 for glutamic acid and pH 11 for lysine, p, ρ_h[5], ρ_h/ρ_0[5], and V_e[5] increase. The data are summarized in Table I.

The helices of poly Glu^{97}Lys^{3} and poly Glu^{63}Lys^{37} (No. 3) show a higher degree of polarization and a longer rotational relaxation time than that of poly Lys (No. 2), and the V_e[5] of the glutamic acid helices is larger. The midpoint of the helix–coil transition in poly Glu^{97}Lys^{3} by polarization measurements (pH 4.5 in 0.2 *M* NaCl + 0.1 *M* phosphate or citrate buffer) is lower than that obtained from titration (pH 5.0 in 0.2 *M* NaCl)[9] and from optical rotation (pH 5.1 in 0.2 *M* NaCl).[9–12] This suggests that the glutamic acid residues assume the helical conformation before the helix reaches its maximal rigidity. The degree of

(1) Supported by the National Science Foundation (GB-940).

(2) G. Weber, *Advan. Protein Chem.*, **8**, 416 (1953).
(3) G. Weber and F. W. J. Teale, *Proteins*, 3, 445 (1965).
(4) R. F. Steiner and H. Edelhoch, *Chem. Rev.*, 62, 457 (1962).
(5) T. J. Gill, III, *Biopolymers*, 3, 43 (1965).
(6) G. S. Omenn and T. J. Gill, III, unpublished data.
(7) The symbols used in these equations are: p, the degree of polarization at temperature T; p_0, the limiting degree of polarization as $T/\eta \rightarrow 0$; R, the gas constant; V, the molar volume; η, the viscosity; τ, the lifetime of the excited state of the conjugated fluorescent dye; and K and a, constants in the exponential equation.
(8) The polypeptide nomenclature is defined in T. J. Gill, III, *Biopolymers*, 2, 283 (1964). The number following the polypeptide formula denotes the preparation; (No. 1) is omitted in all cases.
(9) A. Wada, *Mol. Phys.*, 3, 409 (1960).
(10) M. Idelson and E. R. Blout, *J. Am. Chem. Soc.*, 80, 4631 (1958).
(11) G. Fasman, C. Lindblow, and E. Bodenheimer, *Biochemistry*, 3, 155 (1964).
(12) P. Doty, K. Imahori, and E. Klemperer, *Proc. Natl. Acad. Sci. U.S.*, **44**, 424 (1958).