Table I. Labeling in Leaf Amino Acids of *Nicotiana rustica* L. after Exposure to $^{14}CO_2$

	Light exposure time of 3 min				Light exposure time of 18 min			
	μcuries/ mmole	% of total	μcuries/ mmole	% of total	μcuries/ mmole	% of total	μcuries/ mmole	% of total
Glutamic acid								
Total	1.76		0.54		14.3		39.1	
C-1, COOH		9.1[a]		>20.0[a]		30.4		32.0
C-2, $CHNH_2$		1.9		<1.0		7.1		10.3
C-3, CH_2		2.3		<1.0		7.6		11.0
C-4, CH_2		40.8		38.0		25.3		21.1
C-5, COOH		45.8		40.4		27.4		21.8
Aspartic acid								
Total	3.86		1.62		15.4		58.5	
C-1 + C-4, COOH		79.9		92.4		75.8		70.6

[a] By difference.

Table II. Derivation of Glutamate Carbon Atoms

Pathway	Carbon atoms C-1	C-2	C-3	C-4	C-5
Glyoxylate–malate proposal[8]	a-1[a]	a-2[b]	g[c]	a-2	a-1
Chlorobium thiosulfatophilum[8]	CO_2[d]	CO_2	p-2,3[e]	p-2,3	CO_2
Clostridium tetanomorphum[9,10]	a-1	a-2	p-3	p-2	CO_2
Clostridium kluyveri[11]	a-1	a-2	a-1	a-2	CO_2

[a] a-1 = acetate, C-1. [b] a-2 = acetate, C-2. [c] g = glyoxylate. [d] CO_2 = carbon derived from CO_2 fixation such as pyruvate C-1 and oxalacetate C-1 and C-4. [e] p-2,3 = pyruvate C-2 and C-3.

As the exposure time is lengthened, increasing amounts of label are found in C-2 and C-3 of pyruvate, and almost uniform labeling is achieved.[13] Pyruvate-1-^{14}C and $^{14}CO_2$, *via* the TCA cycle, yield primarily glutamate-1-^{14}C.[14] Therefore, it would be expected that lengthening the exposure time would enhance the incorporation of ^{14}C into C-4 and C-5 of glutamate at the expense of C-1, whereas the opposite effect was observed (Table I).

The rapid formation of symmetrically labeled glycolate, a reaction known to occur in tobacco plants,[6] and its subsequent metabolism *via* glyoxylate, glycine, serine, pyruvate, and the TCA cycle also yield almost uniformly labeled pyruvate. If the results (Table I) are to be explained by carbon reduction, glycolate formation, and the TCA cycle, it must be assumed that symmetrically labeled acetate of high specific activity (relative to that of the middle carbons of oxalacetate) is formed from uniformly labeled pyruvate.

The data presented here can be explained by assuming a rapid formation of symmetrically labeled glycolate and its subsequent conversion to glutamate *via* glyoxylate, oxalmalate, γ-hydroxy-α-ketoglutarate, and α-ketoglutarate.[15] The middle carbons of oxalacetate, formed from pyruvate (carbon reduction cycle) and $^{14}CO_2$, would be labeled more slowly than glyoxylate, thus accounting for the observed increase in activity with time of (C-2 + C-3)/(C-4 + C-5). The formation of α-ketoglutarate from glyoxylate and pyruvate, *via* γ-hydroxy-α-ketoglutarate, is another possibility and would account for the rather low labeling in C-1 in the short-time experiment. Some evidence for the formation of γ-hydroxy-α-ketoglutarate in plants has been recorded.[16,17] In addition to raising several questions concerning glutamate synthesis during CO_2 fixation, the results presented here should also be considered in future studies on the biosynthesis of the pyrrolidine ring of nicotine.

Acknowledgment. We wish to thank Dr. George R. Waller for his interest in this research.

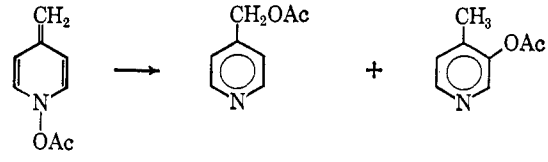
(14) R. E. Koeppe and R. J. Hill, *J. Biol. Chem.*, **216**, 813 (1955).
(15) Y. Sekizawa, M. E. Maragoudakis, T. E. King, and V. H. Cheldelin, *Biochemistry*, **5**, 2392 (1966).
(16) B. Payes and G. G. Laties, *Biochem. Biophys. Res. Commun.*, **13**, 179 (1963).
(17) B. Payes and G. G. Laties, *ibid.*, **10**, 460 (1963).

Linda C. Burns, Robert M. O'Neal, Roger E. Koeppe
Department of Biochemistry, Agricultural Experiment Station Oklahoma State University, Stillwater, Oklahoma 74074
Received January 13, 1967

The Reaction of 4-Picoline N-Oxide with Acetic Anhydride. Trapping of the Cationic Intermediate

Sir:

The reaction of 4-picoline N-oxide with acetic anhydride[1] is generally believed to proceed by way of the anhydrobase intermediate **1**. The rearrangement of **1** appears to be largely intramolecular in the presence of a diluent[2] but intermolecular when it is generated in the neat mixture of reactants.[3] The mechanistic details of these reactions of **1** have been the subject of considerable controversy. A radical-pair mechanism has been suggested to account for the intramolecular path in this and related cases,[2a,3-5] while the intermolecular reaction has been postulated[2b] to occur by attack of acetate ions at ring position 3 and at the 4-methylene group of **1**, with expulsion of acetate ion.



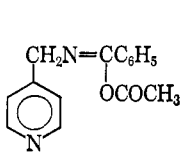
1 **2** **3**

(1) J. A. Berson and T. Cohen, *J. Am. Chem. Soc.*, 77, 1281 (1955); V. Boekelheide and W. J. Linn, *ibid.*, **76**, 1286 (1954); O. H. Bullitt, Jr., and J. T. Maynard, *ibid.*, **76**, 1370 (1954).
(2) (a) V. J. Traynelis and R. F. Martello, *ibid.*, **82**, 2744 (1960); (b) S. Oae, Y. Kitaoka, and T. Kitao, *Tetrahedron*, **20**, 2685 (1964).
(3) S. Oae, T. Kitao, and Y. Kitaoka, *J. Am. Chem. Soc.*, **84**, 3359 (1962).
(4) V. J. Traynelis and A. I. Gallagher, *ibid.*, **87**, 5710 (1965).
(5) S. Oae, S. Tamagaki, and S. Kozuka, *Tetrahedron Letters*, 1513 (1966).

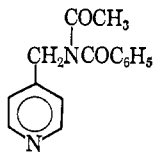
Cohen and Fager[6] have suggested that the N–O bond of **1** might cleave *heterolytically* to produce an acetate anion and a picolyl cation. The latter could then be attacked by internal or external acetate (or acetic acid), thereby accounting for products from both routes *via* a single mode of cleavage of **1**. The production of substantial ester in the reation of the related 2-picoline N-oxide with phenylacetic[6,7] and trichloroacetic[7] anhydrides has been interpreted as supporting a picolyl cation mechanism in that case, although quite similar experimental results[4] in the case of 4-picoline N-oxide have been used to support the radical-pair mechanism.

We have attempted to trap the intermediate picolyl species by performing the reaction of 4-picoline N-oxide with acetic anhydride in the solvents anisole and benzonitrile, and in a 1:1 mixture of the two. The anisole experiment produced a 20% yield of a mixture of three picolylanisoles in addition to the usual ester product. The *m*- and *p*-(4-picolyl)anisoles were identified by comparison with independently prepared samples.[8] The *meta*:*para* ratio, 0.25, is consistent with cationic attack on a ring bearing an *ortho,para*-directing substituent but not with alkyl radical attack, which gives a ratio varying between 5.6 and about 1.4, depending upon the particular radical.[11]

Radical attack is known[11] to occur readily on the benzonitrile ring system, whereas cations attack the nitrogen.[12] In this solvent, the picolyl group was found to attack the nitrogen almost exclusively, producing the imide **5** (11% yield by nmr), probably by way of the intermediate **4**. The merest trace of material which



4



5

could result from attack of a picolyl group on the nucleus was detected by vpc–mass spectrometry. The structure of **5** was determined by vpc–mass spectrometry and by comparison with a specimen prepared by treatment of the benzamide with sodium hydride followed by acetic anhydride.

Corresponding results were obtained in the direct competition between anisole and benzonitrile. Although the latter is known to be much more susceptible to radical substitution than the former,[11] essentially all the substitution occurred in the anisole ring. The only other product involving the solvent was **5**.

While these experiments clearly demonstrate that the reaction of 4-picoline N-oxide with acetic anhydride generates a substantial portion of picolyl cations, it is conceivable that this is a side reaction and that ester formation proceeds by an independent radical-pair process in which recombination is so remarkably efficient that picolyl radicals cannot be trapped by the solvent. However, this possibility must be regarded as highly unlikely, especially in view of the strong contrary evidence in the case of 2-picoline N-oxide.[6,7]

The synthetic implications of this simple method of generation of picolyl cations is now under investigation.

Acknowledgment. We wish to thank the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this work. We also thank the National Institutes of Health for a grant used to purchase the LKB 9000 combined gas chromatograph–mass spectrometer used in this work and Dr. Charles Sweeley for help in determining and interpreting the mass spectra.

Theodore Cohen, Gary L. Deets
Department of Chemistry, University of Pittsburgh
Pittsburgh, Pennsylvania 15213
Received May 16, 1967

(6) T. Cohen and J. H. Fager, *J. Am. Chem. Soc.*, **87**, 5701 (1965).
(7) T. Koenig, *ibid.*, **88**, 4045 (1966).
(8) The *para* isomer was prepared by reduction of *p*-(4-picolyl)-nitrobenzene[9] and thermal decomposition in methanol of the corresponding diazonium salt. A mixture of the *meta* and *para* isomers was prepared by the benzyne method, which involves the reaction of *p*-bromoanisole with sodium amide and 4-picoline.[10]
(9) A. J. Nunn and K. Schofield, *J. Chem. Soc.*, 583 (1952).
(10) P. H. Dirstine and F. W. Bergstrom, *J. Org. Chem.*, **11**, 55 (1946).
(11) J. R. Shelton and C. W. Uzelmeier, *J. Am. Chem. Soc.*, **88**, 5222 (1966).
(12) R. M. Lusskin and J. J. Ritter, *ibid.*, **72**, 5577 (1950).

Anomalous Behavior of 3-*endo*-Hydroxy-3-*exo*-phenyl-2-*endo*-norbornylamine during Deamination[1]

Sir:

An explanation based on torsional effects has recently been advanced[2] to account for the "remarkable stereospecificity of 3,2 shifts" in rearrangements of derivatives of bicyclo[2.2.1]heptane. The stereospecificity requirements are so stringent that 3-*endo*-hydrogens[3] or methyl groups[4] must become *exo* through circuitous routes[3] involving Wagner–Meerwein and 6,1-hydride shifts before they can migrate to the adjacent (*exo*-2) position. The phenyl is another group which is unable to migrate 3 → 2 or 2 → 3 in an *endo–endo* fashion.[3,5] To our surprise, however, there also does not seem to be an example of *exo–exo* migration of phenyl during solvolyses of substituted norbornyl derivatives.[3,5,6]

It is well known that during deamination of aliphatic amines the energy profiles of the various processes which can occur are so compressed that pathways which are unlikely during ordinary solvolyses often become important.[7-9] In 3-*endo*-hydroxy-3-*exo*-phenyl-2-*endo*-

(1) Research sponsored by the U. S. Atomic Energy Commission under contract with the Union Carbide Corp.
(2) P. von R. Schleyer, *J. Am. Chem. Soc.*, **89**, 699 (1967).
(3) C. J. Collins, Z. K. Cheema, R. G. Werth, and B. M. Benjamin, *ibid.*, **86**, 4913 (1964); see also the several pertinent references given in ref 2.
(4) J. D. Roberts and J. A. Yancey, *ibid.*, **75**, 3165 (1953); W. R. Vaughan and R. Perry, Jr., *ibid.*, **75**, 3168 (1953); A. M. T. Finch, Jr., and W. R. Vaughan, *ibid.*, **87**, 5520 (1965).
(5) B. M. Benjamin, B. W. Ponder, and C. J. Collins, *J. Am. Chem. Soc.*, **88**, 1558 (1966); B. M. Benjamin and C. J. Collins, *Tetrahedron Letters*, **45**, 5477 (1966); C. J. Collins and B. M. Benjamin, *J. Am. Chem. Soc.*, **89**, 1652 (1967).
(6) Dr. D. C. Kleinfelter and his co-workers have subjected the four 3-phenyl-2-norbornyl tosylates to exhaustive solvolytic examination. Although isotopic experiments will be required to rigidly exclude the possibility of phenyl migration, neither the kinetic data nor the products isolated on solvolysis require phenyl migration in their interpretation. See also D. C. Kleinfelter, E. S. Trent, J. E. Mallory, and T. E. Dye, *J. Am. Chem. Soc.*, **88**, 5350 (1966).
(7) R. Huisgen and Ch. Rüchardt, *Ann.*, **601**, 1 (1956).
(8) H. Stetter and P. Goebel, *Chem. Ber.*, **96**, 550 (1963).
(9) B. M. Benjamin, P. Wilder, Jr., and C. J. Collins, *J. Am. Chem. Soc.*, **83**, 3654 (1961); B. M. Benjamin and C. J. Collins, *ibid.*, **83**, 3662 (1961).