in deuteriochloroform (0.45 *M*) held at 55° above the P–C–H coalescense temperature for 7 hr experiences a maximum of 5% exchange. This is evidenced by mass spectroscopic examination of the crude reaction product.[14]

We are engaged in further study to elucidate the source of the temperature-dependent observations.

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centered at τ 2.60 (12 hydrogens), singlet at 6.47 (three hydrogens), broad singlet at 7.10 (one hydrogen), singlet at 7.62 (six hydrogens); mass spectrum, *m/e* 376 (M^+, 39%), 343 (100%), 319 (30%).

(13) Aldrich Chemical Co., Milwaukee, Wis.; mp 124–125° (lit.[7] 116–117°).

(14) Referee III has suggested that reversible protonation of the ylide at carbon by traces of acid could account for the variable temperature observations. Proton exchange between triphenylphosphoranes and their conjugate acids is, however, neither facile nor readily reversible under the conditions employed here.[15]

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Labeling Patterns in Glutamic Acid in *Nicotiana rustica* L. from Carbon-14 Dioxide[1]

Sir:

The hypothesis that the pyrrolidine ring of nicotine is formed from glutamic acid *via* a symmetrical intermediate[2] has been challenged by the labeling patterns obtained in the pyrrolidine ring of nicotine following exposure of *Nicotiana glutinosa* to $^{14}CO_2$. First,[3] the partial degradation of the pyrrolidine ring revealed labeling patterns difficult to reconcile with known glutamate biosynthetic pathways. The authors suggested[3] "a new glutamate biosynthesis" involving glyoxylate and acetate *via* malate synthetase and the tricarboxylic acid cycle. Subsequently,[4] more complete degradation of the pyrrolidine ring gave unsymmetrical labeling patterns from $^{14}CO_2$ which are contrary to the symmetrical intermediate hypothesis.[2] Recently[5] the complete degradation of the pyrrolidine ring of nicotine was reported and an earlier degradative error corrected. Again[5] $^{14}CO_2$ gave labeling patterns different from those produced from precursor feedings. It was suggested[5] that since $^{14}CO_2$ exposures represent normal growth conditions, the symmetrical labeling produced during precursor feedings might result from a minor or aberrant pathway.

In view of the paradoxical status of the current knowledge concerning the biosynthetic route and the lack of data concerning labeling in plant glutamate after exposure to $^{14}CO_2$, we have isolated and degraded the free glutamic and aspartic acids from leaves of *Nicotiana rustica* L. exposed to $^{14}CO_2$ in the light for 3 and 18 min.

For these experiments pots containing 3 or 4 plants, 1 month old, were prepared. A biosynthesis chamber of approximately 2 l. volume was fitted with a side arm to hold the radioisotope, and a Geiger–Muller tube connected to a count-rate meter was used to monitor the uptake of $^{14}CO_2$. The plants were exposed to 100 μcuries of ^{14}C-labeled carbon dioxide released from 0.326 mg of labeled sodium bicarbonate. This introduced 0.095 ml of $^{14}CO_2$, an increase of 0.005% above atmospheric level (0.03%). The plants were illuminated (3 or 18 min) with two Sylvania "Gro-Lux" lamps and one Westinghouse 150 V reflector spot. At the end of the biosynthetic period, the leaves (5–14 g) were frozen in liquid nitrogen within 1 min of the time of removal from the chamber. The free amino acids were extracted from the leaves by the method of Zelitch.[6] Aspartic and glutamic acids were isolated from the crude extracts by ion-exchange chromatography and degraded and assayed for ^{14}C as previously described;[7] 1.6 and 1.4 μmoles/g of leaf tissue of glutamate and aspartate, respectively, were obtained. The significant features of the experimental results (Table I) are: (a) the labeling of C-4 and C-5 was nearly equal, as was that of C-2 and C-3; (b) the labeling of C-4 + C-5 was always much larger than that of C-2 + C-3; (c) the labeling of C-1 was always higher than that of C-2 or C-3; (d) shortening the exposure time greatly increased the per cent labeling in C-4 and C-5 at the expense of the other three carbons of glutamate and increased the per cent of radioactivity incorporated into the carboxyl carbons of aspartate; (e) the ratio of the specific activity of glutamate to the specific activity of aspartate increased as the exposure time increased.

These data are not compatible, as shown in Table II, with the following routes of glutamate biosynthesis: (a) the glyoxylate–malate proposal by Alworth, *et al.*;[3] (b) the reductive reversal of the TCA cycle postulated to occur in *Chlorobium thiosulfatophilum*;[8] (c) the reversal of the pathway of glutamate fermentation in *Clostridium tetanomorphum*;[9,10] (d) the synthesis of glutamate in *Clostridium kluyveri*.[11]

The high carboxyl labeling in aspartate and the rather high ratio of activity of C-1 to C-2 + C-3 in glutamate, both decreasing somewhat with time, are readily explained by continuous $^{14}CO_2$ fixation into oxalacetate and a slower formation of symmetrically labeled pyruvate *via* the carbon reduction[12] cycle. However, the C-4 and C-5 data of glutamate are not easily explained by a combination of the carbon reduction[12] and TCA cycles. After a short period of $^{14}CO_2$ fixation *via* the carbon reduction cycle pyruvate would be expected to be labeled primarily in the carboxyl position.[13]

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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.

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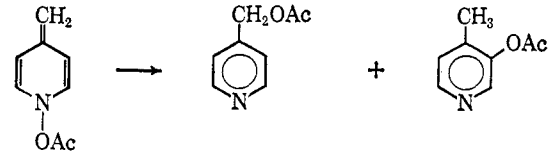
The Reaction of 4-Picoline N-Oxide with Acetic Anhydride. Trapping of the Cationic Intermediate

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.



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