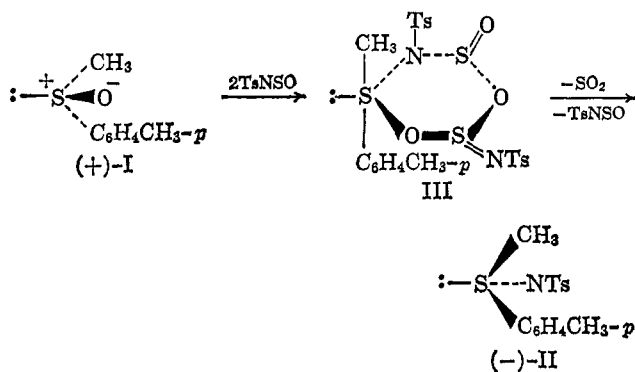tions.[9] Optically pure (+)-I became completely racemic when dissolved in polyphosphoric acid at 25° and recovered.

The relative configurations of (+)-I and (−)-II were determined from the opposite signs of the Cotton effects in their optical rotatory disperison curves (absolute ethanol, Cary Model 60 spectropolarimeter). The curves have very similar shapes except that the center of the Cotton effect in the spectrum of (−)-II lies at shorter wave length than that in the spectrum of (+)-I, possibly due to a greater positive charge on asymmetric sulfur in the sulfilimine. In the curve of (−)-II, principal maxima occur at 243 ([ϕ] −45,500°) and 225 mμ ([ϕ] +35,500°), and [ϕ] is 0° at 234 mμ and 213 mμ. In the curve of (+)-I, principal maxima occur at 256 ([ϕ] +21,000°) and 226 mμ ([ϕ] = −112,000°), and [ϕ] is 0° at 246 and 214 mμ.

The nature of the transition responsible for these Cotton effects is not certain, but a sulfur n → π* transition is thought to be involved with alkyl aryl sulfoxides,[3d] and the close similarity of the two curves indicates the transition of the sulfilimine to be of the same type. Since (+)-I possesses an R configuration,[3d] we may assign the S configuration to (−)-II and conclude that reactions A, B, and C have proceeded with inversion of configuration.[10]

In reaction B sulfur dioxide is formed, and thus the entering and leaving nucleophile must be coupled in a ring system, formally similar to the Wittig reaction.[11] This fact, together with the observed inversion, provides strong evidence for a mechanism involving a trigonal bipyramidal intermediate or transition state such as III in which the entering and leaving groups both occupy radial positions in the trigonal bipyramid,[12] whose formation and decay involve similar bond movements. In the formulation of this mechanism, two moles of N-sulfinylsulfonamide is implicated, a postulate subject to kinetic verification. Preliminary results indicate the reaction is second order in N-sulfinyl-p-toluenesulfonamide. The molecular weight (Signer) of N-sulfinyl-p-toluenesulfonamide in dichloromethane was found to be 223 (monomer mol. wt. 217). The same steric result is predicted whether the methyl, p-tolyl, or electron pair of III occupy the radial posi-

tion. Similar cyclic or noncyclic mechanisms can be formulated for reactions A and C in which the entering and leaving groups both occupy radial positions.

The classical inversion mechanism in which the leaving and entering groups occupy the axial positions of a trigonal bipyramid clearly cannot apply when these groups are in the same ring system (normal size). If the entering and leaving groups are identical in character but different from the other substituents, both entering and leaving groups are more likely to occupy the same kind of position on the trigonal bipyramid (both in radial or both in axial positions). Formation and decay of the trigonal bipyramid should be essentially the microscopic reverse of one another, and inversion of configuration must result. If the entering and leaving groups are sufficiently dissimilar, then one might occupy an axial and the other a radial position of the trigonal bipyramid, with retention of configuration as the stereochemical result. In this case, formation and decay of the trigonal bipyramid would not be the microscopic reverse of one another. Possibly many of the inversion mechanisms observed at second row elements[2,3] involve the incoming and leaving groups at radial positions of a trigonal bipyramid.

Structural analogies for III are found in the structures of sulfur tetrafluoride[13a] and the γ form of solid sulfur trioxide.[13b]

(13) (a) K. Kimura and S. H. Bauer, J. Chem. Phys., 39, 3172 (1963); (b) R. Westrik and C. H. MacGillavry, Rec. trav. chim., 60, 794 (1941).

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Biosynthesis of Nicotine in *Nicotiana glutinosa* from Carbon-14 Dioxide. Labeling Pattern in the Pyrrolidine Ring[1]

Sir:

Since the small fraction of nicotine activity at C-2′ found on short term exposure of *Nicotiana glutinosa* to $^{14}CO_2$[2] was not readily reconcilable with the generally accepted[3] symmetrical intermediate formed via the tricarboxylic acid cycle,[4] nicotine obtained from $^{14}CO_2$ biosynthesis was further degraded.[5] Within the limits of the degradation used, results were consistent with the symmetry postulate in that C-2′ and C-5′ were equally labeled, but a new pathway to glutamic acid from CO_2 was required. However, the symmetrical intermediate hypothesis could not be fully evaluated until a $^{14}CO_2$ biosynthesis was performed from which C-3′ or C-4′, or both, were determined along with C-2′ and C-5′. We now wish to report the results of such experiments.

(9) K. Mislow, T. Simmons, J. T. Melillo, and A. L. Ternay, Jr., J. Am. Chem. Soc., 86, 1452 (1964).

(10) Kresze and Wustrow (ref. 8) based their assignment of an inversion process for hydrolysis of their sulfilimine on the inadequate evidence that starting material and product possessed opposite signs of rotation in the visible region.

(11) Schulz and Kresze (ref. 6) noted this similarity. However, the Wittig reaction occurs with retention of configuration at phosphorus [W. E. McEwen, K. F. Kumli, A. Blade-Font, M. Zanger, and C. A. VanderWerf, J. Am. Chem. Soc., 86, 2378 (1964), and quoted references].

(12) P. C. Haake and F. H. Westheimer, ibid., 83, 1102 (1961).

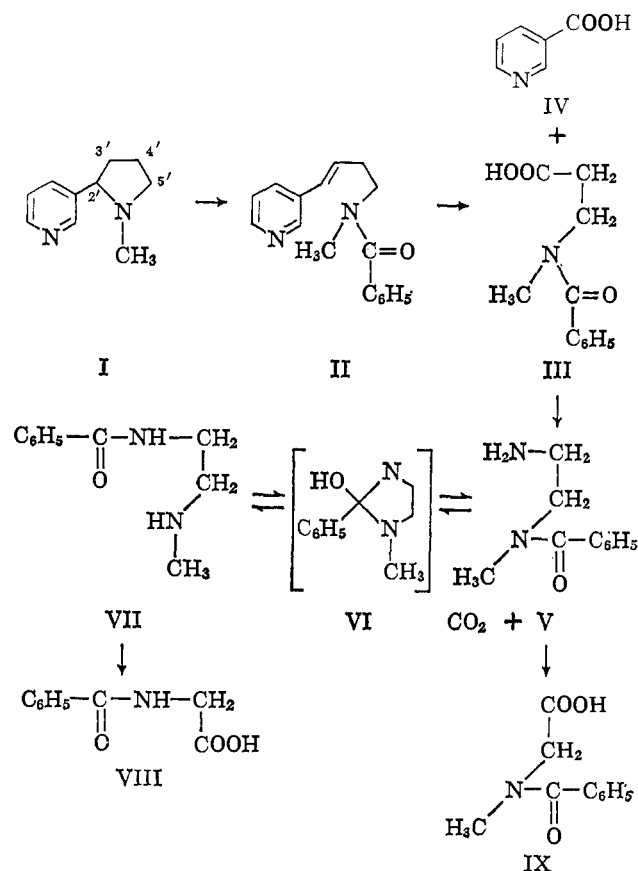(2) W. L. Alworth, R. C. DeSelms, and H. Rapoport, J. Am. Chem. Soc., 86, 1608 (1964).

(3) A. R. Battersby, Quart. Rev. (London), 15, 259 (1961); K. Mothes and H. R. Schutte, Angew. Chem., 75, 265 (1963); E. Ramstad and S. Agurell, Ann. Rev. Plant Physiol., 15, 143 (1964).

(4) E. Leete, J. Am. Chem. Soc., 78, 3520 (1956); 80, 2162 (1958); E. Leete, E. G. Gros, and T. J. Gilbertson, Tetrahedron Letters, 587 (1964); E. Leete, Science, 147, 1000 (1965).

(5) W. L. Alworth, A. A. Liebman, and H. Rapoport, J. Am. Chem. Soc., 86, 3375 (1964).

Existing methods of isolating C-2' and C-5' of nicotine are not adaptable to further unambiguous degradations for either C-3' or C-4'. Therefore, a new procedure was developed from N-benzoylmetanicotine (II)[6,7] which was oxidized with periodate–permanganate[8] to give N-benzoyl-N-methyl-β-alanine (III), m.p. 67–70°,[9] 83%, and nicotinic acid (IV), isolated as methyl nicotinate.[5] The N-benzoyl-N-methyl-β-alanine (III) was decarboxylated (Schmidt reaction) to give C-3'. N-Benzoyl-N-methyl-β-alanine-1-[14]C[10] (5.2 × 10^{5} d.p.m./mmole), when subjected to identical conditions, gave carbon dioxide with 100% of the original activity.[11] Thus the integrity of the carbon dioxide as arising entirely from the β-alanine carboxyl was established.

The amine fraction, characterized only by its infrared absorption (amide bands at 1650 and 1550 cm.⁻¹), was oxidized with permanganate. The resulting acids were separated on silica gel to give hippuric acid (VIII) as the major product and some N-benzoylsarcosine (IX). These results are clearly rationalized by

invoking the intermediate cyclol VI to explain migration of the benzoyl group. The hippuric acid then represents C-4' (methylene) and C-5' (carboxyl) of the pyrrolidine ring.[12]

A [14]CO₂ biosynthesis (XII in the series) was carried out,[5] and the results of degradation of the aerial nicotine are given in Table I. We have previously shown that C-2' and C-5' contain essentially equal amounts of ^{14}C; if the symmetrical intermediate hypothesis is valid, C-3' and C-4' also must contain equal amounts of [14]C. The value found for C-3' in this experiment was lower by a factor of 4 than the value predicted by the symmetrical intermediate hypothesis. Since the hippuric acid obtained from the aerial material did not represent sufficient mass for convenient characterization and activity analysis, an additional biosynthesis experiment (XIII) was performed (Table I) giving results confirmatory with XII and contrary to the symmetry hypothesis.

Table I. Percentage of Total Nicotine Activity Found in Various Degradation Products after [14]CO₂ Biosynthesis[a]

Sample	Specific activity,[b] d.p.m./mmole	Nicotine activity, %
Nicotine, aerial XII	27,652	100
Methyl nicotinate	22,672	82.0
Pyridine	21,973	79.5
C-2'	553	2.0
N-Benzoyl-N-methyl-β-alanine	4,654	16.8
C-3'	473	1.7
N-CH₃	722	2.6
C-4' + C-5' (by difference)		13.1[c]
Nicotine, root XIII	50,198	100
Methyl nicotinate	39,134	78.0
Pyridine	38,050	75.8
C-2'	1.398	2.8
N-Benzoyl-N-methyl-β-alanine	10,410	20.7
C-3'	1.538	3.1
N-CH₃	2,540	5.0
Hippuric acid (C-4' + C-5')	6,785	13.5

[a] Carried out as described previously.[5] [b] Determined by scintillation counting[5] with an error of ±2% of the average counting rate. [c] Average of 12.5, N-benzoyl-N-methyl-β-alanine − [(C-3') + (N-CH₃)], and 13.7, nicotine − [methyl nicotinate + (C-3') + (N-CH₃)].

A possible explanation of this contradiction could have been a species difference, since experiments from which the symmetrical intermediate hypothesis evolved were carried out with species other than *glutinosa*. We tested this possibility by hydroponically feeding ornithine-2-[14]C (50 μcuries, 26 mcuries/mmole) to four mature *N. glutinosa* plants.[2] A modification of the usual technique[13] was employed in that biosynthesis was allowed to proceed for only 6 hr. Uptake was rapid and essentially quantitative, and degradation of the nicotine showed equality of label at C-2' and C-5'. We can only conclude that the pathway from exogenous ornithine to the pyrrolidine ring of nicotine is the same in all *Nicotiana* species tested.

The most significant conclusion that may be drawn from these results is that the labeling pattern of the pyrrolidine ring formed from [14]CO₂ exposure is greatly different from that produced from precursor feedings. Since the [14]CO₂ exposures represent normal growth conditions, it becomes conceivable that the labeling

(6) A. Pinner, *Ber.*, 27, 1053 (1894).
(7) E. Späth and G. Bobenberger, *ibid.*, 77, 362 (1944).
(8) R. U. Lemieux and E. von Rudloff, *Can. J. Chem.*, 33, 1701 (1955).
(9) All isolated compounds were characterized by elemental and spectrophotometric analyses.
(10) J. P. Greenstein and M. Winitz, "Chemistry of Amino Acids," Vol. 3, John Wiley and Sons, Inc., New York, N. Y., 1961, p. 2756; J. L. O'Brien and C. Niemann, *J. Am. Chem. Soc.*, 79, 80 (1957).
(11) Footnote *b*, Table I.
(12) Degradation of hippuric acid *via* dimethyglycine [R. E. Bowman and H. H. Stroud, *J. Chem. Soc.*, 1342 (1950)] followed by lead tetra-

acetate oxidation yields the carboxyl as carbon dioxide and the methylene as formaldehyde. Application of this procedure to radioactive plant material will be reported in the future.

(13) E. Leete and K. J. Siegfried, *J. Am. Chem. Soc.*, 79, 4529 (1957).

pattern *via* a symmetrical intermediate produced from precursor feedings might result from a minor or aberrant pathway. For example, from the results of root XIII the maximum activity at C-4' that could result from a symmetrical intermediate (C-2', C-5' = 2.8%; C-3', C-4' = 3.1%) is only 29% of the total. At the other extreme, no biosynthesis may occur by such a path. Therefore, the labeling pattern produced from short-term $^{14}CO_2$ exposure must result predominantly from an unsymmetrical intermediate, and the question of pyrrolidine ring biosynthesis must now be considered anew.

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Long-Range Mercury-199–Proton Spin–Spin Coupling. I. Substituent Effects in Acyclic Systems

Sir:

Recent interest in organomercury chemistry and in the use of nuclear spin–spin coupling in conformational analysis prompts us to communicate in preliminary form our observations on $Hg^{199}-H^1$ coupling through 4σ bonds. Although spin–spin coupling of mercury with protons separated by two and three bonds (*gem*- and *vic*-coupling, respectively) has been reported by several workers, no mention seems to have been made of coupling through four bonds[1] except in cases involving a π bond (allylic coupling[2]). This in itself seems surprising, since 4σ coupling is readily observable in the oxymercuration products of many simple, acyclic olefins, and can be as large as 50 c.p.s.

Each of the compounds reported below was prepared from equimolar amounts of olefin and mercuric acetate in methanol, followed by neutralization with sodium carbonate and removal of the solvent *in vacuo*. The β-methoxyalkylmercuric acetates were converted to the corresponding chlorides, bromides, and iodides by shaking with excess aqueous potassium halide and subsequent recrystallization. The structures of the adducts followed unequivocally from the observed n.m.r. chemical shifts and relative magnitudes of the large *gem*- and *vic*-$Hg^{199}-H^1$ coupling constants[3]; methoxymercuration of all unsymmetrical olefins took place cleanly in the Markovnikov sense. In each case, assignment of the observed satellites of the high-field methyl protons arising from Hg^{199} 4σ coupling was confirmed by integration ($17 \pm 1\%$ of the total methyl group area) and by conversion to the corresponding iodomercuric derivative, in which the satellites vanished and the relative area of the remaining methyl resonance increased by the expected amount.[4] No coupling

(1) V. G. Klose [*Ann. Physik*, **10**, 392 (1963)] was unable to detect any Hg^{199} satellites about the methyl triplet in di-*n*-propylmercury.

(2) D. May, M. Emerson, and J. P. Oliver, *Inorg. Chem.*, **2**, 1261 (1963).

(3) P. R. Wells and W. Kitching, *Tetrahedron Letters*, 1531 (1963).

through 5σ bonds could be observed in compound **9**; the *t*-butyl group appeared as a sharp singlet with symmetrical relaxation envelope.

The values of $J_{4\sigma}$ ($Hg^{199}-H^1$) for the 2-methyl group in selected compounds are given in Table I.

Table I. Coupling Constants, $J_{4\sigma}$ ($Hg^{199}-CH_3$), for Methoxymercuration Products $CH_3CR_1(OCH_3)CR_2R_3HgCl^a$

Compd.	R_1	R_2	R_3	$J_{4\sigma}$, c.p.s.
1	H	H	H	0
2[b]	H	H	CH_3	7
3[b]	H	CH_3	H	0
4	CH_3	H	H	22
5	CH_3	CH_3	H	22
6	CH_3	CH_3	CH_3	0
7	C_2H_5	H	H	20
8	i-C_3H_7	H	H	17
9	t-C_4H_9	H	H	27
10	p-$C_6H_4OCH_3$	H	H	20
11	CH_2Cl	H	H	30
12	CH_2Br	H	H	33
13	$CH_2C_6H_5$	H	H	39
14	p-$CH_2C_6H_4OCH_3$	H	H	44
15	$2,4,6$-$CH_2C_6H_2(CH_3)_3$	H	H	48

^{a} N.m.r. spectra were recorded at 60 Mc. as 25% solutions in carbon tetrachloride with tetramethylsilane and benzene (2%) as internal references. The estimated uncertainty in $J_{4\sigma}$ values is $\pm 3\%$. ^{b} Compound **2**, prepared from *trans*-2-butene, should be the *erythro* isomer; compound **3**, from *cis*-2-butene, the *threo* [*cf.* J. Chatt, *Chem. Rev.*, **48**, 7 (1951)].

Correlation of the observed magnitudes of $J_{4\sigma}$ ($Hg^{199}-CH_3$) with expected steric effects of substituents reveals that the mechanism of this coupling is indeed complex.[5] It is tempting to speculate on the relative importance of through-space and through-bond interactions[5] and steric and electronic effects on C–Hg and C–C bond hybridizations and angles, and particularly on the effects of delocalization of unshared electrons or C–Hg and C–C bonding pairs into the available mercury orbitals. (Compare, for example, $J_{4\sigma}$ for compounds **8–15** with the positive charge-stabilizing abilities of the groups R_1.) A detailed discussion is deferred until a forthcoming paper, to include studies in progress on cyclic compounds of more precisely known geometry. However, it can easily be seen by examination of selected pairs of compounds in Table I that steric effects are important (*e.g.*, **2** *vs.* **3**), as well as electronic effects (*e.g.*, **7** *vs.* **12**; **13** *vs.* **14**). It is equally clear that empirical rules for the prediction of proton–proton 4σ coupling (the "M" arrangement[5]) and long-range proton–fluorine coupling (the "converging vector rule"[5]) fail badly for $Hg^{199}-H^1$ 4σ coupling when bulky substituents are present, though the former meets with some success in compounds

(4) M. D. Rausch and J. R. Van Wazer [*Inorg. Chem.*, **3**, 761 (1964)] and others have suggested that the absence of *gem*- and *vic*-Hg^{199}-H^1 coupling in alkylmercuric iodides is due to rapid chemical exchange. In support of this assumption, we have observed broadened Hg^{199} satellites in β-methoxyalkylmercuric iodides which possess certain substituents capable of substantially reducing the rate of exchange (*e.g.*, compound **15**). This phenomenon will be discussed in a forthcoming communication.

(5) For a recent discussion of long-range spin–spin coupling mechanisms, see N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, Calif., 1964, Chapter 5.