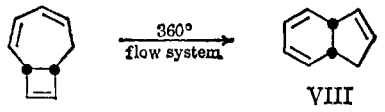
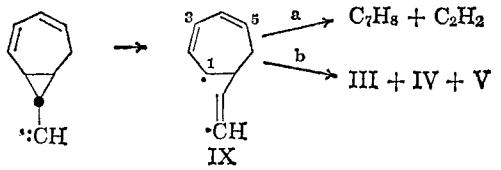
to take place thermally only with difficulty.[14] This path was tested and ruled out as a source of IV and V. Thermal rearrangement of III in a flow system at 360° gave only *cis*-8,9-dihydroindene (VIII) and a minor product of as yet unknown structure. Under these conditions IV is stable and thus does not arise from III. Authentic VIII was synthesized by the method of Vogel,[15] and an infrared spectrum of *trans*-8,9-dihydroindene[16] was kindly provided by Dr. Wolfram Grimme.[5]



III VIII

As III is now eliminated as a source of IV and V, it seems likely that the carbene formed in the decomposition of IIf is involved. The intermediate IX, related to the carbene by the breaking of a carbon–carbon bond, appears capable of yielding the observed products. Path a proceeds by further carbon–carbon bond cleavage to give acetylene and the observed tropilidene. Abundant analogy for this path exists.[17] Path b consists of closure at the 1, 5, and 3 positions to give the three observed products III, IV, and V.



IX

A similar scheme would suffice to explain our earlier[3] observations. In this system the product of closure at the 1 position, bicyclo[6.2.0]deca-2,4,6,9-tetraene (X), is not stable at *ca.* 120° but rearranges to the observed mixture of *cis*- and *trans*-9,10-dihydronaphthalenes in which the *trans* isomer predominates. This rearrangement now has analogy in the conversion of III to VIII. The product of closure at the 5 and/or 3 positions, bicyclo[4.2.2]deca-2,4,7,9-tetraene, is stable and isolable. The preference for the formation of *trans*-9,10-dihydronaphthalene over the *cis* form permits the tentative identification of the cyclodecapentaene intermediate in the transformation from X. The formulations of Woodward and Hoffmann[18] require that *trans*-9,10-dihydronaphthalene be *thermally* related to the cyclodecapentaene with one *trans* double bond and not to the all-*cis* or di-*trans* isomer (XI and XII). In consonance with this is the finding of van Tamelen and Burkoth[19] that *trans*-9,10-dihydronaphthalene is *photochemically* converted to a cyclodecapentaene (XI and/or XII) which closes *thermally* to *cis*-9,10-dihydronaphthalene.

(14) R. B. Woodward and R. Hoffmann, *J. Am. Chem. Soc.*, **87**, 2511 (1965).
(15) E. Vogel, W. Wiedemann, H. Kiefer, and W. F. Harrison, *Tetrahedron Letters*, 673 (1963).
(16) E. Vogel, W. Grimme, and E. Dinné, *ibid.*, 391 (1965).
(17) P. B. Shevlin and A. P. Wolf, *J. Am. Chem. Soc.*, **88**, 4735 (1966), and references therein.
(18) R. B. Woodward and R. Hoffmann, *ibid.*, **87**, 395 (1965).
(19) E. E. van Tamelen and T. L. Burkoth, *ibid.*, **89**, 151 (1967).

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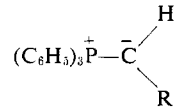
Temperature Dependence of the P–C–H Nuclear Magnetic Resonance Spectra of Methylenetriphenylphosphoranes

Sir:

The hindered internal rotation of alkoxycarbonylalkylidenetriphenylphosphoranes can be investigated by variable-temperature nmr spectroscopy.[1] During the course of such a study it was discovered that phosphorane **1** exhibits a second temperature-dependent feature apparently unrelated to the rotation process.



1a, R = H **c**, R = $CO(CH_2)_2C_6H_5$ **e**, R = CO_2CH_3
b, R = CN **d**, R = $COCH_3$ **f**, R = CHO

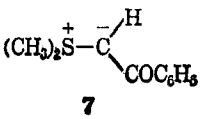
In the absence of further spin–spin interaction the hydrogen α to phosphorus is observed at low temperatures as a well-separated doublet reflecting the expected P–C–H spin–spin coupling. Upon warming the phosphorane solution, however, this doublet gradually coalesces to an unshifted sharp singlet.[2] Three compound classes are to be differentiated.

The simplest case is represented by ylide **1a**,[3] R = H, the temperature-dependent nmr of which is depicted in Figure 1a ($J_{PCH}^{-28°}$ = 7.0 cps[4]). The cyano derivative[5] **1b** exhibits identical proton nmr behavior at τ 8.38 ($J_{PCH}^{-60°}$ = 7.5 cps).

The second variation is displayed by phosphorane **1c**,[6] R = $CO(CH_2)_2C_6H_5$. In this case rotation about the C–R bond is in principle possible, but is not observed. The invariant multiplicity and resonance position of the α-keto ethyl group at τ 7.15 throughout the relevant temperature range establish this point. Figure 1b is illustrative. Nonetheless, the methine hydrogen low-temperature doublet (τ 6.29, $J_{PCH}^{-19°}$ = 25.0 cps) coalesces to a clean unshifted singlet at raised temperatures. Phosphorane **1d**,[6] R = $COCH_3$, behaves likewise ($\delta_H = \tau$ 6.32, $J_{PCH}^{-28°}$ = 24.5 cps).

The final and most complex substitution type is represented by ylide **1e**,[7] R = CO_2CH_3. Hindered internal rotation about the C–R bond is realized, and the *cis*–*trans* conformational isomers **2** and **3** can be distinguished at low temperatures.[1,8] Accordingly, the

(1) H. J. Bestmann, G. Joachim, I. Lengyel, S. F. M. Oth, J. Mereny, and J. Weitkamp, *Tetrahedron Letters*, 3335 (1966). The complete results and structural implications of hindered rotation for stable alkylidenephosphoranes will be reported elsewhere.
(2) All spectra were obtained in deuteriochloroform with TMS as an internal standard on a Japan Electron Optics Laboratory C-60 nmr spectrometer.
(3) G. Wittig and U. Schollkopf, *Chem. Ber.*, **87**, 1318 (1954). We are grateful to H. Liberda, Institut für Organische Chemie, Universitat Erlangen-Nurnberg, for providing a sample of this salt-free ylide in the solid state.
(4) Relative to TMS in chlorobenzene.
(5) Mp 192–193°; S. Trippet and D. M. Walker, *J. Chem. Soc.*, 3874 (1959), report mp 195–196°.
(6) (a) R = $COCH_3$, mp 200–202° (lit.[6b] 200–202°); R = $CO(CH_2)_2C_6H_5$, mp 148–150° (lit.[6b] 148–150°; (b) H. J. Bestmann and B. Arnason, *Chem. Ber.*, **95**, 1513 (1962).
(7) Mp 169.0–169.5°; O. Isler, H. Gutmann, M. Montavon, R. Ruegg, G. Ryser, and P. Zeller, *Helv. Chim. Acta*, **40**, 1242 (1957), report mp 163°.
(8) Ratts and Yao[9] observed a broadened methine proton at room temperature for the sulfonium ylide 7 and the phosphorane **8**. Since



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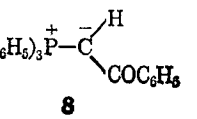

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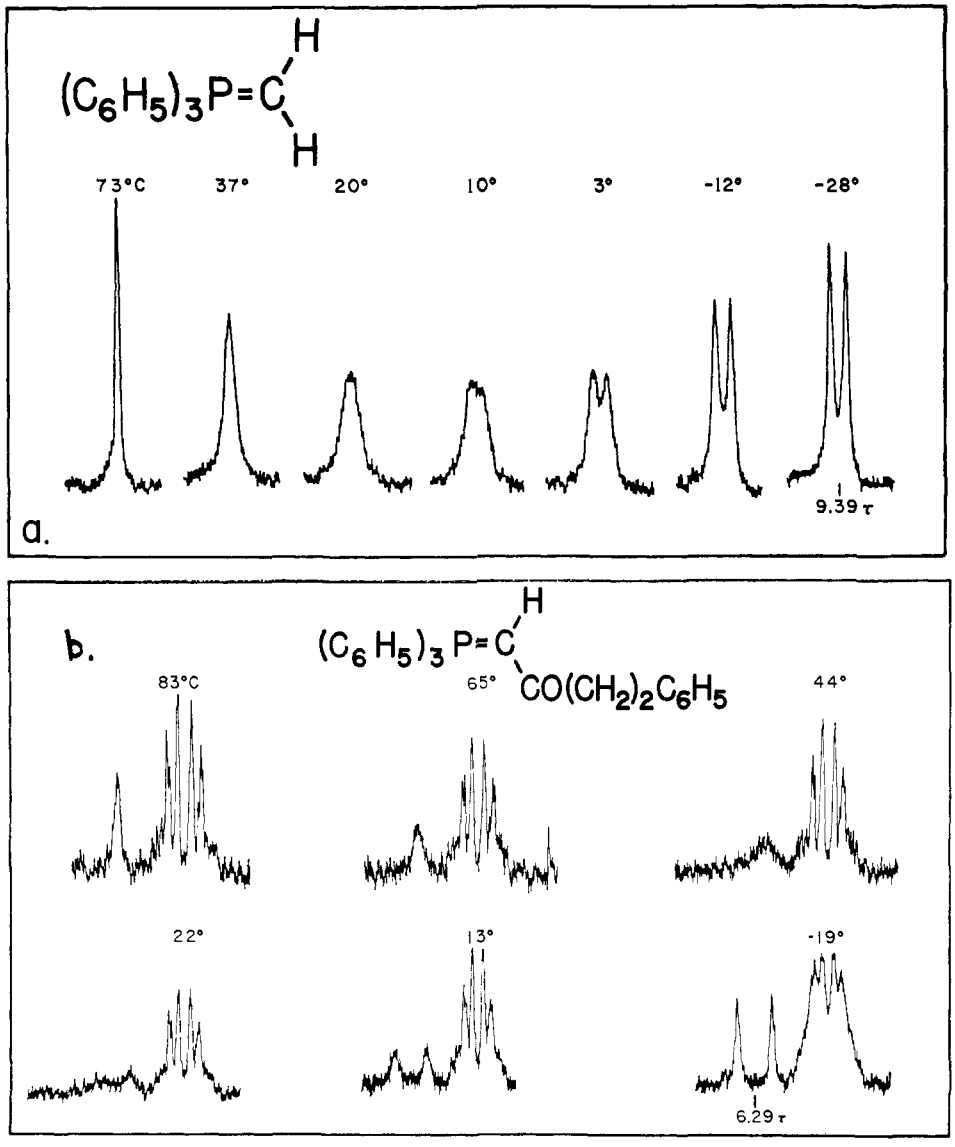
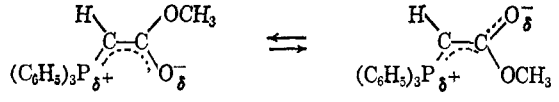


Figure 1. Variable-temperature nmr spectra of (a) methylenetriphenylphosphorane and (b) 2-phenylethylcarbonylmethylenetriphenylphosphorane; the phenyl region is omitted.

methine proton appears in the nmr at −28° as a pair of overlapping symmetrical doublets of unequal intensity centered at τ 6.95 and 7.14 ($J_{PCH}^{-28°}$ = 22.0



2 3

and 20.5 cps, respectively). The four bands merge into a singlet above 30°.[8] Phosphorane **1f**, R = CHO, shows similar activity, but the spectrum is complicated by supplemental P–βH and H–H coupling.[11]

[32]S (natural abundance 99.26%[10]) possesses no nuclear magnetic moment, the methine broadening found for **7** must arise from hindered internal rotation, as they suggest. However, the detection of a broadened hydrogen for ylide **8** is clearly the result of the phenomenon with which this communication is concerned. Compound **8** exhibits the same temperature-dependent nmr effect as the related acyl phosphoranes **1c** and **1d**,[11] but like them presumably does not undergo hindered internal rotation.

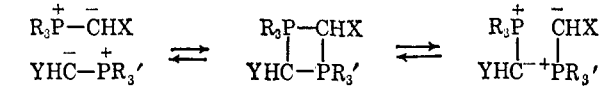
(9) K. W. Ratts and A. N. Yao, *J. Org. Chem.*, 31, 1185 (1966).

(10) J. W. Emsley, J. Feeney, and L. H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," 1st ed, Pergamon Press, 1966.

(11) H. J. Bestmann and J. P. Snyder, unpublished results.

Preliminary studies indicate that the phenomenon observed is independent of concentration but strongly influenced by a change of solvent from nondeuterated media to deuteriobenzene. Phosphorane **1a**, R = H, and phenylmethylenetriphenylphosphorane[3] in the latter solvent possess P–C–H doublets with $J_{PCH}^{20°}$ of 7.5 and 18.5 cps, respectively. In benzene, chlorobenzene, or dioxane the α-hydrogen appears as a broadened singlet for both species at the same temperature.

Of the several mechanistic possibilities, a rapidly reversible dimerization process involving the ylide pairs **4** and **6** equilibrating through diphosphacyclobutane **5** as a transition state or short-lived intermediate can be ruled out. An equimolar mixture of (p-CH_3-



4 5 6

$C_6H_4)_3PCHCO_2CH_3$[12] and $(C_6H_5)_3PCHCO_2CH_2CH_3$[13]

(12) Prepared by the method of Isler, *et al.*,[7] mp 141–142.5° (uncor); infrared λ_{max}^{KBr} 6.16 (C=O), 6.96, and 9.05 μ (C–P); nmr, multiplet

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]

(15) H. J. Bestmann, *Chem. Ber.*, **95**, 58 (1962).

(16) North Atlantic Treaty Organization Postdoctoral Fellow. Author to whom inquiries should be addressed: Yeshiva University, Belfer Graduate School of Science, New York, N. Y.

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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]

(1) Supported in part by Research Grant GM-06765 from the National Institutes of Health.

(2) 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).

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

(4) A. A. Liebman, R. Morsingh, and H. Rapoport, *ibid.*, **87**, 4399 (1965).

(5) A. A. Liebman, B. P. Mundy, and H. Rapoport, *ibid.*, **89**, 664 (1967).

(6) I. Zelitch, *J. Biol. Chem.*, **240**, 1869 (1965).

(7) R. M. O'Neal and R. E. Koeppe, *J. Neurochem.*, 13, 835 (1966).

(8) M. C. W. Evans, B. B. Buchanan, and D F. Arnon, *Proc. Natl. Acad. Sci., U. S.*, **55**, 928 (1966).

(9) A. Munch-Petersen and H. A. Barker, *J. Biol. Chem.*, 230, 649 (1958).

(10) H. A. Barker, R. D. Smyth, E. J. Wawszkiewicz, M. N. Lee, and R. M. Wilson, *Arch. Biochem. Biophys.*, **78**, 468 (1958).

(11) N. Tomlinson, *J. Biol. Chem.*, **209**, 605 (1954).

(12) J. A. Bassham and M. Calvin, "The Path of Carbon in Photosynthesis," Prentice-Hall, Inc., New York, N. Y., 1957, p 39.

(13) E. A. Havir and M. Gibbs, *J. Biol. Chem.*, **238**, 3183 (1963).