because, despite the use of rubber gloves and aprons, the author developed a high sensitivity to 2-bromo-4-chloro-6-methylpyrimidine, resulting in severe contact dermatitis on the hands; but, as a result of the study, it is felt that the diazotization and coupling reactions are general for nontautomeric pyrimidines, it being necessary only to vary the conditions somewhat for the individual compounds.

The large excess of inorganic reagents and long reaction times required in the reverse addition diazotization reactions are evidently due to the fact that the respective amines are only very slightly soluble in water and the contact between the reactants is poor. However, no other solvent system could be found which would adequately dissolve all of the reagents and reactants. In order to maintain a high concentration of nitrous acid for a long reaction time, the hydrobromic acid was introduced at a constant, very slow, drop rate by a pressure-controlled dropping capillary, and the temperature of the reaction vessel was maintained constant with a Wilkens Anderson Low Temp bath.

The infrared spectra of all the pyrimidines, determined on a Perkin-Elmer Model 221 instrument with sodium chloride optics, were consistent with proposed structures. In the visible region, the 4,4′,6,6′-tetramethyl-2,2′-bipyrimidine gave a deep red color upon warming with a dilute solution of Cu(I), but formed no colored complex with Fe(II) or Fe(III). The ultraviolet spectra were determined on a Cary Model 10–11 spectrophotometer in distilled water using a distilled water reference.

Experimental[5]

Preparation of 2-Bromo-4,6-dimethylpyrimidine by Reverse Addition Diazotization.—A 24-g. quantity (0.214 mole) of 2-amino-4,6-dimethylpyrimidine was diazotized by the very slow addition of 114 ml. (1 mole) of concentrated hydrobromic acid to a solution containing the pyrimidine, 300 ml. of water, 300 g. of sodium bromide, and 70 g. (1 mole) of sodium nitrite at −3.2°. The addition of the acid took 24 hr. to completion.

The solution was then cleared of nitrogen oxides by an air stream, made strongly alkaline with cold 30% sodium hydroxide, and filtered. The precipitate and filtrate were each extracted with two 200-ml. portions of carbon tetrachloride and the extracts combined and evaporated to dryness. The residue was taken up in hot petroleum ether (b.p. 90–100°), cooled to 0°, and filtered. The filtrate was evaporated to give 9.4 g. (0.05 mole, 24%) of crystalline 2-bromo-4,6-dimethylpyrimidine. The product was further purified by sublimation *in vacuo* to give white crystals, m.p. 69.5–71.5°, a molecular weight of 183 (theory 187) as determined in chloroform by a Mechrolab osmometer, Model 301 A, and λ_{max} 256 and 217 mμ with log a_m = 3.62 and 3.83, respectively.

Anal. Calcd. for $C_6H_7BrN_2$: C, 38.5; H, 3.75; Br, 42.7; N, 15.0. Found: C, 38.5; H, 3.8; Br, 42.4; N, 15.2.

Scale-up of this reaction failed owing to very marked increase in foaming in the reaction mixture. The 4,6-dimethyl-2-pyrimidinol, also a product of the diazotization reaction, was isolated by adjusting the chloroform-extracted aqueous solution to pH 4.5, evaporating to dryness *in vacuo*, as with 2-pyrimidinol,[3] and extracting with hot ethyl acetate.

Preparation of 2-Bromo-4-chloro-6-methylpyrimidine by Reverse Addition Diazotization.—In like manner 2-amino-4-chloro-6-methylpyrimidine was diazotized by adding 228 ml. (2 moles) of concentrated hydrobromic acid to 40 g. (0.279 mole) of the pyrimidine mixed with 325 ml. of water, 300 g. of sodium bromide, and 140 g. (2 moles) of sodium nitrite at −3.2°. The addition of the acid took 44 hr. to completion.

After adjusting the solution to pH 7, the product was steam distilled, then crystallized from minimum petroleum ether (b.p. 60–70°) and vacuum dried to give 9.5 g. (0.049 mole, 16%) of crystalline 2-bromo-4-chloro-6-methylpyrimidine, m.p. 33–34°, molecular weight in chloroform of 210 (theory 208), and λ_{max} 260 and 217 mμ with log a_m = 3.68 and 3.85, respectively.

Anal. Calcd. for $C_5H_4BrClN_2$: C, 28.9; H, 1.9; Br, 38.1; Cl, 17.2; N, 13.5. Found: C, 28.9; H, 1.7; Br, 38.1; Cl, 17.2; N, 13.6.

The 4-chloro-6-methyl-2-pyrimidinol, also a product of the diazotization, floats to the top of the warm crude reaction mixture before steam distillation and can be skimmed off.

Preparation of 4,4′,6,6′-Tetramethyl-2,2′-bipyrimidine.—The experimental details were similar to those for 2,2′-bipyrimidine,[3] except that no nitrogen was used. A 20-g. (0.3 g.-atom) portion of activated[6] Natural Copper Fine 44-F was added all at once to 10 g. (0.053 mole) of 2-bromo-4,6-dimethylpyrimidine at reflux in 60 ml. of (calcium hydride-distilled) dimethylformamide. After 8 hr. of stirring at reflux, 5 g. of additional activated copper was added. After 24 hr. the mixture was cooled to room temperature, suction filtered, and the residue washed with a little water. The copper-product residue was then twice extracted for 2 min., respectively, with 200 ml. of concentrated ammonium hydroxide saturated with potassium cyanide. Each extraction was separated by suction filtration, and the combined filtrates then extracted with two 500-ml. portions of chloroform. The remaining copper residue was extracted once with chloroform.

The combined chloroform solutions were evaporated to dryness. The tarry residue was dissolved in 200 ml. of hot ethyl acetate, decolorized with Darco, filtered, and evaporated to yield 2.3 g. (0.01 mole, 26%) of tan, amorphous 4,4′,6,6′-tetramethyl-2,2′-bipyrimidine. Sublimation did not give a pure product, but constant m.p. 131–132° was obtained by crystallizing the sublimed material from an ethyl acetate–petroleum ether solution. [The pyrimidine was dissolved in minimum hot ethyl acetate, hot petroleum ether (b.p. 90–100°) added until the solution was cloudy, and the mixture cooled to 0° and filtered.] The molecular weight, determined in chloroform as above, was 213 (theory 214) with λ_{max} at 248 mμ and log a_m = 4.17.

Anal. Calcd. for $C_{12}H_{14}N_4$: C, 67.4; H, 6.6; N, 26.2. Found: C, 65.1; H, 6.9; N, 26.1.

Attempted Synthesis of 4,4′-Dichloro-6,6′-dimethyl-2,2′-bipyrimidine.—It was attempted to prepare this compound by a procedure exactly analogous to the synthesis of 4,4′,6,6′-tetramethyl-2,2′-bipyrimidine, and a reaction definitely took place on two different attempts. However, no one product was isolated, and the author had to give up the project due to his high sensitivity to the starting material. It is felt, however, that 4,4′-dichloro-6,6′-dimethyl-2,2′-bipyrimidine could be obtained, since the chlorine atoms should not interfere.[3]

(5) All melting points are uncorrected.

(6) E. C. Kleiderer and R. Adams, *J. Am. Chem. Soc.*, **55**, 4219 (1933).

Deuterium Exchange in the Pyridoxal-Leucine System[1]

G. A. JUNK AND H. J. SVEC

Institute for Atomic Research and Department of Chemistry, Iowa State University, Ames, Iowa

April 15, 1963

Deuterium exchange from a D_2O solvent is frequently used to confirm reaction mechanisms. The positions at which exchanges occur must be determined, and the total amount of deuterium incorporated at each position should be measured. These values then can be used to support or disprove a particular mechanism. A convenient procedure for accurate and direct determinations of both the location and extent of deuterium incorporated in a molecule uses mass spectral fragmentation data. A detailed discussion of the use

(1) Contribution no. 1297. Work was performed in the Ames Laboratory of the U. S. Atomic Energy Commission.

of these data is given in a recent text by Biemann.[2] This approach was applied to the assay of leucine which had been isolated from the equilibrated reaction mixture of leucine and pyridoxal in D_2O.

$$\text{Leucine} + \text{pyridoxal} \underset{D_2O}{\overset{\text{alum catalyst}}{\rightleftharpoons}} \alpha\text{-keto acid} + \text{pyridoxamine} \quad (1)$$

Extent and specificity of labeling of the leucine was calculated from the mass spectra of pure leucine and that of the isolated product leucine. Pertinent portions of the spectra which were used for these calculations are tabulated in Table I. Discussions of the mass shifts and the information gained from a consideration of these shifts in several mass ranges are given below.

TABLE I

MASS SPECTRA OF LEUCINE AND LEUCINE-d_n

m/e	leu	leu-d_3	m/e	leu	leu-d_3
39[a]	5.6	3.3	84[b]		
40	0.8	2.1	85		
41	12.3	7.5	86	99.1	0.5
42	6.2	3.4	87		1.4
43	19.0	16.8	88	0.7	5.8
44	51.9	4.9	89	0.2	92.3
45	1.6	6.8	90		
46	3.0	7.9			
47		47.5			
			131[d]	100	
			132		
73[c]	1.0		133		5
74	86.0	3.0	134		95
75	13.0	85.0			
76		12.0			
77					

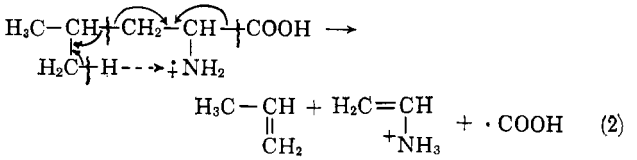
[a] Σ_{39-47} = 31.2%; [b] Σ_{84-90} = 25.0%; [c] Σ_{73-77} = 9.6%; [d] $\Sigma_{131-134}$ = 0.3% of the total ion yield.

Masses 131–134.—The parent ion peak from pure leucine appears at m/e = 131. This peak shifts almost entirely to m/e = 134 in the spectrum of deuterated leucine. One concludes that the sample is apparently 95% leucine-d_3. However, there is a high degree of uncertainty in this conclusion because of the low intensity of the parent ion peak (see footnote *d* of Table I).

Masses 84–90.—The amine fragment $[(CH_3)_2CH\text{-}\overset{+}{C}H_2CH{=}\overset{+}{N}H_2]$ at m/e = 86 is formed by a process whose activation energy is low[3] and no intramolecular mixing of protium and deuterium is expected prior to the bond rupture of the molecule–ion.[4] In addition, large peaks due to the loss of one or more protium atoms from the fragment are not present to complicate the calculations or the interpretation of the observed mass shifts. From the data recorded in this mass range (see Table I), the extent of trideuteration is calculated to be 92%, dideuteration 6%, and monodeuteration 1%. Less than 1% of the original leucine is undeuterated. No information about the position of the deuterium atoms is obtained from the consideration of these fragments. However, Junk and Svec[3] have confirmed that the amino hydrogens are readily exchanged in water at room temperature. Since the sample was recrystallized from water, the only possible distribution of the deuterium atoms is on carbon atoms.

Masses 73–77.—The decompositions which result in the peaks at m/e = 74 $[CH(NH_2)COOH]^+$ and m/e = 75 $[CH_2(NH_2)COOH]^+$ from leucine have been established.[3] These peaks shift to m/e = 75 and 76 in the mass spectrum of leucine-d_n. Thus, most of the α-protium has been replaced with deuterium. The remaining m/e = 74 peak in the mass spectrum of leucine-d_n is a measure of the amount of protium left on the α-carbon atom. This peak and the m/e = 75 are used to calculate the extent of labeling on the α-carbon position. The calculated result is 97% α-deuterated. It has already been shown that the sample is 92% trideuterated. A reasonable assumption is that the trideuterated leucine is mono-α and di-β since one would expect the sample to be leucine-d_2 if the γ-position were activated and leucine-d_7 if the δ-positions were activated.[5] The validity of this assumed distribution (mono-α and di-β) was established by the observation of the mass shifts in the 39–47 mass range.

Masses 39–47.—The fragment causing the peak at m/e = 44 is formed by the process shown in eq. 2.



(2)

This peak from leucine contributes 51.9% of the total peak intensities observed in the 39–47 mass range. The peak at m/e = 47 from leucine-d_n contributes 47.5% of the total peak intensities observed in the same mass range. If the isotope effect[6] is assumed to be negligible, the ratio of these two peaks can be used to calculate the extent of trideuteration. The calculated value is 91.5% trideuteration which agrees with the value calculated from data in 84–90 mass range.

The observed m/e = 47, 46, 45, and 44 peaks cannot be used directly for the calculation of the extent of tri-, di-, mono-, and undeuterated leucine. Suitable corrections must be applied to the observed data because of the presence of interference peaks. These corrections were applied to the observed data using the method of successive approximations. The corrected peaks were then used to calculate the per cents listed in column 2 of Table II. These are compared with the

TABLE II

COMPOSITION OF LEUCINE-d_3

No. of D	39-47	84-90	$\frac{44\ (\text{leu})}{47\ (\text{leu-}d_3)}$	Stat. 97% D
d_0	0.4	0.5		
d_1	4.4	1.4		0.3
d_2	5.6	5.8		8.5
d_3	89.6	92.3	91.5	91.3

(2) K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, pp. 204–250 and references cited there.

(3) G. A. Junk and H. J. Svec, *J. Am. Chem. Soc.*, **85**, 839 (1963).

(4) W. H. McFadden and M. Lounsbury, *Can. J. Chem.*, **40**, 1965 (1962).

(5) It should be noted that the absence of a peak at m/e = 77 in the mass spectrum of leucine-d_n is experimental evidence that the γ-position is not deuterated. The energetically favorable process for formation of the rearranged fragment is migration of the protium (or deuterium if present) from the γ-position to the carboxyl oxygen. The mass of the rearranged fragment would be 77 if deuterium is on the γ-position and 76 if protium is on this position (see Table I).

(6) D. P. Stevenson and C. D. Wagner, *J. Chem. Phys.*, **19**, 11 (1951).

results obtained from a consideration of the mass ranges 84:90 (column 3) and the 44:47 ratio (column 4). Discrepancies between columns 2 and 3 are attributed to errors inherent in the approximations used to calculate the values in column 2 and to the isotope effect.[6] Possibly some mixing of protium and deuterium occurs prior to decomposition of the molecule–ion. Such mixing also would affect the results.

The expected distribution of d_0, d_1, d_2, and d_3 in the $(-C=C-\overset{|}{N}-)^+$ fragment was calculated assuming that the two β-positions had the same deuterium content as that calculated for the α-position (that is, 97%). These calculated values are listed in column 5 of Table II. Rather close agreement exists between these values and those derived from experimental data in the mass ranges 39–47 and 84–90.

Additional critical work would be necessary to unequivocally arrive at a complete interpretation of the observed isotopic exchange. However, the results obtained in this present work confirm that the pyridoxal–metal–amino acid reaction mechanism[7,8] proceeds through formation of the generally accepted Schiff base intermediate. The α-position protiums are replaced with deuteriums through tautomerization of the Schiff base. The β activation occurs by tautomerization of the Schiff base and/or the α-keto acid.

In conclusion, the reaction of amino acids with pyridoxal appears to be generally useful for the selective labeling of amino acids in both the α- and β-positions. Samples so labeled can be assayed rapidly, accurately, and directly using a mass spectral approach similar to that described in this report for leucine-d_3. Less than 0.1 mg. of sample is consumed per assay.

Experimental

Leucine.—Calbiochem grade A leucine which was vacuum sublimed at 170° was used.

Leucine-d_3.—A 6.6-g. sample of leucine and 500 mg. of pyridoxal hydrochloride (Sigma Chemical) were dissolved in 200 ml. of 99.5% D_2O. Ammonium or potassium alum (250 mg.) was added to catalyze the reaction. The mixture was then refluxed for ~24 hr. The deuterated leucine was isolated from the cooled reaction mixture and recrystallized ten times from hot water. The product was further purified by vacuum sublimation at 170°. The yield of purified product was 3.7 g.

Mass Spectra.—A General Electric analytical mass spectrometer, which had been converted for use of the crucible source technique, was used to establish the mass spectra. Instrumental conditions were ion chamber temp., ~105°; electron energy, 70 v.; trap current, 10 μa.; ion accelerating voltage, 2000 v.; and magnetic scanning.

If it is not possible to use the crucible source technique, the deuterium assay of the isolated amino acid can conveniently be accomplished by conversion of the amino acid to a volatile derivative and by subsequent assay using a conventional external heated inlet system.[9,10]

Acknowledgment.—The authors are indebted to L. Levine who prepared the deuterated leucine and to Dr. D. Metzler for helpful discussions.

(7) J. P. Greenstein and M. Winitz, "Chemistry of Amino Acids," John Wiley and Sons, Inc., New York, N. Y., 1961, pp. 593–610.

(8) A. E. Braunstein, "The Enzymes," Vol. 2, Academic Press, New York, N. Y., 1970, pp. 137–148.

(9) C.-O. Andersson, R. Ryhage, and E. Stenhagen, *Arkiv Kemi*, **19**, 417 (1962).

(10) K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, pp. 260–296 and references cited there.

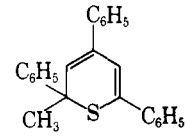
The Nuclear Magnetic Resonance Spectra of Some Thiopyran Derivatives

THYAGARAJA PARASARAN[1] AND CHARLES C. PRICE

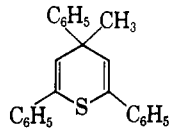
Department of Chemistry, University of Pennsylvania, Philadelphia 4, Pennsylvania

Received July 12, 1963

We[2] have reported earlier on the preparation of isomeric 2- and 4-methyl-2,4,6-triphenylthiopyrans by the coupling of 2,4,6-triphenylthiopyrylium ion with methylmagnesium bromide. The assignment of structure was based on a chemical degradation by desulfurization.



I, m.p. 119°



II, m.p. 97°

The structures have now been confirmed by their n.m.r. spectra in carbon tetrachloride with shifts calibrated by audiofrequency sidebands. Compound II shows single sharp peaks at 8.40 and 4.31, and a broad structured band centered at 2.88 τ in the proper ratio of 3:2:15. Compound I shows single sharp peaks at 8.14, 4.17, and 3.23, and broad structured bands centered at 2.66 and 2.38 τ in the ratio of 3:1:1:10:5.

The n.m.r. shifts in I, compared to II, as well as the ultraviolet absorption at much longer wave lengths for I (λ_{max} 257, 347 mμ)[2] compared to II (λ_{max} 235 mμ)[2] support the view that considerable cyclic conjugation occurs in I which is not possible for II. This would be consistent with the abundant evidence that the dimensions and geometry of 3p and 3d orbitals on sulfur permit conjugation past a single intervening saturated carbon atom.[3]

III

Such cyclic conjugation, not possible for II where cyclic conjugation is blocked cleanly at the 4-position, would explain the ultraviolet spectra and the downfield shifts of *all* hydrogens in I as compared to II. The larger downfield shift for one of the ring hydrogens in I could be explained readily since the 3-carbon is essentially "directly" joined to sulfur leading to a downfield chemical shift. We suggest that the hydrogens of the phenyl group on the saturated 2-carbon in I are those shifted further downfield than those on the 4- and 6-phenyls.

The n.m.r. spectrum of I sulfoxide,[2] m.p. 146–147°, shows sharp bands at 8.01, 4.03, and 3.32, and a relatively sharp band at 2.86 τ in the ratio of 3:1:1:15. The normal position for the aromatic hydrogens suggests there is little of the added cyclic conjugation effect indicated by structure III when the sulfide sulfur is

(1) Supported in part by National Science Foundation Grant No. 19470.

(2) G. Suld and C. C. Price, *J. Am. Chem. Soc.*, **84**, 2090 (1962).

(3) See C. C. Price and S. Oae, "Sulfur Bonding," Ronald Press Co., New York, N. Y., 1962, pp. 51–55.