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.

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The Nuclear Magnetic Resonance Spectra of Some Thiopyran Derivatives

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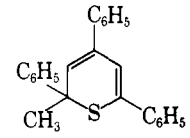
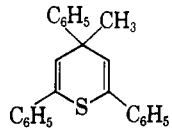
We[2] have reported earlier on the preparation of isomeric 2- and 4-methyl-2,4,6-triphenylthiopyrans by the coupling of 2,4,6-triphenylthiopyrylium ion with methylmagnesium bromide. The assignment of structure was based on a chemical degradation by desulfurization.



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.

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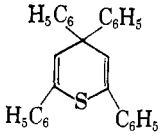
(3) See C. C. Price and S. Oae, "Sulfur Bonding," Ronald Press Co., New York, N. Y., 1962, pp. 51–55.

converted to sulfoxide. The shifted position for the ring hydrogens could then be due to a direct chemical shift by the electron-attacting sulfoxide group. The downfield shift for the methyl group may be analogous to that observed by Morin[4] in penicillin sulfoxide and indicates that the 2-methyl group is *cis* to the sulfoxide oxygen.[5]

The crystalline tetraphenylthiopyran, m.p. 157°, reported earlier[6] has been confirmed as the 2,4,4,6 isomer (IV) by n.m.r. bands at 2.87 and 4.00 τ in the ratio of 10:1.[6] Efforts to isolate a pure sample of the 2,2,4,6 isomer from the yellow oily residues after crystallization of IV were unsuccessful.[7]



IV

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The Polynitration of Indolines. 5,7-Dinitration

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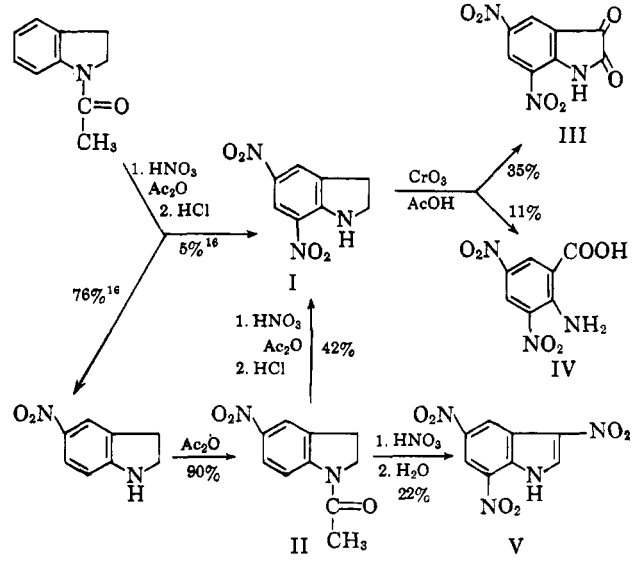
As indoles do not nitrate in the 7-position,[2] synthetic approaches to 7-nitroindoles have depended largely on ring closure to a benzene ring in which a nitro group has been prelocated in the potential 7-position.[3-14] The recently described[15,16] general method for preparation of indoles containing a nitro group in the benzene ring, by nitration of the corresponding indolines following by dehydrogenation, has only been applied to the synthesis of 5- and 6-nitroindoles. An adaptation of the indoline method, involving nitration of the indole–sodium bisulfite adduct and subsequent alkaline hydrolysis, recently has been used, however, for the synthesis of both 5- and 7-nitroindole.[17] Evidence for 5,7-dinitration of an acylindoline is available from an earlier example: Strychnine[18] is degraded by 20% nitric acid[19] (in a reaction which involves nitration, oxidation, and hydrolysis) to 5,7-dinitroindole-2,3-dicarboxylic acid (dinitrostrycholcarboxylic acid),[20] which undergoes decarboxylation to what was proved to be 5,7-dinitroindole-2-carboxylic acid (dinitrostrychol),[4,20,21] or further nitration in fuming nitric acid to 3,5,7-trinitroindole-2-carboxylic acid (trinitrostrychol).[4,20]

Nitration of 1-acetylindoline is reported to give 5-nitroindoline (64[22]–74[16]%), and a dinitroindoline (5%) of melting point 243–244°, which was assumed to be 5,7-dinitroindoline (I).[16] In this paper we report proof that dinitration of 1-acetylindoline and mononitration of the presumed intermediate, 1-acetyl-5-nitroindoline (II), gives 5,7-dinitroindoline (I). Chromic acid oxidation of the dinitroindoline gave as degradation products the known compounds, 5,7-dinitroisatin (III) and 3,5-dinitroanthranilic acid (IV). Attempts to dehydrogenate I to the still unknown 5,7-dinitroindole were unsuccessful, either with palladium on carbon (also tried on the acetyl derivative of I) or with tetrachloro-1,2-benzoquinone, compound I being recovered unchanged in moderate yields.

Addition of 1-acetyl-5-nitroindoline to fuming nitric acid gave a trinitro derivative, which, as indicated by its low hydrogen content, is an indole. The compound is colorless in the solid state, but appears to dissociate as an acid in ethanol solution, with the longest wavelength absorption as a broad band at 413 mμ. The compound is tentatively assigned the structure 3,5,7-trinitroindole (V), and is believed to be formed by dehydrogenation of a probable intermediate, 1-acetyl-



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