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Relative Stabilities of the *N*-Methyldihydropyridines[1]

Sir.

The isomeric 1,2- and 1,4-dihydropyridines have held the attention of chemists for many years.[2] This interest is due in part to the early observation[3] that the dihydropyridine ring system occurs in the ubiquitous reducing agents NADH and NADPH.[4] It was originally believed that the 1,2-dihydropyridine[3] system was present in NADH and NADPH. However, it has been shown conclusively that the isomeric 1,4-dihydropyridine occurs in these reducing agents.[5]

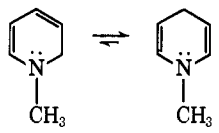
A number of methods are available for the synthesis of these ring systems and much is known about their chemistry.[2] However, there is little information available concerning their relative stabilities. There are scattered data available that indicate the 1,4-dihydropyridine system is more stable. For example, it is known that some 1,2-dihydropyridines are oxidized by silver ion at a faster rate than the 1,4 isomer[6] and Lyle and Gauthier have shown[7] that 1-methyl-3,4,5-tricyano-1,4-dihydropyridine is more stable than the isomeric 1-methyl-2,3,5-tricyano-1,2-dihydropyridine. The magnitude of this difference in stability was not reported.

In many of the previous studies on the dihydropyridine ring system and in the biological reducing agents, there are strong electron-withdrawing groups β to the nitrogen atom. The effect of these substituents on the relative stabilities of the 1,2- and 1,4-dihydropyridine systems is unknown. To date, no work has been reported on either the position or magnitude of the equilibrium between *simple* derivatives of the dihydropyridine systems.

HMO calculations[8] on the π system of the dihydropyridine ring system indicate the 1,2-dihydropyridine system is more stable.

In order to prevent problems of tautomerization we chose the *N*-methyldihydropyridines for our studies. These compounds can be prepared by reduction of the *N*-carbomethoxydihydropyridines[9] with lithium aluminum hydride. Although the dihydropyridines are relatively unstable to oxidation and polymerization they can be handled if care is used to exclude oxygen and contact with acidic surfaces.

Treatment of *either* isomer with 1.0 *M* potassium *tert*-butoxide in dimethyl sulfoxide at 91.6° produces an equilibrium mixture containing 7.7% of *N*-methyl-1,2-dihydropyridine.[10] If statistical factors are taken into



1 **2**

consideration, the *N*-methyl-1,4-dihydropyridine is 2.29 ∓ 0.01 kcal/mol more stable than the 1,2 isomer at this temperature. It is interesting to compare this result with that obtained for the carbocyclic system where 1,4-cyclohexadiene is only slightly less stable (0.07 kcal/mol with statistical correction) than 1,3-cyclohexadiene[11] at 95.0°. These results indicate that electron-withdrawing groups are not needed to stabilize the 1,4-dihydropyridine with respect to the 1,2 isomer.

It can only be speculated as to the origin of the difference in stability of these dihydropyridines. It has previously been suggested that the small difference in stability of the cyclohexadienes is due to special destabilization of the 1,3-diene. Factors such as the "cis effect"[12] and poor orbital overlap[13] have been suggested. We do not believe these effects adequately rationalize the difference in stability of the dihydropyridines. We believe that the 1,4-dihydropyridine ring system is stabilized by a favorable electronic interaction.[14] This assumption is consistent with the observation that the unsubstituted 1,4-dihydropyridine is a remarkably stable enamine.[17] In the absence of oxygen

(1) This work was presented in part at the 163rd National Meeting of the American Chemical Society, Boston, Mass., April 9–14, 1972, Paper ORGN 152.

(2) For an excellent and recent review see U. Eisner and J. Kuthan, *Chem. Rev.*, **72**, 1 (1972).

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(4) The reduced forms of nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate (NAD and NADP). For recent reviews see: (a) S. P. Colowick, J. van Eys, and J. H. Park, *Compr. Biochem.*, **14**, 1 (1966); (*b*) *Enzymes 1959–1963*, 7 (1963); (c) *Enzymes*, **2** (1970).

(5) (a) M. E. Pullman, A. San Pietro, and S. P. Colowick, *J. Biol. Chem.*, **206**, 121 (1954); (b) F. A. Loewns, B. Vennesland, and D. C. Harris, *J. Amer. Chem. Soc.*, 77, 3391 (1955); (c) R. F. Hutton and F. H. Westheimer, *Tetrahedron*, **3**, 73 (1958); (d) H. E. Dubb, M. Saunders, and J. H. Wang, *J. Amer. Chem. Soc.*, 80, 1767 (1958); and (e) K. Wallenfels, "Steric Course of Microbiological Reactions," G. E. W. Wolstenholm and C. M. O'Connor, Ed., Churchill, London, 1959, p 10.

(6) W. Traber and P. Karrer, *Helv. Chim. Acta*, **41**, 2066 (1958).

(7) R. E. Lyle and G. J. Gauthier, *Tetrahedron Lett.*, 4615 (1965).

(8) The parameters used were $\alpha_N = \alpha_C + 1.5\beta$ and $\beta_{C-N} = \beta_{C-C}$ (A. Streitwieser, "Molecular Orbital Theory for Organic Chemists, Wiley, New York, N. Y., 1961) giving DE (delocalization energy) of 1,2-DHP = -0.9157β and DE of 1,4-DHP = -0.8045β.

(9) F. W. Fowler, *J. Org. Chem.*, **37**, 1321 (1972).

(10) The equilibrium mixture was analyzed by glc using a 5 ft × 1/4 in. SE-30 column at 30°. The rate of isomerization in 1.0 *M* KO-*t*-Bu was also measured ($k = 5.40 \times 10^{-5}$ sec^{-1}) using pmr spectroscopy to follow the progress of the reaction. Within experimental error the equilibrium constant using either nmr spectroscopy or glc were the same indicating that no isomerization or partial decomposition occurred during the glc analyses.

(11) R. B. Bates, R. H. Carnighan, and C. E. Staples, *J. Amer. Chem. Soc.*, **85**, 3031 (1963).

(12) S. Staley, Ph.D. Thesis, Yale University, 1964.

(13) M. J. S. Dewar, "The Molecular Orbital Theory of Organic Chemistry," McGraw-Hill, New York, N. Y., 1969, p 293.

(14) Two possibilities are homoaromaticity[15] or hyperconjugation.[16]

(15) S. Winstein, *Chem. Soc., Spec. Publ.*, **No. 21**, 5 (1967).

(16) The symmetries of the highest occupied molecular orbital of the π system and one of the antibonding molecular orbitals of the methylene group allow for effective orbital interaction (R. Hoffmann and R. A. Olofson, *J. Amer. Chem. Soc.*, **88**, 943 (1966)). Also HMO calculations that include hyperconjugation predict the 1,4-dihydropyridine to be the more stable isomer (ref 1, p 3).

(17) A. G. Cook, "Enamines: Synthesis, Structure, and Reactions," Marcel Dekker, New York, N. Y., 1969.

it does not tautomerize, hydrolyze, or polymerize when treated with NaOD–D_2O in acetone-d_6 for several days.[9]

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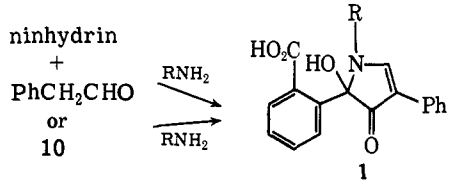
A Novel Reagent for the Fluorometric Assay of Primary Amines

Sir:

The formation of fluorescent pyrrolinones (**1**) from ninhydrin, phenylacetaldehyde, and primary amines provides the basis for a novel assay, which is of particular value in peptide analysis.[1,2] The conditions which are required to impel the "fluorogenic ninhydrin reaction" are severe enough to often impede its wider utility. Frequently, the oxidizing properties of ninhydrin are the cause of limiting side reactions. Therefore, we sought to replace ninhydrin and phenylacetaldehyde by a single reagent which would react with peptides and other primary amines of biological importance, to afford the same, or closely related fluorophors.

We report here the design and synthesis of the novel reagent **10**, which now supersedes the fluorogenic ninhydrin reaction.



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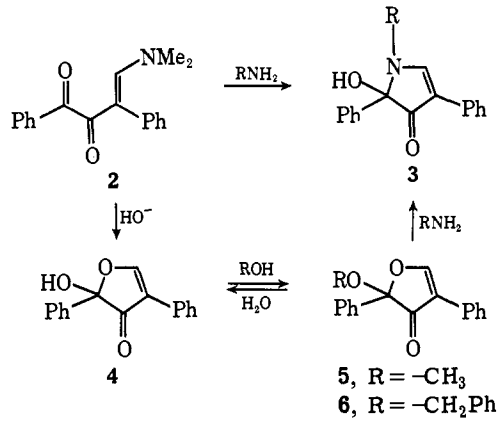
We had previously observed[2] that 1-dimethylamino-2,4-diphenyl-1-butene-3,4-dione (**2**) reacts with primary amines to give fluorescent pyrrolinones of structure **3** (Scheme I). However, the use of this reagent is restricted to nonaqueous systems, since it is rapidly destroyed by hydrolysis.

When the enamine **2** was subjected to alkaline hydrolysis, it was converted to 2-hydroxy-2,4-diphenyl-3-(2*H*)-furanone (**4**) [90%; mp 125°; uv max (MeOH) 244 (ε 18,400) and 292 nm (6,250); nmr ($CDCl_3$) δ 8.59 (s, =C*H*O)].[3] Heating of **4** in methanol afforded the methyl ether **5** [83%; mp 93°; uv max (MeOH) 241 (ε 18,750) and 307 nm (3,500); nmr ($CDCl_3$) δ 8.69 (s, =C*H*O), 3.43 (CH_3O)]. Reaction of **5** with benzyl alcohol at 100° furnished the benzyloxy compound **6** [52%; mp 140°; uv max (MeOH) 240 (ε 21,650) and 300 nm (5,100); nmr (C_6H_6-d_6) δ 4.48 and 4.58 (AB, J = 12 Hz, PhCH_2O)]. The cyclic nature of **4**, **5**, and **6**

Scheme I



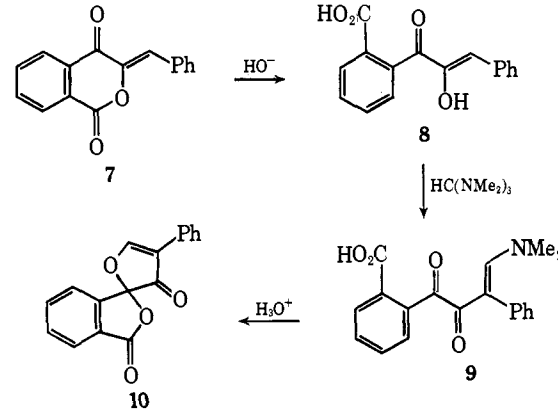
is strongly supported by the nmr spectrum of **6**, which shows that the benzylic methylene protons are nonequivalent and hence in proximity to a chiral center.

The methoxyfuranone **5** reacts rapidly with primary amines in nonaqueous solution to yield fluorescent pyrrolinones. For example, with ethylamine in acetonitrile, it gives the fluorophor **3** [R = -C_2H_5,[2] 85%]. In aqueous systems, however, the methoxyfuranone **5** readily reverts to the unreactive hydroxy compound **4**, and is thus unsuited as a reagent for assay purposes.

We then envisaged the structurally modified reagent **10**. This molecule, which retains the structural features of **5** responsible for fluorogenicity, but also possesses a more reactive leaving group, was anticipated to react with primary amines to yield fluorophors, identical with those of the fluorogenic ninhydrin reaction.

The synthesis of the spirolactone **10** is outlined in Scheme II. Alkaline hydrolysis of 3-benzylidene-1,4-

Scheme II



isochromanedione (**7**)[4] gave *o*-(α-hydroxycinnamoyl)-benzoic acid **8** [75%; mp 106–115° dec; uv max (Et_2O) 315 nm (ε 23,300)]. Formylation of **8** with tris(dimethylamino)methane[5,6] in *N*,*N*-dimethylformamide proceeded to the dimethylaminomethylene derivative

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(2) M. Weigele, J. F. Blount, J. P. Tengi, R. C. Czajkowski, and W. Leimgruber, *J. Amer. Chem. Soc.*, **94**, 4052 (1972).

(3) All new compounds gave satisfactory elemental analyses. Melting points are uncorrected.

(4) J. N. Chatterjea, B. K. Banerjee, and H. C. Jha, *J. Indian Chem. Soc.*, **44**, 911 (1967); E. B. Knott, *J. Chem. Soc.*, 402 (1963).

(5) (a) H. Weingarten and W. A. White, *J. Org. Chem.*, **31**, 2874 (1966); (b) H. Bredereck, F. Effenberger, T. Brendle, and H. Muffler, *Chem. Ber.*, **101**, 1885 (1968).

(6) In connection with an unrelated problem, we observed that this reagent is a powerful formylating agent whose reactivity is similar to that of aminal esters (A. Wick and W. Leimgruber, unpublished results); *cf.* H. Bredereck, F. Effenberger, and H. Botsch, *Chem. Ber.*, **97**, 3397 (1964), and H. Bredereck, G. Simchen, and R. Wahl, *ibid.*, **101**, 4048 (1968).