yields. Their structures were confirmed by paper electrophoresis, paper chromatograms (see Table I), and ultraviolet spectra and by identification of the corresponding nucleotides on hydrolysis.

Acknowledgment. The authors heartily thank Professor Teruaki Mukaiyama for his encouragement and discussion throughout the investigation.

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(5) The trimethylsilyl groups can be removed from nucleotides by a simple treatment of the reaction mixture with water at room temperature for 30 min.

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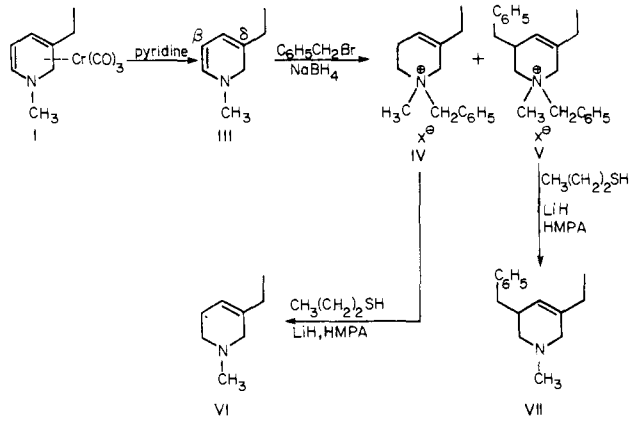
Studies in the Dihydropyridine Series. II.[1] Unstable Dihydropyridines Generated from Their Chromium Complexes and Their C-Alkylation

Sir:

In a previous study[1] we have described the preparation and characterization of two novel and stable complexes of *N*-methyl-3-ethyl-1,2-dihydropyridine (I) and *N*-methyl-3-ethyl-1,6-dihydropyridine (II). It was noted that such complexes might provide a convenient and stable system for the generation of unstable dihydropyridines, many of which are important in synthetic as well as biosynthetic considerations. We wish to describe some results which reveal that such metal carbonyl complexes are indeed useful synthetic intermediates and will undoubtedly serve to investigate much of the unknown chemistry of these interesting systems.

Reaction of *N*-methyl-3-ethyl-1,2-dihydropyridinetricarbonylchromium(I) with pyridine at room temperature provides essentially pure *N*-methyl-3-ethyl-1,2-dihydropyridine (III) which, without further isolation, can be used directly for various studies. Independent isolation and complete characterization of this unstable dihydropyridine was performed in another series of reactions to be described below.

Reaction of III with an excess of benzyl bromide and sodium borohydride in a two-phase system (ethyl ether, aqueous methanol containing sodium hydroxide) provided the mono- and dialkylated products IV and V. Direct debenzylation of IV and V employing *n*-propyl mercaptan, lithium hydride, and hexamethylphosphoramide[2] provided *N*-methyl-3-ethyl-1,2,5,6-tetrahydropyridine (VI) and *N*-methyl-3-ethyl-5-benzyl-1,2,5,6-tetrahydropyridine (VII). Characterization of these products is presented below.



In a complementary series of investigations designed to isolate the above dihydropyridine (III) from a direct reductive process, *N*-methyl-3-ethylpyridinium iodide was allowed to react in a two-phase system (ethyl ether, aqueous methanol containing sodium hydroxide) with sodium borohydride, under a nitrogen atmosphere, for 5 min. Careful evaporation of the ether solution provided III as a nearly colorless oil in 86% yield. Nmr analysis of this product revealed it to be the 1,2-dihydropyridine III of high purity with virtually no contamination by the 1,6-isomer. The nmr spectrum of III (100 MHz in deuteriobenzene) revealed the signals δ 1.0 (CH_3CH_2, t), 1.88 (CH_3CH_2, q), 2.27 (N-CH_3, s), 3.58 (N-CH_2, s), 4.84 (1 H, dd, $J \approx 7$ Hz, olefinic), and 5.73 (2 H, m, olefinic) while the uv spectrum (in methanol) showed a maximum at 327 nm. This product proved identical with that obtained when the metal carbonyl ligand had been removed from I. In this instance, the reductive process provided a more desirable route to III.

During the various alkylation studies with the dihydropyridine system it was found that optimum conditions for the desired C-alkylated product VII (or its salt V) could be obtained when various reagents were allowed to transfer between the layers in a two-phase system. This method provides a convenient isolation of the desired product with little or no complication from side reactions. Interestingly it was found that the ratio of products IV, V, and VII was markedly dependent on the molar ratios of alkylating agent employed.

When *N*-methyl-3-ethylpyridinium iodide was allowed to react in a vigorously stirred mixture of sodium borohydride, benzyl bromide (1.05 equiv) in ether, and aqueous methanol containing sodium hydroxide, the product mixture, after purification, consisted of the C-alkylated base VII (6%) and the quaternary salts IV (X = I), mp 178.5–179.5° (46%), and V (X = I, 17%), mp 204.5–205.5°.[3]

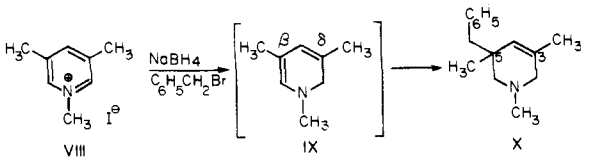
The base VII exhibited the following signals in the nmr spectrum (100 MHz, C_6D_6), δ 0.91 (CH_3CH_2, t), 1.83 (CH_3CH_2, q), 2.10 (N-CH_3, s), 5.34 (olefinic, s), 7.09 (aromatic, s), and 1.95–2.70 (7H, methylene and methine, m), while the mass spectrum revealed fragments at *m/e* 215 (M^+), 200, 143 (base peak), 129, 128, 124, 94, 91 (high resolution measurement, 215.67; calcd for $C_{15}H_{21}N$, 215.167).

On the other hand when *N*-methyl-3-ethylpyridinium iodide was allowed to react in the above manner except that 10 equiv of benzyl bromide were employed, the reaction mixture, as monitored by tlc, consisted almost entirely of salt V which upon direct debenzylation, under conditions described above, provided VII in an overall yield of 48%.

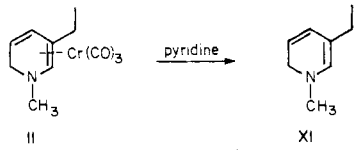
Thus in the above studies, the pyridinium iodide, initially present in the aqueous phase, is reduced and the resulting dihydropyridine III is transferred to the organic phase whereupon it reacts immediately with benzyl bromide to form a salt. The latter intermediate then transfers to the aqueous layer and is reduced with borohydride and the end product finally transfers to the organic layer from where it is extracted. These rapid transfers of the intermediates undoubtedly minimize side reactions in these studies. All of our earlier investigations, prior to the utilization of this technique, led essentially to intractable tars.

The above studies provided the first clear indication that the 1,2-dihydropyridine III was being alkylated at the β-position and in that sense was similar in reaction to that of dienamines studied previously.[4]

To extend the alkylation studies to dihydropyridines bearing an alkyl substituent at the β-position, the above procedure was applied to *N*-methyl-3,5-dimethylpyridinium iodide (VIII) and the C-alkylated product X was isolated in overall 48% yield. In this instance it was unnecessary to expose the reaction mixture to the debenzylation procedure mentioned above. The nmr data, δ 0.86 (C_5-CH_3, s), 1.16 (C_3-CH_3, s), 2.11 (C_6-CH_2, q), 2.28 (N-CH_3, s), 2.63 ($CH_2C_6H_5$, s), 2.71 (C_2-CH_2, q), 5.11 (olefinic, s), and 7.15 (aromatic), and the mass spectrum with fragments at *m/e* 215 (M^+), 172, 158 and 157, (base peak) 124, 123, 122, and 91 substantiated the structure X for the alkylation product.



It was now desirable to prepare the novel 1,6-dihydropyridine system XI for further studies. From various investigations conducted in our laboratory it was not possible to obtain XI directly *via* reduction of *N*-methyl-3-ethylpyridinium iodide. The chromium complex II obtained previously[1] was of particular value here and indeed represents presently the only synthetic route available to such systems. Thus reaction of II with pyridine at room temperature affords a product for which spectral data allow assignment of the expected structure XI. The nmr spectrum (100 MHz, C_6D_6)



with signals at δ 1.02 (CH_3CH_2, t), 1.98 (CH_3CH_2, q), 2.24 (N-CH_3, s), 3.50 (C_6-H, d), 5.18 (C_5-H, m), 5.52 (C_2-H, s), and 5.58 (C_4-H, d) and the uv spectrum (λ_{max} 332 nm) were fully in accord with expectation. Studies on the alkylation and other reactions on XI are now in progress.

In conclusion the above investigations have provided some information about the chemistry of several novel dihydropyridine systems. It is already clear from these preliminary results that such systems will indeed provide some interesting avenues for synthetic and biosynthetic studies in natural products[1] as well as in heterocyclic chemistry. With respect to the latter, for example, extension of the present studies will allow a versatile synthetic pathway to 3- and 3,5-disubstituted pyridines from readily available pyridine systems. There are presently only a limited number of approaches to the direct synthesis of such compounds.[5,6]

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Nuclear Magnetic Resonance Studies of Hemoproteins.[1] Restricted Rotation of a Heme Side Chain Methyl Group in Some Ferric Myoglobin Complexes and Its Implication in van der Waals Contact in the Heme Side Chain Environments

Sir:

The heme in hemoproteins is embedded in a hydrophobic cleft made up by the polypeptide chain. It has been revealed[2] that the porphyrin side chains, directed toward the interior of myoglobin and hemoglobin, are in van der Waals contact with nonpolar groups of the polypeptide chain. Some of the important contact in these hemoproteins has been visualized by the X-ray crystallographic study.[2] Although the nuclear magnetic resonance(nmr) method has been proved[3] to be potentially useful for the studies of electronic structure and conformation of hemoproteins, there has been no nmr work on the interaction between heme side chain and apoproteins. In the present communication we wish to report the nmr studies of restricted rotation of the heme side chain methyl group in some ferric myoglobin complexes and to show that the methyl rotational barrier could serve as a sensitive probe for the studies of this heme-apoprotein interaction.

Wüthrich, *et al.*, have observed[4] at an appropriate temperature doubling of one heme ring methyl signal in sperm whale and gray whale cyanoferrimyoglobins, which was tentatively ascribed to methyl hindered rotation without further verification.[4] Here we have obtained more confirmative evidence in the nmr spectra[5] for methyl hindered rotation in horse ferricyanomyoglobin, which is amenable to numerical estimation of this rotational barrier. We have also studied the effect of some ionic additives or different axial ligands on this methyl restricted rotation in order to shed light on van der Waals contacts in the heme side chain environments.

Figure 1 shows the temperature dependence of hyperfine-shifted signals of a heme side chain group at the lower field side in horse ferricyanomyoglobin($MbCN^-$). The most striking feature of this figure is collapse of one methyl signal (1) when the temperature is lowered. At the temperature above 35°, four methyl signals have the same line width (see Figure 1a). However, the highest field methyl signal (1) at −13.5 ppm behaves itself quite differently from the other three methyl signals below 30° at which it broadens and the signal line becomes unsymmetrical. At