research and Roberta L. Arnold for assistance with certain aspects of this work.

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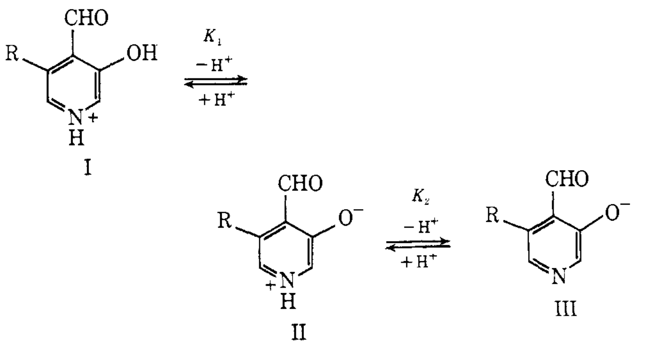
Reduction of Pyridoxal Phosphate (and Analogs) by 1,4-Dihydropyridine

Sir:

Though in enzymatic reactions nicotinamide–adenine dinucleotide (NADH) serves as a reducing agent for aldehydes, *via* direct hydrogen transfer, rather extensive search has led to no aldehyde substrate reducible by 1,4-dihydropyridine in simple model systems operating in aqueous solution at ambient temperature.[1] For horse liver alcohol dehydrogenase the Zn^{II} species present at each of the two NADH-containing active sites has been suggested to facilitate aldehyde reduction by polarization of the carbonyl group through direct interaction with the carbonyl oxygen.[2] Though Creighton and Sigman[3] have quite recently described a Zn^{II}-dependent reduction of 1,10-phenanthroline-2-carboxaldehyde by *N*-propyl-1,4-dihydronicotinamide (NPrNH), this system, being reported only in acetonitrile, is apparently restricted to aprotic solvents. Apparently searches for aldehyde substrates have never been directed to pyridoxal phosphate and analogs. This is rather surprising considering the fact that, as cofactors, NADH and pyridoxal phosphate cohabit in the same cell milieu. We report herein that pyridoxal phosphate (PLP), pyridoxal (PL), and 3-hydroxypyridine-4-aldehyde (PCHO) serve as suitable aldehyde substrates for reduction by the 1,4-dihydropyridines, NPrNH, and 2,6-dimethyl-3,5-dicarbethoxy-1,4-dihydropyridine (Hantzsch ester). Also, we find the reductions to be facilitated by metal ions in aqueous solution. Kinetic studies were carried out spectrophotometrically under N_2 in Thunberg cuvettes employing two media: (a) neat boiling methanol (NPrNH at 354 nm, Hantzsch ester at 372 nm); and (b) 52.1 wt % aqueous methanol at 30°, $\mu = 0.01$ with KCl (NPrNH at 362 nm, Hantzsch ester at 372 nm). Tlc and nmr studies established that the products of reaction of PCHO and NPrNH were *N*-propylnicotinamide and the carbinol formed by reduction of the aldehyde. In refluxing methanol without buffer, the predominant species of pyridine aldehydes present will be II and III. We may assume that the rate of reduction of II $\gg$ III. The second-order rate constants (M^{-1} min^{-1}) for reduction of species II are as follows: 16 [PCHO]-[NPrNH]; 0.74 [PCHO][Hantzsch ester]; 0.28 [PL]-[Hantzsch ester]. The nmr spectrum of the product from reaction of PL and Hantzsch ester in refluxing methanol-*d* had singlet peaks of almost identical integral intensities at 4.63 and 4.82 ppm (δ). This result establishes direct transfer of a hydrogen from the dihydropyridine to the 4-aldehyde of PL. In the buffered aqueous methanol solutions at pH 8.34 the following apparent second-order rate constants were obtained employing Hantzsch ester: PCHO, no reaction; PL, 0.22; PLP, 0.48 M^{-1} min^{-1}. From the pH dependence of the reaction of PLP with NPrNH the rate constants for the ionic forms of PLP corresponding to I, II, and III could be estimated at 17, 2.7, and $\sim 10^{-1}$ M^{-1} min^{-1}, respectively. The order of reactivity of $\mathrm{PLP_I} > \mathrm{PLP_{II}} > \mathrm{PLP_{III}} >$ pyridine-4-aldehyde = 0 is that previously noted for imine formation[4] and finds similar explanation.



One would anticipate enhancement in the rate of reduction of PLP, PL, and PCHO upon complexation by metal ions, much as in the transamination reaction.[5] This was found to be the case. Employing 52.1 wt % methanol–water (30°) buffered by EDTA (pH 7.05–7.10) at 0.02 *M* with metal ion at 0.015 *M*, the following order of catalysis was observed in reduction of PLP by Hantzsch ester: $Ni^{2+}(k_{\rm rel} = 7.2) > Co^{2+}(k_{\rm rel} = 3.4) \geq Zn^{2+}(k_{\rm rel} = 2.8) > Mn^{2+}(k_{\rm rel} = 1.3) = Mg^{2+}(k_{\rm rel} = 1.2)$, and no metal ion ($k_{\rm rel} = 1.0$). Considering that [EDTA] slightly exceeded [metal ion] in these experiments, the metal ion enhancement of rate is appreciable.

At present, it would appear as though the only aldehydes susceptible to 1,4-dihydropyridine reductions in aqueous solutions at ambient temperatures are PLP and its analogs. Also, the metal ion promotion of these reactions apparently represents the only case of metal ion catalysis of aldehyde reduction by a 1,4-dihydropyridine in aqueous solution.

Acknowledgment. This research was supported by grants from the National Institutes of Health and the National Science Foundation.

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(1) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. II, W. A. Benjamin, New York, N. Y., 1966, Chapter 9.

(2) For a review see A. S. Mildvan, *Enzymes*, **2**, 446 (1970).

(3) D. J. Creighton and D. S. Sigman, *J. Amer. Chem. Soc.*, **93**, 6314 (1971).

(4) D. S. Auld and T. C. Bruice, *ibid.*, **89**, 2083 (1967).

(5) For a review of the work of E. E. Snell, D. E. Metzler, and others, see ref 1, Chapter 8.

(6) Postdoctoral fellow, Department of Chemistry, University of California, Santa Barbara, Calif. 93106.

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Nicandrenone, an Insecticidal Plant Steroid Derivative with Ring D Aromatic

Sir:

In 1951, the isolation of a substance termed "nicandrin" from the Peruvian weed *Nicandra physalodes* was reported.[1] It was later found to possess strong insect repellent and mild insecticidal properties, and as it was

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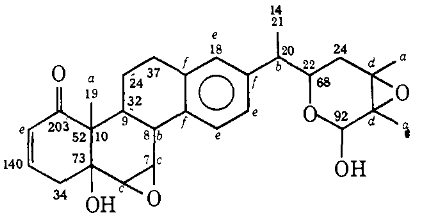
(1) F. V. Gizycki and G. Kotitschke, *Arch. Pharm.* (*Weinheim*), **284** 129 (1951).

found to be an α,β-unsaturated ketone rather than a glycoside, the name "nicandrenone" was suggested.[2] The molecular formula $C_{34}H_{42}O_7$ was indicated. We wish to present spectral evidence[3] that nicandrenone has formula I and is apparently the first natural steroid derivative with ring D aromatic.



Ia,[3] $a = \mathbf{17}, \mathbf{17}, \text{ or } \mathbf{19}$

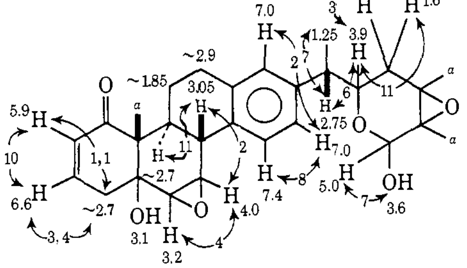
$b = \mathbf{39} \text{ or } \mathbf{43}$

$c = \mathbf{56} \text{ or } \mathbf{57}$

$d = \mathbf{64} \text{ or } \mathbf{65}$

$e = \mathbf{124}, \mathbf{125}, \mathbf{129}, \text{ or } \mathbf{129}$

$f = \mathbf{135}, \mathbf{137} \text{ or } \mathbf{142}$

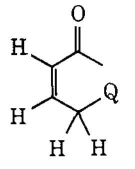


Ib,[3] $a = \mathbf{1.25}, \mathbf{1.35}, \text{ or } \mathbf{1.35}$

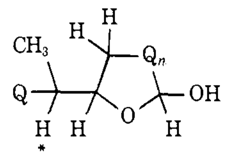
The proton-decoupled cmr spectrum of a benzene-free sample purified by tlc clearly showed the presence of 28 carbons; off-resonance decoupling showed 4 methyls, 4 methylenes, and 12 methinyls, for a total of 32 protons attached to carbon. Two hydroxyl hydrogens rather than one[2] and 6 oxygens complete the molecular formula $C_{28}H_{34}O_6$, consistent with a parent peak in the mass spectrum at *m/e* 466 and suggesting a steroidal origin[4] with one added carbon. The earlier molecular formula[2] was based largely on elemental analyses of samples containing 0.25 mol of benzene of crystallization.

The enone structure could readily be expanded to II (pmr), which fits only in ring A of a steroid. The benzene ring detected earlier[2] has trialkyl substitution (cmr, pmr) with a 1,2,4 pattern (pmr). There are no further sp^2 hybridized carbons (cmr), so seven rings are present.

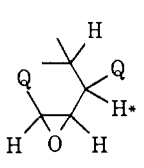
The three oxygens not in hydroxyl or carbonyl groups must be in ether linkages. No methyls or methylenes are attached to oxygen (cmr); one methinyl (cmr, **92**; pmr, δ 5.0) is attached to *two* oxygens, evidently in a



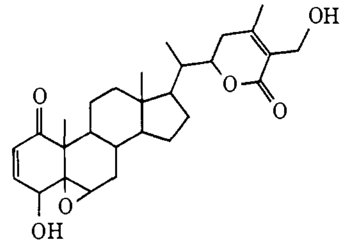
II



III, $n = 1$ or 2



IV



V

hemiacetal grouping since in some samples this methinyl proton is coupled (7 Hz) to a hydroxyl proton.

Only one other methinyl carbon and no quaternary (Q) carbons are attached to nonepoxidal ether oxygens (cmr), and from the coupling pattern observed (pmr) and the ring sizes favoring hemiacetals, part structure III can be written. The other two ether oxygens must be in epoxide groupings, consistent with finding cmr peaks for methinyl carbons at **56** and **57** and quaternary carbons at **64** and **65**. The methinyl protons at δ 4.0 and 3.2 are coupled to one another with $J = 4$ Hz, typical of cis 1,2 protons in an epoxide grouping;[5] the additional splitting involving these protons indicates the presence of part structure IV.

A tetraalkyl-substituted epoxide grouping, three unsplit methyls, two methylenes, and a tertiary hydroxyl group remain to be placed. If the assumption of steroidal origin is made,[4] only structure I is compatible with all of the facts. The structure with the carbonyl at 4 instead of 1 is ruled out by the downfield location of the C-19 methyl protons.[6] The starred hydrogens in part structures III and IV are benzylic, in accord with their downfield positions. The epoxide proton at C-7 is approximately in the plane of the benzene ring, accounting for its downfield location. When this proton was irradiated, an NOE[7] of 20% was observed for the aromatic proton absorbing at δ 7.4, confirming (1) the spatial closeness of these protons and (2) the location of the $C_9H_{15}O_3$ side chain on the aromatic ring. Decoupling experiments showed the methylene protons absorbing at δ ~1.85 to be coupled to protons at 2.9 and 2.7, but due to the complexity of the absorption in these regions, the coupling constants were not sorted out. Strong confirmation of the location of the aromatic ring and composition of the side chain comes from the mass spectrum: the strongest peak (other than those for loss of water and methyl groups) is at *m/e* 323, consistent with cleavage of the C-20 to C-22 bond to give a benzyl (and then tropylium) cation.

The biogenesis of I poses no problems; the aromatization of ring D involves oxidation to the C-18 alcohol, generation of positive charge at C-18, and the appro-

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[2] O. Nalbandov, R. T. Yamamoto, and G. S. Fraenkel, *J. Agr. Food Chem.*, 12, 55 (1964).

[3] Nmr measurements were made at 60 and 100 MHz on $DCCl_3$ solutions. Cmr chemical shifts are given in boldface in parts per million downfield from TMS (δ) on structure Ia; conventional numbering is also given on this structure. Pmr shifts in δ units and coupling constants in Hz are given on structure Ib. All couplings where the chemical shifts differ by 0.3 ppm or less were verified by spin decoupling.

[4] G. Fraenkel, J. Nayar, O. Nalbandov, and R. T. Yamamoto, *Int. Kongr. Entomol. Verh., 11th*, 3, 122 (1960), present evidence from color tests which suggests nicandrenone to be a steroid. Later,[2] a steroidal structure was considered unlikely since the pmr spectrum is so different from that of a typical steroid. We have tried unsuccessfully to find a nonsteroidal type structure for nicandrenone.

[5] *Cf.* styrene oxide: N. S. Bhacca, L. F. Johnson, and J. N. Shoolery, "NMR Spectra Catalog," Varian Associates, Palo Alto, Calif., 1962, Spectrum No. 193.

[6] N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, San Francisco, Calif., 1964, p 19.

[7] F. A. L. Anet and A. J. R. Bourn, *J. Amer. Chem. Soc.*, 87, 5250 (1965).

priate 1,2-alkyl shift. Withaferin A (V),[8] also obtained from a solanaceae, is perhaps the most closely related substance known.

Nicandrenone (I) has 11 asymmetric centers. It probably possesses the usual plant steroid configurations at C-8, C-9, C-10, and C-20 as shown in Ib; the trans-diaxial relationship of the protons attached to C-8 and C-9 is confirmed by the observed coupling constant between them (11 Hz). The 6–3 ring junctures are presumably cis because of excessive strain if trans. The configuration at C-22 is probably the same as in withaferin A (V) but is not yet known. Our attempts to obtain a crystalline derivative of nicandrenone suitable for X-ray analysis have failed, but we are pursuing this approach in the hope of answering the remaining stereochemical questions.

Acknowledgments. We thank Dr. G. S. Fraenkel for seeds of *Nicandra physalodes*, Dr. D. N. Stephens for isolating the nicandrenone used in this work, the Bruker Company for the cmr spectra, and the Public Health Service (GM-12447 and CA-10944) and Sloan Foundation (Fellowship to R. B. B.) for financial support.

(8) D. Lavie, E. Glotter, and Y. Shvo, *J. Chem. Soc.*, 7517 (1965).

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Intramolecular Metal–Double Bond Interaction. III. Intramolecular Cyclization Reactions of Organometallic Compounds

Sir:

Facile cyclization of organometallic compounds by addition across nonconjugated double bonds is presently known only for aluminum derivatives under special conditions,[1,2] and for Grignard reagents.[3,3a] In the former case cyclization takes place on addition of an aluminum hydride to 1,5-hexadiene and in the latter, cyclization or cyclic intermediates have been reported for but-3-enyl, pent-4-enyl, and hex-5-enyl derivatives. No generally successful cyclization process has been outlined, however, for synthetic purposes.

At this time we wish to report that cyclization of hex-5-enyl organometallic compounds is a general reaction and propose that this reaction proceeds through eq 1 and 2 for lithium, magnesium, aluminum, gallium, and indium derivatives. The alkyl group exchange indicated in eq 3 also occurs for the Mg and Li systems

$$\tfrac{n}{2}\mathrm{Hg[(CH_2)_4CH{=}CH_2]_2} + m\mathrm{M} \longrightarrow m\mathrm{M[(CH_2)_4CH{=}CH_2]}_n + \tfrac{n}{2}\,\mathrm{Hg} \quad (1)$$

$$\mathrm{M[(CH_2)_4CH{=}CH_2]}_n \longrightarrow \mathrm{M(CH_2(}c\text{-}\mathrm{C_5H_9))}_n \quad (2)$$

$$\mathrm{M[CH_2(}c\text{-}\mathrm{C_5H_9)]}_n + \mathrm{Hg[(CH_2)_4CH{=}CH_2]_2} \rightleftharpoons \{\mathrm{M(CH_2(}c\text{-}\mathrm{C_5H_9))}_{n-1}\mathrm{[(CH_2)_4CH{=}CH_2]} + \mathrm{Hg(CH_2(}c\text{-}\mathrm{C_5H_9))[(CH_2)_4CH{=}CH_2]}\} \quad (3)$$

$$2\mathrm{Hg(CH_2(}c\text{-}\mathrm{C_5H_9))(CH_2)_4CH{=}CH_2)} \rightleftharpoons \mathrm{Hg(CH_2(}c\text{-}\mathrm{C_5H_9))_2} + \mathrm{Hg[(CH_2)_4CH{=}CH_2]_2} \quad (4)$$

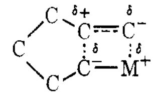
giving rise to formation of the cyclic mercury compound.

The conditions for complete conversion to the methylenecyclopentane derivatives are indicated in Table I.

Table I. Experimental Conditions for Cyclization Reactions of Hex-5-enyl Metal Derivatives

Metal	Time for complete cyclization	Temp, °C	Solvent
Li	8 days	25	C_5H_{10}
Li	96 hr	25	C_6H_6
Li	<1 hr	25	$(C_2H_5)_2O$
Mg	24 hr	110	Neat
Al	48 hr	40	Neat
Al	48 hr	25	C_5H_{10}
Ga	3 weeks	95	Neat
In	3 weeks	110	Neat

The reaction may be depicted as an internal addition across the double bond as indicated in I and appears to proceed more readily for the hex-5-enyl derivatives than for other chain lengths[4] with terminal olefin functionality. It has been demonstrated that the intramolecular cyclization of aluminum derivatives[2] requires a strain-free conformation for facile reaction and it is clear that steric and electronic effects are important. It is also noteworthy that these cyclizations proceed with such facility in contrast to the analogous intermolecular reactions.[5] The cyclization of the lithium derivative has been shown to be solvent dependent; addition of ether results in an increased rate of cyclization. This may be interpreted in terms of stabilization of the polar transition state *via* the solvation of the lithium and from the increased polarity of the lithium–carbon bond also resulting from this solvation.



I

This reaction appears to provide an interesting and readily available procedure for formation of five-membered ring systems which is potentially significant for synthetic purposes. It also provides a convenient system for the examination of the initial stages of addition to π systems. These features are now under further investigation.

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(1) C. Zweifel, G. M. Clark, and R. Lynd, *Chem. Commun.*, 1593 (1971).

(2) G. Hata and A. Miyaki, *J. Org. Chem.*, 28, 3237 (1963).

(3) H. G. Richey, Jr., and W. C. Kossa, Jr., *Tetrahedron Lett.*, 2313 (1969); H. G. Richey, Jr., and T. C. Rees, *ibid.*, 4297 (1966); H. G. Richey, Jr., and A. M. Rothman, *ibid.*, 1457 (1968); M. S. Silver, P. R. Shafer, J. E. Norlander, C. Ruchart, and J. D. Roberts, *J. Amer. Chem. Soc.*, 82, 2646 (1960).

(3a) Note Added in Proof. V. N. Drozd, Yu. A. Ustynyuk, M. A. Tsel'eva, and L. B. Dimitriev, *Zh. Obshch. Khim.*, 38, 2114 (1968), have reported the cyclization of hex-4-enyllithium species to yield methylcyclopentane derivatives similar to those reported in this work.

(4) We have prepared a series of but-3-enyl and pent-4-enyl metal derivatives and have found no cyclization reactions. However, for the pent-4-enyl derivatives we have established the presence of an intramolecular metal–olefin association which we believe to be intimately related to the cyclization processes observed for the hex-5-enyl derivative. A preliminary report has been submitted with respect to the pent-5-enyl derivatives: J. Smart, J. St. Denis, and J. P. Oliver, *J. Organometal. Chem.*, in press.

(5) K. Ziegler in "Organometallic Chemistry," H. H. Zeiss, Ed., Reinhold, New York, N. Y., 1960, pp 220–231.