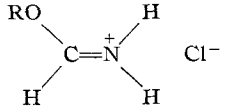
Detection of Some Formiminoesters and the Measurement of Their Rates of Formation Using NMR

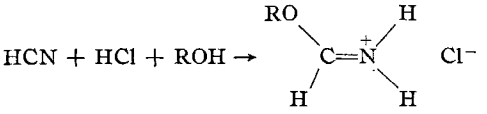
By JAY NEMATOLLAHI* and L. DALLAS TUCK

Formiminoesters have long been postulated as intermediates in the synthesis of formamidines and various heterocyclic compounds. The methyl, ethyl, and isopropyl homologs have been prepared by interaction of hydrogen cyanide with the appropriate alcohol in the presence of dry hydrogen chloride, and the progress of the formation was monitored by NMR at regulated probe temperatures from −25° to 0°. The rate of formation of formiminoesters depends principally on the concentration of hydrogen cyanide. The quartet in the NMR spectrum at a chemical shift near 8 p.p.m. arises from a single proton on a doubly bonded carbon, which is coupled to two nonequivalent protons. This is consistent with the structure:



The lifetime of the nitrogen-proton bond is of the order of 3 sec.

THE FORMIMINOESTER hydrochlorides have long been postulated as intermediates in the syntheses of formamidines and related compounds. These intermediates are presumed to form by interaction of hydrogen cyanide and dry hydrogen chloride with the appropriate alcohol. Pinner (1) established empirical formulas for several of these intermediates and proposed the structure shown in I.



I

Because of the extreme instability of these compounds under ordinary laboratory conditions, their properties have not been explored, and nothing is known about their rates of formation. The authors have confirmed the structure proposed by Pinner using NMR spectrometry and have studied the dependence of the rates of formation on the concentrations of the reactants and on temperature from −25° to 0°.

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The authors wish to dedicate this paper to Dean T. C. Daniels, whose leadership at the University of California has done so much to raise the standards of education and research in the pharmaceutical sciences.

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DISCUSSION

To explore the possibility of a reaction in the absence of hydrogen chloride, a mixture of liquid hydrogen cyanide and each of the alcohols was allowed to stand for 7 days at temperatures ranging from −10° to 25°. The NMR spectra showed that no reaction occurred during this period.

A series of experiments to test the dependence of the reaction on the concentration of hydrogen chloride proceeded as follows. To mixtures of hydrogen chloride with alcohol, ranging from a catalytic amount to 40% by weight, were added varying amounts of hydrogen cyanide. Unless the hydrogen chloride in the alcohol–HCl mixture exceeded 25% by weight, no reaction occurred based on the NMR spectra. At higher concentrations the reaction proceeded at a rate in which there was a minor dependence on HCl concentration. It was concluded that roughly a stoichiometric amount of hydrogen chloride is necessary in order for the reaction to proceed. From this result it would appear that an intermediate complex is formed which is structurally very close to the protonated imino ester which is the final product. More detailed studies on the effect of the concentration of HCl were not pursued because of the difficulties due to the extreme volatility of HCl under the conditions of these experiments.

Dry trifluoracetic acid was tried as a possible substitute for HCl gas. There was no interaction between HCN and alcohol in this solvent; the NMR spectrum showed only the rapid formation of esters of trifluoracetic acid. Unlike the case with HCl, there is no direct interaction between HCN and trifluoracetic acid (see below).

It is well known that HCN and HCl react to produce a solid from which *s*-triazine can be obtained (2). The product of this reaction does not occur in quantity detectable in the NMR spectrum when alcohol is present. Research on this product is continuing.

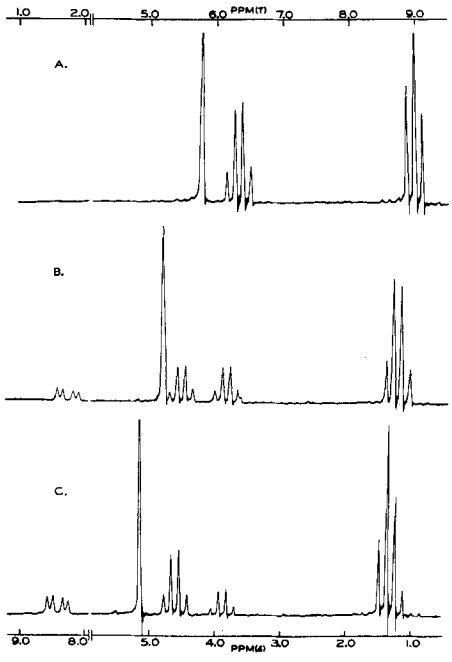

Fig. 1—NMR spectra during the formation of ethyl formimidate at −20°. Key: A, *0 min.;* B, *56 min.;* C, *94 min.*

As long as it remains liquid, the most useful measure of the extent of the formation of the formiminoesters is the integral of the NMR peaks of the imino ester described in a later paragraph. At a late stage of the reaction, a white crystalline solid is precipitated. The solid, which is insoluble in chloroform, reacts instantaneously with water. With dimethylsulfoxide it reacts to produce a brown solution containing incompletely dissolved material. It was desired to know whether the solid was the formiminoester hydrochloride, which would have a limited solubility in the alcoholic medium. The imino ester may react with alcohol in the mixture or with atmospheric water to produce ammonium chloride. Then

$$ROCH{:}NH_2^+Cl^- + 2ROH \rightarrow CH(OR)_3 + NH_4^+Cl^-$$
$$ROCH{:}NH_2^+Cl^- + H_2O \rightarrow HCOOR + NH_4^+Cl^-$$

In both cases soluble compounds with characteristic NMR spectra would be formed—in the former the orthoformate, in the latter, a formate ester. Neither of these compounds was found in the NMR spectrum. The infrared spectrum indicated that the solid was not ammonium chloride. Moreover, the effect of dimethylsulfoxide, together with NMR and I.R. evidence, showed that the solid was different from that observed by direct interaction of HCl and HCN.

NMR Spectrum—The progress of the reaction is illustrated by reference to the ethanol case, the other alcohols differing only in the details of the alkyl portions of the NMR spectrum. In Fig. 1, A, at zero time the triplet at $\delta = 1.0$ ($\tau = 9.0$) and the quartet at $\delta = 3.8$ arise, respectively, from the methyl and methylene portions of ethanol. The sharp singlet at 4.2 is due to HCN, and an additional peak due to mutually exchangeable alcoholic OH and HCl is found offscale to the left. Figure 1,B, shows after a lapse of time a new methylene quartet which grows at the expense of the original quartet. The transformation of the triplet into a quartet of the same over-all intensity is due to the overlap of the ethanol triplet with that of an ethyl group in the product. The fact that the new methylene and methyl multiplets are downfield (left) of ethanol indicates that in the product the ethyl group is associated with a functional group more electronegative than the alcoholic OH. At the same time a new quartet has appeared at $\delta = 8.0$, whose integral is exactly half of the integral of the new CH_2 quartet and is thus due to a single proton. Figure 1,C, shows a continuation of the same process as the product builds up at the expense of the reactants. There is a general downfield shift of the entire spectrum owing to changes in the solvent and acidic properties of the mixture. Table I summarizes the chemical shifts of the various alcohols and the corresponding esters.

TABLE I—CHEMICAL SHIFTS (p.p.m. FROM TETRAMETHYLSILANE) IN THE MIXTURE DURING THE FORMATION OF THE RESPECTIVE FORMIMINOESTER, AS INDICATED

	Methyl Formimidate	Ethyl Formimidate	Isopropyl Formimidate
HCN	3.9–5.4	4.3–5.0	4.1–4.9
Alcohol, αCH	3.0–3.3	3.7–4.1	3.8–4.4
βCH	...	0.9–1.2	1.0–1.2
Ester, αCH	3.9–4.0	4.4–4.8	4.6–5.2
βCH	...	1.1–1.5	1.2–1.35
N=CH—O	7.9–8.3	8.0–8.5	8.0–8.5

The feature of the spectrum which leads to important structural inference is the well-resolved quartet due to a proton on an sp^2 carbon at $\delta = 8$. This indicates the presence of two nonequivalent protons on the nitrogen *cis* and *trans* to the CH. The spin-spin splitting arising from the *cis* coupling is 5 c.p.s. and from the *trans* 14 c.p.s.

The separate lines are sufficiently well resolved that the lifetimes of the states may be calculated directly from their line width, about 2 c.p.s. The net lifetime T_2' is the magnetic lifetime determined by the spin-lattice interaction as modified by the chemical lifetime of the NH protons with respect to exchange (3). A spin-lattice relaxation time T_2 is obtained from the line width of the CH_3 line, and assuming that the same prevails for the NH, the exchange lifetime is found by the reciprocal difference, *viz.*,

$$1/\tau = 1/T_2' - 1/T_2 = 2\pi(\delta\nu_{CH} - \delta\nu_{CH_3}) \approx 3 \text{ sec.}$$

The relative amount of formiminoester formed was determined from the integral of an appropriate section of the NMR spectrum. The methyl singlets of the ester and alcohol were well separated, and their integrals were used as a measure of the extent of the methanol reaction. Because of overlap between the multiplets of the ethyl formimidate and the ethanol, as well as the HCN singlet, it was necessary to use the integral of the downfield peak of the methyl triplet as a measure of the ethanol reaction. In the isopropyl formimidate the integral of the methyl doublet was used. To establish a constant scale

which would cancel out instrumental fluctuations, the relative measure of the formimidate is taken as the ratio of the appropriate integral (see above) to the sum of the integrals of the peaks ascribed to the OH, NH_2, and HCl, which sum should remain constant during the reaction. For each series of measurements on a given mix, the relative concentration of formimidate was plotted against the time of measurement as shown for methyl formimidate in Fig. 2. During the early part of the experiment, the points in these plots describe straight lines, and the initial reaction rates calculated as the slopes are shown in Table II.

The results of the rate measurements of the formation of the methyl formimidate at −10° are the most complete and show a close proportionality with HCN concentration. Little can be said about the relationship of the rate with concentration of HCl or alcohol except the general comments found earlier in this paper. Not enough data were collected from the ethyl and isopropyl experiments to draw similar conclusions, but judging from the qualitative behavior and the time required for precipitation of the solid compound, the same approximate dependence on HCN and HCl prevails. Comparing the three alcohols, it is evident that for a comparable concentration of HCN the rate is higher for the higher homolog.

These temperature dependencies of the rates were used to determine the approximate values for the energy of activation. These are given in Table III, and their respective values seem to indicate a reasonable trend. As one might expect, the simpler structure has a lower energy of activation.

Because of the impossibility of independent adjustment of the concentration of the reactants in a system in which the solvent is itself a reactant, it is difficult to ascertain the dependency of the rate on the concentration of alcohol or HCl. Moreover, in these circumstances, mass action behavior is no longer the primary determinant of rate because of the importance of other variables of the mix, such as viscosity and specific solvation effects. On the other hand, it is the simplicity of the system, particularly the absence of a proton-containing solvent, which makes the study by NMR feasible.

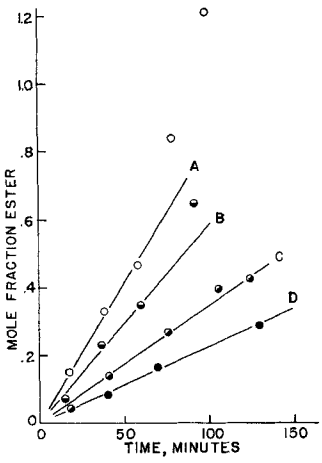

Fig. 2—Mole fraction of product during esterification of methanol at −10° calculated from the NMR integral. The straight lines yield the initial rates. The runs may be readily identified from the summary in Table II.

TABLE II—INITIAL RATES OF FORMATION OF FORMIMINO ESTERS

Temp.	Mole Fractions HCN	HCl	Alcohol	Rate, min.$^{-1}$	Rate/HCN
Methyl Formimidate					
−25	0.52	0.18	0.30	0.00052	0.001
−10	.60	.17	.23	0.0083	0.014
−10	.41	.25	.34	0.0059	0.014
−10	.27	.31	.42	.0035	0.013
−10	.46	.19	.35	.0022	0.048
0	.58	.15	.27	.015	0.026
Ethyl Formimidate					
−20	.53	.245	.22	.0028	0.005
−15	.545	.242	.213	.003	0.005
−10	.57	.222	.201	.0101	0.018
0	.70	.154	.141	Solid forms too fast to measure	...
Isopropyl Formimidate					
−10	.555	.265	.179	.0134	0.024
0	.64	.159	.204	.045	0.070
0	.59	.181	.229	.0275	0.047

TABLE III—ENERGIES (Kcal./mole) OF ACTIVATION OF THE FORMATION OF FORMIMINOESTERS

Methyl	26.4
Ethyl	34.4
Isopropyl	34.6

EXPERIMENTAL

Hydrogen chloride gas, dried by passing through concentrated sulfuric acid, was introduced into a weighed amount of alcohol cooled in a dry ice–acetone mixture. A sintered glass tip was used for the gas dispersion. Three hours were required to produce a solution of 25 to 30% HCl. The amount of hydrogen chloride was determined by the gain in weight. The solution was stored at −20°. The alcohol was dried using magnesium turnings and iodine.

Hydrogen cyanide from a tank was dried by passing through a tower containing $CaCl_2$ and $CaSO_4$ and condensed in an ice–salt mixture at −5°.

To a tared NMR tube was added about 0.5 ml. of HCN, which was then capped, cooled to −20°, and weighed. The tube was placed in a dry ice–acetone bath and to it was added 0.5 ml. of the cooled HCl–alcohol solution. The material was brought to the melting point of HCN just before placing it in the constant-temperature probe of the NMR spectrometer. The first spectrum was run immediately and then at time intervals varying from 5 to 30 min. The tube was not removed from the probe during the experiment.

The solid product remaining in the tube at the end of the experiment was filtered in a dry box under nitrogen atmosphere. The infrared spectrum of this solid was determined in KBr disks.

NMR spectra were run using a Varian A60 spectrometer with a temperature controlled probe. Infrared spectra were determined with a Beckman IR-8 spectrophotometer.

REFERENCES

(1) Pinner, A., and Klein, F., *Ber.*, **10**, 1889(1877); Pinner, A., *ibid.*, **16**, 352, 1643(1883).
(2) Smolin, E. M., and Rapaport, L., "*s*-Triazine and Derivatives," Interscience Publishers, Inc., New York, N. Y., 1959, pp. 6–10.
(3) Pople, J. A., Schneider, W. G., and Bernstein, H. J., "High-Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, p. 218 *ff*.

Binding of Cortisol and Its Degradation Products by Human Serum Albumin

By K. J. KRIPALANI and DONALD L. SORBY

The rate of degradation of the 17-dihydroxyacetone function of cortisol has been measured in a phosphate buffer system, pH 7.4 and ionic strength 0.16, at 15.0°, 25.0°, and 35.0°. The results correspond favorably with data in the literature for degradation of prednisolone under similar conditions. The binding of cortisol and its degradation products to human serum albumin was measured at 25.0° by an equilibrium dialysis procedure. The degradation products were found to be bound more strongly than was cortisol, but did not appear to compete with cortisol for binding sites on the protein molecule. In systems having a constant concentration of total corticosteroid, the apparent binding constant of total steroid increases as the fraction of the material present as degradation products increases. The results of this research are important to the design of experiments which attempt to study the binding of steroids containing the 17-dihydroxyacetone function to serum proteins.

INTERACTIONS with various serum proteins may potentially affect the distribution, metabolism, elimination, and therapeutic effects of a drug. Hence, interactions between drugs and proteins have been subjects for study by many investigators. In his extensive review, Goldstein (1) cites several hundred published studies of drug protein interactions. One particularly active area of interest has been the interactions which occur between various steroid hormones and serum proteins. Such interactions appear to be of major importance to the transport and distribution of these compounds. Cortisol, in particular, has received much attention. Sandberg *et al.* (2), Daughaday (3), Slaunwhite *et al.* (4), Brunkhorst and Hess (5), and Westphal (6), among others, have studied the interaction between cortisol and serum albumin. The literature pertaining to steroid–protein interactions has been reviewed by Daughaday (7).

Two plasma components appear to be involved in binding cortisol (8–10). At physiologic levels, cortisol is bound predominantly by an α globulin commonly termed "transcortin" or "corticosteroid binding globulin" (8, 10). Transcortin is present in relatively low concentrations, however, and rapidly becomes saturated in its binding ability if plasma cortisol concentrations rise much above normal physiologic levels. Serum albumin has a lesser affinity for binding cortisol than does transcortin but plays a major role in binding at elevated serum cortisol levels (11). Effects of cortisol concentration on its binding to plasma proteins have been discussed by Daughaday (7) and by Bush (12). For the most part, research on the steroid–plasma protein interaction to date has employed equilibrium dialysis procedures (7). Equilibration times have varied between 18 and 72 hr. One important criterion which must be met in such a study is that the solute molecules must be sufficiently stable so that chemical degradation does not occur to a significant extent during the equilibration period. Oesterling and Guttman (13) and Guttman and Meister (14) have studied prednisolone degradation. Their data show that the extent of degradation of the 17-dihydroxyacetone function might be significant during the average equilibrium dialysis experiment. While similar information pertaining to cortisol was not available, it was considered that it too should be subject to similar rates of degradation. This research was initiated to determine whether cortisol would undergo significant degradation under conditions of the equilibrium dialysis experiment and whether such degradation would significantly alter the results of

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The authors wish to dedicate this paper to Dean T. C. Daniels, whose leadership at the University of California has done so much to raise the standards of education and research in the pharmaceutical sciences.