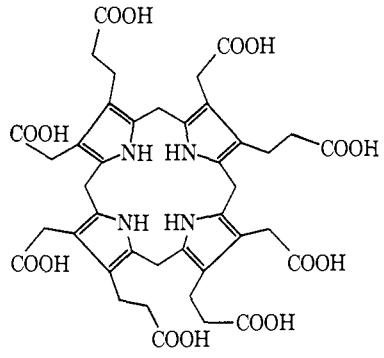
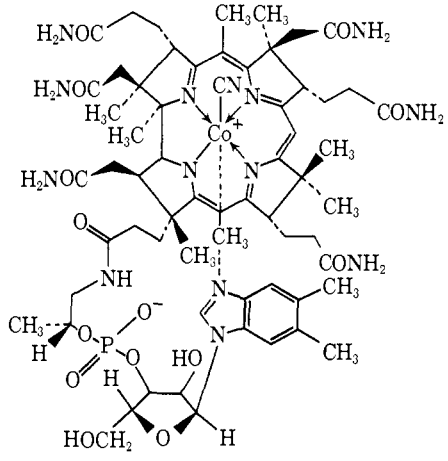
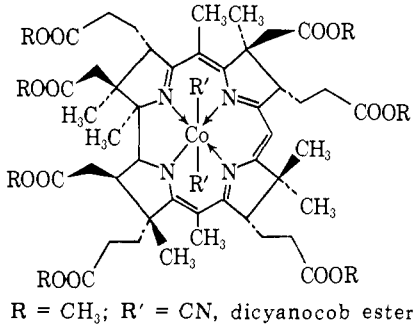


1, uroporphyrinogen III



2, cyanocobalamin



3a, R = CH_3; R′ = CN, dicyanocob ester
b, R = H; R′ = OH, diaquocobyrinic acid

found to be radioactive indicating that the decarboxylative enzymes of urogen metabolism are also present in the cell-free system, in common with similar preparations from bacteria,[10a] avian red cells,[10b] mammalian reticulocytes,[10c] and mouse spleen.[10d] Experiment 4 was carried out with [*methyl*-^{3}H]SAM using [^{14}C]urogen as internal standard. The by-products of the incubation, *viz.* uro, copro, and the partially decarboxylated porphyrins (as their methyl esters), contained ^{14}C but no tritium isotope. On the other hand, repeated crystallization of cobester from experiment 4 gave a constant ^{3}H/^{14}C ratio (in agreement with the separate feeding experiments 3 and 5) providing an internal check that both decarboxylating and methylating systems were operative. Since all of the cell-free assays depend on the purification of cobyrinic acid as the crystalline heptamethyl ester, experiment 4 removes any ambiguity of *in vitro* methyl transfer in the esterification process and also shows that no secondary incorporation of ^{3}H from [*methyl*-^{3}H]SAM occurs during the incubation.

Experiment 5 demonstrates that, in the presence of added ALA, the incorporation of [*methyl*-^{14}C]SAM reaches 36% while the methylating enzyme system is inactivated by boiling (experiment 6).

Using an entirely different assay procedure, the actual biosynthesized corrin was found to be cobyrinic acid (**3b**) (experiment 3). The post-incubation mixture was subjected to phenol extraction after treatment with corrin mixture (as carrier) and the purified solution was separated by electrophoresis (Whatman 3MM and ET 81) and ion exchange paper chromatography (Whatman ET 81). Autoradiographs showed cobyrinic acid to be the only detectable radioactive corrin in all of these separations.

In summary, the stable crude enzyme mixture described in this communication contains the requisite system for converting both ALA and urogen III to cobyrinic acid (**3b**), the prototype of the more complex corrins, in the presence of SAM.

With the establishment of the comparatively rapid assay technique described herein, separation of the component synthetase(s) responsible for the intriguing steps between urogen III and cobyrinic acid is now in progress.

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Received May 26, 1973

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Determination of Relative Glycosyl Bond Strengths in Nucleosides by Chemical Ionization Mass Spectrometry. A Comparative Study of 7- and 9-β-D-Ribofuranosylpurines

Sir:

Several in-depth studies have been recently published of the acid-catalyzed hydrolysis of purine nucleosides.[1-4] It has been suggested[1-4] that these nucleosides hydrolyze by an A-1 mechanism which involves a preequilibrium protonation of the purine followed by a rate-limiting cleavage of the ribosyl–purine bond.

A similar reaction of protonation followed by cleavage of the glycosyl bond has been shown to occur in the vapor phase.[5] The ion–molecule reaction has been studied by chemical ionization mass spectrometry (CIMS)[6,7] and provides a highly effective means of following the reactions of protonated species in the absence of solvent effects, under carefully controlled conditions.

(1) R. P. Panzica, R. J. Rousseau, R. K. Robins, and L. B. Townsend, *J. Amer. Chem. Soc.*, **94**, 4708 (1972).
(2) J. A. Zoltewicz, D. F. Clark, T. W. Sharpless, and G. Grahe, *J. Amer. Chem. Soc.*, **92**, 1741 (1970); J. A. Zoltewicz and D. F. Clark, *J. Org. Chem.*, **37**, 1193 (1972).
(3) L. Hevesi, E. Wolfson-Davidson, J. B. Nagy, O. B. Nagy, and A. Bruylants, *J. Amer. Chem. Soc.*, **94**, 4715 (1972).
(4) E. R. Garrett and P. J. Mehta, *J. Amer. Chem. Soc.*, **94**, 8532 (1972).
(5) M. S. Wilson, I. Dzidic, and J. A. McCloskey, *Biochim. Biophys. Acta*, **230**, 623 (1971).
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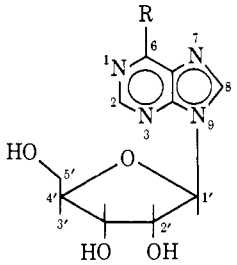
Table I. Principal Ion Abundances; NH_4^+ CIMS of 7- and 9-β-D-Ribofuranosylpurines

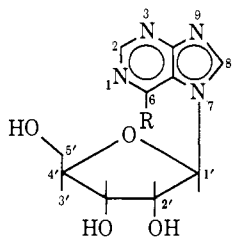
Nucleoside	Rate coefficients[a] (soln), 45°, 10^6k, sec^{-1}	MH^+/BH_2^+	Temp of vaporizn, °C	Base peak, m/e
Nebularine (**1**)	19.6	<2.9[b]	190	253 (MH^+)
7-(β-D-Ribofuranosyl)purine (**6**)	46.3	0.53	190	121 (BH_2^+)
Adenosine (**2**)	4.10	10	230	268 (MH^+)
7-(β-D-Ribofuranosyl)adenine (**7**)	110.0	0.11	230	136 (BH_2^+)
6-Methylamino-9-(β-D-ribofuranosyl)purine (**3**)	4.15	27	195	282 (MH^+)
6-Methylamino-7-(β-D-ribofuranosyl)purine (**8**)	65.6	0.58	195	150 (BH_2^+)
6-Dimethylamino-9-(β-D-ribofuranosyl)purine (**4**)	3.26	37[c]	180	296 (MH^+)
6-Dimethylamino-7-(β-D-ribofuranosyl)purine (**9**)	63.6	0.43[c]	180	164 (BH_2^+)
6-Methylthio-9-(β-D-ribofuranosyl)purine (**5**)	5.26	1.2	187	299 (MH^+)
6-Methylthio-7-(β-D-ribofuranosyl)purine (**10**)	69.0	0.27	187	167 (BH_2^+)

[a] Reference 1. [b] Maximum value due to the presence of protonated purine derived from partial thermal degradation during vaporization, confirmed by thin-layer chromatography of the sample residue. [c] Significant amount of BH^+ also formed; ~50% of BH_2^+.

We have now studied the vapor-phase hydrolysis[8] of nucleosides in order to determine to what extent the primary factors which govern rates of hydrolysis in solution are also reflected in the vapor phase. This initial investigation has dealt with the isomeric 7- and 9-β-D-ribofuranosylpurines **1–10**, primarily because an



1, R = H
2, R = NH_2
3, R = $NHCH_3$
4, R = $N(CH_3)_2$
5, R = SCH_3



6, R = H
7, R = NH_2
8, R = $NHCH_3$
9, R = $N(CH_3)$
10, R = SCH_3

earlier detailed study[1] has provided relative rates, rate coefficients, and kinetic parameters for solution hydrolysis. More important, the relative stabilities of the glycosyl bonds for the 7- and 9-β-D-ribofuranosylpurines can be directly compared in the analogous vapor-phase process. This comparison is simplified by suitable choice of reagent gas such that competing reaction pathways are suppressed. Ammonia was chosen because of its relatively high proton affinity (207 kcal/mol).[9] Consequently, only the protonated molecular ion and the protonated free base (BH_2^+) fragmentation product are observed as CIMS products.

In solution hydrolysis, the solvent serves as the complete source of *all* labile protons. On the other hand, in the gas phase only the initial proton is derived from the reagent gas; results from isotopically labeled reagent gases and nucleosides[5,10] have shown that the second proton is from the sugar hydroxyl groups, in analogy to similar reactions in the electron ionization mass spectra of nucleosides.[11] Examination of Corey–Pauling–Koltun (CPK) models shows that during this step a hydrogen from O-2′ or O-5′ can be transferred to the base without ring opening in the series **1–5** while for the isomers **6–10** severe restrictions on rotation preclude transfer from O-5′ but not from O-2′.

Ion abundance data from the present study and selected hydrolysis rate data from the earlier solution study[1] are given in Table I. Three isomeric pairs from the previous work are excluded. Neither 7- nor 9-(β-D-ribofuranosyl)purine-6-thione exhibited protonated molecular ions of significant abundance using methane or ammonia reagent gases, making it impossible to determine relative glycosyl bond stabilities as represented by the ratios MH^+ (M = nucleoside molecule)/BH_2^+. Attempts to vaporize 7- and 9-(β-D-ribofuranosyl)guanine as well as inosine and its isomer 7-(β-D-ribofuranosyl)hypoxanthine without thermal degradation were unsuccessful due to their low volatility, a common problem with the more polar nucleosides.[12]

The mass spectrometer used was a Varian CH7 instrument, extensively modified for high-pressure operation.[13] Conditions of operation were: accelerating potential, 2 kV; ionizing electron energy, 2.5 kV; repeller voltage, 0.00 V; reagent gas pressure, 0.5 Torr. All samples (1–10 μg) were introduced into the mass spectrometer by direct probe, at minimum ion source temperatures required for vaporization, with the provision that members of each 7- and 9-isomeric pair were vaporized at the same temperature (Table I). Prolonged heating of nucleosides in the ion source in the presence of ammonia produces partial chemical degradation to the corresponding free base. Measurements of ion abundance were therefore made during the period in which a constant ratio of MH^+/BH_2^+ was obtained in the early stages of vaporization.

The most important and useful comparisons afforded by the chemical ionization and solution hydrolysis data are those of the corresponding 7 and 9 isomers. In analogy to their behavior in solution, the 7 isomers which were examined show a greater tendency to fragment than the 9 isomers. Relative glycosyl bond stabilities are therefore clearly reflected in reactions represented in CIMS, in spite of certain differences in the reaction mechanisms involved and basic experimental

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(11) J. A. McCloskey and K. Biemann, *J. Amer. Chem. Soc.*, **84**, 2005 (1962).
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conditions employed. In addition, although at this time the site of attachment of the glycosyl bond to the heterocyclic aglycon (7 *vs.* 9) cannot be established by mass spectrometry if only a single compound is available, the differentiation of isomers does appear feasible on the basis of the data obtained (Table I). It would further appear that CIMS may prove to be useful in predicting the relative rates of hydrolysis of closely related compounds in solution. Particularly advantageous are the speed and simplicity of data acquisition; moreover, much less material is required.

Further refinements in understanding the details of these reactions, as well as the prediction of substituent effects in both the vapor phase and in solution, will require more detailed knowledge of the site(s) of protonation and of the activation energies of the dissociation reactions.

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Structure and Reactivity Relationships in Chromium Carbonyl Photoassisted Hydrogenation of 1,3 Dienes

Sir:

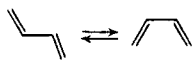
We report herein product studies, relative rates, and selectivity experiments which implicate the importance of the s-cis conformation of 1,3 dienes which undergo hydrogenation under the conditions indicated in reaction 1. Further, our preliminary results reveal that the role of the uv light in reaction 1 is to generate a

$$\text{1,3 diene} \xrightarrow[\substack{\text{uv light}\\ \text{Cr(CO)}_6\text{ (catalytic amount), hydrocarbon solvent}\\ 10^\circ}]{H_2,\ 1\text{ atm}} \text{alkene} \qquad (1)$$

thermally active catalyst *via* photolysis of $Cr(CO)_6$ in the presence of H_2 and 1,3 dienes. The $Cr(CO)_6$ photoassisted hydrogenation of 1,3-cyclohexadiene and 2,3-dimethyl-1,3-butadiene has been reported to give, in synthetic yields, only cyclohexene and 2,3-dimethyl-2-butene, respectively.[1] Thermally catalyzed hydrogenation of 1,3 dienes using (arene)$Cr(CO)_3$[2] or $Fe(CO)_5$[3] requires both elevated temperatures and high H_2 pressure in marked contrast to the mild conditions of reaction 1.

That 2,3-dimethyl-1,3-butadiene yields only 2,3-dimethyl-2-butene reveals that 1,4 hydrogenation has been effected. The generality of this mode of addition is demonstrated by the products obtained from a series of 1,3 dienes:[4] *trans*-1,3-pentadiene yields *cis*-2-pentene, 2-methyl-1,3-butadiene yields 2-methyl-2-butene, and *trans,trans*-2,4-hexadiene gives *cis*-3-hexene. Additionally, when H_2 is replaced by D_2 in reaction 1 we find that *trans,trans*-2,4-hexadiene yields 2,5-dideuterio-*cis*-3-hexene as the exclusive product. The products indicated can be obtained in essentially quantitative yield. No gas chromatographically detectable isomers of the products are obtained with respect to either cis–trans or positional isomers nor does subsequent hydrogenation or isomerization of the alkenes occur under the conditions of reaction 1 using $Cr(CO)_6$.

All of the 1,3 dienes mentioned above have at least one structural feature in common, the ability to easily achieve the s-cis conformation, reaction 2. The sig-



(2)

nificance of this observation is first demonstrated by considering the relative rates of hydrogenation of 2-methyl-1,3-butadiene, *trans*-1,3-pentadiene, and *cis*-1,3-pentadiene under identical conditions. Of these three 1,3 dienes only *cis*-1,3-pentadiene cannot easily achieve the s-cis conformation and it undergoes H_2 addition at an initial rate of less than one-tenth that of the other two. Even for *cis*-1,3-pentadiene, though, the product obtained is *cis*-2-pentene, consistent with 1,4 addition to an s-cis diene, and may actually be due to the selective hydrogenation of the *trans*-1,3-pentadiene which was present as a small impurity in the *cis*-1,3-pentadiene (*vide infra*). We observe that photoassisted *cis- to trans*-1,3-pentadiene conversion using $Cr(CO)_6$ does not occur on the same time scale under the hydrogenation conditions of reaction 1.

A set of selectivity experiments, Table I, lends even more impressive evidence supporting the implication that the s-cis conformation must be available for the $Cr(CO)_6$ photoassisted hydrogenation to occur. Hydrogenation of mixtures containing equal amounts of one diene which can easily achieve the s-cis conformation and one which cannot invariably yields selective hydrogenation of the s-cis diene. Particularly convincing is the fact that essentially total disappearance of *trans*-1,3-pentadiene to yield only *cis*-2-pentene is achieved in the presence of an equimolar amount of *cis,cis*-2,4-hexadiene, and little or no hexene products are formed.

The conformation effect established by the information in Table I is of practical value, and the product

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(4) The $Cr(CO)_6$ photoassisted hydrogenations were generally carried out in either benzene or isooctane solutions of the diene ($\sim$0.1 *M*) and $Cr(CO)_6$ (10^{-3}–10^{-2} *M*) at 10°. The solutions were thoroughly deoxygenated and then simultaneously stirred, exposed to uv light (300–380 nm), and subjected to 1 atm of H_2. Analysis of dienes and alkenes was by vpc where authentic samples of all C_5 and C_6 olefins could be separated; a 25 ft × 1/8 in. 25% $\beta\beta'$-ODPN column at 25° was used. The hydrogenation product of *trans,trans*-2,4-hexadiene has an nmr spectrum superimposable with an authentic sample of *cis*-3-hexene. The 2,5-dideuterio-*cis*-3-hexene was identified by its 100-MHz deuterium decoupled proton nmr.