Electron Spin Resonance Study of Hydrogen-Addition and -Replacement Reactions in Some Pyrimidine Compounds[1]

Janko N. Herak[2] and Walter Gordy

Contribution from the Department of Physics, Duke University, Durham, North Carolina 27706. *Received February 6, 1967*

Abstract: Various derivatives of uracil in powdered form were exposed to gaseous hydrogen atoms, and the free radicals thereby produced in the samples were identified from their electron spin resonances. Only free radicals characteristic of hydrogen-bombarded uracil were observed for 5-nitrouracil and uracil-5-carboxylic acid. This indicates that the NO_2 or COOH group is first replaced by hydrogen atoms to form uracil, which in turn adds hydrogen to form the observed radical. In like manner, it was found that the COOH group in uracil-6-carboxylic acid and the NH_2 group in 6-aminouracil are also replaced by hydrogen atoms. In contrast, 5-aminouracil, uracil-5-sulfamic acid, and 5-acetyluracil were observed to form mainly direct hydrogen-addition radicals, with the addition occurring on C_6, although the substitution reaction also occurred to a lesser degree. Only radicals formed by direct hydrogen addition on C_5 were observed for 6-methyluracil. Proton couplings for the various radicals were measured.

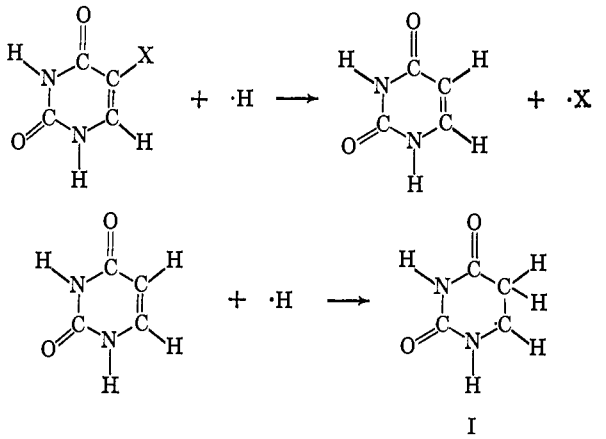
Earlier studies with electron spin resonance (esr) have shown that gaseous hydrogen atoms at thermal energies add directly to purine and pyrimidine constituents of the nucleic acids.[3-5] Similar investigation with 5-halouracils[6] revealed that the hydrogen atom does not add directly to the 5-bromouracil and 5-iodouracil, but simply replaces the halogen to form uracil. This fact was proved by esr detection of radicals formed by hydrogen addition to the resulting uracil. Thus it was found that esr could be used for study of replacement reactions as well as hydrogen-addition reactions in such compounds. At room temperature, 5-chlorouracil showed direct addition radicals, whereas at lower temperatures chlorine replacement was observed. These observations prompted the study of the reactions of gaseous hydrogen atoms with a wider range of pyrimidine compounds. Pyrimidines containing attached methyl, amino, nitro, carboxyl, acetyl, or sulfamino groups were examined. In most of the compounds either direct addition of the hydrogen to the ring or replacement of the attached group by the hydrogen was observed.

Experimental Procedure

Various pyrimidine derivatives obtained from commercial sources were used without further purification. They were bombarded in powdered form with hydrogen atoms produced in an electric discharge of hydrogen gas. Most of the bombardment was done at a temperature of about 200°K (Dry Ice), although some experiments were also performed at both 77 and 300°K. After bombardment at reduced temperatures, the samples were kept under vacuum at liquid nitrogen temperature until observed.

Replacement Reactions

Figure 1 shows the esr spectra of some hydrogen-bombarded uracil derivatives with the spectrum of similarly bombarded uracil shown for comparison. All the compounds were evacuated and were subjected to hydrogen atoms at Dry Ice temperature, which was then lowered to 77°K for the observation. The spectral pattern of each of the derivatives is the same, within the accuracy of the observation, as that of uracil. We therefore conclude that the hydrogen atoms react with these derivatives to produce uracil molecules and that other hydrogen atoms add to these molecules to produce the observed radicals. For the 5-halouracils, the reactions can be represented by



I

Since the X radical is not observed, it is probably eliminated by the reaction X + H → XH. In the uracil derivatives of Figure 1, 5-nitrouracil and uracil-5-carboxylic acid, the X group is NO_2 and COOH, respectively.

The esr pattern characteristic of hydrogen-bombarded uracil was also obtained when uracil-6-carboxylic acid and 6-aminouracil were bombarded with hydrogen atoms at 200°K and observed at 77°K, as is shown by the two lower curves of Figure 1. Thus hydrogen replacement of the COOH group and the NH_2 group on C_6 of uracil is likewise proved. It is hardly conceivable that the uracil-like pattern could be produced in these various compounds without a replacement of the X group by hydrogen.

When 5-nitrouracil and uracil-5-carboxylic acid (previously bombarded at 200°K) were warmed to room temperature, the pattern characteristic of the H–uracil radical at room temperature was obtained. In contrast, the corresponding signals from 6-aminouracil

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(2) On leave of absence from Institute Ruder Boskovic, Zagreb, Yugoslavia.

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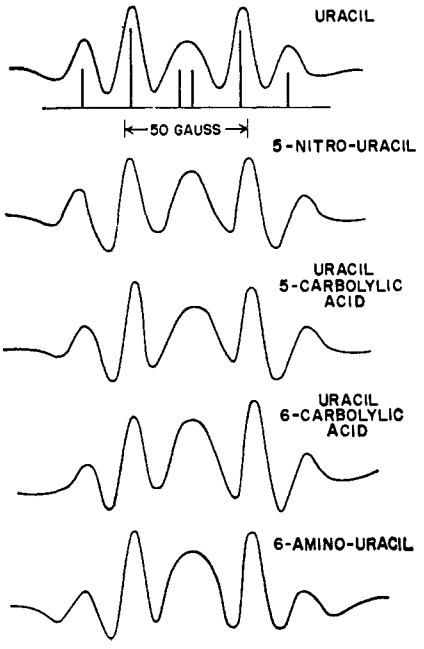
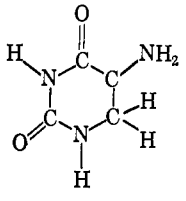


Figure 1. Electron spin resonance (second derivative curves) of hydrogen-bombarded uracil and of various uracil derivatives exposed at 200°K (observed at 77°K).

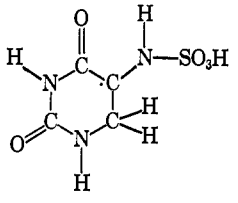
and from uracil-6-carboxylic acid disappeared when the samples were warmed to 300°K. Apparently the latter compounds react quickly with the H–uracil radicals at room temperature, whereas the 5-nitrouracil and uracil-5-carboxylic acid do not.

Hydrogen-Addition Reactions

Unlike 6-aminouracil, 5-aminouracil formed direct hydrogen-addition radicals when subjected to thermal hydrogen atoms. This is shown by the top curve of Figure 2, which should be compared with the bottom curve of Figure 1. The triplet for 5-aminouracil in Figure 2 is that to be expected from the hydrogen-addition radical (II), with the triplet splitting arising from



II



III

the two equally coupling C_6 protons and with broadening from the NH_2 coupling only. The triplet was the predominant signal observed, although a noticeable component arising from the H-uracil radical could be produced by more prolonged bombardment.

The triplet observed for hydrogen-bombarded uracil-5-sulfamic acid (lower curve of Figure 2) is attributed to radical III, which is formed by direct hydrogen addition to C_6. A weaker component of the H–uracil radical could be detected when the sample which had been bombarded at 200°K was observed at 77°K. Like 5-aminouracil, uracil-5-sulfamic acid gives evidence of some substitution of the H for the X group, but the predominant reaction in both is direct hydrogen addition on C_6 without replacement of the X group.

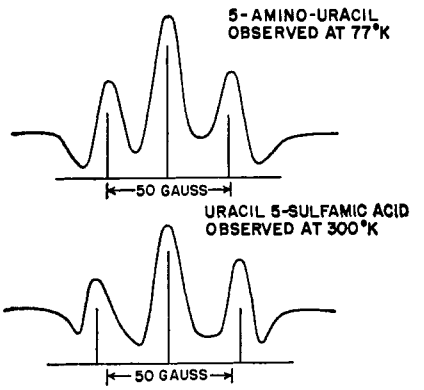


Figure 2. Electron spin resonance of hydrogen-addition radicals in 5-aminouracil and uracil-5-sulfamic acid (200°K).

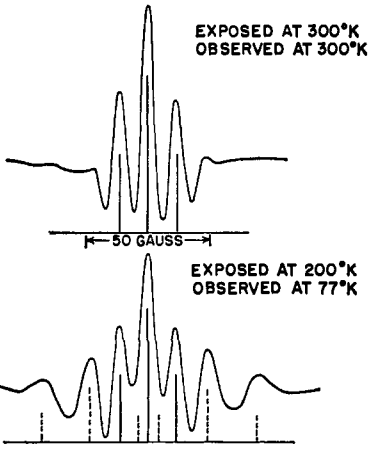
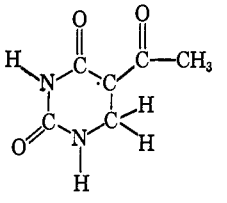


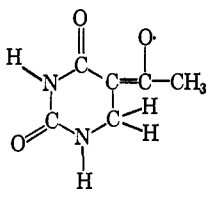
Figure 3. Electron spin resonance of hydrogen-bombarded acetyluracil.

There is a slight asymmetry in the triplet for uracil-5-sulfamic acid, indicating an asymmetry in the g tensor which may arise from a small spin density on the sulfur. This asymmetry is more noticeable when the observations are made at 77°K.

When exposed to hydrogen atoms at room temperature, 5-acetyluracil formed mainly the direct addition radicals indicated by structure IV. However, when 5-



IV



V

acetyluracil is subjected to hydrogen atoms at 200°K, hydrogen substitution for the acetyl group occurred almost as often as direct addition to C_6. The top curve of Figure 3 represents the triplet of radical IV. Its components are noticeably sharper and are more closely spaced than are the triplets of Figure 2. The smaller triplet splitting is probably due to the significant spreading of the π orbital of the unpaired electron onto the acetyl group through conjugation with the CO of the acetyl group, as indicated by structure V. This spreading would diminish the spin density on the ring and

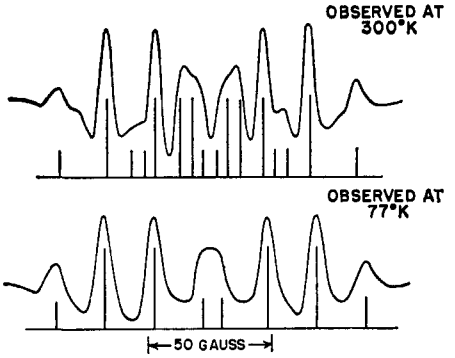


Figure 4. Electron spin resonance of 6-methyluracil after exposure to hydrogen atoms at 200°K.

hence the coupling of the CH_2 group at position 6. The extra sharpness of the lines is caused by the absence of unresolved hyperfine structure which arises from the amino groups in radicals II and III. A comparison of the various proton splittings is given in Table I.

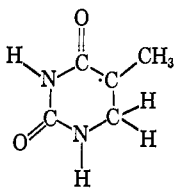
Table I. Observed Proton Couplings in the Various Radicals

H-bombarded compd	Radical	T, °K	Coupling, gauss >$C_\beta H_2$	Coupling, gauss $-C_\beta H_3$
Uracil[a]	I	300	$a_1 = a_2 = 33$	
5-Aminouracil	II	77	$a_1 = a_2 = 24$	
Uracil-5-sulfamic acid	III	77 or 300	$a_1 = a_2 = 28$	
5-Acetyluracil	IV	77 or 300	$a_1 = a_2 = 11$	
5-Methyluracil (thymine)[b]	VI	300	$a_1 = a_2 = 38$	20.5
		77	$a_1 = 34, a_2 = 42$	20.5
6-Methyluracil	VII	300	$a_1 = 20.5, a_2 = 39.5$	19
		77	$a_1 = 0, a_2 = 65$	19

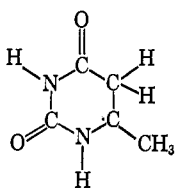
[a] Uracil formed by hydrogen-substitution reactions in 5-nitrouracil, or in uracil-5-carboxylic acid. The same coupling is observed for hydrogen-bombarded powdered uracil observed at 300°K. The coupling for the $C_\alpha H$ proton of the uracil radical is 18.5 gauss at 300°K. [b] From ref 3.

The lower curve of Figure 3 demonstrates that both addition reactions and substitution reactions occur in 5-acetyluracil at 200°K. Although the curve shown was observed at 77°K, the H–uracil component formed at 200°K was found to remain the same when the sample was warmed to room temperature.

That thymine (5-methyluracil) forms the hydrogen-addition radical VI when exposed to gaseous hydrogen atoms has been shown before.[3] Figure 4 demonstrates that 6-methyluracil, when similarly exposed to hydrogen atoms, forms the corresponding radical VII. The



VI

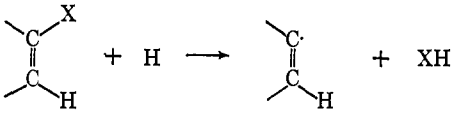


VII

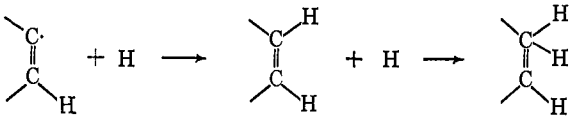
upper curve of Figure 4 represents the esr observed for the sample at room temperature. The CH_2 protons are not symmetrically oriented relative to the π orbital and therefore have unequal couplings of 29.5 and 39.5 gauss with the electron spin. This results in a splitting of the resonance into four equally strong components, each of which is split into a quartet by the CH_3 group, thus making a total of 16 lines, as indicated by the bars. It is of interest that the coupling by one of the CH_2 protons becomes so small that its splitting cannot be resolved when the temperature is lowered to 77°K, whereas the coupling by the other becomes very large, 65 gauss. To make its splitting unresolvable, one of the protons of the ethylene group must be essentially in the plane of the ring; the proton with the 65-gauss coupling must be essentially in the plane of the π orbital of the unpaired electron. The three methyl protons have an equal coupling of 19 gauss at 77 and 300°K. This indicates that the spin density on C_5 is essentially unchanged by the cooling process despite the reorientation of the CH_2 group and that the CH_3 group rotates about the C_6–CH_3 bond even at 77°K. The methyl couplings which are listed in Table I indicate a π spin density on C_5 of $\rho_5 = 0.66$ for the 6-methyluracil as compared with $\rho_6 = 0.71$ for the 5-methyluracil radical.

Comments

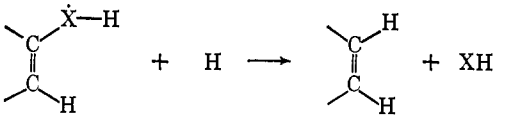
The above results prove that the C–N bond in amino- and nitropyrimidines can be broken by a hydrogen atom and related groups replaced by hydrogen. In the same way, the C–C bond is found to be broken in carboxyl- and acetyl-substituted pyrimidines, but not in methyl-substituted ones. However, the sequence of reactions which lead to the observed radicals is not necessarily that of direct replacement: the replaced group might first be abstracted by an H atom, as, for example



Other H atoms could then react with the unstable primary radical



to produce the observed H–uracil radical. In some of the compounds the hydrogen atom may first become attached to the abstracted group to form a complex which would, in turn, form the uracil as follows.



Since we observe only the final H–uracil radical, we cannot learn the mechanism of the substitution. If intermediary radicals are involved in the process, they are too short-lived to be observed.

The major change of energy is due to the replacement of the C–N or C–C bond by the C–H bond. The fact that the C–H bond is appreciably stronger (98.8 kcal/mole) than either the C–N bond (69.7 kcal/mole) or the C–C bond (83.1 kcal/mole) makes the replacement

energetically possible. Actually, one must take into consideration the differences in energy in the π-electronic system too, but in these compounds they are smaller than the differences between the σ-bond energies. We cannot explain why in some cases only radicals formed by a replacement reaction are observed, whereas in other cases predominantly, or only, radicals formed by hydrogen addition on unsubstituted carbons are observed. Structural indices, bond orders, and free valences are of little help in the explanation. We do not know whether some of the compounds are in the lactam (2,4-dioxy) or lactim (2,4-dihydroxy) form. Whatever the form assumed, the replaceable substituted groups were never attached to the carbons with the largest free valence. In all cases where hydrogen addition occurs, the preferable places are the unsubstituted carbons C_5 or C_6.

The observed uracil-like hydrogen-addition radicals can be attributed to hydrogen addition on either C_5 or C_6 after the replacement. In the lactam form, both electronic indices and localization energies favor hydrogen addition on C_5 of the uracil.[7] Our experiments on 5-iodouracil proved that the hydrogen addition does occur on C_5. For the lactim form there is no experimental evidence. The free valence is slightly larger for C_5 than for C_6, but the localization energies are in favor of addition on C_6—2.55β for C_6 and 2.58β for C_5. The bond orders, free valences, and localization energies for these compounds were calculated in Hückel's approximation of molecular orbital theory, outlined by Pullman and Pullman.[8]

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Electron-Transfer Processes. VII. Formation of Paramagnetic and Condensation Products from Nitrosobenzene in Basic Solution[1]

Glen A. Russell, Edwin J. Geels, F. J. Smentowski, K.-Y. Chang, James Reynolds, and Gerd Kaupp

Contribution from the Department of Chemistry, Iowa State University, Ames, Iowa 50010. *Received October 22, 1966*

Abstract: The formation of nitrosobenzene radical anions and nitroxide-type radicals from nitrosobenzene is considered. In the presence of strong base nitrosobenzene forms the radical anion. The radical anion can be detected in appreciable quantity from mixtures of nitrosobenzene and phenylhydroxylamine in basic solution, and it is argued that the radical anion is an intermediate in the condensation reaction yielding azoxybenzene. The oxidative coupling of aniline by base and oxygen to yield azobenzene is considered and data are presented to show that the coupling proceeds *via* nitrosobenzene as an intermediate. Reactions of nitrosobenzene and phenylhydroxylamine in the absence of added base are shown to involve the formation of phenyl nitroxide. It is suggested that nitrosobenzene can also give rise to a stable species, $C_6H_5N(O\cdot)ONHC_6H_5$, previously assigned the $C_6H_5\dot{N}OH$ structure.

Nitrosobenzene can be converted to a number of paramagnetic species. Photolysis in tetrahydrofuran solution,[2] or reaction with phenylmagnesium halides[3] produces diphenyl nitroxide ($(C_6H_5)_2NO\cdot$). The product of photolysis in tetrahydrofuran may be a result of photolysis of the nitroso dimer.[4a] In dimethyl sulfoxide (DMSO) solution photolysis yields a mixture of phenyl nitroxide and diphenyl nitroxide, perhaps as a result of photolysis of monomeric and dimeric species. Alkylphenyl nitroxides can be prepared by photolysis of mixtures of nitrosobenzene and alkylnitroso compounds with red light.[4b] Upon heating, nitrosobenzene is reported to react with 2,3-dimethyl-2-butene to yield a radical assigned the structure $C_6H_5N(O\cdot)C(CH_3)_2C(CH_3)=CH_2$.[5]

Reduction of nitrosobenzene in strongly basic solutions,[6] or electrochemically,[7] produces the nitrosobenzene radical anion ($C_6H_5NO\cdot^-$) while reduction with titanous ion in aqueous acid yields phenyl nitroxide ($C_6H_5NHO\cdot$).[6] The nitrosobenzene radical anion is also produced by the action of strong bases with nitrosobenzene, particularly in DMSO solution,[8] by the reaction of traces of oxygen with phenylhydroxylamine in basic solution,[9] or by electron transfer between nitrosobenzene and phenylhydroxylamine in basic solution (Figure 1A).[9] In the absence of added base, mixtures of nitrosobenzene and phenylhydroxylamine yield phenyl nitroxide (Figure 1B).[10] Phenyl nitroxide is

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