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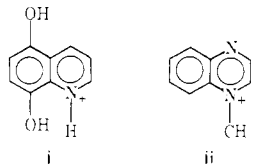

i ii

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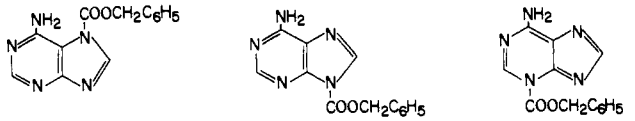
Unequivocal Structural Assignments of N^7- and N^9-Acyladenines

Sir:

Reaction products of chloroformates with adenine have been used as intermediates in the synthesis of naturally occurring ureidopurine derivatives.[1,2] The reaction of adenine with benzyl chloroformate was studied by Altman and Ben-Ishai[3] who obtained two products under different conditions, and for which they suggested the structures benzyl 6-aminopurine-7-carboxylate (I) and benzyl 6-aminopurine-9-carboxylate (II) on the basis of their chemical properties and IR and UV spectral data. The former (I) had UV absorption maximum



(I) (II) (III)

(λ_{max} (CH_3CN)) at 291 nm and the latter (II) at 254 nm. These reactions were later reinvestigated by Dyer and coworkers[4] who concluded that the product II with λ_{max} at 254 nm is indeed benzyl 6-aminopurine-9-carboxylate (II), whereas the other isomer (λ_{max} 291 nm) is, in fact, a 3-carboxylate (III) and not a 7-carboxylate (I). Their reassignment of the structure of the second isomer was based on the known occurrence of N-3 to N-9 alkyl migration of 3-alkyladenine derivatives[5] and on the observation that this compound underwent a facile conversion to the 9-carboxylate. Further, similar to 3-alkyladenines, this compound (λ_{max} 291 nm) showed a large difference (31 Hz) between the shifts of 2 and 8 protons of the purine ring in the NMR spectrum and a relatively high value for the UV absorption maxima.[6]

Several N-acylated purine derivatives have been synthesized in our laboratory,[7] and we were interested in determining the correct structures of these two reaction products of adenine with benzyl chloroformate for an unequivocal assignment of

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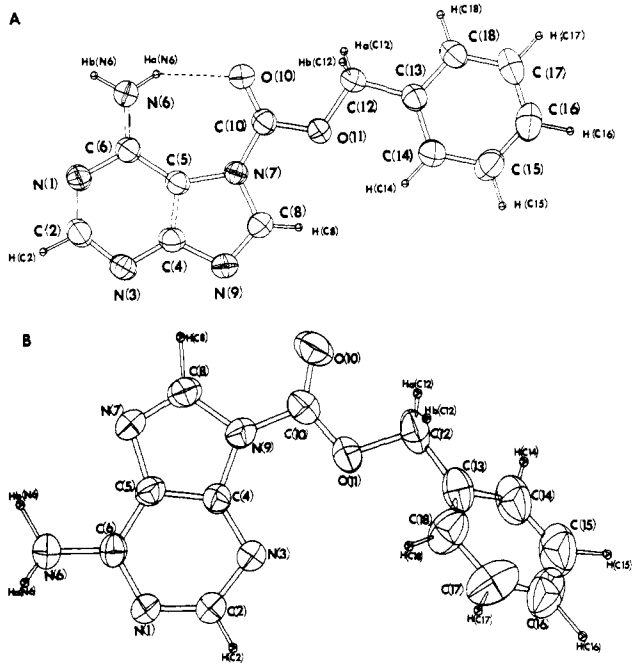

Figure 1. (A) An ORTEP drawing of benzyl 6-aminopurine-7-carboxylate (I). Note the intramolecular hydrogen bonding from the NH_2 to the acyl oxygen. (B) An ORTEP drawing of benzyl 6-aminopurine-9-carboxylate. There are two molecules, **1** and **2**, in the asymmetric unit with slightly differing conformations and the torsion angles O(11)-C(12)-C(13)-C(14) for **1** and **2** are, respectively, ±54.7 and ±45.2°. This figure shows molecule **2**.

Table I. Spectral Data and Physical Properties of Benzyl 6-Aminopurine-7-carboxylate (I) and Benzyl 6-Aminopurine-9-carboxylate[a,b] (II)

	I	II
UV, λ_{max} (CH_3CN), nm (log ϵ)	289 (3.75) [lit.[3] 291]	252 (4.12) [lit.[3] 254]
UV, λ_{max} (aqueous CH_3OH), nm (log ϵ)	288 (3.80)	253 (4.16)
UV, λ_{max} (0.1 N HCl), nm[c] (log ϵ)	273 (4.01)	253 (4.17)
NMR, δ[d]	2-H, 8.33; 8-H, 8.88 ($\Delta\delta_{H_8-H_2}$ = 0.55)	2-H, 8.30; 8-H, 8.57 ($\Delta\delta_{H_8-H_2}$ = 0.27)
mass spectrum	M^+· 315	M^+· 315
IR, ν_{max} (KBr), cm^{-1}	3480 (NH_2), 1740 (C=O), 1640, 1580, 1560 (C=C, C=N)	3510 (NH_2), 1755, (C=O), 1650, 1595, 1560 (C=C, C=N)
mp, °C	157-158 [lit.[3] 158]	165-166 [lit.[3] 162]

[a] Both of these compounds had C, H, and N analyses within ±0.4% of the calculated values. [b] Apparently, no synthesis of a 3-carboxylate has been reported. [c] In alkaline pH both these compounds underwent a rapid degradation to adenine. [d] NMR spectra were run in a Varian XL-100 instrument immediately after dissolving in Me_2SO-d_6.

the structures of our compounds. Our investigation using x-ray crystallographic techniques unambiguously establishes the first product (λ_{max} 289 nm) as benzyl 6-aminopurine-7-carboxylate (I) and the second isomer (λ_{max} 252 nm) as benzyl 6-aminopurine-9-carboxylate (II), as was earlier suggested by Altman and Ben-Ishai.[3] During the course of our study, we also confirmed the observations of Dyer et al.[4] that I undergoes a facile transformation to II on warming in a solution of anhydrous Me_2SO for a short time.

Reaction of adenine with benzyl chloroformate in ethyl acetate in presence of aqueous potassium acetate at room temperature yielded the product I and the same reaction in absolute EtOH in presence of NaOEt furnished II.[3] The spectral data obtained by us on these two products are summarized in Table I. When I was warmed in Me_2SO at 60 °C for 30 min, this product underwent a facile transformation to II. The two products I and II could be readily distinguished by the large difference in the $\Delta\delta$ values (Table I) between the 2-H and 8-H of the purine ring in the NMR spectra of these compounds. Large crystals of I and II, suitable for x-ray analysis, were obtained by recrystallizing them from toluene and chloroform, respectively.

Table II. Crystallographic Data of Benzyl 6-Aminopurine-7-carboxylate (I) and Benzyl 6-Aminopurine-9-carboxylate (II)[a]

	I	II
a, Å	25.448 (9)	12.426 (4)
b, Å	6.052 (1)	6.528 (2)
c, Å	16.975 (6)	30.580 (5)
β, deg	112.05 (5)	
systematic absences	hkl: $h + k$ odd $h0l$: l odd (h, odd)	$h0l$; h odd $0kl$; l odd
space group	$C2/c$	$Pca2_1$
Z	8	8
ρ_{calcd}, g/cm^3	1.48	1.44
ρ_{measd}, g/cm^3	1.49	1.45
λ, Å (Cu $K\alpha_1$)	1.54018	1.54018
no. of reflections ($>2\sigma$)	2545	2557
R	0.068	0.058

[a] For I, $|F(\bar{8}07)|_{obsd}$ is not, as demanded by the space group, equal to zero, but has a value of 15.16. If the structure of I is refined in $C2$, the calculated value of $|F(\bar{8}07)|$ is 12.06, R = 0.066, but such a refinement is invalid (see ref 10). In II, the two independent molecules are related by an approximate center of inversion. Attempts to solve or refine the structure in *Pcam* were not fruitful. These difficulties would not change the molecular structure or conformation, but would affect the accuracy of the bonds and angles.

Using a GE XRD-6 diffractometer equipped with Cu Kα radiation, the unit cell constants (Table II) and the intensity data were obtained following the usual procedure.[8] The crystal structures were solved by direct methods using the program MULTAN[9] and refined by least-squares method to R values of 0.068 for I and 0.058 for II. The hydrogen atoms were located from electron density difference maps and their positional and individual isotropic thermal parameters were also included in the final cycles of refinement.

The molecular structure and conformation of I and II are shown in Figure 1. Both crystals I and II did not contain any solvent of crystallization. I contains an intramolecular N-H··O bond (H··O, 2.04 Å; ∠N-H··O, 145.2°) from the amino nitrogen to the carbonyl oxygen of the acyl group. The base, the acyl group, and the phenyl are all nearly coplanar. Crystals of II contain two independent molecules in the asymmetric unit of slightly differing conformation; the phenyl ring is twisted away from the plane of the base and the acyl group by different amounts (the torsion angles O(11)-C(12)-C(13)-C(14) are ±54.7 and ±45.2° for the two molecules). The details of the structure determination, hydrogen bonding, and stacking will be discussed elsewhere; the lists of atomic coordinates have been deposited (see paragraph at end of paper regarding supplementary material).

This study constitutes the first unequivocal assignment of the structures of N^7- and N^9-acylated adenines. The UV and NMR spectral data may now be used for the assignment of structures of other similar N-acylated purine derivatives.

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Supplementary Material Available: A listing of atomic coordinates for crystals I and II (6 pages). Ordering information is given on any current masthead page.

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Paramagnetic Carbon Monoxide Adducts of Reduced Iron-Sulfur Tetramers: Relevance to the Mechanism of Action of Hydrogenase

Sir:

The reversible oxidation of molecular hydrogen and reduction of protons catalyzed by the bacterial enzyme hydrogenase is conceptually the simplest reaction in biochemistry. Despite the apparent simplicity of the reaction, the detailed mechanism of action has yet to be elucidated. Studies on the enzyme-catalyzed equilibration of H_2 and D_2O demonstrated that molecular hydrogen was cleaved heterolytically,[1] suggesting that the enzyme induces an inequivalence of the two hydrogen atoms of bound H_2. It has been shown[2,3] that a preparation of hydrogenase from *Clostridium pasteurianum*, containing 4Fe and $4S^{2-}$ per molecule, possesses as prosthetic group a single tetranuclear iron-sulfur cluster. (A different preparation with a higher Fe-S content has been shown to contain three tetrameric units per molecule[4].) In addition, Erbes et al.[2] demonstrated that the Fe_4S_4 cluster of hydrogenase could exist in three distinct oxidation states related by transfer of one electron, and that carbon monoxide, a competitive inhibitor of H_2,[5] could bind directly to the iron-sulfur cluster in both the most oxidized and reduced states.

We anticipated that CO would also coordinate to nonprotein bound iron-sulfur clusters in the appropriate oxidation states. Salts of $[Fe_4S_4(SR)_4]^{1-}$, isoelectronic with the most oxidized state of hydrogenase, have not yet been prepared,[6,7] while $[Fe_4S_4(SR)_4]^{2-}$, corresponding to the diamagnetic intermediate oxidation state of hydrogenase, shows no evidence from optical spectra for reaction with CO. Consequently, we have investigated the interaction of CO with the reduced iron-sulfur species, $[Fe_4S_4(SPh)_4]^{3-}$, generated in solution by chemical reduction of the dianion with sodium acenaphthalenide.[8] Inasmuch as the intense absorption of acenaphthalene and its radical anion precluded absorbance measurements, we chose to monitor the reaction using EPR spectrometry. As shown in Figure 1, the reaction of CO with $[Fe_4S_4(SPh)_4]^{3-}$ produces

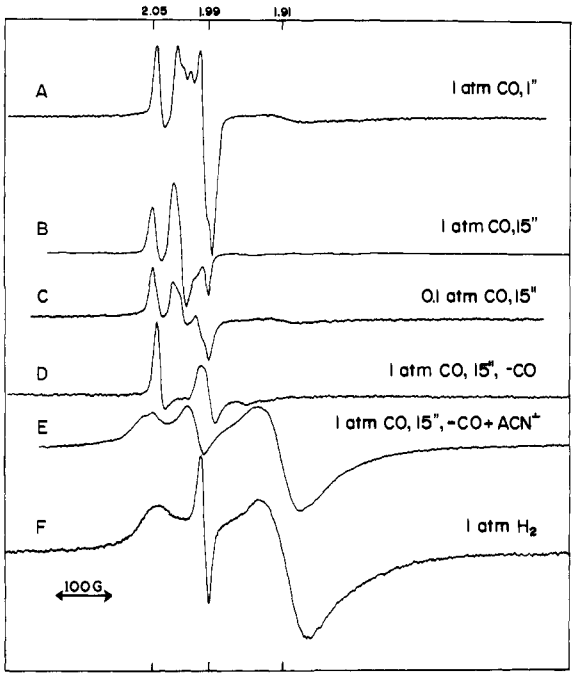

Figure 1. EPR spectra of 0.7–0.8 mM $(Et_4N)_2[Fe_4S_4(SPh)_4]$ in *N,N*-dimethylacetamide treated as follows: A, 1 atm of CO, frozen 1 min after adding 4 equiv of 1 M acenaphthalenide radical ion (ACN^-) in THF; B, same as A, but frozen 15 min after ACN^- addition; C, same as B, but under 0.1 atm of CO; D, same as B, but evacuated after 15 min and CO replaced with argon; E, same as D, but with addition of 4 equiv of ACN^-; F, 1 atm of H_2, frozen 15 min after addition of 4 equiv of ACN^-. The small $g = 2.00$ signal seen in Figure 1F (and also in Figure 2A) is of variable intensity and unknown origin (possibly ACN^-). It is always observed in $[Fe_4S_4(SPh)_4]^{3-}$ samples prepared in this way. The broader signal at $g \sim 2.01$ in Figure 1E may be due to some degradation of the cluster during the cycle. Conditions of EPR spectroscopy: microwave frequency, 9.1 kHz; microwave power, 30 μW; modulation amplitude, 10 G; magnetic field sweep rate, 500 G min^{-1}; time constant, 0.3 s; sample temperature, 10.2 K; instrument gain, 5000 (A), 200 (B), 1000 (C, E), 2000 (D), 3200 (F).

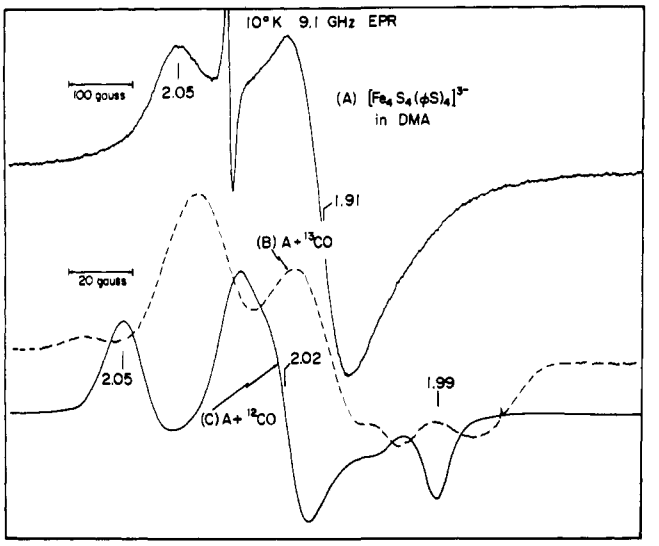

Figure 2. EPR spectra of A, $[Fe_4S_4(SPh)_4]^{3-}$; B, sample as in A, under 0.4 atm of ^{13}CO, frozen at 20 min after ACN^- addition; C, sample as in A, under 1 atm of ^{12}CO, frozen at 15 min after ACN^- addition. Conditions of EPR spectroscopy are as in Figure 1 except the following: magnetic field sweep rate, 100 G min^{-1} (B, C); instrument gain, 1600 (A), 400 (B), 320 (C).

complex EPR spectra which slowly (~15 min) evolve to reach what appears to be an equilibrium mixture, in which ~5–10%

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