Table I. Fractional Atomic Coordinates (× 10^3) and esd's for Nonhydrogen Atoms of Phosphocyclocreatine Molecules. Coordinates for Solvent Molecules (Li^+ and Water) Are Also Listed[a]

	X	Y	Z
C(1)	774 (1)	764 (3)	743 (1)
C(2)	745 (1)	454 (4)	586 (1)
C(3)	817 (1)	453 (3)	614 (2)
C(4)	664 (1)	677 (4)	692 (1)
C(5)	641 (1)	816 (4)	565 (2)
N(3)	827 (1)	681 (3)	696 (1)
N(2)	768 (1)	936 (3)	827 (2)
N(1)	729 (1)	617 (3)	698 (2)
O(1)	932 (1)	780 (2)	637 (1)
O(2)	921 (1)	569 (2)	858 (1)
O(3)	888 (1)	1001 (2)	835 (1)
O(4)	675 (1)	919 (2)	488 (1)
O(5)	588 (1)	939 (4)	569 (2)
P	898 (1)	768 (1)	764 (1)
C(1A)	−773 (1)	−757 (3)	−742 (2)
C(2A)	−749 (1)	−452 (3)	−582 (2)
C(3A)	−813 (1)	−476 (3)	−609 (2)
C(4A)	−665 (1)	−685 (4)	−693 (2)
C(5A)	−639 (1)	−851 (5)	−568 (2)
N(3A)	−825 (1)	−694 (2)	−697 (1)
N(2A)	−770 (1)	−954 (2)	−831 (1)
N(1A)	−726 (1)	−630 (4)	−686 (2)
O(1A)	−935 (1)	−779 (2)	−635 (1)
O(2A)	−920 (1)	−561 (1)	−861 (1)
O(3A)	−889 (1)	−1002 (1)	−838 (1)
O(4A)	−672 (1)	−906 (3)	−479 (1)
O(5A)	−583 (1)	−795 (3)	−549 (1)
P(A)	−898 (1)	−769 (1)	−762 (1)
Li(1)	933 (1)	757 (3)	438 (2)
Li(2)	−935 (1)	−751 (5)	−426 (3)
Li(3)	−001 (2)	497 (3)	748 (3)
Li(4)	475 (1)	592 (5)	157 (3)
O(W1)	979 (1)	755 (1)	100 (1)
O(W2)	−980 (1)	−760 (2)	−103 (1)
O(W3)	553 (1)	678 (3)	893 (2)
O(W4)	473 (1)	001 (3)	693 (2)

[a] The additional designation "A" is for the other molecule in the asymmetric unit and designation "W" for the oxygen atoms of the water molecules.

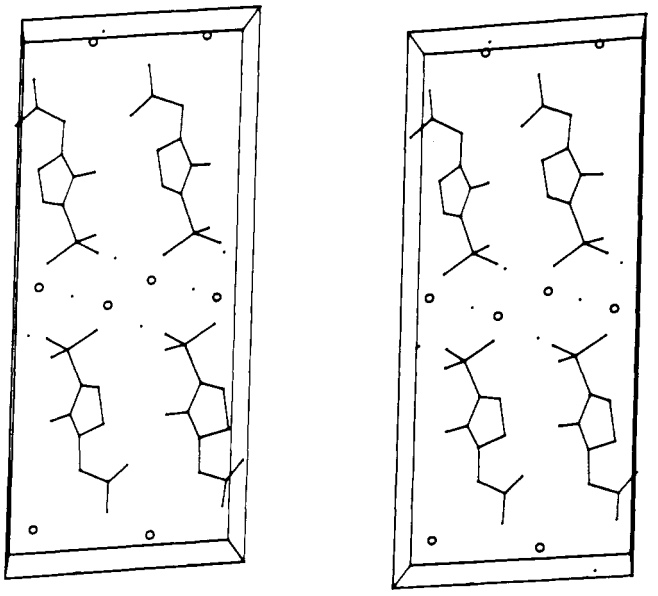

Figure 1. A stereodrawing of the crystal packing of phosphocyclocreatine. The nonbonded solvent atoms are represented as follows: water oxygens, large open circles; Li^+, small filled circles. The unit cell is viewed down the crystallographic x axis.

cated at the center of the cell. The lack of true centrosymmetry is most evident in the region of the carboxymethyl groups.

It is interesting to note that the bond distances and angles of the common structural element of phosphocyclocreatine (this study), phosphocreatine,[10] and creatine[11-12] are not significantly different. Additionally, the phosphocreatine portion in the phosphocyclocreatine compound is similar in conformation to that found in phosphocreatine. This configuration avoids unfavorable steric and electrostatic repulsion between the charged COO^- and PO_3^{2-}.

In reaction 1, which is catalyzed by creatine kinase, phosphorylation of creatine (I) could occur on the free nitrogen either cis or trans to the methyl group. The addition of methylene bridge to form the cyclocreatine II fixes the stereochemistry of the two possible sites of phosphorylation. Since analysis of the enzymatically synthesized phosphocyclocreatine showed the formation of only one isomer,[1,7] establishment of the structure of the active isomer as a 3-phosphono compound III demonstrates unequivocally that creatine kinase catalyzes the stereospecific phosphorylation of creatine at the nitrogen group which is cis to the methyl group. This study further indicates that the positions and angles of the atoms fixed by the ring structure in II are very close to those adopted by creatine in the enzyme-substrate complex.

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Formation of 1-Ethylxanthyl-2,7-diaminomitosene and 1,10-Diethylxanthyl-2,7-diaminodecarbamoylmitosene in Aqueous Solution upon Reduction–Reoxidation of Mitomycin C in the Presence of Potassium Ethylxanthate

Sir:

Mitomycin C is a clinically useful antitumor agent which is produced by several species of *Streptomyces*.[1] Iyer and Szybalski and others have demonstrated that mitomycin C cross links DNA in vivo and, after reduction by sodium dithionite in aqueous buffers, in vitro. An activation mechanism for mitomycin C was proposed by Iyer and Szybalski[2] and by Patrick et al.[3] which was largely based on the acid-catalyzed chemistry of mitomycin C.[4] It was suggested that two electrophilic sites would be generated at positions 1 and 10 in mitomycin C, after loss of the 9a-methoxy substituent, opening

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Table I. UV and IR Spectra of Compounds I and II

UV			
λ_{max}^{MeOH}, nm (ϵ)			
compound I		compound II	
253 (16 400)	315[a] (9400)	253 (19 100)	315[a] (10 700)
279 (18 400)	365 (4300)	279 (14 100)	365 (3400)

IR[b]			
cm^{-1} (KBr)			
compound I		compound II	
3710 (w)	1450 (s)	3710 (w)	1595 (s)
3340 (m)	1205 (s)	3416 (s)	1250 (m)
2340 (w)	1055 (s)	3340 (s)	1205 (m)
1595 (s)	895 (m)	2215 (w)	1080 (s)
1490 (s)	725 (m)	1710 (s)	1040 (s)

[a] Shoulder. [b] s = strong, m = moderate, w = weak.

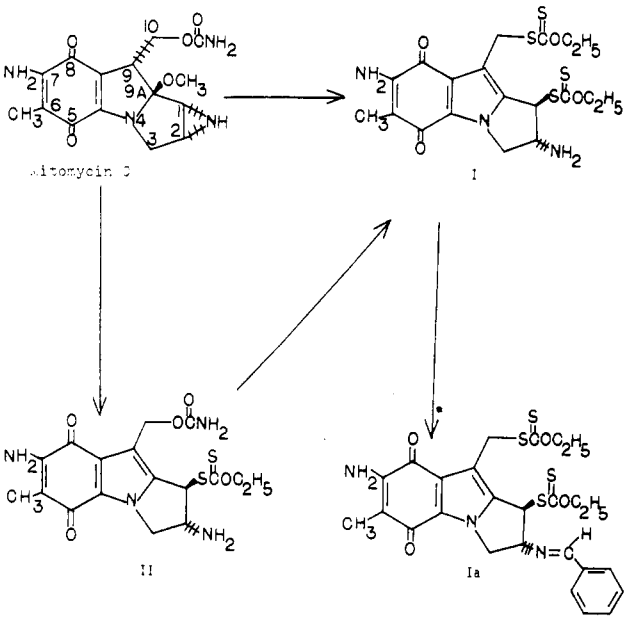
Table II. Field Desorption Mass Spectral Data

compound I			compound II		
	%			%	
m/z	base	likely ion	m/z	base	likely ion
486	35	MH^+	465	100	$[MH_2 + K]^+$
468	100	$[MH - H_2O]^+$	464	92	$[MH + K]^+$
364	49	[M − ethyl-xanthate]$^+$	407	21	$[M - NH_3]^+$
121	9	$[C_3H_5OS_2]^+$	343	10	[MH + K − ethyl-xanthate]$^+$
89	27	$[C_3H_5OS]^+$	29	58	$[C_2H_5]^+$
29	45	$[C_2H_5]^+$			

of the aziridine ring, and release of the carbamate moiety. Concomitant interaction with nucleophiles would lead to the formation of adducts involving carbons 1 and 10. In spite of much experimental effort[1,5] there is a paucity of direct evidence for the proposed mechanism.

Previous studies in this laboratory have been aimed at contributing to the understanding of the chemistry of reduced-reoxidized mitomycin in aqueous solution. These studies led to the isolation of a blue compound, sodium 7-aminomitosane-9a-sulfonate[6] and of red highly polar mitomycin C conversion products of unknown structure. It appeared that $NaHSO_3$ generated from $Na_2S_2O_4$, the reducing agent used for the activation of mitomycin C, reacted as a nucleophile with the reduced antibiotic leading to the formation of highly polar reaction products. The polar nature of these compounds impeded structure elucidation studies. Therefore we explored the addition of nucleophiles to the reaction mixture which when incorporated would produce less polar reaction products. Potassium phthalimide, KCN, KSCN, KCNO, NaN_3, morpholine, Meldrum's acid (sodium salt), and sodium diethylphosphate were not suitable nucleophiles for this system. However, potassium ethylxanthate has proven to be an ideal nucleophile leading to the formation of several relatively volatile and lipophilic reaction products. We here report the structures of two of these reaction products, compounds I and II.

Mitomycin C (50 mg) dissolved in water (50 mL) and cooled to 0–5 °C was purged with nitrogen for 5 min. Cold potassium



ethylxanthate (J. T. Baker) solution (18 mL, 0.05 M) was added, followed by the addition of $Na_2S_2O_4$ (50 mg) freshly dissolved in water (1 mL). The reaction was allowed to proceed for 10 min with nitrogen bubbling and then was stopped by reoxidation with oxygen bubbling for 5 min. The reaction mixture, containing water-insoluble violet-colored material, was extracted three times with ethyl acetate. TLC analysis of the dried and concentrated organic extract showed four major reaction products on silica gel using the system 10:25:25 *n*-octanol–acetone–ligroin (90–115 °C): R_F = 0.11 (mitomycin C), 0.27 (II), 0.74 (I), 0.97, 0.99. Later TLC analysis in other systems showed that the two high R_F spots were composed of several components. In reactions where the pH was closely monitored, it remained between 7.5 and 8.0, and the same family of products was produced. Compounds I and II were also produced when the reaction mixture was reduced for only 30 s and when hydrogen and Pd/C were employed as the reducing agent. In the latter case, substantial formation of un-

Table III. 360-MHz ^1H NMR Data[a] for Compounds I, Ia, and II in $CDCl_3$

compound I	compound Ia	compound II
1.41 (t, 3 H, J = 7 Hz, xanthate CH_3)	1.39 (t, 3 H, J = 7 Hz, xanthate CH_3)	1.45 (t, 3 H, J = 7 Hz, xanthate CH_3)
1.46 (t, 3 H, J = 7 Hz, xanthate CH_3)	1.41 (t, 3 H, J = 7 Hz, xanthate CH_3)	1.74 (br, 2 H, D_2O exchange, C_2 NH_2)
1.80 (br, 2 H, D_2O exchange, C_2 NH_2)	1.85 (s, 3 H, C_6 CH_3)	1.82 (s, 3 H, C_6 CH_3)
1.84 (s, 3 H, C_6 CH_3)	4.36 (dd, 1 H, J = 12, 2 Hz, C_3 H_α)	4.18 (d, 1 H, J = 13 Hz, C_3 H_α)
4.17 (dd, 1 H, J = 12, 2 Hz, C_3 H_α)	4.55 (AB q, 2 H, J = 13 Hz, C_{10} H_2)	4.23 (d, 1 H, J = 5 Hz, C_2 H)
4.24 (ddd, 1 H, J = 5, 2, 1.5 Hz, C_2 H)	4.58 (dd, 1 H, J = 12, 5 Hz, C_3 H_β)	4.38 (dd, 1 H, J = 13, 5 Hz, C_3 H_β)
4.40 (dd, 1 H, J = 12, 5 Hz, C_3 H_β)	4.61 (m, 1 H, C_2 H)	4.62 (br, 2 H, D_2O exchange, carbamate NH_2)
4.53 (AB q, 2 H, J = 13 Hz, C_{10} H_2)	4.66 (m, 4 H, xanthate methylenes)	4.68 (q, 2 H, J = 7 Hz, xanthate methylene)
4.64 (q, 2 H, J = 7 Hz, xanthate CH_2)	4.85 (br, 2 H, C_7 NH_2)	4.89 (s, 1 H, C_1 H)
4.70 (dq, 2 H, J = 7 Hz, xanthate CH_2)	5.13 (d, 1 H, J = 1.5 Hz, C_1 H)	4.92 (br, 2 H, D_2O exchange, C_7 NH_2)
4.92 (br, 2 H, D_2O exchange, C_7 NH_2)	7.45 (m, 3 H, ortho and para benzenoid)	5.23 (AB q, 2 H, J = 13 Hz, C_{10} H_2)
4.95 (d, 1 H, J = 1.5 Hz, C_1 H)	7.75 (m, 2 H, meta benzenoid)	
	8.46 (s, 1 H, azomethine)	

[a] In δ.

characterized polymeric material was noted. No products were formed when the reducing agent was omitted from the reaction mixture and unchanged mitomycin C was recoverable. Both compounds I and II, which were produced in 42 and ~5% yield, respectively, were isolated by applying the organic extract to the top of an HPLPLC (high performance low pressure liquid chromatography)[7] column packed with Whatman LP-1 silica gel. Compound I was eluted with ethyl acetate (35 psi, 20 mL/min), followed by preparative TLC on silica gel developed twice in 1:1 hexanes–ethyl acetate. Its homogeneity was shown in the following systems: *n*-octanol–acetone–ligroin (90–115 °C), R_F = 0.74; 3:2:2 2-propanol–ethyl acetate–hexanes, R_F = 0.69; 1:4 ethyl acetate–$CHCl_3$, R_F = 0.13; 1:4 acetone–ethyl acetate, R_F = 0.86. Compound II was eluted from the HPLPLC column with 1:3 acetone–ethyl acetate and purified by preparative TLC using the system 2:5:5 2-propanol–ethyl acetate–hexanes. It was observed in highest yield (~20% besides much unreacted mitomycin C) when only 6.5 mg of $Na_2S_2O_4$ rather than 50 mg/50 mg of mitomycin C was used. Its homogeneity was shown in the following systems: 10:25:25 *n*-octanol–acetone–ligroin (90–115 °C), R_F = 0.27; 3:2:2 2-propanol–ethyl acetate–hexanes, R_F = 0.36; 4:1 ethyl acetate–acetone, R_F = 0.44.

Compound I melts sharply at 166 °C dec, $[\alpha]_D^{20}$ +15.4° (*c* 0.026, methanol). It is soluble in ethanol, acetone, ethyl acetate, and $CHCl_3$, but not in water or hexanes. The spectroscopic data for compound I (Tables I–III) show it to be 1,10-diethylxanthyl-2,7-diaminodecarbamoylmitosene.[4] The molecular ion plus one proton was observed by field desorption mass spectrometry (FD MS), but not by EI MS nor CI MS, which only yielded fragments analogous to those seen by FD MS. The UV spectrum is compatible with that of a mitosene,[8] but the strong absorption at 279 nm is unusual for mitosenes. The UV spectrum of the model compound *S*-ethyl *O*-ethylxanthate shows λ_{max}^{MeOH} 279 nm (ϵ 11 500). The molar extinction coefficient at 279 nm for I is 18 400, thus indicating a dixanthate. The IR spectrum lacks the 1710-cm^{-1} carbamate carbonyl absorption seen in the IR of mitomycin C[8] and of compound II, while retaining the quinone carbonyl 1595-cm^{-1} absorption. The 360-MHz ^1H NMR spectrum, which is supported by homonuclear decoupling experiments, confirmed the dixanthate structure. However, there remained some uncertainty as to whether the ethylxanthate moiety and the amino group were in positions 1 and 2, respectively, or vice versa, although the chemical shifts of *S*-ethyl *O*-ethylxanthate and ethylamine suggested the former orientation.

To test this possibility the benzaldehyde Schiff base adduct Ia was prepared. Compound I (10 mg) was dissolved in 2.5 mL of 4:1 benzene–$CHCl_3$, 1.1 equiv of freshly distilled benzaldehyde was added, the reaction vessel was filled with 3-Å molecular sieves, and the reaction was allowed to proceed at room temperature for 12 h. Most of the reaction product was adsorbed on the molecular sieves, which were extracted with ethyl acetate, and the extract was combined with the initial supernatant. Analysis of the mixture by TLC showed one red-colored product and unreacted compound I in the systems: 1:1 hexanes–ethyl acetate (R_F = 0.08 (I), 0.68 (Ia)) and 1:4 ethyl acetate–$CHCl_3$ (R_F = 0.13 (I), 0.72 (Ia)). Compound Ia was isolated by preparative TLC on silica gel using 1:1 hexanes–ethyl acetate. It decomposes into compound I in the presence of trace amounts of water; therefore all equipment and reagents were carefully dried. It is apparent from the ^1H NMR data (Table III) of Ia, which are supported by homonuclear decoupling experiments, that the C_2 H signal has shifted downfield δ 0.37 while the C_1 H and C_3 H_2 signals have shifted less than half that amount relative to their positions in the ^1H NMR of I. Irradiation of the C_2 H signal caused a 45% increase in the peak height of the azomethine signal by virtue of abolition of long-range coupling, while irradiation of the C_1 H did not change the height of the azomethine peak. These data are taken as confirmation of the assignment of the amino group of I to C_2.

Compound II melts at 142 °C, $[\alpha]_D^{20}$ −83.3° (*c* 0.026, methanol). It possesses solubility properties similar to those of compound I. The spectroscopic data for compound II (Tables I–III) show it to be 1-ethylxanthyl-2,7-diaminomitosene. The FD MS of II shows an MH + K complex peak but no molecular ion, a situation for which precedence has been reported by Reinhart et al.[9] As was observed for compound I, EI MS and CI MS did not afford a molecular ion, but only fragments analogous to those observed by FD MS. In the UV spectrum the 279-nm absorption is considerably weaker than the 253-nm absorption as opposed to the situation for compound I, indicating that II is a monoxanthate. The IR spectrum shows a strong absorption at 1710 cm^{-1} demonstrating the presence of the carbamate moiety. The 360-MHz ^1H NMR spectrum of II is similar to that of I, except that only one set of xanthate ethyl peaks are observed and an additional D_2O exchangeable two proton peak is present, confirming the monoxanthate structure. The small couplings for the C_1 H, C_2 H, and C_3 H_2 as seen in the ^1H NMR of I were observed for II in acetone-d_6 but not in $CHCl_3$. Assignments were confirmed by homonuclear decoupling experiments.

It was observed that compound II can be converted into compound I by exposing it to conditions similar to those that led to its formation. Compound II (5 mg) was dissolved in 1:1 ethanol–water (5 mL), cooled to 0–5 °C, and purged with nitrogen for 5 min. Cold potassium ethylxanthate solution (0.5 M in 1:1 ethanol–water, 1.8 mL) was added, followed by the addition of $Na_2S_2O_4$ (5 mg in 0.1 mL of water). The reaction was allowed to proceed for 10 min with nitrogen bubbling and then stopped with oxygen bubbling for 5 min. Water (3 mL) was added to the reaction mixture which was then extracted twice with ethyl acetate. The organic extract was separated by preparative TLC on silica gel using 2:5:5 2-propanol–ethyl acetate–hexanes. Both I prepared from II and I prepared from mitomycin C showed superimposable IR and ^1H NMR spectra and identical behavior on TLC in the systems given for compound I.

Taylor and Remers[10] have discussed the problems associated with ^1H NMR based 1,2 stereochemical assignments of mitosenes. In 100-MHz ^1H NMR spectra of some mitosenes which they had isolated, $J_{1,2}$(cis) = 6 Hz while $J_{1,2}$(trans) = 4–5 Hz. In other instances, notably for 1-*O*-acyl-2-aminomitosenes, the NMR method appeared to be useful for assigning configurations. Compounds I and II are closely analogous to 1-*O*-acyl-2-aminomitosenes and $J_{1,2}$ = 1.0–1.5 Hz. Additional studies not reported here in detail have revealed that some of the other mitomycin C–ethylxanthate reaction products appear to contain thiazoline or thiazolidine rings involving the substituents at C_1 and C_2. The 360-MHz ^1H NMR spectra of these compounds, whose structures are not yet fully elucidated, show signals corresponding to C_1 H and C_2 H where $J_{1,2}$ = 5 Hz, which may reflect a 1,2 cis coupling. These considerations suggest a trans relationship for the 1,2 substituents in both compounds I and II. On the basis of the 1,2 trans relationships and the assumption that the 2-amino groups are α,[10] likely absolute configurations for I and II as shown are suggested.

The present investigation constitutes the first report of a 1,10-bis-substituted decarbamoylmitosene arising from a redox reaction sequence in aqueous solution, thus lending credence to the proposed mechanism of action of mitomycin C. The formation of this compound demonstrates that the reduction of mitomycin C activates C_1 and C_{10} equally well which is in marked contrast to the acid-catalyzed reactions of this antibiotic, where C_1 is much more reactive than C_{10}.[8] Thus the propensity for cross-link formation can be expected to be greater for reduced mitomycin C than for acid-activated mi-

tomycin C.[11] It is not clear why the use of a lesser amount of $Na_2S_2O_4$ favors the formation of compound II and it will therefore be of interest to examine if the generation of compound II requires a one-electron reduction, while perhaps the generation of I requires a two-electron reduction.[12] Experiments to study these questions and to elucidate the structures of the other products formed together with I and II are in progress.

The facile formation and isolation of compounds I and II lead us to propose that the use of potassium ethylxanthate in preference to other nucleophiles may be advantageous for investigating other suggested bioreductive alkylating agents.[13]

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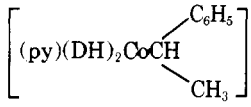
Metal–Alkyl Bond Dissociation Energies in Organocobalt Compounds Related to Vitamin B_{12} Coenzymes

Sir:

Transition metal–alkyl bond dissociation energies are of importance in the context of various homogeneous catalytic processes,[1,2] as well as certain biochemical systems, notably those encompassing the coenzyme B_{12} (5′-deoxyadenosylcobalamin) dependent rearrangements.[3–5] The "strengths" of transition metal–alkyl bonds and the contributions of thermodynamic and kinetic factors to the "stabilization" of such bonds have been issues of some controversy in recent years.[6] Unfortunately, hardly any transition metal–alkyl bond dissociation energies are known reliably, nor have general methods for the determination of such energies been developed.[7]

We describe here an effective new approach to the determination of certain transition metal–alkyl bond dissociation energies and its application to the first direct estimation of cobalt–carbon bond dissociation energies in some organocobalt compounds of possible relevance as coenzyme B_{12} analogues.

Our studies relate to the determination of the equilibrium constants and kinetics of reactions exemplified by eq 1 (where



1

$$\rightleftharpoons [(py)(DH)_2Co^{II}] + C_6H_5CH{=}CH_2 + \tfrac{1}{2}H_2 \quad (1)$$

2

DH_2 = dimethylglyoxime and py = pyridine) which we have found to attain a readily measurable equilibrium at ambient temperatures under H_2 pressures of ~1 atm. Reactions, corresponding to the reverse of eq 1 and analogues thereof (including variants involving the addition of "cobalt hydrides" to activated olefins), have long been recognized as synthetic routes to organocobalt compounds, including organocobalamins.[8–11] The decomposition of alkyl–cobalt compounds to yield olefins also is well-documented qualitatively,[9,12,13] but neither the *equilibrium nor kinetic behavior* of reactions such as eq 1 appears to have previously been examined.

Solutions of $[(py)(DH)_2CoCH(CH_3)C_6H_5]$ in toluene, equilibrated with a constant partial pressure of H_2, decomposed according to eq 1, attaining a measurable equilibrium in ~1 h at ~20 °C. The approach to equilibrium was monitored, and the final equilibrium concentrations of **1** and **2** were determined, spectrophotometrically. Identical results were obtained monitoring either the absorbance decrease at 360 nm (ϵ(**1**) 9.55×10^3, ϵ(**2**) 3.6×10^3 M^{-1} cm^{-1}) or the absorbance increase at 430 nm (ϵ(**1**) 2.1×10^3, ϵ(**2**) 3.5×10^3 M^{-1} cm^{-1}). The value of the equilibrium constant, K_2 (defined by eq 2), at 18.8 °C was determined to be $(6.4 \pm 0.2) \times 10^{-6}$ $M^{3/2}$ and was found to be unaffected by initial concentration variations over the following ranges: $[(py)(DH)_2CoCH(CH_3)C_6H_5]$, 1.1×10^{-4} to 3.0×10^{-4} M; $[H_2]$, 8.5×10^{-4} to 2.6×10^{-3} M (0.32 to 1.0 atm of H_2);[14] excess [py], 0 to 2×10^{-3} M. The same value of the equilibrium constant was determined when the equilibrium was approached from the reverse direction, i.e., starting from $[(py)(DH)_2Co^{II}]$, $C_6H_5CH{=}CH_2$, and H_2. Values of K_2, determined at temperature ranging from 9.8 to 37.0 °C, are listed in Table I.[15] These data yield an excellent linear van't Hoff plot (log K vs. T^{-1}) corresponding to $\Delta H° = 22.1 \pm 0.5$ kcal/mol and $\Delta S° = 51.9 \pm 1.6$ cal/(mol deg).

$$K_2 = \frac{[(py)(DH)_2Co^{II}][C_6H_5CH{=}CH_2][H_2]^{1/2}}{[(py)(DH)_2CoCH(CH_3)C_6H_5]} \quad (2)$$

Using the above value of $\Delta H°$ for reaction 1, in combination with available data for the heats of formation of $C_6H_5CH{=}CH_2$ ($\Delta H_f°$ (25 °C) = 35.2 kcal/mol)[16] and of the $C_6H_5\dot{C}HCH_3$ radical ($\Delta H_f°$ (25 °C) = 33 kcal/mol),[17] the cobalt–carbon bond dissociation energy of $[(py)(DH)_2Co{-}CH(CH_3)C_6H_5]$ can be deduced to be 19.9 kcal/mol using the following thermochemical cycle:

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