anism(s). At [CTAC] < cmc, we suggest that mixed aggregates of F and CTAC are formed with the monomer and excimer fluorescences arising from different aggregates of the general formulas F[CTAC]$_n$ and F$_m$[CTAC]$_n$ ($m \geq 2$), respectively. The negligible rise time of the excimer fluorescence may mean that the aggregates F$_m$[CTAC]$_n$ are small.[7] The development of a rise time (which means a decrease in the encounter frequency between F* and F) *after micellization* probably reflects the larger volume available to F* and F in the CTAC micelle compared with the premicellar aggregates.

The persistence of excimer fluorescence (and the aggregates F$_m$[CTAC]$_n$) below the cmc at high [CTAC]/[F$_T$] is surprising since we expected that F would be dispersed and isolated through the aggregates F[CTAC]$_n$. However, the prevalence of the structures F$_m$[CTAC]$_n$ is consistent with the results of an ESR study on the aggregates F$_m$Q$_n$.

ESR Experiment. The three-line (a_N = 16.9 G, line width 1.7 G) ESR spectrum of Q[1] shows loss of signal intensity with added F as illustrated in Figure 3, upper.[10] We assign the "lost" intensity in the narrow line spectrum to mixed aggregates F$_m$Q$_n$ ($n \geq 2$) in which the ESR spectrum of Q is severely broadened because of spin exchange.[11] The observed narrow line spin intensity, by this reasoning, arises only from monomeric Q (Q or F$_m$Q).

Figure 3, lower, is a spin intensity profile for Q in the absence (curve A[12]) and presence (curve B) of F (3.0×10^{-5} M). A replot of the data as a *spin intensity loss* profile (curve A − curve B = curve C) reveals that the intensity loss levels off near [Q$_T$] = 4.5×10^{-5} M. Eventually, the monomer spin intensity profiles (A and B) reach a plateau at [Q$_T$] = cmc, coincident with the appearance of a severely broadened (~15 G) single-line ESR spectrum due to micellized Q.[1]

We assign the point of discontinuity in curve C as the saturation limit where all F (3.0×10^{-5} M) is aggregated as F$_m$Q$_n$ ($n \geq 2$). This point in curve C ([Q$_T$] = 4.5×10^{-5} M) indicates that F and Q are incorporated into the mixed aggregates responsible for the ESR spin intensity loss in the ratio F/Q ≈ 0.7 with several candidate structures being FQ_2, F_2Q_2, F_2Q_3, F_3Q_4, and F_3Q_5 as well as their multiples such as F_2Q_4.

The ESR spin loss data can be used to predict the fluorescence quenching data for the above candidate structures.[13] The concentrations of the uncomplexed and aggregated Q ([Q] and [Q$_a$], respectively) can be calculated directly from the spin intensity data. In turn, [F] and [F$_a$] are derived from [Q$_a$], the composition of the candidate aggregate, and [F$_T$]. The values of $(\Phi_0/\Phi)_{pred}$ are shown in Table I.

Note that the calculated [F] and [F$_a$] are very sensitive to the values of the percent spin loss for the higher [Q$_T$]s which accounts for the wide range in those $(\Phi_0/\Phi)_{pred}$. With this in mind, it seems that a reasonable correlation is obtained only for the composition F_2Q_3. Further, this composition (F/Q = 0.66) agrees exactly with the graphically observed saturation limit in curve C.

The following conclusions can be drawn. The spectroscopic observations on the above systems indicate that amphipathic compounds of opposite charge efficiently, and to some extent specifically, aggregate in very dilute aqueous solution. We suspect that the relative hydrophobic contents of the components may be important in determining the compositions of the dominant aggregates. Despite the analysis leading to F_2Q_3, we do not believe that only a single aggregate structure is formed in that system as noted by the absence of isosbestic points in the electronic absorption spectra. Rather, we believe that an ensemble of aggregates is formed in which F_2Q_3 is highly favored.[14] Finally, these results suggest experimental approaches for studying the association of amphipathic materials with possible application to biological systems[15] and the design of efficient systems for solar energy utilization.

References and Notes

(1) S. S. Atik, C. L. Kwan, and L. A. Singer, *J. Am. Chem. Soc.*, **101**, 5696 (1979).

(2) "Diffusional quenching" is by collisional or diffusion-controlled encounter of the excited fluorophor and quencher while "static quenching" involves fluorphors and quenchers positioned within effective interaction distance in the ground state. Fluorescence quenching by nitroxyl radicals has been shown to be diffusion limited with an interaction distance of $\sigma \approx$ 5–6 Å.[3]

(3) J. A. Green, II, L. A. Singer, and J. H. Parks, *J. Chem. Phys.*, **58**, 2690 (1973).

(4) For diffusional quenching, the fluorescence lifetimes should follow the relationship $\tau_{obsd}^{-1} = \tau_f^{-1} + k_q[Q_T]$ where τ_{obsd} and τ_f are the lifetimes in the presence and absence of Q.

(5) J. B. Birks, "Photophysics of Aromatic Molecules," Wiley-Interscience, New York, 1979, p 301.

(6) C. L. Kwan, S. Atik, and L. A. Singer, *J. Am. Chem. Soc.*, **100**, 4783 (1978).

(7) A negligible rise time also would be predicted for structures of micelle size where $m \approx n$. However, we would expect that these mixed micelles would have decreasing m and increasing n as [CTAC] was increased leading to a gradual increase in excimer fluorescence rise time rather than a sudden onset at the cmc as is observed. We[8] and others[9] have observed a rise time for pyrene excimer fluorescence in ionic micelles.

(8) S. S. Atik and L. A. Singer, submitted to *Chem. Phys. Lett.*

(9) P. P. Infelta and M. Gratzel, *J. Chem. Phys.*, **70**, 179 (1979).

(10) Precipitation is not observed over the concentration range of this experiment so that the ESR spin intensity loss is due to changes involving solubilized materials.

(11) The ESR spectral features expected for randomly oriented nitroxyl radical moieties in F$_m$Q$_n$ are different from those of rigid nitroxyl biradical systems. For example, see J. Michon and A. Rassat, *J. Am. Chem. Soc.*, **101**, 995 (1979), and references therein.

(12) We previously noted and commented on the less than theoretical intensity observed in the ESR spectra of very dilute (<2.0×10^{-5} M) aqueous solutions of Q.[1] Since the ESR data at [Q$_T$] = 1.0×10^{-5} M are not used in the following analyses, this observation does not jeopardize the conclusions below.

(13) The analysis that follows assumes that aggregates of the general composition F$_m$Q are relatively unimportant. Such structures would contribute to fluorescence quenching but not to the ESR spin intensity loss.

(14) The analyses leading to the results in Table I also indicate that for the overall equilibrium $2F + 3Q \rightleftharpoons F_2Q_3$, $K \approx 3 \times 10^{21}$ M^{-4} which illustrates the remarkable stability of these aggregates.

(15) A possible example of a highly specific aggregation of biological molecules is the association of lecithin and sodium cholate as described by D. G. Dervichian, *Adv. Chem. Ser.*, **No. 84** (1968).

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Farnesyl Pyrophosphate Synthetase. Mechanistic Studies of the 1′-4 Coupling Reaction in the Terpene Biosynthetic Pathway

Sir:

The 1′-4 condensation between isopentenyl pyrophosphate (isopentenyl-PP) and an allylic pyrophosphate is the key building reaction in the terpene biosynthetic pathway. The reaction was discovered in the late 1950s, and its mechanism has been the subject of considerable speculation.[1] An electrophilic condensation between C(1) of the allylic substrate and C(4) of isopentenyl-PP was proposed initially.[2] This mechanism was subsequently supplanted by another (Scheme I) thought to be more compatible with the stereochemistry of the reaction in which displacement of pyrophosphate and formation of the carbon-carbon bond were assisted by a nucleophilic group (X) located in the active site of the enzyme. The reaction was completed by elimination of the X group to generate a new C(2)-C(3) double bond.[3] Although a nucleophilic displacement was preferred for the condensation step, the "X-group" mechanism, when taken in the broadest context, is compatible with a nucleophilic or an electrophilic process. Recently we ruled out the former possibility using fluorinated

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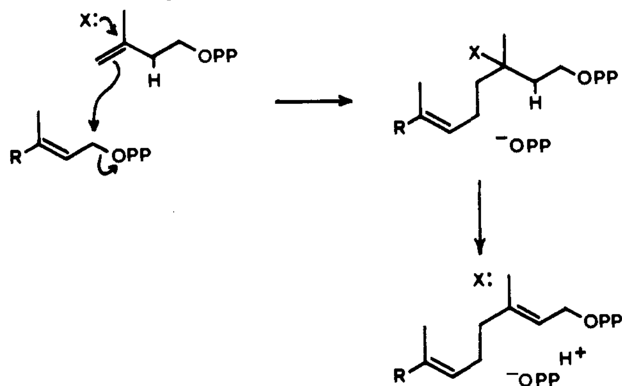
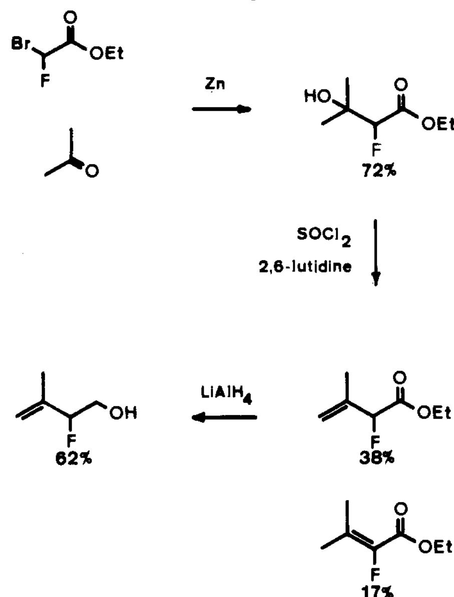
Scheme I. X-Group Mechanism for 1′-4 Condensation



Scheme II. Synthesis of 2-Fluoroisopentenol



analogues of the allylic substrate,[4] and obtained evidence which suggests that ionization of the allylic substrate precedes formation of a carbon-carbon bond between the two substrates. However, it was still possible that the developing positive charge generated at C(3) of the isopentenyl moiety during electrophilic alkylation of the double bond was stabilized by covalent attachment of an X group. Since the final step of this mechanism requires elimination of a proton from C(2) and X from C(3) to generate the new double bond, we reasoned that it would be possible to trap a covalent X-group complex by replacing hydrogen at C(2) of isopentenyl-PP by fluorine. With fluorine in the site normally occupied by the proton which is eliminated (Scheme I), decomposition of the complex to give normal products would be thwarted. We now report experiments with two analogues, 2-fluoroisopentenyl-PP and 2,2-difluoroisopentenyl-PP, which bear on the question of X-group participation in the 1′-4 coupling.

2-Fluoroisopentenol[7] was prepared from ethyl bromofluoroacetate following the route outlined by Machleidt and Wessendorf[8] (Scheme II), and the pyrophosphate was obtained using the procedure reported by Donninger and Popjak.[9] 2,2-Difluoroisopentenol[10] was obtained from ethyl bromodifluoroacetate by a similar sequence except that distillation from phosphorus pentoxide was required for the elimination step. [1-^{3}H]-2-Fluoroisopentenyl-PP (20 Ci/mol) and [1-^{3}H]-2,2-difluoroisopentenyl-PP (20 Ci/mol) were synthesized by reduction of the corresponding esters with lithium aluminum triteride and direct pyrophosphorylation using the Cramer procedure.[11]

Incubation of avian liver farnesyl-PP synthetase[1] (SA 2 μmol min^{-1} mg^{-1}) with isopentenyl-PP and geranyl-PP in the presence of 2-fluoroisopentenyl-PP (**1**) or 2,2-difluoroisopentenyl-PP (**2**) resulted in inhibition of the 1′-4 condensation.[12] Lineweaver-Burke analysis of the initial velocities using the computer techniques developed by Cleland[14] gave noncompetitive patterns[15] for both analogues when isopentenyl-PP was the varied substrate: $K_{is}^1 = 0.46 \pm 0.04$,[13] $K_{ii}^1 = 16 \pm 3$,[13] $K_{is}^2 = 30 \pm 5$, $K_{ii}^2 = 57 \pm 8$ μM. However, all attempts to uncover an irreversible component for the inhibition failed. A 0.01 μM solution of enzyme in buffer[12] containing 0.5 μM geranyl-PP as a control was incubated in parallel with two samples, one with added [1-^{3}H]-2-fluoroisopentenyl-PP (0.8 μM, 20 Ci/mol) and another with added [1-^{3}H]-2,2-difluoroisopentenyl-PP (0.8 μM, 20 Ci/mol). Periodically, samples were diluted into buffer and assayed in the standard way after adding [1-^{14}C]-isopentenyl-PP (10 Ci/mol) and geranyl-PP. Although the activity of the enzyme slowly decayed (~75% of the original activity remained after 73 h), the rate of loss was similar for all three incubations.[16] In another set of experiments, seven tubes, each containing enzyme (6.5 μM, SA 2.5 μmol min^{-1} mg^{-1}), and the following substrates were incubated for 8 days at 37 °C: tube 1, [1-^{3}H]-geranyl-PP (367 μM, 4.7 Ci/mol); tube 2, [1-^{3}H]-geranyl-PP (367 μM, 4.7 Ci/mol) and 2-fluoroisopentenyl-PP (350 μM); tube 3, geranyl-PP (350 μM) and [1-^{3}H]-2-fluoroisopentenyl-PP (430 μM, 20 Ci/mol); tube 4, [1-^{3}H]-2-fluoroisopentenyl-PP (430 μM, 20 Ci/mol); tube 5 [1-^{3}H]-geranyl-PP (367 μM, 4.7 Ci/mol) and 2,2-difluoroisopentenyl-PP (815 μM); tube 6, [1-^{3}H]-2,2-difluoroisopentenyl-PP (450 μM, 20 Ci/mol); and tube 7, geranyl-PP (350 μM) and [1-^{3}H]-2,2-difluoroisopentenyl-PP (450 μM, 20 Ci/mol). At the end of the incubation, blue dextran was added to each tube, and the contents were chromatographed on a 1.5 × 100 cm Sephadex G-25 column eluted with deionized water. In each instance the blue dextran-enzyme fraction[17] eluted first followed by the substrates. A small portion of the radioactivity was found in the enzyme-containing fractions. In the tubes containing [1-^{3}H]-geranyl-PP (1, 2, 5), a small radioactive peak, which corresponded to alkylation of 0.4-1.1% of the available active sites, comigrated with the enzyme. However, this peak does not represent the X-group-bound complex since it formed in the absence of isopentenyl analogues (tube 1), and similar peaks were not observed for labeled isopentenyl analogues (tubes 3, 4, 6, 7).[18]

In a separate set of experiments designed to look for an X-group intermediate not bound to the enzyme, 2,2-difluoroisopentenyl-PP (350 μM, 20 Ci/mol) was incubated at 37 °C with 8×10^{-2} mg of enzyme (SA 2 μmol min^{-1} mg^{-1}) in 1 mL of buffer containing 2.2 mM geranyl-PP for 3 days, during which time the enzyme retained >90% of its original activity. The reaction was terminated upon addition of 100 μL of 1 M lysine buffer, pH 10.4, followed by heating at 80 °C for 30 min. After an additional incubation with calf mucosa alkaline phosphatase (10 mg) at 37 °C for 12 h, the sample was extracted with pentane and the extracts were chromatographed using reverse-phase silica gel-60 plates (55:45 acetone-water). All of the radioactivity comigrated with an isopentenol marker (R_f 0.83), and the C_{15} region (R_f 0.2-0.4) was devoid of activity. In contrast, a similar experiment with [1-^{3}H]-2-fluoroisopentenyl-PP yielded a single new product which comigrated with farnesol (R_f 0.24). The structure of the new product obtained from large-scale incubations was established as 2-fluorofarnesol by comparing NMR and mass spectra of the corresponding benzoate derivative[19] with those of an authentic sample.[20] The fluoro analogue was almost as reactive as isopentenyl-PP in the condensation reaction with an initial rate ratio of 0.24. However, deviations from linearity were noticed at much lower conversions of 2-fluoroisopentenyl-PP, and its conversion into 2-fluorofarnesyl-PP only approached

50% even upon prolonged incubation in the presence of excess geranyl-PP. Since farnesyl pyrophosphate synthetase catalyzes the stereospecific removal of the *pro-R* proton at C(2) of isopentenyl-PP during 1′-4 coupling,[1] it is logical that only (*S*)-2-fluoroisopentenyl-PP is a substrate for 1′-4 condensation,[21] although this point has not been proved.

Experiments with 2-fluoro- and 2,2-difluoroisopentenyl-PP failed to uncover any evidence for X-group involvement. Given the sensitivity with which radioisotopes can be detected and the remarkable stability of the enzyme upon prolonged incubation, the frequency of a chemical event that leads to irreversible inhibition or formation of an X-group bound product not covalently attached to the enzyme relative to the normal reaction under similar conditions must be $<10^{-5}$. It is unlikely that the inability of the fluoro analogues to function as affinity labels can be attributed to poor binding, since both are good inhibitors.[22] It is also unlikely that fluorine has deactivated the double bond to the point where the electrophilically initiated addition of the allylic moiety and X is no longer possible, at least in the 2-fluoro system where the rate of 1′-4 condensation is depressed by only a factor of 4 relative to that for isopentenyl-PP. The simplest explanation for our results is that the 1′-4 condensation does not involve covalent attachment of a nucleophile at C(3) of the isopentenyl moiety,[23] and we suggest that the X-group mechanism[24] be retired until direct support is found.[25]

Acknowledgments. Support from the National Institutes of Health, GM 21328 (C.D.P.) and AM 13140 (H.C.R.), is gratefully acknowledged. We also thank the Givaudan Corporation for a generous sample of geranylacetone.

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(2) (a) F. Lynen, H. Eggerer, U. Henning, and I. Kessel, *Angew. Chem.*, **70**, 738 (1958); (b) H. C. Rilling and K. Bloch, *J. Biol. Chem.*, **234**, 1424 (1959); (c) J. W. Cornforth and G. Popjak, *Tetrahedron Lett.*, **No. 19**, 29 (1959).
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(6) W. S. Johnson and R. A. Bell, *Tetrahedron Lett.*, **No. 12**, 27 (1960).
(7) ^{1}H NMR (δ, $CDCl_3$) 1.78 (3, m, methyl at C(3)), 2.80 (1, s, hydroxyl proton), 3.80 (2, d of d, $^3J_{^1H-^{19}F}$ = 24.5, $^3J_{^1H-^1H}$ = 5.0 Hz, H at C(1)), 4.49 (1, d of t, $^2J_{^1H-^{19}F}$ = 49, $^3J_{^1H-^1H}$ = 5.0 Hz, H at C(2)), and 5.10 ppm (2, m, H at C(4)).
(8) H. Machleidt and R. Wessendorf, *Justus Liebigs Ann. Chem.*, **674**, 1 (1964).
(9) C. Donninger and G. Popjak, *Biochem. J.*, **105**, 545 (1967).
(10) ^{1}H NMR (δ, CCl_4) 1.82 (3, s, methyl at C(3)), 3.71 (3, m which collapses to a 2 H triplet after exchange with D_2O, $^3J_{^1H-^{19}F}$ = 13 Hz, H at C(1) and hydroxyl proton), 5.14 (1, s, H at C(4)), and 5.28 ppm (1, s, H at C(4)).
(11) F. D. Cramer and G. Weimann, *Chem. Ind.* (*London*), 46 (1960).
(12) A standard 0.5-mL assay contained 10 mM PIPES buffer, pH 7.0, 10 mM β-mercaptoethanol, 1 mM magnesium chloride, and 0.1 μM potassium azide. All kinetic measurements were made at 37 °C using the acid lability technique.[13]
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(14) W. W. Cleland, *Adv. Enzymol.*, **29**, 1 (1967).
(15) The noncompetitive patterns are expected since the normal substrate, isopentenyl-PP, can inhibit the enzyme by binding to the geranyl-PP region of the active site.[13]
(16) The activity of the enzyme incubated with 2-fluoroisopentenyl-PP decayed slightly faster than the other samples. However, in a second run at higher concentrations of enzyme (9 μM) and substrates (400 μM), the rate of loss of activity in the presence of 2-fluoroisopentenyl-PP was identical with that of the control.
(17) The fractions containing active enzyme were determined by the standard assay[13] with [1-^{14}C]isopentenyl-PP.
(18) Small amounts of radioactivity in the enzyme-containing fractions of these tubes represented the leading edge of a massive peak for the labeled substrate.
(19) ^{1}H NMR (δ, $CDCl_3$) 1.58 (6, s, methyls at C(7) and C(11)), 1.67 (3, s, methyl at C(11)), 1.73 (3, d, $^4J_{^1H-^{19}F}$ = 3.4 Hz, methyl at C(3)), 1.97–2.15 (8, m, H at C(4), C(5), C(8), and C(9)), 4.92 (2, d, $^3J_{^1H-^{19}F}$ = 22 Hz, H at C(1)), 5.08 (2, m, H at C(6) and C(10)), 7.48 (3, m, meta and para H), and 8.08 ppm (2, m, ortho H's); ^{19}F NMR (ϕ, CCl_4) −119.3 (t of q); mass spectrum (70 eV) 344 (0.8), 222 (5), 207 (7), 105 (72), 77 (40), 69 (100), and 49 (59).
(20) The alcohol was prepared from geranylacetone according to the route previously reported for 2-fluorogeraniol[4] and converted into the benzoate ester by treatment with benzoyl chloride. The stereochemistry of the C(2)–C(3) double bond was established by comparing chemical shifts and ^{1}H–^{19}F coupling constants for the C(3) methyls of methyl (*E*)- and (*Z*)-2-fluorofarnesate with those of methyl 2-fluorogeranate and methyl 2-fluoronerylate.[4]
(21) By implication, (*R*)-2-fluoroisopentenyl-PP is the potential X-group trap.
(22) Although it is not possible to disect out all of the kinetic constants because of the very complex binding properties of the enzyme, the magnitudes of the slopes and intercepts of the double reciprocal plots indicate that both analogues prefer to bind to the isopentenyl-PP site. Since 2,2-difluoroisopentenyl-PP prefers to bind to the isopentenyl site, it is reasonable that (*R*)-2-fluoroisopentenyl-PP also prefers that site. However, it should be emphasized that our arguments do not require that these analogues bind preferentially to the isopentenyl-PP site, only that some fraction of (*R*)-2-fluoroisopentenyl-PP or 2,2-difluoroisopentenyl-PP forms an enzyme–analogue–geranyl-PP complex whose topology approximates that of the normal enzyme-substrate complex.
(23) The lack of condensation observed for 2,2-difluoroisopentenyl-PP and presumably (*R*)-2-fluoroisopentenyl-PP is not inconsistent with our conclusions. For example, it is possible that elimination of a proton from C(2) of the isopentenyl moiety is concerted with electrophilic addition, thereby bypassing a fully developed tertiary cation at C(3). Failure to remove a proton at C(2) would then abort the condensation step and the allylic pyrophosphate would be regenerated by internal return.
(24) The X-group mechanism enjoys widespread popularity for a variety of enzymatic olefin alkylations, although evidence for the process rests solely on stereochemical arguments for 1′-4 condensation catalyzed by farnesyl-PP synthetase.
(25) There are a wide variety of prenyltransferases and only one, farnesyl-PP synthetase, has been studied in detail. Thus, alternate mechanisms may be uncovered as other enzymes are studied.
(26) (a) Alfred P. Sloan Fellow; (b) National Institutes of Health Research Career Development Awardee, HL 00084, 1975–1980.
(27) University of Utah Graduate Research Fellow, 1978–1980.

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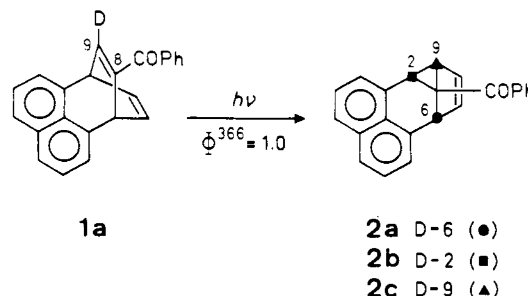
Thermal Interconversion of Naphthobarrelene- and Naphthosemibullvalene-like Compounds. Ground-State Counterpart of a Di-π-methane Photorearrangement

Sir:

We have recently reported that 8-benzoyl-9-deuteriobicyclo[3.2.2]nona[*de*]naphthalene (**1a**) rearranges quantitatively to the tricyclo[4.3.0.0^{2,9}]nona[*de*]naphthalenes **2a–c** in regioselective di-π-methane-type photoreactions (Scheme I).[1] While **2b** and **2c** could possibly have been formed in concerted [$_\pi 2 + _\sigma 2 + _\pi 10$(or 2)] processes, **2a** is not accessible by any photochemically allowed concerted path, and evidence was presented that indeed at least one biradical intermediate intervenes in the photorearrangement of **1a**.[1]

Compounds **1** and **2** have now been found to interconvert thermally in the dark (Scheme II). The transformation **1** → **2** is the first example of a ground-state counterpart of a di-π-methane photorearrangement.[1-3] The interconversion **1** ⇄ **2** combines reaction paths which result in regioselective product formation competing with positional interchanges of the deuterium-labeled carbon atoms. The observed regiose-

Scheme I. Photorearrangement of **1a** to **2a–c**



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