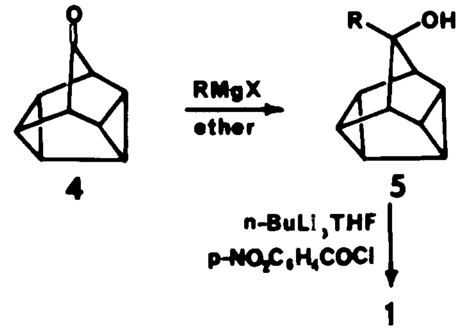
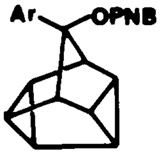
Table II. Comparison of the Rates of Solvolysis of 9-Pentacyclo[4.3.0.0^{2,4}.0^{3,8}.0^{5,7}]nonyl p-Nitrobenzoates with the Corresponding 7-Norbornyl Derivatives

	Relative Rate	
Substituent	**2**[a] (taken as unity)	**1**
p-CH$_3$O	1.00[b]	159
p-H	1.00[b]	5.85×10^3
p-CF$_3$	1.00[b]	4.90×10^6
3,5-(CF$_3$)$_2$	1.00[b]	3.47×10^8
CH$_3$	1.00[b]	9.3×10^7
H	1.00[c]	9.8×10^{13}

[a] Reference 8, 9. [b] Corrected from 70% dioxane to 80% acetone.[9] [c] Corrected from tosylate in acetic acid to p-nitrobenzoate in 80% acetone.[9]



volysis of 7-norbornenyl derivatives (**7**) where π-participation is significant,[8] and much larger than ρ^+ for 7-norbornyl (**8**). Hence the $\pi\sigma$-participation from the cyclopropane ring is a linear function of the electron demand of the incipient carbonium ion center.



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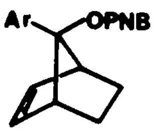

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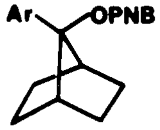

8

ρ^+	−2.05	−2.30	−5.27

The rates of solvolysis of **1** are compared with the 7-norbornyl p-nitrobenzoates (**2**)[8,9] and are listed in Table II. The relative rate data reveal that with increasing electron demand at the cationic center the rates of solvolysis of the pentacyclic derivatives (**1**) increase markedly relative to the rates of the corresponding 7-norbornyl derivatives (**2**).

It is interesting to note that very low 9-methyl/9-hydrogen (127) and 9-phenyl/9-methyl (14) ratios are evident in this system (Table I). These values should be compared with the very large methyl/hydrogen (1.23×10^8)[10] and phenyl/methyl ratios (2.3×10^5)[11] observed in the solvolysis of the parent 7-norbornyl derivatives. The low methyl/hydrogen and phenyl/methyl ratios can be attributed to the fact that the cation is so stabilized by $\pi\sigma$-participation that it makes relatively little demand upon substituents for further stabilization. Similar diminished methyl/hydrogen (420)[12] and phenyl/methyl ratios (9.5)[11] are also observed in the solvolysis of 7-norbornenyl derivatives (**7**) where the solvolysis proceeds through π-participation.

The major product of solvolysis of 9-phenyl-9-pentacyclo[4.3.0.0^{2,4}.0^{3,8}.0^{5,7}]nonyl p-nitrobenzoate (**1**, R = Ph) in 80% aqueous acetone at 75 °C after ten half-lives is the unrearranged tertiary alcohol (95%). The solvolysis also furnished a minor amount, 5%, of the secondary alcohol.

In conclusion, both the application of the tool of increasing electron demand and the low methyl/hydrogen and phenyl/methyl ratios observed in the solvolysis of **1** unambiguously support the earlier conclusion that the solvolysis of this system proceeds with carbon ($\pi\sigma$)-participation. However, it should be pointed out that the application of the same criteria to 2-norbornyl fails to reveal such participation under solvolytic conditions.[13,14]

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Reduction of Excited Singlet State Acetone by 2-Propanol

Sir:

While the intermediacy of the nπ^* triplet states (T$_1$) of alkanones in their photoreductions by hydrogen donors is well established, less is known about the reactivities of the corresponding nπ^* singlet states (S$_1$). Theory[1,2] predicts similar reactivities for S$_1$ and T$_1$, in keeping with the quenching of adamantanone fluorescence by hydrogen donors.[3] The S$_1$ reaction may be less efficient, however, since ground state educts and radical pair product lie on a common energy hypersurface, and return to the ground state can compete with radical pair formation.[1,2] Wagner's finding of no[4] or only little[5] 2-propanol formation during reaction of excited acetone with tributylstannane under conditions where only S$_1$ should react may point to a low efficiency of the S$_1$ reaction. However, this explanation conflicts with the unit quantum yield of 2-propanol formation under conditions where both S$_1$ and T$_1$ states of acetone react with the stannane.[4,5]

Extending our studies[6] on the acetone/2-propanol photosystem we have now observed the formation of the photoreduction product pinacol from a singlet reaction of excited acetone, and confirm that S$_1$ and T$_1$ states are about equally reactive towards 2-propanol.

Samples (560 µl) of three different reaction mixtures consisting of (a) 5 M 2-propanol plus 5 M 2-propanol-d_6 in di-*tert*-butyl peroxide, (b) 1 M acetone plus 10 M 2-propanol-d_6

Table I. Yields of Pinacols

System	$(CH_3)_2CHOH$ (5 M)/ $(CD_3)_2CHOH$ (5 M) in DTBP[a]	$(CH_3)_2CO$ (1 M)/ $(CD_3)_2CHOH$ (10 M) in CH_3CN	$(CD_3)_2CO$ (1 M)/ $(CH_3)_2CHOH$ (10 M) in CH_3CN
$[R_H\text{-}R_H]$[b]	6.72	4.82	6.85
$[R_H\text{-}R_D]$[b]	12.51	11.84	14.49
$[R_D\text{-}R_D]$[b]	5.87	5.99	6.00
$[R_H\text{-}R_D]_C$[c]	−0.08	1.03	1.64
$[R_H\text{-}R_D]^2/[R_H\text{-}R_H][R_D\text{-}R_D]$	3.97	4.86	5.11

[a] Di-*tert*-butyl peroxide. [b] Absolute concentrations in 10^{-3} M, ±5%, relative concentrations ±1%, result of two independent runs and more than two GLC analysis each. [c] Excess pinacol $[R_H\text{-}R_D]_C = [R_H\text{-}R_D] - [R_H\text{-}R_H] - [R_D\text{-}R_D]$.

in acetonitrile, (c) 1 M acetone-d_6 plus 10 M 2-propanol in acetonitrile were exposed for 5 min at (26 ± 1) °C in septum closed NMR tubes to the filtered light of a Philips SP 1000-W lamp[7] ($295 < \lambda < 360$ nm, absorbed flux ca. 10^{17} quanta s^{-1}). The yields of the three pinacols $R_H\text{-}R_H$, $R_H\text{-}R_D$, and $R_D\text{-}R_D$ ($R_H = (CH_3)_2\dot{C}OH$, $R_D = (CD_3)_2\dot{C}OH$) were then determined by quantitative GLC.[8] As evident from Table I, for the photoreduction systems the yield of $R_H\text{-}R_D$ exceeds the sum of the yields of $R_H\text{-}R_H$ and $R_D\text{-}R_D$ by about 10% whereas no excess $R_H\text{-}R_D$ is found in the peroxide/2-propanol/2-propanol-d_6 mixture.

To analyze this finding we first consider the peroxide system. Here R_H and R_D are generated independently from each other by reaction of primary *tert*-butoxy radicals with the propanols. They terminate by combination (rate constants k_{HH}, k_{HD}, and k_{DD}) to the pinacols $R_H\text{-}R_H$, $R_H\text{-}R_D$, and $R_D\text{-}R_D$ and by disproportionation to acetones and 2-propanols (in part via the acetone enols).[6] The ratio of pinacol yields $[R_H\text{-}R_D]^2/[R_H\text{-}R_H]\cdot[R_D\text{-}R_D]$ is given by

$$\frac{[R_H\text{-}R_D]^2}{[R_H\text{-}R_H][R_D\text{-}R_D]} = \frac{k_{HD}^2}{k_{HH}k_{DD}} \frac{(\int R_H R_D \, dt)^2}{\int R_H^2 dt \cdot \int R_D^2 dt} \tag{1}$$

where the integrations extend over the reaction period. Now, since[9]

$$(\int R_H R_D \, dt)^2 \leq \int R_H^2 dt \cdot \int R_D^2 dt \tag{2}$$

we have

$$\frac{[R_H\text{-}R_D]^2}{[R_H\text{-}R_H][R_D\text{-}R_D]} \leq \frac{k_{HD}^2}{k_{HH}k_{DD}} \tag{3}$$

Both sides of eq 2 and 3 are equal if the concentrations of R_H and R_D are proportional to each other,[9] for instance for stationary radical concentrations. In view of the low conversion (Table I) $R_H \sim R_D$ can be safely assumed for the peroxide system. Thus the observed value $[R_H\text{-}R_D]^2/[R_H\text{-}R_H][R_D\text{-}R_D] = 3.97$ should be close to the true value of $k_{HD}^2/k_{HH}k_{DD}$.[10]

For the photoreduction systems the ratios $[R_H\text{-}R_D]^2/[R_H\text{-}R_H][R_D\text{-}R_D]$ ar considerably larger than 3.97 (Table I). Because of eq 3 this must mean that the excess yield of $[R_H\text{-}R_D]$ is not formed in radical reactions describable by usual kinetic rate laws. Since in these systems R_H and R_D are generated simultaneously we conclude that the excess $R_H\text{-}R_D$ is due to combination in primary geminate radical pairs and reflects a cage effect.

The multiplicity of the primary pairs follows from a triplet quenching experiment: Acetonitrile solutions of acetone-d_6 (1 M) and 2-propanol (10 M) containing *cis*-1,3-pentadiene (Q) were exposed as described above. For [Q] = 0.12 M not even traces of $R_H\text{-}R_H$ and $R_D\text{-}R_D$ were formed, yet $R_H\text{-}R_D$ was obtained with a yield of $(1.44 \pm 0.15) \times 10^{-3}$ M. Within the error limits this yield was found unquenchable also for higher quencher concentrations[12] ($0.12 \leq [Q] \leq 1.4$ M, $k_q\tau = (0.04 \pm 0.04)$ M^{-1}), and this proves that it must be formed in a photoreduction from the singlet S_1 state.[13] Further we note that the unquenchable fraction of $R_H\text{-}R_D$ is practically equal to the excess fraction found in the absence of quencher (Table I). This shows that primary pairs derived from the T_1 photoreduction do not lead to effective cage product formation.

The quantum yield of pinacol formation ϕ_P was determined for solutions of 0.1 M acetone in 2-propanol and 0.1 M acetone plus 1.0 M 2-propanol in acetonitrile. Both systems gave $\phi_P = 0.105 \pm 0.005$ at 25 °C and 300 nm.[15] If we accept this value for the systems of Table I then the fractions of excess $R_H\text{-}R_D$ correspond to quantum yields of pinacol formation in S_1 cage reactions of $\phi_P{}^{SC} = 0.0048$ (acetone system) and $\phi_P{}^{SC} = 0.0063$ (acetone-d_6 system), respectively (±10%). Further, knowledge of ϕ_P and the ratio of disproportionation to combination of the radicals (7.8 ± 1.5[16]) leads to a quantum yield for acetone photoreduction to radicals, $\phi_R = 0.92 \pm 0.19$. From these results ranges for the rate constant $k_q{}^S$ of acetone S_1 quenching by 2-propanol I and for the efficiency (probability) P_{rad} of radical pair formation during this process may be estimated. We assume that quenching and intersystem crossing (k^{ST}) are the only reactions available to S_1 and have

$$\phi_P{}^{SC} = \frac{k_q{}^S[I]}{k_q{}^S[I] + k^{ST}} P_{rad}P_{cage}P_{com} \tag{4}$$

where in addition to the previously defined quantities P_{cage} is the probability that the radicals react in the cage rather than diffuse apart and P_{com} is the probability that the radicals couple rather than disproportionate. We further assume that acetone T_1 is completely reduced to radicals, from which

$$\phi_R = 1 - \frac{k_q{}^S[I]}{k_q{}^S[I] + k^{ST}} (1 - P_{rad}) \tag{5}$$

follows. For the evaluation of P_{rad} from eq 4 and 5 we take $0.0045 \leq \phi_P{}^{SC} \leq 0.0065$, $0.73 \leq \phi_R \leq 1.00$, and $0.10 \leq P_{com} \leq 0.14$ as reasonable ranges from the experimental data, and adopt $0.5 \leq P_{cage} \leq 1.0$ as usual for S_1 pair reactions.[17] This gives $0.107 \leq P_{rad} \leq 1$, i.e., an efficiency range of 10 to 100% for the S_1 reaction. With the known rate constant for intersystem crossing[18] $k^{ST} = 5 \times 10^8$ M^{-1} s^{-1} and the above range for P_{rad} the rate constant for quenching then becomes $2.8 \times 10^7 \geq k_q{}^S \geq 2.4 \times 10^6$ M^{-1} s^{-1}, low values for P_{rad} corresponding to high values for $k_q{}^S$ and vice versa.

Our lower limit for $k_q{}^S$ agrees with the rate constant for the quenching of adamantanone fluorescence by 2-propanol[3] (1.9×10^6 M^{-1} s^{-1}) and is similar to the rate constant for photoreduction of acetone T_1 by 2-propanol[14] (1×10^6 M^{-1} s^{-1}). This indicates that the reactivities and efficiencies of acetone S_1 and T_1 may be roughly equal. More generally, acetone S_1 either has a reactivity towards 2-propanol which is similar to that of acetone T_1 and leads to radicals with unit efficiency or it reacts with a higher rate constant but less efficiently. These results differ from those obtained by Wagner[4] for the acetone/tributylstannane system, and we cannot offer an explanation for this difference.

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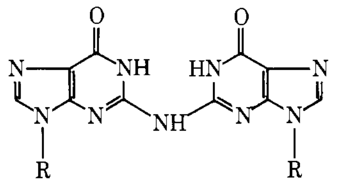
Isolation and Identification of Cross-Linked Nucleosides from Nitrous Acid Treated Deoxyribonucleic Acid

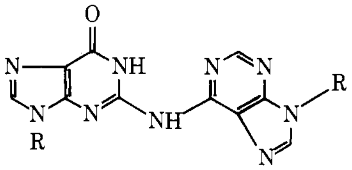
Sir:

Treatment of DNA with nitrous acid covalently cross-links the two strands of the double helix.[1,2] This reaction leads to inactivation[3] and perhaps to deletion mutations[4] in bacteriophage. The chemical structure of the cross-links has not been determined, although this knowledge is needed in the study of DNA repair processes.[5] A recent suggestion that the cross-links arise from the reaction of aldehyde groups, liberated by depurination, with amino groups on the opposite chain[6] has now been withdrawn.[3] We have approached this problem directly, by isolating cross-linked nucleosides from nitrous acid treated DNA. We wish to propose I and tentatively, II, as the structures of two such products.

Calf thymus DNA (500 mg) was treated with 1 M $NaNO_2$ at pH 4.2 and 25 °C for 24 h. At the end of this time, the product (T_m 75 °C) had at least one cross-link per molecule, even after sonication, as measured by the ultraviolet assay for reversible denaturation.[1,2] The product was freed of salt by dialysis, and hydrolyzed with deoxyribonuclease I and snake venom phosphodiesterase. The mixture was fractionated by DEAE-Sephadex chromatography using a LiCl gradient in the presence of 7 M urea.[7] Mononucleotides were eluted, followed by dXMP, and then a series of small peaks containing the cross-linked dinucleotide Ia, and oligonucleotides resulting from inhibition of enzymatic hydrolysis of the modified DNA. Each of these latter peaks was desalted,[8] treated with alkaline phosphatase, and subjected to Sephadex G-25 chromatography, in water. This procedure converted Ia (Ve/Vo 1.0) to Ib (Ve/Vo 1.9). The mobility of the oligonucleotide peaks (Ve/Vo 1.0) was not altered substantially by this treatment, as they retained internal phosphates.



I



II

a, R = 2-deoxy-β-D-ribofuranosyl 5'-phosphate
b, R = 2-deoxy-β-D-ribofuranosyl

Compound Ib (yield 12.5 A_{290} units) was homogeneous in an anion exchange high pressure liquid chromatography system, and had the following properties: λ_{max} (nm) 260, 300 (pH 2.5), 292 (pH 7.0), 250, 260, 290 (pH 13); pK_a values, 5.6 and 10.8, the NMR (in D_2O, Fourier transform) showed an aromatic proton (δ 8.1) and a set of peaks due to deoxyribose[9] in a 1:1 ratio. The compound resisted reduction by sodium dithionite, which excluded the presence of nitro, nitroso, azo, azoxy, or diazoamino functions. The pK_a of Ib was shifted from 5.6 to 7.8 in the presence of glyoxal,[10] which suggested the presence of the adjacent N-1 and amino functions of guanine.

After trimethylsilylation[11] the mass spectrum[12] of Ib showed a molecular weight of 1021, which from comparison with the trimethylsilyl-d_9 derivative[13] (M = 1075) gave a molecular weight of free Ib of 571. Measurement of exact mass (1021.4426, found) supported $C_{20}H_{16}N_9O_8(SiMe_3)_7$ (1021.4436, calcd), corresponding to two deoxyguanosine molecules minus NH_3. The principal fragmentation pathway showed sequential loss of each sugar moiety with hydrogen rearrangement to give *m/e* 761 and 501, which are analogous to common nucleoside reactions[14] and militate against a sugar–sugar linkage. N,O-Permethylation (CD_3)[15] produced a similar mass spectrum (M = 618) in which the base–base linkage was maintained in the principal fragment ions, as required for the proposed structure. Treatment of Ib with D_2O for 1 h at 80 °C resulted in 2 amu shifts for all base-containing ions, in accord with two unsubstituted C-8 moieties.

Structure Ib is consistent with the properties and origin of the compound, as well as the known thermal and base stability of the cross-links induced by nitrous acid in DNA.[16] To confirm its origin, we allowed 500 mg of dGMP to react with nitrous acid under the conditions used for DNA, and isolated 8.2 A_{290} units of Ia, after a DEAE-Sephadex workup. A possible mechanism for the formation of I involves diazotization of a guanine amino group, and attack at the position by a second