55 mμmoles; and adenylyl-(3′–5′)-cytidine 3′-phosphate, 55 mμmoles; Cp:Up:ApCp, 3.02:2:2.

The improved analytical technique involving periodate oxidation and base elimination is based on modifications of the methods described by Neu and Heppel.[3] Cyclohexylamine is used for the elimination reaction in preference to lysine since the latter reacts with periodate. In addition, it is considered advantageous to buffer the elimination reaction mixture at pH 8 with di-*n*-propylmalonic acid. Another modification involves the removal of the phosphatase enzyme. The whole procedure requires that, between the removal of one base and the removal of the next, this enzyme should be added in order to cleave off the terminal phosphate group. It also requires that, prior to the next oxidation step, the enzyme should be completely removed. In this regard it has been found that, in solution, this enzyme can be inactivated *in situ* by treatment with EDTA in the free acid form, and that it does not recover activity when the pH of the solution is brought back to neutrality. This procedure was based on the observation that phosphatase is denatured at low pH and requires the presence of zinc ions for the recovery of activity at neutral pH.[4] The third modification involves the method of analysis of the products formed by the base elimination reaction. In a study of the periodate oxidation of model compounds it was found that, if the reaction mixtures were applied to small DEAE-Sephadex columns and washed through with water, the liberated bases were eluted in the order cytosine, uracil, adenine, and guanine. The bases could then be easily recognized by their position of elution and by their spectra. The column serves a second purpose in that, subsequent to the elution of the base, the polynucleotide fragment can be eluted by a solution containing a salt gradient, and thus the fragment is obtained in a pure condition ready for the next elimination cycle.

In a typical oxidation–elimination cycle the oligonucleotide (10–100 mμmoles) in water (0.1 ml) was treated with EDTA (free acid, 4.5 mg) to inactivate residual phosphatase from the previous cycle. The mixture was allowed to stand for 1 hr with occasional shaking. A buffered cyclohexylamine solution (0.05 ml) and 0.1 *M* sodium periodate (0.05 ml) were added and the mixture was kept at 45° for 90 min. The amine solution was prepared by dissolving di-*n*-propylmalonic acid (5 mmoles) in water and adjusting the pH to 8.0 with sodium hydroxide. To this solution was added cyclohexylamine (10 mmoles) and water such that the final volume was 10 ml. Subsequent to the oxidation reaction the solution (pH 8–8.2) was treated with 0.2 *M* ethylene glycol (0.05 ml). After 20 min water (0.65 ml) and phosphatase solution[5] (0.1 ml, 0.6 unit) were added and the mixture was kept at 37° for 2 hr and then applied to a column (0.6 × 25 cm) of DEAE-Sephadex (HCO_3^- form). The column was eluted with water to recover the liberated base and was then eluted with a linear gradient (600 ml) of 0.2 to 1.0 *M* triethylamine bicarbonate to recover the oligonucleotide fragment. The bases released from six cycles carried out on the fragment from f2 RNA are given in Table I. These results, taken together with the analysis of the ribonuclease digest and together with earlier considerations[1,6] regarding the last two bases of the RNA molecule, are sufficient to define the terminal eleven bases of the RNA as shown above.

Table I. Sequential Periodate Oxidation and Elimination of Bases from the Terminal Fragment of f2 RNA

Cycle	Bases released, mμmoles C	U	A	G
1	109	<2	<5	<3
2	62	<2	<3	<3
3	43	<2	6	<3
4	<4	<2	25	<3
5	17	<2	<3	<3
6	15	<2	<1	<3

Acknowledgment. This work was supported by the National Institutes of Health.

(6) J. C. Lee and P. T. Gilham, *J. Am. Chem. Soc.*, 87, 4000 (1965).

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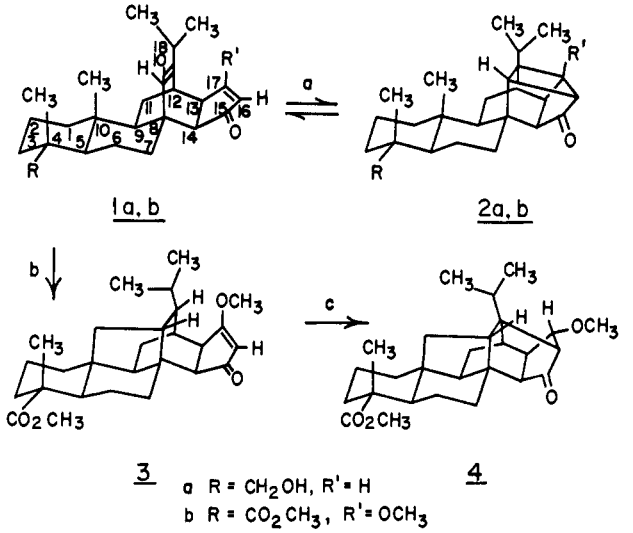
(3) H. C. Neu and L. A. Heppel, *J. Biol. Chem.*, **239**, 2927 (1964).
(4) M. J. Schlesinger, A. Torriani, and C. Levinthal, *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 539 (1963).
(5) Bacterial alkaline phosphatase was obtained from Worthington Biochemical Corp., Freehold, N. J. One unit is that amount of enzyme which liberates 1 μmole of *p*-nitrophenol from *p*-nitrophenyl phosphate per minute under the assay conditions given by A. Garen and C. Levinthal, *Biochim. Biophys. Acta*, **38**, 470 (1960).

A Remarkable Case of Intramolecular Energy Transfer

Sir:

Photolysis of the levopimaric acid–*p*-benzoquinone adduct yields[1] the expected cage product.[2] Similarly, photoisomerization[3] (Pyrex filter) of **1a**[4] [infrared bands at 3475, 1670, and 1600 cm^{-1}, λ_{max} (cyclohexane) 235 and 310 mμ (ϵ_{max} 7340 and 100), significant nmr signals



(1) W. Herz, R. C. Blackstone, and M. G. Nair, *J. Org. Chem.*, **32**, 2992 (1967).
(2) R. C. Cookson, E. Crundwell, and J. Hudec, *Chem. Ind.* (London), 1003 (1958); R. C. Cookson, E. Crundwell, R. R. Hill, and J. Hudec, *J. Chem. Soc.*, 3062 (1964).
(3) Irradiations were carried out in methanol using a Hanovia 679A-36 lamp in a quartz immersion well with or without a Pyrex filter, but proceeded equally well in cyclohexane.
(4) Synthesis and structure proof of **1a**, **1b**, and related compounds will be presented in our detailed paper. All substances analyzed within acceptable limits.

at 7.24 dd (6, 2.5, H-17), 5.98 dd (6, 2, H-16), 5.28 (H-19), 0.96 (C-4 methyl), 0.78 d (7, six protons, isopropyl), and 0.60 ppm (C-10 methyl)] gave exclusively **2a**, 85%, infrared bands at 3470 and 1765 cm^{-1}, no vinyl protons, 0.98 d and 0.88 d (7, isopropyl), 0.79, and 0.75 ppm (C-4 and C-10 methyls).

However, photolysis (Pyrex filter) of **1b**, λ_{max} (ethanol) 237.5 and 295 mμ (ϵ_{max} 11200 and 112), infrared bands at 1750, 1680, and 1600 cm^{-1}, relevant nmr signals at 5.19 br (H-19), 5.02 (H-16), 1.11 (C-4 methyl), 0.82 d (7, six protons, isopropyl), and 0.59 ppm (C-10 methyl), took an unexpected and unprecedented course yielding only 20% of **2b**, infrared bands at 1770 and 1750 cm^{-1}, no vinyl protons, 1.05 d and 1.02 d (6, isopropyl), 1.12 (C-4 methyl), and 0.77 ppm (C-10 methyl), 15% of recovered **1b**, and 60% of a new isomer whose physical properties indicated retention of the cyclopentenone chromophore, λ_{max} 247 and 290 mμ (ϵ_{max} 12500 and 170), infrared bands at 1750, 1675, and 1600 cm^{-1}, nmr singlet at 5.27 (H-16), disappearance of the bridge double bond and disappearance of the methyl group at C-10 [only one methyl singlet at 1.24 (C-4 methyl), methyl doublets at 0.82 and 0.70 ppm (7, isopropyl)]. The only structure which satisfies these properties is **3**.

Further photolysis (no Pyrex filter) of **3** furnished a third completely saturated isomer (65%, infrared bands at 1775 and 1750 cm^{-1}, no vinyl protons) which must be assigned formula **4** because of the appearance in the nmr spectrum of a doublet of doublets at 4.18 ppm (H-17) and the retention of the C-4 methyl singlet (1.26 ppm) and the methyl doublets (0.87 and 0.84 ppm) of the isopropyl group.[5]

Lack of deuterium incorporation when the various photolyses were carried out in CH_3OD precluded an ionic reaction involving intermolecular H^+ transfer. From the absence of products characteristic of hydrogen abstraction from solvents methanol and cyclohexane, it then followed that hydrogen transfers during reactions b and c were completely intramolecular. Since, as in all compounds of this type, the π-electron system of the *endo*-α,β-unsaturated ketone chromophore is close to the π-electron system of the isolated double bond which in turn strongly shields the C-10 methyl groups we infer that the conversion of **1b** to **3** has its origin in internal photosensitization of the isolated double bond by the conjugated chromophore of **1**.[6] Intramolecular hydrogen abstraction from the C-10 methyl group by the resulting diradical is accompanied or followed by a formal hydrogen migration from C-19 to C-18[7] and ring closure to **3**.[10]

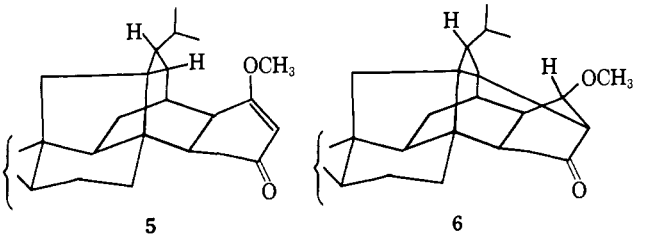
(5) Photolysis of **3** with a Pyrex filter resulted in quantitative recovery of starting material. Photolysis of **1b** without a filter gave **2b** and **4**, the latter being formed from **3** as shown by analysis of aliquots taken at intermediate periods. Since the relative amount of **2b** seemed to decrease with time, the photolysis of **2b** (Pyrex filter) was studied separately. This established that **2b** was in equilibrium with **1b** which in turn was converted to **3**.

(6) The *cis–trans* isomerization of certain olefins reported by H. Morrison (*Tetrahedron Letters*, 3653 (1964); *J. Am. Chem. Soc.*, **87**, 931 (1965)) provides a precedent of sorts.

(7) Since evidence for direct 1,2-hydrogen shifts is meager,[8] we are considering the possibility that H-19 is passed to C-18 *via* C-16 or C-17.[9]

(8) R. Kh. Freidlina, *Advan. Free-Radical Chem.*, **1**, 215 (1965); however, see R. E. K. Winter and R. F. Lindauer, *Tetrahedron Letters*, **25**, 2345 (1967); G. W. Griffin, J. Covell, R. C. Petterson, R. M. Dodson, and G. Close, *J. Am. Chem. Soc.*, **87**, 1410 (1965); D. I. Schuster and I. S. Krull, *ibid.*, **88**, 3456 (1966); A. Padwa, D. Crumrine, R. Hartman, and R. Layton, *ibid.*, **89**, 4435 (1967).

(9) Alternate formulations **5** and **6** (for **3** and **4**, respectively), which would avoid this difficulty, are rejected because of the large distance required for hydrogen transfer from the C-10 methyl to C-18 (~4 A in Dreiding models of **2b**) and from C-19 to C-17 of **5** (~3.8 A) and because **6** cannot be constructed with the models. It seems unreasonable to suppose that bond distortions in the photoexcited states could reduce these distances sufficiently. The possibility of resolving this ambiguity by X-ray analysis is being investigated.



5 **6**

(10) For other examples of photochemically induced hydrogen transfers to double bonds in bridged compounds, see A. M. Parsons and D. J. Moore, *J. Chem. Soc., Sect. C*, 2026 (1966); H. D. Scharf, *Tetrahedron*, **23**, 3057 (1967).

(11) H. E. Zimmerman and D. I. Schuster, *J. Am. Chem. Soc.*, **84**, 4527 (1962); H. E. Zimmerman, *Pure Appl. Chem.*, **9**, 493 (1964); H. E. Zimmerman, L. D. Rieke, and J. R. Scheffer, *J. Am. Chem. Soc.*, **89**, 2033 (1967).

(12) Kindly determined by Mrs. Frances DeTar at 77°K in EPA glass.

(13) (a) V. Ermolaev and A. Terenin, *Opt. i Spektroskopiya*, **1**, 523 (1956); (b) M. Kasha, *Radiation Res. Suppl.*, **2**, 265 (1960).

(14) This appears to be the first demonstration of phosphorescence in a cyclopentenone (private communication from Professor P. de Mayo). See also E. Y. Y. Lam, D. Valentine, and G. S. Hammond, *J. Am. Chem. Soc.*, **89**, 3482 (1967).

(15) In view of the close structural similarity of **1a** and **1b**, the cautionary note expressed by H. E. Zimmerman and J. S. Swenton, *J. Am. Chem. Soc.*, **89**, 906 (1967), is probably not applicable here. See also G. Porter and P. Suppan, *Proc. Chem. Soc.*, 191 (1964); *Pure Appl. Chem.*, **9**, 499 (1964), for a discussion of the effect of electron-donating groups on lowering the energy of the $\pi \rightarrow \pi^*$ triplet.

At first glance it seemed tempting to ascribe this unusual internal photosensitization to the $n \rightarrow \pi^*$ triplet of the cyclopentenone chromophore and to assume that reaction c, which occurs only at $\lambda < 300$ mμ, involves the $\pi-\pi^*$ triplet, although it requires hydrogen abstraction. The effect of the methoxyl group in promoting reaction b at the expense of reaction a by sensitizing the double bond could then be understood in terms of its presumed[11] influence in raising the energy of the **1b** $n \rightarrow \pi^*$ triplet relative to the ground state, although the magnitude of the effect seemed surprising.

This was contraindicated by the emission spectra.[12] That of **1a** exhibited the highly structured phosphorescence spectrum characteristic of emission from $n \rightarrow \pi^*$ triplets (0–0 band 74.5 kcal),[13] whereas the entirely featureless phosphorescence spectra of **1b** and **3** which begin at 74 kcal are indicative of $\pi \rightarrow \pi^*$ triplets.[14,15] Efforts to shed light on the actual mechanism and to clarify related aspects are in progress.

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Thexylborane as a Convenient Reagent for the Cyclic Hydroboration of Dienes. Stereospecific Syntheses *via* Cyclic Hydroboration

Sir:

The simultaneous addition of equimolar quantities of thexylborane (2,3-dimethyl-2-butylborane) and appro-