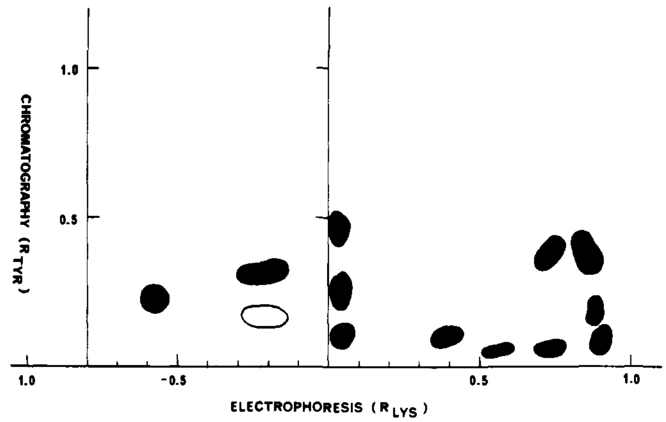

Figure 3. Two-dimensional peptide maps of a tryptic digest of synthetic apoC-I. The protein (1.5 mg) was dissolved in ammonium bicarbonate (0.5%), trypsin (15 μg) was added, and the digestion was performed at 37° for 4 h. The digest was then examined in two dimensions by electrophoresis at pH 6.5 in pyridine:acetic acid:water buffer (25:1:225) and by chromatography in butanol:acetic acid:water (4:1:5, upper phase). The chromatogram was visualized in ninhydrin and chlorine/starch reagent (both reagents gave similar results). The single open spot corresponds to the only Trp containing peptide (uv fluorescence).

polyacrylamide gel electrophoresis at pH 8 in the presence of 0.1% sodium dodecyl sulfate, and pH 8.2 in the presence of 8 M urea (the single band had the same appearance as shown in the Figure 1 inset but with the minor band absent). The synthetic product showed strong cross-reactivity with antiserum specific to native apoC-I.[25] The synthetic apoC-I was assayed by the procedure of Soutar et al.[2] and showed significant activity as an activator of LCAT.[24] Amino acid analysis of an acid hydrolysate was in good agreement with the expected values,[26] i.e., Lys 9.3 (9), Arg 3.1 (3), Trp 0.8 (1), Asp 5.0 (5), Thr 2.8 (3), Ser 7.1 (7), Glu 9.0 (9), Pro 0.9 (1), Gly 1.1 (1), Ala 3.1 (3), Val 2.2 (2), Met 1.0 (1), Ile 2.8 (3), Leu 5.8 (6), Phe 3.0 (3). Peptide maps of tryptic digests of synthetic apoC-I showed the expected number of fragments[4,27] and confirmed that the synthetic product was essentially pure.

Acknowledgment. We wish to thank Dr G. G. Midwinter for numerous amino acid analyses and Miss Marston for expert technical assistance. This investigation was supported in part by University Research Committee (New Zealand) Grants No. 72/214, 73/94, Medical Research Council (New Zealand) Grant No. 74/126, and National Heart Foundation of New Zealand Awards No. 79 and 102. We would also like to express our appreciation to Drs. J. Morrisett and J. T. Sparrow for providing a preprint of ref 4 and helpful discussions on the synthetic work.

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(24) Synthetic apoC-I (15 μmol) activated LCAT to a specific activity of 200 pmol of cholesterol ester formed per hour, which compares favorably with the activity of the native protein.[2]
(25) The antibody assays were kindly performed by Dr. L. Simons, Department of Medicine, University of New South Wales.
(26) HCl (6N), 110 °C, and 24 h, the value for Trp was measured spectrophotometrically.[16]
(27) Based on the published sequence, apoC-I would be expected to give 12 peptide spots on peptide mapping. After three duplicate maps it was not possible to determine if the bottom right spots were one broad or two closely overlapping spots (the latter interpretation is shown and gives a total of 13 spots).
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Low Temperature Studies on Propionyl Benzoyl Peroxide and Propionyl Peroxide. The Ethyl Radical

Sir:

Recently we demonstrated that the infrared spectra of simple free radicals, in particular the infrared spectra of the phenyl and methyl radical, may be obtained by photochemical cleavage of asymmetric acyl peroxides.[1] Here we report that analogously ethyl and phenyl radicals may be obtained by photodestruction of propionyl benzoyl peroxide (I), and that high concentrations of alkyl radicals may be obtained by photolysis of symmetric diacyl peroxides as well. In fact, the concentrations of the radicals produced are so high that the complete vibrational spectrum is obtained.

Compound I was isolated in an argon matrix at 6 K and subsequently irradiated with a medium-pressure mercury lamp in conjunction with a water filter and a Corning No. 053 uv filter with no transmission below 2900 Å. During the irradiation bands due to CO_2 and ethyl benzoate[2] appeared together with an intense band at 710 cm^{-1} characteristic of the phenyl radical,[3] and another band at 541 cm^{-1}. Upon warming the matrix to 30 K the latter two bands disappeared[4] within several seconds, and simultaneously new bands due to ethylbenzene, ethylene, and benzene appeared. Since these new bands can conveniently be explained as being due to the combination and

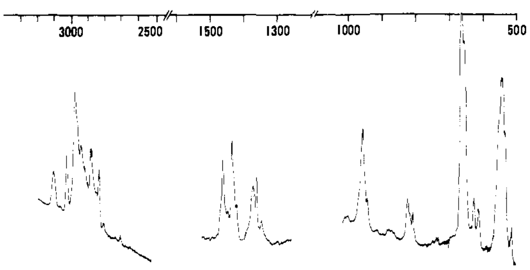


Figure 1. $CH_3CH_2CO_2O_2CCH_2CH_3$ in an argon matrix. Irradiation with light $\lambda > 2900$ Å for $T = 6795$ min. (Scale in cm^{-1}.)

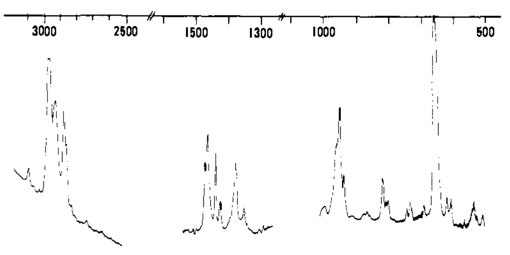


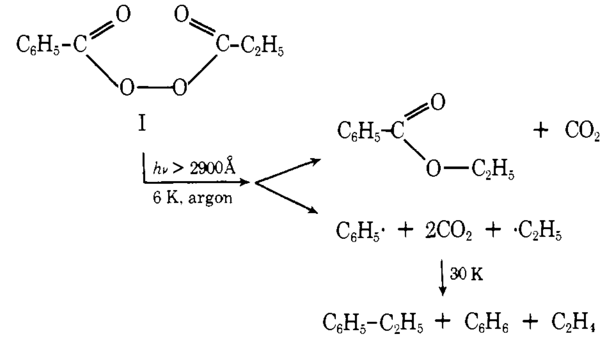
Figure 2. Brief warm-up to 30 K for 1 min after all peroxide ($CH_3CH_2CO_2O_2CCH_2CH_3$) was destroyed by light with $\lambda > 2900$ Å. (Scale in cm^{-1}.)

Table I. A List of the Vibrational Bands Which Are Shown in Figure 1 and Are Assigned to the Ethyl Radical, and Those Due to Ethane, Ethylene, and Butane, the Combination and Disproportionation Products of Ethyl Radicals Shown in Figure 2.

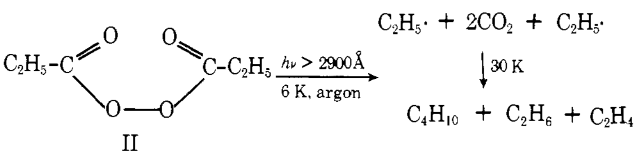
$C_2H_5\cdot$, cm^{-1} (Figure 1)	Combination and disproportionation products, cm^{-1} (Figure 2)	$C_2H_5\cdot$, cm^{-1} (Figure 1)	Combination and disproportionation products, cm^{-1} (Figure 2)
3112.5	3100[a]	1437.5	1379[c]
3032.5	2980[b,c]	1365.8	965[c]
2982.5	2940[b,c]	1186.5	954[a]
2887.5	1471[c]	955.0	821[b]
2840.0	1464[b,c]	814.0	735[c]
1465.0	1439[a]	805.0	
		541.0	

[a] Ethylene. [b] Ethane. [c] Butane.

Scheme I



Scheme II



disproportionation products of a phenyl and an ethyl radical we assign the 541-cm^{-1} band to the out-of-plane bending mode of the ethyl radical. Scheme I summarizes these results.

Dipropionyl peroxide (II) was isolated in an argon matrix and irradiated under the same conditions as I. Upon irradiation, bands due to CO_2 and those listed in Table I appeared. Kinetic plots (band intensities vs. time) show that the rate of CO_2 formation as well as those of all the other bands shown in Figure 1 correlate with the rate of disappearance of II. Upon a brief warm-up to 30 K all of the new bands shown in Figure 1, except CO_2, synchronously disappear with the appearance of vibrational bands due to butane, ethane, and ethylene. The spectrum recorded after the warm-up is shown in Figure 2.

We assign the vibrational spectrum shown in Figure 1 to the ethyl radical because the same spectrum is obtained from I and II. Furthermore, the warm-up products which result from the combination and disproportionation reactions clearly indicate the presence of the ethyl radical. Scheme II summarizes these results.

A qualitative description of the structure of the ethyl radical may be obtained by comparison of its infrared spectrum with CH_2CD_2.[5] The frequencies at 3112.5 and 3032.5 cm^{-1} and the band at 1365.8 cm^{-1} agree favorably with the CH stretching (3095 and 3016 cm^{-1}) and in-plane deformation (scissoring motion at 1384 cm^{-1}) of the CH_2 group in CH_2CD_2.[6] This similarity with ethylene leads us to conclude that the electronic structure of the CH_2 group in the ethyl radical probably exists in an sp^2 configuration.[7] However, the much lower out-of-plane bending frequency (CH_2CD_2 = 943 cm^{-1}, CH_3CH_2 = 541 cm^{-1}) in the ethyl radical is a direct reflection of the absence of the π bond in C_2H_5 and the relative ease with which the radical center may distort.

The frequencies in the CH stretching region below 3000 cm^{-1} are assigned to those of the methyl group in the ethyl radical. The band at 2982.5 cm^{-1} is similar to the asymmetric CH stretch of the CH_3 group in alkanes; however, the band at 2840 cm^{-1} is lower than any CH stretching frequency associated with normal alkanes.[8] This may be a reflection of the low energy required (38 kcal/mol) to break a CH bond in the methyl group of the ethyl radical.[9] Olah and co-workers[10] have observed a similar effect in the isopropylcarbonium ion and have suggested that the low CH stretching frequencies were due to hyperconjugation. We have also observed similar effects in the isopropyl radical.[11]

Studies on deuterated species are being conducted in order to perform a complete normal coordinate analysis. These results will appear shortly.

Acknowledgment. The technical assistance of Mr. F. L. Rodgers is gratefully appreciated.

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Received January 19, 1976