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20 January 1966

Methyl Radicals: Preparation and Stabilization

Abstract. *Methyl radicals were prepared by photolysis of methyl iodide in porous glass and were stabilized for days at room temperature. Their reactivity was investigated.*

Gomberg (*1*) isolated in 1900 a complicated free radical, triphenyl methyl, and opened up a new discipline in organic chemistry—the chemistry of free radicals. The simplest of the free radicals, methyl, could not be produced and stabilized at room temperature. Paneth (*2*) in 1929, using a flow system and a metallic film detection scheme, inferred the transient existence of methyl radicals with lifetimes from 20 to 100 msec. Since then methyl radicals have been produced and stabilized at low temperatures (42° to 77°K) either by embedding them in a frozen matrix or by adsorbing them, after gamma irradiation, on silica and alumina (*3*, *4*). Herzberg and Shoosmith measured, at 2140 Å, absorption spectra of CH_3 and CD_3 in gas phase (*5*).

We now report the preparation and stabilization for days at room temperature of methyl radicals. The methyl radical was produced by the photolysis of methyl iodide adsorbed on porous Vycor glass. The corresponding deuteromethyl and C^{13}-methyl have also been produced. The radicals were identified by their characteristic electron-spin resonance (ESR) spectra. We studied the reactivity of the methyl radical with hydrogen, deuterium, oxygen, nitric oxide, and other gases using the change of ESR spectra to monitor the concentration of the methyl radical.

The methyl iodide and other alkyl iodides were degassed either in a vacuum system or by a purified helium flush; CD_3I and $C^{13}H_3I$ (50 percent) were obtained from Merck, Sharpe and Dohme of Canada, Limited. Helium (General Dynamics grade A) was purified by passing it over activated alumina cooled at liquid-nitrogen temperature. Oxygen (General Dynamics, industrial grade) was used without further purification. The other gases such as H_2, D_2, NO, CH_4, C_2H_6, and n-C_4H_{10} (Matheson) were either CP grade or prepurified grade and were used from lecture-size gas cylinders without purification.

The Vycor porous glass (Corning Glass No. 7930, 96 percent SiO_2 and 3 percent B_2O_3) was treated in oxygen at 600° to 650°C to remove the organic material usually absorbed on it from the contaminants present in the laboratory atmosphere. It was cooled to room temperature in helium and stored in a stoppered glass bottle. After this treatment the Vycor changed its color from yellow-brown to water-white. Vycor glass rods, 25 by 4 mm, weighing 0.5 g, were used. The surface area, as determined by the Brunauer-Emmett-Teller method of nitrogen adsorption at liquid-nitrogen temperature, was 144 m^2/g.

In the flow system a purified helium gas stream was passed through a saturator containing the alkyl iodide. The partial pressure of the alkyl iodide in the gas stream was controlled by the temperature of the saturator. The helium stream then passed over the Vycor porous glass placed in a quartz tube (6.5 mm outside diameter, 5.0 mm inside diameter) which was inserted into the flow system with polyethylene tube connections. The exit end of this quartz tube was also connected to a rotary vacuum pump. The helium was passed over the sample at a high flow rate at a pressure greater than atmospheric. The sample was irradiated outside of the cavity and, after irradiation, was transferred to another portion of the sample tube since a signal developed in the quartz sample tube on irradiation. The system was then evacuated with the rotary pump, and pure helium was passed over the sample. With helium flowing, the exit end of the sample tube was disconnected, was inserted into the microwave cavity, and again connected to the original flow line. For the measurement of the interaction of methyl radicals with other gases, the sample was evacuated after stopping the helium flow. The interacting gas at a certain pressure was introduced from another flow line, and the change in ESR signal was observed under static conditions.

In static experiments, a previously cleaned Vycor glass rod was evacuated at 500°C in a quartz tube (special purity, General Electric Co., 5 mm outside diameter, 4 mm inside diameter) which had a quartz-pyrex graded seal for attachment to a conventional vacuum

Table 1. Characteristics of methyl and deuteromethyl radicals. RT, room temperature; LN, liquid nitrogen; VG, Vycor glass; HFS, hyperfine splitting (separation between individual lines composing spectrum).

Condition	Total spread (gauss)		HFS (gauss)		Line width (gauss)	Ratio of line intensities at peak
	Calc.	Obs.	Calc.	Obs.		
			Methyl radical			
CH_3I Matrix at LN	68	68.8	22.4	23.2	3.6	1.0|2.8|2.9|1.0
Adsorbed on VG at RT		67.8		22.7	1.0	1.0|3.0|3.3|1.1
Adsorbed on VG at LN		68.8		22.9	0.8	1.0|3.7|4.1|1.3
			Deuteromethyl radical			
Adsorbed on VG at RT		21.2		3.5	1.8	1.0|3.3|6.8|7.8|6.5|3.2|1.4

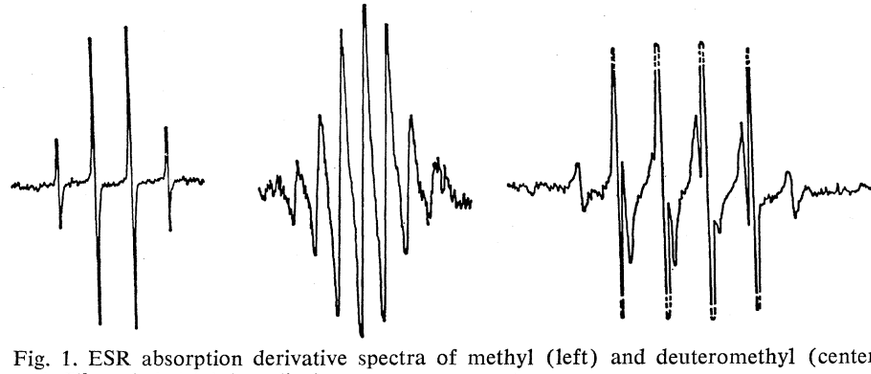

Fig. 1. ESR absorption derivative spectra of methyl (left) and deuteromethyl (center) and C^{13}-methyl (right) radicals at room temperature.

system. The sample system was cooled to room temperature, and a previously well-degassed substrate was distilled into the sample tube in the vacuum system. After the Vycor was completely wet with the substrate and had become transparent, the excess substrate was removed by vacuum distillation and evacuated for about 1 hour at room temperature. The amount of methyl iodide adsorbed under these conditions was estimated to cover 2 percent of the available surface.

Irradiation of the sample was carried out with a low-pressure mercury lamp (110 volt input, 5000 volt output, 500 watts) at a distance of 2 to 3 cm from the sample tube.

A Varian spectrometer (model V4500) operating at 9.5 kmcy/sec and TE102 was employed. The magnetic field was modulated at 100 kcy/sec and was controlled by a Field-Dial regulator V-FR-2200. This enabled us to keep the magnetic field at a predetermined value for a period of time and to observe the variation in signal intensity at this particular field setting.

A sample of frozen methyl iodide was irradiated with ultraviolet light at liquid-nitrogen temperature, and the ESR signal of CH_3 in CH_3I matrix was measured at that temperature (Table 1). A broad line quartet was found, similar to that reported by previous workers.

In both the static and flow systems the signal of the methyl radical appeared as soon as 5 minutes after irradiation of methyl. Representative ESR signals of methyl and deuteromethyl radicals are shown in Fig. 1, and their characteristics are listed in Table 1.

For the methyl radical four sharp lines of the hyperfine structure are due to the three protons $(2nI+1)$, where $n = 3$, and I, the proton spin, is equal to ½, the intensity is in the ratio of 1.0|3.0|3.3|1.4 where the theoretical values are 1|3|3|1.

For the deuteromethyl seven lines were observed, in agreement with the value of I for the deuteron being equal to 1. The intensity ratios were equal to 1.0|3.3|6.8|7.5|6.3|3.2|1.4 where the theoretical values are 1|3|6|7|6|3|1.

The values for hyperfine splitting of the separation between individual lines in the case of the methyl radical was 22.6 gauss and in the case of the deuteromethyl 3.54 gauss, giving a ratio of 6.38; the theoretical ratio, that is, the ratio of gyromagnetic constant of the proton to that of deuteron, is 6.52. It should be noted that McConnell's (*6*)

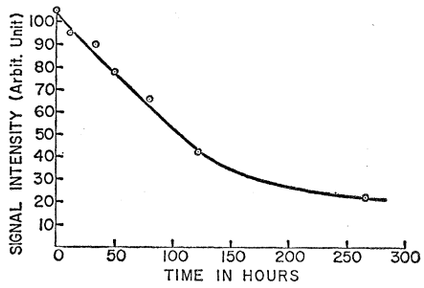

Fig. 2. Decay curve of methyl radicals at room temperature.

calculation for splitting gives 22.5 gauss. The narrowness of the hyperfine components of the radical in Vycor glass as compared to those in CH_3I matrix indicates some kind of motional narrowing of the width which is normally present and due to dipolar and spin-lattice interactions. The width at liquid-nitrogen temperature remains almost the same. This could mean that the motion still persists even at liquid-nitrogen temperature.

The spectrum of the mixture of $C^{13}H_3$ (55 percent) and $C^{12}H_3$ (45 percent) is shown in Fig. 1. The separation and intensities of the lines follow exactly those calculated by Cole, Pritchard, Davidson, and McConnell (*4*). We were able to resolve the lines for which only envelopes were previously obtained. The separation between the ultimate lines is 106.0 gauss and between the penultimate strong lines is 67.5 gauss, giving a difference of 38.5 gauss. Theory predicts for this difference a value of 38 gauss for a planar free-moving radical, 60 to 66 or 4 to 10 gauss for a planar radical rotating around the axis, and 300 gauss for a tetrahedral configuration for CH_3. Since no other signals were observed except those indicated in the Fig. 1, we must conclude that the CH_3 radical is planar and free to move either on the surface or in the pore of the Vycor glass.

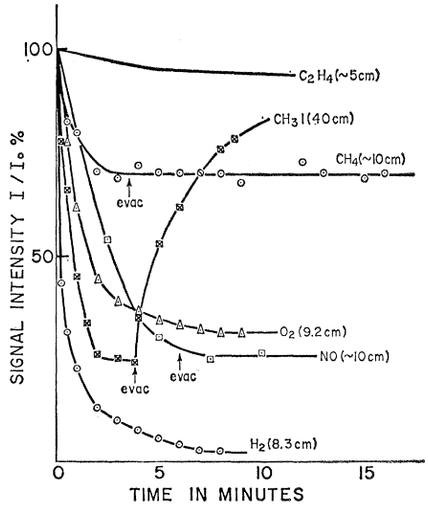

Fig. 3. Interaction of gases with methyl radicals at room temperature.

The methyl radicals are stable for more than a week at room temperature (Fig. 2). Heating to a temperature of 70° to 100°C is necessary to make the signal disappear in 5 minutes.

A 10-percent copper sulfate solution in a 1-cm thick quartz vessel was used to cut out radiation of wavelength shorter than 3000 Å from the quartz lamp. This type of radiation reduced the yield of the free radical to zero. This is consistent with the fact that the low-pressure mercury lamp has a high efficiency at radiation of 2537 Å, and the methyl iodide has an adsorption maximum at 2580 Å.

The production of methyl radicals differed in the static and dynamic systems. In the static system, prolonged irradiation resulted in a constant concentration of the radical, possibly because of a back reaction between the CH_3 and the iodine adsorbed on the surface. In the flow system the yield increased linearly with time of irradiation, reaching a value of 5.8×10^{14} spins in 30 minutes with a helium flow of 3.2 liter/min and methyl iodide in saturation at 36°C. Lowering the concentration of methyl iodide in the helium stream by keeping the temperature of the saturator at 20°C decreased the yield and showed leveling off of the yield.

The interaction of methyl radicals with several gases was studied (Fig. 3). Hydrogen and deuterium decrease the signal. Introduction of H_2 at a pressure of 83 mm cut down the intensity of the methyl signal to half its value in 15 seconds, whereas the reactivity of the deuterium was lower. The times for half reaction of deuteromethyl with hydrogen and deuterium were similar, and of the same magnitude as that of methyl. The signal for H or D atoms, so characteristic for these species in ESR, could not be detected. The H or D atoms undoubtedly produced in these reactions must either recombine or react with the iodine on the surface.

Both oxygen and nitrous oxide react readily, though the rate of reaction is lower than that with hydrogen.

Methane causes a slight decrease in signal intensity which may be due to a

hydrogen impurity. Evacuation of the signal does not restore the loss in signal.

Ethylene surprisingly enough shows no decrease in the signal. This cannot be due to a molecular-sieve action of the pores in the Vycor glass since oxygen, nitric oxide, and methyl iodide readily diffuse to the sites of the methyl radicals.

Introduction of methyl iodide decreases the signal, but this loss in signal strength is recovered on evacuation. The disappearance of the signal may be due either to the gradual diffusion of the methyl iodide into the pores containing CH_3 radical and broadening the signal due to shortening of their lifetime as result of

$$CH_3 + CH_3I \longrightarrow CH_3I + CH_3$$

or to change in the Q of the cavity, as the dielectric constant of CH_3I is 7.

Methyl radicals do not react with ethane, normal butane, and toluene.

Attempts to produce and stabilize methyl radicals from acetone and dimethyl mercury, both good sources of methyl radicals, were unsuccessful both under static and flow conditions, in spite of substantial overall decomposition of these substances under our reaction conditions.

Similarly, no radicals were detected in subjecting the following substances to photolysis under our conditions: ethyl iodide, isopropyl iodide, toluene, and benzyl chloride.

Our study indicates the possibility of preparing and stabilizing methyl radicals for periods long enough to make them useful as organic reagents. Possibly other species such as H atoms, OH, HO_2, NH_2 can be prepared by proper choice of porous substances. Furthermore, the role of free radicals in surface catalysis can be evaluated.

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3 March 1966

Oxygen Dependence of Retinal S-Potential-Producing Cells

Abstract. *Changes in the membrane potential of the S-potential-producing cells (S-cells) in the isolated retina of fish (Gerridae) were correlated with changes in oxygen concentration. During brief hypoxia the changes in potential consisted of initial depolarization and subsequent hyperpolarization to near 70 millivolts. Depolarization occurred when oxygen concentration was reduced to a level of from 13 to 10 percent, and hyperpolarization occurred on reduction from 10 to 2.5 percent; there was variation from cell to cell. The recovery of S-cell function from anoxia was fast in oxygen but slow in air. The results show that the S-cell stops functioning in seconds without oxygen; hence this kind of cellular element in the nervous system is much more sensitive to oxygen deprivation than other cells studied thus far.*

Previous studies have established that the light-induced S-potentials originate in the horizontal and amacrine cells of the fish retina (*1*), and that CO_2, NH_3, temperature change (*2, 3*), alcohols, and volatile anesthetics (*3*) have immediate and drastic effects on the membrane potential of these S-cells. On the other hand, these particular agents and temperature change have much less effect on the neuronal membrane potential of ganglion cells of frog dorsal root (*3*). Further, the S-potential is graded in nature and is sustained as long as the light stimulus lasts, and the membrane potential of the S-cells is not electrically excitable (*2, 4*). These facts suggest that the S-cell membrane potential is maintained by a mechanism different from that of the neuronal membrane potential; the latter is exclusively dependent on transmembrane ionic gradient and gives rise to the all-or-none spike potential.

This paper deals with experiments on hypoxia in the S-cell membrane potential of the fish retina. The retina was dissected from a light-adapted fish (Gerridae) and placed, receptor side up, in a plastic chamber having a volume of 80 cm^3. The mixtures of gas were introduced into the chamber through valve-equipped inlets after they had been bubbled in water bottles; the gas circulated through the entire system at a flow rate of about 200 cm^3/min. Pure O_2 or air was used as the control gas medium; O_2 concentration was easily changed by introduction of other gases or gas mixtures through a by-pass valve system. In our gas exchange system, the total gas content in the chamber could be completely exchanged in 2 minutes. An oxygen macroelectrode (Beckman) mounted inside the chamber monitored the O_2 concentration changes for recording, while a micropipette electrode simultaneously recorded the S-cell membrane potential. An Ag–AgCl wick electrode lying beneath the retina was used as reference. When the tip of the microelectrode is located outside the S-cell, the potential difference between the microelectrode and the reference electrode, and the potential changes under various experimental conditions, are almost negligible in comparison with the S-cell membrane potential and its changes. If the microelectrode is placed on the receptor surface of the retina, a transretinal d-c potential of 3 to 5 mv can be recorded. This d-c potential has been observed to behave opposite in direction to S-cell membrane potential changes under certain conditions (*2, 3*). Identification of the S-cells is based on recent histological studies of various fish retinas in this department (*1*) and on other findings by an electrophoretic dye method for marking individual cells (*5*). Alternating blue (460 nm) and red (630 nm) light stimuli (300-msec flashes) were routinely employed to differentiate the types of S-potentials. The experiments were conducted at room temperature (20° to 22°C).

Typical examples of hypoxia experiments in the isolated fish retina are illustrated in Figs. 1 and 2. In Fig. 1, the continuous recording A–H was obtained from a stellate amacrine cell, which responds with the C-type S-po-