vestigations cast doubt on this structure.[21] Measurements of the integrated absorption intensity of the carbonyl band gave a value of 4.9 units. The intensities for triketones absorbing near 1714 cm.$^{-1}$ lie in the neighborhood of 6.7 units and for diketones absorbing in the same region between 4.0 and 5.3 units (Table II). A dicarbonyl structure is therefore indicated for this hecogenin derivative.

Concluding Remarks

Integrated absorption intensities are more satisfactory than molecular extinction coefficients as a basis for correlation with molecular structure, particularly where small differences of intensity are involved, *e.g.*, less than a factor of 2–3, or if overlapping band systems are being investigated. The integrated absorption intensity is less dependent on slit width corrections and hence is more suitable for comparing results on different instruments.

In comparing the results obtained by the three methods it has been observed that methods II and III should be more accurate than I, since the latter is dependent on measurements at three points on the curve only. In the practical application, the much greater rapidity of method I is a compensatory factor. Methods II and III are tedious if the absorption curves are plotted point-by-point from the experimental records; this however may be obviated in the case of method II by the use of a logarithmic integrator,[22] or in method III by the use of a double beam spectrometer with a linear wave number scale.[8]

For survey work where an accuracy of 10% is sufficient, apparent integrated absorption intensities as determined by method II are probably adequate. If true integrated absorption intensities are required however, a small correction for the finite slit width may be applied. In this connection it is better to make measurements on strong bands, *e.g.*, peak absorption 70%, than on weak bands for the areas may be measured more accurately and the wing corrections minimized.

Although these investigations have been directed to the solution of certain specific problems in steroid chemistry, the application of infrared intensity measurements to other problems of molecular structure is self-evident. Molecules with chromophoric groups producing isolated absorption bands, *e.g.*, O–H, N–H, S–H, $-C\equiv C-H$, $C\equiv N$, $C=C$, are especially suitable for study as also are molecules with a particular absorption band considerably stronger than the neighboring absorption, *e.g.*, the 1240-cm.$^{-1}$ acyl acetate band. The relative intensities of some C–H, C–D, O–H and O–D stretching bands are at present being investigated in connection with the analysis and structure determination of deuterium-labeled compounds.

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OTTAWA, CANADA
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[21] R. B. Wagner, J. A. Moore and R. F. Forker, THIS JOURNAL, **71**, 3856, 4159 (1950).

[22] S. A. Francis, *J. Chem. Phys.*, **18**, 861 (1950).

[CONTRIBUTION FROM THE GIBBS CHEMICAL LABORATORY, HARVARD UNIVERSITY]

Photochemical Decomposition of Ketene. III

BY G. B. KISTIAKOWSKY AND WALTER L. MARSHALL

Methylene diradicals formed in the photochemical decomposition of ketene have been shown to react readily at room temperature with carbon monoxide forming ketene. The reaction appears to be of third order. A mass spectrographic study of the products of reaction of methylene and *sym*-dideuteroethylene favors the intermediate formation of trimethylene diradical rearranging into propylene. Allene has been identified as one of the products of the reaction of methylene with ketene. The over-all mechanism of the photochemical decomposition of ketene is discussed.

Past work[1] has identified several secondary reactions involved in the photochemical decomposition of ketene, but some of the experimental observations still lack adequate explanation. Thus, the rate of pressure rise decreases more on prolonged illumination than is to be expected on the basis of the proposed reaction mechanism; the dependence of the quantum yield of carbon monoxide on the ethylene to ketene ratio is not correctly reproduced; the postulated polymethylene diradicals are in doubt because of the absence of cycloparaffins among the products and so forth. The research reported here is an attempt to elucidate these matters.

Experimental

The reaction took place in a thermostated, dual cylindrical jacketed quartz cell with a G. E. fluorescent lamp (maximum at 3200 Å.) placed axially in the middle compartment of the cell. The reaction was followed by pressure measurements and by analyses of the products. Pressure measurements were made with the aid of a Pearson-type differential manometer.[2]

The preparation, purification and analysis of ketene were the same as described in the previous paper.[1]

Carbon monoxide from two sources was used, without noting any difference in the results. For earlier runs Mathieson compressed carbon monoxide was passed over hot copper and further purified by condensation in liquid nitrogen. Mass spectrographic analysis indicated that this gas contained a small amount of methane. For later runs

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[1] G. B. Kistiakowsky and N. W. Rosenberg, THIS JOURNAL, **72**, 321 (1950).

[2] A. Farkas and H. W. Melville, "Experimental Methods in Gas Reactions," The Macmillan Co., New York, N. Y., 1939.

carbon monoxide was generated from formic acid and passed through a conventional purification train.

Dideuteroethylene was prepared[3] from dideuteroacetylene by reduction with chromous chloride. Its infrared spectrum suggested that it was substantially pure *trans*-CHD=CHD. Dideuteroacetylene was generated by the reaction of thoroughly dehydrated calcium carbide with deuterium oxide.

Heavy carbon monoxide was prepared from Eastman Kodak C^{13} enriched (24%) potassium cyanide. Hydrogen cyanide was generated and hydrolyzed with concentrated hydrochloric acid to form formic acid. The latter yielded carbon monoxide on dehydration.

The Results and Interpretation

The results obtained in this investigation establish the occurrence of the association reaction of methylene diradicals with carbon monoxide, throw additional light on the reactions of methylene with ethylene and with ketene and provide some information on the subsequent fate of the products of these reactions.

The existence and nature of the reaction with carbon monoxide was established by the use of $C^{13}O$. The infrared spectrum of the products of irradiation of ketene and heavy carbon monoxide, taken with the Baird double-beam spectrometer, showed a shoulder in the strong carbonyl band of ketene at 2100 cm.$^{-1}$ displaced to lower frequencies. Upon balancing out the main band with pure ketene in the compensation cell, a well developed band with a maximum at 2050 cm.$^{-1}$ was observed. Neither the shoulder nor the new band were obtained when ketene was irradiated in presence of ordinary carbon monoxide. The products of the reaction with heavy carbon monoxide were then subjected to mass spectrographic analysis in the G. E. Research Laboratory.[4] Table I shows observed relative ion intensities in the region of interest. The corrections to observed intensities were made necessary by the presence of heavier ions (from traces of acetic acid, etc.) and consequently also of their fragments. This table shows that ketene was enriched to the extent of 7.6% in $H_2CC^{13}O$ and since the carbon monoxide contained 22% C^{13}, it follows that the recovered ketene contained 32% of material which is the product of recombination of methylene and carbon monoxide. This calculation assumes that the reactivities of ordinary and $C^{13}O$ are identical. A simultaneous measurement of the rate of ethylene formation showed that under the conditions of these experiments, methylene diradicals reacted five times more frequently with carbon monoxide than with ketene by that mechanism which leads to ethylene formation. In these experiments the average pressures of ketene and of carbon monoxide were 51 and 113 mm., respectively.

TABLE I

RELATIVE ION INTENSITIES FROM KETENE IRRADIATED IN THE PRESENCE OF ORDINARY CARBON MONOXIDE AND CARBON MONOXIDE ENRICHED IN $C^{13}O$

	Ordinary		Enriched		
Mass	Obsd.	Cor.	Obsd.	Cor.	Ion identity
43	7.3	3.4	15.1	11.9	$H_2CC^{13}O$
42	100	100	100	100	$HCC^{13}O$; H_2CCO
41	23	21.1	22.7	21.4	$CC^{13}O$; $HCCO$

The formation of ketene by the reaction of methylene and carbon monoxide explains the previously reported pronounced drop of the rate of pressure rise on protracted irradiation of ketene. To check this point further, experiments were made to determine the ratio (R) of rates of pressure rise in ketene with added carbon monoxide and in pure ketene. Because of comparatively low light intensity and the consequent small pressure changes, the results were somewhat erratic. Table II shows results obtained with mixtures containing approximately twice as much carbon monoxide as ketene. Their quality is typical of results obtained with other mixtures. The table shows not only that R is decreased below unity by the addition of carbon monoxide, but also that this decrease becomes more pronounced with rising total pressure. Moreover, the addition of carbon dioxide, which has no effect on the decomposition of pure ketene,[1] lowers the rate of pressure rise in the presence of carbon monoxide. Evidently total pressure exerts a preferential accelerating effect on the reaction of methylene with carbon monoxide.

TABLE II

RELATIVE RATES OF PRESSURE RISE IN SYSTEMS CONTAINING CARBON MONOXIDE

P_{CO}, mm.	P_{CH_2CO}, mm.	P_{CO_2}, mm.	R
44.3	25.2	...	0.61
92.7	47.3	...	.40
104.0	59.5	...	.28
108.0	51.0	...	.25
120.3	61.2	...	.33
53	27	260	.07
64	32	313	.10
80	40.5	391	.08
99	50	486	.07

It has been shown previously[1] that the association reactions of methylene radicals do not play a significant part in the kinetics of the over-all reaction. In the present experiments the reaction with ethylene was negligible and consequently the observed pressure effect means that the order of the reaction of methylene with ketene is lower than is that of the reaction with carbon monoxide. Nonetheless both could be association reactions if the mechanism of both is

$$\text{(a)}\quad R + X \rightleftarrows RX^*$$

$$\text{(b)}\quad RX^* + M \longrightarrow RX + M^*$$

where R is methylene, X is carbon monoxide or ketene and RX* is the "hot" molecule subsequently stabilized by collision with a molecule M. One needs only to suppose that, in view of the smaller number of internal degrees of freedom, the "hot" H_2CCO has shorter life so that most of the initially formed molecules decompose before stabilization by collision, whereas the adduct of methylene and ketene, $(CH_2)_2CO$, largely survives until the deactivating collision.[5] The observed ratio of over-all

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[3] W. I. Patterson and V. du Vigneaud, *J. Biol. Chem.*, **123**, 327 (1938).

[4] We wish to express our sincere gratitude for this and other mass spectrographic measurements to Dr. F. J. Norton.

[5] R. Gomer and G. B. Kistiakowsky, *J. Chem. Phys.*, **19**, 85 (1951).

rates with carbon monoxide and with ketene shows that step (a) must be considerably faster when X is carbon monoxide. Experiments were made at 18 and 48° to determine whether the ratio of these rates were temperature dependent. They showed that the difference of activation energies of the two reactions must be less than 2 kcal. which, however, does not conflict with the proposed mechanism.

It had been suggested previously[1] that the immediate product of the reaction of methylene and ethylene is the trimethylene diradical. It occurred to us that its formation might be demonstrated by the use of symmetrically substituted dideuteroethylene. If propylene is formed in one elementary step, there must be a transfer of a hydrogen atom in the critical complex, either from ethylene to methylene or from one carbon atom of ethylene to the other. Unless both processes occur with commensurate rates, which is most improbable, one must expect, in the case of dideuteroethylene, either $CH_3CD{=}CHD$ and, in lesser yield, $CH_2DCH{=}CHD$; or $CH_2DCD{=}CH_2$ and, in lesser yield, $CHD_2CH{=}CH_2$ but not all four simultaneously. The presence of these isomers should be detectable by the study of methyl ions derived from them in the mass spectrograph; if propylene is formed in one elementary step, one should find either CH_3 and CH_2D or CH_2D and CHD_2 but not all three. The experiments were complicated by the usual formation of higher hydrocarbons and the necessity to study the products of initial decomposition only, *i.e.*, very small quantities of propylene. The first separation of the reaction products gave a mixture in which dideuteroethylene was present in such a high concentration that the ion CHD^+ obscured the results of the analysis. A second separation was therefore carried out, repeating the bulb-to-bulb distillations longer. Ethylene was completely eliminated and so, unfortunately, was also the major portion of propylene the material being largely allene (*vide infra*). Nonetheless the results of the mass analysis of this sample are significant. Mass 17, 16 and 15 ion intensities, obtained with a sufficient resolving power of the instrument to separate peaks due to NH_2, NH, OH and O (present in the spectrograph) from those due to hydrocarbons, were found to be in the ratio 2:7:12. Mass 17 ion can be only CHD_2 but the relative intensities of the other two peaks are such that they cannot be due entirely to CD_2 and CHD, respectively. Evidently all three methyl ions, CHD_2, CH_2D and CH_3 are formed and hence we conclude that all four isotopic propylenes were present in the sample. This is to be expected if trimethylene radical is formed initially or, more generally, if the formation of propylene proceeds *via* an intermediate in which the identity of the methylene diradical has been lost.

A by-product of the mass spectrographic study of the products of dideuteroethylene was the discovery of allene as another of the secondary products formed in the decomposition of ketene. Table III, listing relative ion currents in the mass range 46 to 32, shows that in addition to ions produced by deuteropropylene those due to allene are very abundant. The intensity ratios are such as to exclude the presence of any significant amounts of deuterated allene. The formation of non-deuterated allene was further confirmed by obtaining its very characteristic infrared spectrum. A reaction of methylene with ketene, rather than ethylene, is clearly indicated as the source of allene. Altogether it now appears that the reaction of methylene with ketene is largely responsible for the complex products of this photochemical process. Thus the polymer tentatively identified in the previous publication[1] as polymethylene has been now definitely shown to contain some carbonyl bonds. The polymer was precipitated on sodium chloride plates inserted into the reaction vessel and then analyzed with the Baird spectrograph. The very thin layers obtained did not show many bands but the clearest of all was one at 1720–1760 cm.$^{-1}$. Noteworthy is also that the yield of higher hydrocarbons relative to that of the main product[1] is much greater from the reaction of methylene with ketene than from the corresponding reaction with ethylene.

TABLE III

RELATIVE ION ABUNDANCE FROM KETENE–DIDEUTEROETHYLENE MIXTURES

Mass	Ketene–ethylene mixture	Allene[6]
46	0.8	...
45	...	...
44	22.1	...
43	23.6	...
42	16.7	...
41	23.6	...
40	100	100
39	87.2	94
38	37.9	40
37	27.3	30
36	24.0	9
32	...	...

Previous work has shown that under the conditions of all these experiments the recombination of methylene radicals into ethylene does not take place to any significant extent. The important reactions of methylene are therefore

$$CH_2 + C_2H_4 \longrightarrow (CH_2)_3^* \qquad (1)$$

$$CH_2 + CO + M \longrightarrow CH_2CO + M \qquad (2)$$

$$CH_2 + CH_2CO \longrightarrow (CH_2)_2CO^* \qquad (3)$$

Reaction 3 is written as an addition because the alternative: $CH_2 + CH_2CO \rightarrow C_2H_4 + CO$ would result in two moles of carbon monoxide produced per mole of ketene decomposed in the primary photochemical step. In mixtures rich in ethylene, on the other hand, one mole only must be obtained as a consequence of reaction 1. Thus the yield of carbon monoxide should decrease by a factor of two when ethylene is added to ketene, which is contrary to observations.[1]

The trimethylene diradical produced in reaction 1 must rearrange readily into propylene, as suggested by the small yield of more complex products when this reaction is predominant. The product of reaction 3 is probably a mixture of diradicals CH_2CH_2CO and CH_2COCH_2, the second of which at any rate should be stable against isomerization and cyclization because of resonance with struc-

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(6) Y. M. Delfosse and W. Bleakney, *Phys. Rev.*, 56, 256 (1939).

tures such as $CH_2C\dot{O}{=}CH_2$. It is very tempting to assume that ethylene, propylene, etc., are formed as the result of a reaction of methylene with these diradicals. Against this, however, is the definite evidence[1] that light intensity has no effect on the course of the reaction in ketene–ethylene mixtures. We have now repeated previous work on this subject arriving at the same conclusion. Therefore, we are led to the proposition that metathetic reactions of two such diradicals account for the olefinic hydrocarbons, whereas their association leads to the polymer. The rearrangement of the first of the two radicals readily accounts for the trace of acrolein observed in previous work,[1] whereas the oxygen of the second diradical should be quite reactive and therefore may be removed (perhaps by methylene) to yield allene.

CAMBRIDGE 38, MASS. RECEIVED JUNE 14, 1951

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND THE STUDY GROUP ON RHEUMATIC DISEASES, NEW YORK UNIVERSITY COLLEGE OF MEDICINE]

Metachromasy of Thiazine Dyes Produced by Chondroitin Sulfate[1]

BY ANNE LEVINE AND MAXWELL SCHUBERT

A spectrophotometric study has been made of the appearance and disappearance of metachromasy in solutions of two thiazine dyes as a function of increasing chromotrope concentration. Purified but amorphous potassium chondroitin sulfate and crystalline calcium chondroitin sulfate were used. The resulting progressive changes in extinction at two critical wave lengths have been compared with the corresponding changes that occur in aqueous solutions of thiazines as their concentration is increased. The results rule out the possibility that metachromasy is due to dye dimerization, but could be interpreted to offer slender support to the theory of dye polymerization. An alternative view is suggested. The effect of electrolytes in destroying metachromasy is also studied. A difference in the chromatropic behavior between amorphous and crystalline chondroitin sulfate is found to occur at high concentrations.

Metachromasy[1a] is the change in color of dilute aqueous solutions of some dyes caused by the addition of certain high molecular weight polyelectrolytes. Lison[2] pointed out that the common substances of plant and animal tissues which cause metachromasy are half sulfate esters of high molecular weight polysaccharides, but it was subsequently observed that hyaluronate, hexametaphosphate[3] and silicates[4] also produce this effect.

Holmes first noticed that the basic dyes which do not conform to Beer's law are capable of producing metachromasy in appropriate tissues, and that this metachromatic color is similar to the color of the more concentrated dye solutions.[5] In the cases of toluidine blue and brilliant cresyl blue, Lison[2] observed a gradual shift in the absorption maxima with increasing dye concentrations approaching the maxima of the dyes in their metachromatic states. Michaelis and Granick,[6] in an extensive study of the absorption of a number of cationic dyes in the presence of agar, concluded that a parallelism exists between the production of metachromasy in dyes and their deviation from Beer's law in aqueous solution. A great advance was made by the work of Lison[2] and of Michaelis and Granick[6] who measured extinction curves in agar gels in contrast to all previous work which had been confined to qualitative observations on stained tissues.

If the metachromatic color change is caused by a mechanism similar to that which causes the color change with increasing dye concentration, then it might be expected that gradually increasing the chromotrope concentration, at a fixed dye concentration, would produce a similar progressive effect on the extinction curve of the dye. The use of chondroitin sulfate to induce metachromasy lends itself to such a study better than most known chromotropes since it has been made in quite pure form and has been crystallized as a calcium salt.[7] It yields perfectly clear, stable solutions that do not gel even at concentrations up to 10% and for quantitative study, therefore, has several advantages over agar. With this substance, a detailed study has been made of the metachromasy produced in aqueous solutions of a few thiazine dyes under a variety of conditions. It is now possible to compare quantitatively the effects due to metachromasy with those due to deviations from Beer's law. Most of the work has been done with methylene blue since deviations from Beer's law have been more intensively studied with this dye than with any other thiazine. Methylene blue does not show metachromasy in histological applications but its metachromatic spectral changes are similar to those of toluidine blue or of thionin which show obvious color changes.

Experimental

Methylene blue was prepared from commercial material by recrystallization three times from hot water. For comparison a preparation was synthesized according to a method already described.[8] Extinction curves of both were the same and were affected similarly by chondroitin sulfate. The position of the peak of the band persistently occurred

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[1] This work was supported in part by the United States Public Health Service and in part by the Masonic Foundation for Medical Research and Human Welfare.

[1a] The word "metachromasy" first appears in English in the work of Holmes (ref. 5). The word "metachromasia" first appears in *C. A.*, (1934) in an abstract of a paper in French by Lison. Only in 1940 does an original paper appear in English using the word "metachromasia," Hempelman, *Anal. Record*, **78**, 197 (1940). Therefore, in spite of common current usage of "metachromasia" in histochemical works the word "metachromasy" is retained here.

[2] L. Lison, *Arch. Biol.*, **46**, 599 (1935).

[3] J. M. Wiame, THIS JOURNAL, **69**, 3146 (1947).

[4] R. C. Merrill and R. W. Spencer, *ibid.*, **70**, 3683 (1948).

[5] W. C. Holmes, *Stain Technol.*, **1**, 116 (1926).

[6] L. Michaelis and S. Granick, THIS JOURNAL, **67**, 1212 (1945).

[7] J. Einbinder and M. Schubert, *J. Biol. Chem.*, **191**, 591 (1951).

[8] L. Michaelis, M. Schubert and S. Granick, THIS JOURNAL, **62** 204 (1940).