

While these effects do not change the conclusions of the earlier work, namely, that  $J_{\text{HF}}$  is absolutely positive in  $\text{CH}_3\text{F}$ ,<sup>6</sup> it seemed desirable to carefully examine the question of chemical shift referencing in liquid-crystal solvents.

Remeasurement of the proton chemical shift anisotropy of  $\text{CH}_3\text{F}$  in *p,p'*-di-*n*-hexyloxyazoxybenzene at 80° internally referenced with TMS gave  $(\sigma_{\parallel} - \sigma_{\perp})^{\text{H}} = -12.9 \pm 1.3$  ppm. The concentration of TMS was roughly equal to that of  $\text{CH}_3\text{F}$ , while in the previous measurement, where a larger value was obtained, the TMS was present in excess. Measurement of the proton chemical shift anisotropy at 80° internally referenced with  $\text{CH}_4$  gave the surprising result that  $(\sigma_{\parallel} - \sigma_{\perp})^{\text{H}} = -0.6 \pm 0.5$  ppm. Moreover, it was found that when  $\text{CH}_4$  and TMS were both dissolved in *p,p'*-di-*n*-hexyloxyazoxybenzene the signal due to TMS was  $0.172 \pm 0.005$  ppm upfield from  $\text{CH}_4$  in the isotropic state and  $0.278 \pm 0.003$  ppm upfield in the nematic state, while the signal of gaseous TMS is  $0.138 \pm 0.004$  ppm upfield from gaseous  $\text{CH}_4$ . We believe these differences are mainly due to what has been called "solvent anisotropy effects."<sup>7</sup> There is at present no definite prescription for deciding which of the two compounds is the best reference to use in a particular situation, although TMS does exhibit a small direct dipolar splitting in a nematic solvent,<sup>8</sup> indicating that it may possibly be exhibiting a small shift anisotropy.

The fluorine chemical shift anisotropy is  $(\sigma_{\parallel} - \sigma_{\perp})^{\text{F}} = -159.3 \pm 0.7$  ppm internally referenced with  $\text{CF}_4$ .

The anisotropy in the magnetic susceptibility of *p,p'*-di-*n*-hexyloxyazoxybenzene,  $\Delta\chi$ , is of interest since it must be known to evaluate the change in the bulk susceptibility correction for the nematic to isotropic phase transition.<sup>5</sup> This can be determined from the width of the nmr signal of nuclei in the annulus of a coaxial sample cell containing the liquid crystal in the central region. The nematic phase has a smaller diamagnetic susceptibility than the isotropic phase and will produce a smaller signal width of the nuclear resonance of material in the annulus. This width has been discussed previously<sup>9-11</sup> and is given by<sup>9</sup>

$$\Delta H = 4\pi H_0 \left[ (\chi_1 - \chi_2) \frac{a^2}{r^2} + (\chi_2 - \chi_3) \frac{b^2}{r^2} \right]$$

where  $a$  and  $b$  are the inner and outer radii of the inner tube of the coaxial cell,  $r$  is the mean radius of the annulus, and  $\chi_1$ ,  $\chi_2$ , and  $\chi_3$  are the magnetic susceptibilities of the contents of the inner tube, the glass, and the contents of the annulus, respectively. The anisotropy in the magnetic susceptibility is then given by

$$\Delta\chi = \frac{3}{8\pi} \frac{r^2}{a^2} \left[ \frac{\Delta H^{\text{iso}} - \Delta H^{\text{nem}}}{H_0} \right]$$

neglecting the small contributions from the volume change accompanying the phase transition. In Table I values of  $\Delta H$  are given for hexafluorobenzene in the

(6) R. A. Bernheim and B. J. Lavery, *J. Am. Chem. Soc.*, **89**, 1279 (1967).

(7) A. D. Buckingham, T. Schaefer, and W. G. Schneider, *J. Chem. Phys.*, **32**, 1227 (1960).

(8) L. C. Snyder and S. Meiboom, *ibid.*, **44**, 4057 (1966).

(9) C. A. Reilly, H. M. McConnell, and R. G. Meisenheimer, *Phys. Rev.*, **98**, 264A (1955).

(10) M. G. Morin, G. Paulett, and M. E. Hobbs, *J. Phys. Chem.*, **60**, 1594 (1956).

(11) J. R. Zimmerman and M. R. Foster, *ibid.*, **61**, 282 (1957).

**Table I.** Measured Widths,  $\Delta H$ ,<sup>a</sup> Magnetic Susceptibility Anisotropies,  $\Delta\chi$ ,<sup>b</sup> Downfield Chemical Shifts,  $\delta_{\text{CF}_4}$ ,<sup>c</sup> and Estimated Change in Solvent Anisotropy Contributions to the Magnetic Shielding,  $\sigma_a$ , for the Isotropic to Nematic Phase Transition<sup>d</sup>

Temp, °C	$\Delta H$ , ppm	$\Delta\chi$ , ppm	$\delta_{\text{CF}_4}$ , ppm	$\Delta\sigma_a$
80	2.74	0.177	105.28	-0.50
100	2.86	0.151	105.27	-0.45
130	3.55	0	105.03	...

<sup>a</sup> Of the  $^{19}\text{F}$  resonance of  $\text{C}_6\text{F}_6$  in the annulus of a coaxial cell containing  $\text{CF}_4$  dissolved in *p,p'*-di-*n*-hexyloxyazoxybenzene. <sup>b</sup> For *p,p'*-di-*n*-hexyloxyazoxybenzene. <sup>c</sup> From  $\text{C}_6\text{F}_6$ . <sup>d</sup> The liquid crystal is isotropic at 130°, nematic at the other two temperatures.

annulus of a coaxial cell containing  $\text{CF}_4$  dissolved in *p,p'*-di-*n*-hexyloxyazoxybenzene. The value of  $a^2/r^2$  was 0.545, and the fluorine resonance frequency was 94.075 MHz. The values of  $\Delta\chi$  compare favorably to the value of 0.158 ppm found for *p*-azoxyanisole.<sup>12</sup>

It is also seen from Table I that there is a downfield shift of the  $^{19}\text{F}$  resonance of  $\text{CF}_4$  when the solvent changes from isotropic to nematic. This is opposite to what would be expected if the anisotropy in bulk susceptibility were the only environmental effect present. Attributing this behavior to changes in the solvent anisotropy shielding, we find for the change in shielding in going from the isotropic to nematic solvent

$$\Delta\sigma = \Delta\sigma_b + \Delta\sigma_a = \frac{4\pi}{9}\Delta\chi + \Delta\sigma_a$$

The values of  $\Delta\sigma_a$  required to produce the observed shifts in  $\text{CF}_4$  are listed in Table I.

Assuming that the magnitude of the correction is the same for  $\text{CH}_3\text{F}$ , the chemical shift anisotropies are  $(\sigma_{\parallel} - \sigma_{\perp})^{\text{H}} = -6.1$  ppm and  $(\sigma_{\parallel} - \sigma_{\perp})^{\text{F}} = -157$  ppm, obtained from the externally referenced spectrum.<sup>4</sup> The agreement for the fluorine chemical shift anisotropy obtained when internally referenced with  $\text{CF}_4$  is quite reasonable.

In conclusion, we believe that the determination of  $^{19}\text{F}$  magnetic shielding anisotropies by internal referencing or by external referencing with bulk susceptibility anisotropy corrections leads to reasonably accurate values. However, proton shielding anisotropies are sharply dependent upon the techniques and references used. Until these various effects are well understood, consistent and well-described experimental technique is suggested.

(12) G. Föex, *Trans. Faraday Soc.*, **29**, 958 (1933).

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## The Formation and Reactions of Monovalent Carbon Intermediates. I. The Photolysis of Diethyl Mercurybisdiazoacetate

Sir:

The chemistry of carbon atom as well as di- and tri-valent carbon intermediates has been thoroughly explored. Little, however, is known about the monovalent carbon intermediates. Methyne (CH) has been detected spectroscopically in numerous energetic reac-

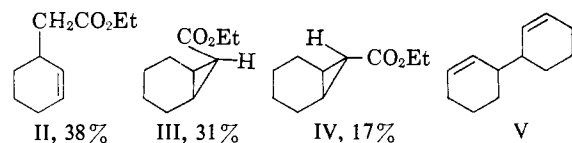
tions<sup>1</sup> and its intermediacy has been postulated in reactions of "hot" carbon atoms with organic substrates,<sup>1a,d,f</sup> although, with the exception of the recent vacuum ultraviolet flash photolysis of methane by Braun, McNesby, and Bass,<sup>1b</sup> no detailed kinetic-mechanistic study has been reported to date.<sup>1</sup> The scarcity of information on this family of carbon intermediates is related to the difficulty in generating them under conditions amenable to mechanistic interpretation.

We have recently initiated a program in our laboratory for the systematic study of monovalent carbon intermediates utilizing the photochemical decomposition of mercury and other metal diazo compounds. The generation and chemical behavior of these intermediates will be exemplified in the present communication through the photolysis of diethyl mercurybis(diazoacetate) in cyclohexene and 2-propanol.

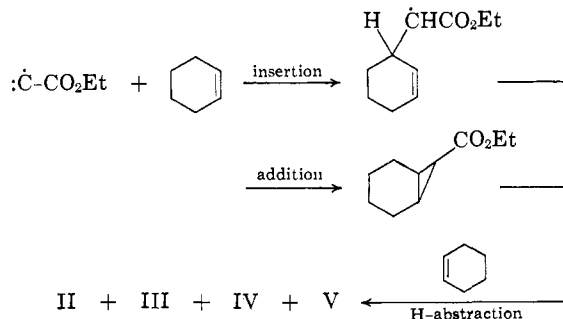
Hg(N<sub>2</sub>CCO<sub>2</sub>Et)<sub>2</sub> (I) was prepared in 85% yield by treating ethyl diazoacetate with yellow mercuric oxide in ether, mp<sup>2</sup> 102–103°; λ<sub>max</sub><sup>EtOH</sup> 264 mμ (ε 2490), 380 (107). The mass spectrum indicated a molecular weight of 428 with a base peak at *m/e* 85.

Photolysis of I in fluorolube glass at –196° developed a single symmetrical esr signal with no hyperfine splitting at *g* = 2.001, indicating the presence of a doublet ground-state species containing no protons adjacent to the radical site<sup>3</sup> (↓↑ C–CO<sub>2</sub>Et).

On photolysis in cyclohexene (2.5 g in 250 ml, 250-w medium-pressure mercury arc with a Vycor filter) elimination of nitrogen and mercury was immediately visible. On bleaching of the yellow color the following products were recovered.



Both the esr spectrum and the products suggest the intervention of carboxymethylene as the reactive intermediate of the photolysis.



Insofar as the spin conservation rule holds, a doublet-state reagent may be expected to undergo insertion

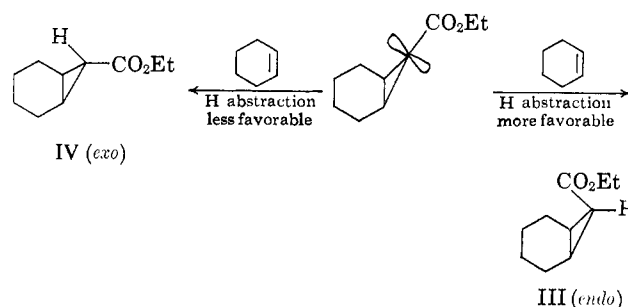
(1) (a) D. E. Clark and A. F. Voigt, *J. Am. Chem. Soc.*, **87**, 5558 (1965); (b) W. Braun, J. R. McNesby, and A. M. Bass, *J. Chem. Phys.*, **46**, 2071 (1967); (c) C. E. Moore and H. P. Broida, *J. Res. Natl. Bur. Std.*, **A63**, 19 (1959); (d) J. Nichols, C. MacKay, and R. Wolfgang, *J. Am. Chem. Soc.*, **88**, 1065 (1966); (e) G. J. Pontrelli, *Sci. Tech. Aerospace, Rept.*, **4** (1), 24 (1966); (f) A. P. Wolf, *Advan. Phys. Org. Chem.*, **1**, 255 (1964).

(2) E. Buchner, *Ber.*, **28**, 215 (1895), mp 104°.

(3) P. S. H. Bolman, H. E. Gunning, O. P. Strausz, and T. DoMinh, to be published.

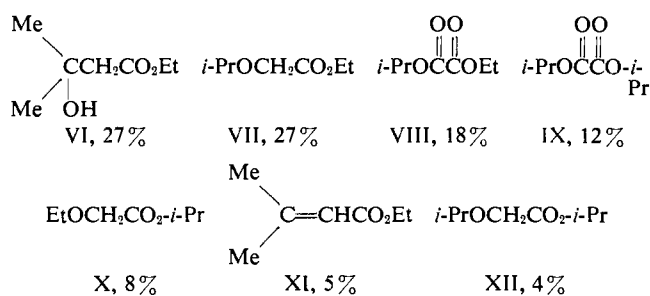
reaction with hydrocarbons (by analogy with singlet carbenes), and quartet-state reagent, hydrogen abstraction (by analogy with triplet carbenes). The esr spectrum indicates a doublet ground state for CCO<sub>2</sub>Et (the ground state of CH is also doublet<sup>4</sup>) which is probably the primary product of the photolysis.

An alternate to the above sequence would be hydrogen abstraction first, followed by insertion and addition of the resulting carboxymethylene. The latter from the photolysis of diazoacetate in cyclohexene has been reported to give similar products with one notable exception: the product ratio IV:III obtained was 1.6 as compared to 0.56 for the present experiment.<sup>5</sup> This militates against the intervention of a carbene intermediate. The fact that the less stable *endo* isomer is the predominant product here can be readily rationalized by a preferential attack of the radical intermediate on the more exposed side of the p orbital, in accordance with the proposed addition-abstraction sequence

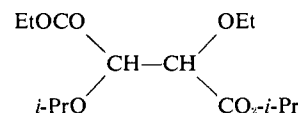


When the yield of bicyclohexenyl was carefully measured and corrected for the competitive disproportionation of cyclohexenyl radical, the mole ratio of addition and insertion products over bicyclohexenyl was found to agree (1.85) with the stoichiometric value (2.0).

Photolysis of I in 2-propanol under similar conditions gave<sup>6</sup> VI–XII, acetone, and a compound tentatively



identified as



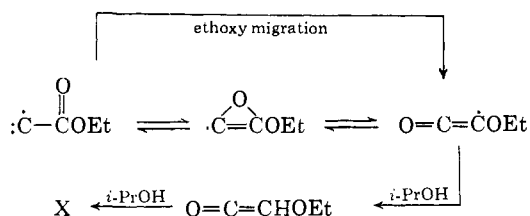
Evidently this reaction is more complex than the cyclohexene reaction. The two oxalates VIII and IX arise from the interaction of a reactive intermediate (possibly the isomeric ketene radical) with dissolved

(4) (a) G. Herzberg, "Spectra of Diatomic Molecules," 2nd ed, D. Van Nostrand Co., Inc., Princeton, N. J., 1961, p 341; (b) P. W. B. Pearce and A. G. Gaydon "The Identification of Molecular Spectra," 3rd ed, John Wiley and Sons, Inc., New York, N. Y., 1963, pp 100–101.

(5) P. S. Skell and R. M. Etter, *Proc. Chem. Soc.*, 443 (1961). We have also examined the *trans* to *cis* product ratios with vinyl ethyl ether. Skell and Etter reported a value of 1.9 for carboxymethylene addition.

(6) Numbers in parentheses represent only relative yields.

oxygen since under anerobic conditions their yield fell drastically. The two principal products VI and VII can then be rationalized as being formed by a C-H insertion and OH insertion or polar addition of carbethoxymethylene, followed by H abstraction. Product XI may form from dehydration of VI. Of particular interest is product X with a net shift of either the ethoxy group or of the carbonyl oxygen. The process may be envisaged as going through a ketene type intermediate.<sup>7</sup>



Again, as with cyclohexene, the product distribution was significantly different from that obtained from the reaction of carbethoxymethylene, indicating that the latter is not a principal transient in the present reaction. (Photolysis of ethyl diazoacetate in 2-propanol gave rise to VI, VII, X, and XII in a ratio of 9:25:29:12).<sup>7</sup>

The insertion reactions of carbethoxymethylene appear to be highly selective, which may partly be attributed to the intervention of polar structures in the transition state analogous to the one postulated by Doering and Knox for carbethoxymethylene.<sup>8</sup>

Further extensive studies on a series of monovalent carbon intermediates, including species like NC-C·, RCO·, PhCO·, CF<sub>3</sub>C·, and :CH itself, are currently underway in our laboratory.

The results of a preliminary esr study of (CF<sub>3</sub>CN<sub>2</sub>)<sub>2</sub>Hg and the photolysis of (PhCOCN<sub>2</sub>)<sub>2</sub>Hg are in good agreement with the proposed intervention of a carbyne intermediate in the photodecomposition of the diazomercurial structure.

Product identifications in the present study were accomplished by high-resolution mass spectrometry, infrared spectra, and nmr spectroscopy in conjunction with unambiguous syntheses and chemical transformations to known derivatives.

**Acknowledgment.** The authors are grateful to the National Research Council of Canada for financial support.

(7) Carbethoxymethylene has been shown to undergo an analogous rearrangement to yield carbethoxy ketene: T. DoMinh, O. P. Strausz, and H. E. Gunning, submitted for publication.

(8) W. von E. Doering and L. H. Knox, *J. Am. Chem. Soc.*, **83**, 1989 (1961).

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## The Chemistry of the Gentamicins. I. Characterization and Gross Structure of Gentamicin A

Sir:

Gentamicin A, a constituent of the gentamicin complex,<sup>1</sup> is a broad-spectrum antibacterial antibiotic.<sup>2</sup>

(1) M. J. Weinstein, G. M. Luedemann, E. M. Oden, and G. H. Wagman, "Antimicrobial Agents and Chemotherapy," American Society for Microbiology, Ann Arbor, Mich., 1963, p. 1.

Its separation from the commercial gentamicin C complex as a coproduced antibiotic<sup>1</sup> and its chromatographic differentiation from other gentamicins and related antibiotics<sup>3</sup> have been described.

Crude gentamicin A can be purified as the N-acetylated derivative by liquid-liquid partition chromatography followed by regeneration to the free base with alkaline hydrolysis.<sup>4</sup> Fractional crystallization from aqueous methanol afforded pure gentamicin A free base as colorless clusters of prismatic needles, [α]<sub>D</sub><sup>23</sup> +136° (c 1.0, H<sub>2</sub>O).

Thin layer chromatography<sup>5</sup> reveals only one component. Crystalline gentamicin A free base has a constant weight at room temperature and 2 mm. *Anal.* Calcd for C<sub>18</sub>H<sub>36</sub>N<sub>4</sub>O<sub>10</sub>·1.5H<sub>2</sub>O: C, 43.63; H, 7.93; N, 11.31; O, 37.13. Found: C, 43.60; H, 7.86; N, 11.56; O, 37.16. The base hydrate sinters at 151–154° with loss of crystal water and decomposes slowly above 200°. The quantitative removal of the crystal water is accomplished with great difficulty. *Anal.* Calcd for C<sub>18</sub>H<sub>36</sub>N<sub>4</sub>O<sub>10</sub>: C, 46.15; H, 7.75; N, 11.96; O, 34.15; mol wt, 468.5. Found (after drying to constant weight at 120° *in vacuo*): C, 45.70; H, 7.60; N, 11.80; O, 34.90; mol wt, 410 (Signer). Conversion to the N-acetyl derivative produced a homogeneous substance.<sup>6</sup> *Anal.* Calcd for C<sub>18</sub>H<sub>32</sub>N<sub>4</sub>O<sub>10</sub>(COCH<sub>3</sub>)<sub>4</sub>: acetyl, 27.04. Found: acetyl, 26.44 (hydrazinolysis). Gentamicin A free base is soluble in water and partly soluble in methanol but practically insoluble in ethanol and less polar solvents. Gentamicin A exhibits positive ninhydrin, Elson-Morgan, and Lemieux-Bauer reactions, but negative Sakaguchi and Fehling reactions. In contrast to kanamycin, neomycin, and paromomycin, gentamicin A does not yield a furfural-type of chromophore when treated with 40% sulfuric acid.

Hydrolysis of gentamicin A in 6 N hydrochloric acid for 6 hr at 100° results in complete destruction of biological activity. Paper chromatography<sup>7</sup> of the hydrolysate and ninhydrin development revealed the presence of four major components. The slowest component (R<sub>f</sub> 0.11) was obtained in crystalline form after charcoal decolorization, concentration to a syrup, and addition of methanol and ethanol. After recrystallization it was found to be identical with 2-deoxystreptamine dihydrochloride isolated from neomycin,<sup>8</sup> kanamycin,<sup>9</sup> and paromomycin.<sup>10</sup> Identity was confirmed by paper chromatography, infrared spectroscopy, and optical rotation measurement. A second crystalline hydrolysis product (R<sub>f</sub> 0.26) obtained from the mother liquor was identified as 2-amino-2-deoxy-D-glucose hydrochloride by paper chromatography, infrared spectroscopy,

(2) M. J. Weinstein, G. M. Luedemann, E. M. Oden, and G. H. Wagman, "Antimicrobial Agents and Chemotherapy," American Society for Microbiology, Ann Arbor, Mich., 1965, p. 816.

(3) H. Maehr and C. P. Schaffner, *J. Chromatog.*, **30**, 572 (1967).

(4) G. H. Wagman and M. J. Weinstein, *J. Med. Chem.*, **7**, 800 (1964).

(5) Silica gel G; chloroform-methanol-28% ammonium hydroxide-water, 1:4:2:1, v/v; R<sub>f</sub> 0.60.

(6) Descending paper chromatography, Whatman No. 1; 1-butanol-pyridine-water, 3:2:2, v/v; R<sub>f</sub> 0.30.

(7) Descending paper chromatography, Whatman No. 1; 1-butanol-pyridine-water-acetic acid, 6:4:3:1, v/v.

(8) D. A. Kuehl, Jr., M. N. Bishop, and K. Folkers, *J. Am. Chem. Soc.*, **73**, 881 (1951).

(9) M. J. Cron, D. L. Johnson, F. M. Palermi, Y. Perron, H. D. Taylor, D. F. Whitehead, and I. R. Hooper, *ibid.*, **80**, 752 (1958).

(10) T. H. Haskell, J. C. French, and Q. R. Bartz, *ibid.*, **81**, 3480 (1959).