

charged molecules due to the ω amino groups in lysine residues and with lipophilic chains through the side chains of leucine residues should lead to the conclusion that this protein has not only different binding capacities for different compounds, but also different mechanisms by which it binds. If bacterial proteins rather than lipids are the compounds with which phenolic disinfectants associate, it should be possible to demonstrate as much binding of the latter to bacterial proteins as to whole cells as was shown by Weinbach and Garbus (10, 11) with mitochondria and mitochondrial protein in their studies with uncoupling halophenols. These possibilities are currently being tested by examining reaction mixtures containing serum proteins or bacterial proteins and phenol derivatives for complexes using Sephadex chromatography, electrophoresis, density gradient ultracentrifugation, difference spectrophotometry, and reduction in bactericidal and bacteriostatic effects. It is felt that an intimate knowledge of the reaction between phenol derivatives and organic matter such as serum proteins would be useful in the design of disinfectants, the activity of which would not be affected by organic matter. An ultimate goal in these studies is a complete knowledge of the site(s) of action of phenolic germicides in bacterial cells.

REFERENCES

- (1) Judis, J., *J. Pharm. Sci.*, **51**, 261(1962).
- (2) *Ibid.*, **52**, 126(1963).

- (3) *Ibid.*, **53**, 196(1964).
- (4) *Ibid.*, **54**, 417(1965).
- (5) *Ibid.*, **54**, 541(1965).
- (6) Commager, H., and Judis, J., *ibid.*, **54**, 1436(1965).
- (7) Dedenken, R. H., *Biochim. Biophys. Acta*, **17**, 494 (1964).
- (8) Gladky, E., and Liss, E., *Biochem. Z.*, **331**, 65(1959).
- (9) Weinbach, E. C., and Garbus, J., *Science*, **145**, 824 (1964).
- (10) Weinbach, E. C., and Garbus, J., *J. Biol. Chem.*, **240**, 1811(1965).
- (11) *Ibid.*, **240**, 169(1966).
- (12) Sykes, G., "Disinfection and Sterilization," D. Van Nostrand Co., Inc., Princeton, N. J., 1958, p. 244.
- (13) Albert, A., "Selective Toxicity," John Wiley & Sons, Inc., New York, N. Y., 1965.
- (14) Foster, J. F., in "The Plasma Proteins," vol. I, Putnam, F. W., ed., Academic Press Inc., New York, N. Y., 1960, p. 188.
- (15) Haurowitz, F., "The Chemistry and Function of Proteins," Academic Press Inc., New York, N. Y., 1963, p. 238.
- (16) Norkin, S. A., Czernobilsky, B., Griffith, E., and Dubin, I. N., *Arch. Pathol.*, **80**, 273(1965).
- (17) McMenamy, R. H., *J. Biol. Chem.*, **240**, 4235(1965).
- (18) Brunkhorst, W. K., and Hess, E. L., *Arch. Biochem. Biophys.*, **111**, 54(1965).
- (19) Tritsch, G. L., and Tritsch, N. E., *J. Biol. Chem.*, **240**, 3789(1965).
- (20) Perkins, H. R., *Bacteriol. Rev.*, **27**, 18(1963).
- (21) Salton, M. R. J., "The Bacterial Cell Wall," Elsevier Press, Amsterdam, The Netherlands, 1964.
- (22) Salton, M. R. J., in "The Bacteria," vol. I, Gunsalus, I. C., and Stanier, R. Y., eds., Academic Press Inc., New York, N. Y., 1960, p. 120.
- (23) Judson, C. M., and Kilpatrick, M., *J. Am. Chem. Soc.*, **71**, 3110(1949).
- (24) Parke, T. V., and Davis, W. W., *Anal. Chem.*, **26**, 642 (1954).
- (25) Vandenbelt, J. M., Henrich, C., and Vanden Berg, S. G., *ibid.*, **26**, 126(1954).
- (26) Condon, F. E., and Meislich, H., "Introduction to Organic Chemistry," Holt, Rinehart and Winston, Inc., New York, N. Y., 1960, p. 615.
- (27) Davis, B., and Dubos, R., *J. Exptl. Med.*, **86**, 215 (1947).

Molecular Orbital Calculations of the Electronic Structure of the Sydnones

By LEMONT B. KIER and EDWARD B. ROCHE*

The electronic structure of the sydnone ring has been calculated from an ω -HMO technique. Several new parameters for the calculation have been derived, in conjunction with previously used parameters. Charge densities derived have yielded accurate calculations of dipole moments. Energy levels have yielded values which correlate well with observed U.V. maxima. A general discussion of the calculated structures is presented.

OUR INTEREST in the medicinal chemical aspects of the mesoionic compounds known as the sydnones has required a further understanding of the chemistry of these aromatic heterocycles (1-6). Recent reviews of these com-

pounds have summarized their chemical and physical properties (7-10) but have not added much basic information concerning their electronic structure and the electronic changes involved in their reactions. This information is essential if the relationships between their structure and pharmacological activity is to be evaluated.

Received February 15, 1966, from the College of Pharmacy, Ohio State University, Columbus.

Accepted for publication May 10, 1966.

This investigation was supported in part by grant GM-13100-01 and predoctoral fellowship I-F1-GM-23,852-01A1 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

The authors acknowledge the Ohio State University Computer Center for the use of the IBM 7094 computer and Mr. Douglas Fleckner, a programmer at the center, for his assistance in modifying the program. The authors also acknowledge Dr. Thomas H. Brown, Department of Chemistry, University of Illinois, Chicago Circle, for his assistance on the convergence problem. We also acknowledge Mr. Harold Brown, Department of Mathematics, Ohio State University, for valuable discussions.

* National Institutes of Health Predoctoral Fellow.

A classical resonance theory or valence bond approach to the electronic picture of the sydnones is not illuminating since, embodied within the definition of a mesoionic compound is the requirement that no covalent structure can be drawn for the system (11). Furthermore, it has been shown that at least 20 polar structures can be drawn for phenylsydnone (12). The total number of such

TABLE I.—PARAMETERS

Atom	h	Bond	k
\dot{N} (pyrrole type)	1.5	C— \dot{N} (pyrrole type)	0.8
H ₃ (conjugated methyl)	-0.5	\dot{N} — \ddot{O}	0.5
$\equiv\text{C}$ — (conjugated methyl)	-0.1	\dot{N} — \dot{N}	0.9
		H ₃ $\equiv\text{C}$ — (conjugated methyl)	2.8

TABLE II.—DIPOLE MOMENTS

Compd.	Calcd. μ (D)	Exptl. μ (D)	Ref.
Pyrrole	1.85	1.80	(25, 26)
<i>N</i> -Methylpyrrole	2.16	1.92	(44)
Toluene	0.38	0.39	(45)
Furazan	3.10	3.38	(46)
Isoxazole	2.79	2.76	(47, 48)
<i>N</i> -Methylpyrazole	2.34	2.28	(49)
<i>N</i> -Phenylpyrazole	2.30	2.0	(49)
3-Phenylsydnone	6.47	6.48	(50)
3-Methylsydnone	7.06	7.31 ^a	...

^a This value was determined by the authors employing the method described by Smyth (51). Dielectric constants were determined on a General Radio Co-type No. 1610-A capacitance bridge.

structures which must be considered in a valence bond approach is many more, and the real problem arises in weighting each structure in the overall equation for the wave function of the molecule. Thus, the valence bond or classical resonance theory approach to the electronic structure of sydnone presents immense difficulties.

A more reasonable approach to the solution of the wave equation of the system is the LCAO-MO (linear combination of atomic orbitals-molecular orbital) method.

The approximations involved and the various techniques employed for solution of electronic structures have been adequately described (13-15). A number of studies of this type have been done on the sydnone, using simple Hückel procedures (16-18). The results of these calculations failed to reproduce physical experience, such as dipole moment. This failure undoubtedly was partly a result of a poor selection of heteroatom parameters, certainly unrefined at the time these calculations were made, and the failure of the simple Hückel method to consider electron correlation.

In previous calculations performed in this laboratory, the authors have empirically treated the effect of electron correlation within the framework of the Hückel method (19, 20). This has been done by employing a disposable parameter which serves to alter the Coulomb integrals of the atoms as a function of the calculated charge densities, q , of the respective atoms (21). The procedure, known as the " ω -technique," produces new values of the Coulomb integral,

in terms of β_0 , the standard resonance integral for the carbon-carbon bond in benzene (22).

$$\alpha_i = \alpha_0 + \omega q_i \beta_0$$

This technique involves iterating the calculation until self-consistency is achieved in the charge densities at each position in the molecule. The value of ω used in this study was 1.4, which corresponds to the value used by several other authors (23, 24) and appears to give results that correlate well with experimental data.

PARAMETERS

An essential feature for the successful use of this method of calculation is the choice of parameters for heteroatoms. The parameter used to modify the Coulomb integral of a heteroatom, h_x , must reflect its modified electronegativity and electronic configuration with respect to an aromatic carbon atom. This modification is again made in terms of the standard resonance integral, β_0

$$\alpha_x = \alpha_0 + h_x \beta_0$$

A second parameter, k_{xy} , is employed to reflect the alteration in electron exchange between the heteroatom in question and its neighbors with respect to an aromatic carbon-carbon bond:

$$\beta_{xy} = k_{xy} \beta_0$$

In previous work (19, 20) the authors have approached this problem with the desire to obtain calculated values of eigenfunctions and eigenvalues of heteroatom-containing molecules which would adequately approximate chemical and physical experience. These calculations have resulted in the close approximation of dipole moments from eigenfunctions (19) and relative chemical reactivities from eigenvalues (20). The ability to produce these results from the same set of heteroatom parameters lends a measure of confidence in their values.

In the present work, these same values have been used with only one modification. In the previous study on dipole moments (19) an experimental value of 1.54 D for pyrrole and a model for the nitrogen atom identical to that for the nitrogen of aniline were used. It is now felt that the value of 1.80 D for pyrrole is a better experimental value (25, 26). Also, the model for the nitrogen atom must be re-evaluated. The unshared electron pair on nitrogen of aniline is not in a pure p orbital; hence the Coulomb integral should reflect a greater electronegativity than that of the nitrogen in pyrrole (27). Furthermore, since the pure p orbital of nitrogen in pyrrole is more available for delocalization, a higher resonance integral should be used for the C-N bond. This improved model with the accompanying parameters has permitted us to calculate suc-

cessfully the dipole moments of pyrrole and *N*-methylpyrrole. (See Table I for parameters and Table II for dipole moments.)

Although the heteroatom model has previously been used for the treatment of a methyl group on an aromatic ring (28), the authors felt that a more realistic model of this group is provided by the conjugative model (29) in which the electron distribution of both the hydrogens and the methyl carbon atom is specifically designated and the methyl hydrogens are collected into a pseudo π orbital. Using the parameter values proposed by Coulson and Crawford (30) for this model, the authors have again successfully calculated the dipole moment of toluene.

For the calculation of the electronic structure of the sydnone molecule, two new parameters had to be introduced, and their values had to be consistent with other parameters employed. These parameters were the resonance integral for the $\dot{N}-\dot{O}$ bond ($k_{\dot{N}\dot{O}}$) and the resonance integral for the $\dot{N}-\dot{N}$ bond ($k_{\dot{N}\dot{N}}$). The value of 0.6 has been derived for $k_{\dot{N}\dot{O}}$ for use in simple Hückel calculations (18). The value of 0.5 was found to reproduce successfully the value of the dipole moments of furazan and isoxazole. For the parameter $k_{\dot{N}\dot{N}}$, the value of 0.87 has been derived from a proportionality to overlap integrals (31). The value of 0.9 has been employed successfully to calculate the dipole moments of *N*-methylpyrazole and *N*-phenylpyrazole.

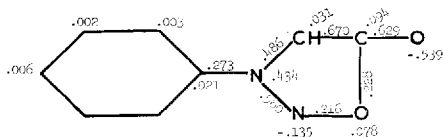


Fig. 1.—3-Phenylsydnone. Numbers shown are calculated π -bond orders and charge densities.

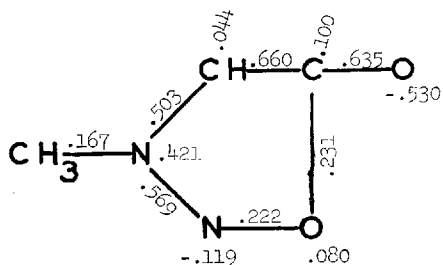


Fig. 2.—3-Methylsydnone. Numbers shown are calculated π -bond orders and charge densities.

CALCULATIONS

The computations were made using the computer program previously described (20). Due to the greater complexity of some of the sydnones, convergence of charge densities to self-consistency required a substantially larger number of iterations. The versatility of this program permitted this with no difficulty. The values from consecutive calculations of the charge densities were tested until an increment no larger than 0.001 electron existed between the final iteration and the average of all preceding calculations. The test was simultaneously applied to all atoms by the program. The calculated charge densities for the ring atoms are shown for 3-phenylsydnone in Fig. 1 and for 3-methylsydnone in Fig. 2. The π -bond order for each of these compounds was calculated from the eigenfunctions and is shown for the appropriate bonds in Figs. 1 and 2.

The dipole moments were calculated from charge densities as previously described (19) and are listed in Table II along with experimental values.

From the eigenvalues of the matrices the $M_m \rightarrow M_{m+1}$ transition energies were calculated. These are recorded in Table III along with the corresponding wavelengths for a series of variously substituted sydnones. The energy levels in units of β for 3-phenylsydnone and 3-methylsydnone are listed in Table IV. The values of ΔM , in units of β , are plotted against the corresponding frequencies of maximum absorption in Fig. 3.

DISCUSSION

As can be seen from Table II, the agreement between the calculated and experimental dipole moments for the sydnones is excellent. This, in the authors' opinion, attests to the reasonableness of the values for the charge densities calculated (32).

Several observations can be made from an inspection of the charge densities and bond orders of 3-phenyl and 3-methylsydnone (Figs. 1 and 2). The exocyclic oxygen atom in both compounds bears a high charge density, being over 0.15 electron higher than what was calculated for butyrolactone (33). This clearly implies significant participation from all ring atoms, in the total delocalization of the π -electrons. The number 3 nitrogen atom bears a high positive charge, particularly in the case of the 3-phenylsydnone. This observation, combined with the highest bond order calculated, between the carbon atoms, indicates the substantial participation of

TABLE III.—MAXIMUM U.V. ADSORPTION OF THE SYDNONE RING AND CALCULATED VALUES OF ΔM

Compd.	$\lambda_{\max.}, m\mu$	$\nu, \text{cm.}^{-1}$	$\Delta M(\beta)$	Ref.
1. 3-Methylsydnone	290	34483	0.481	(41)
2. 3-Phenyl-4-ethylsydnone	307	32573	0.451	(41)
3. 3-(<i>p</i> -Tolyl)-4-methylsydnone	307	32573	0.450	(41)
4. 3-Phenylsydnone	310	32258	0.465	(41)
5. 3-(<i>m</i> -Tolyl)sydnone	310	32258	0.465	(41)
6. 3-(β -Pyridyl)sydnone	312	32051	0.461	(41)
7. 3-(β -Naphthyl)sydnone	315	31746	0.457	(52)
8. 3-Phenyl-4-acetylsydnone	326 ^a	30675	0.417	...
9. 3,4-Di-(<i>p</i> -tolyl)sydnone	338	29586	0.405	(41)
10. 3,4-Di-phenylsydnone	340	29412	0.405	(52)

^a The $\lambda_{\max.}$ of this compound was determined by the authors on a Cary model 15 U.V. spectrophotometer in ethanol.

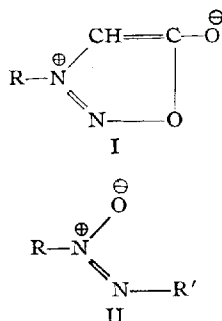
TABLE IV.—ENERGY LEVELS FOR 3-PHENYL AND 3-METHYLSYDNONE IN UNITS OF β

3-Phenylsydnone	3-Methylsydnone
-2.025	
-1.268	
-1.040	-3.164
-0.996	-1.228
-0.060	-0.058
0.405	0.423
0.951	1.381
1.004	2.101
1.380	2.951
1.931	3.294
2.802	
3.218	

valence bond representation, I, in the total wave equation of the molecule, a conclusion also reached by dipole moment studies of Hill and Sutton (34).

A comparison of the two calculated structures indicates that the phenyl group in the 3 position makes a greater mesomeric contribution of electrons to the sydnone ring than the 3-methyl group. The phenyl ring is deactivated at all positions by the sydnone ring; hence electrophilic attack on the benzene ring would be expected to be retarded. This is borne out by experiment (12, 35). This fact may in reality be the result of electron withdrawal by the sydnone ring rather than donation by the phenyl ring. The phenyl ring is known to be an electron withdrawing group in most systems (36).

In the work cited previously (35), the sydnone ring was found to be comparable to the β -azoxy group (II), with respect to its activating effect on nucleophilic displacement reactions on *p*-chlorophenyl derivatives. The proposed transition state in reactions of this type is stabilized by electron-withdrawing substituents (37). The comparison between these two substituents is also observed in electrophilic substitutions on benzene in which both groups are deactivating. This is not true for α -azoxy substituents (35).



This relationship between the sydnone ring and β -azoxy groups lends further support for the charge distributions shown in Fig. 1. A larger bond order between the two nitrogens would also be required for conjugation with the phenyl ring leaving the bond order between nitrogen and oxygen somewhat deficient. This situation might also be expected from electronegativity considerations (35).

Further support for the bond order calculated

between nitrogen and oxygen (Fig. 1) is found in a comparison of bond lengths. An X-ray study of *p*-bromophenyl sydnone has shown (38, 39) the length of the N—O bond to be 1.34 Å. The bond length of the N—O single bond has been reported to be 1.46 Å, while that of an N=O double bond is 1.14 Å. (40). Thus, in general terms, the N—O bond in the sydnone ring is largely of a single bond nature which corresponds favorably with the relatively low π -bond order calculated for these compounds.

To determine the reliability of the eigenvalues obtained from solution of the secular equations, an examination of the ultraviolet spectra of a number of substituted sydrones seemed appropriate. A single peak in the 290 to 350 μ region has been attributed to the sydnone ring (41). This maximum is quite intense ($\epsilon > 4700$) and experiences a bathochromic shift from 3-alkyl to 3-aryl substitution indicating an extended interannular conjugation (Table III) (42). Increase in delocalization also appears to be more effective with the aryl substituent in the 4-position of the sydnone ring than in the 3-position. This is expected from the increased ability of carbon π -orbitals to participate in electron delocalization compared to that of the nitrogen.

Other bands in the spectra of aryl-substituted sydrones appear at lower wavelength and have been attributed to benzenoid *K* absorption (41).

General considerations of ultraviolet spectra of aromatic compounds within the framework of molecular orbital calculations have led to the general conclusion that the more intense peaks at longer wavelengths are due to transitions occurring between the highest filled energy level (m) and the lowest unoccupied level ($m+1$) (43). In empirical LCAO-MO calculations, such as are presented here, the over-all electronic transition energy is considered to be larger than any energy differences due to configuration interaction or singlet-triplet splitting (43). The transition is then taken to be the perpendicular Franck-Condon transition. The frequency of such a transition is given by

$$h\nu = E^* - E$$

where E^* is the energy of the excited state, and E is the energy of the ground state.

In the total π -energy expressions from LCAO-MO calculations, the energy of the two states are given by

$$E^*_\pi = n\alpha + M^*\beta$$

$$E_\pi = n\alpha + M\beta$$

Thus, the frequency should be proportional to the difference in the coefficients of β :

$$h\nu = (M^* - M)\beta = \Delta M\beta$$

This operation is equivalent to taking the difference between M_m and M_{m+1} .

Table III shows the λ_{\max} and ΔM for a number of substituted sydrones, and Fig. 3 shows the relationship between the frequency in cm^{-1} and ΔM .

The correlation of these data is good. The correlation coefficient of 0.931 indicates that about 86.6% of the variance is accounted for. The maximum deviation of the experimental λ_{\max} from that calculated by the correlation equation from Fig. 3

$$\nu(\text{cm}^{-1}) = 60533(\Delta M) + 4794$$

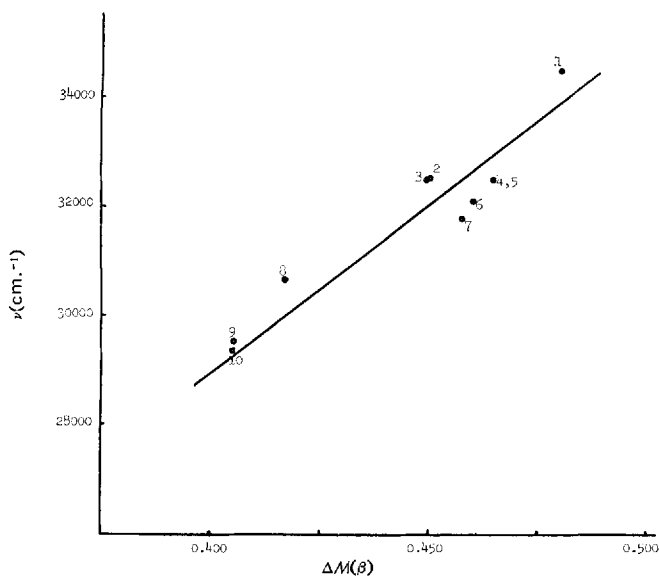


Fig. 3.—Correlation plot of the frequency of maximum absorption of the sydnone ring vs. the calculated ΔM in units of β .

is $\pm 7 \text{ m}\mu$, and the average deviation of points from the line is $\pm 0.010 \Delta M$ units. Thus, it appears that the relative energy levels calculated by the present method are in good agreement with the major electronic transition energy of the substituted sydnone rings.

The deviations from calculated values are not unexpected considering the empirical nature of the calculations. In the ω -HMO technique, no distinction is made between the geometry of the ground and excited state or between singlet and triplet state. A perpendicular Franck-Condon transition is assumed which may involve a promotion to a higher level than the lowest available level in the excited state.

Further error is involved with the nonexplicit consideration of electron repulsion. When the energy difference between the ground and excited state is small, the energy levels will interact. This repulsion will result in a larger separation between the levels. More sophisticated methods employing configuration interaction are required to take this phenomenon into account.

Through an application of the ω -HMO technique in conjunction with parameters previously derived (19) and parameters derived in this present work, the dipole moments of some representative heterocyclic systems including 3-methyl and 3-phenylsydnone have been successfully calculated (Table II). These same parameters have also given relative energy levels that correspond well with the maximum ultraviolet absorptions of the variously substituted sydnone rings (Table III and Fig. 3). Thus, the authors feel that the values of these parameters when employed in this type of an LCAO-MO calculation produce a reasonable electronic structure of the sydnones.

REFERENCES

- (1) Kier, L. B., *et al.*, *Nature*, **195**, 817(1962).
- (2) Kier, L. B., and Dhawan, D., *J. Pharm. Sci.*, **51**, 1058(1962).
- (3) Dhawan, D., and Kier, L. B., *ibid.*, **53**, 83(1964).
- (4) Fregly, M. J., Kier, L. B., and Dhawan, D., *Toxicol. Appl. Pharmacol.*, **6**, 529(1964).
- (5) Kier, L. B., Dhawan, D., and Fregly, M. J., *J. Pharm. Sci.*, **53**, 677(1964).
- (6) Lawson, K. D., Brey, W. S., and Kier, L. B., *J. Am. Chem. Soc.*, **86**, 463(1964).
- (7) Baker, W., and Ollis, W. D., *Quart. Rev. (London)*, **11**, 15(1957).
- (8) Stewart, F. H. C., *Chem. Rev.*, **64**, 129(1964).
- (9) Noel, Y., *Bull. Soc. Chim. France*, **1964**, 173.
- (10) Ohta, M., and Kato, H., *J. Chem. Soc. Japan*, **86**, 661(1965).
- (11) Baker, W., and Ollis, W. D., *Chem. Ind. (London)*, **1955**, 910.
- (12) Baker, W., Ollis, W. D., and Poole, V. D., *J. Chem. Soc.*, **1949**, 307.
- (13) Daudel, R., LeFebvre, R., and Moser, C., "Quantum Chemistry," Interscience Publishers, Inc., New York, N. Y., 1959, Chap. IV.
- (14) Streitwieser, A., "Molecular Orbital Theory for Organic Chemists," John Wiley & Sons, Inc., New York, N. Y., 1961, Chap. 2.
- (15) Pullman, B., and Pullman, A., "Quantum Biochemistry," Interscience Publishers, Inc., New York, N. Y., 1963, Chap. III.
- (16) Hill, R. A. W., Sutton, L. E., and Longuet-Higgins, H. C., *J. Chim. Phys.*, **46**, 244(1949).
- (17) Longuet-Higgins, H. C., *ibid.*, **46**, 246(1949).
- (18) Orgel, L. E., Cottrell, T. L., Dick, W., and Sutton, L. E., *Trans. Faraday Soc.*, **47**, 113(1951).
- (19) Kier, L. B., *Tetrahedron Letters*, **1965**, 3273.
- (20) Kier, L. B., *J. Pharm. Sci.*, **55**, 98(1966).
- (21) Wheland, G. W., and Mann, D. E., *J. Chem. Phys.*, **17**, 264(1949).
- (22) Streitwieser, A., *J. Am. Chem. Soc.*, **82**, 4123(1960).
- (23) Ettinger, R., *Tetrahedron*, **20**, 1579(1964).
- (24) Berson, J. A., Evleth, E. V., and Manatt, S. L., *J. Am. Chem. Soc.*, **87**, 2901(1965).
- (25) Kofod, H., Sutton, L. E., and Jackson, J., *J. Chem. Soc.*, **1952**, 1467.
- (26) Buckingham, A. D., Harris, B., and LeFebvre, R. J. W., *ibid.*, **1953**, 1626.
- (27) Elliott, J. J., and Mason, S. F., *ibid.*, **1959**, 2352.
- (28) Matsen, F. A., *J. Am. Chem. Soc.*, **74**, 5243(1950).
- (29) Streitwieser, A., and Nair, P. M., *Tetrahedron*, **5**, 149(1959).
- (30) Coulson, C. A., and Crawford, V. A., *J. Chem. Soc.*, **1953**, 2052.
- (31) Wheland, G. W., *J. Am. Chem. Soc.*, **64**, 900(1942).
- (32) Streitwieser, A., "Molecular Orbital Theory for Organic Chemists," John Wiley & Sons, Inc., New York, N. Y., 1961, p. 140.
- (33) Kier, L. B., and Roche, E. B., unpublished data.
- (34) Hill, R. A. W., and Sutton, L. E., *J. Chem. Soc.*, **1949**, 746.
- (35) Tin-Lok, C., Miller, J., and Stansfield, F., *ibid.*, **1964**, 1213.
- (36) Jaffe, H., *Chem. Rev.*, **53**, 191(1953).
- (37) Miller, J., and Parker, A. J., *Australian J. Chem.*, **11**, 302(1958).
- (38) Bärnighausen, H., Jellinek, F., and Vos, A., *Proc. Chem. Soc.*, **1961**, 120.
- (39) Bärnighausen, H., *et al.*, *Acta Cryst.*, **16**, 471(1963).
- (40) Gould, E. S., "Mechanism and Structure in Organic Chemistry," Holt, Rinehart and Winston, New York, N. Y., 1959, p. 45.
- (41) Hammick, D. L., and Voaden, D. J., *J. Chem. Soc.*, **1961**, 3303.

- (42) Zuman, P., and Voaden, D. J., *Tetrahedron*, **16**, 130 (1961).
- (43) Daudel, R., LeFebvre, R., and Moser, C., "Quantum Chemistry," Interscience Publishers, Inc., New York, N. Y., 1959, Chap. VIII; Streitwieser, A., "Molecular Orbital Theory for Organic Chemists," John Wiley & Sons, Inc., New York, N. Y., 1961, Chap. 8.
- (44) Kofod, H., Sutton, L. E., and Jackson, J., *J. Chem. Soc.*, **1952**, 1467.
- (45) Schaafs, W., *Z. Physik. Chem.*, **196**, 413 (1951).
- (46) Saegebarth, E., and Cox, A. P., *J. Chem. Phys.*, **43**, 186 (1965).
- (47) Speroni, G., and Pino, P., *Gazz. Chim. Ital.*, **80**, 549 (1950).
- (48) Speroni, G., and Mori, L., *ibid.*, **82**, 269 (1952).
- (49) Hiller, S. A., Mazeika, I. B., and Grandberg, I. I., *Khm. Geterolsikhich Soed.*, **1965**, 107.
- (50) Hill, R. A. W., and Sutton, L. E., *J. Chem. Soc.*, **1949**, 746.
- (51) Smyth, C. P., in "Technique of Organic Chemistry," vol. 1, A. Weissberger, ed., Interscience Publishers, Inc., New York, N. Y., 1960, p. 2599.
- (52) Earl, J. C., LeFebvre, R. J. W., and Wilson, I. R., *J. Chem. Soc.*, **1949**, S103.

Gas Chromatographic Analysis of Trichloroethanol, Chloral Hydrate, Trichloroacetic Acid, and Trichloroethanol Glucuronide

By EDWARD R. GARRETT and HOWARD J. LAMBERT*

A sensitive and specific assay has been developed for trichloroethanol, chloral hydrate, trichloroacetic acid, and trichloroethanol glucuronide utilizing gas chromatography and electron capture detection. Trichloroethanol and chloral hydrate are extracted from alkalized water or urine with ether, and a portion of the ether extract is assayed with chlorobutanol and chloroform as internal standards. Trichloroacetic acid and trichloroethanol glucuronide are not extracted under these conditions. The trichloroacetic acid is decarboxylated as the potassium salt and the resultant chloroform extracted into ether and assayed. The trichloroethanol glucuronide is enzymatically hydrolyzed and the hydrolysate assayed for the trichloroethanol formed. Sensitivities obtained were 0.5 mcg. of trichloroethanol and chloral hydrate (3.3 and 3.02 μmoles , 10^{-9} moles, respectively), 1.0 mcg. of trichloroacetic acid (6.1 μmoles) in 2.0 ml. of sample and 0.5 mcg. of trichloroethanol glucuronide (1.54 μmoles) in 3.0 ml. of sample. Statistical analysis of assays on urinary mixtures of the four compounds demonstrates the reliability of the method.

THERE ARE NO sensitive specific assays for the hypnotic drug trichloroethanol (TCE), its precursor chloral hydrate (CH), or its detoxification products trichloroacetic acid (TCA) and trichloroethanol glucuronide (TCE-G), in the available literature.

Extant methods (1-3) are mostly modifications of the colorimetric method of Fujiwara (4) and were not sufficiently sensitive or specific for a planned pharmacokinetic study of TCE in this laboratory. Friedman and Cooper (2) obtained a sensitivity of 20 μmoles of TCA and CH and 100 μmoles of TCE in mixtures but found that some tissue preparations interfered with chromophore production as much as 25% and lessened the reliability of the Fujiwara procedure. Leibman and Hindman (3) improved the assay sensitivity down to 5 μmoles of TCA and CH and 15 μmoles of TCE, but separation of the

components prior to reaction would be required as the procedure was nonspecific. A gas chromatographic separation of CH, TCA, and chlorobutanol, but not TCE, with a sensitivity of 306, 302, and 281 μmoles , respectively, has also been reported (5). This sensitivity of detection is not as good as the modified Fujiwara (2, 3).

The purpose of this investigation was to develop a specific procedure for the quantitative determination of TCE and its metabolites separately and in mixtures with gas-liquid chromatography and the electron capture detector. If possible, this method would have greater sensitivity than those previously reported.

EXPERIMENTAL

Materials.—The TCE (Calbiochem, Inc.) used in the preparation of calibration curves and synthetic mixtures in water and urine was redistilled at 152° before use.

Chloral hydrate U.S.P., chlorobutanol U.S.P., and TCA (reagent grade and 5% solution) were obtained from the Fisher Scientific Co. Ether (Baker) was redistilled over sodium before use. The purity of these components was verified chromatographically, and only one peak was observed.

The sodium salt of trichloroethanol glucuronide

Received March 11, 1966, from the College of Pharmacy, University of Florida, Gainesville, 32603.

Accepted for publication May 13, 1966.

Presented to the Scientific Section, A.P.H.A., Detroit meeting, March 1965.

This investigation was supported in part by an unrestricted research grant from Smith Kline & French Laboratories, Philadelphia, Pa.

* Recipient of the Lunsford Richardson Pharmacy Award, Region III, 1965.