units glucose-6-phosphate dehydrogenase. Control mixtures were prepared in the same way but with liver preparation which had been boiled for 1 min. A blank mixture was also prepared in like manner but without substrate. The liver mixtures were incubated at 37° for 1 hr.; each mixture was quenched by boiling. The incubated mixtures were extracted with 3 × 1 ml. of ethyl acetate; after drying over anhydrous Na_2SO_4, the ethyl acetate extracts were reduced to dryness and then treated as indicated under GLC. For TLC, *n*-heptane extracts of the ethyl acetate residues were used.

TLC—Plates of 250-μ silica gel GF_{254} were used throughout with the following solvent system: benzene–ethyl acetate, 1:1 v/v; development was 10 cm. Detection was *via* fluorescence quenching in 254-nm. radiation. Attempts to locate phenolic metabolites were with diazotized sulfanilic acid of Axelrod and Pulliam (12).

GLC—A Hewlett-Packard 5750B gas chromatograph, equipped with 3% OV-17 on Gas Chrom Q (100/120 mesh) column (6 mm. i.d. × 180 cm.), was used with 60 ml./min. carrier gas flow and the column temperatures listed in Table II. Phenylpropanols were chromatographed as their acetates following reaction of 10–20 mg. of them in 0.05 ml. of pyridine–acetic anhydride (1:1) for 1 hr. at room temperature.

Partition Studies—UV spectra were recorded with a Beckman DK-2 spectrophotometer. Phenylpropanone derivative, 1–5 mg., dissolved in 25 ml. of *n*-heptane was shaken vigorously for 1.5 hr. with 25 ml. of citrate phosphate buffer (pH 7.0) at 25.0 ± 0.1°. The heptane layer was dried over anhydrous Na_2SO_4, diluted to 50 ml. in a volumetric flask with *n*-heptane, and compared to standard solutions at the maxima indicated in Table I with a Gilford model 240 spectrophotometer.

Pharmacological Evaluation—All compounds were administered as suspensions in less than 1% polysorbate 80. Graded doses were administered intraperitoneally to Swiss-Webster male mice weighing 25–30 g. Groups of 10 mice at each dose level were observed, and the gross effects were recorded. Loss of righting ability was considered positive in animals that could not right themselves for a period of at least 30 sec. The loss of righting ability dose (LRA_{50}) and the lethal dose (LD_{50}) were calculated by the method of Litchfield and Wilcoxon (13).

REFERENCES

(1) J. Harley-Mason, A. H. Laird, and J. R. Smythies, *Confin. Neurol.*, **18**, 152(1958).

(2) M. Goldstein, A. J. Friedhoff, S. Pomerantz, C. Simons, and J. F. Contreara, *J. Neurochem.*, **6**, 253(1961).

(3) A. T. Shulgin, T. Sargent, and C. Naranjo, *Nature*, **221**, 537 (1969).

(4) L. G. Dring, R. L. Smith, and R. T. Williams, *Biochem. J.*, **116**, 425(1970).

(5) C. F. Barfknecht, J. M. Miles, and J. L. Leseney, *J. Pharm. Sci.*, **59**, 1842(1970).

(6) H. W. Culp and R. E. McMahon, *J. Biol. Chem.*, **243**, 848 (1968).

(7) C. Hansch, R. M. Muer, T. Fujita, P. P. Maloney, F. Geiger, and I. M. Streich, *J. Amer. Chem. Soc.*, **85**, 2817(1963).

(8) R. P. Quintana, *J. Pharm. Sci.*, **54**, 462(1965).

(9) R. B. Moffett, A. R. Hanze, and P. H. Seay, *J. Med. Chem.*, **7**, 178(1964), and previous references cited therein.

(10) A. Feldstein, F. H. Chang, and J. M. Kucharski, *Life Sci.*, **9**, 323(1970).

(11) A. Y. H. Lu and M. J. Coon, *J. Biol. Chem.*, **243**, 1331 (1968).

(12) L. R. Axelrod and T. E. Pulliam, *Arch. Biochem. Biophys.*, **89**, 106(1960).

(13) J. T. Litchfield, Jr., and F. W. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99(1949).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 2, 1970, from the *Division of Medicinal Chemistry, College of Pharmacy and Department of Pharmacology, College of Medicine, University of Iowa, Iowa City, IA 52240*

Accepted for publication January 8, 1971.

This work was supported in part by U. S. Public Health Service Grant MS-01396. The technical assistance of V. D. Reif is gratefully acknowledged.

NMR Studies on Enolization of 1-*p*-Chlorophenyl-1-hydroxy-2-propanone

R. J. WARREN, L. J. RAVIN, P. P. BEGOSH, E. G. SHAMI, and J. E. ZAREMBO

Abstract □ NMR spectroscopy was used to study the enolization of 1-*p*-chlorophenyl-1-hydroxy-2-propanone. At 55°, chloroform solutions of this material gave an equilibrium mixture consisting of the starting material, the enol form, and a third material. This third component was isolated and identified by spectral data as 3,6-bis-(*p* - chlorophenyl) - 1,4 - dimethyl - 2,5,7 - trioxabicyclo - [2,2,1]-heptane. The equilibrium mixture was found to contain 70% of the starting material and 30% of the two reaction products.

Keyphrases □ 1-*p*-Chlorophenyl-1-hydroxy-2-propanone—enolization followed by NMR, characterization of equilibrium mixture □ 3,6-Bis(*p*-chlorophenyl)-1,4-dimethyl-2,5,7-trioxabicyclo-[2,2,1]-heptane—isolation, identification from enolization of 1-*p*-chlorophenyl-1-hydroxy-2-propanone □ NMR spectroscopy—enolization of 1-*p*-chlorophenyl-1-hydroxy-2-propanone

In studies on 1-*p*-chlorophenyl-1-hydroxy-2-propanone, it became necessary to determine if enolization occurred and, if so, to what extent, under what set of conditions, and with what products, if any, being formed. NMR was the analytical method of choice because it was capable of following both the disappearance of the starting material and the appearance of the reaction products as they formed. NMR has been shown to be a valuable analytical tool in the study of reactions and reaction mechanisms (1, 2).

EXPERIMENTAL

All NMR spectra were recorded on a Jeolco C60H spectrometer equipped with a variable-temperature probe. Deuterated chloroform was used as the solvent. Chemical shifts were measured relative to tetramethylsilane.

IR spectra were recorded between 4000–625 $cm.^{-1}$ on a Perkin-Elmer model 21 spectrometer with a sodium chloride prism.

NMR Spectra—A sample of Compound I was dissolved in deuterated chloroform and transferred to an NMR sample tube. The tube was flushed with nitrogen and sealed, and an NMR spectrum was immediately run on the sample. After this spectrum was obtained, the sealed sample tube was placed in a constant-temperature bath set at 55°. The sample was withdrawn at selected intervals, and the NMR spectrum was recorded and integrated.

Isolation of Compound III—The solution containing Compounds I, II, and III was streaked onto 0.5-mm. thick silica gel GF plates. The plates, 20 × 20 cm. (8 × 8 in.), were developed in benzene

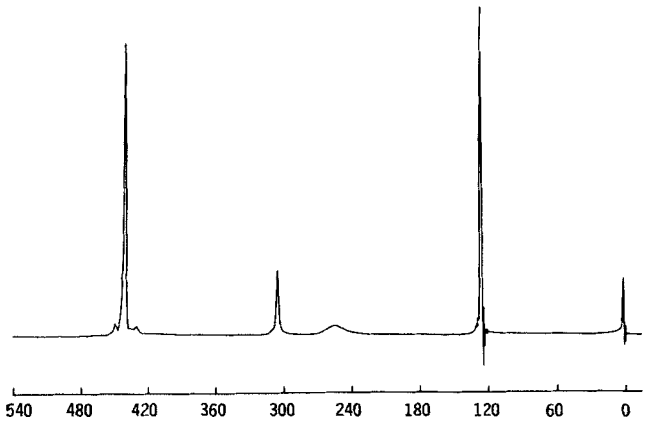


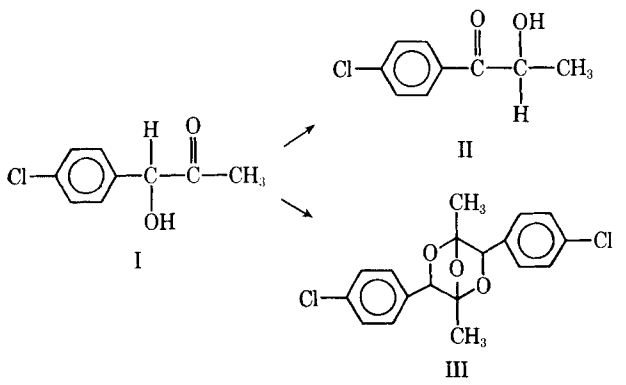
Figure 1—*NMR spectrum of starting material.*

with no equilibration. After the solvent front advanced 10 cm., the plates were dried in a hood and observed under 254-nm. UV. The zones where the various components were located were outlined with a scribe. The silica in the individual zones of interest was scraped from the plate, loaded in a 10-ml. hypodermic syringe fitted with a disk and Whatman No. 3 filter paper, and eluted with ether. The ether solution was evaporated to dryness, and the residue was taken up in 0.5 ml. of deuterated chloroform. An NMR spectrum was obtained on this solution of Compound III.

The IR spectrum was obtained by evaporating the solution used for NMR on a sodium chloride plate.

RESULTS AND DISCUSSION

The enolization of 1-*p*-chlorophenyl-1-hydroxy-2-propanone in chloroform solution at room temperature has been found to proceed very slowly. The process is accelerated with increasing temperature; at 55°, the reaction reaches an equilibrium state after approximately 168 hr. The equilibrium mixture was found to be as shown in Scheme I.



Scheme I

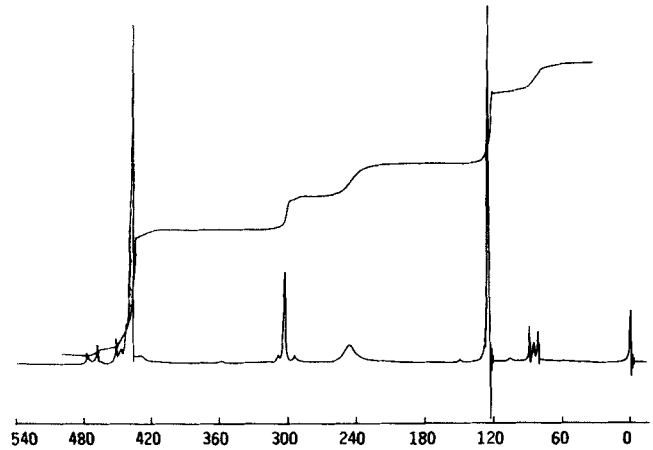


Figure 2—*NMR spectrum after 114 hr. at 55°.*

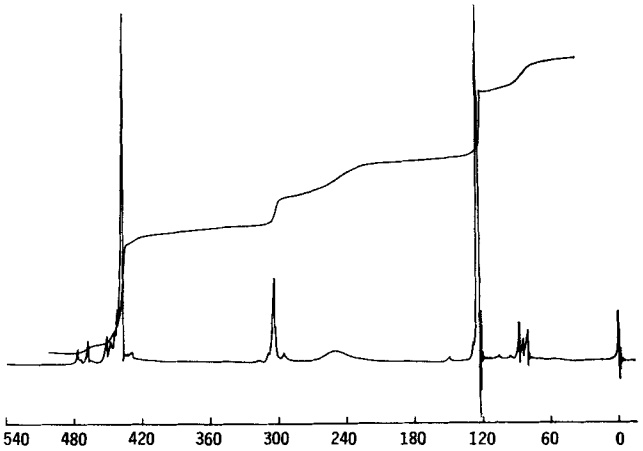


Figure 3—*NMR spectrum of equilibrium mixture.*

NMR Spectra—The NMR spectrum of Compound I is shown in Fig. 1. The NMR absorption peaks may be assigned as follows: methyl group appears as a singlet at 124 Hz., hydroxyl as a broad singlet at 255 Hz., methine as a singlet at 304.5 Hz., and aromatic protons at 439.5 Hz. Figure 2 illustrates a spectrum taken after 114 hr. at 55°. In addition to the peaks present in the original starting material (Fig. 1), additional peaks are evident in the spectrum in Fig. 2. NMR signals at 81, 84.5, and 88.5 Hz. represent the CH_3 groups in products II and III. The weak peaks in the area of 304.5 Hz. represent a quartet due to the CH group in II. The quartet is partially overlapped or masked by the CH in the starting material. A doublet at 450 Hz. and another at 472.5 Hz. arise from the aromatic protons in II. The additional chemical shift downfield is attributable to the increased proximity of the carbonyl group in the enol form. Figure 3 is the spectrum of the mixture at equilibrium. This spectrum, taken after 238 hr. at 55°, shows the increased buildup of the area represented by II and III. After 168 hr., there is no significant increase in intensity of the NMR absorption in these areas.

Identification of Compound III—The spectrum of Compound III (Fig. 4) reveals a singlet absorption peak at 89 Hz. assignable to the isolated methyl groups in the structure proposed for III. Another singlet at 294 Hz. is due to the CH groups, and the remaining signal at 438 Hz. is from the aromatic protons.

The IR spectrum of Compound III (Fig. 5) shows the absence of OH— and C=O functions and the presence of strong ether linkages (8.70, 9.25, and 10.0 μ.) and a *para*-substituted aromatic system (11.9–12.5 μ.).

The mass spectrum gave a molecular weight of 350 and supported the proposed structure.

All of the spectral data are compatible with the structure proposed for Compound III.

Calculation—The amount of remaining starting material and of products II and III at equilibrium may be calculated from the integrated peak intensity of their NMR signals. As mentioned, the signals from the various CH_3 groups in I, II, and III all fall in the area of 70–204 Hz. If one takes the total integrated area for this range to represent 100% of the CH_3 group present, then the amount

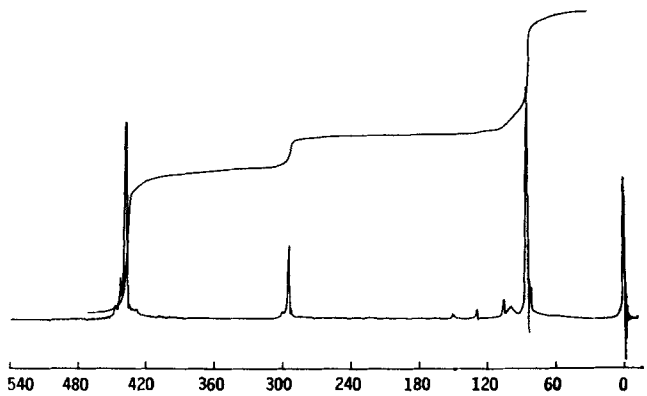


Figure 4—*NMR spectrum of Compound III.*

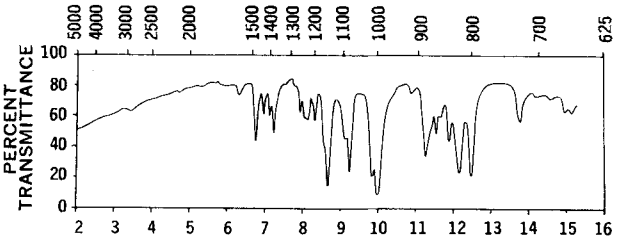
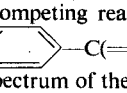


Figure 5—*IR spectrum of Compound III.*

of starting material present at any time may be calculated as:

$$\% \text{ Compound I} = \frac{\text{integral I}}{\text{integral I} + \text{II} + \text{III}} \times 100 \quad \text{(Eq. 1)}$$

Calculations on this basis were made for the spectra run at various time intervals. The data are shown in Table I.

Interferences—If oxygen is not excluded from the system in this experiment, a competing reaction involving the formation of the diketone Cl—  —C(=O)—C(=O)—CH_3 will result. Figure 6 is the NMR spectrum of the diketone. It is obvious from the chemical shift of the methyl group in the diketone spectrum that this material is easily detected in the reaction mixture if it is present.

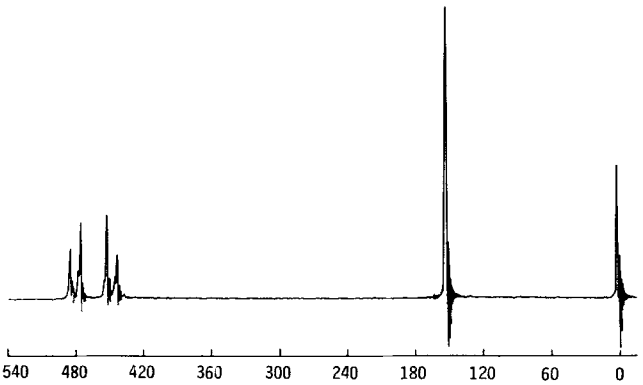


Figure 6—*NMR spectrum of diketone.*

Table I—Percent I, II, and III at Various Time Intervals

Hours	Percent I	Percent II and III
0	100	—
66	83.3	16.7
72	83.3	16.7
91	84.4	15.6
96	82.6	17.4
114	77.3	22.7
120	75.7	24.3
138	75.5	24.5
140	76.0	24.0
144.5	73.1	26.9
166	70.7	29.3
168	70.4	29.6
238	69.9	30.1

CONCLUSION

NMR has been used successfully to monitor the enolization of 1-*p*-chlorophenyl-1-hydroxy-2-propanone. In this study, an unexpected product (III) was formed. This material was isolated and identified by IR, NMR, and mass spectral data.

REFERENCES

(1) R. Warren and J. Zarembo, *J. Pharm. Sci.*, **59**, 840(1970).
(2) *Ibid.*, **60**, 307(1971).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 18, 1970, from the *Research and Development Division, Smith Kline & French Laboratories, Philadelphia, PA 19101*

Accepted for publication January 8, 1971.

The authors are grateful to Dr. L. M. Jackman for helpful discussions of the spectra presented, and to Mr. John Messina for technical assistance in obtaining the spectra presented here.

New Compounds: 2,4-Dinitrobenzenesulfenamides

M. J. KORNET, T. C. HO, and L. ISENBERG

Abstract □ A compilation of 2,4-dinitrobenzenesulfenamide derivatives of 72 amines is given. Fifty-one of the derivatives are described for the first time.

Keyphrases □ 2,4-Dinitrobenzenesulfenamide derivatives—compilation and description of 72 amines □ Amines, 2,4-dinitrobenzenesulfenamide derivatives—description, compilation

In the course of other studies with 2,4-dinitrobenzenesulfenyl chloride (I), the authors found that this reagent is excellent for the derivatization of amines. This observation was made earlier by Billman *et al.* (1) who characterized 14 amines by converting them into the corresponding 2,4-dinitrobenzenesulfenamides (Scheme I):

$$2,4\text{-}(O_2N)_2C_6H_3SCl + 2R_2NH \rightarrow 2,4\text{-}(O_2N)_2C_6H_3SNR_2 + R_2NH \cdot HCl$$

Scheme I

Subsequently, Kharasch (2) showed that I can also be used for the preparation of derivatives of many other functional groups. Tables containing the physical properties of such derivatives have since been published (3).

More recently, I was advocated for the characterization of pharmaceutically important organic compounds such as barbituric acid, phenylbutazone, and saccharin (4). In addition, sulfenamides are valuable precursors for a number of pharmaceutically useful sulfonamides (5). In the biochemical area, Fontana *et al.* (6) and Scoffone *et al.* (7) showed that I may be used to determine both the cysteine and tryptophan content in polypep-