and suprathreshold concentrations. Under treatment of the vagina with the suppository, the respective odorograms became populated by nonobjectionably odorous components and some fragrant ones which may have been derived from the suppository preparation (although its composition and odor qualities provide no support for this statement). In some gas-chromatographic positions which were populated by malodors before treatment, no odorants were found after treatment; the effect of treatment therefore is not a simple masking but rather the result of simultaneous reduction in malodorous components and increased dominance of milder odors and fragrances.

REFERENCES

(1) S. S. Stevens, in "Psychophysics of Sensory Function in Sensory Communication," Rosenblith, Ed., M.I.T. Press, Cambridge, Mass., 1961, p. 1.

(2) S. S. Stevens, *Psychol. Rev.*, **64,** 153(1957).

(3) ASTM Manual on Sensory Testing Methods, ASTM, No. 434, 1968, p. 24.

(4) B. H. Ellis, *Food Sci. Technol.*, **3,** 307(1967).

(5) ASTM, Tentative Test for Odor in Industrial Water and Waste Water, ASTM Spec. Publ. 148-F, ASTM D-1292-617 (1962).

(6) A. Dravnieks and B. K. Krotoszynski, *J. Gas Chromatogr.*, **6,** 144(1968).

(7) A. Dravnieks and B. K. Krotoszynski, to be published.

(8) E. sz Kovats, *Advan. Gas Chromatogr.*, **1,** 229(1965).

(9) A. Dravnieks, B. K. Krotoszynski, W. E. Lieb, and E. Jungermann, *J. Soc. Cosmet. Chem.*, **19,** 611(1968).

(10) A. Dravnieks, B. K. Krotoszynski, R. E. Caseley, and D. Taber, *Proc. Sci. Sect. Toilet Goods Ass.*, **1,** 14(1969).

ACKNOWLEDGMENTS AND ADDRESSES

Received August 27, 1969, from *IIT Research Institute, Chicago, IL 60616*

Accepted for publication December 8, 1969.

* Present address: Department of Urology, Cook County Hospital, and the Chicago Medical School, Chicago, Ill.

Spectral Studies of Trimethylsilyl Ether of Chloramphenicol

MICHEL MARGOSIS

Abstract □ The trimethylsilyl ether of chloramphenicol used in GLC analysis has been characterized by UV, near IR, IR, NMR, and mass spectroscopy, confirming the structure of the *O,O*-ditrimethyl siloxy derivative. Pyridine and acetonitrile, used as solvents in the silylation reaction, promote formation of diastereomers. Addition of hydroxylic solvents promotes the inversion of configuration and causes partial solvolysis. To verify this, two fractions were collected from a GLC column and analyzed by IR and mass spectroscopy. The experimental evidence presented establishes the two fractions as diastereomers.

Keyphrases □ *O,O*-Ditrimethyl siloxy chloramphenicol diastereomers—structure confirmation, effect of pyridine, acetonitrile □ Trimethylsilyl ether—chloramphenicol derivative, effect of pyridine, acetonitrile □ IR—structure, identification □ UV—structure, identification □ NMR—structure, identification □ Mass spectrometry—structure, identification □ GLC—analysis, separation

Chloramphenicol, D(−)-*threo*-*p*-nitrophenyl-2-dichloroacetamido-1,3-propanediol, is a certifiable antibiotic for which neither of the current official assay methods (microbiological and UV procedures) (1) is specific. Gas-liquid chromatographic (GLC) procedures were investigated for chloramphenicol assay; however, when chloramphenicol was chromatographed on a QF-1 column above 200°, poorly resolved peaks were obtained, which were probably due to thermal breakdown products (Fig. 1). When it was chromatographed on a DC-200 or SE-30 column, no peaks of any kind were obtained.

Several workers (2–4) have successfully applied the silylation method of Bentley *et al.* (5) to this determination. However, several problems arose when the method was tried in this laboratory. The principal difficulties included contamination of the anode of the flame-ionization detector and solvent tailing, similar to that previously observed in the GLC of lincomycin (6). To overcome these difficulties, the technique was modified as follows: a previously developed extraction procedure (6) was used; methanol or other hydroxylic solvent was added to the sample immediately before injection into the gas chromatograph; different solvent systems were used; and the reaction mixture was evaporated to dryness and reconstituted in an inert solvent.

The TMS derivative prepared by the procedure of Bentley *et al.* (5) exhibited a single symmetrical chromatographic peak at 5.7 min. (Fig. 2) on each of the three columns (7), and recovery was quantitative. Freshly prepared solutions of the derivative gave no other significant peaks when injected directly. After standing (at least overnight), these solutions began to exhibit secondary peaks at 9.2 min., which reached limiting values of 2–3.5% of the area in the case of ethyl acetate or methylene chloride solutions but increased to about 50% in pyridine or acetonitrile. The addition of small amounts of methanol or other hydroxylic solvents tended to accelerate this reaction. With increasing amounts of methanol, additional peaks at 4.3 min. were noted on the QF-1 column (Fig. 3) but not on the DC-200 or SE-30 columns. When the methanol solution of the derivative was partitioned between water and carbon tetrachloride, the chromatogram of the nonpolar fraction showed two peaks (at 5.7 and 9.2 min.; Fig. 4) attributed to the TMS ether of chloramphenicol and its *erythro* isomer, while the chromatogram of the polar fraction was similar to that of the

Figure 1—*Chloramphenicol on QF-1 column at 238°.*

parent compound, with its major fragment peak at 4.3 min., reverted by solvolysis.

By chromatographing chloramphenicol in the three different columns, it has also been determined that the same TMS derivative is obtained using either the procedure of Bentley *et al.* (5) (see *Experimental*) or *N,O*-bis(trimethylsilyl) acetamide.

In the first phase of this study, the TMS derivative, as originally prepared for GLC analysis, was characterized and identified by UV, IR, NMR, and mass spectroscopy. In the second phase, the two nonpolar chromatographic effluents were trapped with a simple collection system and identified by IR and mass spectroscopy.

EXPERIMENTAL

Instrumental Analysis—*Gas Chromatography*—A Barber-Colman model 5000 was used with a flame-ionization detector and a 1.83-m. (6-ft.) × 3-mm. U-shaped glass column packed with 5% QF-1 on 80–100-mesh Gas Chrom Q. The carrier gas was nitrogen at 23 p.s.i., 58 ml./min., and the column temperature was 238°. For fraction collection, a 23:1 splitter was connected to the exit of the column, with the column temperature at 250° and the collector tube temperature at 240°.

Gas Chromatography–Mass Spectroscopy—An LKB model 9000 combination gas chromatograph–mass spectrometer equipped with a 1.83-m. (6-ft.) × 0.635-cm. (0.25-in.) stainless steel column packed with 3% OV-17 on Gas Chrom Q was used. The temperatures were: flash heater, 270°; column, programmed from 200°; and the separator and source, 300°. The carrier gas was helium and the ionization potential was set at 70 ev.

Mass Spectrometry—The mass spectrometer was an Atlas CH4 equipped with a probe inlet system permitting the sample temperature to be maintained independently from the ion source. The conditions were as follows: ion source regulated at 250°; the ionization potential, 70 ev.; magnetic scanning; accelerating voltage, 3 kv.; and sample temperature, 60–80°.

Nuclear Magnetic Resonance—Varian A-60 spectrometers were used.

Spectrophotometry—UV and near IR spectra were obtained with a Cary 14 spectrophotometer with 1-cm. and 10-cm. cells, respectively. The IR spectra were obtained with Beckman IR-12 and Perkin-Elmer 457 spectrophotometers.

Sample Preparation—*Sample 1 (S1)*—Deuterated chloramphenicol was obtained by treating chloramphenicol with D_2O and freeze-drying.

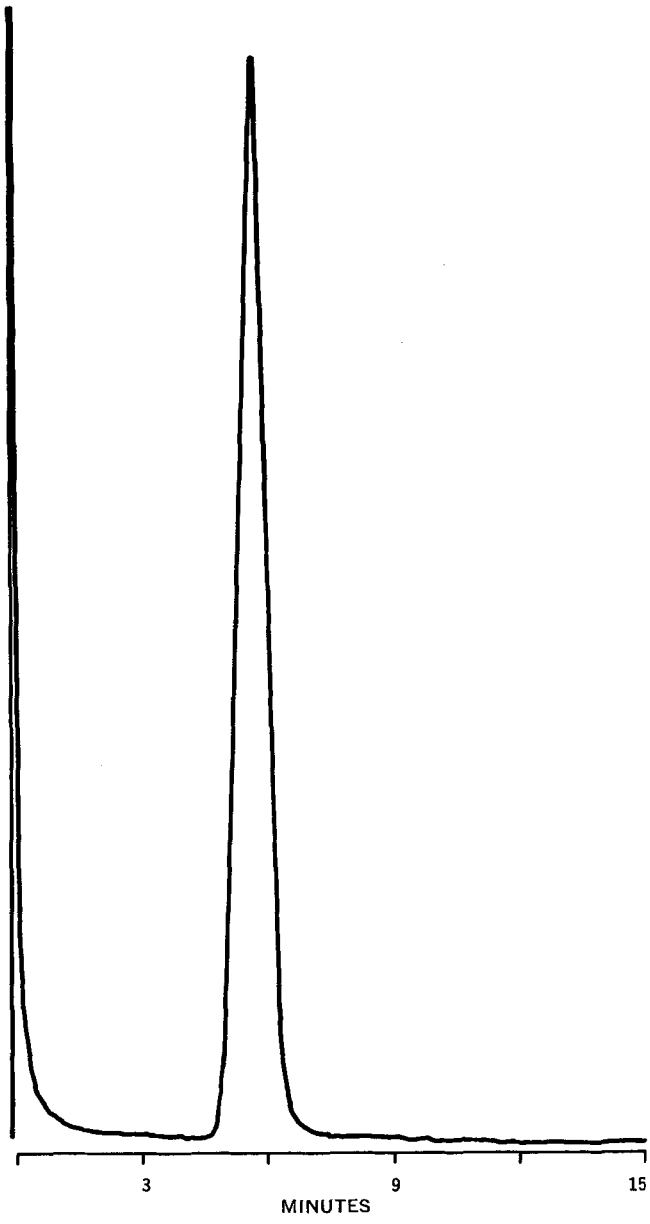


Figure 2—*TMS ether of chloramphenicol (S2) on QF-1 column at 238°.*

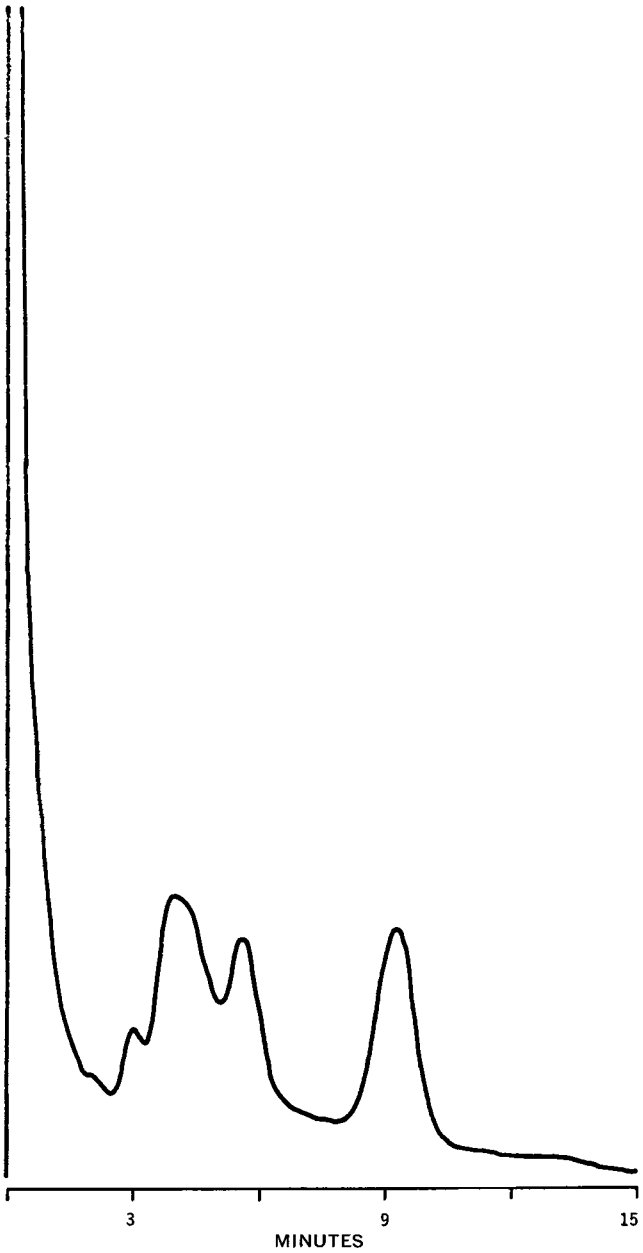


Figure 3—*TMS ether of chloramphenicol with methanol added (S3 before extraction) on QF-1 column at 238°.*

Sample 2 (S2)—The derivative "standard" used for this qualitative analysis was prepared by the Bentley *et al.* (2) procedure which consists of dissolving an appropriate amount of chloramphenicol in pyridine, adding an excess mixture of hexamethyldisilazane and trimethylchlorosilane (9:1), and allowing the mixture to stand 30–60 min. About 75% of the mixture was evaporated with heat and air to dryness; the residue was dissolved in carbon tetrachloride, filtered, and reevaporated to dryness. Solutions were made from this stock sample in appropriate solvents for GLC and spectral analyses.

Sample 3 (S3)—The sample for effluent analysis was prepared as described for S2, but after standing for 30 min., methanol was added to about 5% of original volume, and the solution was allowed to stand overnight. An equal amount of CCl_4 was added and the mixture was shaken. Water was then added and the CCl_4 was extracted. The extract was washed twice with water and passed through a CCl_4-saturated wad of glass wool. About 50 μl. of this CCl_4 solution was injected into the chromatograph. Fraction collection was started as soon as the first peak began to appear by connecting an open-end glass capillary tube to the split end of the column exit with a sleeve of Teflon "spaghetti" tubing and leaving it connected until near the end of the peak. Another glass capillary was similarly connected to collect the second fraction. The separations and collections were repeated until enough material was collected for the various analyses.

The effluent samples were prepared for mass spectrometry by rinsing each capillary tube with 10 μl. of CCl_4 into a gold microcup which was placed in the instrument inlet probe after air evaporation. Samples for IR analyses were prepared by rinsing each capillary tube with 10 μl. of CCl_4 onto a small amount of KBr. The KBr was then ground in a boron carbide mortar until dry, pressed into a 1-mm. disk, and scanned on an IR spectrophotometer equipped with a beam condenser.

RESULTS AND DISCUSSION

Characterization of the TMS Derivative—There are four possible isomers of *p*-nitrophenyl-2-dichloroacetamido-1,3-propanediol: two enantiomers each of the *threo* and of the *erythro* configuration. However, only the D (—)-*threo* conformer (chloramphenicol) possesses therapeutic antibiotic activity.

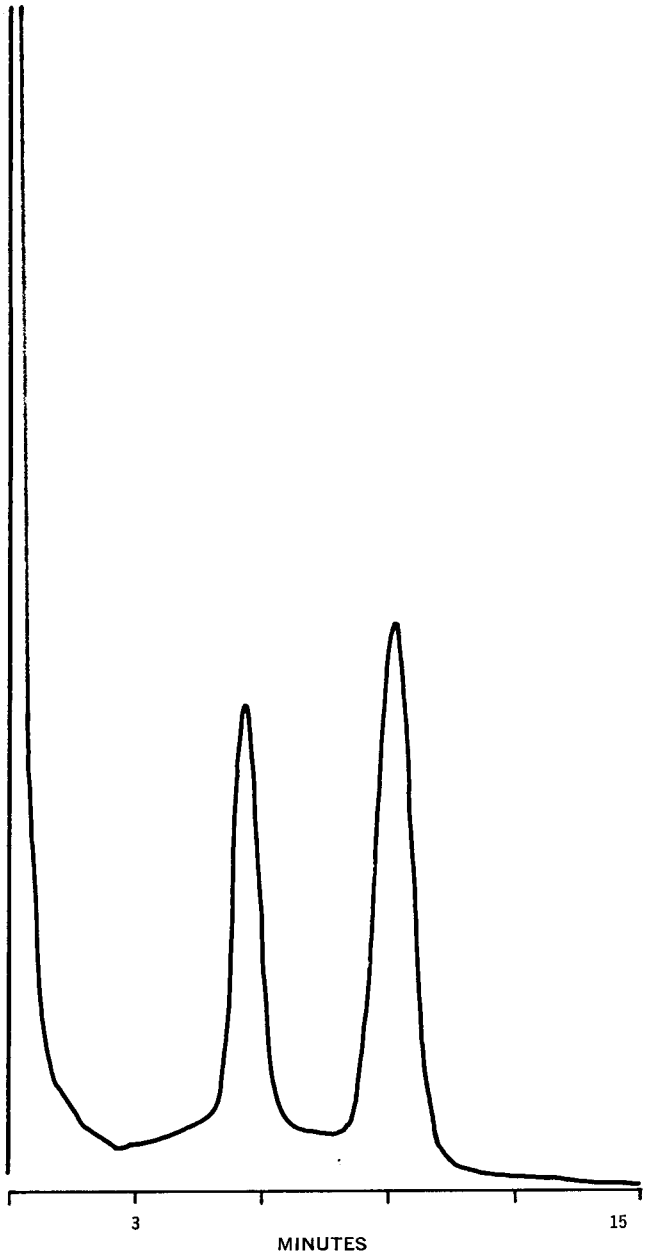


Figure 4—*Carbon tetrachloride extract of mixture in Fig. 3 (S3) on QF-1 column at 238°.*

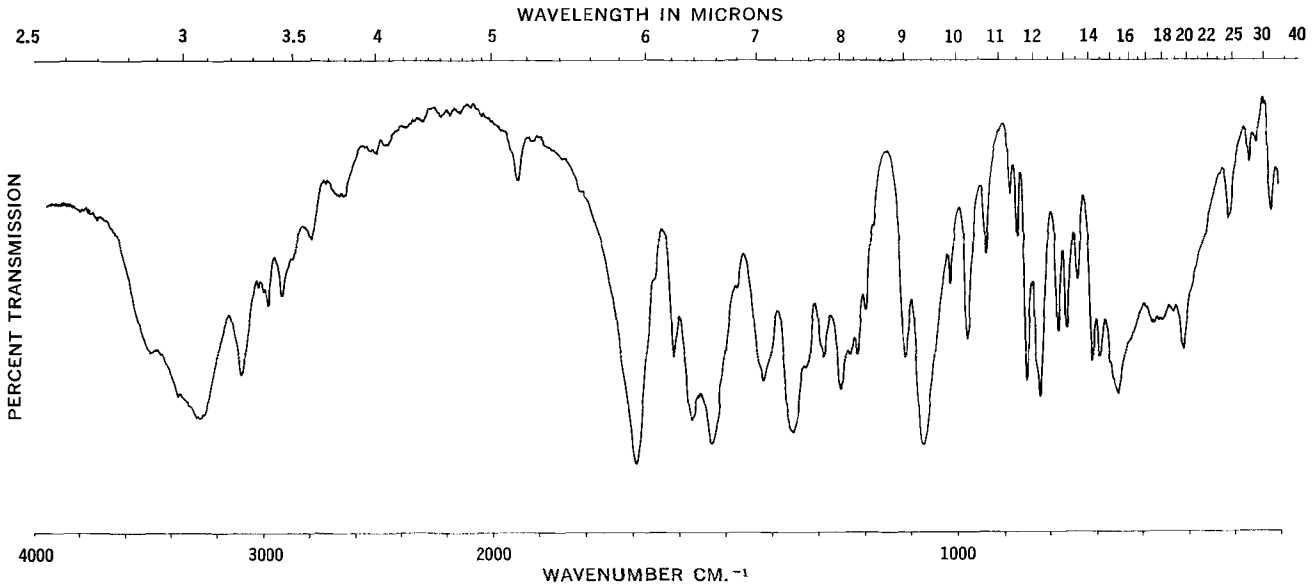
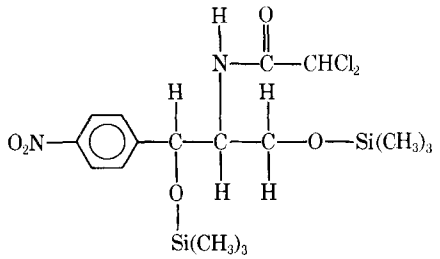


Figure 5—*IR spectrum of chloramphenicol KBr disk.*

The silylation reaction that produces a derivative sufficiently volatile and stable for use in GLC analysis yields the *O,O*-ditrimethylsilyl ether as represented in Structure I. Substantiation of this structure forms the main part of this study.



I

Morphology—The trimethylsilyl ether of chloramphenicol (S2) is a colorless, amorphous solid that crystallizes slowly over a period of weeks and melts below 55°. In contrast, chloramphenicol is a white crystalline solid melting at around 152°.

Ultraviolet Analyses—The UV spectrum of S2 in cyclohexane exhibited a single absorption band with a maximum at 264 mμ and an absorptivity of 10,900 l./mole cm. A methanolic solution showed a maximum at 273 mμ whereas chloramphenicol itself showed a maximum at 278 mμ in water with absorptivity of 9630 l./mole cm.

The UV spectrophotometric method has been demonstrated to be rather nonspecific (8), since the single absorption band near 280 mμ ascribed to the n-π transition of the nitrophenyl nucleus is also exhibited by many structurally related compounds (9, 10). Yamamoto *et al.* (4) reported that chloramphenicol, *p*-nitrophenylserinol (1-phenyl-2-amino-1,3-propanediol), and *N*-acetyl-*p*-nitrophenylserinol were separated on a SE-52 column above 200°. These workers collected the effluents and decided that they were not degradation products on the basis of UV spectrophotometry. This seems to contradict the fact that chloramphenicol was previously shown by thermogravimetry to decompose rapidly at about 160° (11) as well as the author's findings of thermolysis of chloramphenicol on the QF-1 column.

Infrared Analyses—A near IR spectrum of S2 in CCl_4 showed the presence of an amide proton at 6702 and 4926 cm.$^{-1}$, aro-

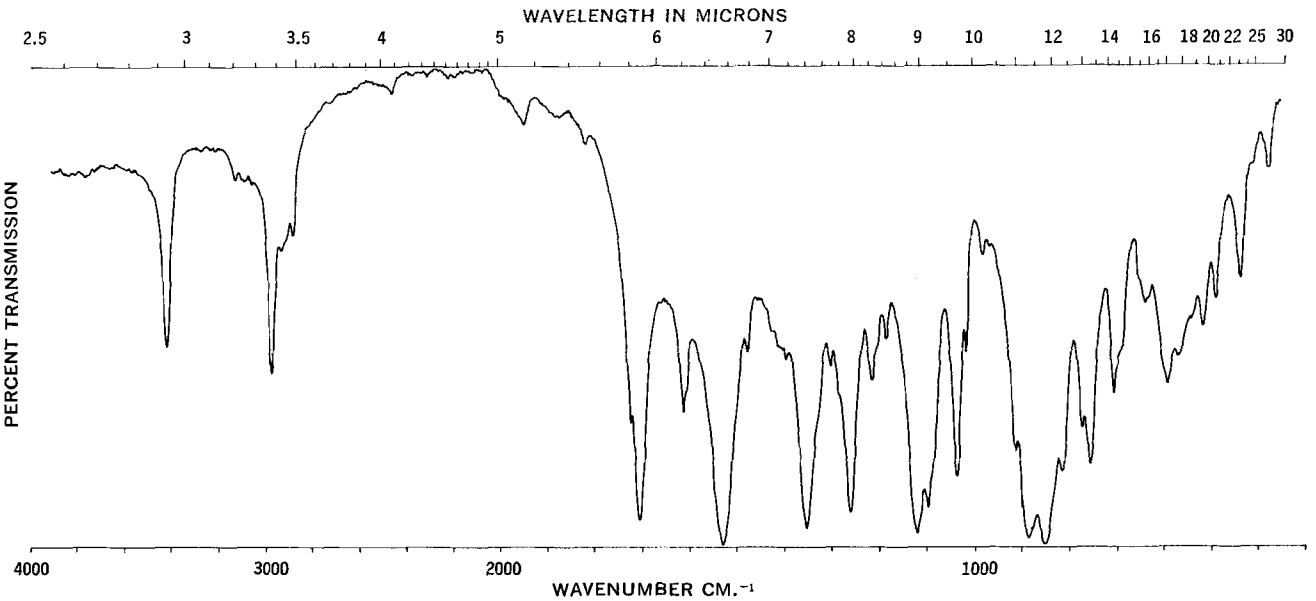


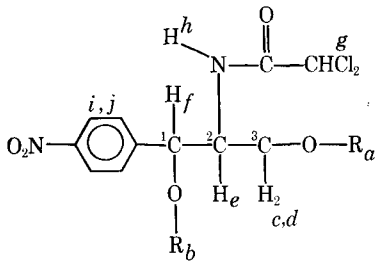
Figure 6—*IR spectrum of TMS ether of chloramphenicol (S2).*

matic protons at 6050, 5907, and 4615 cm.$^{-1}$, and methyl protons at 4355 cm.$^{-1}$

IR spectra of chloramphenicol and S2 were obtained as KBr disks (Figs. 5 and 6). Significant absorption bands common to both spectra were those of the amide proton stretching mode at about 3400 cm.$^{-1}$, the amide carbonyl fundamental at about 1695 cm.$^{-1}$, the aromatic nucleus at 1610 cm.$^{-1}$, the nitro group at 1527 and 1351 cm.$^{-1}$, and the aromatic substitution pattern at 2000–1667 and 758 cm.$^{-1}$

The spectrum of S2 showed additional new intense bands at 2967 and 2882 cm.$^{-1}$ attributed to the symmetric and asymmetric stretching mode of the six methyl groups; at 1259 cm.$^{-1}$ due to the rocking deformation mode of the methyl groups; at 1135 and 1120 cm.$^{-1}$ due to the Si—O—C stretching; at 850 and 755 cm.$^{-1}$ due to the Si—C bond. The position, shape, and intensity of the absorption bands at 1259, 850, and 755 cm.$^{-1}$ are particularly characteristic of Si—$(CH_3)_3$ groups. The absorption bands due to the hydroxyl functions near 3330 cm.$^{-1}$ have disappeared.

Nuclear Magnetic Resonance—The NMR spectra of S2 in CCl_4 and in deuterated acetone were obtained and compared to that of deuterated chloramphenicol (S1) in deuterated acetone. Replacing the three readily exchangeable protons with deuterium clarified the spectrum to some extent by eliminating three peaks and reducing the splitting pattern and peak overlap. A typical spectrum obtained from a CCl_4 solution is shown in Fig. 7 and assignments of peaks are given in Table I.



A comparison of spectra obtained from deuterated acetone solutions shows that the spectrum of S2 was, as expected, similar to that of S1 except for the NH proton at 7.45 p.p.m., and the two trimethylsilyl groups of 0.10 and 0.15 p.p.m.

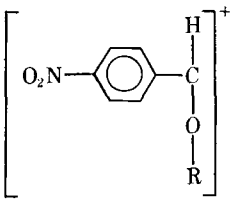
The C_3 proton peaks were found at 3.63 and 3.75 p.p.m. compared with 3.75 and 3.85 p.p.m. in the deuterated compound. Similarly, the C_2 proton peak appeared at 4.07 *versus* 4.25 p.p.m., the C_1 proton peak at 5.35 *versus* 5.33 p.p.m., the $\underline{C}HCl_2$ proton peak at 6.25 *versus* 6.35 p.p.m., and the aromatic proton peaks at 7.95 p.p.m. for both compounds. Thus, the silylation of chloramphenicol results in a variable upfield shift of the peaks, believed to be most likely due to the shielding effects of the silyl function.

The NH proton coupling with the C_2 proton (J = 9 c.p.s.) shows up as a doublet. The aromatic proton splitting is typical of *p*-nitro substitution. The $\underline{C}HCl_2$ and the methyl protons of the siloxy groups at C_1 and C_3 gave rise to singlets. The C_1 proton doublet peak is indicative of a coupling with the C_2 proton. The latter, in turn, exhibited a more complex multiplet because it couples with the amide proton as well as with one at C_1 and two at C_3. The two C_3 protons may also be expected to be nonequivalent because of their adjacent position to an asymmetric carbon.

The two singlet peaks observed for the trimethylsilyl groups are clear evidence of the nonequivalence of the two groups. This nonequivalence may be due partly to the diamagnetic anisotropy of the benzene nucleus. Similarly, the two doublets centered at 4.16 p.p.m. indicate that the two C_3 protons are nonequivalent. The relative values of the integrated peaks are also consistent with the proposed structure.

Mass Spectroscopy—In the electron-impact mass spectrum of chloramphenicol (Fig. 8) and S2 (Fig. 9), some fragmentation may be common to both compounds or may be associated solely with silyl ethers (12–15).

One mode of scission common to both compounds occurs between C_1 and C_2, resulting in two major fragments (I and II), where R is H in chloramphenicol and $Si(CH_3)_3$ in S2.



I

(*a*) R = H, *m/e* 153

(*b*) R = $Si(C_3H)_3$, *m/e* 225

$ROCH_2CH=\overset{+}{N}HCOCHCl_2$

II

(*a*) R = H, *m/e* 170

(*b*) R = $Si(CH_3)_3$, *m/e* 242

In chloramphenicol, the two fragments appear as the base peak at *m/e* 153 (RI = 100%; Σ_{35}, the calculated percentage of total ionization over the range *m/e* 35 to M^+, = 6.16%) and at *m/e* 170 (RI = 56%; Σ_{35} = 3.42%). High resolution has shown the *m/e* 153 peak to be a doublet due to the loss of HO from Fragment II as the main fraction, whereas the other is ascribed to protonated Fragment I*a* (16). S2 is ruptured at the same site with protonated Fragment I*b* at *m/e* 225 (RI = 58%; Σ_{35} = 14.94%), and Fragment II*b* at *m/e* 242 (RI = 4.9%; Σ_{35} = 1.27%).

Fragmentations generally ascribed to trimethylsilyl ethers are expectedly found in the spectrum of S2. The molecular ion is barely perceptible at *m/e* 466 (RI = 0.012%; Σ_{35} = 0.003%) at

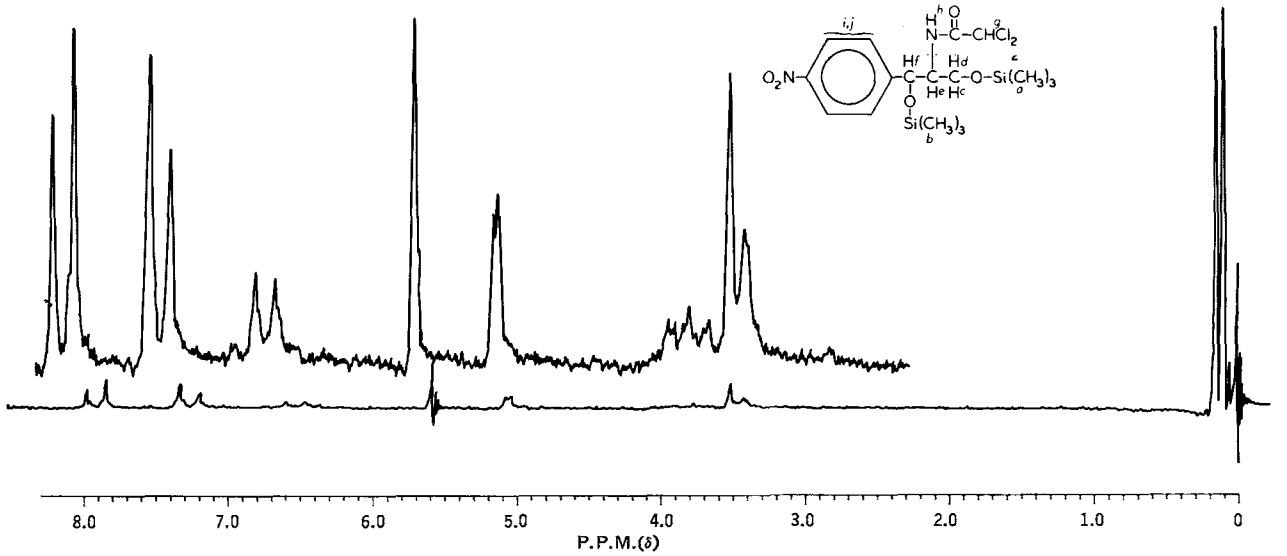


Figure 7—*NMR spectrum of TMS ether of chloramphenicol (S2) in CCl_4.*

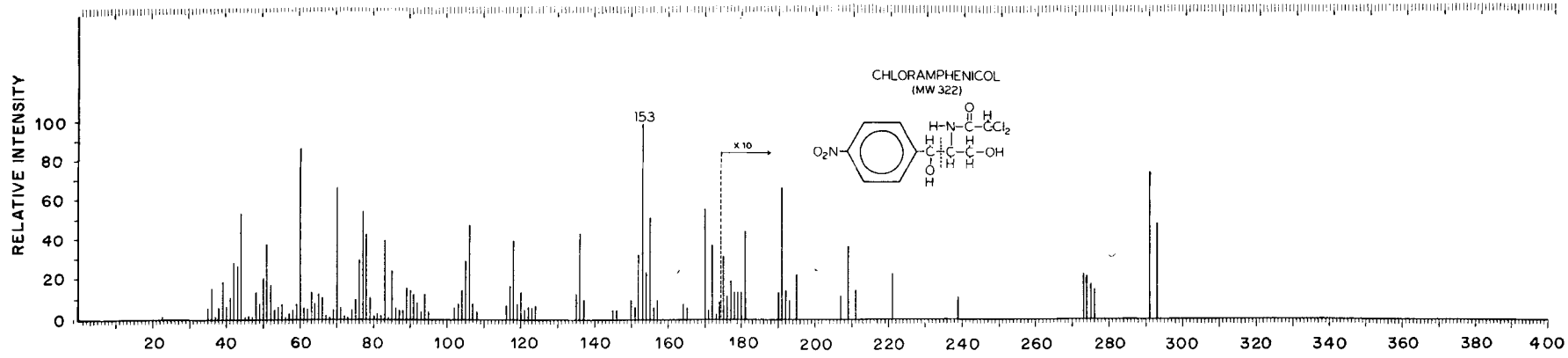


Figure 8—*Mass spectrum of chloramphenicol.*

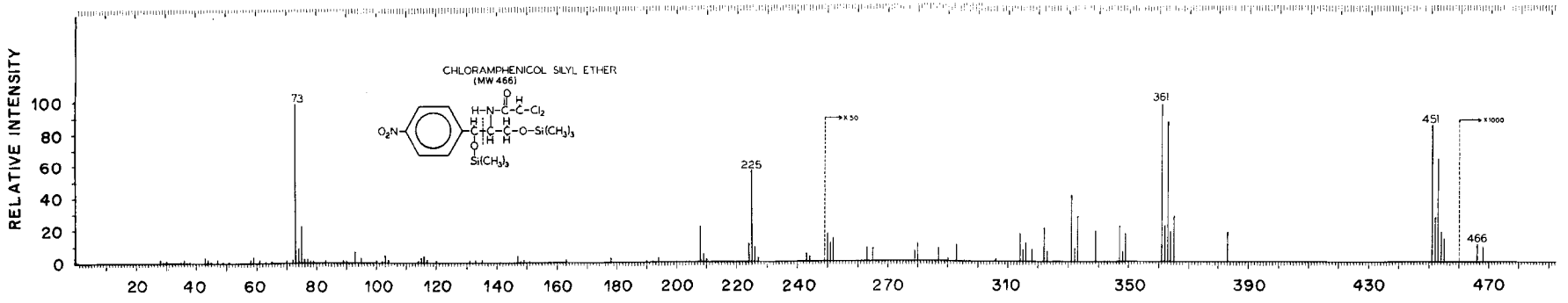


Figure 9—*Mass spectrum of TMS ether of chloramphenicol (S2).*

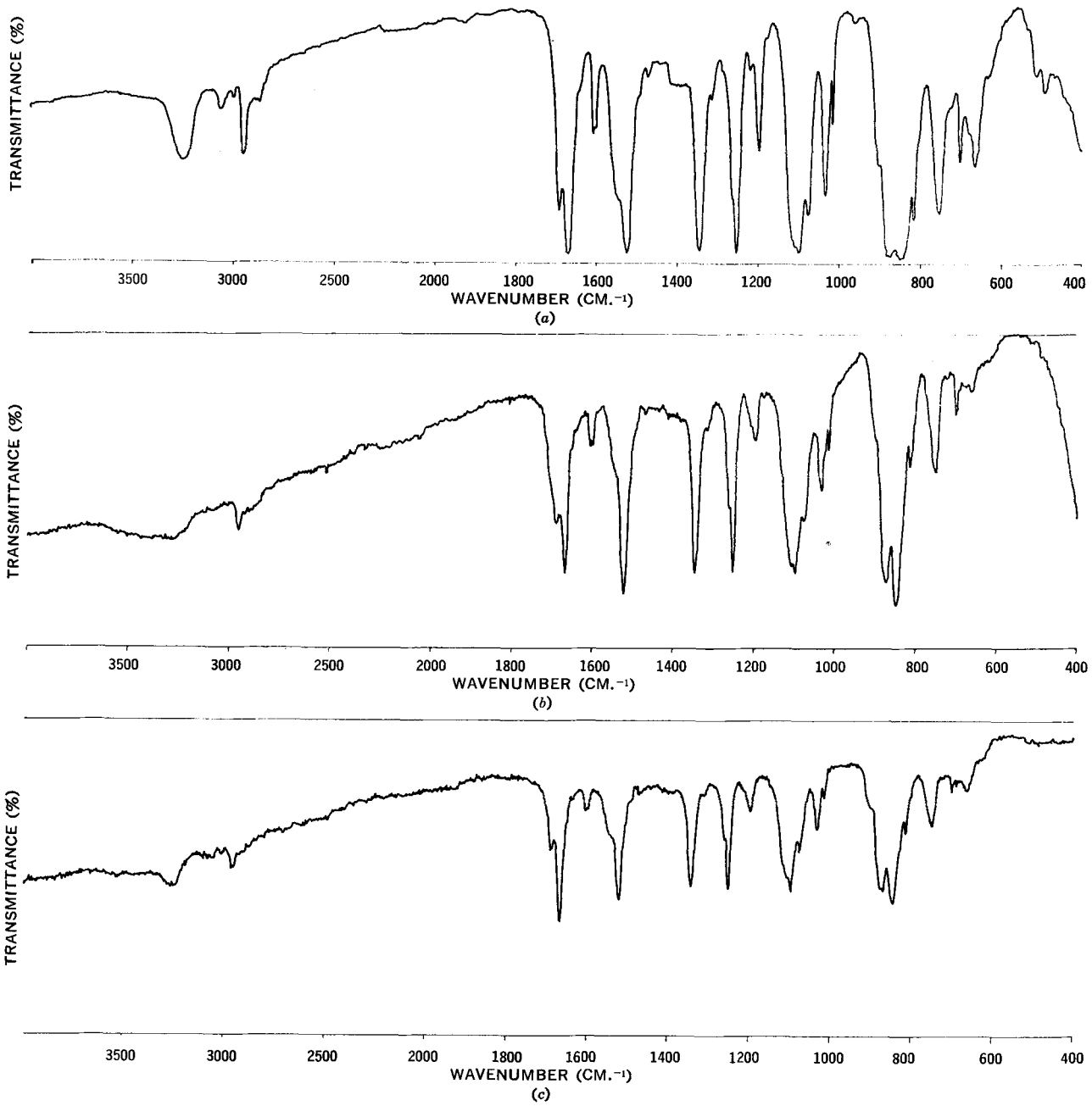


Figure 10—*IR spectra of microsamples:* (a) *Standard TMS ether of chloramphenicol (S2).* (b) *Chromatographic Fraction I from S3.* (c) *Chromatographic Fraction II from S3.*

a high instrument sensitivity. The loss of one methyl group to yield the $(M\text{-}15)^+$ peak at *m/e* 451 (RI = 1.8%; Σ_{35} = 0.45%) and the subsequent elimination of trimethylsilanol to yield a $(M\text{-}15\text{-}90)^+$ peak at *m/e* 361 (RI = 2.0%; Σ_{35} = 0.52%) are particularly useful in establishing the proper location of the molecular ion. A broad metastable peak at *m/e* 290 (calculated $361^2/451 = 288.96$ and $363^2/453 = 290.88$ for the +2 peak), probably due to the coalescence of two metastable peaks from each of the two chlorine isotopes, was observed corresponding to this sequence.

Fragment I*b* (*m/e* 225) seems to undergo losses typical of *O*-silyl ethers, M-15, -73, or -90, in addition to the loss of atomic oxygen from the nitro group to show peaks at *m/e* 194 (RI = 3.3%; Σ_{35} = 0.84%), *m/e* 136 (RI = 2.2%; Σ_{35} = 0.58%), and *m/e* 119 (RI = 0.86%; Σ_{35} = 0.22%), respectively.

Fragment II*b* (*m/e* 242) shows a similar pattern of an *O*-silyl ether with the loss of 15, 30, or 73, simultaneous with the elimination of $COCHCl_2$, to give peaks at *m/e* 116 (RI = 4.3%; Σ_{35} = 1.11%), *m/e* 101 (RI = 0.93%; Σ_{35} = 0.24%), and *m/e* 58 (RI = 2.4; Σ_{35} = 0.61%).

Characterization of Gas Chromatographic Effluents—A preliminary melting point of an evaporated nonpolar mixture of S3 taken before chromatography was about 71–72°, in contrast to about 55° for the standard S2.

An IR spectrum of chloramphenicol obtained with a Beckman IR-12 spectrophotometer matched that reported by Sensi (17) and by Suzuki (18) but differed somewhat from that reported by Suzuki and Shindo (19). However, the latter authors did indicate spectral differences between the *threo* and *erythro* conformers in the crystalline state in the 5.93–5.97, 6.40–6.54, and 10.00–11.10-μ regions. Although these differences were expected to appear in the silyl ethers, none was detected in the KBr spectra of the collected fractions (Figs. 10*a*, *b*, *c*). In fact, both fractions from S3 appeared to be identical to the standard S2.

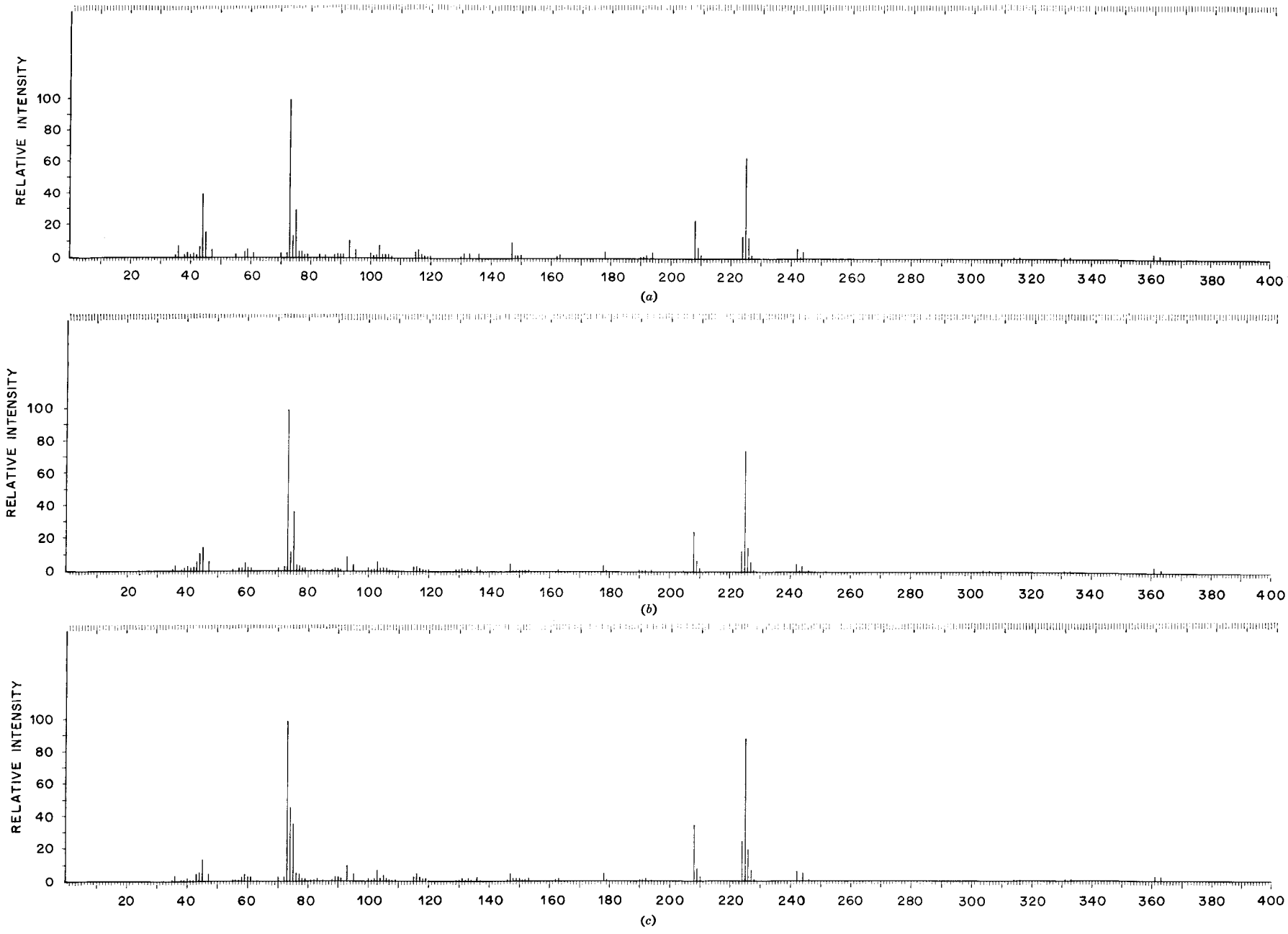


Figure 11—*Mass spectra of microsamples:* (a) *Standard TMS ether of chloramphenicol (S1).* (b) *Chromatographic Fraction I from S3.* (c) *Chromatographic Fraction II from S3.*

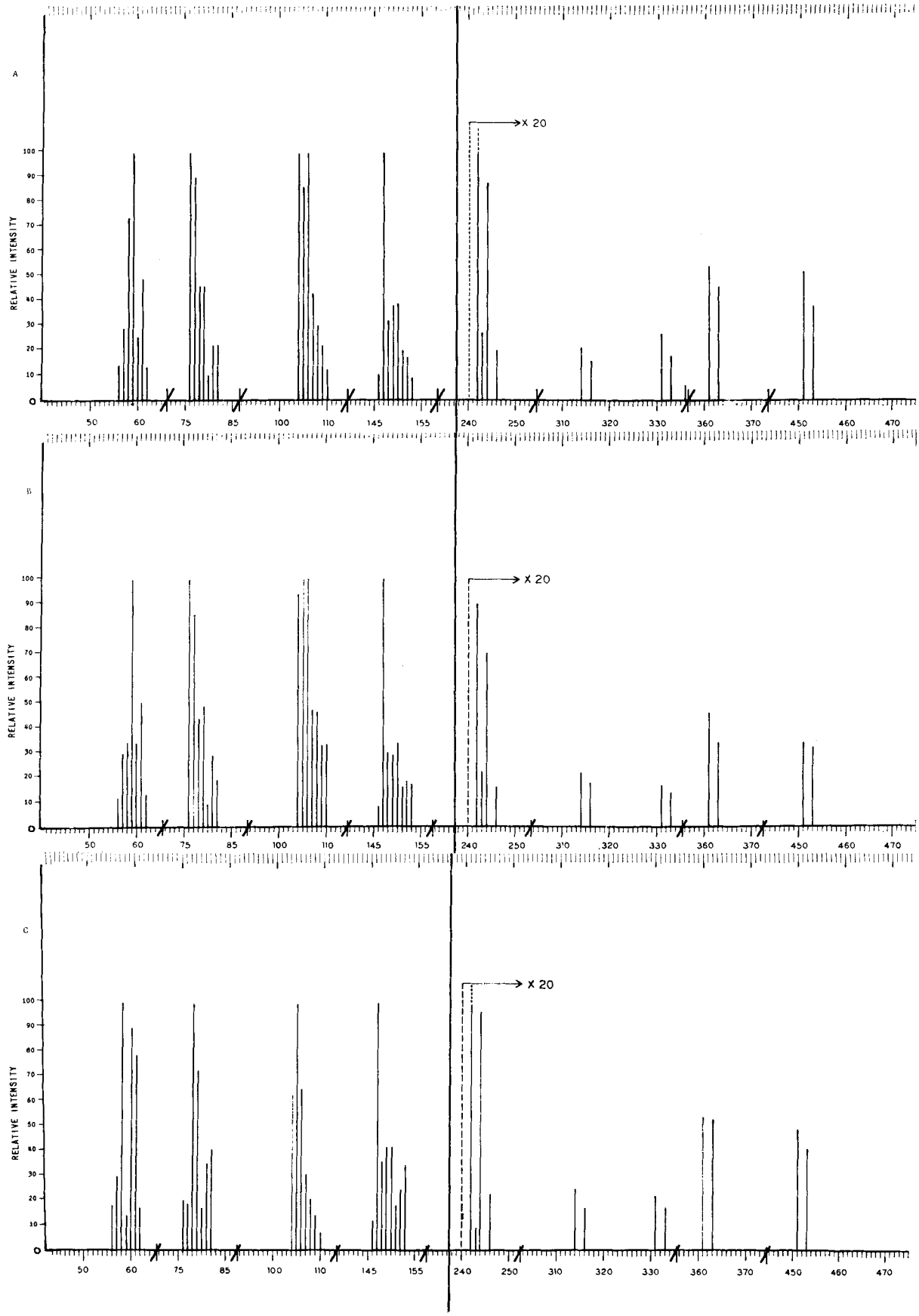


Figure 12—*Partial mass spectra of microsamples:* (a) *TMS ether of standard, S2.* (b) *Chromatographic Fraction I from S3.* (c) *Chromatographic Fraction II from S3.*

Table I—Observed Shift and Splitting of S2 in CCl_4

Assignment	ν^a	δ^a	No. Protons	Splitting
a	6	0.10	18	Singlet
b	9	0.15		Singlet
c	205	3.42	1	Doublet
d	211	3.52	1	Singlet
e	228	3.80	1	Multiplet
f	304	5.06	1	Doublet
g	335	5.58	1	Singlet
h	392	6.53	1	Doublet
i,j	455	7.58	4	Quartet

[a] Chemical shift (ν) measured in c.p.s. from tetramethylsilane used as internal standard in CCl_4 and in p.p.m. (δ).

Standard S2, a fresh derivative solution, and S3 were injected into the LKB 9000 and the Barber-Colman 5000 gas chromatographs. The resultant chromatograms showed identical retention times for standard S2, the fresh derivative solution, and the first fraction from S3. The mass spectra of S2 and of the fresh derivative solution obtained after injection into the LKB 9000 were identical to S2 placed directly into the inlet probe, indicating that the three samples were identical.

The mass spectrum of each collected fraction was compared to that of the S2 and the same fragmentation pattern was obtained peak for peak (Fig. 11), confirming the identity of each as the *O,O*-ditrimethylsilyl ether of *p*-nitrophenyl-2-dichloroacetamido-1,3-propanediol. However, S2, although essentially identical to Fraction I, differed from Fraction II in the relative intensity of several fragment peaks and clusters (Fig. 12).

The conclusion that Fraction II is the diastereomer of chloramphenicol silyl ether is substantiated by the available data as follows: the methanol-treated silyl solution (S3) yielded two GLC peaks and enantiomers are not known to be separable under these conditions; IR spectra showed that both effluent fractions were identical to S2; and mass spectra showed that Fraction I was identical with the S2 but differed from Fraction II only in the relative abundance of some peaks.

Petersson *et al.* (12) and Capella and Zorzut (14) have shown that mass spectra of diastereomers have the same fragmentation pattern but differ in the relative intensities of some fragment peaks. They report that the *m/e* 147 disiloxonium ion exhibits a very high abundance, particularly when the sample material contains more than one trimethylsilyl group. The *m/e* 147 peak was also reported to be more intense in the *erythro* than in the *threo* conformer in the case of oleic and elaidic acid derivatives. With regard to chloramphenicol silyl ether, the *m/e* 147 peak was found to be relatively weak but significant at about RI = 5% and Σ_{35} = 1% for both fractions of S3 as well as for S2. Walden inversions of structures related to chloramphenicol have been reported to occur with phosphorus halides (20), in esterification reactions (21), with heat (22), with hydrolysis (23), and with other nucleophilic attack by solvolysis (24).

REFERENCES

(1) Code of Federal Regulations, Title 21, Part 141d, 301 (1968).

(2) P. D. Shaw, *Anal. Chem.*, **35**, 1580(1963).

(3) G. L. Resnick, D. Corbin, and D. H. Sandberg, *ibid.*, **38**, 582(1966).

(4) M. Yamamoto, S. Iguchi, and T. Aoyama, *Chem. Pharm. Bull.*, **15**, 123(1967).

(5) R. Bentley, C. C. Sweeley, M. Makita, and W. W. Wells, *Biochem. Biophys. Res. Commun.*, **11**, 14(1963).

(6) M. Margosis, *J. Chromatogr.*, **37**, 46(1968).

(7) M. Margosis, FDA, NCAIA, unpublished data.

(8) E. F. Schwarm, C. Dabner, J. W. Wilson, Jr., and M. P. Boghosian, *J. Pharm. Sci.*, **55**, 744(1966).

(9) D. Masterson, Jr., *ibid.*, **57**, 305(1968).

(10) L. Doub and J. M. Vandenbelt, *J. Amer. Chem. Soc.*, **69**, 2714(1947).

(11) W. W. Wendlandt and M. Zief, *Naturwissenschaften*, **45**, 467(1958).

(12) G. Petersson, O. Samuelson, K. Anjou, and E. von Sydow, *Acta Chem. Scand.*, **21**, 1251(1967).

(13) J. Diekman, J. B. Thomson, and C. Djerassi, *J. Org. Chem.*, **32**, 3904(1967).

(14) P. Capella and C. M. Zorzut, *Anal. Chem.*, **40**, 1458(1968).

(15) J. A. McCloskey, R. N. Stillwell, and A. M. Lawson, *ibid.*, **40**, 233(1968).

(16) C. Brunnee, G. Kappus, and K.-H. Maurer, *Fresenius' Z. Anal. Chem.*, **232**, 17(1967).

(17) P. Sensi, *Boll. Soc. Ital. Biol. Sper.*, **29**, 592(1953).

(18) M. Suzuki, *J. Antibiotics (Tokyo), Ser. B*, **14**, 323(1961).

(19) M. Suzuki and H. Shindo, *J. Pharm. Soc. Japan*, **76**, 927 (1956).

(20) A. Iliceto, *Ric. Sci.*, **23**, 1621(1953); through *Chem. Abstr.*, **48**, 12702*c*(1954).

(21) W. Logemann, F. Lauria, and E. Pella, *Gazz. Chim. Ital.*, **83**, 407(1953); through *Chem. Abstr.*, **49**, 222*h*(1955).

(22) T. Taguchi, M. Tomoeda, and I. Aratani, *J. Amer. Chem. Soc.*, **78**, 1468(1956).

(23) M. P. Kerop'yan and A. S. Elina, *Zh. Obshch. Khim.*, **31**, 3298(1961); through *Chem. Abstr.*, **57**, 2114*f*(1962).

(24) L. H. Sommer and F. A. Carey, *J. Org. Chem.*, **32**, 2473 (1967).

ACKNOWLEDGMENTS AND ADDRESSES

Received July 29, 1969, from the *National Center for Antibiotics and Insulin Analysis, Division of Pharmaceutical Sciences, Food and Drug Administration, Department of Health, Education, and Welfare, Washington, DC 20204*

Accepted for publication November 6, 1969.

The author wishes to express his appreciation to Dr. J. Y. T. Chen, Mr. J. N. Damico, and Mrs. B. S. Fisher of the Food and Drug Administration, for obtaining some of the spectra and for most helpful discussions, and to Mr. R. Pittman of the National Institutes of Health, for the determinations with the LKB 9000.

This study was initiated at the National Center for Antibiotics and Insulin Analysis and pursued at the FDA Institute for Advanced Analytical Chemistry at Georgetown University under the supervision of Dr. Charles F. Hammer, Director.