Keyphrases

Prasinomycins—molecular association

Molecular weight—approach-to-equilibrium method

Sedimentation coefficients—schlieren, interference optical systems

Diffusion constants—centrifuge, schlieren optical system

Gas-Liquid Chromatographic Determination of Lincomycin

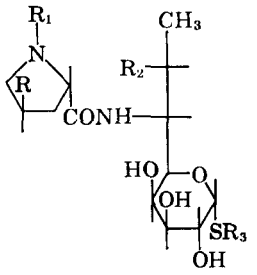
By R. L. HOUTMAN, D. G. KAISER, and A. J. TARASZKA

The gas-liquid chromatography of lincomycin and some of its analogs, determined as trimethylsilyl ethers, is reported. The application of this procedure to quantification of lincomycin in bulk material and pharmaceutical preparations is described.

THE APPLICATION of gas-liquid chromatography to polyhydroxy compounds has been greatly enhanced by the advent of methods for the quantitative conversion of these compounds to their trimethylsilyl ethers (1, 2). The silylation procedure consists of dissolving the material in pyridine and adding hexamethyldisilazane and trimethylchlorosilane.

Lincomycin (I) (3), a medium spectrum antibiotic containing an octopyranose moiety, was silanized by this procedure and chromatographed as the intact tetra trimethylsilyl ether. U-21699 (II), an antibiotic produced at about the 3% level in the microbiological synthesis of lincomycin (4) was chromatographed under the same conditions and gave a retention time of 0.85 relative to lincomycin. This separation enabled the quantification of small amounts of U-21699 in lincomycin as well as the determination of lincomycin in bulk material and pharmaceutical formulations.

Since the preparation of the trimethylsilyl ether derivatives of these compounds is rapid, quantitative, and can be applied on a micro scale, GLC analysis of lincomycin and some of its analogs has become a practical laboratory procedure.



I. Lincomycin
R = —$CH_2CH_2CH_3$
R_1 = —CH_3
R_2 = —OH
R_3 = —CH_3

II. (U-21699)
R = —CH_2CH_3
R_1 = —CH_3
R_2 = —OH
R_3 = —CH_3

III. (U-21251F)
R = —$CH_2CH_2CH_3$
R_1 = —CH_3
R_2 = —Cl
R_3 = —CH_3

IV. (U-11921A)
R = —$CH_2CH_2CH_3$
R_1 = —CH_3
R_2 = —OH
R_3 = —CH_2CH_3

V. (U-11973E)
R = —$CH_2CH_2CH_3$
R_1 = —H
R_2 = —OH
R_3 = —CH_3

VI. (U-24166A)
R = —$CH_2CH_2CH_3$
R_1 = —CH_2CH_3
R_2 = —OH
R_3 = —CH_3

EXPERIMENTAL

Reagents and Materials—The lincomycin and analogs used in this study were prepared by the Research Division of The Upjohn Company. Hexamethyldisilizane and trimethylchlorosilane were obtained from the Dow Corning Co., Midland, Mich. A solution of tetraphenylcyclopentadienone[1] in pyridine (5 mg./ml.) was employed as the internal standard. The column packing utilized for gas-liquid chromatography was 3% (w/w) SE-30 on 100–120 mesh Gas Chrom Q.[2] All solvents were reagent grade and used as supplied.

Instrumentation—An F & M model 402 high efficiency gas chromatograph,[3] equipped with a flame ionization detector and U-shaped glass columns (2 m. long × 3 mm. i.d.) was utilized throughout the study. Helium was used as the carrier gas at a flow rate of 60 ml./min. Air and hydrogen flow rates were adjusted to give maximum response. The column oven was operated isothermally at 240°, the flash heater at 270°, and the detector at 290° Peak areas were measured with an Infotronics 11HSB/42 electronic integrator.[4]

Procedures—In general, the silanization technique of Makita and Wells (5) was used. For quantification of lincomycin, 50 mg. of the bulk drug was accurately weighed and dissolved in 10.0 ml. of the internal standard solution. One milliliter of hexamethyldisilizane and 0.5 ml. of trimethylchlorosilane were added. The solutions were allowed

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[1] Aldrich Chemical Co., Milwaukee, Wis.

[2] Applied Science Laboratories, Inc., State College, Pa.

[3] F & M Scientific Corporation, Avondale, Pa.

[4] Infotronics Corporation, Houston, Tex.

TABLE I—ANALYTICAL DATA OF TMS DERIVATIVES

Lincomycin TMS			U-21251F TMS		
Assay	Theory	Found	Assay	Theory	Found
C	51.82	51.65	C	50.55	50.20
H	9.57	9.58	H	8.94	8.72
N	4.03	4.03	N	4.37	4.14
S	4.61	4.72	S	5.00	4.96
Mol. Wt.[a]	695	695	Cl	5.53	5.66
			Mol. Wt.[a]	640	640

[a] Determined by mass spectrometry.

to stand for 10 min. and then centrifuged prior to chromatography.

Silanizable excipients, such as sucrose and lactose, in formulations required some adjustment in the aforementioned reagent ratios but did not interfere with the quantification of lincomycin. One extreme example was an aqueous solution containing 650 mg. of sucrose and 50 mg. of lincomycin per ml. of formulation. Two milliliters of the solution was lyophilized, dissolved in 10 ml. of internal standard solution, and silanized with 5 ml. of hexamethyldisilazane and 2.5 ml. of trimethylcholorosilane. It was necessary to allow 30 min. for complete reaction. Similarly, centrifuged fermentation beers may be analyzed following lyophilization.

For qualitative examination or determination of analog ratios from fermentation samples, 10 mg. of material was dissolved in 1 ml. pyridine. Hexamethyldisilizane (0.2 ml.) and trimethylchlorosilane (0.1 ml.) were added. Reaction conditions were the same as described for the determination of lincomycin in the bulk drug.

For each series of samples, a reference solution was prepared by accurately weighing an amount of lincomycin reference standard approximately equivalent to that in the sample and treating it identically.

Calculations—The lincomycin concentration in the sample solutions was determined by a direct comparison of peak area ratios.

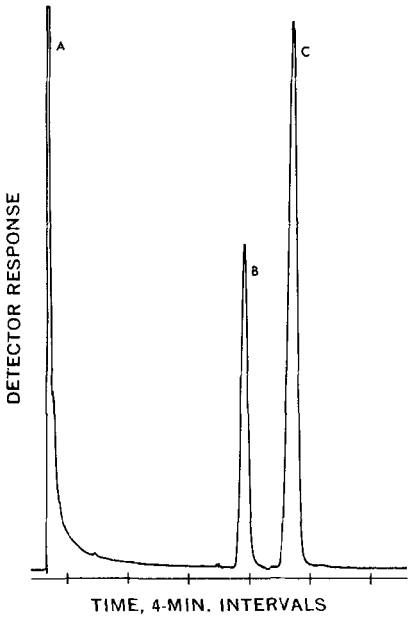

Fig. 1—Chromatogram of U-21699 and lincomycin, A = solvent, B = U-21699, C = lincomycin.

Characterization of Derivatives—Samples of the trimethylsilyl derivatives were collected at the exit port of the chromatograph for characterization. The collected samples were compared either by infrared spectroscopy or mass spectrometry to materials isolated from the reaction solutions. In the case of lincomycin and U-21251F (III) (6) sufficient sample was isolated to enable complete elemental and spectral analysis. These data are summarized in Table I.

RESULTS AND DISCUSSION

Quantification of U-21699 in Lincomycin—In the search for a suitable liquid phase for the separation and quantification of U-21699 in lincomycin, it was observed that any of the nonpolar liquid phases were acceptable. The SE-30 column described in the procedure was selected for its stability and general availability. After establishing conditions

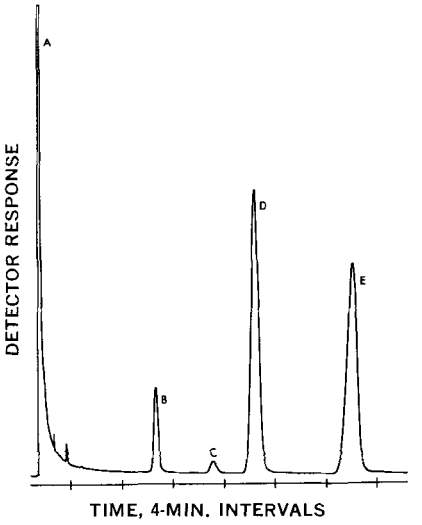

Fig. 2—Chromatogram of an assay preparation from a hard-filled capsule: A = solvent, B = lactose, C = U- 21699 and lactose, D = lincomycin, E = internal standard.

TABLE II—EXCIPIENT RETENTION TIMES

Compound	Relative Retention Time
Sorbitol	0.08
Sucrose	0.56
Lactose	0.81 and 0.54
Lincomycin	1.0
Internal standard (tetraphenylcyclopentadienone)	1.46

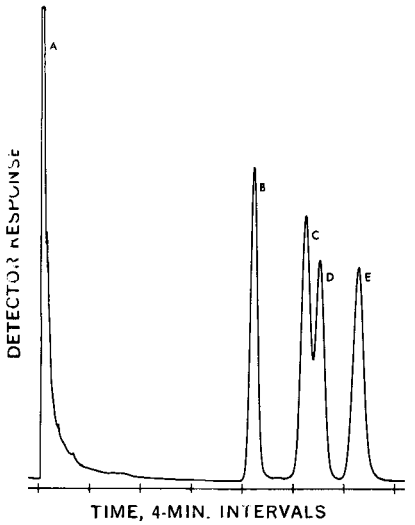

Fig. 3—Chromatogram of lincomycin analogs. A = solvent, B = U-21699, C = lincomycin, D = U-11921, E = U-11973.

for chromatography, the response factors for lincomycin and U-21699 were determined by silylating and chromatographing a 50–50 mixture of the two compounds. The difference in response per mg. for the two materials was less than 1%.

This small difference was insignificant in the determination of small amounts of U-21699 in lincomycin. The comparable response for lincomycin and U-21699 might have been predicted from the similarity of the compounds (I and II). One milligram each of lincomycin and U-21699, when weighed as the monohydrate hydrochloride, yield 0.781 and 0.778 mg. of carbon, respectively, as the trimethylsilylethers. This response relationship was further established by a recovery study. Eight prepared mixtures ranging from 0–10% U-21699 in lincomycin by weight were assayed. Statistical analysis of the data indicated a slope of 0.99.

A chromatogram of a production lot of lincomycin containing 2.7% of U-21699 is presented in Fig. 1. Note the change in attenuation allowing a measurable area for the minor component.

Twenty replicate assays, each on different days, of a sample of lincomycin containing 2.33% U-21699 gave a standard deviation of 0.108% for U-21699. This represents a coefficient of variation of 4.8% for the minor constituent.

Quantification of Lincomycin in Bulk Drug and Formulations—Tetraphenylcyclopentadienone, the internal standard used in the assay, had a retention time relative to lincomycin of 1.46 which minimized the interference of possible decomposition products. The relative retention times of common excipients used in various formulations were established and are shown in Table II.

Table III—Retention Times of Lincomycin Analogs

Compd.	Relative Retention Time	Derivative
II (U-21699)	0.81	Tetra TMS ether
III (U-21251F)	0.86	Tri TMS ether
I (Lincomycin)	1.00	Tetra TMS ether
IV (U-11921A) (7)	1.05	Tetra TMS ether
V (U-11973E) (8)	1.20	Tetra TMS ether
VI (U-24166A) (9)	1.22	Tetra TMS ether
Tetraphenylcyclopentadienone	1.46	...

Precision studies conducted on several formulations indicated that the coefficients of variation were all less than 1%. A chromatogram of an assay preparation from a hard-filled capsule is shown in Fig. 2.

Chromatography of Other Analogs—Figure 3 is a chromatogram of a synthetic mixture of lincomycin and three analogs to illustrate the degree of separation. It should be noted, however, that this is a hypothetical mixture and that only U-21699 is normally produced in the microbiological synthesis of lincomycin. Two other analogs, U-21251F and U-24166A, not shown in Fig. 2, were chromatographed using this technique and a summary of the retention times relative to lincomycin are shown in Table III.

REFERENCES

(1) Sweeley, C. C., Bentley, R., Makita, M., and Wells, W. W., *J. Am. Chem. Soc.*, **85**, 2497(1963).
(2) Bentley, R., Sweeley, C. C., Makita, M., and Wells, W. W., *Biochem. Biophys. Res. Commun.*, **11**, 14(1963).
(3) Mason, D. J., Dietz, A., and Deboer, C., *Antimicrobial. Agents Chemotherapy*, **1962**, 554.
(4) Argoudelis, A. D., Fox, J. A., and Eble, T. E., *Biochemistry*, **4**, 698(1965).
(5) Makita, M., and Wells, W. W., *Anal. Biochem.*, **5**, 523(1963).
(6) Birkenmeyer, R. D., and Kagan, F., Fifth Interscience Conference on Antimicrobial Agents and Chemotherapy and Fourth International Congress of Chemotherapy, October 17–21, 1965, Washington, D. C., p. 17 (abstracts).
(7) Argoudelis A. D., and Mason, D. J., *Biochemistry*, **4**, 704(1965).
(8) Argoudelis, A. D., Fox, J. A., and Mason, D. J., *ibid.*, **4**, 710(1965).
(9) Magerlein, B. J., Birkenmeyer, R. D., and Kagen, F., *J. Med. Chem.*, **10**, 355(1967).

Keyphrases

Lincomycin—analysis
Trimethylsilyl ether—lincomycin
Analogs, lincomycin—characterized
GLC—analysis