LOMOFUNGIN, A NEW BROAD SPECTRUM ANTIBIOTIC*

ISOLATION AND CHARACTERIZATION

M. E. BERGY

Research Laboratories, The Upjohn Company, Kalamazoo, Michigan, U.S.A.

(Received for publication January 24, 1969)

Lomofungin is a new antibiotic which is extracted from the fermentation broth of *Streptomyces lomondensis* var. *lomondensis* with methyl ethyl ketone and purified by crystallization. It is an acidic olive-yellow colored compound with a molecular formula $C_{15}H_{10}N_2O_6$ and a molecular weight of 314. It exhibits ultraviolet and visible absorption maxima at 221, 270, 311, 375 and 439 mμ in 0.01 N methanolic hydrochloric acid, and at 239, 265, 299, 342, 478 in 0.01 N aqueous sodium hydroxide. Lomofungin does not melt below 320°C. It is soluble in dimethylformamide, alkaline water, acidic acetone and acidic methyl ethyl ketone but only slightly soluble in water, methanol, cyclohexane, acetone, ether or ethyl acetate.

This paper describes the isolation and characterization of the new crystalline antibiotic, lomofungin, which is produced in the broth of *Streptomyces lomondensis* var. *lomondensis*. Its production and biological properties are described by JOHNSON and DIETZ[1].

Isolation and Purification

Lomofungin was extracted from the fermentation broth filtrate with methyl ethyl ketone at pH 2.0 and recovered from a concentrate of the extract by crystallization. The impure antibiotic was purified by recrystallization from a mixture of dimethylformamide and water.

Characterization

Lomofungin is an olive-yellow crystalline compound which is soluble in dimethylformamide, alkaline water, acidic acetone and acidic methyl ethyl ketone. It is only slightly soluble in water, methanol, cyclohexane, acetone, ether and ethyl acetate. It does not melt below 320°C.

Analytical data are consistent with the molecular formula $C_{15}H_{10}N_2O_6$ (M. W. 314). The molecular weight determined by mass spectrometry was 314.

Lomofungin shows the following ultraviolet and visible absorption:

Methanol:	0.01 N Methanolic HCl:	0.01 N Aqueous NaOH:
Max. @ 219 mμ, E=25312	Max. @ 221 mμ, E=27500	Max. @ 239 mμ, E=19518
Infl. @ 260 mμ, E=26527	Max. @ 270 mμ, E=50388	Max. @ 265 mμ, E=34857
Max. @ 284 mμ, E=35240	Max. @ 311 mμ, E=13615	Max. @ 299 mμ, E=28263
Max. @ 316 mμ, E=13147	Max. @ 375 mμ, E= 9404	Max. @ 342 mμ, E=34945
Infl. @ 330 mμ, E=12547	Max. @ 439 mμ, E= 4754	Infl. @ 390 mμ, E= 8393
Max. @ 372 mμ, E= 8893	Infl. @ 465 mμ, E= 4004	Max. @ 478 mμ, E= 4735
Max. @ 441 mμ, E= 4556		
Infl. @ 463 mμ, E= 4189		

* Formerly known as lomondomycin

The ultraviolet absorption spectra in 0.01 N methanolic hydrochloric acid and in 0.01 N aqueous sodium hydroxide are shown in Fig. 1. The infrared absorption spectrum is shown in Fig. 2. Absorption peaks in the nuclear magnetic resonance spectrum, observed in deuterated dimethylformamide at −30°C, are presented in Table 1 (chemical shifts expressed from tetramethylsilane).

Fig. 1. Ultraviolet absorption spectra of lomofungin in 0.01 N methanolic hydrochloric acid and 0.01 N aqueous sodium hydroxide.

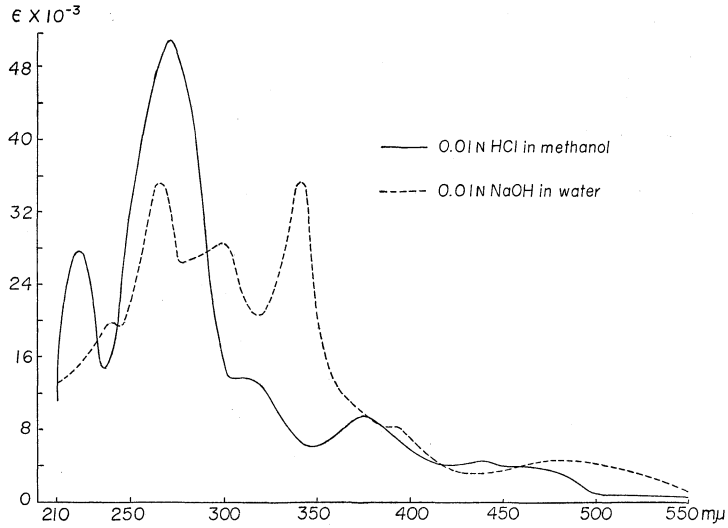

Fig. 2. Infrared absorption spectrum of lomofungin as a Nujol mull.

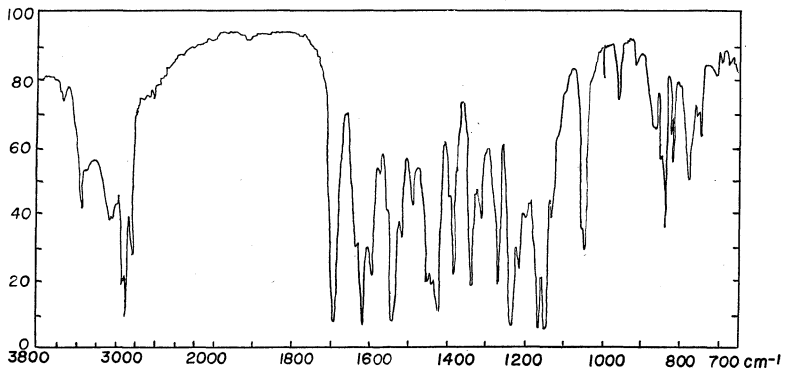

Table 1. N.M.R. spectrum of lomofungin

Chemical shift (δ) (ppm)	Number of hydrogens	Shape of peaks	Suggested structural features
3.98	3	singlet	$-\overset{O}{\overset{\|}{C}}-OCH_3$
6.95	1	singlet	aromatic or =CH-
7.42	1	doublet (J=8 cps)	adjacent aromatic hydrogens
8.33	1	doublet (J=8 cps)	
11.22	1	singlet	probably not exchangeable
11.56	1	singlet	exchangeable
13.08	1	broad	
14.55	1	singlet	

Experimental

Isolation from Fermentation Broth

The whole broth (1,640 ml, 220 mcg/ml) was mixed with 5 % (w/v) diatomaceous earth filter aid and filtered. A water wash (200 ml) of the cake was combined with the clarified broth.

An aliquot (66 %) of the combined filtrate and wash was adjusted to pH 2.0 with sulfuric acid and mixed with methyl ethyl ketone (950 ml). The solvent phases were separated. The aqueous phase (1,300 ml) was discarded but the methyl ethyl ketone extract was concentrated *in vacuo* to a volume of 20 ml, mixed with acetone (80 ml), and concentrated *in vacuo* until crystallization occurred. The mixture was refrigerated overnight, and the crystalline lomofungin was removed by filtration, washed with water and dried *in vacuo* to a constant weight (198 mg). This preparation contained 890 mcg of lomofungin per mg.

Recrystallization

Impure lomofungin (10 g, 810 mcg/mg) was dissolved in dimethylformamide (40 ml) at room temperature and the solution was clarified by filtration. Water (80 ml) was added slowly and the solution was mixed until crystallization started. The mixture was refrigerated for 12 hours. The crystals were filtered, washed with dimethylformamide and water (5 : 1) followed by methanol, and dried *in vacuo* to a constant weight (7.24 g). One further recrystallization gave essentially pure lomofungin (5.67 g).

Analysis: $C_{15}H_{10}N_2O_6$ Calculated: C 57.33, H 3.21, N 8.92, O 30.55
Found: C 56.89, H 3.35, N 8.90, O 30.28

Thin-Layer Chromatography

Lomofungin was analyzed by thin-layer chromatography on 10×20 cm plates prepared with silica gel HF_{254} (E. Merck, A. G.-Darmstadt, Germany) suspended in a solution of buffer salts (pH 6.7) composed of equal volumes of 0.2 M Na_2HPO_4 and 0.2 M KH_2PO_4. The plates were air-dried but not activated. The antibiotic (25 mcg) was applied to the plate in methanol and chromatographed with the system methyl ethyl ketone and methanol (94 : 6). Recrystallized lomofungin gave a single spot (Rf, 0.4) when observed with visible light or by potassium permanganate-sodium metaperiodate spray[2].

Acknowledgements

The author wishes to thank the members of The Upjohn Company who contributed to this work. In particular, D. R. WAIT for competent technical assistance and N. H. KNIGHT and G. SLOMP and their associates for microanalyses and absorption spectra.

References

1) JOHNSON, L. E. & A. DIETZ: Lomofungin, a new antibiotic produced by *Streptomyces lomodensis* sp. N. Appl. Microbiol. May 1969.
2) LEMIEUX, R. U. & H. F. BAUER: Spray reagent for the detection of carbohydrates. Anal. Chem. 26 : 920, 1954.