THE ISOLATION AND CHARACTERIZATION OF NARASIN, A NEW POLYETHER ANTIBIOTIC

DAVID H. BERG and ROBERT L. HAMILL

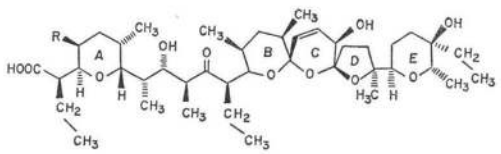
The Lilly Research Laboratories, Eli Lilly and Company
Indianapolis, Indiana 46206, U.S.A.

(Received for publication October 14, 1977)

Narasin is a new polyether antibiotic produced by a strain of *Streptomyces aureofaciens*. It is purified by organic solvent extraction and silica gel chromatography. Narasin is active *in vitro* against gram-positive bacteria, anaerobic bacteria, and fungi and is effective in protecting chickens from coccidial infections.

Narasin, a new polyether antibiotic, is produced by *Streptomyces aureofaciens* NRRL 5758. The discovery and fermentation of narasin[1], the elucidation of its structure (Fig. 1)[2], and biosynthetic studies using ^{13}C-precursors[3] have been reported recently. Narasin belongs to the rapidly growing family of polyether antibiotics which includes monensin[4], A204A[5], lasalocid (X537A)[6], salinomycin[7], dianemycin[8], nigericin[9], and lonomycin[10]. Narasin is closely related structurally to salinomycin. This paper reports the isolation of narasin, narasin methyl ester, and narasin B; their physical-chemical and biological properties; and the preparation of derivatives of narasin.

Fig. 1.



Salinomycin R = H $C_{42}H_{70}O_{11}$
Narasin R = CH_3 $C_{43}H_{72}O_{11}$

Isolation

A flow diagram for the isolation of narasin, narasin B, and narasin methyl ester from broth filtrate and mycelial filter cake is given in Fig. 2. An alternate method utilizing the insolubility of narasin in water is presented in Fig. 3. Narasin and narasin B are obtained as crystalline acids by both procedures.

The factors present in the crude narasin preparations obtained from either procedure were separated by silica-gel chromatography. Crude narasin was dissolved in benzene and applied to a silica gel (Grace-Davison, Grade 62) column packed in benzene. A benzene wash removed inactive oils, and elution with benzene - ethyl acetate (9: 1) eluted narasin methyl ester and narasin B in separate fractions. Narasin was eluted with benzene - ethyl acetate (4: 1). The elution of factors was monitored by TLC using vanillin-H_2SO_4 spray (see Table 1). The fractions containing only narasin methyl ester and narasin B, respectively, were combined and concentrated to an oily residue which was dissolved in acetone; water was added to effect crystallization. The crystals were recovered by filtration and dried *in vacuo* to yield narasin methyl ester (mp 160~162°C) and narasin B free acid (mp 150~153°C). The fractions containing narasin were concentrated to a residue which was dissolved in acetone. An equal volume of water was added, the pH of the mixture was adjusted to

Fig. 2. Isolation and purification of narasin

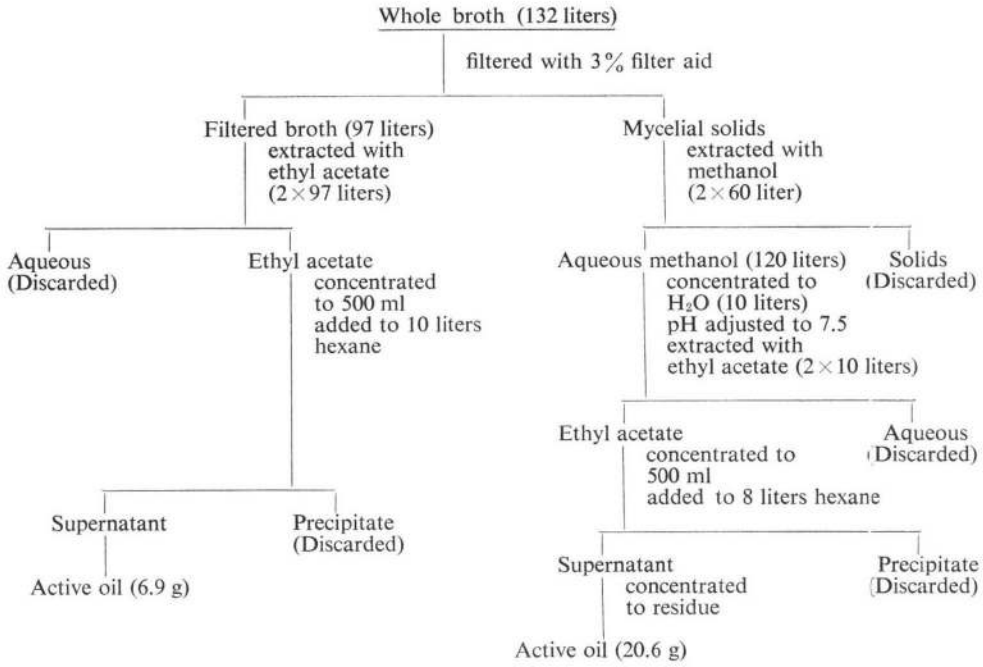


Fig. 3. Isolation and purification of narasin

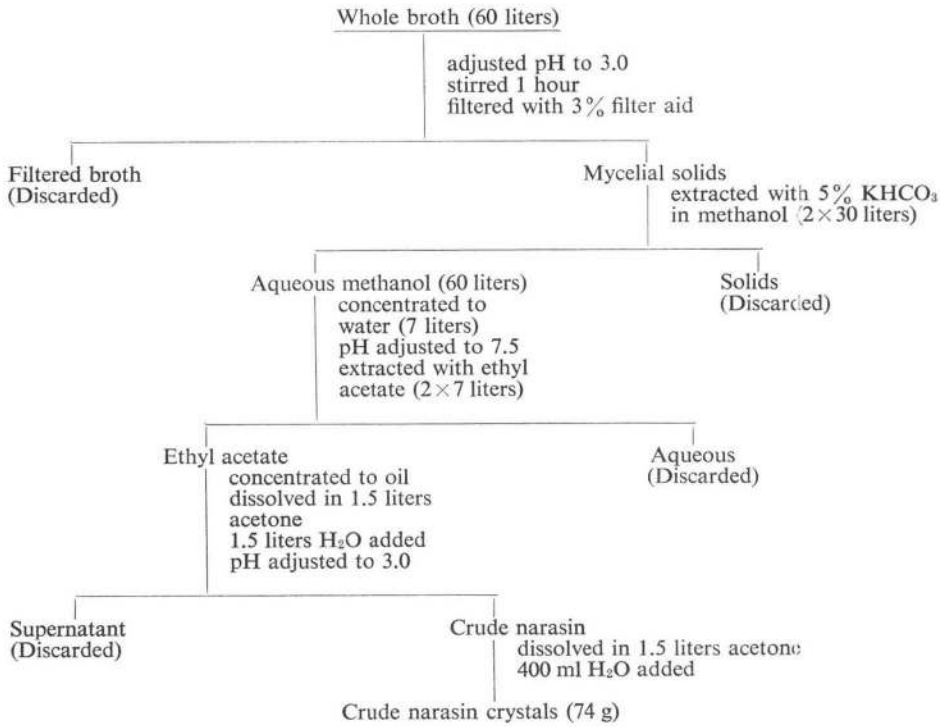


Table 1. Paper and thin-layer chromatography of narasin

Paper chromatography

System	Rf*		
	Narasin	Narasin B	Salinomycin
H_2O satd with methyl isobutyl ketone	0.11	0.09	0.32
H_2O satd with methyl isobutyl ketone plus 2% *p*-toluenesulfonic acid and 1% piperidine	0.41	0.16	0.57
Benzene satd with H_2O	0.24	0.51	0.31
17.4 g K_2HPO_4 plus 30 ml ethanol in 1 liter H_2O	0.15	0.33	0.32

Thin-layer chromatography

Support	System	Rf			
		Narasin*	Narasin B*	Narasin methyl ester**	Salinomycin*
cellulose	Water saturated with methyl isobutyl ketone	0.18	0.06	—	0.55
cellulose	Water - methanol - acetone (12: 3: 1) solution is adjusted to pH 10.3 with NH_4OH, then to pH 7.5 with HCl	0.25	0.31	—	0.53
silica gel	Ethyl acetate	0.19	0.35	0.54	0.24
silica gel	Ethyl acetate - diethylamine (95: 5)	0.34	0.20	0.66	0.43

* Bioautography using *Bacillus subtilis* ATCC nutrient agar, pH 6.8

** 3% Vanillin - 0.5% H_2SO_4 spray, 100°C

3 with dilute HCl, and crystallization was allowed to occur. The crystals were collected and recrystallized from acetone - water (2: 1) (mp 98~100°C, remelts at 195~200°C).

Narasin, narasin B, and narasin methyl ester are readily separated by thin-layer and paper chromatography as shown in Table 1.

Derivatives

The sodium salt of narasin was prepared by dissolving narasin in dioxane (25 mg/ml), adding a volume of 2.5 N NaOH and stirring for 2 hours. One volume each of water and ethyl acetate were mixed with the solution and the organic phase was separated and dried over Na_2SO_4. The organic phase was concentrated *in vacuo* to an oil which was dissolved in 1/10 volume of hot acetonitrile. The solution was cooled to 5°C and crystalline narasin sodium salt was recovered (mp 158~160°C).

Narasin reacts with a number of acid anhydrides in pyridine to form the appropriate esters. Examples of the crystalline esters prepared are: acetyl (mp 100~103°C), *n*-propionyl (mp 96~98°C), *n*-butyryl (mp 79~81°C), *n*-valeryl (mp 173~175°C), and *n*-caproyl (163~167°C). The structures of these derivatives were confirmed by ^{1}H-NMR spectra, IR spectra, and mass spectrometry.

Characterization

Physico-chemical properties of narasin are listed in Table 2. The IR spectrum of narasin in chloroform, shown in Fig. 4, indicates the presence of –OH (3500 cm^{-1}), –C–H (3000 cm^{-1}, 1460 cm^{-1}), C=O (1710 cm^{-1}), and a carboxyl C=O (1685 cm^{-1}, shoulder). The 100 MHz ^{1}H-NMR spectrum of

narasin in $CDCl_3$ is shown in Fig. 5. The resonance at 6.0 ppm, unique to narasin and salinomycin among the polyether antibiotics, is attributed to the allylic protons in the middle ring of the tricyclic spiroketal. The peak represents the two center peaks of the AB portion of an ABX system with the two outside peaks being observable in a 220 MHz spectrum. Narasin B, $C_{43}H_{70}O_{11}$, has a keto function in place of the hydroxyl group on the middle ring; mp 150~153°C; UV λ_{max}^{EtOH} 220 nm (ε=10,477). In addition to the absorptions for narasin A indicated above, the infrared spectrum of narasin B indicates a third C=O (1740 cm^{-1}). The nmr spectrum of narasin B lacks the peak at δ 6.0; the allylic protons are represented by four peaks (δ 6.15, 6.25, 7.09, 7.20; J_{AB}=11 Hz).

Table 2. Physical properties of narasin

Empirical formula	$C_{43}H_{72}O_{11}$
Elemental analysis	Obs. C, 65.69; H, 9.85; O, 23.10
	Calc. C, 67.51; H, 9.49; O, 23.00
Molecular weight (EIMS)	764
Ultraviolet maximum	285 nm, ε=58
Titration	pKa=7.9 (80% DMF)
Optical rotation	$[\alpha]_D^{25}$ −54° (*c* 0.2, MeOH)
Melting point	98~100°C, remelt
	198~200°C

Soluble in alcohols, acetone, chloroform, ethyl acetate. Insoluble in water.

Fig. 4. Infrared spectrum of narasin in $CHCl_3$

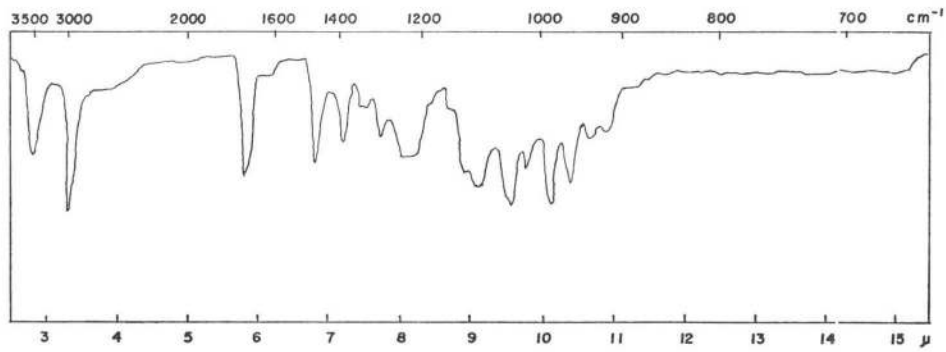
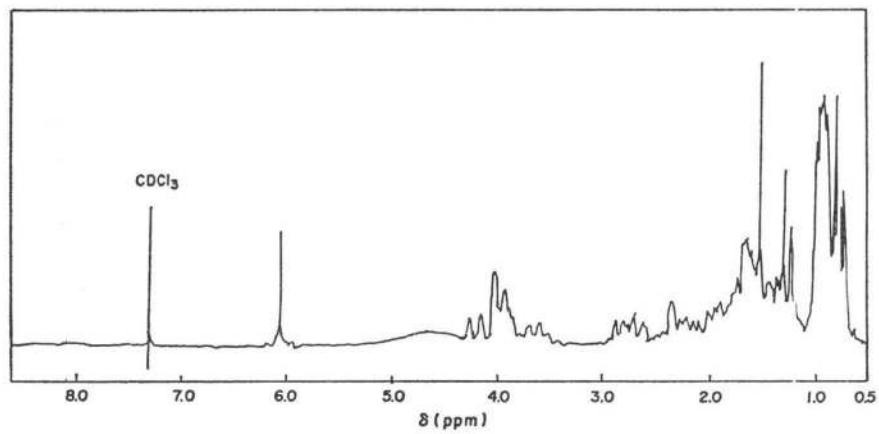


Fig. 5. 100 MHz NMR spectrum of narasin in $CDCl_3$



The physical-chemical properties of the isolated narasin methyl ester and the narasin methyl ester prepared by reacting narasin and diazomethane in ether were identical.

Biological Properties

Narasin is active *in vitro* against gram-positive bacteria, anaerobic bacteria, and fungi-(Table 3). Narasin B possesses similar biological activity and narasin methyl ester is inactive. The acyl esters of narasin are more active than narasin in a turbidometric assay using *Staphylococcus aureus*; the activity increases with increasing chain length.

When administered in the feed to chickens, narasin provides a high degree of protection against coccidial infections and does not depress weight gain (Table 4). The antibiotic increases feed efficiency in ruminants. Narasin is active against a number of viruses including vaccinia virus, herpes virus, type III poliovirus, transmissible gastroenteritis, Newcastle disease virus, and infectious bovine rhinotracheitis virus[11].

The (ip) LD_{50} for narasin in mice is ~7 mg/kg. The subcellular effects of narasin on ion transport have been reported by WONG *et al.*[12]

Table 3. Biological properties of narasin

Organism	MIC (μg/ml)
Staphylococcus aureus	100
Streptococcus faecalis	100
Candida tropicalis	100
Trichophyton mentagrophytes	12.5
Ceratocystis ulmi	50
Mycoplasma gallisepticum	12.5
Mycoplasma hyorhinis	12.5
Mycoplasma synoviae	6.25
Mycoplasma hyopneumoniae	6.25
Mycoplasma hyosynoviae	6.25
Anaerobic organisms	
Actinomyces bovis	<0.5
Clostridium innocuum	<0.5
Clostridium perfringens	<0.5
Eubacterium aerofaciens	1.0
Peptococcus anaerobius	<0.5
Propionibacterium acenes	2.0
Fusobacterium symbiosum	<0.5
Bacteroides fragilis	8

Narasin also exhibits antiviral activity against transmissible gastroenteritis, Newcastle disease, and infectious bovine rhinotracheitis viruses.

LD_{50} (i.p., mouse): 7 mg/kg

Table 4. Effect of narasin on coccidial* infestation of chickens

Treatment	ppm	No. birds	% Mortality**	% Wt. gain***
Normal controls	0	20	0	100
Infected controls	0	20	25	65
Narasin	100	20	0	89
Narasin	80	20	0	95
Narasin	60	20	0	92
Narasin	40	20	0	86

* *Eimeria tenella*

** % mortality due to coccidiosis

*** For survivors. Weight gain for narasin-treated birds is not significantly different from normal controls.

References

1) BOECK, L. D.; M. M. HOEHN, R. E. KASTNER, R. W. WETZEL, N. E. DAVIS & J. E. WESTHEAD: Narasin, a new polyether antibiotic: discovery and fermentation studies. Develop. in Industr. Microbiol. 18: 471~485, 1977

2) (a) OCCOLOWITZ, J. L.; D. H. BERG, M. DEBONO & R. L. HAMILL: The structure of narasin and a

related ionophore. Biomed. Mass Spectrom. 3: 272~277, 1976
(b) SETO, H.; T. YAHAGI, Y. MIYAZAKI & N. ŌTAKE: Studies on the ionophorous antibiotics. IX. The structure of 4-methylsalinomycin (narasin). J. Antibiotics 30: 530~532 1977

3) DORMAN, D. E.; J. W. PASCHAL, W. M. NAKATSUKASA, L. L. HUCKSTEP & N. NEUSS: The use of ^{13}C-NMR spectroscopy in biosynthetic studies. II. Biosynthesis of narasin, a new polyether ionophore from fermentation of *Streptomyces aureofaciens*. Helv. Chim. Acta 59: 2625~2634, 1976
4) HANEY, M. E. & M. M. HOEHN: Monensin, a new biologically active compound. I. Discovery and isolation. Antimicr. Agents & Chemoth.-1967: 349~352, 1968
5) HAMILL, R. L.; M. M. HOEHN & M. GORMAN: A204, a new biologically active compound. I. Discovery and isolation. Abst. 15, 10th Intersci. Conf. Antimicr. Agents & Chemoth. (Oct. 18~21, 1970, Chicago).
6) BERGER, J.; A. I. RACHLIN, W. E. SCOTT, L. H. STERNBACH & M. W. GOLDBERG: The isolation of three new crystalline antibiotics from *Streptomyces*. J. Am. Chem. Soc. 73: 5295~5298, 1951
7) MIYAZAKI, Y.; M. SHIBUYA, H. SUGAWARA, O. KAWAGUCHI, C. HIROSE, J. NAGATSU & S. ESUMI: Salinomycin, a new polyether antibiotic. J. Antibiotics 27: 814~821, 1974
8) HAMILL, R. L.; M. M. HOEHN, G. E. PITTENGER, J. CHAMBERLIN & M. GORMAN: Dianemycin, an antibiotic of the group affecting ion transport. J. Antibiotics 22: 161~164, 1969
9) HARNED, R. L.; P. H. HIDY, C. J. CORUM & K. L. JONES: Nigericin, a new crystalline antibiotic from an unidentified *Streptomyces*. Antibiot. & Chemoth. 1: 594~596, 1951
10) ŌMURA, S.; M. SHIBATA, S. MACHIDA & J. SAWADA: Isolation of a new polyether antibiotic, lonomycin. J. Antibiotics 29: 15~20, 1976
11) GALE, C. & L. R. MCDOUGALD: Antiviral methods in animals. U. S. Patent 3,995,027, 1976
12) WONG, D. T.; D. H. BERG, R. L. HAMILL & J. R. WILKINSON: The ionophorous properties of narasin, a new polyether monocarboxylic acid antibiotic, in rat liver mitochondria. Biochem. Pharmacol. 26: 1373~1376, 1977