Communications to the editor

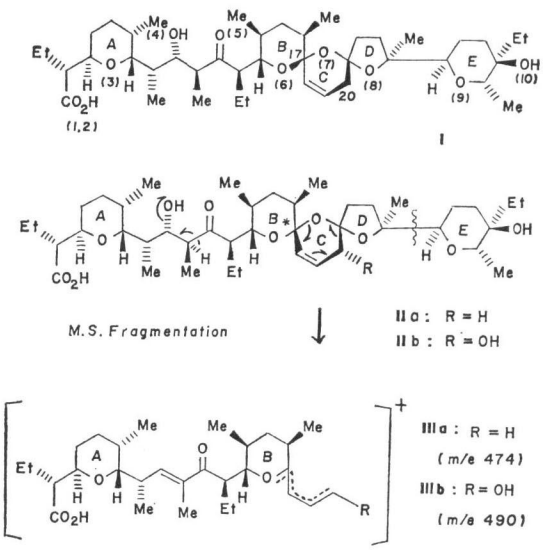
C-17 EPIMERS OF DEOXY-(O-8)-SALINOMYCIN FROM *STREPTOMYCES ALBUS* (ATCC 21838)

Sir:

Salinomycin[1] is a member of the polyether family of antibiotics[2] which include nigericin[3], lasalocid[4], antibiotic X-206[5] and monensin[6]. The salinomycin-producing organism was identified as *Streptomyces albus* (ROSSI-DORA) WAKSMAN and HENRICI and deposited with ATCC.[7] In this paper, we describe the isolation and characterization of two novel polyether antibiotics from the same salinomycin-producing culture of *S. albus* (ATCC 21838).

Using a different culture medium[8] from that described for salinomycin[7], the major product isolated in crystalline form was shown by X-ray analysis (see Fig. 1) of the free acid to be deoxy-(O-8)[9]-*epi*-17-salinomycin (**I**), m.p. 180°C, $[\alpha]_D$ −59° (*c* 1, $CHCl_3$); ν_{max} (KBr) 970, 1030, 1115 (C-O-C), 1710(CO_2H), 3420, 3530 cm^{-1} (OH); λ_{max} (EtOH) 280 nm (ε74); n.m.r. ($CDCl_3$) at δ5.4 (H, d of d, *cis* CH=CH, *J*=10.5 Hz) and 5.85 (H,m, *cis* CH=CH). Mass spectrometry of **I** gave a molecular ion at *m/e* 734 consistent with a formula of $C_{42}H_{70}O_{10}$. Other major peaks at *m/e* 716 and 698 are the result of dehydrations and a small peak at *m/e* 591 probably arises from loss of the *E* ring. The base peak at *m/e* 474 (**IIIa**, see Scheme) is the most useful in distinguishing **I** from salinomycin (**IIb**) which has a molecular formula $C_{42}H_{70}O_{11}$. Mass spectrometry of an authentic sample of **IIb**[10] gave a base peak at *m/e* 490 (**IIIb**) suggesting the extra oxygen present in **IIb** is contained in this principal fragment. The mechanism proposed in the Scheme consists of a dehydration and cleavage of the *C* ring resulting in the loss of rings *D* and *E*.

Scheme 1.



Crystals of **I** ($C_{42}H_{70}O_{10}$, M=735.01) are orthorhombic, space group $P2_12_12_1$, with *a*= 7.206(4), *b*=23.708(7), *c*=25.342(7) Å, and d_{calcd} = 1.127 g cm^{-3} for z=4. The intensity data were measured on a Hilger-Watts four-circle diffractometer (θ-2θ scans, Ni-filtered CuKα radiation). Of the 4985 accessible reflections for $\theta < 76°$, 3399 were considered observed [$I > 2.5\ \sigma(I)$].

The structure was solved by a multiple solution procedure.[11] The absolute stereochemistry of **IIb** was assumed for **I** [C(17) excepted]. Block diagonal least squares, in which the matrix was partitioned into four blocks, was used for the final refinement. The hydrogen atoms were included at their calculated positions but were not refined. The final discrepancy indices are R= 0.049 and wR=0.047 (hydrogens isotropic, heavier atoms anisotropic).

A second, minor product was also isolated in approximately one-tenth the yield of **I** as an

Fig. 1.

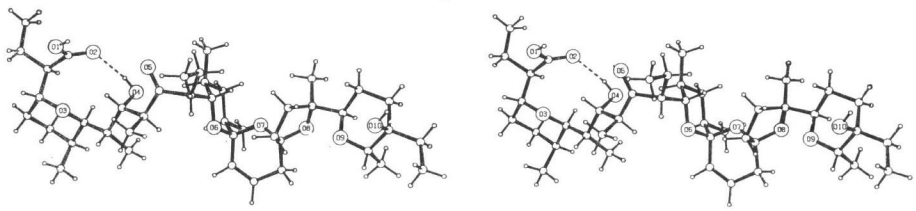


Table 1. *In vitro* antimicrobial spectra of salinomycin and related compounds (M.I.C. values in mcg/ml) using the agar diffusion well technique

Name of organism	ATCC number	Compounds		
		I[a]	IIa[a]	IIb[a]
Sarcina lutea	9341	25	12.5	3.13
Bacillus megaterium	8011	6.25	3.13	0.79
Bacillus subtilis	558[b]	6.25	6.25	0.79
Staphylococcus albus	6538P	>25	>25	3.13
Bacillus TA	27860	6.25	6.25	1.57
Mycobacterium phlei	355	500	250	6.25
Actinomyces cellulosae	3313	250	62.5	3.13
Paecilomyces varioti	26820	15.7	31.3	0.39
Candida albicans	155	2000	2000	>25
Bacillus E	27859	6.25	—	0.2

a: I = Deoxy-(O-8)-*epi*-17-salinomycin.
IIa = Deoxy-(O-8)-salinomycin.
IIb = Salinomycin.
b: NRRL Culture

amorphous powder. N.m.r. and mass spectral studies were consistent with this compound being deoxy-(O-8)-salinomycin (**IIa**), $[\alpha]_D - 19.4°$ (*c* l, $CHCl_3$); n.m.r. ($CDCl_3$) at δ 0.7, 1.5 (many C-methyl groups), δ 6.06 (2H, s, *cis* CH=CH) which was very similar to the reported[7] n.m.r. spectrum of salinomycin (**IIb**). In contrast, the mass spectrum of **IIa** was almost identical to that of **I**, with a molecular ion at *m/e* 734 ($C_{42}H_{70}O_{10}$) and the same fragment ions including *m/e* 474 (**IIIa**) showing **IIa** to be an epimer of **I** with the same stereochemistry as **IIb** at C-17.

The structures of the two epimers, **I** and **IIa** are both lacking the allylic hydroxyl group present in **IIb** at C-20, suggesting that the cyclization mechanism leading to rings *B* and *C*, which probably occurs late in the biosynthesis, takes place to give **I** and **IIa** before oxidation at C-20 has occurred. No evidence of *epi*-17-salinomycin was found in the cultures which indicates that the allylic hydroxyl group at C-20 plays a role in directing the enzymatic cyclization to give only the sterically less favored epimer, **IIb**. An alternative explanation is that the oxidation occurs after cyclization, but that only **IIa** is affected by a substrate-specific enzyme in *S. albus* that leaves **I** unchanged.

The antimicrobial spectra are shown in Table 1.

JOHN W. WESTLEY
JOHN F. BLOUNT
RALPH H. EVANS, Jr.
CHAO-MIN LIU

Chemical Research Development
Hoffmann-La Roche Inc.
Nutley, New Jersey 07110
U.S.A.

(Received April 18, 1977)

References

1) KINASHI, H.; N. ŌTAKE, H. YONEHARA, S. SATO & Y. SAITO: The structure of salinomycin, a new member of the polyether antibiotics. Tetrahedron Lett. 1973: 4955~4958, 1973
2) WESTLEY, J. W.: The polyether antibiotics. Monocarboxylic acid ionophores. Ann. Rep. Med. Chem. 10: 246~256, 1975
3) STEINRAUF, L. K.; M. PINKERTON & J. W. CHAMBERLIN: The structure of nigericin. Biochem. Biophys. Res. Commun. 33: 29~31, 1968
4) WESTLEY, J. W.; R. H. EVANS, Jr., T. WILLIAMS & A. STEMPEL: Structure of antibiotic X-537A. Chem. Commun. 1970: 71~72, 1970
5) BLOUNT, J. F.; R. H. EVANS, Jr., C.-M. LIU, T. HERMANN & J. W. WESTLEY: X-Ray structure of Ro 21-6150; a polyether antibiotic related to dianemycin. Chem. Commun. 1975: 853~855, 1975
6) AGTARAP, A.; J. W. CHAMBERLIN, M. PINKERTON & L. K. STEINRAUF: The structure of monensic acid, a new biologically active compound. J. Amer. Chem. Soc. 89: 5737~5739, 1967
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) Details of the microbiology will be published

elsewhere.

9) The numbering systems for carbon and oxygen are according to the proposal of J. W. WESTLEY. J. Antibiotics 29: 584~586, 1976

10) A gift kindly provided by the Institute of Applied Microbiology, University of Tokyo, Japan

11) GERMAIN, G.; P. MAIN & M. M. WOOLFSON: The Application of phase relationships to complex structure. III. The optimum use of phase relationships. Acta Cryst. A27: 368~376, 1971