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STUDIES ON PHOSPHONIC ACID ANTIBIOTICS. I.
STRUCTURE AND SYNTHESIS OF 3-(N-ACETYL-N-HYDROXYAMINO)PROPYLPHOSPHONIC ACID (FR-900098) AND ITS N-FORMYL ANALOGUE (FR-31564)

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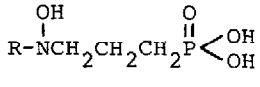
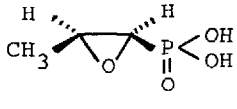
Summary: The structure elucidation of FR-900098 (1) isolated from a microorganism source and the syntheses of FR-900098 and its N-formyl analogue, FR-31564 (2), are described. The latter possesses a superior antimicrobial activity.

A group of microbial metabolites containing the phosphonic acid function has been received a current attention, because of their unique biological properties. As part of our continuing program directed toward the discovery of new antibiotics, we have now synthesized 3-(N-acetyl-N-hydroxyamino)propylphosphonic acid (FR-900098) and its N-formyl congener (FR-31564). The former (1) has been recently isolated from a microorganism source during the course of a screening program for cell wall biosynthesis inhibitors in our laboratories[1], while the latter (2) has been first designed on the basis of a biogenetic consideration as part of the chemical modification of this class of antibiotics and more recently



1 R=$COCH_3$
2 R=CHO
3 R=H



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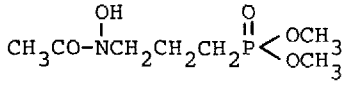

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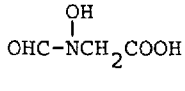


ii

shown to be also present in nature[2]. This latter antibiotic (2) has a superior and promising activity[3] against gram-negative bacteria including Pseudomonas species[4]. In this communication, we wish to report the structure elucidation of FR-900098 and syntheses of FR-900098 and FR-31564.

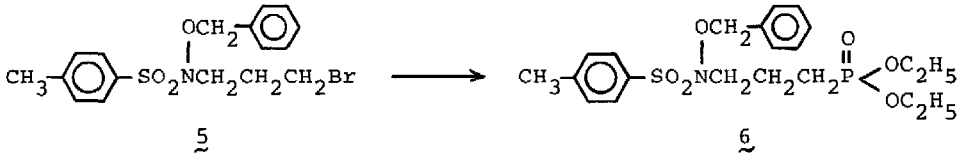
Antibiotic FR-900098 (1), $C_5H_{11}O_5NPNa$ (Na salt), mp. 193-194°(dec), was isolated from the fermentation broth of Streptomyces rubellomurinus. Methylation of 1 (free acid) with CH_2N_2 gave dimethyl ester 4 (positive $FeCl_3$ test), whose mass spectrum showed the molecular ion peak at m/e 225.0760 (Calcd. 225.0766), establishing the molecular formula of 1.

Potentiometric titration (pKa 2.0, 7.2, 9.5) and color tests (positive reaction to phosphomolybdate and $FeCl_3$ reagents) suggested that 1 contains phosphonic acid and hydroxamic acid functions. The phosphonic acid function was corroborated by the following characteristic NMR coupling data[6]: in the ^1H NMR spectrum ($CDCl_3$) of 4, the methyl signal (6H) appeared as a doublet (J=10 Hz) at δ 3.70; the proton-decoupled ^{13}C NMR spectrum (D_2O) of 1 showed a doublet (J= 207.5 Hz) attributed to the C-1 carbon at δ 24.32, while the C-2 and C-3 carbons appeared as doublets (J=3.1 and 18.3 Hz) at δ 21.47 and 54.44, respectively.

The ^1H NMR spectrum (D_2O) of 1 showed, at δ 2.16, an acetyl signal, which in the spectrum of 4, appeared at δ 2.13. This acetyl group was readily hydrolyzed by treatment of 1 with 1N HCl (reflux, 1 hr) to give hydroxylamine 3 [mp. 160-6° (dec), positive triphenyltetrazolium reaction]. These data clearly indicated the presence of the acetyl hydroxamic acid function. The structure of FR-900098 was hence deduced to be 3-(N-acetyl-N-hydroxyamino)propylphosphonic acid (1).

For confirmation of this structure, 1 was synthesized, as follows, starting from 3-(N-tosyl-N-benzyloxyamino)propyl bromide (5)[7]. Michaelis-Becker reaction of 5 with sodium diethylphosphonate in benzene (reflux, 5 hr) gave the condensation product 6 (oil), which was then hydrolyzed with conc. HCl-AcOH (1:2) (reflux, 50 hr) to give in 63 % overall yield hydroxylamine 3, identical with the sample derived from the natural product. The final acetylation of 3 with Ac_2O/ H_2O (room temp, 1 hr) gave 83 % yield of 1. This synthetic sample was proved to be identical with the naturally occurring material, establishing the structure of

FR-900098 as being 1.



5 6

→ 3 → 1 and 2

The structural feature of this antibiotic is a new addition to the naturally occurring products bearing the hydroxamic acid or phosphonic acid functions. From a viewpoint of biogenesis of these metabolites, we anticipated that some congeners of 1 might be present in the culture broth of the same or other strain of organisms. In particular, we were interested in the acyl analogues in relation to the biosynthesis of hadacidin (ii)[10], formyl hydroxamic acid antibiotic, having a unique biological property. Thus, we prepared the formyl derivative, FR-31564 (2). Acylation of 3 with acetic-formic anhydride (room temp, 30 min) gave 70 % yield of 2 [mono Na salt, mp. 189-191°(dec)][11].

This antibiotic FR-31564, which has been very recently found to be produced by a Streptomyces[2], is active against a variety of gram-negative bacteria and shows an especially high potency against Pseudomonas species[2,3] while the parent product FR-900098 is substantially less active[1]. It should be noted that the activity of FR-31564 is more significant in vivo than in vitro and also orally effective[3].

References and Notes

1. M. Okuhara, Y. Kuroda, T. Goto, M. Okamoto, H. Terano, E. Iguchi, M. Kohsaka, H. Aoki, and H. Imanaka, J. Antibiot., submitted for publication.
2. (a) M. Okuhara, Y. Kuroda, Y. Goto, M. Okamoto, H. Terano, M. Kohsaka, H. Aoki, and H. Imanaka, J. Antibiot., submitted for publication. (b) Y. Kuroda, M. Okuhara, T. Goto, M. Okamoto, H. Terano, M. Kohsaka, H. Aoki, and H. Imanaka, J. Antibiot., submitted for publication.
3. (a) Y. Mine, T. Kamimura, S. Nonoyama, and M. Nishida, J. Antibiot., submitt-

ed for publication; (b) H. Kojo, Y. Shigi, and M. Nishida, J. Antibiot., submitted for publication; (c) T. Murakawa, H. Sakamoto, S. Fukada, T. Hirose, S. Nakamoto, and M. Nishida, J. Antibiot., submitted for publication

4. Antibiotic FR-31564 has a superior potency especially against e.g. Pseudomonas species as compared with fosfomycin (i)[5], a representative of the class of phosphonic acid antibiotics.
5. (a) D. Hendelin, E.O. Stapley, M. Jackson, H. Wallick, A.K. Miller, F.J. Wolf, T.W. Miller, L. Chaiet, F.M. Kahan, E.L. Foltz, H.B. Woodruff, J.M. Mata, S. Hernandoz, and S. Mochales, Science, 166, 122 (1969); (b) B.G. Christensen, W.J. Jeanza, T.R. Beattie, A.A. Ratchett, B.H. Arison, R.E. Ormond, F.R. Kuehl Jr., G. Albers-Schonberg, and O. Jardetzky, Science, 166, 123 (1969); (c) E.J. Glamkowski, G. Gal, R. Purick, A.J. Davidson and M. Sletzinger, J. Org. Chem., 35, 3510 (1970).
6. For the proton-phosphorus and carbon-phosphorus coupling data, see e.g. (a) L.M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", Pergamon Press, 1969, pp 353-356; (b) J.B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, 1972, pp 400-407.
7. Y. Isowa, T. Takashima, M. Ohmori, H. Kurita, M. Sato, and K. Mori, Bull. Chem. Soc. Japan, 45, 1461 (1972).
8. As will be discussed in the following paper of this series, we speculated that the origin of the 3-(N-hydroxyamino)propylphosphonic acid skeleton is 3-phosphonopyruvic acid as in the case of the fosfomycin biosynthesis[9]. Based on this speculation, we have also synthesized some analogues with other carbon skeletons. This will also be the subject in the following paper.
9. T.O. Rogers and J. Birnbaum, Antimicrob. Ag. Chemother. 5, 121 (1974).
10. R.L. Stevens and T.F. Emery, Biochemistry, 5, 74 (1966).
11. In the ^{1}H NMR spectrum (D_2O) of 2, the formyl proton was doubled in a ratio of 4 : 1 at δ 8.00 and 8.35 presumably due to a rotational restriction of the formyl group. At 90°C, this pair of signals coalesced to a singlet at δ 8.16.

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