A New Antibiotic. Furanomycin, an Isoleucine Antagonist

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Received November 15, 1966
Revised Manuscript Received August 9, 1967

An antibiotic, a neutral amino acid analog, was isolated from a culture filtrate of *Streptomyces* L-803 (ATCC 15795) and designated furanomycin. A competitive reversal of furanomycin inhibition by isoleucine is observed in *Escherichia coli* with an inhibition index of 10 over a 100-fold range of increasing substrate concentrations. Furanomycin was found to inhibit the growth of T-even coliphage. On the basis of chemical and physical evidence, the structure of furanomycin was determined to be (+)-α(*R*)-amino(2,5-dihydro-5(*R*)-methyl)-furan-2(*R*)-acetic acid (XV) or one of its diastereoisomers (XVII).

In a screening program for new antibiotics, it was observed that culture filtrates of *Streptomyces* L-803 (ATCC 15795) which resembles *Streptomyces flaveolus* inhibited the growth of coliphage T2. An active principle, designated as furanomycin, was extracted from the fermentation liquor, was purified by chromatography, and showed higher inhibition against certain microorganisms on a synthetic medium than on a nutrient agar. Further studies of the nature of inhibition revealed that its activity was antagonized by L-isoleucine.

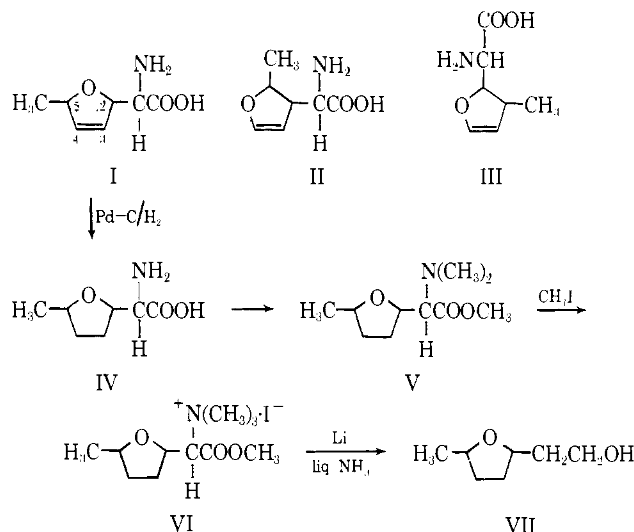
Structure of Furanomycin.—Furanomycin, $C_7H_{11}NO_3$, which decolorized an aqueous potassium permanganate solution and bromine, shows an absorption maximum at 196 mμ (ϵ 6300) in its ultraviolet spectrum. Hydrogenation of furanomycin on Pd–C gave dihydrofuranomycin, $C_7H_{13}NO_3$. These facts prove the presence of a double bond in furanomycin.

Furanomycin and dihydrofuranomycin gave a reddish orange ninhydrin reaction. The infrared spectra of furanomycin and dihydrofuranomycin shown in Figure 1 exhibit typical bands characteristic of α-amino acids (see Experimental Section). Although the C=C stretching band is obscured in the infrared spectrum of furanomycin, a band at 683 cm^{-1} can be ascribed to the bending absorption band arising from *cis*-disubstituted isolated double bond. The hydrochloride of furanomycin, $C_7H_{11}NO_3 \cdot HCl$, also displays absorption bands characteristic of an α-amino acid hydrochloride (see Experimental Section).

The pmr spectrum of furanomycin in deuterium oxide at 100 Mc (see Figure 2) shows a doublet (J = 6.4 cps) at τ 8.77 (3 H) corresponding to a secondary methyl group, a doublet (J = 2.6 cps) at 6.18 (1 H) arising from a methine proton on the α-amino acid, a multiple quintet at 4.81 (1 H), a multiplet at 4.58 (1 H), and an AB-type quartet (J = 6.3 cps) at 4.17 and 3.84 (2 H) each of which is further split into quartets. In the spectrum of dihydrofuranomycin in D_2O at 100 Mc shown in Figure 3, the AB-type quartet at the lowest field disappears, and instead, complex multiplets (4 H) appear around τ 7.7–8.1, but a doublet (J = 6.2 cps) at 8.81 (3 H) corresponding to secondary methyl group and a doublet (J = 6.3 cps) at 6.34 (1 H) arising from the methine proton still remain. These facts demonstrate that furanomycin has a *cis*-disubstituted double bond,[1] a >$CHCH_3$ group, and a >$CHCH(NH_2)COOH$ group. If furanomycin were not an α-amino acid, two signals of protons on amino-bearing and carboxyl-bearing carbons would appear separately. As a result, the possible plain structures for furanomycin can be formulated as I–III. However, in view of the chemical shifts of the two nonolefinic methine protons in the ring, the structures II and III can readily be excluded.

In order to confirm the assumed structure I, dihydrofuranomycin methyl ester was treated with sodium borohydride in formaldehyde and methanol to give a dimethylamino derivative (V). Its methiodide (VI) was reduced with lithium in liquid ammonia so as to eliminate the dimethylamino grouping.[2] The product was an alcohol and assumed to be 5-methyltetrahydrofuran-2-ethanol (VII) (Scheme I).

SCHEME I



Compound VII was synthesized as shown in Scheme II. 1,4-Dihydroxypentane (VIII) was acetylated with 1 mole of acetic anhydride in pyridine to give a monoacetate (IX). Its tetrahydropyranyl ether (Xa) afforded an aldehyde (XI) by saponification followed by oxidation of Xb with chromium trioxide in pyridine. Reformatsky reaction of XI with methyl α-bromoacetate gave a hydroxy ester (XII), which was converted into a diol (XIII) by treatment with 80% acetic acid at room temperature. When a solution of XIII and

(1) For the coupling constant between *cis*-protons on a double bond, the value of 6.3 cps is somewhat small, but this value falls in the usual range for an *endo* double bond in a five-membered ring, as stated later. See (a) O. L. Chapman, *J. Am. Chem. Soc.*, **85**, 2014 (1963); (b) G. V. Smith and H. Kriloff, *ibid.*, **85**, 2016 (1963); (c) P. Laszlo and P. von R. Schleyer, *ibid.*, **85**, 2017 (1963).

(2) A. J. Birch and H. Smith, *Quart. Rev.* (London), **12**, 32 (1958).

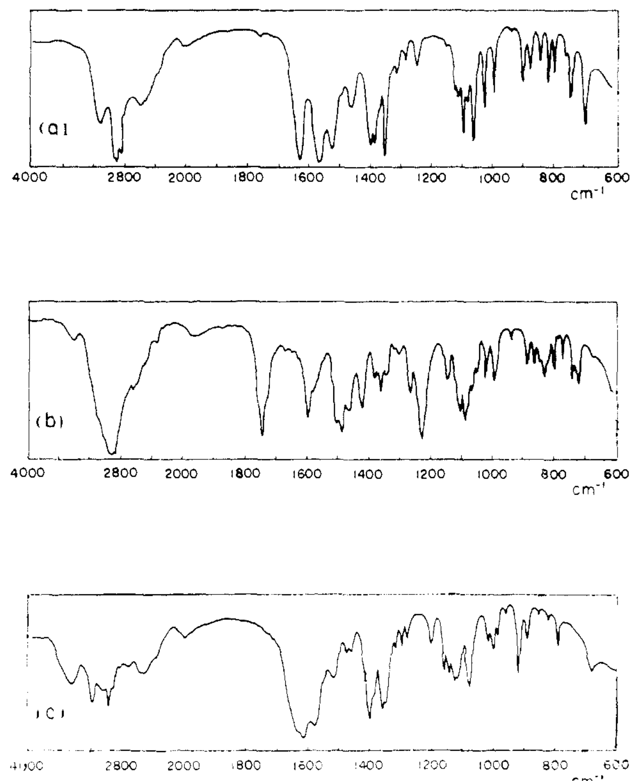
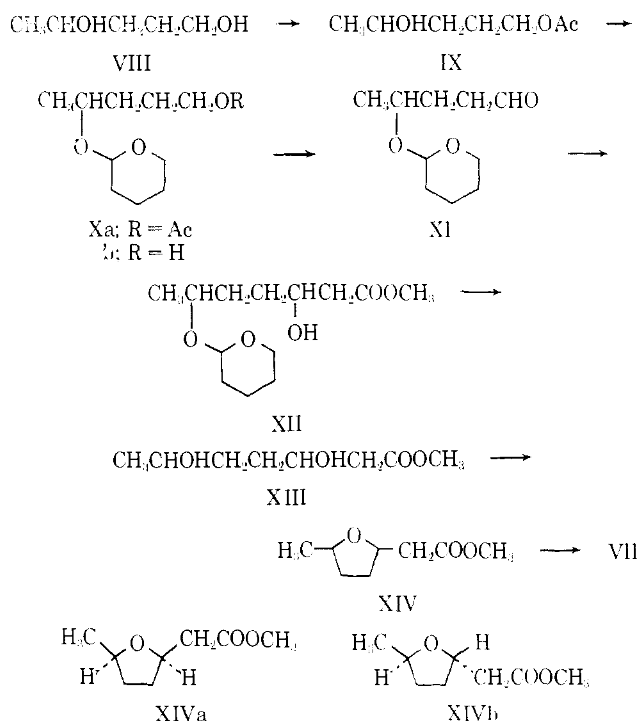


Figure 1.—Infrared absorption spectra of furanomycin (a, in Nujol), furanomycin hydrochloride (b, in Nujol), and dihydrofuranomycin (c, KBr tablet).

toluene-*p*-sulfonyl chloride (1.3 moles) in pyridine was left overnight at room temperature, methyl 5-methyltetrahydrofuran-2-acetate (XIV) was obtained. This ester showed two peaks at retention times of 8.3 and 9.5 min in a ratio of 1:1 in the gas chromatogram. Since these two peaks should correspond to a *cis* (XIVa) and a *trans* derivative (XIVb), they were separated into each compound by preparative gas chromatography. The ester having a peak at retention time of 9.5 min was reduced with lithium aluminum hydride to give 5-methyltetrahydrofuran-2-ethanol, retention time of 4.4 min, which was identical with VII derived from furanomycin by comparison with their infrared spectra and gas chromatographic retention times. Therefore, furanomycin was determined to be α-amino-(2,5-dihydro-5-methyl)furan-2-acetic acid as represented by formula I.

SCHEME II



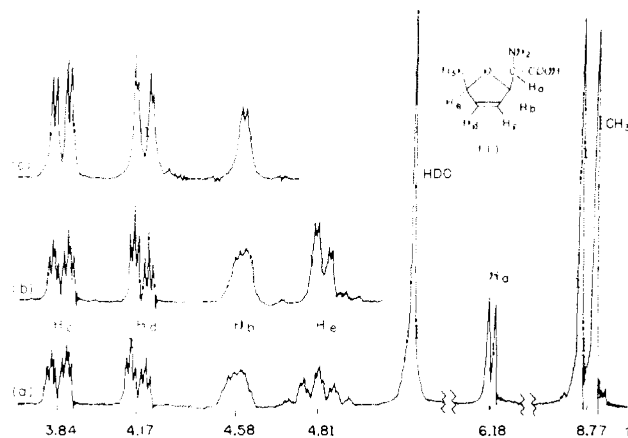


Figure 2.—Pmr spectra of furanomycin in D_2O solutions (DSS internal reference) at 100 Mc: (a) normal spectrum, (b) double-irradiated spectrum at the methyl-proton resonance frequency, (c) double-irradiated spectrum at the proton H_e resonance frequency.

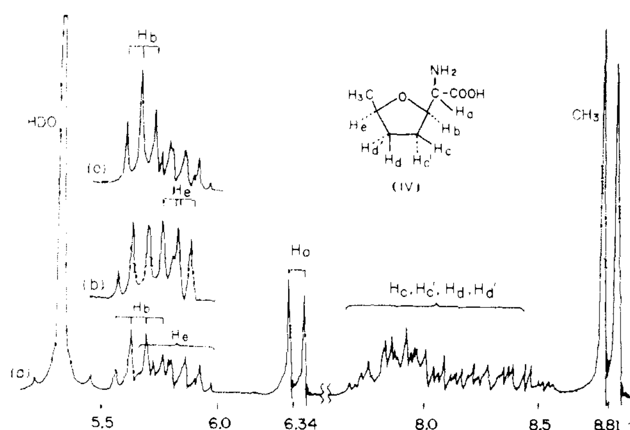


Figure 3.—Pmr spectra of dihydrofuranomycin in D_2O solutions (DSS internal reference) at 100 Mc: (a) normal spectrum, (b) double-irradiated spectrum at the methyl-proton resonance frequency, (c) double-irradiated spectrum at the proton H_b resonance frequency.

Stereochemistry of Furanomycin.—The fact that the circular dichroism spectra of I and dihydrofuranomycin (IV) show positive Cotton effects ($[\theta]_{216}$ +26,000 and $[\theta]_{215}$ +5000, respectively) in aqueous 6 *N* HCl implies that these α-amino acids belong to the L series as shown in structures I and IV.

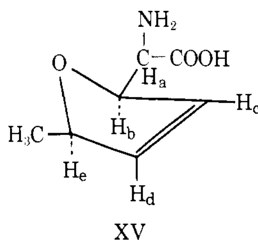
In order to confirm the assignments of the pmr signals and determine the steric structures of I and IV, we made proton spin-decoupling experiments as shown in Figures 2 and 3. As can be seen in Figure 2(b), on double irradiation at the methyl-proton resonance frequency, the multiple quintet at τ 4.81 collapses to a multiple doublet (J = 5.7 cps), and each peak of the higher field half of the AB-type quartet at τ 4.17 becomes somewhat sharper. Further, on double irradiation at the methine-proton frequency (τ 6.18)

(see Figure 2(c)), the doublet of quartets at the lowest field (τ 3.84) collapses to a quartet, the signal at τ 4.17 alters into a doublet further split into multiplets, and the broad multiplet at τ 4.58 becomes narrower. From these facts, the assignment of all the signals exhibited by I can be made as indicated in Figure 2. These spin-decoupling experiments also revealed all the coupling constants between protons of I as listed in Table I. Particularly, a large long-range coupling

TABLE I
APPARENT COUPLING CONSTANTS (J, cps) IN FURANOMYCIN

	H_d	H_c	H_b	H_a	CH_3
H_e	1.9	1.7	5.7	~0	6.4
H_d	...	6.3	1.5	~0	~0.3
H_c		...	2.1	$\ll$0.3	~0
H_b			...	2.6	~0

constant (J = 5.7 cps) was demonstrated between the protons H_b and H_e. In general, a homoallylic coupling constant, whose magnitude depends on the degree of $\sigma-\pi$ overlap and the amount of π-electron localization on the intervening double bond, falls in a range of about 1–3 cps.[3] The large value of the homoallylic coupling constant implies that the protons H_b and H_e are *cis* in relation to each other, and that the molecule I takes a boatlike envelope conformation[4] as shown in formula XV because only in this case is the degree of



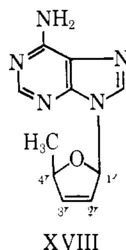
XV

$\sigma-\pi$ overlap between the π electrons and the σ bonds, C_1-H_b and C_4-H_e, extremely high. If the two substituents on the 2,5-dihydrofuran ring were *trans*, the ring should take a more flattened conformation, and, hence, the degree of $\sigma-\pi$ overlap between the π electrons and either σ bond C_1-H_b or C_4-H_e must be minimal.[5,6] This conclusion was confirmed by the spin-decoupling experiments on IV. As shown in Figure 3, on double irradiation at the methyl-proton resonance frequency,

(3) For a review, see S. Sternhell, *Rev. Pure Appl. Chem.*, **14**, 15 (1964).

(4) K. Tori, *Chem. Pharm. Bull.* (Tokyo), **12**, 1439 (1964), and references cited therein.

(5) Recently the pmr spectrum of 2′,3′,5,-trideoxy-2′,3′-didehydroadenosine (XVIII) was reported by J. R. McCarthy, Jr., M. J. Robins, L. B.

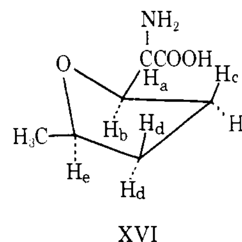


XVIII

Townsend, and R. K. Robins, *J. Am. Chem. Soc.*, **88**, 1549 (1966). The signals of protons on C-2′, C-3′, and C-4′ are quite similar to those in I.

(6) Extremely large values for long-range spin couplings have frequently been observed when there is more than one path between a pair of coupling protons. For leading references, see (a) K. Tori and M. Ohtsuru, *Chem. Commun.*, 886 (1966); (b) W. G. Woods and P. L. Strong, *J. Am. Chem. Soc.*, **88**, 4667 (1966), and references cited therein.

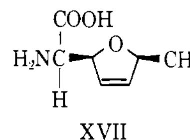
the multiplet at about τ 5.83 collapses to a quartet with the spacings of 5.8, 1.0, and 5.8 cps, whereas the quartet at τ 5.67 collapses to a triplet with the spacings of 6.6 cps by the spin decoupling of the methine proton on α-carbon of the amino acid. Thus the assignment was made as indicated in Figure 3. No long-range spin coupling between the protons H_b and H_e was discernible in IV. These results confirm that IV is a *cis*-2,5-disubstituted tetrahydrofuran formulated as XVI. If the substituents were *trans*, the signal arising from the



XVI

protons H_b or H_e should appear as a narrower triplet or quartet than those shown in Figure 3(b) or (c) according to the well-known Karplus equation[7] even used in a qualitative sense because either protons H_b or H_e might be expected to be equatorial-like.

Thus, we concluded that furanomycin is (+)-α(R)-amino(2,5-dihydro-5(R)-methyl)furan-2(R)-acetic acid or one of its diastereoisomers (XVII), but which absolute configuration this molecule has remains to be established.



XVII

Biological Activities.—Furanomycin inhibits the growth of several microorganisms when examined on a chemically defined medium by the agar streak dilution method. The results are shown in Table II. Furanomycin (100 μg/ml), however, inhibits none of these bacteria tested on nutrient agar.

TABLE II
ANTIMICROBIAL SPECTRUM OF FURANOMYCIN ON SYNTHETIC MEDIUM[a]

Test microorganism	MIC, μg/ml
Shigella paradysenteriae Komagome B_1	5
Shigella paradysenteriae Ohara	2
Salmonella paratyphi A	5
Salmonella paratyphi B	>50
Salmonella paratyphi C	>50
Escherichia coli Harshey	5
Pseudomonas aeruginosa	>50
Klebsiella pneumoniae	>50
Bacillus subtilis PCI219	1
Mycobacterium tuberculosis H37Rv	20

[a] H. Nishimura, K. Nakajima, and N. Shimaoka, *Ann. Rept. Shionogi Res. Lab.*, **5**, 560 (1955).

Antagonism.—All amino acids contained in the nutrient medium were examined for the effects of antagonizing the inhibitory activity of furanomycin. Furanomycin at a concentration of 0.5–2 μM inhibits the

(7) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959); *J. Am. Chem. Soc.*, **85**, 2870 (1963).

TABLE III

REVERSAL OF FURANOMYCIN[a] BY L-AMINO ACIDS IN *E. coli* H[b]

	Absorbances at 590 mμ						
	Concn of amino acids, μM						
Supplements	0	0.1	1	10	100	1000	10,000
Isoleucine	0.00	0.00	0.05	0.19	0.19		
Valine	0.00	0.00	0.00	0.19	0.19		
Leucine	0.00			0.00	0.19		
Threonine	0.00				0.00	0.00	0.19
Methionine					0.00	0.00	0.19

[a] Furanomycin was supplied in all cultures at a concentration of 1 μM. [b] Organism was incubated at 37° for 18 hr in glucose–inorganic salts medium, consisting of glucose (3 g), $(NH_4)_2HPO_4$ (1 g), $MgSO_4 \cdot 7H_2O$ (0.4 g) in 1 l. of distilled water (pH 7.0).

growth of *E. coli* H in the glucose–inorganic salts medium. The growth inhibition of furanomycin was found to be reversed extensively by isoleucine and valine and, to a lesser extent, by leucine, but all the others exerted virtually no reversal effect. The effect of increasing concentrations of isoleucine, valine, leucine, threonine, and methionine on the activity of furanomycin at a concentration of 1 μM is indicated in Table III. The ratio of the concentration of furanomycin to isoleucine necessary for complete inhibition of growth was about 10 at concentrations of isoleucine from 0.1 to 10 μM as can be seen in Table IV. Valine at concentrations of 0.2 and 0.5 μM is as effective as isoleucine in reversal of the inhibition; however, higher concentrations of valine fail to prevent the inhibition by furanomycin at the higher concentration (20 μM). Although valine is almost as active as isoleucine in reversing inhibitory action by furanomycin, the nature of reversal is different. From these results, furanomycin appears to be a competitive antagonist of L-isoleucine. Cyclopentaneglycine is known as an isoleucine antagonist. It was suggested that stereochemical resemblance of two groups, cyclopentyl and *sec*-butyl, is responsible for the activity.[8] Similarities in the size and shape of dihydrofuryl and cyclopentyl groups would imply that furanomycin inhibits L-isoleucine for the same reason.

TABLE IV

REVERSAL OF ANTIBACTERIAL ACTIVITY OF FURANOMYCIN BY L-ISOLEUCINE IN *E. coli* H

	Absorbances at 590 mμ									
Furanomycin, μM	Concn of L-isoleucine, μM									
	0	0.1	0.2	0.5	1	2	5	10	20	50
0	0.21	0.21	0.21	0.21	0.23	0.23	0.23	0.23	0.23	0.23
0.5	0.21	0.21	0.21							
1	0.00	0.00	0.24	0.21						
2			0.00	0.21						
5				0.05	0.23					
10					0.00	0.23				
20					0.00	0.12	0.23	0.23	0.23	
50						0.00	0.00	0.10	0.08	0.23
100								0.00	0.00	0.00
	Concn of L-valine, μM									
	0	0.1	0.2	0.5	1	2	5	10	20	50
0	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
1	0.18	0.18	0.18							
2	0.00	0.00	0.00	0.18						
5				0.00	0.18					
10				0.00	0.18					
20					0.00	0.00	0.00	0.00	0.00	0.05

Antiphage Activity.—Furanomycin at a level of 0.3 μg/ml inhibited phage production by 48% and, at a level of 1 μg/ml, by more than 90%. In neither case was free phage inactivated (Table V). When viable cell counting was made 60 min after the addition of antibiotic to growing cultures of *E. coli* H, furanomycin was found to be ineffective to the host organism at a level of 1 μg/ml. Furanomycin at a level of 1 μg/ml was used to determine the sensitivity of other types of phage. Differences in the effectiveness of furanomycin on T2, T3, T4, and T5 phages were found to be 89, 59, 95, and 43% inhibition of phage growth, respectively. Thus, T2 and T4 phages are more sensitive to furanomycin than T3 and T5.

(8) W. M. Harding and W. Shive, *J. Biol. Chem.*, **206**, 401 (1954).

Experimental Section

All melting points are corrected and were taken on a Monoscop VS Hansbock apparatus. The ultraviolet absorption spectrum of I was recorded on a Beckman DK-2 automatic recording spectrophotometer in an aqueous solution. Infrared spectra were recorded on a Nippon Bunko DS-201B spectrometer in Nujol mulls or in a KBr tablet. Pmr spectra were taken with a Varian HA-100 spectrometer operating at 100 Mc in about 5% (w/v) solutions in D_2O containing about 1% sodium dimethyl-2-silapentane-5-sulfonate (DSS) as an internal reference. Spin-decoupling experiments were performed in the frequency-swept and DSS-locked mode by using a Hewlett-Packard HP-200ABR oscillator. Circular dichroism spectra were run on a Nippon Bunko ORD/UV-5 automatic recording spectrophotopolarimeter in aqueous 6 N HCl solutions. Paper chromatography employed Tōyō Roshi No. 50 filter paper in the following systems: A, *n*-BuOH–AcOH–H_2O (4:1:6, by vol., upper phase); B, *n*-BuOH saturated with 2 N NH_4OH; C, pyridine–AcOH–H_2O (10:7:3, by vol.). Thin layer chromatography was done using Merck silica gel G with thickness of 250 μ; the solvent systems used were D, *n*-PrOH–H_2O (7:3, by vol.); E, *n*-BuOH–AcOH–H_2O,

TABLE V

PHAGE-GROWTH-INHIBITION CONCENTRATIONS OF FURANOMYCIN

Furanomycin, μg/ml	Growth of T2 phage,[a] pfu/ml	Inhib, %	Growth of *E. coli* H,[b] cfu/ml	Inhib, %
0	1.8×10^{10}		2.2×10^{9}	
0.1	1.8×10^{10}	0		
0.3	9.3×10^{9}	48		
1	2.0×10^{9}	89	2.8×10^{9}	0
3	1.6×10^{9}	91	2.6×10^{8} [c]	88
10	1.3×10^{9}	94	1.3×10^{8} [c]	94

[a] The average burst size of T2 phage was 92 at 30 min in one-step growth experiment. [b] Inoculum size of *E. coli* H was 7×10^{8} cells/ml. [c] Decrease of viable cells below inoculum.

(3:1:1, by vol.); F, $CHCl_3$-MeOH-17% NH_3 (2:2:1, by vol., upper phase). In antagonism experiment the amount of growth was determined photometrically at 590 mμ with a Coleman nephocolorimeter, Model 9.

Fermentation of *Streptomyces* L-803 (ATCC 15795).—Slant cultures were prepared on Bennet's medium (glucose, 10 g; N-Z amine A, 2 g; yeast extract, 1 g; beef extract, 1 g; agar, 20 g in H_2O (1 l.) and adjusted to pH 7.2 with 1 *N* NaOH). After incubation at 28° for 1 week, the slant cultures were used to inoculate flasks for the preparation of vegetative inoculum. After 40 hr of incubation at 28° on a reciprocatory shaker in flasks (containing the following ingredients in g/l.: starch, 20; soybean meal, 10; corn steep liquor, 5; NaCl, 5; $CaCO_3$, 3.5), a 5% inoculum was transferred into stirred-jar fermentors containing the above medium. The medium was adjusted to pH 7.0 before sterilization. These were incubated for 4–5 days and antibiotic concentration reached a maximal value of 20–35 mg/l. The concentration of furanomycin in a cultured broth was determined by the disk diffusion method with *Escherichia coli* H on a glucose–inorganic salt agar.

Purification of Furanomycin (I).—A cultured broth of furanomycin fermentation (16 l.) was adjusted to pH 3 with concentrated HCl and filtered with suction. The filtrate was decolorized with Darco-G 60 (160 g). The clear solution was passed through a column containing Amberlite IR-120, H^+ form (4 × 35 cm). After washing with distilled water, materials containing furanomycin were eluted from the resin with 0.5 *N* NH_4OH. The distribution of antibiotic was detected by bioassay and found in fractions from 600 to 900 ml. The alkaline eluates were concentrated to dryness under reduced pressure to remove NH_3. The residues were dissolved in H_2O (20 ml) and added to Me_2CO (200 ml). The precipitates were filtered and dried (500 mg). This material was dissolved in 70% *n*-PrOH-H_2O, and the solution was added to the top of a column containing 60 g of silica gel (2.8 × 20 cm) packed in the same solvent. The column was eluted with 70% *n*-PrOH, and the eluates were collected in 10-ml fractions. The distribution of furanomycin was determined by thin layer chromatography. The fractions containing furanomycin were combined and concentrated to dryness *in vacuo*. Rechromatography of this material (320 mg) was carried out by the same procedure to obtain 130 mg of crude crystals. This material was dissolved in water and allowed to stand at room temperature overnight to get colorless plates: mp 220–223° dec; $[\alpha]^{27}D$ +136.1 ± 2° (*c* 1, H_2O); $\lambda_{max}^{H_2O}$ 196 mμ (ε 6300); ν_{max}^{Nujol} 3120, 2565, 2000, 1635, 1570, 1525, 1405, 1307, 1087, 1053, 683 cm^{-1}; pK_1' = 2.4 ± 0.2 and pK_2' = 9.1 ± 0.2. Furanomycin thus purified showed a clear single spot on paper chromatography in systems A (R_f 0.35), B (R_f 0.12), and C (R_f 0.69) and on thin layer chromatography in systems D (R_f 0.41), E (R_f 0.41), and F (R_f 0.64).

Anal. Calcd for $C_7H_{11}NO_3$: C, 53.49; H, 7.05; N, 8.91. Found: C, 52.94; H, 7.17; N, 8.91, mol wt, 163 (titration method) and 164 (osmometric method).

Furanomycin hydrochloride melted at 190–192° dec, white needles (from EtOH), ν_{max}^{Nujol} 3400, 2623, 2473, 2340, 1745, 1598, 1488, 1420, 1225 cm^{-1}.

Anal. Calcd for $C_7H_{12}ClNO_3$: C, 43.41; H, 6.20; N, 7.25; Cl, 18.34. Found: C, 43.62; H, 6.38; N, 7.37; Cl, 18.19.

N-2,4-Dinitrophenylfuranomycin.—Furanomycin (100 mg) was 2,4-dinitrophenylated by the usual procedure; yield 88%. Recrystallization from EtOH-H_2O gave yellow needles, mp 159–160°.

Anal. Calcd for $C_{13}H_{13}N_3O_7$: C, 48.30; H, 4.05; N, 13.00. Found: C, 48.40; H, 4.35; N, 12.86.

Dihydrofuranomycin (IV).—Furanomycin (100 mg) was hydrogenated on 5% Pd-C in H_2O (7 ml), H_2 (17.7 ml) being absorbed. The reaction mixture was filtered, washed (H_2O), and dried to yield crude crystals (72 mg), which were recrystallized twice from EtOH-H_2O to give 22 mg of colorless needles: mp 227–229.5° dec; ν_{max}^{KBr} 3440, 3197, 2532, 1995, 1635, 1615, 1575, 1517, 1397, 1355, 1070 cm^{-1}.

Anal. Calcd for $C_7H_{13}NO_3$: C, 52.81; H, 8.23; N, 8.80. Found: C, 52.75; H, 8.22; N, 9.06.

N-Dimethyldihydrofuranomycin Methyl Ester (V).—Formaldehyde (40%, 0.5 ml) was added to a solution of dihydrofuranomycin methyl ester (130 mg) in MeOH (10 ml) with stirring, and stirring was continued for 4 hr at room temperature. $NaBH_4$ (400 mg) was added in small portions to this mixture at 10–20° in an ice bath with stirring, and stirring was continued for an additional 3 hr at the same temperature. Then, Me_2CO (3 ml) was added to this mixture to decompose excess $NaBH_4$, and the mixture was poured into ice–water and extracted with ether. The extract was washed with water, dried (Na_2SO_4), and evaporated, leaving an oily residue (130 mg), which was distilled at 140° (bath) (20 mm) to give a colorless oil (V): 100 mg; ν_{max}^{film} 1735, 1167, and 1080 cm^{-1}.

Anal. Calcd for $C_{10}H_9NO_3$: C, 59.67; H, 9.52; N, 6.96. Found: C, 59.34; H, 9.71; N, 6.70.

Conversion of V into VII.—A solution of V (90 mg) and MeI (0.5 ml) in dry MeOH (2 ml) was heated under reflux for 2 hr and the solvent was removed under reduced pressure. The residue was dissolved in water (2 ml), alkalized with 2 *N* K_2CO_3, and extracted with ether. The aqueous layer was saturated with K_2CO_3 and extracted ($CHCl_3$) to give a methiodide, a viscous oil (80 mg). The methiodide was dissolved in liquid NH_3 (15 ml), and to this solution was added Li (250 mg), with stirring for 40 min. Then, NH_4Cl (1.5 g) was added to this mixture with stirring, and stirring was continued for 2 hr. Liquid NH_3 was evaporated, and the residue was extracted with ether. The extract was washed with water, dried (Na_2SO_4), and evaporated, leaving a colorless oil (VII) (30 mg), which was distilled at 100–105° (bath) (30 mm) to give a colorless oil (VII) (19 mg): $\nu_{max}^{CHCl_3}$ 3440, 1105, 1070, 1035, 928, 870, and 836 cm^{-1}; retention time[9] 4.4 or 13.5 min.

Anal. Calcd for $C_7H_{14}O_2$: C, 64.58; H, 10.84. Found: C, 64.29; H, 10.67.

1-Acetoxy-4-hydroxypentane (IX).—A solution of Ac_2O (8.8 g, 1 equiv) in dry pyridine (35 ml) was added dropwise to a solution of 1,4-dihydroxypentane (VIII) (9.0 g) in dry $CHCl_3$ (90 ml) with stirring in an ice bath and left for 17 hr at 5°. The solution was diluted with ether (200 ml) and washed with saturated NaCl solution, dried (Na_2SO_4), and evaporated, leaving an oil (11.0 g). The residue was chromatographed on alumina to give a monoacetate (IX), 6.4 g, bp 80° (0.7 mm), ν_{max}^{film} 3500 and 1732 cm^{-1} (*Anal.* Calcd for $C_7H_{14}O_3$: C, 57.55; H, 9.65. Found: C, 57.60; H, 9.58), and a mixture of the diacetate and IX (*ca.* 4:1).

4-(1-Acetoxypentyl) 2-Tetrahydropyranyl Ether (Xa).—A solution of IX (6.3 g), dihydropyran (5.25 g), and toluene-*p*-sulfonic acid (200 mg) in dry ether (100 ml) was stirred for 4 hr at room temperature. The solution was poured into ice–water containing Na_2CO_3 and extracted with ether. The extract was washed with water, dried (Na_2SO_4), and evaporated leaving an oily residue (9.8 g), which was distilled at 85–86° (0.4 mm) to give a colorless oil (Xa): 9.55 g; ν_{max}^{film} 1741, 1242, and 1024 cm^{-1}.

Anal. Calcd for $C_{12}H_{22}O_4$: C, 62.58; H, 9.63. Found: C, 62.63; H, 9.61.

4-(1-Hydroxypentyl) 2-Tetrahydropyranyl Ether (Xb).—A solution of Xa (9.5 g) in 10% KOH in MeOH (100 ml) was heated under reflux for 1 hr. The solvent was evaporated, and the residue was extracted with ether, washed with water, dried (Na_2SO_4), and evaporated leaving an oil (7.8 g), which was distilled at 95° (0.5 mm) to give a colorless oil (Xb), 7.6 g, ν_{max}^{film} 3540 and 1024 cm^{-1}.

Anal. Calcd for $C_{10}H_{20}O_3$: C, 63.79; H, 10.71. Found: C, 63.56; H, 10.63.

γ-Valeraldehyde 2-Tetrahydropyranyl Ether (XI).—A solution of Xb (7.5 g) in dry pyridine (30 ml) was added dropwise to a mixture of CrO_3 (7.5 g) in pyridine (100 ml) with stirring in an ice bath and left overnight at room temperature. The mixture was poured onto ice–water and extracted with ether. The extract was washed with water, dried (Na_2SO_4), and evaporated, leaving an oily residue (6.9 g). The residue was chromatographed on neutral alumina (140 g) to give the starting material (Xb), 4.0 g, and an aldehyde (XI), 1.8 g, colorless oil, bp 65–70° (0.5 mm), ν_{max}^{film} 2750, 1726, and 1023 cm^{-1}.

Anal. Calcd for $C_{10}H_{18}O_3$: C, 64.49; H, 9.74. Found: C, 64.08; H, 9.67.

Reformatsky Reaction of XI with Methyl Bromoacetate.—A solution of methyl bromoacetate (4.25 g) in dry benzene (5 ml) was added dropwise to a mixture of XI (1.7 g), Zn dust (1.8 g), and one crystal of I_2 in dry C_6H_6 (25 ml) with stirring under reflux and heated under reflux for 1.5 hr with stirring. Water was added to this mixture and extracted with ether. The extract was washed with water, dried (Na_2SO_4), and evaporated, leaving a yellow mobile oil (2.9 g). The residue was chromatographed on

(9) A column, 10 ft × 3/8 in., consisting of 5% diethylene glycol succinate or 5% Carbowax on Chromosorb W (45–60 mesh) was, respectively, operated at 150° with a flow rate of 100 ml/min of He.

alumina to give a hydroxy ester (XII), 1.9 g, a colorless oil: bp 119–120° (0.3 mm); ν_{max}^{film} 3495, 1730, and 1020 cm^{-1}.

Anal. Calcd for $C_{13}H_{24}O_5$: C, 59.98; H, 9.29. Found: C, 60.29; H, 9.30.

Hydrolysis of XII.—A solution of XII (1.7 g) in 80% AcOH (10 ml) was allowed to stand for 5 hr at room temperature. The solution was poured into ice–water and extracted with ether. The extract was washed (2 *N* Na_2CO_3, H_2O), dried (Na_2SO_4), and evaporated, leaving an oily residue (1.2 g). The residue was chromatographed on alumina to give a diol ester (710 mg), which was distilled at 99–100° (0.2 mm) to give a colorless oil (XIII), 700 mg, ν_{max}^{film} 3370 and 1725 cm^{-1}.

Anal. Calcd for $C_8H_{16}O_4$: C, 54.53; H, 9.15. Found: C, 54.32; H, 9.07.

Methyl 5-Methyltetrahydrofuran-2-acetate (XIV).—Toluene-*p*-sulfonyl chloride (780 mg, 1.3 equiv) was added to a solution of XIII (600 mg) in dry pyridine (4.0 ml) with stirring in an ice bath and left overnight at room temperature. The mixture was poured onto ice–water and extracted with ether. The extract was washed (2 *N* H_2SO_4, 2 *N* Na_2CO_3, H_2O), dried (Na_2SO_4), and evaporated, leaving an oily residue (506 mg). The residue was chromatographed on alumina to give XIV, a colorless oil: 170 mg; bp 80° (30 mm); ν_{max}^{film} 1738, 1200, 1168, and 1085 cm^{-1} (*Anal.* Calcd for $C_8H_{14}O_3$: C, 60.74; H, 8.92. Found: C, 60.82; H, 8.99). This ester showed two peaks at retention times of 8.3 and 9.5 min in a ratio of 1:1 on the gas chromatogram,[10] and was separated into each compound by preparative gas chromatography, XIV having a peak at retention time of 8.3 min (colorless oil; $\nu_{max}^{CHCl_3}$ 1730, 1160, and 1079 cm^{-1}), XIV having a peak at retention time of 9.5 min (colorless oil; $\nu_{max}^{CHCl_3}$ 1730, 1158, 1070, and 1003 cm^{-1}).

5-Methyltetrahydrofuran-2-ethanol (VII).—A solution of XIV, retention time 9.5 min (15 mg), in dry ether (1 ml) was added to a suspension of $LiAlH_4$ (20 mg) in dry ether (1 ml) with stirring and stirring was continued for 3 hr at room temperature. To this mixture was added ether (3 ml) containing water and filtered. The ether solution was dried (Na_2SO_4) and evaporated, leaving an oily alcohol (7.5 mg), which was distilled at 100–105° (bath) (30 mm) to give VII, a colorless oil: $\nu_{max}^{CHCl_3}$ 3442, 1103, 1072, 1036, 929, 870, and 835 cm^{-1}; retention time[9] 4.4 or 13.5 min, which was identical with VII obtained from foramomycin by comparison with their infrared spectra and gas chromatographic retention times.

Anal. Calcd for $C_7H_{14}O_2$: C, 64.58; H, 10.84. Found: C, 64.41; H, 10.78.

XIV, retention time 8.3 min (21.2 mg), was reduced ($LiAlH_4$) under the same conditions to give 5-methyltetrahydrofuran-2-ethanol (VII) having a retention time[9] of 4.1 or 12.5 min; $\nu_{max}^{CHCl_3}$ 3440, 1063, 1030, 930, and 867 cm^{-1}.

Acknowledgment.—We are indebted to Drs. H. Otsuka and J. Shoji for their assistance in the preparation of the derivatives and Dr. K. Kuriyama for the measurements of the circular dichroism spectra. The authors also wish to express their thanks to Dr. K. Sato and his colleagues, who carried out evaluation of antiphage activity.

(10) A column, 10 ft × 3/8 in. consisting of 5% diethylene glycol succinate on Chromosorb W (45–60 mesh) was operated at 120° with a flow rate of 100 ml/min of He.

Notes

Synthesis and Evaluation of the Local Anesthetic Activity of a Series of 2-Alkoxy-4-(ω-alkylaminoacylamino)benzoic Acid Esters[1,2]

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Received April 29, 1967

In a previous communication,[3] the synthesis and evaluation of the local anesthetic activity of a series of 4-(ω-alkylaminoacylamino)salicylic acid esters was reported. Compared to lidocaine, these compounds were generally more irritating, less toxic, and less active. Those compounds which had local anesthetic activity approaching that of lidocaine were extremely irritating. Einhorn and Oppenheimer[4] reported that nirvanine, methyl 5-diethylaminoacetamidosalicylate, which possessed strong local anesthetic activity and low toxicity was also extremely irritating.

Derivatives of alkoxyaminobenzoates, *e.g.*, 2- and 3-alkoxy derivatives of diethylaminoethyl 4-aminobenzoate,[5] 2-alkoxy derivatives of procaine,[6,7] and dialkylaminoethyl esters of 2-, 5-, and 6-alkoxy-3-aminobenzoic acids,[8] have been reported to possess local anesthetic activity. In addition, Clinton and co-workers[9] reported local anesthetic activity in a number of dialkylaminoacylamino derivatives of some 2-alkoxybenzoic acid esters. These studies suggested that etherification of the derivatives, reported in the previous communication,[3] might result in local anesthetic agents devoid of the observed irritancy.

Chemistry.—Treating an ester of 2-alkoxy-4-aminobenzoic acid with chloroacetyl or 3-chloropropionyl chloride and subsequently heating the intermediate 4-(ω-chloroacylamino) derivative (*cf.* Table I) with excess amine in ethanol produced ethyl, *n*-butyl, and 2-diethylaminoethyl esters of 2-ethoxy- and 2-*n*-butoxy-4-(ω-alkylaminoacylamino)benzoates as the hydrochloride salts (*cf.* Tables II and III).

(1) This investigation was supported by a research grant from the Royal Hellenic Research Foundation.

(2) A preliminary report of this work has been presented at the 25th International Congress of Pharmaceutical Sciences, Prague, Czechoslovakia, Aug 21–27, 1965. This paper comprises a portion of a thesis presented by D. K. at the University of Athens.

(3) G. Tsatsas, C. Sandris, D. Kontonassios, J. F. Zaroslinski, R. K. Browne, and L. H. Possley, *J. Med. Chem.*, **10**, 235 (1967).

(4) A. Einhorn and M. Oppenheimer, *Ann. Chem.*, **311**, 154 (1900).

(5) J. Buchi, E. Stunzi, M. Fleury, R. Hirt, P. Labbart, and L. Ragaz, *Helv. Chim. Acta*, **34**, 1002 (1951).

(6) F. P. Luduena and J. O. Hoppe, *J. Pharmacol. Exptl. Therap.*, **104**, 40 (1952).

(7) F. P. Luduena and J. O. Hoppe, *ibid.*, **117**, 89 (1956).

(8) E. Epstein and M. Meyer, *J. Am. Chem. Soc.*, **77**, 4059 (1955).

(9) R. O. Clinton, S. C. Laskowski, U. J. Salvador, H. G. Bates, and P. M. Carroll, *ibid.*, **79**, 2285 (1957).