# NOVEL POLYETHER ANTIBIOTICS X-14873A, G AND H PRODUCED BY A *STREPTOMYCES*: TAXONOMY OF THE PRODUCING CULTURE, FERMENTATION, BIOLOGICAL AND IONOPHOROUS PROPERTIES OF THE ANTIBIOTICS

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Novel polyether antibiotics X-14873A, X-14873G, and X-14873H are produced by the fermentation of *Streptomyces* sp. X-14873 (ATCC 31679). This report presents taxonomic studies and fermentation conditions for the antibiotic producing culture. The antibiotics are mainly active against Gram-positive bacteria. The ionophore properties of X-14873A are also characterized.

Intensive search in our laboratory[1~4] and others[5~7] in the past decade has revealed that the biosynthesis of carboxylic acid polyether antibiotics is a widespread property among streptomycetes. In this paper, we report the discovery of another novel polyether antibiotic X-14873A and its descarboxyl derivatives, X-14873G and X-14873H (Fig. 1). This report presents the taxonomy of the producing culture *Streptomyces* sp. X-14873, fermentation conditions for the antibiotic production, and biological as well as the ionophorous properties of X-14873A. The isolation and characterization of the antibiotics and other metabolites are described in the accompanying paper[8].

## Taxonomy of the Producing Culture

The streptomycetes culture producing antibiotic X-14873A, X-14873G, and X-14873H was isolated from a soil sample collected near a sagebrush plant in Crandall Creek, Wyoming. The culture was designated strain X-14873 and has been deposited at American Type Culture Collection where it was assigned the number ATCC 31679. *Streptomyces* sp. X-14873 also elaborates a novel actinomycin complex, of which three of the antibiotic components designated actinomycins X-14873B, X-14873C, and X-14873D have been isolated and characterized[8]. The culture has the following morphological, physiological, and chemical characteristics.

### Microscopic and Macroscopic Examination

Strain X-14873 produces a substrate mycelium which does not fragment into spores, and an aerial mycelium forming *Rectus-Flexibilis* spore chains with 10~20 spores per chain. Spores are smooth and range in size from $1.0\times0.52$ $\mu$m to $1.25\times0.75$ $\mu$m (Fig. 2). Table 1 summarizes the amount of growth, degree of sporulation, spore mass color, color of the reverse-substrate mycelium, and presence of soluble pigment produced by strain X-14873 on various agar media.

### Physiological and Chemical Characteristics

The carbon utilization and other metabolic characteristics of strain X-14873 are shown in Tables 2 and 3. The culture hydrolyzes casein, starch, gelatin, adenine, xanthine, hypoxanthine, tyrosine,

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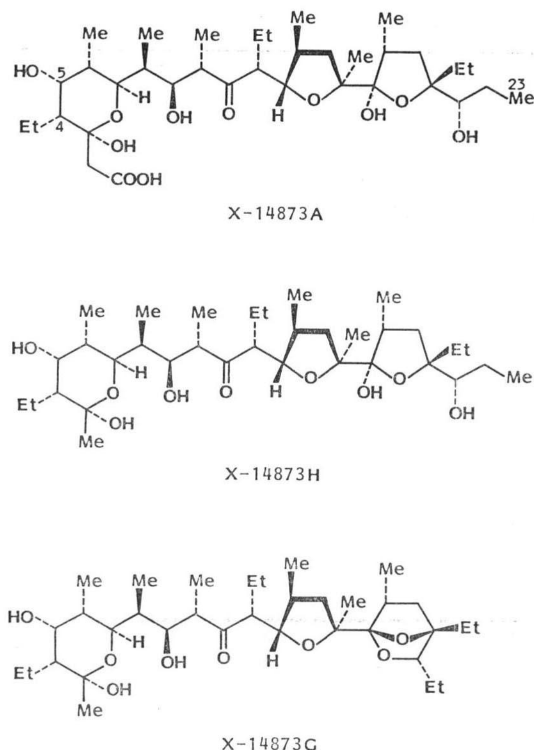



Fig. 1. The structure of antibiotic X-14873A, X-14873H, and X-14873G.

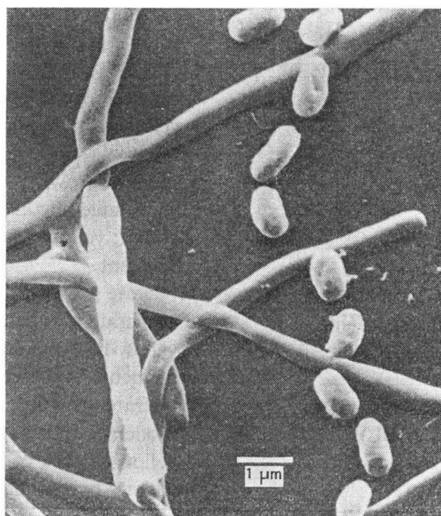



Fig. 2. *Streptomyces* sp. X-14873.

Smooth spores and a chain of undifferentiated spores on ISP 2 after 14 days of incubation at 28°C. Treatment included glutaraldehyde fixation and critical point drying. Bar represents 1 μm in length.

and urea by the method of GORDON[9]. As shown in Table 2, strain X-14873 does not grow on cellulose or sucrose. The analysis of the isomers of diaminopimelic acid in the cell wall of strain X-14873 was carried out according to the method of BECKER *et al.*[10]. The cell wall contains the LL-isomer of diaminopimelic acid which, together with the above characteristics, places this organism in the genus *Streptomyces*[11]. According to BERGEY's Manual[12], *Streptomyces* strain X-14873 resembles *S. chrysomallus*, *S. parvus*, and *S. globisporus*. All but *S. globisporus* produce actinomycin. These known cultures possess a yellow-colored spore mass, while strain X-14873 is mainly gray with only a hint of yellow. Strain X-14873 produces a yellow soluble pigment as do the other strains. The carbon utilization patterns are very similar for all the strains. Since the cultures all hydrolyze starch, gelatin, casein, and urea, and decompose adenine, xanthine, hypoxanthine, and tyrosine, it is difficult to distinguish between these cultures based on the above criteria.

## Fermentation

Spores of strain X-14873 were used to inoculate a 500-ml Erlenmeyer flask containing 100 ml of inoculum medium having the following composition (in g/liter): tomato pomace 5.0, distiller's soluble 5.0, meat peptone (Type-SB, Marco Development Corp., Hackensack, NJ, U.S.A.) 5.0, debittered dried yeast 5.0, Eclipse N starch (A.E. Staley, Decatur, IL, U.S.A.) 20.0, $CaCO_3$ 1.0, and $K_2HPO_4$ 1.0. The pH of the medium was adjusted to 7.0 before autoclaving. The inoculated flask was incubated for 96 hours at 28°C on a rotary shaker (5-cm gyration) operating at 250 rpm. One ml of the resulting vegetative growth was used as inoculum to start fermentation in a 500-ml Erlenmeyer flask containing 50 ml of production medium (in g/liter): glycerol 40.0, Cerelose (technical grade glucose)

Table 1. Cultural characteristics of strain X-14873.

| Agar medium | Amount of growth, degree of sporulation | Spore mass color[a] | Color of reverse-substrate mycelium[a] |
|---|---|---|---|
| Yeast - malt extract (ISP 2)[b] | Moderate to abundant growth; moderate sporulation; brown soluble pigment | c (light gray) where sporulated; 3ni (clove brown) where not sporulated | 3ie (camel) and 2gc (light tan) edge |
| Oatmeal (ISP 3)[b] | Moderate growth; sparse sporulation; yellow soluble pigment | b (oyster white) where sporulated; 2gc (bamboo) where not sporulated | 2ec (biscuit) |
| Inorganic salts - starch (ISP 4)[b] | Abundant growth; well sporulated; hydrolyzes starch; yellow soluble pigment | 3cb (sand) | 3lc (amber) and 3le (cinnamon) |
| Glycerol - asparagine (ISP 5)[b] | Moderate growth; moderate sporulation; yellow-brown soluble pigment; hygroscopic | b (oyster white) | 3le (cinnamon) |
| Czapek - Dox (BBL) | Moderate growth; moderate sporulation; yellow soluble pigment; slightly hygroscopic | b (oyster white) | 2ic (honey gold) |

a The color scheme used was Color Harmony Manual, 4th Ed., 1958, Container Corporation of America, Chicago, IL, U.S.A.

b Media recommended by the International Streptomyces Project[16].

Table 2. Comparison of carbon utilization by strain X-14873 and related strains on ISP 9 medium.

| Carbon source | Result[a] | | | |
|---|---|---|---|---|
| | X-14873 | *S. chrysomallus* NRRL 225D | *S. parvus* NRRL B-1455 | *S. globisporus* ATCC 15864 |
| No carbon control | − | − | − | − |
| D-Glucose | ++ | ++ YS | ++ YS | ++ YS |
| D-Xylose | ± | + | ++ YS | + |
| L-Arabinose | + | + | + (+) | + |
| L-Rhamnose | ++ YS | ++ YS | ++ | ++ |
| D-Fructose | ++ | ++ | ++ | ++ |
| D-Galactose | ++ | ++ YS | ++ YS | ++ |
| Raffinose | ± | − | − | − |
| D-Mannitol | ++ YS | ++ YS | ++ YS | ++ |
| *i*-Inositol | ± | − | − | − |
| Salicin | + | ± | ± | ± |
| Sucrose | − | − | − | − |
| Cellulose | − | − | − | − |
| Maltose | ++ | ++ YS | ++ intense YS | ++ |
| Glycerol | ++ | ++ | ++ intense YS | ++ |
| Starch | ++ | ++ YS | | ++ |
| Ribose | ++ | ++ YS | ++ YS | ++ YS |

a −: Negative response; ±: doubtful response; +: more growth than on carbon control but less than on glucose; +(+): positive response, nearly equal to growth on glucose. ++: positive response equal to growth on glucose. YS: yellow soluble pigment.

All tests were performed by the method of Shirling and Gottlieb[16].

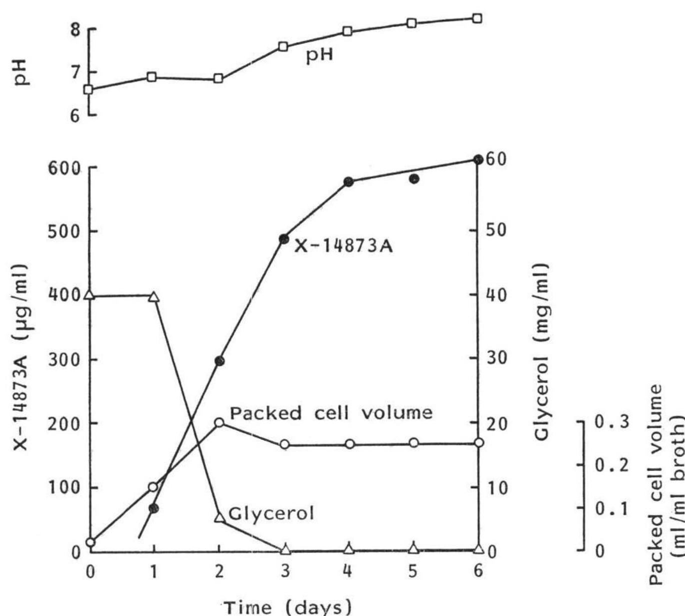
Table 3. Metabolic characteristics of X-14873.

| Test | Result[a] | Test | Result[a] |
|---|---|---|---|
| ISP 6, darkening | − | Streptomycin sensitivity (10 μg disc) | +13 mm |
| Melanin, ISP 7 | − | | |
| ISP 1, darkening | − | Lysozyme sensitivity[e] | + |
| Gelatin hydrolysis[b] | + | Nitrate reduction[c] | + |
| Casein hydrolysis[c] | + | Hygroscopic property | + |
| Starch hydrolysis[d] | + | Antibiotic production | Polyether antibiotics |
| NaCl (%) tolerance[c] | 5 | | X-14873 A, G, & H; |
| Growth range temp (°C) | 10~26 | | Actinomycins |
| Reverse-side pigment | − | | X-14873 B, C, & D |
| Soluble pigment | Yellow | | |

[a] All tests were done at 28°C.
[b] Test was performed by the method of SKERMAN[17].
[c] Test was performed by the method recommended in GORDON and SMITH[18].
[d] Determination was made after growth on agar of Actinomyces broth (Difco) with 0.25% soluble starch by flooding the plates with iodine-KI solution.
[e] Test was performed by the method of GORDON[19].

Fig. 3. Time course of X-14873A fermentation in shake flask.

The fermentation was carried out in 500-ml Erlenmeyer flasks containing 50 ml of production medium under the condition described in the text.




2.0, meat peptone (Type-SB, Marco Development Corp., Hackensack, NJ, U.S.A.) 5.0, potato starch 5.0, toasted Nutrisoy (Archer Daniels Midland Co., Decatur, IL, U.S.A.) 5.0, NaCl 5.0, and $CaCO_3$ 2.0. The medium pH was adjusted to 6.4 before sterilization. The fermentation was carried out at 28°C on a rotary shaker (5-cm gyration) operating at 250 rpm.

The amount of antibiotic X-14873A produced in the fermentation broth was determined by ethyl acetate extraction and high pressure liquid chromatography. The analysis was carried out on a reverse phase $C_{18}$-Nova Pak (15 cm × 4.5 mm, Waters Assoc., Milford, MA, U.S.A.) using a solvent sys-

Table 4. *In vitro* antimicrobial activity of antibiotic X-14873A, X-14873G, and X-14873H.

| | Organism | Minimal inhibitory concentration (μg/ml)[a] | | |
|---|---|---|---|---|
| | | X-14873A | X-14873G | X-14873H |
| G(−) rods | *Pseudomonas aeruginosa* ATCC 8705 | >1,000 | >1,000 | >1,000 |
| | *Proteus vulgaris* ATCC 6380 | >1,000 | >1,000 | >1,000 |
| | *Escherichia coli* ATCC 27856 | >1,000 | >1,000 | >1,000 |
| | *Klebsiella pneumoniae* ATCC 27858 | >1,000 | >1,000 | >1,000 |
| | *Serratia marcescens* ATCC 27857 | >1,000 | >1,000 | >1,000 |
| | *Acinetobacter calcoaceticus* ATCC 10153 | >1,000 | >1,000 | >1,000 |
| G(+) cocci | *Streptococcus faecium* ATCC 8043 | 0.23 | 62.5 | 3.9 |
| | *Staphylococcus aureus* ATCC 6538P | 3.9 | 1,000 | 7.9 |
| | *Micrococcus luteus* ATCC 9341 | 7.9 | 1,000 | 3.9 |
| G(+) rods | *Bacillus megaterium* ATCC 8011 | 7.9 | 1,000 | 3.9 |
| | *Bacillus* sp. E ATCC 27359 | 0.23 | 7.9 | 3.9 |
| | *B. subtilis* NRRL 558 | 1.9 | >1,000 | 7.9 |
| | *Bacillus* sp. TA ATCC 27860 | 1.9 | >1,000 | 7.9 |
| | *Mycobacterium phlei* ATCC 355 | 31.3 | >1,000 | >1,000 |
| G(+) filament | *Streptomyces cellulosae* ATCC 3313 | 15.7 | >1,000 | 62.5 |
| Molds | *Paecilomyces varioti* ATCC 25820 | 62.5 | >1,000 | >1,000 |
| | *Penicillium digitatum* ATCC 26821 | >1,000 | >1,000 | >1,000 |
| Yeasts | *Candida albicans* NRRL 477 | 31.3 | >1,000 | >1,000 |
| | *Saccharomyces cerevisiae* ATCC 4226 | >1,000 | >1,000 | >1,000 |

[a] Lowest two-fold dilution still showing zone of inhibition by an agar well diffusion assay.
G(+): Gram-positive; G(−): Gram-negative.

tem composed of acetonitrile and 1% aq acetic acid (85: 15) with a flow rate of 0.9 ml/minute. Antibiotic X-14873A was observed for the flow time at 6 minutes ($K'$ 4.5) using a RI detector (ERC-7510, Erma Optical Works, Tokyo, Japan). Glycerol content in fermentation broth was determined by a colorimetric method[13]. The cell growth was estimated by measuring the packed-mycelia volume in broth by centrifugation at 1,600 rpm for 10 minutes in a graduated glass centrifuge tube (13×130 mm) with a Sorvall GLC-2B bench-top centrifuge. A typical time course of X-14873 is shown in Fig. 3.

## Biological and Ionophore Properties

As an antibiotic, X-14873A is mainly active against Gram-positive bacteria and exhibits no activity against all the Gram-negative rods tested. The antimicrobial spectra of X-14873A, X-14873G, and X-14873H are shown in Table 4. It is interesting to note that X-14873H, the descarboxyl molecule of X-14873A, is also active against most of the Gram-positive bacteria tested while the other descarboxyl derivative, X-14873G, is practically inactive. These results indicate that the carboxylic function of the polyether antibiotic molecule is not required for the antimicrobial activity, even though the ionized carboxylic group plays an important role in complexation of cations by carboxylic acid polyether antibiotics[1].

Antibiotic X-14873A also induces a change in the proportion of volatile fatty acids (acetate, propionate, and butyrate) produced in the rumen by increasing the molar proportion of propionate in the rumen fluid. This activity has been implicated as the mechanism for increased feed utilization in ruminants by the polyether antibiotics, lasalocid and monensin[14], and suggests that antibiotic X-14873A is a potential candidate as a ruminant performance enhancer.

X-14873A is structurally similar to lysocellin[15] and exhibits similar ionophorous properties[2].

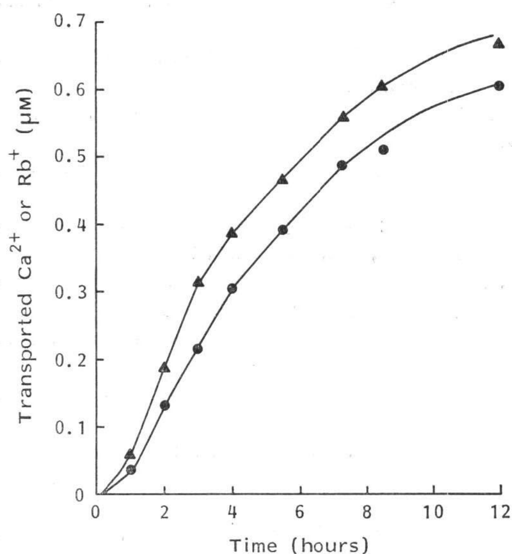



Fig. 4. Time course of $Ca^{2+}$ and $Rb^{+}$ transport by antibiotic X-14873A in a *U*-tube.

The *U*-tube system described by ASHTON and STEINRAUF[21] was employed for the assay. Five ml of a $CHCl_3$ solution of antibiotic ($1\times10^{-4}$ M) were added to a glass *U*-tube. Two ml of an aqueous Tris-HCl buffer (20 mM, pH 9.5) containing 1 mM [$^{45}$Ca]calcium chloride or [$^{86}$Rb]rubidium chloride was placed in one arm of the *U*-tube and an equal volume of the same buffer solution with unlabelled calcium or rubidium chloride in the other arm. The chloroform phase separating the two aqueous phases was then gently stirred with a magnetic stirrer. The rate of appearance of radioactive calcium or rubidium in the label-free side was determined by counting samples (50 μl) taken from both aqueous phases with 10 ml Aquasol (New England Nuclear, Boston, Mass., U.S.A.) in an Intertechnique liquid scintillation spectrometer.

●: $Ca^{2+}$; ▲: $Rb^{+}$.

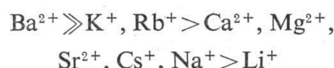
Table 5. Displacement of $^{86}Rb^{+}$ or $^{45}Ca^{2+}$ from X-14873A-cation complex by other cations.

| Displacing cation | Cation radius (Å) | $^{86}Rb^{+}$ or $^{45}Ca^{2+}$ remaining in complex (%) | |
|---|---|---|---|
| | | $^{86}Rb^{+}$ | $^{45}Ca^{2+}$ |
| None | — | 62.3 | 77.7 |
| $Mg^{2+}$ | 0.82 | 47.7 | 43.7 |
| $Ca^{2+}$ | 1.18 | 48.0 | 43.8 |
| $Sr^{2+}$ | 1.12 | 54.5 | 39.8 |
| $Ba^{2+}$ | 1.34 | 39.1 | 13.7 |
| $Li^{+}$ | 0.68 | 59.2 | 72.8 |
| $Na^{+}$ | 0.97 | 46.4 | 50.5 |
| $K^{+}$ | 1.33 | 32.9 | 33.0 |
| $Rb^{+}$ | 1.47 | 34.5 | 35.3 |
| $Cs^{+}$ | 1.67 | 40.5 | 43.8 |

The cation selectivity sequence of X-14873A was determined by the method of LIU and HERMANN[20]. Two μmol of antibiotic (in 2 ml of 70% toluene-30% butanol) were equilibrated by shaking for 2 hours at 20°C with 2 μmol of $^{86}$RbCl or 1 μmol of $^{45}CaCl_2$ (in 1 ml of dimethylglycine-tetramethylammonium hydroxide buffer, 25 mM pH 10.0). Complex formation was measured by liquid scintillation counting of both phases (see Fig. 4).

Displacement of labeled cation from the ionophore complexes was determined by the addition of unlabeled cations (2 μmol of monovalent or 1 μmol of divalent metal chloride).

It complexes and transports both monovalent and divalent cations as demonstrated in the experiments shown in Table 5 and Fig. 4. The cation binding sequence of X-14873 as determined by an ion-competition experiment (Table 5) is:



$$Ba^{2+} \gg K^{+}, Rb^{+} > Ca^{2+}, Mg^{2+}, Sr^{2+}, Cs^{+}, Na^{+} > Li^{+}$$

As expected, the descarboxylic derivatives of X-14873A, *i.e.*, X-14873G and X-14873H, do not bind and transport either rubidium or calcium ions in the two-phase distribution system described in Table 5.

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