ent on qualitative color tests and on R_f. The periodate oxidation information given is difficult to interpret in terms of a unique structure. Quantitative data on the amounts of formaldehyde liberated during periodate oxidation before and after reduction with borohydride would be useful in determining whether the compound tested and found to be inactive in hemagglutination inhibition tests in the blood group B system was galactose linked α to the 6 carbon of N-acetylglucosamine; inhibition of precipitation, which is a more sensitive test, was not carried out.

The fucose in blood group B substance from ovarian cyst appears to be linked very differently from the fucose in blood group A and O(H) substances from hog gastric mucin. Only 45 mg. of free fucose (2.6%) of 1.7 g. dialysable constituents were found when ovarian cyst B substance was hydrolyzed at *p*H 1.6 for 2 hr. at 100°, whereas 47 to 95% of the fucose in the dialysate was free when hog gastric mucin was treated similarly.[10] A blood group A substance derived from human ovarian cyst fluid and partially hydrolyzed at *p*H 1.9 also liberated most of the dialysable fucose as free fucose.[35] These observations would suggest that part of the fucose in blood group A substance is linked quite differently from that in blood group B substance. Thus if the hypothesis of Watkins and Morgan[36] were correct that A and B substances are derived from H substance under the influence of the A and B genes, respectively, these genes would also have to modify the fucose portion of the H substance in addition to introducing the specific A and B oligosaccharides side chains to account for the differences in lability of the fucose. Moreover, the findings that the P1 fractions[7] liberated by mild acid hydrolysis of B and A substance give rise to antibodies which were different in specificity from each other and from the original A and B substances also indicate that the genes determining A or B specificity control other steps in the synthesis of their respective substances in addition to the synthesis of the specific oligosaccharide side chains which are recognized using anti-A and anti-B serum.

(35) Unpublished observations.

(36) W. M. Watkins and W. T. J. Morgan, *Vox Sanguinis*, **4**, 97 (1959).

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[CONTRIBUTION FROM JOHN L. SMITH MEMORIAL FOR CANCER RESEARCH]

E-73: An Antitumor Substance. Part I. Isolation and Characterization[1]

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The isolation of E-73 with antitumor properties together with two diastereoisomeric forms of cycloheximide, fungicidin and two inert crystalline compounds from the culture filtrates of *Streptomyces albulus* is described.

In our cancer-screening program, a new species of actinomycete was isolated from the soil by our microbiology division. Broths from this culture, when tested at the Sloan–Kettering Institute as well as at our laboratories showed significant activity against mouse Sarcoma 180 and against human tumor transplants grown in rats. This organism was designated as *Streptomyces albulus*,[2] and the isolation of the active principle is reported here.

The organism is grown in the usual natural media in submerged culture for 3–6 days. The broths are active against rodent tumors such as S-180 and HS#1 and against various fungi, especially yeast-like organisms *in vitro*. It appeared early in the work that the antitumor activity might parallel the activity against yeasts. The conventional disk–plate assay with *Saccharomyces cerevisiae* grown in glucose–yeast extract-agar was used during the isolation work. The samples were plated at serial dilutions and the last dilution which gave a distinct zone was expressed as the number of dilution units per ml. The antitumor activity of the various fractions was checked from time to time against Sarcoma 180 in mice as well as by the heterologous screens of the type HS#1 and HEp#3 in rats.[3]

Extraction of the broths with 1-butanol and concentration gave a product designated as A-73 and which agreed in its properties with fungicidin.[4] Ethyl acetate extracts of the broth on processing gave products which were free from fungicidin and showed antitumor activity at levels at 0.5 to 2.0 mg./kg. in S-180 tumors. Paper chromatography showed possible presence of cycloheximide.[5] However, the latter is known to be active only at levels of 30–80 mg./kg. against Sarcoma 180.[6] Thus, it appeared that the concentrates from *Streptomyces albulus* contained a highly active compound in addition to the possible cycloheximide.

Partition chromatography on a silica gel column was employed for the separation of the active components. Aqueous methanol and diisopropyl ether were selected as stationary and mobile phases, respectively. When the progress of the column was followed by gravimetric and microbiological

(1) Presented at the 134th Meeting of the American Chemical Society, Chicago, Ill., September, 1958.

(2) The organism was characterized by Dr. J. B. Routien, Mycology Department, Chas. Pfizer & Co., Inc.

(3) The antitumor assays were carried out by our Assay Department. For methods see H. W. Toolan, *Cancer Research*, **14**, 660 (1954), and ref. 5.

(4) J. D. Dutcher, G. Boyack and S. Fox, *Antibiotics Annual*, 191 (1953–1954).

(5) J. H. Ford and B. E. Leach, THIS JOURNAL, **70**, 1223 (1948).

(6) H. C. Reilly, C. C. Stock, S. M. Buckley and D. A. Clark, *Cancer Research*, **13**, 684 (1953).

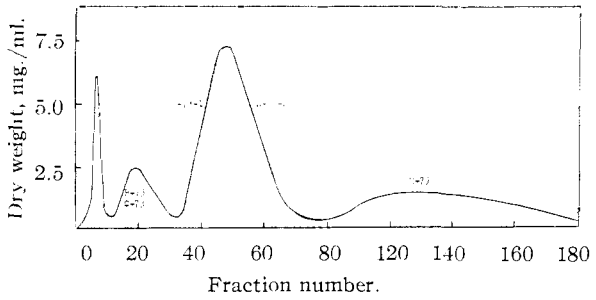

Fig. 1.—Separation of the components of *Streptomyces albulus*.

methods, four distinct bands could be detected as shown in Fig. 1.

The first band (10–15% of total solids) yielded no crystalline product and had no antitumor activity. The second band (5–10% of the total solids) gave two crystalline solids designated as B-73 and C-73, neither of which showed any significant antitumor activity.

The next and the major band (40–50% of total solids) which represented the bulk of the activity against yeast, gave two types of crystals designated as D-73-1 and D-73-2. These two are shown to be two stereoisomeric forms of cycloheximide. D-73-1 shows a positive Cotton effect and D-73-2 a negative Cotton effect.[7] Both the fractions D-73-1 and D-73-2 showed antitumor activity at levels of 30–80 mg./kg. body weight against Sarcoma 180 and at levels of 0.5 – 1.0 mg./kg. against human tumor transplants grown in rats as is expected of cycloheximide.

The final band (15–20% of total solids) represented the rest of the material active against yeast. This fraction, designated as E-73 crystallized from ethanol as colorless needles. Its activity against yeast was only about 20–30% of that of cycloheximide, but against Sarcoma 180 in mice it is 200–400 times as much. E-73 thus accounts for most of the antitumor activity of the broths. Samples of E-73 produced strong irritation on normal skin as dust or as 1% solutions. Table I lists some of the physical properties of E-73.

Experimental

The culture is grown submerged in a medium composed of glucose 1%, soy bean meal 2%, K_2HPO_4 0.5% and NaCl 0.2% and adjusted to *p*H 7.0. The fermentations are harvested at 60–85 hours.

A-73.—The beer is filtered through Hyflo Supercel and the broth as well as the mycelial cake extracted with 1-butanol. Concentration of the combined extract and leaving for 2–6 days at 5° gave a semi-crystalline solid which is recrystallized from 1-butanol saturated with water. It appeared as small needles and melted in the range 150–160°. *Anal.* Found: C, 58.57; H, 8.10; N, 1.55. Fungicidin was purified by a similar manner from commercially available preparations. The two products gave identical infrared spectra and showed the same R_f values in the system 70% aqueous isopropyl alcohol.

(7) Since the completion of this work the existence of two isomeric forms of cycloheximide has also been described by T. Okuda, M. Suzuki, T. Egawa and K. Ashimo, *Chem. & Pharm. Bull (Japan)*, **6**, 328 (1958).

TABLE I

PROPERTIES OF E-73

	Property	E-73
1	Melting point, °C.	140–141
2	$[\alpha]^{25}D$ (*c* 1% in methanol)	−8.8°
3	Ultraviolet absorption spectrum	Weak max, at 285 mμ, ε 20
4	Infrared spectrum (KBr pellet), μ	2.95, 5.80, 5.90, 7.25, 7.75, 7.92
5	Empirical formula	$C_{17}H_{25}O_6N$
6	Activity against *Sacch. cerevisiae*	200–300 dilution units/mg.

For the isolation of the other fractions the broth is extracted with one-half volume of ethyl acetate and the extract concentrated and freed from oils by treatment with heptane.

The solvent system for the partition chromatography consisted of 55% aqueous methanol and isopropyl ether. Silica gel (28–200 mesh) is impregnated with the aqueous phase and packed in a suitable column. The sample (1 g. per 25–50 g. of silica gel) is added to the column and developed with the mobile phase.

B-73.—Fractions from the second band (Fig. 1) are combined and concentrated. The crystalline product recrystallized twice from ethyl acetate. B-73 separates out as colorless narrow rectangular plates which melt at 275–276°. *Anal.* Calcd. for $C_{15}H_{16}O_2N_2$: C, 70.29; H, 6.29; N, 10.92. Found: C, 70.03; H, 6.20; N, 10.40.

C-73.—The product from B-73 mother liquors is crystallized several times from a mixture of methanol and methylene chloride. Pure C-73 separates out as very pale yellow long needles which melt at 198–199°. *Anal.* Calcd. for $C_{15}H_{17}O_4N$: C, 65.44; H, 6.22; N, 5.09. Found: C, 65.57; H, 6.33; N, 5.10.

Both B-73 and C-73 are rather slightly soluble in common organic solvents.

D-73-1 and D-73-2.—Fractions from band III (Fig. 1) after standing for several days deposited the two types of crystals D-73-1 and D-73-2. Sufficient quantities of these crystals are separated manually and used as seed for subsequent batches.

The fractions from band III are combined, concentrated to dryness, dissolved in 1:9 methylene chloride–ether and seeded with D-73-1. The crystalline product is filtered and recrystallized from ethanol–ether. D-73-1 formed colorless feathery plates which melted at 100–105°. *Anal.* Calcd. for $C_{15}H_{23}O_4N$: C, 64.03; H, 8.24; N, 4.98. Found: C, 64.65; H, 8.35; N, 5.31.

The filtrate from D-73-1 is seeded with D-73-2 and the product recrystallized from ethanol–ether. D-73-2 formed large colorless rectangular prisms which melted at 118–119°. *Anal.* Calcd. for $C_{15}H_{23}O_4N$: C, 64.03; H, 8.24; N, 4.98. Found: C, 63.57; H, 8.22; N, 5.23.

E-73.—Fractions from band IV (Fig. 1) are combined and concentrated to dryness. The colorless glassy solid is crystallized first from ethanol and then from a mixture of ethanol–ether (1:9). E-73 separates out as colorless narrow rectangular plates which melt at 141–142°. *Anal.* Calcd. for $C_{17}H_{25}O_6N$: C, 60.16; H, 7.43; N, 4.13. Found: C, 59.86, 59.97; H, 7.56, 7.70; N, 4.27, 4.16.

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