8. A kind of a curious anomaly may be noted here. The streptomycetes are recognized as a specific group of actinomycetes. Nevertheless the old generic name *Actinomyces* (which is now used only for the anaerobic group of actinomycetes), rather than *Streptomyces*, is still used.
9. This would be comparable to the activity of grisein reported by Kuehl (*6*), if one considers the greater sensitivity of staphylococci than *E. coli* to this antibiotic.
10. M. G. Brazhnikova, N. N. Lomakina, L. I. Muravieva, *Compt. rend. acad. sci. U.R.S.S.* 99, 827 (1954); V. A. Shorin and Y. O. Sazykin, *Compt. rend. acad. Sci. U.R.S.S.* 96, 645 (1954).
11. L. P. Garrod and P. M. Waterworth, *Brit. Med. J.* 1956, II, 61 (1956).
12. E. O. Stapley and R. E. Ormond, *Science*, this issue.

Similarity of Albomycin and Grisein

Edward O. Stapley and Robert E. Ormond

In the course of an investigation into the nature of albomycin, an antibiotic described by Gause (*1*) as a product of *Actinomyces subtropicus*, considerable data have been obtained indicating similarity of this product to grisein, which has been described by Reynolds, Schatz, and Waksman (*2*) as a product of *Streptomyces griseus*. We have also obtained information on the existence of several components in partially purified grisein which have not been described previously.

Information on the chemical constitution of grisein and albomycin published by Kuehl, Bishop, Chaiet, and Folkers (*3*) and Gause (*1*), respectively, suggests possible similarity, since both antibiotics are described as red-colored, amino-acid containing, iron complexes. Both substances are very active on a weight basis against sensitive bacteria.

Materials and Methods

We have had available ampules of albomycin obtained from international sources and also a preparation of albomycin obtained directly from G. F. Gause through S. A. Waksman. The potency of the albomycin employed in our studies was 91,000 units per milligram. Comparison was made with two partially purified concentrates of grisein prepared at Merck and Company, Inc., that were active at 41,000 and 22,000 units per milligram, and with a crude preparation of grisein prepared in the laboratory of S. A. Waksman in 1948, which proved to be active at 312 units per milligram in a recent assay. All assays were performed by the agar diffusion method with *Escherichia coli* as the test culture and a standard based on an assigned value of 300,000 units per milligram for the pure grisein of Kuehl *et al.* (*3*).

Paper-strip chromatograms were developed on Whatman No. 1 filter paper. Bioautographs were obtained by placing air-dried paper strips on the surface of large baking dishes of nutrient agar seeded with *Escherichia coli* W followed by incubation at 25°C for 18 hours. Ascending paper chromatograms were developed at room temperature until the solvent front had moved 25 to 30 centimeters. Descending paper chromatograms were run so that the fastest moving component had traveled approximately 24 centimeters in 52 hours when they were developed at 28°C with a solvent mixture of butyl alcohol (4 parts), acetic acid (1 part), and water (5 parts). The strips were developed with the solvent phase after a 3-hour equilibration with an atmosphere saturated with the water phase.

Column partition chromatography was accomplished by pouring a solution of 1.1 grams of grisein (22,000 units per milligram) in 40 milliliters of upper phase from an *n*-butyl alcohol (4 parts), acetic acid (1 part), and water system (5 parts) over 800 grams of pulverized paper wet with lower phase. The column was developed with upper phase. Starting just before the first yellow eluate came off the column, 25-milliliter aliquots were collected, absorption at 4250 A was determined, and the reading was plotted. The peak fractions were combined, concentrated in a vacuum, and lyophilized. Repartition of 134 milligrams of a combined fraction (Fig. 1, tubes 71 to 150) with 60,000 units per milligram containing mainly component C was carried out in the same fashion as the original column partition, using 300 grams of pulverized paper. Eluate aliquots of 9 milliliters each were taken by a fraction collector, and the ultraviolet absorption at 4250 A was measured.

Data from the column partition were compared with a 39-plate countercurrent distribution of grisein (41,000 units per milligram) employing 2*M* phosphate buffer at *p*H 6.7 and 10 grams of solid phenol diluted to 100 milliliters with chloroform. The countercurrent fraction where $K = 0.26$ corresponded to the fraction from which pure grisein was originally obtained by Kuehl *et al.* (*3*).

Results and Discussion

In our tests of grisein and albomycin, we noted that the inhibition zones produced on disk or cup-plate assays were very similar, especially with regard to the rapid appearance of resistant colonies within the inhibition zone. The capacity of grisein to permit exceptionally rapid development of resistance and the characteristic hazy appearance of inhibition zones produced as a result of rapid development of resistance have not been

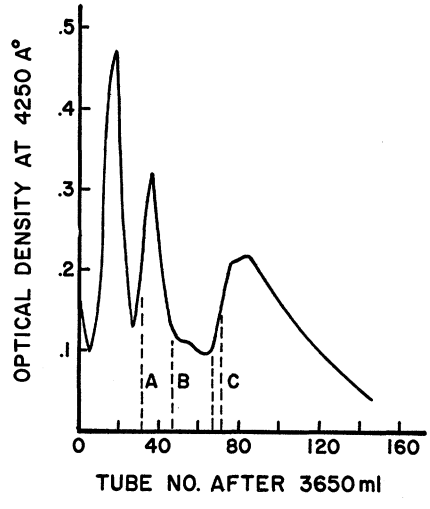

Fig. 1. Column: partition chromatography of grisein. The curve shows the ultraviolet absorption at 4250 A of 25-milliliter aliquots eluted from a cellulose column that was charged with grisein (22,000 units per milligram).

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Table 1. Comparison of the cross resistance of albomycin and grisein. The test of cross resistance of albomycin and grisein was made using filter paper disks 13 millimeters in diameter on nutrient agar plates seeded with *Escherichia coli* W and 12 antibiotic-resistant strains obtained from *E. coli* W.

E. coli W	Zone of inhibition (mm)	
	Albomycin (10 μg/ml)	Grisein (10 μg/ml)*
Sensitive parent	24	22
Streptomycin resistant	22	21
Streptothricin resistant	27	23
Cycloserine resistant	26	23
Pleocidin resistant	35	30
Chloramphenicol resistant	35	28
Chlortetracycline resistant	35	31
Oxytetracycline resistant	35	26
Neomycin resistant	35	26
Tetracycline resistant	35	31
Viomycin resistant	0	0
Grisein resistant	0	0
Albomycin resistant	0	0

* 41,000-unit/mg preparation.

Table 2. Antibacterial activities of albomycin and grisein by the cup-diffusion method.

Test culture	Zone diameters (mm)	
	Albomycin (5 μg/ml)	Grisein* (5 μg/ml)
Micrococcus pyogenes var. *aureus*	25.5†	15.0†
Diplococcus pneumoniae	23.5	21.0
Klebsiella pneumoniae	24.0	18.0†
Salmonella schottmülleri	24.0†	22.0†
S. typhosa	§	§
Pseudomonas aeruginosa	±‡	§
Proteus vulgaris	§	§

* 41,000-unit/mg preparation.
† Resistant colonies present in zone of inhibition.
‡ Inhibition zone beneath the cup only.
§ No inhibition zone.

noted for other antibiotics produced by actinomycetes. Rapid development of resistance to the antibiotic action of albomycin has been noted also by Garrod and Waterworth (*4*).

Cross-resistance tests have revealed a close relationship between albomycin and grisein in their biological activity. The results of testing both antibiotics against 12 antibiotic-resistant strains of *Escherichia coli* W are presented in Table 1. These data demonstrate that albomycin and grisein are mutually cross-resistant and that both are cross-resistant with viomycin, while neither is cross-resistant with any of nine other antibiotics produced by actinomycetes.

Table 3. Paper-strip chromatograms of albomycin and grisein. The antibiotics were compared by ascending paper chromatography on Whatman No. 1 filter-paper strips, bioautographed against *Escherichia coli* W. The figures in parentheses in column 1 indicate the proportions of the components in the solvent systems.

No.	Solvent system	R_f values	
		Albomycin (0.4 μg)	Grisein (0.5 μg)
1	*n*-Butanol (4), H_2O (2), acetic acid (1)	0.14, 0.33, 0.46	0.14, 0.37, 0.46
2	*n*-Butanol (1), H_2O (2), acetic acid (1)	0.83	0.83
3	Methanol (3), 0.1*N* HCl (1)	0.68	0.68
4	*n*-Propanol (10), 2.5% NaCl (8), acetic acid (1)	0.79	0.78
5	*n*-Butanol (25), ethanol (25), H_2O (47), acetic acid (3)	0.75, 0.92	0.97
6	Acetone (60), H_2O (37), acetic acid (3)	0.71	0.75

A comparison of the activities of albomycin and grisein against various pathogenic bacteria, as reported in Table 2, is consistent with the other observations on the similarity of albomycin and grisein. A low degree of activity, if any, against *Salmonella typhosa, Pseudomonas aeruginosa,* and *Proteus vulgaris* has been reported for grisein by Reynolds *et al.* (*2*) and in our laboratories has been found to be characteristic of purified grisein and of fermentation broths that contain grisein.

A comparison of albomycin and grisein by ascending paper chromatography with several solvent systems is presented in Table 3. It may be noted that there is a remarkable similarity in R_f values obtained for the two substances, especially in systems 2, 3, and 4. The results obtained with solvent system 1 suggest that both antibiotics are made up of several components. This point has been confirmed by comparison of the antibiotics on descending paper chromatograms with a higher resolving power, as is reported in Table 4. Since the solvent front moves off the strip during the prolonged development time, results are reported as mobilities with respect to the distance traversed by the fastest moving component. The center of spot A on these strips was approximately 24 centimeters from the origin in the case of both albomycin and grisein.

Albomycin appears to be made up of four antibiotic components, three of which are present at detectable levels in the crude grisein (312 units per milligram) produced in 1948 and all of which are found in the partially purified grisein (41,000 and 22,000 units per milligram). Table 5 presents an estimation of the relative antibiotic activities of the four components in crude and partially purified preparations. We have found that component C, the major antibiotic moiety of albomycin, is present in partially purified grisein and is the major component of crude grisein.

Pure grisein as previously described (*3*) is characterized by a paper-strip mobility corresponding to that of component A. Purification studies on grisein product 5 of Table 5 (22,000 units per milligram), a partially purified grisein in which the quantitative distribution of components closely resembles that of albomycin, have demonstrated that component C is converted to component A during the purification process. The mechanism whereby this takes place is unknown; treatment of component C with oxygen, ferric chloride, or the acid alcohol system failed to convert it completely to A.

The results of column chromatography of grisein product 5 (22,000 units per milligram) are presented in Fig. 1. The eluate fractions showing ultraviolet absorption, labeled A, B, and C, correspond to the antibiotic components A, B, and C of grisein. The first optical density peak represents antibiotically inert mate-

Table 4. Comparison of the relative mobility of the components of albomycin and grisein by descending paper-strip chromatography.

Antibiotic	Relative mobility of components			
	A	B	C	D
Albomycin	1.0	0.73	0.59*	0.27
Grisein (41,000 unit/mg)	1.0*	0.75	0.60	0.28
Grisein (22,000 unit/mg)	1.0	0.81	0.58*	0.30
Grisein (312 unit/mg)	1.0		0.65*	0.35

* Indicates location of most active antibiotic zone.

Table 5. Comparison of the relative importance of the four antibiotic components in several grisein preparations and albomycin obtained from paper chromatograms.

No.	Antibiotic*	Potency (unit/mg)	Grisein components			
			A	B	C	D
1	Albomycin	91,000	+++	++	++++	+
2	Grisein	41,000	++++	+++	+	+
3	Grisein	312	+	−	++++	+
4	Grisein	94	++	−	++++	+
5	Grisein	22,000	+++	++	++++	+

* Sample 2 was prepared by Kuehl from *Streptomyces griseus* 25G (culture obtained from S. A. Waksman). Sample 3 was obtained from Waksman. Sample 4 was obtained from Compañía Española de la Penicilina y Antibióticos, S.A., Madrid, Spain (culture isolated in their laboratories). Sample 5 was prepared in our laboratories from the Spanish culture; this was the sample used for purification studies.

Table 6. Comparison of chromatographic and countercurrent distribution fractions of grisein.

Fraction tube Nos.	Wt. of residue (mg)	Activity (unit/mg)	Components by paper chromatogram	Optical absorption	
				$\lambda_{max.}$ (A)	$E_{1\ cm}^{1\%}$
29–46*	51.3	100,000	A	2650	96
				4200	20
47–65*	38.4	20,000	A, B		
71–110*	91.8	72,000	A, B, C	2750	87
				4300	20
111–150*	49.3	40,000	A, B, C	2750	76
				4300	12
Plates					
7–10 ($K = 0.26$)†		80,000	A	2650	70
				4200	18
34–39 ($K = 18$)†		inactive	D	2750	80

* Data on chromatographic column eluate described in Fig. 1.
† Fractions from a 39-plate countercurrent distribution of grisein (41,000 unit/mg).

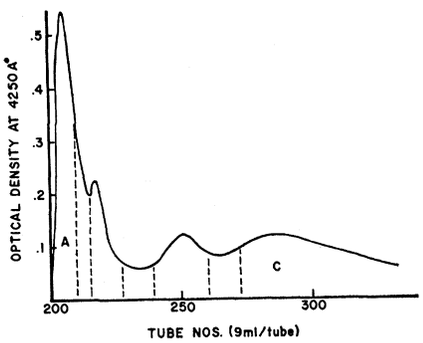

Fig. 2. Repartition of grisein component C. The curve shows the ultraviolet absorption at 4250 A of 9-milliliter aliquots eluted from a cellulose column that was charged with grisein C (60,000 units per milligram).

rial, and component D was not eluted from the column. Determinations of weight, activity, and optical absorption characteristics were made on the lyophilized fractions, and these data, together with the results of paper chromatography, are presented in Table 6. Data from a countercurrent distribution of grisein are included in Table 6 for comparison with the fractions obtained by column partition. The fractions which are composed of component A or D behave as single antibiotic substances on paper chromatograms. However, the B and C antibiotic components obtained in fractions from column chromatography segregate further when they are subjected to paper chromatography.

Repartition on a cellulose column of the fractions that contained mainly component C (tubes 71 to 150) gave the absorption data recorded in Fig. 2. Other data obtained on the peak fractions are included in Table 7. Repartition of component C resulted in separation of other grisein components from C. The highest peak representing the purest material was again component A. The A component behaved on paper strips as a single substance, whereas the C component again segregated into both the A- and C-type antibiotics.

An area 1 centimeter wide was cut from the exact center of the visible orange spot corresponding to antibiotic component C resulting from paper chromatography of 500 micrograms of fraction 272–350 and was suspended in 0.5 milliliter of water. Restripping of 0.01 milliliter of this component on paper, followed by bioautographing with *Escherichia coli* W again showed the segregation of the antibiotic activity into approximately 30 percent component C and 70 percent A and B. It is concluded from these results that component C is converted partly to a more stable form, A, by the step of cellulose column or paper chromatography with *n*-butanol, acetic acid, and water development systems.

Table 7. Data on fractions obtained by repartition of component C (described in Fig. 2).

Fraction tube Nos.	Wt. of residue (mg)	Activity (unit/mg)	Components by paper-gram
200–210	15.8	180,000	A
216–227	12.3	90,000	A*
239–260	14.8	80,000	A*, C
272–350	36.2	90,000	A*, C

* Components A and B were not clearly resolved on these paper strips.

Infrared spectra have been obtained on albomycin and on grisein of activity 300,000 units per milligram. The results are consistent with the interpretation that albomycin and grisein are related compounds, taking into consideration the differences in purity of the two materials. The spectra indicate the presence of the same functional groups in each substance.

Summary

Albomycin and grisein were found to be composed of four antibiotically active substances of which A, a strongly active component, and D, a very weakly active component, appear to be stable to purification, while C has been observed to break down continually with concomitant appearance of more A. On the basis of published reports and of studies made in our laboratories, it is concluded that albomycin and grisein are chemically very similar and identical with respect to antimicrobial activity (*5*).

References and Notes

1. G. F. Gause, *Brit. Med. J.* 1956, II, 1177 (1955).
2. D. M. Reynolds, A. Schatz, S. A. Waksman, *Proc. Soc. Exptl. Biol. Med.*, 64, 50 (1947).
3. F. A. Kuehl *et al.*, *J. Am. Chem. Soc.* 73, 1770 (1951).
4. L. P. Garrod and P. M. Waterworth, *Brit. Med. J.* 1956, II, 61 (1956).
5. We are indebted to F. A. Kuehl and N. R. Trenner for making available some chemical data and to B. M. Frost for the antibacterial tests reported in Table 2.