United States Patent [19]

DeBoer et al.

[11] **3,907,643**

[45] **Sept. 23, 1975**

[54] **PROCESS FOR PRODUCING THE ANTIBIOTIC U-44,590**

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[73] Assignee: **The Upjohn Company,** Kalamazoo, Mich.

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Related U.S. Application Data

[62] Division of Ser. No. 471,322, May 20, 1974.

[52] **U.S. Cl.** ..... **195/80 R**

[51] **Int. Cl.²** **C12D 9/00**

[58] **Field of Search** ..... 195/80 R

[56] **References Cited**

UNITED STATES PATENTS

3,462,414 8/1969 Wechter 260/211.5

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[57] **ABSTRACT**

New antibiotic U-44,590 and derivatives thereof, produced by the controlled fermentation of the new microorganism *Streptomyces platensis* var. *clarensis* var nova, NRRL 8035. This antibiotic and its derivatives are active against Gram-negative bacteria. Accordingly, they can be used in various environments to eradicate or control such bacteria.

3 Claims, No Drawings

PROCESS FOR PRODUCING THE ANTIBIOTIC U-44,590

CROSS REFERENCE TO RELATED APPLICATION

This application is a division of our co-pending application Ser. No. 471,322, filed on May 20, 1974.

BRIEF SUMMARY OF THE INVENTION

The novel antibiotic of the invention, U-44,590 is obtained by culturing *Streptomyces platensis* var. *clarensis*, NRRL 8035, in an aqueous nutrient medium under aerobic conditions. Various derivatives of U-44,590 can be made as disclosed infra. U-44,590 and its derivatives have the property of adversely affecting the growth of Gram-negative and Gram-positive bacteria, for example, *Streptococcus hemolyticus*, *Klebsiella pneumonia*, Salmonella Sp., Serratia marcescens, Pasteuralla multocida, *Hemophilus Sp.*, *Proteus morgani* and *Proteus rettgeri*. According, U-44,590 and its derivatives can be used along or in combination with other antibiotic agents to prevent the growth of or reduce the number of bacteria, as disclosed above, in various environments.

U-44,590 and its derivatives are also active against DNA viruses, for example, the Herpes virus and, thus, can be used to control such virus where its presence is not desired.

DETAILED DESCRIPTION OF THE INVENTION

Chemical and Physical Properties of U-44,590

Elemental Analysis: Calcd. for $C_9H_{15}N_3O_5$: C, 44.08; H, 6.17; N, 17.13. Found: C, 44.14; H, 6.08; N, 17.36.

Molecular Weight: 245 (Determined by mass spectrometry)

Melting Point Range: 141° – 142° C.

Specific Rotation: $[\alpha]_D^{25} = -5°$ (c, 0.9030 in H_2O)

Solubilities: Highly soluble in water, and lower alcohols, for example, methanol and ethanol; relatively insoluble in Me_2CO, EtOAc, hydrocarbons, CH_2Cl_2 and $CHCl_3$.

Infrared Absorption Spectra: U-44,590 has a characteristic infrared absorption spectrum when suspended in a mineral oil mull. Peaks are observed at the following wave lengths expressed in reciprocal centimeters:

Band Frequency (Wave Numbers)	Intensity
3440	M
3440	M
3400	M
3340	M
3190	M
3080	M
3000	W
2960 (N = Nujol)	S
2930 (N)	S
2860 (N)	S
1695 sh.	S
1683	S
1510	M
1503	M
1483	M
1463 (N)	S
1440	S
1421	M
1407	M
1396	M
1375 (N)	W
1350	M
1315	W
1300	W
1280	W
1276	W

-Continued

Band Frequency (Wave Numbers)	Intensity
1262	W
1243	S
1230 sh.	M
1195	W
1165	W
1133	W
1093	M
1085	W
1060	S
1011	S
985	M
971	W
943	M
885	W
872	W
849	W
826	W
792	M
755	M
735	W

Note. sh means a shoulder band.

U-44,590 also has a characteristic infrared absorption spectrum when pressed in a KBr disc. Peaks are observed at the following wave lengths expressed in reciprocal centimeters:

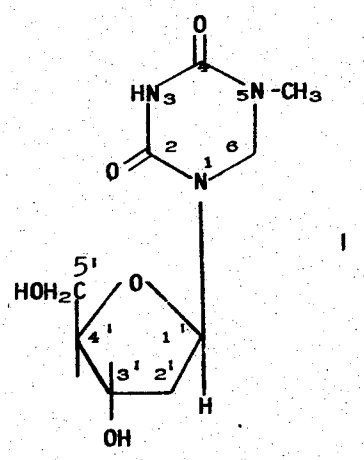
Band Frequency (Wave Numbers)	Intensity
3440	S
3200	M
3080	M
3000	W
2970	W
2960	W
2935	W
2920	W
2870	W
1697 sh.	S
1685	S
1510	M
1482	M
1461	S
1437	M
1420	M
1406	M
1396	M
1349	W
1310	W
1298	W
1290	W
1275 sh.	M
1263	M
1243	S
1195	W
1165	W
1133	W
1097	M
1085	M
1060	M
1010	M
985	W
971	W
942	M
883	W
870	W
847	W
827	W
791	M
754	W
733	W

Note: sh means a shoulder band.

Infrared band intensities, throughout this disclosure, are indicated as S, M, and W respectively and are approximated in terms of the backgrounds in the vicinity of the bands. An S band is of the same order of intensity as the strongest in the spectrum; M bands are between ⅓ and ⅔ as intense as the strongest band; and, W bands are less than ⅓ as intense as the strongest band. These

estimates are made on the basis of a percent transmission scale.

The following is considered to be the structure of U-44,590:



I

Thus, U-44,590 can be referred to by the trivial name 5,6-dihydro-5-azathymidine, or by its chemical name 1-(2-deoxy-β-D-erythro-pentofuranosyl)-5,6-dihydro-5-methyl-s-triazine-2,4(1H,3H)-dione.

Antibacterial Activity of U-44,590

Organism	No. of Strains	Inhibition (μg/ml)
Staphylococcus aureus	1	>1000
Streptococcus hemolyticus	1	15.2
Diplococcus pneumoniae	1	500.
Klebsiella pneumoniae	5	2.0 – >1000
Salmonella sp.	4	15.6 – >1000
Serratia marcescens	2	125
Pseudomonas aeruginosa	5	>1000
Pasteurella multocida	1	125
Hemophilus sp.	5	31.2 – >1000
Proteus vulgaris	3	>1000
Proteus mirabilis	3	>1000
Proteus morgani	3	62.5 – 250
Proteus rettgeri	3	31.2 – >1000

The above antibacterial spectrum was obtained by a standard agar dilution test with the following media and conditions:

Diffo Brain Heart Infusion Medium was used for all test bacteria except *P. multocida* and *Hemophilus* species which were grown in Difco Blooc Agar Base with 5% defibrinated rabbit blood. All were grown aerobically at 37° C. (except *Hemophilus* species, grown anaerobically) 16–18 hours. Inocula were grown overnite (16–18 hours) at 37° C. and used to seed agar at the rate of 0.025 ml. of 10^{-3} dilution (approximately 2500 to 25,000 bacteria per drop of inoculum).

In vivo testing of U-44,590 in mice infected with selected microorganisms is as follows:

Activity (CD_{50} in mg/kg)

Organism	Mice, IP Challenge LD_{50}	Subcutaneous Route	Oral Route
Salmonella flexneri	40	38 (25–57)	62.5
Escherichia coli	79	141 (116–172)	218 (154–307)
Proteus mirabilis	1259	152 (96–240)	101 (66–156)
Proteus vulgaris	79	100 (66–152)	<62.5

-Continued

Activity (CD_{50} in mg/kg)

Organism	Mice, IP Challenge LD_{50}	Subcutaneous Route	Oral Route
	150		~30
Streptococcus hemolyticus	100	—	>160

ANTIVIRAL ACTIVITY OF U-44,590

The following is an example of the antiviral activity of antibiotic U-44,590. The antibiotic is administered subcutaneously to mice which are inoculated intravenously with *Herpes simplex* virus. Treatment is initiated two hours prior to viral infection and is followed by treatment four times daily for five consecutive days. A detailed account of the materials and methods and results are as follows:

Male mice, weighting approximately 20 gm. each, are divided into 4 groups of 20. Group 1 is treated with saline, Group 2 with 400 mg./kg/dose (mkd) U-44,590, Group 3 with 200 mkd U-44,590, and Group 4 with 100 mkd U-44,590. The antibiotic is dissolved in saline and administered subcutaneously in the nape of the neck at 8 a.m., 12 noon, 4 p.m., and 8 p.m. on days 0, **1, 2, 3,** and 4. Herpes virus at $10^{-1.5}$ dilution, 0.05 ml/mouse, equivalent to a viral dose of 40 LD_{50}s, is inoculated into the tail vein at 10 a.m. on day 0. Paralysis and death are recorder daily.

Hind leg paralysis usually preceded death by 1-2 days. All mice died that became paralyzed. Death pattern of the 4 groups, as shown in the curves which follow, illustrates the dose response obtained. Statistical analysis of the results at day 11 indicates that all 3 treated groups are significantly different from the control group (1).

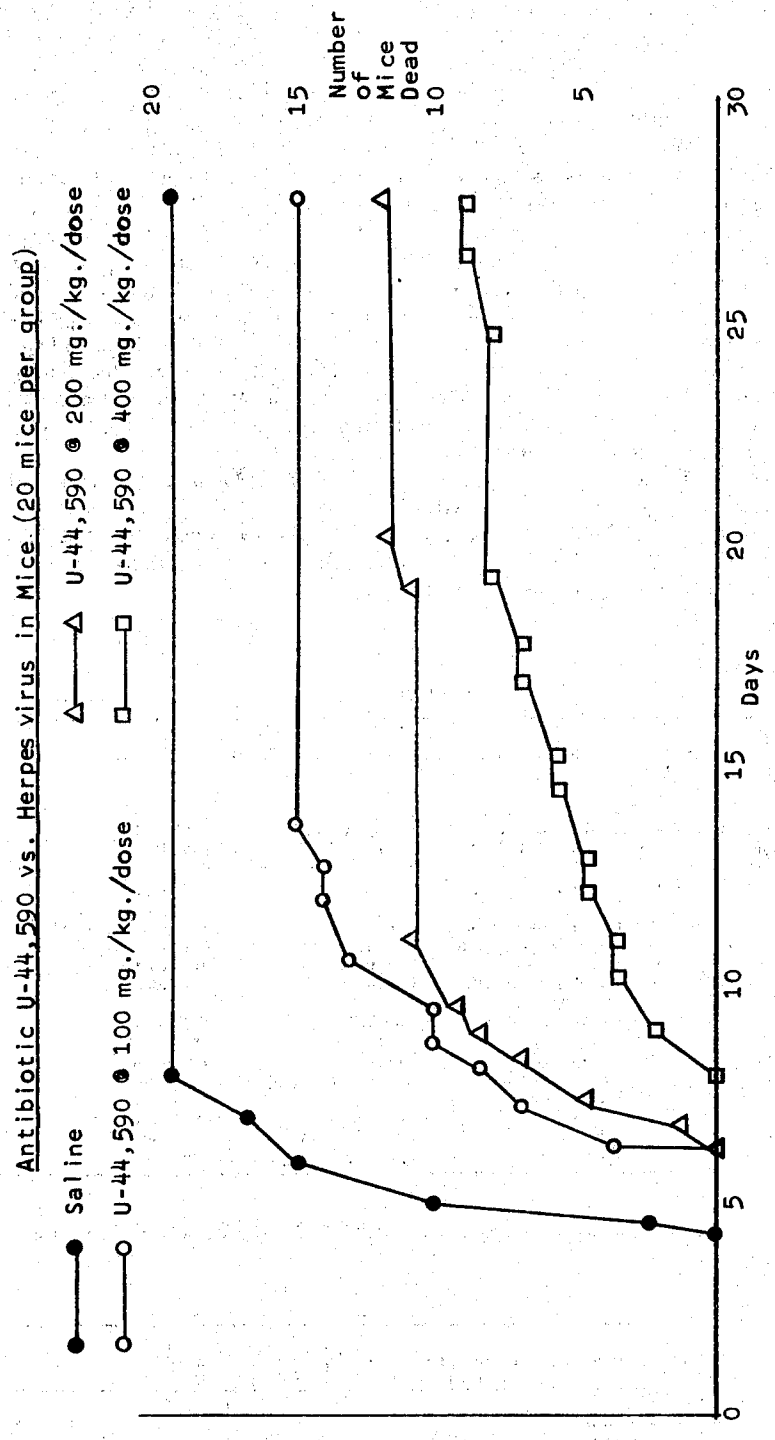
THE MICROORGANISM

The microorganism used for the production of U-44,590 is *Streptomyces platensis* var. *clarensis*, NRRL 8035. A permanent collection of the Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Illinois, U.S.A.

The microorganism of this invention was studied and characterized by Alma Dietz of the Upjohn Research Laboratories.

A new soil isolate with hygroscopic spore masses, but with smooth, hat-shaped (crescent) or brazil-nut-shaped (elliptical) spores, has been found to differ in certain characteristics from the type culture *Streptomyces platensis*. An outstanding difference of the new culture is the production of antibiotic U-44,590. The new isolate can be recognized as a variant of *Streptomyces platensis* by its cultural, microscopic, and biochemical characteristics. Therefore, it is proposed that this new isolate be designated *Streptomyces platensis* var. *clarensis* Dietz var. nova. Rule 7 of the International Code of Nomenclature of Bacteria [International Code of Nomenclature of Bacteria. 1966. Edited by the Editorial Board of the Judicial Commission of the International Committee on Nomenclature of Bacteria. Int. J. Syst. Bacteriol. 16: 459-490] was applied in designating the variety epithet.

Streptomyces platensis var. *clarensis* is compared with the type species *Streptomyces platensis* Pittenger and



Gottlieb [Shirling, E.B., and D. Gottlieb. 1968. Cooperative description of type cultures of Streptomyces III. Additional species descriptions from first and second studies. Int. J. Syst. Bacteriol. 18:279-392] [Tresner, H. D., E. J. Backus, and Jean A. Hayes. 1967. Morphological spore types in the *Streptomyces hygroscopicus*-like complex. Appl. Microbiol. 15:637-639] NRRL 2369, and two recently characterized strains: *Streptomyces platensis* NRRL 3593 [Evans, Ralph Henry Jr., and Samuel Owen Thomas. 1971. Antibiotics AH272α_2 and AH272β_2 and process for producing same. U.S. Patent 3,592,925] and *Streptomyces platensis* NRRL 3761 [Okuda, Tomohau, and Shigemi Awatagouchi. 1973. Antibiotics YL 704 and preparation thereof. U.S. Patent 3,718,742].

Color characteristics: Aerial growth white to yellow to gray. Moist black hygroscopic patches on some media. Melanin-negative. Appearance on Ektachrome [Dietz, A. 1954. Ektachrome transparencies as aids in actinomycete classification. Ann. N.Y. Acad. Sci. 60:152-154] is given in Table 1. Reference color characteristics are given in Tables 2 and 3. The new culture may be placed in the White (W), Yellow (Y), and Gray (GY) color series of Tresner and Backus [Tresner, H.

D., and E. J. Backus. 1963. System of color wheels for streptomycete taxonomy. Appl. Microbiol. 11:335-338].

Microscopic characteristics: Spore chains in tight spirals uncoiling to long open spirals. Spore chains spiral (S) in the sense of Pridham et al. [Pridham, T. G., C. W. Hesseltine, and R. G. Benedict. 1958. A guide for the classification of streptomycetes according to selected groups. Placement of strains in morphological sections. Appl. Microbiol. 6:52-79]. Spore hat-shaped (crescent) or Brazil-nut (elliptical) shaped. Spores are the platenis-type of Tresner and Backus [Tresner, H. D., E. J. Backus, and Jean A. Hayes. 1967. Morphological spore types in the *Streptomyces hygroscopicus*-like complex. Appl. Microbiol. 15:637-639]. Spore silhouette smooth by direct observation with the electron microscope. Spore surface ridged with surface markings by the carbon replication technique of Dietz and Mathews [Dietz, A. and J. Mathews. 1962. Taxonomy by carbon replication. I. An examination of *Streptomyces hygroscopicus*. Appl. Microbiol. 10:258-263].

Cultural and biochemical characteristics: See Table 4, infra.

Carbon utilization: Growth on carbon compounds was determined in the synthetic medium of Pridham and Gottlieb [Pridham, T. G., and D. Gottlieb. 1948. The utilization of carbon compounds by some Actinomycetales as an aid for species determination. J. Bacteriol. 56:107-114], Table 5 and in the synthetic medium of Shirling and Gottlieb [Shirling, E. B. and D. Gottlieb. 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16:313-340], Table 6.

Temperature: The cultures grew well as 18° – 37° C. on Bennett's, Czapek's sucrose, maltose-tryptone, and Hickey-Tresner agars. Optimum growth was at 24° – 37° C. The new culture and the type culture did not grow at 45° – 55° C. The cultures designated NRRL 3593 and NRRL 3761 grew at 45° C. but not at 55°C.

Antibiotic-producing properties: See Table 7, infra.

Source: Soil

Type culture: *Streptomyces platensis* Pittenger and Gottlieb NRRL 2364.

Type variety: *Streptomyces platensis* var. platensis NRRL 2364. Variety: Streptomyces platensis var. clarensis Dietz var. nova.

Table 1

Appearance of Streptomyces platensis Cultures on Ektachrome*

Agar medium	Determination	S. platensis var. clarensis NRRL 8035	S. platensis NRRL 2364	S. platensis NRRL 3593
Bennett's	S	Lavender-gray with black patches	Lavender-gray	Lavender-gray
	R	Cream-yellow-pink	Pink-tan	Yellow
Czapek's sucrose	S	Lavender-gray-white	Lavender-gray	Lavender-gray-white
	R	Cream-yellow	Pale pink-gray	Bright yellow
Maltose-tryptone	S	Lavender-gray with black patches	Lavender-gray	Lavender-gray
	R	Cream-yellow-pink	Pink-tan	Yellow-tan
Peptone-iron	S	Trace gray-white	Trace lavender-gray	Trace gray
	R	Cream yellow	Yellow	Yellow-tan
0.1% Tyrosine	S	Trace gray with black patches	Pale lavender-gray	Trace gray
	R	Pale yellow	Pink-tan	Colorless
Casein starch	S	Lavender-gray	Lavender-gray	Lavender-gray
	R	Pale cream	Pale gray-yellow	Cream

S = Surface
R = Reverse
*Dietz, A. 1954. Ektachrome transparencies as aids in actinomycete classification. Ann. N.Y. Acad. Sci. 60:152-154

Table 2

Reference Color Characteristics of Streptomyces platensis Cultures
Color Harmony Manual 3rd ed. 1948*

Agar Medium	Determination	S. platensis v. clarensis NRRL 8035	S. platensis NRRL 2364	S. platensis NRRL 3593	S. platensis NRRL 3761
Bennett's	S	2ih(g) to d(g)	2fe(m)	2dc(m)	2dc(m)
	R	2fb(g)	3ge(g)	1½ge(g)	2ec(g)
	P	—	—	—	—
Czapek's sucrose	S	a(g) to 2ba(g)	3ge(m)	2ba(m)	b(m)
	R	2fb(g)	3ge(g)	1½ic(g)	2ca(g)
	P	—	—	—	—
Maltose-tryptone	S	3fe(g)	2ge(m) to b(m)	2dc(m)	2dc(m)
	R	1½fb(g)	2ge(m)	1½ge(g)	2ge(m)
	P	—	—	—	—
Hickey-Tresner	S	3li(m) to 3fe(m)	3ig(m)	2dc(m)	3ge(m)
	R	lec(g) to 2ie(g)	2ec(m)	1½ge(g)	2ec(m)
	P	—	—	—	—
Yeast extract-malt extract (ISP-2)	S	3ig(m)	3ge(m)	2dc(m) to 2ba(m)	3ge(m)
	R	lec(g)	3ie(g)	1½lc(g)	2ge(m)
	P	—	—	1½lg(m)	—
Oatmeal (ISP-3)	S	3ig(m)	3ig(m)	3ge(m)	3li(m)
	R	lec(g)	3ie(g)	2ge(g)	2ca(g)
	P	—	—	—	—

Table 2 – Continued

Agar Medium	Determination	S. platensis v. clarensis NRRL 8035	S. platensis NRRL 2364	S. platensis NRRL 3593	S. platensis NRRL 3761
		Reference Color Characteristics of Streptomyces platensis Cultures Color Harmony Manual 3rd ed. 1948*			
Inorganic salts-starch (ISP-4)	S	3li(m) to 3ge(m)	3li(m)	2dc(m)	2ge(m)
	R	2fb(g)	2ca(m) to 3ie(m)	2ec(g)	2ec(g)
	P	2ge(g)	3ie-trace	—	—
Glycerol-asparagine (ISP-5)	S	b(m)	3ge(m)	2ba(g)	2ba(m)
	R	2ec(g)	2ge(m)	2fb(m)	3ca(g)
	P	—	—	—	—

*Jacobson, E., W. C. Granville, and C. E. Foss. 1948. Color harmony manual. 3rd ed. Container Corporation of America. Chicago, Illinois

Agar Medium	Determination	S. platensis v. clarensis NRRL 8035	S. platensis NRRL 2364	S. platensis NRRL 3593	S. platensis NRRL 3761
		NBS Circular 553, 1955**			
Bennett's	S	112m,113g	94g,112gm	93gm	93gm
	R	87g,89m	76gm	102g,105gm	90gm
	P	—	—	—	—
Czapek's sucrose	S	263gm,92gm	79m,94m	92gm	263gm,92gm
	R	87g,89m	79m,94m	87gm	89gm
	P	—	—	—	—
Maltose-tryptone	S	63gm	94m,109gm, 263m,264g	93gm	93gm
	R	87g	90gm	102g,105gm	90gm
	P	—	—	—	—
Hickey-Tresner	S	80m,95g	80m,95g	93gm	79m,94m
	R	121m,122g,91gm 94g,106g	90gm	102g,105gm	90gm
	P	—	—	—	—
Yeast extract-malt extract (ISP-2)	S	80m,95g	79m,94m	93gm,92gm	79m,94m
	R	121m,122g	76m,77g	87m	90gm
	P	—	--	—	—
Oatmeal (ISP-3)	S	80m,95g	80m,95g	79m,94m	80m,95g
	R	121m,122g	76m,77g	90gm	89gm
	P	—	—	—	—
Inorganic salts-starch (ISP-4)	S	80m,95g	80m,95g	93gm	94m,109g
	R	87g,89m	89gm,76m,77g	90gm	90gm
	P	90gm	76m,77g	—	—
Glycerol-asparagine (ISP-5)	S	263m,264g	79m,94m	92gm	92gm
	R	90gm	90gm	87g,89m	73gm
	P	—	—	—	—

**Kelly, K. L., and D. B. Judd. 1955. The ISCC-NBS method of designating colors and a dictionary of color names. U.S. Dept. Comm. Circ. 553

S = Surface

R = Reverse

P = Pigment

(g) = glossy chip surface

(m) = matte chip surface

Table 3

Color Code for Table 2

Color Harmony Manual 3rd ed. 1948* Color Chip	Color Name	NBS Circular 553, 1955** Color Chip	Color Name
a	White	263gm	White
b	Oyster white	263m	White
		264g	Light gray
lec	Light citron gray, putty	121m	Pale yellow-green
		122g	Grayish yellow-green
1 ½fb	Pastel yellow	87g	Moderate yellow
1 ½ge	Dusty yellow	102g	Moderate greenish yellow
		105gm	Grayish greenish yellow
1 ½ie	Light antique gold	87gm	Moderate yellow
1 ½lc	Gold		
1 ½lg	Golden olive	107g	Moderate olive
2ba	Pearl, shell tint	92gm	Yellowish white
2ca	Light ivory, eggshell	89gm	Light yellow
2dc	Natural, string	93gm	Yellowish gray
2ec	Biscuit, ecru, oatmeal, sand	90gm	Grayish yellow
2fb	Bamboo, buff, straw, wheat	87g	Moderate yellow
		89m	Pale yellow
2fe	Silver gray	94g	Light olive brown
		112gm	Light olive gray
2ge	Bamboo, chamois	90gm	Grayish yellow
2ge	Covert tan, griege	90gm	Grayish yellow
2ie	Light mustard tan	91gm	Dark grayish yellow
		94g	Light olive brown
		106g	Light olive
2ih	Dark covert gray	112m	Light olive gray
		113g	Olive gray

Table 3 – Continued

Color Code for Table 2			
Color Harmony Manual 3rd ed. 1948*		NBS Circular 553, 1955**	
Color Chip	Color Name	Color Chip	Color Name
3ca	Pearl pink, shell	73gm	Pale orange yellow
3fe	Silver gray	63gm	Light brownish gray
3gc	Light tan	76gm	Light yellowish brown
3ge	Beige, camel	79m	Light grayish yellowish brown
		94m	Light olive brown
3ie	Camel, maple sugar, tan	76m	Light yellowish brown
		77g	Moderate yellowish brown
3ig	Beige brown, mist brown	80m	Grayish yellowish brown
		95g	Moderate olive brown
3li	Beaver	80m	Grayish yellowish brown
		95g	Moderate olive brown

*Jacobson, E. W. C. Granville, and C. E. Foss. 1948. Color harmony manual. 3rd ed. Container Corporation of America, Chicago, Illinois

**Kelly, K. L., and D. B. Judd. 1955. The ISCC-NBS method of designating colors and a dictionary of color names. U.S. Dept. Comm. Circ. 553

Table 4

Cultural and Biochemical Characteristics of *Streptomyces platensis* Cultures

Medium	Determination	*S. platensis v. clarensis* NRRL 8035	*S. platensis* NRRL 2364	*S. platensis* NRRL 3593	*S. platensis* NRRL 3761
Agar					
Peptone-iron	S	Pale gray	—	Pale gray	Pale gray-pink
	R	Pale olive-tan	Tan	Yellow-tan	Yellow
	P	—	—	Yellow	—
	O	Melanin-negative	Melanin-negative	Melanin-negative	Melanin-negative
Calcium malate	S	Pale gray	Pale tan	Pale gray	Pale gray-white
	R	Gray	Pale tan	Gray	Gray
	P	—	—	—	—
	O	Malate not solubilized	Malate not solubilized	Malate not solubilized	Malate slightly solubilized
Glucose asparagine	S	Trace pale gray	Pale pink-tan	Pale gray-pink	Gray-white
	R	Pale olive-tan	Pink-tan	Pale yellow-tan	Pale salmon
	P	Pale yellow	—	—	—
Skim milk	S	Pale gray-pink	Pale pink on edge	Pale gray on edge	Trace white on edge
	R	Orange-tan	Pale orange	Pale orange	Yellow
	P	Orange-tan	Pale orange	Yellow-orange	Yellow
	O	Casein not solubilized	Casein not solubilized	Casein not solubilized	Casein not solubilized
Tyrosine	S	Pale gray-pink	White	Pale gray-cream	Pale gray-pink
	R	Yellow	Yellow	Yellow	Orange-tan
	P	Yellow	Yellow	Yellow	Orange-tan
	O	Tyrosine solubilized	Tyrosine solubilized	Tyrosine solubilized	Tyrosine solubilized
Xanthine	S	Pale gray-pink	White	Pale gray	Pale gray-pink
	R	Yellow	Pale yellow	Pale yellow	Yellow
	P	—	—	Pale yellow	—
	O	Xanthine slightly solubilized under growth	Xanthine slightly solubilized under growth	Xanthine not solubilized	Xanthine solubilized under growth
Nutrient starch	S	Pale gray-pink	Pale pink	Pale gray-pink	Pale gray-pink
	R	Yellow	Yellow	Lemon-yellow	Pale yellow
	P	—	—	Yellow	—
	O	Starch hydrolyzed	Starch hydrolyzed	Starch hydrolyzed	Starch hydrolyzed
Yeast extract-malt extract	S	Gray with black patches	Pale pink-tan	White	Pale gray-pink
	R	Yellow-tan	Red-tan	Deep yellow	Yellow
	P	Yellow	Yellow	Yellow	—
Bennett's	S	Lavender-gray with black patches	Lavender-gray with black patches	Pale lavender-gray	Heavy gray
	R	Olive-gray	Red-tan	Light olive	Cream-tan
	P	Pale yellow-olive	Pink-tan	Light olive	—
Czapek's sucrose	S	Gray-white	Gray-black in center, light gray on edge	White	Sparse gray-white
	R	Yellow	Gray-green	Yellow	Cream
	P	Pale yellow	—	Yellow	—
Maltose-tryptone	S	Lavender-gray	Pale gray	Gray-white	Gray-white
	R	Olive-green	Cream-yellow-pink	Olive	Pale olive-cream
	P	Olive-yellow	Pale yellow	Pale olive	Pale tan
Hickey-Tresner (modified)	S	Gray with black center	Black with gray edge	Deep gray-white	Gray with black patches
	R	Olive	Olive-tan	Light olive	Pale olive-cream
	P	—	Pale olive	Light olive	—
Peptone-yeast extract-iron (ISP-6)	S	Pale gray	—	Trace white	—
	R	Yellow	Pale yellow	Yellow	Yellow
	O	Melanin-negative	Melanin-negative	Melanin-negative	Melanin-negative

Table 4 – Continued

Cultural and Biochemical Characteristics of *Streptomyces platensis* Cultures

Medium	Determination	*S. platensis v. clarensis* NRRL 8035	*S. platensis* NRRL 2364	*S. platensis* NRRL 3593	*S. platensis* NRRL 3761
Tyrosine (ISP-7)	S	Gray with black patches	Gray with black patches	Pale gray-white	Pale salmon
	R	Pale olive	Pale cream-pink	Pale olive	Pale red-tan
	O	Melanin-negative	Melanin-negative	Melanin-negative	Melanin-negative
Gelatin					
Plain	S	White	Colorless vegetative growth	Colorless vegetative growth	Colorless vegetative growth
	P	Yellow	Trace tan	Trace brown	--
	O	Trace liquefaction	No liquefaction	No liquefaction	No liquefaction
Nutrient	S	Trace vegetative growth	Colorless vegetative growth	White	White
	P	—	—	Trace yellow	—
	O	Trace liquefaction	Trace to no liquefaction	No liquefaction	No liquefaction
Broth					
Synthetic nitrate	O	Trace bottom growth Nitrate not reduced to nitrite	Colorless surface pellicle and bottom growth Nitrate not reduced to nitrite	Trace bottom growth Nitrate not reduced to nitrite	Trace bottom growth Nitrate reduced to nitrite
Nutrient nitrate	O	Trace white surface ring Flocculent bottom growth Nitrate not reduced to nitrite	Trace white surface ring Flocculent bottom growth Nitrate not reduced to nitrite	Trace bottom growth Nitrate not reduced to nitrite	Trace bottom growth Nitrate reduced to nitrite
Litmus milk	O	surface pellicle Litmus reduced Partial peptonization coagulation pH 7.0	Surface pellicle No change pH 6.8	Surface pellicle Partial peptonization pH 7.0	Surface pellicle Partial peptonization pH 6.8

S = surface
R = reverse
P = pigment
O = other characteristics

Table 5

Utilization of Carbon Compounds by *Streptomyces platensis* Cultures in the Synthetic Medium of Pridham and Gottlieb*

	S. platensis v. clarensis NRRL 8035	*S. platensis* NRRL 2364	*S. platensis* NRRL 3593	*S. platensis* NRRL 3761
CONTROL (no carbon compound added)	(−)	(+)	(−)	(−)
1. D-Xylose	+	+	(+)	(+)
2. L-Arabinose	(+)	(+)	(+)	(−)
3. Rhamnose	(−)	(+)	(−)	(−)
4. D-Fructose	+	+	+	+
5. D-Galactose	+	+	+	+
6. D-Glucose	+	+	+	+
7. D-Mannose	+	+	+	+
8. Maltose	+	+	+	+
9. Sucrose	+	+	+	+
10. Lactose	+	+	+	+
11. Cellobiose	+	+	+	(−)
12. Raffinose	+	(+)	+	+
13. Dextrin	+	+	+	+
14. Inulin	(−)	(+)	(−)	(−)
15. Soluble starch	+	+	+	+
16. Glycerol	+	+	+	+
17. Dulcitol	(−)	(+)	(−)	(−)
18. D-Mannitol	+	+	+	+
19. D-Sorbitol	+	+	+	+
20. Inositol	+	+	+	+
21. Salicin	−	−	−	(−)
22. Phenol	−	−	−	−
23. Cresol	−	−	−	−
24. Na Formate	−	−	−	−
25. Na Oxalate	(−)	(−)	−	−
26. Na Tartrate	(−)	(−)	(−)	(−)
27. Na Salicylate	−	−	−	−
28. Na Acetate	(+)	(+)	(−)	(−)
29. Na Citrate	+	(+)	(+)	(+)
30. Na Succinate	+	(+)	(+)	(+)

Table 6

Utilization of Carbon Compounds by *Streptomyces platensis* Cultures in the Synthetic Medium of Shirling and Gottlieb*

	S. platensis v. *clarensis* NRRL 8035	*S. platensis* NRRL 2364	*S. platensis* NRRL 3593	*S. platensis* NRRL 3761
CONTROLS				
Negative-basal medium	Slight growth	Slight growth	Slight growth	Slight growth
Positive-basal medium plus D-glucose	Good growth	Good growth	Good growth	Good growth
CARBON COMPOUNDS				
L-Arabinose	++	++	++	+
Sucrose	++	++	++	++
D-Xylose	+	+	±	±
Inositol	++	++	++	++
D-Mannitol	++	++	++	++
D-Fructose	++	++	++	++
Rhamnose	±	±	±	±
Raffinose	++	++	++	++
Cellulose	—	—	—	—

++ Strong utilization
+ Positive utilization
± Utilization doubtful
— Utilization negative

*Shirling, E. B., and D. Gottlieb. 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16:313–340

Table 7

Antibiotics Produced by *Streptomyces platensis* Cultures

Antibiotic	*S. platensis* v. *clarensis* NRRL 8035	*S. platensis* NRRL 2364	*S. platensis* NRRL 3593	*S. platensis* NRRL 3761
U-44,590	+			
Oxytetracycline		+		
AH272α_2			+	
AH272β_2			+	
YL-704				+

The new compound of the invention is produced when the elaborating organism is grown in an aqueous nutrient medium under submerged aerobic conditions. It is to be understood, also, that for the preparation of limited amounts surface cultures and bottles can be employed. The organism is grown in a nutrient medium containing a carbon source, for example, an assimilable carbohydrate, and a nitrogen source, for example, as assimilable nitrogen compound or proteinaceous material. Preferred carbon sources include glucose, brown sugar, sucrose, glycerol, starch, cornstarch, larctose, dextrin, molasses, and the like. Preferred nitrogen sources include cornsteep liquor, yeast, autolyzed brewer's yeast with milk solids, soybean meal, cottonseed meal, cornmeal, milk solids, pancreatic digest of casein, fish meal, distiller's solids, animal peptone liquors, meat and bone scraps, and the like. Combinations of these carbon and nitrogen sources can be used advantageously. Trace metals, for example,, zinc, magnesium, manganese, cobolt, iron, and the like, need not be added to the fermentation media since tap water and unpurified ingredients are used as components of the medium prior to sterilization of the medium.

Production of the compound of the invention can be effected at any temperature conducive to satisfactory growth of the microorganism, for example, between about 18° and 40° C., and preferably between about 20° and 32° C. Ordinarily, optimum production of the compound is obtained in about 5 to 15 days. The medium normally remains neutral during the fermentation. The final pH is dependent, in part, on the buffers present, if any and in part on the initial pH of the culture medium.

When growth is carried out in large vessels and tanks, it is preferable to use the vegetative form, rather than the spore form, of the microorganism for inoculation to avoid a pronounced lag in the production of the new compound and the attendant inefficient utilization of the equipment. Accordingly, it is desirable to produce a vegetative inoculum in a nutrient broth culture by inoculating this broth culture with an aliquot from a soil, liquid N_2 agar plug, or a slant culture. When a young, active vegetative inoculum has thus been secured, it is transferred aseptically to large vessels or tanks. The medium in which the vegetative inoculum is produced can be the same as, or different from, that utilized for the production of the new compound, so long as a good growth of the microorganism is obtained.

A variety of procedures can be employed in the isolation and purification of the compound of the subject invention, for example, solvent extraction, partition chromatography, silica gel chromatography, liquid-liquid distribution in a Craig apparatus, absorption on resins, and crystallization from solvents.

In a preferred recovery process the compound of the subject invention is recovered from the culture medium by separation of the mycelia and undissolved solids by conventional means, such as by filtration or centrifuga-

tion. The antibiotic is recovered from the filtered or centrifuged broth by adsorption on activated carbon. The activated carbon is then washed with water to remove some impurities. This is followed by elutions with acetone: water solutions which remove the antibiotic from the activated carbon. The acetone in the eluates is removed, advantageously by evaporation, and the remaining aqueous residue is lyophilized to afford a crude preparation of antibiotic U-44,590.

A preferred purification procedure is to subject a crude preparataion of U-44,590, as described above, to chromatography on silica gel from which U-44,590 is eluted. Fractions which show activity against the bacterium *Klebsiella pneumoniae* on a standard agar plate test, are pooled and taken to dryness to yield a relatively pure preparation of U-44,590. Further purification is achieved by acetylation to a crystalline diacetate derivative of U-44,590. Zemplen [G. Zemplen and E. Pacsu, Ber., 62, 1613 (1929)] de-esterification (transesterification) with sodium methoxide in methanol, and neutralization of the catalytic amount of base with carbon dioxide gives the free antibiotic U-44,590 which crystallizes readily from methanol-ethyl acetate to give a pure preparation of U-44,590.

Antibiotic U-44,590 is active against *Streptococcus hemolyticus* and, thus, can be used to disinfect instruments, utensils or surfaces when contaminated with this microorganism, where the inactivation of this microorganism is desirable. Also, U-44,590 is active against *Escherichia coli* and can be used to reduce, arrest, and/or eradicate slime production in papermill systems because of its antibacterial action against this bacterium. Antibiotic U-44,590 can also be used to prolong the life of cultures of *Trichomonas foetus*, *Trichomonas hominis*, and *Trichomonas vaginalis* by freeing them of *Escherichia coli* contamination. Further, U-44,590 can be used to inhibit the growth of *E. coli* in hospital flower vases where it has been reported to exist and present a hazard to hospital patients. See Clinical Medicine, February, 1974, Page 9.

Novel acyl derivatives of U-44,590, as disclosed herein, can be used for the same antibiotic purposes as U-44,590 in environments possessing means to deacylate the compound to U-44,590. Thus, the acyl derivatives of U-44,590 can be used to treat laboratory mice infected with a Gram-negative bacteria, for example *E. coli*, as disclosed herein. Further, acyl derivatives of U-44,590 can be used, advantageously, to upgrade U-44,590. This is accomplished by acylating U-44,590, recovering the acylated compound relatively free of impurities, then deacylating the acylated U-44,590 in a more purified form.

The following examples are illustrative of the process and products of the present invention but are not to be construed as limiting. All percentages are by weight and solvent mixture proportions are by volume unless otherwise noted.

EXAMPLE 1

Part A. Fermentation

A soil stock of *Streptomyces platensis* var. *clarensis*, NRRL 8035 is used to inoculate a series of 500-ml. Erlenmeyer flasks, each containing 100 ml. of sterile seed medium consisting of the following ingredients:

Glucose monohydrate	10 Gm/l
Bacto Peptone (Difco)	10 Gm/l
Bacto Yeast Extract (Difco)	2.5 Gm/l
Deionized water	Balance

The flasks are grown for 2 days at 28° C. on a Gump rotary shaker operating at 250 r.p.m.

Seed inoculum, described above, is used to inoculate a series of 500 ml. Erlenmeyer flasks each containing 100 ml. of sterile fermentation medium. The inoculation rate is 5 ml. of seed inoculum per 100 ml. of fermentation medium. The fermentation medium consists of the following ingredients:

Brer Rabbit Molasses (RJR Foods Inc., N.Y., N.Y. 10017)	20 ml
Yeast Extract (Difco) Detroit. Michigan	1 Gm/l
Glucose monohydrate	10 Gm/l
Dextrin (Corn Products Co. International Inc., International Plaza, Englewood Cliffs, New Jersey 07632)	10 Gm/l
Proteose Peptone No. 3 (Difco)	10 Gm/l
Tap water q.s.	Balance

The presterilization pH is 7.0. The inoculated fermentation flasks are incubated at a temperature of 28° C. on a Gump rotary shaker operating at 250 r.p.m. with a 2½ inch stroke. Ucon antifoam (a synthetic defoamer supplied by Union Carbide, N.Y., N.Y.) is used if needed. Harvest is usually after 5 to 12 days of fermentation.

The antibiotic titer of the fermentation beer can be monitored by an agar plate disc assay using the bacterium *Klebsiella pneumoniae*. This bacterium is inoculated into the assay agar (Streptomycin Assay Agar, BBL, Cockeysville, Maryland, 21030) of the following composition:

Beef Extract	1.5 Gm/l
Yeast Extract	3.0 Gm/l
Gelysate Peptone, supplied by Baltimore Biological Laboratories	6.0 Gm/l
Agar	15.0 Gm/l
Deionized water adjust pH to 7.9	Balance

Sterilize at 121° C. (15 No. steam pressure) for 15 minutes.

Phosphate buffer (0.1N pH 6.0) is used as the diluent. The agar plates are incubated at 37° C. for 16–18 hours. Presence of antibiotic U-44,590 is evidenced by the zone of inhibition around a paper disc to which a fermentation sample was previously applied. The diameter of the zone of inhibition reflects the potency of the antibiotic sample. Thus, a 20 mm. zone of inhibition using a 12.7 mm. paper disc to which 0.08 ml. of antibiotic sample has been applied is expressed as one bio unit per ml. (1 BU/ml.).

Part B. Recovery

Whole fermentation beer (ca 1600 ml. assaying 5 BU/ml. against *K. pneumoniae*), obtained as described above, is filtered using diatomaceous earth as a filter aid. The filter cake is washed with water. The clear beer and wash (1800 ml.) is then passed through an activated carbon column. The column measures 2.8 × 44 cm. and contains 126 grams of activated carbon. The carbon column is washed with 1750 ml. water and the wash is discarded. The column is then washed with 1 liter each of of 1%, 2% and 5% acetone:water concentration. These eluates are also discarded. The column is then eluted with 1 liter each of 10%, 25% and 50% acetone:water concentration. These eluates, which contain antibiotic U-44,590, are pooled and the acetone is removed on a rotatory evaporator at 30° C./15

mm. Hg. The resulting acetone-free preparation is shell-frozen to an aqueous residue and then lyophilized, yield, 3.55 grams assaying 2 BU/mg. of U-44,590 against *K. pneumoniae*. This preparation, labeled for convenience as *Solid A*, is then subjected to further recovery procedures as follows.

A silica gel (Merck-Darmstadt Car. 7734) column is prepared from 420 grams of silica gel packed in methanol: chloroform (1:1 v/v.). The column measures 3.8 × 88 mm. *Solid A*, obtained as described above, is added on the top of the column and the column is then eluted with methanol: chloroform (1:1 v/v). Active fractions, as determined by the above-described *K. pneumoniae* assay, are pooled and the solvent is removed from said pooled fractions by use of a rotary evaporator at 30° C./15 mm. Hg.; yield, 830 mg. assaying 7.5 BU/mg. of antibiotic U-44,590.

Part C. Purification No. 1

A preparation of antibiotic U-44,590, obtained as described above in Part B., is subjected to chromatography on silica gel using the solvent system ethyl acetate: methanol (6:1 v/v) to give a purer preparation containing U-44,590. The procedure for this purification step is as follows:

A column of silica gel (Merck-Darmstadt, 115 grams/gram of the U-44,590 preparation being chromatographed) in ethyl acetate: methanol (6:1 v/v) is prepared by pouring a slurry of silica in the solvent into the column to give a height-diameter ratio of 10:1 after being packed. The U-44,590 preparation, obtained as described above in Part B, is dissolved in methanol, silica is added (three times the weight of the U-44,590 preparation used), and this is then taken down to a dry powder on a rotatory evaporator at 40 /15 mm. Hg. The resulting dry solid is added to the top of the silica column through a small head of the solvent ethyl acetate:methanol (6:1 v/v). After a forerun of 4 liters, 50 ml. fractions are collected and assayed for activity against *K. pneumoniae*. Active fractions are also tested for solids content. Fractions greater than 50 BU/mg are pooled and then taken to dryness in a rotary evaporator at 40° C./7 mm. Hg. to yield a syrup. Fractions and their *K. pneumoniae* (K.p.) activity and solids from a usual run are as follows:

Fraction Number	Zone of Inhibition (using 12.7 mm. discs)	Wt. of solid in Fraction (mgm)
110	16	34.5
115	30	
120	33	30.9
125	35	
130	37	40.8
135	37	
140	36	35.7
145	35	
150	35	27.0
155	34	
160	33	21.7
165	32	
170	31	21.8
175	30	
180	29	21.8
185	28	
190	28	17.9
195	28	
200	27	17.5
205	27	
210	26	14.4
215	26	
220	26	12.1
225	26	
230	26	10.0
235	25	
240	25	11.5
245	24	
250	24	11.7
255	23	
260	23	12.6
265	23	
270	23	15.4

Fractions 120–180, incl. are pooled and taken to dryness on a rotatory evaporator at 40°/7 mm. Hg. to give a syrup, wt. 2.66g, assaying 54 K.p. BU/mg (Fractions 181–240, incl. give a syrup, 830 mg. 32 BU/mg., and fractions 241–300, incl. give a syrup, wt. 710 mg., assaying 11 BU/mg.) The standard assayed 4 BU/mg. against the usual assay for this standard of 6 BU/mg.

Part D. Purification No. 2

The preparations of U-44,590 obtained as described in Part C., can be further purified to a preparation of essentially pure U-44,590 by passage over another silica gel column using this time the solvent system methanol: methylene chloride (1:8 v/v). The procedure is as follows:

A U-44,590 preparation, as obtained in Part C., (2,28 grams) is dissolved in methol and 7 grams of silica gel, as described in Part C., is added. The solvent from this mixture is removed on a rotary evaporator at 40° C./7 mm. Hg. The resulting solid is added to a column of silica get [750 g., 4.8 × 96 cm, hold-up volume 1500 ml., made up in MeOH-CH_2Cl_2 (1:8 v/v)]. A forerun (1100 ml.) is collected, followed by 50 ml. fractions. Fractions 141–200, inclusive, weight 390 mg. when taken to dryness in the form of a syrup. This material is shown to be almost pure U-44,590 by thin layer chromatography (tlc).

The tlc is conducted on silica gel plates using the solvent system MeOh-Ch_2Cl_2 (1:9 v/v). Zones of the antibiotic are detected by spraying the plates with $10^{-}{}_4$/M-$nO^{-}{}_4$ spray, and with 50% aq. H_2SO_4 followed by heating at 110° C. for ca 10 min. The Rf of the active material in this solvent system is 0.11.

Part E. Purification No. 3

The preparation of antibiotic U-44,590 obtained in Part D can be further purified by acetylation of the preparation followed by deacetylation and crystallization. The procedure for acetylation is as follows:

A sample (ca. 22 g.) of U-44,590 preparation, prepared as described in Part D and assaying 160 BU/mg, is dissolved in pyridine (300 ml), and to this solution, stirred magnetically, is added acetic anhydride (150 ml) over the course of 45 min. After standing overnight at room temperature, volatile materials are removed as completely as possible on a rotatory evaporated at 40°/15 mm. Hg., and finally under high vacuum, to give a tan syrup.

This syrup is stirred with CH_2Cl_2 (200 ml), and a colorless, flocculent precipitate is removed by filtration and washed with CH_2Cl_2 until the washings are colorless. The precipitate is discarded. THe combined filtrate and washings are washed with aqueous HCl (N/20, 100 ml) twice, the aqueous layer being acidic after the second wash. The aqueous layers are dis-

carded. The organic phase is then washed with water (100 ml), saturated aqueous $NdHCO_3$ (100 ml), again with water (100 ml), and dried (NA_2SO_4). The aqueous layers are discarded.

Removal of solvent on a rotary evaporator at 40° and 15 mm. Hg. gives a dark syrup (21.10 g), which is dissolved in EtOAc (50 ml) by warming on a steam-bath. On cooling, crystallization occurs; the solid is removed by filtration, washed with EtOAc, and dried at 60°.15 mm. Hg., to give essentially pure 3',5'-di-O-acetylated U-44,590 (12.0l g., m.p. 123°–124.5° C.). Recrystallization from the same solvent gives U-44,590 diiacetate, having a melting point 124°–125° C. This compound is then labeled U-44,474.

U-44,474 is deacetylated to afford essentially pure U-44,590 by the Zemplen procedure which is as follows.

The crystalline diacetate U-44,474 (24.90 g) is stirred magnetically in methanol (400 ml), and methanolic sodium methoxide (Stauffer Chem. Co. 25%, 5 drops) is added. Stirring is continued till the solid has dissolved (Drierite tube), and the solution allowed to stand at room temperature for about 2 hours. Solid carbon dioxide, in small pieces, is then added cautiously, with stirring, to neutralize the methoxide, and the solvent is removed on a rotatory evaporator at 40° and 15 mm. Mg., giving a colorless oil.

The residue is dissolved in methanol (50 ml) by warming on a steambath and diluted with ethyl acetate (50 ml). Crystallization occurs on cooling. The solid (12.39 g) is collected on a sintered filter at the pump, washed with methanol, and dried in a vacuum oven at 60°/15 mm. Hg. Antibiotic U-44,590 crystallizes in colorles prismatic needles, m.p. 141°–142°. Removal of solvent from the filtrate plus washings on the evaporator and crystallization from methanol-ethyl acetate gives additional material (1.91 g, m.p. 140.5°–141.5°).

EXAMPLE 2

The acylating procedure described in Example 1, Part E can be substituted by acylating U-44,590 with any readily-available acylating agent to give acylated U-44,590. This acylated U-44,590 product can then be deacylated by methods well known in the art to yield a purified preparation of U-44,590. Readily-available acylating agents which can be used to acylate U-44,590, and which are within the scope of this invention, are as disclosed in U.S. Pat. No. 3,426,012, Columns 5 and 6.

EXAMPLE 3

As disclosed in Example 2, various acylates of U-44,590 can be made, and these acylates are useful to upgrade U-44,590. By following the procedure of Example 1, Part E, the 3',5'-di-esters of U-44,590 are formed.

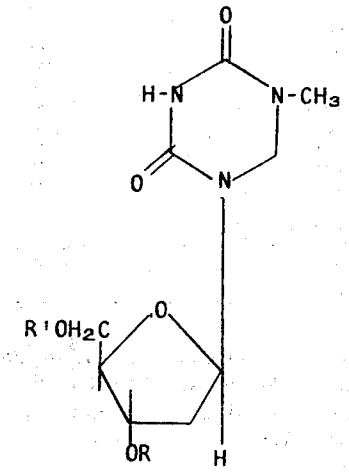
The 5'-mono-esters can be formed by standard procedures using a minimum amount of acylating agent.

The 3'-mono-esters and phosphate can be formed by tritylating U-44,590 to give the 5'-trityl derivative, acylating this compound with the desired acylating agent, selected from those disclosed above, to give the 3'-mono-ester 5'-trityl derivative, which then can be converted to the 3'-mono-ester by removal o the trityl group. The tritylation procedure disclosed in U.S. Pat. No. 3,426,012, Columns 4 and 5, or other standard tritylation procedures can be employed. The trityl group can be removed by using the procedure disclosed in U.S. Pat. No. 3,426,012, Column 6.

EXAMPLE 4

The 5'-phosphate of U-44,590 can be prepared by procedures as disclosed in the work of D. Mitsunobu, K. Kato, and J. Kimura [J. Amer. Chem. Soc., 91, 6510 (1969)]. This compound can be used for the same purposes as U-44,590.

The compounds, described above, being the derivatives of U-44,590 which are within the scope of the subject invention, can be shown by the following structural formula



wherein R and R' are selected from the group consisting of a carboxylic acid acyl radical of from 2 to 18 carbon atoms inclusive; or a halo-, nitro-, hydroxy-, amino-, cyano-, thiocyano-, and lower alkoxy-substituted hydrocarbon carboxylic acid acyl radical of from 2 to 18 carbon atoms, inclusive; R is hydrogen and R' is as defined above or phosphate; or R' is hydrogen and R is a carboxylic acid acyl radical of from 2 to 18 carbon atoms, inclusive; or a halo-, nitro-, hydroxy-, amino-, cyano-, thiocyano-, and a lower alkoxy-substituted hydrocarbon carboxylic acid acyl radical of from 2 to 18 carbono atoms, inclusive, or phosphate.

Additional characterization data for U-44,474, prepared as disclosed in Example 1, Part E, is as follows:

Elemental Analysis: Calcd. for $C_{13}H_{19}N_3O_7$ Found: C, 47.41; H, 5.82; N, 12.76; O, 34.0l.

Molecular Weight: 329 (Determined by mass spectrometry)

Infrared Absorption Spectra: U-44,474 has a characteristic infrared absorption spectrum when suspended in a mineral oil mull. Peaks are observed at the following wave lengths expressed in reciprocal centimeters:

Band Frequency (Wave Numbers)	Intensity
3210	M
3080	M
2960 (oil)	S
2930 (oil)	S
2860 (oil)	S
1750	S
1732	S
1702	S
1520	S
1468 (oil)	S

-Continued

Band Frequency (Wave Numbers)	Intensity
1411	M
1379 (oil)	S
1358	W
1327	W
1318	W
1310	W
1298	W
1280	M
1250	S
1227	S
1188	M
1148	W
1113	W
1097	S
1057	M
1030	S
1011	M
1000	M
987	M
964	M
957	M
950	M
936	W
892	M
863	M
828	M
791	M
775	M
755	M
740	W
721 (oil)	W
675	W
668	W

U-44,474 also has a characteristic infrared absorption spectrum when pressed in a KBr disc. Peaks are observed at the following wave lengths expressed in reciprocal centimeters:

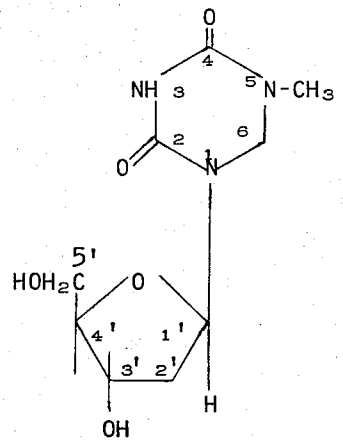
Band Frequency (Wave Numbers)	Intensity
3420 (water)	W
3210	M
3080	M
2970	W
2960	W
2930	W
2880	W
2830	W
1750	S
1732	S
1703	S
1517	M
1468	S
1410	M
1382	M
1370	M
1248	S
1227	S
1186	M
1145	W
1095	M

-Continued

Band Frequency (Wave Numbers)	Intensity
1055	M
1030	M
1011	M
999	M
986	M
963	M
949	M
932	W
890	M
863	M
825	M
790	M
773	M
754	M
739	W
668	W

We claim:

1. A process for preparing antibiotic U-44,590 having the structure



which comprises cultivating *Streptomyces platensis* var. *clarensis*, having the identifying characteristics of NRRL 8035, in an aqueous nutrient medium under aerobic conditions until substantial antibiotic activity is imparted to said medium by the presence of antibiotic U-44,590.

2. A process, according to claim 1, wherein said aqueous nutrient medium contains a source of assimilable carbohydrate and assimilable nitrogen.

3. A process, according to claim 1, wherein said antibiotic U-44,590 is isolated from the fermentation broth.

* * * * *

UNITED STATES PATENT OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 3,907,643

DATED : September 23, 1975

INVENTOR(S) : Clarence DeBoer and Brian Bannister

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 1, line 19, for "According" read -- Accordingly --; line 50, for " 3440 M " read --

3440 M 3440 M

3400 M 3400 M

--.

Column 3, line 48, for "Diffo" read -- Difco --; line 50, for "Blooc" read -- Blood --; line 55, for "10^{3}" read -- 10^{-3} --. Column 4, line 31, for "recorder" read -- recorded --; line 44, for "A permanent" read -- A subculture of this microorganism can be obtained from the permanent --. Column 5, line 60, for "18.279-392]" read -- 18:279-392 --. Column 8, line 10, for "as" read -- at --. Columns 13 and 14, at bottom of Table 5, Missing:

\+ = Good utilization
(+) = Poor utilization
(-) = Doubtful utilization
\- = No utilization

* Pridham, T.G., and D. Gottlieb. 1948. The utilization of carbon compounds by some Actinomycetales as an aid for species determination. J. Bacteriol. 56:107-114.

Column 15, line 47, for " as assimilable" read -- an assimilable --; line 50, for "larctose" read -- lactose --; line 62, for "to steriliization" read -- to sterilization --. Column 17, line 52, for "U-44,590 in" read -- U-44,590 to give U-44,590 in --. Column 18, line 40, for "(15 No. " read -- (15 # --; line 63, for "each of of 1%" read -- each of a 1% --; line 65, for "each of 10%" read -- each of a 10% --. Column 19, line 7, for "Car. 7734)" read -- Cat. 7734) --; line 35, for "40/15 mm." read -- 40°/15 mm. --; line 42, for "rotary" read -- rotatory --. Column 20, line 42, for "MeOh-" read -- MeOH- --; line 58, for "evaporated" read -- evaporator --. Column 21, line 2, for

UNITED STATES PATENT OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 3,907,643

DATED : September 23, 1975

INVENTOR(S) : Clarence DeBoer and Brian Bannister

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

"$NdHCO_3$" read -- $NaHCO_3$ --; line 3, for "(NA_2SO_4)" read -- (Na_2SO_4) --; line 5, for "rotary" read -- rotatory --; line 9, for "60°.15 mm." read -- 60°/15 mm. --; lines 34-35, for "colerles" read -- colorless --; line 68, for "o" read -- of --.

Signed and Sealed this

thirteenth Day of *April 1976*

[SEAL]

Attest:

RUTH C. MASON
Attesting Officer

C. MARSHALL DANN
Commissioner of Patents and Trademarks