IV and benzyl chloride, by the method described for the preparation of VIIIb.

Anal. Calcd for $C_{13}H_{15}NO_3S$: C, 58.85; H, 5.70; N, 5.28; O, 18.09; S, 12.08. Found: C, 58.96; H, 5.79; N, 5.15; O, 18.30; S, 12.27.

2-(Carboxy)-5-[N-(3,4-dichlorobenzyl)carbamoyl]tetrahydrothiophene (VIIIc) was similarly obtained in 77% yield (mp 98.5–99.5° from methanol–water).

Anal. Calcd for $C_{13}H_{13}Cl_2O_3NS$: C, 46.71; H, 3.91; Cl, 21.21; N, 4.23. Found: C, 46.70; H, 3.91; Cl, 21.92; N, 4.23.

All the compounds in the VIII series had infrared absorption bands at 3330, 3160, 1730, and 1655–1635 cm^{-1}.

8-Thia-3-azabicyclo[3.2.1]octane-2,4-Dione 8,8-dioxide (IX).—A mixture of IV (4.0 g, 0.025 mole) and 30% H_2O_2 (3.6 ml) in glacial acetic acid (15 ml) was gradually heated to 70° and held at this temperature for 2 hr. On cooling slowly, white crystalline material (2.4 g, 50%) separated. Two recrystallizations from methanol gave the analytically pure IX: mp 284.5°; ν_{max} 3200, 3100, 1740, 1690, 1325, and 1170 cm^{-1}.

Anal. Calcd for $C_6H_7NO_4S$: C, 38.09; H, 3.72; N, 7.40; O, 33.83; S, 16.94. Found: C, 38.37; H, 3.66; N, 7.75; O, 33.70; S, 16.59.

Structure of Antibiotic C-73X[1]

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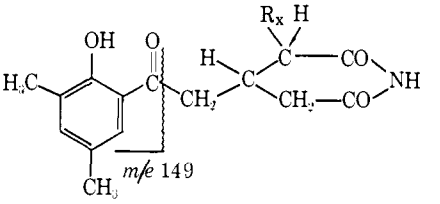
Three biologically active compounds have been isolated from the fermentation products of a strain of *Streptomyces griseus* originally obtained in a soil sample and included in the Culture Collection of the Squibb Institute for Medical Research (SC 3675). One compound was the previously identified actidione,[3] a second was the known antibiotic C-73[4] [3-(2-hydroxy-3,5-dimethylphenacyl)glutarimide], and a third, designated by the authors as C-73X, was found to be closely related to antibiotic C-73. Based on elemental analysis and ultraviolet, infrared, nmr, and mass spectra, the structure of C-73X is assigned as 2-hydroxy-3-(2-hydroxy-3,5-dimethylphenacyl)glutarimide. The procedure for isolation of C-73X is shown in Chart I.

The ultraviolet spectrum of C-73X showed two λ_{max}^{MeOH} values similar to those for C-73. Structural similarity of the two compounds was also demonstrable from the infrared, nmr, and mass spectra.

Relevant nmr spectral data for C-73, C-73X, and their acetates are shown in Table I. In addition to those proton resonances assignable to the structure of C-73, C-73X shows a resonance [τ 5.94, $(CD_3)_2SO$] attributable to a tertiary proton on a carbon atom to which is connected an electronegative atom. The acetylated derivative of C-73X likewise has a resonance attributable to the corresponding tertiary proton [τ 4.59, $CDCl_3$].

In the infrared spectrum, C-73X shows a band at 2.95 μ (KBr), indicating the presence of a hydroxyl group, in addition to those bands present in the infrared spectrum of C-73.

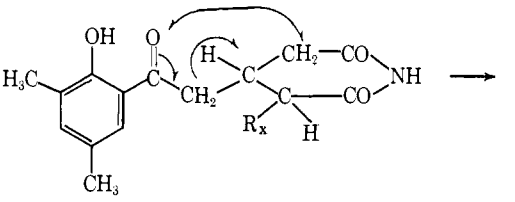
The mass spectra of C-73 and C-73X are shown in Figure 1. The parent ion (M^+) of C-73 appears at *m/e* 275. The M^+ of C-73X appears at *m/e* 291, leading us to conclude that C-73X is a hydroxylated derivative of C-73. The prominent (M − 18) peak at *m/e* 273 in the mass spectrum of C-73X suggests a facile dehydration of this compound. The most intense peak in the mass spectra of both compounds is at *m/e* 149, which suggests a fragment formed by cleavage of the bond to the aryl carbonyl (I).



I

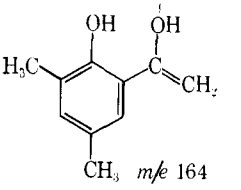
C-73, R_x = H
C-73X, R_x = OH

The peak at *m/e* 164 in the mass spectra of both compounds is attributable to the fragment formed by the proton rearrangement reaction shown below (II).

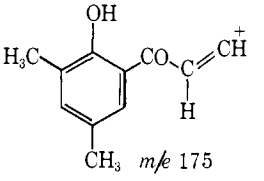


II

C-73, R_x = H
C-73X, R_x = OH



The peak at *m/e* 175, also shown by the mass spectra of both compounds, is probably attributable to the fragment formed in a proton rearrangement (III).



III

The intensity ratios for the peaks at *m/e* 164 and 175 are similar for both compounds. These similarities of intensity suggest that the part of the molecule between the aromatic ring and the glutarimide ring is the same in both compounds. Were the hydroxyl group in C-73X on the carbon α to the aryl carbonyl group, rather than on the carbon α to the imido carbonyl group, a significant change in the intensity ratio of the *m/e* 175 and 164 peaks from that found in C-73 might have been expected, arising from a different fragmentation process.

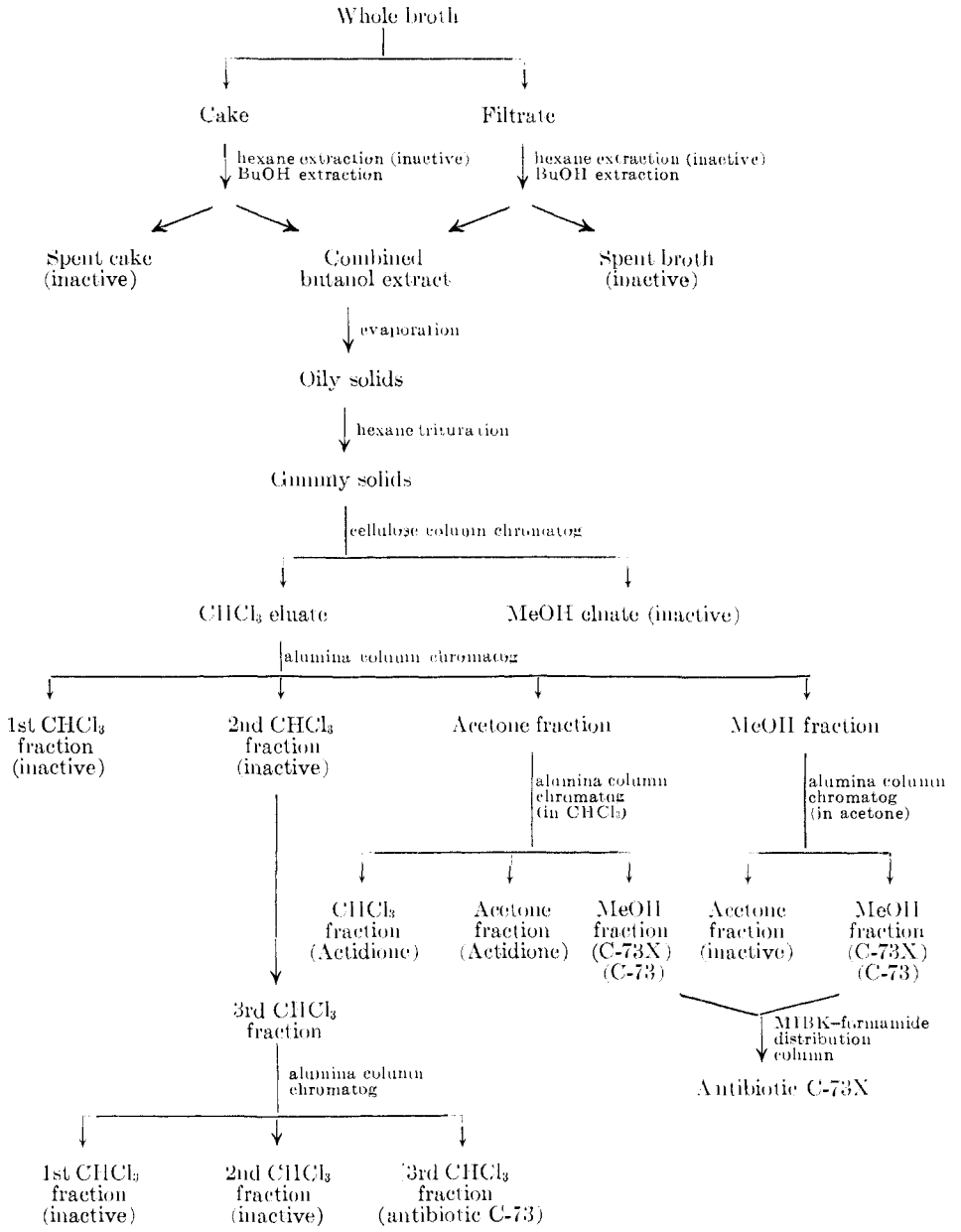
(1) This work was carried out as part of the effort on Cancer Chemotherapy National Service Center contract SA-43-ph-3041.

(2) Olin Mathieson Chemical Corp., New Haven, Conn.

(3) J. H. Ford and B. E. Leach, *J. Am. Chem. Soc.*, **70**, 1223 (1948).

(4) K. V. Rao, *J. Org. Chem.*, **25**, 661 (1960).

CHART I
PROCEDURE FOR ISOLATION OF ANTIBIOTIC C-73X



The doublet at *m/e* 217 and 216 in the mass spectrum of C-73 appears to involve the simultaneous rearrangement of one or two protons, the loss of the $-CH_2CONH-$ group, and the loss of one or two protons. The mass spectrum of C-73X has a similar doublet at *m/e* 217 and 216, but the relative intensities within the doublet are reversed. Loss of two protons would give rise to the peaks at *m/e* 215 and 214 which are common to the mass spectra of C-73 and C-73X, but are more intense in the latter. This greater intensity of the 215, 214 doublet in C-73X may be attributable to a substantial thermal dehydration of C-73X before impact.[5]

The greater intensity of the peak at *m/e* 112 (glutarimide fragment) in C-73 than in C-73X also reflects a secondary fragmentation of the dehydrated glutarimide ring in C-73X and provides further substantiation of the location of the hydroxyl group of C-73X on the carbon atom α to the imido carbonyl group.

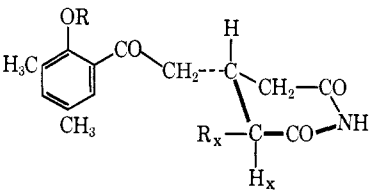
Assuming the glutarimide ring to be in the half-chair conformation, it would follow from the nmr spectral data that the vicinal protons are *trans* diaxial and other functional groups attached to the ring are *trans* equatorial. Antibiotic C-73X and its diacetate exhibit one-proton multiplets at τ 4.94, $(CD_3)_2SO$, and at τ 4.59, $CDCl_3$, respectively. These multiplets, each involving a proton on a carbon atom to which is connected an oxygen atom, exclude the possibility of a tertiary hydroxyl group. The vicinal coupling of 10 Hz for the proton resonating at τ 4.59 implies a dihedral angle between the coupled protons of approximately 180°.[6] It has been observed[7] that when an acetoxyl group in the equatorial conformation

(5) "Interpretation of Mass Spectra of Organic Compounds," H. Budzikiewicz, C. Djerassi, and D. H. Williams, Ed., Holden-Day, Inc., San Francisco, Calif., 1964, p 33.

(6) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

(7) A. I. Cohen, unpublished results.

TABLE I
NMR SPECTRAL DATA FOR C-73, C-73X, AND THEIR ACETATES



V

R	R_x	Solvent[a]	Chemical shifts (τ[b]) OR	Ar-Me	Ar-H	H_x	R_x	NH
H	H	$CDCl_3$	...	7.83 (C-5)	2.76 (m)	...	...	...
				7.68 (C-3)				
Ac	H	$CDCl_3$	7.68	7.83 (C-5)	2.76 (m)	...	...	1.6[c]
				7.68 (C-3)				
Ac	OAc	$CDCl_3$	7.64	7.82 (C-5)	2.73 (m)	4.59 (d, J = 10 Hz)	7.82	1.9[c]
				7.67 (C-3)				
H	H	$(CD_3)_2SO$	−2.2	7.83 (C-5)	2.73 (C-4)	...	...	0.7[c]
				7.73 (C-3)	2.41 (C-6)			
H	OH	$(CD_3)_2SO$	−2.2	7.83 (C-5)	2.71 (C-4)	5.94 (m)[d]	4.24[d]	...
				7.73 (C-3)	2.40 (C-6)			
H	H	C_5H_5N	...	7.81 (C-5)	...	...	...	...
				7.72 (C-3)				
H	OH	C_5H_5N	...	7.80 (C-5)	...	...	...	...
				7.73 (C-3)				

[a] Containing $(CH_3)_4Si$ as an internal reference. [b] In parentheses, m = multiplet, d = doublet, C-3–C-6 = position assignment. [c] Tentative assignment. [d] On exchange with D_2O, peak at τ 4.24 disappears and peak at τ 5.94 becomes a doublet.

is vicinal to a carbonyl group, the methyl proton resonance occurs near τ 7.8. When the acetoxyl group is in the axial conformation, the resonance occurs at τ >7.9. Since the acetoxyl methyl proton resonance in C-73X diacetate is at τ 7.82, the acetoxyl group is probably in the equatorial conformation and the geminal proton is axial (Table I, R_x = OAc and H_x = H).

Biological Data.—The biological (antitumor) activity of antibiotic C-73X was determined against Sarcoma 180 in Swiss albino mice in tests at Hazleton Laboratories.[8] Though C-73X inhibits the growth of Sarcoma 180 in Swiss albino mice, the therapeutic index is poor, since an intraperitoneal dose of 200 mg/kg/day is toxic to mice, whereas intraperitoneal doses smaller than 100 mg/kg/day are inactive.

The biological activity spectrum of C-73X is similar to that of C-73. The compound does not inhibit the growth of gram-positive or gram-negative bacteria, mycobacteria, or *Saccharomyces cerevisiae*. Of five strains of *Candida*, one is inhibited by a concentration of C-73X of 31 μg/ml; the other four strains are not suppressed by the antibiotic.

Experimental Section[9]

2-Hydroxy-3-(2-hydroxy-3,5-dimethylphenacyl)glutarimide was prepared from the harvested broth of a *Streptomyces griseus* SC 3675 culture. The broth was filtered and both the filtrate and filter cake were extracted with hexane. The hexane extracts, found to be biologically inactive, were discarded. After extraction of filtrate and filter cake with butanol, the spent cake and spent broth, also found to be biologically inactive, were discarded.

Volatile substances were evaporated *in vacuo* from the combined butanol extracts and the residual oily material was poured into 15 vol. of hexane. After the mixture had remained at 5° overnight, the supernatant liquid was decanted. The gummy residue (*ca.* 200 g from a 600-l. fermentation batch) was dissolved in $CHCl_3$ and applied to a cellulose column for elution with chloroform. Eluted fractions which showed any color were pooled, evaporated to a small volume, and chromatographed on a Merck acid-washed alumina column. Elution with $CHCl_3$, acetone, and methanol, respectively, was carried out until the eluate was colorless after each solvent. The acetone and methanol eluates were evaporated to dryness *in vacuo*. The original acetone fraction, dissolved in chloroform, was rechromatographed on a Merck acid-washed alumina column and eluted again with $CHCl_3$, acetone, and methanol, sequentially. The original methanol fraction, dissolved in acetone, was similarly rechromatographed and eluted with acetone and methanol, sequentially. Concentration of the methanol eluates of both original fractions yielded crystalline C-73. Further concentration of the eluates yielded crystalline C-73X.

The approximately 200 mg of crude C-73X crystals obtained from a 600-l. fermentation batch was further purified on an isobutyl methyl ketone (MIBK)–formamide (20:1) distribution column, with cellulose as a support. For chromatography, the C-73X crystals were dissolved in 5 ml of formamide and the solution was mixed with cellulose. The resulting wet powder was put on top of a packed cellulose column which had been previously wetted with formamide (cellulose suspended in formamide and filtered with suction). After elution of the column with MIBK, only those fractions shown by paper chromatography to contain pure C-73X were retained. The solvents were evaporated *in vacuo* from these fractions and the residual solids were recrystallized from methanol. The yield was approximately 50 mg; mp 200°; λ_{max}^{MeOH} [mμ ($\epsilon \times 10^{-3}$)], 262 (11.7), 347 (4.3); infrared spectrum significant bands: λ_{max}^{KBr} 2.95, 3.15, 3.25, 3.45, 5.90, 6.10, 6.25, 7.45, 7.72, 7.83, 7.95, 8.25, 8.85, 9.65, 11.80, 11.90, 12.75, 13.34, 13.70 μ; paper chromatography (MIBK–formamide, 20:1): R_f 0.67 (we determined the R_f of antibiotic C-73 to be 0.80).

Anal. Calcd for $C_{15}H_{17}NO_5$: C, 61.85; H, 5.87; N, 4.80. Found: C, 61.26; H, 6.29; N, 4.87.

(8) Falls Church, Va.

(9) The ultraviolet spectra were obtained with a Cary Model 15 spectrophotometer from methanol solutions. The nmr spectra were those of samples dissolved in appropriate solvents (Me_4Si as internal reference), with Varian Model A-60 nmr spectrometer. The infrared spectra were obtained with a Perkin-Elmer Model 21 spectrophotometer (KBr). The mass spectra were obtained with a Consolidated Electrodynamic Corp. Model 103 C spectrometer; solid samples were introduced through the heated inlet (190°); the ionization voltage was 70 ev. Corrected melting points were observed on a Fisher-Johns hot stage.

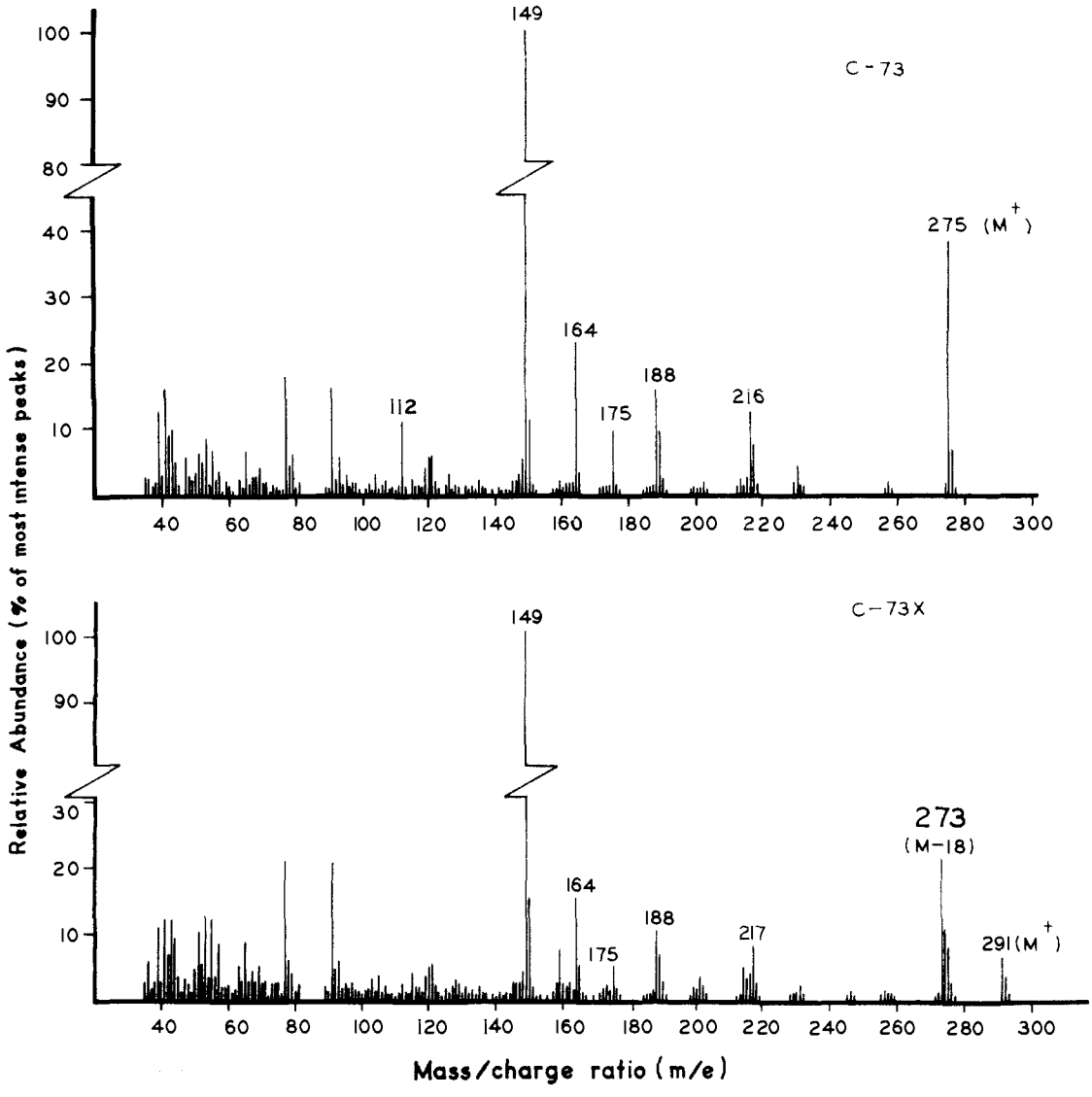


Figure 1.—Mass spectra of antibiotics C-73 and C-73X.

2-Hydroxy-3-(2-hydroxy-3,5-dimethylphenacyl)glutarimide Diacetate.—A total of 200 mg of antibiotic C-73X was dissolved in 7 ml of pyridine, then 5 ml of acetic anhydride was added. The solution was kept at room temperature for 3 days. Addition of 30 ml of hexane caused precipitation. The precipitate was crystallized from an ether–hexane mixture. The yield was 150 mg; mp 149°; mass spectrum, M^+ *m/e* 375.

Anal. Calcd for $C_{19}H_{21}NO_7$: C, 60.80; H, 5.60; N, 3.73. Found: C, 60.40; H, 5.91; N, 3.52.

Acknowledgments.—The authors are indebted to the following staff members of The Squibb Institute for Medical Research—to J. Alicino, for the elemental analyses; to Miss B. T. Keeler, for interpretation of the infrared spectra; to Dr. R. S. Robison, for the fermentation; and to Dr. D. Frost, for editorial assistance.