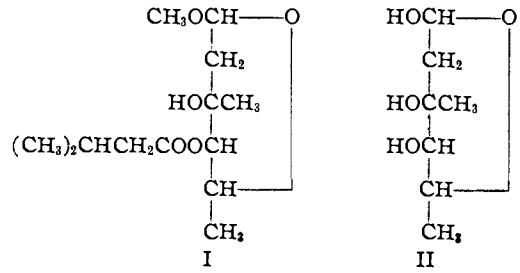


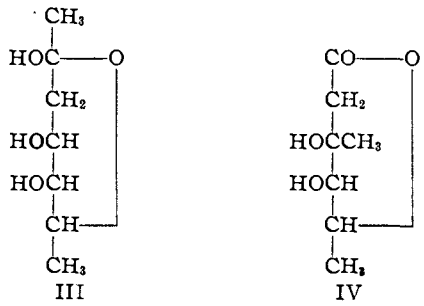
I II

Alkaline hydrolysis of the cleavage product yields isovaleric acid (*p*-nitrobenzyl ester, infrared spectrum, R_F value) and a mixture of anomeric methyl mycarosides, which is separable by fractionation *in vacuo* into a crystalline isomer, m.p. 60.5–61° [b.p. 65° (1.1 mm.), $[\alpha]^{25}D$ − 141° (*c*, 1, $CHCl_3$), *Anal.* Calcd. for $C_8H_{16}O_4$: C, 54.63; H, 9.15; OCH_3, 17.62. Found: C, 54.74; H, 9.18; OCH_3, 17.94], and an oily isomer [b.p. 107° (1.1 mm.), $n^{25}D$ 1.4649, $[\alpha]^{25}D$ + 54° (*c*, 2.3, $CHCl_3$), *Anal.* Found: C, 54.71; H, 9.01; OCH_3, 17.82].

Aqueous acid hydrolysis of the methyl mycarosides yields mycarose as a crystalline solid, m.p. 128–129° $[[\alpha]^{25}D$ − 31.1° (*c*, 4, H_2O), *Anal.* Calcd. for $C_7H_{14}O_4$: C, 51.84; H, 8.70; CCH_3 (2), 18.58. Found: C, 52.07; H, 8.72; CCH_3, 12.69]. Mycarose reduces hot Fehling solution very slowly, contains two methyl groups bound to carbon, shows three active hydrogen atoms in the Zerewitinoff determination, and exhibits only end absorption in the ultraviolet.

Mycarose consumes *two* moles of periodate, and yields one mole each of acetaldehyde and formic acid, as well as lesser amounts of 1,3,5-triacetylbenzene, m.p. 162–163° [mixture melting point with an authentic sample[5] not depressed]. When the reaction mixture from the oxidation of mycarose with *one* mole of periodate is treated with 2,4-dinitrophenylhydrazine, 1-(2,4-dinitrophenyl)-3(*or* 5)-methylpyrazole,[6] m.p. 139–140° [*Anal.* Calcd. for $C_{10}H_8N_4O_4$: C, 48.39; H, 3.25; N, 22.57; CCH_3 (1), 6.05. Found: C, 48.75; H, 3.21; H, 22.65; CCH_3, 3.02], identical with a sample prepared from synthetic acetoacetaldehyde and 2,4-dinitrophenylhydrazine, is produced.

The formation of acetoacetaldehyde, acetaldehyde, and formic acid from mycarose on periodate oxidation, taken with the characterization data, requires that the sugar be formulated as (II) or (III).



III IV

(5) L. Claisen and N. Stylos, *Ber.*, **21**, 1145 (1888).

(6) L. Claisen and P. Roosen, *Ber.*, **24**, 1888 (1891), describe the formation of both possible methylphenylpyrazoles from acetoacetaldehyde and phenylhydrazine.

The latter is excluded by the smooth formation from mycarose, by hypobromite oxidation, of a *lactone*, $C_7H_{12}O_4$, m.p. 108–109° $[[\alpha]^{25}D$ − 35.0° (*c*, 1.86, H_2O), *Anal.* Calcd. for $C_7H_{12}O_4$: C, 52.49; H, 7.55; mol. wt., 160. Found: C, 52.35; H, 7.46; sap. eq., 154], clearly of the structure (IV).[4]

Since the methyl isovalerylmycaroside obtained from magnamycin is not attacked by periodic acid, the isovaleryl residue must be attached at position 4, as in (I).

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MANGANESE REVERSAL OF AUREOMYCIN INHIBITION OF BACTERIAL CELL-FREE NITRO-REDUCTASE

Sir:

There have been several reports indicating that aureomycin inhibits oxidations and/or coupled phosphorylations mediated by various mammalian tissues.[1–3] Van Meter, *et al.*,[4] have reported the inhibition of the respiration of rat liver mitochondria by aureomycin and the reversal of this inhibition by added magnesium. Until recently, there have been no reports of cell-free bacterial systems inhibited by aureomycin. It has been reported from this laboratory[5] that cell-free extracts of *Escherichia coli* (E-26) reduce the nitro groups of chloramphenicol and *p*-nitrobenzoic acid to the corresponding arylamines. Aureomycin in low concentrations markedly inhibited these reductions. The present communication shows that in the partially resolved nitro reductase system, Mn^{++} significantly reversed the inhibitory activity of aureomycin on the reduction.

Cells of *E. coli* (E-26) were grown and harvested as previously described. Arylamine formation was determined by the Bratton–Marshall technique. Cell-free extracts were prepared by suspending 4 g. wet weight of *E. coli* in 20 ml. of cold, distilled water. The suspension was placed in the 9KC Raytheon sonic oscillator for 60 minutes. The extract was then centrifuged in the cold at 27,000 × g. Untreated extracts were capable of reducing nitro groups actively and aureomycin markedly inhibited the reduction. The extracts were dialyzed with stirring for 96 hours at 5° *versus* 4 liters of frequently changed distilled water. After dialysis the extracts were completely inactive in reducing nitro groups. The activity could be almost completely restored by adding to the reaction mixture L-cysteine, diphosphopyridine nucleotide (DPN) and L-malic acid. In this system 1.2×10^{-4} *M* Mn^{++} further stimulated the formation of arylamine. Higher concentrations were

(1) W. F. Loomis, *Science*, **111**, 474 (1950).

(2) T. M. Brady and J. A. Bain, Trans. Fall Meeting, Am. Soc. Pharmacol. and Exp. Therap., 5 (1951).

(3) J. C. Van Meter and J. J. Oleson, *Science*, **113**, 273 (1951).

(4) J. C. Van Meter, A. Spector, J. J. Oleson and J. H. Williams, *Proc. Soc. Exp. Biol. Med.*, **81**, 215 (1952).

(5) A. K. Saz and J. Marmur, *Proc. Soc. Exp. Biol. Med.*, **82**, 783 (1953).

inhibitory. The degree of inhibition of arylamine formation in the fortified system, by low concentrations of aureomycin, was similar to the inhibition in original untreated extracts. It was found that dihydrodiphosphopyridine nucleotide (DPNH) could replace the requirement of the system for L-malate and DPN. In this system, aureomycin was considerably less effective in inhibiting arylamine formation. When 10^{-3} *M* L-malate, 2×10^{-4} *M* DPN and 0.05 ml. dialyzed extract were incubated together in 0.05 *M* tris-(hydroxymethyl)-aminomethane buffer, *p*H 7.5 and the formation of DPNH determined by absorption at 340 mμ in the Beckman DU spectrophotometer, it was observed that no formation of DPNH occurred unless 6×10^{-3} *M* Mn^{++} was added to the reaction mixture. These results indicated that aureomycin inhibited arylamine formation by preventing the formation of DPNH and consequently the transfer of hydrogen to nitro groups. Since Mn^{++} was essential for the formation of DPNH, it seemed possible that aureomycin could prevent the formation of DPNH by binding Mn^{++}.[6] If such were the case, excess Mn^{++} added to the reaction might be expected to reverse the inhibitory activity of aureomycin. Table I shows that Mn^{++} reverses aureomycin inhibition of arylamine formation.

TABLE I

The tubes were incubated at 37° for 120 minutes. Each tube contained (final concentration) 0.05 *M* tris-(hydroxymethyl)-aminomethane buffer, *p*H 7.5; 3×10^{-4} *M* chloramphenicol, 1×10^{-3} *M* L-malate, 1×10^{-5} *M* DPN; 5×10^{-3} *M* L-cysteine; 0.30 ml. dialyzed extract, final volume 1.5 ml.

Aureomycin concn., micrograms/ml.	Micrograms arylamine formed/ml. No Mn^{++}	6×10^{-3} *M* Mn^{++}
0	17.0	14.6
90	1.1	6.3
45	1.7	8.3
18	2.8	9.4
9	4.0	10.8

In the present system, it would seem that aureomycin inhibits by binding Mn^{++}; possibly this effect is due to the formation of a chelate.[7] Other biological reactions with a need for metal activators are being studied in order to determine whether the inhibition by aureomycin is a general phenomenon of cation-requiring reactions. The implication of the findings in terms of antibiotic activity of aureomycin are under investigation.

(6) Due to the high absorption of aureomycin in the spectrophotometer at 340 mμ, it has not been possible to show directly the inhibition of DPNH formation by aureomycin.

(7) The binding of metallic cations by aureomycin and terramycin was reported very recently by A. Albert, *Nature*, **172**, 201 (1953).

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CORRELATION OF RATES OF SOLVOLYSIS

Sir:

We wish to point out the usefulness of the two-parameter equation

$$\log (k/k^0)_A - \log (k/k^0)_{A_0} = ab$$

where A and A^0 refer to any compound and to a standard compound (*e.g.*, methyl bromide) respectively, k is the first-order rate constant for solvolysis of A or A^0 in any solvent, k^0 is the corresponding rate constant in a standard solvent (*e.g.*, 80% ethanol), a is a constant characteristic of only the compound, and b is a constant characteristic of only the solvent.

Those data on the solvolysis of organic bromides and chlorides in which at least three solvents have been investigated for each compound were used to test the equation; these are for 124 reactions of 15 compounds and 19 solvents, including 18 reactions which form substituted amines or quaternary ammonium salts. After assigning $a = 0.00$ for methyl bromide (A^0), $a = 1.00$ for *t*-butyl chloride, and $b = 0.00$ for 80% ethanol–20% water by volume, the best values of a and b for other compounds and solvents may be found without any complicated methods or equipment.

The average error in $\log (k/k^0)_{calcd.} - \log (k/k^0)_{obs.}$ is 0.18, corresponding to a factor of 1.52 in k itself, excluding 33 standard cases where the error is zero. The maximum error, which corresponds to a factor of 7.6, occurs with benzhydryl chloride in 90% acetone. This is a more than satisfactory fit considering the wide range of rates being correlated; *e.g.*, the ratios of the fastest to the slowest rate measured are 2.8×10^6, 8.7×10^4 and 3.4×10^5 for methyl bromide, benzhydryl chloride and *t*-butyl chloride, respectively.

The 15 compounds and their a values are picryl chloride (−0.42), *p*-nitrobenzoyl chloride (− 0.37), phenacyl bromide (− 0.04), methyl bromide (0.00), benzoyl chloride (+ 0.06), ethyl bromide (+ 0.15), *i*-butyl bromide (+ 0.16), *n*-butyl bromide (+ 0.18), benzyl chloride (+ 0.19), *p*-methylbenzoyl chloride (+ 0.41), *i*-propyl bromide (+ 0.42), α-phenylethyl chloride (+ 0.64), benzhydryl chloride (+ 0.78), *t*-butyl bromide (+ 0.93) and *t*-butyl chloride (+ 1.000).

The 19 solvents and their b values are triethylamine (− 17.3), *n*-butylamine (− 10.2), pyridine (− 9.66), aniline (− 4.78), 100%, 96.7% and 69.5% methanol (−0.94, −0.51, +0.61), 100%, 90%, 80%, 60% and 50% ethanol (−0.79, −0.52, 0.00, +0.88, +1.14), 90%, 80%, 70% and 50% acetone (−0.72, +0.04, +0.42, +1.02), water (+2.95), acetic acid (+0.57) and formic acid (+4.00).

The success of this correlation is due to effective cancellation of kinetic energy and entropy terms which restrict the applicability of many linear free-energy relationships.

Strictly, this type of approach should be confined to compounds whose leaving group is the same or closely similar to the leaving group of the standard compound; furthermore, the atom which is the reaction site should be of the same species in both the standard compound (A^0) and the compound being studied (A). In all the cases above, a bromide or chloride ion is the leaving group, and the reaction is a simple displacement at a carbon atom.

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