in pseudoecgonine the hydroxyl is *cis* to the nitrogen, as in pseudotropine, and that the carboxyl is *trans* to both the nitrogen and the hydroxyl group (IV).[6] Therefore also, the carboxyl group of ecgonine itself is *cis* to these two functions. Ecgonine may accordingly be called, the nitrogen atom being used as the point of reference, 2-*cis*-carboxy-3-*cis*-hydroxytropane (I).

The failure of N-acetylnorecgonine ethyl ester to rearrange to the O-acetyl isomer was considered by Fodor to favor Willstätter's opinion. This failure is, however, negative evidence, and it has been found here that O-benzoylnorecgonine [*Anal.* Calcd. for $C_{15}H_{17}NO_4$: C, 65.44; H, 6.24; N, 5.09. Found: C, 65.30; H, 6.24; N, 5.20], m.p. 250° (hydrochloride, m p. 219–221°[8]) rearranges in dilute aqueous potassium carbonate to N-benzoylnorecgonine [*Anal.* Calcd. for $C_{15}H_{17}NO_4$: C, 65.44; H, 6.24; N, 5.09. Found: C, 65.67; H, 6.19; N, 4.87], m.p. 163.5°. The neutral O-benzoyl isomer (Nujol mull) has broad weak absorption from *ca.* 3.65 to 5.5 μ attributable to NH_2^+ of a zwitterion[9] and maxima at 5.80 μ and 6.45 μ ascribable to benzoate and carboxylate ion,[9] respectively. The acidic N-benzoyl isomer (Nujol mull) has absorption maxima at 3.12 and 5.76 μ assignable to bonded hydroxyl and the carboxyl group, respectively, and a double maximum at 6.21 and 6.26 μ attributable to the disubstituted amide linkage.

Ecgonine methyl ester, cocaine, pseudoecgonine methyl ester, and pseudococaine are, in view of the foregoing considerations, to be represented by II, III, V and VI. I shall present a more detailed account of this investigation in the near future.

(8) A. Einhorn, *Ber.*, **21**, 3029 (1888).
(9) L. Larsson, *Acta Chem. Scand.*, **4**, 27 (1950).

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RECEIVED JULY 13, 1953

OXIDATION-REDUCTION POTENTIALS OF HORSERADISH PEROXIDASE

Sir:

A systematic, potentiometric study of horseradish peroxidase (HRP), organized as a joint project of the Department of Biochemistry, Medical Nobel Institute, and the Department of Physiological Chemistry, The Johns Hopkins University School of Medicine, has now been carried to a first point of general interest.

Our studies to date indicate that the oxidation–reduction potentials of the ferri HRP/ferro HRP system are much more negative than the corresponding potentials that have been determined for other hemoproteins. Detailed data over a large range of *p*H are not yet available, but measurements made between *p*H 6 and 8 indicate that here the values of E_0' are more negative even than those reported for free iron protoporphyrin IX. The contrasts are shown in the table.

System	Temp., °C.	*p*H	E_0', volt	Ref.
ferri HRP/ferro HRP	30	6.1	−0.21	
		7.3	−0.27	
ferri protoporphyrin IX/ ferro protoporphyrin IX	30	7.0	−0.14[a]	1
metmyoglobin/myoglobin	30	7.0	+0.05	2
methemoglobin/hemoglobin	30	7.0	+0.14	3
ferri cytochrome c/ ferro cytochrome c	30	7.0	+0.25	4, 5

[a] Value found by extrapolation of experimental data on the basis of an estimated pK_a' value.

It would appear that the different ferri hemoprotein/ferro hemoprotein systems range from among the most positive biological oxidation–reduction systems known to among the most negative systems known. It seems reasonable to ask now whether the well-known resistance to reduction displayed by free catalase might not be at least in part the result of a very negative oxidation–reduction potential for the ferri catalase/ferro catalase system.

The author wishes to acknowledge the great aid of Dr. Hugo Theorell and Dr. Karl-Gustav Paul, who directed the preparation of HRP in crystalline form. Dr. W. Mansfield Clark has lent invaluable advice, and has supplied the equipment for the potentiometric measurements.

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RECEIVED AUGUST 7, 1953

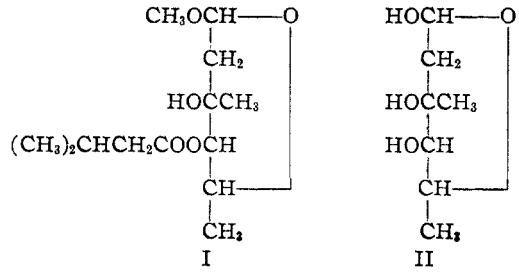
(1) J. Shack and W. M. Clark, *J. Biol. Chem.*, **171**, 143 (1947).
(2) J. F. Taylor and V. J. Morgan, *ibid.*, **144**, 15 (1942).
(3) J. F. Taylor and A. B. Hastings, *ibid.*, **131**, 649 (1939).
(4) F. L. Rodkey and E. G. Ball, *ibid.*, **182**, 17 (1950).
(5) K. G. Paul, *Arch. Biochem.*, **12**, 441 (1947).
(6) Public Health Service Research Fellow of the National Institutes of Health, 1951–1953. These studies supported in part by a grant from Eli Lilly and Company.

MAGNAMYCIN.[1] II. MYCAROSE, AN UNUSUAL BRANCHED-CHAIN DESOXYSUGAR FROM MAGNAMYCIN

Sir:

Methanolysis of the antibiotic Magnamycin[2,3] by 1 *N* methanolic hydrochloric acid yields a crystalline *base* of the formula $C_{29-30}H_{47-49}NO_{12}$ and an oily *neutral substance*, $C_{13}H_{24}O_5$ [b.p. 116° (1.1 mm.), $n^{25}D$ 1.4493, $[\alpha]^{25}D$ − 10.7° (*c* 9, $CHCl_3$), *Anal.* Calcd. for $C_{13}H_{24}O_5$: C, 59.98; H, 9.29; OCH_3, 11.90; mol. wt., 260. Found: C, 60.04; H, 9.40; OCH_3, 11.70; sap. eq., 263]. We wish to record evidence which proves that the neutral substance is the 4-isovaleryl methyl glycoside (I)[4] of a new sugar, *mycarose*, of the structure (II).[4]

(1) Magnamycin is the registered trade name of Chas. Pfizer and Company for the antibiotic carbomycin.
(2) F. W. Tanner, A. R. English, T. M. Lees and J. B. Routien, *Antibiotics and Chemotherapy*, **2**, 441 (1952).
(3) R. L. Wagner, F. A. Hochstein, K. Murai, H. Messina and P. P. Regna, THIS JOURNAL, in press.
(4) These formulas should be regarded as devoid of configurational implications. The stereochemistry of mycarose is now under investigation.



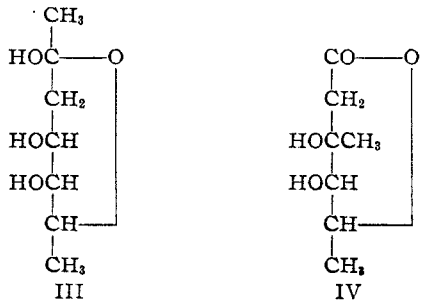
I II

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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RECEIVED AUGUST 14, 1953

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).