$KHCO_3$ under the conditions used[5,10] leads to the more stable 2α-hydroxy epimer.

(10) R. L. Clarke, K. Dobriner, A. Mooradian and C. Martini, THIS JOURNAL, **77**, 661 (1955).

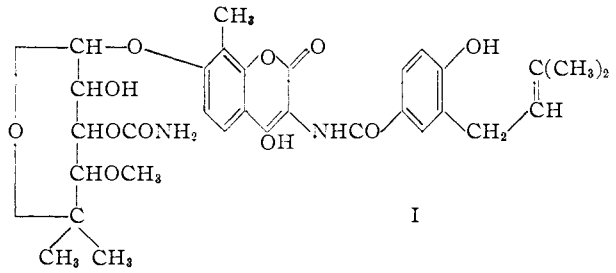
THE WORCESTER FOUNDATION
FOR EXPERIMENTAL BIOLOGY
SHREWSBURY, MASSACHUSETTS, AND
THE LABORATORY OF PATHOLOGY SHLOMO BURSTEIN
NATIONAL CANCER INSTITUTE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND

RECEIVED MARCH 2, 1956

NOVOBIOCIN. II. STRUCTURE OF NOVOBIOCIN

Sir:

Formula I represents the structure of novobiocin (I).



I

Review statements on the isolation of novobiocin in three laboratories have been made,[1] and initial structural studies have been described.[1,2] A recent communication[3] presents a partial structure of novobiocin which is in agreement with structure I.

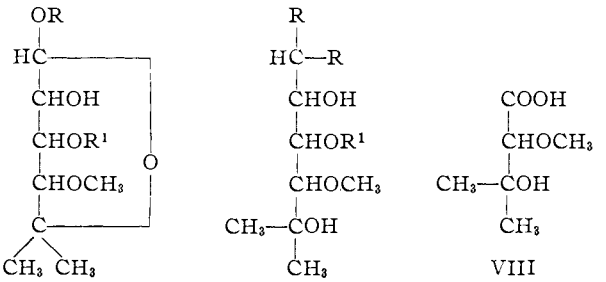
Cleavage of novobiocin with methanolic hydrogen chloride yielded methyl 3-carbamyl-4-methylnovobioside,[1] $C_{10}H_{19}NO_6$ (II), m.p. 191–192°. This glycoside did not react with sodium periodate. Hydrolysis of II with dilute hydrochloric acid gave 3-carbamyl-4-methylnovobiose (III) (Calcd. for $C_9H_{17}NO_6$: N, 5.96. Found: N, 5.98), which reacted with one mole of sodium periodate. Thus, an hydroxyl group is present on the carbon adjacent to the glycosidic carbon. Alkaline hydrolysis of II formed ammonia, carbon dioxide and methyl 4-methylnovobioside (IV) (Calcd. for $C_9H_{18}O_5$: C, 52.41; H, 8.80. Found: C, 52.70; H, 8.31). These products show the presence of a urethane group in II, and the infrared spectrum is consistent with this formulation. Periodate oxidation of IV (one mole of periodate consumed) yielded glyoxal.

Reaction of methyl 3-carbamyl-4-methylnovobioside (II) with ethyl mercaptan and hydrogen chloride gave the mercaptal (V), m.p. 143–145° (Calcd. for $C_{13}H_{27}NO_5S_2$: C, 45.72; H, 7.97; S, 18.78. Found: C, 45.04; H, 7.22; S, 19.15). Treatment of V with Raney nickel gave VI, m.p. 117–118°, (Calcd. for $C_9H_{19}NO_5$: C, 48.55; H, 8.66; N, 6.33. Found: C, 49.10; H, 8.00; N,

(1) E. A. Kaczka, C. H. Shunk, J. W. Richter, F. J. Wolf, M. Gasser, and K. Folkers, THIS JOURNAL, in press.

(2) H. Hoeksema, J. L. Johnson and J. W. Hinman, *ibid.*, **77**, 6710 (1955).

(3) J. W. Hinman, H. Hoeksema, E. L. Caron and W. G. Jackson, *ibid.*, **78**, 1072 (1956).

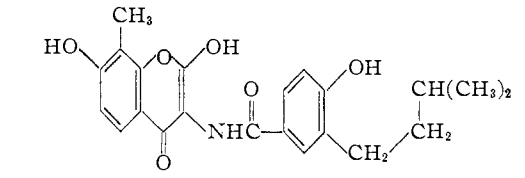


II, R = CH_3; R^1 = $CONH_2$
III, R = H; R^1 = $CONH_2$
IV, R = CH_3; R^1 = H

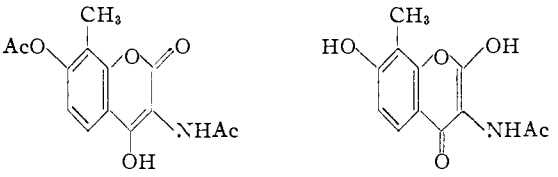
V, R = SEt; R^1 = $CONH_2$
VI, R = H; R^1 = $CONH_2$
VII, R = R^1 = H

VIII

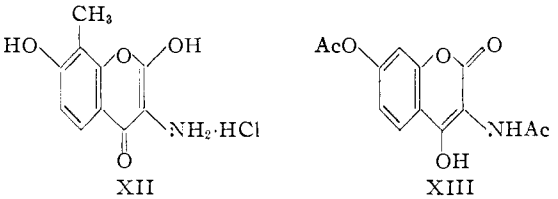
6.68) which did not react with sodium periodate. Alkaline hydrolysis of VI yielded VII, which reacted with one mole of sodium periodate giving a mole of acetaldehyde. Periodate oxidation of VII followed by bromine oxidation of the reaction product yielded (−)-α-methoxy-β-hydroxyisovaleric acid (VIII), which formed a crystalline N,N′-dibenzylethylenediamine salt, m.p. 119–120° (Calcd. for $C_{28}H_{41}N_2O_8$: C, 62.67; H, 8.27; N, 5.22. Found: C, 62.93; H, 8.07; N, 4.99). The infrared spectrum of this salt was identical with that of its optical antipode, which was synthesized from (−)-α,β-dihydroxyisovaleric acid.[4]



IX



X XI



XII XIII

Cleavage of dihydronovobiocin[1,2] with hydrochloric acid in methanol gave dihydronovobiocic acid (IX) (Calcd. for $C_{22}H_{23}NO_6$: C, 66.49; H, 5.83; N, 3.53; O, 24.2. Found: C, 66.66; H, 5.68; N, 3.84; O, 23.4). Treatment of IX with hydrogen bromide–acetic acid–acetic anhydride gave X, pK_a = 4.9, (Calcd. for $C_{14}H_{13}NO_6$: C, 57.72; H, 4.50; N, 4.81; CH_3CO, 29.6. Found: C, 57.72; H, 4.11; N, 4.90; CH_3CO, 24.8). Alkaline deacetylation of X gave XI, pK_a = 5.3 and 11.1 (Calcd. for $C_{12}H_{11}NO_5$: C, 57.83; H, 4.45;

(4) J. R. Sjolander, K. Folkers, E. A. Adelberg and E. L. Tatum, *ibid.*, **76**, 1085 (1954).

N, 5.62; CH_3CO, 17.27. Found: C, 57.79; H, 4.71; N, 5.45; CH_3CO, 20.0).

The infrared spectrum of X was consistent with an O,N-diacetyl compound containing an unsaturated lactone structure; the spectrum of XI showed amide, but neither ester nor unsaturated lactone absorption. Hydrolysis of XI in hydrochloric acid–dioxane gave XII. Titration data (pK_a = 2.9) and a fluorescein chloride color test[5] both indicated that XII is an aromatic amine. Acetylation of compounds XI and XII gave X. Thus, no rearrangement during the deacetylation of X had occurred. 2,4-Dihydroxy-3-methylbenzoic acid,[6] m.p. 220–224°, and 2,4-dihydroxy-3-methylphenylglyoxylic acid, m.p. 139–140°, were formed by alkaline degradation of cyclonovobiocic acid.[1,3] The glyoxylic acid was identical with a sample prepared by reaction of 2-methylresorcinol with ethyl oxalyl chloride followed by hydrolysis.

The desmethyl analog XIII was synthesized for comparison with X. 4,7-Dihydroxycoumarin[7] was converted by nitrous acid into the oximino derivative which was hydrogenated. The product was acetylated to XIII, m.p. 256–260°. The infrared absorption spectra of XIII and X were essentially identical between 5 and 6.5 μ.

It has been shown that the sugar portion of the molecule is attached to the coumarin moiety.[2] The 4-hydroxy-3-(3-methyl-2-butenyl)-benzoic acid[1,2] may be attached to XII only through an ester or amide linkage. Absence of ester-carbonyl absorption in the infrared spectrum of IX shows that the *p*-hydroxybenzoyl moiety is attached by an amide linkage.

(5) F. Feigl, "Qualitative Analysis by Spot Tests," 3rd ed., Elsevier Publishing Company, Inc., Houston, Texas, 1946, p. 371.

(6) Identical with synthetic sample prepared by procedure of R. C. Shah and M. C. Laiwalla, *J. Chem. Soc.*, 1828 (1938).

(7) A. Sonn, *Ber.*, **50**, 1299 (1917).

CONTRIBUTION FROM THE
MERCK SHARP & DOHME
RESEARCH LABORATORIES
DIVISION OF MERCK & CO., INC.
RAHWAY, NEW JERSEY

CLIFFORD H. SHUNK
CHARLES H. STAMMER
EDWARD A. KACZKA
EDWARD WALTON
CLAUDE F. SPENCER
ANDREW N. WILSON
JOHN W. RICHTER
FREDERICK W. HOLLY
KARL FOLKERS

RECEIVED MARCH 23, 1956

ZINC, A COMPONENT OF RABBIT MUSCLE LACTIC DEHYDROGENASE

Sir:

Zinc has been found as a functional component in the apoenzyme molecules of glutamic[1] and alcohol dehydrogenases.[2,3,4,5] DPN dependent glutamic dehydrogenation takes an exceptional place in metabolism, among the known pyridine nucleotide dependent reactions, since it dehydrogenates an amino acid with an accompanying deamination. It seemed important to enlarge upon the implications of the presence of zinc in alcohol dehydrogenases by investigating a second dehydrogenation reaction devoid of concomitant deamination.

Crystalline Lactic Dehydrogenase (LDH) was prepared from rabbit muscle by the method of Beisenherz, *et al.*[6] Quantitative emission spectrography and microchemical analysis[2] established the presence of significant quantities of zinc in the crystals of this enzyme. The concentration of all other metals was found to be small and variable as compared to that of zinc in the many preparations examined. Zinc was the only element consistently present in high concentrations, the exact amount being a function of purity of the preparation. Both spectrographic and microchemical data for zinc are shown in Table I. The differences between them are within the limits of the methods when milligram quantities of enzyme are analyzed but their zinc concentration is expressed per gram of protein.

The molecular weight and the DPN-binding characteristics of this enzyme are not on record. The calculation of stoichiometric proportionality of zinc and LDH are contingent upon the isolation of an enzyme, homogeneous by physical-chemical criteria. This has not been achieved for this enzyme as yet, though the quantities of zinc present are significant. The molecular weight of LDH obtained from beef heart muscle is known to be 137,000[7] and preliminary analytical and kinetic data on this enzyme also indicate that it is a zinc metalloenzyme.[8]

The zinc is thought to be a functional component of LDH of rabbit muscle. In enzymatic preparations of comparable purity, activity and zinc content, both the oxidation of lactate at *p*H 8.8 and the reduction of pyruvate at *p*H 7.0 are inhibited markedly by metal-binding agents such as sodium diethyldithiocarbamate, 1,10-phenanthroline, sodium azide, sulfide, 8-hydroxyquinoline and

TABLE I

EMISSION SPECTROGRAPHIC ANALYSIS OF TWICE-CRYSTALLIZED LACTIC DEHYDROGENASE OF RABBIT MUSCLE

All values are given as μg. of metal per gram of protein.

	Prepn. 1	Prepn. 2	Prepn. 3
Zinc	368	746	576
Zinc[a]	491	554	746
Aluminum	29	16	15
Magnesium	1100	72	71
Calcium	230	251	185
Strontium	7	0	trace
Barium	9	12	6
Manganese	[b]	[b]	24
Iron	109	344	232
Cobalt	[b]	[b]	[b]
Copper[a]	27	[c]	[c]
Lead	[b]	[b]	trace

[a] Chemical analysis. [b] Not detected; and also beryllium, cadmium, lithium, molybdenum, nickel, potassium, silver, tin. Sodium was present but not determined. [c] Lost.

(1) B. L. Vallee, S. J. Adelstein and J. A. Olson, THIS JOURNAL, **77**, 5196 (1955).

(2) B. L. Vallee and F. L. Hoch, *ibid.*, **77**, 821 (1955).

(3) B. L. Vallee and F. L. Hoch, *Proc. Natl. Acad. Sci. (U. S.)*, **41**, 327 (1955).

(4) H. Theorell, A. P. Nygaard and R. Bonnichsen, *Acta. Chem. Scand.*, **9**, 1148 (1955).

(5) B. L. Vallee, in "Advances in Protein Chemistry," Vol. 10, p. 317 (1955).

(6) G. Beisenherz, H. J. Boltze, T. Bucher, R. Czok, K. H. Garbade, E. Meyer-Arendt and G. Pfleiderer, *Z. Naturforsch.*, **8b**, 555 (1953).

(7) J. B. Neilands, *J. Biol. Chem.*, **199**, 373 (1952).

(8) B. L. Vallee and W. E. C. Wacker, unpublished observations.