Acknowledgment.—We would like to express our deep appreciation to Professor E. M. Purcell for a valuable discussion on several of the above points. This work was made possible by generous grants from E. I. du Pont de Nemours and Co., Inc., and from the Esso Education Foundation.

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RECEIVED DECEMBER 28, 1955

PARAMAGNETIC RESONANCE OF OCTACYANOTUNGSTATE(V)[1]

Sir:

The paramagnetic resonance of crystalline potassium octacyanotungstate and its aqueous solutions has been observed. A polycrystalline sample of composition $K_3W(CN)_8 \cdot 0.55H_2O$ yields a single almost symmetrical resonance at room temperature. The g value at the center of the peak is 1.98. The breadth of the peak between points of extreme slope is 30 oersteds.

An aqueous solution, approximately 0.01 M in $W(CN)_8^{---}$, yields a symmetrical resonance of three lines. The central intense line occurs at $g = 1.972$ with breadth between points of extreme slope of 9.3 oersteds. The two satellites, each of intensity $7 \pm 3\%$ of that of the central peak are separated by 52 oersteds. The central peak in all probability arises from ions containing W^{184} which has zero nuclear spin and is 86% abundant; the satellites are the hyperfine components associated with W^{183} which has spin $^1/_2$ and is 14% abundant.

The spectrum of the aqueous solution is unusual in its sharpness. Owing to rapid relaxation processes, most paramagnetic compounds of heavy elements have lines so broad at room temperature, that the resonances are not easily observed. The hyperfine coupling constant is unusually large, corresponding to a magnetic field of 6×10^5 oersteds at the tungsten nucleus. The results suggest the possible usefulness of $W(CN)_8^{---}$ in experiments involving alignment of the nuclei of some of the radioactive isotopes of tungsten.

The conventional description of $W(CN)_8^{---}$ ascribes the paramagnetism to an unpaired electron occupying a d orbital. The isotropic hyperfine interaction here described requires admixture of a configuration containing unpaired electrons in s orbitals.[2] Further experiments with dilute solid solutions of $W(CN)_8^{---}$ in single crystals of a diamagnetic substance are required for more complete determination of the nature of the electronic wave function.

An attempt was made to measure the rate of electron exchange between $W(CN)_8^{---}$ and $W(CN)_8^{---}$ by the observation of the spectrum of the former in the presence of the latter. At concentrations of $W(CN)_8^{---}$ lower than 0.02 M no broadening of the hyperfine components of $W^{183}(CN)_8^{---}$ was observed. With $W(CN)_8^{---}$ at 0.04 M a slight broadening, of the order of one oersted, was observed. If the broadening arises from the electron exchange reaction, the rate constant for the bimolecular exchange is 4×10^8 liter mole^{-1}sec.$^{-1}$. The absence of broadening at lower concentrations establishes this value as an upper limit, *i.e.*, $k \leqslant 4 \times 10^8$ liter mole^{-1}sec.$^{-1}$. Previous work with radioactive tracers[3,4] has indicated that $k > 4 \times 10^4$ liter mole^{-1}sec.$^{-1}$.

(1) This work has been supported in part by the United States Air Force through the Office of Scientific Research of the Air Research and Development Command and by the United States Atomic Energy Commission under Contract AT(11-1)-34 with the University of California.

(2) A. Abragam, J. Horowitz and M. H. L. Pryce, *Proc. Roy. Soc. (London)*, **A230**, 169 (1955).

(3) E. L. Goodenow and C. S. Garner, THIS JOURNAL, **77**, 5272 (1955).

(4) H. Baadsgaard and W. D. Treadwell, *Helv. Chim. Acta*, **38**, 1669 (1955).

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RECEIVED FEBRUARY 6, 1956

THE PARTIAL STRUCTURE OF NOVOBIOCIN (STREPTONIVICIN).[1] II.

Sir:

The isolation and properties of novobiocin have been described.[2-4] The present studies, added to those previously reported,[5] indicate that it is a C_9 sugar attached glycosidically to the 7-position of 3-[4-hydroxy-3-(3-methyl-2-butenyl)-benzamido]-4,7-dihydroxy-8-methylcoumarin.

Novobiocin (I) ($C_{31}H_{36}N_2O_{11}$), is cleaved by the action of hot acetic anhydride to yield 4-acetoxy-3-(3-methyl-2-butenyl)-benzoic acid (II) and a neutral compound, $C_{23}H_{26}N_2O_{10}$ (III).[5] Hydrolysis of I by 4 N hydrochloric acid in 60% ethanol gave an optically inactive acid, $C_{22}H_{21}NO_6$ (IV), which upon cleavage with hot acetic anhydride yielded 2,2-dimethyl-6-chromancarboxylic acid (VI) and an optically inactive neutral compound, $C_{14}H_{11}NO_5$ (VII).[5]

In addition to IV, the acid hydrolysis affords an

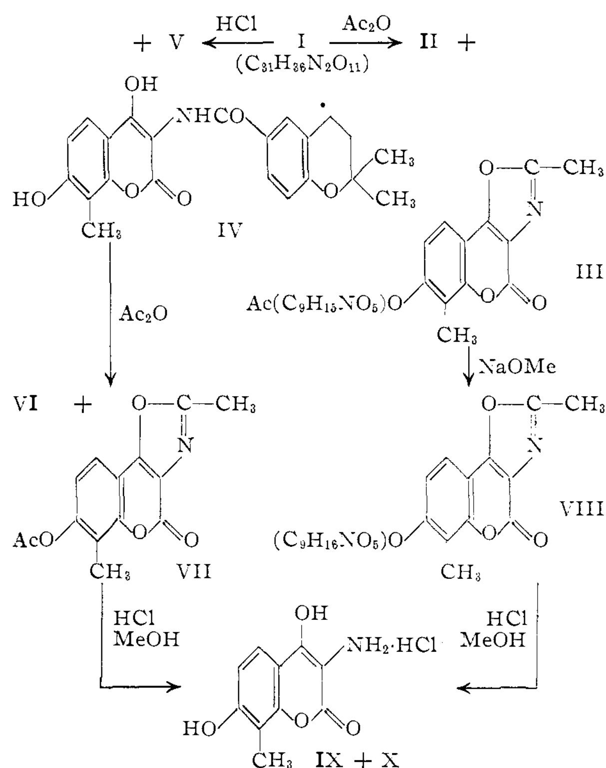
(1) The Upjohn Company Registered Trade Mark for novobiocin is Albamycin. Our previous Communication[5] on the structure of this antibiotic is listed under our former generic name, streptonivicin, now abandoned. The isolation of the same material by the Merck group has been described in THIS JOURNAL, **77**, 6404 (1955). The comparisons establishing identity are described by Henry Welch and W. W. Wright in *Antibiotics and Chemotherapy*, **5**, 670 (1955).

(2) (a) Streptonivicin, A New Antibiotic. I. Discovery and Biologic Studies, C. G. Smith, A. Dietz, W. T. Sokolski and G. M. Savage, *Antibiotics and Chemotherapy*, in press, February, 1956. (b) II. Isolation and Characterization, H. Hoeksema, M. E. Bergy, W. G. Jackson, J. W. Shell, J. W. Hinman, A. E. Fonken, G. A. Boyack, E. L. Caron, J. H. Ford, W. H. DeVries, and G. Crum, *ibid.* (c) III. *In Vitro* and *In Vivo* Evaluation, J. R. Wilkins, C. Lewis and A. R. Barbiers, *ibid.* (d) IV. A Biological Assay for Body Tissues and Fluids, R. M. Taylor, W. T. Sokolski, G. M. Savage and M. J. Vander Brook, *ibid.* (e) V. Absorption, Distribution and Excretion, R. M. Taylor, W. L. Miller and M. J. Vander Brook, *ibid.* (f) VI. Toxicology, E. John Larson, N. E. Connor, O. F. Swoap, R. A. Runnells, M. C. Prestrud, T. E. Eble, W. A. Freyburger, W. Veldkamp and R. M. Taylor, *ibid.*, March, 1956.

(3) Streptonivicin (Albamycin) A New Antibiotic; Preliminary Report, F. R. Heilman, D. R. Nichols, W. E. Wellman, and J. E. Geraci, *Proc. Staff Meetings Mayo Clinic*, **30**, 540 (1955).

(4) Streptonivicin, Laboratory and Clinical Studies in the Pediatric Age Group, Feng-Kai Lin and L. L. Coriell, Third Annual Symposium on Antibiotics, November 2-4, 1955; "Antibiotics Annual, 1955-1956," Welch and Marti-Ibanez, Medical Encyclopedia, Inc., New York, N. Y., in press.

(5) Herman Hoeksema, James L. Johnson, and Jack W. Hinman, THIS JOURNAL, **77**, 6710 (1955). New analytical data for I and III have been obtained as follows: Calcd. for $C_{31}H_{36}N_2O_{11}$ (I): C, 60.77; H, 5.92; N, 4.58. Found: C, 60.62; H, 5.91; N, 4.54. Calcd. for $C_{23}H_{26}N_2O_{10}$ (III): C, 56.33; H, 5.35; N, 5.71. Found: C, 56.53; H, 5.33; N, 5.68.



optically active neutral substance (V), m.p. 173–175° (calcd. for $C_{11}H_{21}NO_6$: C, 50.18; H, 8.04; N, 5.32; 1 OCH_3, 11.79; 1 OEt, 17.11. Found C, 50.68; H, 8.16; N, 5.25; OCH_3, 11.71; OEt, 17.01). The reaction with acetic anhydride thus apparently involves an amide linkage and the acid hydrolysis cleaves a glycosidic linkage.

Deacetylation of III with sodium methoxide in boiling methanol yields a neutral substance (VIII), m.p. 276–280°, with ultraviolet absorption essentially the same as III (calcd. for $C_{21}H_{24}N_2O_9$: C, 56.34; H, 5.40; N, 6.25. Found: C, 56.07; H, 5.54; N, 6.12). Hot methanolic hydrogen chloride cleaves VIII to amphoteric IX and neutral X, separable by fractional crystallization from acid methanol and ether. Similar treatment of VII affords IX. Under appropriate hydrolytic conditions, VII is converted to 7-acetyl-IX or to N-acetyl-IX, each subsequently convertible to IX. Recrystallization of IX from water or aqueous ethanol gives the free base (calcd. for $C_{10}H_9NO_4$: C, 57.99; H, 4.38; N, 6.77; C-CH_3, 7.3. Found C, 57.69; H, 4.32; N, 6.25; C-CH_3, 6.25). Compound IX and its free base decompose gradually above 200°. The acidic benzoyl derivative is obtained under Schotten-Baumann conditions, m.p. 309–310° (calcd. for $C_{17}H_{13}NO_5$: C, 65.59; H, 4.21; N, 4.50. Found: C, 65.63; H, 4.19; N, 4.42). When this is treated with hot acetic anhydride and pyridine, VII is obtained in good yield.

The reactions VII → N-acetyl-IX → IX, IX → VII, and IX → N-benzoyl-IX → VII proceed identically with those carried out on model compounds prepared from 3-amino-4-hydroxycoumarin generously supplied by Prof. K. P. Link, or prepared according to his procedures.[6] The oxazole prepared from 3-amino-4-hydroxycoumarin has characteristic ultraviolet fine structure identical with that of III, VII and VIII. The spectra of III, VII, VIII and IX differ only slightly from those of the analogous models, and in a manner attributable to the effect of a phenolic group on the aromatic ring. Compound IX gives positive Benedict and ninhydrin reactions and nitrous acid converts it to a diazo compound analogous to that reported by Link.[6]

The location of the 7-hydroxyl and 8-methyl groups was established by degradation of IX to known compounds. Fusion of IX with potassium hydroxide yields an ether-extractable dihydroxytoluene, m.p. 117–119° (calcd. for $C_7H_8O_2$: C, 67.73; H, 6.49. Found: C, 67.89; H, 6.21). The latter is not oxidized at a rotating platinum or a dropping mercury electrode in direct contrast to hydroquinone, *p*-toluquinol, and 3-methylgentisic acid. Identification of the compound as 2-methylresorcinol[7] was confirmed by direct comparison with an authentic sample. Treatment of IX with 1 *N* sodium hydroxide for several days at room temperature or with hot Benedict reagent gives an ether-extractable acid purifiable by chromatography over Florisil (ether) and sublimation. This dihydroxytoluic acid (calcd. for $C_8H_8O_4$: C, 57.14; H, 4.8; C-CH_3 8.99. Found: C, 57.3; H, 5.2; C-CH_3, 9.18) melts with sublimation at about 200°, and was identified as 2,4-dihydroxy-*m*-toluic acid by comparison with an authentic sample prepared by the procedure of Shah and Laiwalla.[8]

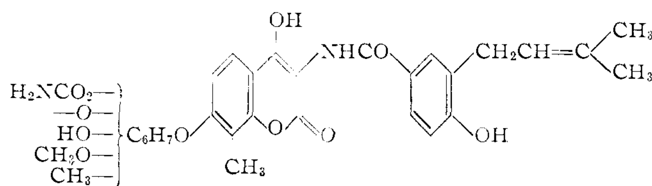
The optically active, neutral substance, V, appears to be the ethyl glycoside of a methoxy sugar, and X appears to be the corresponding methyl glycoside (calcd. for $C_{10}H_{19}NO_6$: C, 48.19; H, 7.68; N, 5.62; 2 O-CH_3, 24.9; 1 C-CH_3, 6.03. Found: C, 48.11; H, 7.71; N, 5.61; O-CH_3, 21.8; C-CH_3, 1.9). Infrared bands at 1702 and 1625 cm.$^{-1}$ are indicative of a urethan grouping. Boiling methanolic hydrogen chloride causes elimination of the neutral nitrogen of X as ammonium chloride with the formation of another neutral substance (XI) (calcd. for $C_{10}H_{16}O_6$: C, 51.72; H, 6.95; 2 O-CH_3, 26.73. Found: C, 51.97; H, 6.91; O-CH_3, 27.59) with infrared adsorption and chemical properties indicative of a cyclic carbonate ester. Reaction with barium hydroxide in aqueous solution at room temperature causes precipitation of the theoretical amount of barium carbonate with formation of XII. The nitrogen of the glycoside moiety appears, therefore, in the form of a carbamate. The single acetylatable hydroxyl (indicated by the transformation III → VIII), the methoxyl group and a bridge oxygen account for all of the remaining functionality of X. The low C-CH_3 analyses are consistent with the demonstration of twin infrared bands of *gem*-dimethyl groups at 1382 and 1366 cm.$^{-1}$ in X, XI and XII. The facile conversion of X to the carbonate ester (XI) and the ready reduction of one mole of periodate by XII limit the acetylatable hydroxyl and carbamate groups to positions on adjacent carbon atoms.

(6) C. F. Heubner and K. P. Link, THIS JOURNAL, **67**, 99 (1945).

(7) E. T. Jones and A. Robertson, *J. Chem. Soc.*, 1690 (1932).

(8) R. C. Shah and M. C. Laiwalla, *ibid.*, 1828 (1938).

These data permit the following partial structure for novobiocin:



We acknowledge with gratitude the contributions of Drs. M. Calvin, H. E. Carter, D. J. Cram, J. L. Johnson and E. C. Olson, Mrs. A. E. Fonken and Mr. W. A. Struck to this work.

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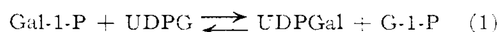
RECEIVED FEBRUARY 9, 1956

DIPHOSPHOPYRIDINE NUCLEOTIDE, A COFACTOR FOR GALACTO-WALDENASE[1]

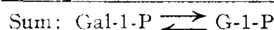
Sir:

The interconversion of Gal-1-P and G-1-P in galactose adapted yeast[2,3] and in mammalian tissue[4] has been found to occur according to the equations



$$\text{Gal-1-P} + \text{UDPG} \rightleftarrows \text{UDPGal} + \text{G-1-P} \quad (1)$$

$$\text{USPGal} \rightleftarrows \text{UDPG} \quad (2)$$



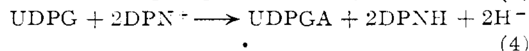
$$\text{Sum: Gal-1-P} \rightleftarrows \text{G-1-P}$$

Reaction (1), catalyzed by a specific uridyl transferase[3,4] is a reversible transfer of the uridyl group from G-1-P to Gal-1-P. Reaction (2) is an inversion at the C4 of the monosaccharide moiety catalyzed by galacto-waldenase.[3]

Galacto-waldenase has now been purified about 30-fold from a water extract of calf liver acetone powder. The extract was adjusted to *p*H 5.5 and acetone (−10°) was added to a final concentration of 25%. The precipitate was dissolved in glycine buffer, *p*H 8.0, and fractionated with alkaline ammonium sulfate (active fraction 45–65% saturation). This precipitate was dissolved in water and ammonium sulfate was added to 35% saturation. The active protein was then precipitated by adjusting the *p*H to 4.9. Further purification was obtained by absorption and elution from calcium phosphate gel followed by a final alkaline ammonium sulfate fractionation.

The activity of the fractionated preparation of galacto-waldenase was measured by the rate of DPN reduction when UDPGal[4] was added as substrate and UDPG dehydrogenase[5,6] and DPN were present as indicators of the inversion.

$$\text{UDPGal} \longrightarrow \text{UDPG} \quad (3)$$



$$\text{UDPG} + 2\text{DPN}^+ \longrightarrow \text{UDPGA} + 2\text{DPNH} + 2\text{H}^- \quad (4)$$

When purified galacto-waldenase was incubated with UDPGal in the absence of the indicator system, the inversion reaction did not proceed. It could be initiated, however, by adding catalytic amounts of DPN. This is illustrated in Table I. That the cofactor is DPN and not some impurity in the preparation is borne out by experiments using purified *Neurospora*, DPNase.[7] DPN preincubated with DPNase until it was no longer active when assayed with alcohol dehydrogenase was not active in the galacto-waldenase system. A control with DPN similarly preincubated with heat inactivated DPNase was active. DPNH was inactive in the system unless it was oxidized by preincubation with acetaldehyde and alcohol dehydrogenase. DPN could not be replaced by TPN.

TABLE I

EFFECT OF DPN ON GALACTO-WALDENASE REACTION

Reaction mixture consisted of 0.065 μmole of UDPGal containing 0.009 μmole UDPG as impurity, 4.0 μmoles of cysteine, purified galacto-waldenase (12 μgm. protein), and DPN or TPN as indicated in a total volume of 0.5 ml. of 0.1 *M* glycine buffer, *p*H 8.7. The reaction mixture was inactivated after 15 min. incubation at room temperature by heating at 100° for 20 sec.; 200-μl. aliquots were analyzed for UDPG with DPN and UDPG dehydrogenase.[6] UDPGal remaining was determined in the same cuvette by the subsequent addition of galacto-waldenase. A blank of 0.009 μmole due to UDPG originally present in the UDPGal has been subtracted.

	Nucleotide added, μmole	UDPG formed, μmole	UDPGal utilized, μmole
	None	0.000	0.000
DPN	0.0005	.006	.005
DPN	.002	.012	.012
DPN	.02	.026	.023
DPN	.016 (DPNase treated)	.000	.002
DPN	.016 (Heated DPNase treated)	.024	.023
DPNH	.014	.000	.000
DPNH	.014 (enzy. reoxidized)	.022	.021
TPN	.02	.002	. . .
TPN	.20	.004	.005

The marked effect of catalytic amounts of DPN suggests that the inversion occurs by an oxidation and subsequent reduction at the C4 of the carbohydrate. The structure of the hypothetical oxidized intermediate remains to be determined. Attempts to accumulate such an intermediate are in progress.

Acknowledgment.—The author wishes to express sincere appreciation to Dr. Herman M. Kalckar, in whose laboratory these experiments were performed, for his constant and valuable advice.

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RECEIVED FEBRUARY 3, 1956

(1) The following abbreviations have been used: Gal-1-P for α-D-galactose-1-phosphate, G-1-P for α-D-glucose-1-phosphate, UDPG for uridine diphosphoglucose, UDPGal for uridine diphosphogalactose, DPN for diphosphopyridine nucleotide, DPNH for reduced diphosphopyridine nucleotide, TPN for triphosphopyridine nucleotide; UDPGA for uridine diphosphoglucuronic acid.

(2) L. Leloir, *Arch. Biochem. Biophys.*, **33**, 186 (1951).

(3) H. M. Kalckar, B. Braganca and A. Munch-Petersen, *Nature*, **172**, 1039 (1953).

(4) E. S. Maxwell, H. M. Kalckar and R. M. Burton, *Biochim. Biophys. Acta*, **18**, 444 (1955).

(5) J. L. Strominger, H. M. Kalckar, J. Axelrod and E. S. Maxwell, THIS JOURNAL, **76**, 6411 (1954).

(6) J. L. Strominger, H. M. Kalckar, E. S. Maxwell, "Methods in Enzymology," Vol. III, Edited by S. P. Colowick and N. O. Kaplan, Academic Press Inc., in press.

(7) N. O. Kaplan, "Methods in Enzymology," Vol. II, p. 664, Academic Press, Inc., 1955. This preparation was kindly supplied by Mr. Francis Stolzenbach, McCollum Pratt Institute, Johns Hopkins University, Baltimore, Maryland.