[CONTRIBUTION FROM THE UPJOHN COMPANY AND THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Further Characterization of Neomycin B and Neomycin C

BY JARED H. FORD, MALCOLM E. BERGY, A. A. BROOKS, EDWARD R. GARRETT, JOSEPH ALBERTI, JOHN R. DYER[1] AND H. E. CARTER[2]

RECEIVED MAY 14, 1955

Neomycin B and neomycin C sulfates have been purified by carbon chromatography and converted to the corresponding free bases and hydrochlorides. Methanolysis studies indicate that equimolar amounts of neamine and methyl neobiosaminides are produced and suggest an empirical formula of $C_{23}H_{46}N_6O_{12}$ for both neomycins. Elementary analyses and potentiometric titrations do not disprove this composition but are in somewhat better agreement with a $C_{23}H_{48}N_6O_{13}$ formula.

Neomycin B and neomycin C were first separated as discrete components of the neomycin complex by Dutcher, Hosansky, Donin and Wintersteiner.[3] The neomycin B and C hydrochlorides were separated by chromatography in 80% methanol over alumina and were characterized by bioassay and specific rotation. Methanolysis experiments yielded the following products: (A) an amorphous non-reducing hydrochloride, identical from neomycin B and neomycin C, for which an empirical formula of $C_9H_{19}N_3O_5 \cdot 3HCl$ was proposed on the basis of analytical data for its crystalline acyl derivatives; (B) non-identical methyl glycosidic moieties which were named methyl neobiosaminides B and C. The analysis of a purified polyacetate of methyl neobiosaminide B was best compatible with the composition $C_{11}H_{16}N_2O_6(OCH_3)(COCH_3)_5$. On vigorous hydrolysis, methyl neobiosaminide C yielded the crystalline hydrochloride of a reducing diamine, $C_6H_{14}N_2O_3 \cdot 2HCl$. A *deoxy* function for both methyl neobiosaminides was indicated from these data. The remainder of the neobiosaminide fragment was accounted for by a pentose, as evidenced by the formation of furfural from both neobiosaminides on acid hydrolysis. Therefore, neobiosamines B and C appear to be isomeric bases having an empirical formula of $C_{11}H_{22}N_2O_7$. From their 1.85 weight ratio of non-reducing fraction to methyl neobiosaminide fraction, Dutcher and co-workers[3] were led to believe that the neomycins liberated two moles of the C_9 base and one mole of C_{11} neobiosamine on acid hydrolysis. Hence they proposed an empirical formula of $C_{29}H_{58}N_8O_{16}$ for both neomycins B and C. Subsequently, Dutcher and Donin[4] converted the amorphous non-reducing hydrochloride (A) to a crystalline base which was proven to be identical with neamine, a compound which Leach and Teeters[5] had obtained by acid hydrolysis of a neomycin B preparation, and with neomycin A which Peck and co-workers[6] had isolated from crude neomycin concentrates. Further studies by Peck and co-workers[7] indicated that this base had an empirical formula of $C_{12}H_{26}N_4O_6$ and was identical with neamine.

In the present investigation neomycin B and C sulfates have been separated by carbon chromatography using the method described by Leach, DeVries, Nelson, Jackson and Evans.[8] Our best neomycin C preparation is believed to contain less than 0.06% of neomycin B. This was determined biologically by means of a coccobacterium which was not inhibited by 50,000 mcg./ml. of neomycin C or neamine, while 15–30 mcg./ml. of neomycin B prevented it from growing.[9] At present we have no comparable method for determining the neomycin C content of our best neomycin B preparation.

We have subjected purified neomycin B and neomycin C sulfates to methanolysis by the conditions of Dutcher and co-workers[2] and have obtained weight ratios of 1.39 and 1.37 for the neamine and methyl neobiosaminide hydrochloride fractions. The calculated weight ratio, based on one mole of neamine hydrochloride ($C_{12}H_{26}N_4O_6 \cdot 4HCl$: 468.2) and one mole of methyl neobiosaminide hydrochloride ($C_{11}H_{21}N_2O_6(OCH_3) \cdot 2HCl$: 381.3) is 1.23. Papergram analyses indicated that the neamine fractions contained traces of unreacted neomycin and that the neobiosaminide fractions contained small amounts of neamine and neomycin. The filtrates from the collection of the neobiosaminides contained additional neobiosaminide. These data clearly show that neomycins B and C yield equimolar amounts of neamine and the respective methyl neobiosaminides on methanolysis.

We have found another property of neomycins B and C which shows the presence of one mole of neamine per mole of neomycin. Treatment of neamine with boiling 48% hydrobromic acid results in the appearance of a characteristic ultraviolet spectrum with a maximum at 265 mμ. Furfural, produced from the neobiosamines by mild acid hydrolysis,[10] is destroyed under these drastic conditions. When equimolar amounts of neamine, neomycin B and neomycin C are subjected to the action of 48% hydrobromic acid the intensity of absorption at 265 mμ is essentially identical. These data strongly support the conclusion that neomycins B and C each contains only one neamine residue. Furthermore, Leach and Teeters[5] reported that 1.0 g. of neomycin sulfate produced 0.33 g. of neamine. Assuming molecular weights of 910 for neomycin sulfate and 324 for neamine, the theoretical yield of neamine would be 0.35 g.

Assuming the $C_{12}H_{26}N_4O_6$ formula for neamine and the $C_{11}H_{22}N_2O_7$ formula for the neobiosamines the neomycin B and neomycin C bases would have the composition $C_{23}H_{46}N_6O_{12}$. Elementary analyses

(1) Upjohn Company. (2) University of Illinois.

(3) J. D. Dutcher, N. Hosansky, M. N. Donin and O. Wintersteiner, THIS JOURNAL, **73**, 1384 (1951).

(4) J. D. Dutcher and M. N. Donin, *ibid.*, **74**, 3420 (1952).

(5) B. E. Leach and C. M. Teeters, *ibid.*, **73**, 2794 (1951).

(6) R. L. Peck, C. E. Hoffhine, Jr., P. H. Gale and K. Folkers, *ibid.*, **71**, 2590 (1949).

(7) R. L. Peck, C. E. Hoffhine, Jr., P. H. Gale and K. Folkers, *ibid.*, **75**, 1018 (1953).

(8) B. E. Leach, W. H. DeVries, H. A. Nelson, W. G. Jackson and J. S. Evans, *ibid.*, **73**, 2797 (1951).

(9) O. K. Sebek, *Bacteriol. Proc.*, 78 (1955).

(10) J. D. Dutcher, N. Hosansky and J. H. Sherman, *Antibiotics and Chemotherapy*, **3**, 534 (1953).

TABLE I

ANALYTICAL DATA[a]

	C	H	N	S	Cl	Eq. wt.
Calcd. for $C_{23}H_{46}N_6O_{12}$	46.14	7.74	14.04			99.8
Calcd. for $C_{23}H_{48}N_6O_{13}$	44.79	7.85	13.63			102.8
Found (B base)	45.18	7.88	13.36			103.0,[b] 104.2[c]
Found (C base)	45.10	7.70	13.33			102.8,[b] 104.2[c]
Calcd. for $C_{23}H_{46}N_6O_{12}\cdot 6HCl$	33.79	6.41	10.28		26.03	
Calcd. for $C_{23}H_{48}N_6O_{13}\cdot 6HCl$	33.06	6.52	10.06		25.46	
Found (B hydrochloride)	33.51	6.88	9.52		25.25	
Found (C hydrochloride)	33.13	6.95	9.76		25.04	
Calcd. for $C_{23}H_{46}N_6O_{12}\cdot 2.7H_2SO_4$[d]	32.00	6.00	9.73	10.02		
Calcd. for $C_{23}H_{48}N_6O_{13}\cdot 2.7H_2SO_4$[d]	31.34	6.11	9.53	9.81		
Found (B sulfate)	31.18	6.14	8.93	9.90		
Calcd. for $C_{23}H_{46}N_6O_{12}\cdot 2.9H_2SO_4$[d]	31.28	5.91	9.52	10.53		
Calcd. for $C_{23}H_{48}N_6O_{13}\cdot 2.9H_2SO_4$[d]	30.66	6.02	9.33	10.31		
Found (C sulfate)	31.18	5.91	8.71	10.52		

[a] All samples were dried to constant weight at 50° and about 0.2 mm. pressure. No corrections have been made for the residue on ignition (0.39% for B, 0.46% for C), except in the data on equivalent weights. [b] Potentiometric titration in glacial acetic acid. [c] Potentiometric titration in water. [d] Composition estimated from *p*H of its solution using Fig. 1, curve A.

on our chromatographed neomycin sulfates and on the free bases and hydrochlorides which were prepared from them do not disprove this $C_{23}H_{46}N_6O_{12}$ formula but are in somewhat better agreement with a $C_{23}H_{48}N_6O_{13}$ formula (see Table I). These data suggested a monohydrate, but attempts to remove one mole water by rigorous drying procedures or to demonstrate its presence by Karl Fischer moisture determinations have been unsuccessful. Molecular weight determinations[11] on neomycin B base (ebullioscopic in methanol) have given values ranging from 507 to 669 and the calculated value for the $C_{23}H_{48}N_6O_{13}$ formula is 616.7. Further studies on the degradation products will be required to establish the correct empirical formula of neomycins B and C.

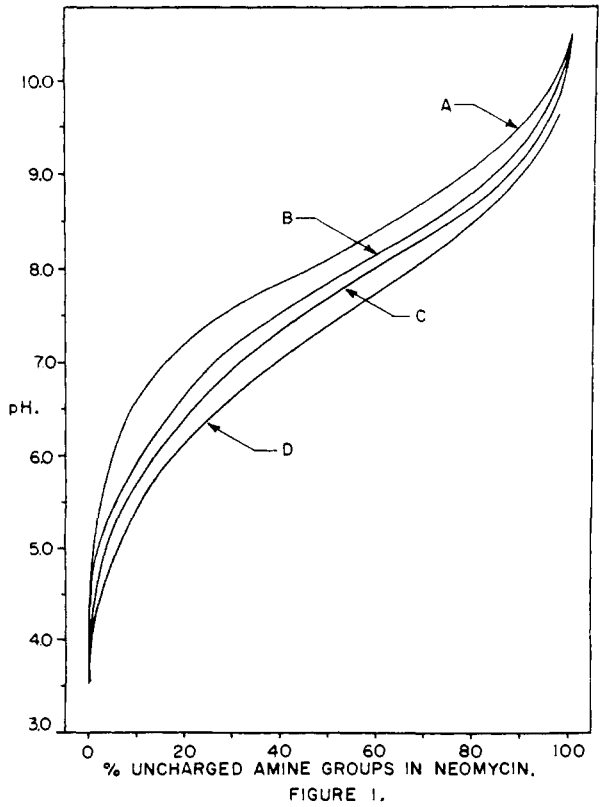

Fig. 1.—Titration curves: A, neomycin B as sulfate, 0.026 *M* in neomycin, titrated with 0.1 *N* NaOH; B, neomycin B as hydrochloride, 0.035 *M* throughout; C, neomycin C as hydrochloride, 0.035 *M* throughout; D, neomycin C as hydrochloride, 0.010 *M* throughout.

The equivalent weight determinations (see Table I) also tend to favor the $C_{23}H_{48}N_6O_{13}$ formula. Typical titration curves of aqueous solutions of neomycins B and C are given in Fig. 1. The difference between the curves obtained with hydrochloric and sulfuric acids is of interest.[12]

Our values indicate specific rotations of +83° and +121° for the B and C bases, respectively, in acid solution. These values (see Table II) are somewhat higher than those reported by Dutcher and co-workers.[2] The specific rotations are somewhat lower in alkaline solutions than in acid solutions. There is a gradual increase in rotation as the bases are neutralized, but the rotation is independent of H^+ concentration between 10^{-6} and 10 *N*, provided that the readings in 10 *N* acid are taken before hydrolysis becomes appreciable. The specific rotations have been found to be independent of concentration over the range from 10 to 100 g./l. The optical rotation is a function of the temperature for acid solutions.

For neomycin B

$$[\alpha]^t_D = [\alpha]^{25}_D [1 - 0.00171 (t - 25)] \qquad 0 < t < 80°$$

For neomycin C

$$[\alpha]^t_D = [\alpha]^{25}_D [1 + 0.000112 (t - 25)^2] \qquad 0 < t < 25°$$

$$[\alpha]^t_D = [\alpha]^{25}_D \qquad 25° < t < 80°$$

The above equations have not been corrected for the dimensional changes for the cell or solvent (0.1 *N* H_2SO_4). Measurements were made in 4% solutions. The equation for neomycin B was determined by a least squares fit.

(11) Determinations by Clark Microanalytical Laboratories, Urbana, Ill.

(12) The unusual physico-chemical characteristics of neomycin and its derivatives will be discussed in more detail in a future publication by E. R. Garrett.

It is interesting to note that the specific rotations for neamine and neamine hydrochloride which were reported by Peck and co-workers[6] indicate that neamine has the same rotation in acid and basic solutions. This suggests that one or both of the nitrogens in the neobiosamine moieties are involved in the change of rotation which the neomycins undergo between acid and alkaline solutions.

TABLE II
SPECIFIC ROTATIONS[a]

Compd.	Solvent	$[\alpha]^{25}D$, degree	Calcd. $[\alpha]^{25}D$ for base,[b] degree
B base	0.02 *N* NaOH	+71	....
B base	.2 *N* H_2SO_4	+83	+83
B base	.2 *N* HCl	+83	+83
B hydrochloride	H_2O	+63	+85
B hydrochloride[c]	H_2O	+54	+73
B sulfate[d]	0.2 *N* H_2SO_4	+56	+80
C base	.02 *N* NaOH	+110	....
C base	.2 *N* H_2SO_4	+121	+121
C hydrochloride	H_2O	+88	+121
C hydrochloride[c]	H_2O	+80	+110
C sulfate[e]	0.2 *N* H_2SO_4	+82	+120

[a] Corrected for ash and moisture. Concentrations from 1 to 2% (see text). [b] Calculated on basis of $C_{23}H_{48}N_6O_{13}$ formula for base. [c] Data of Dutcher and co-workers.[3] [d] Calculated as $C_{23}H_{48}N_6O_{13}\cdot 2.7H_2SO_4$. [e] Calculated as $C_{23}H_{48}N_6O_{13}\cdot 2.9H_2SO_4$.

Experimental[13,14]

Neomycin B Sulfate, Reduction of Ash Content.—The starting material which had been prepared by the method of Leach and co-workers[8] had the following composition: residue on ignition, 3.8%; moisture, 5.7%; $[\alpha]D$ +54°, bioassay, 650 mcg./mg. Nine hundred grams was dissolved in 4.0 l. of water and the solution was passed through a column containing 20 l. of sodium hydroxide-regenerated Ionac A-300[15] and then through a column containing 0.5 l. of acid-regenerated Ionac C-240.[16] A portion of the resulting solution was decolorized batchwise with Darco G-60 and freeze-dried to give a product having the following properties: residue on ignition, 0.54%; $[\alpha]D$ +54°; bioassay, 714 mcg./mg. The remainder of the solution was dried and chromatographed as described in the following paragraph.

Carbon Chromatography of Neomycin B Sulfate.—An aqueous slurry containing 2 kg. of Darco G-60 and 1 kg. of Celite 545 was adjusted to *p*H 2.0 with sulfuric acid, stirred for two hours and packed into a column of 4-inch Pyrex pipe under pressure, giving a bed depth of 42 inches. The column was washed with 13.5 l. (about 2 holdup volumes) of distilled water. The starting material, 400 g. of the above-described preparation, was dissolved in sufficient water to give 760 ml. of solution and adjusted to *p*H 2.5 with sulfuric acid. This solution was slurried with 200 g. of acid-washed Darco G-60 and 100 g. of acid-washed Celite 545 and introduced into the top of the column. The column was developed with water at a flow rate of 10 ml./min. After the removal of 7.1 l. of optically-inactive liquid holdup, the active fractions were collected in 50-ml. cuts. A graph showing observed and specific rotations of the eluates is given in Fig. 2. The specific rotation remained constant from 0.8 to 3.1 l. These fractions (indicated by the arrows on Fig. 2) were pooled and adjusted[17] to *p*H 6.4 with a

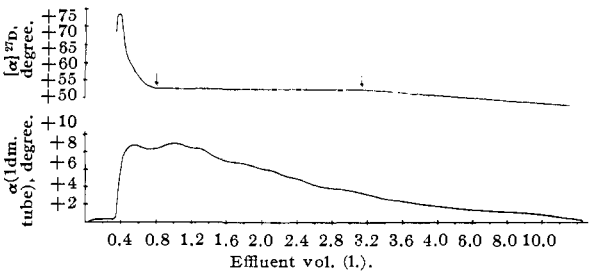

Fig. 2.—Carbon chromatography of neomycin B sulfate.

column of Ionac A-300[15] and freeze-dried. The resulting white amorphous solid (257 g.) represented a 58% recovery of the optical activity. It had the following properties: *p*H (72 mg./ml. aqueous solution), 6.4; residue on ignition, 0.39%; $[\alpha]D$ +56°; bioassay, 738 mcg./mg.

A small amount of material having a higher specific rotation was eluted before the main neomycin B fraction. This was believed to be mainly neomycin C.

Neomycin C Sulfate, Reduction of Ash Content.—The starting material was obtained by collecting forefractions from a large number of carbon chromatograms. These were dissolved in water to give a 10% solution, adjusted to *p*H 10.1 with sodium hydroxide and diluted with 3 volumes of acetone. The resulting ash precipitate was filtered off and the filtrate was adjusted to *p*H 7.2 with sulfuric acid. The precipitate of neomycin sulfate was filtered, dissolved in water, decolorized with carbon (Nuchar C-190) and dried from the frozen state. This material had the following composition: residue on ignition, 3.4%; moisture, 6.3%; $[\alpha]D$ +78°.

One kilogram of this material was further purified by the use of Ionac A-300 and Ionac C-240 ion-exchange resins using the same process that was described under Neomycin B Sulfate, Reduction of Ash Content. This product had the following composition: residue on ignition, 0.49%; $[\alpha]^{25}D$ +79°; *p*H, 6.4; bioassay, 368 mcg./mg.

Carbon Chromatography of Neomycin C Sulfate.—The column was packed with 1780 g. of Darco G-60 and 890 g. of Celite 545 using the method described under Carbon Chromatography of Neomycin B Sulfate. The starting material was 360 g. of the above described neomycin C sulfate which was added to the column as a slurry with 200 g. of Darco G-60 and 100 g. of Celite 545. A graph showing the observed and specific rotations of the eluates is given in Fig. 3. A small fraction which contained ash and neomycin C sulfate was obtained shortly after the first holdup volume had been removed. The specific rotation then remained constant and the fractions between the arrows on Fig. 2 were pooled and adjusted to *p*H 6.2 with Ionac A-300 and dried from the frozen state. The yield was 298 g. of a white amorphous solid and represented an 80% recovery of the optical activity. It had the following properties: *p*H (33 mg./ml. aqueous solution), 6.2; residue on ignition, 0.46%; $[\alpha]D$ +82°; bioassay, 348 mcg./mg.

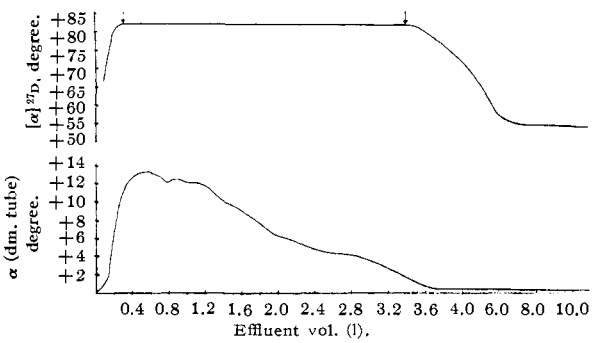

Fig. 3.—Carbon chromatography of neomycin C sulfate.

Near the end of the chromatogram the specific rotation of the eluates decreased and then leveled off at approximately +54°, the specific rotation of neomycin B sulfate.

Neomycin B Base.—Six grams of the chromatographed neomycin B sulfate was dissolved in 100 ml. of boiled dis-

(13) Unless otherwise specified, all specific rotations are measured in 20 mg./ml. aqueous solutions and are corrected for ash and moisture.

(14) Bioassays are reported in mcg. of neomycin base/mg. as determined by *K. pneumoniae* turbidimetric assay using the United States Food and Drug Administration method. This method employs a neomycin B sulfate standard.

(15) An "intermediate base" anion-exchange resin sold by The Permutit Co.

(16) A sulfonated polystyrene cation-exchange resin sold by The Permutit Co.

(17) The *p*H adjustment was made in order to conform with the United States Food and Drug Administration specification (*p*H 5.0 to 7.5 for a 33 mg./ml. solution).

tilled water and passed through a column containing 100 ml. of Dowex-2[18] which had been regenerated with carbonate-free sodium hydroxide. The effluent was freeze-dried and suitable precautions were taken to minimize contact with carbon dioxide from the air. The yield was 3.8 g. of a white amorphous solid having the following properties: residue on ignition, 0.38%; carbonate, none; sulfate, none; chloride, trace; loss on drying at 50° in high vacuum, 6.88%; water (Karl Fischer), 7.3%; water (Karl Fischer) on sample dried at 50° in high vacuum, 0.64%.

Neomycin B Hydrochloride.—Neomycin B base was dissolved in a slight excess of dilute hydrochloric acid and the solution was freeze-dried to give a white amorphous solid.

Neomycin C Base.—It was prepared from the chromatographed neomycin C sulfate by the same process that was used for neomycin B base and was found to have the following properties: carbonate, none; sulfate, none; chloride, trace; loss on drying at 50° in high vacuum, 5.34%; water (Karl Fischer), 5.7; water (Karl Fischer) on sample dried at 50° in high vacuum, 0.51%.

Neomycin C Hydrochloride.—Neomycin C base was dissolved in a slight excess of dilute hydrochloric acid and freeze-dried to give an amorphous solid.

Effect of Concentration on Specific Rotation.—A preparation of neomycin B sulfate was found to have a specific rotation of +53.2 ± 0.3° (average deviation) when measured at concentrations from 10 mg./ml. to 100 mg./ml. in water at 22°. The data showed no trend with concentration.

Potentiometric Titrations.—The aqueous titrations were run under oxygen-free nitrogen with a Precision-Dow Recordomatic Titrator and the maximum probable error in a specific *p*H reading is ±0.1 *p*H unit. The titrations in glacial acetic acid were made with 200–400 mcg./ml. solutions of the base *vs.* 0.1 *N* perchloric acid using a Beckman Model G *p*H meter equipped with glass (Beckman No. 1190-42) and silver–silver chloride (Beckman No. 1264) electrodes. End-points were determined from a plot of relative millivolts *vs.* acid volume, using second derivatives for greater accuracy.

Methanolysis of Neomycin B and C.—A mixture of 1.0 g. of neomycin B sulfate ($[\alpha]^{25}D$ +54°; residue on ignition, 3.8%) and 120 ml. of anhydrous methanol 0.38 *N* in dry hydrogen chloride was refluxed for 2.5 hours. Complete solution occurred after one hour. The colorless solution was chilled in an ice-bath and was diluted with 40 ml. of anhydrous ether. The flocculent, white precipitate of neamine hydrochloride which formed was removed by filtration on a tared sintered glass funnel, washed with 5 ml. of anhydrous ether, and dried *in vacuo* over phosphorus pentoxide to yield 476 mg. of material. The filtrate was evaporated *in vacuo* to a volume of 10 ml., chilled in an ice-bath, and diluted with 100 ml. of anhydrous ether. The flocculent precipitate of methyl neobiosaminide B hydrochloride which formed was removed by filtration on a tared sintered glass funnel, washed with 10 ml. of anhydrous ether, and dried *in vacuo* over phosphorus pentoxide to yield 343 mg. of material.

The above procedure was exactly repeated using 1.0 g. of neomycin C sulfate ($[\alpha]^{25}D$ +78°; residue on ignition, 3.4%). The yield of neamine hydrochloride was 495 mg.; the yield of methyl neobiosaminide C hydrochloride was 361 mg.

Vigorous Acid Hydrolysis of the Neomycins.—Solutions of 50 mg. of neamine, 100 mg. of neomycin B sulfate ($[\alpha]^{25}D$ +54°; residue on ignition, 3.8%), and 100 mg. of neomycin C sulfate ($[\alpha]^{25}D$ +78°; residue on ignition, 3.4%) in 5 ml. of 48% hydrobromic acid were refluxed for 10 hours. Each hydrolysate rapidly became dark maroon in color. Each hydrolysate was concentrated to dryness by distillation *in vacuo* and two 5-ml. portions of distilled water were distilled *in vacuo* from the hydrolysis residues. Dilutions for ultraviolet[19] spectral analysis of the respective hydrolysates were made such as to contain 63 mcg. (0.20 micromole) of parent neamine per ml., 115 mcg. (0.19 micromole) of parent neomycin B per ml., and 115 mcg. (0.19 micromole) of parent neomycin C per ml.

Acknowledgments.—We wish to express our thanks to Dr. A. D. Cooper for the potentiometric titrations in glacial acetic acid, to Mr. W. A. Struck and associates for the microanalyses, to Mrs. Jean W. Snyder and associates for the microbiological assays, and to Dr. O. K. Sebek for the microbiological estimation of the neomycin B content of our neomycin C.

(18) A strongly basic anion-exchange resin obtained from the Dow Chemical Co.

(19) The ultraviolet spectra were measured on a Beckman Spectrophotometer, model D.

KALAMAZOO, MICHIGAN
URBANA, ILLINOIS

[CONTRIBUTION FROM COBB CHEMICAL LABORATORY, UNIVERSITY OF VIRGINIA]

Nuclear Substituted Analogs of Norepinephrine, Dihydroxyphenylalanine and Adrenochrome

BY EDWIN D. HORNBAKER[1] AND ALFRED BURGER

RECEIVED MAY 14, 1955

Syntheses of 2-methylnorepinephrine, (±)4-methylnoradrenochrome, 1-(2-chloro-3,4-dimethoxyphenyl)-2-aminoethanol, β-(2-methyl-3,4-dihydroxyphenyl)-α-alanine and β-(2-chloro-3,4-dihydroxyphenyl)-α-alanine are described.

As part of a program designed to investigate the pharmacological effects of nuclear substitution in epinephrine precursors, the study of certain nuclear substituted derivatives of 3,4-dihydroxyphenylalanine (DOPA) and norepinephrine appeared desirable since DOPA and norepinephrine may play a role in the biosynthesis of epinephrine.[2] Structural analogs of DOPA produced by side chain alterations have been shown to cause inhibition of mammalian DOPA decarboxylase,[3] and modifications of the side chain in norepinephrine are known to produce valuable pharmacodynamic properties.[4] The effects of nuclear substitution of epinephrine have been studied only for the (±)6-methyl homolog.[5] Hydroxy[6] and amino[7] derivatives of 3,4-dihydroxyphenylethanolamine have been described but their biological properties have not been divulged. Likewise, syntheses of 2-methyl-,[8] 5-

(1) National Science Foundation Fellow, 1953–1955.

(2) K. H. Beyer, "Chemical Factors in Hypertension," A.C.S. Advances in Chemistry Series, No. 2, 1950, p. 37.

(3) T. L. Sourkes, *Arch. Biochim. Biophys.*, **51**, 444 (1954); G. A. Stein, H. A. Bronner and K. Pfister, 3rd, THIS JOURNAL, **77**, 700 (1955); G. J. Martin, R. Brendel and J. M. Beiler, *Exper. Med. & Surg.*, **8**, 5 (1950).

(4) A. M. Lands and M. L. Tainter, *Arch. exper. Path. Pharmakol.*, **219**, 76 (1953); A. M. Lands, Natl. Research Council, Natl. Acad. Sci., Washington, D. C., Chem. Biol. Coördination Center, Pub. No. 206, 73–123 (1951).

(5) R. S. Grewal, *Brit. J. Pharmacol.*, **7**, 338 (1952).

(6) O. Hinsberg, *Ber.*, **56**, 852 (1923); A. Dornow and G. Petsch, *Arch. Pharm.*, **284**, 160 (1951).

(7) C. Mannich and G. Berger, *ibid.*, **277**, 147 (1939).

(8) R. I. T. Croumartie and J. Harley-Mason, *J. Chem. Soc.*, 1052 (1952).