They do not react with the indicator sprays used. In general, R_f values are high in phenol (0.38 to 0.8) and low in butanol (below 0.3). Eleven such spots are evident at 100°, and nineteen at 56.5°.

Activities.—Activities are low but in the aggregate may represent an appreciable percentage of the total. Activity is derived from glucose, and in some cases from glycine.

Organic Acids.—Non-volatile acids were not detected by the procedures used, in chromatograms of the final reaction mixtures.

Dialysis Residues.—The dialysis residue is highly pigmented (dark brown), insoluble in water and on chromatography differs from spot 10 in that it does not move from the origin in phenol–water or in butanol mixtures. With labeled glucose, the specific activity at both temperatures approximates that of the original mixture. With labeled glycine, the specific activity at 100° was 20%, and at 56.5°, 65 to 70% that of the original.

Discussion

The reaction between glucose and glycine is complex, involving the formation of numerous compounds, as shown in Fig. 1 where the presence of more than two dozen compounds has been demonstrated. Other compounds are present in small amount, indicated by weak ninhydrin or *m*-phenylenediamine reactions, but they are not demonstrable radiographically in significant amount. They have therefore been ignored in the present discussion.

Compound 5 requires further study because of its absorption maximum in the 270–300 mμ region where furfurals show high absorption. It derives its activity solely from the glucose and its role in browning also requires evaluation.

Compound 2 has many characteristics similar to the compounds separated by Gottschalk and Partridge.[4] We hope to isolate and purify this compound, the major reaction product, except for brown pigment, and to evaluate its possible role as an intermediate in browning.

Compound 8, identified with HMF is clearly of interest because furfurals have been shown to play a part in browning of natural products.[7]

As shown by analysis of dialysis residues, more carbon is incorporated into brown pigment at the lower temperature, from glycine carboxyl carbon, than at the higher temperature. This coincides with the fact that at the lower temperature more pigment is produced per millimole of carbon dioxide from the glycine molecule.[5]

(7) E. R. Stadtman, *Advances in Food Research*, 1, 325 (1948).

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The Identity of Neomycin A,[1] Neamine[2] and the Methanolysis Product of Neomycin B and C[3]

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A comparison of the reported chemical and biological properties of these products[1–3] suggested that they might be identical and hence a fuller and more direct comparison was made, with the result that their identity has been adequately established. The free base of the methanolysis product 1 obtained from neomycins B and C[3] crystallized readily when subjected to the conditions reported for the crystallization of neamine,[2] the product isolated from the hydrolysis of neomycin with 6 *N* sulfuric acid. From the comparison of the properties of the bases and their derivatives shown in Table I there can be no doubt as to their identity. In view of the observation that both neamine and neomycin A possess antibacterial activity, of low order in the broth test but high in the agar diffusion assay,[2] and that in many of our neomycin preparations there could be demonstrated an antibacterial component with the same mobility in papergrams as neamine, it was suspected that neamine might be identical with neomycin A. A sample of the purified crystalline *p*-(*p*'-hydroxyphenylazo)-benzenesulfonate of neomycin A[4] was compared with the corresponding salt of neamine with the results also shown in Table I.

Although the identity of these products is apparent from their properties, the composition is not yet certain. The empirical formula $C_6H_{12\text{-}14}O_3N_2$ has been proposed for neamine[2] whereas the composition $C_9H_{19}O_5N_3$ has been advanced for the methanolysis product 1.[3] Since neomycin A has been found to yield on hydrolysis at 140° with 6 *N* hydrochloric acid a product with the proven composition $C_6H_{14}O_3N_2$,[5] it would therefore appear that the original product contains more than 6 carbon atoms and has either the C_9 composition or the C_{12} dimeric composition suggested.[2] Papergram studies have shown that an additional moiety formed by this further hydrolysis with strong acid is a ninhydrin positive-alkaline silver reducing material with an R_f similar to that of the diaminodesoxyhexose fragment obtained from the hydrolysis of methyl neobiosaminide.[3] Further studies of these products are being made.

Experimental

Methanolysis of Neomycin B.—A solution of 2.81 g. of neomycin B hydrochloride in 280 ml. of anhydrous methanol was refluxed for 2.5 hours with 60 ml. of 1.8 *N* methanolic hydrogen chloride. The addition of 100 ml. of anhydrous ether to the cooled solution caused the precipitation of 1.62 g. of amorphous white solid (methanolysis product 1). The mother liquor from the precipitation was concentrated to 25 ml. and diluted with 250 ml. of ether, yielding 1.00 g. of amorphous methanolysis product 2. Papergrams of these fractions show that each contains small amounts of the other but that subsequent purification results in homogeneous products.

Crystallization of Methanolysis Product 1.—A solution of 1.60 g. of the amorphous hydrochloride in 1.8 ml. of concentrated ammonium hydroxide was diluted with 146 ml. of methanol, and ammonia gas was bubbled through the solution until crystallization had begun. This solution was refrigerated for 2.5 hours and then the crystals were collected on a Buchner funnel, washed with cold absolute methanol, and dried *in vacuo*; yield 605 mg. An additional 187 mg. obtained on concentration of the mother liquor. The base was recrystallized for analysis from an ethanol–water mixture. It darkens at 230° with softening at 235° and decomposes above 280°; $[\alpha]^{24}D$ +123° (*c*, 0.7 in water).

Anal. Calcd. for $(C_6H_{14}O_3N_2)_x$: C, 44.43; H, 8.70;

(1) R. L. Peck, C. E. Hoffhine, Jr., P. Gale and K. Folkers, THIS JOURNAL, **71**, 2590 (1949).

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

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

(4) The sample of neomycin A *p*-(*p*'-hydroxyphenylazo)-benzenesulfonate was obtained through the courtesy of Dr. R. L. Peck, Merck and Co., Rahway, N. J.

(5) F. A. Kuehl, Jr., M. N. Bishop and K. Folkers, THIS JOURNAL, **73**, 881 (1951).

TABLE I

A COMPARISON OF NEOMYCIN A, NEAMINE AND METHANOLYSIS PRODUCT 1 FROM NEOMYCIN B

Properties	Methanolysis Product 1	Neamine[a]	Neomycin A
Of free base:			
Melting point, °C.	Darkens at 230 with softening	256 (dec.)	
ca. 253 no distinct m. p. but dec. above 280			.....
$[\alpha]_D$ in H_2O	+123°	+123°	.....
Analyses, %	C, 44.90; H, 7.64; N, 16.93	C, 44.92; H, 8.06; N, 16.95	.....
	45.09 7.98 17.07	44.48 8.13 17.35	
Infrared	Experimentally obtained curve was very similar to published curve[2]		.....
Antibacterial potency (Ratio of minimum-inhibiting concentration in Y–B broth *vs. E. coli*:*Staph. aureus*:*K. pneumoniae*)	1.0:0.15:0.50	1.0:0.13:0.50	.....
Of amorphous hydrochloride:			
Melting point, °C.	Starts to darken 165, dec. *ca.* 250		Darkens *ca.* 220° and melts with dec. 250–260°[1]
$[\alpha]_D$ in H_2O	+84°	+85° (calcd.)	+83°[1]
Of crystalline *p*-(*p'*-hydroxyphenylazo)-benzenesulfonate:			
Melting point, °C.	Starts dec. 222		Dec. at 240 but does not melt up to 300[1]
Analyses, %	C, 50.63; H, 4.64; N, 11.40	.....	C, 50.42; H, 5.01; N, 11.2[b]
$E^{1\%}_{1\ cm}$ at 3700 Å. in *M*/20 phosphate buffer at *p*H 8.0	397	.....	410
Antibacterial potency Diameter of zone on Y–B agar *vs. S. aureus* after 27 hours diffusion at 5°	8 mm.	.....	8 mm.
Papergram:			
Descending in *n*-BuOH(50)–H_2O(25)–acetic acid (25), 30 hr.	12.5 cm. from origin	.....	12.5 cm. from origin
Ascending in *n*-propanol(50)–H_2O(45)–acetic acid (5)	R_f 0.32	.....	R_f 0.32
Of polyacetate:			
Melting point, °C.	260–262	261	.....
Analyses, %	C, 49.77; H, 6.90; N, 8.33; O-acetyl, 25.97; total acetyl, 43.75	C, 52.08; H, 6.62; N, 7.68; O-acetyl, 34.0; total acetyl, 48.1	.....
Mol. wt. in camphor (Rast)	543, 512	502, 657	.....
Of N-benzoate:			
Melting point, °C.	>300	327–328	.....
Analyses, %	C, 64.54; H, 6.02; N, 7.43	C, 64.80; H, 5.91; N, 7.50	.....

[a] All data from ref. 2. [b] Data presented by R. L. Peck at A.A.A.S. meeting, Cleveland, Ohio, Dec. 1950: see also *Ann. Review Biochemistry*, XX, 376 (1951).

N, 17.28. Calcd. for $C_9H_{19}O_5N_3$: C, 43.36; H, 7.68, N, 16.86. Found: C, 44.90, 45.09; H, 7.64, 7.98; N, 16.93, 17.07.

Crystalline *p*-(*p'*-Hydroxyphenylazo)-benzenesulfonate of Methanolysis Product 1.—To a solution of 78.4 mg. of amorphous hydrochloride in 5 ml. of water was added 5 ml. of a 5% aqueous solution of *p*-(*p'*-hydroxyphenylazo)-benzenesulfonic acid. The yellow precipitate which formed was collected by filtration and recrystallized four times from hot water. The fine needles began to decompose at 222° but showed no definite melting point.

Anal. Calcd. for $C_{30}H_{34}O_{11}N_6S_2$: C, 50.13; H, 4.77; N, 11.69; S, 8.92. Calcd. for $C_{45}H_{49}O_{17}N_9S_3$: C, 49.85; H, 4.56; N, 11.63; S, 8.87. Found: C, 50.63; H, 4.64; N, 11.40; S, 9.05.

Crystalline N-Acetate of Methanolysis Product 1.—A mixture of 0.700 g. of amorphous hydrochloride, 1.777 g. of silver acetate, 1.5 ml. of acetic anhydride and 35 ml. of absolute methanol was shaken in the dark for 6 hours and then allowed to stand at room temperature overnight. The reaction mixture was refluxed on the steam-bath for 5 minutes and then filtered free of silver chloride. The filter cake was washed well with hot water. Hydrogen sulfide was bubbled through the filtrate to precipitate excess silver ions. After filtration, the clear water-white solution was concentrated to dryness *in vacuo*, yielding 808 mg. of a white solid. This crystallized from methanol as fine needles which sintered at 260° and decomposed at 300°; $[\alpha]^{22}_D$ +84° (*c*, 0.5 in water). Two recrystallizations from methanol raised the specific rotation to +87° (H_2O).

Anal. Calcd for $(C_{10}H_{18}O_5N_2)_x$: C, 48.77; H, 7.37; N, 11.38; acetyl, 34.95. Calcd. for $C_{15}H_{25}O_8N_3$: C, 48.00; H, 6.71; N, 11.20; acetyl, 34.41. Found: C, 47.76, 47.94; H, 6.95, 7.36; N, 12.01; acetyl, 30.60.

Crystalline Polyacetate a Methanolysis Product 1.—A mixture of 98.8 mg. of the N-acetate, 3.0 ml. of dry pyridine and 1.0 ml. of acetic anhydride was shaken for 48 hours, by which time complete solution had been effected. The residue which remained after evaporation of the solvent crystallized as prisms from acetone; yield 87.8 mg. The crystalline polyacetate softened at 165°, resolidified at 215°, and melted at 260–262°, $[\alpha]^{24}_D$+ 38° (*c*, 0.6 in water).

Anal. Calcd. for $C_{16}H_{24}O_8N_2$: C, 51.61; H, 6.49; N, 7.52; O-acetyl, 34.7; total acetyl, 57.8; mol. wt., 372.37. Calcd. for $C_{21}H_{31}O_{11}N_3$: C, 50.29; H, 6.23; N, 8.38; O-acetyl, 25.75; total acetyl, 51.50; mol. wt., 501.48. Found: C, 49.77; H, 6.90; N, 8.33; O-acetyl, 25.97; total acetyl, 43.75; mol. wt. (Rast in camphor), 543, 512.

Crystalline N-Benzoate of Methanolysis Product 1.—A 242-mg. portion of amorphous hydrochloride was dissolved in 5 ml. of *N* sodium hydroxide solution and treated with 2 ml. of benzoyl chloride and 4 ml. of 25% sodium hydroxide solution according to the Schotten–Bauman procedure. The resulting pasty solid was collected by centrifugation, washed well with water and with ether, and dissolved in chloroform. Evaporation of the chloroform solution yielded 562 mg. of amorphous polybenzoate. This, combined with 70 mg. from a previous preparation, was dissolved in 15 ml. of 0.5 *N* methanolic sodium hydroxide solution and refluxed for 4 hours. The methanolic solution was filtered, brought to *p*H 7.0 by the addition of 2 *N* hydrochloric acid, and concentrated to dryness *in vacuo*. The solid was extracted with methanol, and the methanolic solution was concentrated to dryness *in vacuo*. The residue was washed well with water and ether and then crystallized as fine needles from hot aqueous methanol; yield 311 mg. A sample for analysis was recrystallized from hot methanol–water mixture; $[\alpha]^{24}_D$ +70° (*c*, 0.5 in methanol).

Anal. Calcd. for $C_{30}H_{22}O_5N_2$: C, 64.85; H, 5.99; N, 7.56. Calcd. for $C_{30}H_{31}O_8N_3$: C, 64.14; H, 5.56; N, 7.49. Found: C, 64.54; H, 6.02; N, 7.43.

Papergrams.—Two systems which have been found to separate neomycin and neamine on the papergram are (1) *n*-butanol 50 parts by volume, water 25 parts, acetic acid 25 parts, and (2) *n*-propanol 50 parts by volume, water 45 parts, acetic acid 5 parts. Resolution of the bases is obtained whether these are applied as the hydrochlorides or as the salts of *p*-(*p'*-hydroxyphenylazo)-benzenesulfonic acid. The location of the bases is determined either by spraying the dried sheet with ninhydrin reagent or by incubating the developed sheet on an agar plate seeded with a microorganism. The crystalline *p*-(*p'*-hydroxyphenylazo)-benzenesulfonates of neomycin A and methanolysis product 1 (neamine) were run side by side on Whatman paper number 1 in descending fashion in the butanol system and the distance from the origin determined after a 30-hour period of time. This distance was identical as shown in Table I. The same compounds were run ascendingly in the propanol system and gave identical R_f values as listed in Table I.

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Derivatives of Sulfenic Acids. VII. Addition of Sulfenyl Halides to Olefins

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In a previous study,[1] the characterization of a series of olefins *via* 2,4-dinitrobenzenesulfenyl chloride (I) was reported, and the reaction involved was discussed. Six new examples of this reaction are now included in Table I. The excellently crystalline products may serve as useful derivatives.

While additions of sulfenyl chlorides to olefins are already well known, formation of 2-chloroethyl 2'-bromocyclohexyl sulfide from 2-chloroethanesulfenyl bromide and cyclohexene appears to be the only recorded addition of a sulfenyl bromide to an olefin.[2] The adducts of 2,4-dinitrobenzenesulfenyl bromide (II) with cyclohexene and styrene, as well as the adduct of 2-nitrobenzenesulfenyl bromide (III) with 1,4-dihydronaphthalene, are now reported.

Independent proofs of structure for the products of Table I were not made. The structures of the 1:1 adducts of I and III with 1,4-dihydronaphthalene, as well as of II with cyclohexene, may be specified with assurance to be the expected β-halo sulfides, *e.g.*, 2-bromocyclohexyl 2'-nitrophenyl sulfide. Application of Markownikoff's rule, considering the polarity of the sulfenyl halides as $ArS^{\delta+}X^{\delta-}$, suggests the most probable structures for the 1:1 adducts to the unsymmetrical open-chain olefins of Table I.

2,4-Dinitrobenzenesulfenyl bromide was obtained by reaction of I with potassium bromide (82%); and by action of bromine on 2,4-dinitrothiophenol (75–80%). The preparation of this sulfenyl bromide, in low yield, by brominolysis of 2,4-dinitrophenyl disulfide, was mentioned previously,[3] but its analysis and alternate syntheses have not been recorded.

Experimental

Adducts of Table I.—Known procedures were used to prepare 2,4-dinitrobenzenesulfenyl chloride[3] and 2-nitrobenzenesulfenyl bromide.[4] The preparation of 2,4-dinitrobenzenesulfenyl bromide is given below. Additions to the olefins were made in glacial acetic acid by the general technique previously described.[1] With the sulfenyl bromides, the reaction mixtures were heated on the steam-bath for five to ten minutes, then let stand at room temperature for one day, rather than heating until a negative test for the sulfenyl bromide was obtained. The olefins used were either the purified commercial products, or were prepared by standard methods. Yields of the crude adducts were generally 60–80%, and purifications were effected by recrystallization from alcohol or benzene.

2,4-Dinitrobenzenesulfenyl Bromide.—A solution of 7.2 g. of I in 25 ml. of benzene was shaken with 30 g. of dry potassium bromide for 24 hours. The orange solution was filtered, the salt cake washed with three 15-ml. portions of benzene, and the filtrate and washings combined.

Evaporation of solvent gave 7 g. (82%) of orange crystals, m.p. 103–105°. The analytical sample (m.p. 104.5–105.5°) was obtained by two recrystallizations from carbon tetrachloride.

Anal. Calcd. for $C_6H_3O_4N_2SBr$: C, 25.81; H, 1.08. Found: C, 25.96; H, 1.29.

By another route: 2,4-dinitrothiophenol[5] (9.6 g., 0.048 mole) was added with stirring during 1.5 hours to 4.8 ml. (0.094 mole) of bromine, in 250 ml. of chloroform, in a 1-liter 3-neck flask—under anhydrous conditions. Evaporation of solvent and excess bromine gave 11.6 g. (85%) of excellent needles, melting at 101–104°. This product was

TABLE I

REACTIONS OF SULFENYL HALIDES WITH OLEFINS

Olefin	Sulfenyl[a] halide	Product	M.p., °C.[b]	Analyses,[d] % Calcd. C	Calcd. H	Found C	Found H
Allyl bromide	I	$C_9H_5O_4N_2BrClS$	110–111[c]	30.40	2.27	30.48	2.32
Allyl chloride	I	$C_9H_5O_4N_2Cl_2S$	110–111	34.74	2.59	34.41	3.08
p-Chlorostyrene	I	$C_{14}H_{10}O_4N_2Cl_2S$	150–151	45.05	2.70	45.31	2.48
Cyclohexene	II	$C_{12}H_{13}O_4N_2BrS$	117–118	39.89	3.60	39.96	3.66
1,4-Dihydronaphthalene	I	$C_{16}H_{13}O_4N_2ClS$	156–157	52.68	3.59	52.71	3.71
1,4-Dihydronaphthalene	III	$C_{16}H_{14}O_2NBrS$	94–96	52.75	3.84	52.88	3.81
Indene	I	$C_{15}H_{11}O_4N_2ClS$	126.5–127	51.36	3.16	51.37	3.27
Styrene	II	$C_{14}H_{11}O_4N_2BrS$	142–143	43.86	2.87	44.06	3.13
Vinylacetic acid	I	$C_{10}H_9O_6N_2ClS$	167–169	37.45	2.83	37.49	2.79

[a] I, 2,4-Dinitrobenzenesulfenyl chloride; II, 2,4-dinitrobenzenesulfenyl bromide; III, 2-nitrobenzenesulfenyl bromide. [b] Melting points are not corrected. [c] The melting point of a mixture of the adducts of I to allyl chloride and allyl bromide was not depressed—suggesting formation of mixed crystals. Analogous behaviors appear to be involved in the adducts of I and II with cyclohexene and styrene. The melting points of the adducts of I with these olefins, previously recorded, are essentially the same (117–118° and 143–143.5°)[1] as those of the similar adducts of II (see above); melting points of mixtures of the corresponding pairs were not appreciably depressed. [d] We are indebted to Dr. A. Elek and Mr. J. Pirie for the microanalyses.

(1) N. Kharasch and C. M. Buess, THIS JOURNAL, **71**, 2724 (1949).
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