[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE PYRIDIUM CORPORATION]

Tuberculostatic Compounds. II. N⁵-Derivatives of 2-Butoxy-5-aminopyridine

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In the first paper of this series,[1] various ethers of 2-hydroxy-5-aminopyridine which possessed *in vitro* tuberculostatic activity were described. Of these, 2-butoxy-5-aminopyridine was shown to possess maximum tuberculostatic activity. We have now prepared derivatives of this compound in which one or both of the hydrogens of the 5-amino group have been replaced.

All of the compounds, described in Table I, are new with the exception of the acetyl derivative. Standard methods of preparation are given by references in the footnotes to the table. Preparations which deviated from the usual, or contained new elements of procedure, are described in the experimental part.

Many of the derivatives prepared possessed high *in vitro* activity but none possessed higher activity than that of the parent amino ether.[2] The possibility that certain of the derivatives owe their activity to regeneration of the amino group has been considered. It was not possible by means of the Marshall method[3] to detect free diazotizable primary aromatic amine during the period of incubation. These observations, together with the fact that certain of the active derivatives are non-hydrolyzable, indicate that a free amino group is not a necessary condition for activity in compounds of this type.

The results of these experiments, and toxicity studies reported elsewhere,[4] indicate that optimum properties are attained in the formaldehyde bisulfite derivative. This and several other compounds were selected for further study *in vivo*.[5]

Experimental

Compound numbers are those used in Table I.

2-Butoxy-5-butylaminopyridine, 2.—A solution of 18 g. of 2-butoxy-5-aminopyridine and 7 g. of *n*-butyl bromide was refluxed for twenty hours (112°). The resulting dark brown material was poured into water and the solution made alkaline. The mixture was extracted with ether and the fraction boiling at 150–180° at 3 mm. was collected. This was redistilled and the fraction boiling at 141–143° at 1.2 mm. was retained. This distillate was dissolved in ether and treated with dry hydrogen chloride gas. The resulting hydrochloride was reconverted to the base and redistilled. The product, b. p. 155–156° at 2.7 mm., was obtained as a green liquid which turned crystalline. It was then recrystallized from petroleum ether to give a low yield of white crystals, m. p. 51–52°.

2-Butoxy-5-dimethylaminopyridine, 3.—On cooling to 50° a hot solution of 11.6 g. of 2-butoxy-5-aminopyridine in 200 cc. of 1 *N* sulfuric acid, the sulfate precipitated in part. To this suspension was added 19 g. of 37% formaldehyde solution diluted to 70 cc.; complete solution resulted. Forty grams of zinc dust was added portionwise with stirring at 50–80°. The temperature was then maintained at 95–100° for one and one-half hours. After cooling, the solution was decanted from the zinc dust. No product was found in this solution. Dilute sodium hydroxide was added to the zinc sludge and the pasty mass extracted with ether. On evaporation of the ether, 13 g. of solid residue remained. Crystallization from methanol gave 5.9 g. of white needles, m. p. 73–75°.

Anal. Calcd. for $C_{11}H_{18}N_2O$: C, 68.1; H, 9.3. Found: C, 68.4; H, 9.0.

2-Butoxy-5-hydroxyethylaminopyridine, 4.—Ten grams of ethylene oxide and 38.5 g. 2-butoxy-5-aminopyridine were mixed and let stand at room temperature for one week. The solution was then heated at 80° for three hours. The resulting liquid was distilled and the fraction of b. p. 177–210° at 1.2 mm. retained. Redistillation gave 7.3 g. of viscous liquid, b. p. 178–181° at 2.5 mm.

2-Butoxy-5-(sulfinomethylamino)-pyridine,[6] 5.—To 206 g. of 2-butoxy-5-aminopyridine, dissolved in 3200 cc. of isopropanol, there was added 206 g. of powdered sodium formaldehyde sulfoxylate. The whole was refluxed, with stirring, for five and one-half hours. The flask was partially insulated and the slurry allowed to stand overnight. The solid was centrifuged off, washed with acetone and recentrifuged. The highly water-soluble product weighed 282 g. after drying *in vacuo* and assayed 88% purity (76% yield).

6-Butoxy-3-pyridylaminomethyl Sodium Sulfite,[6] 6.—Freshly distilled 2-butoxy-5-aminopyridine (442 g.) was added to a solution of 310 g. of sodium bisulfite and 203 cc. of 37% formaldehyde solution in 1300 cc. of water. This was heated at 80–85° for one hour; complete solution of the amine was effected in the first ten minutes. After cooling, the iridescent plate-like crystals were separated, washed with 300 cc. of ice-water and then with 500 cc. of ice-cold methanol. The resulting product, a dihydrate, was converted to the anhydrous form by drying at 110°. The yield was 611 g. (80%); solubility about 12% in water at room temperature.

2-Butoxy-5-phthaloylamino-(and phthalimido)-pyridine, 15, 16.—A solution of 53 g. of 2-butoxy-5-aminopyridine and 75 g. of phthalic anhydride in 500 cc. of absolute ethanol was refluxed one-half hour. Water was added to the cooled reaction mixture to maximum precipitation. The solid was resuspended in water and sodium hydroxide added to *p*H 8. The insoluble phthalimido compound was separated, 17 g., m. p. 162–163°. The filtrate was acidified to give the phthaloyl derivative, 33.7 g., m. p. 159–159.5°.

2-Butoxy-5-guanylaminopyridine, 20.—2-Butoxy-5-aminopyridine dihydrochloride (11.9 g.), cyanamide (2.2 g.) and methanol (40 cc.) were heated in a pressure bottle at 100° for four hours. After evaporation to dryness, water was added and then potassium hydroxide to strong alkalinity. The oil which precipitated resisted crystallization. It was therefore precipitated from ethereal solution with carbon dioxide gas. The 4.1 g. of the prod-

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(1) Friedman, Braitberg, Tolstoouhov and Tisza, THIS JOURNAL, **69**, 1204 (1947).

(2) The antibacterial activities in the Table are those obtained when using M. Tbc 607. In a personal communication, Charles J. Duca reports that 2-butoxy-5-aminopyridine and its formaldehyde bisulfite derivative displayed identical activities when three strains of bovine tubercle bacilli, namely, ATCC 599, ATCC 8420 and B1 were grown in Proskauer-Beck medium; but these compounds displayed activity only in concentrations over 64 mg. per cent. when the same organisms were grown in Dubos' medium (Dubos and Davis, *J. Exptl. Med.*, **83**, 409 (1946)).

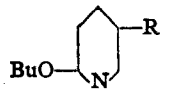
(3) Bratton, Marshall, *et al.*, *J. Biol. Chem.*, **128**, 537 (1939).

(4) Feinstone, Friedman, Rothlauf, Kelly and Williams, *J. Pharmacol.*, **89**, 153 (1947).

(5) Feinstone, *Proc. Soc. Exptl. Biol. Med.*, **63**, 153 (1946).

(6) The nomenclature for these compounds was kindly supplied by Mr. Stemen of Chemical Abstracts.

TABLE I



	R	Empirical formula	M. p., °C.	B. p. °C.	Mm.	Nitrogen, % Calcd.	Found	Tbc[s] stasis mg. %
1	—NH_2	$C_9H_{14}N_2O$						1/32
2	—NHC_4H_9	$C_{13}H_{22}N_2O$	51–52	155–156	2.7	12.6	12.4	1/2
3	—$N(CH_3)_2$	$C_{11}H_{18}N_2O$	73–75	205–207	0.15	14.5	14.5	4
4	—$NHCH_2CH_2OH$	$C_{11}H_{18}N_2O$		178–181	3	13.4	13.5	1/4
5	—$NHCH_2SO_2Na$	$C_{10}H_{15}N_2O_3SNa$	[n]			(See experimental)		1/8
6	—$NHCH_2SO_3Na$	$C_{10}H_{15}N_2O_4SNa$	[n]			9.94	9.85	1/16
7	—$NHCH(CH_3)SO_3Na$[a]	$C_{11}H_{17}N_2O_4SNa$	[n]			8.45	8.54[o]	1/32
8	—$N{=}CHC_6H_5$[b]	$C_{16}H_{18}N_2O$	53.5–54.5			10.9	11.0	1/8
9	—$NHC_6H_{11}O_5$[c]	$C_{15}H_{24}N_2O_6$	137–137.5			8.55	8.53	1/16
10	—$NHC_6H_{12}O_6(SO_3Na)$[d]	$C_{15}H_{25}N_2O_9SNa$	[d]			[d]		1/8
11	—$NHCOCH_3$[e]	$C_{11}H_{16}N_2O_2$	84–85.5	164–166	0.5			1/16
12	—$NHCOC_3H_7$[f]	$C_{13}H_{20}N_2O_2$	69–71	182–184	1.5	11.9	12.1	1/8
13	—$NHCOC_6H_4NH_2$[g]	$C_{16}H_{19}N_3O_2$	144–146			14.8	14.9	>4
14	—$NHCOCH_2CH_2COOH$[h]	$C_{12}H_{18}N_2O_4$	148–149			266	264[p]	1/4
15	—$NHCOC_6H_4COOH$-*o*	$C_{17}H_{18}N_2O_4$	159–159.5			8.93	9.01	1/8
16	—$N{=}(CO)_2{=}C_6H_4$	$C_{17}H_{16}N_2O_3$	164–164.5			9.45	9.44	1
17	—$NHCONH_2$[i]	$C_{10}H_{15}N_3O_2$	153–154			20.1	20.3	16
18	—$NHCONHC_6H_5$[j]	$C_{16}H_{19}N_3O_2$	142–144			14.7	14.9	>16
19	—$NHCSNHC_6H_5$[k]	$C_{15}H_{19}N_2OS$	123.5–124			14.0	13.9	1
20	—$NHC{=}NH(NH_2)]_2{\cdot}H_2CO_3$	$(C_{10}H_{16}N_4O)_2H_2CO_3$	[n]			21.1	21.1[q]	1/8
21	—$NHCH_2COOH$	$C_{11}H_{16}N_2O_3$	125–126			12.5	12.6	1/8
22	—$NHSO_2C_6H_4CH_3$[l]-*p*	$C_{16}H_{20}N_2O_3S$	73–75			8.74	8.80	4
23	—$NHSO_2C_6H_4$*p*-$NHCOCH_3$[m]	$C_{17}H_{21}N_3O_4S$	173–174.5			11.6	11.4	
24	—$NHSO_2C_6H_4$*p*-NH_2[m]	$C_{15}H_{19}N_3O_3S$	139–140			13.1	13.2	>4
25	—$NHSO_3Na$	$C_6H_{13}N_2O_4SNa$	[n]			10.3	10.3	>64

[a] Prepared as the preceding methylene sulfonate, see experimental. [b] Prepared by warming the amine with benzaldehyde in alcohol. [c] Method of Kuhn and Birkofer, *Ber.*, **71**, 621 (1938). [d] Method of U. S. Patent 2,287,071 (prepared and used in solution, on drying a small amount *in vacuo*, a pinkish white hygroscopic solid resulted. The linkage is assumed to be the same as in Promin). [e] Described in German Patent 607,662 (*C. A.*, 29, 4026[3] (1935)). [f] Prepared by warming with butyric anhydride and subsequent vacuum distillations. [g] Prepared by reaction of the amine with *p*-nitrobenzoyl chloride in pyridine followed by reduction in 50% methanol with iron and acetic acid. [h] Prepared by refluxing the amine and succinic anhydride in alcohol. [i] Prepared by adding potassium cyanate to an aqueous solution of the amine monohydrochloride. [j] From the amine and phenyl isocyanate. [k] From the amine and phenyl isothiocyanate. [l] Prepared by reaction of the amine and *p*-tolylsulfonyl chloride in pyridine. [m] Prepared by reaction of the amine in pyridine with acetyl-sulfanilyl chloride and subsequent hydrolysis in alkali. [n] All these compounds were obtained as white crystalline solids. All decomposed on heating to high temperatures. [o] Calculated for a dihydrate. [p] Molecular weight (by alkali titration). [q] Calculated for a tri-hydrate. [r] Calculated for a di-hydrate. [s] Tested against avirulent strain 607 (see ref. 3).

uct on recrystallization from water with Darco gave 2.5 g. of white lustrous crystals. On the basis of the nitrogen analysis and a positive qualitative test for water (Karl Fischer reagent), the compound is assumed to be $(C_{10}H_{16}N_4O_2)_2{\cdot}H_2CO_3{\cdot}3H_2O$.

2-Butoxy-5-carboxymethylaminopyridine, 21.—A solution of 16.6 g. of 2-butoxy-5-aminopyridine and 3.6 g. concentrated hydrochloric acid in 50 cc. of water was added to a solution of 9.5 g. chloroacetic acid in 20 cc. of 40% sodium hydroxide and the mixture was refluxed for sixteen hours. The solution was made strongly alkaline and excess amine was extracted with ether. The aqueous solution was adjusted to *p*H 5 whereupon the product precipitated. Recrystallized from water, the product melted at 125–126° (yield, 3 g.).

2-Butoxy-5-sodium Sulfaminopyridine, 25.—To a solution of 15 g. of sodium triphosphate (hydrate) and 80 g. of sodium hydrosulfite in 150 cc. of water 12 g. of 2-butoxy-5-nitropyridine was added and the mixture was heated to 75–85° for one and one-half hours. Upon cooling and concentrating the reaction mixture, 8 g. of product was obtained. After recrystallization from water, the yield was 4.2 g.

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Summary

The preparation of a new series of N^5-substituted derivatives of 2-butoxy-5-aminopyridine is described. Tuberculostatic activity is not dependent upon the presence of a free primary amino group. Of the compounds reported, the formaldehyde bisulfite derivative appears to combine optimum properties of maximum activity with lowered toxicity.

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