THIOUREA DERIVATIVES

XIII. PREPARATION AND TUBERCULOSTATIC ACTION OF SODIUM SALTS OF 2-[(ARYLTHIOCARBAMOYL)AMIDO]ETHYL DIHYDROGEN PHOSPHATES

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A number of 1,3-disubstituted thioureas have found application in clinical medicine as antitubercular and antileprotic agents. Among examples of such drugs may be mentioned the following p,p'-disubstituted thiocarbanilides [1-5]: p-butyloxy-p'-dimethylamino- (thiambutosine), p,p'-diisoamyloxy- (isoxyl), p,p'-diethoxy- (ethoxyd), and p-butyloxy-p'-(4-methylpiperazino)thiocarbanilide (butomelide). The value of these preparations is however somewhat limited by the fact that they are all nearly insoluble in water, and for this reason it appeared of interest to attempt to prepare for antitubercular examination a series of 1,3-disubstituted thioureas containing salt-forming groups. Compounds of this type could be expected to have a greater solubility in water and to present special features when undergoing metabolic processes. We have in fact already described [6, 7] the preparation of 1,3-disubstituted thioureas containing carboxyl, carboxyacyl, and methylenesulfonic acid groups; however, in spite of the presence of these functions, the resulting products proved, with certain exceptions, to be sparingly soluble in water; the exceptional cases were the diethylamine and isonicotinoylhydrazine salts of p-alkoxythiocarbanilide-p'-carboxylic acids, and aminothiocarbanilides carrying a methylenesulfonic acid residue.

Continuing our work along these lines, we have now prepared and examined for their tuberculostatic action 13 1,3-disubstituted thioureas containing the phosphoric acid residue as salt-forming group (III-XV; see Table 1). The compounds in question are the disodium salts of 2-[(arylthiocarbamoyl)amido]ethyl dihydrogen phosphates, and their preparation was effected by condensing 2-aminoethyl dihydrogen phosphate (II) with a 10-20% excess of each of the 13 aryl isothiocyanates (I) in the presence of two moles of sodium hydroxide.

$$\underset{\text{I}}{R\text{NCS}} + \underset{\text{II}}{H_2\text{NCH}_2\text{CH}_2\text{OPO(OH)}_2} \xrightarrow{\text{NaOH}} \underset{\text{III-XV}}{R\text{NHCSNHCH}_2\text{CH}_2\text{OPO(ONa)}_2}$$

In each case, the pH of the alkaline reaction medium was maintained between the limits 9.0-10.0 during the greater part of the process but allowed to fall to 8.0 towards the end of the operation.

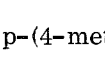
The alkoxyphenyl isothiocyanates (I; R = p-alkoxy$-C_6H_4-$) needed for the preparation of the seven cases (V-XI) were obtained by the method previously described [6, 8], in which the corresponding symmetrically disubstituted thiocarbanilides, p-alkoxy$-C_6H_4NH-C(S)-NHC_6H_4-$alkoxy-p, served as starting materials. The compound, p-(4-methylpiperazino)phenyl isothiocyanate (I: R = p-Me  $-C_6H_4-$), needed for the preparation of (XIV), was obtained by warming p-(4-methylpiperazino)phenylthiourea, [p-Me  $-C_6H_4NH-C(S)-NH_2$], in chlorobenzene [9]. p-Nitrophenyl isothiocyanate (I; R = p-$O_2N-C_6H_4-$), the starting material for (XIII), was prepared from p-nitroaniline and tetraethylthiuram disulfide by the

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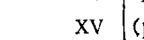
TABLE 1. Sodium Salts of 2-[(Arylthiocarbamoyl)amido]ethyl Dihydrogen Phosphates and Their Tuberculostatic Activity

Compound	R	Yield, %	Found, %					Empirical formula	Calculated, %					Min. tuberculostatic concn. (μg/ml) using the H-37 Rv strain of the organism in a Sutton med.	
			C	H	N	S	H_2O		C	H	N	S	H_2O	absence of serum	presence of serum
III*	C_6H_5	90	34,10	3,58	8,29	10,4	—	$C_9H_{11}N_2Na_2O_4PS$	33,75	3,46	8,74	10,01	—	250	500
IV	$p\text{-}CH_3C_6H_4$	70	33,90	4,43	7,44	8,56	5,32	$C_{10}H_{13}N_2Na_2O_4PS\cdot H_2O$	34,09	4,29	7,95	9,10	5,15	64	1000
V	$p\text{-}CH_3OC_6H_4$	83	32,90	4,02	7,84	8,36	4,13	$C_{10}H_{13}N_2Na_2O_4OS\cdot H_2O$	32,91	4,10	7,60	8,70	4,90	250	1000
VI	$p\text{-}C_2H_5OC_6H_4$	45	33,36	4,13	7,21	8,87	4,44	$C_{11}H_{15}H_2Na_2O_5PS\cdot H_2O$	34,55	4,48	7,32	8,41	4,70	4	250
VII	$p\text{-}C_3H_7OC_6H_4$	37	34,02	4,86	6,80	5,68	7,50	$C_{12}H_{17}N_2Na_2O_5PS\cdot H_2O$	34,78	5,11	6,76	7,74	8,33	8	>1000
VIII	$p\text{-iso }C_3H_7OC_6H_4$	54	36,44	4,36	7,24	7,68	3,71	$C_{12}H_{17}N_2Na_2O_5PS\cdot H_2O$	37,66	4,47	7,32	8,34	4,16	125	
IX	$p\text{-}C_4H_9OC_6H_4$	65	37,33	4,84	6,88	7,84	5,46	$C_{13}H_{19}N_2Na_2O_5PS\cdot H_2O$	38,00	5,10	6,82	7,90	4,40	0,5	500
X	$p\text{-iso }C_4H_9OC_6H_4$	48	39,80	5,20	7,31	8,31	—	$C_{13}H_{19}N_2Na_2O_5PS\cdot H_2O$	39,79	4,88	7,14	8,17	—	8	64
XI	$p\text{-iso }C_5H_{11}OC_6H_4$	66	40,43	5,32	6,94	—	—	$C_{14}H_{25}N_2Na_2O_5PS$	41,37	5,20	6,89	—	—	8	125
XII*	$p\text{-}BrC_6H_4$	79	26,03	2,78	7,32	8,26	4,54	$C_9H_{10}BrN_2Na_2O_4PS\cdot H_2O$ †	25,90	2,88	6,70	7,67	4,31	250	>1000
XIII*	$p\text{-}NO_2C_6H_4$	52	27,52	3,03	10,31	9,35	8,53	$C_9H_{10}N_3Na_2O_6PS\cdot H_2O$	28,20	3,15	10,96	8,36	9,40	64	250
XIV		53	38,58	4,75	12,30	7,12	3,25	$C_{14}H_{21}N_4Na_2O_4PS\cdot H_2O$	38,52	5,31	12,84	7,34	4,12	250	1000
XV	$(p\text{-}BrC_6H_4)_2CH$	85	34,06	2,93	5,16	6,11	—	$C_{16}H_{15}Br_2N_2Na_2O_4PS$ ‡	33,82	2,66	4,93	5,64	—	16	

*Previously described in [13].

† Found %: Br 18.50; P 7.67. Calculated %: Br 19.20; P 7.43.

‡ Found %: Br 27.75. Calculated %: Br 28.13.

method of Marquardt [10]. The two bromo derivatives, (XV) and (XII), were prepared from p,p'-dibromobenzhydryl isothiocyanate [I; R = $(p\text{-}BrC_6H_4)_2CH$] and p-bromophenyl isothiocyanate (I; R = $p\text{-}BrC_6H_4$), respectively, obtained by the interaction of the hydrochloride of p,p'-dibromobenzhydrylamine (or of p-bromoaniline, as the case may be) with thiophosgene in a chloroform or dichloroethane medium under conditions worked out by Kalamař et al. [11] for the preparation of 3-methyl- and 4-methoxy-benzhydryl isothiocyanates.

2-Aminoethyl dihydrogen phosphate (II) was prepared by warming ethanolamine with pyrophosphoric acid at 110°C in accordance with the method given by Cherbuliez and Rabinowitz [12].

All the products (III-XV) listed in Table 1 are crystalline compounds infusible below 300°. The compounds (V), (VII), and (VIII) are readily soluble in water; (III), (IX), (XI), (XII), (XIII), (XIV), and (XV) are moderately soluble; and (IV), (VI), and (X) are sparingly soluble in water.

The compounds described in this paper have been examined for their bacteriostatic activity against mycobacterium tuberculosis. The strain of the organism employed was H-37 R_V, and the experiments were conducted in vitro using the Sutton nutritive medium. The experiments show that the introduction of alkoxy groups (but not methoxy or isopropyloxy) into the molecule of 2-[(phenylthiocarbamoyl)amido]ethyl dihydrogen phosphate increases its antitubercular action. The compounds (VI), (VII), (IX), (X), and (XI), the structures of which contain the ethoxy, propyloxy, butyloxy, isobutyloxy, or isopentyloxy groups, respectively proved to have pronounced tuberculostatic properties (they could arrest the development of the test organism at concentrations 8-0.5 μg/ml), but their activity fell sharply when the nutritive medium was reinforced by a protein adjuvant.*

EXPERIMENTAL

2-Aminoethyl Dihydrogen Phosphate (II). Monoethanolamine (6.1 g) was added in small portions to 29.36 g of pyrophosphoric acid. The reaction mixture was stirred at 110°C, and the stirring continued so long as the ethanolamine could be detected chromatographically by means of a marker spot. The hot mixture was then poured into water, stirred, neutralized by the addition of barium carbonate to saturation point (about 24 g), and finally made alkaline to phenolphthalein by the gradual addition of a saturated solution of barium hydroxide. The resulting precipitate was filtered off, washed repeatedly with water, and the filtrate evaporated in a vacuum to a final volume of 200 ml. The concentrate was freed from barium ions by the addition of diluted sulfuric acid to a pH value of 2.0, and the barium sulfate so precipitated filtered off and the filtrate evaporated in a vacuum to a volume of 100 ml. This was treated with 400 ml of ethanol which caused the solution to become cloudy. The solid (II) formed on standing for 3 h was filtered off; yield, 2.61 g (18.6%); mp 235-236°. Cherbuliez et al. [12, 13, 14] give mp 233-235° and 237°. Chromatography was carried out on cross-grained weave paper of medium porosity (grade M-20 of the Leningrad firm of suppliers); the method of descending flow was used, and the solvent system was butanol–acetic acid–water (4 : 1 : 5); the marker spots were developed by means of a freshly prepared solution of ferric chloride, followed, after drying the chromatogram, by a freshly prepared solution of sulfosalicyclic acid.

Sodium Salt of 2-[(p-Butyloxyphenylthiocarbamoyl)amido]ethyl Dihydrogen Phosphate (IX). An aqueous 1.0 N solution of sodium hydroxide (14.2 ml) and 20 ml of dioxane were added to 1 g of (II). The resulting mixture, which had pH 9.0, was warmed to 40° and stirred. A solution of p-butyloxyphenyl isothiocyanate (I; R = $p\text{-}C_4H_9OC_6H_4$) (1.46 g) in dioxane (10 ml) was now slowly added and the temperature then maintained at 40° for 7 h. At the end of this process the reaction medium had pH 8.0. The solvent was removed in a vacuum, the residue warmed with ether, the ethereal solution decanted off, and this final residue dissolved in methanol. The solid which remained undissolved, and which consisted of the sodium salt of (II), was filtered off and the filtrate evaporated in a vacuum. The residue was crystallized by trituration with petroleum ether, and this furnished, after drying in a vacuum at 100°, 2 g of (IX).

The compounds (IV), (V), (VI), (VII), (VIII), (X), and (XI) were obtained in an analogous manner.

β-[{p-(4-Methylpiperazino)phenylthiocarbamoyl}amido]ethyl Dihydrogen Phosphate (XIV). A mixture of (II) (0.8 g), an aqueous 1.0 N solution of sodium hydroxide (11.36 ml), and dioxane (15 ml) was warmed to 40°, stirred, and gradually treated with a solution of p-(4-methylpiperazino)phenyl isothiocyanate (1.3 g) in dioxane (25 ml). The temperature was held at 40° during 20 h. The solvent was then removed in a vacuum and the residue worked up twice with anhydrous ether and methanol in the manner described in the preceding paragraph. Yield, 1.12 g.

The compound (XV) was obtained in an analogous manner.

* Horse serum was used for this purpose.

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