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PREPARATION AND BIOLOGICAL ACTIVITY OF THE DERIVATIVES OF PHENYLSUCCINIC ACID. II.* PREPARATION AND ANTIBACTERIAL SCREENING OF SOME NITROGEN DERIVATIVES OF *p*-HALOPHENYLSUCCINIC ACID

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Opisano otrzymywanie i własności amidów, hydrazydów i soli sodowych kwasów hydroksamowych pochodnych wszystkich czterech kwasów *p*-chlorowcofenylobursztynowych. W badaniach bakteriologicznych jedynie hydrazydy wykazały umiarkowaną czynność przeciwbakteryjną.

Описано получение и свойства амидов, гидразидов и натриевых солей гидроксамовых производных всех четырех *n*-галоидофенилянтарных кислот. Только гидразиды показывают умеренное протибактериальное действие.

Preparation and properties are described of amides, hydrazides, and sodium hydroxamates derived from all four *p*-halophenylsuccinic acids. In bacteriological tests, only the hydrazides revealed a moderate antibacterial activity.

In 1947, Barry and Twomey [1] reported that some esters and amides of alkylsuccinic acids showed *in vitro* an outstanding antituberculous activity. This kind of activity is a rather rare feature of compounds containing no nitrogen, as is the case of the above mentioned esters, but amides and other simple nitrogen compounds such as hydrazides and hydroxamic acids, have been often claimed to be potent antitubercular. The bacteriological tests with both hydrazides and hydroxamic acids may be considered as particularly encouraging [2–4].

In the earlier paper of this series [5] we have described the preparation of all four *p*-halophenylsuccinic acids and methyl esters thereof. It seemed now advisable to prepare some new biologically active compounds by converting these acids into some nitrogen-bearing derivatives. To this end a series of *p*-halophenylsuccinic amides, hydrazides, and hydroxamic acids have been prepared.

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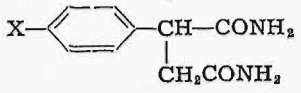
* Part I, see ref. 5.

The amides were prepared in the conventional way by treating the corresponding methyl esters with ammonium hydroxide. In the case of fluorine and chlorine derivatives, the reaction was complete within a few days at room temperature; the bromine and iodine analogues underwent ammonolysis in very drastic conditions when heated in a sealed tube. Partial of full homogenization of the reaction mixture by adding some alcohol improved neither rate nor yield of the process; the average yields ranged within the limits of 70 to 80%. The physical and analytical data are recorded in Table 1.

Table 1

p-Halophenylsuccinamides

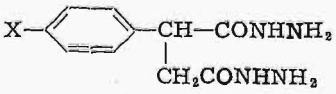


No	X	Formula	% Yield	M. p., °C	% N	
					calcd.	found
I	F	$C_{10}H_{11}N_2O_2F$	73	207 — 9	13.3	13.5
II	Cl	$C_{10}H_{11}N_2O_2Cl$	81	237 — 40	12.4	12.1
III	Br	$C_{10}H_{11}N_2O_2Br$	76	243 — 5	10.3	10.2
IV	I	$C_{10}H_{11}N_2O_2I$	71	259 — 60	8.8	8.9

The hydrazides were prepared similarly by refluxing the appropriate esters with 2.5 moles of 40 or 80% hydrazine hydrate. In this case, too, addition to alcohol appeared to give no effect. The corresponding physical and analytical data are collected in Table 2.

Table 2

p-Halophenylsuccinic hydrazides

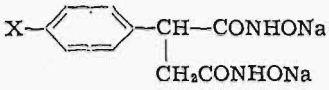


No	X	Formula	% Yield	M. p., °C	% N	
					calcd.	found
V	F	$C_{10}H_{13}N_4O_2F$	78	178 — 80	23.35	22.95
VI	Cl	$C_{10}H_{13}N_4O_2Cl$	83	176 — 8	21.8	21.6
VII	Br	$C_{10}H_{13}N_4O_2Br$	85	182 — 4	18.6	18.6
VIII	I	$C_{10}H_{13}N_4O_2I$	91	191 — 3	16.1	15.9

The procedure we have used in preparing the corresponding hydroxamic acids also followed the most general method, i.e. treatment of the appropriate esters with hydroxylamine; the latter was added in a slight excess. Recrystallization of the precipitated sodium hydroxamates failed; they were completely insoluble in the usual organic solvents, whereas in water they were very readily soluble. In dilute solvents, even minute amounts of water prevented the formation of crystals. Sodium assays suggested that the crude sodium hydroxamates, even after thorough washing and desiccation, contained one molecule of ethanol; this observation is in perfect agreement with that of Hurd and Botteron[6], and Hurd, Buess, and Bauer[7], who reported the same phenomenon in the case of sodium succinohydroxamate. The analytical data concerning the sodium hydroxamates prepared are recorded in Table 3.

Table 3

Sodium *p*-halophenylsuccinohydroxamates



No	X	Formula	% Yield [b]	% Na		
				calcd. [a]	calcd. [b]	found
IX	F	$C_{10}H_9N_2O_4FNa_2$	88	16.1	13.85	13.5
X	Cl	$C_{10}H_9N_2O_4ClNa_2$	92	15.3	13.2	12.9
XI	Br	$C_{10}H_9N_2O_4BrNa_2$	90	13.25	11.7	11.45
XII	I	$C_{10}H_9N_2O_4INa_2$	94	11.7	10.45	10.1

a) Calculated for compound containing no ethanol.
b) Calculated for compound containing one molecule of ethanol.

The sodium hydroxamates were converted in the usual way into copper chelates which were decomposed with hydrogen sulphide to yield free hydroxamic acids as yellowish waxy products. Failure of the recrystallization attempts evidenced further analogy with reports of Hurd et al.[6,7] Preparation of crystalline ammonium salts was similarly unsuccesful. The results of elemental analyses were invariably erratic; nevertheless, identification of these products seems to be doubtless, since they gave a positive hydroxamic test with ferric chloride and on treatment with sodium ethoxide yielded readily the corresponding sodium hydroxamates which proved to be identical with those obtained directly.

All the compounds prepared as well as their analogues containing no ring substituent were tested for their antibacterial activity againts *Mycobacterium smegmatis*, *Mycobacterium tuberculosis*, strains 607, 279, 209P,

and $H_{37}Rv$, *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi,* and *Shigella flexneri.* All amides and hydroxamates were inactive. The hydrazides showed a moderate activity; detailed results are present in Table 4. In general, the results of bacteriological screening are to be regarded as negative.

Table 4

Antibacterical activity of *p*-halophenylsuccinic hydrazides.
All determinations made in propylene glycol solution.

No	X	Minimum concetration (in mg %) inhibiting the growth of							
		Mycobact. smegmatis	*Mycobact. tuberculosis* 279	*Mycobact. tuberculosis* 607	*Mycobact. tuberculosis* 209 P	*Escherichia coli*	*Klebsiella pneumoniae*	*Salmonella typhi*	*Schigella flexneri*
V	F	31.2	31.2	31.2	62.5	125.0	125.0	125.0	125.0
VI	Cl	31.2	31.2	15.6	62.5	62.5	62.5	125.0	62.5
VII	Br	31.2	15.6	15.6	62.5	62.5	62.5	125.0	125.0
VIII	I	31.2	31.2	15.6	125.0	125.0	125.0	125.0	125.0
—	H	31.2	31.2	31.2	62.5	62.5	62.5	125.0	62.5

EXPERIMENTAL

Preparation of *p*-halophenylsuccinamides

Procedure A. Methyl *p*-fluorophenylsuccinate (12 g, 0.05 moles) was treated with 20 ml of 27% ammonium hydroxide and the mixture was left to stand 3 days at room temperature. The separated crystals were collected and recrystallized from water to give 7.65 g (73%) *p*-fluorophenylsuccinamide, m. 207—9°.

The chlorine derivative was prepared in a similar way.

Procedure B. Methyl *p*-iodophenylsuccinate (10.45 g, 0.03 moles) and 30 ml 27% ammonium hydroxide was heated 5 hrs at 100° in a sealed tube. The crystals were treated as above to yield 6.8 g (71%) *p*-iodophenylsuccinamide, m. 259—60°.

The bromine derivative was prepared analogously.

Preparation of *p*-halophenylsuccinic hydrazides

Procedure A. Methyl *p*-chlorophenylsuccinate (5.14 g, 0.02 moles) was refluxed 4 hrs with 4 ml (0.5 moles) 80% hydrazine hydrate and 25 ml ethanol. The crude product was recrystallized from dilute ethanol (5 : 1) to yield 4.27 g (83%) *p*-chlorophenylsuccinic hydrazide, m. 176—8°.

The fluorine and iodine derivatives were prepared analogously.

Procedure B. Methyl *p*-bromophenylsuccinate (6 g, 0.02 moles) was refluxed with 9 ml (0.06 moles) 37% hydrozine hydrate. The product was isolated as above to give 5.1 g (85%) *p*-bromophenylsuccinic hydrazide, m. 182—4°.

Preparation of *p*-halophenylsuccinohydroxamic acids

The ethanolic solution of hydroxylamine was prepared by adding sodium ethoxide (2.55 g, i.e. 0.11 moles sodium in 50 ml ethanol) to a suspension of 7.65 g hydroxylamine hydrochloride (0.11 moles) in 90 ml ethanol and filtering. To this solution, 12 g (0.05 moles) methyl *p*-fluorophenylsuccinate was added under constant stirring and immediately followed by sodium ethoxide solution prepared from 2.3 g (0.1 moles) sodium and 45 ml ethanol. Precipitation of light yellow sodium hydroxamate was almost instantaneous; the temperature rose spontaneously to 35—8°. The mixture was left overnight, filtered, and the precipitate washed thoroughly with ethanol and ether. After vacuum desiccation, 14.6 g sodium *p*-fluorophenylsuccinohydroxamate was obtained; the analysis for sodium suggested that the product contained one molecule of ethanol (cf. table 3). The yield calculated on this basis was 88%.

The sodium salt (3.3 g, 0.01 moles) was dissolved in 25 ml water and treated with a saturated solution of copper acetate. The greenish precipitate was centrifuged off and washed repeatedly by cetrifuging with ethanol and ether to give 2.6 g dry copper chelate. This was suspended in 40 ml methanol and hydrogen sulfide was introduced into the suspension. The filtrate was evaporated to dryness to yield a yellow wax which failed to solidify even on prolonged vacuum desiccation. It gave a positive hydroxamic test (deep red color) with ferric chloride solution. The product was reconverted into sodium *p*-fluorophenylsuccinohydroxamate by treatment with ethanolic sodium ethoxide.

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OTRZYMYWANIE I AKTYWNOŚĆ BIOLOGICZNA POCHODNYCH KWASU FENYLOBURSZTYNOWEGO. II. OTRZYMYWANIE I CZYNNOŚĆ PRZECIWBAKTERYJNA AZOTOWYCH POCHODNYCH KWASÓW *p*-CHLOROWCOFENYLOBURSZTYNOWYCH

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Nawiązując do pracy Barryego i Twomeya[1], którzy stwierdzili wysoką czynność przeciwgruźliczą szeregu estrów i amidów pochodnych kwasów alkilobursztynowych, otrzymaliśmy i poddaliśmy badaniom bakteriologicznym amidy i hydrazydy wszystkich czterech kwasów *p*-chlorowcofenylobursztynowych oraz odpowiednie kwasy hydroksamowe. Amidy i hydrazydy otrzymaliśmy stosując konwencjonalną metodę amonolizy lub hydrazynolizy estrów; dane fizyczne i analityczne podano odpowiednio w tablicach 1 i 2. Kwasy hydroksamowe otrzymaliśmy i opisaliśmy (tablica 3) pod postacią trwałych soli sodowych, otrzymanych również standardową metodą; oznaczenie zawartości sodu wskazuje na obecność jednej cząsteczki etanolu, co pokrywa się ze spostrzeżeniami Hurda i współprac.[6,7] odnośnie soli sodowej kwasu sukcynohydroksamowego. Wolne kwasy hydroksamowe otrzymaliśmy w postaci substancji woskowatych, których nie udało się nam przeprowadzić w formę krystaliczną. Badania bakteriologiczne wykazały, że amidy i pochodne hydroksamowe są pozbawione czynności przeciwbakteryjnej, natomiast hydrazydy wykazują czynność umiarkowaną; odpowiednie dane podano w tablicy 4.