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# PREPARATION AND ANTIBACTERIAL PROPERTIES OF CERTAIN DERIVATIVES OF HYDROQUINONE *

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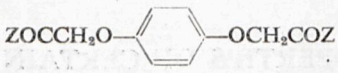
Amides, hydrazides and hydroxamic acids derived from carboxylic acids are still being explored as compounds with potential biological activity. A particularly great number of papers has so far been devoted to the preparation and antibacterial (mostly antituberculotic) derivatives. Much of this work was inspirated by the well-known discovery of the strong *in vitro* and *in vivo* inhibition of the growth of tuberculosis bacteria by isoniazid [8] and by the subsequent introduction of this compound as the key-drug in the treatment of tuberculosis. In certain more recent investigations a number of hydrazides, derived from aliphatic, aromatic and heterocyclic carboxylic acids, showed an interesting *in vitro*, and in single cases also *in vivo* antituberculotic activity, though therapeutical application has so far been unsuccessful. An excellent review of the biologically active hydrazine derivatives, covering the literature data up to 1963, was presented by Jucker [10].

Investigations on hydroxamic acids as antibacterial agents date back to the observation of *Mycobacterium tuberculosis* inhibition by salicylohydroxamic acid [17]. Later research revealed 5-bromosalicylohydroxamic acid to be a still more active tuberculostatic [19] whose positive effects in the treatment of human tuberculosis, particularly inhibition of the development of isoniazid-resistant strains, was also confirmed in quite recent reports [9, 22]. Interesting *in vitro* activities were similarly observed for a number of other hydroxamic acids [2, 18, 20, 21], of which some were found to be active fungicides [1] and agents capable of reducing serum cholesterol level [4].

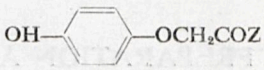
The present paper is concerned with the preparation of amides, hydrazides and hydroxamic acids derived from two hydroquinone derivatives: (p-phenyl-

* Paper XL in the series „Research on new antituberculotic compounds".

enedioxy)-diacetic acid (I) and p-hydroxyphenoxyacetic acid (II). The choice of such structures was determined first and foremost by the interesting biological features of phenoxyacetohydroxamic acids [6]. On the other hand, most positive results in biological screening of hydroxamic acids referred to salicylic acid derivatives, i.e., compounds with a phenolic group. Such a regularity was presumably connected with the well-known detoxication effect of the OH group [12].



I Z = OH
V Z = $NH_2$
VI Z = $NHNH_2$
VII Z = NHOH



II Z = OH
XI Z = $NH_2$
XII Z = $NHNH_2$
XIII Z = NHOH

It seemed also worthwile to investigate the antibacterial activity of the compounds derived from I, in which the functional groups supposedly responsible for biological activity were doubled.

The preparation of the compounds under investigation was effected by the routine method, involving simple aminolysis of the appropriate esters with ammonia, hydrazine hydrate and hydroxylamine, respectively.

$$RCOOR' + HZ \rightarrow RCOZ + R'OH$$

where Z = $NH_2$, $NHNH_2$ and NHOH

Most of the synthetic work concerned, therefore, the corresponding carboxylic acids.

In the case of (p-phenylenedioxy)-diacetic acid, duplication of the procedure developed by Ettel et al. [7], was successful only in small-scale runs. With more than 0·5 mole of hydroquinone the yields were considerably lower and variable, presumably owing to increased hydrolytic formation of glycollic acid *. (p-Phenylenedioxy)-diacetic acid was prepared in a constant yield of 80—85% by modification given in experimental part. Crude I prepared in this way was pure enough to make the isolation and laborious purification of its sodium salt dispensable.

Equally encouraging results were noted in an indirect method, in which hydroquinone was condensed with chloroacetonitrile in an anhydrous medium in the presence of potassium carbonate to give diacetonitrile (III), subsequently hydrolysed to the acid I. Data presented by Djerassi and Scholz [5] for certain other aryloxyacetonitriles were taken into account in the first step of this synthesis. The over-all yield of the acid was approximately 70% (Fig. 1).

* This side-reaction was apparently favoured in large scale runs where much more time was required to warm up the reaction mixture to the temperature of 80—90°, at which the condensation could be effected.

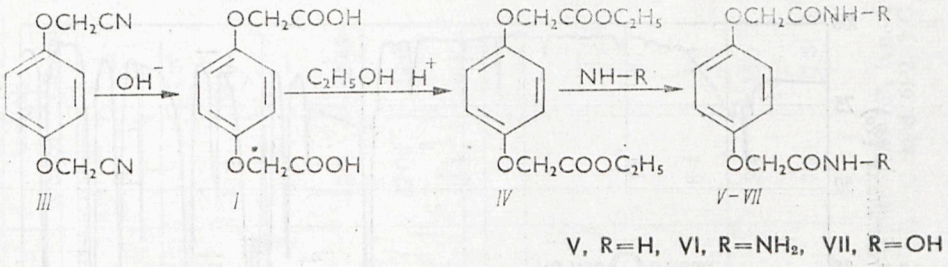



*Fig. 1.* Synthetic route to (p-phenylenedioxy)-diacetic acid derivatives.

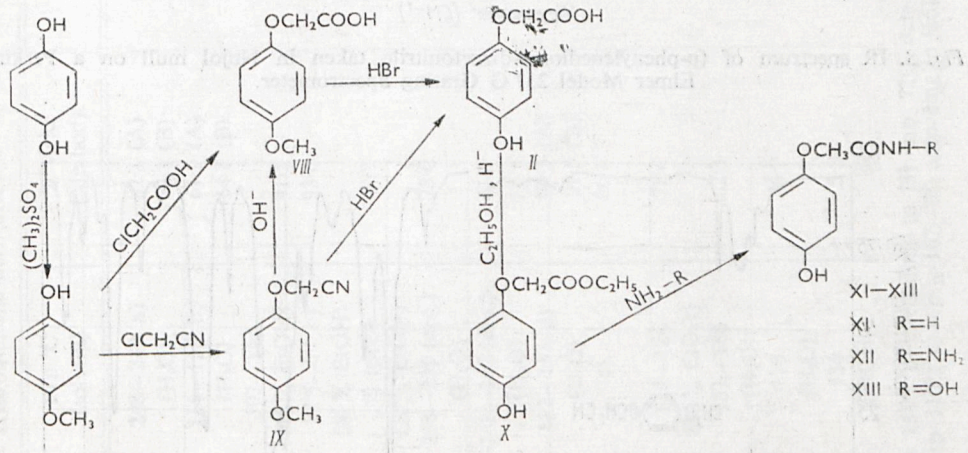



*Fig. 2.* Synthetic route to p-hydroxyphenoxyacetic acid derivatives.

Condensation of hydroquinone methyl ether [14] with chloroacetic acid was effected analogously to yield over 80% of p-methoxyphenoxyacetic acid (VIII), subsequently demethylated by heating with 40% hydrobromic acid. Demethylation according to Sobotka and Austin [15] gave slightly inferior yields and required pressure vessels.

Similarly, p-hydroxyphenoxyacetic acid (II) was prepared in high yield by condensing hydroquinone methyl ether with chloroacetonitrile and subsequently hydrolysing the intermediate p-methoxyphenoxyacetonitrile (IX). Hydrolysis with 40% hydrobromic acid gave II in one step (Fig. 2).

It seems very interesting that the IR spectra of both aryloxyacetonitriles involved (III and IX) revealed no bands in the 2240—2260 $cm^{-1}$ region, which is generally assumed to be very characteristic of the C≡N stretching vibrations in nitriles. The intensity of the nitrile bands in IR spectra is known to depend to a considerable extent on the nature of the adjacent groups. Particularly low intensities have been observed [11] in nitriles with oxygen atom of the ether

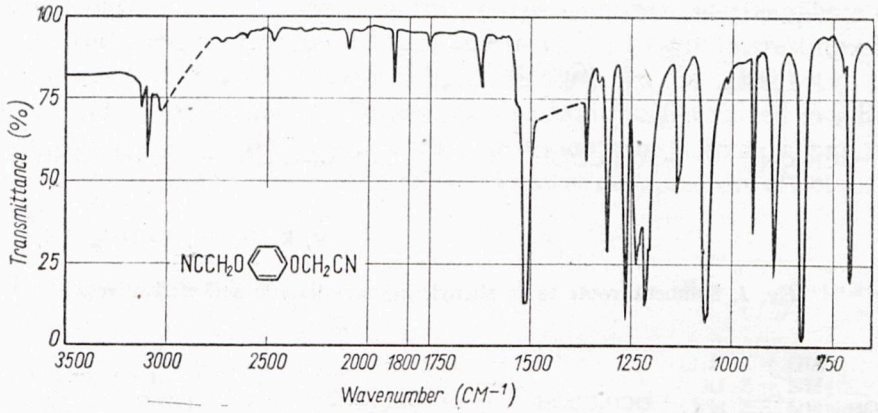



*Fig. 3.* IR spectrum of (p-phenylenedioxy)-diacetonitrile taken in Nujol mull on a Perkin-Elmer Model 237 G Grating Spectrometer.

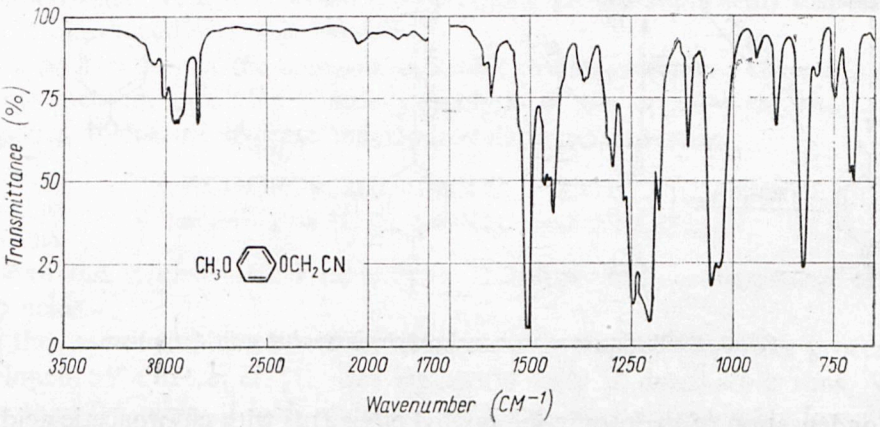



*Fig. 4.* IR spectrum of p-methoxyphenoxyacetonitrile taken in capillary film (apparatus as in *Fig 3*).

or hydroxyl type attached to the same carbon atom as the nitrile group. Nevertheless, a complete absence of the nitrile band * has so far been reported in a few cases with multiple oxygen-bearing substituents.

Some physical and chemical data on the compounds prepared are given in Table 1. Bacteriological screening of the amides, hydrazides and hydroxamic acids against several Gram-positive and Gram-negative strains showed only moderate or even weak activity (Table 2) **.

* A similar phenomenon was observed quite recently by Z. Eckstein for a wide series of other aryloxy nitriles.

** Biological tests were carried out in the Pharmacology Department, Institute of Drugs, Warsaw, under the supervision of Doc. Dr. J. Venulet, whose kind co-operation is hereby gratefully acknowledged.

*Table 1.* Hydroquinone derivatives

| No. | Compound | Mp °C (solvent) | % yield (Method) | Formula (mol. wt.) | Analysis N calcd. | N found |
|---|---|---|---|---|---|---|
| I | (p-Phenylenedioxy) diacetic acid | 248—249 a) ($H_2O$) | 82·1 (A) 87·3 (B) | $C_{10}H_{10}O_6$ (226·2) | — | — |
| II | p-Hydroxyphenoxyacetic acid | 154—155 b) ($H_2O$) | 61·9 (A) 64·3 (B) | $C_8H_8O_4$ (168·2) | — | — |
| III | (p-Phenylenedioxy) diacetonitrile | 102—144 (60% EtOH) | 91·5 | $C_{10}H_8N_2O_2$ (188·2) | 14·9 | 15·0 |
| IV | Ethyl (p-phenylenedioxy) diacetate | 72·5—74 c) (60% EtOH) | 93·0 | $C_{14}H_{18}O_6$ (282·3) | — | — |
| V | (p-Phenylenedioxy) diacetamide | 249 (dec.) (50% EtOH) | 60·9 | $C_{10}H_{12}N_2O_4$ (224·2) | 12·5 | 12·45 |
| VI | (p-Phenylenedioxy)-bis (acetylhydrazine) | 238—239 (dec.) ($H_2O$) | 75·0 | $C_{10}H_{14}N_4O_4$ (254·2) | 22·05 | 22·0 |
| VII | (p-Phenylenedioxy) diacetohydroxamic acid | 161—162 (dec.) ($H_2O$) | 41·0 | $C_{10}H_{12}N_2O_6$ (256·2) | 10·9 | 10·6 |
| VIII | p-Methoxyphenoxyacetic acid | 112—113 d) ($H_2O$) | 80·5 (A) 91·5 (B) | $C_9H_{10}O_4$ (182·2) | — | — |
| IX | p-Methoxyphenoxyacetonitrile | e) | 86·8 | $C_9H_9NO_2$ (163·2) | — | — |
| X | Ethyl p-hydroxyphenoxyacetate | 124—124 (50% EtOH) | 71·4 | $C_{10}H_{12}O_4$ (196·2) | f) | f) |
| XI | p-Hydroxyphenoxyacetamide | 157—158 ($H_2O$) | 62·3 | $C_8H_9NO_3$ (167·2) | 8·4 | 8·6 |
| XII | p-Hydroxyphenoxyacetylhydrazine | 178 ($H_2O$) | 76·9 | $C_8H_{10}N_2O_3$ (182·2) | 15·4 | 15·3 |
| XIII | p-Hydroxyphenoxyacetohydroxamic acid | 134 (EtOH) | 5·8 | $C_8H_9NO_4$ (183·2) | 7·65 | 7·6 |

a) According to [7] mp 248°; b) according to [15] mp 154°; c) according to [3] mp 72°; data given in [13] (mp 108—16°, b.p.$_{2\cdot6}$ 197—200°) are apparently erroneous; d) according to [15] mp 110°; e) b.p.$_{18}$ 167—9°; according to [16] b.p.$_5$ 142—5°; f) %C calcd. 61·2, %C found 61·3; %H calcd. 6·1, %H found 5·9.

*Table 2.* Bacteriological screening data

| Compound \ Strain | Minimum concentration (in mg%) inhibiting the growth of | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Mycobacterium smegmatis* | *Mycobacterium tuberculosis* strain 279 | *Mycobacterium tuberculosis* strain 607 | *Mycobacterium tuberculosis* strain 209P | *Escherichia coli* | *Klebsiella pneumoniae* | *Salmonella typhi murium* | *Shigella flexneri* |
| V | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 |
| VI | 3·9 | 500 | 3·9 | 500 | 500 | 500 | 500 | 500 |
| VII | 62·5 | 62·5 | 62·5 | 125 | 250 | 250 | 250 | 250 |
| XI | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 |
| XII | 31·2 | 62·5 | 31·2 | 500 | 500 | 500 | 500 | 500 |
| XIII | 125 | 62·5 | 125 | 500 | 500 | 500 | 500 | 500 |

Liquid medium according to Youmans was used in all tests.The compounds tested were applied in aqueous or aqueous-alcoholic solutions.

## EXPERIMENTAL *

*(p-Phenylenedioxy) diacetonitrile (III).* To a refluxed and stirred mixture of 11 g (0·1 mole) of hydroquinone, 27 g (0·195 mole) of anhydrous potassium carbonate and 30 ml of anhydrous 2-butanone a solution of 16·6 g (0·22 mole) of chloroacetonitrile in 20 ml of 2-butanone was added dropwise within 2 hrs. The latter solution was prepared 24 hrs earlier and activated by adding a few crystals of potassium iodide. The mixture was refluxed for another 2-hr period, the solvent distilled off, the residue treated with 125 ml of water, the organic layer separated and the aqueous layer repeatedly extracted with ether; the extracts were combined, washed with 8% NaOH and then with water, dried, and the solvent distilled in vacuo to yield 17·2 g of crude III.

*p-Methoxyphenoxyacetonitrile (IX)* was prepared analogously from 24·8 g (0·2 mole) of p-hydroxyanisol and 16·6 g (0·22 mole) of chloroacetonitrile.

### *(p-Phenylenedioxy) diacetic acid (I)*

A. Condensation of hydroquinone with chloroacetic acid. A solution of 55 g (0·5 mole) of hydroquinone in 235 ml of 15% sodium hydroxide was heated with stirring under nitrogen to 90° and solutions of 94·5 g (1 mole) of chloroacetic acid in 250 ml of water and 40 g (1 mole) of sodium hydroxide in 230 ml of water were simultaneously added

* Taken in part from the graduation thesis by O. Kaltenberg.

dropwise from two separate dropping funnels. The rate of addition was adjusted so that the pH value of the reaction mixture did not drop below 8. The mixture was refluxed 5—10 min with some activated carbon, filtered, and the filtrate acidified with hydrochloric acid to yield upon cooling 93 g of I.

B. Hydrolysis of III. A mixture of 5 g (0·0266 mole) of III and 35 ml of 20% sodium hydroxide was refluxed until evolution of ammonia ceased (approximately 7 hrs). Treatment with charcoal, filtration and acidification with hydrochloric acid yielded 5·25 g of I.

*p-Methoxyphenoxyacetic acid (VIII)*

A. Condensation of p-hydroxyanisole with chloroacetic acid. The reaction was similar to that used in the preparation of I by the procedure A. No nitrogen atmosphere was, however, necessary in this case. From 62 g (0·5 mole) of p-hydroxyanisole and 47·5 g (0·5 mole) of chloroacetic acid 91 g of the sodium salt of VIII was obtained, subsequently converted into 73·2 g of VIII by treatment with hydrochloric acid.

B. Hydrolysis of IX. The procedure used in the preparation of I (method B) yielded 91·5% of VIII.

*p-Hydroxyphenoxyacetic acid (II)*

A. Demethylation of VIII. A mixture of 9·1 g (0·05 mole) of VIII and 30 ml of 40% hydrobromic acid was refluxed 5 hrs and the excess of the acid was distilled off to give 5·2 g of II.

B. Hydrolysis and demethylation of IX. A mixture of 8·15 g (0·05 mole) of IX and 35 ml of 40% hydrobromic acid refluxed 6 hrs. and worked up as above gave 5·4 g of II.

*Ethyl (p-phenylenedioxy)-diacetate (IV).* A mixture of 45·2 g (0·2 mole) of I, 146 ml (2·5 moles) of anhydrous ethanol and 2·5 ml of concd. $H_2SO_4$ refluxed 6 hrs, cooled, and diluted with water yielded 52·5 g of IV, purified by distillation and recrystallisation.

*Ethyl p-hydroxyphenoxyacetate (X).* A mixture of 33·6 g (0·2 mole) of II, 120 ml (2 moles) of anhydrous ethanol and 3 ml of concd. $H_2SO_4$ was refluxed 45 min, cooled, neutralised with saturated aq. $NaHCO_3$, diluted by adding 100 ml of water to yield crystalline X. After recrystallisation from 50% ethanol the yield was 28 g.

*(p-Phenylenedioxy)-diacetamide (V).* A solution of 6·2 g (0·022 mole) of IV in 30 ml of ethanol was mixed with 30 ml of concd. aq. ammonia, the mixture refluxed 15 min and left overnight at room temp. Crude V (3 g) was filtered off and purified by recrystallisation from 50% ethanol.

*p-Hydroxyphenoxyacetamide (XI).* A mixture of 5 g (0·025 mole) of X and 50 ml of concd. aq. ammonia was refluxed a few min and left overnight at room temp. After evaporation in vacuum the residue was dissolved in a minimum amount of $H_2O$, the solution decolorized with activated carbon and thoroughly cooled to yield 2·6 g of XI.

*(p-Phenylenedioxy)-bis-(acetylhydrazine) (VI).* A solution of 10 g (0·035 mole) of IV in 50 ml of ethanol was added portionwise with stirring to 20 g of 80% hydrazine hydrate. After thorough cooling the product was filtered off to yield 6·1 g of VI.

*p-Hydroxyphenoxyacetylhydrazine (XII)* was prepared analogously.

*(p-Phenylenedioxy)-diacetohydroxamic acid (VII).* Ethanolic solution of hydroxylamine: (0·55 g = 0·024 mole of Na in 20 ml of ethanol added to 1·66 g = 0·024 mole of hydroxylamine hydrochloride in 50 ml of ethanol and NaCl filtered off) was mixed with 2·82 g (0·01 mole) of IV in 20 ml of ethanol and the solution was treated with 0·46 g (0·02 mole) of Na in 20 ml of ethanol. After 24 hrs at room temp. the sodium hydroxamate was filtered off, dissolved in a small amount of $H_2O$ and acidified with 10% $H_2SO_4$. A crystalline product (1·05 g) was obtained after prolonged cooling. A characteristic red color was observed with $FeCl_3$.

*p-Hydroxyphenoxyacetohydroxamic acid (XIII).* Sodium hydroxamate prepared as above from 10 g (0·05 mole) of X was dissolved in a minimal amount of water, acidified with 20% acetic acid and treated with an excess of saturated aqueous copper acetate. The green precipitate of the copper chelate was filtered off, washed with water, ethanol and ether, suspended in 200 ml of dry ethanol and decomposed with gaseous $H_2S$. CuS was filtered off through a layer of activated carbon and the filtrate was concentrated in vacuo to yield 0·53 g of XIII. The product gave the characteristic color reaction with $FeCl_3$.

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## OTRZYMYWANIE I WŁASNOŚCI ANTYBAKTERYJNE NIEKTÓRYCH POCHODNYCH HYDROCHINONU

*Streszczenie*

Amidy, hydrazydy i kwasy hydroksamowe pochodne kwasu (p-fenylenodwuoksy)-dwuoctowego (V, VI i VII) oraz kwasu p-hydroksyfenoksyoctowego (XI, XII i XIII) otrzymano drogą aminolizy odpowiednich estrów za pomocą amoniaku, hydratu hydrazyny i hydroksyloaminy. Metody otrzymywania wyjściowych kwasów karboksylowych (ryc. 1 i 2) zmodyfikowano, uzyskując wysokie i powtarzalne wydajności. Badania bakteriologiczne (tab. 2) wykazały słabą lub umiarkowaną czynność antybakteryjną otrzymanych pochodnych.

Na podkreślenie zasługuje fakt, że widma w podczerwieni (p-fenylenodwuoksy)-dwuacetonitrylu (III) i p-metoksyfenoksyacetonitrylu (IX) wykazały całkowity brak pasma 2260—2240 $cm^{-1}$, charakterystycznego dla drgań rozciągających C≡N (ryc. 3 i 4).

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## ПОЛУЧЕНИЕ И ПРОТИВОБАКТЕРИЙНЫЕ СВОЙСТВА НЕКОТОРЫХ ПРОИЗВОДНЫХ ГИДРОХИНОНА

*Содержание*

Амиды, гидразиды и гидроксамовые кислоты производные (р-фениленодиокси)-диуксусной кислоты (V, VI и VII) а также р-гидроксифеноксиуксусной кислоты (XI, XII и XIII) получены путем аминолиза соответственных эфиров при помощи аммиака, гидрата гидразина и гидроксилоамина. Методы получения исходных карбоновых кислот (Черт. 1 и 2) видоизменены, благодаря чему получены высокие и повторимые производительности. Бактериологические исследования показали (Таблица 2) слабое или умеренное противобактерийное действие полученных производных.

Следует подчеркнуть, что спектры в инфракрасной части (р-фениленодиокси)-диацетонитрила (III) и р-метоксифеноксиацетонитрила (IX) показали абсолютное отсутствие полосы 2260—2240 $см^{-1}$, характерной для растягивающих колебаний C ≡ N (Черт. 3 и 4).

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