in the body. Tolerance of the drug in animals following intravenous administration is satisfactory. The use of the conjugate is best indicated in cases requiring prolonged fibrinolytic activity.

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# SYNTHESIS AND ANTIVIRAL ACTIVITY OF 3-NITRO-5-HYDROXYBENZOFURAN DERIVATIVES


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Recently we proposed a new preparative method of synthesizing previously unknown derivatives of 3-nitrobenzofuran [3]. In this work we studied some conversions of 2-methyl-3-nitro-5-hydroxybenzofurans (Ia, b) and the antiviral activity of derivatives produced from them.

It is known that acetals of amides readily condense with compounds possessing active methylene and methyl groups with the formation of enamines [4]. The presence of a nitro group in the 3-position in compounds Ia, b so activates the neighboring methyl group that the interaction of these derivatives with diethylacetals of dimethylformamide and diethylacetamide (IIa, b) proceeds smoothly at room temperature, and the corresponding β-(benzofuranyl-2) enamines (IIIa-c) are formed with high yields (see scheme on following page).

One of the most characteristic reactions of enamines is the transamination reaction [4]. The interaction of the enamine IIIb with amines proceeds under mild conditions


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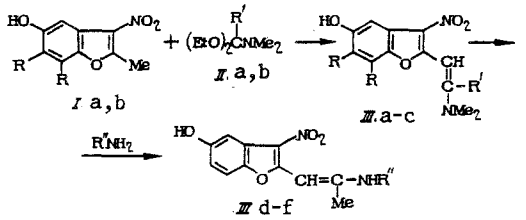
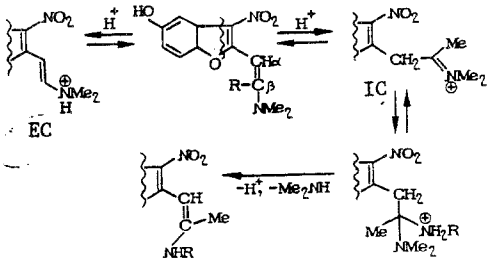


Ia, IIIa, b: R=H; Ib, IIIc: R=Cl; IIa, IIIa: $R^1$=H; IIb, IIIb, c: $R^1$=Me; IIId: R″=H; IIIe: R″=$CH_2Ph$; IIIf: R″=$(CH_2)_2C_6H_4(OMe)_2$-3,4.

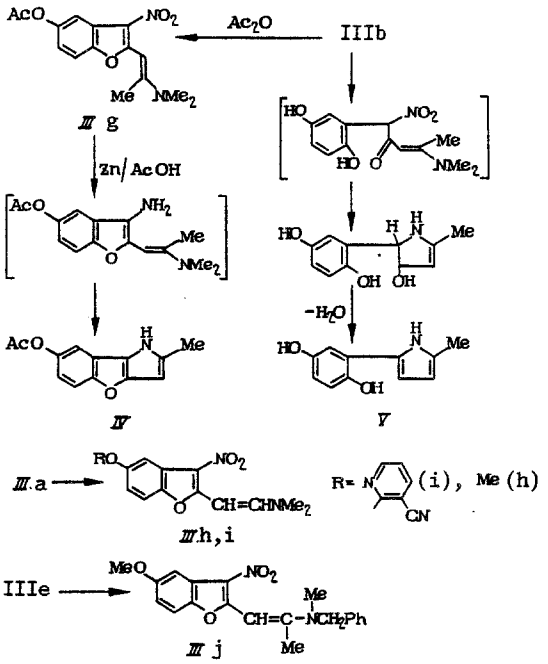
(DMFA, 20°C) with the formation of secondary enamines (IIId-f), but requires the presence of an acid agent – an equimolar amount of p-toluenesulfonic acid – for its successful implementation. Without the acid process of transamination slows down sharply. We should note that transamination of the enamine IIIa, which does not contain a β-methyl group, could not be conducted under the indicated conditions.



Such a difference in the behavior of such structurally similar compounds IIIa and IIIb may be evidence of a difference in the site of their protonation under the conditions of the process. It is known [1] that enamines possessing a β-methyl group are characterized by C-protonation, whereas their desmethyl analogs are characterized by N- and O-protonation. If we assume that the patterns are preserved in our case, then it is understandable that compound IIIb, for which protonation with the formation of an immonium cation (IC) is realized, has a substantially greater tendency for transamination reactions in acid medium than the enamine IIIa, which under these conditions is subjected primarily to N-protonation (enammonium cation – EC).

The acetylation of IIIb to Ac20 leads smoothly to the 5-acetoxy derivative (IIIg), which is then used for the synthesis of pyrrolo[3,2-b]-benzofuran (IV). The latter was produced by reducing acetoxybenzofuran IIIg with zinc in AcOH, which is accompanied by an intramolecular transamination with the formation of a pyrrole ring. It is interesting that the direction of the reduction process is radically changed when it is conducted with the aid of sodium hydrosulfite. Unexpectedly, the only reaction product isolated from the reduction of IIIb (yield 11.6%) proved to be 2-(2,5-dihydroxyphenyl)-5-methylpyrrole (V), the structure of which follows from the data of the mass and PMR spectra. In the PMR spectrum of compound V in $CDCl_3$, signals characteristic of the 2,5-disubstituted pyrrole ring are observed: signals of protons at the 3- and 4-carbon atoms (δ = 5.88 ppm and δ = 6.39 ppm, t), singlet signals of the protons of the nitrogen atom (δ = 9.97 ppm) and methyl group (δ = 2.31 ppm). The presence of singlet signals of the two hydroxyl groups (δ = 8.01, 8.75 ppm) in the PMR spectrum and the signals of the protons of the benzene ring substituted in positions 1, 2, and 4 in the region δ 6.51-7.09 ppm is evidence of the presence of a hydroquinone fragment in the molecule. The proposed scheme of formation of the pyrrole V is presented below (see scheme on following page).

Considering that a number of substances possessing substantial antiviral activity have been found among the alkylaryl and diaryl oxides [5, 6], we studied the alkylation and hetarylation of some of the derivatives obtained. Methylation and pyridylation (using 2-chloro-3-cyanopyridine) of the enamine IIIa proceeds under rather rigorous conditions – during boiling of the reagents in DMFA in the presence of potash. As a result, the 2-β-dimethylaminovinyl-3-nitro-5-methoxy derivative (IIIh) and the 5-(3-cyanopyridyl-2)-hydroxy derivative (IIIi) were isolated with high yields.



We should mention that methylation of the secondary enamine IIIe under these conditions proceeds not only to the 5-hydroxy group but also to the secondary enamine NH group with the formation of an N,N-disubstituted derivative (IIIj).

The structure of the compounds obtained was confirmed by the data of the PMR spectra (see the Experimental section).

## EXPERIMENTAL (CHEMICAL)

The PMR spectra were obtained on a Varian XL-200 spectrometer with a TMS internal standard. The characteristics of the compounds are presented in Table 1. The values found in elementary analyses correspond to those calculated.

2-(2-Dimethylaminovinyl)-3-nitro-5-hydroxybenzofuran (IIIa). To a solution of 0.58 g (3 mmoles) of 2-methyl-3-nitro-5-hydroxybenzofuran in 10 ml of DMFA we added 2.2 ml (15 mmoles) of the diethyl acetal of dimethylformamide. The mixture was exposed for 10 min, then diluted with 100 ml of water, the precipitate filtered off, washed with water, and dried. Yield 0.6 g of compound IIIa. PMR spectrum, δ, ppm, DMSO: 3.03 s, 3.29 s ($NMe_2$), 6.14 d (J = 12.8 Hz) (α = CH), 6.63 g ($J_1$ = 9 Hz, $J_2$ = 2.5 Hz) ($H^6$), 7.17 d (J = 9 Hz) ($H^7$), 7.31 d (J = 2.5 Hz) ($H^4$), 8.07 d (J = 12.8 Hz) (β = CH), 9.37 s (OH).

2-(2-Dimethylaminopropen-1-yl)-3-nitro-5-hydroxybenzofuran (IIIb) was produced analogously. PMR spectrum, δ, ppm, DMSO: 2.56 s (β = $CH_3$), 3.20 s ($NMe_2$), 6.47 s (α = CH), 6.62 g ($J_1$ = 9 Hz, $J_2$ = 2.5 Hz) ($H^6$), 7.22 d (J = 9 Hz) ($H^7$), 7.34 d (J = 2.5 Hz) ($H^4$), 9.35 s (OH).

2-(2-Dimethylaminopropen-1-yl)-3-nitro-5-hydroxy-6,7-dichlorobenzofuran (IIIc). To a suspension of 1 g (3.8 mmoles) of 2-methyl-3-nitro-5-hydroxy-6,7-dichlorobenzofuran in 15 ml of DMFA we added 3.06 ml of the diethyl acetal of dimethylacetamide. The mixture was allowed to stand for 15 min. The precipitate that formed was filtered off, boiled in 50 ml of DMFA, and again filtered, washed with water, and dried. Yield 1 g of compound IIIc.

2-(2-Aminopropen-1-yl)-3-nitro-5-hydroxybenzofuran (IIId). To a solution of 2.62 g (10 mmoles) of compound IIIb in 170 ml of DMFA we added 1.72 g (10 mmoles) of p-toluenesulfonic acid and 10 ml of ammonia. The mixture was exposed for one day. It was diluted with 1000 ml of water, the precipitate filtered off, washed with DMFA, with water, and dried. Yield 1 g of compound IIId.

2-(2-Benzylaminopropen-1-yl)-3-nitro-5-hydroxybenzofuran (IIIe) and 2-(2-homoveratryl-aminopropen-1-yl)-3-nitro-5-hydroxybenzofuran (IIIf) were produced analogously. PMR spectrum of compound IIIe, δ, DMSO: 2.52 s (β = $CH_3$), 4.45 d (J = 5.6 Hz) ($CH_2$), 6.48 s (α = CH),

6.62 g ($J_1$ = 9 Hz, $J_2$ = 2.5 Hz) ($H^6$), 7.24 d (J = 9 Hz) ($H^7$), 7.30-7.45 m ($H^4$, $C_6H_5$), 8.86 t (NH), 9.37 s (OH). PMR spectrum of compound IIIf, δ, ppm, DMSO: 2.48 s (β = $CH_3$), 2.88 t, 3.38 t ($CH_2CH_2$), 3.73 s, 3.79 s ($OCH_3$), 6.55-7.35 m (aromatic protons), 8.54 t (NH), 9.36 s (OH).

2-(2-Dimethylaminopropen-1-yl)-3-nitro-5-acetoxybenzofuran (IIIg). A solution of 5.24 g (20 mmoles) of compound IIIb in 250 ml of $Ac_2O$ was boiled for 1 h. The $Ac_2O$ was evaporated under vacuum. The residue was recrystallized from alcohol. Yield 5 g of compound IIIg. PMR spectrum, δ, ppm, DMSO: 2.33 s (OCOMe), 2.54 s (β = $CH_3$), 3.18 s ($NMe_2$), 6.53 s (α = CH), 6.90 g ($J_1$ = 9 Hz, $J_2$ = 2.5 Hz), 7.23 d (J = 9 Hz) ($H^7$), 7.80 d (J = 2.5 Hz) ($H^4$).

2-(2-Dimethylaminovinyl)-3-nitro-5-methoxybenzofuran (IIIh). To a solution of 0.5 g (2 mmoles) of compound IIIa in 10 ml of DMFA we added 0.56 g (4 mmoles) of anhydrous $K_2CO_3$ in 2.8 g (20 mmoles) MeI. The mixture was boiled for 1 h. The solution was filtered off, and DMFA was evaporated under vacuum. The residue was recrystallized from alcohol. Yield 0.3 g of compound IIIh. PMR spectrum, δ, ppm, DMSO: 3.05 s ($NMe_2$), 3.80 s (OMe), 6.17 d (J = 12.8 Hz) (α = CH), 6.80 g ($J_1$ = 9 Hz, $J_2$ = 2.5 Hz) ($H^6$), 7.30 d (J = 9 Hz) ($H^7$), 7.40 d (J = 2.5 Hz) ($H^4$), 8.12 d (J = 12.8 Hz) (β = CH).

2-(2-Dimethylaminovinyl)-3-nitro-5-(3-cyanopyrid-2-yloxy)indole (IIIi). To a solution of 2 g (8 mmoles) of compound IIIa in 80 ml of DMFA we added 2.25 g (16 mmoles) of anhydrous $K_2CO_3$ and 1.7 g (12 mmoles) of 2-chloro-3-cyanopyridine and boiled for 2 h. The solution was filtered off, DMFA was evaporated under vacuum, and the residue recrystallized from dioxane. Yield 1.85 g of compound IIIi. PMR spectrum, δ, ppm, DMSO: 3.08 s ($NMe_2$), 6.19 d (J = 12.8 Hz) (α = CH) 7.11 g ($J_1$ = 9 Hz, $J_2$ = 2.5 Hz) ($H^6$), 7.30 g ($H^5$), 7.44 d (J = 9 Hz) ($H^7$), 7.62 d (2.5 Hz) ($H^4$), 8.29 d (12.8 Hz) (β = CH), 8.39-8.45 m ($H^{4'}$, $H^{6'}$).

2-(2-Benzylmethylaminopropen-1-yl)-3-nitro-5-methoxybenzofuran (IIIj). A mixture of 0.25 g (0.77 mmole) of compound IIIe, 0.21 g (1.54 mmoles) $K_2CO_3$, 1.1 g (7.7 mmoles) MeI, and 5 ml of DMFA was boiled for 1 h. The reaction mass was diluted with 100 ml of water, the precipitate formed was filtered off, dried, boiled in petroleum ether, and the solution decanted and evaporated. The residue was recrystallized from alcohol. Yield 0.08 g of compound IIIj. PMR spectrum, δ, ppm, DMSO: 2.48 s (β = $CH_3$), 3.80 s ($OCH_3$), 4.86 s ($CH_2$), 6.64 s (α = CH), 6.84-7.44 m (aromatic protons). The signal of the $NCH_3$ group is overlapped by the signals of the solvent.

2-Methyl-7-acetoxypyrrolo[3,2-b]benzofuran (IV). To a boiling solution of 4 g (13 mmoles) of compound IIIg in 55 ml of glacial AcOH we added 8.45 g (130 mmoles) of zinc dust in portions, then boiled for 10 min and filtered. The mother liquor was diluted with 500 ml of water. The precipitate formed was filtered off, washed with water, dried, and recrystallized from benzene. Yield 0.75 g of compound IV. PMR spectrum, δ, ppm, $CDCl_3$: 2.36 s (2-$CH_3$, $CH_3COO$), 5.89 g ($H^3$), 6.78 g ($J_1$ = 9 Hz, $J_2$ = 2.5 Hz) ($H^6$), 7.11 d (J = 2.5 Hz) ($H^8$), 7.34 d (J = 9 Hz) ($H^5$).

2-(2,5-Dihydroxyphenyl)-5-methylpyrrole (V). To a boiling suspension of 2.62 g of compound IIIb in 150 ml of dioxane, we added a saturated aqueous solution of sodium hydrosulfite until a light-yellow solution formed. It was extracted with $CHCl_3$, the extract evaporated, and the residue recrystallized from $CHCl_3$. Yield 0.22 g of compound V. PMR spectrum, δ, ppm, $CDCl_3$: 2.31 s (2 = $CH_3$), 5.88 m ($H^3$), 6.39 t ($H^4$), 6.51 g ($H^{4'}$), $J_1$ = 9 Hz, $J_2$ = 3 Hz), 6.75 d (J = 9 Hz) ($H^{3'}$), 7.09 d (J = 3.0 Hz) ($H^{6'}$), 8.01 br. s., 8.75 br. s., (OH), 9.97 br. s. (NH). Mass spectrum: $(M)^+$ 189.

## EXPERIMENTAL (BIOLOGICAL)

The virus-inhibiting activity of the compounds was studied in a primary cell culture of chick embryo fibroblasts (CEF) with respect to the influenza virus A/FPV ($H_7N_7$), Waybridge strain, Venezuelan equine encephalomyelitis virus (VEEV) strain 230, and herpes simplex virus (HSV) I antigenic type, strain $L_2$.

A monolayer of a culture of CEF cells was infected with 10-100 $TCD_{50}$ (50% tissue cytopathic doses) of the virus, and after adsorption of the virus, which was conducted for 1 h at 37°C, the substances to be studied were introduced.

The compounds were used in concentrations constituting 1/4 and 1/8 of the maximum permissible concentration (MPC), which was determined in a study of the cytotoxic action of the substances on the cells. The virus-inhibiting activity was determined according to

TABLE 1. Characteristics of Compounds IIIa-j, IV, and V

| Compound | mp, °C | Yield, % | Gross formula |
|---|---|---|---|
| III a | 264 (with dec.) | 81 | $C_{12}H_{12}N_2O_4$ |
| III b | 258 (with dec.) | 75 | $C_{13}H_{14}N_2O_4$ |
| III c | 330 (with dec.) | 79,3 | $C_{13}H_{12}Cl_2N_2O_4$ |
| III d | 292 (with dec.) | 42,7 | $C_{11}H_{10}N_2O_4$ |
| III e | 198—199 | 66,4 | $C_{18}H_{16}N_2O_4$ |
| III f | 236—238 (with dec.) | 62,8 | $C_{21}H_{22}N_2O_6$ |
| III g | 210—212 | 82,2 | $C_{15}H_{15}N_2O_5$ |
| III h | 153—154 | 57,2 | $C_{13}H_{14}N_2O_4$ |
| III i | 287—289 | 65,6 | $C_{18}H_{14}N_3O_4$ |
| III j | 122—123 | 29,5 | $C_{20}H_{20}N_2O_4$ |
| IV | 154—155 | 25,2 | $C_{13}H_{11}NO_3$ |
| V | 158—159 | 11,6 | $C_{11}H_{11}NO_2$ |

Note. Compound IIIa was recrystallized from dioxane, IIIb from a mixture of DMFA—ethyl acetate, 1:1, IIIc from DMFA, IIId, g, h, and j from alcohol, IIIe, i, and IV from benzene, IIIf from 50% aqueous acetone, and V from $CHCl_3$.

the inhibition of the cytopathic effect of the virus on the cells, the decrease in its infectious titer in comparison with the control, and was expressed in log $TCD_{50}$.

The chemotherapeutic activity of the substances was studied on models of influenzal pneumonia and generalized herpes in mice, produced by intranasal inoculation of influenza virus A/Bethesda/63 (H2N2) and herpes simplex virus type I, respectively.

The substances were administered per os once a day for five days, using 1/2, 1/4, and 1/8 of the MPC. The activity was judged according to the decrease in the mortality rate of the treated animals in comparison with the control.

In a study of the interferon-inducing activity, the compounds were administered per os to noninbred white mice weighing 18-20 g (20 mice in a group). Titration of interferon in the blood serum of the animals was performed 24 and 48 h after the administration of the compounds studied in a continuous line of murine lymphoid cells L-929 with vesicular stomatitis test virus. The interferon activity unit used was the reciprocal of the value of the dilution providing 50% protection of the cells from the cytopathic action of 100 $TCD_{50}$ of the virus.

Of the eight derivatives of 3-nitro-5-hydroxybenzofuran studied, three compounds exhibited virus-inhibiting effects on the reproduction of VEE virus in a CEF cell culture: IIIa, b, and i. The infectious titer of the virus was decreased by 1-2 log $TCD_{50}$ when these compounds were used in a concentration of 2.5-5 μg/ml in comparison with the control. Compound IIIb, which in a concentration of 5 μg/ml inhibited the reproduction of VEE virus and herpes simplex virus, lowering the infectious titer by 2 and 1.25 log $TCD_{50}$, respectively, was the most active. However, the influence of this compound on the reproduction of FPV virus in CEF cell culture was negligible — a 0.5 log $TCD_{50}$ decrease in the infectious titer of the virus.

The rest of the 3-nitro-5-hydroxybenzofuran derivatives studied had no virus-inhibiting effect on influenza and herpes simplex viruses.

In model experiments on animals and in a study of the interferon-inducing activity, the substances studied were inactive.

Thus, among the 3-nitro-5-hydroxybenzofuran derivatives, substances possessing antiviral activity were found. The spectrum of antiviral activity of these compounds includes both RNA-genome (VEE, influenza) and DNA-genome (herpes) viruses. The highest activity was noted with respect to VEE virus (the arbovirus family).

The results obtained, as well as the antiviral activity of 5-hydroxybenzofuran derivatives that we had detected previously [2], suggest that the search for compounds with antiviral activity among benzofuran derivatives will be promising.

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