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SYNTHESIS OF 6-NITRO- AND 6-AMINO- DERIVATIVES OF 2-(2,4-DIOXO-1,3-DIAZASPIRO[4.5]DECAN-3-YL)-1*H*-BENZO[*DE*]ISOQUINOLINE-1,3(2*H*)-DIONE

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Abstract: *This article describes the synthesis of 6-nitro- and 6-amino- derivatives of 2-(2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione. The structures of the obtained compounds were proved by physicochemical parameters, elemental analysis, IR, ^{1}H and ^{13}C NMR spectral data. The antimicrobial activity of the synthesized compounds against various microorganisms was studied.*

Keywords: *1,3-phenalenediones, isoquinolines, spirohydantoins, antimicrobial activity*

INTRODUCTION

The amino derivatives of 2-phenyl-2,3-dihydrophenalene-1,3-dione have biological (physiological) activity, in particular affecting the central nervous system (Britze *et al*., 1966). A large number of photoresist dyes based on 1,8-naphthalic anhydride have also been synthesized (Chakravarti, 1925). Recently, substances have been synthesized that have aldose reductase inhibitory ability, especially in the fight against diabetes, which affects the peripheral nerves, the lens of the eye, the retina, the cornea, the iris and the kidneys (Kador, 1988; Kador *et al*., 1988; Kador *et al*., 1989; Lee *et al*., 1994).

The question remains what will be the biological activity of compounds with substituents in the naphthalene nucleus in their interaction with imidazolidine derivatives. That is why we have focused our research in this direction.

EXPOSITION

Experimental

All used chemicals were purchased from Merck and Sigma-Aldrich. The melting points were determined by a SMP-10 digital melting point apparatus. The purity of the compounds was checked by thin layer chromatography on Kieselgel 60 F_{254}, 0.2 mm Merck plates, eluent system (vol. ratio): acetone : *n*-pentane = 1 : 1. The IR spectra were taken on Perkin-Elmer FTIR-1600 spectrometer in KBr discs. The NMR spectra were taken on a Bruker DRX-250 spectrometer, operating at 250.13

and 62.90 MHz for ^{1}H and ^{13}C, respectively, using the standard Bruker software. The chemical shifts were referenced to tetramethylsilane (TMS). The measurements in DMSO solutions were carried out at ambient temperature (300 K). Typical conditions for ^{1}H NMR spectra were: pulse width 30º, 1 s relaxation delay, 16K time domain points, zero-filled to 64K, hard pulse with 90º pulse width of 11.8 μs; ^{13}C NMR spectra: pulse width 30º, 1 s relaxation delay, 16K time domain points, zero-filled to 32K, hard pulse with 90º pulse width of 6.4 μs at a power level of 3 dB below the maximum output.

1. Synthesis of 5-nitro-1,2-dihydroacenaphthylene (I)

In a 250 mL three-neck flask equipped with a stirrer and a reflux condenser, 20 g of acenaphthene (1,2-dihydroacenaphthylene) were dissolved in 40 mL of dichloroethane. With stirring and cooling to 10-15°C, 24 mL of 48% nitric acid were added dropwise over 30 minutes. The reaction mixture was stirred at this temperature for an hour, then filtered off and washed with water from the excess nitric acid. After drying at 40°C under vacuum, the final product (I) was recrystallized from 85% acetic acid. Yield 98%. M. p. 100-101°C.

2. Synthesis of 4-nitro-1,8-naphthalic anhydride (6-nitro-1H,3H-naphtho[1,8-cd]pyran-1,3-dione) (II)

In a 500 mL three-neck flask equipped with a reflux condenser and a stirrer, 21.5 g of 5-nitro-1,2-dihydroacenaphthylene (I) were dissolved in 220 mL of glacial acetic acid. At 80°C, 71 g of sodium dichromate were added in several portions over two hours, then the temperature was raised to 95°C and heated for 5 hours. The thick reaction mixture was poured into 1L of water. The precipitate was filtered off and washed thoroughly with water from the chromium salts to a colourless filtrate. The precipitate was dissolved in 300 mL of 5% aqueous sodium hydroxide solution. The undissolved particles were filtered off and a precipitate (II) was formed from the filtrate by acidification with hydrochloric acid to pH = 4, which was filtered off and dried at 90°C. Yield 84%. M. p. 222-223°C.

3. Synthesis of 3-aminospirohydantoins (III)

3-Amino-1,3-diazaspiro[4.5]decane-2,4-dione (IIIa) and 3-amino-8-methyl-1,3-diazaspiro[4.5]decane-2,4-dione (IIIb) were obtained by a modified method (Naydenova *et al*., 2002).

4. Interaction of 4-nitro-1,8-naphthalic anhydride (II) with 3-aminospirohydantoins (III)

A mixture of 4-nitro-1,8-naphthalic anhydride (8.5 g, 0.0035 mol) and the corresponding 3-aminospirohydantoin (IIIa and IIIb) (0.04 mol) was refluxed in 50 mL of glacial acetic acid for 4 hours. The mixture was cooled down to room temperature and poured into 250-300 mL of cold water. The precipitate formed (IVa and IVb) was filtered off, washed with water and, after drying, recrystallized from benzene.

5. Reduction of compounds IV to the corresponding amino derivatives (V)

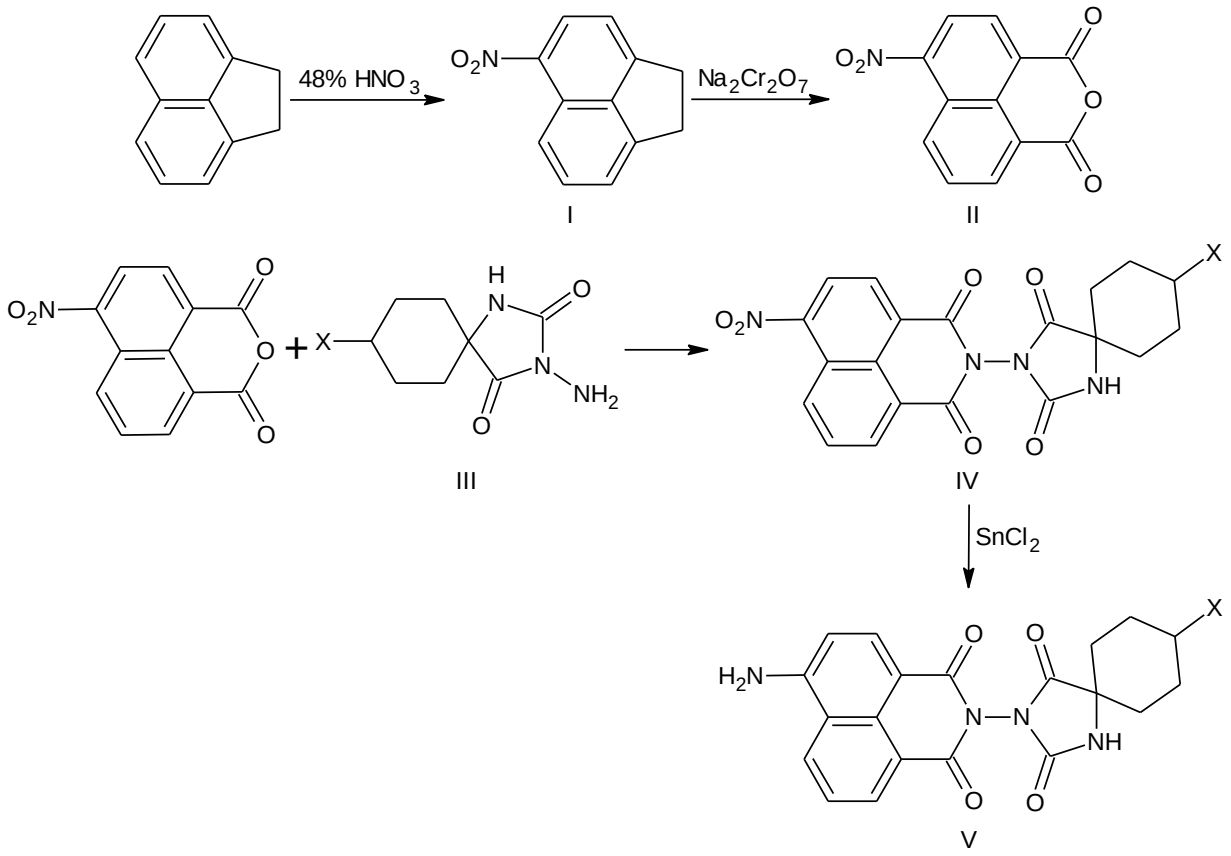
The corresponding product IV (0.0053 mol) was dissolved in 30 ml of absolute ethanol in a 250 ml three-neck flask equipped with a reflux condenser. The mixture was refluxed and 5.2 g of $SnCl_2$ dissolved in 5.2 mL of concentrated hydrochloric acid was added portionwise at reflux. After the addition of the entire amount of $SnCl_2$, stirring was continued for another hour. After cooling to room temperature, the precipitate formed (Va and Vb) was filtered off, washed with water and dried at 100°C.

6. Determination of antimicrobial activity

To determine the antimicrobial activity of the substances, the method of diffusion in agar and test microorganisms was used: Gram-positive bacteria *Staphylococcus aureus ATCC 6538*, *Bacillus subtilis ATCC 6633*, Gram-negative bacteria *Escherichia coli ATCC 8739*, *Pseudomonas aeruginosa ATCC 9027* and *Salmonella abony* NTCC 6017 (Balouiri *et al*., 2016).

RESULTS AND DISCUSSION

We started the experimental part with the synthesis of 5-nitro-1,2-dihydroacenaphthylene (I). The reaction was carried out at 10-15°C with 48% HNO_3. The separated product was recrystallized from glacial acetic acid and oxidized with $Na_2Cr_2O_7$ to obtain 4-nitro-1,8-naphthalic anhydride (II), which was reacted with 3-aminospirohydantoins (III) in dimethylformamide (DMF) medium to give the corresponding imido derivatives (IV), which were reduced with $SnCl_2$ to the corresponding amino derivatives (V).



a) X = H, b) X = CH_3

The physicochemical and spectral data of the obtained compounds are as follows:

2-(2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)-6-nitro-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (IVa): Yield 91%. M. p. 271-272°C. R_f = 0.68. IR (KBr): ν (cm^{-1}) 3242 (NH), 3076 (arom.), 2927 (aliph.), 2862, 1802 (C=O), 1739, 1708, 1696, 1536 (NO_2), 1340 (NO_2). ^{1}H NMR (DMSO-d_6): δ (ppm) 9.49 (s, 1H, NH).

2-(8-methyl-2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)-6-nitro-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (IVb): Yield 90%. M. p. 289-290°C. R_f = 0.72. IR (KBr): ν (cm^{-1}) 3255 (NH), 3061 (arom.), 2931 (aliph.), 2873, 1798 (C=O), 1741, 1710, 1695, 1544 (NO_2), 1345 (NO_2). ^{1}H NMR (DMSO-d_6): δ (ppm) 9.43 (s, 1H, NH).

6-amino-2-(2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (Va): Yield 84%. M. p. > 300°C. R_f = 0.44. ^{1}H NMR (DMSO-d_6): δ (ppm) 9.28 (s, 1H, NH), 6.92-6.90 (d, 2H, NH_2). ^{13}C NMR (DMSO-d_6): δ (ppm) C=O (174.2, 161.5, 160.0), spiro C-atom (61.3), CH (33.8), CH_2 (31.0), CH_3 (29.2), CH_2 (22.5). ^{13}C DEPT 135 (DMSO-d_6): δ (ppm) 135.4, 132.6, 131.1, 124.5, 109.0, 33.6, 24.3, 20.7.

6-amino-2-(8-methyl-2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (Vb): Yield 89%. M. p. > 300°C. R_f = 0.49. ^{1}H NMR (DMSO-d_6): δ (ppm) 9.21 (s, 1H, NH), 6.89-6.85 (d, 2H, NH_2). ^{13}C NMR (DMSO-d_6): δ (ppm) C=O (173.6, 161.2, 159.7), spiro C-atom (60.9), CH_2 (33.6, 24.3, 20.7). ^{13}C DEPT 135 (DMSO-d_6): δ (ppm) 135.7, 132.9, 131.5, 124.8, 109.3, 33.8, 31.0, 29.2, 22.5.

Table 1. Antimicrobial action of substances IVa, IVb, Va and Vb

Test microorganisms	Inhibition zone diameter (mm)			
	IVa	**IVb**	**Va**	**Vb**
Staphylococcus aureus ATCC 6538	12.3	13.5	22.5	24.4
Bacillus subtilis ATCC 6633	13.2	14.1	23.8	25.3
Escherichia coli ATCC 8739	11.2	12.9	18.3	19.4
Pseudomonas aeruginosa ATCC 9027	13.3	14.2	19.1	20.3
Salmonela abony NCTC 6017	15.6	17.3	23.7	25.4

Studies on the antimicrobial activity of the synthesized substances show that compounds Va and Vb have high activity against Gram-positive bacteria *S. Aureus* and *B. subtilis*. The inhibition zones diameters are 22.5 mm (Va) and 24.4 mm (Vb) in *S. aureus* and 25.4 mm (Va) and 25.3 mm (Vb) in *B. subtilis*. Of the Gram-negative bacteria, *S. abony* is also highly sensitive to the action of the two substances: 23.7 mm (Va) and 25.4 mm (Vb). *E. coli* and *P. aeruginosa* are less sensitive to these two substances.

The gram-negative bacterium *S. abony* is sensitive to compounds IVa and IVb, the growth inhibition zones are 15.6 mm and 17.3 mm, respectively. Gram-positive test microorganisms *S. aureus* and *B. subtilis*, as well as Gram-negative *E. coli* and *P. aeruginosa* are slightly sensitive to these substances.

CONCLUSION

The interaction of 4-nitro-1,8-naphthalic anhydride (6-nitro-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione) with 3-aminospirohydantoins has been described. Upon their reduction with $SnCl_2$, the corresponding amino derivatives were obtained. The compounds not described so far in the literature were characterized by IR and NMR. The microorganisms used in the experiments were sensitive to the action of substances Va and Vb and slightly sensitive to substances IVa and IVb.

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