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SYNTHESIS OF SUBSTITUTED ANILIDES OF THE ALKALOID CYTISINE AND MOLECULAR STRUCTURE OF *N*-(2′,6′-DICHLORO-4′-NITROPHENYL)-2-*N*-CYTISINOACETAMIDE

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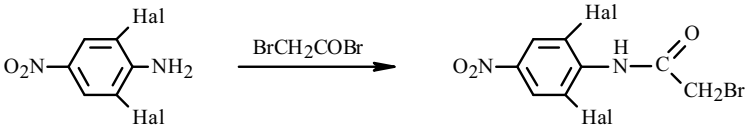
*Anilides of cytisinylacetic acid were synthesized by reaction of the alkaloid cytisine with 2-bromo-*N*-(2,6-dihalo-4-nitrophenyl)acetamides. The compositions and structures of the products were confirmed by IR and PMR spectroscopy, mass spectrometry, and x-ray structure analysis.*

Key words: alkaloid cytisine, 2,6-dihalo-4-nitroanilides, PMR spectroscopy, x-ray structure analysis.

Chemical design of biologically active compounds, including natural alkaloids, is performed by combining in a molecule several pharmacophoric fragments that results rather frequently not only in strengthening the basic therapeutic effect but also in the manifestation of new and sometimes unexpected types of activity.

Herein we examine several methods of introducing into the structure of the physiologically active alkaloid cytisine a substituent containing a nitro group and a halogen atom because many nitro compounds exhibit high antibacterial, insecticidal, growth-stimulating, and other types of biological activity [1–3]. Introduction of halogens increases the lipophilicity of drugs and facilitates their passage through biomembranes [4]. The simultaneous presence of halogen and nitro groups in an aromatic core as, for example, in derivatives of phenylsalicylanilides with 2-halogen and 4-nitro groups, imparts high antihelmintic activity and produces a powerful disjunctive effect [5, 6].

Therefore, we devised the following scheme of transformations. 2,6-Dichloro-4-nitroaniline (**1**) [7] and 2,6-dibromo-4-nitroaniline (**2**) [8] were synthesized by known methods; 2,6-diiodo-4-nitroaniline (**3**), by a scheme developed by us that differed favorably from the published scheme [9]. These were acylated by bromoacetic acid bromide using Scheme 1.



1 - 3 **4 - 6**

Hal = Cl (**1, 4**); Br (**2, 5**); I (**3, 6**)

Scheme 1

Because the amino group in 2,6-dihalo-4-nitroanilines **1**–**3** was less active and sterically hindered for nucleophilic attack, the acylation occurred under rather forcing conditions, refluxing for 12–15 h in toluene. Higher yields of the starting products were also obtained under milder conditions for acylation of 2,6-dihalo-4-nitroanilines **1**–**3** in anhydrous DMF.

IR spectra of **4**–**6** contained a strong absorption band for carbonyl at 1695 cm^{-1}; nitro, 1515 and 1345; and N–H, 3200.

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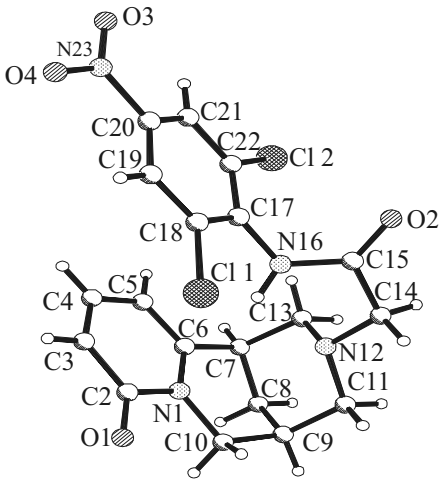
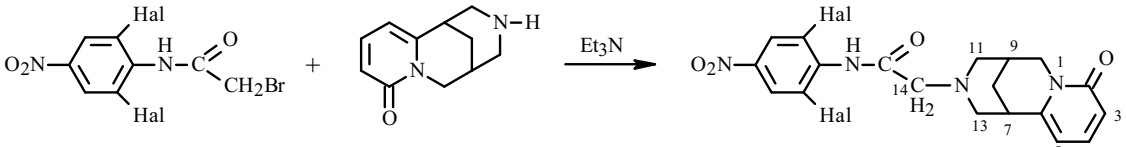


Fig. 1. Molecular structure of *N*-(2′,6′-dichloro-4′-nitrophenyl)-2-*N*-cytisinoacetamide (**7**).

PMR spectra of **4**–**6** showed methylene protons of C$\underline{H}_2$Br as a narrow singlet at 4.15 ppm. Aromatic protons were also recorded as a singlet at rather weak field at 8.40–8.60 ppm.

The resulting 2,6-dihalo-4-nitrobromoacetanilides **4**–**6** were rather reactive alkylating reagents. Thus, alkylation by **4**–**6** of the alkaloid cytisine in anhydrous benzene or toluene in the presence of a two-fold excess of triethylamine produced the corresponding 2,6-dihalo-4-nitroanilides of *N*-cytisinylacetic acid **7**–**9**.



4 - 6 Hal = Cl (**4, 7**); Br (**5, 8**); I (**6, 9**) **7 - 9**

The synthesized compounds **7–9** were yellowish crystalline compounds that were soluble in DMF and moderately soluble in hot polar solvents. The compositions and structures of **7–9** were confirmed by elemental analysis, IR and PMR spectroscopy, and mass spectrometry.

IR spectra of **7**–**9** contained a strong absorption band for carbonyl at 1690 cm^{-1} (1650 for the amide fragment N–C=O of cytisine) and for nitro at 1525 and 1344.

PMR spectra of **7**–**9** showed protons of the alkaloid at their characteristic regions. Aromatic protons for **7**–**9** were recorded at weak field at 8.37–8.58 ppm as a singlet. Methylene protons of the carbonyl group of **7** and **8**, in contrast with starting **4**–**6**, **9**, and simple analogous derivatives based on morpholine or piperidine, were nonequivalent and observed as a doublet of doublets at 3.15 ppm with SSCC 15.2 Hz.

The molecular structure of the synthesized compounds were established by solving the x-ray structure of **7** (Fig. 1).

It was found that the bond lengths and angles in the cytisine backbone of **7** were similar to the standard values [10] and corresponded with those of earlier structural studies such as those of *N*-acetonylcytisine [11] and *N*-methylcytisine [12]. The dihydropyridine ring was planar within ±0.007 Å, carbonyl atom O1 was almost in the plane of the other atoms, deviating by 0.06 Å The tetrahydropyridine ring N1C6C7C8C9C10 adopted a distorted half-chair conformation (ΔC_s^8 = 9.99 Å) with bridging C8 deviating from the average plane of the other atoms by 0.73 Å (I). The piperidine ring had an almost ideal chair conformation (ΔC_s^{13} = 0.75 Å). The bulky substituent on N12 was oriented euqatorially relative to the piperidine ring (torsion angle C11N12C14C15 = 161.7°). The Cl and N atoms were in the plane of the benzene ring (coplanar within ±0.04 Å). The O atoms deviated from this plane by ±0.13 Å. The 2,6-dichloro-4-nitrophenyl fragment was twisted and located in a plane almost parallel to the plane of the dihydropyridine ring (interplanar distance 3.746 Å). Such placement of the bulky substituent can be explained by crystal packing effects and intermolecular interaction between O4 (x, y, z) and Cl2 (1 + x, y, z) (distance O4...Cl2 = 3.208 Å), which pulled the nitro group of a neighboring molecule toward the cytisine framework and formed infinite chains along the *a* axis.

EXPERIMENTAL

PMR spectra were recorded in DMSO-d_6 on a Bruker DRX500 spectrometer at frequency 500 MHz relative to TMS internal standard. Mass spectra were recorded in a Finnigan Mat. Incos 50 instrument with direct sample introduction at ionization energy 70 eV. Melting points were determined on a Boetius instrument. TLC was performed on Sorbfil plates with detection by iodine vapor.

X-ray Structure Analysis. Cell constants and intensities of 3857 independent reflections were measured on a CCD Xcalibur^{TM} R diffractometer (Oxford Diffraction) using Cu K_α-radiation, graphite monochromator, ω/2θ-scanning, and scan range $6 \le \theta \le 151.5°$. Crystals were monoclinic ($0.3 \times 0.2 \times 0.4$ mm), $a = 8.833(1)$, $b = 7.596(1)$, $c = 14.834(1)$ Å, $\beta = 96.79(9)°$, V = 988.37(18) Å^3, $Z = 2(C_{19}H_{18}Cl_2N_4O_4)$, $d_{calc} = 1.469$ g/cm^3, space group $P2_1$.

A total of 3462 independent reflections with $I > 2\sigma(I)$ was used in the calculations. The structure was solved by direct methods and refined by anisotropic full-matrix least-squares methods for nonhydrogen atoms. H atoms were fixed geometrically and refined by the riding model. Absorption corrections were applied using multi-scan. The final agreement factors were $R[F^2 > 2\sigma(F^2)] = 0.0401$, $\omega R(F^2) = 0.1050$. Atomic coordinates and geometric parameters were deposited in the Cambridge Crystallographic Data Centre (CCDC 711612). All calculations were carried out using the SHELXL-97 programs.

2-Bromo-*N*-(2,6-dichloro-4-nitrophenyl)acetamide (4). A solution of 2,6-dichloro-4-nitroaniline (3.20 g, 0.0155 mol) in DMF (10 mL) was stirred by a magnetic stirrer, treated dropwise at room temperature with bromoacetic acid bromide (3.17 g, 0.0157 mol), heated with constant stirring at 55–60°C for 3 h, left overnight, and poured into a beaker with water (100 g) and ice (100 g). The resulting yellowish precipitate was filtered off, washed with water, and dried at 90°C to afford **2** (4.78 g, 94%), mp 198–200°C (propanol-2), $C_8H_5BrCl_2N_2O_3$. PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 4.15 (2H, s, C$\underline{H}_2$), 8.40 (2H, s, H-Ar), 10.73 (1H, s, N–H).

2-Bromo-*N*-(2,6-dibromo-4-nitrophenyl)acetamide (5) was prepared analogously to **4** from 2,6-dibromo-4-nitroaniline (5.92 g, 0.02 mol) and bromoacetic acid bromide (4.24 g, 0.021 mol) in 96% yield. Two recrystallizations from 2-propanol:EtOAc (1:1) gave mp 211–212°C, $C_8H_5Br_3N_2O_3$. PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 4.17 (2H, s, C$\underline{H}_2$), 8.52 (2H, s, H-Ar), 10.62 (1H, s, N–H).

2-Bromo-*N*-(2,6-diiodo-4-nitrophenyl)acetamide (6) was prepared analogously to **4** from 2,6-diiodo-4-nitroaniline (3.90 g, 0.01 mol) and bromoacetic acid bromide (2.22 g, 0.011 mol) in 91% yield. Recrystallization from 2-propanol:DMF (1:1) gave mp 225–227°C, $C_8H_5BrI_2N_2O_3$. PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 4.15 (2H, s, C$\underline{H}_2$), 8.63 (2H, s, H-Ar), 10.31 (1H, s, N–H).

***N*-(2′,6′-Dichloro-4′-nitrophenyl)-2-*N*-cytisinoacetamide (7).** A suspension of **4** (0.71 g, 2.2 mmol) in anhydrous toluene (10 mL) was treated with triethylamine (0.50 g, 5 mmol) and cytisine (0.42 g, 2.2 mmol) and refluxed with stirring for 3 h. The hot solution was filtered to remove the precipitate of triethylamine hydrobromide, which was washed several times with hot benzene. The combined mother liquors were evaporated to afford a light-yellow crystalline compound (0.60 g, 63%), mp 214–215°C (benzene:2-propanol, 5:1), $C_{19}H_{18}Cl_2N_4O_4$. Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 437 (5) $[M]^+$, 203 (100), 146 (27), 58 (98), 42 (43). PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.77 (2H, dd, $J_{8,7} = 12.6$, $J_{8,9} = 12.7$, H-8), 2.45 (1H, br.s, H-9), 2.60 (2H, m, H-11), 2.96 (1H, m, H-7), 3.06 (2H, m, H-13), 3.17 (2H, dd, $J_{a,b} = 15.2$, H-14), 3.75 (1H, m, H-10a), 3.88 (1H, d, $J_{10e,10a} = 15.3$, H-10e), 6.09 (1H, d, $J_{5,4} = 6.8$, H-5), 6.14 (1H, d, $J_{3,4} = 9.0$, H-3), 7.24 (1H, dd, $J_{4,5} = 6.8$, $J_{4,3} = 9.0$, H-4), 8.37 (2H, s, H-Ar), 9.45 (1H, s, N–H).

***N*-(2′,6′-Dibromo-4′-nitrophenyl)-2-*N*-cytisinoacetamide (8)** was prepared analogously to **7** from **5** (1.60 g, 3.8 mmol) and cytisine (0.73 g, 3.8 mmol) in 79% yield. Recrystallization from 2-propanol:hexane (5:1) gave mp 195–197°C, $C_{19}H_{18}Br_2N_4O_4$. Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 524, 528 (3) $[M]^+$, 203 (100), 160 (33), 43 (32). PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.77 (2H, dd, $J_{8,7} = 12.6$, $J_{8,9} = 12.7$, H-8), 2.45 (1H, br.s, H-9), 2.61 (2H, m, H-11), 3.01 (1H, m, H-7), 3.08 (2H, m, H-13), 3.14 (2H, dd, $J_{a,b} = 15.1$, H-14), 3.77 (1H, m, H-10a), 3.91 (1H, d, $J_{10e,10a} = 15.2$, H-10e), 6.10 (1H, d, $J_{5,4} = 6.7$, H-5), 6.13 (1H, d, $J_{3,4} = 8.9$, H-3), 7.24 (1H, dd, $J_{4,5} = 6.7$, $J_{4,3} = 8.9$, H-4), 8.48 (2H, s, H-Ar), 9.42 (1H, s, N–H).

***N*-(2′,6′-Diiodo-4′-nitrophenyl)-2-*N*-cytisinoacetamide (9)** was prepared analogously to **7** from **6** (0.51 g, 1 mmol) and cytisine (0.19 g, 1 mmol) in 80% yield. Recrystallization from 2-propanol gave mp 241–242°C, $C_{19}H_{18}I_2N_4O_4$. Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 418 (23), 203 (100), 58 (71), 42 (27). PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.80 (2H, dd, $J_{8,7} = 12.8$, $J_{8,9} = 13.1$, H-8), 2.45 (1H, br.s, H-9), 2.68 (2H, m, H-11), 3.04 (1H, m, H-7), 3.09 (2H, m, H-13), 3.12 (2H, s, H-14), 3.76 (1H, m, H-10a), 3.95 (1H, d, $J_{10e,10a} = 15.3$, H-10e), 6.09 (1H, d, $J_{5,4} = 6.3$, H-5), 6.11 (1H, d, $J_{3,4} = 8.4$, H-3), 7.23 (1H, dd, $J_{4,5} = 6.3$, $J_{4,3} = 8.4$, H-4), 8.58 (2H, s, H-Ar), 9.30 (1H, s, N–H).

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