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SYNTHESIS AND HIV-1 RNase H-ACTIVITY OF NEW ALIZARIN ACETONYL DERIVATIVES

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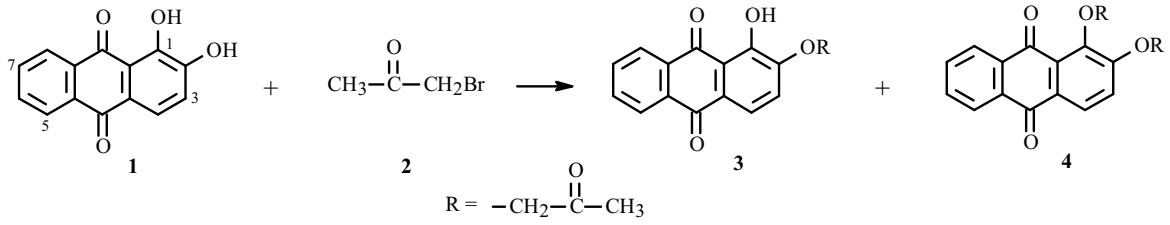
Alkylation of 1,2-dihydroxyanthraquinone (alizarin) by α-bromoacetone was studied and its β-acetonyl derivative was chemically modified. The composition and structure of the products were confirmed by elemental analysis; UV, IR, PMR, and ^{13}C NMR spectroscopy; and mass spectrometry. The synthesized derivatives were tested as inhibitors of HIV-1 RNase H.

Key words: alizarin, *β*-acetonyl derivative of alizarin, HIV-1 RNase H-activity.

Anthraquinones are widely distributed in nature [1] and are used in various branches of industry and medicine [2–4]. 1,2-Dihydroxyanthraquinone (alizarin, **1**) is one of the most common natural hydroxyanthraquinones [5]. It and its methyl, methoxy, and acetoxy derivatives in addition to a glycoside, ruberitrinic acid, have been isolated from various *Rubia* species and other plants [6, 7]. Considering the variety of biological activity of anthraquinone derivatives and the fact that anthraquinone derivatives have been reported as potential HIV-1 inhibitors [8–10], we studied the chemical modification of alizarin and the activity of the resulting derivatives as inhibitors of HIV-1 RNase H.

Introduction into **1** of fragments with a carbonyl group enabled a wide range of its polyfunctional derivatives to be prepared and opened pathways for using them in further syntheses. Alkylation of **1** with *α*-bromoacetone (**2**), which was prepared by the literature method [11] using brominating agent dioxane dibromide, was used to prepare *O*-acetonyl derivatives.



Reaction of **1** with **2** could form both mono- (**3**) and di-substituted (**4**) products depending on the synthesis method because **1** contains two hydroxyls. The reaction of **1** in acetone with added K_2CO_3 was studied. As shown earlier [12], alkylation of the *β*-hydroxyl (**3**) occurred first in all instances with an equimolar ratio of reagents and with an excess of the *α*-bromoketone. Formation of product **4** was not observed with an equimolar ratio of reagents. Performing the reaction with a two-fold excess of the bromide and increasing the time (to 78 h) formed a mixture consisting of the mono- and di-substituted derivatives (**3** and **4**), which were separated by column chromatography over silica gel. Formation of the mono- and di-substituted products is easily followed by TLC using the color of the compounds (**3** is yellow whereas **4** is light yellow), by chromatographic mobility, and by the presence or absence of the characteristic color from the Borntreger reaction (ammonia vapor). The maximum yield of **4** was 54% for the reaction with a three-fold excess of the bromide for 60 h.

The purity of the products was confirmed by TLC. They were identified using elemental analysis and UV, IR, PMR, ^{13}C NMR, and mass spectra. They were crystalline compounds that were very soluble in polar organic solvents and insoluble in water.

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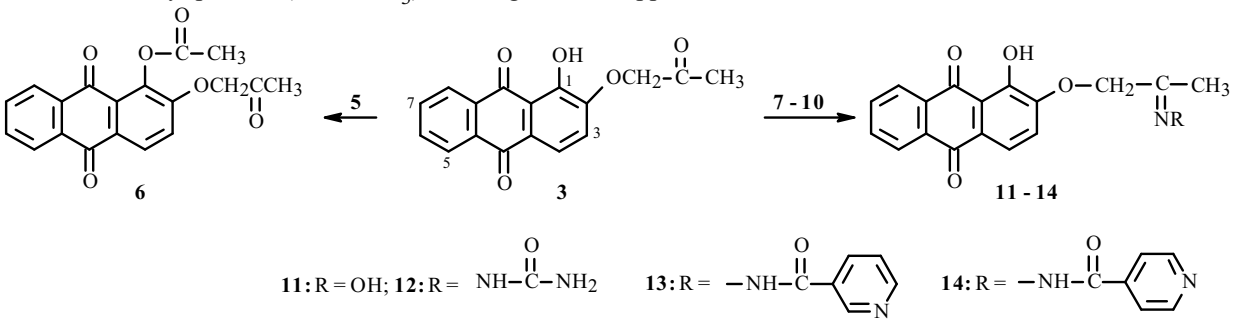
IR spectra of **3** and **4** exhibited characteristic stretching vibrations for carbonyls in the 9- and 10-positions of anthraquinone at 1616–1680 cm^{-1} and for the acetonyl substituent at 1716–1722. The C=C stretching vibrations of the anthraquinone aromatic rings were found at 1575–1591 cm^{-1}. Introduction of the acetonyl fragment was seen clearly in the PMR spectra. Thus, two singlets at 5.01 ppm (–CH_2–) and 2.26 (–CH_3) were seen instead of the resonance of the β-hydroxyl proton in the spectrum of **3**. The α-hydroxyl proton of **3** corresponded to a resonance at 12.91. The aromatic anthraquinone α- and β-protons of **3** and **4** appeared in the range 7.30–8.31.

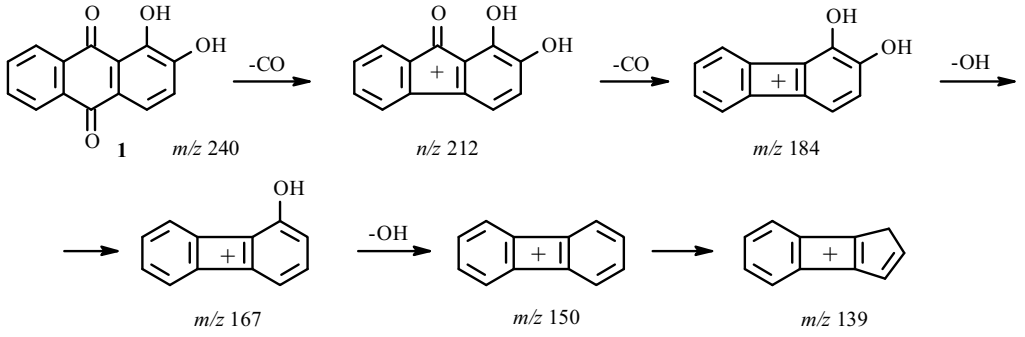
Synthesis of **3** was also confirmed by preparing acylated product **6** by reaction of **3** with acetic anhydride (**5**). The IR spectrum of **6** contained absorption bands due to C=O stretching vibrations at 1661–1774 cm^{-1}. Its PMR spectrum had a resonance for methyl protons (–OCO$\underline{C}H_3$) as a singlet at 2.50 ppm.



Hydroxylamine and hydrazine derivatives are typical reagents for carbonyls that are often used to identify aldehydes and ketones. Several compounds based on them are widely used in medicine, agriculture, and industry [13, 14]. A study of the biological activity of the oxime, hydrazone, and phenylhydrazone of chrysophanic acid (4,5-dihydroxy-2-methyl-9,10-anthraquinone) showed that they were mildly toxic and had moderate antitumor activity whereas 4,5-dihydroxy-2-methylanthra-9-oxime stimulated growth of connective tissue and epithelial tumors [15].

We studied the reaction of **3** with hydroxylamine (**7**), semicarbazide (**8**), nicotinic hydrazide (**9**), and isonicotinic acid (**10**). IR spectra of **11**–**14** lacked a carbonyl absorption band of the acetonyl substituent. Stretching vibrations of the hydroxyl group of oxime **11** appeared at 3292 cm^{-1}; the primary and secondary amines, at 3198–3485. The carbonyls in the 9,10-positions of the anthraquinone system were found at 1633–1669; the semicarbazone carbonyl of **12**, at 1749. PMR spectra of **11**–**14** exhibited resonances of aromatic protons at 7.48–10.10 ppm. Singlets at 12.75–12.86 corresponded to the α-hydroxyl proton. The hydroxyl proton of oxime **11** appeared as a singlet at 10.95.

The base peak in the mass spectrum of **1** was the molecular ion, which fragmented according to the following scheme:



Elimination of CO occurred first to form an ion with *m*/*z* 212. Then, a second CO molecule was eliminated to form a dihydroxyfluorene ion with *m*/*z* 184. Next, hydroxyls were eliminated to form hydroxybiphenyl ion with *m*/*z* 167 and biphenyl with *m*/*z* 150.

Although the molecular ion was the base peak in the mass spectrum of **1** ($[M]^+$, *m*/*z* 240, I = 100%), it was weak for **3**, **4**, and **11**–**14**. For these, the bond to the substituent cleaved first. For **3** and **4**, loss of [–$C(O)CH_3$] from the acetonyl substituent was typical. This formed a corresponding ion with *m*/*z* 43 and $[M - C(O)CH_3]^+$ ions with *m*/*z* 253 (for **3**) and *m*/*z* 309 (for **4**). Successive elimination of the OH group of the oxime to form $[M - OH]^+$ with *m*/*z* 294 and of [–$C(N)CH_3$] to give an ion with *m*/*z* 251 were typical of **11**. Mass spectra of **13** and **14** typically had ions with *m*/*z* 254 that were formed by elimination of [CH_3–C=N–NH–$C(O)C_5H_4N$]. Subsequent decomposition for all derivatives gave an ion with *m*/*z* 240.

TABLE 1. Effect of Acetonylated Alizarins (**3**, **4**, **6**, **11**–**14**) on HIV-1 RNase H-Activity

Derivative	[a]IC_{50}, μM	Derivative	[a]IC_{50}, μM
	HIV-1 RNase H		HIV-1 RNase H
Alizarin **1**	>100 (92%)	Compound **11**	>100 (74%)
Compound **3**	>100 (100%)	Compound **12**	>100 (100%)
Compound **4**	>100 (91%)	Compound **13**	90
Compound **6**	>100 (90%)	Compound **14**	>100 (67%)

[a]Concentration of derivative lowering HIV-1 RNase H activity by 50%.

Acquired immunodeficiency syndrome (AIDS) is a consequence of infection by human immunovirus-1 (HIV-1), clinical treatment of which uses strong and selective inhibitors of such enzymes as reverse transcriptase (RT) and protease (PR). RT of HIV-1 is an enzyme that catalyzes transformation of RNA into double-stranded DNA. RT fulfills two different activities in order to carry out this process. These are DNA polymerase activity, which recognizes RNA and DNA templates, and destructive activity, called RNase H, which hydrolyzes the RNA component of the heterodoublet RNA:DNA intermediate.

The synthesized series of alizarin acetonyl derivatives (**3**, **4**, **6**, **11**–**14**) were tested biochemically for the ability to inhibit HIV-1 RNase H-activity. However, the results showed that the compounds were either inactive or weakly active (Table 1).

EXPERIMENTAL

The course of reactions and purity of products were monitored by TLC on Silufol UV-254 plates using various solvent systems. UV spectra were recorded on a Perkin—Elmer Lambda 35 UV/Vis spectrometer. IR spectra were recorded in KBr disks on a Nicolet 5700 spectrometer. PMR and ^{13}C NMR spectra were obtained on a Bruker-500 spectrometer at room temperature with TMS internal standard. Mass spectra (EI, 70 eV) were measured in a Finnigan MAT 8200 instrument. Melting points were determined on a Boetius instrument. Products were separated over Silica Gel 60 (Merck, Germany) with elution by hexane:EtOAc (gradient from 100:0 to 40:60 by volume). Elemental analysis of the derivatives agreed with those calculated. The synthesized compounds were tested as inhibitors of RNase H-activity according to the published method [16].

1,2-Dihydroxyanthraquinone (alizarin) (1). $C_{14}H_8O_4$, mp 289–290°C. IR spectrum (KBr, ν, cm^{-1}): 3355 (β-OH), 1663, 1632 ($C=O_{anth}$), 1586 (Ar). PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 7.20 (d, J = 8, H-3), 7.60 (d, J = 8, H-4), 7.88 (m, H-6,7), 8.11, 8.16 (m, H-5,8), 10.82 (s, β-OH), 12.58 (s, α-OH).

^{13}C NMR spectrum (DMSO-d_6, δ, ppm): 150.6 (C-1), 152.6 (C-2), 126.3 (C-3), 120.9 (C-4), 126.5 (C-5), 134.9 (C-6), 133.8 (C-7), 126.3 (C-8), 188.6 (C-9), 180.3 (C-10), nodal atoms: 133.4, 132.6, 123.6, 116.0.

Mass spectrum (EI, 70 eV): 240 (100), 212 (8), 184 (5), 138 (6), 128 (5), 92 (6), 77 (6).

1-Hydroxy-2-*O*-acetonylanthraquinone (3). A solution of alizarin (2.4 g, 0.01 mol, **1**) in acetone (200 mL) at room temperature was treated with α-bromoacetone (0.01 mol, **2**) and K_2CO_3 (0.01 mol). The reagents were mixed. The solution was refluxed vigorously and stirred. The course of the reaction was monitored by TLC. After the reaction was finished, a part of the solvent was distilled off. The mixture was treated with water acidified with HCl. The resulting precipitate was filtered off and dried. The product was isolated by column chromatography over silica gel. Yield 79%, crystalline orange compound, mp 218–220°C (acetone), $C_{17}H_{12}O_5$, R_f 0.46 (hexane:acetone, 2:1), 0.19 (CCl_4:ether, 6:5). UV spectrum (MeCN, λ_{max}, nm): 200 (0.73), 230 (0.58), 249 (0.78), 280 (0.37), 304 (0.10), 420 (0.18). IR spectrum (KBr, ν, cm^{-1}): 1722 ($C=O_{sub}$), 1665, 1637 ($C=O_{anth}$), 1591 (Ar).

PMR spectrum (acetone-d_6, δ, ppm, J/Hz): 7.30 (1H, d, J = 8.4, H-3), 7.73 (1H, d, J = 8.4, H-4), 7.92 (2H, m, H-6,7), 8.24 (1H, m), 8.31 (1H, m, H-5,8), 12.91 (1H, s, α-OH), 5.01 (2H, s, CH_2), 2.25 (3H, s, CH_3).

^{13}C NMR spectrum ($CDCl_3$, ppm): 151.45 (C-1), 153.02 (C-2), 119.17 (C-3), 120.57 (C-4), 126.76, 127.30 (C-5,8), 133.67, 134.72 (C-6,7), 189.10 (C-9), 181.10 (C-10), nodal atoms: 133.16, 133.02, 126.34, 116.55; substituent in β-position: 205.05 (C=O), 83.00 ($-CH_2-$), 25.37 ($-CH_3$).

Mass spectrum (EI, 70 eV): 296 (26) $[M]^+$, 253 (100), 240 (8), 225 (31), 211 (15), 195 (13), 167 (13), 139 (70), 43 (98).

1,2-*O*-Diacetonylanthraquinone (4) was prepared by the above method using an excess of bromoacetone. Yield 54%, crystalline yellow compound, mp 176–178°C (acetone), $C_{20}H_{16}O_6$, R_f 0.16 (hexane:EtOAc, 2:1), 0.24 (CCl_4:ether, 6:5). UV spectrum (MeCN, λ_{max}, nm): 224 (0.38), 253 (0.45), 370 (0.12). IR spectrum (KBr, ν, cm^{-1}): 1716 ($C=O_{sub}$), 1680, 1616 ($C=O_{anth}$), 1575 (Ar).

PMR spectrum (acetone-d_6, δ, ppm, J/Hz): 7.35 (1H, m, H-3), 7.85 (1H, m, H-4), 7.92 (2H, m, H-6,7), 8.16 (2H, m, H-5,8), 4.43 (2H, s, CH_2), 4.68 (2H, s, CH_2), 2.65 (3H, s, CH_3), 2.86 (3H, s, CH_3).

Mass spectrum (EI, 70 eV): 309 (34), 279 (20), 251 (36), 240 (15), 211 (11), 152 (6), 139 (18), 43 (100).

1-*O*-Acetoxy-2-*O*-acetonylanthraquinone (6). A weighed portion of **3** (0.6 g, 0.002 mol) was dissolved in a mixture of acetic anhydride (**5**) and pyridine (10:1, v/v), treated with NaOAc, and refluxed for 1.5 h. After the reaction was finished, water was poured into the flask. The resulting precipitate was filtered off, washed, and dried. Recrystallization from $CHCl_3$, yield 97%, $C_{19}H_{14}O_6$, mp 165–167°C, R_f 0.24 (CCl_4:ether, 6:5), 0.27 (hexane:EtOAc, 2:1). IR spectrum (KBr, ν, cm^{-1}): 1774 [C=O in $-OC(O)CH_3$], 1726 ($C=O_{sub}$), 1672, 1661 ($C=O_{anth}$), 1587 (Ar).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 7.35 (1H, m, H-3), 7.80 (3H, m, H-4, H-6,7), 8.22 (2H, m, H-5,8), 4.65 (2H, s, CH_2), 2.23 (3H, s, CH_3), 2.50 [3H, s, $OC(O)\underline{CH_3}$].

General Method for Preparing Derivatives 11–14. A four-necked flask equipped with a stirrer, thermometer, dropping funnel, and condenser was charged with **3** (0.89 g, 0.003 mol) dissolved in MeOH:dioxane (50 mL, 1:1), stirred, and treated with hydroxylamine hydrochloride (**7**), semicarbazide (**8**), nicotinic acid hydrazide (**9**), or isonicotinic acid hydrazide (**10**) in ratios from 1:1 to 1:3 g/mol. The catalyst for preparing **11** and **12** was NaOAc; **13** and **14**, HCl. The reaction was carried out with vigorous stirring for 1.5-5 h at 50–65°C. After the reaction was finished, a part of the solvent was distilled off in a rotary evaporator. The contents of the flask were precipitated by water acidified with HCl. The resulting precipitate was filtered off, dried, and recrystallized from $CHCl_3$:dioxane (5:1).

1-Hydroxy-2-*O*-acetonylanthraquinone Oxime (11). Yield 89%, $C_{17}H_{13}NO_5$, mp 211–213°C, R_f 0.34 (hexane:EtOAc, 2:1). IR spectrum (KBr, ν, cm^{-1}): 3292 (OH), 1669, 1635 ($C=O_{anth}$), 1592 (Ar).

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 7.48 (1H, d, J = 8.50, H-2), 7.77 (1H, d, J = 8.47, H-4), 7.93 (2H, m, H-6,7), 8.18 (1H, m), 8.24 (1H, m, H-5,8), 12.86 (1H, s, α-OH), 5.12 (2H, s, CH_2), 1.80 (3H, s, CH_3), 10.95 (1H, s, NOH).

Mass spectrum (EI, 70 eV): 3.11 (10) $[M]^+$, 294 (7), 251 (17), 240 (100), 211 (36), 183 (14), 167 (13), 155 (10), 139 (14), 127 (20).

1-Hydroxy-2-*O*-acetonylanthraquinone Semicarbazone (12). Yield 91%, mp 223–225°C, $C_{18}H_{15}N_3O_5$, R_f 0.08 (hexane:EtOAc, 2:1). IR spectrum (KBr, ν, cm^{-1}): 3485, 3298, 3198 (NH_2, NH), 1749 ($C=O_{semicarb}$), 1666, 1633 ($C=O_{anth}$), 1590 (Ar).

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 7.49 (1H, d, J = 8.4, H-2), 7.74 (1H, d, J = 8.4, H-4), 7.94 (2H, m, H-6,7), 8.19 (1H, m), 8.24 (1H, m, H-5,8), 12.76 (1H, s, α-OH), 5.11 (2H, s, CH_2), 2.03 (3H, s, CH_3), 6.35 (2H, s, NH_2), 9.24 (1H, s, NH).

Mass spectrum (EI, 70 eV): 353 (2) $[M]^+$, 240 (66), 211 (14), 184 (14), 155 (9), 138 (20), 128 (23), 77 (28), 44 (100).

1-Hydroxy-2-*O*-acetonylanthraquinone Nicotinic Acid Hydrazone (13). Yield 97%, mp 221–223°C, $C_{23}H_{17}N_3O_5$, R_f 0.04 (hexane:EtOAc, 2:1). IR spectrum (KBr, ν, cm^{-1}): 3246 (NH), 1664, 1640 ($C=O_{anth}$), 1590 (Ar).

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 7.55 (1H, d, J = 8.0, H-2), 7.72 (1H, d, J = 8.0, H-4), 7.96 (2H, m, H-6,7), 8.21 (1H, m), 8.27 (1H, m, H-5,8), 12.76 (1H, s, α-OH), 4.96 (2H, s, CH_2), 2.10 (3H, s, CH_3), 7.78 (1H, m, Ar), 8.70 (1H, m, Ar), 8.76 (1H, m, Ar), 10.10 (1H, s, Ar), 11.00 (1H, s, NH).

Mass spectrum (EI, 70 eV): 415 (4) $[M]^+$, 254 (15), 240 (100), 212 (10), 184 (7), 176 (15), 162 (10), 106 (58), 78 (25), 51 (14).

1-Hydroxy-2-*O*-acetonylanthraquinone Isonicotinic Acid Hydrazone (14). Yield 95%, mp 229–231°C, $C_{22}H_{17}N_3O_5$, R_f 0.03 (hexane:EtOAc, 2:1). IR spectrum (KBr, ν, cm^{-1}): 3245 (NH), 1665, 1638 ($C=O_{anth}$), 1592 (Ar).

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 7.57 (1H, d, J = 8.0, H-2), 7.74 (1H, d, J = 8.0, H-4), 7.95 (2H, m, H-6,7), 8.20 (1H, m), 8.26 (1H, m, H-5,8), 12.75 (1H, s, α-OH), 4.95 (2H, s, CH_2), 2.13 (3H, s, CH_3), 8.46 (2H, m, Ar), 8.84 (2H, m, Ar), 10.54 (1H, s, NH).

Mass spectrum (EI, 70 eV): 415 (5) $[M]^+$, 254 (32), 240 (100), 212 (10), 184 (5), 176 (17), 162 (11), 106 (59), 78 (29), 51 (16).

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