SEARCH FOR NEW DRUGS

SYNTHESIS AND CYTOTOXIC ACTIVITY OF 2-ACETYLCYCLOPENT-4-ENE-1,3-DIONES AND THEIR DERIVATIVES

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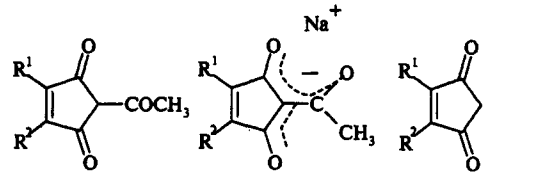
2-Acetylcyclopent-4-ene-1,3-diones (I) are known to exhibit high reactivity [1] and possess a broad spectrum of biological action [2].

In solution, triketones I represent a mixture of four enolic forms [2]. Two of these forms contain both a carbonyl-conjugated 1'-hydroxyethylidene group and the 2-ene-1,4-dione fragment, which makes them structurally similar to cyclopentane-based antitumor antibiotics such as pentenomycins [3, 4] (including sarcomycin [5] used in clinical practice), as well as clavulones, chloro(boromo, iodo)vulones, and punaglandins [6, 7]. As is known, 2-alkyl(aryl)idene derivatives of some 4,5-substituted cyclopent-4-ene-1,3-diones exhibit high antitumor activity *in vitro* with respect to ascites sarcoma 180 [8] and cell lines of some malignant human tumors [6]. In this context, it was of interest to study the cytotoxicity of triketones Ia – Ih and their sodium salts (II) and deacylated derivatives (III).



Ia – Ih IIa – IIe, IIg, IIh IIIa – IIIe, IIIg –IIIi

$R^1 = R^2 = H$ (a), Me (e), Ph (f);
$R^1 = H$, $R^2 =$ Me (b), Cl (c), Br (d), OH (i);
$R^1 =$ Me, $R^2 =$ Cl (g).

The synthesis and properties of triketones Ia – Ih were reported previously [2]. Sodium salts IIa – IIe, IIg, and IIh were obtained in quantitative yield by treating the corresponding triketones I with an equimolar amount of MeONa in MeOH. The proposed structures were confirmed by the results of IR and ^{1}H NMR spectroscopic measurements (Table 1). The fact that the bands due to carbonyl stretching vibrations above 1700 cm^{-1} (present in the spectra of triketones I and diketone III) are missing from the IR spectra of the DMSO solutions of salts II allow us to assign these salts a structure with the negative charge uniformly distributed over three oxygen atoms. Salts II appear as crystalline substances of bright-yellow color, which are well soluble in water, ethanol, DMSO, and DMF.

In the previous work [2], it was noted that removal of the COMe group (i.e., the transformation of triketones I into diketones III) leads to a sharp drop in antimicrobial activity of the resulting derivatives. In order to study the influence of this transformation on the cytotoxicity, we have synthesized the known diketones IIIa [9], IIIb [10], IIIh [11], and IIIi [12] and a series of previously undescribed compounds (IIIc – IIIe, IIIg). Most of diketones III were obtained by the reaction of diluted hydrocloric acid with triketones under the conditions described in [10]. Note that the splitting of triketones Ia, Ib, Ie, and Ig proceeded at a rather slow rate (requiring 3 – 10 h), while the same process in compounds Ic, Id, and Ih was much faster (20 – 30 min). Under these conditions, triketones Ic and Id yielded a mixture of the same products (IIIc and IIIi). Apparently, the deacylation of ketone IIId is accompanied by rapid nucleophilic substitution of for chlorine for bromine, followed by partial displacement of bromine with hydroxy groups. The target diketone IIId was obtained by acid hydrolysis of triketone Id with diluted acetic acid under the conditions described in [13]. The structure of diketones III was confirmed by IR and ^{1}H NMR spectra (Table 2), which confirmed that the samples dissolved in $CHCl_3$ occur entirely in the diketone form. Diketones III appear as colorless crystalline substances. These compounds are readily evaporated and are well soluble in DMSO, DMF, AcOH, and ethanol, but insoluble in water.

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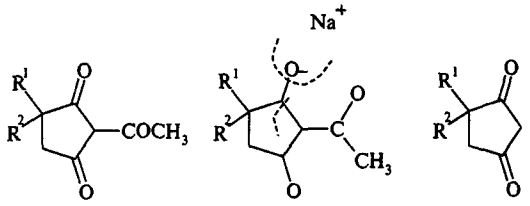

IV: $R^1 = R^2 = H$, V: $R^1, R^2 = CH_2$;
VI: $R^1 = R^2 = H$, VII: $R^1, R^2 = CH_2$;
VIII: $R^1 = R^2 = H$, IX: $R^1, R^2 = CH_2$.

For the comparative study of the cytotoxic activity of various structures, we have acylated isopropenyl acetate with succinic and itaconic anhydrides [2] to obtain triketones IV (saturated analog of compound Ia) and V (isomer of compound Ib), respectively. Using these triketones, we synthesized the corresponding sodium salts (VI, VII) and diketone VIII; we failed to obtain a diketone (IX) corresponding to triketone V, since the acid hydrolysis of compound V in all variants led to triketone IIIb formed as a result of the double bond migration into the cycle.

EXPERIMENTAL CHEMICAL PART

The IR spectra were measured on a Specord M-82 spectrophotometer (Germany). The ^1H NMR spectra were measured on a Bruker WM-250 (250 MHz) spectrometer (Germany) using TMS as the internal standard. The melting temperatures were determined using a Boethius heating stage. The data of elemental analyses of the synthesized compounds agreed with the results of analytical calculations. The purity of the reaction products was checked by TLC on Silufol UV-254 plates eluted in a hexane – acetone (2 : 1) system. Individual compounds were isolated from the reaction mixtures by preparative TLC on 20 × 20 cm plates with unfixed SiO_2 layer (5 – 40 μm thick) eluted in the same solvent system.

2-Acetylcyclopentane-1,3-dione (IV). Colorless crystals; yield, 52%; m.p., 72 – 73°C (reported, 73 – 74°C [14]); IR spectrum in $CHCl_3$ (ν_{max}, cm^{-1}): 1595, 1635, 1710, 2210 – 2730 (OH); ^1H NMR spectrum in $CDCl_3$ (δ, ppm): 2.52 (m, 2H, CH_2), 2.53 (s, 3H, COMe), 2.75 (m, 2H, CH_2), 15.67 (bs, 1H, OH).

2-Acetyl-4-methylenecyclopentane-1,3-dione (V). Light-yellow crystals; yield, 27%; m.p., 93 – 94°C; IR spectrum in $CHCl_3$ (ν_{max}, cm^{-1}): 1566, 1584, 1628, 1656, 1706, 2310 – 2710 (OH); ^1H NMR spectrum in $CDCl_3$ (δ, ppm): 2.58 (s, 3H, COMe), 3.15 (t, 2H, J 0.8 Hz, CH_2), 5.61 (t, 1H, J 0.8 Hz, $=CH_2$), 6.21 (t, 1H, J 0.8 Hz, $=CH_2$), 13.46 (bs, 1H, OH).

2-Acetyl-4-chlorocyclopent-4-ene-1,3-dione sodium salt (IIc). To a solution of 172 mg (1 mmole) of triketone Ic in 2 ml of methanol was added 0.6 ml of a 10% MeONa solution in methanol (1 mole MeONa) and the mixture was stirred for 1 h to obtain 190 mg (98%) of salt IIc (Table 1). Similar procedures were used to obtain salts IIa, IIb, IId, IIe, IIg, IIh, and compounds VI and VII.

4-Chlorocyclopent-4-ene-1,3-dione (IIIc) and 4-hydroxycyclopent-4-ene-1,3-dione sodium salt (IIIi). A solution of 172 mg (1 mmole) of triketone Ic in 16 ml of 15% aqueous hydrochloric acid was boiled for 20 min, cooled to room temperature, diluted with 40 ml of water, and extracted with ethyl acetate (3 × 10 ml). The extracts were combined, dried over Na_2SO_4, and evaporated to dryness. The residue, representing a mixture of diketones IIIc and IIIi, was sepa-

TABLE 1. Physicochemical Characteristics of Sodium Salts of Triketones IIa – IIe, IIg, IIh, VI, and VII

Compound	R^1	R^2	M.p., °C	Empirical formula	IR spectrum (DMSO): ν, cm^{-1}	^1H NMR spectrum (CD_3OD): δ, ppm (J, Hz)	
						COMe (s)	Other
IIa	H	H	>320 (decomp.)	$C_7H_5O_3Na$	1547.1580, 1622.1684*	2.24	6.26(s, 2H, H^4 and H^5)
IIb	H	Me	265 – 268	$C_8H_7O_3Na$	1545, 1560, 1619, 1681*	2.24	1.87 (d, 3H, J 1.6 C_4-Me), 5.96 (q, 1H, J 1.6, H^5)
IIc	H	Cl	215 (decomp.)	$C_7H_4ClO_3Na$	1561.1634, 1688*	2.25	6.24 (s, 1H, H^5)
IId	H	Br	213 – 217 (decomp.)	$C_7H_4BrO_3Na$	1560.1574, 1631, 1688*	2.25	6.49 (s, 1H, H^5)
IIe	Me	Me	197 – 199	$C_9H_9O_3Na$	1542.1553, 1619, 1684*	2.24	1.78 (s, 6H, C^4-Me, C^5-Me)
IIg	Me	Cl	202 – 205 (decomp.)	$C_8H_6ClO_3Na$	1560.1630, 1656, 1688*	2.25	1.86 (s, C^5-Me)
IIh	Cl	Cl	>190 (decomp.)	$C_7H_3Cl_2O_3Na$	1566.1578, 1624, 1647, 1700*	2.26	
VI	H	H	>350(decomp.)	$C_7H_7O_3Na$	1561,1608, 1675*	2.16	2.30 (bs, 2H, CH_2), 2.32 (bs, 2H, CH_2)
VII	CH_2		201 – 205	$C_8H_7O_3Na$	1560, 1611, 1624, 1663*, 1675*	2.20	2.83 (s, 2H, 2 × H^5), 5.18 (s, 1H, $=CH_2$), 5.75 (s, 1H, $=CH_2$)

* Low-intensity bands, probably, due to interrelated vibrations of ionized C–O bonds.

TABLE 2. Physicochemical Characteristics of Diketones IIa – IIe, IIg – IIi, and VIII

Compound	R^1	R^2	Yield, %	M.p., °C	Empirical formula	R_f	IR spectrum (DMSO): ν, cm^{-1}	^1H NMR spectrum ($CDCl_3$): δ, ppm (J, Hz)	
								CH_2 (s)	Other
IIIa	H	H	28	39 – 40[1]	$C_5H_4O_2$	0.41	1565, 1646, 1718, 1751	2.90	7.30 (s, 2H, H^2, H^3)
IIIb	H	Me	70	33[2]	$C_2H_6O_2$	0.44	1615, 1705, 1740	2.90	2.12 (d, 3H, J 1.6, C^2-Me), 7.01 (q, 1H, J 1.6, H^3)
IIIc	H	Cl	53	57 – 58	$C_5H_3ClO_2$	0.47	1569, 1703, 1725, 1760	3.11	7.32 (s, 1H, H^3)
IIId	H	Br	35	63 – 65	$C_5H_3BrO_2$	0.47	1563, 1723, 1757	3.10	7.53 (s, 1H, H^3)
IIIe	Me	Me	24	48	$C_7H_8O_2$	0.46	1640, 1648, 1700, 1744	2.87	2.04 (s, 6H, C^2-Me, C_3-Me)
IIIg	Me	Cl	45	60 – 61	$C_6H_5ClO_2$	0.50	1616, 1628, 1718, 1756	3.06	2.15 (s, 3H, C^2-Me)
IIIh	Cl	Cl	98	158 – 160[3] (decomp.)	$C_5H_2Cl_2O_2$	0.52	1568, 1578, 1721, 1760	3.25	–
IIIi	H	OH	24	149 – 152[4] (decomp.)	$C_5H_4O_3$	0.27	1588, 1603, 1703, 1756, 3421 (OH)	2.99	6.40 (s, 1H, H^3)
VIII	H	H	73	147 – 149[5]	$C_5H_6O_2$	0.29	1589, 1600, 1691, 3215 (OH)[6]		2.37 (m, 2H, CH_2), 2.51 (m, 2H, CH_2), 5.04 (s, 1H, H^2)[7]

Notes: [1] 36 – 37°C [9]; [2] 29 – 30°C [10]; [3] 163°C [11]; [4] 172 – 173°C [12]; [5] 151 – 153°C [13]; [6] solvent, CH_3CN; [7] solvent, CD_3OD.

rated into individual components by TLC on a SiO_2 plate. Extraction of the band with $R_f = 0.47$ yielded 69 mg (53%) of diketone IIIc; extraction of the band with $R_f = 0.27$ yielded 20 mg (18%) of diketone IIIi (Table 2). Similar procedures were used to obtain diketones IIIa, IIIb, IIIe, IIIg, and IIIh.

4-Bromocyclopent-4-ene-1,3-dione (IIId). A solution of 172 mg (1 mmole) of triketone Id in 10 ml of 0.1 M aqueous AcOH was kept for 20 min at 100°C, cooled to room temperature, diluted with 10 ml of water, and extracted with ethyl acetate (3 × 4 ml). The extracts were combined, dried over Na_2SO_4, and evaporated to dryness. The residue was separated by TLC on a SiO_2 plate. Extraction of the band with $R_f = 0.45$ yielded 61 mg (35%) of diketone IIId (Table 2). A similar procedure was used to obtain diketone VIII.

EXPERIMENTAL BIOLOGICAL PART

The cytotoxic activity of compounds I – VIII was studied on fertilized egg cells of a sea urchin (*Strongylocentrotus intermedius*) as described in [15]. Triketones Ic, Ig, and Ih, salt IIh, and diketone IIIh were also tested on Ehrlich's ascites carcinoma cells [16]. Carminomycin was used as the reference drug [17].

It was found that, among triketones I, a most pronounced effect upon sea urchin egg cells was produced by compounds Ic, Id, and especially Ih (Table 3). Both removal of the double bond in positions 4(5) of triketones I and the migration of this bond out of the cycle led to a decease in the activity (cf. data for pairs Ia, IV and Ib, V). Similar results were obtained upon the conversion of triketones I, IV, and V into the corresponding salts (II, VI, VII). At the same time, a reverse effect was observed upon going from triketones I to the corresponding diketones III, where the most active compounds were IIIc, IIIg, and IIIh (i.e., derivatives with the same substituents as in triketones): these compounds induced anomalies and produced complete inhibition of the ovicell cleavage at a concentration as small as 1.56 μg/ml.

Diketone VIII saturated in position 4(5) was, similarly to the case of diketones III versus compounds I, more active than the corresponding triketone. However, the cytotoxic ac-

TABLE 3. Cytotoxic Effect of Compounds I – VIII *in vitro*

Compound	MIC_{100} *, μg/ml	Compound	MIC_{100} *, μg/ml	Compound	MIC_{100} *, μg/ml
Ia	100.0	IIa	> 100	IIIa	3.12
IIb	50.0	IIb	> 100	IIIb	12.5
IIc	25.0	IIc	50.0	IIIc	1.56
IId	12.5	IId	25.0	IIId	1.56
Ie	100.0	IIe	> 100	IIIe	12.5
IIf	25.0	–	–	–	–
IIg	50.0	IIg	> 100	IIIg	3.12
Ih	6.25	IIh	12.5	IIIh	1.56
IV	> 100	VI	100.0	IIIi**	25.0
V	> 100	VII	> 100	VIII	50.0
Carminomycin	1.0				

* Incubation time, 2 h.

** 1,3-Diketone IIIi obtained by acid hydrolysis of triketone Ic.

TABLE 4. Antiviral Effect of Compounds Ih and IIIh *in vivo*

Compound	Number of animals tested	Dose, mg/kg	Number of drug injections	Inoculation (12th day), %	Loss, %
Ih	10	10.0	2	50	0
Ih	10	5.0	2	100	0
Ih	10	5.0	5	20	0
IIIh	10	5.0	2	100	0
IIIh	10	5.0	5	100	20
Control	10	—	—	100	0

tivity of compound VIII was markedly (by a factor of 16) lower as compared to that of the saturated analog IIIa.

Compounds Ic, Ig, Ih, IIh, and IIIh suppressed viability of the Ehrlich carcinoma cells, the corresponding MIC_{50} values being 7.2×10^{-6}, 1.35×10^{-5}, 6.0×10^{-6}, 2.4×10^{-5}, and 7.6×10^{-6} M, respectively (carminomycin, 1.35×10^{-5} M). As seen from data for the series Ih – IIh – IIIh, the passage from triketone to its salt decreases the activity to 1/4 of the initial level (i.e., the drop is more pronouced than in the test with sea urchin egg cells), while deacylation decreases the triketone activity by a factor of 1.3. The latter result is at variance with that observed for the sea urchin egg cells, where the cytotoxic effect was much more pronounced in 1,3-diketones than in the base ketones.

The above data indicate that dichlorotriketone Ih produces a stronger cytotoxic action upon the tumor cells than upon the sea urchin egg cells, whereas dichlorodiketone IIIh exhibits the inverse pattern. Since these two compounds were most active in the entire series studied, the antitumor properties of compounds Ih and IIIh were additionally tested *in vivo*.

The experiment was performed on a group of white mongrel mice with inoculated Ehrlich's ascites tumor [18]. The antitumor activity was assessed by the extent of tumor transfer determined on the 12th day after cell inoculum injection.

As seen from the data presented in Table 4, triketone Ih showed a considerable antitumor activity: after five injections of this compound at a dose of 5.0 mg/kg, the tumor growth was observed only in 20% of the inoculated animals. At the same time, diketone IIIh exhibited no antitumor action while possessing a higher acute toxicity as compared to that of triketone Ih.

The above results indicate good prospects in the search for new antitumor agents among 2-acetylcyclopent-4-ene-1,3-diones and their derivatives.

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