# SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME 2-ACETYLCYCLOPENT-4-ENE-1,3-DIONES

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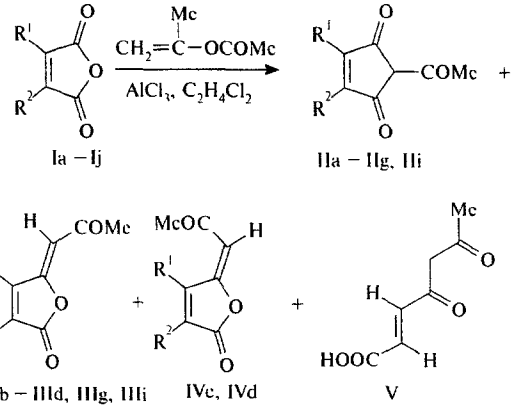
As is known [1 – 3], the acylation of α-methylvinyl acetate with maleic anhydrides (I) yields 2-acetylcyclopent-4-ene-1,3-diones (II). This series of compounds offer an interesting object for investigation because they are closely related to the relatively rare group of natural cyclopentene β,β'-triketones [1 – 3] and can be used as multipurpose reagents for the synthesis of various carbo- and heterocyclic compounds. Indeed, even the simplest representative of this series contains 10 (sic!) reactive centers, which is explained by the susceptibility to tautomerism. The presence of a conjugated double 4(5)-bond in the structure of II makes these compounds capable of participating as acceptors in Michaelis nucleophilic addition reactions involving biological molecules. The presence of a γ-hydroxy-β-en-α-one fragment (vinyl carboxy group) imparts acylating ability to these compounds with respect to biological substrates. Finally, the presence of a enolized β,β'-triketone group allows these compounds to form chelate complexes by binding the ions of microelements controlling the activity of metal-dependent enzymes. However, despite the large potential, the chemical and biological properties of triketones II are still almost unstudied.

There are two possible approaches to the synthesis of these compounds, which have been described in the literature: via acylation of α-methylvinyl acetate (MVA) with maleic anhydrides I [4] or via a catalytic rearrangement of 4-ylidenebutenolides III [5, 6]. The former approach was used [4] in the synthesis of triketones IIa – IIc and IIe, although the yields were rather low (5 – 12%). The latter pathway was more effective for obtaining triketone IIe (~90%), but poorly suited for the synthesis of IIb (~7%).

The aim of this work was to assess more thoroughly possibilities of the first approach to the synthesis of triketones II. Another purpose was to characterize these compounds with respect to antimicrobial activity and study their inhibiting effect upon $Na^+/K^+$ – ATPase.

The probability that triketones II might possess antimicrobial properties was rather large because many of the saturated cyclopentane [7, 8] and cyclohexane [9 – 11] β,β'-triketones exhibited high antimicrobial activity, related to the presence of a β,β'-triketone pharmacophore in their structures. Taking into account that the first barrier to biologically active substances penetrating into animal cells is the plasma membrane, it was also interesting to study the interaction of triketones II with $Na^+/K^+$ – ATPase, one of the most important membrane enzymes, having an active center containing a cysteine residue with a free SH group. It was expected that the interaction of triketones II with this nucleophilic functional group would lead to irreversible inhibition of the enzyme.

The reaction of maleic anhydrides (Ia – Ij) with MVA in a boiling 1,2-dichloroethane solution in the presence of $AlCl_3$ led to the formation of a mixture of products (II – V). Table I gives optimum conditions providing the best yield of the target β,β'-triketones II. Fumarylacetone V is obtained only in the case of the MVA reaction with unsubstituted maleic anhydride Ia. Use of the halogen-substituted anhydrides Ic, Id, and Ig – Ij leads to the formation of a mixture of triketones II with *Z*- and *E*-4-ylidenebutenolides III and IV. The formation of β,β'-triketones from bromine-containing anhydrides Ih and Ii was accompanied by the loss of bromine.




$R^1 = R^2 = H$ (Ia, IIa), Me (Ic, IIc, IIIc), Ph (If, IIf), Cl (Ii, IIi, IIIi), Br (Ij, IIIj);

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$R^1$ = H, $R^2$ = Me (Ib, IIb, IIIb), Cl (Ic, IIc, IIIc, IVc), Br (Id, IId, IIId, IVd); $R^1$ = Me, $R^2$ = Cl (Ig, IIg, IIIg), Br (Ih).

The presence of anhydrous $AlCl_3$ allowed the mixtures of *Z*- and *E*-4-ylidenebutenolides (IIIc + IVc, IIId + IVd) and butenolide IIIi to be rearranged with the formation of the corresponding triketones IIc (yield, 30%), IId (31%), and IIi (73%). The total yields of these products were 22, 35, and 42% (calculated relative to the corresponding initial anhydrides I). As a result, the yields of triketones IIa – IIc and IIe increased by a factor of 2 – 8 as compared to the values reported in [4]. The β,β′-triketones IId, IIf, IIg, and IIi were synthesized for the first time.

Compounds IIa – IIg and IIi appear as crystalline substances of a light-yellow color (see the physicochemical characteristics in Table 2). These compounds are readily soluble in DMSO, DMF, and AcOH, moderately soluble in EtOH and $Me_2CO$, and poorly soluble in water on heating. In solution, triketones II exhibit complete enolization and comprise a mixture of various enol forms. These compounds, being strong CH-acids (vinylogs of carboxylic acids), may readily form salts (e.g., upon dissolving in an aqueous sodium bicarbonate solution) and chelate complexes with multivalent metal ions.

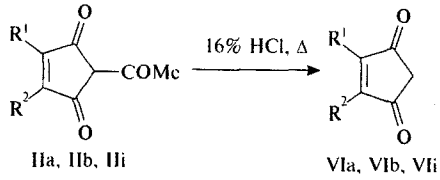
The proposed structures of triketones IIa – IIg and IIi were confirmed by the results of elemental analyses and the data of IR and $^1$H NMR spectroscopies. The IR spectra obtained in $CHCl_3$ solutions contain absorption bands due to stretching vibrations of C=O groups (1558 – 1590, 1655 – 1672, and 1714 – 1730 $cm^{-1}$), C=C bonds (1616 – 1634 $cm^{-1}$), and enol OH groups (2300 – 3350 $cm^{-1}$, diffuse band).

In order to elucidate the influence of the COMe group upon the character of activity of the target compound, we have carried out the acid hydrolysis of triketones IIa, IIb, and IIi to obtain the well-known deacylated non-enolyzed 1,3-diketones VIa (yield, 28%) [12], VIb (70%) [13], and VIi (98%) [14]. The deacylation reaction was conducted as described in [13].

**TABLE 1.** Conditions and Results of α-Methylvinyl Acetate (MVA) Acylation with Maleic Anhydrides

| Initial anhydride | Reagent ratio I : MVA : $AlCl_3$ | Reaction time, h | Reaction products (yield, %) |
|---|---|---|---|
| Ia | 1.0 : 1.5 : 3.4 | 1 | IIa (39), V (5) |
| Ib | 1.0 : 1.5 : 3.4 | 1 | IIb (73) |
| Ic | 1.0 : 1.5 : 2.0 | 2 | IIc (20), IIIc (9), IVc (6) |
| Id | 1.0 : 1.0 : 2.0 | 1 | IId (26), IIId (31), IVd (4) |
| Ie[1)] | 1.0 : 1.0 : 2.0 | 3 | IIe (18), IIIe (2) |
| If[2)] | 1.0 : 1.5 : 3.4 | 8 | IIf (3) |
| Ig[3)] | 1.0 : 1.0 : 2.0 | 3 | IIg (30), IIb (9), IIIg (8) |
| Ih[4)] | 1.0 : 1.5 : 2.0 | 1 | IIb (3), IIIb (6) |
| Ii | 1.0 : 1.0 : 2.0 | 1 | IIi (6), IIIi (58) |
| Ij | 1.0 : 1.0 : 2.0 | 2.5 | IId (28), IIId (42), IIIi (9) |

**Notes.** Degree of anhydride conversion: 1) 90%; 2) 12%; 3) 55%; 4) 45%.




IIa, IIb, IIi VIa, VIb, VIi

$R^1$ = $R^2$ = H (a), Cl (b); $R^1$ = H, $R^2$ = Me (b).

## EXPERIMENTAL CHEMICAL PART

The IR spectra were recorded on a Specord 75IR spectrophotometer. The $^1$H NMR spectra were measured with a Bruker WM-250 spectrometer operated at a working frequency of 250 MHz with TMS as the internal standard. In order to facilitate the exchange processes and simplify the pattern, the $^1$H NMR spectra of triketones were measured in $CDCl_3$ in the presence of $Et_3N$ (Table 2). The data of elemental analyses agree with the results of analytical calculations.

**TABLE 2.** Physicochemical Characteristics of Triketones IIa – IIg and IIi

| Compound | $R^1$ | $R^2$ | Yield, % | M.p., °C | Empirical formula | $^1$H NMR spectrum: δ, ppm (J, Hz) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | COMe (s) | OH (bs) | Other |
| IIa | H | H | 39 | 83 (83 – 84 [4]) | $C_7H_6O_3$ | 2.43 | 12.30 | 6.88 (d, 1H, $J_{H,H}$ 6.4, $H^4$), 6.97 (d, 1H, $J_{H,H}$ 6.4, $H^5$) |
| IIb | H | Me | 73 | 43 – 47 (45 – 50 [4]) | $C_8H_8O_3$ | 2.39 | 11.40 | 2.08 (d, 3H, $J_{CH_3,H}$ 1.6, $C^4$-Me), 6.61 (q, 1H, $J_{CH_3,H}$ 1.6, $H^5$) |
| IIc | H | Cl | 22* | 70 – 75, 81 – 84 (85 – 87 [4]) | $C_7H_5ClO_3$ | 2.45 | 12.03 | 6.91 (s, 1H, $H^5$) |
| IId | H | Br | 35* | 86 – 89 | $C_7H_5BrO_3$ | 2.47 | 12.00 | 7.15 (s, 1H, $H^5$) |
| IIe | Me | Me | 18 | 51 – 52 (51 – 52 [4]) | $C_9H_{10}O_3$ | 2.41 | 12.20 | 1.99 (s, 6H, $C^4$-Me, $C^5$-Me) |
| IIf | Ph | Ph | 3 | 128 – 130 | $C_{19}H_{14}O_3$ | 2.52 | 12.74 | 7.38 (m, 10H, $H_{arom}$) |
| IIg | Me | Cl | 30 | 44 – 46 | $C_8H_7ClO_3$ | 2.46 | 12.01 | 2.09 (s, $C^5$-Me) |
| IIi | Cl | Cl | 42* | 96 – 97 | $C_7H_4Cl_2O_3$ | 2.49 | 11.80 | – |

* Total yield of the MVA acylation and butenolide III rearrangement reactions.

The purity of the target products was checked by TLC on Silufol UV-254 plates eluted in benzene – acetone (10 : 1 to 2 : 1) systems. Column chromatography was performed using a hexane – acetone (H / A) solvent mixture of various compositions.

**α-Methylvinyl acetate (MVA) acylation with brominated maleic anhydride (Id).** To a suspension of 15.6 g (88 mmole) anhydride Id and 23.5 g (176 mmole) anhydrous $AlCl_3$ in 200 ml of absolute 1,2-dichloroethane in a flask cooled with ice-cold water was added dropwise over 20 min with intensive stirring 8.8 g (88 mmole) of freshly distilled MVA. Then the reaction mixture was stirred for 1 h at the boiling temperature of the solvent, cooled to room temperature, and poured into a mixture of 200 ml 15% hydrochloric acid with 100 g crushed ice. The organic layer was washed with a 5% hydrochloric acid solution (3 × 80 ml). The aqueous phase was extracted with chloroform. The organic phases ($CHCl_3$ and $C_2H_4Cl_2$) were combined, washed with water until a neutral pH reaction of the wash liquid, and dried over anhydrous $Na_2SO_4$. Finally, the solvent was removed and the residue chromatographed on a $SiO_2$ column. Elution with a 30 : 1 H / A mixture yielded 3.9 g (26%) 2-acetyl-4-bromocyclopent-4-ene-1,3-dione (IId) in the form of light-yellow crystals; m.p., 86 – 89°C (hexane). The 20 : 1 H / A mixture eluted 4.7 g (31%) *Z*-4-acetylmethylene-2-bromo-2-buten-1-olide (IIId); $C_7H_5BrO_3$; m.p., 107 – 109°C (hexane); IR spectrum ($\nu_{max}$, $cm^{-1}$): 1802 (C=O), 1698 (C=O), 1670 (C=O), 1645 (C=C), 1624 (C=C), 1569 (C=C); $^1H$ NMR spectrum (δ, ppm): 2.56 (s, 3H, COMe), 5.62 (s, 1H, $H^5$), 7.61 (s, 1H, $H^3$). The 4 : 1 H / A mixture eluted 0.6 g (4%) *E*-4-acetylmethylene-2-bromo-2-buten-1-olide (IVd); $C_7H_5BrO_3$; m.p., 105 – 107°C (hexane – ethanol, 2 : 1); IR spectrum ($\nu_{max}$, $cm^{-1}$): 1796 (C=O), 1696 (C=O), 1620 (C=C), 1564 (C=C); $^1H$ NMR spectrum (δ, ppm): 2.36 (s, 3H, COMe), 6.26 (s, 1H, $H^5$), 8.43 (s, 1H, $H^3$).

Analogous procedures under the conditions indicated in Table 1 yielded the known triketones IIa – IIc and IIe and the previously unreported 2-acetyl-4,5-diphenylcyclopent-4-ene-1,3-dione (IIf), 2-acetyl-4-methyl-5-chlorocyclopent-4-ene-1,3-dione (IIg), and 2-acetyl-4,5-dichlorocyclopent-4-ene-1,3-dione (IIi).

**Rearrangement of a mixture of *Z*- and *E*-4-ylidenebutenolides (IIId + IVd) into β,β′-triketone (IId).** A solution of the mixture of butenolides IIId + IVd (5.3 g, 23.85 mmole) and 6.5 g (47.7 mmole) anhydrous $AlCl_3$ in 200 ml of absolute 1,2-dichloroethane was stirred for 2.5 h at 80 – 90°C, cooled to room temperature, and poured into a mixture of 150 ml 15% hydrochloric acid with 10 g crushed ice. The organic layer was separated and the aqueous phase was extracted with chloroform (5 × 30 ml). The organic phases were combined and treated as described above. Chromatography on a $SiO_2$ column eluted with a 30 : 1 H / A mixture yielded 1.64 g (31%) of bromotriketone IId identical with that obtained as described above.

## EXPERIMENTAL BIOLOGICAL PART

The acute toxicity ($LD_{50}$) of the synthesized compounds was determined by a conventional method [15] on white mongrel mice weighing 20 – 22 g. The compounds were intraperitoneally injected with a mixture of physiological solution and ethanol (100 : 1, v / v).

The antimicrobial activity of the synthesized compounds with respect to the standard yeasts *Saccharomyces carlsbergensis* race 11, thread fungi *Trichophyton mentagraphytes* (dermatomycetes), *Staphylococcus aureus* P-209, and *Escherichia coli* K-13 was studied by a conventional method of double serial dilutions in liquid nutrient media [16] of the compositions reported previously [17]. The incubation mixture contained 0.99 ml of the test microbial cell suspension

**TABLE 3.** Acute Toxicity and Antimicrobial Activity of Compounds IIa – IIg, IIi, VIa, VIb, and VIi

| Compound | $LD_{50}$, mg / kg | MIC, μg / ml | | | | $Na^+/K^+$- ATPase inhibition (%), for concentratrion (M) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *S. carlsb.* | *T. mentagr.* | *S. aureus* | *E. coli* | $5 \times 10^{-4}$ | $1 \times 10^{-4}$ | $5 \times 10^{-5}$ | $2.5 \times 10^{-5}$ | $5 \times 10^{-6}$ | $1.5 \times 10^{-6}$ |
| IIa | 110 | 12.5 | 50.0 | 12.5 | > 100 | – | – | – | – | – | – |
| IIb | 190 | > 100 | 50.0 | 50.0 | > 100 | – | – | – | – | – | – |
| IIc | 60 | 100.0 | 6.25 | 6.25 | 12.5 | 100 ± 0 | 95 ± 0 | 75 ± 3 | 50 ± 3 | 35 ± 3 | 15 ± 3 |
| IId | 65 | > 100 | 6.25 | 6.25 | 12.5 | 100 ± 0 | 100 ± 0 | 90 ± 1 | 80 ± 2 | 65 ± 2 | 25 ± 2 |
| IIe | 210 | > 100 | 50.0 | > 100 | > 100 | 10 ± 1 | – | – | – | – | – |
| IIf | 250 | > 100 | 6.25 | 3.12 | > 100 | 50 ± 3 | – | – | – | – | – |
| IIg | 90 | > 100 | 50.0 | 100.0 | > 100 | – | – | – | – | – | – |
| IIi | 25 | 100.0 | 12.5 | 1.56 | 50.0 | 30 ± 2 | – | – | – | – | – |
| VIa | 120 | 50.0 | 100.0 | 50.0 | > 100 | – | – | – | – | – | – |
| VIb | 280 | > 100 | > 100 | > 100 | > 100 | – | – | – | – | – | – |
| VIi | 50 | 100.0 | 1.56 | 100.0 | > 100 | 10 ± 1 | – | – | – | – | – |
| Tetracycline | – | > 100 | > 100 | 0.78 | 3.12 | | | | | , | |
| Griseofulvin | – | > 100 | 0.78 | > 100 | > 100 | | | | | , | |
| Ouabain | | | | | | 100 ± 0 | 100 ± 0 | 100 ± 0 | 90 ± 1 | 70 ± 2 | 50 ± 2 |

(microbial load, $1 \times 10^6$ units) and 0.01 ml of an ethanol solution of the triketone studied.

The antimicrobial activity with respect to yeasts and bacteria was evaluated from the results of turbidimetric measurements performed after 24 and 48 h incubation. The action upon the dermatomycete cultures was also monitored visually by detecting the presence or absence of colonies in the medium after 7-day incubation. The activity was expressed as the minimum inhibiting concentration (MIC, μg / ml) representing the maximum dilution at which the compound completely suppressed the growth of test microbes. A threshold concentration corresponded to a dose of 100.0 μg / ml. The reference drugs were the well-known antibiotics tetracycline and griseofulvin.

The enzyme-inhibiting effect of triketones was studied with respect to $Na^+/K^+$-ATPase isolated from the large-hemisphere cerebral cortex of rats. The enzyme preparations were isolated using the method of Klodos et al. [18], and the $Na^+/K^+$-ATPase activity was determined as described in [19]. The reference compound was the cardiac glycoside ouabain, known to be a specific $Na^+/K^+$-ATPase inhibitor.

The experimental data presented in Table 3 indicate that most of the triketones studied exhibit no activity with respect to yeasts (except for compound IIa) and *E. coli* (except for IIc and IId). At the same time, the compounds tested act upon dermatomycetes and Gram-positive bacteria. Deacylation of the cyclopentene β,β′-triketones markedly decreased their antimicrobial activity. The only exception was observed upon the conversion of triketone IIi to 1,3-diketone IVi, whose activity toward dermatomycetes was comparable with that of griseofulvin.

The inhibiting action of triketones IIc and IId with respect to $Na^+/K^+$-ATPase differs from the effect of other triketones studied. This is apparently related to a different mechanism of the action of these compounds (IIc, IId) containing β-halide vinyl ketone groups (i.e., fragments of a vinylog halogen anhydride), which probably interact with the SH group of the substrate by the mechanism of nucleophilic substitution (impossible for the other triketones).

Thus, the results of our investigation show that 2-acetylcyclopent-4-ene-1,3-diones may be of interest as antimicrobial agents with a broad spectrum of antimicrobial activity. Some of these compounds (IIc and IId) are also effective as $Na^+/K^+$-ATPase inhibitors.

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