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Mendeleev Commun., 2019, 29, 372–374

Mendeleev Communications

Simple antitumor model compounds for cross-conjugated cyclopentenone prostaglandins

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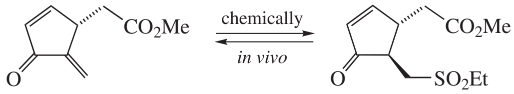
DOI: 10.1016/j.mencom.2019.07.003

Unstable methyl (5-methylidene-4-oxocyclopent-2-en-1-yl)-acetate, having a ring moiety of J-type prostaglandins (PGJs) and similar cytotoxicity, reacts with EtSH and other thiols with formation of mono- and bis-adducts, which have been further converted by oxidation with mCPBA into corresponding stable mono-sulfones possessing cytotoxic effect most probably due to *in vivo* regeneration of the starting dienone. Thus, the derived sulfones can be used as transport forms for original dienone as a pharmacologically important moiety of Δ^{12}-PGJ_2 and similar prostaglandins.

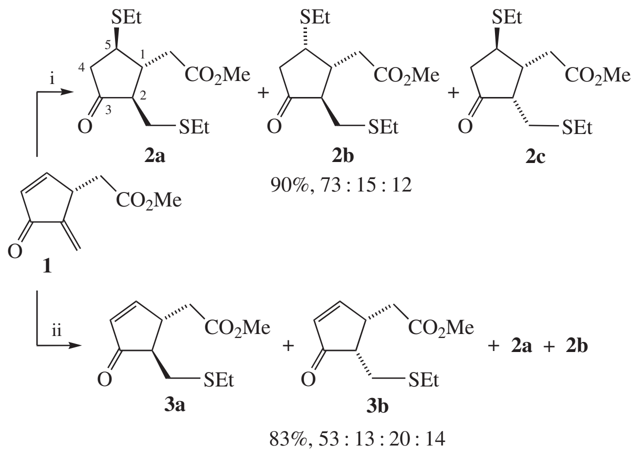


Cyclopentenone prostaglandins (CyPGs)[1–3] attract attention due to their cytotoxicity as well as potential antiviral and anti-inflammatory effects.[4–9] Their cross-conjugated di- and triene systems are mainly responsible for the bioactivity profile. CyPGs readily penetrate into cells and are accumulated in the nucleus where they undergo covalent Michael binding, through the more reactive endocyclic double bond, with the cysteine sulfhydryl groups of nucleus proteins.[10] This alkylation of cysteine residues leads to the loss of protein biochemical functions. Since α,β-unsaturated carbonyl groups in CyPGs represent soft electrophiles, they poorly alkylate the weak nucleophilic centers on the surface of non-protein biomolecules, such as DNA.[11] In the series of CyPGs, it is worth to mention Δ^{12}-PGJ_3 with remarkable antileukemic properties,[12,13] 1,15-lactone of Δ^{12}-PGJ_3[14] with promising cytotoxicity as well as 15-deoxy-$\Delta^{12,14}$-PGJ_2, which inhibits PPARγ nucleus receptors participating in cell division, oncogenesis, virus replication and other processes.[15] 15-Deoxy-$\Delta^{12,14}$-PGJ_2 also participates in the modulation of inflammatory processes[2] and is considered as an ‘anti-inflammatory prostaglandin’.[16]

In a search for simple, stable and readily available CyPG analogues with retained biological activity, we focused on the cross-conjugated cyclopentenone **1** obtained[17] from (±)-Corey lactone diol.[18]

The ring part of cyclopentenone **1** is functionally similar to those of natural cross-conjugated cyclopentenones Δ^{12}-PGJ_3 and $\Delta^{12,14}$-PGJ_2. Taking into account that their bioactivity originates from the covalent Michael binding to SH and NH_2 groups of nucleic acids and proteins, we studied the reactions of compound **1** with various thiols.

Reaction of dienone **1** with EtSH (5 equiv.) and Et_3N (10 equiv.) resulted in the formation of a mixture of bis-adducts **2a–c** in a high yield and 5 : 1 : 0.8 ratio, calculated from the intensity of OMe signals in the [1]H NMR spectrum (Scheme 1).[†] According to the spectra of individual compounds isolated by chromatography, they are stereoisomers formed by addition of two EtSH molecules to one molecule of dienone **1**. It is known that the configuration assignment by spectral and computational means for substituted cyclopentanes is typically complicated by the conformation uncertainty of the five-membered ring and the existence of several conformations close to each other. Therefore, to make the assignment easier, we divided the process of formation of bis-



Scheme 1 *Reagents and conditions*: i, EtSH (5 equiv.), Et_3N, CH_2Cl_2, room temperature; ii, EtSH (1.1 equiv.), Et_3N, THF, room temperature.

[†] All compounds in this work are racemic, but only single enantiomers with absolute configurations corresponding to those of natural prostaglandins are depicted in Schemes.

adducts **2** into steps and carried out the reaction of dienone **1** with only 1.1 equiv. of EtSH. The products of this step were stereoisomeric mono-adducts **3a** and **3b** in 4 : 1 ratio as well as just two bis-adduct by-products **2a** and **2b**. Contrary to the cross-conjugated natural CyPGs like Δ^7-PGA_1[10] and $\Delta^{12,14}$-PGJ_2, where it is the endocyclic double bond that reacts preferably with thiols, in case of compound **1**, the exocyclic unsubstituted methylene double bond is more reactive as a Michael acceptor due to its spatial accessibility. Thus, the formation of bis-adducts **2** was found to proceed through the intermediate mono-adducts **3**.

Both isomeric bis-adducts **2a** and **2b**, formed at *ca*. 30% conversion of the intermediate mono-adducts **3**, were derived from a single mono-adduct, which was afterwards shown to be the major intermediate **3a**. An alternative supposition, namely the formation of one bis-adduct **2a** or **2b** from the minor intermediate **3b**, would imply the predominant stereoselective formation of the corresponding single stereoisomer of **2a** or **2b** from each mono-adduct **3a** or **3b**, which is impossible because of the absence of stereoselectivity in this reaction, as confirmed by formation of three stereoisomeric bis-adducts **2a**, **2b** and **2c** upon total conversion of mono-adducts **3**. The forth possible stereoisomer of compound **2** has not been identified due to its small amount. The predominance of bis-adduct **2a** (73% of the total **2a–c**) proves its formation, as well as **2b**, from the major mono-adduct **3a**.

For the major mono-adduct **3a**, the *trans* configuration was established by NOE effect in the corresponding sulfone **5** obtained after its oxidation (Figure 1), namely the interactions between C^1–CH_2 and C^5–H as well as their absence between C^1–H and C^5–H. This result also implies the *cis* configuration for the minor mono-adduct **3b** as well as *trans* configuration for bis-adducts **2a**,**b** and *cis* configuration for bis-adduct **2c**.

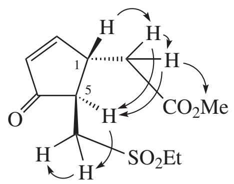
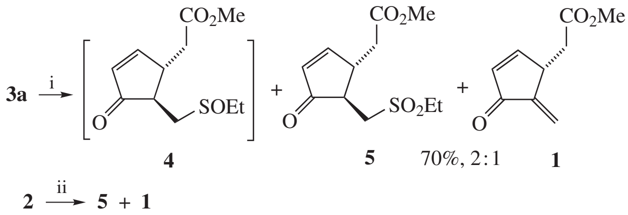


Figure 1 NOE correlations in sulfone **5**.

The assignment of the 1,5 configuration for compounds **2a–c** was made on the basis of ^{1}H NMR spectra. Using a simulation program, the chemical shifts and coupling constants of all protons of these stereoisomers were reliably established. For compound **2a** with *trans* configuration, the values of coupling constants $J_{1,2}$ and $J_{1,5}$ between the protons of the cycle are 10.9 and 10.3 Hz, respectively, *i.e.* almost equal. This means that the 1,2 and 1,5 configurations are similar and stereoisomer **2a** has a *trans–trans* configuration.

For compound **2b**, the value of $J_{1,2}$ equal to 11.1 Hz coincides with those for stereoisomer **2a**, but $J_{1,5}$ is much smaller, namely 5.8 Hz, which confirms *trans–cis* configuration for compound **2b**. As for stereoisomer **2c**, its coupling constants $J_{1,2}$ 8.0 Hz and $J_{1,5}$ 3.6 Hz are somewhat similar to those for compound **2b**, in spite of definitely opposite configuration. The probable reason for these coupling constant values appears to be the difference in conformations of the cyclopentane ring, which compensate the distinction between stereoisomers in NMR data. The *cis–trans* configuration is accepted for stereoisomer **2c** as the main bis-adduct formed from the *cis* mono-adduct **3b**, due to the preponderance of trans products **2a** and **3a** in the thia-Michael addition.

Adducts **2** and **3** are of interest as stabilized derivatives of reactive dienone **1**, which are convenient for storage and further application. As an example, these compounds could be used in biomedical studies after their conversion into the corresponding sulfoxides and sulfones, which should ensure their penetration into cells and fast *in vivo* delivery to targets. In the areas of inflammation or proliferation, the corresponding sulfoxides and sulfones are expected to undergo the reverse Michael reaction with regeneration of dienone **1**. To check this assumption, we investigated the oxidation of bis-adduct **2a** with 3 equiv. of mCPBA at –30 °C. The ^{1}H NMR analysis of the crude reaction mixture revealed formation of sulfoxide **4**, sulfone **5** and dienone **1** in 0.7 : 1 : 1 ratio. Compounds **5** and **1** were isolated from this mixture in total yield of ~70%. Sulfoxide **4** appeared to be unstable and underwent a conversion into dienone **1** during isolation. Under similar conditions, oxidation of mono-adduct **3a** with mCPBA resulted in sulfone **5** (Scheme 2) and dienone **1**.

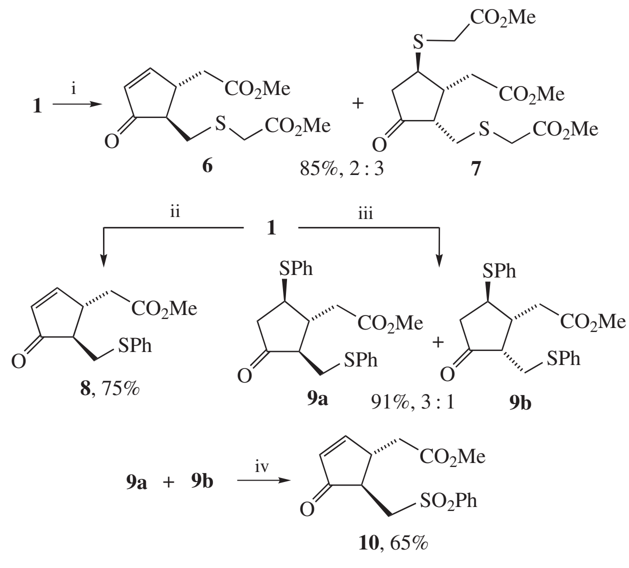


Scheme 2 *Reagents and conditions*: i, mCPBA, CH_2Cl_2, –30 °C, 2 h; ii, mCPBA, CH_2Cl_2, –30 °C → room temperature.

To expand the scope of compounds bearing SH groups, we performed reactions of dienone **1** with thiophenol and methyl thioacetate in CH_2Cl_2 using 3 equiv. of both mercaptan and NEt_3. The additions proceeded slowly and required ~12 h. The reaction with methyl thioacetate led to mono-adduct **6** and bis-adduct **7** in ~2 : 3 ratio. Under the same conditions, thiophenol reacted with dienone **1** affording only mono-adduct **8**. The use of 20 equiv. of both thiophenol and NEt_3 in THF under UV irradiation resulted in a good yield of bis-adducts **9a** and **9b** in ~3 : 1 ratio as determined by ^{1}H NMR.

We failed to separate isomers **9a** and **9b** on silica gel, hence we used their mixture in the next step of oxidation by mCPBA. Successive oxidation and elimination reactions, as detected by TLC, afforded sulfone **10** as the final product (Scheme 3).

The cytotoxic properties of the representative compounds were evaluated using three human cancer cell lines and condi-



Scheme 3 *Reagents and conditions*: i, $HSCH_2CO_2Me$ (3 equiv.), CH_2Cl_2, Et_3N, 20 °C; ii, PhSH (3 equiv.), Et_3N, CH_2Cl_2; iii, PhSH (20 equiv.), Et_3N, THF, UV irradiation; iv, mCPBA, CH_2Cl_2, 20 °C.

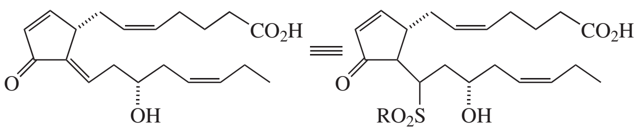
Table 1 *In vitro* cytotoxic activity of compounds **1**, **2a**, **3**, **5**, **6** and **8**.

Compound	IC_{50}/μM			
	HEK293	A549	MCF-7	SH-SY5Y
1	6.17±1.63	58.77±3.32 (p = 0.00003)	2.95±0.93	2.44±0.20
2a	31.90±8.34	>100 (p = 0.000005)	>100 (p = 0.000005)	5.58±1.97 (p = 0.00003)
3	46.23±16.22	>100	35.25±1.29	17.49±1.00 (p = 0.007)
5	7.07±2.38	43.90±5.54 (p = 0.00008)	6.21±1.13	2.10±0.45
6	7.60±1.95	78.87±10.97 (p = 0.000005)	48.98±4.02 (p = 0.00001)	2.57±0.88
8	3.55±1.0	62.31±4.58 (p = 0.000005)	10.97±1.82 (p = 0.05)	2.76±0.34

tionally normal HEK293 cells.[‡] It has been demonstrated (Table 1) that all tested compounds, except for mono-adduct **3**, exhibit higher activity for both neuroblastoma SH-SY5Y cells and embryonic kidney HEK293 cells compared with lung carcinoma A549 cells. Compounds **1**, **5** and **8** reveal cytotoxic potential against breast adenocarcinoma MCF-7 cells as well. In general, the most active are dienone **1**, sulfone **5** and mono-adduct **8**, whereas adducts **2a** and especially **3** are less cytotoxic. The less activity of adduct **2a** compared with sulfone **5** can be explained by hindering of formation of the cross-conjugated cyclopentenone system responsible for cytotoxicity, because SEt is known as a poor leaving group.

In general, the antitumor activity of cyclopentenones is lower than that of compounds with exomethylidene cyclopentenone topology.[10,19,20] It can be assumed that under the conditions of the cytotoxicity assay sulfone **5** is converted to more active dienone **1** (*cf.* data for compounds **1** and **3** in Table 1). These results can be used in the search for prodrugs or stabilized forms of labile cross-conjugated CyPGs by replacing the Δ^{12} double bond with ethanesulfonyl group, for example using the structure **11** as a bioisostere of Δ^{12}-PGJ_3 (Scheme 4).

In summary, in this work new mono- and bis-adducts of dienone **1** with EtSH, PhSH and $HSCH_2CO_2Me$ have been described. Sulfoxides obtained from the thioadducts are unstable, while the corresponding sulfones are sufficiently stable and suitable for subsequent investigation of biological properties. Oxidation of thia-Michael bis-adduct of EtSH and compound **1** with excess of mCPBA results in decomposition of the adduct resulting in dienone **1** and sulfone **5**. The suggestion to use sulfoxides and sulfones as chemically stabilized forms and vehicles for the delivery of dienones like compound **1** to biological targets has been confirmed with sulfone **5** as an example. The cytotoxicity of sulfone **5** is comparable to that of dienone **1**.



Δ^{12}-PGJ_3 **11**

Scheme 4

[‡] The compounds were dissolved in DMSO as 100 mM stock solutions, diluted in complete DMEM and then added to the assay plates. After 24 h of culturing, the cells were treated with compounds solutions at final concentrations of 1, 10 and 100 μM for 48 h. The final concentration of DMSO in samples and controls was 0.1%. Data are presented as means ± SEM from three independent experiments performed in triplicate. The p-values indicate comparison of results for A549, MCF-72 and SH-SY5Y cells with those for HEK293 cells using one-way ANOVA and Dunnett's post-hoc test.

This work was carried out within the flamework of the state task program no. AAAA-A17-117011910032-4 and supported by the Russian Foundation for Basic Research (grant no. 18-53-00011).

The spectral and analytical studies were performed using the equipment of the 'Khimiya' Joint Center, Ufa Institute of Chemistry, Ufa Federal Reserch Center, Russian Academy of Sciences.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2019.07.003.

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Received: 24th December 2018; Com. 18/5787