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Rapid report

Inhibition of photosystem II electron transport by acyl derivatives of 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid)

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Abstract

In the course of the synthesis of γ-pyrones, well-known inhibitors of photosystem II electron transport, it turned out that the starting material, acyl derivatives of 2,2-dimethyl-1,3-dioxane-5,6-dione (Meldrum's acid) are potent inhibitors of photosystem II electron transport. Thus, in a simple one-step synthesis from commercial available substances, highly potent photosystem II inhibitors are generated. The biological activity of the acyl derivatives is in a parabolic fashion dependent from the length of the alkyl side chain.

Keywords: 2,2-dimethyl-1,3-dioxane-4,6-dione; Pyrandione; γ-Pyrone; Photosystem II; Inhibitors

Electron transport at the reducing site of photosystem II, i.e., the Q_B-site, can be interrupted by a variety of substances, some of which are used as commercial herbicides. The herbicides have a higher affinity to the Q_B-site than the native plastoquinone itself. Consequently, they displace plastoquinone from its binding site. A wide variety of substances, however, are known which are excellent inhibitors of photosystem II in vitro, but bear not at all any in vivo activity. In fact, most of the compounds belong to this type. Of simple six-membered ring systems, 1,4-benzoquinones [1,2], γ-pyrones [3,4] and γ-pyridones [4] have been recognized as efficient photosystem II inhibitors. In order to study the structure–activity relationship of γ-pyrones as inhibitors of photosystem II electron transport, we have synthesized a series of γ-pyrones. The synthesis of γ-pyrones starts with 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) which is an adduct of acetone and malonic acid. In the course of the synthesis of the γ-pyrones, the commercially available Meldrum's acid is acylated by means of acid chlorides [5]. By testing the inhibitory activity of the acyl derivatives of 2,2-dimethyl-1,3-dioxane-4,6 diones on photosystem II electron transport, we found that they are efficient inhibitors with pI_{50}-values of >6. In their inhibitory activity, they are comparable to commercial herbicides. Thus, in a simple one-step synthesis, an effective photosystem II inhibitor is generated, which is even more active than the from the synthesis resulting γ-pyrones.

2,2-Dimethyl-1,3-dioxane-4,6-dione was acylated with an acid chloride in the presence of pyridine to yield the acyl derivative 1 (Fig. 1). The acyl derivative 1 was refluxed in dry benzene with *n*-butyl-vinyl ether for 2 h to form the pyrandione 2 (Fig. 1). The solvent was removed under reduced pressure and the residue was taken up in a mixture of tetrahydrofuran/water (4:1, by volume). *p*-Toluolsulfonic acid was added and the mixture refluxed for 16–20 h. The volume was reduced to 1/3 and the residue taken up in CH_2Cl_2. The mixture was extracted with distilled H_2O and saturated aqueous NaCl, and then the combined aqueous layers were extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude γ-pyrone 3 was purified by flash chromatography [5].

Chloroplasts from spinach leaves were prepared according to [6]. Uncoupled photosystem II electron flow from water to 2,6-dichlorophenol-indophenol (DCIP) in the presence of 2′,4,4′-trinitro-2′-iodo-3′-methyl-6′-isopropyl-dipenylether (DNP-INT, [7]) was measured as described recently [8]. The inhibition constants of the inhibitors (I_{50}-values, i.e., the concentration which inhibits electron transport by 50%) were determined

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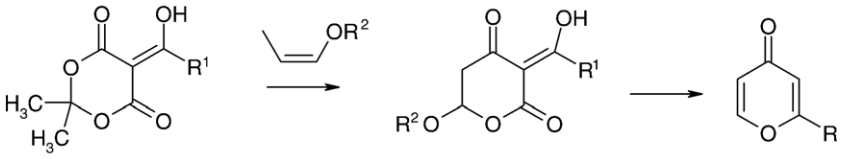

Fig. 1. Synthesis of γ-pyrones 3 from acyl derivatives of Meldrum's acid 1 via pyrandiones 2.

graphically. The correlation coefficient r of the linear regression needed for graphical determination of the I_{50}-value was >0.9. Each value was determined at least twice, in most cases three times, and the average value was taken. The pI_{50}-value is the negative decadic logarithm of the I_{50}-value.

The length of the alkyl side chain was determined by measuring the distance using the molecular modelling software Hyper Chem (MM+Kraftfeld). Multiple linear regression was performed using Microsoft Excel.

Table 1 lists the pI_{50}-values in photosystem II electron transport for the acyl derivatives of Meldrum's acid, pyranediones and γ-pyrones. As reported in the literature [3,4], the γ-pyrones are indeed inhibitors of photosystem II electron transport. The best inhibitor is the *n*-dodecyl derivative, which exhibits a pI_{50}-value of 5.08 (Table 1). Fig. 2 shows that the pI_{50}-value depends on the chain length of the alkyl side group in a parabolic fashion. The maximum is reached at 12 carbon atoms which corresponds to about 16 Å. The parabola is described by Eq. (1):

$$pI_{50} = -0.055L^2 + 1.647L - 7.392 \quad (1)$$

where the number of compounds $n=6$ and the correlation coefficient $r=0.96$.

The pyrandiones 2 are only weak inhibitors with moderate activity (Table 1). Surprisingly, the starting material for the synthesis of pyrandiones and γ-pyrones, the acyl derivatives of Meldrum's acid, were excellent inhibitors (Table 1). Thus, in a simple one-step synthesis from commercial available substances, efficient photosystem II inhibitors with high pI_{50}-values are created. Like the γ-pyrones, the acyl derivatives of Meldrum's acid reach their maximal inhibitory activity at a length of the alkyl side chain of 12 C atoms (16 Å) with a pI_{50}-value of 6.48.

Table 1
pI_{50}-values of Meldrum's acid derivatives 1, Pyrandiones 2, and γ-Pyrones 3

No.	r	Length (Å)	pI_{50}-value acyl derivative of Meldrum's acid	pI_{50}-value Pyrandione	pI_{50}-value γ-Pyrone
1	*n*-C_7H_{15}	8.55	4.50	4.41	<4
2	*n*-C_8H_{17}	9.80	4.70	4.47	<4
3	*n*-C_9H_{19}	11.10	5.19	<4	4.33
4	*n*-$C_{10}H_{21}$	12.36	6.13	4.33	4.37
5	*n*-$C_{11}H_{23}$	13.66	6.26	4.83	4.72
6	*n*-$C_{12}H_{25}$	16.22	6.48	4.42	5.08
7	*n*-$C_{13}H_{27}$	17.48	6.00	<4	4.82
8	*n*-$C_{15}H_{31}$	18.76	5.92	4.00	4.03
9	*n*-$C_{17}H_{35}$	21.32	4.82	<4	<4

The parabolic dependence of the pI_{50}-value from the length of the alkyl side chain can be described by Eq. (2):

$$pI_{50} = -0.055L^2 + 1.647L - 7.392 \quad (2)$$

where $n=9$ and $r=0.85$.

A similar maximal activity at a chain length of about 15 Å has been observed for the inhibition of photosystem II electron transport by alkyl-4-quinolones and their *N*-Oxides [9] and for the inhibition of electron transport through the cytochrome b_6/f-complex as well [9]. Similarly, the same compounds at a maximum chain length of 15 Å also inhibit mitochondrial electron transport through complexes I and III [10]. For alkyl acridones in mitochondrial systems the biological activity peaks at around 10 Å [11,12]. If one takes into account that the acridones as compared to the quinolones bear an additional aromatic moiety, the overall distance from the imino group to the end of the carbon chain is about the same for both types of compounds. This further corroborates the notion that the hydrophobic pocket of the quinone/inhibitor binding protein can only accommodate an alkyl side chain up to an optimal length.

All commercial photosystem II herbicides contain nitrogen (see [13]), whereas acyl derivatives of Meldrum's acid and γ-pyrones do not. A common structural element –N–(C═X), where X═N or O has been recognized in photosystem II herbicides which is necessary for biological activity [14]. Acyl derivatives of Meldrum's acid and γ-pyrones because of the lack of nitrogen do not contain this structural element. They have to be considered as a new class of photosystem II

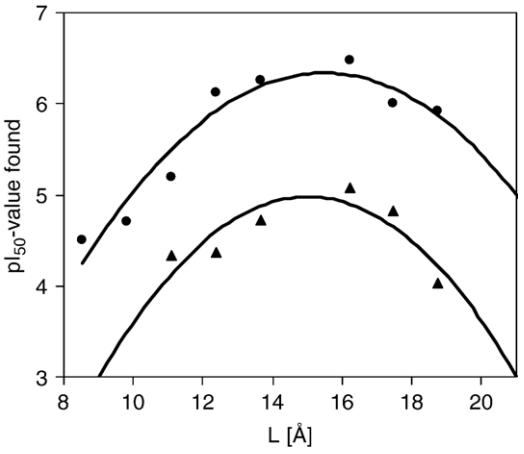

Fig. 2. Dependence of the pI_{50}-value of γ-pyrones (▲—▲) and acyl derivatives of Meldrum's acid (■—■) from the length of the alkyl side chain.

inhibitors which belong neither to the class of Ser_{264} nor the His_{215} family [15].

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