The Oxidized Products of 8-Methoxypsoralen (8-MOP) with H_2O_2–NaOCl

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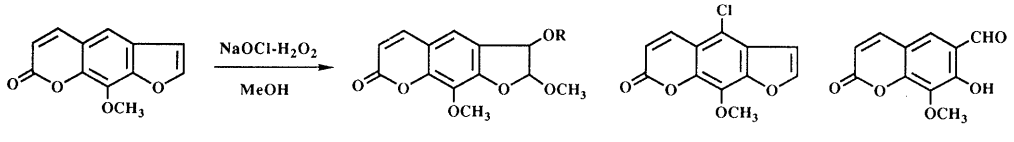
In order to obtain adducts of 8-methoxypsoralen (8-MOP, 1) and singlet oxygen ($^1O_2$), the oxidation of 1 with chemically generated singlet oxygen in H_2O_2–NaOCl was undertaken. Bioassay-directed fractionation of the crude oxidized products has led to the isolation and characterization of a novel derivative of 1, 2,3-dihydro-2,9-dimethoxy-3-hydroxy-7*H*-furo[3,2-*g*][1]benzopyran-7-one (2) as a substance inhibiting chemotactic activity of polymorphonuclear neutrophils toward anaphylatoxin $C5_a$ des Arg. The structure of 2 was determined from the spectroscopic data and by correlation with its acetate (2a). Furthermore, the oxidation of 1 in H_2O_2–NaOCl afforded 5-chloro-9-methoxy-7*H*-furo[3,2-*g*][1]benzopyran-7-one (3) and 6-formyl-7-hydroxy-8-methoxy-2*H*-1-benzopyran-2-one (4) along with 1, but they exhibited no such activity.

Keywords furocoumarin; 8-methoxypsoralen; singlet oxygen ($^1O_2$); photoadduct; chemotactic activity of polymorphonuclear neutrophil; anaphylatoxin $C5_a$ des Arg; 2,3-dihydro-2,9-dimethoxy-3-hydroxy-7*H*-furo[3,2-*g*][1]benzopyran-7-one

Linear furocoumarins, commonly named psoralens, are naturally occurring compounds which exhibit various biological activities associated with their sensitivity to light, and they have proved useful as drugs for the treatment of skin disease.[1] Their recent use in PUVA (psoralen + UVA-light) therapy for the treatment of psoriasis has generated much interest in their biological properties.[2] Previously, Mizuno *et al.* reported that the photoadduct of 8-methoxypsoralen (8-MOP, **1**) inhibited psoriasis leukotactic factor (PLF) activity,[3] a finding which was presumably related to the biological activity of **1**. Although the photoadduct of **1** with singlet oxygen may be involved in the biological activity,[4] the photoreaction of **1** with singlet oxygen has been little investigated. So far, Wasserman's and Logani's groups have isolated only 6-formyl-7-hydroxy-8-methoxy-2*H*-1-benzopyran-2-one (**4**) as the photoadduct of **1** with singlet oxygen.[5] They have not characterized other photoadducts because of low yields and have not examined the biological activity of **4**. Thus, we investigated the reaction of **1** and singlet oxygen chemically generated by H_2O_2–NaOCl,[6] and isolated a novel 8-MOP derivative (**2**) inhibiting chemotactic activity of polymorphonuclear neutrophilis (PMN) toward anaphylatoxin $C5_a$, by means of bioassay-directed fractionation of oxidized products of 8-MOP with H_2O_2–NaOCl, along with known derivatives (**3** and **4**). In addition, 6-formyl-7-hydroxy-8-methoxy-2*H*-1-benzopyran-2-one (**4**) was also prepared by the ozonolysis of **1** to examine its pharmacological activity. In this paper, we describe the structural elucidation of the 8-MOP (**1**) oxidized product, which inhibits chemotactic activity of PMN toward anaphylatoxin $C5_a$.

First of all, we examined the reactions of **1** with chemically generated singlet oxygen under various conditions such as decomposition of triphenylphosphite ozonide[7] and 9,10-diphenylanthracene peroxide,[8] and H_2O_2–NaOCl treatment.[6] 8-MOP (**1**) did not react and was recovered quantitatively under the former two conditions, but in the last case, **1** was consumed completely. The oxidation of **1** in methanol with 30% aqueous H_2O_2 and 5% aqueous NaOCl furnished a mixture of several 8-MOP oxidized products. Since extraction of the reaction mixture with organic solvents gave secondarily modified compounds on thin layer chromatographic (TLC) analysis, the resulting mixture was extracted using commercially available prepacked Extrelute 20 (Merck Co., Ltd.) (see Fig. 1). Namely, the reaction mixture was absorbed on Extrelute 20, then the column was eluted with EtOAc and MeOH successively. The EtOAc eluate with activity to inhibit PLF activity was further separated by preparative thin layer chromatography to afford six fractions. An active fraction 3 was purified by normal-phase high performance liquid chromatography (HPLC) to furnish **2** as a substance inhibiting chemotactic activity of PMN toward anaphylatoxin $C5_a$ des Arg.[9] On the other hand, fractions 1 and 2 were purified by silica gel column chromatography to yield **3** and **4**, respectively.

Compound **2** was shown to have the molecular formula $C_{13}H_{12}O_6$ from its electron impact mass spectrum (EI-MS) and high resolution mass spectra. The infrared (IR) spectrum of **2** showed a hydroxyl absorption band (3690, 3590 cm^{-1}), while the proton nuclear magnetic resonance (^{1}H-NMR) spectrum indicated the retention of the α-pyrone and aromatic moieties and the disappearance of the furan unit in **1**. In addition, one methoxyl group signal (δ 3.61, 3H, s) and two oxymethine signals (δ 5.04, 1H, s; δ 5.54, 1H, s) were newly observed in the ^{1}H-NMR



8-MOP (1) 2 : R=H 2a : R=Ac 3 4

Chart 1

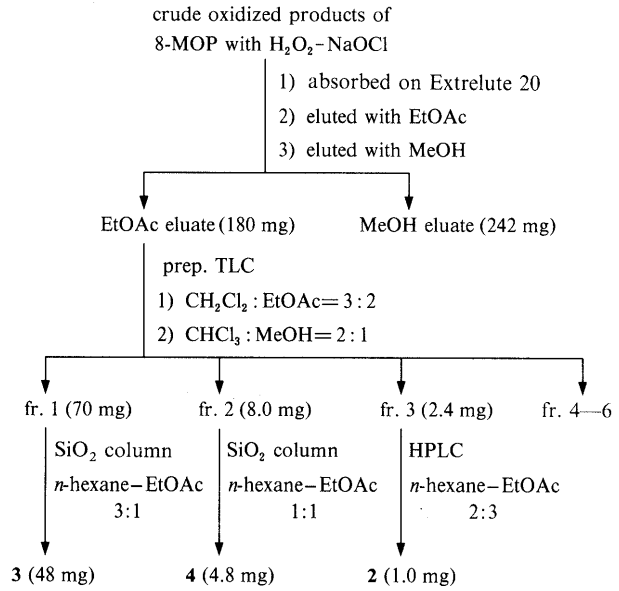


Fig. 1. Fractionation and Isolation of 8-MOP Oxidized Products

TABLE I. ^{13}C-NMR Data for **1**, **2**, and **2a** [δ_c, 100 MHz, $(CD_3)_2CO$]

	1	2	2a
2	148.1	116.3	112.7
3	107.8	76.5	77.5
3a	127.1	128.3	123.8
4	115.3	120.0	121.4
4a	117.6	115.3	115.7
5	145.5	145.2	145.1
6	114.5	113.6	113.9
7	160.5	160.5	160.2
8	148.5	149.2	149.8
9	133.4	132.7	132.9
9a	144.1	154.1	154.7
2-OMe		56.9	56.9
9-OMe	61.5	60.1	61.1
OAC			170.6 20.7

spectrum of **2**. Detailed comparison of the ^{13}C-NMR data for **2** with those for **1** has shown that two methine carbons (δ_c 116.3, 76.5) bearing oxygen are newly formed, which is indicative of the presence of a ketal moiety. Thus, the oxidized active product (**2**) was presumed to have added hydroxyl and methoxyl groups to the furan moiety of **1**.

In order to verify the positions of methoxyl and hydroxyl groups in **2**, it was acetylated with Ac_2O and pyridine. The ^{1}H-NMR spectrum of its acetate (**2a**) showed the signal due to 3-H (δ 5.93, 1H, s) shifted downfield in comparison with that of **2** (δ 5.04, 1H, s). However, the 2-H signals of **2a** and **2** appeared at similar chemical shifts. Consequently, the newly formed hydroxyl group was determined to be at C-3 and the structure of **2** was elucidated as 2,3-dihydro-2,9-dimethoxy-3-hydroxy-7*H*-furo[3,2-*g*][1]benzopyran-7-one, which is also substantiated by its ^{13}C-NMR spectrum (Table I). The relative stereochemistry of 2-OCH_3 and 3-OH was proved to be *trans* because of the absence of coupling between 2-H and 3-H.

The major product (**3**) in the reaction was indicated to contain chlorine in the molecule from its EI-MS, and it has the molecular formula $C_{12}H_7ClO_4$ from the high resolution mass spectrum. The ^{1}H-NMR spectrum of **3** is nearly the same as that of **1** except for the signal due to 5-H in **1**. Compound **3** was, therefore, identified as 5-chloro-9-methoxy-7*H*-furo[3,2-*g*][1]benzopyran-7-one.[10] Compound **4** was obtained as colorless needles having the molecular formula $C_{11}H_8O_5$. The IR spectrum of **4** showed a hydroxyl absorption band (3540 cm^{-1}) and two carbonyl absorption ones due to a lactone group and a formyl group (1740, 1660 cm^{-1}). The ^{1}H-NMR spectrum of **4** exhibited signals due to the formyl (δ 9.93, 1H, s) and the phenolic hydroxyl group (δ 11.30, 1H, br s) as well as ones due to α-pyrone and aromatic moieties, but it lacked signals ascribable to the furan unit. Thus, compound **4** was assigned as 6-formyl-7-hydroxy-8-methoxy-2*H*-1-benzopyran-2-one. On the other hand, the oxidative cleavage reaction of the double bond of the furan unit in **1** was examined under various conditions in order to obtain sufficient samples for bioassay. In consequence, we found that the ozonolysis of **1** in CH_2Cl_2 afforded **4** in 62% yield as a major product.

TABLE II. Effect of Chemotactic Activity of PMN toward Anaphylatoxin $C5_a$ des Arg

Compound	Migrated PMN
2	52±5
3	98±5
4	99±4

In order to examine inhibition of chemotactic activity of PMN toward anaphylatoxin $C5_a$ des Arg, the PMN migration test was performed by Boyden's method as modified by Snyderman and Pike.[11] The number of migrated PMN was expressed as a percentage of the absolute control and the test was run in triplicate. The PMN migration test result of each compound (**2**—**4**) is given in Table II.

In conclusion, we found that 2,3-dihydro-2,9-dimethoxy-3-hydroxy-7*H*-furo[3,2-*g*][1]benzopyran-7-one (**2**) inhibits PMN activity, by means of bioassay-directed separation of the products of 8-MOP in H_2O_2–NaClO. The compound was presumably formed *via* either an oxetane derivative[12] or 2-chloro-2,3-dihydro-3-hydroxy-9-methoxy-7*H*-furo-[3,2-*g*][1]benzopyran-7-one, but the pathway of formation of **2** was ambiguous. It should be noted that this is a first example of isolation of an 8-MOP derivative inhibiting chemotactic activity of PMN toward anaphylatoxin $C5_a$ des Arg.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. The EI-MS and high-resolution mass spectra were measured with a JEOL JMX DX-300 mass spectrometer, and the IR spectra with a JASCO IRA-2 spectrometer. The ultraviolet (UV) spectra were recorded with a Shimadzu UV 2100 spectrometer, and the ^{1}H-NMR and ^{13}C-NMR spectra with JEOL FX-100 and JEOL GSX-400 spectrometers using tetramethylsilane as an internal standard. The following abbreviations are used: s, singlet; d, doublet. TLC was performed on Merck precoated Kieselgel 60F_{254} plates. Column chromatography was carried out on Silica gel BW-200 (Fuji Davison Chemicals Co., Ltd.). For HPLC, a JASCO twincle-type instrument with a UV detector (JASCO UVIDEC 100-III) was used. Preparative TLC was run on Kieselgel 60PF_{254} plates preparated by ourselves. Extrelute 20 were purchased from Merck Co., Ltd.

Oxidation of 8-MOP with H_2O_2–NaOCl A 5% aqueous solution of NaOCl (35 ml) was added dropwise over 30 min to a water-cooled

solution of 8-MOP (600 mg) in MeOH (60 ml) and 30% aqueous H_2O_2 (4.5 ml), then the whole mixture was stirred at room temperature for 30 min. After removal of MeOH from the whole mixture under reduced pressure, the residual solution was absorbed on an Extrelute 20 column. The column was eluted with EtOAc (300 ml) and removal of the solvent from the eluate under reduced pressure gave crude 8-MOP oxidized products (180 mg).

Purification of the 8-MOP Oxidized Products The crude product (180 mg) was subjected to preparative TLC (CH_2Cl_2 : EtOAc = 3 : 2–$CHCl_3$: MeOH = 2 : 1) to give six fractions. Fraction 1 (70 mg) was purified by column chromatography (SiO_2 5 g, *n*-hexane : EtOAc = 3 : 1) to furnish **3** (48 mg). Fraction 2 (8.0 mg) was chromatographed on silica gel (3 g) with *n*-hexane : EtOAc = 1 : 1 to yield **4** (4.8 mg). Fraction 3 was subjected to normal-phase HPLC (Develosil 100-5, *n*-hexane : EtOAc = 2 : 3) to furnish **2** (1.0 mg).

2: An amorphous powder. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3690, 3590, 2925, 2850, 1730, 1720, 1620, 1580. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 3.61 (3H, s, 2-OMe), 4.10 (3H, s, 9-OMe), 5.04 (1H, s, 3-H), 5.54 (1H, s, 2-H), 6.25 (1H, d, J = 9.5 Hz, 6-H), 7.21 (1H, s, 4-H), 7.62 (1H, d, J = 9.5 Hz, 5-H). UV λ_{max}^{MeOH} nm (ε): 260 (6200), 321 (16000). MS *m/z* (%): 264 (M^+, 63), 232 ($M^+ - CH_3OH$, 16). High resolution MS *m/z*: Calcd for $C_{13}H_{12}O_6$: 260.063; Found: 260.064.

3: Colorless needles, mp 154—155 °C (EtOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2940, 2845, 1730, 1620, 1585. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 4.28 (3H, s, 9-OMe), 6.46 (1H, d, J = 10.0 Hz, 6-H), 6.93 (1H, d, J = 2.2 Hz, 3-H), 7.72 (1H, d, J = 2.2 Hz, 3-H), 8.17 (1H, d, J = 10.0 Hz, 5-H). UV λ_{max}^{MeOH} nm (ε): 253 (13000), 266 (12000), 305 (8000). MS *m/z* (%): 252 (M^+, 34), 250 (M^+, 100), 237 ($M^+ - CH_3$, 16), 235 ($M^+ - CH_3$, 46). High resolution MS *m/z*: Calcd for $C_{12}H_7{}^{37}ClO_4$: 252.637. Found: 252.637. Calcd for $C_{12}H_7{}^{35}ClO_4$: 250.640. Found: 250.638.

4: Colorless needles, mp 185—187 °C (EtOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 2945, 2850, 1740, 1625, 1585. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 4.07 (3H, s, 8-OMe), 6.34 (1H, d, J = 9.5 Hz, 3-H), 7.49 (1H, s, 5-H), 7.66 (1H, d, J = 9.5 Hz, 4-H), 9.93 (1H, s, 6-CHO), 11.3 (1H, s, 7-OH). UV λ_{max}^{MeOH} nm (ε): 264 (20000), 313 (10000). MS *m/z* (%): 220 (M^+, 100). High-resolution MS *m/z*: Calcd for $C_{11}H_8O_5$: 220.183. Found: 220.182.

Acetylation of 2 Ac_2O (0.5 ml) was added dropwise to an ice-cooled solution of **2** (5.0 mg) in pyridine (1.0 ml) and the whole mixture was allowed to stand at room temperature for 10 h. The reaction mixture was poured into ice-water, then the whole was extracted with EtOAc. The EtOAc extract was washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl, then dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a product which was purified by column chromatography (SiO_2 5 g, *n*-hexane : EtOAc = 5 : 1) to furnish **2a** (5.8 mg, quant.).

2a: A white powder, mp 108—109 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2940, 2840, 1740, 1730, 1620, 1580. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.10 (3H, s, OAc), 3.61 (3H, s, 3-OMe), 4.11 (3H, s, 9-OMe), 5.61 (1H, s, 2-H), 5.93 (1H, s, 3-H), 6.27 (1H, d, J = 9.6 Hz, 6-H), 7.30 (1H, s, 4-H), 7.61 (1H, d, J = 9.6 Hz, 5-H). UV λ_{max}^{MeOH} nm (ε): 262 (6800), 315 (14000). MS *m/z* (%): 306 (M^+, 61), 219 (100). High resolution MS *m/z*: Calcd for $C_{15}H_{14}O_7$: 306.074. Found: 306.074.

Preparation of 4 by Ozone Oxidation of 1 A solution of **1** (300 mg) in CH_2Cl_2 (50 ml) was bubbled through with ozonized oxygen at −78 °C for 2 h. The cooled solution was then bubbled through with nitrogen to remove excess ozone. After warming gradually to room temperature, the reaction mixture was treated with $(CH_3)_2S$ (1.0 ml) at room temperature for 10 min. The reaction mixture was poured into ice-water, then the whole was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl and dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a product which was purified by column chromatography (SiO_2 10 g, *n*-hexane : EtOAc = 1 : 1) to furnish **4** (189 mg, 62%). The product was identical with **4** obtained in the previous H_2O_2–NaOCl treatment of **1** based upon a comparison of the physical data.

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