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Oxidation and Degradation Products of Papaverine, Part II[1]: Investigations on the Photochemical Degradation of Papaverine Solutions

The structure of the final degradation product formed in papaverine solutions in either water or chloroform was found to be a 2,3,9,10-tetramethoxy-12-oxo-12*H*-indolo[2,1-a]isoquinolinylium salt (a dibenzo[b,g]pyrrocolonium derivative). Its formation from papaverine oxidation products that is papaverinol, papaveraldine, and papaverine-*N*-oxide chloroform solutions under the influence of UV light, was investigated and possible reaction pathways are discussed.

Keywords: Papaverine oxidation; Photochemical degradation; Structure elucidation

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Introduction

The formation of papaverinol **3** and papaveraldine **5** from aqueous solutions of papaverine has been known for a long time, however, there was polarographic evidence that **5** is not the final product in this oxidation sequence [2]. In 1959, Machovičová and Parrák reported on their analysis of 4 % aqueous papaverine hydrochloride solutions which were stored for a prolonged period of time; using paper chromatography, a more polar structure – named "compound X" [3] – was found besides **3** and **5**. Later, one of us succeded to isolate compound X from a chloroform solution of papaverine hydrochloride (**1 · HCl**) stored for about one year at daylight condition in a yield of nearly 50 % [4]. To our knowledge, no structure for "compound X" has been proposed in the literature. Pfeiffer et al. investigated the decomposition of a number of alkaloids upon storage under various conditions [5–7]. With papaverine **1** they found **3** and **5** and also two *N*-oxygenated derivatives, namely papaverine-*N*-oxide **2** and 6,7-dimethoxyisoquinoline-*N*-oxide [5–7]. Here, we report the elucidation of the structure of compound X **8** and some results investigating the mechanism of the photochemical oxidation of papaverine.

Results and discussion

Material for investigation

8 · Cl can be obtained within a few hours in a yield of up to 40 % by irradiating a chloroform solution of papaverinol with a mercury low pressure lamp at 254 nm [8]. The crude material was purified by treatment with methyl iodide in hot methanol to afford **8 · I** as a black powder [8]. This material and the original sample [4] were used for spectroscopic analysis.

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Structure elucidation

The structure of the brownish-yellow **8 · I** as a cationic derivative of **1** could be deduced from mass spectrometric experiments comparing the results of the electron impact (EI) mass spectrum on the one hand and the electrospray ionization (ESI) mass spectra in either ammonium acetate buffer or in deuterium oxide on the other. A molecular ion at 352 Th was found in all cases. Interestingly, the EI mass spectra are more complicated, as cleavage of methyl iodide, or methyl chloride for **8 · Cl** respectively, is observed, together with methylation of **8** (affording a signal at 367 Th) presumably during the mass spectrometric evaporation process. Of cause, these reactions are not occurring during electrospray ionization. When performing MS^2 experiments, the fragments observed in EI-MS or in ESI-MS indicated that the methylene bridge is no longer present, independent on its oxidation state as major fragmentation position. The structure of the sparingly soluble **8 · I** was assembled using ^1H and ^{13}C NMR data in dimethyl sulfoxide affording the structure shown in Scheme 1. Similar NMR spectra were obtained for **8 · Cl**, which is even less soluble; the ESI-MS data and retention times in HPL chromatography were identical. Two-dimensional NMR experiments were performed on **8 · I** in a mixture of trifluoroacetic acid and deuterium oxide. The structure is in accordance with all the other spectroscopic data obtained (IR and UV/VIS). The number of compounds with a dibenzopyrrocoline skeleton reported in the literature is rather small, Radchenko et al. [9] reported on the synthesis of some

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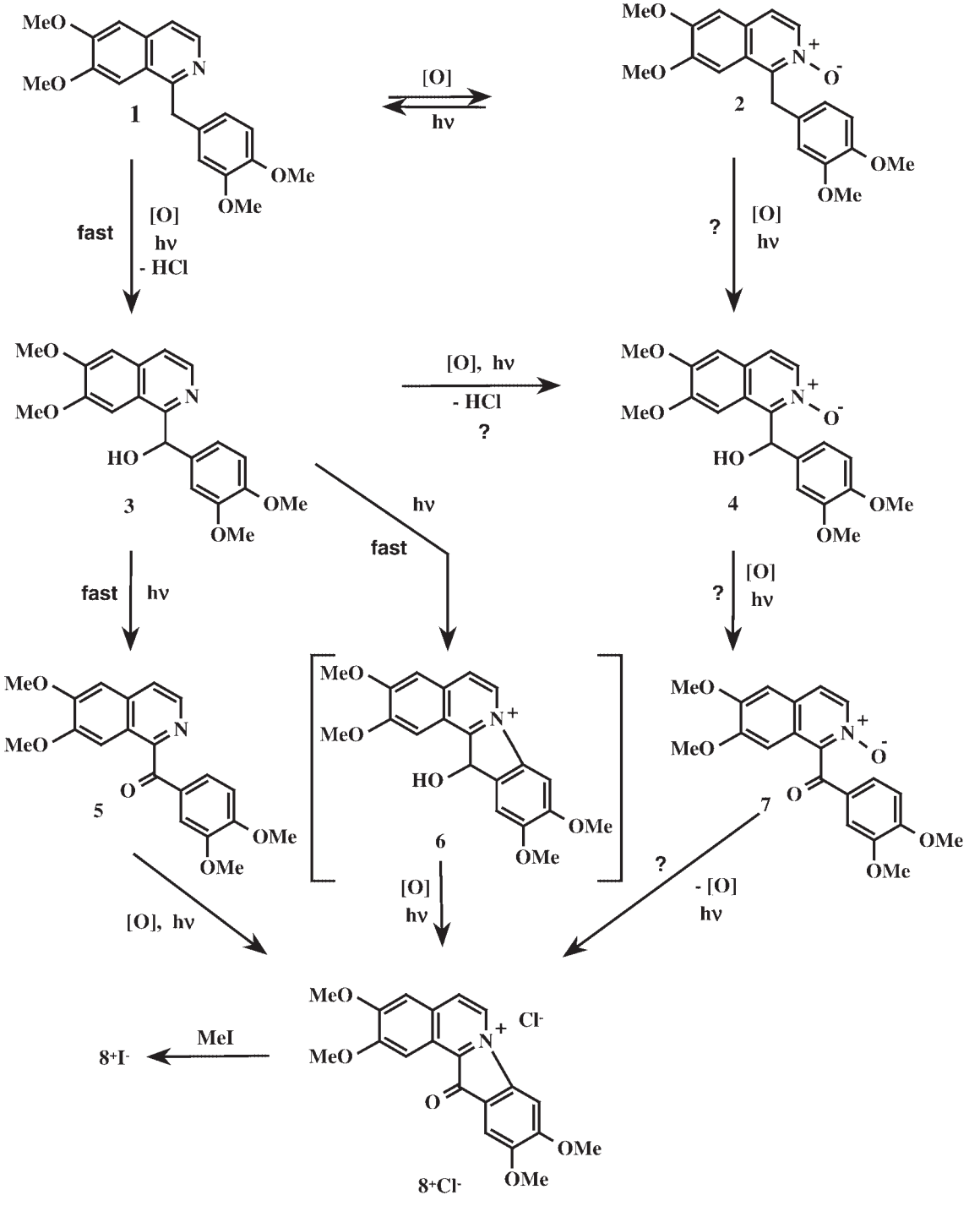
methoxy and hydroxy derivatives together with pharmacological data; Pabuccuoglu et al. [10] described the formation of a 2,3-dimethoxy-12,12a-dihydro-12-oxodibenzopyrrocolonium salt as a side product in lysicamine synthesis, giving an X-ray structure of the picrate salt. Another observation, i.e. the disappearance of the absorption bands of **8·I** between 311 and 400 nm upon addition of a strong base can be explained by nucleophilic addition of the hydroxyl group to position 12a of the aromatic ring, destroying the conjugation of the two ring systems [11].



Scheme 1. Possible intermediates in papaverine oxidation leading to pyrrocolonium salt **8**$^{+}$**Cl**$^{-}$.

Mechanism

Solutions of **1**, **1·HCl**, **2**, **3**, and **5** in chloroform were subjected to weak irradiation at 254 nm using a low-power TLC plate detection lamp under aerobic conditions in order to obtain more information on the mechanism of the formation of **8**. These irradiated chloroform solutions were investigated by RP-LC/MS coupling using electrospray ionization, the details are given in the "Experimental"-section.

First, **1 · HCl** was irradiated and analyzed, because the original sample of **8 · Cl** has been obtained from such a solution. Furthermore, we propose a similar way of decomposition of papaverine hydrochloride in aqueous solutions, which are used as injections [12].

Investigation of the data obtained showed that UV light exposition at 254 nm leads to photooxidation of the methylene bridge, first, to the secondary alcohol **3** and, subsequently, to the ketone **5** and to **8** (Scheme 1). Furthermore, there was mass spectroscopic evidence for the formation of various demethylated derivatives of **1 · HCl** (Figure 1; peaks A, B, C), and also for a remarkable amount of a compound with a molecular weight of 383 Da having a papaverine skeleton according to MS^2 experiments (Figure 1; peak D). However, the reaction rate of **1 · HCl** oxidation is rather low. In order to obtain more insight into the formation of **8**, solutions of **1**, **3**, **5**, and **2** were also irradiated.

After exposition of a solution of **1** in chloroform to UV light **3**, **5**, **8**, and the demethylation derivatives mentioned above were observed. In addition, *N*-oxygenation of **1** to **2** was found, the identification of which was accomplished with an independently synthesized sample. Additionally, up to ten different addition products of dichlorocarbene to papaverine were found. Compared to the photodegradation of hydrochloride **1**, the free base is decomposed much faster.

Previous experiments [2] suggested that **8 · Cl** is an oxidation product of papaveraldine **5**, so **5** was synthesized according to [13]. Our analysis confirmed the appearance of **8**, and it was formed much faster than starting from **1 · HCl**.

Quite remarkably, of all the compounds under investigation (compare Scheme 1) the photodegradation of papaverinol (**3**) (synthesized according to [14]) to **8** proceeded with the fastest rate [8]. Besides **5**, the apolar compound D was found in the irradiated solutions as well, together with many other compounds. Papaverine-*N*-oxide (**2**) was obtained according to Pfeifer et al. [6] by reacting **1** with aqueous H_2O_2. However, the time required for the reaction at 70 °C was longer and amounted

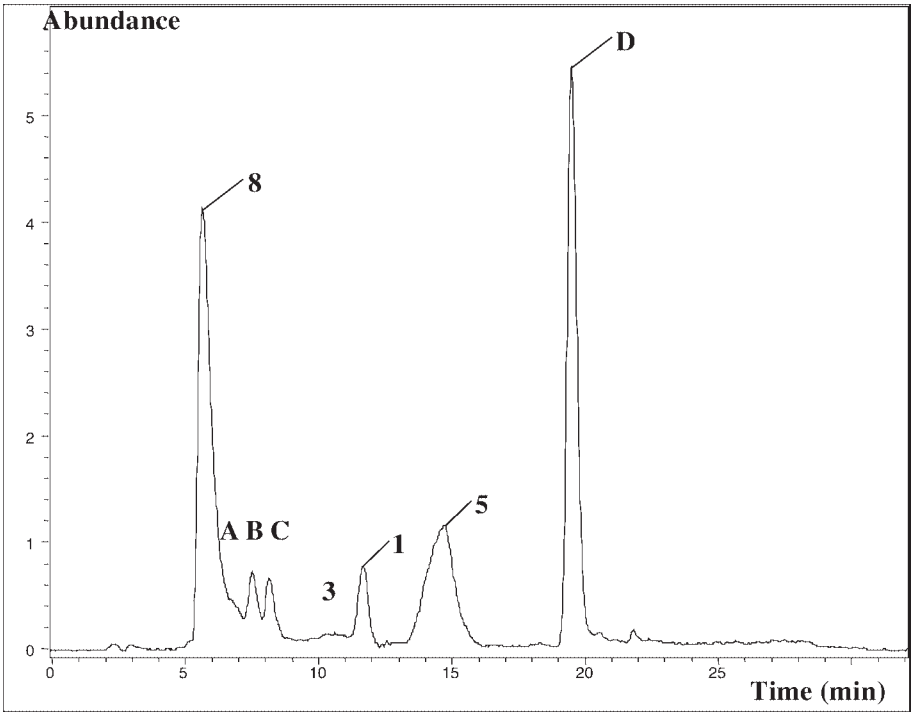


Figure 1. Reconstructed ion chromatogram (ion of papaverine with 340 Th excluded) of an irradiated solution of **1 · HCl** in chloroform.

to 10 hours. After exposition to UV light a solution of **2** contained a large number of different oxidation products (for example up to ten isomers with a molecular weight of 355 Da were found). Only a comparatively small amount of **8** and also **1**, **3**, and **5** could be detected. Photochemical reactions of **2** have been reported by Intestrosa et al. [15] only recently. Except for hydroxylated derivatives of papaverine they observed ring enlargement of the *N*-oxide to a diazepine derivative in deoxygenated solvents.

Therefore, we can not exclude that **8 · Cl** is formed through the reaction cascade from **2** to **4** to **7** rather than the proposed alternative way through **3** and **5**. The high reactivity of papaverinol to photooxygenation can also be explained by the intermediate formation of **6**, arising from oxidation of the nitrogen atom with higher electron density in **3** compared to **5**, but **6** might have only a very short lifetime under the reaction conditions.

The observed rate of formation of **8** can be given as **3** > **5** > **1** > **2** ≫ **1 · HCl**, not taking other side reactions like carbene insertion into account. In conclusion, the results obtained from the above described experiments indicate that the oxidation of **1 · HCl** in aerobic chloroform solutions occurs via **3** and **5** to **8 · Cl**.

The question of the importance of the yellowish hydrochlorides of **3** and **5** as photosensitizers for the reactions discussed above in order to explain papaverine decomposition at daylight conditions can not be answered with the results obtained, requiring instead detailed kinetic experiments. Furthermore, the regioselective ring closure of the two ring systems in **3** or **5** is of interest, only the attack on position 6 of the 3,4-dimethoxyphenyl ring is observed, only one isomer of **8** was found with all the analytical methods employed.

Experimental

Melting points were determined on a Boëtius microscope and are not corrected. EI (70 eV) mass spectra were recorded on a HP-MS engine 5989 A (Agilent Technologies, Waldbronn, Germany). Electrospray mass spectra were recorded on an Bruker Esquire LC. 300 MHz ^{1}H- and 75 MHz ^{13}C NMR spectra were recorded on a Bruker ARX 300 spectrometer (Bruker, Ettlingen, Germany). Chemical shifts are given in ppm and TMS was used as an internal standard for the spectra obtained in DMSO-d_6. UV/Vis data were obtained with a HP 8452 UV/Vis spectrometer. IR spectra were recorded with a FT IR Raman Magna 760 spectrometer (Nicolet, Thermo-Nicolet, Madison, WO, USA). Two Hanau Fluotest lamps with emission at 254 nm (15 Watt each) were used for the irradiation experiments. HPLC analysis was performed using a HP1090 LC series II together with UV detection at 255 nm with a HP 1050 VWD. A Zorbax Eclipse XDB-C8 column 4.6 × 150 mm was used together with a precolumn (Agilent Technologies, Waldbronn, Germany). The mobile phase consisted of a mixture of 35 % of acetonitrile and 65 % (v/v) of 50 mM aqueous ammonium acetate, pH 6.5 with a flow rate of 0.8 ml/min and a split ratio of 1:7 to the ESI mass spectrometer.

Synthesis of **8 · I** [8]:

50 ml of a 0.3 % solution of **3** in chloroform (150 mg, 0.42 mmol) were irradiated with a low pressure mercury lamp "original Hanau" TNN 1532 for 4 h while cooling the solution. After irradiation, the solvent was evaporated and the remainder was dissolved in 5 ml of boiling methanol, then 0.2 ml of methyl iodide (Fluka, Buchs, Switzerland) was added. After cooling to room temperature, a few drops of water were added, the solution became turbid and a precipitate formed upon storing at 5 °C for a few hours.

The solid was collected by filtration and recrystallized from methanol to form a black powder; yield 62 mg (0.13 mmol, 31 %) with mp. 225 °C, decomp. Calculated for $C_{20}H_{18}NO_5I$: C, 50.12; H, 3.79; N, 2.92; found C, 50.37, H, 3.96; N, 2.87. UV/Vis (MeOH, λ_{max}, lg ε, ε in l · mol^{-1} · cm^{-1}): 222 (4.5), 256 (4.3), 310 (4.6), 398 (4.1); in 0.1 M NaOH: 224 (4.8), 256 (4.5), 322 (4.1). IR (KBr pellet): ν/cm^{-1} 1719 (m), 1625 (m), 1589 (m), 1495 (s), 1318 (s), 1206 (s). EI-MS of **8 · Cl**: m/z 367 ([M + Me]$^+$, 1), 352 (M$^+$, 22), 337 (10), 336 (9), 322 (8), 308 (5), 292 (4), 176 (4), 172 (4) 52 (Me^{37}Cl, 31), 50 (Me^{35}Cl, 100). EI-MS of of **8 · Cl**: m/z 367 ([M + Me]$^+$, 1), 352 (M$^+$, 21), 337 (74), 336 (32), 322 (69), 308 (14), 293 (25), 292 (23), 172 (32) 142 (MeI$^+$, 100), 127 (I$^+$, 30).

ESI-MS in buffered ammonium acetate solution with enhanced fragmentation afforded ions with m/z 352, 336, 322, 308, 292, 291. ^{1}H NMR (300 MHz, DMSO-d_6, TMS as internal standard) of **8 · Cl**: δ 3.93/4.07/4.08/4.12 (4 × s, 4 × 3 H, 4 × OCH_3), 7.16/7.34/7.47/8.28 (4 × s, 4 × 1 H, H-1/4/8/11) 8.53/9.57 (2 × d, 2 × 1 H, *J* = 6.2 Hz, H-5/6).

^{1}H NMR (300 MHz, DMSO-d_6, TMS as internal standard) of **8 · I**: δ 3.97/4.09/4.12/4.16 (4 × s, 4 × 3 H, 4 × OCH_3), 7.50/7.82/8.14/8.27 (4 × br s, 4 × 1 H, H-1/4/8/11) 8.70/9.39 (2 × br s, 2 × 1 H, *J* = 6.2 Hz, H-5/6). ^{13}C NMR (75 MHz, DMSO-d_6, TMS as internal standard) of **8 · I**: δ 56.57, 56.64, 57.2, 57.3, 100.0, 101.4, 106.6, 107.4, 115.3, 121.3, 125.3, 126.7, 133.5, 140.8, 142.4, 150.6, 155.5, 156.3, 158.4, and 182.4.

^{1}H NMR (300 MHz, TFA/D_2O, sodium 3-trimethylsilylpropionate as internal standard) of **8 · CF_3CO_2**: δ 4.03/4.15/4.19 (3 × s, 2 × 3 H, 1 × 6 H, 3/9/10/2-OCH_3), 7.49/7.49/7.60/8.48 (4 × s, 4 × 1 H, H-11/H-4/H-8/H-1), 8.25/8.76 (2 × d, *J* = 6.9 Hz, 2 × 1 H, H-6/5). ^{13}C NMR (75 MHz, TFA/D_2O, sodium 3-trimethylsilylpropionate as internal standard) of **8 · CF_3CO_2**: δ 59.7 (3-OCH_3), 60.1 (9-OCH_3), 60.3 (2/10-OCH_3, two overlapping signals), 101.6 (C-8), 105.7 (C-1), 109.5 (C-11), 111.4 (C-4), 119.0 (C-11a), 126.5 (C-12b), 128.6 (C-6), 129.4 (C-5), 136.1 (C-4a), 146.0 (C-12a), 146.2 (C-11a), 155.0 (C-10), 160.5 (C-9), 161.0 (C-2), 163.6 (C-3), and 186.3 (C-12).

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