

## PHOTOOXIDATION OF PAPAVERINE, PAPAVERINOL AND PAPAVERALDINE IN THEIR CHLOROFORM SOLUTIONS

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**Abstract:** Papaverine hydrochloride, papaverinol, and papaveraldine chloroform solutions were exposed to UV light of 254 nm in atmospheric, aerobic and anaerobic (helium) conditions. The same degradation products appear (TLC) in the above papaverine hydrochloride chloroform solutions. However, the rate of papaverine hydrochloride degradation processes is enhanced as a function of oxygen pressure. Papaverinol and papaveraldine photooxidation products are essentially not different from those observed in the above papaverine hydrochloride solutions. However, the amount of an unknown brown degradation product (X) is the greatest in the papaverinol chloroform solution degraded. That brown compound was previously observed in papaverine either hydrochloride or sulfate injection solutions on their storage even when protected from daylight. The preliminary X product structure development was undertaken (TLC, molecular weight, elemental analysis, UV/VIS, IR and <sup>13</sup>C MAS NMR spectroscopy).

**Keywords:** papaverine, papaverinol, papaveraldine, photooxidation, thin-layer chromatography, structure development of X, methyl ester of X.

Papaverine is susceptible to oxidation in its aqueous (injection) and nonaqueous solutions (1,2, 3,4,5). The methylene bridge in papaverine molecule is vulnerable to oxygen attack because lack of any steric hindrance around it. It is oxidized to a secondary alcohol – papaverinol and subsequently to a ketone – papaveraldine (6). The above oxidation products contaminate to some extent also papaverine salts (hydrochloride, sulfate). Their toxicity is increased and pharmacological action is lowered if compared to papaverine (6).

Paper chromatographic analysis of yellowish injection solutions of papaverine sulfate lets to identify its degradation products papaverinol, papaveraldine and an unknown brown product X (7). The product X has been also detected in injection solutions of papaverine hydrochloride degraded (8) and isolated from chloroform papaverine hydrochloride solutions exposed to sun- and daylight (2) but its chemical structure is still unknown. Photoalkylation reaction has been observed in alcoholic papaverine solutions resulting in either 1-methyl- or 1-ethyl- 6,7-dimethoxyisoquinoline (9). Papaverine N-oxide has been isolated from papaverine solutions in nonaqueous solvents (acetone, chloroform, benzene) as well as 6,7-dimethoxyisoquinoline N-oxide was isolated from its solutions in chloroform

exposed to sun- and daylight (3,4,5). Very efficient syntheses were developed for papaverinol (10) and papaveraldine (11).

Our project concentrates on the exposition not only of papaverine hydrochloride but also of its degradation products (papaverinol and papaveraldine) chloroform solutions to UV light of 254 nm in aerobic and anaerobic conditions. The above photodegradation processes were followed using thin-layer chromatography and UV/VIS spectrometry. The structure of the unknown product X was supposed to be elucidated to a greater extent.

## EXPERIMENTAL

## Materials

Papaverine hydrochloride M.p. 219–225°C with decomposition (lit. 218–223°C FPV) (FARM-IMPEX sc, Gliwice, Poland); papaveraldine C<sub>20</sub>H<sub>19</sub>NO<sub>5</sub> (synthesised according to Tsatsas (11), M.p. 210–211°C [lit. 209–210°C (2); 206–207°C (6); 207–209°C (5)]; papaveraldine hydrochloride C<sub>20</sub>H<sub>19</sub>NO<sub>5</sub> HCl 2.5 H<sub>2</sub>O [synthesised according to Tsatsas (11)], M.p. 211–212°C [lit. 210°C (11)]; papaverinol C<sub>20</sub>H<sub>21</sub>NO<sub>5</sub> (synthesised according to Gadamer and Schulemann (6)), M.p. 136–137°C [lit. 137°C (6)]; 139–140°C (5); 137°C (12)].

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All the above authentic samples produced only one spot on thin-layer chromatography procedures described below.

#### Exposition to UV light

Chloroform papaverine hydrochloride ( $5.32 \cdot 10^{-2}$  M), papaveraldine hydrochloride (5.13 mM) and papaverinol (5.63 mM) solutions were exposed to UV light 254 nm (a low vacuum mercury lamp Originau Hanau TNN 15/32, Germany) at the ambient temperature. The time of exposition of the above papaverine hydrochloride solution in atmospheric, aerobic and anaerobic (helium) conditions was equal to 65, 40 and 30 h, respectively. That time for papaveraldine hydrochloride and papaverinol solutions was equal to 55 and 7 h respectively, both only in the atmospheric conditions.

#### Thin-layer chromatography

Precoated 0.2 mm silica gel 60 plastic foils (Merck, Germany) were spotted with 100  $\mu$ g of each: papaverine hydrochloride, papaveraldine hydrochloride, papaverinol, product X, its methyl ester and 13-(3,4-dimethoxyphenyl)-2,3,8,9-tetramethoxy-6a,12a-diazadibenzo[a,g]fluorenylium chloride (13). Two mobile phases were developed. The first solvent system consisted of chloroform – toluene – methanol – glacial acetic acid (13:4:3:1, V/V) which advanced 12 cm in 40 min at the ambient temperature. The second one was an alkaline solvent obtained as the upper layer of the mixture: toluene – acetone – 7.1 M ammonia (13:4:1, V/V). It advanced 12 cm in 30 min. It was mainly used for the identification of product X (100  $\mu$ g) and its methyl ester. The spots of the compounds separated were visualised in day and ultra – violet light (366 nm UV lamp, Hanau, Germany).

#### Spectroscopy of the compound X

UV/VIS spectra of the compound X ( $6.52 \cdot 10^{-7}$  M) and its methyl ester in methanol and 0.1 M NaOH were recorded in 10 mm matched silica cells in a Specord M 40 (Carl Zeiss, Jena, GDR). The IR spectrum was read for a KBr pellet (1 mg/200 mg) on a FT-IR-RAMAN MAGNA 760, (Nicolet, USA) machine equipped with the Fourier's transformation. A heteronuclear Bruker instrument with a 7 mm rotor was used to record 100.62 MHz  $^{13}$ C CP MAS NMR spectrum of the compound X in the solid phase. 3-(Trimethylsilyl)-tetradeutero sodium propionate (TSP) was applied as the internal standard.

#### Preparation of X methyl ester

0.1 g of X in 10 ml of methanol was dissolved on boiling under a reflux condenser and 5 ml

methyl iodide was added. The solution was maintained at boiling for 40 min and filtered. A few drops of water were added until the solution became turbid and was allowed to stand in a refrigerator overnight. Black, brilliant crystals were filtered off, washed and dried (M.p. 225°C with decomposition).

#### Molecular weight determination of the compound X and its methyl ester

Aqueous solution of compound X (0.2%) and its methyl ester (0.08%) were prepared and their molecular weight was determined in an osmometer Oakley's type equipped with a membrane Capran Nylon 6 film (General Chemical Division, Morristown, N.J., USA).

## RESULTS AND DISCUSSION

#### Photooxidation of papaverine, papaverinol and papaveraldine

A suitable mobile phase for thin-layer chromatography was developed. The main problems to overcome were: first, the difficulty to separate papaverine and papaverinol and second to move the compound X from the starting point of the chromatogram. Those requirements could meet a mobile phase of an acidic pH, because the compound X is a heterocycle base (2) whose solubility increases as a salt in a hydrophilic solvent. Therefore, the mobile phase containing glacial acetic acid produced a spot of compound X of  $R_f = 0.20$  (Table 1), far enough from the starting point.

Papaverinol, papaverine hydrochloride and papaveraldine were very well separated in the above conditions and are characterised with  $R_f$  values of 0.47, 0.55 and 0.75, respectively (Table 1).

#### Papaverine hydrochloride

Seven degradation products were found in papaverine hydrochloride chloroform solutions in aerobic (A), atmospheric (B) as well as in anaerobic (C) conditions (Table 1). A product of  $R_f = 0.22$  was observed in atmospheric (B) and anaerobic (C) conditions. A blue fluorescence spot ( $R_f = 0.31$ ) appears in aerobic (A) and anaerobic (C) conditions. Three additional spots were observed for the solution exposed to UV light in aerobic (A) conditions of  $R_f = 0.21$  (orange – red fluorescence) and of a blue fluorescence (0.41, 0.73) (Table 1). Another spot was separated on a chromatogram of the papaverine hydrochloride solution exposed to UV light in atmospheric (B) conditions ( $R_f = 0.35$ ). A product of  $R_f = 0.60$  appears only in the solution exposed to UV light in anaerobic (C) conditions

Table 1. Mean  $R_f$  values of papaverine hydrochloride, papaveraldine hydrochloride and papaverinol photodegradation products separated on TLC silica gel foils

Name or number of a spot	Colour in UV light	A	B	C	D	E
1	blue	—	—	—	—	0.09
2	yellow	—	—	—	—	0.11
3	blue	0.14	0.13	0.13	0.15	—
4 (X)	yellow (VIS)	0.19	0.20	0.19	0.20	0.20
5	orange – red	0.21	—	—	—	—
6	blue	—	0.22	0.22	—	—
7	orange	—	—	—	0.24	—
8	blue	0.30	—	0.31	0.31	0.32
9	orange – red	—	0.35	—	—	—
10 (FC)	violet	—	—	—	—	0.38
11	blue	0.41	—	—	—	—
Papaverinol	orange	—	—	—	—	0.47
12	yellow	—	—	0.60	—	—
13	yellow (VIS)	0.63	0.65	0.64	0.64	0.64
14	blue	0.67	0.69	0.66	0.67	—
15	blue	0.73	—	—	0.73	—
Papaveraldine	yellow (VIS)	0.75	0.75	0.74	0.75	0.75
16	blue	0.80	0.79	0.81	0.79	0.79
17	blue	—	—	—	—	0.83
18	blue	0.86	0.87	0.87	—	—

Chloroform papaverine hydrochloride ( $5.32 \cdot 10^{-2}$  M) solutions exposed to UV light 254 nm:

A – aerobic conditions (40 h); B – atmospheric conditions (65 h); C – anaerobic conditions (30 h); D – chloroform papaveraldine hydrochloride (5.13 mM) solution, atmospheric conditions (55 h); E – chloroform papaverinol (5.63 mM) solution, atmospheric conditions (7 h); FC – a fluorenylium chloride authentic sample.

(Table 1). Papaverinol was not observed at all, because it oxidises very rapidly to papaveraldine ( $R_f = 0.75$ ), which was observed as a predominant product (approx. 50%) in all solutions exposed to UV light. It indicates that papaveraldine is a relatively stable compound during photooxidation processes. Among unidentified degradation products of papaverine could be found papaverine N-oxide, 6,7-dimethoxyisoquinoline N-oxide. However, they could not be identified because we did not have a standard sample of these degradation products. We possessed a 1-methyl-6,7-dimethoxyisoquinoline standard, but its  $R_f$  value did not correspond to any of papaverine, papaverinol and papaveraldine degradation products specified in Table 1.

#### Papaveraldine hydrochloride

Eight photodegradation products of papaveraldine ( $R_f$ : 0.15, 0.20, 0.31, 0.64, 0.67, 0.73, 0.75, 0.79) are also observed in papaverine hydrochloride solutions degraded. One spot of  $R_f = 0.24$  (orange fluorescence) was not previously detected in papaverine hydrochloride solution degraded.

#### Papaverinol

Five products of papaverinol degradation were also detected in all papaverine and papaveraldine solutions degraded ( $R_f$ : 0.20, 0.32, 0.64, 0.75, 0.79). One product of  $R_f = 0.38$  of violet fluorescence is formed only from papaverinol after 7 h elapsed. It seems to be 13-(3,4-dimethoxyphenyl)-2,3,8,9-tetramethoxy-6a,12a-diazadibenzo[a,g]fluorenylium chloride which was previously synthesised as a minor side product from papaverine on its oxidation with mercury(II) acetate to papaverinol (13). A state near equilibrium has been reached between papaverinol, papaveraldine, the substance of blue fluorescence ( $R_f = 0.83$ ) and compound X between 1 and 10 h exposition to UV light. Thirty h were needed to elapse in order to diminish significantly the area of papaverinol spot and its intensity.

#### Compound X

The compound X appears in all papaverine hydrochloride solutions (A,B,C columns, Table 1) examined but in rather minute amounts. Compound

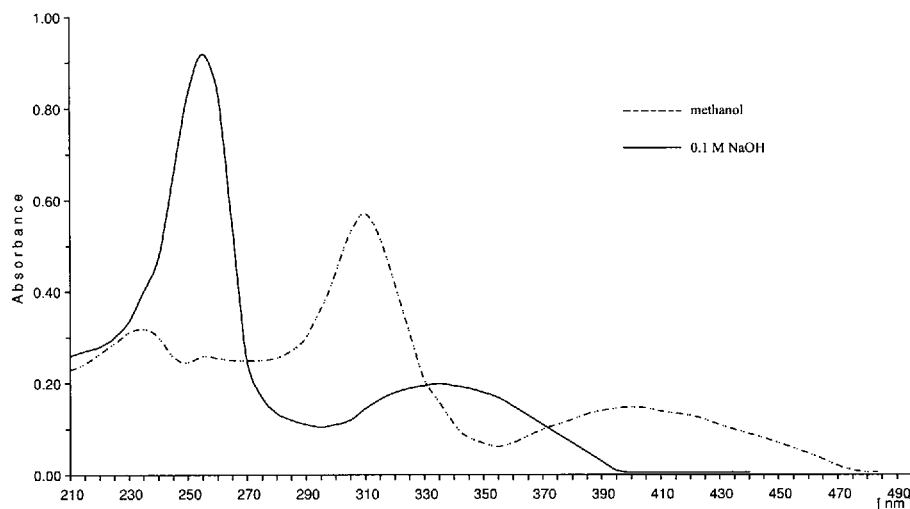


Figure 1. UV/VIS spectra of the compound X ( $6.52 \cdot 10^{-7}$  M) in methanol and 0.1 M NaOH (10 mm silica cells, Specord M 40, Carl Zeiss, Jena, GDR).

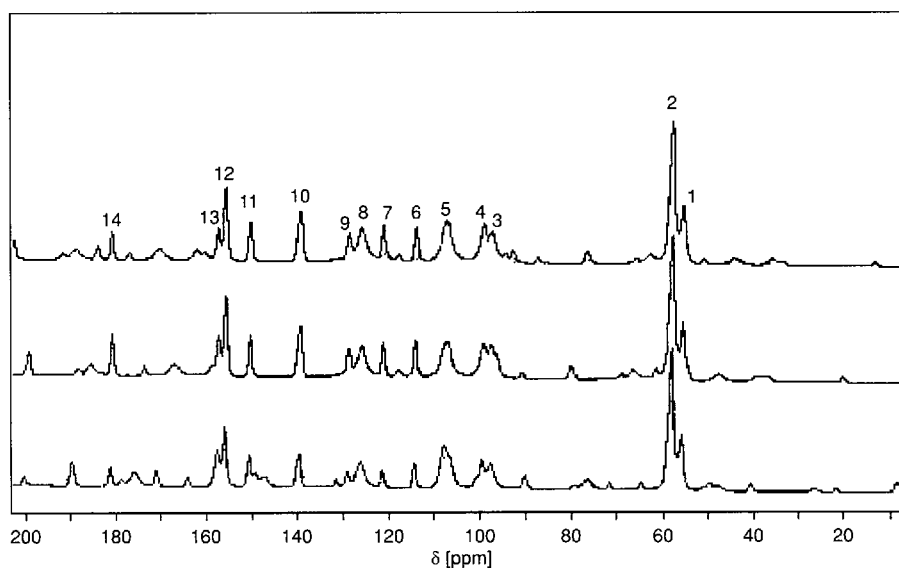


Figure 2. The 100.62 MHz  $^{13}\text{C}$  CP MAS NMR spectrum of compound X recorded on a Heteronuclear Bruker instrument with a 7 mm rotor (internal standard TSP). Frequency of rotor rotation: 5.0, 6.0 and 6.35 kHz for the lower up to the upper spectrum, respectively.

X ( $R_f = 0.20$ ) is also observed in papaveraldine hydrochloride solution (D) but its amount is not too large either. However its extremely large amounts (approx. 50%) are observed in a papaverinol chloroform solution (E) exposed to UV light in atmospheric conditions for a relatively short period of time (1 h).

#### Chemical characterisation of compound X

The compound X seems to be an amphoteric species because it moves also in an alkaline mobile

phase further. Its  $R_f$  increases from 0.2 (acidic pH) to 0.55. As an alkaline mobile phase the upper layer of the following system: toluene – acetone – 0.1 M NaOH (13:7:1) was used. However, the separation of papaverine hydrochloride, papaverinol, X and papaveraldine hydrochloride is not good enough in the above system ( $R_f$ : 0.46, 0.50, 0.55, 0.58, respectively). Nevertheless, it is very interesting that the brown compound X becomes colourless in 0.1 M NaOH of aquamarine fluorescence in

Table 2. Results of molecular weight determination (D) and elemental analysis (%) of compound X and its methyl ester

Symbol of a compound	Elemental analysis				Number average molecular weight
	N	C	H	O	
X	2.41	52.63	4.978	39.98	9200
Methyl ester of X	3.55	56.65	4.344	35.66	3200

UV light 254 nm and the brown colour is recovered on acidification. In its UV/VIS spectrum in neutral solvents (Figure 1) (e.g. methanol) 4 maxima of absorption are observed (235, 483282, 255, 398773, 311, 889724, 398, 236810 –  $\lambda$  [nm] and  $\epsilon$  [ $\text{cm}^{-1} \cdot \text{mol}^{-1} \cdot \text{l}$ ], respectively). A bathochromic shift is observed of the main peak of papaverine from 242 nm ( $\epsilon = 56742 \text{ cm}^{-1} \cdot \text{mol}^{-1} \cdot \text{l}$ ) to 311 nm ( $\epsilon = 889724 \text{ cm}^{-1} \cdot \text{mol}^{-1} \cdot \text{l}$ ) (2). However, the solution of that compound in 0.1 mol/l NaOH is colourless and a hypsochromic shift of the main  $\lambda_{\text{max}}$  (311 nm) to 256 nm ( $\epsilon = 1410123 \text{ cm}^{-1} \cdot \text{mol}^{-1} \cdot \text{l}$ ) is observed (Figure 1). The second  $\lambda_{\text{max}}$  is observed at 334 nm ( $\epsilon = 287423 \text{ cm}^{-1} \cdot \text{mol}^{-1} \cdot \text{l}$ ). UV/VIS spectrum of the compound X methyl ester in methanol (235, 214723, 255, 153374, 311, 338344 and 398, 89877 –  $\lambda$  [nm] and  $\epsilon$  [ $\text{cm}^{-1} \cdot \text{mol}^{-1} \cdot \text{l}$ ], respectively) and in 0.1 M NaOH (256, 499387, 334, 98313 –  $\lambda$  [nm] and  $\epsilon$  [ $\text{cm}^{-1} \cdot \text{mol}^{-1} \cdot \text{l}$ ], respectively) are similar to the parent compound X. It means that there are minor differences in the structure of compound X and its methyl ester. IR spectra of compound X (2) and its methyl derivative are also similar:

IR (1 mg/200 mg KBr): 3395 (w, v N–H or v O–H), 3097 (m, v C–H), 3051 (m, v C–H), 3022 (m, broad, v O–H), 1719 (s, v C=O), 1704 (m, v C=O), 1626 (m, v C=C), 1606 (s, v C=C), 1588 (s, v C=C), 1558 (s, v C=C), 1495 (s, v C=N), 1471 (s, v C=C), 1462 (s, v C=C), 1433 (s,  $\delta$  CH<sub>3</sub>), 1420 (s,  $\delta$  CH<sub>3</sub>), 1405 (m,  $\delta$  CH<sub>3</sub>), 1317 (s, v C–O), 1272 (m, v<sub>as</sub> C–O–C), 1259 (m, v<sub>as</sub> C–O–C), 1244 (m, v<sub>as</sub> C–O–C), 1233 (m, v<sub>as</sub> C–O–C), 1208 (s, v C–O), 1072 (m, v<sub>s</sub> C–O–C), 1055 (s, v<sub>s</sub> C–O–C), 1015 (m, v<sub>s</sub> C–O–C), 1013 (m, v<sub>s</sub> C–O–C), 992 (s,  $\delta$  C–H), 987 (s,  $\delta$  C–H), 894 (s,  $\delta$  C–H), 864 (s,  $\delta$  C–H), 756 (s,  $\delta$  C–H).

Analysis of the above spectrum indicates 2 carboxylic groups in the structure of X, because 2 frequencies at 1719  $\text{cm}^{-1}$  and 1704  $\text{cm}^{-1}$  are observed for C=O stretch together with 3022  $\text{cm}^{-1}$  (O–H stretch, bonded) and 1317  $\text{cm}^{-1}$  (C–O stretch) frequencies. Isoquinoline ring is observed (1495  $\text{cm}^{-1}$ ) and some other bands typical for an aromatic structure of papaverine. Numerous methoxy groups

of papaverine are also seen in the structure of X (v C–O–C stretch out-of-plane, 1272, 1244, 1233  $\text{cm}^{-1}$  and planar stretch 1072, 1055 and 1015  $\text{cm}^{-1}$ ). It also possesses conjugated double bonds with an aromatic ring at frequency 1626  $\text{cm}^{-1}$  and strong deformational out-of-plane frequencies (992 and 978  $\text{cm}^{-1}$ ) – typical for alkenes (14).

<sup>13</sup>C CP MAS NMR confirms some of the above structural features (Figure 2). Two peaks between 50 and 60 ppm argue for aliphatic carbons in methoxy groups of different steric locations. Four peaks between 90 to 100 ppm indicate a conjugated double bond chain. Five signals between 120 – 150 ppm confirm aromatic carbons. Two peaks resonate between 155 – 160 ppm and argue for the presence of either two kinds of carboxylic groups or situated in different steric environment. The last of 14 isotropic signals at 182 ppm is of unknown origin. It could be a carbon of a ketone or an ether. The sample of compound X seems to be pure and homogeneous also from NMR point of view, because its <sup>13</sup>C MAS NMR spectrum provides narrow signals. It contains some repeatable fragments of a polymer of rather simple and mobile (not rigid) molecule.

#### Molecular weight determination and elemental analysis

Number average molecular weight of the compound X and its methyl ester confirm their oligomeric structure (Table 2). Its methyl ester molecule possesses two times smaller number average molecular weight (3200 D). It means that during the above derivatization the molecule chain is also cut into shorter fragments and the carboxylic groups mentioned are methylated. The elemental analysis and the number average molecular weight determination allow to propose the molecular formulas of X and its methyl ester, respectively: (C<sub>26</sub>H<sub>26</sub>O<sub>13</sub>N)<sub>16</sub> and (C<sub>20</sub>H<sub>20</sub>O<sub>10</sub>N)<sub>n</sub> n = 7. The methyl ester contains more carbon and nitrogen atoms and less hydrogens atoms (Table 2). The above conclusions seem to be logical for an ester whose chain is partly cut off. The compound X demonstrates a remarkable Tyndall effect. Its molecules (Table 2) are not

monodispersional, because a slight transport across the membrane is observed. However, the Tyndall effect of its methyl ester is very insignificant. It means that a small number of its molecules which crosses the membrane exists in colloidal system.

### CONCLUSIONS

1. Aerobic and anaerobic conditions of papaverine photolysis do not have a significant effect on the kind of products obtained but basically influence the time of their formation.

2. Papaverine and papaveraldine photooxidation products are not significantly different.

3. Substance X can be obtained from papaveraldine and from papaverinol but the yield is much greater when papaverinol is the substrate.

4. The substance X is an oligomer consisting of 2 carboxylic groups, isoquinoline and benzene rings and some methoxy groups. The aromatic rings should be conjugated with a chain of double bonds.

5. Further spectroscopic examinations are needed to solve completely the structure of the compound X and its methyl ester.

### Acknowledgements

The present authors appreciate Prof. Bernd W. Müller's, Head of the Department of Pharmaceutical Technology and Biopharmacy of Christian Albrecht University (CAU) of Kiel in Germany, support in order to make  $^{13}\text{C}$  CP MAS NMR in the CAU Department of Inorganic Chemistry.

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*Received: 9.04.2002*