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Oxidation and Degradation Products of Papaverine. Part I: Gadamer and Schulemann's Papaverinol Synthesis Revisited

In 1915 Gadamer published in this journal [1] a procedure for the synthesis of papaverinol **2** from papaverine **1** in excellent yield. However, he did not investigate the formation of a violet fluorescence produced upon crystallization of papaverinol **2** from ethanol. The compound responsible for this fluorescence was isolated and identified as the yet unknown quaternary ammonium ion **4**, a 6a,12a-diazadibenzo-[*a,g*]fluorenylium derivative. The isolation of **4** and its structure determination by spectroscopic methods are described. However, its formation mechanism is unknown.

Keywords: Papaverine oxidation; Diazafluorene derivative; Hg^{2+} cations; Column chromatography; Structure evaluation

Received: September 19, 2001 [FP632]

Introduction

Papaverinol **2** and papaveraldine **3** are papaverine oxidation products, which contaminate authentic samples and are also found in its injection solutions [2–4]. Papaverine *N*-oxide and 6,7-dimethoxyisoquinoline *N*-oxide were identified as products of papaverine photooxidation [5]. Polarography provided evidence that papaveraldine **3** is further oxidized to a brown compound of polymeric nature under the influence of sun- and daylight [6, 7]. Different methods were used for synthesis of papaverinol from papaverine [1, 2, 8]. Gadamer and Schulemann [1] oxidized papaverine **1** to papaverinol **2** in excellent yield (93.6 %) [2]. They used mercury(II) acetate [1] as oxidant. However, they did not investigate the side products (6.4 %), the structures of which have not been analyzed up to now. We focussed our attention on the major side product detected by virtue of its violet-blue fluorescence which developed on crystallization of papaverinol **2** from ethanol, and is also observed on washing of HgS precipitated upon addition of thioacetamide to the reaction mixture in order to separate all the mercury salts present. Therefore, the goal of our experiments was the isolation and identification of the compound responsible for the violet-blue fluorescence mentioned above.

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Synthesis

Mercury(II) acetate was used to oxidize the base **1**. Mercury(I) acetate was filtered off and the excess of Hg^{2+} ions was removed. Then the filtrate was alkalized and extracted with chloroform. The extract was first desiccated and then evaporated to dryness. Papaverinol **2** was recrystallized from ethanol. The filtrate was evaporated to dryness and the residue was separated by column chromatography. After careful elution of any residual papaverinol with a mobile phase consisting of chloroform-methanol (19:1 and 18:2), a mixture of chloroform and methanol (1:1, *v/v*) was used to isolate the band of violet fluorescence of the compound under investigation, which turned out to be 13-(3,4-dimethoxyphenyl)-2,3,8,9-tetramethoxy-6a,12a-diazadibenzo[*a,g*]fluorenylium chloride **4.** The above compound base (mp 228–232 °C) was identified by means of UV/vis, fluorescence, ^{1}H and ^{13}C NMR spectroscopy, and mass spectrometry. It was also crystallized as chloride (mp 226–238 °C, decomp.). The above crystallization procedure permits removal of a red-colored impurity. Other salts of **4**, such as the picrate (mp 299–302 °C) and the perchlorate (mp 324–327 °C), can also be obtained.

Spectroscopic evaluation of the structure of 4

The UV/vis and fluorescence spectrum (in methanol) confirmed the optical properties, **4** showing the absorption maximum of longest wavelength at 393 nm. The fluorescence maximum was observed at 430 nm (uncorrected), when the excitation wavelength was set to either

Note

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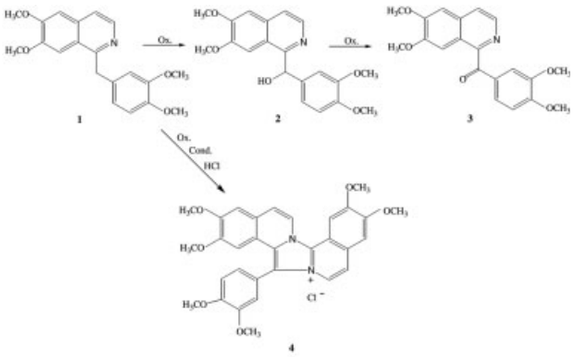

Scheme 1

285 or 395 nm. The appearance of these spectra, however, resembled those observed for papaverine hydrochloride (242, 56742; 280, 7539; 313, 5118, and 329, 4792, λ_{max} [nm] and ε [mol^{-1} cm^{-1} L], respectively) being bathochromically shifted, suggesting that a dimethoxy substituted isoquinoline moiety is still present. However, a hyperchromic effect is observed for the absorption maximum at the longest wavelength pointing towards the presence of an additional isoquinoline moiety in structure **4**.

The electron impact mass spectrum did not allow the determination of the molecular mass due to strong pyrolysis in the inlet system, thus the electrospray technique (ESI, pos. ion) was used, affording a molecular ion at *m/z* 525, and an isotope pattern (*m/z* 525:526:527) = 100:36.5:8.5 %. Interestingly, this ion was obtained as well when dissolving **4** in D_2O. Enhancement of the ion yield by addition of appropriate buffers was not observed, evidencing that the compound under investigation already contains a quaternary nitrogen atom and is not ionized by the solvent system. Furthermore MS/MS experiments in the ion trap of the mass spectrometer indicated a very stable and presumably aromatic structure as only small neutral fragments (CH_4, H_2O) were lost, with the major fragmentation series at *m/z* 525 → 509 → 493 → 475.

NMR spectroscopy confirmed the mass spectrometric data, affording the molecular formula $(C_{31}H_{29}O_6N_2)^+Cl^-$, (isotopic pattern calculated 100:36.1:7.5 %), which was confirmed by elemental analysis (Found/Calcd, C 61.27/66.37, H 5.425/5.210, N 4.655/4.990, Cl 6.23/6.32). All the hydrogen and carbon atoms resonate at different frequencies, no exchangeable protons were present in **4**. The structure of **4** was assembled using two-dimensional NMR techniques such as H,H- and C,H long range correlation. The assignments of the ^1H and ^{13}C resonance are given in the experimental section. Unfortunately, the ^1H resonance of the aromatic protons of the 3,4-dimethoxyphenyl group are partially overlapping in all of the solvents tested (perdeuterated acetone, chloroform, and dimethyl sulfoxide), making the assignment of these signals arbitrary.

Discussion

On Gadamer and Schulemann's procedure [1] papaverine **1** is mainly oxidized (93.6 %) to papaverinol (6,7-dimethoxyisoquinoline-1-(3,4-dimethoxyphenyl)-1-carbinol). Among the remaining 6.4 %, an unknown oxidation product 4 has been identified as the major constituent. The mechanism of its formation is still unknown. However, it can be obtained neither from papaverinol **2** nor from papaveraldine **3** with mercury(II) acetate. It is formed as a minor product only (0.6 %) of a parallel reaction of papaverine **1** oxidation with mercury(II) acetate according to the major pathway of Gadamer and Schulemann's synthesis leading to papaverinol (94 %) which is further oxidized to papaveraldine **3** via a very minor sequential reaction (< 1 %) (Scheme 1). We have also proved that an equimolar mixture of papaverine **1** and 1-methyl-6,7-dimethoxyisoquinoline can be oxidized to **4** with mercury(II) acetate, but the yield is still very low (0.6 %), identical to that yield of the reaction without 1-methyl-6,7-dimethoxyisoquinoline. It should also be mentioned that the presence of 1-methyl-6,7-dimethoxyisoquinoline in a mixture with either papaverinol **2** or papaveraldine **3** does not generate at all the new compound **4** with mercury(II) acetate. It seems to us that a radical mechanism may be involved in the above reaction of **4** formation. It is found in the literature [9] that papaverine possesses catalytic properties in the indigo carmine oxidation reaction with hydrogen peroxide. It is postulated that free radicals can be formed from papaverine and could be responsible for its catalytic properties. Furthermore, hydrogen atoms of the papaverine methylene group presumably form free radicals, which activate oxygen in hydrogen peroxide [10]. Therefore, condensation of a free radical of papaverine with 6,7-dimethoxyisoquinoline, formed from another molecule of the papaverine free radical, and subsequent dehydrogenation would afford the base of the isolated product **4**. The above radical pathway is only a hypothesis, requiring further investigation and is therefore not depicted in Scheme 1. The parent heterocycle was prepared according to Brown et al. from 2-bromopyridine and 2-bromomethyl pyridine [11, 12]. All the physical chemical properties of **4**, i.e. its solubility in organic solvents as a base and its spectroscopic data, are in accordance with the postulated structure.

Further investigations will concentrate on a synthetic approach to **4** in order to explain the mechanism of its for-

mation and to obtain sufficient quantities to evaluate the pharmacological properties of this interesting structure.

Acknowledgement

Prof. T. W. Hermann appreciates the scholarship obtained for one month from the Christian-Albrechts-University at Kiel and all excellent research facilities provided at the Departments of Pharmaceutical Chemistry (Prof. B. Clement) and Pharmaceutical Technology and Biopharmacy (Prof. B. W. Müller).

Experimental section

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 and are consistent with the assigned structure **4**. ESI mass spectra and MS^n experiments were performed with a Bruker Esquire~LC mass spectrometer. 300 MHz ^{1}H- and 75 MHz ^{13}C NMR spectra were recorded on a Bruker ARX 300 spectrometer. Chemical shifts are given in ppm and TMS was used as an internal standard for the spectra obtained in DMSO-d_6 and $CDCl_3$. The IR spectrum was recorded as KBr pellet on a Perkin Elmer 16 PC FT-IR instrument. A fluorescence spectrometer Perkin Elmer LS 50 B was used. UV/vis spectra were recorded on a Specord M-40 (Carl Zeiss, Jena) and **4** showed maxima at 242, 280, 374, and 393.5 nm (ε [mol^{-1} cm^{-1} L], 41480, 24383, 11883, 11883, respectively). Column chromatography was performed on aluminium oxide (ICN Alumina B-Super I), whose absorption activity III was obtained by addition of 7 % of water and was employed for the isolation of **4** from the reaction mixture. The purity of the fractions was monitored by TLC on silica gel plates (Polygram® 40 × 80 mm SIL G/UV_{254}) with chloroform-methanol (18 : 2, *v/v*). Compound **4** was visualized on the column and the TLC plates with a Hanau Fluotest Heraeus lamp at emissions at 254 and/or 366 nm. It shows a blue fluorescence when excited at 366 nm.

Synthesis of **4**

Papaverine base (5.1 g, mp 147 °C, lit. 147 °C) was dissolved in a solution of water (25 mL) and glacial acetic acid (5 mL) on heating in a water bath. Mercury(II) acetate (10.2 g) was dissolved in water (25 mL) and 2 mol/L acetic acid (5 mL). The above solutions were combined and heated until 65 °C was reached. The solution was left at room temperature for 24 h and then heated to 70 °C in a water bath for 2 h. The precipitate of $Hg_2(CH_3COO)_2$ was filtered off and washed first with water and secondly with ethanol, and the ethanol filtrate was put aside. The cool aqueous filtrate was acidified with 2 mol/L HCl (50 mL) and from it was precipitated mercury(II) sulfide by addition of thioacetamide (2.4 g) and heating in a water bath. The black-brown precipitate was filtered off and washed with small volumes of water and then with ethanol. The ethanol filtrates were combined and put aside. The aqueous reaction filtrate was alkalized with a saturated aqueous solution of sodium carbonate. The alkaline solution was extracted with chloroform and evaporated to dryness in vacuo after drying. Papaverinol **2** was crystallized from the residue obtained in ethanol. This ethanol filtrate was combined with the previously obtained ethanol solutions and was evaporated to dryness in vacuo and the residue was purified by the column chromatography procedure mentioned above. Mobile phases consisting of chloroform, chloroform-methanol (19 : 1, 18 : 2, and 1 : 1, *v/v*) were used, respectively. The chromatographic fractions with pure compound **4** were combined and evaporated to dryness in vacuo and the rose-white residue (25 mg, 0.64 %) was analyzed by spectroscopic means specified above. The chloride of 4 could also be crystallized from methanol with a few drops of 10 % HCl and a few drops of benzene or toluene to produce a white turbidity. The mixture was first cooled to room temperature and then in a refrigerator to obtain thin, long white-yellowish needles (12 mg) which decompose when melted at 226–236 °C. Compound **4** develops an intensive green color on reaction with concentrated sulfuric acid.

^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 3.44, 3.81, 3.93, 3.94, 4.04, 4.24 (6 × s, 6 × 3H, 8-, 3′-, 9-, 4′-, 3-, 2-OCH_3), 7.08 (s, 1H, H-7), 7.40 (m, 2H, H-5′, H-6′), 7.45 (m, 1H, H-2′), 7.60 (d, *J* = 6.0 Hz, 1H, H-11), 7.67 (s, 1H, H-10), 7.92 (s, 1H, H-4), 7.96 (d, *J* = 6.5 Hz, 1H, H-5), 8.20 (s, 1H, H-1), 8.25 (d, *J* = 6.5 Hz, 1H, H-6), 9.26 (d, *J* = 6.0 Hz, 1H, H-12).

^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 54.82, 55.78, 55.83, 55.88, 56.10, 56.65 (8-, 3′-, 9-, 4′-, 3-, 2-OCH_3), 102.97 (C-1), 104.74 (C-7), 109.14 (C-4), 109.21 (C-10), 111.97 (C-13b), 113.02 (C-2′), 114.59 (C-5′), 115.55 (C-6c), 116.84 (C-11), 117.14 (C-1′), 117.71 (C-13), 118.69 (C-5), 119.86 (C-6), 120.70 (C-12), 122.79 (C-10a), 124.99 (C-6′), 125.26 (C-6b), 127.72 (C-4a), 128.21 (C-13a), 149.74 (C-8), 150.00 (C-3′), 150.83 (C-9), 151.01 (C-4′), 151.37 (C-2), 152.04 (C-3)

References

[1] J. Gadamer, *Arch. Pharm.* **1915**, *253*, 284–287

[2] I. Racz, D. Varsanyi, *Pharmaz. Zhalle* **1962**, *101*, 18–25.

[3] A. S. Labenskij, *Zhurn. Obšc. Khim. (USSR)* **1952**, *22*, 886–889.

[4] F. Machovicova, V. Parrak, *Pharmazie* **1959**, *14*, 10–12.

[5] S. Pfeifer, G. Behnsen, L. Kühn, R. Kraft, *Pharmazie* **1972**, *27*, 734–738.

[6] J. Krepinskij, *Ceskoslov. Farm.* **1958**, *7*, 13–16.

[7] E. Pawelczyk, T. Hermann, *Chem. Analit. (Warsaw)* **1968**, *13*, 617–625.

[8] L. Stuchlik, *Mh. Chem.* **1900**, *21*, 813–830.

[9] A. Krause, P. Meteniowski, *Pharmazie* **1966**, *21*, 378–379.

[10] A. Krause, P. Meteniowski, *Naturwissenschaften* **1965**, *52*, 641.

[11] B. R. Brown, J. Humphreys, *J. Chem. Soc.* **1959**, 2040–2042.

[12] T. R. Jones, F. L. Rose, *J. Chem. Soc. Perkin Trans. I* **1987**, 2585–2592.