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Vilsmeier formylation of praziquantel: synthesis and application for polarographic assay

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Dedicated to Prof. Dr. Dr. h.c. mult. H. Oelschläger, Jena, on the occation of his 80th birthday

A simple and sensitive method for the polarographic determination of praziquantel (**1**) after derivatization using Vilsmeier formylation is described. The polarographically active compound obtained by this procedure has been separated, identified and prepared using *N*,*N*-dimethylformamide and phosphorus oxychloride.

1. Introduction

Praziquantel (**1**) is one of the most effective anthelmintics for the treatment of trematodiasis and cestodiasis [1–3]. Various analytical methods have been reported for the determination of this drug [4–9]. Thus, **1** was hydrolyzed by Putter [4] with potassium hydroxide after extraction from plasma and urine with organic solvents and he used dansylchorid as a chromogenic reagent for indirect fluorometric assay. A similar procedure for colorimetric determination was developed by Saleh [5] including derivatization using 4-chloro-7-nitrobenzofurazan after hydrolysis. Feasibility of quantitative determination of praziquantel (**1**) as a crystalline substance by spectral and chemical analytic procedures has been investigated by Lopatin et al. [6]. The chemical methods based on nitrogen measurement in the samples were shown to lack precision which is obligatory for quantitative drug analysis. The analytic procedures based on UV spectrophotometry were of low precision and selectivity. Lopatin et al. found that IR spectroscopy was the only method to determine the concentration of praziquantel that meets the requirements for drug substance measurement. ^{1}H NMR spectroscopic analysis has been developed by comparing the sharp singlet of maleinic acid as an internal standard at $\delta = 6{,}3$ ppm with the multiplet of praziquantel at $\delta = 7{,}3$ ppm [7]. Selective gc methods have been applied for determination in body fluids by Diekmann [8] and modified by Högemann [9]. Several chromatographic procedures based on HPLC using different detectors have been developed [10–16]. HPLC methods [12–16] and capillary electrophoresis [17] have been applied for stereoselective biotransformation studies of the main metabolites. Furthermore, pharmacokinetic parameters have been investigated by biological [18] methods and additionally macroautoradiography [19] was used for the determination of metabolites in plasma, urine, and other body fluids.

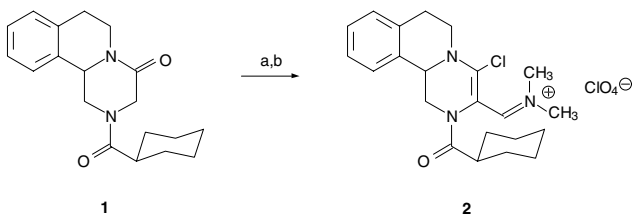
To our best knowledge, electrochemical procedures for the praziquantel assay have not yet been described. High sensitivity and good reproducibility make polarography suitable for the quantitative determination of drugs. Unfortunately, the praziquantel molecule does not contain any polarographically active group. Otherwise, it should be possible to introduce such a group by Vilsmeier formylation of its lactam structure. In the present study, effective conditions for the quantitative polarographic determination of praziquantel were examined combined with the attempt to clarify the mechanism of the reduction at the mercury electrode.

2. Investigations and results

2.1. The Vilsmeier reaction of praziquantel

3-Chloropropeniminium salts have attained synthetic importance as versatile synthons [20]. Although nucleophilic substitution of halogen atoms bound to vinylic carbons is difficult, they react easily as 1,3-bifunctional electrophilic systems with different nucleophilic reagents to form, in particular, heterocyclic compounds. When electrophilic substitution with *N,N*-dimethylformamide and phosphorus oxychloride is carried out at suitable carboxamide derivatives such as *N,N*-disubstituted acetamides [21] or lactams [22–25] 3-amino substituted salts can be obtained. We found that the Vilsmeier reaction could be successfully applied to praziquantel (**1**) since one methylene group in the piperazine moiety is sufficiently activated by the lactam carbonyl to be attacked. The product is conveniently isolated as the perchlorate **2** and the structure was unequivocally confirmed by usual spectroscopic methods. Thus, the IR-spectrum shows an intensive band in the range of 1080 to 1120 cm^{-1} for the perchlorate anion as well as signals at 2950 and 1630 cm^{-1} for aliphatic CH as well as C=N and C=O, respectively. The UV-spectrum is characterized by a maximum at 354 nm due to the cyanine structure of the molecule. Furthermore, the ^{1}H NMR spectrum shows, in addition to the expected signals for the protons of the educt [26], typical singlets for the (N−CH$_3$) protons at $\delta = 3.09$ and 3.45 ppm and a further singlet for the methine proton at $\delta = 8.29$ ppm, all characteristic for Vilsmeier products.

Scheme



1 **2**

a: DMF/$POCl_3$; b: $HClO_4$

2.2. Polarographic studies

2.2.1. Determination of praziquantel via Vilsmeier reaction

As mentioned above, praziquantel is a polarographically inactive substance and a direct assay by this electrochemi-

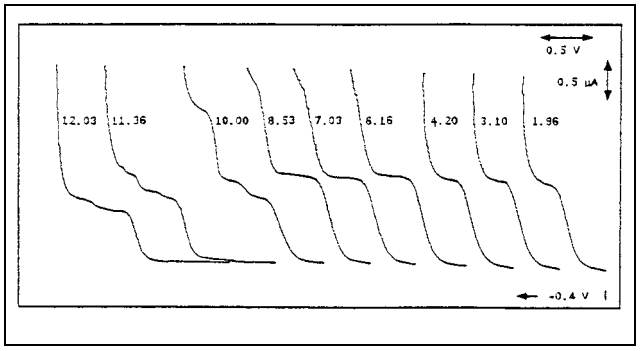


Fig. 1: DC_{rapid}-Polarograms of PVP reaction mixture in BR-buffer containing 9.4% DMF at different pH-values ($c = 1.36 \times 10^{-4}$ mol/l)

cal method seems to be impossible. But a precise and reliable procedure might be developed after derivatization of the drug by introducing an iminium ion moiety as polarographically sensitive functional group via Vilsmeier reaction (VM reaction) (Scheme).

As a result, the derivatization product **2** is characterized by polarographically high sensitivity. Using Differential Pulse Polarography (DPP_{50}) the reaction mixture of **1** and the VM reagent shows three characteristic peak potentials at pH 9.2 at −0.85 V, −1.39 V and −1.77 V, respectively. The praziquantel Vilsmeier product (PVP) **2** shows almost the same polarograms as the reaction mixture (**1** and the VM reagent) over the entire pH range under the same conditions. The pH-effects on this mixture are shown in Fig. 1.

In alkaline media, the reduction process differs from that in acid and in neutral media. Thus, in the range of pH 8.5–11.0, the pH-dependence of the mixture was carefully determined at intervals of 0.2 pH units. As a result, the half wave potential of the first wave, which was mainly investigated in this study, appears at ca. −0.75 V in the range of up to ca. pH 9.3, with almost the same wave height, another one decreases and is observed no longer above pH 10.3.

Investigating the height of the first wave dependent on the temperature, the buffer capacity and ion strength, the salt concentration and the height of the mercury reservoir we were able to show that the limiting current is diffusion controlled**.** In the range of 6×10^{-3} mol/l–6×10^{-5} mol/l, a linear calibration curve was obtained according to differential pulse polarography (DPP_{50}). For the determination of the reproducibility of this method, eight measurements were carried out under equal conditions with a relative SD of 0.72%.

This method has also been successfully applied for the analysis of praziquantel in commercially available Droncit® tablets. The experiment was carried out in strong acidic solution, because, in this pH range, no dilution process is required and the polarogram stays constant in the range of strong acidic to weak alkaline medium. The results obtained were reproducible and quantitative.

2.2.2. Investigations to clarify the mechanism

First of all, the stability of the reaction mixture (**1** + VM reagent) as well as PVP **2** in 1 N−HCl was determined by polarography and UV-spectroscopy, respectively. The results indicate that they are sufficiently stable for polarographic and UV-spectroscopic studies. Two electrons per molecule of **2** are consumed by reduction at a macroscale mercury electrode in BR-buffer (pH 6.42) containing 10% DMF; one electron is consumed in methanolic acetate buffer.

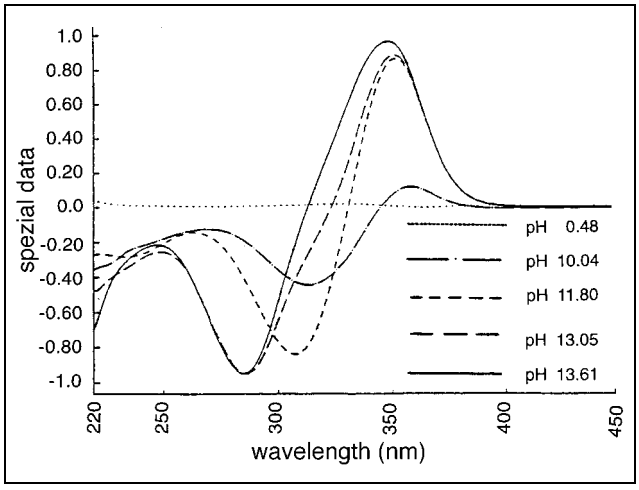


Fig. 2: Difference spectra of PVP solution containing 1.0% acetonitrile in various pH-ranges ($c = 3.0 \times 10^{-5}$ mol/l)

The analysis solutions were extracted and determined with TLC after electrolysis. The Rf-value was changed from 0.91 (before the electrolysis) to 0.55 in BR-buffer pH 6.42 containing 10% DMF. However, four spots were observed in the chromatogram using acetate buffer, namely at Rf 0.97, 0.79, 0.73, and 0.53. Therefore, it was assumed that different reactions occurred in the two analytical methods investigated. Obviously, the compound was decomposed on the thin layer plate.

The UV spectrum of **2** showed an absorption maximum at 354 nm (ε = 34230) while that of **1** had its maxima at 263 nm (ε = 355.5) and 271 nm (ε = 317.2). The pH dependence of **2** showed the same result in the UV spectrum as in the polarogram. Absorption differences between standard solutions of **2** over the entire pH range are illustrated in Fig. 2. No variation of the UV-spectrum was observed from strong acidic to slightly basic solution (pH 9.2). The absorption at 354 nm decreased above pH 10, accompanied by a hypsochromic shift.

Since the reactivity of 3-chloropropeniminium salts towards nucleophilic reagents is well-known, variations in the reactive behaviour in alkaline medium was expected. To determine the reversibility of the reaction in alkaline medium, the solution of **2** is alkalized first to pH 13 with

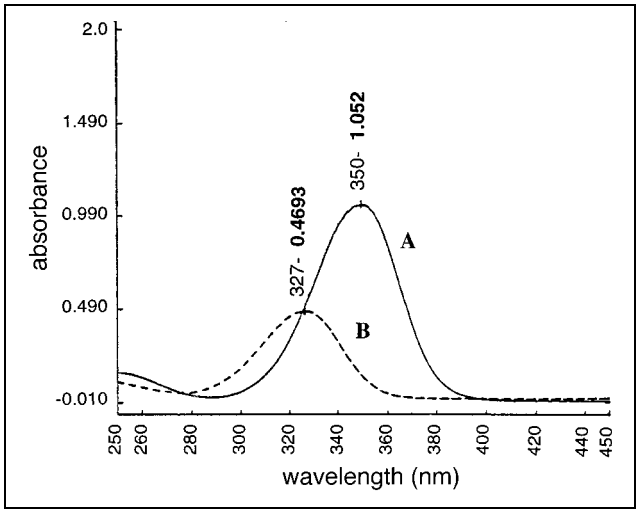


Fig. 3: UV/VIS-spectra of PVP solution containing 1.0% acetonitrile ($c = 3.0 \times 10^{-5}$ mol/l) A: at pH 1.0 B: acidified solution after alkalization with 1 N−NaOH (pH = 1.0)

1 N NaOH and then acidified to pH 1.0 with 1 N−HCl (solution B). The UV-spectrum (B) of solution B was compared with (A) of solution A, i.e. solution of **2** at pH 1.0 in the same concentration (Fig. 3). A hypsochromic shift and a remarkable decrease of the ε_{max} at 354 nm were observed. Therefore, it was assumed that an irreversible substitution process had occurred in this reaction.
The dissociation constant of **2** was determined by UV-spectrophotometry and potentiometry. No change in the UV-spectrum was visible in the range of pH 1.0–pH 9.0. Due to the insolubility of **2** in DMSO/water mixture (1 : 1), the measurement was carried out in pure DMSO; the pKa value (DMSO) of **2** was 7.85. Therefore, the relative value of the dissociation constant could only be estimated taking in account a correlation coefficient of 0.75 between the $pKa_{(water)}$ and the $pKa_{(DMSO)}$ [27].

3. Discussion

The results of the electrochemical determinations of the reaction mixture (**1** + VM reagent) and **2** lead to some conclusions. The limiting current of the first wave, which was investigated in our experiments, is diffusion controlled. It is assumed that this wave is caused by the reduction of the C=N-bond of a cyanine partial structure.
The decrease of the first wave height above pH 9.2 is caused by the hydrolysis of the iminium group. This observation could be confirmed by the UV-spectroscopic determinations. The absorption band at 354 nm, probably caused by the cyanine partial structure, decreases above pH 9.2. 3-Chloropropeniminium salts can be easily hydrolyzed [28–31] to form β-chlorovinyl carbonyl compounds, which correspond to the merocyanine partial structure of **2**.
The proposed polarographic method is simple, rapid, and suitable for routine analysis, especially if expensive equipment (HPLC) is not available.

4. Experimental

The m.p. of **2** was uncorrected and determined on a Reichert microhotstage. Elementary analysis was determined by the Microanalytical Laboratory of Ilse Beetz, D-96317 Kronach. The IR spectrum was recorded on Beckman Acculab 10 using the KBr Wafer technique. UV/VIS Spectra were recorded on Hewlett Packard 8450A. The NMR spectrum was recorded on a Bruker AM 400 spectrometer. Chemical shifts are reported in δ (ppm) relative to TMS (δ=0) for ^{1}H NMR. Polarographic recordings were carried out using a Polarecord E 506 connected to a Polarography Stand E 505, Metrohm, Herisau, Switzerland. Potentiometric experiments were carried out using a Titroprocessor E 636, Metrohm with Dosimat E 635 and Stirrer E 649. For the determination of praziquantel in tablets the VM reagent was prepared by adding 40 mmol (2,92 g) of *N*,*N*-dimethylformamide dropwise to 22 mmol (3.37 g) of phosphorylchloride under stirring and ice cooling. After 1 h at ambient temperature the VM reagent was ready for use.

4.1. Synthesis of 4-Chlor-2-(cyclohexylcarbonyl)-2,6,7,11b-tetrahydro-1H-pyrazino[2,1-a]isoquinoline-3-yldimethyliminiummethine perchlorate (**2**)

N,*N*-Dimethylformamide (40 mmol, 2,92 g) was added to 22 mmol (3.37 g) of phosphorus oxychloride dropwise with stirring. After the addition, the ice bath was removed, the mixture was stirred at room temperature for 1 h and then 10 mmol (3.12 g) of praziquantel was added in small portions at 60 °C. After 5 h the reaction mixture was dissolved in 50 ml of methanol under cooling and stirring and to the resulting solution 2.5 ml of perchloric acid (70%) were added. The yellow crystalline product was filtered, washed thoroughly with methanol and ether and recrystallized from glacial acetic acid (**2**, 7.4 g, 95%) melting at 225–226 °C.
UV λ_{max} nm, (ε): 354 (34230); IR (KBr): ν 620, 1080–1120 (ClO_4), 1630, 1670 (C=N$^+$, C=O), 2950 cm^{-1} (CH); ^{1}H NMR (DMSO-d_6): δ 1.06–2.05 (m, 10 H, cyclohexyl), 2.85–3.26 (m, 3 H, H-7a, H-7e, CHCON), 3.09 (s, 3 H, CH_3), 3.46 (s, 3 H, CH_3), 3.60–3.90 (m, 2 H, H-1a, H-6a), 4.35–4.50 (m, 1 H, H-1e), 4.82–4.97 (m, 1 H, H-11b), 5.32–5.44 (m, 1 H, H-6e), 7.25–7.44 (m, 4 H, Ph), 8.29 (s, 1 H, CH=N$^+$) ppm.
$C_{22}H_{29}N_3O_5Cl_2$ (486.4) calcd. C 54.32 H 6.02 N 8.64 found C 54.23 H 5.99 N 8.64

4.2. Analytical procedures

4.2.1. Dilution process for the polarographical studies of the effect of the pH value

One ml of DMF containing 53 mg of praziquantel was given into a 7 ml screw cap glass tube and then was mixed with 0.5 ml of VM reagent in a thermostated waterbath at 60 °C for 30 min. The contents of the glass tube was quantitatively transferred to a 25 ml measuring flask and completed with BR-buffer containing 10% DMF.

4.2.2. Determination of praziquantel in Droncit® tablets

In order to determine **1** in Droncit® (50 mg, Bayer) tablets, each tablet was extracted with 5 ml of DMF in a screw cap glass tube for 15 min in an ultrasonic bath (Bandelin, Sonorex RK 514). Then the whole suspension was filtered into a 25 ml volumetric flask and filled with DMF. One ml of this stock solution contained 2 mg of praziquantel (**1**). In a 7 ml screw cap glass tube, to 1 ml of this solution was added 0.2 ml of VM reagent and the resulting solution was warmed at 60 °C for 30 min. The mixture was then quantitatively transferred to a 25 ml volumetric flask and completed with 1.5 ml of DMF and BR-buffer. Then the polarograms were recorded.

4.2.3. Determination of $pKa_{(DMSO)}$-value

Ca. 0.27 mmol of PVP and ca. 0.56 mmol of tetrabutylammonium perchlorate were dissolved in 20 ml of DMSO and titrated with 0.1 N propanolic KOH.

4.2.4. UV-spectroscopy

Solutions with an absorption of 1.0 were prepared by using "solvents for spectroscopy". All experiments were carried out in 1 cm cells.

4.2.5. UV/VIS-difference spectroscopy

In order to determine the reaction of **2** at various pH ranges, a 3.1×10^{-3} mol/l stock solution of **2** in acetonitrile was prepared. One ml of the stock solution was diluted in two steps with BR-buffer in order to reduce the concentration of acetonitrile to 1% and then determined UV/VIS-spectroscopically. For all buffer solutions the ion strengths were 1.0. The spectra obtained were multiplied with a correction factor so that the absorption maxima indicated the same extinction as the standard solution. The difference spectra then resulted from the difference between the spectrum of the standard solution and that of each sample solution. Continuously the difference spectra were plotted, overlapped, and projected over the entire pH-range.

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