J. Clin. Chem. Clin. Biochem.
Vol. 24, 1986, pp. 565–570

Jaffe' Reaction Products

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(Received November 1, 1985/February 27, 1986)

Summary: Polarographic and spectrophotometric studies of the interaction of creatinine with alkaline picrate have been undertaken in sodium hydroxide concentrations ranging between 0.95 and 4.5 mol/l. Red colored 1 : 1 and orange colored 2 : 1 creatinine-picrate complexes readily formed along with orange colored 1 : 1 : 1 creatinine-picrate-hydroxide complexes. The red and orange colored complexes were easily identified by their corresponding absorption maxima near 490 nanometers and 390 nanometers, respectively.

Alkaline picrate polarograms showed three well-defined nitro group reduction waves with approximate half-wave potentials of −0.60 volts, −0.77 volts, and −0.91 volts. Increased concentrations of hydroxide and/or creatinine resulted in a decreased diffusion current for reduction waves 1−3 and the appearance of a fourth reduction wave, with an approximate half-wave potential of −1.24 volts. Further increases in base and/or creatinine concentration resulted in the disappearance of reduction waves 1−3, with only reduction wave 4 remaining. Based upon the experimental data, a tri-nitro anion structure has been assigned for the 2 : 1 complex.

Produkte der Jaffe-Reaktion

Zusammenfassung: Polarographische und spektrophotometrische Untersuchungen der Wechselwirkung von Kreatinin mit alkalischem Pikrat wurden in Natriumhydroxidlösungen in einem Konzentrationsbereich zwischen 0,95 und 4,5 mol/l durchgeführt. Rot gefärbte 1 : 1- und orange gefärbte 2 : 1-Kreatinin-Pikrat-Komplexe bildeten sich leicht neben orange gefärbten 1 : 1 : 1-Kreatinin-Pikrat-Hydroxid-Komplexen.

Die rot und orange gefärbten Komplexe wurden durch ihre entsprechenden Absorptionsmaxima bei 490 bzw. 390 nm leicht identifiziert. Polarogramme von alkalischem Pikrat zeigten drei gut begrenzte Reduktionswellen von Nitrogruppen mit angenäherten Halbwellenpotentialen von −0,60 V, −0,77 V und −0,91 V. Zunehmende Konzentrationen von Hydroxid und/oder Kreatinin führten zu einem abnehmenden Diffusionsstrom für die Reduktionswellen 1−3 und zum Auftreten einer 4. Reduktionswelle mit einem angenäherten Halbwellenpotential von −1,24 V. Eine weitere Zunahme der Basen- und/oder Kreatinin-Konzentration führte zu einem Verschwinden der Reduktionswellen 1−3, wobei nur die 4. Reduktionswelle übrig blieb.

Auf der Grundlage der experimentell ermittelten Daten wird eine Tri-nitro-anion-Struktur für den 2 : 1-Komplex benannt.

Introduction

The room temperature reaction between creatinine and alkaline sodium picrate, commonly known as the *Jaffe'* reaction (1), forms a red colored product which absorbs maximally near 490 nanometers.

Major products of the *Jaffe'* reaction have been investigated under a variety of reaction conditions i. e. pH, temperature, time, and reagent concentrations. The structure of the 1 : 1 creatinine-picrate product (2) formed by attachment of the methylene group of

creatinine to the meta position of the picric acid is presented in figure 1. Thereafter, similar structures have also been published by *Butler* (3), *Breckner* (4), *Kohashi* et al. (5), and *Kovar & Rupp* (6), and others. In the presence of excess creatinine, a 2 : 1 creatinine-picrate product of similar attachment has been described (7) (see fig. 2). While for heated reaction conditions, *Archibald* (8) claimed that the major products were picramic acid and methyl guanidine. These heated reaction products have recently been confirmed (9).

The formation of a 2 : 1 orange *Jaffe'* product was first described in 1928 by *Greenwald* (10). In 1975, *Butler* (7) postulated the structure of a 2 : 1 complex as presented in figure 2. *Kohashi* et al. (11), in 1977, proposed a 2 : 1 complex containing nitro anion formation in the para position (see fig. 3). Thereafter, *Kohashi* et al. (5) further claimed that in the presence of excess creatinine the colour observed during the *Jaffe'* reaction was initially due to the isomers of the 1 : 1 and finally attributed to three isomers of the 2 : 1 creatinine-picrate. While more recently, *Kovar & Rupp* (6) have described the formation of an orange colored 1 : 1 : 1 creatinine-picrate-hydroxide species as presented in figure 4. The present paper relates to detailed polarographic and spectrophotometric studies of the 2 : 1 *Jaffe'* reaction product.

Materials and Methods

Creatinine was purchased from Sigma Chemical Co., St. Louis, MO 63178. Additional reagents and apparatus were previously described (12).

A 56.7 mmol/l creatinine solution was prepared by adding 0.642 gram of creatinine to a 100-milliliter volumetric flask which was filled to volume with distilled water. A saturated picric acid solution, a 5 mol/l sodium hydroxide solution, and reagent blank alkaline picrate solutions A, B, C, D, E, F, G, H, and I, where prepared as previously described (12). Reagent blank solutions of alkaline picrate contained 0.2 milliliters of saturated picric acid and sodium hydroxide at 0.95, 1.05, 1.25, 1.50, 1.75, 2.00, 2.50, 3.75, and 4.50 mol/l, respectively.

A 50-milliliter aliquot of blank solution A was transferred to a 25 °C water-jacketed electrolysis vessel. The solution was purged with nitrogen gas and direct current polarographic analysis was initiated at exactly 30 minutes of incubation time. The reference, counter, and working electrodes were a saturated calomel electrode, a wire platinum electrode, and a dropping mercury electrode, respectively. The natural drop time characteristics of the dropping mercury electrode were: $m = 2.242$ mg s^{-1}; $t = 3.80$ s; $m^{2/3}t^{1/6} = 2.140$ mg $s^{-1/2}$. The height of the mercury column was 56.0 centimeters. Direct current polarography was performed within a potential range of -0.40 to -1.90 volts, with a scanning time of 2.5 minutes, and employing a mercury drop rate of 0.5 seconds/drop. A second 50-milliliter aliquot of blank solution A was maintained in a 25 °C water bath. Spectrophotometric analysis of the second aliquot was initiated at exactly 30 minutes of incubation time. Spectrophotometric analysis was performed between 550 nanometers and 350 nanometers versus a distilled water blank. The scanning time was 4.0 minutes. A 0.1 milliliter volume of creatinine solution was added to each aliquot of solution A. This produced

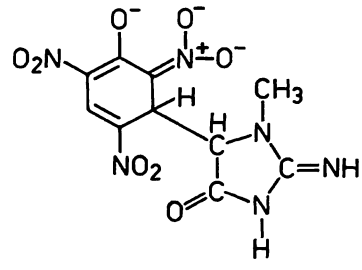

Fig. 1. 1:1 creatinine-picrate product as proposed by *Blass* et al. (2).

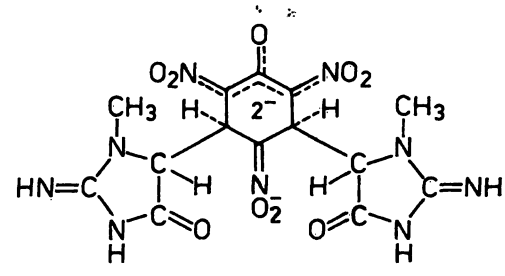

Fig. 3. 2:1 creatinine-picrate product as proposed by *Kohashi* et al. (11).

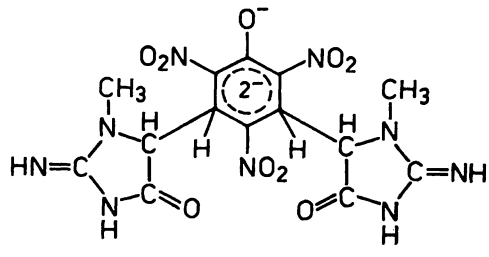

Fig. 2. 2:1 creatinine-picrate product as proposed by *Butler* (7).

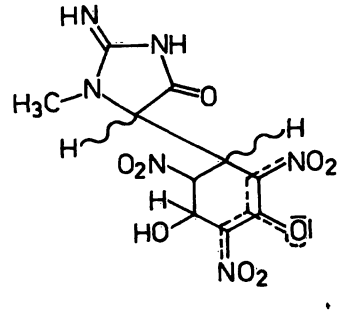

Fig. 4. 1:1:1 creatinine-picrate-hydroxide species as proposed by *Kovar & Rupp* (6).

a test solution containing an approximate 1 : 1 creatinine-picrate molar ratio. Polarographic and spectrophotometric analyses of the respective test aliquots were initiated at exactly 30 minutes of incubation time. Approximate 2 : 1 and 3 : 1 molar ratio test solutions were similarly prepared by adding 0.1 milliliter volumes of creatinine solution to 1 : 1 and 2 : 1 test solutions, respectively. Polarographic and spectrophotometric analyses were performed after 30 minutes of incubation time, as described above. Polarographic and spectrophotometric studies were similarly performed for blank and test aliquots of solutions B through I, inclusively. The above procedure was reproduced in duplicate.

Results

Three well-defined reduction waves with half-wave potential ($E_{1/2}$) values of −0.60 volts, −0.78 volts, and −0.92 volts, were observed for polarograms of alkaline picrate blank solution in the presence of 0.95 mol/l sodium hydroxide. A fourth diffuse not well-defined reduction wave of more negative potential was also observed but not measured. Upon the addition of creatinine, to produce a 1 : 1 creatinine-

Tab. 1. Polarographic results of the nitro reduction waves of alkaline picrate and 1:1, 2:1 and 3:1 creatinine-picrate molar ratio solutions at varying sodium hydroxide concentrations.

NaOH concentration (mol/l)	Creatinine-Picrate molar ratio	Picrate reduction waves[1])							
		1		2		3		4	
		$E_{1/2}$	I_d	$E_{1/2}$	I_d	$E_{1/2}$	I_d	$E_{1/2}$	I_d
0.95	[3])	−0.60	1.00	−0.78	0.94	−0.92	1.44	[2])	[2])
	1 : 1	−0.60	0.49	−0.77	0.52	−0.91	0.78	−1.24	1.10
	2 : 1	[2])	[2])	−0.77	0.31	−0.92	0.38	−1.23	2.06
	3 : 1	[2])	[2])	−0.75	0.33	−0.96	0.28	−1.24	2.20
1.05	[3])	−0.60	1.03	−0.77	1.00	−0.91	1.28	[2])	[2])
	1 : 1	−0.60	0.50	−0.77	0.60	−0.91	0.84	−1.24	1.00
	2 : 1	−0.59	0.13	−0.76	0.29	−0.92	0.35	−1.23	2.00
	3 : 1	[2])	[2])	−0.72	0.22	−0.95	0.23	−1.24	2.31
1.25	[3])	−0.60	1.03	−0.77	0.97	−0.91	1.52	−1.22	0.88
	1 : 1	−0.59	0.45	−0.76	0.56	−0.91	0.75	−1.24	1.36
	2 : 1	−0.59	0.13	−0.76	0.33	−0.90	0.25	−1.24	2.20
	3 : 1	[2])	[2])	[2])	[2])	[2])	[2])	−1.24	2.19
1.50	[3])	−0.59	0.88	−0.75	0.78	−0.91	1.34	−1.23	1.13
	1 : 1	−0.59	0.33	−0.76	0.45	−0.90	0.50	−1.24	1.69
	2 : 1	−0.58	0.11	−0.76	0.27	−0.92	0.23	−1.22	2.27
	3 : 1	[2])	[2])	[2])	[2])	[2])	[2])	−1.22	2.53
1.75	[3])	−0.58	0.75	−0.75	0.72	−0.90	0.97	−1.23	1.34
	1 : 1	−0.58	0.23	−0.75	0.35	−0.90	0.36	−1.24	1.63
	2 : 1	[2])	[2])	[2])	[2])	[2])	[2])	−1.24	2.00
	3 : 1	[2])	[2])	[2])	[2])	[2])	[2])	−1.24	2.00
2.00	[3])	−0.58	0.66	−0.75	0.66	−0.91	1.06	−1.25	1.97
	1 : 1	−0.58	0.24	−0.74	0.34	−0.90	0.36	−1.25	2.05
	2 : 1	[2])	[2])	[2])	[2])	[2])	[2])	−1.25	2.28
	3 : 1	[2])	[2])	[2])	[2])	[2])	[2])	−1.25	2.28
2.50	[3])	−0.58	0.31	−0.74	0.44	−0.89	0.66	−1.26	2.75
	1 : 1	−0.57	0.15	−0.73	0.18	−0.87	0.34	−1.25	2.44
	2 : 1	[2])	[2])	[2])	[2])	[2])	[2])	−1.25	2.25
	3 : 1	[2])	[2])	[2])	[2])	[2])	[2])	−1.25	2.20
3.75	[3])	[2])	[2])	[2])	[2])	[2])	[2])	−1.24	2.87
	1 : 1	[2])	[2])	[2])	[2])	[2])	[2])	−1.25	2.81
	2 : 1	[2])	[2])	[2])	[2])	[2])	[2])	−1.25	2.56
	3 : 1	[2])	[2])	[2])	[2])	[2])	[2])	−1.26	2.13
4.50	[3])	[2])	[2])	[2])	[2])	[2])	[2])	−1.25	2.63
	1 : 1	[2])	[2])	[2])	[2])	[2])	[2])	−1.26	2.47
	2 : 1	[2])	[2])	[2])	[2])	[2])	[2])	−1.26	2.25
	3 : 1	[2])	[2])	[2])	[2])	[2])	[2])	−1.27	2.00

[1]) The $E_{1/2}$ and I_d values presented above are reported in volts versus a saturated calomel electrode and microamperes, respectively.
[2]) The nitro reduction waves were either not well-defined or of insufficient size for measurement.
[3]) Alkaline picrate blank solutions.

picrate test solution, two polarogram changes were observed. Firstly, the diffusion current (I_d's) of reduction waves 1, 2, and 3 decreased by 40 to 50% (see tab. 1). Secondly, a well-defined 4th reduction wave of $E_{1/2}$ = −1.24 volts was observed. Subsequent addition of creatinine, to produce a 2 : 1 creatinine-picrate test solution, resulted in a further decrease of reduction waves 1, 2, and 3 with an increase for reduction wave 4. Similar I_d decreases were observed for 1 : 1 and 2 : 1 creatinine-picrate test solutions containing from 1.05 to 2.0 mol/l sodium hydroxide. Typical polarograms depicting a 1.25 mol/l sodium hydroxide blank, an alkaline picrate blank, and a 2 : 1 creatinine-picrate test are presented in figure 5. Once the sodium hydroxide concentration exceeded 2.0 mol/l, the addition of creatinine produced further I_d decreases for reduction waves 1, 2, and 3, however in contrast the I_d of reduction wave 4 started to decline slightly (see tab. 1). Further decreases in the I_d of reduction wave 4 were observed for creatinine-picrate solutions containing 3.75 and 4.5 mol/l sodium hydroxide.

Selected absorbance spectra are presented in figure 6. Alkaline picrate solutions containing 0.95 mol/l sodium hydroxide had an absorbance band near 360 nanometers.

For the 1 : 1 creatinine-picrate test solution, in 0.95 mol/l sodium hydroxide, the absorbance band near 360 nanometers had disappeared with the formation of two absorbance bands near 390 and 490 nanometers. Subsequent addition of creatinine to the 1 : 1 test solution resulted in a further increase in absorbance near 390 nanometers with a slight decrease in absorbance near 490 nanometers. Similar spectral changes were observed for creatinine-picrate test solutions containing 1.05 to 1.5 mol/l sodium hydroxide. The addition of creatinine to alkaline picrate blank solutions containing 1.75 and 2.0 mol/l sodium hydroxide resulted in slight decreases in the absorbance near 490 nanometers with relatively large increases in absorbance near 390 nanometers (see fig. 6). However, 1 : 1 and 2 : 1 creatinine-picrate test solutions containing 2.5 to 4.5 mol/l sodium hydroxide showed decreases in the 390 nanometer absorbance band.

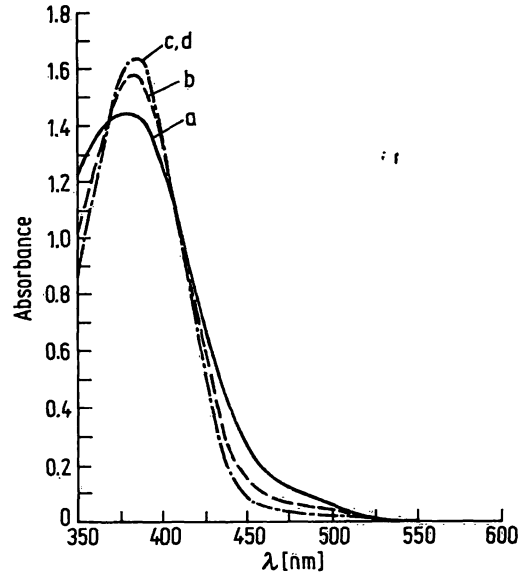

Fig. 6. Visible spectra of alkaline picrate solutions containing 2.0 mol/l sodium hydroxide with the following creatinine-picrate molar ratio: a, 0 : 1; b, 1 : 1; c, 2 : 1; d, 3 : 1.

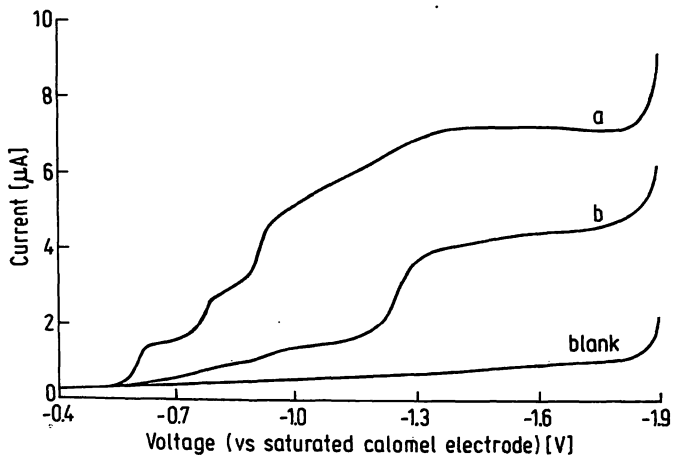

Fig. 5. Polarograms of an aqueous 1.25 mol/l sodium hydroxide blank, an alkaline picrate blank (a), and a 2 : 1 creatinine-picrate test (b) solution. Current and voltage are expressed in microamperes (μA) and volts (V) versus a saturated calomel electrode, respectively.

Discussion

Polarograms of alkaline picrate blank solutions showed three well-defined nitro group reduction waves. The addition of creatinine resulted in a decreased diffusion current for reduction waves 1—3, with the appearance of a fourth reduction wave of $E_{1/2}$ approximately −1.24 volt. Further increase in creatinine resulted in the disappearance of reduction waves 1—3, with only reduction wave 4 remaining. Under our laboratory conditions, polarographic reduction waves 1—3 were quantitatively recovered by treating the test solutions with HCl, followed by NaOH to a pH near 12.8. Recovery of the nitro reduction waves indicates that the nitro groups are not displaced from the aromatic ring. The disappearance of reduction waves 1—3 reflects involvement of the nitro groups which are not destroyed during the reaction process. Wave 4 has been attributed to the

reduction of a nitro anion species (12), of which related species have previously been described (13–15). On this basis, the appearance of a single reduction wave 4 indicates the formation of a trinitro anion complex.

In the present spectrophotometric study, the 1 : 1 red and 2 : 1 orange colored complexes were easily identified by their corresponding absorption maxima near 490 nanometers and 390 nanometers, respectively (6). Upon the addition of excess creatinine to alkaline picrate blank solutions, a mixture of 1 : 1 and 2 : 1 complexes readily formed. According to *Kohashi* et al. (5), in alkaline medium with excess creatinine, the 2 : 1 complexes are formed via the isomers of 1 : 1 complexes. This phenomenon is clearly observed in the spectrophotometric study by the decreased absorbance around 490 nanometers and the increased absorbance around 390 nanometers (see fig. 6). However, further addition of creatinine to the 2 : 1 complex test solution, showed a decrease in the absorption of 390 nanometers. Polarographic studies of this phenomenon showed a corresponding decrease in diffusion current for reduction wave 4. This phenomenon may be associated with the formation of a new 3 : 1 complex, of which a similar species has previously been described by *Abe* (16). Although the latter observation correlated well with the observed decrease of the 390 nanometer absorbance maxima, the polarographic response is presently unexplained.

Polarograms of alkaline picrate blank solutions confirmed the reactivity of hydroxide with picrate. As the sodium hydroxide concentration was increased, the diffusion current for reduction waves 1–3 decreased, while the diffusion current of reduction wave 4 increased (tab. 1). Spectrophotometric studies of this phenomenon have confirmed the presence of 1 : 1 red and 2 : 1 orange colored hydroxide-picrate complexes (12). Subsequent addition of creatinine would likely result in further reactivity of the 1 : 1 hydroxide-picrate to form a 1 : 1 : 1 hydroxide-picrate-creatinine complex, as postulated by *Kovar & Rupp* (6). Whereas, reactivity of 2 : 1 hydroxide-creatinine complexes would result in the formation of a colorless species. Colorless species formation is accompanied by a decrease of reduction wave 4.

It is not customary to present a detailed description of the difficulties encountered during research, however such may be surmised from a brief overview of the literature pertaining to the polarographic reduction of alkaline picrate. Researchers have had difficulty in establishing and agreeing upon the number of electrons involved during the electrochemical reduction of picric acid (17). Values of 16, 17, 17.1 and 18 electrons have been cited. Furthermore, under alkaline conditions even the number of reduction waves is in dispute. Three distinct reduction waves have been reported (18) for an alkaline solution of picric acid at pH 9.8, whereas only two were reported at pH 12.0. While other investigators (19) have reported three reduction waves at pH 11.7. In contrast, the present highly alkaline picrate studies support a total of four reduction waves; comprised of three nitro group waves and one nitro anion wave. It is likely that many past investigators had not taken into consideration the diffuse nitro anion reduction wave 4 observed under alkaline conditions *e. g.*, polarogram depicted in figure 5a.

Conclusion

In summary, the experimental data substantiates the formation of a 1 : 1 red colored species, a 2 : 1 orange colored species and likely a 3 : 1 colorless species depending upon the creatinine-picrate molar ratio. Based on the polarographic and spectrophotometric data, the 390 nanometer orange 2 : 1 creatinine-picrate species contains three nitro anions as depicted in figure 7.

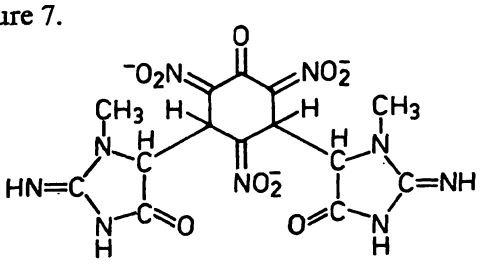

Fig. 7. Structure of the 2 : 1 creatinine-picrate product.

Acknowledgement

The authors wish to thank the Natural Sciences and Engineering Research Council of Canada for financial support of this work.

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