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The influence of reaction conditions on the derivatization of carbidopa by β-dicarbonyl compounds for enhanced optical rotation

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Abstract

Enhancement of the optical rotation of carbidopa by reaction of the hydrazine moiety with β-dicarbonyl compounds has been reported. The influence of reaction conditions on the extent and rate of pyrazole formation by the carbidopa reaction with 2,4-pentadione, 1,1,1-trifluoro-2,4-pentadione or malondialdehyde was investigated. The reaction was found to be sensitive to pH, temperature, ionic strength and the pK_a of the β-dicarbonyl compound. The extent of optical rotation enhancement was dependent on pH and the wavelength used in the polarimetric analysis. Recommendations for reaction conditions are given.

Key words: Carbidopa; β-Dicarbonyl compound; Optical rotation enhancement; pH effect; Temperature effect; Ionic strength effect; Pyrazole formation

1. Introduction

The IUPAC name for carbidopa is (*S*)-(−)-α-hydrazino-3,4-dihydroxy-α-methylbenzenepropanoic acid monohydrate (Fig. 1). Carbidopa (CD) is a decarboxylase inhibitor (Porter et al., 1962) used in combination with levodopa in Sinemet™, which is effective in the treatment of Parkinson's disease (Pakkenberg et al., 1976; Diamond et al., 1978). Carbidopa prevents many of the adverse side effects which occur as a result of decarboxylation of levodopa in the periphery (Pinder et al., 1976; Diamond et al., 1978).

Measurement of the optical rotation of CD is a means to evaluate its optical purity; however, the drug itself exhibits very low optical rotation. The US Pharmacopeia test directs chelation of CD by aluminum, using aqueous aluminum chloride as the solvent. Under these conditions, the USP requirement is a specific rotation, $[\alpha]_D^{25}$, between −21.0 and −23.5° at pH 1.5 (USP XXII, 1990). Ceccarini and Maione (1987) proposed hydrazone formation at 25°C by reacting CD with *p*-dimethylaminobenzaldehyde in a mixture of equal volumes of concentrated formic and acetic acids. The derivative exhibited a specific rotation

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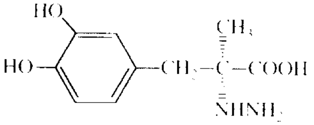

Fig. 1. The chemical structure of carbidopa.

of about + 190° after 10 min, and observed rotations were linear from 0.6 to 3% (w/v).

Because catechols are unstable at alkaline pH, the reaction of the hydrazine moiety of CD with an excess of 2,4-pentadione under aqueous acidic conditions has been recommended (Neau et al., 1992). The pseudo-first order reaction rate constant was 0.34 min^{-1} at 25°C when the solvent was 1 N HCl. The specific rotation of the product was reported to be − 139° at the mercury 365 nm wavelength, and a linear relation was found between the observed final rotation and the initial concentration of CD in the range 0.1–1.0% (w/v). The present study reports the reaction kinetics of the pyrazole derivatization of CD using 2,4-pentadione, 1,1,1-trifluoro-2,4-pentadione or malondialdehyde. The influence of reaction pH, temperature, ionic strength, and wavelength were investigated and are reported.

2. Materials and methods

2.1. Materials

Carbidopa was a gift from Merck, Sharp and Dohme Research Laboratories (Rahway, NJ) and was used without further purification. The β-dicarbonyl compounds used in this study were 2,4-pentadione (acetylacetone, AA, Sigma Chemical Co., St. Louis, MO), 1,1,1-trifluoro-2,4-pentadione (TFAA, Aldrich Chemical Co., Milwaukee, WI) and malondialdehyde (MD). Because of its instability as such, malondialdehyde was generated from its diacetal, 1,1,3,3-tetraethoxypropane (Sigma Chemical Co., St. Louis, MO), by exposure to aqueous acidic conditions and then dilution with the appropriate buffer prior to the reaction. The pH was controlled using 1.0 N HCl to obtain a pH near 0, a 0.04 M chloride buffer at pH 1.5, a 0.04 M phthalate buffer at pH 3.5 and a 0.04 M acetate buffer at pH 5.5.

2.2. Optical rotation

Optical rotation was measured at the sodium D-line at 589 nm or the mercury line at 365 nm using a water-jacketed 1 dm cell in a Perkin-Elmer Model 241 polarimeter. The polarimeter cell was rinsed and the instrument zeroed with the respective buffer solution. CD and β-dicarbonyl solutions were prepared separately in the appropriate buffer. For each reaction, the known molar concentration of the β-dicarbonyl compound was approx. 10 times the CD molar concentration to force pseudo-first order kinetics. Aliquots of CD and β-dicarbonyl solutions were mixed and the polarimeter cell was filled as quickly as possible. Monitoring the time course of the reaction began at the time of mixing. Optical rotations and the respective times were recorded. Reactions were monitored until no further change in optical rotation was observed.

2.3. Reaction kinetics

There are actually two contributions to the optical rotation that must be considered, a contribution by CD and a contribution from the product:

$$a = a_{\mathrm{CD}} + a_{\mathrm{product}} \qquad (1)$$

where a is the observed optical rotation. Since the reaction follows first-order kinetics, the loss of CD should be exponential, defining the pseudo-first-order reaction rate constant, k:

$$a_{\mathrm{CD}} = a_{\mathrm{o}} e^{-kt} \qquad (2)$$

where a_{o} is the initial optical rotation by CD alone. The appearance of product should likewise be exponential and can be given by:

$$a_{\mathrm{product}} = a_{\mathrm{f}}(1 - e^{-kt}) \qquad (3)$$

where a_{f} is the asymptotic observed rotation. The observed rotation is the sum of these contributions:

$$a = a_{\mathrm{o}} e^{-kt} + a_{\mathrm{f}}(1 - e^{-kt}) \qquad (4)$$

Fitting this equation to the experimental data using a Gauss iterative program available through the SAS 6.06 software on a VAX 6000 model 540 yielded estimates of k and a_f. From Eq. 4 it follows that the difference between the observed optical rotation at any time t and the asymptotic observed rotation should be an exponential function of time:

$$a - a_f = (a_0 - a_f)e^{-kt} \quad (5)$$

but the natural logarithm of that difference is a linear function with respect to time:

$$\ln(a - a_f) = \ln(a_0 - a_f) - kt \quad (6)$$

2.4. Specific rotation calculations

Specific rotations were estimated after the reaction had come to equilibrium. They were calculated using the asymptotic observed rotation:

$$[\alpha]_\lambda^T = 100a_f/lc \quad (7)$$

where $[\alpha]$ is the specific rotation in degrees at temperature T when measured using the wavelength λ, a_f denotes the asymptotic observed rotation in degrees, l is the length of the polarimeter cell in decimeters and c represents the initial concentration of CD in % (w/v).

2.5. Effect of wavelength

CD reactions with each of the β-dicarbonyl compounds were conducted using a mercury lamp as the source for four additional wavelengths of the incident light. The optical rotations and respective times were recorded as before.

2.6. Effect of temperature

Temperature-controlled CD and AA reactions in 1 N HCl at 15, 25 and 35°C were accomplished using a Neslab RTE-110 circulating water bath. Other reactions were conducted at room temperature, which was approx. 22°C.

2.7. Effect of ionic strength

The effect of the ionic strength on the reaction of CD with AA was examined by adding sodium chloride to alter the ionic concentrations, yet maintaining the pH and the initial AA concentration. For an aqueous solution at 25°C with an ionic strength between 0.01 and 0.10:

$$\log k = \log k_0 + 1.02Z_aZ_b\left[\sqrt{\mu}/\left(1 + \sqrt{\mu}\right)\right] \quad (8)$$

where Z is the charge on a reactant, μ denotes the ionic strength, k is the observed pseudo-first order reaction rate constant, and k_0 represents the constant when the ionic strength is zero (Carstensen, 1970).

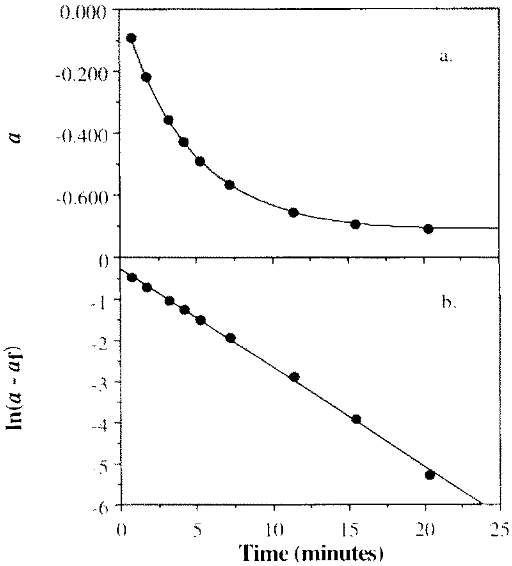

Fig. 2. Sample experimental data (a) for the room temperature reaction of carbidopa with 2,4-pentadione in 1 N HCl using the 365 nm wavelength. Linear data (b) confirms pseudo-first order kinetics.

3. Results and discussion

3.1. Confirmation of pseudo-first order kinetics

An example of the exponential data generated using this system is presented in Fig. 2. The linear relation as described in Eq. 6 confirms that keeping the β-dicarbonyl concentration essentially constant allowed a pseudo-first order reaction.

3.2. pH and pK_a effect

The pseudo-first order reaction between CD and β-dicarbonyl compounds was affected by pH

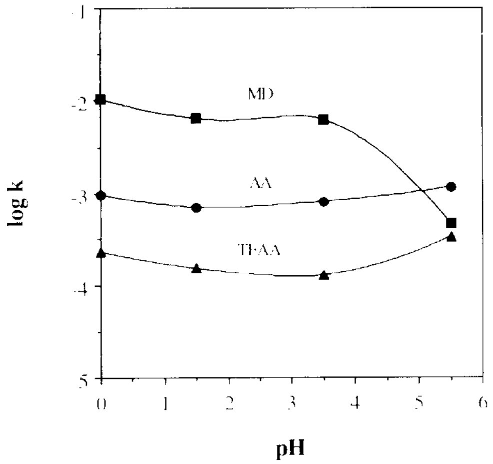

Fig. 3. Room temperature pseudo-first order rate constant for the reaction of carbidopa with beta-dicarbonyl compounds as a function of pH.

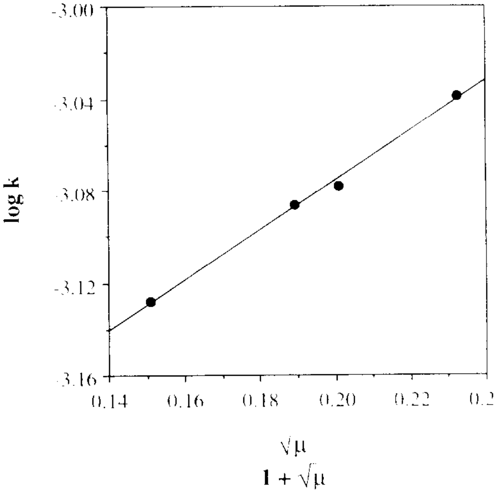

Fig. 4. Ionic strength effect on the pH 1.5 reaction of carbidopa with 2,4-pentadione at room temperature.

(Fig. 3). The most profound result, the reduced reaction rate observed with MD at pH 5.5, may be due to the weak acid nature of the methylene between the carbonyl groups. The conjugate bases apparently do not react with CD. The pK_a of each of the three dicarbonyl compounds was measured potentiometrically at room temperature. Malondialdehyde, with a pK_a of 4.5, revealed a markedly slower rate at pH 5.5 due to 90% deprotonation. The pK_a values are 8.9 and 6.5 for AA and TFAA, respectively, and these acidic conditions would allow only negligible deprotonation.

3.3. Ionic strength effect

Ionic strength had an effect on the rate of the reaction between CD and AA at pH 1.5 (Fig. 4). The equation for the linear regression line is:

$$\log k = -3.29 + 1.08\left[\sqrt{\mu}/\left(1 + \sqrt{\mu}\right)\right]$$

From Eq. 8, the charges on both CD and AA would be + 1. No ionic strength effect was observed at pH 3.5 or 5.5 indicating that the β-dicarbonyl reactant is uncharged. The effect could not be examined in 1.0 N HCl by this technique because the ionic strength already exceeded 0.1.

3.4. Temperature effect

An Arrhenius plot indicates that the reaction is faster at elevated temperature (Fig. 5). A mean activation energy of 5.7 kcal/mol was calculated from the data for reactions of AA with CD in 1 N HCl at the 365 nm wavelength.

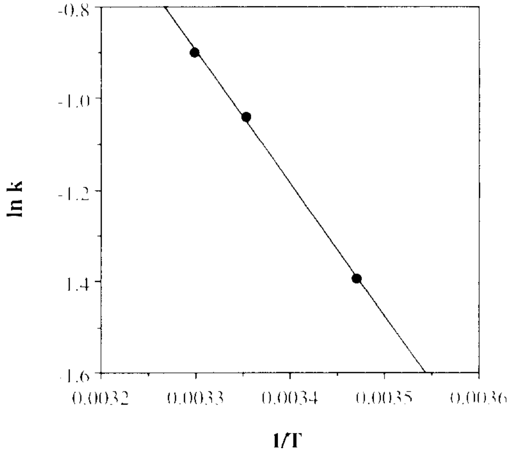

Fig. 5. Arrhenius plot of the data for the 1 N HCl reaction of carbidopa with 2,4-pentadione.

3.5. Effect of the wavelength and pH on optical rotation enhancement

The enhancement of optical rotation by CD as a result of reaction with a β-dicarbonyl compound is evident and has been discussed elsewhere (Neau et al., 1992). Chafetz (1991) has recommended use of the mercury line wavelength of 436 or 365 nm instead of the sodium D-line because the magnitude of observed rotations at these wavelengths is usually doubled or tripled, respectively, and because the mercury lamp is more stable. The dependence of specific rotation on wavelength has been described by the Drude equation (Drude, 1933; Crabbé, 1965):

$$[\alpha]_\lambda = \sum_i \frac{\kappa_i}{\lambda^2 - \lambda_i^2}$$

where κ_i are constants, λ denotes the wavelength at which the specific rotation was measured, and λ_i are the wavelengths for the maximum absorbance for the i-th optically active absorption band. For a compound with one chiral chromophore, with a maximum absorbance centered at λ_0, the equation reduces to a single term (Schirmer, 1982; Goodall, 1990):

$$[\alpha]_\lambda = \frac{\kappa}{\lambda^2 - \lambda_0^2}$$

Rearrangement yields a linear relationship between the square of the experimental wavelength and the inverse of the specific rotation:

$$\lambda^2 = \lambda_0^2 + \frac{\kappa}{[\alpha]_\lambda}$$

κ, then, is a measure of the sensitivity of the specific rotation to the wavelength of the incident light. From the slopes, intercepts and parameter values reported in Table 1, it is evident that the product from the reaction of CD with AA is less sensitive to the wavelength than the other products. The values for λ_0 are less than the mercury lamp wavelengths, indicating that the 365 nm wavelength yields the highest specific rotations using this system. Estimates of the enhanced specific rotation as a function of pH are presented in Table 2. Specific rotations were tripled when the optical rotation was measured using the mercury lamp at 365 nm rather than the sodium lamp at 589 nm as the light source.

Table 1
Data from the linear Drude relation for the product of the reaction of carbidopa and beta-dicarbonyl compounds at room temperature

Parameter	AA	MD	TFAA
Slope ($\times 10^7$)	−1.50	−2.65	−2.67
Intercept ($\times 10^4$)	2.67	4.05	5.70
r	0.999	0.993	0.995
λ_0 (nm)	163	201	239
κ ($\times 10^7$)	−1.50	−2.65	−2.67

Table 2
Specific rotation of the product of the reaction of carbidopa with 2,4-pentadione at room temperature

pH	589 nm	365 nm
0	−47	−140
1.5	−35	−102
3.5	−25	−76
5.5	−12	−34

3.6. Effect of carbidopa concentration

The asymptotic observed rotation using the sodium D-line was consistent for at least 4 h and

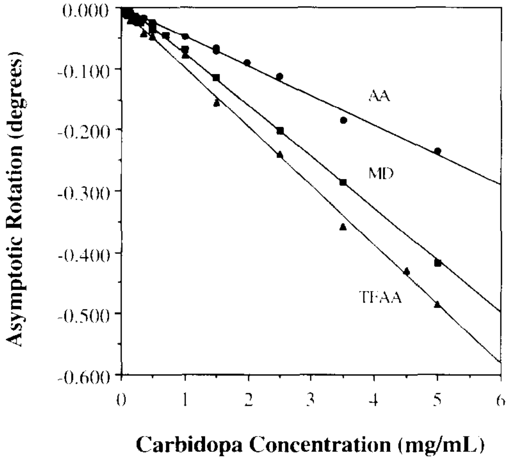

Fig. 6. Room temperature asymptotic optical rotation as a function of the initial carbidopa concentration in 1 N HCl using the D-line.

linear with respect to the initial CD concentration over the range 0.01–0.5% (w/v) in 1 N HCl room temperature reaction mixtures (Fig. 6). The linearity and the stability revealed no degradation or side reactions and indicated that the reaction can be treated quantitatively under these conditions. From the results it can be concluded that the product of these reactions adheres to the Biot relation presented in Eq. 7.

Since 1,1,3,3-tetraethoxypropane must be converted to malondialdehyde prior to reaction, the extent of conversion may be suspect. 1,1,1-Trifluoro-2,4-pentadione may actually provide two products, depending on which carbonyl reacts with the terminal nitrogen of the hydrazine moiety, and the reaction conditions may alter the ratio of the two products and, hence, the equilibrium optical rotation. It is recommended that 2,4-pentadione be used as the β-dicarbonyl reactant.

The recommended solvent is 1 N HCl, which would also maintain a reasonably constant ionic strength. Using 2,4-pentadione would provide a single product and a reasonably fast reaction with CD in 1 N HCl. Room temperature is sufficient for practical analysis; the reaction is complete within 20 min. Measurement using the 365 nm mercury line yields larger optical rotations and, therefore, is recommended.

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