Acid Dissociation Constants of Some Novel Isatin Thiosemicarbazone Derivatives

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The acid dissociation constants (pK_a) of five novel biologically active isatin thiosemicarbazone derivatives were determined by a UV−visible spectrophotometric technique at (25 ± 0.1) °C. The deprotonations for all studied molecules were found to occur at the substituted α-amino group, whereas the protonation seems to take place at the oxo group of the five-membered ring.

Introduction

Isatin or 1*H*-indole-2,3-dione is an indole derivative and was first obtained in 1841 by the oxidation of indigo with nitric and chromic acids by Erdmann[1] and Laurent.[2] Isatin is an endogenous human compound that is found to possess a wide range of biological activities. Thiosemicarbazones appear to take place in the structural class with antipox virus activity.[3] Five isatin derivatives such as methisazone (marboran), the *β*-thiosemicarbazone of *N*-methyl isatin, have been described as smallpox chemoprophylactic agents.[4]

Thiosemicarbazones basically are Schiff bases and are obtained by the condensation of an aldehyde or a ketone with a thiosemicarbazide. They are broadly classified as monothiosemicarbazones and bis-thiosemicarbazones.[5] Thiosemicarbazones constitute an important class of donor ligands which possess nitrogen and sulfur atoms, and their coordination chemistry was initially explored during the early 1960s.[6] Karali et al.[7] synthesized a series of 5-fluoro-1*H*-indole-2,3-dione-3-thiosemicarbazones and a series of 5-fluoro-1-morpholino/piperidinomethyl-1*H*-indole-2,3-dione-3-thiosemicarbazones and evaluated them for antituberculosis activity against *Mycobacterium tuberculosis* H37Rv using the microplate alamar blue assay (MABA) or the BACTEC 460 radiometric system in BACTEC 12B. Kandemirli and co-workers synthesized and characterized 5-methoxyisatin-3-(*N*-phenyl)thiosemicarbazone, 5-methoxyisatin-3-(*N*-benzyl) thiosemicarbazone, and 5-methoxyisatin-3-(*N*-(4-chlorophenyl))thiosemicarbazone.[8] Sağdinç and co-workers synthesized the zinc(II) complex of 5-fluoroisatin-3-(*N*-benzylthiosemicarbazone) and studied its properties using theoretical and IR spectroscopic methods.[9]

The acidity concept has been used in various areas of research, such as stereochemical and conformational structure determinations, the directions of nucleophilic and electrophilic attack, the stabilities of intermediates, the size of activation energies in organic reactions, and the determination of the active sites of enzymes in biochemistry.[10−13] We are now reporting on the acidity/basicity behavior of five novel isatin thiosemicarbazone derivatives.[14,15]

Experimental Section

Materials and Solutions. The studied compounds (Table 1) were synthesized, and the procedures of synthesis are described elsewhere.[8] Methanol, ethanol, KOH, H_2SO_4, HCl, CH_3COOH, CH_3COONa, NaOH, KH_2PO_4, Na_2CO_3, $NaHCO_3$, phenolphthalein indicator, and standard buffer solutions were from Aldrich and were not purified further.

Table 1. Nomenclature and Formulas of the Studied Molecules 1 to 5

molecule	IUPAC name
1	5-isatin-3-(*N*-phenyl)thiosemicarbazone
2	5-methoxyisatin-3-(*N*-benzyl)thiosemicarbazone
3	5-methoxyisatin-3-(*N*-phenyl)thiosemicarbazone
4	5-methoxyisatin-3-(*N*-(4-chlorophenyl))thiosemicarbazone
5	5-methoxyisatin-3-(*N*-cyclohexyl)thiosemicarbazone

Apparatus. pH measurements were performed using a glass electrode. pH values of the standard buffer solutions were 4, 7, and 9, and they were used in the calibration of the Orion pH/ion analyzer. The Ohaus Advanturer balance and a UNICAM UV2 PC UV−visible scanning spectrophotometer were used for measurements.

Procedure. CO_2-free NaOH solutions were prepared with NaOH pellets [(1 to 16.4) mol·dm^{-3}] in water.[16] Buffer solutions were prepared with procedures described by Perrin.[17] The potentiometric measurements were performed by measuring the hydrogen ion concentration (under nitrogen atmosphere) at (25 ± 0.1) °C, and ionic strengths of the media were maintained at 0.1 mol·L^{-1} using NaCl.

Spectrophotometry is an ideal method[18] when a substance is not soluble enough for potentiometry or when its pK_a value is particularly low or high (e.g., less than 2 or more than 11).

The proton gain of a weak base can be defined as follows:[19]

$$HX + SH \rightleftharpoons X^- + SH_2^+ \qquad (1)$$

where SH is the solvent. Then the equilibrium constant might be expressed in terms of concentration and activity coefficients:

$$K_a = \frac{a_{X^-} a_{SH_2^+}}{a_{HX}}$$

where $a = c\gamma$; a = activity constant; γ = activity coefficient; c = concentration:

$$K_a = \frac{[X^-]}{HX}\frac{\gamma_{X^-}}{\gamma_{HX}} a_{SH_2^+} = h_x \frac{[X^-]}{[HX]} \qquad (2)$$

Therefore, eq 2 can be rearranged as follows:

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Table 2. UV−Visible Spectral Data and the Acidity Constants, pK_{a1} Values, of Compounds 1 to 5 for the Deprotonation Process

	spectral maximum λ/nm		acidity measurements				
compound	neutral species[a] (log ϵ_{max})	anion[b] (log ϵ_{max})	λ[c]/nm	pH$^{1/2}$[d]	m[e]	pK_{a1}[f]	corr.[g]
1	359 (3.37)	417 (3.21)	359	9.70 ± 0.09	0.65	6.30 ± 0.09	0.98
2	360 (3.40)	365 (3.26)	377	10.50 ± 0.04	0.42	4.41 ± 0.04	0.99
3	360 (3.32)	406 (3.20)	474	9.49 ± 0.08	0.42	3.99 ± 0.08	0.99
4	362 (3.35)	400 (3.19)	403	9.62 ± 0.06	0.35	3.37 ± 0.06	0.99
5	357 (3.37)	369 (3.23)	409	10.19 ± 0.09	0.45	4.58 ± 0.09	0.99

[a] Measured in pH = 7 buffer solution. [b] Measured in pH = 13 buffer solution. [c] The analytical wavelength for pK_a determination. [d] Half deprotonation values ± uncertainties refer to the standard error for the deprotonation. [e] Slopes of the log I − pH plot.[18] [f] Acidity constant value. [g] Correlation of the log I − pH plot.

Table 3. UV−Visible Spectral Data and the Acidity Constants, pK_{a2} Values, of Compounds 1 to 5 for Proton-Gain Process

	spectral maximum λ/nm		acidity measurements				
compound	neutral[a] species (log ϵ_{max})	monocation[b] (log ϵ_{max})	λ[c]/nm	$H^{1/2}$[d]	m[e]	pK_{a2}[f]	corr.[g]
1	359 (3.37)	358 (3.34)	389	4.00 ± 0.09	0.78	3.12 ± 0.09	0.98
2	360 (3.40)	359 (3.41)	360	5.80 ± 0.06	0.84	4.87 ± 0.06	0.99
3	360 (3.32)	361 (3.21)	361	6.60 ± 0.05	0.52	3.43 ± 0.05	0.99
4	362 (3.35)	362 (3.06)	391	4.70 ± 0.09	0.87	4.09 ± 0.09	0.99
5	357 (3.37)	357 (3.33)	357	6.47 ± 0.05	1.02	6.92 ± 0.05	0.98

[a] Measured in pH = 7 buffer solution. [b] Measured in pH = 1 buffer solution. [c] The analytical wavelength for pK_a determination. [d] Half deprotonain values ± uncertainties refer to the standard error for the protonation. [e] Slopes of the log I − pH plot.[18] [f] Acidity constant value. [g] Correlation of the log I − pH plot.

$$H_x = -\log h_x = \mathrm{p}K_a - \log \frac{[HX]}{[X^-]} \qquad (3)$$

A plot of log I against H_x does not yield the pK_a at log I = 0, unless it is a Hammett base, but yields the pH at half-proton-gain value ($H_x^{1/2}$). The general eq 3 may therefore be applied, and eq 4 can be derived.

$$\mathrm{p}K_a = mH_x^{1/2} \qquad (4)$$

where $H_x^{1/2}$ describes the half-proton-gain value with respect to an appropriate acidity function and m is the slope of the line which passes through the origin (as $y = mx$).

The general procedure applied was as follows. A stock solution of the compound under investigation was prepared by dissolving the compound [about (10 to 20) mg] in water of known strength (25 mL) volumetric flasks. Aliquots (1 mL) of this solution were transferred into 10 mL volumetric flasks and diluted to the mark in buffers of various pH. The pH values were measured before and after addition of the new solution. The optical density of each solution was then measured in 1 cm cells, against solvent blanks, using a constant temperature cell holder UNICAM UV2. A scanning spectrophotometer was thermostatted at 25 °C (to within ± 0.1 °C). The wavelengths

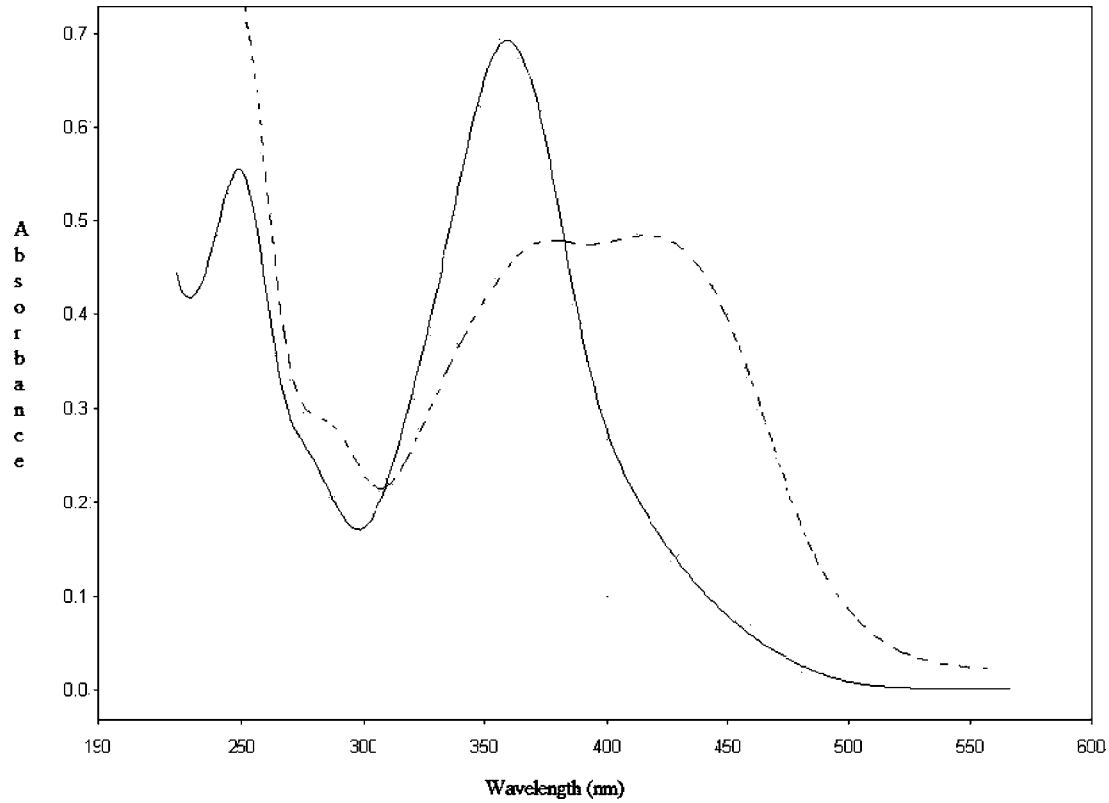


Figure 1. UV−visible spectrum of compound **1**. —, neutral molecule in pH = 7; ----, anion in pH = 13.

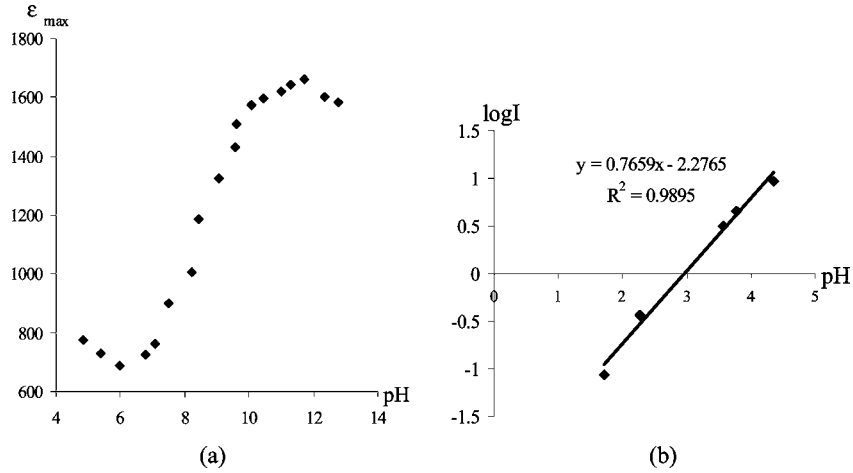
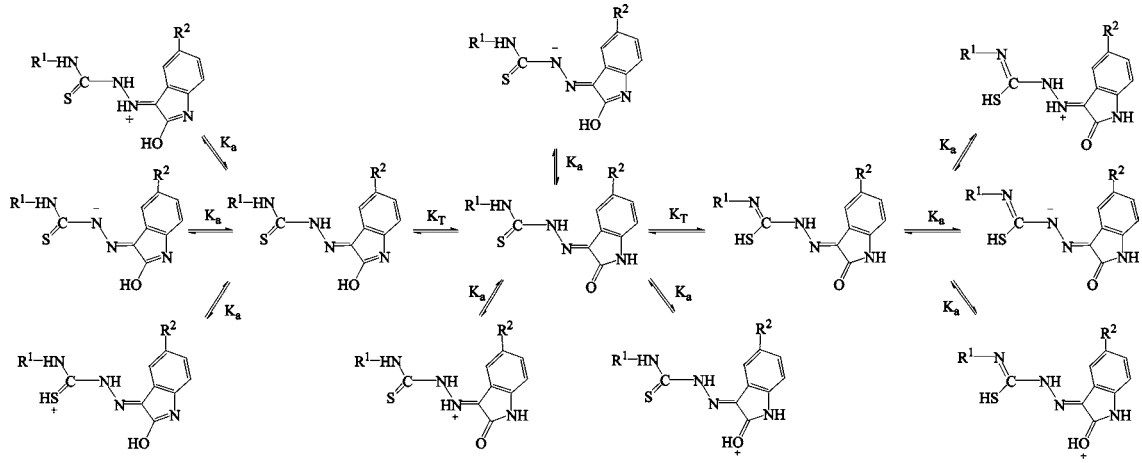


Figure 2. (a) pH − ϵ_{max} plot; (b) pH and log *I* (at 403.0 nm) plot for molecule **4** for the protonation.

Scheme 1. Possible Tautomerization and Protonation Patterns for Molecules 1 to 5



R^1= C_6H_5–, C_6H_5-CH_2–, p-Cl-C_6H_5–, C_6H_{11}–
R^2= H-, *m*-CH_3O–

were chosen such that the fully cationic or anionic form of the substrate had a much greater or a much smaller extinction coefficient than the neutral form. The analytical wavelengths, the half-proton-gain values, and the UV absorption maxima for each substrate are depicted in Tables 2 and 3.

Calculations of half-proton-gain values were carried out as follows. The sigmoid curve of optical density or extinction coefficients at the analytical wavelength (OD, λ) was first obtained (Figure 1). The optical density of the fully protonated molecule (OD_{ca}, optical density of the conjugated acid) and the pure free base (OD_{fb}, optical density of the free base) at an acidity were then calculated by linear extrapolation of the arms of the curve. Equation 5 provides the ionization ratio where the OD_{obs} (the observed optical density) was in turn converted into molar extinction ϵ_{obs} using the Beers law of OD = ϵbc (*b* = cell width, cm; *c* = concentration, mol·dm^{-3}):

$$I = \frac{[BH^+]}{[B]} = \frac{(OD_{obs} - OD_{fb})}{(OD_{ca} - OD_{obs})} = \frac{(\epsilon_{obs} - \epsilon_{fb})}{(\epsilon_{ca} - \epsilon_{obs})} \quad (5)$$

A linear plot of log *I* against pH, using the values $-1.0 < \log I < 1.0$, with a slope *m*, yields the half-proton-gain value as $pH^{1/2}$ at log *I* = 0. The pK_a values were calculated by using eq 6 (Figure 2).

Table 4

compound	**3**	**4**	**1**	**5**	**2**
half protonation ($pH^{1/2}$)	9.49	9.62	9.70	10.19	10.50
slope (*m*)	0.42	0.32	0.65	0.45	0.42
pK_{a1}	3.99	3.37	6.30	4.58	4.41
→ increasing basicity					

$$pK_a = m \cdot pH^{1/2} \quad (6)$$

Results and Discussions

The nomenclature and obtained acidity constants of studied 3-substitued indol-2-one derivatives are depicted in Tables 1 to 3. The deprotonation and protonation behaviors were evaluated in the following manner (Scheme 1).

Tautomerism. Aminohydrazon/iminohydrazin tautomerism of isatin 2-thiosemicarbazone was studied earlier, and the predominance of the amino form is well-established.[20] Therefore, we have concentrated on this form in the discussion of our results.

Deprotonation Process. The trend in Table 4 was obtained when we put the deprotonation pK_a values in order.

Since the indole part of the molecules **2** to **5** have the same structure (i.e., containing 5-methoxy-2-indolinone), the basicity seems to be affected only by the substituent on the thiosemicarbazide moiety (i.e., R^1), which means deprotonation occurs

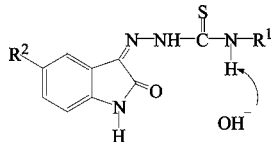


Figure 3. Possible deprotonation pathway for the studied molecules **1** to **5**.

Table 5

compound	**1**	**4**	**2**	**5**	**3**
half protonation ($pH^{1/2}$)	4.00	4.70	5.80	6.47	6.60
slope (*m*)	0.78	0.87	0.84	1.02	0.52
pK_{a2}	3.12	4.09	4.87	6.92	3.43
→ increasing basicity					

at the nitrogen atom which possesses the substituent R^1. Presumably in molecule **3** the phenyl group withdraws (i.e., $\sigma_{m\text{-phenyl}} = 0.06$ and $\sigma_{p\text{-phenyl}} = -0.01$)[21] electrons mesomerically and increases the acidity of the −NH− group. Consequently, molecule **3** becomes more acidic or less basic compared to the other molecules within the group. So the protonation occurs more easily at the C_6H_5−NH− group in basic media. On the contrary, molecule **2** has become the most basic or least acidic because the electron-withdrawing effect of the phenyl group was filtered by a $-CH_2-$ bridge. For the rest of the series we can say that the presence of the cyclohexyl ring makes the molecule **5** more basic or less acidic than that of molecule **2** but is less basic or more acidic than that of molecule **1** due to the phenyl group. In molecule **4** the presence of a chlorine atom at the para position of the phenyl ring increases the electron-withdrawing effect of R^1, and the molecule becomes more acidic or less basic as expected because the σ_p value of chlorine is very small (i.e., $\sigma_{p\text{-Cl}} = 0.23$).[21] Furthermore, it seems that the mezomeric electron-donating effect of the methoxy group (CH_3O), which

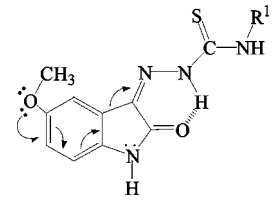
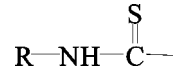


Figure 4. Conjugation and possible H-bonding formation in studied molecules.

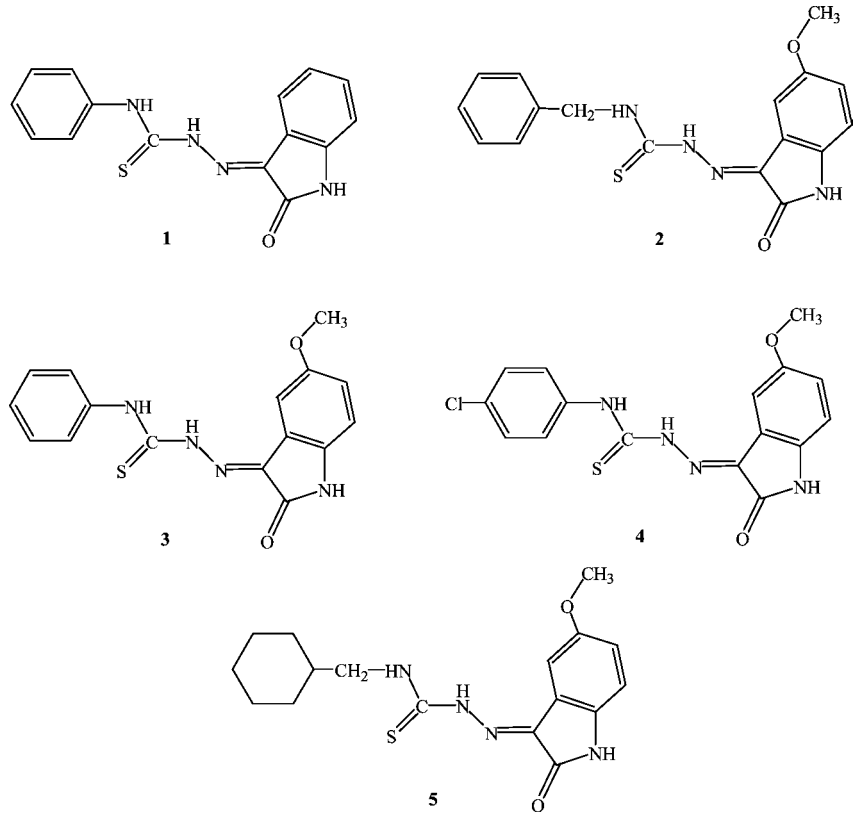
is located at position 5 of the studied molecules, is reflected in molecules **1** to **4** by making them more basic compared to molecule **5**, which has no substituent at the benzenoid moiety of indole. Since the deprotonation pK_a value for indole was reported as 16.97,[22] we can conclude that the deprotonation for all studied molecules occurs at the group probably with



the mechanism in Figure 3.

The pK_a values of Schiff bases derived from isatin with aryl hydrazines were found as slightly basic (i.e., about 10).[23] So we can compare our pK_a values of Schiff bases derived from istain with thiosemicarbazide which also has a slightly basic character (Table 1), and we can conclude that a deprotonation mechanism occurs with the same mechanism as we have shown in Scheme 1. The pK_a values suggest that the proton dissociates from the NH group adjacent to the thione (C═S) group rather than from the NH proton of the isatin moiety, which may dissociate at basic pH values. However, a similar conclusion was obtained by Hassan et al.[24,25]

Scheme 2. Formula of Studied Molecules 1 to 5



Proton-Gain Process. For the protonation process the trend in Table 5 was obtained when we put the protonation pK_a values in order.

The highest basic character of molecule **3** obviously indicates the mezomeric electron-donation effect of the methoxy group which is located at the meta position of the benzenoid ring and can have a through conjugation effect over the imine nitrogen which is located at the five-membered pyrrole ring of indole. Furthermore, a possibility of H-bonding is the least compared to the other molecules (Figure 4).

On the other hand in molecule **1** there exists no substituent on the benzenoid ring. Consequently, there is no conjugative or inductive effect in this molecule. Besides, the H-bonding possibility is the strongest in this molecule. Therefore, molecule **1** becomes the least basic among the studied molecules. The lesser effectiveness of the *m*-methoxy group in molecules **2**, **4**, and **5** may rise out from the changing strength of the possible hydrogen bonding which may change with the R^1 group. Obviously an H-bond is stronger in molecule **4** and less strong in molecule **2**, and in turn it is even less strong in molecule **5**.

Since the tautomeric equilibrium studies have indicated the favorability of the keto form for the 2-indolinone derivative,[26] we can predict that the pK_a value for the protonation of the studied compounds should be close to the protonation acidity constants of 2-indolinone. The pK_a value of α-protonation for indole has been reported as p$K_a = -3.5$ which is close enough to pK_a values of α-protonation for 1-methyl indole, indicating a similar protonation mechanism for these two molecules.[22] The pK_a values of studied molecules **1** to **5**, however, have pK_a values ranging from 4 to 7, suggesting a different protonation pathway from indole and 1-methyl indole. So we can suggest an oxo (carbonyl) protonation for the studied molecules **1** to **5** as depicted in Scheme 2.

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