# 1
# Tautomerism: Introduction, History, and Recent Developments in Experimental and Theoretical Methods

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## 1.1
## The Definition and Scope of Tautomerism: Principles and Practicalities

*Prototropic tautomerism*, defined by one of its early investigators as ''the addition of a proton at one molecular site and its removal from another'' [1], and hence clearly distinguished from ionization, is one of the most important phenomena in organic chemistry despite the relatively small proportion of molecules in which it can occur. There are several reasons for this. Enantiomers, or cis and trans isomers, possess a formulaic identity just as tautomers do but are difficult to interconvert and hence easy to isolate. Tautomers are different. Tautomers are the chameleons of chemistry, capable of changing by a simple change of phase from an apparently established structure to another (not perhaps until then suspected), and then back again when the original conditions are restored, and of doing this in an instant: intriguing, disconcerting, perhaps at times exasperating. And a change in structure means changes in properties also. A base may be replaced by an acid and *vice versa*, or more to the point perhaps, a proton acceptor group by a proton donor, as, for instance, carbonyl by hydroxyl. Hence, if the major tautomer has biological activity, the replacement of this structure by another may result in a total mismatch in terms of receptor binding or the partition coefficient. It therefore becomes vital, on the most elementary level, to know which tautomer is the major one, since not only the structure but also the chemical properties are bound up with this. This problem is compounded by another: there is no automatic guarantee that, if the great majority of known compounds in a given category exist chiefly as one tautomer, the next one to be investigated will follow their lead. Examples of this sort will be described below. Hence an understanding of the factors that give rise to this problem becomes more important as time goes by.

Except for proton transfers on and off carbon,[1)] whose rate depends on pH and can sometimes take weeks. Proton transfer in the course of tautomerization is typically a very fast process. The equilibrium between tautomers is dynamic.

1) An excellent, up-to-date account of tautomerism involving carbon – hydrogen bonds can be found in [2].

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$$\text{Enol} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} \text{Keto} \tag{1.1}$$

where the equilibrium constant $K_T$ is given by

$$K_T = \frac{k_f}{k_r} \tag{1.2}$$

Since the sum of the forward and reverse rates ($k_{obs} = k_f + k_r$) determines the measured rate, as indicated in Eq. (1.1), whichever is the faster will dominate the process. With the exception noted above, $k_{obs} \geq 10^6 s^{-1}$ when $k_f$ and $k_r$ are similar in magnitude and rises toward the relative diffusion limit as the imbalance between them increases; that is, as $K_T \gg 1$ or $K_T \ll 1$ in Eq. (1.2) is approached. At such speeds, there is simply no hope of "freezing" the process, and worse, no way of isolating a minor tautomer, as on attempting isolation it would instantly be transformed into the major one. The classic way around this is to use the properties, for example, $pK_a$ of "model compounds," chosen that are as close electronically as possible to those of the minor tautomer. This is described in Chapter 12, along with certain pitfalls in their use which are often neglected. Another technique that can sometimes bypass the problem is to use linear solvation energy relationship (LSER) methods, which are described in some detail in Chapter 11. The reader is referred to both these chapters for further details.

On the other hand, the small differences in free energy between the components make them very useful. In biological systems, delicate and subtle control is needed for the organization of chains of reactions. Life is the controlled motion of electrons and protons. The thermodynamics and kinetics of electrons are to a large extent governed by redox centers, and the equally important motion of protons can be viewed as an extended series of tautomerization reactions. DNA is built from bases all of which have a number of different tautomers. There are even a few enzymes, called tautomerazes, that enable rapid tautomerization between keto and enol forms of molecules [3]. Tautomers are interesting for many reasons, technological as well as fundamental. Their optical properties make them suitable as signaling molecules in sensors, as they can rapidly switch between states. Many biologically important molecules have several tautomers. Adenine, for instance, an important moiety in DNA and adenosine triphosphate (ATP), comes in three varieties, the main one–according to some people–chosen by nature to avoid fluorescence. One of the more interesting and complicating properties is that tautomeric equilibria in the ground state are often vastly different from those in the excited states. In addition, tautomeric equilibria are easily shifted by the environment.

Tautomers are also the prime molecules for studying proton transfer. Initially this was thought to be an advantage: Lapworth and Hann [1], in one of the earlier kinetic studies of tautomerization, state:

> Of the various types of isomeric change, that which involves a change of position of one hydrogen atom only, as in a simple desmotropic change, would, for various reasons, appear to be the most simple, and probably the most easy to investigate.

Of course, this was before the invention of quantum mechanics, and they could not have foreseen the enormous literature that proton transfer reactions would generate in the next century and the conceptual problems this seemingly simple reaction would engender. The fact that the proton is at the borderline between classical and quantum mechanics is another complicating factor, of which some of the issues will be explored in Chapter 9.

In the next few sections, we describe the influence of a number of parameters: aromatic resonance, lone-pair and dipolar repulsion, internal hydrogen bonding, electronegative substituents, and the surrounding solvent on the relative stability of tautomeric forms. The remainder of this chapter is devoted to a brief history of tautomeric equilibria and tautomer dynamics.

## 1.2
## Causes of Reversal in Tautomeric Form: Aromatic Resonance

Change of aromaticity in a ring system influences the position of the equilibrium between tautomers. This may be the reason for the excited-state proton transfer reactions in *ortho*-hydroxybenzaldehyde derivatives, where the excited state loses aromaticity, but it is also evident in the ground state of a number of compounds.[2)]

This can happen in a number of contexts but we present just one case. Figure 1.1 displays the effect on **1a**, in which the enol **1b** is a minor tautomer [5, 6] of inserting carbon–carbon double bonds to give **2** [7], thus allowing the ring to become aromatic in **2b**, while the accompanying reversal in the energetics of the tautomers, at $\Delta \log K_E \approx 17.4$, is equivalent to $\Delta G \approx -23.6$ kcal mol$^{-1}$, that is, a value not far short of current estimates for the resonance energy $\Delta H$ of benzene.

Here, an important contrast is with **3** and **4**, where an estimate for piperidine-2-one **3** is contrasted with the (corrected) value for 2-pyridone **4**.[3)] In that case,

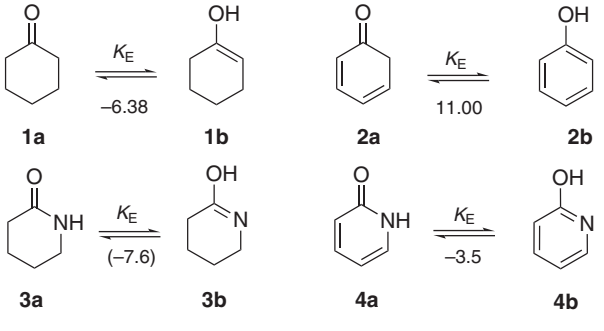



**Figure 1.1** Tautomerism in some alicyclic and aromatic ketones and amides.

2) Often, a tautomerization constant $K_T$ is defined on the basis of the dominant tautomer in the gas phase. In [4], it is argued that this is not always good practice, and we prefer to use the enolization constant $K_E$ for that reason.
3) See Chapter 12.

the estimated rise in log $K_E$ is only $\Delta \log K_E \approx 4$, leaving the latter's amide character reduced but intact. The explanation must lie in the conjugation present in 2-pyridone **4a** itself, which, while less than the aromaticity of its iminol **4b**, is sufficient for the purpose, in contrast to **2a** which possesses no through conjugation at all. Katritzky and coworkers [8, 9] have drawn attention to the aromaticity still present in 2-pyridone and related compounds and have made attempts to quantify it.

## 1.3
## Causes of Reversal in Tautomeric Form: Lone-Pair and Dipolar Repulsion

Figure 1.2 is a diagrammatic representation of the repulsion between two π-acceptors (**5**), two π-donors (**7**), and the compromise position (**6**) with one of each, which is always taken up except when, as can happen with linked equilibria, the overall result is a less favorable energetic position than before. Note (i) that lone-pair repulsion in **5** disappears on twisting one nitrogen atom through 90°, as in azobenzene, but (ii) that bond angle does not appear to matter in **7** as far as present evidence goes [4]. Also note that NH is not the only π-donor that can be involved; if one NH is replaced by an O, the effect is considerably greater, and S is also capable of causing dipolar repulsion when contiguous with NH, though its position in the ''pecking order'' is probably closer to NH than to O [4]. Finally, note that the effect of replacing one NH by NR is dependent on the electronic effect of R on N; while NH and NMe are roughly on the same level, NPh lies about half way between either and O in its overall effect [4].

Figure 1.3 displays, in **8** and **9**, the extreme ends of the scale in $K_E$ for the effects of dipolar repulsion on amide tautomerism. It starts with *N*-methylacetamide **8**, where none exists, and proceeds by stages, via 2-piperidinone, 2-pyrrolidinone, and the $\Delta^4$–C=C derivative of the latter, to 3-hydroxyisoxazole **9**,[4] all in aqueous solution. Both $K_E$ values are estimates, but neither should be badly wrong. A range in log $K_E$ of nearly 11 is not quite comparable to that found for **1**→**2** but is still the next largest that we have encountered [4]. Two individual compounds are also shown. The quinoxalinones **10a** ($R_1 = H$ or $CO_2R$, $R_2 = CO_2R$ or CN) were examined by Kurasawa *et al.* [10] using NMR in dimethyl sulfoxide (DMSO) and DMSO–TFA (trifluoroacetic acid) mixtures and were found to go 100% to **10b** despite loss of aromaticity, presumably because of dipolar repulsion in **10a**; no quantitative data were reported, however. Pit'ha and coworkers [11] examined

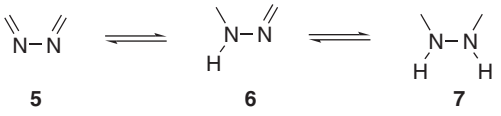



**Figure 1.2** Diagrammatic representation of lone-pair **5** and dipolar **7** repulsion.

4) P.J. Taylor (2012), The Fault Line in Prototropic Tautomerism, manuscript under preparation.

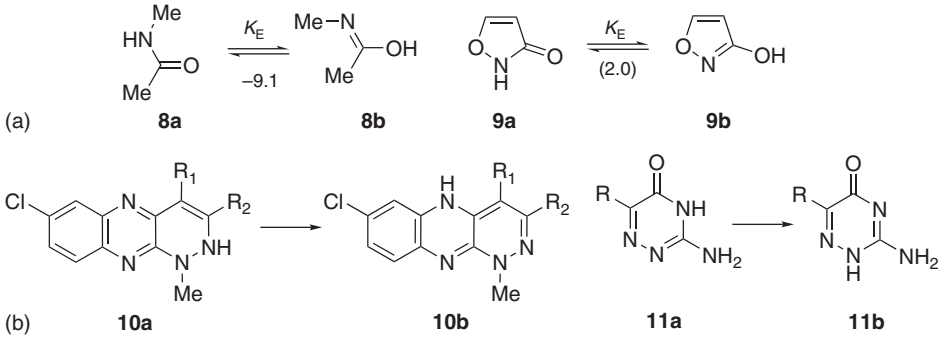



**Figure 1.3** (a) The most extreme examples known, **8** and **9**, of $K_E$ for dipolar repulsion in related amides. (b) An unquantified case of dipolar repulsion, **10**, set against a quantified case, **11**, of lone-pair repulsion.

**11** in aqueous solution and, using the "basicity method" (see Chapter 12), found log $K = 2.40$ in favor of **11b** for R = H and 2.34 for R = Me; this is in line with other examples of lone-pair repulsion in six-membered rings, for which we [4] find $\Delta \log K = -2.2 \pm 0.2$ for six examples. This is one of the few examples of lone-pair repulsion to have been quantified so far.

## 1.4
## Causes of Reversal in Tautomeric Form: Selective Stabilization Through "Far" Intramolecular Hydrogen Bonding

Although a shift in the real tautomeric equilibrium can be achieved as a rule by changes in the environment and, as rule again, it is difficult to be done in controlled manner, there are some cases where controlled switching is possible through structural modifications that do not directly influence the tautomeric skeleton. This happens in **12** (Figure 1.4), where, compared to the parent compound **13**, the intramolecular hydrogen bonding between the tautomeric OH group and the basic nitrogen from the side arm leads to the disappearance of the keto tautomer [12, 13]. The situation changes upon protonation (or complex formation) – the basic nitrogen is protonated and a new hydrogen bonding, this time between protonated sidearm and tautomeric carbonyl group, shifts the equilibrium toward the keto form **12H$^+$**. In this way, by changing pH (or salt concentration) of the solution, controlled shift in the position of the enol–keto equilibrium can be achieved. Unfortunately, the efficiency of the switching system crucially depends on tautomerism in the parent skeleton: it works in the case of the azonaphthols **13** and **14** [14] and the heterocyle **15** [15], but does not in azophenol **16** or azoanthranol **17**, where the tautomeric equilibrium is strongly shifted a priori. The replacement of the side arm, as it is in **18** [16] and **19** [17], does not stabilize the enol tautomer because of keto dimer formation (**18**) or double hydrogen bonding stabilization of the keto tautomer (**19**).

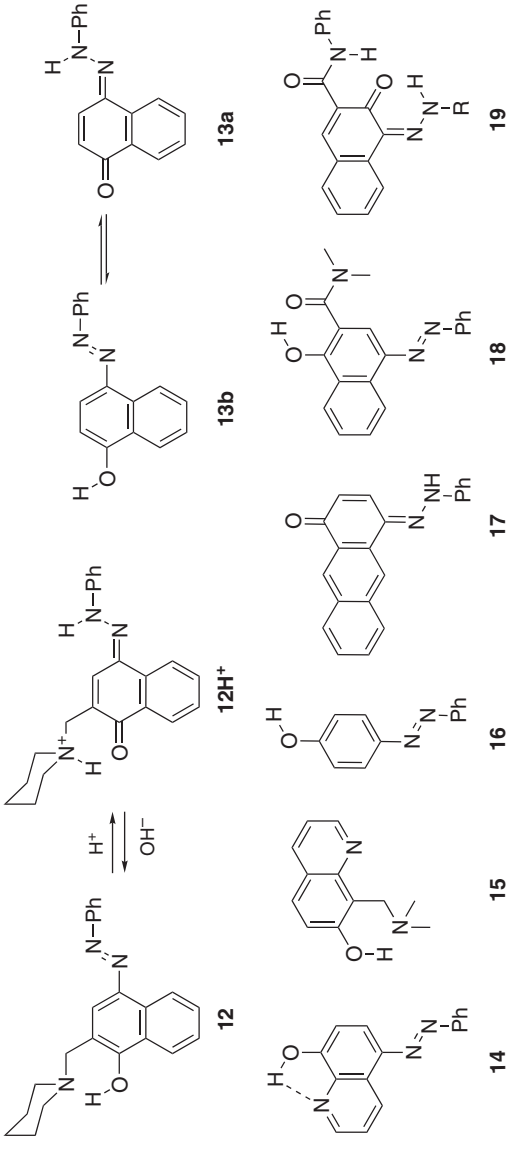



**Figure 1.4** Intramolecular hydrogen bonding with a side chain group.

## 1.5
## Changes in Tautomeric Form Brought About by Electronegative Substituents

This problem, which is specially prominent in oxoheterocycles, is caused largely by the effect of electronegative substituents in engineering a switch from the oxo to the less polar hydroxyl tautomer. There is little good documentation on this subject in the literature so we have generated our own [4]. The type of equation we have tried, and which works well enough to be provisionally worth pursuing, typically takes the following form:

$$\log\ K_T(\text{obs}) = \log\ K_T(\text{parent}) - \sum(\sigma) \tag{1.3}$$

where $\log\ K_T$(parent) is that of the parent oxoheterocycle, $\log\ K_T$(obs) refers to that resulting from substitution, and $\sum(\sigma)$ lists the relevant σ-values for the substituent, of which there may be more than one. We have so far distinguished four situations, each with its own governing equation. Positions adjacent to NH are much more sensitive to substitution than any others, and two equations are required each with two terms, one for lactams (e.g., 2-pyridone) and the other for vinylogous amides (e.g., 4-pyridone):

$$\text{For 2-pyridone:}\quad 3\sigma_m - 8\sigma_I \tag{1.4}$$

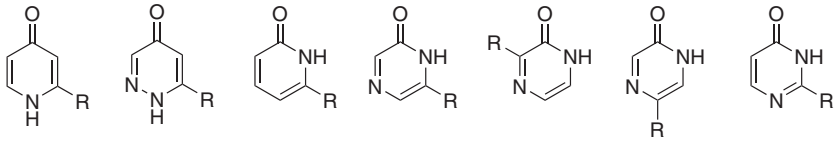
$$\text{For 4-pyridone:}\quad 3\sigma_m - 10.5\sigma_I \tag{1.5}$$

where $\sigma_m$ and $\sigma_I$ are the so-called electrical effect substituent constants used in correlation analysis for the equilibrium constant [18]. Here, $\sigma_I$ for 4-pyridones is derived from Taft's equation [19] for the $pK_a$ values of 2-substituted pyridines, dominated by the term $-10.5\sigma_I$, while $-8\sigma_I$ resulted from a trial-and-error approach which suggested that a similar but less extreme value should fit the corresponding equation for 6-substitution into its 2-hydroxy derivative. The $\sigma_m$ term with its opposite sign monitors the partially cancelling effect of the 2-pyridone tautomer. The apparently universal use of $\sigma_m$ here and elsewhere, and never $\sigma_p$, is at first sight surprising but may be due to the considerable reduction of resonance transmission in heterocycles relative to purely benzenoid structures. Only for one other position in 2-pyridone do we possess adequate data for both the NH and (as OMe) the OH tautomers, but these give $\sigma_m$ values of $-2.7$ and $-6.1$ respectively, leading to $-3.4$ for $\log\ K_T$, which, rounded off to $-3$, also fits the scattered data for 3- and 5-substituted 2-pyridones and, very accurately, the 5-position of 4-pyrimidone [4]. On this suggestive, though fundamentally inadequate, evidence, we provisionally adopt Eq. (1.6) for all but one of the substituent positions in any monocyclic oxoheterocycle not covered by Eqs. (1.4) and (1.5):

$$\log\ K_T(\text{obs}) = \log\ K_T(\text{parent}) - 3\sigma_m \tag{1.6}$$

$$\log\ K_T(\text{obs}) = \log\ K_T(\text{parent}) - 0.7\sigma_m \tag{1.7}$$

The exception is 3(5)-substituted 4-pyridones, to which Eq. (1.7) applies. Katritzky and coworkers [20] have drawn attention to the extreme shallowness of this response, which could be due to the symmetrical positioning of $R^3/R^5$ between



| –1.3 | –2.0 | 0.3 | –1.3 | –0.3 | –0.3 | 0.3 |
|---|---|---|---|---|---|---|
| **20** | **21** | **22** | **23** | **24** | **25** | **26** |

**Figure 1.5** For R=$NO_2$, monocyclic oxoheterocycles likely take up the hydroxyl form.

the O and N cations. If so, there may be other such positions in oxoheterocycles waiting to be discovered. The force of Eq. (1.7) is that even the nitro group should reduce the dominance of the oxo form by only $\Delta \log K_T \approx 0.5$.

Figure 1.5 contains calculations, above the compound number, of log $K_T$ for R=$NO_2$ in all those monocyclic oxoheterocycles of known parent log $K_T$ which are seriously at risk of going over to the hydroxyl tautomer in aqueous solution – in any other solvent this will be more likely. The nitro group was chosen as the most electronegative of common aromatic substituents, but it should be noted that multiple substitutions can make the risk even greater. Particular attention should be drawn to perhalogenation, a very common feature among such molecules and likely to prove a particularly lethal one. Benzofusion, which leads to a considerable rise in log $K_T$, $\Delta \log K_T$ 1.0 or 1.8 according to its position with respect to ring NH,[5] will clearly reduce the risk, so no examples are considered. Also note that aminoheterocycles are not at risk at all, since these are less polar than their imino tautomers, and electronegative substituents can only increase their dominance.

## 1.6 The Influence of Solvent on Tautomeric Form

This is a large subject which, so far as it can be quantified, is treated in Chapter 11. Here, however, the approach is qualitative and a broad sweep is attempted. Katritzky *et al.* [21] have enunciated the principle that the most polar tautomer of a given compound is generally the one to be found in the solid state; an equally valid principle is that liquid tautomers are generally dominated by the least polar tautomer. The latter situation may be exemplified by the invariable finding that aliphatic and some other simple thiones exist in the thiol form in liquids [4]. In fact, careful work in aqueous solutions suggests a typical gap of $\sim 10^6$ between $K_E$ for ketones and thioketones in water [22], so that if $10^{-7} - 10^{-8}$ is taken as typical for a ketone, $10^{-1} - 10^{-2}$ becomes the corresponding value for a thioketone. Kresge and Meng [23] studied **27** (Z = O, Figure 1.6) and **27** (Z = S) in aqueous solution and found log $K_E = -6.92$ and $-0.94$, respectively; both are crowded molecules and probably nonplanar, thereby helping the stability of the thione but perhaps causing mildly distorted $K_E$ values. However, they make their point.

5) See Chapter 12.

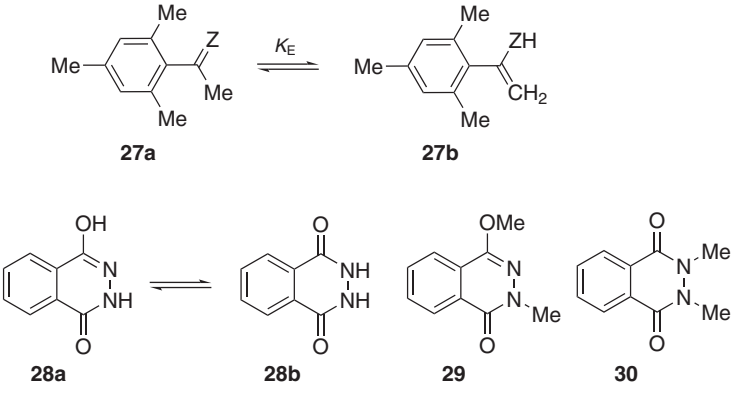



**Figure 1.6** Some effects of the structure and solvent on tautomer preference.

The other persistent source of trouble is hydrogen bonding in the solid state, which may not correspond to what happens in solution. Elvidge and Redman [24] studied the tautomerism of **28** and concluded that it exists as **28a**, the same dominant tautomer as for the related ''maleic hydrazide,'' both in aqueous solution and the solid state. In fact, while this is true for the solid state, with a strong $\nu$(OH) peak and a solitary strong $\nu$(C=O), it is not true for aqueous solution, in which the UV spectrum of **28** much more closely resembles that of **30** than of **29** [24]. So why the difference? A likely reason is that, instead of the amide dimers that **28b** might be expected to form in the solid state, **28a** will form iminol dimers, which are generally much stronger.[6] While this might not be sufficient to generate a tautomeric switch if the energy difference between **28a** and **28b** were great enough, in the present case the gap is probably small enough to allow it. We have encountered other cases [4] in which this situation probably occurs.

## 1.7
## Tautomeric Equilibrium: Historical Overview of an Analytical Problem

It is difficult to trace when and how exactly tautomerism was discovered. In the first tautomeric book, written by Baker [25], the priority is given to Berzelius, who in 1832 used the term ''metamerism'' to explain reciprocal conversion of cyanic and cyanuric acid. In Ingold's [[26], Chapter 11] review on tautomerism, ethyl acetoacetate, discovered in 1863 by Geuther [27], is mentioned as the first tautomeric compound described (Figure 1.7).

The real fact is that many compounds were discovered in the second half of the nineteenth century, whose properties and behavior were impossible to explain with the available concepts at that time in structural chemistry. Here we can mention some of them: preparation of *p*-bromodiazoaminobenzene **31** in two ways (Griess,

6) See Chapter 11.

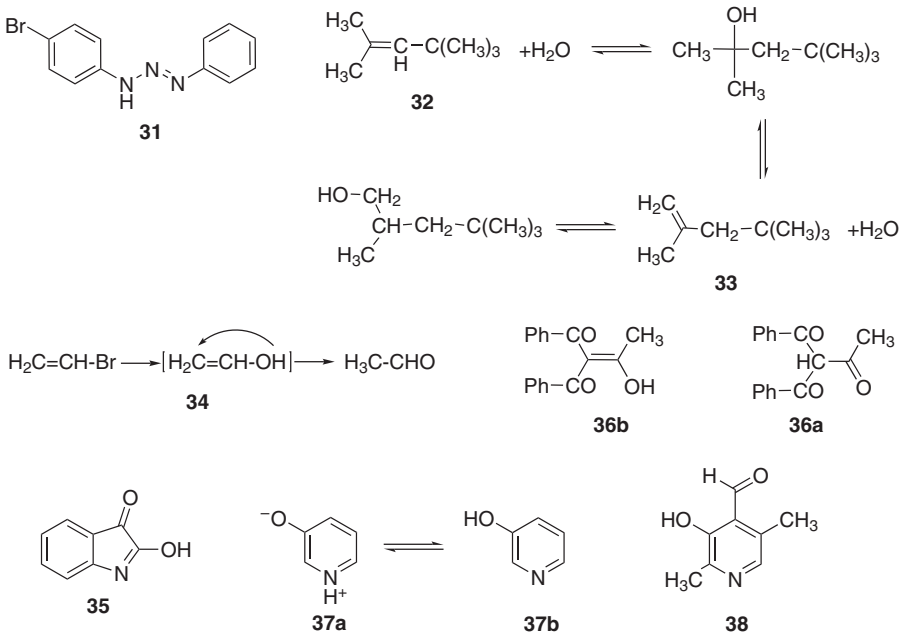



**Figure 1.7** Historically relevant compounds. The tautomeric structures are presented as they were in the original papers.

1874, [28]); the interaction between sulfuric acid and thrimethylcarbinol giving two isomeric di-isobutylenes **32** and **33**, that is, isomerizing through addition and elimination of water (Butlerov, 1877, [29]); attempts to isolate alcohols in which the hydroxyl group is attached directly to a double-bonded carbon atom as in **34**, giving, however, always isomeric carbonyl compounds (Erlenmeyer, 1880, [30]); and ethyl malonate (Conrad and Buschoff, 1880, [31]).

In 1882, Baeyer and Oekonomides [32] found out that isatin **35** gives two isomeric (*N*- and *O*-) methyl derivatives. They explained this fact with *pseudomerie* [33] – the possibility of one compound to have more than one structure obtained in the process of interaction, which, being unstable, converts very fast to the stable configuration.

In 1884, Zincke and Bindewald [34] obtained the same orange dye by coupling benzenediazonium chloride with 1-naphthol and by condensing phenylhydrazine with 1,4-naphthoquinone. They supposed that a mobile equilibrium existed between two forms, namely azo (**13b**) and quinonehydrazone (**13a**), a phenomenon classified by them as *ortisomerie*.

Obviously, it was time for summarizing the results and formulating some rules in this business. This was done by Laar in 1885 [35, 36] with the paper ''Ueber die Möglichkeit mehrerer Strukturformeln für dieselbe chemische Verbindung,'' where the existing examples of compounds that combine properties of two isomers are discussed in terms of the uncertainty of the position of one hydrogen atom and a double bond. He defines these systems as triadic (HX–Y=Z and X=Y–ZH) and

postulates that they cannot be separated experimentally, being two border cases of one intramolecular oscillation. The process was named *tautomerie*. As result, the question about the real existence of the isomers gave rise to two contradictory theories: *pseudomerie/ortisomerie* or *tautomerie*.

The dispute was in fact resolved in 1896 by Claisen [37], who isolated acetyldibenzoylmethane as two separate solid forms, each with different melting points and chemical properties (interaction with metallic salts). Claisen correctly diagnosed them as the enol and keto forms having the structures **36b** and **36a**, respectively. More important still was the observation that, if either the keto or the enol form was heated in a solvent such as alcohol, or fused in the absence of solvents, a mixture was obtained from which both the keto and enol forms could be isolated. As result of this discovery, the *pseudomerie/ortisomerie* theory about the real existence of the isomers was proven to be correct. Ironically, the term *tautomerism* came into use to describe the process. In some natural way, according to the early reviews [38–46], tautomerism was considered and it is still considered in most of the cases as an equilibrium[7] between forms coexisting in solution,[8] and was defined as ''one of the most difficult subjects of experimental science.''

It is worth remarking here that the pioneers of tautomerism were not armed with some extraordinary equipment. They had to trust mainly their eyes and their abilities to reach conclusions based on a limited amount of experimental information, which, actually, was enough for them to correctly define the factors influencing tautomerism in solution: the chemical structure (main tautomeric skeleton and substituents) and the environment (solvents, temperature, acidity, salt additions).

The problem is that each of these factors brings two questions: *how* and *to what extent* the tautomeric equilibrium is affected. The first question is qualitative; it brings as answer a descriptive explanation of the effects or a relative description, comparing to other compounds. Such a study can be done (and it was in the beginning) even without equipment by looking for visual changes (color change, precipitation, etc.). In terms of molecular spectroscopy methods, which are traditionally used for stationary state study of tautomeric systems (UV–vis absorption, fluorescence, IR, NMR), it means change in the registered instrumental signal.

The second question is quantitative. Its answer requires the values of the equilibrium constants (and related parameters) to be estimated in the terms of analytical chemistry. Following this, the concept for quantitative instrumental analysis postulates that the individual responses of the components of a mixture must be previously measured, that is, be known. However, taking into account that even if the individual tautomers are isolated in the solid state, in solution they always convert to a mixture, and such a requirement cannot be easily fulfilled. This

7) This is the line of the discussion below. Only real tautomeric systems existing as a mixture in solution are considered. Otherwise, we talk about potentially tautomeric compounds.

8) Information about tautomers avoiding effects of the solvent can be obtained in the gas phase. It is also possible either using computational chemistry (see Chapters 10 and 13) or through, for instance, NMR [47], mass spectrometry [48, 49], electron diffraction [50], or double resonance techniques (cf. Chapter 7).

contradiction has left a mark on the studies of tautomeric systems even today. Many compounds have been studied, but the conclusions are approximate and do not allow exact treatment of environmental effects and structure–tautomeric property relations. Of course, there have been attempts to mimic instrumental responses of individual tautomers by using model fixed compounds, where the movable proton is replaced by a methyl group, or by using compounds whose structure approximates the structure of the tautomers under investigation. As described in Chapters 2, 5, and 12, these approaches work reasonably well in some limited cases, but they always remain semiquantitative, because there is no physical ground for full correspondence between instrumental signals (as both the shape and intensity) of the model and of real tautomers.

In the first, ''descriptive,'' period of tautomeric studies, UV–vis, IR, and NMR spectroscopy, which are considered in Chapters 2–6, became the basic experimental tools. To trace the development, it is worth mentioning the first review devoted to absorption spectroscopy (from the UV to the IR region) in organic chemistry and particularly in elucidating structures of tautomeric compounds, written by Dobbie *et al*. in 1921 [51]. According to this review, the first study using absorption spectroscopy was performed by Hartley and Dobbie [52] in 1899, who proved the constitution of isatin and other tautomeric compounds by comparison of their absorption curves with those of their nitrogen and oxygen methyl derivatives. In 1908, von Liebig [53] studied the fluorescence of organic dyes, some of which are tautomeric. In 1931, Raman effect was discussed in relation to the tautomerism of acetoacetic ester by Dadieu and Kohlrausch [54]. According to Kol'tsov and Kheifets [55], the first study in which NMR was employed to investigate tautomerism was published in 1953 [56]. Although mass spectrometry is not related to solution, we have to recognize the first attempts (in 1967) devoted to its application in the structural study of tautomeric compounds [57].

As seen from the publication trend shown in the Preface, the number of scientific articles dealing with tautomeric compounds boomed after World War II. The main reason, along with the rapid developments in organic chemistry, was the commercialization of scientific equipment, which allowed reproducible spectral investigations to be performed on accessible, user-friendly equipment. In addition, the development of electronics allowed digitalization, storage, and processing of experimental data. It was a time of transition and hope in the 1960s and 1970s, when the traditional *spectral charts*, containing beautiful pictures of shifting a tautomeric equilibrium by changing factors influencing it, became *spectral files*, ready for processing. The ''quantitative'' phase of tautomeric research commenced with the development of chemometric methods to obtain analytical signals of the individual tautomers in a mixture even though these are never present in their pure form [58, 59]. Availability of this information makes it possible to obtain thermodynamic and kinetic parameters needed for an exact description of the environmental effects and defining the structure-tautomeric property relations needed for modeling tautomeric processes. The quantitative analysis of tautomeric systems is discussed in Chapters 2 and 5, because UV–vis and NMR spectroscopies are the major experimental tools in this respect.

However, in the end, we have to pay attention to the first chemometric work in 1973 by Metzler and collaborators [60, 61], who studied strictly quantitatively the two-component tautomeric equilibrium between the neutral form **37b** and the dipolar ion **37a** of 3-hydroxypyridine in water and water/methanol binary mixtures at various temperatures by using band-shape analysis. The same approach was applied in the case of the three-component tautomeric system 5-deoxypyridoxamine **38**, showing that the tautomeric equilibrium is not an analytical problem anymore.

## 1.8
## Short Historical Overview of Tautomerization Dynamics

Elucidation of reaction mechanisms requires the study of reaction kinetics. Investigation of equilibria as a function of temperature can give insight into the differences in free energy, enthalpy, and entropy between tautomers but, in order to clarify the way tautomers are converted into one another, detailed information about the dynamics of transformation is needed. Tautomers present a particular difficulty, both for equilibrium as well as dynamic studies, in that it is impossible to separate them and create a good starting point for the study of kinetics. The study of the dynamics of prototropic tautomerization reactions therefore fall in two classes. Before the 1970s, only a few papers were published, and these dealt mainly with acid- or base-catalyzed tautomerization reactions where the conditions could be chosen to bring the rates into the measurable range. Very few papers dealt directly with intramolecular ground-state proton transfer reactions. That changed in the second half of the twentieth-century. In the late 1950s, Weller's [62, 63, 64] experiments on salicylic acid led him to propose an intramolecular excited state proton transfer reaction (ESIPT), and the advent of fast pulsed (pico and femtosecond) lasers later that century made it possible to study this directly. This resulted in numerous papers on ESIPT on a large variety of compounds, as well as numerous theoretical studies.

The study of prototropic tautomerization is intimately related to the study of proton transfer reactions. The study of the dynamics of proton transfer is as old as the study of reaction kinetics itself. Indeed, the first reactions studied, that is, the inversion of sugar by Wilhelmy in 1850 [65], involves a proton transfer as the elementary step in the reaction. In the first studies on the dynamics of tautomerization, primarily keto–enol tautomerization in acetone-like compounds were studied, which is a slow process involving a number of reaction steps of which the acid catalyzed keto–enol conversion was taken as the rate determining one [66]. In the past century, since 1910, nearly 2000 papers have been published on the kinetics of tautomerization, and in the first 60 years most of those were devoted to the ground-state reactions of the keto–enol type involving a C atom. Until the mid-1950s, only a handful of papers can be found; this was obviously due to experimental limitations. Two things are needed: a method to start the reaction, and a method to follow it. In Dawson's experiments [66], the rate could be influenced by the amount of acid present, and the reaction could be followed because the enol produced

reacts rapidly with iodine, and the disappearance of iodine due to formation of iodoacetone can easily be followed using optical spectroscopy. Reaction times were on the order of hours to days. And the situation almost remained like that until the 1960s. Several faster techniques became available around that time.

One of the topics much under discussion in the early years of kinetics research was the nature of the two tautomeric forms, or where the proton actually resided. Laar [35] had proposed a so-called oscillatory model, where a hydrogen atom vibrates continuously between the two possible positions. Other early observations include dielectric effects: the polarity of the solvent could help release a proton from one position, thus making a transfer possible [1]. Although a tautomerization reaction is not an ionization (Section 1.1), an ionization step does play a crucial role, and may in many cases be the rate-determining step.

Apart from NMR, in which the equilibrium fluctuations of a reaction can be monitored in order to make an estimate of the reaction rates, until the mid-1970s basically three methods were available to measure direct proton transfer. The first is the temperature jump technique, where a rapid (of the order of microseconds) jump in temperature shifts the equilibrium, and the decay of the system to this new equilibrium can be followed with optical techniques. This technique was again mainly used in tautomerizations involving an ionization step [67], but in the mid-1970s the technique was also used for intramolecular studies [68, 69] in order to get insight into the question of whether ground-state proton transfer was as rapid as the proton transfers suggested in the excited state, which at that time were thought to be faster than 1 ns. These experiments did not give conclusive evidence of a direct intramolecular proton transfer step, although for the fitting of the data it was necessary to take the possibility into account, as was done by Ahrens [70] in an earlier paper who suggested the, rather unlikely, possibility shown in Figure 1.8, which is reminiscent of Laar's ideas [35].

A second method for studying tautomerization rates makes use of the fact that in vapor or in different solvents the equilibrium constant between the tautomers can be vastly different. Watarai *et al.* [71, 72, 73, 74] studied the tautomerization rate of acetelacetone (Figure 1.9) in a variety of solvents and solvent mixtures. Their method makes use of the fact that, in the vapor, the enol content is 93.3%, whereas in water the enol fraction is only 0.15. The reaction can be followed by UV spectroscopy, since the enol has an absorptivity $\varepsilon = 11\,000$ at 273 nm, whereas the

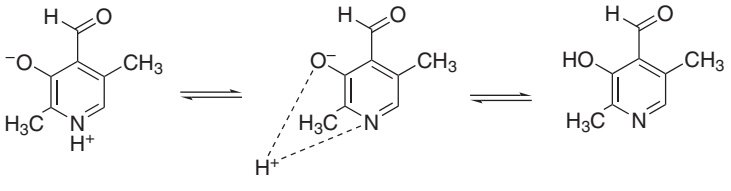



**Figure 1.8** Ahrens's proposition for intramolecular proton transfer in 5′-deoxypyridoxal. It is still considered a two-step process, where a collision first is needed to produce the intermediate.


**Figure 1.9** (a) Enol and (b) diketo form of acetylacetone. In the vapour, the enol form is dominant, and in water the diketo form.

keto form barely absorbs at all ($\varepsilon = 249$). The reaction is initiated by injecting the vapor into the solvent.

The third type of experiment is photolysis, where the product is one of a tautomer pair [2, 7, 75]. Again, almost all reactions studied are keto–enol tautomerizations where the proton transfer is not direct but in a number of steps via the solvent. Since the first step is often an ionization (proton transfer to solvent molecule), which is thought to be diffusion-controlled [67], it does give some insight into proton transfer reactions, but exact elucidation is hard, since often there are numerous possibilities for reaction mechanisms and roles of solvent molecules and internal vibrations [76, 77]. In view of the lack of understanding of proton transfer reactions, it would be much better to have a simpler and more direct way to initiate intramolecular proton transfer. This possibility is offered by looking at intramolecular proton transfer reactions in the excited state, which can be initiated much faster and followed on a much shorter timescale than ground-state reactions.

The vast majority of papers devoted to tautomerization dynamics deal with ESIPT reactions. Since Weller's suggestion that the large Stokes shift he measured for salicylic acid fluorescence was caused by rapid proton transfer in the excited state [62], and the development of techniques to study this on a femtosecond timescale, the field has blossomed. Most of the 2000 papers on tautomerization dynamics is on ESIPT, from both an experimental and a theoretical point of view. The number of compounds exhibiting ESIPT is far too large to discuss here. It ranges from molecules as simple as malonaldehyde to systems as complicated as 3-hydroxyflavone or 2-(2′-hydroxyphenyl)benzothiazole. In particular, substituted salicylic acids and *ortho*-hydroxybenzaldehydes have attracted much attention from both experimentalists and theoreticians. Weller's idea is depicted in Figure 1.10.

In the case of salicylic acid, only one tautomer (E) is present in the ground state. Upon excitation of this tautomer, a rapid proton transfer takes place in the excited state. Traditionally, this was attributed to the idea that in the excited state the phenolic OH becomes acidic, and the carboxyl group instead acquires more basic properties, providing the driving force due to the change in free energy in the excited state. This rapid conversion competes with normal fluorescence to the point where that is no longer observed, and only decay from the T* state results, which, as the diagram shows, has a considerable red-shifted – often of the order of 10 000 $cm^{-1}$ – fluorescence. Since in fluorescence experiments no accumulation in the T state is observed, the back reaction to E is also considered fast, although no direct experiments have confirmed this so far.

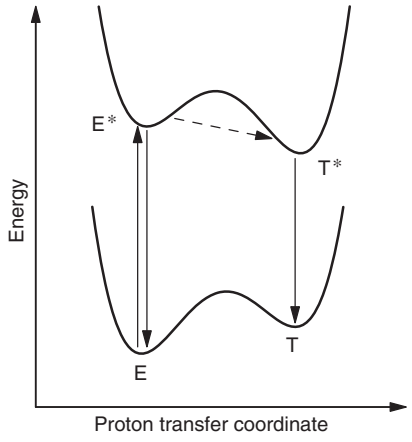



**Figure 1.10** Weller's explanation for the red-shifted fluorescence found in salicylic acid. The dashed arrow indicates the excited-state proton transfer. There are a few cases of dual emission from both the normal (E) and tautomeric (T) forms. Salicylic acid is not one of them in most solvents.

This observation has led to many other cases in which a large red shift is found, and where ESIPT is invoked to explain this. Since absorption and emission wavelengths can be modified by substituents at various places in the ring system, and there is a considerable dependence on the solvent or other environment (protein, membranes), many reporter systems have been designed on the basis of this idea. Salicylic acid and the related *ortho*-hydroxybenzaldehyde derivatives have attracted most attention in the literature for fundamental research, but there are a few other groups of ESIPT molecules that have attracted attention as well.

Although it is not a commonly used molecule for experiments, a few words can be said about one of the simplest of tautomeric molecules, namely malonaldehyde, shown in Figure 1.11.

This molecule is often thought of as model system for tautomeric proton transfer [78], although experimentally it does not give many possibilities for study. Only gas-phase measurements of the tunneling frequencies between the two equilibrium states have been reported [79, 80]. Although in the picture the molecule looks symmetric, in fact it is not. We discuss this molecule here to point out another problem with calculations in tautomers. Accurate ground-state calculations of tautomeric ratios have been shown to be exceedingly hard because

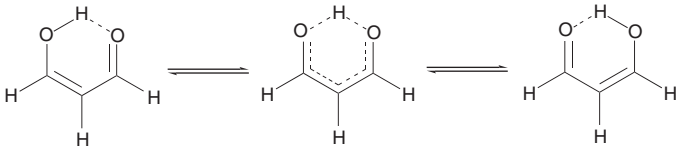


**Figure 1.11** Proton transfer in malonaldehye. In the gas phase, the proton is thought to tunnel through the transition state (middle) barrier.

of the small free energy differences between the tautomeric forms [81, 82]; the situation with calculations of barriers between the tautomeric forms is not much better. Even apart from the question of whether it is just the barrier heights that are needed to estimate the tunneling frequencies – this idea probably derives from Arrhenius and the transition-state theory, but in tunneling other parameters are also relevant – no consensus can be found between various methods to calculate these. Kar *et al.* [83] reported a comparison of a number of calculations where the barrier heights in the ground state vary from $-1.8\,\mathrm{kcal\,mol^{-1}}$ (not a barrier at all) to $+20.2\,\mathrm{kcal\,mol^{-1}}$. In the excited states, this is even worse. In the first excited singlet state, the value ranges from $-18.6$ to $+12.3\,\mathrm{kcal\,mol^{-1}}$, and in other excited states it can vary by as much as $60\,\mathrm{kcal\,mol^{-1}}$. It is not immediately obvious whether such calculations in these and in the much more complicated molecules that are of real interest contribute to our understanding of proton transfer dynamics in the ground and excited states.

The situation is somewhat better for experimental results, since trial and error as well as careful modeling using measurable properties of solvents and parameterization of substituents can lead to the design of molecules with desired properties. Flavones (Figure 1.12), for instance, form a large group of biologically relevant molecules whose properties can be modulated by various substituents to make them sensitive to properties of the environment [84, 85, 86]. They absorb visible wavelengths up to about 450 nm, and emit above 500 nm.

Molecules like this can be used as a platform structure to design probes for microenvironments [87, 88]. In some of these cases, both tautomers are present in the ground state, and dual emission takes place so that monitoring the color of the emission gives direct insight into the local environment, for instance in electric field strength inside membranes or proteins. Proton transfer in these compounds, although not as favorable geometrically as in salicylic acid or similar molecules, is still very fast so as to apparently allow equilibration of the excited state before the emission takes place. Although in the 1990s some hope was expressed that these compounds could also be used to "... demonstrate the accuracy and applicability of our direct *ab initio* dynamics approach for studying quantal effects in proton transfer reactions and also to establish a reference point for our future studies of proton transfer reactions in biological systems" [89]; further theoretical work on this type of compounds appears to be very limited (Figure 1.12).

Research of a more fundamental nature – not directly geared toward finding useful applications – has been reported on two other groups of molecules. 7-Azaindole is another biologically relevant molecule since it is closely related to indole, the core of the amino acid tryptophan. Tryptophan is an important reporter molecule in protein spectroscopy, and replacement of the indole group by an azaindole makes it even more suitable for its simpler decay characteristics and red-shifted spectrum [90]. It was also extensively investigated by Kasha and coworkers [91], and has been the subject of much theoretical work [92]. The tendency of 7-azaindole to form dimers in particular solvents has also led to the study of double proton transfer reactions in the excited state [93, 94]. Some of these issues are complicated by the possible presence of anion fluorescence [95] (Figure 1.13).

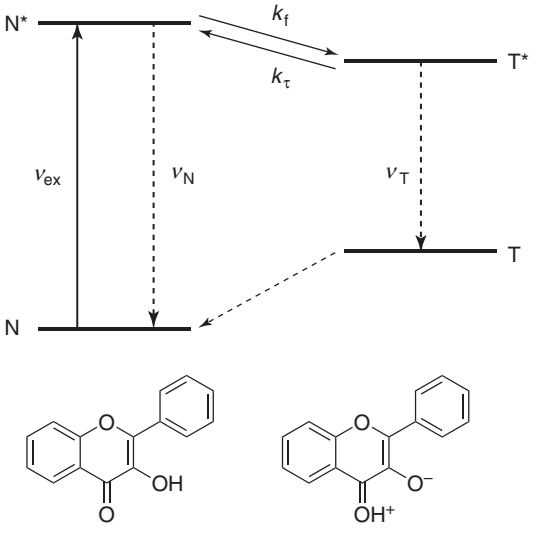



**Figure 1.12** 3-Hydroxyflavone (flavonol) exhibits ESIPT as a result of motion of the OH proton to the nearby oxygen in the excited state. (a) The generic level scheme used to describe this type of reaction. In the flavones, often both compounds are present in the excited state, and dual emission can occur, leading to the possibility of using the color change as indicator. The forward and reverse rates ($k_f$ and $k_r$) in the excited state are usually much faster than the emission rates. The tautomer emission ($\nu_T$) wavelength has a large Stokes shift because of the lower excited state and higher ground state of the tautomer. This adds to the Stokes shift as a result of vibrational and solvent relaxation. The backtransfer (dashed arrow) to the normal (N) ground state is usually also thought to be fast. (b) In the tautomer, the positive charge is likely to be delocalized so that the ring system remains aromatic.

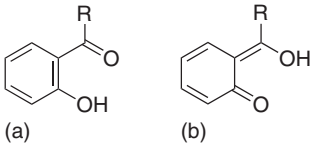



**Figure 1.13** (a) Enol and (b) keto forms of *ortho*-hydroxybenzaldehyde (R=H) and salicylic acid (R=OH). Another compound often investigated is methoxysalicylic acid ($R_1 = OCH_3$). Many substitutions on the ring are also investigated. For most of these molecules, the enol form is the only one present in the ground state, and dual emission is rare. Salicylic acid is present as the anion is water. It also exhibits ESIPT [96, 97].

The most extensively investigated class of molecules is the *ortho*–hydroxybenzaldehyde derivatives (Figure 1.13). Almost every conceivable technique has been used to probe its properties in the gas phase as well as in a large variety of solvents and solvent mixtures. Starting with the work of Weller, both steady-state and time-resolved fluorescence remain the most commonly used techniques [96]. Femtosecond spectroscopy gives details of proton transfer

on a very short timescale [98, 99]. The available literature on these compounds is too vast to be treated here. It ranges from very low temperature high-resolution spectroscopy to gas-phase photoelectron spectroscopy, from steady state to femtosecond fluorescence upconversion, and a variety of other nonlinear optical techniques. Numerous different solvents and substituents on the ring or carboxyl group have also been the topics of investigation. In addition, it has been the subject of multiple theoretical investigations, both for ground- and excited-state properties, up to and including exploration of the ''path'' the proton takes.

The conclusion we can draw from all this research is that there is still no coherent picture of intramolecular ground and excited-state proton transfer reactions in tautomers. The topic is complicated from an experimental as well as a theoretical point of view, and many questions remain. Intramolecular ground-state proton transfer is hard to study directly, and although femtosecond pulsed lasers allow initiating and following proton transfers in the excited state on a very short time scale, these methods bring their own complications to the interpretation of the results.[9)]

## 1.9 Conclusions and Outlook

In the foregoing sections, we have outlined some of the difficulties in the study of tautomerism, which, as indicated, have been present from the very first until the most recent investigations. The small free energy difference between tautomers and the low barrier between them make it impossible to study them in isolation and make them very sensitive to the properties of the local environment and to parameters like pH, temperature, and salt concentration – indeed almost anything that influences the energy and entropy of the molecule in solution. Accurate calculation of the properties in the ground and excited states is equally problematic as long as the current accuracy of numerical methods is not at least improved by one or two orders of magnitude. Quantum aspects of the proton transfer reaction present a particular theoretical challenge. Most of the work has a high phenomenological content, and the parameters used in solvent descriptions (dielectric constant, proton donating, or accepting properties) are themselves hard to calculate from first principles. The study of tautomers will remain a challenging field for some time to come.

In this book, we have tried to put together a number of approaches to these topics, which, on one hand, highlight these problems, and, on the other, try to offer solutions to at least a few of them. In a number of chapters, tools are presented for the experimental and theoretical study of tautomerism: absorption in combination with chemometrics to unravel the composition of a tautomeric mixture (Chapter 2); steady-state and time-resolved optical techniques to investigate transfer dynamics (Chapters 3 and 4); the use of NMR to elucidate equilibrium properties

9) Some of these issues are explored in Chapter 9.

(Chapters 5 and 6); the properties of tautomers in nonsolvent environments: biological molecules, gas phase, and solids (Chapters 7 and 8); some theoretical investigations into proton transfer and electronic properties of tautomers (Chapters 9, 10, and 13); and a number of techniques to classify solvent and substituent effects on the position of tautomeric equilibria, and methods to investigate properties of the individual components (Chapters 11 and 12).

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