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Low barrier hydrogen bonds within salicylate mono-anions

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Abstract—Progressive incorporation of electron-withdrawing substituents into the aromatic ring of salicylic acid selectively acidifies the ArOH group, until the intrinsic pK_a values of the ArOH and $ArCO_2H$ groups become matched, as in the case of 3-chloro-5-nitrosalicylic acid. The corresponding salicylate mono-anion at −50°C in aqueous deuterioacetone solution exhibits a low barrier hydrogen bond intramolecularly linking the adjacent anionic oxygens, according to NMR evidence (δ 18.1 ppm for bridging proton, H–D fractionation factor ϕ 0.4, versus δ 14 and ϕ 0.7 for a more typical asymmetric salicylate H-bond). Induction of this unique linkage correlates with a 1.4 pK-unit increase in acidity for pK_{a1} of a substituted salicylic acid, while not perturbing pK_{a2}. For the postulated occurrence of such anomalous H-bonds in the course of enzymic catalysis, the implications are that only ~2 kcal/mol of special transition-state stabilization energy might be available from this source, and that it should become manifested chiefly in a facilitation of general acid–base proton transfers.

1. Introduction

Interest in especially robust hydrogen-bonding has been stimulated by recent suggestions that kinetic accelerations characterizing enzymic reactions can be partly explained by the phenomenon.[1–4] The proposition has been advanced that enzyme-substrate noncovalent binding forces can be marshalled at an active site so as to induce anomalously potent H-bond linkages therein. These can be uniquely stabilizing within the transition state for an enzymically abetted reaction, thereby explaining some of the reduction in activation energy that constitutes catalysis. To improve understanding of the nature of such special H-bonds, we have undertaken to examine their occurrence within a relevant model system.[5]

Low barrier hydrogen bonds, acronym LBHB, are postulated to arise between appropriately linked electronegative heteroatoms when certain restrictions are met. The necessary conditions are a compressed interatomic distance between those participating heteroatoms, less than the sum of interpenetrating van der Waals radii, and a comparable proton affinity for each; i.e. the individual pK_a for their uncoupled conjugate acids should be equivalent. Under these constraints, a bridging proton can be expected to migrate toward an unique position equidistant between the electronegative atoms, yielding a 'low barrier' linkage. The latter arrangement contrasts with the normal pattern for hydrogen bonding that involves substantially unequal bond distances to the intervening H-atom, even when the heteroatoms have similar proton affinity, with the linking proton primarily associated with a single heteroatom. A sizeable energy barrier delineates that structure from its correspondent tautomer, which would entail protonation on the less basic heteroatom of the pair. Low barrier H-bonds have sometimes been alternatively labelled 'short and strong hydrogen bonds', a categorization which this article questions. At dispute is the energetic advantage, if any, of the more symmetrical arrangement in the case of a low barrier linkage.

The mono-anion of salicylic acid, **1**, offers an opportunity to examine systematically this type of H-bonding in a model system. Structurally, the phenolic-OH group spontaneously couples intramolecularly to the adjacent oxyanionic carboxylate group in an appropriate coplanar array. The interatomic gap between these oxygen atoms is held to a distance of 2.5 Å according to crystallographic evidence,[6] due to the rigidity of the interconnecting molecular framework. That spacing satisfactorily falls within the range characterized as yielding more symmetrical H-bonds. Although phenol itself is considerably less prone to ionization than is a carboxylic acid, substituents placed on the aromatic ring are capable of increasing phenolic acidity to the range of benzoic acid (which is itself not so susceptible to pK_a-perturbation by aryl substituents). Hence, the stipulations for a LBHB may be realized in substituted salicylate mono-anions, **1**.[†]

Keywords: hydrogen bonding; acidity; energy.

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† A previous study claiming LBHB in this system relied upon insufficiently acidic salicylates, and so did not actually detect the phenomenon.[7]

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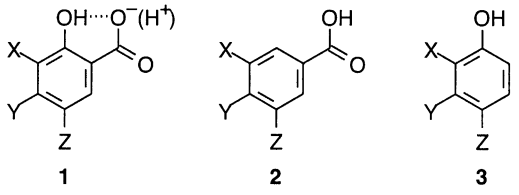
Table 1. NMR chemical shift and pK_a values (consensus constants from the literature or practical pK_a values herein obtained by spectrophotometric titration, employing calibrated pH meter readings in buffered solution. Sources used: Ref. 17) for salicylates and models (**1**–**3**)

Substituents (X, Y, Z)	**1**, pK_{a1}	**1**, pK_{a2}	**2**, pK_a	**3**, pK_a	**1**, δ NMR[a] (mono-anion)
H, H, CH_3	3.23	≥14.0[b]	4.27	10.26	13.6
H, H, H	2.97	13.8[b,c]	4.20	10.00	14.1
H, H, Cl	2.56	13.02[b]	3.84	9.35	14.1
Cl, H, H	2.36	12.44[b]	3.84	8.49	15.2
H, H, $COOCH_3$	2.51	11.91	3.89	8.34	15.5
Cl, H, Cl	2.05	11.60	3.44	7.85	15.6
H, H, SO_2CH_3	2.10	10.88	3.53	7.83	15.5
H, H, CHO	2.19	10.91	3.77	7.60	15.7
H, H, NO_2	1.98	10.34	3.46	7.15	16.1
NO_2, H, H	1.78	10.29	3.46	7.23	17.2
Cl, H, $COOCH_3$	1.84	10.39	3.53[d]	6.88	17.1
Cl, H, NO_2	1.33	8.85	3.13	5.45	18.1
NO_2, H, CHO	0.58[e]	7.91	3.03[d]	4.58	18.3
NO_2, H, NO_2	0.13[e]	7.36	2.82	4.00	17.8
NO_2, Cl, NO_2	0.00[e]	6.96	2.69	3.23	16.6

[a] In aqueous deuterioacetone 1:9, −50°C, ^{1}H 400 MHz; reproducibility ±0.2 ppm.
[b] Tetramethylammonium hydroxide employed in titration in order to suppress chelational perturbation pK_a by metal ions.
[c] Literature value pK_a 13.9, with extrapolation of metal ion concentration to zero: Ref. 18.
[d] Substituent *meta* group-additive estimates of pK_a, which is a technique established as reliable for benzoic acids: Ref. 19.
[e] Data employing H_0 scale (H_2SO_4), plus verified continuity with calibrated pH meter readings.



2. Results

2.1. Perturbation of NMR signal

One of the signatures of a LBHB is an anomalous ^{1}H NMR chemical shift for the bridging proton. Strong deshielding by mutual distancing from the participating pair of electronegative heteroatoms characteristically yields for the hydrogen nucleus a δ-value of ≥17 ppm.[8] Appropriate spectral data obtained from a series of substituted salicylic acids, **1**, are recorded in Table 1 along with pK_a data for **1** and for reference substances **2** and **3**, which are suitable for aiding detection of the LBHB phenomenon. Entries have been sorted according to decreasing pK_a value of phenolic protons.

Experimental values for the chemical shift of the proton engaged in H-bond bridging in salicylate mono-anions were secured at low temperature in 10%-aqueous deuterioacetone solution, in which medium H^+-exchange with solvent is slow enough to yield a discrete signal from that hydrogen nucleus. The ^{1}H NMR δ values so obtained at −50°C have been plotted versus an estimate of ΔpK_a for the respective oxygen atoms that anchor the H-bond, as obtained nominally by subtracting pK_a values for equivalently substituted model substrates **2** (benzoic acids) from those for **3** (phenols). A satisfactory least-squares fit to a conventional double sigmoidal expression results, as the bell-shaped curve in Fig. 1 depicts. This indicates a smooth transition from 'normal' H-bonding in salicylic acid itself (δ ~14 ppm) over to a LBHB (δ ≥18 ppm), which takes place when the apparent pK_a difference associated with the individual anchoring oxygens of **1** has appropriately decreased.[7] With regard to increasing the chemical shift, the locus of maximization corresponds to 2.1 pK units between ionizations of models **2** and **3** at 25°C, as marked by the high point upon the bell-shaped curve. However, because the ΔpK_a scale on the lower horizontal axis of Fig. 1 relies on acidities of surrogates, it needs to be corrected for the reciprocal inductive and conjugative effects of the carboxylate and phenol groups upon one another in **1**, excluding intramolecular H-bonding insofar as possible. For 4-hydroxybenzoic acid, which has substituent conjugation similar to

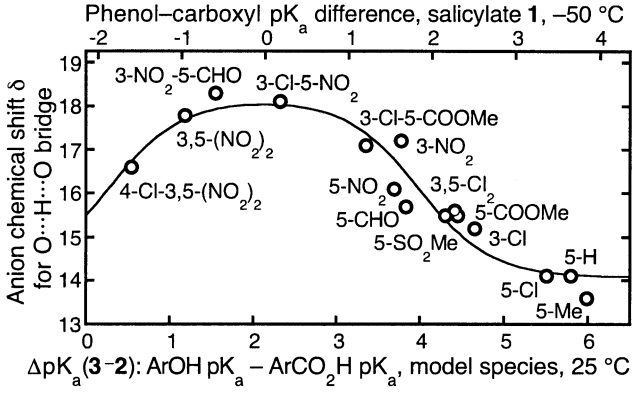


Figure 1. ^{1}H NMR chemical shift δ of bridging proton within substituted salicylate mono-anions **1** at −50°C in aqueous deuterioacetone, as a function of ΔpK_a(**3**–**2**) for anchoring oxygen atoms as derived from models. Symbol labels in plot refer to substituent positions for **1** or **2**. Curve is described by the following equation (pertaining to lower horizontal axis): $\delta(\text{apparent}) = \delta_1 + (\delta_2 - \delta_1)/\{[1 + 10^{-\Delta pK_1}/10^{-\Delta pK_a(\mathbf{3-2})}][1 + 10^{-\Delta pK_a(\mathbf{3-2})}/10^{\Delta pK_1 - 2\Delta pK_2)}]\}$ for which the fitted parameters are: $\delta_1 = 14.1 \pm 0.3$ ppm (limiting value, normal H-bond); $\delta_2 = 18.1 \pm 0.3$ ppm (limiting value, quasi-symmetrical H-bond); $\Delta pK_1 = 4.0 \pm 0.2$ (right-flank half-maximum point); and $\Delta pK_2 = 2.1 \pm 0.1$ (apogee of bell-shaped curve). Auxiliary scale on upper horizontal axis resets to zero the value of ΔpK_2 for **1**, from the estimate derived of models **2** and **3** (see text). Tolerances listed are standard errors from least-squares analysis.

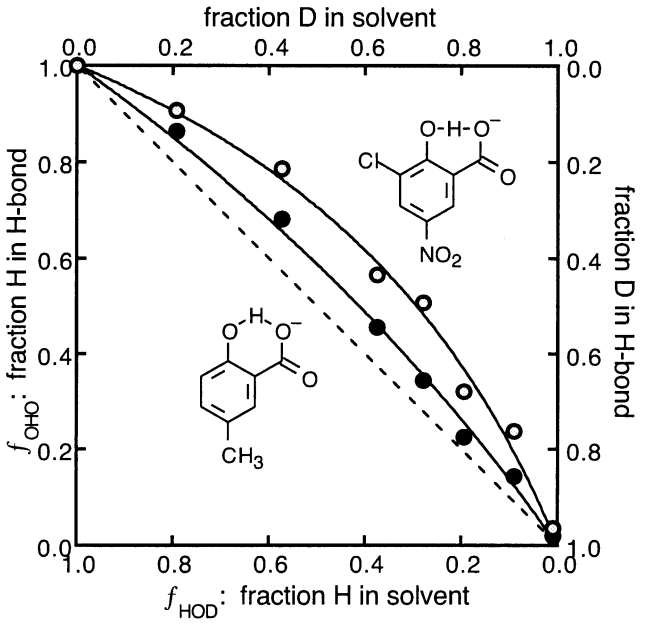


Figure 2. For H_2O–D_2O mixtures, mole-fraction of protons (f_{OHO}) found in H-bond versus fraction of available protons present in solvent (f_{HOD}), for 3-chloro-5-nitrosalicylate anion (open circles) and 5-methylsalicylate anion (filled circles), as measured by NMR signal intensity (at −50°C, in aqueous deuterioacetone). Curves are described by the following equation: H-bond f_{OHO}(apparent) = $1/(1-\phi+\phi/f_{HOD})$, for which the fitted parameter ϕ (H–D fractionation factor, see text) is 0.41 ± 0.03 for 3-chloro-5-nitrosalicylic acid, and 0.70 ± 0.03 for 5-methylsalicylic acid. Dashed-line diagonal corresponds to *no* fractionation ($\phi = 1$).

salicylic acid but for which an internal H-bond is impossible, pK_{a1} is 4.6 and pK_{a2} is 9.3, representing a convergence from the models employed, $C_6H_5CO_2H$ pK_a 4.2 and C_6H_5OH pK_a 10.0, all at 25°C. Accordingly, the implied correction should be a systematic diminution in ΔpK_a (**3**–**2**) on the horizontal axis for Fig. 1, of at least 1 pK unit. Most plausibly from internal evidence, the crest amounting to greatest chemical shift on the bell-shaped curve as drawn (the point with lower abscissa registering ~2.1 ΔpK units) correlates with a quasi-symmetrical and hence maximally favorable LBHB as evinced for the 3-chloro-5-nitrosalicylate anion.[‡] The ^{1}H NMR δ value of 18.1 ppm is appropriate for an H-bond length of 2.5 Å,[8] which is the crystallographically determined interatomic distance between participating oxygen atoms within a salicylate mono-anion. In other words, the phenolate and carboxylate oxygens actually attain a near-identical basicity in the case of the 3-chloro-5-nitrosalicylate anion. Any further electron-withdrawal by substituents on the aromatic ring in **1** results in the bridging proton becoming biased toward the carboxylate residue, which would have emerged as more basic relative to the phenolate oxygen atom (hence, the extrapolated downturn on the left flank of the curve in Fig. 1).[§] An auxiliary scale positioned at the top of Fig. 1 has been provided to reflect such as renormalization of ΔpK_a for **1**.

Conformation of a LBHB may be found in a protium–deuterium partitioning experiment. A low barrier H-bond is characterized by a wider and shallower energy-potential well relative to that of a normal H-bond. The zero-point vibrational frequency of a deuteron is less sensitive to the shape of such a well, because of its greater mass. As a consequence, protons tend to be relatively favored thermodynamically within a LBHB.[8] For a solvent-exchangeable proton within a substrate, the fractionation factor (ϕ) is defined as the equilibrium constant for exchange of deuterium from the solvent into the labile position: $\phi = [D_{substrate}][H_{solvent}]/[H_{substrate}][D_{solvent}]$. A direct experimental comparison between the mono-anions of 3-chloro-5-nitrosalicylate (δ 18.1) and 5-methylsalicylate (δ 13.6) was carried out. A series of NMR spectra for an equimolar mixture of the two anions in various combinations of H_2O–D_2O and deuterioacetone was secured. Relative signal intensities at low temperature for each of the bridging protons were obtained by integration (versus that of the nonexchanging proton in the 4-position of 3 chloro-5-nitrosalicylate), and the results are summarized in Fig. 2. Curve fitting to the appropriate expression yields for the fractionation factor ϕ values of 0.4 for 3-chloro-5-nitrosalicylate, and 0.7 for methylsalicylate. Therefore, the 3-chloro-5-nitrosalicylate anion appears to favor an intramolecular H-bond over a D-bond by a factor of 1.7, relative to the corresponding bridge occurring within the 5-methylsalicylate anion. According to previously furnished calibration data, a ϕ value of 0.4 is appropriate for an overall bond length of 2.5 Å (*OHO* interatomic distance), as pertains for the salicylate mono-anion.[8]

It is possible to interpret the data of Fig. 1 so as to estimate an incremental strengthening for the LBHB in the present case. The half-maximum point on the right-flank of the fitted sigmoidal curve occurs at a ΔpK_a of +1.9 units subsequent to correction (upper axis). This position would then correlate with the anion-basicity difference that is formally capable of yielding LBHB and normal H-bonds that are energetically equivalent within the salicylate. This two pK-unit offset from the center of the bell-shaped curve equates thermodynamically to a free energy difference of 2 kcal/mol at 220 K [$\Delta\Delta G^0 = RT\ln(K/K_0)$]. The LBHB situation should be more stable by approximately that amount; i.e. it takes free-energy bias in proton affinity of such magnitude to balance exactly the special favorability of the quasi-symmetrical LBHB against that of an asymmetric counterpart arising from the preference of the proton to associate most strongly with a more basic oxygen atom (standard H-bond). However, the application of a sigmoidal curve should not be taken to indicate exclusive two-state behavior as might be inferred from the previous deduction. From theoretical considerations the transitional species more probably consists of a single state of intermediate geometry, rather than an either-or choice between extreme cases. Although it may be questionable to assign conventional sigmoidal behavior to the ΔpK_a(**3**–**2**) dependence of δ in Fig. 1; that has been done for simplicity, with allowance that another function might fit the data as well.[8] Nonetheless, thermodynamic effects are independent of mechanism,

[‡] By the term 'quasi-symmetrical' we seek to avoid distinguishing, in the instance of a 2.5 Å (*OHO*) linkage, between a limiting case of two equal *O–H* bond lengths of 1.25 Å, versus an H-bonding situation with discrete but equivalent energy wells ~0.5 Å apart, separated by a vanishingly small barrier, which pattern may be more plausible theoretically.

[§] We have observed a chemical shift of δ 17.6 ppm at low temperature for the bridging proton in the 1,8-naphthalenediol mono-anion, for which the anionic oxygens are necessarily equivalent in basicity.

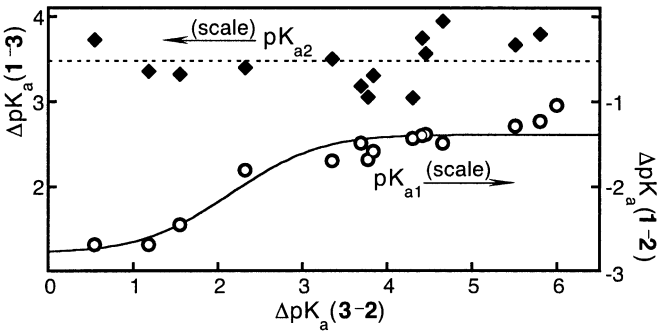


Figure 3. Perturbation of pK_a values for substituted salicylic acids (**1**) at 25°C by LBHB formation. Same horizontal axis is employed as in Fig. 1, i.e. ΔpK_a(**3**–**2**) for anchoring oxygen atoms as derived from models, with vertical axes showing difference between pK_{a1} or pK_{a2} of a salicylic acid and corresponding pK_a of model **2** or **3** (right and left, respectively). Fitted equation describing lower curve (circles) is a follows: Right-hand axis $\Delta pK_a(\text{apparent}) = \log\{10^{[\Delta\Delta pK+\Delta pK(\text{lim})]} + 10^{\Delta pK(\text{lim})}/[1 + 10^{-\Delta pK_a(\mathbf{3}-\mathbf{2})}/10^{-\Delta pK(\text{infl})}]\}$, for which the fitted parameters are: $\Delta pK(\text{lim}) = -1.38 \pm 0.17$ (right-hand vertical axis intercept, pK_{a1} of substituted salicylic acid **1** minus pK_a of correspondingly substituted benzoic acid **2**, for a normal H-bond); $\Delta\Delta pK = -1.40 \pm 0.06$ (vertical axis sigmoidal range, equals extra perturbation of same pK_{a1}, associated with a quasi-symmetrical H-bond in the salicylate bridge); and $\Delta pK(\text{infl}) = 2.8 \pm 0.2$ (horizontal axis, inflection point marking downturn in curve). The dashed straight line fitting upper set of data points (diamonds) corresponds to left-hand axis intercept $\Delta pK(\text{lim}) = 3.48 \pm 0.07$ (pK_{a2} of substituted salicylate **1** minus pK_a of correspondingly substituted phenol **3**, difference exhibiting no systematic variation).

so the energy estimate should not be vulnerable to this uncertainty in any event, and a similar result would emerge from any model for the transition occurring at this pK_a differential. Given the chemical shift magnitudes, and with due cognizance of certain inherent assumptions,[¶] the so-derived formal energy increment for engendering LBHB in salicylates should apply regardless, a $\Delta\Delta G^0$ of approximately −2 kcal/mol.

2.2. Perturbation of pK_a

The foregoing analysis might also be questioned for its reliance upon NMR evidence. A variety of factors influence δ, and the obligatory presence of *o*-chloro and *o*-nitro aryl groups in those substrates purportedly exhibiting LBHB is a cause for concern, even though less acidic salicylates having these substituents adjacent to the aryl-OH appear to register in line with other members of the series, according to Table 1. An alternative (and more insightful) way of estimating energetics for low barrier hydrogen bonding in salicylates is to examine pK_a perturbations of the carboxyl and phenol functional groups. The spread between pK_{a1} and pK_{a2} for the various salicylic acids contains a direct reflection of the strength of the intramolecular phenol-carboxylate hydrogen bond within the mono-anionic species. Introduction of an especially stable LBHB should a priori have the dual consequences of raising pK_{a2}, since it could be harder to dissociate the bridging proton, and of lowering pK_{a1}, because the more acidic proton must depart in order for the LBHB to form. Moreover, this reciprocal pK_a perturbation associated with the LBHB ought to be convertible directly into a thermodynamic free energy difference, applicable to the question of H-bond stabilization: $\Delta\Delta G^0 = 2.3RT(\Delta pK_{a2} - \Delta pK_{a1})$.

In Fig. 3 are plotted the apparent substituent-induced pK_a perturbations in **1**, with reliance on reference models **2** and **3** for scaling as before. As regards the substantial difference in pK_a between phenolic ionization of a salicyate **1** and the corresponding deprotonation of an equivalently substituted phenol **3**, the data from Table 1 display no systematic variation with respect to substituent pattern (diamond symbols, representing pK_{a2} of **1** minus pK_a of **3**, fitted by upper horizontal line within Fig. 3). The direction of the observed uniform pK_a offset is positive, reflecting the fact that intramolecular enlistment of even a normal H-bond to an *ortho*-carboxylate de-acidifies the phenol donor within the salicylate mono-anion, by about +3.5 p*K* units (the energetic dependence of a normal salicyl H-bond upon phenolic pK_a previously has been shown to be slight in aqueous solution[5,7]). However, no special imprint of a LBHB is evident anywhere in the ΔpK_{a2} profile.

In a complementary fashion, the increment between pK_{a1} of a salicylate **1** and the pK_a of a cognate benzoic acid **2** does not vary from a fixed −1.4 units, until approximate values of pK_{a1} and pK_{a2} of the salicylate (from models **2** and **3**, not renormalized) approach one another to within 3 p*K* units, as brought about by electron-withdrawing substituents on the aryl ring. Thereupon, the carboxyl residue of the salicyclic acid becomes progressively acidified, resulting in the relative divergence between pK_{a1} and pK_{a2} values expected for the substrate, a perturbation ascribed to LBHB formation. This realizes the anticipated behavior, albeit solely with respect to carboxyl group acidity, and the results which have been plotted in the lower portion of Fig. 3 (as circles) are to be interpreted commensurately with the previous NMR manifestation. In analogy to Fig. 1 an eventual upturn on the left flank of the profile would be expected here, but is insufficiently supported by data, so the applied curve-fit is a

[¶] Implicity, the pK_a differentials at 25°C in water ought to have been corrected to −50°C in aqueous acetone. The acetone-concentration dependences of pK_a for PhCOOH and PhOH are similar.[9] As regards temperature coefficient, phenols are known to have a more positive enthalpy of ionization than carboxyls;[10] however, no suitable data exist for extrapolations of <0°C. These considerations should matter only if salicylate ionization coefficients behave differently from those of the models. Additionally, a supposition of constant *O···O* interatomic distance for the H-bond throughout the series of salicylates might be questioned. The *C–O* bonds (phenol and carboxyl) are coplanar and essentially parallel in the available crystal structure for the parent salicylate mono-anion (involving a normal H-bond).[6] The oxygens therein are not splayed apart, nor is the carboxyl group rotated out-of-plane, as would otherwise suggest existence of significant angle strain resulting from *O···O* compression. Since the normal H-bond appears not to entail a native distortion that could be relieved by LBHB development, an assumption of constant geometry appears credible.

mono-phasic sigmoid. In this instance the maximal perturbation within pK_{a1} amounts to an additional −1.4 p*K* units of acidification, and the inflection marking its onset occurs at ΔpK_a (**3**–**2**) value of 2.8 units on the horizontal axis, as defined by uncorrected reference pK_a values. This profile accords with the pattern previously seen in Fig. 1. An apparent displacement toward the left of the inflection within the overall curve, relative to the same abscissa scale in Fig. 1, is in part an artifact of presentation. Fig. 3 is a log–log plot, whereas in Fig. 1 chemical shifts were presented linearly on the vertical axis. The actual horizontal deviation as secured by curve fitting is more like 1 p*K* unit, which nonetheless suggests that attainment of a LBHB linkage requires greater electron-withdrawal from the aryl ring by substituents in the case of Fig. 3. Such behavior can be rationalized. The NMR chemical shift measurements of Fig. 1 were for a temperature of −50°C in aqueous acetone, whereas all pK_a measurements are for +25°C in water, in each case out of experimental necessity. The discrepancy in location of the sigmoidal wave between Figs. 1 and 3 may be explicable by a greater temperature coefficient characteristic of phenolic ionization relative to that of a carboxyl, and possibly from a solvent medium effect.

Especially notable is the extent of the perturbation of pK_{a1} in those substrates which attain the status to LBHB in aqueous solution. The −1.4 p*K* units of extra acidification here seen for carboxyl ionization in Fig. 3 again translates thermodynamically into ~2 kcal/mol of special stabilization energy ($-\Delta\Delta G^0$) for the symmetrically bridging H-bond at the higher temperature of 298 K, a magnitude which may be equated with the transition from a normal to a low barrier hydrogen bond in this instance. The curious circumstance that for salicylic acids the LBHB-induced perturbation manifests itself entirely within pK_{a1}, with no corresponding deviation in the pK_{a2} profile, is addressed subsequently.

3. Discussion

3.1. Generalization of results

Evidently a LBHB can be attained in a substituted salicylate mono-anion when specified constraints of bond length and pK_a differential are realized. Two independent methods of estimation suggest that when this linkage occurs within its most favorable salicylate case, it represents approximately 2 kcal/mol of greater stabilization than for a normal H-bond. It is pertinent to ask to what extent the example can be generalized. First, it should be acknowledged that what we call a normal H-bond in the salicylates may also be especially strong. Even in that case the oxygen atoms are held in close proximity to one another, and the limiting value for the ^{1}H NMR chemical shift of δ 14.1 ppm, as well as an H–D fractionation factor ϕ of 0.7, for an asymmetrically bridging proton under our measurement conditions may actually reflect an extra strong link. Hence, the potential incremental advantage of a LBHB might be underestimated in our analysis, relative to less contracted linkages. However, salicylates were chosen for examination precisely for this reason. To establish the contribution of LBHB-character, it is essential that all extraneous variables be held constant. Answering the question of stabilization energy requires that *only* the pK_a values of the participating oxygen atoms be varied until they match, and that other factors such as *O···O* interatomic distance be held constant. The experimental system and its data analysis were designed toward that end. Secondly, it is commonly recognized that most effective H-bonding ordinarily entails a linear array of the participating elements, due to an electrostatic and dipolar nature of the phenomenon. In order for normal H-bonds to acquire an optimal length in salicylates, they must become bowed (by NMR calibration, an overall *O–H···O* bent-bond distance of 2.6 ± 0.1 Å according to Harris and Mildvan,[8] requiring a calculated *OHO* bond angle of ~147° in the salicylate structure, and in agreement with a ϕ value of 0.7). The shorter low barrier H-bond attained in the instance of matched pK_a values for anchoring oxygens also may not achieve complete *O···H···O* colinearity within the anion, because of preferred bond angles. This complication is an unavoidable consequence of holding the oxygen-to-oxygen interatomic distance to a rigid dimension of 2.5 Å by the scaffolding of a salicylate, since it is not simple to fashion a practical alternative unimolecular system allowing comparably fixed linear H-bonds. Estimates are that extreme bending may weaken an H-bond by a factor of ≥2-fold, but data are scarce.[11] Thirdly, solvent-leveling is a concern, particularly in aqueous solution. Concurrent H-bonding to oxyanions by external H_2O molecules, when that depends on basicity, could perturb pK_a values unequally.[5,7] However, our experimental design, namely, the taking of differences involving close analogues, was constructed so as to render this an auto-cancelling factor (cf. the level pK_{a2} profile in Fig. 3). The observation that a similar stabilization energy was derived of data from both aqueous acetone as well as water is confirmatory in that regard.

3.2. Enzyme-catalytic significance

The present observation of LBHB formation in salicylates (^{1}H NMR δ ~18 ppm, and H–D fractionation factor ϕ ~0.4), associated with not more than 2 kcal/mol of incremental stabilization energy, deters inference of a greater magnitude for $\Delta\Delta G^0$ for any similar NMR observation in a biochemical system. Comparable model systems demonstrating a greater effect should be provided to substantiate such claims. Consequently, the LBHB phenomenon might amount only to a minor catalytic contributor in enzymes.

Low barrier hydrogen bonds do not replace normal H-bonds spontaneously in polar media, but only as a consequence of interatomic compression. Such a strain-induction mechanism for engendering LBHB in the course of the enzyme-catalytic process (paid for by noncovalent binding energy of substrate that has been gained at neighboring enzymic locales) means that the phenomenon can have no effect on the specificity constant V_{max}/K_m. The latter kinetic parameter, representing the reaction velocity with concentrations of substrate that are much less than saturating, is the truest index of enzyme efficiency but it is insensible to strain-activation of substrate.[12] Hydrogen bonding is known to be more potent in a medium of low dielectric constant, where less dipole competition exists for charge stabilization from H-bonding, which of course consists of a dipolar effect

as well. Hence, it has been suggested that some enzymic active sites are intrinsically nonpolar (a questionable speculation in itself[13]), so that LBHB effects can emerge as especially important in such an environment, where strong H-bonds can form spontaneously.[4] However, this low dielectric constant bond-perturbation argument may be misleading, since any enzymic rate acceleration ought to be referenced to the uncatalyzed reaction in (highly polar) aqueous solution, from whence substrates are ordinarily bound to the enzyme; i.e. it is similarly disallowed as a V_{max}/K_m effect. Any transition-state stabilization from a LBHB in this situation will be negated by formal energy of transfer of transition state between polar and nonpolar environments, with no net catalytic gain. The enzymic low polarity environmental contrivance is just another 'electrostatic strain' argument for engendering LBHB, as elaborated subsequently.[‖] Regardless of polarity, LBHB participation can matter as regards the kinetic parameter V_{max} itself (the reaction velocity when enzyme becomes saturated with substrate), which has some relevance since enzymes characteristically are found to operate in vivo at a substrate concentration that is comparable to their Michaelis constant K_m. However, in such a case LBHB realization depends upon the capability of an enzyme to bind a reacting substrate in a manner producing a compressed H-bond. But a substrate of any flexibility would tend to respond to such a vectorial force by relaxing to a nonproductive binding mode, restricting the effectiveness of this type of strain-activation, with loss of benefit to V_{max}. It seems unlikely that any hypothesized gain of reaction-intermediate stabilization from the LBHB phenomenon could net more than the few kcal/mol that we have detected in the salicylate model.

3.3. Incremental H-bond strengthening?

Additionally and perhaps most significantly, comment is needed on the curious observation in Fig. 3 that the incremental LBHB stabilization energy, when it arises in the salicylic acids, is thrown entirely into the initial ionization pK_{a1}, with no discernable perturbation of pK_{a2} (a dissociation of the H-bonding proton). That selectivity may be a peculiarity which is unique to salicylates, but it seems to say that the LBHB is actually not any stronger than the corresponding normal H-bond, by the measure of being able to abstract the bridging proton with an external base. The energy release in forming a LBHB goes entirely into acidification within the first protonic ionization, an observation which can be rationalized. Nothing requires that the sum of two bridging 'half bonds' in a LBHB needs to be stronger than a single normal *O–H* bond engaged in highly asymmetric bridging, so long as the overall thermodynamics of ionization is satisfied. On theoretical grounds this behavior could well be a general property of low barrier hydrogen bonds; i.e. in principle an anomalous and quasi-symmetrical H-bond produced by compression cannot logically be stronger than the normal H-bond it replaces, when that latter equilibrium linkage constitutes the spontaneously formed species in the absence of compression.[14] Microscopically, it takes energy input to compress any bond. Were that not so, then all H-bonds would be low barrier. No more energy can be gotten back out than was put in by compression. Withdrawing a normal H-bond from polar medium (H_2O) and introducing it into a 'hydrocarbon-like' active site where it spontaneously becomes low barrier, as in the enzymic activation-stratagem previously propounded,[4] merely amounts to destabilization of the asymmetric H-bond, resulting in environmental compression. The normal (elongated) H-bond may adopt its interatomic distance because of the existence of external dipoles and similar factors near at hand, but when those are taken away, what is left becomes globally weaker, so the bond shortens enough to compensate.

The realization that LBHB energy ought to appear only in pK_{a1} has important ramifications as regards enzyme mechanisms, for it constitutes an eminently desirable feature. As a concrete example, one might imagine enzymic conversion of a substrate containing a reactive ketone into the corresponding enolate reaction-intermediate, with oxy-anion-stabilization of the latter within ES by means of a LBHB donated by the enzyme, as has been proposed in several instances.[1–4] The pattern here observed for salicylate ionization would suggest that enolate formation could be sped-up, since that deprotonation step corresponds to pK_{a1}, but that the resulting carbanion would lose none of its reactivity as a consequence of LBHB formation. Relative stabilization of the hypothetical enolate anion itself within an enzyme complex could actually be undesirable, because that would reduce its reactivity towards a subsequent electrophile, for completion of the enzymic reaction. The LBHB mechanism appears to offer the promise of facilitating formation of a reaction intermediate, without diminishing its chemical activity within the ES complex.

But the Pauling principle of catalysis[15] introduces an important qualification for optimum enzymic effectiveness in such a case. The passing stabilization from a LBHB, entailing a critical matching of pK_a values of donor and acceptor heteroatoms, should occur within the transition state for proton transfer, rather than for the eventual enolate intermediate produced. At least this situation should prevail for instances where proton-abstraction comprises the slow step in catalysis. However, that circumstance should only arise where the resulting enolate subsequently becomes more basic than the H-bond donor residue, since the transition state-pK_a of the enzymically conducted substrate ought to fall between that of reactant and product. In such a case, a general-acid catalysis by the H-bond donor should happen,[16] with the immediate product of the catalytic step an actual enol arising from outright proton transfer from its now more acidic H-bond donor, rather than the yielding of a stabilized enolate. A 'late' transition state as has previously been advocated[2] would not yield special kinetic benefit when the low barrier H-bond itself is actually no more stable than its uncompressed counterpart, as in the salicylates. That raises a question of whether the phenomenon of LBHB in enzymic reactions should ever amount to more than a participant within incipient general acid–base catalysis, from which it would seem to be inextricable by

[‖] We do not dismiss the possibility that active sites may be nonpolar (for catalyzing reactions in which the transition state is less polar than the ground state); that circumstance would not apply where T–S H-bonding is critical, since it is by nature a dipolar interaction.

mechanistic analysis. These aspects of low barrier hydrogen bonding deserve more attention.

4. Experimental

4.1. Materials

Salicylic acid substrates were purchased or prepared for this study according to literature procedures (with each representative known before), as were model substituted benzoic acids and phenols in instances where satisfactory pK_a values were not previously available. Structures were verified spectroscopically for all substrates.

4.2. Measurements

Low temperature ^{1}H 400 MHz NMR measurements were obtained upon salicylate specimens dissolved in H_2O–CD_3COCD_3 in the ratio 1:9 (v/v) at approximately −50°C, with addition of ≥1.0 equiv of a suitable proton acceptor, *N*-(2-hydroxyethyl)morpholine. At this temperature a single, moderately sharp, bridging-proton resonance of appropriate intensity emerged within the spectral region δ 14–18 ppm consequent to addition of the amine (Table 1). An internal chemical shift reference consisting of benzaldehyde (CHO, δ 10.0) was employed. The signal arising from the resulting mono-anions generally broadened and disappeared (reversibly) on warming, due to dynamic exchange with solvent. Protium–deuterium partitioning ratios as regards the H-bond were secured analogously, from a direct comparison experiment. Low temperature spectra were obtained for an equimolar mixture of 3-chloro-5-nitrosalicylate and 5-methylsalicylate mono-anions in aqueous deuterioacetone incorporating various amounts of D_2O. Signal intensities for the respective bridging protons were estimated by integration, relative to the intensity for the 4-position ring proton of 3-chloro-5-nitrosalicylate (δ 8.6). A comparative enrichment of proton within the bridge was noted for 3-chloro-5-nitrosalicylate, in amount appropriate for a LBHB (Fig. 2).

Acidities of substrates were determined in buffered aqueous solution by spectrophotometric titration. The recorded pK_a values are practical constants for minimal ionic-strength solution, as secured by fitting visible or UV absorption measurements for a suitable fixed wavelength (adjoining an isosbestic where available), procured at various pH values, to an appropriate sigmoidal expression by the method of nonlinear least squares. Standard errors of pK_a values so obtained (Table 1) were generally ±0.1–0.2 units. For especially acidic salicylic acids (pK_{a1} <1), an extrapolation into the H_0 acidity function scale (aqueous H_2SO_4) had to be employed. In such cases contiguous pH meter readings for substrate solutions (at pH ≥1) were also taken to generate internal consistency, and a satisfactory fit of spectral absorptions to the appropriate sigmoidal expression incorporating both H_0 and pH was secured. Also, for salicylate pK_{a2} values that were especially high (>12) tetramethylammonium hydroxide was employed in the spectral titration, to avoid a perturbation due to chelation of metal ions by the salicylate di-anion. The same titrimetric procedure was employed for model benzoic acids and phenols, where it was necessary to obtain experimental pK_a values. In several instances for the models only, available consensus literature values taken from compilations were adopted, normalized for internal consistence if necessary, to pK_a 4.2 for benzoic acid, or pK_a 10.0 for phenol (see Table 1).

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