# Secondary Hydrogen Isotope Effects. Part 5.[1] Acid and Base Strengths: Corrigendum and Addendum

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The effect of deuteriation of the methylene group on the acid strength of phenylacetic acid and the base strength of benzylamine are reinvestigated, using both the differential potentiometric method described in Part 1 of this series and a newly developed differential n.m.r. method, which is described. The differential potentiometric method is established to be sound, but the $\Delta pK_a$ values reported earlier could not be reproduced and are withdrawn in favour of the following values, obtained by the old method and confirmed by the new: $PhCD_2CO_2H$–$PhCH_2CO_2H$, $\Delta pK_a$ ($\equiv pK_a^D - pK_a^H$) = 0.005 ± 0.003; $PhCD_2NH_3^+$–$PhCH_2NH_3^+$, $\Delta pK_a$ = 0.032 ± 0.001.

In Part 1 of this series[2] two sets of secondary hydrogen isotope effects were reported: (1) the effect of methyl and methylene deuteriation on the strength of acetic, propionic, and phenylacetic acids and on the base strength of benzylamine; (2) that of methyl deuteriation on *N*-methyl-2,4-dinitroaniline and *N*-methyl-2,4,6-trinitroaniline. The latter set, determined spectrophotometrically, was confirmed soon thereafter and correlated with kinetic isotope effects on *N*-nitration.[3] In contrast, the reliability of the former, obtained by a differential potentiometric method specificially designed for the purpose, was soon called in question.

The value reported for acetic acid at 298 K was $\Delta pK_a (\equiv pK_a^{CD_3} - pK_a^{CH_3}) = 0.026$, corresponding to $K_a^{CH_3}/K_a^{CD_3} = 1.06$, whereas Klein and Streitwieser,[4] using a conductimetric method, found $\Delta pK_a = 0.014$ ($K_a^{CH_3}/K_a^{CD_3} = 1.03$). Eventually, the exhaustive potentiometric investigation of Paabo, Bates, and Robinson[5] resolved the discrepancy in favour of the latter, lower, value.

The comparative unreliability of the results obtained by our differential potentiometric method could be rationalised as resulting from its requirement that the samples of the two acids being compared must be exactly equivalent, a particularly severe limitation in the case of acetic and propionic acids, which were difficult to purify and retain pure, and which had to be weighed either as liquids or as their uncrystallized sodium salts. However, similar considerations would scarcely be expected to invalidate measurements carried out on the easily purified phenyl[αα-$^2H_2$]acetic acid and [αα-$^2H_2$]-benzylammonium chloride, our report on which ($\Delta pK_a$ = 0.048 and 0.054 respectively) constituted the first published examples of secondary hydrogen isotope effects on chemical equilibrium.[6] Consequently, the results of Barnes and Scott's conductimetric[7] investigation, according to which the acidity of phenylacetic acid and several of its derivatives is virtually unaffected by methylene deuteriation, were profoundly disturbing.

The present reinvestigation of the isotope effects on the acidity of phenylacetic acid and the basicity of benzylamine was designed to answer the following two questions: (1) Are the earlier results reproducible? To clear up this point, it was necessary to repeat the differential potentiometric measurements, adhering as closely as possible to the procedure specified in the earlier report. (2) Is the differential potentiometric method for the measurement of small differences in acid and base strength a reliable one? For this purpose, the results obtained with this method, whether or not they reproduce the earlier ones, had to be compared with determinations made by some entirely different method, preferably one that is neither potentiometric nor conductimetric.

We chose an n.m.r. technique, which is a modification of the method which Saunders and Vogel[8] had successfully applied to secondary isotope effects on equilibria involving carbonium ions. In this method, the acid and its deuteriated analogue are both present in the same solution, so that it is insensitive to minor discrepancies in the preparation of samples and to other possible systematic errors. $^{13}C$ N.m.r. was chosen in order to take advantage of the relatively large chemical shifts of this nucleus.

*The N.M.R. Method.*—The n.m.r. spectrum allows the determination of the concentration ratio of two species undergoing fast exchange, provided that the chemical shifts of corresponding sites in the two species are known. Measuring, in this way, the relative concentrations of an acid and its conjugate base in a solution of known hydrogen ion concentration allows the evaluation of the acid dissociation constant.[9]

During the direct determination of the isotope effect on such a dissociation constant, the four species present in solution are the two isotopically different acid–base pairs, each of which produces a distinct average signal. The chemical shift between these two lines, suitably corrected for isotope effects on their chemical shifts, allows the evaluation of $\Delta pK_a$.

The n.m.r. measurements are taken in three pH regions: (A) and (B) acidic and basic solutions respectively in which either the acid or the conjugate base of each isotopic pair is present exclusively and the isotope effect on its chemical shifts could be measured; (K) an intermediate solution, pH $\cong$ p$K$, so $\Delta pK_a$ could be evaluated with the equation:

$$\Delta pK_a = \log_{10}\left\{1 - \frac{4[\delta - (\delta_A + \delta_B)/2]}{\Delta}\right\} \quad (1)$$

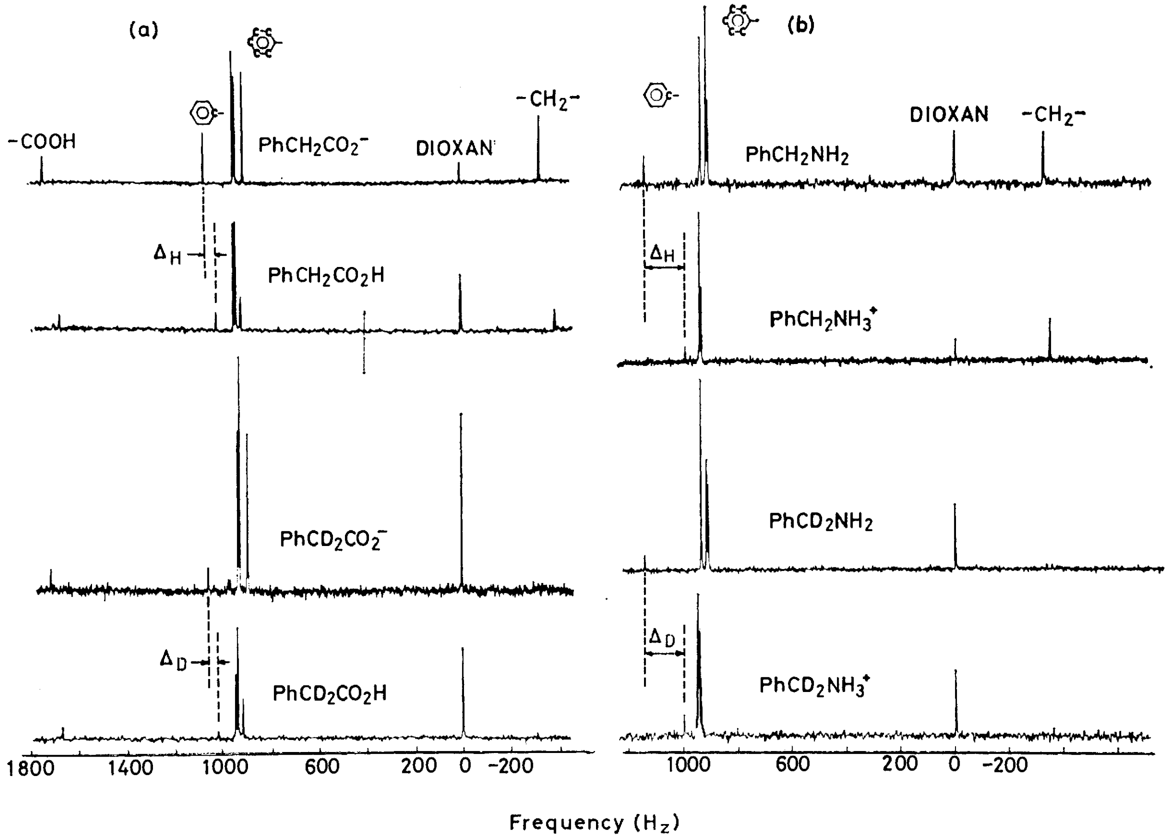

FIGURE 1 $^{13}C$ N.m.r. spectrum of the isotopic acid–base pairs: (a) phenylacetic acid and (b) benzylamine

The terms in equation (1), which is derived in the Appendix, are defined as follows:

$\delta_A = \nu_A^H - \nu_A^D$, the chemical shift between the protio- and deuterio-acids (determined in a solution of pH $\cong 2.5$).
$\delta_B = \nu_B^H - \nu_B^D$, the chemical shift between their respective conjugate bases (pH $\cong 13.7$).
$\delta = \nu^H - \nu^D$, the chemical shift between the average signals of the protio- and deuterio-species in a solution at which pH $\cong pK_a$.
$\Delta = \nu_A^H - \nu_B^H \cong \nu_A^D - \nu_B^D$, the chemical shift between the acid (A) and its conjugate base (B), the isotope effect on which is small enough to be neglected.

Figure 1 depicts the superposed $^{13}C$ spectra of the four relevant species of (a) phenylacetic acid and of (b) benzylamine. The signal of the substituted carbon atom on the benzene ring, which is well separated from those of the other aromatic carbon atoms, was chosen because of its relatively large chemical shift between the acid and its conjugate base. It can be seen that this chemical shift is isotope-insensitive ($\Delta_H \cong \Delta_D$) in both systems, so that the use in equation (1) of a common average value, $\Delta$, is justified.

The determination of the very much smaller $\delta_A$, $\delta_B$, and $\delta$ had to be carried out in a single measurement, in which the acids and/or bases of both isotopic species are present together in solution. Because the corresponding signals of the two isotopic species overlap, a spectrum simulation was performed in which the chemical shift between the two lines and the $^{13}C$ deuterium coupling

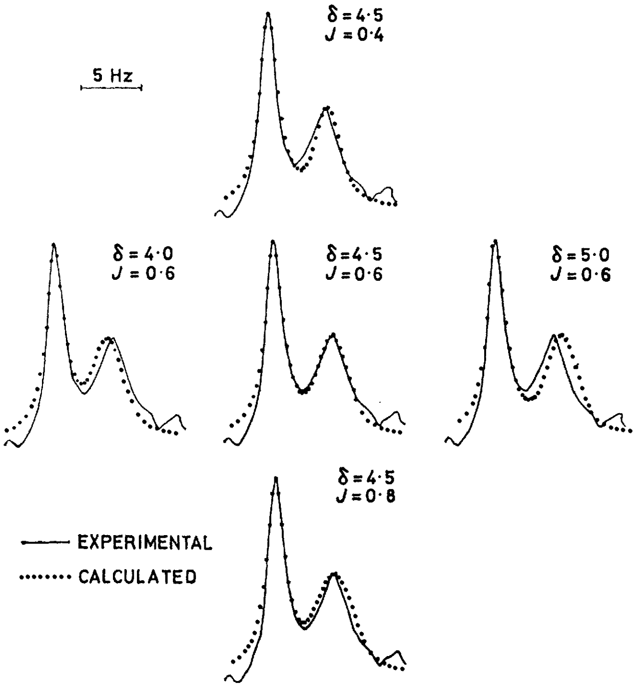

FIGURE 2 Effect of $\delta$ and $J$ on the fit of calculated and experimental spectra. (Benzylamine; pH $\cong pK_a$; LB = 0.6)

constant ($J$) were introduced as parameters. As demonstrated in Figure 2 for benzylamine at pH $\cong$ p$K$, the choice of $J$ affects the width of the peak of the deuteriated base, but not its position. The latter is sensitive only to the chemical shift $\delta$, which could be easily fixed to within 0.1 Hz. In fact, the accuracy of the fit appeared to be limited by the number of data points (4K) used in the Fourier-transform calculation. Figure 3 shows three simulations of the benzylamine and [$\alpha\alpha$-$^2H_2$]benzylamine spectrum at pH $\cong$ p$K_a$, with optimized values of the parameters $\delta$ and $J$, but with different values of the mathematical filter (LB) that modifies the line width in the Fourier-transform calculation. In all cases a Lorentzian line shape of width 0.5 Hz and a statistical (1 : 2 : 3 : 2 : 1) intensity distribution of the components of the peak of the deuteriated species were assumed. The quality of the fit is evidently quite insensitive to LB.

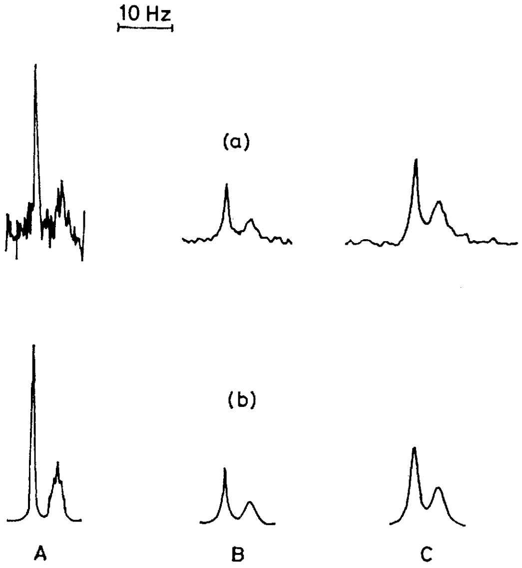

FIGURE 3 Comparison of experimental (a) and calculated (b) spectra with different values of LB. (Benzylamine; pH $\cong$ p$K_a$; $\delta = 4.5$; $J = 0.6$)

## EXPERIMENTAL

*Materials.*—Benzoic acid (Koch-Light, Puriss.), phenylacetic acid (Fluka, Puriss.), and phenyl[$\alpha\alpha$-$^2H_2$]acetic acid (Merck, Sharpe, and Dohme) were each recrystallized twice from light petroleum (b.p. 60—80 °C) and dried at 60 °C *in vacuo* over $P_2O_5$. The chemical purity of each acid was confirmed by its i.r. spectrum (Perkin-Elmer 257). The isotopic purity ($\geqslant$99% D) of phenyl[$\alpha\alpha$-$^2H_2$]acetic acid was established by the absence of a proton signal in the methylene region of its n.m.r. spectrum (Varian T-60).

When required as its sodium salt, the appropriate acid was neutralized with NaOH (EKA, Puriss.) to pH 7 and the solution was evaporated to dryness. Excess of free acid was washed away with diethyl ether (Matheson, Coleman, and Bell, Absolute) and the salt was dried at 100 °C *in vacuo* over $P_2O_5$.

Benzylamine (Fluka, Puriss.) was neutralized to pH 2 with 10M-HCl (Frutarom, Analytical Grade). The hydrochloride was recrystallized from ethanol and dried at 100 °C *in vacuo* over $P_2O_5$.

[$\alpha\alpha$-$^2H_2$]Benzylamine was prepared by the $LiAlD_4$ reduction of benzonitrile.[19] The hydrochloride was isolated and recrystallized as described above. Its chemical and isotopic ($\geqslant$99% D) purity was confirmed as described above for phenyl[$\alpha\alpha$-$^2H_2$]acetic acid.

*The Differential Potentiometric Method.*—The procedure previously described [2] was followed nearly without change, except for the use of an El-Hama PBS9 digital pH-meter, with Activion glass (model 101), and calomel (model 602) electrodes.

A working stock solution of each acid (benzoic, phenylatic, and phenyl[$\alpha\alpha$-$^2H_2$]acetic) was prepared immediately before carrying out a series of comparisons by dissolving an accurately weighed 5 mmol sample in 0.1 N-NaOH (20 ml) and making the solution up to 250 ml with triply distilled $CO_2$-free water.

A set of $\Delta$p$K_a$ determinations was carried out as follows. A 50 ml aliquot of the first acid was brought to thermal equilibrium at 25.0 °C while dry $N_2$ gas was bubbled through it. Some 20 successive 0.50 ml portions of 0.2M-NaOH were added, the pH being recorded after each addition. The instrument read to 0.01 units of pH, so a reading that fluctuated between two successive values, $\gamma$ and $\gamma + 0.01$, was recorded as $\gamma + 0.005$. The entire procedure was repeated with an aliquot of the second acid, followed by another one of the first acid, and so on. The pH differences between corresponding points (equal additions of NaOH) of consecutive runs were recorded as $\Delta$p$H$. On the rare occasion when there was a drift of $\Delta$pH along the run, the run was discarded. Ordinarily, however, $\Delta$pH remained constant, and its standard deviation over the 20 or so points of successive runs was in the range 0.002—0.004.

In the case of each of the above acid pairs the reverse procedure was also adopted. Stock solutions of the sodium salts were prepared, and the aliquot portions were titrated against HCl. The benzylamine–[$\alpha\alpha$-$^2H_2$]benzylamine determinations were carried out similarly, aliquot portions of the stock solutions of the hydrochlorides being titrated against 0.2M-NaOH. In these latter experiments $N_2$ gas was not bubbled through the solution in order to avoid partial volatilization of the free benzylamine.

*The N.M.R. Method.*—The 'water' solvent was invariably a 90 : 10 mixture of $H_2O$ and $D_2O$, the solutions in which were as follows.

(a) *Phenylacetic and phenyl*[$\alpha\alpha$-$^2H_2$]*acetic acid*. A saturated solution of each acid and a mixed solution, in which the saturated solution of the protio-acid was combined with twice the amount of that of the deuterio-acid.

(b) *Sodium phenylacetate and phenyl*[$\alpha\alpha$-$^2H_2$]*acetate*. To a weighed 0.7 mmol sample of each acid 0.5 ml of 2M-NaOH was added and the solution made up to 3 ml with 'water.' The isotopic mixture of salts was similarly prepared from 0.6 mmol of the protio- and 1.3 mmol of the deuterio-acid, to which 1 ml of 2M-NaOH was added and the solution made up to 5 ml.

(c) *Isotopic phenylacetate mixture at* pH $\cong$ p$K_a$. Weighed samples of the protio- (1 mmol) and deuterio- (2 mmol) acids were dissolved in 5 ml 0.3M-NaOH, and the solutions made up to 25 ml.

(d) *Benzylammonium and* [$\alpha\alpha$-$^2H_2$]*benzylammonium hydrochlorides*. A 2.25 mmol portion of each was dissolved in 5 ml 'water.' The isotopic solution was a 1 : 3 ($CH_2$ : $CD_2$) mixture of these two solutions.

(e) *Benzylamine and* [$\alpha\alpha$-$^2H_2$]*benzylamine*. A 2.5 ml portion of each of the solutions of hydrochloride was neutralized with 0.2 ml of 10M-NaOH. The isotopic mixture was prepared from weighed samples of the protio-(0.7 mmol) and deuterio-(1.3 mmol) hydrochlorides, which were treated with 0.2 ml of 10M-NaOH and the solutions made up to 3 ml.

(f) *Isotopic benzylamine mixture at* pH $\cong pK_a$. 2 mmol samples of each of the two hydrochlorides were combined, treated with 1 ml 2M-NaOH, and the solution was made up to 5 ml.

Proton-decoupled $^{13}C$ spectra were obtained on a Bruker WP-60 Fourier-transform n.m.r. spectrometer operating at 15.08 MHz. The temperature was adjusted to 25 °C by a Bruker B-ST 100/700 Temperature Control Unit. The external magnetic field was locked on the internal $D_2O$ signal. The measuring time was *ca.* 12 h for benzylamine samples, and up to 48 h for those with phenylacetic acid.

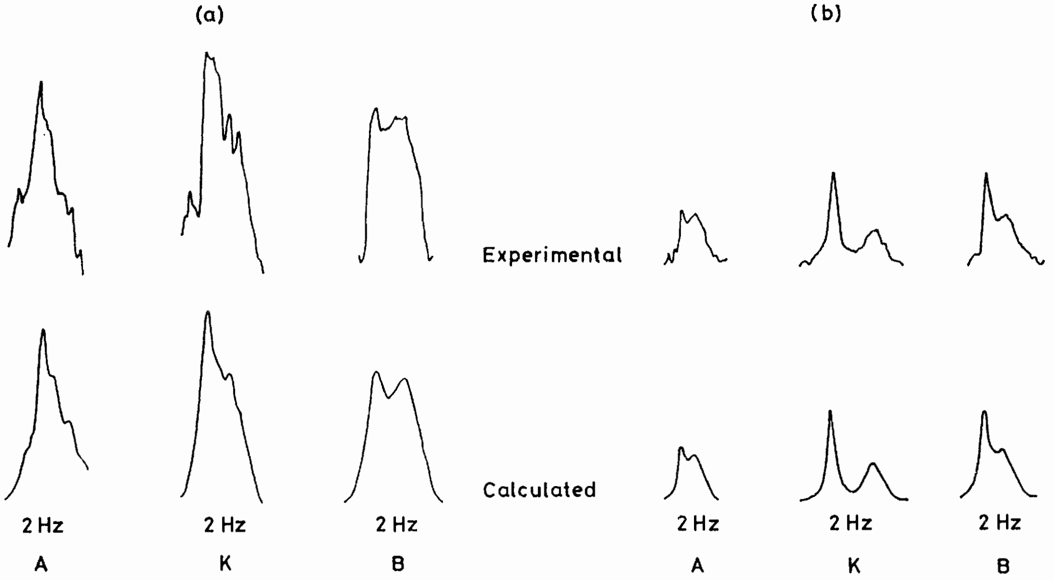

FIGURE 4 Spectra of isotopic mixture in solutions of varying pH. (a) phenylacetic acid; (b) benzylamine. In both cases: (A) acids; (B) conjugate bases; (K) pH $\cong pK_a$

RESULTS AND DISCUSSION

Eleven differential potentiometric comparisons of phenylacetic and benzoic acids yielded $\Delta pK_a = 0.104 \pm 0.002$, in good agreement with recently published absolute $pK_a$ values: $pK_a$ (phenylacetic) [7a] = 4.307 ±0.001 and $pK_a$ (benzoic) [11] = 4.201 ± 0.004, and thus providing *prima facie* evidence for the reliability of the method. The results of five separate sets of comparisons of phenylacetic acid *vs.* phenyl[$\alpha\alpha$-$^2H_2$]acetic acid and of four such sets with benzylamine *vs.* [$\alpha\alpha$-$^2H_2$]benzylamine are listed in the appropriate column of Table 1. The originally reported [2] isotope effect on the acidity of phenylacetic acid cannot be reproduced, the very much smaller value observed being consistent, within experimental error, with Barnes and Scott's conductimetric results.[7] The newly obtained $\Delta pK_a$ value for benzylammonium ion is in only semi-quantitative agreement with the older value, being 60% as large.

TABLE 1

Secondary deuterium isotope effects on acid strength

| | Previously published data | | This investigation | |
|---|---|---|---|---|
| Acids | Diff. potent. (ref. 2) | Conduct. (ref. 7a) | Diff. potent. | N.m.r. |
| $C_6H_5CD_2CO_2H$–<br>$C_6H_5CH_2CO_2H$ | 0.048<br>±0.005 | $0.002_5$<br>±0.002 | $0.005_5$<br>±0.003 | $0.004_7$<br>±0.006 |
| $C_6H_5CD_2NH_3^+$–<br>C H CH NH + | 0.054<br>±0.001 | | $0.032_3$<br>±0.001 | $0.033_1$<br>±0.002 |

The relevant region of the $^{13}C$ spectra of the two systems under study in the three pH regions is shown in Figure 4. Table 2 shows the best-fit parameters obtained by the n.m.r. method, along with the visually estimated error of each. The $\Delta pK_a$ values obtained with these parameters are entered in the appropriate column of Table 1. The accuracy of the phenylacetic acid result is limited by the low value of $\Delta$, so that $\Delta pK_a$ is no larger than its estimated error. Even so, there is no doubt that the n.m.r. measurements, within their rather large experimental error, confirm the present, rather than the original potentiometric results and, of course, also the conductimetric data.[7] The more precise result for benzylamine is in excellent agreement with that obtained by the potentiometric method in this investigation.

TABLE 2

Best-fit parameters of n.m.r. method *

| Acid–base pair | $\delta$ | $\delta_A$ | $\delta_B$ | $\Delta$ |
|---|---|---|---|---|
| Phenylacetic | 1.4<br>±0.1 | 1.84<br>±0.06 | 0.70<br>±0.10 | $-47.9_6$<br>±1.2 |
| Benzylamine | 4.5<br>±0.1 | 1.65<br>±0.10 | 1.62<br>±0.07 | $-144.8_7$<br>±0.8 |

* All values in Hz.

It must be concluded that, for reasons that cannot be ascertained at this time, the $\Delta pK_a$ values originally reported for phenylacetic acid and, to a less serious extent, those for benzylamine as well, are unreliable. They are therefore withdrawn, as are also those for acetic and propionic acids listed with them in Table 2 of Part 1 of this series.[2] On the other hand, the differential potentiometric method described in that communication is confirmed to be sound.

The n.m.r. method herein described would seem to have general applicability. Two of its limitations, which turned out to be consequential in the case of phenylacetic acid, were the low solubility of the acid in water and the rather small chemical shift ($\Delta$) between the corresponding sites in the acid and its conjugate base. It should not be difficult to overcome these limitations by using samples enriched in $^{13}$C or by working with an instrument with higher frequency resolution and/or a larger data-storage capacity. A more sophisticated simulation program, though unnecessary for the purposes of the present study, might prove useful if the precision of the method were to be improved as suggested.

The conclusions drawn in Part 1 about the origin of secondary isotope effects in these and similar systems are not substantially affected. That on the base strength of benzylamine is a manifestation of the greater effective electropositivity of $CD_2$ than of $CH_2$. In phenylacetic acid, this effect is counterbalanced by hyperconjugative interaction of the methylene and carboxy-groups, which has now to be given more weight than was assumed in Part 1 on the basis of the earlier erroneous result. The relation to conventional isotope effect theory of this interpretation of secondary isotope effects, as outlined in Part 1 and amplified in a contemporary review,[12] is now well established.[13]

The authors are pleased to acknowledge the suggestion of Professor M. Saunders, which triggered the development of the n.m.r. method described in this paper. They are also grateful to Professor A. Loewenstein for valuable discussions.

## APPENDIX

*Derivation of Equation* (1).—We let $A^i$ and $B^i$ represent the acid and conjugate base of an isotopic species (i = H or D) and the mole fraction of $A^i$ be: $x_i = [A^i]/([A^i] + [B^i])$. The ratio between the dissociation constants of the protio- and deuterio-acids in the same solution becomes:

$$\frac{K_a^H}{K_a^D} = \frac{x_D(1 - x_H)}{x_H(1 - x_D)} \qquad \text{(A–1)}$$

Introducing $\bar{x} = x_H - x_D$ and rearranging converts this equation into

$$\frac{K_a^H}{K_a^D} = \left(1 - \frac{\bar{x}}{x_H}\right)\Big/\left(1 + \frac{\bar{x}}{1 - x_H}\right) \qquad \text{(A–2)}$$

Since $\bar{x} \ll 1 - x_H$, the denominator can be expanded in series and the higher terms neglected, leading to

$$\frac{K_a^H}{K_a^D} = 1 - \frac{\bar{x}}{x_H(1 - x_H)} \qquad \text{(A–3)}$$

In the region pH $\cong$ p$K_a$, we can, with sufficient accuracy, set $x_H \cong (1 - x_H) \cong \frac{1}{2}$ from which it follows easily that

$$\Delta pK_a = pK_a^D - pK_a^H = \log_{10}(1 - 4\bar{x}) \qquad \text{(A–4)}$$

In the case of fast exchange between $A^i$ and $B^i$

$$\delta = \nu_B^H + (\nu_A^H - \nu_B^H)x_H - \nu_B^D - (\nu_A^D - \nu_B^D)x_D \qquad \text{(A–5)}$$

Algebraic manipulation of equation (A–5), followed by the assumption: $x_D \cong \frac{1}{2}$, leads to

$$\bar{x} = [\delta - (\delta_A + \delta_B)/2]/\Delta \qquad \text{(A–6)}$$

introduction of which into equation (A–4) yields equation (1) in the text.

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REFERENCES

[1] Part 4, E. A. Halevi and B. Ravid, *Israel J. Chem.*, 1968, **6**, 723.

[2] Part 1, E. A. Halevi, M. Nussim, and A. Ron, *J. Chem. Soc.*, 1963, 866.

[3] Part 3, E. A. Halevi, A. Ron, and S. Speiser, *J. Chem. Soc.*, 1965, 2560.

[4] H. S. Klein and A. Streitwieser, *Chem. and Ind.*, 1961, 180; A. Streitwieser and H. S. Klein, *J. Amer. Chem. Soc.*, 1963, **85**, 2759.

[5] M. Paabo, R. G. Bates, and R. A. Robinson, *J. Phys. Chem.* 1966, **70**, 540.

[6] E. A. Halevi and M. Nussim, *Bull. Res. Council Israel*, 1956, **5A**, 263.

[7] (*a*) D. J. Barnes and J. M. W. Scott, *Canad. J. Chem.*, 1973, **51**, 411; (*b*) D. J. Barnes, P. D. Golding, and J. M. W. Scott, *ibid.*, 1974, **52**, 1966.

[8] M. Saunders, M. H. Jaffe, and P. Vogel, *J. Amer. Chem. Soc.*, 1971, **93**, 2558; M. Saunders and P. Vogel, *ibid.*, pp. 2559, 2561.

[9] E. Grunwald, A. Loewenstein, and S. Meiboom, *J. Chem Phys.*, 1957, **27**, 641.

[10] R. F. Nystrom and W. G. Brown, *J. Amer. Chem. Soc.*, 1948, **70**, 3738.

[11] B. M. Lowe and D. G. Smith, *J.C.S. Faraday I*, 1975, **71**, 389.

[12] E. A. Halevi, *Progr. Phys. Org. Chem.*, 1963, **1**, 109.

[13] See *e.g.* W. A. Van Hook, in ' Isotope Effects in Chemical Reactions,' C. J. Collins and N. S. Bowman, eds., Van-Nostrand Reinhold, Amsterdam, 1970, p. 46.