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EXCHANGE OF ACTIVE HYDROGEN FOR DEUTERIUM IN SPECTROSCOPIC STUDIES

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Replacement of active hydrogen by deuterium in alcohols, carboxylic acids and amines causes a great shift of the infrared absorption band of the O-H or N-H stretching mode, but this property has been used very infrequently for structural studies. Exchange has generally been accomplished by repeated evaporation of the sample from a solution of deuterium oxide, or in the case of compounds with low water solubility, from deuterium oxide-dioxan mixtures or from deuterated alcohols. Elaborate dry-box techniques are necessary to prevent back exchange of the deuterated compounds due to traces of moisture in solvents and adsorbed on apparatus.[1] This problem is particularly acute at the low solute concentrations necessary for infrared hydrogen bonding studies. The fact that the exchange process is essentially

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[1] H. M. Randall, R. C. Fowler, M. Fuson and J. R. Dangyl, Infrared Determination of Organic Structures. D. Van Nostrand Co., Inc., 1949, p. 8.

instantaneous[2,3,4] and that the direction of exchange is controlled by whether D_2O or H_2O is present in excess led to the development of the following simple and successful technique. A small droplet of deuterium oxide is placed on the top of the solution in the infrared absorption cell (generally 1 cm. path carbon tetrachloride solvent). In double beam techniques a small droplet is also added to the reference cell. Exchange of O-H or N-H in the solute for O-D or N-D then occurs rapidly by diffusion and is essentially quantitative within 15 minutes and without shaking the cells. Shaking is avoided because droplets of water adhere to the cell walls and cause scattering. In this way sodium chloride cells can be used with little damage, but "Infrasil Quartz" cells[5] which are transparent in the OH-OD region are especially convenient.

Figure 1 illustrates the technique with an example from Amaryllidaceae alkaloids. Buphanamine,[6,7] which contains one hydroxyl group, exhibits two bands in the O-H region at 3584 cm^{-1} and 3613 cm^{-1} and it was desired to prove that the bands were neither the result of overtones nor Fermi resonance but rather due to hydrogen bonding. After exchange with deuterium

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[2] H. Kwart, L. P. Kuhn and E. L. Bannister, J. Am. Chem. Soc. 76, 5998 (1954).

[3] A. I. Brodsky, J. Gen. Chem. 24, 421 (1954).

[4] J. Hine and C. H. Thomas, J. Amer. Chem. Soc. 75, 739 (1953).

[5] Quaracell Products, Inc., 401 Broadway, New York 13, N. Y.

[6] H. A. Lloyd, E. A. Kielar, R. J. Highet, S. Uyeo, H. M. Fales and W. C. Wildman, Tetrahedron Letters No. 3, 104 (1961).

[7] H. M. Fales and W. C. Wildman, J. Org. Chem. 26, 881 (1961).

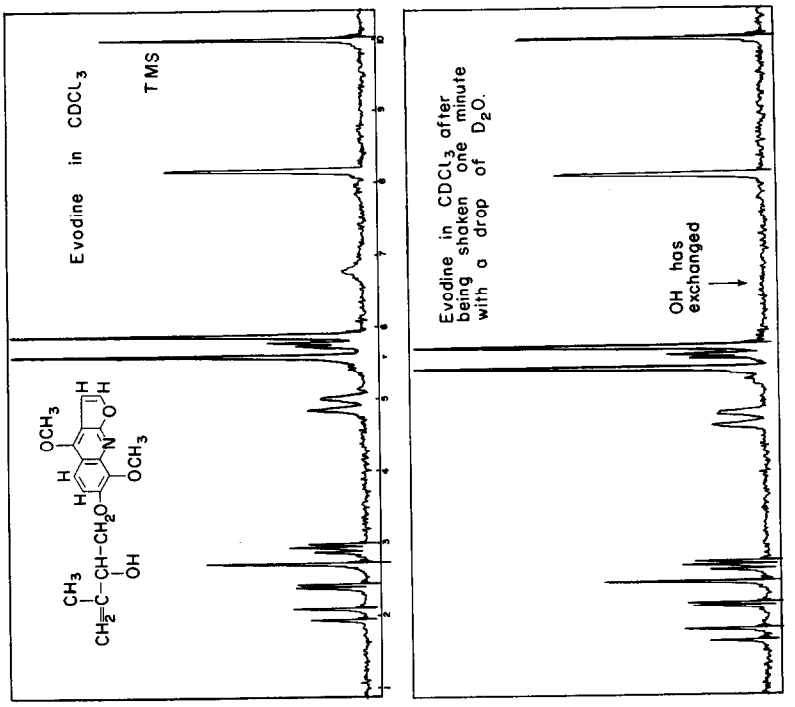
Evodine in $CDCl_3$
TMS
Evodine in $CDCl_3$ after being shaken one minute with a drop of D_2O.
OH has exchanged

Fig. 2

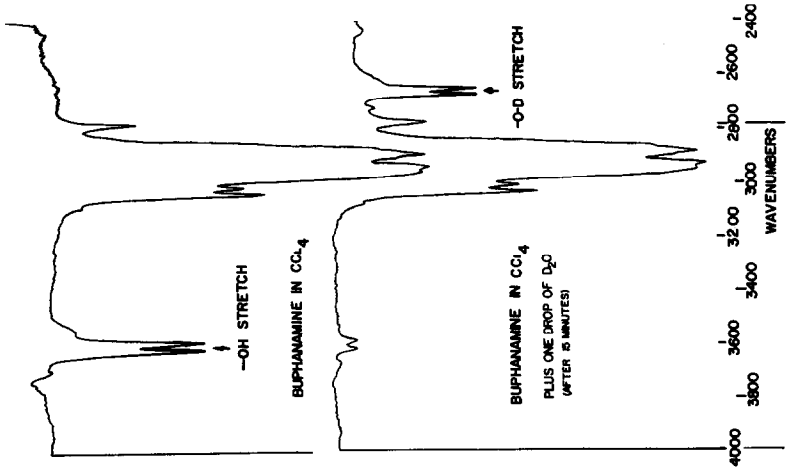
-OH STRETCH
BUPHANAMINE IN CCl_4
-O-D STRETCH
BUPHANAMINE IN CCl_4
PLUS ONE DROP OF D_2O
(AFTER 15 MINUTES)
4000
3800
3600
3400
3200
3000
2800
2600
2400
WAVENUMBERS

Fig. 1

oxide as above the absorption has shifted to the O-D region at 2665 and 2644 cm^{-1} and the general band shape is preserved. A small imbalance in either water or deuterium oxide results in rather broad bands at 3702 and 3611 cm^{-1} or at 2748 and 2640 cm^{-1}.[8] The low frequency band (symmetric HOH or DOD stretching) of each pair is in the region assigned to alcohols and must be avoided by cancellation in the reference beam.

For NMR studies, deuteration of active hydrogen by the repeated evaporation method is feasible because much larger specimens are used compared with infrared samples, and back exchange with adsorbed moisture then becomes negligible.[9] Nevertheless, the procedure is time consuming and wasteful of deuterated solvents. The infrared technique above has been adapted for NMR as follows. After taking the NMR spectrum (usually in $CDCl_3$ solution) the solution is returned to the vial in which it was prepared and shaken briefly with a drop of D_2O. The organic layer is transferred again to the NMR probe tube and the spectrum rerun. In general, less than one minute of shaking by hand is sufficient to exchange OH or NH protons quantitatively and the appropriate signals disappear completely from the spectrum. No great care needs to be taken to keep droplets of heavy water out of the probe tube because the spinning of the sample separates the layers.

Figure 2 illustrates the technique with the furoquinoline alkaloid evodine.[10] The low broad peak for the hydroxyl proton near $\tau = 6.8$ complete-

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[8] L. B. Borst, A. M. Buswell and W. H. Rodebush, J. Chem. Phys. 6, 61 (1938).

[9] e.g., L. A. Cohen, J. W. Daly, H. Kny and B. Witkop, J. Amer. Chem. Soc. 82, 2184 (1960).

[10] R. H. Prager, E. Ritchie, A. V. Robertson and W. C. Taylor, Austral. J. Chem. in press.

ly disappears after deuteration by the above treatment. The rapidity and simplicity of the process encourages its use on a routine basis with new compounds, both as a means of identifying signals from active hydrogens and to expose fine structure of bands which overlap such signals. The method fails for hydroxyl groups known to be strongly bonded intramolecularly. However such negative results have valuable structural implications for compounds of unknown constitution. In another case interesting rate effects have been observed; the signal from the amide protons of 3,4-dehydroprolinamide[11] disappeared after shaking for one minute whereas that from the secondary amino proton was only slightly reduced in intensity. Thirty minutes' shaking was necessary for complete exchange of the latter proton. This is surprising in view of the accepted "rapid" mechanism of exchange of hydrogens on amino type nitrogen since steric hindrance can hardly be a kinetic factor for this molecule as it can be in cases such as proteins.[12] Nor can the heterocyclic nitrogen be of the ammonium type (which does exhibit "slow" exchange[3]) because the N-H signal is of one proton area as would be expected in neutral solution. The reason for the behaviour of this proton in 3,4-dehydroprolinamide is being investigated. The present technique thus has obvious application to rate studies on the mechanism of hydrogen exchange.

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[11] A. V. Robertson and B. Witkop, J. Amer. Chem. Soc. 82, 5008 (1960).

[12] e.g., S. O. Nielsen, W. P. Bryan and K. Mikkelsen, Biochim. et Biophys. Acta 42, 550 (1960).