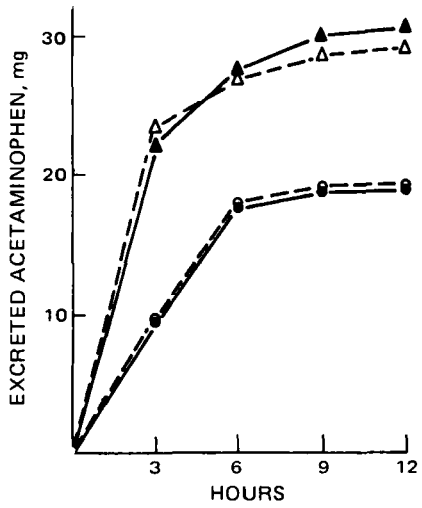

Figure 4—*Cumulative urinary excretion of free acetaminophen in two healthy subjects as determined by HPLC (○, △) and radioisotope (●, ▲) methods.*

mination, the paper chromatography system of Shahidi (6) was used to separate the ^{14}C-acetaminophen from its metabolites. The overall difference between the two methods was less than 6%.

This HPLC method is rapid and simple for the determination of acetaminophen in biological fluids. It involves no silylation of the compound as is required in GLC determinations. Because the extraction procedure is simple and complete, the use of an internal standard is not necessary; reproducible results to within 4% were achieved. The limit of detectability for acetaminophen by this method is 1 μg/ml. Below this concentration, the reproducibility of acetaminophen estimations is more variable.

This HPLC technique also could be used for the determination of acetaminophen metabolites. The estimation of the glucuronide and sulfate of acetaminophen is usually carried out by analyzing the free acetaminophen generated from enzymatic cleavage of these metabolites (7). Likewise, the cysteine and mercapturate metabolites could be estimated after chemical cleavage with Raney nickel (8).

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[x] To whom inquiries should be directed.

Enthalpies of Hydrogen Bonding in Psychotropic Drugs

TIMOTHY B. PATRICK [x] and RANDALL R. BRESEE

Abstract □ The enthalpy of hydrogen bonding of some antipsychotic, antidepressant, anticonvulsant, and antianxiety agents with phenol, as determined from IR and NMR spectroscopic measurements, was shown not to be responsible for differences in activity within the drug classes. These results support a theoretical prediction advanced for anticonvulsant activity.

Keyphrases □ Enthalpy—hydrogen bonding between various psychotropic drugs with phenol, IR and NMR spectral measurement, related to differences in activity □ Hydrogen bonding—various psychotropic drugs with phenol, IR and NMR spectral measurement, related to differences in activity □ Psychotropic drugs—enthalpy of hydrogen bonding with phenol, IR and NMR spectral measurement, related to differences in activity

Hydrogen bonding is often mentioned when the mechanism of drug action is considered. However, little quantitative information concerning this subject has been reported (1, 2). Therefore, the enthalpy of hydrogen bonding of several psychotropic drugs with phenol was investigated to obtain factual information for testing the theoretically based conclusion that hydrogen bond acceptance is not responsible for variations in anticonvulsant activity (3). Furthermore, hydrogen bond strengths of some tranquilizing and anticonvulsant drugs were examined so that information regarding the importance of hydrogen bonding in diazepam (V), which acts as both a tranquilizer and an anticonvulsant (4), could be evaluated.

DISCUSSION

Hydrogen-bonding enthalpies with phenol were obtained using the IR and NMR spectral techniques developed by Drago and coworkers (5, 6). The IR technique relies on a linear relationship between the enthalpy of hydrogen bonding and the hydroxyl frequency difference of free phenol and hydrogen-bonded phenol. The NMR method is based on a linear relationship between hydrogen-bonding enthalpy and the chemical shift of a hydroxyl proton in a hydrogen-bonded phenol–base adduct. The IR procedure is better because measurements are straightforward and small sample concentrations are used; the NMR method requires many sample variations and an estimate of anisotropic corrections (6).

The IR–enthalpy relationship obtained was:

$$-\Delta H \text{ (kcal/mole)} = 0.010\,(\Delta\nu) + 3.67 \pm 0.04 \qquad r = 0.986 \quad \text{(Eq. 1)}$$

The NMR–enthalpy relationship is:

$$-\Delta H \text{ (kcal/mole)} = 1.89\,(\delta \text{ adduct}) - 9.14 \pm 0.52 \qquad r = 0.991 \quad \text{(Eq. 2)}$$

Errors are reported at the 99% confidence level (7).

Table I—Hydrogen-Bonding Enthalpies for Phenol–Drug Adducts

Compound	$\Delta\nu$, cm^{-1}	$-\Delta H$, kcal/mole	δ Adduct, ppm	$-\Delta H$, kcal/mole	Activity[a]
I: Promazine	367	6.91	8.97	7.81	—
II: Amitriptyline	336	6.63	8.94	7.76	—
III: Imipramine	308	6.35	8.80	7.49	—
IV: Chlorpromazine	262	5.89	8.25	6.45	—
V: Diazepam	154	5.72	7.32	4.69	0.04[b]
VI: Phenobarbital	207	5.34	7.23	4.52	0.10
VII: Dimethadione	194	5.21	7.30	4.66	2.6
VIII: Phenytoin	170	4.97	7.01	4.10	0.04
IX: Bemegride	137	4.62	7.19	4.45	Convulsant

[a] Supramaximal electroshock data. Activity is in dose (millimoles per kilogram) that protects 50% of mice from the tonic extensor phase (3). [b] L. H. Sternbach, L. O. Randall, and S. R. Gustafson, in "Psychopharmacological Agents," vol. I, M. Gorden, Ed., Academic, New York, N.Y., 1968, p. 138.

The data in Table I clearly show that the hydrogen-bonding enthalpies found by IR measurements for the anticonvulsant compounds (VI–IX) have a mean value of 5.04 kcal/mole; the spread of enthalpies is within the ±0.40-kcal/mole experimental error. Enthalpies measured by NMR spectroscopy for VI–IX show a mean value of 4.60 kcal/mole, with the enthalpy spread within the ±0.52-kcal/mole experimental error. According to these results, enthalpies of hydrogen bonding of phenol with the anticonvulsants studied are essentially the same and support the theoretical prediction that hydrogen bond acceptance alone cannot account for differences in anticonvulsant activity (3).

The antipsychotic and antidepressant agents (I–IV) exhibit enthalpy values of 1.41 (IR) and 2.77 (NMR) kcal/mole higher than the anticonvulsants V–IX. The enthalpy spread in this class is also within experimental error, suggesting that differences in activity are not the result of differences in hydrogen-bonding strength. Hydrogen bond acceptance has been suggested to be an important factor in chlorpromazine's activity (8).

Diazepam, which exhibits both tranquilizing and anticonvulsant activities (4) and belongs to both drug classes, displays an enthalpy of hydrogen bonding intermediate between that of the two classes. The significance of this observation is not apparent at present and must await further quantitative studies of other factors important for physiological activity.

In summary, the evidence presented shows that hydrogen bond acceptance alone is not responsible for the differences in activity within drug classes.

EXPERIMENTAL[1]

Materials—All solvents were spectrograde quality and were stored over 4A molecular sieves[2]. Phenol was distilled and then freshly sublimed before each run. Amitriptyline[3], chlorpromazine[4], promazine[5], and imipramine[6] were obtained as their hydrochloride salts. The free amines, obtained by neutralization of the salts with ammonia, were purified by sublimation. Diazepam[7] was obtained as the free amine. Phenytoin, dimethadione, bemegride, and phenobarbital were obtained commercially[8]. All compounds had melting points and spectral data consistent with reported properties. All materials were sublimed directly before use.

Procedures—*IR Spectroscopy*—To a 0.02 *M* solution of phenol in carbon tetrachloride was added the base until IR absorbances due to both free phenol and complexed phenol could be observed. This procedure was repeated at several concentrations, and the results were extrapolated to infinite dilution. The frequency difference between free phenol and complexed phenol ($\Delta\nu$) was correlated with the enthalpies for the compounds listed in Table II to give Eq. 1. The procedure was repeated with the drugs listed in Table I to obtain ($\Delta\nu$); Eq. 1 was used to determine ΔH.

[1] IR measurements were made in carbon tetrachloride solution using a Perkin-Elmer model 621 spectrophotometer. NMR spectra were run in methylene chloride solution at 35° employing a Varian T-60 instrument locked on tetramethylsilane (0.0 Hz).
[2] Linde.
[3] Merck Sharp and Dohme.
[4] Smith Kline and French.
[5] Wyeth.
[6] Geigy.
[7] Hoffmann-La Roche.
[8] Sigma Chemical Co.

Table II—Chemical Shifts and Frequency Differences for Phenol–Base Adducts

Base	$\Delta\nu$ (CCl_4), cm^{-1}[a]	δ (Adduct) (CH_2Cl_2), ppm[b]	$-\Delta H$, kcal/mole[c]
Triethylamine	592	9.41	9.1
Pyridine	484	9.10	8.0
Dimethyl sulfoxide	357	8.53	6.9
N-Methylpyrrolidinone	325	8.00	6.1
N,N-Dimethylacetamide	312	8.45	6.8
Acetamide	193	7.89	5.0
Ethyl acetate	149	7.15	4.8

[a] ±10 cm^{-1}. [b] Correction of −1.1 ppm included except for triethylamine and dimethyl sulfoxide. [c] Reference 5.

NMR Spectroscopy—A dilution study of phenol in methylene chloride showed that only monomeric phenol was present at concentrations below 0.05 *M*. Solutions containing 0.50–0.25 *M* base and 0.25 *M* phenol in methylene chloride were observed at 35°. The concentration of uncomplexed phenol under these conditions was less than 0.05 *M*. The base concentration was increased in 0.05 *M* increments.

Chemical shift dependence of the phenolic hydroxyl proton signal as a function of concentration was recorded. A previously described computerized method (9) was used to determine the chemical shift of the 1:1 phenol–base adduct (5). A plot of the chemical shift of adducts (δ adduct) for the compounds listed in Table II *versus* the known enthalpy values provided Eq. 2. The adduct chemical shift for the drugs listed in Table I was obtained in the same manner, and the hydrogen bonding enthalpy was determined from Eq. 2. Anisotropic corrections of −1.1 ppm were used for the compounds containing carbonyl groups (5).

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[x] To whom inquiries should be directed.