# Chemical and Spectroscopic Analysis of a Phenobarbital–Ephedrine Complex

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**Abstract** □ A complex of phenobarbital and ephedrine was prepared and characterized. The complex was differentiated from physical mixtures of the two drugs by TLC and IR spectroscopy. NMR spectroscopy was used to verify the proportion of each drug in the complex and to confirm the existence of intermolecular binding. Mass spectrometry was employed to establish that all fragments characteristic of the individual drug entities could be isolated from the complex. Elemental analysis confirmed the chemical composition of the complex. A possible chemical structure for the complex was hypothesized on the basis of the chemical and spectroscopic data.

**Keyphrases** □ Phenobarbital—complex with ephedrine prepared, chemical and spectroscopic analysis □ Ephedrine—complex with phenobarbital prepared, chemical and spectroscopic analysis □ Complexes—phenobarbital with ephedrine, preparation, chemical and spectroscopic analysis

One widely used method to measure complexation has been to monitor a change in the curve produced for some additive property such as spectrophotometric absorbance. Titration techniques have been used whenever a change in pH accompanied the complexation reaction. The change in absolute solubility in a given solvent was used to measure quantitatively the production of caffeine complexes (1), and a phase solubility technique was used to study the formation of complex salts of triamterene with several acids (2).

The color reaction of local anesthetics with 1,3,5-trinitrobenzene was utilized to study the stoichiometry of complex formation (3). NMR spectroscopy was used (4) to study the structure of the caffeine–sodium benzoate complex, and differential thermal analysis was used (5) to follow the interaction of aminopyrine and barbital. A combination of NMR and IR spectroscopy was employed to investigate the extent of hydrogen bonding resulting in increased solubility of paraben combinations (6).

A number of molecular phenobarbital complexes were investigated. Phase solubility studies of phenobarbital and polyethylene glycol complexes (7), an IR study of phenobarbital association with purines (8), and IR studies of phenobarbital complexes with quinine, quinidine, and hydroquinidine (9) were reported. The isolation of a 1:1 phenobarbital–ephedrine complex formed by acid–base neutralization in ether also was reported (10).

The objective of this study was to apply chemical and spectroscopic analytical techniques to determine the nature and structure of a phenobarbital–ephedrine complex.

## EXPERIMENTAL

Phenobarbital was recrystallized from boiling water, and the melting point of the recrystallized and dried product was determined by differential thermal analysis to be 174–176°. Ephedrine hemihydrate was made anhydrous by azeotroping with benzene, and the resulting solid was dried and stored under vacuum. The melting point determined by differential thermal analysis was 33–36°. Spectral grade chloroform was used for the IR analysis. All other reagents were analytical grade. The recrystallized complex was subjected to elemental analysis[1].

**Table I—$R_f$ Values for Ephedrine, Phenobarbital, and Complex**

| Solvent System | Plates | $R_f$ Ephedrine | $R_f$ Phenobarbital | $R_f$ Complex |
|---|---|---|---|---|
| 50% Ethanol | Alumina | 0.38 | 0.75 | 0.70 |
| Anhydrous methanol | Alumina | 0.57 | 0.31 | 0.23 |
| 50% Ethanol | Silica | 0.10 | 0.80 | 0.80 |
| | | | | 0.03 |
| Anhydrous methanol | Silica | 0.18 | 0.71 | 0.71 |
| | | | | 0.11 |

**Synthesis of Complex**—Exactly 1.65 g (10 mmoles) of anhydrous ephedrine was added to 2.70 g (12 mmoles) of phenobarbital. The mixture was dissolved in approximately 15 ml of anhydrous ether, which had been stored over metallic sodium. Any excess phenobarbital was removed by filtration. The resulting solution was crystallized by chilling in an acetone–dry ice bath. The sides of the flask were scratched with a sharp glass rod to induce crystallization.

A "dry box" was prepared from an air-tight glove box containing phosphorus pentoxide under an atmosphere of nitrogen and was fitted with a suction hose for filtration. A specially prepared funnel was made by cutting a hole for a 9-cm büchner funnel in a 15-cm polyethylene büchner funnel. The holes in the outer funnel were sealed with a resin, and the space between the two funnels was filled with crushed dry ice. The funnel and filter paper were allowed to equilibrate in the dry box for at least 0.5 hr prior to filtration.

The crystals were collected in the central filter, and the ether was removed as rapidly as possible by vigorous suction aided by a positive pressure from nitrogen gas being introduced into the box. The filter system, including the dry ice and the crystalline material, was rapidly removed to a vacuum desiccator containing sulfuric acid and phosphorus pentoxide. The desiccator was immediately evacuated, and the crystals were left to dry for not less than 24 hr.

**TLC**—TLC was performed on both silica[2] and alumina[3] fluorescent plates for phenobarbital, ephedrine, and the complex. The plates were spotted with an ether solution of each compound, and spots were visualized with a UV lamp and iodine vapors. The following solvents were used: anhydrous methanol, 95% ethanol, 70% ethanol, anhydrous ether, 75% benzene–25% ethyl acetate, and 50% benzene–50% ethyl acetate.

**IR Spectroscopy**—IR spectra were obtained[4] for ephedrine, phenobarbital, and the complex using approximately 10% (w/v) samples dissolved in spectral grade chloroform.

**NMR Spectroscopy**—NMR spectroscopy[5] was used to examine the proton resonance structures of ephedrine, phenobarbital, the complex, ephedrine acetate, and phenobarbital triethylamine solutions in deuterochloroform (approximately 5% w/v). The spectra were determined in the field-sweep mode using an internal tetramethylsilane standard.

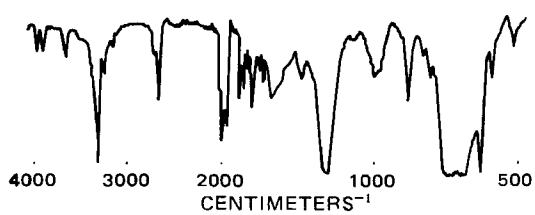



**Figure 1**—*IR spectrum of phenobarbital.*

[1] Carbon and hydrogen analyses were performed by Micro-Tech Laboratories, Skokie, Ill. Nitrogen analysis was obtained using a Coleman nitrogen analyzer.
[2] Merck silica gel 60 F254.
[3] Merck aluminum oxide type T F254.
[4] Perkin-Elmer 337 grating spectrophotometer.
[5] Varian T-60A NMR spectrometer equipped with Fourier transform.

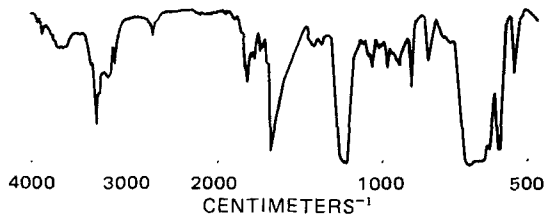



**Figure 2**—*IR spectrum of ephedrine.*

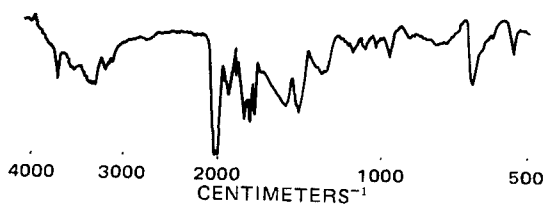



**Figure 3**—*IR spectrum of complex.*

**Mass Spectrometry**—Mass spectrograms for ephedrine, phenobarbital, and the complex were obtained[6].

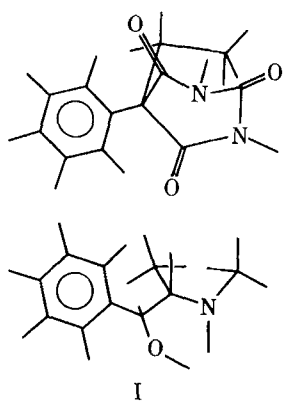
## RESULTS AND DISCUSSION

The results of Babin and Coustou (10) for the preparation of the complex by titration could not be duplicated in this laboratory. Only by removing all sources of water from the reagents and from the atmosphere surrounding them could a crystalline substance be isolated. It was then necessary to dry the crystals in a chilled, evacuated vessel to prevent further absorption of water and subsequent liquefaction. Once the crystals were completely free of solvent, they exhibited no further hygroscopic tendency. Differential thermal analysis showed the complex to have a wide melting range, centering at approximately 70°.

The results of TLC studies performed on the complex are summarized in Table I. Since the manufacturer's plates were used without subsequent activation, hydration of the silica plates cannot be ruled out as a cause of the decomposition of the complex that occurred on the silica plates but not on the alumina plates.

IR spectroscopy of phenobarbital in spectral grade chloroform yielded values of 3690 and 3630 $cm^{-1}$ for NH and of 1750 and 1730 $cm^{-1}$ for CO (Fig. 1). Ephedrine yielded peaks at 3670 and 3620 $cm^{-1}$ for the phenolic hydrogen and a broad peak between 3500 and 3300 $cm^{-1}$ for the hydrogen-bonded COH group (Fig. 2). The IR spectrum of the complex differed greatly from spectra of the individual compounds. An NH peak was found at approximately 3375 $cm^{-1}$ with broad and ill-defined peaks surrounding it, indicating that hydrogen bonding existed in this area of the molecular complex (Fig. 3). The 3600–2600-$cm^{-1}$ region for the complex more closely resembled that of the salts of ephedrine than of the alkaloid itself. A CO peak was found at 1750 $cm^{-1}$, as in the spectrum of phenobarbital.

To gain further evidence of complex formation, the NMR spectra of ephedrine, phenobarbital, and the product of their reaction were examined. The formation of a complex (Structure I) might be expected to affect the magnetic environment of the hydrogens of the aromatic rings of both compounds and the protons on the carbon atom of ephedrine adjacent to the methylamino group. It is assumed that the reaction proceeds first with salt formation, followed by the formation of a hydrogen bond between phenobarbital and the hydroxyl group of ephedrine and an alignment of the phenyl groups one on top of the other.

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The NMR spectra of phenobarbital and ephedrine are shown in Figs. 4 and 5, respectively. The spectrum of the product of the reaction between phenobarbital and ephedrine (Fig. 6) appears to exist as a superimposition of one spectrum on another. A closer examination indicates an appreciable change in the chemical shift of the protons of the aromatic ring of phenobarbital, the benzylic proton of ephedrine, and the methine proton adjacent to the methylamino group of ephedrine (Table II). Since these shifts might result from the protonation of ephedrine by phenobarbital or the formation of the anion of phenobarbital, the spectra of these species were examined. The anion was formed by treating a solution of phenobarbital in deuterochloroform with excess triethylamine (Fig. 7). The spectrum of the protonated species of ephedrine (Fig. 8) was obtained by treating a deuterochloroform solution of ephedrine with excess acetic acid. The significant chemical shifts of these species are summarized in Table II.

In the complex, the aromatic protons of ephedrine and phenobarbital overlapped, representing an upfield shift of the aromatic protons of phenobarbital to 7.28 ppm. Formation of the anion of phenobarbital caused a downfield shift of the aromatic protons to 7.41 ppm. Protonation of ephedrine caused a downfield shift of the aromatic protons. The benzylic proton of ephedrine appeared as a doublet at 4.65 ppm. When protonated with excess acetic acid, it was slightly shifted downfield to 4.83 ppm. In the complex, this proton gave a doublet at 5.14 ppm. Protonation of the hydrogen adjacent to the methylamino group of ephedrine caused a downfield shift from 2.69 to 3.15 ppm. In the complex, this multiplet was found at 2.78 ppm.

The chemical shifts reported in Table II represent several spectral examinations and did not result from concentration differences. The findings indicate that the product of the reaction of phenobarbital and ephedrine is held together by weak intermolecular forces as well as an ionic bond. The changes in chemical shifts of aliphatic protons between ephedrine, the complex, and the protonated species were all to a lower magnetic field, and the complex was shifted the most. In the case of the aromatic protons of phenobarbital, however, the aromatic protons of the anion were shifted downfield from phenobarbital while those of the complex were shifted upfield. Integration of the NMR signal as well as

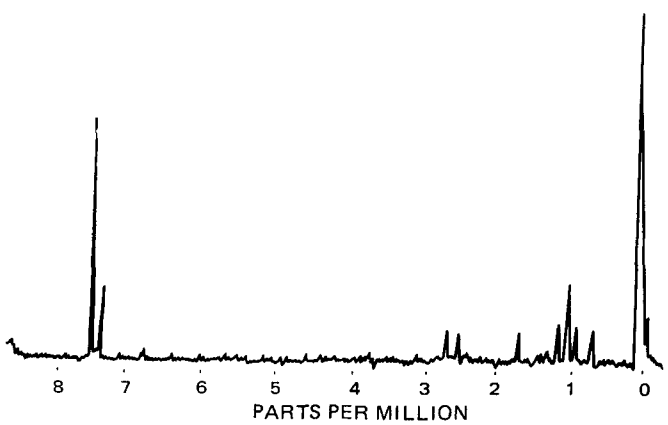


**Figure 4**—*NMR spectrum of phenobarbital.*

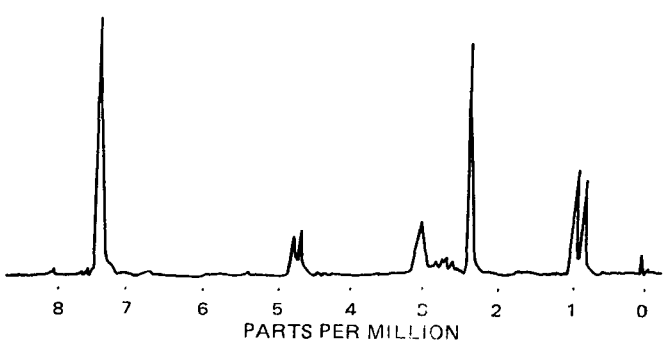


**Figure 5**—*NMR spectrum of ephedrine.*

[6] Hitachi Perkin-Elmer RMU-60 spectrometer.

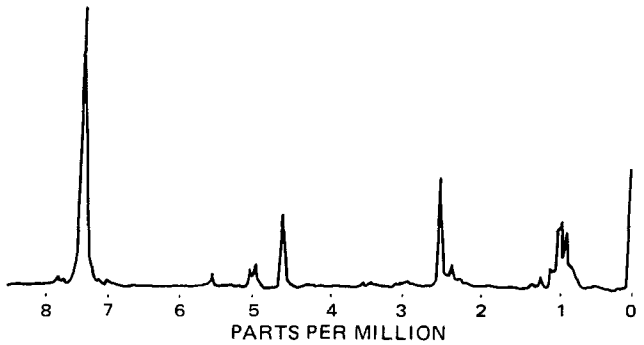


**Figure 6**—*NMR spectrum of complex.*

elemental analysis suggests that the complex is composed of equimolecular quantities of phenobarbital and ephedrine.

While it is not possible to elucidate completely the structure of the complex, the spectral data suggest the protonation of ephedrine by phenobarbital followed by the formation of a hydrogen bond between the alcoholic hydroxyl of ephedrine and a suitable group of phenobarbital. A structure resembling I would place the phenyl groups of one molecule above the other and provide an opportunity for hydrophobic bonding. Such an arrangement might provide a conformational change in phenobarbital, which would account for the change in chemical shift of the aromatic protons.

The observed fragmentation pattern for phenobarbital as determined by mass spectrometry yielded two different primary fragments: one from the loss of an NH group, which had an *m/e* ratio of 217, and another from the loss of a CO group with an *m/e* of 204. Both gave rise to a secondary fragment of *m/e* 189 in which both a CO and an NH were missing. This fragment was broken down further by loss of a second CO to give an ion with *m/e* 161. A second NH was then lost to produce a fragment of *m/e* 146. The mass spectrometry data show that ephedrine broke down into four primary fragments with *m/e* ratios of 132, 105, 91, and 58. Furthermore, the fragment at 105 split to produce an ion with an *m/e* ratio of 77. The fragmentation pattern observed from the mass spectrometry of the complex was the combination of the two patterns for phenobarbital and ephedrine.

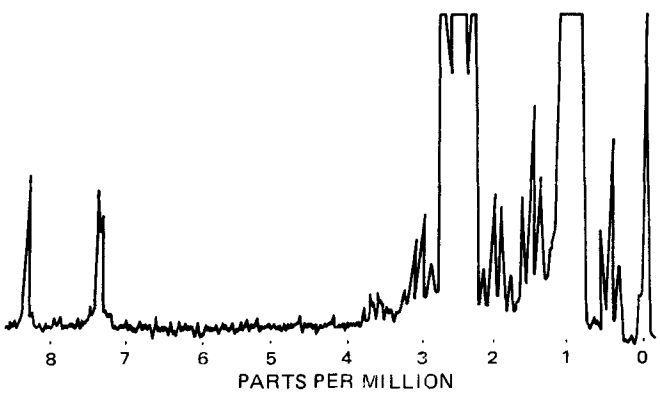


**Figure 7**—*NMR spectrum of phenobarbital plus triethylamine.*

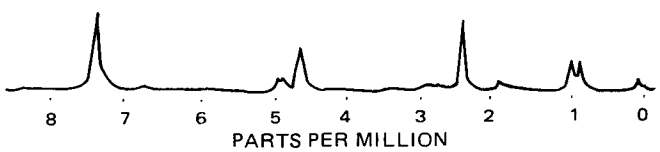


**Figure 8**—*NMR spectrum of ephedrine plus acetic acid.*

**Table II—Pertinent Chemical Shifts from NMR Spectra**

| Compound | Chemical Shifts, ppm: Aromatic Proton | Benzylic Proton of Ephedrine | Methine Proton Adjacent to Nitrogen of Ephedrine |
|---|---|---|---|
| Phenobarbital | 7.33 | — | — |
| Phenobarbital triethylamine | 7.41 | — | — |
| Complex | 7.28 | 5.14 | 2.78 |
| Ephedrine acetate | 7.32 | 4.83 | 3.15 |
| Ephedrine | 7.28 | 4.65 | 2.69 |

*Anal.*—Calc. for 1:1 complex: C, 67.37; H, 7.18; N, 10.27. Found: C, 66.99; H, 7.23; N, 10.25.

## CONCLUSIONS

A complex of phenobarbital and ephedrine was isolated and demonstrated to have different physical and chemical properties from the individual drugs or from simple admixtures of the drugs. It is postulated that the phenobarbital–ephedrine complex forms through a combination of bonding types rather than simply being a salt formed as the product of a neutralization reaction between an acid and a base. The complex does, however, exhibit some characteristics of a salt, and salt formation is proposed as one bonding mechanism involved.

Other types of bonds are proposed as an explanation for some of its unique properties. Face-to-face stacking of the aromatic rings may occur and allow $\pi$ forces to interact between the rings. Hydrogen bonding may also occur at several sites. In addition to dimer formation, it is also possible that trimers, tetramers, and higher aggregates such as base-stacked tetramers, spirals, and indefinite linear aggregates may occur.

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