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Characterization of Hydrogen Bonding between Selected Barbiturates and Polyethylene Glycol 4000 by IR Spectral Analysis

B. L. CHANG, N. O. NUESSLE, and W. G. HANEY, Jr. [x]

Abstract □ Several barbiturates and primidone were equilibrated with polyethylene glycol 4000 in pyridine. IR spectral properties of these samples indicate that seven disubstituted barbiturates complex with polyethylene glycol 4000 while five disubstituted barbiturates and two trisubstituted barbiturates as well as primidone do not. Forces responsible for complexation of barbiturates with polyethylene glycol 4000, as inferred from spectral data, consist of hydrogen bonds formed between N^1 and N^3 hydrogens of the barbiturate ring and two oxygen atoms of the —O—CH_2CH_2—O— moiety. Also, there appear to be three configurations of intermolecular hydrogen bonding sites between disubstituted barbiturates. Several factors affect the barbiturate–polyethylene glycol 4000 interaction, including the nature of the solvent, C_5 substituents, the number of hydrogen bonds formed between reactants, and the 2-carbonyl group of the barbiturate ring. Complexes of polyethylene glycol 4000 with phenobarbital, butabarbital, and cyclobarbital are stable in water at 26° or below, but complexes of polyethylene glycol 4000 with butethal, cyclopentenyl allylbarbituric acid, pentobarbital, and probarbital are not.

Keyphrases □ Barbiturates—IR characterization of hydrogen bonding with polyethylene glycol 4000 □ Primidone—interaction with polyethylene glycol 4000 □ Hydrogen bonding of barbiturates and primidone with polyethylene glycol—characterized by IR spectrophotometry □ Complexes, barbiturates and polyethylene glycol 4000—IR characterization □ IR spectrophotometry—characterization, hydrogen bonding between barbiturates and polyethylene glycol 4000

Table I—N—H Absorption Bands in Spectra of Barbiturates and Their Complexes

Compound	N—H Absorption Bands, cm^{-1}				
Phenobarbital	3310	3200			3090
Phenobarbital complex	3220			3110	
Pentobarbital		3210	3170		3090
Pentobarbital complex	3240			3110	
Probarbital		3220			3100
Probarbital complex	3270			3120	
Butabarbital		3220			3095
Butabarbital complex	3260			3120	
Cyclobarbital		3220			3100
Cyclobarbital complex	3280			3120	
Butethal		3200			3090
Butethal complex	3220			3100	
Cyclopentenyl allylbarbituric acid		3210	3160		3080
Cyclopentenyl allylbarbituric acid complex		3210			3080

Barbiturates may interact with various organic molecules by hydrogen bonding to form soluble or insoluble complexes such as phenobarbital–theophylline (1), phenobarbital–polyvinylpyrrolidone (2), phenobarbital–caffeine (3), barbital–aminopyrine (4), and phenobarbital–poly-*N*-vinyl-5-methyl-2-oxazolidinone (5). Application of such complexation to pharmaceutics has been discussed in the literature (1–7).

In 1968, Kyogoku *et al.* (8) found that barbiturates also form specific and strong hydrogen bonds to adenine derivatives. Since adenine derivatives exist in a number of biochemicals, *e.g.*, coenzymes and adenosine triphosphate, this interaction formed the basis of a hypothesis explaining the molecular mechanism underlying the pharmacological mode of action of barbiturates. Kyogoku and Yu (9–11) extended the research from ethyladenine to nicotinamide adenine dinucleotide (NAD) and flavine adenine dinucleotide (FAD) and postulated that barbiturates inhibit biological oxidation by interaction with the adenine moiety of NAD and FAD. Thus, an understanding of hydrogen bonding properties is important in barbiturate dosage form development and may allow explanation of the mechanism by which barbiturates inhibit biological oxidation. It is of interest, therefore, to study hydrogen bonding properties of the barbiturate ring in detail.

It was shown (8) that phenobarbital–9-ethyladenine has an association constant of 1200 M^{-1}, whereas barbital–9-ethyladenine and pentobarbital–9-ethyladenine have association constants of 1000 M^{-1}. The larger association constant of phenobarbital–9-ethyladenine was attributed to the lower pKa of phenobarbital (7.3) as compared to that of barbital (7.8) and pentobarbital (8.0). However, since pentobarbital–9-ethyladenine has the same association constant

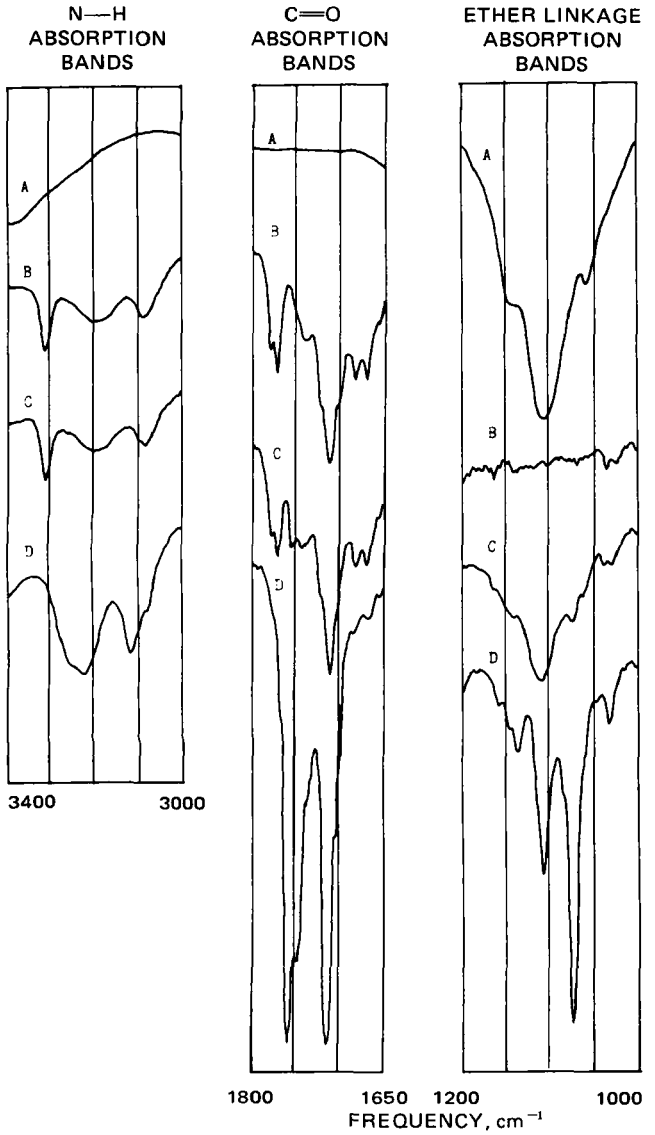


Figure 1—*IR spectra of phenobarbital–polyethylene glycol 4000 from water. Key: A, polyethylene glycol 4000; B, phenobarbital; C, physical mixture of phenobarbital and polyethylene glycol 4000; and D, phenobarbital–polyethylene glycol 4000 complex from water.*

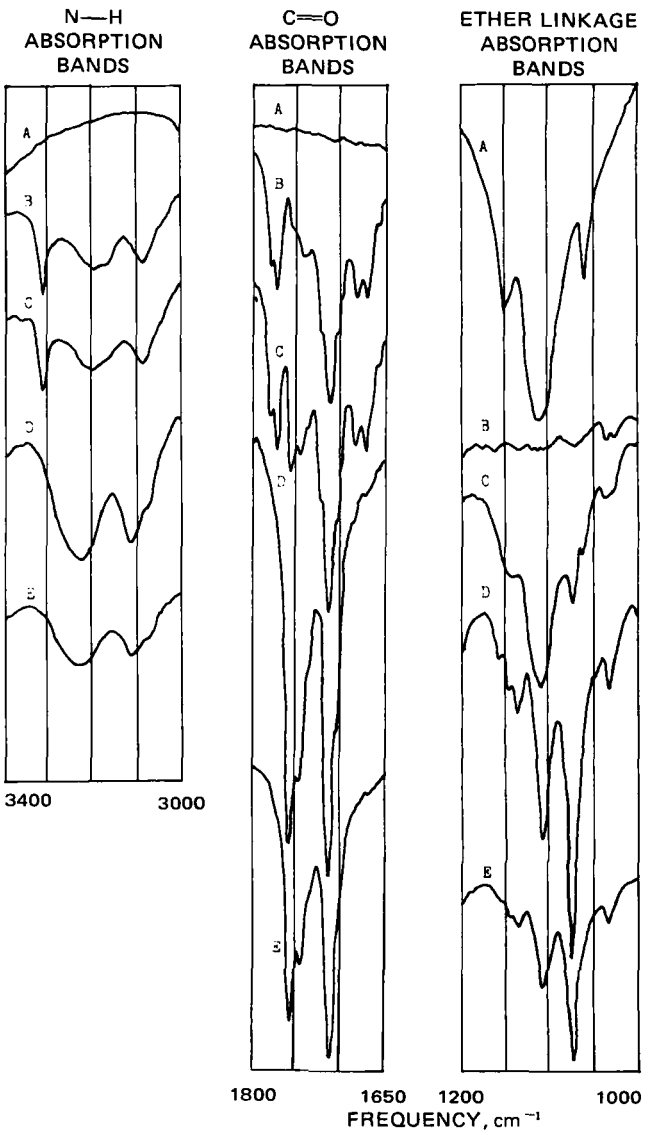


Figure 2—*IR spectra of phenobarbital–polyethylene glycol 4000 from pyridine. Key: A, polyethylene glycol 4000; B, phenobarbital; C, physical mixture of phenobarbital and polyethylene glycol 4000; D, equilibrated phenobarbital–polyethylene glycol 4000; and E, aqueous-washed equilibrated phenobarbital–polyethylene glycol 4000 shown in D.*

as barbital–9-ethyladenine, other factors (*e.g.*, the steric effect of the C_5 substituent) must contribute to the strength of hydrogen bonds formed between barbiturates and 9-ethyladenine.

A particularly interesting observation (7) related to hydrogen bonding properties of barbiturates is that phenobarbital interacts with polyethylene glycol 4000 to form a water-insoluble complex with a 1:2 stoichiometric ratio while barbital and pentobarbital do not form this complex. To account for this observation, it was suggested that the interaction mechanism is influenced primarily by the dipole–dipole interaction of reactants and secondarily by the "squeezing-out" effect of water. However, barbital and, especially, the lipophilic pentobarbital are not as favorably affected by the squeezing-out effect as is phenobarbital. Negative results obtained with barbital and pentobarbital were attributed in part to a stereochemical effect and in part to the absence of the aromatic ring, which participates more readily in complexation. It is of interest, therefore, to identify the stereochemical factors and the mechanism by which the phenyl substituent favorably affects the interaction.

This investigation was undertaken to examine the interaction between barbiturates and polyethylene glycol 4000 and to elucidate factors contributing to this interaction using IR spectral analysis. In barbiturates, the hydrogen bond donor is the =N—H group and the hydrogen bond acceptor is the >C=O group. In polyethylene glycol 4000, the oxygen atom of the ether linkage is the acceptor and the contribution of terminal hydroxy groups, present in low proportion, may be neglected. These functional groups have characteristic absorption bands in the IR region.

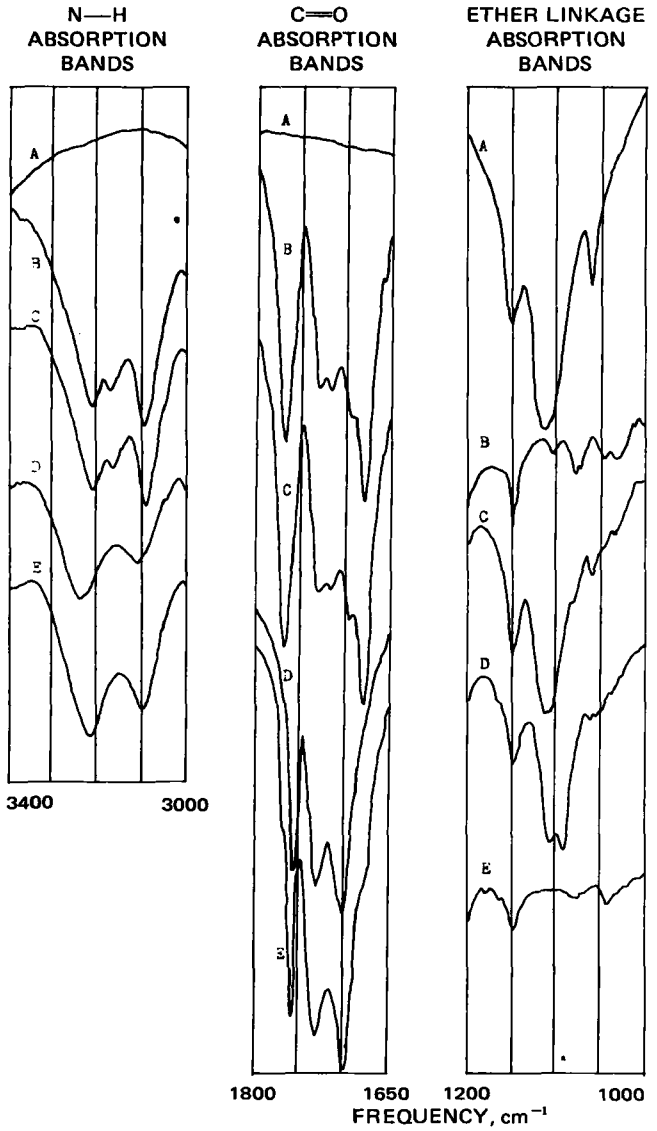


Figure 3—*IR spectra of pentobarbital–polyethylene glycol 4000 from pyridine. Key: A, polyethylene glycol 4000; B, pentobarbital; C, physical mixture of pentobarbital and polyethylene glycol 4000; D, equilibrated pentobarbital–polyethylene glycol 4000; and E, aqueous-washed equilibrated pentobarbital–polyethylene glycol 4000 shown in D.*

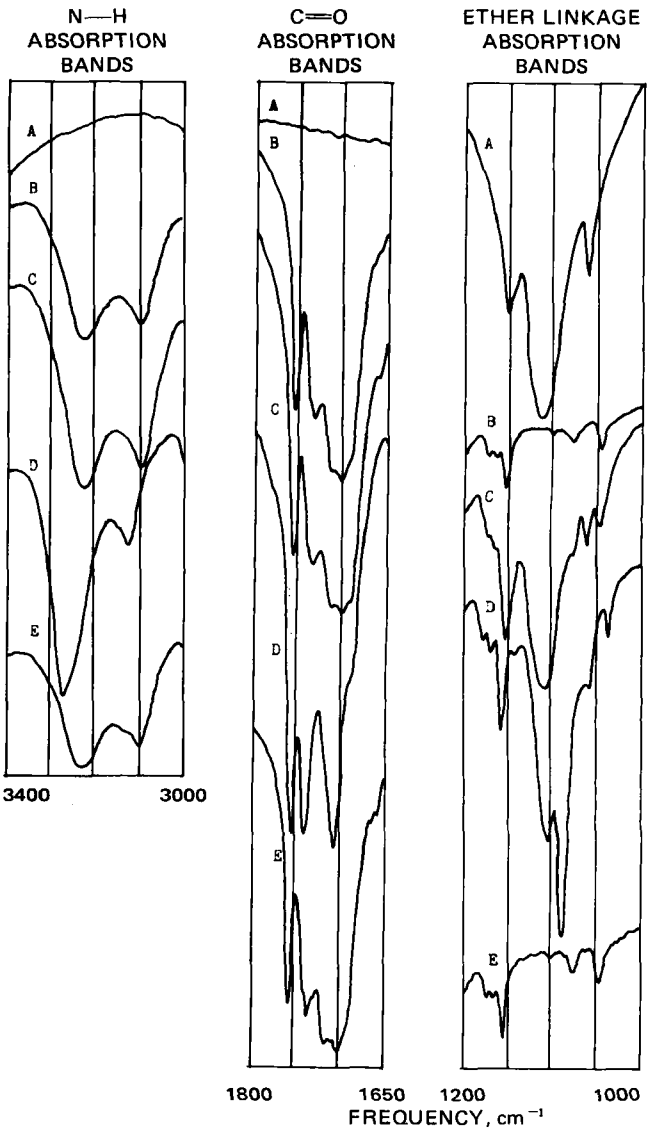


Figure 4—*IR spectra of probarbital–polyethylene glycol 4000 from pyridine. Key: A, polyethylene glycol 4000; B, probarbital; C, physical mixture of probarbital and polyethylene glycol 4000; D, equilibrated probarbital–polyethylene glycol 4000; and E, aqueous-washed equilibrated probarbital–polyethylene glycol 4000 shown in D.*

Stretching and bending force constants for NH, C=O, and ether linkages are changed after hydrogen bond formation, resulting in a shift of the characteristic absorption bands (12). Based on this principle, characteristic C=O, NH, and ether linkage bands in the IR spectra of the barbiturate, polyethylene glycol 4000, and barbiturate–polyethylene glycol 4000 equilibrated sample were examined for shifts. These variations were, in turn, used to characterize the interaction between barbiturates and polyethylene glycol 4000.

EXPERIMENTAL[1]

Reagents—The reagents included phenobarbital, barbital, butabarbital sodium, amobarbital, probarbital sodium, pentobarbital sodium, cyclobarbital, talbutal[2], hexobarbital sodium[2], butethal, vinbarbital, cyclopentenyl allylbarbituric acid[3], allobarbital, primidone, mephobarbital, polyethylene glycol 4000, and pyridine[4].

Preparation of Free Barbiturates from Salts—Butabarbital, hexobarbital, and pentobarbital were obtained as sodium salts. To prepare the free barbituric acid derivative, an aqueous solution of the salt was acidified with 10% hydrochloric acid, and the mixture was cooled and filtered. The white crystalline precipitate was washed with water until the washings were free from chloride ion, and the solid was dried at 105°. The butabarbital so obtained had a melting range of 165–169°, the hexobarbital had a melting range of 145–147°, and the pentobarbital had a melting range of 127–133°.

Probarbital and cyclobarbital were obtained as calcium salts. The calcium salt was suspended in 10% hydrochloric acid, and the

[1] IR spectra were obtained on a Perkin-Elmer 457 grating spectrophotometer.

[2] Sterling-Winthrop Research Institute, Rensselaer, N.Y.
[3] Cyclopal, The Upjohn Co., Kalamazoo, Mich.
[4] J. T. Baker Co., Phillipsburg, N.J.

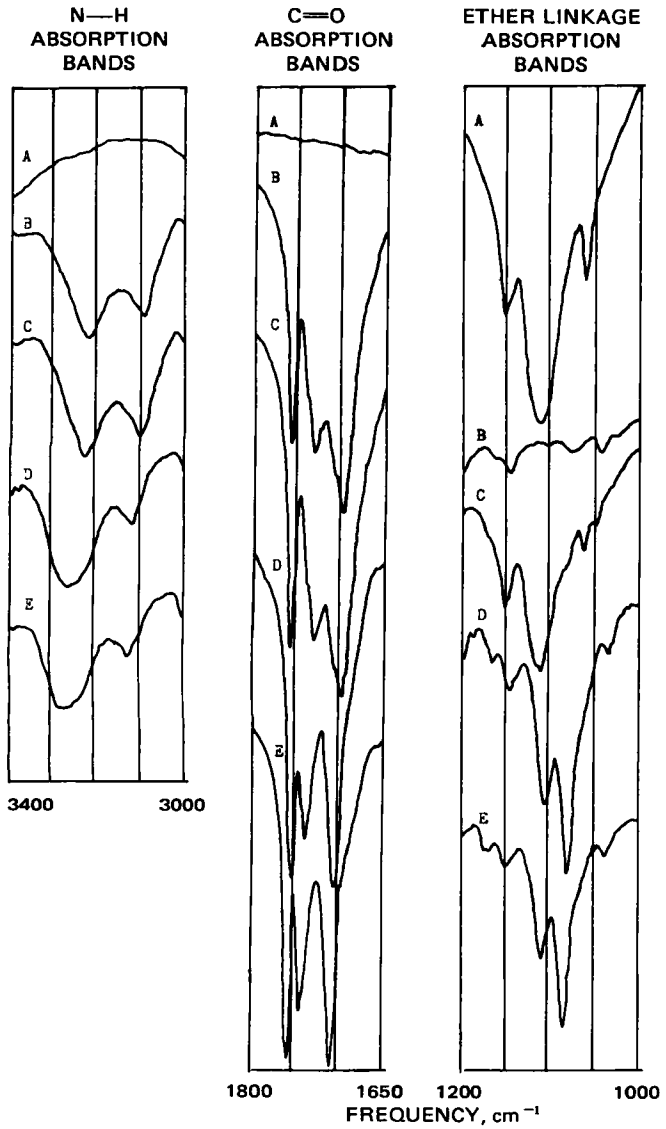


Figure 5—*IR spectra of butabarbital–polyethylene glycol 4000 from pyridine. Key: A, polyethylene glycol 4000; B, butabarbital; C, physical mixture of butabarbital and polyethylene glycol 4000; D, equilibrated butabarbital–polyethylene glycol 4000; and E, aqueous-washed equilibrated butabarbital–polyethylene glycol 4000 shown in D.*

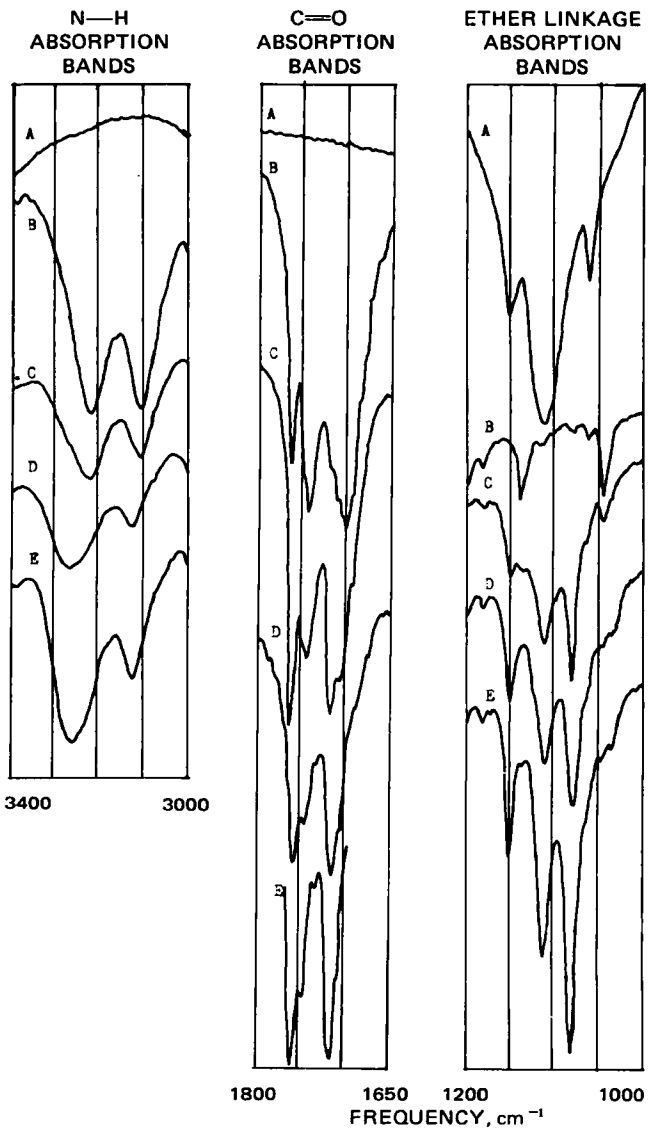


Figure 6—*IR spectra of cyclobarbital–polyethylene glycol 4000 from pyridine. Key: A, polyethylene glycol 4000; B, cyclobarbital; C, physical mixture of cyclobarbital and polyethylene glycol 4000; D, equilibrated cyclobarbital–polyethylene glycol 4000; and E, aqueous-washed equilibrated cyclobarbital–polyethylene glycol 4000 shown in D.*

mixture was stirred for 10 min. The mixture was extracted three times with ether, and the combined ether extracts were washed with water and evaporated to dryness in a current of air. The residue was dried at 105°. Probarbital so obtained had a melting range of 200–202°, and cyclobarbital had a melting range of 170–174°.

Solvent Selection for Complexation Studies—Initially, a procedure was required for the preparation of barbiturate–polyethylene glycol 4000 complexes. The phenobarbital–polyethylene glycol 4000 complexation in water appears to be a special case, since other barbiturates do not undergo this interaction. Complexes might, however, be prepared from other solvents.

Since the primary mechanism of complexation appears to involve hydrogen bond formation, the ideal solvent should possess only weak hydrogen bond acceptor and donor properties but still dissolve the barbiturate and polyethylene glycol 4000. In addition, IR spectral characteristics of the phenobarbital–polyethylene glycol 4000 complex prepared from this solvent must be the same as those of the complex prepared from water (Fig. 1, curve D). Finally, the solvent should be easily evaporated at room temperature under reduced pressure.

Pyridine was found to be useful for the preparation of barbiturate–polyethylene glycol 4000 complexes. It dissolves both phenobarbital and polyethylene glycol 4000, and after evaporation the complex has an IR spectrum (Fig. 2, curve D) identical to that of the complex obtained from water.

Preparation of Barbiturate–Polyethylene Glycol 4000 Equilibrated Samples from Pyridine—One millimole of the barbiturate and 2 mEq of polyethylene glycol 4000 were dissolved in 3.0 ml of pyridine. The solvent was evaporated in a current of air, and the residue was placed in a reduced-pressure desiccator for 24 hr. The residue was ground and passed through a No. 80 sieve.

Crystallization of Reactants from Pyridine—Polymorphism of barbiturates is well known and has been extensively investigated (13–16). An important contributor to the formation of polymorphs is the solvent of crystallization. Cleverley and Williams (13) and Mesley (16) prepared a series of polymorphs for each barbiturate using different solvents and found that each form exhibited different IR absorption bands. The presence of different polymorphic forms would increase the difficulty of properly interpreting IR spectral changes. Therefore, all constituents of the study

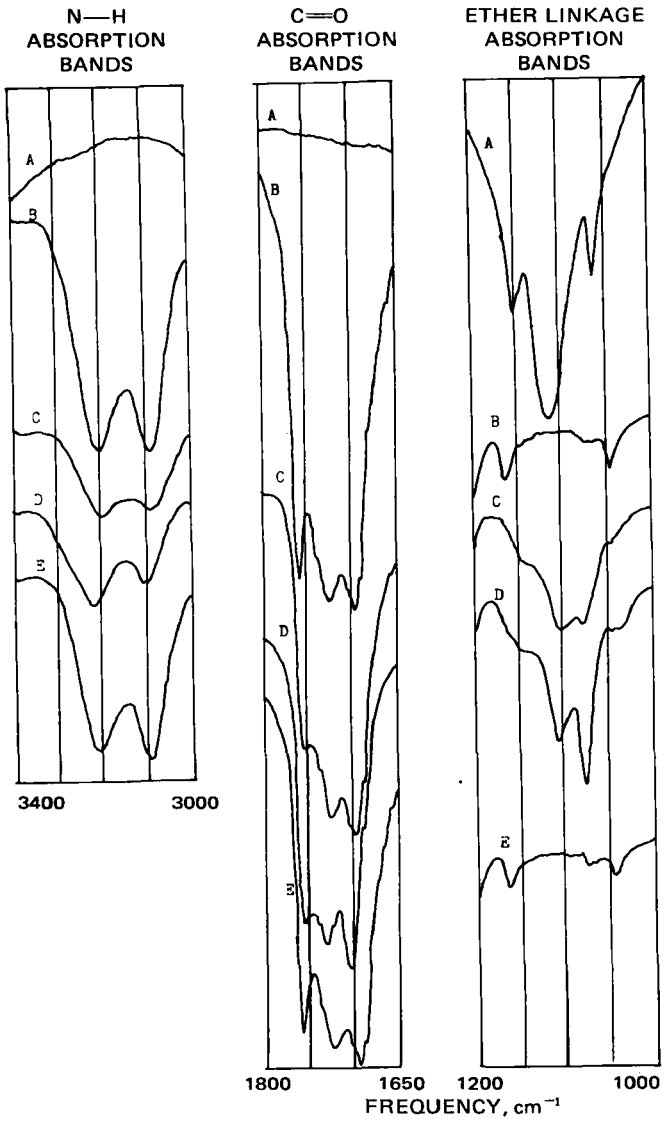


Figure 7—*IR spectra of butethal–polyethylene glycol 4000 from pyridine. Key: A, polyethylene glycol 4000; B, butethal; C, physical mixture of butethal and polyethylene glycol 4000; D, equilibrated butethal–polyethylene glycol 4000; and E, aqueous-washed equilibrated butethal–polyethylene glycol 4000 shown in D.*

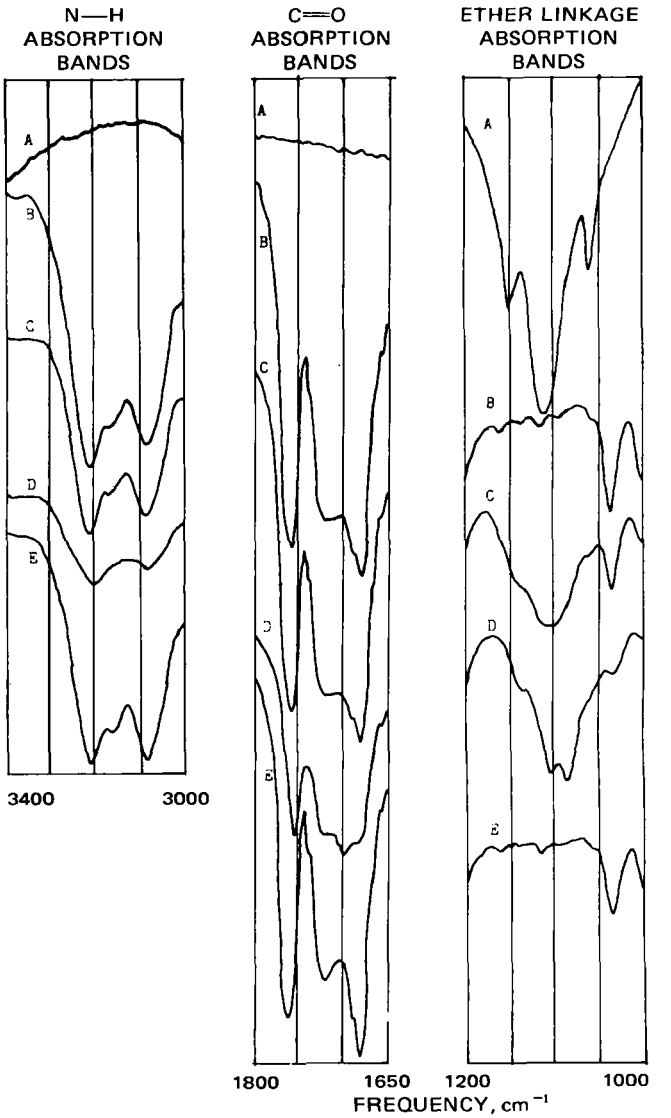


Figure 8—*IR spectra of cyclopentenyl allylbarbituric acid–polyethylene glycol 4000 from pyridine. Key: A, polyethylene glycol 4000; B, cyclopentenyl allylbarbituric acid; C, physical mixture of cyclopentenyl allylbarbituric acid and polyethylene glycol 4000; D, equilibrated cyclopentenyl allylbarbituric acid–polyethylene glycol 4000; and E, aqueous-washed equilibrated cyclopentenyl allylbarbituric acid–polyethylene glycol 4000 shown in D.*

were recrystallized from a single solvent (pyridine) prior to IR spectral analysis.

Determination of Aqueous Stability of Complexes—A 0.5-g quantity of the complex was suspended in 10.0 ml of water in a screw-capped vial. The vial was fastened in a horizontal position on an agitator, and the shaking speed was adjusted to agitate the vial contents actively. After shaking for 2 hr at 26°, the mixture was filtered and the solids were washed with 20.0 ml of water. The solid was dried at room temperature in a vacuum desiccator for 3 days, and the IR spectrum was taken. Differences between the spectra of the complex before and after treatment with water indicate the stability of the complex.

Preparation of Potassium Bromide Dispersion—The sample and potassium bromide were obtained in proportions by weight of 1:200. The mixture was triturated in a small mortar and pressed in a die under 24,000 psi.

RESULTS AND DISCUSSION

Discussion will focus on the regions from 3400 to 3000 [NH stretching bands (16–19)], from 1800 to 1650 [C=O stretching bands (16, 20)], and from 1200 to 1000 [aliphatic ether bands (21)] cm^{-1}. The 3000–2700-cm^{-1} region will not be discussed since it appears to be of no analytical significance (17). No bands occur between 2700 and 1800 cm^{-1} in any of the tested samples. In the areas from 1650 to 1200 and from 1000 to 250 cm^{-1}, each barbiturate has a characteristic series of bands. Accurate assignment of these bands is difficult with currently available information (16), particularly after they are coupled with absorption bands of polyethylene glycol 4000 in these regions.

In Figs. 1 and 2, N—H stretching absorption bands of the physical mixture of phenobarbital and polyethylene glycol 4000 (curve C) are the same as those of phenobarbital alone (curve B). There are three absorption bands: 3310, 3200, and 3090 cm^{-1}. According to Levi and Hubley (17) and Mesley (16), 3310 cm^{-1} is assigned as a free N—H stretching absorption band while the remaining bands are intermolecular hydrogen-bonded N—H stretching absorption bands. The intermolecular hydrogen bond is between the N—H and C=O of different barbiturate molecules (16).

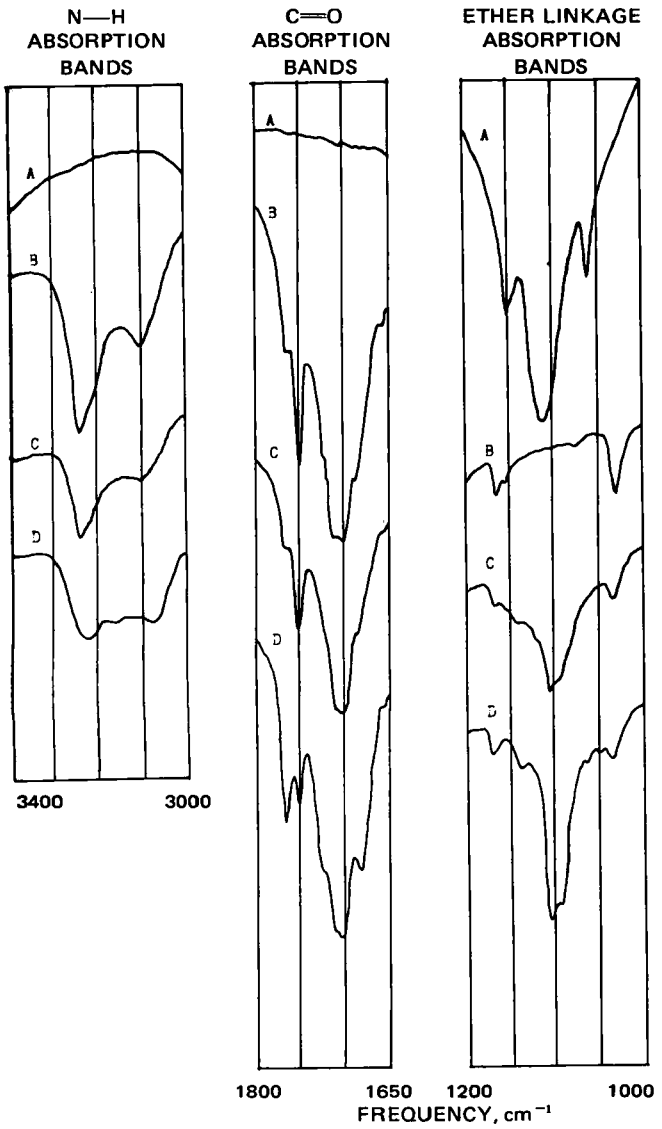


Figure 9—*IR spectra of barbital–polyethylene glycol 4000 from pyridine. Key: A, polyethylene glycol 4000; B, barbital; C, physical mixture of barbital and polyethylene glycol 4000; and D, equilibrated barbital–polyethylene glycol 4000.*

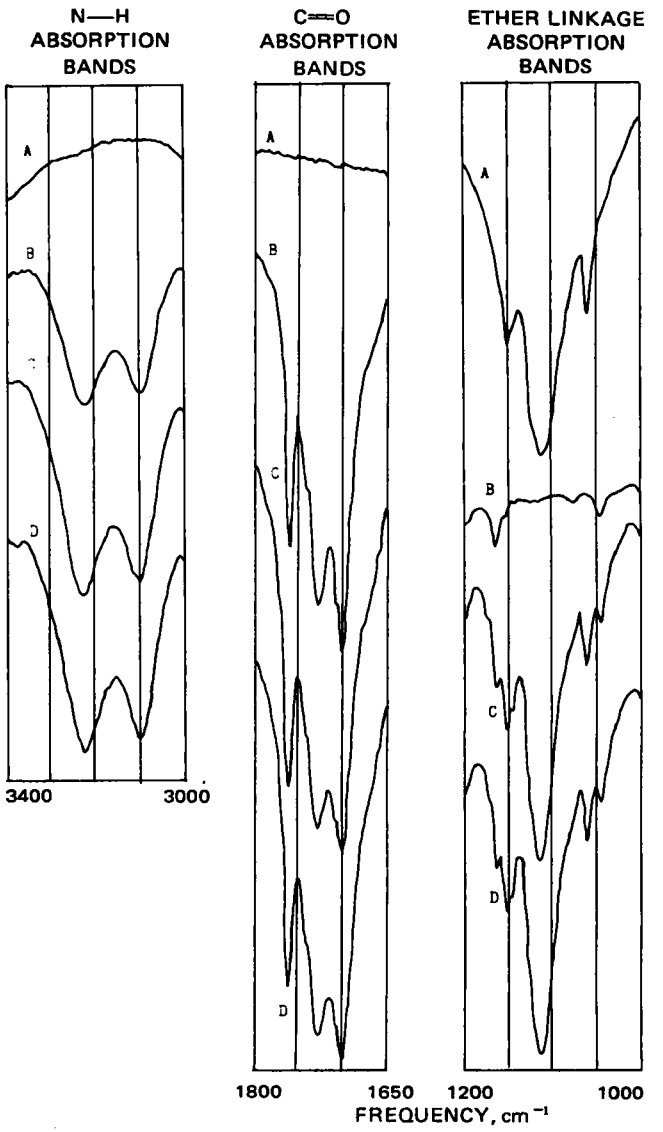


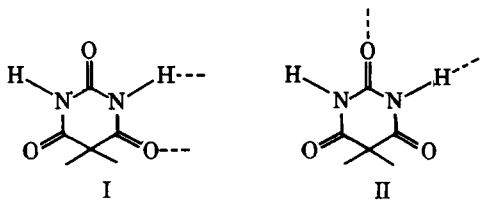
Figure 10—*IR spectra of amobarbital–polyethylene glycol 4000 from pyridine. Key: A, polyethylene glycol 4000; B, amobarbital; C, physical mixture of amobarbital and polyethylene glycol 4000; and D, equilibrated amobarbital–polyethylene glycol 4000.*

The N—H stretching absorption regions of the phenobarbital–polyethylene glycol 4000 complex from water (Fig. 1, curve D) and equilibrated phenobarbital–polyethylene glycol 4000 from pyridine (Fig. 2, curve D) differ from that of phenobarbital alone (Figs. 1 and 2, curves B). In the spectrum of the complex, the band at 3220 cm^{-1} is stronger and broader than the one at 3110 cm^{-1}. Loss of the free N—H stretching absorption band (present in the spectrum of phenobarbital) in the spectra of the complex from water and the equilibrated samples from pyridine can be attributed to hydrogen bond formation. The 3220- and 3110-cm^{-1} N—H bands of the complex and equilibrated sample have higher frequencies compared to the 3200- and 3090-cm^{-1} N—H bands of phenobarbital. This shifting can be attributed to hydrogen bonds formed between phenobarbital and polyethylene glycol 4000, which displace hydrogen bonds formed between phenobarbital molecules. Since the acceptor molecules are different, the N—H stretching vibration frequency is changed in the complex and equilibrated sample.

In Figs. 3–13, the N—H stretching absorption region of each barbiturate shows the same pattern: two absorption bands, one in the range from 3270 to 3200 cm^{-1} and the other in the range from 3100 to 3090 cm^{-1}. These two bands are assigned as intermolecular hydrogen-bonded N—H stretching bands (16). In Figs. 3–8, the higher frequency band of the equilibrated system is more intense than the lower frequency band, and both bands occur at a higher frequency than those of the barbiturate alone.

The N—H absorption bands of equilibrated samples and the corresponding barbiturates are summarized in Table I. The observed spectral differences indicate that pentobarbital, butabarbital, cyclobarbital, butethal, and cyclopentenyl allylbarbituric acid interact with polyethylene glycol 4000. Mesley (16) already noted the possible existence of intermolecular hydrogen bonds in barbiturate crystals, and Craven and coworkers (22–24) proved it by X-ray diffraction analysis.

Since the phenobarbital spectrum has both free and hydrogen-



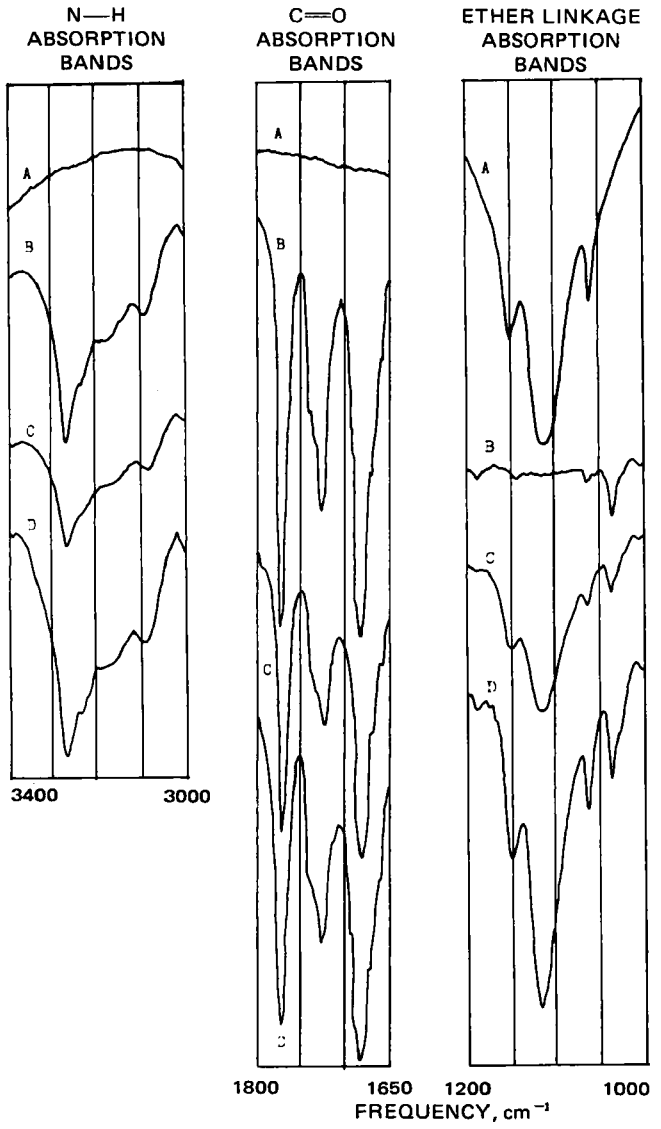


Figure 11—*IR spectra of vinbarbital–polyethylene glycol 4000 from pyridine. Key: A, polyethylene glycol 4000; B, vinbarbital; C, physical mixture of vinbarbital and polyethylene glycol 4000; and D, equilibrated vinbarbital–polyethylene glycol 4000.*

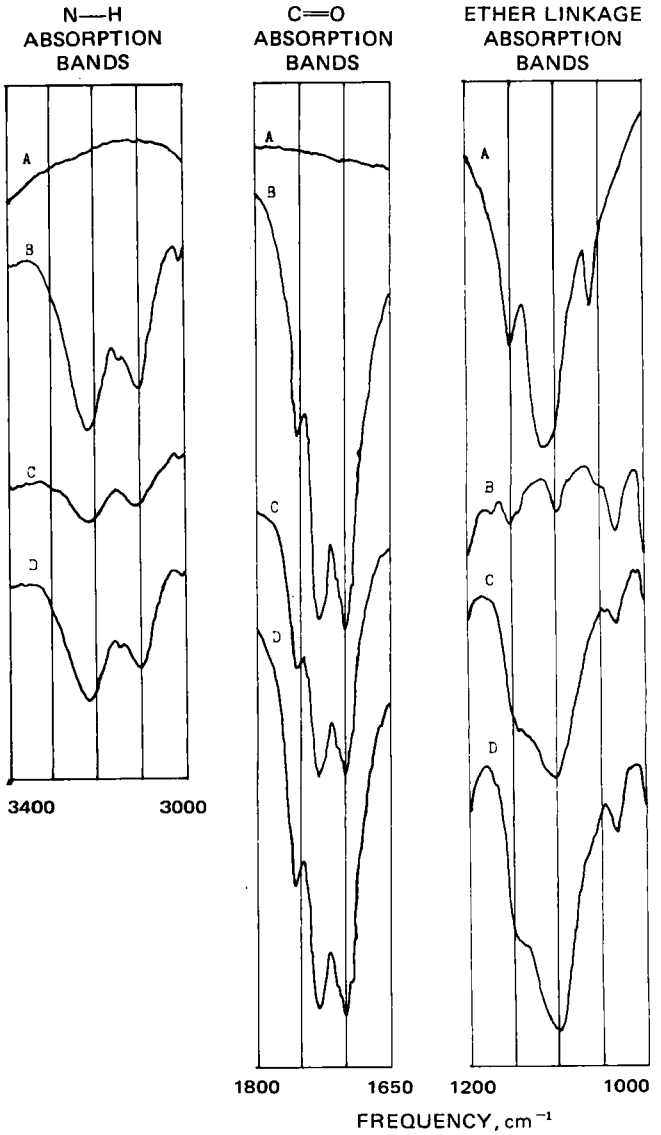
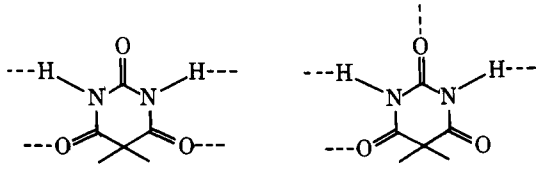


Figure 12—*IR spectra of talbutal–polyethylene glycol 4000 from pyridine. Key: A, polyethylene glycol 4000; B, talbutal; C, physical mixture of talbutal and polyethylene glycol 4000; and D, equilibrated talbutal–polyethylene glycol 4000.*

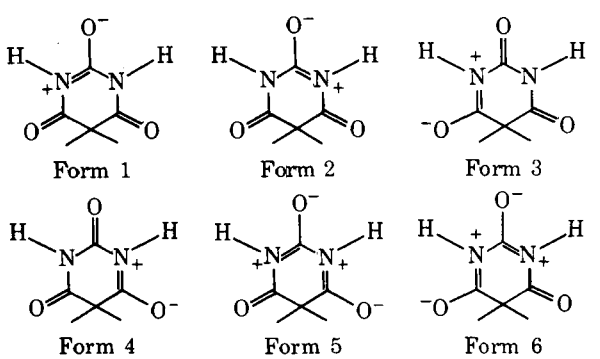
bonded N—H stretching absorption bands, the conformation of hydrogen bonding sites in the phenobarbital crystal would be as in I or II. The lack of free N—H stretching absorption bands in the spectra of the remaining barbiturates implies a conformation of hydrogen bonding sites as in III or IV. Upon complexation, phenobarbital also assumes a hydrogen bonding arrangement as in III or IV, as indicated by the loss of free N—H stretching.

The N—H stretching absorption bands of the barbital–polyethylene glycol 4000 equilibrated sample (Fig. 9) occur at a lower frequency than those of barbital. If hydrogen bond formation between barbiturates and polyethylene glycol 4000 shifts N—H stretching to a higher frequency than intermolecular hydrogen bond formation (as previously discussed), then shifting (Fig. 9) to a lower frequency must not be due to hydrogen bond formation between barbital and polyethylene glycol 4000. Instead, the change may be attributed to an alteration in the configuration of hydrogen bonding sites upon crystallization of barbital from pyridine in the presence of polyethylene glycol 4000.

Hydrogen bonding increases the stability of resonance forms contributing to the resonance stabilization of barbital by decreas-



III IV



Form 1 Form 2 Form 3

Form 4 Form 5 Form 6

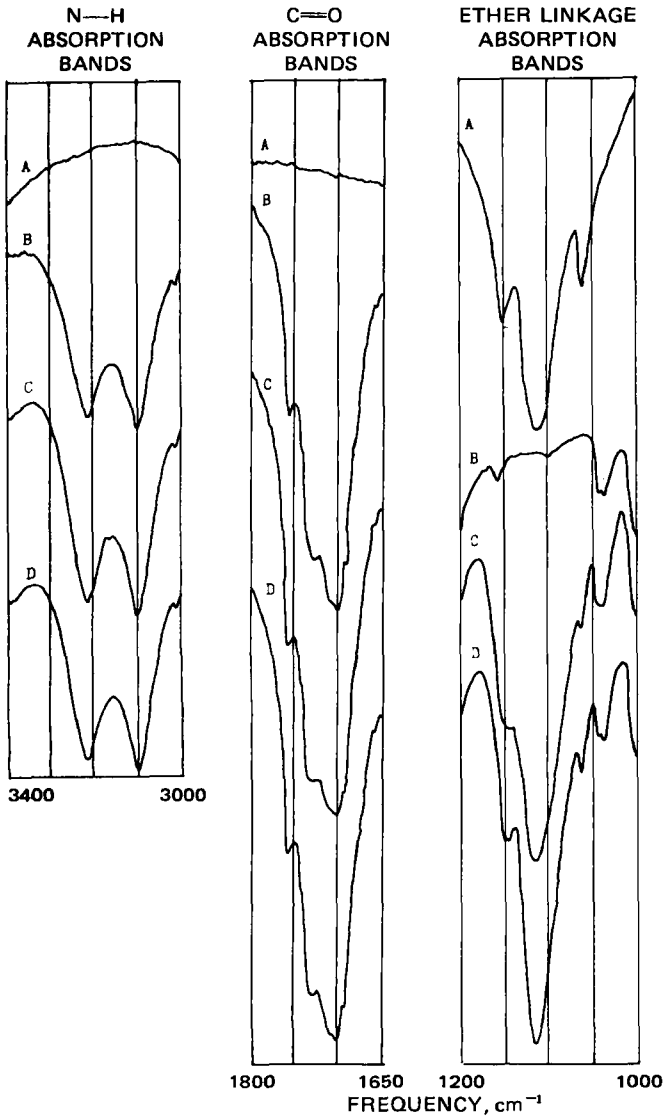


Figure 13—*IR spectra of allobarbital–polyethylene glycol 4000 from pyridine. Key: A, polyethylene glycol 4000; B, allobarbital; C, physical mixture of allobarbital and polyethylene glycol 4000; and D, equilibrated allobarbital–polyethylene glycol 4000.*

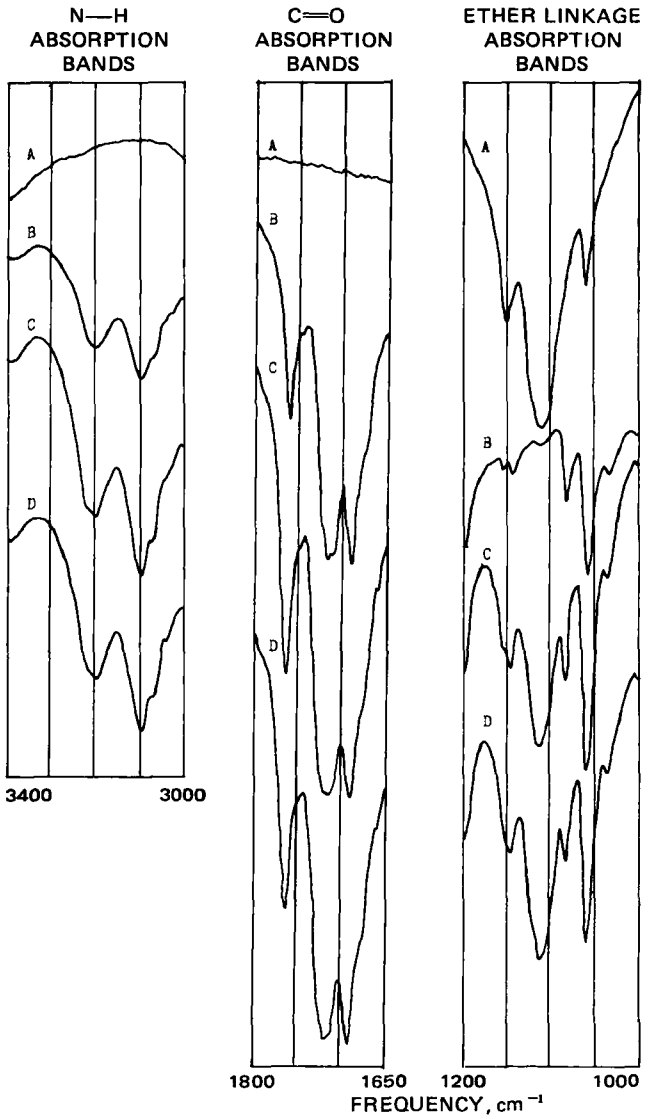
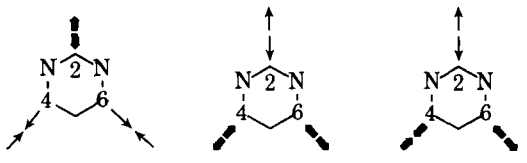


Figure 14—*IR spectra of mephobarbital–polyethylene glycol 4000 from pyridine. Key: A, polyethylene glycol 4000; B, mephobarbital; C, physical mixture of mephobarbital and polyethylene glycol 4000; and D, equilibrated mephobarbital–polyethylene glycol 4000.*

ing the negative charge on the oxygen and the positive charge on the nitrogen. Thus, those canonical forms stabilized by hydrogen bonding in III (Forms 3 and 4) would contribute most to the stabilization energy of barbital molecules hydrogen bonding as in III, while forms stabilized by hydrogen bonding in IV (Forms 1, 2, 5, and 6) contribute most to molecules having bonding sites arranged as in IV. As a result, the N—H stretching of barbital molecules hydrogen bonding as in IV is at a higher frequency due to the more positive character of the nitrogens in these molecules. Based on this rationale and the fact that the N—H absorption of the equilibrated sample is at a lower frequency than barbital, barbital crystallized from pyridine has hydrogen bonding sites as in III, and barbital crystallized from pyridine with polyethylene glycol 4000 hydrogen bonds as in IV.

The N—H stretching absorption bands of amobarbital, vinbarbital, talbutal, allobarbital, mephobarbital, hexobarbital, and primidone (Figs. 10–16) show no evidence of pattern change when equilibrated with polyethylene glycol 4000, indicating that no interaction occurs.

In the carbonyl stretching absorption area of Figs. 1 and 2, the IR spectrum of phenobarbital (curve B) shows three absorption bands: 1780–1770, 1720–1710, and 1680–1670 cm^{-1}. According to Price *et al.* (20), the three carbonyl bonds act as coupled oscillators, giving rise to the following vibration modes:



Vibration 1 (associated mainly with 2-carbonyl vibration) was assigned as the low frequency band because the 2-carbonyl has two neighboring nitrogens to participate in tautomerism and thus the most single bond (C—O^-) character.

Vibration 2 (associated mainly with 4- and 6-carbonyl symmetric vibration) has a higher frequency since the associated carbonyls have only one nitrogen that participates in tautomeric resonance.

Vibration 3 (associated with 4- and 6-carbonyl asymmetric stretching) has a higher frequency than Vibration 2 because the combined force of carbonyl unsymmetric stretching is perpendicular to the 2-carbonyl stretching, and the stretching force of the 2-carbonyl has little influence on it. In contrast, 2-carbonyl stretch-

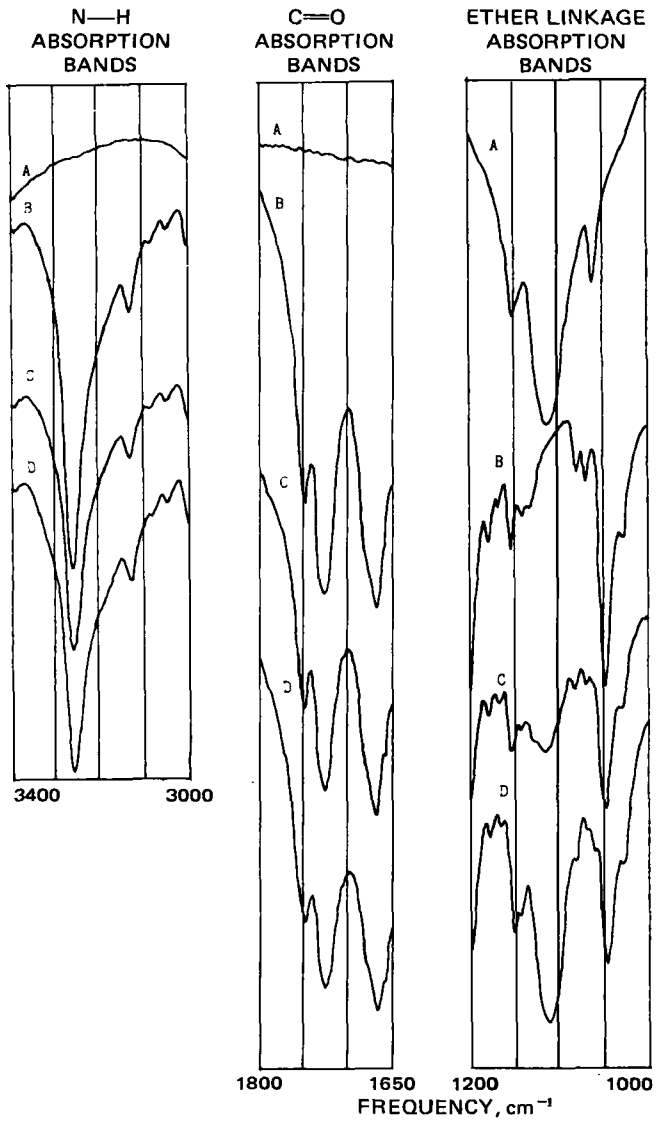


Figure 15—*IR spectra of hexobarbital–polyethylene glycol 4000 from pyridine. Key: A, polyethylene glycol 4000; B, hexobarbital; C, physical mixture of hexobarbital and polyethylene glycol 4000; and D, equilibrated hexobarbital–polyethylene glycol 4000.*

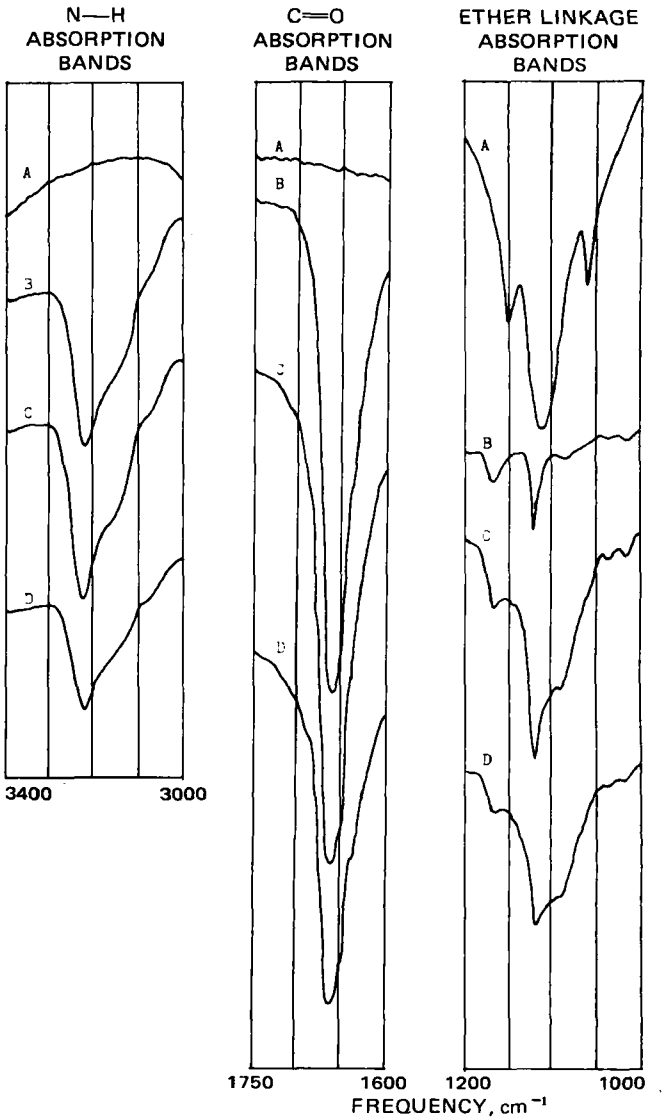


Figure 16—*IR spectra of primidone–polyethylene glycol 4000 from pyridine. Key: A, polyethylene glycol 4000; B, primidone; C, physical mixture of primidone and polyethylene glycol 4000; and D, equilibrated primidone–polyethylene glycol 4000.*

ing in Vibration 2 opposes the combined force of 4- and 6-carbonyl stretching. However, Goenechea (25) assigned the high frequency band to the 2-carbonyl vibration (Vibration 1) because the high frequency band disappears in the 2-thio analog.

Mesley (16) contrasted the frequency of carbonyl absorption bands of barbiturates as determined in ether and in the solid state and showed average changes of 25, 22, and 8 cm^{-1} for the low, middle, and high frequency bands, respectively. Since the 2-carbonyl is free both in the solid state and in ether, the frequency change should be small. The 4- and 6-carbonyl groups, hydrogen bonded in the solid state but free in ether solution, should show much greater frequency changes, leading to an assignment in agreement with that of Goenechea (25).

Although assignment of the high frequency band to the 2-carbonyl is strongly supported by Goenechea's data and in conflict with the assignment of Price *et al.* (20), the coupling oscillator theory stated by the latter is still a powerful method for analyzing force factors of barbiturate vibrations resulting in carbonyl stretching absorption bands. If the assignment of the high frequency band to 2-carbonyl stretching (Vibration 1, or 2-, 4-, and 6-carbonyl unsymmetric in-phase stretching) is accepted, then the middle frequency band must be assigned to 4- and 6-carbonyl out-of-phase stretching (Vibration 3) and the low frequency band must be assigned to 4- and 6-carbonyl symmetric in-phase stretching (Vibration 2), since the latter has the lowest frequency according to this interpretation (20).

The absorption frequencies of carbonyl bands of the barbiturates and their complexes are summarized in Table II. These data indicate that the 2-carbonyl stretching band is shifted to a lower frequency while the 4- and 6-carbonyl out-of-phase stretching and symmetric in-phase stretching are shifted to a higher frequency upon complexation. The 2-carbonyl band of phenobarbital undergoes a large shift upon complexation, indicating a large change in the electronic environment of the 2-carbonyl upon complexation. The direction of the shift indicates that the 2-carbonyl is free in the phenobarbital crystal (I) but hydrogen bonded in the complex (IV).

The shift of 4- and 6-carbonyl out-of-phase stretching and symmetric in-phase stretching to a higher frequency is explicable if the 4- or 6-carbonyl is hydrogen bonded in the barbiturate crystal (III) but free in the complex (IV). Electron density on the free carbonyl is greater than on the hydrogen-bonded carbonyl, giving more double bond character to the carbon–oxygen bond. Thus, both 4- and 6-carbonyl in-phase and out-of-phase stretching are shifted to higher frequencies.

Figures 3–8 have the same pattern of carbonyl absorption bands

Table II—Carbonyl Absorption Bands in Spectra of Barbiturates and Their Complexes

Compound	2-Carbonyl Stretching Out-of-Phase, cm^{-1}		2-, 4-, and 6-Carbonyl Symmetric Stretching In-Phase, cm^{-1}		2-, 4-, and 6-Carbonyl Unsymmetric Stretching In-Phase, cm^{-1}	
Phenobarbital	1780			1710	1680–1670	
Phenobarbital complex		1765	1748		1712	
Pentobarbital	1770		1730–1720			1680
Pentobarbital complex		1760	1730		1700	
Probarbital	1755			1730		1700
Probarbital complex		1752	1740		1705	
Butabarbital	1755			1730		1697
Butabarbital complex		1750	1740		1705	
Cyclobarbital	1762			1742		1700
Cyclobarbital complex		1758	1748		1712	
Butethal	1758			1724		1695
Butethal complex		1752	1730		1700	
Cyclopentenyl allylbarbituric acid	1755			1720		1680
Cyclopentenyl allylbarbituric acid complex		1752	1720		1700	

when absorption of the equilibrated sample (curve D) is compared to that of the free barbiturate (curve B).

This discussion indicates that the phenobarbital crystal may be represented as I while pentobarbital, probarbital, butethal, and cyclopentenyl allylbarbituric acid have crystal forms such as III. Upon complexation, each barbiturate should be represented as IV.

A comparison of the carbonyl absorption areas in Figs. 10–16 of the barbiturate alone (curve B), the physical mixture (curve C), and the equilibrated sample (curve D) indicates that amobarbital, vinbarbital, allobarbital, talbutal, mephobarbital, and hexobarbital do not interact with polyethylene glycol. Changes observed in the absorption patterns of the barbital system (Fig. 9) may again be rationalized on the basis of a change in hydrogen bond configuration upon crystallization with polyethylene glycol 4000, and complexation need not be implicated.

The ether linkage of polyethylene glycol has a characteristic absorption band at 1110 cm^{-1} (Figs. 1 and 2). Upon complexation, however, a new band is formed at a lower frequency (Figs. 1–8, curves D). The decreased frequency is attributable to the formation of a hydrogen bond upon complexation, in which the oxygen of the ether linkage serves as a hydrogen acceptor.

The aqueous stability of the seven barbiturates found to interact with polyethylene glycol was determined (Figs. 2–8, curves E). The differences between the spectra before (curve D) and after (curve E) water washing are summarized in Table III.

Barbiturates that complex with polyethylene glycol can be classified into three groups on the basis of the water stability of the complexes:

1. Water-stable complexes in which the hydrogen bond between the constituents of the complex is stronger than that between water and the constituents of the complex. Phenobarbital, butabarbital, and cyclobarbital give such complexes.
2. Water-unstable complexes with the barbiturate returning to the original crystal state. Probarbital, butethal, and cyclopentenyl allylbarbituric acid give such complexes, as evidenced by the similarity between curves E and A.
3. Water-unstable complexes with the barbiturate having a different crystal state. Pentobarbital gives such a complex, and the similarity between curves D and E indicates that the hydrogen bonding configuration in the complex is carried over into that of the barbiturate (IV).

SUMMARY

Several factors affecting the barbiturate–polyethylene glycol 4000 interaction have been identified. Since only phenobarbital interacts with polyethylene glycol in water while phenobarbital, butabarbital, butethal, cyclobarbital, cyclopentenyl allylbarbituric acid, pentobarbital, and probarbital interact in pyridine, the nature of the solvent must play an important role. Seven disubstituted barbiturates interact while five disubstituted barbiturates do not, so the nature of the C_5 substituents is critical. Trisubstituted barbiturates do not interact, so two hydrogen bonds are required for complexation. Finally, the fact that primidone does not interact indicates that the 2-carbonyl has an important role in complexation.

Evidence from this study suggests that phenobarbital crystallized from pyridine has hydrogen bonding sites arranged as in I, whereas other barbiturates that complex have bonding sites as in III. All barbiturates, upon complexation, are represented by IV.

Of the seven barbiturate complexes studied, probarbital, butethal, cyclopentenyl allylbarbituric acid, and pentobarbital complexes were unstable in water at 26°. Pentobarbital retains the IV arrangement, but the other three barbiturates revert to III after water washing.

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Table III—Variation of Absorption Bands of Barbiturate Complexes after Water Washing

Equilibrated Sample	N—H Absorption	C=O Absorption	Ether Linkage Absorption
Phenobarbital–polyethylene glycol 4000	No change	No change	No change
Butabarbital–polyethylene glycol 4000	No change	No change	No change
Cyclobarbital–polyethylene glycol 4000	No change	No change	No change
Pentobarbital–polyethylene glycol 4000	No change	No change	Loss of ether absorption band
Probarbital–polyethylene glycol 4000	Bands same as probarbital alone	Bands same as probarbital alone	Loss of ether absorption band
Butethal–polyethylene glycol 4000	Bands same as butethal alone	Bands same as butethal alone	Loss of ether absorption band
Cyclopentenyl allylbarbituric acid–polyethylene glycol 4000	Bands same as cyclopentenyl allylbarbituric acid alone	Bands same as cyclopentenyl allylbarbituric acid alone	Loss of ether absorption band

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Binding of Metronidazole and Its Derivatives to Plasma Proteins: An Assessment of Drug Binding Phenomenon

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Abstract □ Metronidazole and four derivatives were studied *in vitro* to investigate the differences in the extent of their binding to plasma proteins. Modification at the terminal portion of the alkyl side chain resulted in wide differences in the extent of binding. Molecular orbital calculations were performed by the CNDO and MINDO/2 methods to estimate the frontier electron density on the hetero atom at the 3′-position of the alkyl side chain. A linear correlation between the protein binding parameter ($\log_e P$) and the frontier electron density (q_r) was observed for the binding of this group of trichomonicidal drugs. NMR spectroscopy was used to demonstrate that the alkyl side chain participated in the binding of these compounds to plasma proteins.

Keyphrases □ Metronidazole and four derivatives—plasma protein binding, correlation with frontier electron density □ Plasma protein binding—metronidazole and four derivatives, correlation with frontier electron density □ Electron density, frontier—metronidazole and four derivatives, correlation with plasma protein binding parameters

Several reports provided information on the theoretical, biological, and clinical aspects of drug–protein binding phenomena (1–6). The majority of these investigations was concerned with the drug–serum albumin interaction, since it is the predominant phenomenon occurring in humans. Quantitative correlation has been found between physicochemical parameters such as lipophilicity, pKa, and electronic charge density and the strength of drug binding to serum albumin (7–10).

It is well recognized that the therapeutic activity of a drug is primarily dependent upon the availability of its free (unbound) permeable species at the effective receptor sites located either in the vascular or extravascular compartments of the body (1, 11–14). Recent investigations demonstrated that any structural modification in a functional group can influence significantly both the activity and the extent of protein binding of disopyramide phosphate and its derivatives (14–16).

Metronidazole[1] has been shown to be an effective antiprotozoal agent with a broad spectrum of activity against anaerobes (17–21). In this study, it was observed that the extent of plasma protein binding of

[1] Flagyl, G. D. Searle & Co., Chicago, IL 60648