Identification of Complexes of Phenobarbital with Quinine, Quinidine, or Hydroquinidine in Pharmaceutical Dosage Forms

By W. N. FRENCH and J. C. MORRISON*

Complexes of phenobarbital with quinine, quinidine, or hydroquinidine have been prepared and characterized. By means of infrared spectroscopy, these complexes were differentiated from physical mixtures of their respective components when the drug was examined either alone or in pharmaceutical dosage forms. Of four commercial preparations examined, only three were found to contain true complexes. In contact with simulated gastric juice, the complexes were shown to be readily decomposed, regenerating the sedative and alkaloid from which they were formed.

PHENOBARBITAL is known to react with organic bases such as 2-amino-1-phenylpropane, papaverine, theophylline, caffeine, and quinine to form complexes containing various molecular ratios of reactants (1–5). One to one ratios have been observed in all cases, but 2 to 1 ratios of barbiturate to base have also been noted for caffeine (4), theophylline (5), and strychnine (5), as well as a 3 to 1 ratio of barbiturate with the latter base (5). The complexes of pharmacological interest would appear to have been prepared in order to combine the therapeutic effects of the individual components as well as to alter the solubility of the barbiturate.

A number of products containing phenobarbital with either quinidine or hydroquinidine have recently appeared on the Canadian market. In some cases the labeled declaration does not clearly indicate whether the active ingredients are present as a complex or a physical mixture of its individual components. Since a procedure for distinguishing between these two possibilities would be of value to regulatory agencies concerned with the control of such products, a study was undertaken to develop such a procedure and to investigate the nature of the complexes of phenobarbital with quinidine and hydroquinidine. The previously reported complex of phenobarbital with quinine (1) also has been included in the investigation.

EXPERIMENTAL

Preparation of Complexes of Phenobarbital with Quinine, Quinidine, and Dihydroquinidine.—One mmole of the quinine alkaloid (324 mg. of quinine or quinidine, 326 mg. of hydroquinidine) and 1.2 mmole of phenobarbital (279 mg.) were dissolved in 5 ml. of hot absolute ethanol and the solution cooled to 0°. Crystalline complexes of the sedative with quinine formed in 1 to 2 hr., but those of the sedative with quinidine or hydroquinidine took several hours or even days, and reaction flasks had to be shaken intermittently to induce crystallization. The complexes were collected by filtration and washed with small amounts of cold ethanol, thus affording yields of 70 to 80%. The complex of phenobarbital with quinine melted sharply at 184–185°, whereas those of phenobarbital with quinidine and with hydroquinidine first sintered at 130 to 145° and melted gradually from 146–150°. Titrimetric analyses (*vide infra*) showed that in each of the complexes the ratio of phenobarbital to alkaloid was 1 to 1. Specific rotations observed for each product were −94° for phenobarbital–quinine, +138° for phenobarbital–quinidine, and +130° for the phenobarbital–hydroquinidine complex. Repeated crystallizations from aqueous ethanol did not alter the chemical composition, melting points, or optical rotations of the isolated products.

Assay of Reaction Products.—The alkaloidal moiety of the complexes was determined by titrating samples (about 60 mg.) dissolved in 50 ml. of glacial acetic acid with perchloric acid in dioxane using crystal violet as indicator. Phenobarbital was estimated by dissolving known samples of about 50 mg. in 50 ml. of chloroform to which 1 ml. of methanol was added, and titrating the solution with potassium hydroxide in methanol using thymol blue as indicator.

Polarimetric Analyses.—Optical rotatory measurements were made with a model N200 Rudolph photoelectric polarimeter using 1% w/v ethanolic solutions in tubes of 10 cm. pathlength.

Infrared Measurements.—Infrared spectra were measured by the potassium bromide disk technique using a Beckman IR5 spectrophotometer. Analytical samples were prepared by mixing approximately 0.8 mg. of drug, or a portion of finely powdered tablet material containing this amount of the active constituent, with 300 mg. of potassium bromide, shaking the mixture with a Wig-L-Bug amalgamator[1] for 5 sec., and pressing specimens thus obtained at a pressure of 20,000 p.s.i.

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[1] Manufactured by Crescent Dental Mfg. Co., Chicago, Ill.

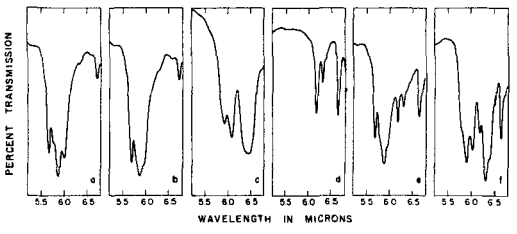


Fig. 1.—Infrared spectra of recrystallized phenobarbital (a), commercially available phenobarbital (b), sodium phenobarbital (c), quinine (d), physical mixture of phenobarbital and quinine (e), and complex of phenobarbital with quinine (f).

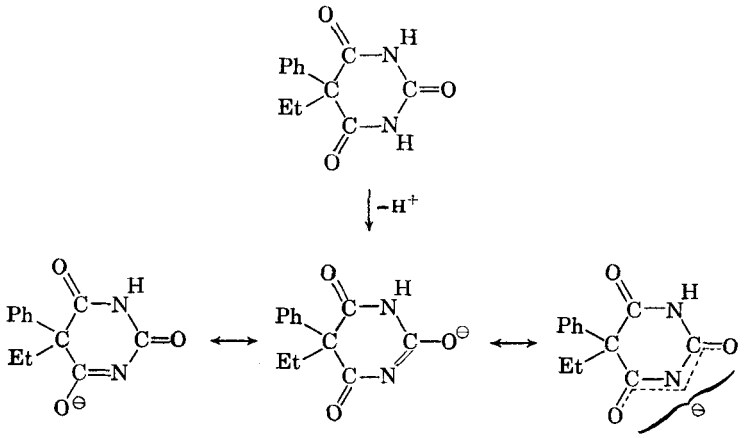
RESULTS AND DISCUSSION

The complexes of phenobarbital with quinine, quinidine, and hydroquinidine were readily generated by allowing concentrated ethanolic solutions of reactants to stand in the cold for varying periods of time. Although a crystalline complex separated when equimolar quantities of phenobarbital and quinine were used, excess of the sedative with quinidine and hydroquinidine was required to prevent the preferential separation of the alkaloids. Hence a 20% excess of phenobarbital was arbitrarily chosen for the preparation of all three products. Larger molar ratios did not change the composition of the resulting product. In each case the molar ratio of phenobarbital to alkaloid in the product was 1 to 1.

The sharp melting point of the phenobarbital–quinine complex (185°), which was above that of either reactant (174° for phenobarbital, 177° for quinine), contrasted with the rather poor melting point characteristics (sintering at 130–145°, melting at 146–150°) observed for the complexes of phenobarbital with quinidine or hydroquinidine. The melting points of the latter complexes were also considerably lower than those of their components (174° for quinidine, 169° for hydroquinidine). Interestingly, complex formation in the compounds studied appeared to take place in the absence of solvent under the influence of heat. Heating an equimolar mixture of phenobarbital with the respective alkaloid gave rise to the same melting points observed with the complex prepared in the solvent. Ultraviolet analyses of each complex (in ethanol) at the point of maximum absorption (232 mμ) showed that the observed absorptions corresponded to the additive values of each component at this wavelength. Similarly, specific rotations were found to correspond to the proportion of the alkaloid in the complex and were not affected by adding varying amounts of phenobarbital to a solution of the complex. Therefore, none of these techniques were of value in differentiation of complexes from physical mixtures of their components.

Differentiation was found to be possible, however, by means of infrared spectrophotometry. Phenobarbital crystallized from ethanol–water displayed three absorption peaks throughout the carbonyl region of its infrared spectrum (Fig. 1a) reflecting vibrational characteristics of the carbonyl groups acting either alone or as coupled oscillators (6,7). Commercially available phenobarbital (Brickman and Co., Montreal) displayed only two of these bands (5.67 and 5.88 μ) as shown in Fig. 1b. The different absorption characteristics of the two samples may be attributed to the different crystalline modifications of the compound in the solid state (8). The infrared spectrum of sodium phenobarbital (Fig. 1c) showed a strong absorption band near 5.9 μ as well as in the 6.3–6.4-μ region, but the spectrum was devoid of sharp absorption at 5.67 μ. The spectral differences between phenobarbital and its sodium salt may be attributed to ionization of the molecule during salt formation, as illustrated in Scheme I.

The three quinine alkaloids exhibited sharp absorptions near 6.2 and 6.3 μ (Fig. 1d), while the spectra of physical mixtures of commercial phenobarbital and these alkaloids displayed additive absorptions of their individual components (Fig. 1e) in the 5.5 to 6.5-μ region of the spectrum. In the spectra of the complexes (Fig. 1f), the peak at 5.67 μ indicative of the presence of unionized phenobarbital is no longer observed and a strong absorption indicative of the presence of the barbiturate ion appeared in the 6.3 to 6.4-μ region, masking the absorptions of the alkaloid at 6.2 and 6.3 μ. By



Ionization of Phenobarbital
Scheme I

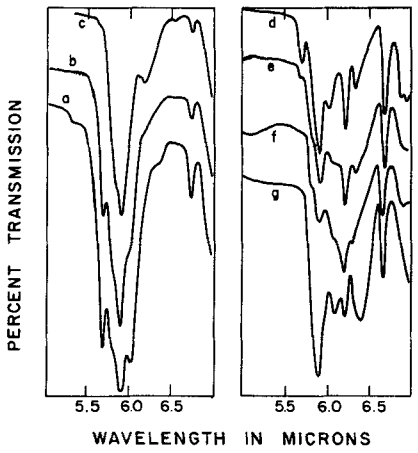


Fig. 2.—Effect of shaking in the Wig-L-Bug on infrared spectra. Phenobarbital shaken alone for 2 min. (a), with KBr for 30 sec. (b), and with KBr for 120 sec. (c). Physical mixture of phenobarbital and quinine shaken with KBr for 30 sec. (d), 120 sec. (e), and 300 sec. (f). Complex of phenobarbital and quinine shaken for 300 sec. with KBr (g).

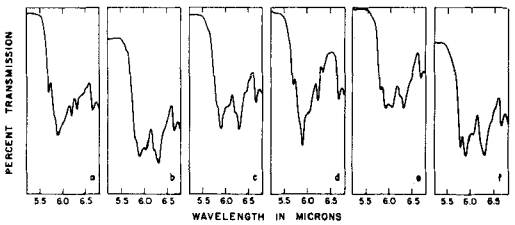


Fig. 3.—Infrared spectra of tablet formulations. Physical mixture (a) and complex (b) of phenobarbital and quinidine compressed as tablets. Commercial preparations labeled quinidine phenylethylbarbiturate (c, d). Commercial preparations labeled dihydroquinidine phenylethylbarbiturate (equimolar synergy) (e) and hydroquinidine phenobarbital (f).

making use of these spectral differences—presence of strong absorption in the 6.3 to 6.4-μ region, and absence of sharp absorption near 5.67 μ—the complexes may be distinguished from physical mixtures of their respective components.

Although the infrared data indicates that phenobarbital exists as the phenobarbiturate ion in the complex, appreciable dissociation of the complex into true ions apparently does not occur in solution as indicated by its relative insolubility in water and solubility in organic solvents. Nevertheless, only weak bonding is involved in the complex since thin-layer chromatography using silica gel as adsorbant and a variety of solvents resulted in the formation of separate spots corresponding to phenobarbital and the alkaloid in every instance.

Examination of infrared spectra of variously prepared samples showed that spectral changes were often dependent on sample treatment. Thus, the characteristic band observed in the spectrum of phenobarbital at 5.67 μ disappeared when mixtures of the sedative and potassium bromide were shaken for about 2 min. in the Wig-L-Bug (Fig. 2b and c). No band appeared, however, throughout the 6.3 to 6.4-μ region, indicating that ionization of the molecule did not occur (the emerging weak peak at 6.2 μ of Fig. 2c is attributed to moisture picked up during prolonged shaking of the sample). No change in the infrared spectrum was observed when phenobarbital was treated similarly but in the absence of potassium bromide. The disappearance of the band at 5.67 μ may be due to formation of a solid solution of phenobarbital with potassium bromide, since chloroform solution spectra of phenobarbital did not display this band. Spectral changes have been noted previously upon shaking diethylbarbital with potassium bromide, but these were attributed to conversion of the barbiturate from one crystalline form to another (9). Spectral effects observed upon shaking the complex or an equimolar mixture of phenobarbital and quinine with potassium bromide are shown in Fig. 2d–g. In the phenobarbital–quinine mixture, the band at 5.67 μ characteristic of phenobarbital disappeared after shaking for 2 min., but the resultant spectrum was not identical with that of the complex treated similarly.

Infrared analysis of the phenobarbital–quinine complex following exposure to saliva showed that the complex was stable in this medium. However, treatment with simulated gastric juice, with or without enzymes, caused a breakdown of the complex into a physical mixture of phenobarbital and quinine dihydrochloride. These conclusions were reached by spectroscopically examining the residue obtained after freeze drying a solution of the complex which had been kept at 37° for 15 min. in a small amount of gastric juice. The infrared spectrum of the residue was identical to that of a physical mixture of phenobarbital and quinine dihydrochloride.

To extend the scope of the spectral analyses, complexes of phenobarbital with 2-naphthylamine, quinoline, 8-hydroxyquinoline, and amphetamine were examined. These complexes were prepared by dissolving equimolar quantities of reactants in a small amount of ethanol and pouring the solution into cold water to form the crystalline product. By titrimetric analyses, each product was found to contain an equimolar ratio of phenobarbital to base. Weak bonding appears to occur in these complexes, however, since treatment in a drying pistol at 56° and 0.001 mm. pressure resulted in the loss of the basic moiety. Thus, the phenobarbital–2-naphthylamine complex, which initially contained 61% phenobarbital and melted at 111–115°, changed after 30 min. to material containing 93% phenobarbital and melting at 169–172°. The weak bonding was also reflected by their indistinct melting points: 87–90° for the phenobarbital–quinoline complex, 73–78° for the phenobarbital–8-hydroxyquinoline complex, and 166–168° for the phenobarbital–amphetamine complex. Nevertheless, each product displayed strong absorption in the 6.3 to 6.4-μ region of the spectrum and absence of absorption at 5.67 μ, in accord with the observations made with the quinine complexes.

To determine the applicability of the described method to commercial preparations, tablets were prepared containing either the complex of phenobarbital with the quinine-type alkaloid or an equimolar physical mixture of the respective components. Lactose was used as filler, starch paste as binder, and magnesium stearate and talc as lubricants. The tablets were powdered and aliquots con-

taining approximately 1 mg. of active ingredient subjected to infrared analysis. As expected, the spectra of the tablets containing the physical mixture displayed absorption bands throughout the carbonyl region (5.67, 6.2, and 6.3 μ) typical of the individual components (Fig. 3a), while those of the tablets containing complexes displayed peaks characteristic of the pure complex (Fig. 3b). Thus, the presence of the tablet excipients did not interfere with this method of identification. The results also showed that complex formation did not occur during formulation of the tablet, nor during powdering or compression of the specimen in preparing the potassium bromide disk.

Four commercial products containing phenobarbital and quinidine or hydroquinidine alkaloids were subsequently examined by this method. Results obtained are shown in Fig. 3c–f. Only one of the preparations displayed absorption at 5.67 μ and lacked the intense absorption near 6.4 μ (Fig. 3d), indicating the presence of free phenobarbital and hence the presence of a mere physical mixture of the active ingredients. All other spectra (Fig. 3c,e,f) were characteristic of complexes.

By means of the experimental data presented, phenobarbital–quinine type complexes may be differentiated from physical mixtures of their components. This should prove of value in applying uniformity of legislation regarding the manufacture and sale of these pharmaceutical dosage forms.

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Chemical Examination of a Toxic Extract of *Indigofera endecaphylla*

The Endecaphyllins

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Chromatography on silica gel of a toxic acetone extract of leaves and stems of *Indigofera endecaphylla* Jacq. has provided, in addition to 3-nitropropanoic acid, ethyl 3-nitropropanoate, succinic acid, and methyl β-D-glucopyranoside, a series of nine 3-nitropropanoate esters of glucose. Six of these, endecaphyllins A, A_1, A_2, B, B_1, and C are isomeric triesters while three, C_1, D, and E, are diesters. A fourth nitro-containing glucose diester, endecaphyllin I, was also obtained by chromatography and is suggested to contain as an esterifying acid a nitro-acid other than 3-nitropropanoic. The eleventh member of this group, endecaphyllin X, was isolated directly from the crude extract and found to be a glucose tetra-(3-nitropropanoate) ester. Some chemical and physical properties of these compounds are described.

INTEREST in the chemical constituents of *Indigofera endecaphylla* Jacq. (creeping or trailing indigo), a tropical legume which had achieved status as a forage crop (2), stems from observations (3) of its production of acute toxic symptoms, frequently fatal, in cattle and other animals (4). Earlier chemical examination of this plant resulted in the isolation and identification of 3-nitropropanoic acid (5, 6) which previously had been shown (7) to be identical with hiptagenic acid, a hydrolysis product of hiptagin (8) and karakin (9–11). These latter two substances, along with 3-nitropropanoic acid and 2-phenyl-1-nitroethane (12), appear to be the only naturally occurring aliphatic nitro-compounds which have, until now, been reported (1, 13).[1]

Toxicity studies with *I. endecaphylla* extracts as well as with pure 3-nitropropanoic acid indicated that, while both showed high activity in chick feeding tests (5, 6, 14), the acid did not produce the severe liver damage in other animals which was found to be a characteristic effect of the

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The authors acknowledge the technical assistance of Mrs. Ursula Mueller.
For a preliminary account of some of these results, see *Reference 1.*
* Present address: Esso Research and Engineering Co., Linden, N. J.

[1] A more complete dosier on naturally occurring nitro-compounds is given by Mueller, W. H., Thesis, The Ohio State University, Columbus, 1964. *Added in proof:* Just recently, Burrows, B. F., Mills, S. D., and Turner, W. B., *Chem. Comm.*, **1965**, 75, have observed 1-amino-2-nitrocyclopentane carboxylic acid to be a metabolite of *Aspergillus wentii.*