An Infrared Study of the Reaction of Barbiturates with p-Nitrobenzyl Chloride

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Abstract

In alkaline media barbiturates of the general formula $HNCONHCOCR'R''CO$ react with *p*-nitrobenzyl chloride to give derivatives of the composition $NCONCOCH_2C_6H_4NO_2CR'R''COCH_2C_6H_4NO_2$. It is postulated that the process of complex formation proceeds via the interaction of a negatively charged enolized barbituric acid ion with a positively charged *p*-nitrobenzyl ion. The infrared absorption spectra of the derivatives are in accord with this mechanism and suggest that in the complex the barbiturate is bonded to the *p*-nitrobenzyl moiety through the carbonyl oxygens in the 4 and 6 positions The compounds show unique features throughout the region studied, 4000 - 650 cm^{-1}, and hence the method affords a high degree of specificity for detecting and characterizing these sedative drugs

Introduction

Numerous procedures for the qualitative and quantitative analysis of barbiturates can be found in the literature. The methods reported include microcrystal tests, complex formation, color reactions, titrimetric procedures and a variety of modern instrumentation techniques as cited by Penprase and Biles (*17*).

The present paper records the authors' findings of a method based upon the reaction of barbiturates with *p*-nitrobenzyl chloride and infrared analysis of the reaction products. The application of this reaction followed by determination of the melting points of the isolated complexes as a means of characterizing malonyl urea derivatives has been reported by several authors (*1, 2, 5, 6, 7, 8, 16*). Gonzales, Vance, Helpern and Umberger refer to it in their widely recognized text on *Legal Medicine, Pathology and Toxicology* (*4*) and Lilliman modified the general procedure to permit the detection of semimicro quantities of sedative drugs in biological materials (*14*). The method is included in the latest edition of the United States Pharmacopeia and thus become an official test for the identification of barbiturates (*18*).

Examination of the experimental data as summarized by Allport (*1*), Castle and Poe (*2*), and Jespersen and Larsen (*8*) shows, however, that the melting points of the derivatives do not differ sufficiently from one another to permit unequivocal identification of all the clinically important barbiturates. It was felt, therefore, that the infrared spectra of these compounds may offer additional parameters for distinguishing between the various members of this important class of compounds. Also, in view of previous work carried out in these laboratories on barbiturate drugs (*10, 11, 12, 13*), it was anticipated that useful information regarding the mechanism of reaction governing the process of complex formation might be obtained.

Experimental

The barbiturates used in this investigation were obtained by recrystallization of commercial products from dilute aqueous ethanol.

The *p*-nitrobenzyl derivatives were prepared in accordance with the procedure described by Castle and Poe (*2*). Sodium carbonate (0.04 mole) and barbituric acid (0.02 mole) were dissolved in a minimum amount of boiling water. *p*-Nitrobenzyl chloride (0.04 mole) in ethanol was added to this solution, the volume of the aqueous to the nonaqueous phase being maintained at a 1:2 ratio. The reaction vessel was placed on a hot plate and the solution refluxed for about ½ hour. After cooling to room temperature the precipitate was filtered off and washed thoroughly with water to remove unreacted starting materials (barbiturate, carbonate, *p*-nitrobenzyl halide) as well as any monosubstituted *p*-nitrobenzyl derivative which might have formed.

The products were purified by dissolving in hot acetone, adding sufficient ethanol to induce precipitation and cooling in a refrigerator for several hours. Because of their low solubility in acetone, the method proved unsuitable for purifying the derivatives of Sigmodal,® Dial® and Alurate®, which compounds were recrystalized from chloroform.

Melting points were determined using a Fisher-Johns apparatus and following the procedure described for Class I in the United States Pharmacopeia (*18*).

Preparation of Samples for Infrared Analysis

The materials were finely powdered in a mechanical grinder until they would pass through a 250-mesh sieve (U. S. Standard Sieve Series No. 230). Accurately weighed amounts were then mixed intimately with sufficient A.C.S. reagent grade potassium bromide—treated similarly and dried overnight at 125°C—to obtain preparations of known composition. Aliquots of 200 mg were subjected *in vacuo* to a pressure of 10,000 lbs/sq inch for about 5 minutes and the absorbancies of the clear discs thus produced measured over the frequency range extending from 4000-650 cm^{-1}. A potassium bromide disc prepared under comparable conditions was placed in the path of the reference beam to compensate for absorption by the reagent. After each experiment the discs were weighed in order to determine the amount of sample present. The weight variations between different discs never exceeded 1%.

Generally 0.25% concentrations were used for recording the spectral curves. Since all barbiturates absorb strongly in the 1750 - 1650 cm^{-1} region, their spectra were in some instances also scanned using 0.125% concentrations. Similarly, in order to characterize more accurately the fingerprint region of the complexes, their spectra were traced at both 0.25% and 1.0% concentrations throughout the 1350 - 650 cm^{-1} range.

A Perkin Elmer Double Beam Model 21 Spectrophotometer equipped with rock salt optics was used to record the spectra.

Results and Discussion

The melting points of the derivatives were generally in good agreement with those recorded in the literature. Only the Mebaral® and Rutonal® compounds, when prepared in accordance with the procedure described,

TABLE 1. MELTING POINTS OF BARBITURATES AND THEIR p-NITROBENZYL DERIVATIVES

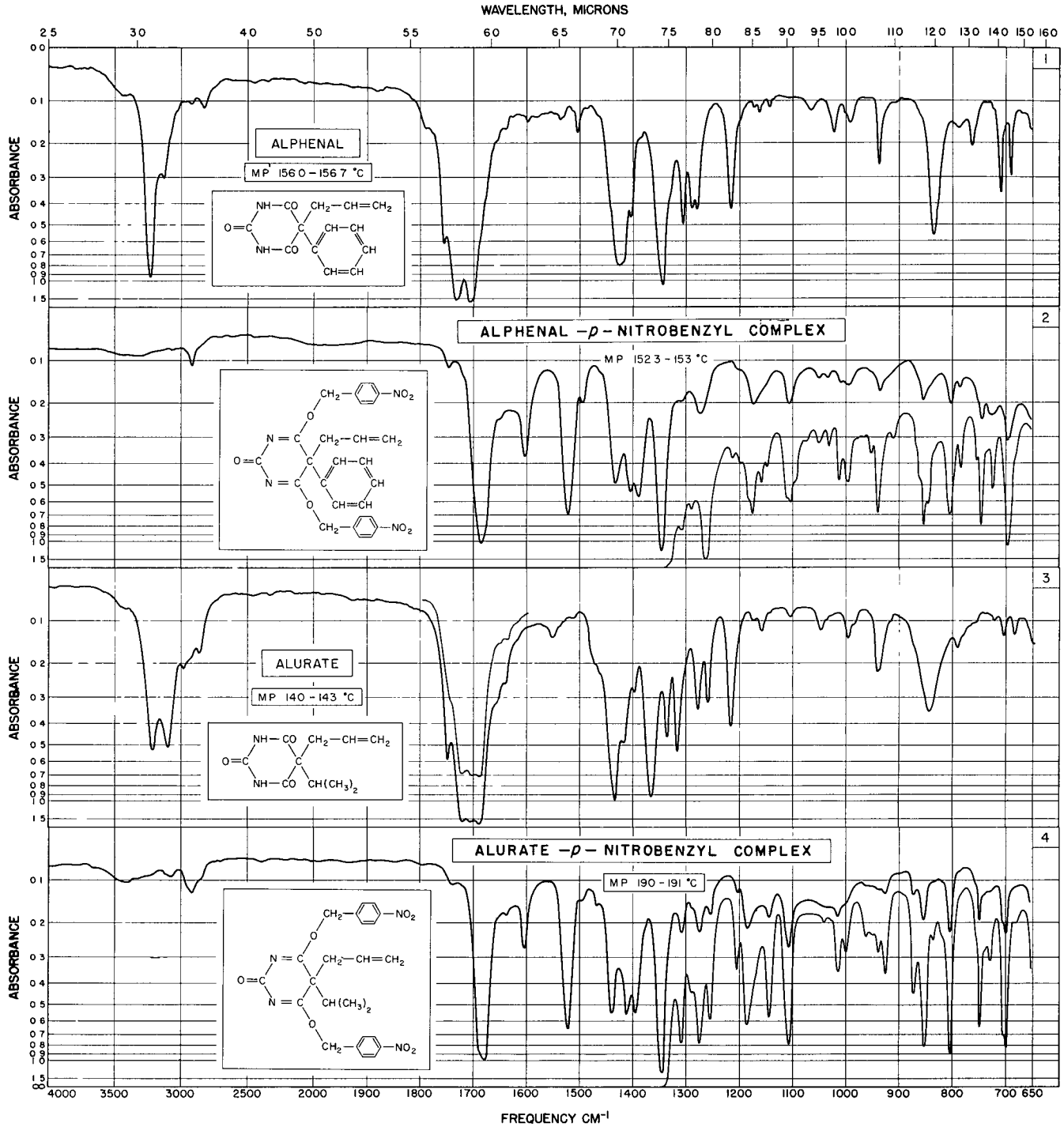
Compound			Melting Point (°C)	
Chemical Name	*Trade Name*	*Name in Standard Texts**	*Barbituric Acid*	*Complex*
5-Allyl-5-phenylbarbituric acid	Alphenal	———	156.0 - 156.7	152.3 - 153.0
5-Allyl-5-isopropylbarbituric acid	Alurate	Aprobarbital (N.N.R.)	140.0 - 143.0	190.0 - 191.0
5-Ethyl-5-isoamylbarbituric acid	Amytal	Amobarbital (N.F.) Amylobarbitone (B.P.C.)	156.3 - 156.9	151.3
5-Allyl-5-(1-cyclopenten-2-yl) barbituric acid	Cyclopal	———	139.8 - 140.3	185.0 - 185.5
5-Ethyl-5-(1-methyl-1-butenyl) barbituric acid	Delvinal	Vinbarbital (N.N.R.)	166.0 - 166.9	131.0 - 132.0
5,5-Diallylbarbituric acid	Dial	Diallylbarbituric acid (N.N.R.) Allobarbitone (B.P.C.)	172.5 - 173.0	190.5 - 191.5
5-(1-Cyclohexen-1-yl)-3,5-dimethylbarbituric acid	Evipal	Hexobarbitone (B.P.) Hexobarbital (N.N.R.)	144.8 - 145.2	114.5 - 115.0
5-Ethyl-5-isopropylbarbituric acid	Ipral	Probarbital (N.N.R.)	201.2 - 201.7	159.0 - 159.8
5-Ethyl-5-phenylbarbituric acid	Luminal	Phenobarbital (U.S.P.) Phenobarbitone (B.P.)	174.1 - 174.9	183.0 - 183.5
5-Ethyl-5-(1-cyclohepten-1-yl) barbituric acid	Medomin	———	171.0 - 172.2	165.0 - 165.8
5-Ethyl-5-(1-methylbutyl) barbituric acid	Nembutal	Pentobarbital (U.S.P.) Pentobarbitone (B.P.)	128.8 - 129.4	149.5 - 150.5
5-Ethyl-5-n-butylbarbituric acid	Neonal	Butethal (N.N.R.) Butobarbitone (B.P.C.)	127.4 - 128.5	147.0 - 148.0
5-(2-Bromoallyl)-5-isopropylbarbituric acid	Nostal	Propallylonal (N.N.R. '47)	180.3 - 181.2	204.5 - 205.0
5-(2-Bromoallyl)-5-(1-methylpropyl) barbituric acid	Pernoston	Butallylonal (N.N.R.)	131.5 - 132.2	192.0 - 192.5
5-Ethyl-5-(1-cyclohexen-1-yl) barbituric acid	Phandorn	Cyclobarbital (N.N.R.) Cyclobarbitone (B.P.C.)	171.8 - 172.2	195.5 - 196.5
5-Methyl-5-phenylbarbituric acid	Rutonal	———	225.5 - 226.2	196.3 - 197.0
5-Allyl-5-(1-methylbutyl) barbituric acid	Seconal	Secobarbital (N.N.R.) Quinalbarbitone (B.P. Add. '51)	96.1 - 96.9	159.0 - 160.0
5-(2-Bromoallyl)-5-(1-methylbutyl) barbituric acid	Sigmodal		167.3 - 168.2	180.5 - 181.5
5,5-Diethylbarbituric acid	Veronal	Barbital (U.S.P.) Barbitone (B.P.)	189.6 - 190.4	193.0 - 194.0

* U.S.P.—United States Pharmacopeia, B.P.—British Pharmacopeia, N.N.R.—New and Nonofficial Remedies, B.P.C.—British Pharmaceutical Codex.

TABLE 2. INFRARED ABSORPTION CHARACTERISTICS OF BARBITURATE-p-NITROBENZYL DERIVATIVES

Compound (p-Nitrobenzyl derivative of)	*Melting Point °C*	*Regions of Infrared Absorption (cm^{-1})*			
		1240 - 1230	*1200 - 1100*	*1000 - 900*	*800 - 710*
Neonal®	147.0 - 148.0	Sharp Band	Triplet (intensity of bands increasing with wavelength)	One weak band One inflection	One strong band, one weak band, two inflections
Nembutal®	149.5 - 150.5	No Band	Triplet (central band of lowest intensity)	Medium band (broad)	One strong band One inflection
Amytal®	151.3	Sharp Band	Three intense bands	3 weak bands	Strong band with shoulder
Alphenal®	152.3 - 153.0	No Band	Two strong bands, two weak bands, one inflection	One strong band, one med. band, two weak bands	Two strong bands, one med. band, one inflection
		1205 - 1195	*1180*	*1085*	*995*
Ipral®	159.0 - 159.8	Strong Band	Inflection	Weak band	No band
Seconal®	159.0 - 160.0	Weak Band	Strong band	No band	Weak band
		1205	*950*	*895*	*790 - 720*
Sigmodal®	180.5 - 181.5	Weak Band	Triplet	Medium band	One strong band, one weak band, one inflection
Luminal®	183.0 - 183.5	Inflection	One weak band	No band	Three strong bands One medium band
Cyclopal®	185.0 - 185.5	Medium Band	Two inflections	Inflection	One intense band, one weak band, one inflection
		1260 - 1140	*950 - 920*	*900 - 800*	*790 - 715*
Alurate®	190.0 - 191.0	Three strong bands One medium band	One inflection, one weak band followed by medium band	Two strong bands, one med. band, one weak band (preceding the second strong band), one inflection	One strong band One weak band
Dial®	190.5 - 191.5	One strong band, Two weak bands	One strong band One weak band	Two strong bands, one medium band, one weak band, one inflection	Two strong bands
Pernoston®	192.0 - 192.5	One strong band, two weak bands, one inflection	One medium band One weak band	Two strong bands, one med. band, one weak band	One strong band, one weak band, one inflection
Veronal®	193.0 - 194.0	Two strong bands One weak band	One strong band	Three strong bands, one weak band (preceding the strong bands), one inflection	One strong band, one med band, one weak band
Phanodorn®	195.5 - 196.5	One strong band (broad) with sholder, one med. band	Doublet	Two strong bands One shoulder	One strong band, One inflection
Rutonal®	196.3 - 197.0	Two strong bands (One broad)	No band	Two strong bands (one with three shoulders) One medium band (broad)	Two strong bands (each with one shoulder) One medium band

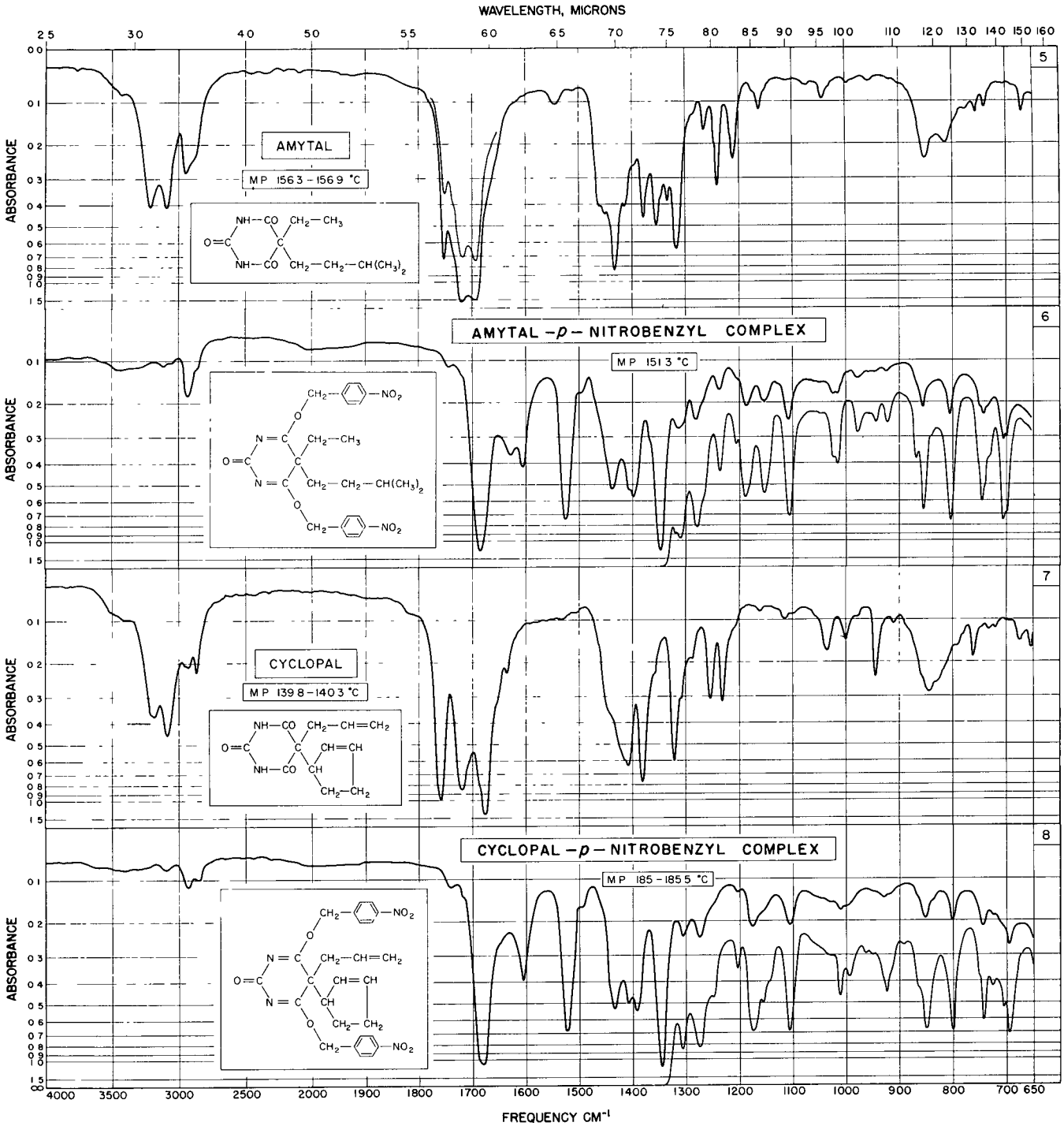
FIG. 1. INFRARED SPECTRA OF BARBITURATES AND THEIR p-NITROBENZYL DERIVATIVES



melted considerably higher (M.P. 140 - 141.5°C and 223 - 224°C respectively) than had previously been reported. However, when using a waterbath instead of a hot plate for refluxing the reactants—as suggested by Jespersen and Larsen (5)—products were isolated whose physicochemical properties were in close agreement with those cited in the literature. Evidently the rate of heating does, in these instances, become a critical factor co-determining the course of the reaction.

The experimental data, as summarized in Table I for both the free barbituric acids and their *p*-nitrobenzyl complexes, show that Alphenal® and Amytal® (M.P. 156-157°C.) as well as their derivatives (M.P. 151 - 153°C.) cannot be distinguished on the basis of their melting points. Likewise, Alurate® and Cyclopal® both melt in the 140°C region and the melting points of their *p*-nitrobenzyl complexes, although considerably higher, lie only a few °C apart (185=191°C). It would also be difficult to distinguish Nembutal® from Neonal® (M.P. 127 - 129°C) and even the preparation of their *p*-nitrobenzyl derivatives (M.P. 147 - 150°C) would be of little value in this respect. On the other hand Pernoston® (M.P. 131 - 132°C) may readily be distinguished from these two barbiturates via its *p*-nitrobenzyl derivative (M.P. 192°C)

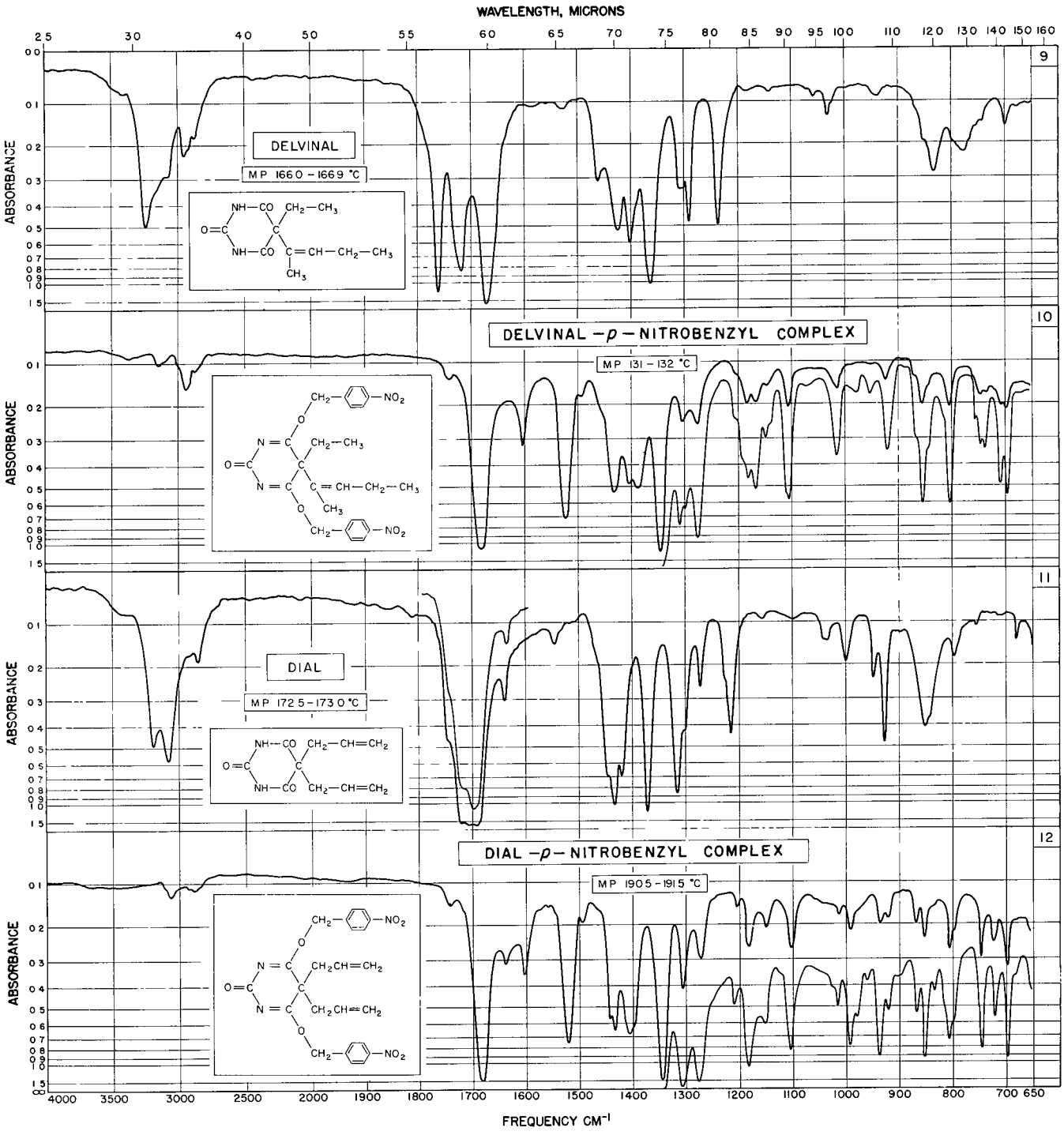
SPECTRA OF BARBITURATES AND DERIVATIVES, CONTD.



Similarly Delvinal® and Sigmodal® (M.P. 166 - 168°C) are easily identified by preparing their *p*-nitrobenzyl derivatives whose melting points lie 50°C apart. It would likewise be of advantage to characterize Medomin®, Luminal® and Dial® (M.P. 171-175°C) as their *p*-nitrobenzyl derivatives whose melting points range from 165 - 191°C.

As far as the authors are aware, no procedure has as yet been reported for regenerating the barbiturate from its *p*-nitrobenzyl complex. Unless the free acid is therefore first isolated from the sample under investigation and sufficiently characterized, the preparation of a *p*-nitrobenzyl derivative followed by a determination of the melting point of the isolated product as the sole criterion of identity becomes a highly suspect procedure. It can be seen, for example, that the derivatives of Alphenal®, Amytal®, Nembutal® and Neonal® all melt in the 147 - 153°C range. Similarly, the complexes of Cyclopal®, Luminal® and Sigmodal® (M.P. 180-185°C) and of Alurate®, Dial®, Pernoston®, Phanodorn®, Rutonal® and Veronal® (M.P. 190 - 197°C) all melt within relatively narrow temperature regions. Practically identical melting points are exhibited by the *p*-nitrobenzyl compounds of Ipral® and Seconal® (M.P. 159-160°C.)

SPECTRA OF BARBITURATES AND DERIVATIVES, CONTD.



These observations clearly indicate the need for additional criteria of identity for these substances in order to enhance the value of the reaction to the toxicologist and forensic chemist as a means of detecting and characterizing the barbiturate drugs.

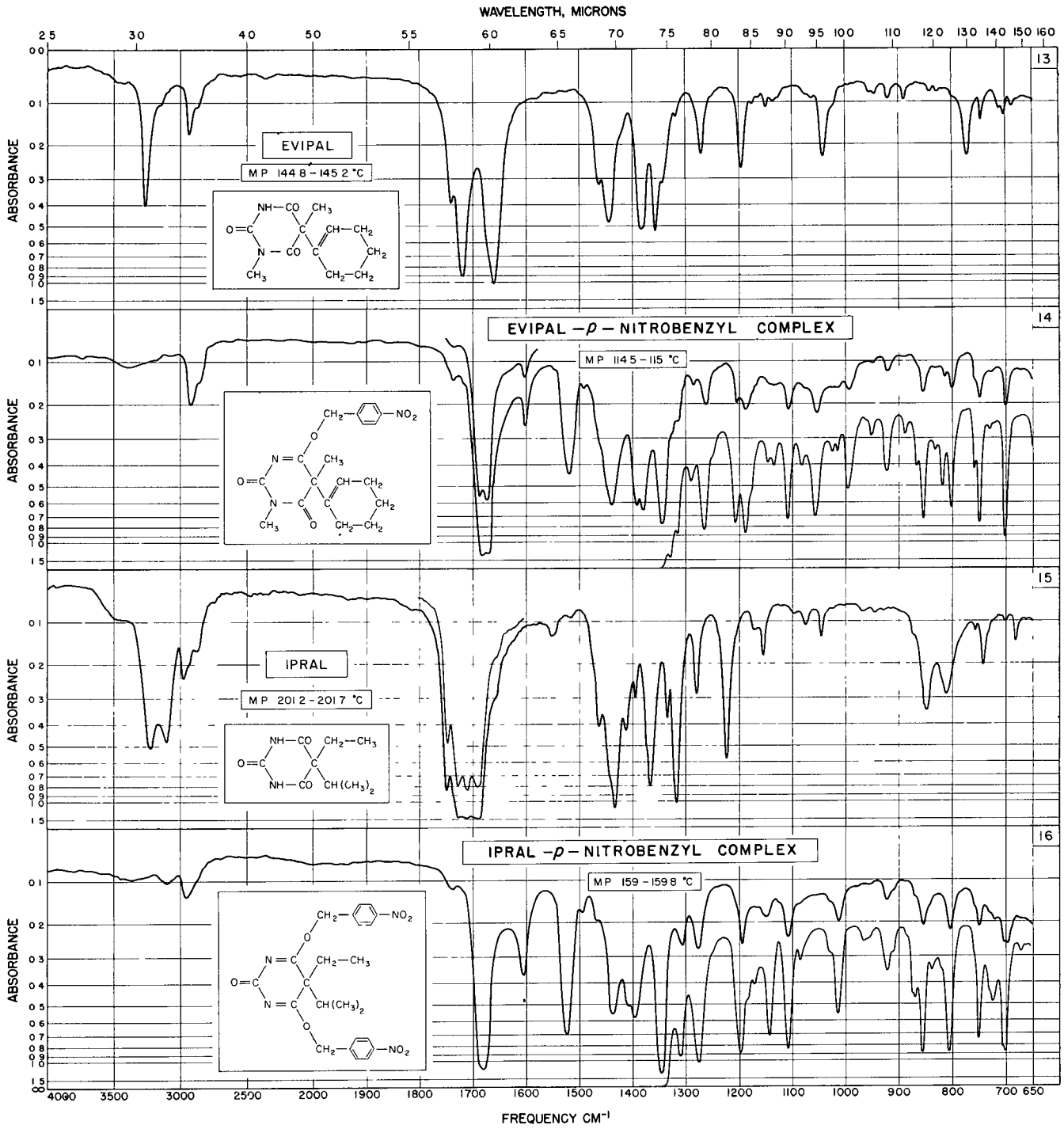
Such criteria are found in the infrared spectra of the derivatives as shown in Figure 1, along with those of the free barbituric acids.

It can be seen that generally the *p*-nitrobenzyl compounds are much richer in structure than the corresponding barbituric acids, and they may all be readily distinguished from one another by their IR spectral characteristics. Table II illustrates this feature in a qualitative manner with regard to those representatives of the series which, as earlier described, cannot be differentiated on the basis of their melting points.

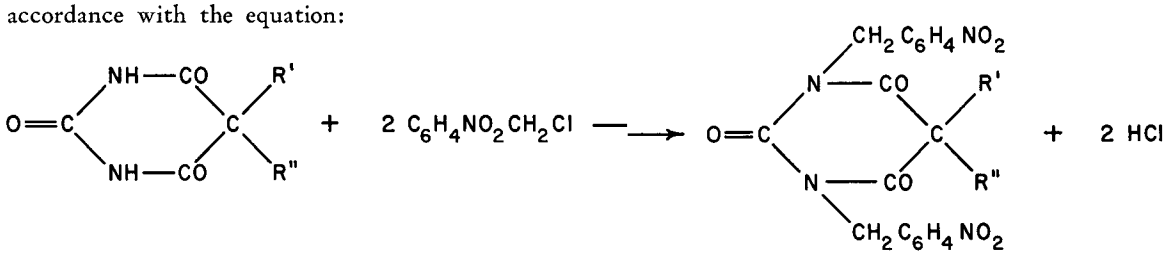
In addition to their value for differentiating and identifying the various barbituric acids included in this study, the spectral curves shown in Fig. 1 provide considerable information regarding the mechanism of the process of complex formation.

Lyons and Dox (*16*) considered the reaction to take

SPECTRA OF BARBITURATES AND DERIVATIVES, CONTD.

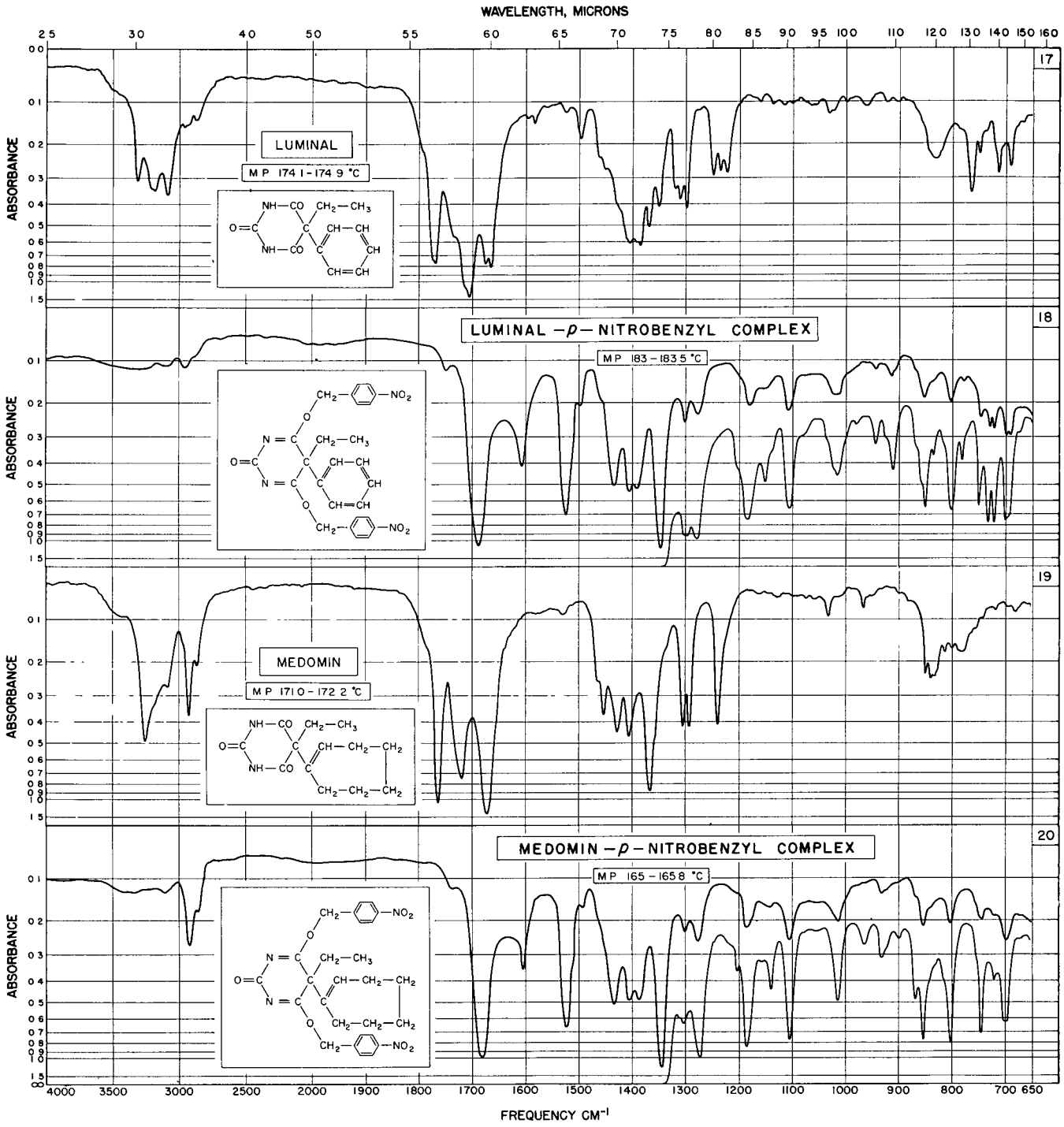


place in accordance with the equation:

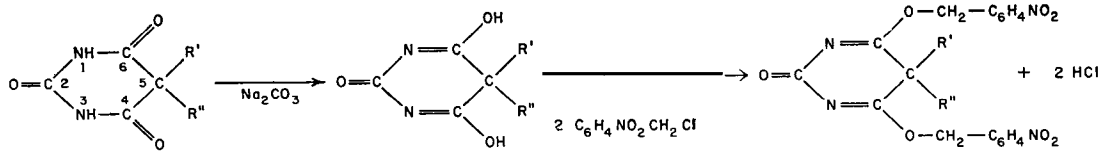


and their view was later shared by Jespersen and Larsen (*8*). No experimental data were given by these authors, however, in support of this mechanism. The spectral curves presented in Figure 1 strongly suggest that the process

SPECTRA OF BARBITURATES AND DERIVATIVES, CONTD.



does not take such a course but is in accord with the following sequence of reactions:

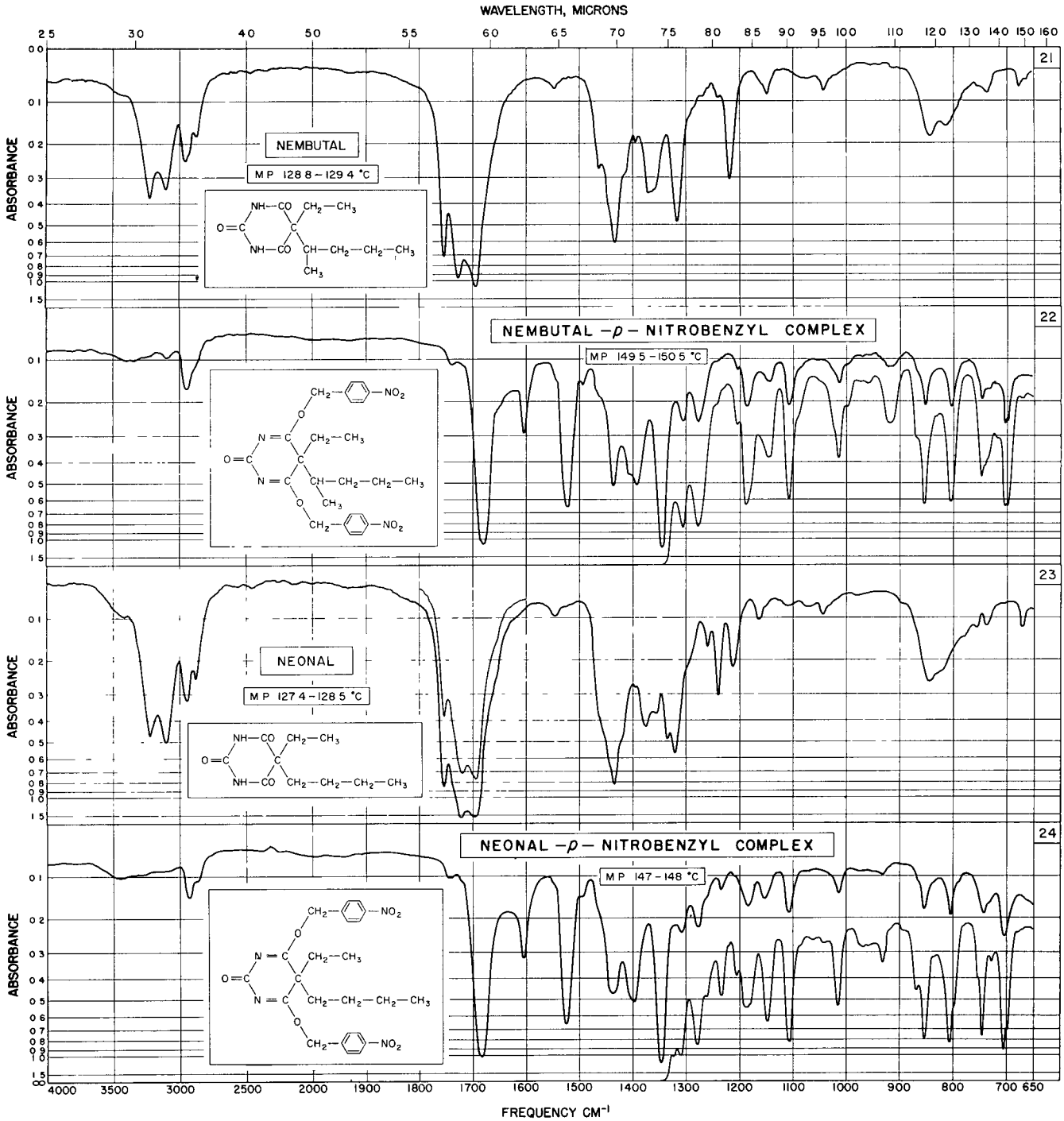


BARBITURATE

BARBITURATE —p— NITROBENZYL DERIVATIVE

This mechanism visualizes preliminary ionization and enolization of the barbiturates in the alkaline medium followed by interaction of the generated anions with the halide. The enolization of barbituric acids in aqueous systems has been investigated by Fox and Shugar (*3*), Loofbourow and Stimson (*15*), Stuckey (*20*), and Wood

SPECTRA OF BARBITURATES AND DERIVATIVES, CONTD.

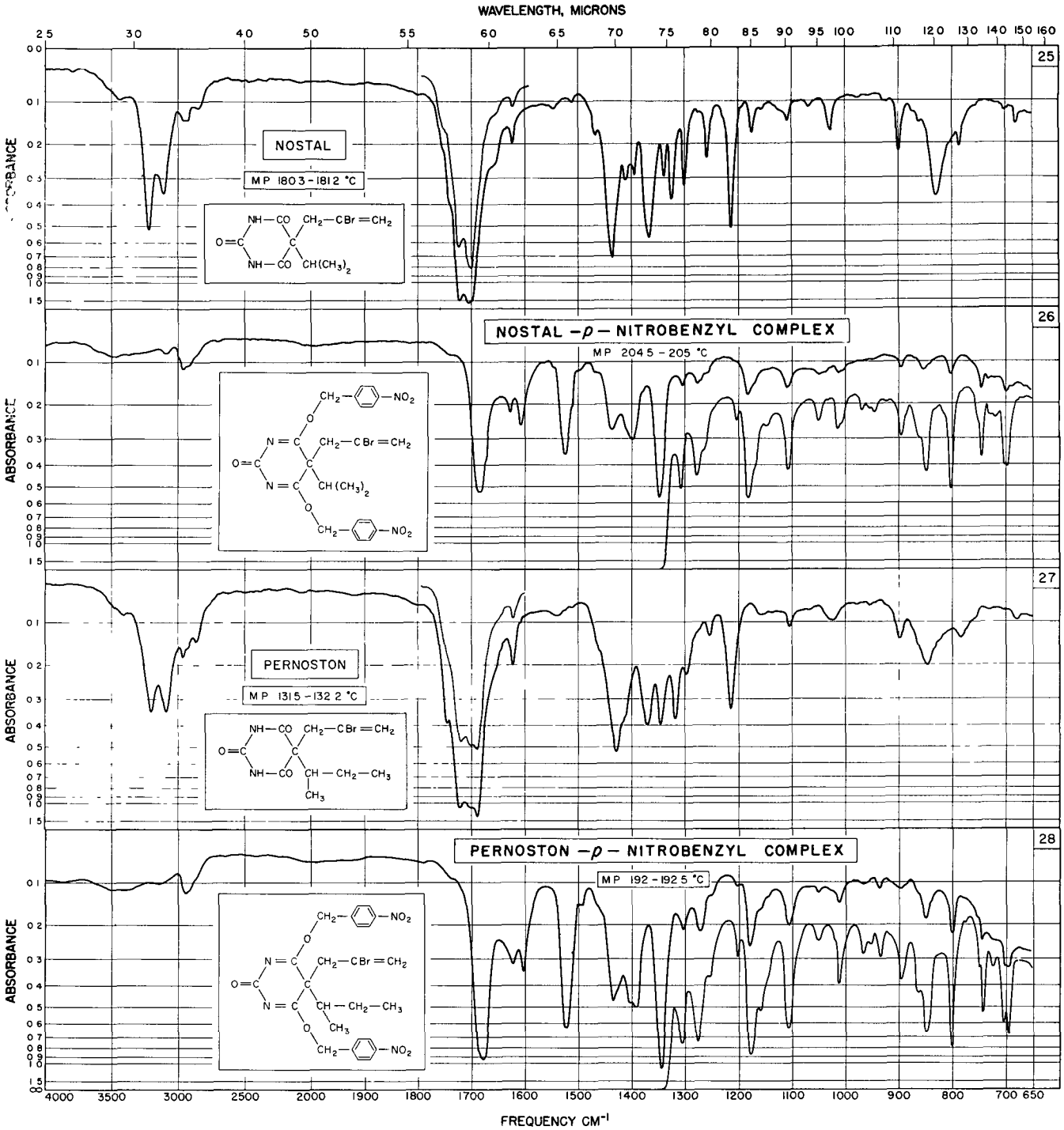


(21), and strong infrared evidence for the occurrence of the process was recently presented by Levi and Hubley (12).

Examination of the spectra of the free barbituric acids recorded in Figure 1 shows generally three distinct bands occurring in the 1750 - 1650 cm^{-1} region. They reflect the vibrational characteristics of the three carbonyl groups of these molecules as either individual or coupled oscillators (19). The low frequency band is associated with vibrations of the carbonyl bond in the 2 position, the central band corresponds to motions of the carbonyl bonds in the 4 and 6 positions, attenuated because of simultaneous coaxial out-of-phase oscillations of the carbonyl linkage in the 2 position, and the high-frequency band reflects the oscillations—perpendicular to the axis of symmetry of the molecule—of the 4 and 6 bonds exclusively.

In contrast to these three marked absorptions, seen in the spectra of the barbituric acids, only one strong band is observed in the spectra of the *p*-nitrobenzyl derivatives throughout the 1750 - 1650 cm^{-1} region. This observation clearly reflects the presence of but one carbonyl linkage in these molecules. The position of this band (1685 cm^{-1} region), furthermore, strongly suggests that the carbonyl

SPECTRA OF BARBITURATES AND DERIVATIVES, CONTD.

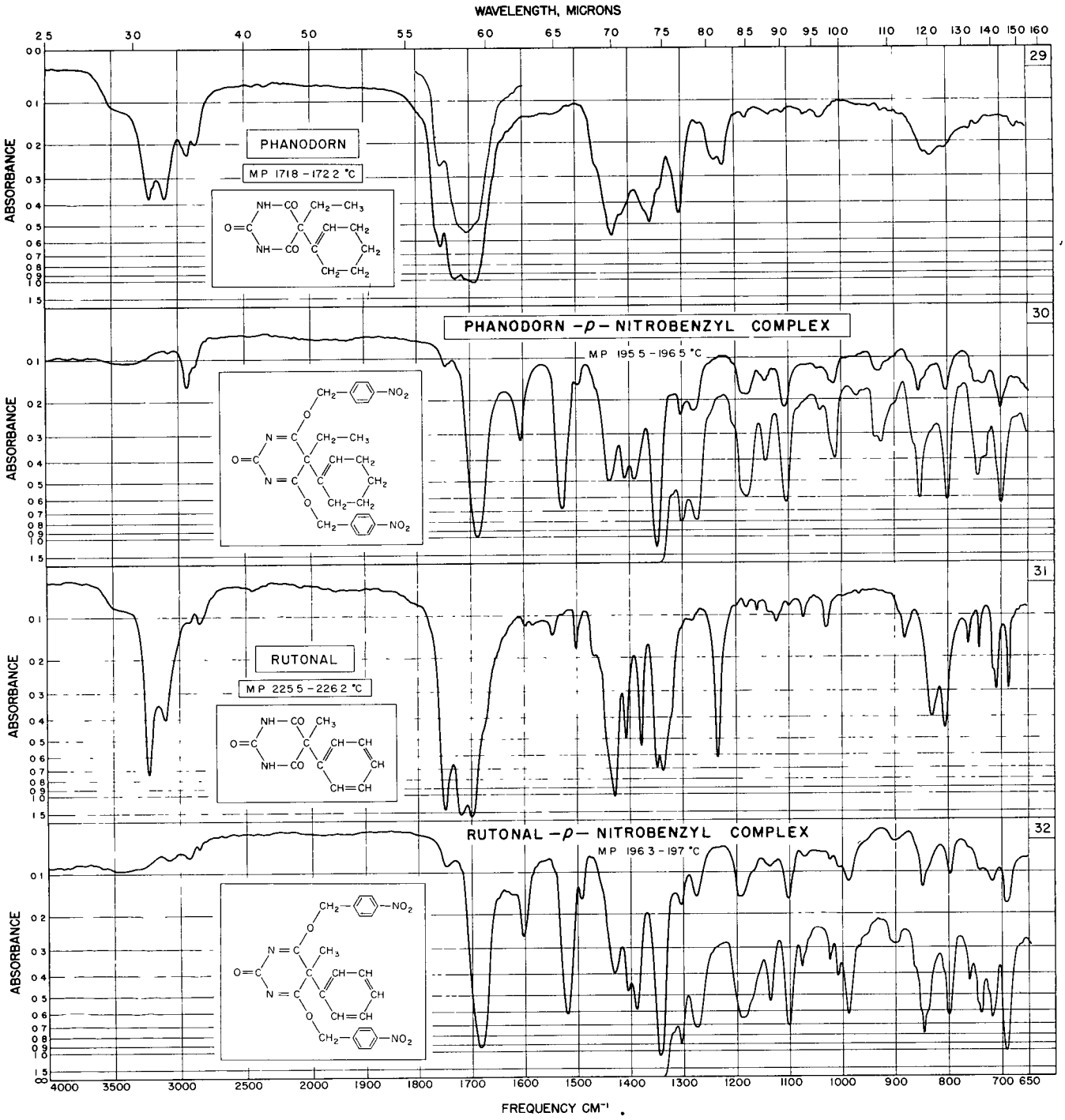


linkage in the 2 position is not involved in the enolization process and that complex formation is accompanied by establishment of two new bonds via the carbonyls in the 4 and 6 positions.

Further support for this mechanism stems from an examination of the spectrum of the Evipal® complex (Spectrum No. 14). The parent compound is an N-methyl substituted barbituric acid. Hence enolization can involve but one imino hydrogen and but one carbonyl bond of the molecule. Two of the three C=O linkages should therefore remain intact, and this is seen to be the case—the infrared spectrum of the derivative displaying a distinct doublet in the carbonyl frequency region (1690 and 1675 cm^{-1} respectively).

One should further note that the characteristic N-H (bonded) absorptions occurring in the spectra of all the barbiturates throughout the 3350 - 3050 cm^{-1} region are no longer observed in the spectra of the complexes. This finding, too, is in accord with the structures assigned to the derivatives and the reaction mechanism described which visualizes conversion of the $O{=}C(-NH-)_2$ linkages of the barbituric acids to $O{=}C(-N{=}C-)_2$ bonds in the complexes. Both the position (1683 ± 4 cm^{-1}) and intensity of the carbonyl band observed in the

SPECTRA OF BARBITURATES AND DERIVATIVES, CONTD.



spectra of the derivatives further support these conclusions.

Examination of the spectrum of *p*-nitrobenzyl chloride (Figure 1; No. 39) shows prominent bands near 1610, 1540, 1350, 1270, 1105, 860, 800 and 705 cm^{-1}. Marked absorptions in these regions are also observed in the spectra of the derivatives. The 1350 cm^{-1} band representing the nitro symmetric stretching vibration and the 1540 cm^{-1} band representing the nitro asymmetric stretching vibration (9) are in all instances particularly sharp and intense. Thus, the presence of a *p*-nitrobenzyl group as a common moiety of the complexes is in full agreement with the spectral characteristics of these molecules and the postulated reaction mechanism.

Acknowledgment

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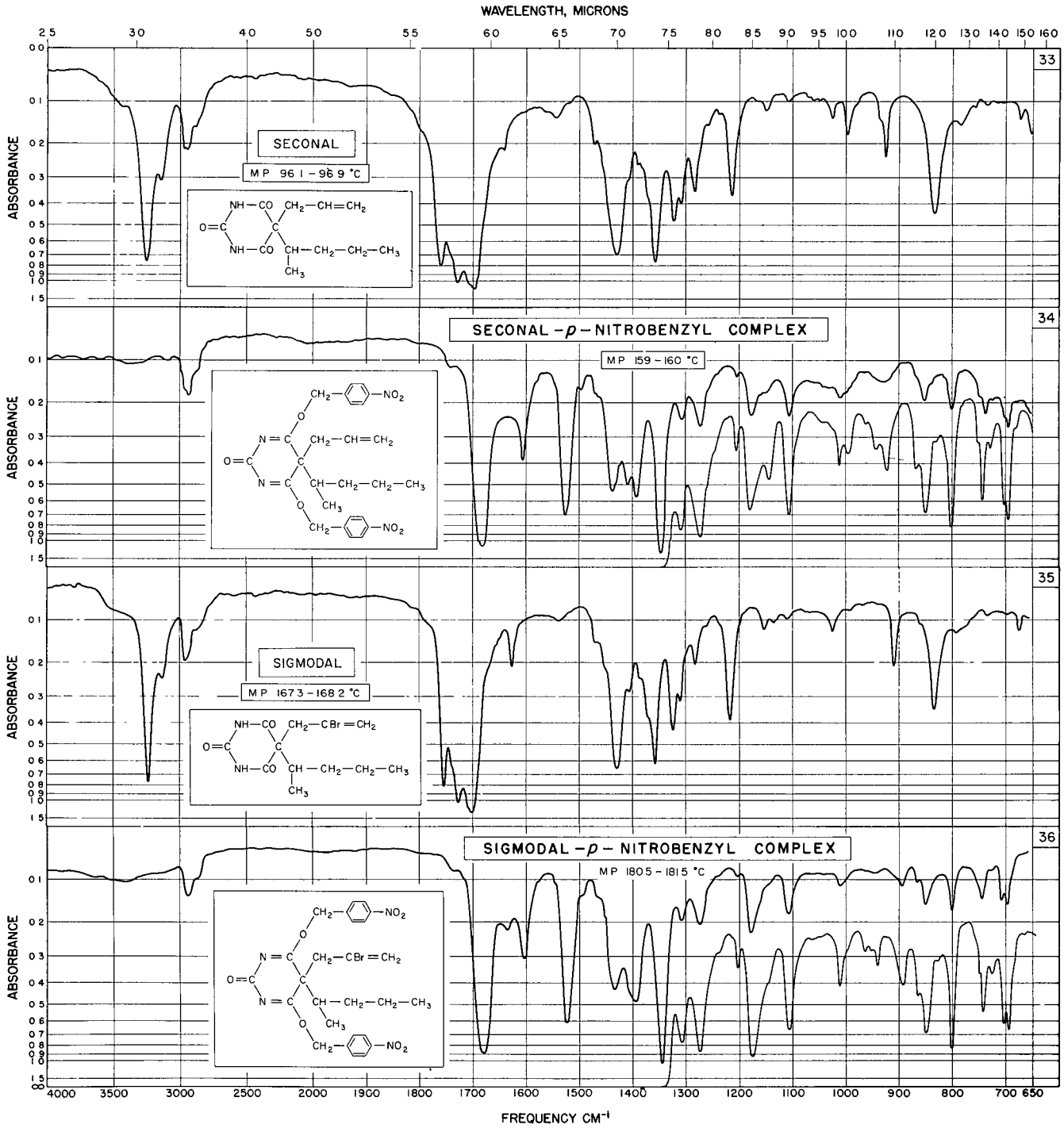
SPECTRA OF BARBITURATES AND DERIVATIVES, CONTD.



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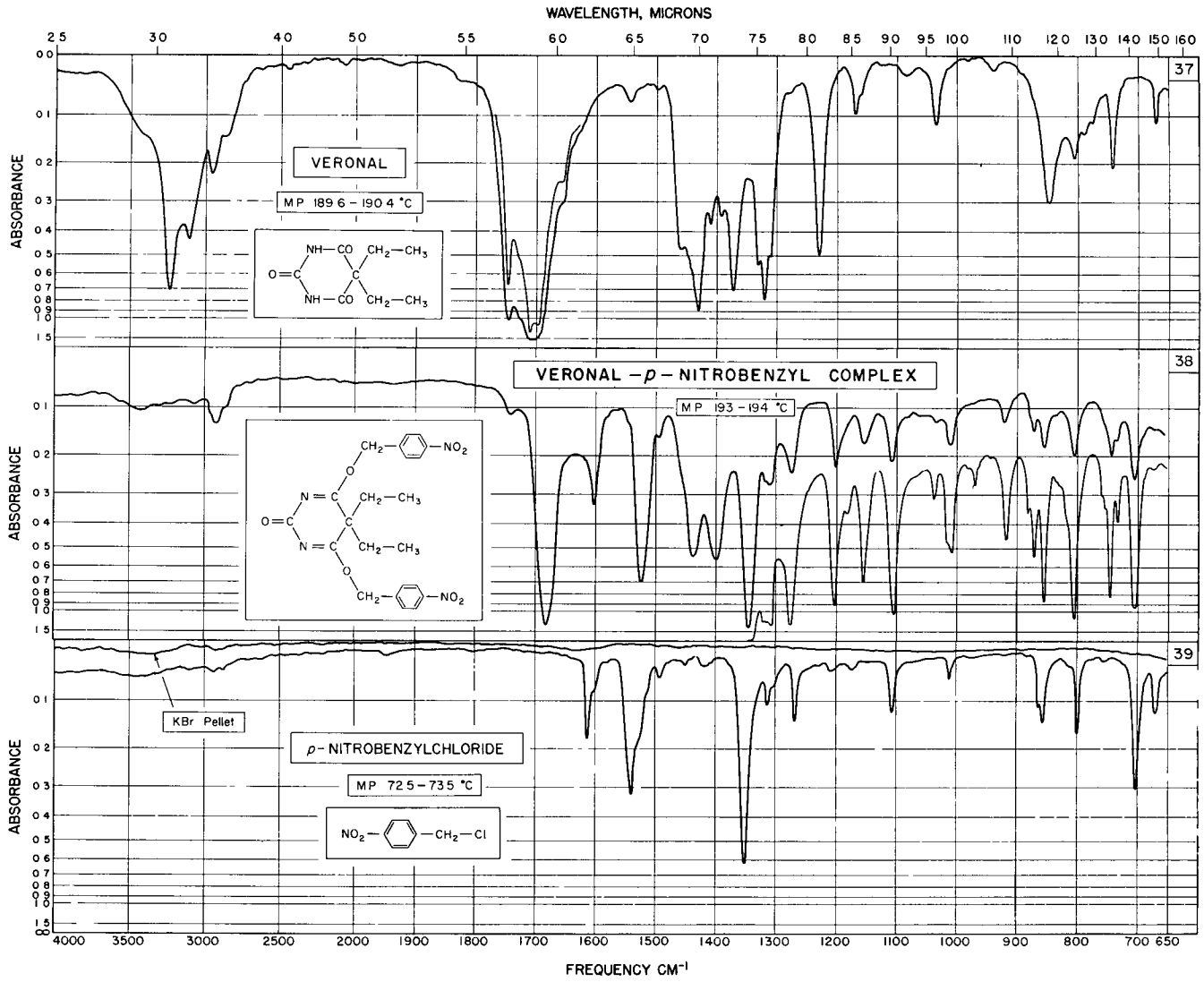

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Note

Deuteration of Acrylonitrile†

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During preparation of acrylonitrile-1-d, $CH_2{=}CHD{-}CN$, by D_2O exchange, as described by Leitch (*1*), it was found that a linear relationship obtained between absorbance values for the 1165 cm^{-1} band and the degree of exchange indicated by mass spectrometer data. This note contains a brief description of the exchange experiment, spectra of acrylonitrile before and after deuteration, and a table of IR and mass spectral data recorded at intervals during the exchange.