Complexation Between 1,3,5-Trinitrobenzene and Several Local Anesthetic Compounds

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The color-producing molecular reactions of local anesthetic compounds with 1,3,5-trinitrobenzene have been studied to determine the stoichiometry of the complexes formed and to elucidate the nature of the reaction. The resulting complexes appear to be characterized by the amine groups in the local anesthetic moiety. In general, 1:1 complexes were formed in dilute solutions.

THE COLORED complexes or molecular addition compounds of many organic chemicals with 1,3,5-trinitrobenzene have been known and studied for many years (1–3). Data pertaining to the mechanism of these reactions have been presented by Mulliken (4), Lewis (5), and Foster *et al.* (6). Recently, attention has been given to the hypothesis that a relationship may exist between this type of reversible reaction and the molecular pharmacology of drug molecules (7). The purpose of this work was to study the colored complexing reactions between 1,3,5-trinitrobenzene and several representative local anesthetic compounds by spectral absorption methods.

1,3,5-Trinitrobenzene is frequently referred to as a Lewis acid because it reacts with electron donor molecules to form colored compounds, known variously as complexes, molecular addition compounds, Lewis salts, etc. The forces responsible for these combinations of molecules arise principally from the paucity of electrons on the unsubstituted carbon atoms of the 1,3,5-trinitrobenzene brought about by the inductive effect of the nitro groups, resulting in a partial positive charge on the unsubstituted carbon atoms. These partial positive charges are attracted to π molecular orbitals of certain aromatic hydrocarbons or to electron donor substituent groups of molecules, such as amine groups.

In this work, the principle mechanism of molecular attraction involved aliphatic and aromatic amine portions of local anesthetic molecules as electron donors. Amines, in general, react with 1,3,5-trinitrobenzene by virtue of the unshared pair of electrons on the nitrogen atom which are available for sharing with an electropositive carbon of the trinitrobenzene.

A study of the reactions considered here may have value in elucidating the ultimate mechanism of biologic reactions to drug molecules. In addition, these reactions may yield data capable of being utilized in the identification and estimation of organic medicinals.

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EXPERIMENTAL

Materials.—In all instances, the base form of the local anesthetic compound was used. Isolation of the base from hydrochloride or sulfate salt forms was carried out by alkalinizing an aqueous solution of the salt and extracting with chloroform. The compounds ordinarily were recrystallized one time; however, those which remained liquid at room temperature were used as the oily liquid obtained on evaporating the solvent chloroform. 1,3,5-Trinitrobenzene (EK 639) was used as purchased without further purification. The solvents employed were absolute ethanol and reagent grade chloroform and carbon tetrachloride.

Equipment.—The spectral absorption data obtained in this study were determined on a Beckman model DU spectrophotometer, a Beckman model DB spectrophotometer fitted with a Sargent SRL recorder, and a Bausch & Lomb Spectronic 20 colorimeter.

Procedure.—Preliminary testing to determine complexing tendencies was carried out by placing samples of approximately 100 mg. of local anesthetic base on one end of a common microscope slide. Approximately 100 mg. of trinitrobenzene was placed on the other end. A portion of the two materials, in roughly equal quantities, was drawn into the middle of the slide and mixed. The slide then was placed on a hotplate and heat applied until the compounds melted. If a colored compound appeared in the melt, complex formation was indicated. If no color developed in the melt of mixed compounds, compared to the pure reactants, it was presumed that no complex formed. Table I lists the color of the fused reactants obtained in this manner.

Stoichiometry of the complexes was studied by spectral absorption characteristics. The strong color of complexes in ethanol and chloroform solution was conclusive evidence of their existence; however, the authors were unable to isolate good crystals from these solutions. The molecular ratio of complexes was found by determining the spectral absorption as a function of varying concentrations of the two reactants. Table II and Fig. 1 summarize the data found in the trinitrobenzene–procaine complex study. This method was described origi-

TABLE I.—LOCAL ANESTHETICS USED IN THE EXPERIMENTAL PROCEDURES AND THE COLOR OF COMPLEXES FORMED WITH 1,3,5-TRINITROBENZENE

Local Anesthetic	Color of Fused Reactants in Screening Procedure	Color of Soln. of Complexes in Ethanol and Chloroform
Benoxinate	Brown	Orange
Benzocaine	Orange	Yellow
Butacaine	Black	Yellow
Butyl amino-benzoate	Red	Yellow
Cyclo-methycaine	Yellow	Amber
Dibucaine	Black	Yellow
Dimethisoquin	Green	Green
Diperodon	Green	Yellow
Hexylcaine	Brown-green	Amber
Lidocaine	Green	None
Phenacaine	Amber	Orange
Pramoxine	Amber-orange	Yellow
Procaine	Black	Yellow
Tetracaine	Brown-red	Orange

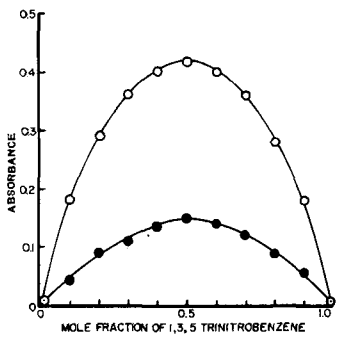


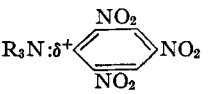
Fig. 1.—Net absorbance of procaine–trinitrobenzene complex at 400 mμ and of benzocaine–trinitrobenzene complex at 475 mμ in absolute ethanol. Maximum absorption at 0.5 mole fraction (equimolar 0.02 mole/L.) indicated 1:1 molecular ratios. Key: O, procaine; ●, benzocaine.

nally by Job (8), and the graphs of these data are commonly called Job plots. In general, these plots indicated that 1:1 complexes were formed at the concentrations employed in both ethanol and chloroform solutions. Exceptions were noted in the case of lidocaine, which was indeterminate, and of hexylcaine, which yielded a skewed curve indicating the formation of a mixture of two ratios of complexes—one molecule of hexylcaine and two of trinitrobenzene in addition to the typical 1:1 ratio.

The solutions became colored immediately on mixing the reactants, indicating an almost instantaneous formation of the complex in all cases except lidocaine. Frequently, a slow secondary reaction was noted, which appeared to be associated with the tertiary amine group when present in the compound. While the magnitude of absorption was increased by this secondary reaction, the Job plots indicated the general 1:1 ratio remained unchanged, regardless of the time that elapsed between initial mixing of reactants and determination of absorbance.

DISCUSSION

Of the several postulates set forth to describe these reactions, that presented by Foster *et al.* (6) most nearly corresponds to the results of this study. The initial reaction between local anesthetic amines and trinitrobenzene may be depicted as



This concept may be an oversimplification. For example, Lewis (5) suggested that hydrogen bonds may form with oxygen atoms of the nitro groups and hydrogen atoms of amine groups to enhance the initial reaction. In addition, others have suggested that the character of these compounds indicate resonance between no-bond and charge transfer or ionic structures. Since the tertiary amine is more basic than the aryl amine, it is probable that the tertiary amine takes precedence in this reaction, where both groups are present, as in the case of procaine. This hypothesis was substantiated by studying the reaction between procaine and trinitrobenzene compared with other tertiary amines. Foster *et al.* (6) and others have called attention to the slow secondary irreversible reaction that takes place between trinitrobenzene and tertiary amines in ionizable solvents, such as ethanol. Experience indicates that this secondary reaction occurs in procaine and other local anesthetics with tertiary amines present, regardless of the solvent. It occurred in carbon tetrachloride solution in the same order as in ethanol. This secondary reaction has not been elucidated satisfactorily. It occurred at varying rates with the different local anesthetic

TABLE II.—DETERMINATION OF THE STOICHIOMETRY OF PROCAINE–TRINITROBENZENE COMPLEX BY THE METHOD OF JOB IN ETHANOL AT A WAVELENGTH OF 400 mμ[a]

Mole Fraction of Trinitrobenzene in Soln.	Concn. of Procaine in Soln., mole/L.	Concn. of Trinitro-benzene in Soln., mole/L.	Absorbance of Soln.	Absorbance of Trinitroben-zene Blank	Net Absorbance of Complex
0	0.040	0	0	0	0
0.1	0.036	0.004	0.26	0.08	0.18
0.2	0.032	0.008	0.45	0.16	0.29
0.3	0.028	0.012	0.58	0.22	0.36
0.4	0.024	0.016	0.68	0.28	0.40
0.5	0.020	0.020	0.75	0.33	0.42
0.6	0.016	0.024	0.79	0.39	0.40
0.7	0.012	0.028	0.80	0.44	0.36
0.8	0.008	0.032	0.76	0.48	0.28
0.9	0.004	0.036	0.70	0.52	0.18
1.0	0	0.040	0.56	0.56	0

[a] The greatest absorbance of the complex occurred at 0.5 mole fraction, an indication of a 1:1 ratio. (See Fig. 1.)

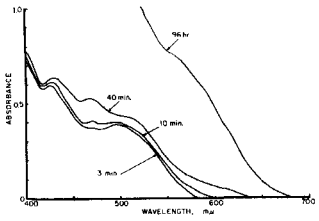


Fig. 2.—Changes in spectral absorbance with time due to the secondary reaction of trinitrobenzene and triethyl amine in absolute ethanol.

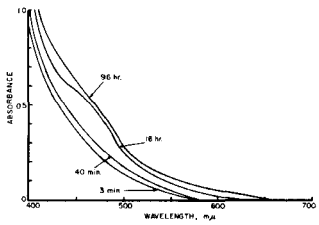


Fig. 3.—Changes in spectral absorbance with time due to secondary reaction of trinitrobenzene and procaine in absolute ethanol.

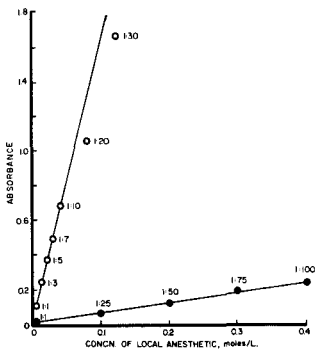


Fig. 4.—Relation between molecular ratios (shown at each point) and absorbance of solutions in absolute ethanol at 460 mμ. Concentration of trinitrobenzene was constant at 0.004 mole/L. Key: O, procaine; ●, benzocaine.

compounds tested. One of the faster reactions was found with phenacaine, which reached a maximum absorption in 60 to 90 min. in ethanol solution. On the other hand, butacaine required 5 days at 25° to reach maximum absorption.

The secondary reactions of triethyl amine (Fig. 2) and of procaine (Fig. 3) were studied as a function of time at room temperature (approximately 25°). Equimolar concentrations of 0.04 mole/L. were employed in this study. It should be noted that the rate of the secondary reaction was notably slower with the local anesthetics than with the free tertiary amine. In comparison, the benzocaine–trinitrobenzene complex formed immediately on mixing, as was typical. However, no secondary reaction was

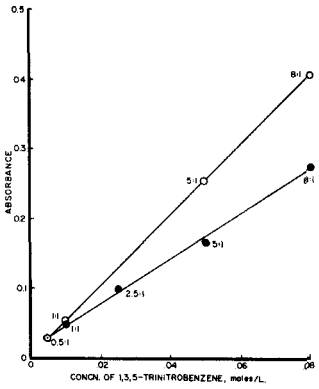


Fig. 5.—Relation between molecular ratios (shown at each point) and absorbance of complex in solutions as the proportion of trinitrobenzene was increased. Absorbance of trinitrobenzene was subtracted to yield net absorbance of complex in solution. Concentration of procaine and of benzocaine was constant at 0.01 mole/L. Determined at 460 mμ in absolute ethanol. Key: O, procaine; ●, benzocaine.

noted, even after 3 days at 25°. In this study, absorbances of all solutions of complexes were determined immediately after mixing unless otherwise noted.

The increasing absorbance of the solutions with increased concentrations of reactants, shown in Figs. 4 and 5, indicate that higher orders of complex ratios formed, particularly in the cases of polyamine local anesthetic compound. However, the methods used here did not permit establishment of molecular ratios in these more concentrated solutions. In this work, the absorbance of complexes decreased regularly from 400 mμ to zero near 600 mμ. (See Fig. 3.) Therefore, it was necessary to choose an arbitrary point on the slope of the absorbance *versus* wavelength curve, where the absorption of the individual reactants was negligible. Consequently, the 460 mμ wavelength was used frequently in this study.

CONCLUSION

Local anesthetic amine bases form complexes immediately on mixing in solution or when fused with 1,3,5-trinitrobenzene. In dilute solutions, 1:1 ratios commonly were found. The amine group is the primary site of reaction, with the tertiary amine taking precedence when present.

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