For accurate quantitative use of the Folin phenol reagent, either its response as related to a standard decomposition curve must be considered or its activity must be correlated with a known amount of standard protein or amino acid.

It follows that to get sensitivity with the reagent with microgram quantities of protein, the state of decay of the reagent must be known. If deterioration is sufficiently great, either fresh reagent must be used or the quantity must be increased.

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Nuclear Magnetic Resonance Spectra of Amines II. Identification of *N*-Phenyl Amines

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The effects of a strongly acidic solvent on the chemical shift and spin-spin splitting of the phenyl protons in *N*-phenyl amines are characteristic for this functional group. These effects provide the basis for the identification of the *N*-phenyl group in primary, secondary, and tertiary amines.

THE EFFECT of protonation of amines containing *N*-methyl groups has been recently reported by these laboratories (1). The authors wish to report on the *N*-phenyl group as characteristically identified by examination of the NMR spectrum of free base in deuterated chloroform and the spectrum in trifluoroacetic acid.

EXPERIMENTAL

All NMR spectra were recorded on a Varian A-60 spectrometer using Varian sample tubes. Deuterated chloroform and trifluoroacetic acid were used as solvents. Spectra were obtained on samples at room temperature at a concentration of 50 mg./ml.

The *N*-phenyl amines used were Eastman organic chemicals as purchased from Distillation Products Industries, Rochester, N. Y., or K and K chemicals as purchased from the K and K Laboratories, Plainview, N. Y.

RESULTS AND DISCUSSION

The pronounced change in the aromatic proton pattern of an *N*-phenyl amine free base on conversion to the amine cation is illustrated in Fig. 1, curve A, diphenylamine in deuterated chloroform, and curve B, diphenylamine in trifluoroacetic acid. This phenomenon, the collapse of a complex A_2B_2C aromatic pattern into a simple peak (or narrow band of peaks), is general for any protonatable *N*-phenyl group with no other substituents on the *N*-phenyl ring. The collapse of this pattern can be attributed to equalization of the chemical shifts of the phenyl protons. The principal cause of inequality of chemical shifts for the phenyl protons in an amine free base is conjugation of the amine group with the phenyl ring. Protonation of the amine blocks this conjugation and results in nearly uniform chemical shifts for the protons on the benzene ring. The reduction of conjugation of an *N*-phenyl amine on formation of the amine cation is well established in the theory of ultraviolet spectra for anilines (2).

The authors have found the collapse of the A_2B_2C spectral pattern of the *N*-phenyl group useful for determining whether one or more *N*-phenyl groups in an unknown compound have other substituents on the *N*-phenyl ring. For example 3-chloro-*N*-phenyl aniline shows 2 species of protons in trifluoroacetic acid due to the *meta* substituted benzene ring.

The NMR data for 5 representative *N*-phenyl reference compounds are listed in Table I. It should be noted that acidic solvents, such as aqueous hydrochloric and sulfuric acids, all influence the NMR absorption pattern of the *N*-phenyl group in the same fashion, regardless of whether the amine is primary, secondary, or tertiary.

If the *N*-phenyl group is close to another aromatic ring, the asymmetric magnetic field generated by the second aromatic ring may prevent observation of collapse of the A_2B_2C pattern on cation formation. For example, the NMR spectra for *N*-methyl-*N*-phenyl-benzylamine (Fig. 2) show a more complex pattern for the ion (Fig. 2, B) than for the free base (Fig. 2, A). The authors have observed a similar

TABLE I.—NMR CHEMICAL SHIFTS FOR *N*-PHENYL GROUPS IN ANILINE AND *N*-SUBSTITUTED ANILINES

Compd.	Appropriate Range of Complex A_2B_2C Aromatic Pattern in $CDCl_3$, p.p.m.	Chemical Shift Downfield from Tetramethylsilane for Single Aromatic *N*-Phenyl Peak in CF_3COOH, p.p.m.
Aniline	6.7–7.4	7.52
Diphenylamine	6.5–7.5	7.60
N,N-Dimethyl-aniline	6.3–7.4	7.62
N-Methylaniline	6.6–7.4	7.55
N-Methyldi-phenylamine	6.7–7.4	7.65

Received November 3, 1965, from Smith Kline & French Laboratories, Philadelphia, Pa.
Accepted for publication January 4, 1966.

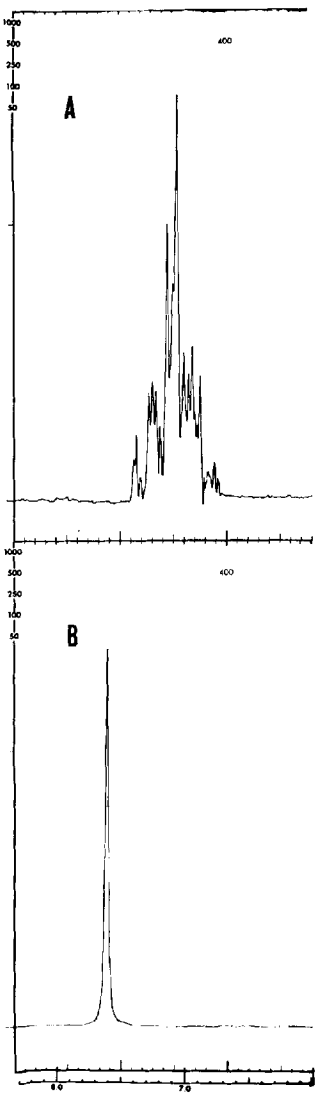

Fig. 1.—NMR spectra of diphenylamine. Key: A, solvent, deuterated chloroform; B, solvent, trifluoroacetic acid.

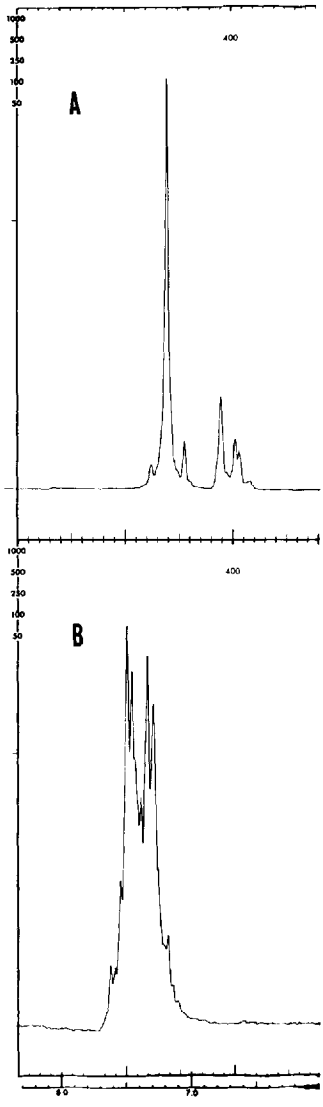

Fig. 2.—NMR spectra of *N*-methyl-*N*-phenlybenzylamine. Key: A, solvent, deuterated chloroform; B, solvent, trifluoroacetic acid.

lack of collapse for the *N*-phenyl protons of *N*-phenylbenzylamine.

CONCLUSION

The difference between the NMR spectrum of the *N*-phenyl amine free base and that of the *N*-phenyl amine ion is a useful diagnostic tool for structure determinations of these compounds. This difference makes it possible to establish the presence of the *N*-phenyl structures in primary, secondary, and tertiary amines. Interference with identification of the *N*-phenyl group may occur from other aromatic rings in the molecule. It is conceivable that long-range effects of any functional group possessing magnetic anisotropy could cause some interference; however, no examples of this effect were noted in this study.

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