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Foundations of chemical microscopy, 2 Derivatives of primary phenylalkylamines with 5-nitrobarbituric acid

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Abstract

5-Nitrobarbituric acid (dilituric acid) was extensively used with great success as a chemical microscopic reagent for the qualitative identification of primary phenylalkylamines. This methodology was based on the characterization of observed crystal morphologies, since a unique crystal habit could be associated with each adduct product. To understand the scientific foundations which permitted chemical microscopy to function as a useful analytical technique, the products formed between dilituric acid and a series of primary phenylalkylamines were characterized using polarizing optical microscopy, powder X-ray diffraction, thermal analysis, and solid-state nuclear magnetic resonance. It was deduced that the origins of the different crystal morphologies associated with each of the crystalline adducts arose from the ability of the systems to form differing structural types and/or hydrates upon crystallization. The degree of hydration in the crystalline phenylalkylamine adducts appeared to increase as additional carbon atoms were added between the aromatic ring and the terminal amine group of the aliphatic sidechain.

Keywords: Microscopy; Phenylalkylamines; 5-Nitrobarbituric acid; Dilituric acid

1. Introduction

An exceedingly important analytical technique which was extensively used during the first half of this century for the qualitative identification of inorganic and organic compounds was that of chemical microscopy. For such work, derivatives of the analyte species were prepared, crystallized, and identified by means of the morphological characteristics of the resulting adducts [1–3].

During the development of chemical microscopy as an analytical technique, it was reported that 5-nitrobarbituric acid (dilituric acid, whose structure is shown in Fig. 1) surpassed all the available nitro-enolic reagents for the isolation and identification of basic compounds [4]. In a series of works, Dewy and Plein established this compound as the reagent of choice for the identification of aliphatic amines [5], primary aromatic

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amines [6], and secondary aromatic amines [7]. Most important to the success of the technique was the high degree of crystallinity and ease of recrystallization associated with the adduct compounds.

To more fully understand the scientific foundations which permitted chemical microscopy to function as a useful analytical technique, detailed investigations into the solid-state behavior of the crystalline adducts formed by dilituric acid and substrates have been conducted. In the first study, the salts formed by the reagent with Group IA and IIA cations were thoroughly characterized with the aim of deducing the underlying principles governing the production of characteristic crystal morphologies [8]. It was found in this work that the origins of the different crystal morphologies associated with each of the adducts arose from the ability of the systems to form various hydrate species, which could also contain structural variations due to cation/dilituratе packing patterns.

To establish the generality of the conclusions reached in the first paper of this series, the line of investigation has been continued. In the present work, results obtained using polarizing optical microscopy, powder X-ray diffraction, thermal analysis, and solid-state nuclear magnetic resonance are reported for the crystalline adducts formed by dilituric acid and a series of primary phenylalkylamines.

2. Experimental

2.1. Chemicals

5-Nitrobarbituric acid (dilituric acid, or DLA), aniline (AN), benzylamine (BA), phenethylamine (PEA), 3-phenyl-1-propylamine (PPA), and 4-phenyl-1-butylamine (PBA) were obtained from Aldrich (Milwaukee, WI) in the highest purity available, and were used without subsequent purification.

2.2. Preparation of cation adducts

Derivatives were prepared by suspending 1 mmol of 5-nitrobarbituric acid and 1 mmol of phenylalkylamine in 5–10 ml of deionized water, shaking vigorously for 1 min, and then heating at 60°C until the contents were completely dissolved. Upon cooling to room temperature, the derivatives precipitated as crystalline products which were allowed to air-dry prior to their analysis. Each adduct was prepared in duplicate, and the optical microscopic characteristics of each lot compared. In every instance, identical adduct products were produced.

2.3. Equipment

2.3.1. Polarizing optical microscopy

Optical microscopic investigations were conducted on either a Nikon Microphot SA or a Nikon Labophot-2 compound microscope system, at magnifications between $\times 40$ and $\times 200$ (depending on the particle size). Samples were immersed in mineral oil, and held on the slide under a cover glass. The samples were viewed using ordinary illumination, and between crossed polarizers to evaluate the degree of crystallinity. When

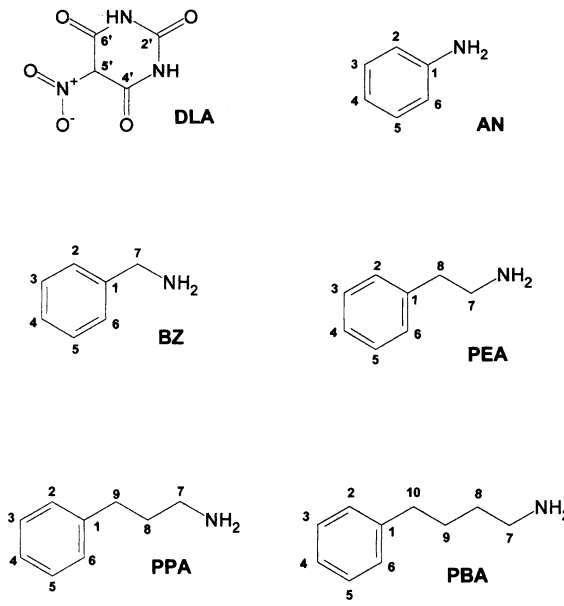


Fig. 1. Structures and carbon numbering system for 5-nitrobarbituric acid (dilituric acid, or DLA), aniline (AN), benzylamine (BA), phenethylamine (PEA), 3-phenyl-1-propylamine (PPA), and 4-phenyl-1-butylamine (PBA).

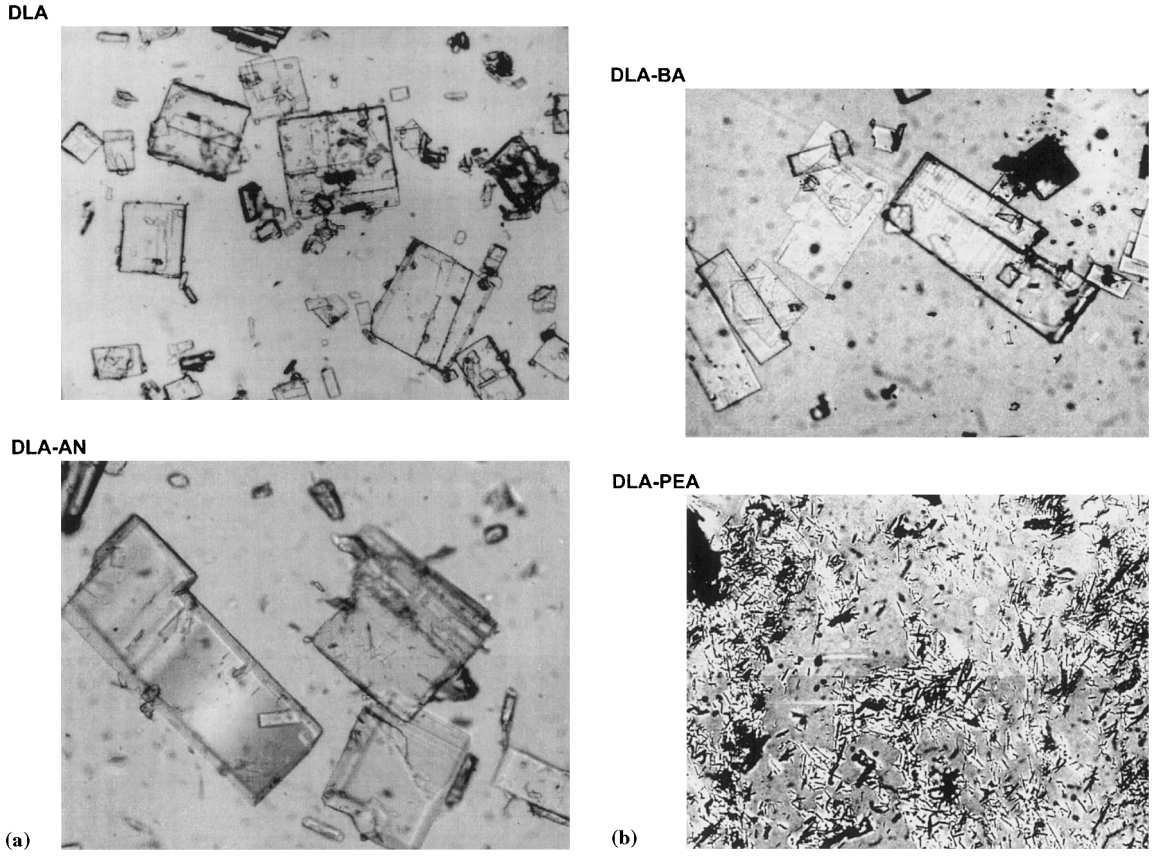


Fig. 2. Crystal morphology of dilituric acid (DLA), obtained using optical microscopy at a magnification of × 200. Also shown are the crystal morphologies of the products formed by dilituric acid with aniline (DLA–AN, magnification of × 100), benzylamine (DLA–BA, magnification of × 100), phenethylamine (DLA–PEA, magnification of × 200), 3-phenyl-1-propylamine (DLA–PPA, magnification of × 100), and 4-phenyl-1-butylamine (DLA–PBA, magnification of × 200).

required, hot-stage microscopic characterizations were carried out using a simple Kofler device.

2.3.2. Thermal analysis

Measurements of thermogravimetry were obtained using either a TA Instruments 2100 thermal analysis system or a Perkin-Elmer TGA-7 thermal analysis system. For the DSC work, approximately 2–4-mg samples were accurately weighed into DSC pans, the pans hermetically sealed, and a pinhole punched into the pan lids. For TG determinations, approximately 5–10 mg of sample was placed on the pan, and inserted in the TG furnace. For either measurement, the samples were heated at a rate of 10°C min^{-1}, up to a final temperature of 300°C.

2.3.3. Powder X-ray diffraction

The X-ray powder patterns of the samples were obtained using a Philips model APD 3720 powder diffraction system, equipped with a vertical goniometer configured in the $\theta/2\theta$ geometry. The Kα emission of copper (1.544390/1.540562 Å) was used as the radiation source. Each sample was scanned between 2 and 40° 2θ, at a scan rate of 0.02° 2θ s^{-1}.

2.3.4. Solid-state NMR

Solid-state ^{13}C NMR spectra were either acquired at Spectral Data Services (Champaign, IL) at a field strength of 270 MHz, or using a Bruker AM-250 spectrometer equipped with a solids probe. All spectra were obtained using the cross-

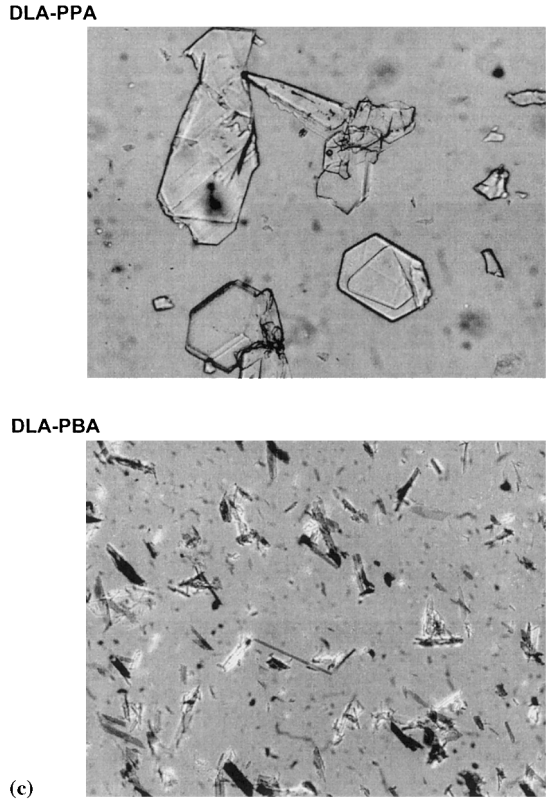


Fig. 2. (*Continued*)

polarization, magic-angle spinning (CP/MAS) pulse sequence.

3. Results and discussion

The series of aliphatic phenylalkylamines studied in the present work consists of a sequence of primary amines for which one carbon unit is successively inserted between the primary amine group and the phenyl group. Structures of these ligands, and a carbon numbering system for them, are found in Fig. 1.

3.1. Particle morphology

The crystal morphology of the dilituric acid reagent itself has been studied [8]. When the pure substance itself was recrystallized according to the protocol used for generation of the adduct precipitates, it was shown to be obtained as tabular plates which exhibited only first-order birefringence owing to their thinness.

As evident in Fig. 2, each of the crystalline products formed between the dilituric acid reagent and the phenylalkylamine substrates differed significantly from each other with respect to their morphological properties. The DLA–AN adduct formed as thick tabular plates, which exhibited surprisingly weak birefringence given their evident thickness. This behavior stands in contrast to the DLA–BA adduct, which formed as thin rectangular plates, but which exhibited moderately strong birefringence. The DLA–PEA adduct crystallized as exceedingly fine needles, which exhibited extremely strong birefringence. Irregularly shaped hexagonal plates were formed upon crystallization of the DLA–PPA adduct, and these exhibited strong multiple order birefringence. Finally, the DLA–PBA adduct also crystallized as small

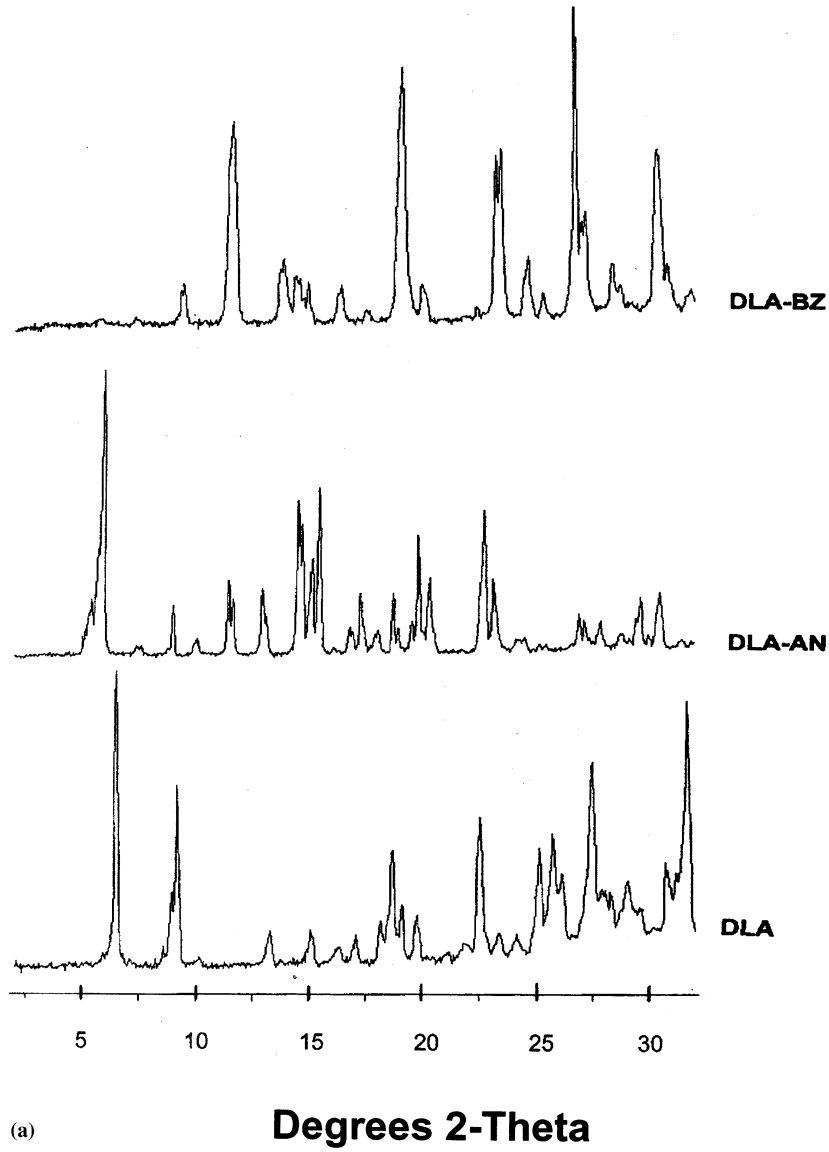
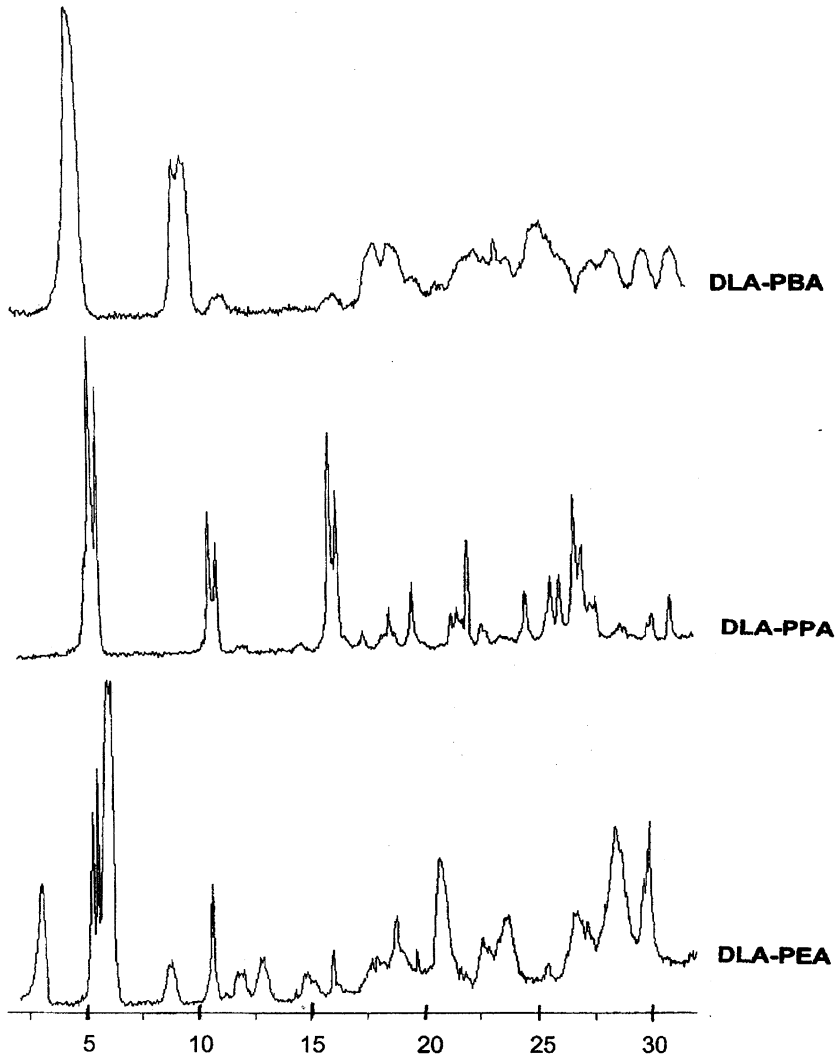


Fig. 3. Powder X-ray diffraction pattern of dilituric acid (DLA), as well as the powder patterns of the products formed by dilituric acid with aniline (DLA–AN), benzylamine (DLA–BA), phenethylamine (DLA–PEA), 3-phenyl-1-propylamine (DLA–PPA), and 4-phenyl-1-butylamine (DLA–PBA).

needles, but these exhibited relatively weak birefringence.

3.2. Powder X-ray diffraction

Dilituric acid forms a well-defined trihydrate phase, whose structure has been reported [9]. In the literature structure, the dilituric acid molecules form hydrogen-bonded sheets which are essentially coplanar, and parallel to the (010) plane. The water molecules are hydrogen-bonded in the channels formed by the three-dimensional placement of the sheet planes. Dilituric acid processed in a manner identical with that used to generate



(b)

Fig. 3. (*Continued*)

the adducts of the present study was obtained as a highly crystalline product, and yielded a characteristic X-ray powder diffraction pattern [8].

As illustrated in Fig. 3, each of the isolated adducts yields a unique powder pattern, which is completely non-equivalent to the pattern of DLA itself. Given the definitive nature of powder X-ray diffraction, this finding demonstrates the existence of a unique crystal structure for each adduct species.

3.3. Thermal analysis

The DSC thermogram of the DLA reagent consists of a dehydration endotherm at 102°C, and a decomposition exotherm at 178°C [8].

Table 1
Differential scanning calorimetry analysis of the adducts formed by dilituric acid with aliphatic phenylalkylamines

Phenylalkylamine	Observed maximum for the low-temperature DSC endotherm (°)	Observed maximum for the high-temperature DSC endotherm (°)
None	102	178
Aniline	155	261
Benzylamine	Not observed	273
Phenethylamine	95	262
3-Phenyl-1-propylamine	75	261
4-Phenyl-1-butylamine	94	268

These assignments are supported by the TG data, which featured a 23.5% weight loss by 150°C and a further loss of 37.9% by 200°C. The first weight loss was assigned to the loss of lattice water, and correlates well with the theoretical weight loss of 23.9% for a trihydrate phase. The second weight lost is certainly associated with the exothermic decomposition of the compound, which was observed to take place without the observation of a melting endotherm.

A summary of the thermal events noted during the performance of differential scanning calorimetry thermal analysis on the phenylalkylamine derivatives of dilituric acid is provided in Table 1. Most of the adducts yielded low temperature endotherms around 100°C attributable to loss of water, although the dehydration of the DLA–AN isolate did not occur until substantially higher temperatures. The exothermic decomposition of each adduct species took place above 250°C, which represents a substantial increase relative to that noted for the free DLA reagent.

The solvation state of each adduct was established using thermogravimetry and Karl Fisher titration, and the results of this work are found in Table 2. In most cases, application of the two methods yielded equivalent results, and a facile deduction of the hydration state. The DLA–AN adduct was determined to be a hemihydrate, the DLA–PEA adduct to be a monohydrate, the DLA–PBA adduct to be a dihydrate, and the DLA–BA adduct to be an anhydrate.

The thermal analysis of the DLA–PPA adduct could not be interpreted without using the results of the Karl Fisher titration. While the TG thermogram indicated a total volatile content of 50.5%, the Karl Fisher titration result revealed that the water content was actually 7.7%. This latter finding indicated that DLA–PPA adduct was obtained as a sesquihydrate, since the theoret-

Table 2
Thermogravimetric analysis and Karl Fisher titration of the adducts formed by dilituric acid with aliphatic phenylalkylamines

Phenylalkylamine	Total volatile content by thermogravimetry (%)	Water content by Karl Fisher titration (%)	Theoretical volatile content for the *n*-hydrate species	Hydration state of solvate formed
None	23.8	23.9	23.9	Trihydrate
Aniline	3.7	3.3	3.28	Hemihydrate
Benzylamine	0.6	0.3	0.0	Anhydrate
Phenethylamine	5.5	5.8	5.79	Monohydrate
3-Phenyl-1-propylamine	50.5	7.7	7.76	Sesquihydrate
4-Phenyl-1-butylamine	9.8	10.1	10.08	Dihydrate

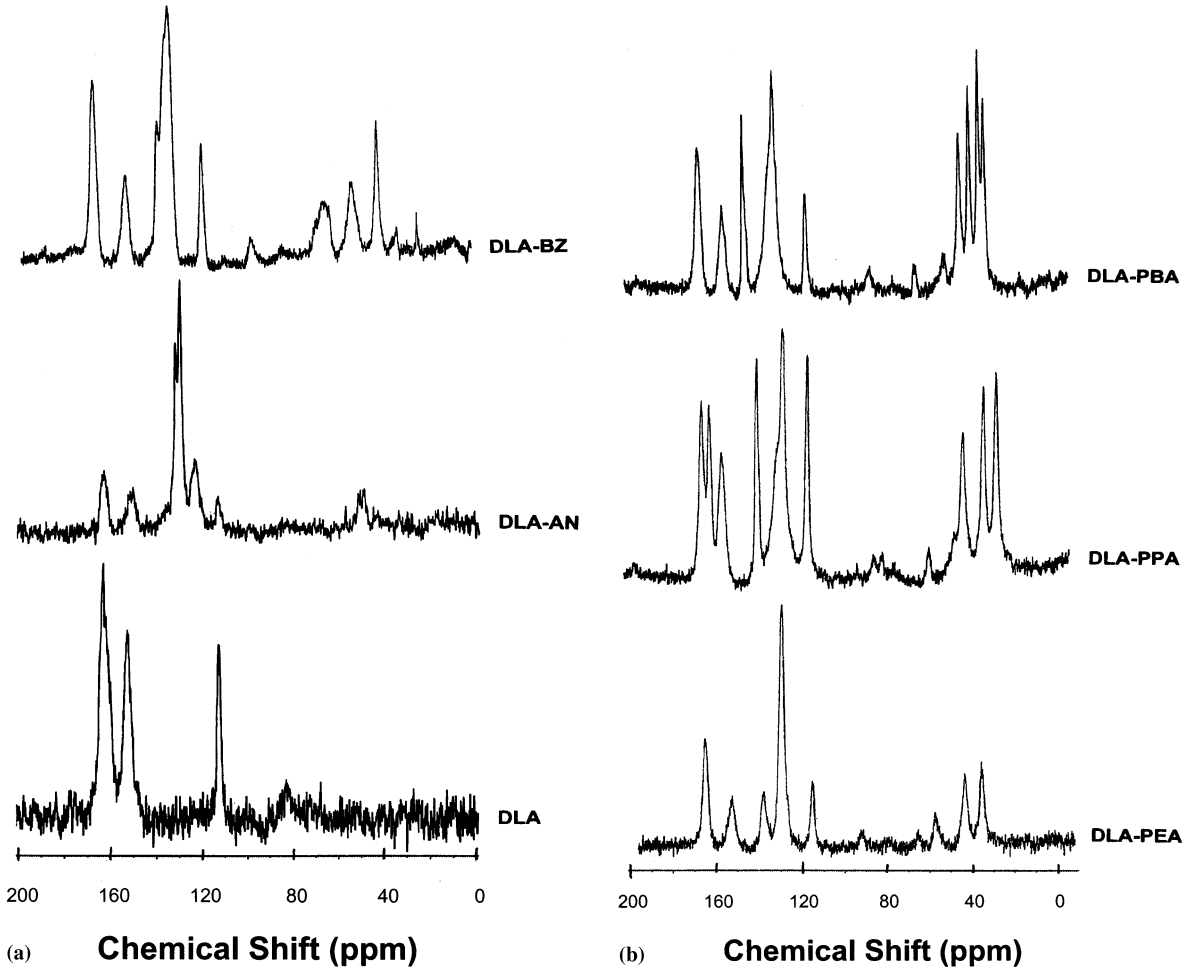


Fig. 4. Solid-state ^{13}C NMR spectra obtained for dilituric acid (DLA), as well as the spectra of the products formed by dilituric acid with aniline (DLA–AN), benzylamine (DLA–BA), phenethylamine (DLA–PEA), 3-phenyl-1-propylamine (DLA–PPA), and 4-phenyl-1-butylamine (DLA–PBA).

ical water content of DLA–PPA·sesquihydrate would be 7.76%. The difference between the TG volatile content and the water titration result was then calculated as 42.8%, which agrees excellently with the theoretical amine content of 42.84% in DLA–PPA·sesquihydrate. Apparently, both the water of hydration and the amine included in the solid adduct can be evolved through heating.

3.4. Solid-state ^{13}C NMR

As illustrated in Fig. 4, the solid-state ^{13}C NMR spectrum of dilituric acid consists of three resonances observed at 112.7, 152.4 and 163.1 ppm, the assignment of which was made in the preceding work [8]. Following the numbering system of Fig. 1, the resonance at 112.7 ppm is assigned to carbon-5′, the resonance at 152.4 ppm is assigned to carbon-2′, and the resonance at 163.1 ppm is assigned to the magnetically equivalent nuclei at carbon-4′ and carbon-6′. These assignments were based on conclusions deduced from solution-phase studies, where it was concluded that C-5′ resonated at 112.9 ppm, C-2′ at 149.9 ppm, and the magnetically equivalent C-4′/C-6′ pair at 159.6 ppm [10].

Table 3
Solid-state ^{13}C NMR bands obtained for the carbon nuclei of dilituric acid in its adducts with aliphatic phenylalkylamines

Cation	Chemical shift, carbon-5′ (ppm)	Chemical shift, carbon-2′ (ppm)	Chemical shift, carbons-4′,6′ (ppm)
None	112.7	152.4	163.1
Aniline	114.4	151.7	163.5
Benzylamine	115.0	150.8	166.0
Phenethylamine	115.2	153.3	165.2
3-Phenyl-1-propyl-amine	114.8	155.1	160.6/164.4
4-Phenyl-1-butyl-amine	114.4	153.4	164.8

The solid-state ^{13}C NMR spectra of the adduct species formed between the aliphatic phenylalkylamines and dilituric acid (also found in Fig. 4) consisted of three resonance bands attributable to the dilituric moiety and additional peaks attributable to the phenylalkylamine moiety. In all cases, the dilituric peaks were assignable through a comparison with the spectrum of the free DLA reagent, while the phenylalkylamine peaks could be assigned on the basis of known solution phase spectra [11] and the established assignments for these [12].

A summary of the dilituric resonance bands is collected in Table 3. As had been previously noted for the cation adducts of dilituric acid [8], the resonance positions of carbon-2′ were not significantly shifted upon formation of the adducts, remaining essentially invariant around 152 ppm. In addition, the magnetically equivalent nuclei at carbon-4′ and carbon-6′ were found to barely differ from the analogous resonances of the DLA reagent itself. The only exception to this latter trend was noted for the DLA–PPA adduct, where the C4′/C6′ band was split into a doublet analogous to the situation noted for the Sr(II) adduct of DLA [8]. In that case, the splitting suggested that the DLA-Sr adduct crystallized with more than one molecule per unit cell, and an equivalent explanation is suggested for the DLA–PPA adduct.

All of the adducts formed between dilituric acid and the phenylalkylamines exhibited a definite downfield shift of the resonance associated with carbon-5′ (the carbon containing the nitro group), as had been previously noted for the adducts between DLA and group IA and IIA cations [8]. These NMR results indicate that the bonding between the dilurate moiety and the phenylalkylamines also originates with the oxygens of the nitro group at the 5′-position, and not with the ketone oxygens of the barbiturate ring.

Table 4 contains the chemical shifts observed for the carbon atoms of the phenylalkylamine substrates in their dilurate adducts, as well as the chemical shifts of the free amines in the solution phase. In every case, the chemically equivalent carbon-3/carbon-5 and carbon-2/carbon-6 nuclei pairs were found to resonate at essentially the same chemical shift values, indicating little perturbation by the adduct formation. However, the chemical shifts of carbon-1 and carbon-4 were found to increase upon adduct formation, suggesting potential sites of interaction with the dilurate reagent.

On the aliphatic sidechain of the amine substrates, only the carbons adjacent to carbon-1 of the aromatic ring yielded solid-state resonance bands which differed between the solution phase and the solid state. This finding provides additional support for the conclusion that the dilurate reagent interacts primarily with the portion of the aromatic ring most polarized by the aliphatic amine substrate. The observation that carbon-7 of the DLA–PEA, DLA–PPA, and DLA–PBA adducts (as well as carbon-8 of the DLA–PBA adduct) undergoes essentially no change in chemical shift upon formation of the solid adducts suggests that the terminal amine group is not involved in an interaction with the dilurate reagent. This would leave the amine

Table 4
Solid-state ^{13}C NMR bands obtained for the carbon nuclei of the phenylalkylamine substrates in their adducts with dilituric acid

Phenylalkylamine substrate	Carbon number	Solution-phase chemical shift [11] (ppm)	Solid-state chemical shift (ppm)
Aniline	C-1	146.35	151.7
	C-3, C-5	129.19	130.0, 131.6
	C-4	118.39	123.4
	C-2, C-6	115.01	113.4
Benzylamine	C-1	143.30	139.4
	C-3, C-5	128.42	131.7
	C-2, C-6	126.96	
	C-4	126.65	
	C-7	46.47	35.2
Phenethylamine	C-1	139.8	138.1
	C-2, C-6	128.76	129.6
	C-3, C-5	128.35	
	C-4	126.05	
	C-7	43.60	43.6
	C-8	40.16	35.6
3-Phenyl-1-propylamine	C-1	142.08	138.5
	C-2, C-6	128.28	126.7
	C-3, C-5	128.25	
	C-4	125.67	
	C-7	41.78	41.5
	C-9	35.43	32.2
	C-8	33.24	25.8
4-Phenyl-1-butylamine	C-1	142.40	144.5
	C-2, C-6	128.31	130.1
	C-3, C-5	128.18	
	C-4	125.61	
	C-7	42.11	42.4
	C-10	35.74	38.1
	C-8	33.47	33.4
	C-9	28.73	30.5

group free to interact with lattice water, thereby promoting new packing patterns for the crystalline solids. Hence, one finds that the DLA–PEA adduct is obtained as a monohydrate, the DLA–PPA adduct as a sesquihydrate, and DLA–PBA adduct as a dihydrate.

4. Conclusions

As had been deduced in the previous work [8], the variety of morphologies observed for the phenylalkylamine adducts with 5-nitrobarbituric acid (dilituric acid) have been shown to arise from the ability of this system to form differing structural types and/or hydrates upon crystallization. The non-equivalence observed for the powder X-ray diffraction patterns demonstrates that each isolated adduct exhibits a unique crystal structure, which in turn yields differing crystal morphologies. The degree of hydration in the crystalline phenylalkylamine adducts appears to increase as additional carbon atoms are added between the aromatic ring and the terminal amine group of the aliphatic sidechain.

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