THIN-LAYER CHROMATOGRAPHY OF STEREOISOMERIC 2,4-DINITROPHENYLHYDRAZONES OF ALIPHATIC ALDEHYDES

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A number of articles have been published recently on chromatographic methods of separating aliphatic aldehyde 2,4-dinitrophenylhydrazones (2,4-DNP)[1–4], with no mention of encountering stereoisomeric forms. Theoretically, two stereoisomeric forms of each aldehyde 2,4-DNP and unsymmetrical ketone 2,4-DNP are possible. There are reports in the literature of the occurrence of two forms of 2,4-dinitrophenylhydrazones[5–8]. BRYANT[5] reported two distinct crystalline forms of acetaldehyde 2,4-DNP designated as stable and metastable, distinguished mainly by optical crystallographic constants. GORDON and coworkers[6] noted that the 2,4-DNP of normal chain aldehydes and methyl ethyl ketone gave two bands when column chromatography was used to separate them. RAMIREZ AND KIRBY[7] utilized crystallization to obtain the *syn* and *anti* form of 2-bromo-acetophenone 2,4-DNP and proved their difference by melting point studies and spectrographic studies. VAN DUIN[8] separated two forms, designated the α-isomer and the β-isomer, of the 2,4-DNP of α-keto-acid esters by reverse-phase column chromatography, using a nitromethane–silica gel stationary phase and light petroleum ether as a mobile phase.

While developing a method for localization of double bonds in fatty acids by identification of 2,4-DNP derivatives of reduced ozonides by thin-layer chromatography[9], it was noted that two bands always appeared for each aldehyde 2,4-DNP derivative. This communication presents observations on the occurrence of these two bands and evidence that they are stereoisomeric forms of these derivatives.

EXPERIMENTAL

Materials

The aliphatic aldehydes (propanol through nonanal) were obtained from Fluka AG, Switzerland, and were of extremely high purity (> 95 %) as determined by gas–liquid chromatography on a silicone column. The ketones used were older products that had been carefully distilled and stored and were of high purity (> 90 %) by chromatography. The 2,4-DNP were prepared from these products by mixing 0.5 g of the aldehyde or ketone with 0.8 g 2,4-dinitrophenylhydrazine, 4 ml of concentrated sulfuric acid, 50 ml of ethanol and 3 ml of distilled water. The 2,4-DNP were then twice recrystallized from ethanol. Uncorrected melting points were within 2° of values reported in the literature.

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Chromatographic systems

All of the systems used were reverse-phase thin-layer chromatography.

1. Stationary phase: *n*-undecane (applied to plate as 10% *n*-undecane in petroleum ether); moving phase: 75% methanol–25% distilled water; plate coating: Kieselgel G according to STAHL.

2. Stationary phase: silicone oil (petroleum ether); moving phase: 75% methanol–25% distilled water; plate coating Kieselgel G according to STAHL.

3. Stationary phase: 2-phenoxyethanol (absolute ethanol); moving phase: *n*-heptane; plate coating: Kieselgur G. This is the system developed by URBACH[4].

4. Stationary phase: dimethylformamide (absolute ethanol); moving phase: *n*-heptane; plate coating: (a) for separation of homologous series and isomers of aliphatic aldehydes, Kieselgur G, (b) for maximum separation of isomers and much less separation of homologous series, Aluminum Oxide according to STAHL.

Smooth glass plates either 6.66 × 6.66 cm, or 20 × 20 cm were used with the plate coating 0.25 mm or 0.50 mm thick respectively. The plates were allowed to air dry for at least 24 h after pouring. They were impregnated with the stationary phase immediately before use either by dipping into the solution or by allowing the plates to develop in a tank containing 2–3 cm of the stationary phase solution. They were removed, allowed to air dry and all samples applied with a microsyringe. The plates were then developed by the ascending method in regular tanks.

When the developing solution was methanol–water the plates were developed only one time. However, when heptane was the mobile phase, they were developed two or three times.

For routine laboratory study, the spots were observed under ultraviolet light, no detecting agent being required. Spraying with a small amount of 0.2% of 4,5-dichlorofluorescein in 96% ethanol often sharpened the spots and made for better detection of small spots. The plates were sprayed with aqueous 5 *N* sodium hydroxide solution before photographs were taken.

Removal from chromatographic plates

A. When the non-polar stationary phases were used, the spots were scraped from the plates and placed in elution tubes. The material was extracted twice with 2–5 ml of petroleum ether (the diameter of the elution tube and amount of petroleum ether used depended on the amount of material removed from the plate) to remove most of the stationary phase material. The 2,4-DNP was then removed by two extractions with 5 ml of acetone.

B. When the polar solvents were the stationary phase the spots were removed to the elution tube and the 2,4-DNP eluted with two extractions of 5 ml of acetone. Since this also contained all the stationary phase further extraction was necessary to obtain the pure compound. This involved taking the acetone solution to dryness, dissolving the 2,4-DNP in a large volume of petroleum ether, extraction of this with water several times, drying with anhydrous sodium sulfate, filtration and reduction of volume.

RESULTS AND DISCUSSION

When the purified crystalline 2,4-DNP of aliphatic aldehydes were dissolved in

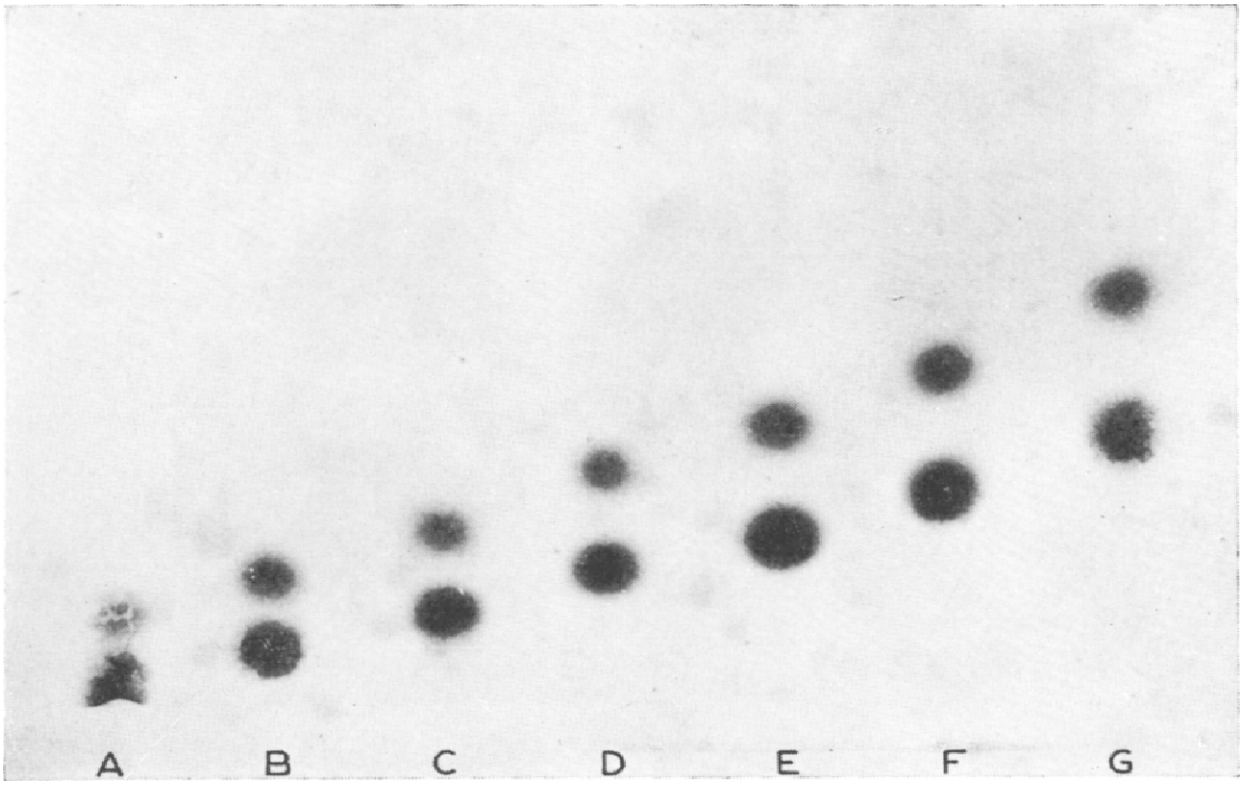

Fig. 1. TLC separation of stereoisomers of aliphatic aldehyde 2,4-DNP using phenoxyethanol on Kieselgur and developing two times with *n*-heptane. The 2,4-DNP were dissolved in chloroform for placing on the plate. A = propanal; B = butanal; C = pentanal; D = hexanal; E = heptanal; F = octanal; G = nonanal. Note the separation of both isomers and homologous series. The *syn* form is the lower form.

chloroform and then separated by thin-layer chromatography in the phenoxyethanol system, each phenylhydrazone showed two spots (Fig. 1). This separation had been observed in three other thin-layer reverse-phase chromatographic systems, consisting of either non-polar stationary phases such as undecane and silicone oil or the polar phase dimethylformamide. For reasons to be discussed later, the dark lower spot (Fig. 1) will be called the *syn* form and the lighter upper spot the *anti* form. When these phenylhydrazones were separated on thin-layer systems with non-polar stationary phases, the short chain aldehyde 2,4-DNP traveled farthest from the origin and the *syn* form traveled further than the *anti*. These two forms were apparently normal products of the reaction to produce the hydrazones from the hydrazine. Direct chromatography of reaction mixtures of chloroform, acetone or petroleum ether extracts of the reaction mixtures showed two forms.

When 2,4-DNP recrystallized several times from ethanol were placed in solution in petroleum ether, heptane, acetone, methanol, or ethanol, only the *syn* form was apparent from chromatography. When the same crystalline material was placed in solution in chloroform, two forms were apparent from chromatography (Fig. 2, D and E). When a small amount of hydrochloric acid was added to an acetone or methanol solution of crystals that were only the *syn* form, racemization took place and the two forms appeared. It is believed that the racemization that took place in the chloroform solution (laboratory distilled chloroform with 1.5% methanol added as a stabilizer) was due to small amounts of hydrochloric acid formed by

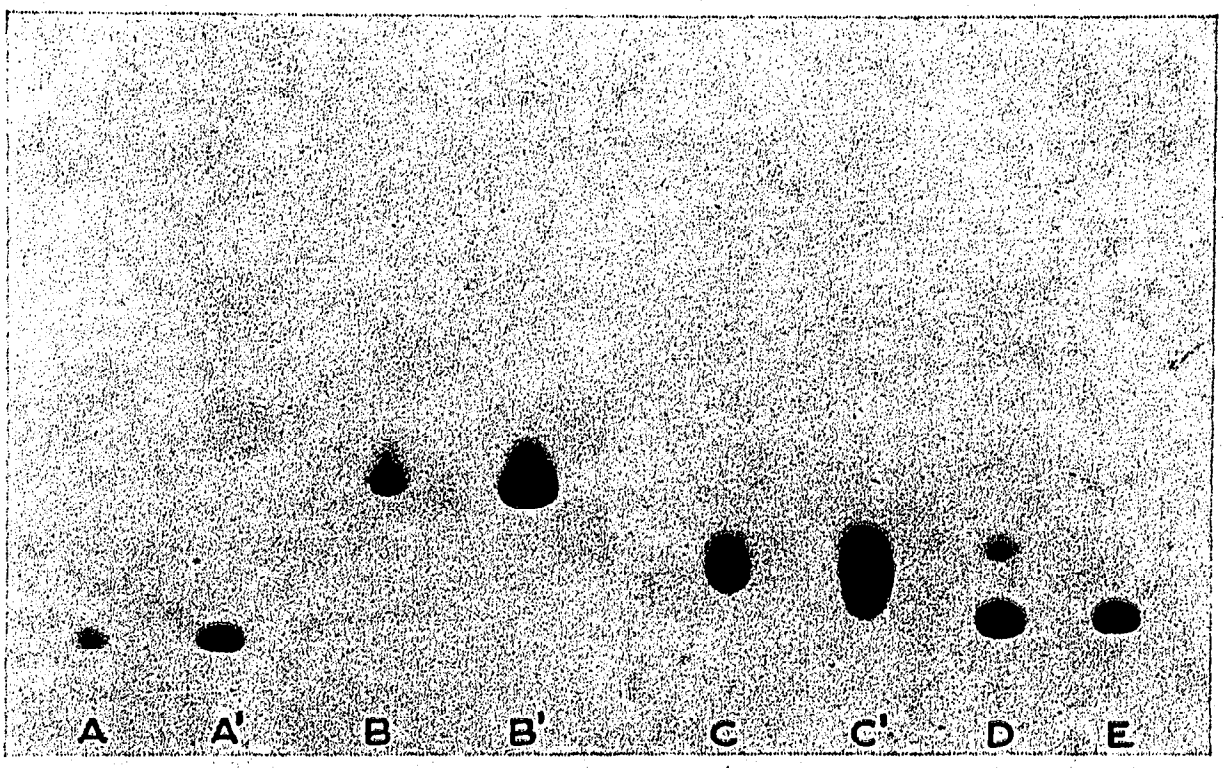

Fig. 2. TLC separations of 2,4-dinitrophenylhydrazones using phenoxyethanol on Kieselgur and developing two times with *n*-heptane. A and A′ = acetone; B and B′ = methyl *n*-amyl ketone; C and C′ = methyl *n*-propyl ketone; D = crystalline 2,4-DNP of pentanal dissolved in chloroform for placing on the plate; E = same as D, but dissolved in acetone for placing on the plate.

chloroform breakdown. No success was obtained in attempts to force the equilibrium so that only the *syn* or *anti* form was produced in a solution that originally contained the two forms. While hydrochloric acid addition caused racemization, this proceeded only to an equilibrium of 75 % *syn* form and 25 % *anti*. Larger amounts of acid or refluxing for several hours with additional acid had no further effect. The addition of sodium hydroxide caused an acetone solution containing both the *syn* and *anti* forms or the *syn* form alone to turn dark purple or black. There was no effect on the equilibrium between the two forms and no conversion of the *syn* form to the *anti* form when it was the only form present. Since VAN DUIN[8] reported that ultraviolet light stimulated the conversion of the *anti* to *syn* (he reported α-isomers to β-isomers), this procedure was subjected to preliminary investigation. These observations were at first interpreted to indicate that a small amount of irradiation decreased the *anti* form. With greater irradiation, it was apparent that destruction was taking place and that both forms were being destroyed. While the ratio remained constant, the decrease in the intensity of the faint spot (*anti* form) was first observed. This gave the false impression that it was being converted to the *syn* form.

The equilibrium of all of the 2,4-DNP of aldehydes tested was constant in chloroform solution (75 % *syn* and 25 % *anti* form). This was not the ratio observed in reaction mixtures where the solvents were primarily ethanol with small amounts of sulfuric acid and water. When these mixes were analyzed directly or when the 2,4-DNP were extracted into petroleum ether and then analyzed, the ratio was approximately 90 % *syn* and 10 % *anti* form.

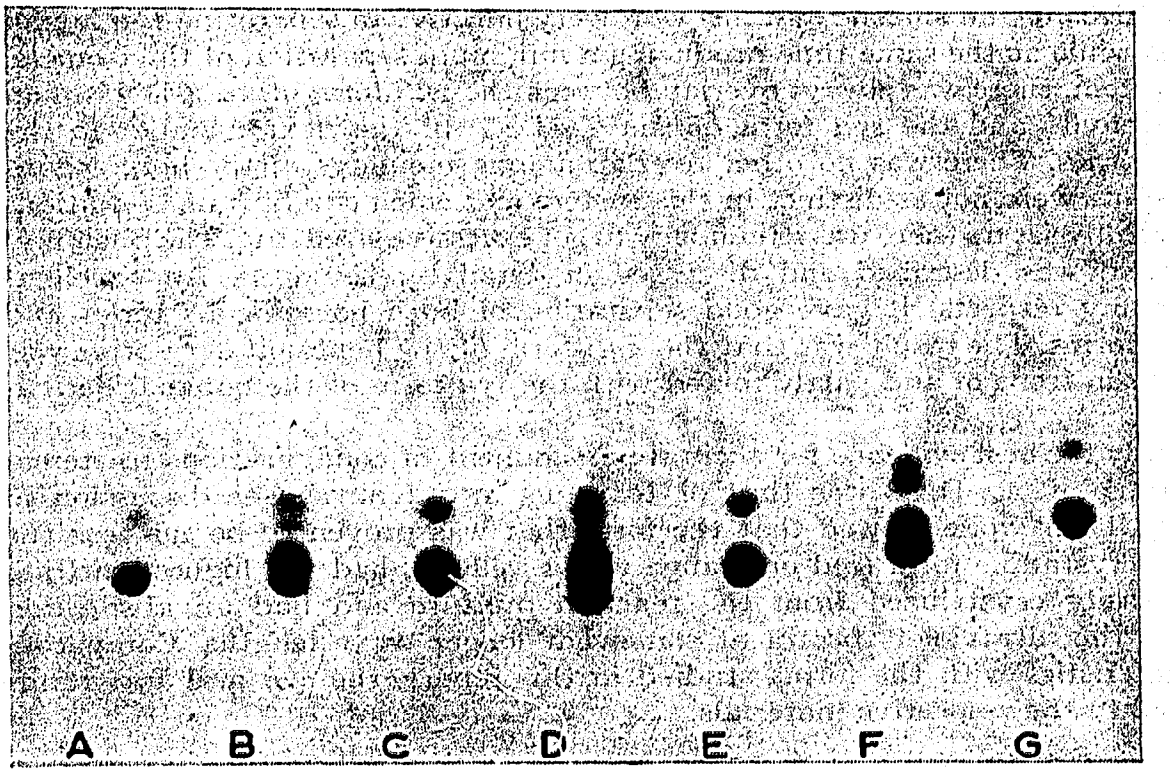

Fig. 3. TLC separation of stereoisomers of some aliphatic aldehyde 2,4-DNP using dimethylformamide as a stationary phase on Aluminum Oxide and developing two times with *n*-heptane. A = pentanal; B = pentanal and heptanal; C = heptanal; D = pentanal, heptanal and nonanal; E = heptanal; F = heptanal and nonanal; G = nonanal. Note the very good separation of isomers, but limited separation of homologous series.

Pure *anti* form 2,4-DNP of a particular aldehyde was easily obtained by elution of the band scraped from the thin-layer plate. The material isolated was a light yellow liquid or liquid-crystal, as contrasted to the *syn* form which was an orange crystal. The material was chromatographically pure and had a slight difference in adsorption maximum from the *syn* form ((352 mμ for the *anti* form of the 2,4-DNP of pentanal as compared to 358 mμ for the *syn* form).

The 2,4-DNPs produced from methyl *n*-amyl ketone also gave two spots when chromatographed, but they did not separate as well as the aliphatic aldehyde derivatives. Since a symmetrical ketone such as dimethyl ketone (acetone) should produce a 2,4-DNP of only one form, this was tested and the results of such a comparison are presented in Fig. 2. It is apparent from this chromatoplate that the 2,4-DNP of acetone gives only one spot, while that of methyl *n*-amyl ketone or methyl *n*-propyl ketone gives two spots.

A chromatographic system was found that gives good separation of the two stereoisomeric forms of aliphatic aldehyde 2,4-DNP while giving very poor separation of the homologous series. This system utilizing dimethylformamide as a stationary phase and heptane as the mobile phase was unique in that if Kieselgur G was used instead of Aluminum Oxide, all other things equal, the system separated both isomers and homologous series equally well. Fig. 3 shows a chromatoplate using this system. This system used in conjunction with the phenoxyethanol system was of value in resolving mixtures of *syn* and *anti* forms of mixtures of aliphatic aldehyde 2,4-DNP.

The undecane and silicone systems were very effective in separating the homologous series while at the same time producing a minimum separation of the isomers. With this system, there was always overlap between the *anti* form of a 2,4-DNP of an aliphatic aldehyde and the *syn* form of the 2,4-DNP of the next member in the homologous series. There was no other overlap among members of the homologous series. The spots were not as distinct in this system and when equilibrium conditions were not carefully set up there was a tendency to get excessive streaking. The phenoxyethanol system was described and discussed in detail by URBACH[4]. An excellent system in many respects, it gave good separation of both homologous series and *syn* and *anti* forms. The result was that the *anti* form of the heptanal derivative was always partly in front of and partly mixed with the *syn* form of the nonanal derivative.

Probably the best evidence for accurate assignment of configurations to stereoisomeric forms of 2,4-DNP was that of RAMIREZ AND KIRBY[7] (see discussion in WHELAND[10]). Their work showed that the form that was proven to be *syn* was the darker color crystalline form (red or orange *versus* yellow), had the highest melting point, was easily crystallized from the reaction mixture and had an absorption maximum in the ultraviolet spectra at somewhat longer wave lengths. Comparing these characteristics with the forms studied in this report, the *syn* and *anti* terms were assigned to the respective materials.

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SUMMARY

The stereoisomeric forms of 2,4-dinitrophenylhydrazones (2,4-DNP) of some aliphatic aldehydes and unsymmetrical ketones were separated by several reverse-phase thin-layer chromatographic procedures. Evidence indicated that the reaction to produce the 2,4-DNP produces both the *syn* and *anti* form, but that repeated recrystallization in ethanol yielded the pure *syn* crystalline form. Racemization of the crystalline *syn* form could be stimulated by dissolving it in chloroform or by the addition of a small amount of hydrochloric acid to an acetone or methanol solution. Certain thin-layer chromatographic systems separated the homologous series to the greatest extent and the isomers the least (undecane/75 % methanol–25 % water). Another system separated the isomers to a considerable extent but was not too effective in separating the homologous series (dimethylformamide/heptane). Combinations of these systems should be of value in resolving mixtures of *syn* and *anti* forms of mixtures of aliphatic aldehyde 2,4-DNP.

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