

REACTION CHROMATOGRAPHY

II. GAS-LIQUID/THIN-LAYER CHROMATOGRAPHIC DERIVATIZATION TECHNIQUE FOR THE IDENTIFICATION OF ALCOHOLS*

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(Received December 16th, 1966)

The preceding paper¹ outlined requirements for successful application of the gas-liquid/thin-layer chromatographic (GLC/TLC) derivatization technique. The procedure was developed for use with compounds containing reactive group functions and successfully applied to carbonyl compounds. Components eluted from the GLC exhaust into appropriate reagents spotted on thin-layer plates form derivatives which then may be chromatographed and removed for subsequent examination.

This work extends the concept to alcohols with the use of 3,5-dinitrobenzoate (DNB) and *o*-nitrophenylurethan (ONPU) derivatives. Thin-layer chromatographic migration behavior of these derivatives on several new systems is tabulated.

EXPERIMENTAL

Reagents and apparatus

The apparatus and methods employed are essentially those reported previously except that a thermometer was wrapped in contact with the GLC exhaust with heating tape to allow exhaust temperature measurement. GLC stationary phases employed to separate the alcohols were Carbowax 4000 and Apiezon L.

3,5-Dinitrobenzoyl chloride and *o*-nitrophenylisocyanate (Eastman Organic Chemicals) were used without purification to prepare reagents as follows:

ONPU. *o*-Nitrophenylisocyanate (10.0 g) was dissolved by heating in 100 ml of benzene redistilled over calcium oxide.

DNB. 3,5-Dinitrobenzoyl chloride (1.0 g) was dissolved in 7.5 ml *p*-xylene and 1.0 ml tetrahydrofuran.

TLC plates were prepared and activated as described previously¹. Additionally, Silica Gel G (SGG) plates were coated with vaseline by immersing in a 5% (w/v) vaseline-heptane solution, removing, and allowing the heptane to evaporate at room temperature for 20 min. Fluorescent SGG plates for DNBS were prepared by adding 200 μ g 2',7'-dichlorofluorescein (Eastman Organic Chemicals) per 20 \times 20 cm plate during adsorbent slurry preparation.

All alcohols used were commercial samples available in our laboratory.

* Part of a thesis submitted by J. P. MINYARD in partial fulfillment of requirements for the Ph.D. degree from Mississippi State University, State College, Mississippi.

Formation of standard derivatives

DNBs and ONPUs were prepared according to SHRINER AND FUSON², except for the terpene ONPUs which were prepared as described by ATTAWAY *et al.*⁴. The DNBs were purified by recrystallization from aqueous alcohol or preparative TLC on system A (see later). The ONPUs were purified by preparative TLC on system B (see later), since many were very slow in crystallizing from the reaction mixture; their authenticity was established by comparing paper chromatographic R_F values with those reported by ATTAWAY *et al.*^{3,4}.

Formation of derivatives from gas chromatograph

The reagent spotting and GLC derivatization were accomplished as before¹. In some instances, a 20-gauge hypodermic needle 1 cm long was attached to the Luer-Lok GLC exhaust port to cool the emerging compounds for better derivatization.

Control of the exhaust port temperature for optimum derivatization was necessary with the DNBs. Primary and all terpene alcohols investigated reacted better at an exhaust temperature of 185° with the needle off; the secondary alcohols gave much better yields at 155° with the needle on. ONPUs were prepared with the needle off and the outlet at 185°.

After formation of the DNBs, excess reagent was hydrolyzed and fixed at the TLC plate starting line by adding one or two drops of 10 % aqueous sodium hydroxide to each spot. The ONPUs ran well ahead of the unexpended reagent on system B (see later) so no post-reaction treatment before TLC was necessary.

Thin layer chromatography

Samples spotted 1.5 cm from the bottom edge of the plate and 2 cm apart were developed to a height of 10 cm in solvent vapor equilibrated tanks. Systems used to separate the derivatives were:

(A) Benzene-petroleum ether (50-68°) (1:1); solid adsorbent—SGG with 200 μg /plate of 2',7'-dichlorofluorescein incorporated.

(B) Heptane-acetone (4:1); solid adsorbent—SGG.

(C) Methanol-water (9:1); solid adsorbent—polyamide powder.

(D) Methanol-water (95:5); solid adsorbent—SGG coated with 5 % vaseline.

The DNBs appeared as light pink spots on the fluorescent plates (system A) by daylight and as dark purple absorbing spots on a yellow green background under U.V. light. On polyamide plates, they were initially colorless by daylight but dark purple U.V. absorbers. If the polyamide plates were exposed to U.V. light for more than about 2 min, the DNBs changed appearance and began to fluoresce deep fuchsia. Afterwards, they were clearly visible by daylight as light violet spots, the color persisting for several hours but gradually fading. The ONPUs were visible as yellow spots on all systems and even more distinct as strong absorbers under U.V. radiation.

Test mixture (Desaga, Heidelberg) was spotted on each plate and R_F values were calculated relative to Butter Yellow for adjacent standard and GLC derivatives.

All reactions were performed on SGG plates. The DNBs were developed in system A and the ONPUs in system B, with reagent blanks run on each derivative plate. The derivative spot from each sample was scraped from the plate and eluted with methylal. The DNBs then were spotted and run on systems A and C, the ONPUs on systems B, C, and D, and R_Y values obtained.

RESULTS AND DISCUSSION

The hydrolysis of unexpended DNB reagent with aqueous alkali illustrates the requirement mentioned in the previous paper¹, *i.e.*, the derivative should be separable from unexpended reagent by some TLC system. This principle should be applicable generally when large quantities of unused reagent overload the TLC adsorbent and mask the derivative spot upon development. This was the case with the DNBs as well as with the α -naphthylurethans which were examined initially. In the latter case, derivatization was successful, but since all the derivatives had nearly the same R_F values in all TLC systems tried, the work was terminated. The use of mildly acidic or basic aqueous solutions was preferred for the hydrolysis since the salts formed were unremoved by the organic developing solvents.

Certain secondary and tertiary alcohols, particularly some of the terpenoids, are quite sensitive to thermal or acid catalyzed rearrangements and dehydrations. The reaction conditions for DNB formation (localized HCl production plus elevated temperature) may be too severe for such sensitive compounds, especially since non-deactivated silica gel has been shown to be an effective catalyst for terpenoid rearrangements⁵. However, the ONPUs seem to form without any major attendant rearrangements or degradation. Whenever the identity of the GLC prepared derivative is uncertain due to possible rearrangements, standard and unknown derivatives should be prepared in an identical manner by GLC/TLC for comparison.

The ONPUs and DNBs prepared by GLC/TLC agreed in R_F values in all systems with the standards ($\pm 3\%$ relative).

tert.-Butyl and *tert.*-amyl alcohols, the only two nonterpenoid tertiary compounds tested, did not form DNB or ONPU derivatives from the gas chromatograph at the 10 μ l level, either because of lowered reactivity or degradation occurring in the GLC separation. The yields of derivatives from linalool and α -terpineol by GLC/TLC also were considerably smaller than those from secondary and primary alcohols. Thus this technique may have limited use in identification of tertiary alcohols.

DNBs of primary and secondary alcohols were formed in detectable amounts from 0.5 mg or slightly less of compound. Primary alcohols consistently gave better yields than secondary alcohols. Tertiary alcohols which formed DNBs did so only at the 5 mg or higher level. Primary and secondary alcohols formed ONPUs from 0.05 mg of sample, but the tertiary terpene alcohols required a minimum of 0.5 mg to give detectable spots. Primary alcohols formed ONPUs in better yields than secondary alcohols. The ONPUs thus seem to be excellent derivatives for primary, secondary, and some tertiary alcohols.

Tables I and II give relative TLC behavior of representative alcohols in several systems. All values reported for a given system and derivative type were obtained from runs of all samples on single plates and thus are internally consistent.

DNB R_F values in two systems are listed in Table I. Values for the derivatization system (A) were calculated from plates on which the derivative had been respotted and developed. Because unhydrolyzed 3,5-dinitrobenzoyl chloride (or the acid) streaked from the starting line up to about R_F 2.0, much above all the derivatives, the alkaline hydrolysis postreaction step was necessary. All the DNBs ran well ahead of the hydrolyzed reagent, which stayed at the starting line.

The DNBs can be visualized with a 1% ethanolic α -naphthylamine spray⁶, but

TABLE I

TLC R_F ^a VALUES OF 3,5-DINITROBENZOATES (DNB)

Solvent systems: (A) Silica Gel G with 2',7'-dichlorofluorescein; benzene-petroleum ether (38-50°) (1:1). (C) Polyamide; methanol-water (9:1).

DNB Compound	R_F	
	A	C
1-Propanol	0.68	2.01
1-Butanol	0.82	1.90
1-Pentanol	0.96	1.71
1-Hexanol	1.04	1.57
1-Heptanol	1.08	1.42
1-Octanol	1.15	1.28
1-Nonanol	1.19	1.17
1-Decanol	1.24	1.03
1-Undecanol	1.28	0.91
1-Dodecanol	1.35	0.78
2-Propanol	0.79	2.08
2-Butanol	0.92	2.02
2-Pentanol	1.05	1.89
2-Heptanol	1.15	1.77
2-Octanol	1.23	1.54
3-Pentanol	1.05	1.91
4-Heptanol	1.42	1.72
2-Methyl-1-butanol	1.01	1.76
3-Methyl-1-butanol	0.96	1.84
3-Hexen-1-ol	0.94	1.73
Phenylethyl alcohol	0.61	1.54
Cinnamyl alcohol	0.66	1.31
α -Terpineol	1.15	1.17
Geraniol	1.04	1.44
Citronellol	1.08	1.40
Linalool	1.07	1.46
Menthol	1.46	1.44

^a R_F is travel ratio of unknown to Butter Yellow dye.

sprayed derivatives run at slightly low R_F values when rechromatographed in the same or other systems. The 2',7'-dichlorofluorescein dye from system A plates did not affect the TLC behavior of recovered DNBs on rechromatography.

Other TLC systems^{6,7} and paper chromatographic systems⁸⁻¹¹ have been recommended for DNB separations. GALETTO, KEPNER AND WEBB recently reported successful GLC of DNBs on an SE-30 column¹².

Table II gives TLC R_F values for ONPUs of representative alcohols on systems B, C, and D. TLC of ONPUs has not been reported previously, to our knowledge. Again, system B (reaction system) values were obtained by rechromatography of the derivatives removed from the reaction plate. Because the derivatives run with nearly the same R_F values and because of its handling properties during derivatization and elution, system B is desirable for the initial formation of ONPU's but is of little use for distinguishing between alcohols.

Urethan derivatives appear to form readily by GLC/TLC, especially if activating *ortho* or *para* electron withdrawing groups are present in the aryl isocyanate. The structure of the residue attached to the isocyanate function appears to have a large influence on the TLC separability of the urethans formed, judging by our experience

TABLE II

TLC R_f ^a VALUES OF *o*-NITROPHENYLURETHANS (ONPU)

Solvent systems: (B) Silica Gel G; heptane-acetone (4:1). (C) Polyamide; methanol-water (9:1). (D) Silica Gel G coated with 5% vaseline; methanol-water (95:5).

ONPU Compound	R_f		
	B	C	D
1-Propanol	0.98	2.05	1.19
1-Butanol	1.08	1.94	1.11
1-Pentanol	1.21	1.80	1.04
1-Hexanol	1.21	1.61	1.00
1-Heptanol	1.23	1.45	0.91
1-Octanol	1.28	1.30	0.84
1-Nonanol	1.30	1.12	0.75
1-Decanol	1.30	1.00	0.66
1-Undecanol	1.30	0.87	0.52
1-Dodecanol	1.30	0.75	0.41
2-Propanol	1.06	2.30	1.26
2-Butanol	1.09	2.22	1.19
2-Pentanol	1.15	2.16	1.07
2-Hexanol	1.23	1.86	1.05
2-Heptanol	1.25	1.70	0.98
2-Octanol	1.31	1.54	0.86
4-Heptanol	1.27	1.89	0.93
2-Methyl-1-butanol	1.14	1.96	1.10
3-Methyl-1-butanol	1.16	1.82	1.13
3-Hexen-1-ol	1.28	1.76	1.02
Phenylethyl alcohol	0.89	1.70	1.19
α -Terpineol	1.23	1.35	0.84
Geraniol	1.20	1.38	0.73
Citronellol	1.27	1.34	0.63
Linalool	1.17	1.55	0.88
Menthol	1.28	1.35	0.72
Fenchyl alcohol	1.45	1.35	0.88

^a R_f is travel ratio of unknown to Butter Yellow dye.

with the α -naphthylurethans and the work of ATTAWAY *et al.*^{3,4}. *p*-Phenylazophenyl-, *m*-nitrophenyl-, and *p*-nitrophenylurethans also might provide effective GLC/TLC derivatives. Traces of water present a major difficulty in the formation of urethans in test tube reactions, but this can be avoided nicely by the GLC/TLC technique if the SGG plates are used immediately after activation and if the reagent solvent is carefully dried. Urethans also avoid the possibly detrimental effect of acidic reagents in terpenoid derivatizations.

ACKNOWLEDGEMENTS

We thank Mrs. MODERIA PETTY and Mrs. LAVENIA MILES for technical assistance.

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SUMMARY

The technique for the identification of reactive compounds by derivative formation on thin-layer plates at the exhaust of a gas chromatograph (gas-liquid/thin-layer chromatography) was applied to alcohols. Advantages, limitations, and reaction conditions for 3,5-dinitrobenzoates (DNBs) and *o*-nitrophenylurethans (ONPUs) as derivatives of representative alcohols used in this study are discussed and thin-layer chromatographic behavior in several systems is tabulated. DNBs are readily formed from 0.5 mg and ONPUs from 0.05 mg of the same primary and secondary alcohols. Some tertiary alcohols failed to give either derivative from 10 mg of reactant, while 0.5-5 mg of others reacted discernibly.

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