

Examples of differently reacting steroids

Some examples of the use of the above location reagents are given in Table I.

Note added in proof

The tetrazolium reagent is also effective on silica gel, provided that an 8% (2 N) solution of sodium hydroxide is used to ensure alkaline conditions on the adsorbent.

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Reverse phase thin-layer chromatography of 2,4-dinitrophenylhydrazones of *n*-alkanals and *n*-alkan-2-ones*

URBACH¹ of C.S.I.R.O. (Melbourne) has recently published a comprehensive thin-layer chromatographic (TLC) study of the 2,4-dinitrophenylhydrazones (DNPH's) of the aliphatic monocarbonyls. The author briefly reviewed the pertinent literature, and presented methods for both class and homolog separations. Our purpose in this communication is to briefly describe an alternate method for the separation by TLC of an homologous series of the DNPH's of aliphatic aldehydes and ketones. In URBACH's system, chain-length separation is achieved on Kieselguhr G plates impregnated with 2-phenoxyethanol; the impregnated plates are spotted with derivatives, and developed several times with 4% diethyl ether in light petroleum. Resolution of the DNPH's of C₁-C₁₄ *n*-alkanals and C₃-C₁₃ *n*-alkan-2-ones was achieved; the higher members of the series moved the fastest.

In our method we have adapted the KLEIN AND DE JONG² paper chromatographic procedure for TLC. Glass plates 5 × 20 cm were coated with a 250 μ layer of silica gel G using the Brinkmann apparatus. After allowing about 15 min for the adsorbent to set, the plates were placed in an oven and heated at 110° for at least 1 h. The plates were then cooled to room temperature and very slowly immersed in petroleum ether (boiling range 30-60°) containing 10% (v/v) of Shell Ondina 27 mineral oil. After impregnation, the petroleum ether was allowed to evaporate at room temperature, and the plates were then spotted. The plates were given a single development with

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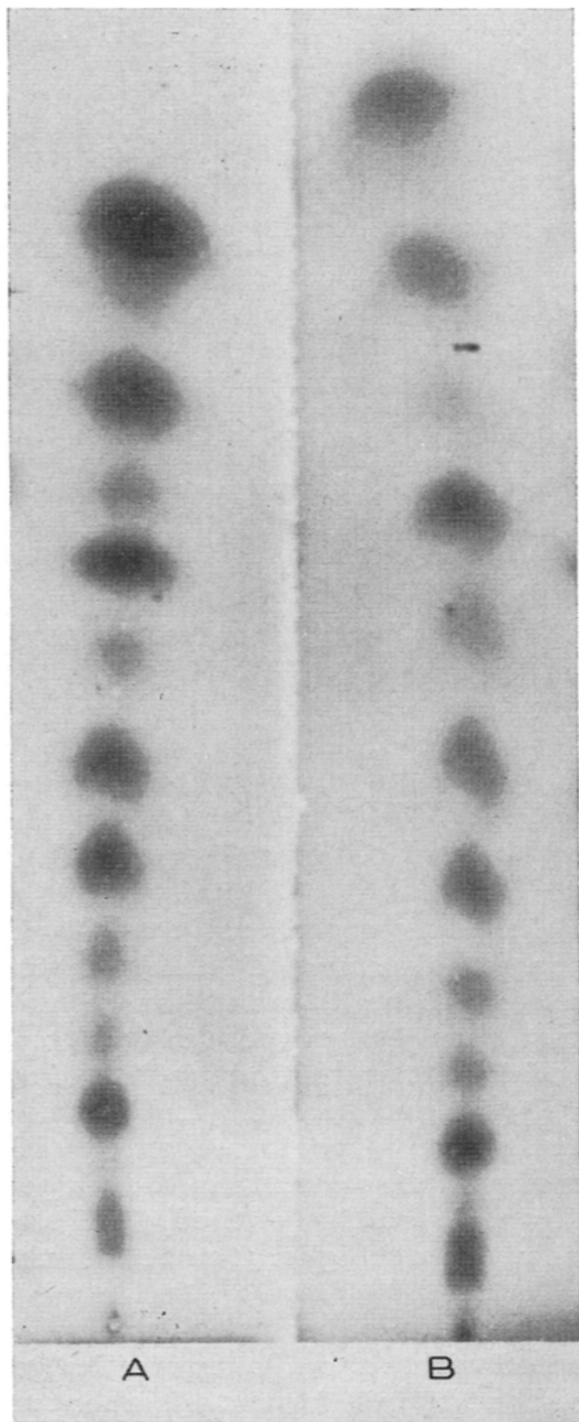


Fig. 1. TLC of C_1 - C_{14} *n*-alkanal DNPH's (A and B are from two different runs). Developed for 7 h with dioxane-water (65:35, v/v) using the ordinary TLC cylinder as a chromatographic chamber. The C_1 derivative moved the fastest, the C_{14} the slowest.

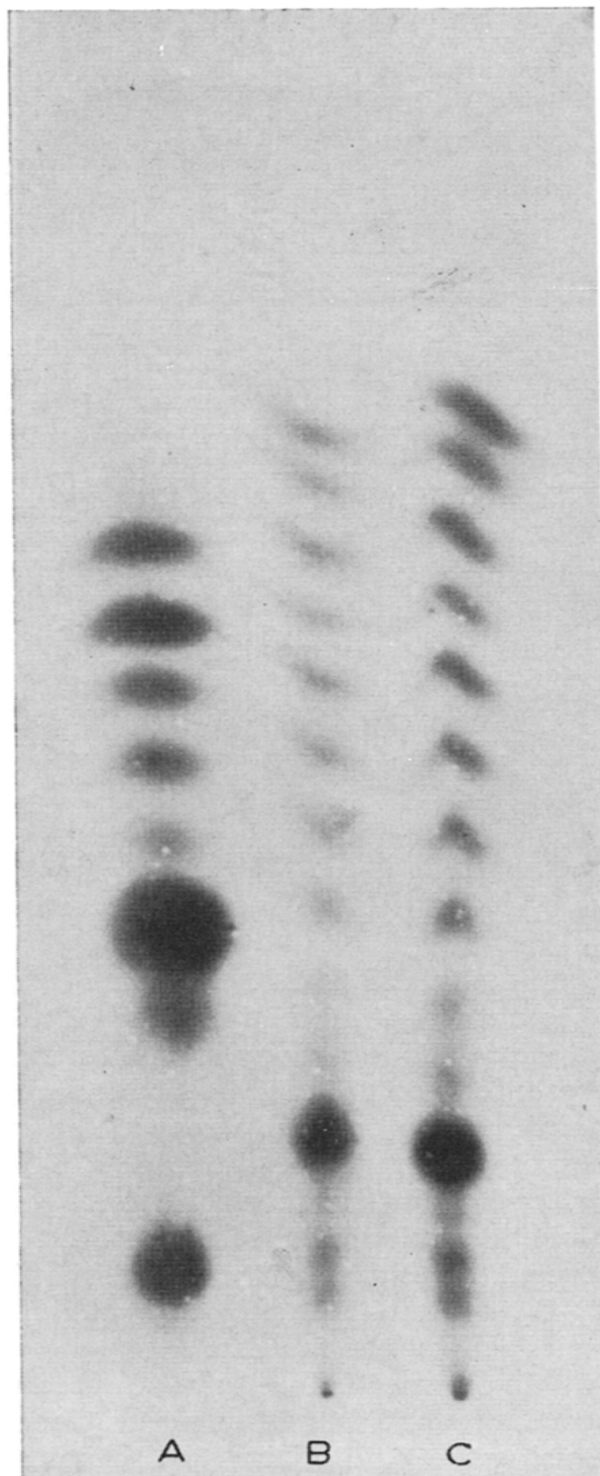


Fig. 2. TLC using "continuous development". Developed for 7 h with dioxane-water (65:35, v/v) in a 1000 ml beaker covered with Saran film; 3-4 cm of the top of the plate was exposed to the atmosphere through a slit in the film. (A) C_3 - $C_{9,13}$ *n*-alkan-2-one DNPH's. (B) and (C) C_1 - C_{14} *n*-alkanal DNPH's.

dioxane-water (65:35, v/v). A single development took about 6 h at 25°; multiple development, in this particular system, did not result in further resolution. Fig. 1 shows a photograph, taken under U.V. light, of the TLC of the C₁-C₁₄ *n*-alkanal DNPH's using the technique described. We have observed that the best separation is achieved with the middle members of the C₁-C₁₄ series; the C₁ and C₂ derivatives tend to run together, and the C₁₃ and C₁₄ derivatives are often not well separated.

Although multiple development did not enhance resolution, we have used what we call "continuous development" to increase resolution. In this technique the derivatives are spotted into the impregnated plates, and the plates are developed with the dioxane-water system with 3-4 cm of the top of the plates exposed to the atmosphere. This is conveniently done in a Saran*-covered 1000 ml beaker, with a slit cut in the Saran film for the plate. Using this technique the slow and medium-mobility fractions are usually well resolved. Overdevelopment, however, can cause the "piling-up" of the fast-moving fractions at the top of the plate. Fig. 2 shows a plate that had been run with "continuous development".

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* Saran is a trade name for polyvinylidene chloride.

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Thin-layer chromatography of tetra- and pentacyclic triterpenes

Thin-layer chromatography has occasionally been applied to the separation of triterpenes, e.g. by TSCHESCHE^{1,2} to those of *Bredemeyera floribunda*, by THOMAS³ to those of *Commiphora glandulosa* and by HUNECK⁴ to those of *Sorbus torminalis*.

In experiments with Israeli peat, which will be reported elsewhere, we have developed a system that proved useful in the separation of triterpenoid compounds and permitted their easy identification. The solvent mixture used was heptane-benzene-ethanol (50:50:0.5), applied to alumina G. This mixture has the advantage that an increase in the alcohol concentration increases and a decrease in its concentration decreases the rate of migration. For example, the R_F values for betulin (No. 3) are 0, 0.14, 0.73 for 0%, 0.5% and 2% alcohol, for lupeol (No. 10) 0.16, 0.37, 0.94 for the same three alcohol concentrations.

A systematic study has given the following results, which will be extended by further investigations: *epi*- β -Amyrin (No. 15) and *epi*-lupeol (14) can be separated from their diastereoisomers β -amyrin (No. 6) and lupeol (10); the *epi*-compounds have higher R_F values.

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