Boiling points of cyclohexane, cyclohexene and benzene are similar and differences in their net retention volumes have been shown to be indicative of their degrees of interaction with the column adsorbents[6]. Chromatograms obtained for a mixture of these compounds are shown in Fig. 1. An additional double bond in 1,4-hexadiene compared to 1-hexene causes greater retention for the former compound on the columns in Table I, even though they possess similar boiling points.

Interaction of adsorbents with unsaturated compounds decreases in the order Ag-2-pyr > Ag-pyr > Ag-4-pyr. The placement of the Ag-3-pyr complex coated support in the above order is difficult due to inconsistancies in retention volumes observed for this column. The presence of a third ligand molecule in this complex may be the cause for this effect. Attempts to remove a single molecule of ligand from this complex failed.

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Reaction thin-layer chromatography: the reduction of tropinone

Although Willstatter first synthesized tropinone, SCHOPF AND LEHMANN[1] prepared tropinone under "physiological conditions"—suggesting the biogenetic synthesis of tropinone and tropane alkaloids. Tropine and pseudotropine, the reduction products of tropinone, have been found in the roots and leaves of numerous plant species[2–5]. They often appear as the undesirable hydrolysis products of their esters, which are physiologically more significant. The hydrolysis of atropine, for example, yields tropine, while the hydrolysis of tropacocaine yields pseudotropine. The modes of action of these epimers and their derivatives on mammals have been studied[6], adding to the total picture of stereospecificity in pharmacological response.

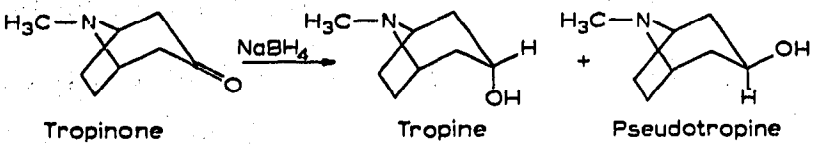
The stereochemical configurations of tropine and pseudotropine have been established[7]; namely that in pseudotropine the hydroxyl group is *cis* to the methylimino bridge, while in tropine these groups are *trans*.

The reduction of tropinone on the macro level has been studied under various conditions. SCHMIDT *et al.*[8] hydrogenated over Raney nickel, FRIESS *et al.*[6] hydrogenated over platinum, and BECKETT *et al.*[9] used borohydrides.

The products of reduction, tropine and pseudotropine, have also been separated

and estimated by a variety of laborious procedures: gold salt formation[10], electrophoresis[11–13], gas chromatography[14], fractional distillation under reduced pressure[6], infrared spectrophotometry[5,9], column chromatography[5], paper chromatography[5,13], thin-layer chromatography[5], and esterification with tigloyl chloride[3]. Acetone–ammonium hydroxide (97:3) had been used earlier[15] as a general developing solvent for the thin-layer chromatographic separation of tropane alkaloids. By altering the ratio of the solvents to 80:20, tropinone and its reduction products can be separated satisfactorily within 30 min.

The reduction of tropinone may be represented by the following equation:



Tropinone Tropine Pseudotropine

Experimental

Equipment and chemicals

Thin-layer plates 20 × 20 cm, coated with a 0.25 mm layer of Silica Gel G using the Desaga–Brinkmann Applicator (from Brinkmann Instruments, Inc., Westbury, N.Y.), activated at 120° for 2 h.

Chromatography tank. 21 × 10 × 22 cm (Chromaflex K-41615 from Kontes, Vineland, N.J.).

Sodium borohydride. From Fisher Scientific Co., Fair Lawn, N.J.

Dragendorff reagent[15].

Test compounds. Tropine (from K and K Labs., Inc., Plainview, N.Y.); pseudotropine, tropinone and *l*-hyoscyamine (from Sandoz Ltd., Basle, Switzerland).

Procedure

Reduction. Using a micropipet, 50 μg of tropinone and of *l*-hyoscyamine, dissolved in chloroform, were spotted at positions 1 and 5, respectively, on a thin-layer plate. The plate was placed in a spraying chamber in a well-ventilated hood and covered with a sheet of absorbent paper (Whatman No. 120 Drop Reaction Paper) in such a manner that only the spotting area was exposed. This area was then sprayed thoroughly with an 0.8% aqueous solution of sodium borohydride. The plate was placed in a circulating-air oven, at 50°, for 15 min. It was then placed in a desiccator, containing silica gel, for 15 min. Ten micrograms of the test compounds—tropine, pseudotropine, tropinone and *l*-hyoscyamine (dissolved in chloroform)—were applied to positions 2, 3, 4 and 6, respectively. These served as control spots for identifying starting materials and products.

Separation and detection. The chromatogram was developed using 100 ml of acetone–ammonium hydroxide (80:20), until the solvent ascended 15 cm. A current of cool air from a hair dryer was used to dry the plate and remove residual ammonia (which impeded detection with Dragendorff reagent). After spraying the chromatogram thoroughly with Dragendorff reagent it was heated at 105° for 5 min. Spots became even more pronounced by subsequently exposing the chromatogram to short-wave ultraviolet radiation for about 20 min, and re-spraying.

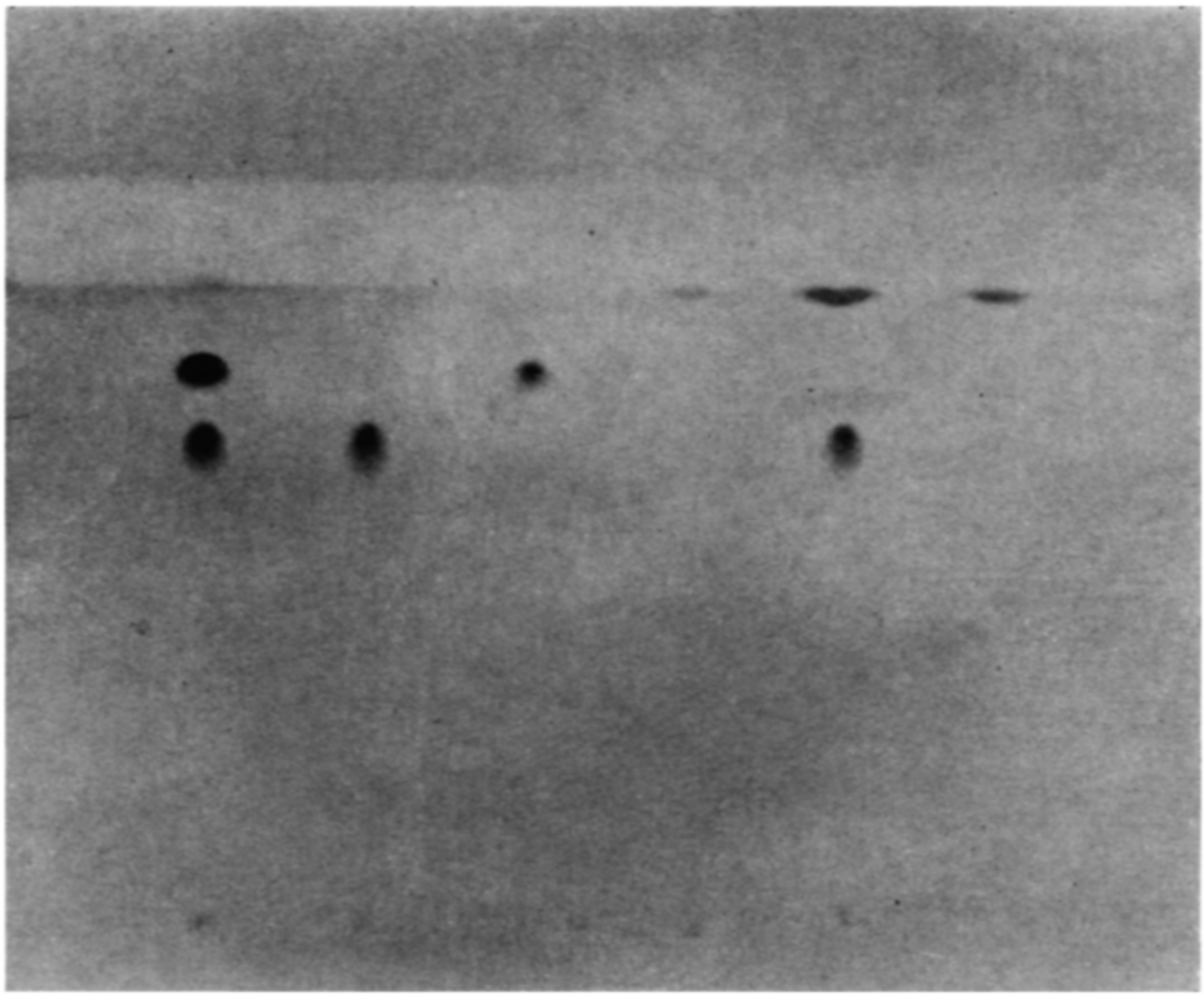


Fig. 1. Reduction of tropinone. 1 = Products from tropinone; 2 = tropine; 3 = pseudotropine; 4 = tropinone; 5 = products from *l*-hyoscyamine; 6 = *l*-hyoscyamine.

Results and discussion

Fig. 1 is a chromatogram of the products and the control compounds. The products are easily distinguishable by their reddish color; the starting materials are orange-yellow. The reduction of tropinone yielded pseudotropine and tropine in approximately a 2:1 ratio. This agrees favorably with results reported by BECKETT *et al.*[9]. These investigators had performed the reaction (on the macro-scale) in aqueous sodium borohydride solution under varying conditions of time and temperature, but invariably obtained this same proportion of isomers.

It is interesting to note that the reaction between *l*-hyoscyamine and sodium borohydride did not yield a reduction product, but a hydrolysis product (tropine). The alkalinity of the sodium borohydride solution promoted hydrolysis. The thin-layer plate thus serves as a vehicle for conveniently and rapidly determining the nature of the interaction between reactants. Reaction and separation of products can be performed on the same medium in less than 2 h.

The degree of reaction was measured visually, by considering both size and intensity of spots. Quantitative determinations are also possible: the separated

compounds can be extracted into a suitable solvent and a spectrophotometer then employed to measure the absorbance of the solution[16].

Acetone–ammonium hydroxide (97:3) has been found[15] to be applicable as a general developing solvent for the tropane alkaloids. By changing the proportion of the constituents (to 80:20), the solvent system has been extended to the separation of tropine and pseudotropine. Considering the quantity and variety of methods that have been proposed for the separation of these epimers, the TLC method provides apparent advantages.

It has been demonstrated that a reaction can be studied on the thin-layer plate, using microgram quantities of reactants. The results compared favorably with those obtained on the macro-scale.

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