150 mg palladium chloride are dissolved in 100 ml 0.2 *N* hydrochloric acid.

(*12*) *Antimony pentachloride.* 20 ml antimony pentachloride are mixed with 80 ml carbon tetrachloride. The plate is heated for 10 min at 105°.

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Received March 1st, 1966

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The cytogenetics of *Lotus*

XII. Thin-layer chromatography in the separation of secondary phenolic compounds in *Lotus* (Leguminosae)

The successful separation of secondary phenolic compounds in *Lotus* through the use of the Shandon thin-layer chromatographic equipment and silica gel G as the coating material has recently been reported by GRANT AND WHETTER[1]. The availability of commercially prepared coated plates would eliminate the initial time needed to learn the technique required in order to obtain a satisfactory coating on the plates as well as the time required for the messy preparation of the silica gel coating on the plates. This note reports the results obtained in the separation of secondary phenolic compounds in *Lotus* using the techniques reported in the earlier paper by GRANT AND WHETTER[1] but using prepared Eastman Chromagram sheets, Type K301R with a fluorescent indicator, and the Eastman Chromagram Developing Apparatus (Eastman Organic Chemicals, Distillation Products Industries, Rochester, New York).

Preliminary tests

Samples of fresh leaves of *Lotus* were prepared by weighing out 0.08 g and leaving them in 0.5 ml of 1 % hydrochloric acid in methanol at room temperature, in the dark, overnight. The plates were prepared for development by applying an approximately 7 μl spot of sample solution with a micropipette at a distance of 2.0 cm from the base. Two spots were run for each sample. Ascending development was carried

out at room temperature in the Eastman Chromagram developing apparatus. Cyclohexane–ethyl acetate (1:1, v/v) was used as the first solvent and allowed to pass twice up the layer to a height of 15 cm, the plate being dried between runs. A second solvent, methanol–chloroform (30:70) was then allowed to run up only to R_F 0.5. The plates were examined between runs for spots, but the results were very unsatisfactory as the color was extremely faint and very poor resolution was obtained.

Final tests

From the preliminary results it was considered that the leaf extract used was too weak. Plates were then prepared by using extracts from both 0.08 g and 0.16 g of fresh leaves (in 0.5 ml of 1 % hydrochloric acid in methanol) and spotting the plates with 7, 10 and 15 μl from each extract. When the plates with the highest concentration of extract (0.16 g and 15 μl) were examined the fluorescence was still only approximately half as bright as that from the glass plates prepared with silica gel G. Consequently, the chromagrams were considered unsatisfactory for proper diagnosis. Depending on the species of *Lotus*, three to nine leaves (including leaflets) are required for the preparation of 0.08 g of extract. If the quantity is exceeded beyond the doubled amount, that is 0.16 g, then this would require most of the leaves on a young plant, and certainly would not be satisfactory for the analyses of herbarium material[1]. Therefore, we do not consider the Eastman Chromagram sheets suitable for our particular requirements, as to use increased amounts of leaf material would with certain species, such as *Lotus micranthus*, use up most of the leaves on a single plant. In addition, we found that it required 1.5 h for each single development with the Eastman Chromagram sheets using the Eastman Chromagram developing apparatus, while a single development with the glass plates and silica gel G required only 30 to 40 min. Therefore, for our analyses of the phenolic compounds of *Lotus* we have abandoned the commercially prepared sheets in favor of the standard glass plates and the silica gel G coating method which was reported previously[1].

This work is supported through an Extramural Research Grant to the senior author by the Canada Department of Agriculture and is gratefully acknowledged.

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Received February 24th, 1966