Sprague-Dawley rats weighing about 180 g were used. After depilation of the abdominal skin, the animals were anaesthetized with ethyl ether and injected intravenously with azovan blue (3 ml/kg of a 0·4% solution). Immediately, 0·1, 1 and 10 μg of 5-HT were injected intradermally, each in 0·1 ml of saline, into each animal. 30 min later, the rats were killed, the diameters of the coloured areas measured, and the pieces of skin removed and submitted to the azovan blue extraction and determination, according to the method of Beach & Steinetz (1961). Two antagonists of 5-HT, 1-methyl-lysergic acid butanolamide (UML 491—Sandoz) (Berde, Doepfner & Cerletti, 1960) and cyproheptadine (Periactin—Merck) (Stone, Wenger, Ludden, Stavorski & Ross, 1961), which had been shown to have a strong inhibitory activity in this test (Jori, Bentivoglio & Garrattini, 1961), were injected intravenously 30 min before azovan blue, at doses of 3 μg/kg and 100 μg/kg respectively. Fig. 1 shows that the curves of the dye concentration (blueing) and of the coloured surface area measurements are congruent. Therefore, at least in our experimental conditions, measurement of the surface of the coloured area is adequate to evaluate the degree of the dye infiltration.

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Paper chromatography and identification of *Magnolia acuminata* L. alkaloids

SIR,—In a survey of plants for steroidal sapogenins and other constituents Wall, Fenske, Garwin, Willaman, Jones, Schubert & Gentry (1959) screened some plants of the magnoliaceae family. Of the four species of the American magnolias examined, presence of alkaloids was reported in stems and leaves of *Magnolia acuminata* L. (Cucumber-tree). However, these compounds have not been isolated and characterized.

We have extracted the stems of *M. acuminata* and isolated fractions containing alkaloids. Descending paper chromatography of the quaternary chlorides was performed using three different solvent systems. Five quaternary bases (A to E) were detected (Table 1) after development with solvent system 1 and spraying with reagent I. Four bases have been identified and checked by running them with authentic specimens. A is choline chloride; B is magnoflorine chloride, D is salicifoline chloride and E is magnocurarine chloride.

Chromatograms developed with solvent systems 2 and 3 showed distinct coloured spots of salicifoline and magnocurarine chlorides when both IIa and IIb were sprayed. Thus reagent II affords a useful means of identification of each of the three quaternary alkaloids magnoflorine, magnocurarine and salicifoline, which are known to occur in some species of magnolias (Tomita & Nakano, 1957).

TABLE 1. Rf VALUES AND COLOUR REACTIONS OF QUATERNARY BASES FROM STEMS OF *M. acuminata*

Quaternary base	Rf values system			Colour reaction after systems	
	1	2	3	Reagent I	Reagent II *
A	0·14	0·10	0·27	Violet	—
B†	0·16	0·21	0·58	Orange	Red then yellow-orange
C†	0·26	0·31	0·62	Orange	Red then yellow-orange
D	0·41	0·22	0·57	Orange	Yellow then orange
E	0·70	0·51	0·67	Orange	Light brown then dark brown

Solvent systems 1 = Ethyl acetate : pyridine : water (750 : 310 : 165).
2 = n-Butanol : water : acetic acid (100 : 100 : 5).
3 = n-Butanol : water : acetic acid (50 : 40 : 10).
Reagent I = Modified Dragendorff reagent (Munier & Macheboeuf, 1951).
Reagent II = Modified Pauly's reagent (Ames & Mitchell, 1952).

* Colour changes indicate shades observed when sprayed with IIa (diazotized sulphanilic acid) followed by IIb (20% sodium carbonate solution). Distinct changes were seen only on papers developed with sytem 1.
† Shows purple fluorescence under ultra-violet light.

Chromatographic identification of compound D and E was confirmed by isolating crystalline picrates of the two bases. Mixed melting point determination of picrate D with salicifoline picrate showed no depression and its infra-red spectrum was identical with that of the latter. Compound E was similarly characterized as magnocurarine chloride.

Quaternary alkaloidal fraction of leaves of *M. acuminata* has been isolated and studied by chromatographic technique. Results suggest the presence of only one major phenolic quaternary compound with Rf values identical with magnoflorine chloride.

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