easily expandable clays possessing exchangeable interlamellar cations should be equally applicable.

It is clear that the use of modified organo-clays as gas chromatographic stationary phases may have wide possibilities. Their application is certainly not limited to the separation of the C_6–C_9 aromatics described here, and many naphthenic and substituted hydrocarbon mixtures for which separation by conventional gas liquid partition chromatography has involved extended retention times may be more rapidly resolved on columns containing modified organo-clay packings.

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Thin Layer Chromatography of the Gibberellins

Paper chromatography of the gibberellins has been investigated by many investigators but no comprehensive study of all the known gibberellins has been reported. MacMillan, Seaton and Suter[1] described four solvent systems which separate gibberellins A_5, A_6, A_8 and A_9 but not the pairs gibberellin A_1 and gibberellic acid (A_3) or gibberellins A_4 and A_7. The solvent systems used by Takahashi *et al.*[2] and Phinney *et al.*[3] do not separate gibberellins A_1, A_2 and A_3. Gibberellins A_1 and A_3 can be separated on paper which is developed by overflow elution with benzene/acetic acid/water (4 : 1 : 2); this method is lengthy when conducted under gravity as described by Bird and Pugh[4] and resolution is poor when development is hastened by centrifugal force[5].

Thin layer chromatography of gibberellins A_1 and A_3 has been described by Kutacek, Rosmus and Deyl[5]; good resolution was obtained on a layer of aluminium oxide, developed by overflow elution with benzene/acetic acid (100 : 23).

We have found that the nine known gibberellins can be separated by thin layer chromatography (Table 1).

Table 1. R_F values of the gibberellins

Gibberellin	Silica gel		Kieselguhr	
	Solvent system 1	Solvent system 2	Solvent system 3	Solvent system 2
A_1	0·11	0·0	0·54	0·26
A_2	0·04	0·0	0·64	0·30
A_3	0·11	0·0	0·42	0·18
A_4	0·37	0·82	1·0	1·0
A_5	0·31	0·35	1·0	0·88
A_6	0·25	0·21	0·95	0·76
A_7	0·37	0·70	1·0	1·0
A_8	0·04	0·0	0·28	0·06
A_9	0·75	1·0	1·0	1·0

For solvent systems, see legend to Table 2.

Thin layers of silica-gel *G* or kieselguhr *G* were prepared[6] on glass plates; they were spotted with not more than 10 μg gibberellic acid and the solvent was run for 15 cm at room temperature (17°–21° C). Running times for solvent system 1 were 25–50 min and for solvent systems 2 and 3 were 45–70 min. With solvent systems 2 and 3 plates were equilibrated overnight with lower phase then developed with upper phase.

Two sprays were used: (*a*) ethanol/concentrated sulphuric acid (95 : 5); (*b*) water/concentrated sulphuric acid (30 : 70).

After spraying with (*a*) the plates are heated at 120° for 10 min. The gibberellins are then visible in ultra-violet light. Gibberellins A_1, A_3, A_5, A_6 and A_8, which possess a 7-hydroxyl group, give blue fluorescent spots of different intensities; gibberellins A_2, A_4, A_7 and A_9, which have no 7-hydroxyl group, give purple spots.

With spray (*b*) gibberellins A_3 and A_7 are visible in ultra-violet light as yellow-green fluorescent spots before heating. After heating at 120° for 10 min, all are visible in ultra-violet light. Gibberellins A_1, A_3, A_6 and A_8 give green-blue fluorescent spots; gibberellins A_2, A_4 and A_9 give blue-purple fluorescent spots; gibberellins A_5 and A_7 are exceptions and give light yellow and brown fluorescent spots respectively.

Both sprays show similar sensitivity ranging from 0·00025 μg gibberellin A_3 to 0·01 μg gibberellin A_6. In mixtures 0·5 per cent gibberellin A_1 can be detected in 1 μg gibberellin A_3 and 0·05 per cent gibberellin A_1 in 10 μg gibberellin A_3. Similar quantities of gibberellin A_4 can be detected in gibberellin A_7.

Methyl esters (Table 2) give the same coloured spots as the corresponding acids but with weaker fluorescence.

Table 2. R_F values of Methyl Esters of Gibberellins

Methyl ester	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9
Solvent system 2	0·33	0·34	0·26	1·0	1·0	1·0	1·0	0·1	1·0
Solvent system 1 (2% for 5% acetic acid)	0·18	0·08	0·16	0·50	0·38	0·31	0·48	0·08	0·80

Solvent systems: (1) Di-isopropyl ether/acetic acid (95 : 5); (2) Benzene/acetic acid/water (8 : 3 : 5); (3) Benzene/propionic acid/water (8 : 3 : 5).

No one solvent system separates all the gibberellins or their methyl esters. Gibberellin A_5, A_6 and A_8 are separated from each other and from the unresolved pairs gibberellins A_2 and A_8, A_1 and A_3, and A_4 and A_7.

Gibberellins A_4 and A_7 are separated on silica-gel *G* by solvent system 2. The pairs, gibberellins A_2 and A_8 and gibberellins A_1 and A_3, are separated on kieselguhr *G* by solvent system 2; all four (A_1, A_2, A_3 and A_8) are separated on kieselguhr *G* by solvent system 3; but this system is unreliable since streaking of the plates frequently occurs for reasons unknown.

Gibberellins A_3 and A_7 can be further distinguished from the other gibberellins by means of spray (*b*); they give characteristic yellow-green fluorescent spots without heating while the other gibberellins fluoresce only after heating for 10 min at 120°.

Indolyl-3-acetic acid has an R_F value of 0·60 on silica gel in solvent system 1 and so can be separated from the gibberellins.

All methyl esters except those of gibberellins A_4 and A_7 can be resolved on silica-gel *G* using solvent systems 1 and 2.

Advantages of these methods are convenience, speed and sensitivity. The sulphuric acid sprays, which cannot be applied to paper chromatograms, are to be preferred to the perchloric acid spray[5] and are more sensitive and specific than the 0·5 per cent aqueous potassium permanganate spray[1]. The latter reagent does not detect gibberellin A_2 or less than 5 μg of the other gibberellins.

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