

easily expandable clays possessing exchangeable inter-lamellar 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 C₆-C₉ 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.

We thank the Directors of the British Petroleum Co., Ltd., for permission to publish this communication.

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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 Suter¹ described four solvent systems which separate gibberellins A₅, A₆, A₈ and A₉ but not the pairs gibberellin A₁ and gibberellic acid (A₃) or gibberellins A₄ and A₇. The solvent systems used by Takahashi *et al.*² and Phinney *et al.*³ do not separate gibberellins A₁, A₂ and A₃. Gibberellins A₁ and A₃ 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 Pugh⁴ and resolution is poor when development is hastened by centrifugal force⁵.

Thin layer chromatography of gibberellins A₁ and A₃ has been described by Kutacek, Rosmus and Deyl⁶; 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 prepared⁶ 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 A₁, A₃, A₅, A₆ and A₈, which possess a 7-hydroxyl group, give blue fluorescent spots of different intensities; gibberellins A₂, A₄, A₇ and A₉, which have no 7-hydroxyl group, give purple spots.

With spray (b) gibberellins A₃ and A₇ 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 A₁, A₃, A₆ and A₈ give green-blue fluorescent spots; gibberellins A₂, A₄ and A₅ give blue-purple fluorescent spots; gibberellins A₅ and A₇ are exceptions and give light yellow and brown fluorescent spots respectively.

Both sprays show similar sensitivity ranging from 0.00025 μg gibberellin A₂ to 0.01 μg gibberellin A₆. In mixtures 0.5 per cent gibberellin A₁ can be detected in 1 μg gibberellin A₃ and 0.05 per cent gibberellin A₁ in 10 μg gibberellin A₃. Similar quantities of gibberellin A₄ can be detected in gibberellin A₇.

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 A₅, A₆ and A₈ are separated from each other and from the unresolved pairs gibberellins A₂ and A₈, A₁ and A₃, and A₄ and A₇.

Gibberellins A₄ and A₇ are separated on silica-gel G by solvent system 2. The pairs, gibberellins A₂ and A₈ and gibberellins A₁ and A₃, are separated on kieselguhr G by solvent system 2; all four (A₁, A₂, A₃ and A₈) 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 A₃ and A₇ 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 A₄ and A₇ 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 spray⁵ and are more sensitive and specific than the 0.5 per cent aqueous potassium permanganate spray¹. The latter reagent does not detect gibberellin A₂ or less than 5 μg of the other gibberellins.

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