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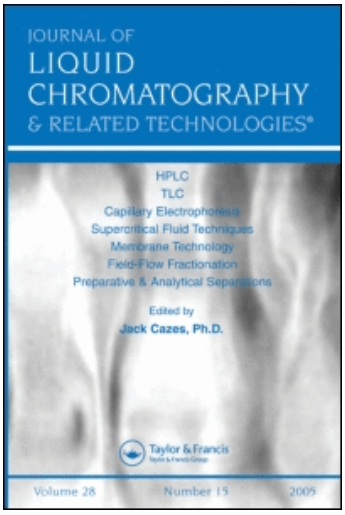

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Argentation Thin-Layer Chromatography of the p-Nitrobenzyl Esters of Gibberellins and Their Precursors

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ARGENTATION THIN-LAYER CHROMATOGRAPHY OF THE p-NITROBENZYL ESTERS OF GIBBERELLINS AND THEIR PRECURSORS

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ABSTRACT

Thirteen p-nitrobenzyl esters of gibberellins and their precursors have been separated by thin-layer chromatography on silver nitrate-impregnated silica gel. The fluorescence produced by sulfuric acid was used for their detection.

INTRODUCTION

Thin layer chromatography (TLC) is one of the most important analytical techniques for biochemical research on gibberellins. In earlier work, reviewed by Paleg (1), difficulties were encountered in resolving, by adsorption, pairs of compounds differing only by the presence or absence of a double bond. Partition systems have solved this problem in some instances (2-6), but argentation TLC, which is generally used for such pairs in the lipid field (7), has so far not been applied to the gibberellins. Having accomplished difficult separations of this kind by

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argentation high-pressure liquid chromatography (8), we are now reporting that closely related gibberellin analogs (Fig. 1) (9) are easily resolved on silver nitrate-impregnated silica gel plates.

Because carboxylic acids are too strongly adsorbed by silver nitrate, they were chromatographed in the form of their p-nitrobenzyl esters. The esters are as easily detected as the free acids by spraying the plates with dilute sulfuric acid, and they are useful derivatives for mass spectrometry (8).

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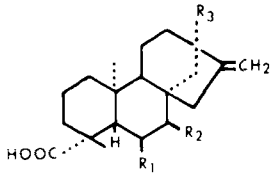
MATERIALS

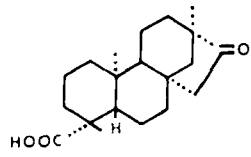
Thin-layer plates, 5 x 20 cm, were ordered to contain 10% $AgNO_3$ on a silica gel G layer, 250 μm thick (10). All solvents were spectroquality (11).

The preparation of the p-nitrobenzyl esters was carried out in a Reacti-Vial System (12), consisting of an electrically heated metal block (Reacti-Therm heating module) and 1-ml vials with conical wells (Reacti-Vials), which were sealed with Teflon-lined screw caps. The reagent was p-Nitrobenzyl8 (12), a 0.1 M solution of O-p-nitrobenzyl-N,N'-diisopropylisourea in dichloromethane.

The chromatograms were viewed under long-wave ultraviolet light (365 nm) in a dark-cabinet (13).



	R_1	R_2	R_3
STEVIOL	H	H	OH
DHK	OH	OH	H



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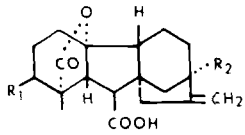
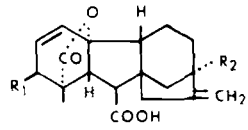


GA	R_1	R_2
9	H	H
4	OH	H
20	H	OH
1	OH	OH



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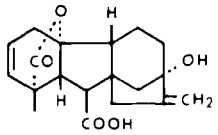
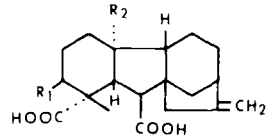
7	OH	H
3	OH	OH



GA_5



GA	R_1	R_2
14	OH	Me
25	H	COOH
13	OH	COOH

FIGURE 1

Structures of Gibberellin Analogs

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METHODS

The esterfication reaction is outlined for gibberellic acid in Fig. 2. It was routinely performed by heating the dry gibberellin analog with the p-Nitrobenzyl8 reagent (2 µl of reagent per µg) at 80°C for 2 hr in the Reacti-Vial System (14).

The reaction mixture, equivalent to 1 µg of gibberellin analog, was applied to the thin-layer plates without purification. The plates were stored and developed in the dark. All chromatograms started 2 cm from the bottom edge of the plates and developed over a distance of 17 cm. The solvent

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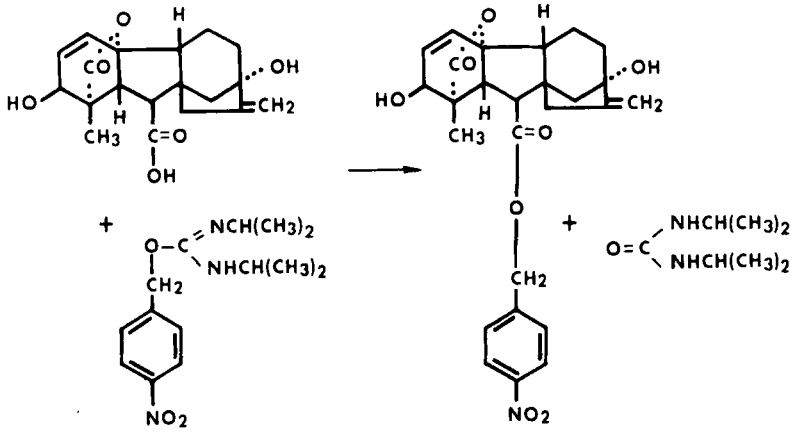

FIGURE 2

Formation of the GA3 p-Nitrobenzyl Ester

systems were: 1, carbon tetrachloride-acetone (3:1); 2, n-pentane-acetone (7:3); 3, n-hexane-ethyl acetate (1:1).

After they had been sprayed with sulfuric acid-water (1:1), the chromatograms were heated on a hot plate at low heat (ca. 100°C) for 0-20 min and examined under long-wave UV light at intervals.

RESULTS

Table 1 shows the hR_F values of the p-nitrobenzyl esters of gibberellin analogs in three solvent systems and their fluorescence behavior in the sulfuric acid reaction, i.e. the time of appearance and color of the zones.

Although the limit of detection was 10 ng in most cases, the data in Table 1 are based on chromatograms of 1 µg of each compound.

The fluorescence colors of several esters changed considerably as they were heated. The yellow fluorescence of GA7 and the gray fluorescence of GA3 appeared in about 5 min at room temperature. Upon heating, GA7 charred within 5 min, whereas the fluorescence of GA3 turned to yellow after 5 min, then to light blue after 10 min. The gray fluorescence of GA4 became

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TABLE 1

TLC of p-Nitrobenzyl Esters of Gibberellin Analogs

Compound	hR_F Values in Solvent Systems*			Fluorescence	
	1	2	3	Time (min)	Color
Isosteviol	100	87	92	17	brown
GA9	92	78	88	7	dark blue
GA25	90	69	91	12	brown
Steviol	71	68	76	17	brown
GA14	72	59	73	10	light blue
DHK**	62	64	50	7	gray
GA13	66	50	71	12	brown
GA4	63	56	57	10	yellow
GA7	56	51	56	0	yellow
GA20	51	46	45	10	light blue
GA5	49	41	41	10	light blue
GA1	31	23	18	15	light blue
GA3	26	19	15	0	gray

*Solvent systems: 1, CCl_4 - Me_2CO (3:1); 2, C_5H_{12} - Me_2CO (7:3); 3, C_6H_{14} - EtOAc (1:1).

**DHK = 6β, 7β- dihydroxykaurenoic acid

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yellow, while the gray fluorescencene of GA1 and DHK became light blue, and the light blue fluorescence of GA14 turned to gray.

DISCUSSION

The sensitivity and specificity of the sulfuric acid detection method for the p-nitrobenzyl esters was similar to previous results for the methyl esters and free gibberellins (2). GA3 and GA7 were easily distinguished by their fluorescence at

room temperature. The dark blue fluorescence of GA9 distinguished it from other nonpolar gibberellin analogs.

The zones were compact in Solvents 1 and 2, but somewhat diffuse in Solvent 3. The mobility of the esters was mainly influenced by the number of hydroxyl groups, but DHK is a notable exception. The order of migration depended on the solvent system. For instance, GA9 moved ahead of GA25 in Solvents 1 and 2, but in Solvent 3 the order was reversed. The order of GA13 and GA4 was likewise reversed between Solvent 2 and Solvents 1 and 3. Such reversals of order are useful for resolving complex mixtures.

Pairs of gibberellin esters differing by the presence or absence of a double bond migrated similarly, but the unsaturated analog was always more retarded than the saturated one. The best separation of GA3 and GA7 from their analogs, GA1 and GA4, respectively, was achieved in Solvent 1, but GA5 was best separated from GA20 in Solvent 2. Thus, esterification is a useful device for analysis by TLC as well as mass spectrometry (8).

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REFERENCES

1. Paleg, L. G., Annu. Rev. Plant Physiol., 16, 291, 1965.
2. MacMillan, J. and Suter, P. J., Nature, 197, 790, 1963.
3. Kagawa, T., Fukinbara, T. Sumiki, Y., Agr. Biol. Chem. 27, 598, 1963.
4. Jones, K. C., J. Chromatogr., 52, 512, 1970.
5. Stainier, R. L., Landbowwet., Rijksuniv. Gent, 40, 881, 1975.
6. LePage - Degivry, M.-T., Garello, G., Isaia, A., Barthe, P. and Bulard, C., Physiol. Veg., 15, 343, 1977.
7. Dallas, M. S. J., Morris, L. J. and Nichols, B. W., Chromatography, A Laboratory Handbook of Chromatographic and Electrophoretic Methods, Heftmann, E., ed., Van Nostrand Reinhold, New York, 3rd ed., 1975, p. 540.
8. Heftmann, E., Saunders, G. A. and Haddon, W. F., J. Chromatogr., in press.
9. DHK = 6β, 7β-dihydroxykaurenoic acid.
10. Analtech, Newark, Del., U.S.A. Reference to a company and/or a product named by the Department is only for the purpose of information and does not imply approval or recommendation of the product named to the exclusion of others which may also be suitable.
11. "Distilled in Glass" quality, Burdick & Jackson, Muskegon, Mich., U.S.A.
12. Pierce Chemical Co., Rockford, Ill., U.S.A.

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13. Chromato-Vue, Ultra-Violet Products, Inc., San Gabriel, Calif., U.S.A.

14. Knapp, D. R. and Kruger, S., Anal. Lett., 8, 603, 1975.

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