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THIN-LAYER CHROMATOGRAPHY, ULTRAVIOLET AND FLUORESCENCE SPECTRA OF SOME FLUORENE-9-CARBOXYLIC ACID DERIVATIVES (MORPHACTINS)*

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SUMMARY

A chromatographic method for the separation and identification of some fluorene-9-carboxylic acid (morphactin) derivatives, down to 0.5 or 1.0 μg, was developed. The ultraviolet spectra of these compounds as well as the fluorescence emission spectra of the *n*-butyl derivative prior to and after exposure to ultraviolet light was determined.

INTRODUCTION

A new class of plant growth substances collectively called morphactins, derivatives of fluorene-9-carboxylic acid, are of practical interest as herbicides, plant growth retardants, and regulators and inhibitors of senescence of detached plant parts[1–9]. The effect of these compounds on geotropism and phototropism of plants[2, 10] is of particular interest as a novel and basic phenomenon. These properties of morphactins may aid in the understanding of causes of plant tropisms and may be of horticultural importance *i.e.* to change plant growth habit from upright growing to one of creeping character. The low mammalian toxicity and low persistence in soils[2, 4] of these compounds is an important consideration in any potential use in controlling plant growth.

The literature describing the economical use and biological effects of these compounds does not provide information on their detection and analysis. This may be due to the inherent analytical difficulties in resolving closely related materials. The parent compound, fluorene may be detected chromatographically using acetylated paper[11]. The purpose of this paper is to describe a thin-layer chromatographic method for separation and identification of *n*-butyl 9-hydroxyfluorene-9-carboxylate, methyl 2-chloro-9-hydroxyfluorene-9-carboxylate, methyl 2,7-dichloro-9-hydroxyfluorene-9-carboxylate, methyl 9-hydroxyfluorene-9-carboxylate, and 9-fluorenone. The U.V. fluorescence spectra of the derivatives are also given.

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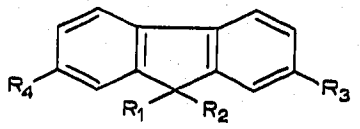
TABLE I

STRUCTURAL FORMULAE OF SOME FLUORENE-9-CARBOXYLIC ACID DERIVATIVES



	R_1	R_2	R_3	R_4
(1) 9-Fluorenone	=O		–H	–H
(2) Methyl 9-hydroxyfluorene-9-carboxylate	–OH	$-COOCH_3$	–H	–H
(3) Methyl 2-chloro-9-hydroxyfluorene-9-carboxylate	–OH	$-COOCH_3$	–Cl	–H
(4) Methyl 2,7-dichloro-9-hydroxyfluorene-9-carboxylate	–OH	$-COOCH_3$	–Cl	–Cl
(5) *n*-Butyl 9-hydroxyfluorene-9-carboxylate	–OH	$-COO(CH_2)_3CH_3$	–H	–H

MATERIALS

9-Fluorenone was purchased from E. H. Sargent and Co.; the other compounds were donated by E. Merck, A. G. Darmstadt (Table I). Commercially available reagent grade chemicals were used in the analyses. For chromatography, the compounds were dissolved in benzene (1 mg per 2 ml). The samples were spotted (1–10 μl, or 0.5–5 μg) on 0.25 mm thick Silica Gel G thin-layer chromatographic plates (20 cm × 20 cm). The slurry was made up with hot water containing 0.5 g of caffeine per plate[10]. To avoid possible cooling and recrystallization of the added caffeine during the coating of the chromatographic plate, the applicator (Desaga) was also heated briefly on a hot plate. The plates were either air-dried, or air-dried and activated for 30 min at 105°, and developed (ascending) in a solvent mixture of ethyl ether and petroleum ether (1:1, v/v; boiling range 30–60°). The developed plates were dried in an air system and photographed in U.V. according to JACKSON[13]. Aluminum Oxide-G, pH 7.4, (according to STAHL) coated thin-layer chromatographic plates were prepared by dissolving 2,4,7-trinitro-9-fluorenone (15 mg per plate) in a small amount of acetone[10], adding the required amount of water and aluminum oxide, preparing the slurry and coating the plates at a thickness of 0.25 mm. The plates were activated for 30 min at 100°, spotted and developed (ascending) with methylene chloride solvent or the ethyl ether–petroleum ether (1:1, v/v) mixture.

RESULTS

The compounds on the developed Silica Gel G plates were localized in visible light by spraying the plates with concentrated sulphuric acid containing 0.2 ml of 36% (by wt.) formaldehyde per 10 ml of acid. After the spray the *n*-butyl 9-hydroxyfluorene-9-carboxylate spot appeared bright turquoise. The blue methyl 2-chloro-9-hydroxyfluorene-9-carboxylate spot appeared a few seconds later. Colour development may be facilitated by heating the sprayed plate in an oven for 10 min at 100°. In such a case, the methyl 2,7-dichloro-9-hydroxyfluorene-9-carboxylate appeared as a light spot against the very light grey background. The grey methyl 9-hydroxyfluorene-9-carboxylate spot appeared on standing after 5–10 min. The spray reagent deteriorates on standing and should be freshly prepared.

Use of solvents of the eluotropic series: petroleum ether–cyclohexane–carbon

TABLE II

R_F VALUES OF SOME FLUORENE-9-CARBOXYLIC ACID DERIVATIVES CHROMATOGRAPHED ON SILICA GEL G PLATES

(The plates are developed in ethyl ether–petroleum ether, 1:1, v/v.)

Compound	R_F
(1) 9-Fluorenone	0.50
(2) Methyl 9-hydroxyfluorene-9-carboxylate	0.38
(3) Methyl 2-chloro-9-hydroxyfluorene-9-carboxylate	0.47
(4) Methyl 2,7-dichloro-9-hydroxyfluorene-9-carboxylate	0.57
(5) *n*-Butyl 9-hydroxyfluorene-9-carboxylate	0.51

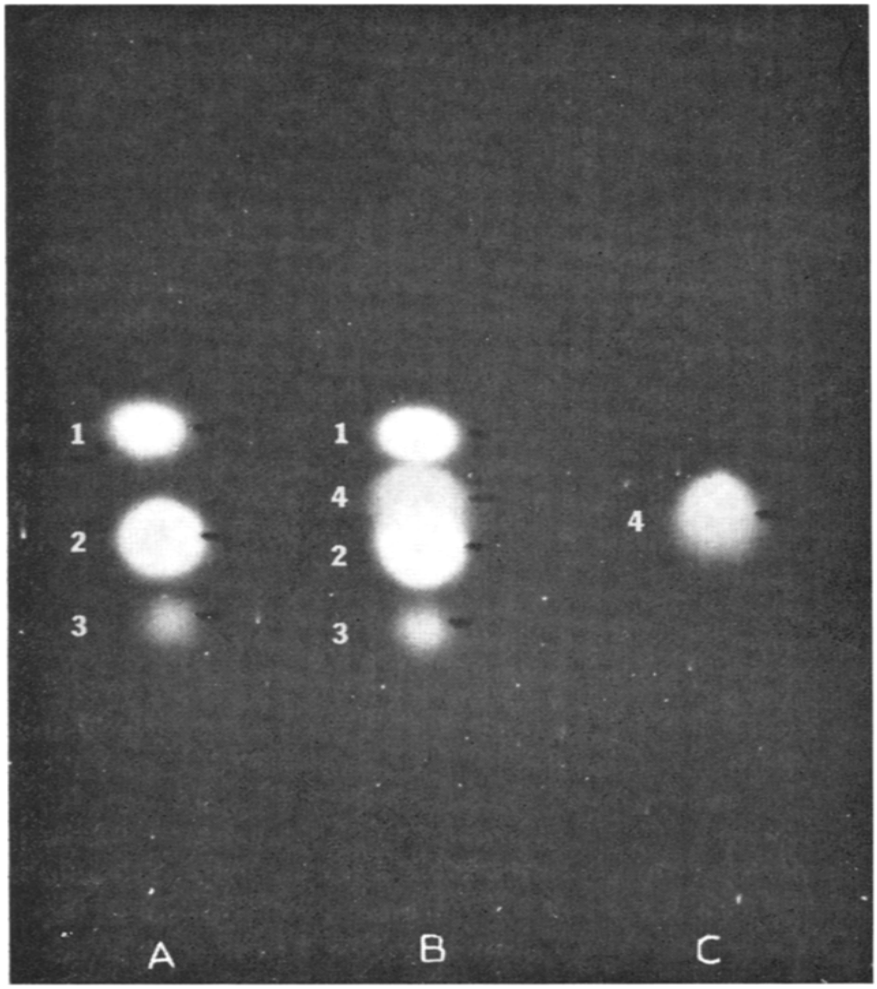


Fig. 1. U.V. photograph of thin-layer chromatogram of a mixture of fluorene-9-carboxylic acid derivatives. (1) Methyl 2,7-dichloro-9-hydroxyfluorene-9-carboxylate; (2) methyl 2-chloro-9-hydroxyfluorene-9-carboxylate; (3) methyl 9-hydroxyfluorene-9-carboxylate; (4) *n*-butyl 9-hydroxyfluorene-9-carboxylate.

tetrachloride–methylene chloride–ethyl ether showed the greatest R_F's with ethyl ether and the smallest with petroleum ether as the developing solvents; the remaining solvents gave intermediate values. Methylene choride gave R_F values similar to those shown by the ethyl ether–petroleum ether (1:1, v/v) mixture except that with methylene chloride the separation of compounds was poor because of tailing of spots. The absence of caffeine in the absorbing layer resulted in poor chromatographic resolution but may be used in order to obtain the compounds for spectrophotometric tests.

The R_F values of the compounds developed in the ethyl ether–petroleum ether mixture are shown in Table II; and Fig. 1. The movement of 9-fluorenone in the ethyl ether–petroleum ether solvent was very similar to that of the *n*-butyl carboxylate derivative (R_F 0.50 *vs.* 0.51) but was distinguishable from the latter by its yellow colour in the visible and yellow fluorescence under U.V. light. The limit of detection of the tested compounds by chromatography on Silica Gel G coated plates was from 0.5–1.0 μg.

TLC separation of the tested compounds was poor on Aluminum Oxide G coated plates with either of the developing solvents used — the R_F of the *n*-butyl derivative was 0.4 (top of an elongated spot) but the difference in response of the *n*-butyl and the methyl 2-chloro derivatives towards the U.V. light was of interest. Both compounds absorbed U.V. light (254 mμ) initially and appeared as dark spots; after a few minutes the *n*-butyl derivative began to fluoresce a yellowish green while

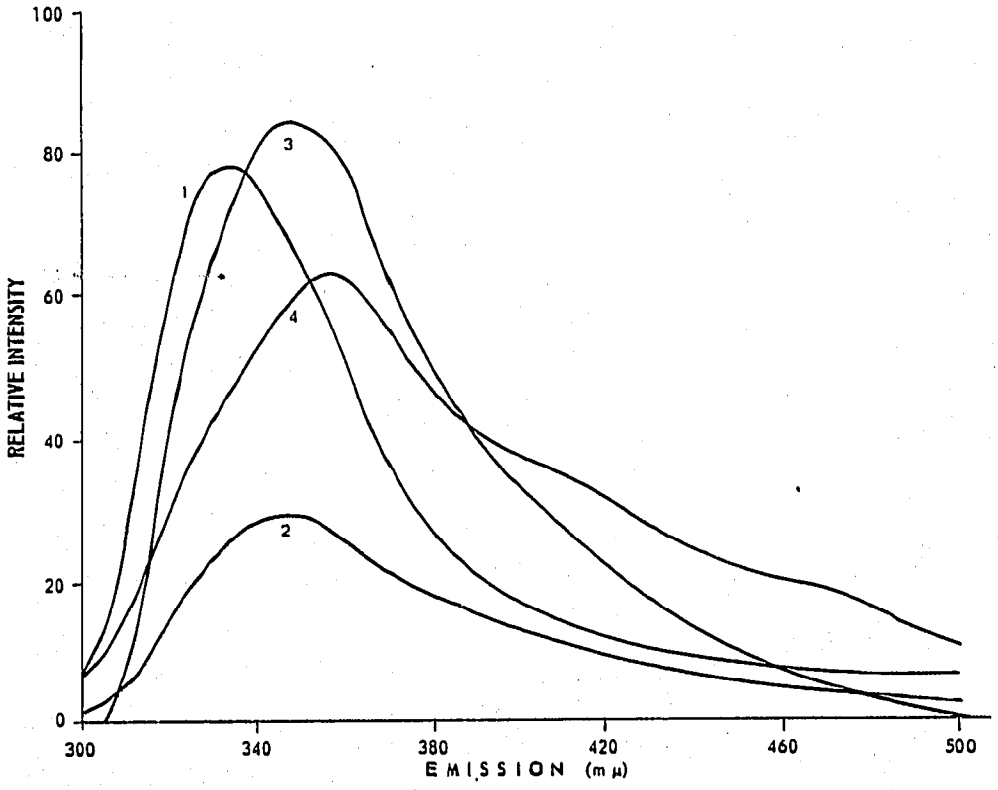


Fig. 2 (a) Fluorescence emission spectra of *n*-butyl 9-hydroxyfluorene-9-carboxylate after exposure to U.V. light. Instrument setting and concentrations as in (b). Number of curves, time of irradiation and meter multiplier settings: (1) 30 min, 0.01; (2) 4 h, 0.01; (3) 4 h, 0.003; (4) 18 h, 0.001. Excitation wavelength 281 mμ.

the methyl 2-chloro derivative remained a dark spot. This approach may be used to differentiate between the two compounds.

On exposure of the developed but non-sprayed Silica Gel G plates to U.V. light (350 mμ) the spots appeared dark initially. After a few minutes of continuous U.V. light (either 254 or 350 mμ) the spots began to fluoresce: at the 254 mμ wavelength the methyl 2,7-dichloro-9-hydroxyfluorene-9-carboxylate spot appeared blue, the other derivatives appeared bluish green; at the 350 mμ wavelength the derivatives were yellowish green against a violet background.

Prolonged exposure, up to 18 h, of the *n*-butyl derivative to U.V. light (Pen-Ray, Fisher) changed the fluorescence emission spectrum of this compound: the maximum of the spectrum changed from 335 mμ after 30 min exposure to 347 mμ and 355 mμ after 4 h and 18 h exposure, respectively (Fig. 2a). The long exposure did not change appreciably the R_F value of this compound when chromatographed on Silica Gel G. However, in this case there was a marked decrease in fluorescence at original R_F but an intensely fluorescing spot remained at the origin. The typical turquoise colour reaction of this compound when sprayed with the sulphuric acid–formaldehyde mixture changed to one of a very weak mauve after a long exposure to U.V. light. Similar changes were observed also with the other derivatives. Changes caused by U.V. light have been reported with other plant growth regulators[14], and may be considered of biological importance when plant growth regulators are used or tested under conditions where they may be exposed to U.V. light.

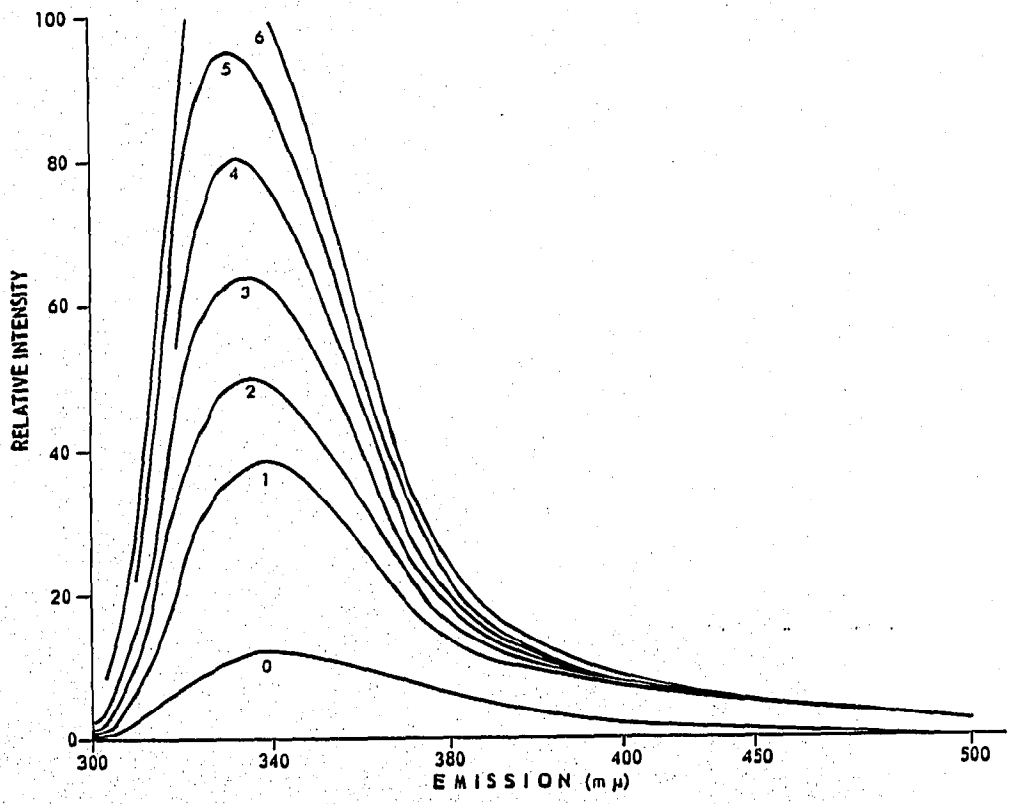


Fig. 2(b). Fluorescence emission spectra of *n*-butyl 9-hydroxyfluorene-9-carboxylate exposed to U.V. light. Aminco-Bowman fluorometer, PM tube: 1P21; solvent: 95% ethanol; concentration: 8 μg/ml, meter multiplier: 0.001; slit arrangement number 3, 1200 G/mm; sample tube; 10 mm × 10 mm I.D. Number of curves and time: (0) 0 min; (1) 12 min; (2) 14.5 min; (3) 17 min; (4) 19.5 min; (5) 22 min; (6) 24.5 min. Excitation wavelength: 281 mμ.

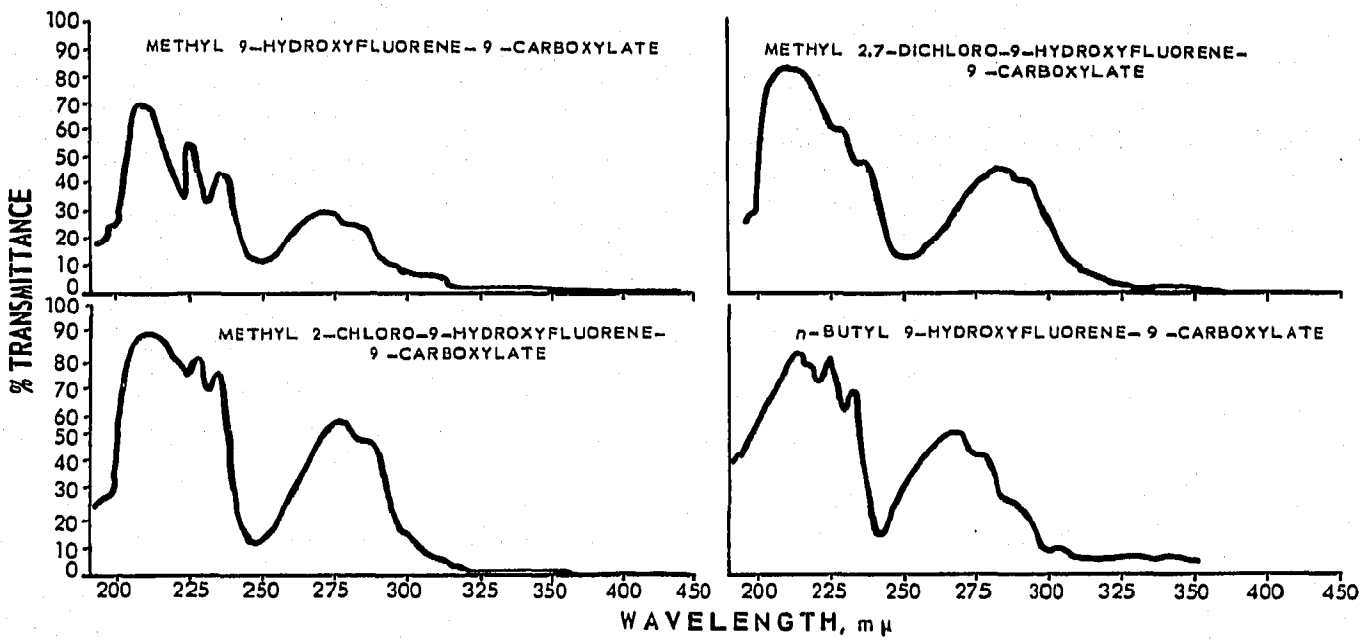


Fig. 3. U.V. spectra of some fluorene-9-carboxylic acid derivatives.

An increase in fluorescence was observed when the *n*-butyl derivative was dissolved in ethanol and exposed to U.V. light (281 mμ) in a spectrofluorometer (Aminco-Bowman) (Fig. 2b).

The U.V. absorption curves of the various derivatives (8 μg/ml. 95% ethanol, Beckman DK Recording Spectrophotometer) showed several typical absorption maxima and minima (Fig. 3). Methyl 2,7-dichloro-9-hydroxyfluorene-9-carboxylate had absorption maxima at 210, 229, 236 and 281 mμ, and minima at 227, 234, and 248 mμ. Methyl 2-chloro-9-hydroxyfluorene-9-carboxylate had peaks at 212, 229, 235, 277 and 287 mμ, and minima at 225, 232 and 247 mμ. Methyl 9-hydroxyfluorene-9-carboxylate had peaks at 210, 227, 234 and 272 mμ and minima at 223, 231 and 246 mμ. The *n*-butyl 9-hydroxyfluorene-9-carboxylate had major peaks at 272, 235, 227 and 211 mμ and minor peaks at 305, 282 and 220 mμ. The U.V. absorption curve of the *n*-butyl derivative after exposure to U.V. light for 18 h was different: there was loss of absorption at 272, 235 and 227 mμ.

ACKNOWLEDGEMENT

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