Phosphorescence Characteristics of Several Plant Growth Hormones

Phosphorescence excitation and emission wavelength peaks, lifetimes, limits of detection, and concentration ranges of analytical usefulness of 17 plant growth hormones in rigid (77° K) ethanolic solution were determined. Twelve of the hormones produced analytically useful phosphorescence, whereas the remaining five were of limited or no analytical use.

Phosphorimetry is becoming more widely accepted and used as an analytical tool as its wide range of utility becomes known for the study and analysis of compounds of biochemical interest (Winefordner *et al.*, 1967). It has previously been used to determine the nicotine alkaloids in tobacco (Winefordner and Moye, 1965), drugs in biological fluids (Winefordner and Latz, 1963; Winefordner and Tin, 1964; Hollifield and Winefordner, 1965, 1966; Stille and Szent-Gyorgyi, 1957), pesticides in foods and in biological fluids (Moye and Winefordner, 1965a,b), and anti-metabolites of plant and animal growth (Sanders *et al.*, 1969). In this study, the phosphorescence characteristics of 17 plant growth hormones were determined and the possible application of phosphorimetric techniques to the analysis of 12 of these compounds in biological materials is indicated. The plant growth hormones studies are of considerable importance in plant and animal growth.

EXPERIMENTAL

Apparatus. All phosphorimetric measurements were taken with an Aminco-Bowman spectrophotofluorometer (No. 4-8202) with a phosphoroscope attachment (No. C 27-62140), a xenon lamp (No. 901 C-1), and a Varian nmr spinner attachment for the sample tube (Zweidinger and Winefordner, 1970); all items (except Varian spinner) were manufactured by American Instrument Co., Inc., Silver Spring, Md. All phosphorimetric studies were made with the spectrometer slit program: A 4 mm, B 3 mm, C 3 mm, D 4 mm, and E 3 mm. All spectra were recorded with an Aminco X-Y recorder (No. 1620-827, American Instrument Co., Inc.).

Reagents. All plant growth hormones were purchased in a growth factor analog kit (Nutritional Biochemicals Corp., Cleveland, Ohio). Absolute ethanol (Union Carbide Corp.), purified as previously described (Winefordner and Tin, 1964), was used as the solvent for all phosphorimetric measurements. Stock ethanolic solutions (approximately 10^{-3} *M*) of each compound were prepared; solutions of lower concentrations were prepared by successive dilution. Ethanol was used as the solvent because it is inexpensive, can be prepared in a highly pure state, and freezes to a clear, rigid glass at 77° K.

Procedures. Analytical curves (logarithm of phosphorimetric signal *vs.* logarithm of concentration) and limits of detection were obtained to determine whether a compound was analytically useful. The limit of detection was defined (St. John *et al.*, 1967) as the concentration giving a signal-to-noise ratio of $t\sqrt{2}/\sqrt{n}$. The noise is estimated to be one-fifth of the range of fluctuation in the background signal, t is the Student t, and n is the number of combined blank and sample measurements made at the limit of detection. With n equal to 6 and a 99% confidence level, the signal-to-noise ratio is approximately 2.

The lifetime, τ, was measured by terminating the exciting radiation with a manual shutter and plotting the phosphorimetric signal *vs.* time with the X–Y recorder. The response of the recorder prevented accurate τ measurements shorter than 0.5 sec.

RESULTS AND DISCUSSION

Twelve plant growth hormones having analytically useful phosphorescence are given in Table I. In addition to these 12 compounds, gibberelic acid, maleic acid hydrazide, β-(2-furyl)-acrylic acid and methyl-L-naphthalene acetate phos-

Table I. Phosphorescence Characteristics of Several Plant Growth Hormones

Hormone	Excitation maximum,[a,b] nm	Emission maximum,[a,b] nm	Lifetime,[c] sec	Concentration range of near linearity	Limit of detection (μg/ml)	Limit of detection (botanical test values) (μg/ml)
β-Naphthoxyacetic acid	328	497	2.6	10^{3d}	6×10^{-3}	1×10^{0e}
o-Chlorophenoxyacetic acid	280	518	0.7	10^{3d}	2×10^{-1}	2×10^{0f}
p-Chlorophenoxyacetic acid	283	396	<0.5	$10^{5d,g}$	4×10^{-3}	2×10^{-1f}
2,4-Dichlorophenoxyacetic acid	289	490	<0.5	$10^{5d,g}$	2×10^{-3}	2×10^{-2f}
Indole-3-acetic acid	290	438	<0.5	$10^{4d,g}$	2×10^{-2}	$1 \times 10^{-2f,h,i}$
3-Indolebutyric acid	284	510	0.6	$10^{5d,g}$	4×10^{-3}	2×10^{-2h}
3-Indolepropionic acid	290	440	0.6	10^{5d}	2×10^{-3}	
Ethyl 3-indoleacetate	290	440	3.3	$10^{4d,g}$	2×10^{-2}	
α-Naphthalene acetamide	297	513	2.5	10^{3d}	4×10^{-3}	
Naphthaleneacetic acid	295	510	2.8	10^{5d}	4×10^{-4}	1×10^{-2f}
2,4,5-Trichlorophenoxyacetic acid	294	473	1.1	$10^{6d,g}$	5×10^{-4}	2×10^{-3g}
2,4,5-Trichlorophenoxypropionic acid	294	467	<0.5	$10^{5d,g}$	3×10^{-3}	

[a] Peaks are uncorrected for instrumental characteristics. [b] Uncertainty wavelength of peaks is ± 5 nm. [c] Limiting value of lifetime (0.5 sec) is determined by recorder response time. [d] Lower concentration (limit of detection) is on linear portion of analytical curve in all cases. [e] Tomato ovary test (Leopold, 1955). [f] *Avena* straight growth test (Leopold, 1955). [g] Upper concentration (limit of detection) is on linear portion of analytical curve, and so actual useful upper concentration is greater than listed here. [h] Split pea test (Leopold, 1955). [i] *Avena* test (Leopold, 1955).

phoresced, but too weakly to be considered analytically useful. The plant growth hormone, 2-dodecenedioic acid, did not phosphoresce.

The limits of detection of the plant growth hormones listed in Table I compare favorably with those attained by currently accepted colorimetric and enzymic procedures discussed by Leopold (1955) and Nitsch and Nitsch (1956). Previous studies of conjugated ring system analogs show that phosphorescence can be enhanced by selective substitution on the ring(s) with corresponding improvement of the limits of detection. Therefore, the limits of detection found in Table I indicate promise and need for further work on analogs of pharmacological interest.

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