

(UV, IR, NMR, and mass) were consistent with Alkaloid 2 being lauroilsine. This was confirmed by direct comparison (TLC, UV, and IR spectra) with an authentic sample of lauroilsine and by TLC identification of the product of *M*-methylation as boldine. Attempts to crystallize Alkaloid 2 or its picrate were unsuccessful.

Isolation of Alkaloid 6—TLC examination showed Alkaloid 6 to be present mainly in Solution iii, with smaller amounts in Solution iv and traces in Solution ii. The alkaloids in Solution iii were partially resolved by column chromatography on sodium hydroxide-treated silicic acid (1) using benzene-methanol mixtures as eluant. Fractions containing Alkaloid 6 in admixture with traces of boldine, isoboldine, and norisocorydine were combined. The alkaloid was then obtained chromatographically pure by preparative TLC and was precipitated from methanolic solution by *n*-hexane as a light brown solid. Attempts to crystallize it from other solvents were unsuccessful. Further amounts were obtained similarly from Solutions ii and iv. Yield 133 mg. (0.003%). The UV spectrum [$\lambda_{\text{max}}^{\text{ethanol}}$ 282, 303, 312 (inflection) m μ] was markedly similar to that of Alkaloid 2 and was typical of published spectra of aporphines unstrained by lack of substitution at Position 11 (8). The alkaloid was shown to be phenolic by color tests, chemical behavior, and bathochromic shift of the UV maxima in alkali. The NMR spectrum indicated that the compound was a noraporphine [No. 3 proton peak at 2.35–2.55 δ (9)] and an *O*-methyl derivative of Alkaloid 2. This was confirmed by *O*-methylation of each alkaloid with diazomethane to a common nonphenolic product (characterized by TLC). That Alkaloid 6 is laurotetanine was confirmed by the identical

behavior on TLC of the product of *N*-methylation and an authentic sample of *N*-methyl laurotetanine. A picrate of Alkaloid 6 was prepared and crystallized from water as orange-yellow needles, m.p. 152–154° [lit. m.p. laurotetanine picrate 148° (10)].

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Keyphrases

Alkaloids—*Peumus boldus*
 Laurotetanine— isolation, identification
 Lauroilsine— isolation, identification
 Column chromatography— separation
 TLC— separation, identity
 UV spectrophotometry— identity
 IR spectrophotometry— identity
 NMR, mass spectroscopy— structure

Polyamide Layer Chromatography of Organophosphorus Pesticides

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Thin-layer chromatographic system and spray reagents are described for rapid differentiation of 11 organophosphorus pesticides.

THE USE of paper or thin-layer chromatography to determine and identify the organophosphorus pesticides had been widely studied and reviewed by Zweig (1). Recently, several workers used the formamide impregnated paper (2) and thin-layer (3) to separate organophosphorus pesticides. In previous reports (4, 5) better separation of opium alkaloids was obtained with polyamide layer, therefore, this method was applied to separate the

organophosphorus pesticides. This note describes the results obtained.

EXPERIMENTAL

Material—Eleven organophosphorus pesticides, FDA standard were used. The solvents and chemicals are the reagent grade of Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Thin-Layer Plates—All plates used were 15 × 15 cm. of polyamide layer sheet described by Wang (6).

Chromatographic Procedure—The standard techniques of ascending thin-layer chromatography (7) were employed.

Visualization—Four methods were employed: (a) iodine vapor exposing, (b) 5% alcoholic potassium hydroxide solution spraying, (c) iodoplatinate reagent spraying (8), (d) first spraying 5% alcoholic potassium hydroxide solution on the plate, allowing the plate to dry, then exposing the plate with iodine vapor for 5 min., followed by exposing ammonia vapor.

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TABLE I—CHROMATOGRAPHIC DATA

Pesticides	R_f Value Solvent Systems ^a				Color of Spot Visualization ^b			
	I	II	III	IV	1	2	3	4
2-Iso-propoxyphenyl- <i>N</i> -methylcarbamate	0.80	0.77	0.70	0.82	+	Y	—	W
Isopropylmethylpyrimidil diethylthiophosphate	0.87	0.75	0.45	0.59	+	Y	—	—
<i>O,O</i> -Diethyl- <i>S</i> -2 (ethylthio) ethylphosphorodithioate	0.62	0.25	0.30	0.36	+	Y	—	—
<i>O</i> -Ethyl- <i>p</i> -nitrophenyl-thiobenzene phosphate	0.40	0.12	0.10	0.20	+	—	Y	B
<i>O,O</i> -Dimethyl- <i>S</i> -4-oxo-1,2,3-benzotriazin-3-(4 <i>H</i>)-ylmethyl phosphorodithioate	0.65	0.48	0.35	0.54	+	Y	—	B
Phthalimido-methyl- <i>O,O</i> -dimethyl phosphorodithioate	0.65	0.45	0.85	0.54	+	Y	—	W
Dimethyl-paranitrophenylthiophosphate	0.58	0.38	0.25	0.50	+	—	Y	Y
<i>S</i> -1,2-Bis-(ethoxycarbonyl)-ethyl- <i>O,O</i> -dimethylphosphorodithioate	0.77	0.57	0.73	0.68	+	—	—	W
<i>O,O</i> -Dimethyl-(<i>O</i> -2,4,5-trichloro-phenyl) phosphorothioate	0.48	0.14	0.11	0.03	+	—	—	—
<i>S</i> -(<i>p</i> -Chlorophenylthiomethyl)- <i>O,O</i> -diethyl phosphorodithioate	0.35	0.09 ^c	0.10 ^c	0.11 ^c	+	Y	—	—
Diethyl-paranitrophenylthiophosphate	0.55	0.28	0.16	0.37	+	—	Y	B

^a I, isopropanol-water-formic acid (2:3:1); II, formaldehyde-formic acid (10:1); III, water-methanol-ammonia (4:5:2); IV, acetone-water-formamide (1:2:1); Loading, 1.0 mcg. in 0.01 ml. methanol. ^b Y, yellow; W, white; (+) positive action to detection reagent; (—) negative action to detection reagent; B, brown spot on yellowish background. ^c Tailing.

RESULTS AND DISCUSSION

R_f values for the organophosphorus pesticides are in Table I. The detection limit of each compound is approximately 0.1 mcg. with iodine vapor. It is possible to differentiate each compound by using the different response to the detection reagents. For example, 2-iso-propoxyphenyl-*N*-methylcarbamate failed to give a color spot when sprayed with alcoholic potassium hydroxide solution, but it gave a yellow spot with the iodoplatinate reagent. Also, 2-iso-propoxyphenyl-*N*-methylcarbamate was detected by using visualization method number four described above. This method produced a chromatogram characterized by a white spot on a brown-yellow background.

The experiment indicates that this method is very useful for identification of organophosphorus pesticides. It is convenient in handling the chromatograms and is highly reproducible. Another important feature of this polyamide layer chromatogram is a possible direct filing. The development of this

method as official to detect pesticides residues in vegetables is currently under investigation.

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 **Keyphrases**

Organophosphorus pesticides—analysis
TLC, polyamide—analysis