excess oxychloride was then removed *in vacuo* and the residue dissolved in water (5 ml.). After standing overnight, crystals were deposited; these were collected by filtration (0.42 g.). After recrystallization from dilute hydrochloric acid, the compound appeared as colorless prisms, m.p. 209–210° dec. The color reactions, m.p. and mixed melting point and the ultraviolet absorption spectrum were identical with those of an authentic sample of dihydroanhydroberberine methochloride.[2]

Treatment of the same base with acetic anhydride followed by recrystallization from dilute hydrochloric acid gave the same product.

Oxidation of Base-A with Potassium Permanganate.—The base (1 g.) was dissolved in acetone (500 ml.), and to this solution was added dropwise with stirring, a solution of potassium permanganate (0.31 g.) in acetone (200 ml.). The addition took 2 hr., and the solution was stirred for a further hour. The manganese dioxide was filtered off and washed with a little chloroform. The acetone solution and the chloroform wash were combined and evaporated to small bulk (*ca.* 100 ml.). The product started to separate at this point and the separation became complete on cooling. In all, 0.7 g. was collected. After recrystallization from methanol–ethyl acetate, it formed needles, m.p. 267° (dec. after darkening from *ca.* 245°).

Anal. Calcd. for $C_{21}H_{25}O_6N$: C, 65.1; H, 6.5; N, 3.6; 2 OMe, 16.1. Calcd. for $(C_{21}H_{24}O_4N)_2C_4H_6O_6$: C, 66.5; H, 6.5; N, 3.3; 4 OMe, 15.0. Found: C, 65.8, 65.6; H, 6.3, 6.3; N, 3.7, 3.5; OMe, 15.6.

A solution of the above compound in methanol gave, with picric acid, a picrate, m.p. 245–246° dec. This proved to be identical with an authentic sample of β-tetrahydromethylberberine picrate, m.p. 245° dec.

Anal. Calcd. for $C_{27}H_{26}O_{11}N_4$: C, 55.7; H, 4.5; N, 9.5. Found: C, 55.8; H, 4.6; N, 9.3.

With picrolonic acid a picrolonate was obtained, m.p. 244° (dec. after blackening from 230–235°).

Anal. Calcd. for $C_{31}H_{31}O_9N_5$: C, 60.4; H, 5.0; N, 11.3. Found: C, 60.6; H, 5.2; N, 11.6.

p-Toluenesulfonic acid gave a *p*-toluenesulfonate, m.p. 252°.

Anal. Calcd. for $C_{28}H_{31}O_7NS$: C, 64.6; H, 5.9; N, 2.7. Found: C, 64.4; H, 6.0; N, 2.5.

Oxidation of Base-A with Iodine and Silver Acetate.[16]—Silver carbonate (0.85 g.) was treated with glacial acetic acid (20 ml.) containing water (0.1 ml.). To this was added base-A (1 g.), and then iodine (0.72 g.) was added over 1 hr. The mixture was warmed at 90° for 2 hr. on the steam-bath. After filtration, the acetic acid was removed *in vacuo*. The residue was dissolved in water (*ca.* 15 ml.) and basified with sodium hydroxide solution. The solution was then extracted with chloroform and the aqueous layer acidified with concentrated hydrochloric acid, dihydroanhydroberberine methochloride (0.9 g.) separated. This material was identical in every respect with the previously described samples. The chloroform solution contained a little material, but no pure compound was isolated.

Conversion of the Amine Oxide (VIII) to *Allo*cryptopine (IV).—The amine oxide (1.0 g.), prepared as described by Haworth and Perkin[9] (except that monoperphthalic acid was used in place of perbenzoic acid) was dissolved in glacial acetic acid (5 ml.) and concentrated hydrochloric acid (2.5 ml.) added. The mixture was heated for 1 hr. on a rapidly boiling water-bath. The acids were then evaporated *in vacuo* and the residue dissolved in water and basified with sodium hydroxide. The precipitated solid was extracted with chloroform, the chloroform solution was washed and the chloroform removed. The residue was dissolved in methanol (*ca.* 2 ml.), the methanol solution diluted with ether (*ca.* 15 ml.) and acidified with a solution of hydrogen chloride in methanol. On scratching, a hydrochloride separated, m.p. 188–190° (dec. sinters 170°) (0.77 g. or 70%). The base was obtained by basification of this hydrochloride; it crystallized from ether in small colorless needles, m.p. 160–161°. The melting point was unchanged on admixture with an authentic sample of *allo*cryptopine.

The aqueous solution from the chloroform extraction was acidified with concentrated hydrochloric acid. On standing, dihydroanhydroberberine methochloride (0.1 g.) separated.

When the above procedure was carried out with the glycol VIII, m.p. 197° (0.61 g.), no basic material was obtained. The aqueous solution, on acidification with concentrated hydrochloric acid, deposited dihydroanhydroberberine methochloride (0.5 g.).

Paper Chromatography.—*Allo*cryptopine (0.02 mg.) in dry chloroform was spotted onto chromatogram paper, the spot was developed with butanol–water (86–14) in the usual manner overnight. Examination in ultraviolet light revealed a spot R_f 0.39, which fluoresced blue. Protopine gave a yellowish fluorescent spot R_f 0.24. A mixture of the two alkaloids could be separated on the paper. The synthetic *allo*cryptopine and the total basic fraction obtained from the amine oxide conversion showed only the blue spot R_f 0.39.

Ultraviolet Absorption Spectra.—Were measured with a Beckman model DU quartz spectrophotometer. The spectra were measured at a concentration of 10 mg./l. (cell length 1 cm.).

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The Basic Esters of Some Plant Growth Regulators

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The hydrochlorides and methohalides of the β-diethylaminoethyl esters of several plant growth regulators and of one inhibitor of plant growth hormonal activity have been synthesized. These compounds have been tested as plant growth regulators, as esterase substrates and as spasmolytic agents. In general, they have plant growth hormonal activity equal to or less than the parent acids, are not substrates for the non-specific esterases of rat serum and are much less effective than atropine in reducing acetylcholine-induced spasms.

Most spasmolytic agents are, like atropine, basic esters of carboxylic acids; many are basic esters of α,α-disubstituted acetic acid. In the acid moiety of such compounds there is often a region of high electron density insulated by a single carbon atom from the carbonyl double bond. Most of the plant growth regulators are α-monosubstituted acetic acids in which a region of high electron density is insulated by one or more carbon atoms or by a combination of oxygen and carbon atoms from the carbonyl double bond. The chemical and biological properties and the relationships between these two, for both spasmolytic agents and plant growth hormones, have been the subject of much research.[1,2]

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(2) J. Bonner, Harvey Lectures, Ser. 48, 1 (1954).

TABLE I

BASIC ESTERS OF SOME PLANT GROWTH REGULATORS

Ester: Acidic part	Alcoholic part[a]	M.p., °C.[b]	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Plant growth[c] activity	Spasmolytic[d] activity
2,4-Dichlorophenoxyacetic	—HCl	140–141[e]	47.1	47.0	5.65	5.64	=	<0.1
2,4-Dichlorophenoxyacetic	—CH_3I	102	39.0	39.4	4.80	4.98	=	..[f]
4-Fluorophenoxyacetic	—HCl	113	55.0	55.2	6.92	7.27	=	<0.1
4-Fluorophenoxyacetic	—CH_3I	134.5	43.8	43.3	5.64	5.53	=	..[f]
3-Indoleacetic	—HCl	112.5–113.5	61.8	61.9	7.46	7.35	<	<0.1
3-Indoleacetic	—CH_3Br	138–139	55.3	55.7	6.82	7.18	$\ll$ to <	0.3
3-Indolepropionic	—HCl	140.5–141.5	62.9	62.8	7.76	7.71	=	<0.1
α-Naphthaleneacetic	—HCl	128–129[g]	67.2	67.0	7.52	7.80	=	0.1–0.2
α-Naphthaleneacetic	—CH_3Br	147–149	60.0	59.7	6.89	7.19	0 to $\ll$	0.1–0.2
β-Naphthoxyacetic	—HCl	118.5–119.5	64.0	64.4	7.15	7.20	=	<0.1
β-Naphthoxyacetic	—CH_3I	70–74	51.5	51.3	5.91	5.88	<	..[f]
2,3,5-Triiodobenzoic	—HCl	183–185[h]	24.6	24.6	2.70	2.82	[i]	0.1–0.2
2,3,5-Triiodobenzoic	—CH_3I	187–188	22.7	23.1	2.58	2.79	[i]	..[j]

[a] — = $CH_2CH_2N(C_2H_5)_2$ in all cases. [b] Fisher–Johns melting point apparatus. [c] Relative to parent acid. [d] Atropine = 100. [e] Reported[4] m.p. 140–141°. [f] Unstable in water. [g] Reported[5] m.p. 128–130°. [h] Reported[3] m.p. 184–185°. [i] Inhibited plant growth activity of 3-indoleacetic acid to about same degree as parent compound, 2,3,5-triiodobenzoic acid. [j] Insoluble in water.

In the present investigation, the basic ester hydrochlorides of six plant growth regulators and the quaternary derivatives of five of these have been synthesized. In addition, the basic ester hydrochloride and the quaternary derivative of an inhibitor of plant growth activity (2,3,5-triiodobenzoic acid) have been synthesized. Most of these thirteen compounds have been tested as spasmolytic agents, as plant growth regulators and as substrates for the non-specific esterases of rat serum. The results of these tests are included in this report. Three of the compounds have been prepared previously: the β-diethylaminoethyl ester hydrochlorides of 2,3,5-triiodobenzoic acid,[3] 2,4-dichlorophenoxyacetic acid[4] and α-naphthaleneacetic acid.[5]

The properties of the compounds synthesized are given in Table I. None of the quaternary compounds was found to be enzymatically hydrolyzed by rat serum. However, the quaternary derivatives of the three aryloxyacetates were found to be unstable in water. Their hydrolyses appeared to proceed as first-order reactions. This aqueous instability probably accounts for the reduced yield of these three compounds as well as for the observation that the three had plant growth regulating properties equal or nearly equal to those of the parent acids. By comparison, the quaternary derivatives of 3-indoleacetic and α-naphthaleneacetic acids exhibited little or no epinasty. None of the compounds tested exhibited sufficient spasmolytic activity to be of pharmacological interest. These results indicate that, although there appears to be some structural resemblance between the plant growth regulators and the acid portions of many spasmolytic agents, such a resemblance is probably more fortuitous than fundamental.

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Experimental

Syntheses.—The basic ester hydrochlorides were prepared from freshly distilled β-diethylaminoethyl chloride and the appropriate carboxylic acid according to the procedure of Blicke and Kaplan.[6] Repeated washing of the crude product with anhydrous ether, recrystallization from absolute ethanol–ether and desiccation gave yields of 60 to 80% in all cases except that involving 2,3,5-triiodobenzoic acid where the yield on two occasions was not above 50%. For further purification the basic esters were released from the hydrochlorides, ether extracted, dried and precipitated from ethereal solutions with dry hydrogen chloride. The products were washed, recrystallized and desiccated as before. All yields were considerably lower, especially those of the aryloxyacetate esters.

The quaternary derivatives were prepared by the reaction of the released basic esters with an excess of either methyl bromide or methyl iodide in anhydrous ether. The products precipitated while standing for several days at room temperature and several days in the cold. They were washed with anhydrous ether, recrystallized from absolute ethanol–ether and desiccated. Yields were 70% or better except those involving the aryloxyacetate esters.

Biological Testing.—The plant growth hormonal activity of the synthesized compounds was compared to that of the parent carboxylic acid.[7] The quaternary derivatives were tested in the Warburg manometric apparatus[8] as substrates for the non-specific esterases of rat serum. Nine of the compounds were tested for their ability to prevent the spasms caused in the isolated guinea-pig ileum by acetylcholine chloride. The results were compared to those obtained using atropine sulfate.

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