An isomer with a lower melting point pptd from the Et_2O filtrate (283 mg, mp 197–202°). *Anal.* both isomers ($C_{24}H_{19}Cl_3N_4 \cdot HCl$), C,H,N.

3-(4-Chlorophenyl)crotonaldehyde.—This experiment follows the general procedure of Schmidle and Barnett.[15] $POCl_3$ (7.7 g, 0.050 mole) was added dropwise to 14.6 g (0.020 mole) of ice-cooled DMF at a rate that did not permit the temp of the mixture to rise above 20°. To this was added 7.6 g (0.05 mole) of α-methyl-*p*-chlorostyrene (freshly distilled). The temp of the mixture was raised slowly to 80°. After 1 hr at 80°, the mixture was cooled and 30 g of NaOAc, in a minimum amount of H_2O was added. Stirring was contd for 15 min and the mixture was reheated to 80° for 15 min. After chilling, the crude product was extd from the dark reaction mixture with Et_2O. Purification was effected by chromatography over silica gel, using $CHCl_3$ elution. The yield of yellow, oily product was 2.0 g (22%). It was not characterized beyond a favorable ir spectrum.

1,5-Bis(4-chlorophenyl)hexa-2,4-dien-1-one.—A mixture of 2.0 g (11 mmoles) of 3-(4-chlorophenyl)crotonaldehyde and 4.6 g (11 mmoles) of 4-chlorobenzoyltriphenylphosphonium methylide in dioxane was refluxed under N_2 for 12 hr. The solvent was removed *in vacuo* and the residue, in $CHCl_3$ soln, was chromatographed over silica gel. A dark orange, oily substance, probably another geometrical isomer (ir spectrum), preceded the yellow, cryst product from the column; yield, 0.5 g (14%). An anal. sample, mp 110–113°, was obtained by prep tlc and subsequently recrystd from petroleum ether. *Anal.* ($C_{18}H_{14}Cl_2O$) C, H.

1,1,3-Tris(4-chlorophenyl)-1-propen-3-one.—This procedure is a modification of the general method of Bergmann, *et al.*[16] A soln of 17 g (0.0683 mole) of 1,1-bis(chlorophenyl)ethylene and 13.1 g (0.0751 mole) of 4-chlorobenzoyl chloride was heated to 240°. At this temp HCl was evolved. The acid was swept from the flask by a slow stream of N_2 gas and monitored by bubbling through a NaOH soln (phenolphthalein). After about 18 hr, acid evolution had ceased. The resulting black, solid reaction mixture was extd with EtOH several times and the extracts were filtered. The solvent was removed from the combined filtrate and the residue was chromatographed on silica gel. Elution with CCl_4 yielded starting materials and by-products. Elution with $CHCl_3$ then gave 5.15 g of moderately pure product, which was recrystd several times with EtOH to yield 2.0 g of pure product, mp 135–140°. *Anal.* ($C_{21}H_{13}Cl_3O$) C, H.

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Antispasmodic Agents. 1. Syntheses and Pharmacological Activity of Aminoalkyl 3-Substituted Phenylacetates

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The pronounced pharmacological activities of atropine, scopolamine, and other aminoalkyl phenylacetate esters have stimulated the preparation and evaluation of numerous analogs of such compounds for their antispasmodic properties. Although molecular modifications of atropine and scopolamine have been prepared in the hope of improving pharmacological properties of the drugs, most of them are toxic and have side effects such as mydriasis, thirst, and flushing of cheeks. Since few studies[1,2] on the syntheses of aminoalkyl phenylacetates with substituents in the benzene ring have been reported, we synthesized 48 analogs with substituents such as OH, AcO, and MeO at the 3 position in order to examine the effects of substitution in the benzene ring for antispasmodic activities *in vitro*.

The 3-substituted phenylacetic acids were prepared by alkaline hydrolysis of the substituted phenylacetonitriles. Among these acids, VIII[3] and XIII,[4] respectively, were identical with authentic samples. The nitriles were prepared in good yield by the benzyne reaction[5a,b] between 2-chloroanisole (II) and the required nitrile in the presence of $NaNH_2$ in liquid NH_3. α-*sec*-Butyl-3-hydroxyphenylacetic acid(XV) was synthesized by the condensation of 3-methoxyphenylacetonitrile (VII) with *sec*-BuBr in the presence of $NaNH_2$, followed by hydrolysis with methanolic KOH. On the usual work-up of VII with acid or alkali, only starting material was recovered. O-Methylation of XV with Me_2SO_4 afforded XII. These 3-substituted phenylacetic acids were converted into the corresponding aminoalkyl esters as follows; (A) condensation of carboxylic acids with aminoalkyl halide with the use of NaOEt; (B) condensation of acid chlorides (XVI) with aminoalkylcarbinol; and (C) condensation of haloalkyl ester (XVII), prepared from XVI, with secondary amines. Compounds **47** and **48** were acetylated with Ac_2O to give the corresponding *O*-acetates. The 3-OH compounds were converted into the 3-OCH_3 derivatives by CH_2N_2.

Pharmacology.—Table I gives the results of screening for antispasmodic and anticholinergic activities. The compounds were tested by the Magnus guinea pig ileum screen.[6] Although all the compounds were inferior to atropine sulfate in anticholinergic activity, almost half of them showed a stronger antispasmodic effect than papaverine hydrochloride. Among them, three compounds, **7**, **13**, and **30** were 10 times more effective than papaverine·HCl.

Experimental Section[7]

3-Methoxyphenylacetonitriles. General Procedure.—To a stirred solution of $NaNH_2$ (prepared from 3 moles of Na in 1.5 l. of liq NH_3 with $FeCl_3$) was added carefully 1.7–1.8 moles of nitrile within 5–10 min, and 0.8 mole of 2-chloroanisole was then added rapidly. After the mixture had been stirred for another 1.5 hr, excess $NaNH_2$ was decompd by addn of 100–120 g of NH_4Cl. The resultant mixture was poured into H_2O and extracted (PhH). The extract was evapd to give a brown oil, which was dissolved *in vacuo* to afford the corresponding phenylacetonitrile. Yields and physical constants of the compds prepared are shown in Table II.

α-*sec*-Butyl-3-methoxyphenylacetonitrile (VII).—A stirred mixture of 17 g of 3-methoxyphenylacetonitrile (III) and 5.4 g of

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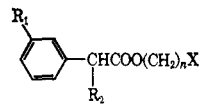
(7) Melting points were measured in capillary tubes in a bath and uncorrected. Ir spectra were determined on a Shimazu spectrometer and nmr data on a JNM-MH-60 instrument (TMS).

TABLE I: SYNTHESES AND CHARACTERISTICS OF AMINOALKYL 3-SUBSTITUTED PHENYLACETATE



Compd	R_1	R_2	X	n	Derivative	Mp or bp, °C (mm)	Method	Formula[i]	Relative activity[a] *in vitro*: Activity relative to papaverine·HCl = 1	Relative activity[a] *in vitro*: Anticholinergic activity relative to atropine = 1
1	OCH_3	H	$N(CH_3)_2$	2	OX[f]	124–125	A	$C_{13}H_{19}NO_3 \cdot C_2H_2O_4$	0.123	
2	OCH_3	H	$N(C_2H_5)_5$	2	Oil	140–145 (0.5)	B	$C_{15}H_{23}NO_3$	0.275	0.009
3	OCH_3	H	C_4H_8N[b]	2	OX[f]	151–153	B	$C_{15}H_{21}NO_3 \cdot C_2H_2O_4$	0.152	0.003
4	OCH_3	H	$C_5H_{10}N$[c]	2	OX[f]	92–93	C	$C_{16}H_{23}NO_3 \cdot C_2H_2O_4$	0.561	0.002
5	OCH_3	H	C_4H_8NO[d]	2	Oil	182–186 (1.5)	C	$C_{15}H_{21}NO_4$	1.727	0.001
6	OCH_3	H	$C_6H_5CH_2NCH_3$	2	OX[f]	95–96	C	$C_{19}H_{23}NO_3 \cdot C_2H_2O_4$	0.156	0.002
7	OCH_3	H	$N(CH_3)_2$	3	OX[f]	113–114	A	$C_{14}H_{21}NO_3 \cdot C_2H_2O_4$	11.148	0.012
8	OCH_3	H	$N(C_2H_5)_2$	3	OX[f]	73–74	B	$C_{16}H_{25}NO_3 \cdot C_2H_2O_4$	2.657	0.007
9	OCH_3	H	C_4H_8N[b]	3	OX[f]	107–108	C	$C_{16}H_{23}NO_3 \cdot C_2H_2O_4$	0.031	
10	OCH_3	H	$C_5H_{10}N$[c]	3	OX[f]	138.5–140	C	$C_{17}H_{25}NO_3 \cdot C_2H_2O_4$	1.041	0.005
11	OCH_3	H	C_4H_8NO[d]	3	OX[f]	122–123.5	C	$C_{16}H_{23}NO_4 \cdot C_2H_2O_4$	1.838	0.032
12	OCH_3	H	$C_6H_5CH_2NCH_3$	3	HCl	95–96.5	C	$C_{20}H_{25}NO_3 \cdot HCl$	4.284	0.029
13	OCH_3	CH_3	$N(CH_3)_2$	2	OX[f]	137–138	A	$C_{14}H_{21}NO_3 \cdot C_2H_2O_4$	14.038	0.011
14	OCH_3	CH_3	$N(C_2H_5)_2$	2	Oil	150–155 (0.5)	A	$C_{16}H_{25}NO_3$	1.004	0.035
15	OCH_3	CH_3	C_4H_8N[b]	2	OX[f]	120–121	A	$C_{16}H_{23}NO_3 \cdot C_2H_2O_4$	0.232	
16	OCH_3	CH_3	$N(CH_3)_2$	3	OX[f]	88–90	A	$C_{15}H_{23}NO_3 \cdot C_2H_2O_4$	0.645	0.002
17	OCH_3	CH_3	$N(C_2H_5)_2$	3	OX[f]	81–83	A	$C_{17}H_{27}NO_3 \cdot C_2H_2O_4$	1.373	0.003
18	OCH_3	CH_3	C_4H_8N[b]	3	OX[f]	72–73	A	$C_{17}H_{25}NO_3 \cdot C_2H_2O_4$	1.446	
19	OCH_3	C_2H_5	$N(CH_3)_2$	2	OX[f]	113–115	A	$C_{15}H_{23}NO_3 \cdot C_2H_2O_4$	4.865	
20	OCH_3	C_2H_5	$N(C_2H_5)_2$	2	OX[f]	75–77	A	$C_{17}H_{27}NO_3 \cdot C_2H_2O_4$	0.796	0.012
21	OCH_3	C_2H_5	C_4H_8N[b]	2	OX[f]	128–130	A	$C_{17}H_{25}NO_3 \cdot C_2H_2O_4$	3.892	0.012
22	OCH_3	*sec*-C_4H_9[e]	$N(CH_3)_2$	2	OX[f]	143–144	A	$C_{17}H_{27}NO_3 \cdot C_2H_2O_4$	0.048	0.019
23	OCH_3	*sec*-C_4H_9[e]	$N(C_2H_5)_2$	2	OX[f]	89–91	A	$C_{19}H_{31}NO_3 \cdot C_2H_2O_4$	1.386	0.069
24	OCH_3	*sec*-C_4H_9[e]	C_4H_8N[b]	2	OX[f]	155–156.5	A	$C_{19}H_{29}NO_3 \cdot C_2H_2O_4$	3.288	0.017
25	OCH_3	*sec*-C_4H_9[e]	$N(CH_3)_2$	3	OX[f]	113–114.5	A	$C_{18}H_{29}NO_3 \cdot C_2H_2O_4$	1.262	0.059
26	OCH_3	*sec*-C_4H_9[e]	$N(C_2H_5)_2$	3	OX[f]	78–80	A	$C_{20}H_{33}NO_3 \cdot C_2H_2O_4$	0.772	0.058
27	OCH_3	*sec*-C_4H_9[e]	C_4H_8N[b]	3	OX[f]	82–84	A	$C_{20}H_{31}NO_3 \cdot C_2H_2O_4$	1.093	0.023
28	OCH_3	C_6H_5	$N(CH_3)_2$	2	OX[f]	157–159	A	$C_{19}H_{23}NO_3 \cdot C_2H_2O_4$	0.240	0.053
29	OCH_3	C_6H_5	$N(C_2H_5)_2$	2	OX[f]	106–108	A	$C_{21}H_{27}NO_3 \cdot C_2H_2O_4$	2.222	0.021
29					HCl	92–94		$C_{21}H_{27}NO_3 \cdot HCl$		
29					CH_3Br	119–121		$C_{21}H_{27}NO_3 \cdot CH_3Br$		
30	OCH_3	C_6H_5	C_4H_8N[b]	2	OX[f]	148–149	A	$C_{21}H_{25}NO_3 \cdot C_2H_2O_4$	16.132	0.121
30					HCl	95–96.5		$C_{21}H_{25}NO_3 \cdot HCl$		
30					CH_3Br	119–121		$C_{21}H_{25}NO_3 \cdot CH_3Br$		
31	OCH_3	C_6H_5	$N(CH_3)_2$	3	OX[f]	130–132	A	$C_{20}H_{25}NO_3 \cdot C_2H_2O_4$	0.499	0.213
32	OCH_3	C_6H_5	$N(C_2H_5)_2$	3	OX[f]	73–75	A	$C_{22}H_{29}NO_3 \cdot C_2H_2O_4$	1.638	0.025
33	OCH_3	C_6H_5	C_4H_8N[b]	3	OX[f]	146–147	A		1.555	0.018
34	OH	H	$N(CH_3)_2$	2	Me[g,h]		A		0.044	0.001
35	OH	H	$N(C_2H_5)_2$	2	Me[g,h]		A		0.624	0.002
36	OH	H	C_4H_8N[b]	2	Me[g,h]		A		0.079	
37	OH	H	$N(CH_3)_2$	3	Me[g,h]		A		0.007	
38	OH	H	$N(C_2H_5)_2$	3	Me[g,h]		A		0.106	0.001
39	OH	H	C_4H_8N[b]	3	Me[g,h]		A		0.018	
40	OH	*sec*-C_4H_9[e]	$N(CH_3)_2$	2	Me[g,h]		A		1.079	0.014
41	OH	*sec*-C_4H_9[e]	$N(C_2H_5)_2$	2	Me[g,h]		A		2.116	0.017
42	OH	*sec*-C_4H_9[e]	C_4H_8N[b]	2	Me[g,h]		A		3.192	0.013
43	OH	*sec*-C_4H_9[e]	$N(CH_3)_2$	3	Me[g,h]		A		1.102	0.025
44	OH	*sec*-C_4H_9[e]	$N(C_2H_5)_2$	3	Me[g,h]		A		1.794	0.732
45	OH	*sec*-C_4H_9[e]	C_4H_8N[b]	3	Me[g,h]		A		0.279	0.022
46	OH	C_6H_5	$N(C_2H_5)_2$	2	CH_3Br[h]	159–162	A	$C_{20}H_{25}NO_3 \cdot CH_3Br$	0.312	0.022
47	$OCOCH_3$	*sec*-C_4H_9[e]	$N(C_2H_5)_2$	2	Oil	145–149 (0.2)	A	$C_{20}H_{31}NO_4$	3.327	0.041
48	$OCOCH_3$	C_6H_5	$N(C_2H_5)_2$	2	Oil		A	$C_{22}H_{27}NO_4$	1.344	0.012

[a] Papaverine·HCl depressed the contraction (50%) at 4×10^{-4} *M* $BaCl_2$ = 1. Ratio of papaverine·HCl vol of test sample showing the same effect is its antispasmodic activity. For anticholinergic activity, atropine sulfate which depressed the contraction at 1×10^{-6} *M* of ACh to the extent of 50% = 1; the ratio between the test samples and the standard was measured as above. [b] C_3H_8N; pyrrolidino. [c] $C_5H_{10}N$; piperidino. [d] C_4H_8NO; morpholino. [e] *sec*-C_4H_9; *sec*-Bu. [f] Oxalate. [g] These compounds did not solidify nor did several of salts. Therefore, after methylation of these compds with CH_2N_2, they were identified spectroscopically. [h] Compounds **34–46** were prepd by method A; since a slight excess of NaOEt (1.1 moles) was used, these oily substances were purified by repeated silica gel chromatography and showed one spot on tlc. They were very hygroscopic and tested for pharmacological activity as HCl salts. [i] All compounds (except **34–45**, see footnotes *g*, *h*) were analyzed for C, H, N.

SCHEME I

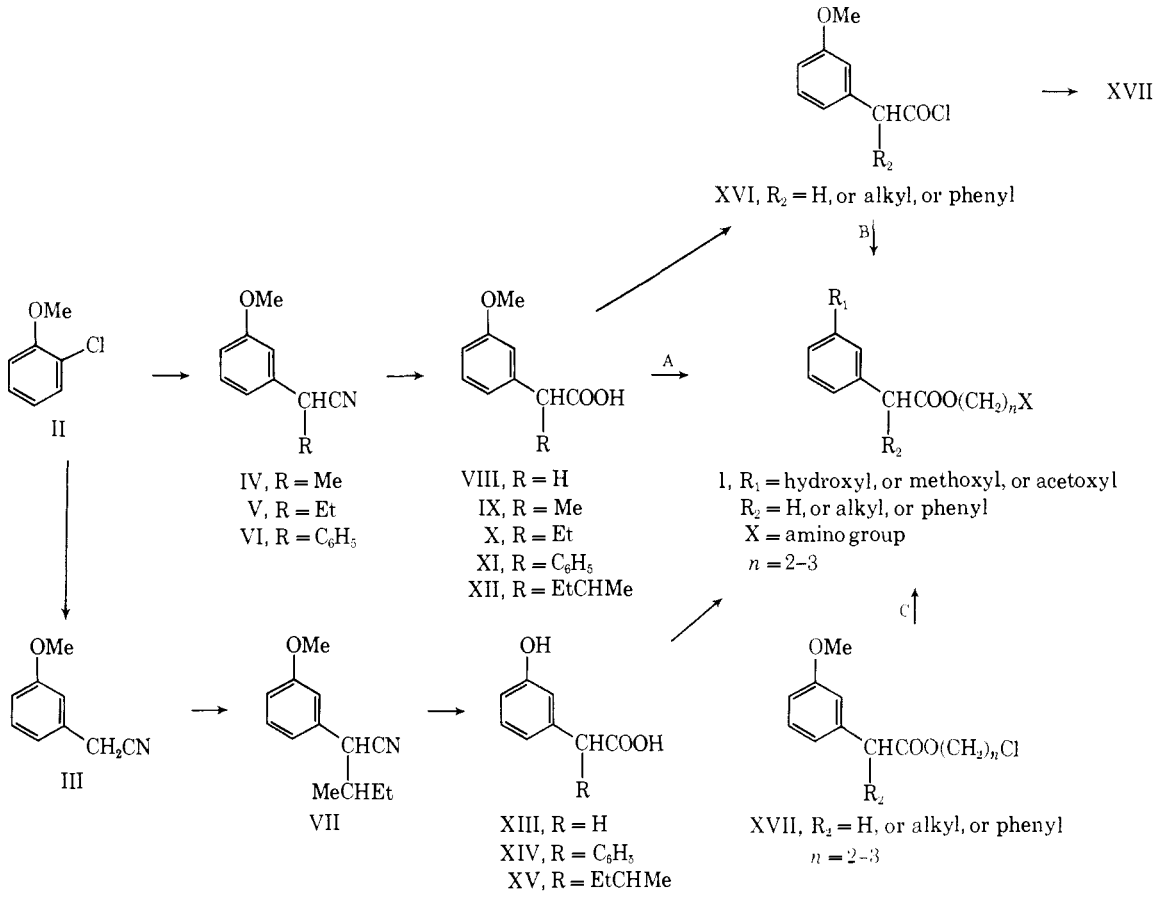
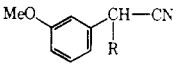


TABLE II
BENZYNE REACTION OF 2-CHLOROANISOLE



Compd	R	Bp, °C (mm)	Yield (%)
III	H	128–131 (4)[a]	55.6
IV	Me	120–123 (4)[a]	32.5
V	Et	104–106 (0.2)[a]	27.5
VI	Pr	165–170 (0.2)[b]	30.5

[a] Colorless oil. [b] Pale yellow oil.

$NaNH_2$ in 150 ml of dry PhH was refluxed for 2.5 hr, to which was added dropwise 20 g of *sec*-BuBr. The stirring was then continued for 4 hr. After cooling, excess $NaNH_2$ was decompd with H_2O and the material extd (PhH). The extract was washed (H_2O), dried (Na_2SO_4), and evapd. The resulting residue was distilled *in vacuo* to give 18 g (76%) of VII as a yellowish oil: bp 135–139° (0.25 mm); ir (liquid) 2230 cm^{-1} (C≡N); nmr ($CDCl_3$) δ 7.45–6.65 (m, 4 H, ArH), 3.79 (s, 3 H, OCH_3), 3.65 (d, 1 H, CHCN), 2.0–0.6 [m, 9 H, $CHCH_3(CH_2CH_3)$].

2-(3-Methoxyphenyl)propionic Acid (IX).—A stirred soln of 68 g of 2-(3-methoxyphenyl)propionitrile (IV) in 260 ml of 35% KOH was heated at 160° for 4 hr. After cooling, the mixture was dild with H_2O and washed (PhH). The aq layer was acidified (HCl) and extd (Et_2O). The extract was washed (H_2O), dried (Na_2SO_4), and evapd. The remaining residue was distd *in vacuo* to give 50 g (66%) of IX as a pale yellowish oil: bp 178–180° (0.5 mm); ir (liquid) 1705 cm^{-1} (C=O); nmr ($CDCl_3$) δ 11.1 (broad s, 1 H, COOH), 7.6–6.9 (m, 4 H, ArH), 3.88 (s, 3 H, OCH_3), 3.78 (q, 1 H, >$CHCH_3$, J = 7 Hz), 1.50 (d, 3 H, >$CHCH_3$, J = 7 Hz).

2-(3-Methoxyphenyl)butyric Acid (X).—A stirred mixture of 18 g of 2-(3-methoxyphenyl)butyronitrile (V) and 80 ml of 40% KOH was heated at 160° for 10 hr. After the same work-up as IX, evapn of the extract, followed by distn of the resulting residue *in vacuo*, gave 9.6 g (48%) of X as a colorless oil: bp 155–156° (0.4 mm); ir (liquid) 1705 cm^{-1} (C=O); nmr ($CDCl_3$) δ 11.0 (broad s, 1 H, COOH), 7.4–6.5 (m, 4 H, ArH), 3.88 (s, 3 H, OCH_3); 3.34 (t, 1 H, $CHCH_2$, J = 7 Hz), 1.85 (q, 2 H, CH_2CH_3, J = 7 Hz), 0.90 (t, 3 H, CH_2CH_3, J = 7 Hz).

2-(3-Methoxyphenyl)phenylacetic Acid (XI).—A stirred suspension of 150 g of 2-(3-methoxyphenyl)phenylacetonitrile (VI) in 150 ml of 60% KOH was heated under reflux for 6 hr. After cooling, the mixture was diluted with H_2O and washed (Et_2O). The aq layer was made acidic (HCl) and extd (Et_2O). The extract was washed (H_2O), dried (Na_2SO_4), and evapd. The resulting residue was recrystd from PhH–petr ether to give 142 g (87.5%) of XI as colorless prisms: mp 104–105°; ir (KBr) 1705 cm^{-1} (C=O). *Anal.* ($C_{15}H_{14}O_3$) C, H.

α-*sec*-Butyl-3-methoxyphenylacetic Acid (XII).—To a stirred soln of 10 g of α-*sec*-butyl-3-hydroxyphenylacetic acid (XV) in 10 ml of 33% KOH was added 10 ml of Me_2SO_4. After the stirring had been continued for 30 min, 10 ml of 33% KOH and 10 ml of Me_2SO_4 were added, after 30 min, the same treatment was repeated, and then 40 ml of 33% KOH was added. After another hour, the reaction mixture was extd (Et_2O). The extract was washed (H_2O), dried (Na_2SO_4), and evapd. A suspension of the remaining residue in 50 ml of 10% ethanolic KOH was refluxed for 3 hr. After the usual work-up, the crude product was recrystd from hexane to give 8.3 g (79%) of XII as colorless prisms: mp 78–80°; ir (KBr) 1703 cm^{-1} (C=O). *Anal.* ($C_{13}H_{18}O_3$), C, H.

2-(3-Hydroxyphenyl)phenylacetic Acid (XIV).—A mixture of 7 g of XI, 20 ml of 47% HBr, and 20 ml of AcOH was refluxed for 2 hr. After the same work-up as above, recrystn of the crude product from PhH–EtOH afforded 5.6 g (85%) of XIV as colorless prisms, mp 143–144° (lit.[8] mp 144°).

α-*sec*-Butyl-3-hydroxyphenylacetic Acid (XV).—A stirred mixture of 10 g of VII, 20 g of KOH, 20 ml of H_2O, and 60 ml of MeOH was heated at 160–180° in a sealed tube (pressure, 25 kg/cm^2) for 20 hr. The mixture was washed (Et_2O) after an addition of H_2O. The resulting aq layer was made acidic (concd HCl) and extd (Et_2O). The extract was washed (H_2O), dried (Na_2SO_4), and evapd to give 7.5 g (76%) of XV as a pale yellowish oil: ir (liquid) 1700 cm^{-1} (C=O); nmr ($CDCl_3$) δ 8.7 (broad s, 2

(8) I. N. Somin. *Zh. Obsch. Khim.*, **32**, 3788 (1962).

H, OH, COOH), 7.40–6.66 (m, 4 H, ArH), 3.23 (d, 1 H, CHCOO), 2.27–0.5 (m, 9 H, $CHCH_3CH_2CH_3$).

3-Substituted Phenylacetyl Chloride (XVI).—To a soln of 3-substituted phenylacetic acid (1 mole) in dry Et_2O was added $SOCl_2$ (3 moles), and the mixture was refluxed for 3 hr. Evaporation of the solvent gave the crude chloride (XVI) which was used for the following reaction without purification.

Haloalkyl 3-Substituted Phenylacetate (XVII).—To a soln of chloride XVI (1 mole) in dry PhH was added a mixture of haloalkylcarbinol (1.1 moles) and pyridine (1 mole) and the resultant mixture was refluxed for 5 hr. After the evapn of the solvent, the resulting residue was extd (Et_2O) after addn of H_2O. The extract was washed (H_2O), dried (Na_2SO_4), and evapd. The residual oil was distd *in vacuo* to give XVII.

Amino 3-Substituted Phenylacetates (I). General Procedure. A.—A mixture of carboxylic acid (1 mole), aminoalkyl chloride (1.1 moles), and NaOEt (prepared from 1.1 g-atoms of Na) in EtOH was refluxed for 3 hr, and the solvent was evapd. The resulting residue was extd (Et_2O) after addition of H_2O. The extract was washed (H_2O), dried (Na_2SO_4), and evapd to give the crude compd I, which was purified by distillation *in vacuo*, or column chromatography, or formation of salt such as the oxalate.

B.—A mixture of acid halide (XVI, 1 mole), pyridine (1 mole), and aminoalkylcarbinol (1.1 moles) in PhH was refluxed for 5 hr. The solvent was evapd and the resulting residue was extd (Et_2O) after the addn of H_2O and 5% NaOH. Work-up as in method A gave I.

C.—A mixture of the ester (XVII, 1 mole) and secondary amine (2 moles) was heated on a water bath for 5 hr. Treatment as in B gave I.

O-Methylation of 3-Hydroxy Derivatives.—To a soln of aminoalkyl 3-hydroxyphenylacetate in Et_2O was added excess CH_2N_2 in Et_2O and the mixture was allowed to stand in the refrigerator for 3 days. Evaporation of the solvent, followed by purification by column chromatography on silica gel, gave the 3-methoxy derivatives.

Diethylaminoethyl 2-(3-Acetoxyphenyl)phenylacetate.—A mixture of 1 g of diethylaminoethyl 2-(3-hydroxyphenyl)phenylacetate, 5 ml of Ac_2O, and 1 drop of pyridine was heated on a water bath for 2 hr. Excess Ac_2O was evapd and the residue was extd (Et_2O). The extract was washed (H_2O), dried (Na_2SO_4), and evapd. The remaining residue was chromatographed on silica gel to give 0.7 g (62.5%) of the acetate as a pale yellowish oil.

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Inhibition of Leucine Aminopeptidase by Halide Complexes of Platinum

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Leucine aminopeptidase levels have been found to be increased in tumors.[1] Recently Rosenberg,[2] *et al.*, reported that some halide complexes of Pt inhibited sarcoma 180 and leukemia L1210 in mice. In order to find a chemical basis for the sarcoma 180, leukemia L1210 inhibition, we have tested some Pt–halide complexes for inhibitory action on purified swine leucine aminopeptidase[3] (LAP).

LAP is a metal-requiring enzyme which has been shown to be active in the Mg^{2+} or Mn^{2+} form and more recently in the Zn^{2+} form by Himmelhoch.[4] The enzyme catalyzes stepwise hydrolysis from the N-terminal end of a polypeptide chain liberating free amino acids.

In the enzyme assay procedure with KBr added in the same concentration as the $PtBr_2$ complexes, no inhibition was observed in 50 hr at 37°. The 5×10^{-3} *M* tetrabromo complex of Pt inhibited completely the LAP within 1 hr. The ethylenediamine dibromo complex, Pt(En)Br_2, of Pt (5×10^{-3} *M*) resulted in over 80% inhibition of the LAP in 50 hr. The diethylenetriamine monobromo, Pt(Dien)Br^{1+} complex, of (5×10^{-3} *M*) resulted in about 20% inhibition of LAP in 50 hr.

Rosenberg *et al.*,[2] found that the most active antitumor compounds were *cis*-Pt$(NH_3)_2Cl_4$; *cis*-Pt$(NH_3)_2Cl_2$; Pt$(NH_2CH_2CH_2NH_2)Cl_2$ and Pt$(NH_2CH_2CH_2NH_2)Cl_4$. Spikes and Hodgson[5] also found the $PdCl_2$ inhibited chymotrypsin and trypsin, but did not inhibit catalase, lysozyme, peroxidase, and ribonuclease at 1×10^{-3} *M* Pd^{2+} concentrations.

The structures of the Pt complexes employed in this study are illustrated in Figure 1. It has been frequently observed that halide ligands are more labile than amine ligands in substitution reactions of Pt(II) complexes. In fact many workers have utilized this effect in order to study substitution reactions of halide ligands. For example, Gray[6] has reported the results of substitution studies for a single halide ligand in Pt complexes in which the other 3 coordination positions were blocked by the tribasic amine, diethylenetriamine. It is very noticeable that the rates of inhibition of LAP by the 3 platinum complexes used in this study increase with an increasing number of halide ligands. As can be seen in Figure 2, $PtBr_4^{2-}$ completely deactivates the enzyme much more rapidly than Pt(En)Br_2. Pt-

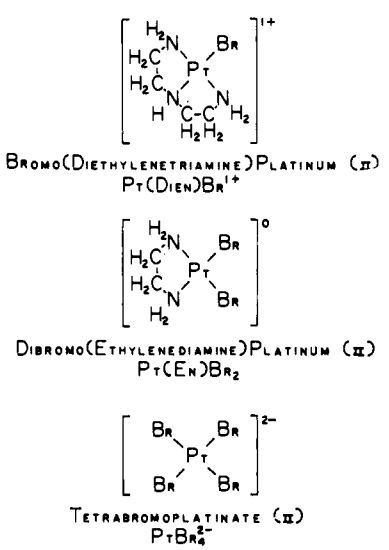

Figure 1.

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