latter. Our agreement with Cragwall's work is illustrated in Fig. 1.

Excess recrystallized *p*-aminophenol was added to previously boiled distilled water (to expel dissolved oxygen) in a 200-ml. flask placed in a thermostated oil-bath. The system was again boiled by evacuation (to remove oxygen in the vapor phase), sealed off and allowed to equilibrate with occasional hand agitation. Samples were taken in a tared pipet and weighed, and the amine content was determined by the nitrite procedure.[5]

The solubility of *p*-aminophenol in methyl ethyl ketone (2-butanone) at 58.5° was determined in a manner similar to that above except that no effort was made to exclude oxygen. The solubility was found to be 9.1 and 9.3 weight % by successive determinations.

(5) A 0.5-g. *p*-aminophenol sample was dissolved in 600 ml. of distilled water containing 10 g. of potassium bromide and 25 ml. of concentrated hydrochloric acid. Tenth-normal sodium nitrite was added in 5-ml. portions until indication of an excess (starch–iodide paper), persistent for 15 minutes, was obtained. The solution was then back titrated with 0.1 *N* sulfanilic acid and finally adjusted with more of the 0.1 *N* sodium nitrite. A faint positive starch–iodide test one minute after the last addition was taken as the end-point.

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Preparation of Testosteronephosphoric Acid[1]

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Testosteronephosphoric acid was required for testing as a possible intermediate in the metabolism of testosterone by prostatic tissue. Testosteronephosphoric acid had been obtained before from the reaction of testosterone with phosphorus pentachloride,[2] from 4-androsten-3,17-dione in a four-step sequence[3] and from the phosphorylation of testosterone with phosphorus oxychloride in pyridine.[4] The first two methods, although carefully defined, did not appear particularly attractive, whereas the third method, although of possible value, was published barren of detail. We have now tried the phosphorus oxychloride method and have found it to be satisfactory. A description of this direct phosphorylation, which makes testosteronephosphoric acid readily and conveniently available from testosterone, is given below.

Experimental[5]

Into a 250-ml. round-bottomed flask provided with a magnetic stirrer were placed 1.160 g. (4.00 millimoles) of testosterone, 5 ml. of pure dry pyridine and 50 ml. of sodium-dry ether. With the flask in a bath at −15 to −10° and with continuous stirring, a solution of 0.40 ml. (*ca.* 8.0 millimoles) of freshly distilled phosphorus oxychloride in 25 ml. of sodium-dry ether was added dropwise. The reaction mixture was protected from atmospheric moisture by a calcium chloride tube. After the addition, which required one-half hour, the mixture was stirred at −10° for two hours and was then allowed to come to room temperature (25°) and to stand at this temperature for an additional four hours. Addition of 100 ml. of ice-cold distilled water dissolved the white precipitate. After stirring the hydrolysis mixture for one hour, 50 ml. of ether was added. The ether layer was removed, and was washed with a 50-ml. portion of 0.4% sodium hydroxide solution. The sodium hydroxide washings were combined with the first aqueous phase. Acidification of the aqueous alkaline solution with 2% hydrochloric acid afforded a white precipitate, which was removed by filtration and dried in a desiccator. The crude testosteronephosphoric acid (1.250 g.) was dissolved in approximately 60 ml. of 0.5% aqueous sodium hydroxide, the solution was filtered and the clear filtrate was acidified with 2% hydrochloric acid. The white solids were collected on the filter, and were crystallized twice from approximately 50-ml. portions of 50–60% aqueous methanol. The pure white testosteronephosphoric acid monohydrate so obtained (1.010 g. or 65% yield) melted with vigorous evolution of bubbles at 157–159°. Shrinking and softening was noted at 135–138°, and transformation to an opalescent semi-solid was observed at 138–143°.

Anal. Calcd. for $C_{19}H_{29}O_5P \cdot H_2O$; C, 59.05; H, 8.08; P, 8.01. Found: C, 59.31; H, 8.14.

A 2×10^{-5} *M* solution of the testosteronephosphoric acid in 95% ethanol showed an absorption maximum at 240 mμ (ϵ 1.69×10^4). The material (0.0094 g. in 1.00 ml. of absolute methanol) showed $[\alpha]^{27.6}D$ 72.6°. The melting point of testosteronephosphoric acid monohydrate has been reported before as 160° dec.,[2] 155–157° dec.[3] and 150°,[4] and the specific rotation as $[\alpha]^{20}D$ 71.9°.[3] **Dimethyl testosterone phosphate**, as a 10^{-4} *M* ethanolic solution, was reported before with λ_{max} 238 mμ and ϵ 1.58×10^4 (approximate values).[3]

A sample of the testosteronephosphoric acid that had been crystallized a third time showed no change in melting point behavior. The material was dried at 50° *in vacuo* for two hours before analysis.

Anal. Found: C, 59.27; H, 7.81; P, 7.80.

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Antispasmodics. I. α-Amino-α-phenylacetamides

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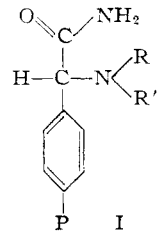
As part of a pharmacological screening program involving various basic phenylacetonitriles and derivatives, a number of amides, represented by the general formula I, where R = alkyl or H, R′ = alkyl, and P = H, CH_3 or OCH_3, were prepared.



P I

The amides recorded in Table I possess musculotropic antispasmodic properties. The *p*-methoxy-substituted compounds are more active than the unsubstituted or *p*-methyl-substituted analogs. α-Dibutylamino-α-(*p*-methoxyphenyl)-acetamide

(1) This work was supported by an Institutional Grant from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council, and was carried out as part of a coöperative program on cancer, with the Departments of Chemistry and Biology and the Boston University Medical School participating.

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(5) Elementary analyses were performed by Dr. Stephen M. Nagy and his staff at the Massachusetts Institute of Technology Microchemical Laboratory, Cambridge, Mass.

TABLE I

	Substituents P	R	R′	Mol. formula	M.p., °C.	Mol. wt. Found	Mol. wt. Calcd.	N, % Found	N, % Calcd.	Crude yield[a] Proc. A	Crude yield[a] Proc. B	Ref.
1	H	H	Cyclohexyl	$C_{14}H_{20}N_2O$	115–117	237	232.3	11.88	12.06	15%	33%	...
2	H	CH_3	CH_3	$C_{10}H_{14}N_2O$	150–153	178	178.2	15.63	15.72		60	...
3	H	CH_3	Cyclohexyl	$C_{15}H_{22}N_2O$	158–160	248	246.3	11.48	11.37		62	...
4	H	C_2H_5	C_2H_5	$C_{12}H_{18}N_2O$	140–142	206	206.3	13.72	13.58		69	6, 12
5	H	$-(CH_2)_3-CH_3$	$-(CH_2)_3-CH_3$	$C_{16}H_{26}N_2O$	145–146	264	262.4	10.88	10.68	68	97	6
6	H	$-CH_2-CH-(CH_3)_2$	$-CH_2-CH-(CH_3)_2$	$C_{16}H_{26}N_2O$	109–110	260	262.4	10.59	10.68		80	...
7	H	Piperidino		$C_{13}H_{18}N_2O$	153–154	218.3	218.3	12.65	12.83	79	85	2, 3, 4
8	H	Morpholino		$C_{12}H_{16}N_2O_2$	155–157	226	220.3	12.68	12.72		82	9
9	OCH_3	CH_3	CH_3	$C_{11}H_{16}N_2O_2$	174	204	208.3	13.10	13.45		58	...
10	OCH_3	C_2H_5	C_2H_5	$C_{13}H_{20}N_2O_2$	150–152	238	236.3	11.90	11.86	58	62	...
11	OCH_3	$-(CH_2)_2-CH_3$	$-(CH_2)_2-CH_3$	$C_{15}H_{24}N_2O_2$	128	264	264.4	10.60	10.59		73	...
12	OCH_3	$-CH-(CH_3)_2$	$-CH-(CH_3)_2$	$C_{15}H_{24}N_2O_2$	133–134	259	264.4	10.10	10.59		76	...
13	OCH_3	$-(CH_2)_3-CH_3$	$-(CH_2)_3-CH_3$	$C_{17}H_{28}N_2O_2$	130	292.4	292.4	9.56	9.58	72	77	...
14	OCH_3	$-CH_2-CH-(CH_3)_2$	$-CH_2-CH-(CH_3)_2$	$C_{17}H_{28}N_2O_2$	106–108	289	292.4	9.31	9.58		65	...
15	OCH_3	CH_3	Cyclohexyl	$C_{16}H_{24}N_2O_2$	110	280	276.4	10.42	10.14		32	...
16	OCH_3	Piperidino		$C_{14}H_{20}N_2O_2$	182–184	250	248.3	11.15	11.28	56	75	...
17	OCH_3	H	$-(CH_2)_3-CH_3$	$C_{13}H_{20}N_2O_2$	104	236	236.3	11.90	11.86		58	...
18	OCH_3	H	Cyclohexyl	$C_{15}H_{22}N_2O_2$	115	264	262.3	10.82	10.67		30	...
19	CH_3	Piperidino		$C_{14}H_{20}N_2O$	148–150	232	232.3	11.83	12.06	555	62	...
20	CH_3	Morphilino		$C_{13}H_{18}N_2O_2$	152–153	232	234.3	11.75	11.96		58	...

[a] Based on the amount of benzaldehyde, anisaldehyde or *p*-methylbenzaldehyde used.

equals papaverine in this respect. It is however several times less toxic. A detailed description of the pharmacological properties of this series of amides will be published elsewhere.[1]

Experimental Part

The amines, obtained in good yield by concentrated sulfuric acid hydrolysis of the corresponding nitriles for 10 to 60 minutes at 100°, were purified with charcoal in boiling acid solution, followed by repeated crystallization from ethanol–ether mixtures. Hydrolysis does not proceed beyond the amide stage in these conditions.

The corresponding α-amino-α-phenylacetonitriles were prepared by one or both of the following general procedures adapted from reported methods.[2-13] **First Procedure:** from the amine hydrochloride (0.1 mole), the corresponding benzaldehyde (0.1 mole) and potassium cyanide (0.11 mole).[2] **Second procedure:** from the amine (0.12 mole), the bisulfite addition product of the corresponding benzaldehyde (0.1 mole) and potassium cyanide (0.1 mole).[3,11,12]

The following analytical methods were used (Table I).

(a) Melting points were determined with the micro-apparatus described by Kofler.[14]

(b) Total nitrogen content was determined using a semi-micro Kjeldahl method.[15]

(c) The molecular weight was determined by potentiometric perchloric acid titration of the amine function of the amides, dissolved in anhydrous acetic acid, using a Metrohm Titroskop.[16]

(d) Ultraviolet spectrophotometry (Beckman DU spectrophotometer) served as an important criterion of purity. The ultraviolet spectra of the amides in isopropyl alcohol (2 mmoles per liter) were recorded at 20 ± 1° between 210 and 300 mμ. The molar absorption spectra of the amides listed in the table fall into three groups, depending on the nature of the substituent P. Within these three groups, identical spectra were recorded for the amides with varying N-alkyl-substituents R and R′.

The following examples illustrate these two procedures.

A.—0.1 mole of dibutylamine (12.9 g., 97% pure by titration) was neutralized with 20% hydrochloric acid; anisaldehyde (0.1 mole, 13.6 g.; b.p. 134–135° (12 mm.)) was added at once and potassium cyanide (0.11 mole, 7.16 g.) dissolved in 25 ml. of water added dropwise to the stirred mixture at room temperature. After a two-hour heating period (100–120°), the oily α-dibutylamino-α-(*p*-methoxyphenyl)-acetonitrile, which separated from the cooled solution, was mixed with 30 ml. of concentrated sulfuric acid. This mixture was heated at 100° for 10 minutes, cooled for one hour at room temperature, and treated with three volumes of water at 0°. When this solution was neutralized with concentrated ammonia, a brown solid precipitated immediately. It was collected on a filter, washed with 200 ml. of water, dried, weighed[17] and dissolved in 100 ml. of 10% hydrochloric acid. After addition of 2 g. of charcoal, the suspension was heated under reflux for 20 minutes and neutralized with ammonia. The white precipitate was collected by filtration, washed with 200 ml. of water and repeatedly recrystallized from ethanol containing 10% of ethyl ether. Three crystallizations were necessary to obtain pure α-dibutylamino-α-(*p*-methoxyphenyl)-acetamide (white rods, m.p. 125–127°, 14.6 g., 50%).

B.—To 13.6 g. (0.1 mole) of ice-cooled anisaldehyde, a saturated aqueous solution of 12.5 g. (0.12 mole) of sodium bisulfite was added. After the subsequent dropwise addition of 15.5 g. (0.12 mole) of dibutylamine, 6.5 g. (0.1 mole) of solid potassium cyanide was poured at once into the reaction mixture. The oily nitrile, which separates from the solution at room temperature, was converted to the pure amide as described in the first procedure; 17.0 g. (58%) of pure α-dibutylamino-α-(*p*-methoxyphenyl)-acetamide was obtained.

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(17) The crude yields recorded in the table are based on this weight.

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The Conversion of 5-Hydroxykynurenine to 6-Hydroxykynurenic Acid and 6,4-Dihydroxyquinoline with Liver Homogenates

BY KATASHI MAKINO AND HITOSHI TAKAHASHI

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5-Hydroxy-D,L-kynurenine (I), which was recently synthesized in our laboratory, has been con-