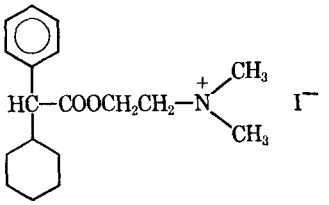
α-Phenyl-α(2,3-cyclohexenyl) Acetic Acid Derivatives as Potential Antispasmodics

By HEINO A. LUTS, J. F. GRATTAN, S. Z. HAIDRI, and W. L. NOBLES*

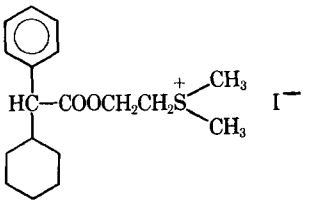
A group of compounds having the (2,3)-cyclohexene moiety has been prepared for pharmacological evaluation. The preparation and biological activities of these compounds are presented.

MANY α-phenyl-α-cyclohexyl acetic acid derivatives have been prepared (1–4) for pharmacological evaluation. They are generally very active antispasmodics, and are often superior to the diphenyl analogs.

Protiva and Exner (5) first reported the active sulfonium compound, thiospasmin, in which the nitrogen of compound I below has been replaced by the sulfur of compound II. This agent demonstrated exceptional antispasmodic activity; it was regarded as being 10 times as active as atropine.



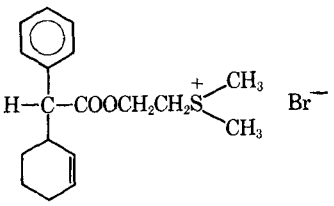
I



II

Thiospasmin has found successful clinical application in Czechoslovakia and in the Union of Soviet Socialist Republics. In radioactive studies Francova and his associates (6) found that orally administered thiospasmin was distributed in the rats in the following order: liver, kidney, lungs, spleen, heart, plasma, muscle, blood cells, skin, and brain. The maximum activity was found after 4 hr. in the gastrointestinal tract.

While proceeding with their studies on the interdependence and effectiveness of substances, Votava and his associates (7) then synthesized α - phenyl - α - cyclohexyloxyacetoxyethyldimethylsulfonium iodide and α-phenyl-α-*n*-propylhydroxyacetoxydimethylsulfonium iodide. Both compounds proved to be superior to thiospasmin in activity and were also less toxic. Since 1953, several structural modifications have been made. In 1956, Neesby and his associates (8) synthesized a series of compounds in which the substituent on the α-carbon atom and the nature of the ionic radical were altered. They found that dimethylsulfonium ethylphenyl α-(2, 3-cyclohexenyl) acetic acid bromide (III) completely voids the xerostomic effect without decreasing the antispasmodic activity.



III

It is noteworthy that in their studies the addition of a hydroxyl group to the α-carbon atom did not increase the activity, which is the generally accepted rule.

To our knowledge, this selectivity does not exhibit itself in any other antispasmodic compound reported to the present. It is speculated that possible hydroxylation takes place in the detoxification process on the 2,3 position of the cyclohexene ring.

In order to explore more extensively the 2,3 cyclohexene analog structures of this type, we have prepared a group of them for pharmacological evaluation (Table I).

MATERIALS AND METHODS

Compound II was made by the standard method of adding *trans*-1,2-dibromocyclohexane to the sodium salt of phenyldiethylmalonate, and the α-phenyl-α-(2,3-cyclohexene) acetic acid (III) was accomplished by hydrolysis and partial decomposition

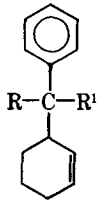
Received May 22, 1967, from Structure-Activity Research, Inc., College Hill, Oxford, MS 38655, and Smith, Miller and Patch, Inc., New York, NY

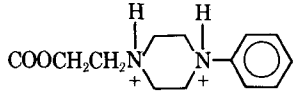
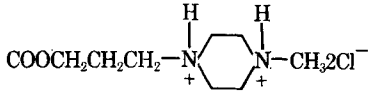
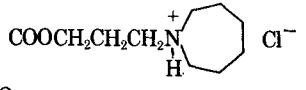
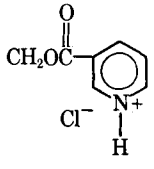
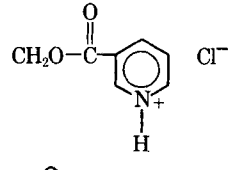
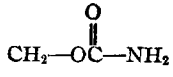
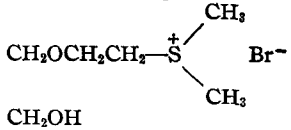
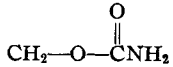
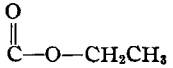
Accepted for publication October 18, 1967.

Presented to the Medicinal Chemistry Section, APhA Academy of Pharmaceutical Sciences, Las Vegas meeting, April 1967.

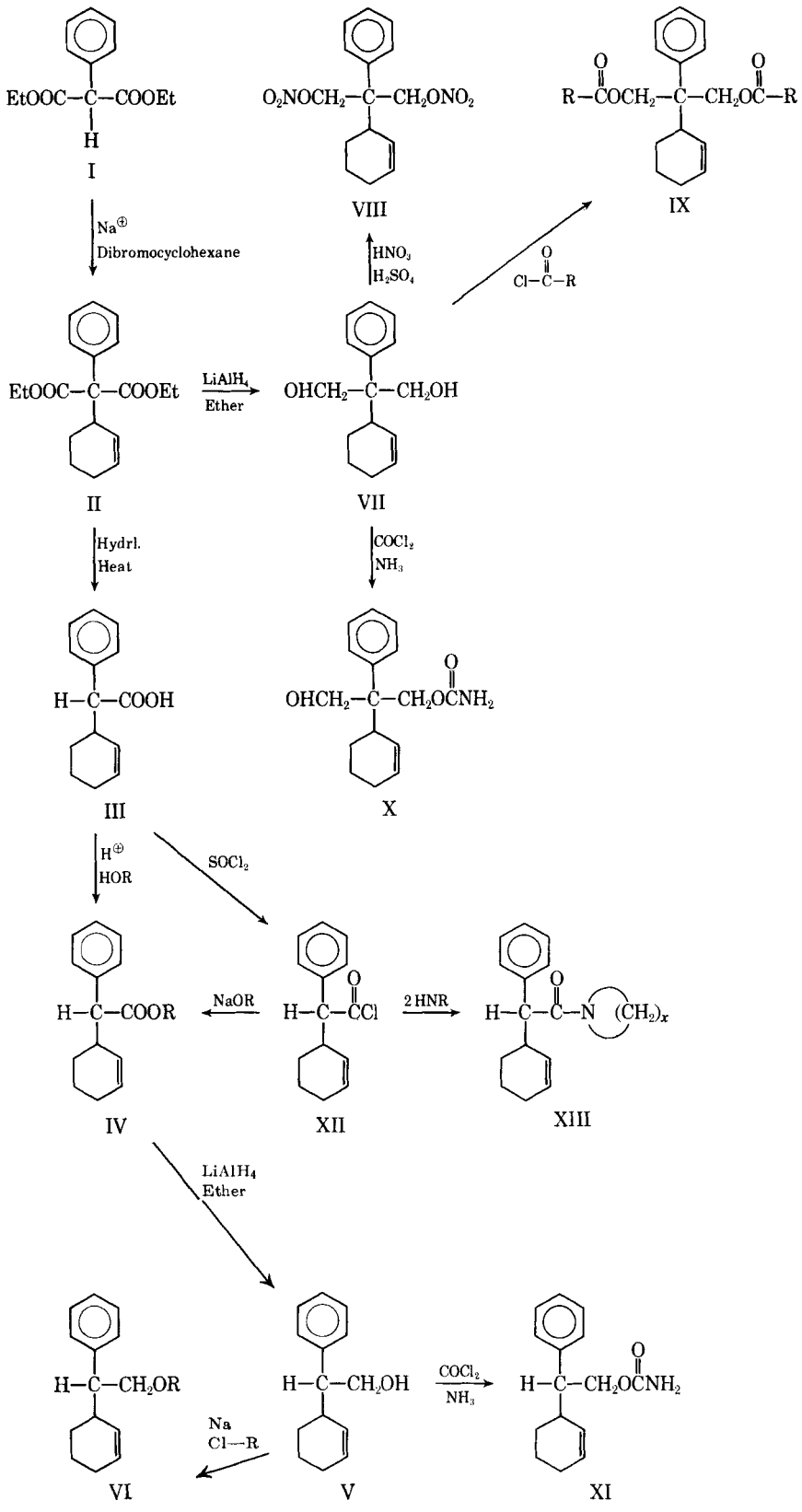
* School of Pharmacy, The University of Mississippi, University, MS 38677

TABLE I—2,3-Cyclohexane Analogs



No.	R	R¹	M.p., °C.	Formula	Calculated C	Calculated H	Calculated N	Found C	Found H	Found N
1	H		179–180	$C_{26}H_{26}N_2O_2Cl$	63.96	5.58	6.18	63.82	5.64	6.15
2	H		222	$C_{22}H_{34}N_2O_2Cl_2$	61.67	8.00	6.67	61.78	8.10	6.59
3	H		160–161	$C_{23}H_{34}NO_2Cl$	61.40	8.76	3.57	61.27	8.72	3.63
4	CH_2ONO_2	CH_2ONO_2	58–159	$C_{15}H_{19}N_2O_4$	55.68	5.92	8.66	55.41	5.78	8.78
5			182–183	$C_{27}H_{30}N_2O_4Cl_2$	56.47	6.27	5.81	56.40	6.30	5.72
6	H		100–101	$C_{15}H_{19}NO_2$	73.84	7.81	5.71	73.96	7.86	5.77
7	H		167–168	$C_{18}H_{27}OSBr$	58.21	7.35	8.9(S)	58.01	7.49	9.20(S)
8	CH_2OH	CH_2OH	75–76	$C_{15}H_{20}O_2$	77.54	8.67	...	77.24	8.60	...
9	CH_2OH		134	$C_{15}H_{23}NO_3$	67.89	8.73	5.27	67.98	8.58	4.99
10	H	CH_2OH	112–117 0.3 mm.[a]	$C_{14}H_{18}O$	83.11	8.96	...	82.82	8.99	...
11	H		117–130 1.2 mm.[a]	$C_{16}H_{20}O_2$	78.65	8.24	...	78.52	8.30	...

[a] Boiling points.


EtOOC–C(H)–COOEt
I
Na⊕
Dibromocyclohexane
EtOOC–C(H)–COOEt
II
LiAlH4
Ether
OHCH2–C(H)–CH2OH
VII
HNO3
H2SO4
O2NOCH2–C(H)–CH2ONO2
VIII
Cl–C(=O)–R
R–COCH2–C(H)–CH2OC–R
IX
Hydrl.
Heat
H–C(H)–COOH
III
COCl2
NH3
OHCH2–C(H)–CH2OCNH2
X
H⊕
HOR
SOCl2
H–C(H)–COOR
IV
NaOR
H–C(H)–C(=O)Cl
XII
2 HNR
H–C(H)–C(=O)–N (CH2)x
XIII
LiAlH4
Ether
H–C(H)–CH2OR
VI
Na
Cl–R
H–C(H)–CH2OH
V
COCl2
NH3
H–C(H)–CH2OCNH2
XI

Scheme I

of the diacid formed. Compound IV was obtained by esterifying the acid; the alcohol V, was prepared by reducing the acid with $LiAlH_4$. The alcohol was then converted to the ether as indicated in the accompanying synthetic scheme. Reducing compound II with $LiAlH_4$ gave compound VII as the main product and the corresponding nitrate (VIII) was accomplished by nitration. Compound IX was made by the addition of 2 moles of the corresponding acid chloride. The 2-hydroxycarbamate (X) was obtained by reaction with phosgene and ammonia. The monocarbamate XI was prepared from compound V by reacting with phosgene and ammonia and compound XII by reacting compound III with thionyl chloride. The acid chloride was then converted to amides (XIII) by reaction with the corresponding amines (Scheme 1).

PROCEDURES

Phenylquinone Writhing Test in Mice—The principle of this test is that phenylquinone, when administered subcutaneously to mice, will induce painful writhing movements. Analgesics will significantly reduce these movements.

Four groups of fasted male mice of the Swiss-Webster strain (body weight 15–17 Gm.) were utilized in this experiment. The drug in question was administered orally to three groups of mice at dose levels of 25, 50, and 100 mg./Kg., respectively, in a volume of 0.2 ml. per mouse. The fourth group which was used as the control received 0.01 ml./Gm./body weight of saline. Five animals per group were employed. Phenylquinone at 20 mg./Kg. was injected subcutaneously to all groups of mice 30 min. after the administration of the tested compound.

Tremorine Test—Five groups of fasted male mice (body weight 15–17 Gm.) were utilized in this experiment. The drug was administered at the standard levels of 25, 50, and 100 mg./Kg. For ready comparison, it was tested with a standard antitremorine drug, in this instance trihexyphenidyl HCl[1] (5 mg./Kg.). Untreated controls were utilized at 0.9% saline. All drugs were prepared in distilled water. All drugs were administered orally 30 min. prior to the tremorine which was administered intraperitoneally.

Hexobarbital-Induced Sleep in Mice—Four groups of fasted male mice of the Swiss-Webster strain (body weight 15–17 Gm.) were utilized. The drug was administered orally at 25, 50, and 100 mg./Kg. concentrations. Hexobarbital was administered intraperitoneally at 100 mg./Kg. 30 min. after the initial administration of the tested drug. The animals were then observed for the time intervals of the onset and duration of sleep. The tested comporids were prepared in distilled water; however, hexobarbital was prepared in 5% sodium hydroxide in the final volume of 9 ml. of water, making a total volume of 10 ml.

Antipyretic Activity—Male rats of the Sprague-Dawley strain (body weight 100–125 Gm.) were utilized. They were given a 20% yeast solution at a volume of 2 ml. per rat, subcutaneously, 24 hr. prior to the administration of the tested compound.

After the yeast treatment, the animals were fasted for 24 hr. and a rectal temperature was taken each hour for 2 successive hr. prior to the administration of the drug (each animal served as its own control). After the drug was given, the rectal temperatures were taken each hour for 4 consecutive hr. to determine whether the tested drugs reduced the body temperature.

Ganglionic-Blocking Action—It has been found through previous experiments that the cat gives the greatest response with respect to this test; therefore, a cat was used. A cat of either sex was anesthetized with diallylbarbituric acid[2] with urethan solution (0.7 ml./Kg.), administered intraperitoneally. The surgery included the exposition of the carotid sheath (the carotid artery and the vagus nerve). Also, included was the exposition of the right femoral vein for the purpose of injection and the left femoral artery for the recording of the blood pressure. There was a unilateral severing of the vagus nerve and an exposition of the superior cervical ganglion, for the purpose of controlling the nictitating membrane. Sustained contraction of the nictitating membrane that disappears after intravenous injection of a ganglion-blocking agent is indicative of preganglionic electrical stimulation.

Hypotensive Activity—This test was conducted on five species of animals—rat, guinea pig, rabbit, cat, and dog.

The rat and the guinea pig were anesthetized with urethan, 1.2 Gm./Kg., administered intraperitoneally. The right sublingual vein was exposed, and cannulated for injection of the drug. The left carotid artery was exposed, and cannulated for recording blood pressure. The blood pressure was measured by means of a mercury manometer and recorded directly on a kymograph. In both species, the pressure was allowed to stabilize approximately 30 min. to 1 hr., depending upon the responses of the individual animals. There was a 30-min. interval between each dose of drug or until the blood pressure returned to the base line.

Heparin was given in the amount of 500 USP units per animal, to prevent coagulation.

In order to test the cholinergic effects of the tested compound, acetylcholine bromide at 2 mcg./Kg. was administered. After the characteristic hypotensive tracing had been obtained, acetylcholine bromide was administered a second time; however, this second injection was preceded by the tested compound.

A slight modification of the above procedures was used to prepare the cat for cardiovascular study. Because of the ready accessibility of the vessels, the left femoral artery and right femoral vein were used to measure blood pressure and to inject the tested drug, respectively.

Terminology—

Phenylquinone writhing test in mice:

good activity	>40% decrease of writhing movements as compared to untreated controls.
slight activity	10–40% decrease of the writhing movements as compared to the untreated controls.

hexobarbital-induced sleep in mice:

good potentiation	>40% increase in sleeping time over the untreated controls.

[1] Marketed as Artane by Lederle Labs, Pearl River, N. Y.

[2] Dial, Ciba Pharmaceutical Products, Inc., Summit, N. J.

slight potentiation	10–40% increase in sleeping time over the untreated controls.

Tremorine test in mice:
Induced Tremors (T), Lacrimation (L), and Salivation (S)

good activity	One or more factors (T, L, S) decreased by 75–100% as compared with untreated controls.
slight activity	One or more factors (T, L, S) decreased by 50–75% as compared with untreated controls.

Preliminary Results—Initial pharmacological screening of some of the compounds in this series has demonstrated the following:

Compound I exhibited a mild depressant effect, slight activity in the phenylquinone test, and slight antipyretic activity. In the hexobarbital-induced sleep test in mice, it demonstrated good potentiation of the barbiturate.

Compound II behaved in the manner of CNS depressant drugs, generally, and also produced tremor in some instances. This compound demonstrated a good sympathomimetic activity, transient hypotensive activity, and slight activity in the phenylquinone test.

Compound III displayed good sympathomimetic activity and produced a mild depressant response. Administration of the drug provoked transient lowering of the blood pressure. It also exhibited a slight analgesic effect with the typical "hot plate" test. Slight activity in the phenylquinone test was noted.

Compound V proved to be active as a sympathomimetic agent and produced a good effect in the phenylquinone writhing test. It decreased hexobarbital-induced sleeping time. It also exhibited a slight antagonism to tremorine-induced tremors and exerted a slight response as an antipyretic agent.

Compound VIII behaved as a mild depressant and exhibited cholinergic activity. It did not have an influence on the response of the organism to epinephrine. The compound also produced a mild hypothermia.

Compound X exhibited depressant qualities and demonstrated anticholinergic activity. It had no effect on body temperature and did not interfere with or potentiate the action of epinephrine. Some data obtained suggest that the compound is possibly a vasodilator. In general, this compound was less active than compound VIII in comparative tests.

Compound XI acted as a mild stimulant. It demonstrated atropine-like action, as well as ganglionic-blocking activity. A slight hyperthermic response was also observed.

EXPERIMENTAL[3]

3-Hexamethyleneiminopropyl α-(2-cyclohexene)-phenylacetate Hydrochloride—To the mixture of 345 mg. (0.015 mole) of sodium in 75 ml. of isopropanol was added 3.24 Gm. (0.15 mole) of α-phenyl-α-cyclohexene acetic acid. The mixture was refluxed for 30 min. and 2.42 Gm. (0.015 mole) 3-chloro-*N*-propylhexamethylimine was added. This mixture was refluxed 4 hr. The salt was removed by filtration and the residue evaporated to dryness. The residue was extracted with 75 ml. of ether and the extract acidified with hydrochloric acid. The crystals thus formed were separated by filtration; yield 3.0 Gm., m.p. 160–161°.

2 - Phenyl - 2 - cyclohexenyl - 1,3 - propanediol—Twenty grams (0.08 mole) of phenylcyclohexenediethylmalonate was dissolved in 250 ml. of anhydrous ether and 8.0 Gm. of $LiAlH_4$ was added in small portions. The reaction mixture was refluxed for 8 hr. and allowed to stand overnight. The excess $LiAlH_4$ was decomposed with water and the ether layer separated. The aqueous residue was extracted twice with 100-ml. portions of ether and the ether layers combined. The ethereal solution was dried over anhydrous calcium sulfate[4]; the ether was then removed to yield a residue; yield 14 Gm., m.p. 75–76°.

Ethyl - α - (2 - cyclohexenyl) - α - phenylacetate—Twenty-five grams (0.115 mole) of α-phenyl-α-cyclohexene acetic acid was refluxed 2 hr. with absolute ethanol in the presence of hydrochloric acid. The ester thus formed distilled in the range 117–130° (1.2 mm.); yield 17 Gm., n_D^{28} 1.519.

2 - (2 - Cyclohexenyl) - 2 - phenylethanol—Seventeen grams (0.08 mole) of the ethyl ester of α-phenyl-α-cyclohexene acetic acid was reduced with 6 Gm. of $LiAlH_4$ in 250 ml. of ether. After refluxing for 6 hr., the excess $LiAlH_4$ was decomposed with a water–ether mixture and the ether layer separated. The residue was extracted twice with 100-ml. portions of ether and the ether layers were combined; the ethereal solution was dried over anhydrous calcium sulfate and the ether was removed. The residual 12 Gm. distilled at 112–117° 0.3–0.35 mm., n_D^{27} 1.5447.

2-Phenyl-2-cyclohexenyl-1,3-propanediol Nicotinate—To 6.96 Gm. (0.03 mole) of 2-phenyl-2-cyclohexenyl-1,3-propanediol, 10.63 Gm. (0.06 mole) of nicotinylchloride hydrochloride was added and the mixture heated at 100° for 20 min.; the mixture was stirred constantly during this period. After cooling, the residue was dissolved in water and made strongly basic with concentrated ammonia water. The solid precipitate was extracted twice with 50-ml. portions of ether. The solution was dried over anhydrous calcium sulfate and then acidified with anhydrous hydrogen chloride. The product thus formed was collected by filtration; yield 16.5 Gm., m.p. 182–183°.

N-Methylpiperazino-N'-propyl α-(2-cyclohexene)-phenylacetate Dihydrochloride—To a solution of 6.5 Gm. (0.03 mole) of α-phenyl-α-cyclohexene acetic acid and 100 ml. of absolute isopropanol, 700 mg. (0.03 mole) of sodium was added. After the salt formation, 5.5 Gm. (0.03 mole) of α-chloropropyl-*N*-methylpiperazine was added and the mixture refluxed for 6 hr. The solvent was removed by distillation and the residue dissolved in 50 ml. of ether. The ether was then treated with anhydrous hydrogen chloride and the product was collected by filtration; yield 7.9 Gm., m.p. 222°.

N-Phenylpiperazinoethyl α - (2 - cyclohexene)-phenylacetate Dihydrochloride—Three grams (0.015 mole) of *N*-α-hydroxy-ethyl-*N*-phenylpiperazine was reacted with 300 mg. (0.015 mole) of sodium in 75

[3] Microanalysis was performed by Mr. G. Roberts, Jr., Florham Park, New Jersey. All melting points are uncorrected.

[4] Drierite, W. A. Hammond Drierite Co., Xenia, Ohio.

ml. of xylene; 3 Gm. (0.015 mole) of α-phenyl-α-cyclohexene acid chloride was added in small portions and the mixture was refluxed for 4 hr. The salt was removed by filtration and the residue distilled to dryness in high vacuum. The residue was dissolved in 60 ml. of ether, the solution decolorized with pulverized carbon and acidified with anhydrous hydrogen chloride. The product thus formed was collected by filtration and dried; yield, 5.0 Gm., m.p. 179–180°.

2 - Phenyl - 2 - cyclohexenyl - 1,3 - propanedinitrate—To 4.6 Gm. (0.02 mole) of 2-phenyl-2-cyclohexenyl-1,3-propanediol was added 20 ml. of 100% nitric acid at 0–5°. (The acid was previously prepared by distilling it over 100% sulfuric acid.) To the reaction mixture was added 20 ml. of 100% sulfuric acid and the mixture was left standing overnight. The solution was then poured into ice; at this point crystals were formed. They were collected by filtration and washed with water. The product was dissolved in methanol and 2% sodium bicarbonate solution added until the crystals separated. The crysals were collected again by filtration and dried; yield 4.1 Gm., m.p. 58–59°.

2 - Cyclohexenyl - 2 - phenylethoxy - 1 - ethylmethylsulfide Methiodide—Six grams (0.03 mole) of 2-(2-cyclohexenyl)-2-phenylethanol was reacted with 690 mg. (0.03 mole) of sodium in xylene. Three and four tenths grams (0.03 mole) of α-chloroethylmethyl sulfide was added over a period of 15 min. and the reaction mixture was refluxed for 12 hr. After filtering off the salt, the xylene was distilled and discarded. To the residue was added 20 ml. of methyl bromide, the mixture was allowed to stand for 2 days and then worked up at room temperature. Ten milliliters of absolute methanol was added and the mixture was refluxed for 2 hr. To this mixture 25 ml. of absolute ether was added; on cooling, the crystallized product formed; yield 3.6 Gm., m.p. 167–168°.

2-Cyclohexenyl-2-phenylethylcarbamate—To a solution of 2.4 Gm. (0.012 mole) of 2-(2-cyclohexenyl)-2-phenylethanol in 25 ml. of benzene was added twice the molar quantity of phosgene in 25 ml. of benzene. This mixture was placed in a pressure bottle and warmed to 50° for 4 hr. The excess phosgene was boiled off and the residue treated with dry ammonia gas. The reaction mixture was reduced to dryness under reduced pressure. The residue was dissolved in alcoholic ammonia and filtered. The clear solution was poured on ice; at this point fine crystals were formed. They were collected and washed with water.

The crystals were recrystallized from an ethanol–water mixture to give a compound, m.p. 100–101°; yield 2.4 Gm.

2-Phenyl-2-cyclohexenyl-1,3-propanediol Carbamate—To a solution of 12.0 Gm. (0.05 mole) of 2-(2-cyclohexenyl)-2-phenylethanol in 50 ml. of benzene, 150 ml. of 12% phosgene in benzene was added. The mixture was placed in a pressure bottle and kept 4 days in sunlight. The volume of the mixture was reduced by one-half and anhydrous ammonia was added. The precipitated crystals were filtered off and discarded. The benzene layer was then concentrated to dryness with the aid of high vacuum; to that residue 50 ml. of concentrated ammonia was added and the mixture was boiled for 1 hr. The solution was cooled and the ammonia was removed by blowing air over it. The crystals were separated and recrystallized from the ethanol–water mixture, m.p. 134°; yield 11.9 Gm.

REFERENCES

(1) Graham, J. S. P., and Lazarus, S., *J. Pharmacol.*, **69**, 331(1940).
(2) Plummer, A. J., Barret, W. E., Rutledge, R., and Yonkman, F. F., *ibid.*, **108**, 292(1943).
(3) Ehrenberg, M. H., Ramp, J. A., Blanchard, E. W., and Treves, G. R., *ibid.*, **106**, 141(1952).
(4) Hockstra, J. B., and Dickison, H. L., *ibid.*, **98**, 14 (1950).
(5) Protiva, M., and Exner, P., Czechoslovakian pat. 83205 (Feb. 1955).
(6) Francova, Z., Svatos, A., Franc, Z., and Hais, I. M., *Physiol. Bohemsloven*, **10**, 390(1961).
(7) Votava, Z., Metysch, I., Shramkova, F., and Vanechek, M. M., *Farmakol. i Toksikol.*, **20**, 35(1957).
(8) Neesby, T. E., Furhman, R., Pircio, A. W., and Luts, H. A., U.S. pat. 2,989,561.

Keyphrases

Antispasmodics—potential
α-Phenyl-α(2,3-cyclohexenyl) acetic acid derivatives—synthesis
Pharmacological screening
Microanalysis—identity