measured using the potentiometric method, titrating the amine hydrochloride with sodium hydroxide. The value for **1** is based upon 55 points distributed among three concentrations with an average deviation of 0.039 pK_a units. For **2**, 54 points distributed among three concentrations were used. The average deviation was 0.043 pK_a units.

Acknowledgment.—The authors are grateful to Mr. Don Lee for the pK_a measurements. The authors are indebted to Dr. B. Pendleton of the Psychology Department, San Jose State College, for aid in the studies using the ECG and EEG.

Cyclopropane Analogs of Choline Ethers[1]

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Although many esters and ethers of choline have been examined for their activity in cholinergic and cholinesterase systems, the effect of conformational changes of the –O–C–C–N segment has not yet been defined. A transition from cisoid to transoid shapes has been considered as a possible source of the stimulatory and depressant components of such compounds.[2] Branching of the carbon chain with small alkyl groups greatly prolongs the activities, probably by steric interference with the reactions of the respective compounds at biocatalytical sites.[3] In such branched compounds, marked differences in the activities of optical isomers and diastereoisomers have been observed.[4] We are now recording the synthesis and biological evaluation of some 2-N-alkoxycyclopropyl-N,N,N-trimethylammonium salts in which the methyl group of the α- and β-methylcholine ethers, or the methylene group of the muscarinic agent, (2-methoxyallyl)trimethylammonium hydroxide[5] have been incorporated in a small rigid ring. In the case of N-(2-benzyloxycyclopropyl)-N,N,N-trimethylammonium iodides, both the *cis* and *trans* forms have been isolated and tested.

These compounds were synthesized by subjecting the corresponding 2-alkoxycyclopropanecarboxylic acids to modified Curtius degradations and quaternizing the respective 2-alkoxycyclopropylamines with methyl iodide. During the Curtius procedures, the azides were rearranged either to the isocyanates, or better, transformed to the corresponding benzyl carbamates; the latter could be hydrolyzed or hydrogenolyzed to the amines.

The configurations assigned to the N-(2-benzyloxycyclopropyl)-N,N,N-trimethylammonium iodides are supported by the n.m.r. spectra of these salts (see Table I). In the *trans* isomer, the positive nitrogen may be expected to cause a larger shift of the proton peak in the 2-position due to its higher shielding effect. Furthermore, the peak of the benzyl α-protons should be shifted less in the case of the *trans* isomer. As shown in the Experimental section, the *trans* isomer predominates in the reaction mixture.

TABLE I

NUCLEAR MAGNETIC RESONANCE SPECTRA OF *cis*- AND *trans*-N-(2-BENZYLOXYCYCLOPROPYL)-N,N,N-TRIMETHYLAMMONIUM IODIDES IN DEUTERATED DIMETHYL SULFOXIDE[a]

cis isomer,[b] τ-values	*trans* isomer,[c] τ-values	
2.54	2.57	5 Phenyl protons
5.29	5.35	2 Benzyl protons
6.3–6.7	5.74	1 Proton at 2-position of cyclopropane ring; blurred for *cis* by the solvent peak
6.76	6.89	9 N-CH_3 protons
8.3–9.1	8.2–9.0	CH_2 protons of 3-position in cyclopropane ring

[a] Tetramethylsilane as the internal standard. [b] More soluble; m.p. 164–168° dec. [c] Less soluble; m.p. 168.5–169°.

Arguments supporting the *trans* configuration of the only isomer isolated in the case of N-(2-butoxycyclopropyl)-N,N,N-trimethylammonium iodide are presented in the Experimental section.

Pharmacology.—The methodology for tests with *cis*-2-N-benzyloxycyclopropyl-N,N,N-trimethylammonium iodide (I), the *trans* isomer (II), and *trans*-N-(2-*n*-butoxycyclopropyl)-N,N,N-trimethylammonium iodide (III) is summarized in the Experimental section.[6] On the isolated ileum of the guinea pig, I had no cholinergic activity up to 10^{-2} mg./ml. At $1–2 \times 10^{-3}$ mg./ml. it reduced acetylcholine-caused contractions by 50%. At 10^{-2} mg./ml. II showed neither a cholinergic nor anticholinergic action, while III at 5×10^{-3}–2.5×10^{-2} mg./ml. led to contractions of short duration. This compares with a similar effect of acetylcholine at 2.5×10^{-6} mg./ml. In contrast to acetylcholine, the effect of III was not cancelled by atropine but was abolished by 10^{-3} mg./ml. of hexamethonium.

At 0.25 mg./kg., I raised the blood pressure briefly in the anesthetized cat; a rise of 60 mm. occurred after 0.5 mg./kg., but after 1 mg./kg. it amounted to only 30 mm., and could no longer be demonstrated after 2 mg./kg. The pressor effect was not affected by hexamethonium, but was greatly decreased by 1 mg./kg. of phentolamine. By contrast, the *trans* isomer (II) caused no effect up to 1 mg./kg., and 5 mg./kg. led to an acute drop in pressure with full recovery within 5 min. Compound III (0.1 mg./kg.) produced a strong pressor effect of short duration which could be decreased moderately by atropine. Phentolamine or hexamethonium greatly decreased the pressor effect of large doses of III, and abolished it after low doses. A combination of the two agents always cancelled the pressor effect. Pretreatment with reserpine (see Experimental section) decreased the pressor action of III; a clear pressor effect was achieved with 0.5

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(6) We are grateful to Dr. H. H. Frey, Leo Pharmaceutical Products, Ballerup, Denmark, for these data.

mg./kg. Neostigmine (0.1 mg./kg.) did not alter the action of III.

In the chloralosed cat, I (up to 1 mg./kg.) did not affect the contraction of the nictitating membranes evoked by preganglionic stimulation of the sympathetic nerve. A dose of 2 mg./kg. reduced this effect by 40%, but already 3 min. later the contraction had recovered to 90% of the control value. Compound III (0.1–1 mg./kg.) caused contractions of the nictitating membranes which seemed to be slightly diminished after acute gangliectomy. Depending on the dose of III, the effects were reduced or abolished by hexamethonium or phentolamine. After chronic denervation of one nictitating membrane by removal of the ganglion 5–10 days before the experiment, the denervated side was more sensitive to III, perhaps due to hypersensitivity of the denervated nictitating membrane towards endogenously liberated epinephrine or norepinephrine from the adrenals and other tissues.

In the preparation of Chen, *et al.*,[7] III behaved qualitatively like 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP); the blood pressure rose due to a stimulation of sympathetic ganglia, and the tension of the urinary bladder increased by parasympathetic ganglionic excitation. Quantitatively, III was 10–40 times less potent than DMPP, however. Hexamethonium decreased or abolished the effects of either substance.

Up to a dose of 2 mg./kg., I showed no influence on neuromuscular transmission but immediately after injection of 4 mg./kg. the myogram was reduced by 90%; full recovery took 3–4 min. Compound II (5 mg./kg.) had no effect, and neither had III (0.01–5 mg./kg.).

The i.v. LD_{50} (mouse) dose was: I, 4.5 mg./kg.; and III, 6.8 mg./kg. The animals died from neuromuscular paralysis after I, and after strong muscular fibrillation from respiratory paralysis after injection of III.

Experimental[8]

Ethyl *cis,trans*-2-Benzyloxycyclopropanecarboxylate.—To a stirred suspension of 0.5 g. of copper powder (Merck) in 22.0 g. (0.16 mole) of benzyl vinyl ether[9] was added dropwise, at 100°, 19 g. (0.16 mole) of ethyl diazoacetate, the temperature being maintained at 95–105°. After completion of the addition (75 min.), the mixture was distilled, a fraction of b.p. 99–114° (0.3 mm.) being collected (26 g.). Redistillation furnished 23.5 g. (64%) of a colorless oil, b.p. 95–99° (0.2 mm.).

Anal. Calcd. for $C_{13}H_{16}O_3$: C, 70.86; H, 7.32. Found: C, 70.91; H, 7.56.

Alkaline hydrolysis as described for the *n*-butoxy analog below gave 2-benzyloxycyclopropanecarboxylic acid in 98% yield as a viscous oil.

***cis,trans*-2-Benzyloxycyclopropylamine. A.**—Working by the general method of Weinstock,[10] an anhydrous stirred solution of 7.9 g. (0.041 mole) of oily 2-benzyloxycyclopropanecarboxylic acid in 33 ml. of acetone, was treated with 60 ml. of a dry acetone solution of 5.0 g. (0.050 mole) of triethylamine at −5° followed by a solution of ethyl chlorocarbonate (6.0 g., 0.055 mole) in 23 ml. of acetone. After stirring at −5° for 30 min., a solution of 4.18 g. (0.064 mole) of sodium azide in 6 ml. of water was added, stirring was continued at −5 to 0° for 1 hr., and the azide was worked up as usual.[10] Freshly distilled benzyl alcohol (25 ml.) was added, the mixture was refluxed for 8 hr., and benzene and excess benzyl alcohol were removed (0.2 mm.). The crude benzyl 2-benzyloxycyclopropanecarbamate was a viscous oil, yield, 10.2 g. (0.034 mole, 83%). It was refluxed with 57 ml. (0.114 mole) of 2 *N* KOH in 135 ml. of ethanol for 7 hr., the mixture was cooled and acidified with 30 ml. of 4 *N* HCl, and ethanol was removed under reduced pressure. After further addition of 5 ml. of 4 *N* HCl the solution was extracted with ether, made alkaline with 2 *N* KOH solution, and again extracted with three 60-ml. portions of ether. After drying ($MgSO_4$) and treatment with charcoal, the ether was removed from the remaining oily amine (3.8 g., 69%). The infrared spectrum showed cyclopropane bands at 1030 and 1018 cm.$^{-1}$. The amine was converted to the cyclohexylsulfamate salt in ethyl acetate–tetrahydrofuran (10:1). After recrystallization from the same solvent, the salt (colorless needles) melted at 138.5–140.5°.

Anal. Calcd. for $C_{16}H_{26}N_2O_4S$: C, 56.11; H, 7.65. Found: C, 55.86; H, 7.56.

B.—When a benzene solution of 2-benzyloxycyclopropanecarboxazide was refluxed for 8 hr. and evaporated, an 88% yield (based on starting acid) of 2-benzyloxycyclopropyl isocyanate was obtained. The oily material absorbed strongly at 2230 cm.$^{-1}$. A solution of 0.95 g. (0.005 mole) of this oil was refluxed with 1.4 g. (0.025 mole) of KOH in 8 ml. of 60% aqueous ethanol for 2 hr. and worked up as described for the carbamate above. The yield of amine was 0.3 g. (40%). The infrared spectrum showed bands at 1030 and 1018 cm.$^{-1}$ which may be ascribed to the *cis* and *trans* isomers, respectively. The cyclohexylsulfamate salt melted at 138–140° and did not depress the melting point of a sample prepared by method A. The infrared spectra of the two salts were identical.

N-(2-Benzyloxycyclopropyl)-N,N,N-trimethylammonium Iodides.—Oily 2-benzyloxycyclopropylamine (3.6 g., 0.022 mole) was added to a stirred mixture of 3.9 g. (0.028 mole) of anhydrous potassium carbonate, methyl iodide (93.7 g., 0.66 mole), and 53 ml. of absolute ethanol. After refluxing for 6 hr., the mixture was cooled and filtered from 10.2 g. of solids which were washed with absolute ethanol. The material was also insoluble in chloroform. The precipitated solid was treated with 15 ml. of water and filtered. The residue weighed 3.7 g. (50%). Recrystallization from absolute ethanol gave colorless crystals, m.p. 168.5–169.5°; infrared absorptions at 1012 and 850 cm.$^{-1}$.

Anal. Calcd. for $C_{13}H_{20}INO$: C, 46.86; H, 6.05; N, 4.21. Found: C, 47.00; H, 5.72; N, 4.37.

On the basis of n.m.r. spectral comparisons, this salt has been assigned the *trans* configuration (see Table I).

The mother liquors from the sparingly soluble solid salt were evaporated to dryness (2.8 g.). Extraction with 80 ml. of chloroform dissolved 2.2 g. Evaporation of the chloroform solution and washing of the residue with ethyl acetate led to 2.0 g. (27%) of material which was recrystallized from absolute ethanol and washed with a little water. The pure isomer (1.6 g.) melted at 164–168° dec.; infrared absorptions at 1030 and 855 cm.$^{-1}$.

Anal. Calcd. for $C_{13}H_{20}INO$: C, 46.86; H, 6.05; N, 4.21. Found: C, 46.75; H, 6.23; N, 4.05.

A mixture of the two isomeric methiodides melted at 147–150°.

2-*n*-Butoxycyclopropylamine. 1.—Ethyl 2-(*n*-butoxy)cyclopropanecarboxylate (18.6 g., 0.1 mole), obtained in 70% yield according to D'yakonov,[11] was refluxed in a solution containing 5.65 g. (0.1 mole) of KOH in 200 ml. of ethanol and 50 ml. of water for 30 min. Ethanol was removed under reduced pressure; the solution was extracted with ether, acidified, and extracted with three 100-ml. portions of ether. Drying ($MgSO_4$) and evaporation gave 15.4 g. (97%) of oily 2-(*n*-butoxy)cyclopropanecarboxylic acid.

2.—The oily acid was converted to the oily N-benzyl carbamate as described for the benzyloxy analog above, in 50% yield, b.p. 145–150° (0.3 mm.).

3a.—The alkaline hydrolysis of this carbamate was performed as described for the benzyloxy analog above. The yield of oily amine was 70%. The cyclohexylsulfamate salt crystallized from a 10:1 mixture of ethyl acetate and tetrahydrofuran, m.p. 134–139°. A mixture melting point with the salt prepared by method **3b** showed no depression.

(7) G. Chen, R. Portman, and A. Wickel, *Arch. Internat. Pharmacodyn.*, **96**, 291 (1954).

(8) Melting points were determined on a Fisher-Johns apparatus and are corrected. Boiling points are uncorrected. Infrared spectra were measured with a Perkin-Elmer 137 spectrophotometer, n.m.r. spectra with a Varian A-60 spectrometer; microanalyses by Mrs. W. E. Coyne.

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3b.—A solution of 0.5 g. (2 mmoles) of benzyl 2-*n*-butoxycyclopropanecarbamate in 20 ml. of ethyl acetate, containing 125 mg. of 10% Pd on C, was hydrogenated at 25° at atmospheric pressure. After completing the hydrogen absorption the amine was worked up and converted to the cyclohexylsulfamate salt, m.p. 134–138° (from ethyl acetate–tetrahydrofuran).

Anal. Calcd. for $C_{13}H_{28}N_2O_4S$: C, 50.63; H, 9.15. Found: C, 50.63; H, 9.34.

The infrared spectra of the salts prepared by methods 3a and 3b were identical.

***trans*(?)-N-(2-*n*-Butoxycyclopropyl)-N,N,N-trimethylammonium Iodide.**—The oily 2-*n*-butoxycyclopropylamine (6.1 g., 0.047 mole) was quaternized with methyl iodide as described for the 2-benzyloxy analog above. The reaction mixture was filtered, the filtrate was evaporated, the residue (16.8 g.) was extracted with 120 ml. of chloroform, and the extract was cleared with charcoal. Evaporation of the chloroform and washing of the residue with ethyl acetate left 13.4 g. of a solid which crystallized from ethanol–ether and chloroform–ether, yielding 10.8 g. (76%), m.p. 108–110°; infrared absorptions at 1020 and 838 cm.$^{-1}$.

Anal. Calcd. for $C_{10}H_{22}INO$: C, 40.14; H, 7.41; N, 4.68. Found: C, 40.06; H, 7.35; N, 4.58.

In the n.m.r. spectrum, the one-proton peak at τ 5.8 was considered characteristic of the hydrogen at position 2 (*cf.* Table I, *trans* isomer).

No isomer of this quaternary salt was obtained. While it is possible that stereoisomeric intermediates may have been lost during the synthesis, the appearance of one major carbonyl peak in the infrared spectrum (1725 cm.$^{-1}$) of ethyl 2-*n*-butoxycyclopropanecarboxylate, with only a small shoulder at 1735 cm.$^{-1}$, implies that one isomer, probably the *trans* form, predominated already in this ester. A similar observation has been substantiated in the case of the isomeric ethyl 2-ethoxycyclopropanecarboxylates[12]; in analogous cases the lower frequency has been assigned to the *trans* isomer.[13,14]

Pharmacology.—Compounds I, II, and III were tested in the following systems: (a) isolated guinea pig ileum *in vitro*, at concentrations of 10^{-2} to 10^{-3} mg./ml.; (b) blood pressure effects in cats (for III also in rats). Vagotomized animals were used under chloralose or pentobarbital anesthesia. Pressor effects were also measured after pretreatment with reserpine (2 doses of 0.3 mg./kg. on the two days preceding the experiment), neostigmine (0.1 mg./kg.), and phentolamine and hexamethonium (1 mg./kg.). (c) Ganglionic effects in the cat under urethane or chloralose anesthesia; effects on the blood pressure and urinary bladder tension were measured by the method of Chen, *et al.*[7] The effect of preganglionic sympathetic stimulation was determined by the contraction of the nictitating membrane of the cat. (d) Effects on neuromuscular transmission were measured by stimulating the sciatic nerve and registering the contraction of the gastrocnemii in rats. (e) The LD_{50} was determined in mice by intravenous injection.

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Derivatives of 1-(5-Nitrofurfurylideneamino)hydantoin. Synthesis and Some Biological Properties

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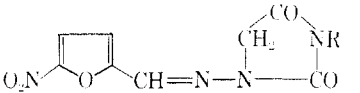
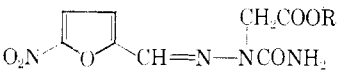
1-(5-Nitrofurfurylideneamino)hydantoin[1] has been widely used in the therapy of urinary tract infections. High urinary concentrations follow oral administration of this drug, but blood levels are unimportant even after intravenous administration of its sodium salt.[2] Attempts to modify its structure have not given satisfactory biological results.[3–5] The effects of 3-substituents in the hydantoin ring on the antibacterial activity, toxicity, and protein-binding capacity of the drug have now been investigated.

We have examined compounds with the following general formula



I, R = CH_2COOH V, R = $CH_2CH_2N(C_2H_5)_2$
II, R = $CH_2COOC_2H_5$ VII, R = *m*-ClC_6H_4
IV, R = C_6H_5

and the two semicarbazidoacetic acid derivatives III and VI.



III, R = C_2H_5
VI, R = H

Compound I was prepared according to Jack.[4] Compounds II, III, and VI already have been described,[4,6,7] but we have prepared II by a modified procedure; II and V were obtained by reaction of the sodium salt of 1-(5-nitrofurfurylideneamino)-hydantoin with ethyl chloroacetate and β-diethylaminoethyl chloride, respectively. Compounds IV and VII were obtained from the corresponding 1-aminohydantoins, prepared from 4-substituted semicarbazides by reaction with ethyl chloroacetate and sodium ethoxyde,[8] while III and VI were obtained simply from 5-nitro-2-furaldehyde and semicarbazidoacetic acid or its ethyl ester.

Materials and Methods. Antibacterial Spectrum.—Sensitivity determinations were carried out by the broth dilution method using phenol red mannitol broth (Difco) for the genera *Salmonella, Staphylococcus, Escherichia,* and *Aerobacter;* phenol red dextrose broth (Difco) for the genera *Bacillus, Shigella, Streptococcus,* and *Proteus;* and nutrient broth (Difco) for *Klebsiella pneumoniae, Alkaligenes faecalis,* and *Sarcina lutea.* Nitrofuran solutions were prepared by dissolving the compound in a 1:1 (v./v.) dimethylformamide–physiological saline mixture and Seitz filtering the solution. The inocula were prepared by making a 1:100 dilution of 18-hr. cultures grown in brain–heart infusion broth (Difco).

Acute Toxicity.—Swiss white mice (19–20 g.) were injected intraperitoneally with 1 ml. of solution. Deaths were noted over 7 days and the LD_{50} values were calculated by the method of Litchfield and Wilcoxon.[9]

Plasma Binding.—The ultrafiltration method[10] was used. About 10–15 ml. of plasma was pipetted into a dialyzer tubing (diameter inflated to 3 cm.) that was soaked in physiological saline and dried at room temperature. The cellophane sack was placed in a heavy-walled glass tube (9 × 2.9 cm.) about 2 cm. from the bottom. The tube was then centrifuged for about 3 hr. at 3000 r.p.m. Of the ultrafiltrate, 0.6 ml. was used for the

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