trans-2-Acetoxycyclobutyltrimethylammonium Iodide, a Cyclobutane Analog of "*trans*-ACTM"

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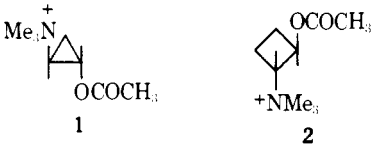
The (±) title compound was prepared to evaluate prior observations that certain acetylcholine congeners derived from cyclobutane are devoid of muscarinic effects. It was prepared by a multistep sequence from *trans*-2-carbomethoxycyclobutyl methyl ketone. In a guinea pig ileum assay, it was 0.02 times as active as AcCh, and in a dog blood pressure assay, it was 0.09 times as active. In these assays, its (±)-cyclopropane congener and AcCh were equally active. This is the first cyclobutane-derived AcCh congener possessing significant muscarinic effect.

Prior communications from this laboratory have described synthesis[1] and muscarinic effects[2] of (1*S*,2*S*)-*trans*-2-acetoxycyclopropyltrimethylammonium iodide (1, "*trans*-ACTM") which is somewhat more potent, on a molar basis, than acetylcholine. Comparison of a series of cyclopropane- and cyclobutane-derived congeners of acetyl-γ-homocholine and 4-acetoxy-*n*-butyltrimethylammonium for muscarinic and nicotinic effects[3,4] consistently revealed low orders of potency for all compounds, and every cyclobutane-derived system investigated was less potent than the corresponding cyclopropane system and was much less potent than the open-chain prototypes. It was concluded that, for this series, muscarinic inactivity seems an inexplicable property of the cyclobutane congeners.



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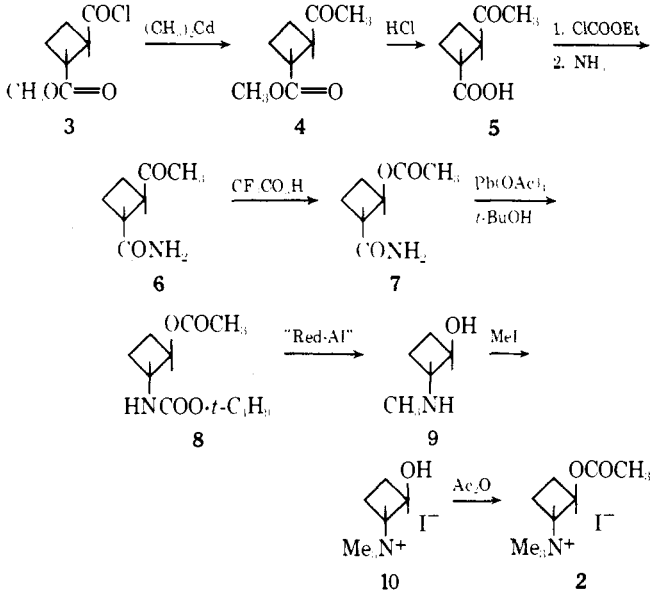
It seemed useful further to test this conclusion by preparation of the cyclobutane analog 2 of *trans*-ACTM, one of the most potent muscarinic agonists known. Synthesis of (±)-2 from *trans*-2-carbomethoxycyclobutanecarbonyl chloride 3 is outlined in Scheme I. As an alternate procedure, 5 could be prepared by treatment of *cis*-cyclobutane-1,2-dicarboxylic anhydride with dimethylcadmium; in the course of the reaction and/or in purification of the product, the material isomerized from cis to trans. A prior communication from this laboratory[4] stated that the pure cis isomer was isolated from this reaction. On the basis of the present work, it is concluded that improper assignment of stereochemistry was made in the earlier study and that the purported *cis*-2-acetylcyclobutanecarboxylic acid was actually trans. Spectral data on all compounds were consistent with the proposed structures.

Scheme I. Preparation of (±)-*trans*-2-Acetoxycyclobutyltrimethylammonium Iodide



Pharmacology. The potency of (±)-1 and (±)-2 relative to acetylcholine was calculated following two bioassay procedures evaluating muscarinic activity. (1) Superfusion of guinea pig ilea using Tyrode's solution was used as an in vitro assay. The doses of the compounds were varied by 0.3 log intervals and were injected into the superfusing medium. Both (±)-1 and (±)-2 induced maximal contractions equal to acetylcholine. The contractions induced by all compounds were antagonized by atropine sulfate. The order of administration of doses was randomized. Five ilea were used for each compound. (2) The depressor response of arterial pressure in five dogs anesthetized with baribtal sodium (250 mg/kg) was used for an in vivo assay. The route of administration was intravenous. Compounds and dosages were randomized. Atropine sulfate inhibited the responses. For both assays, the relative potencies and their 95% confidence intervals were calculated using Finney's[5] 2 × 2 parallel line bioassay. All criteria for a valid parallel line bioassay were met (see Table I).

Discussion

Compound 2 is the first cyclobutane derivative encountered in this laboratory which has exhibited significant muscarinic effect. However, it is decidedly less potent than (±)-1, its cyclopropane congener. While definitive conclusions and theories do not seem warranted, it may be noted that incorporation of a cyclobutane ring into these systems does not a priori lead to abrogation of muscarinic effect. It is not apparent why (±)-2 exhibited markedly different potencies in the two assays employed.

These data are consistent with our prior proposals[2,6] that a transoid disposition of the acetylcholine molecule is significant in interactions with the muscarinic receptor.

Experimental Section

Melting points were determined in open capillaries on a Thomas-Hoover Uni-Melt apparatus and are corrected. Boiling points are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn., and by the Microanalytical Service, College of Pharmacy, University of Iowa. Where analyses are indicated by symbols of the elements, the analytical results were within ±0.4% of the theoretical values. Ir spectra were recorded on a Perkin-Elmer 267 instrument, and NMR spectra were recorded on a Varian Associates T-60 instrument (Me_4Si).

Table I. Relative Muscarinic Activities of Acetylcholine Congeners

Compound	Guinea pig ileum, rel potencies (95% C.I.)	Dog blood pressure, rel potencies (95% C.I.)
Acetylcholine	1.0	1.0
(±)-1	1.01 (0.52–1.72)	0.93 (0.45–1.90)
(±)-2	0.02 (0.005–0.08)	0.09 (0.05–0.17)

(±)-*trans*-2-Acetoxycyclopropyltrimethylammonium Iodide (1). This was prepared by the method of Armstrong.[7]

(±)-*trans*-2-Carbomethoxycyclobutyl Methyl Ketone (4). An ether solution of dimethylcadmium (ca. 0.045 mol), prepared from 11 g (0.06 mol) of $CdCl_2$ and 0.12 mol of MeMgCl in 250 ml of ether, was siphoned (leaving the insoluble $MgCl_2$ behind) into a flask containing 14.1 g (0.08 mol) of *trans*-2-carbomethoxycyclobutanecarbonyl chloride **3**[3] in 100 ml of anhydrous ether. The mixture was stirred under N_2 overnight; then excess dimethylcadmium was hydrolyzed with excess 5% HCl and the aqueous phase was extracted three times with $CHCl_3$. The combined organic phases were washed with saturated $NaHCO_3$, dried (Na_2SO_4), and concentrated. The crude product was distilled through a spinning band column, bp 55–57° (0.2 mm), to afford 9.4 g (75%) of product [lit.[4] bp 99–101° (12 mm)].

(±)-*trans*-2-Acetylcyclobutanecarboxylic Acid (5). Method A. Compound **4** (10 g, 0.065 mol) was refluxed with 100 ml of 20% HCl for 6 hr. The cooled mixture was extracted three times with $CHCl_3$; the extracts were washed with a small amount of H_2O, dried (Na_2SO_4), and concentrated. Distillation of the residue at 100–102° (0.2 mm) gave 6.3 g (68%) of product [lit.[4] bp 105–108° (0.2 mm)].

Method B. An ether solution of dimethylcadmium (ca. 0.045 mol), prepared as described for **4**, was siphoned into a flask containing 10.1 g (0.08 mol) of *cis*-cyclobutane-1,2-dicarboxylic anhydride (Aldrich Chemical Co.), 7 g (0.08 mol) of anhydrous LiBr, and 50 ml of anhydrous THF. The mixture was stirred overnight under N_2; then the liquid portion of the reaction mixture was decanted, leaving behind a gelatinous solid. The solid was treated with 60 ml (0.08 mol) of 5% HCl and the resulting solution was extracted three times with $CHCl_3$. The combined extracts were washed with H_2O, dried (Na_2SO_4), and concentrated to afford an oil which was distilled at 100–102° (0.2 mm) to yield 4.5 g (40%) of a product whose spectral (ir and NMR) and physical properties were identical with those of the product of method A.

(±)-*trans*-2-Acetylcyclobutanecarboxamide (6). To an ice-cooled solution of 8.4 g (0.06 mol) of **5** in 200 ml of $CHCl_3$ was added slowly 10 ml (0.065 mol) of triethylamine. After 10 min, 8.0 g (0.072 mol) of ethyl chloroformate in 25 ml of $CHCl_3$ was added dropwise. The mixture was stirred at 0° for 1 hr. A slow stream of NH_3 was passed through the solution for 0.75 hr; then the mixture was stirred at room temperature for 2 hr. Evaporation of the solvent left a white solid which was extracted with benzene in a Soxhlet apparatus for 16 hr. The cooled benzene extract was filtered and evaporation of the filtrate gave a solid which was recrystallized from benzene to yield 6.3 g (75%) of product, mp 93–95°. Anal. ($C_7H_{11}NO_2$) C, H, N.

(±)-*trans*-2-Acetoxycyclobutanecarboxamide (7). Trifluoroperacetic acid was prepared by adding 14 ml (0.1 mol) of trifluoroacetic anhydride (Aldrich Chemical Co.) in 40 ml of CH_2Cl_2 to 2.4 ml (0.08 mol) of 90% H_2O_2 in 50 ml of CH_2Cl_2 at 0°. After 0.5 hr, this solution was added dropwise to a mixture of 5.6 g (0.04 mol) of **6**, 28.4 g (0.2 mol) of anhydrous Na_2HPO_4, and 120 ml of CH_2Cl_2; then the mixture was refluxed for 1 hr. The inorganic salt was removed and was washed with CH_2Cl_2. The combined organic phases were washed with saturated $NaHCO_3$ and dried (Na_2SO_4). Evaporation of the solvent and recrystallization of the solid residue from benzene afforded **7**. Additional product was obtained by Soxhlet extraction of the inorganic salt portion of the reaction mixture with benzene. The total yield of **7** was 4.2 g (67%), mp 105–106°. Anal. ($C_7H_{11}NO_3$) C, H, N.

(±)-*tert*-Butyl *trans*-2-Acetoxycyclobutanecarbamate (8). A mixture of 5.0 g (0.032 mol) of **7**, 20.0 g of lead tetraacetate, 300 ml of benzene, and 200 ml of *tert*-butyl alcohol was stirred under reflux for 4 hr. Triethylamine (40 ml) in 50 ml of benzene was then added dropwise. This mixture was then refluxed for 1 hr; then it was cooled and poured into 500 ml of EtOAc. The solid which separated was removed by filtration and was washed with EtOAc. The combined filtrate and washings were washed with saturated $NaHCO_3$ and dried (Na_2SO_4). Evaporation of the solvent left a dark oil which slowly crystallized and was recrystallized from hexane to yield 6.1 g (83%) of **8**, mp 77–79°. Anal. ($C_{11}H_{19}NO_4$) C, H, N.

(±)-*trans*-2-Hydroxycyclobutylmethylamine (9). To 14.4 ml (0.05 mol) of a 70% benzene solution of Red-Al (Aldrich Chemical Co.) in 40 ml of benzene was added 2.29 g (0.01 mol) of **8** in 20 ml of benzene, and this mixture was refluxed for 24 hr. Excess H_2O was added dropwise, and the resulting mixture was transferred to a separatory funnel containing 10 ml of saturated NaCl. After the aqueous layer separated, it was extracted three times with $CHCl_3$. The combined organic phases were dried (Na_2SO_4) and the volatiles were removed. Distillation of the oily residue through a short-path apparatus afforded 0.68 g (67%) of **9**, bp 57–58° (0.2 mm), mp 47–49°. Anal. ($C_5H_{11}NO$) C, H, N.

(±)-*trans*-2-Hydroxycyclobutyltrimethylammonium Iodide (10). MeI (12 g, 0.085 mol) was added to a cooled (0°) mixture of 4.3 g (0.043 mol) of **9**, 7.9 g (0.043 mol) of tributylamine, and 120 ml of EtOAc. This mixture was stirred in the dark at room temperature overnight. It was then filtered under N_2 and the solid on the filter was washed with EtOAc. Recrystallization of this solid from 1-butanol–heptane yielded 8.1 g (74%) of product, mp 178–181° dec. Anal. ($C_7H_{16}INO$) C, H, N.

(±)-*trans*-2-Acetoxycyclobutyltrimethylammonium Iodide (2). Compound **10** (2.9 g, 0.011 mol) in 30 ml of Ac_2O was heated to 80° for 16 hr. Upon cooling, a white solid separated; anhydrous ether was added until precipitation of the solid was complete. This material was collected on a filter, washed with ether, and recrystallized twice from 1-butanol–heptane to give 2.6 g (79%) of **2**, mp 164–166° dec. Anal. ($C_9H_{18}INO_2$) C, H, N.

Acknowledgment. This investigation was supported in part by Grant NS-06100, National Institute of Neurological Diseases and Stroke.

References and Notes

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