In Vivo Hydrolysis of Isomeric Bis(1,1,1-trichloro-2-propyl) 1,2-Cyclobutanedicarboxylates

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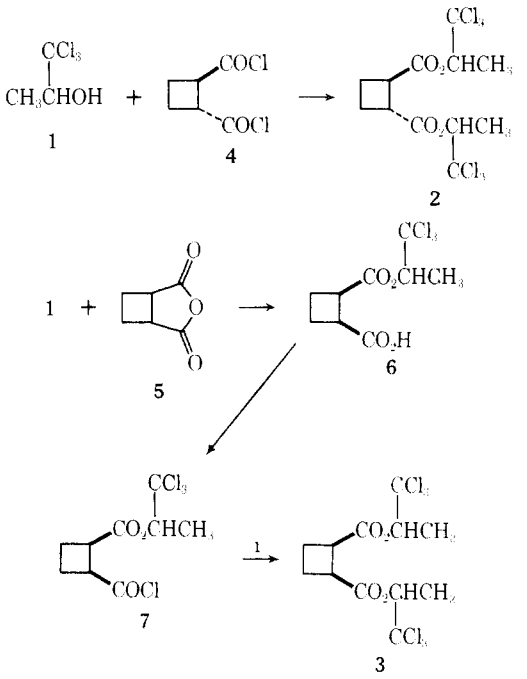
Both the *trans* (**2**) and *cis* isomers (**3**) of the title diester possess CNS depressant properties. Evidence has been obtained to show that the diesters are hydrolyzed *in vivo* in the rat to release 1,1,1-trichloro-2-propanol (**1**) which is responsible for the CNS depressant activity of the parent diesters. Rats receiving a 500 mg/kg oral dose of **2** were sedated 30 min after dosing and exhibited loss of the righting reflex after 1 hr, whereas those receiving the same dose of **3** were only slightly lethargic during the same experimental period. The serum concentration of **1** found in rats receiving **2** at a given period of time was markedly higher than that found in rats receiving **3**. The present results establish that the *trans* isomer **2** has a faster *in vivo* hydrolysis rate than the *cis* isomer **3**. This is in agreement with the chemical hydrolysis rates of isomeric diethyl 1,2-cyclobutanedicarboxylates.

Like 2,2,2-trichloroethanol, 1,1,1-trichloro-2-propanol (**1**) is a potent hypnotic. Its carbamate is also known to be a strong hypnotic agent.[1] We have prepared a series of esters of carbonic acid[2] and aliphatic acids and tested for CNS depressant activity. Evidence has been obtained that these esters are cleaved *in vivo* to release **1**. The onset, duration, and degree of hypnotic action are directly related to the rate of hydrolysis and the amount of **1** released. Therefore, **1** is responsible for the CNS depressant activity of the esters. This paper reports the difference in the metabolic rates, and hence, in the hypnotic responses, of two stereoisomers in the rat, bis(1,1,1-trichloro-2-propyl)-*trans*- and -*cis*-1,2-cyclobutanedicarboxylate (**2** and **3**, respectively).

The *trans* diester **2** was prepared from the corresponding acid chloride **4**. Treatment of the *cis* anhydride **5** with **1** (in molar equivalent or excess) afforded the half-ester **6**, which was converted into the acid chloride **7** with oxalyl chloride. Compound **3** was obtained by treating **7** with **1** (Scheme I).

Scheme I



Twelve male rats (Sprague Dawley strain) (250–300 g) were divided into two groups of 6 rats each. One group received **2** and the other group received **3** at an oral dose of 500 mg/kg of body weight. One and two hours later, 3 rats from each group were placed under light ether anesthesia and blood samples were obtained by aortic puncture. An additional group of three rats was administered vehicle (0.2 ml of a 3.3% v/v "Tween 20" solution in H_2O per 100 g of body weight) and blood obtained after 1.5 hr. The blood samples were allowed to clot at 0–4° for 3 hr and centrifuged to obtain serum. Aliquots of EtOAc extracts of the serum samples were analyzed for **1**, **2**, and **3** by gas–liquid partition chromatography. The rats were also observed visually for signs of drug effect during the period before sacrifice. The results of this experiment are tabulated in Table I. There were no detectable amounts of parent esters (<5 μg/ml) present in the serums. Even when three times the normal amounts of extracts of several samples were chromatographed, no **2** or **3** was detected. The concentration of **1** in the serum of rats was highest after the administration of **2**. Rats receiving **2** were sedated 30 min after dosing and exhibited loss of the righting reflex after 1 hr, which extended to the time of termination of the experiment at 2 hr. Rats receiving **3** were only slightly lethargic during this same experimental period.

The results clearly show that **1** is present in the serum of rats after administration of **2** or **3** and suggest that the *trans* isomer **2** is metabolized faster than the *cis* isomer **3** by the rat. The serum concentrations of **1** correlate very well with the hypnotic and lack of hypnotic effect of **2** and **3**, respectively. This suggests that **1** is responsible for the CNS depressant effect of both esters.

(1) L. Yoder, *J. Amer. Chem. Soc.*, **45**, 475 (1923).

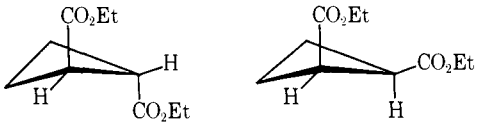
(2) (a) J. P. Li and J. H. Biel, to be published; (b) Clinical study of chlorethate has been reported by W. J. Beard and S. M. Free, Jr. [*J. Clin. Pharmacol.*, **7**, 41 (1967)].

TABLE I

Treatment	Serum concentration of 1 (μg/ml) 1 hr (n)[a]	2 hr (n)
2	81.5 ± 6.4[b] (3)	122.0 ± 21.6 (3)
3	12.2 ± 3.1 (3)	19.1 ± 2.0 (3)
Significance of difference in serum concn of 1, (2 *vs.* 3)	2P < 0.001	2P < 0.01

[a] *n* Denotes the number of individual rats treated. [b] Values are means ± standard errors.

Chemical hydrolysis of the *trans* (**8**) and *cis* isomer (**9**) of diethyl 1,2-cyclobutanedicarboxylate was studied by Gelin, *et al.*[3] They have found the rate constants k_1 and k_2 for **8** to be larger than those for **9** (at 25°, $k_1(\mathbf{8})/k_1(\mathbf{9}) \cong 5$, $k_2(\mathbf{8})/k_2(\mathbf{9}) \cong 38$). Their results suggest that the ethoxycarbonyl groups have a diaxial configuration in **8** and an axial–equatorial configuration in **9**. Hence, the ester functions in **8** are more exposed to attack by OH^-. Our *in vivo* hydrolysis results on **2** and **3** are in excellent agreement with Gelin's findings. The ester functions in **2** likewise must be in diaxial dispositions, so that they are more susceptible to enzymic hydrolysis due to smaller steric hindrance.



8 **9**

Experimental Section[4]

Quantitation of 1, 2, and 3 in Rat Serum.—Water-saturated AcOEt (0.6 ml) was added to 3 ml of serum (pH 7) in 15 × 125 mm tubes which were sealed with Teflon-lined screw caps. The tubes were shaken on a reciprocal shaker for 15 min and centrifuged. The AcOEt layers were transferred to 2-ml vials which were then sealed with Teflon-lined rubber closures. Compounds 1, 2, and 3 were quantitated in the extracts by gas–liquid partition chromatography. Concentration of extracts by solvent evaporation was avoided since it was determined in a preliminary extraction procedure that such a process caused marked losses of 1, 2, and 3 due to volatilization.

Glpc analyses were carried out on an F & M Model 810 analytical gas chromatograph. Coiled glass columns (6 ft × 6 mm o.d.) were employed. Column 1, 3% by wt of FFAP (Carbowax 20-M-terephthalic acid, Wilkins) on 100–120 mesh Gas Chrom-Q (Applied Sci.), operated at 92° isothermal at an attenuation of 10-1; and column 2, 3% by wt OV-17 (Applied Sci.), on 100–120 mesh Gas Chrom-Q, operated at 225° isothermal at an attenuation of 10-2. He was used as the carrier gas (flow rate 60 ml/min). A hydrogen flame detector was employed (hydrogen, 10 psig and air, 40 psig).

1,1,1-Trichloro-2-methyl-2-propanol was used as an internal standard for the quantitation of 1 in serum extracts. Solutions were prepared in AcOEt, containing 0.15 mg of internal standard and from 0.55 to 0.50 mg of 1 per ml and 1 μl of solution was chromatographed on column 1. Peak areas were determined by the height times the width at half-height method. The concentration of 1 in the solutions was plotted against the ratio of peak areas of **1** to peak areas of the internal standard. The plot was linear through the origin for the range covered. Aliquots of the serum extracts were spiked with internal standard solution and 1 was quantitated as for the standard curve. Recoveries of 1 from spiked serum were 100 ± 4%.

Solutions of **2** and **3** were prepared in AcOEt and 1 μl of each was chromatographed on column 2. The limit of detection of **2** or **3** was 0.025 μg. Since 1 μl of the serum extracts was chromatographed, the limit of detection is 5 μg of **2** or **3** per ml of serum. Recoveries of **2** and **3** from spiked serum was 100 ± 4%.

No peaks were observed at the retention times of 1, **2**, or **3** when extracts of serums obtained from rats treated with drug vehicle solution were chromatographed.

Bis(1,1,1-trichloro-2-propyl) *trans*-1,2-Cyclobutanedicarboxylate (2).—A solution of *trans*-1,2-cyclobutanedicarbonyl chloride (**4**, 4.5 g, 25 mmoles) in anhydrous C_6H_6 (5 ml) was added slowly into a stirred solution of 1,1,1-trichloro-2-propanol (1, 8.2 g, 50 mmoles) and pyridine (6 ml) in C_6H_6 (10 ml). After stirring at room temperature for 24 hr, the reaction mixture was diluted with more C_6H_6, washed first with dilute HCl, then with saturated $NaHCO_3$, and finally with H_2O. The dried ($MgSO_4$) solution was evaporated *in vacuo*, affording the product as a colorless syrup (11 g, quantitative yield). Pure **2** was obtained by kugelrohr distillation, collected at 165° and 1 × 10^{-3} mm, $n^{20}D$ 1.4954. *Anal.* ($C_{12}H_{14}Cl_6O_4$) C, H, Cl.

***cis*-2-(1,1,1-Trichloro-2-propoxycarbonyl) Cyclobutanecarboxylic Acid (6). Procedure A.**—A mixture of 1 (16.3 g, 0.1 mole), *cis*-1,2-cyclobutanedicarboxylic anhydride (**5**, 6.3 g, 0.05 mole), and pyridine (25 ml) was refluxed for 3 hr. After removing the pyridine *in vacuo*, the residue was mixed with iced H_2O, acidified with HCl, and extracted with $CHCl_3$. Work-up of the extract afforded an oily residue. Kugelrohr fractionation afforded unreacted 1 (7 g), sublimed and collected at 50–140° (0.05 mm), and **6** (13.6 g, 94% yield), collected at 165–170° (1 × 10^{-3} mm). Redistillation on kugelrohr gave the analytical sample, collected at 154–58° and 1 × 10^{-3} mm, $n^{20}D$ 1.4962. *Anal.* ($C_9H_{11}Cl_3O_4$) C, H, Cl.

Procedure B.—A mixture of 1 (16.3 g, 0.1 mole), **5** (6.3 g, 0.05 mole, C_6H_6 (50 ml), and concd HCl (1 drop) was refluxed for 4 hr, washed with H_2O, dried ($MgSO_4$), and evaporated *in vacuo*. Kugelrohr fractionation of the liquid residue at 1 × 10^{-3} mm afforded unreacted 1 (4.8 g), sublimed and collected at 50–100°, and **6** (15 g, quantitative yield), collected at 150°. Redistillation on kugelrohr gave the analytical sample of **6**, collected at 147–50° (1 × 10^{-3} mm), as a colorless syrup which crystallized to a white, waxy solid upon standing. The ir spectrum in $CHCl_3$ solution was superimposable upon that of **6** obtained by procedure A.

1,1,1-Trichloro-2-propyl *cis*-2-Chlorocarbonylcyclobutanecarboxylate (7).—A mixture of **6** (5.3 g, 18.2 mmoles), oxalyl chloride (9.3 g, 72.8 mmoles), and anhydrous C_6H_6 was stirred at room temperature for 18 hr. Evaporation of the solution *in vacuo* afforded **7** as a colorless liquid, ir (neat) 5.56, 5.72 μ. The crude product was used in subsequent reactions without further purification.

Bis(1,1,1-trichloro-2-propyl) *cis*-1,2-Cyclobutanedicarboxylate (3).—This compound was prepared in quantitative yield from 1 (10 mmoles) and **7** (10 mmoles; prepared from **6** obtained by procedure A) by the same procedure as is used in synthesizing **2**. Pure **3** was obtained by kugelrohr distillation and collected at 165° (1 × 10^{-3} mm), $n^{20}D$ 1.5010. *Anal.* ($C_{12}H_{14}Cl_6O_4$) C, H, Cl.

A sample of **3**, prepared, in the same manner, from **6** obtained by procedure B, had a superimposable ir spectrum in liquid state (neat) and an identical retention time in glpc.

(3) R. Gelin, S. Gelin, and C. Boutin, *C. R. Acad. Sci. Ser. C*, **262**, 1084 (1966).

(4) All temperatures given in kugelrohr distillations were not boiling points but were of the air bath during collection of the compounds. Microanalyses were performed by the Analytical Division, Aldrich Chemical Co., Inc. Determinations of C and H were conducted on an F & M Model 185 CHN analyzer.