and water until the time of the experiments. All animals were anesthetized with sodium pentobarbital solution (45 mg/kg, Abbott) prior to injection with labeled drug.

Dose and Route of Administration. Immediately after synthesis [^{82}Br]bromperidol was dissolved in 10.0 mL of the injection mixture, which consisted of propylene glycol–ethanol–water (40:20:40) and had its pH adjusted to 4 using concentrated HCl. An aliquot of the solution was counted to determine the exact dose of ^{82}Br radioactivity. Each animal received an intravenous bolus injection in the jugular vein of 19–22 μCi (0.2 mg/kg) of [^{82}Br]bromperidol.

Collection and Counting of Tissue Samples. After injection of [^{82}Br]bromperidol, rats were sacrificed by cardiac puncture at time intervals of 15, 30, 45, 60, and 120 min. A sample of blood was first collected with a syringe, followed by removal of liver, kidneys, lungs, and brain. All samples were placed in preweighted glass scintillation vials and counted in a Packard γ counter by monitoring the 777-keV peak. Counts were corrected for decay, and all samples were weighed after counting.

Acknowledgment. The authors express their thanks to Drs. C. Issidoridis and J. Chaney for invaluable suggestions and encouragement during the course of this work. This work has been partially supported by a grant from the National Institute of Mental Health (1 RO1 MH31477-01).

Notes

Glycerides as Prodrugs. 2. 1,3-Dialkanoyl-2-(2-methyl-4-oxo-1,3-benzodioxan-2-yl)glycerides (Cyclic Aspirin Triglycerides) as Antiinflammatory Agents[1]

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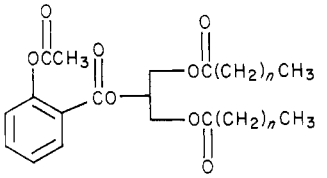
A series of 1,3-dialkanoyl-2-(2-methyl-4-oxo-1,3-benzodioxan-2-yl)glycerides ("cyclic aspirin triglycerides") was synthesized. They demonstrated essentially all the systemic antiinflammatory activity associated with aspirin in the carrageenin-induced rat paw edema test. Examination of the rat stomachs showed that the 1,3-didecanoyl derivative did not cause gastric lesions.

The antiinflammatory activity and the absence of gastric irritation properties of a series of 1,3-dialkanoyl-2-(*O*-acetylsalicyloyl)glycerides (1) having aspirin at the 2



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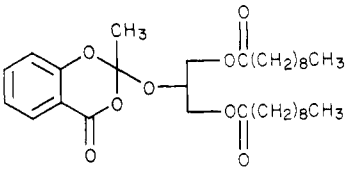
position of glycerol and fatty acids at the 1 and 3 positions have been reported in a previous paper.[3]

The most active member of this series was the 1,3-didecanoyl derivative (**1**, $n = 8$). During the purification of this compound by column chromatography, a byproduct was isolated and characterized as 1,3-didecanoyl-2-(2-methyl-4-oxo-1,3-benzodioxan-2-yl)glyceride (**2b**).

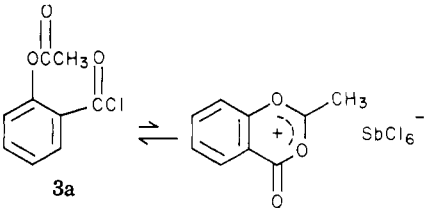
We now report the synthesis and the pharmacological properties of a series of glycerides in which aspirin in **1** is replaced by a 2-methyl-4-oxo-1,3-benzodioxan-2-yl moiety in the 2 position. For convenience, we will refer to this



2b

series of compounds as the "cyclic aspirin triglycerides".

Chemistry. It was reported by Brinkman and Rüchardt in 1972[4] that *O*-acetylsalicyloyl chloride (**3a**) exists as an



3a 3b

equilibrium mixture with the cyclic structure **3b**, which was isolated as the hexachloroantimonate salt.

In 1974, Rüchardt and Rochlitz[5] reported the isolation of the cyclic compound 4 as the main product obtained when a solution of *O*-acetylsalicyloyl chloride and alcohol (or phenol) was heated in tetrahydrofuran in the presence of a base.

(1) This work has been presented in part at the 176th National Meeting of the American Chemical Society, Miami Beach, Fla., Sept 11–15, 1978.
(2) Corresponding Address: Abbott Laboratories, North Chicago, Ill. 60064.
(3) Paris, G. Y.; Garmaise, D. L.; Cimon, D. G.; Swett, L.; Carter, G. W.; Young, P. *J. Med. Chem.* **1979**, *22*, 683.
(4) Brinkman, H.; Rüchardt, C. *Tetrahedron Lett.* **1972**, 5221.
(5) Rüchardt, C.; Rochlitz, S. *Justus Liebigs Ann. Chem.* **1974**, 15.

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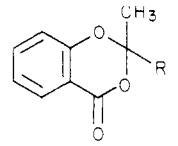
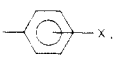


4, R = OC_2H_5, $OC_4H_9(n)$, 

X = H, 2-Cl, 4-Cl, 2-CH_3, 4-CH_3

A series of 1,3-dialkanoylglycerides (**5**) was prepared by the general method of Bentley and McCrae.[6] Extended refluxing of **5** and *O*-acetylsalicyloyl chloride (**6**) in $CHCl_3$ (for 24 h) in the presence of pyridine favored the formation of "cyclic aspirin triglycerides" (**2**; Scheme I).

Three cyclic aspirin triglycerides were prepared by this method and are described in Table I. The products were characterized by the ^1H and ^{13}C NMR, IR spectroscopy, and mass spectrometry.

The structure proof of **2** rested primarily on their NMR spectra. ^1H NMR spectra of **1** (n = 8) and **2b** were determined in $CDCl_3$. The 2-methyl group of the cyclic derivatives (**2**) resonated at a higher field (1.85 ppm) than the corresponding $-C(=O)CH_3$ in compound **1** (2.35 ppm). These results were in agreement with the values previously reported for structurally related esters.[5]

Further evidence for the assigned structure was gained from the ^{13}C NMR spectrum. The ^{13}C NMR spectrum of **2b** was completely consistent with the proposed structure, as determined by comparing the carbon resonances with compound **1** (n = 8). The most pertinent points were (a) the large upfield shift of the CH_3COOO- group and (b) the difference in the six phenyl carbons and the methyl ^{13}C NMR resonances (Table II).

Table III outlines the results of the reaction of 1,3-didecanoylglyceride (**5**, n = 8) with *O*-acetylsalicyloyl chloride (**6**) under various conditions. The reaction of **5** with **6** in the presence of a slight excess of pyridine in refluxing $CHCl_3$ afforded a 9.5:0.5 mixture of **2b** and **1**, in 75% yield, after 24 h. An 8:2 mixture and a 3:7 mixture were obtained by replacing $CHCl_3$, respectively, by CH_2Cl_2 and CCl_4. The use of a larger excess of pyridine, or a larger excess of pyridine combined with a lower temperature, did not favor the formation of the cyclic product. The replacement of pyridine by triethylamine was ineffective. In all these reactions a mixture was obtained.

Investigation of alternative approaches to the synthesis of the cyclic derivatives led to the observation that treatment of acetylsalicylic acid with trifluoroacetic anhydride, followed by reaction of the mixed anhydride with 1,3-didecanoylglyceride, gave **2b** in 76% overall yield.[7]

Biological Activity and Discussion

1. **Antiedema Efficacy and Ulcerogenicity.** A modification of the method described by Winter et al.[8] was used to test compounds **2** for antiinflammatory activity in the carrageenin rat paw edema assay; the results are presented in Table IV.

Potency. As can be seen in Table IV, compound **2b** was equipotent to aspirin, when compared on a molar basis, in the carrageenin-induced rat paw edema model. The chain length of the substituting fatty acids seems to have a modest effect on the antiinflammatory activity; the C_2 and the C_{16} compounds were approximately 60% as potent as aspirin.

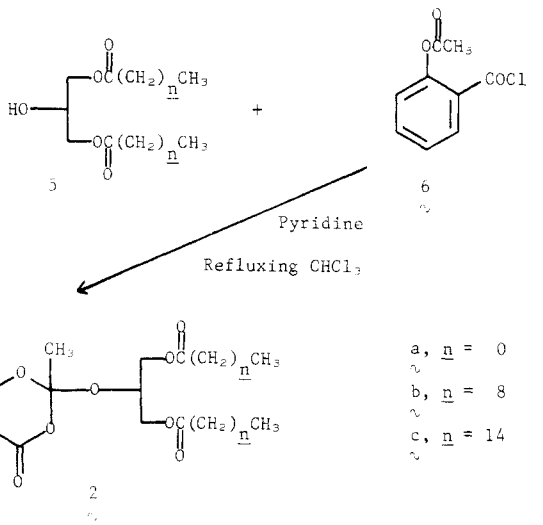
2-Hexadecyl-1,3-benzodioxan-4-one was prepared by refluxing cetyl alcohol and *O*-acetylsalicyloyl chloride in $CHCl_3$. In contrast to the cyclic aspirin triglycerides, this simple ester was found to be inactive in the rat paw edema test.

Ulcerogenicity. Compound **2b** was tested for its tendency to produce gastric irritation[9] in fasted rats. The compound produced a dose-related incidence of macroscopic lesions at oral doses of 995 to 7962 μmol/kg. The dose calculated to produce 50% incidence of lesions (UD_{50}) was 4102 μmol/kg by probit analysis. A comparative dose of 500 μmol/kg of aspirin produced 100% incidence.

2. **Salicylate Blood Level Determination.** The plasma salicylate levels following the oral administration of aspirin and compound **2b** were compared in rats (Figure 1). The plasma salicylate level obtained from **2b** at 414 μmol/kg, after 5 h, was approximately 70% that of aspirin, at the same molar dose.

The plasma salicylate levels and the antiedema activities of cyclic aspirin triglycerides obtained in this study are consistent with the results previously reported[1] for the aspirin triglycerides, as evidenced by the following: (a) compound **2b** has shown all the systemic activity associated with the cyclic aspirin incorporated in the molecule;

Scheme I



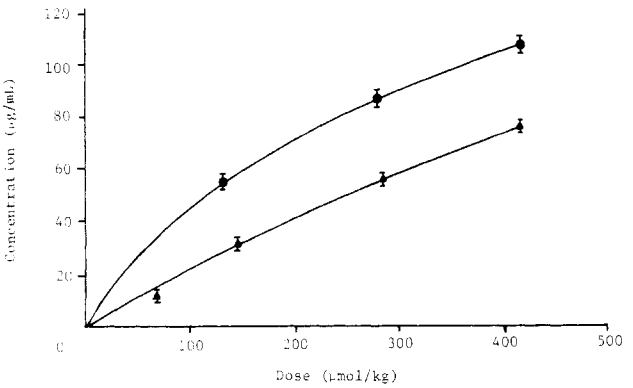
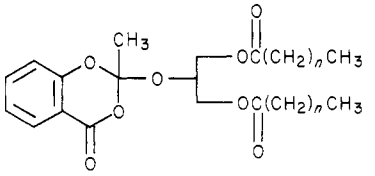


Figure 1. Rat plasma salicylate levels, 5 h after dosing, resulting from the oral administration of 1,3-didecanoyl-2-(2-methyl-4-oxo-1,3-benzodioxan-2-yl)glyceride (**2b**, ▲–▲) or plain aspirin (●–●). Values plotted are the mean ± SE of N = 6.

(6) Bentley, P. H.; McCrae, W. *J. Org. Chem.* **1970**, *35*, 2082.
(7) We are indebted to Mr. Frank Fisher of Abbott Laboratories, North Chicago, for the observation that led to the development of this method.
(8) Winter, C. A.; Risley, E. A.; Nuss, G. W. *Proc. Exp. Biol. Med.* **1962**, *111*, 544.
(9) Brodie, D. A.; Hansen, H. M. *Gastroenterology* **1960**, *38*, 353.

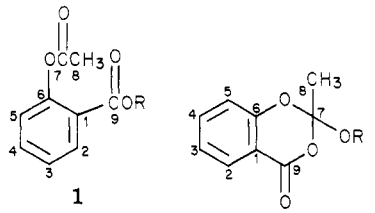
Table I. 1,3-Dialkanoyl-2-(2-methyl-4-oxo-1,3-benzodioxan-2-yl)glycerides



no.	n	M_r	aspirin, %	yield, %	physical form	formula	anal.	MS (M^+)
2a	0	338.32	53	65	66.5-67[a]	$C_{16}H_{18}O_8$	C, H	338
2b	8	562.74	32	31[b]	liquid	$C_{32}H_{50}O_8$	C, H	562
2c	14	731.08	24	36	semisolid[c]	$C_{44}H_{74}O_8$	C, H	730

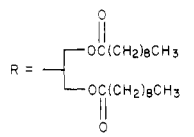
[a] Recrystallized from MeOH. [b] A 76% yield of pure **2b** was obtained by method B (see Experimental Section). [c] Recrystallized from petroleum ether (bp 30-60 °C).

Table II. ^{13}C NMR Spectra[a] of Compounds **1** and **2b**



1

2b



Ph

no.	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9
1	122.9	131.8	126.0	134.1	124.0	157.0	169.2[b]	21.0	163.4[b]
2	117.3	129.6	123.3	136.5	117.0	159.8[c]	113.2	23.6	154.9[c]

[a] All spectra were determined in $CDCl_3$ and the shifts are given in parts per million from Me_4Si as an internal standard. [b] These values can be interchanged. [c] These values can be interchanged.

Table III. Reaction Conditions of 1,3-Didecanoylglyceride with *O*-Acetylsalicyloyl Chloride[a]

expt no.	solvent[b]	base equiv[c]	yield,[d] %	c-asp/ asp TG[d,e]
1	$CHCl_3$	1.1	75	9.5:0.5
2	CH_2Cl_2	1.1	35	8:2
3	CH_2Cl_2	1.5	35[f]	6:4
4	CH_2Cl_2	1.5	35	4:6
5	CCl_4	1.1	55	3:7
6	CCl_4	1.1[g]	30	5:5
7[h]	CCl_4	2.2	90	1:9

[a] In all experiments, 1 equiv of 1,3-didecanoylglycerol and 1 equiv of *O*-acetylsalicyloyl chloride (commercial product) were used except as specified. [b] All reactions were done in the refluxing solvents for 24 h. [c] Pyridine was used as base, except where specified. [d] Calculated from high-performance LC analysis [μ-Bondapak C_{18}, using CH_3CN-H_2O (78:22) as solvent]. [e] Ratios of cyclic aspirin/aspirin triglyceride. [f] Done at 25 °C. [g] Triethylamine was used as base. [h] Two equivalents of acid chloride was used.

Table IV. Antiedema Efficacy of 1,3-Dialkanoyl-2-(2-methyl-4-oxo-1,3-benzodioxan-2-yl)-glycerides Relative to Aspirin

compd	act. as % of aspirin[a]
2a	62
2b	100
2c	62
aspirin	100

[a] Male Sprague-Dawley rats weighing 160-190 g were fasted overnight but allowed water ad libitum. Aspirin and cyclic aspirin triglycerides were administered orally 2 h prior to the subplantar injection of 0.1 mL of 1.5% carrageenin in saline. Paw volumes were measured, prior to and 3 h after injection, by immersion in a mercury-containing vessel connected to a volumetric transducer and a polygraph. The doses expected to produce 25% inhibition (ED_{25}) were calculated by simultaneous linear-regression analyses, and the activity reflects the potency comparison between the four compounds. The ED_{25} value for aspirin was 402 μmol/kg with 95% confidence limits of 326-499 μmol/kg.

(b) based on the low degree of gastric ulceration produced by compound **2b** as compared to aspirin, we assume that very little salicylate is liberated in the stomach [the ratio between ulcerogenicity and antiedema activity (UD_{50}/ED_{25}) is 10.6 for this compound, whereas the previously reported[10] index for aspirin is 0.17]; (c) the replacement of a fatty acid in position 2 of a triglyceride by a cyclic aspirin moiety does not alter the natural metabolic pathway of triglycerides.

Experimental Section

Melting points (uncorrected) were taken on a Thomas-Hoover capillary apparatus. Microanalyses were performed by the Analytical Research Department, Abbott Laboratories, North Chicago, Ill.; IR, UV, and NMR were in agreement with the assigned structures; NMR spectra were recorded with a Varian

(10) Carter, G. W.; Young, P. R.; Swett, L. R.; Paris, G. Y. *Agents Actions*, in press.

EM 360 (Me_4Si). All solvents were dried and used without further purification. The 1,3-dialkanoylglycerols were prepared by literature procedures.[6] TLC's were performed on fluorescent silica gel GF plates; the spots were detected by UV or with a potassium permanganate solution. The purity of the products was also checked by high-performance LC.

1,3-Didecanoyl-2-(2-methyl-4-oxo-1,3-benzodioxan-2-yl)-glyceride (2b). Method A. A solution of 1,3-didecanoylglyceride (10.0 g, 0.025 mol), acetylsalicyloyl chloride (4.96 g, 0.025 mol), and pyridine (2.2 mL, 0.028 mol) in dry ethanol-free $CHCl_3$ was refluxed for 24 h. The reaction mixture was treated with 100 mL of H_2O. The $CHCl_3$ layer was decanted, washed with 100 mL of 1% HCl, 100 mL of 1% aqueous $NaHCO_3$, and 2 × 100 mL of H_2O, and dried over $MgSO_4$, and the solvent was removed in vacuo. The oily product was chromatographed on 500 g of silica gel (previously deactivated with wet ether) with petroleum ether–ether (85:15). This procedure gave 4.8 g (31%) of **2b**: ^1H NMR ($CDCl_3$) δ 1.85 (s, 3 H, $OOOCCH_3$), 4.1 (m, 4 H, 2 × CH_2O), 4.4 (m, 1 H, CH). Compounds **2a** and **2c** were prepared in a similar manner except that they were purified by crystallization.

Method B. Trifluoroacetic anhydride (10.5 g, 0.05 mol) was added to a stirred suspension of acetylsalicylic acid (8.94 g, 0.045 mol) in dry C_6H_6 (60 mL). The reaction mixture was heated at 45 °C until a clear solution was obtained (1–2 min). The stirring was continued at room temperature for 30 min and 1,3-didecanoylglycerol (18.03 g, 0.045 mol) was added. The reaction mixture was stirred for 1 h at room temperature and cooled in an ice bath. To the cold solution was added 5% $NaHCO_3$ (about 150 mL), until the medium was neutral or slightly basic. The organic layer was decanted, washed with water (2 × 100 mL) and brine (100 mL), and dried over $MgSO_4$. Removal of C_6H_6 yielded crude 1,3-didecanoyl-2-(2-methyl-4-oxo-1,3-benzodioxan-2-yl)-glyceride (23.0 g, 92%). The clear oil was purified by chromatography as described in method A (19 g, 76% yield).

2-Hexadecyl-1,3-benzodioxan-4-one. *O*-Acetylsalicyloyl chloride (993 mg, 5 mmol) was added to a solution of cetyl alcohol (1.21 mg, 5 mmol) in 50 mL of dry $CHCl_3$ (ethanol free), and the solution was refluxed for 17 h. The reaction product was isolated as described for compound **2b**. The oily product was chromatographed on silica gel with petroleum ether–ether (95:5): yield 700 mg (35%); mp 36–38 °C (MeOH); ^1H NMR ($CDCl_3$) δ 1.80 (s, 3 H, $OOOCCH_3$), 3.65 (m, 2 H, OCH_2); MS m/e 404 (M^+).

Acknowledgment. The authors are most grateful to Dr. S. Huckin and Mr. G. Nettleship for high-performance LC and ^1H NMR spectra. We also thank Dr. R. Egan, Ms. R. Stanaszek and Dr. M. Levenberg, and Ms. S. Mueller of Abbott Laboratories, North Chicago, for ^{13}C NMR and mass spectra, respectively, and to the staff of the Analytical Research Department of Abbott Laboratories for elemental analysis.

Cholecystokinin (Pancreozymin). 5.[1] Hormonally Active Desamino Derivative of Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

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Acylation of the 6-peptide derivative L-methionylglycyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine amide with the *N*-hydroxysuccinimide ester of desaminotryosine afforded 3-(4-hydroxyphenyl)propionyl-L-methionyl-glycyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine amide. The phenolic hydroxyl group in this compound was esterified by treatment with SO_3–pyridine complex in dimethylformamide–pyridine. The sulfate ester was purified by chromatography and by countercurrent distribution. The desamino analogue of the C-terminal 7-peptide segment of cholecystokinin (DA-CCK-7) was tested for its abilities to stimulate amylase secretion from dispersed pancreatic acini in vitro, to increase protein secretion from cat pancreas in vivo, and to cause contraction of guinea pig gall bladder in situ. In increasing amylase secretion in vitro, the desamino heptapeptide was equal in efficacy with but approximately one-tenth as potent as the unaltered heptapeptide, whereas when tested in vivo or in situ, these two peptides were approximately equal in biological activity. It is evident that the N-terminal amino group of the C-terminal heptapeptide of CCK is not essential for its biological activities. The difference between the biological activity of the desamino compound and the unaltered heptapeptide seen in vitro and the absence of a substantial difference in vivo or in situ may indicate that the N-terminal amino group of CCK-7 is important in influencing its rate of disposition from the circulation. Additional evidence for this possibility is our finding that the desamino 7-peptide had a longer duration of action on gall bladder contraction in situ than did the unaltered peptide.

In our earlier studies[1,3] on the biologically active C-terminal 7-peptide segment of cholecystokinin (pancreozymin),[4] we observed that the partially protected intermediate in which the N-terminal *tert*-butyloxycarbonyl group was still present (Boc-CCK-7) showed somewhat

Boc–Tyr(SO_3H)–Met–Gly–Trp–Met–Asp–Phe–NH_2
Boc–CCK–7

more activity than the free 7-peptide (CCK-7). In fact,

(1) For the preceding paper in this series, which also summarizes the discovery, structure determination, and biological activities of cholecystokinin (pancreozymin), cf. M. Bodanszky, J. Martinez, G. P. Priestley, J. D. Gardner, and V. Mutt, *J. Med. Chem.*, **21**, 1030 (1978).

(2) Visiting scientist on leave from Equipe de Recherche No. 195 du Centre National de la Recherche Scientifique, Ecole Nationale Superieur de Chimie, Montpellier, France.

(3) M. Bodanszky, S. Natarajan, W. Hahne, and J. D. Gardner, *J. Med. Chem.*, **20**, 1047 (1977).

(4) V. Mutt and J. E. Jorpes, *Biochem. Biophys. Res. Commun.*, **26**, 392 (1967); *Eur. J. Biochem.*, **6**, 156 (1968); *Proc. Int. Union Physiol. Sci.*, **6**, 193 (1968); J. W. Jorpes, *Gastroenterology*, **55**, 157 (1968); V. Mutt and J. E. Jorpes, *Biochem. J.*, **125**, 57 (1971).

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