6×10^{-3} g/mL of K^+, to obtain a dose–response curve for calcium, which was determined in the presence of the test compound. On the basis of difference between the responses, the pA_2 value of the calcium antagonistic activity of the test compound was computed.[29] The results are shown in Table IV.

Effect of Intraduodenal Administration on Coronary Flow and Blood Pressure in Dogs. Dogs weighing 12–22 kg were anesthetized with pentobarbital sodium (35 mg/kg, ip), and under supportive respiration, at left thoracotomy was performed at the fourth intercostal space. The pericardium was incised to expose the heart and to facilitate measurement of the blood flow through the circumflex branch of the left coronary artery by means of an electromagnetic flowmeter (Nihon Kohden Co. Ltd., MF-26). The blood pressure was measured via a cannula inserted into the carotid artery connected to a pressure transducer (Nihon Kohden Co. Ltd., MPU-0.5), while the heart rate was calculated from the electrocardiogram. The abdomen was then sutured, leaving the end of the cannula outside of the body, and the test compound was administered. The test compound was diluted with 0.5% CMC to a concentration of 10 mg/mL and administered at a dose of 10 mg/kg.

(29) Nabata, H. *Jpn. J. Pharmacol.* **1977**, *27*, 239.
(30) Weil, C. S. *Biometrics* **1952**, *8*, 249.

Acknowledgment. We are indebted to Dr. T. Nose, director of this laboratory, for his support and encouragement. We thank Dr. M. Moriyama for helpful discussion. Thanks are also due to S. Fukada and K. Goto for skillful technical assistance.

Agents for the Treatment of Brain Edema. 2. [(2,3,9,9a-Tetrahydro-3-oxo-9a-substituted-1*H*-fluoren-7-yl)oxy]alkanoic Acids and Some of Their Analogues

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Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486, and Division of Neurosurgery and Department of Neurology, Albany Medical College, Albany, New York 12208. *Received July 22, 1985*

Our initial paper discussed brain edema resulting from traumatic head injury and the need for specific and effective agents to treat the disorder and disclosed a novel approach for the discovery of a drug of this kind. The current study describes the synthesis of a series of [(2,3,9,9a-tetrahydro-3-oxo-9a-substituted-1*H*-fluoren-7-yl)oxy]alkanoic acids and their analogues. These compounds were evaluated in an in vitro cerebrocortical tissue slice assay for their relative potencies in inhibiting $K^+ + HCO_3^-$ induced swelling. Structural modification at a number of sites in the "lead" compound revealed that significant biological activity was inherent only within a very narrow range of structural types. The observation that nearly all the biological activity resided in one of the two enantiomers demonstrated the marked stereospecificity of the most active compounds. One of the most potent compounds, (*R*)-(+)-[(5,6-dichloro-2,3,9,9a-tetrahydro-3-oxo-9a-propyl-1*H*-fluoren-7-yl)oxy]acetic acid ((+)-**5c**), exhibited a dose–response relationship in the in vivo acceleration/deceleration brain edema assay, and the data from the two highest doses were statistically significant. Electron microscopic examination demonstrated that the perivascular astroglial swelling that arises from this procedure is abolished in the animals treated with (+)-**5c**. This compound is currently being evaluated for its clinical efficacy and safety in the treatment of traumatic head injury.

Our first report[1] on the design and development of agents for treating brain injury emphasized the high incidence of this disorder, the present lack of effective, specific therapeutic agents to treat this problem, and other potential medical applications for a drug of this type. The rationale for a novel approach to drug therapy for this disorder was delineated. The concept was based on the sequence of events believed to follow traumatic insult to the brain: edema (including cellular swelling or cellular edema), ischemia, hypoxia, neuronal death, and necrosis, which sometimes leads to irreversible coma and death.[1]

Prevention of the cellular edema was viewed as a logical place to focus therapeutic intervention. Gray matter was considered to be the major site of clinical importance, with the astrocyte being the specific cell type involved.[2-4] The swelling was shown to result from a chloride plus cation transport into astrocytes accompanied by an osmotic equivalent of water.[3,4] The investigation was facilitated by the development of in vitro assays using cat cerebrocortical tissue slices in which inhibition of chloride transport and/or inhibition of swelling, all or a significant part of which is due to astrocytes, could be readily measured.[1,3]

Our initial studies involved an investigation of a variety of loop diuretics such as ethacrynic acid and (indanyloxy)acetic acids, which were known to owe at least part of their saliuretic activity to the inhibition of Cl^--transport in Henle's loop. The examination of several series of (aryloxy)alkanoic acids that had been designed as salidiuretic agents, including their nonsalidiuretic members, was instituted. It soon became obvious that the effects of these compounds on Cl^- transport in the kidney and the brain did not always run parallel. Of greatest interest were those compounds that exhibited marked Cl^--transport inhibitory activity in brain slices but displayed little or no effect in the kidney. Some (indanyloxy)alkanoic acids possessing these properties were described in our first paper[1] along with a discussion of the structural features that appeared to be responsible for the separation of effects.

Subsequent to the observation of specific anti brain edema activity in certain (indanyloxy)alkanoic acids, it was

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(1) Cragoe, E. J., Jr.; Gould, N. P.; Woltersdorf, O. W., Jr.; Ziegler, C.; Bourke, R. S.; Nelson, L. R.; Kimelberg, H. K.; Waldman, J. B.; Popp, A. J.; Sedransk, N. *J. Med. Chem.* **1982**, *25*, 567.
(2) Kimelberg, H. K.; Ransom, B. R. In *Astrocytes*; Fedoroff, S., Vernadakis, A., Eds.; Academic, in press.
(3) Bourke, R. S.; Kimelberg, H. K.; Daze, M.; Church, G. *Neurochem. Res.* **1983**, *8*, 5.
(4) Kimelberg, H. K.; Bourke, R. S. In *Handbook of Neurochemistry*; Lajtha, A., Ed.; Plenum: New York, 1982; p 31.

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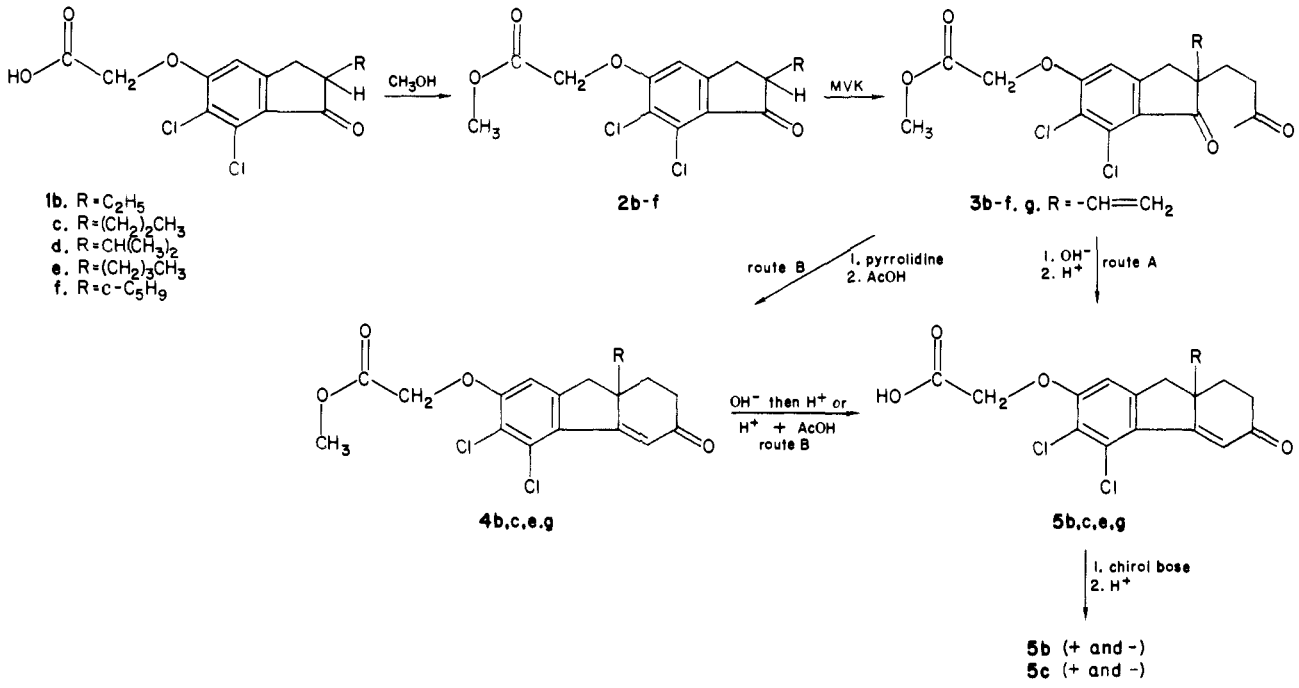
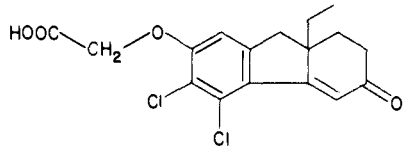
Scheme I



observed that potent in vitro inhibition of tissue swelling, as measured by the cat cerebrocortical tissue slice assay, occurred with [(tetrahydrofluoren-7-yl)oxy]acetic acid (**5b**).

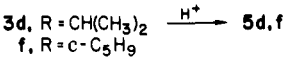


5b

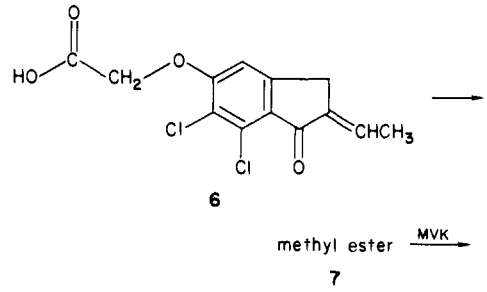
Cerebrocortical slices from the rat and guinea pig also were examined in this assay. Guinea pig tissue displayed comparable sensitivity to swelling inhibition by these analogues while rat tissue was relatively insensitive. This paper emphasizes the use of this assay to probe for an optimal compound of this structural class suitable for in vivo evaluation as a potential clinical candidate. One of these compounds, *R*(+)-[(5,6-dichloro-9a-propyl-2,3,9,9a-tetrahydro-3-oxo-1*H*-fluoren-7-yl)oxy]acetic acid ((+)-**5c**), was selected on this basis for evaluation in the cat brain edema model.

Chemistry. The molecules of concern in this series were assembled in various sequences depending on the accessibility of intermediates and the nature of the substituents. Scheme I discloses the synthesis of a number of compounds of type **5** starting with [(2-R-2,3-dihydro-1-oxo-1*H*-inden-5-yl)oxy]acetic acids (**1**).[1,5] Esterification of **1** to **2** followed by reaction with methyl vinyl ketone (MVK) to give **3** and then cyclization to **4** with concomitant saponification provided the desired product **5**. Simple tetrahydrofluorenones of this type have been described in which somewhat similar synthetic routes were used.[6-9]

Scheme II



Scheme III



Selected racemates of type **5** were resolved by fractional crystallization of salts of chiral bases. Thus, the pure enantiomers of both **5b** and **5c** were prepared.

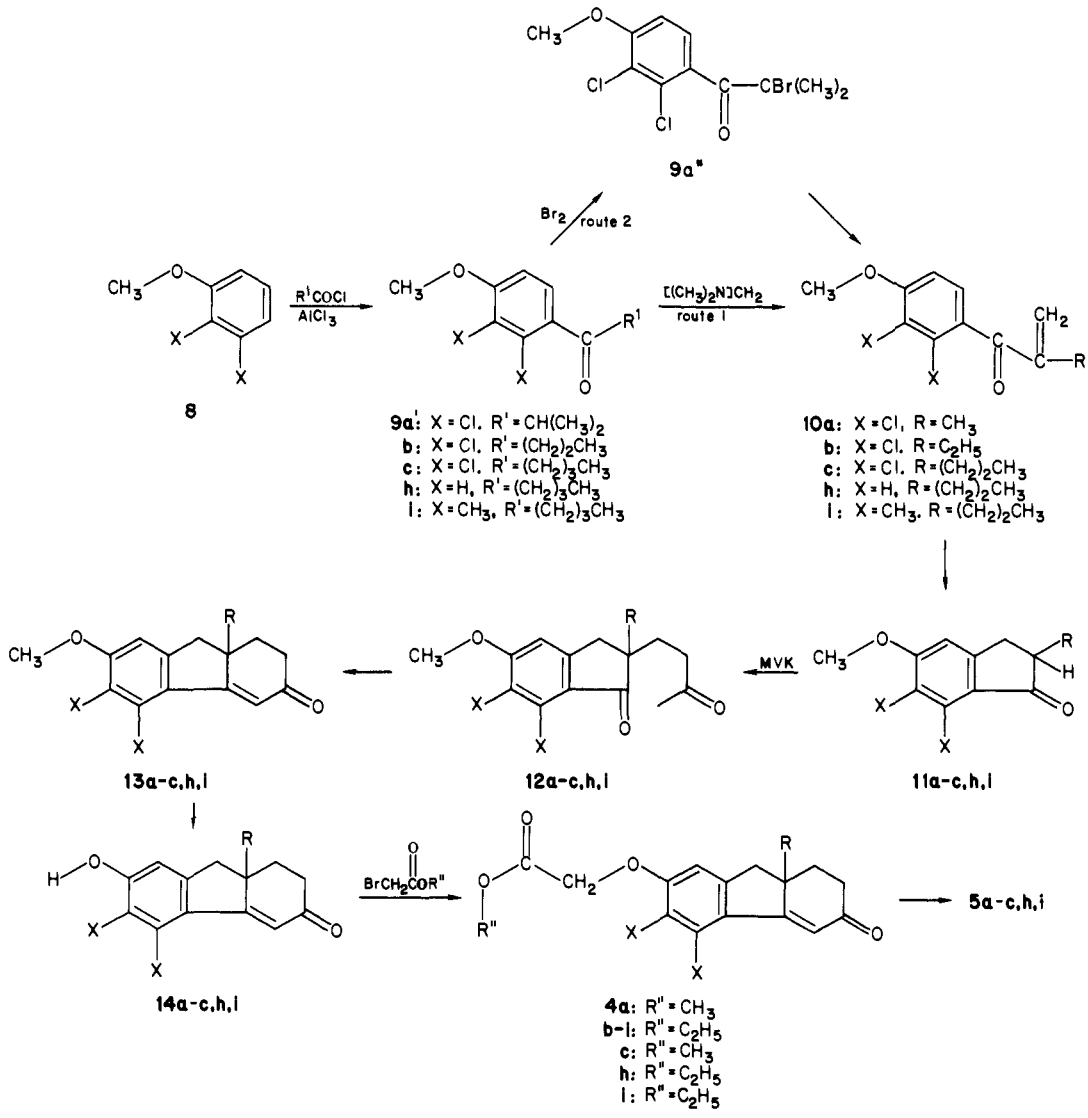
Base-catalyzed cyclization of intermediates of type **3** proceeds poorly, giving some unwanted annulation when R is branched (e.g., isopropyl or cyclopentyl). However, cyclization to the desired product(s) occurs in acidic milieu (Scheme II).

An alternate route (Scheme III) to the 9a-ethenyl compound **5g** is employed using the 2-ethylidenyl compound **6**. Esterification of this compound to give **7** followed by reaction with MVK produced the desired intermediate **3g** that could then be cyclized and saponified to **5g**.

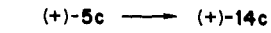
Another route to compounds of type **5** is shown in Scheme IV. A Friedel–Crafts reaction involving an appropriate anisole (**8**) and acid chloride produced the desired alkanophenone (**9**). The 2-methylenealkanophenones (**10**) could be prepared by either of two routes: (a) bromination to the 2-bromo derivative (**9a''**) followed by dehydrobromination to **10** or (b) a Mannich type reaction with *N,N,N',N'*-tetramethyldiaminomethane in the presence of acetic anhydride.[10] Annulation of compounds of type **10** to **11** proceeds well with concentrated sulfuric acid.

(5) Cragoe, E. J., Jr.; Woltersdorf, O. W., Jr. U.S. Patent 3 704 314, Nov 28, 1972.
(6) Davey, J.; Keene, B. R. T.; Mannering, G. *J. Chem. Soc., Sec. C Org. Chem.* **1967**, 120.
(7) Brown, E.; Ragault, M.; Touel, J. *Bull. Soc. Chim. Fr.* **1971**, 2195.
(8) Brown, E.; Touel, J.; Ragault, M. *Bull. Soc. Chim. Fr.* **1972**, 212.
(9) Kos, Y.; Loewenthal, H. J. E. *J. Chem. Soc.* **1963**, 605.
(10) deSolms, S. J.; Woltersdorf, O. W., Jr.; Cragoe, E. J., Jr.; Watson, L. S.; Fanelli, G. *J. Med. Chem.* **1978**, *21*, 437.

Scheme IV



Scheme V



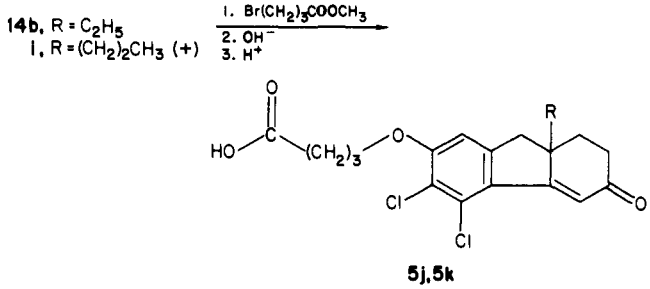
Reaction of indanones of type **II** with MVK produced the 2-(3-oxobutyl) derivatives (**12**) that were cyclized to the tetrahydrofluorenones (**13**) in base and the methyl ether function was cleaved by pyridine hydrochloride to produce the corresponding phenols (**14**). Reaction of **14** with ethyl bromoacetate produced the expected esters (**4**) that were saponified to yield the corresponding carboxylic acids (**5**).

To produce the enantiomeric phenol (+)-**14c**, the enantiomeric oxyacetic acid ((+)-**5c**) was cleaved by the use of pyridine hydrochloride (Scheme V).

For the synthesis of the oxybutyric acid homologues (**5j** (+)-**5k**) of **5b** and (+)-**5c**, **14b** and (+)-**14c** were treated with methyl 4-bromobutyrate in the presence of K_2CO_3 followed by basic hydrolysis of the resulting ester (Scheme VI).

A variety of esters (**16**) of the carboxylic acids of type **5** were prepared either by the classical acid-catalyzed reaction of **5** with the appropriate alcohol, R^2OH (method A), or by generating the acyl-1-imidazole (**15**) from **5** and 1,1'-carbonyldiimidazole followed by reaction with R^2OH (method B) (Scheme VII). Amides of type **17** were afforded by reaction of **15** with the appropriate amine (R^3NH_2).

Scheme VI



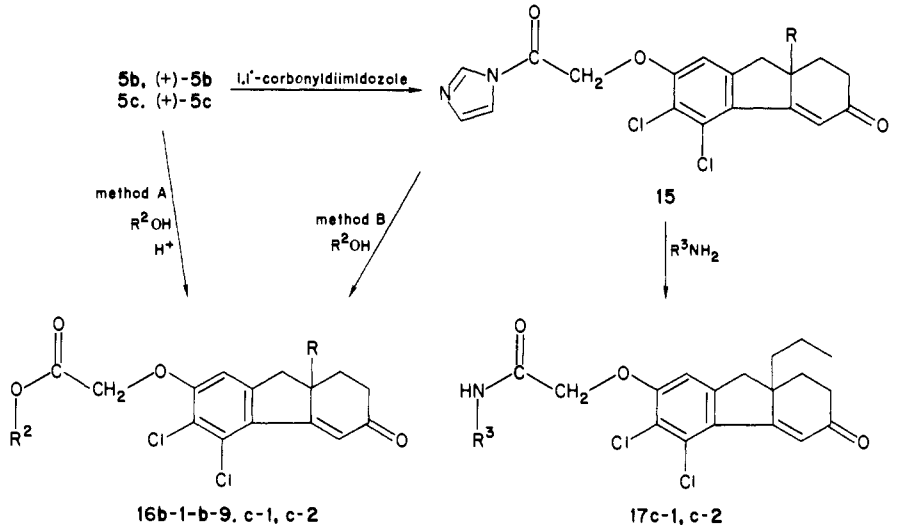
The 5-tetrazolyl analogue (**19**) of **5b** was prepared by the usual reaction sequence: generation of the phenol **14b** for reaction with chloroacetonitrile to produce the desired nitrile **18** that upon treatment with sodium azide and ammonium chloride produced tetrazole **19** (Scheme VIII).

The 4-methyl derivative (**21**) of **5c** was prepared from indanone **2c** by reaction with ethyl vinyl ketone in the presence of a catalytic amount of 1,5-diazabicyclo[4.3.0]-non-5-ene (DBN) to generate adduct **20** that was cyclized in acid to give **21** (Scheme IX).

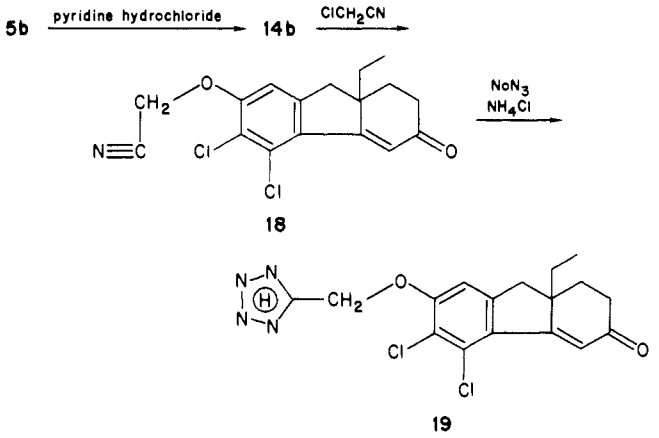
The 1,2-dehydro derivative of **5b** was synthesized from **5b** by bromination to **22** followed by dehydrobromination to **23** with DBN (Scheme X).

The homologue (**28**) of **5c** lacking the 1-methylene group was afforded by the five-step synthesis shown in Scheme

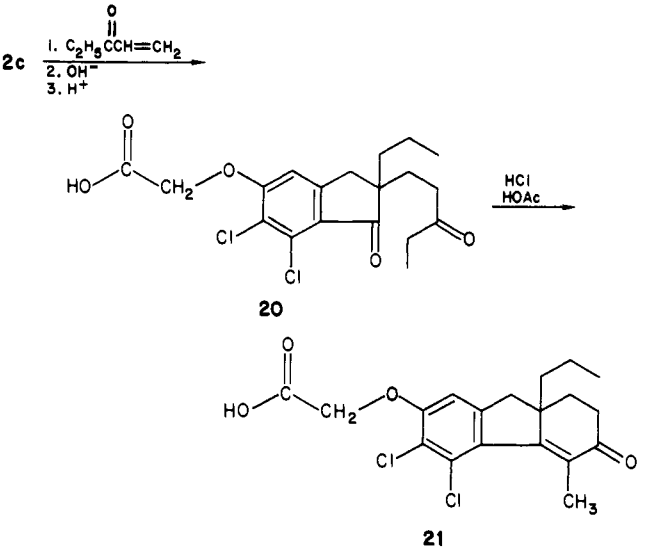
Scheme VII



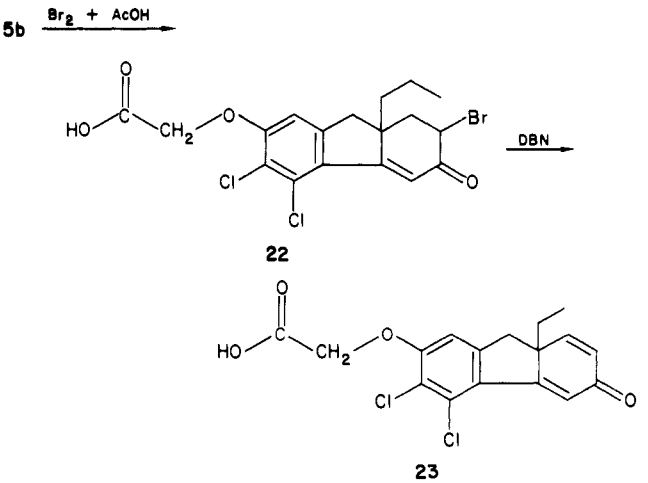
Scheme VIII



Scheme IX



Scheme X



XI. Indanone **11c** was alkylated with allyl bromide to obtain **24** that was oxidized[11] to ketone **25** with benzoquinone and $PdCl_2$. Compound **25** was demethylated with pyridine hydrochloride to give **26** that was cyclized under basic conditions to **27**. Etherification of **27** with methyl bromoacetate followed by saponification gave **28**.

A homologue of **5c** wherein the carbonyl function was exocyclic (**31**) was prepared as shown in Scheme XII. Alkylation of **11c** with 2-methyl-2-(3-iodopropyl)-1,3-dioxolane yielded **29**, which was demethylated and cyclized with pyridine hydrochloride to **30**, which was etherified with methyl bromoacetate and saponified to give **31**.

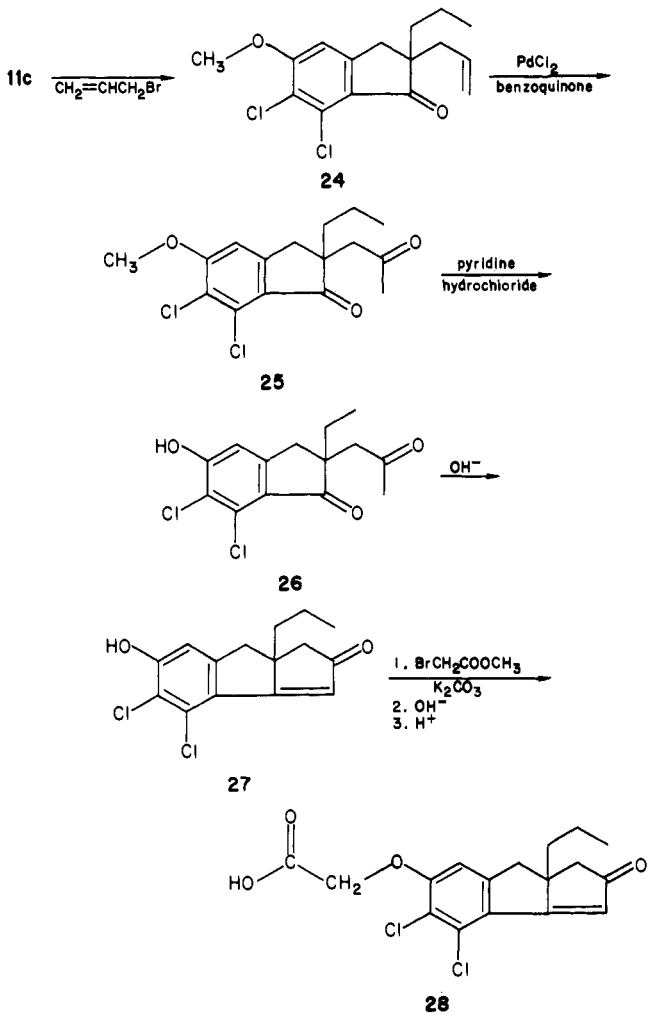
The synthesis of the thio analogue (**37**) of **5c** is illustrated by Scheme XIII. Reaction of indanol **14c** with dimethylthiocarbamoyl chloride afforded thiocarbamate **32** that was thermally rearranged to **33**. Compound **34** was produced by aqueous methanolic KOH cleavage of **33**. Etherification of **34** with methyl bromoacetate yielded ester **35**. Reaction of **35** with MVK produced adduct **36** that was simultaneously saponified and cyclized in aqueous alcoholic base to give **37**.

X-ray structure analysis[12] of crystalline (+)-**5c** using Hamilton *R*-factor values revealed that this enantiomer had the 9a *R* absolute configuration. A similar study on (+)-**16b**-1, the ethyl ester of (+)-**5b**, revealed this enantiomer also has the 9a *R* absolute configuration.

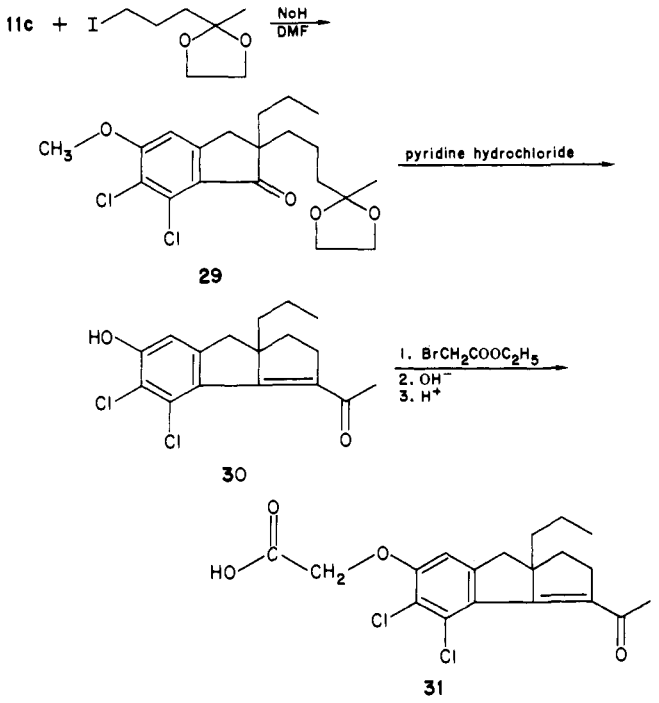
(11) Clement, W. H.; Selwitz, C. M. *J. Org. Chem.* **1964**, *29*, 241.
(12) Springer, J. P.; Krenz, H., unpublished data.

Biology. Tables I and II show the I_{50} values for inhibition of the HCO_3^--dependent component of swelling of cat brain slices (which also requires elevated K^+ in the medium),[1,3] for a number of 2,3,9,9a-tetrahydro-3-oxo-7,9a-disubstituted-1*H*-fluorenones and their analogues. Values for the pK_a and the octanol/water distribution for these compounds are also presented. The conditions of this assay were as described in the previous paper[1] and

Scheme XI



Scheme XII



elsewhere[3] and are also briefly recapitulated in the Experimental Section. It can be seen that a number of the compounds were extremely effective with I_{50} values in the range of 10^{-10} M. Some of these compounds exhibited

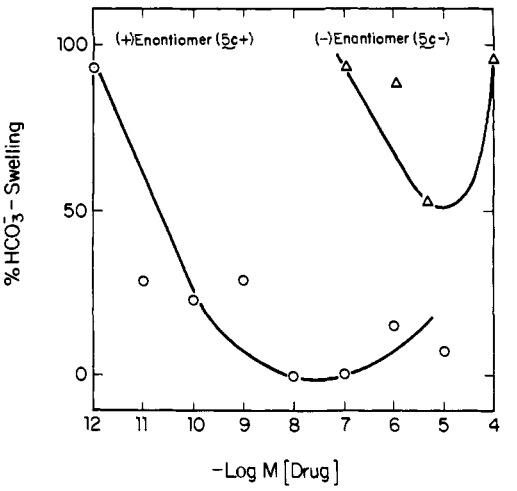


Figure 1. In vivo concentration–response curve for (+)-**5c** and (−)-**5c** using the cat cerebrocortical slice assay. This is the swelling dependent on added HCO_3^- in the presence of raised K^+ (27 mM) in the medium. See the Experimental Section for further details.

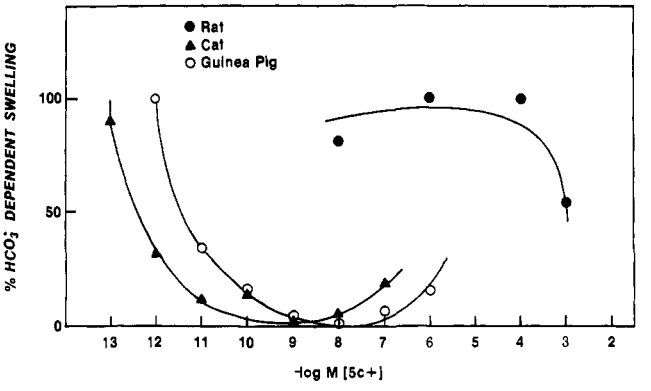
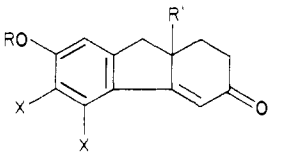


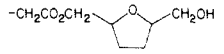
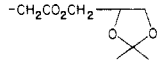
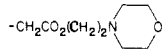
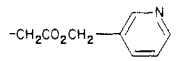
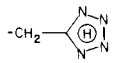
Figure 2. Dose–response curve for inhibition by the compound (+)-**5c** of HCO_3^--dependent swelling of cerebrocortical slices from cat, guinea pig, and rat. The assays were essentially the same for all species, but some modifications in cutting the slices and removal of excess fluid due to the greater fragility of rat and especially guinea pig slices had to be introduced (see the Experimental Section).

remarkable stereospecificity. A complete dose–response curve for the (+) and the (−) enantiomers of **5c** is shown in Figure 1. The (+) enantiomer shows 100% inhibition at 10^{-8}–10^{-7} M with an I_{50} of about 2×10^{-11} M. This 100% inhibition reduces the swelling to the values seen in the absence of bicarbonate, and these drugs do not affect the swelling in the absence of bicarbonate. The (−) enantiomer only shows a maximum of 50% inhibition at 10^{-5} M and then shows increased swelling again at 10^{-4} M.

We also found a remarkable species specificity in regard to the responsiveness of cerebrocortical slice swelling and ion uptake to inhibition by these drugs. This is shown in Table III and Figure 2 where it can be seen that while inhibition of HCO_3^--dependent swelling (see the Experimental Section for definition) was almost equally sensitive to inhibition by (+)-**5c** in both cat and guinea pig, rat brain slices were quite insensitive to inhibition. Data for the swelling and the associated changes in the ion content of slices from these three species demonstrating both stimulation due to HCO_3^- and inhibition of such stimulation by the varying concentrations of the drugs, including the appropriate p values, are shown in Table III. The data for rat slices reveal that the inhibition of swelling at 10^{-3} M (+)-**5c** is, in fact, associated with decreased K^+ and increased Na^+ content, suggesting inhibition of the Na^+,K^+ pump. Figure 3 shows data for inhibition of steady-state

Table I. Comparison of the Structure and Physical Data of Various 2,3,9,9a-Tetrahydro-3-oxo-7,9a-disubstituted-1*H*-fluorenes with Their in Vitro Activity



compd no.	R	X	R^1	enantiomer	% distribn in[a] octanol vs. pH 7.4 buffer	pK_a in[b] 30% ethanol	in vitro assay:[c] I_{50}, M
5a	$-CH_2CO_2H$	Cl	$-CH_3$	±	62	3.75	10^{-8}
5b	$-CH_2CO_2H$	Cl	$-C_2H_5$	±	81	3.6	3×10^{-8}
(+)-5b	$-CH_2CO_2H$	Cl	$-C_2H_5$	+	81	3.6	10^{-8}
(−)-5b	$-CH_2CO_2H$	Cl	$-C_2H_5$	−	81	3.6	3×10^{-5}
5c	$-CH_2CO_2H$	Cl	$-(CH_2)_2CH_3$	±	93	3.6	4×10^{-11}
(+)-5c	$-CH_2CO_2H$	Cl	$-(CH_2)_2CH_3$	+	93	3.6	2×10^{-11}
(−)-5c	$-CH_2CO_2H$	Cl	$-(CH_2)_2CH_3$	−	93	3.6	10^{-5}
5d	$-CH_2CO_2H$	Cl	$-CH(CH_3)_2$	±	89	3.83	$>10^{-7}$
5e	$-CH_2CO_2H$	Cl	$-(CH_2)_3CH_3$	±	98	3.5	$>10^{-8}$
5f	$-CH_2CO_2H$	Cl	$c\text{-}C_5H_9$	±	97	3.83	10^{-9}
5g	$-CH_2CO_2H$	Cl	$-CH{=}CH_2$	±	70	3.5	$>10^{-8}$
5h	$-CH_2CO_2H$	H	$(CH_2)_2CH_3$	±	49	4.05	5×10^{-8}
5i	$-CH_2CO_2H$	CH_3	$(CH_2)_2CH_3$	±	49	4.30	10^{-7}
5j	$-(CH_2)_3CO_2H$	Cl	C_2H_5	±	99.2	3.7	$>10^{-5}$
5k	$-(CH_2)_3COOH$	Cl	$(CH_2)_2CH_3$	+	99.8	3.7	3×10^{-9}
4b	$-CH_2CO_2CH_3$	Cl	C_2H_5	±	99.5		2×10^{-8}
16b-1	$-CH_2CO_2C_2H_5$	Cl	C_2H_5	±	>99.8		2×10^{-10}
(+)-16b-1	$-CH_2CO_2C_2H_5$	Cl	C_2H_5	+	>99.9		10^{-10}
(−)-16b-1	$-CH_2CO_2C_2H_5$	Cl	C_2H_5	−	>99.9		$\gg 10^{-5}$
16b-2	$-CH_2CO_2(CH_2)_3OH$	Cl	C_2H_5	±	99.3		7×10^{-10}
16b-3		Cl	C_2H_5	±	99.7		10^{-6}
16b-4		Cl	C_2H_5	±	99.9		$>10^{-8}$
16b-5	$-CH_2CO_2CH_2COCH_3$	Cl	C_2H_5	±	>99.9		10^{-9}
16b-6		Cl	C_2H_5	±	98.6	5.08 ($+H^+$)	$>10^{-8}$
16b-7		Cl	C_2H_5	±	>99.9	3.32 ($+H^+$)	10^{-10}
16b-8	$-CH_2CO_2CH_2CO_2H$	Cl	C_2H_5	±	91	3.80	6×10^{-9}
(+)-16b-8	$-CH_2CO_2CH_2CO_2H$	Cl	C_2H_5	+	83	3.80	3×10^{-9}
16b-9	$-CH_2CO_2C(CH_3)_2CO_2H$	Cl	C_2H_5	±	95	3.80	10^{-9}
(+)-16b-9	$-CH_2CO_2C(CH_3)_2CO_2H$	Cl	C_2H_5	+	96	3.80	5×10^{-10}
4-c	$-CH_2CO_2CH_3$	Cl	$(CH_2)_2CH_3$	±	>99.9		2×10^{-12}
(+)-16c-1	$-CH_2CO_2C(CH_3)_2CO_2H$	Cl	$(CH_2)_2CH_3$	+	98	3.80	4×10^{-12}
(+)-16c-2	$-CH_2CO_2C(C_2H_5)_2CO_2H$	Cl	$(CH_2)_2CH_3$	+	>99.9	3.40	4×10^{-11}
17c-1	$-CH_2CONH(CH_2)_2N(CH_3)_2$	Cl	$(CH_2)_2CH_3$	±	99.7	7.7 ($+H^+$)	$>10^{-6}$
17c-2	$-CH_2CONHN(CH_3)_2$	Cl	$(CH_2)_2CH_3$	±	99.7	3.90 ($+H^+$)	$>10^{-7}$
19		Cl	C_2H_5	±	95	3.72	$>10^{-7}$

[a] These data[16] were obtained by E. L. Cresson, M. M. Zrada, and S. J. Smith. [b] These data[17] were obtained by Y. C. Lee. [c] These data were obtained by the method described earlier[1] or are described in the Experimental Section-Biology under Cat Cerebrocortical Slice Assay and consist of the molarity required for 50% inhibition of swelling of cat cerebrocortical slices as determined by the concentration vs. response curve with values of $p = 0.005$. The > sign indicates that less than 50% inhibition occurred at the given concentration while the ≫ sign indicates that no inhibition was seen at the given concentration.

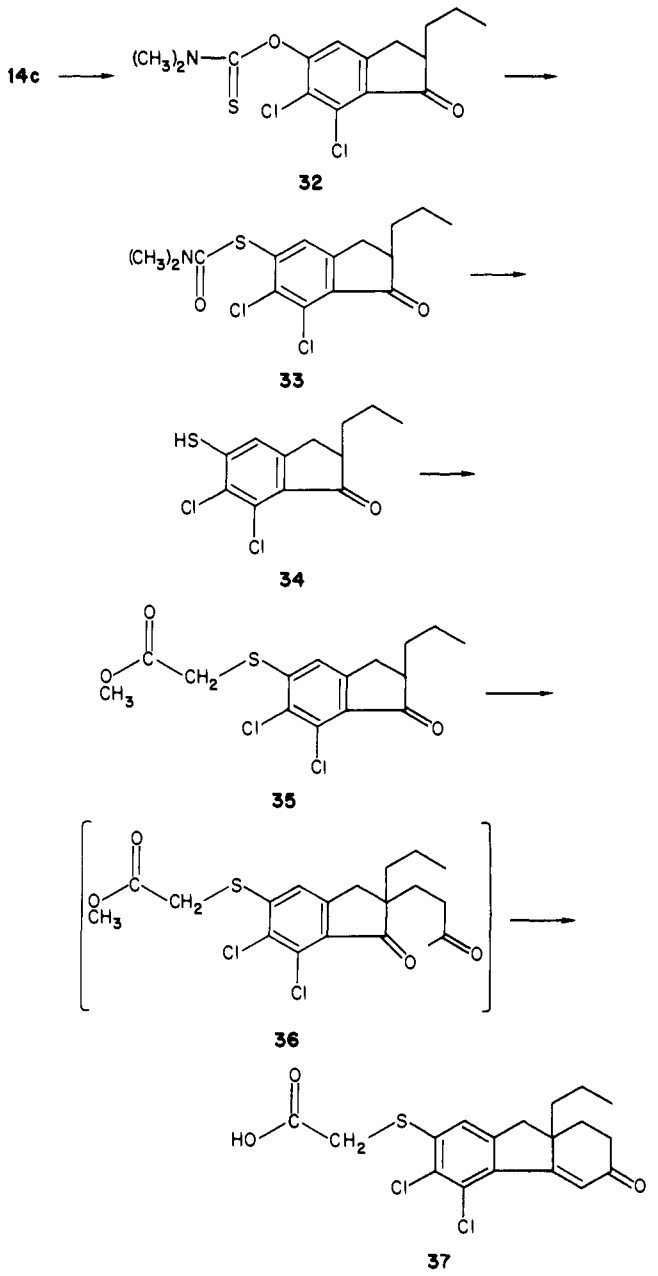
Table II. Comparison of the Structure and Physical Data of 2,3,9,9a-Tetrahydro-3-oxo-7,9a-disubstituted-1*H*-fluorene Analogues with Their in Vitro Activity

compd no.	% distribn octanol vs. pH 7.4 buffer	pK_a in 30% ethanol	in vitro assay:[a] I_{50}, M
(+)-14c	11	6.18	$>10^{-5}$
21	98	3.50	$>10^{-7}$
23	79	3.70	$>10^{-8}$
28	88	3.84	10^{-7}
31	95	4.10	10^{-7}
37	99	4.70	$>10^{-6}$

[a] See Table I, note *c*.

uptake of $^{36}Cl^-$ by rat astrocyte cultures under control conditions of normal $[K^+]_0$ in HCO_3^--buffered medium (see figure legend and the Experimental Section). Two separate experiments are shown in the two panels of a dose–response study for the effects of (+)-**5c**. The first value, shown as open bars, is a 2-min uptake to measure unidirectional influx; the second (hatched) bars represent a 40-min uptake to measure the steady-state Cl^- content when $^{36}Cl^-$ inside has equilibrated with the outside, i.e. reached the same specific activity. Consistent with the insensitivity seen in rat brain slices, inhibition of $^{36}Cl^-$ influx in the primary astrocyte cultures was only seen at 10^{-3} to 5×10^{-3} M concentrations of the drug. There was

Scheme XIII



a large increase in Cl^- content at 40 min in one culture at 5×10^{-3} M that may have been due to influx of NaCl due to a toxic effect. The apparent stimulation of Cl^- steady-state content at 10^{-6}–10^{-4} M of (+)-**5c** might be due to inhibition of coupled HCO_3^-/Cl^- exchange where the entry of HCO_3^- from the medium would lead to a lower steady-state $^{36}Cl^-$ content. Thus, selective inhibition of this process would tend to increase $^{36}Cl^-$ content.

One of the most potent compounds tested in the cat cerebrocortical slice assay, (+)-**5c**, was evaluated for its effectiveness in the acceleration–deceleration assay for brain edema in the cat developed at Albany Medical College.[13,14] Compound (+)-**5c** exhibited activity in this assay, showing a clear dose–response relationship between 1 and 10 mg/kg, with the reductions in mortality at the two highest doses being statistically significant (Table IV).

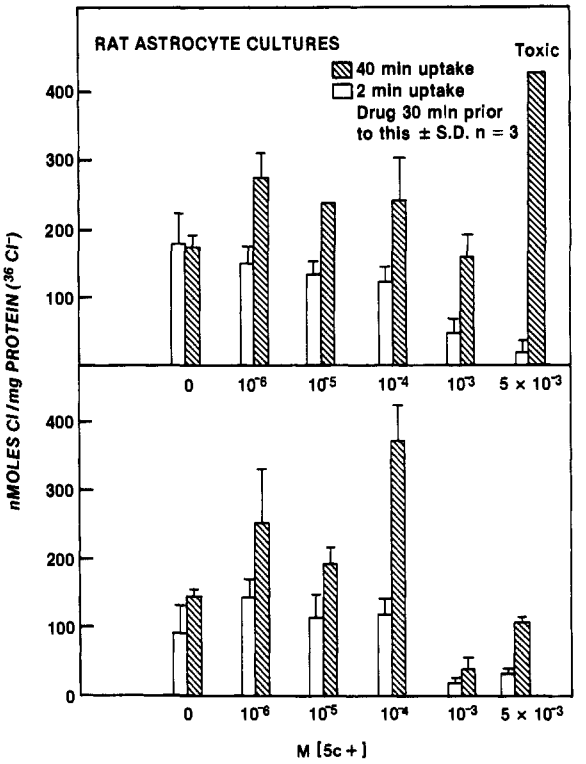


Figure 3. Inhibition of uptake of $^{36}Cl^-$ under normal conditions in HCO_3^--buffered medium in primary rat astrocyte cultures. Effects on unidirectional influx of $^{36}Cl^-$ measured at 2 min and steady-state levels measured at 40 min are shown as a function of the concentration of (+)-**5c**. The results are expressed as micromoles of Cl^- based on the specific activity of $^{36}Cl^-$ present in the medium. At 40 min a plateau is reached so that the specific activity of $^{36}Cl^-$ inside the cell and in the outside medium is assumed to be the same. Since these cells are in a steady state, total Cl^- influx equals efflux, and when the specific activities inside and outside the cell are the same, $^{36}Cl^-$ influx also equals $^{36}Cl^-$ efflux. Upper and lower panels represent two separate experiments on different cultures. n = three separate wells for each bar.

We have previously reported that perivascular astrocytic swelling occurs in this model as seen in electron micrographs[14] and we have attributed the HCO_3^--dependent component of swelling in vitro to swelling of the same cells. This type of swelling as produced in the acceleration–deceleration model is illustrated in Figure 4 where each panel shows a brain capillary surrounded by neuropil. Capillary lumens are present approximately in the center of all three panels of the figure. Compare the control, nontraumatized animal in Figure 4A with an animal perfuse-fixed 40 min after trauma in Figure 4B where the perivascular astroglial processes are markedly swollen. Figure 4C illustrates that when the animals were treated with 10 mg/kg of (+)-**5c** 20 min after the acceleration–deceleration event and then perfuse-fixed 20 min later, the perivascular region and the remainder of the neuropil are unswollen and appear normal.

Discussion

Structure–Activity Relationship (SAR). The in vitro data shown in Tables I and II consist of I_{50} (M) values for inhibition of K^+-dependent HCO_3^--stimulated swelling of cat cerebrocortical tissue slices. It had been shown previously[1] that these data provided a measurement of intrinsic activity and, therefore, furnished a useful basis

(13) Nelson, L. R.; Bourke, R. S.; Popp, A. J.; Cragoe, E. J., Jr.; Signorelli, A.; Foster, V. V.; Creel, W. In *Neural Trauma*; Popp, A. J., Nelson, L. R., Bourke, R. S, Kimelberg, H. K., Eds.; Raven: New York, 1979; p 297.

(14) Nelson, L. R.; Auen, E. L.; Bourke, R. S.; Barron, K. D.; Malik, A. B.; Cragoe, E. J., Jr.; Popp, A. J.; Waldman, J. B.; Kimelberg, H. K.; Foster, V. V.; Creel, W.; Schuster, L. In *Head Injury: Basic and Clinical Aspects*; Grossman, R. G., Gildenberg, P. L., Eds.; Raven: New York, 1982; p 117.

Table III. K^+-Stimulated, HCO_3^--Dependent Swelling and Ion Uptake in Cat, Guinea Pig, and Rat Cerebrocortical Slices and Its Inhibition by (+)-**5c**[a]

media	% swelling	Cl^-	Na^d	K^+	n
		Cat			
27 mM K^+ HEPES	17.9 ± 1.0	87.4 ± 1.1	80.2 ± 1.5	84.5 ± 1.4	8
27 mM K^+ HEPES + 5 mM HCO_3^-	28.3 ± 1.1[c]	98.0 ± 1.0[c]	102.3 ± 2.7[c]	85.8 ± 1.4	5
(+)-**5c**, M					
10^{-7}	20.0 ± 2.1[e]	86.9 ± 2.9[e]	86.0 ± 2.3[e]	83.9 ± 3.3	4
10^{-8}	18.6 ± 2.9[d]	89.1 ± 2.1[e]	87.2 ± 2.3[a,e]	83.8 ± 3.2	4
10^{-9}	18.3 ± 0.9[f]	89.3 ± 2.7[e]	85.6 ± 2.2[a,f]	86.0 ± 1.0	6
10^{-10}	19.5 ± 1.9[e]	91.9 ± 2.5	86.9 ± 2.1[a,e]	86.9 ± 0.9	5
10^{-11}	19.1 ± 1.7[e]	87.9 ± 3.1[d]	86.4 ± 3.0[e]	88.8 ± 1.7	4
10^{-12}	21.3 ± 0.8[a,e]	90.5 ± 3.5	96.4 ± 2.2[c]	90.9 ± 0.8[b,d]	4
10^{-13}	27.3 ± 1.2[c]	98.5 ± 3.0[b]	107.3 ± 2.4[c]	87.6 ± 1.9	4
		Guinea pig			
27 mM K^+ HEPES	26.1 ± 0.8	107.7 ± 1.7	110.4 ± 1.6	86.7 ± 1.4	27
27 mM K^+ HEPES + 5 mM HCO_3^-	30.6 ± 1.0[c]	118.7 ± 2.5[c]	128.2 ± 2.4[c]	94.4 ± 2.1[b]	23
(+)-**5c**, M					
10^{-6}	26.8 ± 1.2[d]	103.8 ± 2.5[f]	115.0 ± 3.2[e]	95.0 ± 3.1[a]	8
10^{-7}	26.4 ± 0.9[d]	115.4 ± 1.8[b]	125.4 ± 1.6[c]	88.1 ± 1.8[d]	16
10^{-8}	24.4 ± 2.8[d]	103.8 ± 3.6[e]	108.6 ± 3.4[f]	90.8 ± 3.3	5
10^{-9}	26.2 ± 1.0[e]	105.2 ± 0.9[f]	110.0 ± 2.1[f]	95.9 ± 2.0[c]	4
10^{-10}	26.8 ± 1.7	101.4 ± 3.1[f]	108.3 ± 3.6[e]	101.3 ± 2.2[c]	8
10^{-11}	27.6 ± 0.9	94.8 ± 3.2	103.4 ± 2.4	88.9 ± 2.9	5
10^{-12}	30.9 ± 1.2[c]	100.5 ± 2.5	104.7 ± 3.0	87.7 ± 1.9	8
		Rat			
27 mM K^+ HEPES	15.6 ± 1.1	92.9 ± 1.5	101.5 ± 1.4	77.2 ± 1.5	18
27 mM K^+ HEPES + 5 mM HCO_3^-	22.1 ± 1.1[c]	104.7 ± 1.5[c]	123.1 ± 2.2[c]	76.7 ± 1.9	15
(+)-**5c**, M					
10^{-3}	19.1 ± 1.8	106.3 ± 1.2[c]	132.4 ± 4.4[c]	60.2 ± 1.7[c,f]	6
10^{-4}	22.8 ± 2.7[b]	92.3 ± 2.2[f]	112.1 ± 2.6[b,e]	73.7 ± 1.7	6
10^{-6}	22.7 ± 0.9[c]	101.5 ± 2.3[b]	127.0 ± 3.6[c]	71.9 ± 2.9	5
10^{-8}	20.9 ± 1.2[b]	101.4 ± 2.1[b]	118.9 ± 3.7[c]	75.4 ± 3.1	6

[a] See experiments as shown in Figure 2. See the Experimental Section for further details of the assay, such as duration of the test, low and high HCO_3^- concentration and definition of % swelling. All data are mean values ± SEM. Drugs were always added in the presence of HCO_3^-. The drugs had no effect in the absence of added HCO_3^- (see reference 3). Key: a = $p < 0.025$ vs. 27 K; b = $p < 0.005$ vs. 27 K; c = $p < 0.0005$ vs. 27 K; d = $p < 0.025$ vs. 27 K + HCO_3^-; e = $p < 0.005$ vs. 27 K + HCO_3^-; f = $p < 0.0005$ vs. 27 K + HCO_3^-. All statistical values were determined by the two-tailed student's t-test.

Table IV. In Vivo Activity of (+)-**5c** in the Acceleration–Deceleration Head Injury Assay[a]

dose, mg/kg	treated animals: deaths/total	treated animals: % mortality	control animals: deaths/total	control animals: % mortality	Δ%
1	12/25	48	14/25	56	8
2.5	5/21	24	8/19	42	18
5	1/19	5[a]	7/22	32	27[a]
10	6/16	37.5[b]	15/19	79	41.5[b]

[a] Level of significance: a = $p < 0.05$, b = $p < 0.025$ by χ^2 analysis. For each study pairs were matched according to sex and run simultaneously with one animal randomly chosen to receive the intravenously administered drug and the other used as a control. Deaths were recorded over a period of 24 h following trauma, excluding the animals that died prior to application of hypoxia. Δ% refers to differences in mortality between control and treated animals.

for selection of compounds for in vivo evaluation.

Examination of the contribution of the 9a substituent (R^1 in the structure shown in Table I) to biological activity reveals its fundamental importance. Decreasing the chain length of the lead compound (**5b**) from ethyl to methyl (**5a**) had little effect on activity, but increasing the chain length to propyl (**5c**) produced a dramatic, 750-fold increase in activity. Compounds **5b** and **5c** were resolved to determine the influence of chirality on in vitro biological activity. In each instance, the enantiomer having the 9a *R* configuration was twice as active as the racemate. The apparent weak activity of the 9a *S* enantiomer, (−)-**5c**, at high concentrations may be a nonspecific effect or due to a trace of the (+)-**5c** enantiomer. The rigidity of these molecules and the importance of the 9a substituent in the expression of biological activity are consistent with the large differences in enantiomeric potency observed here.

Branching the propyl group (**5c**) to isopropyl (**5d**) or lengthening it to butyl (**5e**) produced a profound reduction in activity. However, when R^1 was cyclopentyl (**5f**), relatively good activity was exhibited (30-fold that of the lead **5b**). An alkenyl analogue (**5g**) of **5b** was markedly less active than its saturated counterpart.

From these data, we postulate a hydrophobic binding site above the plane of the hydrocarbon backbone that is optimally occupied by the methyl group of the 9a-propyl moiety of **5c** in its extended conformation. Molecular modeling revealed that only the cyclopentyl substituent (of **5f**) approached the efficiency of the *n*-propyl group in filling this spatial region.

Introduction of a 4-methyl group (**21**) in the highly active compound **5c** produced a dramatic reduction in activity. Introduction of a 1,2 double bond (**23**) in **5b** also resulted in a marked reduction in activity.

Replacement of the 5- and 6-chloro substituents of **5c** by H (**5h**) or CH_3 (**5i**) produced a corresponding 1250- and 2500-fold reduction in activity. The structural features of the 7-position side chain (R of Table I) were highly restrictive for maintenance of biological activity. Replacement of the acetic acid moiety of (+)-**5c** by H, (+)-**4c**, eliminated significant activity; likewise, homologation of **5b** and (+)-**5c** from CH_2COOH to $(CH_2)_3COOH$ (**5j**, (+)-**5k**), either eliminated or markedly reduced activity.

A number of esters of **5b** and **5c** were prepared and assayed. However, the relative insolubility of esters that were devoid of salt-forming moieties made their assay difficult and precluded their use by intravenous admin-

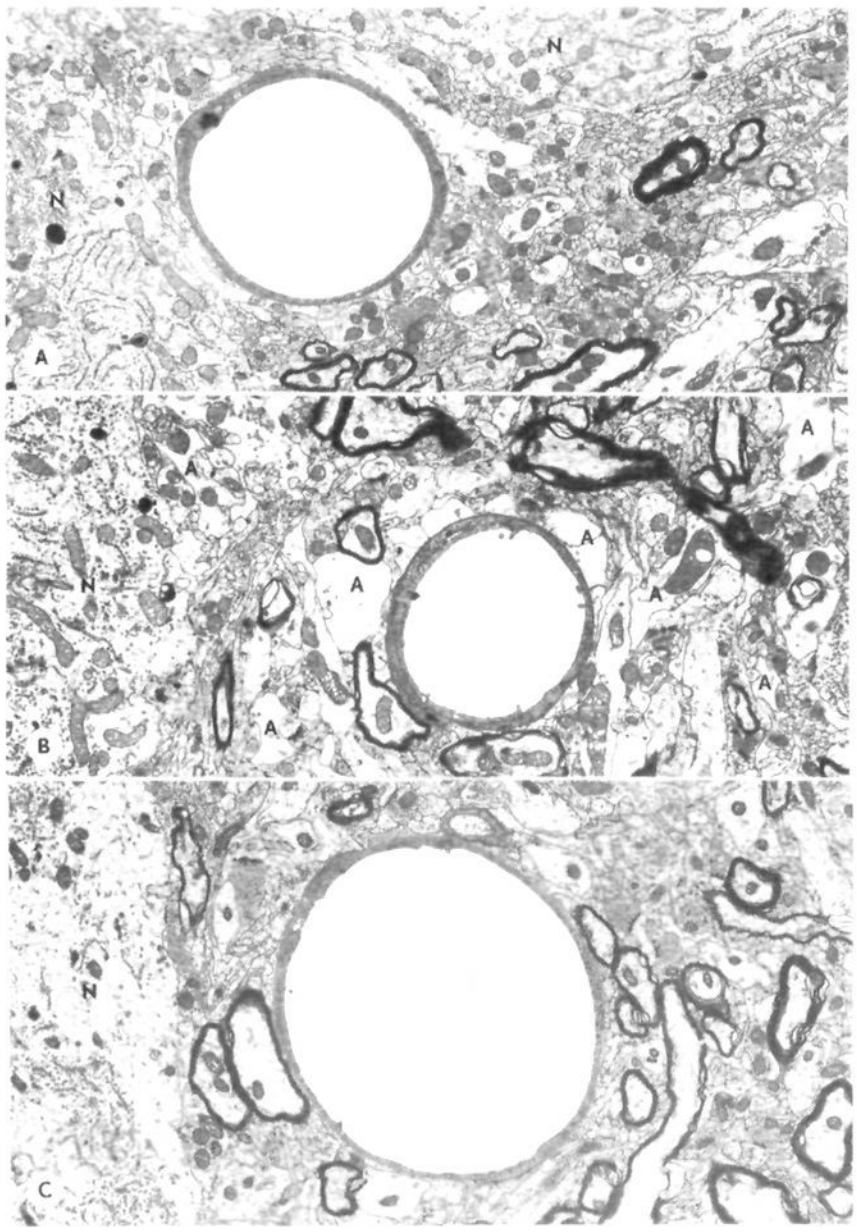


Figure 4. Effect of (+)-**5c** on trauma-induced astroglial swelling in the cat. The three figures were derived form the Betz cell layer of feline sensorimotor cortex. A: Normal animals. Note compact pericapillary neuropil. Neuronal cytoplasm at N. 8590×. B: Animal killed 40 min after acceleration–deceleration injury. Note electron-lucent, expanded astrocytic profiles, some of which are labeled A. Neuron at N. 8590×. C: Animal injected with (+)-**5c** at 10 mg/kg 20 min after shake injury and killed 20 min later. Astrocytic edema has been prevented, and the neuropil resembles that seen in A. 8590×. Animals were perfused, fixed, and brain tissue prepared for electron microscopy as previously described.[29]

istration to animals. The methyl ester (**4b**) was equipotent to the parent acid **5b**. However, the ethyl ester (**16b-1**) was much more potent than **5b** and, as expected, the major activity resided in the *R*(+) enantiomer ((+)-**16b-1**). The methyl ester (**4c**) of **5c** was 20-fold more potent than the parent carboxylic acid (**5c**). The 3-hydroxypropyl (**16b-2**) and 2-oxopropyl ester (**16b-5**) exhibited enhanced activity over that of **5b**, but two other types of esters were ineffective (**16b-3**, **16b-4**).

Two esters of **5b** bearing basic substituents were prepared; the 2-(4-morpholinyl) ester (**16b-6**) showed little activity, but the less basic 3-pyridylmethyl ester (**16b-7**) was 200-fold more active than **5b**.

To provide esters bearing a solubilizing carboxy function on the ester moiety, the glycolic acid esters **16b-8** and (+)-**16b-8** derived from **5b** and (+)-**5b** were prepared. These esters were slightly more active than their parent carboxylic acids, but their sodium salts displayed some tendency to hydrolyze. However, the corresponding 1-carboxy-1-methylethyl esters **16b-9** and (+)-**16b-9** were not only more active but more stable to hydrolysis. Similar enhancement of activity was observed with the 1-carboxy-1-methylethyl ester, (+)-**16c-1**, of (+)-**5c**. However, a higher homologous ester, (+)-**16c-2**, did not exhibit increased activity over the parent carboxylic acid, (+)-**5c**.

An amide of **5c** bearing a basic substituent (**17c-1**) and a hydrazide (**17c-2**) were markedly less active than the parent carboxylic acid.

The 5-tetrazolyl analogue of a biologically active carboxylic acid often displays similar or enhanced activity. However, this was not true of the 5-tetrazolyl analogue (**19**) of **5b**.

Replacement of the ether oxygen of loop diuretics with sulfur has little influence on activity;[15] however, application of this maneuver to the highly active **5c** to produce **37** resulted in a dramatic reduction in activity.

Two compounds were prepared in which the cyclohexanone ring of compound **5c** was five-membered rather than six-membered. The first one was the 1-demethylene analogue **28**. The second one was similar, being a homologue with the carbonyl group exocyclic, i.e. in the form of a 3-acetyl moiety (**31**). In each instance, a 2500-fold reduction in activity was observed.

In order to discover physical parameters that might relate to biological activity, a number of physical measurements, including pK_a[16] and water/lipid distribution[17] (as shown in Tables I and II), were made for each compound. Our earlier study suggested that pK_a values consistent with minimal ionization at physiological pH were preferable. In the present investigation there is some evidence of this trend, e.g. with **16b-8**, **16b-9**, (+)-**16c-1**, etc., but other factors may also contribute to the increased activity. Within a narrow series, increased lipid solubility appears to contribute to intrinsic biological activity. This is most obvious when carboxylic acids like **5b**, **5b**, **5c**, and (+)-**5c** were converted to the corresponding esters **16b-1**, **16b-2**, **16b-7**, **16b-9**, (+)-**16b-1**, (+)-**16b-9**, **4c**, and (+)-**16c-1**.

Biological Assay Systems and Chloride Transport Studies. The data in Figure 2 show a remarkable species specificity for the action of one of the most potent of the present series of drugs, (+)-**5c**. Guinea pig brain slices were only about 1 order of magnitude less sensitive than cat brain slices when assayed by inhibition of cerebrocortical slice swelling and ion uptake (see also Table III). However, in marked contrast rat brain slices only showed inhibition of swelling at 1 mmol, or about 9 orders of magnitude less than that seen in cat brain slices. Examination of the effect of 1 mmol of (+)-**5c** on the ion content of rat brain slices disclosed a marked drop in K^+ and a gain in Na^+ content at this concentration (Table III). This is consistent with inhibition of the (Na^+K^+) pump and may also explain the U-shaped curves for (+)-**5c** and its enantiomer (−)-**5c** observed for inhibition of cat cerebrocortical slice swelling (Figure 1). The more active enantiomer, (+)-**5c**, shows a broad range of inhibitory effects in cat brain slices at 10^{-9}–10^{-7} M with a slight increase in swelling at $>10^{-6}$ M. Measurements of the ion content of cat brain slices (Table III) were only carried to 10^{-7} M (+)-**5c**, and at this concentration content of Na^+ and Cl^- was still depressed. In contrast, the less active enantiomer, (−)-**5c**, shows a much shorter trough and only 50% maximum inhibition of HCO_3^--dependent swelling at 10^{-5} M. The marked increase in swelling at 10^{-4} M (−)-**5c** suggests that the (Na^+K^+) pump inhibitory effect is operative while the inhibition of swelling at 10^{-5} M (−)-**5c** suggests little or no pump inhibition.

The insensitivity of the swelling and ion uptake of rat

(15) Cragoe, E. J., Jr., Ed. *Chemistry and Pharmacology of Drugs*; Wiley: New York, 1983; Vol. 2, pp 212, 259.
(16) Albert, A.; Serjeant, E. P. In *Ionization Constants of Acids and Bases*; Wiley: New York, 1962.
(17) Scherrer, R. A.; Howard, S. M. *J. Med. Chem.* **1977**, *20*, 53.

cerebrocortical slices to (+)-**5c** was also found when unidirectional influx and steady-state levels of $^{36}Cl^-$ were measured in primary rat astrocyte cultures. However, it should be noted that these assays were done under conditions of normal K^+ concentration (4.5 mmol) in HCO_3^--buffered medium and thus do not represent the high K^+-stimulated swelling of brain slices that is associated with astroglial swelling.[3] Elsewhere we have discussed possible stimuli for such swelling,[2-4] including release of transmitters, lactic acid, and a high CO_2/HCO_3^- value either as single effectors or in combination. It seems unlikely that the compounds whose tests are described in this paper, and the (indanyloxy)alkanoic acids studies previously,[1] would interact with such effectors, and it is more likely that they inhibit ion-transport mechanisms activated by such conditions. It will be of considerable interest to examine guinea pig primary astrocytic cultures to see whether "resting" unidriectional $^{36}Cl^-$ influx in these cells is more sensitive to inhibition. It would also be of interest to determine whether $^{36}Cl^-$ influx stimulated by transmitters, lactate, or high K^+ is sensitive to inhibition in guinea pig and also rat cultures. As we have discussed,[2-4] these transport mechanisms could include coupled Na^+/H^+ and Cl^-/HCO_3^- exchange, leading to net uptake of NaCl with H^+ and HCO_3^- recycling via membrane-permeable CO_2 and H_2CO_3. Under conditions of hypoxia there will be a decreased capacity for the (Na^+K^+) pump to pump out the Na^+ accumulated, due to impairment of energy metabolism. In addition, these compounds could also inhibit uptake of Na^+ and Cl^- on a coupled $Na^+ + Cl^-$ ($+K^-$) cotransport system against which loop diuretics such as furosemide have been shown to be effective.[18] All these transport systems have been shown to be present in brain slices and primary astrocyte cultures,[3,4,19] and recently we have shown some involvement of a furosemide-sensitive system in volume regulation, due to changes in medium osmolarity, in rat astrocyte cultures.[20] Because of the strategic location of astrocytic processes around capillaries and neurons,[2-4,21,22] such swelling could have profound effects on neuronal function by exacerbating ischemia due either to partial occlusion of blood flow or to increasing O_2 diffusion path length (reviewed in ref 2). Also, uptake of Na^+ will decrease the inwardly directed Na^+ gradient and decrease the ability of astrocytes to take up and terminate the action of released transmitters such as amino acids[23] and catecholamines and serotonin.[24] Inhibition of astrocyte swelling, a major component of cellular edema,[2,25] and maintenance of normal transmembrane ion gradients early in the evolving posttrauma state, could thus be expected to have a marked beneficial effect.

Summary. The structural requirements that have been determined for the tetrahydrofluorenones described in this study are very narrow. The single 4,4a-double bond and 3-oxo group appear in each of the active compounds. Introduction of a 1,2 double bond decreased activity. Likewise, introduction of an alkyl substituent in the 4-position had a strong negative effect on activity. Replacement of the 5- and 6-chloro groups by H or CH_3 or the replacement of the ether oxygen by sulfur provided analogues with considerably reduced potency. Homologation of the acetic acid moiety to butyric acid has a detrimental effect on activity. Certain esters of the highly active carboxylic acids, particularly those bearing carboxy functions, exhibited enhanced activity.

The 9a substituent has a profound effect on intrinsic activity as measured by the in vitro assay. Peak activity among the alkyl substituents is seen with the propyl compound. Branching or unsaturation of the alkyl groups decreased activity. Chirality at the 9a position strongly influenced potency, with the *R* enantiomer possessing by far the greater activity.

Table IV lists the data obtained by using analogue (+)-**5c** in the in vivo acceleration–deceleration assay. This analogue displayed a dose-related increase in prevention of mortality of the animals, with the 5 and 10 mg/kg doses being statistically significant. Figure 4 shows the electron microscopic view of the perivascular astroglial swelling that occurs as a result of the acceleration–deceleration procedure and the important fact that treatment with a 10 mg/kg intravenous dose of (+)-**5c** just after the procedure abolishes this pathological response. Compound (+)-**5c** is currently being evaluated for its clinical efficacy and safety in the treatment of traumatic brain injury.

Experimental Section

Biology. Rat Primary Astrocyte Cultures and $^{36}Cl^-$ Uptake. Primary astrocyte cultures were started from the dissociated cerebral hemispheres of 1-day-old rats essentially as previously described but with some modifications, particularly the use of the neutral bacterial protease dispase for the initial tissue dissociation.[26] They were grown in 12 well plastic multiwells (Costar), with each well used for one determination. We have shown by immunocytochemical staining for the astroglial-specific marker glial fibrillary acidic protein (GFAP) that the proportion of cells in these cultures that are astrocytes is at least 90%.[26]

Uptake of $^{36}Cl^-$ was measured as previously described.[1,19] Essentially, the growth medium was replaced with 2 mL of HCO_3^--buffered medium of the following composition (mmol): NaCl, 122; KCl, 4.5; $NaHCO_3$, 25; $CaCl_2$, 1.3; KH_2PO_4, 1.2; $MgSO_4$, 0.4; Glucose, 10; Sucrose, 60. It was then equilibrated for 30 min at 37 °C in 5% CO_2/95% air atmosphere to pH 7.4, and 1–2 μCi of $^{36}Cl^-$ was then added to each well in 0.5 or 1.0 mL of medium. After varying periods of time, the dishes were rapidly washed seven times within 20 s with 2 mL/well of ice-cold 0.32 M sucrose solution, each wash using a repipet plus immediate vacuum aspiration. The cells were then dissolved in 1 N NaOH, and portions were used for determining $^{36}Cl^-$ radioactivity and total protein content of each well.

Swelling and Ion Transport in Cerebrocortical Tissue Slices. This was done essentially as previously described.[1,3] For cats, pial surface slices (~0.5 mm thick) were cut from the cerebral convexities with a Stadie–Riggs microtome without any moistening. Each slice was then weighed on a torsion balance and immediately placed in a Warburg flask containing 2 mL of incubation medium at room temperature (22 °C). The basic composition of the incubation medium (mmol/L) was as follows: KCl, 27; NaCl, 100; glucose, 10; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 1.3; HEPES (*N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid) (Sigma). This solution was titrated with NaOH to pH 7.8 and saturated with oxygen by bubbling the gas through the solution.

To obtain HCO_3^--dependent swelling, a solution of $NaHCO_3$ in the above medium was added from the side arm of the flask

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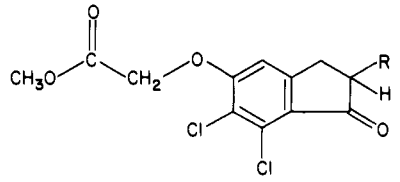
Table V. Compounds of Type 2

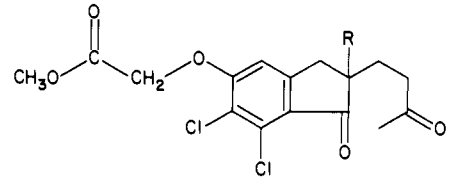


2

compd no.	R	% yield	recrystn solvent	mp, °C	formula	anal.
2b	C_2H_5	95	MeOH	128–130	$C_{14}H_{14}Cl_2O_4$	C, H
2c	$(CH_2)_2CH_3$	98	MeOH	126–128	$C_{15}H_{16}Cl_2O_4$	C, H
2d	$CH(CH_3)_2$	95	MeOH	96–97	$C_{15}H_{16}Cl_2O_4$	C, H
2e	$(CH_2)_3CH_3$	96		*a*	$C_{16}H_{18}Cl_2O_4$	*a*
2f	c-C_5H_{11}	99	MeOH	121–122	$C_{17}H_{16}Cl_2O_4$	*a*

[a] Used in the next step without purification.

Table VI. Compounds of Type 3



3

compd no.	R	% yield	recrystn solvent	mp, °C	formula	anal.
3b	C_2H_5	78	*a*	84–85	$C_{18}H_{20}Cl_2O_5$	C, H, Cl
3c	$(CH_2)_3CH_3$	66	Et_2O	91–93	$C_{19}H_{22}Cl_2O_5$	C, H, Cl
3d	$CH(CH_3)_2$	69	*a*	liquid	$C_{19}H_{22}Cl_2O_5$	*b*
3e	$(CH_2)_3CH_3$	77	MeOH	92–93	$C_{20}H_{24}Cl_2O_5$	C, H
3f	c-C_5H_{11}	68	*a*	101–102	$C_{21}H_{24}Cl_2O_5$	C, H
3g	$CH{=}CH_2$	49	THF–Et_2O–petroleum ether	79–82	$C_{18}H_{18}Cl_2O_5$	C, H

[a] Purified by column chromatography on silica gel using CH_2Cl_2–THF (50:1). [b] Identified by NMR, purity confirmed 99.9% by HPLC.

to slices that had been incubated in 2 mL of medium without added HCO_3^- at 37 °C for 20 min to achieve a final concentration of 5 mmol. The slices were then incubated for an additional 40 min. For control experiments, 0.5 mL of the medium without HCO_3^- was similarly added from the side arm. Inhibitors, when used, were present with the slices throughout the 60-min incubation period. After 60 min, the tissue slices were separated from the medium by careful vacuum filtration, reweighed, then homogenized (10% w/v suspension) in 1 N perchloric acid, and then centrifuged. The supernatant was then assayed for electrolytes as previously described.[1,3]

The tissue content of electrolytes is always expressed as micromoles per gram of the initial wet weight. Percent swelling was calculated as [(final wet weight minus initial wet weight)/initial wet weight] × 100, and HCO_3^--dependent swelling would be the increased swelling of slices due to added HCO_3^- in media containing 28–29 mmol of K^+. Such HCO_3^--stimulated swelling is not seen in medium with a normal K^+ concentration (4–5 mmol).[3]

For rat brain slices, pial slices from adult Sprague–Dawley male rats were cut, one from each cerebral hemisphere, with the Stadie–Riggs guide bar but with a jeweler's saw with a tightly stretched 4-0 size surgical steel strand (Ethicon DS-32 monofilament type B noncapillary) instead of a Stadie–Riggs blade. This was found to be less injurious to the more fragile rat brain slices, possibly because the tissue was not compressed by the large surface area of the regular blade. Other experimental conditions were identical with those described for the cat.

The brain of male guinea pig (body weight approximately 750 g) was found to be more fragile and delicate than that of the rat. Slices were cut with the jeweler's saw and steel strand as for the rat, but removal of excess fluid after incubation was by blotting on a glass plate[27] instead of aspiration under vacuum. Two slices could be obtained from each cerebral hemisphere.

Acceleration–Deceleration Procedure. This was performed as previously described.[1,13,14] Drugs tested in this paradigm were administered intravenously 20 min after the acceleration–deceleration event and prior to the period of hypoxemia. Mortality was based on a postinjury 24-h survival period excluding that which occurred during the acceleration–deceleration procedure or the hypoxemia period.

Chemistry. Melting points were determined on a Thomas–Hoover capillary melting point apparatus and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained are within ±0.4% of theoretical values. ^1H NMR spectra were recorded in $CDCl_3$, unless otherwise noted, on either a Varian T-60 or EM-390 spectrometer. Chemical shifts are reported in parts per million relative to Me_4Si as the internal standard. Carbon, hydrogen, and nitrogen analyses were carried out on a Perkin-Elmer Model 240 elemental analyzer. Chlorine analyses were conducted on a Metrohm Model E336 potentiograph. The synthetic routes are disclosed in Schemes I–XIII. Detailed experimental procedures are given only for selected compounds, which serve to illustrate the general synthetic methods employed. The remaining data are summarized in Tables V–XIV.

Compound of Type 2. Methyl [(6,7-Dichloro-2-ethyl-2,3-dihydro-1-oxo-1*H*-inden-5-yl)oxy]acetate (2b). Compound **1b**[5] (52.5 g, 173.2 mmol) was dissolved in MeOH (200 mL), and concentrated H_2SO_4 (0.65 mL) was added. The mixture was stirred and refluxed for 2 h, cooled, and poured onto ice (1 kg). The product was separated by filtration, dried, and recrystallized. Further data on this compound and the other members of this series are summarized in Table V: ^1H NMR ($CDCl_3$) δ 0.97 (t, J = 7 Hz, 3 H, CH_3), 1.30–1.97 (m, 2 H, CH_2CH_3), 2.37–3.50 (m, 3 H, $^2CH^3CH_2$), 3.83 (s, 3 H, CO_2CH_3), 4.82 (s, 2 H, OCH_2), 6.73 (s, 1 H, Ar*H*).

Compounds of Type 3. Via Scheme I. Methyl [[6,7-Dichloro-2-ethyl-2,3-dihydro-1-oxo-2-(3-oxobutyl)-1*H*-inden-5-yl]oxy]acetate (3b). Compound **2b** (15.8 g, 50 mmol) was suspended in THF (25 mL), and 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) (1.24 g, 10 mmol) was added. Methyl vinyl ketone (MVK) (7 g, 100 mmol) was added dropwise with vigorous stirring. The temperature rose to 55 °C, and dissolution of the starting material

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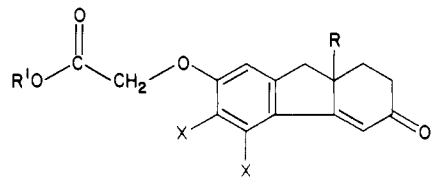
Table VII. Compounds of Type 4

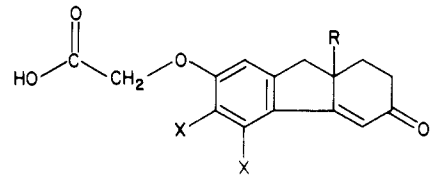


4

compd no.	R^1	X	R	synth scheme	% yield	recrystn solvent	mp, °C	emp formula	anal.
4a	CH_3	Cl	CH_3	4	47	*a*	225–227	$C_{17}H_{16}Cl_2O_4$	C, H
4b	CH_3	Cl	C_2H_5	1	66	THF	185–186	$C_{18}H_{18}Cl_2O_4$	C, H
4b-1	C_2H_5	Cl	C_2H_5	4	69	*a*	165–166	$C_{19}H_{20}Cl_2O_4$	C, H
4c	CH_3	Cl	$(CH_2)_2CH_3$	1	25	*a*	158–159	$C_{19}H_{20}Cl_2O_4$	C, H
4e	CH_3	Cl	$(CH_2)_3CH_3$	1	56	*a*	153–154	$C_{20}H_{22}Cl_2O_4$	C, H
4g	CH_3	Cl	$CH{=}CH_2$	*b*	77	*a*	180–182	$C_{18}H_{16}Cl_2O_4$	C, H
4h	C_2H_5	H	$(CH_2)_2CH_3$	4	48	*c*		$C_{20}H_{24}O_4$	
4i	C_2H_5	CH_3	$(CH_2)_2CH_3$	4	53	*c*		$C_{20}H_{28}O_4$	

[a] Purified by chromatography using silica gel and CH_2Cl_2–THF (50:1, v/v). [b] Prepared by esterification of 5 g. [c] Used in the next step without further purification.

Table VIII. Compounds of Type 5

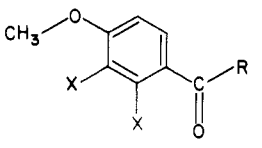


5

compd no.	X	R	synth scheme	% yield	recrystn solvent	mp, °C	formula	anal.
5a	Cl	CH_3	4	43	*a*	241–243	$C_{16}H_{14}Cl_2O_4$	C, H, Cl
5b	Cl	C_2H_5	4	57	AcOH–H_2O	237–239	$C_{17}H_{16}Cl_2O_4$	C, H
5b	Cl	C_2H_5	1B	84				
(+)-5b	Cl	C_2H_5	1B	48[c]	THF–Et_2O	235–237	$C_{17}H_{16}Cl_2O_4$	C, H
(−)-5b	Cl	C_2H_5	1B	63[d]	THF–Et_2O–petroleum ether	237–238	$C_{17}H_{16}Cl_2O_4$	C, H
5c	Cl	$(CH_2)_2CH_3$	1B	87	*a*	>240	$C_{18}H_{18}Cl_2O_4$	C, H
(+)-5c	Cl	$(CH_2)_2CH_3$	1B	71[e]	BuCl	178	$C_{18}H_{18}Cl_2O_4$	C, H
(−)-5c	Cl	$(CH_2)_2CH_3$	1B	78[f]	CH_2Cl_2–hexane	174–176	$C_{18}H_{18}Cl_2O_4$	C, H
5d	Cl	$CH(CH_3)_2$	2	24	AcOH–H_2O	252–254	$C_{18}H_{18}Cl_2O_4$	C, H
5e	Cl	$(CH_2)_3CH_3$	4	61	EtOH	213–214	$C_{19}H_{20}Cl_2O_4$	C, H, Cl
5f	Cl	c-C_5H_9	2	35	AcOH	219	$C_{20}H_{20}Cl_2O_4$	C, H
5g	Cl	$CH{=}CH_2$	1B	56	*g*	230–232	$C_{17}H_{14}Cl_2O_4$	C, H
5h	H	$(CH_2)_2CH_3$	4	56	EtOAc		$C_{18}H_{20}O_4 \cdot {}^1/_4$EtOAc	C, H
5i	CH_3	$(CH_2)_2CH_3$	4	46	MeOH–EtOAc		$C_{20}H_{24}O_4 \cdot {}^2/_3C_4H_9N$	C, H, N

[a] Purified by chromatography on silica gel using CH_2Cl_2–THF–HOAc (50:1:1, v/v/v). [b] Prepared by resolution of the racemate. [c] α^{25}_D = +151.5° (*c* 1, THF). [d] α^{25}_D = −149.3° (*c* 1, THF). [e] α^{25}_D = +155.4° (*c* 1, THF). [f] α^{25}_D = +154.6° (*c* 1, THF). [g] Purified by chromatography on silica gel using CH_2Cl_2–MeCO–HOAc (90:5:5, v/v/v).

Table IX. Compounds of Type 9



9

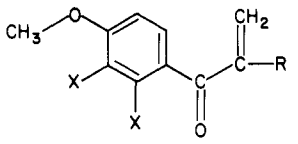
compd no.	X	R	% yield	recrystn solvent	(bp, °C/mm) or mp, °C	formula	anal.
9a′	Cl	$CH(CH_3)_2$	79		(137–142/0.5)	$C_{11}H_{12}Cl_2O_2$	C, H
9b	Cl	$(CH_2)_2CH_3$	90			$C_{11}H_{12}Cl_2O_2$	*a*
9c	Cl	$(CH_2)_3CH_3$	84	petroleum ether	61–63	$C_{12}H_{14}Cl_2O_2$	C, H
9h	H	$(CH_2)_3CH_3$	100		oil	$C_{12}H_{16}O_2$	C, H
9i	CH_3	$(CH_2)_3CH_3$	96		68–69	$C_{14}H_{20}O_2$	C, H

[a] Used without further purification.

occurred. After standing at ambient temperature for 16 h, the reaction mixture was poured into ice water, extracted with Et_2O, and dried over Na_2SO_4. Concentration and cooling of the extract gave 13.3 g of **3b**, which was purified by recrystallization. Further data on this compound and other members of the series are given in Table VI: ^1H NMR ($CDCl_3$) δ 0.78 (t, *J* = 7 Hz, 3 H, CH_3CH_2), 1.37–2.60 (m, 6 H, $COCH_2CH_2$, CH_3CH_2), 2.08 (s, 3 H, CH_3CO), 2.87 (br s, 2 H, $^3CH_2$), 3.82 (s, 3 H, CO_2CH_3), 4.82 (s, 2 H, OCH_2), 6.72 (s, 1 H, Ar*H*).

Via Scheme III. Methyl [(6,7-Dichloro-2-ethylidene-2,3-dihydro-1-oxo-1*H*-inden-5-yl)oxy]acetate (7). Compound 6[5] (10.8 g 35.9 mmol) was dissolved in MeOH (250 mL) containing *p*-toluenesulfonic acid (0.2 g), and the mixture was refluxed for 4 h. The product that separated upon cooling was removed by

Table X. Compounds of Type 10

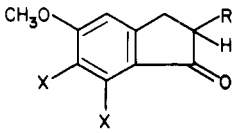


10

compd no.	X	R	% yield	recrystn solvent	mp, °C	formula	anal.
10a	Cl	CH_3	98		liquid	$C_{11}H_{10}Cl_2O_4$	*a*
10b	Cl	C_2H_5	94		liquid	$C_{12}H_{12}Cl_2O_4$	*a*
10c	Cl	$(CH_2)_2CH_3$	92	petroleum ether	58–60	$C_{13}H_{14}Cl_2O_4$	C, H
10h	H	$(CH_2)_2CH_3$	93		liquid	$C_{13}H_{16}O_4$	*a*
10i	CH_3	$(CH_2)_2CH_3$	95		liquid	$C_{15}H_{20}O_4$	*a*

[a] Used directly in the next step without purification but the structure confirmed by NMR.

Table XI. Compounds of Type 11

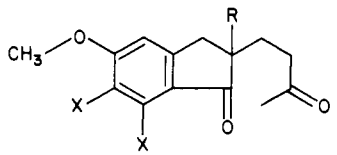


11

compd no.	X	R	% yield	recrystn solvent	mp, °C	emp formula	anal.
11a	Cl	CH_3	71	EtOH-H_2O	129	$C_{11}H_{10}Cl_2O_2$	C, H
11b	Cl	C_2H_5	93	Et_2O	141–141.5	$C_{12}H_{12}Cl_2O_2$	C, H
11c	Cl	$(CH_2)_2CH_3$	84	Et_2O	115–117	$C_{13}H_{14}Cl_2O_2$	C, H
11h	H	$(CH_2)_2CH_3$	35		liquid	$C_{13}H_{16}O_2$	*a*
11i	CH_3	$(CH_2)_2CH_3$	80		liquid	$C_{15}H_{20}O_2$	*a*

[a] Used in the next step without further purification.

Table XII. Compounds of Type 12

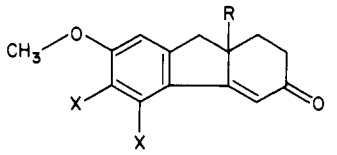


12

compd no.	X	R	% yield	recrystn solvent	mp, °C	formula	anal.
12a	Cl	CH_3	70	Et_2O	112–113	$C_{15}H_{16}Cl_2O_3$	C, H
12b	Cl	C_2H_5	75	$(CH_2)_6$	88–90	$C_{16}H_{18}Cl_2O_3$	C, H
12c	Cl	$(CH_2)_2CH_3$	100	*a*		$C_{17}H_{20}Cl_2O_3$	
12h	H	$(CH_2)_2CH_3$	100	*a*		$C_{17}H_{22}O_3$	
12i	CH_3	$(CH_2)_2CH_3$	53	*a*		$C_{19}H_{26}O_3$	

[a] Used in the next step without purification.

Table XIII. Compounds of Type 13



13

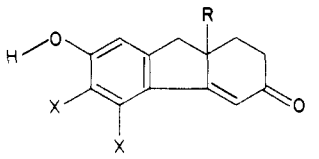
compd no.	X	R	% yield	recrystn solvent	mp, °C	formula	anal.
13a	Cl	CH_3	39	*a*		$C_{15}H_{14}Cl_2O_2$	*b*
13b	Cl	C_2H_5	42	*a*	160–161.5	$C_{16}H_{16}Cl_2O_2$	C, H
13c	Cl	$(CH_2)_2CH_3$	51	EtOH	156–158	$C_{17}H_{18}Cl_2O_2$	*b*
13h	H	$(CH_2)_2CH_3$	61	*b*		$C_{17}H_{20}O_2$	
13i	CH_3	$(CH_2)_2CH_3$	40[c]	MeOH	128–129	$C_{19}H_{24}O_2$	C, H

[a] Purified by column chromatography on silica gel using CH_2Cl_2–THF (50:1). [b] Used in the next step without further purification. [c] The cyclization was carried out with HCl in AcOH as described for the conversion of **4b** to **5b**.

filtration and was submitted to column chromatography with silica gel (350 g); CH_2Cl_2 was employed for development and finally CH_2Cl_2–THF (4:1, v/v) for elution. The yield of **7** was 9.4 g (83%); mp 171–173 °C. Anal. ($C_{14}H_{12}Cl_2O_4$) C, H.

Methyl [[6,7-Dichloro-2-ethenyl-2,3-dihydro-1-oxo-2-(3-oxobutyl)-1*H*-inden-5-yl]oxy]acetate (3g). Compound **7** (3.2 g, 10 mmol) in THF (25 mL) was stirred and treated with MVK (1.4 g, 20 mmol) and DBN (10 drops). After stirring for 3 h at ambient temperature, the reaction mixture was poured into H_2O (100 mL), acidified with HCl, and extracted with Et_2O. The Et_2O extract was dried over Na_2SO_4, filtered, and evaporated in vacuo. The residue was purified by column chromatography with silica gel (150 g), eluting with toluene–Me_2CO–AcOH (85:10:5, v/v/v) to give 1.9 g (49%) of **3g**.

Table XIV. Compounds of Type 14



14

compd no.	X	R	% yield	recrystn solvent	mp, °C	formula	anal.
14a	Cl	CH_3	73	*b*		$C_{14}H_{12}Cl_2O_2$	
14b	Cl	C_2H_5	86	EtOAc	265–267	$C_{15}H_{14}Cl_2O_2$	C, H
14c	Cl	$(CH_2)_2CH_3$	90	EtOAc	>250	$C_{16}H_{16}Cl_2O_2$	C, H
14h	H	$(CH_2)_2CH_3$	78	*a*	214–215	$C_{16}H_{18}O_2$	C, H
14i	CH_3	$(CH_2)_2CH_3$	83	*b*		$C_{18}H_{22}O_2$	

[a] Purified by chromatography using a silica gel column, eluting with CH_2Cl_2–EtOAc. [b] Used in the next step without further purification.

Compounds of Type 4. Via Scheme I, Route B. Methyl [(5,6-Dichloro-9a-ethyl-2,3,9,9a-tetrahydro-3-oxo-1*H*-fluoren-7-yl)oxy]acetate (4b). Compound **3b** (5.81 g, 15 mmol) was dissolved in dry toluene (100 mL); pyrrolidine (1.07 g, 15 mmol) and AcOH (0.9 g, 15 mmol) were added. The mixture was heated and stirred at 85 °C for 1.5 h, then cooled, washed with H_2O, and dried over $MgSO_4$. The solvent was removed by evaporation in vacuo and chromatographed over silica gel with CH_2Cl_2–THF (2:5 v/v) to obtain **4b**. Further data on this compound and other members of the series are given in Table VII: ^{1}H NMR ($CDCl_3$) δ 0.87 (t, J = 7 Hz, 3 H, CH_3CH_2), 1.30–2.60 (m, 6 H, $COCH_2CH_2$, CH_3CH_2), 2.83 (d, J = 7 Hz, 2 H, $^3CH_2$), 3.80 (s, 3 H, $COCH_3$), 4.73 (s, 2 H, OCH_2), 6.67 (s, 1 H, C=CH), 6.72 (s, 1 H, Ar*H*).

Compounds of Type 5. Via Scheme I, Route A. [(5,6-Dichloro-9a-ethyl-2,3,9,9a-tetrahydro-3-oxo-1*H*-fluoren-7-yl)oxy]acetic Acid (5b). Compound **3b** (31.7 g, 100 mmol) was suspended in EtOH (100 mL), and NaOH (11.9 g, 298 mmol) in H_2O (130 mL) was added with cooling and stirring. The mixture was stirred at 25 °C for 48 h and then poured into crushed ice (350 g) and acidified with HCl. The solid was removed by filtration, dispersed into very dilute HCl, filtered, washed with H_2O, dried, and recrystallized. Further data on this compound and other members of the series are given in Table VIII.

Via Scheme I, Route B. Compound 5b. Compound 4b (5 g, 12.1 mmol) was treated with AcOH (40 mL) and 5% HCl (15 mL), and the mixture was heated at 100 °C and stirred for 1 h. The reaction mixture was cooled and poured into crushed ice (100 g) to give **5b**, which was removed by filtration, washed with H_2O, and recrystallized (see Table VIII): ^{1}H NMR (Me_2SO-d_6) δ 0.78 (t, J = 7 Hz, 3 H, CH_3), 1.17–2.00 (m, 2 H, CH_2CH_3), 2.00–2.63 (m, 4 H, $^1CH_2{}^2CH_2$), 2.83 (d, J = 7 Hz, 2 H, $^9CH_2$), 4.83 (s, 2 H, OCH_2), 6.45 (s, 1 H, Ar*H*), 7.02 (s, 1 H, C=CH).

Resolution of 5b. Compound **5b** (33 g, 93 mmol) in MeCN (2.5 L) was heated to boiling, and cinchonine (27.4 g, 93 mmol) was added. The solution was stirred at 5 °C for 24 h, the solid that separated was filtered off and washed with MeCN, and the filtrate (I) was saved. The salt was recrystallized from MeCN and the product removed by filtration, dried, treated with 1 N HCl (450 mL), and extracted with 20% THF in Et_2O. The extract was dried over $MgSO_4$; the solvent was evaporated in vacuo, and the residue was recrystallized to give (+)-**5b**.

Filtrate I was evaporated in vacuo, treated with 2 N HCl (400 mL), and extracted with 20% THF in Et_2O, and the extract was dried over $MgSO_4$. The solvent was evaporated in vacuo, the residue dissolved in MeCN (2.5 L), the resultant mixture heated to boiling, and cinchonidine (27.4 g, 93 mmol) added. The solution was cooled to 5 °C and stirred for 24 h. The solid that separated was recrystallized and worked up as described for the (+) enantiomer to give (−)-**5b**. Further data on (+)-**5b**, (−)-**5b**, as well as (+)-**5c** and (−)-**5c**, which were resolved in a similar fashion, are given in Table VIII.

Via Scheme II. [[5,6-Dichloro-2,3,9,9a-tetrahydro-9a-(1-methylethyl)-3-oxo-1*H*-fluoren-7-yl]oxy]acetic Acid (5d). Compound **3d** (3.85 g, 10 mmol) in a mixture of AcOH (40 mL) and 6 N HCl (40 mL) was refluxed for 24 h, poured into ice H_2O, extracted with EtOAc, washed with H_2O and then brine, and finally dried over $MgSO_4$. Evaporation of the solvent in vacuo and recrystallization of the residue from AcOH-H_2O gave 900 mg (24%) of **5d** (see Table VIII).

Via Scheme IV. Compound **5b** can also be prepared by alkaline hydrolysis of **4b** under conditions similar to that described for the production of **5b** and **3b** except that the reaction time is reduced to 2 h.

Via Scheme VI. 4-[(5,6-Dichloro-9a-ethyl-2,3,9,9a-tetrahydro-3-oxo-1*H*-fluoren-7-yl)oxy]butyric Acid (5j). Compound **14b** (6 g, 20 mmol), ethyl 4-bromobutyrate (5.5 g, 28 mmol), and K_2CO_3 (8.3 g, 60 mmol) in DMF (35 mL) were stirred at 55 °C for 18 h and then poured into ice H_2O. The ester was separated by filtration, washed with H_2O, dried, and recrystallized from benzene–cyclohexane; mp 141–142 °C. This compound, the ethyl ester of **5j** (5 g, 12.1 mmol), was dissolved in a mixture of AcOH (40 mL) and 5% aqueous HCl (15 mL) and stirred and heated on a steam bath for 1 h. H_2O (5 mL) was added and the mixture chilled to give 4.3 g (93%) of crude **5j** that, upon recrystallization from THF, gave **5j**, mp 200–202 °C. Anal. ($C_{19}H_{20}Cl_2O_4$) C, H.

A similar reaction was carried out using (+)-**5c** to produce (+)-**5k** as a $^3/_4$ CH_3CN solvate, mp 163–165 °C. Anal. ($C_{19}H_{23}Cl_2O_4 \cdot {}^3/_4CH_3CN$) C, H, N.

Compounds of Type 9. Via Scheme IV. 1-(2,3-Dichloro-4-methoxyphenyl)-1-pentanone (9c). Compound **8c** (35.4 g, 200 mmol) and valeryl chloride (25 g, 207 mmol) in CH_2Cl_2 (100 mL) were stirred and treated portionwise with $AlCl_3$ (28 g, 210 mmol) over a period of 0.5 h while cooling in an ice bath. After standing at ambient temperature for 15 h, the reaction mixture was poured onto crushed ice (250 g). After vigorous stirring, the CH_2Cl_2 layer was separated and the aqueous layer extracted with more CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with 5% NaOH and then with H_2O and finally dried over $MgSO_4$. Removal of the solvent in vacuo gave **9c** which was triturated with petroleum ether and then recrystallized. Further data on this compound and other members of the series are given in Table IX: ^{1}H NMR ($CDCl_3$) δ 0.92 (m, 3 H, CH_3), 1.05–1.93 (m, 4 H, $^1CH_2{}^2CH_2$), 2.87 (t, J = 7 Hz, 2 H, $COCH_2$), 3.88 (s, 3 H, OCH_3), 6.77 (d, J = 7 Hz, 1 H, Ar*H*), 7.28 (d, J = 9 Hz, 1 H, Ar*H*).

Compounds of Type 10. Via Scheme IV. Route A. 1-(2,3-Dichloro-4-methoxyphenyl)-2-bromo-2-methyl-1-propanone (9a″). Compound **9a′** (45 g, 183 mmol) was dissolved in AcOH (150 mL) and Br_2 (30 g, 187 mmol) added dropwise with stirring. After standing for 16 h, the mixture was poured into ice H_2O containing a little $NaHSO_3$. The solid that separated was removed by filtration, washed with H_2O, and dried. The solid was dissolved in Et_2O, the Et_2O solution washed with H_2O and dried over $MgSO_4$, and the solvent removed by distillation in vacuo. The resulting **9a″**, 57 g (96%), was used directly in the next step.

1-(2,3-Dichloro-4-methoxyphenyl)-2-methylene-1-propanone (10a). Compound **9a″** (57 g, 175 mmol) was dissolved in DMF (300 mL), LiBr (31 g, 357 mmol) was added, and the mixture heated at 95 °C with stirring for 3 h in an N_2 atmosphere. Most of the solvent was removed by distillation in vacuo, and the residue was cooled and added to ice H_2O. The mixture was extracted with Et_2O and the extract washed with H_2O and dried over $MgSO_4$. Evaporation of the solvent at reduced pressure gave **10a** as a viscous oil (42 g, 98%) which was used directly in the next step: ^{1}H NMR ($CDCl_3$) δ 2.0 (br s, 3 H, C=CCH_3), 3.86 (s,

3 H, OCH_3), 5.50 (br s, 1 H, C=CH_2), 5.87 (br s, 1 H, C=CH_2), 6.73 (d, J = 9 Hz, 1 H, Ar*H*), 7.07 (d, J = 9 Hz, 1 H, Ar*H*).

Route B. 1-(2,3-Dichloro-4-methoxyphenyl)-2-methylene-1-pentanone (10c). Compound **9c** (39 g, 150 mmol) and *N,N,N',N'*-tetramethyldiaminomethane (22.5 g, 220 mmol) were warmed to 50 °C and stirred without further heating while Ac_2O (30 mL) was added at such a rate that the temperature rose to 98–100 °C. After the addition was complete, the reaction temperature was maintained at 95 °C by heating for another 2 h. After cooling to ambient temperature and allowing to stand for 15 h, the reaction mixture was poured onto crushed ice (250 g) containing 10% NaOH (200 mL). The product was extracted with Et_2O and dried over Na_2SO_4, and the solvent was removed in vacuo. The product can be used per se or recrystallized. Further data on this compound and other members of the series are given in Table X: ^{1}H NMR ($CDCl_3$) δ 0.95 (t, J = 7 Hz, 3 H, CH_3), 1.15–1.90 (m, 2 H, CH_2CH_3), 2.42 (t, J = 7 Hz, 2 H CH_2C=C), 3.96 (s, 3 H, OCH_3), 5.63 (s, 1 H, C=CH), 5.91 (s, 1 H, C=CH), 6.85 (d, J = 9 Hz, 1 H, ArH), 7.17 (d, J = 9 Hz, ArH).

Compounds of Type 11. 6,7-Dichloro-2,3-dihydro-5-methoxy-2-propyl-1*H*-inden-1-one (11c). Compound **10c** (27.3 g, 100 mmol) was added portionwise with vigorous stirring and mild cooling to concentrated H_2SO_4 (100 mL). The temperature rose to 55 °C during the addition. After standing at ambient temperature for 15 h, the mixture was added to crushed ice (500 g) with stirring. The product was removed by filtration, resuspended in H_2O, filtered again, and washed with H_2O until free of acid. After thorough drying, the product can be used in the next step or recrystallized. Further data on this compound and other members of the series are given in Table XI: ^{1}H NMR ($CDCl_3$) δ 0.95 (m, 3 H, CH_3), 1.15–3.53 (m, 7 H, CH_2CH_2, $^2CH^3CH_2$), 3.95 (s, 3 H, OCH_3), 6.77 (s, 1 H, Ar*H*).

Compounds of Type 12. 6,7-Dichloro-2-ethyl-2,3-dihydro-5-methoxy-2-(3-oxobutyl)-1*H*-inden-1-one (12b). Compound **11b** (26 g, 100 mmol) was dissolved in THF (500 mL) and 40% benzyltrimethylammonium hydroxide in MeOH (8 mL) added. MVK (10 g, 140 mmol) was added with stirring over 10 min, and then the solution was allowed to stand for 2.5 h. The solvent was evaporated in vacuo and the residue treated with H_2O (100 mL) and acidified with HCl. The solid that separated was removed by filtration, washed with H_2O, dried, and recrystallized. Further data on **12b** and related compounds are given in Table XII: ^{1}H NMR ($CDCl_3$) δ 0.78 (t, J = 7 Hz, 3 H, CH_3), 2.08 (s, 3 H, $COCH_3$), 1.07–2.65 (m, 6 H, CH_2CH_2CO, CH_3CH_2), 2.90 (br s, 2 H, $^3CH_2$), 3.98 (s, 3 H, OCH_3), 6.88 (s, 1 H, Ar*H*).

Compounds of Type 13. Via Scheme IV. 5,6-Dichloro-1,2,9,9a-tetrahydro-7-methoxy-9a-propyl-3*H*-fluoren-3-one (13c). Compound **12c** (5 g, 14.6 mmol) was dissolved in dioxane (60 mL) and treated with 2% aqueous NaOH (60 mL), and the mixture was stirred and refluxed for 16 h. The solution was cooled, poured into H_2O (200 mL), and extracted with CH_2Cl_2 and then with Et_2O. The combined organic extracts were washed with aqueous HCl and then with H_2O and, finally, dried over $MgSO_4$. The solvents were removed by evaporation, and the residual **13c** was purified by recrystallization. Further data on this compound and other members of the series are given in Table XIII.

Compounds of Type 14. Via Scheme IV. 5,6-Dichloro-9a-ethyl-1,2,9,9a-tetrahydro-7-hydroxy-3*H*-fluoren-3-one (14b). Compound **13b** (32.5 g, 100 mmol) was added to a stirring melt of pyridine hydrochloride (115.6 g, 1 M) at 190 °C. The mixture was stirred and heated at this temperature until the TLC indicated **13b** had all reacted (ca. 30 min). The mixture was poured with stirring into crushed ice (1 kg), and the resulting solid was removed by filtration. The solid was slurried in a Waring Blendor with more H_2O, filtered, washed with H_2O until the filtrate tested negative for chloride, dried, and purified. Further data on this compound and other members of the series are given in Table XIV: ^{1}H NMR (Me_2SO-d_6) δ 0.80 (t, J = 7 Hz, 3 H, CH_3), 1.13–2.63 (m, 6 H, CH_2CH_2CO, CH_2CH_3), 2.81 (d, J = 8 Hz, 2 H, $^3CH_2$), 6.53 (s, 1 H, Ar*H*), 6.93 (s, 1 H, C=CH).

Via Scheme V. (*R*)-(+)-5,6-Dichloro-1,2,9,9a-tetrahydro-7-hydroxy-9a-propyl-3*H*-fluoren-3-one ((+)-14c). Compound (+)-**5c** (7.38 g, 20 mmol) was added to a stirring melt of pyridine hydrochloride (73.8 g, 630 mmol) at 190 °C and heated at 190 °C for 15 min. The mixture was quickly poured, with stirring, into crushed ice (400 g), and the resulting solid was separated by filtration, washed with H_2O, resuspended in H_2O, refiltered, thoroughly washed with H_2O, and dried. The yield of (+)-**14c** was 6.4 g (100%); this product was purified by extraction with EtOAc via a Soxhlet, the solvent evaporated, and the residue washed with Et_2O to give material: mp 253–255 °C; $[\alpha]^{25}_D$ +177.4 (*c* 1, THF). Anal. ($C_{16}H_{16}Cl_2O_2$) C, H.

Compounds of Type 16. Via Scheme VII. Method A. Ethyl [(5,6-Dichloro-9a-ethyl-2,3,9,9a-tetrahydro-3-oxo-1*H*-fluoren-7-yl)oxy]acetate (16b-1). Compound **5b** (15 g, 42.2 mmol) was refluxed for 1 h with EtOH (60 mL) and H_2SO_4 (1 mL). The solution was poured into ice H_2O, extracted with Et_2O, dried over $MgSO_4$, and evaporated in vacuo and the residue recrystallized to give **16b-1**. Further data on this compound and other members of the series are given in Table XV.

Method B. 1-Carboxy-1-methylethyl [(5,6-Dichloro-9a-ethyl-2,3,9,9a-tetrahydro-3-oxo-1*H*-fluoren-7-yl)oxy]acetate (16b-9). Compound **5b** (7.1 g, 20 mmol) was dissolved in THF (20 mL). 1,1'-Carbonyldiimidazole (3.2 g, 20 mmol) was added, and the mixture was stirred at 0 °C for 1 h. $HOC(CH_3)_2COOH$ (2.10 g, 20 mmol) was added with stirring, and then the mixture was allowed to stand for 18 h at ambient temperature. The solvent was evaporated in vacuo and the residue purified by chromatography followed by recrystallization. Further data on this and related compounds are given in Table XV: ^{1}H NMR ($CDCl_3$) δ 0.82 (m, 3 H, CH_3), 1.23–2.67 (m, 6 H, $^1CH_2{}^2CH_2$, CH_2CH_3), 1.62 (s, 6 H, $CO_2C(CH_3)_2CO_2$), 2.80 (d, J = 6 Hz, 2 H, $^3CH_2$), 4.73 (s, 2 H, OCH_2), 6.68 (s, 1 H, Ar*H*), 8.85 (br s, 1 H, COOH).

Compounds of Type 17. Via Scheme VII. *N*-[2-(Dimethylamino)ethyl][(5,6-dichloro-2,3,9,9a-tetrahydro-3-oxo-9a-propyl-1*H*-fluoren-7-yl)oxy]acetamide (17c-1). Compound **5a** (3.7 g, 10 mmol) was suspended in dry THF (50 mL). 1,1'-Carbonyldiimidazole (1.7 g, 11 mmol) was added, and the mixture was stirred for 10 min. The solution became clear, and **5a** had disappeared as evidenced by TLC. 2-(Dimethylamino)-ethylamine (1.6 g, 20 mmol) was added and stirring continued for 16 h. The solid that separated was removed by filtration, washed with a THF–Et_2O mixture, and dried. The yield of **17c-1** was 2.11 g (48%) which, after recrystallization from THF–Et_2O, melted at 159–161 °C. Anal. ($C_{22}H_{28}Cl_2N_2O_3$) C, H, N.

Compound **17c-2** was prepared by a similar procedure; mp 217–219 °C. Anal. ($C_{20}H_{24}Cl_2N_2O_3$) C, H, N.

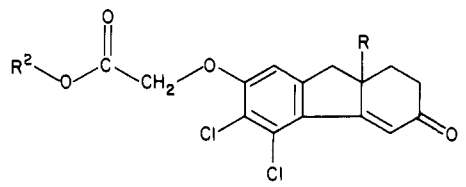
Compound 19. Via Scheme VIII. [(5,6-Dichloro-9a-ethyl-2,3,9,9a-tetrahydro-3-oxo-1*H*-fluoren-7-yl)oxy]acetonitrile (18). Compound **14b** (5.94 g, 20 mmol), chloroacetonitrile (1.66 g, 22 mmol), K_2CO_3 (4.14 g, 30 mmol), KI (500 mg), and dry acetone (1 L) were stirred and refluxed for 16 h. The mixture was filtered and the solvent removed by distillation in vacuo. The residue was triturated with a little Et_2O to give 6.2 g (92%) of **18** which was recrystallized from MeCN to give material, mp 171–172 °C. Anal. ($C_{17}H_{15}Cl_2NO_2$) C, H, N.

5-[[(5,6-Dichloro-9a-ethyl-2,3,9,9a-tetrahydro-3-oxo-1*H*-fluoren-7-yl)oxy]methyl]tetrazole (19). Compound **18** (4.14 g, 12.3 mmol), NaN_3 (955 mg, 14.7 mmol), and NH_4Cl (786 mg, 14.7 mmol) in DMF (30 mL) were stirred and heated at 80 °C for 1 h, cooled, and poured into ice H_2O (200 mL). The solid that separated was removed by filtration, washed with H_2O, and dried. The yield of **19** was 2 g (44%) which, after recrystallization from 2-methoxyethanol, melted at 246–247 °C. Anal. ($C_{17}H_{16}Cl_2N_4O_2$) C, H, N.

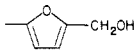
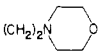
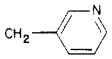
Compound 21. Via Scheme IX. [[6,7-Dichloro-2,3-dihydro-1-oxo-2-(3-oxopentyl)-2-propyl-1*H*-inden-5-yl]oxy]-acetic Acid (20). To a solution of **2c** (6.6 g, 20 mmol) in THF (10 mL) was added DBN (100 μL). A solution of ethyl vinyl ketone (2 mL) in THF (5 mL) was added over 15 min. The reaction mixture was stirred for 4 h at 55 °C, and then the solvent was evaporated in vacuo. The residue was dissolved in a Et_2O–CH_2Cl_2 mixture, washed with H_2O, and dried over $MgSO_4$, and the solvent was evaporated in vacuo. The residue was chromatographed on silica gel (140 g), eluting with CH_2Cl_2–AcOH–THF (50:1:1, v/v/v), affording 500 mg of **20** as an oil which was used directly in the next step.

[(5,6-Dichloro-2,3,9,9a-tetrahydro-4-methyl-3-oxo-9a-propyl-1*H*-fluoren-7-yl)oxy]acetic Acid (21). A solution of **20** (500 mg, 1.25 mmol) in AcOH (10 mL) and 6 N HCl (10 mL) was refluxed for 18 h, cooled, and treated with ice H_2O (50 mL). The solid that separated was filtered, washed with H_2O, dried,

Table XV. Compounds of Type 16



16

compd no.	R^2	R	synth method	% yield	recrystn solvent	mp, °C	formula	anal.
16a-1	C_2H_5	C_2H_5	A	98	THF-Et_2O-petroleum ether	164–166	$C_{19}H_{20}Cl_2O_4$	C, H
(+)-16b-1	C_2H_5	C_2H_5 (+)	A	46	Me_2CO-petroleum ether	94–96	$C_{19}H_{20}Cl_2O_4$	C, H, Cl
(−)-16b-1	C_2H_5	C_2H_5 (−)	A	44	THF-Et_2O-petroleum ether	94–97	$C_{19}H_{20}Cl_2O_4$	C, H
16b-2	$(CH_2)_3OH$	C_2H_5	*a*	55	*b*	114–117	$C_{20}H_{22}Cl_2O_5$	C, H
16b-3		C_2H_5	*c*	41	*i*-PrOH	146–147	$C_{23}H_{22}Cl_2O_6$	C, H
16b-4		C_2H_5	A	40	THF-Et_2O-petroleum ether	130–135	$C_{23}H_{26}Cl_2O_6$	C, H
16b-5	CH_2COCH_3	C_2H_5	*d*	22	*b*	153–155	$C_{20}H_{20}Cl_2O_5$	C, H
16b-6		C_2H_5	B	62	*e*	133–133.5	$C_{23}H_{27}Cl_2NO_5$	C, H, N
16b-7		C_2H_5	*f*	70	BuCl	120–121	$C_{23}H_{21}Cl_2NO_4$	C, H, N
16b-8	CH_2CO_2H	C_2H_5	*g*	94	*i*-PrOH	184–186	$C_{19}H_{18}Cl_2O_6$	C, H, Cl
(+)-16b-8	CH_2CO_2H	C_2H_5 (+)	*g*	90	CH_2Cl_2	100–103	$C_{19}H_{18}Cl_2O_6$	C, H, Cl
16b-9	$C(CH_3)_2CO_2H$	C_2H_5	B	58	*h*-THF-DMF	215–216	$C_{21}H_{22}Cl_2O_6$	C, H
(+)-16b-9	$C(CH_3)_2CO_2H$	C_2H_5 (+)	B	59	*h*	117–120	$C_{21}H_{22}Cl_2O_6$	C, H
(+)-16c-1	$C(CH_3)_2CO_2H$	$(CH_2)_2CH_3$ (+)	B	46	*i*	166–168	$C_{22}H_{24}Cl_2O_6$	C, H
(+)-16c-2	$C(C_2H_5)_2CO_2H$	$(CH_2)_2CH_3$ (+)	B	50	*h*	157–158	$C_{24}H_{28}Cl_2O_6$	C, H

[a] Prepared from **5b**, $Br(CH_2)_3OH$, K_2CO_3, and DMF. [b] Purified by chromatography on silica gel using AcOH-Me_2CO-MeC_6H_5 (5:5:90, v/v/v). [c] Prepared from **5b** + Et_3N + $ClCO_2Et$ + THF followed by 2,5-bis(hydroxymethyl)furan. [d] Prepared from **5b** + chloroacetone + K_2CO_3 in DMF. [e] Purified by chromatography on silica gel using CH_2Cl_2–*i*-PrOH (20:1, v/v). [f] Prepared from **4b**, 3-(hydroxymethyl)pyridine + NaOMe, and molecular sieves (3A) in MeC_6H_5. [g] Prepared from **5b** + benzyl 2-bromoacetate + K_2CO_3 in DMF to give the benzyl ester of **16b-8** which was then hydrogenolyzed using H_2 + Pd/C (10%) in THF. [h] Purified by chromatography on silica gel using CH_2Cl_2–THF–AcOH (100:2:1, v/v/v). [i] Purified by Waters reversed-phase HPLC using a C_{18} column and 50:50 mixture of MeCN + 0.5% AcOH-H_2O.

and triturated with MeCN (3 mL) to give **21**: 180 mg (38%); mp 206–208 °C. Anal. ($C_{19}H_{20}Cl_2O_4$) C, H.

Compounds of Type 23. Via Scheme X. [(2-Bromo-5,6-dichloro-9a-ethyl-2,3,9,9a-tetrahydro-3-oxo-1*H*-fluoren-7-yl)oxy]acetic Acid (22). Compound **5b** (1.24 g, 3.5 mmol) was dissolved in AcOH (50 mL), and Br_2 (560 mg, 3.5 mmol) in AcOH (25 mL) was added dropwise with stirring. After stirring an additional 1 h, the solution was poured into H_2O (250 mL) and the resulting solid was removed by filtration, washed with H_2O, and dried. The yield of **21** was 1.4 g (92%) which, after recrystallization from THF–Et_2O–petroleum ether, melted at 207–209 °C. Anal. ($C_{17}H_{15}BrCl_2O_4$) C, H, Br, Cl.

[(5,6-Dichloro-9a-ethyl-9,9a-dihydro-3-oxo-3*H*-fluoren-7-yl)oxy]acetic Acid (23). Compound **21** (1.3 g, 3.2 mmol) was dissolved in Me_2SO (10 mL), and DBN (800 mg, 6.4 mmol) in Me_2SO (5 mL) was added. The mixture was stirred and warmed on a steam bath for 30 min, cooled, and poured into ice H_2O (100 mL). The mixture was acidified with HCl and extracted with 20% THF in Et_2O. The organic extract was dried over Na_2SO_4, and the solvent was removed by distillation in vacuo. The residue was triturated with EtOAc, filtered, and dried. The yield of **23** was 800 mg (71%) which, after recrystallization from THF–Et_2O–petroleum ether, melted at 198–200 °C. Anal. ($C_{17}H_{14}Cl_2O_4$) C, H.

Compound 28. Via Scheme XI. 6,7-Dichloro-2,3-dihydro-5-methoxy-2-(2-propenyl)-2-propyl-1*H*-inden-1-one (24). Compound **11c** (34.1 g, 125 mmol) in 1:1 toluene–DMF (180 mL) was added dropwise, with stirring under N_2, to a suspension of 56% NaH in mineral oil (6 g, 140 mmol) in 1:1 toluene–DMF (220 mL). After the mixture was stirred 1.75 h postaddition, allyl bromide (24.2 g, 200 mmol) was added dropwise. After an additional 3.5 h of stirring, the excess NaH was destroyed by addition of MeOH; then, the mixture was poured into crushed ice (1200 mL) and extracted with Et_2O. The organic extract was washed with H_2O, dried over $MgSO_4$, evaporated in vacuo, and chromatographed on silica (1 kg), eluting with hexane–$CHCl_3$ (2:3, v/v) to obtain **24** (32.5 g, 83%), mp 54–56 °C, which was used in the next step without further purification.

6,7-Dichloro-2,3-dihydro-5-methoxy-2-(2-oxopropyl)-2-propyl-1*H*-inden-1-one (25). $PdCl_2$ (1 g, 5.64 mmol)[8] and H_2O (0.75 mL) were added to a solution of **24** (8.83 g, 28.2 mmol) and benzoquinone (3.05 g, 28.2 mmol) in DMF (30 mL). The mixture was stirred and heated at 65 °C while H_2O (0.75 mL portions) was added after 15, 45, and 135 min. After 2.5 h, the mixture was poured into H_2O (250 mL) and extracted with Et_2O and then with CH_2Cl_2. The combined organic extracts were washed with H_2O, dried over $MgSO_4$, and evaporated in vacuo. The residue was dissolved in C_6H_6 (150 mL), and hexane (75 mL) was added. The solid that separated was removed by filtration and the filtrate diluted with C_6H_6, washed with 0.1 N NaOH and then with H_2O, and dried over $MgSO_4$, and the solvent was evaporated in vacuo. The residue was dissolved in *n*-BuCl (20 mL) and petroleum ether (100 mL) added to give crude **25** (6.8 g, 73%), mp 128–130 °C, which was used without further purification.

6,7-Dichloro-2,3-dihydro-5-hydroxy-2-(2-oxopropyl)-2-propyl-1*H*-inden-1-one (26). Compound **25** (6.8 g, 20.7 mmol) was added to molten pyridine hydrochloride (35.9 g, 310 mmol) under N_2 with stirring. After 2 h at 175–185 °C, the mixture was poured onto crushed ice (400 mL) and extracted with CH_2Cl_2 and then Et_2O. The combined organic extracts were extracted with a 2 N NaOH solution, and the aqueous portion was acidified with HCl. The product was extracted with CH_2Cl_2, then Et_2O and the combined extracts were washed with H_2O and dried over $MgSO_4$, and the solvent was removed to give **26** which was used directly in the synthesis of **27**.

4,5-Dichloro-8,8a-dihydro-6-hydroxy-8a-propylcyclopent[*a*]inden-2(1*H*)-one (27). Compound **26** (4.5 g, 14.3 mmol) was refluxed under N_2 for 24 h in a solution of KOH (20 g) in H_2O (175 mL) and EtOH (25 mL). The reaction mixture was cooled, treated with H_2O (300 mL), and filtered. The filtrate was made just acid with HCl and extracted first with EtOAc and then with *n*-BuOH. The combined organic extracts were washed with

brine, dried over $MgSO_4$, and concentrated. The **27** that crystallized was removed by filtration, washed with a little *i*-PrOH, and dried. The yield of **27** was 2.1 g (49%), mp 268–272 °C. This material was used directly in the synthesis of **28**.

[(4,5-Dichloro-8,8a-dihydro-2-oxo-8a-propyl-1*H*-cyclopent[*a*]inden-6-yl)oxy]acetic Acid (28). Compound **27** (2.07 g, 6.96 mmol) was stirred in DMF (25 mL) containing K_2CO_3 (2 g, 14.5 mmol) for 20 min at 60 °C. Methyl bromoacetate (2.13 g, 13.9 mmol) was added, and the mixture was stirred and heated at 75 °C for 3.75 h, then cooled, poured into ice H_2O (200 mL), and extracted first with CH_2Cl_2 and, finally, with Et_2O. The combined organic extracts were washed with H_2O, dried over $MgSO_4$, and evaporated in vacuo. The residue was stirred at ambient temperature for 16 h in a mixture of EtOH (25 mL), H_2O (10 mL), and 1 N NaOH (21 mL). The solution was diluted with H_2O (350 mL), acidified with HCl, and extracted with CH_2Cl_2 and then with Et_2O. The combined organic extracts were washed with H_2O, the solvents were evaporated in vacuo and the residue was chromatographed on silica gel (140 g), eluting with CH_2Cl_2–AcOH (40:1, v/v) to obtain **28**: 2.2 g (85%); mp 213–216 °C. Anal. ($C_{17}H_{16}Cl_2O_4 \cdot {}^1/_4AcOH$) C, H.

Compound 30. Via Scheme XII. 6,7-Dichloro-2,3-dihydro-2-[3-(2-methyl-1,3-dioxolan-2-yl)propyl]-5-methoxy-2-propyl-1*H*-inden-1-one (29). Compound **11c** (13.66 g, 50 mmol) in toluene (80 mL) was added with stirring under N_2 to 56% NaH in mineral oil (2.28 g, 53.3 mmol) in DMF (65 mL) and toluene (15 mL), and the mixture was stirred for 1.5 h. 2-Methyl-2-(3-iodopropyl)-1,3-dioxolane[28] (13.4 g, 53.3 mmol) in toluene (10 mL) was then added over 15 min, and the suspension was heated and stirred for 16 h at 55 °C. The mixture was poured into ice H_2O (600 mL) and extracted with Et_2O. The organic extract was washed with brine, dried over $MgSO_4$, evaporated in vacuo, and chromatographed on silica gel (450 g), eluting with 0.7% THF in CH_2Cl_2 to give **29** (12.3 g, 61%), mp 80–80.5 °C, which was used directly in the next step.

3-Acetyl-4,5-dichloro-8a-propyl-1,2,8,8a-tetrahydrocyclopent[*a*]inden-6-ol (30). Compound **29** (8.3 g, 20.5 mmol) was added to molten pyridine hydrochloride (90 g, 779 mmol) and heated at 175 °C for 2 h, then poured onto crushed ice (500 g), and extracted with Et_2O. The organic extract was washed with H_2O, dried over $MgSO_4$, and evaporated in vacuo and the residue chromatographed on silica gel (275 g), eluting with THF–CH_2Cl_2 (1:150, v/v) to obtain **30** (3.8 g, 57%) which was used directly in the next step.

[(3-Acetyl-4,5-dichloro-8a-propyl-1,2,8,8a-tetrahydrocyclopent[*a*]inden-6-yl)oxy]acetic Acid (31). By a procedure similar to that described for the preparation of **28**, **30** (3.45 g, 10.6 mmol) was used to prepare **31**. The yield was 3.2 g (79%) which melted at 188–190 °C after recrystallization from AcOH. Anal. ($C_{19}H_{20}Cl_2O_4$) C, H.

Compound 37. Via Scheme XIII. 6,7-Dichloro-2,3-dihydro-5-[(dimethylthiocarbamoyl)oxy]-2-propyl-1*H*-inden-1-one (32). Compound **14c** (23.5 g, 90.7 mmol) was stirred with K_2CO_3 (20.7 g, 150 mmol) suspended in DMF (65 mL) at 55 °C for 15 min. Dimethylthiocarbamoyl chloride (12.7 g, 102.7 mmol) was added, and the mixture was heated at 60 °C for 2 h with stirring, then poured into ice H_2O (700 mL), and extracted with Et_2O. The organic extract was washed with H_2O, dried over $MgSO_4$, and concentrated in vacuo. The residue was recrystallized from *i*-PrOH to give **32** (16 g, 51%), mp 106–108 °C. Anal. ($C_{15}H_{17}Cl_2NO_2S$) C, H, N.

6,7-Dichloro-2,3-dihydro-5-[(dimethylcarbamoyl)thio]-2-propyl-1*H*-inden-1-one (33). Compound **32** (5 g, 14.4 mmol) was heated and stirred under N_2 for 45 min at 195–205 °C. The melt was cooled and triturated with *i*-PrOH, and the resulting solid was collected by filtration to obtain **33** (5 g, 100%), mp 129–131 °C. Anal. ($C_{15}H_{17}Cl_2NO_2S$) C, H, N.

5,6-Dichloro-2,3-dihydro-5-mercapto-2-propyl-1*H*-inden-1-one (34). Compound **33** (11 g, 31.7 mmol) was refluxed with stirring under N_2 for 4 h with MeOH (80 mL), H_2O (125 mL), and KOH (12 g). The mixture was cooled, diluted to 700 mL with H_2O, and filtered through Super-Cel. The filtrate was acidified with HCl and extracted first with CH_2Cl_2 and then with Et_2O. The combined organic extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give **34** (8.1 g, 93%), mp 103–105 °C. Anal. ($C_{12}H_{12}Cl_2OS$) C, H.

Methyl [(6,7-Dichloro-2,3-dihydro-1-oxo-2-propyl-1*H*-inden-5-yl)thio]acetate (35). Compound **34** (6.8 g, 25 mmol) was dissolved in DMF (60 mL) and heated with stirring under N_2 with K_2CO_3 (6.15 g, 44.5 mmol) at 50 °C for 15 min. Methyl bromoacetate (5.81 g, 38 mmol) was added, and the mixture was heated at 60–65 °C for 1 h. The mixture was cooled, poured into ice H_2O (250 mL), and then extracted, first with $CHCl_3$ and finally with Et_2O. The combined organic extracts were washed with H_2O, dried over $MgSO_4$, and concentrated in vacuo. The oily residue was triturated with hexane to give **35** which was removed by filtration to give 8.0 g (92%), mp 92.5–94.5 °C. Anal. ($C_{15}H_{16}Cl_2O_3S$) C, H.

[(5,6-Dichloro-2,3,9,9a-tetrahydro-3-oxo-9a-propyl-1*H*-fluoren-7-yl)thio]acetic Acid (37). Compound **35** (7.87 g, 22.7 mmol) in THF (25 mL) was stirred and treated, first with DBN (0.1 mL) and then with MVK (1.25 mL, 15.1 mmol) at 50 °C. Amounts of DBN and MVK identical with those used initially were added after 10 and 20 min. After 3.25 h, the mixture was poured into ice H_2O (200 mL), made just acid with HCl, and extracted, first with CH_2Cl_2 and then with Et_2O. The combined organic extracts were washed with H_2O, dried over $MgSO_4$, and concentrated in vacuo. The residue (**36**) was dissolved in EtOH (25 mL) and treated with a solution of NaOH (2.4 g) in H_2O (25 mL). The mixture was stirred at 25 °C for 18 h, diluted to 350 mL with H_2O, acidified with HCl, and extracted with Et_2O. The organic extract was washed with H_2O, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in MeCN (20 mL) and chilled; the precipitate that formed was filtered and washed with MeCN. There was obtained 1.3 g (15%) which, after recrystallization from a mixture of AcOH (12 mL) and H_2O (2 mL), gave pure **37**, mp 235–237 °C. Anal. ($C_{18}H_{18}Cl_2O_3S$) C, H.

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