Synthesis and Antitumor Activity of Some Aromatic Seleno Lactones

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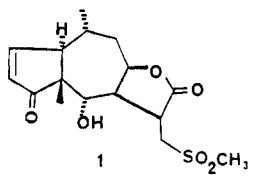
Received September 19, 1983

Several aromatic seleno lactones have been synthesized and shown to possess significant inhibitory activity against human colon tumor-8r cells in culture at concentrations lower than 1 mM. Although all of the compounds tested were found to be active, 5-hydroxy-3-[(phenylseleno)methyl]hydrocoumarinoctanoate (**3d**) and 5-hydroxy-3-[(phenylseleno)methyl]hydrocoumarindecanoate (**3e**) were found to be the most effective in inhibiting cell growth. In situ formation of the corresponding α-methylene lactones is postulated to account for the cytotoxic activity in this class of compounds.

Naturally occurring α-methylene γ- and δ-lactones have attracted considerable interest in recent years because of their diverse biological properties. Many natural and synthetic lactones of this type have been found to possess pronounced cytotoxic[1] antiinflammatory,[2] antibacterial,[3] antihyperlipidemic,[4] and allergenic[5] activity. The biological activity of these compounds resides primarily in their ability to react with nucleophilic cellular components in Michael addition type reactions. Unfortunately, the moiety that is responsible for the biological activity of these compounds is also responsible for their high reactivity. Thus, their use as pharmacological agents has been severely curtailed because of their high toxicity arising from indiscriminate reactions with nucleophilic cellular components. Several attempts have been made in the past to achieve selectivity of action by improving the transport properties of these compounds via attachment to an appropriate carrier, such as a carbohydrate[6] or steroid[7] moiety, or by utilizing derivatives of these compounds, such as simple vinyl and α-methylene-γ-butyrolactone sulfonate esters, silyl enol ethers,[8] and others.[9-16] Because of the inherent instability and high reactivity of these compounds,[17,18] we have sought to circumvent the problem of toxicity through the synthesis of precursor molecules that might function as "masked" α-methylene lactones. It is interesting to note that such masked α-methylene lactones occur naturally,[19] an example being sulferalin (1).



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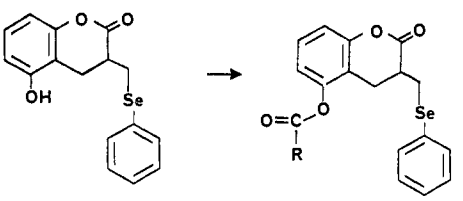
We have recently reported an efficient method for the synthesis of aromatic α-methylene-δ-valerolactones in protected form.[17,18] Several seleno lactones **3** of varying lipophilicity were prepared starting from **2**, and their cytotoxic activity was investigated.

Chemistry. Compound **2** of Table I was readily made as described in the Experimental Section, starting with 1,3-cyclohexanedione. The compounds listed in Table I were then obtained by reacting compound **2** with the appropriate anhydride, acid chloride, or dihydropyran (Scheme I).

Biological Results and Discussion

The compounds listed in Table I were evaluated for cytotoxic activity as described in the Experimental Section,

Scheme I



2

	R
3a	CH_3
b	CH_3CH_2-
c	n-C_3H_7-
d	n-C_7H_{15}-
e	n-C_9H_{19}-

and the results are summarized in Table II. 6-Mercaptopurine was used for comparative purposes. The

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0022-2623/84/1827-0548$01.50/0

Table I. Aromatic Seleno Lactones

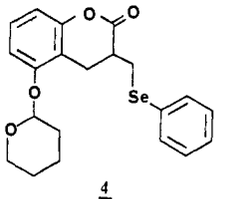
no.	mp, °C	recrystn solv	yield, %	mol formula	anal.
2	108-109	hexane/$CHCl_3$	75	$C_{16}H_{14}O_3Se$	C, H; Se[a]
3a	104.5-105	hexane/CH_2Cl_2	36	$C_{18}H_{16}O_4Se$	C, H, Se
3b	108-109	petr ether/CH_2Cl_2	72	$C_{18}H_{18}O_4Se$	C, H, Se
3c	105-106	hexane/CH_2Cl_2	76	$C_{20}H_{20}O_4Se$	C, H, Se
3d	102-103		67	$C_{24}H_{28}O_4Se$	C, H, Se
3e	103-104		72	$C_{26}H_{32}O_4Se$	C, H, Se
4	147-150	hexane/CH_2Cl_2	56	$C_{21}H_{22}O_4Se$	C, H, Se

[a] Se: calcd, 23.71; found, 22.93.

Table II. Inhibition of Human Colon Tumor-8r Cells by Aromatic Seleno Lactones in Vitro

compd	LD_{50}, mM, at the following incubation times		
	24 h	48 h	72 h
2	0.33	0.28	0.23
3a	0.29	0.24	0.18
3b	0.31	0.25	0.21
3c	0.37	0.27	0.32
3d	0.30	0.23	0.18
3e	0.23	0.15	0.14
4	0.26	0.19	0.15
6-mercaptopurine	0.76	0.35	0.28

data in Table II demonstrate clearly that compounds **2a–e** and **4** possess significant activity. The cytotoxic activity



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of these compounds probably arises from the in situ generation of the corresponding α-methylene lactones. The facile breakdown of selenoxides is well documented.[20] This hypothesis is supported by previous findings[1] that indicate that saturation of the exocyclic C=C bond abolishes all biological activity in this class of compounds. However, further experimentation is needed in order to establish unequivocally the identity of the active species. More significantly, virtually all the seleno lactones were found to have superior cytotoxic activity to 6-mercaptopurine at earlier time intervals, i.e., 24 h. These compounds seem to function as precursors of the corresponding α-methylene lactones and as such offer a means of circumventing the problem of toxicity associated with α-methylene lactones.

Experimental Section

The ^1H NMR spectra were recorded on a Varian T-60 spectrometer. Infrared spectra were recorded on a Perkin-Elmer 283 infrared spectrophotometer. Melting points were determined on a Hoover melting point apparatus and are uncorrected. Elementary analyses were performed by the Microanalytical Services Laboratory, Department of Chemistry, University of Illinois-Urbana.

Synthesis of Compound 2.[21] A mixture of 0.4 g (1 mmol) of **3a**,[18] methanesulfonic acid (0.10 g, 1.04 mmol), and 3 mL of formic acid was stirred under reflux for 5 h. The solution was cooled to room temperature and poured into 50 mL of methylene chloride in a separatory funnel. The solution was then extracted with brine (3 × 25 mL) and dried over anhydrous sodium sulfate. Evaporation of the solvent in vacuo left a yellow solid, which was purified by recrystallization from hexane/chloroform (50:1). The crystallized white product was obtained in 75% yield (0.24 g) and melted at 108–109 °C.

Synthesis of Compounds 3d,e. General Procedure. Compound **2** (8 mmol) in 20 mL of $CHCl_2$ was mixed with 16 mmol of the appropriate acid chloride and 8 mmol of dry pyridine. The mixture was refluxed for 2 h under a nitrogen atmosphere. The solution was transferred to a separatory funnel, washed with water (25 mL), 10% $NaHCO_3$ (25 mL), and water (25 mL), and dried over anhydrous sodium sulfate. Evaporation of the solvent in vacuo left a crude solid, which was purified by recrystallization.

Compound **3b** was prepared by reacting **2** with propionic anhydride.

Synthesis of Compound 4. Compound **2** (1.28 g, 4 mmol) was mixed with dihydropyran (0.67 g, 8 mmol) in 3 mL of dry THF and 1 drop of concentrated HCl. After the mixture was stirred overnight at room temperature, the precipitated solid was collected by suction and recrystallized from CH_2Cl_2/hexane.

Biology. Cell Growth Inhibition Technique. Target Cell Setup. (a) An established cell line of human colon tumor-8r cells were grown to confluency in a 75-cm^2 tissue culture flask with Eagles' MeM with 10% fetal calf serum. The cells were trypsinized for 10 min at 37 °C with a 0.25% trypsin/EDTA solution. The trypsinized cells were washed twice in complete media (MeM, 10% newborn calf serum), counted, and resuspended in MeM (10% LCS) to a concentration of 10^5 cells/mL. One milliliter was then seeded in each of the 12 wells of a Corvax 12-well plate. The plates were placed in a 37 °C incubator with 5% CO_2 and allowed to incubate for 24 h.

Compound Dilution and Addition to Wells. Cell dilution of the compounds were made in complete MeM (10% LCS). At the end of the 24-h incubation period, 1 mL of the appropriate dilution was added to each of the three wells (each dilution was tested in triplicate). One milliliter of complete MeM (10% LCS) was added to three wells to serve as the control. The plates were returned to the incubator for 24 h, at which time the first cell count was taken. Three different concentrations (0.33, 0.5, and 1.0 mM) of each compound were used in each experiment.

Counting Cells. The cells were incubated with the compounds for 24, 48, and 72 h. At the end of each incubation period the culture medium was aspirated, and 1 mL of normal saline was added to each cell. The plates were swirled, the saline was aspirated, and 1 mL of warm trypsin/EDTA solution was added

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to each cell. After 10 min, the trypsin was aspirated and placed in a centrifuge tube (15 mL) containing 1 mL of complete media (10% NBCS). This process was repeated for the content of each cell. The wells were washed with saline, and the washes were added to the tube. The tube was vortexed and then placed in the centrifuge and spun for 800*g* for 5 min. The media was decanted, and 1 mL of a 0.1% Trypsin blue in PBS was added to each tube. The tubes were vortexed, and the cells were counted with a hemocytometer.

Registry No. **2**, 88703-35-9; **3a**, 74254-89-0; **3b**, 88703-36-0; **3c**, 88703-37-1; **3d**, 88703-38-2; **3e**, 88703-39-3; **4**, 88703-40-6; decanoyl chloride, 112-13-0; dihydropyran, 110-87-2; octanoyl chloride, 111-64-8; propanoic anhydride, 123-62-6.

Synthesis and Pharmacological Activity of Some 9-Substituted Δ^8-Tetrahydrocannabinol Analogues[1]

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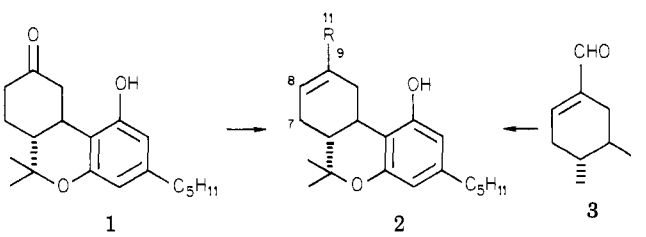
Several 9-substituted Δ^8-tetrahydrocannabinol (Δ^8-THC) analogues were synthesized and evaluated for biological activity in mice. Compounds with phenyl (**2b**) and butyl (**2c**) substituents were prepared by the addition of phenyllithium and *n*-butyllithium, respectively, to (−)-9-nor-9-oxohexahydrocannabinol (**1**), followed by dehydration, whereas, isopropyl (**2d**), $PhCH_2$ (**2e**), and $Ph(CH_2)_2$ (**2f**) derivatives were synthesized via the Grignard reaction with subsequent dehydration. Compounds with $C_2H_5CH(OH)$ (**2g**) and $CH_3CH(OH)$ (**2h**) substituents at C-9 were prepared from (−)-9-nor-9-formyl-Δ^8-tetrahydrocannabinol acetate (**3**) by the reaction of ethyl and methyl Grignard reagents, respectively. Biological activity indicated that a methyl group at the C-9 position is, thus far, optimum for producing hypoactivity and hypothermia in mice. In addition, hydroxyethyl substitution at position 9 reduced the antinociceptive activity of Δ^8-THC, in contrast to the increased activity reported for hydroxymethyl substitution.

The current interest in cannabinoids as potential therapeutic agents has given new direction to their structure–activity relationships (SAR). With the possibility that cannabinoids may eventually be useful in the treatment of a variety of diseases,[2] there is a need to develop cannabinoids that are more specific in their effects. It is well documented that Δ^9- and Δ^8-THC's are rapidly metabolized in vivo to their corresponding 11-hydroxy derivatives.[3] They are biologically potent with a pharmacological profile similar to the parent compounds. In addition, it has been reported that 9-nor-Δ^8-THC has a pharmacological profile similar to that of Δ^8-THC.[4]

In an attempt to further characterize the hydrocarbon substitution pattern at C-9 for optimum biological activity, we synthesized several 9-substituted Δ^8-THC analogues. They were evaluated for their ability to alter spontaneous activity, antinociceptive activity, and body temperature in mice in an effort to separate behavioral effects from other pharmacological effects. The results of these studies are presented in this paper.

Chemistry. The various Δ^8-THC analogues **2b** to **2h** were conveniently synthesized from either the known (−)-9-nor-9-oxohexahydrocannabinol (**1**)[5] or the (−)-9-nor-9-formyl-Δ^8-tetrahydrocannabinol acetate (**3**)[6] (Scheme I). The compounds **2b** and **2c** were prepared by the addition of phenyllithium and *n*-butyllithium, respectively, to the ketone **1**, followed by dehydration, whereas **2d** to **2f** were synthesized via the Grignard reaction (prepared from the appropriate halide), followed by dehydration. Compounds **2g** and **2h** were synthesized from **3** by the reaction of ethyl and methyl Grignard reagents, respectively, followed by careful decomposition with NH_4Cl to avoid dehydration.

Scheme I



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Pharmacological Results and Discussion

The discovery that the 11-hydroxy metabolite of Δ^9-THC exhibited potent cannabinoid activity[3a] served to demonstrate the importance of the methyl substituent at C-9. Similar results were observed in the Δ^8 series.[3b] Removal of the methyl group at C-9 in both the Δ^9 and Δ^8 series does not abolish behavioral activity but merely reduces it by 50–60%.[4] Similarly, replacement of the methyl by CH_2CH_3, CH_2OCH_3, or CH_2NH_2 at C-9 reduces but does not eliminate the behavioral activity in the Δ^8 series.[7] On the other hand, when the double bond is exocyclic ($\Delta^{9(11)}$) rather than endocyclic, as in Δ^9- or Δ^8-

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