Potential Antitumor Agents.[1a] Synthesis of Bifunctional α-Methylene-γ-butyrolactones[1b]

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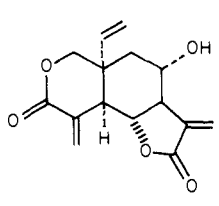
A series of alkoxy-substituted di- and monolactones including the (*E*,*E*)-3,3'-(dioxaalkanediylidene)bis[dihydro-2(3*H*)-furanones] (**17a–f**), monolactones **6**, **7**, **8**, **14**, and **15**, and dilactone **19** was synthesized by reaction of enolate **4** with appropriate alkyl halides (**6**, **7**, **17a–f**, **19**), tosyl chloride (**8**), or acid chlorides (**14**, **15**). Reaction of enolate **9** with tosyl chloride gave both *Z* and *E* isomers which allowed unequivocal assignment of stereochemistry to **8**, **14**, and **15**. A series of open-chain bis(α-methylene-γ-butyrolactones) (**21a–d**) was also prepared by a Reformatsky-type reaction between ethyl α-(bromomethyl)acrylate and appropriate dialdehydes. These compounds were tested for antitumor activity as part of a study of the influence of β-substituents and distance between alkylating sites on the antitumor activity of α,β-unsaturated lactones. The testing was carried out in standard NCI screens and selected compounds were tested against the Walker tumor. The alkoxy-substituted lactones were inactive in L1210 and were not cytotoxic. The open-chain bis(α-methylene-γ-butyrolactones) showed borderline activity in 9KB and were inactive in L1210. Compound **21a** gave a 45% inhibition of the Walker tumor at 18.75 mg/kg and was toxic at 37.5 mg/kg.

In the search for antitumor agents of plant origin a number of sesquiterpene lactones having significant in vivo activity have been reported.[2] The biological activity of these complex natural products appears to be associated with their ability to act as alkylating agents by virtue of conjugate addition of biological nucleophiles to the α-methylene lactone moiety.[3-6] Unfortunately, active sesquiterpene lactones such as vernolepin (**1**),[7] elephantopin



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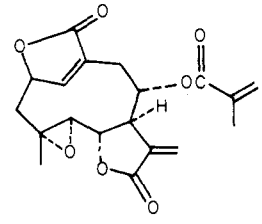

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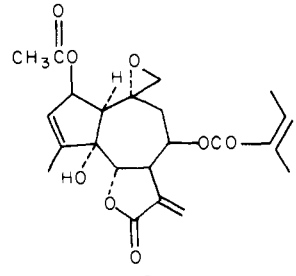

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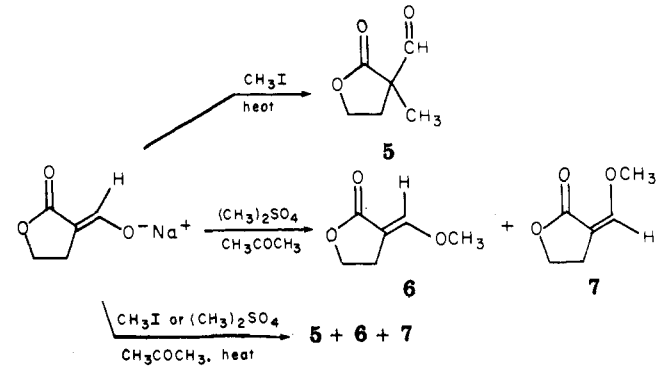
(**2**),[8] and euparotin acetate (**3**)[9] have therapeutic indices which prelude their clinical use. It has been established that the α-methylene lactone is the most reactive chemical functionality in both **2** and **3**, with no reaction being observed between cysteine and the epoxide in **2** and **3** or the endocyclic α,β-unsaturated lactone in **2**.[3,4] Model synthetic compounds containing the unsubstituted α-methylene-γ-lactone moiety were recently shown to be highly cytotoxic to normal human lymphoid cells as well as to human lymphoblastic leukemia cells.[10] However, vernolepin, which contains no functionalities other than α-methylene lactones, shows significant in vivo activity.[7] This raises the possibility that the toxicity associated with the sesquiterpene lactones may arise from the pronounced (and indiscriminant) chemical reactivity of the α-methylene lactone moiety, whereas the antitumor activity may arise from concurrent reaction of this moiety with biological nucleophiles of significance to tumor growth. These views are in accord with the theory of tumor inhibition by selective alkylation of biological macromolecules which has been advanced by Kupchan and coworkers.[3-6,11,12]

Scheme I

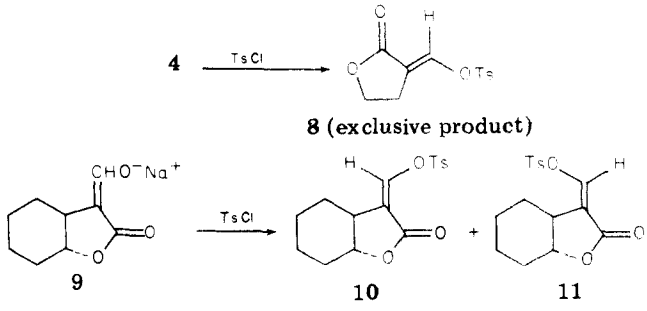


Whether the chemical reactivity of the α-methylene lactone moiety can be modified to the extent that antitumor activity is maintained and toxicity is minimized has not yet been established. Our initial attempts[13] to modify the activity in simple synthetic α-methylene lactones by substitution of an alkyl group at the β position (alkylating site) resulted in compounds which were not significantly cytotoxic. We now wish to report results obtained for a series of acyclic bis(α-methylene-γ-butyrolactones) and alkoxy-substituted α-methylene-γ-lactones which were prepared as potential antitumor agents. The synthesis of difunctional compounds has been emphasized because of the apparent enhancement in activity which has been attributed to difunctionality in alkylating agents.[14,15] Several monofunctional compounds were prepared to serve as models for comparison of monofunctional vs. difunctional activity.

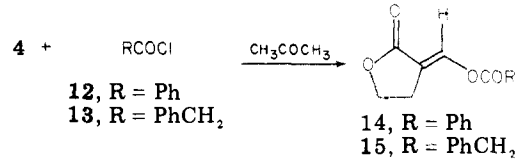
Synthesis. The alkoxy lactones were synthesized by extension of the method developed by Arjungi and coworkers.[16] Reaction of enolate **4**[17] with ethyl iodide or tosyl chloride was reported to give the ethoxy- and tosyloxy-α-methylene-γ-butyrolactones with *Z* configuration. We have found, however, that the major and almost exclusive products in our alkylation and tosylation reactions have the *E* configuration.

The composition of the product from alkylation of **4** can be controlled by choice of reaction conditions. As shown in Scheme I, reaction of **4** with dimethyl sulfate in acetone at room temperature followed by distillation of the crude reaction mixture gave a mixture of isomeric O-alkylated products consisting of **6** (*E* isomer, 85%) and **7** (*Z* isomer, 15%). Assignment to the *Z* and *E* isomers was made based

Scheme II



Scheme III



on the position of the vinyl protons in the NMR spectrum of the mixture, with the *Z* isomer showing a vinyl proton signal at δ 6.65 and the *E* isomer at δ 7.3.[18] The values are in reasonable agreement with those calculated (δ 7.3 and 6.8) by adding the δ 1.18 alkoxy deshielding parameter given by Pasquel[19] to the δ 6.13 and 5.6 vinyl proton signals of α-methylene-γ-butyrolactone. In contrast, reaction of **4** with refluxing methyl iodide gave exclusively the C-alkylated product **5**. Mixtures of **5**, **6**, and **7** were obtained on refluxing **4** in acetone with either methyl iodide or dimethyl sulfate.

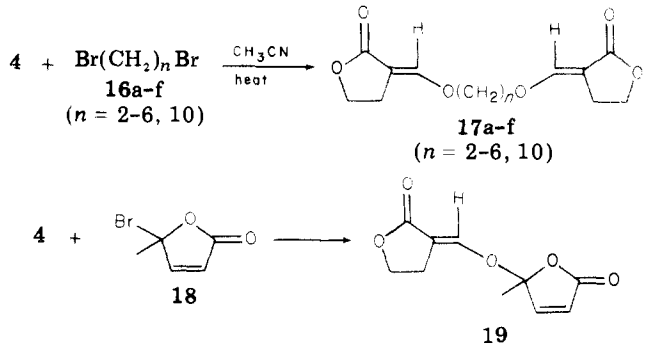
Reaction of **4** with tosyl chloride gave a product which was identical in melting point with and showed vinyl proton absorption (at δ 7.55) in good agreement with that reported by Arjungi.[16] The assignment to the *Z* isomer by Arjungi was questionable based on the position of the signal and the fact that in all of the O-alkylations of **4** which we have carried out the *E* isomer is the predominant product. Further evidence that this product was in fact the *E* isomer was gained by reaction of the enolate **9** with tosyl chloride (Scheme II). In this case both *Z* and *E* isomers **10** and **11** were formed and these compounds were separated and analyzed by NMR. The *Z* isomer **10** showed as expected a vinyl proton signal at δ 6.70 in contrast to the *E* isomer which had a downfield signal at δ 7.48. On this basis the products **14** and **15** resulting from reaction of **4** with acid chlorides **12** and **13** were assigned the *E* configuration (Scheme III).

Reaction of **4** with dibromides **16a–f** in refluxing acetonitrile gave the desired bis(alkoxylactones) **17a–f**. Although a small amount of *Z* isomer was present in the spectra of the crude reaction mixtures, recrystallization invariably resulted in the isolation of the pure *E* isomers as evidenced by vinyl proton signals at δ 7.3–7.5 in the NMR (Scheme IV).

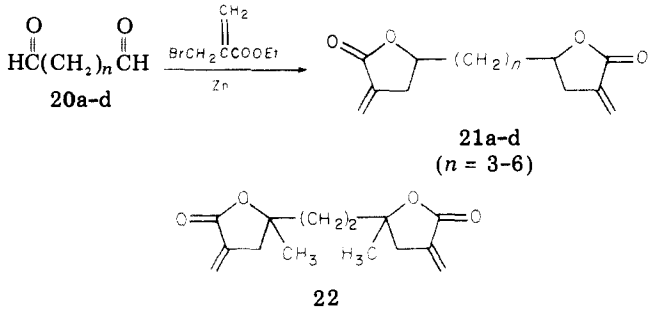
Reaction of **4** with bromobutenolide **18** gave the dilactone **19** which is related to the potent seed germination stimulant strigol.[18]

A series of bis(α-methylene-γ-butyrolactones) was prepared by reaction of the dialdehydes **20a–d** with ethyl α-(bromomethyl)acrylate in a Reformatsky-type reaction. This convenient method of α-methylene-γ-butyrolactone synthesis was developed by Öhler and co-workers[20] and has also recently been extended to the synthesis of potential lactone tumor inhibitors by Rosowsky and coworkers who prepared compound **22** by reaction of 2,5-hexanedione with the acrylate (Scheme V). This compound showed significant cytotoxicity and represents the

Scheme IV



Scheme V



n = 2 chain length in this series of compounds. Lee and co-workers[21] have also applied this reaction to produce several steroidal α-methylene-γ-lactones in which one lactone group is spiro-linked to C-3 of the steroid nucleus. Our series extends to include compounds with n = 3 (**21a**), n = 4 (**21b**), n = 5 (**21c**), and n = 6 (**21d**). The dilactones showed the characteristic signals for the α-methylene protons as triplets at about δ 6.2 and 5.6 due to coupling with the allylic protons on the butyrolactone ring.[22] In each reaction a major product was produced which was purified to homogeneity. Further studies are underway to determine the exact stereochemistry of these compounds which may be either the meso or racemic forms of the molecules.

Biological Activity. All of the compounds have been submitted for screening according to standard NCI protocols[23] and in addition several of the compounds were tested against the Walker carcinosarcoma in the rat at the Chester Beatty Research Institute.

The alkoxylactones **8**, **14**, **15**, and **17a–f** exhibited no in vivo activity against the L1210 lymphoid leukemia at doses between 100 and 400 mg/kg. Compounds **17a,c,e**, **8**, and **15** were also inactive in vitro in the 9KB cell culture system. Apparently alkoxy substitution directly at the carbon involved in the alkylation process lowers the chemical reactivity of α-methylene-γ-lactones below that required for significant biological effects; however, other factors such as changes in solubility, transport, or metabolism may be involved. Compound **17e** was also tested against the Walker tumor in rats and was nontoxic and inactive. The strigol analog **19** which contains both a butenolide and alkoxylactone was cytotoxic to HeLa cells at 5 μg/ml and was toxic at 240 mg/kg in the rat. This latter compound also gave a slight inhibition (27%) of the Walker tumor at 120 mg/kg.

The series of open-chain bis(α-methylene-γ-butyrolactones) showed borderline cytotoxicity in testing against cells derived from the human carcinoma of the nasopharynx (KB) with ED_{50} values ranging from 4.0 μg/ml for **21a** to as high as 32 μg/ml for **21b** with intermediate values of 15 μg/ml for **21c** and 7 μg/ml for **21d**. None of

these compounds were active in the L1210 assay and only compound **21a** showed any activity against the Walker tumor giving an inhibition of 45% at 18.75 mg/kg. The parent compound in this series, α-methylene-γ-butyrolactone, has an ED_{50} of 16 μg/ml in the 9KB assay[13] which leads to the conclusion that in this series of open-chain α-methylene-γ-lactones neither cytotoxicity nor in vivo antitumor activity is significantly increased by coupling two of these pharmacophores. The possibility remains that distance between the two groups is critical and it does appear that the activity is increasing as the distance between the groups is decreasing. In this regard it should be noted that Rosowsky's compound **22**, with a shortened distance between the lactone rings, is significantly cytotoxic to a human lymphoblastic leukemia cell line in vitro;[10] however, no animal tumor data have been reported. Most bifunctional alkylating agents show a relationship between activity and distance between the alkylating sites.[24] Further work exploring the structure–activity relationships of α-methylene-γ-lactones and related compounds is in progress.

Experimental Section

All melting points are determined on a Mel-Temp apparatus and are uncorrected. The structures of compounds are supported by their ir, NMR, and uv spectra. Solutions were concentrated under reduced pressure on a rotary evaporator. Variations in yield largely reflect care taken in recovering product from the mother liquor during recrystallization. Where analyses are indicated only by the symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. NMR were run on either a Varian A-60 or EM-360 in $CDCl_3$ unless otherwise indicated.

Sodium Salt of 3-(Hydroxymethylene)dihydro-2(3*H*)-furanone (4).[12] A 1-l., three-neck, round-bottomed flask equipped with mechanical stirrer, addition funnel, and reflux condenser was placed in a hood behind a shield. The condenser was fitted with a drying tube which was connected to a mineral oil bubble chamber so hydrogen evolution could be monitored. After the NaH (44 g of 57% oil dispersion, 1.05 mol) was washed with n-C_6H_{14} (2 × 100 ml), filtered with brief suction drying, and transferred to the flask, sufficient diethyl ether (Mallinckrodt anhydrous AR) was added to cover the resulting solid. A catalytic amount of C_2H_5OH (ca. 0.5 ml) was added directly to the Et_2O–NaH suspension and dropwise addition without stirring of a solution of γ-butyrolactone (86.1 g, 1.0 mol) and ethyl formate (74.1 g, 1.0 mol) in Et_2O (100 ml) was started. As soon as the Et_2O began to reflux, additional Et_2O (500–600 ml) was rapidly added through the reflux condenser and stirring was begun. Lactone–formate addition was complete in 1 hr, and stirring was continued for an additional 22 hr. Filtration, washing with Et_2O, and vacuum drying gave **4** (140.1 g, 95.5%) as a fine powdery solid.

3-Methyldihydro-2(3*H*)-furanon-3-ylcarboxaldehyde (5). A suspension of freshly prepared **4** (17.9 g, 0.13 mol) in CH_3I (32 ml, 0.51 mol) was refluxed 21 hr with stirring in a 100-ml round-bottomed flask equipped with a heating mantle, magnetic stirring bar, and a Drierite protected reflux condenser. The reaction mixture was cooled to room temperature and filtered, and the flask and residue were washed with $CHCl_3$ (25 ml). Concentration at reduced pressure gave 13.8 g (82.6%) of crude liquid **5** which was pure by NMR: δ 9.55 (s, 1, O=CH), 4.35 (t, 2, $-OCH_2-$), 3.05 and 1.9 (m, 1, $-OCH_2CH_2-$), 1.5 (s, 3, $-CH_3$). Two distillations gave an analytical (C, H) sample, bp 63.5° (0.05 mm).

3-(Methoxymethylene)dihydro-2(3*H*)-furanone Mixture (6 and 7).[18,25] A suspension of **4** (13.6 g, 0.10 mol) and $(CH_3)_2SO_4$ (11.4 g, 0.09 mol) in acetone (100 ml) was stirred 2 days at room temperature. Filtration, concentration, and vacuum distillation gave 7.1 g [70% based on $(CH_3)_2SO_4$] of a colorless liquid containing a mixture of **6** and **7** by NMR: δ 7.30 and 6.65 (t, ratio 6:1, 1, $-C=CH-$), 4.33 (t, 2, $-OCH_2-$), 3.90 (s, 3, $-OCH_3$), 2.88 (triplet of doublets, 2, $-OCH_2CH_2-$).

If the suspension of **4** is cooled (ice water bath) during the slow addition of $(CH_3)_2SO_4$ and purified by chromatography on 2 mm Merck 60, F-254 silica gel plates (hexane–Et_2O–EtOAc, 1:1:1, double run) then compound **7** is formed only in trace amounts. The *E* isomer **6** can be separated in almost quantitative yield as a clear colorless liquid which solidifies on standing in the refrigerator: mp 33–35°; ir ($CHCl_3$) 1735 (C=O), 1670 cm^{-1} (C=C); NMR (Varian EM-360, 60 MHZ, $CDCl_3$) δ 2.85 (d of t, 2 H), 3.90 (s, 3 H), 4.35 (t, 2 H), and 7.30 (t, 1 H); mass spectrum (high resolution) calcd for $C_6H_8O_3$, 128.047; found 128.048.

Monolactones 8, 14, and 15. A suspension of **4** (8.2 g, 0.06 mol) was swirled with cooling (ice bath) in acetonitrile (50 ml) or acetone (175 ml, for preparation of **8**) while an equimolar solution of the acid chloride in acetonitrile (75 ml) or acetone (100 ml for preparation of **8**) was added. After addition was complete (10 min) the mixtures were stirred for 1 day, filtered, concentrated, and recrystallized to give **8**, **14**, and **15** (see Table I). Vinyl NMR signals appeared as triplets centered at δ 8.55 (**14**), 8.2 (**15**), and 7.65 (**8**).

***trans*-8-Oxo-9-oxabicyclo[4.3.0]nonane Used in Preparation of 9.** A modification of the method of Newman and Vander Werf[26] was used for preparation of the saturated lactone precursor of **9**. Sodium metal (12.7 g, 0.55 mol) was carefully added with stirring to absolute EtOH (300 ml) in a 1-l. flask equipped with vented (to hood!) reflux condenser, addition funnel, mechanical stirrer, and cooling bath (tap water). After reaction of the Na was complete, diethyl malonate (88.9 g, 0.55 mol) was added dropwise with stirring. The resulting suspension was stirred an additional 0.5 hr before dropwise addition (15 min) of cyclohexene oxide (54.2 g, 0.55 mol). After stirring 2.25 hr at room temperature, the mixture was heated (heating mantle) until it became too viscous for efficient stirring (1 hr) and then allowed to sit overnight at room temperature before adding a solution of KOH (41.5 g, 0.74 mol) in water (200 ml). The aqueous solution was distilled until ca. 500 ml of distillate was collected, 450 ml of water was added, and distillation was continued until a second ca. 500-ml portion of distillate was collected. The resulting solution was acidified with concentrated HCl (90 ml, pH ~1) and refluxed for 12 hr. After cooling to room temperature the oil layer was removed and the H_2O layer was saturated with NaCl and extracted with Et_2O (300 ml). The oil and extracts were combined, washed (2 × 20 ml of saturated NaCl solution), dried, filtered, concentrated, and distilled under high vacuum to give 48.2 g (62.4%) of *trans*-8-oxo-9-oxabicyclo[4.3.0]nonane[26] as a clear colorless liquid.

Sodium Salt (9) of 7-(Hydroxymethylene)-*trans*-8-oxo-9-oxabicyclo[4.3.0]nonane. Sodium salt **9** (52.1 g, 80%) was obtained by addition of a solution of ethyl formate (25.2 g, 0.34 mol) and *trans*-8-oxo-9-oxabicyclo[4.3.0]nonane (47.4 g, 0.39 mol) in Et_2O as described for the preparation of **4**.

(*Z*)- and (*E*)-7-[(*p*-Tosyloxy)methylene]-*trans*-8-oxo-9-oxabicyclo[4.3.0]nonane (10 and 11). A mixture of sodium salt **9** (9.5 g, 0.05 mol) and TsCl (9.5 g, 0.05 mol) in CH_3COCH_3 (50 ml) was swirled with slight initial cooling. After stirring for 4 days at room temperature, the mixture was filtered and the filtrate cooled in a dry ice–acetone bath to give **10** (2.9 g, 18%) as a white solid. Partial concentration of the filtrate and filtration gave an additional 1.3 g (8%) of crude **10**. Recrystallization from acetone gave an analytical sample (Table I), which had a vinyl proton signal (1 H, d, J = 3.0 Hz) at δ 6.7 in the NMR.

Concentration of the mother liquor from which **10** was crystallized followed by filtration through a column (54 g of Mallinckrodt AR, 100 mesh silicic acid, 1 × 2.5 in.) using $CHCl_3$ (750 ml) as eluent gave 3.3 g of **11** as a crude oil containing **10** as a minor component. This oil was chromatographed further on silica gel plates (Merck, 2 mm, 60, F-254) using hexane–Et_2O–EtOAc (50:50:2). Removal was accomplished by extraction with EtOAc and concentration gave **11** as a clear, colorless liquid which solidified on standing in the freezer (see Table I): ir (KBr) 1760 (=O), 1690 (C=C), 1600 cm^{-1} (aromatic); NMR (JEOL PFT-100, $CDCl_3$) δ 1.18–2.48 (m, 9 H), 2.48 (s, 3 H), 6.47 (m, 1 H), 7.48 (d, 1 H, J = 2.9 Hz), 7.40 (d, 2 H), and 7.80 (d, 2 H) (the doublet at δ 7.48 was assigned to the vinyl proton); mass spectrum (high resolution) calcd for $C_{16}H_{18}O_5S$, 322.087; found 322.084.

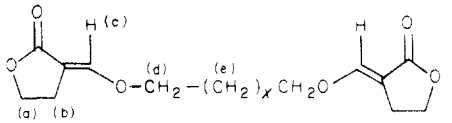
(*E*)-Bis(alkoxylactones) (17a–f). A mixture of **4** (16.3 g, 0.12 mol) and the appropriate dibromide (**16a–f**, 0.05 mol) was refluxed for 3 days with stirring in CH_3CN (130 ml). Cooling to room temperature, filtration, concentration, and recrystallization gave dilactones **17a–f** in the indicated yields (Table I). In all cases

Table I

Compd	Yield, %	Recrystn solvent[a]	Mp, °C[b]	Formula	Analyses
(*E*)-3-[(*p*-Tosyloxy)methylene]dihydro-2(3*H*)-furanone (8)[c]	77	CH_3COCH_3	118–119	$C_{12}H_{12}O_5S$	C, H, S
(*Z*)-7-[(*p*-Tosyloxy)methylene]-*trans*-8-oxo-9-oxabicyclo-[4.3.0]nonane (10)	26	CH_3COCH_3	158–159	$C_{16}H_{18}O_5S$	C, H, S
(*E*)-7-[(*p*-Tosyloxy)methylene]-*trans*-8-oxo-9-oxabicyclo-[4.3.0]nonane (11)	20 (crude)	Solid on standing	82–83	$C_{16}H_{18}O_5S$	C, H
(*E*)-3-[(Benzoyloxy)methylene]dihydro-2(3*H*)-furanone (14)	37	EtOAc	149–151	$C_{12}H_{10}O_4$	C, H
(*E*)-3-[(Phenylacetyloxy)methylene]dihydro-2(3*H*)-furanone (15)	65	EtOAc	75–76	$C_{13}H_{12}O_4$	C, H
(*E,E*)-3,3′-(2,5-Dioxa-1,6-hexanediylidene)bis[dihydro-2(3*H*)-furanone] (17a)	33	EtOAc	125–126	$C_{12}H_{14}O_6$	C, H
(*E,E*)-3,3′-(2,6-Dioxa-1,7-heptanediylidene)bis[dihydro-2(3*H*)-furanone] (17b)	36	EtOAc	95–96	$C_{13}H_{16}O_6$	C, H
(*E,E*)-3,3′-(2,7-Dioxa-1,8-octanediylidene)bis[dihydro-2(3*H*)-furanone] (17c)	14[d]	CH_3CN, EtOAc, Me_2CO	147–150	$C_{14}H_{18}O_6$	C, H
(*E,E*)-3,3′-(2,8-Dioxa-1,9-nonanediylidene)bis[dihydro-2(3*H*)-furanone] (17d)	49	CH_3CN, EtOAc	78–79	$C_{15}H_{20}O_6$	C, H
(*E,E*)-3,3′-(2,9-Dioxa-1,10-decanediylidene)bis[dihydro-2(3*H*)-furanone] (17e)	23	EtOAc	111–113	$C_{16}H_{22}O_6$	C, H
(*E,E*)-3,3′-(2,13-Dioxa-1,14-tetradecanediylidene)bis[dihydro-2(3*H*)-furanone] (17f)	34	EtOAc	66–68	$C_{20}H_{30}O_6$	C, H
(*E*)-3-[(2,5-Dihydro-2-methyl-5-oxo-2-furanyl)oxy]methylenedihydro-2(3*H*)-furanone (19)	78	EtOAc-Et_2O	88–89	$C_{10}H_{10}O_5$	C, H
1,3-Bis(2-methylene-4-butyrolactonyl)propane (21a)	77	EtOAc	37–39	$C_{13}H_{16}O_4$	C, H
1,4-Bis(2-methylene-4-butyrolactonyl)butane (21b)	72	EtOAc-Et_2O-hexane	54–60 and 72–78	$C_{14}H_{18}O_4$	C, H
1,5-Bis(2-methylene-4-butyrolactonyl)pentane (21c)	73	Syrup		$C_{15}H_{20}O_4$	C, H
1,6-Bis(2-methylene-4-butyrolactonyl)hexane (21d)	72	EtOAc-Et_2O-hexane	55–60 and 64–66	$C_{16}H_{22}O_4$	C, H

[a] Solvent mixtures were used; where several solvents are listed recrystallization was from first one and then each successive solvent. [b] For analytical samples. [c] Prepared by Arjungi et al.[16] but reported as the *Z* isomer. [d] For 18 hr reaction time.

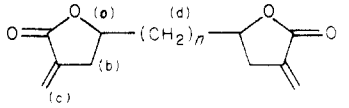
Table II. NMR and Ir Data for Alkoxymethylenedilactones 17a-f



17a-f (x = 0–4, 8)

Compd	NMR ($CDCl_3$) δ[c]	δ[a,d]	δ[b]	δ[e]	Ir (KBr) C=CH	C=O	C=C
17a	7.32 (t, 2 H)	4.3 (m, 8 H)	2.85 (m, 4 H)			1750	1685
17b	7.35 (t, 2 H)	4.25 (m, 8 H)	2.85 (m, 4 H)	2.1 (m, 2 H)		1745	1680
17c	7.48 (t, 2 H)	4.3 (m, 8 H)	2.9 (m, 4 H)	1.82 (m, 4 H)	2860	1750	1680
17d	7.35 (t, 2 H)	4.2 (m, 8 H)	2.85 (m, 4 H)	1.7 (m, 6 H)	2860	1750	1680
17e	7.37 (t, 2 H)	4.15 (m, 8 H)	2.83 (m, 4 H)	1.55 (m, 8 H)	2860	1750	1680
17f	7.35 (t, 2 H)	4.15 (m, 8 H)	2.8 (m, 4 H)	1.55 (m, 16 H)	2860	1750	1680

Table III. Tabulated NMR, MS, and Ir Data for Bis(α-methylene-γ-butyrolactones)



21a-d (n = 3–6)

Compd	NMR ($CDCl_3$) δ[a]	δ[b]	δ[c]	δ[d]	hrms m/e	Calcd for	Ir C=O	C=C
21a	4.6 (m, 2 H)	2.35–3.42 (m, 4 H)	6.23 (2 H, t, J = 3), 5.72 (2 H, t, J = 3)	1.7 (br s, 6 H)	218.096	$C_{13}H_{16}O_4 - H_2O$, 218.094	1755 ($CHCl_3$)	1660
21b[a]	4.53 (m, 2 H)	2.45–3.21 (m, 4 H)	6.23 (2 H, t, J = 2.7), 5.64 (2 H, t, J = 2.4)	1.63 (br s, 8 H)	232.109	$C_{14}H_{18}O_4 - H_2O$, 232.110	1750 (KBr)	1655
21c	4.56 (m, 2 H)	2.3–3.33 (m, 4 H)	6.25 (2 H, t, J = 3), 5.68 (2 H, t, J = 3)	1.5 (br s, 10 H)	246.124	$C_{15}H_{20}O_4 - H_2O$, 246.125	1750 ($CHCl_3$)	1660
21d[a]	4.52 (m, 2 H)	2.44–3.19 (m, 4 H)	6.22 (2 H, t, J = 2.8), 5.63 (2 H, t, J = 2.6)	1.48 (br s, 12 H)	278.159	$C_{16}H_{22}O_4$, 278.159	1745 (KBr)	1650

[a] Run on JEOL PFT-100 NMR; 21a and 21c were run on a Varian EM-360 NMR.

the vinyl NMR absorption appeared as a 2 H triplet in the range of δ 7.32–7.48 (see Table II).

Dilactone 19. A mixture of 1.3 g (9.55 mmol) of 4 and 1.3 g (7.34 mmol) of 18 was stirred at room temperature for 45 hr. The mixture was filtered through a sintered glass funnel; the filtrate was concentrated and eluted from a column of silicic acid using

$CHCl_3$ (900 ml). The $CHCl_3$ was concentrated to a homogeneous syrup, 1.2 g (78%). An analytical sample was prepared by preparative layer chromatography on silica gel (Merck 60F-254) using EtOAc–Et_2O–hexane (1:1:1, three times). The plate was extracted with EtOAc and **19** was crystallized from EtOAc–Et_2O: mp 88–89°; uv λ_{max} 230 nm (ϵ 14,000); ir ($CHCl_3$) 1776, 1751, 1680 cm^{-1}; NMR ($CDCl_3$) δ 1.83 (3 H, s), 2.92 (2 H, m, J = 2.5, 7.5), 4.38 (2 H, t, J = 7.5), 6.37 (1 H, d, J = 5.5), 7.3 (2 H, m); mass spectrum (low resolution) m/e 210 (weak, M^+), 97 (strong, methylbutenolide fragment, $C_5H_5O_2$). See Table I for additional data.

Bis(α-methylene-γ-butyrolactones) (21a–d). Activated zinc,[27] 2.75 g (20 mesh, Mallinckrodt AR), was placed in a dry round-bottomed flask equipped with a magnetic stirrer, N_2 inlet, and addition funnel. A septum was attached to the addition funnel and in sequence (through septum via syringe) a solution of 0.02 mol of dialdehyde (**20a–d**)[13] in 25 ml of dry (distilled from $LiAlH_4$) THF followed by 0.042 mol of bromomethylacrylic ester was added. About 1 ml of the mixture was then added into the flask and the flask was warmed with a small flame. On initiation of the reaction, stirring was initiated and the solution was added at a rate which maintained the temperature of the reaction mixture at 40–45°. The mixture was stirred for an additional 2 hr in a water bath (40–43°), then poured into ice-cold dilute H_2SO_4, and extracted with EtOAc. The EtOAc was washed with a cold, dilute solution of $NaHCO_3$ and water, dried (Na_2SO_4), and evaporated to a syrup. Chromatography on a column of Florisil using hexane–ether (8:2) mixtures followed by preparative layer chromatography on silica gel plates (Merck 60, F254) using hexane–ether–EtOAc (1:1:1) gave the pure dilactones **21a–d**. Data on the individual compounds are given in Tables I and III.

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References and Notes

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Potential Central Nervous System Antitumor Agents. Aziridinylbenzoquinones. 1

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A series of 3,6-substituted 2,5-diaziridinyl-1,4-benzoquinones was prepared as potential CNS antitumor agents. Activity was evaluated in the murine leukemia L1210 system. The diurethane derivative **9** was found to have significant activity in that system as well as in the intraperitoneal P388 and B16 tumor models. Marginal Lewis lung activity was observed. Reproducible activity was seen in the intracerebral L1210 and P388 systems. Multiple cures were observed in the murine ependymoblastoma brain tumor model. The effect of substituent type on aziridinylquinone activity is discussed.

A recent analysis of murine antitumor test data obtained by the National Cancer Institute on quinone derivatives indicated that the aziridinylquinones, as a family, possessed significant activity against lymphoid leukemia L1210 as well as other test systems.[1] While the L1210 results were all obtained on an intraperitoneally implanted tumor, the molecular properties of the compounds appeared to fit some of the requirements suggested by Rall and Zubrod[2] as important for CNS penetration. Subsequent testing of these compounds at the NCI in several intracerebral (ic) tumor systems indicated that the aziridinylquinones possessed substantial ic antitumor activity.[3]