for 1 h, ice cooled, carefully decomposed with 4 mL of H_2O followed by 2 mL of 8% NaOH, and filtered from the inorganic salts. The Et_2O solution was dried (Na_2SO_4) and evaporated. The oily base was characterized as the hydrochloride (yield 85–90%) (Table III).

***N*-(3,3-Dimethyl)allyl Derivatives α- and β-1f, α- and β-3f.** A mixture of 2 g (ca. 10 mmol) of the corresponding secondary amine, 2.3 mL (20.4 mmol) of 1-chloro-2-methyl-2-butene, 2 g of K_2CO_3, and 25 mL of EtOH was refluxed for 4 days [TLC monitoring, Al_2O_3 G Merck, petroleum ether–Et_2O (2:1)] and evaporated to dryness in vacuo. The residue was distributed between H_2O and Et_2O. The ethereal extract was dried (Na_2SO_4) and evaporated. The oily residue was characterized as the hydrochloride (yield 80–85%) (Table III).

***N*-Acetyl Derivatives α- and β-3g.** A mixture of α-**3a** (400 mg, ca. 2 mmol) in 12 mL of dry C_6H_6, 0.3 mL of pyridine, and 0.6 mL of acetyl chloride was left overnight at room temperature. The excess of chloride was decomposed with water and the organic layer separated, washed with 10% $NaHCO_3$ and dried (Na_2SO_4). Evaporation under vacuum afforded an oil which slowly solidified. The solid was crystallized to yield white crystals (350 mg, 73%). β-**3g** was prepared similarly as a clear oil starting from 100 mg of β-**3a** (Table III).

***O*-Demethyl Derivatives α-4b, α-4c, and α-4d.** A solution of 2 g of the corresponding methoxyphenyl derivative in 10 mL of 48% HBr was refluxed for 1 h under nitrogen. The cooled solution was made alkaline with aqueous 10% $NaHCO_3$ and extracted with Et_2O or $CHCl_3$. The organic layer was dried (Na_2SO_4) and evaporated, and the base was converted to the HCl or HBr salts (yields 55–60%) (Table III).

***O*-Demethyl Derivatives α- and β-2e, α- and β-2f, α-4e, and α-4f.** To a stirred solution of the corresponding methoxyphenyl derivative (2.5 g, 8–9 mmol) in 15 mL of CH_2Cl_2 cooled at −60 °C was added a solution of BBr_3 (1.90 mL, 20 mmol) in 10 mL of CH_2Cl_2 cooled at the same temperature. After stirring at −60 °C for 1 h and at room temperature for 30 min, the mixture was cooled again at −60 °C, the boron–ether complex decomposed with MeOH, and the solvent evaporated under vacuum. The crude gummy solid was triturated with Et_2O, the Et_2O decanted, and the solid dissolved in hot EtOH and allowed to cool, affording 1.7–1.8 g (ca. 55%) of pink crystals of the corresponding phenolic derivative as hydrobromide. The hydrobromide was dissolved in water, made alkaline with NH_4OH, extracted with Et_2O, and dried (Na_2SO_4). Evaporation afforded the oily base which was converted to the HCl or HBr salts (Table III).

Acknowledgment. We wish to thank the Committee on Problems of Drug Dependence, Inc., for their tests for narcotic antagonism in monkeys and Dr. K. Schellekens of Janssen Pharmaceutica for the antagonist tests in rats.

References and Notes

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(20) After alkylation the higher R_f compound was the α isomer [Al_2O_3 G Merck, petroleum ether–Et_2O (1:1)]. Similar behavior was observed for the α/β pairs of the *m*-methoxy series.

Potential Antitumor Agents. Synthesis, Reactivity, and Cytotoxicity of α-Methylene Carbonyl Compounds

John M. Cassady,* S. R. Byrn,* I. K. Stamos, S. M. Evans, and A. McKenzie

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907. *Received February 9, 1978*

The α-methylene lactones **9**, **12**, **21**, and **24** were prepared by a mild, convenient α-methylenation process using the α-ethyloxalyl derivatives in nonoptimized yields ranging from 23 to 90%. The rates of reaction of these and several other lactones with cysteine at pH 7.4 and their KB toxicities were measured. These studies showed that neither the strained trans-fused α-methylene lactone **12** nor the hydroxy-α-methylene lactones **5** and **6** reacted with cysteine with rates comparable to elephantopin. Based on these limited studies, the rate of cysteine addition appears to be relatively insensitive to changes in strain energy and neighboring groups. In addition, the rate constant for reaction with cysteine did not correlate with cytotoxicity.

A number of sesquiterpene lactones or ketones containing an enone system have cytotoxic and, in some cases, significant antitumor activity.[1,2] The activity of these compounds apparently derives from their chemical reactivity which primarily involves the conjugate addition of various nucleophilic thiols resulting in alkylation. Compounds which contain the α-methylene γ-lactone or 2-cyclopentenone groups have demonstrated reactivity with thiol-rich enzymes, including phosphofructokinase[3] and glycogen synthetase,[4] and with simple model thiols

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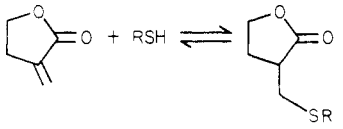
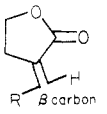
such as cysteine.[5-10] This research[5-8] and recent reports by Lee and co-workers[9,10] have established that compounds of this type can be viewed as a group of alkylating agents which exert their biological effects by inhibiting cellular enzyme activity and metabolism and not by alkylating and impairing DNA function.

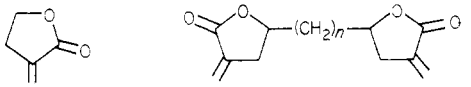


Although none of the naturally occurring compounds, such as vernolepin,[11] elephantopin,[12] euparotin acetate,[13] and helenalin,[10] which have shown in vivo antitumor activity in animal systems, has demonstrated enough selectivity to be considered for clinical use, these compounds are of interest as leads to the synthesis of analogues which may achieve the necessary selectivity. Our group is particularly interested in determining whether the chemical reactivity of the α-methylene lactone and other enone groups can be modified to the extent that antitumor activity is maintained and toxicity is minimized.

Previous work in our laboratory has shown that substitution of alkyl, alkoxy, or alkamino groups at the β carbon (alkylation site) resulted in compounds that were not significantly cytotoxic.[14-16]



Synthesis of unsubstituted acyclic dilactones of the general structure shown below did lead to cytotoxic



9KB, ED_{30} 15 μg/mL $n = 3$, ED_{50} 4 μg/mL

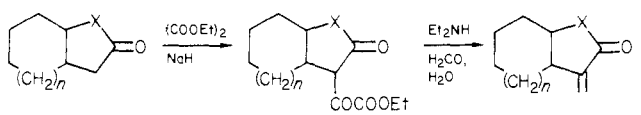
compounds (n = 2, 3);[15,17] however, none of these compounds achieved the level of activity exhibited by certain natural products.

In addition, the reactivity of these compounds toward cysteine was very low in comparison with most of the natural products, and none of the bifunctional compounds showed in vivo antitumor activity.

We have extended these studies to a series of β-unsubstituted α-methylene lactones in hopes of developing compounds of varying chemical reactivity. Tests of these compounds would therefore reveal whether improved selectivity could be obtained by modulating the structures of chemically reactive enones.

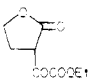
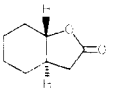
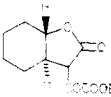
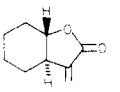
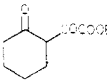
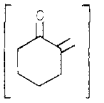
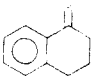
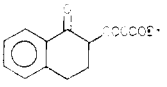
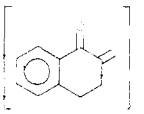
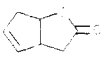
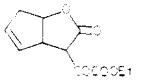
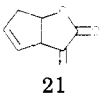
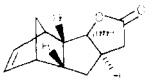
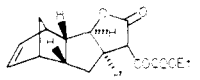
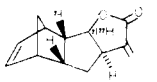
Chemistry. The open-chain α,β-unsaturated ester, ethyl α-(hydroxymethyl)acrylate (**1**), was prepared according to a published procedure.[18] Compounds **2**–**4** have been previously reported from our laboratory.[14,15] Compounds **5** and **6** were reported by Grieco and co-workers.[19]

The remaining β′-unsubstituted α-methylene lactones **9**, **12**, **21**, and **24** were prepared from the corresponding lactones by a two-step α-methylenation process involving the intermediate α-ethyloxalyl derivatives (Table I). This method, which is outlined below, was also extended to the



synthesis of α-methylene ketones **15** and **18**. Compound **21** has been prepared by an alternate procedure reported by Ali and Roberts.[20]

Table I. α-Methylenecarbonyl Compounds Prepared

carbonyl compd	ethyloxalyl derivative	α-methylene derivative	yield,[a] %
 7	 8	 9	50
 10	 11	 12	23
 13	 14	 15	66
 16	 17	 18	89
 19	 20	 21	70
 22	 23	 24	91

[a] Yield based on overall yield for two steps from lactone precursor.

Numerous methods have been developed for the α-methylenation of lactones due to the interest aroused by the biological activity of compounds which contain this group and these methods have been reviewed.[21,22] McMurray has recently reported a procedure which also involves formation of an ethyloxalyl derivative, followed by reaction with aldehyde to give a diketo lactone which is cleaved by base to give the α-methylene carbonyl compound.[23] This method failed in attempts to synthesize fused-ring α-methylene lactones. Previous reports outlined the conversion of esters[24] and lactams[25] to the corresponding α-methylene derivatives and served as the basis for developing the general procedure we have applied to ketones and lactones. This method is mild, convenient, and efficient, giving a variety of enones, including the fused-ring type. Although yields have not been optimized for all of the compounds reported, we have achieved quantitative yields in each step of the synthesis of the bicyclolactone **24**.

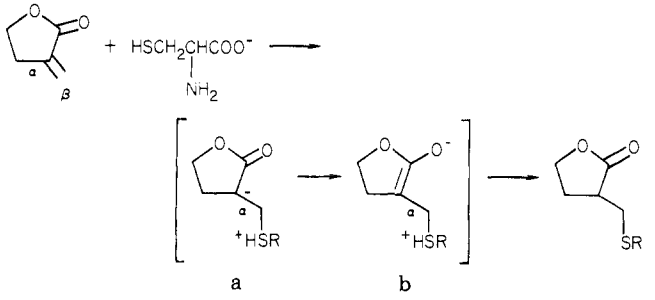
Correlation of Structure and Reactivity. The rates of reaction with cysteine of a series of α-methylene γ-lactones were measured using essentially the methods of Kupchan et al.[1b] The concentrations were treated assuming second-order kinetics. The alternative possibility of a reversible second-order reaction (Lac + HSR ⇄ Add) such as that observed by Esterbauer[26] for the reaction of α,β-unsaturated aldehydes with thiols was tentatively ruled out for this preliminary study because (1) it had been shown that elephantopin reacted completely with an equimolar amount of cysteine in 60 min, thus ruling out any reversibility for this model α-methylene γ-lactone;[27] (2) the elephantopin reaction followed second-order kinetics for greater than 70% of the reaction; and (3) the kinetic equations for a second-order process and a reversible second-order process are different, and a reversible reaction would not be expected to fit second-order kinetics

with a correlation coefficient of greater than 0.99.

The reproducibility of our method is best reflected in the average and standard deviation of the rate constant for elephantopin (**25**),[12,27] $k = 2974 \pm 117$ L M^{-1} min^{-1}, which were calculated from 31 kinetic runs made on different days during the course of determination of the other rates. Study of the rates of the reaction of elephantopin with cysteine in the ratios 1:1, 1:2, and 2:1 revealed that the reaction was second order. In addition, it should be noted that we have not studied the pH or buffer dependence of the rate constants; however, earlier studies have shown that at pH 7.4 the reaction of cysteine with α,β-unsaturated nitriles and ketones is due almost entirely to reaction of the $HSCH_2CH(NH_2)COO^-$ species. At present we are planning more detailed studies of the effects of pH, buffers, and the stability of the adducts on the rates of these reactions.

Table II reports the results of our rate studies on compounds **1–6**, **9**, **12**, **24**, and **25**. The rates are reported as average ± standard deviation and were determined from at least four measurements. Control experiments showed that the rate of cysteine decomposition in the absence of lactone was approximately 21 ± 7 L M^{-1} min^{-1}. All of the compounds reported (Table II) react with cysteine at a measurable rate. However, with the exception of elephantopin (**25**), none of these compounds have rates of reaction faster than the simplest α-methylene γ-lactone, **9**, and none had a reactivity comparable to that of elephantopin. Substitution at the site of alkylation as in **2–4** produced rates of reaction slightly slower than the simplest α-methylene γ-lactone, **9**. Similarly, OH groups adjacent to the α-methylene group as in **5** and **6** did not accelerate the rate of reaction relative to **9**, in contrast to a previous suggestion.[16]

The strain energy in the α-methylene γ-lactone ring also appeared to have little effect on the rates of reaction or cytotoxicity of the α-methylene γ-lactones. Based on our previous studies[16] the trans-fused α-methylene γ-lactone **12** was expected to be the most highly strained compound in the series according to calculations and the presence of this functionality in the reactive natural products such as vernolepin. If the transition state of cysteine addition has substantially more sp^3 character at the β carbon, as shown in resonance structure a, then one would expect that strain



a b

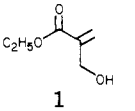
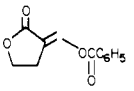
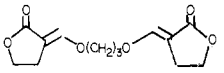
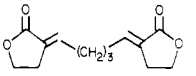
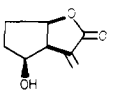
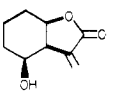
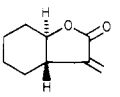
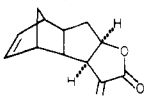
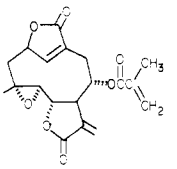
energy relief would play a role in these reactions and that lactone **12** should be quite reactive. This was not the case as seen in Table II. Furthermore, the rates of cysteine addition to fused and unfused lactones were essentially the same.

Correlation of Structure and Cytotoxicity. Our results (Table II) on model compounds are completely consistent with the observations of Kupchan et al. that reactivity and cytotoxicity are not directly related.

Conclusion

These studies indicate that the rate of cysteine addition to α-methylene γ-lactones of essentially the same electronic structure is insensitive to changes in the other obvious structural parameters (strain and neighboring groups) in the starting material. This conclusion is best explained by assuming that the transition state of the reaction is dissimilar from the starting material or that the accelerated rate in the natural products is due to a complex combination of a number of factors such as electronic, strain, neighboring group, and steric effects.

Table II. Comparison of Structure with Rate of Reaction with Cysteine

compd	rate, L M^{-1} min^{-1}	estd[a] O···C distance, Å	cytotoxicity (ED_{50}, KB), μg/mL
 1	66 ± 17	*b*	
 2	110 ± 6		100
 3	106 ± 18		>100
 4	133 ± 13		>100
 5	284 ± 8	2.8	cytotoxic[f]
 6[c]	96 ± 11	2.8–3.8[d]	cytotoxic[f]
 9	296 ± 74		16
 12	78 ± 16		38
 24	144 ± 60		1
 **25** (elephantopin)[e]	2974 ± 117	2.9	1

[a] Measured from molecular models. [b] Depends on conformation. [c] A sample of this compound was kindly provided by Professor Paul A. Grieco, University of Pittsburgh, Pittsburgh, Pa. [d] Exact value depends on conformation of the six-membered ring. [e] A sample of this compound was kindly provided by the late Professor S. M. Kupchan, University of Virginia, Charlottesville, Va. [f] See ref 19.

Experimental Section

Melting points were taken in open capillary tubes and are

uncorrected. UV spectra were determined in 95% EtOH using a Cary Model 17 spectrophotometer. IR spectra were determined in KBr using a Beckman Model 33 spectrometer. NMR spectra were recorded in $CDCl_3$ either on a Varian Model EM-360 or A-60 or JEOL PFT-100 spectrometer; chemical shifts were recorded in parts per million downfield from Me_4Si which was used as an internal standard. Mass spectra were obtained on a Hitachi Perkin-Elmer RMU-6A or a CEC 21-110 mass spectrometer.

α-Ethyloxalyl Compounds 8, 11, 14, and 17. α-Ethyloxalyl-γ-butyrolactone (**8**),[23] α-ethyloxalyl-*trans*-8-oxo-9-oxabicyclo[4.3.0]nonane (**11**),[28,29] ethyl cyclohexanone-2-glyoxalate (**14**),[23,30] and ethyl dihydro-1(2*H*)-naphthalenone-2-glyoxalate (**17**)[31] were prepared by published routes.

Synthesis of *cis*-α-Ethyloxalyl-2-hydroxycyclopent-4-ene-1-acetic Acid γ-Lactone (20). To a stirred suspension of 0.32 g (13.2 mmol) of degreased NaH in 50 mL of anhydrous Et_2O under N_2 was added dropwise a solution of 0.93 g (7.5 mmol) of *cis*-2-hydroxycyclopent-4-ene-1-acetic acid γ-lactone (**19**), prepared by literature methods,[32,33] and 2.35 g (16.1 mmol) of redistilled diethyl oxalate (Matheson, Coleman and Bell) in 30 mL of anhydrous Et_2O. The reaction mixture was refluxed overnight and then allowed to cool to room temperature. The tan salt suspended in the ether was collected by suction filtration, washed with Et_2O (1 × 50 mL), then treated with dilute H_2SO_4, and extracted with Et_2O (3 × 100 mL). The combined ether extracts were dried over $MgSO_4$, concentrated, and filtered through a column of silica gel using Et_2O. Evaporation of the solvent under reduced pressure on a rotary evaporator yielded 1.46 g (87%) of **20** as a yellow oil (similar treatment of the ether filtrates yielded an additional 788.5 mg of crude **20** as an orange oil), homogeneous by thin-layer chromatography [ether–CH_2Cl_2 (2.5:97.5); visualization with $Ce(SO_4)_2$ and heat]: IR (neat) 3500–2800 (OH, partially obscured by CH stretch), 1740 with shoulders at 1755 and 1730 (γ-lactone C=O), 1700 with shoulder at 1715 (ester C=O), 1635 and 1645 cm^{-1} (strong, C=C); NMR ($CDCl_3$) δ 10.03 (br s, 1 H, D_2O exchangeable, –C=COH), 5.83 (s, 2 H, –CH=CH–), 5.33 (m, 1 H, γ-lactone –OCH–), 4.27–4.77 (br, m, 3 H, –CH–, $-OCH_2CH_3$), 2.80 (m, 2 H, $-CH_2-$), 1.40 (t, 3 H, $-OCH_2CH_3$); UV (MeOH) λ_{max} 278 nm (ϵ_{max} 3383), 212 (1583); mass spectrum M^+ (EI) 224 (*m/e* of M^+ = 224.068 obsd, 224.068 calcd). An analytical sample was prepared by molecular distillation at 85 °C (0.5 mm). The compound crystallized on standing, mp 52–54 °C. Anal. ($C_{11}H_{12}O_5$) C, H.

Synthesis of (3aα,4aβ,5β,8β,8aβ,8bα)-3,3a,4,4a,5,8,8a,8b-Octahydro-3-ethyloxalyl-5,8-methano-2*H*-indeno[1,2-*b*]furan-2-one [(1*R,2*S**,3a*S**,4*R**,7*S**,7a*R**)-3a,4,7,7a-Tetrahydro-α-ethyloxalyl-1-hydroxy-4,7-methanoindan-2-acetic Acid γ-Lactone] (23).** To a stirred suspension of 0.74 g (30 mmol) of degreased NaH in 100 mL of anhydrous Et_2O under N_2 was added dropwise a solution of 3.83 g (20 mmol) of the saturated bicyclolactone **22**[32,34] and 5.89 g (40 mmol) of redistilled diethyl oxalate (Matheson, Coleman and Bell) in 50 mL of anhydrous Et_2O. The reaction mixture was gently refluxed overnight and then allowed to cool to room temperature. The white salt, suspended in ether, was rapidly collected by suction filtration and washed with Et_2O (1 × 100 mL) and then treated with dilute H_2SO_4, followed by extraction with ether (3 × 200 mL). The ether extracts were combined and dried over Na_2SO_4. Removal of solvent under reduced pressure on a rotary evaporator gave a white solid which was dried under high vacuum and twice recrystallized from hexane, yielding 5.33 g (91%) of **23** as a white powder, mp 86.5–87 °C, homogeneous by thin-layer chromatography [ether–$HCCl_3$ (1:1); visualization with $Ce(SO_4)_2$ and heat]: IR (KBr) 2800–3600 (OH, partially obscured by CH stretch), 1730 (γ-lactone C=O), 1695 with shoulder at 1680 (ester C=O), 1630 cm^{-1} (strong, C=C); NMR ($CDCl_3$) δ 10.73 (br s, 1 H, D_2O exchangeable, –C=COH), 6.23 (m, 2 H, –CH=CH), 4.52 (d, 1 H, γ-lactone –OCH), 4.33 (q, 2 H, $-OCH_2CH_3$), 3.62 (br q, 1 H, –CH–), 2.73–3.17 (br m, 4 H, –CH–), 2.02 (m, 2 H, $-CH_2-$), 1.23–1.47 (m, 5 H, $-CH_2-$, and triplet centered at 1.52, $-OCH_2CH_3$); UV (MeOH) λ_{max} 278 nm (ϵ_{max} = 6341) and λ (end absorption) 210 nm (ε 3828); mass spectrum M^+ (EI) 290 (*m/e* of M^+ = 290.116 obsd, 290.115 calcd). Anal. ($C_{16}H_{18}O_5$) C, H.

α-Methylene Carbonyl Compounds. α-Methylene-γ-butyrolactone (9). To a solution of 2 g (10.7 mmol) of crude **8** in 10 mL of dioxane was added a catalytic amount of sodium acetate and a mixture of 4 mL of aqueous H_2CO (37%), Mallinckrodt AR, and 1.5 g (20.5 mmol) of Et_2NH (Aldrich). The mixture was stirred at room temperature for 35 h, then treated with dilute HCl, saturated with NaCl, extracted with EtOAc, and dried. Concentration and filtration through a column of silica gel using ether, followed by preparative-layer chromatography, gave 528 mg (50%) of **9**.[14]

2-(*trans*-2-Hydroxycyclohexyl)propenoic Acid Lactone (12). To a solution of 11 in 15 mL of dioxane was added 3 mL of aqueous H_2CO (37%) and then dropwise 1.25 g (17.1 mmol) of Et_2NH. The reaction mixture was stirred for 24 h at room temperature and then 2 h at 65 °C, then treated with dilute HCl, saturated with NaCl, extracted with ether, and dried (Na_2SO_4). Concentration and filtration on a column containing Florisil in the lower half and silica gel in the upper half using ether, followed by preparative-layer chromatography on silica gel plates (F-254) using hexane–ether (1:1), gave 0.325 g (23%) of **12** as a colorless syrup.[35]

α-Methylenecyclohexanone (15). A solution of 2 mL of aqueous H_2CO (37%) and 0.5 g (6.8 mmol) of Et_2NH in 2 mL of dioxane was added dropwise into 0.99 g (5 mmol) of ethyl cyclohexanone-2-glyoxalate (**14**) in 5 mL of dioxane. The mixture was stirred at room temperature for 40 h, then poured into ether, washed with dilute HCl, and dried (Na_2SO_4). Filtration through Florisil gave a syrupy oil, 0.365 g (66.5%), which was the dimer according to the NMR spectrum.[36]

α-Methylene-α-tetralone (18). A solution of 2 mL of aqueous H_2CO (37%) and 0.4 g (5.5 mmol) of Et_2NH in 5 mL of dioxane was added dropwise to 1.09 g (4.43 mmol) of ethyl 3,4-dihydro-1(2*H*)-naphthalenone-2-glyoxalate (**17**) in 5 mL of dioxane. The mixture was stirred at room temperature for 48 h, then poured into ether, washed with dilute HCl, dried (Na_2SO_4), and concentrated. Purification by preparative-layer chromatography on silica gel plates (Merck 60, F-254) using hexane–ether (1:1) gave a colorless syrup, 0.575 g (89%), which solidified (dimerized) slowly on standing at room temperature: mp 105–107 °C (lit.[37,38] mp 106–107 °C).

Synthesis of *cis*-2-Hydroxy-α-methylenecyclopent-4-ene-1-acetic Acid γ-Lactone (21).[20] A solution of 1 mL of Et_2NH and 2 mL of 37% H_2CO in 2 mL of 1,4-dioxane was added dropwise to a stirred solution of 905.5 mg (4.0 mmol) of the α-ethyloxalyl γ-lactone **20** in 5 mL of 1,4-dioxane under N_2. The solution was allowed to stir at room temperature overnight (about 12 h), then was diluted with Et_2O, and washed with a dilute HCl solution (2 × 100 mL) and distilled water (1 × 100 mL). The ether layer was dried over Na_2SO_4 and filtered twice through a column of silica gel using ether. Evaporation of the solvent under reduced pressure on a rotary evaporator yielded 486 mg (88%) of **21** as a hygroscopic, pale yellow oil, homogeneous by thin-layer chromatography [ether–CH_2Cl_2 (5:95); visualization with $Ce(SO_4)_2$ and heat]: IR ($CHCl_3$) 1755 (γ-lactone C=O), 1660 (exocyclic C=C), 1600 cm^{-1} (endocyclic C=C); NMR ($CDCl_3$) δ 6.18 (d, *J* = 2 Hz, 1 H, –C=CH), 5.67 (d, *J* = 2 Hz, 1 H, –C=CH), 5.65 (m, 2 H, –CH=CH), 5.07 (m, 1 H, γ-lactone –OCH–), 4.00 (m, 1 H, –CH–), 2.65 (m, 2 H, $-CH_2-$); UV (MeOH) λ_{max} 208 nm (ϵ_{max} 12 040); mass spectrum M^+ (EI) 136 (*m/e* of M^+ = 136.053 obsd, 136.052 calcd). An analytical sample was prepared by molecular distillation at 45 °C (1.2 mm) to give a colorless oil. This compound was previously reported.[20]

Synthesis of (3aα,4aβ,5β,8β,8aβ,8bα)-3,3a,4,4a,5,8,8a,8b-Octahydro-3-methylene-5,8-methano-2*H*-indeno[1,2-*b*]furan-2-one [(1*R,2*R**,3a*R**,4*S**,7*R**,7a*S**)-3a,4,7,7a-Tetrahydro-1-hydroxy-α-methylene-4,7-methanoindan-2-acetic Acid γ-Lactone] (24).** A solution of 2.00 g (6.9 mmol) of the α-ethyloxalyl bicyclolactone **23** in 10 mL of 1,4-dioxane was added dropwise to a stirred solution of 2 mL of Et_2NH and 3 mL of 37% aqueous H_2CO in 5 mL of 1,4-dioxane. The reaction mixture was allowed to stir overnight (about 11 h) at 50 °C, during which time the clear solution turned yellow. After the solution cooled to room temperature, it was diluted to 200 mL with Et_2O and washed with a dilute HCl solution (2 × 100 mL) and distilled water (1 × 100 mL). The ether layer was dried over Na_2SO_4 and filtered twice through a column of silica gel using ether. Evaporation of the solvent under reduced pressure on a rotary evaporator gave 1.40 g (100%) of **24** as a pale oil, homogeneous by thin-layer chromatography [ether–CH_2Cl_2 (3:97); visualization

with $Ce(SO_4)_2$ and heat]: NMR ($CDCl_3$) δ 6.23 (m, 2 H, -CH=CH-), 6.05 (d, J = 2 Hz, 1 H, -C=CH), 5.48 (d, J = 2 Hz, 1 H, -C=CH), 4.45 (d, 1 H, γ-lactone -OCH-), 2.75-3.45 (br m, 5 H, -CH-), 1.18-1.92 (br m, 4 H, $-CH_2-$). The oil solidified on standing at room temperature; subsequent sublimation at 45 °C (1.2 mm) gave an analytical sample of **24** as a white powder: mp 55-56 °C; IR (KBr) 1740 (γ-lactone C=O), 1655 (exocyclic C=C), 1630 cm^{-1} (endocyclic C=C); UV (MeOH) λ_{max} 208 nm (ϵ_{max} 10 334); mass spectrum M^+ (EI) 202 (m/e of M^+ = 202.101 obsd, 202.099 calcd). Anal. ($C_{13}H_{14}O_2$) C, H.

Measurements of the Rate of Cysteine Addition. The rates of cysteine addition to α-methylene γ-lactones were measured following a modified version of the procedure of Kupchan et al.[1b] To 50 mL of 10^{-4} cysteine in 0.067 M phosphate buffer (pH 7.4) under a N_2 atmosphere was added 0.5 mL of a 10^{-2} M THF solution of the lactone. At intervals of approximately 3 min 3.6 mL of the reaction mixture was removed and assayed according to the procedure of Grassetti and Murray using 2,2'-dithiopyridine.[39]

The phosphate buffer was prepared using doubly distilled water. Spectral grade THF and Me_2SO were used and the THF was distilled from $LiAlH_4$ before use. The cysteine and 2,2'-dithiopyridine were obtained from Aldrich Chemical Co.

The results were analyzed using least-squares curve-fitting procedures with all rates based on the best line through at least six points. The lines through the points used all had correlation coefficients of 0.99 assuming second-order kinetics.

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Antitumor Agents. 32.[1] Synthesis and Antitumor Activity of Cyclopentenone Derivatives Related to Helenalin

Kuo-Hsiung Lee,* Eng-Chun Mar, Masao Okamoto, and Iris H. Hall

Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27514.
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Several new cyclopentenones related to helenalin have been synthesized as potential alkylating antitumor agents. The procedure involved the transformation of 2-methyl-2-carbethoxycyclopentanone (**2**) to an ethylene ketal **3**, bromination of **3** followed by dehydrobromination to yield a ketal olefin **5**, reduction of **5** to the alcohol **6**, conversion of **6** to the corresponding hydroxycyclopentenone **7**, and esterification of **7** to afford the cyclopentenone esters **8**-**11**. Biological assays indicated that only cyclopentenones possessing a conjugated ester side chain, such as **9** and **10**, demonstrated significant in vitro cytotoxicity against the growth of tissue culture cells originating from human epidermoid carcinoma of the larynx (H.Ep.-2) as well as in vivo antitumor activity in Walker 256 carcinosarcoma in rats and P-388 lymphocytic leukemia in mice.

Previous papers of this series demonstrated that a β-unsubstituted cyclopentenone ring of helenalin (**1**) related sesquiterpene lactones, such as plenolin and tenulin, contributes significantly to in vitro cytotoxicity (H.Ep.-2)

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