R. S. Pardini, *Biochem. Biophys. Res. Commun.*, **43**, 102 (1971).
(18) J. Boler, R. Pardini, H. Mustafa, K. Folkers, R. Dilley, and F. Crane, *Proc. Nat. Acad. Sci. U. S.*, **69**, 3713 (1972).
(19) K. C. Agrawal, B. A. Booth, and A. C. Sartorelli, *J. Med. Chem.*, 11, 700 (1968).
(20) A. L. Smith, *Methods Enzymol.*, **10**, 81 (1967).
(21) E. Layne, *Methods Enzymol.*, **3**, 447 (1957).
(22) W. K. Anslow, J. N. Ashley, and H. Raistrick, *J. Chem. Soc.*, 439 (1938).
(23) K. Sato, S. Inoue, and H. Sato, *Bull. Chem. Soc. Jap.*, **45**, 3455 (1972).
(24) L. S. Silbert and D. Swern, *J. Amer. Chem. Soc.*, 81, 2364 (1959).

Cysteine Scavengers. 2. Synthetic α-Methylenebutyrolactones as Potential Tumor Inhibitors†

Andre Rosowsky,* Nickolas Papathanasopoulos, Herbert Lazarus, George E. Foley, and Edward J. Modest

The Children's Cancer Research Foundation and the Departments of Biological Chemistry and Pathology, Harvard Medical School, Boston, Massachusetts 02115. *Received December 19, 1973*

Synthetic α-methylenebutyrolactone derivatives related to the naturally occurring antitumor sesquiterpene lactones of plant origin were synthesized as potential experimental antitumor agents on the basis of their possible action as cysteine scavengers. Although these compounds were cytotoxic to cysteine-requiring human lymphoblastic leukemia cells (CCRF-CEM) in continuous culture at doses of 10^{-6}–10^{-7} mol/l., they displayed comparable cytotoxicity against normal human lymphoid cells, and their cytotoxic action did not appear to be a consequence of selective cysteine scavenging.

Human lymphoblastic leukemia cells grown in culture have been shown by Foley and coworkers[1] to possess an absolute nutritional requirement for L-cysteine (L-cystine) which is independent of both their state of ploidy and their population density. In contrast, normal human lymphoid cells in culture are capable of maintaining normal growth in the absence of L-cysteine if they are supplied with L-cysteine precursors such as L-serine and L-homocysteine, or L-cystathionine.[2] That the dependence of various lymphoblastic leukemic cell sublines on exogenous L-cysteine is probably a consequence of one or more enzymatic defects in the methionine–cystathionine–cysteine biosynthetic pathway was indicated by experiments demonstrating a marked deficiency in cystathionase levels in such cells.[2] Accordingly, it was suggested[1,3,4] that rational chemotherapeutic approaches based on the concept of "cysteine scavenging" might be possible, since cultured normal cells can apparently biosynthesize L-cysteine whereas lymphoblastic leukemic cells cannot.[1]

In this context, as part of a larger search for classes of chemical agents capable of blocking exogenous L-cysteine uptake by leukemic cells, we were interested in α-methylenebutyrolactones. Compounds of this type are widely prevalent among terpenoid natural products of plant origin[5,6] and are being discovered with continuing regularity.[7-15] They are noted for their cytotoxic properties, which have been ascribed to a rapid and essentially irreversible 1,4-addition reaction involving the SH group of L-cysteine, either as the free amino acid[16,17] or as part of a peptide chain or protein.[18] Susceptibility to attack by amines has also been demonstrated.[19,20] Some plant lactones containing the α-methylenebutyrolactone moiety have aroused interest in recent years on the basis of encouraging antitumor activity *in vitro*, and efforts to deduce meaningful structure–activity correlations have been reported by two independent groups of investigators.[21,22] Papers describing simple synthetic α-methylenebutyrolactones have likewise appeared intermittently in the literature[23-37] but the biological properties of these small molecules have not been studied to nearly the same extent as those of the more complex terpenoid lactones of natural origin.

In searching for cysteine scavengers that could be used therapeutically, we were especially attracted by the possibility that simple synthetic α-methylenebutyrolactones might be relatively nontoxic because they lack the multifunctional character and complex stereochemistry that Kupchan and coworkers[18,21] have suggested may account for the remarkable toxicity of natural products containing the α-methylenebutyrolactone moiety. According to this view, simple α-methylenebutyrolactones with just the proper degree of chemical reactivity might combine irreversibly with intracellular or extracellular cysteine without interacting with complex biological macromolecules such as respiratory enzymes or membrane proteins.

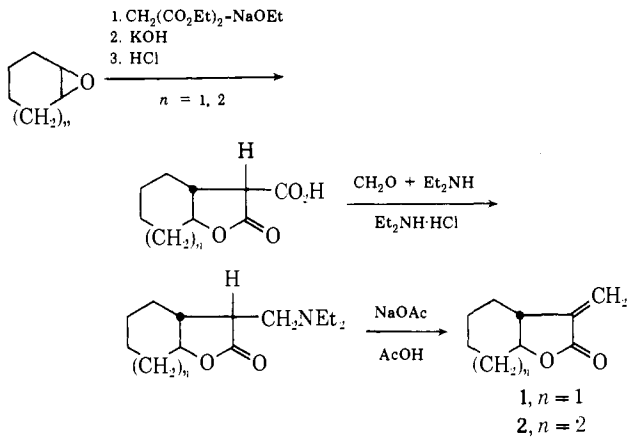
In the work reported herein, a group of nine synthetic α-methylenebutyrolactones was assayed against human lymphoblastic cells in culture. The compounds were found to be more toxic than expected and, in fact, proved to be no less cytotoxic than several complex α-methylenebutyrolactones of natural origin which were evaluated for comparison.

Chemistry. A modified procedure involving a combination of several published routes[27-29] was employed for the preparation of *trans*-9-methylene-7-oxabicyclo[4.3.0]nonan-8-one (**1**) and *trans*-10-methylene-8-oxabicyclo[5.3.0]decan-9-one (**2**) from cyclohexene oxide and cycloheptene oxide, respectively (Scheme I). The epoxides were condensed with diethyl malonate as described by Newman and VanderWerf[38] and the resultant lactone acids were treated with CH_2O and Et_2NH in the presence of $Et_2NH{\cdot}HCl$,[29] and then with NaOAc in glacial AcOH.[28] Overall yields of **1** and **2** from the epoxides were 15–25%. The identity of each product was established by microanalysis and comparison of ir and nmr spectral data with published values.[27]

3-Methylene-1-oxaspiro[4.5]decan-2-one (**3**) and 3-methylene-1-oxaspiro[4.6]undecan-2-one (**4**) were synthesized conveniently *via* a Reformatsky-type reaction between ethyl α-bromomethacrylate[39] and cyclohexanone or cycloheptanone. This novel α-methylenebutyrolactone synthe-

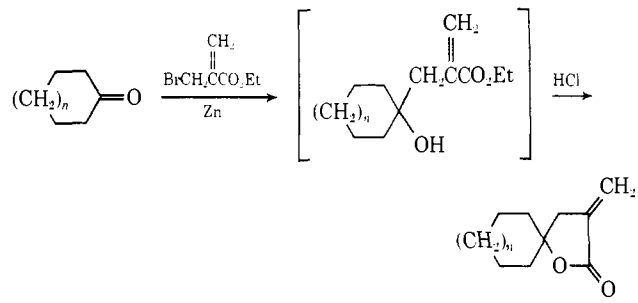
†This work was supported in part by research grant C6516, research career award K6-CA-22,150 (to G. E. F), and research career development award K3-CA-22,150 (to E. J. M) from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md. For paper 1 of this series, see ref 3.

Scheme I



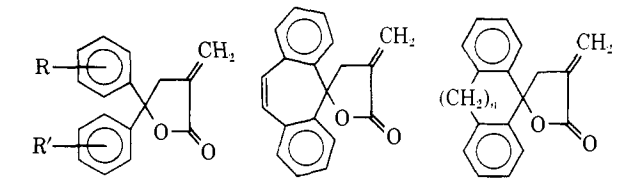
1, $n = 1$
2, $n = 2$

sis has been described recently by Öhler and coworkers with several other ketones and aldehydes[30] and appears to be of general preparative value. Our procedure is adapted from their method, with one minor difference (see Experimental Section) stemming from the observation that heating of the initially formed hydroxy ester is sometimes necessary in order to effect ring closure to the lactone.



3, $n = 1$
4, $n = 2$

Application of the foregoing reaction with benzophenone, 4,4′-dichlorobenzophenone, and 3,4-dimethylbenzophenone led to the *gem*-diaryl α-methylenebutyrolactones **5**–**7**, respectively, in yields of 60–70%. Similar use of 5*H*-dibenzo[*a*,*d*]cyclohepten-5-one, its 10,11-dihydro derivative, and 9-fluorenone yielded the bridged analogs **8** (73%), **9** (94%), and **10** (40%), respectively.‡



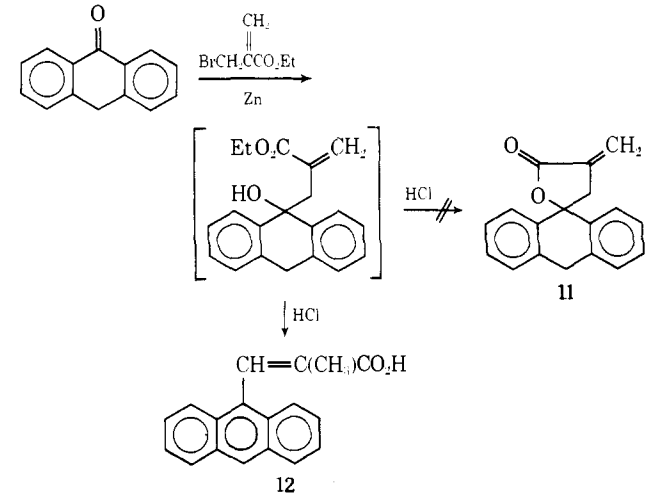
5, R = R′ = H
6, R = R′ = 4-Cl
7, R = 3,4-Me_2; R′ = H

8

9, $n = 2$
10, $n = 0$
11, $n = 1$

Preparation of the α-methylenebutyrolactone **11** from anthrone *via* the ethyl α-bromomethacrylate route was also attempted. However, instead of **11**, an acidic product was isolated (18% yield) whose ir and nmr spectra showed the α-methylenebutyrolactone moiety to be absent, and whose uv spectrum [λ_{max} (EtOH) 255, 331, 347, 364, 383 nm] revealed that aromatization of the anthracene ring had occurred. On the basis of the spectral and microchemical evidence the product was formulated as α-methyl-9-anthraceneacrylic acid (**12**). The failure of **11** to form in the reaction of anthrone and ethyl α-bromomethacrylate may be rationalized on the basis that dehydration and concomitant aromatization is energetically more favorable than lactonization of the initial hydroxy ester adduct (Scheme II) on treatment with acid.

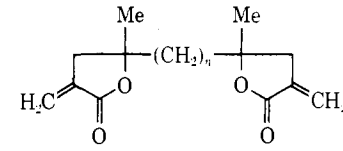
Scheme II



11

12

Lastly, the novel bifunctional α-methylenebutyrolactone **13** was synthesized in 56% yield from 2,5-hexanedione and ethyl α-bromomethacrylate. Lactone **13** was viewed with particular interest as a possible prototype for a new class of "two-armed" reagents reminiscent of the bifunctional epoxides and sulfonate esters (*e.g.*, busulfan).[40]



13, $n = 2$

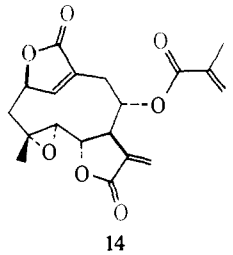
Biological Results and Discussion. Nine of the synthetic lactones described in this paper were evaluated for growth-inhibitory activity against the CCRF-CEM cysteine-dependent human leukemic cell line in continuous culture. The assay procedure has been described previously[41] and is summarized briefly in the Experimental Section. Also tested were three antitumor plant products containing the α-methylenebutyrolactone moiety, namely elephantopin (**14**),[42] vernolepin (**15**),[43] and eupatundin (**16**).[44] Samples of the latter compounds were kindly provided for comparison by Dr. Harry B. Wood, Jr., Drug Development Branch, Division of Cancer Treatment, Drug Research and Development, National Cancer Institute. The data for these compounds as well as the synthetic lactones **1**, **3**–**10**, and **13** are presented in Table I.

All the lactones tested (**1**, **3**–**10**, **13**–**16**) proved to be inhibitory to CCRF-CEM cells at rather low concentrations, in the range of 10^{-6}–10^{-7} *M*. Cytotoxicity against this cell line was of comparable magnitude to that reported by Kupchan and coworkers[21] against human nasopharyngeal carcinoma (KB) cells in culture. Among the synthetic lactones, no particular correlation of structure–activity was evident, other than a possible favorable effect of lipophilic aromatic rings in the molecule. Interestingly, the simple synthetic lactones were approximately as cytotoxic as the

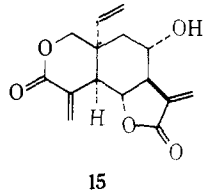
‡Following the completion of this work, an alternative method of preparation of compounds **5** and **10**, based on the classical route to α-methylenebutyrolactones,[25,26] was reported by Dalton and coworkers.[32,33] The physical constants given by these authors are in agreement with our values.

Table I. Cytotoxic Activity of α-Methylenebutyrolactones against Human Lymphoblastic Leukemia (CCRF-CEM) Cells in Culture

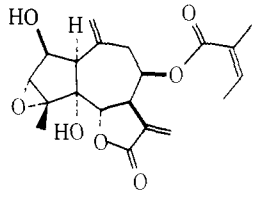
Compd	ID_{50} ($\times 10^{-6}$ mol/l.)	Compd	ID_{50} ($\times 10^{-6}$ mol/l.)
1	5.0	**8**	0.4
3	7.0	**9**	1.6
4	7.0	**10**	0.7
5	0.8	**13**	1.0
6	1.5	**14**	0.2
7	1.3	**15**	1.4
		16	2.0



14



15



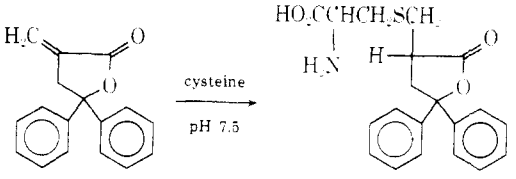
16

more complex natural products. The results supported the tentative conclusion that, in cell culture as opposed to whole animal assays, the α-methylenebutyrolactone moiety is sufficient to confer cytotoxic properties upon the molecule even in the absence of other reactive functionalities.

As a predictive measure of potential therapeutic usefulness, lactones **1**, **3–10**, and **13** were also assayed simultaneously against a line of normal human lymphocytes which did not require exogenous cysteine for growth in continuous culture. A significant difference in cytotoxicty toward normal and leukemic cells did not emerge among this group of agents. It was concluded from these results, as well as the essential observation that cytotoxicity occurred at lactone concentrations well below the level of cysteine in the culture medium, that the mechanism of growth inhibition involves a mechanism other than (or in addition to) scavenging of free cysteine. One possibility is rapid, irreversible S-alkylation of various metabolically essential intracellular proteins (*e.g.*, respiratory or other enzymes), as suggested by the work of Hanson and coworkers[18] on the inhibition of phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, E.C. 2.7.1.11) by plant lactones such as vernolepin (**15**). A second possibility is S-alkylation of sulfhydryl groups in cell surface proteins, as has been suggested recently for the action of 6,6′-dithionicotinic acid and related cytotoxic pyridine disulfides.[45]

As anticipated, reaction of several representative lactones *in vitro* with sulfhydryl compounds did prove to be extremely rapid. For example, treatment of lactone **5** with mercaptoacetic acid in deuteriochloroform solution containing a catalytic amount of triethylamine and periodic examination of the nmr spectrum revealed the complete disappearance of the vinyl proton AB quartet after 4 hr. Similar addition of mercaptoacetic acid to lactone **8** proved to be even more rapid, the vinyl proton nmr signal being no longer discernible after only 20 min.

Direct evidence for the ability of the lactones to form adducts with cysteine itself was also obtained. In a typical instance lactone **5** was allowed to react overnight at room temperature with an equimolar amount of cysteine in aqueous methanol at pH 7.5. A product was isolated in 66% yield whose microanalytical data and spectral properties were consistent with structure **17**.



5 17

The high degree of chemical reactivity of the lactones chosen in this initial study, together with their small molecular size and uncomplicated chemical structure, makes it appear likely that these agents would give rise to nonspecific S-alkylation at many different sites *in vivo*. Future work on the design of such compounds for therapeutic evaluation will need to take greater cognizance of this problem.§

Antitumor assays in tumor-bearing mice are in progress with lactones **4–10** and **13**, and the results will be reported elsewhere.

Experimental Section

Ir spectra were determined with a Perkin-Elmer Model 137B recording spectrophotometer, uv spectra with Cary Model 11 and Model 15 spectrophotometers, and nmr spectra on a Varian A-60 instrument with $CDCl_3$ as the solvent and Me_4Si as the reference. Melting points were measured in Pyrex capillary tubes by means of a Mel-Temp apparatus (Laboratory Devices Inc., Cambridge, Mass.) and are uncorrected. Microanalyses were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn. Found values are within ±0.4% of theory.

trans-**10-Methylene-8-oxabicyclo[5.3.0]decan-9-one** (**2**). To a stirred solution of NaOEt prepared by dissolving Na metal (34 g, 1.48 g-atoms) in absolute EtOH (1 l.) were added dropwise diethyl malonate (226 g, 1.41 mol) and cycloheptene oxide (145 g, 1.29 mol). After being heated under reflux for 24 hr, the mixture was cooled, diluted with H_2O (400 ml), treated with a solution of KOH (150 g) in H_2O (600 ml), and distilled until 1 l. of EtOH was removed. The remaining aqueous mixture was stirred under reflux for 30 min, cooled again, diluted with ice–water, and acidified with concentrated HCl. Extraction with CH_2Cl_2 (1800 ml), drying ($MgSO_4$), and evaporation under reduced pressure left the lactone acid (Scheme I, n = 2) as a yellow oil (154 g). The crude acid was dissolved in dioxane (250 ml), and the solution was cooled while 37% aqueous formalin (200 ml), Et_2NH (190 ml), and Et_2NH·HCl (280 g) were added successively with vigorous stirring. After being allowed to stand for 3 days, the mixture was saturated with solid K_2CO_3 and the product was taken up in CH_2Cl_2. Extraction of the CH_2Cl_2 solution with several portions of 1 *N* HCl, basification of the combined acid extracts to pH 10 with saturated Na_2CO_3, reextraction with CH_2Cl_2, drying ($MgSO_4$), and evaporation under reduced pressure left the amino lactone (Scheme I, n = 2) as a pale yellow oil (107 g). This was dissolved in glacial AcOH (300 ml), anhydrous NaOAc (9 g) was added, and the mixture was refluxed for 5 hr. Evaporation to dryness under reduced pressure, addition of H_2O, extraction with CH_2Cl_2, washing with saturated Na_2CO_3 and saturated NaCl, drying ($MgSO_4$), solvent removal, and vacuum distillation of the residue gave **2** as a colorless liquid (46.4 g, 22% overall yield starting from cycloheptene oxide): bp 100–104° (0.25 mm) [lit.[27] bp 60° (bath temperature) (0.03 mm)].

trans-**9-Methylene-7-oxabicyclo[4.3.0]nonan-8-one** (**1**) was obtained in 15% overall yield from cyclohexene oxide: bp 68–70°

§After this manuscript was written an abstract appeared which described the synthesis of other types of reactive unsaturated lactones as potential antitumor agents on the basis of the plant product analogy.[46]

(0.05 mm) [lit.[27] bp 70° (bath temperature) (0.1 mm)]. Essentially the same procedure was used as in the synthesis of **2** except that MeOH, rather than dioxane, was used in the reaction of the lactone acid (Scheme I, n = 1) with 37% aqueous formalin, Et_2NH, and $Et_2NH \cdot HCl$.

3-Methylene-1-oxaspiro[4.5]decan-2-one (3). A solution of ethyl α-bromomethacrylate (10.6 g, 0.055 mol) in dry THF (30 ml) was added dropwise with stirring to a suspension of Zn metal (3.5 g, 0.054 g-atom) in cyclohexanone (4.9 g, 0.05 mol) and THF (15 ml),‡ the internal temperature being raised gradually to 40–50° during addition. After 1 hr at 50° the mixture was poured into 0.25 *N* HCl (200 ml), and the product was extracted into Et_2O. The Et_2O solution was washed with saturated $NaHCO_3$, rinsed with saturated NaCl, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. The resultant liquid hydroxy ester [ir ν (thin film) 3450 (OH), 1710 (ester C=O), 1640 cm^{-1} (=CH_2, alicyclic)] was dissolved in a mixture of absolute EtOH (450 ml) and concentrated HCl (25 ml) which was refluxed for 30 min, diluted with H_2O (1 l.), neutralized carefully with Na_2CO_3, and extracted with Et_2O. Drying ($MgSO_4$), solvent removal, and vacuum distillation of the oily residue gave 2.6 g (31% overall yield) of colorless liquid: bp 80–85° (0.1 mm); ir ν (thin film) 1750 (five-membered lactone C=O), 1660 cm^{-1} (=CH_2 on a five-membered ring); nmr τ ($CDCl_3$) 3.96 and 4.38 (2 triplets, 2 H, =CH_2),** 7.29 (triplet, 2 H, allylic CH_2),** 8.0–9.0 (10 H, 5 methylenes). Traces of adventitious water are difficult to remove from this compound (and also from the other spirolactone, **4**) despite care in the distillation of the analytical sample. *Anal.* ($C_{10}H_{14}O_2 \cdot 0.5H_2O$) C, H.

3-Methylene-1-oxaspiro[4.6]undecan-2-one (4). A solution of ethyl α-bromomethacrylate (19.3 g, 0.1 mol) in THF (60 ml) was added dropwise to a suspension of Zn metal (6.54 g, 0.1 g-atom) in a mixture of cycloheptanone (11.2 g, 0.1 mol) and THF (60 ml) under reflux. When addition was complete, the mixture was stirred under reflux for another hour, then cooled, treated with 2% HCl (200 ml), and refluxed again for 30 min. Extraction with CH_2Cl_2, washing with 5% $NaHCO_3$, rinsing with H_2O, drying ($MgSO_4$), solvent removal, and vacuum distillation of the yellow residue yielded analytically pure material (6 g, 33%): bp 82–83° (0.005 mm); ir ν (thin film) 1770, 1660 cm^{-1}; nmr τ ($CDCl_3$) 3.80 and 4.38 (2 triplets, 2 H, =CH_2), 7.25 (triplet, 2 H, allylic CH_2), 8.0–8.7 (12 H, 6 methylenes). *Anal.* ($C_{11}H_{16}O_2 \cdot 0.1H_2O$) C, H.

2-Methylene-4,4-diphenylbutyrolactone (5). A solution of ethyl α-bromomethacrylate (19.3 g, 0.1 mol) in dry THF (60 ml) was added dropwise to a refluxing mixture of benzophenone (18.2 g, 0.1 mol) and Zn metal (6.54 g, 0.1 g-atom) in THF (60 ml). After being stirred under reflux for 3 hr, the mixture was cooled to room temperature, treated with 3% HCl (200 ml), and extracted with Et_2O. Drying ($MgSO_4$), solvent removal, and crystallization of the residue from EtOH gave colorless prisms (18 g, 72%): mp 101–103° (lit.[32] mp 109°); ir ν (KCl) 1770, 1670 cm^{-1}; nmr τ ($CDCl_3$) 2.50–2.85 (10 H, aromatic protons), 3.74 and 4.37 (2 triplets, 2 H, =CH_2), 6.37 (triplet, 2 H, allylic CH_2). *Anal.* ($C_{17}H_{14}O_2$) C, H.

4,4-Bis(*p*-chlorophenyl)-2-methylenebutyrolactone (6). The reaction of 4,4′-dichlorobenzophenone with ethyl α-bromomethacrylate and Zn metal was performed as in the preceding experiment, except that refluxing was extended to 4 hr and HCl treatment was followed by extraction with CH_2Cl_2 instead of Et_2O. The crude product (4.5 g, 71%) was recrystallized from petroleum ether (bp 30–50°) in the form of colorless prisms: mp 60–62°; ir ν (KCl) 1780, 1670 cm^{-1}; nmr τ ($CDCl_3$) 2.7 (singlet, 8 H, aromatic protons), 3.70 and 4.30 (2 triplets, 2 H, =CH_2), 6.42 (triplet, 2 H, allylic CH_2). *Anal.* ($C_{17}H_{12}Cl_2O_2$) C, H, Cl.

2-Methylene-4-phenyl-4-(3′,4′-xylyl)butyrolactone (7). The reaction of 3,4-dimethylbenzophenone with ethyl α-bromomethacrylate and Zn metal was carried out as above. The crude product was triturated with EtOH at −60° (Dry Ice–acetone), the EtOH was decanted, and the semisolid residue was triturated with petroleum ether (bp 30–50°) until crystallization occurred. Recrystallization of this material, representing a 61% yield, from EtOH gave small colorless needles: mp 60–62°; ir ν (KCl) 1770, 1670 cm^{-1}; nmr τ ($CDCl_3$) 2.5–3.0 (8 H, aromatic protons), 3.75 and 4.35 (2 triplets, 2 H, =CH_2), 6.39 (triplet, 2 H, allylic protons), 7.78 (singlet, 6 H, Me protons). *Anal.* ($C_{19}H_{18}O_2 \cdot 0.3H_2O$) C, H.

3-Methylene-6,7:10,11-dibenz-1-oxaspiro[4.6]undec-8-en-2-one (8). Reaction of 5*H*-dibenzo[*a,d*]cyclohepten-5-one with ethyl α-bromomethacrylate and Zn metal was carried out as above, except that the crude product was triturated with a small volume of warm EtOH prior to recrystallization from EtOH, which gave colorless needles (73% yield): mp 166–168°; ir ν (KCl) 1760, 1670 cm^{-1}; nmr τ ($CDCl_3$) 2.0–3.0 (multiplet, 10 H, aromatic and CH=CH protons), 4.00 and 4.55 (2 triplets, 2 H, =CH_2), 6.80 (triplet, 2 H, allylic CH_2). *Anal.* ($C_{19}H_{14}O_2$) C, H.

3-Methylene-6,7:10,11-dibenz-1-oxaspiro[4.6]undecan-2-one (9). Reaction of 10,11-dihydro-5*H*-dibenzo[*a,d*]cycloheptan-5-one with ethyl α-bromomethacrylate and Zn metal was carried out as in the preceding experiment except that the crude product (94% yield) was recrystallized from petroleum ether (bp 30–50°) directly: colorless needles; mp 172–174°; ir ν (KCl) 1770, 1665 cm^{-1}; nmr τ ($CDCl_3$) 2.2–2.9 (multiplet, 8 H, aromatic protons), 3.70 and 4.41 (2 triplets, 2 H, =CH_2), 6.39 (triplet, 2 H, allylic CH_2), 6.75 (A_2B_2 multiplet, 4 H, CH_2CH_2). *Anal.* ($C_{19}H_{16}O_2$) C, H.

3-Methylene-6,7:8,9-dibenz-1-oxaspiro[4.4]nonan-2-one (10) was obtained in 40% yield from 9-fluorenone, ethyl α-bromomethacrylate, and Zn metal: colorless needles; mp 142–143° (lit.[33] mp 142°); ir ν (KCl) 1770, 1665 cm^{-1}; nmr τ ($CDCl_3$) 2.2–2.8 (multiplet, 8 H, aromatic protons), 3.54 and 4.18 (2 triplets, 2 H, =CH_2), 6.61 (triplet, 2 H, allylic CH_2). *Anal.* ($C_{17}H_{12}O_2$) C, H.

α-Methyl-9-anthraceneacrylic Acid (12). A solution of ethyl α-bromomethacrylate (10 g, 0.052 mol) in THF (60 ml) was added dropwise to a refluxing mixture of anthrone (9.5 g, 0.049 mol) and Zn metal (3.5 g, 0.054 g-atom) in THF (40 ml). After being stirred under reflux for 5 hr, the mixture was cooled to room temperature, treated with 3% HCl (200 ml) for 5 min, and extracted with Et_2O. Drying ($MgSO_4$) and solvent removal left an orange oil which was dissolved partially by trituration with boiling 5% KOH. The insoluble portion was filtered off, the filtrate was treated with charcoal and acidified, and the resultant solid was filtered. Recrystallization from EtOH gave needles (2.3 g, 18%): mp 188–191° dec; ir ν (KCl) 3400–2500 (br), 1700, 1660 cm^{-1}; nmr τ (DMSO-d_6) 1.4 (singlet, 1 H, C-10 aromatic proton), 1.7–2.6 (multiplet, 8 H, C-1 to C-8 aromatic protons), 3.95 (doublet, J = 1 Hz, 1 H, -CH=), 5.36 (singlet, 3 H, =C(Me)CO_2H). *Anal.* ($C_{18}H_{14}O_2 \cdot 0.2H_2O$) C, H.

1,2-Bis(2-methylene-4-methyl-4-butyrolactonyl)ethane (13). The reaction of 2,5-hexanedione with ethyl α-bromomethacrylate and Zn metal was carried out as described above, except that the crude product was triturated with H_2O and then petroleum ether (bp 30–50°) before being recrystallized from 1:4 EtOH–H_2O: colorless needles (56% yield); double mp 73–80° and 100–102°; ir ν (KCl) 1760, 1665 cm^{-1}; nmr τ ($CDCl_3$) 3.76 and 4.33 (2 triplets, 4 H, =CH_2), 7.22 (triplet, 4 H, allylic CH_2), 8.23 (singlet, 4 H, CH_2CH_2), 8.59 (singlet, 6 H, Me protons). *Anal.* ($C_{14}H_{18}O_4$) C, H.

Reaction of Lactone 5 with Cysteine. Lactone **5** (5.0 g, 0.02 mol) and cysteine hydrochloride monohydrate (3.5 g, 0.02 mol) were suspended in 50% aqueous MeOH (100 ml), the pH was adjusted to approximately 7.5 (indicator paper) by dropwise addition of 1 *N* NaOH (30 ml), and the mixture was stirred 18 hr at room temperature under nitrogen. After dilution with MeOH (100 ml) and water (100 ml) the gelatinous precipitate was collected, washed generously with MeOH, rinsed with Et_2O, and dried to a white powder (4.9 g, 66%). The analytical sample, mp 185–190° (effervescing), was prepared by digesting a portion of this material with boiling water and then with 95% EtOH, rinsing with Et_2O, and drying for 48 hr at 90° (0.05 mm) over P_2O_5. *Anal.* ($C_{20}H_{21}NO_4S$) C, H, N, S.

Cell Culture Assay. In this assay,[41] actively dividing CCRF-CEM cells are prepared by suspending cells in Eagle's minimal essential medium (MEM) supplemented with 10% whole fetal calf serum and incubating at 37° to give a final cell population of approximately 5 × 10^5 cells per milliliter of medium at the time of addition of the test compound. The test compound is dissolved in MEM, with the aid of some DMSO if necessary, and ID_{50} values are determined from plots of mean cell counts at 48 hr. Assays are carried out in replicate series with appropriate controls.

‡The Zn metal used in this and other ethyl α-bromomethylacrylate reactions was granular 20 mesh grade and was acid-washed as prescribed for the Reformatsky reaction.[47] The THF was dried over Linde 3A molecular sieves for several days prior to use.

**All =CH_2 and allylic CH_2 triplets reported in this paper had $J \sim 2.5$ Hz, in agreement with the published literature (see, for example, ref 27).

Acknowledgment. The authors are indebted to Mrs. S. O. Oppenheim, Laboratories of Cellular Physiology, The Children's Cancer Research Foundation, for performing the *in vitro* assays reported herein.

References

(1) G. E. Foley, E. F. Barell, R. A. Adams, and H. Lazarus, *Exp. Cell Res.*, **57**, 129 (1969).
(2) H. Lazarus, E. F. Barell, and S. O. Oppenheim, manuscript in preparation.
(3) M. Israel, M. A. Whitesell, E. J. Modest, G. E. Foley, H. Lazarus, and F. Bergel, *J. Med. Chem.*, **15**, 337 (1972).
(4) M. Israel, E. J. Modest, G. E. Foley, H. Lazarus, and F. Bergel, *Proc. Amer. Ass. Cancer Res.*, **13**, 7 (1972).
(5) S. M. Kupchan, *Pure Appl. Chem.*, **21**, 227 (1970).
(6) T. A. Geissman and M. A. Irwin, *Pure Appl. Chem.*, **21**, 167 (1970).
(7) R. W. Doskotch and C. D. Hufford, *J. Org. Chem.*, **35**, 486 (1970).
(8) R. W. Doskotch and F. S. El-Feraly, *J. Org. Chem.*, **35**, 1928 (1970).
(9) S. M. Kupchan, M. Maruyama, R. J. Hemingway, J. C. Hemingway, S. Shibuya, T. Fujita, P. D. Cradwick, A. D. U. Hardy, and G. A. Sim, *J. Amer. Chem. Soc.*, **93**, 4915 (1971).
(10) S. M. Kupchan, V. H. Davies, T. Fujita, M. R. Cox, and R. F. Bryan, *J. Amer. Chem. Soc.*, **93**, 4916 (1971).
(11) K.-H. Lee, D. C. Anuforo, E.-S. Huang, and C. Piantadosi, *J. Pharm. Sci.*, **61**, 626 (1972).
(12) K.-H. Lee, H.-C. Huang, E.-S. Huang, and H. Furukawa, *J. Pharm. Sci.*, **61**, 629 (1972).
(13) K.-H. Lee, H. Furukawa, and E.-S. Huang, *J. Med. Chem.*, **15**, 609 (1972).
(14) K.-H. Lee, R. Meck, C. Piantadosi, and E.-S. Huang, *J. Med. Chem.*, **16**, 299 (1973).
(15) S. M. Kupchan, T. Fujita, M. Maruyama, and R. W. Britton, *J. Org. Chem.*, **38**, 1260 (1973).
(16) S. M. Kupchan, D. C. Fessler, M. A. Eakin, and T. J. Giacobbe, *Science*, **168**, 376 (1970).
(17) S. M. Kupchan, T. J. Giacobbe, I. S. Krull, A. M. Thomas, M. A. Eakin, and D. C. Fessler, *J. Org. Chem.*, **35**, 3539 (1970).
(18) R. L. Hanson, H. A. Lardy, and S. M. Kupchan, *Science*, **168**, 378 (1970).
(19) S. V Hiremath, G. H. Kulkarni, G. R. Kelkar, and S. C. Bhattacharyya, *Indian J. Chem.*, **6**, 339 (1968).
(20) T. Kawamata and S. Inayama, *Chem. Pharm. Bull.*, **19**, 643 (1971).
(21) S. M. Kupchan, M. A. Eakin, and A. M. Thomas, *J. Med. Chem.*, **14**, 1147 (1971).
(22) K.-H. Lee, E.-S. Huang, C. Piantadosi, J. S. Pagano, and T. A. Geissman, *Cancer Res.*, **31**, 1649 (1971).
(23) C. J. Cavallito and T. H. Haskell, *J. Amer. Chem. Soc.*, **68**, 2332 (1946).
(24) E. R. H. Jones, T. Y. Shen, and M. C. Whiting, *J. Chem. Soc.*, 230 (1950).
(25) E. E. Van Tamelen and S. R. Bach, *J. Amer. Chem. Soc.*, **77**, 4683 (1955).
(26) E. E. Van Tamelen and S. R. Bach, *J. Amer. Chem. Soc.*, **80**, 3079 (1958).
(27) J. A. Marshall and N. Cohen, *J. Org. Chem.*, **30**, 3475 (1965).
(28) J. Martin, P. C. Watts, and F. Johnson, *Chem. Commun.*, 27 (1970).
(29) E. S. Behare and R. B. Miller, *Chem. Commun.*, 402 (1970).
(30) E. Öhler, K. Reininger, and U. Schmidt, *Angew. Chem., Int. Ed. Engl.*, **9**, 457 (1970).
(31) J. W. Patterson and J. E. McMurry, *Chem. Commun.*, 488 (1971).
(32) L. K. Dalton and B. C. Elmes, *Aust. J. Chem.*, **25**, 625 (1972).
(33) L. K. Dalton, B. C. Elmes, and B. V. Kolczynski, *Aust. J. Chem.*, **25**, 633 (1972).
(34) K. Yamada, M. Kato, and Y. Hirata, *Tetrahedron Lett.*, 2745 (1973).
(35) P. F. Hudrlik, L. R. Rudnick, and S. H. Korzeniowski, *J. Amer. Chem. Soc.*, **95**, 6848 (1973).
(36) P. A. Grieco and K. Hiroi, *J. Chem. Soc., Chem. Commun.*, 500 (1973).
(37) P. A. Grieco and M. Kiyachita, *J. Org. Chem.*, **39**, 120 (1974).
(38) M. S. Newman and C. A. VanderWerf, *J. Amer. Chem. Soc.*, **67**, 233 (1945).
(39) A. F. Ferris, *J. Org. Chem.*, **20**, 780 (1955).
(40) J. A. Montgomery, T. P. Johnston, and Y. F. Shealy in "Medicinal Chemistry," 3rd ed, Part I, A. Burger, Ed., Wiley-Interscience, New York, N. Y., 1970, pp 698–700.
(41) G. E. Foley and H. Lazarus, *Biochem. Pharmacol.*, **16**, 659 (1967).
(42) S. M. Kupchan, Y. Aynehchi, J. M. Cassady, H. K. Schnoes, and A. L. Burlingame, *J. Org. Chem.*, **34**, 3867 (1969).
(43) S. M. Kupchan, R. J. Hemingway, D. Werner, and A. Karim, *J. Org. Chem.*, **34**, 3903 (1969).
(44) S. M. Kupchan, J. E. Kelsey, M. Maruyama, J. M. Cassady, J. C. Hemingway, and J. R. Knox, *J. Org. Chem.*, **34**, 3876 (1969).
(45) D. R. Grassetti and J. F. Murray, Jr., *Biochem. Pharmacol.*, **19**, 1836 (1970).
(46) G. A. Howie, P. E. Manni, and J. M. Cassady, Abstracts, 166th National Meeting of the American Chemical Society, Chicago, Ill., Aug 27, 1973, MEDI-1.
(47) R. L. Shriner, *Org. React.*, **1**, 1 (1942).

Potential Antimalarials. 8. Some 10-Substituted 9-Phenanthrenemethanols[1,†]

Lee C. Washburn and D. E. Pearson*

Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37235. *Received June 26, 1972*

Starting with phenanthrene or fluorenone, a series of 10-substituted and 2,7,10-trisubstituted 9-phenanthrenemethanols has been synthesized and tested for antimalarial activity by the Rane *Plasmodium berghei* test in mice. 2,7-Dibromo- (and dichloro-) 10-methoxy-9-(2-dibutylamino-1-hydroxyethyl)phenanthrene hydrochloride, which showed 4/5 cures and 2/5 cures, respectively, at 80 mg/kg, were the most active compounds tested. Selective butyllithium exchange with the 10-bromine atoms of 2,7,10-tribromo-9-methoxy- and -9-methylphenanthrene is reported.

The revived interest in 9-phenanthrenemethanols as curative agents in malaria led to the synthesis of a large number of these compounds, mostly *via* a Perkin condensation followed by a Pschorr ring closure.[2] It was our purpose to develop new routes to 9-phenanthrenemethanols *via* phenanthrenes or other simple polynuclear compounds which would avoid ring closures and lead to antimalarials not available by the ordinary method of synthesis.

Earlier studies on 2-*p*-chlorophenyl-7-quinolinemethanols[3] indicated that antimalarial activity was enhanced by flanking the 2-dialkylamino-1-hydroxyethyl side chain with halogens. Thus, 9-phenanthrenemethanols substituted at the 10 position were of special interest.‡

† Contribution No. 1251 to the Army Research Program in Malaria.

‡ To completely confirm or refute the earlier lead in the 9-phenanthrenemethanol series, substituents at both the 10 and 8 positions should be present.