Synthesis of Mono- and Bifunctional α-Methylene Lactone Systems as Potential Tumor Inhibitors[1a]

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Synthetic mono- and bifunctional α-methylene lactone derivatives including deoxyvernolepin and dihydrodeoxyvernolepin were tested as inhibitors of the growth of CCRF-CEM human lymphoblastic leukemia cells in culture. The range of ID_{50} values for compounds 1–7 (ca. 10^{-5}–10^{-6} M) was roughly comparable to the doses observed earlier in the CCRF-CEM cell system with synthetic α-methylene-γ-butyrolactones. Of significance is that dihydrodeoxyvernolepin and deoxyvernolepin were at least an order of magnitude more active than natural vernolepin.

The potent cytotoxic action of many sesquiterpene plant products and their ability to inactivate certain selected enzymes in vitro have been attributed to the presence of an α-methylenebutyrolactone moiety.[2] The extreme ease with which this functionality reacts with thiols and other biological nucleophiles is well documented[3-5] and is in fact sufficient to allow these compounds to be viewed as a special class of naturally occurring alkylating agents, though the exact biological role these agents might have in plants remains undefined.

Interest in α-methylenebutyrolactone derivatives as medicinal agents has been stimulated by the possibility that some of them might show enough selective toxicity against neoplastic cells to be of therapeutic value as anticancer agents. A number of sesquiterpenes containing the α-methylenebutyrolactone moiety have shown high levels of cytotoxicity against tumor cells in vitro,[6-11] and studies have been made which suggest that an important requirement for biological activity is the simultaneous presence of more than one chemically reactive group in the molecule (e.g., a five- or six-membered α-methylene lactone, an α,β-unsaturated ester, or an epoxide).[7] It has also been demonstrated,[9] however, that synthetic α-methylenebutyrolactone derivatives containing no other reactive functional groups can have growth-inhibitory activity comparable to that of multifunctional products. The advent of versatile new synthetic methods for the preparation of α-methylenebutyrolactones[12] has now made it possible to evaluate the therapeutic potentialities of many more compounds of this type than has been possible previously.

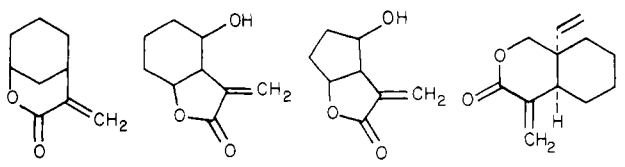
In the work described here, nine heretofore unknown α-methylene lactone derivatives (1–9) were synthesized and tested as inhibitors of the growth of CCRF-CEM human lymphoblastic leukemia cells in culture.[13] These cells have been characterized[14] as having an absolute nutritional requirement for exogenous L-cysteine, stemming from a deficiency of the enzyme cystathionase which in normal cells converts L-cystathionine into L-cysteine, α-ketobutyrate, and ammonia. In the presence of a "cysteine scavenger" (i.e., a molecule capable of reacting irreversibly with L-cysteine), cells that contain cystathionase can grow in an L-cysteine-free medium because they can manufacture all the L-cysteine they need from L-cystathionine.[13,14] Cystathionase-deficient cells lack this ability and therefore cannot grow in a medium that has not been supplemented with L-cysteine. The use of CCRF-CEM cells to assay the growth-inhibitory properties of compounds 1–9 was based on the reasonable expectation that these α-methylenebutyrolactones and α-methylenevalerolactones would be powerful Michael acceptors and would therefore be able to function as "cysteine scavengers".[9]

Table I. Growth Inhibition of CCRF-CEM Human Lymphoblastic Leukemia Cells in Culture by Unsaturated Lactones

Compound	ID_{50}, μM[a] Expt 1	Expt 2
4-Hydroxy-3,4-dimethyl-2-methylenepentanoic acid lactone (1)	21	
2-(*cis*-3-Hydroxycyclohexyl)propenoic acid lactone (2)	16	
2-(*cis,cis*-2,6-Dihydroxycyclohexyl)propenoic acid lactone (3)	15	
2-(*endo*-3-Hydroxymethylbicyclo[2.2.1]heptan-*endo*-3-yl)propenoic acid lactone (4)	7.0	
2-(*cis,cis*-2,5-Dihydroxycyclopentyl)propenoic acid lactone (5)	2.7	
2,2′-(*trans*-2-Hydroxy-*cis,cis*-1,3-cyclohexyl)dipropenoic acid monolactone monomethyl ester (6)	2.1	
2-(*cis*-2-Hydroxymethyl-*trans*-2-vinylcyclohexyl)propenoic acid lactone (7)	0.68	
Dihydrodeoxyvernolepin (8)	0.084	0.034
Deoxyvernolepin (9)	0.083	0.034
Vernolepin (10)[b]		0.43

[a] Assays were carried out as previously described.[9,13] [b] A sample of this compound was kindly provided by Professor S. M. Kupchan, University of Virginia, Charlottesville, Va.

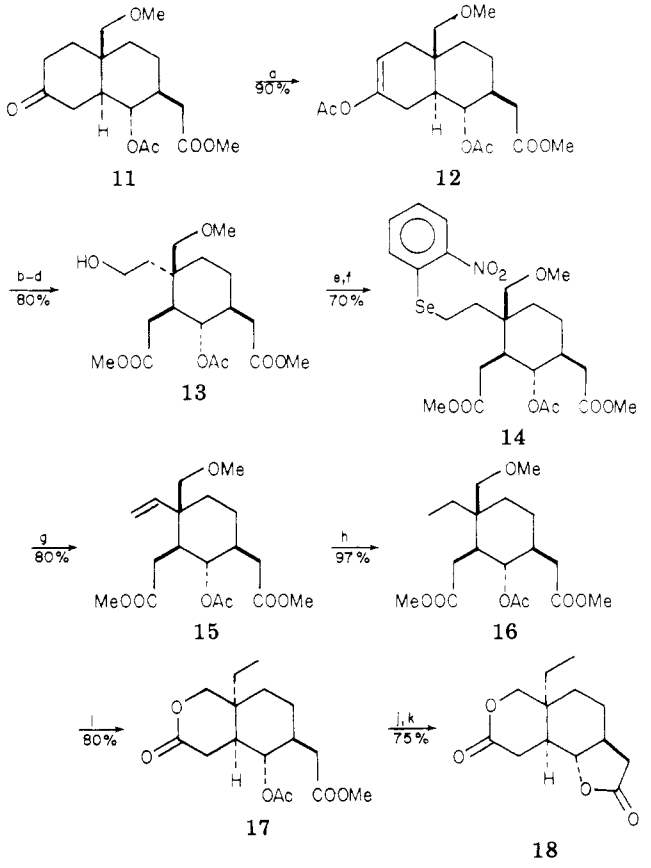
Chemistry. The majority of α-methylene lactones reported in Table I were prepared according to procedures developed in our laboratory. The synthesis of the novel 1,3-fused α-methylene-δ-valerolactone **2**,[15] as well as the synthesis of the oxygenated α-methylene-γ-butyrolactones **3** and **5**,[16] and the vernolepin AB model **7**[17] has recently been published in detail and will not be reviewed here.



2 **3** **5** **7**

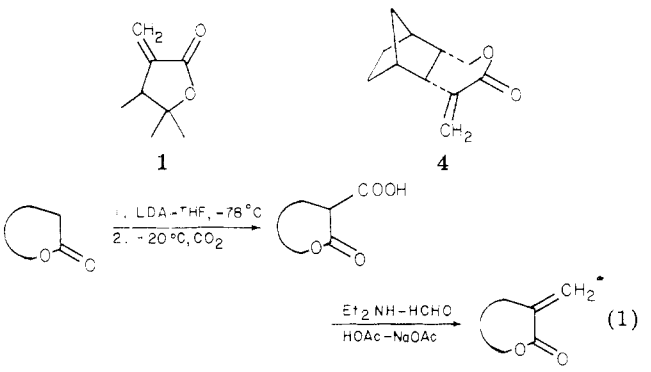
The α-methylene-γ-butyrolactone 1 and the fused bicyclic α-methylene-δ-valerolactone 4 were prepared via α-carboxylation[18] of their corresponding lactone enolates followed by decarboxylative methylenation (eq 1). The

Scheme I. Synthesis of Bisnordihydrodeoxyvernolepin (18)



11 12 13 14 15 16 17 18

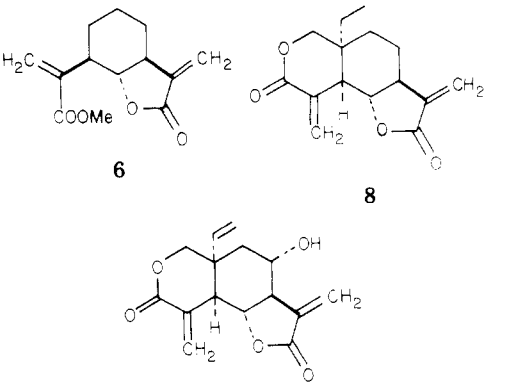
a, $CH_2=C(OAc)CH_3$, TsOH; b, O_3, CH_2Cl_2; c, BH_4^-, OH^-, MeOH; d, CH_2N_2; e, MsCl, Py, 5 °C; f, o-$NO_2C_6H_4SeCN$, BH_4^-, DMF; g, 50% H_2O_2, THF; h, H_2, Pd/C; i, BBr_3, CH_2Cl_2, −78 to −20 °C; j, K_2CO_3, MeOH; k, TsOH, C_6H_6, reflux.



1 4

overall yields of 1 and 4 from their preformed lactones were 71 and 85%, respectively (see Experimental Section).

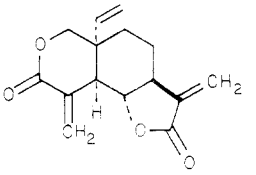
It appeared to us that the bifunctional bis-α-methylenated systems 6 and 8 (dihydrodeoxyvernolepin) could be easily constructed via bis-α-hydroxymethylation of appropriate dienolate systems followed by β elimination. The recently reported total synthesis of vernolepin (10)[19] established the feasibility of bis-α-methylenation utilizing the α-hydroxymethylation procedure.[20] The tricyclic dilactone 18 needed for the synthesis of dihydrodeoxyvernolepin (8) was prepared as outlined in Scheme I. The known decalone 11[21] was treated under thermodynamically controlled conditions with isopropenyl acetate containing p-toluenesulfonic acid at reflux. After 9 h, a near quantitative yield of enol acetate 12 was obtained. It was indeed to our advantage that none of the $\Delta^{3,4}$-enol acetate (steroid numbering) was produced. Apparently the



6 8 10

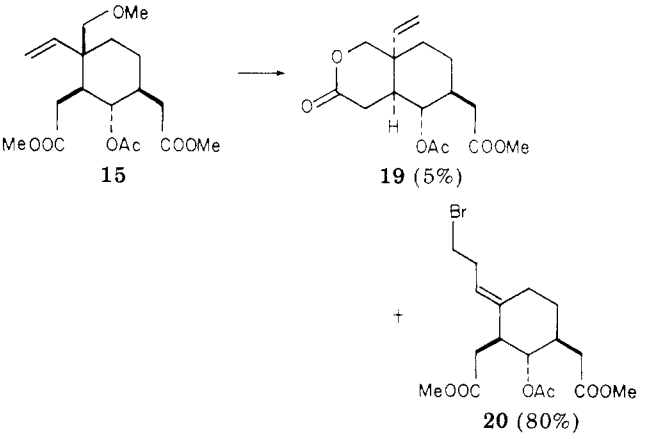
presence of the α-acetoxy function at C-6 interacts unfavorably with the C-4 hydrogen of the $\Delta^{3,4}$ double bond. We have observed similar effects with other α-substituents at C-6.

Ozonolysis of enol acetate 12 followed by reductive work-up (sodium borohydride, sodium hydroxide) and esterification with ethereal diazomethane provided an 80% yield of the hydroxyethyl compound 13. As indicated in Scheme I compound 13 was converted into the olefinic diester 15 via elimination of the o-nitrophenyl selenoxide derived from selenide 14.[22] It was our initial hope that 15 would serve as a key intermediate for deoxyvernolepin (9)



9

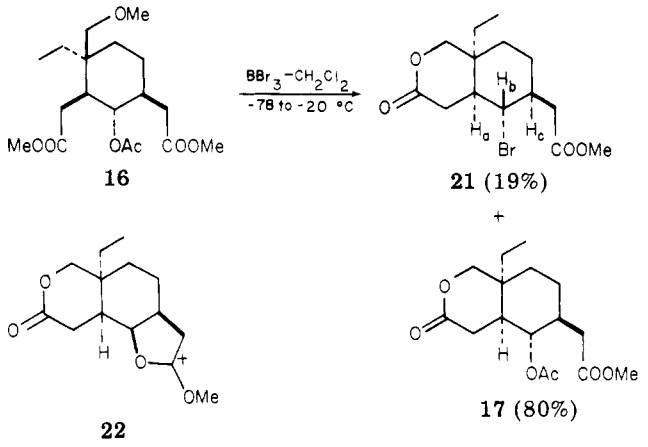
as well as dihydrodeoxyvernolepin (8). This was not to be the case since demethylation of 15 with boron tribromide gave the desired lactone 19 in ca. 5% yield and the rearranged homoallylic bromide 20 as the major product (80%).[23] Many attempts were made to alter the product ratios; however, they were unsuccessful.



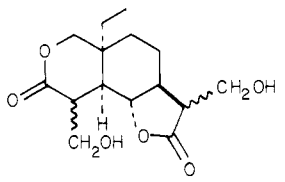
15 19 (5%) 20 (80%)

On the other hand, catalytic hydrogenation of the vinyl substituent provided the dihydro derivative 16 which was demethylated (80%) using excess boron tribromide in methylene chloride at temperatures below −20 °C. Cleavage of the methyl ether was accompanied by simultaneous lactonization to 17. In addition, a bromine containing δ-lactone 21 was isolated (19%) as a crystalline compound, mp 113–114 °C. Appreciable amounts of compound 21 are produced when the reaction is carried out at 0 °C. We speculate that compound 21 is formed

via the intermediacy of species **22**. Support for incorporation of a bromine atom was provided by high-resolution mass spectral data which indicated M^+ and M + 2 ions of roughly equal intensity. Evidence that the bromine atom was introduced with complete retention of configuration was obtained by examination of the NMR[24] spectrum of **21** which exhibited a triplet centered at δ 4.12 for H_b with $J_{ab} = J_{bc} = 11$ Hz.



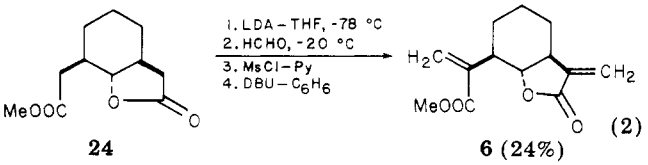
With the dihydrodeoxy derivative **17** in hand, we focused our attention on its conversion to the tricyclic dilactone **18**. Treatment of **17** with anhydrous potassium carbonate in methanol provided a hydroxy ester which was smoothly transformed into dilactone **18**. Bis-α-hydroxymethylation of dilactone **18** in tetrahydrofuran containing 10% hexamethylphosphoramide gave the bis-α-hydroxymethylated adduct **23**. The use of hexamethylphosphoramide was essential in order to solubilize the dilactone enolate. In the absence of hexamethylphosphoramide only very poor yields of adduct **23** could be realized. Treatment of the mesylate derived from **23** with pyridine at elevated



23

temperatures afforded crystalline dihydrodeoxyvernolepin (**8**), mp 146–147 °C. The deoxyvernolepin (**9**) employed in the bioassay was synthesized from its corresponding tricyclic dilactone[21] employing the above bis-α-methylenation sequence.

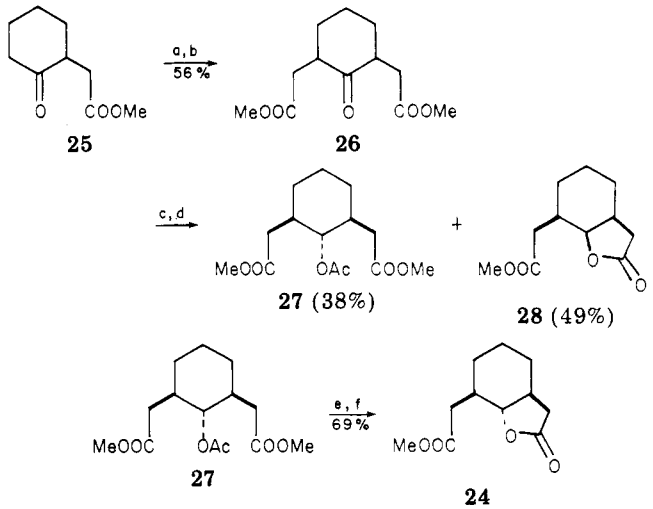
A modified bis-α-methylenation sequence was applied to the ester lactone system **24** (eq 2) which did not require the use of hexamethylphosphoramide during dienolate formation and employed 1,5-diazabicyclo[5.4.0]undec-5-ene in benzene at room temperature for the β-elimination step, thus avoiding the use of refluxing pyridine. The preparation of intermediate **24** is outlined in Scheme II.



24 **6** (24%) (2)

Methyl 2-oxocyclohexaneacetate (**25**), prepared by reaction of the pyrrolidineenamine of cyclohexanone with methyl bromoacetate,[25] was converted to its pyrrolidineenamine. Treatment of the latter enamine with methyl bromoacetate afforded dimethyl 2-oxo-1,3-cyclohexanediacetate (**26**). Reduction of the oxo function in compound **26** was followed by treatment with acetic anhydride in pyridine in order to facilitate separation of the *all-cis*-lactone **28** from the desired all-trans compound **27**. Methanolysis of the acetate function and subsequent lactonization gave the ester lactone **24**.

Scheme II. Synthesis of Ester Lactone 24



a, pyrrolidine; b, $BrCH_2COOMe$; c, BH_4^-, MeOH; d, Ac_2O, Py; e, K_2CO_3, MeOH; f, TsOH, C_6H_6.

Bioassay Results. The growth-inhibitory activity of compounds **1**–**9** against CCRF-CEM cells in culture is shown in Table I. It is of interest to note that the compounds used in this work were of two types. Compounds **8** and **9** could be viewed as close structural analogues of the natural product vernolepin (**10**). The other compounds exemplify a broad range of molecular structure bearing no special relationship to vernolepin with the possible exception of compound **7** which represents the A and B rings of the natural product.

The range of ID_{50} values for compounds **1**–**7** (ca. 10^{-5}–10^{-6} M) was roughly comparable to the doses observed earlier[9] in the CCRF-CEM cell system with synthetic α-methylenebutyrolactones. Structure–activity correlations are not readily constructed from this list of structures, but a few tentative conclusions may be drawn. It seems clear that the six-membered α-methylenevalerolactone moiety (as in **2** or **4**) is not necessarily less active than the five-membered α-methylenebutyrolactone moiety, despite its larger ring size. The 5.5-fold greater activity of compound **5** relative to compound **3** is also of interest and is perhaps attributable to the increased strain associated with the fusion of two five-membered rings. This suggests that a B-nor analogue of vernolepin might be a worthwhile target for chemical synthesis. Also noteworthy is the moderately high activity of compound **7**, which retains the two-carbon bridgehead substituent of vernolepin but lacks ring C. Compound **7** was three times more active than compound **6** despite the fact that the latter contains two Michael acceptor groups, rather than one.

Of greatest interest, among the data in Table I, is that compounds **8** and **9** were more active than vernolepin by at least one order of magnitude. Since the OH group in ring C of vernolepin is absent in compounds **8** and **9**, it would seem that attention should be focused on this structural feature in designing further analogues. The enhanced activity of the deoxyvernolepins **8** and **9** may be due to improved transport across the cell membrane because of increased lipophilicity or to sterically more

favorable conditions for Michael addition of L-cysteine or other biological nucleophiles to the α,β-unsaturated lactone system.

It should be pointed out that all the lactones in Table I had ID_{50} values of less than 3×10^{-5} M, whereas the concentration of L-cysteine in the medium was 1×10^{-4} M (a tenfold difference). Thus, the activity of these compounds cannot be due only to cysteine scavenging. Indeed, if a compound were inhibiting cell growth only via this mechanism, its ID_{50} value would presumably have to be in excess of 5×10^{-5} M. In order to test this point, compounds **1–3** (the only ones whose ID_{50} values were greater than 1×10^{-5} M) were assayed against a cell line (CCRF-SLT) known to contain cystathionase and capable of growth in a medium supplemented with cystathionine. Since the growth-inhibitory effect of compounds **1–3** against CCRF-SLT cells was the same in the presence or the absence of cystathionine in the medium, it would appear that these lactones are not acting as cysteine scavengers to any significant degree. Rather, as has been suggested by other workers, these potent Michael acceptors may be acting via alkylation of thiol and other nucleophilic groups on enzyme molecules.[4] Reaction with thiol groups on the cell surface[26] has also been proposed as a possible mechanism of action for these compounds.[9]

Experimental Section

Melting points were determined on a Fisher-Johns hot-stage melting point apparatus. All melting points and boiling points are uncorrected. Infrared (IR) spectra were determined on a Perkin-Elmer 247 grating infrared spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded at either 60 MHz (Varian A-60D or T-60 spectrometer) or at 250 MHz as indicated. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (Me_4Si) (δ_{Me_4Si} = 0.00 ppm) as an internal standard. Low-resolution mass spectra were recorded on an LKB-9000 instrument. High-resolution spectra were recorded on a Varian MAT CH5-DF instrument. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

Reactions were run under an atmosphere of nitrogen. "Dry" solvents were dried immediately before use. Tetrahydrofuran and dimethoxyethane were distilled from lithium aluminum hydride; dimethylformamide, hexamethylphosphoramide, and pyridine were distilled from calcium hydride. Ether was distilled from sodium metal. Dichloromethane was passed through a column of alumina prior to use.

4-Hydroxy-3,4-dimethyl-2-carboxypentanoic Acid Lactone. To a solution of lithium diisopropylamide in THF [prepared by the addition of *n*-butyllithium (0.81 ml of a 1.6 M solution in hexane) to a solution of diisopropylamine (131 mg, 1.3 mmol) in 5 ml of a dry THF at −20 °C] cooled to −78 °C was added dropwise over a period of 10 min a solution of 4-hydroxy-3,4-dimethylpentanoic acid lactone (128 mg, 1.0 mmol) in dry THF (2.0 ml). After stirring at −78 °C for 30 min, the temperature was raised to −20 °C and carbon dioxide was passed into the reaction vessel for ca. 15 min. After an additional 30 min, the reaction was quenched with 10% HCl. The product was isolated by ether extraction followed by washing of the combined ether extracts with a saturated solution of $NaHCO_3$. The resulting aqueous layer was separated and acidified with concentrated HCl. Ether extraction afforded, after drying ($MgSO_4$) and concentration in vacuo, 150 mg of crude product which crystallized on standing. Recrystallization from benzene gave 129 mg (75%) of pure 4-hydroxy-3,4-dimethyl-2-carboxypentanoic acid lactone: mp 125 °C (lit.[27] 126–126.5 °C); IR ($CHCl_3$) 1771, 1718 cm^{-1}; NMR δ ($CDCl_3$) 10.44 (s, 1 H), 3.40 (d, 1 H), 2.72 (m, 1 H), 1.50 (s, 3 H), 1.30 (s, 3 H), 1.18 (d, 3 H).

4-Hydroxy-3,4-dimethyl-2-methylenepentanoic Acid Lactone (1). 4-Hydroxy-3,4-dimethyl-2-carboxypentanoic acid lactone (200 mg, 1.2 mmol) was treated at 60 °C with a solution prepared from 1.0 ml of 37% aqueous formaldehyde and 402 mg of diethylamine. Stirring was continued for 30 min followed by the addition of NaOAc (100 mg) and glacial acetic acid (1.0 ml). The resulting solution was heated further for 15 min at 75 °C. The ether extract was washed with 10% HCl, saturated $NaHCO_3$, and concentrated NaCl solution. Drying ($MgSO_4$) and removal of the solvent under reduced pressure gave 160 mg of crude product. Chromatography on 7.0 g of silica gel (elution with benzene) provided 150 mg (95%) of pure α-methylene lactone **1**: IR ($CHCl_3$) 1762, 1662 cm^{-1}; NMR δ (CCl_4) 6.02 (d, 1 H, J = 3 Hz), 5.34 (d, 1 H, J = 3Hz), 2.72 (m, 1 H), 1.46 (s, 3 H), 1.18 (s, 3 H), 1.15 (d, 3 H, J = 7 Hz). Anal. Calcd for $C_8H_{12}O_2$: C, 68.55; H, 8.63. Found: C, 68.40; H, 8.58.

2-(*endo*-3-Hydroxybicyclo[2.2.1]heptan-*endo*-3-yl)-propenoic Acid Lactone (4). The lactone[28] (179 mg, 1.08 mmol) in 2.0 ml of dry THF was added dropwise over a period of 10 min to a solution of lithium diisopropylamide in dry THF [prepared from 0.81 ml of 1.6 M *n*-butyllithium in hexane and 131 mg (1.30 mmol) of diisopropylamine in 5.0 ml of dry THF at −78 °C] cooled to −78 °C. After ca. 30 min, the reaction was warmed to −20 °C and carbon dioxide was passed into the reaction vessel for ca. 15 min. After an additional 30 min, the reaction was quenched at −20 °C by the addition of 10% aqueous HCl. The product was isolated by ether extraction. The combined ether extracts were washed with saturated $NaHCO_3$ solution and the combined aqueous layer was acidified with concentrated HCl. Extraction with ether followed by drying ($MgSO_4$) and evaporation of solvent under reduced pressure gave 227 mg (99%) of crude crystalline acid. Recrystallization gave 210 mg (95%) of the desired acid: mp 101–102 °C; IR ($CHCl_3$) 1718, 1740 cm^{-1}.

The above acid (115 mg, 0.55 mmol) was treated at 60 °C with a solution prepared from 0.5 ml of 37% formaldehyde solution and 252 mg of diethylamine. After ca. 30 min, 63 mg of NaOAc and 0.63 ml of glacial acetic acid were added. The resulting solution was heated for 15 min at 75 °C. Standard work-up as above gave 86 mg of pure α-methylene lactone **4**: IR ($CHCl_3$) 1720, 1625 cm^{-1}; NMR δ (CCl_4) 6.11 (t, 1 H, J = 1.5 Hz), 5.38 (t, 1 H, J = 1.5 Hz), 4.14 (d, 2 H). Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 73.95, H, 7.93.

Enolacetylation of Decalone 11. A mixture of decalone **11** (696 mg, 2.14 mmol)[21] and 22 ml of isopropenyl acetate containing 130 mg of *p*-toluenesulfonic acid was refluxed (bath temperature, 96 °C) for 9 h. Upon cooling of the reaction mixture, solid $NaHCO_3$ was added to neutralize the acid present. The solvent was evaporated in vacuo on a rotary evaporator and the residue was taken up in ether and washed with concentrated NaCl solution. Drying of the organic layer (Na_2SO_4) and solvent evaporation gave 1.07 g of crude product which crystallized on standing (780 mg, 98%). Recrystallization from ethyl acetate–hexane provided pure enol acetate: mp 120 °C; IR ($CHCl_3$) 1735, 1690 cm^{-1}; NMR δ (CCl_4) 5.20 (m, 1 H), 4.80 (m, 1 H), 3.60 (s, 3 H), 3.40 (s, 2 H), 3.30 (s, 3 H), 2.03 (s, 3 H), 1.98 (s, 3 H). Anal. Calcd for $C_{19}H_{28}O_7$: C, 61.94; H, 7.66. Found: C, 62.13; H, 7.69.

Ozonolysis of Enol Acetate 12. A solution of enol acetate **12** (459 mg, 1.25 mmol) in 150 ml of CH_2Cl_2 cooled to −78 °C was treated with 1 equiv of ozone. After addition of ozone, 100 ml of absolute MeOH was added at −78 °C. Stirring was continued at that temperature for 15 min followed by addition of 47.4 mg (1.25 mmol) of $NaBH_4$. At 15-min intervals for ca. 1 h, an equal amount of $NaBH_4$ was added (−78 °C). The reaction was warmed to room temperature (ca. 45 min) and 2.2 ml of 1 N aqueous NaOH was added. After an additional 30 min, the solvent was removed under reduced pressure and the residue was taken up in water and washed with ether. The aqueous layer was cooled (0 °C), acidified carefully with 37% HCl, and extracted exhaustively with EtOAc. The combined organic layers were evaporated to leave a solid (414 mg) which was dissolved in ether and treated (0 °C) with an ethereal solution of diazomethane. There was obtained 395 mg of crude diester which was purified by column chromatography on 7.0 g of silica gel. Elution with hexane–ethyl acetate (3:2) followed by ethyl acetate gave 372 mg (80%) of pure **13**: IR ($CHCl_3$) 3450, 1730 cm^{-1}; NMR[24] δ ($CDCl_3$) 4.94 (t, 1 H, J = 10 Hz), 3.76 (m, 2 H), 3.71 (s, 6 H), 3.40 (s, 3 H), 3.38 (AB q, 2 H, J = 9 Hz, $\Delta\nu_{AB}$ = 49.2 Hz), 2.05 (s, 3 H). Anal. Calcd for $C_{18}H_{30}O_8$: C, 57.74; H, 8.08. Found: C, 57.63; H, 8.07.

Preparation of *o*-Nitrophenyl Selenide 14.[29] Methanesulfonyl chloride (78 mg, 0.68 mmol) was added to a solution of alcohol **13** (215 mg, 0.57 mmol) in 3.0 ml of dry pyridine cooled to 0 °C. After 30 min at 0 °C, the reaction temperature was raised

to 25 °C and stirring was continued for an additional 30 min. The solvent was removed under high vacuum and the residue was taken up in ether and washed with water. The combined ether extracts were dried over anhydrous $MgSO_4$. Filtration followed by removal of the solvent in vacuo gave 242 mg (94%) of crude mesylate: IR (film) 1725, 1340, 1165 cm^{-1}; NMR δ ($CDCl_3$) 4.80 (m, 1 H, CHOAc), 4.32 (t, 2 H, $MsOCH_2$), 3.61 (s, 6 H), 3.25 (br s, 5 H, CH_2OCH_3), 2.99 (s, 3 H, CH_3SO_2), 1.95 (s, 3 H, OAc).

The above crude mesylate (242 mg) in 2.4 ml of dry DMF was added dropwise to a solution of *o*-nitrophenyl selenide ion prepared by addition of $NaBH_4$ (54 mg) to *o*-nitrophenyl selenocyanate[30] (170 mg, 0.75 mmol) in 3.6 ml of dry DMF cooled to 15 °C. After 20 h, the reaction mixture was taken up in ether and washed with water. The aqueous layer was further extracted with ether. Drying (anhydrous $MgSO_4$) of the combined organic washes and evaporation in vacuo left 371 mg of crude selenide. Purification on 60 g of silica gel using hexane–ether (2:1) gave 241 mg of pure *o*-nitrophenyl selenide **14** (75% overall yield from alcohol **13**): IR ($CHCl_3$) 1730, 1590, 1565, 1518, 1336 cm^{-1}; NMR δ ($CDCl_3$) 8.20 (d, 1 H), 7.40 (m, 3 H), 4.81 (m, 1 H), 3.60 (s, 6 H), 3.35 (s, 3 H), 3.25 (br s, 2 H), 2.78 (m, 2 H), 1.92 (s, 3 H). Anal. Calcd for $C_{24}H_{33}NO_9Se$: C, 51.61; H, 5.96. Found: C, 51.54; H, 5.90.

***trans*-2-Acetoxy-*trans*-4-vinyl-*cis*-4-methoxymethyl-*cis*,*cis*-1,3-cyclohexanediacetic Acid Dimethyl Ester (15).** A solution of 720 mg (1.29 mmol) of *o*-nitrophenyl selenide (**14**) in 17 ml of THF cooled to 0 °C was treated dropwise with 0.35 ml of 50% H_2O_2. After addition was complete, the reaction was warmed to room temperature and stirring was continued for 20 h. The reaction mixture was concentrated in vacuo and the residue was dissolved in ether and washed with water. The aqueous layer was extracted exhaustively with ether. The combined ether extracts were dried over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. There was obtained 505 mg of crude olefin. Purification on silica gel [elution with hexane–ethyl acetate (10:1)] gave 370 mg (81%) of crystalline **15**: mp 69–71 °C (ether–hexane); IR ($CHCl_3$) 3080, 1735, 1638 cm^{-1}; NMR^{24} δ (CCl_4) 5.06–5.75 (typical vinyl eight-line pattern, 3 H), 4.90 (t, 1 H, CHOAc), 3.55 (s, 6 H), 3.45 (AB q, 2 H, J = 10 Hz, $\Delta\nu_{AB}$ = 33.5 Hz), 3.30 (s, 3 H, OCH_3), 1.88 (s, 3 H, OAc). Anal. Calcd for $C_{18}H_{28}O_7$: C, 60.66; H, 7.92. Found: C, 60.54; H, 7.89.

***trans*-2-Acetoxy-*trans*-4-ethyl-*cis*-4-methoxymethyl-*cis*,*cis*-1,3-cyclohexanediacetic Acid Dimethyl Ester (16).** Olefin diester **15** (395 mg, 1.11 mmol) was hydrogenated in 40 ml of MeOH at 1 atm for 18 h using 200 mg of 5% Pd/C. Filtration followed by evaporation of the solvent in vacuo gave 387 mg (97%) of the dihydro derivative **16** which was essentially pure by NMR and TLC analysis [hexane–ethyl acetate (7:3), two developments]: IR ($CHCl_3$) 1735 cm^{-1}; NMR^{24} δ (CCl_4) 4.87 (t, 1 H), 3.64 (s, 6 H), 3.34 (s, 3 H), 3.29 (AB q, 2 H, J = 9 Hz, $\Delta\nu_{AB}$ = 20.1 Hz), 1.88 (s, 3 H), 0.88 (t, 3 H, J = 7 Hz). Anal. Calcd for $C_{18}H_{30}O_7$: C, 60.32; H, 8.44. Found: C, 60.09; H, 8.40.

Boron Tribromide Cleavage of Methyl Ether 16. To a solution of methyl ether **16** (348 mg, 0.96 mmol) in 20 ml of dry CH_2Cl_2 cooled to −78 °C was added slowly dropwise 1.08 ml of BBr_3. After addition was complete, the reaction was warmed to −20 °C over a 45-min period. Stirring was continued for 30 min at −20 °C followed by quenching with 20 ml of ether. After an additional 10 min, still at −20 °C, 2.0 ml of 5% $NaHCO_3$ solution was added. The reaction mixture was taken up in EtOAc and washed with saturated NaCl solution. Drying of the organic layer (anhydrous $MgSO_4$) and evaporation under reduced pressure left 336 mg of crude product. Chromatography on silica gel [elution with ether–hexane (1:2)] gave 64 mg of a less polar crystalline substance identified as bromo lactone **21**: mp 113–114 °C (carbon tetrachloride); IR ($CHCl_3$) 1730 cm^{-1}; NMR^{24} δ (CCl_4) 4.15 (AB q, 2 H, J = 12 Hz, $\Delta\nu_{AB}$ = 131.4 Hz), 4.12 (t, 1 H, J = 11 Hz), 3.68 (s, 3 H), 0.94 (t, 3 H, J = 7 Hz). Anal. Calcd for $C_{14}H_{21}O_4Br$: m/e 334.0603. Found: 334.0579.

Continued elution provided 244 mg of pure lactone **17**: IR ($CHCl_3$) 1730 cm^{-1}; NMR^{24} δ (CCl_4) 4.84 (t, 1 H, J = 11 Hz), 4.18 (AB q, 2 H, J = 12 Hz, $\Delta\nu_{AB}$ = 149.5 Hz), 3.65 (s, 3 H), 2.15 (s, 3 H), 0.95 (t, 3 H, J = 7 Hz). Anal. Calcd for $C_{16}H_{24}O_6$: C, 61.52; H, 7.74. Found: C, 61.48; H, 7.76.

Bisnordihydrodeoxyvernolepin (18). A solution of acetate **17** (238 mg, 0.76 mmol) in 10 ml of MeOH containing 105 mg (0.76 mmol) of finely powdered anhydrous K_2CO_3 was stirred 16 h at room temperature. The reaction was quenched by the addition of 10% HCl and the resulting solution was concentrated in vacuo. The residue was dissolved in EtOAc and washed with concentrated NaCl solution. Drying of the organic layer ($MgSO_4$) and solvent evaporation under reduced pressure left 206 mg of crude hydroxy ester [IR ($CHCl_3$) 3450, 1728 cm^{-1}] which was used directly in the next reaction.

A solution of the above hydroxy ester in 35 ml of benzene containing *p*-toluenesulfonic acid (80 mg) was refluxed for 1.5 h. The reaction mixture was diluted with EtOAc, washed with saturated NaCl solution, and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure gave 190 mg of crystalline tricyclic dilactone **18**. Recrystallization from ether–hexane (2:1) gave 136 mg (75% overall) of pure crystalline dilactone: mp 145–146 °C; IR ($CHCl_3$) 1780, 1728 cm^{-1}; NMR^{24} δ ($CDCl_3$) 4.22 (AB q, 2 H, J = 12 Hz, $\Delta\nu_{AB}$ = 84.1 Hz), 4.00 (t, 1 H), 0.96 (t, 3 H, J = 7 Hz). Anal. Calcd for $C_{13}H_{18}O_4$: C, 65.53; H, 7.61. Found: C, 65.32; H, 7.59

Dihydrodeoxyvernolepin (8). A solution of dry diisopropylamine (63 μl, 0.45 mmol) in dry THF (1.0 ml) cooled to 0 °C was treated dropwise with *n*-butyllithium (0.25 ml of a 1.58 M solution in hexane). After 15 min, the resultant solution of lithium diisopropylamide was cooled to −78 °C and to it was added dropwise over 30 min via a syringe pump a solution of bisnordihydrodeoxyvernolepin **18** (41.3 mg, 0.173 mmol) in 2.0 ml of dry THF containing 0.3 ml of dry HMPA. After addition was complete, stirring was continued at −78 °C for 10 min, followed by warming to −20 °C. Formaldehyde [generated by depolymerization of paraformaldehyde (200 mg) at 150 °C (bath temperature)] was passed into the cooled (−20 °C) reaction vessel with the aid of a stream of nitrogen. After complete depolymerization (ca. 20 min) the reaction mixture was stirred for an additional 30 min (−20 °C). The reaction was quenched by the addition of 1.5 ml of 5% HCl. The product was extracted with EtOAc. The combined organic extracts were washed with water, saturated $NaHCO_3$, and concentrated NaCl solution. Drying over anhydrous $MgSO_4$ and removal of the solvent in vacuo left 317 mg of very crude product still containing HMPA.

The crude mixture of diols from above (317 mg) was diluted with 0.2 ml of dry pyridine and treated at 0–5 °C with methanesulfonyl chloride (47.7 mg, 2.4 equiv). Stirring at 5 °C was continued for 9 h. Addition of EtOAc (30 ml) followed by washing with cold water, cold 5% HCl, and concentrated NaCl solution provided, after drying ($MgSO_4$) and evaporation of solvent under reduced pressure, 81 mg of crude dimesylate which was used directly in the next reaction.

The mixture of crude dimesylate (81 mg) was dissolved in 2.0 ml of dry pyridine and refluxed for 20 h. After cooling to room temperature, the reaction was diluted with 30 ml of EtOAc and washed with water, 5% HCl, saturated $NaHCO_3$, and concentrated NaCl solution. The organic layer was dried ($MgSO_4$) and the solvent was evaporated in vacuo leaving 46 mg of crude material. Chromatography on 700 mg of silica gel [elution with ethyl acetate–hexane (1:1)] gave 8 mg of pure crystalline dihydrodeoxyvernolepin, mp 146–147 °C, and 6 mg of recovered starting material. The overall yield based on unrecovered starting material was 21%. Dihydrodeoxyvernolepin exhibited the following spectral characteristics: IR ($CHCl_3$) 1770, 1715, 1670, 1620 cm^{-1}; NMR^{24} δ ($CDCl_3$) 6.72 (s, 1 H), 6.14 (d, 1 H, J = 3 Hz), 5.88 (s, 1 H), 5.46 (d, 1 H, J = 3 Hz), 4.22 (AB q, 2 H, J = 13 Hz, $\Delta\nu_{AB}$ = 99.3 Hz), 3.90 (t, 1 H, J = 11 Hz), 3.72 (m, 1 H), 0.94 (t, 3 H, J = 7 Hz). Anal. Calcd for $C_{15}H_{18}O_4$: m/e 262.1205. Found: 262.1205.

2-Oxo-*cis*-1,3-cyclohexanediacetic Acid Dimethyl Ester (26). A solution containing 2-oxocyclohexaneacetic acid methyl ester (6.0 g, 0.035 mol) and pyrrolidine (5.04 g, freshly distilled from KOH) in 110 ml of dry benzene was stirred at reflux for 22 h with removal of water via a Dean-Stark trap. The solvent was removed under reduced pressure and the residue was dissolved in 100 ml of dry benzene and methyl bromoacetate (8.03 g, 0.052 mol) was added. After 20 h at reflux, the reaction was treated with 30 ml of water containing several drops of acetic acid and refluxing was continued for an additional 2 h. The resulting cooled mixture was washed with 100 ml of 5% HCl. The combined aqueous washes were extracted with ether, and the combined

organic extracts were dried ($MgSO_4$) and filtered. Solvent evaporation in vacuo left 9.44 g of crude product. Chromatography on silica gel [400 g, elution with ether–hexane (1:3)] gave 4.71 g (56%) of pure diester **26**: bp 136–138 °C (0.5 mmHg); IR (film) 1740, 1712 cm^{-1}; NMR δ ($CDCl_3$) 3.65 (s, 6 H). Anal. Calcd for $C_{12}H_{18}O_5$: *m/e* 242.1154. Found: 242.1155.

trans-**2-Acetoxy**-*cis,cis*-**1,3-cyclohexanediacetic Acid Dimethyl Ester (27).** A solution of keto diester **26** (300 mg, 1.2 mmol) in 5.0 ml of MeOH was treated dropwise over a 10-min period at −20 °C with a solution of $NaBH_4$ (150 mg) in 2.5 ml of DMF. Stirring was continued at −20 °C for 2 h followed by quenching with 0.5 ml of acetone. The reaction was concentrated in vacuo and the residue was dissolved in ether and washed with water. Evaporation of the ether provided 280 mg of crude product which was directly acetylated by dissolving in 4.0 ml of dry pyridine containing 500 mg of acetic anhydride. After ca. 20 h, the reaction mixture was concentrated to dryness under high vacuum and the crude product (260 mg) was purified on a column of silica gel. Elution with ether–hexane (1:4) gave 125 mg (49%) of a compound identified as *cis*-lactone **28**: IR ($CHCl_3$) 1770, 1728 cm^{-1}; NMR δ (CCl_4) 4.42 (m, 1 H), 3.68 (s, 3 H). Continued elution provided 130 mg (38%) of pure acetate **27**: IR ($CHCl_3$) 1725 cm^{-1}; NMR[24] δ (CCl_4) 4.40 (t, 1 H, J = 10 Hz), 3.62 (s, 6 H), 1.92 (s, 3 H).

trans-**2-Hydroxy**-*cis,cis*-**1,3-cyclohexanediacetic Acid Monolactone Monomethyl Ester (24).** A solution of acetate **27** (340 mg, 1.2 mmol) in 7.5 ml of MeOH containing 165 mg (1.2 mmol) of finely powdered anhydrous K_2CO_3 was stirred 3 h at room temperature. The reaction was quenched by the addition of 10% HCl. Concentration of the resulting solution under reduced pressure left 225 mg of an alcohol which was dissolved in 25 ml of dry benzene containing 75 mg of *p*-toluenesulfonic acid and heated under reflux for 2 h. The reaction mixture was diluted with EtOAc, washed with concentrated NaCl solution, and dried ($MgSO_4$). Evaporation of the solvent in vacuo gave the crude lactone which was chromatographed in silica gel. Elution with ether–hexane (3:2) gave 175 mg (69%) of pure lactone **24**: IR ($CHCl_3$) 1780, 1730 cm^{-1}; NMR δ (CCl_4) 3.60 (s, 3 H), 3.49 (br t, 1 H). Anal. Calcd for $C_{11}H_{16}O_4$: *m/e* 212.1049. Found: 212.1052.

2,2′-(*trans*-**2-Hydroxy**-*cis,cis*-**1,3-cyclohexyl)dipropenoic Acid Monolactone Monomethyl Ester (6).** A solution of dry diisopropylamine (136 mg, 1.34 mmol) in anhydrous THF (3.0 ml) cooled to 0 °C was treated dropwise with *n*-butyllithium (0.83 ml of a 1.56 M solution in hexane). After 15 min the solution of lithium diisopropylamide was cooled to −78 °C and treated dropwise over 30 min via a syringe pump with a solution of the lactone ester **24** (95 mg, 0.45 mmol) in 3.0 ml of dry THF. After addition was complete, stirring at −78 °C was continued for 10 min followed by warming to −20 °C. Formaldehyde (as described above) was passed into the reaction vessel at −20 °C for ca. 20 min. After 30 min at −20 °C the reaction was quenched by the addition of 1.5 ml of 5% HCl. The product was extracted with EtOAc. The combined organic extracts were washed with water, saturated $NaHCO_3$, and concentrated NaCl solution. Drying over anhydrous $MgSO_4$ and removal of the solvent under reduced pressure afforded a crude bis adduct which was chromatographed on 30 g of silica gel. Elution with ethyl acetate–hexane (5:1) gave 50 mg of pure diol which was dissolved in 0.5 ml of dry pyridine and treated at 5 °C with methanesulfonyl chloride (80 μl). Stirring at 5 °C was continued for 20 h. Addition of EtOAc followed by washing with concentrated NaCl solution provided, after drying ($MgSO_4$) and evaporation of solvent in vacuo, 61 mg of crude dimesylate which was used directly in the next reaction.

The above dimesylate (61 mg) was dissolved in 0.5 ml of benzene containing 80 mg of 1,5-diazabicyclo[5.4.0]undec-5-ene and stirred at 25 °C for 30 min. The reaction was concentrated in vacuo and the residue was chromatographed on 20 g of silica gel. Elution with hexane–ether (2:1) gave 25 mg (24% overall) of pure bis-methylenated compound **24**: IR ($CHCl_3$) 1768, 1712, 1670, 1628 cm^{-1}; NMR[24] δ ($CDCl_3$) 6.27 (s, 1 H), 6.06 (d, 1 H, J = 3 Hz), 5.67 (s, 1 H), 5.38 (d, 1 H, J = 3 Hz), 3.91 (t, 1 H, J = 12 Hz), 3.74 (s, 3 H), 2.98 (m, 1 H), 1.78 (m, 1 H). Anal. Calcd for $C_{13}H_{16}O_4$: *m/e* 236.1049. Found: 236.1045.

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References and Notes

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