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# Antitumor Agents—CLI. Bis(helenalinyl)Glutarate and Bis(Isoalantodiol-B)Glutarate, Potent Inhibitors of Human DNA Topoisomerase II

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**Abstract**—Evaluation of a number of cytotoxic antitumor sesquiterpene lactones and their derivatives has led to the discovery of bis(helenalinyl)glutarate (**4**) and bis(isoalantodiol-B)glutarate (**10**) as potent inhibitors of human-derived topoisomerase II. Unlike etoposide, which inhibits by preventing the DNA rejoining process, compounds **4** and **10** inhibit topoisomerase II without causing DNA breakage. The structure–activity relationships of **4**, **10**, and related compounds are discussed.

## Introduction

Type II topoisomerases are enzymes which catalyze DNA strand passage through transient double strand breaks in the DNA. The resulting change in linking number of DNA allows these enzymes to mediate DNA interconversions, such as supercoiling and relaxation of supercoiling, catenation and decatenation, knotting and unknotting.[2,3] These enzymes have been implicated in a number of vital cellular processes, including DNA replication and transcription and chromosomal segregation.[4] The prime importance of this enzyme makes it a critical target for the action of a wide variety of anticancer drugs, including the clinically useful natural products, etoposide and teniposide,[5–10] and their analogs.[11–15] As a result of our screening of natural products for potential antitumor agents using this enzyme as a target, the bis(helenalinyl)glutarate (**4**) was found to be a potent inhibitor of human-derived DNA topoisomerase II. We report herein on the structure–activity relationships among **4**-related compounds.

## Results and Discussion

Sesquiterpene lactones, including the pseudoguaianolide helenalin (**1**), the germacranolide eupatolide (**5**) and the eudesmanolide isoallantolactone (**12**), are a diverse group of secondary plant metabolites that exhibit cytotoxic antitumor and other pharmacological activities.[16–18] Helenalin (**1**) and its derivatives, such as bis-(helenalinyl)malonate (**2**), bis(helenalinyl)succinate (**3**), and bis(helenalinyl)glutarate (**4**), are potent cytotoxic antitumor agents.[19]

Compounds **1–4** contain an O=C–CH=CH alkylating center that affords a Michael-type addition to functional thiol groups of enzymes, thereby inhibiting the activities of thiol-bearing DNA polymerase α, ribonucleotide reductase and IMP dehydrogenase in the L-1210, P-388, KB, and Ehrlich ascites cells,[20–22] as well as the eIF-3 function and activated eIF-2 kinase activity in the initiation process of protein synthesis of P-388 cells and rabbit reticulocytes.[23–26] Since DNA topoisomerase II possesses the thiol group, a screening of these compounds as inhibitors of this enzyme was carried out. The results of these studies along with the cytotoxicity (KB) data are summarized in Table 1. In the enzyme inhibition assay, all compounds were screened at 100 μM concentration, and the protein-linked DNA breakage assay was performed at 10 μM concentration.

For all compounds tested, the *in vitro* cytotoxicity appears to have no correlation with the inhibitory activity of human DNA topoisomerase II. For the bis(helenalinyl)-diester series, the length of linkage between two subunits seems to be important for the inhibition of topoisomerase II unknotting activity. Thus, only the glutarate diester **4** demonstrated high (>75%) activity. The other diesters (**2** and **3**) and the parent alcohol (**1**) were inactive.

Both eupatolide (**5**) and its diester (**6**) have no enzyme-inhibitory effect. The structure and stereochemistry of this series is quite different from the above active series and the following bis(isoalantodiol)-diester series. In the latter series, the enzyme-inhibitory activity depends highly upon the stereochemistry at C-4. Demonstration of this stereoselectivity is reflected in the 4-β-hydroxy isomer (**10**) which shows the highest (>>75%) activity when compared with the intermediate activity of the 4-α-hydroxy isomer (**8**). A similar stereoselectivity is also observed in their parent alcohols where the 4-β-hydroxy alcohol (**9**) is weakly active whereas the corresponding 4-α-isomer (**7**) is inactive.

[1]For paper 150 in this series, see Ref. 1.

**Table 1.** Biological evaluation of **1–10**

| Compound | Cytotoxicity[a] ($ED_{50}$, μM) | | | % Inhibition of DNA-topoisomerase II activity (100 μM) | % Cellular protein DNA Complex formation (10μM) |
|---|---|---|---|---|---|
| | KB | KB 1.0c | KB 20a | | |
| **1** | 0.91 | | | –[b] | –[b] |
| **2** | 0.07 | | | – | – |
| **3** | 0.06 | | | – | – |
| **4** | 0.21 | | | >75 | – |
| **5** | 2.30 | | | – | – |
| **6** | >10.00 | | | – | – |
| **7** | >10.00 | | | – | – |
| **8** | >10.00 | | | 50 | – |
| **9** | 8.00 | | | <50 | – |
| **10** | 37.00 | 63.00 | 31.00 | >>75 | – |
| **etoposide** | 1.60 | 4.70 | 30.60 | >75 | 100 |

[a] $ED_{50}$ is the concentration of drug which affords 50% reduction in cell number after 3 days incubation. KB1.0c and KB20a are etoposide-resistant cells.
[b] "–": No activity.

The mechanism of action of **4** and **10** is different from that of etoposide, which breaks the DNA linkage by blocking the rejoining process. Compounds **4** and **10** had no apparent cleavage of DNA as reflected by comparison of their autoradiograms with the background cleavage caused by the enzyme alone. It is noteworthy that **10** shows inhibition against the growth of etoposide-resistant KB1.0c and KB20a cells. The underlying mechanism of cytotoxic action of **4** and **10** related to their inhibition of Topo II is still unclear and being investigated. However, their mechanisms could be very different from that of **1–3**.

## Experimental Section

All melting points were taken on a Fischer–Johns melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer 1320 spectrophotometer. UV spectra were taken on a Varian 220 UV-VIS spectrophotometer, and $^1$H-NMR spectra (Table 2) were obtained from a Varian 400 MHz NMR spectrophotometer. All chemical shifts are reported in parts per million from $(CH_3)_4Si$. Mass spectral analyses were determined on a V.G. Micromass 70-70 instrument at 70 eV with a direct inlet system. Analytical TLC was carried out on Merck precoated silica gel 60F-254. EM Kieselgel 60 (230–400 mesh ASTM) was used for column chromatography. Preparative TLC was performed on Analtech precoated silica gel GF (500 μm, 20 x 20 cm).

*Test compounds*

Helenalin (**1**),[19] bis(helenalinyl)malonate (**2**),[19] bis-(helenalinyl)succinate (**3**),[19] bis(helenalinyl)glutarate (**4**),[19] and eupatolide (**5**)[27] were obtained from the previous studies of this laboratory. Etoposide was a gift from Bristol-Myers Company, Wallingford, CT.

*Bis(eupatolyl)glutarate (6)*

A mixture of eupatolide (**5**, 44.1 mg, 0.18 mM) in anhydrous benzene (2 mL) and glutaryl chloride (38.9 mg, 0.23 mM) was refluxed overnight and worked up in the usual way to give a colorless residue (33.4 mg). Purification of this residue over PTLC (silica gel) using $CHCl_3$–EtOAc (5:1) as developing solvent yielded **6** as an amorphous compound (19.3 mg): $[\alpha]_D^{24} = +4.67$ (c 0.45, $CHCl_3$); IR: ν max (KBr) $cm^{-1}$: 1763 (s) (CO), 1603, 1520, 1417, 1216, 1017, and 929; EIMS m/z (%): 264 (2.6), 248 (21.4), 233 (21.2), 215 (17.4), 204 (11.4), 187 (13.7), 179 (26.0), 161 (29.0), 149 (22.0), 137 (37.0), 119 (25.9), 109 (59.2), 91 (63.8), 81 (55.6), 69 (92.3), and 55 (100.0); FABMS (positive mode): 593 $(M + H)^+$ and 615 $(M + Na)^+$ for $C_{35}H_{44}O_8$ ($M^+$, 592).

*Isoallantolactone epoxide (11)*

A solution of isoallantolactone (**12**, 0.94 g, 4.0 mM) in $CH_2Cl_2$ (8 mL) was added dropwise to a solution of *m*-chloroperbenzoic acid (3 g, 15 mM) in $CH_2Cl_2$ (20 mL) and the resulting solution was kept at room temperature for 3 h. The reaction mixture was then diluted with $CHCl_3$ and washed successively with 10% $NaHSO_3$, 10% $Na_2CO_3$, and $H_2O$. The lower, non-aqueous layer was dried over anhydrous $Na_2SO_4$ and evaporated to yield a white crystalline product (1.05 g). Recrystallization from $Me_2CO$ yielded **11** as prisms: m.p. 113–114 °C; $[\alpha]_D^{24} = +2.65$

Table 2. $^1$H NMR spectral data ($CDCl_3$, 400 MHz) for compounds 7–11

| H | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|
| 1a | | | 1.49m* | 1.50m | 1.67m |
| 1b | | | 1.80m* | overlap | 1.70m |
| 2a | | | 1.70m | 1.69m | 1.35dddd (12.7, 1.6) |
| 2b | | | 1.49m* | 1.50m | 1.19ddd (12.7, 9) |
| 3a | | | 1.80m* | 1.80m | 1.88m |
| 3b | | | 2.17m* | overlap | 1.7 |
| 5 | | | 2.91dd (13.5, 7.4) | 2.88dd (13.6, 7.4) | 1.6-1.7 |
| 6a | | | 1.69m | 1.69m | 1.61ddd (12.9) |
| 6b | | | 2.02dd (13.5, 11.3) | 2.02 | 0.92dd (12.9) |
| 7 | 3.07m | 3.03ddd (12.1, 6.8, 5.1) | 3.09m | 3.08m | 2.90ddd (11.8, 6.5, 5.8) |
| 8 | 4.56m | 4.55dd (5.1, 3) | 4.52dd (13.2, 7.2) | 4.51dd (13.2, 6.5) | 4.47dd (4.8, 1.6) |
| 9a | | | 1.69m* | 1.69m | 1.50dd (15.2, 4.8) |
| 9b | | | 2.17m* | 2.18m | 2.19d (15.2) |
| 13a | 5.64d (1.2) | 5.79d (1.6) | 5.63d (2.4) | 5.65d (0) | 5.55d (0) |
| 13b | 6.15d (1.2) | 6.15d (1.6) | 6.25d (2.4) | 6.26d (0) | 6.10d (0) |
| 14a | 4.06ABq (12) | 4.49ABq (12.1) | 4.12ABq (11.5) | 4.59ABq (11.9) | 2.67dd (4.5, 1.9) |
| 14b | 4.06ABq (12) | 4.49ABq (12.1) | 4.12ABq (11.5) | 4.59ABq (11.9) | 2.55d (4.5) |
| 15 | 0.91s | 0.91s | 1.12s | 1.13s | 0.98s |
| 2' | | | | 2.45m | |
| 3' | | | | 1.97m | |
| 4' | | | | 2.45m | |

*Data were obtained from COSY. All chemical shifts and coupling constants were expressed in δ and Hz, respectively.

(c 1.17, $CHCl_3$); IR: ν max (KBr) $cm^{-1}$: 2937, 1762 (s) (CO), 1634, 1520, 1346, 1264 (s), 1147 (s), and 965; EIMS m/z (%): 249 (6.2), 248 (5.9, $M^+$), 231 (44.1), 230 (20.3), 185 (13.8), 159 (9.8), 143 (12.7), 137 (14.2), 131 (13.3), 121 (22.8), 119 (43.0), 105 (26.3), 91 (36.2), 86 (64.5), 84 (100.0), 79 (28.0), 67 (26.2), and 55 (34.8); HRMS m/z ($M^+$) found: 248.1421, calcd for $C_{15}H_{20}O_3$: 248.1423. The constitution of **11** was unequivocally confirmed by a single crystal X-ray analysis *(vide infra).*

*Isoallantodiol-A (7) and isoallantodiol-B (9)*

A solution of **11** (2.82 g, 11.4 mM) in tetrahydrofuran (30 mL) was treated with 30% $HClO_4$ (20 mL). After the mixture had been kept at 48 °C for 6 h, it was diluted with $H_2O$ and extracted with $CHCl_3$ (50 mL x 3). The combined $CHCl_3$ layers were washed with $H_2O$ and dried over anhydrous $Na_2SO_4$ to furnish a pale brown oil (3.22 g). Column chromatography on silica gel (150 g) of this oil in $CHCl_3$ with increasing concentration of EtOAc yielded **7** (0.36 g) and **9** (0.32 g) as amorphous compounds.

*Compound 7.* $[\alpha]_D^{24}$ = + 4.12 (c 1.02, $Me_2CO$); IR: ν max (KBr) $cm^{-1}$: 3446 (s) (OH), 2931 (s), 1756 (s) (CO), 1649, 1519, 1348, 1265, 1158, and 909; EIMS m/z (%): 262 (5.6, $M^+$ - 4), 248 (14.3), 230 (17.8), 217 (20.6), 203 (11.5), 177 (12.0), 171 (16.1), 161 (18.5), 149 (34.2), 131 (43.8), 119 (43.4), 105 (63.2), 91 (94.6), 79 (73.0), 67 (55.5), and 55 (100); FABMS (negative mode): 265 for $C_{15}H_{21}O_4$ ($M^+$ - H).

*Compound 9.* $[\alpha]_D^{24}$ = +3.37 (c 1.08, $Me_2CO$); IR: ν max (KBr) $cm^{-1}$: 3441 (s) (OH), 2933 (s), 1762 (s) (CO), 1638, 1523, 1408, 1352, 1264, 1169, and 966; EIMS m/z (%): 262 (2.2, $M^+$ - 4), 246 (8.9), 230 (24.0), 217 (14.9), 187 (15.0), 185 (15.3), 171 (18.2), 156 (42.6), 139 (69.8), 131 (29.9), 119 (31.4), 111 (61.3), 105 (48.0), 91 (80.8), 77 (60.5), 67 (47.9), and 55 (100.0); FABMS (negative mode): 265 for $C_{15}H_{21}O_4$ ($M - H)^+$.

*Bis(isoallantodiollyl-A)glutarate (8)*

A solution of **7** (214 mg, 0.8 mM) in anhydrous benzene (4 mL) and $CHCl_3$ (5 mL) was refluxed with a solution of glutaryl chloride (178 mg, 1 mM) in anhydrous benzene (3 mL) for 2 h. After dilution with benzene, water was added and the reaction mixture was washed with 5% $NaHCO_3$ and saturated NaCl solutions. The organic layer was dried over anhydrous $Na_2SO_4$ to give a residue (350 mg). Purification of this residue by PTLC [(silica gel, 1 mm thickness, $CHCl_3$–$Me_2CO$ (5:1)] yielded **8** (46 mg) in an amorphous form: $[\alpha]_D^{24}$ = +3.22 (c 0.97, $CHCl_3$); IR: ν max (KBr) $cm^{-1}$: 1735 (s) (CO), 1640, 1410, 1145, 940; EIMS m/z (%): 266 (1.1), 230 (68.7), 215 (23.2), 197 (10.0), 185 (32.7), 169 (15.8), 151 (14.9), 143 (26.7), 131 (26.6), 119 (100.0), 105 (42.6), 91 (56.5), 87 (45.2), 79 (42.1), 67 (24.0), and 55 (67.0); FABMS: (positive mode) 629 (M + $H)^+$ and 651 (M + $Na)^+$ for $C_{35}H_{48}O_{10}$ ($M^+$, 628).

*Bis(isoallantodiollyl-B)glutarate (10)*

A solution of **9** (114 mg, 0.43 mM) in anhydrous benzene (2 mL) was refluxed with a solution of glutaryl chloride (95 mg, 0.56 mM) in benzene (1.5 mL), and following work-up in the same manner as described above for the preparation of **8** from **7**, **10** (62 mg) was obtained as an amorphous compound: $[\alpha]_D^{24}$ = + 1.42 (c 1.35, $CHCl_3$); IR: ν max (KBr) $cm^{-1}$: 1731 (s) (CO), 1638, 1523, 1410, 1196, 1162, 958; EIMS m/z (%): 282 (1.0), 266 (14.5), 230 (67.5), 215 (25.3), 197 (9.8), 185 (32.3), 169 (20.8), 159 (35.6), 143 (46.3), 131 (32.9), 119 (71.5), 105 (48.8), 91 (67.3), 87 (49.3), 79 (46.8), 67 (34.0), and 55 (100.0); FABMS (positive mode): 629 (M + $H)^+$ and 651 (M + $Na)^+$ for $C_{35}H_{48}O_{10}$ ($M^+$, 628).

*X-Ray crystallographic analysis of isoallantolactone epoxide (11)*

For X-ray measurements, a crystal of dimensions *ca.* 0.20 x 0.25 x 0.40 mm was mounted on the end of a thin-glass fiber. Preliminary space group information and unit-cell parameters were obtained from oscillation and Weissenberg photographs. Intensity measurements were made on an Enraf–Nonius CAD-4 diffractometer equipped with a graphite monochromator. Crystal data: $C_{15}H_{22}O_3$, $M$ = 250.34, orthorhombic, $a$ = 12.030 (1) Å, $b$ = 12.158 (1) Å, $c$ = 8.767 (1) Å (by least-squares treatment of the diffractometer setting angles for 25 reflections widely separated in reciprocal space, 43° < θ < 47°), $V$ = 1320.2 (4) $Å^3$, $Z$ = 4, $d_{calcd}$ = 1.259 g $cm^{-3}$, μ(Cu–Kα radiation, λ = 1.5418 Å) = 6.5 $cm^{-1}$. The space group $P2_12_12_1$ was established uniquely by the systematic absences: $h00$ when $h \neq 2n$, $0k0$ when $k \neq 2n$, $00l$ when $l \neq 2n$. One octant of intensity data (1373 reflections) was recorded by use of Cu–Kα radiation [ω - 2θ scans, scanwidth (1.15 + 0.14 tanθ)°]. Two reference reflections, monitored every 2 h during data collection, showed no significant variation (< ± 1%). Those 1269 reflections with $I > 3.0\ \sigma(I)$ were retained for the analysis following application of the usual Lorentz and polarization corrections; the small effects of absorption were ignored.

The crystal structure was solved by direct methods (MULTAN11/82). Initial carbon and oxygen atom positions were obtained from an $E$-map evaluated by use of that set of phases which yielded the highest combined-figure-of-merit. Several rounds of full-matrix least-squares adjustment of atomic positional and thermal parameters (at first isotropic, then anisotropic) were followed by evaluation of a difference Fourier synthesis, which yielded positions for all hydrogen atoms. Continuation of the least-squares iterations with hydrogen atom positional and isotropic thermal parameters, and latterly an extinction correction ($g$), included as variables, led to convergence (max shift < 0.02 σ in the final least-squares cycle) at $R$ = 0.031 [$Rw$ = 0.049, GOF = 1.25, $g$ = 6.2(2) x $10^{-6}$]. A final difference Fourier synthesis contained no unusual features [Δρ (e/$Å^3$) max 0.16; min -0.14]. Final atomic positional parameters are listed in Table 3,[28] while a view of the structure is presented in Figure 2. Bond lengths and angles, shown in Figure 3, are in accordance with expectations.[29]

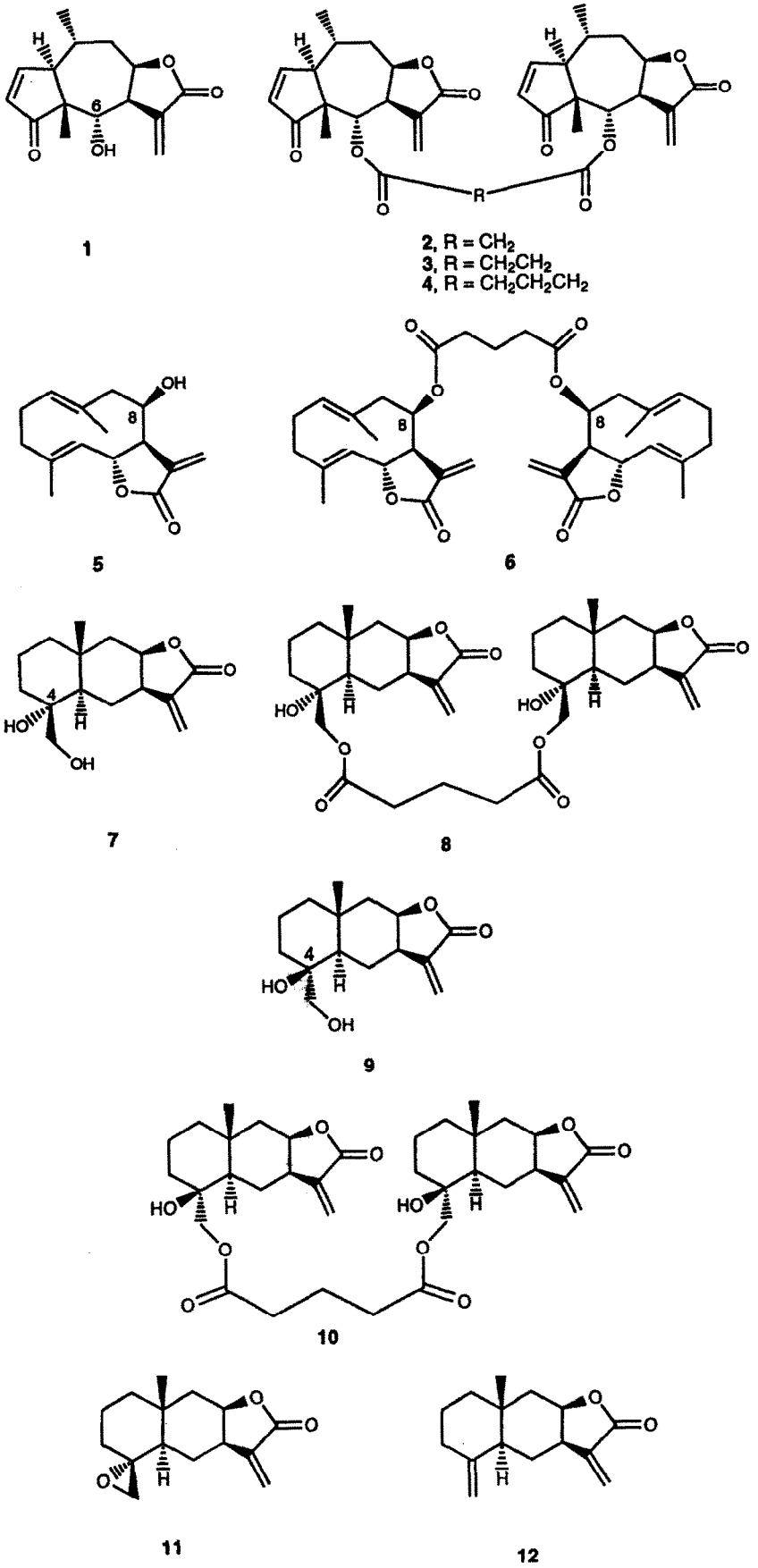


**Figure 1.** Structures for compounds 1–12

**Table 3.** Fractional atomic coordinates and equivalent isotropic thermal parameters[a] for isoallantolactone epoxide (**11**), with estimated standard deviations in parentheses

| Atom | *x* | *y* | *z* | $B_{eq}$ (Å$^2$) |
|---|---|---|---|---|
| C(1) | 0.3274(2) | 0.4174(1) | 0.1530(2) | 3.94(4) |
| C(2) | 0.3601(2) | 0.4306(2) | 0.3199(3) | 4.68(4) |
| C(3) | 0.3271(2) | 0.3324(2) | 0.4129(2) | 4.36(4) |
| C(4) | 0.3730(2) | 0.2322(1) | 0.3405(2) | 3.53(3) |
| C(5) | 0.3382(1) | 0.2183(1) | 0.1750(2) | 2.97(3) |
| C(6) | 0.3759(2) | 0.1137(1) | 0.1037(2) | 3.31(3) |
| C(7) | 0.3270(1) | 0.0969(1) | -0.0569(2) | 3.27(3) |
| C(8) | 0.3392(2) | 0.1965(1) | -0.1576(2) | 3.47(3) |
| C(9) | 0.3245(2) | 0.3037(1) | -0.0806(2) | 3.46(3) |
| C(10) | 0.3753(1) | 0.3160(1) | 0.0792(2) | 3.06(3) |
| C(11) | 0.3967(1) | 0.0182(1) | -0.1432(2) | 3.47(3) |
| C(12) | 0.4798(2) | 0.0817(2) | -0.2303(2) | 3.70(3) |
| C(13) | 0.3948(2) | -0.0876(2) | -0.1433(2) | 4.49(4) |
| C(14) | 0.4740(2) | 0.1833(2) | 0.4023(2) | 4.38(4) |
| C(15) | 0.5026(2) | 0.3273(1) | 0.0684(2) | 3.78(3) |
| O(16) | 0.3666(1) | 0.1377(1) | 0.4358(2) | 4.60(3) |
| O(17) | 0.4516(1) | 0.1864(1) | -0.2220(1) | 3.79(2) |
| O(18) | 0.5615(1) | 0.0523(1) | -0.2971(2) | 4.99(3) |
| H(1A) | 0.351(2) | 0.476(1) | 0.097(2) | 2.00(4) |
| H(1B) | 0.247(2) | 0.416(2) | 0.143(3) | 3.30(5) |
| H(2A) | 0.324(2) | 0.500(2) | 0.359(3) | 4.20(6) |
| H(2B) | 0.443(2) | 0.446(2) | 0.329(3) | 2.70(5) |
| H(3A) | 0.243(2) | 0.327(1) | 0.418(3) | 2.40(4) |
| H(3B) | 0.355(2) | 0.341(1) | 0.523(3) | 3.10(5) |
| H(5) | 0.257(2) | 0.219(2) | 0.171(3) | 2.00(4) |
| H(6A) | 0.358(2) | 0.055(2) | 0.165(3) | 2.30(4) |

**Table 3.** *Continued*

| Atom | $x$ | $y$ | $z$ | $B_{eq}$ (Å$^2$) |
|---|---|---|---|---|
| H(6B) | 0.453(2) | 0.109(1) | 0.098(3) | 2.00(4) |
| H(7) | 0.248(2) | 0.075(2) | -0.049(3) | 2.00(4) |
| H(8) | 0.288(2) | 0.194(2) | -0.246(3) | 2.70(5) |
| H(9A) | 0.243(2) | 0.315(1) | -0.072(3) | 2.00(4) |
| H(9B) | 0.356(2) | 0.359(1) | -0.147(2) | 1.80(4) |
| H(13A) | 0.346(2) | -0.124(1) | -0.087(3) | 3.20(5) |
| H(13B) | 0.454(2) | -0.128(1) | -0.205(3) | 3.00(5) |
| H(14A) | 0.506(2) | 0.213(1) | 0.493(2) | 3.00(5) |
| H(14B) | 0.521(2) | 0.140(1) | 0.328(3) | 2.50(4) |
| H(15A) | 0.524(2) | 0.396(2) | 0.014(3) | 3.70(6) |
| H(15B) | 0.540(2) | 0.327(2) | 0.164(3) | 2.50(4) |
| H(15C) | 0.533(2) | 0.267(2) | 0.009(3) | 2.60(5) |

[a]Hydrogen atoms were refined isotropically.

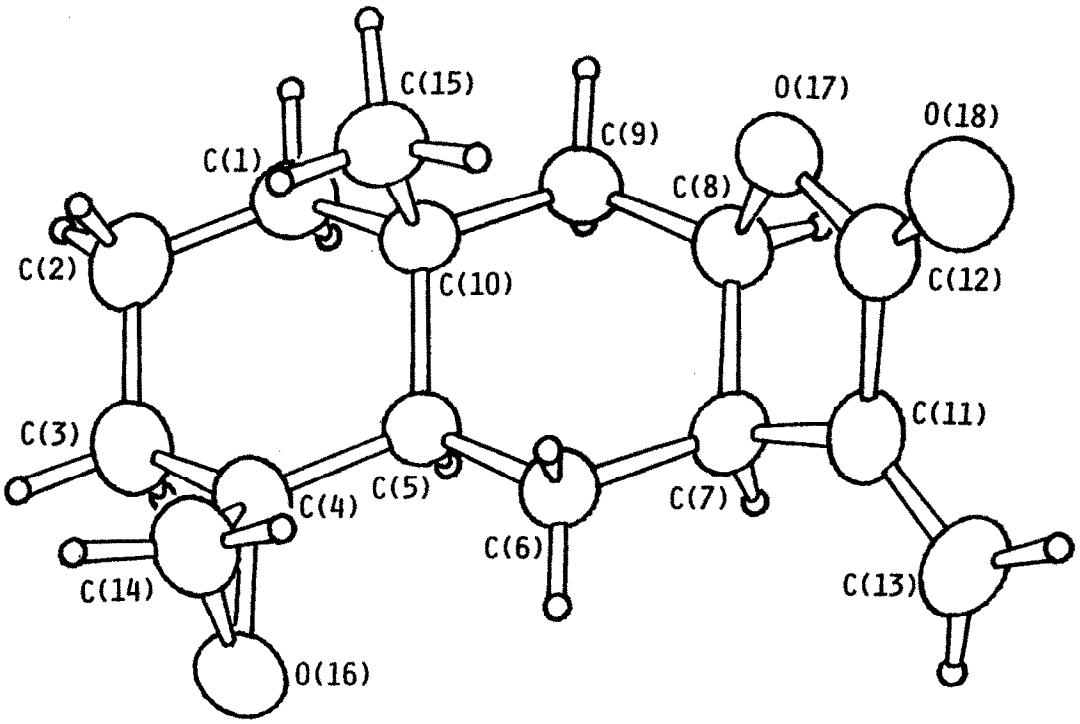


**Figure 2.** Structure and solid-state conformation of isoallantolactone epoxide (11); small circles represent hydrogen atoms

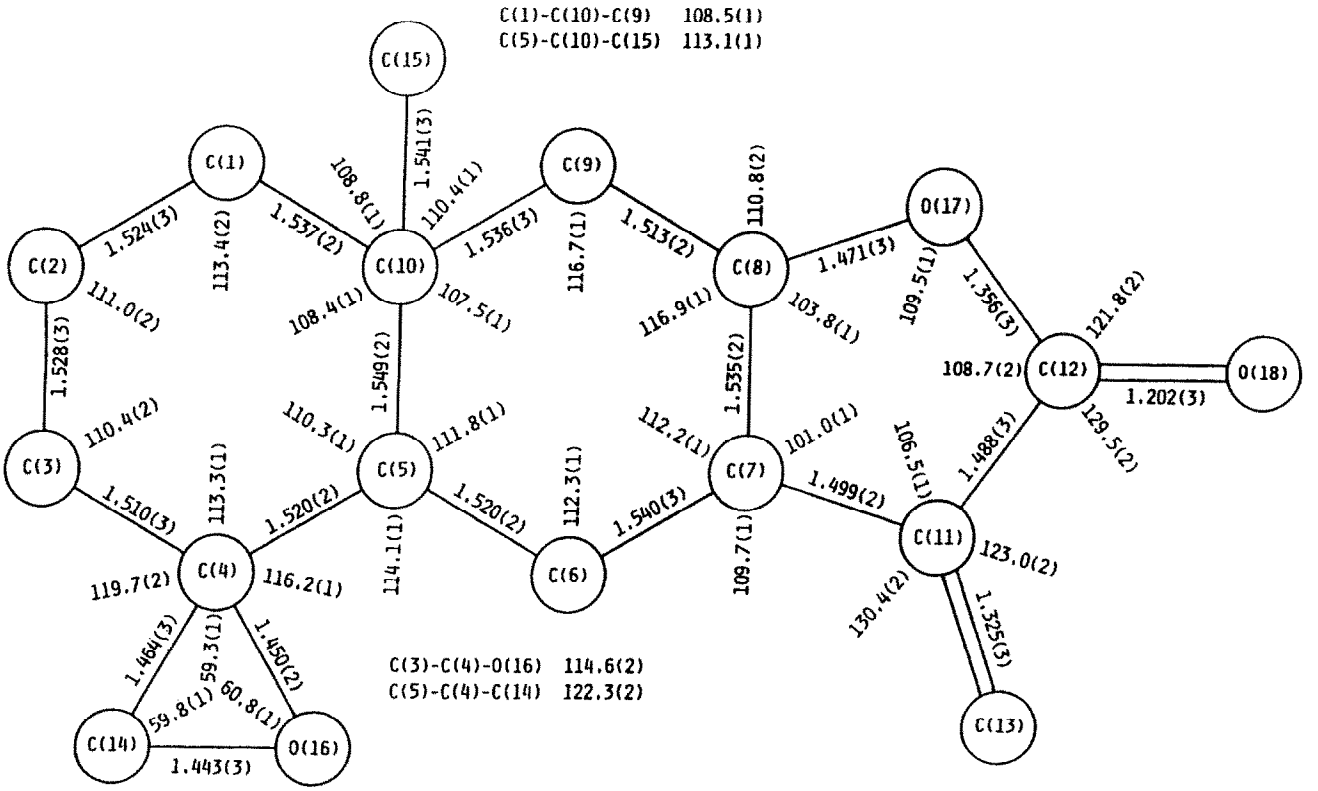


**Figure 3.** Bond lengths (Å) and angles (°) for isoallantolactone epoxide (**11**), with estimated standard deviations in parentheses; C–H distances range from 0.89(2)–1.03(3) Å

Crystallographic calculations were performed on PDP11/44 and MicroVAX computers by use of the Enraf–Nonius Structure Determination Package (SDP) incorporating the direct methods program MULTAN-1/82. For structure–factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from Ref. 30. In the least-squares iterations, $\Sigma w\Delta^2$ [$w = 1/\sigma 2$ ($|F_o|$), $\Delta = (|F_o| - |F_c|)$] was minimized.

*Isolation of human DNA topoisomerase II*

Human DNA topoisomerase II was isolated from peripheral blast cells of a patient with acute leukemia. The isolation procedure is a partial combination of the procedures of Goto *et al.*[31] and Halligan *et al.*[32] which have been detailed in our previous work.[11–15]

*DNA topoisomerase assay*

Drugs were dissolved in $Me_2SO$ at a concentration of 20 mM as the stock solution and diluted before use with $H_2O$ to the desired concentration of each drug prior to use. The P4 unknotting reaction was a modification of the procedure reported by Hsieh.[33]

The reaction mixture (20 μL), which contained 50 mM HEPES (pH 7.0), 50 mM KCl, 100 mM NaCl, 0.1 mM EDTA, 10 mM $MgCl_2$, 1.0 mM ATP, 50 μg/mL bovine serum albumin, 0.4 μg P4 knotted DNA, and enzyme, was incubated with and without drugs.

Incubation of the reaction mixture at 37 °C for 30 min was followed by termination of the reaction by addition of 5.0 μL of a quenching solution (2% sodium dodecyl sulfate, 20% glycerol, 0.05% bromophenol blue). The samples were loaded onto a 1% agarose gel and subjected to electrophoresis at 55 V overnight using a buffer that contained 90 mM Tris-boric acid (pH 8.3) and 2.5 mM EDTA. At completion, the gel was stained in 0.5 μg/mL of ethidium bromide. A photograph was taken of the DNA bands visualized with fluorescence induced by a long-wavelength ($\lambda_{max}$ 340 nm) UV lamp. Estimation of the degree of inhibition of different drugs on human type II DNA topoisomerase was achieved by comparison of the relative densities of unknotted and knotted P4 DNA on agarose gel with those obtained by use of different dilutions of enzyme alone.

*K-SDS precipitation assay for protein–DNA complexes*

The effect of different drugs on the formation of protein-linked DNA breakage in drug-treated KB cells was determined by K-SDS assay. In this assay, the intracellular formation of covalent topoisomerase II–DNA complexes was quantitated using the potassium SDS precipitation assay, a procedure adapted from the method of Rowe *et al.*[34] The KB cells used in this study were obtained from the American Type Culture Collection (ATCC) and were prelabeled with 0.05 mCi/mL {$^{14}$C}thymidine (specific activity 50.5 mCi/mmol) for 18 h. A final concentration of 5 x $10^5$ cells/sample was treated with 10 mM of the

drugs at 37 °C for 1 h, and the procedure described in Rowe *et al.*[34] was used to detect the protein-linked DNA levels.

*Cytotoxicity assay*

The cytotoxicity (KB) assay was carried out according to the procedure described in Ferguson *et al.*[35]

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## References and Notes

1. Li, L.; Wang, H. K.; Kuo, S. C.; Lednicer, D.; Lin, C. M.; Hamel, E.; Lee, K. H. *J. Med. Chem.*, in press.

2. Wang; J. C. *Annu. Rev. Biochem.* **1985**, *54*, 665.

3. Maxwell, A.; Gellert, M. *Adv. Protein Chem.* **1986**, *38*, 69.

4. Holm, C.; Goto, T.; Wang, J. C.; Botstein, D. *Cell* **1985**, *41*, 553.

5. Lock, R. B.; Ross, W. E. *Anti-Cancer Drug Design* **1987**, *2*, 151.

6. Chen, G. L.; Yang, L.; Rowe, T. C.; Halligan, B. D.; Tewey, K.; Liu, L. F. *J. Biol. Chem.* **1984**, *259*, 13560.

7. Ross, W.; Rowe, T.; Glisson, B.; Yalowich, J.; Liu, L. F. *Cancer Res.* **1984**, *44*, 5857.

8. Rowe, T.; Kuppfer, G.; Ross, W. *Biochem. Pharmacol.* **1985**, *43*, 2483.

9. Tewey, K. M.; Chen, G. L.; Nelson, E. M.; Liu, L. F. *J. Biol. Chem.* **1984**, *259*, 9182.

10. Doyle, T. W. In *Etoposide (VP-16)—Current Status and New Developments*, p. 15, Issell, B. F.; Muggia, F. M.; Carter, S. K., Eds.; Academic Press; New York, 1984; and pertinent references cited therein.

11. Thurston, L. S.; Irie, H.; Tani, S.; Han, F. S.; Liu, Z. C.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1986**, *29*, 1547.

12. Thurston, L. S.; Imakura, Y.; Haruna, M.; Li, D. H.; Liu, Z. C.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1989**, *32*, 604.

13. Lee, K. H.; Imakura, Y.; Haruna, M.; Beers, S. A.; Thurston, L. S.; Dai, H. J.; Chen, C. H.; Liu, S. Y.; Cheng, Y. C. *J. Nat. Prod.* **1989**, *52*, 606.

14. Liu, S. Y.; Hwang, B. D.; Haruna, M.; Imakura, Y.; Lee, K. H.; Cheng, Y. C. *Mol. Pharmacol.* **1989**, *36*, 78.

15. Lee, K. H.; Beers, S. A.; Mori, M.; Wang, Z. Q.; Kuo, Y. H.; Li, L.; Liu, S. Y.; Cheng, Y. C. *J. Med. Chem.* **1990**, *33*, 1364.

16. Lee, K. H.; Huang, E. S.; Piantadosi, C.; Pagano, J. S.; Geissman, T. A. *Cancer Res.* **1971**, *31*, 1649.

17. Lee, K. H.; Hall, I. H.; Mar, E. C.; Starnes, C. O.; ElGebaly, S. A.; Waddell, T. G.; Hadgraff, R. I.; Ruffner, C. G.; Weidner, I. *Science* **1977**, *196*, 533.

18. Lee, K. H. Program and Abstracts—*16th Natl Med. Chem. Symp., Am Chem. Soc.*, pp. 43–58, June 18–22, Kalamazoo, Michigan, 1978.

19. Lee, K. H.; Ibuka, T.; Sims, D.; Muraoka, O.; Kiyokawa, H.; Hall, I. H.; Kim, H. L. *J. Med. Chem.* **1981**, *24*, 924.

20. Lee, K. H.; Yamagishi, T. *Abs. Chin. Med.* **1987**, *1*, 606; and literature cited therein.

21. Hall, I. H.; Lee, K. H.; Grippo, A. A.; Chaney, S. G.; Holbrook, D. J. *Pharm. Res.* **1987**, *4*, 509.

22. Hall, I. H.; Williams, Jr, W. L.; Grippo, A. A.; Lee, K. H.; Holbrook, D. J.; Chaney, S. G. *Anticancer Drugs* **1988**, *8*, 33.

23. Williams, Jr, W. L.; Hall, I. H.; Grippo, A. A.; Oswald, C. B.; Lee, K. H.; Holbrook, D. J.; Chaney, S. G. *J. Pharm. Sci.* **1988**, *77*, 178.

24. Williams, Jr, W. L.; Chaney, S. G.; Willingham, III, W.; Considine, R. T.; Hall, I. H.; Lee, K. H. *Biochim. Biophys. Acta* **1983**, *740*, 152.

25. Williams, Jr, W. L.; Chaney, S. G.; Hall, I. H.; Lee, K. H. *Biochemistry* **1984**, *23*, 5637.

26. Liou, Y. F.; Hall, I. H.; Lee, K. H.; Williams, Jr, W. L.; Chaney, S. G. *Biochim. Biophys. Acta* **1983**, *739*, 190.

27. Lee, K. H.; Huang, E. S.; Furukawa, H. *J. Pharm. Sci.* **1972**, *61*, 629.

28. Anisotropic temperature factor parameters, individual bond lengths involving hydrogen atoms, and a listing of observed and calculated structure amplitudes for **11** are available from one of the authors (ATMcP).

29. Allen, F. H.; Kennard, O.; Watson, D. G.; Brammer, L.; Orpen, A. G.; Taylor, R. *J. Chem. Soc., Perkin Trans 2* **1987**, S1.

30. *International Tables for X-Ray Crystallography, Vol. IV*, Ibers, J. A.; Hamilton, W. C., Eds.; Kynoch; Birmingham (Present Distributor Kluwer Academic Publishers; Dordrecht), 1974.

31. Goto, T.; Laipia, P.; Wang, J. *J. Biol. Chem.* **1984**, *259*, 10422.

32. Halligan, B.; Edwards, K.; Liu, L. F. *J. Biol. Chem.* **1985**, *260*, 2475.

33. Hsieh, T. *J. Biol. Chem.* **1983**, *258*, 8413.

34. Rowe, T. C.; Chen, G. L.; Hsiang, Y. H.; Liu L. F. *Cancer Res.* **1986**, *46*, 2021.

35. Ferguson, P. J.; Fisher, M. H.; Stephenson, J.; Li, D. H.; Zhou, B. S.; Cheng, Y. C. *Cancer Res.* **1988**, *48*, 5956.

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