Reaction of Irofulven with Zinc and Acid

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Reaction of antitumor agent irofulven (**1**) with zinc and acetic acid yielded several new indene derivatives (**6**, **7**, **8**, **10**) as well as the known indene (**9**). These all have greatly reduced toxicity to human leukemia (HL60) cells compared to irofulven.

Irofulven (**1**) is a promising antitumor agent that is currently in phase II clinical trials for several cancers including ovarian, prostate, and liver cancers.[1] The compound is derived from the toxic sesquiterpene illudin S (**2**), which on treatment with dilute H_2SO_4 gives acylfulvene (**3**). Reaction of **3** with formaldehyde and dilute H_2SO_4 yields **1**.[2]

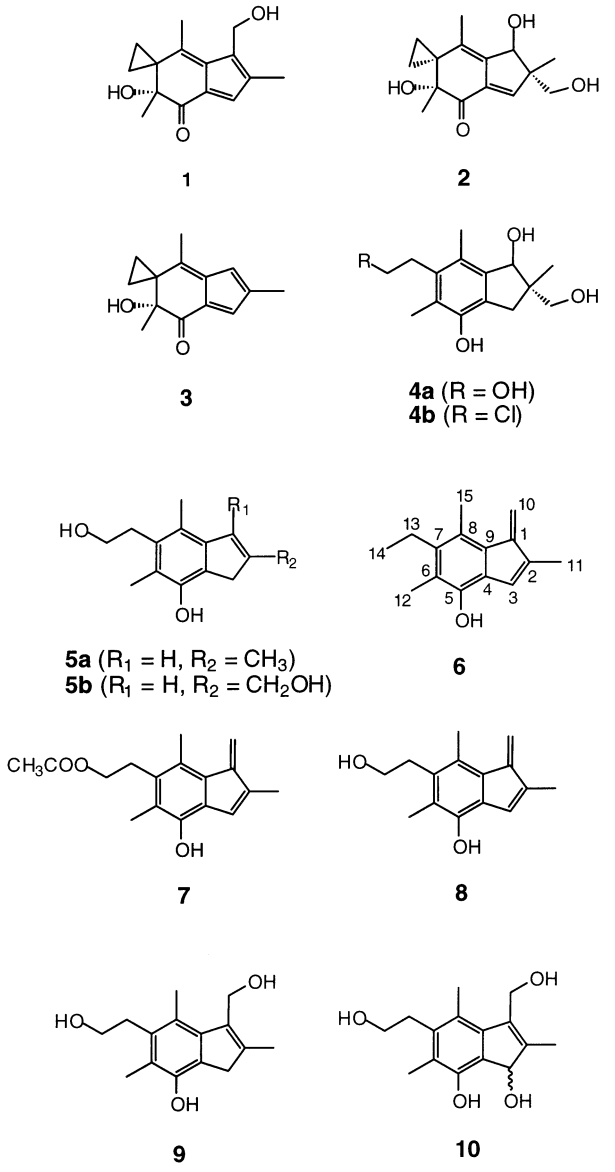
The toxicity and antitumor activity of compounds **1**, **2**, and **3** can be explained by their behavior as alkylating agents. As shown in Scheme 1, Michael-type addition to the $\alpha\beta$-unsaturated ketone gives a cyclohexadiene intermediate that is highly reactive and undergoes cyclopropane ring opening with attack by nucleophiles (protein, DNA, H_2O). The driving force in this reaction is relief of ring strain when the cyclopropane ring is cleaved and also the formation of the stable aromatic structure.[3]

The nucleophile in the Michael reaction can be a thiol such as cysteine or glutathione, or the thiol group of a protein.[4] It has also been found that reduction by a cytosolic NADPH-dependent enzyme can lead to similar aromatic products in the case of all three compounds[4] (**1**, **2**, **3**). Thus compounds **4a**, **4b**, **5a**, **5b**, and **9** have been identified in reduction with NADPH and cytosol from rat liver.

The metabolite **9** of irofulven is of special interest since it has been isolated from the plasma of rats to which irofulven was administered. We have therefore studied reactions of irofulven under reductive conditions in the hope of producing adequate amounts of metabolites for biological tests. Reactions were carried out simply by stirring a solution of **1** with acetic acid and zinc dust at room temperature. Standard workup followed by chromatography yielded five indene compounds (**6**, **7**, **8**, **9**, **10**). Their structures were determined by HREIMS and NMR spectroscopy (^{13}C, ^{1}H, NOESY, COSY, HMBC) as detailed in the Experimental Section and Supporting Information. The major product isolated was compound **8** with smaller amounts of **10**. Similar products (except for **7**) were obtained with dilute H_2SO_4 instead of acetic acid.

Formation of **8** may involve Michael-type reaction of **1** to give **9** followed by 1,4-elimination of water. A more likely route is zinc attack on the ketone leading to elimination of the protonated allylic hydroxyl. Cyclopropane ring opening and aromatization completes the sequence. A plausible pathway is shown in Scheme 2. Consistent with this pathway is the observation that **9** was unaffected when exposed to zinc and acid under the same conditions. Although only an ionic mechanism is given, the zinc reactions probably involve radical species as well, particularly in the production of **6**, where C−H bond formation must occur on opening of the cyclopropane.[6]

It is unlikely that **6**, **7**, and **8** are formed in the cell. Nevertheless it was of interest to test their biological activity. The toxicity of these compounds as well as **9** and



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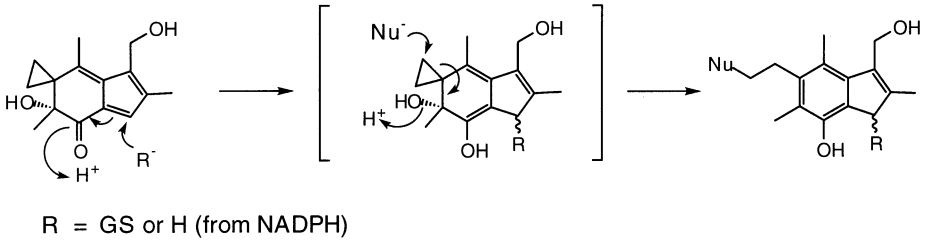
Scheme 1



Scheme 2

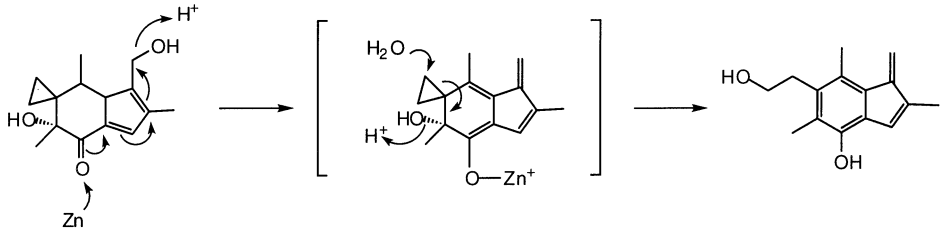


Table 1. Drug Cytotoxicity (IC_{50}) in HL 60 Cells (48 h) Trypan Blue Exclusion Assay

	ηM
illudin S	3 ± 1
irofulven	73 ± 8
6	140×10^3
7	110×10^3
8	130×10^3
9	120×10^3
10	114×10^3

[a] The IC_{50} is reported as a mean ± SD for three to seven experiments. For cytotoxicity tests, the compounds were dissolved in DMSO (1 mg/mL stock solution) and the solutions were diluted in 20% DMSO/phosphate buffered saline just prior to addition to cultures of HL 60 cells. Control cells received equal amounts of the DMSO/phosphate buffered saline. After incubation for 48 h the cells were washed, trypan blue was added, and the cells were counted. These values correlate closely with those determined by colony-forming assay.[7]

10 to HL 60 cells was determined. The results (Table 1) indicate that the compounds are far less toxic than the precursor **1**. In particular, **9**, the known metabolite of **1**, was more than 5000 times less toxic than **1**. These results emphasize the importance of the cyclopropyl methyl carbinol and $\alpha\beta$-unsaturated ketone structures in the toxicity of **1**, **2**, and **3**.

Experimental Section

General Experimental Procedures. All chromatography was carried out with silica gel (230−425 mesh). Analytical TLC was carried out on Whatman 4420 222 silica gel plates. Reactions were routinely monitored by TLC. Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR were measured at 400 and 100 MHz, respectively (Varian Mercury 400 spectrometer). Mass spectra were determined at the University of California, Riverside Mass Spectrometry Laboratory.

Reactions. (A) Zinc powder (7 g) was added to a solution of **1** (2.03 g) in acetone (40 mL) and H_2O (40 mL). Glacial HOAc (15 mL) was then added to the vigorously stirred mixture. After 60 min the reaction mixture was diluted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ and brine and dried over anhydrous $MgSO_4$. Concentration and chromatography (1:1 hexane/EtOAc, EtOAc alone, 10% MeOH in EtOAc) afforded **6** (10 mg), **7** (15 mg), **8** (360 mg, recrystallized from CH_2Cl_2), **9** (17 mg, triturated from MeOH/EtOAc), and **10** (108 mg, triturated from MeOH/EtOAc).

(B) Zinc powder (8 g) was added to a solution of **1** (2.33 g) in acetone (40 mL) and H_2O (40 mL). Dilute H_2SO_4 (1 M, 14 mL) was then added to the vigorously stirred mixture. After 30 min the reaction mixture was diluted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ and brine and dried over anhydrous $MgSO_4$. Concentration and chromatography (CH_2Cl_2 to 20% MeOH in CH_2Cl_2) afforded **6** (81 mg), **8** (680 mg, triturated with hexane/EtOAc), **9** (10.8 mg, triturated from MeOH/EtOAc), and **10** (40 mg, triturated from MeOH/EtOAc).

Indene 6: mp 153−154 °C; TLC R_f (1:1 hexane/EtOAc) 0.74; ^{1}H NMR (CD_3OD, 400 MHz) δ 1.08 (3H, t, J = 7.2 Hz, H-14), 2.08 (3H, s, H-11), 2.19 (3H, s, H-12), 2.37 (3H, s, H-15), 2.71 (2H, q, J = 7.2 Hz, H-13), 5.71 (1H, s, H-10), 5.95 (1H, d, J = 2 Hz, H-10), 6.68 (1H, br s, H-3); ^{13}C NMR (CD_3OD, 100 MHz) δ 11.1 (q, C-12), 11.6 (q, C-11), 13.5 (q, C-14), 14.6 (q, C-15), 22.3 (t, C-13), 113.0 (t, C-10), 123.5 (s, C-6), 124.2 (s, C-8), 125.2 (d, C-3), 127.4 (s, C-4), 132.0 (s, C-9), 135.3 (s, C-2), 139.1 (s, C-7), 145.0 (s, C-5), 151.3 (s, C-1); HRMS (DCI, isobutane), m/z 214.1355 (M^+ calcd for $C_{15}H_{18}O$, 214.1358).

Indene 7: mp 135−136 °C; TLC R_f (1:1 hexane/EtOAc) 0.63; ^{1}H NMR (CD_3OD, 400 MHz) δ 2.03 (3H, s, CH_3CO), 2.09 (3H, d, J = 2 Hz, H-11), 2.24 (3H, s, H-12), 2.41 (3H, s, H-15). 3.06 (2H, t, J = 8 Hz, H-13), 4.10 (2H, t, J = 8 Hz, H-14), 5.75 (1H, s, H-10), 5.98 (1H, d, J = 2 Hz, H-10), 6.70 (1H, br s, H-13); ^{13}C NMR (CD_3OD, 100 MHz) δ 11.4 (q, C-12), 11.6 (q, C-11), 14.9 (q, C-15), 19.7 (q, CH_3CO), 28.6 (t, C-13), 63.4 (t, C-14), 111.5 (t, C-10), 124.9 (s, C-6), 125.09 (d, C-3), 125.12 (s, C-8), 128.4 (s, C-4), 131.8 (s, C-9), 132.0 (s, C-7), 136.4 (s, C-2), 145.1 (s, C-5), 151.1 (s, C-1), 171.7 (s, CO); HRMS (DCI, isobutane) m/z 272.1408 (M^+, calcd for $C_{17}H_{20}O_3$, 272.1412).

Indene 8: mp 155−156 °C; TLC R_f (1:1 hexane/EtOAc) 0.37; UV (MeOH) λ_{max} (log ϵ) 248 (4.38), 347 (3.51) nm; ^{1}H NMR (CD_3OD, 400 MHz) δ 2.08 (3H, d, J = 1.6 Hz, H-11), 2.23 (3H, s, H-12), 2.40 (3H, s, H-15), 2.97 (2H, t, J = 8 Hz, H-13), 3.56 (2H, t, J = 8 Hz, H-14), 5.74 (1H, s, H-10), 5.97 (1H, d, J = 1.6 Hz, H-10), 6.69 (1H, br s, H-3); ^{13}C NMR (CD_3OD, 100 MHz) δ 11.4 (q, C-12), 11.6 (q, C-11), 14.9 (q, C-15), 32.8 (t, C-13), 61.3 (t, C-14), 113.3 (t, C-10), 124.7 (s, C-6), 125.0 (s, C-8), 125.1 (d, C-3), 128.0 (s, C-4), 132.9 (s, C-8), 136.1 (s, C-2), 145.0 (s, C-5), 151.2 (s, C-1); HRMS (DCI, isobutane) m/z 230.1306 (M^+, calcd for $C_{15}H_{18}O_2$, 230.1307).

Indene 9: mp >230 °C; TLC R_f (EtOAc) 0.47; ^{1}H NMR (CD_3OD, 400 MHz) δ 2.13 (3H, s, H-11), 2.23 (3H, s, H-12), 2.54 (3H, s, H-15), 2.97 (2H, t, J = 8 Hz, H-13), 3.17 (2H, s, H-3), 3.56 (2H, t, J = 8 Hz, H-14), 4.61 (2H, s H-10); ^{13}C NMR (CD_3OD, 100 MHz) δ 11.1 (q, C-12), 12.9 (q, C-11), 13.6 (q, C-15), 33.2 (t, C-13), 39.52 (t, C-3), 55.7 (t, C-10), 61.1 (t, C-14), 120.1 (s, C-6), 121.2 (s, C-8), 126.1 (s, C-4), 134.3 (s, C-7), 137.2 (s, C-2), 142.40 (s, C-1), 142.44 (s, C-9), 147.8 (s, C-5).

Indene 10: mp >230 °C; TLC R_f (EtOAc) 0.15; ^{1}H NMR (DMSO-d_6 + CD_3OD, 400 MHz) δ 1.23 (3H, s, H-11), 2.38 (3H, s, H-12), 2.61 (3H, s, H-15), 3.03 (2H, t, J = 8 Hz, H-13), 3.61

(2H, t, $J = 8$ Hz, H-14), 4.46 (2H, s, H-10), 4.61 (1H, s, H-3); ^{13}C NMR (DMSO + CD_3OD, 100 MHz) δ 12.1 (q, C-11), 12.8 (q, C-12), 14.2 (q, C-15), 33.9 (t, C-13), 48.7 (d, C-3), 55.2 (t, C-10), 61.0 (t, C-14), 120.8 (s, C-6), 121.2 (s, C-8), 130.0 (s, C-4), 135.4 (s, C-7), 137.6 (s, C-2), 142.2 (s, C-9), 145.0 (s, C-1), 148.4 (s, C-5).

Supporting Information Available: NOESY, HMBC, and additional physical constant data for compounds **6**−**10** are available free of charge via the Internet at http://pubs.acs.org.

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