161.2, 180.4, 186.4. Anal. Calcd for $C_{16}H_{12}O_3$: C, 76.19; H, 4.76. Found: C, 75.98; H, 5.00.

(±)-1,4-Ethano-5-methoxy-1,4-dihydro-9,10-anthraquinone (10). A suspension of **20** (50 mg, 0.2 mmol), Ag_2O (460 mg, 1.98 mmol), and MeI (0.4 mL, 6.42 mmol) in 10 mL of dry CH_2Cl_2 was vigorously stirred at rt. After 24 h, the solution was filtered and the solvent evaporated to afford compound (±)-**10** (49 mg, 93% yield): mp 148–149 °C (methanol).

(+)-(1*R*,4*S*)-1,4-Ethano-1-methoxy-1,4-dihydro-9,10-anthraquinone (12a). Compound (+)-**12a** was obtained following method D from 89 mg of **1a** (56 mg, 78% yield): mp 124–125 °C (methanol); $[\alpha]^{20}_D$ +114° (*c* = 1, $CHCl_3$); IR (KBr) 1660, 1590, 1280, 720 cm^{-1}; MS *m/z* (relative intensity) 266 (M^+, 3), 238 (100), 209 (46), 181 (20), 152 (32); ^{1}H-NMR δ 1.40–1.90 (4 H, m, H_{11a}, H_{11b}, H_{12a}, and H_{12b}), 3.70 (3 H, s, CH_3O), 4.50 (1 H, ddt, *J* = 1.6, 6.1, and 2.6 Hz, H_4) 6.42 (1 H, dd, *J* = 6.1 and 7.8 Hz, H_3), 6.63 (1 H, dd, *J* = 1.6 and 7.8 Hz, H_2), 7.70 (2 H, m, H_6 and H_7), 8.07 (2 H, m, H_5 and H_8); ^{13}C-NMR δ 24.8, 31.0, 33.4, 55.6, 85.0, 125.7, 126.3, 130.0, 131.2, 132.9, 133.6, 135.1, 136.3, 147.5, 150.4, 180.8, 181.1. Anal. Calcd for $C_{17}H_{14}O_3$: C, 76.69; H, 5.26. Found: C, 76.80; H, 5.25.

(+)-(1*R*,4*S*)-1,4-Ethano-1,5-dimethoxy-1,4-dihydro-9,10-anthraquinone (12b). Compound (+)-**12b** was obtained following method D from 98 mg of **1b** (63 mg, 77% yield): 122–123 °C (methanol); $[\alpha]^{20}_D$ +55° (*c* = 0.5, $CHCl_3$); IR (KBr) 1651, 1585, 1472, 1290, 1057 cm^{-1}; MS *m/z* (relative intensity) 296 (M^+, 4), 268 (42), 253 (100), 209 (23), 180 (21), 152 (35), 139 (15); ^{1}H-NMR δ 1.30–1.90 (4 H, m, H_{11a}, H_{11b}, H_{12a}, and H_{12b}), 3.67 and 3.99 (6 H, 2s, $2CH_3O$), 4.50 (1 H, m, H_4), 6.38 (1 H, dd, *J* = 6.1 and 7.8 Hz, H_3), 6.60 (1 H, dd, *J* = 1.6 and 7.8 Hz, H_2), 7.23 (1 H, dd, *J* = 1.4 and 8.2 Hz, H_6) 7.63 (1 H, dd, *J* = 7.7 and 8.2 Hz, H_7), 7.73 (1 H, dd, *J* = 1.4 and 7.7 Hz, H_8); ^{13}C-NMR δ 25.1, 31.2, 33.7, 55.6, 56.4, 84.9, 117.0, 119.2, 119.3, 131.5, 134.7, 135.3, 135.6, 145.4, 152.1, 159.3, 180.7, 180.8. Anal. Calcd for $C_{18}H_{16}O_4$: C, 72.97; H, 5.41. Found: C, 73.11; H, 5.50.

(+)-(1*R*,4*S*)-1,4-Ethano-1,8-dimethoxy-1,4-dihydro-9,10-anthraquinone (12c). Compound (+)-**12c** was obtained following method D from 98 mg of **1c** (65 mg, 80% yield): mp 120–121 °C (methanol); $[\alpha]^{20}_D$ +76° (*c* = 0.5, $CHCl_3$); IR (KBr) 1660, 1580, 1470, 1295, 1050 cm^{-1}; MS *m/z* (relative intensity) 296 (M^+, 3), 268 (36), 253 (100), 236 (12), 209 (12), 180 (13), 152 (27), 139 (28); ^{1}H-NMR δ 1.30–1.90 (4 H, m, H_{11a}, H_{11b}, H_{12a}, and H_{12b}), 3.69 and 3.97 (6 H, 2s, $2CH_3O$), 4.41 (1 H, m, H_4), 6.38 (1 H, dd, *J* = 6.1 and 7.8 Hz, H_3), 6.59 (1 H, dd, *J* = 1.6 and 7.8 Hz, H_2), 7.26 (1 H, dd, *J* = 1.4 and 8.2 Hz, H_7), 7.59 (1 H, dd, *J* = 7.6 and 8.2 Hz, H_6), 7.69 (1 H, dd, *J* = 1.4 and 7.6 Hz, H_5); ^{13}C-NMR δ 25.2, 31.4, 33.3, 55.7, 56.6, 85.4, 123.8, 124.9, 125.5, 130.1, 131.5, 133.9, 135.5, 137.1, 140.2, 148.0, 181.1, 181.5. Anal. Calcd for $C_{18}H_{16}O_4$: C, 72.97; H, 5.41. Found: C, 72.80; H, 5.59.

1,5-Dimethoxy-9,10-anthraquinone (14). Compound **12b** (55mg, 0.2 mmol) was heated at 110 °C in vacuo (6 mmHg) to give, after crystallization of the residue, compound **14** (39 mg, 72% yield): mp 234–235 °C (methanol) (lit.[24] mp 237 °C); MS *m/z* (relative intensity) 268 (M^+, 38), 253 (100), 152 (35), 139 (39); ^{1}H-NMR δ 4.04 (6 H, s, $2CH_3O$), 7.28 (2 H, dd, *J* = 1.2 and 8.4 Hz, H_2 and H_6), 7.70 (2 H, dd, *J* = 7.8 and 8.4 Hz, H_3 and H_7), 7.91 (2 H, dd, *J* = 1.2 and 7.8 Hz, H_4 and H_8); ^{13}C-NMR δ 56.4 (2 C), 116.7 (2 C), 119.7 (2 C), 123.9 (2 C), 135.0 (2 C), 137.4 (2 C), 159.7 (2 C), 182.7 (2 C). Anal. Calcd for $C_{16}H_{12}O_4$: C, 71.64; H, 4.48. Found: C, 71.48; H, 4.33.

1,8-Dimethoxy-9,10-anthraquinone (15). Compound **12c** (55 mg, 0.2 mmol) was heated at 120 °C in vacuo (8 mmHg) to give, after crystallization of the residue, compound **15** (40 mg, 75% yield): mp 222–223 °C (methanol) (lit.[25] mp 223 °C); MS *m/z* (relative intensity) 268 (M^+, 100), 254 (59), 237 (50), 209 (31), 181 (24), 152 (57), 139 (70); ^{1}H-NMR δ 4.01 (6 H, s, $2CH_3O$), 7.30 (2 H, dd, *J* = 1.2 and 8.4 Hz, H_2 and H_7), 7.63 (2 H, dd, *J* = 7.8 and 8.4 Hz, H_3 and H_6), 7.85 (2 H, dd, *J* = 1.2 and 7.8 Hz, H_4 and H_5); ^{13}C-NMR δ 56.4 (2 C), 118.0 (2 C), 118.8 (2 C), 124.0 (2 C), 133.8 (2 C), 134.7 (2 C), 159.4 (2 C), 182.8, 183.9. Anal. Calcd for $C_{16}H_{12}O_4$: C, 71.64; H, 4.48. Found: C, 71.71; H, 4.43.

Acknowledgment. We thank Dirección General de Investigación Científica y Técnica (Grant PB88-0176) for financial support and Mr. José M. Mata for the synthesis of compounds **2a,b** and **3a,b**.

Supplementary Material Available: ^{1}H NMR spectra of **2a–c**, **3a–c**, and **11a,b** (11 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Structure–Activity Relationships of Illudins: Analogs with Improved Therapeutic Index

Trevor C. McMorris,*[1a] Michael J. Kelner,[1b] Wen Wang,[1a] Leita A. Estes,[1b] Mark A. Montoya,[1b] and Raymond Taetle[1c]

Departments of Chemistry and Pathology, University of California, San Diego, La Jolla, California 92093

Received May 22, 1992

Illudin S and M are extremely toxic sesquiterpenes produced by *Omphalotus illudens*. At low pH they behave as bifunctional alkylating agents, but at physiological pH they do not react with oxygen or nitrogen nucleophiles. Illudins react spontaneously with sulfur nucleophiles, glutathione or cysteine, at or slightly below pH 7, and toxicity to HL 60 cells can be modulated by altering glutathione levels in cells. Analogs of illudin M, e.g. the deoxy and, particularly, the dehydro derivatives, are less reactive to thiols and correspondingly less toxic to HL 60 cells than the parent compound. Dehydroilludin M has been found to be quite effective at inhibiting tumor growth in vivo at doses well tolerated by athymic nude mice.

Introduction

The poisonous nature of the jack-o'-lantern mushroom, *Omphalotus illudens* (formerly *Clitocybe illudens*), has been known for a long time. For example, there were reports in the *New York Botanical Garden Journal* in 1938 and 1939 of persons who became ill after eating the mushroom.[2] Fortunately, they vomited, and so recovered quickly. In a report in *Nature* in 1963 on the isolation of an antitumor substance from *Lampteromyces japonicus* (synonymous with *O. illudens*), a bioluminescent mushroom,[3] it was stated that the mushroom was known in

(1) (a) Department of Chemistry, University of California—San Diego, La Jolla, California 92093. (b) Department of Pathology, University of California—San Diego, La Jolla, California 92093. (c) Present address: Division of Hematology, Arizona Cancer Center, 1501 N. Campbell Ave., University of Arizona, Tucson, Arizona 95724.

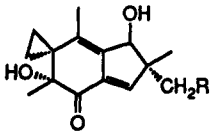
(2) Seaver, F. J. *J. N.Y. Botan. Garden* **1938**, 263. Seaver, F. J. *J. N.Y. Botan. Garden* **1939**, 236.

(3) Nakanishi, K.; Tada, M.; Yamada, Y.; Ohashi, M.; Komatsu, N.; Terekawa, H. *Nature* **1963**, *197*, 292.

0022-3263/92/1957-6876$03.00/0

Japan for its toxicity, which had occasionally caused fatal accidents due to its similarity in appearance to edible mushrooms. Cases of poisoning by the mushroom *Clitocybe olearia* Maire (syn. *Pleurotus olearius* Gillet, *Omphalotus olearius* Sing.) in Yugoslavia have been reported.[4,5] In one report, all 23 patients complained of nausea and vomiting following ingestion of the mushroom. Similar cases of nausea and vomiting were reported recently among campers in Maryland, who ingested jack-o'-lantern mushroom.[6]

A survey of wood-destroying fungi for antibacterial activity was carried out at the New York Botanical Garden in 1943, and *O. illudens* was found to inhibit the growth of *Staphylococcus aureus*.[7] Isolation of crystalline antibiotic substances illudin S (1) and illudin M (2) from

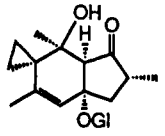


1. Illudin S (R=OH)
2. Illudin M (R=H)

culture liquids of the fungus was reported in 1950,[8] and elucidation of their structures was reported in 1963.[9,10] Illudin S was also isolated from the mushroom *L. japonicus* by two groups in Japan and was found to be the toxic factor in the mushroom.[11,12] The toxicity to mice was reported to be 5 mg/kg on intraperitoneal injection. In an earlier report, illudin S and illudin M were found to be lethal to mice at 15.6 mg/kg when a 0.9% saline solution of either compound was injected into a tail vein.[8]

Illudins and certain derivatives have been evaluated for antitumor activity in the National Cancer Institute Developmental Therapeutics Program. Illudin M significantly increased the life span of rats with Dunning leukemia and had activity in another leukemia model, but had a low therapeutic index in solid tumor systems.[13]

A number of compounds related to illudins have been isolated from *O. illudens* and other basidiomycetes.[14] Rather surprisingly, similar compounds have also been found in certain ferns, including bracken, *Pteridium aquilinum*,[15] a widely distributed fern, said to be the most economically important of ferns.[16] One bracken metabolite named ptaquiloside (3) was found to be carcinogenic



3. Ptaquiloside

Table I. IC_{50} Values for Illudin Analogs and Some Known Compounds When Tested in HL-60 Cells

compounds	nM
illudin S (1) or M (2)	3 ± 1 (0.8 ng/mL)
illudin S monoacetate	8 ± 2
illudin S mono 3,5-dinitrobenzoate	13 ± 2
illudin S mono (fluorosulfonyl)benzoate	60 ± 10
illudin S diacetate	240 ± 40
dihydroilludin S (6) or M (7)	>100 000
isoilludin M (8)	37 000 ± 200
dehydroilludin M (18)	296 ± 8 (73 ng/mL)
deoxyilludin M (5)	31 ± 4 (7 ng/mL)
dehydropterosin B acetate (10)	410 ± 20 (106 ng/mL)
pterosin C (13)	11 500 ± 900 (2,7 μg/mL)
chloroindantriol 11 or 12	5 500 ± 200
chloroindantriol 12 or 11	25 100 ± 400
2,5,6,7-tetramethylindenone 14	490 ± 40 (91 ng/mL)
ptaquiloside (3)	7 400 ± 700 (2.9 μg/mL)
tetrahydrofurano analog 22	1 947 ± 200
chlorodeoxyilludin M (23)	6 500 ± 800
anhydroilludin M (24)	>15 000
keto aldehyde 25	>25 000
DNA polymerase inhibitor: aphidicolin	1 800 ± 100
alkylating agent: BCNU	16 000 ± 2800
cross-linking agent: cisplatin	>550 ± 14
alkylating agent: MNNG	>15 000
protein synthesis inhibitor: ricin	0.2

and to be a toxic factor responsible for acute cattle bracken poisoning.[17] The fundamental feature of this toxicity is severe depression in bone marrow activity, causing aplasia of the granulocytic series with hypoplasia of the erythrocytic series.

For the past 12 years, we have been studying reactions of illudins and related sesquiterpenes from bracken in order to determine the reasons for their toxicity.[18] In addition, we have felt that clarification of the mechanism of toxicity would enable us to design analogs of illudins which would be less toxic but still retain antitumor properties.

Among our more important findings are the following: (1) Illudin S and illudin M are toxic to a wide range of tumor cells and normal cells after prolonged exposure (≥48 h), but show selective toxicity on short exposure (≤2 h) for human myelocytic leukemia and epidermoid, lung, ovarian, and breast carcinoma cells of various species of origin.[19] (2) Of great significance is the finding that the apparent histologic specificity of illudin S toxicity is based on an energy-dependent transport mechanism present in sensitive cells, but absent in cells relatively resistant to illudin S.[19] Many tumor cells possess this mechanism, whereas it appears to be absent from normal cells so far tested. (3) Illudin S is equally effective against CEM T-lymphocyte leukemia cells expressing the multidrug resistance phenotype associated with M_r 180 000 glycoprotein and the parental cell line. CEM cells resistant to doxorubicin, epipodophyllotoxins, and 1-β-D-arabinofuranosylcytosine show only a 2-fold increased resistance to illudin S.[20] (4) Cytokinetic experiments with HL 60 cells indicate that illudin S exerts a primary effect on DNA synthesis. Illudin S caused a complete block at the G_1–S phase interface of the cell cycle.[20]

The above results give strong indication of the potential of illudins as useful antitumor agents. However, because of their extreme toxicity and consequent low therapeutic

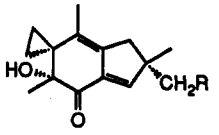
(4) Maretić, Z. *Toxicon* **1967**, *4*, 263.
(5) Maretić, Z.; Russell, F.; Golobić, V. *Toxicon* **1975**, *13*, 379.
(6) French, A. L.; Garrettson, L. K. *Clin. Toxicol.* **1988**, *26*, 81.
(7) Robbins, W. J.; Kavanagh, F.; Hervey, A. *Proc. Natl. Acad. Sci. U.S.A.* **1947**, *33*, 171.
(8) Anchel, M.; Hervey, A.; Robbins, W. *J. Proc. Natl. Acad. Sci. U.S.A.* **1950**, *36*, 300.
(9) McMorris, T. C.; Anchel, M. *J. Am. Chem. Soc.* **1963**, *85*, 831.
(10) McMorris, T. C.; Anchel, M. *J. Am. Chem. Soc.* **1965**, *87*, 1594.
(11) Nakanishi, K.; Ohashi, M.; Tada, M.; Yamada, Y. *Tetrahedron* **1965**, *21*, 1231.
(12) Matsumoto, T.; Shirahama, H.; Ichihara, A.; Fukuoka, Y.; Takahashi, Y.; Mori, Y.; Watanabe, M. *Tetrahedron* **1965**, *21*, 2671.
(13) Results from the NCI DTP Screening Program. Data may be obtained from the authors of this paper.
(14) Ayer, W. A.; Browne, L. M. *Tetrahedron* **1981**, *37*, 2199.
(15) Ng, K.-M. E.; McMorris, T. C. *Can. J. Chem.* **1984**, *62*, 1945.
(16) Tryon, R. M.; Tryon, A. F. *Ferns and Allied Plants*; Springer-Verlag: New York, 1981; p 387.
(17) Ojika, M.; Wakamatsu, K.; Niwa, H.; Yamada, K. *Tetrahedron* **1987**, *43*, 5261.
(18) McMorris, T. C.; Kelner, M. J.; Wang, W.; Moon, S.; Taetle, R. *Chem. Res. Toxicol.* **1990**, *3*, 574.
(19) Kelner, M. J.; McMorris, T. C.; Taetle, R. *J. Natl. Cancer Inst.* **1990**, *82*, 1562.
(20) Kelner, M. J.; McMorris, T. C.; Beck, W. T.; Zamora, J. M.; Taetle, R. *Cancer Res.* **1987**, *47*, 3186.

index, we have modified the structures to reduce cytotoxicity without compromising antitumor activity. In this report, we describe the preparation, biological activity, and analysis of analogs which has led to new compounds with improved therapeutic index.

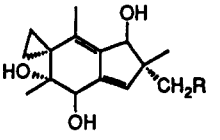
Results

Role of Functional Groups. Cytotoxicity determinations were carried out with human leukemia (HL 60) cells exposed to the compounds for 48 h. The results are shown in Table I. The IC_{50} values found for illudin S and illudin M to HL 60 cells are about the same, i.e. 3 nM (0.8 ng/mL), which implies that the primary hydroxyl in the former compound is not important for biological activity. Illudin S monoacetate and mono 3,5-dinitrobenzoate had an IC_{50} of the same order of magnitude as the parent compound, and the mono (fluorosulfonyl)benzoate was slightly less toxic. Illudin S diacetate was about 1/100 as toxic as the parent compound, suggesting that the free secondary hydroxyl in the five-membered ring is important for high toxicity. Isolation of deoxyilludin S (4) and M (5) was reported recently,[21] and the LD_{50} in mice of the latter was stated to be 5 mg/kg, greater than the 1.0 mg/kg that we found for illudin M. This indicates that the secondary hydroxyl enhances toxicity. The reason for the lower toxicity of illudin S diacetate may thus be lack of the free secondary hydroxyl.

As will be evident from following discussion, the tertiary hydroxyl plays a vital role in the unique reactivity of illudins. The carbonyl group is also most important for toxicity of illudins. Reduction of illudin S and illudin M with sodium borohydride rapidly gives the corresponding dihydro derivatives (6 and 7), which are at least 4 orders



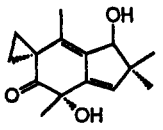
4. Deoxyilludin S (R=OH)
5. Deoxyilludin M (R=H)



6. Dihydroilludin S (R=OH)
7. Dihydroilludin M (R=H)

of magnitude less toxic than the parent compounds (Table I).

The α,β-unsaturated carbonyl moiety in illudins would be expected to behave as a Michael acceptor, and the product from reaction with a nucleophile would be expected to undergo further nucleophilic attack leading to stable aromatic species. Thus, the greatly reduced toxicity of dihydroilludin correlates with its inability to partake in the Michael reaction. Another derivative, the acyloin rearrangement product of illudin M, isoilludin M (8), is



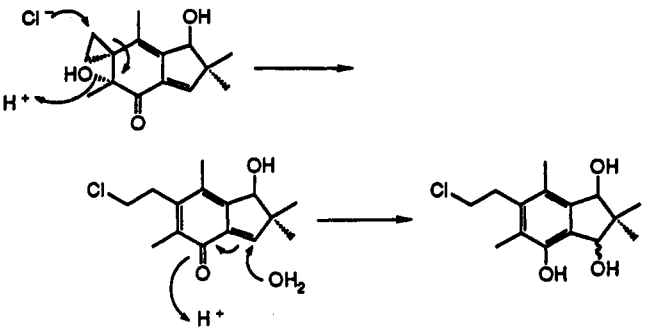
8. Isoilludin M

far less toxic than the parent compound, as anticipated.

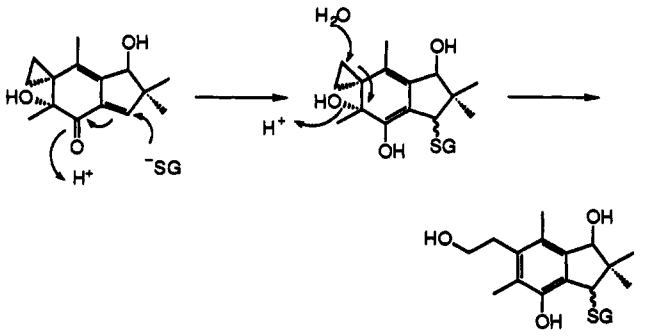
In seeking support for the hypothesis that toxicity is due to alkylating ability, reaction of illudins with various nucleophiles was examined. It was found that at low pH illudin M behaves as a bifunctional alkylating agent. The first reaction, however, involves loss of the tertiary hydroxyl and concomitant opening of the cyclopropane ring

(21) Hara, M.; Yoshida, M.; Morimoto, M.; Nakano, H. *J. Antibiot.* 1987, *40*, 1643.

Scheme I



Scheme II



by the nucleophile. The intermediate formed in this reaction is a quinone methide which rapidly reacts with water acting as nucleophile to give the aromatic product[22] (Scheme I).

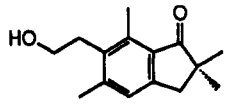
At neutral pH, illudins are completely unreactive to oxygen, nitrogen, or halogen nucleophiles. However, thiols react readily at room temperature, adding to the α,β-unsaturated carbonyl giving an intermediate which rapidly undergoes opening of the cyclopropane and loss of the tertiary hydroxyl.[18] The overall result is addition of two nucleophiles (Scheme II).

Significance of Indan Skeleton. A question that needs to be addressed at this point is the following: are the aromatic products formed by reaction of illudins with two nucleophiles cytotoxic, and do they possess antitumor activity? Aromatic compounds derived from illudins are related to indanones found in bracken fern *Pteridium aquilinum* and other ferns. These indanones called pterosins were reported to be cytotoxic and to possess antibacterial activity.[23] The two most cytotoxic compounds were pterosin Z (9) and dehydropterosin B acetate (10), both of which had an IC_{50} value of 10 μg/mL when tested on HeLa cells. Pterosin C (13) had an IC_{50} of 320 μg/mL.[23]

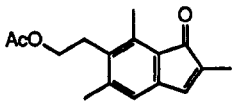
The chloroindantriols (11 and 12) derived from treatment of illudin M with dilute HCl were found in our studies to have IC_{50} values of 1.6 and 7.1 μg/mL. While these values are much higher than that for illudin M, they are comparable to the value found for the anticancer alkylating agent BCNU. It thus appears that the indan-type structures are inherently somewhat cytotoxic. We find pterosin C (synthetic) to be more toxic to HL 60 cells (IC_{50} 2.7 μg/mL) than reported for the 2*S*,3*S* isomer to HeLa cells.[23] Likewise dehydropterosin B acetate was more toxic to HL 60 cells (IC_{50} 0.1 μg/mL). A possible explanation

(22) McMorris, T. C.; Kelner, M. J.; Chadha, R. K.; Siegel, J. S.; Moon, S.; Moya, M. *Tetrahedon* 1989, *45*, 5433.

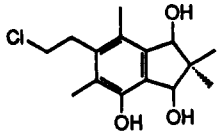
(23) Yoshihira, K.; Fukuoka, M.; Kuroyanagi, M.; Natori, S.; Umeda, M.; Morohoshi, T.; Enomoto, M.; Saito, M. *Chem. Pharm. Bull.* 1978, *26*, 2346.



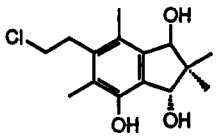
9. Pterosin Z



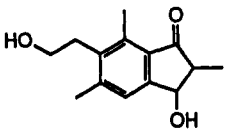
10. Dehydropterosin B acetate



11.

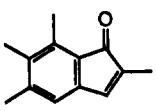


12.

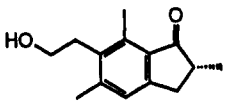


13. Pterosin C

for the increased toxicity of the latter compound compared to pterosin C is that it can act as a Michael acceptor in an alkylating reaction. We have found that the synthetic compound, 2,5,6,7-tetramethylindenone (14) is also quite toxic (IC_{50} 0.09 μg/mL). These indenones have been found to react with thiols near neutral pH giving the expected Michael adducts.



14.



15.

The major pterosin found in bracken fern is pterosin B (15). This compound may be an artifact, being formed by decomposition of an unstable precursor, ptaquiloside (3), which has also been isolated from bracken.[17,24] Ptaquiloside as mentioned earlier is a factor responsible for the toxicity to cattle when the fern is ingested. Ptaquiloside very readily loses a molecule of glucose giving an unstable dienone which behaves as a monofunctional alkylating agent. It reacts with water forming pterosin B. When tested against HL 60 cells, ptaquiloside had an IC_{50} of 2.9 μg/mL. It is therefore much less toxic than illudin S.

Reactivity with Thiols. The reaction of illudins with thiols is pH dependent, the optimum pH for reaction of illudin M with glutathione being 6.1.[18] This suggested that reaction with glutathione might occur in cells and that toxicity of illudins is modulated by glutathione. Experiments with HL 60 cells pretreated with agents that depress or enhance glutathione levels confirmed that illudins were more toxic to cells with depressed glutathione levels and less toxic to cells with enhanced glutathione levels.[18]

Thus, we were led to the hypothesis that toxicity of illudins is due partly to reaction in the cell with vital thiol enzymes. The intermediate formed in the reaction of illudins and the thiol group of a protein is highly reactive and is instantly attacked by solvent medium. However, it is possible that other nucleophiles, DNA or protein, in the immediate environment will also react, leading to cross-linking of protein to DNA or protein to protein.

Another possible way in which illudins might be activated is by hydrogenation of the α,β-unsaturated ketone

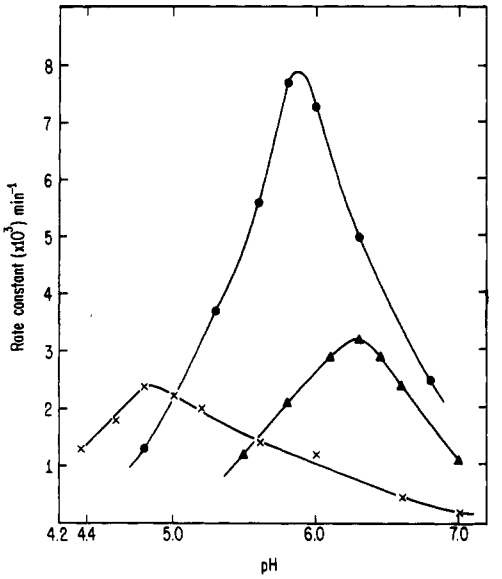
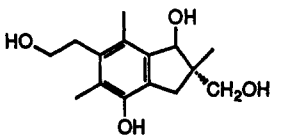
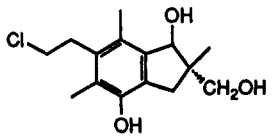


Figure 1. Dependence of rate constant on pH for reaction of methyl thioglycolate with illudin M (●), deoxyilludin M (▲), and dehydroilludin M (×) in methanol–water (1:1). In each case, a 10-fold molar excess of thiol was used.

to a cyclohexadiene intermediate. This intermediate, like the one in Scheme II, would be a powerful alkylating agent, reacting instantly with nucleophiles in the cell to give stable aromatic products. (Hydrogenation can be readily effected in the laboratory with a palladium catalyst or by reaction of illudins with zinc and dilute acid.[10]) Support for this possibility comes from the very recent report that illudin S is metabolized to aromatic products 16 and 17



16.



17.

by a rat liver cytosol preparation, with NADPH in phosphate buffer containing $MgCl_2$.[25,26] These products accounted for 50–60% of the substrate, the remainder, presumably, having reacted with biomacromolecules. A third possibility for activation of illudins would be conversion of the tertiary hydroxyl into a better leaving group, e.g. by phosphorylation. One could envisage such a molecule behaving as a bifunctional alkylating agent at physiological pH.

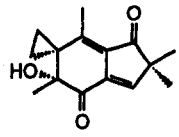
Since reactivity with thiols appears to be an indicator of extreme toxicity, we have examined analogs of illudins which retain the cyclopropyl tertiary carbinol and α,β-unsaturated carbonyl moieties but which are less reactive to thiols than the parent compounds.

One such analog is readily made by oxidizing the secondary hydroxyl of illudin M. The product, dehydroilludin M (18) is less toxic to HL 60 cells and has an IC_{50} of ~296 nM (73 ng/mL). This diketone reacts with dilute HCl giving the chloroindanone 19. However, the reaction is slower than the corresponding reaction with illudin M. The latter undergoes a pseudo first-order reaction with $k = 4.7 \times 10^{-3}$ min^{-1}, $t_{1/2}$ = 148 min at pH 0. Dehydroilludin M also shows first-order kinetics, but with $k = 2 \times 10^{-4}$ min^{-1}, $t_{1/2}$ = 2765 min. In this reaction no intermediate

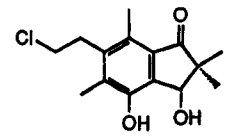
(24) Van der Hoeven, J. C. M.; Lagerweij, W. J.; Posthumus, M. A.; van Veldhuizen, A.; Holterman, H. A. J. *Carcinogenesis* **1983**, *4*, 1587.

(25) Tanaka, K.; Inoue, T.; Kadota, S.; Kikuchi, T. *Xenobiotica* **1990**, *20*, 671.

(26) Tanaka, K.; Inoue, T.; Kadota, S.; Kikuchi, T. *Xenobiotica* **1992**, *22*, 33.



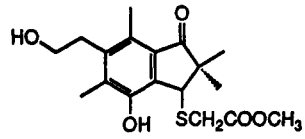
18. Dehydroilludin M



19.

could be detected by NMR spectroscopy, unlike the reaction of illudin M with dilute HCl.[22] Presumably it is formed too slowly and is too short-lived to be detected.

Dehydroilludin M reacts with methyl thioglycolate giving the expected product **20**, but the reaction is slower than

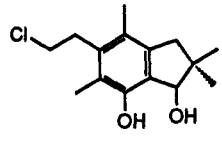


20.

that of illudin M and methyl thioglycolate. The rate of reaction was found to depend on the pH of medium as illustrated in Figure 1. It is also apparent that the optimum pH for reaction of the former compound is about 4.8 rather than 5.8 in the case of illudin M. Presumably, conjugation of the cyclopropane to the ketone (an "electron sink") reduces the tendency for delocalization of electrons toward the incipient carbocation resulting from departure of the tertiary hydroxyl, i.e. the carbocation is higher in energy and thus less easily formed than in illudin M.

The decreased reactivity of dehydroilludin M raises questions about the mechanism of cyclopropane ring opening in illudin M itself. At low pH, it is assumed that this occurs concomitantly with loss of the protonated tertiary hydroxyl. An alternative mechanism would involve ring opening, but with loss of the protonated secondary hydroxyl. Water or some other nucleophile could then add back at this position with loss of the tertiary hydroxyl. The resulting quinone methide would then undergo Michael reaction, leading to the same final product (Scheme III).

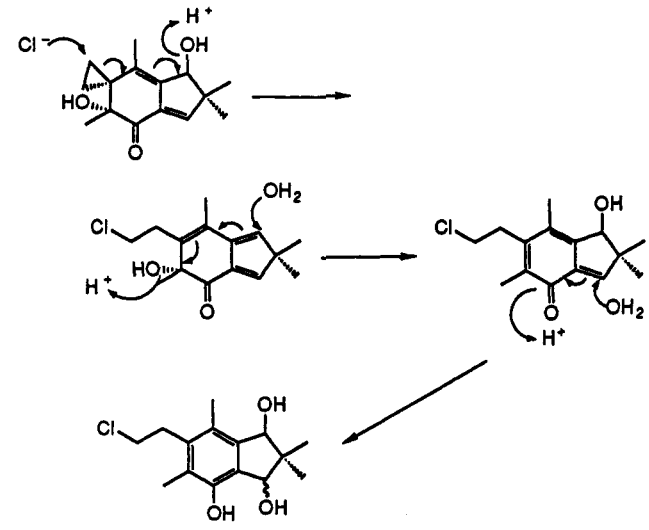
The availability of deoxyilludin M (**5**) allowed us to test this mechanism. We were unable to obtain natural deoxyilludin M. However, we have been able to convert illudin M to the deoxy analog by treatment with triethylsilane and boron trifluoride etherate in dichloromethane.[27] Deoxyilludin M was found to react in dilute HCl giving the chloroindandiol (**21**). The reaction was



21.

somewhat slower than the corresponding one with illudin M, suggesting that the mechanism involving loss of the secondary hydroxyl may also be operating, in the conversion of illudin M to the chloroindantriols (**11** and **12**). It is worth noting that because of poor solubility in water, the kinetic experiments with deoxyilludin M were carried out in methanol–water (1:1). In this medium at pH 0.18 (dilute HCl) the pseudo first-order rate constant was $k = 6.3 \times 10^{-4}$ min^{-1}. The corresponding values for illudin M and dehydroilludin M were 1.4×10^{-3} min^{-1} and 8.5×10^{-5} min^{-1}, respectively. (The rate constants were considerably

Scheme III



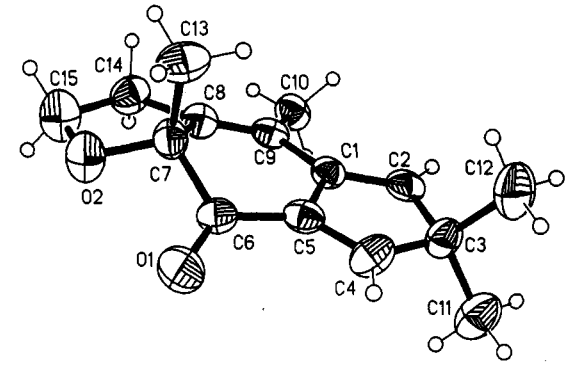


Figure 2. ORTEP view of X-ray molecular structure of the tetrahydrofurano analog **22**.

less than those determined in pure water, vide supra). Deoxyilludin M reacted with methyl thioglycolate in a pH-dependent manner the fastest rate occurring at pH 6.1 (Figure 1). As mentioned earlier, the toxicity of deoxyilludin M (LD_{50} in mice) was reported to be 5 mg/kg. We have confirmed that deoxyilludin M (**5**) is less toxic than illudin M but more toxic than dehydroilludin M (**18**, Table I). Thus, toxicity correlates with reactivity to thiols in the pH range 6–7, near the physiological pH. The increase in reactivity of all three compounds as the pH is lowered from 7 to 6 may be a factor in their selective toxicity to tumor cells over normal cells in vivo. It is known that the average extracellular pH is about 0.5 pH units less than in normal tissues (pH 7.0–7.5), and values of 6.0 or lower have been detected in some tumors.[28]

A byproduct from the reaction which yielded deoxyilludin M was found to have the structure **22** as established by X-ray crystallographic analysis (Figure 2). The latter is formed presumably by elimination of the secondary hydroxyl and opening of the cyclopropane ring on nucleophilic attack by the neighboring tertiary hydroxyl.

Two further analogs of illudin M were obtained in an attempt to prepare the mesyl derivative of the secondary hydroxyl by reaction with mesyl chloride in pyridine at −78 °C. Apparently the mesylate was so unstable that only products of elimination (**23** and **24**) could be isolated. Structure **23** in fact corresponds to an intermediate in Scheme III. As expected it was unstable, and it decomposed even when kept at 0 °C. Compound **24** was more

(27) Adlington, M. G.; Orfanopoulos, M.; Fry, J. L. *Tetrahedron Lett.* **1976**, *34*, 2955.

(28) Rotin, D.; Wan, P.; Grinstein, S.; Tannock, I. *Cancer Res.* **1987**, *47*, 1497.

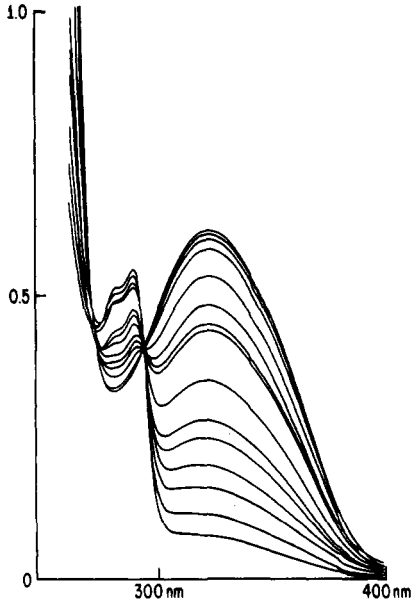
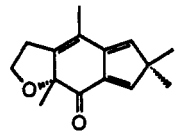
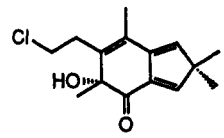


Figure 3. Change in UV absorption as illudin M (λ_{max} 320 nm) reacts with methyl thioglycolate giving the aromatic adduct (λ_{max} 287 nm) (see Scheme II).

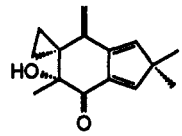
stable and exhibited low toxicity. It would not be expected to be as susceptible to nucleophilic attack leading to a stable aromatic compound as is the case with illudin M.



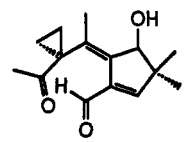
22.



23.



24.



25.

One additional compound, a keto aldehyde (**25**) derived by periodate cleavage of dihydroilludin M, was tested and was found to have very low toxicity as anticipated. Although this compound contains an α,β-unsaturated aldehyde, it reacted very slowly with methyl thioglycolate, indicating that the quaternary center inhibits Michael reaction at the adjacent double bond. The fact that reaction with thiols occurs so readily with illudin M is clearly a reflection of the powerful driving force to aromatization on the Michael adduct (Scheme II). This unstable intermediate has only a fleeting existence and could not be detected by low temperature NMR measurements even at high concentrations of thiol and relatively high pH values (pH 6–8). Consistent with the transient nature of the Michael adduct was the sharp isosbestic point observed in the reaction of illudin M with methyl thioglycolate (Figure 3).

Antitumor Activity. Deoxyilludin M is reported to cause increase of life span when tested against murine leukemia P388.[21] An ILS of 24% was obtained with a daily dose of 5 mg/kg for 5 days (ip). Illudin S and its 6-deoxy analog were found to be ineffective.[21] We have found dehydroilludin M (**18**) to be considerably more effective than illudin M when tested on Molt-4 (human myeloid leukemia) xenografts established in 4-week-old athymic Balb/c nu/nu mice.[29] Tumor growth was greatly inhibited (by about 75%) in treated animals compared to control animals. The doses, 3 mg/kg (iv, six doses in 14 days), were well tolerated by the animals. Thus, this compound possesses a greatly improved therapeutic index compared to illudin S or M. We are carrying out further in vivo experiments with various other xenografts, and details of all biological experiments will be published elsewhere. We are also preparing new analogs following guidelines indicated by the structure–activity studies in the hope of obtaining even more effective antitumor agents.

Experimental Section

^1H and ^{13}C NMR spectra were obtained at 300 or 500 MHz and 75 or 125 MHz, respectively. Spectra were taken as solutions in $CDCl_3$ with Me_4Si as internal standard or in D_2O with sodium (trimethylsilyl)propanesulfonate. Melting points were determined with a Kofler hot stage apparatus. Column chromatography was carried out with silica gel (Davisil 100–200 mesh and 230–425 mesh, Fisher Scientific). Analytical TLC was carried out on Whatman 4410 222 silica gel plates. Reactions were routinely monitored by TLC.

Preparation of Illudins and Analogs. Illudin S (**1**) and illudin M (**2**) were isolated from cultures of *O. illudens* (formerly *C. illudens*) as described previously.[8]

Illudin S monoacetate and diacetate were obtained by dissolving illudin S (13 mg) in pyridine (0.25 mL) and adding acetic anhydride (5.5 mg). The solution was kept overnight, pyridine and acetic acid were removed under reduced pressure, and the residue was chromatographed on silica gel with hexane–ethyl acetate 1:1. The monoacetate, diacetate, and unreacted illudin S were isolated and their structures confirmed by determination of spectra.[10]

Illudin S mono 3,5-dinitrobenzoate was obtained from reaction of 3,5-dinitrobenzoyl chloride and illudin S as reported earlier.[11] Similarly illudin S mono and bis[(fluorosulfonyl)benzoate] were obtained from the reaction with (fluorosulfonyl)benzoyl chloride and illudin S.

Dihydroilludin M (**7**) was obtained by reduction of illudin M with sodium borohydride.[10] A mixture of isomers was formed (9:1), and the major isomer was obtained by recrystallization of the mixture from ethyl acetate: mp 142–144 °C.

Isoilludin M (**8**) was prepared as reported previously, as were chloroindantriols **11** and **12**.[22]

Dehydropterosin B acetate (**10**), pterosin C (**13**), 2,5,6,7-tetramethylindenone (**14**), and pterosin B (**15**) were prepared as reported previously.[15] Ptaquiloside (**3**) was a gift from Professor K. Yamada, Nagoya University, Japan.

Dehydroilludin M (18). A mixture of illudin M (200 mg) and pyridinium dichromate (1 g) in CH_2Cl_2 (60 mL) was stirred under Ar at rt for 20 h. The reaction mixture was diluted with diethyl ether (20 mL) and filtered through a short column of silica. The column was further eluted with more diethyl ether and the combined filtrate was concentrated, giving a residue which was chromatographed on silica with hexane–ethyl acetate (10:1) yielding dehydroilludin M (**18**) (140 mg) as white crystals: mp 64–65 °C; ^1H NMR δ 0.62–1.05 (m, 4 cyclopropane H), 1.19 (s, 3 H, 1.22 (s, 3 H), 1.34 (s, 3 H), 2.05 (s, 3 H), 3.65 (s, OH), 6.84 (s, 1 H); UV (methanol) 288 nm (1.8×10^4); MS m/z 246.1257 calcd for $C_{15}H_{18}O_3$ 246.1256.

Chloroindanone 19. Dehydroilludin M (50 mg, 0.20 mmol) was dissolved in acetone (5 mL) at rt, and 6 N HCl (5 mL) was added. The solution was kept overnight during which the crystalline indanone precipitated. Removal of the acetone by warming gave a quantitative yield of **19** (56 mg): mp 160–163 °C; ^1H NMR δ 1.11 (s, 3 H), 1.28 (s, 3 H), 2.28 (d, J = 7.2 Hz, 1 H), 2.33 (s, 3 H), 2.59 (s, 3 H), 3.20 (t, J = 8.3 Hz, 2 H), 3.53 (t, J = 8.3 Hz, 2 h), 5.12 (d, J = 7.2 Hz, 1 H), 7.03 (s, 1 H); MS m/z 284 ($M + 2)^+$, 282.1024 (M^+), 266, 264 ($M^+ - H_2O$), 251, 249 ($M^+ - H_2O - CH_3$), 215 ($M^+ - Cl - CH_2 - H_2O$), 187 ($M^+ - ClCH_2 - H_2O - CO$). Calcd for $C_{15}H_{19}O_3Cl$ 282.1024.

Adduct of Dehydroilludin M with Methyl Thioglycolate (20). Dehydroilludin M (30 mg, 0.12 mmol) was dissolved in THF (1 mL) and H_2O (10 mL), and methyl thioglycolate (33 μL, 0.36

(29) Taetle, R.; Honeysett, J. M.; Rosen, F.; Shoemaker, R. *Cancer* **1986**, *58*, 1969.

mmol) was added. After 4 h at rt, the solution was extracted with Et_2O, and the extract was washed with brine and dried over $MgSO_4$. Removal of solvent gave a quantitative yield of adduct **20** as an oil: ^1H NMR δ 1.21 (s, 3 H), 1.39 (s, 3 H), 2.38 (s, 3 H), 2.59 (s, 3 H), 3.04 (t, J = 7.5 Hz, 2 H), 3.45 (ABq, J_{AB} = 17.4, 28.7 Hz, 2 H), 3.76 (t, J = 7.5 Hz, 2 H), 3.77 (s, OH), 3.86 (s, 3 H), 4.08 (s, 1 H), 7.99 (br, OH); MS m/z 352 (M^+), 279 ($M^+ - CH_2COOCH_3$), 247 ($M^+ - SCH_2COOCH_3$).

Upon adding $CCl_3CON{=}C{=}O$, a new triplet appeared at δ 4.31 (2 H), indicating esterification of the CH_2OH group, with disappearance of the triplet at δ 3.76.

Kinetics of Reaction of Dehydroilludin M with Dilute HCl. An aqueous solution of dehydroilludin M (0.2 mM) was adjusted to pH 0 with dilute HCl, and the reaction was allowed to proceed at rt (25 °C), monitored by measuring the decrease in intensity of the long wavelength UV absorption (λ_{max} 292 nm) of dehydroilludin M with time. Measurements were made for at least three half-lives. A good first-order plot was obtained with rate constant $k = 2.4 \times 10^{-4}$ min^{-1} and half life $t_{1/2}$ = 2765 min.

Deoxyilludin M (5). Boron trifluoride etherate (0.11 mL, 1.0 mmol) was added to a solution of illudin M (50 mg, 0.2 mmol) and triethylsilane (0.32 mL, 2.0 mmol) in 6 mL of CH_2Cl_2 at −78 °C under Ar.[27] The mixture was kept for 20 min at −78 °C, then quenched with water, and extracted with CH_2Cl_2. The organic layer was neutralized with saturated $NaHCO_3$, washed with water and brine, and dried over $MgSO_4$. Chromatography on silica with hexane and ethyl acetate (10:1) yielded 30 mg (64%) of a colorless oil (**5**) and 8 mg of a white solid (**22**). Deoxyilludin M (**5**) had the following properties: ^1H NMR δ 0.33 to 1.06 (m, 4 H), 1.16 (s, 3 H), 1.19 (s, 3 H), 1.33 (s, 3 H), 1.46 (s, 3 H), 2.46 (s, 2 H), 3.59 (s, 1 H), 6.54 (s, 1 H); MS m/z 232 (M^+), 218 ($M^+ - CH_2$), 217 ($M^+ - CH_3$), 203 ($M^+ - CH_3 - CH_2$), 189 ($M^+ - CH_3 - CH_2CH_2$), 175 ($M^+ - CH_3 - CH_2CH_2 - CH_2$). The tetrahydrofurano analog **22** mp 129–131 °C (recrystallized from CH_2Cl_2–hexanes); ^1H NMR δ 1.24 (s, 3 H), 1.29 (s, 3 H), 1.33 (s, 3 H), 1.92 (s, 3 H), 2.75 (m, 2 H), 3.99 (m, 1 H), 4.17 (m, 1 H), 5.96 (br. s, 1 H), 7.07 (d, J = 2.1 Hz, 1 H); MS m/z 230 (M^+), 215 ($M^+ - CH_3$), 187 ($M^+ - CH_3 - CH_2CH_2$), 159 ($M^+ - CH_3 - CH_2CH_2 - CO$); UV 244 nm ($8.0 \times 10^3$). An ORTEP view of the X-ray structure of the compound is shown in Figure 2.

Adduct of Deoxyilludin M with Methyl Thioglycolate (26). Deoxyilludin M (50 mg, 0.22 mmol) was dissolved in 2 mL of methanol, $HSCH_2COOCH_3$ (0.2 mL) was added, and the reaction mixture was stirred for 20 h. It was concentrated and chromatographed on silica with hexane–ethyl acetate (10:3) followed by further purification with HPLC with CH_3CN, CH_3OH, and H_2O (9:1:10), giving the noncrystalline adduct **26**: ^1H NMR δ 1.11 (s, 3 H), 1.36 (s, 3 H), 2.12 (s, 3 H), 2.25 (s, 3 H), 2.51 (d, J = 15.6 Hz, 1 H), 2.80 (d, J = 15.6 Hz, 1 H), 2.94 (t, J = 7.5 Hz, 2 H), 3.33 (m, 2 H), 3.38 (s, 3 H), 3.41 (t, J = 7.5 Hz, 2 H), 3.83 (s, 3 H), 3.92 (s, 1 H), 7.43 (s, 1 H); MS m/z 352 (M^+), 279 ($M^+ - CH_2COOCH_3$), 247 ($M^+ - SCH_2COOCH_3$), 201 ($M^+ - SCH_2COOCH_3 - H - CH_3O - CH_2$).

Chloroindandiol 21. Deoxyilludin M (10 mg, 0.04 mmol) was dissolved in 2 mL of acetone, and 5 N HCl (2 mL) was added. The reaction mixture was stirred for 1 h and extracted with ethyl acetate. The extract was washed with saturated $NaHCO_3$ solution, water, and brine, and dried over $MgSO_4$. Chromatography with hexane and ethyl acetate (10:3) yielded a white solid (**21**): mp: 119–120 °C; ^1H NMR δ 0.99 (s, 3 H), 1.27 (s, 3 H), 2.00 (d, J = 7.8 Hz, 1 H), 2.12 (s, 3 H), 2.21 (s, 3 H), 2.62 (q_{AB}, J_{AB} = 15.3, 20.1 Hz, 2 H), 3.11 (m, 2 H), 3.51 (m, 2 H), 5.03 (d, J = 7.5 Hz, 1 H), 6.87 (s, 1 H).

Kinetics of Reactions of Deoxyilludin M, Illudin M, and Dehydroilludin M with HCl in Methanol–H_2O (1:1). Solutions of illudin M (**2**), deoxyilludin M (**5**), and dehydroilludin M (**18**) (0.2 mM in MeOH–H_2O = 1:1) were acidified to pH 0.18 with dilute HCl. Reactions were allowed to proceed at rt and were monitored by measuring the decrease in intensities of the long wavelength absorption bands (λ_{max} 320, 335, and 290) of **2**, **5**, and **18** with time. Good first-order plots were obtained which gave the following rate constants: illudin M $k = 1.4 \times 10^{-3}$ s^{-1}, deoxyilludin M $k = 6.3 \times 10^{-4}$ s^{-1}, and dehydroilludin M $k = 8.5 \times 10^{-5}$ s^{-1}.

Kinetics of Reactions of Deoxyilludin M, Dehydroilludin M, and Illudin M with Methyl Thioglycolate. A series of methanol–water (1:1) buffer solutions were prepared by using sodium acetate–acetic acid (for pH values in the range of 4.0–5.4), 2-(*N*-morpholino)ethanesulfonic acid (MES, pK_a 6.15, for pH values in the range of 5.6–6.4), and piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES, pK_a 6.8, for pH values above 6.5). The pH of the solutions was measured with a pH meter. In all cases the buffer concentration was 50 mM. Deoxyilludin M, dehydroilludin M, and illudin M were dissolved in the buffer solutions to give in each case a concentration of 0.2 mM, and to each 10-mL solution was added methyl thioglycolate (1.8 μL). This concentration of methyl thioglycolate (2.0 mM) was chosen so as to obtain first-order kinetics. The reactions were allowed to proceed at rt and were monitored in the way described above. Good first-order plots were obtained in all cases. The results are shown in Figure 1.

Reaction of Illudin M with Methanesulfonyl Chloride. CH_3SO_2Cl (0.3 mL, 4 mmol) was added dropwise to a solution of illudin M (150 mg, 0.6 mmol) and triethylamine (0.75 mL) in CH_2Cl_2 (15 mL) at −78 °C. It was kept at that temperature for 1 h. Saturated $NaHCO_3$ solution was added, the mixture was shaken, and then the organic layer was dried over $MgSO_4$ and concentrated. Chromatography with hexane and ethyl acetate (10:1) gave two compounds (**23** and **24**). Compound **23** (18 mg) was a colorless oil: ^1H NMR δ 1.27 (s 3 H), 1.29 (s, 3 H), 1.32 (s, 3 H), 1.97 (s, 3 H), 2.84 (m, 2 H), 3.53 (m, 1 H), 3.64 (s, 1 H), 3.72 (m, 1 H), 6.06 (d, J = 2.1 Hz, 1 H), 7.14 (d, J = 2.1 Hz, 1 H); ^{13}C NMR δ 14.57, 21.92, 22.56, 29.41, 31.90, 43.45, 53.90, 78.00, 125.89, 134.12, 136.89, 138.26, 138.54, 153.05, 198.63; MS m/z 268 ($M + 2)^+$, 266 (M^+), 225, 223 ($M^+ - CH_3 - CO$), 175 ($M^+ - Cl - CH_2 - CH_2 - CO$); UV 244 nm ($4.8 \times 10^3$). Compound **24** (21 mg) was a white solid: mp 46–48 °C; ^1H NMR δ 0.19–1.02 (m, 4 H), 1.29 (s, 6 H), 1.32 (s, 3 H), 3.62 (s, 1 H), 4.94 (s, 1 H), 5.42 (s, 1 H), 6.39 (d, J = 2.5 Hz, 1 H), 7.20 (d, J = 2.5 Hz, 1 H). Coupling between the latter two protons (δ 6.39 and 7.20) was established by both decoupling experiments and 2D COSY NMR; MS m/z 230 (M^+), 215 ($M^+ - CH_3$), 202 ($M^+ - CH_2CH_2$), 187 ($M^+ - CH_2CH_2 - CH_3$); ^{13}C NMR δ 3.96, 11.98, 21.69, 21.92, 24.68, 30.54, 53.57, 75.37, 107.61, 135.79, 137.58, 138.46, 140.89, 154.25, 199.41; UV 210 nm (1.9×10^4), 227 nm (1.3×10^4), 247 nm (5.9×10^3).

Keto Aldehyde 25. Dihydroilludin M (200 mg, 0.80 mmol) was dissolved in ethyl acetate (8 mL) and a solution of $NaIO_4$ (400 mg, 1.87 mmol) in 8 mL of H_2O was added. The mixture was stirred vigorously for 1 h. The organic layer was separated and washed with H_2O and brine and dried over $MgSO_4$. Recrystallization of the product from ethyl acetate–hexane gave keto aldehyde **25** (150 mg, 75%): mp 102–103 °C; ^1H NMR δ 0.79–1.76 (m, 4 H), 1.02 (s, 3 H), 1.23 (s, 3 H), 2.10 (s, 3 H), 2.24 (s, 3 H), 4.27 (broad s, 1 H), 6.79 (s, 1 H), 9.70 (s, 1 H).[10]

Adduct of Dehydropterosin B with Methyl Thioglycolate (27). Dehydropterosin B[30] (50 mg, 0.23 mmol) was dissolved in THF (3 mL), 1 mL of $HSCH_2COOCH_3$ and 0.5 mL of saturated $NaHCO_3$ solution were added, and the mixture was stirred for 3 h. The mixture was extracted with Et_2O, and the extract was dried over $MgSO_4$ and concentrated. Chromatography with hexane–ethyl acetate (10:1) gave the adduct **27** as an oil (61 mg) with a yield of 81%: ^1H NMR δ 1.36 (d, J = 7.5 Hz, 3 H), 1.49 (t, J = 5.7 Hz, 1 H), 2.46 (s, 3 H), 2.68 (s, 3 H), 2.96 (q, J = 7.5 Hz, 1 H), 3.02 (t, J = 7.2 Hz, 2 H), 3.15 (ABq, J = 14.7, 21.3 Hz, 2 H), 3.72 (s, 3 H), 3.77 (m, 2 H), 4.63 (d, J = 7 Hz, 1 H), 7.36 (s, 1 H); MS m/z 322 (M^+), 292 ($M^+ - 2\ CH_3$), 249 ($M^+ - CH_2COOCH_3$), 217 ($M^+ - SCH_2COOCH_3$).

Adduct of 2,5,6,7-Tetramethylindenone with Methyl Thioglycolate (28). $HSCH_2COOCH_3$ (0.5 mL) was added to a THF solution (2 mL) containing 50 mg (0.27 mmol) of tetramethylindenone[30] and 0.5 mL of saturated $NaHCO_3$ solution. After 15 min the bright yellow color disappeared. The solution was partitioned between Et_2O and H_2O. The organic layer was washed with H_2O, brine, dried over $MgSO_4$, and concentrated. Chromatography with hexane–ethyl acetate (10:1) gave an adduct (**28**) as an oil in 80% yield: ^1H NMR δ 1.36 (d, J = 7.5 Hz, 3 H), 2.20 (s, 3 H), 2.38 (s, 3 H), 2.61 (s, 3 H), 2.96 (m, 1 H), 3.16 (ABq, J_{AB} = 15.0, 11.1 Hz, 2 H), 3.72 (s, 3 H), 4.64 (d, J = 6.9 Hz, 1 H), 7.33 (s, 1 H).

(30) Ng, K.-M. E. Ph. D Thesis, 1984, University of California, San Diego.

Cytotoxicity Tests. For cytotoxicity tests the compounds were dissolved in DMSO (1 mg/mL stock solution) and the solutions 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.[20]

Acknowledgment. This investigation was supported in part by grant 1-KT-02 from the Tobacco-related Disease Research Program of the University of California. X-ray crystallographic analysis was carried out by Dr Peter Gantzel, Department of Chemistry, University of California, San Diego.

Registry No. 1, 1149-99-1; 1 monoacetate, 144156-55-8; 1 diacetate, 1099-42-9; 1 mono(3,5-dinitrobenzoate), 3677-16-5; 1 mono(fluorosulfonylbenzoate), 144156-56-9; 1 bis(fluorosulfonylbenzoate), 144156-57-0; 2, 1146-04-9; 3, 87625-62-5; 4, 112953-13-6; 5, 112953-12-5; 6, 25532-76-7; 7, 34338-99-3; 8, 30950-46-0; 10, 35938-45-5; 10 alcohol, 93525-52-1; 11, 125392-73-6; 12, 125472-36-8; 13, 35938-43-3; 14, 137247-02-0; 15, 34175-96-7; 18, 28282-65-7; 19, 144156-45-6; 20, 144156-46-7; 21, 144156-47-8; 22, 144156-48-9; 23, 144156-49-0; 24, 144156-50-3; 25, 144156-51-4; 26, 144156-52-5; 27, 144156-53-6; 28, 144156-54-7; $HSCH_2COOCH_3$, 2365-48-2.

Supplementary Material Available: Experimental details of the X-ray structure determination of 22, tables of atomic coordinates and equivalent isotropic displacement coefficients, bond lengths, bond angles, and anisotropic displacement coefficients, and ^1H NMR spectra for compounds 5, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, and 28 (21 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Reductive Cyclization of Quinone Methides

Steven R. Angle* and Jon D. Rainier

Department of Chemistry, University of California, Riverside, California 92521-0403

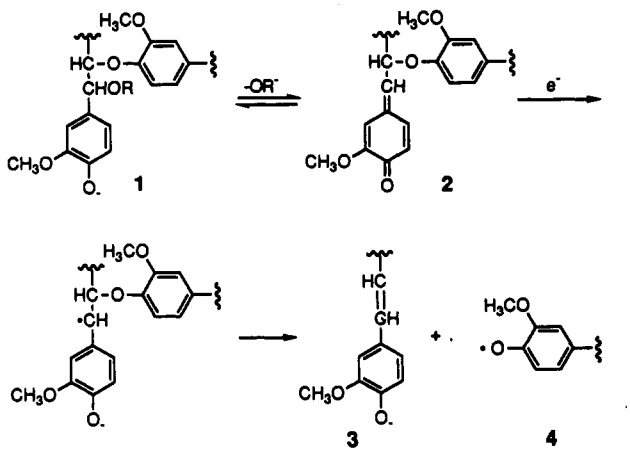
Received July 21, 1992

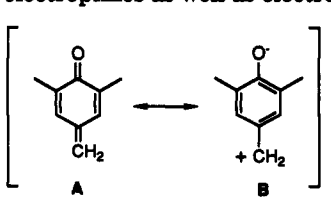
The first use of reduced quinone methides for the formation of carbon–carbon bonds in cyclization reactions is described. Treatment of quinone methides with SmI_2 resulted in intramolecular reaction with internal activated multiple bonds to afford 5- and 6-membered carbocycles in good yields. The activated multiple bonds used are aldehydes and α,β-unsaturated esters and nitriles.

Introduction

Simple *p*-quinone methides such as *p*-benzoquinone methide **A** are highly reactive compounds that readily undergo 1,6-addition of nucleophiles.[1,2] Aromatization of quinone methide **A** produces resonance structure **B** which illustrates the dipolar character of *p*-quinone methides. Upon O-protonation, or complexation with a Lewis Acid, the electrophilicity of the exocyclic alkylidene is enhanced to the point that even simple alkenes can act as nucleophiles toward quinone methides.[1,2] Quinone methides have been proposed as intermediates in a number of biological transformations and synthetic reactions.[1-4] Thus far, the synthesis applications of *p*-quinone methides have exploited their electrophilic character in reactions with nucleophiles, whereas the biological processes where quinone methides have been proposed as intermediates call for them to act as electrophiles as well as electron acceptors.[3]

Scheme I. Proposed Mechanism of Lignan Fragmentation[3]





Lignin fragmentation in pulping processes is proposed to involve the reduction of transient quinone methide intermediates.[4] In support of this notion, anthraquinone has been observed to accelerate fragmentation of lignans containing β-aryl ether linkages such as **1**. The acceleration

(1) (a) Angle, S. R.; Turnbull, K. D. *J. Am. Chem. Soc.* **1990**, *112*, 3698. (b) Angle, S. R.; Turnbull, K. D. *J. Am. Chem. Soc.* **1989**, *111*, 1136. (c) Angle, S. R.; Louie, M. S.; Mattson, H. L.; Yang, W. *Tetrahedron Lett.* **1989**, *30*, 1193. (d) Angle, S. R.; Arnaiz, D. O. *J. Org. Chem.* **1990**, *55*, 3708.

(2) For reviews on quinone methides see: (a) Turner, A. B. *Quart. Rev.* **1964**, *18*, 347. (b) Wagner, H. U.; Gompper, R. In *The Chemistry of Quinonoid Compounds*; Patai, S., Ed.; John Wiley and Sons: New York, 1974; Vol. 1, pp 1145–1178. (c) Gruenanger, P. In *Houben-Weyl Methoden der Organischen Chemie*; Mueller, E., Bayer, O., Eds.; G. Thieme Verlag: Stuttgart, 1979; Vol. VII/36, pp 395–521. (d) Peter, M. G. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 555. (e) Volod'kin, A. A.; Ershov, V. V. *Russ. Chem. Rev.* **1988**, *57*, 336.

(3) For recent reviews and leading references to lignans and neolignans see: (a) Macrae, W. D.; Towers, G. H. N. *Phytochemistry* **1984**, *23*, 1207. (b) Whiting, D. A. *Nat. Prod. Rep.* **1990**, *7*, 349. (c) Whiting, D. A. *Nat. Prod. Rep.* **1987**, *4*, 499.

(4) (a) Smith, D. A.; Dimmel, D. R. *J. Org. Chem.* **1988**, *53*, 5428 and references cited therein. (b) Pardini, V. L.; Smith, C. Z.; Utley, J. H. P.; Vargas, R. R.; Viertler, H. *J. Org. Chem.* **1991**, *56*, 7305.

0022-3263/92/1957-6883$03.00/0