Steroidal α-Methylene δ-Lactones as Potential Antitumor Agents

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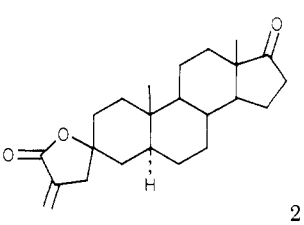
Four novel steroidal α-methylene δ-lactones have been synthesized and shown to be active against human nasopharyngeal carcinoma (KB) cells in culture. The syntheses involved the use of known α-methylenation procedures. In addition, the lactone **6** was directly methylenated by reaction with CH_2O/KOH or $Et_2NH/CH_2O/Et_2NH{\cdot}HCl$. The formation of a cysteine adduct (**15**) with the α-methylene lactone **10** is reported.

The achievement of selectivity is a major problem in cancer chemotherapy. One rationale of designing a less toxic drug is to attach an alkylating mustard moiety to a biological carrier. In this way, steroidal mustards have been developed and shown to be highly active against various experimental tumors.[1]

The α-methylene butyrolactone grouping found in several terpenoid compounds of natural origin is a highly active alkylating function and confers powerful cytotoxic activity on these compounds. Thus, the activity of elephantopin is ascribed to a rapid and essentially irreversible Michael-type addition to biological nucleophiles.[2-4]

Only compounds containing additional functional groups have activity in vivo,[5] but this is associated with poor therapeutic indices. Efforts to reduce toxicity by producing simpler butyrolactones have resulted in compounds cytotoxic in vitro[6,7] but, when tested, lacking useful activity in vivo.[7]

We have synthesized potential target-specific alkylating agents by introducing the α-methylene lactone function into the A and D rings of the steroid nucleus and evaluated these compounds against human nasopharyngeal carcinoma (KB) cells in culture. Recently, and after the start of this work, a series of steroidal α-methylene spirolactones (e.g., **1**) have been shown to be strongly cytotoxic.[8]



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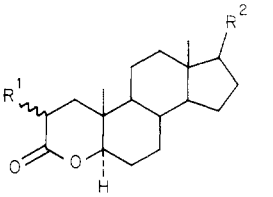
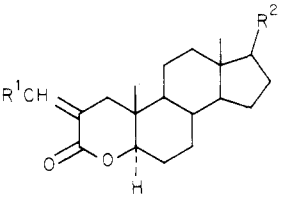
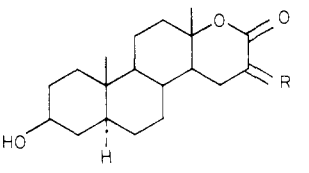


2, R^1 = H; R^2 = C_8H_{17}
3, R^1 = H; R^2 = OH
8, R^1 = CH_2NEt_2; R^2 = C_8H_{17}
9, R^1 = CH_2NEt_2; R^2 = OH
15, R^1 = $CH_2SCH_2CH(NH_3^+)CO_2^-$; R^2 = C_8H_{17}



4, R^1 = OH; R^2 = C_8H_{17}
5, R^1 = R^2 = OH
6, R^1 = NEt_2; R^2 = C_8H_{17}
7, R^1 = NEt_2; R^2 = OH
10, R^1 = H; R^2 = C_8H_{17}
11, R^1 = H; R^2 = OH
12, R^1 = H; R^2 = OAc



13, R = H
14, R = CH_2

Chemistry. α-Methylenation of the lactones **2**[9] and **3**[9] was achieved using the procedure of Yamada and coworkers.[10] Reaction of the lactone **2** with NaH/HCO_2Et in ether gave the hydroxymethylene lactone **4**[11] which was generally used without purification, owing to its relative instability. Conversion of the hydroxymethylene lactone (**4**) to the diethylaminomethylene lactone **6**,[11] followed by catalytic hydrogenation, gave the diethylaminomethyl derivative **8** and some α-methylene lactone **10**. Heating the mixture with NaOAc/AcOH gave the α-methylene lactone **10**. A similar procedure with the lactone **3** led through the hydroxymethylene lactone **5**,[11] the diethylaminomethylene lactone **7**,[11] and the diethylaminomethyl lactone **9** to the α-methylene lactone **11** and its acetylated derivative **12**. The acetylation leading to the latter compound occurs in the final elimination step.

Reaction of the lactone **13**[12] with HCHO/KOH in aqueous ethanol gave the α-methylene lactone **14** directly in modest yield (27%) with some recovery of starting lactone **13** (34%). This direct reaction demonstrates that it is not necessary to preform[13,14] the enolate of the lactone **13** in order to achieve reaction with formaldehyde. Direct α-methylenation of the lactone **13** was also achieved using a Mannich reaction with $HCHO/Et_2NH/Et_2NH{\cdot}HCl$. The conversion was relatively low (27%), and considerable starting lactone **13** (33%) was isolated. Even so, the reaction is of interest since direct application of the Mannich reaction to simple lactones has not been reported and, quite recently, the reaction of their preformed enolates with Eschenmoser's salt has been reported as a synthetic method for α-methylene lactones.[15,16] The generality of

(1) F. I. Carroll, A. Philip, J. T. Blackwell, D. J. Taylor, and M. E. Wall, *J. Med. Chem.*, **15**, 1158 (1972).
(2) S. M. Kupchan, D. C. Fessler, M. A. Eakin, and T. J. Giacobbe, *Science*, **168**, 376 (1970).
(3) R. L. Hanson, H. A. Hardy, and S. M. Kupchan, *Science*, **168**, 378 (1970).
(4) C. H. Smith, J. Larner, A. M. Thomas, S. M. Kupchan, *Biochim. Biophys. Acta*, **276**, 94 (1972).
(5) S. M. Kupchan, M. A. Eakin, and A. M. Thomas, *J. Med. Chem.*, **14**, 1147 (1971).
(6) A. Rosowsky, N. Papathanosopoulos, H. Lazarus, G. E. Foley, and E. J. Modest, *J. Med. Chem.*, **17**, 672 (1974).
(7) G. A. Howie, I. K. Stamos, and J. M. Cassidy, *J. Med. Chem.*, **19**, 309 (1976).
(8) K.-H. Lee, T. Ibuka, S.-H. Kim, B. R. Vestal, I. H. Hall, and E. S. Huang, *J. Med. Chem.*, **18**, 812 (1975).
(9) G. R. Pettit and T. Kasturi, *J. Org. Chem.*, **26**, 4557 (1961).
(10) K. Yamada, M. Kato, and Y. Hirata, *Tetrahedron Lett.*, 2745 (1973).
(11) These compounds are mixtures of *Z* and *E* isomers in solution, as indicated by the presence of high- and low-field signals for the olefinic proton in the ^1H NMR spectra. In the solid state, the compounds **4** and **5** appear to be largely the *E* isomers (IR).
(12) H. Levy and R. P. Jacobson, *J. Biol. Chem.*, **171**, 71 (1947).
(13) P. A. Grieco and K. Hiroi, *J. Chem. Soc., Chem. Commun.*, 1317 (1972).
(14) D. H. Lucast and J. Wemple, *Synthesis*, 724 (1976).
(15) J. L. Roberts, P. S. Borromeo, and C. D. Poulter, *Tetrahedron Lett.*, 1299, 1621 (1977).

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Table I. Effects of Steroidal α-Methylene Lactones and Related Compounds on Inhibition of Cell Growth

compd	LD_{50}, μg/mL KB	LD_{50}, μg/mL HEp-2[a]
elephantopin[b]	0.30[c]	0.48[e]
1		0.58[d]
10	0.82	
11	0.76	
12	0.72	
14	1.00	
testosterone	1.27	0.44, 0.58[e]
5α-pregnane-3,20-dione		0.84[d]

[a] Taken from literature sources as indicated. [b] Sample kindly provided by Professor A. T. Sneden, University of Virginia. [c] CCNSC data quotes 0.32 μg/mL (ref 21). [d] See ref 8. [e] See ref 22.

this direct Mannich reaction and the HCHO/KOH reaction has not been investigated.

The α-methylene lactone **10** readily forms the adduct **15** with L-cysteine and, although it was not possible to obtain a satisfactory NMR spectrum, owing to low solubility, the structure was supported by the IR and mass spectra.

Biological Results and Discussion

The steroidal α-methylene lactones **10–12** and **14** were evaluated for cytotoxicity against human nasopharyngeal carcinoma (KB) cells in culture. All of the compounds show inhibition of growth at concentrations of 1 μg/mL or less (Table I), that is, in the same concentration range as the plant product elephantopin.

The α-methylene lactone **10** readily reacts with L-cysteine in vitro (see Chemistry Section and Experimental Section), and the compounds may act in vivo in this way. However, scavenging of cysteine is probably not important, but more so is the rapid irreversible alkylation of thiol groups of cell-surface proteins[17] or essential intracellular proteins, possibly those involved in cell replication and differentiation.[18] None of our α-methylene lactones are active in the Ames mutagenicity tests,[19] suggesting that they do not modify DNA. Sesquiterpene α-methylene lactones do not alkylate nucleic acid bases,[18] although it has been recently suggested that the germacranolide *eupahyssopin* does react with DNA, as measured by UV absorption.[20]

The activity of testosterone against KB cells and H.Ep-2 cells and of 5α-pregnane-3,20-dione against the H.Ep-2 cells raises the possibility that the steroid nucleus may have activity of its own, in addition to the activity of the alkylating function. Certainly many of the digitalis glycosides are highly active against KB cells.[21] However, although only a few structures were tested, the degree of activity appears to be independent of whether the α-methylene lactone function is part of the A ring or of the D ring, suggesting that the alkylating function itself is sufficient to confer cytotoxic properties on the molecule. This is further confirmed by the inactivity (up to 50 μg/mL) of the lactone **2**.

Further work is planned to investigate the effects of the compounds in vivo and the possibility of specificity of action toward tumors of the breast and prostate.

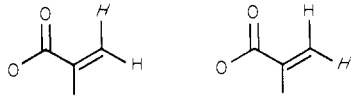
Experimental Section

Solutions were dried over anhydrous magnesium sulfate, and solvents were removed in vacuo on a rotary evaporator. Plates (1 m × 0.5 mm thick) of Kieselgel PF 254 (Merck) were used for preparative TLC. IR spectra were determined with Perkin-Elmer 257 and 177 spectrophotometers. UV spectra were determined with a Unicam SP 8000 spectrophotometer. ^1H NMR spectra were determined for solutions in $CDCl_3$, unless specified otherwise, at 60 MHz with a Varian EM360A spectrometer or at 90 MHz with a Perkin-Elmer R32 spectrometer. Mass spectra were determined with AEI MS902 and Kratos (AEI) MS 50 spectrometers. Rotations were measured for solutions in $CDCl_3$ at ambient temperature with a Bendix polarimeter 143C. Melting points were determined on a Kofler hot-stage apparatus.

2-(Hydroxymethylene)-4-oxa-5α-cholestan-3-one (4). The lactone **2** (388 mg, 1.0 mmol) was added to a stirred suspension of NaH (from 960 mg of a 50% dispersion in oil, 20 mmol) in dry ether (200 mL) under an atmosphere of nitrogen. After 5 h, ethyl formate (1.58 g, 20 mmol) was added and the reaction mixture was kept at ambient temperature for 70 h. The reaction mixture was carefully acidified with dilute hydrochloric acid, and the ether layer was washed with water (three times), dried, and evaporated to give the hydroxymethylene lactone 4 (374 mg, 84%), which was used directly in the next stage. A small sample which was crystallized from ethanol had mp 238–240 °C; $[\alpha]_D$ +104° (*c* 0.12); MS 416.3293 (M, $C_{27}H_{44}O_3$).

2-[(*N*,*N*-Diethylamino)methylene]-4-oxa-5α-cholestan-3-one (6). Diethylamine (1.46 g, 20 mmol) was added to a solution of the hydroxymethylene lactone 4 (374 mg, 0.9 mmol) in dry benzene (150 mL), and the reaction mixture was heated under reflux using a Dean–Stark trap. After 85 h, the solution was evaporated and the residue was crystallized from acetone–petroleum ether (bp 60–80 °C) to give the diethylaminomethylene lactone **6** (382 mg, 90%), which was used directly in the next stage. A small sample was recrystallized from acetone–petroleum ether (bp 60–80 °C) and gave mp 156–158 °C; $[\alpha]_D$ −16.2° (*c* 0.46); MS 471.4076 (M, $C_{31}H_{53}NO_2$).

2-Methylene-4-oxa-5α-cholestan-3-one (10). A solution of the diethylaminomethylene lactone **6** (375 mg, 0.8 mmol) in glacial acetic acid (40 mL) was stirred in an atmosphere of hydrogen in the presence of PtO_2 (50 mg) until the uptake of hydrogen ceased. The catalyst was removed by filtration and the filtrate was evaporated. The residue was taken up in acetic acid (40 mL) and sodium acetate (400 mg) was added, after which the mixture was heated under reflux until elimination was complete (TLC). After partial evaporation of the reaction mixture, excess saturated aqueous sodium bicarbonate was added and the aqueous mixture was extracted with ether (three times). The combined ether extracts were washed with water, dried, and evaporated. Crystallization of the residue from methanol gave the α-methylene lactone **10** (243 mg, 76%): mp 105–106 °C; $[\alpha]_D$ +90.5° (*c* 0.16); IR (KBr) 1728 (C=O), 1620 (C=C) cm^{-1}; UV λ_{max} (EtOH) 213 nm (ε 7190); ^1H NMR δ 6.5 (1 H, m, i), 5.57 (1 H, m, ii), 4.04 (1



i ii

H, q, $J \approx 11$ and 5 Hz, 5-H), 0.92 (3 H, s, 10-Me), 0.70 (3 H, s, 13-Me); MS 400.3341 (M, $C_{27}H_{44}O_2$). Anal. ($C_{27}H_{44}O_2$) C, H.

17β-Hydroxy-2-methylene-4-oxa-5α-androstan-3-one (11) and 17β-Acetoxy-2-methylene-4-oxa-5α-androstan-3-one (12). Lactone **3** was similarly converted via 17β-hydroxy-2-(hydroxymethylene)-4-oxa-5α-androstan-3-one [**5**; 87%; mp 221–223 °C; $[\alpha]_D$ +95°; MS 320.1986 (M, $C_{19}H_{28}O_4$)] and 17β-hydroxy-2-[(*N*,*N*-diethylamino)methylene]-4-oxa-5α-androstan-3-one [**7**; 93%; mp 121–123 °C; $[\alpha]_D$ +42°; MS 375.2771 (M, $C_{23}H_{37}NO_3$)] to the α-methylene lactone **12** [30%; mp 141–143 °C; $[\alpha]_D$ +72°;

(16) S. Danishefsky, T. Kitahata, R. McKee, and P. F. Schuda, *J. Am. Chem. Soc.*, **98**, 6715 (1976).
(17) D. R. Grassetti, and J. F. Murray, Jr., *Biochem. Pharmacol.*, **19**, 1836 (1970).
(18) K.-H. Lee, Y.-S. Wu, and I. K. Hall, *J. Med. Chem.*, **20**, 911 (1977).
(19) P. Hotten, R. J. Stretton, and J. R. Traynor, unpublished observations.
(20) I. H. Hall, K.-H. Lee, and S. A. Eigebaly, *J. Pharm. Sci.*, **67**, 1232 (1978).
(21) J. L. Hartwill and B. J. Abbott, *Adv. Pharmacol. Chemother.*, **7**, 117 (1969).

MS 346.2147 (M, $C_{21}H_{30}O_4$). Anal. ($C_{21}H_{30}O_4$) C, H] and the α-methylene lactone **11** [28%; mp 139–140 °C; MS 304.2071 (M, $C_{19}H_{28}O_3$)], which were separated by preparative TLC eluting with chloroform–methanol (85:15).

3β-Hydroxy-16-methylene-17a-oxa-*D*-homo-5α-androstan-17-one (14). a. A solution of the lactone **13** (53 mg, 0.16 mmol), paraformaldehyde (30 mg, 0.8 mmol), and KOH (10 mg) in ethanol (1 mL) and water (0.5 mL) was heated under reflux for 10 min and diluted with water (3 mL). After it was left standing overnight at ambient temperature, the reaction mixture was cooled to 10 °C, acidified with dilute HCl, diluted with water (5 mL), and extracted with ether (three times). The combined ether extracts were washed with water, dried and evaporated. TLC of the residue, eluting two times with ether–chloroform (80:20), gave the lactone **13** (18 mg, 34%) and a less polar fraction which crystallized from methanol to give the α-methylene lactone **14** (15 mg, 27%): mp 259–260 °C; $[\alpha]_D$ +89°; IR (KBr) 3420 (OH), 1715 (C=O), 1630 (C=C) cm^{-1}; UV λ_{max} (EtOH) 214 nm (ε 3760); ^1H NMR δ 6.48 (1 H, m, i), 5.70 (1 H, m, ii), 5.63 (1 H, m, 3-H), 1.29 (3 H, s, 13-Me), 0.78 (3 H, s, 10-Me); MS 318.2241 (M, $C_{20}H_{30}O_3$).

b. A solution of the lactone **13** (136 mg, 0.44 mmol), 37% aqueous formaldehyde (0.5 mL, 4.84 mmol), diethylamine (150 mg, 2.05 mmol), diethylamine hydrochloride (170 mg, 1.55 mmol), and dioxane (10 mL) was heated under reflux for 8 h under a nitrogen atmosphere. The solution was made alkaline by the addition of sodium hydroxide (2 N) solution and extracted with benzene (three times). The combined benzene extracts were washed with NaCl solution, dried, and evaporated. TLC of the residue as in **a** gave the lactone **13** (45 mg, 33%) and the α-methylene lactone **14** (38 mg, 27%).

Reaction of L-Cysteine with 2-Methylene-4-oxa-5α-cholestan-3-one (10). A solution of the α-methylene lactone **10** (40 mg, 0.4 mmol) and L-cysteine (12 mg, 0.4 mmol) in 60% aqueous ethanol (5 mL) was heated under reflux for 1 h and allowed to cool to ambient temperature. The resultant white precipitate was collected by filtration and recrystallized from aqueous ethanol to give the cysteine adduct **15** (41 mg, 79%): mp 186–189 °C; IR (KBr) 3700–2300 (COOH and NH_2), 1725 (C=O of carboxylic acid and lactone), 1630 (CO_2^-) cm^{-1}; MS 402.3492 (M – $C_3H_5NO_2S$, $C_{27}H_{46}O_2$), 401.3388 (M – $C_3H_6NO_2S$, $C_{27}H_{45}O_2$), 400.3333 (M – $C_3H_7NO_2S$, $C_{27}H_{44}O_2$).

Cell Culture Assay. KB cells were cultivated in Eagle's minimal essential medium (MEM) supplemented with 10% fetal calf serum.

Preliminary screening was performed using a rapid microtitre-plate assay.[22] Active compounds were then assayed following the Cancer Chemotherapy National Service Centre (CCNSC) protocol for KB cells.[23] Cell numbers were determined using a Coulter counter, and LD_{50} values were determined from plots of mean cell counts at 48 h. In all cases, drugs were added in Me_2SO, which did not exceed a final concentration of 0.5%.

(22) E.-S. Huang, K.-H. Lee, C. Piantadosi, T. A. Geissman, and J. S. Pagano, *J. Pharm. Sci.*, **61**, 1960 (1972).
(23) *Cancer Chemother. Rep.*, **25**, 22 (1962).

Pyrido[2,1-*b*]quinazolinecarboxylic Acids as Orally Active Antiallergy Agents

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A series of 8-substituted pyrido[2,1-*b*]quinazoline-2-carboxylic acids was prepared by the nickel carbonyl mediated carboxylation of the corresponding bromides. The activities of these compounds in the rat PCA test are comparable to those of the corresponding 2-substituted pyrido[2,1-*b*]quinazoline-8-carboxylic acids.

Bronchial asthma is a chronic and debilitating disease which in its severe forms can be life threatening. Traditionally, three classes of drugs have been employed to combat the symptoms of this disease: β-sympathomimetic agents, phosphodiesterase inhibitors, and corticosteroids. All three classes have serious liabilities[1] and the introduction of disodium cromoglycate in 1967 was considered a major breakthrough.[2,3] This novel drug inhibits release, from sensitized mast cells, of the mediator substances responsible for the clinical manifestations of bronchial asthma while lacking bronchodilator activity.[3] Although disodium cromoglycate is not orally active and must be administered by insufflation, it has become an important drug in the prophylactic therapy of this state.

A great deal of synthetic work has been directed toward the discovery of more potent and orally active compounds. Recently, an interesting series of 2-substituted pyrido[2,1-*b*]quinazoline-8-carboxylic acids has been described.[4] We are therefore prompted to describe one aspect of our work in this area which constitutes an extension of the reported results.[4]

Results and Discussion

The 2-bromo derivatives **1–4** (Table I) were prepared by thermal condensation of 5-bromoanthranilic acid[5] with the appropriate 5-substituted 2-chloropyridine either neat or as a triglyme suspension in the presence of a catalytic amount of potassium iodide. The oxime **5** was readily available from the ketone **4** by reaction with methoxyamine. The 2-chloro-5-(1-methylethyl)pyridine (**17**) necessary for the preparation of **3** was synthesized through the intermediates **15** and **16** (Scheme I).

In order to convert the bromides **1–5** to the corresponding carboxylic acids, we employed a variation of a

(1) N. Svedmyr and B. G. Simonsson, *Pharmacal. Ther., Part B*, **3**, 397 (1978).
(2) H. Cairns, C. Fitzmaurice, D. Hunter, P. B. Johnson, J. King, T. B. Lee, G. H. Lord, R. Minshull, and J. S. G. Cox, *J. Med. Chem.*, **15**, 583 (1972).
(3) J. S. G. Cox in "Proceedings of Symposium of Disodium Cromoglycate in Allergic Airways Disease", J. Pepys and A. W. Frankland, Ed., Butterworths, London, 1970, pp 13–27.
(4) C. F. Schwender, B. Sunday, D. J. Herzig, E. K. Kusner, P. R. Schumann, and D. L. Gawlak, *J. Med. Chem.*, **22**, 748 (1979); C. F. Schwender, B. Sunday, and D. J. Herzig, *J. Med. Chem.*, **22**, 114 (1979).
(5) A. S. Wheeler and W. M. Oats, *J. Am. Chem. Soc.*, **32**, 770 (1910); A. S. Wheeler, *J. Am. Chem. Soc.*, **31**, 565 (1909).

0022-2623/80/1823-0092$01.00/0