Antitumor Agents. 3 †,[1] Synthesis and Cytotoxic Activity of Helenalin Amine Adducts and Relate Derivatives

Kuo-Hsiung Lee,* Hiroshi Furukawa,

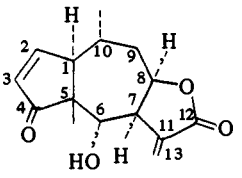
Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27514

and Eng-Shang Huang

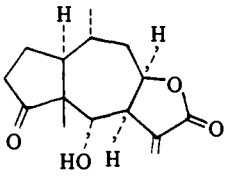
Department of Bacteriology and Immunology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514.
Received November 23, 1971

2,3-Dihydrohelenalin (II) and a series of Michael-type secondary amine adducts of helenalin (I) have been prepared in an effort to evaluate the potential significance of the α,β-unsaturated ketonic moiety in addition to the α-methylene-γ-lactone system of helenalin for cytotoxic activity against the growth of tissue culture cells derived from human epidermoid carcinoma of larynx (H. Ep.-2). The synthesis of II was accomplished by a modified procedure involving the use of dimethylamine for the protection of the highly reactive α-methylene-γ-lactones. The results of the cytotoxicity test of the compounds studied indicate that the α,β-unsaturated ketonic moiety in helenalin plays an important role in the maintenance of the high level of cytotoxicity.

In the previous paper,[2] the cytotoxicities of a number of naturally occurring sesquiterpene lactones against 3 different cell lines have been reported. The results obtained show that the most immediate and direct factor responsible for cytotoxicity of the compounds studied is the introduction of the O=C–C=CH_2 system. Helenalin (I), a linear pseudoguaianolide containing, in addition to the α-methylene-γ-lactone moiety, an α,β-unsaturated ketone system, *i. e.*, 2 alkylating functions in the molecule, has been shown[2,3] to possess potential significant inhibitory activity of the *in vitro* growth of tissue culture cells derived from human epidermoid carcinoma of larynx (H. Ep.-2) and human carcinoma of the nasopharynx (KB). In order to investigate structure-activity relationships, it appeared important to elucidate the role of the above mentioned 2 structural features on the cytotoxicity of helenalin. The present paper deals with the synthesis and cytotoxicity of 2,3-dihydrohelenalin (II) as well as a series of Michael-type secondary amine adducts of helenalin.



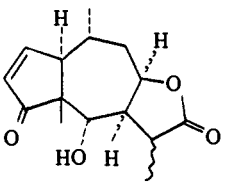
I



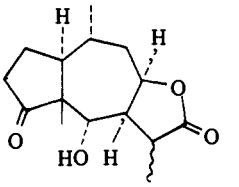
II

Chemistry. The synthesis of 2,3-dihydrohelenalin (II) was undertaken as an example for an evaluation of the potential significance of the α,β-unsaturated ketonic moiety. Direct selective reduction of the α,β-unsaturated ketonic function of helenalin (I) has been shown to be elusive, since earlier studies have indicated that reduction of helenalin (I) led either to 11,13-dihydrohelenalin (III)[4] or 2,3,11,13-tetrahydrohelenalin (IV).[4,5] The synthesis of II exemplifies a modified facile method for the protection of the highly reactive α-methylene-γ-lactones, a structural unit commonly found in many naturally occurring sesquiterpene lactones.[6]

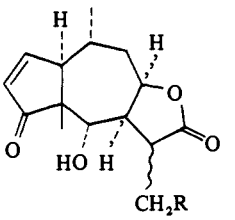
The Michael-type Me_2NH adduct Va was prepared in 80% yield from helenalin (I) by treating its abs EtOH solution with excess of anhyd Me_2NH at 5° overnight (method A).



III



IV

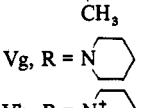


Va, R = $N(CH_3)_2$
Vb, R = $N^+(CH_3)_3$, I^-
Vc, R = $N(C_2H_5)_2$

Vd, R = N

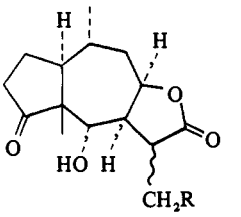
Ve, R = N

Vf, R = N^+(CH$_3$), I^-

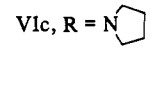


Vg, R = N

Vh, R = N^+(CH$_3$), I^-



VIa, R = $N(CH_3)_2$
VIb, R = $N^+(CH_3)_3$, I^-



VIc, R = N

Catalytic reduction of Va with 5% Pd/C in EtOAc afforded 2,3-dihydrohelenalin Me_2NH adduct (VIa) in quantitative yield (method B). Conversion of VIa to the corresponding methiodide VIb was obtained almost quantitatively by conventional procedures (method C). The final step in the synthesis of 2,3-dihydrohelenalin (II) could be achieved in quantitative yield from any one of the following 3 methods: (a) by heating the aqueous methiodide (VIb) solution on a steam bath for 30 min,‡ (b) by stirring the aqueous methiodide (VIb) solution with freshly prepared Ag_2O for 30 min at room temp, and (c) by triturating the aqueous methiodide (VIb) solution with 5% $NaHCO_3$ at room temp as reported.[7] The resulting product (II) showed the characteristic low-

†This work was generously supported by U.S. Public Health Service Research Grant 1-R01-CA-12360-01 from the National Cancer Institute and the University Research Council (Grant VF 170-1511) of the University of North Carolina.

‡It is interesting to note that the use of water for the trituration of the methiodides of some pulchellin alicyclic amine adducts has been mentioned with no experimental data by Kawamata and Inayama.[9]

Table I

No.	Formula	Anal.[a]	Method	Mp, °C	Recryst solvent	Ir, cm^{-1}	ED_{50}, μg/ml,[g] H. Ep.-2
I	$C_{15}H_{18}O_4$			170–172[c]	Benzene	3340, 1755, 1700, 1570, *ca.* 1650	0.083[h]
II	$C_{15}H_{20}O_4$	C, H	a, b, c	154–155	CH_2Cl_2–hexane	1760, 1735, 1660[f]	3.835
III	$C_{15}H_{20}O_4$			225–227[d]	Me_2CO	3500, 1745, 1710, 1575	0.814
IV	$C_{15}H_{22}O_4$			171–173[e]	Ethyl *n*-butyrate	3480, 1750, 1736	>40
Va	$C_{17}H_{25}O_4N$	C, H, N	A	214 dec	$CHCl_3$–EtOH	3380, 1765, 1699, 1580	0.604
Vb	$C_{18}H_{28}O_4NI$	C, H, N[b]	C	243–245	Aqueous EtOH	3360, 1780, 1710, 1586	0.600
Ve	$C_{19}H_{27}O_4N$	C, H, N[b]	A	200–202 dec	EtOH–CH_2Cl_2	3380, 1764, 1700, 1580	0.754
Vf	$C_{20}H_{30}O_4NI$	C, H, N[b]	C	179–181	MeOH–Me_2CO	3300 (br), 1780, 1700, 1580	0.765
Vg	$C_{20}H_{29}O_4N$	C, H, N		203–205	CH_2Cl_2–Et_2O	3360, 1760, 1696, 1575	0.540
Vh	$C_{21}H_{32}O_4NI$	C, H, N[b]	C	172–174	Me_2CO	3320 (br), 1780, 1700, 1580	0.434
VIa	$C_{17}H_{27}O_4N$	C, H, N	A	161–162.5	CH_2Cl_2–Et_2O	3440, 1774, 1724	6.040
VIb	$C_{18}H_{30}O_4NI$	C, H, N	C	195	Me_2CO–Et_2O	3340, 1764, 1700	3.460
VIc	$C_{19}H_{29}O_4N$	C, H, N[b]	A	168–169	CH_2Cl_2–Et_2O	3400, 1760, 1726	2.540

[a] Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. [b] Nmr spectra were also consistent with the assigned structures. [c] Lit.[13] reported mp 169–172° (benzene). [d] Lit.[4] reported mp 224–225° (EtOAc). [e] Lit.[5] reported mp 174–175° (*n*-butyl ether). [f] (Taken in $CHCl_3$. In Nujol, it showed absorption bands at 3540 (free OH), 3480 (hydrogen bonded OH), 1750, 1650 (α-methylene-γ-lactone), 1735 (cyclopentanone), and 1723 cm^{-1} (hydrogen bonded cyclopentanone). [g] The values of ED_{50} are used for expressing the potency of cytotoxicity which is the calculated effective dose that inhibits the net cell growth to 50% of control growth. [h] It should be noted that the ED_{50} value of helenalin (0.18) reported in ref 2 is incorrect and has to be changed to 0.083.

field doublets corresponding to the γ-lactone-α-methylene protons at δ 6.40 (1 H, J = 3 cps) and 5.88 (1 H, J = 3 cps) and the disappearance of the olefinic protons at C_2 and C_3 as seen in the nmr spectrum of helenalin.

A recent communication has reported the use of Me_2NH addition as a protecting reaction for the preparation of some inaccessible conjugated α-methylene-γ-lactones.[8] However, higher temperatures are needed in the latter method for the pyrrolysis of the methiodide in order to remove the protecting group. This method has been shown to be incomplete or less effective[9] than ours, especially since the higher temperatures required restrict the practical application toward the synthesis of heat-sensitive lactones.[10]

Our finding indicates that the facile elimination of quaternary N in the methiodide of VIb in a *neutral* medium as described in the foregoing (methods a and b) is apparently more effective and particularly useful for the synthesis of lactones bearing alkali-sensitive groups in comparison with previously reported methods,[7-12] in which the removal of the protecting groups was carried out in a dilute *basic* medium. Furthermore, studies of the relative ease with which some other secondary amines, such as Et_2NH, aziridine, pyrrolidine, and piperidine, undergo this Michael-type addition with helenalin (I) (giving Vc, Vd, Ve, and Vg) have indicated that Me_2NH is a superior protecting group for the above-mentioned lactones, since under identical reaction conditions as described above for the preparation of Va (method A), the products (Vc, Vd, Ve, and Vg) isolated are either a mixture or in low yield.

Cytotoxicity and Structure-Activity Relationship. 2,3-Dihydrohelenalin (II) and the Michael-type secondary amine adducts prepared in this study were screened for their cytotoxicity against the growth of tissue culture cells derived from human epidermoid carcinoma of larynx (H. Ep.-2) according to a rapid microtiter method described.[2,13] Comparison of the ED_{50} values for the cytotoxicity of the compounds listed in Table I disclosed that the α,β-unsaturated ketonic moiety in helenalin shows remarkable effect on cytotoxicity since selective reduction of this α,β-unsaturated ketonic moiety results in a 46-fold diminution in cytotoxicity as seen in 2,3-dihydrohelenalin (II). Modification of the α-methylene-γ-lactone by either hydrogenation or Michael-type amine addition yields compounds (III, Va,b,e-h) which are about 5- to 10-fold less active than the parent molecule (I). Modification of both α-methylene-γ-lactone and α,β-unsaturated ketonic system as in IV and VIa-c, leads to the derivatives which are either much less active or essentially inactive.

The deamination reaction for the quaternary salt of the amine adducts which takes place with relative ease *in vitro* with H_2O as mentioned above does not seem to occur in tissue culture, since none of these amine adducts (Va,b,e-h) has potency equal to that of helenalin (I).

Experimental Section

Unless otherwise specified, melting points were detd on a Thomas-Hoover melting point apparatus and are corrected. Ir spectra were detd in Nujols mulls with a Perkin-Elmer 257 grating ir spectrophotometer. Nmr spectra were measured in $CDCl_3$ with a Jeolco C 60 HL spectrometer (TMS), and chemical shifts reported in δ (ppm) units; s, singlet, d, doublet, t, triplet, and m, multiplet and the J values in cps. Silica gel for column chromatography refers to Baker A. R. No. 3405 and silica gel for the tlc refers to Merck silica gel G developed with MeOH and visualized with I_2 vapor. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, Ga.

Helenalin (I). Helenalin (I) was isolated from the extn of *Helenium microcephalum* M. A. Curt. ex Gray according to an exact lit. procedure.[2,14]

Method A. Helenalin Dimethylamine Adduct (Va). A soln of helenalin (I) (100 mg) in abs EtOH (3 ml) was treated with excess of anhyd Me_2NH at 5° overnight. The resulting crystals were filtered, washed (EtOH), and recrystd from $CHCl_3$–EtOH to yield Va as colorless needles (93 mg): mp 214° dec; ir 3380 (OH), 2825, 2810, and 2775 (CH stretching in $N(CH_3)_2$), 1765 (γ-lactone) and 1699 (cyclopentenone); nmr 7.68 (1 H, dd, J = 6 cps, 2 cps, H-2), 6.10 (1 H, dd, J = 6 cps, 3 cps, H-3), 4.92 (1 H, m, H-8), 4.38 (1 H, br s, H-6), 2.36 (6 H, s, $N(CH_3)_2$), 1.27 (3 H, d, J = 6 cps, C_{10}-CH_3) and 1.17 (3 H, s, C_5-CH_3), and the absence of the characteristic exocyclic CH_2 signals in the low-field region as seen in the nmr spectrum of helenalin (I).

Method B. 2,3-Dihydrohelenalin Dimethylamine Adduct (VIa). Compd Va (400 mg) in EtOAc (8 ml) was hydrogenated in the presence of prereduced 5% Pd/C (100 mg) at room temp and atm pressure. The uptake of H_2 ceased after 20 min. After removal of the catalyst, the filtrate was concd *in vacuo* and the residue was purified in CH_2Cl_2 by passing through a column of silica gel (1 g) to give VIa in quantitative yield: mp 161–162.5°; nmr 4.86 (1 H, m, H-8), 4.03 (1 H, d, J = 4.5 cps, H-6), 2.30 (6 H, s, $N(CH_3)_2$), 1.10 (3 H, overlapped d, J = 6 cps, C_{10}-CH_3) and 1.02 (3 H, s, C_5-CH_3) and the disappearance of the signals corresponding to the olefinic protons at C_2 and C_3.

Method C. 2,3-Dihydrohelenalin Dimethylamine Adduct Methiodide (VIb). A soln of VIa (300 mg) in MeOH (10 ml) was treated

with excess MeI at 5° overnight. The reaction mixt was evapd to dryness and the residue was crystd from Me_2CO-Et_2O to furnish colorless needles of VIb in almost quantitative yield: mp 195°; the quaternary *N*-Me group signaled at 3.30 (9 H, s) in the nmr (D_2O) spectrum.

2,3-Dihydrohelenalin (II). 2,3-Dihydrohelenalin could be readily synthesized in quantitative yield from any one of the following 3 methods.

(a) A soln of VIb (30 mg) in H_2O (1 ml) was heated on a steam bath for 30 min. The reaction mixt was acidified with 5% HCl and extd with $CHCl_3$, washed with H_2O, dried (Na_2SO_4), and evapd. The residue was purified in $CHCl_3$ by passing through a column of silica gel (0.6 × 3.5 cm) to provide II as fine colorless silky needles after one recrystn from CH_2Cl_2–hexane: mp 154–155°; nmr 4.88 (1 H, t, J = 7.5 cps, H-8), 4.30 (1 H, d, J = 3 cps, H-6), 1.08 (3 H, d, J = 6 cps, C_{10}-CH_3) and 0.78 (3 H, s, C_5-CH_3).

(b) A soln of VIb (67 mg) in MeOH (20 ml) was treated with excess of freshly prepared Ag_2O and stirred for 30 min at room temp. The reaction mixt was filtered and evapd *in vacuo* to yield II as an oil. This was chromatogd in $CHCl_3$ on silica gel (0.3 × 3 cm) to give colorless needles (35 mg) after one recrystn from PhH–EtOH: mp 154–155°.

(c) A soln of VIb (18 mg) in 5% aq $NaHCO_3$ soln (5 ml) was stirred at room temp for 1 hr. The reaction mixt was acidified with dil HCl and extd with $CHCl_3$. The $CHCl_3$ ext was washed with H_2O, dried (Na_2SO_4), and evapd to yield 10 mg of II as colorless needles after 1 recrystn from benzene–EtOH: mp 154–155°.

Helenalin from Method b. Treatment of Vb (20 mg) in a similar manner as described for II (method b) afforded I as colorless needles. The identity of this compd with helenalin was established by tlc, ir comparison, and mmp determination.

11,13-Dihydrohelenalin (III) was prepd according to the method of Adams and Herz.[4]

2,3,11,13-Tetrahydrohelenalin (IV) was prepd by the method of Clark[5] and melted at 171–173°.

Helenalin Piperidine Adduct (Vg). A soln of helenalin (I) (100 mg) in freshly distd piperidine (1 ml) was allowed to stand at room temp overnight. The reaction mixt was dild with H_2O and extd with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried (Na_2SO_4), and evapd under reduced pressure to yield a residue (30 mg) which crystd upon addn of Et_2O. Recrystn from CH_2Cl_2-Et_2O gave colorless needles (Vg): mp 203–205°; nmr 7.70 (1 H, dd, J = 6 cps, 1.5, H-2), 6.10 (1 H, dd, J = 6.3 cps, H-3), 4.95 (1 H, m, H-8), 4.55 (1, H, br, s, H-6), 1.30 (3 H, d, J = 6 cps, C_{10}-CH_3) and 1.18 (3 H, s, C_5-CH_3).

Acknowledgment. The authors wish to thank Professor C. Piantadosi for his encouragement and interest in this work.

References

(1) K.-H. Lee, H.-C. Huang, E.-S. Huang, and H. Furukawa, *J. Pharm. Sci.* (paper 2), in press.
(2) K.-H. Lee, E.-S. Huang, C. Piantadosi, J. S. Pagano, and T. A. Geissman, *Cancer Res.*, 31, 1649 (1971), and the references cited therein.
(3) J. L. Hartwell and B. J. Abbott, *Advan. Pharmacol. Chemother.*, 7, 117 (1969).
(4) R. Adams and W. Herz, *J. Amer. Chem. Soc.*, 71, 2554 (1949).
(5) E. P. Clark, *ibid.*, 58, 1982 (1936).
(6) T. A. Geissman and M. A. Irwin, *Pure Appl. Chem.*, 21, 167 (1970).
(7) E. E. van Tamelen and S. R. Bach, *J. Amer. Chem. Soc.*, 77, 4683 (1955).
(8) N. R. Unde, S. V. Hiremath, G. H. Kulkarni, and G. R. Kelkar, *Tetrahedron Lett.*, 4861 (1968).
(9) T. Kawamata and S. Inayama, *Chem. Pharm. Bull.*, 19, 643 (1971).
(10) T. C. Jain, C. M. Banks, and J. E. McCloskey, *Tetrahedron Lett.*, 841 (1970).
(11) S. M. Kupchan, J. J. Giacobbe, and I. S. Krull, *Tetrahedron Lett.*, 2859 (1970).
(12) E.-S. Huang, K.-H. Lee, C. Piantadosi, T. A. Geissman, and J. S. Pagano, in preparation.
(13) R. Adams and W. Herz, *J. Amer. Chem. Soc.*, 71, 2546 (1949).
(14) K.-H. Lee and T. A. Geissman, *Phytochemistry*, 9, 403 (1970).

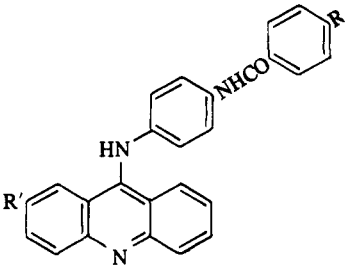
Potential Antitumor Agents. 12. 9-Anilinoacridines

G. J. Atwell, B. F. Cain,* and R. N. Seelye

Cancer Chemotherapy Laboratory, Cornwall Hospital, Auckland, New Zealand. *Received November 8, 1971*

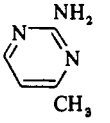
Development of a series of L1210 active 9-(R-substituted-anilino)acridines is described. R should be an electron-donating substituent and should be placed at either or both of the 3′ and 4′ positions. It is suggested that the 9-(R-anilino)acridines described can be considered as nonquaternary analogs of the 5-alkyl-6-phenylphenanthridinium salts.

A consideration of the mode of binding and factors governing the distribution of antileukemic bisquaternary salts[1] described earlier led us to prepare the L1210 active nonquaternary base **1a**. It has been postulated[2] that the corresponding base **1b** was inactive in the L1210 system because at physiological pH values the amount of un-ionized species present is too small to allow penetration of cellular barriers at sufficiently high rates by passive diffusion to elicit the required biological response. The higher percentage of neutral form present in the more weakly basic pyrimidine **1a** allows readier distribution and thus the intrinsic activity of the molecular type can be demonstrated. To investigate the contribution made to biologic activity by the weakly basic pyrimidine function in **1a** the simpler **1c** was prepared; suitable intermediates for this being already at hand.[1] Compd **1c** was only slightly less active in the early ip L1210 test than **1a**. The progressively simpler molecules **1d** then **1e** were prepared and found to have low but significant levels of antileukemic activity. Further simplification to the anilinoacridine **2** provided a molecule with a

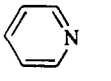


1

a, R′ = H; R = –NH



b, R′ = H; R = –NH



c, R′ = NH_2; R = NH_2
d, R′ = NH_2; R = H
e, R′ = R = H