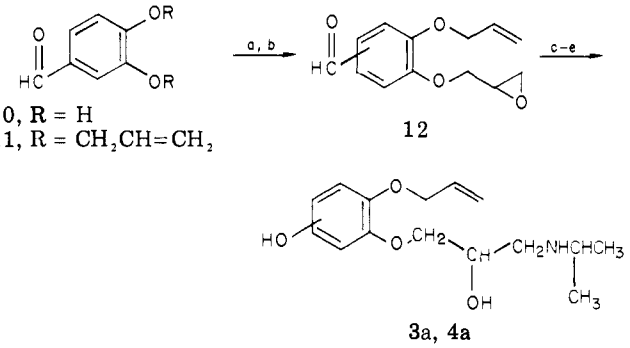
Scheme II[a]



10, R = H
11, R = $CH_2CH{=}CH_2$

12

3a, 4a

[a] Step a, NBS, wet dioxane; b, NaOH-H_2O; c, *m*-Cl-$C_6H_4CO_3H$; d, NaOH-H_2O; e, *i*-$PrNH_2$.

increased length of exposure to CH_2N_2.

A mixture of 4- and 5-hydroxyoxprenolol (**3a** and **4a**) was prepared from 3,4-dihydroxybenzaldehyde (**10**, Scheme II) in order to study the methylation conditions. Diallyl ether **11** was converted to a mixture of epoxides **12** by treatment with 1 equiv of *N*-bromosuccinimide, followed by NaOH. Baeyer–Villiger oxidation and hydrolysis followed by treatment with *i*-$PrNH_2$ afforded a mixture of **3a** and **4a**. After methylation, the mixture was shown by GC (TFA derivatives) to be approximately a 1:1 mixture of **3b** and **4b**.

Determination of metabolites was done by GC–CIMS monitoring *m/e* 488 (M + 1) and/or 374 (M + 1 – CF_3COOH)[10] at the appropriate retention times. By monitoring the total ion current at these masses, a ratio of approximately 1:4 of 5-methoxy- to 4-methoxyoxprenolol (**4b** to **3b**) as TFA derivatives was determined. No 3- or 6-methoxyoxprenolol (**5b** and **6b**) was found.

A 6-h human urine sample of one subject administered (orally) a mixture of 63 mg of oxprenolol-d_6[11] and -d_0 (1:1) was sequentially subjected to hydrolysis with aqueous 1 N HCl at 37 °C for 12 h and with β-glucuronidase (18 000 units, overnight, pH 5.0). From an EtOAc extract obtained identically with the EtOAc extract from rat urine, approximately a 1:4 mixture of 5- and 4-methoxyoxprenolol (**4b** and **3b**) was found.

These data confirm the formation of 4- and 5-hydroxyoxprenolol as important pathways of aromatic hydroxylation of oxprenolol in the rat and in man. Under these conditions neither 3- nor 6-hydroxylation was noted, although the absence of these potential metabolites cannot be completely assured. If formed, they may have been present in such small amounts that they are not detected by these analyses or possibly could be more slowly methylated by CH_2N_2 and thus not detected.

The confirmation of the presence of hydroxylated oxprenolol metabolites coupled with the observation that some of the pharmacological effects of oxprenolol in man are not well correlated with blood levels of oxprenolol[12] could suggest that these hydroxylated metabolites may contribute to observed effects of oxprenolol. Synthesis of sufficient quantities of these hydroxylated oxprenolols is underway to allow investigation of this possibility. Mechanistic aspects of this hydroxylation process are also under study.

Acknowledgment. The authors acknowledge support of Terrence R. Burke, Jr., by the Edwin Leigh Newcombe Memorial Fellowship from the American Foundation for Pharmaceutical Education.

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Received July 27, 1978

Plant Antitumor Agents. 17. Structural Requirements for Antineoplastic Activity in Quassinoids[1]

Sir:

The initial finding[2] that the novel quassinoid holacanthone (**1**) had antineoplastic activity prompted an extensive search for other active quassinoids[3–8] with the result that some of the structural requirements for antineoplastic activity, particularly in the P-388 mouse leukemia system, are known. These have been recently reviewed.[9,10] It is well established that the Δ^3-2-oxo moiety in ring A, the lactone moiety in ring D, and ester groups at C-6 and/or C-15 are required for biological activity.[7–10] However, the role of the C-1, C-11, and C-12 hydroxyl groups and the epoxymethano bridge between C-8 and C-11 in most quassinoids or between C-8 and C-13 as in the bruceantin (**2**) series is unknown. This information is vital in planning the total synthesis of active quassinoids or analogues. We wish to present recent studies which indicate the absolute necessity of the epoxymethano bridge and the desirability of the C-1 and C-12 hydroxyl moieties for biological activity.

6α-Senecioyloxychaparrinone (**3**) [mp 254–257 °C dec; $[\alpha]^{22}_D$ +203.8° (*c* 0.210, MeOH); IR ($CHCl_3$) 1740 (δ-lactone), 1715 (α,β-unsaturated ester), 1680 (α,β-unsaturated ketone) cm^{-1}; UV (MeOH) 228 nm (log ε 4.34); ^1H NMR ($CDCl_3$) δ 1.29 (3 H, s, C-10), 1.93, 2.20 (6 H, s, C-4′, C-5′), 2.74 (1 H, s, C-9), 3.66, 4.16 (2 H, d, *J* = 8 Hz, CH_2O),

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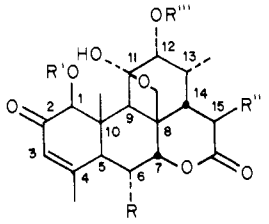
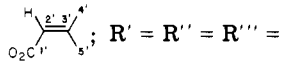
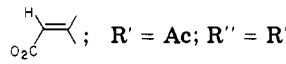


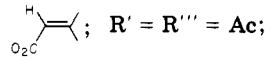
1, R = R′ = R‴ = H; R″ = OAc



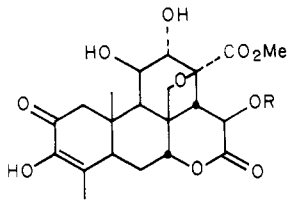
3, R = ; R′ = R″ = R‴ = H

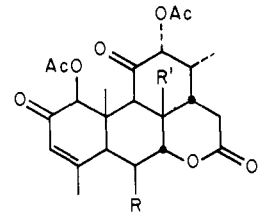


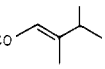
4, R = ; R′ = Ac; R″ = R‴ = H



6, R = ; R′ = R‴ = Ac; R″ = H





2, R = 

5, R =  ; R′ = CH_2OAc

4.16 (1 H, s, C-1), 4.54 (1 H, d, J = 2 Hz, C-7), 5.49 (1 H, d of d, J = 2, 12 Hz, C-6), 5.79 (1 H, s, C-2′), 6.14 (1 H, s, C-3); m/e 476.2046 ($C_{25}H_{32}O_9$)] has recently been isolated in our laboratories[7] and has potent antileukemic activity in the P-388 mouse leukemia system, indicating for the first time that the presence of a C-6 ester moiety can be an important structural feature in the absence of C-15 esterification.

Acetylation of **3** at low temperature resulted in selective acetylation at C-1 to give the acetate **4** [mp 231–234 °C; $[\alpha]^{21}_D$ +139.9° (c 0.25, MeOH); IR ($CHCl_3$) 1770 (acetate) cm^{-1}; UV (MeOH) 228 nm (log ϵ 4.33), 240 sh (4.25); ^1H NMR ($CDCl_3$) δ 1.45 (3 H, s, C-10), 2.24 (3 H, s, C-1 OAc), 5.39 (1 H, s, C-1), the rest of the spectrum was similar to that of **3**; MS showed a weak M^+ at m/e 518, $M^+ - C_2H_2O$, m/e 476.2042 ($C_{27}H_{34}O_{10} - C_2H_2O$)].

Exhaustive acetylation gave the triacetate **5** [mp 266–269 °C dec; $[\alpha]^{22}_D$ +101.5° (c 0.10, MeOH); IR ($CHCl_3$) 1750 (br, δ-lactone, three acetate esters), 1725 (sh, α,β-unsaturated ester, saturated ketone), 1690 (α,β-unsaturated ketone) cm^{-1}; UV (MeOH) 226 nm (log ϵ 4.32), 238 sh (4.27), 245 (4.20); ^1H NMR ($CDCl_3$) δ 1.55 (3 H, s, C-10), 2.00 (3 H, s, CH_2OAc), 3.44 (1 H, s, C-9), 4.04, 4.70 (2 H, d, J = 12 Hz, CH_2O), 4.96 (1 H, d, J = 2 Hz, C-12), 5.20 (1 H, s, C-1), the rest of the spectrum was similar to that of **3**; m/e 602.2367 ($C_{31}H_{38}O_{12}$)].

Selective hydrolysis[9] of **5** with triethylamine in a mixture of aqueous methanol and chloroform gave the 1,12-diacetate **6** [mp 257–259 °C dec; $[\alpha]^{24}_D$ +108.6° (c 0.12, MeOH); IR and UV spectra were similar to those of **4**; ^1H NMR ($CDCl_3$) δ 0.86 (3 H, d, J = 7 Hz, C-13), 2.06 (3 H, s, C-12 OAc), 3.76, 4.18 (2 H, d, J = 8 Hz, CH_2O), 4.94 (1 H, d, J = 4 Hz, C-12), 5.32 (1 H, s, C-1), the rest of the spectrum was similar to that of **3**; m/e 560.2254 ($C_{29}H_{36}O_{11}$)]. Table I presents the P-388 data for the parent compound 6α-senecioyloxychaparrinone (**3**), monoacetate **4**, triacetate **5**, and the 1,12-diacetate **6**. It will be noted that **5** and **6** are completely inactive and the C-1 monoester **4**, while still retaining partial activity, is of a much lower order of potency than **3**.

Table I. Antileukemic Activity of the Quassinoid Derivatives against the P-388 System

compd	dose, mg/kg	survival	wt diff	T/C, %
3	8	0/6	−1.3	
	4	4/6	−4.0	
	2	6/6	−3.7	191
	1	6/6	−2.3	198
	0.5	6/6	−2.8	161
	0.25	6/6	−2.7	145
4	8	6/6	−1.0	142
	4	6/6	−1.7	142
	2	6/6	0.0	132
	1	6/6	0.5	121
	0.5	6/6	−1.5	118
5	8	6/6	−1.2	102
	4	6/6	−0.3	101
	2	6/6	−1.2	102
	1	5/6	−0.8	97
	0.5	6/6	−1.2	100
6	10	6/6	−0.8	107
	5	6/6	−0.7	107
	2.5	6/6	−0.9	107
	1.25	6/6	+0.4	104

The epoxymethano bridge between C-8 and C-11 produces a pronounced flattening of ring B around C-8 and C-9.[6] Exhaustive acetylation destroys this bridge so that the stereochemistry of **5** differs markedly from that of **3** or **4**. It is possible that the molecular configuration of quassinoids with the epoxymethano bridge is an important requirement for biological activity. However, other considerations may be of equal importance.[11] Marked reduction of activity resulting from the monoacetylation of hydroxyl at C-1 may be due to the absence of intramolecular hydrogen bonding between the C-1 and C-11 hydroxyl groups.[6] It should, however, be noted that hydrogen bonding to the acetyl oxygen still exists. Reduction in activity may also be due to unfavorable alterations in hydrophilic–hydrophobic balance required for maximal activity, as indicated by the reduced P-388 leukemic activity of the 1-monoacetate **4** and the complete inactivity of the 1,12-diacetate **6**. Moreover, in the case of bruceantin **2**, one of the most potent tumor inhibitors, the C-1 hydroxyl, is replaced by the C-3 hydroxyl. Although the potential for hydrogen bonding is absent in **2**, the hydrophilic–hydrophobic balance is approximately the same as in **3**.

Acknowledgment. This investigation was supported by Contract No. N01-CM-67089 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare. The authors thank H. L. Taylor and J. B. Thompson for expert technical assistance and Dr. D. Rosenthal and F. Williams for mass spectral data obtained from the Research Triangle Center for Mass Spectrometry.

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Articles

Collagenase-Sensitive Peptidyl-Nitrogen Mustards as Potential Antitumor Agents

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Contribution No. 2124 from the Central Research Department, Experimental Station, E. I. du Pont de Nemours and Company, Wilmington, Delaware 19898. Received March 16, 1978

Attempts to design an agent which would release cytotoxic nitrogen mustards within collagenase-producing tumors led to the synthesis of Cbz-L-Pro-L-Leu-Gly-L-Pro-Gly-$NHC_6H_4N(CH_2CH_2Cl)_2$ (**10**). **10** was cleaved in vitro by bacterial and tumor-associated collagenase as expected at the peptide bond joining L-leucine and glycine to give Gly-L-Pro-Gly-$NHC_6H_4N(CH_2CH_2Cl)_2$ which was over six times more toxic, on a molar basis, than **10**. In vivo tests of **10** against well-advanced Sarcoma-180 gave disappointing results. The lack of specific antitumor activity may be accounted for by the presence of competing cleavage reactions by collagenases in certain normal tissues.

The usefulness of most antitumor agents has been severely limited by their lack of specificity. One approach to circumvent this involves administration of antitumor agents in inactive or latentiated forms.[1-3] Harper[4] has defined drug latentiation as the chemical modification of a biologically active compound to form a new compound which liberates this active compound on in vivo enzymatic attack.

In order to achieve the maximum benefit in cancer therapy, the enzyme which acts upon the latentiated agent should be associated with the targeted tumor tissue.[5] A proposal for using peptide-latentiated nitrogen mustards was originally made by Danielli in 1953.[5b] Unfortunately, most studies of such latentiated antitumor agents have failed to exploit qualitative or quantitative differences between tumor and normal tissue. This study attempts to exploit tumor-associated collagenase[6-12] by using a nitrogen mustard inactivated by a collagenase-sensitive peptide.

Collagenase is a highly specific[13,14] collagen-degrading enzyme[15] which is elaborated by many, if not all, slow-growing tumors.[6-12] It has been suggested that the enzyme aids tumor infiltration of healthy tissue by degrading the surrounding connective tissue.[7,8] This mechanism may be important in tumors of mesodermal origin since collagenase is absent from the normal striated muscle and fibrous connective tissue from which these collagenase-containing tumors arise.[11]

The foregoing suggests that a synthetic agent may have tumor-specific activity if it can be activated within the tumor by either endogenous or artificially introduced[16] collagenase. We therefore synthesized a nitrogen mustard peptide derivative designed to serve as a substrate for tumor-localized collagenase.

The mustard portion of this compound was derived from *N,N*-bis(2-chloroethyl)-*p*-phenylenediamine (**2**). This parent mustard was selected because it is highly cytotoxic but, as suggested initially by Danielli,[5b] can be rendered relatively nontoxic by acylation with groups which are not easily removable by normal mammalian enzymes. (Such acylating groups include alkyl- or arylcarboxylic acid residues[18,19] as well as amino acids[20,21] or serum proteins.[22])

In the current study we report on several of the properties of compound **10** of Table I. In this compound the acyl moiety is Cbz-L-Pro-L-Leu-Gly-L-Pro-Gly. The first four amino acids, starting at the N-terminus, have been sequentially arranged the same way as they are in the Wünsch–Heidrich substrate,[23] which has been shown to be cleaved by a tumor-associated collagenase.[8] The bond joining the L-leucyl and glycyl residues in this peptide is sensitive to cleavage by collagenase. The fifth amino acid, glycine, was added to move the sensitive bond further from the sterically bulky M. The Cbz moiety was attached to the N-terminus of the peptide to prevent premature chain degradation by aminopeptidases. We expected that collagenase cleavage of the acylated mustard would result in liberation of a tripeptide nitrogen mustard (Gly-L-Pro-Gly-M) which could then serve as a substrate for aminopeptidases. If such were the case, the removal of the remaining two glycyl residues and the prolyl residue would lead to the liberation of the highly cytotoxic HM (**2**, Table I).

Whole animal toxicity studies of model compounds in mice (Table I) indicated that this sequence of events should take place with **10**. The molar toxicities of peptidyl nitrogen mustard compounds fell into two distinct classes: those whose toxicities were similar to that of the parent mustard (HM) and those which showed significantly reduced toxicities. The factor which appears to determine the class into which a peptidyl nitrogen mustard falls is the ease of removal of the group attached to the N-terminus of the peptidyl chain by mammalian enzymes. Relatively resistant groups include Cbz, *t*-Boc, D-phenylalanine, and D-lysine while normal L-configuration

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