Michael-Type Reactions of Tenulin, a Biologically Active Sesquiterpene Lactone

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Abstract □ The antitumor pseudoguaianolide tenulin has been exposed to a wide variety of biological and model nucleophilic reagents and has been shown to react exclusively with sulfur nucleophiles in a Michael-like fashion. The biological implications of these results are discussed.

Keyphrases □ Tenulin—sesquiterpene lactone, Michael-type reactions, nucleophilic reagents □ Sesquiterpene lactones—tenulin, Michael-type reactions, nucleophilic reagents □ Michael-type reactions—tenulin with nucleophilic reagents, biologically active sesquiterpene lactones

The sesquiterpene lactone tenulin (I) (1) is the major bitter principle of the medicinal herb *Helenium amarum* (2). This compound has received considerable attention with regard to its antitumor (2–5), anti-inflammatory (6, 7), and antihyperlipidemic (8) activity. Recent structure–activity studies have related the biological properties of tenulin to the presence of the cyclopentenone moiety (4, 7, 8). The potential of this unit to act as an electrophile in Michael-like addition reactions is reminiscent of the *in vivo* role of the α-methylene-γ-lactone group (II) as a sulfhydryl (SH) acceptor in cytotoxic natural products (9). Indeed, the efficient Michael-like addition of cysteine (SH) to the tenulin enone has been reported (3). The α-methylene-γ-lactone group is reactive toward a variety of sulfur (9), oxygen (10), and nitrogen (11) nucleophiles. Therefore, it is important to the understanding of the mechanism of action to establish systematically the reactivity and selectivity of the cyclopentenone group of tenulin toward a variety of S, N, and O nucleophilic reagents. Such a study is of broad significance since the cyclopentenone unit appears in several antitumor terpenoids (12, 13).

RESULTS AND DISCUSSION

Table I presents a summary of this work and, for completeness, includes data taken from earlier work (entries 4–8 and 22). Reactions were attempted in aqueous alcohol solutions at room temperature and observed for periods up to 7 days. With the exception of entries 4–8, where spectral methods were used (3), negative reaction results were determined by TLC.

Nucleotides and nucleic acids exposed the cyclopentenone unit of tenulin to various nitrogen and oxygen nucleophiles. However, in no case was any reaction observed. Indeed, cyclopentenone itself does not react with adenosine 5′-monophosphate. Similarly, the amino acids alanine, serine, cysteine, histidine, and lysine contain nitrogen, oxygen, and sulfhydryl nucleophiles. Methanolic solutions of serine, histidine, or lysine (phosphate buffer, pH 7.2), when treated with tenulin, resulted only in the retroaldol conversion of tenulin to isotenulin (III) (14). In striking contrast, the efficient Michael addition of cysteine (SH) to the enone of tenulin (and cyclopentenone) to produce the adduct IV has been reported (3). The results summarized above suggest a strong Michael selectivity of the electrophilic cyclopentenone group for sulfur nucleophiles.

This notion was further tested (entries 14–22) by treating tenulin and cyclopentenone with glutathione (SH) and small model nucleophiles. Tenulin and methylamine interacted in aqueous methanol to produce the Schiff base, tentatively assigned structure V. Compound V was insoluble in water but readily soluble in dilute hydrochloric acid. The IR spectrum showed ν_{max} at 1770 br (γ-lactone and ester) and 1660 cm^{-1} (C=N), but lacked OH and enone absorption. The Schiff base portion of the molecule was confirmed by the UV spectrum where λ_{max} 246 nm (methanol) shifted to 265 nm on addition of two drops of 50% HCl. This bathochromic shift is characteristic of conjugated azomethines (15). An analogous reaction of 2-cyclopentenone with methylamine to form the Schiff base was demonstrated spectrophotometrically (see *Experimental*). Thus, the nucleophilic nitrogen of methylamine prefers the electrophilic carbonyl group of the cyclopentenone unit rather than the β-carbon. Methanol and ethanol do not react with tenulin even at reflux,

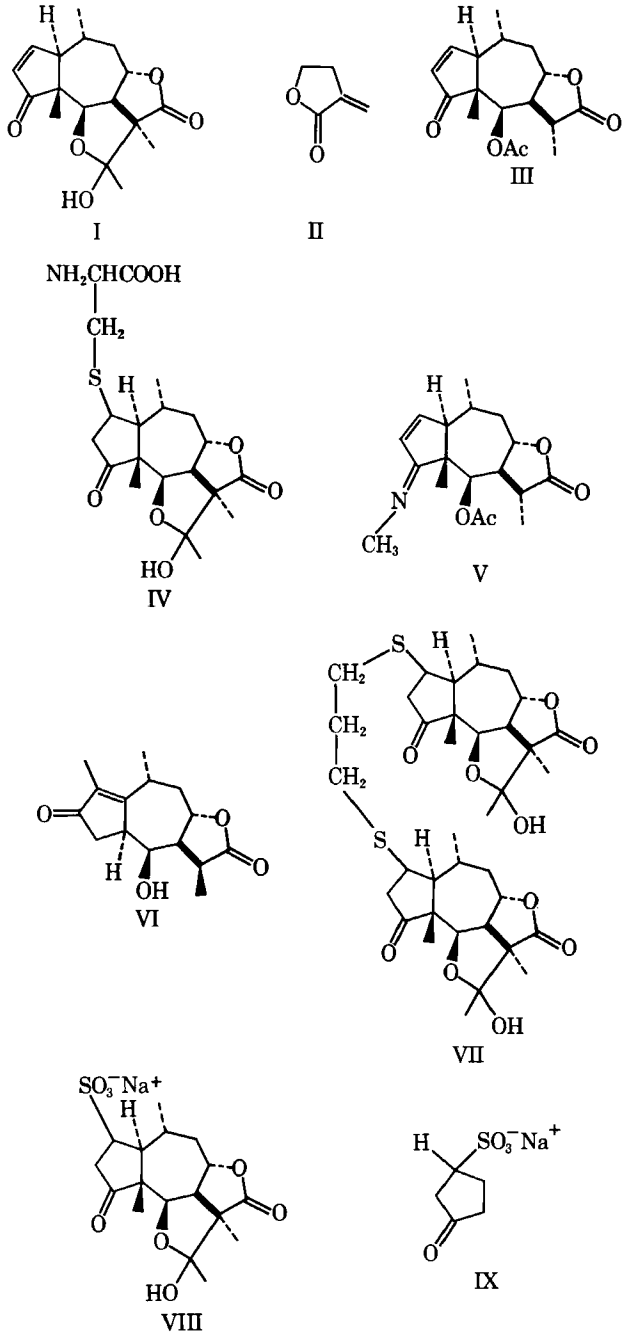

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Table I—Reactions of Tenulin and 2-Cyclopentenone with Nucleophiles

Entry	Nucleophilic Reagent	With Tenulin	With 2-Cyclopentenone
1	Adenosine 5′-monophosphate	No reaction	No reaction
2	Adenosine	No reaction	
3	Adenine	No reaction	
4	Deoxyguanosine 5′-monophosphate	No reaction (3)	
5	Deoxyguanosine 5′-triphosphate	No reaction (3)	
6	Deoxyadenosine 5′-monophosphate	No reaction (3)	
7	Deoxyadenosine 5′-triphosphate	No reaction (3)	
8	Deoxyribonucleic acid	No reaction (3)	
9	L-Alanine	No reaction	
10	L-Serine	Conversion to isotenulin	
11	L-Cysteine	Michael addition (3)	Michael addition (3)
12	L-Histidine	Conversion to isotenulin	No reaction
13	L-Lysine	Conversion to isotenulin	No reaction
14	Methylamine	Schiff base	Schiff base
15	Sodium methoxide	Skeletal rearrangement	
16	Methanol	No reaction	
17	Ethanol	No reaction	
18	Thiourea	No reaction	No reaction
19	1,3-Propanedithiol	Bis Michael addition	
20	Glutathione (SH)		Michael addition (3)
21	Sodium bisulfite	Michael addition	Michael addition
22	1-Propanethiol		Michael addition (18)

whereas the more reactive sodium methoxide affects the known skeletal rearrangement of tenulin to deacetylneotenulin (VI) (16).

The reactivity of tenulin toward several types of sulfur nucleophiles is summarized in entries 18–22 (Table I). Although the sulfur atom of thiourea has been shown to act as a nucleophile in Michael-like additions (*cf.* 17), this reagent does not react with tenulin or 2-cyclopentenone. In contrast, cyclopentenone does form Michael adducts with the sulfhydryl-containing glutathione (3) and 1-propanethiol (18). The high reactivity of the cyclopentenone toward SH groups is further demonstrated in the reaction of tenulin with one mole equivalent of 1,3-propanedithiol where the major product is the crystalline bis adduct VII, $C_{37}H_{52}O_{10}S_2 \cdot H_2O$. Compound VII showed IR absorption at 3300–3500 (OH), 1780 (γ-lactone), and 1740 cm^{-1} (cyclopentanone). Sulfhydryl absorption near 2550 cm^{-1} was absent. The UV spectrum lacked the characteristic enone absorption of tenulin and chemical analysis for SH (19) was negative.

The known reactivity of sodium bisulfite as a sulfur nucleophile in Michael-like additions (20) prompted the examination of this reagent as a potential nucleophile. The reaction of tenulin with excess sodium bisulfite in aqueous methanol cleanly produced the water-soluble Michael adduct VIII. The IR spectrum of VIII lacked enone carbonyl absorption near 1700 cm^{-1}. A broad band centered at 1740–1770 cm^{-1} indicated γ-lactone and cyclopentanone functional groups. Further evidence for Michael addition was seen in the NMR spectrum of VIII which featured the absence of olefinic proton signals and the presence of a new one proton multiplet at 3.70 ppm (H-2, $HCSO_3Na$). In an analogous manner, 2-cyclopentenone forms the Michael adduct IX when treated with either one or two equivalents of sodium bisulfite.

These experiments have demonstrated that the cyclopentenone unit of tenulin is highly selective for sulfur nucleophiles (SH, bisulfite) in Michael-like addition reactions. This selectivity is not surprising in view of the classification of sulfur and enone as soft Lewis base and Lewis acid, respectively, whereas nitrogen and oxygen nucleophiles are considered to be hard Lewis bases (21).

There are biological implications of this new chemistry of tenulin. The hypothesis that the *in vivo* activity of cyclopentenone-containing natural products may be due to the enone unit alkylating essential sulfhydryl groups of cellular enzymes (3) now stands more firmly with this additional support.

EXPERIMENTAL

Attempted Reactions of Tenulin with Model Nucleophiles—In separate experiments, tenulin (50 mg) was treated with 2 ml of an aqueous methanol solution containing one equivalent of thiourea, adenine, or adenosine. Analogously, in 2 ml of methanol–phosphate buffer (pH 7.2) tenulin was treated with one equivalent of adenosine 5′-monophosphate, L-serine, L-histidine, L-lysine, or L-alanine. Reaction vessels were stoppered, left at room temperature for several days, and monitored by TLC[1]. No reactions were observed. Quantitative yields of tenulin (or isotenulin) were recovered on extraction with chloroform, drying over anhydrous magnesium sulfate, filtration, and evaporation of the filtrate.

Attempted Reactions of 2-Cyclopentenone with Model Nucleophiles—In separate experiments, 2-cyclopentenone (50 mg) was treated with 2 ml of an aqueous methanol solution containing one equivalent of thiourea, L-histidine, L-lysine, or adenosine 5′-monophosphate (in methanol–phosphate buffer, pH 7.2). Reaction vessels were stoppered, left at room temperature for several days, and monitored by TLC. No reactions were observed.

Isotenulin Methylimine (V)—To a solution of 0.051 g (0.17 mmole) of tenulin in 0.5 ml of methanol was added 0.5 ml (excess) of 40% aqueous methylamine. After 2 days at room temperature, the reaction solution was poured into 20 ml of water and extracted twice with 15 ml of chloroform. The combined chloroform extract was dried (magnesium sulfate), filtered, and evaporated to dryness at room temperature. Ether trituration yielded a crystalline compound (8 mg) tentatively assigned as V, mp 155–156° dec.[2] IR[3] ($CHCl_3$): 1770 (γ-lactone and ester) and 1660 cm^{-1} (C=N); UV[4]: 246 nm (MeOH) and 265 nm (MeOH + 2 drops 50% HCl). Compound V was water insoluble but readily soluble in dilute hydrochloric acid.

Deacetylneotenulin (VI)—To a solution of 0.100 g (0.32 mmole) of tenulin in 3 ml of methanol was added dropwise a sodium methoxide solution prepared by dissolving 4 mg (0.17 mmole) of sodium in 2 ml of methanol. After 80 min the reaction mixture was diluted to 50 ml with distilled water and extracted with three 25-ml portions of chloroform. The combined organic layer was dried (magnesium sulfate), filtered, and evaporated to dryness to give 0.075 g (87% yield) of a colorless gum. Ether trituration and recrystallization from acetone–ether gave deacetylneotenulin (VI), mp 242–245° dec. [lit. (22) mp 239–240°]. IR and UV spectra of VI were identical to published data.

Tenulin-1,3-propanedithiol Bis Adduct (VII)—To 0.304 g (0.99 mmole) of tenulin in 2 ml of methanol was added 0.10 ml (0.99 mmole) of 1,3-propanedithiol. The solution was swirled, deoxygenated under a nitrogen stream, sealed, and left at 0° for 3 days. After careful evaporation of the solvent, the yellow oily product was taken up in chloroform–ether whereupon a crystalline solid (136 mg) slowly separated in several crops. Recrystallization from hot methanol gave the bis adduct VII (69 mg, 19% yield), mp 244–248°. IR (nujol): 1780 (γ-lactone) and 1740 cm^{-1} (cyclopentanone); UV(MeOH): 205 mm.

Anal[5].—Calc. for $C_{37}H_{52}O_{10}S_2 \cdot H_2O$: C, 60.14; H, 7.36, S. 8.68. Found: C, 60.27; H, 7.55; S, 9.09.

Tenulin–Sodium Bisulfite Adduct (VIII)—Tenulin (0.052 g, 0.17 mmole) and sodium bisulfite (0.034 g, 0.33 mmole) were dissolved in 1.5 ml of aqueous methanol. After 4 days at room temperature, the reaction solution was allowed to slowly evaporate on a watchglass. The remaining crystalline material was taken up in methanol and filtered through a 1-cm

[1] EM Reagents precoated plates, silica gel 60, 0.25 mm thickness.

[2] Thomas-Hoover apparatus. All melting points are corrected.
[3] Perkin-Elmer 710B.
[4] Hitachi 100-80.
[5] Galbraith Laboratories, Knoxville, Tenn.

column of silica gel. Evaporation of the solvent left a clear oil which crystallized to yield 45 mg (65% yield) of water-soluble VIII, mp gradual dec. up to 108°. IR (nujol): 1740–1770 cm^{-1} (γ-lactone and cyclopentanone). ^{1}H-NMR(CD_3OD)[6]: δ 5.10 (br t, 1, H-8), 4.00 (d, 1, H-6), 3.70 (br, 1, H-2), and 1.20–1.60 ppm (12, 4 Me groups).

2-Cyclopentenone–Sodium Bisulfite Adduct (IX)—2-Cyclopentenone (0.055 g, 0.67 mmole) and sodium bisulfite (0.063 g, 0.61 mmole) were taken up in 2 ml of distilled water. Methanol (0.5 ml) was added after 2 hr at room temperature, the mixture was poured onto a watchglass and the solvent was allowed to evaporate slowly. Recrystallization of the solid residue from methanol–ether yielded 57 mg (46% yield) of the adduct IX, mp 163–166° [lit. (23) mp 165°]. IR (nujol): 1740 cm^{-1} (cyclopentanone). ^{1}H-NMR (D_2O): δ 3.70 (m, 1, $HCSO_3Na$) and 2.10–3.00 ppm (6, complex). Identical results were obtained when this experiment was repeated using a 1:2 molar ratio of ketone to sodium bisulfite.

2-Cyclopentenone Methylimine (Schiff Base)—Excess methylamine was added to a reference 2-cyclopentenone–methanol solution in a UV cuvette. After 18 hr, the $\pi \rightarrow \pi^*$ absorption of the enone carbonyl (310 nm) disappeared. Addition of 2 drops of 50% HCl caused a bathochromic shift in the original $\pi \rightarrow \pi^*$ (230 nm) to 250 nm. A control experiment without methylamine revealed no changes in the original cyclopentenone UV spectrum. The above results are consistent with the formation of the cyclopentenone–methylamine Schiff base (15).

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