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STRUCTURE–ANTIMICROBIAL ACTIVITY RELATIONSHIPS AMONG THE SESQUITERPENE LACTONES AND RELATED COMPOUNDS*

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Key Word Index—Helenalin derivatives and related sesquiterpene lactones; structure–antimicrobial activity relationships.

Abstract—Thirty-six sesquiterpene lactones and related compounds were evaluated for antimicrobial activity against six strains of bacteria. The results obtained show that the *beta* unsubstituted cyclopentenone ring moiety contributes to moderate antimicrobial activity against Gram positive bacteria. The corresponding saturated compounds gave a more than ten-fold decrease in activity. The significant antimicrobial activity appears to be independent of the presence or absence of an α-methylene-γ-lactone moiety. A more than ten-fold diminution in antimicrobial activity was also observed when the *beta* position of the cyclopentenone ring was substituted. A similar result was found when the *beta* unsubstituted enone system was present in a six-membered ring. Enhanced activity was obtained by esterification of the hydroxyl group of helenalin as well as epoxidation of mexicanin-A.

INTRODUCTION

Naturally occurring unsaturated lactones of simpler chemical structure, such as α- and β-angelicalactones [1], patulin [1], protoanemonin [1, 2], as well as the α-methylene butyrolactone from *Erythronium americanum* [3] are known for their antimicrobial activity. More recently, some sesquiterpene lactones have also been shown to possess this activity [2, 4, 5], e.g. cnicin was reported to have weak antibacterial properties [6], mikanolide and dihydromikanolide inhibited the growth of *Staphylococcus aureus* and *Candida albicans* [7], parthenin was reported to inhibit sporangial germination and zoospore mobility in *Sclerospora graminicola* [8], and helenalin (**1**) was shown to exhibit activity against the human pathogenic fungi, *Trichophyton mentagrophytes*, *T. acriminatum* and *Epidermophyton* sp. [9].

During the course of an investigation of the relationship between the sesquiterpene lactone structure and the cytotoxic/antitumor activities, it was found that sesquiterpene lactones which bear a *beta* unsubstituted cyclopentenone ring, a structural requirement for *in vitro* cytotoxicity, also show antimicrobial activity, and the significant antimicrobial activity appears to be independent of the presence or absence of an α-methylene-γ-lactone moiety [10]. This report details and extends the result of these observations and although the resulting compounds were only moderately active against Gram positive bacteria, subsequent studies on modified β-unsubstituted cyclopentenone bearing analogs may afford potentially useful antimicrobial agents.

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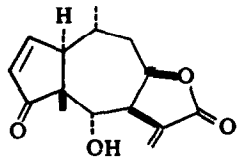
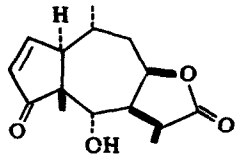
RESULTS AND DISCUSSION

Antimicrobial activity and structure–activity relationships

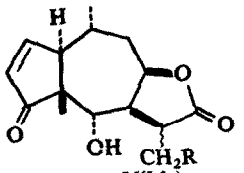
Helenalin derivatives and related sesquiterpene lactones used in this study were assayed for their antimicrobial activity according to Mitscher's method [11]. A comparison of the values for the minimum inhibitory concentration of the compounds listed in Table 1 clearly disclosed that these compounds were exclusively active against Gram positive bacteria, such as *S. aureus* and *B. subtilis*, and that a *beta* unsubstituted cyclopentenone ring moiety, such as in compounds **1**–**14**, contributes to the antimicrobial activity. The corresponding saturated compounds (**15**, **17**, **21** and **24**) gave approximately at least a 10-fold decrease in activity. The significant antimicrobial activity appears to be independent of the presence or absence of an α-methylene-γ-lactone or α-methyl-γ-lactone moiety (compare compounds **1** and **2**,



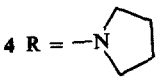
1 (helenalin)



2 (plenolin)

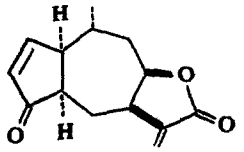


3 R = —N(Me)$_2$

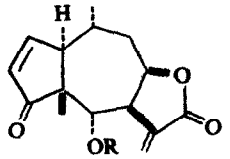


4 R = —N

5



6 (mexicanin-E)

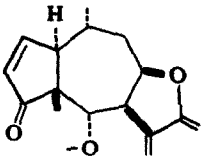


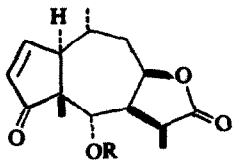
7 R = —COCH=CMe$_2$
8 R = —COCH$_2$CHMe$_2$
9 R = —COMe

10 R = —COCH=CH—

11 R = —COCH$_2$CH$_2$—COCH=CH—

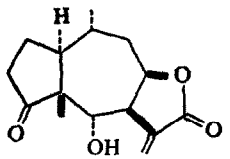
12 R = —COCH$_2$CH$_2$COR′ R′ =





13 R = —COCH=CH—

14 R = —COCH$_2$CH$_2$—



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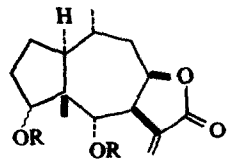
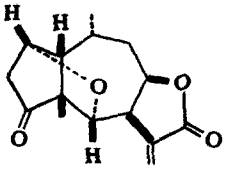


16 R = —COCH=CH—



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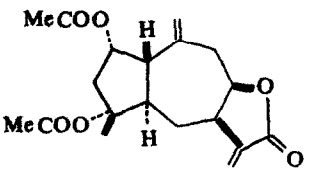
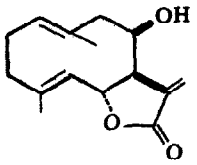
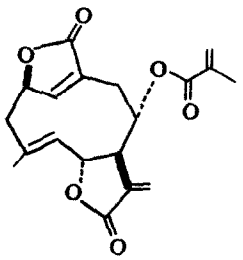


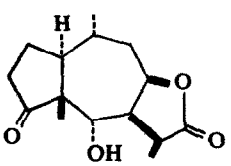
18 (florilenalin diacetate)



19 (eupatolide)



20 (deoxyelephantopin)



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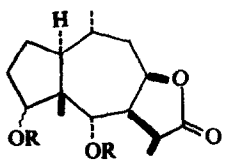
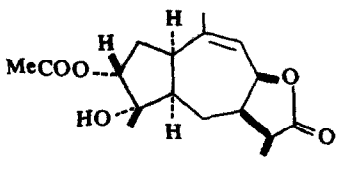


22 R = —COCH=CH—



23 (carolenalin monoacetate)

as well as the fact that many α-methylene-γ-lactone or α-methyl-γ-lactone bearing compounds, such as **15** to **23** were inactive (minimum inhibitory concentration > 1000 μg/ml), i.e. if no *beta* unsubstituted cyclopentenone ring was found in these molecules). The fact that compounds **5** and **6** were less active than compounds **1–4** might be suggestive of the importance of the stereochemistry of the cyclopentenone ring junction as well as the presence of a C-6 oxygenated function with respect to the enhanced activity. Esterification of the cyclopentenone bearing helenalin and plenolin led to compounds (**7–14**) which were more potent than the parent molecules (**1** and **2**), among which the conjugated cinnamate ester was the most active. Further rearrangement of helenalin (**1**) to the beta substituted cyclopentenone ring system (compounds **25** and **26**) resulted in the loss of activity as expected. Similar results were also obtained from deacetoxymatricarin (**27**) and the simple 3-methyl-2-cyclopenten-1-one (**28**).

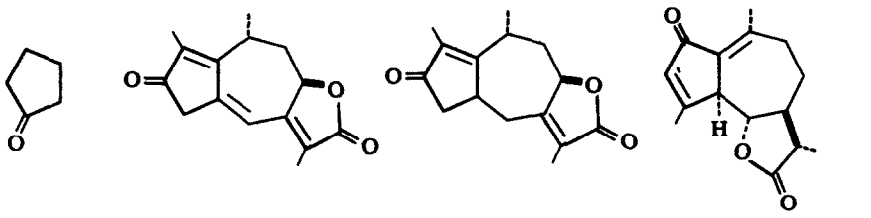
Previously it had been suggested that the mechanism of antibacterial action of the unsaturated ketone and lactone was by reaction with enzyme —SH groups [3, 12]. More recently we had also demonstrated that one of the possible mechanisms of cytotoxic antitumor action of the cyclopentenone bearing sesquiterpene lactones and related compounds was due to their facile reaction with the sulfhydryl containing bionucleophiles [13, 14]. It is likely that a similar mechanism of antimicrobial action may be applicable toward the cyclopentenone bearing sesquiterpene lactones. Unsubstitution at the *beta* position of this five-membered ring enone system, appears to provide a readily accessible site for alkylation by the bionucleophile —SH groups. In the case of *beta* substitution this alkylation reaction would be greatly hindered. The *beta* unsubstituted enone system seems to possess antimicrobial activity only with a five-membered ring since the corresponding six-membered sesquiterpene lactones (compounds **29–31**), which are chemically less reactive toward Michael addition, were inactive although the introduction of an exocyclic double bond conjugated to the enone system, such as **32** and **33**, enhanced the activity. Further modification of the cyclopentenone ring by epoxidation led to mexicanin-A 1,2-α-epoxide (**34**) which was more active than compounds **1** and **2**. However, the corresponding 2,3-epoxides (**35** and **36**) were less active.

In summary, the results indicate that a *beta* unsubstituted cyclopentenone is one of the structural requirements for antimicrobial activity. Other naturally occurring antibiotics involving this moiety isolated recently were pentenomycins-I (**37**) and -II (**38**) [15, 16] which were moderately active against Gram-positive and Gram-negative bacteria.

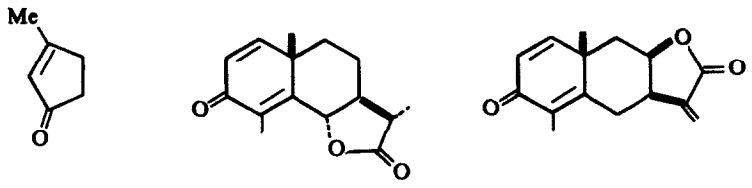
EXPERIMENTAL

Material. The naturally occurring sesquiterpene lactones and related compounds used in this study are all analytical samples obtained from previous studies (Table 1). The selection of these compounds was based mainly upon the availability of the samples as well as their characteristic structural types.

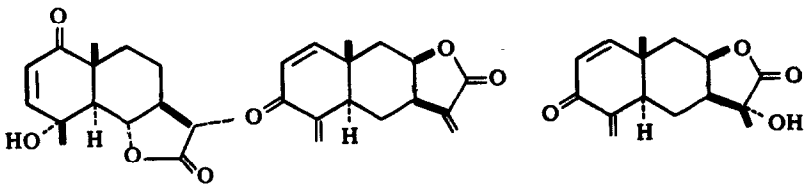
Organisms. The microorganisms used in this study included (a) *Staphylococcus aureus* PS 80–81 (Gram positive), (b) *Bacillus subtilis* PCI 219 (Gram positive), (c) *Escherichia coli* ATCC



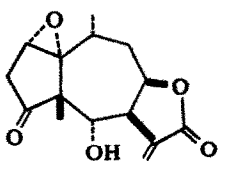
24 **25** **26** **27** (deacetoxymatricarin)



28 **29** (α-santonin) **30** (yomogin)



31 (vulgarin) **32** (encelin) **33** (farinosin)



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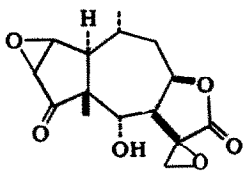

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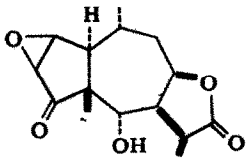

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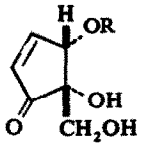

37 R = —H
38 R = —COMe

12765 (Gram negative), (d) *Salmonella enteritidis* ATCC 13076 (Gram negative), (e) *Klebsiella pneumoniae* ATCC 13883 (Gram negative) and (f) *Candida albicans* ATCC 934 (Yeast).

Antimicrobial testing. This was carried out according to ref. [11].

Table 1. Antimicrobial activity* of the sesquiterpene lactones and related compounds

Compound	Reference	MIC† (μg/ml) *Staphylococcus aureus* PS 80–81	MIC† (μg/ml) *Bacillis subtillis* PCI 219
1–4	17–19	100	100
5	‡	100	600
6	20	300	400
7	21	75	50
8	21	100	75
9	21	50	50
10	21	25	10
11	21	50	25
12	§	50	10
13	22	75	25
14	22	100	100
15	19	>1000	>1000
16	‖	>1000	>1000
17	23, 24	>1000	>1000
18–20	25–27	>1000	>1000
21–24	19, 28, 29, ‡, ‖	>1000	>1000
25	¶	>1000	>1000
26	¶	>1000	>1000
27–31	17, 30, 31, ‡	>1000	>1000
32	17, 32	400	100
33	17, 33	300	300
34	34	50	50
35	34	400	400
36	34	300	200

* Antimicrobial activity was determined according to ref. [11].

† Minimum inhibitory concentration. Compounds **1–36** were inactive against Gram negative bacillus, such as *E. coli*, *S. enteritidis*, and *K. pneumoniae* at 1000 μg/ml level. All compounds were also inactive against the yeast-like bacteria, *C. albicans* at 1000 μg/ml level except for compounds **6**, **12**, **34**, **35** and **36** which had MIC of 100–1000 μg/ml.

‡ The NMR spectrum indicated that this compound was homogeneous.

§ Lee, K. H., Ibuka, T. and Hall, I. H., unpublished data.

‖ Lee, K. H. and Kim, S. H., unpublished data.

¶ Lee, K. H., Ibuka, T. and Wu, R. Y., unpublished data.

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