

Antimycobacterial Plant Terpenoids

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Abstract: Tuberculosis (TB), mainly caused by *Mycobacterium tuberculosis*, is the leading killer among all infectious diseases worldwide and is responsible for more than two million deaths annually. For over thirty years no antitubercular agents with new mechanisms of action have been developed. The recent increase in the number of multi-drug resistant clinical isolates of *M. tuberculosis* has created an urgent need for the discovery and development of new antituberculosis leads. This review covers recent reports on plant-derived terpenoids that have demonstrated moderate to high activity in *in vitro* bioassays against *M. tuberculosis*. In this review, mono-, sesqui-, di- and triterpenes, and sterols, their structural analogs and semisynthetic derivatives will be discussed, with particular emphasis on the structural features essential for antimycobacterial activity.

Key words: Terpenoids, *Mycobacterium tuberculosis*, antituberculosis, tuberculostatic, antimycobacterial.

Introduction

It is estimated that one-third of the world's population is infected with the tubercle bacillus (1). While only a small percentage of infected individuals will develop clinical tuberculosis, each year there are approximately eight million new cases and two million deaths. *M. tuberculosis* is thus responsible for more human mortality than any other single bacterial species. The HIV pandemic has exacerbated the problem by providing a large reservoir of highly susceptible individuals (2).

A number of efficacious antitubercular agents were discovered in the late 1940's and 1950's with the last, rifampin, introduced in the 1960's (3), (4). These agents have reasonable efficacy and when used in combination should preclude the development of drug resistance. The use (or in most cases misuse) of these drugs has led over the years to an increasing prevalence of multiple-drug resistant (MDR) strains and there is now an urgent need to develop new effective agents (5), (6).

There have been a number of practical obstacles to the development of new anti-TB agents, among them a lack of econom-

ic incentive due to the predominance of disease in the developing world. The very slow growth and highly contagious nature of *M. tuberculosis* have also served to discourage the drug discovery effort.

Nonetheless, several drugs with interesting anti-TB activity have been identified in the past few years; three such compounds are the rifamycins. Rifabutin, with less P450 activating activity than rifampicin, has activity against a small percentage of rifampin-resistant strains (7), (8) and is active clinically (9), (10), (11), (12). Rifapentene provides a much longer serum half-life and thus holds the promise of allowing for intermittent dosing but exhibits complete cross-resistance with rifampin (13). KRM-1648 appears to be the most potent among the rifamycins and has demonstrated activity in phase II trials in tuberculosis (14); however, it also exhibits partial cross-resistance with rifampin. Fluoroquinolones such as ofloxacin and levofloxacin have demonstrated clinical activity (15), (16), (17), (18); however, the recently approved (in the U.S.A. for non-TB respiratory indications) moxifloxacin appears to be the most active fluoroquinolone against *M. tuberculosis in vitro* and in the mouse (19), (20), (21). Two members of a new class of antimicrobials, the oxazolidinones, showed modest *in vivo* activity against *M. tuberculosis* (22). Finally a nitroimidazopyran has demonstrated interesting *in vitro* and *in vivo* activity (23). While all of these compounds show potential, none have yet to demonstrate activity in clinical trials (with the exception of KRM-1648 and the older quinolones), thus there continues to be a need to identify new agents.

The following review surveys the literature for plant-derived terpenoids that have demonstrated moderate to significant biological activity against *M. tuberculosis*. It covers active compounds of five major groups of terpenoids: monoterpenes, sesquiterpenes, diterpenes, triterpenes, as well as phytol and its derivatives and structural analogs. Nearly all of the compounds discussed were screened using the BACTEC 460 system (24), (25) except where indicated otherwise. Compounds that did not demonstrate a minimum inhibitory concentration (MIC) of 64 µg/ml or lower and were unrelated to active terpenoids, have been omitted. Comparisons between chemical structures and their biological activities must be treated with caution, especially when different bioassay techniques have been used.

Table 1 Minimum inhibitory concentrations (MICs) of selected plant terpenoids against *M. tuberculosis* (H₃₇Rv)

Compound Class Name	Source	MIC (µg/ml) ^a	Reference
Antituberculosis Drugs			
isoniazid	Sigma-Aldrich Co.	0.05	(40)
rifampin	–	0.25	(40)
streptomycin	Sigma-Aldrich Co.	2.0	(40)
ethambutol	Sigma-Aldrich Co.	3.8	(40)
pyrazinamide	Sigma-Aldrich Co.	100	(40)
Monoterpenes			
citronellol (1)	Sigma-Aldrich Co.	64	(26)
nerol (2)	Sigma-Aldrich Co.	128	(26)
geraniol (3)	Sigma-Aldrich Co.	64	(27)
Sesquiterpenes			
costunolide (4)	<i>Saussurea lappa</i>	32	(28)
11βH-dihydrocostunolide (5)	synthetic analog	128	(28)
parthenolide (6)	<i>Magnolia grandiflora</i>	16	(28)
11βH-dihydroparthenolide (7)	<i>Ambrosia artemisiifolia</i>	128	(28)
1,10-epoxycostunolide (8)	synthetic analog	64	(28)
1,10-epoxydihydrocostunolide (9)	synthetic analog	128	(28)
1,10-epoxyparthenolide (10)	synthetic analog	128	(28)
1,10-epoxydihydroparthenolide (11)	synthetic analog	128	(28)
α-cyclocostunolide (12)	synthetic analog	64	(26)
santamarine (13)	<i>Ambrosia confertiflora</i>	64	(28)
11βH,13-dihydrosantamarine (14)	<i>Sonchus hierrensis</i>	>128	(28)
β-cyclocostunolide (15)	synthetic analog	64	(26)
reynosin (16)	<i>Ambrosia confertiflora</i>	64	(28)
11βH,13-dihydroreynosin (17)	synthetic analog	>128	(28)
α-santonin (18)	<i>Artemisia ramosa</i>	>128	(26)
reynosin triol derivative (19)	–	>128	(28)
11,13-dihydro triol of reynosin (20)	–	>128	(28)
santamarine triol derivative (21)	–	>128	(28)
dehydrocostuslactone (22)	<i>Saussurea lappa</i>	2	(30)
7-hydroxydehydrocostuslactone (23)	<i>Podachenium eminens</i>	>128	(30)
zaluzanin C (24)	<i>Zaluzania triloba</i>	>128	(30)
dehydrocostuslactone, 4α(15)-epoxide (25)	synthetic analog	32	(30)
dehydrocostuslactone, 10β(14)-epoxide (26)	synthetic analog	32	(30)
dehydrocostuslactone, 10α(14)-epoxide (27)	synthetic analog	64	(30)
dehydrocostuslactone, 4β(15),10α(14)-diepoxide (28)	synthetic analog	64	(30)
dehydrocostuslactone, 4α(15),10α(14)-diepoxide (29)	synthetic analog	128	(30)
dehydrocostuslactone, 4α(15),10β(14)-diepoxide (30)	synthetic analog	128	(30)
micheliolide (31)	–	50	(30)
pumilin (32)	<i>Berlandiera texana</i>	128	(30)
damsin (33)	<i>Ambrosia maritima</i>	32	(26)
deacetylconfertiflorin (34)	<i>Ambrosia confertiflora</i>	128	(41), (26)
confertiflorin (35)	<i>Ambrosia confertiflora</i>	128	(41), (26)
deacetylisconfertiflorin (36)	synthetic analog	128	(26)
parthenin (37)	<i>Parthenium hysterophorus</i>	64	(26)
tenulin (38)	<i>Helenium amarum</i>	128	(26)
peruvlin (39)	<i>Ambrosia peruviana</i>	128	(26)
peruvlin acetate (40)	synthetic analog	>128	(26)
burrocin (41)	<i>Ambrosia dumosa</i>	128	(42)
aromaticin (42)	<i>Helenium aromaticin</i>	16	(26)
alantolactone (43)	<i>Inula helenium</i>	32	(31)
isoalantolactone (44)	<i>Inula helenium</i>	32	(31)
encelin (45)	<i>Montanoa speciosa</i>	16	(31), (43)
1,2-dehydro-3-epi-isotelekin (46)	<i>Montanoa speciosa</i>	32	(31), (43)
ivalin (47)	<i>Iva imbricata</i>	64	(26)
11αH,13-dihydroisoalantolactone (48)	<i>Inula helenium</i>	>128	(31)
isoalantolactone (49)	<i>Rudbeckia mollis</i>	128	(44), (31)
alantolactone (50)	<i>Rudbeckia subtomentosa</i>	32	(31)
3-oxoalantolactone (51)	<i>Rudbeckia subtomentosa</i>	128	(31)
4α-epoxyisoalantolactone (52)	synthetic analog	32	(31)
5α-epoxyalantolactone (53)	synthetic analog	8	(31)

Table 1 cont.

Compound Class Name	Source	MIC ($\mu\text{g/ml}$) ^a	Reference
11,13-dihydroxylantolactone (54)	synthetic analog	>128	(31)
6- <i>epi</i> -deacetyl-laurenobiolide (55)	<i>Montanoa grandiflora</i>	16	(45)
4,5-epoxy-6- <i>epi</i> -deacetyl-laurenobiolide (56)	synthetic analog	16	(26)
curcuphenol (57)	<i>Euthamia leptocephala</i>	16	(Robbs, S.L., 1997 unpublished)
nerolidol (58)	<i>Magnolia acuminata</i>	32	(26)
farnesol (59)	Sigma-Aldrich Co.	8	(27)
Diterpenes			
sandaracopimara-8(14)-15-diene-7 α ,18-diol (60)	<i>Tetradenia riparia</i>	25–100 ^d	(33)
sandracopimarinic acid (61)	<i>Juniperus excelsa</i>	15.0 ^b	(34)
sclareol (62)	–	6.0 ^b	(34)
12-demethylmulticauline (63)	<i>Salvia multicaulis</i>	0.46 ^c	(32)
multicaulin (64)	<i>Salvia multicaulis</i>	5.6 ^c	(32)
12-demethylmultiorthoquinone (65)	<i>Salvia multicaulis</i>	1.2 ^c	(32)
multiorthoquinone (66)	<i>Salvia multicaulis</i>	2.0 ^c	(32)
12-methyl-5-dehydrohorminone (67)	<i>Salvia multicaulis</i>	1.2 ^c	(32)
12-methyl-5-dehydroacetylhorminone (68)	<i>Salvia multicaulis</i>	0.89 ^c	(32)
salvipimarone (69)	<i>Salvia multicaulis</i>	7.3 ^c	(32)
9,12-cyclomulin-13-ol (70)	<i>Azorella madreporica</i>	20	(35)
juniperexcelsic acid (71)	<i>Juniperus excelsa</i>	14.4 ^b	(34)
Triterpenes			
ergosterol-5,8-endoperoxide (72)	<i>Ajuga remota</i>	1	(36)
ergosterol-5,8-endoperoxide acetate (73)	synthetic analog	8	(36)
ergosterol (74)	Sigma-Aldrich Co.	>128	(36)
12 β -hydroxykylactone (75)	<i>Melia volkensii</i>	16	(37)
6 β -hydroxykylactone (76)	<i>Melia volkensii</i>	4	(37)
kulonate (77)	<i>Melia volkensii</i>	16	(37)
(24 <i>R</i>)-24,25-epoxycycloartan-3-one (78)	<i>Borrchia frutescens</i>	8	(25)
(3 β ,24 <i>R</i>)-24,25-epoxycycloartan-3-ol (79)	<i>Borrchia frutescens</i>	8	(25)
(3 β ,24 <i>R</i>)-24,25-epoxycycloartan-3-ol acetate (80)	<i>Borrchia frutescens</i>	>128	(25)
(23 <i>R</i>)-3-oxolanosta-8,24-dien-23-ol (81)	<i>Borrchia frutescens</i>	64–128	(25)
compound 82	synthetic analog	>128	(25)
fusidic acid (83)	Sigma-Aldrich Co.	4	(25), (46)
zeorin (84)	<i>Sarmienta scandens</i>	8 ^e	(38)
7 β -acetyl-22-hydroxyhopane (85)	<i>Sarmienta scandens</i>	>128 ^e	(38)
7 β ,22-dihydroxyhopane (86)	<i>Sarmienta scandens</i>	>128 ^e	(38)
oleanolic acid (87)	<i>Baccharis patagonica</i>	64 ^e	(38)
erythodiol (88)	<i>Baccharis patagonica</i>	64 ^e	(38)
3- <i>epi</i> oleanolic acid (89)	<i>Junellia tridens</i>	16	(39)
oleanonic acid (90)	<i>Junellia tridens</i>	16	(39)
lupeol (91)	<i>Chuireira ulicina</i>	64 ^e	(38)
betulinic acid (92)	–	32 ^e	(38)
betulin (93)	–	32 ^e	(38)
<i>epi</i> -betulinic acid (94)	<i>Monttea aphylla</i>	64 ^e	(38)
lupeol acetate (95)	<i>Chuireira ulicina</i>	>128 ^e	(38)
lupenone (96)	<i>Chuireira ulicina</i>	>128 ^e	(38)
3-hydroxynorlupen-2-one (97)	<i>Chuireira ulicina</i>	>128 ^e	(38)
3-acetoxynorlupen-2-one (98)	<i>Chuireira ulicina</i>	>128 ^e	(38)
ursolic acid (99)	<i>Aspidosperma quebracho-blanco</i>	32 ^e	(38)
uvaol (100)	–	32 ^e	(38)
pomolic acid (101)	<i>Acaena pinnatifida</i>	64 ^e	(38)
pomolic acid acetate (102)	<i>Acaena pinnatifida</i>	32 ^e	(38)
tormentic acid (103)	<i>Acaena pinnatifida</i>	32 ^e	(38)
2- <i>epi</i> -tormentic acid (104)	<i>Acaena pinnatifida</i>	>128 ^e	(38)
euscaphic acid (105)	<i>Acaena pinnatifida</i>	128 ^e	(38)
niga-ichigoside F1 aglycone (106)	<i>Acaena pinnatifida</i>	>128 ^e	(38)
Phytol, derivatives, and analogs			
(<i>E</i>)-phytol (107)	<i>Lucas volkensii</i>	2	(27)
(<i>E</i>)-phytyl acetate (108)	synthetic analog	16	(27)
(<i>E</i>)-phytyl methyl ether (109)	synthetic analog	16	(26)
phytyl amine (110)	synthetic analog	32	(26)

Table 1 cont.

Compound Class Name	Source	MIC ($\mu\text{g/ml}$) ^a	Reference
phytyl amine (110)	synthetic analog	32	(26)
phytyl diisopropylamine (111)	synthetic analog	64	(26)
(<i>Z</i>)-phytol (112)	Sigma-Aldrich Co.	2	(27)
(3 <i>R</i> ,5 <i>R</i> ,7 <i>R</i> ,11 <i>R</i>)-phytanol (113)	synthetic analog	2	(27)
(<i>E</i>)-phytol epoxidation epimers (114)	synthetic analog	8	(27)
(3 <i>R</i> ,5 <i>R</i> ,7 <i>R</i> ,11 <i>R</i>)-phytanic acid (115)	synthetic analog	>128	(27)
2-phytylphenol (116)	synthetic analog	32	(26)
phytantriol (117)	Sigma-Aldrich Co.	16	(26)
2-hexadecanol (118)	Sigma-Aldrich Co.	8	(26)

^a Biological activity determined using BACTEC radiorespirometric bioassay except where noted otherwise.

^b Biological activity determined using disc-diffusion method.

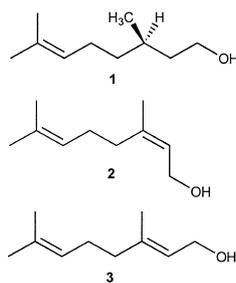
^c Biological activity determined using broth microdilution method.

^d Biological activity determined by conventional proportion method. Clinical isolates of *M. tuberculosis* were used in the bioassay.

^e Concentration reported in μM .

Monoterpenes

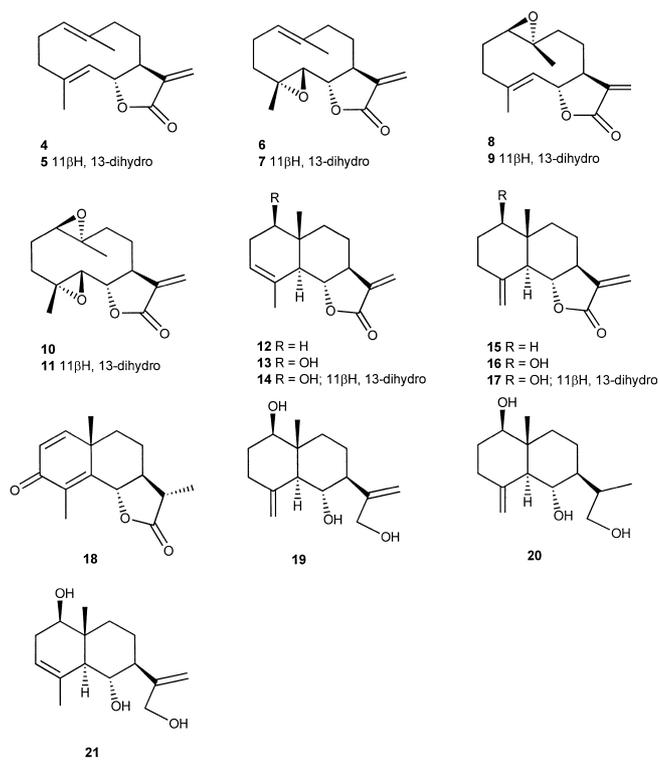
Due to the small number of monoterpenes with reported biological data and their structural similarity to phytol and its analogs, a more detailed structure-activity analysis will be discussed together with the results on phytol. It should be briefly pointed out, however, that among the open-chain monoterpenes, citronellol (**1**), nerol (**2**), and geraniol (**3**) gave MICs of 64, 128 and 64 $\mu\text{g/ml}$, respectively (Table 1) (26), (27). Compounds **2** and **3** represent respective *Z*- and *E*-isomers, and **1** being saturated at C-2; this suggests that the presence of a C-2 double bond has only minor influences upon the activity.



Sesquiterpenes

Among the over fifty sesquiterpenes, mainly sesquiterpene lactones of the germacranolide, guaianolide, and eudesmanolide type, tests resulted in MICs ranging from 2 $\mu\text{g/ml}$ to >128 $\mu\text{g/ml}$. A series of α -methylene- γ -lactone containing germacranolides showed MICs at or below 64 $\mu\text{g/ml}$ (28). The relatively lipophilic costunolide (**4**) gave an MIC of 32 $\mu\text{g/ml}$ while its 4-epoxide derivative, parthenolide (**6**), showed an increased activity against *M. tuberculosis* (MIC 16 $\mu\text{g/ml}$). The 1(10)-epoxycostunolide (**8**) was less active than **4** and **6** with an MIC of 64 $\mu\text{g/ml}$, suggesting that the position of the epoxide within the medium ring has a distinct influence on the activity. With the exception of the non-active diepoxide 1,10-epoxy parthenolide (**10**), all the sesquiterpene lactones in this series with an α -methylene- γ -lactone moiety (**4**, **6**, **8**, **13**, **16**, **12** and **15**) have activities with MICs at 64 $\mu\text{g/ml}$ or below. In contrast, their 11 β H,13-dihydro derivatives (**5**, **7**, **9**, **14**, and **17**)

as well as the sesquiterpenes obtained by reductive opening of the lactone ring (**19**, **20**, and **21**) (29) showed no activity against *M. tuberculosis* at concentrations below 128 $\mu\text{g/ml}$, suggesting that the presence of the exocyclic α -methylene- γ -lactone moiety is essential, but not sufficient, for activity.



Parthenolide, with an MIC of 16 $\mu\text{g/ml}$, was the most active germacranolide tested and exhibited a higher MIC than costunolide (MIC = 32 $\mu\text{g/ml}$). Moreover, 1(10)-epoxycostunolide (**8**) and the diepoxide **10**, although both with an α -methylene- γ -lactone moiety and an epoxide at C-1(10), neither compound showed activity comparable with parthenolide (**6**). It has therefore been suggested that the higher antimycobacterial activity of parthenolide (**6**) may be due to the presence of two sites of alkylation, one site being the α -methylene- γ -lac-

tone moiety and the other site at C-10, the electron deficient center being generated by a transannular cyclization with the C-1(10) double bond being the donor and the C-5 epoxide the nucleophilic receptor (28).

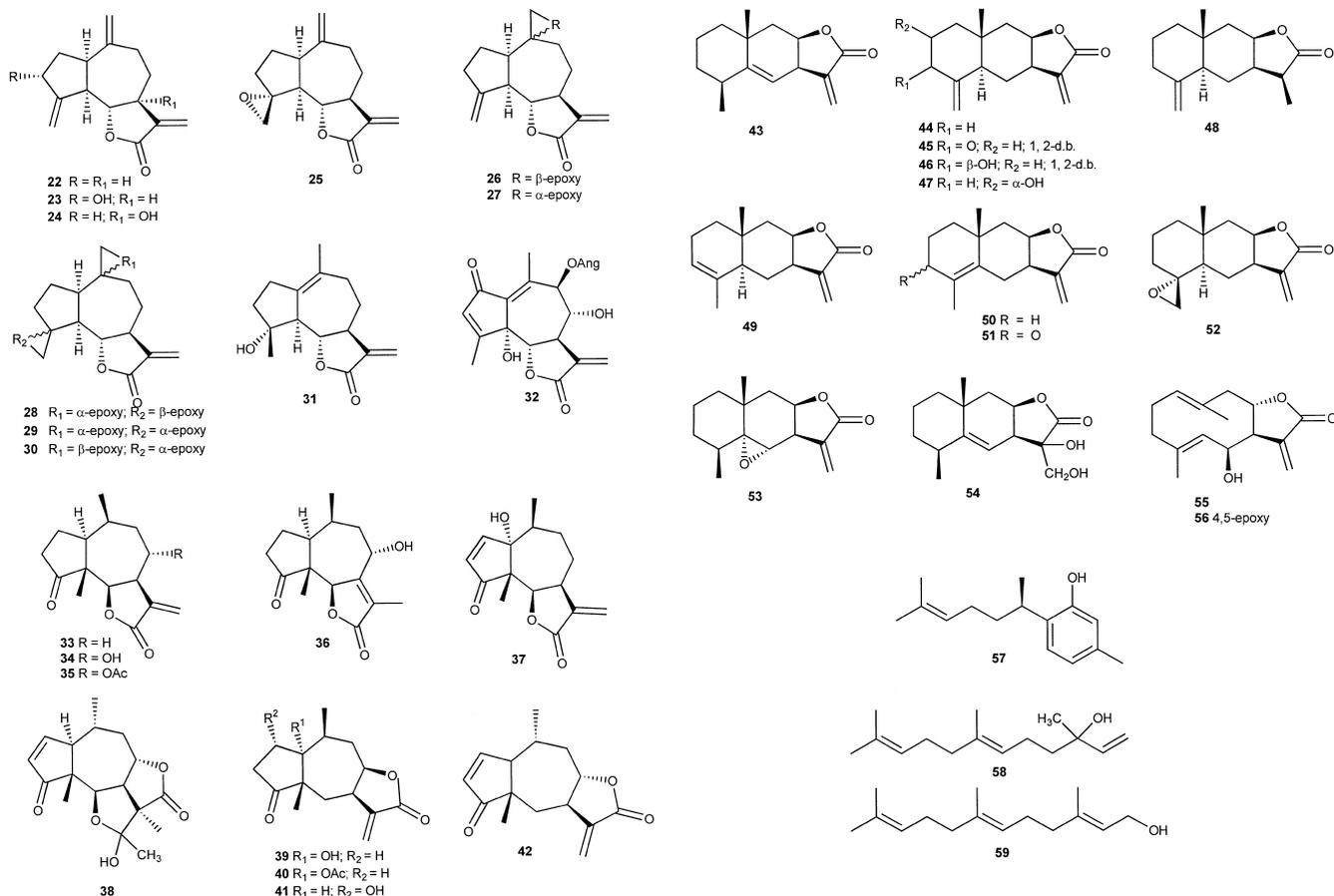
A series of guaianolides screened against *M. tuberculosis* demonstrated MICs ranging from 2 to >128 $\mu\text{g}/\text{ml}$, with the lipophilic dehydrocostuslactone (**22**) (MIC of 2 $\mu\text{g}/\text{ml}$) being the most active (30). Semi-synthetic mono- and diepoxide derivatives of **22** (**25–30**), as well as its hydroxy analogs showed very distinct activity trends. Monoepoxides **25**, **26**, and **27** were more active than the more polar diepoxides **28**, **29**, and **30**, which in turn were more active than the more polar hydroxy analogs **23** and **24**. It has been suggested that the low MIC of **22** may be due not only to its exocyclic methylene lactone moiety but also to its lipophilic nature (30). This was supported by the decreased activity from **22** to the more polar monoepoxides via the diepoxides to the most polar hydroxy analogs **23** and **24**. Micheliolide (**31**) (MIC 50 $\mu\text{g}/\text{ml}$) holds a unique position, possibly due to the ability to undergo an intramolecular substitution involving the 1(10)-double bond as a nucleophilic donor and the C-4 hydroxy as a leaving group, thus allowing a double alkylation as in parthenolide (**6**).

The pseudoguaianolides damsin (**33**), parthenin (**37**), and aromaticin (**42**) showed antimycobacterial activity with MICs of 32, 64, and 16 $\mu\text{g}/\text{ml}$, respectively. This suggests that two alkylating sites within the molecule such as **42**, increase activity. Again, the more polar C-8 hydroxy (**34**) and acetoxy (**35**) analogs of **33** gave MICs of 128 $\mu\text{g}/\text{ml}$, that is, much lower activity than **33**, which may be due to the more polar nature of **34** and

35. Also, tenulin (**38**) differs from **42** in its absence of an α -methylene- γ -lactone moiety. Consequently, its MIC of 128 $\mu\text{g}/\text{ml}$ further supports the necessity of this lactone group for significant activity.

Bioassay-directed investigations of *Inula helenium* and *Rudbeckia subtomentosa* resulted in the isolation of eudesmanolides, with MICs ranging from 8 to >128 $\mu\text{g}/\text{ml}$. Moderate activities were observed for alantolactone (**43**), isoalantolactone (**44**), 11 α H,13-dihydroisoalantolactone (**48**), alloalantolactone (**50**), and 3-oxoalloalantolactone (**51**) (31). Compounds **43**, **44**, and **50** all gave MICs of 32 $\mu\text{g}/\text{ml}$, while **48** and **51** were not active (MICs >128 and 128 $\mu\text{g}/\text{ml}$, respectively). Stereoselective epoxidation of **43** gave the eudesmanolide 5 α -epoxyalantolactone (**53**) with an MIC of 8 $\mu\text{g}/\text{ml}$ while oxidation of **43** with OsO_4 resulted in the inactive dihydroxy derivative **54**. Analogs of **44** include the monoepoxide **52**, the C-2 hydroxy derivative **47**, the conjugated ketone **45**, and alcohol **46** with MICs of 32, 64, 16, and 32 $\mu\text{g}/\text{ml}$, respectively.

As seen above with the various structural types of sesquiterpene lactones, the α -methylene- γ -lactone moiety appears to be an essential, but not sufficient, structural requirement for significant activity. The necessity of the presence of an α -methylene- γ -lactone group is supported by the moderate to high activity of α -methylene- γ -lactone bearing sesquiterpenes, when compared with the inactive 11 α H,13-dihydro derivatives with values of 128 $\mu\text{g}/\text{ml}$ or higher. The presence of a second alkylating site, such as an α,β -unsaturated carbonyl group and/or an epoxide function together with moderate to high lipophilicity, seems to enhance the *in vitro* antimycobac-

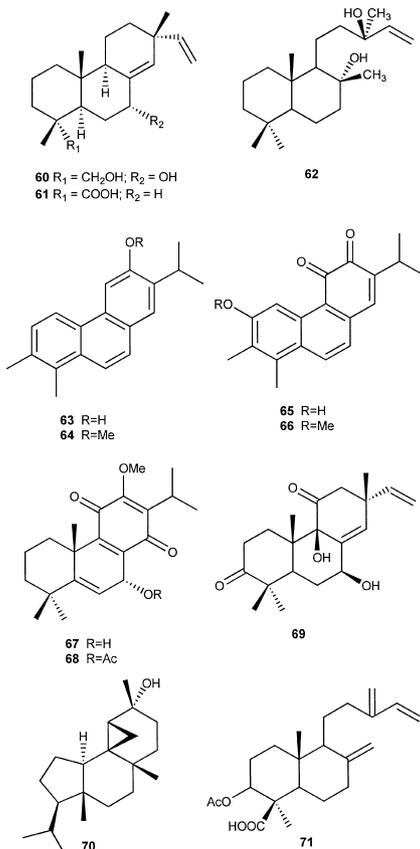


terial activity. For instance, encelin (**45**) is more active than **44** and 5α -epoxyalantolactone (**53**), with an MIC of $8\ \mu\text{g/ml}$, is significantly more active than its precursor **43** with an MIC of $32\ \mu\text{g/ml}$.

The sesquiterpenes curcuphenol (**57**), nerolidol (**58**), and farnesol (**59**) gave MICs of 16, 32, and $8\ \mu\text{g/ml}$, respectively. Compounds **58** and **59** are constitutional isomers differing in the positioning of the allylic alcohol. Due to the structural similarity of **57**, **58**, and **59** with phytol and its analogs, a more detailed discussion of their biological activities will follow later.

Diterpenes

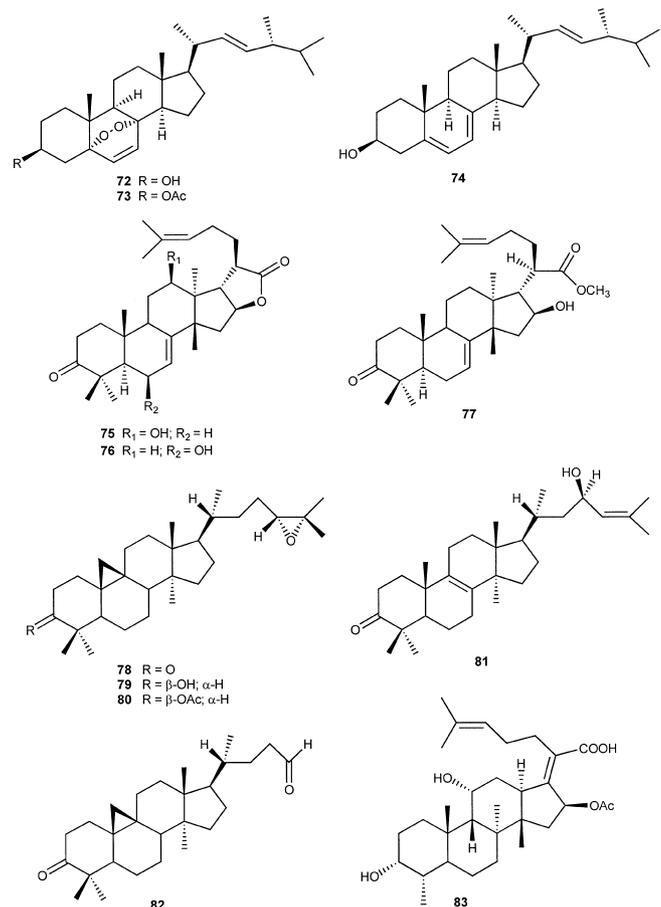
A limited number of diterpenes have been tested for antituberculosis activity and some have demonstrated remarkable biological activities against *M. tuberculosis* with MICs below $1\ \mu\text{g/ml}$. The most active among the diterpenes were recently reported by Ulubelen et al. from *Salvia multicaulis*, the norditerpenoid 12-demethylmulticauline (**63**) showing a remarkable MIC of $0.46\ \mu\text{g/ml}$ and its C-12 methoxy analog, **64**, with an MIC of $5.6\ \mu\text{g/ml}$ (32). Similarly, the phenolic *o*-quinone (**65**) gave an MIC of $1.2\ \mu\text{g/ml}$ while its C-2 methoxy analog had an MIC of $2.0\ \mu\text{g/ml}$. Interestingly, the abietane diterpenoid **67** had an MIC of 1.2 while its C-12 acetate **68** was more active with an MIC of $0.89\ \mu\text{g/ml}$ (32). Compounds **60** (33), **61** (34), **62** (34), **69** (32), **70** (35), and **71** (34) gave MICs of 25–100, 15, 6, 7.3, 20, and $14.4\ \mu\text{g/ml}$, respectively. Biological activities of the diterpene phytol, its derivatives and structural analogs will be discussed later.



Triterpenes and Sterols

Numerous triterpenoids and sterols have been tested with MICs ranging from 1 to $>128\ \mu\text{g/ml}$. Bioassay-guided investigations of active fractions from *Ajuga remota* led to the isolation of the most active triterpenoid, ergosterol-5,8-endoperoxide (**72**), with an MIC of $1\ \mu\text{g/ml}$ (36). Acetylation of **72** provided the acetate, **73**, with an MIC of $8\ \mu\text{g/ml}$. In contrast, the parent compound, ergosterol (**74**), gave an MIC of $>128\ \mu\text{g/ml}$. Compound **72** was prepared in a one step synthesis from commercially available ergosterol (**74**) (36) making it an ideal candidate for future structure-activity relationship studies. Within this set of ergosterol derivatives, it appears that the presence of a C-3-OH combined with the endoperoxide group is necessary for high antimycobacterial activity.

Bioactivity-guided investigations of *Melia volkensii* resulted in the isolation of 12β -hydroxykukulactone (**75**), 6β -hydroxykukulactone (**76**), and kulonate (**77**). Compounds **75** and **77** both had MICs of $16\ \mu\text{g/ml}$ while compound **76** was more active with an MIC of $4\ \mu\text{g/ml}$ (37). From this set of limited data, it appears that a hydroxy group at C-6 gives rise to higher activity than a C-12 hydroxy group. Based on the same activity of **75** and **77**, there appears to be little or no effect on biological activity upon methanolysis of the lactone moiety.



In another bioassay-guided investigation, *Borrchia frutescens* afforded a number of antimycobacterial cycloartanes (25). The most active of these compounds were (24R)-24,25-epoxycycloartan-3-one (**78**) and (3 β ,24R)-24,25-epoxycycloartan-3-

ol (**79**) with MICs of 8 $\mu\text{g/ml}$, whereas the acetate **80** was inactive (MIC >128 $\mu\text{g/ml}$). Unsuccessful attempts to oxidize **79** to **78** resulted in the synthesis of the inactive degradation product **82** (MIC >128 $\mu\text{g/ml}$), and the isolate **81** gave an MIC of 64–128 $\mu\text{g/ml}$. Correlations of structural features and the MICs of these five triterpenes suggest that the presence of the C-3 keto and/or β -hydroxy group, the cyclopropane ring and the epoxide moieties, as in **78** and **79**, seem to play a major role in the *in vitro* antituberculosis activity. Both, the cyclopropane and epoxide functions are absent in **81**, resulting in the loss of activity. Also, the loss of activity by acetylation of the C-3 hydroxy group (MIC >128 $\mu\text{g/ml}$) strongly suggests that either a free hydroxy or a keto group at C-3 is required for significant activity.

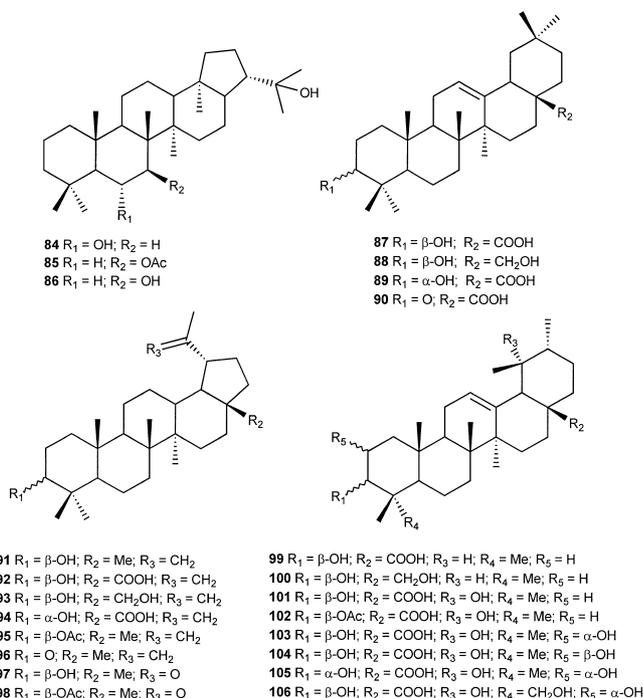
Several pentacyclic triterpenoids have been isolated with activities ranging from 8 to >128 μM . The most active of these compounds was zeorin (**84**), isolated by bioassay-guided fractionation of *Sarmienta scandens* and shown to have an MIC of 8 $\mu\text{g/ml}$ (38). 7 β ,22-Dihydroxyhopane (**86**) contains a β -hydroxy group at C-7 rather than an α -hydroxy at C-6 in **84**, resulting in a loss of activity (MIC >128 μM). In addition, the C-7 acetoxy derivative of **86**, 7 β -acetyl-22-hydroxyhopane (**85**), was inactive (38). 3-Epooleanic acid (**89**) and oleanonic acid (**90**), which differ in the functional group present at C-3, were both isolated from *Junellia tridens* and shown to have MICs of 16 $\mu\text{g/ml}$ (39). Compound **89** contains an α -hydroxy group at C-3 while **90** bears a ketone. Oleanolic acid **87** differs from **89** by the presence of a C-3 β rather than an α -hydroxy group, resulting in a less active derivative with an MIC of 64 μM . The dihydroxy analog of **87**, erythrodiol (**88**), gave a similar MIC value of 64 $\mu\text{g/ml}$. Similarly, ursolic acid (**99**) and betulinic acid (**92**) gave MIC values of 32 μM , identical to those of uvaol (**100**) and betulin (**93**) (38). In contrast to the results obtained when comparing the MICs of compounds **87** and **89**, betulinic acid (**92**) with its β -hydroxy group at C-3 is more active than its C-3 epimer, *epi*-betulinic acid (**94**) (MIC 64 μM). Also, re-

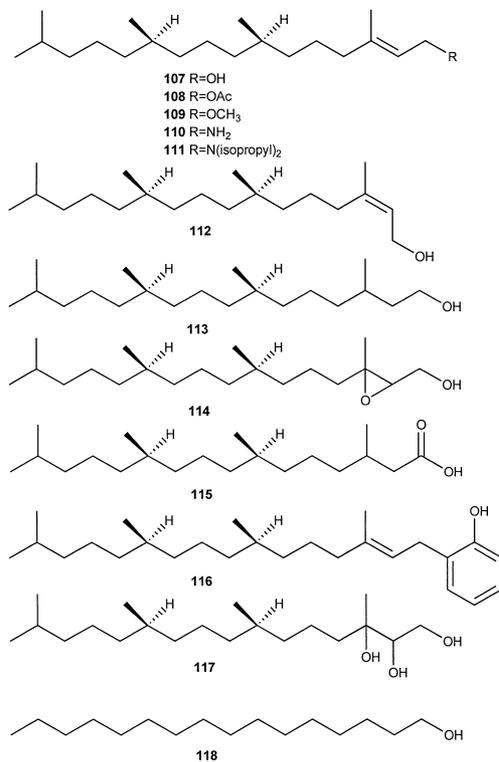
placement of the 3-hydroxy group of lupeol (**91**) with either an acetate or a ketone leads to complete loss of activity in lupeol acetate (**95**) and lupenone (**96**). A similar result was observed for the C-3 acetoxy derivative **80** when compared to **79**. However, the 3-oxo derivative **78** gave the same MIC as **79** which is in contrast to the correlations mentioned above. Further complicating the situation was the observation that pomolic acid 3-acetate (**102**) was more active than pomolic acid (**101**) itself. Comparison of ursolic acid (**99**) with pomolic acid (**101**), tormentic acid (**103**), 2-*epi*-tormentic acid (**104**), euscaphic acid (**105**) and the aglycone of niga-ichigoside (**106**) showed that additional hydroxy groups in rings A and E can, in some cases, reduce activity (38). These conflicting results observed among the various groups of triterpenoids makes it difficult to predict structural requirements for antimycobacterial activity.

Phytol, Derivatives and Analogs

This group of open chain diterpenes covers phytol, its derivatives and structural analogs. Also included are the previously mentioned linear monoterpenes **1**, **2**, and **3** and diterpenes **57**, **58**, and **59**. Within this set of structural analogs, all but two compounds showed MICs at or below 64 $\mu\text{g/ml}$. One of the most active compounds in this series, (*E*)-phytol (**107**) (MIC 2 $\mu\text{g/ml}$) was isolated from *Lucas volkensii*, using a bioassay-guided fractionation (27). In addition, the analogs (*Z*)-phytol (**112**) and (3*R,S,7R,11R*)-phytanol (**113**) demonstrated MICs of 2 $\mu\text{g/ml}$, suggesting that the 2,3-double bond may not be essential for bioactivity. However, (*E*)-phytyl acetate (**108**) and (*E*)-phytyl methyl ether (**109**) showed MICs of 16 $\mu\text{g/ml}$ implying that a free hydroxy group, as present in **107**, is required for significant activity. Due to their structural and biosynthetic similarities to (*E*)-phytol, geraniol (**3**) and farnesol (**59**) were also tested against *M. tuberculosis*. The monoterpene alcohol geraniol had an MIC of 64 $\mu\text{g/ml}$ while the more lipophilic farnesol with a 15-carbon chain gave a significant increase in activity with an MIC of 8 $\mu\text{g/ml}$. Citronellol (**1**) and nerol (**2**) were tested due to their structural similarity to geraniol and gave MICs of 64 and 128 $\mu\text{g/ml}$, respectively. The identical MICs of 64 $\mu\text{g/ml}$ between geraniol and citronellol further support the observation above for phytol and its reduced isomer (3*R,S,7R,11R*)-phytanol that the 2-double bond is not essential for biological activity. Additional derivatives and analogs tested include curcuphenol (**57**), nerolidol (**58**), phitylamine (**110**), phityldiisopropylamine (**111**), (*E*)-phytol epoxidation epimers (**114**), (3*R,S,7R,11R*)-phytanic acid (**115**), 2-phytylphenol (**116**), phytantriol (**117**), and 2-hexadecanol (**118**) giving MICs of 16, 32, 32, 64, 8, >128, 32, 16, and 8 $\mu\text{g/ml}$, respectively (27), (26). It is significant to note that a complete loss of activity is found when the C-1 hydroxy of (3*R,S,7R,11R*)-phytanol (**113**) is oxidized to the carboxylic acid, (3*R,S,7R,11R*)-phytanic acid (**115**), again suggesting that the free hydroxy is essential for high activity.

The most interesting observation among the above group of compounds is the relationship between the calculated log P values and the MIC. Higher lipophilicity generally results in higher antimycobacterial activity, within a series of structurally similar compounds. For example, the more lipophilic 20-carbon phytol (log P = 8.66) is more active than the 15-carbon farnesol (log P = 5.31) which is more active than the 10-carbon geraniol (**3**) (log P = 3.28). However, there appears to





be a point at which further increasing the lipophilicity results in lower antimycobacterial activity (Fig. 1). Replacing the hydroxy of phytol with nitrogen containing functional groups strongly reduces activity in spite of an increase in lipophilicity. For example, replacing the hydroxy of phytol with a diisopropylamine as in **111** (log P = 11.14) results in a more lipophilic molecule that is less active. Figure 1 is a plot of calculated log P values versus their respective MIC's, for a selected group of phytol derivatives and analogs.

Conclusions

A broad range of plant terpenoids from various classes have been evaluated for their *in vitro* antimycobacterial activity. The most active terpenoid presented in this review is the norditerpenoid 12-demethylmulticauline (**63**), isolated from the roots of *Salvia multicaulis*, with an MIC of 0.46 µg/ml. It is more active than the first line tuberculosis drug ethambutol and nearly as active as rifampin. Further terpenoids with high activities are the sesquiterpene dehydrocostuslactone (**22**) with an MIC of 2 µg/ml, the sterol ergosterol-5,8-endoperoxide (**72**) (MIC 1 µg/ml), and (*E*)-phytol (**107**) with an MIC of 2 µg/ml.

Distinct similarities between different classes of terpenes and the structural features important for high activity within each class have been observed. In each series it was shown that more lipophilic compounds are significantly more active than their more polar analogs. This was observed for the sesquiterpene lactone dehydrocostuslactone (**22**) which gave an MIC of 2 µg/ml, when compared to its C-3 and C-7 hydroxy analogs, both with MICs of >128 µg/ml. Furthermore, higher MICs were found for the monoepoxide derivatives of **22**, followed by even higher MICs of the diepoxides. Additional support for a correlation between lipophilicity and antimycobacterial activ-

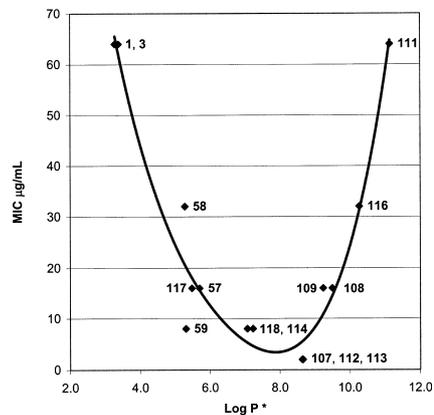


Fig. 1 The relationship between calculated Log P values and antimycobacterial activity of phytol, its derivatives, and structural analogs. Plotted compounds include: **1**, **3**, **57**, **58**, **59**, **107**, **108**, **109**, **111**, **112**, **113**, **114**, **116**, **117**, **118**. Curve fitting was performed using a polynomial fourth order equation. *Log P values were estimated using ACD Labs Log P module (47).

ity was found in the (*E*)-phytol series in which the highest activity was observed for the least polar 20-carbon (*E*)-phytol (**107**) followed by the 15-carbon analog farnesol (**59**), which was more active than the more polar 10-carbon geraniol (**3**).

Among phytol (**107**), its acetate (**108**), and methyl ether analog **109**, both derivatives gave distinct decreases in activity. Also, a decrease of activity was found for the more lipophilic nitrogen containing phytol analogs **110** and **111**. Similar relationships were observed among the triterpenes and sterols where a free hydroxy or carbonyl at C-3 appears to be essential for high activity. For instance, compounds **72** and **79** were both more active than their corresponding C-3 acetoxy derivatives, **73** and **80**, respectively.

The resurgence of infectious diseases such as drug-resistant tuberculosis necessitates more intense future efforts in the discovery of new specific drugs from natural and synthetic sources. The recent development of efficient and reproducible bioassays plays a significant role in the present and future discovery and development of new anti-TB leads. A number of compounds covered in this review possess *in vitro* antimycobacterial activities comparable to standard anti-TB drugs. They certainly warrant further investigations on the long path from the initial activity findings to the development of new antituberculosis drugs.

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