Sterols and Triterpenoids with Antiviral Activity

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Abstract: Terpenoid compounds of natural origin with antiviral activities, namely triterpenes and steroids, include compounds with potential future medical use as well as substances in different stages of clinical research and testing. These natural compounds have often been synthetically modified, the modification including both changes in the basic structure or mere substituent modifications.

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INTRODUCTION

Modern medical science is constantly searching for new and more powerful agents to prevent, treat or retard viral infections and cure the diseases they cause. Viral infections of humans and domestic animals cause vast sums of money to be spent each year by pharmaceutical companies to identify, characterize, and produce new antivirals. Yet, despite the costs and efforts to identify treatments for viral infections, such as hepatitis and AIDS, effective therapies remain elusive. Prevention of some viral infections, such as hepatitis B virus, is possible through vaccination, which is, however, not effective in treating those already infected, i.e. carriers and patients. With others, such as hepatitis C virus, no effective immunization is currently available and the disease can only be controlled by preventive measures such as improvement of hygiene and sanitary conditions. Likewise, there is still no cure for the deadly AIDS and its causative agent HIV. Even when effective, many of current antivirals lead to the development of viral resistance coupled with the problem of side effects, recurrence and viral latency. A world-wide search for new antivirals of natural origin is therefore underway. An important part of the research in this field is the application of biosynthetic knowledge to the synthesis of interesting, natural products (biomimetic synthesis). Our review focuses on triterpenes and steroids with antiviral activities, their sources, structure-activity relationships and possible synthetic modifications. Typical representatives of these compounds are betulinic acid [1] and betulinol [2]. Both compounds possess the basic skeleton of lupine and have therefore been often synthetically modified [3]. Two recent reviews [4,5] summarize the advances in this field including hundreds of other compounds of natural origin.

TRITERPENES AND STEROLS

Terpenes are a large and varied class of hydrocarbons, produced primarily by a wide variety of living organisms. The name "terpene" is derived from the word "turpentine". In addition to their roles as end-products in many organisms, terpenes are major biosynthetic building blocks within nearly every living creature. Sterols are derivatives of the triterpene squalene.

When terpenes are modified chemically, such as by oxidation or rearrangement of the carbon skeleton, the resulting compounds are generally referred to as terpenoids. Terpenoids are the primary constituents of the essential oils of many types of plants and flowers. Essential oils are used widely as natural flavor additives for food, as fragrances in perfumery, and in traditional and alternative medicines such as aromatherapy. Synthetic variations and derivatives of natural terpenes and terpenoids also greatly expand the variety of aromas used in perfumery and flavors used in food additives.

Terpenes are derived biosynthetically from units of isoprene, which has the molecular formula C_5H_8. The basic molecular formulae of terpenes are multiples of that, $(C_5H_8)_n$, where n is the number of linked isoprene units. This is called the isoprene rule or the C_5 rule. The isoprene units may be linked together "head to tail" to form linear chains or they may be arranged to form rings. One can consider the isoprene unit as one of nature's common building blocks.

As chains of isoprene units are built up, the resulting terpenes are classified sequentially by size as hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, tetraterpenes, and polyterpenes, where a prefix in the name indicates the number of terpene units needed to assemble the molecule. A single terpene unit is formed from two molecules of isoprene, so that a monoterpene consists of two isoprene units.

Sesquiterpenes consist of three isoprene units; diterpenes are composed for four isoprene units. They derive from geranylgeranyl pyrophosphate. Diterpenes also form the basis for biologically important compounds such as retinol, retinal, and phytol. They are known to be antimicrobial and antiinflammatory.

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Sesterterpenes, terpenes having 25 carbons (five isoprene units), are rare relative to the other sizes. The sester- prefix means half to three, i.e. two and a half.

Triterpenes consist of six isoprene units. The basic structure, i.e. squalene is processed biosynthetically to generate either lanosterol or cycloartenol, the structural precursors to all the steroids.

Tetraterpenes contain eight isoprene units and biologically important tetraterpenes include the acyclic lycopene, the monocyclic γ-carotene, and the bicyclic α- and β-carotenes.

Polyterpenes consist of long chains of many isoprene units. Natural rubber consists of polyisoprene in which the double bonds are *Z* (*cis*). Some plants produce a polyisoprene with *E* (*trans*) double bonds, known as gutta-percha.

Sterols, or steroid alcohols are a subgroup of steroids with a hydroxyl group at the 3-position of the A-ring. The overall molecule is quite flat. The hydroxyl group on the A ring is polar. The rest of the aliphatic chain is non-polar. Sterols and related compounds play essential roles in the physiology of eukaryotic organisms. For example, cholesterol forms part of the cellular membrane in animals, where it affects the cell membrane's fluidity and serves as secondary messenger in developmental signaling. Phytosterols may block cholesterol absorption sites in the human intestine thus helping to reduce cholesterol in humans. Plant sterols and stanols are naturally occurring substances found in plants. They are present in small quantities in many fruits, vegetables, vegetable oils, nuts, seeds, cereals and legumes.

3-β-Hydroxy-lup-20(29)-en-28-oic acid (betulinic acid) (**1**) is a pentacyclic lupane-type triterpene that is widely distributed throughout the plant kingdom. Among its many biological activities it is highly regarded for its anti-HIV-1 activity and specific cytotoxicity against a variety of tumor cell lines. Interest in developing even more potent anti-HIV agents based on betulinic acid has led to the discovery of a host of highly active derivatives. The targets of betulinic acid derivatives are varied, depending primarily on the side chain structures of the compounds [6, 7].

Betulinic acid (**1**) and platanic acid (**2**) exhibited anti-HIV activity in H9 lymphocyte cells at an IC_{50} of 1.4 and 6.5 μM, respectively (selectivity index: 9.3 and 14, respectively). Betulinic acid demonstrated an anti-HIV activity with an EC_{50} value of 1.4 μM and an IC_{50} value of 13 μM. Dihydrobetulinic acid (**3**) showed EC_{50} and IC_{50} values of 0.9 and 13 μM respectively [8]. Modification of betulinic acid and dihydrobetulinic acids has successfully increased anti-HIV potency. Esterification at C-3 hydroxyl resulted in more potent compounds with greatly improved TI values. 3-*O*-(3,3′-dimethylsuccinyl) betulinic acid (**4**, DSB, PA-457) had an $EC_{50} < 3.5\ 10^{-4}$ μM and TI > 20,000) [9]. PA-457, which was discovered by Panacos Corp. scientists, works by a mechanism different from that of any approved drug or other drugs under development, by blocking a key step in the processing of a viral core protein called capsid. Preclinical studies have shown that PA-457 retains full activity against drug-resistant virus, is effective in an animal model of HIV infection and should be suitable for use in combination therapy with other drugs. Also 3-*O*-(3′,3′-dimethylsuccinyl) dihydrobetulinic acid (**5**) has remarkably high anti-HIV activity and selectivity; IC_{50} <0.35 nM; selectivity index >14 000, respectively [9].

A different derivative of the betulinic acid RPR 103611 (**6**) represents the only non-peptidic low-molecular-weight compound known to block HIV-1 infection through interaction with gp41. This triterpene derivative has been found to inhibit the infectivity of a number of HIV-1 strains in the 10 nM concentration range [10], apparently through interference with a post-binding, envelope-dependent step involved in the fusion of the virus with the cell plasma membrane. The exact mode of action of RPR 103611 remains to be elucidated. Resistance to this compound appears to be associated with the emergence of two amino acid substitutions within gp41. The antiviral efficacy of RPR 103611 therefore depends on the sequence, and accessibility, of the gp41 loop region [11].

A stereoisomer of RPR 103611, namely IC 9564 (4*S*-[8-(28-betuliniyl)amino octanoylamino]-3*R*-hydroxy-6-methylheptanoic acid) (**7**), has been found to inhibit HIV replication through interference with the viral envelope-induced membrane fusion [12].

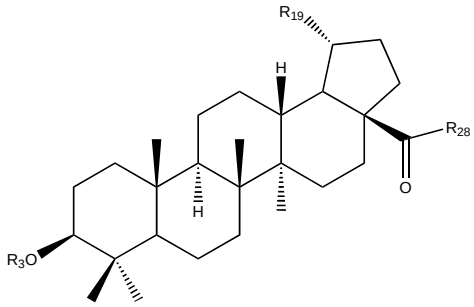
Other betulinic acid derivatives such as LH15 (**8**) and LH55 (**9**) exhibit the above activities combined, i.e. they are antientry like IC9564 and antimaturation like PA-457 [7].

Frodin is a principal ingredient of an herbal tea (*Schefflera heptaphylla*) formulation widely used for the treatment of common cold in southern China [13]. An extract of the long leafstalk of the compound leaf of *S. heptaphylla* exhibited the most potent antiviral activity against respiratory syncytial virus. Further antiviral-guided fractionation and isolation of the leafstalk extract of *S. heptaphylla* yielded two highly active pure triterpenoids, namely dioic acid (**10**) and 3-O-sulfate (**11**). An antiviral assay using a cytopathic effect reduction method showed that the two triterpenoids possessed broader antiviral activity against respiratory syncytial virus (IC_{50} 6.25 μg/mL), influenza A (H1N1) virus (IC_{50} 25 and 31.3 μg/mL), Coxsackie B3 virus (IC_{50} 12.5 and 20 μg/mL) and herpes simplex virus type 1 (IC_{50} 18.8 and 25 μg/mL), respectively.

Lupenone (**12**) from *Euphorbia segetalis* exhibited strong viral plaque inhibitory effect against HIV-1 and HIV-2 [14].

Oleanolic acid (**13**) was identified as an anti-HIV principle from several plants, including *Rosa woodsii* (leaves), *Prosopis glandulosa* (leaves and twigs), *Phoradendron juniperinum* (whole plant), *Syzygium claviflorum* (leaves), *Hyptis capitata* (whole plant) and *Ternstromia gymnanthera* (aerial part). It inhibited HIV-1 replication in acutely infected H9 cells with an EC_{50} value of 1.7 μg/mL and inhibited H9 cell growth with an IC_{50} value of 21.8 μg/mL (TI 12.8) [15]. Like betulinic acid, esterification at C-3 hydroxyl of oleanolic acid resulted in 3-oxotirucalla-7,24-dien-21-oic acid (**14**) with improved activity (EC_{50} 0.0039 μg/mL, TI 3750). It also inhibited HIV protease with an IC_{50} value of 10 μg/mL.

Pomolic acid (**15**), isolated from *R. woodsii* and *H. capitata*, was also identified as an anti-HIV agent (EC_{50} 1.4 μg/mL, TI 16.6). A new triterpene (**16**) (1α-hydroxy-2-oxopomolic acid) was also isolated from the $CHCl_3$-soluble fraction of *R. woodsii*, though it showed no anti-HIV activity [16].



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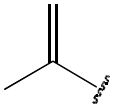
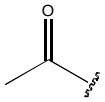
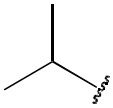
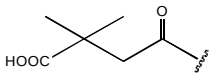
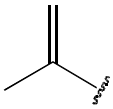
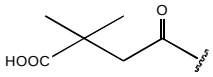
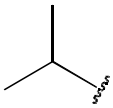
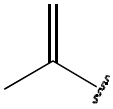
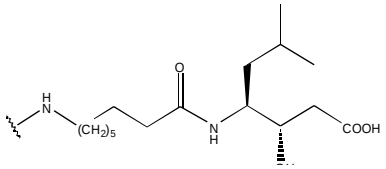
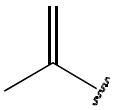
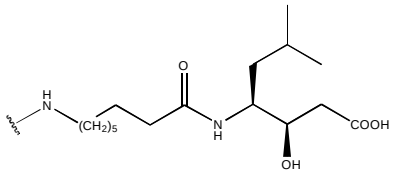
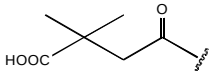
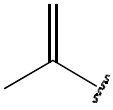
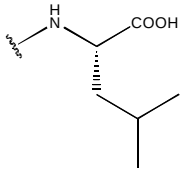
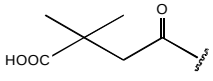
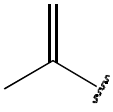
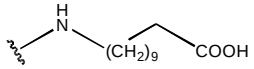
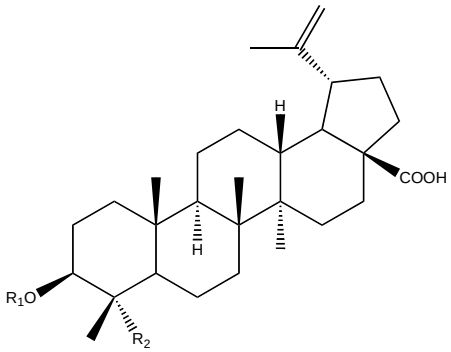
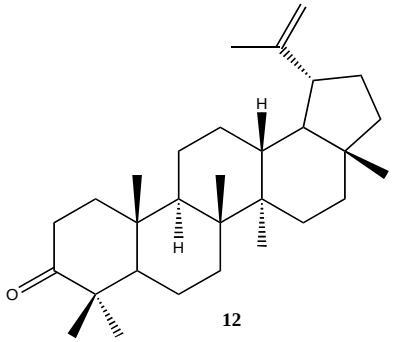
1	H		OH
2	H		OH
3	H		OH
4			OH
5			OH
6	H		
7	H		
8			
9			

Uvaol (**17**) and ursolic acid (**18**) isolated from the methanolic extract of leaves of *Crataegus pinatifida* [17], showed potent inhibitory activity against HIV-1 protease at a concentration of 100 μg/mL. Maslinic acid (**19**) isolated from *Geum japonicum* [18] showed potent inhibitory activity against HIV-1 protease at a concentration of 17.9 μg/mL. Moronic

acid (**20**) isolated from *Myrceugenia euosma* showed significant anti-HIV activity with therapeutic index of more than 186 [19]. Pentacyclic triterpenes, 1β-hydroxymaprounic 3-*p*-hydroxybenzoate (**21**), and 2α-hydroxymaprounic 2,3-bis-*p*-hydroxybenzoate (**22**) isolated from the roots of *Maprounea africana* [20], inhibited HIV-1 RTase with an IC_{50} value of 3.7 μM. Celasdin B (**23**) isolated from ethanolic extract of *Celastrus hindsii* exhibited anti-HIV replication activity in H9 lymphocyte cells *in vitro* [21]. Oxygenated triterpenes, such as α-ganoderic acid (**24**), isolated from methanolic extract of *Ganoderma lucidum*, were found to inhibit HIV-1 induced cytopathic effects in MT-4 cells and also possessed HIV-1 protease inhibitory activity [22].



	R_1	R_2
10	H	COOH
11	SO_3H	Me



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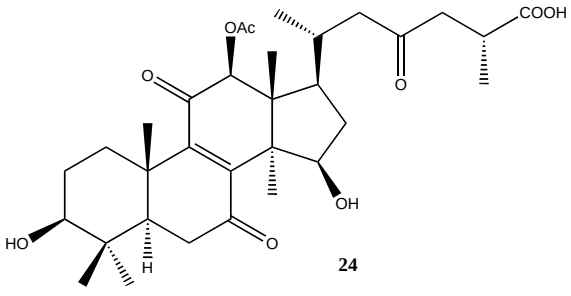

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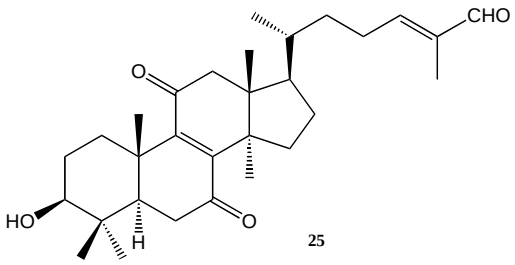
Three new triterpenes were isolated from the fruiting bodies of *Ganoderma pfeifferi*, lucialdehyde D, (**25**), ganoderone A, (**26**), and ganoderone C, (**27**). Ganoderone A exhibited strong inhibitory activities against Vero cells (IC_{50} was 0.3 μg/mL) [23].



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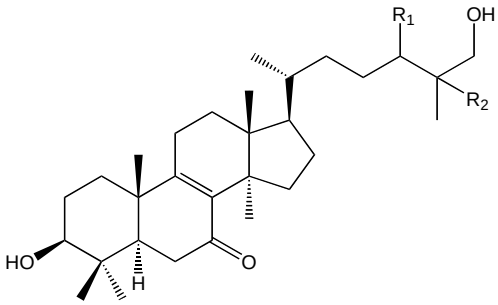
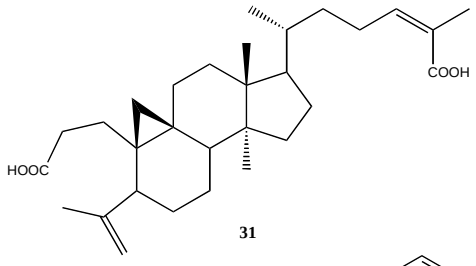


	$R_1 - R_2$
26	Δ^{24}
27	-O-

Lanostane-type triterpene, suberosol (**28**), isolated from ethanolic extract of the stems and leaves of *Polyalthia suberosa* showed anti-HIV replication activity in H9 lymphocyte cells [24]. The protostanes, garcisaterpenes A (**29**) and B (**30**) isolated from ethyl acetate extract of bark and stems of *Garcinia speciosa*, showed significant inhibitory activities against HIV-1 RTase and in the syncytium assay [25]. A ring-secocycloartene triterpenoid, nigranoic acid (**31**), isolated from the stems of *Schisandra sphaerandra* [26], inhibited HIV-1 RTase and HIV-2 RTase. Triterpene lactone, lancilactone C (**32**) isolated from stems and roots of *Kadsura lancilimba*, also possessed inhibitory activity against HIV replication in H9 lymphocytes [27].



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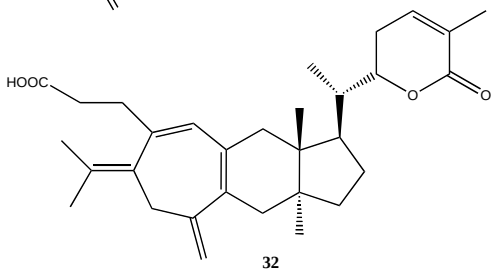

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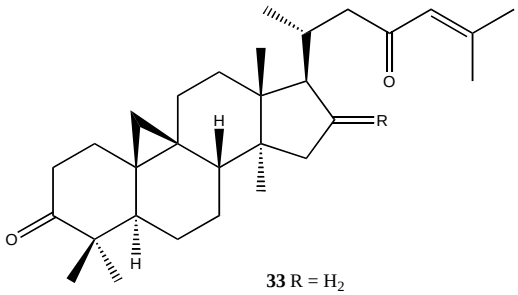
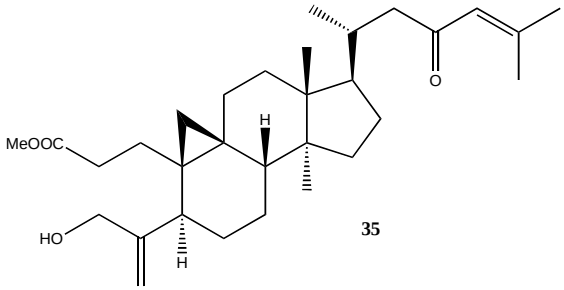
Pure compounds (**33-35**) from *Gardenia obtusifolia* were evaluated for cytotoxic effects against a panel of cultured mammalian cell lines [28]. The results including their an-

timitotic activities are shown in Table **1**. Moderate to high cytotoxicity was found in compound **35**. All of the isolated and modified compounds were also tested employing HIV-1 reverse transcriptase (RT), and a syncytium assay using DTat/RevMC99 virus and 1A2 cell line system (see Table **2**). It was found that only **35** showed potent inhibitory activity (99.9% inhibition at 200 mg/mL) against HIV-1 RT (fagaronine chloride was used as a positive control). However, compound **33** was found to be very toxic to the cell lines used in this assay. It is noteworthy that a semisynthetic derivative, which was modified from the isolated **34**, gave the best therapeutic index (TI 32.1; EC_{50} 3.9 μg/mL; IC_{50} 125 μg/mL) comparable with other compounds.

Callus tissue cultures induced from an axenic leaf of *Eriobotrya japonica* produced triterpenes in large amounts [29]. Nine triterpenes were characterized as oleanolic acid (**13**), ursolic acid (**18**), maslinic acid (**19**), 2α-hydoxyursolic acid (**36**), tormentic acid (**37**), 2α,19α-dihydroxy-3-oxo-urs-12-en-28-oic acid (**38**), hyptadienic acid (**39**) and a mixture of 3-*O*-cis-p-coumaroyltormentic acid (**40**) and 3-*O*-trans-p-coumaroyltormentic acid (**41**). The triterpene composition in the callus tissues was noticeably different from that in intact leaves. All of the triterpenes isolated from the callus tissues showed an inhibitory effect comparable to (-)-epigallocatechin gallate (EGCG) (main polyphenol of green tea) on the activation of Epstein-Barr virus early antigen (EBV-EA) induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Compound **38** was the most potent inhibitor among them andcaused a significant delay of two-stage carcinogenesis on mouse skin. The results are shown in Table **3**. Most of the tory effects comparable to or much stronger (more than 65% triterpenes except for compounds **36** and **37** exhibited inhibi



33 R = H_2
34 R = O



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inhibition) than that of a positive control, epigallocatechin gallate at a concentration of 500 mol ratio/TPA. Compound **38** having an oxo group showed the most potent activity among the compounds tested (76.3% inhibition at 500 mol ratio/TPA).

A new lanostane-type triterpenoid (**42**) was isolated as a new natural product for the first time from the mushroom *Scleroderma citrinum* [30]. Compound **42** exhibited strong

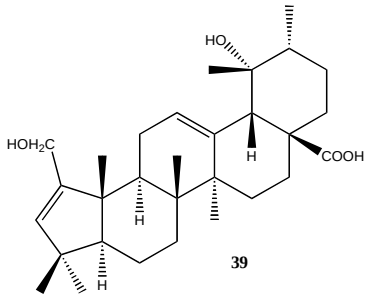
Table 1. Cytotoxic and Antimitotic Activities (ASK Assay) of Isolated Compounds 33-35

Compound	Cytotoxicity					
	Cell line					
	P-388	KB	Col-2	BCA-1	Lu-1	ASK
33	>20	>20	>20	>20	>20	>20
34	>20	>20	>20	>20	>20	>20
35	3.3	16.4	9.1	10.9	5.8	10.9

Table 2. Anti-HIV Activities of the Isolated 33-35

Compound	Anti-syncytium (MC99+1A2)				Anti-HIV-1 RT	
	IC_{50} (μg/mL)	EC_{50} (μg/mL)	TI (IC_{50}/EC_{50})	Activity %	Inhibition	Activity
33	215	–	–	Ia	12.0	I
34	>250	146.2	>1.7	Aa	7.0	I
35	<3.9	–	–	T	99.9	A

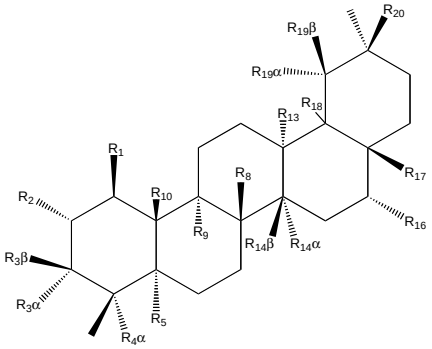
Syncytium assay: A = active in the assay for inhibition of syncytium formation by MC99-infected cells, Aa = active in the assay for reduction of syncytium formation by MC99 virus, I = inactive in the sanctum inhibition assay, Ia = inactive in the reduction assay, T = toxic; Radioisotopic RT assay: A = very active (>70% inhibition), I = inactive (<30% inhibition).

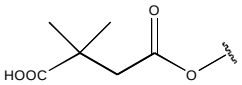
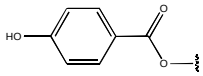


39

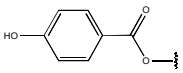
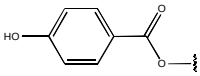
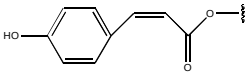
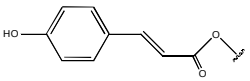
Table 3. Relative Ratio of EBV–EA Activation with Respect to Positive Control (100%) in the Presence of Triterpenes from Callus Tissues of *Eriobotrya japonica*

Compound	Concentration (mol ratio/TPA)			
	1000	**500**	**100**	**10**
13	8.4±0.5	29.8±1.1	72.5±2.0	93.7±0.5
18	0±0.3	28.2±1.3	66.6±1.4	88.3±0.7
19	10.3±0.4	25.4±1.3	70.3±1.9	91.2±0.3
36	8.5±0.5	49.0±1.5	79.6±1.8	100.0±0.3
37	6.3±0.5	45.5±1.8	72.9±1.8	100.0±0.4
38	0±0.2	23.7±1.0	63.4±1.5	85.2±0.7
39	15.2±0.4	33.9±1.2	72.1±1.7	100.0+0.3
40/41	13.7±0.5	30.5±1.5	73.8±1.5	100.0±0.3

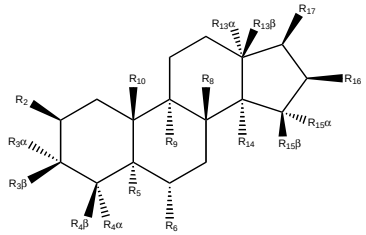


No	R_1	R_2	$R_{3\alpha}$	$R_{3\beta}$	$R_{4\alpha}$	R_5	R_8	R_9	R_{10}
13	H	H	H	OH	Me	H	Me	H	Me
14	H	H	H		Me	H	Me	H	Me
15	H	H	H	OH	Me	H	Me	H	Me
16	OH	=O	H	OH	Me	H	Me	H	Me
17	H	H	H	OH	Me	H	Me	H	Me
18	H	H	H	OH	Me	H	Me	H	Me
19	H	OH	H	OH	Me	H	Me	H	Me
20	H	H	=O		Me	H	Me	H	Me
21	OH	H	H		Me	H	Me	H	H

(Table 3) Contd....

No	R_1	R_2	$R_{3\alpha}$	$R_{3\beta}$	$R_{4\alpha}$	R_5	R_8	R_9	R_{10}
22	H		H		Me	H	Me	H	H
23	H	H	=O		H	Me	H	Me	H
36	H	OH	H	OH	Me	H	Me	H	Me
37	H	OH	H	OH	Me	H	Me	H	Me
38	H	OH	=O		Me	H	Me	H	Me
39	H	OH	H		Me	H	Me	H	Me
40	H	OH	H		Me	H	Me	H	Me

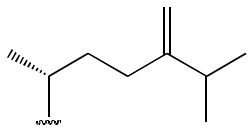
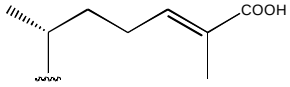
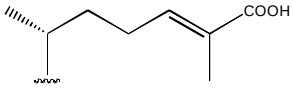
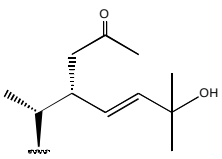
No	R_{13}	$R_{14\alpha}$	$R_{14\beta}$	R_{16}	R_{17}	R_{18}	$R_{19\alpha}$	$R_{19\beta}$	R_{20}	Δ^{12}	Δ^{14}	Δ^{18}
13	-	Me	-	H	COOH	H	H	H	Me	=	-	-
14	-	Me	-	H	COOH	H	H	H	Me	=	-	-
15	-	Me	-	H	COOH	H	OH	Me	H	=	-	-
16	-	Me	-	H	COOH	H	OH	Me	H	=	-	-
17	-	Me	-	H	CH_2OH	Me	H	H	H	=	-	-
18	-	Me	-	H	COOH	Me	H	Me	H	=	-	-
19	-	Me	-	H	COOH	H	H	H	Me	=	-	-
20	H	Me	-	H	COOH	-	-	-	Me	-	-	=
21	Me	-	-	H	COOH	H	H	H	Me	-	=	-
22	Me	-	-	H	COOH	H	H	H	Me	-	=	-
23	Me	-	Me	OH	CH_2OH	H	H	H	Me	-	-	-
36	-	Me	-	H	COOH	H	H	Me	H	=	-	-
37	-	Me	-	H	COOH	H	OH	Me	H	=	-	-
38	-	Me	-	H	COOH	H	OH	Me	H	=	-	-
39	-	Me	-	H	COOH	H	OH	Me	H	=	-	-
40	-	Me	-	H	COOH	H	OH	Me	H	=	-	-



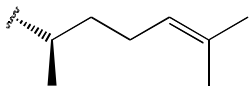
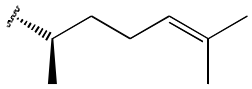
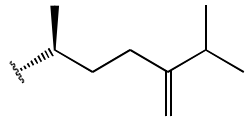
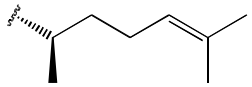
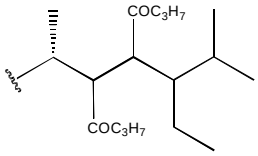
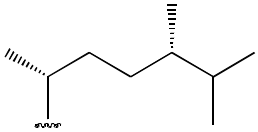
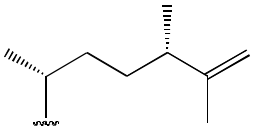
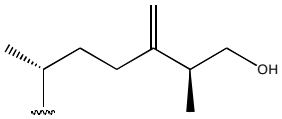
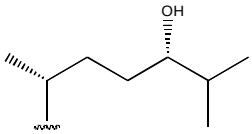
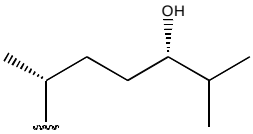
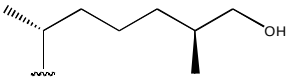
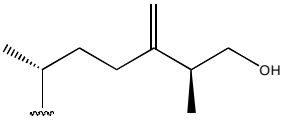
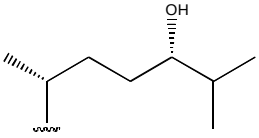
No	R_2	$R_{3\alpha}$	$R_{3\beta}$	$R_{4\alpha}$	$R_{4\beta}$	R_5	R_6	R_8	R_9	R_{10}	$R_{13\alpha}$
28	H	H	OH	Me	Me	H	H	-	-	Me	-
29	H	H	AcO	Me	Me	H	H	H	Me	Me	-

(Table 3) Contd....

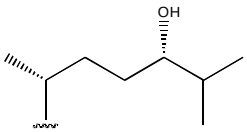
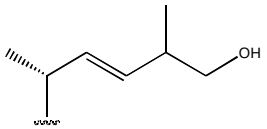
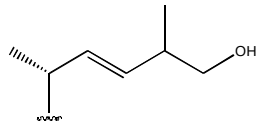
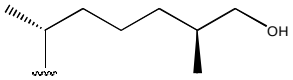
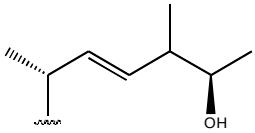
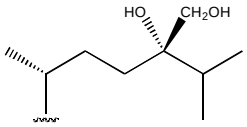
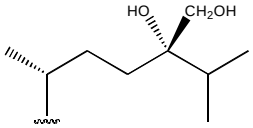
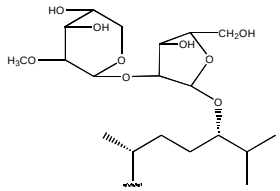
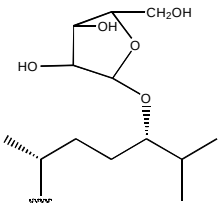
No	R_2	$R_{3\alpha}$	$R_{3\beta}$	$R_{4\alpha}$	$R_{4\beta}$	R_5	R_6	R_8	R_9	R_{10}	$R_{13\alpha}$
30	H	H	OH	Me	Me	H	H	H	Me	Me	-
42	H	=O		Me	Me	H	H	-	-	Me	-
46	H	H	OH	Me	Me	H	H	-	-	Me	Me
47	H	H	OH	Me	Me	-	-	H	Me	H	Me
48	H	H	OH	Me	Me	H	H	-	-	Me	Me
49	H	H	OH	Me	Me	H	H	-	-	Me	Me
78	OSO_3Na	OSO_3Na	H	H	OH	H	H	H	H	Me	-
79	OSO_3Na	OSO_3Na	H	H	H	Me	OSO_3Na	H	H	Me	-
80	OSO_3Na	OSO_3Na	H	H	H	Me	OSO_3Na	H	H	Me	-
81	H	H	OH	H	OH	H	OH	OH	H	Me	-
82	H	H	OH	H	OH	H	OH	OH	H	Me	-
83	H	H	OH	H	H	H	OH	OH	H	Me	-
84	H	H	OH	H	OH	H	OH	-	H	Me	-
85	H	H	OH	H	OH	H	OH	-	H	Me	-
86	H	H	OH	H	H	H	OH	-	H	Me	-
87	H	H	OH	H	OH	H	OH	-	H	Me	-
88	H	H	OH	H	OH	H	OH	OH	H	Me	-
89	H	H	OH	H	OH	H	OH	H	H	Me	-
90	H	H	OH	H	OH	H	OH	OH	H	Me	-
91	H	H	OH	H	OH	H	OH	OH	H	Me	-
92	H	H	OH	H	OH	H	OH	H	H	Me	-
93	H	H	OH	H	H	H	OH	H	H	Me	-
94	H	H	OH	H	OH	H	OH	H	H	Me	-
95	H	H	OH	H	OH	H	OH	H	H	Me	-

No	$R_{13\beta}$	R_{14}	$R_{15\alpha}$	$R_{15\beta}$	R_{16}	R_{17}	Δ^5	Δ^7	Δ^8	$\Delta^{8,14}$	$\Delta^{9,11}$
28	H	H	H	OH	H		-	=	-	-	=
29	H	Me	H	H	H		-	-	-	-	-
30	H	Me	H	H	H		-	-	-	-	-
42	Me	Me	H	H	H		-	-	=	-	-

(Table 3) Contd....

No	$R_{13\beta}$	R_{14}	$R_{15\alpha}$	$R_{15\beta}$	R_{16}	R_{17}	Δ^5	Δ^7	Δ^8	$\Delta^{8,14}$	$\Delta^{9,11}$
46	-	Me	H	H	H		-	=	-	-	=
47	-	Me	H	H	H		=	-	-	-	-
48	-	Me	H	H	H		-	-	=	-	-
49	-	Me	H	H	H		-	-	=	-	-
78	Me	H	H	OAc	OH		-	-	-	-	-
79	Me	H	H	H	H		-	-	-	-	-
80	Me	H	H	H	H		-	-	-	-	-
81	Me	H	=O		H		-	-	-	-	-
82	Me	H	=O		H		-	-	-	-	-
83	Me	H	=O		H		-	-	-	-	-
84	Me	-	=O		H		-	-	-	=	-
85	Me	-	=O		H		-	-	-	=	-
86	Me	-	=O		H		-	-	-	=	-

(Table 3) Contd....

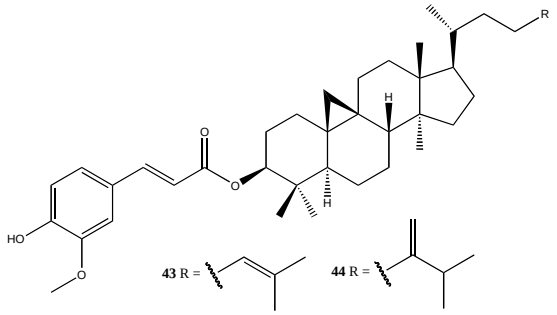
No	$R_{13\beta}$	R_{14}	$R_{15\alpha}$	$R_{15\beta}$	R_{16}	R_{17}	Δ^5	Δ^7	Δ^8	$\Delta^{8,14}$	$\Delta^{9,11}$
87	Me	-	=O		H		-	-	-	=	-
88	Me	H	OH	H	H		-	-	-	-	-
89	Me	H	OH	H	H		-	-	-	-	-
90	Me	H	OH	H	H		-	-	-	-	-
91	Me	H	OH	H	H		-	-	-	-	-
92	Me	H	OH	H	H		-	-	-	-	-
93	Me	H	OH	H	H		-	-	-	-	-
94	Me	H	OH	H	H		-	-	-	-	-
95	Me	H	OH	H	H		-	-	-	-	-

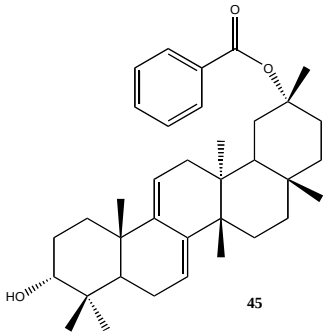
antiviral activity against herpes simplex type 1 (Anti-HSV-1 (IC_{50} 5.2 μg/mL)).

Cycloartenol ferulate (**43**) (IC_{50} = 2.2 μM), 24-methylenecycloartanol ferulate (**44**) (IC_{50} = 1.9 μM), and karounidiol 29-benzoate (**45**) (IC_{50} = 2.2 μM) inhibited purified HIV-1RT and have been suggested as potential lead compounds [31].

Three triterpene alcohols isolated from the latex of *Euphorbia antiquorum* were established to be antiquol C (**46**), antiquol B (**47**), and euphorbol (**48**) [32]. Compounds **46**-**48** and four other known compounds isolated from the latex, euphol (**49**), lemmaphylla-7,21-dien-3β-ol (**50**), isohelianol (**51**), and camelliol C (**52**), showed potent inhibitory effects. The inhibitory effects of compounds **46**-**52** on EBV-EA (Epstein-Barr virus early antigen) activation induced by TPA (12-*O*-tetradecanoylphorbol-13-acetate) were examined for the primary screening of antitumor-promoting activities and the results are shown in Table **4**. All compounds, with the exception of a monocyclic compound **52**, showed potent inhibitory effects while preserving high viability of Raji

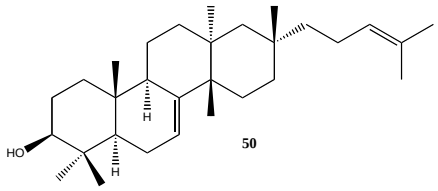
cells. In this assay, compounds **46-48** and **51** showed 100% inhibition of activation at 1000 mol ratio/TPA. The inhibitory effects against EBV-EA activation have been demonstrated to be closely parallel to those against tumor promotion *in vivo*. Therefore, the triterpenes from *E. antiquorum* may be useful as chemopreventive agents.

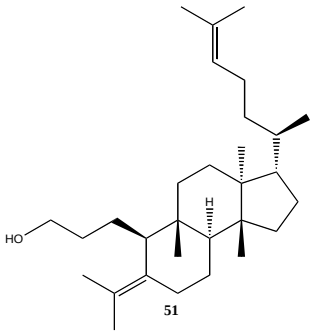


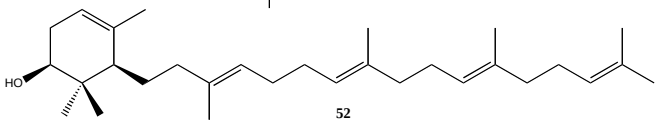


Values represent relative percentages to the positive control value. TPA (32 pmol, 20 ng) 100%. Values in parentheses are viability percentages of Raji cells.

Glycyrrhizin (**53**), from licorice root (*Glycyrrhiza radix*), has been known for some time as an antiviral agent, its IC_{50} for HIV-1(IIIB) in MT-4 cells being 0.15 mM. Although the site of interaction of glycyrrhizin (at the envelope glycoprotein) has not been further characterized [33], its action may at least partially be attributed to an interference with virus-cell binding. Two mechanisms were proposed for the anti-HIV activity of glycyrrhizin: inhibition of viral adsorption to target cells by interference with protein kinase C activity and/or disruption of the initial stages of viral replication caused by non-specific interactions of the compound with the viral membrane.







Some of the chemically modified glycyrrhizin derivatives (salts, amides, glycopeptides) were potent HIV-1 and HIV-2 inhibitors *in vitro*. An example of these is niglizin (penta-*O*-nicotinate of GL) [34, 35].

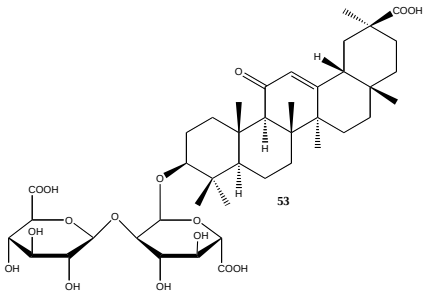
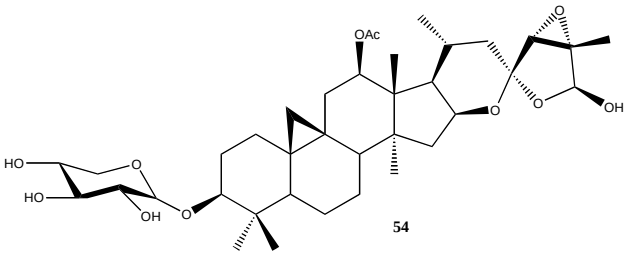


Table 4. Percentage of Epstein-Barr Virus Early Antigen Induction in the Presence of Compounds 46-52 with Respect to a Positive Control (100%)

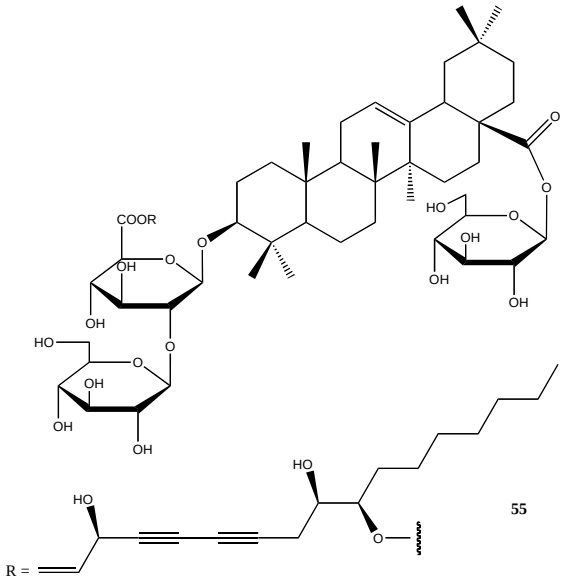
Compound	Concentration (mol ratio/TPA)			
	1000	**500**	**100**	**10**
46	0 (70)	28.2	75.0	92.8
47	0 (70)	34.7	78.4	96.5
48	0 (70)	32.6	77.9	95.8
49	0 (70)	29.8	75.7	93.5
50	2.2 (70)	33.8	79.4	97.2
51	0 (70)	25.8	72.4	95.7
52	20.1 (70)	51.5	86.3	100
β-carotene	8.6 (70)	34.2	82.1	100

Actein (**54**), a tetracyclic triterpenoid saponin isolated from the rhizome of *Cimicifuga racemosa* (black cohosh), showed potent anti-HIV activity [36]. Soybean saponins isolated from soybean seeds inhibited HIV-1 replication in MT-4 cells. They possess narrow therapeutic index and did not inhibit HIV-1 RTase. Escins, the triterpenoid saponin mixture extracted from the seeds of *Aesculus chinensis*, were found to show moderate anti-HIV-1 protease activity [37].



54

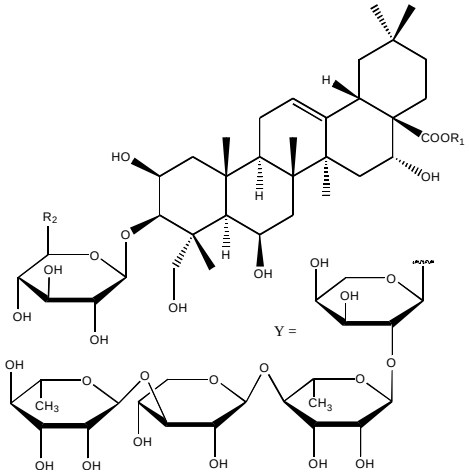
A new oleanolic acid-derived saponin, polyacetylenegin-senoside-Ro (**55**), was isolated from the roots of *Panax ginseng* [38]. Compound **55** is the first example of a polyacetylene-containing ginsenoside and was found to inhibit the replication of HIV-1 with an IC_{50} value of 13.4 μg/mL (11.1 μM). The compound was nontoxic toward the cultured cells up to a concentration of 16.4 μM. The reported role of this compound suggests that the polyacetylene moiety of **55** may well be responsible for its higher antiviral potency relative to that of the other published HIV-1 inhibitory saponins.



55

Arganine C (**56**) and a new saponin, tieghemelin (**57**), were isolated from *Tieghemella heckelii* fruits [39]. Arganine C was not significantly cytotoxic to HeLa-CD4(+) cells at the level required to reduce the syncytium count to zero, suggesting it to be a promising candidate for further study as an antiviral drug. A cell-cell fusion assay was used to measure the anti-HIV activity. At 20 μM, arganine C caused 100% inhibition of syncytium formation. Tieghemelin was about half as effective, whereas the removal of the four-sugar chain gave compounds **58** and **59**, which were devoid of activity.

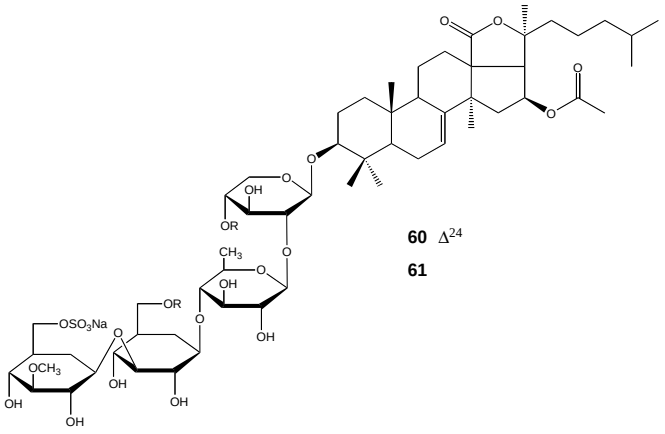
Two new trisulfated triterpene glycosides, liouvillosides A (**60**) and B (**61**), have been isolated from the Antarctic sea



Y =

	R_1	R_2
56	Y	CH_2OH
57	Y	COOH
58	H	CH_2OH
59	H	COOH

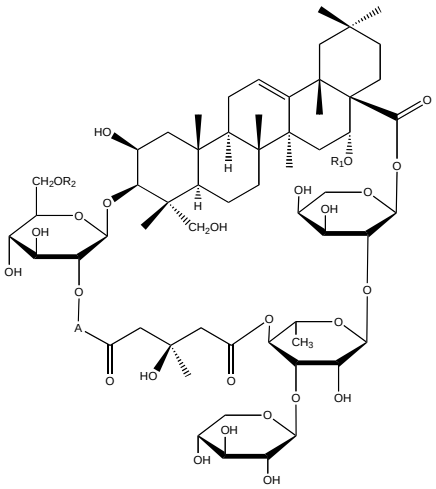
cucumber *Staurocucumis liouvillei* [40]. Liouvillosides A and B are two new examples of a small number of trisulfated triterpene glycosides from sea cucumbers belonging to the family Cucumariidae. The cytotoxicity of saponins **60** and **61** was evaluated in Vero cells at concentrations ranging from 6.25 to 50 μg/mL with increasing time periods. Little or no cytotoxicity was detected within 8 h of cell exposure to the compounds, but both saponins were cytotoxic following prolonged incubation periods. According to the results obtained in the cytotoxicity test, the virucidal activity of glycosides **60** and **61** was then evaluated by incubation of a suspension of HSV-1 with the compounds at concentrations below 10 μg/mL for 1 h at 37 °C. Both saponins exerted an irreversible virucidal effect on HSV-1, but with different effectiveness: liouvilloside A produced a weak inactivation of HSV-1 since at the maximum concentration tested the residual infectivity was 24% with respect to the control virus sample, whereas after treatment with liouvilloside B in the same experimental conditions the remaining infectivity was 10-fold lower (2.5%).

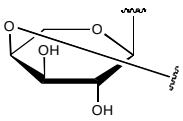
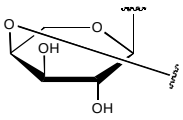
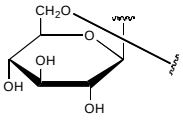
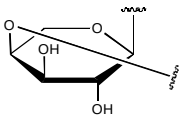


60 Δ^{24}

61

Nine new triterpenoid saponins were isolated from the bulbs of *Bolbostemma paniculatum*. In addition, four known triterpenoid saponins were isolated [41]. From the results of preliminary tests of antiviral activity of these compounds (Table **5**), the TC_{50} values to Vero cells of the compounds **62-65** were 1.37, 0.45, 2.45, and 4.11 μg/mL, respectively, and their IC_{50} values to the virus were not available, which indicated that the four compounds have a strong cytotoxic activity while their antiviral activity cannot be assayed by this method. The IC_{50} values to the virus of compounds **67-72** were not available from their TC_{50} to the cells, which led to the conclusion that the six compounds did not exhibit anti HSV-I activity in this experiment.

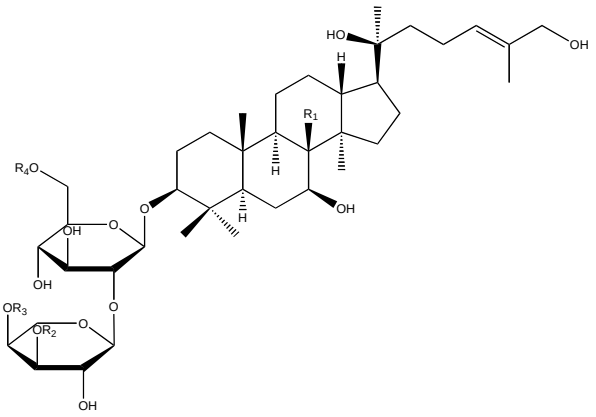


	R_1	R_2	A
62	H	CH_3-$(CH_2)_{14}$-CO-	
63	OH	H	
64	OH	H	
65	H	H	

It has been known for decades that semi-purified extracts from the bark of the South American tree, *Quillaja saponaria Molina*, exhibit remarkable immunoadjuvant activity [42, 43]. The most active components of these extracts, designated QS-21A, were identified to be a mixture of two principal isomeric triterpene glycoside saponins, QS-21A-api (**72**) and QS-21A-xyl, each incorporating a quillaic acid triterpene core, flanked on either side by complex oligosaccharides and a stereochemically rich glycosylated fatty acyl chain. The potency of QS-21A and its favorable toxicity profile in more than 80 recent and ongoing vaccine clinical trials (melanoma, breast cancer, small cell lung cancer, prostate cancer, HIV-1, malaria) have established it as one of the most promising new adjuvants for immune response potentiation and dose-sparing.

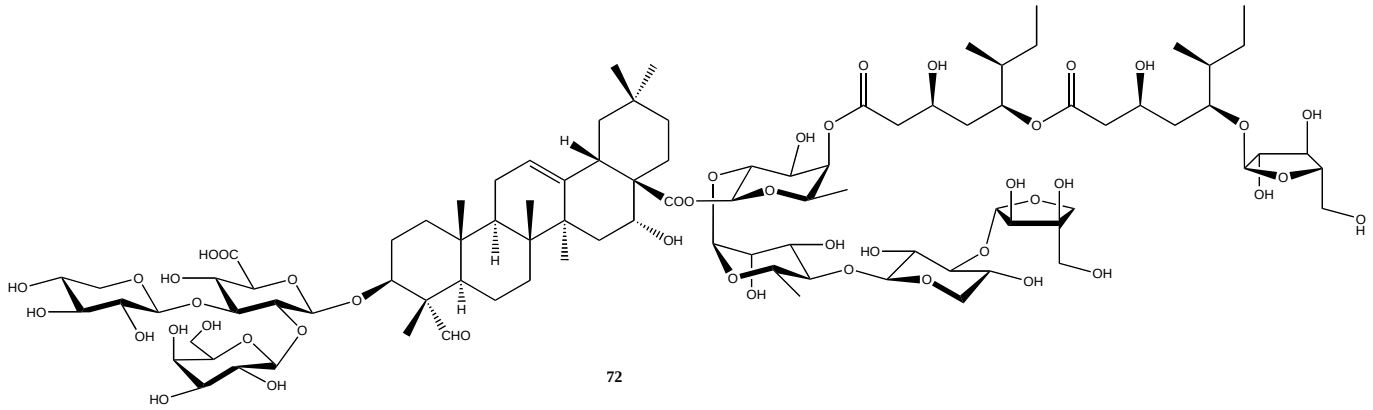
Table 5. Antiviral Activity of Compounds 62-71

Compound	TC_{50} (μg/mL)	IC_{50} (μg/mL)	TI
62	1.37	-	-
63	<0.45	-	-
64	2.45	-	-
65	4.1	-	-
66	577	-	-
67	>2000	-	-
68	1704	-	-
69	1704	-	-
70	>2000	-	-
71	852	-	-
Acyclovir	>1000	2.60	384



	R_1	R_2	R_3	R_4
66	CH_2OH	H	H	H
67	CH_2OH	H	H	Ac
68	CH_3	Ac	H	H
69	CH_3	H	Ac	H
70	CHO	Ac	H	H
71	CHO	H	Ac	H

Six new triterpenoid saponins of the ursane types were isolated from the MeOH extract of the leaves of *Ilex oblonga*. They were oblonganoside A (**73**) and further oblonganosides (B-F) together with known triterpenoid saponins [44]. Compound **73** showed appreciable inhibitory activity against TMV replication with EC_{50} value 0.074 μmol.



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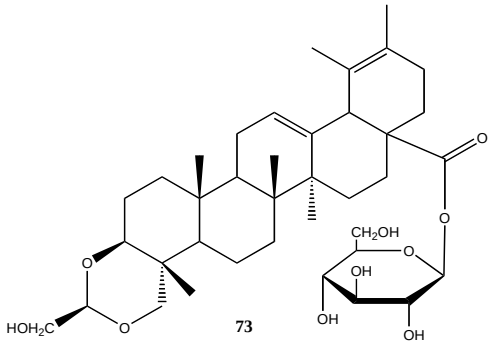
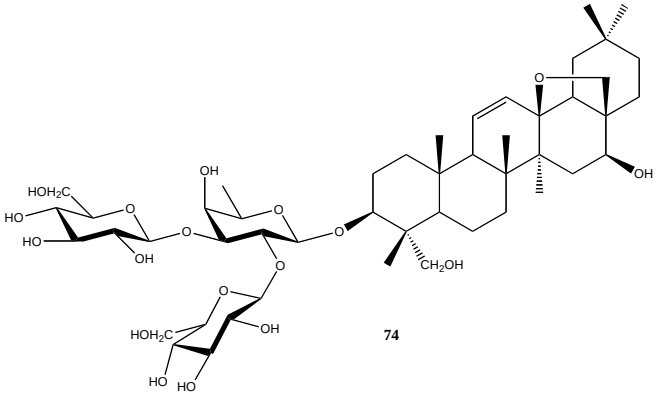


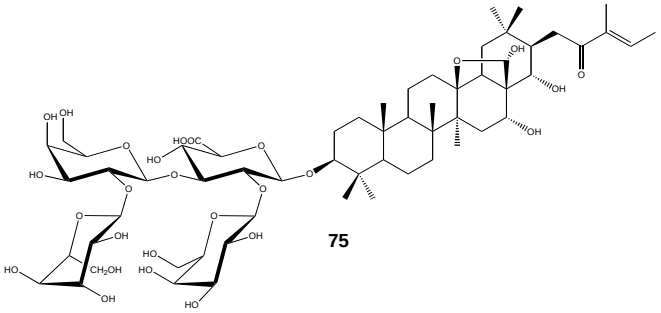
73

As part of screening of antiviral agents from medicinal plants, three saikosaponins of plant origin (*Bupleurum rigidum* and *Scrophularia scorodonia*), were tested *in vitro* against herpes simplex type 1 (HSV-1), vesicular stomatitis virus (VSV) and poliovirus type 1 [45, 46]. The percentages of cellular viability at the non-toxic limit concentrations of the active compound buddlejasaponin IV (**74**) were 56.9% at 25 μg/mL. None of the saikosaponins were active against HSV-1.



74

Ten saponins isolated from the leaves of *Maesa lanceolata* were tested for their antiviral, haemolytic and molluscicidal activities [47]. The influence of the substitution pattern of these acylated triterpenoid saponins on their biological activities was investigated and structure-activity relationships were established. Maesasaponin VI_2 (3β-*O*-[[α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)]-[β-D-galactopyranosyl-(1→2)]-β-D-glucopyranuronyl]-21β, 22α-diangeloyloxy-13β,28-epoxyolean-16α,28α-diol) (**75**), the most potent molluscicidal compound (LC_{50} = 0.5 ppm), also showed virucidal and haemolytic activity. In general, 21,22-diacylation appeared to be associated with a virucidal (reduction factor of the viral titer ≥ 10^3 at 50 μg/mL) and haemolytic activity ($HC_{50} \leq 1$ μg/mL).



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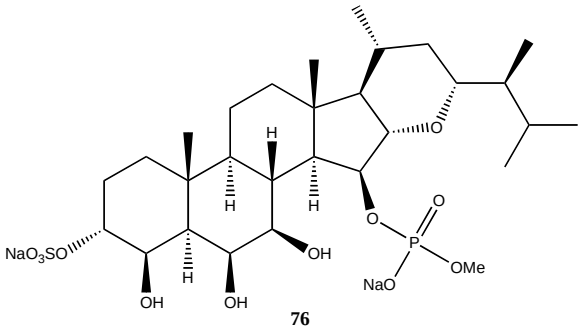

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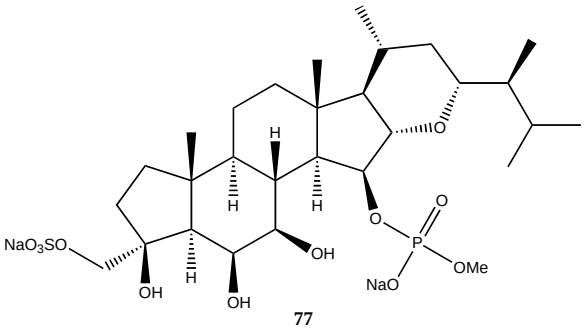


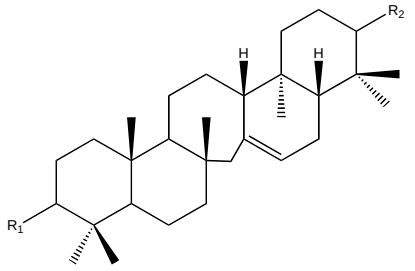
77

Marine sponges are known to produce a variety of interesting and unconventional steroids among which polyoxygenated steroids have received greatest attention due to their remarkable biological and pharmacological activities. In particular, sulfated steroids have been examined for their potential as inhibitors of HIV. Haplosamates A (**76**) and B

(**77**), sulfated sterols isolated from Philippine sponge *Xestospongia* sp. inhibited HIV integrase [48]. The MeOH-EtOAc extract of a Red Sea sponge, *Clathria* sp. was shown to be active as a novel inhibitor of human immunodeficiency virus type 1 reverse transcriptase, [49]. Bioassay-guided fractionation of the extract yielded a novel sterol sulfate, clathsterol (**78**), which is responsible for the activity and is active at a concentration of 10 μM. Halistanol sulphates G (**79**) and H (**80**) isolated from marine sponge *Pseudoaxinissa digitata* were cytoprotective against HIV-1 in the anti-HIV screen [50].

Twelve new (**81-92**) polyhydroxysterols and two new saponins (**93** and **94**) were isolated from the starfish *Certonardoa semiregularis* by activity-guided fractionation [51]. Compounds **81-86** are rare examples of 15-keto steroids from starfish. The compounds were evaluated for cytotoxicity against a small panel of human solid tumor cell lines (Table **6**). Compound **86** displayed the highest potency, which is comparable to that of doxorubicin. The potency of the compounds might be partly governed by the polarity of the compounds, since the number of sugar units (**93**, **94**) or the degree of oxygenation of the nucleus (**303**-**306**) makes a difference.

Two other new triterpenoids, (**95**), and (**96**), were isolated from the stem bark of *Picea glehni* [52] together with three known triterpenoids, 3-α-methoxyserrat-14-en-21-beta-ol (**97**), 3-β-methoxyserrat-14-en-21-β-ol (**98**), and piceanonol A (**99**) [52]. The inhibitory effects of **95-99**, a synthetic sample 3-α-methoxyserrat-13-en-21-β-yl formate **100** and the control substance, oleanolic acid on Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) were examined as a preliminary evaluation of anti-tumor-promoting activities. Compounds **95-98** and **100** exhibited potent inhibitory effects (100 % inhibition of induction at 1000 mol ratio/TPA, and about 75 % inhibition at 500 mol ratio/TPA, and 40-20% inhibition at 100 mol ratio/TPA, respectively) on EBV-EA induction by TPA. Among them, compounds **95**, **96** and **100** showed inhibitory effects almost equivalent to that of oleanolic acid. It is therefore interesting to note that the OMe and OCOH groups in the serratane skeleton seemed to enhance the antitumor-promoting activity.



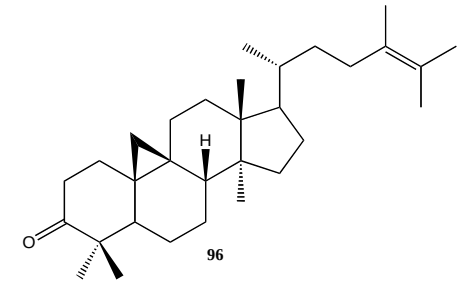
	R_1	R_2
95	α-OMe	β-HCOO
97	α-OMe	β-OH
98	β-OMe	β-OH

Geumonoid (**101**), a new triterpene, was isolated from *Geum japonicum* [53]. Compound **101** was tested for HIV-1 protease inhibitory effects with a recombinant enzyme and exhibited strong activity with 89% inhibition at the concentration of 17.9 μg/mL.

Table 6. Cytotoxicity Data of 81-92, and 94 Against Human Solid Tumor Cells[a]

Compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
81	6.94	6.85	>20	4.10	5.82
82	5.43	12.3	7.30	12.6	13.6
83	3.80	4.10	3.40	2.90	>20
84	>30	>30	>30	>30	>30
85	0.43	0.22	0.17	0.12	0.48
86	4.58	6.65	4.66	3.80	7.10
87	>30	>30	>30	>30	>30
88	12.5	12.1	7.13	14.7	10.4
89	11.7	16.2	5.24	18.6	20.3
90	17.0	16.4	5.70	28.4	19.3
91	>30	36.6	7.50	>30	>30
92	3.65	2.80	0.82	0.52	>20
94	>30	>30	8.3	>30	>30
Doxorubicin	0.04	0.12	0.05	0.12	0.18

[a] Data as expressed in ED_{50} values (μg/mL). A549: human lung cancer; SK-OV-3: human ovarian cancer; SK-MEL-2: human skin cancer; XF498: human CNS cancer; HCT 15: human colon cancer.



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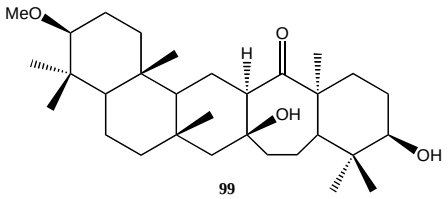

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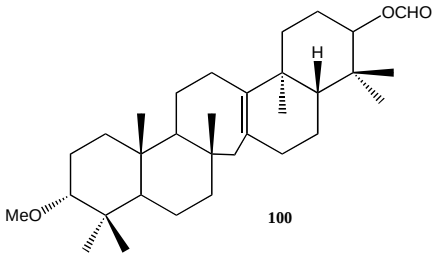

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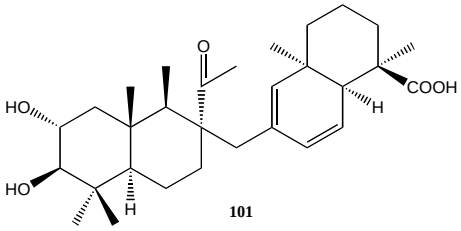

101

CONCLUDING REMARKS

Biological effects of triterpenes include, apart from the antiviral properties discussed in this review, also anti-inflammatory and antitumor effects, anti-nociception and immunoregulatory effects. The anti-inflammatory activity of some triterpenes may be due to their anti-complement activity. Some triterpenes have a glucocorticoid-like structure, and may have apoptotic effects. They may also be used for treating hypercholesterolemia or atherosclerosis. Triterpenes also have antioxidant effects, reducing lipid peroxidation and stimulating antioxidant systems in tissues. Some triterpenes have been shown to reduce the hepatotoxicity of various compounds by inducing the synthesis of certain intracellular proteins that is caused by the enhanced expression of several genes. Synthetic derivatives of triterpenes have been shown to be more effective than the natural parent compounds.

Our review focuses on triterpenes and steroids with antiviral activities, their sources, structure-activity relationships and possible synthetic modifications. Important natural triterpenes such as betulinic acid and betulinol have been often synthetically modified. These efforts have yielded a number of more potent antiviral agents often acting by mechanisms different from that of the parent compound or any other approved drug.

ACKNOWLEDGEMENT

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LIST OF ABBREVIATIONS

EBV-EA	=	Epstein-Barr virus early antigen
EC_{50}	=	Half maximal effective concentration
ED_{50}	=	Half maximal effective dose
EGCG	=	Epigallocatechin gallate
GL	=	Glycyrrhizin
HC_{50}	=	Half maximal haemolytic activity
IC_{50}	=	Half maximal inhibiting concentration
LC_{50}	=	Half maximal lethal concentration
TC_{50}	=	Toxic concentration
TI	=	Therapeutic Index
TMV replication	=	Tobacco mosaic virus replication
TPA	=	12-*O*-tetradecanoylphorbol-13-acetate
VSV	=	Vesicular stomatitis virus

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