Medicinal chemistry

Mehanna et al., Med chem 2014, 4:10
DOI: 10.4172/2161-0444.1000217

Research Article Open Access

Natural Bioactive Sterol 5 α ,8 α -endoperoxides as Drug Lead Compounds

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Abstract

The natural product sterol 5α,8α-endoperoxides are structural different from general sterols. These compounds belong to the group of oxidized sterol derivatives and contain a 5α,8α-endoperoxide bond in addition to the fragments characteristic of original sterols. Many researches have reported that sterol 5α,8α-endoperoxides have potential bioactivities, including antioxidant, antimicrobial, anti-tumor activity, immunomodulatory activity, inhibitory hemolytic activity and anti-inflammatory activity etc. The review discussed the structures, properties, bioactivity and synthetic methods of sterol 5α,8α-endoperoxides. The natural peroxides are valuable sources in the development of novel bioactive agents.

Keywords: Sterol; Endoperoxide; Peroxide bond; Bioactivity

List of Abbreviations: EP: Ergosterol Peroxide; ERGO: Ergosterol; U266, RPMI-8226, PMI-8226: Multiple Myeloma cell Line; SNU-1: Human Gastric Tumor Cell Line; Hep3B, HepG2, SUN-354, BEL-7402, LO2: Human Hepatoma Cell Line; SUN -C4: Human colorectal tumor cell line; HL60, K562, P388, K462: Human Leukaemia Cell Line; DU-145, LNCAP: Human Prostate Cancer Cell line; HT29, COLO-205: Human Colorectal Tumor Cell; HCT-8: Human Colorectal Cancer Cell Line; SF-295: Human Glioblastoma Cancer Cell Line; WM-1341: Human Malignant Melanoma Cell Line; SGC-7901: Human Gastric Adenocarcinoma Cell Line; HeLa: Human Cervical Carcinoma Cancer Cell Line; KB: Human Nasopharyngeal Epidermoid Carcinoma Cell Line; FL: Human Follicular Lymphoma Cell Line; Hep-2: Human Epithelial Cancer Cell; HTLV-I: Human T-Cell Leukemia/Lymphotropic Virus Type I; MCF7WT, MDA-MB-231, MDA-MB435, MCF-7: Human breast cancer cell line; A549: Human Lung Cancer Cell Line; SK-OV-3: Human Ovarian Cancer Cell Line; SK-MEL-2: Human Skin Melanoma Cancer Cell Line; XF498: Human Central Nerve System Cancer Cell Line; HCT15: Human Colic Cancer Cell Line; WI38: Human Lung Fibroblast Cell Line; H37Rv: Mycobacterium tuberculosis; OVCAR-3, OVCAR-8: Ovarian Cancer Cell Line; MOLT-4, Jurkat: Human lymphoid cancer cell line; JAK2: Janus kinase 2; STAT: Signal Transducer and Activator of Transcription; ATCC: American Type Culture Collection; MyD88: Myeloid Differentiation factor 88; VCAM-1: Vascular Cell Adhesion Molecule 1; NF-kB: Nuclear factor kappa B; RAW264.7: Macrophages; C/EBPβ: Enhancer-Binding Protein β; p38: Mitogen-activated protein kinase; JNK: Jun N-Terminal Kinase; ERK: Extracellular singal-Regulated Kinase; MAPKs: Mitogen-Activated Protein Kinases; CDKN1A: Cyclin-Dependent Kinase Inhibitor; iNOS: Inducible Nitric Oxide Synthase (enzyme); COX-2: Cyclo-oxygen-ase; PGE2: Prostaglandin E2

Introduction

Natural products, especially bioactive molecules as drug lead compounds, have attracted extensive attention in health promotion and in drug discovery and development. It is essential to understand the structures and functional mechanisms of these lead molecules prior to drug development. Since the natural peroxides artemisinin and Yingzhaosu which have excellent antimalarial activity are found, and their peroxide bonds are key to antimalarial activity, natural products containing peroxide bonds have began to attract scientists' attention [1-4].

Among natural sterols, there are some chemical entities which the reasons for the existence and fine biological roles in plants and animals have so far remained unexplored. These highly functionalized sterols have recently attracted considerable attention because of their biological and pharmacological activities.

Sterol 5α,8α-endoperoxides belong to the group of oxidized sterol derivatives and contain a 5α,8α-endoperoxide grouping in addition to the fragments characteristic of such derivatives. This structural element arises as the result of the addition of an oxygen molecule to a 5,7-diene system in the molecule of the initial sterols, for example, ergosterol, 7-dehydrocholesterol and 9(11)-dehydroergosterol.

Up to now, several excellent reviews have been published on the structure and distribution of natural endoperoxides, but there is little information on the biological activity of the sterol 5α,8α-endoperoxides in recent years. This review brings together information on the structures, bioactivities and chemical synthetic methods of the natural sterol 5α,8α-endoperoxides reported from 2000 to now. In some other scientific literatures "steroid peroxides" or "5α,8α-epidioxysteroids" are also usually used to name these compounds. Therefore, sterol 5α,8α-endoperoxides are discussed in order of similar carbon skeleton in the review. We hope the review could attracted considerable attentions to sterol peroxides synthesis pathway or cultivation methods research. The sterol peroxides are valuable sources in the development of new drug agents.

The structures and properties of sterol 5α,8α-endoperoxides

Ergosterol 5α,8α-endoperoxide (EP, 1) is the best-known representative of the group of sterol 5α,8α-endoperoxides. The ubiquitous ergosterol peroxide continues to be isolated from a number of natural sources. The newly identified natural sources for the compound are summarized in Table 1. The proof of the structure of the compound caused no difficulties since it proved to be identical with a specimen obtained in the photooxidation of ergosterol in the presence of the sensitizer (Figure 1).

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Received June 27, 2014; **Accepted** September 10, 2014; **Published** September 15, 2014

Citation: Ming Bu, Yang BB, Hu L (2014) Natural Bioactive Sterol 5α,8α-endoperoxides as Drug Lead Compounds. Med chem 4: 709-716. doi:10.4172/2161-0444.1000217

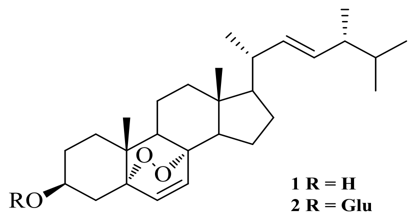
Source	Ref.	Source	Ref.
Agrocybe chaxingu	5	*Melia azedarach*	42
Amanita subjunquillea	6	*Morchella esculenta*	43
Anemone rivularis Buch.-Ham.	7	*Momordica charantia*	44
Antrodia camphorate	8	*Naematoloma fasciculare*	45
Armillaria mellea	9	*Neoplaconema napellum*	46
Azadirachta indica	10	*Nomuraea rileyi*	47
Bulgaria inquinans	11	*Paecilomyces variotii*	48
Chaetomium longirostre	12	*Penicillium janthinellum*	49
Ciocalapata sp.	13	*Penicillium oxalicum*	50
Cordyceps cicadae	14	*Pisonia aculeate*	51
Cordyceps militaris	15	*Pleurotus eryngii*	52
Datura stramonium L.	16	*Pleurotus ostreatus*	53
Euphorbia lagascae	17	*Pycnoporus sanguineus*	54
Ficus nervosa	18	*Pycnoporus cinnabarinus*	55
Ganoderma applanatum	19	*Radermachera boniana*	56
Ganoderma lucidum	20-22	*Ramaria botrytis*	57
Ganoderma sinense	23	*Rhizopus sp.*	58
Gomphus clavatus	24	*Sarcodon aspratus*	59-61
Grifola frondosa	25	*Sarcodon imbricatus*	62
Halichondria sp.	26	*Sarcodon joedes*	63
Helianthus tuberosus	27	*Sarcographa tricosa*	64
Hericum erinaceum	28	*Sargassum pallidum*	65
Heritiera littoralis bark	30	*Sellaginella tamariscina*	66
Hygrophorus russula	31	*Sinularia flexibilis*	67
Inonotus obliquus	32	*Solanum violaceum*	68
Junceella fragilis	33	*Sporotrichosis*	69
Lactarius hatsudake	34	*Stereum hirsutum*	70
Laetiporus sulphureus	35	*Stereum subtomentosum*	71
Lentinus edodes	36	*Strychnos toxifera*	72,73
Lentinus polychrous	37	*Suillus luteus*	74
Lobophytum crassum	38	*Topsentia sp.*	75
Macrolepiota neomastoidea	39	*Trichosanthes kirilowii*	76
Magnolia kachirachirai	40	*Verticillium sp.*	77
Mallotus macrostachyus	41	*Volvariella bombycina*	78
Melandrium firmum	32	*Volvariella volvacea*	79

Table 1: Sources of ergosterol peroxide 1.

A number of biological activities have been attributed to ergosterol peroxide, such as anti-tumor activity, immunomodulatory activity, inhibitory hemolytic activity and anti-inflammatory activity, anti-viral activity et al. Ergosterol peroxide has shown to exert anti-tumor activity in multiple myeloma U266 cells partly with anti angiogenic activity targeting JAK2/STAT3 signaling pathway as a potent cancer preventive agent for treatment of multiple myeloma cells [5]. Ergosterol peroxide is also against Walker carcinosarcoma and human mammary adenocarcinoma cell lines *in vitro*, as well as against human gastric tumor cell line (SNU-1), human hepatoma cell line (SUN-354), human colorectal tumor cell line (SUN-C4), and murine sarcoma-180. Recent studies showed that the cytotoxicity of ergosterol peroxide completely inhibited growth and induced apoptosis of HL60 human leukaemia cells at a concentration of 25 μM. It also inhibited TPA-induced inflammation and tumour promotion in mice and suppressed proliferation of mouse and human lymphocytes stimulated with mitogens [6]. The IC_{50} value of the compound based on the cell viability of Hep3B was 16.7 μg/mL [7]. Ergosterol peroxide exhibited an inhibitory effect on androgen-sensitive (LNCaP) and androgen-insensitive (DU-145) human prostate cancer cells at micromolar concentrations [8]. Moreover, ergosterol peroxide appeared to suppress cell growth and STAT1 mediated inflammatory responses by altering the redox state in HT29 cells [9]. Biological evaluation revealed that the compound inhibited the relaxation of supercoiled DNA (pBR322) induced by DNA topoisomerase I, and also showed marginal, selective cytotoxic activity against human colon tumor cells [10]. Ergosterol peroxide displayed potent activity against the cancer cell lines MDA-MB435, HCT-8 and SF-295 [11]. It was also demonstrated that ergosterol peroxide produced greater activity inducing death of miR-378 cells. With future clinical development, ergosterol peroxide represents a promising new reagent that can overcome the drug-resistance of tumor cells [12].

Immunosuppressive activity was found in ergosterol peroxide isolated from several species. Ergosterol peroxide exhibited significant inhibitory activities against leishmaniasis, tuberculosis, *Mycobacterium tuberculosis* H37Rv and *M. avium* [13]. It also played an important role in inhibiting the hemolytic activity of human serum against erythrocytes [14]. It was also shown to be devoid of any activities against an antibiotic sensitive ATCC strain of Staphylococcus aureus [15]. This suggests its potential application in medicinal use as an antivenom and anti-inflammatory agent. Ergosterol peroxide significantly blocked MyD88 and VCAM-1 expression, and cytokine (IL-1β, IL-6 and TNF-α) production in LPS-stimulated cells. It also effectively inhibited NF-*k*B activation, which was further confirmed with siRNA treatment. The above-mentioned data indicated that ergosterol peroxide may play an important role in the immunomodulatory activity of GF through inhibiting the production of pro-inflammatory mediators and activation of NF-*k*B signaling pathway [16]. In addition, ergosterol peroxide suppressed LPS-induced DNA binding activity of NF-*k*B and C/EBPβ, and inhibited the phosphorylation of p38, JNK and ERK MAPKs. It down-regulated the expression of low-density lipoprotein receptor (*LDLR*) regulated by C/EBP, and HMG-CoA reductase (*HMGCR*) in RAW264.7 cells. Furthermore, ergosterol peroxide induced the expression of oxidative stress-inducible genes, and the cyclin-dependent kinase inhibitor *CDKN1A*, and suppressed *STAT1* and interferon-inducible genes [17]. It was found that ergosterol peroxide possess markedly activity against PGE2 release with an IC_{50} value of 28.7 μM. The mechanism in transcriptional level of ergosterol peroxide was found to down-regulate mRNA expressions of iNOS and COX-2 in dose-dependent manners. In addition, a glycosylated derivative of ergosterol peroxide **2** has been obtained from *Cordyceps sinensis*. The glycosylated form of ergosterol peroxide was found to be a greater inhibitor to the proliferation of K462, Jurkat, WM-1341, HL-60 and RPMI-8226 tumor cell lines by 10 to 40% at 10 μg/mL [18] (Figure 2, Table 2).

Sterol 5α,8α-endoperoxides 3-7 are different from 1 with saturated



1 R = H
2 R = Glu

Figure 1: Steroidal endoperoxides 1 and2.

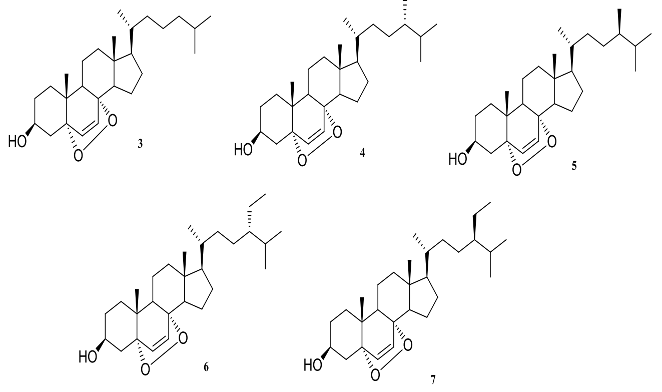


Figure 2: Steroidal endoperoxides 3 -7.

Comp.	Source	Ref.
3	Aplidium constellatum	81
	Bathymodiolus septemdierum	82
	Cynthia savignyi	83
	Didemnum salary	87
	Eunicella cavolini	88
	Glyptocidaris crenularis	89
	Helianthus tuberosus	27
	Hyrtios erectus	89
	Luffariella cf. variabilis	94
	Oscarella	91
	Trididemnum inarmatum	88
	Tripneustes gratilla	92
	Didemnum salary	87
4	Lactarius hatsudake	34
	Meretrix lusoria	93
	Luffariella cf. variabilis	94
	Didemnum salary	87
5	Luffariella cf. variabilis	94
	Meretrix lusoria	93
	Didemnum salary	87
6	Luffariella cf. variabilis	94
	Didemnum salary	87
7	Eunicella cavolini	88
	Luffariella cf. variabilis	94
	Trididemnum inarmatum	88

Table 2: Steroidal endoperoxides 3-7 and their natural sources.

side-chain. The newly identified natural sources are summarized in Table 2. The compound 5α,8α-epidioxycholest-6-en-3β-ol 3 displayed cytotoxicity toward various cancer cell lines. It was evaluated for cytotoxicity against three human tumor cell lines, and showed mild cytotoxicity against SGC-7901, HepG2 and HeLa cells with IC_{50} values of 99, 65 and 94 μg/mL, respectively. While compound 3 did not show cytotoxicity against human normal hepatocytes LO2 (215 μg/mL), and the corresponding results showed 3 was safe to human normal hepatocytes in the therapeutic dosages [19]. In addition, compound 3 possesses antifungal activity against three tomato pathogenic fungi, *Botrytis cinere*a, *Fusarium oxysporum* and *Verticillium albo atrum* and antibacterial activity against *Agrobacterium tumefaciens, Escherichia coli*, *Staphylococcus faecalis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. It showed significant toxicity against brine shrimp larvae with an LD_{50} value of 4.5 μg/mL [20,21].

5α,8α-epidioxy-24(*S*)-methylcholest-6-en-3β-ol (4) and 5α,8α-epidioxy-24(*R*)-methylcholest-6-en-3β-ol (5) identified in hard clam (*Meretrix lusoria*). Compounds 4 and 5 showed apoptosis-inducing activity against the human leukemia HL-60 cells [22]. Compound 4 showed selective inhibitory activity against *Crotalus adamenteus* venom phospholipase A_2 (PLA_2) enzyme with an ED_{50} value of 100 μg/mL, but not against *Apis mellifcra* bee venom PLA_2 (ED_{50} >400 μg/mL) [23]. 5α,8α-epidioxy-24(*S*)-ethylcholest-6-en-3β-ol (6), 5α,8α-epidioxy-24(*R*)- ethylcholest-6-en-3β-ol (7) together with 4 and 5 were also isolated from the tunicate *Didemnum salary* and *Luffariella cf. variabilis*. The obtained mixture of the four steroids showed inhibitory activity against the human T-cell leukemia/lymphotropic virus type I (HTLV-I) and also displayed cytotoxic activity against the human breast cancer cell line(MCF_7WT) [24,25] (Figure 3).

Sterol 5α,8α-endoperoxides 8-14 were isolated from the gorgonian *Eunicella cavolini* and the ascidian *Tridemnum inarmatum*. Compounds 8-14 were identified by comparison of their spectroscopic and physical characteristics as (22*E*)-5α,8α-epidioxy-24-nor-cholesta-6,22-dien-3β-ol (8), (22*E,24S*)-5α,8α-epidioxy-24-methyl-cholesta-6,22-dien-3β-ol (9), (22*Z*)-5α,8α-epidioxy-24ξ-methyl-27-nor-cholesta-6,22-dien-3β-ol (10), 5α,8α- epidioxy-24-methyl-cholesta-6,24(28)-dien-3β-ol (11), (22*E*)-5α,8α-epidioxy- cholesta-dien-3β-ol (12), (22*E,24S*)- 5α,8α-epidioxy-24-ethyl-cholesta-6,22-dien-3β-ol (13), and (22*E*)-5α,8α-epidioxy-24-ethyl-cholesta-6,22(28)- dien-3β-ol (14) [26]. Compounds 8-14 were evaluated for cytotoxicity against a panel of five human solid tumor cell lines (A549, SK-OV-3, SK-MEL-2, XF498 and HCT15), all compounds exhibited weak cytotoxicity. No clear correlations between structure and cytotoxicity could be delineated due to diverse variations of the side chain [27] (Figure 4).

A sterol 5α,8α-endoperoxides sulfate (16) and its desulfated derivative (17) were isolated from the cultured diatom *Odontella aurita* (NIES 589), and its structure was elucidated by spectroscopic methods. Compound 16 was evaluated for its cytotoxicity against P388, HL-60, A549 and BEL-7402 cell lines, the activity data suggested that it was more active against P338(IC_{50}=5.9 μM) and HL-60 (IC_{50}=8.7 μM) than against A549 and BEL-7402(IC_{50}>100 μM) [28]. Compounds 18 and 19 were obtained as an inseparable mixture of C-24 stereoisomers in the form of a colorless solid from a Palauan marine sponge, *Lendenfeldia chondrodes*. Compounds 18 and 19 showed any antifouling effect

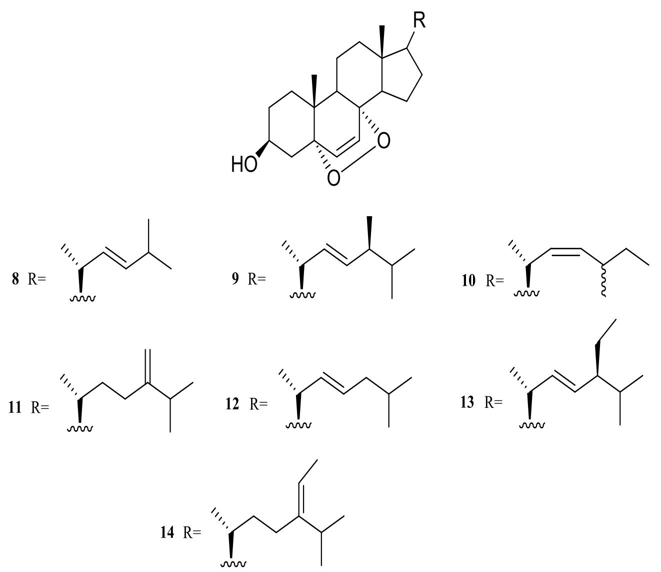


Figure 3: Steroidal endoperoxides 8 -14.

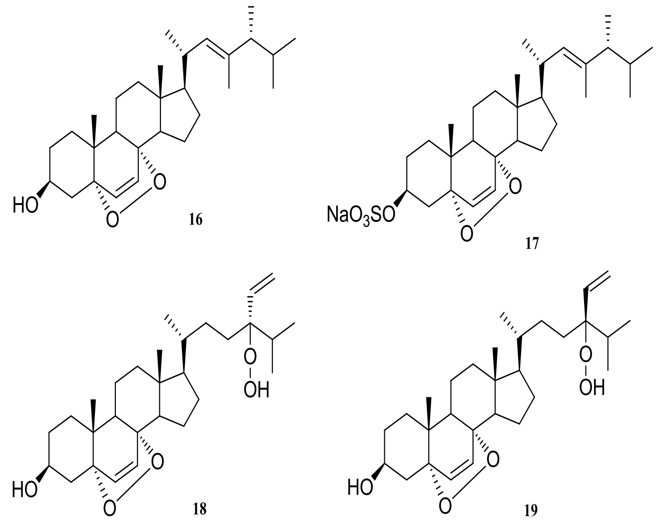


Figure 4: Steroidal endoperoxides 16 -19.

against the Blue Mussel [29].

A second structural type of sterol endoperoxides includes compounds which contain a 9(11)-double bond in addition to a 3β-hydroxy, a 5α,8α-epidioxide and a 6-double bond. Formally, these compounds may be considered as oxidation products of steroids having double bonds in the $\Delta^{5,7}$ and $\Delta^{9(11)}$ positions, such as 9,11-dehydroergosterol (Figure 5).

(22*E*,24*R*)-5α,8α-epidioxyergosta-6,9(11),22-trien-3β-ol (20) was isolated from fermentation mycelia of *Ganoderma lucidum*, edible mushroom *Sarcodon aspratus* (Berk.). The IC_{50} value of 20 based on the cell viability of human hepatocellular carcinoma cells (Hep 3B) was 16.7 μg/mL [8]. Flow cytometric analysis also suggested that it inhibited the growth of human breast adenocarcinoma MCF-7 cells by inducing cell apoptosis [24]. It has also been shown to inhibit HT29 cell growth selectively but not WI38 normal human fibroblasts by inducing CDKN1A expression, thus causing cell cycle arrest and apoptosis [30]. Moreover, it showed potent activity against the HepG2, A549 and MDA-MB-231 cancer cell lines (IC_{50}=7.73~16.74 μg/mL) [31] (Figure 6, Table 3).

Sterol 5α,8α-endoperoxides 22 and 23 were isolated from *Stereum hirsutum*. Both of them exhibited a killing activity with MIC of 16 μg/mL against *M. tuberculosis* H37Rv reference strain [32]. 5α,8α-epidioxy-24-methyl- cholesta-6-en-3β-ol (24) was isolated from the a Palauan marine sponge, *Lendenfeldia chondrodes*. It showed no activity against the blue mussel *Mytilus edulis galloprovincialis* [26]. Sterol 5α,8α-endoperoxide 25 was isolated from the marine sponge *Neopetrosia exigua* (formerly called *Xestospongia exigua*) collected in Palau. Cytotoxicity against the human leukemia cells HL-60 and antimicrobial activity of compounds were examined. The IC_{50} value of compound 25 was 9.6 μM against HL-60. It did not inhibit the growth of *Escherichia coli*, *Staphylococcus aureus*, *Saccharomyces cerevisiae*, *Mucor hiemalis*, and marine bacterium *Ruegeria atlantica* even at 100 μg/disc [33]. 5α,8α-epidioxy-23,24(*R*)-dimethyl-cholesta-6,9(11),22-trien-3β-ol (26) was isolated from the marine-derived fungus *Rhizopus* sp. 26 was evaluated for its cytotoxicity against P388, HL-60, A549 and BEL-7402 cell lines, the activity data suggested that it was more active against P338 (IC_{50}=7.9 μM) and HL-60 (IC_{50}=2.7 μM) than against A549 and BEL-7402 (IC_{50}>100 μM) [34] (Figure 7).

An ergostane-type sterol 9(11)-dehydroaxinysterol (27) was isolated from a sponge of the genus *Axinyssa* along with axinysterol 28. The molecular formula of compound 27, $C_{28}H_{40}O_3$, was determined by HR-EI-MS analysis. The growth inhibitory properties of 27 against cancer cells were examined with a disease-oriented panel of 39 human cancer cell lines (HCC panel). Compound 27 exhibited a strong growth inhibitory effect against some ovarian cancer cells such as OVCAR-3 at IC_{50} 0.19 μg/mL (logGI_{50} -6.20) and OVCAR-8 at IC_{50} 0.22 μg/mL (logGI_{50} -6.14), as shown in Table 3, and also indicated significant growth inhibition in 21 human cancer cell lines at less than 0.60 μg/mL [34] (Table 4).

Sterol 5α,8α-endoperoxides 29-34, each containing a three-membered ring in the side-chain together with 5α,8α-epidioxide grouping. Compound 29 was identified as (22*E*,24*R*,25*R*)-5α,8α-epidioxy-24,26-cyclo-cholesta- 6,22-dien-3β-ol, and was isolated from the gorgonian *Eunicella cavolini* and the ascidian *Trididemnum inarmatum*. Compound 29, bearing a cyclopropyl moiety in the side chain, exhibited significant growth inhibitory effects against MCF-7 human breast cancer cells [26]. Compound 30 was isolated from a marine sponge *Topsentia* sp., the structure of compound 30 was defined as (24*R*,25*R*,27*R*)-5α,8α-epidioxy-26,27-cyclo-24,27-dimethylcholest- 6-en-3β-ol [26]. Two compounds, (22*R*,23*R*,24*R*)-5α,8α-epidioxy-22,23-methylene-24-methlycholest-6-en-3β-ol (31) and (22*R*,23*R*,24*R*)-5α,8α-epidioxy-22,23-methylene-24-methlycholest-6,9(11)-dien-3β-ol (32) were isolated from the soft coral *Sinularia gaweli* and *Lobophytum cacrassum*. Compound 31 exhibited significant cytotoxicity toward the growth of P-388, KB, A549, and HT-29 cells (ED_{50}=0.4, 2.1, 2.7 and 1.4 μg/mL respectively) [35]. Compound 32 had no cytotoxicity against K562 or MOLT-4 tumor cells, but exhibited cytotoxicity toward the growth of HL-60 (12.14 μg/mL) [36]. Two compounds, 5α,8α-epidioxygorgosta-6-en-3β-ol (33) and 5α,8α- epidioxygorgosta-6,9(11)-dien-3β-ol (34) were isolated from the methanolic extract of the marine soft coral, *Sinularia flexibilis* [37] (Figure 8).

The isolation of two new sterol 5α,8α-endoperoxides, 5α,8α-epidioxy-22β,23β-epoxyergosta-6-en-3β-ol (35) and, 5α,8α-epidioxy-22α,23α-epoxyergosta-6-en-3β-ol (36), were new addition to the molecular diversity of *H. tuberosus*, which exhibited weak antibacterial activity and toxicity against brine shrimp [38] (Figure 9).

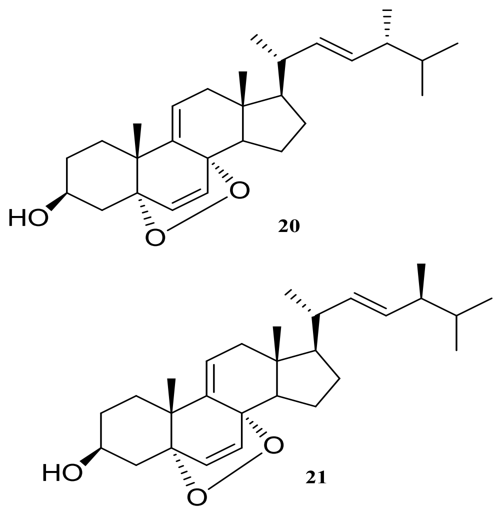


Figure 5: Steroidal endoperoxides 20 and 21.

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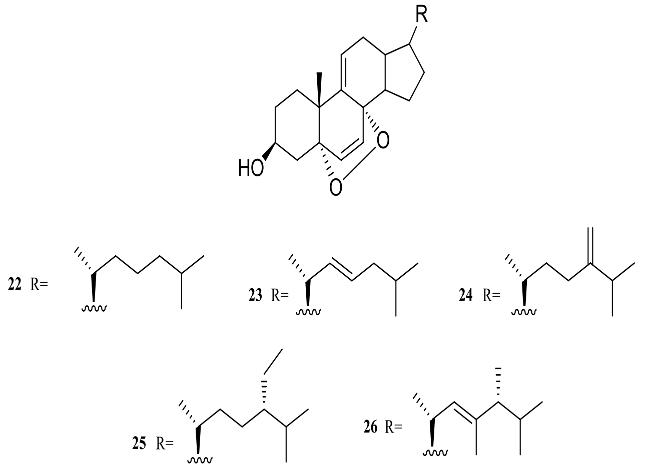


Figure 6: Steroidal endoperoxides 22 - 26.

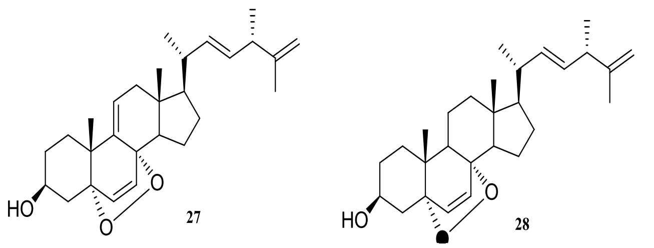


Figure 7: Steroidal endoperoxides 27 and 28.

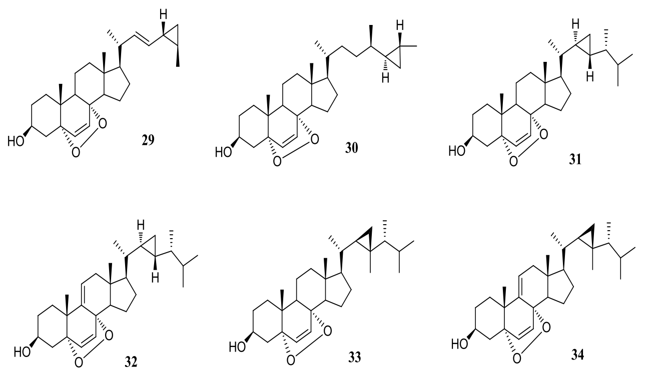


Figure 8: Steroidal endoperoxides 27 and 28.

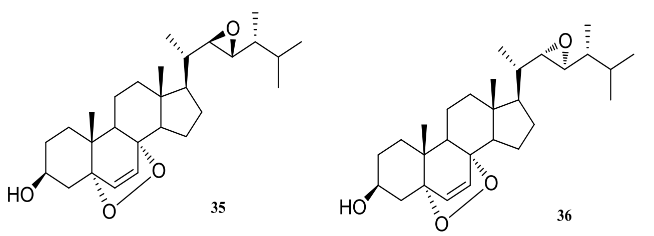


Figure 9: Steroidal endoperoxides 35 and 36.

Synthesis

On the basis of above discussions, it may be concluded that the isolation of sterol 5,8-endoperoxides into the pure state from natural sources is a fairly complex and laborious process. In a number of cases their chemical synthesis appears more convenient, especially if the initial $\Delta^{5,7}$– or $\Delta^{5,7,9(11)}$–sterols are available. This is also favored by the circumstance that chemical synthesis of 5,8-epidioxides are based on the well-studied photochemical oxidation of 5,7-diene group in sterols (Figure 10).

The reaction of ergosterol with singlet oxygen *in vitro* was studied by using different combinations of the photosensitizers (i.e. rose bengal and eosine) and solvents (i.e. pyridine, ethanol and methyl tert-butyl ether) and all the products obtained were isolated and fully characterized. In pyridine, the expected (22*E*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol (1) together with the keto derivative (22*E*)-3β-hydroxyergosta- 5,8(9),22-trien-7-one (KE) were obtained. In ethanol, the expected 1 and main products (22*E*)-ergosta-5,7,9,22-tetraen-3β-ol (DHE) and by-product (22*E*)-5α,8α-epidioxyergosta-6,9,22-trien-3β-ol (EEP9(11)), (22*E*)-ergosta-6,9,22-triene-3β,5α,8α-triol (DHOE) were obtained. In methyl tert-butyl ether, a complex mixture of 1, KE, DHOE, EEP9(11), DHE, together with (22*E*)-7α-hydroperoxyergosta-5,8(9),22-trien-3β-ol (EHP) and (22*E*)-ergosta-5,8(9),22-triene-3β,7α-diol (EH) were obtained. The minor products were characterized and showed strong dependence on the reaction medium (Scheme 1). The method has been used for the synthesis of ergosterol 5,8-epidioxide, 7-dehydrocholesterol 5,8-epidioxide, 7,9(11)- dehydrocholesterol 5,8-epidioxide, and 9(11)-dehydrocholesterol 5,8-epidioxide [39] (Scheme 1).

In cases where the initial $\Delta^{5,7}$–sterols are unavailable, special schemes of synthesis must be developed for obtaining the 5,8-epidioxides. The synthesis of endoperoxide 3 started from cholesterol, cholesterol was converted to cholesterol-3-acetate to protect the hydroxy group [40]. Cholesterol-3-acetate allylic benzoyloxylation and further reduction and esterification of to cholesterol-3,7-diacetate as shown by Scheme 2 stepts I, II and III. Following this procedure 50% yield was obtained [41]. The second route employed for the synthesis of cholesterol-3,7-diacetate was chromium or cobalt catalyzed allylic oxidation in the presence of ter-butyl hydroperoxide to produce 7-oxocholesterol-3-acetate which is further reduced to 7-hydroxycholesterol-3-acetate and in turn to cholesterol-3,7-diaceate. The final yield of acetate obtained by cobalt and chromium allylic oxidation was almost similar (45%), but due to the hazardous nature of chromium catalyst cobalt allylic oxidation step is not frequently-used [42]. Cholesterol-3,7-diacetate formation is also reported by using electrochemical oxidation of cholesterol [43]. Considering the most productive and less hazardous route, the third route is employed in major in study. On the allyl bromination of cholesterol-3-acetate with N-bromosuccinimide in the presence of 2,2-azobis (isobutyronitrile) followed by dehydrobromination with β-collidine in boiling xylene, the cholesterol-3-acetate-5,7-diene was obtained with an overall yield of 54%. Hydrolysis of the acetoxy group with potassium carbonate in methanol led to 7-dehydrocholesterol, the photooxidation of which in the presence of Rose Bengal enabled the endoperoxide 3 to be obtained with an overall yield of 20% from cholesterol [44,45] (Scheme 2).

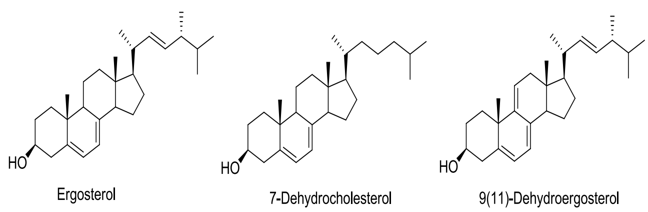
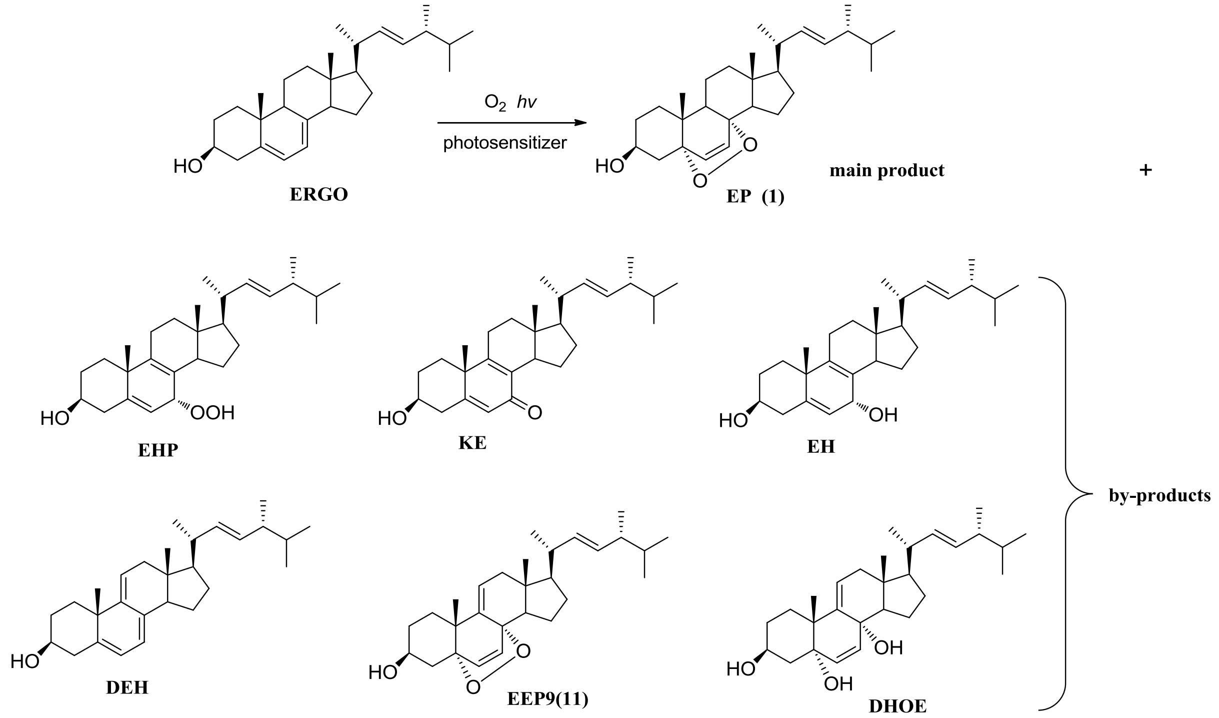
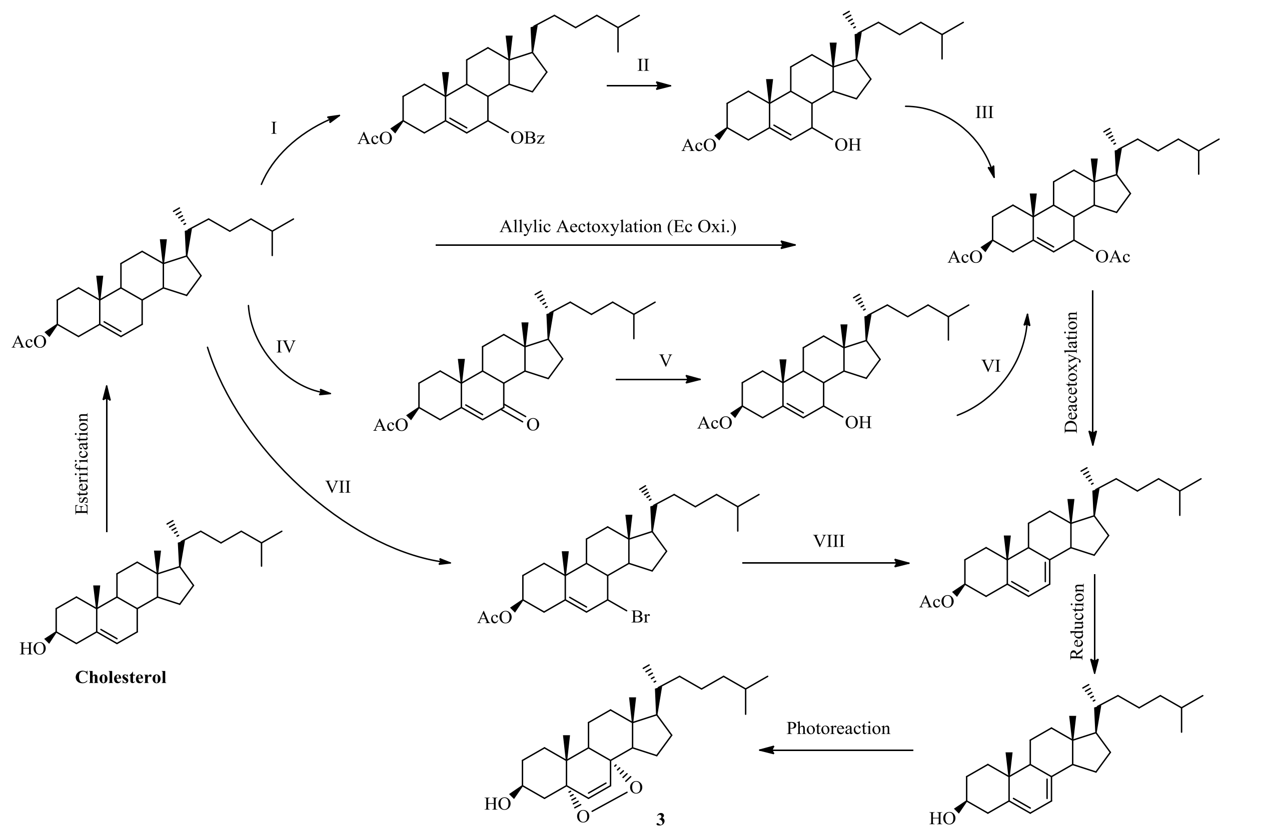


Figure 10: Steroidal endoperoxides 35 and 36.

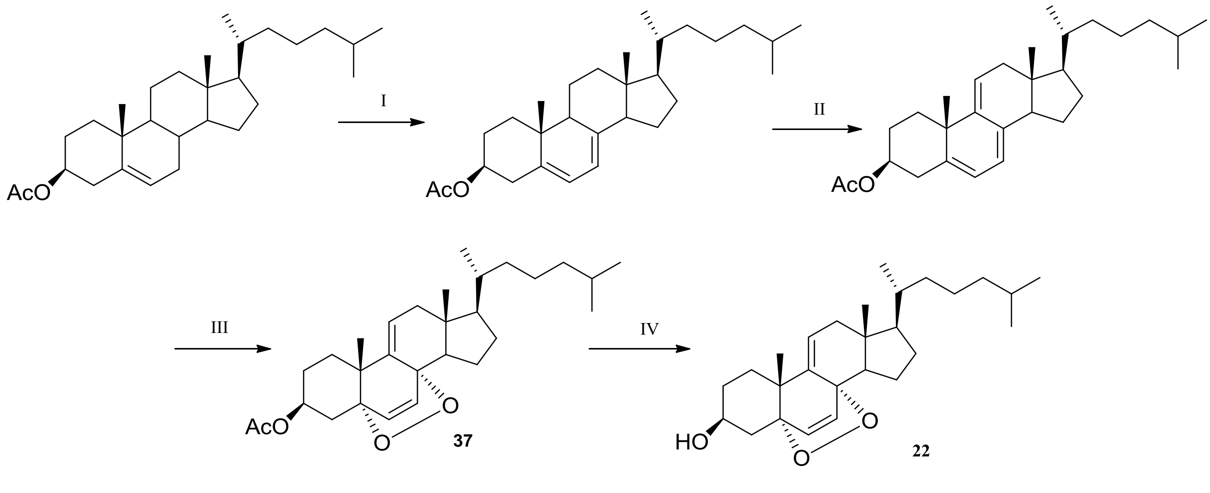


Scheme 1: Steroidal endoperoxides 35 and 36.



I) CuBr t-BuOOCOPh; II) NaBH4/methanol; III, VI) acetic anhydride, pyridine; IV) CrO3, TBHP, or Co(Ac)2/silica TBHP; V)NaBH4, CeCl3 in 30%THF/methanol; VII) NBS; VIII) r-collidine, xylene. Ec Oxi:Electrochemical Oxidation

Scheme 2: Synthesis endoperoxide 3 from cholesterol.



I) NBS, hexane, then xylene, r-collidine; II) (CH3COO)2Hg, dioxane,CH3COOH; III)O2, Rose Bengal, hv

Scheme 3: Synthesis endoperoxide 22 from cholesterol.

If the initial $\Delta^{5,7,9(11)}$–sterols is not available, special synthesis route should be developed(see Scheme 3). The synthesis of endoperoxide 22 also started from cholesterol-3-acetate. The reaction of the cholesterol-3-acetate-5,7-diene could obtained as shown in Scheme 3. Cholesterol-3-acetate-5,7-diene with mercury(II) acetate in dioxane and acetic acid led with a yield of 35% to the cholesterol-3-acetate-5,7,9(11)-triene the photooxidation of which gave a 61% yield of the endoperoxide 37. Hydrolysis of the 37 acetoxy group with potassium carbonate in methanol gave the endoperoxide 22 with a yield of 80% [46] (Scheme 3).

There have been a fairly large number of studies in which sterol 5α,8α-endoperoxides have been used to obtain compounds with various structures. However, up to now, these studies have not led to any compounds bearing practical utilities. For this reason they will not be discussed in details here.

Conclusion

In this review we have shown that most of the natural sterol 5,8-endoperoxides display *in vitro* antimicrobial, anti-tumor activity, immunomodulatory activity, and anti-inflammatory activity even in the nanomolar range. These allow us to assume that sterol 5,8-endoperoxides may be involved in ecological, most probably nutritional, interactions between plants, fungi, and animals, similarly to the situation with other steroids (cardiac glycosides, steroid saponins and alkaloids, ecdysteroids, withanolides, ect.). All these bioactivity natural sterol 5,8-endoperoxides were isolated from terrestrial sources and marine sources (such as plants, fungi and sponge). It is noted that isolation and purification of these natural peroxides in the pure state from natural sources is a fairly complex and laborious process. As a result, it is essential to develop methods for chemical synthesis to increase the efficiency for research and drug development. Likely, increasing investigations on synthesis pathways or cultivation methods of sterol 5,8-endoperoxides will hopefully increase the possibilities of a full pharmacological evaluation and a possible introduction in therapy as lead structures for the development of new drug agents.

Acknowledgement

This work was supported by the National Science Foundation of China (21272020, 21272021).

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