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ETHERIFICATION, OXIDATION, ALKYLATION AND SKELETAL TRANSFORMATIONS OF ECDYSTEROIDS

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Abstract

Phytoecdysteroids (PEs) are naturally occurring polyhydroxylated compounds with a structure similar to that of insect molting hormone and the plant hormone brassinosteroids. PEs have a four-ringed skeleton composed of 27, 28, 29, or 30 carbon atoms (derived from plant sterols). The carbon skeleton of ecdysteroid is known as cyclopentanoperhydrophenanthrene and has a β-sidechain on C-17. ECs are polar steroids, and their solubility is identical to that of sugar molecules; thus, they are lipophilic and soluble in aqueous mediums. However, mammalian steroidal hormones are relatively non-polar and have variable structures.

Keywords

phytoecdysteroids, ecdysteroids, etherification, ecdysone, 20-hydroxyecdysteroid.

1. Introduction

The name ecdysteroids (ECs) originates from the Ancient Greek word ecdysis, which means “stripping”, ‘‘the shedding of an exoskeleton in insects”. Butenandt and Karlson [1] isolated the first EC—ecdysone—from silkworm pupae. Later, its structure was reported via X-ray crystallography [2]. ECs are steroidal hormones initially found in animals that control insect molting or ecdysis and other important metamorphotic processes in arthropods [3]. ECs have a polyhydroxylated four-ringed skeleton bearing 27–30 carbon atoms derived from cholesterol or other sterols. Depending on the natural source, ECs are subdivided into three groups: phytoecdysteroids (PEs), zooecdysteroids, and mycoecdysteroids. The naturally occurring ECs found in plants are differentiated from the ECs found in animals. However, 20-hydroxyecdysone (20-HE) is the most common and widely used [4,5,6,7]. Many ECs are present in plants and animals, such as 20-HE, ecdysone, ajugasterone C, polypodine B, and cyasterone (Figure 1).

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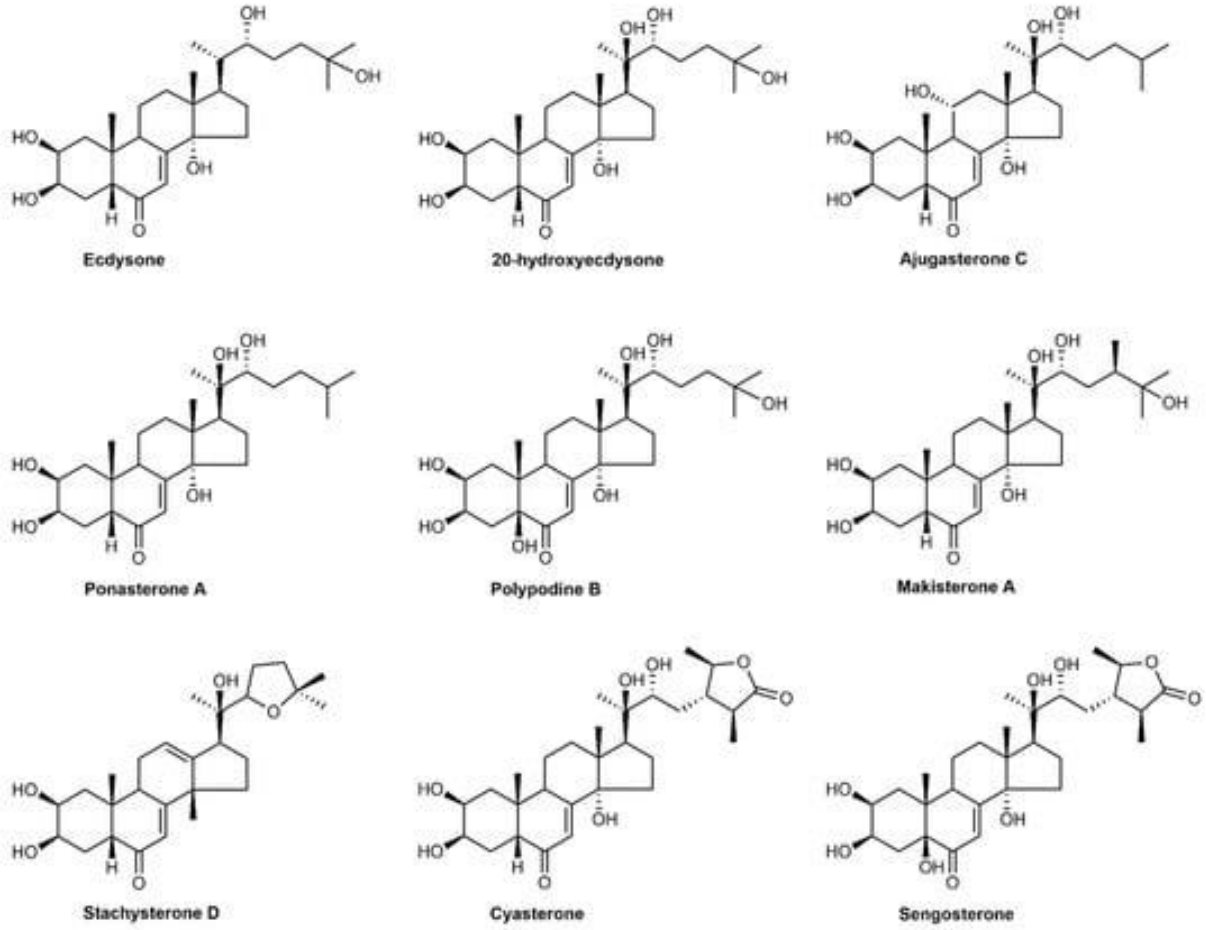

Figure 1. Structures of widely distributed phytoecdysteroids.

This review highlights the latest knowledge on PE distribution, chemistry, isolation, identification, biosynthesis, and regulation. The recent identification of 207 new PEs in 17 plant families is presented. We also highlight chemical transformations and stereochemistry of ECs on the synthesis of EC analogs via esterification, oxidation, reduction, and alkylation. However, complete records on their biosynthesis, distribution, regulation, and role in mammals and plants are scarce, demanding a summary and compilation of present knowledge of PEs. We critically analyze the role of PEs with regard to their biological, pharmacological, and medicinal properties to understand the impact of these phytoconstituents on health and disease. Furthermore, the physio–biochemical roles of PEs in plants and their defensive role against insects, nematodes, fungi, heavy metals, and salinity are highlighted. Additionally, we discuss crosstalk between PEs and phytohormones such as auxins, cytokinins (CKs), gibberellins (GAs), brassinosteroids (BRs), jasmonic acid (JA), and ethylene (ET).

2. Structure of Phytoecdysteroids

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The carbon skeleton of ECs is known ascyclopentanoperhydro-phenanthrene, and it contains a β-sidechain at carbon-17. The important features of ECs are the presence of the cis-(5β-H) junction of rings A and B, the 7-en-6-one chromophore, and the trans-(14α-OH) junction of rings C and D. The sterol structure modulates and synthesizes ECs; subsequently, the trans-A/B ring in sterols undergoes conversion into the junction of the cis-A/B in ECs. These structures are C_{27}, C_{28}, C_{29}, or C_{30} chemical polyhydroxy steroids that bear 14α-hydroxy-7-en-6-one chromophore and the A/B-cis ring. 20-hydroxyecdysteroids have been identified for the first time in arthropods, in which they are the main bioactive ECs [8]. ECs are polar steroids, and their solubility is identical to that of sugar molecules; thus, they are lipophilic and soluble in aqueous mediums. However, mammalian steroidal hormones are relatively non-polar and have variable structures. For example, they do not contain polyhydroxylated side-chain features. Additionally, invertebrates cannot synthesize ECs; rather, they consume phytosterols and convert them into ECs. On the other hand, plants produce ECs via mevalonic acid (MVA) and cholesterol [9,10,11,12]. PEs are found in free-state or conjugated form with sugars (e.g., xylose, glucose, and galactose) as glycosides or with organic acids as esters (such as acetate, cinnamate, benzoate, crotonate, and p-coumarate), sulfates, or isopropylidene. Steroid ring structure shows variation, which is not significant; substantial variations are found in the number, positioning, and orientation of hydroxyl groups and conjugating groups. In some cases, the oxo group may be located at different carbon positions, such as C-2, C-12, C-17, C-20, or C-22, along with the required C-6 position. Several structural modifications are present in different plant families, probably due to different uses of metabolites [4,8,13,14]. EC synthesis, stereochemistry, and transformation via etherification, esterification, oxidation, reduction, alkylation, amination, and fluorination (Figure 2) are discussed below.

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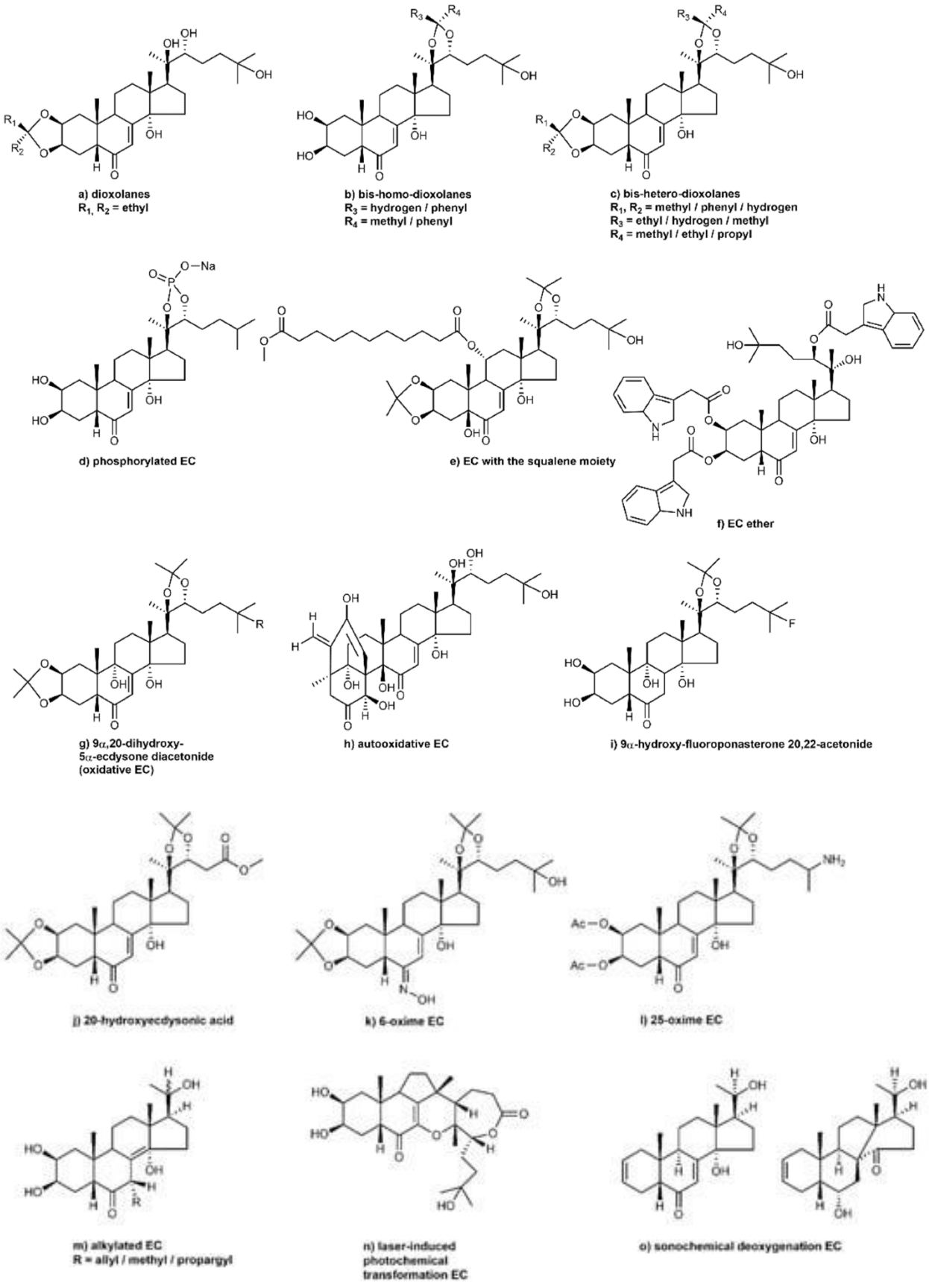

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Figure 2. Structural modifications of ccdysteroids via etherification, esterification, oxidation, amination, fluorination and alkylation.

3. Etherification of Ecdysteroids

2,3-mono- and 2,3:20,22-bis-dioxolane are new 20-HE derivatives; they are prepared by aldehyde and ketone acid-catalyzed condensation (Figure 2a,b) [15]. When EC is dissolved in methanol (MeOH) prior to the addition of a carbonyl component, new polar EC compounds are formed: 2,3-dioxolane, 20,22-dioxolanes, 2,3:20,22-bis-homodioxolanes, and 2,3:20,22-bis-heterodioxolanes [16]. A similar standard procedure is used for the preparation of 20,22-dioxolanes, bis-homo-dioxolanes, and bis-hetero-dioxolanes (Figure 2a–c). It has been observed that reaction selectivity to cyclic acetal is because of the higher reactivity of the 20,22-diol moiety in contrast to the 2,3-diol moiety. Moreover, in the synthesis of heterodioxolanes, it was observed that the 2,3-dioxolane ring is not synthesized if bulky molecules with benzene-substituted rings are used as reagents [17]. NMR spectroscopy is used to obtain several 20-HE products, including isolated and characterized diastereomers [18].To extend the opportunity for the production of low-polarity ECs and to investigate their chemosensitizing characteristics, poststerone 2,3-dioxolanes have been produced. It has been reported that the treatment of poststerone with methyl isobutyl ketone has two pathways, giving two epimers in equal proportions [19]. It has been observed that in the formation of the stereogenic center at the C-22 position, the bulkiest substituent was located at the β-position. The acyl derivative is a byproduct of the preparation of dioxolane. Therefore, acid-catalyzed condensation is used for the synthesis of nonpolar mono- and bis-dioxolanes of 20-HE and poststerone [15].

A new water-soluble phosphorylated EC analog with a high glycemic index has been synthesized from 20-HE [20]. The synthesis strategy was based on the selective protection of 2,3,20,22-vicinal diol groups and the tertiary 25-OH group. The alkaline oxidation of boronate with hydrogen peroxide and phosphorylation with phosphorous oxychloride in pyridine form 20-HE 20,22-phosphoric acid (Figure 2d) with an overall yield of 67% [15]. The structure–activity relationship showed a large number of semisynthetic EC analogs with the presence of a 2,3-acetonide group, which has chemosensitizing properties against both multi-drug resistant and drug-susceptible cancer cell lines [21]. Moreover, the latest approach to develop nanoscale materials based on EC–squalene conjugates has been reported (Figure 2e) [22]. Many new EC ethers and esters have been synthesized via alkylation and condensation of naturally occurring EC and with the help of several

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aldehydes and ketones for testing their inhibitory activity against transmembrane protein ABCB1. A large library of new EC ethers and esters has been synthesized by O-alkylation or condensation of natural ECs with various aldehydes and ketones for testing of their ability to inhibit the ABCB1 transmembrane protein in vitro (Figure 2f) [23].

4. Oxidation of Ecdysteroids

The autooxidation of ECs under alkaline conditions was reported several years ago [15]. It has been reported that 20-hydroxyecdysone and ponasterone A diacetonides in 10% methanol (MeOH) solution of sodium hydroxide (NaOH) at room temperature for 3 h via column chromatography resulted in 9α-hydroxy-5α-derivatives isolation, i.e., 9α,20-dihydroxy-5α-ecdysone diacetonide and 9α-hydroxy-5α-ponasterone A diacetonide [24]. Moreover, when NaOH is replaced by potassium carbonate, then it is changed into a 85:15 mixture of initial compounds and its 5α-epimer, which are not easily separated. Furthermore, when the mixture is treated with a 10% MeOH solution of NaOH, it is completely converted into 9α,20-dihydroxy-5α-ecdysone diacetonide. Thus, it can be concluded that hydroxylation takes place after 5β-EC epimerization into the 5α-epimer. Hydrolysis of compounds 9α,20-dihydroxy-5α-ecdysone diacetonide (Figure 2g) and 9α-hydroxy-5α-ponasterone A diacetonide in 10% perchloric acid gives 20,22-monoacetonides and deprotected ECs. One- and two-dimensional NMR spectroscopy are used to determine 9α-OH and 5α-H configurations. Alkaline autooxidation of 5β-ECs continues as stereoselective oxidation and produces its 5α, 9α-hydroxy analogs [15]. Autooxidation of 20-HE with alkaline MeOH solution gives abeo-steroid, which has a skeletal rearrangement of 20-HE with cleavage of the bond between the C-3 and C-4 atoms (Figure 2h) [25]. EC oxidation with an ozone or oxygen mixture via pyridine continues chemo- and stereoselectivity to produce natural 2-dehydro-3-epi-20-HE, which has been isolated from the fly Calliphora erythrocephala. Furthermore, its hydride reduction yields a diastereomeric mixture of 2α,3α-alcohols and their 2β,3β-analogs. EC 25-fluoroponasterone A diacetonide reaction with lithium in liquid ammonia gives 5β type 9α-hydroxy-EC, which then gives 9α-hydroxy-fluoroponasterone 20,22-acetonide (Figure 2i) [15].

The oxidation of aldehyde with ozone in pyridine and diazomethane solution in diethyl ether synthesizes 23-methoxycarbonyl-25,26,27-tris-nor-20-hydroxyecdysone, which is an analog of 20-hydroxyecdysonic acid (Figure 2j) [15]. Phenyliodine(III) diacetate (PIDA) is used for the oxidative cleavage of EC C20-C22 bonds [26]. Use of PIDA induces production of poststerone from 20E with a yield of

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up to 81.41%, whereas iodobenzene I,I-bis(trifluoroacetate) (PIFA) used for this reaction yields only 57.8% [19]. Use of 2-deoxy-20-HE, polypodine B, ajugasterone C, calonysterone, and PIDA reagent provides a higher amount of C-21 E and a lower quantity of byproducts than using PIFA [26]. It has been reported that PIFA acts as a more aggressive reagent due to the release of trifluoroacetic acid, which decreases the reaction chemoselectivity [15].

5. Oximes of Ecdysteroids

Oximes are used diversely in organic synthesis and display several biological activities. Recently, the synthesis of steroidal oximes has increased. The synthesis of oximes from 20-HE and their later rearrangement into lactams has been studied [27]. Further, several EC oximes are prepared via 20-HE diacetonide using alkoxyamines [28]. It has been found that, depending on the nature of the reaction mixture, this reaction synthesizes either mixtures of ECs (Z)- and (E)-oximes or their 14,15-anhydrous derivatives; 6(E)-oximes are converted to lactam (Figure 2k). The oximation of ketones with hydroxylamine hydrochloride in pyridine and triethylamine (100 °C, 3 h) leads to the formation of several oximes, such as 20- and 25-oximes that had E-configuration, identified via X-ray diffraction (Figure 2l) [29].

6. Alkylation of Ecdysteroids

Alkylation is the major process for steroid-compound modification, e.g., an alkyl group addition at the 7-position. 20-HE treatment with an alkyl halide in lithium ammonia solution forms stereospecific 7α-alkyl 20-hydroxyecdysone derivatives such as 7α-methyl, 7α-ethyl, and 7α-allyl. Allyl derivative synthesis has been used to evaluate the stereospecificity of EC alkylation via X-ray diffraction and NMR. 20-HE reacting with propargyl bromide produces O-alkylated, 7α-monoalkylated, and 7,7-dialkylated products. When the amount of propargyl bromide increases, C-alkylation products are produced: 7,7-bis(2-propyl-1-yl)-14-deoxy-Δ8(14)-20-HE and O-monoalkylation ethers. When the methylation reaction occurs with poststerone, having carbonyl groups at the C-6 and C-20 atoms, the formation of stereoselective 7α-alkyl derivatives takes place, with the reduction of the 20-oxo group producing an equimolar mixture of 20R- and 20S-hydroxy derivatives; separated by HPLC, these have 94% total yield (Figure 2m). Reaction of excess halide with poststerone gives a diastereomeric mixture of 20-hydroxy-7,7-bis-alkyl EC derivatives. Therefore, alkylation of ECs can lead to a few medicinally potent compounds [30,31,32].

7. Skeletal Transformations

Few studies have shown that ECs undergo skeletal transformations. Irradiation with UV rays on 20-HE (aqueous solution) synthesized abeo-EC [33].

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Similarly, the use of laser radiation also caused phototransformation of ECs [34]. Laser irradiation of 20-HE and its diacetonide at 226 nm caused the synthesis of complex mixtures (Figure 2n) and photochemical transformation; apart from synthesizing poststerone, stachysterone B, 14α,15α-epoxy-14,15-dihydrostachysterone B, 14-epi-20-HE, and 14α-hydroperoxy-20-HE, this reaction also gave new products such as lactone, 6-carbaldehyde, and tetrahydroxepine ring-containing skeletal rearrangement products [35]. Ultrasonic reactions have recently been used for the transformation of steroids (Figure 2o). These treatments may influence the conversion process, reaction chemoselectivity, product purity, and yield; further, they may reduce reaction time, inhibit byproduct formation, and reduce catalyst presence [36].

It has been found that sonochemical deoxygenation of poststerone 2,3-dimesylate gives products such as abeo-steroid and target products. Treatment using ultrasound rays on poststerone 2,3-dimesylate with a sodium iodide-zinc-dimethylformamide reagent yields 3-dideoxy-Δ2(3)-poststerone and/or its (8R)-13(14→8)-abeo-isomer, which are formed by an intramolecular rearrangement. Reaction of abeo-pregnanes with complex metal hydrides has been reported for 6- and 20-oxo-groups; however, the 14-oxo-group reactivity was decreased. In 6,20-dioxo-Δ2,3-pregnane and 6,20,14-trioxo-13(14→8)-abeo-isomer structures, the reduction of hydride is stereospecific, giving 6α,20R-diols [37].

8. Isolation and Identification of Phytoecdysteroids

Isolation of PEs from plants involves several procedures, including extraction, separation, purification, and identification. The polar nature of PEs makes them difficult to isolate from other major polar plant materials such as chlorophyll, steroids, amino acids, terpenoids, phenols, and pigment constituents. Thus, different chromatographic techniques, such as thin-layer chromatography, normal- and reversed-phase column chromatography, flash chromatography, droplet counter-current chromatography, gel chromatography, and high-performance column chromatography (HPLC) are used for isolation. Rotation planar chromatography (RPC) is an effective preparative method for the separation of ECs that is faster and more effective than preparative TLC [45]. PEs are isolated by solvent extraction of dried plant parts with MeOH or ethanol, followed by a partition with water and hexane. Further, an aqueous portion of the material can be exposed to column chromatography via silica gel, Sephadex® LH-20 (Sigma-Aldrich, Saint Louis, MO, USA), or Diaion® HP-20 (Sigma-Aldrich, Saint Louis, MO, USA). The fraction recovered goes through reverse-phase HPLC using silica gel as the stationary phase. Moreover, normal-phase HPLC, rotation locular

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countercurrent chromatography, and droplet countercurrent chromatography are also used for PE isolation [38,39]. PEs can be identified using 13C- NMR and 2D-NMR; further, they give heteronuclear single quantum coherence or correlation, correlation, heteronuclear multiple bond correlation, rotating frame nuclear Overhauser effect, and nuclear Overhauser effect in spectroscopy. Additionally, carbon resonances of C-2 and C-3, C-14 and C-7, and C-8 lie near δC 67–69, 83–85, 121–123, and 162–165, respectively. PE chromophores, i.e., 14α-hydroxy-7-en-6-one, can be identified using UV absorption in MeOH at the wavelength (λmax) 240–245 nm [8,40].

Achyranthes bidentata (Amaranthaceae) contains four furanoECs that have been isolated from the ethanolic extract [41]. The methanolic extracts of Polypodium vulgare and Serratula coronate roots [15] and Callisia fragrans stems [44] have been used for the isolation of PEs. In Aerva javanica, the structures of new PEs have been established based on 1D and 2D 1H NMR and 13C NMR spectroscopy and HREIMS [42]. A new family of zooecdysteroids, called ecdysone lactones, has been isolated from an extract (CH2Cl2-MeOH, 1:1) of freeze-dried Antipathozoanthus hickmani. Ponasterones have been isolated from Podocarpus nakaii and Alcyonidium gelatinosum via freeze-dried extract (CH2Cl2-MeOH, 1:1); for their identification, 1D and 2D NMR were applied [15]. Many ECs have been isolated from seeds of Serratula chinensis via butanol extract [43].

9. Conclusion

PEs are naturally occurring polar steroidal secondary metabolites that have versatile uses in invertebrates, animals, and plants. Invertebrates cannot synthesize ECs, so they consume phytosterols and convert them into ECs; however, plants produce ECs via MVA and cholesterol. The polar nature of PEs makes them difficult to isolate from other major polar plant materials such as chlorophyll, steroids, amino acids, terpenoids, phenols, and pigment constituents. Thus, different chromatographic techniques, such as thin-layer chromatography, normal- and reversed-phase column chromatography, flash chromatography, droplet counter-current chromatography, gel chromatography, and high-performance column chromatography (HPLC) are used for isolation.

Abbreviations

20-HE	20-hydroxyecdysone
EC	ecdysteroid
HPLC	high-performance column chromatography
MeOH	methanol

MVA	mevalonic acid
PE	phytoecdysteroid
NMR	nuclear magnetic resonance
PIDA	phenyliodine(III) diacetate
NaOH	sodium hydroxide

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