Terpenoids and Glycolipids from Euphorbiaceae

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Abstract: The family *Euphorbiaceae* is widely distributed throughout both hemispheres and ranges in morphological form from large desert succulents to trees and even small herbaceous types. Many species contain a milky juice which is more or less toxic, especially for cold-blooded animals, and can produce a dermatitis similar to that from poison ivy.

Separation procedures and characterization of the less polar fractions of the plant extracts have been widely described in the literature for their content in diterpene derivatives.

In the continuing research on biologically active compounds from Euphorbiaceae, a series of studies on the isolation and structure elucidation of glyceroglycolipids (GGLs) and glycosphingolipids (GSLs) have been carried out in order to develop the novel medicinal resources from natural Euphorbiaceae products.

Glyceroglycolipids are major constituents of the chloroplast membrane in the plant kingdom. Recently, glycolipids were found to possess antitumor-promoting activity while glyceroglycolipids isolated from Euphorbiaceae have shown an interesting anti-inflammatory activity *in vivo*.

Glycosphingolipids are present at the outer layer of the lipid-bilayer in biological membranes and are thought to partecipate in antigen-antibody reactions and transmission of biologically informations. Sphingolipid breakdown products, sphingosine and lysosphingolipids, inhibit protein kinase C, a pivotal enzyme in cell regulation and signal transduction. Sphingolipids and lysosphingolipids affect significantly cellular responses and exhibit antitumor promoter activities in various mammalian cells. These molecules may function as endogenous modulators of cell function and possibly as second messengers.

Key Words: Euphorbiaceae, glyceroglycolipids, glycosphingolipids, diterpenes, fatty acids, sphingosines, long-chain bases, digalactosyldiacylglycerols, monogalactosylmonoacylglycerols, monogalactosyldiacylglycerols.

1. INTRODUCTION

Euphorbiaceae plants have been the object of chemical and pharmacological investigations because of the irritant and carcinogenic properties of their lattices [1-4]. These biological properties have been traced back in many cases to the presence of certain types of diterpenes, most particularly phorbol derivatives, which have the tigliane framework [4, 5].

The isolation and final structure elucidation of the tumour-promoting phorbol-12,13-diesters from the seed oil of *Croton tiglium* by ERICH HECKER [6], nearly 30 years ago, initiated intensive research into the pro-inflammatory and tumor-promoting diterpenes of the plant families Euphorbiaceae. These pure compounds have been instrumental in furthering our understanding of the diseases of cancer and inflammation.

The toxic diterpenes of the plant family Euphorbiaceae are hydrocarbons which belong to the tigliane, daphnane and ingenane groups [7].

Further macrocyclic diterpenes belonging to other skeletal types, present in the plant as oxygenated derivatives, esterified at one or more positions have been found in plants of the family Euphorbiaceae and are also characterized by similarly strong pharmacological effects [8]. These various types are based upon the hydrocarbons casbane [9], jatrophane [10, 11, 12], lathyrane [13, 14], jatropholane [15], crotofolane [16] and rhamnifolane [17], respectively (Fig. (**1**)).

Modern studies have highlighted the wide spread use of several of these plants to treat cancerous conditions in the traditional medicine of many areas of the world and of recent interest is the observation that certain of these diterpenes may possess antitumour activity [18-20].

While the separation procedures and characterisation of the less polar fractions of the whole plant extracts have been widely described in the literature for the presence of diterpene derivatives, the analysis of the latex of *Euphorbia biglandulosa* Desf. has led to the isolation of the calcium salt of 5,5-dimethyl-5,6-dihydro-α-pyrone-3,4-dicarboxylic acid (**1**) from the aqueous phase of the coagulated latex sap of the plant [21] which inhibits the oxidative phosphorylation of heart and liver mitochondria. A mixture of the calcium salts of two isomeric 3-O-(β-hydroxy-β-methylglutaroyl) shikimic acid (**2**) and 5-O-(β-hydroxy-β-methylglutaroyl) shikimic acid (**3**) also occurred in the aqueous phase of the coagulated latex sap of *Euphorbia biglandulosa* Desf. [22, 23] (Fig. (**2**)). From the lipophilic phase of the latex sap of *Euphorbia biglandulosa* Desf. have been isolated new diterpene derivatives which inhibit the oxidative phosphorylation of isolated heart mitochondria and possess an irritating action on the skin of mice and toxicity on fishes [24].

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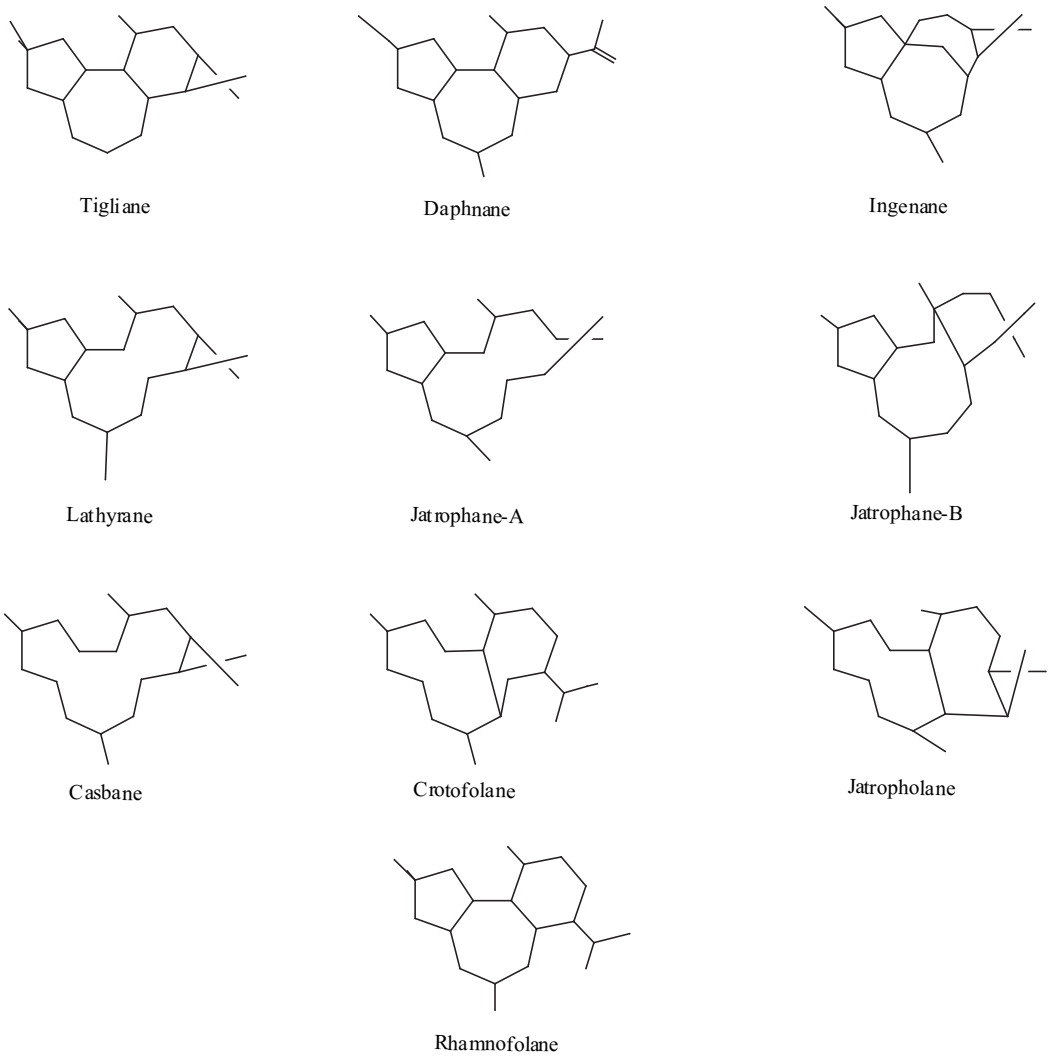


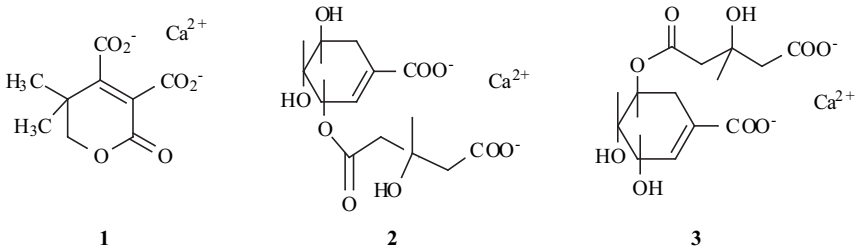
Fig. (1). Structural types of diterpenes from Euphorbiaceae.

In Sicily (Italy) *Euphorbia biglandulosa* Desf. and *Euphorbia characias* L. have been used for centuries for fishery in the rivers of the isle. The toxic activity of the latex of *E. biglandulosa* is due to the 4-deoxyphorbol and ingenol derivatives, which have shown a high toxicity on fishes (0.01 ppm) with effects on respiratory and central nervous systems.

In the continuing research on biologically active compounds from Euphorbiaceae, a series of studies on the isolation and structure elucidation of glyceroglycolipids (GGLs) and glycosphingolipids (GSLs) have been carried out in order to develop the novel medicinal resources from natural Euphorbiaceae products [25-27].

Chemically, GGLs contain a glycerol unit glycosylated at one primary alcoholic function, with the remaining hydroxyl groups acylated by a fatty acid and with a stereogenic center at the C-2 position of the glycerol moiety (Fig. (**3**)).

The structure of a representative GSL is shown in Fig. (**4**). A carbohydrate chain and a fatty acyl group are linked to a long-chain aminoalcohol called long-chain base (LCB). The fatty acyl chain is linked with an amide bond to the LCB, and together they constitute the ceramide; the monosaccharide or oligosaccharide group is bound to the primary alcoholic function of the ceramide. Sphingosine (**4**) is the LCB most commonly found in nature, so that LCBs



1 **2** **3**

Fig. (2). Chemical structures of the compounds isolated from the latex of *Euphorbia biglandulosa* Desf.

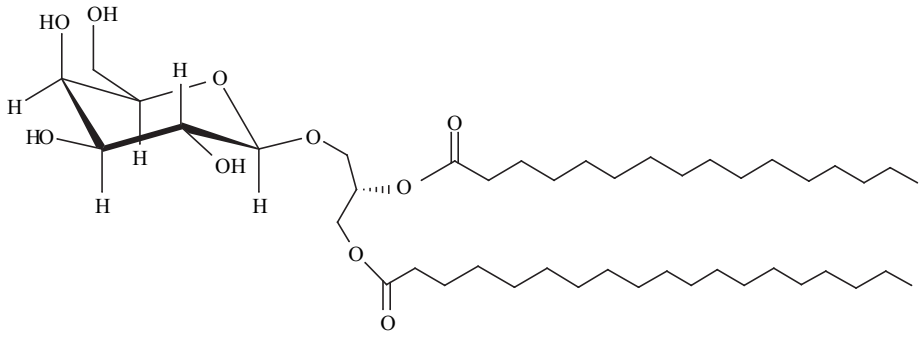


Fig. (3). Structure of a representative glycoglycerolipid.

are often referred to as sphingosines. In plant glycolipids, the trihydroxylated LCB phytosphingosine (**5**) is frequently found and the name phytosphingosine is often used to denote any trihydroxylated LCB (Fig. (**5**)), while tetrahydroxysphingosines are rarely found and recently have been isolated for the first time from *Euphorbia characias* L.

Only starting from 1973, some glycolipids were isolated from a marine cyanobacterium and the structures unambigously determined [28] and previously published reviews deal with marine glycolipids from invertebrates [29, 30] and algae [31]. The present review deals mainly with the two classes of compounds, namely GGLs and GSLs isolated for the first time from Euphorbiaceae.

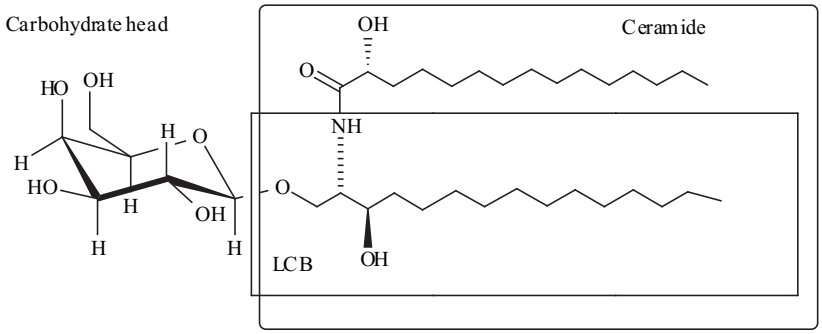


Fig. (4). Structure of a representative glycosphingolipid.

2. BOTANICAL CONSIDERATIONS

The family Euphorbiaceae is widely distributed throughout both hemispheres and ranges in morphological form from large desert succulents to trees and even small herbaceous types. Many species are weeds and they invade cultivated land as secondary growth and provide a health hazard both to humans and grazing livestock.

Within the family Euphorbiaceae, the sixth largest among flowering plants, the genus *Euphorbia* L. alone accounts for almost a sixth of the whole group [32, 33]. The largest genera are *Euphorbia* consisting of over 1600 species and Croton of about 700 species. Thirteen other genera contain over 100 species each [33]: these include *Phyllantus* (480 species), *Acalypha* (430 species), *Glochidon* (280 species), *Macaranga* (240 species), *Manihot* (160 species), *Jatropha* (150 species) and *Tragia* (140 species). The family has been divided into four sub-families:

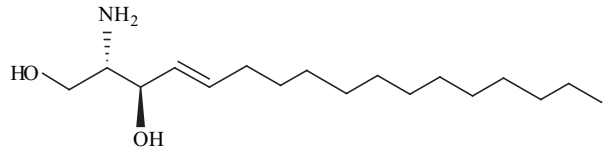
Phyllanthoideae

Crotonoideae

Poranteroideae

Ricinocarpoideae

Several of these genera contain species characterised by the occurrence of highly irritant latex [34]. From the tribe Crotonae, which contains the genus *Croton*, the first phorbol- 12, 13-diesters were isolated [1]. Nevertheless, several diterpenes have been frequently reported from the plant of the family Euphorbiaceae because of their diverse biological activities [35]. Recently, ingols have attracted considerable interest as antineoplastic agents [18, 36].



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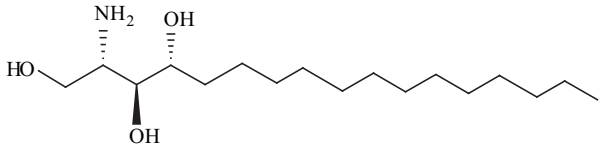

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Fig. (5). Structures of representative sphingosines.

3. ISOLATION PROCEDURES

Glycosphingolipids, sphingosine derivatives and analogues have been isolated from a number of marine sources, including sea stars [37-40], sea sponges [41-43], shellfishes [44, 45], algae [46, 47], fungi [48-50] and plants [51-53].

Many glycolipids (GGLs, GSLs) occur as mixture of homologues with different lengths in the alkyl side chains of the lipid portion of the molecule.

Generally, every class of the mixture of homologous glycolipids (GGLs, GSLs) shows a single spot on silica gel TLC (thin-layer chromatography), while the TLC on RP-18 gives different spots due to the presence of different alkyl side chains of the sphingoid bases (GSLs) and of the fatty acids (GGLs). Besides, the nature of the fatty acids and/or the sphingoid bases is subsequently determined by chemical degradation following by chemical reactions, GC (gas chromatography) and MS (mass spectrometry) analysis.

Many species of Euphorbiaceae are characterised by the presence of the latex so that the isolation procedures are different in the case of a species rich in latex or for a whole plant. *Euphorbia biglandulosa* Desf. is an example of a plant with latex sap which for coagulation provides an aqueous phase (50%) and a lipophilic phase (50%).

The aqueous fraction of the coagulated latex sap contains derivatives of shikimic acid and of 5,5-dimethyl-2-oxo-5,6-dihydro-2H-pyrane-3,4-dicarboxylic acid [21, 22]. The lipophilic phase contains triterpene esters, triterpens alcohols, ingenol derivatives and 4-deoxyphorbol triesters

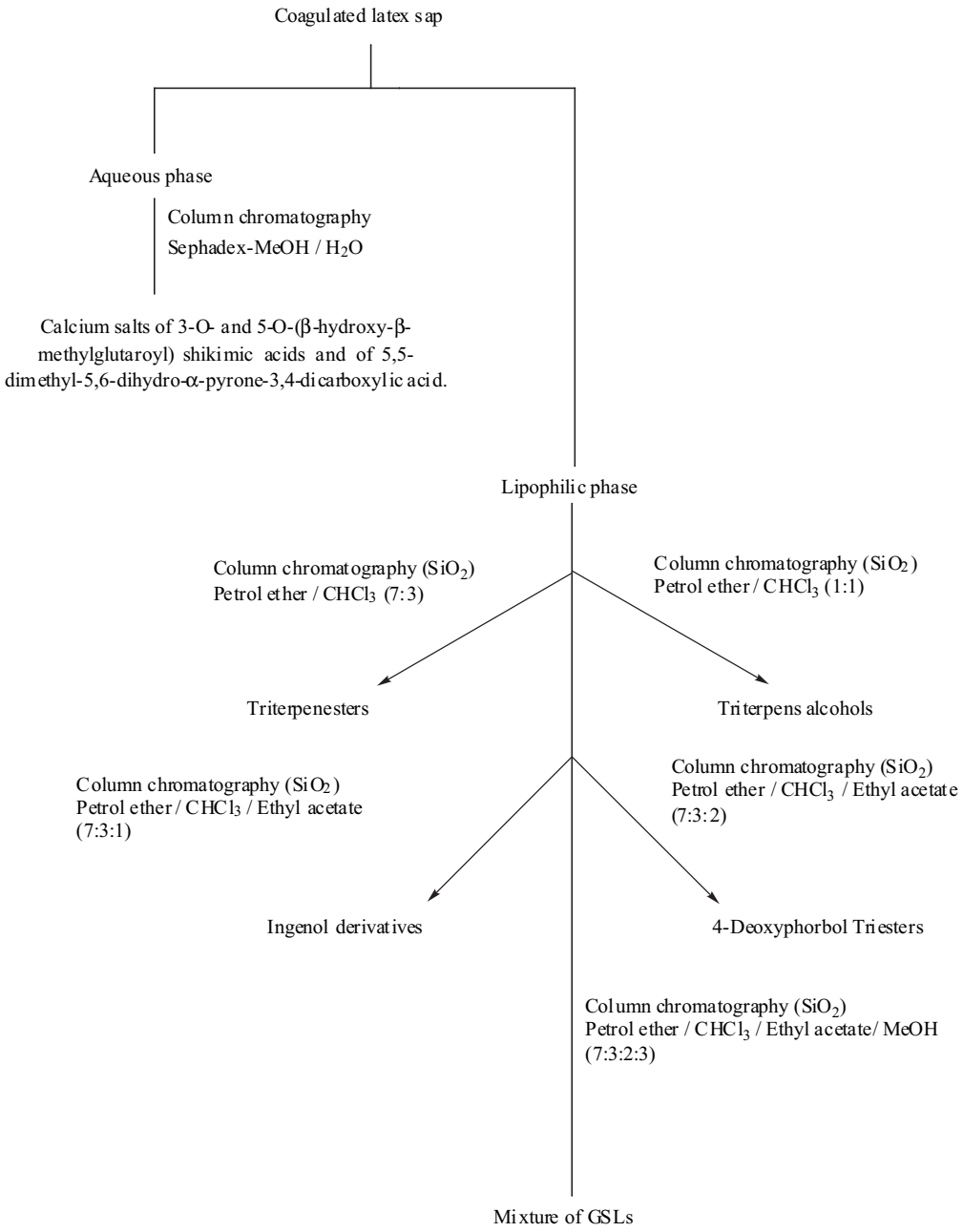


Fig. (6). General isolation procedure for GSLs from the latex sap of Euphorbiaceae.

[54-56]. Further elution of the lipophilic phase with petrol ether / Chloroform / Ethyl acetate / Methanol (7 : 3 : 2 : 3 / v:v) provides a complex mixture rich in GSLs [57] (Fig. (**6**)).

In the case of plants of the family of Euphorbiaceae poor in latex content, the most recent procedures include the extraction of the whole plant material with methanol. The most commonly used techniques include absorption chromatography (SiO_2) and reversed-phase chromatography.

An illustrative example of the isolation of glycolipids is represented by the purification of GGLs and GSLs from *Euphorbia peplis* L. [58] (Fig. (**7**)).

Methanol extract of plant material has been evaporated to dryness below 45°C and after removal of solvent the residue is dissolved in methanol and filtered through a silica gel column using chloroform as eluent. The chloroform fraction contains hydrocarbons, steroids, triterpenoids and macrocyclic diterpenes. Silica gel column chromatography has been used as first step in the purification of the crude extract in order to remove apolar products.

The glycolipid material can be recovered in the polar fraction of the methanol extract of the plant. The crude residue obtained after evaporation of the solvent is subjected to different chromatographic separations including absorption chromatography (SiO_2) and reversed-phase chromatography (RP-18). Glycolipids are isolated from the polar fraction of the methanol extract of the plant by using silica gel column chromatography eluted with solvents at increasing polarity and reversed-phase chromatography to obtain GGLs as monogalactosyldiacylglycerols (MGDGs), digalactosyldiacylglycerols (DGDGs), monogalactosylmono-acylglycerols (MGMGs) and glycosphingolipids (GSLs) [26, 27, 58] (Fig. (**7**)).

Other products have been isolated from Euphorbiaceae which cannot be classified either as glycoglycerolipids or glycosphingolipids but they are structurally composed of a carbohydrate linked through a glycosidic bond to a moiety with different polarity. Glucoclionasterol is an example of this class of compounds [58] (Fig. (**7**)), but Euphorbiaceae are also known for their content of tannins. Yoshida and co-workers [59] have isolated and chemically characterised a series of dimeric hydrolyzable tannins of a new class having a geraniin moiety as a monomeric unit from various species of Euphorbia [60].

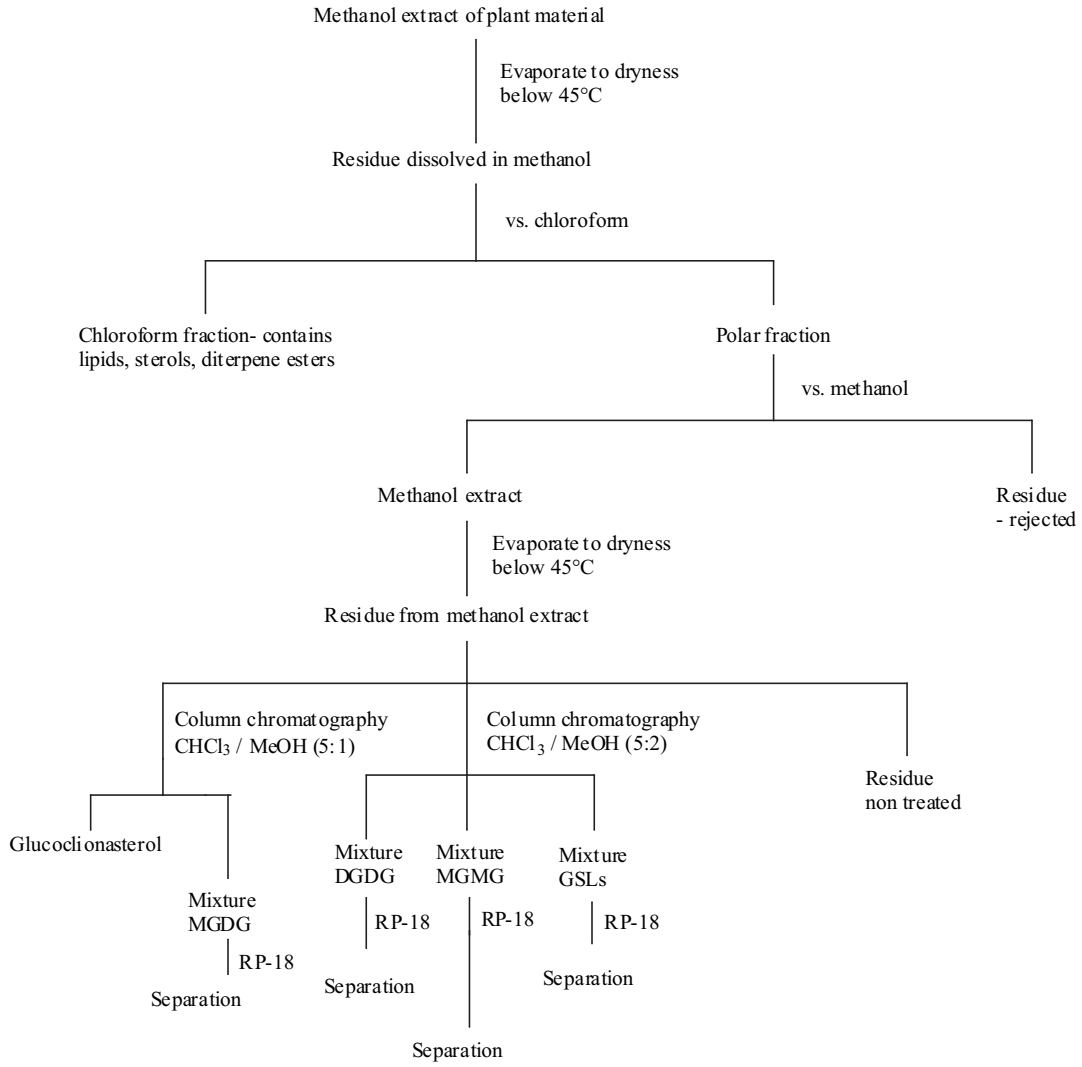


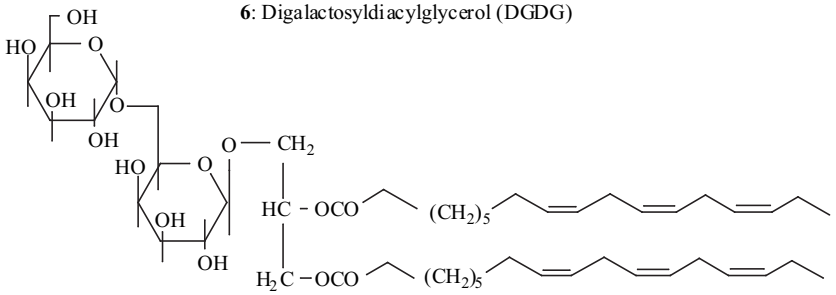
Fig. (7). General isolation procedure for GGLs and GSLs from methanol extract of plant material.

4. ANALYSIS AND STRUCTURE ELUCIDATION OF GLYCEROGLYCOLIPIDS

The glyceroglycolipids (GGLs) found in the family of Euphorbiaceae are digalactosyldiacylglycerols (DGDGs), monogalactosylmonoacylglycerols (MGMGs), monogalactosyldiacylglycerols (MGDGs). The identification has been performed using chemical and spectroscopic analysis. FAB-MS spectrometry (the negative ion mode is generally used), ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}H COSY, DQF-COSY, ^{1}H-^{13}C COSY experiments were useful in providing informations for their structure elucidation.

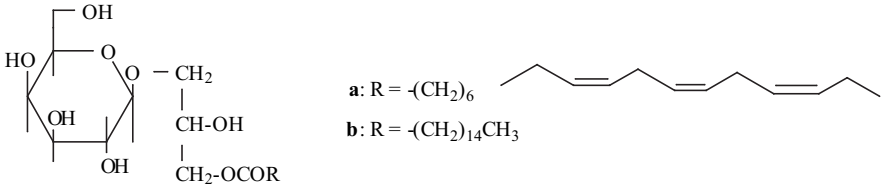
Digalactosyldiacylglycerols (DGDGs) are commonly found in photosynthetic organisms such as algae [61-63] and for the first time in *Euphorbia peplis* L. [27]. In order to determine the location of the fatty acid residues in the compound **6**, the glycolipid was subjected to hydrolysis

6: Digalactosyldiacylglycerol (DGDG)



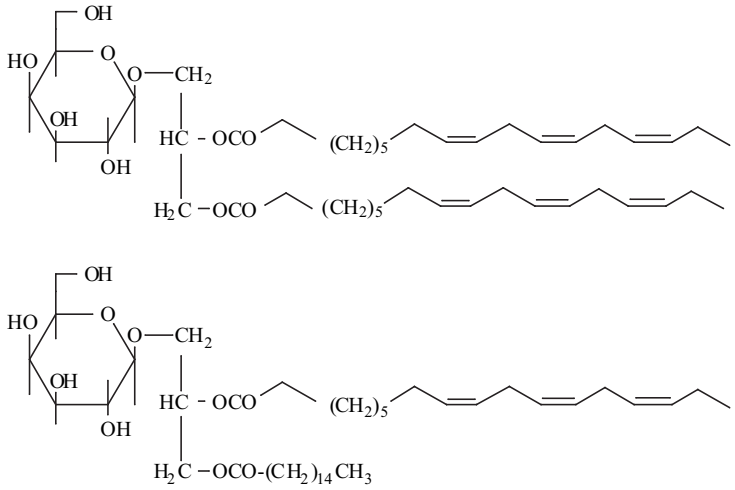
Occurence: *Euphorbia peplis* L. [27], photosynthetic organisms [61-63]
Physical Data: ^{1}H-NMR [27], ^{13}C-NMR [27], FAB-MS [27].

7 : Monogalactosylmonoacylglycerols (MGMG)



Occurence: *Euphorbia peplis* L. [58], Euphorbia cyparissias L. [65]
Physical Data: ^{1}H-NMR [58, 65], ^{13}C-NMR [58, 65], FAB-MS [58, 65].

8, 8a: Monogalactosyldiacylglycerols (MGDG)



Occurence: *Euphorbia cyparissias* L. [67], photosynthetic organisms, mainly algae [63-65, 69], marine sponges [68] and cyanobacteria [70].
Physical Data: ^{1}H-NMR [67], ^{13}C-NMR [67], FAB-MS [67].

Fig. (8). Chemical structures of GGLs isolated from Euphorbiaceae.

with a 0.5 N NaOH solution in CH_3OH and then with BF_3/CH_3OH to give a mixture of fatty acid methyl esters [64]. The GC analysis of the methyl esters in **6** indicates the presence of a kind of ester, methyl linolenate (Fig. (**8**)).

Monogalactosylmonoacylglycerols (MGMGs) have been found in *Euphorbia peplis* L. [58] (**7a**) and *Euphorbia cyparissias* L. [65] (**7b**) (Fig. (**8**)). The absence of the multiplet at $\delta = 5.60$ ppm in the ^{1}H-NMR spectrum and the signal at $\delta = 68.76$ ppm in the ^{13}C-NMR spectrum suggest that the hydroxyl group in C-2 position of the glycerol moiety is free. If the free hydroxyl group will be in C-3 position, instead of C-2 of the glycerol moiety, the signal of the carbon C-3 in the ^{13}C-NMR spectrum was moved at 60 ppm. Detailed analysis of the homonuclear decoupling spectra defined β-D-galactopyranoside. The doublett at 4.90 ppm in the ^{1}H-NMR spectrum and the signal at 105.6 ppm in the ^{13}C-NMR spectrum confirm the β-glycosidic bond of the sugar moiety in C-1 position of glycerol.

Galactosyldiacylglycerols (MGDGs) are present as metabolites virtually in every photosynthetic organism and particularly in marine plants (mainly algae). MGDGs are often present as a mixture of homologues differing in the acyl chains. While MGDGs in marine algae are often found with tetra- and pentaunsaturated fatty acids [62], MGDGs from higher plants are characterised by a high content of triunsaturated fatty acids [65]. Monogalactosyldiacylglycerols isolated from Euphorbiaceae (*Euphorbia cyparissias* L.) have been separated using reversed phase column chromatography, and two MGDGs have been obtained (**8**, **8a**) [65] (Fig. (**8**)). Alkaline treatment of MGDGs and GC analysis of the methyl esters of the fatty acids mixture obtained, indicated the presence only of a $C_{18:3\omega3}$ fatty acid for the compound **8** and a mixture (1:1) of $C_{18:3\omega3}$ and palmitic acids for the compond **8a**. The ^{13}C-NMR and FAB-MS analysis of the two compounds enabled us to identify the location of the fatty acid residues to be at C-2 ($C_{18:3\omega3}$) and C-3 (C_{16}) of the glycerol moiety.

5. BIOLOGICAL ACTIVITIES OF GLYCEROGLYCOLIPIDS

Galactolipids are major constituents of the chloroplast membrane in the plant kingdom. The biological functions as well as occurrence and distribution of galactolipids have been an area of intense interest and investigation [66]. Galactosyl diacylglycerols are present in photosynthetic tissue of plants and are structural components of thylakoid membrane [67]. Furthermore, they are concerned with the electron transport chain in photosynthesis as well as being involved in the construction of chloroplasts.

Concerning the biological activity of glycolipids, from the Okinawan marine sponge *Phyllospongia foliascens* Pallas [68] have been isolated galactolipids, which exhibit anti-inflammatory activity. Recently, an examination of the antialgal activities of the isolated glyceroglycolipids from *Phormidium tenue* revealed that the monogalactosyl diacylglycerols induced the lysis of this cyanobacterium and showed anti-tumour activity [69].

The digalactosyldiacylglycerol **6** isolated from *Euphorbia peplis* L. has been tested for its topical anti-inflammatory activity, evaluated as inhibition of the Croton oil-induced ear oedema in mice [27]. The compound **6**, administered at the same single dose level (1 μM/cm^2), revealed a significant anti-inflammatory activity, reducing the oedematous response by 82.9%, whereas 0.25 μM/cm^2 of the reference drug indomethacin induced about 50% oedema reduction. Furthermore, the monogalactosylmonoacylglycerols (**7a**, **7b**) isolated from *Euphorbia peplis* L. [58] and *Euphorbia cyparissias* L. [65] and the monogalactosyldiacylglycerols (**8**, **8a**) isolated from *Euphorbia cyparissias* L. [65] have shown an interesting anti-inflammatory activity *in vivo* (Table 1).

Table 1. Anti-Inflammatory Activity of Compounds 6-8

Compound	Dose (mM)	n+	Oedema (mg) M ± S.E.	%++
Control	---	10	7.0 ± 0.4	---
Indomethacin	0.25	10	3.6 ± 0.4*	48.6
6	1.00	10	1.2 ± 0.3*	82.9
7a, 7b	1.00	10	2.4 ± 0.5*	61.0
8	1.00	10	0.6 ± 0.1*	92.0
8a	1.00	10	1.1 ± 0.2*	85.0

p< 0.05 at the Student's t-test
+: Number of animals
++: % = Percentage of oedema reduction

It was recently shown that glycoglycerolipid analogues have a promising inhibitory effect on Epstein-Barr virus early antigen (EBV-EA) activation induced by the tumour promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) [70-72]. The mechanism of glycoglycerolipid inhibitory effect on tumour promoting activity is still not known, but it could be that inhibitors interfere with the TPA-induced promotional events, interacting with the receptor of tumour promoters, the protein kinase C [73].

Glycoglycerolipids constitute a family of glycolipids with apparently very restricted expression in human tissues. Nevertheless, two glycoglycerolipids were isolated from the HT29 human colon carcinoma cell line [74]. The presence of glycoglycerolipids in a human colon carcinoma cell line indicates that glycolipids may serve as differentiation antigens in various normal tissues and in tumour development. The Galalpha1-4Gal epitope was previously identified as a receptor for bacterial adhesins and toxins. The finding that this epitope is also linked to a glycerolipid moiety opens up new possible roles for this carbohydrate receptor in intracellular signalling.

Besides, glycoglycerolipids were isolated from an aquatic bacterium, *Corynebacterium aquaticum* having virus-neutralizing activities [75] and this constitutes the first evidence of the binding- and neutralizing-abilities of native glycoglycerolipids as to influenza viruses.

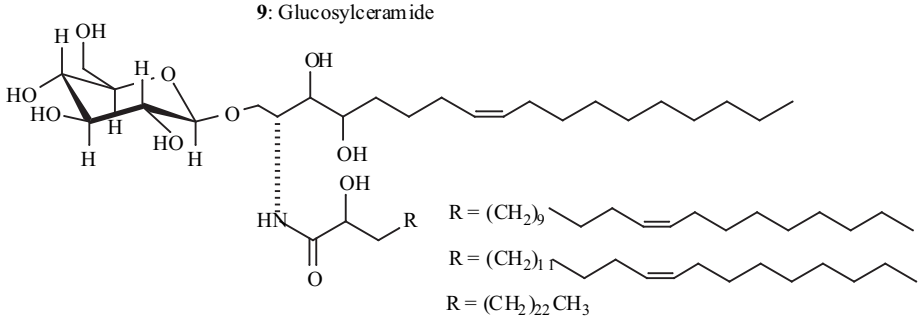
6. ANALYSIS AND STRUCTURE ELUCIDATION OF GLYCOSPHINGOLIPIDS

Glycosphingolipids (GSLs) are membrane constituents of animals and plants and are believed to possess a wide range

of biological activities, including modulation of growth and regulation of differentiation. They are involved in membrane phenomena, such as cell-cell recognition, cell-cell adhesion, antigenic specificity and other kinds of transmembrane signalling [76-78].

Neutral GSLs, commonly referred to as cerebrosides, are widely distributed in organisms belonging to a number of different taxa and have been isolated from the polar fraction of extracts of Euphorbiaceae.The structure and distribution of plant sphingolipids having unsaturated hydroxy fatty acids are poorly understood. The isolation and structure elucidation of cerebrosides from Euphorbiaceae is one of the few examples in literature of identification of the fatty acids [41, 52].

9: Glucosylceramide



Occurence: *Euphorbia biglandulosa* Desf. [57], marine animals [45], fungi [48].
Physical Data: ^{1}H-NMR [57], ^{13}C-NMR [57], FAB-MS [57].

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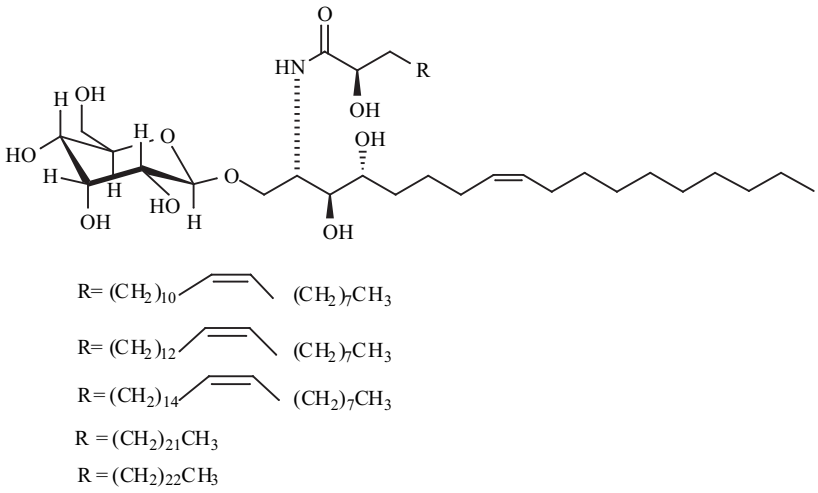

Occurence: *Euphorbia wulfenii* Hoppe ex Hock [79], *Euphorbia characias* L. [80], marine animals [45], fungi [48].
Physical Data: ^{1}H-NMR [79, 80], ^{13}C-NMR [79, 80], FAB-MS [79, 80].

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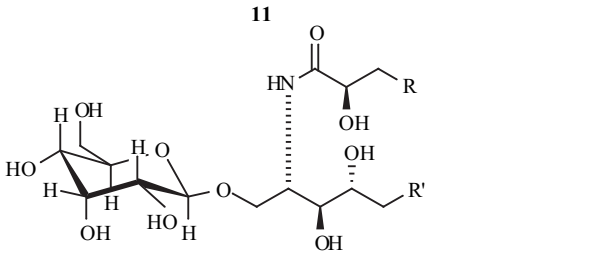

Occurence: *Euphorbia biglandulosa* Desf. [81], marine animals [45], fungi [48].
Physical Data: ^{1}H-NMR [81], ^{13}C-NMR [81], FAB-MS [81].

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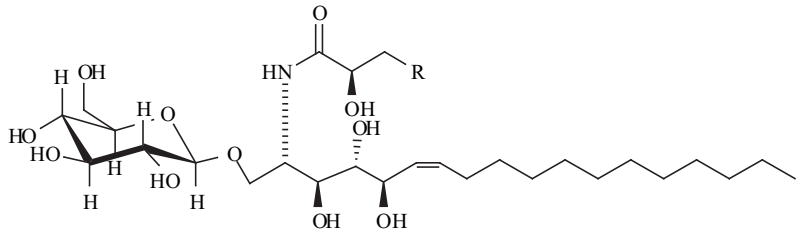

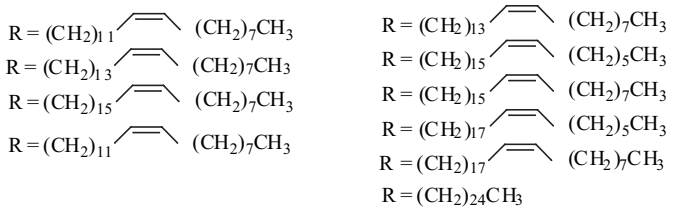

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Occurence: *Euphorbia characias* L. [25], *Euphorbia wulfenii* Hoppe ex Hock [26],marine animals [45], fungi [48].

Physical Data: ^{1}H-NMR [25, 26], ^{13}C-NMR [25, 26], FAB-MS [25, 26].

Fig. (9). Chemical structures of GSLs isolated from Euphorbiaceae.

From *Euphorbia biglandulosa* Desf, *Euphorbia wulfenii* Hoppe ex Koch and *Euphorbia characias* L. has been isolated a series of cerebrosides characterised by a trihydroxylated sphingosine in their structure (**9**-**11**), while from *Euphorbia characias* L. and *Euphorbia wulfenii* Hoppe ex Koch a new class of cerebrosides containing a tetrahydroxylated sphingosine (**12**) has been isolated (Fig. (**9**)).

FAB-MS and FD-MS spectrometry [82-84], ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HETCOR, DQF-COSY analysis and chemical reactions were useful in providing informations for the structure elucidation of GSLs. The ^{1}H-NMR spectra showed signals assignable to –NH proton at δ = 8.5 ppm, to olefinic protons at δ = 5.5 ppm and one anomeric proton (δ = 4.8- 4.9 ppm, d, J = 8.0 Hz) and complex signals due to methylene protons, while the ^{13}C-NMR spectra showed signals due to C-N at δ = 52.3 ppm, to olefinic carbons with Z geometry at 130 ppm, one amide carbon at 175.7 ppm, and the glucosyl carbons at 105.6 ppm and between 62.6-78.6 ppm. 1D and 2D ^{1}H-NMR spectroscopy, DQF-COSY and HMQC indicated that the head group consists of a single glucose residue in the β configuration. The glucose configuration was determined by the characteristic chemical shifts, the spin-spin splitting and the multiplicity of the characteristic resonance of the H-4 proton, as well as by the splittings of the other ring protons.

Fast-atom-bombardment mass spectrometry (FAB-MS) spectra show molecular ion peaks $(M + Na)^{+}$ and fragments at $(M - 179)^{+}$ and $(M + Na - FA)^{+}$. The analysis of the above data indicates that generally $C_{26:0}$, $C_{27:0}$, $C_{30:0}$ are the major saturated hydroxylated fatty acids, while $C_{24:1}$, $C_{25:1}$, $C_{26:1}$, $C_{28:1}$ are the major unsaturated hydroxylated fatty acids.

For determining of the structure and the location of the double bonds in the long-chain parts, the isolated cerebrosides were methanolized, according to the Gover-Sweely method [85], with methanolic hydrochloric acid to yield fatty acid methyl esters (FAMs), a long chain base (LCB) and methyl-D-glucopyranosid (Fig. (**10**)).

The FAMs obtained from the methanolysis of the cerebrosides exhibit ^{13}C-NMR signals at about 175.9, 129.9, 70.5, 51.6 ppm expected for monounsaturated fatty acid methyl esters. The resonance at about 27.2 ppm confirms the Z geometry of the double bond in the long-chain fatty acids.

The position of the double bond in the monounsaturated fatty acid methyl esters was determined by EI-MS analysis of the corresponding dimethyl disulfide (DMDS) derivatives [86]. The characteristic fragment at m/z= 173, obtained from the cleavage between the sulphide carbons, indicates the position of the double bond (Fig. (**11**)).

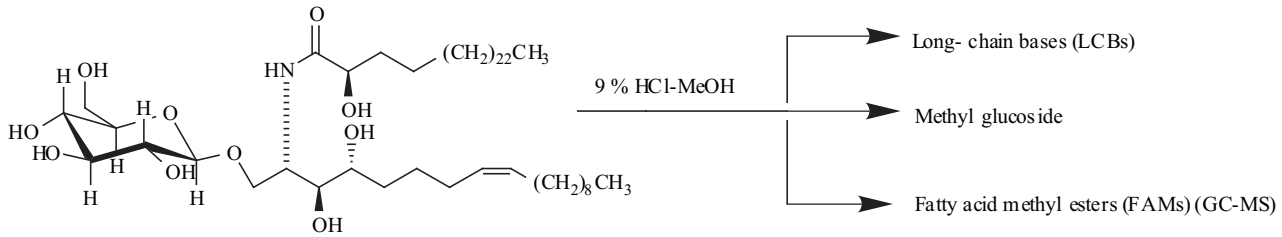

Fig. (10). Methanolysis of a cerebroside.

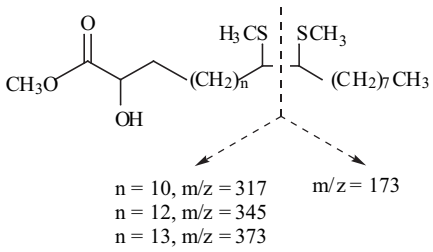

Fig. (11). Principal fragments in EI-MS spectra of some FAM-DMDS derivatives of cerebrosides.

7. BIOLOGICAL ACTIVITIES OF GLYCOSPHINGOLIPIDS

Glycosphingolipids are ubiquitous components of the membranes of all eukaryotic cells and are particularly abundant in plasma membranes. In animals, they play important roles in general membrane function, cell-to-cell contact, cell recognition, and regulation of cell growth, differentiation, and apoptosis [76-78]. Sphingolipids modulate transmembrane signal transduction via their effects on protein kinases associated with growth factor receptors and on protein kinase C, thereby regulating cell proliferation and inducing cell differentiation and apoptosis [87, 88]. The discovery that breakdown products of cellular sphingolipids are biologically active has generated interest in the role of these molecules in cell physiology and pathology. Sphingolipid breakdown products, such as sphingosine, inhibit protein kinase C (PKC), a pivotal enzyme in cell regulation and signal transduction. Sphingolipids affect significant cellular responses and exhibit antitumor promoter activities in various mammalian cells. These molecules may function as endogenous modulators of cell function and possibly as second messengers [89].

Recently, it was found that α-glucosylceramides as well as α-galactosylceramides markedly stimulate proliferative response of spleen cells, but their corresponding β-anomers have little stimulatory effects [90]. In a recent investigation the *in vitro* and *in vivo* natural killer (NK) cell activity enhancing effects of α- and β-galactosylceramides and α- and β-glucosylceramides were examined [91]. The results indicated that the α-types show stronger enhancing effects than β-types, with the α-galactosylceramides possessing the most potent activity.

Furthermore, an α-galactosylceramide, KRN-7000, activates the immune system via the enhancement of the antigen-presenting cell (APC) function of dendritic cells (DC), which play a critical role as professional APCs in the primary immune response [92, 93] (Fig. (**12**)).

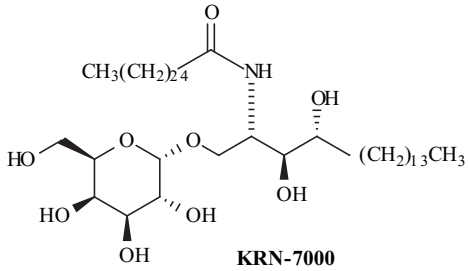

Fig. (12). Chemical structure of KRN-7000.

The antimetastatic effect of the α-galactosylceramide is mediated by NK1.1(+)T (NKT) cells; however, the mechanisms behind this process are poorly defined. Although it has been shown to involve NK cells and interferon-gamma (IFN-gamma) production, the way these factors collaborate to mediate effective tumour rejection and the importance of other factors characteristic of NKT cell and NK cell activation are unknown [94, 95].

Besides, it has been shown that galactosylceramide is an alternative receptor allowing human immunodeficiency virus (HIV)-1 entry into CD4-negative cells of neural and colonic origin. Several lines of evidence suggest that this glycosphingolipid recognises the V3 region of HIV-1 surface envelope glycoprotein gp120 [96]. Since the V3 loop plays a key role in the fusion process driven by HIV-1, various soluble analogues of galactosylceramide were also prepared with the aim to develop a new class of anti-HIV-1 agents that could neutralise HIV-1 infection through masking of the V3 loop. This investigation allowed a study of the relationships between the structures and the bioactivities of galactosylceramide analogues.

Cerebrosides have also been investigated biochemically in connection with lipidoses such as Gaucher's disease [97], a lysosomal storage disorder characterised by a deficiency of the enzyme acid β-glucosidase.

New bioactive cerebrosides have been isolated from *Arisaema amurense* and from *Lycium chinense* which displayed significant antihepatotoxic activity [98, 99]. Recently, a mixture of cerebrosides was purified from Phytolaccae Radix (Phytolaccaceae) and characterised as 1-O-β-D-glucopyranosides of phytosphingosine type ceramides with a common long chain base (2S, 3S, 4R, 8Z)-2-amino-8-octadecene-1,3,4-triol and fatty acids. The cerebrosides isolated inhibited the cyclooxygenase-2 dependent phase of prostaglandin D2 generation in bone marrow-derived mast cells in a concentration dependent manner with an IC50 of 6.2 μg / ml [100].

8. CONCLUSIONS

The Euphorbiaceae have proven to be a rich source of compounds that might be useful for the development of new pharmaceutical agents. These compounds range in structural types from macrocyclic diterpenes, some of which have anti-tumour activity, to the tigliane diterpenes which are well known tumour-promoting phorbol esters of *Croton* oil to the glyceroglycolipids with anti-inflammatory activity, glycosphingolipids with interesting biological properties, sterols and tannins.

Recent studies conducted for the considerable interest and importance of discovering new biologically active compounds from Euphorbiaceae have centred upon the isolation and structure elucidation of complex mixtures of glyceroglycolipids and glycosphingolipids. In this context, nuclear magnetic resonance and mass spectrometry studies have been very useful in gaining new informations about structural elucidation and besides, a number of novel glycosphingolipids have been reported to possess interesting biological properties (DNA polymerase α-inhibitory , antiviral, antifungal, cytotoxic, antihepatotoxic activity).

Important applications have been registered in the design of synthetic analogues of galactosylceramides which have been shown to possess immunostimulatory and antitumor activity and the sulphated analogues have been shown to be active against HIV-1, most likely by binding to the viral envelope protein gp120. These findings have promoted an increasing interest for these compounds for biological investigation and future work will involve the elucidation of their mechanism of action and the characterisation of their receptor sites on cell membrane surface.

ABBREVIATIONS

GGLs	=	Glyceroglycolipids
GSLs	=	Glycosphingolipids
LCB	=	Long-chain base
TLC	=	Thin-layer chromatography
GC	=	Gas chromatography
MS	=	Mass spectrometry
GC-MS	=	Gas chromatography-mass spectrometry
FAB-MS	=	Fast-atom-bombardment mass spectrometry
FD-MS	=	Field desorption mass spectrometry
EI-MS	=	Electron-impact mass spectrometry
MGDG	=	Monogalactosyldiacylglycerol
DGDG	=	Digalactosyldiacylglycerol
MGMG	=	Monogalactosylmonoacylglycerol
1H-NMR	=	Proton nuclear magnetic resonance
13C-NMR	=	Carbon-13 nuclear magnetic resonance
DQF-COSY	=	Double quantum filtered-correlated spectroscopy
HMQC	=	Heteronuclear multiple-quantum coherence
HETCOR	=	Heteronuclear correlation
EBV-EA	=	Epstein-Barr virus early antigen
TPA	=	12-O-Tetradecanoylphorbol-13-acetate
FAMs	=	Fatty acid methyl esters
DMDS	=	Dimethyl disulfide derivatives
PKC	=	Protein kinase C
APC	=	Antigen-presenting cell
DC	=	Dendritic cells
NK	=	Natural Killer cell
IFN-gamma	=	Interferon-gamma
HIV-1	=	Human immunodeficiency virus-1
GP120	=	Glycoprotein 120

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