Immunomodulatory Triterpenoids from the Oleogum Resin of *Boswellia carterii* Birdwood

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The immunomodulatory bioassay-guided fractionation of the oleogum resin of frankincense (*Boswellia carterii* Bird wood) resulted in the isolation and identification of 9 compounds; palmitic acid and eight triterpenoids belonging to lupane, ursane, oleanane, and tirucallane skeleta were isolated form the resin. These triterpenoids are lupeol, β-boswellic acid, 11-keto-β-boswellic acid, acetyl β-boswellic acid, acetyl 11-keto-β-boswellic acid, acetyl-α-boswellic acid, 3-oxo-tirucallic acid, and 3-hydroxy-tirucallic acid. The structures of the isolated compounds were deduced based on spectroscopic evidences. The lymphocyte transformation assay of the isolated compounds proved that the total extract retained more activity than that of any of the purified compounds.

Key words: Boswellia carterii, Triterpenoids, Immunomodulation

Introduction

Frankincense oleogum resin is obtained by incision of the bark of several species of *Boswellia*, Burseraceae. It is known as Olibanum, Luban Dakar, Bakhor or Kendar (In Arabic), and Salai Guggal (In Ayurvedic medicine) (Wallis, 1967; Evans, 1996). The plant is native to India, Arabian Peninsula (Yemen, Sultanate of Oman), Red Sea region of North-East Africa (Somalia, Eritrea) (Dietrich, 1900; Leung and Foster, 1996; Maupetit, 1984; Tschrich and Stock,1935). *Boswellia* resin and its individual components has shown various biological activities; including anti-inflammatory activity (Sharma *et al.*, 1989); leukotriene biosynthesis-inhibitory activity (Ammon *et al.*, 1991; Gupta *et al.*, 2001); and anti-tumor activity (Shao *et al.*, 1998). Frankincense oleo gum resin is a complex mixture containing a series of mono-, sesqui-, di-, and triterpenoids (El-Khadem *et al.*, 1972; Pradhy and Bhattacharyya, 1978). Both the alcoholic extract of the oleogum resin and boswellic acids (a mixture of triterpenoids obtained from the oleogum resin *Boswellia serrata*), influenced both cellular and humoral immune responses in rats and mice (Sharma *et al.*, 1988 and 1996). The defatted alcoholic extract of *Boswellia serrata* caused almost total inhibition of the classical complement pathway of the immune system (Wagner, 1989) while β-boswellic acid demonstrated a marked inhibitory effect on both classical and alternate complement systems (Knaus and Wagner, 1996). The extracts of *Boswellia serrata* gum resin and its constituents, the boswellic acids (BAs), activated the mitogen-activated protein kinase (MAPK) p42 and (MAPK) p38 in isolated human polymorphonuclear leukocytes (PMNLs) (Altmann *et al.*, 2002). In view of such activities of the oleogum resin and its components, we initiated a bioassay-guided fractionation to monitor the immunomodulatory activity of the frankincense resin obtained from *Boswellia carterii* Birdwood.

Results and Discussion

The isolated compounds were separated adopting bioassay-guided fractionation from the oleogum resin of *Boswellia carterii* Birdwood. Lymphocyte proliferation (mitogenesis) assay involves the study of a specific immune response. The assay investigates the mitogenic effect of the crude drug on T-lymphocyte proliferation. Anti-proliferative activity on T-lymphocyte culture indicates immunosuppression while promotion of T-lymphocyte proliferative response means immunostimulation (Sairam *et al.*, 1997). The results of the assay expressed in terms of the % lymphocyte transformation are shown in Table I. The total alcoholic ex-

tract of frankincense oleogum resin and the volatile oil have shown a significant immunostimulant action on T-lymphocytes (90% lymphocyte proliferation) that is comparable to the standard immunostimulants *viz. Echinaceae purpurea* extract and (*S*)-2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]thiazole hydrochloride (levamisole). The individual components of the resin elicited mild to moderate immunostimulant activity. LTA proved that the total extract exhibited a better activity than that of any of the isolated pure compounds. This may be partly attributed to the synergistic effect among the different components of the total extract.

The spectral data of compound **1** indicated that it is palmitic acid, based on the EI/MS molecular ion peak at *m/z* 256 confirming the molecular formula of $C_{16}H_{32}O_2$. The IR spectrum showed absorption bands at 1701 cm^{-1} (C=O), and 3321 cm^{-1} (O–H). The ^{13}C-NMR spectrum showed the presence of 16 signals, the multiplicities of which were determined using APT experiment, that revealed the presence of one methyl signal at δ 14.2 that could be assigned to a terminal methyl (C-16), and fourteen methylene signals, one quaternary carbon signal. The ^{13}C-NMR signal at δ 22.7, 24.7, 31.9, and 34.0 could be assigned to methylene carbons (C-15), (C-3), (C-14), and (C-2), respectively. The remaining methylene carbon signals from 29.1 to 29.7 ppm could be assigned to carbons from (C-4) to (C-13). The remaining quaternary carbon signal at δ 179.6 could be assigned to the carboxylic acid function (C-1). ^{1}H-NMR of compound **1** showed the following signals, one methyl triplet at δ 0.83 (3H, *t*) which could be assigned to terminal methyl group (H-16). A multiplet at δ 1.23 ppm that could be assigned to protons (H-4) to (H-15). A multiplet at δ 1.6 ppm (2H, *m*) which could be assigned to methylene protons (H-3). A triplet at δ 2.3 ppm (2H, *t*) which could be assigned to methylene protons (H-2). The EI/MS mass spectrum confirmed the above assignments showing a base peak at *m/z* 60 that resulted from Mclafferty rearrangement (Silverstein *et al.*, 1991). The rest of the EI/MS spectrum consists of a homologous series of hydrocarbon clusters at intervals 14 mass units ($-CH_2$ group) due to gradual breakdown of the hydrocarbon side chain. Other characteristic fragments are those of $[CH_3(CH_2)_4]^+$ at *m/z* 71, and $[(CH_2)_2COOH]^+$ at *m/z* 73. The above mentioned data, co-chromatography with a series of fatty acids and previously reported literature data (Pouchert and Behnke, 1993) made us to figure out compound **1** to be the long-chain fatty acid, *n-hexadecanoic acid* (*palmitic acid*). To the best of our knowledge, this is the first report of isolation of palmitic acid from the genus *Boswellia*.

The ^{13}C-NMR spectrum of compound **2** (Table II), showed the presence of 30 carbons, the multiplicities of which were determined using an APT experiment, that revealed the presence of seven methyl, six methine, eleven methylene, and six quaternary carbon signals. Compound **2** showed an EI/MS molecular ion peak at *m/z* 426 suggesting a molecular formula of $C_{30}H_{50}O$. More evidences came from the ^{13}C-NMR signals at δ 150.9 (C-20), and 109.5 (C-29), as well as ^{1}H-NMR signals for two vinylic protons at 4.57, and

Table I. Results of lymphocyte transformation (proliferation) assay.

Extract, or compound	Concentration	% Transformation	TC_{50}*
Total alcoholic extract	1.00 mg/ml	90	0.55 mg/ml
Gum	1.00 mg/ml	20	2.50 mg/ml
Volatile oil	1.00 mg/ml	90	0.55 mg/ml
1 (*palmitic acid*)	0.0039 μM	10	0.0195 μM
2 (*lupeol*)	0.0023 μM	40	0.0029 μM
3 (*acetyl-β-boswellic acid*)	0.0020 μM	20	0.0050 μM
4 (*acetyl-α-boswellic acid*)	0.0020 μM	35	0.0029 μM
5 (*3-oxo-tirucallic acid*)	0.0022 μM	10	0.0110 μM
6 (*acetyl-11-keto-β-boswellic acid*)	0.0019 μM	45	0.0022 μM
7 (*β-boswellic acid*)	0.0022 μM	50	0.0022 μM
8 (*3-hydroxy-tirucallic acid*)	0.0022 μM	20	0.0055 μM
9 (*11-keto-β-boswellic acid*)	0.0021 μM	25	0.0043 μM

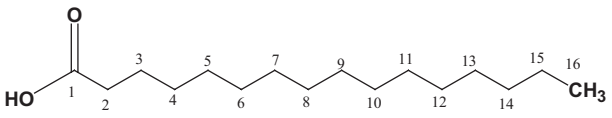
* Concentration showing 50% lymphocyte transformation.

Table II. ^{13}C-NMR spectral data of the isolated triterpenoids (**2–9**).

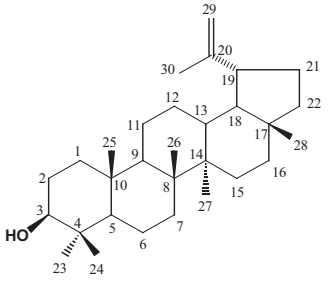
C #	Lupane derivative	Ursane derivatives				Oleanane derivative	Tirucallane derivatives	
	2	**3**	**6**	**7**	**9**	**4**	**5**	**8**
1	37.8	34.9	35.0	34.3	34.3	34.9	35.7	35.7
2	27.7	24.0	23.9	26.6	26.6	24.0	34.6	27.9
3	76.5	73.6	73.6	71.8	70.7	73.6	216.9	79.4
4	40.3	47.1	46.7	47.6	47.5	47.1	47.3	39.3
5	50.5	51.0	50.8	49.6	49.2	51.0	51.6	51.3
6	18.5	19.9	19.2	20.1	19.0	19.9	20.2	19.3
7	34.4	33.5	31.3	33.5	31.3	33.5	28.8	29.2
8	40.8	40.2	43.9	39.8	44.1	40.2	134.4	134.4
9	49.3	47.2	60.7	47.3	60.8	47.2	132.2	132.6
10	36.9	37.2	37.6	37.7	37.7	37.5	37.2	37.7
11	21.1	23.8	199.6	23.8	199.8	23.8	25.9	26.3
12	24.2	124.9	130.8	124.9	130.6	122.2	32.5	32.8
13	38.3	139.9	165.3	139.8	165.4	145.5	43.9	44.3
14	42.5	40.4	45.3	39.9	45.3	42.3	49.7	50.0
15	25.9	28.5	33.2	28.5	33.3	26.5	29.4	29.7
16	35.9	26.9	27.6	26.9	27.6	27.4	27.5	28.2
17	43.3	33.7	34.2	33.5	34.1	33.2	47.0	47.3
18	48.6	59.6	59.4	59.6	59.4	47.2	21.2	20.4
19	48.3	40.1	39.7	40.1	39.7	47.8	15.9	15.9
20	150.9	40.0	39.6	40.0	39.7	31.5	47.6	48.1
21	29.5	31.7	27.9	31.7	27.7	34.2	182.4	183.1
22	33.5	41.9	41.3	41.9	41.3	37.9	21.4	21.9
23	31.7	24.1	24.3	24.6	24.7	24.1	26.9	27.3
24	16.3	183.1	182.2	183.6	182.9	183.1	123.5	124.0
25	22.4	13.7	13.6	13.7	13.6	13.5	132.7	133.7
26	19.6	17.3	18.8	17.3	18.7	17.2	25.7	24.8
27	14.5	21.7	20.9	21.8	20.9	26.3	19.7	18.1
28	28.5	23.7	21.5	23.6	21.5	28.9	24.3	28.4
29	109.5	17.8	17.8	17.8	17.8	33.7	17.6	16.2
30	30.4	29.2	29.3	29.2	29.3	23.7	26.6	26.1
CH_3CO	–	21.7	21.7	–	–	21.8	–	–
CH_3**C**O	–	170.6	170.5	–	–	170.8	–	–

4.68 ppm (1H, *d*, *J* = 2 each), the ^{13}C-NMR signal for an oxygenated carbinylic carbon signal at 76.5 that was assigned to C-3. All the above evidences; suggested the presence of a lupane triterpene skeleton. The 3-hydroxyl group is β-configurated as reflected by the broad peak half-height width $W_{1/2}$ (12 Hz) of axially oriented H-3 signal at 3.42 ppm (1H, *dd*, *J* = 19.5, 3.3) (Mahato and Kundu, 1994). Such axial orientation of H-3 is corroborated by the presence of the relatively large coupling constant characteristic to H-2, H-3 axial-axial interaction. From the above data, and through comparison with literature data (Reynolds *et al*., 1986); it was concluded that compound **2** is *lup-20(29)-en-3β-ol* (*lupeol*), that has been previously isolated from the closely related species *Boswellia frereana* Birdwood (Fattorusso *et al*., 1985).

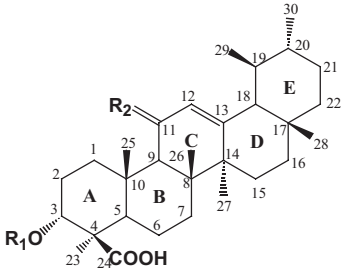
The ^{13}C-NMR spectra of compound **3** (Table II) showed the presence of 32 signals, the multiplicities of which were determined, by DEPT experiment, into seven methine, eight methyl, nine methylene, and eight quaternary carbons. The EI/MS molecular ion peak at *m/z* 498 suggests a molecular formula of $C_{32}H_{50}O_4$ thus giving a double bond equivalent of 8, five of which were assigned to the 5 rings of a pentacyclic triterpene skeleton. Two equivalents were assigned to an acetyl carbonyl (δ 170.5), and a carboxylic acid carbonyl (δ 183.1). The last equivalent was assigned to a double bond that was consistent with that of Δ^{12}-ursane skeleton based on its ^{13}C-NMR resonances, δ 124.9 (C-12), and 139.9 (C-13). The appearance of a vinylic proton at δ 5.30 (H-12) as a broad triplet, appearance of H-18 as a doublet at δ 1.31 (*J* = 13.6), in addition to the appearance of a two methyl groups signals, each as a doublet at δ 0.8, and 0.85 assigned for 29, and 30 positions, respectively, unambiguously confirmed the presence of



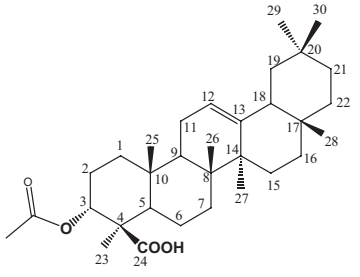
1 (Palmitic acid)



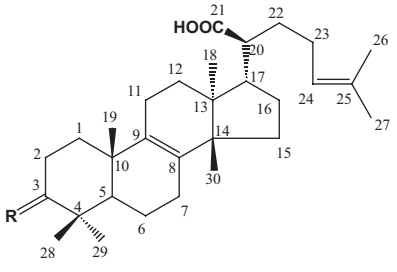
2 (Lupeol)



	R_1	R_2
3 (acetyl β-boswellic acid)	Ac	2 H
6 (acetyl 11-keto-β-boswellic acid)	Ac	O
7 (β-boswellic acid)	H	2 H
9 (11-keto-β-boswellic acid)	H	O



4 (acetyl-α-boswellic acid)



	R
5 (3-oxo-tirucallic acid)	O
8 (3-hydroxy-tirucallic acid)	β-OH, α-H

Fig. 1. The immunomodulatory triterpenoids isolated from the oleogum resin of *Boswellia carterii* Birdwood.

Δ^{12}-ursane skeleton. The location of the carboxylic acid group was found to be at 24-position based on the downfield shift of C-4 resonating at δ 47.6 ppm relative to similar compounds cited in literature (Mahato and Kundu, 1994). The ^{13}C-NMR signal for an oxygenated methine carbon signal at 73.6 was assigned to C-3 on biosynthetic (Kojima and Ogura, 1989) and analogy grounds (Pradhy and Bhattacharyya, 1978), as well as HMQC correlations. The latter revealed crosspeaks between methyl protons (H-23) resonating at δ 1.24 (3H, s) and the oxygenated carbinylic carbon (C-3) at δ 73.6, quaternary carbon (C-4) at δ 47.1, and methine carbon (C-5) at δ 51.0. In addition, HMBC showed strong contour between the carboxyl carbon (C-24) at δ 183.1 and methyl protons (H_3-23) at δ 1.24. These established connectivities supported the substitution pattern of ring A. The interaction crosspeaks between the vinylic proton (H-12) at δ 5.15 (1H, *t*), and methine carbon (C-9) at δ 47.2, methyl carbon (C-27) at δ 21.7 (weak $^4J_{CH}$ interaction); between proton (H-9) at δ 1.61 (1H, *m*) and both the (C-11) at δ 23.8, and the methyl carbon (C-26) at δ 17.3 confirmed the substitution pattern of rings B, C. The interaction crosspeaks between the methyl protons (H_3-27) at δ 0.90, and the quaternary carbons (C-8) at δ 40.2 ppm, as well as, (C-13) at δ 139.9, and (C-14) at δ 40.4 confirmed the substitution pattern of ring D. The presence of a 3-acetate group is evident from the downfield shift of *ca.* 2–3 ppm of (C-3), the upfield shift of *ca.* 3 ppm of (C-2), and the upfield shift of *ca.* 1 ppm of (C-4) relative to non-acetylated compounds *viz.* **7** and **9** (Mahato and Kundu, 1994). The 3-acetoxyl group was found to be α-configurated, as reflected by the narrow peak half-height width $W_{1/2}$ (5 Hz) of the equatorially-oriented H-3 signal at 5.3 ppm (1H, *t*) (Siddiqui *et al*., 1989). Such equatorial orientation of H-3 is corroborated by the absence of the relatively large coupling constant characteristic to H-2, H-3 axial-axial interaction, and this also was confirmed by the most recent X-ray crystallographic analysis (Rajnikant *et al*., 2001). Assignment of other atoms was made by comparison with other separated compounds, referring to reported compilation data of a variety of similar compounds (Mahato and Kundu, 1994). The above evidences revealed that compound **3** is *acetyl-β-boswellic acid*.

EI/MS of compound **6** showed [M$^+$] peak at *m/z* 512, suggesting the molecular formula; $C_{32}H_{48}O_5$. The analysis of all the spectral data for compound **6** indicated its similarity to compound **3**, but it contains an additional α,β-unsaturated oxo function, an enone system, that was confirmed from IR spectrum where a carbonyl group absorption band was observed at 1658 cm^{-1}, and by the appearance of a ^{13}C-NMR carbonyl signal at 199.6 ppm. The location of this oxo group was concluded to be at 11-position, since H-12 proton signal was downfield shifted from δ 5.3 to 5.54, and appeared as singlet, rather than broad triplet. Furthermore, ^{13}C-NMR signals of (C-9), (C-12), (C-13) were downfield shifted from δ 47.2, 124.9, and 139.9 to δ 60.7, 130.8, and 165.3, respectively. Further structure connectivities, and assignments by HMQC, HMBC, and COSY spectra confirmed that compound **6** is *acetyl-11-keto-β-boswellic acid*.

The spectral data for compound **7** was found to be similar to that of compound **3**, but lacking the 3-acetate group. This was proved through the absence of ^{13}C-NMR signals at δ 21.7, and 170.5, as well as, ^{1}H-NMR signal at δ 2.07, and the appearance of the 2 ppm upfield shifted hydroxylated carbinylic carbon (C-3) signal at δ 71.8, relative to that in compound **3**. EI/MS spectrum of compound **7** showed a molecular ion peak [M]$^+$ at *m/z* 456 suggesting a molecular formula of $C_{30}H_{48}O_3$, confirming the absence of a 3-acetate group. Therefore, compound **7** was concluded to be β-boswellic acid.

EI/MS of compound **9** showed a [M$^+$] peak at *m/z* 470 suggesting a molecular formula of $C_{30}H_{46}O_4$. It was found to be the 11-keto derivative of compound **7**, as deduced from the appearance of carbonyl carbon signal at δ 199.8 (C-11), and based on a similar arguments as mentioned under compound **6**. Therefore, it was confirmed that compound **9** is *11-keto-β-boswellic acid*.

The EI/MS of compound **4** showed a molecular ion peak at *m/z* 498 suggesting a molecular formula of $C_{32}H_{50}O_4$. The spectral data of **4** are more or less similar to that of compound **3**, but with few differences. Firstly, the two ^{1}H-NMR doublets for methyls at 29, and 30 positions are absent, and appeared as singlets at δ 0.87, and 1.11, respectively; referring to the possible presence of an oleanane skeleton. This was corroborated by the upfield shift of C-12 signals to δ 122.2, and down-

field shift of C-13 to δ 145.5 (27). Accordingly, compound **4** has been identified as *acetoxy-olean-12-ene-24β-oic acid* known as *acetyl-α-boswellic acid*.

The analysis of the spectral data of compound **5**, concluded its structure as *3-keto-tirucall-8,24-diene-21-β-oic acid*, known as *3-oxo-tirucallic acid*. The ^{13}C-NMR spectral data of **5** (Table II) showed the presence of 30 carbon signals, the multiplicities of which were determined using an APT experiment, that revealed the presence of seven methyl, ten methylene, four methine, and nine quaternary carbon signals. The EI/MS molecular ion peak at *m/z* 454 suggests a molecular formula of $C_{30}H_{46}O_3$ thus giving a DBE of 8, four of which were assigned to the 4 rings of the tetracyclic tirucallane triterpene skeleton. Two of the remaining 4 double bond equivalents were assigned to 2 double bonds, C_8-C_9 double bond that is evident from the two quaternary ^{13}C-NMR signals at δ 134.4 (C-8), and 132.2 (C-9), and $C_{24}-C_{25}$ double bond reflected by the ^{13}C-NMR signals at δ 123.5 (C-24) and 132.7 (C-25), the two remaining DBEs were assigned to 2 carbonyl groups, the ketonic function (C-3) at δ 216.9, and the carboxyl carbonyl (C-21) at δ 182.4. The carboxylic group (C-21) is linked to (C-20) indicated by the 10–12 ppm downfield shift of that carbon relative to that closely related compounds lacking C-21 carboxyl (Benosman *et al*., 1995; Puripattanavong *et al*., 2000). The ketonic function is definitely (C-3) as revealed by the 7 ppm downfield shift of (C-2) in comparison with similar compounds with hydroxyl group at (C-3) such as compound **7**. Assignment of other atoms was made by comparison with literature data (Benosman *et al*., 1995; Puripattanavong *et al*., 2000). EI/MS fragments corroborated the above assignments showing a molecular ion peak $[M]^+$ at *m/z* 454 corresponding for the molecular formula $C_{30}H_{46}O_3$. The base peak at *m/z* 439 resulted from the loss of one methyl group. Subsequent loss of a water molecule resulted in the peak at *m/z* 421. Fragment at *m/z* 257 resulted from the retro-Diels Alder (RDA) fragmentation characteristic of several triterpenoids (Budzikiewicz *et al*., 1964), followed by decarboxylation. The proposed EI/MS fragmentation pattern of compound **5** is depicted in Fig. 2.

Compound **8** was analyzed for $C_{30}H_{48}O_3$ from EI/MS spectrum. It was found to be the 3-hydroxy analogue of compound **5**. This was confirmed by appearance of ^{13}C-NMR signal at δ 79.4 ppm that was assigned to an oxygenated methine carbo (C-3). The 3-hydroxyl group is β-configurated reflected by the wide peak half-height width $W_{1/2}$ (16 Hz) of the axially oriented H-3 signal at 3.23 ppm (1H, *dd, J* = 11.5 and 4.4) (Siddiqui *et al*., 1989). Such axial orientation of H-3 is corroborated by the relatively large coupling constant characteristic to H-2, H-3 axial-axial interaction. The aforementioned data suggested that compound **8** is *3-hydroxy-tirucall-8,24-diene-21-β-oic acid* known as *3-hydroxy-tirucallic acid*.

In conclusion, eight triterpenoids, in addition to a fatty acid isolated for the first time from the resin, were isolated from the oleogum resin of *Boswellia carterii* Birdwood. Compounds **3–9** have been previously isolated from the oleogum resin of *Boswellia carterii* Birdwood (El-Khadem *et al*., 1972) and *Boswellia serrata* Roxb. (Pradhy and Bhattacharyya, 1978; Mahajan *et al*., 1995). All the isolated compounds exhibited immunostimulant activity as reflected by a lymphocyte transformation assay. Interestingly, it was found that the immunostimulant activity of the total extract (90% Lymphocyte transformation) is much greater than that of the individual components; accordingly it is advisable to use the total extract of the oleogum resin in herbal preparations intended for immunostimulation. These results suggested that frankincense could be a promising herbal immunostimulant that may be used in various immune disorders.

Materials and Methods

Materials for chromatographic study

Silica gel $G60F_{254}$ for TLC (E. Merck, Germany), silica gel for column chromatography (70–230 mesh) (E. Merck, Germany), reversed phase silica (RP-C_{18}) for column chromatography (E. Merck, Germany), precoated silica gel GF_{254} plates, aluminium and plastic sheets for TLC (E. Merck and Macherey-Nagel, Germany), precoated reversed phase silica plates for TLC (E. Merck, Germany).

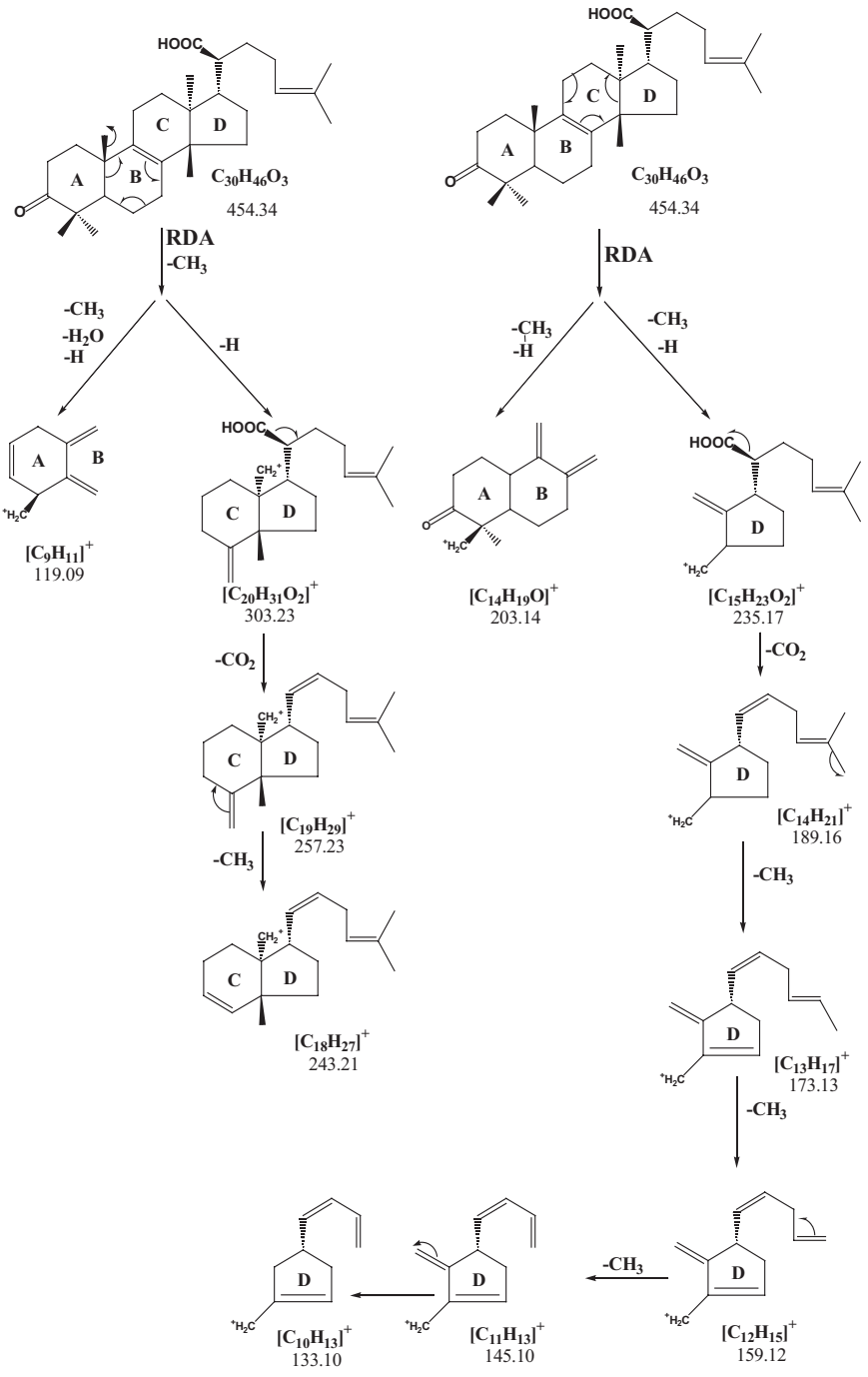


Fig. 2. Proposed EI/MS fragmentation pattern for compound **5**.

Reagents for lymphocyte transformation assay

Heparinized peripheral venous blood was obtained from healthy volunteers from the blood bank of Mansoura University Hospital; Ficoll/Hypaque obtained from Amersham Pharmacia, Uppsala, Sweden; phytohaemagglutinin (PHA) obtained from Difco, Detroit, MI, USA; Concanvalin A (ConA) obtained from Merck, Germany; Hank's balanced salt solution (HBSS); foetal calf serum (FCS); glutamine; HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid)-buffer and RPMI-1640 medium obtained from Gibco BRL, Life Technologies, Pailsey, Scotland; crystalline penicillin G and streptomycin obtained from El-Nile Pharmaceutical Co., Cairo, Egypt. *Echinacea Purpurea* extract (Immulone®) obtained from Sekem Pharmaceutial Co., Cairo, Egypt. Levamisole (Ketrax®) obtained from Elkahira Pharmaceutial Co., Cairo, Egypt (manufactured under license from AstraZeneca, Wilmington, Delaware, USA). Cyclophosphamide (Endoxan®) obtained from ASTA Medica AG, Frankfurt, Germany. Cyclosporin (Sandimmune Neoral®) obtained from Novartis Pharma, Switzerland.

General instrumentation

UV spectra were recorded in MeOH using a Shimadzu 1601-PC UV/Visible spectrophotometer, IR spectra were recorded on Buck model 500 Infra red spectrophotometer, NMR spectra were recorded using Bruker AM-300 spectrometer, Drx-400, and Varian Mercury-300 spectrometer using $CDCl_3$, and DMSO-d_6 as solvents and TMS as internal standard. Chemical shifts (δ) are expressed in ppm. DEPT, APT, COSY, HMQC, and HMBC experiments were conducted under standard conditions. EI/MS were performed using a Finningan Mat SSQ 7000 mass spectrometer with a Digital DEC 3000 workstation.

Plant material

The oleogum resin of *Boswellia carterii* Birdwood (Bursearceae) was purchased from the local herbal stores in Mansoura on March 1999. It was authenticated by comparison with a genuine sample kept in the Drug Museum of Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

Extraction

The finely ground oleogum resin (500 grams) was extracted with methylene chloride (5 liters). The extract was concentrated under reduced pressure to yield 330 grams of semisolid oleoresin.

Isolation and identification

The total extract (15 grams) was applied onto the top of a glass column (120 × 5 cm) previously packed with silica gel (300 g) in petroleum ether (b.p. 60–80 °C). The extract was gradiently eluted with petroleum ether containing increasing proportions of ethyl acetate. The effluent was collected in 250-ml fractions. Each fraction was concentrated, *in vacuo*. Fractions (51–70) gave white waxy crystals (400 mg) which were further purified by column chromatography (60 × 1.5 cm) using (20 g) silica gel. Elution was adopted using petroleum ether (b.p. 60–80 °C) containing gradually increasing proportions of ethyl acetate. Sub-fractions (8–10) afforded 329 mg of **1**. Fractions (71–100) were subjected to reversed phase Rp-C_{18} flash column chromatography using isocratic elution with methanol–water (8:2 v/v) to afford **2** (11.9 mg). Factions (101–130) were further purified by preparative TLC using petroleum ether–ethyl acetate (9:1 v/v) as solvent system affording 30 mg of **3**. Fractions (131–160) were further purified by reversed phase Rp-C_{18} flash column chromatography using methanol–water (95:5 v/v) to afford **4** (86 mg). Upon crystallization, fractions (161–190) gave **5** (14 mg). Fractions (191–220), (221–250), and (251–280) were separately purified by reversed phase Rp-C_{18} flash column chromatography adopting isocratic elution with methanol–water (9:1 v/v), to afford **6** (52 mg), **7** (130 mg), and **8** (12.1 mg), respectively. Fractions (311–340) were purified in the same manner but using methanol–water (80:20 v/v) as solvent system to afford **9** (28 mg).

Compound **1***: hexadecanoic acid; palmitic acid*

$C_{16}H_{32}O_2$; white waxy crystals; m.p. 57–62 °C; UV λ_{max} (MeOH) nm, 202.5; IR (KBr) υ_{max} cm^{-1}: 723, 940, 1297, 1414, 1464, 1701 (C=O), 2851, 2955, and 3321 (O–H); EI/MS *m/z* (rel. int.): 256 (56.9) $[M]^+$, 228 (15.5), 213 (19.4), 185 (22.7), 157 (17.5), 129 (58.9), 97 (39.2), 83 (44.6), 73 (94.7), and 60

$[C_2H_4O_2]^+$ (100); ^{1}H-NMR (300 MHz, $CDCl_3$, δ in ppm, *J* = Hz): 0.83 (3 H, *t*, H-16), 1.23 (24 H, *m*, H-4–15), 1.58 (2 H, *m*, H-3), and 2.30 (2 H, *t*, H-2); ^{13}C-NMR (75 MHz, $CDCl_3$, δ in ppm): 14.2 (C-16), 22.7 (C-15), 24.7 (C-3), 29.1 (C-4), 29.3 (C-5), 29.4 (C-6), 29.6 (C-7), 29.7 (C-8 to C-11), 31.9 (C-14), 34.0 (C-2), and 179.6 (C-1).

Compound **2***: lup-20(29)-en-3β-ol; lupeol*

$C_{30}H_{50}O$; short fine colorless needles; m.p. 214–217 °C; UV λ_{max} nm (MeOH), 203.5; IR υ_{max} cm^{-1}: 1534, 1618 (C=C), 2366, 2970, and 3490 cm^{-1} (O–H); EI/MS *m/z* (rel. int.): 426 (61.7) $[M]^+$, 411 (32.1) $[M-CH_3]^+$, 393 (15.7) $[M-CH_3-H_2O]^+$, 316 (13.0), 274 (12.1), 229 (10.6), 218 (100), 189 (90.6), 175 (29.4), 135 (54.1), 95 (61.6), and 69 (57.9); ^{1}H-NMR (300 MHz, $CDCl_3$, δ in ppm, *J* = Hz): 0.79 (3H, *s*, H-25), 0.94 (3H, *s*, H-27), 0.96 (3H, *s*, H-24), 1.04 (3H, *s*, H-26), 1.29 (3H, *s*, H-28), 1.33 (3H, *s*, H-23), 1.68 (3H, *s*, H-30), and 3.42 (1H, *dd*, 19.5, 3.3, H-3), 4.57, 4.68 (2H, *d*, 2, 2, H-29). The ^{13}C-NMR data of compounds **2–9** are shown in Table I.

Compound **3***: 3α-acetoxy-urs-12-ene-24β-oic acid; acetyl-β-boswellic acid*

$C_{32}H_{50}O_4$; colorless needles; m.p. 250–252 °C; UV λ_{max} (MeOH) nm, 206; IR (KBr) υ_{max} cm^{-1}: 1275, 1376, 1456, 1618 (C=C), 1702 (–COOH), 1727 (CH_3CO–), 2965, and 3400 (O–H); EI/MS *m/z* (rel. int.): 498 (4.5) $[M]^+$, 438 (0.7), 394 (0.42), 379 (0.17), 255 (3.0), 203 (17.3), 175 (5.1), and 119 (6.0); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm, *J* = Hz): 0.80 (3H, *d*, 12.5, H-29), 0.85 (3H, *d*, 13, H-30), 0.90 (3H, *s*, H-25), 0.90 (3H, *s*, H-27),1.02 (2H, *dd*, 16.0, 2.0, H-16), 1.04 (3H, *s*, H-26), 1.12 (3H, *s*, H-28), 1.24 (3H, *s*, H-23), 1.28 (2H, *dd*, 10.2, 2.0, H-21), 1.31 (1H, *d*, 13.6, H-18), 1.49 (1H, *dd*, 13.0, 1.3, H-5), 2.09 (3H, *s*, H-3, Ac), 5.15 (1H, *br t*, H-3), and 5.30 (1H, *br t*, H-12).

Compound **4***: 3α-acetoxy-olean-12-ene-24β-oic acid; acetyl-α-boswellic acid*

$C_{32}H_{50}O_4$; colorless needles; m.p. 247–250 °C; UV λ_{max} (MeOH) nm, 204.5; IR (KBr) υ_{max} cm^{-1}: 1275, 1376, 1456, 1618 (C=C), 1708 (–COOH), 1727 (CH_3CO–), 2965, and 3450 (O–H); EI/MS *m/z* (rel. int.): 498 (0.55) $[M]^+$, 423 (0.59), 343 (0.03), 280 (0.16), 255 (2.57), 218 (100), 203 (22.5), 189 (8.9), 133 (6.4), and 43 (3.6); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm, *J* = Hz): 0.80 (3H, *s*, H-27), 0.80 (3H, *s*, H-28), 0.87 (3H, *s*, H-29), 0.90 (3H, *s*, H-25), 1.04 (3H, *s*, H-26), 1.11 (3H, *s*, H-30), 1.49 (1H, *dd*, 13.0, 1.3, H-5), 2.09 (3H, *s*, H-3 Ac), 5.14 (1H, *br t*, H-3), and 5.20 (1H, *t*, H-12).

Compound **5***: 3-keto-tirucall-8, 24-diene-21-β-oic acid; 3-oxotirucallic acid*

$C_{30}H_{46}O_3$; colorless needles; m.p. 263–265 °C; UV λ_{max} (MeOH) nm, 206; IR (KBr) υ_{max} cm^{-1}: 1193, 1347, 1420, 1448, 1620 (C=C), 1708 (C=O), 1710 (–COOH), 2954, and 3450 (O–H); EI/MS *m/z* (rel. int.): 454 (64.1) $[M]^+$, 439 (100) $[M-CH_3]^+$, 421 (14.3), 393 (17.2), 311 (4.6), 297 (23.7), 243 (5.8), 173 (7.3), 159 (10.7), 119 (14.0), and 95 (12.6); ^{1}H-NMR (300 MHz, $CDCl_3$, δ in ppm, *J* = Hz): 0.83 (3H, *s*, H-18), 0.91 (3H, *s,* H-19), 1.04 (3H, *s,* H-30), 1.05 (3H, *s,* H-28), 1.09 (3H, *s,* H-29), 1.29 (1H, *dd*, 9.0, 6.3, H-5), 1.54 (2H, *dd*, 9.0, 6.3, H-16), 1.59 (3H, *s,* H-27), 1.68 (3H, *s,* H-26), 2.3 (1H, *dd*, 12.5, 5.1, H-17), and 2.5 (2H, *ddd*, 15.5, 10.8, 4.0, H-2).

Compound **6***: 3α-acetoxy-urs-12-ene-11-keto-24β-oic acid; acetyl-11-keto-β-boswellic acid (AKBA)*

$C_{32}H_{48}O_5$; colorless needles; m.p. 274–276 °C; UV λ_{max} (MeOH) nm, 250; IR (KBr) υ_{max} cm^{-1}: 1238, 1274, 1379, 1457, 1622 (C=C), 1658 (α,β-unsaturated C=O), 1706 (–COOH), 1728 (CH_3COO–), 2864, 2970, and 3350 (O–H); EI/MS *m/z* (rel. int.): 512 (0.01) $[M]^+$, 408 (14.5), 393 (5.9), 353 (2.7), 273 (41.8), 232 (77.7), 189 (12.1), 175 (14.3), 161 (27.9), 119 (35.1), 105 (49.5), 91 (37.5), 55 (49.8), and 43 (100); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm, *J* = Hz): 0.78 (3H, *d*, 6.3, H-29), 0.80 (3H, *d*, 7.4, H-30), 0.93 (3H, *s*, H-28), 1.01 (2H, *dd*, 11.8, 3.0, H-21), 1.12 (3H, *s*, H-25), 1.17 (3H, *s*, H-26), 1.21 (3H, *s*, H-23), 1.33 (3H, *s*, H-27), 1.37 (1H, *d*, 12.3, H-18), 1.38 (1H, *dd*, 12.0, 2.0, H-5), 2.07 (3H, *s*, H-3 Ac), 2.39 (1H, *s*, H-9), 5.28 (1H, *t*, H-3), and 5.54 (1H, *s*, H-12).

Compound **7***: 3α-hydroxy-urs-12-ene-24β-oic acid; β-boswellic acid*

$C_{32}H_{48}O_5$; colorless needles; m.p. 226–228 °C; UV λ_{max} nm (MeOH), 204; IR (KBr) υ_{max} cm^{-1}:

1367, 1456, 1629 (C=C), 1708 (–COOH), 2918, and 3400 (O–H); EI/MS *m/z* (rel. int.): 456 (5.2) $[M]^+$, 441 (1.6) $[M-CH_3]^+$, 379 (0.1), 326 (0.2), 293 (0.1), 238 (13.2), 218 (100), 203 (24.8), 159 (8.3), 133 (15.5), 119 (23.0), 95 (18.7), 69 (18.0), and 57 (15.9); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm, *J* = Hz): 0.78 (3H, *d*, 5.67, H-29), 0.79 (3H, *d*, 3.25, H-30), 0.81 (2H, *d*, 12.3, H-15), 0.89 (3H, *s*, H-25), 0.91 (3H, *s*, H-27), 1.02 (2H, *dd*, 16.0, 2.0, H-16), 1.03 (3H, *s*, H-26), 1.33, (1H, *d*, 8.9, H-18), 1.34 (3H, *s*, H-23), 1.48 (1H, *dd*, 11.8, 3.1, H-5), 1.68 (2H, *dd*, 14.1, 3.0, H-6), 2.22 (2H, *dd*, 14.0, 4.0, H-2), 4.08 (1H, *t*, H-3), and 5.14 (1H, *t*, H-12).

Compound **8***: 3β-hydroxy-tirucall-8,24-diene-21-β-oic acid; 3β-hydroxytirucallic acid*

$C_{30}H_{48}O_3$; colorless long very fine needles; m.p. 258–260 °C; UV λ_{max} nm (MeOH), 206; IR (KBr) υ_{max} cm^{-1}: 1466, 1622 (C=C), 1702 (–COOH), 2942, and 3416 (O–H); EI/MS *m/z* (rel. int.): 456 (67.9) $[M]^+$, 441 (85.6) $[M-CH_3]^+$, 423 (93.3), 395 (10.9), 341 (7.4), 299 (12.9), 281 (32.9), 187 (70.9), 119 (65.2), and 82 (100); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm, *J* = Hz): 0.74 (3H, *s*, H-19), 0.82 (3H, *s*, H-28), 0.87 (3H, *s*, H-30), 0.93 (3H, *s*, H-18), 0.99 (3H, *s*, H-29), 1.11 (1H, *dd*, 11.8, 2.0, H-5), 1.2 (2H, *dd*, 9.9, 3.1, H-1), 1.37 (2H, *dd*, 12.2, 6.8, H-16), 1.53 (2H, *dd*, 14.4, 7.3, H-7), 1.58 (3H, *s*, H-27), 1.67 (3H, *s*, H-26), 1.75 (2H, *dd*, 12.8, 3.0, H-12), 2.27 (1H, *dd*, 12.1, 3.5, H-17), and 3.23 (1H, *dd*, 11.5, 4.4, H-3).

Compound **9***: 3α-hydroxy-urs-12-ene-11-keto-24β-oic acid; 11-keto-β-boswellic acid*

$C_{32}H_{48}O_5$; colorless needles; m.p. 195–197 °C; UV λ_{max} nm (MeOH), 249.5; IR υ_{max} cm^{-1}: 1235, 1457, 1625, 1669 (α,β-unsturated C=O), 1708 (–COOH), 2921, and 3455 (O–H); EI/MS *m/z* (rel. int.): 470 (1.81) $[M]^+$, 425 (3.1), 408 (5.6), 287 (4.9), 273 (54.4), 232 (100), 189 (12.6), 175 (14.3), 148 (13.9), 135 (33.7), 119 (25.9), 105 (41.2), 69 (34.8), and 55 (74.9); ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm, *J* = Hz): 0.78 (3H, *d*, 6.3, H-29), 0.79 (3H, *d*, 8.6, H-30), 0.93 (3H, *s*, H-28), 1.12 (3H, *s*, H-25), 1.17 (3H, *s*, H-26), 1.30 (3H, *s*, H-27), 1.33 (3H, *s*, H-23), 1.46 (1H, *dd*, 13.0, 3.0, H-5), 1.53 (1H, *d*, 11.2, H-18), 2.42 (1H, *s*, H-9), 2.49 (2H, *dd*, 13.0, 1.2, H-1), 4.07 (1H, *t*, H-3), and 5.54 (1H, *s*, H-12).

Assessment of the immunomodulatory activity: Lymphocyte blast transformation (mitogensis) assay (Stites, 1987; Nores *et al*. 1997)

The lymphocyte blast transformation (mitogensis) or proliferation assay was applied. The assay was adapted as a test for cell-mediated immunity. The cell-mediated immune response was determined in the peripheral blood lymphocytes (PBL) in response to mitogenic stimulation using either phytohaemagglutinin (PHA) or concanavalin A (Con A) as mitogens that stimulate human T and B cells but T-cells more vigorously.

A) Separation of peripheral blood lymphocytes (PBL)

Lymphocytes were separated from peripheral human venous blood by Ficoll/Hypaque gradient technique (Stites, 1987). For each sample, 5 ml of heparinized blood was diluted with equal volume of Hank's balanced salt solution (HBSS) in a sterile plastic centrifuge tube. Diluted blood (6 ml) was carefully overlaid on 4 ml Ficoll/Hypaque solution gradient without allowing the solution to become mixed by keeping the pipette against the tube wall 5–10 mm above the fluid meniscus. The tube was centrifuged at 1200 rpm at room temperature. The lymphocytes were localized as a whitish layer on the upper meniscus of the gradient solution. Using a fine pasteur pipette, the zone containing lymphocytes was taken and washed twice in HBSS (10 min at 1200 rpm). The residue is a buffy coat of polymorphonuclear leucocytes (PMNLs).

B) Lymphocyte transformation assay

The viable lymphocytes were adjusted to a concentration of 2×10^6 cells/ml in RPMI-1640 medium supplemented with 600 µl penicillin, 0.1 ml streptomycin, 1% glutamine, 25% HEPES-buffer, and 20% foetal calf serum (FCS). The lymphocytes were plated into 96-well tissue culture plates (or Ependorff tubes). The test solution (100 µl) in DMF (100 µl/ml) and 20 µg of the mitogen (PHA) were added to each well. Cell cultures were incubated at 37 °C in 5% CO_2 atmosphere for 72 hrs, during which the mitogen produced its maximal effect on DNA synthesis. After culture, cell films were stained by Giemsa stain and the average

count of percentage of transformed (proliferated) blasts was determined. Aqueous *Echinaceae Purpurea* extract (Immulone®) and Levamisole (Ketrax®) were used as positive control (standard immunostimulant) while Cyclophosphamide (Endoxan®) and cyclosporin (Sandimmune Neoral®) were used as negative control (standard immunosuppressant) 100 μg/ml of each drug in DMSO.

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