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MARGOSINOLIDE AND ISOMARGOSINOLIDE, TWO NEW TETRANORTRITERPENOIDS FROM *AZADIRACHTA INDICA* A.JUSS (MELIACEAE)

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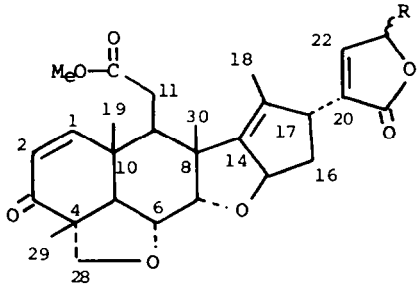
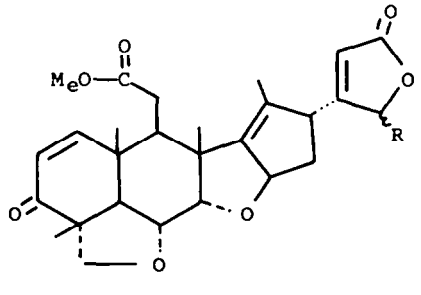
Abstract — Margosinolide (1) and isomargosinolide (3), two new ring - C seco, bitter tetranortriterpenoid γ-hydroxybutenolides have been isolated from the fresh, green twigs of *Azadirachta indica* (neem) and their structures elucidated through chemical and spectral studies. It may be noted that no chemical study has been carried out on the twigs prior to this work.

Chemical investigations of the limonoids and their biogenetic congeners, present in the ethanolic extract of the fresh neem fruits[1-5] and leaves[3,6-8] have led to the isolation and structure elucidation of a series of new triterpenoids with euphane (tirucallane) skeleton. In pursuance of these studies, the work undertaken for the first time on neem twigs has resulted in the isolation of two new ring - C seco, bitter limonoids margosinolide and isomargosinolide, from the acidic fraction of the methylene chloride extract. The structures of these tetranortriterpenoids have been deduced as 1 and 3 respectively, through spectral studies and chemical reactions. Margosinolide (1) and isomargosinolide (3) are of potential biological importance as other γ-hydroxybutenolides have been shown to possess insect growth regulating[3] and insect antifeeding[9] properties. Moreover, it has been observed earlier that many of the most potent insect feeding deterrent limonoids are ring - C seco type[10] and also that the growth inhibiting activity of limonoids with a salannin skeleton is enhanced by the presence of an α,β-unsaturated



1 : R = OH
2 : R = OAc



3 : R = OH
4 : R = OAc

ketone or lactone in the ring-A[11], and 1 and 3 possessing these features may prove to share these properties.

RESULTS AND DISCUSSION

The residue from methylene chloride extract of the fresh, undried, green, spring twigs of neem was divided into acidic and neutral fractions. After usual work-up, the acidic fraction was subjected to prep. TLC, ultimately yielding two crystalline bitter limonoids margosinolide (1) and isomargosinolide (3).

Margosinolide (1) has molecular formula $C_{27}H_{32}O_8$ (high resolution mass). Its UV spectrum showed absorption at 223 nm while IR spectrum showed peaks at 3400 (-OH), 1760 (α,β-unsaturated-γ-lactone), 1740 (carbomethoxy), 1660 (cyclohexenone), 1640 and 820 (trisubstituted double bond), 1155 and 1075 cm^{-1} (ether linkage). The ^{1}HNMR spectrum of 1 (Table 1) showed the presence of three angular methyls at δ 1.10, 1.18 and 1.30; a vinylic methyl at δ 1.73; two one-proton multiplets at δ 6.87 and 5.99 due to 22-H and 23-H respectively; four one-proton doublets at δ 4.22 (J=3.3Hz), 3.80 (J=7.2Hz), 3.71 (J=7.2Hz) and 2.73 (J=12.6Hz) for 7-H, 28-Hα, 28-Hβ and 5-H respectively; a one-proton double doublet at δ 4.10 (J=12.6, 3.3Hz) attributable to 6-H; and a one-proton quartet of double doublet at δ 5.43 (J=1.5, 6.5 and 6.5Hz) have been assigned to 15-H. These values are comparable with those reported for the same protons in salannolide.[12] However, the signals of tiglyl (at C-1) and acetyl ester (at C-3) functions were missing in the ^{1}HNMR of 1 and two AB doublets were exhibited instead, at δ 7.05 and 5.88 (J=10.0Hz, 1-H and 2-H respectively), which are consistent with ring-A 1-en-3-one, a usual feature of meliacins.[1-3,5-7] This was further supported by IR (1660 cm^{-1}), a fragment at m/z 137.0959 ($C_9H_{13}O$) resulting from characteristic cleavage of ring-A and signals observed in the ^{13}C NMR spectrum of 1 [δ 152.2 (C-1), 130.0 (C-2) and 202.4 (C-3)]. The assignments made so far were confirmed through ^{1}H-^{1}H homonuclear decoupling experiments, thus irradiation at δ 7.05 collapsed the doublet at δ 5.88 into a singlet and vice versa. Irradiation at δ 4.22 collapsed a double doublet at δ 4.10 to a doublet (J=12.6Hz) while, irradiation at δ 4.10 collapsed two doublets at δ 4.22 and 2.73 into each singlet. Irradiation at δ 2.73 collapsed the double doublet at δ 4.10 into a doublet (J=3.3Hz) while irradiation at δ 3.80 collapsed a doublet at δ 3.71 to a singlet and vice versa. Moreover, irradiation at δ 2.11 (ddd, J=12.0, 8.8 and 6.5Hz)

collapsed the double doublet (J=12.0, 6.5Hz) at δ 2.31 to a doublet (J=6.5Hz), the doublet (J=8.8Hz) at δ 3.55 to a singlet and the quartet of double doublet (J=1.5, 6.5 and 6.5Hz) at δ 5.43 to a quartet of doublet (J=1.5, 6.5Hz). Irradiation at δ 2.31 collapsed the doublet of double doublet at δ 2.11 to a double doublet (J=8.8, 6.5Hz) and the quartet of double doublet at δ 5.43 to a double quartet (J=6.5, 1.5Hz). When the quartet of double doublet at δ 5.43 was irradiated, the double doublet at δ 2.31 collapsed to a doublet (J=12.0Hz), the doublet of double doublet at δ 2.11 collapsed to a double doublet (J=8.8, 12.0Hz) while the doublet (J=1.5Hz) at δ 1.73 was converted into a singlet. In the light of these observations, the signals at δ 5.43, 3.55, 2.31, 2.11 and 1.73 have been assigned to 15-H, 17-H, 16-Hα, 16-Hβ and 18-H respectively.

Acetylation of 1 yielded the monoacetyl derivative 2 in the ^{1}HNMR (Table 1) of which, the signals of 22-H and 23-H shifted to δ 6.95 and 6.83 respectively, and a three-protons singlet appeared at δ 2.12 for the acetoxy methyl. The presence of double signals for C-15 to C-17 and C-20 to C-23 in the ^{13}CNMR spectrum of 1 (Table 2) indicated that it is epimeric at C-23, an observation also noted for other γ-hydroxybutenolides.[3,9]

The high resolution mass of isomargosinolide (3) showed its molecular formula as $C_{27}H_{32}O_8$ and the signals at δ 5.98 (21-H) and 6.02 (22-H) in the ^{1}HNMR spectrum (Table 1) indicated the 21-hydroxybut-20(22)-ene-γ-lactone side chain. These signals shifted to δ 6.84 and 5.98 respectively upon acetylation. This side chain was supported by the signals at δ 164.5 (C-20), 97.5 (C-21), 119.7 (C-22) and 170.0 (C-23) in the ^{13}CNMR spectrum of 3 (Table 2). The C-21 epimeric nature of isomargosinolide (3) was also indicated by the presence of double signals for the side chain carbons. The spectral data of 3 further showed that the rest of the molecule is identical with that of 1.

The stereochemistry of various centres of margosinolide (1) has been established through NOESY spectrum, which exhibited the spatial connectivities of 15-H with 9-H and 22-H; 5-H with 9-H and 28-Hα; 30-H with 6-H, 7-H, 17-H, 18-H and 19-H; 6-H with 19-H, 29-H and 28-Hβ; and also of 1-H with 2-H, and 22-H with 23-H. These

observations showed that ring-A and ring-B are trans fused and the spatial proximity of 15-H with 22-H showed that the side chain at C-17 has α disposition.

The NOESY spectrum of isomargosinolide (3) showed the spatial connectivities of 17-H with 11-Hβ and 30-H; 30-H with 6-H, 7-H, 18-H and 19-H; 5-H with 9-H and 28-Hα; 29-H with 19-H and 28-Hβ; and also of 1-H with 2-H. These spatial connectivities revealed that 3 also has the typical trans A/B ring junction. Further, the spatial connectivity of 17-H with 30-H and 11-Hβ showed that the side chain at C-17 of isomargosinolide (3) is also α oriented.

The unique feature of margosinolide (1) and isomargosinolide (3) is the ether linkage between C-28 and C-6 with the 1-en-3-one system, the characteristic ring-A of meliacins (loc.cit). A number of meliacins are reported in literature with this ether linkage but they lack 1-en-3-one system and instead possess oxygen substituents at C-1 and C-3.[12-15] On the other hand, the hydroxybutenolide side chain of 1 and 3 may be regarded as the intermediate in the formation of furan ring of meliacins.[16] These characters suggest that 1 and 3 may be the precursors of salannin[17] and the formation of ring-A of the latter can be rationalized through hydration of the double bond between C-1 and C-2 followed by reduction of the ketone function and esterification.

It is noteworthy in this context that margosinolide (1) and isomargosinolide (3) were initially obtained as a mixture showing a single spot on TLC (silica gel, benzene-ethyl acetate 5:95). However, the ^{1}HNMR spectrum recorded on a 300 MHz instrument and double signals observed in the ^{13}CNMR spectrum indicated that it is a mixture of two isomeric compounds. TLC (silica gel, chloroform-methanol 95:5) of the acetylated product of this mixture clearly revealed its isomeric nature showing two distinct spots with very close Rf values, which were ultimately separated and characterized as 2 and 4. After a great deal of effort towards the separation of isomeric mixture prior to its acetylation, it could eventually be resolved into margosinolide (1) and isomargosinolide (3) on plates coated with aluminium oxide (choloroform-methanol 85:15).

EXPERIMENTAL SECTION

General Experimental. Mps. were recorded in glass capillary tubes and are uncorrected. IR (in $CHCl_3$) and UV (in MeOH) spectra were measured on JASCO IRA-I and Pye-Unicam SP-800 spectrometers respectively; mass spectra were recorded on

Finnigan MAT 112 and 312 double focussing mass spectrometers. NMR spectra were recorded in $CDCl_3$ on a Bruker AM 300 spectrometer, operating at 300 MHz for ^1H and 75 MHz for ^{13}C nuclei and the chemical shifts are reported in δ (p.p.m). The ^{13}CNMR spectral assignments have been made partly through DEPT experiments and partly through comparison with published data.[3,12] Optical rotations were measured at 24°C in $CHCl_3$, on a Polartronic-D polarimeter. Merck kieselgel 60 PF_{254} and aluminium oxide 60 PF_{254} coated on glass plates were used for analytical (thin layer) and preparative (thick layer) chromatography.

Materials and method. 6 kg of the fresh, undried, uncrushed, spring twigs of neem were repeatedly extracted out with methylene chloride at room temperature. The methylene chloride layer was separated from a small quantity of the aqueous extractive and freed of the solvent under reduced pressure. The greenish brown residue obtained was partitioned between ethyl acetate and water and the former was repeatedly extracted out with 4% Na_2CO_3 to separate the acidic and neutral fractions. The combined Na_2CO_3 phase was acidified with dilute HCl, extracted out with ethyl acetate which was washed, dried (Na_2SO_4 anhydrous), charcoaled and filtered. The charcoal was successively eluted with ethyl acetate and benzene-methanol (1:1), and the ethyl acetate eluate was combined with the filtrate and freed of the solvent. The residue obtained was subjected to prep. TLC (silica gel, benzene - ethyl acetate 5:95) to yield a crystalline product which was ultimately separated into 1 and 3 by prep. TLC on plates coated with aluminium oxide (chloroform - methanol 85:15).

Margosinolide (1). It crystallized from chloroform as plates (25 mg, 0.0007% on the dry wt. basis); mp 130°C; $[\alpha]_D$ + 50° (c, 0.02, $CHCl_3$); UV λ_{max} nm: 223 (ε 7066); IR ν_{max} cm^{-1}: 3400, 1760, 1740, 1660, 1640, 1155, 1075 and 820; HR-MS m/z (%): 484.2090 (M^+, calc. for $C_{27}H_{32}O_8$: 484.2096) (6), 466.1984 (M-H_2O) (10), 454.1964 (M-CH_2O) (4), 434.1719 (M-CH_3OH-H_2O) (8), 419.1510 (434-CH_3) (5), 349.1439 ($C_{22}H_{21}O_4$) (4) and 137.0959 (ring A + H) (32).

Isomargosinolide (3). It crystallized from chloroform as rods (50 mg, 0.0014% on the dry wt. basis); mp 125°C; $[\alpha]_D$ + 14.28° (c 0.07, $CHCl_3$); UV λ_{max} nm: 220 (ε 7025); IR ν_{max} cm^{-1} : 3400, 1765, 1740, 1660, 1640, 1150, 1070 and 820; HR-MS m/z (%) : 484.2078 (M^+, calc for $C_{27}H_{32}O_8$: 484.2096) (4), 466.1980 (M-H_2O) (15), 454.1969 (M-CH_2O) (5), 434.1725 (M-CH_3OH-H_2O) (8); 419.1529 (434-CH_3) (4), 349.1440 ($C_{22}H_{21}O_4$) (6) and 137.0959 (ring A+H) (32).

Acetylation of margosinolide (1). Acetic anhydride (2ml) was added to a solution of 1 (8 mg) in pyridine (1 ml) and the reaction mixture was kept overnight at room temperature. On usual work-up 2 was obtained as a colourless crystallizate which crystallized from chloroform as fine needles, mp 105 - 108°C; UV λ_{max} nm: 225 (ε 6078); IR ν_{max} cm^{-1}: 1760 (α,β-unsaturated-γ-lactone), 1745 (carbomethoxyl), 1720 (ester carbonyl), 1665 (cyclohexenone), 1640 and 825 (trisubstituted double bond), 1150 and 1075 (ether linkage); HR- MS m/z (%): 526.2187 (M^+, calc. for $C_{29}H_{34}O_9$: 526.2201) (3), 494.1927 (M-CH_3OH) (5), 466.1984 (M-CH_3COOH) (12), 451.1754 (466-CH_3) (4), 434.1715 (466-CH_3OH) (8) and 409.1637 (M-$HCOOCH_3$-CH_3-C_2H_2O) (14).

Acetylation of isomargosinolide (3). Compound 3 (8mg) was acetylated in the same manner as 1 to yield 4, which crystallized from chloroform as clusters of rods, mp 100-102°C; UV λ_{max} nm : 222 (ε 6255); IR ν_{max} cm^{-1}: 1765 (α,β-unsaturated-γ-lactone), 1740 (carbomethoxyl), 1725 (ester carbonyl), 1665 (cyclohexenone), 1645 and 820 (trisubstituted double bond), 1150 and 1070 (ether linkage); HR-MS m/z (%): 526.2195 (M^+, calc. for $C_{29}H_{34}O_9$: 526.2201) (5), 494.1930 (M-CH_3OH) (4), 466.1990 (M-CH_3COOH) (10), 451.1754 (466-CH_3) (6), 434.1721 (466-CH_3OH) (4) and 409.1645 (M-$HCOOCH_3$-CH_3-C_2H_2O) (7).

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Table 1. ^{1}H NMR Spectral data of tetranortriterpenoids (δ_H p.p.m and J/Hz)

Assignment	1	2	3	4
1-H	7.05 (d) $J_{1,2}$ 10.0	7.03 (d) $J_{1,2}$ 10.0	7.06 (d) $J_{1,2}$ 9.6	7.02 (d) $J_{1,2}$ 9.7
2-H	5.88 (d) $J_{2,1}$ 10.0	5.85 (d) $J_{2,1}$ 10.0	5.87 (d) $J_{2,1}$ 9.6	5.83 (d) $J_{2,1}$ 9.7
5-H	2.73 (d) $J_{5,6}$ 12.6	2.75 (d) $J_{5,6}$ 12.6	2.72 (d) $J_{5,6}$ 12.8	2.74 (d) $J_{5,6}$ 12.5
6-H	4.10 (dd) $J_{6,5}$ 12.6 $J_{6,7}$ 3.3	4.09 (dd) $J_{6,5}$ 12.6 $J_{6,7}$ 3.5	4.12 (dd) $J_{6,5}$ 12.8 $J_{6,7}$ 3.6	4.08 (dd) $J_{6,5}$ 12.5 $J_{6,7}$ 3.4
7-H	4.22 (d) $J_{7,6}$ 3.3	4.21 (d) $J_{7,6}$ 3.5	4.25 (d) $J_{7,6}$ 3.6	4.18 (d) $J_{7,6}$ 3.4
9-H	2.45 (dd) $J_{9,11\alpha}$ 6.0 $J_{9,11\beta}$ 8.0	2.55 (t) $J_{9,11\alpha}$ 5.4 $J_{9,11\beta}$ 5.4	2.50 (dd) $J_{9,11\alpha}$ 6.3 $J_{9,11\beta}$ 7.8	2.55 (t) $J_{9,11\alpha}$ 5.3 $J_{9,11\beta}$ 5.3
11-Hα	3.18 (dd) J_{gem} 16.0 $J_{11\alpha,9}$ 6.0	3.26 (dd) J_{gem} 16.4 $J_{11\alpha,9}$ 5.4	3.18 (dd) J_{gem} 16.6 $J_{11\alpha,9}$ 6.3	3.28 (dd) J_{gem} 15.4 $J_{11\alpha,9}$ 5.3
11-Hβ	2.39 (dd) J_{gem} 16.0 $J_{11\beta,9}$ 8.0	2.33 (dd) J_{gem} 16.4 $J_{11\beta,9}$ 5.4	2.34 (dd) J_{gem} 16.6 $J_{11\beta,9}$ 7.8	2.36 (dd) J_{gem} 15.4 $J_{11\beta,9}$ 5.3
15-H	5.43 (ddq) $J_{15,16\alpha}$ 6.5 $J_{15,16\beta}$ 6.5 $J_{15,18}$ 1.5	5.36 (ddq) $J_{15,16\alpha}$ 6.4 $J_{15,16\beta}$ 6.4 $J_{15,18}$ 1.5	5.40 (m)	5.33 (ddq) $J_{15,16\alpha}$ 6.4 $J_{15,16\beta}$ 6.4 $J_{15,18}$ 1.5
16-Hα	2.31 (dd) J_{gem} 12.0 $J_{16\alpha,15}$ 6.5	2.20 (dd) J_{gem} 12.1 $J_{16\alpha,15}$ 6.4	2.32 (dd) J_{gem} 12.0 $J_{16\alpha,15}$ 6.8	2.15 (dd) J_{gem} 12.0 $J_{16\alpha,15}$ 6.4
16-Hβ	2.11 (ddd) J_{gem} 12.0 $J_{16\beta,15}$ 6.5 $J_{16\beta,17}$ 8.8	2.10 (ddd) J_{gem} 12.1 $J_{16\beta,15}$ 6.4 $J_{16\beta,17}$ 8.0	2.10 (m)	2.06 (ddd) J_{gem} 12.0 $J_{16\beta,15}$ 6.4 $J_{16\beta,17}$ 7.8
17-H	3.55 (d) $J_{17,16\beta}$ 8.8	3.55 (d) $J_{17,16\beta}$ 8.0	3.57 (d) $J_{17,16\beta}$ 7.2	3.56 (d) $J_{17,16\beta}$ 7.8
21-H	-	-	5.98 (m)	6.84 (m)
22-H	6.87 (m)	6.95 (m)	6.02 (m)	5.98 (m)

Table 1. (Contd.)

Assignment	1	2	3	4
23-H	5.99 (m)	6.83 (m)	-	-
28-Hα	3.80 (d) $J_{28\alpha,28\beta}$ 7.2	3.77 (d) $J_{28\alpha,28\beta}$ 7.2	3.79 (d) $J_{28\alpha,28\beta}$ 7.2	3.77 (d) $J_{28\alpha,28\beta}$ 7.2
28-Hβ	3.71 (d) $J_{28\beta,28\alpha}$ 7.2	3.68 (d) $J_{28\beta,28\alpha}$ 7.2	3.71 (d) $J_{28\beta,28\alpha}$ 7.2	3.69 (d) $J_{28\beta,28\alpha}$ 7.2
OH	3.60 (m)	-	3.62 (m)	-
OAc	-	2.12 (s)	-	2.08 (s)
OMe	3.71 (s)	3.63 (s)	3.75 (s)	3.63 (s)
18-H	1.73 (d) $J_{18,15}$ 1.5	1.72 (d) $J_{18,15}$ 1.5	1.73 (d) $J_{18,15}$ 1.5	1.73 (d) $J_{18,15}$ 1.5
19-H	1.10 (s)	1.14 (s)	1.11 (s)	1.16 (s)
29-H	1.18 (s)	1.19 (s)	1.18 (s)	1.20 (s)
30-H	1.30 (s)	1.32 (s)	1.31 (s)	1.31 (s)

Table 2. ^{13}C NMR spectral data of margosinolide (1) and isomargosinolide (3)

Carbons	1	3	Carbons	1	3
C-1	152.2	152.3	C-17	48.9	48.7
C-2	130.0	130.2		50.0	48.9
C-3	202.4	202.7	C-18	14.5[b]	14.4[b]
C-4	39.0	38.9	C-19	16.8	17.1
C-5	41.3[a]	41.5[a]	C-20	137.0	164.5
C-6	72.4	72.4		138.0	164.9
C-7	87.4	87.2	C-21	171.4	97.5
C-8	46.3	46.0		171.5	97.7
C-9	40.0[a]	40.0[a]	C-22	142.4	119.7
C-10	42.1	42.2		142.6	119.9
C-11	32.1	32.5	C-23	96.9	170.0
C-12	174.9	175.0		97.1	170.5
C-13	133.2	133.2	C-28	79.4	79.4
C-14	147.5	148.7	C-29	20.5	20.5
C-15	85.4	85.5	C-30	13.2[b]	13.0[b]
	85.7	85.7	$-\overset{O}{\overset{\|}{C}}-OCH_3$	52.4	51.8
C-16	40.0	39.8			
	40.1	40.0			

a,b: assignment may be interchanged.

All values are in δ (p.p.m.)