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TRITERPENOIDS FROM *MANGIFERA INDICA*

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Key Word Index—*Mangifera* indica; Anacardiaceae; triterpenoids.

Abstract-A new pentacyclic triterpenoid, hopane-1β,3β,22-triol, was isolated from the neutral fraction of the *n*-hexane extract of the stem bark of *Mangifera indica*. From the acidic fraction of the same extract four new tetracyclic triterpenoids, 3α,22(*R* or S)-dihydroxycycloart-24E-en-26-oic acid, 3β,22(*R* or *S*)-dihydroxycycloart-24*E*-en-26-oic acid, 3β,23(*R* or S)-dihydroxycycloart-24E-en-26-oic acid and 3α,27-dihydroxycycloart-24*E*-en-26-oic acid were isolated. The structures were elucidated by spectroscopic and chemical methods.

INTRODUCTION

In our previous papers [1–3] we have reported the triterpenoids from various parts of the Banganpalli variety of *Mangifera indica* L. In continuation of our studies on the triterpenoid constituents from different varieties of *M. indica, we* have now investigated the stem-bark of the Neelum variety. From this material five new and several known triterpenoids have been isolated.

RESULTS AND DISCUSSION

The neutral fraction of the n-hexane extract of *M. indica* afforded several known compounds, namely cycloartenol, β-amyrin, a-amyrin, sitosterol, 3β-hydroxycycloart-24-en-26-al, the C-24 epimers of cycloart-25-ene-3β,24-diol, 24-methylenecycloartane-3β,26-diol, cycloart-23-ene-3β,25-diol, cycloartane-3β,25-diol (**1**), epi-ψ-taraxastane-3β,20-diol (**2**), the C-24 epimers of cycloartane-3β,24,25-triol, the C-24 epimers of cycloart-25-ene-3β,24,27-triol and a new pentacyclic triterpenoid (3). The acidic fraction of the same extract on column chromatography gave four new tetracyclic triterpenoids (4, **5a**, **5b**, 6) besides mangiferonic acid, isomangiferolic acid, mangiferolic acid and hydroxymangiferolic acid.

Compound A, mp 183–184°, analysed for $C_{30}H_{52}O_2$ [M^+ at m/z 4443. Its ^{1}H NMR displayed no olefinic protons and showed signals for a secondary hydroxyl [δ 3.18 (*m*, 3-H)], seven methyls (6 1.23–0.84) and a cyclopropane methylene [δ 0.57 and 0.33 (ABq, *J* = 4 Hz)]. The downfield shift of the two methyls (6 1.23) indicated a substituted propan-2-ol system. On acetylation, compound A gave two products, the more polar compound was the diol monoacetate (la) and the less polar compound, mp 98–100°, was a monoacetate (lb) possessing a terminal methylene [IR: 890 cm^{-1}; ^{1}H NMR; 64.78 (s, H_2-26)]. The above data suggested the structure of compound A as cycloartane-3β,25-diol (**1**) [4]. Compound **lb** is the dehydration product of la. Dehydration of the C-25 hydroxyl with acetic anhydride–pyridine at room temperature has not been observed previously in cycloartanes. Cycloartane-3β,25-diol (**1**) has not been isolated previously from any part of *M. indica.*

Compound B, mp 260–262°, analysed for $C_{30}H_{52}O_2$. Its ^{1}H NMR spectrum displayed a multiplet at δ 3.18 (H-3) and eight methyls between δ 0.76 and 1.09. It formed a monoacetate (**2a**), mp 267-268° [δ 1.98 and 4.42 (H-3)] whose IR spectrum still exhibited the presence of hydroxyl (3600 cm^{-1}) which presumably is tertiary as it resisted acetylation. The above data indicated that compound B was a saturated dihydroxy pentacyclic triterpenoid.

Dehydration of **2a** with thionyl chloride in pyridine yielded a compound, mp 242–244°, which was readily identified as ψ-taraxasteryl acetate (**2b**) by direct comparison with the authentic sample (mmp, co-TLC and ^{1}H NMR). Therefore, compound B must be one of the C-20 epimers of ψ-taraxastane-3β,20-diol (2) [3, 5, 63. The mmp of compound B and authentic ψ-taraxastane-3β,20-diol was depressed. The physical and spectral characteristics of compound B and its acetate agree closely with those of *epi*-ψ-taraxastane-3β,20-diol (2) and its acetate (2a) [5, 6], respectively. Therefore, compound B is *epi*-ψ-taraxastane-3β,20-diol (2). The ^{13}C NMR of 2 was assigned by comparison with taraxasterol(7) [7] (Table 1). *epi*-ψ-Taraxastane-3β,20-diol (**2**) has not been previously reported from any part of *M. indica* and this is the second report of its natural occurrence.

Compound C was crystallized from $CHCl_3$–MeOH as colourless flakes mp 253-255° and analysed for $C_{30}H_{52}O_3$. Its IR spectrum indicated the presence of hydroxyl (3550 cm^{-1}). Its ^{1}H NMR spectrum could not be secured due to its insolubility in common NMR solvents. Acetylation of compound C afforded a trio1 diacetate (**3a**), mp 262-264° whose IR spectrum showed an acetate carbonyl (1725 cm^{-1}) and a hydroxyl (3600 cm^{-1}). Its ^{1}H NMR spectrum lacked olefinic protons and displayed two secondary acetates [δ 4.57 and 4.65 (both *dd*, *J* = 5 and 12 Hz)] and eight methyls (60.71-1.18). The downfield shift of the two methyls (6 1.18 and 1.16) suggested an hydroxyisopropyl group in the molecule. The above evidence indicated that compound C was a trihydroxy pentacyclic triterpenoid with a hopane or lupane skeleton.

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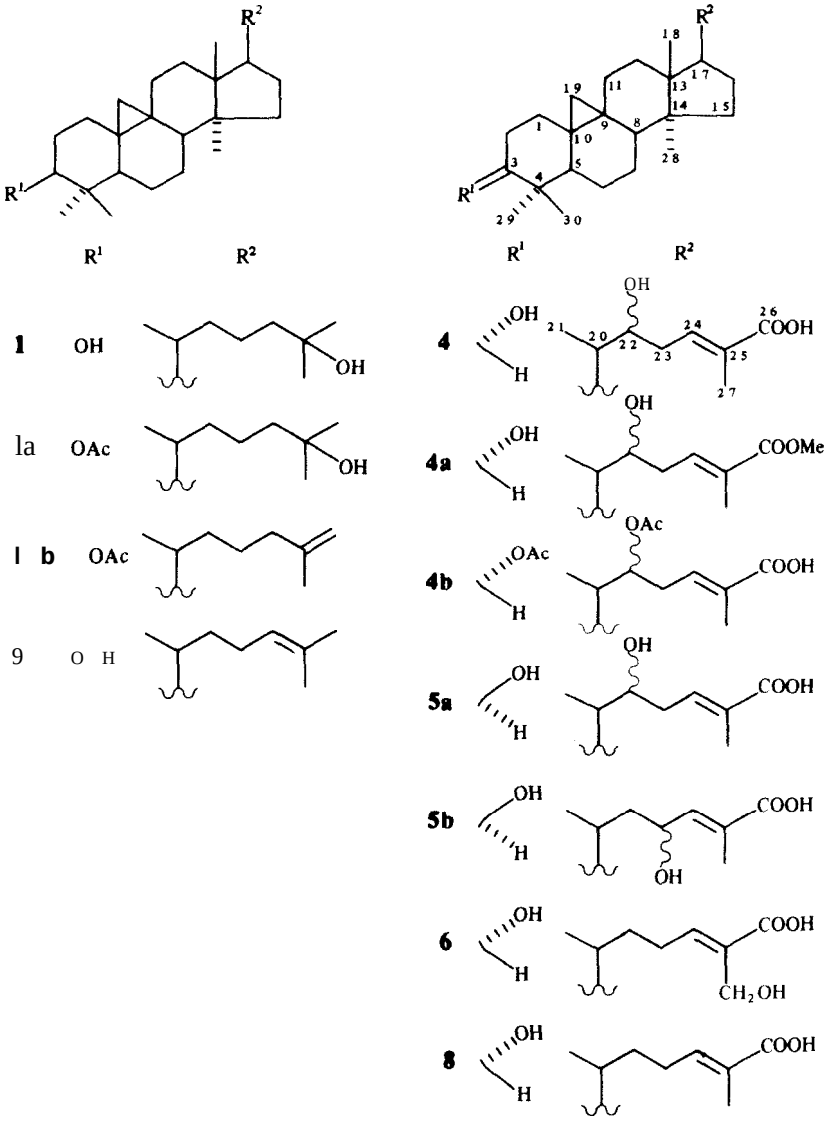


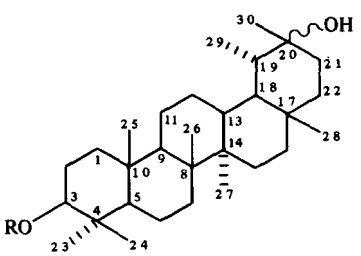
The mass spectrum of compound C showed no molecular ion but showed the ions at ***m/z*** 442 $[M-18]^+$, 424 $[M-18-18]^+$ and 406 $[M-3\times18]^+$. The fragments at *m/z* 207,189 and 149 are typical of 22-hydroxyhopanes [8] or 20-hydroxylupanes [9] and suggested that the other two hydroxyls were in rings AB. Ions at ***m/z*** 171 and 157 restricted the placement of these two hydroxyls to ring A.

A literature survey of various trihydroxyhopane and lupane derivatives revealed that the physical and spectral data of compound C acetate were in very close agreement with those of 1β,3β-diacetoxyhopan-22-ol (3a) [10]. Thus, compound C was hopane-1β,3β,22-triol (3).

Hopane-1β,3β,22-triol (**3**) has been previously reported as a saponification product of 3β-acetoxyhopane-1β,22-diol (3b) isolated from the lichen ***Pseudoparmelia texana*** [10]. This is the first report of its natural occurrence and it is also the first new pentacyclic triterpenoid from *M. indica*.

Compound D, mp 218–220°, analysed for $C_{30}H_{48}O_4$. Its IR spectrum exhibited the presence of hydroxyls (3500 cm-') and a&unsaturated carboxyl group (3300–2500, 1690 and 1640 cm ^{1}). Its 'H NMR spectrum displayed signals for a cyclopropane methylene (60.32 and 0.50, ABq, $J=4.2$ Hz), five methyls (0.84–0.97), a vinylic methyl (1.82), two secondary hydroxyls (3.41 *br s* and 3.75 ***m)*** and an olefinic proton (6.90 t). The broad singlet at 3.41 is characteristic of a proton geminal to a 3α-axial hydroxyl in cycloartanes. On treatment with diazomethane, compound D formed a methyl ester (**4a**), mp 161–163°, whose ^{1}H NMR spectrum showed 3β-H at δ 3.45 and a four proton broad singlet at 3.78 assignable to the geminal proton of a secondary hydroxyl and three protons of a carbomethoxyl group. On acetylation with acetic anhydrideepyridine at room temperature, compound D formed a diacetate (**4b**), mp 92–95°, the ^{1}H NMR spectrum of which showed two acetate methyls at 6 2.02 and 2.06 and the corresponding geminal protons at 4.68 (3β-H) and 5.02. The above data indicated that compound D was a new 9,19-cycloartane derivative having two secondary hydroxyls and an x,/i-unsaturated carboxyl group.

The mass spectral fragmentation of compound D showed a molecular ion at m/z 472 and the corresponding ions due to loss of water at *m/z* 454 and 436. Ions at ***m/z***



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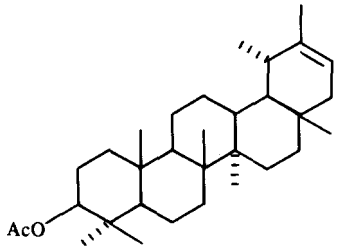
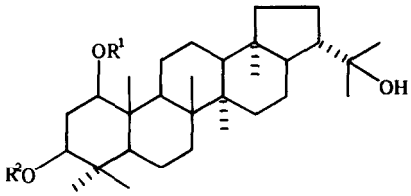
2a R = Ac



2b



3 $R^1 = R^2 = H$
3a $R^1 = R^2$ = Ac
3b R^1 = H, R^2 = Ac

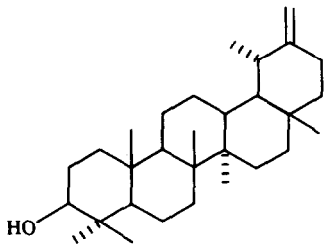


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Table 1. ^{13}C NMR signals of compounds 2 and 7 ($CDCl_3$, TMS as int. standard)

C	2	7
1	38.8	38.7
2	27.4	27.3
3	79.0	78.9
4	38.3	38.7
5	55.1	55.3
6	18.4	18.2
7	34.4	34.0
8	41.2	40.8
9	49.5	50.4
10	36.9	37.0
11	21.3	21.3
12	26.5[a]	25.5[a]
13	38.6	38.7
14	42.0	41.9
15	26.5	26.6
16	40.2[b]	39.1[b]
17	35.6	34.4
18	47.9	48.5
19	37.8	38.2
20	75.3	154.4
21	21.4[a]	25.4[a]
22	43.0[b]	39.2[b]
23	28.0	27.9
24	14.7	15.2
25	15.4	15.8
26	16.1	16.1
27	14.7	14.6
28	28.5	26.1
29	17.4	19.3
30	27.4	107.0

[a,b] Values bearing the same superscript may be interchanged.

332, 315 and 175 were the characteristic fragments of a 9,19-cycloartane triterpenoid [11] with one hydroxyl in rings AB and the other hydroxyl in a side chain bearing an @-unsaturated carboxyl group. An ion at *m/z* 385, due to loss of carbons 2, 3 and 4, suggested that the hydroxyl of rings AB should be placed in ring A, probably at C-3. The hydroxyl present in the side chain must be either at C-22 or C-23. From the multiplicity of the olefinic proton (triplet) in the ^{1}H NMR spectrum of compound D and its derivatives, the hydroxyl was located at C-22. It is well known that the β-olefinic proton *cis* to the carboxylic group is significantly more deshielded than the *trans* [12,13]. Therefore, the downfield shift of the β-olefinic proton in the ^{1}H NMR spectra of compound D and its derivatives clearly suggested the *E*-configuration of the double bond. Thus, all the above data suggested that compound D was 3α,22(*R* or *S*)-dihydroxycycloart-24E-en-26-oic acid (4).

The structure of compound D was further supported by its ^{13}C NMR spectrum. The ^{13}C chemical shifts of compound D were compared with those of isomangiferolic acid (8) and this is also the first report of the assignment of the ^{13}C NMR data of the latter. The carbon resonances of the nucleus of compound D were in close agreement with those of compound 8 confirming the 3α-axial hydroxyl at C-3. The chemical shift of the C-21 methyl was observed at δ 12.3 since it is shielded by the C-22 hydroxyl group. The signal of C-27 methyl (6 11.9) clearly supported the E-configuration of the double bond since it is shielded by the steric interaction with C-23 methylene.

Mixture I, mp 220–223°, analysed for $C_{30}H_{48}O_4$ and showed a single spot on TLC in various solvent systems. Its IR absorption spectrum displayed bands for hydroxyl (3500 cm^{-1}), carboxyl (3300–2500 cm^{-1}) and α,β-unsaturated carbonyl (1690, 1640 cm^{-1}). Methylation of mixture I with diazomethancproved unsuccessful due to formation of diazomethane adducts. The ^{1}H NMR spectrum of mixture I (in $CDCl_3$–DMSO-d_6) showed signals at 60.30 and 0.50 for cyclopropane methylene, methyls between δ 0.78 and 0.98, two signals for vinylic methyls at

δ 1.82 and 1.85, two olefinic protons at δ 6.57 and 6.90 as a doublet and a triplet, respectively. The region of the chemical shifts of protons geminal to hydroxyls was buried under DMSO-d_6 and water peaks.

The mass spectrum of mixture I showed the molecular ion at *m/z* 472 and corresponding ions due to loss of water at *m/z* 454 and 436 indicating the presence of two hydroxyls. Ions at *m/z* 332, 315 and 175 were observed which suggested that the components of the mixture I bears one hydroxyl in rings AB and the other hydroxyl in the side chain which possesses an a&'-unsaturated carboxy1 group.

Thus, from the above evidence, it was concluded that mixture I consists of two isomeric dihydroxycycloartane derivatives both possessing a cycloart-24-en-26-oic acid system. Attempts to separate the mixture into its constituents were not successful.

The ^{13}C NMR spectrum (Table 2) of mixture I further confirmed the presence of two compounds and gave much evidence regarding their structures. Comparison of the ^{13}C signals of mixture I with those of isomangiferolic acid (8) and cycloartenol (9) [14] revealed that both the compounds of the mixture had the same nucleus and variation was observed only in the nature of the side chain. It showed three hydroxyl bearing carbons at 6 79.4, 74.0 and 67.4 as doublets. The chemical shifts of C-l (6 33.8), C-5 (δ 47.3), C-29 (6 26.4) and C-30 (δ 14.5) indicated that the hydroxyl at C-3 was equatorial (β) in both the compounds. Doubling of carbon resonances in the side chain suggested that these two compounds differ only in the position or configuration of the secondary hydroxyl. The multiplicities of the olefinic proton at δ 6.90 as a triplet and 6.57 as a doublet in the ^{1}H NMR of mixture I clearly indicated the presence of a hydroxyl at C-22 in one compound and at C-23 in the other. Further, the downfield shift of the olefinic protons confirmed the E-configuration of the double bond in both the compounds. Thus, the structures of the two new compounds of mixture 1 were established as 3β,22(*R* or *S*)-dihydroxycycloart-24*E*-en-26-oic acid (**5a**) and 3β,23(*R* or *S*)-dihydroxycycloart-24*E*-en-26-oic acid (5b).

Compound E, mp 2055207 ', analysed for $C_{30}H_{48}O_4$ and showed the presence of hydroxyl (3550 cm^{-1}) and α,β-unsaturated carboxyl (3300–2600, 1695 and 1645 cm-') in its IR spectrum. Its ^{1}H NMR spectrum showed signals for a cyclopropane methylene (6 0.33 and 0.53, AB*q*, *J* = 4.2 Hz) and five methyls between δ 0.86 and 0.99 besides a triplet at δ 7.0 for an olefinic proton. It further displayed a broad singlet at δ 3.48 assignable to the proton geminal to 3α-axial hydroxyl and a two-

Table 2. ^{13}C NMR signals of compound D (**4**), mixture I (**5a**, **5b**), compound E (**6**), 8 and 9 (TMS as int. standard with solvent system indicated in parentheses)

C	**4*** ($CDCl_3$–CD_3OD)	**5a**/**5b** (CD_3OD)	**6*** ($CDCl_3$–DMSO-d_6)	**8*** ($CDCl_3$)	9
1	27.3	33.8	26.8	27.4	32.1
2	28.3	31.2	28.0	28.5	30.5
3	76.8	79.4	75.5	77.1	78.9
4	39.4	42.0	38.8	39.5	40.6
5	40.9	47.3	40.1	41.1	47.3
6	20.9	22.4	20.3	21.0	21.2
7	29.6	29.5	27.3	28.1	28.2
8	47.9	49.0	48.1	48.0	48.0
9	19.6	21.2	18.9	19.8	20.1
10	26.3	27.8	25.5	26.4	26.2
11	25.6	27.4	25.8	26.2	26.1
12	32.7	34.7	32.9	32.9	33.0
13	45.5	46.4	44.5	45.3	45.4
14	48.3	49.8	47.3	48.9	48.9
15	35.5	35.2	34.7	35.5	35.7
16	26.0	27.4	24.9	25.9	26.6
17	49.1	51.5, 51.6	51.3	52.1	52.4
18	19.2"	20.0"	18.6[a]	19.3"	19.4"
19	29.9	29.8	29.0	29.8	29.9
20	42.2	44.4, 37.6	35.2	35.9	36.0
21	12.3	12.8. 20.0	11.4"	18.0"	18.0
22	72.8	74.0, 37.6	34.6	34.8	36.5
23	27.1	28.2, 67.4	24.6	21.0	25.0
24	141.0	144.8, 144.0	145.0	145.7	125.4
25	129.0	131.2	127.0	126.7	130.8
26	170.5	173.2	173.0	173.0	25.7
27	11.9	12.4	56.0	11.9	17.6
28	17.7"	18.4"	17.4"	18.1"	18.3"
29	21.1	26.4	20.6	21.2	25.5
30	25.6	14.5	25.4	25.8	14.0

*Multiplicities assigned from DEPT spectrum.

"Assignments in any vertical column may be. interchanged.

proton singlet at δ 4.36 attributable to an allylic primary hydroxyl. The above spectral data can be accommodated only if compound E has the structure **3α,27-dihydroxycycloart-24*E*-en-26-oic** acid (6). This structure was further confirmed by its mass spectrum which lacked a molecular ion but showed an ion at ***m/z*** 436 [M- 18 $-18]^+$. The ion at m/z 314 $(332-18]^+$ and the corresponding fragment due to loss of side chain at m/z 175 $[332-C_8H_{13}O_3]^+$ clearly confirmed the presence of one hydroxyl in rings AB and the other hydroxyl in the side chain terminating with an a&unsaturated carboxyl group.

Firm evidence in support of the proposed structure of this new compound E (6) was obtained from its ^{13}C NMR spectrum (Table 2). The chemical shifts of C-l (6 **26.8**), C-5 (6 **40.1**), C-29 (6 20.6) and C-30 (25.4) clearly confirmed the a-axial hydroxyl at C-3. The triplet at δ 56.0 confirmed the allylic primary hydroxyl at C-27.

It is noteworthy that there is great variation in the triterpenoid constituents of the stem bark of the two varieties (Banganpalli and Neelum) of ***M. indica*** which we have examined so far. One of the significant variations is that several dammarane triterpenoids have been isolated from Banganpalli variety **[3]** but no dammaranes could be detected in the Neelum variety.

EXPERIMENTAL

Mps: **uncorr.** The plant material was collected from an aged tree at Lakshmaneswaram, near Narasapur in Andhra Pradesh, India.

Extraction and isolation procedure. The dried and powdered stem-bark (6 kg) was extracted successively with n-hexane (bp **60–80°**) and **MeOH** in a large aspirator bottle. The dark brown **coloured** n-hexane extract was evapd under red. **pres.** and the resultant gummy residue (70 **g**) was separated into acidic, **phenolic** and neutral fractions by adopting the procedure of ref **[15]**. The neutral fraction (30 **g**) was chromatographed on a silica gel (500g) column and eluted successively with n-hexane, ***n*-hexane–C_6H_6**, **C_6H_6** and **C_6H_6–EtOAc** mixtures. The results of the chromatography are shown in Table 3. The acidic fraction (20 **g**) was subjected to CC and the results are given in Table 4. The **MeOH** extract yielded only mangiferin (15 **g**) **[3]**, mp **269–270.**

Identification of the known compounds was based on their physical and spectral characteristics and comparison (TLC, ^1H NMR, mmp) with the authentic samples wherever possible.

***Compound* A.** Identified as **cycloartane-3*β*,25-diol (1).** Recrystallized from **C_6H_6** as colourless prisms, mp **183–84°**, $[\alpha]_D^{30}$ **+47° (CHCl$_3$**; c 1) (Found: C, 80.95; H, 11.75. **Calc.** for **$C_{30}H_{52}O_2$**: C, 81.08; H, 11.71%). **^1H NMR** (90 MHz, **$CDCl_3$**): 60.33 and 0.57 **(2H, AB*q*, *J* = 4** Hz), **0.84 (6H,** s), 0.93 **(6H,** s), 0.98 **(3H, *s*),** 1.23 **(6H,** s), 3.18 **(1H, *m*),** MS m/z (rel. int.): 444 [M]$^+$ **(10), 429 (15), 426 (50), 411 (15), 408 (18), 397 (8), 383 (28), 357 (18), 339 (25),** 315 **(52),** 304 **(68),** 297 **(52),** 203 **(30),** 175 **(45),** 95 (100).

Acetylation ***of compound* A.** Compound **A (50** mg) was **acetylated** with 2 ml **Ac_2O–pyridine** (1: 1) at room temp. overnight. Usual work-up gave a product which showed two spots on TLC **(*R*,** 0.8 and 0.41 in **C_6H_6**). They were separated by CC on silica gel **eluting** with ***n*-hexane–C_6H_6 (4:1)** to yield 4mg of dehydration product **(Ib),** mp 98-100"; IR ν_{max}^{KBr} **cm^{-1}**: 1735, 890. ^1H NMR (90 MHz, **CCl_4**): 60.32 and 0.57 **(2H, AB*q*, *J* = 4** Hz), **0.86 (6H, *s*), 0.89 (6H, *s*), 0.97 (3H, *s*), 1.80 (3H, *s*), 1.97 (3H, *s*), 4.46 (1H,** m), 4.78 **(2H,** s) and with benzene to give 40mg of diol monoacetate **(Ia),** mp **136–138°,** [a]$' + 52" (CHCI,; c 0.7).

***Compound* B.** Identified as **epi-*ψ*-taraxastane-3*β*,20-diol** (2). Recrystallized from **C_6H_6** as colourless crystals, mp **260–262°**, $[\alpha]_D^{30} \pm 0°$ (CHCI,; c 0.8). (Found: C, 80.82; H, 11.60. **Calc.** for C$_{30}$H$_{52}$O$_2$: C, 81.08; H, 11.71%). **IR** ν_{max}^{KBr} **cm^{-1}**: 3500, 3000, **1480, 950, 915, 890** and 860. ^1H NMR (90 MHz, CDCI,): δ 0.76 **(3H, *s*), 0.84 (3H, *s*), 0.90 (3H, *s*), 0.94 (3H, *s*), 0.97 (3H, *s*),** 1.04 **(6H,** s), 1.09 **(3H,** s), 3.18 **(1H, *m*).**

Acetylation ***of compound* B.** Compound B (25 mg) was treated with 1 ml **Ac_2O–pyridine** (1: 1) at room temp. overnight. The usual work-up followed by recrystallization from **$CHCl_3$–MeOH** gave colourless plates **(2a,** 18 **mg),** mp **267–268°**, $[\alpha]_D^{30}$ **+22.5°** (CHCI,; c 0.8). ^1H NMR (90 MHz, CDCI,): 60.78 **(3H,** s), 0.85 **(3H, *s*),** 0.89 **(3H,** s), 0.92 **(3H,** s), 0.94 **(6H,** s), 1.02 **(3H,** s), 1.10 **(3H, *s*),** 1.98 **(3H,** s), 4.42 **(1H,** m).

***Action of thionyl chloride on* 2a.** A **soln** of **2a** (12 mg) in dry

Table 3. Triterpenoids isolated from the neutral fraction of the ***M. indica*** extract

Eluent	Fractions	Compound	Yield (g)
n-Hexane	1–30	Waxes	5.0
***n*-Hexane–C_6H_6 (4: 1)**	31-38	Cycloartenol	4.2
***n*-Hexane–C_6H_6 (4: 1)**	39-50	a- And *β*-Amyrins	0.65
***n*-Hexane–C_6H_6 (4: 1)**	51–60	*β*-Sitosterol	0.2
***n*-Hexane–C_6H_6 (7: 3)**	61-65	Intractable gum	—
***n*-Hexane–C_6H_6 (3: 2)**	6670	3*β*-Hydroxycycloart-24-en-26-al [3]	0.06
***n*-Hexane–C_6H_6 (1: 1)**	71-80	Intractable gum	
***n*-Hexane–C_6H_6 (1: 3)**	81-85	Intractable gum	
C_6H_6	86-92	C-24 Epimers of cycloart-25-ene-3*β*,24-diol [16]	0.15
C_6H_6	93-98	24-Methylenecycloartane-3*β*,26-diol [17]	0.08
C_6H_6	99-106	Cycloart-24-ene-3*β*,26-diol [3]	0.12
C_6H_6	107-125	Cycloart-23-ene-3*β*,25-diol [4]	3.0
C_6H_6	126-131	Compound **A**	0.5
C_6H_6–EtOAc (19: 1)	132-135	Intractable gum	—
C_6H_6–EtOAc (9: 1)	136140	Compound B	0.03
C_6H_6–EtOAc (4: 1)	141-150	C-24 Epimers of cycloartane-3*β*,24,25-triol [3]	0.05
C_6H_6–EtOAc (4: 1)	151-157	C-24 Epimers of cycloart-25-ene-3*β*,24,27-triol [3]	0.4
C_6H_6–EtOAc (7: 3)	158-160	Compound C	0.01
C_6H_6–EtOAc (1: 1)	161-170	Intractable gum	

Table 4. Triterpenoid acids isolated from acidic fraction of *M.indica* extract

Eluent	Fractions	Compound	Yield (g)
n-Hexane–C_6H_6 (1 : 1)	1-20		
C_6H_6	21-30		
C_6H_6–EtOAc (19: 1)	31-50	Mangiferonic acid [3]	4.5
C_6H_6–EtOAc (19: 1)	51-55	Intractable gum	
C_6H_6–EtOAc (9: 1)	56–68	Isomangiferolic acid [3]	0.4
C_6H_6–EtOAc (9: 1)	69-100	Mangiferolic acid [3]	7.0
C_6H_6–EtOAc (9: 1)	101-105	Intractable gum	
C_6H_6–EtOAc (4: 1)	106–115	Compound D	0.1
C_6H_6–EtOAc (4: 1)	116-120	Mixture I	0.03
C_6H_6 -EtOAc (4: 1)	121-126	Intractable gum	
C_6H_6–EtOAc (7: 3)	127-132	Compound E	0.06
C_6H_6–EtOAc (7:3)	133-136	Hydroxymangiferolicacid [15]	0 . 0 2
C_6H_6–EtOAc (1: 1)	137–145	Intractable gum	

pyridine (1 ml) was cooled to 5' and freshly distilled $SOCl_2$ (0.2 ml) was added dropwise with shaking. The usual work-up followed by recrystallization from $CHCl_3$–MeOH gave colourless needles, mp 242–244°, $[\alpha]_D^{30}$ + 51" (CHCl,; *c* 1) found identical in all respects with authentic ψ-taraxasteryl acetate (2b).

Compound C. Identified as hopane-1β,3β,22-triol (3). Recrystallized from $CHCl_3$–MeOH as colourless needles, mp 253-255". (Found: C, 78.35; H, 10.36. $C_{30}H_{50}O_3$ requires: C, 78.60; H, 10.48%). IR ν_{max}^{KBr} cm- ': 3550. MS m/z (ref. int.): 442 $[M-18]^+$ (3), 427 (1), 424 (2), 406 (1), 399 (2), 355 (2), 287 (1), 223 (1), 207 (6), 205 (6), 193 (4), 189 (39), 187 (9), 175 (14), 171 (2), 167 (1), 157 (2), 153 (1), 149 (29), 139 (1), 135 (26), 121 (45), 59 (100).

Acetylation of compound C. Compound C (7 mg) was dissolved in pyridine (0.5 ml) and acetylated with Ac_2O (0.5 ml) at room temp. overnight. The usual work-up followed by recrystallization from $CHCl_3$–MeOH gave colourless plates (**3a**, 6 mg), mp 262–264°, $[\alpha]_D^{30}$ + 35" ($CHCl_3$; c 0.5). IR ν_{max}^{KBr} cm ^{1}: 3600, 1725. ^{1}H NMR(270 MHz, $CDCl_3$): SO.71 (3H, *s*), 0.82 (6H, *s*), 0.91(3H, *s*), 0.95 (3H, s), 0.99 (3H, *s*), 1.16 (3H, *s*), 1.18 (3H, *s*), 1.96 (3H, *s*), 2.00 (3H, *s*), 4.65 (I H, ***dd), 4.57*** (1 H, ***dd).*** MS *m/z* (rel. int.): ***526*** [M −18]$^+$(2), 511 (1), 483(1), 466 (1), 457 (2), 406 (3), 391 (1), 337 (3), 295 (1), 255 (1), 227 (2), 207 (1), 205 (3), 203 (5), 201 (6), 189 (19), 175 (10), 161 (12), 147 (40), 133 (12), 121 (23), 95 (19), 81 (20), 59 (18), 43 (100).

Compound D. Identified as 3α,22(*R* or *S*)-dihydroxycycloart-24*E*-en-26-oic acid (4). Recrystallized from $CHCl_3$–MeOH as colourless threads, mp 218–220°, $[\alpha]_D^{30}$ + 27.5" ($CHCl_3$; *c* 0.8). (Found: C, 76.18; H, 10.11; $C_{30}H_{48}O_4$ requires: C, 76.27; H, 10.17%). IR ν_{max}^{KBr} cm^{-1}: 3500, 3300–2500, 1690, 1640. UV λ_{max}^{MeOH} nm: 218 (ε 9800). 'HNMR (200 MHz, CDCl,): 60.32 and 0.50 (2H, AB*q*, *J* = 4.2 Hz), 0.84 (3H, *s*), 0.86 (3H, s), 0.92 (6H, s), 0.97 (3H, *s*), 1.82 (3H, *s*), 3.41 (1H, *br s*), 3.75 (1H, *m*), 6.90 (1H, *t*). MS m/z (rel. int.): 472 $[M]^+$ (6), 457 (4), 454 (11), 440 (4), 436 (3), 421 (3), 411 (3), 385 (2), 355 (6), 332 (8), 315 (3), 297 (6), 257 (2). 255 (2), 232 (4), 209 (48), 403 (38), 189 (20), 175 (62). 123 (48), 109 (50), 95 (100), 55 (82).

Methylation of compound D. Compound D (40 mg) in Et_2O (3 ml) was treated with CH_2N_2 at 0° overnight. The usual work-up followed by repeated recrystallization from $CHCl_3$–MeOH gave colourless plates (**4a**, 25 mg), mp 161–163°, $[\alpha]_D^{30}$ + 12.4" (CHCl,; *c* 1.5). ^{1}H NMR (90 MHz, $CDCl_3$): 60.35 and 0.55 (2H, AB*q*, *J* = 4 Hz), 0.89 (6H, s), 0.97 (6H, *s*), 1.0 (3H, *s*), 1.89 (3H, s), 3.45 (1H, *br* s), 3.78 (4H, *br s*).

Acetylation ***ofcompound*** D. Compound D (15 mg) was treated with 1 ml Ac_2O–pyridine (1: 1) at room temp. overnight. The usual work-up followed by recrystallization from $CHCl_3$–MeOH gave the diacetate (4b) as colourless plates (10 mg). mp 92–95°, ^{1}H NMR (90 MHz, CDCI,): SO.32 and 0.55 (2H, AB*q*, *J* = 4 Hz), 0.85 (3H, *s*), 0.92 (6H, s), 0.97 (6H, *s*), 1.87 (3H, s), 2.02 (3H, *s*), 2.06 (3H, *s*), 4.68 (1H, *br* s). 5.02 (1H, *m*), 6.85 (1H, *t*).

Mixture I. This was a mixture of 3β,22(*R* or *S*)-dihydroxycycloart-24*E*-en-26-oic acid (**5a**) and 3β,23(*R* or *S*)-dihydroxycycloart-24*E*-en-26-oic acid (5b): mp 220–223°, IR ν_{max}^{KBr} cm^{-1}: 3500, 3300–2500, 1690, 1640. ^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6): 60.30 and 0.50 (cyclopropane methylene), 0.78, 0.88, 0.91, 0.94, 0.98 (methyls), 1.82 (3H, s), 1.85 (3H. s), 3.25, 3.45, 3.60 and 3.70 (DMSO-d_6 peaks and geminal protons of hydroxyls), 6.57 (1H, ***d),*** 6.90 (1H, r). MS m/z (rel. int.): 472 $[M]^+$ (3), 457 (3), 454 (10), 441 (6), 440 (6), 436 (2), 421 (6), 411 (5), 408 (2), 385 (5), 357 (8), 355 (6), 354 (5), 340 (6), 332 (10), 315 (5). 314 (5), 297 (8), 257 (5), 232 (10), 227 (11), 215 (10), 209 (17), 205 (5), 204 (8), 203 (27), 202 (11), 189 (20), 175 (48), 149 (28), 135 (56). 123 (39), 121 (71), 95 (100), 55 (80).

Compound E. Identified as 3α,27-dihydroxycycloart-24*E*-en-26-oic acid (6). Recrystallized from C_6H_6–EtOAc as colourless short needles, mp 205–207°, $[\alpha]_D^{30}$ + 21.5° (CHCI,; *c* 0.8). (Found: C, 76.12; H, 10.09, $C_{30}H_{48}O_4$ requires: C, 76.27; H, 10.17%). IR ν_{max}^{KBr} cm^{-1}: 3550, 3300–2600, 1695 and 1645: UV λ_{max}^{MeOH} nm: 215 (ε 11200). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.33 and 0.53 (2H AB*q*, *J* = 4.2 Hz), 0.86 (3H, *s*), 0.88 (3H. *s*), 0.96 (6H, *s*), 0.99 (3H, s), 3.48 (1H, ***br s),*** 4.36 (2H, s), 7.0 (1H, *t*). MS m/z (rel. int.): 436 $[M-18-18]^+$ (4), 423 (2), 413 (2). 412 (3), 411 (6), 408 (2), 315 (9), 314 (15), 215 (9), 203 (34), 175 (46). 129 (6), 109 (61), 108 (22). 107 (100), 105 (61), 57 (6).

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