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Triterpenoids from *Mangifera indica*[1]

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Abstract

Two new triterpenoids, 25(*R*)-3-oxo-24-methylene cycloartan-26-ol and ψ-taraxastanonol have been isolated from the neutral fraction of the stem-bark of *Mangifera indica* (var/cv sarikhas). The acidic fraction of the same extract yielded three new tetracyclic triterpenoids, 3-oxo-23(*R* or *S*)-hydroxy cycloart-24-en-26-oic acid and both C-23 epimers of 3β,23-dihydroxy cycloart-24-en-26-oic acid. The structures were elucidated by spectroscopic and chemical methods.

Keywords: Mangifera indica; Anacardiaceae; Stem-bark; Triterpenoids and steroids

1. Introduction

Chemical examination of various varieties namely Banganpalli (Anjaneyulu, Harischandra Prasad, Ravi, & Connolly, 1985) Neelum, (Anjaneyulu, Ravi, Harischandra Prasad, & Connolly, 1989), Himayaddin (Anjaneyulu, Suresh Babu, Murali Krishna, & Connolly, 1993) and Himasagar (Anjaneyulu, Suresh Babu, & Connolly, 1994) of *Mangifera indica* in these laboratories have yielded new triterpenoids of cycloartane, dammarane, taraxastane and hopane skeletons. The variation of triterpenoid and steroid constituents of several varieties collected from different regions of India prompted the examination of yet another variety from West Bengal State. In this communication, we report the isolation and structure elucidation of five new triterpenoids along with many other known compounds.

2. Results and discussion

The neutral fraction of the *n*-hexane extract of *Mangifera indica* yielded two new triterpenoids (compound A and B) besides several known triterpenoids namely cycloartenone (Anjaneyulu, Suresh Babu, & Murali Krishna, 1992), 24-methylene cycloartanone (Ohta, 1960), friedelin (Anjaneyulu, Prasad, & Rao, 1982), taraxerone (Anjaneyulu et al., 1982), friedelan-3β-ol (Anjaneyulu et al., 1982), α-amyrin (Anjaneyulu et al., 1985), β-amyrin (Anjaneyulu et al., 1985), cycloartenol (Anjaneyulu et al., 1985), 24-methylene cycloartanol (Corsano & Mincione, 1967), sitosterol (Anjaneyulu et al., 1985), 11α,12α-oxido-taraxerol (Anjaneyulu, Suresh Babu, & Jyothi, 1994), 6β-hydroxy-stigmast-4-en-3-one (Anjaneyulu et al., 1992), 24-methylene cycloartane-3β,26-diol(Anjaneyulu et al., 1985) and C-24 epimeric mixture of cycloartane-3β,24,25-triol (Anjaneyulu et al., 1985). The acidic fraction of the same extract on column chromatography gave three new triterpenoids (compound C, D and E) besides mangiferonic acid (Anjaneyulu et al., 1989), ambonic acid (Corsano & Mincione, 1966, 1967), isomangiferolic acid (Anjaneyulu et al., 1989), mangiferolic acid (Anjaneyulu et al., 1989), ambolic acid (Corsano & Mincione, 1966, 1967) and 3α-22(*R* or *S*)-dihydroxy cycloart-24-en-26-oic acid (Anjaneyulu et al., 1989).

Compound A, m.p. 145–146°C, analysed for $C_{31}H_{50}O_2$ (M^+ at m/z 454). Its IR spectrum showed a carbonyl band at 1700 cm^{-1}, hydroxyl at 3600 cm^{-1} and a terminal methylene at 890 cm^{-1}. The ^1H NMR spectrum displayed a characteristic cyclopropane methylene protons at δ 0.57, 0.75 (ABq, $J=4$ Hz), two secondary methyls (δ 0.97 and 1.06) and four tertiary methyls (δ 0.82, 0.92 and 1.10). It also displayed a vinyl methylene at δ 4.82 and 4.88 each integrating for one proton and two broad singlets at δ 3.51 and 3.59 assignable to hydroxymethylene protons. The ^{13}C NMR speetrum showed three sp^2 carbon resonances at δ 152.2 (s), 109.5 (t) and 216.4 (s) confirming the presence of vinyl methylene and

[1] Part III in the series "Triterpenoids from *Mangifera indica*". For part II see Anjaneyulu et al., 1989.

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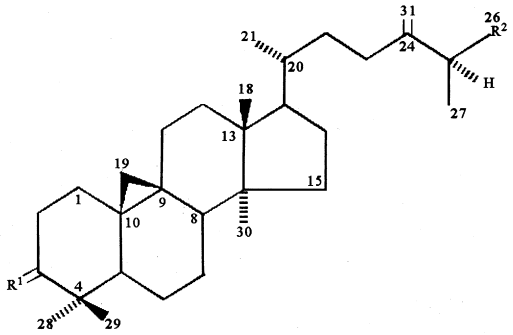
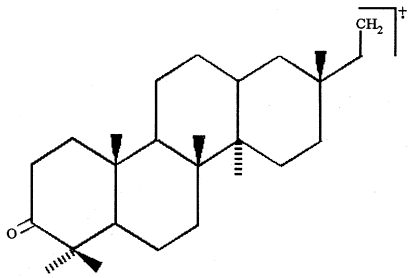
carbonyl groups, respectively, in compound A. It also displayed a sp^3 carbon resonance at δ 66.0 (t) which can be assignable to hydroxy methylene group. The mass spectral fragmentation of compound A showed the characteristic fragments at m/z 316 and 175 for 9,19-cyclolanostane derivatives (Alpin & Hornby, 1966; Audier, Bengelmans, & Das, 1966). The fragment ions at m/z 313 and 316 showed the presence of carbonyl group in the nucleus and a nine carbon side chain with hydroxy methylene group. The characteristic McLafferty type cleavage fragments (Wyllie & Djerassi, 1968; Bortolotto, Brackmann, Daloze, & Tursch, 1976) due to C_{22}–C_{23} bond fission at m/z 354 (M^+-$C_6H_{12}O$) and 216 (316-$C_6H_{12}O$) indicated the presence of 24-methylene side chain. These two fragment ions locate the position of hydroxyl group at C-26. Based on the above spectral data, the structure, 3-oxo-24-methylenecycloartan-26-ol (**1**) is assigned to compound A.

The carbon resonances (Table 1) of compound A were in close agreement with those of 24-methyl-enecycloartanone (**2**) except C-24 to C-27 of the side chain. The C-26 hydroxyl group exhibits the following effects: α (C-26, +44.1); β (C-25, +2.4) and γ (C-27, −5.5 and C-24, −4.2) which are in close agreement with



1. $R^1 = O$, $R^2 = CH_2OH$
2. $R^1 = O$, $R^2 = CH_3$
3. $R^1 =$  , $R^2 = CH_2OH$
4. $R^1 = O$, $R^2 = CO_2H$
15. $R^1 =$  , $R^2 = CO_2H$



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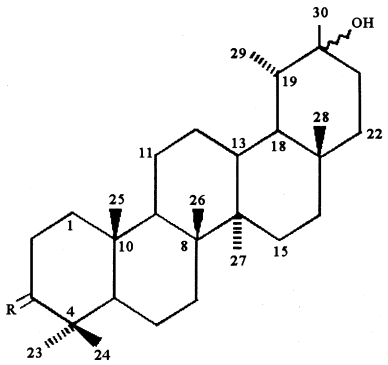
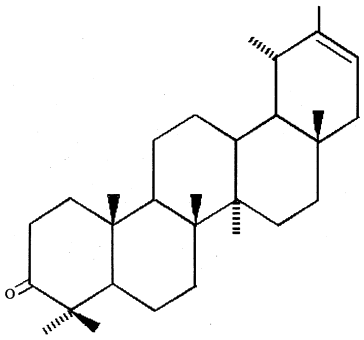


6. $R = O$
8. $R =$ 



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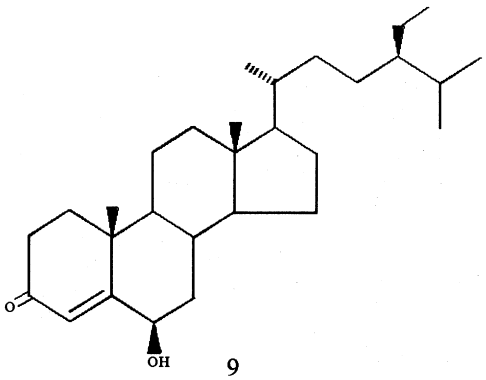

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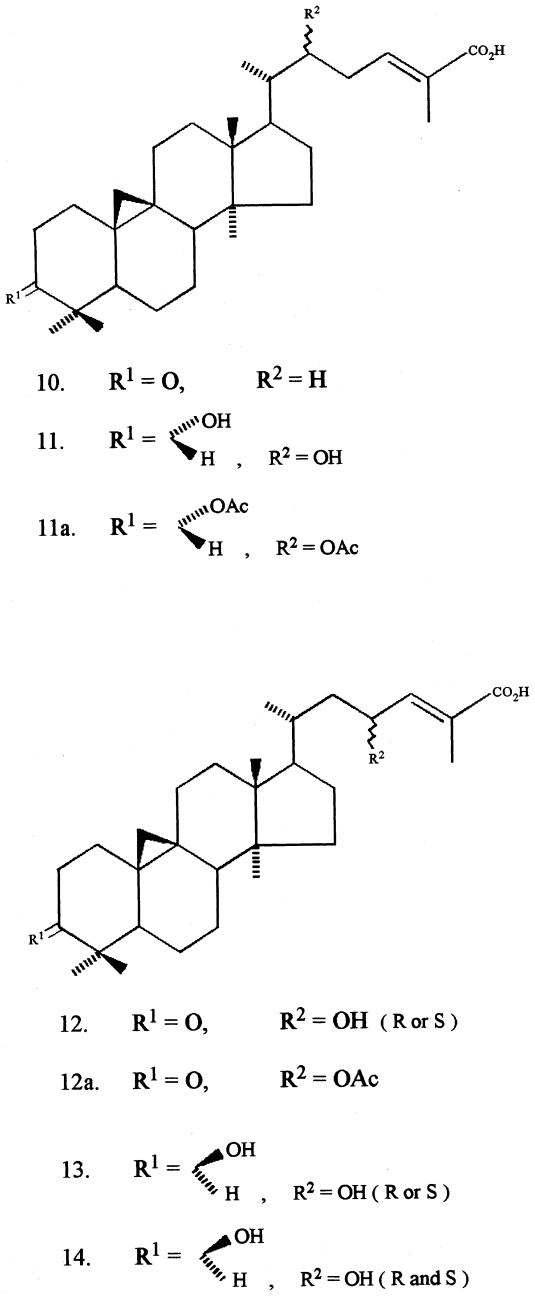
Structure 1.

Table 1
^{13}C NMR spectral data of compounds **1**, **2**, **3**, **6** and **9** (22.4 MHz, $CDCl_3$, TMS as internal standard)

C	1[a]	2	3[a]	6	9[a]
1	33.4	33.4	32.0	39.6	37.1
2	37.4	37.4	30.4	33.8	34.2
3	216.4	216.2	78.9	218.0	206.1
4	50.2	50.2	40.5	47.4	126.1
5	48.5	48.4	47.2	54.8	169.0
6	21.5	21.5	21.2	19.8	73.1
7	28.2	28.1	28.2	34.1	38.6
8	47.9	47.9	48.0	41.4	29.8
9	21.2	21.1	20.1	49.1	53.7
10	25.9	25.9	26.2	36.7	38.0
11	26.1	26.0	26.1	22.2	21.0
12	32.9	32.9	33.0	26.7	39.6
13	45.4	45.4	45.4	38.2	42.5
14	48.8	48.8	48.9	39.2	55.9
15	35.6	36.6	35.6	26.8	24.2
16	26.8	26.6	26.5	38.9	28.2
17	52.3	52.3	52.3	35.6	56.0
18	19.3	19.3	19.4	47.3	12.0
19	29.5	29.5	29.9	43.3	19.5
20	36.0	36.1	36.2	73.5	36.1
21	18.1	18.1	18.1	37.8	18.8
22	35.0	34.1	35.0	35.2	34.0
23	31.4	31.3	31.4	26.8	26.2
24	152.2	156.4	152.2	21.1	45.9
25	36.2	33.8	36.2	16.2	29.2
26	66.0	21.9	66.0	16.1	19.1
27	16.4	21.9	16.4	14.8	19.8
28	18.4	18.3	18.4	29.5	23.1
29	25.9	25.9	25.5	17.8	12.0
30	20.8	20.7	14.0	30.3	
31	109.5	105.9	109.5		

[a] Multiplicities assigned from DEPT spectrum.



Structure 2.

those recorded for such effects in literature (Anjaneyulu et al., 1985). The down-field shift of C-31 resonance in compound A might be due to the proximity of the hydroxy methylene group. The structure of compound A is confirmed by converting it into compounds of known structure. LAH reduction gave a diol, m.p. 150–152°C, identified as 24-methylene cycloartane 3β,26-diol (**3**) from its ^1H NMR data and also by direct comparison with an authentic sample. Chromium trioxide oxidation of compound A gave a keto carboxylic acid, m.p. 150–152°C characterised as ambonic acid (**4**) from its spectral data and also by direct comparison with an authentic sample. The absolute configuration of ambonic acid (**4**) at C-25 has already been established as '*R*' by Corsano and Mincione (1968). Therefore, the absolute configuration at C-25 in compound A is also deduced as '*R*'. Thus the structure of compound A is deduced as 25(*R*)-3-oxo-24-methylenecycloartan-26-ol (**1**) and it is a new addition to triterpenoid literature.

Compound B, m.p. 275–278°C and analysed for $C_{30}H_{50}O_2$. Its IR spectrum showed hydroxyl absorption at 3600 cm^{-1} and carbonyl absorption at 1695 cm^{-1}. The ^1H NMR spectrum showed the absence of olefinic protons, hydroxy methine protons and exhibited one secondary methyl (δ 0.89, $J=7$ Hz) and seven tertiary methyls (δ 0.97, 1.03, 1.07, 1.17). It resisted acetylation under normal conditions indicating the presence of a tertiary hydroxyl group. The low-field methyl signal at δ 1.17 may be assigned to the methyl on the carbon bearing the tertiary hydroxyl group. The ^{13}C NMR spectrum of compound B showed only one sp^2 carbon resonance at δ 218 (s) assignable to carbonyl group and one sp^3 carbon resonance at δ 73.5 (s) assignable to carbon bearing ter-

tiary hydroxyl group. Therefore, compound B is a ketohydroxy derivative of a saturated pentacyclic triterpenoid, probably of ursane or taraxastane skeleton. The methyl pattern in ^{1}H NMR of compound B indicated that it might be a taraxastane derivative. The mass spectrum of compound B showed no molecular ion but the heaviest fragment is at m/z 424 (M^+-18) which corresponds to the loss of a molecule of water from the molecular ion. The prominent mass fragment ions of saturated pentacyclic triterpenes (Budzikiewicz, Wilson, & Djerassi, 1963) at m/z 205 due to the cleavage of ring C indicates the carbonyl group in ring A/B and 189 containing the ring D/E with the loss of a molecule of water. The base mass fragment ion at m/z 371 [M^+-C_4H_7O] (**5**) and its formation can be explained by onium cleavage (Anjaneyulu et al., 1985). This fragment locates the tertiary hydroxyl in ring E at C-19 or C-20. However, the presence of Retro-Diel's-Alder fragment ion at m/z 82 in its mass spectrum fixes the hydroxyl at C-20 of ring E of the taraxastane skeleton. Based on the above spectral data, the structure 3-oxo-taraxastane-20(*R* or *S*)-ol (**6**) is assigned to compound B.

Dehydration of compound B with thionyl chloride gave colourless needles, m.p. 175–176°C identified as ψ-taraxastenone (**7**) by direct comparison with an authentic sample (Anjaneyulu & Row, 1965). On reduction with LAH it afforded a diol, m.p. 270–274°C, characterised as ψ-taraxastane-3β, 20-diol (**8**) by direct comparison with an authentic sample (Anjaneyulu et al., 1985) and also from ^{1}H NMR spectrum. Bhattacharya et al. (Hinge, Paknikar, Das, Bose, & Bhattacharya, 1966) reported the isolation of epi-ψ-taraxastanonol, m.p. 257–259°C, $[\alpha]_D$ +25.3° from Indian black dammar resin. Careful comparison of physical constants and spectral data of compound B and the reported ketol (**11**) revealed that these two were identical, the only difference being that the tertiary hydroxyl groups at C-20 were epimeric. Hence compound B is a new compound and named as ψ-taraxastanonol (**6**). The carbon resonances of compound B were assigned in Table 1 by comparing the carbon resonances with those of taraxasterol (Amarendra, Mukhopadhyay, & Mitra, 1981) and taraxarone (Sakurai, Yaguchi, & Inoue, 1987) and also taking C-20 hydroxy substitution effects into consideration.

The isolation of 6β-hydroxy stigmast-4-en-3-one (**9**) and 24-methylenecycloartane-3β,26-diol (**3**) are reported for the first time from the stem-bark of *M. indica* and its ^{13}C NMR data presented for the first time in Table 1.

Compound C has been isolated as semisolid and resisted crystallisation from several solvent systems. Attempts to separate the mixture into its constituents by column chromatography were unsuccessful. Therefore, it was acetylated with acetic anhydride–pyridine at room temperature for 24 h. Compound C acetate showed a ketonic carbonyl (1705 cm^{-1}), α,β-unsaturated acid carbonyl (1690 cm^{-1}) and a conjugated double bond (1640 cm^{-1}) besides a broad band at 1745 cm^{-1} for ester carbonyl in its IR spectrum. A close examination of its ^{1}H NMR spectrum revealed that it might be a mixture of at least two compounds. The presence of cyclopropane methylene protons at δ 0.35, 0.55 and 0.77 as three doublets indicated that it might be a mixture of 3-oxo (δ 0.55 and 0.77) and 3-acetoxy (δ 0.35 and 0.55) derivatives of cycloartane skeleton. It is further confirmed by the observation of two vinyl methyls (δ 1.82 and 1.85), two olefinic protons (δ 6.65 (t) and 6.70 (d)) and three acetoxy methyls (δ 2.05, 2.10 and 2.11). Further the ^{13}C NMR spectrum of compound C acetate displayed 3-acetoxy carbonyl signals (δ 171.1, 170.9 and 170.2), two carboxyl carbon resonances at δ 172.5 and 170.5 and one carbonyl carbon resonance (δ 216.6). Hence it was concluded that compound C acetate is a mixture of ketol monoacetate and diol diacetate of cycloart-24-en-26-oic acid.

The ^{13}C NMR data of compound C acetate was compared with those of mangiferonic acid (**10**) and 3α,22(*R* or *S*)-dihydroxy cycloart-24-en-26-oic acid (**11**) (Anjaneyulu et al., 1989) in Table 2. One set of carbon resonances are in close agreement with those of known **11**. The other set of carbon resonances of the nucleus were identical with those of **10** and those of the side chain differ from both those of **10** and **11**. Hence the second component of compound C may be a new keto hydroxy carboxylic acid. The position of the acetoxyl group in the side chain of the second component of compound C acetate is deduced as being at C-23 by the multiplicity of one of the olefinic protons as doublet at δ 6.70 in its ^{1}H NMR spectrum. The side chain carbon resonances of the second constituent of compound C acetate are assigned by placing the acetoxyl group at C-23 and taking substitution effects into consideration. Hence it is concluded that compound C is a mixture of a known **11** and a new 3-oxo-23(*R* or *S*)-hydroxycycloart-24-en-26-oic acid (**12**).

Compound D, m.p. 279–281°C analysed for $C_{30}H_{48}O_4$. Its IR spectrum showed absorption bands for hydroxyl (3600 cm^{-1}), α,β-unsaturated carboxyl (3300–2500, 1695 cm^{-1}) and conjugated double bond (1640 cm^{-1}). Its absorption at 217 nm in the UV region confirmed that it is an α,β-unsaturated carboxylic acid. The ^{1}H NMR spectrum of compound D exhibited characteristic ABq (δ 0.35 and 0.55) for cyclopropane methylene protons. A vinyl methyl at δ 1.86 as singlet, five methyls at δ 0.80, 0.85 and 0.95 and an olefinic proton as doublet at δ 6.60. It also showed an axial hydroxy methine proton at δ 3.20 as a multiplet and another hydroxy methine proton buried under the DMSO peak. The ^{13}C NMR spectrum showed two sp^3 resonances at δ 78.0 (d) and δ 66.5 (d) which confirmed the presence of two secondary hydroxyls in the molecule. Based on the above data, it was deduced that compound D is a dihydroxy derivative of cycloart-24-en-26-oic acid.

The mass spectrum of compound D showed no molecular ion, but the fragment ion at m/z 454 (M^+-H_2O)

Table 2
^{13}C NMR spectral data of compounds **10**, **11**, **11**a, **12**a, **13**, **14**, **15** and **4** (22.4 MHz, $CDCl_3$, TMS as internal standard)

C	**10**	**11**	**11**a	**12**a	**13**[a,b]	**14**[b]	**15**[a]	**4**
1	33.4	27.3	33.4	26.0	31.9	31.9	32.0	33.4
2	37.4	28.3	37.4	31.6	30.3	30.3	30.3	37.4
3	216.3	76.8	216.6	77.3	78.0	78.1	78.9	216.7
4	50.2	39.4	50.2	39.4	40.4	40.4	40.5	50.2
5	48.4	40.9	48.4	40.3	47.1	47.1	47.1	48.5
6	21.5	20.9	21.5	20.0	21.0	21.0	21.1	21.5
7	28.1	29.6	28.2	29.7	28.3	28.3	28.1	28.1
8	47.9	47.9	47.7	47.8	47.7	47.8	48.0	47.9
9	21.0	19.6	21.0	19.9	19.8	19.8	20.1	21.1
10	25.9	26.3	26.0	26.3	26.3	26.4	26.1	25.9
11	25.6	25.6	25.6	25.8	25.9	25.9	26.1	26.0
12	32.8	32.7	32.8	32.7	32.8	32.8	33.0	32.9
13	45.3	45.5	45.3	45.4	45.2	45.2	45.4	45.4
14	48.7	48.3	48.8	48.4	48.8	48.8	48.9	48.8
15	35.5	35.5	35.4	35.5	35.4	35.4	35.6	35.6
16	26.7	26.6	26.7	26.2	26.1	26.1	26.5	26.8
17	52.2	49.1	52.7	52.6	52.8	52.9	52.3	52.3
18	18.1	19.2	18.0	19.2	19.4	19.2	19.4	19.4
19	29.5	29.9	29.5	29.7	29.7	29.7	29.9	29.5
20	35.9	42.2	35.4	42.1	33.4	33.4	36.0	36.0
21	18.1	12.3	18.3	12.9	19.2	19.2	18.1	18.1
22	34.9	72.8	38.7	74.0	44.0	44.0, 43.1	34.6	34.6
23	22.2	27.1	69.0	25.4	66.5	66.6, 65.6	31.7	31.7
24	145.5	141.0	142.1	140.9	144.3	144.2, 145.6	148.7	148.6
25	126.7	129.0	128.2	130.1	128.2	128.2, 128.2	45.7	45.8
26	173.2	170.5	172.5	170.5	172.5	172.5, 170.5	179.7	180.3
27	12.0	11.9	12.5	12.5	12.9	12.9, 12.5	16.3	16.4
28	18.1	17.7	18.1	18.0	17.9	17.9	18.3	18.3
29	25.5	21.1	25.8	21.3	25.6	25.5	25.5	25.9
30	20.7	25.6	20.8	26.0	14.1	14.1	14.0	20.8
31							111.1	111.1
$\underline{C}H_3COO$			22.8					
			21.3					
			21.1					
—O—C(=O)—CH_3			17.2					
			170.9					
			170.1					

[a] Multiplicities as from DEPT spectrum.
[b] Spectra recorded in $CDCl_3$ + one drop of DMSO-d_6.

indicated the loss of a water molecule from it. Its mass spectral fragmentation exhibited characteristic of 9,19-cyclo triterpenoid skeleton (Alpin & Hornby, 1966; Audier et al., 1966). The characteristic fragment ions at *m/z* 332, 175 (332-side chain) are formed by the cleavage of ring B and loss of ring A and 315 (M^+-side chain) indicated the location of one hydroxyl group in the side chain and another hydroxyl in ring A/B. The multiplicity of the olefinic proton as a doublet at δ 6.60 in its ^{1}H NMR spectrum of compound D locates the hydroxyl at C-23 in the side chain. Thus the structure 3β,23(*R* or *S*)-dihydroxycycloart-24-en-26-oic acid (**13**) is assigned to compound D.

Previously **13** has been detected by ^{13}C NMR spectral data in a mixture along with 3β,22(*R* or *S*)-dihydroxycycloart-24-en-26-oic acid from the Neelum variety (Anjaneyulu et al., 1989) of *M. indica*. This is the first record of its isolation in the pure state. The carbon resonances of compound D (**13**) are assigned in Table 2.

Compound E, m.p. 240–242°C and found to be isomeric with compound D (**13**) from its elemental analysis. Its IR and UV data indicated it to be an α,β-unsaturated carboxylic acid. The ^{1}H NMR spectrum of compound E is almost identical with that of **13** except for the presence of an extra methyl at δ 1.92 and an olefinic proton at δ 6.75 (d) in the former. The doubling of the side chain carbon resonances C-22 to C-27 in the ^{1}H NMR spectrum of **13** and the multiplicities of two olefinic protons as doublets in the ^{1}H NMR spectrum indicated that it is a mixture of 23-epimers. Hence compound E is a 23-epi-

meric mixture of 3β,23-dihydroxycycloart-24-en-26-oic acid (**14**). The carbon chemical shifts of compound E were assigned by comparing with those of 3β,23(*R* or *S*)-dihydroxycycloart-24-en-26-oic acid (**13**) in Table 2. One set of side chain carbon resonances are in close agreement with those of **13** and another set differ slightly and were assigned to its 23-epimer. The 23-epimer of **13** is a new addition to the literature of triterpenoids.

Ambonic acid (**4**) and ambolic acid (**15**) are isolated for the first time from the stem-bark of *M. indica* and their ^{13}C NMR data were recorded for the first time in Table 2.

It is very interesting to note the co-occurrence of 3β,23(*R* or *S*)-dihydroxycycloart-24-en-26-oic acid (**13**), the C-23 epimers of 3β,23-dihydroxycycloart-24-en-26-oic acid (**14**) and 3-oxo-23(*R* or *S*)-hydroxycycloart-24-en-26-oic acid (**12**) in the Sarikhas variety of *M. indica*. The present investigation on stem-bark of this variety secured from West Bengal yielded eight triterpenoids containing 3-oxo group and six triterpenoids of 24-methylenecycloartane skeleton. Previous investigations on different varieties of *M. indica* in these laboratories afforded only one 24-methylenecycloartane derivative, 24-methylenecycloartane-3β,26-diol (**3**). The isolation of several 24-methylenecycloartane triterpenoids and co-occurrence of many pairs of 3-oxo triterpenoids and their corresponding reduced hydroxy derivatives from Sarikhas variety is of some chemotaxonomic significance.

3. Experimental

M.p.s: uncorr. The plant material was secured from M/s United Chemical and Allied Products, Calcutta. The compounds were dried for analysis at room temp. or 100°C, 0.2 mm^{-1} Hg for 6 h. ^{1}H NMR, ^{13}C NMR: 90 and 22.4 MHz (JEOL), respectively, with TMS as an int. standard; IR: Shimadzu model 408 spectrophotometer; UV: Milton Roy Spectronic 1201; CC: silica gel (100–200 mesh, Acme).

3.1. Extraction and isolation

The dried and powdered stem-bark (8 kg) was extracted with *n*-hexane (b.p. 60–80°C) and MeOH in a big aspiratory bottle. The dark brown coloured *n*-hexane extract was evapd under red. pres. and the resultant gummy residue (194 g) was separated into acidic, phenolic and neutral fractions (Anjaneyulu et al., 1985). The neutral fraction (110 g) was chromatographed over a column of silica gel (900 g) and successively eluted 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 Tables 3 and 4.

Fraction A showed a broad single spot on TLC and was further rechromatographed over silica gel column. Elution with *n*-hexane–Me_2CO (9.9:0.1) afforded compound **1**. Further elution with *n*-hexane–Me_2CO (9.8:0.2) yielded compound **2** and 11α,12α-oxido taraxerol. Identification of the known compounds was based on their physical and spectroscopic characteristics and by direct comparison (TLC, ^{1}H NMR, mmp) with authentic samples wherever possible.

3.2. Compound A: 25(R)-3-oxo-24-methylenecycloartan-26-ol(*1*)

Recrystallised from $CHCl_3$–MeOH as colourless needles, m.p. 145–146°C, $[\alpha]_D^{30}$ +175° ($CHCl_3$; *c* 0.6) (found: C, 81.82; H, 11.10; requires: C, 81.94; H, 11.01%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1640, 890. ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.57, 0.75 (2H, ABq, J=4 Hz, H_2-19), 0.82 (3H, s), 0.92 (3H, s), 0.97 (3H, d, J=6 Hz), 1.06 (3H, d, J=7 Hz), 1.10 (6H, s), 3.51, 3.59 (each 1H, two br s, H_2-26), 4.82, 4.88 (each 1H, two br s, H_2-31). MS *m*/*z* (rel. int): M^+ 454 (3), 354 (3), 316 (3), 313 (11), 216 (3), 175 (15), 140 (23), 121 (38), 100 (46), 95 (75.8), 69 (65), 55 (100).

3.3. LAH reduction of compound A

To a cooled soln of LAH (50 mg) in dry THF (10 ml), a soln of compound A (20 mg) in THF (10 ml) was added drop by drop. The reaction mixture was stirred under cooling for 1 h and then refluxed for 6 h. Unreacted LAH was removed by adding moist Et_2O and then by H_2O. Removal of the solvent and crystallisation from benzene gave 24-methylenecycloartane-3β,26-diol (**3**) as colourless long needles (30 mg), m.p. 151–152°C; $[\alpha]_D^{30}$ +52° ($CHCl_3$; *c* 1). IR ν_{max}^{KBr} cm^{-1}: 3500, 895. ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.30, 0.54 (2H, ABq, J=4 Hz, H_2-19] 0.77, 0.89, 0.94, 0.98, 1.06 (18H, 6×Me), 1.61 (1H, s, –OH), 3.22 (1H, m, H-3α), 3.48 (2H, d, J=7 Hz, H_2-26), 4.80 (2H, d, J=5 Hz, H_2-31).

3.4. Chromium trioxide oxidation of compound A

Compound A (30 mg) in benzene was stirred with chromium trioxide (30 mg) in A.R acetic acid (5 ml) and H_2O (1 ml) for 3 h at room temp. The usual workup followed by crystallisation from $CHCl_3$–MeOH to give ambonic acid (**4**) as shining plates (20 mg), m.p. 150–152°C; $[\alpha]_D^{30}$ +9.6° ($CHCl_3$; *c* 1.5). IR ν_{max}^{KBr} cm^{-1}: 1735, 1701, 1650, 900. ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.55, 0.75 (2H, ABq, J=4 Hz, 19-H_2), 0.90, 1.0, 1.05, 1.10 (15H, 5×CH_3), 1.27 (3H, d, J=7 Hz, H_3-26), 3.12 (1H, q, J=4 Hz, H-25), 4.85, 4.92 (each 1H, two br s, H_2-31).

3.5. Compound B: 3-oxo taraxastan-20(R or S)-ol(*6*)

Recrystallised from $CHCl_3$–MeOH as shining needles, m.p. 275–278°C; $[\alpha]_D^{30}$ −9.0°; (found: C, 81.3; H, 11.1. calc. for $C_{30}H_{50}O_2$; C, 81.45; H, 11.31%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}:

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

Eluent	Fractions	Compound	Yield (g)
n-Hexane	1–17	waxes	6.0
n-Hexane–C_6H_6 (9:1)	18–21	cycloartenone	0.5
n-Hexane–C_6H_6 (9:1)	22–40	cycloartenone and 24-methylenecycloartanone	3.1
n-Hexane–C_6H_6 (4:1)	41–44	friedelin	0.12
n-Hexane–C_6H_6 (4:1)	45–46	taraxerone	0.08
n-Hexane–C_6H_6 (3:2)	47–49	friedelan-3β-ol	0.07
n-Hexane–C_6H_6 (2:3)	50–64	α-amyrin, β-amyrin	1.2
n-Hexane–C_6H_6 (2:3)	65–87	cycloartenol	2.6
n-Hexane–C_6H_6 (2:3)	88–93	24-methylenecycloartanol	0.5
n–Hexane–C_6H_6 (1:4)	94–114	β-sitosterol	2.1
C_6H_6	115–129	fraction A	1.9
C_6H_6	130–134	6β-hydroxystigmast -4-en-3-one	0.2
C_6H_6–EtoAc (9:1)	135–146	24-methylenecycloartane -3β,26-diol	0.3
C_6H_6–EtoAc (9:1)	147–152	intractable gum	–
C_6H_6–EtoAc (4:1)	153–158	C-24 epimeric mixture of cycloartane-3β,24,25-triol	0.18
C_6H_6–EtoAc (3:2)	159–165	uncharacterised	0.005
C_6H_6–EtoAc (2:3)	166–170	intractable gum	–
EtoAc	171–182	intractable gum	–

Table 4
Triterpenoid acids isolated from the acidic fraction of the *Mangifera indica* extract

Eluent	Fractions	Compound	Yield (g)
n-Hexane	1–10	fatty acids	–
n-Hexane–C_6H_6 (1:1)	11–16	intractable gum	–
C_6H_6	17–42	mangiferonic acid	2.8
C_6H_6–EtoAc (19:1)	43–47	ambonic acid	0.6
C_6H_6–EtoAc (9:1)	48–67	isomangiferolic acid	2.4
C_6H_6–EtoAc (4:1)	68–97	mangiferolic acid	3.2
C_6H_6–EtoAc (7:3)	98–105	mangiferolic acid, ambolic acid	0.08
C_6H_6–EtoAc (3:2)	106–115	compound 3	0.03
C_6H_6–EtoAc (2:3)	116–120	compound 4	0.08
C_6H_6–EtoAc (1:4)	121–140	compound 5	0.04
EtoAc	141–150	intractable gum	–

3600, 1695. ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.89 (3H, d, $J=7$ Hz), 0.97 (6H), 1.03 (3H), 1.07 (9H), 1.17 (3H). MS m/z (rel. int.): M^+ 424 (25), 371 (35), 205 (30), 189 (11), 151 (36), 123 (35), 95 (58), 82 (58), 43 (100).

3.6. Dehydration of compound B with thionyl chloride

Compound B (20 mg) in dry pyridine (5 ml) was cooled in ice and freshly distilled thionyl chloride (0.5 ml) was added drop-wise followed by stirring. The mixture was stirred for 10 min. The contents were poured into water and extracted with Et_2O. The Et_2O extract was dried (over anhydrous Na_2SO_4). Removal of the solvent and crystallisation from $CHCl_3$–MeOH gave ψ-taraxastenone (**7**) as colourless needles (15 mg), m.p. 175–176°C, $[\alpha]_D^{30}$ +81° ($CHCl_3$, *c* 1.2).

3.7. LAH reduction of compound B

To a cooled soln of LAH (50 mg) in dry THF (10 ml) a solution by compound B (20 mg) in THF (10 ml) was added drop by drop. The mixture was cooled with constant stirring for 1 h and then refluxed for 5 h. The unreacted LAH is decomposed by adding H_2O. Removal of solvent and crystallisation from benzene gave ψ-taraxastan-3β,20-diol (Anjaneyulu et al., 1985) as colourless plates (30 mg), m.p. 272–274°C; $[\alpha]_D^{30}$ −10.2° ($CHCl_3$, *c* 0.5). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480 (br). ^{1}H NMR (100 MHz,

$CDCl_3$): δ 0.80, 0.83, 0.90, 0.92, 0.96, 1.05, 1.07 (24 H, $8 \times CH_3$), 3.19 (1H, m, H-3α).

*3.8. Compound C: mixture of 3α,22(*R *or* S*)-dihydroxycycloart-24-en-26-oic acid (**11**) and 3-oxo-23(*R *or* S*)-dihydroxycycloart-24-en-26-oic acid (**12**)*

It was contaminated with gummy material and showed a streak on TLC and resisted crystallisation in various solvent systems. Hence it was acetylated. Compound C (25 mg) in pyridine (1 ml) was treated with Ac_2O (2 ml) and kept overnight at room temp. After the usual workup, it afforded a semisolid (20 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300–2500, 1745 (br), 1705, 1690 and 1640. UV $\lambda_{max}^{CHCl_3}$ 217 nm ($\varepsilon = 9800$). ^1H NMR (90 MHz, $CDCl_3$): δ 0.35, 0.55, 0.77 (three d, $J = 3.6$ Hz), 1.81 (br.s), 1.82, 1.85 (two s, each 3H), 2.05, 2.10, 2.11 (3H, s), 4.75 (1H, br s), 5.0 (1H, d, $J = 3$ Hz) 5.65 (1H, t, $J = 8$ Hz, H-23), 6.65 (1H, t, $J = 8$ Hz), 6.7 (1H, d, $J = 8$ Hz).

*3.9. Compound D: 3β,23(*R *or* S*)-dihydroxycycloart-24-en-26-oic acid (**13**)*

It was recrystallised from $CHCl_3$–MeOH as shining crystals, m.p. 279–281°C, $[\alpha]_D^{30}$ +49° (MeOH, *c* 0.52) (found: C, 96.75; H, 12.88 requires: C, 96.77; H, 12.9%). IR ν_{max}^{KBr} cm^{-1}: 3600, 3300–2500, 1695 and 1640; UV λ_{max}^{EtOH} 217 nm. ^1H NMR (90 MHz, $CDCl_3$ + a drop of DMSO-d_6): δ 0.35, 0.55 (2H, ABq, $J = 4$ Hz, H_2-19), 0.80, 0.85, 0.95 (15 H, $5 \times$ Me), 1.86 (3H, s, H_3-27), 3.20 (1H, m, H-3β), 4.4–4.7 (1H, broad, buried under DMSO peak, H-23), 6.60 (1H, d, $J = 4.5$ Hz, H-24). MS *m*/*z* (rel. int): 454 (2), 439 (2), 332 (6), 315 (3), 297 (7), 175 (7).

*3.10. Compound E: 23-epimeric 3β,23-dihydroxycycloart-24-en-26-oic acid (**14**)*

It was recrystallised from $CHCl_3$–MeOH as shining crystals, m.p. 240–242°C (found: C, 96.6; H, 12.8 calc. for $C_{30}H_{48}O_4$: C, 96.77; H, 12.9%). IR ν_{max}^{KBr} cm^{-1}: 3590, 3200–2500, 1690, 1635. UV λ_{max}^{EtOH} 218 nm. ^1H NMR (90 MHz, $CDCl_3$ + 1 drop of DMSO-d_6): δ 0.35, 0.55 (2H, ABq, $J = 4$ Hz, H_2-19), 0.75, 0.80, 0.90, 0.95 (15H, $5 \times$ Me), 1.85, 1.92 (each 3H, two br.s, 27-H_3), 3.2 (1H, m, H-3β), 4.55 (1H, m, H-23), 6.65, 6.75 (each 1H, two d, $J = 4.5$ Hz, H-24).

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