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TRITERPENOIDS FROM *MANGIFERA INDICA*

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

Abstract—From the neutral fraction of the *n*-hexane extract of stem bark of *Mangifera indica*, (var./cv Banganpalli) six new tetracyclic triterpenoids, cycloart-24-ene-3β,26-diol, the C-24 epimers of cycloart-25-ene-3β,24,27-triol, the C-24 epimers of cycloartane-3β,24,25-triol and 3-ketodammar-24*E*-ene-20*S*,26-diol together with the known compounds cycloartenol, α-amyrin, β-amyrin, sitosterol, 3β-hydroxycycloart-25-en-26-al, dammarenediol II, the C-24 epimers of cycloart-25-ene-3β,24-diol, 24-methylenecycloartane-3β,26-diol, ψ-taraxastane-3β,20-diol and ocotillol II have been isolated. The acidic fraction of the same extract, on esterification with diazomethane followed by chromatography, yielded methyl mangiferonate, methyl isomangiferolate, methyl mangiferolate and the diazomethane adduct of methyl mangiferolate. The structures were elucidated by spectroscopic and chemical methods.

INTRODUCTION

Previous investigations of *Mangifera indica* L. reported the presence of free sugars, phenolic compounds and mono-, sesqui- and triterpenoids in the, e.g. leaves [1–3], fruits [4], flowers [5], bark [6] and resin [7–13] of this species.

As part of our continued interest in the triterpenoids of the plants belonging to the Anacardiaceae, we undertook a systematic chemical examination of *M. indica* [14] with the objective of isolating the triterpenoid components and establishing their pattern of biogenesis in this plant. In this paper we report six new and several known triterpenoids from the stem bark of *M. indica*.

RESULTS AND DISCUSSION

Compound A was crystallized from methanol as colourless shining crystals, mp 107–111°. Its IR spectrum showed absorption bands for a cyclopropane methylene (3042 cm^{-1}), an acetate carbonyl (1735 cm^{-1}) and a terminal methylene (890 cm^{-1}). Its ^1H NMR spectrum suggested that it was a mixture. Its high resolution mass spectrum gave ions at *m/z* 540.4179 ($C_{35}H_{56}O_4$) and 526.4034 ($C_{34}H_{54}O_4$) indicating that it was a mixture of homologues. In fact, three compounds, namely the C-24 epimers of cycloart-25-ene-3β,24-diol diacetate (**1a**) and 24-methylenecycloartane-3β,26-diol diacetate (**1b**) were shown to be present by a thorough examination of its DEPT spectrum (Table 1). This was confirmed by GC which revealed three peaks with R_ts 10.89, 10.99 and 14.86 min. Under the same conditions the R_ts of the authentic C-24 epimers of cycloart-25-ene-3β,24-diol diacetate (**1a**) were 10.87 and 11.02 min. These C-24 epimers were first isolated from *Euphorbia trigona* [15].

The presence of compounds **1a** and **1b** was further confirmed by difference mass spectroscopy. Substraction of the mass spectrum of the authentic mixture of C-24 epimers of **1a** from that of compound A afforded the spectrum of the higher homologue, 24-methylenecycloartane-3β,26-diol diacetate (**1b**).

The physical separation of compounds **1a** and **1b** was not achieved, but the above spectroscopic data leave no doubt as to the structure of the components of compound A. 24-Methylenecycloartane-3β,26-diol has been previously isolated from the resin of *M. indica* by Corsano and Mincione [12]. The C-24 epimers of cycloart-25-ene-3β,24-diol have not been found previously in *M. indica*. It is interesting to note that compound A, even though a mixture of **1a** and **1b**, melts at a higher temperature than that of 24-methylenecycloartane-3β,26-diol diacetate (**1b**), mp 83–86° [16].

Compound B, mp 154–155°, was crystallized as colourless shining needles from benzene and analysed for $C_{30}H_{50}O_2$ ($[M]^+$ at *m/z* 442). Its IR spectrum exhibited bands for hydroxyl (3400 cm^{-1}) and a cyclopropane methylene (3042 cm^{-1}). The ^1H NMR spectrum revealed the presence of an olefinic proton [δ5.33 (*t*, *J* = 7 Hz)], a secondary hydroxyl group [δ3.23 (*m*, H-3)] and an allylic primary alcohol [δ3.96 (*br s*, H_2-26)]. The ^1H NMR spectrum also showed an ABq (*J* = 4 Hz) at δ0.53 and 0.32, characteristic of a cyclopropane methylene, in addition to a vinylic methyl (δ1.65) and five methyls between δ0.95 and 0.80. Acetylation afforded the diacetate **2a** [δ4.4 (*m*, H-3), and 4.31 (*br s*, H_2-26)]. From the above ^1H NMR data it was deduced that compound B is a dihydroxy 9,19-cyclotriterpenoid. The mass spectral fragmentation [17, 18] suggested the presence of a OH-3 (*m/z* 315 and 297) and a second hydroxyl in the side chain (*m/z* 302 and 175).

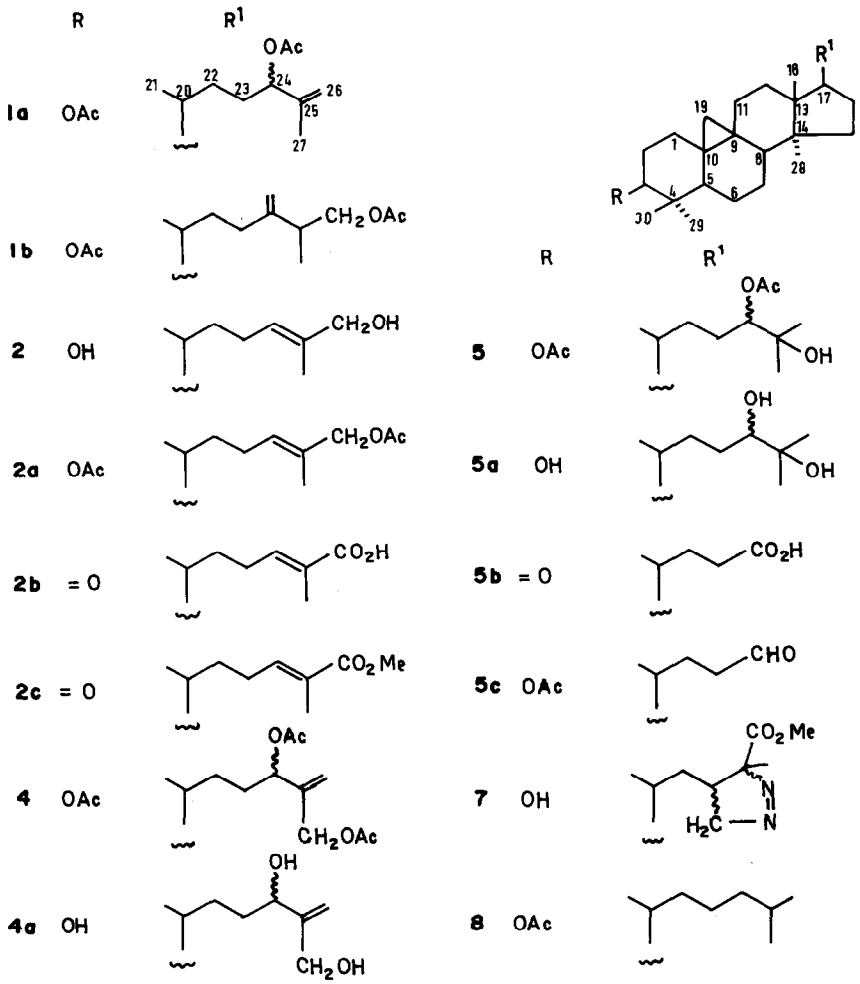
Oxidation of B with chromium trioxide–acetic acid in benzene gave a keto acid, mp 186–188°, $[\alpha]_D$ +24°, which was readily identified as mangiferonic acid (**2b**) (mmp, co-TLC and co-IR). The ^{13}C NMR chemical shifts of the corresponding methyl ester, methyl mangiferonate (**2c**), are listed in Table 1. It follows, therefore, that compound B is cycloart-24-ene-3β,26-diol (**2**). In the ^{13}C NMR spectrum of compound B (Table 1), the olefinic carbons

Table 1. ^{13}C NMR signals of compounds A (**a**, **1b**), B (**2**), D (**4**), E (**5**), (**5a**), (**2c**), (**5b**), G (**7**) and (**8**) (deuterochloroform, TMS as int. standard)

Carbon No.	**1a, 1b***	**2**	**4***	**5***	**5a***	**2c**	**5b**	**7***	**8**
1	31.7	32.0	31.6	31.6	31.9	33.4	33.4	32.3	31.5
2	26.8	30.4	26.8	26.8	30.3	37.4	37.4	30.8	26.7
3	80.6	78.8	80.6	80.6	78.8	216.3	216.7	78.9	80.3
4	39.4	40.5	39.4	39.4	40.9	50.2	50.2	40.8	39.4
5	47.1	47.2	47.1	47.1	47.0	48.4	48.4	47.5	47.0
6	21.0	21.1	20.9	20.9	21.1	21.5	21.5	21.5	20.8
7	28.0	28.2	28.0	28.0	28.1	28.1	28.0	28.4	28.0
8	47.8	48.0	47.8	47.8	48.0	47.9	47.9	48.4	47.6
9	20.1	20.0	20.1	20.1	19.9	21.0	21.0	20.3	20.1
10	25.9	26.1	25.9	25.9	26.0	25.9	25.8	26.5	26.0
11	25.8	26.1	25.8	25.8	26.0	25.6	25.8	26.4	25.8
12	35.5	35.6	35.5	35.5	35.5	35.5	35.6	35.9	35.4
13	45.3	45.4	45.3	45.3	45.3	45.3	45.3	45.6	45.1
14	48.8	48.8	48.8	48.8	48.8	48.7	48.7	49.2	48.6
15	32.8	33.0	32.8	32.8	32.9	32.9	32.8	33.2	32.8
16	26.5	26.5	26.4	26.4	26.4	26.7	26.6	26.7	26.5
17	52.1	52.3	52.1	52.1	52.4	52.2	52.1	52.5	52.2
			52.0		52.3			52.4	
18	17.9†	18.1†	17.9†	18.0†	18.1†	18.1†	18.0†	18.2†	17.9†
19	29.7	29.9	29.7	29.7	29.9	29.5	29.5	30.1	29.6
20	36.8	36.0	35.8	36.7	36.4	35.9	37.0	36.7	36.0
			35.7	36.3	35.9			36.5	
21	18.3†	18.3†	18.2†	18.4†	18.4†	18.1†	18.0†	18.5†	18.3†
				18.1†	18.1†			18.3†	
22	31.6	36.0	30.1	32.7	33.5	34.9	37.4	35.9	36.4
			29.9	32.3	33.1			35.8	
23	34.7	25.1	31.6	26.4	28.7	22.2	22.2	25.2	24.1
	29.4			26.1	28.4				
	29.1								
24	151.6	127.0	75.5	80.8	79.6	143.1	179.8	40.0	39.4
	78.1		74.9	80.1	78.2			39.7	
	77.6								
25	143.0	134.4	142.4	72.5	73.2	127.1	—	94.1	28.0
	36.0		142.0		73.2			93.9	
26	113.1	69.0	115.6	25.0	23.2	168.7	—	15.1	22.5
	112.4		115.2	24.9	23.1			14.9	
	68.1								
27	18.2	13.7	63.8	25.8	26.5	12.3	—	172.5	22.7
	17.8		64.0	24.9	26.5				
	17.0								
28	19.3†	19.4†	19.3†	19.3†	19.3†	19.3†	19.2†	19.4†	19.2†
29	25.4	25.1	25.4	25.4	25.4	25.9	25.8	25.6	25.3
30	15.1	14.0	15.1	15.1	14.0	20.7	20.8	14.2	15.1
31	109.0	—						81.9	
								81.6	
O<u>C</u>OMe	171.1	—	171.0	171.3	—	—	—	—	170.0
	170.9		170.6	171.0					
			170.2						
OCO<u>M</u>e	21.3	—	21.3	21.3	—	—	—	—	21.2
	21.2		21.1	21.0					
			20.9						
COO<u>M</u>e	—	—	—	—	—	51.6	—	52.9	—

*Multiplicities assigned from DEPT spectrum.

†Assignments in any vertical column may be interchanged.



occur at δ127.0 (*d*) and 134.4 (*s*) and the carbons bearing oxygen at δ78.8 (*d*) and 69.0 (*t*). The chemical shift of the Me-27 (δ13.7) clearly supports the *E*-configuration of the double bond, since it is shielded by steric interaction with the C-23 methylene. Cycloart-24-ene-3β,26-diol (**2**) has been reported as a synthetic product obtained by lithium aluminium hydride reduction of mangiferolic acid [7] and also by sodium borohydride reduction of 3β-hydroxy-24-cycloarten-26-al [13]. This is the first report of its occurrence as a natural product.

Compound C was crystallized from methanol as colourless needles, mp 270–273° and analysed as $C_{30}H_{50}O_2$. The ^{1}H NMR spectrum lacked olefinic protons and displayed a multiplet at δ3.18 (H-3), a singlet at δ1.58, which disappeared upon the addition of D_2O and eight methyls between δ0.76 and 1.07. It formed a monoacetate (**3a**), mp 278–280° [δ4.42 (*m*, H-3)], whose IR spectrum showed the presence of an acetate carbonyl (1730 cm^{-1}) and a hydroxyl (3500 cm^{-1}). The above data indicated that compound C was a saturated pentacyclic triterpenoid containing two hydroxyl groups, one secondary and one tertiary. The low field shift of one methyl signal (δ1.07) suggested that it was due to a methyl on a carbon bearing the tertiary hydroxyl.

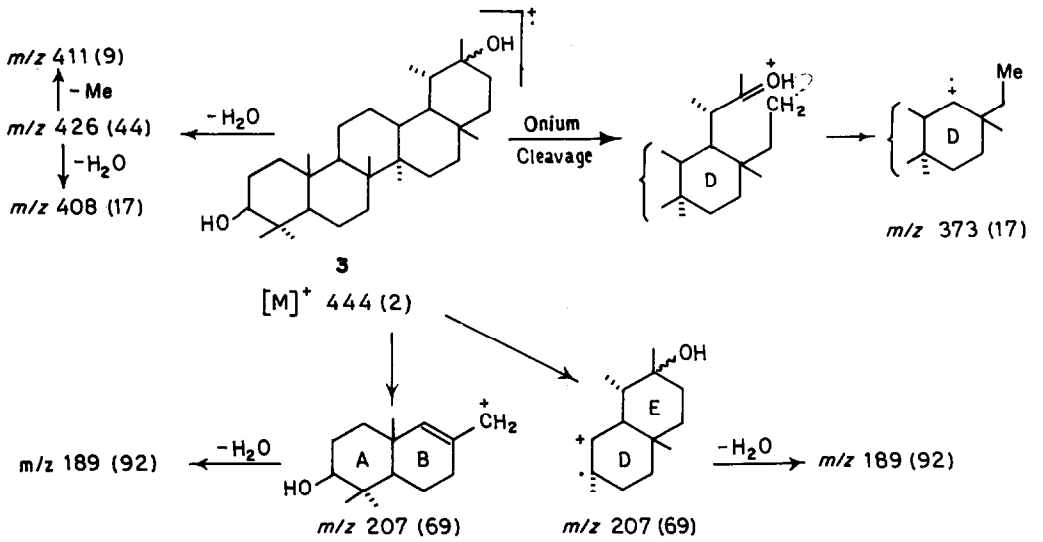
Dehydration of **3a** with thionyl chloride in pyridine gave a compound, mp 246–248°, readily identified as ψ-taraxasteryl acetate (**3b**) by ^{1}H NMR, mmp and co-TLC with authentic ψ-taraxasteryl acetate. Thus, compound C must be ψ-taraxastane,3β,20-diol (**3**).

In the literature, two C-20 epimers of ψ-taraxastane-3β,20-diol have been reported. Morice and Simpson [19, 20] isolated ψ-taraxastane diol, mp 270–272°, $[\alpha]_D$ −10.9°, from *Manila elemi* resin and Hinge *et al.* [21, 22] isolated its C-20 epimer, epi-ψ-taraxastane diol, mp 261–263°, $[\alpha]_D$ ±0°, from Indian black dammar resin. Comparison of the physical characteristics of **3** and **3a** with those of the literature compounds and their acetates indicated that compound C is ψ-taraxastane diol (**3**).

In the mass spectrum of compound C the molecular ion was observed at *m/z* 444 and the ions corresponding to loss of water, *m/z* 426, 408, were also observed. The presence of an ion at *m/z* 207 and the corresponding ion due to the loss of water at *m/z* 189 clearly showed the presence of one hydroxyl on ring A or B and the other hydroxyl on ring D or E. The ion at *m/z* 373 [M $-C_4H_7O]^+$ is significant and its formation can be explained by onium cleavage as shown in Scheme 1. The ion at *m/z* 373 locates the second hydroxyl in ring E at C-19 or C-20.

ψ-Taraxastane diol (**3**) has not been isolated previously from *M. indica* and this is the second report of its natural occurrence.

Compound D was crystallized from methanol as colourless buttons, mp 94–96°, and analysed as $C_{36}H_{56}O_6$



Scheme 1. Mass spectral fragmentation of compound C (3).

([M]$^+$ at m/z 584.4052). Its IR spectrum showed the presence of a cyclopropane methylene (3042 cm^{-1}), acetate carbonyls (1735 cm^{-1}) and a terminal methylene (890 cm^{-1}). The ^{1}H NMR spectrum displayed resonances for a cyclopropane methylene [δ0.32 and 0.56 (ABq, J = 4.2 Hz)], five methyls (δ0.83–0.94), three acetates (δ2.04, 2.05 and 2.08), two secondary [δ4.57 (m, H-3) and 5.22 (m, H-24)] and one primary [δ4.59 (*br s*, H_2-27)], and a terminal methylene [δ5.22 (*s*, H_2-26)]. Hydrolysis of compound D afforded the triol **4a** [δ3.25 (m, H-3) and 4.25 (m, H-24 and H-27)]. These data indicated that compound D is a triacetoxy cycloartene derivative.

The mass spectral fragmentation of compound D [17, 18] suggested the presence of one OAc-3 (m/z 455 and 357) and two acetoxyls in the side chain (m/z 402, 343, 283 and 175). The low field shift (δ5.22) of the side chain secondary acetoxyl proton suggested it was allylic. This can be rationalized by placing one secondary acetoxyl at C-24 in a cycloart-25-ene system, the remaining secondary acetoxyl at C-3 and the primary acetoxyl at C-27. Thus, compound D was assigned the structure cycloart-25-ene-3β,24,27-triol triacetate (**4**).

The ^{13}C NMR spectrum of compound D showed doubling of certain resonances (Table 1). Comparison of the ^{13}C NMR chemical shifts with those of cycloartanyl acetate (**8**) [23] revealed doubling of the resonances associated with C-24 and its adjacent carbons C-17, C-20, C-22 and C-25–C-27. The doubling of the signals for these carbons is consistent with the presence of a mixture of C-24 epimers in compound D. Biosynthetically, compound D may arise from the C-24 epimers of cycloart-25-ene-3β,24-diol, which accompany it in *M. indica* by hydroxylation of the side chain terminal methyl.

Compound E was crystallized from *n*-hexane as colourless shining plates, mp 160–163° and analysed as $C_{34}H_{56}O_5$ ([M]$^+$ at m/z 544.4153). Its IR spectrum exhibited bands for hydroxyl (3600 cm^{-1}), cyclopropane methylene (3040 cm^{-1}) and acetate carbonyl (1735 cm^{-1}) absorption. Its ^{1}H NMR spectrum revealed the presence of a cyclopropane methylene [δ0.32 and 0.55 (ABq, J = 4 Hz)], seven methyls (δ1.17–0.82) and two secondary acetates [δ2.02, 2.08, 4.55 (m, H-3) and 4.75 (m, H-24)]. Of the seven methyl groups in the ^{1}H NMR spectrum, two were at appreciably lower field (δ1.17) than the others. The only structural feature capable of such deshielding was oxygen substitution on the carbon atom bearing these methyl groups. Thus, compound E is a cycloartane derivative with two secondary acetates and one tertiary hydroxyl. The mass spectral fragmentation [17, 18] suggested the presence of one OAc-3 (m/z 455, 357 and 297) with the second acetoxyl and tertiary hydroxyl in the side chain (m/z 302 [362 – 360]$^+$ and 175).

Alkaline hydrolysis of compound E yielded a triol (**5a**), $C_{30}H_{52}O_3$, mp 154–156° [δ3.26 (m, H-3, H-24)]. The triol gave a positive periodic acid test for 1,2-glycols. On the above evidence, compound E was assigned the structure of cycloartane-3β,24,25-triol diacetate (**5**). The ^{13}C NMR of compound E (**5** and **5a**) (Table 1) showed doubling of the resonances associated with C-24 and adjacent carbons. The doubling of these signals is consistent with the presence of a mixture of C-24 epimers in **5** and **5a**.

The presence of C-24 epimers was confirmed by oxidation of **5a** with chromium trioxide–acetic acid in benzene to give a keto acid, mp 187–190°, which was readily identified as 25,26,27-trisnor-3-ketocycloartan-24-oic acid (**5b**) [24] by IR, ^{1}H NMR and ^{13}C NMR spectra. As expected, no doubling of resonances in the side chain was observed in its ^{13}C NMR spectrum (Table 1).

The structure **5a** was further confirmed by sodium metaperiodate oxidation followed by acetylation to give a compound, mp 155–157°, identical in all respects with an authentic sample of 25,26,27-trisnor-24-oxocycloartanyl acetate (**5c**), kindly supplied by Dr. Sukh Dev.

24*R*- and 24*S*-Cycloartane-3β,24,25-triols have been reported as synthetic products obtained by *m*-chloroperbenzoic oxidation of cycloartenyl acetate, followed by cleavage of the resulting epimeric epoxides with dilute sulphuric acid–tetrahydrofuran [25]. This is the first report of their occurrence as natural products.

Compound F was crystallized from *n*-hexane as colourless shining crystals, mp 109–111° and analysed as $C_{32}H_{52}O_2$. Its IR spectrum (γ_{max} cm^{-1}: 3620, 1708 and

1741) indicated that it was a ketodiol monoacetate. The ^{1}H NMR spectrum showed signals for five tertiary methyls (δ1.08, 1.05, 1.00, 0.95 and 0.89), a methyl on a carbon bearing oxygen (δ1.15), a vinyl proton [δ5.42 (*t*, *J* = 7 Hz)], a methylene adjacent to a carbonyl [δ2.46 (2H, *m*)], a primary acetate [δ2.05, 4.42 (2H, *br s*)] and a vinyl methyl (δ1.65). The pattern of the tertiary methyls suggested a dammarane derivative [26].

Alkaline hydrolysis of compound F gave the ketodiol **6a**, $C_{30}H_{50}O_3$, which crystallized from benzene as colourless shining needles, mp 115–118° [δ4.0 (2H, *br s*)]. It exhibited IR absorption bands for hydroxyl (3400 cm^{-1}) and cyclohexanone (1710 cm^{-1}).

Analysis of the mass spectrum of compound F (Scheme 2) revealed the nature of the tetracyclic skeleton. Cleavage of ring C gave a peak at *m/z* 205 which limited the choice of carbon skeleton to the dammarane group and excluded the euphane and lanostane skeletons [21, 27]. Rupture of the C-17–C-20 bond gave a peak at *m/z* 315 for the tetracyclic part of compound F [28]. Allylic cleavage of the C-22–C-23 bond was not observed but, surprisingly, cleavage of the C-20–C-22 bond was indicated by the presence of fragments at *m/z* 359.2941 ($C_{24}H_{39}O_2$) and *m/z* 141.0926 ($C_8H_{13}O_2$). The cleavage of the C-20–C-22 bond can be explained by an onium cleavage. This type of cleavage has not been observed previously in the mass spectral fragmentation of dammaranes.

The above data suggested that compound F is 3-ketodammar-24-ene-20,26-diol-26-monoacetate. This was further supported by oxidation of **6a** with chromic acid–acetic acid to give the ketotrisnorlactone (**6b**) [29] identical in all respects with an authentic sample prepared from ocotillol.

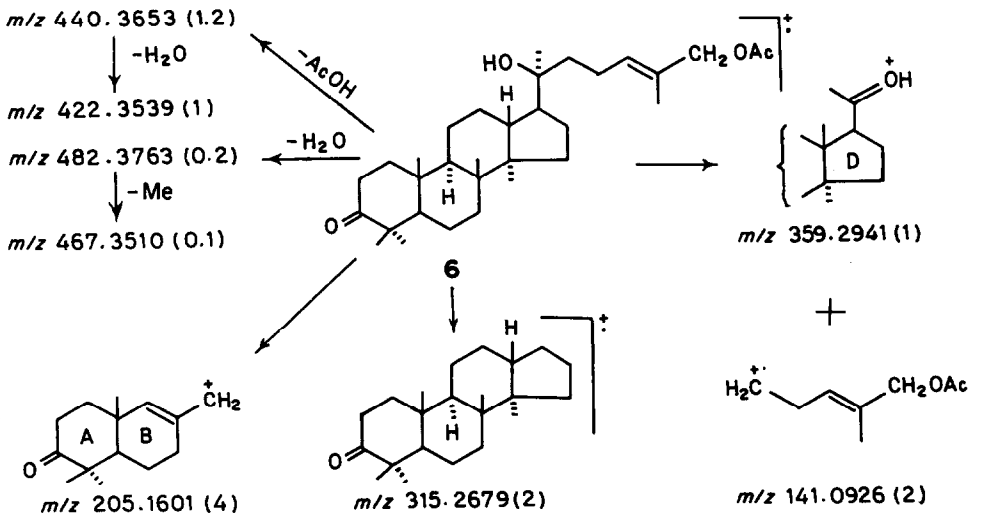
A comparison of the ^{13}C NMR shifts of compound F with those of the hydroxy dammarenones I (**9**) and II (**9a**) [30] is made in Table 2. The chirality at C-20 and the configuration of the double bond follow from the ^{13}C NMR spectrum. The chemical shifts of C-20–C-22 are similar to those of **9a**, indicating that the configuration at C-20 is *S*. The signals at δ129.8 (*d*) and 130.0 (*s*) confirmed the presence of a Δ^{24}-double bond. The singlet resonances at δ50.2, 47.4, 40.2 and 36.8 (C-14, C-4, C-8 and C-10, respectively) in conjunction with a ketonic carbonyl at δ218.0 are consistent with a 3-keto-dammarane skeleton for compound F. The signals at δ70.2 (*t*) and 13.9 (*q*) can be attributed, respectively, to C-26 and a vinylic methyl (C-27), thus characterizing the nature of the side chain. The chemical shift of the Me-27 (δ13.9) supports the *E*-configuration of the double bond since it is shielded by steric interaction with the C-23 methylene, as in the case of cycloart-24-ene-3β,26-diol (**2**). Thus, compound F is 3-ketodammar-24*E*-ene-20*S*,26-diol-26-monoacetate (**6**).

Compound G, $C_{32}H_{52}O_3N_2$, mp 159–163° (IR ν_{max} cm^{-1}: 3400, 1735) had signals in its ^{1}H NMR spectrum for six methyl groups (δ1.25–0.75), a cyclopropane methylene [δ0.32 and 0.55 (AB*q*, *J* = 4 Hz)], a secondary hydroxyl group [δ3.15 (*m*, H-3)], a carbomethoxyl (δ3.7) and a methylene group attached to nitrogen [δ4.66 (*dd*, *J* = 17, 8 Hz) and 3.82 (*dd*, *J* = 17, 8 Hz)]. These data suggest that compound G is the diazomethane adduct of methyl mangiferolate, which is presumably formed during esterification of the crude acidic extract. The ^{13}C NMR spectrum of compound G supports this structural assignment and shows that a mixture of C-24 and C-25 epimers is present. The mass spectral data are also consistent with the proposed structure (**7**).

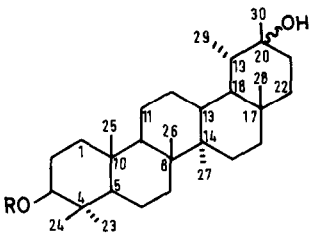
EXPERIMENTAL

All the mps are uncorr. The compounds were dried for analysis at room temp. or 100°/0.2 mmHg for 6 hr. The IR spectra were run on a Shimadzu 408 IR spectrophotometer and the UV spectra on a Beckmann DB-G spectrophotometer. ^{1}H NMR spectra were run either on a Perkin–Elmer 90 MHz NMR spectrometer with TMS as int. standard or on a Bruker 200 MHz instrument with reference to $CHCl_3$ at δ2.75. ^{13}C NMR spectra were obtained on a Bruker instrument with reference to $CDCl_3$ at δ77.0. silica gel (100–200 mesh) was used for CC.

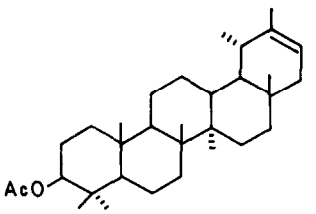
Isolation procedure. The stem bark of *M. indica*, collected from



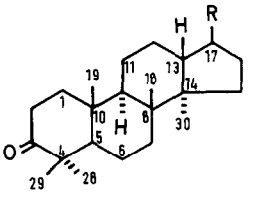
Scheme 2. Mass spectral fragmentation of compound F (**6**).

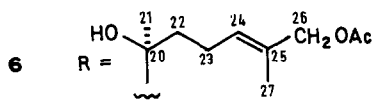


3 R = H
3a R = Ac

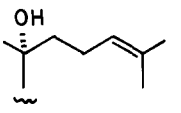


3b

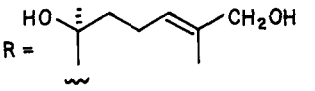




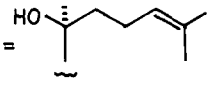
6 R =



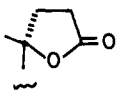
9 R =



6a R =



9a R =



6b R =

an aged tree at Seetanagaram near Rajahmundry, south India was dried and powdered. The dry powder (10 kg) was extracted successively with *n*-hexane (bp 60–80°) and MeOH in a big aspirator bottle. The dark brown *n*-hexane extract was evaporated under red. pres. and the resulting gummy residue (90 g) was separated into acidic, phenolic and neutral constituents by adopting the procedure used by Corsano and Mincione [10].

The neutral fraction (35 g) was chromatographed over a column of silica gel (700 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 Table 3.

The acidic fraction (10 g) after esterification with CH_2N_2 was subjected to CC and the results are shown in Table 4.

The MeOH extract yielded mangiferin (30 g), mp 279–281° [32].

Identification of known compounds is based on their physical and spectroscopic characteristics and comparison (TLC, ^{1}H NMR, mp or ORD) with authentic samples wherever possible.

Fraction A showed two close running spots on TLC (R_f 0.37 and 0.28 in C_6H_6–EtOAc (9:1). Attempts to separate this mixture by fractional crystallization from various solvents or further CC over silica gel were unsuccessful. Separation could be achieved only following acetylation (Ac_2O–pyridine at room temp., 24 hr). CC of the resultant acetate mixture and elution with *n*-hexane–C_6H_6 (1:1) afforded compound A. Further elution with *n*-hexane–C_6H_6 (1:2) yielded the monoacetate of dammarenediol II, mp 133–135°, $[\alpha]_D^{30}$ +38° ($CHCl_3$; *c* 1), fraction B showed several spots on TLC and resisted crystallization and was, therefore, acetylated. The acetate mixture was subjected to CC. Elution with *n*-hexane–C_6H_6 (1:1), C_6H_6 and C_6H_6–EtOAc (9:1) afforded compounds D, E and F, respectively.

Compound A. This was a mixture of the C-24 epimers of cycloart-25-ene-3β,24-diol diacetate (**1a**) and 24-methylenecycloartane-3β,26-diol diacetate (**1b**): mp 107–111°; IR ν_{max}^{KBr} cm^{-1}: 1735, 890; high resolution MS *m/z* (rel. int.): 540.4179 ($C_{35}H_{56}O_4$) (6), 526.4034 ($C_{34}H_{54}O_4$) (5), 525 (1), 511 (1), 481 (7), 480 (15), 466 (22), 465 (6), 452 (1), 451 (5), 406 (1), 391 (2), 358 (6), 357 (4), 344 (6), 299 (4), 297 (8), 284 (3), 203 (14), 175 (17), 147 (14), 107 (24), 95 (33), 43 (100); difference MS (**1b**) = MS of A – MS of authentic C-24 epimers of cycloart-25-ene-3β,24-diol diacetate (**1a**) *m/z* (rel. int.): 540 $[M]^+$ (32), 525 (7), 482 (11), 481 (40), 480 (100), 465 (47), 437 (13), 411 (17), 385 (8), 359 (10), 358 (28), 339 (3), 326 (8), 299 (8), 298 (9), 283 (2), 277 (10), 244 (7), 218 (9), 217 (5), 216 (1), 190 (1), 189 (3), 145 (7), 144 (1), 137 (2).

Compound B. Identified as cycloart-24-ene-3β,26-diol (**2**): mp 154–155°; $[\alpha]_D^{30}$ +51° ($CHCl_3$; *c* 1). (Found: C, 81.39; H, 11.22. Calc. for $C_{30}H_{50}O_2$: C, 81.45; H, 11.31%.) IR ν_{max}^{KBr} cm^{-1}: 3400, 3042; ^{1}H NMR ($CDCl_3$): δ5.33 (1H, *t*, *J* = 7 Hz), 3.96 (2H, *br s*), 3.23 (1H, *m*), 1.65 (3H, *s*), 0.95 (6H, *s*), 0.80 (3H, *s*), 0.53 and 0.32 (2H, ABq, *J* = 4 Hz); MS *m/z* (rel. int.): 442 $[M]^+$ (19), 427 (16), 424 (45), 410 (16), 409 (50), 391 (8), 381 (18), 355 (13), 315 (7), 302 (23), 297 (11), 287 (4), 284 (15), 203 (27), 175 (23), 173 (23), 161 (31), 148 (45), 135 (48), 133 (43), 121 (53), 109 (70), 107 (73), 95 (100).

Acetylation of compound B. Compound B (50 mg) was acetylated with Ac_2O–pyridine (1 ml, 1:1) at room temp. overnight. The mixture was poured into ice–H_2O and the ppt was recrystallized (MeOH) to give colourless needles (**2a**, 40 mg); mp

Table 2. ^{13}C NMR signals of compounds 9, 9a and F (6) (deuterochloroform, TMS as int. standard)

Carbon No.	9	9a	6*
1	39.8	39.8	39.8
2	34.0	34.0	34.0
3	217.7	217.6	218.0
4	47.3	47.3	47.4
5	55.3	55.3	55.3
6	19.6	19.6	19.6
7	34.6	34.5	34.5
8	40.2	40.2	40.2
9	49.9	49.9	49.9
10	36.7	36.7	36.8
11	21.9	22.0	22.0
12	25.2	25.4	24.8
13	42.2	42.3	42.4
14	49.9	50.2	50.2
15	31.0	31.1	31.1
16	27.4	27.5	27.5
17	49.4	49.7	49.8
18	15.9†	15.9†	16.0†
19	15.4†	15.2†	15.2†
20	75.6	75.1	75.1
21	23.5	24.7	25.4
22	41.8	40.5	39.8
23	22.2	22.5	22.2
24	124.6	124.7	129.8
25	131.3	131.3	130.0
26	25.7	25.7	70.2
27	17.6	17.6	13.9
28	26.6	26.6	26.6
29	21.0	21.0	21.0
30	16.2	16.2	16.3
OCOMe	—	—	170.9
OCOMe	—	—	21.0

*Multiplicities assigned from DEPT spectrum.

†Assignments in any vertical column may be interchanged.

74–77°; $[\alpha]_D^{30}$ +107.7° ($CHCl_3$; *c* 0.7); IR ν_{max}^{KBr} cm^{-1}: 1735, 3041; ^1H NMR (CCl_4): δ5.32 (1H, *m*), 4.4 (1H, *m*), 4.31 (2H, *br s*), 1.96 (3H, *s*), 1.93 (3H, *s*), 1.6 (3H, *s*), 0.93 (3H, *s*), 0.88 (6H, *s*), 0.84 (6H, *s*), 0.52 and 0.32 (2H, AB*q*, *J* = 4 Hz).

Chromium trioxide oxidation of compound B. Compound B (50 mg) in C_6H_6 (10 ml) was shaken with CrO_3 (40 mg) in HOAc (5 ml) and H_2O (1 ml) for 3 hr at room temp. Usual work-up followed by recrystallization ($CHCl_3$–MeOH) gave colourless needles: mp 186–188°; $[\alpha]_D^{30}$ +24° ($CHCl_3$; *c* 1); mmp undepressed with authentic mangiferonic acid (**2b**) [14].

Compound C. Identified as ψ-taraxastane-3β,20-diol) (**3**). Crystallized from MeOH as colourless needles: mp 270–273°; $[\alpha]_D^{30}$ −8° ($CHCl_3$; *c* 0.8). (Found: C, 80.97; H, 11.42. Calc. for $C_{30}H_{52}O_2$: C, 81.08, H, 11.71%.) IR ν_{max}^{KBr} cm^{-1}: 3500; ^1H NMR ($CDCl_3$): δ3.18 (1H, *m*), 1.07 (3H, *s*), 1.02 (6H, *s*), 0.95 (3H, *s*), 0.92 (3H, *s*), 0.89 (3H, *s*), 0.83 (3H, *s*), 0.76 (3H, *s*), 1.58 (1H, *s*) which disappeared upon the addition of D_2O. MS *m/z* (rel. int.): 444 $[M]^+$ (2), 426 (44), 411 (9), 408 (17), 393 (9), 373 $[M-C_4H_7O]^+$ (17), 365 (16), 355 (16), 236 (2), 222 (3), 218 (19), 207 (69), 205 (25), 203 (23), 191 (33), 189 (92), 175 (36), 95 (100).

Acetylation of compound C. Compound C (50 mg) was acetylated with Ac_2O–pyridine (1 ml, 1:1) at room temp. overnight. The usual work-up followed by recrystallization ($CHCl_3$–MeOH) afforded colourless needles (**3a**, 40 mg): mp 278–280°; $[\alpha]_D^{30}$ ±0° ($CHCl_3$; *c* 1); IR ν_{max}^{KBr} cm^{-1}: 3500, 1735; ^1H NMR (CCl_4): δ4.42 (1H, *m*), 1.96 (3H, *s*), 1.08 (3H, *s*), 1.02 (6H, *s*), 0.92 (3H, *s*), 0.89 (3H, *s*), 0.86 (3H, *s*), 0.83 (3H, *s*), 0.81 (3H, *s*); MS *m/z* (rel. int.): 486 $[M]^+$ (2), 468 (34), 453 (4), 426 (7), 415 (21), 408 (22), 393 (11), 365 (11), 355 (9), 339 (5), 249 (20), 229 (15), 218 (15), 207 (5), 205 (24), 189 (100), 95 (100).

Action of thionyl chloride on compound **3a**. A soln of **3a** (30 mg) in dry pyridine (5 ml) was cooled to 5° and freshly distilled $SOCl_2$ (0.5 ml) added dropwise with shaking. The reaction mixture was poured into H_2O and extracted with Et_2O. The Et_2O extract was washed with H_2O and dried (dry Na_2SO_4). Removal of the solvent afforded a solid which, on recrystallization ($CHCl_3$–MeOH) gave colourless needles: mp 246–248°; $[\alpha]_D^{30}$ +52° ($CHCl_3$; *c* 1); mmp undepressed with authentic ψ-taraxasteryl acetate (**3b**); IR ν_{max}^{KBr} cm^{-1}: 1730, 780; ^1H NMR ($CDCl_3$): δ5.15 (1H, *d*, *J* = 7 Hz), 4.35 (1H, *m*), 1.92 (3H, *s*), 1.60 (3H, *s*), 0.70 (3H, *s*), 0.80 (3H, *s*), 0.84 (3H, *s*), 0.87 (3H, *s*), 0.94 (6H, *s*), 1.04 (3H, *s*).

Compound D. This was a mixture of the C-24 epimers of cycloart-25-ene-3β,24,27-trioltriacetate (**4**): mp 94–96°; $[\alpha]_D^{30}$

Table 3. Triterpenoids from the neutral fraction

Eluent	Fractions	Compound	Yield (g)
n-Hexane	1–30	Waxes	2
n-Hexane–C_6H_6 (3:1)	31–50	Cycloartenol	1.1
n-Hexane–C_6H_6 (1:1)	51–82	α- and β-Amyrins	2
n-Hexane–C_6H_6 (1:3)	83–90	Intractable gum	—
n-Hexane–C_6H_6 (1:3)	91–100	β-Sitosterol	2
C_6H_6	101–105	3β-Hydroxy cycloarten-24-26-al [13]	0.1
C_6H_6	106–109	Intractable gum	—
C_6H_6–EtOAc (9.5:0.5)	110–114	Dammarenediol II	0.2
C_6H_6–EtOAc (9.5:0.5)	115–127	Fraction A	0.6
C_6H_6–EtOAc (9:1)	128–135	Compound B	2
C_6H_6–EtOAc (9:1)	136–142	Compound C	0.2
C_6H_6–EtOAc (4:1)	143–145	Intractable gum	—
C_6H_6–EtOAc (4:1)	146–151	Ocotillol II [26]	0.5
C_6H_6–EtOAc (7:3)	152–175	Fraction B	1.6
EtOAc	176–200	Intractable gum	—

Table 4. Triterpenoids from the acidic fraction

Eluent	Fractions	Compound	Yield (g)
n-Hexane	1–10	Fatty acid methyl esters	2
n-Hexane–C_6H_6 (9:1)	11–15	Methyl mangiferonate [14]	0.2
n-Hexane–C_6H_6 (7:3)	16–20	Methyl isomangiferolate [9, 10]	0.1
n-Hexane–C_6H_6 (1:1)	21–40	Mangiferolate [14]	2
C_6H_6	41–50	Compound G	0.4

+28.2° ($CHCl_3$; *c* 2.4). (Found: C, 73.81; H, 9.39. Calc. for $C_{36}H_{56}O_6$: C, 73.97; H, 9.59%.) IR ν_{max}^{KBr} cm^{-1}: 1735, 890; ^1H NMR ($CDCl_3$): δ0.32 and 0.56 (2H, AB*q*, *J* = 4.2 Hz), 0.83 (3H, *s*), 0.85 (3H, *d*, *J* = 6 Hz), 0.87 (6H, *s*), 0.94 (3H, *s*), 2.04 (3H, *s*), 2.05 (3H, *s*), 2.08 (3H, *s*), 4.59 (2H, *s*), 4.57 (1H, *m*), 5.22 (2H, *s*); MS *m/z* (rel. int.): 584.4052 $[M]^+$ ($C_{36}H_{56}O_6$) (3), 525 (18), 524 (48), 509 (10), 481 (4), 465 (3), 464 (8), 449 (5), 357 (7), 355 (4), 342 (24), 297 (15), 203 (18), 175 (21), 107 (38), 95 (39), 43 (100).

Alkaline hydrolysis of compound D. Compound D (20 mg) in C_6H_6 (10 ml) was refluxed with 6% alcoholic KOH for 4 hr. The usual work-up followed by crystallization (C_6H_6–hexane) afforded colourless needles (**4a**, 12 mg): mp 190–192°; $[\alpha]_D^{30}$ +18.8° ($CHCl_3$; *c* 1.2); ^1H NMR ($CDCl_3$): δ5.12 (2H, *m*), 4.25 (3H, *m*), 3.25 (1H, *m*), 1.78 (1H, *s*), 0.93 (6H, *s*), 0.87 (6H, *s*), 0.79 (3H, *s*), 0.55 and 0.30 (2H, AB*q*, *J* = 4 Hz).

Compound E. This was a mixture of the C-24 epimers of cycloartane-3β,24,25-triol diacetate (**5**): mp 160–163°; $[\alpha]_D^{30}$ +42.5° ($CHCl_3$; *c* 0.7). (Found: C, 74.82; H, 10.12. Calc. for $C_{34}H_{56}O_5$: C, 75.0, H, 10.29%.) IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600, 3040, 1735; ^1H NMR ($CDCl_3$): δ4.75 (1H, *m*), 4.55 (1H, *m*), 2.08 (3H, *s*), 2.02 (3H, *s*), 1.17 (6H, *s*), 0.92 (3H, *s*), 0.86 (6H, *s*), 0.82 (6H, *s*), 0.55 and 0.32 (2H, AB*q*, *J* = 4 Hz); MS *m/z* (rel. int.): 544.4153 $[M]^+$ ($C_{34}H_{56}O_5$) (2), 529 (2), 526 (2), 511 (2), 484 (25), 471 (14), 469 (15), 466 (10), 425 (35), 424 (100), 412 (21), 411 (62), 420 (22), 409 (66), 382 (10), 381 (32), 357 (15), 355 (24), 302 (47), 297 (51), 203 (65), 175 (56), 95 (96).

Alkaline hydrolysis of compound E. Compound E (55 mg) in C_6H_6 (10 ml) was refluxed with 6% alcoholic KOH for 4 hr. The usual work-up followed by crystallization (C_6H_6) gave colourless buttons (**5a**, 40 mg): mp 154–156°; $[\alpha]_D^{30}$ ±0° ($CHCl_3$; *c* 0.8); IR ν_{max}^{KBr} cm^{-1}: 3500, 3043; ^1H NMR ($CDCl_3$): δ3.26 (2H, *m*), 1.18 (3H, *s*), 1.12 (3H, *s*), 0.93 (6H, *s*), 0.87 (6H, *s*), 0.78 (3H, *s*), 0.53, 0.29 (2H, AB*q*, *J* = 4.3 Hz).

Chromium trioxide oxidation of compound **5a**. Compound **5a** (20 mg) in C_6H_6 (10 ml) was shaken with CrO_3 (15 mg) in HOAc (3 ml) and three drops of H_2O for 3 hr at room temp. The usual work-up gave a solid (mp 177–180°) which, upon two more crystallizations from C_6H_6–hexane, afforded colourless needles (**5b**, 11 mg): mp 187–190°. (Found: C, 78.12; H, 10.11. Calc. for $C_{27}H_{42}O_3$: C, 78.26; H, 10.14%.) IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3042, 1710; ^1H NMR ($CDCl_3$): δ1.22 (3H, *s*), 1.08 (3H, *s*), 1.02 (3H, *s*), 0.98 (3H, *s*), 0.89 (3H, *s*), 0.79, 0.55 (2H, AB*q*, *J* = 4 Hz).

Sodium metaperiodate oxidation of compound **5a**. An aq. soln of $NaIO_4$ (70 mg) was added to a soln of **5a** (15 mg) in EtOAc–EtOH (1:3, 5 ml) and stirred for 4 hr at room temp. The soln was kept at room temp. for 36 hr. Evaporation of the solvent with gradual addition of H_2O and extraction with $CHCl_3$ gave a crude product which was acetylated with Ac_2O–pyridine (0.5 ml, 1:1). Crystallization of the product (Me_2CO–H_2O) afforded white plates (**5C**, 8 mg): mp 155–157°; mmp undepressed with authentic 24-oxo-25,26,27-trisnorcycloartanyl acetate (**5c**).

Compound F. Identified as 3-ketodammar-24*E*-ene-20*S*,26-diol-26-monoacetate (**6**): mp 109–111°; $[\alpha]_D^{30}$ +64.9° ($CHCl_3$; *c* 1.0). (Found: C, 76.48; H, 9.97. Calc. for $C_{32}H_{52}O_4$: C, 76.80; H, 10.4%.) IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3620, 1708, 1741; ^1H NMR (CCl_4): δ5.43 (1H, *t*, *J* = 7 Hz), 4.42 (2H, *br s*), 2.46 (2H, *m*), 2.05 (3H, *s*), 1.65 (3H, *s*), 1.15 (3H, *s*), 1.08 (3H, *s*), 1.05 (3H, *s*), 1.00 (3H, *s*), 0.95 (3H, *s*), 0.89 (3H, *s*); high resolution MS *m/z* (rel. int.): no $[M]^+$, 482.3763 $[M-H_2O]^+$ ($C_{32}H_{50}O_3$) (0.2), 467.3510 ($C_{31}H_{47}O_3$) (0.1), 440.3653 ($C_{30}H_{48}O_2$) (1.2), 422.3539 ($C_{30}H_{46}O$) (1), 359.2941 ($C_{24}H_{39}O_2$) (1), 358 (1), 316 (2), 315.2679 ($C_{22}H_{35}O$) (2), 313 (1), 279 (1), 245 (1), 220 (1), 219 (1), 205.1601 ($C_{14}H_{21}O$) (4), 149 (2), 147 (3), 141.0926 ($C_8H_{13}O_2$) (2), 125 (100), 94 (91).

Alkaline hydrolysis of compound F. Compound F (30 mg) in C_6H_6 (10 ml) was hydrolysed by refluxing with 6% alcoholic KOH on a steam bath for 4 hr. The usual work-up gave a solid which, on crystallization (C_6H_6–hexane) afforded colourless crystals (**6a**, 21 mg): mp 116–118°; $[\alpha]_D^{30}$ +30.6° ($CHCl_3$; *c* 0.6); IR ν_{max}^{KBr} cm^{-1}: 3400, 1710; ^1H NMR ($CDCl_3$): δ5.43 (1H, *t*, *J* = 7 Hz), 4.0 (2H, *br s*), 2.46 (2H, *m*), 1.68 (3H, *s*), 1.16 (3H, *s*), 1.08 (3H, *s*), 1.02 (3H, *s*), 1.00 (3H, *s*), 0.93 (3H, *s*), 0.89 (3H, *s*).

Jones oxidation of compound **6a**. To a soln of compound **6a** (15 mg) in Me_2CO (5 ml) was added Jones reagent. After 2 hr the mixture was filtered, diluted with H_2O (10 ml) and extracted with Et_2O (3 × 50 ml). Evaporation of the washed and dried extract gave a solid (9 mg): mp 52–58° showing one main spot on TLC (R_f 0.26 in $CHCl_3$). Repeated crystallization from absolute EtOH–hexane gave colourless prisms (**6b**, 5 mg): mp 180–182°; $[\alpha]_D^{30}$ +70° ($CHCl_3$; *c* 1); mmp undepressed with authentic ketotrisnorlactone prepared from ocotillol II; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1695.

Compound G. Identified as the diazomethane adduct of mangiferolic acid methyl ester (**7**): mp 159–163°. (Found: C, 74.89; H, 10.29. Calc. for $C_{32}H_{52}O_3N_2$: C, 74.99; H, 10.16%.) IR ν_{max}^{KBr} cm^{-1}: 3400, 1735; ^1H NMR (CCl_4): δ4.75, 4.56 (1H, *dd*, *J* = 17, 8 Hz), 3.92, 3.72 (1H, *dd*, *J* = 17, 8 Hz), 3.72 (3H, *s*), 3.12 (1H, *m*), 1.25 (3H, *s*), 0.92 (6H, *s*), 0.87 (6H, *s*), 0.75 (3H, *s*), 0.55, 0.32 (2H, AB*q*, *J* = 4 Hz); MS *m/z* (rel. int.): 484 $[M-N_2]^+$ (7), 469 (7), 468 (6), 467 (29), 466 (73), 453 (8), 452 (27), 451 (71), 435 (8), 423 (23), 398 (8), 397 (22), 344 (18), 339 (5), 315 (7), 297 (23), 229 (10), 227 (12), 215 (13), 203 (43), 189 (32), 175 (53), 95 (100).

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