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# MARAGENINS I, II AND III, NEW PENTACYCLIC TRITERPENES FROM *MARAH MACROCARPUS*

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**Abstract**—Maragenins I, II and III, new pentacyclic triterpenes from *Marah macrocarpus* (Greene) Greene, are shown to be respectively $\Delta^{12}$-, $\Delta^{12,17}$- and $\Delta^{17}$-3β-hydroxy-16-oxo-28-norlocananes on the basis of chemical and spectroscopic evidence. The structural proposal for maragenin I is confirmed by synthesis from echinocystic acid.

In addition to the cucurbitacins,[1] members of the Cucurbitaceae are known to contain several tetracyclic and pentacyclic triterpenoids,[2] including oleanolic and echinocystic acids, and sterols.[3] Indeed, echinocystic acid was first isolated[4] from a cucurbit—*Echinocystis fabacea*—and a unique sapogenin, momordic acid (3β-hydroxy-1-oxo-olean-12-en-28-oic acid), has been found in *Momordica cochinchinensis*.[5] Previous work on the genus *Marah* however has only reported the isolation of some cucurbitacins.[6,7]

## DISCUSSION

In the present work, mild acid hydrolysis of a methanolic extract of *Marah macrocarpus* fruits yielded a mixture of four closely related triterpenoid sapogenins, one of which was isolated as the diacetate of the methyl ester of echinocystic acid (1; R = $COCH_3$; $R_1$ = H; $R_2$ = $OCOCH_3$; $R_3$ = $CO_2CH_3$), identified by comparison with an authentic sample. Small scale experiments showed that this compound was present in the plant as the free alcohol-acid (1; R = $R_1$ = H; $R_2$ = OH; $R_3$ = $CO_2H$).

The other compounds did not have carboxyl or ester groups and the major one, maragenin I (1; R = $R_3$ = H; $R_1$ = $R_2$ = =O), m.p. 218–220°, on high resolution mass spectrometry gave a molecular ion peak at *m/e* 426. Accurate mass measurements showed the composition of this peak to be $C_{29}H_{46}O_2$, suggesting a nortriterpenoid skeleton. A prominent peak for the loss of a side-chain fragment was not present in the mass spectrum indicating that the compound was probably pentacyclic. Maragenin I formed a mono-acetate (1; R = $COCH_3$; $R_1$,$R_2$ = =O; $R_3$ = H) and the IR spectra of both maragenin I and 1 (R = $COCH_3$; $R_1$,$R_2$ = =O; $R_3$ = H) showed a band at about 1700 $cm^{-1}$ suggesting a 6 membered ring ketone, confirmed by the formation of a de-oxy compound (1; R = $COCH_3$; $R_1$ = $R_2$ = $R_3$ = H) by Wolff–Kischner reduction which lacked CO absorption in the IR. This reaction proceeded smoothly in good yield indicating the unhindered nature of the ketone group. The NMR spectrum of maragenin I showed signals for seven Me groups, all singlets, demonstrating the absence of a secondary Me function.

The most likely skeleton is thus that of a nor-β-amyrin, emphasised by the co-occurrence in the plant of echinocystic acid. The NMR spectrum of 1 (R = $R_3$ = H; $R_1$,$R_2$ = =O) also showed a signal for one hydrogen at δ 5.46 (doublet of doublets, J = 4 Hz, 6 Hz) similar to the corresponding signal in the spectrum of β-amyrin itself (δ 5.26)[8] indicating the presence of a trisubstituted double bond with an adjacent methylene group, confirmed by the synthesis of a dihydro derivative on hydrogenation with platinum oxide catalyst. There was no strong UV absorption hence the CO group and the double bond are not conjugated. Since the compound has no acid function this suggests that the C atom lacking from the β-amyrin skeleton is 28 and the proposed structure for maragenin I is thus 3β-hydroxy-16-oxo-12-en-28-noroleanane (1; R = $R_3$ = R; $R_1$,$R_2$ = =O).

In order to verify this proposal, an authentic specimen of echinocystic acid (1; R = $R_1$ = H; $R_2$ = OH; $R_3$ = $CO_2H$) was acetylated to yield the mono-acetate (1; R = $COCH_3$; $R_1$ = H; $R_2$ = OH; $R_3$ = $CO_2H$). Treatment of this compound with chromium trioxide and sulphuric acid in acetone gave, in almost quantitative yield, a substance which lacked OH absorption in the IR but showed a CO band at 1703 $cm^{-1}$. This material was identical with the acetate of maragenin I (1; R = $COCH_3$; $R_1$,$R_2$ = =O; $R_3$ = H).

The optical rotatory dispersion curve of maragenin I showed a strongly negative Cotton effect (Fig. 1a).

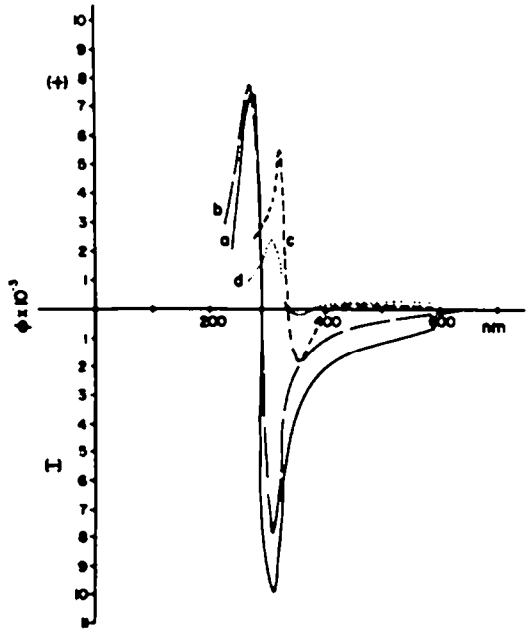



Fig. 1. Optical rotatory dispersion curves of (a) natural maragenin I, (b) synthetic maragenin I acetate, (c) maragenin II acetate and (d) maragenin III acetate.

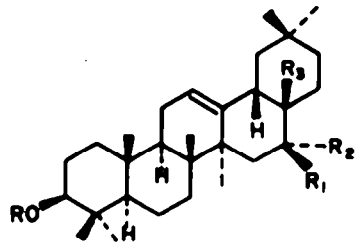
Examination of Dreiding models showed that such an effect would only be observed in the 17β-isomer, i.e. with a *cis* D/E ring junction. The synthetic product on the other hand showed a reduced negative Cotton effect (Fig. 1b). Examination of this material by glc showed it to consist of two compounds, one of which (the major peak, accounting for about 95% of the product) corresponded in retention time with the natural substance. The other compound was presumably the 17α-epimer which, from models, is expected to give a slightly positive Cotton effect, accounting for the reduced magnitude in the ORD curve of the synthetic product. Maragenin I is thus 3β-hydroxy-16-oxo-12-en-28-noroleanane with the stereochemistry shown in 1 (R = $R_3$ = H; $R_1R_2$ = =O).

Another crystalline material from the fraction which also gave the echinocystic acid methyl ester could only be purified after acetylation and preparative argentation tlc, when it was separated into two components. One of these, acetyl maragenin II, had a molecular ion peak in the mass spectrum at *m/e* 466 (i.e. at two mass units lower than that of maragenin I acetate) but still showed in the NMR spectrum a signal for only one vinyl hydrogen (at δ 6.12–doublet of doublets, J = 4 Hz, 6 Hz). The IR spectrum however showed a peak at 1668 $cm^{-1}$ indicating a conjugated CO group, confirmed by examination of the UV spectrum which showed absorption at 298 nm for a highly unsaturated ketone. Since only one oxygen (in addition to that at C-3) is present and this is likely to be at C-16, the most probable structure of maragenin II is 3β-hydroxy-16-oxo-12,17-dien-28-noroleanane (2; R = H). Calculation of the theoretical UV maximum for such an α,β,γ,δ-unsaturated ketone gives 308 nm, providing additional evidence for the proposed structure. Inspection of a Dreiding model shows that the expected Cotton effect is much less than that of maragenin I, but still negative, as is observed (Fig. 1c).

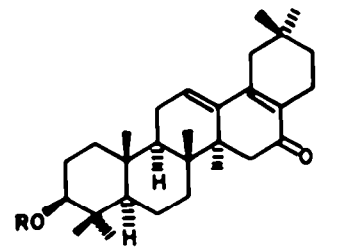
The other compound isolated by argentation tlc, maragenin III acetate, gave a molecular ion peak in the mass spectrum at *m/e* 468 showing that maragenin III is an isomer of maragenin I. However the CO band in the IR spectrum of the former compound (1665 $cm^{-1}$) was similar to that of maragenin II, again indicating unsaturation but examination of the NMR spectrum showed no vinyl absorption. Thus it is likely that maragenin III is the conjugated isomer, 3β-hydroxy-16-oxo-17-en-28-noroleanne (3; R = H) of maragenin I. The configuration at C-13 may be deduced from the Cotton effect in the ORD curve (Fig. 1d) which is seen to be only slightly negative. For models, this is more compatible with the 13α rather than the 13β configuration but since there is some degree of mobility in ring D the assignment is not certain. However in the 13β isomer, ring E is forced into a boat, making the 13α compound the more thermodynamically favoured.

It is interesting to speculate as to whether maragenin I is a genuine compound present in the plant or is an artefact arising by decarboxylation of the corresponding 16-CO derivative of echinocystic acid (1; R = H; $R_1R_2$ = =O; $R_3$ = $CO_2H$) during the acid treatment of the saponin mixture. This suggestion is supported by consideration of the means of synthesis of margenin I, since chromium trioxide treatment of the 3β-acetate of echinocystic acid (1; R = $COCH_3$; $R_1$ = H; $R_2$ = OH; $R_3$ = $CO_2H$) in the presence of acid did cause spontaneous decarboxylation. On the other hand, it must be mentioned that in the case of the corresponding $\Delta^{13(18)}$-isomers, albigenin (4; R = $R_1$ = H) and albigenic acid (4; R = H; $R_1$ = $CO_2H$) isolated from *Albizzia lebbeck*,[9,10] treatment of the keto-ester 4 (R = $COCH_3$; $R_1$ = $CO_2CH_3$) with acid did not cause decarboxylation. However, Belous[11,12] states that decarboxylation did occur in the chromium trioxide oxidation of echinocystic acid, as observed in the present work, but that the product was the 3-ketone corresponding with albigenin, i.e. norechinocystenolone, implying that in this case the double bond migrated to the 13(18) position. However no NMR data is given and it is hence uncertain whether Belous's product was in fact the albigenin or the maragenin I derivative. Similarly it is also possible that maragenin III (3; R = H) is produced by rearrangement from a $\Delta^{13(18)}$ compound (i.e. albigenin) but this is discounted because albigenin acetate was isolated from an acid hydrolysed extract from *Albizzia* and was also completely stable under saponification conditions.[11,12]

It is thus likely that maragenin I is indeed a natural product, perhaps produced by enzymatically controlled decarboxylation of the keto-acid 1 (R = H; $R_1R_2$ = =O; $R_3$ = $CO_2H$), in a manner analogous to that known to occur in the loss of the 4-Me functions during the conversion of lanosterol to cholesterol.[13]




1

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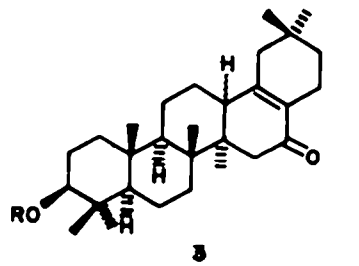


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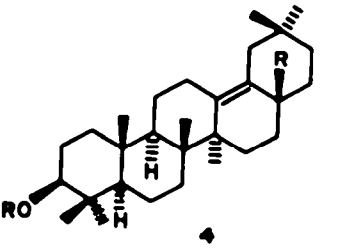


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## EXPERIMENTAL

M.ps are uncorrected. NMR spectra were determined in $CDCl_3$ soln on a Perkin–Elmer R-12 instrument operating at 90 MHz and chemical shift values are quoted in ppm downfield from TMS as internal standard. Mass spectra were obtained on an A.E.I. 902 high resolution instrument having a direct inlet system and operating at 70 eV. ORD curves were measured on a Polarmatic 62 system in EtOH or MeOH soln.

Fruits of *Marah macrocarpus* (Greene) Greene were collected at Summer Canyon near La Jolla, California, U.S.A. in March 1977. Twenty-five specimens from which the seeds had been removed were dried at room temp. and the powdered material (72.4 g) was exhaustively extracted with light petroleum b.p. 40–60° and then with MeOH. Evaporation of the solvents under reduced pressure yielded a yellowish oil (1.36 g) and a dark brown foam (13.7 g) respectively. The methanolic extract was refluxed with 2N HCl in MeOH (150 ml) for 2 hr, most of the solvent removed under reduced pressure, diluted with a large volume of water, neutralised with $NaHCO_3$ and repeatedly extracted with $CHCl_3$. The dried $CHCl_3$ residue (5.2 g) was placed on a column of silica gel (130 g) and this was eluted with light petroleum b.p. 40–60°, then with increasing concentrations of EtOAc in light petroleum b.p. 40–60° and finally with MeOH. Elution with 10% EtOAc in light petroleum b.p. 40–60° gave a colourless oil which crystallised from EtOAc as white rosettes (100 mg, 0.014%) of *3β-hydroxy-16-oxo-12-en-28-norolean*ane (maragenin I) (1; R = $R_3$ = H; $R_1$,$R_2$ = =O), m.p. 218–220°; $\lambda_{max}^{MeOH}$ 288 nm (log ε 1.95); $[\phi]_{589}^{17}$ − 690°, $[\phi]_{511}^{17}$ − 9928° tr., $[\phi]_{492}^{17}$ 0°, $[\phi]_{472}^{17}$ + 7504° pk (*c* = 0.0007, MeOH); $[\theta]_{328}$ 0, $[\theta]_{306}$ − 16178, $[\theta]_{300}$ 0 (*c* = 0.00086, MeOH); $\nu_{max}^{Nujol}$ 3640, 1695, 1385, 1368; δ 5.46 (1H, q.d, J, 4 Hz, 6 Hz), 3.30 (1H, t, J, 8 Hz), 2.52 (1H, d, J, 16 Hz), 1.45 (1H, s, exchangeable with $D_2O$), 1.15 (3H, s), 0.99 (3H, S), 0.96 (3H, s), 0.93 (3H, s), 0.87 (3H, s), 0.85 (3H, s), 0.78 (3H, s); *m/e* (rel. int.) 426 (9), 411 (5), 393 (3), 286 (2), 273 (3), 272 (3), 257 (3), 219 (26), 218 (100), 203 (14), 190 (52), 189 (14), 176 (19), 175 (26), 147 (17), 121 (23), 69 (52), 43 (43), 41 (61); Accurate mass measurement— Found: 426.3478. $C_{29}H_{46}O_2$ requires: 426.3498.

Later fractions eluted with 10% EtOAc in light petroleum b.p. 40–60° gave a compound (7 mg, 0.01%), m.p. 198–200°, identical in every respect with the methyl ester of echinocystic acid diacetate (1; R = $COCH_3$; $R_1$ = H; $R_2$ = $OCOCH_3$; $R_3$ = $CO_2CH_3$). Further elution of the column with 10% EtOAc in light petroleum b.p. 40–60° gave a product which was shown to be a mixture but could not be purified by crystallisation. Accordingly, acetylation in the normal way and preparative chromatography on silica gel G (0.75 mm) layers impregnated with 20% $AgNO_3$ (tlc solvent, cyclohexane: EtOAc, 95 : 5, run six times), allowed isolation of two mono-acetates. One compound, *3β - acetoxy - 16 - oxo - 12,17 - dien - 28 - norolean*ane (maragenin II acetate) (2; R = $COCH_3$) (3.7 mg, 0.005%) had m.p. 215–217°, $\lambda_{max}^{EtOH}$ 298 nm (log ε 3.87); $[\phi]_{589}^{17}$ + 58°; $[\phi]_{485}^{17}$ 0°, $[\phi]_{357}^{17}$ − 1916° tr., $[\phi]_{345}^{17}$ − 1166°, $[\phi]_{321}^{17}$ 0°, $[\phi]_{315}^{17}$ + 3415°, $[\phi]_{304}^{17}$ + 5455° pk, $[\phi]_{312}^{17}$ + 3498° (*c* = 0.0007, MeOH); $\nu_{max}^{Nujol}$ 1730, 1668, 1383, 1370, 1245 $cm^{-1}$; δ 6.12 (1H, q.d, J, 4 Hz, 6 Hz), 4.25 (1H, t, J, 8 Hz), 2.33 (1H, d, J, 14 Hz), 2.05 (3H, s), 1.13 (3H, s), 1.00 (6H, s), 0.95 (6H, s), 0.93 (3H, s), 0.90 (3H, s); *m/e* (rel. int.) 466 (29), 451 (4), 423 (2), 406 (4), 391 (5), 365 (1), 363 (2), 293 (6), 216 (45), 204 (71), 203 (100), 201 (22), 190 (14), 189 (31), 160 (14), 149 (27), 69 (39), 57 (39), 55 (31), 43 (52), 41 (52). Accurate mass measurement - Found: 466.3452. $C_{31}H_{46}O_3$ requires: 466.3447.

The other compound isolated by argentation tlc was *3β - acetoxy - 16 - oxo - 17 - en - 28 - norolean*ane (maragenin III acetate) (3, R = $COCH_3$), m.p. 216–218°; $\lambda_{max}^{EtOH}$ 251 nm (log ε 3.74); $[\phi]_{589}^{17}$ + 201°, $[\phi]_{490}^{17}$ + 101°, $[\phi]_{374}^{17}$ 0°, $[\phi]_{357}^{17}$ − 201° tr., $[\phi]_{346}^{17}$ 0°, $[\phi]_{322}^{17}$ + 2013°, $[\phi]_{312}^{17}$ + 2415° pk, $[\phi]_{294}^{17}$ + 1711° (*c* = 0.0006, EtOH); $\nu_{max}^{Nujol}$ 1728, 1665, 1383, 1370, 1245 $cm^{-1}$; δ 4.34 (1H, t, J, 7 Hz), 2.33 (1H, d, J, 13 Hz), 2.05 (3H, s), 1.03 (3H, s), 0.96 (3H, 3H, s), 0.91 (12H, s), 0.87 (3H, s); *m/e* (rel. int.) 468 (47), 453 (10), 425 (2), 408 (13), 393 (20), 365 (6), 316 (9), 260 (20), 218 (23), 217 (20), 216 (27), 205 (70), 203 (80), 201 (33), 190 (47), 189 (67), 178 (30), 125 (27), 161 (27), 137 (80), 69 (60), 57 (20), 55 (53), 43 (100), 41 (57). Accurate mass measurement—Found: 468.3593. $C_{31}H_{48}O_3$ requires: 468.3603.

*Maragenin I acetate.* Acetylation of maragenin I (29 mg) with $Ac_2O$ in pyridine and crystallisation from EtOAc gave *3β - acetoxy - 16 - oxo - 12 - en - 28 - norolean*ane (1; R = $COCH_3$; $R_1$,$R_2$ = =O; $R_3$ = H) (22 mg), m.p. 203–206°; $\lambda_{max}^{MeOH}$ 292 nm (log ε 1.74); $\nu_{max}^{Nujol}$ 1723, 1703, 1380, 1365, 1228; δ 5.47 (1H, q.d, J, 4 Hz, 6 Hz), 4.50 (1H, t, J, 8 Hz), 2.54 (1H, d, J, 16 Hz), 2.03 (3H, s), 1.04 (3H, s), 0.97 (6H, s), 0.82 (12H, s); *m/e* (rel. int.) 468 (6), 453 (2), 408 (6), 393 (5), 365 (2), 315 (2), 272 (3), 250 (4), 249 (6), 219 (22), 218 (100), 203 (24), 191 (24), 190 (64), 189 (32), 176 (20), 175 (28), 135 (24), 121 (24), 119 (24), 69 (44), 55 (32), 43 (64), 41 (36). Accurate mass measurement—Found: 468.3594. $C_{31}H_{48}O_3$ requires: 468.3603.

*Dihydromaragenin I acetate.* Hydrogenation of maragenin I acetate (18 mg) in EtOAc (5 ml) with excess $PtO_2$ at room temp. for 20 hr gave a product which was purified by chromatography on $AgNO_3$ impregnated (20%) silica gel G plates (0.25 mm) (tlc solvent: cyclohexane: EtOAc, 95 : 5, run three times) and crystallised from EtOAc as colourless needles of *3β - acetoxy - 16 - oxo - 28 - norolean*ane, m.p. 280–283°, $\lambda_{max}^{MeOH}$ 288 nm (log ε 2.31); $\nu_{max}^{Nujol}$ 1730, 1698, 1248 $cm^{-1}$; δ 4.48 (1H, t, J, 8 Hz), 2.58 (1H, d, J, 16 Hz), 2.02 (3H, s), 0.92 (3H, s), 0.88 (6H, s), 0.84 (9H, s), 0.65 (3H, s); *m/e* (rel. int.) 470 (5), 410 (36), 395 (29), 367 (9), 301 (5), 249 (12), 218 (19), 205 (16), 203 (13), 190 (36), 189 (100), 175 (12), 135 (22), 121 (24), 69 (48), 43 (51), 41 (32). Accurate mass measurement—Found: 470.3773. $C_{31}H_{50}O_3$ requires: 470.3760.

*Deoxymaragenin I acetate.* Maragenin I acetate (30 mg) was heated under reflux with diethylene glycol (10 ml) and 100% hydrazine hydrate soln (1.2 ml) for 90 min. Anhyd KOH (1 g) was added and the excess hydrazine and water removed by distillation, the temp. raised to 200° and the mixture heated for 1 hr. Usual work up followed by acetylation of the crude product and preparative chromatography on silica gel G plates (0.25 mm thickness) (tlc solvent: EtOAc, 4 : 1) gave a colourless oil which crystallised from EtOAc as needles of *3β - acetoxy - 12 - en - 28 - norolean*ane (1; R = $COCH_3$; $R_1$ = $R_2$ = $R_3$ = H), m.p. 195–198°; $\nu_{max}^{Nujol}$ 1735, 1380, 1370, 1243 $cm^{-1}$; δ 5.26 (1H, broad qu), 4.32 (1H, t, J, 8 Hz), 2.02 (3H, s), 0.95 (3H, s), 0.90 (3H, s), 0.89 (3H, s), 0.87 (9H, s), 0.81 (3H, s); *m/e* (rel. int.) 454 (10), 439 (4), 394 (2), 393 (2), 379 (2), 362 (1), 293 (10), 204 (90), 190 (27), 189 (42), 175 (23), 149 (33), 69 (77), 57 (73), 55 (67), 43 (100), 41 (67). Accurate mass measurement—Found: 454.3811. $C_{31}H_{50}O_2$ requires: 454.3811.

*Synthesis of maragenin I acetate.* Echinocystic acid (1; R = $R_1$ = H; $R_2$ = OH; $R_3$ = $CO_2H$) (30 mg) was acetylated in the usual way for 1 hr and the crude product dissolved in acetone (5 ml). This was titrated with Jones reagent[14] at room temp. until an orange colour persisted. Dilution with a large volume of water and extraction into $CHCl_3$ allowed isolation of a white residue which was purified by preparative tlc on silica gel G plates (0.25 mm thickness) with benzene: EtOAc, 85 : 15 as solvent. Recrystallisation from MeOH/EtOAc afforded white needles (12 mg), m.p. 187–189°, identical on tlc with natural maragenin I acetate. Lc on a column (165 × 0.4 cm i.d.) of 1% SE 30 on Chromosorb 80/100 at a temp. programmed to rise from 70° to 275° at 5°/min showed there to be two components in this material, of which the major one (*ca.* 95%) exactly corresponded in retention time with natural maragenin I acetate.

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