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TRITERPENOIDS FROM *DESFONTAINIA SPINOSA*

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Key Word Index—*Desfontainia spinosa*; Loganiaceae; triterpenoids; triterpene glucosides; ursolic acid; ursolic acid acetate; pomolic acid; 23-hydroxytormentic acid; 19α-hydroxyasiatic acid; 23-hydroxytormentic acid ester glucoside; niga-ichigoside Fl; 24-hydroxytormentic acid; 24-hydroxytormentic acid ester glucoside; 7α-hydroxytormentic acid; 7α-hydroxytormentic acid ester glucoside; 7α,23-dihydroxytormentic acid; 7α,23-dihydroxytormentic acid ester diglucoside.

Abstract—The leaves and stem of *Desfontainia spinosa* were found to contain eleven urs-12-ene triterpene compounds. The major compound present was ursolic acid. Four other known compounds were isolated, i.e. ursolic acid acetate, pomolic acid, 23-hydroxytormentic acid and nigaichigoside Fl, its ester glucoside. The other six compounds are novel and have been identified as 24-hydroxytormentic acid, 7α-hydroxytormentic acid, 7α,23-dihydroxytormentic acid and their corresponding ester glucosides. It is unlikely that these compounds contribute to the reputed narcotic properties of this plant.

INTRODUCTION

Desfontainia spinosa Ruiz & Pav. is reputed to be used as a hallucinogen but has only recently been subjected to phytochemical investigation. Loganin and related compounds have been isolated from the leaves [1, 2] and also a cytostatic cucurbitacin-type triterpenoid (1) [3]. During a general phytochemical investigation of this plant large amounts of material were precipitated when methanolic extracts were concentrated. This paper describes the identification of this substance as ursolic acid (2) and the isolation and identification of other glucosidal and non-glucosidal triterpenes.

RESULTS AND DISCUSSION

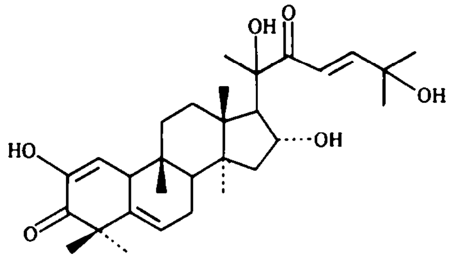
Identification of triterpenes

Triterpenes were identified by the red–violet colour given by the Liebermann–Burchard test. Their characterization as urs-12-ene derivatives was made by examining their MS fragmentation pattern [4, 5], their ^{1}H NMR spectra [6–8] and their ^{13}C NMR spectra [9]. The presence of the 28-COOH was shown by the formation of a methyl ester upon treatment with diazomethane.

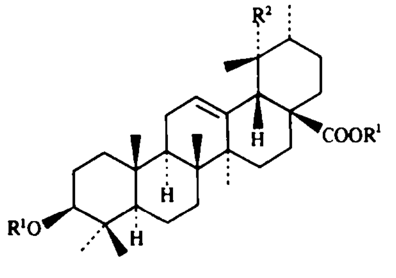
Ursolic acid (2), ursolic acid acetate (3), pomolic acid (4), 23-hydroxytormentic acid (5) and 23-hydroxytormentic acid ester glucoside (6) were identified by comparison of their spectral and chromatographic features with authentic samples and literature values [4–11]. It should be noted that 5 has also been called 19α-hydroxyasiatic acid [12] and that 6 is also known as niga-ichigoside Fl [13].

24-Hydroxytormentic acid (7) showed many features in its IR, mass and ^{1}H NMR spectra which indicated that it was closely related in structure to 5, having the same M_r. The ^{1}H NMR spectrum of 7 showed an upfield shift of the H-3 α signal (δ2.96) compared with 5 (δ3.28). This shift can be interpreted as due to the loss of the deshielding effect which the 23-CH_2OH function exerts in 5. The primary hydroxyl group cannot therefore be at C-23 in 7 but must be at C-24, C-25 or C-26. The last possibility is very uncommon and therefore unlikely. The assignment of the hydroxyl group to C-24 was made by examination of the ^{1}H NMR spectrum of the acetylation product of 7. A triacetate was formed and two of the three positions could be identified as C-2 and C-3 by the downfield shifts at δ5.17 and 4.85 of the H-2β and H-3α signals respectively. The AB quartet seen at δ3.28 and 3.94 in 7 can be assigned to the alcoholic methylene group; in the triacetate this disappears and is replaced by a two-proton singlet at δ4.22. This is more characteristic of a 24-CH_2OAc, such as is shown by sericic acid acetate (8) [14], than an acetate at C-25 where the singlet is seen at δ4.32 [15]. The postulated position of the 24-CH_2OH was confirmed by noting that the methyl signal at δ0.61 for C-24 seen in the spectrum for 5 was missing in that for 7; in contrast a tertiary methyl signal at δ1.09 observed in the spectrum of 7 could not be seen in that of 5. This signal is due to a C-23 methyl group. Final confirmation of assigning the CH_2OH group to C-24 was provided by the NOE difference spectra of 7 which showed enhancement between the AB quartet signals of 24-CH_2OH and the C-25 methyl signal at δ0.90. Dreiding models showed that in the 24-CH_2OH compound the C-24 methylene and C-25 methyl groups were spatially close to one another so giving the NOE effect.

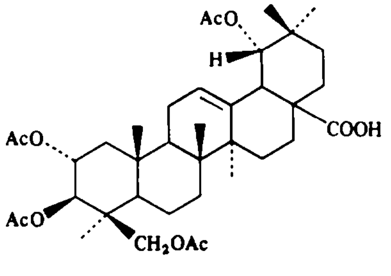
24-Hydroxytormentic acid glucoside (9) gave similar mass and ^{1}H NMR spectra to 7, with additional signals which could be assigned to a sugar. The doublet at δ5.22 (the anomeric proton) indicated that the sugar was probably glucose attached by a β linkage through an ester group [16]. This view was supported by the ester carbonyl peak at 1726 cm^{-1} in the IR spectrum as opposed to that at 1685 cm^{-1} seen in C-28 free acids. Enzyme hydrolysis products were shown to be glucose and 24-hydroxy-



1 Deoxycucurbitacin



		R¹	R²
2	Ursolic acid	H	H
3	Ursolic acid acetate	Ac	H
4	Pomolic acid	H	OH



8 Sericic acid acetate

tormentic acid by chromatographic comparison with authentic material and **7** respectively. Additional proof of the glucosidal linkage being through the C-28 carbonyl is given by the fact that **9** could not be methylated.

7α-Hydroxytormentic acid (**10**) showed peaks at m/z 222, 204 and 186 in its MS which correspond to retro-Diels–Alder breakdown fragments from urs-12-ene triterpenes with three hydroxyl groups in rings A and B. In the ^{1}H NMR spectrum the 2β and 3α carbinol methine signals are seen at δ3.67 and δ2.85 respectively. A third carbinol methine signal is seen at δ4.47. Since it showed no signs of coupling to the other two protons mentioned it was presumed that this carbinol methine must be partner to a secondary hydroxyl in ring B. Cold acetylation of **10** gave only a diacetate whose ^{1}H NMR spectrum showed that acetylation had taken place at C-2 and C-3. Hot acetylation produced a triacetate whose ^{1}H NMR showed a shift of the signal originally at δ4.47–5.55. This indicated steric hindrance of the secondary hydroxyl group in the B ring. Steric models of urs-12-enes indicate that such a hindrance could occur if the hydroxyl group is in the 6β, 7α or 11β positions. Examination of the splitting pattern of **10** and spin-decoupling experiments showed that there was no coupling of the C-12 olefinic proton signal at δ5.32 with the δ4.47 signal which would be expected if the hydroxyl group was at C-11. The two-dimensional COSY spectrum also showed no coupling of the δ4.47 signal with the split triplet at δ2.53. This triplet has been assigned to H-5α and due to the lack of coupling it seems unlikely that the carbinol methine is next to it at C-6β. By this process of elimination the secondary hydroxyl group is seen to be at the 7 position. Further proof for this is given by considering the ^{13}C NMR spectrum of **10**. In ursenes unsubstituted at C-6 and C-7 the ^{13}C signals are at about δ18.3 and 32.6 respectively [9]. These signals cannot be seen in the ^{13}C NMR spectrum of **10** but two other signals are seen at δ66.9 and 27.7. DEPT experiments showed that these were due to $-CH-O-$ and $-CH_2-$ respectively. 6β-Hydroxy ursenes have been shown to give a shift of about δ67 for C-6 and about δ41 for C-7 [17], i.e. a downfield shift of about 10 ppm for the adjacent carbon atom. Since the lower value observed was only δ27.7 it is unlikely that this is given by C-7. Literature data is not available for 7α-hydroxy ursenes but by analogy it is reasonable to assume that they would give values of about δ67 and 28 for C-7 and C-6 respectively. This agrees well with the spectrum for **10**. The presence of the 7α-hydroxyl group is also shown by the downfield shift of about 0.30 ppm for the C-27 methyl singlet in the ^{1}H NMR spectrum of **10** compared to the non-hydroxylated compound. This shift can be explained by deshielding of the methyl by the hydroxyl if they are in a 1:3 diaxial relationship [17]. This is the case if ring B has the 7-hydroxyl group in the α position.

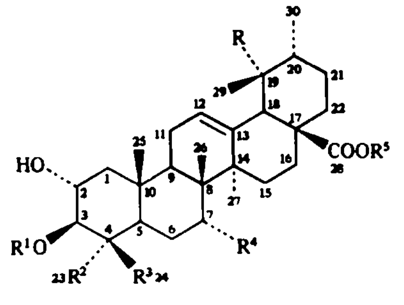
7α-Hydroxytormentic acid ester glucoside (**11**) was identified by noting its many spectral similarities with **10**. Extra signals in the ^{1}H and ^{13}C NMR spectra could be assigned to glucose protons. Differences in the IR spectrum and the inability to form a methylation product indicated that **11** was an ester glucoside with the same linkage as **9**. Confirmation of the nature of **11** was obtained by TLC comparison of its enzyme hydrolysis products with authentic glucose and **10**.

7α,23-Dihydroxytormentic acid (**12**) showed spectral features similar to both **5** and **10**, notably the AB quartet of the C-23 methylene at δ3.23 and 3.48 and the C-7β carbinol methine multiplet at δ4.25. Hot acetylation gave a tetraacetate confirming the presence of an extra hydroxyl group compared with either **5** or **10**. It was therefore presumed that hydroxyl groups were present at both C-7 and C-23. Confirmation of this was seen in the ^{13}C NMR spectrum which showed signals at δ27.7 and 67.0 attributed to C-6 and C-7 as discussed for **10** and a signal at 64.5 ($-CH_2O-$) which was assigned to C-23 as in **5**.

7α,23-Dihydroxytormentic acid ester glucoside (**13**) was identified by considering the differences in its spectral features compared with **12**, by the attempted formation of derivatives and by examination of its hydrolysis products. The salient points noted were identical to those discussed above for **6**, **9** and **11**.

General considerations

All the triterpenes present in the samples analysed by us were of the ursene type rather than the deoxocucurbitacin



		R	R¹	R²	R³	R⁴	R⁵
5	23 - Hydroxytormentic acid	OH	H	CH_2OH	Me	H	H
6	23 - Hydroxytormentic acid ester glucoside	OH	H	CH_2OH	Me	H	D - Glucose
7	24 - Hydroxytormentic acid	OH	H	Me	CH_2OH	H	H
9	24 - Hydroxytormentic acid ester glucoside	OH	H	Me	CH_2OH	H	D - Glucose
10	7α - Hydroxytormentic acid	OH	H	Me	Me	OH	H
11	7α - Hydroxytormentic acid ester glucoside	OH	H	Me	Me	OH	D - Glucose
12	7α 23 - Dihydroxytormentic acid	OH	H	CH_2OH	Me	OH	H
13	7α 23 - Dihydroxytormentic acid ester glucoside	OH	H	CH_2OH	Me	OH	D - Glucose

reported recently by Amokar *et al.* [3]. It should be noted that the deoxocucurbitacin was isolated as a result of a screening process for cytotoxicity and in fact only in 0.003% yield. By comparison our investigation was orientated towards the compounds occurring in greatest amounts and biological activity was not tested. Ursene derivatives may in fact constitute the major part of the triterpenoids present in Amokar's sample but have no cytotoxicity. Alternatively the difference may be due to intraspecific variation dependent on geographical or genetic factors; the material used by Amokar came from Costa Rica whereas ours was grown in Scotland, probably from a Chilean source.

It has been noted that the iridoids isolated from *Desfontainia* cannot explain its reputed CNS activity [1, 2]. Similarly little is known of the actions of ursene triterpenoids on the CNS apart from the sedative action shown by some constituents of *Centella asiatica* [18]. Because of such lack of evidence it would be necessary to perform pharmacological tests before any basis for activity could be ascribed to these compounds.

EXPERIMENTAL

Biological material. Fresh leaves and stem of *D. spinosa* were obtained from the Younger Botanic Garden, Argyll, Scotland. The material was authenticated at source and a specimen voucher is deposited in the herbarium of the Chelsea Department of Pharmacy.

Isolation of triterpene glycosides. Fractionation of the aq. extract of the leaves has been described previously [2]. The methanolic and subsequent $MeOH–CHCl_3$ eluates from the charcoal bed showed several zones on TLC (systems a, c and d) which gave different colour reactions from the iridoids present. These compounds were monitored in the fractions obtained by DCCC separation (system 1) and fractions showing a particular compound were bulked and individual compounds isolated by prep. TLC [silica gel GF_{254} pre-washed with MeOH, 1 mm thick, $CHCl_3$-MeOH(5:1), EtOAc–MeOH(3:1). Bands were detected by spraying the edge of the plate with anisaldehyde reagent and heating the exposed strip]. The fractions showing the glucosides were 14–27, 36–65 and 66–93. Compounds were identified as outlined above.

23-Hydroxytormentic acid ester glucoside (**6**). Obtained as a white amorphous powder (25 mg) from fractions 36–65. It gave a blue colour with the TLC detection method. Spectral features were identical to those published [13].

24-Hydroxytormentic acid ester glucoside (**9**). Obtained as a white amorphous solid (40 mg) from fractions 66–93. It gave a blue colour with the TLC detection reagent. IR ν_{max}^{KBr} cm^{-1}: 3401(OH), 1726 (C=O), 1662 (C=C), 1461, 1383, 1278, 1231, 1164, 1076, 1032, 936, 856; EIMS (probe) 35 eV, *m/z* (rel. int.): 458 $[M - C_6H_{10}O_5 - HCOOH]^+$ (4), 264 (3), 260 (15), 246 (16), 222 (8), 218 (15), 203 (20), 201 (46), 187 (15), 146 (36), 57 (100); ^1H NMR (360 MHz, CD_3OD): δ5.22(H, *d*, *J* = 8 Hz, H-1′), 5.21 (1H, coarse *t*, *J* = 4 Hz, H-12), 3.93 (1H, *d*, *J* = 12 Hz, H-24a), 3.70 (2H, *m*, H-2β, H-6′a), 3.58 (1H, *dd*, *J* = 12, 4 Hz, H-6′b), 3.28 (1H, *d*, *J* = 12 Hz, H-24b), 3.20 (4H, *m*, H-2′ to H-5′) 2.95 (1H, *d*, *J* = 10 Hz, H-3α), 2.51 (1H, split *t*, *J* = 13, 4.5 Hz, H-5α), 2.41 (1H, *br s*, H-18β), 1.22, 1.13, 1.10, 0.89, 0.65 (3H each, *s*, Me-23, 25-29), 0.83 (3H, *d*, Me-30).

24-Hydroxytormentic acid glucoside heptaacetate. EIMS (probe) 18 eV, *m/z* (rel. int.): 960 $[M]^+$ (1), 900 $[M - HOAc]^+$ (2), 840 $[M - 2HOAc]^+$ (1), 780 $[M - 3 \times HOAc]^+$ (2), 720 $[M - 4 \times HOAc]^+$ (2), 600 $[M - 5 \times HOAc]^+$ (3), 587 (7), 331 (90), 272 (10), 188 (13), 169 (100), 135 (20); ^1H NMR (360 MHz, $CDCl_3$): δ5.51 (1H, *d*, *J* = 8 Hz, H-1′), 5.37 (1H, coarse *t*, *J* = 4 Hz, H-12), 5.25–5.09 (4H, *m*, H-2β, H-2′, H-3′, H-4′), 4.83 (1H, *d*, *J* = 10 Hz, H-3α), 4.28 (1H, *dd*, *J* = 12, 4 Hz, H-6′b), 4.21 (2H, *s*, H-24a, H-24b), 4.04 (1H, *dd*, *J* = 12 Hz, H-6′a), 3.78 (1H, *m*, H-5′), 2.53 (2H, *br s* overlapping split *t*, H-18β, H-5α), 2.07, 2.06, 2.05, 2.02 (3H each) 2.01 (6H) (*s*, 2 × OAc), 1.26, 1.20, 1.08, 1.03, 0.71 (3H each, *s*, Me-23, 25-29), 0.93 (3H, *d*, *J* = 6 Hz, Me-30).

7α-Hydroxytormentic acid ester glucoside (**11**). Obtained as a white amorphous powder (50 mg) from fractions 36–65. It gave a purple colour with the detection spray. IR ν_{max}^{KBr} cm^{-1}: 3401 (OH), 1728 (C=O), 1630 (C=C), 1567, 1408, 1263, 1229, 1065, 928, 803; EIMS (probe) 18 eV, *m/z* (rel. int.): 468 $[M - C_6H_{10}O_5 - H_2O]^+$ (12), 440 (12), 368 (45), 264 (28), 247 (27), 236 (51), 218 (24), 210 (30), 184 (57), 169 (100); ^1H NMR (360 MHz, CD_3OD): δ5.34

(1H, coarse *t*, *J* = 4 Hz, H-12), 5.31 (1H, *d*, *J* = 8 Hz, H-1′), 4.46 (1H, *m*, H-7β), 3.82 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 3. 67 (2H, *m*, H-2, H-6′b), 3.30 (4H, *m*, H-2′ to H-5′), 2.85 (1H, *d*, *J* = 10 Hz, H-3α), 2.60 (1H, split *t*, *J* = 13, 4.5 Hz, H-5α), 2.53 (1H, *br s*, H-18β), 1.37, 1.30, 1.20, 1.18, 1.07, 1.04 (3H each, *s*, Me-23 to 29), 0.93 (3H, *d J* = 6 Hz, Me-30).

7α-Hydroxytormentic acid ester glucoside hexaacetate. ^{1}H NMR (360 MHz,CDCl$_3$): δ5.54 (1H, *d*, *J* = 8 Hz, H-1′), 5.42 (1H, coarse *t*, *J* = 4 Hz, H-12), 5.24 (1H, *t*, *J* = 10 Hz, H-3′), 5.17 (2H, *m*, H-2β), 5.12 (1H, *t*, *J* = 10 Hz, H-2′), 4.69 (1H, *d*, *J* = 10 Hz, H-3α), 4.54 (1H, *m*, H-7β), 4.27 (1H, *dd*, *J* = 12, 4 Hz, H-6′b), 4.04 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 3.78 (1H, *m*, H-5′), 2.55 (1H, *br s*, H-18β), 2.52 (1H, split *t*, *J* = 13, 4.5 Hz, H-5α), 2.10, 2.07, 2.06, 2.03, 2.02, 1.98, (3H each, *s*, acetate Me), 1.45, 1.30, 1.24, 1.21, 1.02, 0.98 (3H each, *s*, Me-23 to 29), 0.94 (3H, *d*, *J* = 6 Hz, Me-30).

7α,23-Dihydroxytormentic acid ester glucoside (**13**). Obtained as a white amorphous powder (30 mg) from fractions 14–27. It gave a dark mauve colour with the detection reagent. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3443 (OH), 1728 (C=O),1631 (C=C), 1580, 1408, 1385, 1264, 1232, 1148, 1037, 927, 825; EIMS (probe) 35 eV, *m/z* (rel. int.): 452 [M − C$_6$H$_{10}$O$_5$ − 2H$_2$O − MeOH]$^+$ (3), 262 (10), 246 (15), 201 (13), 145 (12), 125 (60), 111 (35), 70 (100); ^{1}H NMR (360 MHz, CD$_3$OD): δ5.25 (1H, coarse *t*, *J* =4 Hz, H-12), 5.20 (1H, *d J* = 8 Hz, H-1′), 4.28 (1H, *m*, H-7β), 3.70 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 3.64 (1H, split *t*, *J* = 10, 4 Hz, H-2β), 3.58 (1H, *dd*, *J* = 12, 4 Hz, H-6′b), 3.48 (1H, *d*, *J* = 12 Hz, H-23a), 3.32 (1H, *d*, *J* = 10 Hz, H-3α), 3.26 (1H, *d*, *J* = 12 Hz, H-23b), 3.20 (4H, *m*, H-2′ to 5′), 2.50 (1H, split *t*, *J* = 13, 4.5 Hz, H-5α), 2.43 (1H, *br s* H-18β), 1.22, 1.19, 1.11, 0.96, 0.95 (3H each, *s*, Me-24 to 29), 0.83 (3H, *s*, Me-30).

7α,23-Dihydroxytormentic acid ester glucoside heptaacetate. EIMS (probe) 18 eV, *m/z* (rel. int.): 976 [M]$^+$ (1), 958 [M − H$_2$O]$^+$ (1), 916 [M − HOAc]$^+$ (1), 736 [M − 4 × HOAc]$^+$ (2), 676 [M − 5 × HOAc]$^+$ (3), 576 (15), 332 (60), 331 (100), 289 (50), 264 (6), 260 (48), 259 (90), 246 (20), 229 (50), 217 (31), 211 (43), 187 (53); ^{1}H NMR (360 MHz, CDCl$_3$): δ5.53 (1H, *d*, *J* = 8 Hz, H-1′), 5.43 (1H, coarse *t*, *J* = 4 Hz, H-12), 5.23 (3H, *m*, H-2β, H-3′, H-4′), 5.13 (1H, *t*, *J* = 10 Hz, H-2′), 5.03 (1H, *d*, *J* = 10 Hz, H-3α), 4.35 (1H, *m*, H-7β), 4.29 (1H, *dd*, *J* = 12, 4 Hz, H-6′a), 4.05 (1H, *dd*, *J* = 12, 2 Hz, H-6′b), 3.96 (1H, *d*, *J* = 12 Hz, H-23a), 3.79 (1H, *m*, H-5′), 3.71 (1H, *d*, *J* = 12 Hz, H-23b), 2.56 (1H, *br s*, H-18b), 2.53 (1H, split *t*, *J* = 13, 4.5 Hz, H-5α), 2.08 (6H, *s*, 2 × OAc), 2.04 (6H, *s*, 2 × OAc), 2.03 (3H, *s*, OAc), 2.02 (3H, *s*, OAc), 1.99 (3H, *s*, OAc), 1.48, 1.26, 1.24, 1.22, 1.01 (3H each, *s*, Me-24 to 29), 0.94 (3H, *d*, *J* = 6 Hz, Me-30).

A similar extraction of the stems yielded the same compounds. Since some non-glucosidic triterpenes were detected in other fractions of both stems and leaves it was decided that a less polar extraction process would be more efficient for them.

Isolation of triterpene aglycones. Powdered dried leaves (100 g) were extracted with 2 × 1 l. hot MeOH in a Soxhlet apparatus. The extract was concd to dryness to give 14.6 g residue. It was noted that a ppt formed as the extract was being concd. The extract was dissolved in 2 l. of a mixture of CHCl$_3$–MeOH–H$_2$O (3:2:1). The lower layer was taken to dryness to give 6.4 g residue. Three grams of the residue were separated by DCCC (descending mode CHCl$_3$–MeOH–H$_2$O, 9:13:8) with 5 ml fractions being collected and monitored by TLC (systems b and e). Not all the sample dissolved in the two-phase mixture used to introduce the sample into the DCCC process—the insoluble mixture was filtered off, dried and weighed (1.7 g). This residue was found to consist of very non-polar waxy material, chlorophyll and ursolic acid. Individual compounds were isolated by combining fractions containing them and performing prep. TLC (as above, solvents b and e).

Ursolic acid (**2**). 40 mg from DCCC fractions 1–7, 170 mg from insoluble portion. Isolated as white crystals, mp 285° (MeOH). It gave a purple colour with the detecting system. Spectral and chromatographic features were identical with an authentic sample obtained from Dr. P. J. Hylands.

Ursolic acid acetate (**3**). From fractions 1–7, isolated as white crystals (12 mg), mp 286° (MeOH). It gave a purple colour with the detecting reagent. Spectral and chromatographic features were identical to those shown by the acetylation product of authentic ursolic acid.

Pomolic acid (**4**). From fractions 1–7 isolated as a white amorphous powder (10 mg). It gave a reddish purple colour with the detection reagent. Spectral and chromatographic features were identical with those of an authentic sample of pomolic acid supplied by Prof. Brieskorn [9].

23-Hydroxytormentic acid (**5**). From fractions 19–39, isolated as a white amorphous powder (45 mg). It gave a blue colour with the detection system. Spectral features were identical with published values [11, 12].

24-Hydroxytormentic acid (**7**). From fractions 8–18, isolated as a white amorphous powder (55 mg). It gave a blue colour with the detecting reagent. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3422–2626 (broad, COOH, OH), 1685 (COOH), 1628 (C=C), 1595, 1457, 1381, 1268, 1235, 1156, 1109, 1074, 1050, 1033, 969, 803; EIMS (probe) 35 eV, *m/z* (rel. int.): 458 [M − COOH]$^+$ (7), 410 (15), 293 (23), 260 (15), 246 (16), 218 (16), 205 (17), 203 (21), 201 (46), 200 (15), 191 (12), 187 (15), 179 (27), 146 (35); ^{1}H NMR (360 MHz, CDCl$_3$): δ5.18 (1H, coarse *t*, *J* = 4 Hz, H-12) 3.94 (1H, *d*, *J* = 12 Hz, H-24a), 3.70 (1H, split *t*, *J* = 10, 4 Hz, H-2β), 3.28 (1H, *d*, *J* = 12 Hz, H-24b), 2.96 (1H, *d*, *J* = 10 Hz, H-3α), 2.44 (1H, split *t*, *J* = 13, 4.5 Hz, H-5α), 2.40 (1H, *br s*, H-18β), 1.24, 1.14, 1.09, 0.90, 0.69 (3H each, *s*, Me-23), 25-29), 0.83 (3H, *d*, *J* = 6 Hz, Me-30); ^{13}C NMR spectrum: see Table 1.

24-Hydroxytormentic acid triacetate. EIMS (probe) 35 eV, *m/z* rel int.): 586 [M − CO$_2$]$^+$ (2), 584 [M − HCOOH]$^+$ (3), 570 [M − HOAC]$^+$ (2), 521 [M − 2 × HOAc]$^+$ (25), 450 [M − 3 × HOAc]$^+$ (7), 264 (23), 246 (46), 218 (34), 205 (15); ^{1}H NMR (360 MHz, CDCl$_3$): δ5.34 (1H, coarse *t*, *J* = 4 Hz, H-12), 5.17 (1H, split *t*, *J* = 10, 4 Hz, H-2β), 4.85 (1H, *d*, *J* = 10 Hz, H-3α), 4.22 (2H, *s*, H-24a, H-24b), 2.55 (2H, *br s* overlapping split *t*, H-18β, H-5α), 2.07, 2.06, 1.98 (each 3H, *s*, OAc), 1.25, 1.20, 1.08, 1.04, 0.76 (each 3H, *s*, Me-23, 25 to 29), 0.96 (3H, *d*, *J* = 6 Hz, Me-30).

7α-Hydroxytormentic acid (**10**). From fractions 23–46, isolated as a white amorphous powder (65 mg). It gave a blue colour with the detecting reagent. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3421–2500 (broad, COOH, OH), 1688 (COOH), 1630 (C=C), 1455, 1382, 1266, 1239, 1215, 1153, 1106, 1069, 1044, 1020, 927, 862, 803; EIMS (probe) 35 eV, *m/z* (rel. int.): 486 [M − H$_2$O]$^+$ (3), 468 [M − 2 × H$_2$O]$^+$ (9), 458 [M − HCOOH]$^+$ (7), 450 (10), 440 (26), 422 (32), 264 (11), 246 (47), 231 (18), 222 (28), 218 (26), 208 (13), 204 (33), 201 (64), 200 (41), 196 (71), 187 (46), 186 (36), 185 (62), 146 (100); ^{1}H NMR (360 MHz, CD$_3$OD): δ5.32 (1H, coarse *t*, *J* = 4 Hz, H-12), 4.47 (1H, *m*, H-7β), 3.67 (1H, split *t*, *J* = 10, 4 Hz, H-2β), 2.85 (1H, *d*, *J* = 10 Hz, H-3α), 2.54 (1H, *br s*, H-18), 2.53 (1H, split *t*, *J* = 13, 5 Hz, H-5α), 1.90 (2H, *dd*, *J* = 11, 4 Hz, H-11), 1.35, 1.32, 1.20, 1.18, 1.09, 1.08 (3H each, *s*, Me-23 to 29), 0.93, *d*, *J* = 6 Hz, Me-30); ^{13}C NMR: see Table 1.

7α-Hydroxytormentic acid diacetate. EIMS (probe) 35 eV, *m/z* (rel. int.): 542 [M − HCOOH]$^+$ (1), 524 [M − HCOOH − H$_2$O]$^+$ (2), 470 [M − 2 × HOAc]$^+$ (3), 279 (14), 264 (4), 246 (9), 222 (2), 218 (6), 205 (5), 201 (9), 185 (14); ^{1}H NMR (360 MHz, CDCl$_3$): δ5.37 (1H, coarse *t*, *J* = 4 Hz, H-12), 5.17 (1H, split *t*, *J* = 10, 4 Hz, H-2β), 4.69 (1H, *d*, *J* = 10 Hz, H-3α), 4.54 (1H, *m*, H-7β), 2.54 (1H, *br s*, H-18β), 2.52 (1H, split *t*, *J* = 13, 4.5 Hz, H-5α), 2.07, 1.98 (3H each, *s*, OAc), 1.44, 1.30, 1.23, 1.20, 1.04, 0.98 (3H, *s*, Me-23 to 29), 0.94 (3H, *d*, *J* = 6 Hz, Me-30).

Table 1. ^{13}C NMR spectral data for some of the triterpenoids isolated

	2	7	10	12
1	38.7	46.1	48.4	48.2
2	23.5	67.7	67.7	67.8
3	79.0	84.2	82.9	76.6
4	39.6	42.5	41.2	42.8
5	52.7	53.3	53.3	47.2
6	18.3	18.0	27.7	27.9
7	33.0	32.6	66.9	67.0
8	39.1	40.8	39.2	38.3
9	47.6	46.8	47.4	47.1
10	36.7	39.2	38.4	36.7
11	23.7	24.9	22.8	23.8
12	125.8	127.2	127.6	126.7
13	138.0	138.2	137.5	138.5
14	42.0	41.1	41.2	41.4
15	29.4	28.6	28.7	28.7
16	23.3	25.4	25.4	25.5
17	47.9	47.7	47.9	47.9
18	55.3	55.3	55.3	54.0
19	30.6	71.9	71.8	72.4
20	30.4	41.3	41.1	41.1
21	27.3	27.7	24.8	25.3
22	37.0	37.2	37.1	37.7
23	23.4	22.9	25.2	64.5
24	17.0	64.2	16.6	17.1
25	17.0	21.8	16.8	22.3
26	15.5	14.6	16.6	14.8
27	24.2	22.3	27.0	25.9
28	176.0	180.7	180.6	178.4
29	21.1	23.0	22.9	23.0
30	23.4	15.7	14.6	13.3

Spectra were run in $CDCl_3$ at 100.63 Hz. Chemical shifts are given downfield from TMS. Assignments were made with the aid of the DEPT spectra of the compounds.

7α-Hydroxytormentic acid triacetate. EIMS (probe) 35 eV *m/z* (rel. int.): 615 $[M-Me]^+$ (2), 586 $[M-CO_2]^+$ (26), 570 $[M-HOAc]^+$ (19), 567 (30), 513 (23), 450 (15), 280 (21), 264 (22), 246 (66), 233 (92), 218 (34), 201 (78), 187 (94), 185 (60), 173 (42); ^{1}H NMR (360 MHz, $CDCl_3$): δ5.55 (1H, *m*, H-7β), 5.34 (1H, coarse *t*, *J* = 4 Hz, H-12) 5.10 (1H, split *t*, *J* = 10, 4 Hz, H-2β), 4.66 (1H, *d*, *J* = 10 Hz, H-3α), 2.54 (1H, *s*, H-18β), 2.52 (1H, split *t*, *J* = 13, 4.5 Hz, H-5α), 2.15, 2.04, 1.95 (each 3H, *s*, OAc), 1.38, 1.27, 1.17, 1.02, 0.94, 0.90 (each 3H, *s*, Me-23 to 29), 0.89 (3H, *d*, *J* = 6 Hz, Me-30).

7α,23-Dihydroxytormentic acid (**12**). Isolated from the stationary phase fraction as a white amorphous powder (40 mg). It gave a dark blue–purple colour with the detection reagent. IR ν_{max}^{KBr} cm^{-1}: 3445–2575 (broad, COOH, OH), 1688 (COOH), 1634 (C=C), 1576 (1411, 1385, 1351, 1268, 1237, 1216, 1043, 895, 763; EIMS (probe) 35 eV, *m/z* (rel. int.): 452 $[M-2H_2O-MeOH]^+$ (3), 430 (8), 397 (8), 332 (3), 270 (12), 262 (4), 260 (100), 245 (19), 229 (7), 218 (17), 201 (13), 189 (10), 146 (5), 132 (32); ^{1}H NMR (360 MHz, CD_3OD): δ5.22 (1H, coarse *t*, *J* = 4 Hz, H-12), 4.28 (1H, *m*, H-7β), 3.64 (1H, split *t*, *J* = 10, 4 Hz, H-2α), 3.48 (1H, *d*, *J* = 12 Hz, H-23a), 3.35 (1H, *d*, *J* = 10 Hz, H-3α), 3.23 (1H, *d*, *J* = 12 Hz, H-23b), 2.56 (1H, *br s*, H-18), 2.31 (1H, split *t*, *J* = 13, 4.5 Hz, H-5α), 1.28, 1.22, 1.11, 1.02, 0.96 (each 3H, *s*, Me-24 to 29), 0.83 (3H, *d*, *J* = 6 Hz, Me-30). ^{13}C NMR: see Table 1.

7α,23-Dihydroxytormentic acid triacetate. EIMS (probe) 35 eV, *m/z* (rel. int.): 600 $[M-HCOOH]^+$ (12), 528 $[M-2\times HOAc]^+$ (8), 466 $[M-3\times HOAc]^+$ (6), 264 (12), 259 (43), 246 (42), 231 (23), 218 (34), 203 (23), 201 (46), 187 (23), 173 (21), 146 (100); ^{1}H NMR (360 MHz, $CDCl_3$): δ5.38 (1H, coarse *t*, *J* = 4 Hz, H-12), 524 (1H, split *t*, *J* = 10, 4 Hz, H-2β), 5.02 (1H, *d*, *J* = 10 Hz, H-3α), 4.35 (1H, *m*, H-7β), 3.95 (1H, *d*, *J* = 12 Hz, H-23a), 3.74 (1H, *d*, *J* = 12 Hz, H-23b), 2.55 (1H, *br s*, H-18β), 2.53 (1H, split *t*, *J* = 13, 4.5 Hz, H-5α), 2.09, 2.05, 2.00 (each 3H, *s*, OAc), 1.46, 1.28, 1.23, 1.22, 1.03 (each 3H, *s*, Me-24 to 29), 0.96 (3H, *d*, *J* = 6 Hz, Me-30).

A similar extraction and separation of stem material yieded the same compounds.

TLC. Silica gel GF_{254}. Solvents: (a) $CHCl_3$–MeOH (5:1), (b) $CHCl_3$–MeOH (10:1), (c) EtOAc–MeOH (3:1), (d) EtOAc–MeOH (6:1), (e) $CHCl_3$–EtOAc (1:1). Detection of zones was by examination in daylight after spraying with 0.5% anisaldehyde in HOAc–H_2SO_4–MeOH (2:1:17) and heating at 105° for 10 min. The triterpenoids appear as blue or purple spots.

Cold acetylation was carried out using standard procedures at room temp. overnight.

Hot acetylation was carried out by refluxing on a water bath for 3 hr.

Methylation was performed using the Aldrich Mini-Diazald kit.

Enzyme hydrolysis. A few mg of the sample was suspended in water and elaterase incubated at 37° for 3 days. The reaction mixture was then extracted with 3 × 50 ml $CHCl_3$–MeOH (3:2), the organic layers combined and taken to low vol. under red. pres. The concd extract was examined by TLC and products identified by comparison with authentic compounds run on the same plate.

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