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# TRITERPENE ACIDS FROM SOME PAPUA NEW GUINEA *TERMINALIA* SPECIES

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**Key Word Index**—*Terminalia brassii*; *T. impediens*; *T. complanata*; Combretaceae; wood; triterpenoids; arjunolic acid; asiatic acid; terminolic acid; chemotaxonomy.

**Abstract**—The trihydroxylated triterpene acids arjunolic acid and asiatic acid were found in ether extracts of the woods of *Terminalia brassii* and of *T. complanata*. Asiatic acid has not previously been reported as a constituent of the genus *Terminalia*. A similar extract of *T. impediens* yielded the tetrahydroxy acid terminolic acid as the major constituent; this acid was also detected as a minor constituent of *T. complanata*. Terminolic acid, however, does not occur in glycosidic combination in *Centella asiatica*, as has been recently reported.

## INTRODUCTION

In reforestation/plantation studies carried out during the last 30 years, the Papua New Guinea (PNG) Office of Forests has established many trial plots of native hardwoods throughout PNG. Eight species, namely, *Acacia auriculiformis*, *Albizia falcataria*, *Eucalyptus deglupta*, *E. tereticornis*, *Gmelina arborea*, *Terminalia brassii*, *T. complanata* and *T. impediens*, which showed high growth rates and high yields of wood per hectare, were chosen for pulping studies, and for a study of the correlation of their gross chemical composition with their pulping properties [1]. As an extension of this, we now report on some extractives of the three *Terminalia* species, *T. brassii*, Exell, *T. complanata* Schumann and *T. impediens* Coode.

## RESULTS AND DISCUSSION

The finely milled wood of each of the three *Terminalia* species was subjected to sequential extraction with hexane, ether, acetone and methanol. The hexane extracts of each species yielded sitosterol, and the acetone extracts of each species yielded ellagic acid. The crude residues from the ether extracts of *T. brassii*, *T. impediens* and *T. complanata* constituted 1.2, 0.25 and 0.21%, respectively, of the dried woods. A combination of trituration with acetone and chromatography on silica gel of the *T. brassii* extract yielded mixtures of arjunolic acid (**1a**) and asiatic acid (**2a**) which could not be separated by crystallization, nor by chromatography of their methyl esters. Pure samples of the two triterpene acids (**1a**) and (**2a**) were obtained by using the separation procedure of Lewis and Tucker [2].

HPLC analysis of the residue from the ether extract of *T. impediens* revealed that it contained arjunolic acid (**1a**), but the major constituent was a more polar compound which upon isolation by column chromatography on silica gel proved to be the tetrahydroxy acid terminolic acid (**3a**), further characterized as its methyl ester (**3b**) and by conversion into methyl terminolate triacetate (**3c**). HPLC of the residue from the ether extract of *T. complanata* wood showed that it contained mainly arjunolic acid (**1a**) and asiatic acid (**2a**), together with some terminolic acid (**3a**).

## CHEMOTAXONOMY

Of the approximately 21 species of *Terminalia* recognized in PNG [3], and 29 species in Australia [4], very few have heretofore been subjected to even cursory chemical study. On the other hand, the woods of a relatively large number of *Terminalia* species from outside the Australia, Papua New Guinea region have been examined, and they characteristically contain, *inter alia*, a range of polyhydroxylated triterpene acids, some of these also being present as glycosides. Arjunolic acid (**1a**) has been isolated from the bark and wood of *T. arjuna* (Roxb.) Wight and Arnot [5–7] and *T. alata* Heyne ex Roth [8–10], and from the wood of *T. tomentosa* W. et A. [11]. On the other hand, our isolation of asiatic acid (**2a**) from the wood of the PNG species *T. brassii* is the first reported occurrence of this trihydroxy triterpene acid in a *Terminalia* species. In fact, the only previous report on the isolation of an ursane derivative from this genus is the recent discovery of 2α-hydroxymicromeric acid (**4**) in the leaves of *T. chebula* Retz [12]. Terminolic acid (**3a**) has previously been isolated from the woods of *T. alata* [13], *T. avicennioides* Guill. et Perr. [14], *T. catappa* [15], *T. glaucescens* Planeh. ex Benth [14], *T. laxiflora* Engl. [14], and *T. macroptera* Guill. et Perr. [15], and from both the wood and bark of *T. ivorensis* A. Cher. [16, 17].

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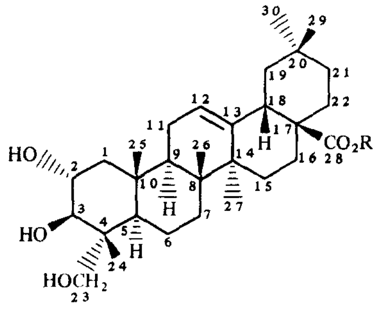
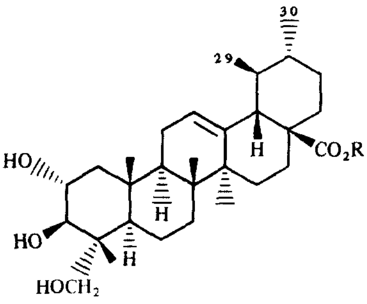
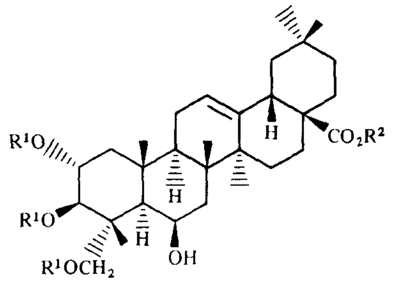


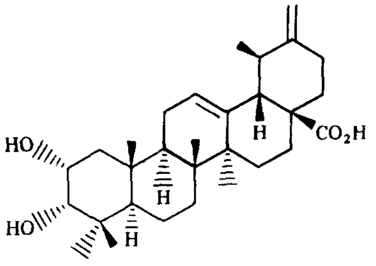
| | R |
|---|---|
| **1a** | H |
| **1b** | Me |



| | R |
|---|---|
| **2a** | H |
| **2b** | Me |



| | R¹ | R² |
|---|---|---|
| **3a** | H | H |
| **3b** | H | Me |
| **3c** | Ac | Me |



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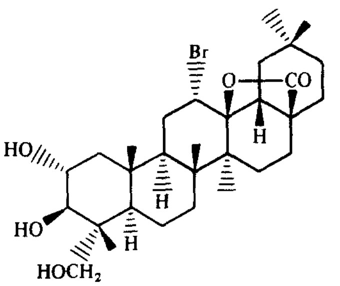

**5**

*NMR spectroscopy—a literature correction*

The $^{13}$C NMR spectra of methyl arjunolate (**1a**) and methyl asiatate (**2a**) have been assigned previously [18], and our data are in accord with these assignments. The $^{1}$H NMR spectrum of methyl terminolate 2α,3β,23-triacetate (**3c**) was essentially identical with that reported by Mallavarapu and Muralikrishna [13] except that their reported δ value (1.254, *s*) for one of the angular methyl groups was an incidental error, and should read 1.46 (Mallavarapu, G. R., personal communication) in agreement with our figure; this signal is attributed to the Me-10β group which is in a 1,3-diaxial relationship with the 6β-axial hydroxyl group. Our assignment of the $^{1}$H NMR spectrum of methyl terminolate 2α,3β,23-triacetate (**3c**) was made by comparison with that of methyl arjunolate (**1b**), and from chemical shift considerations. The $^{13}$C NMR spectra of terminolic acid (**3a**), methyl terminolate (**3b**) and of methyl terminolate 2α,3β,23-triacetate (**3c**) were assigned from DEPT spectra and by comparison with the spectrum of methyl arjunolate (**1b**), taking into account the chemical shift effects of introducing a 6β-axial hydroxyl group. The $^{13}$C NMR data for compounds **1b**–**3c** are compared in Table 1.

A recent report [19] that hydrolysis of a triterpenoid glycoside from *Centella asiatica* afforded terminolic acid (**3a**) is incorrect. The $^{1}$H NMR spectrum reported [19] for the tetrahydroxylated triterpene acid from this source, differs markedly from our data; similarly, the reported $^{1}$H and $^{13}$C NMR data [19] for the triacetylated methyl ester are quite different from our data, and from the previously published $^{1}$H NMR spectrum [13].

## EXPERIMENTAL

Mps: uncorr. TLC was performed on silica gel. $^{1}$H and $^{13}$C NMR spectra were measured at 200 or 300 MHz, and 50 or 75 MHz, respectively. The column used for HPLC analysis of the triterpene acids was a Waters Resolve 5 μm C-18 Radial Pak 8 mm × 10 cm cartridge; the eluent was 72% MeOH–$H_2O$ adjusted to pH 4.7 with HOAc.

*Wood samples.* A sample of *T. brassii* obtained from Oomsis, Morobe Province was a mixture from three 12-year-old plantation trees—voucher specimens P7, P8 and P9. The *T. com-*

*planata* sample was a composite chip mixture from three natural growth trees aged 5–6 years from the Gogol Valley—voucher specimen P10. The *T. impediens* sample was a composite chip mixture of three natural growth trees aged 8–10 years from the Gogol Valley—voucher specimen P12.

*Extraction procedure.* The finely milled wood (~1 kg) was extracted successively with hexane, $Et_2O$ and $Me_2CO$ (~10 l each). The extracts were concd and the residues examined as described below.

Terminalia brassii *wood: isolation of arjunolic acid* (**1a**) *and asiatic acid* (**2a**) *from the ether extract.* The residue (12.0 g) from the $Et_2O$ extract of *T. brassii* wood was triturated with $Me_2CO$ (500 ml). The insoluble residue (3.47 g) was recrystallized from $Me_2CO$ (300 ml) to give a solid (1.9 g), HPLC analysis of which showed it to consist mainly of arjunolic acid (**1a**) and asiatic acid (**2a**). Treatment of this mixture with $Br_2$–HOAc–NaOAc [2] converted the arjunolic acid (**1a**) ($2\alpha,3\beta$,23-trihydroxyolean-12-en-28-oic acid) into its bromolactone (**5**) ($2\alpha,3\beta$,23-trihydroxy-$12\alpha$-bromoolean-13,28-lactone), which was separated from the asiatic acid (**2a**) ($2\alpha,3\beta$,23-trihydroxyurs-12-en-28-oic acid) by chromatography; arjunolic acid (**1a**) was regenerated from the pure bromolactone (**5**).

Methylation of the pure asiatic acid (**2a**) with $CH_2N_2$ and recrystallization from $Me_2CO$ gave methyl asiatate (**2b**), mp 155–160°, resolidified, then 220–224° $[\alpha]_D$ +48° ($CHCl_3$; *c* 0.9) (lit. [18] 222–224°, +49°). $^1H$ NMR (50 MHz, $CDCl_3$): $\delta$0.73 (*s*, H-26), 0.78 (*s*, H-24), 0.88 (*s*, H-25), 0.96 (*s*, H-29), 1.05 (*s*, H-30), 1.10 (*s*, H-27), 2.22 (*d*, $J = 14$ Hz, H-18), 3.38 (*t*, $J = 10$ Hz, $CH_2OH$), 3.51 (*s*, $CO_2Me$), 3.75 (*br s*, H-3), 3.95 (*br s*, H-2), 5.25 (*s*, H-12). EIMS (probe) 70 eV (rel. int.): *m/z* 502 ($[M]^+$, 2), 263 (21), 262 (100), 203 (64), 204 (14), 189 (16), 133 (36), 119 (16), 105 (13). The $^{13}C$ NMR spectrum is given in Table 1.

Methylation ($CH_2N_2$) of pure arjunolic acid (**1a**), and crystallization of the product from hexane–$Et_2O$ afforded pure methyl arjunolate (**1b**), mp 210–215°, resolidified, then 250–255°; $[\alpha]_D$ +36.6° ($CHCl_3$; *c* 0.93) (lit. [18] 250–252°; +53.1°). $^1H$ NMR (50 MHz, $CDCl_3$): $\delta$0.69 (*s*, H-26), 0.74 (*s*, H-24), 0.88 (*s*, H-25), 0.91 (*s*, H-29), 0.97 (*s*, H-30), 1.11 (*s*, H-27), 2.87 (*d*, $J = 14$ Hz, H-18), 3.35 (*t*, $J = 12$ Hz, $CH_2OH$), 3.51 (*s*, $CO_2Me$), 3.91 (*br s*, H-3), 4.07 (*br s*, H-2), 5.26 (*s*, H-12). EIMS (probe) 70 eV (rel. int.): *m/z* 502 ($[M]^+$), 263 (16), 262 (81), 204 (16), 203 (100), 189 (24), 133 (24), 119 (14), 105 (13). The $^{13}C$ NMR spectrum is given in Table 1.

Terminalia impediens *wood: isolation of terminolic acid* (**3a**) *from the ether extract.* Concn of the $Et_2O$ extract yielded a pale brown crystalline solid (2.5 g). Chromatography of this material (2.0 g) on silica gel and elution with $CHCl_3$ containing increasing amounts of MeOH afforded a solid which upon recrystallization from MeOH, then from EtOAc, gave pure terminolic acid (**3a**) ($2\alpha,3\beta,6\beta$,23-tetrahydroxyolean-12-en-28-oic acid) mp 333–334°, $[\alpha]_D$ +34° (MeOH; *c* 1.0) (lit. [16] 335–336°, +40°). $^1H$ NMR (200 MHz, $d_5$-pyridine): $\delta$0.93 (*s*, Me), 1.01 (*s*, Me), 1.24 (*s*, Me), 1.65 (*s*, Me), 1.77 (*s*, Me), 1.80 (*s*, Me), 3.35 (*d*, $J = 10$ Hz, H-18), 4.07 (*d*, $J = 10$ Hz), and 4.27 (*d*, $J = 9$ Hz, H-23, H-23), 4.43 (*d*, $J = 10$ Hz, H-3), 5.12 (*s*, H-6), 5.58 (apparent *s*, H-2 and H-12). $^{13}C$ NMR (50 MHz, $d_5$-pyridine): $\delta$14.2 (C-24), 16.9 (C-25 or C-26), 17.2 (C-26 or C-25), 22.0 (C-16), 22.3 (C-30, C-11), 24.5 (C-27), 26.5 (C-15), 28.8 (C-20), 29.2 (C-22), 31.5 (C-29), 32.5 (C-21), 36.5 (C-10), 37.6 (C-8), 39.3 (C-7), 40.3 (C-18), 41.0 (C-14), 42.8 (C-4), 44.7 (C-1), 44.9 (C-19), 47.0 (C-17), 47.1 (C-9), 48.3 (C-5), 64.4 (C-23), 65.8 (C-6), 67.3 (C-2), 76.5 (C-3), 122.1 (C-12), 142.5 (C-13), 178.5 (C-28). EIMS (probe) 70 eV, *m/z* (rel. int.): 504 ($[M]^+$, 5), 248 (100), 203 (98), 204 (23), 188 (24), 133 (26), 119 (19).

Treatment of terminolic acid (**3a**) (300 mg) with $CH_2N_2$, and recrystallization of the product from hexane–$Et_2O$ yielded methyl $2\alpha,3\beta,6\beta$,23-tetrahydroxyolean-12-en-28-oate (methyl terminolate (**3b**) (220 mg), mp 159–160°, $[\alpha]_D$ +34.7° ($CHCl_3$; *c* 0.97) (lit. [16] 165–168°, +40°). $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$0.91, 0.95, 0.99 (each *s*, 3 × Me), 1.10 (overlapping *s*, 2 × Me), 1.35 (*s*, Me), 2.90 (*d*, $J = 11$ Hz, H-18), 3.33 (*m*, H-2 and H-3), 3.62 (*s*, $CO_2Me$), 3.75 (*m*, $CH_2OH$), 4.39 (apparent *s*, H-6), 5.33 (*s*, H-12). EIMS (probe) 70 eV, *m/z* (rel. int.): 518 ($[M]^+$, 2), 302 (2), 262 (90), 255 (2), 238 (6), 203 (100), 202 (24), 189 (29), 133 (22). The $^{13}C$ NMR spectrum is given in Table 1.

Table 1. $^{13}C$ NMR spectral data for compounds **1b**, **2b**, **3b** and **c** (in $CDCl_3$, 50 MHz)

| C | **1b** | **2b** | **3b** | **3c** |
|---|---|---|---|---|
| 1 | 46.2 | 46.4 | 45.8 | 45.5 |
| 2 | 68.0 | 68.0 | 68.7 | 69.8 |
| 3 | 78.8 | 78.8 | 78.1 | 74.9 |
| 4 | 42.7 | 42.7 | 43.5 | 42.3 |
| 5 | 48.0 | 48.0 | 48.3 | 48.2 |
| 6 | 18.1 | 18.1 | 68.1 | 67.7 |
| 7 | 32.1 | 32.4 | 40.3 | 40.6 |
| 8 | 39.2 | 39.4 | 38.4 | 38.4 |
| 9 | 48.0 | 47.3 | 47.7 | 47.8 |
| 10 | 38.0 | 38.0 | 37.6 | 37.3 |
| 11 | 23.4 | 23.3 | 23.4 | 23.3 |
| 12 | 122.0 | 125.2 | 122.4 | 122.2 |
| 13 | 143.8 | 138.2 | 143.1 | 142.9 |
| 14 | 41.6 | 42.0 | 42.2 | 42.0 |
| 15 | 27.6 | 27.9 | 27.6 | 27.5 |
| 16 | 23.0 | 24.1 | 23.0 | 22.9 |
| 17 | 46.6 | 47.9 | 46.7 | 46.6 |
| 18 | 41.2 | 52.8 | 41.2 | 41.2 |
| 19 | 45.8 | 39.0 | 45.8 | 45.7 |
| 20 | 30.6 | 38.8 | 30.7 | 30.6 |
| 21 | 33.8 | 30.6 | 33.9 | 33.8 |
| 22 | 32.3 | 36.6 | 32.4 | 32.3 |
| 23 | 68.6 | 68.6 | 66.6 | 65.2 |
| 24 | 13.2 | 13.1 | 14.7 | 15.2 |
| 25 | 16.8 | 16.8 | 18.0[a] | 17.9[a] |
| 26 | 17.0 | 17.0 | 18.6[a] | 18.3[a] |
| 27 | 26.1 | 23.7 | 26.2 | 25.7 |
| 28 | 178.3 | 178.5 | 178.0 | 178.1 |
| 29 | 33.1 | 17.2 | 33.2 | 33.0 |
| 30 | 23.6 | 21.2 | 23.7 | 23.5 |
| $CO_2Me$ | 51.5 | 51.4 | 51.7 | 51.6 |
| OCO**Me** | | | | 20.7 |
| " | | | | 20.8 |
| " | | | | 21.0 |
| O**C**OMe | | | | 170.3 |
| " | | | | 170.4 |
| " | | | | 170.9 |

[a] These assignments may be reversed.

Methyl terminolate (**3b**) (90 mg) was treated with $Ac_2O$ (1 ml) in pyridine (1 ml) at room temp. overnight to yield methyl $2\alpha,3\beta$,23-triacetoxy-$6\beta$-hydroxyolean-12-en-28-oate (methyl terminolate triacetate) (**3c**), mp 162–164°, $[\alpha]_D$ +10° ($CHCl_3$; *c* 0.5) (lit. [20] 160–162°, +19°). $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$0.91 (*s*, H-29), 0.94 (*s*, H-30), 1.02 (*s*, H-26), 1.09 (*s*, H-27), 1.28 (*s*, H-24), 1.46 (*s*, H-25), 1.99, 2.04, 2.07 (each *s*, 3 × OCOMe), 2.89 (*d*, $J = 10$ Hz, H-18), 3.62 (*s*, $CO_2Me$), 3.71 [(*d*, $J = 12$ Hz) and 3.95 (*d*, $J = 12$ Hz), $CH_2OAc$], 4.35 (apparent *s*, H-6), 5.01 (*d*, $J = 10$ Hz, H-3), 5.23 (*m*, H-2), 5.33 (*br s*, H-12) (cf. [13]). The $^{13}C$ NMR spectrum is given in Table 1.

Terminalia complanata *wood: the ether extract.* The ether extract (2.1 g) was shown by HPLC to contain largely arjunolic acid (**1a**) and asiatic acid (**2a**) in addition to a small amount of terminolic acid (**3a**). There was also a minor unidentified component of greater polarity. This extract was not examined further.

*Isolation of sitosterol.* Repeated recrystallizations from MeOH of the residues from the hexane extracts of each of the three species *T. brassii*, *T. impediens* and *T. complanata* yielded pure sitosterol (24$\beta$-ethylcholest-5-en-3$\beta$-ol), mp 135–136.5°, $[\alpha]_D$ −33.2° (MeOH; *c* 1.0) (lit. [18] 138–138.5°, −32.5°); it showed a single peak on HPLC analysis. The $^1$H and $^{13}$C NMR spectra were identical with the reported data [21]. Acetylation gave $\beta$-sitosteryl acetate, mp 120–122°, $[\alpha]_D$ −36.7° ($CHCl_3$; *c* 1.04), (lit. [18] 120–121°, −36.7°).

*Isolation of ellagic acid.* The residues from the $Me_2CO$ extracts of the woods of both *T. brassii* and *T. complanata* were triturated with a small volume of $Me_2CO$ to give an insoluble solid, mp >350° which was identified as ellagic acid by comparison of its IR spectrum with that of an authentic specimen. $^{13}$C NMR (50 MHz, $d_6$-DMSO): $\delta$ 106.9 (C-1, 1′), 109.9 (C-5, 5′), 112.4 (C-6, 6′) 136.3 (C-2, 2′), 140.3 (C-3, 3′), 148.2 (C-4, 4′), 159.2 (C-7, 7′).

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