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TWO NEW TRITERPENOIDS FROM *RHODOMYRTUS TOMENTOSA*

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Key Word Index—*Rhodomyrtus tomentosa*; Myrtaceae; triterpenoids; 3β-hydroxy-21αH-hop-22(29)-en-30-al; 21αH-hop-22(29)-en-3β,30-diol; 3β-acetoxy-11α,12α-epoxyoleanan-28,13β-olide; 3β-acetoxy-12-oxo-oleanan-28,13β-olide; 3β-acetoxy-12α-hydroxyoleanan-28,13β-olide; triterpenoid acids.

Abstract—Repetition of an investigation of the petrol extracts of *Rhodomyrtus tomentosa* has led to the isolation of two new triterpenoids, R_4 from the leaves and R_5 from the stems besides R_1, R_2, R_3 and the other known compounds already reported. R_1 and R_4 were proved to be 21αH-hop-22(29)-en-3β,30-diol and 3β-hydroxy-21αH-hop-22(29)-en-30-al respectively, and R_2, R_3 and R_5 are 3β-acetoxy-11α,12α-epoxyoleanan-28,13β-olide, 3β-acetoxy-12α-hydroxyoleanan-28,13β-olide and 3β-acetoxy-12-oxo-oleanan-28,13β-olide respectively. The ethanol extract of the leaves contained betulinic, ursolic and aliphitolic acids and that of the stems betulonic, betulinic and oleanolic acids.

INTRODUCTION

The petrol extracts of the leaves and stems of *Rhodomyrtus tomentosa* have been examined in this laboratory [1]. Lupeol, β-amyrin, 3β-hydroxyolean-12-en-11-one, betulin and an unidentified diol R_1 ($C_{30}H_{50}O_2$) were isolated from the former, and friedelin, lupeol, α-amyrin, taraxerol, betulin-3-acetate, betulin and two lactones, R_2 ($C_{32}H_{48}O_5$) and R_3 ($C_{32}H_{50}O_5$) from the latter. On repetition of the examination in order to obtain larger quantities of R_1, R_2 and R_3 for structural work, two other triterpenoids have now been isolated, R_4 ($C_{30}H_{48}O_2$) from the leaves and R_5 ($C_{32}H_{48}O_5$) from the stems. We describe here the structure determinations of these five compounds. The acidic triterpenoids from this plant are also reported.

RESULTS AND DISCUSSION

The diol R_1 (**1**), $C_{30}H_{50}O_2$ (M^+, *m/e* 442) [1] formed a diacetate (**2**), $C_{34}H_{54}O_4$ (M^+, *m/e* 526). Compound **1** contained a free rotating CH_2OH group [NMR: δ 4.12 (2 H, broad *s*) shifted to δ 4.67 in that of **2**]. Both **1** and **2** possessed a $C{=}CH_2$ group [δ 4.92 (2 H, broad *s*)], the low field character of which could be explained by the partial structure $CH_2{=}\overset{\overset{|}{C}}{C}{-}CH_2OR$ (R=H or Ac) which was also indicated in the IR spectra of both compounds at ν_{max} 1650, 915 cm^{-1} [2]. The second OH group in **1** appeared to be secondary [δ 3.19 (1 H, *m*)].

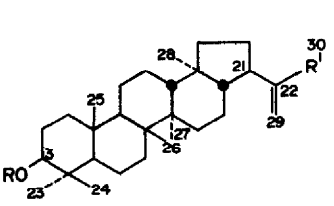
Compound R_4 (**3**), $C_{30}H_{48}O_2$ (M^+, *m/e* 440), which was less polar than **1**, contained an OH function (γ_{max} 3320 cm^{-1}), which was secondary and equatorial [δ 3.19 (1 H, *q*, *J*=7 and 10 Hz)], and a $CH_2{=}\overset{\overset{|}{C}}{C}{-}CHO$ group [ν_{max} 2845, 2720, 1690, 1630 cm^{-1}, δ 5.90, 6.30 (1 H ea., both ~ *s*), δ 9.68 (1 H, *s*); λ_{max} 228 nm (ε9700)]. It formed a monoacetate (**4**), $C_{32}H_{50}O_3$, and the presence of its terminal double bond was confirmed by the formation of formaldehyde upon ozonolysis.

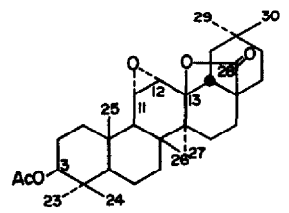
Oxidation of the allylic OH function in **1** with MnO_2 yielded a conjugated aldehyde identical with **3**, while reduction of **3** with $NaBH_4$ gave the diol **1**. Thus the two compounds are inter-related.

The presence of a substituted isopropenyl group together with 6 tertiary Me signals indicated in the NMR spectra of the compounds **1–4** suggested either a hop-22(29)-ene or a lup-20(29)-ene skeleton. The former was proved to be correct by Wolff–Kishner reduction of **3**, which yielded moretenol (21αH-hop-22(29)-en-3β-ol) (**5**). Hence **3** is 3β-hydroxy-21αH-hop-22(29)-en-30-al and **1** is the corresponding 30-hydroxy compound. These structures were further confirmed by partial syntheses from moretenyl acetate (**6**) through oxidation with SeO_2 and $Pb(OAc)_4$ separately in glacial acetic acid which gave the acetates **4** and **2** respectively. Alkaline hydrolysis of the former yielded **3** and of the latter gave **1**.

The 6 tertiary methyl NMR signals of compounds **1–4** are assigned as shown in Table 1.

Compound **3** appears to be the third naturally occurring pentacyclic triterpenoid containing a conjugated aldehyde function. The other two are 3-acetoxyurs-20-en-al from *Stemmadenia donell-smithii* [3] and filic-3-





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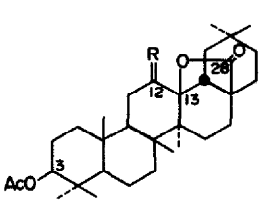

(8) R=α-OH, β-H
(9) R=O

Table 1. NMR spectra of the triterpenoids from *Rhodomyrtus tomentosa*

Compound	C-23	C-24	C-25	C-26	C-27	C-28
(1)	0.96	0.76	0.83	1.01	0.93	0.74
(2)	0.83	0.83	0.83	1.01	0.92	0.76
(3)	0.97	0.76	0.82	1.01	0.93	0.82
(4)	0.85	0.85	0.85	1.02	0.92	0.79

Table 2. NMR spectra of the triterpenoids derived from *Rhodomyrtus tomentosa*

Compound	C-23	C-24	C-25	C-26	C-27	C-29	C-30
(7)	0.88	0.88	0.99	1.04	1.07	0.88	0.92
(8)	0.87	0.87	0.93	0.95	1.30	0.95	0.95
(9)	0.87	0.87	0.98	1.14	1.30	0.90	0.90

en-23-al from *Adiatum pedatum* [4]. However, such a function in the substituted isopropenyl side chain in triterpenoids has not been reported previously.

The two compounds previously obtained from the stems, R_2 (7) and R_3 (8), both contained an acetoxy group and a γ-lactone ring, and the latter also had an OH function [1]. The third related compound R_5 (9), $C_{32}H_{48}O_5$ (M^+ 512), mp 287–289°, which was between 7 and 8 in polarity, similarly contained an acetoxy group (ν_{max}1740, 1247 cm^{-1}) and a γ-lactone ring (ν_{max}1775, 1175 cm^{-1}). A carbonyl function (ν_{max} 1720 cm^{-1}), which was not found in either 7 or 8, was also present.

The NMR spectra of compounds 7–9 each showed 7 tertiary methyl signals; an equatorial secondary OCOMe group in the enviroment $CH_2\underline{C}HOCOC\underline{H}_3$ [δ 2.04 (3 H, *s*) and 4.50 (1 H, *q*, J=7 and 9 Hz)]. That of 8 also revealed a CH_2CHOH group in which the OH function was axially orientated [δ 3.88 (1 H, *t*, J=2 and 3 Hz)], and that of 9 a CH_2CO group [δ 2.50 (2 H, *m*)]. That compound 9 was the corresponding ketone of the alcohol 8 was proved by oxidation of 8 with Jones' reagent, which yielded a carbonyl compound, identical with 9.

The fifth oxygen atom in 7 appeared as an α,β-disubstituted epoxy function [ν_{max} 875 cm^{-1}, δ 3.00 (2 H, ~ *s*)], as on acid treatment, it yielded not an aldehyde, but a ketone identical with 9. The chemical shifts of the two epoxide protons agreed well with that reported for 11α,12α-epoxy-28,13β-olides of oleanane triterpenoids [5]. Assuming the acetate function to be in the usual C-3 position, 7 was probably 3β-acetoxy-11α,12α-epoxy-oleanan-28,13β-olide, 8 was the corresponding 12α-hydroxy compound and 9 was the 12-oxo compound. These three compounds have never been isolated as natural products, however, Barton *et al.* [6] and Yosioka *et al.*[7] have both prepared the 12α-hydroxy compound and the latter also synthesized 7 and 9. Compounds 7 and 8 were proved to be identical with authentic samples of the epoxide and 12α-hydroxy compound respectively kindly supplied by Professor Yosioka. Following Barton's method of reacting acetyl oleanolic acid with *m*-chloroperbenzoic acid, we also obtained a sample of the alcohol, identical with 7.

The 7 tertiary methyl NMR signals of compounds 7–9 are assigned as shown in Table 2.

After extraction with petrol, both the leaves and stems were subsequently extracted with EtOH. These extracts each gave an acidic fraction, which was methylated and chromatographed on alumina. That from the leaves gave methyl betulinate, ursolate and aliphitolate (methyl 2α,3β-dihydroxy-lup-20(29)-en-28-oate), while that from the stems gave methyl betulonate, betulinate and oleanolate.

Rhodomyrtus tomentosa is the only species of the genus *Rhodomyrtus* identified in Hong Kong, and other *Rhodomyrtus* species do not appear to have been examined for triterpenoids and steroids. However, with the isolation of 18 different triterpenoids and 3 steroids [1] from a single species, it shows further work of this kind on other species of this genus is worthwhile.

EXPERIMENTAL

IR spectra were recorded for KBr discs, UV spectra in 95% EtOH, NMR spectra in $CDCl_3$ were determined at 60 MHz using TMS as internal standard, and optical rotations in $CHCl_3$. Petrol had bp 60–80°. Known compounds were identified by TLC, mmp and IR spectral comparisons with authentic samples.

Neutral compounds from leaves. Milled air-dried leaves (44 kg) were extracted 2× with petrol. The combined extracts were conc and chromatographed on Al_2O_3 (4 kg). Elution with petrol and petrol–C_6H_6 (1:1) gave the same compounds in the early fractions as reported previously [1], but gave before betulin, fine needles of (3) (0.08 g), mp 243–246° (from petrol); $[\alpha]_D$ +10.7°. (Found: C, 81.7; H, 11.15. $C_{30}H_{48}O_2$ requires: C, 81.8; H, 11.0%.) It formed a monoacetate (4), mp 227–229° (from petrol–$CHCl_3$); $[\alpha]_D$ +32.8 (Found: M^+ 482. $C_{32}H_{50}O_3$ requires: M^+ 482); ν_{max} (cm^{-1}): 2825, 2720, 1690, 1630 (CH_2=C–CHO), 1740, 1250 (OAc); NMR: δ 2.02 (3 H, *s*, OCOMe), 4.47(1 H, *q*, J=7 and 10 Hz, axial $CH_2\underline{C}HO$-COMe), 6.23, 5.90, 9.42 (1 H ea, *s*, $C\underline{H}_2$=C–C$\underline{H}$O). Elution with C_6H_6–$CHCl_3$ (1:1) afforded needles of (1) (0.01 g), mp 253–254° (formerly reported as 237–239° [1]); $[\alpha]_D$ +6.5°. (Found: C, 81.3; H, 11.5; $C_{30}H_{50}O_2$ requires: C, 81.4, H, 11.4%.) It formed a diacetate (2), mp 197–199° (from aq. MeOH); (Found: M^+ 526, $C_{34}H_{54}O_4$ requires: M^+ 526); ν_{max} (cm^{-1}): 1745, 1245 (OAc), 1650, 915 (CH_2=C–CH_2O).

MnO_2 oxidation of 1. Compound 1 (0.03 g) was shaken with MnO_2 (0.1 g) in $CHCl_3$ (25 ml) for 3 days. The product was recrystallized from petrol to give needles (0.02 g), mp 244–246°; ν_{max} (cm^{-1}): 3320, 2845, 2720, 1690, 1630, identical with 3.

$NaBH_4$ reduction of 3. Compound 3 (15 mg) was refluxed with $NaBH_4$ (0.1 g) in THF (20 ml) for 2 hr. The product was recrystallized from MeOH to give needles (11 mg), mp 252–254°; ν_{max} (cm^{-1}): 3330, 1650, 915, identical with 1.

Wolff–Kishner reduction of 3. A soln of 3 (35 mg), NaOH (0.2 g) and hydrazine hydrate (0.2 ml) in diethylene glycol (30 ml) was heated at 120° for 1 hr, then at 210° for 6 hr. The product was purified by PLC to give needles, mp 235–237° (from petrol); $[\alpha]_D$ +25.0°; M^+ 426; ν_{max} (cm^{-1}) 3500 (OH), 3080, 1650, 885 (C=CH_2); forming an acetate, mp 282–284°, identical with moretenol (5), and its acetate (6) respectively.

Partial synthesis of 1. Moretenyl acetate (6) (0.1 g) was heated with $Pb(OAc)_4$ (0.1 g) in HOAc (25 ml) on the steam bath for 4 hr. The product was extracted in Et_2O, dried and chromatographed on Al_2O_3 (15 g) in petrol to give first unreacted (6) (0.06 g), then fine needles (13 mg), mp 198–200° (from $CHCl_3$–MeOH); M^+ 526; ν_{max} (cm^{-1}): 1745, 1245, 1650, 915, identical with 2, which on hydrolysis with 5% methanolic KOH (30 ml) gave needles, mp 253–254°, identical with 1.

Partial synthesis of 3. Moretenyl acetate (6) (0.1 g) was refluxed with SeO_2 (0.5 g) in HOAc (25 ml) for 4 hr. The product was extracted in Et_2O and chromatographed on Al_2O_3 (10 g) in petrol to give unchanged 6 (0.05 g), then needles (15 mg), mp 225–228°; M^+ 482; ν_{max} (cm^{-1}): 3320, 2845, 2720, 1690, 1630, identical with 4, which on hydrolysis with 5% methanolic KOH (30 ml) gave a product, mp 242–244°, identical with 3.

Acidic compounds from leaves. The leaves, after extraction with petrol, were extracted 2× with 95% EtOH. The combined extracts were distilled to give a dry residue, which was thoroughly extracted with Et_2O. The combined extracts were repeatedly shaken with NaOH soln (1 M). The aq. layers on acidification gave a solid mixture (20 g) which was methylated with CH_2N_2 in Et_2O and chromatographed on Al_2O_3 (500 g) in petrol. Elution with petrol–C_6H_6 (1:1) gave prisms of methyl betulinate (0.2 g), mp 228–230° (from $CHCl_3$); $[\alpha]_D$ +7.0°; M^+ 470, ν_{max} (cm^{-1}): 3550 (OH), 1720, 1174 (COOMe), 3080, 1650, 880 (C=CH_2), then methyl ursolate (0.1 g), mp 168–170°; $[\alpha]_D$ +63.0°; M^+ 470; ν_{max} (cm^{-1}): 3350 (OH), 1740, 1200 (COOMe), 1640, 820 (C=CH). Elution with $CHCl_3$ afforded needles (0.1 g) of methyl aliphitolate, mp 234–236° (from $CHCl_3$–MeOH); M^+ 486; ν_{max} (cm^{-1}): 3300 (OH), 1740, 1160 (COOMe), 3080, 1650, 885 (C=CH_2). It formed a diacetate, mp 231–232° (from MeOH); $[\alpha]_D$ −14.0°; M^+ 570; ν_{max} (cm^{-1}): 1750, 1250 (OAc), 1730, 1160 (COOMe), 3080, 1650, 885 (C=CH_2), identical with an authentic sample of methyl aliphitolate diacetate.

Neutral compounds from stems. Air-dried stems (61 kg) were extracted with petrol and the extract chromatographed on Al_2O_3 (3 kg) as for the leaf extract. Elution with C_6H_6 gave before betulin, as reported previously [1], compound **7** (0.05 g), mp 329–331°; $[\alpha]_D$ +45.3°, and after betulin, compound **9** (0.03 g), mp 287–289°; $[\alpha]_D$ +3.8°; (Found: M^+ 512. Calc. for $C_{32}H_{48}O_5$: M^+ 512), followed by compound **8** (0.01 g), mp 284–286°; $[\alpha]_D$ +39.7°.

Oxidation of **8**. Compound **8** (0.025 g) was oxidized with Jones' reagent. The product was recrystallized from petrol–$CHCl_3$ to give long needles (0.017 g), mp 287–289°, identical with **9**.

Synthesis of **8** *from acetyl oleanolic acid.* Acetyl oleanolic acid (0.05 g) was treated with *m*-chloroperbenzoic acid (0.05 g) in $CHCl_3$ (50 ml) at 0° for 20 hr. The product was recrystallized from $CHCl_3$ to give plates (0.02 g), mp 284–286°, identical with **8**.

Acid isomerization of **7**. Compound **7** (0.025 g) was treated with conc H_2SO_4 (1 ml) in EtOH (25 ml) at room temp. for 12 hr. The product was purified by PLC to give needles (0.01 g), mp 287–289° (from petrol–$CHCl_3$), identical with **8**.

Acidic compounds from stems. Stems, after extraction with petrol, were extracted with 95% EtOH and the methylated acid mixture (8 g) was obtained as for the leaves. Chromatography of this on Al_2O_3 (170 g), afforded in the petrol fractions, prisms (0.04 g), mp 166–168° (from petrol), $[\alpha]_D$ +37.0°; M^+ 468; ν_{max} (cm^{-1}): 1720 (C=O), 1740, 1160 (COOMe), 3080, 1650, 880 (C=CH_2), identical with a sample of methyl betulonate prepared by oxidation of methyl betulinate with Jones' reagent. Elution with petrol–C_6H_6 yielded methyl betulinate (0.1 g) and then methyl oleanolate (0.03 g), mp 198–201° (from C_6H_6); M^+ 470; ν_{max} (cm^{-1}): 3380 (OH), 1730, 1160 (COOMe), 3030, 1650, 850 (C=CH).

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