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TRITERPENES FROM *SIMABA MULTIFLORA*

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Key Word Index—*Simaba multiflora*; Simaroubaceae; canthin-6-one; 8-*O*-methylretusin; vanillic acid; hispidol B; 3*S*,23*R*,25-trihydroxytirucall-7-en-24-one; X-ray analysis.

Abstract—A new triterpene has been isolated from *Simaba multiflora* (Simaroubaceae) and determined to have the structure 3*S*,23*R*,25-trihydroxytirucall-7-en-24-one (**1**). Hispidol B was confirmed to be 3*S*,23*S*,24*R*,25-tetrahydroxytirucall-7-ene (**2**) by X-ray crystallographic analysis. Canthin-6-one, 8-*O*-methyl retusin and vanillic acid were also obtained in the course of this work.

INTRODUCTION

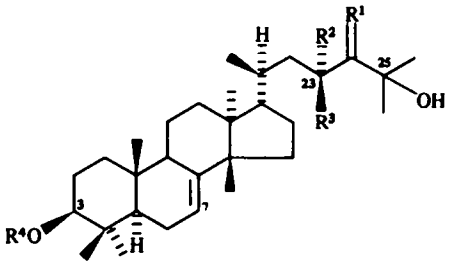
Simaba multiflora A. Juss. (Simaroubaceae) has previously afforded several quassinoids [1–4], canthin-6-ones [4, 5], canthin-2,6-diones [5], coumarins [4, 5], a coumarinolignan [6], two phenolic compounds [4] and β-sitosterol-β-D-glucoside [2]. The quassinoids are of interest because of their anticancer activity, and biogenetically the skeleton is derived from a tirucall-7-ene derivative by way of an apoeuphol rearrangement [7, 8]. However, tirucall-7-ene derivatives have been obtained only occasionally from Simaroubaceous plants [9–12] and we report here on the isolation and structure determination of a new member of this series.

RESULTS AND DISCUSSION

In a continuing search for anticancer agents from the wood of *Simaba multiflora* [2, 4–6], a new triterpene was isolated and determined to be (3*S*,23*R*,25)-trihydroxytirucall-7-en-24-one (**1**). Hispidol B (**2**) was also obtained and confirmed to be 3*S*,23*S*,24*R*,25-tetrahydroxytirucall-7-ene by X-ray analysis.

Compound **1**, mp 208–213°, molecular formula $C_{30}H_{50}O_4$ by high resolution mass spectrometry, exhibited IR absorption bands at 3500, 3250 (OH) and 1690 cm^{-1} (C=O). The ^1H NMR spectrum of **1** indicated the presence of seven tertiary methyl groups, one secondary methyl group, two multiplets for oxymethine protons and one olefinic proton. These signals were regarded as being characteristic of the tirucall-7-ene system having a 3-hydroxy group [12–14]. A singlet for two methyl groups shifted downfield to δ1.30 (6H) suggesting attachment to an oxygenated carbon, i.e. C-25. An oxymethine proton was shifted to δ3.83 indicating the presence of a vicinal diol, and an ion at *m/z* 459 $[M - Me]^+$ for loss of the C-14 methyl supports the assignment of a double bond to C-7. LAH reduction of **1** afforded **3**, the C-23, C-24 isomer of **2**, in which both the 23-H and 24-H were shifted substantially upfield. From the coupling constant between these two protons in **4** and the negative CD curve for **1**, the stereochemistry of C-23 [15] was assigned as *R*. Consequently, the structure of **1** was assigned as 3*S*,23*R*,25-trihydroxytirucall-7-en-24-one.

Compound **2**, mp 252–254°, was found to be the known compound hispidol B, whose structure was previously assigned in ref. [14]. In order to thoroughly establish the stereochemistry in the side chain. X-Ray crystallographic analysis of this compound was performed, through which its structure was confirmed to be 3*S*,23*S*,24*R*,25-tetrahydroxytirucall-7-ene. Canthin-6-one [16] and 8-*O*-methylretusin [17] were identified by comparison with spectral data. Vanillic acid was identified by direct comparison with an authentic sample.



	R^1	R^2	R^3	R^4
1	O	H	OH	H
2		OH	H	H
3		H	OH	H
4		OAc	H	Ac
5		H	OAc	Ac

EXPERIMENTAL

Mps were determined by means of a hot plate and are uncorr. ^{1}H NMR and ^{13}C NMR spectra were recorded at 200 MHz and 50.3 MHz, respectively (TMS: int. standard, chemical shifts: δ).

Preliminary processing and isolation. Details concerning the collection, identification and preliminary fractionation of the wood of *Simaba multiflora* A. Juss. (Simaroubaceae) have been described previously [2, 4–6]. In a continution of the previous study [6], the mother liquor after the isolation of emodin was rechromatographed on silica gel eluting with hexane–EtOAc (1:5) to afford 8-*O*-methylretusin (10 mg), and canthin-6-one (3 mg). Droplet countercurrent chromatography (DCCC) of the combined fractions 11 and 12, using a biphasic mixture of $CHCl_3$–C_6H_6–MeOH–H_2O (3:1:5:2) afforded tricin, 8-hydroxycanthin-6-one and simalikalactone D as previously reported [6], and hispidol B (2, 12 mg), 3*S*,23*R*,25-trihydroxytirucall-7-en-24-one (1, 5 mg) and vanillic acid (6 mg).

3*S*,23*R*,25-*Trihydroxytirucall-7-en-24-one* (1). Recrystallized from Me_2CO gave colourless needles, mp 208–213°. CD: $[\theta]_{295}$ −3330° ($CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3500, 3250, 2950, 2910, 1690, 1460, 1450, 1380, 1360 and 1060; ^{1}H NMR ($CDCl_3$): δ0.83 (3H, *s*, 18-H_3), 1.01 (6H, *s*, 19-H_3 and 30-H_3), 1.03 (3H, *d*, J = 6.5 Hz, 21-H_3), 1.05 (3H, *s*, 29-H_3), 1.12 (3H, *s*, 28-H_3), 1.30 (6H, *s*, 26-H_3 and 27-H_3), 2.69 (1H, *m*, 22-H), 3.28 (1H, *m*, 3-H), 3.83 (1H, *m*, 23-H) and 5.32 (1H, *m*, 7-H); MS *m/z*: 474 $[M]^+$, 459 $[M-Me]^+$, 441 $[M-Me-H_2O]^+$, 423 $[M-Me-2H_2O]^+$, 369, 351, 325, 271, 189, 121, 72 and 55. Mass measurement, obs.: 474.3697. calc. for $C_{30}H_{50}O_4$, 474.3706.

Hispidol B (2). Recrystallized from MeOH gave colourless needles, mp 252–254°. IR ν_{max}^{KBr} cm^{-1}: 3340, 2950, 1450, 1370, 1160, 1070, 1030, 990, 825 and 795; ^{1}H NMR (pyridine-d_5): δ0.83 (3H, *s*, 18-H_3), 0.90 (3H, *s*, 19-H_3), 1.02 (3H, *s*, 30-H_3), 1.12 (3H, *s*, 29-H_3), 1.14 (3H, *d*, J = 6.8 Hz, 21-H_3), 1.18 (3H, *s*, 28-H_3), 1.62 (3H, *s*, 26-H_3), 1.64 (3H, *s*, 27-H_3), 1.88 (2H, *m*, 6-H_2), 2.35 (1H, *dd*, J = 9.5, 12 Hz, 22-H), 3.49 (1H, *dd*, J = 8.0 Hz, 3α-H), 3.68 (1H, *br s*, 24-H), 4.60 (1H, *m*, J = 4.0, 9.5 Hz, 23-H), 5.34 (1H, *m*, 7-H), 5.53, 5.74, 5.89 and 6.08 (each, 1H, m, exchangeable with D_2O, OH); MS *m/z*: 476 $[M]^+$, 461 $[M-Me]^+$, 458 $[M-H_2O]^+$, 433 $[M-Me-H_2O]^+$, 425 $[M-CH_3-2H_2O]^+$, 407, 400, 385, 372, 353 and 327. Mass measurement, obs.: 476.3838. calc. for $C_{30}H_{52}O_4$, 476.3866; ^{13}C NMR (pyridine-d_5): 13.38 (*q*, 18-C), 15.53 (*q*, 29-C), 18.37 (*t*, 11-C), 19.46 (*q*, 19-C), 24.35 (*t*, 6-C), 27.04 (*q*, 26-C), 27.43 (*q*, 30-C), 27.66 (*q*, 27-C), 28.24 (*q*, 28-C), 28.36 (*t*, 16-C), 28.72 (*t*, 2-C), 34.13 (*t*, 12-C), 34.26 (*t*, 15-C), 34.26 (*d*, 20-C), 35.12 (*s*, 10-C), 37.57 (*t*, 1-C), 39.44 (*s*, 4-C), 42.03 (*t*, 22-C), 43.77 (*s*, 13-C), 49.25 (*d*, 9-C), 51.07 (*d*, 25-C), 51.43 (*s*, 14-C), 54.28 (*d*, 17-C), 69.29 (*d*, 23-C), 73.69 (*s*, 5-C), 76.50 (*d*, 24-C), 78.20 (*d*, 3-C), 118.32 (*d*, 7-C) and 145.95 (*s*, 8-C). These spectral data are consistent with the published values for hispidol B (2) [14]. Identity was established through direct comparison with an authentic sample.

Acetylation of 2. Compound 2 (5 mg) was dissolved in Ac_2O–pyridine (1:1, 1 ml) at room temp. overnight, dried *in vacuo* and chromatographed over silica gel eluting with $CHCl_3$ to afford a crystalline triacetate 4 from Et_2O–hexane, mp 146–148°: ^{1}H NMR ($CDCl_3$): δ0.76 (3H, *s*, 19-H_3), 0.80 (3H, *s*, 18-H_3), 0.85 (3H, *s*, 30-H_3), 0.96 (3H, *s*, 29-H_3), 0.97 (3H, *s*, 28-H_3), 0.98 (3H, *d*, J = 6.8 Hz, 21-H_3), 1.19 (3H, *s*, 26-H_3), 1.25 (3H, *s*, 27-H_3), 2.06, 2.07 and 2.20 (each, 3H, *s*, OAc), 4.53 (1H, *m*, 3α-H), 4.89 (1H, *m*, 24-H), 5.23 (1H, *br s*, 7-H) and 5.23 (1H, *br s*, 7-H) and 5.42 (1H, *m*, 23-H); MS *m/z*: 602 $[M]^+$, 569, 542, 527, 509, 484, 467, 449, 425, 407, 389, 369, 353, 335 and 309.

Reduction of 1 *with LAH.* To a THF soln of 1 (4 mg), LAH (1 mg) was added and the mixture refluxed for 1 hr. The reaction mixture was worked up as usual to afford a reduction product (3, 1.3 mg) as colourless needles, mp 142–144° (Me_2CO). ^{1}H NMR ($CDCl_3$): δ0.75 (3H, *s*, 19-H_3), 0.83 (3H, *s*, 18-H_3), 0.86 (3H, *s*, 30-H_3), 0.97 (6H, *s*, 28 and 29-H_3), 1.03 (3H, *d*, J = 5.8 Hz, 21-H_3), 1.30 (6H, *s*, 26 and 27-H_3), 3.25 (1H, *dd*, J = 5.3, 10.9 Hz, 3α-H), 3.28 (1H, *d*, J = 5.7 Hz, 24-H), 3.83 (1H, *ddd*, J = 3.2, 5.7, 5.7 Hz, 23-H) and 5.26 (1H, *dd*, J = 0.8, 2.2 Hz, 7-H); MS *m/z*: 476 $[M]^+$, 461, 443, 425, 408, 400, 386, 371, 353, 327 and 309; Mass measurement, obs.: 476.3858. calc. for $C_{30}H_{52}O_4$, 476.3863.

Acetylation of 3. The reduction product 3 (1 mg) was dissolved in Ac_2O–pyridine (1:1, 0.3 ml) at room temp. overnight, dried *in vacuo* and chromatographed over silica gel to afford a triacetate 5, mp 152–155° (MeOH). ^{1}H NMR ($CDCl_3$): δ0.76 (3H, *s*, 19-H_3), 0.79 (3H, *s*, 18-H_3), 0.85 (3H, *s*, 30-H_3), 0.93 (3H, *s*, 29-H_3), 0.94 (3H, *d*, J = 5.1 Hz, 21-H_3), 0.96 (3H, *s*, 28-H_3), 1.20 (3H, *s*, 26 or 27-H_3), 1.25 (3H, *s*, 27 or 26-H_3), 4.51 (1H, *dd*, J = 5.3, 9.3 Hz, 3α-H), 4.93 (1H, *d*, J = 2.2 Hz, 7-H), 5.23 (1H, *m*, J = 3.6 Hz, 23-H) and 5.24 (1H, *m*, 24-H); MS *m/z*: 602 $[M]^+$, 587, 569, 542, 527, 509, 484, 482, 467, 449, 425, 409, 407, 389, 369, 353, 325 and 309.

Crystal data of 2. $C_{30}H_{52}O_4$, M = 476.4. Orthorhombic, a = 10.605(39), b = 14.455(75), c = 18.203(31) Å, U = 2790.4 Å^3, Z = 4, D_c = 1.13 gcm^{-3}, F(000) = 1056, Mo-K_α radiation, λ = 0.7108 Å, μ = 0.40 cm^{-1}. Space group $P2_12_12_1$.

Crystallographic analysis of 2. The X-ray intensities were collected on a Nicolet P3 automated diffractometer using monochromatized MoK_α radiation. Integrated relative intensities for 1375 independent reflexions with $2\theta \leqslant 40°$ were measured as θ-2θ scans; all reflexions were used in subsequent calculations. The structure was elucidated using MITHRIL [18] and the H atoms were observed in electron density maps calculated at intermediate stages of structure refinement. The co-ordinates for all atoms and anisotropic thermal parameters for the non-hydrogen atoms were varied in least-squares calculations using SHELX [19]. The C–H and O–H bonds were constrained to be equal to 1.00 Å and the hydroxy, methyl and remaining hydrogen were given common temperature factors during refinement. Unit weights were employed and refinement converged at R 7.2%. Final positional parameters are listed in Table 1, bond lengths in Table 2, valency angles in Table 3 and torsion angles in Table 4. Thermal parameters are listed in a Supplementary Publication.

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