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# 14α-METHYLZYMOSTEROL AND OTHER STEROLS FROM *WRIGHTIA TINCTORIA* SEEDS

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**Key Word Index**—*Wrightia tinctoria*; Apocynaceae; sterol; 14α-methylzymosterol; desmosterol; clerosterol; 24-methylene-25-methylcholesterol; 24-dehydropollinastanol; seeds.

**Abstract**—The structure of a new sterol isolated from the unsaponifiable lipid of *Wrightia tinctoria* seed lipid was shown to be 14α-methylzymosterol by comparison with a synthetic authentic compound. Four uncommon sterols, desmosterol, clerosterol, 24-methylene-25-methylcholesterol and 24-dehydropollinastanol, in addition to several usual phytosterols, were isolated and identified.

## INTRODUCTION

*Wrightia tinctoria* R. Br. is a small deciduous tree of the family Apocynaceae distributed in Central India, Burma and Timor. The seeds are carminative, astringent, aphrodisiac and tonic, and are given for infections of the chest, asthma, colic and diuresis [1]. The therapeutic properties of *W. tinctoria* seeds are the same as those of the seeds of *Holarrhena antidysenterica*, another apocynaceous plant which contains several steroidal alkaloids [1, 2]. This paper describes an investigation on the sterols of *W. tinctoria* seeds which led to the isolation and characterization of a new sterol 14α-methylzymosterol (**3b**), in addition to four rare plant sterols, desmosterol (**1b**), clerosterol (**1h**), 24-methylene-25-methylcholesterol (**1i**) and 24-dehydropollinastanol (**2b**).

## RESULTS AND DISCUSSION

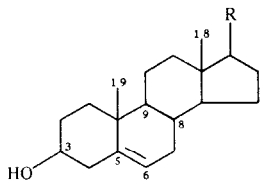
The sterol **3b** was isolated as the acetyl derivative from *W. tinctoria* seeds by the procedure described in the Experimental section. The mass spectrum of **3b**-acetate showed $[M]^+$ at *m/z* 440, corresponding to $C_{30}H_{48}O_2$, accompanied by fragmentation ions at *m/z* 435 $[M-Me]^+$, 365 $[M-HOAc-Me]^+$ and 327 $[M-C_8H_{15}$(side chain)$-2H]^+$ indicating that it was an acetate of a $C_{28}$-sterol with two double bonds, one of which was in the $C_8$-side chain and the other was in the skeleton [3]. Other ions at *m/z* 287 $[M-$side chain$-C_3H_6$(ring D)$]^+$ and 273 $(287-CH_2)$ were characteristic of 14α-methylsterols [4, 5]. The skeletal $^1$H NMR signals of **3b**-acetate at δ 0.706 (3H, *s*, 18-$H_3$), 0.884 (3H, *s*, 30-$H_3$), 0.959 (3H, *s*, 19-$H_3$), 2.021 (3H, *s*, 3β-OAc) and 4.700 (1H, *tt*, $J=10.8$, 5.2 Hz, 3α-H), together with the lack of a skeletal olefinic proton signal, suggested the 14α-methyl-3β-acetoxy-5α-cholest-8-ene skeletal structure [6]. The $^1$H NMR spectrum showed the side chain proton signals at δ 0.918 (3H, *d*, $J=6.1$ Hz, 21-$H_3$), 1.603 (3H, *s*, 27-$H_3$), 1.685 (3H, *s*, 26-$H_3$) and 5.101 (1H, *tt*, $J=7.2$, 1.4 Hz, 24-H) which is consistent with the C-24 unsaturated cholestane side chain [7]. Thus, the sterol **3b** was regarded to have the structure 14α-methyl-5α-cholesta-8,24-dien-3β-ol (14α-methylzymosterol). This was confirmed by comparison (as the acetate) of the chromatographic (GC and HPLC) and spectral (MS and $^1$H NMR) data with those of an authentic sample of **3b** synthesized from 24-dehydropollinastanol (**2b**) by acid-catalysed 9β,19-cyclopropane ring opening. (The $^1$H NMR data of both natural and synthetic **3b**-acetate were shown in Table 1).

Ten other sterols were isolated from *W. tinctoria* seeds and were identified as described in Table 2 by mp, GC and HPLC [8–12]. The composition of the sterol fraction, determined by GC, and the mp and chromatographic (GC and HPLC) data of the sterols from *W. tinctoria* seeds are shown in Table 2. The identification of desmosterol (**1b**) [11], clerosterol (**1h**) [11], 24-methylene-25-methylcholesterol (**1i**) [10] and 24-dehydropollinastanol (**2b**) [8] was supported by the MS and $^1$H NMR data of which the $^1$H NMR data are shown in Table 1.

This study has, thus, demonstrated the occurrence of a 14α-methylsterol **3b**, which is considered to be a new sterol, in the seeds of *W. tinctoria* as the minor sterol constituent. This study represents, moreover, the first instance of the detection of a 14α-methylsterol with a $\Delta^8$-bond in a higher plant though three higher homologues of **3b**, i.e. 14α,24-dimethyl-5α-cholest-8-en-3β-ol (**3c**) and 14α-methyl-24-methylene-5α-cholest-8-en-3β-ol (**3d**) [13], and 14α-methyl-24-ethyl-5α-cholest-8-en-3β-ol (**3e**) [14], have been detected in triparanol-treated cell cultures of *Chlorella emersonii*, a green alga.

The occurrence of **1b** and **2b** is rare in higher plants and it is interesting to note that **1b** has so far been found in the seeds of two *Funtumia* species which are members of Apocynaceae [15], in addition to some Cucurbitaceae [11, 12] and the floral buds and anthers of cotton (*Gossypium hirsutum*) [16]. Sterol **2b** has previously been found only in a mixture of pollens [8] and cotton floral buds and anthers [16]. Taking into account the considerations of sterol biogenesis [17], the co-occurrence of **1a**, **1b**, **2b** and **3b** in *W. tinctoria* seeds may be explained by the presence of the following biosynthetic pathway of sterol in the seeds: **2b**→**3b**→**1b**→**1c**. Sterol **1b** is, on the other hand, a possible intermediate of the biosynthesis of

Skeletons



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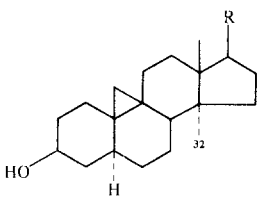

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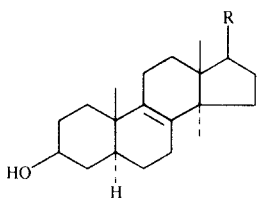

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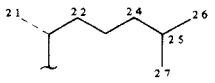
Side chains (R)



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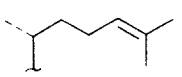

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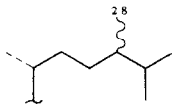
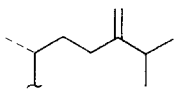


c (24ξ)



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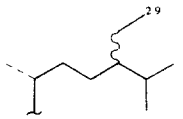
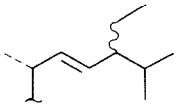
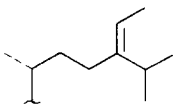


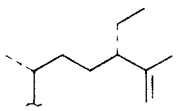
e (24ξ)



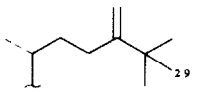
f (22E,24ξ)



g (24Z)



h (24β)



i

Table 1. $^1$H NMR data of the acetates of sterols isolated from *Wrightia tinctoria* seeds (400 MHz, $CDCl_3$, TMS as int. standard)

| | Acetate | | | | | |
|---|---|---|---|---|---|---|
| H | **1b** | **1h** | **1i** | **2b** | **3b** | **3b*** |
| 18-$H_3$ (*s*) | 0.678 | 0.669 | 0.688 | 0.956 | 0.706 | 0.706 |
| 19-$H_2$/$H_3$ | 1.018 (*s*) | 1.016 (*s*) | 1.020 (*s*) | 0.077 (1H, *d*, 4.6)† 0.441 (1H, *d*, 3.9) | 0.959 (*s*) | 0.959 (*s*) |
| 32-$H_3$ (*s*) | — | — | — | 0.889 | 0.884 | 0.882 |
| 21-$H_3$ (*d*) | 0.936 (6.6) | 0.905 (6.6) | 0.964 (6.6) | 0.885 (6.7) | 0.918 (6.1) | 0.918 (6.6) |
| 26-$H_3$ (*s*) | 1.681 | 1.567 | 1.057 | 1.685 | 1.685 | 1.683 |
| 27-$H_2$/$H_3$ (*s*) | 1.601 | 4.640 (1H, *d*, 2.4) 4.726 (1H, *d*, 2.8) | 1.057 | 1.604 | 1.605 | 1.604 |
| 29-$H_3$ | — | 0.801 (*t*, 7.4) | 1.057 (*s*) | — | — | — |
| 3β-OAc (*s*) | 2.030 | 2.030 | 2.031 | 2.024 | 2.021 | 2.023 |
| 3α-H (*tt*) | 4.60 (*m*) | 4.60 (*m*) | 4.60 (*m*) | 4.796 (11.0, 4.8) | 4.700 (10.8, 5.2) | 4.700 (10.8, 5.2) |
| 6-H (*m*) | 5.37 | 5.37 | 5.37 | — | — | — |
| 24-H (*tt*) | 5.090 (7.1, 1.4) | — | — | 5.100 (7.2, 1.4) | 5.101 (7.2, 1.4) | 5.101 (7.2, 1.4) |
| 28-$H_2$ | — | — | 4.660 (1H, *d*, 1.1) 4.833 (1H, *d*, 0.8) | — | — | — |

*Authentic sterol.
†Figures in parentheses denote *J* values (Hz).

the irehdiamine (3β-diamino-20α-pregn-5-ene) type steroidal alkaloids in the *Funtumia* seeds [15].

Although sterols **1h** [11, 12, 18, 19], a 24β-ethylsterol with a $\Delta^{25}$-bond, and **1i** [10, 12, 19], a 24-methylene-25-methylsterol, have been detected only in some restricted plants, the detection of these sterols in *W. tinctoria* seeds in this study, as well as in *Phaseolus vulgaris* (Leguminosae) seeds in our recent study [20], may suggest their widespread occurrence in higher plants though as the minor sterol constituents.

## EXPERIMENTAL

Mp: uncorr. Prep. TLC: silica gel, developed ×3 with hexane–EtOAc (6:1); argentation TLC: silica gel–$AgNO_3$ (4:1)

Table 2. Composition of sterol fraction, and mp and chromatographic data of sterols from *Wrightia tinctoria* seeds

| Sterol | | Composition (%) | Acetate | | |
|---|---|---|---|---|---|
| | | | Mp (°) | $RR_t$ (GC)* | $RR_t$ (HPLC)* |
| **1a** | Cholesterol (cholest-5-en-3β-ol) | 1.1 | 112–115 | 1.00 | 1.00 |
| **1b** | Desmosterol (24-dehydrocholesterol) | 6.7 | 95–97 | 1.21 | 0.66 |
| **1c** | 24-Methylcholesterol (24ξ) | 16.1 | 142–145 | 1.31 | 1.13 |
| **1d** | 24-Methylenecholesterol | 2.0 | 135–137 | 1.35 | 0.80 |
| **1e** | 24-Ethylcholesterol (24ξ) | 58.7 | 122–125 | 1.63 | 1.27 |
| **1f** | 24-Ethyl-22*E*-dehydrocholesterol (24ξ) | 6.9 | 144–146 | 1.44 | 1.10 |
| **1g** | Isofucosterol (24*Z*-ethylidenecholesterol) | 4.3 | 136–137 | 1.81 | 1.00 |
| **1h** | Clerosterol (24β-ethyl-25-dehydrocholesterol) | 1.4 | 122–124 | 1.63 | 0.90 |
| **1i** | 24-Methylene-25-methylcholesterol | 0.2 | 145–148 | 1.68 | 0.96 |
| **2b** | 24-Dehydropollinastanol (14α-methyl-9β,19-cyclo-5α-cholest-24-en-3β-ol) | 0.7 | 75–78 | 1.43 | 0.74 |
| **3b** | 14α-Methylzymosterol (14α-methyl-5α-cholesta-8,24-dien-3β-ol) | 1.4 | — | 1.22 | 0.69 |
| Others, unidentified | | 0.5 | — | — | — |

*$RRt$ were expressed relative to **1a**-acetate.

developed ×3 with $CCl_4$–$CH_2Cl_2$ (5:1); HPLC: Altex Ultrasphere ODS column (Beckman; 5 μm; 25 cm × 10 mm i.d.), MeOH as mobile phase (flow rate, 4 ml/min); GC: OV-17 SCOT glass capillary column (30 m × 0.3 mm i.d.), column temp. 255°. $RR_t$ on HPLC and GC expressed relative to cholesterol (**1a**) acetate. EIMS (70 eV): probe; $^1$H NMR: 400 MHz, $CDCl_3$, TMS as int. standard; acetylation: $Ac_2O$–pyridine at room temp. overnight. The acetates of the following sterols were used as the reference specimens: **1a**–**1i** [11, 12], and **2b** kindly donated by Dr M. J. Thompson (Insect and Nematode Hormone Laboratory, Agricultural Research Service, USDA, Beltsville, MD, U.S.A.). The authentic sample of **3b**-acetate was prepared from **2b**-acetate by gaseous HCl catalysed isomerization in $CHCl_3$ as described previously [7]. The seeds of *Wrightia tinctoria* were collected locally in India.

*Isolation of sterols.* Air-dried and powdered seeds (300 g) of *W. tinctoria* were extracted with petrol in a Soxhlet extractor. After removal of solvent, the extracted lipid (14 g) was refluxed with 5% KOH in EtOH for 4 hr and then extracted with $Et_2O$ which gave unsaponifiable lipid (535 mg). Prep. TLC of the unsaponifiable lipid gave a sterol fraction (50 mg). This was acetylated, and the acetate mixture (43 mg) was subjected to argentation TLC to give five bands (referred to as bands 1–5 in the order of polarity, beginning with the least polar). The least polar fraction (19 mg) from band 1 ($R_f$ 0.57–0.70) was a mixture of the acetates of **1a**, **1c** and **1e**. Band 2 ($R_f$ 0.45–0.51) afforded a mixture (2 mg) contained **1f**-acetate as the dominant component. Band 3 ($R_f$ 0.34–0.43) yielded a mixture (2 mg) of the acetates of **2b** and **3b**. Band 4 ($R_f$ 0.12–0.24) gave a mixture (6 mg) of the acetates of **1b**, **1g** and **1h**, and band 5 ($R_f$ 0.07–0.10) afforded a mixture (3 mg) of the acetates of **1d** and **1i**. Isolation of each component from individual fractions was performed by HPLC. The MS data of the acetates of **1b**, **1h**, **1i**, **2b** and **3b** isolated from *W. tinctoria* seeds in this study are as follows.

*Desmosterol* (**1b**) *acetate.* MS: *m/z* (rel. int.) 426 $[M]^+$ (0.3), 411 (0.5), 366 (100), 351 (12), 313 (1), 253 (21), 245 (7), 228 (4), 213 (7).

*Clerosterol* (**1h**) *acetate.* MS: *m/z* (rel. int.) 454 $[M]^+$ (5), 439 (3), 394 (100), 379 (9), 313 (3), 296(4), 281 (5), 255 (7), 253 (12), 228 (10), 213 (20), 201(5).

24-*Methylene*-25-*methylcholesterol* (**1i**) *acetate.* MS: *m/z* (rel. int.) 454 $[M]^+$ (0.3), 394 (58), 379 (7), 296 (39), 281 (8), 253 (11), 228 (7), 213 (11), 211 (5), 55 (100).

24-*Dehydropollinastanol* (**2b**) *acetate.* MS: *m/z* (rel. int.) 440.3628 $[M]^+$ (5, $C_{30}H_{48}O_2$, requires 440.3652), 425.3353 (2, $C_{29}H_{45}O_2$), 380.3417 (28, $C_{28}H_{44}$), 311.2697 (3, $C_{23}H_{35}$), 297.2561 (2, $C_{22}H_{33}$), 286.2647 (2, $C_{21}H_{34}$), 269.2267 (6, $C_{20}H_{29}$), 267.2102 (5, $C_{20}H_{27}$), 231.2102 (3, $C_{17}H_{27}$), 227.1816 (3, $C_{17}H_{23}$), 213.1670 (3, $C_{16}H_{21}$), 205.1962 (6, $C_{15}H_{21}$), 201.1617 (5, $C_{15}H_{21}$), 43.0197 (100, $C_2H_3O_1$).

14α-*Methylzymosterol* (**3b**) *acetate.* MS: *m/z* (rel. int.) 440.3665 $[M]^+$ (17, $C_{30}H_{48}O_2$), 425.3433 (33, $C_{29}H_{45}O_2$), 365.3169 (9, $C_{27}H_{41}$), 327.2296 (1, $C_{22}H_{31}O_2$), 287.1968 (2, $C_{19}H_{27}O_2$), 273.1832 (7, $C_{18}H_{25}O_2$), 261.1874 (3, $C_{17}H_{25}O_2$), 227.1834 (4, $C_{17}H_{23}$), 213.1642 (4, $C_{16}H_{21}$), 211.1507 (6, $C_{16}H_{19}$), 201.1646 (6, $C_{25}H_{21}$), 69.0715 (100, $C_5H_9$).

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