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THREE STEROID GLYCOSIDES OF THE STIGMASTANE TYPE FROM *DENDROBIUM OCHREATUM**

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Key Word Index—*Dendrobium ochreatum*; Orchidaceae; steroid glycosides; stigmastane steroids; dendrosteroside; ochreasteroside; *epi*-ochreasteroside.

Abstract—The isolation of dendrosteroside and two new steroid glycosides, ochreasteroside and *epi*-ochreasteroside, from *Dendrobium ochreatum* Lindl. is described. The two new glycosides are shown to be the 3-*O*-β-D-glucopyranosides of 3β,16β,22ξ-trihydroxy-6-oxo-5α,14α,17ξ,20ξ,24ξ,25ξ,-stigmastan-26-oic acid δ-lactone and its 16α epimer respectively.

INTRODUCTION

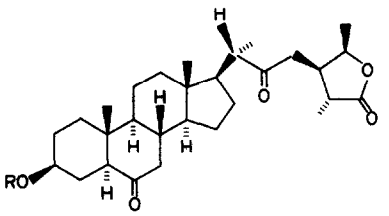
In an earlier communication [2] we reported the constitution and relative configuration of dendrosteroside (**1**), a steroid glycoside from *Dendrobium ochreatum* Lindl. The structure of the aglycone dendrosterone (**3**) was determined by an X-ray diffraction analysis. We now report the structural determination of two closely related glycosides, named ochreasteroside (**4**) and *epi*-ochreasteroside (**7**), found in the same plant.

RESULTS

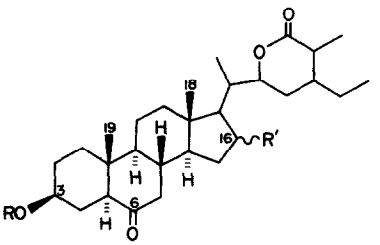
The three glycosides were separated by gel permeation on Sephadex LH-20. Dendrosteroside (**1**), which contains a disaccharide moiety, was eluted first, followed by *epi*-ochreasteroside (**7**) and ochreasteroside (**4**). The successful separation of the latter two glycosides by gel permeation is unexpected, since they differ only in the configuration at C-16.

Spectrochemical and elemental analyses of ochreasteroside (**4**), *epi*-ochreasteroside (**7**) and their corresponding aglycones indicate that both glycosides have the molecular formula $C_{35}H_{56}O_{10}$. Sugar [3,4] and methylation [5] analyses show **4** and **7** to be glucopyranosides. On enzymatic hydrolysis with β-D-glucosidase from almonds, **4** and **7** give the corresponding aglycones, named ochreasterone (**5**) and *epi*-ochreasterone (**8**) respectively. These results, together with the application of Hudson's rules of isorotation [6], indicate that **4** and **7** are β-D-glucopyranosides.

The ^{13}C-NMR spectrum of **5** ($C_{29}H_{46}O_5$) exhibits two peaks at δ 174.6 and 209.8, which in combination with a single IR absorption band in the carbonyl stretching region at 1705 cm^{-1} shows the presence of one δ-lactone and one keto group in **5**. Acetylation of **5** produces a diacetate (**6**). The ^{1}H-NMR spectrum of **5** shows two 1H multiplets at δ 3.6 and 4.4. These are displaced to δ 4.7 and 5.3 in the diacetate (**6**), which shows that both hydroxyl groups in **5** are secondary.



(**1**) R = β-gentiobiosyl
(**2**) R = β-D-glucopyranosyl
(**3**) R = H



(**4**) R = β-D-glucopyranosyl, R' = β-OH
(**5**) R = H, R' = β-OH
(**6**) R = COMe, R' = β-OCOMe
(**7**) R = β-D-glucopyranosyl, R' = α-OH
(**8**) R = H, R' = α-OH
(**9**) R = COMe, R' = α-OCOMe

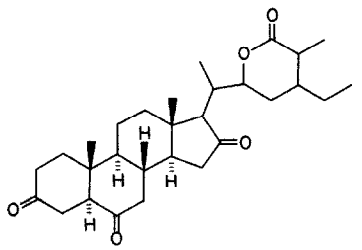
The CD spectra of **3** and **5** exhibit negative Cotton effects of about the same amplitude at 294 and 293 nm respectively, which strongly indicates that the latter compound is also a 6-keto steroid with *trans* junctions between the rings A/B, B/C and C/D.

The structure of the side chain of **5** was indicated by NMR measurements. A doublet of quartets (1 H) at δ 2.61 in the NMR spectrum of **5** is assigned to H_A in the partial structure **A** and a multiplet (1 H) at δ 4.8

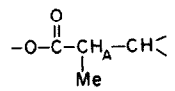
* Part 6 in the series "Studies on Orchidaceae Glycosides". For part 5 see ref. [1].

to H_B in the partial structure **B** or **C**. Since **5** contains an ethyl group (3 H triplet at δ 0.86) the structure of the side chain should be either **D** or **E**. On biogenetic grounds structure **D** is preferred.

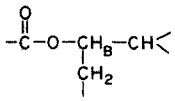
Oxidation of **5** produces the triketo derivative **10**. The mass spectral fragmentation pattern of **10** is in accordance with that expected for a 16-keto steroid [7], and consequently a hydroxyl group is situated at C-16 in the aglycone **5**. The position of the remaining hydroxyl group was determined by NMR measurements. Since the methine proton at the hydroxyl group appears as a complex multiplet, it must be situated at C-2, C-3, C-4 or C-11. The chemical shifts of H-19 in **5** (δ 0.78), **6** (δ 0.78) and **10** (δ 0.99) show good agreement with those reported or calculated for the androstan-6-one derivatives which are substituted at C-3 and C-16 with hydroxy, acetoxy or oxo groups (Table 1) [8]. The C-3 proton in **5** resonates at δ 3.6 and hence the hydroxyl group at C-3 is equatorial [9].



(10)



(a)



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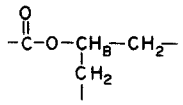
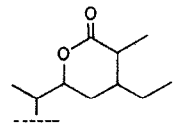
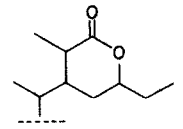


(c)



(d)



(e)

Oxidation of ochreasterone (**5**) and *epi*-ochreasterone (**8**) yield the same triketo derivative (**10**), and they are therefore epimers. From the chemical shift (δ 3.6) of the C-3 proton in **8** it follows that the hydroxyl group at C-3 is equatorial [9], and consequently **5** and **8** are C-16 epimers.

The difference in specific rotation of some 16α and β hydroxy steroids has been investigated [10]. The compounds with a hydroxyl group β-oriented were found to be more dextrorotatory than their α-epimers. The specific rotations (578 nm, MeOH) of ochreasterone (**5**) and *epi*-ochreasterone (**8**) are $+13.5°$ and $-15.9°$ respectively, which indicates that the hydroxyl group at C-16 in **5** is β-oriented and that in **8** it is α-oriented. It has been shown that H-18 in 16β-hydroxy steroids resonates at a lower field (*ca* 0.24 ppm) than in the 16α-epimers [8]. In **5** and **8**, H-18 resonates at 0.90 ppm and 0.69 ppm respectively, which gives further support for the assigned configurations at C-16.

The location of the glucose moieties in **4** and **7** was determined by NMR measurements on the penta-acetates of the glucosides and the diacetates of the aglycones (**6** and **9**). The C-16 hydrogen atoms in **6** and **9** appear at δ 5.2–5.4 and δ 4.9–5.1 respectively. The C-3 hydrogen atoms in **6** and **9**, which are axially oriented, appear at a higher field (δ 4.5–4.9). In the region δ 4.5–4.8, the NMR spectra of the penta-acetates of **4** and **7** exhibit only signals due to the anomeric hydrogen atoms (1 H, doublets). This evidence shows that the sugar moieties in **4** and **7** are located at C-3.

EXPERIMENTAL

General conditions were the same as in an earlier communication [11]. The CD spectra were measured on a Jasco J-40 spectropolarimeter. Plates precoated with Si gel F_{254} (Merck) were used for TLC, and the spots were developed by spraying with H_2SO_4 followed by heating to 120°. For column chromatography on Sephadex, system A was used: Sephadex LH-20 (8 × 56 cm, EtOH–H_2O 1:1).

Plant material. Dendrobium ochreatum Lindl. was delivered from Mr N. Prakash, Chandra Orchid and Bulb Nurseries, 8 1/2 miles P.O. Kalimpong, West Bengal, India.

Isolation of dendrosteroside (**1**), *ochreasteroside* (**4**) *and epi-ochreasteroside* (**7**). Fresh plants of *D. ochreatum* Lindl. (13 kg) were extracted with MeOH (25 l). The soln was concentrated to 3 l. and extracted with CCl_4 (4 × 0.5 l.). One fourth of the aq. layer was evaporated to dryness leaving a brown residue which was chromatographed in system A. The progress of the separation was followed by TLC on Sil gel ($CHCl_3$–MeOH–H_2O 65:35:10, lower phase). A part (2.5 g) of the fraction containing dendrosteroside (**1**, 20.3 g) was chromatographed in system A giving crude **1** (1.40 g). Purification on Si gel (5 × 11 cm, $CHCl_3$–EtOH 2:1), gave dendrosteroside

Table 1. Calculated or reported * chemical shifts of H-19 in various 5α,14α-androstanederivatives

	Y	Chemical shift of H-19			
		C-2	C-3	C-4	C-11
Y,16β-dihydroxy-5α,14α-androstan-6-one	α-OH	0.77	0.76*	0.77	0.87
	β-OH	1.01	0.77*	0.99	0.98
Y, 16β-di-*O*-acetyl-5α,14α-androstan-6-one	α-*O*-acetyl	0.83	0.77	0.81	0.83
	β-*O*-acetyl	0.91	0.80	0.98	—
5α,14α-androstan-Y,6,16-trione	>C=O	0.76	1.01*	0.75	1.00*

(1, 0.35 g). A fraction (3.7 g) from the first column which contained ochreasteroside (4) and *epi*-ochreasteroside (7) was chromatographed in system A. Two fractions were collected. The second one contained crude ochreasteroside (4, 1.24 g), which was purified by crystallisation. The first fraction (1.20 g) which contained a mixture of ochreasteroside (4) and *epi*-ochreasteroside (7) was chromatographed in system A giving crude 7 (0.52 g). Purification on Si gel (5 × 11 cm, $CHCl_3$–MeOH 9:1), gave *epi*-ochreasteroside (7, 0.17 g).

Dendrosteroside (1). Needles (H_2O), mp 239–245°(decomp.); $[\alpha]_{578}^{23}$ −32.5° (*c* 1.27, MeOH). (Found: C 62.0; H 8.16; O 29.8. $C_{41}H_{64}O_{15}$ requires: C 61.8; H 8.03; O 30.1) IR: ν_{max}^{KBr} 3700–3000(*s*), 1760(*s*), 1705(*s*) cm^{-1}.

Ochreasteroside (4). Needles (H_2O–MeOH 1:1), mp 292–296° (decomp.); $[\alpha]_{578}^{22}$ −21.0° (*c* 0.36, MeOH). (Found: C 66.0; H 8.78; O 25.1 $C_{35}H_{56}O_{10}$ requires: C 66.0; H 8.87; O 25.1) IR: ν_{max}^{KBr} 3700–3050(*s*), 1705(*s*) cm^{-1}.

epi-*Ochreasteroside* (7). Needles (H_2O–MeOH 1:1), mp 267–275°(decomp.); $[\alpha]_{578}^{22}$ −38.4° (*c* 0.32, MeOH). (Found: C 65.1; H 8.76; O 25.8. $C_{35}H_{56}O_{10} \times 1/2\ H_2O$ requires: C 65.1; H 8.90; O 26.0) IR: ν_{max}^{KBr} 3700–3500(*s*), 1725(*s*), 1710(*s*) cm^{-1}.

Hydrolysis of dendrosteroside (1). Dendrosteroside (1, 0.45 g) was dissolved in a mixture of MeOH (50 ml) and sodium hydrogen phthalate buffer (pH 5.25, 450 ml) and β-D-glucosidase from almonds (0.11 g) was added. The soln was kept at 37° for 22 hr and was thereafter extracted with $CHCl_3$–EtOH (2:1, 5 × 100 ml). The organic phase was dried and the solvent evaporated. The residue was chromatographed on Si gel (2.5 × 9 cm, $CHCl_3$–MeOH 9:1), giving dendrosterone (3, 69 mg). Needles (MeOH), mp 240–243° (decomp.); $[\alpha]_{578}^{22}$ +19.6° (*c* 0.74, MeOH). (Found: C 73.7; H 9.28; O 17.1. $C_{29}H_{44}O_5$ requires: C 73.7; H 9.38; O 16.9) IR: ν_{max}^{KBr} 3560(*s*), 1760(*s*) cm^{-1}. 1715(*s*), 1705(*s*). CD: $\lambda_{extrema}^{MeOH}$ (Δε) 220 (−1.17), 248 (−0.14), 294 (−1.95). ^1H NMR ($CDCl_3$): δ *inter alia* 0.72 (*s*, 3H), 0.77 (*s*, 3 H), 1.19 (*d*, 3 H, *J* 6.7 Hz), 1.22 (*d*, 3 H, *J* 6.7 Hz), 1.30 (*d*, 3 H, *J* 7.0 Hz), 3.3–3.7 (*m*, 1 H) 4.7–5.0 (*m*, 1 H). ^{13}C NMR (pyridine-d_5): δ 212.7, 210.0, 178.3. MS *m/e* (rel. int.): 472 (M^+, 26). A later fraction contained the monoglucoside 2 (55 mg). Needles (EtOH), mp 235–240° (decomp.); $[\alpha]_{578}^{22}$ −12.5° (*c* 1.39, MeOH). (Found: C 65.9; H 8.74; O 25.3. $C_{35}H_{54}O_{10}$ requires: C 66.2; H 8.57; O 25.2. IR: ν_{max}^{KBr} 3590(*m*), 3500(*m*), 3350(*m*), 1745(*s*), 1705(*s*) cm^{-1}.

Hydrolysis of ochreasteroside (4). Ochreasteroside (4, 0.47 g) was dissolved in a mixture of MeOH (100 ml) and sodium hydrogen phthalate buffer (pH 5.25, 900 ml) and β-D-glucosidase from almonds (0.20 g) was added. The soln was kept at 37° for 40 hr and was thereafter extracted with $CHCl_3$–EtOH (2:1, 5 × 200 ml). The organic phase was dried (Na_2SO_4) and the solvent evaporated. Residue was chromatographed on Si gel (2.5 × 11 cm, $CHCl_3$–MeOH 9:1), giving ochreasterone (5, 162 mg). Needles (MeOH), mp 256–261° (decomp.); $[\alpha]_{578}^{22}$ +13.5° (*c* 0.21, MeOH). (Found: C 73.4; H 9.87; O 16.7. $C_{29}H_{46}O_5$ requires: C 73.4; H 9.77; O 16.9) IR: ν_{max}^{KBr} 3620(*w*), 3480(*m*), 1705(*s*) cm^{-1}. CD: $\lambda_{extrema}^{MeOH}$ (Δε) 222 (−1.56), 254 (−0.14), 293 (−1.62). ^1H NMR (pyridine d_5): δ *inter alia* 0.80 (*s*, 3 H), 0.86 (*t*, 3 H, *J* 7 Hz), 1.03 (*s*, 3 H) 1.12 (*d*, 3 H, *J* 7 Hz), 1.26 (*d*, 3 H, *J* 7 Hz). ^1H NMR ($CDCl_3$): δ *inter alia* 0.78 (*s*, 3 H), 0.90 (*s*, 3 H), 2.61 (*dq*, 1 H), 3.4–3.7 (*m*, 1 H), 4.2–4.5 (*m*, 1 H), 4.7–4.9 (*m*, 1 H): ^{13}C NMR (pyridine-d_5): δ 209.8, 174.6.MS *m/e* (rel. int.): 474 (M^+, 12). A later fraction from the Si gel column contained unhydrolysed glycoside (4, 0.22 g).

Diacetylochreasterone (6). Ochreasterone (5, 18 mg) was treated with Ac_2O–C_5H_5N at room temp. for 16 hr. The diacetate (6) was purified by chromatography on Si gel (1 × 15 cm, $CHCl_3$–MeOH 9:1), when it crystallised from MeOH, needles, mp 226–234° (decomp.); $[\alpha]_{578}^{22}$ +25.3° (*c* 0.64, MeOH). (Found: C 71.1; H 8.9; O 20.3. $C_{33}H_{50}O_7$ requires: C 70.9; H 9.0; O 20.0) IR: ν_{max}^{KBr} 1735(*s*), 1705(*s*), cm^{-1}. ^1H NMR ($CDCl_3$): δ *inter alia* 0.78, (*s*, 3 H), 0.87 (*s*, 3 H), 2.02 (*s*, 3 H), 2.04 (*s*, 3 H), 4.2–4.5 (*m*, 1 H) 4.5–4.9 (*m*, 1 H), 5.2–5.4 (*m*, 1 H). MS *m/e* (rel. int.): 558 (M^+, 0.8), 168 (11), 141 (67).

Hydrolysis of epi-*ochreasteroside* (7). *epi*-Ochreasteroside (7, 0.39 g) was hydrolysed as described for dendrosteroside (1). The soln was kept at 37° for 5 days and was thereafter extracted with $CHCl_3$–EtOH (2:1, 5 × 200 ml). The organic phase was dried and the solvent evaporated. Residue was chromatographed on Si gel (2.5 × 10 cm, $CHCl_3$–MeOH 9:1), giving *epi*-ochreasterone (8, 59 mg). Needles (MeOH), mp 250–256° (decomp.); $[\alpha]_{578}^{22}$ −15.9° (*c* 0.34, MeOH). (Found: C 73.3; H 9.7. $C_{29}H_{46}O_5$ requires: C 73.4; H 9.8) IR: ν_{max}^{KBr} 3600–3300(*s*), 1705(*s*) cm^{-1}. CD: $\lambda_{extrema}^{MeOH}$ (Δε) 222 (−1.36), 254 (−0.25), 293 (−1.61). ^1H NMR ($CDCl_3$): δ *inter alia* 0.69 (*s*, 3 H), 0.74 (*s*, 3 H) 0.94 (*t*, 3 H, *J* 6.5 Hz), 1.01 (*d*, 3 H, *J* 5 Hz), 1.20 (*d*, 3 H, *J* 7 Hz), 3.3–3.8 (*m*, 1 H), 3.9–4.2 (*m*, 1 H), 4.8–5.1 (*m*, 1 H). A later fraction from the Si gel column contained unhydrolysed glycoside (7, 0.19 g).

Diacetyl-epi-*ochreasterone* (9). This was prepared as described for diacetylochreasterone (6). Needles (MeOH), mp 210–230° (decomp.); $[\alpha]_{578}^{22}$ −30° (MeOH). (Found: C 70.8; H 9.1. $C_{33}H_{50}O_7$ requires: C 70.9; H 9.0) IR: ν_{max}^{KBr} 1728(*s*) cm^{-1}. ^1H NMR ($CDCl_3$): δ *inter alia* 0.72 (*s*, 3 H) 0.77 (*s*, 3 H), 2.02 (*s*, 3 H) 2.04 (*s*, 3 H), 4.44 (*dd*, 1 H, J_1 11 Hz, J_2 5.5 Hz), 4.50–4.87 (*m*, 1 H), 4.87–5.12 (*m*, 1 H). MS *m/e* (rel. int.): 558 (M^+, 3), 498 (10), 329 (16), 328 (22), 298 (12), 168 (16), 141 (100).

3,16-*Dioxo-ochreasterone* (10). A soln of ochreasterone (5, 25 mg) in Me_2CO (20 ml) was cooled to 0° and excess of Jones reagent [12] was added. The reaction mixture was stirred at 0° for 20 min, sodium hydrogen sulphite was added and the mixture filtered. The filtrate was concentrated to dryness and the residue was chromatographed on Si gel (0.5 × 4 cm, Me_2CO) giving 10. Crystallisation from Me_2CO-hexane (1:10) gave needles which melted at 158°, solidified and melted again at 217–224° (decomp.), $[\alpha]_{578}^{22}$ −107° (*c* 0.41, MeOH). (Found: C 74.2; H 9.1. $C_{29}H_{42}O_5$ requires: C 74.0; H 9.0) IR: $\nu_{max}^{CHCl_3}$ 1715(*s*) cm^{-1}. ^1H NMR ($CDCl_3$): δ *inter alia* 0.82 (*s*, 3 H) 0.99 (*s*, 3 H). MS *m/e* (rel. int.): 470 (M^+, 2), 372 (26), 357 (20), 329 (30), 287 (24), 169 (100). Oxidation of *epi*-ochreasterone (8) gave a product indistinguishable (mp, TLC, IR, NMR, optical rotation) from 10.

Ochreasteroside penta-acetate. Ochreasteroside (4, 23 mg) was treated with Ac_2O–CH_5N at room temp. for 16 hr. The penta-acetate was purified by chromatography on Si gel (1.5 × 7 cm, $CHCl_3$–MeOH 9:1), and was obtained as an amorphous solid (26 mg), $[\alpha]_{578}^{22}$ +3.1° (*c* 0.55, MeOH). ^1H NMR ($CDCl_3$): δ *inter alia* 1.99 (*s*, 3 H), 2.00 (*s*, 3 H), 2.01 (*s*, 3 H) 2.06 (*s*, 3 H) 2.07 (*s*, 3 H), 3.30–3.79 (2 H), 3.90–4.50 (3 H), 4.60 (*d*, 1 H, *J* 7 Hz), 4.78–5.41 (4 H).

epi-*Ochreasteroside penta-acetate.* This was prepared similarly and obtained as an amorphous solid, $[\alpha]_{578}^{22}$ −38.1° (*c* 0.24, MeOH). ^1H NMR ($CDCl_3$): δ *inter alia* 1.98 (*s*, 3 H), 1.99 (*s*, 3 H), 2.00 (*s*, 3 H), 2.06 (*s*, 3 H), 2.07 (*s*, 3 H), 3.36–3.78 (2 H), 3.98 4.52 (3 H), 4.60 (*d*, 1 H, *J* 7 Hz) 4.82–5.32 (4 H).

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REFERENCES

1. Behr, D., Dahmén, J. and Leander, K. (1976) *Acta Chem. Scand.* **B30**, 309.
2. Behr, D., Berg, J.-E., Karlsson, B., Leander, K., Pilotti, A.-M. and Wiehager, A.-C. (1975) *Acta Chem. Scand.* **B29**, 401.
3. Sawardeker, J. S., Sloneker, J. H. and Jeanes, A. (1965) *Anal. Chem.* **37**, 1602.
4. Golovkina, L. S., Chizhov, O. S. and Wulfsson, N. S. (1966) *Bull. Acad. Sci. U.S.S.R.* **11**, 1853.
5. Björndahl, H., Hellerqvist, C. G., Lindberg, B. and Svensson, S. (1970) *Angew. Chem. Int. Ed. Engl.* **9**, 610.

6. Hudson, C. S. (1909) *J. Am. Chem. Soc.* **31**, 66.
7. Beard, C., Wilson, J. M., Budzikiewicz, H. and Djerassi, C. (1964) *J. Am Chem. Soc.* **86**, 269.
8. Bridgeman, J. E., Cherry, P. C., Clegg, A. S., Evans, J. M., Jones, E. R. H., Kasal, A., Kumar, V., Meakins, G. D., Morisawa, Y., Richards, E. E. and Woodgate, P. D. (1970) *J. Chem. Soc.* (*C*) 250.
9. Shoolery, J. N. and Rogers, M. (1958) *J. Am. Chem. Soc.* **80**, 5121.
10. Fukushima, D. K. and Gallhager, T. F. (1951) *J. Am. Chem. Soc.* **73**, 196.
11. Aasen, A., Behr, D. and Leander, K. (1975) *Acta Chem. Scand.* **B29**, 1002.
12. Bowers, A., Halshall, T. G., Jones, E. R. H. and Lemin, A. J. (1953) *J. Chem. Soc.* 2555.