EXPERIMENTAL

The air-dried plant material, collected in Feb. 1981 in Transvaal (voucher 81/22, deposited in the Botanic Research Institute, Pretoria), was extracted with Et_2O–petrol(1:2) and the resulting extracts were separated by CC (Si gel) and further by repeated TLC (Si gel). The roots (110 g) gave 5 mg stigmasterol and 4 mg sitosterol, while the aerial parts (290 g) afforded 60 mg caryophyllene, 50 mg germacrene D, 20 mg α-humulene and 15 mg **1** (C_6H_6–CH_2Cl_2–Et_2O, 1:1:1).

3β-Hydroxystilpnotomentolide-8-O-(*5-acetoxysenecioate*) (**1**). Colourless gum, IR $\nu_{max}^{CCl_4}$, cm^{-1}: 3520 (OH, hydrogen bonded), 1775 (γ-lactone), 1750 (OAc), 1700 (C=CCO_2R, C=O): MS *m/z* (rel. int.): 494 $[M]^+$ (0.15), 337.129 $[M-O_2CR]^+$ (3) ($C_{17}H_{21}O_7$), 336 $[M-RCO_2H]^+$ (0.5), 276 $[336-HOAc]^+$ (10), 258 $[276-H_2O]^+$ (6), 99 $[HOCH_2C(Me)=CHCO]^+$ (100);

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436\,nm}{-100 \quad -105 \quad -123 \quad -214} \quad (CHCl_3;\ c\ 0.8).$$

A part of **1** during purification was adsorbed on Si gel for 2 hr. After extraction with MeOH 2 mg of **3** were obtained, colourless solid, mp ~ 165° MS *m/z* (rel. int.): 435 $[M-OAc]^+$ (0.5), 337 $[M-O_2CR]^+$ (1), 277 $[377-HOAc]^+$ (8), 99 $[HOCH_2C(Me)=CHCO]^+$ (100).

6 mg **1** on acetylation (Ac_2O, 1 hr, 70°) afforded 6 mg **2**, colourless gum, IR $\nu_{max}^{CCl_4}$, cm^{-1}: 1780 (γ-lactone), 1750 (OAc), 1720 (C=CCO_2R, C=O); MS *m/z* (rel. int.): 536 $[M]^+$ (0.2), 379 $[M-O_2CR]^+$ (1) 378 $[M-RCO_2H]^+$ (0.5), 99 $[HOCH_2C(Me)=CHCO]^+$ (100).

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BESHORNIN AND BESHORNOSIDE, STEROIDAL SAPONINS OF *BESHORNERIA YUCCOIDES*

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Key Word Index—*Beshorneria yuccoides*; spirostanol glycosides; furastanol glycosides; beshornin; beshornoside.

Abstract—Two new saponins beshornin and beshornoside have been isolated from the methanolic extract of *Beshorneria yuccoides* leaves and their structures elucidated. Beshornin is 3-*O*-{[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1 → 2)-]-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl}-(25*R*)-5α-spirostan-3β-ol, whereas beshornoside is 3-*O*-{[α-L-rhamnopyranosyl-(1 → 4)-β-D-glycopyranosyl-(1 → 2)]-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranosyl} 26-*O*-[β-D-glucopyranosyl]-(25*R*)-5α-furostan-3β,22α,26-triol.

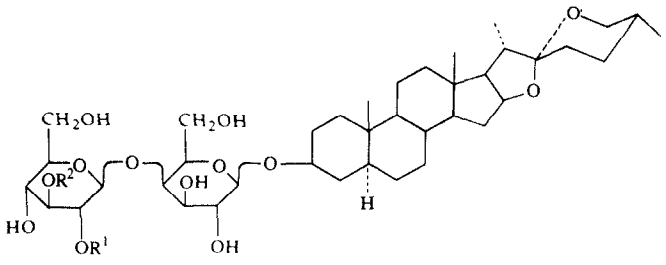
INTRODUCTION

Previous workers [1] have shown the presence of tigogenin based saponins in *Beshorneria yuccoides*. We now report the structure of two new saponins isolated from this plant.

RESULTS AND DISCUSSION

Beshornin and beshornoside were isolated from the leaves of *B. yuccoides* and purified by chromatography. Hydrolysis of both afforded tigogenin and the sugars galactose, glucose and rhamnose in the ratios 1:3:2 for beshornin and 1:4:2 for beshornoside. The fact that beshornoside gave a positive colour with Ehrlich's reagent [2] and was converted to beshornin by β-glucosidase indicated that it was the 26-*O*-(β-D-glucopyranoside) of the furostanol form of beshornin. This was confirmed by chromium trioxide oxidation of beshornoside peracetate to give tetra-acetyl glucosyl-δ-hydroxy-γ-methyl-*n*-valerate [3–5].

The type of glycosidic linkage in compound **1** was proved by methylation [6]. The methylated products were identified by TLC and GLC as methyl-2,3,4-tri-*O*-methyl-L-rhamnopyranoside (**3**), methyl-2,3,6-tri-*O*-methyl-D-glucopyranoside (**4**), methyl-2,3,6-tri-



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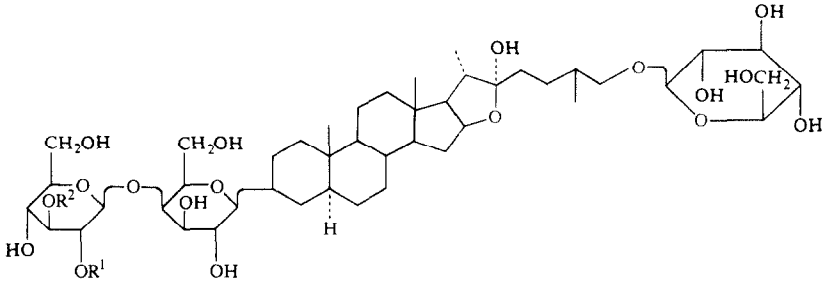

2

1, 2 $R^1 = R^2 =$ α-L-Rhamnopyranosyl-(1→4)-β-D-Glucopyranosyl
9 $R^1 = R^2 = H$
10 $R^1 = H$, $R^2 =$ β-D-Glucopyranosyl
11 $R^1 = R^2 =$ β-D-Glucopyranosyl
12 $R^1 =$ β-D-Glucopyranosyl, $R^2 =$ α-L-Rhamnopyranosyl-(1→4)-β-D-Glucopyranosyl

O-methyl-D-glucopyranoside (**4**), methyl-2,3,6-tri-*O*-methyl-D-galactopyranoside (**5**) and methyl-4,6-di-*O*-methyl-D-glucopyranoside (**6**). After methylation and methanolysis compound **2** gave the same products and additionally methyl-2,3,4,6-tetra-*O*-methyl-D-glucopyranoside (**7**). The sequence of the sugars in **1** was proved by partial hydrolysis which gave a monoglycoside (**8**), diglycoside (**9**), triglycoside (**10**), tetraglycoside (**11**) and pentaglycoside (**12**). Acid hydrolysis of **8** gave galactose; **9–11** gave galactose and glucose in the ratios 1:1, 1:2 and 1:3 respectively; **12** gave galactose, glucose and rhamnose in the ratio 1:3:1. After methylation of **9–12** followed by methanolysis the following were obtained: (a) from **9** compounds **5** and **7**; (b) from **10** compounds **5**, **7** and methyl-2,4,6-tri-*O*-methyl-D-glucopyranoside (**13**); (c) from **11** compounds **5–7**; (d) from **12** compounds **3–7**. Partial hydrolysis of beshornoside led to the formation of **1**, **8–12** and tigogenin. The configuration at C-1 of the monosaccharides was determined with the help of Klyne's rule [7]. From the above results it follows that beshornin is 3-*O*-{[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl (1 → 2)]-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-L-galactopyranosyl}-(25*R*)-5α-spirostan-3β-ol. Beshornoside is 3-*O*-{[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 2)]-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl (1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranosyl}-26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostan-3β, 22α, 26-thiol.

EXPERIMENTAL

Separation of B. yuccoides *saponins.* Dry leaves (100 g) of *B. yuccoides* were extracted with MeOH (3 × 500 ml) at 65° for 4 hr. From the extract, after evaporation of the solvent, a mixture of saponins was isolated by chromatography on Si gel ($CHCl_3$–MeOH–H_2O, 65:35:7), yielding 3.2 g beshornin mp 237–239°, $[\alpha]_D^{20} = -32°$ (DMSO; *c* 1.0) and 4.3 g beshornoside mp 219–221°, $[\alpha]_D^{20} = -25°$ (H_2O; *c* 1.0).

Hydrolysis of **1** *and* **2**. Compounds **1** and **2** (50 mg) were hydrolysed with 5% H_2SO_4 at 110° for 10 hr. Tigogenin was obtained from both glucosides and purified by TLC ($CHCl_3$–MeOH, 9:1) mp 204–206°, $[\alpha]_D^{20} = -65°$ ($CHCl_3$; *c* 1.0) MS: *m/z* 416 $[M]^+$. Monosaccharides were identified in the hydrolysate from both glucosides by PC and by GLC of their aldonenitryls [8]. Aldonenitryl and methyl derivatives of sugars were separated using a 2 m glass column of 5% XE-60 or 3% SE-30 on chromaton N-AW-DMCS (0.16–0.20 mm), temp. program (100°, 5°/min to 220°), He carrier-gas (45 ml/min), a flame ionization detector.

Methylation and methanolysis of permethylated products. Compounds **1** and **2** (0.5 g) were methylated by the Kuhn method to yield permethylated beshornin and beshornoside. They were methanolysed with 72% $HClO_4$ in MeOH (1:10) for 5 hr at 105°. After neutralization by anionic Dowex 1 × 8, TLC on Si gel (Me_2CO–C_6H_6, 1:2) showed four and five products respectively for **1** and **2**. All methylated products were identified by TLC and GLC with the aid of authentic sample compounds.

Enzymic hydrolysis with β-glucosidase of Helix pomatia. Compound **2** (500 mg) in 100 ml H_2O was incubated with the enzyme for 24 hr, at room temp. After 24 hr the mixture

was extracted ×3 with 50 ml BuOH and the extract chromatographed on a column of Si gel to yield 350 mg **1**.

Partial hydrolysis. Compounds **1** and **2** (1.0 g) were heated in 50 ml 1.5 N HCl for 2 hr at +90° with H_2O and extracted with 3 × 30 ml BuOH. The BuOH extracts were chromatographed on Si gel ($CHCl_3$–MeOH–H_2O, 65:25:10). From **1** was obtained compound **8** (20 mg) mp 198–201°, $[\alpha]_D^{20} = -25°$ (DMSO; *c*1.0), **9** (70 mg) mp 251–253°, $[\alpha]_D^{20} = -5°$ (DMSO; *c*1.0), **10** (110 mg) mp 201–203°, $[\alpha]_D^{20} = -17°$ (DMSO; *c*1.0), **11** (320 mg) mp 224–226°, $[\alpha]_D^{20} = -8.5°$ (DMSO; *c*1.0), **12** (60 mg) mp 206–209°, $[\alpha]_D^{20} = -12°$ (DMSO; *c*1.0). From **2** beshornin was obtained in addition to **8–12**. Methylation of 0.05 g of each product (**9–12**) and methanolysis were carried out and the products identified by TLC and GLC.

Oxidation of compound **2**. Acetylated compound **2** (1.0 g), obtained by reaction with HOAc, was dissolved in 10 ml HOAc and 200 mg NaOAC was added [4]. The oxidation was carried out as described in ref. [4] to produce tetra-acetylglucoside methyl ester of δ-hydroxy-γ-methyl-*n*-valeric acid (**14**), which showed the characteristic MS peaks for acetylated glucose, as well as fragment peaks at *m/z* 331, 243, 242, 200, 169, 157, 145, 141, 115, 109 and peaks for the acidic residue at *m/z* 129, 97, 89, 81 [3–5, 9].

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STRUCTURE OF VERSICOLORONE ISOLATED FROM *ASPERGILLUS VERSICOLOR*

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Key Word Index—*Aspergillus versicolor*; fungal metabolite; anthraquinone derivative; structure elucidation.

Abstract—A new anthraquinone metabolite, versicolorone, has been isolated from *Aspergillus versicolor*.

In previous studies averufin[1], versicolorin B[1], averufanin[2], deoxyaverufinone[3], dehydroaverufin[3] and 1, 3, 6, 8-tetrahydroxyanthraquinone[4]were isolated from *Aspergillus versicolor* (Vuillemin) Tiraboschi (strain ATCC 34508). In a continuation of our investigation of anthraquinone metabolites produced by this fungus, a fourth new metabolite has been isolated and named versicolorone (**1**). The structure of versicolorone suggests a relationship between this metabolite and versiconal acetate[5].

Versicolorone, $C_{20}H_{16}O_7$, had UV and IR data which indicated the 1,3,6,8-tetrahydroxyanthraquinone structure[6]. The carbonyl region of the IR spectrum showed a non-chelated carbonyl band at 1670 cm^{-1}, a chelated carbonyl band at 1620 cm^{-1} and an additional carbonyl band at 1700 cm^{-1}. The electron impact mass spectrum of versicolorone lacked the expected $[M]^+$ ion (*m/z* 368, $C_{20}H_{16}O_7$). A prominent ion at *m/z* 310 ($[M-58]^+$, $C_{17}H_{10}O_6$) was initiated by a McLafferty rearrangement. In addition, other peaks of interest were observed at *m/z* 325 ($[M-43]^+$, $C_{18}H_{13}O_6$), 297 ($[M-71]^+$, $C_{16}H_9O_6$), 58 and 43.

The ^1H NMR spectrum of versicolorone confirmed the presence of three aromatic protons: an AX system at δ 6.65 and 7.17 ($^2J = 2.5$ Hz, 7-H and 5-H respectively) and a singlet at δ 7.88 (4-H). The spectrum further showed two sharp one-proton signals at δ 12.33 and 12.89 ascribed to strongly hydrogen-bonded hydroxyl groups (OH-8 and OH-1, respectively), and broad one-proton absorption at δ 11.81 attributed to an unbonded hydroxyl group (OH-6). The three-proton singlet at δ 2.07 was assigned to the methyl group (MeCO). H_2-1′, H-2′, H_2-3′ and H_2-4′ appeared as multiplets at δ 3.41, 3.80, 2.37 and 2.40 respectively.

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