EXPERIMENTAL

The air-dried plant material (Botanic Garden, Pretoria, voucher 81/179, deposited in the Herbarium of the Botanic Research Institute, Pretoria) was extracted with Et_2O–petrol, 1:2 and the resulting extracts separated by CC (Si gél) and further by repeated TLC (Si gel). Compounds were identified by comparing the ^1H NMR, UV and MS spectra with those of authentic material. The roots (100 g) afforded 20 mg aplotaxene 1 mg **1**, 3 mg **2**, 5 mg **3**, 1 mg **4**, 1 mg **5**, 8 mg **6**, 2 mg **7**, 7 mg **8**, 2 mg **9**, 5 mg **12**, 4 mg **13** and 2 mg **14**, yellow gum, IR $\nu_{max}^{CCl_4}$, cm^{-1}: 3600–2700, 1630 (hydrogen bonded PhC=O); MS *m/z* (rel. int.): 324.136 $[M]^+$ (22) ($C_{20}H_{20}O_4$), 256 $[M-isoprene]^+$ (64), 255 $[M-CH_2CH{=}CMe_2]^+$ (62), 179 $[256-C_6H_5]^+$ (83), 152 $[255-CH{=}CHPh]^+$ (44), 69 $[Me_2CH{=}CHCH_2]^+$ (100); ^1H NMR ($CDCl_3$, 400 MHz): 7.61 *m* (H-2, H-6), 7.38 *m* (H-3, H-4, H-5), 7.81 *d* (H-7, $J = 16$ Hz), 8.02 *d* (H-8, $J = 16$ Hz), 5.98 *s* (H-3′, H-5′), 4.52 *br s* (H-1″, $J = 7$Hz), 5.46 *tqq* (H-2″, $J = 7, 1, 1$ Hz), 1.79 *br s* (H-4″), 1.73 *br s* (H-5″), 9.40 *br s* (OH). The aerial parts (200 g) gave 10 mg germacrene *D*, 8 mg lupeol, 6 mg of its Δ-12,13-isomer, 10 mg lupeyl acetate, 8 mg of its Δ-12,13-isomer, 0.1 mg **1**, 1 mg **3**, 1 mg **4**, 5 mg **5**, 10 mg **10** and 2 mg **11**

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A BITTER MONOTERPENE GLUCOSIDE FROM *VIBURNUM PHLEBOTRICHUM*

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Key Word Index—*Viburnum phlebotrichum*; Caprifoliaceae; monoterpene glucoside; phlebotricoside.

Abstract—From the methanol extract of the leaves of *Viburnum phlebotrichum*, a new bitter monoterpene glucoside has been isolated in addition to three known compounds, *p*-hydroquinone, arbutin and glucoluteolin. The structures were elucidated by spectroscopic and chemical methods.

INTRODUCTION

The shrub *Viburnum phlebotrichum* is widely distributed in Japan, and its leaves are remarkably bitter. In the course of the investigation on bitter constituents of *Viburnum* species, a new bitter monoterpene glucoside (**1**) was isolated from *V. phlebotrichum* together with three known compounds *p*-hydroquinone, arbutin and glucoluteolin [1]. The bitter glucoside **1**, phlebotricoside, has now been characterized.

RESULTS AND DISCUSSION

The glucoside **1** was obtained upon ether extraction of the methanol extract of fresh leaves and subsequent fractionations by Si gel CC. **1** was optically active and elemental analysis suggested the formula $C_{22}H_{30}O_9$, but no molecular ion corresponding to this was observed in the mass spectrum. The IR spectrum showed strong hydroxyl and aromatic absorptions at 3500 and 1515 cm^{-1}, respectively. In addition, the occurrence of an α,β-unsaturated ester was recognized by the characteristic IR absorptions at 1710 and 1650 cm^{-1} and the absorption at 920 cm^{-1} typical of a terminal methylene group was also observed. The ^1H NMR spectrum of **1** revealed the presence of a *p*-disubstituted phenyl group [δ6.81 and 7.00(4H, A_2B_2, $J = 8$ Hz)], a mono-substituted double bond [5.04–5.28 and 5.91 (3H, ABX system)], a tertiary methyl group attached to a carbon bearing a hydroxyl group [1.30 (3H, *s*)] and an olefinic methyl group [1.80 (3H, *br s*)].

Acetylation of **1** with acetic anhydride–pyridine

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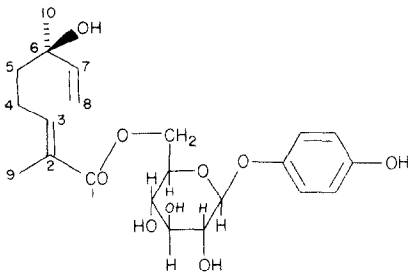

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gave a tetra-acetate (**2**) $C_{30}H_{38}O_{13}$. The ^1H NMR spectrum revealed the presence of four acetyl groups (δ2.02–2.69) and a tertiary hydroxyl group was also recognized by the characteristic IR absorption at 3500 cm^{-1}. Upon alkaline hydrolysis, **2** afforded a monoterpenoid acid (**3**) and arbutin, which was identical with an authentic sample by IR and UV spectra, were obtained. The IR spectrum of **3** showed hydroxyl absorption at 3400 cm^{-1} together with the absorption of α,β-unsaturated carboxyl group at 1695 and 1650 cm^{-1}.

The ^1H NMR spectrum of **3**, with double-resonance experiments, enabled most of the structural features of the compound [6-hydroxy-2,6-dimethyl-2(*E*),7-octadienoic acid] to be elucidated. Two three-proton singlets at δ1.30 and 1.88 were assigned to a tertiary methyl group attached to a carbon (C-6) bearing a hydroxyl group and an allylic methyl group (C-9), respectively. Three protons of monosubstituted double bond appeared at δ5.20, 5.35 and 5.86 as typical ABX system signals (J = 2, 10 and 18 Hz; H-7 and H-8). Another olefinic proton appeared at δ6.71 as quartet of triplet (J = 1 and 7.5 Hz, H-3). This feature of the signal was caused by coupling to a methylene group [2.50(*m*, H-4)] and to an allylic methyl group (1.88). It was supported by the fact that irradiation at H-4 caused the feature at δ6.71 to change to a triplet (J = 1 Hz), and similarly irradiation at H-9 caused a marked change in the multiplet structure of H-4 at 2.50.

Table 1. ^{13}C NMR data of compounds **1** and **2** (25.1 MHz)

Carbon	1	2
C-1	170.8	170.2
C-2	128.0	127.1
C-3	145.7	144.5
C-4	24.6	23.6
C-5	41.0	40.7
C-6	74.6	72.9
C-7	144.9	143.5
C-8	113.6	112.2
9-Me	12.8	12.3
10-Me	27.3	28.1
Glucose		
C'-1	102.2	99.4
C'-2	74.1*	71.1*
C'-3	76.9	72.2*
C'-4	71.5	68.7
C'-5	74.8*	72.8*
C'-6	65.0	62.4
Aromatic ring		
C''-1	152.4†	154.5
C''-2,6	119.4	117.8
C''-3,5	117.2	112.5
C''-4	151.4†	146.2
OAc	—	21.1 and 20.6 × 3

Spectrum of **1** was recorded in D_2O (dioxane), and **2** in $CDCl_3$ (TMS).

*,†Assignments may be interchanged.

E-configuration of the olefinic bond at carbon-2 was determined by the chemical shift and the coupling constant of the proton at C-3 [2]. Another point supporting the structure of **3** was that lithium aluminium hydride reduction of **3** or **2** yielded a known diol (**4**) which was identified with authentic 9-hydroxylinalol on the basis of comparison of IR and ^1H NMR spectra. The configurations of **3** and **4** were determined as the *R*-form, because of the sign of specific rotation of **4** ($[\alpha]_D$ −4°), being in agreement with that of synthetic diol ($[\alpha]_D$ −1.2°) which was prepared from (−)-linalol [3] with SeO_2 [4].

The configuration of 9-hydroxylinalol [5] ($[\alpha]_D$ −1.5°), which was obtained from *Betula alba* by Tschesche *et al.* had not been determined. Now, the configuration of this compound should also be the *R*-form on account of the same sign of its $[\alpha]_D$ as of **4**.

In order to elucidate the location of the ester linkage in **1**, compound **1** was methylated by the Purdie method to give the tetramethyl ether (**5**). Upon alkaline hydrolysis followed by acid hydrolysis, **5** yielded the single 2,3,4-tri-*O*-methyl-D-glucose. This indicated that the ester linkage in compound **1** was located at C-6 of D-glucose.

On the basis of these data, the structure of the new glucoside was determined as **1**. Additionally, this structure was supported by the ^{13}C NMR spectra (Table 1) of **1** and **2**.

EXPERIMENTAL

Isolation of a bitter glucoside from Viburnum phlebotrichum. Fr. leaves of *V. phlebotrichum* (2 kg) were extracted with MeOH (4 l. × 2). After concn of the combined MeOH extracts *in vacuo*, H_2O (500 ml) was added and the insol. material filtered off. The filtrate was extracted with Et_2O (2 l). The Et_2O extract (25 g) was chromatographed on a Si gel (1 kg) column and eluted with $CHCl_3$–MeOH with increasing MeOH content. The fractions eluted with $CHCl_3$–MeOH (19:1) were combined (7 g) and re-chromatographed on Si gel (300 g). Fractions eluted with $CHCl_3$–MeOH (24:1) were evaporated to give a crude bitter glucoside **1** (1.1 g). From the fractions diluted with $CHCl_3$–MeOH (20:1–10:1), *p*-hydroquinone, arbutin (mp 171°) and glucoluteolin (mp 226–228°), which were identified with authentic samples by comparison of the IR and ^1H NMR spectra, were afforded successively. Later, a simple procedure to separate the bitter component from colored impurities, was carried out, i.e. the Et_2O soln was re-extracted with H_2O, then the aq. layer was again extracted with EtOAc. The extracts were evaporated to dryness and the residue crystallized from hot C_6H_6, re-crystallized from MeOH–C_6H_6 to give **1** as pure colorless plates (680 mg), mp 140–142°, $[\alpha]_D$ −37.5° (EtOH; *c* 0.016) UV λ_{max}^{MeOH} nm (ϵ) 220 (13 000) and 285 (1500); IR ν_{max}^{nujol} cm^{-1} 3500, 3350, 1710, 1650, 1515 and 920; ^1H NMR (100 MHz CD_3COCD_3) δ 1.30 (3H,

s), 1.80 (3H, *br s*), 3.67–5.00 (7H, *m*), 5.04–5.28 and 5.91 (3H, ABX, $J = 2$, 10 and 18 Hz), 6.81 and 7.00 (4H, A_2B_2, $J = 8$ Hz); MS *m/z* no $[M]^+$, 136 (100%) (Found: C, 60.21; H, 6.91%. Calc. for $C_{22}H_{30}O_9$: C, 60.26; H, 6.90%).

Tetra-acetate **2**. **1** (130 mg) was acetylated with Ac_2O and pyridine at room temp. and the product recrystallized from MeOH–H_2O to give **2** (100 mg) as colorless needles mp 99–99.5°, UV λ_{max}^{MeOH} nm (ϵ) 218 (45 000), 270 (2500) and 278 (1800); IR ν_{max}^{nujol} cm^{-1} 3500, 1760, 1640, 1500 and 920; ^1H NMR (100 MHz $CDCl_3$) δ 1.29 (3H, *s*), 1.82 (3H, *d*, $J = 1.3$ Hz), 2.02, 2.03, 2.05 and 2.69 (3H × 4, OAc), 4.3–5.0 (7H), 5.07, 5.21 and 5.91 (3H, ABX, $J = 1$, 12 and 16 Hz), 6.78 (1H, *br t*, $J = 7.5$ Hz) and 6.97 (4H, *s*) (Found: C, 59.43; H, 6.32%. Calc. for $C_{30}H_{38}O_{13}$: C, 59.39; H, 6.31%).

Alkaline hydrolysis of **2**. **2** (273 mg) was dissolved in a small amount of MeOH and to this soln was added 5 ml 2 N NaOH. After refluxing for 2 hr, the reaction soln was extracted with Et_2O to remove the neutral material. After acidification of the aq. layer with dil. HCl, extraction with Et_2O was followed by extraction with EtOAc. The Et_2O extracts were evaporated to give a carboxylic acid **3** (76 mg). The EtOAc extracts were evaporated to give arbutin (191 mg).

6-*Hydroxy*-2,6-*dimethyl*-2(*E*),7-*octadienoic acid* **3**. Colorless oil, IR ν_{max}^{film} cm^{-1} 3400, 2900, 1695, 1640 and 920; ^1H NMR (100 MHz $CDCl_3$) δ 1.30 (3H, *s*), 1.68 (2H, *br t*, $J = 7$ Hz), 1.88 (3H, *br s*), *ca* 2.50 (2H, *m*), 5.20, 5.35 and 5.86 (3H, ABX, $J = 2$, 10 and 18 Hz) and 6.71 (1H, *tq*, $J = 1$ and 7.5 Hz) (Found: C, 65.25; H, 8.48%. Calc. for $C_{10}H_{16}O_3$: C, 65.19; H, 8.75%). Arbutin was re-crystallized from MeOH–$CHCl_3$ to give colorless needles, mp 171° which was identical with an authentic sample. UV λ_{max}^{MeOH} nm (ϵ) 218 (7000) and 280 (2100); IR ν_{max}^{nujol} cm^{-1} 3400, 1600, 1520 and 1220.

Permethylation of **1**. **1** was methylated with MeI and Ag_2O in DMF at 5° (2 days), to give tetramethyl ether **5**, IR ν_{max}^{film} cm^{-1} 3400; ^1H NMR (100 MHz CD_3COCD_3) δ 3.46, 3.52, 3.56 and 3.68 (3H × 4). **5** was hydrolysed with 2N NaOH followed by with 2 N HCl to give a methylated D-glucose which was identical with an authentic sample of 2,3,4-tri-*O*-methyl-D-glucose (IR, ^1H NMR and TLC).

Reduction of **2** *or* **3**. $LiAlH_4$ (4.2 mmol) in dry Et_2O (13.4 ml) was stirred for 10 min at room temp. The dry Et_2O soln of sample (1 mmol) was added dropwise to this and the reaction mixture stirred for 10 min at room temp. After cooling with ice–H_2O, H_2O (0.2 ml) and 15% NaOH, in turn, were added dropwise to this soln, then stirred overnight at room temp. After the mixture was filtered off, the filtrate was diluted with H_2O and extracted with Et_2O (30 ml × 3). The Et_2O extracts were dried and evaporated to give a diol **4** (53% yield) after purification by chromatography on Si gel, colorless oil, $[\alpha]_D - 4°$ (MeOH; *c* 0.14); IR ν_{max}^{film} cm^{-1} 3300, 2900, 1660, 1640, 1000 and 920; ^1H NMR (100 MHz $CDCl_3$) δ 1.27 (3H, *s*), 1.64 (3H, *br s*), 1.84 (2H, *m*), 2.20 (2H, *m*), 3.95 (2H, *s*), 5.03, 5.16 and 5.93 (3H, ABX, $J = 1$, 12 and 18 Hz) (1H (*m*) of C-3 was obscure in the range of 5.00–6.00). **4** was identified with the diol derived from (−)-linalol by comparison of the IR and ^1H NMR spectra.

Oxidation of (−)-*linalol with* SeO_2 *to the diol* **4**. The soln of 1 mmol SeO_2, which was purified by sublimation, in 1 ml H_2O was added to the dioxane soln (10 ml) containing (−)-linalol (2 mmol). The reaction soln was refluxed for 5 hr. After cooling with ice–H_2O, the soln was filtered off. The filtrate was diluted by 100 ml H_2O and extracted with Et_2O. The Et_2O extracts were evaporated to give the synthetic diol (24% yield) which was further purified by Si gel chromatography. Colorless oil, $[\alpha]_D - 1.2°$ (MeOH; *c* 0.13). IR (film) and ^1H NMR ($CDCl_3$) spectra were superimposable with those of the diol from natural product, respectively.

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