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Studies on Orchidaceae Glycosides 4.* The Structures and Absolute Configurations of *cis*- and *trans*-Crassinodine, Two Glucosides from *Dendrobium crassinode* B. & Rf.

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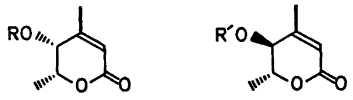
Two glucosides, *cis*- and *trans*-crassinodine (*1* and *2*), have been found in *Dendrobium crassinode* B. & Rf. Compound *1* was isolated as its tetraacetate. The glucosides *1* and *2* are shown to be the 4*R*, 5*R* and 4*S*, 5*R* isomers, respectively, of 4-β-D-glucopyranosyloxy-3-methylhex-2-en-5-olide.

Two glucosides, *cis*- and *trans*-crassinodine (*1* and *2*) have been found in *Dendrobium crassinode* B. & Rf. *trans*-Crassinodine (*2*) was isolated from the glucosidic mixture by fractional crystallisation. The *cis* isomer (*1*), which could not be obtained in a pure state, was isolated and characterised as its tetraacetate (*3*).



1: R = β-D-glucopyranosyl
2: R' = β-D-glucopyranosyl
3: R = 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl
4: R' = 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl
5: R = H
6: R' = H
7: R' = propionyl

Spectrochemical and elemental analyses indicate that *cis*- and *trans*-crassinodine (*1* and *2*) have the composition $C_{13}H_{20}O_8$. Sugar [2,3] and methylation [4] analyses show *1* and *2* to be glucopyranosides. Since β-glucosidase (emulsin) hydrolyses the glucosides, the sugar moieties in *1* and *2* must be D-glucose, β-linked to the aglycones. The β-configuration of the glucosidic bonds in *1* and *2* is also evident from the chemical shifts (δ 4.66 and 4.64) and the large coupling constants (8 Hz) of the anomeric protons in the corresponding tetraacetyl derivatives (*3* and *4*).[5]

Enzymatic hydrolysis of the mixture of glucosides with emulsin gave the aglycones *5* and *6*, which were separated by preparative TLC. When *2* was treated with emulsin, only aglycone *6* was obtained. The spectral properties (UV, IR and NMR) of *5* and *6* are in accordance with those reported for racemic *cis*- and *trans*-3-methyl-4-hydroxyhex-2-en-5-olide, respectively.[6]

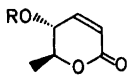
The propionyl derivative of *6* (*7*) has a specific rotation of +98° (in methanol). Since the 4*R*, 5*S* isomer of 3-methyl-4-propionyloxyhex-2-en-5-olide has been reported [7] to have the specific rotation −94.1° (in ethanol), it follows that the aglycone *6* has the 4*S*,5*R* configuration, and hence that the glucoside *2* is (4*S*,5*R*)-4-β-D-glucopyranosyloxy-3-methylhex-2-en-5-olide.

The influence of an allylic oxygen atom on the sign of the Cotton effect associated with the $\pi \rightarrow \pi^*$ transition in UV, located in the 205−230 nm region for enelactones, has been described.[8] A right-handed chirality in the allylic oxygen-olefinic double bond system results in a positive contribution to the CD curve, while a left-handed chirality results in a negative contribution. This sign/chirality re-

* For paper 3 in this series, see Ref. 1.

lationship is illustrated by the CD curves of *2*, *4* and *6* which show a positive effect at 226.5, 224 and 231.5 nm, respectively, consistent with the 4*S* configuration. In the similar compound (4*R*,5*S*)-4-hydroxyhex-2-en-5-olide, osmundalactone [9] (*8*), the corresponding effect at 238 nm is negative.

The CD curves of *3* and *5* show negative Cotton effects at 220 and 218 nm, respectively, which indicate a 4*R* configuration. This assignment is further supported by the application of Mills' rule for cyclic allylic alcohols [10] to *5* and *6*.[11] This rule states that an allylic alcohol with a left-handed chirality of the allylic hydroxyl-double bond helix is more laevorotatory at the sodium D line than its epimer. The aglycones *5* and *6* have specific rotations of −259 and 0°, respectively. Hence the aglycone *5* has the 4*R*,5*R* configuration and the glucoside *1* is (4*R*,5*R*)-4-β-D-glucopyranosyloxy-3-methylhex-2-en-5-olide.



8 : R = H
9 : R = ß-D-glucopyranosyl

Lactones similar to *5* and *6* have been found in some fungi [12–14] and higher plants.[9,15] One of these, osmundalactone (*8*), occurs as the aglycone in the glucoside osmundalin (*9*), isolated from two ferns, *Osmunda japonica* and *O. regalis*.[9] Those of fungal origin show some antibiotic activity [12,14] whereas osmundalin (*9*) may be carcinogenic.[9]

EXPERIMENTAL

General conditions were the same as in an earlier communication.[16] The 60 MHz NMR spectra were measured on a Varian A-60A spectrometer and the CD spectra on a Jasco J-40 spectropolarimeter.

Isolation of the glucosidic mixture (1 *and* 2). Fresh plants of *Dendrobium crassinode* B. & Rf. (2.5 kg) were extracted with methanol (9.5 l). The extract was concentrated to 0.5 l, acidified (pH 4) with hydrochloric acid and washed with carbon tetrachloride (7 × 60 ml). The aqueous phase was made alkaline (pH 8) with sodium hydrogen carbonate, washed with ether (3 × 50 ml), neutralized with hydrochloric acid and evaporated to dryness. The residue was extracted with methanol (2 × 300 ml) and the combined extracts were concentrated to 200 ml. Ethanol (400 ml) was added and the precipitate filtered off. The ethanolic solution was evaporated and the residue was extracted with ethanol-methanol (3:1, v/v, 300 ml). The extract was evaporated and the residue filtered through a column of silica gel (5 × 15 cm, methanol). The eluate was evaporated giving an amorphous residue (18 g).

A portion (5 g) of this residue was chromatographed on silica gel (5 × 15 cm, chloroform-ethanol 2:1, v/v) yielding a mixture of *1* and *2* (800 mg).

Characterisation of 2. Part of this mixture (400 mg) was recrystallised four times from ethyl acetate-methanol giving *2* as needles (40 mg) which started to melt at 94.5 °C, solidified, and melted again at 177.5–182 °C; $[\alpha]_{578}^{25}$ +39° (*c* 0.7, methanol). UV, λ_{max} (methanol) (log ε): 215.5 (4.0) nm. CD, $\lambda_{extrema}$ (methanol) (Δε): 226.5 (+8.45), 256 (−1.92) nm. IR, ν_{max} (KBr): 3480 (s), 3280 (s), 1730 (s), 1640 (w) cm^{-1}. ^{1}H NMR, 60 MHz, δ (pyridine-d_5): 1.46 (d, 3 H), 2.08 (m, 3 H), 3.58–5.00 (m, 9 H), 5.70–7.25 (4 H, exchangeable in D_2O), 5.92 (m, 1 H). (Found: C 51.0; H 6.9; O 42.2. Calc. for $C_{13}H_{20}O_8$: C 51.3; H 6.6; O 42.1).

Acetylation of the glucosidic mixture. A solution of the glucosidic mixture (28 mg), pyridine (1 ml) and acetic anhydride (0.5 ml) was left at room temperature for 20 h. The reaction mixture was poured into ice-water and extracted with chloroform (2 × 3 ml). The chloroform extracts were combined, dried and evaporated. The acetylated glucosides were separated by preparative TLC on silica gel. The plate was developed twice with benzene-acetone (4:1, v/v), giving two bands, R_F 0.45 (fraction I) and R_F 0.50 (fraction II).

Tetra-O-*acetyl*-cis-*crassinodine* (3). Fraction I (10 mg) was crystallised from ethyl acetate giving *3* (6 mg), m.p. 162–163 °C; $[\alpha]_{578}^{21}$ −101° (*c* 0.2, methanol). UV, λ_{max} (methanol) (log ε): 214.5 (4.04) nm. CD, $\lambda_{extrema}$ (methanol) (Δε): 220 (−15.7), 257.5 (−1.48) nm. IR, ν_{max} ($CHCl_3$): 1759 (s), 1650 (w) cm^{-1}. ^{1}H NMR, 100 MHz, δ ($CDCl_3$): 1.41 (d, 3 H, J=7 Hz), 1.96–2.24 (15 H), 3.56–3.82 (m, 1 H), 4.13–4.27 (m, 1 H), 4.19 (d, 2 H, J=4 Hz), 4.46 (m, 1 H), 4.66 (d, 1 H, J=8 Hz), 4.91–5.36 (m, 3 H), 5.91 (m, 1 H). (Found: C 53.3; H 5.9; O 40.6. Calc. for $C_{21}H_{28}O_{12}$: C 53.4; H 6.0; O 40.6).

Tetra-O-*acetyl*-trans-*crassinodine* (4). Fraction II (23 mg) was crystallised from ethyl acetate-light petroleum giving *4* (20 mg), m.p. 171–173 °C: $[\alpha]_{578}^{25}$ +28° (*c* 0.3, methanol). UV, λ_{max} (methanol) (log ε): 213.5 (4.06) nm. CD, $\lambda_{extrema}$ (methanol) (Δε): 201 (−7.45), 224 (+10.1), 254.5 (−1.32) nm. IR, ν_{max} (KBr): 1757 (s), 1725 (m), 1660 (w) cm^{-1}. ^{1}H NMR, 100 MHz, δ ($CDCl_3$): 1.43 (d, 3 H, J=7 Hz), 1.95–2.12 (15 H), 3.60–3.84 (m, 1 H), 4.08

(d, 1 H, $J=7.5$ Hz), 4.22 (d, 2 H, $J=4$ Hz), 4.44 (m, 1 H), 4.64 (d, 1 H, $J=8$ Hz), 4.90–5.40 (m, 3 H), 5.84 (m, 1 H). (Found: C 53.5; H 6.1; O 40.6. Calc. for $C_{21}H_{28}O_{12}$: C 53.4; H 6.0; O 40.6).

Enzymatic hydrolysis of the glucosidic mixture. A mixture of the glucosides (*1* and *2*, 52 mg) was dissolved in a potassium hydrogen phthalate-sodium hydroxide buffer solution (pH 5.2, 4 ml) and emulsin (5 mg) was added. The mixture was kept at 37 °C for 12 h, and then extracted with ether (6 × 1 ml). The ether solutions were combined, dried and evaporated, leaving a mixture (16 mg) of the aglycones *5* and *6*. These were separated by preparative TLC on silica gel. The plate was developed twice with chloroform-ethyl acetate (1:1, v/v), giving two bands, R_F 0.45 (*5*) and R_F 0.50 (*6*).

(4R,5R)-4-Hydroxy-3-methylhex-2-en-5-olide (5). Needles, m.p. 101–102 °C (toluene); $[\alpha]_D^{25}$ −259°, $[\alpha]_{578}^{25}$ −278° (*c* 0.11, methanol). CD, $\lambda_{extrema}$ (methanol) ($\Delta\varepsilon$): 218 (−11.0), 256.5 (−1.83) nm. The UV, IR and NMR spectra were in accordance with those of the racemate.[6] (Found: C 58.9; H 7.1; O 33.4. Calc. for $C_7H_{10}O_3$: C 59.1; H 7.1; O 33.8).

(4S,5R)-4-Hydroxy-3-methylhex-2-en-5-olide (6). Plates, m.p. 121–122 °C (toluene); $[\alpha]_{578}^{26}$ −1.0° (*c* 0.5, methanol); $[\alpha]_{578}^{26}$ −38° (*c* 0.5, acetone). CD, $\lambda_{extrema}$ (methanol) ($\Delta\varepsilon$): 207 (−11.2), 231.5 (+5.28), 256 (−3.49) nm. The UV, IR and NMR spectra are in accordance with those of the racemate.[6] (Found: C 59.0; H 7.0; O 33.5. Calc. for $C_7H_{10}O_3$: C 59.1; H 7.1; O 33.8).

(4S,5R)-3-Methyl-4-propionyloxyhex-2-en-5-olide (7). A solution of *6* (19 mg), pyridine (0.5 ml) and propionyl chloride (0.5 ml) was left at 0 °C for 1 h. Ice-water was added and the reaction mixture was extracted with ether (3 × 1 ml). The ether extracts were combined, washed with hydrochloric acid (0.5 M, 1 ml), dried and evaporated. The residue was purified by preparative TLC on silica gel (chloroform). The plate was developed twice and *7* was isolated as an oil (14 mg); $[\alpha]_D^{21}$ +98° (*c* 1.4, methanol) ($[\alpha]_D^{26}$ −94.1° (ethanol) for the enantiomer[7]). CD, $\lambda_{extrema}$ (methanol) ($\Delta\varepsilon$): 223 (+8.52), 252.5 (−1.06) nm. The IR and NMR spectra were in accordance with those of the enantiomer.[7]

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