Acta Chemica Scandinavica B 29 (1975) 627—639

Short Communications

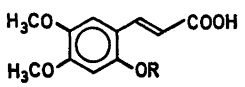
Studies on Orchidaceae Glycosides. 3.* A New Glycoside, 2-(β-D-Glucopyranosyloxy)-4,5-dimethoxy-*trans*-cinnamic Acid (Densifloroside), from *Dendrobium densiflorum* Wall.

JAN DAHMÉN, KURT LEANDER and JAN ROSENBLOM

Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-104 05 Stockholm, Sweden

A small number of phenolic glycosides of some *o*-hydroxycinnamic acids have been reported to occur in Nature.[2–5] Results indicating the presence of glycosides of this type in *Orchidaceae* have also been reported. Thus, extracts from some orchids have been shown to give coumarin upon acid hydrolysis or treatment with emulsin.[6] It has further been demonstrated that acid hydrolysis of an extract from *Dendrobium thyrsiflorum* Rchb. f. gives rise to 6,7-dimethoxycoumarin, 6,7-methylenedioxycoumarin, and 7-hydroxy-6-methoxycoumarin.[7] It was suggested that these coumarin derivatives were formed from glycosides of the corresponding *o*-hydroxy-*trans*-cinnamic acids.

In this communication we report the isolation and characterisation of 2-(β-D-glucopyranosyloxy)-4,5-dimethoxy-*trans*-cinnamic acid, named densifloroside (I), and the corresponding *cis* isomer (II).



I

R=β-D-glucopyranosyl

The glycoside I was isolated from a methanolic extract of *D. densiflorum* by gel filtration, chromatography on silica gel and crystallisation. Spectrochemical and elemental analysis indicated that I has the molecular formula $C_{17}H_{22}O_{10}$. Sugar [8,9] and methylation analysis [10] showed I to be a glucopyranoside. Since an aqueous solution of the sugar showed a positive rotation, the sugar moiety in I is D-glucose. Hydrolysis of I gave 6,7-dimethoxycoumarin, and hence the aglycone of I must be either *cis* or *trans* 2-hydroxy-4,5-dimethoxycinnamic acid. The NMR spectrum of densifloroside (I) shows *inter alia* two doublets at δ 6.46 and 7.97 with a coupling constant of 16 Hz, which established that the double bond has the *trans* configuration.[11]

The IR spectrum of I shows a strong absorption at 1705 cm^{-1}, attributed to an α,β-unsaturated carboxylic acid group. After treatment of I with triethylamine, the absorption band at 1705 cm^{-1} was absent but a new band appeared at 1545 cm^{-1}. The absorption bands in the UV spectrum of I showed a hypsochromic shift of 7 nm when the conditions in the solution were changed from acid to alkaline. These results show that I is a phenolic glycoside and not an ester of D-glucose. The β-configuration of the glucopyranose residue is evident from the large negative specific rotation [12] and the chemical shift (δ 4.88) together with the coupling constant (6 Hz) of the anomeric proton.

Another glycoside was found in the methanolic extract. The amount isolated was too small to permit a complete characterisation but, according to its NMR spectrum, it should be the *cis* isomer of densifloroside (I). At present it is not known whether the *cis* or the *trans* isomer prevails in the plant, since the *cis* glycoside was found to isomerize to the corresponding *trans* isomer during the isolation procedure. The proportions of the *cis* and the *trans* isomers may also be influenced by the conditions under which the plants have grown. It has been shown that the ratio of the *cis* and *trans* isomers of 2-(β-D-glucopyranosyloxy)-cinnamic acid in *Melilotus albus* depends on the light during cultivation.[13] If the plants were grown in sunlight, the *cis* isomer was formed, whereas in the shade only the *trans* isomer was formed. On the other hand if the plants were grown first in sunlight and then for a short period in the shade, a mixture of the *cis* and *trans* isomers was found.

Acid hydrolysis of the crude aqueous extract of *D. densiflorum* gave coumarin, 6,7-dimethoxycoumarin, and 6,7-methylenedioxycoumarin. As these compounds do not occur as such in the plant, it seems reasonable to assume that other glycosides of the *o*-hydroxycinnamic acid

* For paper 2 in this series, see Ref. 1.

type are present in the plant in addition to I and II.

Experimental. General conditions were the same as in the previous communication.[1]

Isolation of densifloroside (I). Fresh plants of *Dendrobium densiflorum* Wall. (8.8 kg) were extracted with methanol (20 l). The extract was concentrated to 0.75 l and washed with chloroform (4 × 0.2 l). Part of the crude aqueous extract (200 ml) was evaporated to dryness and the residue was filtered through Sephadex LH-20 (7.5 × 65 cm) using ethanol-water (1:1) as eluent. The progress of the separation was followed by TLC. The fraction containing the glycosides was evaporated to dryness and the residue (4.0 g) was chromatographed on silica gel (5.0 × 35 cm) using ethanol as eluent. The fraction containing crude densifloroside (I) was evaporated to dryness and the residue was recrystallised from water giving white needles, (0.15 g), m.p. 225 – 227 °C; $[\alpha]_D^{22}$ – 119° (*c* 0.23, methanol). (Found: C 49.7; H 6.0; O 44.3. Calc. for $C_{17}H_{22}O_{10}.1\frac{1}{2}H_2O$: C 49.4; H 6.1; O 44.5). UV, nm (ε): λ_{max} (methanol) 332 (8900), 284 (8550), 234 (7650). IR: ν_{max} (KBr) 3550 (m), 3500 – 2450 (m), 1705 (s), 1640 (m), 1612 (m). NMR (DMSO-d_6): δ 3.0 – 3.65 (7 H), 3.76 (s, 3 H), 3.79 (s, 3 H), 4.3 – 5.6 (4 H, exchangeable in D_2O), 4.88 (d, 1 H, J = 6 Hz), 6.87 (s, 1 H), 7.19 (s, 1 H), 6.46 and 7.97 (2 H, AB pattern. J_{AB} = 16 Hz).

Isolation of the cis-*isomer (II).* Part of the concentrated plant extract (50 ml) was fractionated on Sephadex LH-20 as above. The fraction containing II was chromatographed on silica gel (2.8 × 29 cm) using chloroform:methanol:water (65:35:10) as eluent. Preparative TLC in the same system of the eluent fraction containing II from the silica gel column gave a small amount of II (2 mg, R_F 0.19) together with some I (R_F 0.29). NMR (CD_3OD): δ 3.50 – 3.77 (~6 H, partly overlapping with the CD_2HOD signal) 3.81 (s, 3 H), 3.84 (s, 3 H) 4.80 (anomeric proton and HDO signal), 5.96 and 6.76 (2 H, AB pattern, J_{AB} = 13 Hz), 6.91 (s, 1 H) 7.49 (s, 1 H).

Sugar analysis. Densifloroside (I) (11 mg) was hydrolysed in sulfuric acid (0.25 M, 4 ml) at 100 °C for 15 h. The hydrolysate was neutralised with barium carbonate, filtered and washed with chloroform (5 × 1 ml). The solution was concentrated to 1.00 ml and the optical rotation measured (α_D^{22} + 0.133). To a part of this solution (0.400 ml), xylose (0.405 mg) and arabinose (0.500 mg) were added as internal standards and the sugars were converted into the corresponding alditol acetates and analysed by GLC[8] – MS.[9] The amount of D-glucose was determined from the gas chromatogram and found to be 2.63 mg/ml. The specific rotation was found to be + 51°, in good agreement with the published value for D-glucose.

Acid hydrolysis. The crude aqueous extract (100 ml) was refluxed with hydrochloric acid (2 %, 100 ml) overnight. After cooling, the reaction mixture was extracted with chloroform (5 × 50 ml). The combined chloroform extracts were evaporated to an oily syrup which was chromatographed on silica gel (4.5 × 24 cm) using chloroform as eluent. The first fraction yielded coumarin (110 mg, R_F 0.43) which was recrystallised from hexane/ethanol, m.p. 69.5 – 70 °C (Lit.[14] m.p. 67 – 67.5 °C). The second fraction (770 mg) contained a mixture of coumarin, 6,7-dimethoxycoumarin (R_F 0.23), and 6,7-methylenedioxycoumarin (R_F 0.35). Evaporation of the solvent and recrystallisation of the residue from methanol yielded 6,7-dimethoxycoumarin (140 mg), m.p. 143 – 145 °C (Lit.[15] m.p. 144 – 146 °C). From the mother liquor 6,7-methylenedioxycoumarin (3 mg) was isolated by preparative TLC on silica gel using dichloromethane as eluent; m.p. 228 – 229.5 °C (Lit.[16] m.p. 231 – 232 °C) after recrystallisation from ethylacetate. The identities of coumarin, 6,7-dimethoxycoumarin and 6,7-methylenedioxycoumarin were further confirmed by NMR and MS.

Acknowledgements. We are indebted to Dr. Björn Lüning for his interest in this work. We thank the Swedish Natural Science Research Council for support.

1. Aasen, A., Behr, D. and Leander, K. *Acta Chem. Scand. B 29* (1975). *In press.*
2. Charaux, C. *Bull. Soc. Chim. Biol. 7* (1925) 1056.
3. Kosuge, T. *Arch. Biochem. Biophys. 95* (1961) 211.
4. Brown, S. *Phytochemistry 2* (1963) 137.
5. Nikonov, G. K., Vandyshev, V. V., Pimenov, M. G. and Shadrina, L. S. *Rast. Resur 6* (2) (1970) 177.
6. Herissey, H. and Delauney, P. *J. Pharm. Chim.* (7) *25* (1922) 248.
7. Wrigley, T. C. *Nature (London) 188* (1960) 1108.
8. Sawardeker, J. S., Sloneker, J. H. and Jeanes, A. R. *Anal. Chem. 37* (1965) 1602.
9. Chizhov, O. S., Golovkina, L. S. and Wulfsson, N. S. *Bull. Acad. Sci USSR 11* (1966) 1853.
10. Björndahl, H., Hellerqvist, C. G., Lindberg, B. and Svensson, S. *Angew. Chem. Int. Ed. Engl. 9* (1970) 610.
11. Bishop, E. O. and Richards, R. E. *Mol. Phys. 3* (1960) 114.
12. Hudson, C. S. *J. Amer. Chem. Soc. 31* (1909) 66.
13. Kahnt, G. *Naturwissenschaften 49* (1962) 207.
14. Perkin, W. H. *J. Chem. Soc. 21* (1868) 53.
15. Tiemann, F. and Will, W. *Ber. Deut. Chem. Ges. 15* (1882) 2072.
16. Späth, E., Bose, P. F. and Schläger, J. *Ber. Deut. Chem. Ges. 70* (1937) 702.

Received April 14, 1975.