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A LIGNAN GLUCOSIDE FROM *EUPHRASIA ROSTKOVIANA**

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Key Word Index—*Euphrasia rostkoviana*; Scrophulariaceae; structure elucidation; lignan glucoside; dihydrobenzofuran lignan; dehydrodiconiferyl alcohol-4-β-D-glucoside; ^{13}C NMR.

Abstract—A new lignan glucoside has been isolated from *Euphrasia rostkoviana*. Its structure was elucidated by spectroscopic means and by correlation with dehydrodiconiferyl alcohol to be dehydrodiconiferyl alcohol-4-β-D-glucoside.

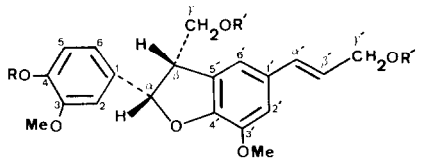
INTRODUCTION

During the isolation of iridoids from *Euphrasia rostkoviana* [2], we encountered a non-iridoid glycoside that has now been identified as a new lignan glucoside. In this paper we describe the isolation and structure determination of this compound, namely dehydrodiconiferyl alcohol-4-β-D-glucoside.

RESULTS AND DISCUSSION

The water-soluble part of *E. rostkoviana* was filtered through a column of neutral alumina and the water eluates were lyophilized and fractionated over a Si gel column. The appropriate eluates (TLC) were combined and further chromatography over C_{18} reversed phase column gave pure dehydrodiconiferyl alcohol-4-β-D-glucoside (**1**).

The molecular formula $C_{26}H_{32}O_{11}$ of **1** is based on field desorption–mass spectrometry (M^+, 520), the number of signals in the PND ^{13}C NMR and their multiplicities in the SFORD and integration of the ^{1}H NMR spectra. The ^{1}H NMR (360 MHz) spectrum showed five aromatic protons, the coupling constants of which gave the substitution pattern indicated in the formula, two olefinic protons (H-α′ and H-β′) which appeared as an AB part of an ABX_2 system, two OMe groups and an anomeric β-D-glucose proton. The large coupling constant ($J_{1'',2''} = 7.5$ Hz) proves the β-configuration of the anomeric centre. The region of the remaining glucoside protons had the expected splitting patterns and integral value. The ^{1}H NMR spectrum of the hexaacetate **2** was similarly related. From the above data, structure **1** (disregarding the site of glucosidation) can be proposed for the new compound.



1 R = β-D-Glucose; R′ = H
2 R = Tetraacetyl-β-D-glucose; R′ = Ac
3 R = R′ = H

Further confirmation of the structure of **1** as well as the site of glucosidation came from an analysis of its ^{13}C NMR spectrum which showed, apart from the six signals due to β-D-glucose, signals corresponding to 18 carbon atoms (excluding the signals due to 2 × $O\underline{C}H_3$), consistent with a lignan structure. The assignment of the signals based on the literature precedent [3] are given in Table 1. The δ values quoted for **3** from ref. [3] are for a solution in acetone-d_6/D_2O and are comparable with spectra recorded in CD_3OD. Out of 18 signals in **3**, 15 signals are virtually identical to signals observed in the ^{13}C NMR of **1**. An expected upfield shift of the signal of C-4 in **1**, due to the glucosidation, is not observed in respect to **3**. However, significant downfield shifts of the signals of the *ortho*- (C-3 and C-5) and *para*- (C-1) related carbons are observed, thereby locating [4, 5] the glucose moiety at C-4. Consequently, the compound **1** is dehydrodiconiferyl alcohol-4-β-D-glucoside.

Table 1. ^{13}C NMR data of dehydrodiconiferyl alcohol-4-β-D-glucoside (**1**) and its aglycone **3**

C atom	1*	3†	C atom	1*	3†
1	137.5	134.3	1′	129.7	130.4
2	110.9	110.7	2′	111.7	111.8
3	150.4	148.6	3′	145.0	145.1
3-OMe	56.4	‡	3′-OMe	56.4	‡
4	147.1	147.3	4′	148.7	148.9
5	117.5	115.8	5′	132.2	132.0
6	119.5	119.5	6′	116.1	116.2
α	88.4	88.3	α′	131.6	130.9
β	54.7	54.6	β′	127.3	128.0
γ	64.5	64.6	γ′	63.5	63.3
1″	102.2	—	4″	70.8	—
2″	74.4	—	5″	77.3	—
3″	77.6	—	6″	62.1	—

*Run at 25.2 MHz in CD_3OD with TMS as an internal standard.

†Data from ref. [3], in Me_2CO-d_6–D_2O (9:1).

‡Not mentioned in lit. [3].

*Part 2 in the series "Glycosides of *Euphrasia* species". For Part 1 see ref. [1].

†Part of a Ph.D. thesis to be submitted to the ETH, Zürich.

Compound **1** has not been reported before in nature although its aglycone **3** was known long ago [6]. Also, this is the first demonstration of the occurrence of a lignan in the genus *Euphrasia*.

EXPERIMENTAL

The general methods are the same as reported in ref. [7].

Extraction. Dried and milled whole plant (1 kg) of *E. rostkoviana* Hayne, available commercially from Siegfried AG (Lot No. 19279), Zofingen, Switzerland, was extracted with MeOH at 40° (4 × 5 l.). After concn of the combined extracts *in vacuo*, H_2O (1.5 l.) was added and the H_2O-insoluble material removed by filtration through Celite. The filtrate was extracted with petrol (60–80°, 4 × 1 l.) and the petrol-soluble part rejected. The aq. layer was concd (200 ml) and the aq. concn filtered through a prewashed (H_2O) neutral Al_2O_3 (500 g) column eluting with H_2O. The aq. eluate was concd and lyophilized to give the crude glycoside fraction (45 g). A portion of the residue (25 g) was chromatographed over Si gel (400 g) eluting with CH_2Cl_2–MeOH–H_2O, 40:10:1 (3 l.), 70:30:3 (3 l.), 14:10:1 (2 l.) and five fractions A–E were collected.

Isolation of dehydrodiconiferyl alcohol-4-β-D-glucoside (**1**). Fraction B (1.8 g) was further chromatographed over Si gel (100 g) eluting with EtOAc–*n*-PrOH–H_2O (4:2:7, upper layer) and two fractions B_1 and B_2 were collected. Low pressure liquid chromatography (reversed phase C_{18} with MeOH–H_2O, 1:3) of B_1 (200 mg) gave pure **1** (*ca.* 0.013%), $[\alpha]_D^{20}$ −71.2° (*c* = 0.56, MeOH). UV λ_{max}^{MeOH} nm: 274 (log ε = 4.04) and 217 (log ε = 4.35). IR ν_{max} cm^{-1}: 3380 (*br* OH), 1600, 1500, 1460 (aromatic ring). ^{1}H NMR (360 MHz, DMSO-d_6): δ 7.07 (1 H, *d*, *J* = 8.5 Hz, H-5), 6.96 (1 H, *d*, *J* = ~2 Hz, H-2′), 6.93 (2 H, *J* = ~2 Hz, H-2/6′), 6.84 (1 H, *dd*, *J* = 8.5/2 Hz, H-6), 6.47 (1 H, *d*, *J* = 16 Hz, H-α′), 6.23 (1 H, *dt*, *J* = 16/5 Hz, H-β′), 5.51 (1 H, *d*, *J* = 6.5 Hz, H-α) 4.88 (1 H, *d*, *J* = 7.5 Hz, H-1″), 3.82 (3 H, *s*, 3-OMe), 3.75 (3 H, *s*, 3′-OMe), ~3.75 (1 H, partly merged with the 3′-OMe signal, H-β), 3.65 (2 H, *m*, 2H-γ′), 3.44 (2J, *dd*, *J* = 6/6 Hz, 2H-γ); ^{13}C NMR (Table 1). EIMS 70 eV *m/z* (rel. int.): 520 [M]$^+$ (1.6), 358 [M − 163]$^+$ with H transfer (17), 342 [M − 179]$^+$ (28), 340 [M − (163 + H_2O)]$^+$ (28), 324 [M − (179 + H_2O)]$^+$ (56), 312 [M$^+$ − (179 + 30)]$^+$ (28), 151 (11), 137 (28), 115 (17), 85 (22), 73 (89), 60 (100), 43 (83), 30 (33), 29 (89), 18 (39).

Dehydrodiconiferyl alcohol-4-β-D-glucoside-hexaacetate (**2**). Acetylation of **1** with Ac_2O–pyridine at room temp for 18 hr followed by usual work-up gave the hexaacetate **2** as an amorphous powder, $[\alpha]_D^{20}$ −2° (*c* = 0.87, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 100 MHz): δ 7.06 (1 H, *d*, *J* = 8 Hz, H-5), 6.96–6.82 (4 H, H-2, 2′, 5,6), 6.58 (1 H, *d*, *J* = 16 Hz, H-α′), 6.12 (1 H, *dt*, *J* = 16/6 Hz, H-β′), 5.48 (1 H, *d*, *J* = 7 Hz, H-α), 4.69 (2 H, *d*, *J* = 6 Hz, H-γ′), 4.4–4.14 (*m*, 2 H-γ), 3.90 (3 H, *s*, 3-OMe), 3.77 (3 H, *s*, 3′-OMe), ~3.74 (1 H, *m*, partly merged with the 3′-OMe signal, H-β), 2.12–1.96 (18 H, 6 × OAc).

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