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HYGROPHILOSIDE, AN IRIDOID GLUCOSIDE FROM *HYGROPHILA DIFFORMIS* (ACANTHACEAE)

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Key Word Index—*Hygrophila difformis*; Acanthaceae; iridoid glucoside; hygrophiloside; 'Cardanthera-Pseudoindican'.

Abstract—A new iridoid glucoside, hygrophiloside, has been isolated from *Hygrophila difformis*. Hygrophiloside is apparently identical to the so-called 'Cardanthera-Pseudoindican'. Its structure has been established by spectroscopic means and by reduction to isoaucubin.

INTRODUCTION

Despite the fact that iridoids are very common in Scrophulariaceae and other related families, iridoid glucosides have been detected in only a few species of the Acanthaceae [1]. Thus shanzhiside methyl ester, barlerin (8-*O*-acetylshanzhiside methyl ester) and acetyl barlerin (6,8-di-*O*-acetylshanzhiside methyl ester) are known from *Barleria prionitis* [2, 3], and catalpol from *Mackaya bella* [1]. In a paper discussing the systematic distribution of iridoids [4], however, a supposed iridoid, 'Cardanthera-Pseudoindican' was detected in leaves of *Hygrophila difformis* (L. fil.) Blume (= *Cardanthera triflora* Buch.-Ham.). *H. difformis* is a commonly used tropical freshwater aquarium plant, and we have now isolated and determined the structure of the iridoid glucoside, for which we propose the name hygrophiloside.

RESULTS AND DISCUSSION

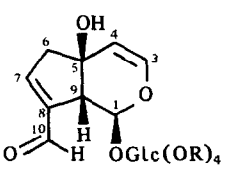
Reversed-phase chromatography of an aqueous extract from whole plants of *H. difformis* gave a single iridoid glucoside, hygrophiloside (**1**). Its ^{1}H NMR spectrum showed the presence of an aldehydic proton at δ9.70 (*s*) and of a vinylic proton at 7.32 (*br d*, J = 2 Hz), suggesting the conjugated system CH=C–CHO. A vinyl ether system –CH=CH–O, devoid of additional coupling, was evident from signals at δ6.48 and 5.27 (J = 6.5 Hz). These features, together with signals at δ6.12 (*d*, J = 3 Hz) and at δ3.05, the latter a multiplet integrating for two protons, were all in keeping with the structure **1**. The ^{13}C NMR spectrum of hygrophiloside exhibited signals corresponding to 15 carbon atoms, six of which could be attributed to a β-glucopyranosyl moiety, while the remaining nine were in good accordance with the proposed structure of the aglucone. An alternative attachment of the aldehyde group to C-4 could be excluded because of the doublet nature of the signal at δ109.5, ascribable to C-4.

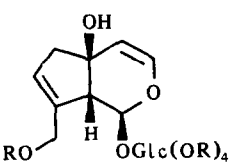
The glucoside was rather unstable and turned yellow on standing or heating and degradation products became visible in the NMR spectra. Acetylation, however, provided a stable crystalline tetra-acetate (**1a**) the spectral data of which (see Experimental) was in agreement with the proposed structure. A chemical proof of structure **1** was provided by sodium borohydride reduction to give, after acetylation, isoaucubin penta-acetate (**2a**) of known absolute configuration [5], identical to an authentic sample.

EXPERIMENTAL

Microanalyses were performed at NOVO Microanalytical Laboratory, Bagsværd, Denmark. Mps are corr. *H. difformis* was grown at 21° in an aquarium in which daylight was supplemented by a 'Gro-lux' fluorescent tube. A voucher (IOK-16/83) has been deposited at The Botanical Museum, Copenhagen.

Isolation of hygrophiloside from Hygrophila difformis. Frozen plants (335 g) were homogenized in EtOH (1.5 l.) The filtrate was taken to dryness and partitioned in H_2O–Et_2O. The aq. fraction was passed through a column of neutral Al_2O_3 (300 g) and the column washed with H_2O (750 ml). Evaporation gave 2.2 g of an extract which was applied to a reversed-phase silica gel column [Merck Lobar RP-8 (C)] and eluted with H_2O–MeOH (4:1), 22 ml/min, UV detection. The first fraction consisted of carbohydrates while the second was almost pure hygrophiloside (**1**, 180 mg, 0.05%). Upon standing, **1** quickly turned yellow and, after a few days, impurities were evident from the ^{1}H NMR spectrum. Consequently, the compound was characterized solely by NMR. ^{1}H NMR (90 MHz, D_2O): δ9.70 (*s*, CHO-10), 7.32 (*br d*, $J_{7,9}$ = 2 Hz, H-7), 6.48 (*d*, $J_{3,4}$ = 6.5 Hz, H-3), 6.12 (*d*, $J_{1,9}$ = 3 Hz, H-1), 5.27 (*d*, $J_{3,4}$ = 6.5 Hz, H-4), *ca* 3.5 (obscured, H-9), 3.05 (*m*, CH_2-6); ^{13}C NMR (22.6 MHz, D_2O): δ193.9 (*d*, C-10), 157.5 (*d*, C-7), 142.9 (*s*, C-8), 141.1 (*d*, C-3), 109.5 (*d*, C-4), 93.4 (*d*, C-1), 74.5 (*s*, C-5), 53.0 (*d*, C-9), 46.1 (*t*, C-6), 99 2, 73.3, 76.2, 70.5, 77.1, 61.5 (C-1–C-6 in the β-glucopyranosyl moiety).





1 R = H
1a R = Ac

2 R = H
2a R = Ac

Hygrophiloside tetra-acetate (**1a**). Prepared by acetylation of **1** (Ac_2O, pyridine, room temp., 2 hr). Crystallization from EtOH gave pure **1a**, mp 148–148.5°; $[\alpha]_D^{20} - 69°$ ($CHCl_3$; *c* 0.9); ^1H NMR (90 MHz, $CDCl_3$): δ9.60 (*s*, CHO-10), 6.78 (*m*, H-7), 6.23 (*d*, *J* = 2 Hz, H-1), 6.12 (*d*, *J* = 6.5 Hz, H-3), 4.92 (*d*, *J* = 6.5 Hz, H-4), 3.39 (*m*, H-9), 3.12 (*s*, OH), 2.91 and 2.68 (*br* AB-system, *J* = 19 Hz, CH_2-6), 2.11, 2.03, 2.00 and 1.98 (4 × OAc); ^{13}C NMR (22.6 MHz, $CDCl_3$): δ189.3 (C-10), 152.3 (C-7), 142.9 (C-8), 139.4 (C-3), 110.0 (C-4), 91.4 (C-1),73.1 (C-5), 52.7 (C-9), 46.0 (C-6), 96.0, 71.0, 71.8, 68.2, 71.8, 61.5 (C-1–C-6 in the β-glucopyranosyl moiety). (Found: C, 52.7; H, 5.5. $C_{23}H_{28}O_{13} \cdot 1/2\ H_2O$ requires: C, 53.0; H, 5.6%.)

Conversion to isoaucubin penta-acetate (**2a**). To a stirred soln of **1** (90 mg) in MeOH (5 ml) was added $NaBH_4$ (60 mg). After 45 min the mixture was taken to dryness and the residue acetylated as above. Work-up gave, after prep. TLC, **2a** (78 mg) as crystals from EtOH, mp and mmp 121–122°; $[\alpha]_D^{20} - 91°$ ($CHCl_3$; *c* 0.7), lit. [5] mp 125°; $[\alpha]_D^{28} - 46°$ (EtOH; *c* 0.95); an authentic sample, kindly supplied by Dr. Endo, exhibited the rotation $[\alpha]_D^{20} - 90°$ ($CHCl_3$; *c* 0.2).

The ^1H and ^{13}C NMR spectra were as reported [5].

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A NOR-SESQUITERPENE-γ-LACTONE FOUND IN *CREPIS PYGMAEA*

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Key Word Index—*Crepis pygmaea*; Compositae; sesquiterpenoid lactones; 1,2–4,5-tetrahydro-11-nor-11-hydroxy-$\Delta^{7,11}$-santonin.

Abstract—The isolation and structural elucidation of a novel nor-sesquiterpene-γ-lactone are reported.

INTRODUCTION

We have recently reported the isolation and structural elucidation of an unusual nor-sesquiterpene-γ-lactone (**1**) found in *Crepis pygmaea* L.‡ [1], whose structure has been definitively confirmed through synthesis [2]. During the isolation of **1** from the chloroform extract of the whole plant, a number of minor by-products were observed.

The present report describes the characterization of one of these products as the novel nor-sesquiterpenoid, 1,2–4,5-tetrahydro-11-nor-11-hydroxy-$\Delta^{7,11}$-santonin (**2**) (or its mirror image).

‡Plant material was collected in July–August on Vettore mountain, Umbria, Italy. A specimen (voucher Nos. 2115/01) has been deposited at The University of Perugia, Perugia, Italy.

RESULTS AND DISCUSSION

Compound **2** was obtained by chromatographic fractionation of the chloroform extract of *C. pygmaea* as previously described [1]. In particular, repeated fractionation on silica gel (Kieselgel 60 Merck) columns of the crude fraction containing **1** [1] yielded, besides **1**, chromatographically pure **2** (80 mg), which crystallized from ether as white cubes.

Compound **2**, mp 177–179°; R_f (TLC) 0.39 (pre-coated Merck plates, *n*-hexane–CH_2Cl_2–*i*-PrOH, 34:6:10), $[\alpha]_D$ +25.7° ($CHCl_3$; *c* 2.9); MS *m/z* 250 $[M]^+$; UV λ_{max}^{EtOH} nm (ε): 237 (8420); molecular formula $C_{14}H_{18}O_4$ (Found: C, 66.92; H, 7.32. $C_{14}H_{18}O_4$ requires: C, 67.18; H, 7.25%). The IR spectrum ($\nu_{max}^{CHCl_3}$ cm^{-1}: 3520, 1755 and 1710) suggested the presence of an α,β-unsaturated γ-lactone containing a cyclohexanone ring and an additional alcoholic function. The ^1H NMR spectrum