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STRUCTURE AND CONFIGURATION OF UNEDIDE, AN IRIDOID GLUCOSIDE FROM *ARBUTUS UNEDO*

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Key Word Index—*Arbutus unedo*; Ericaceae; iridoid glucosides; unedide; 6,7-dihydro-6β-hydroxymonotropein.

Abstract—Unedide, a novel iridoid glucoside isolated from *Arbutus unedo* (Ericaceae), has been established to be 6,7-dihydro-6β-hydroxymonotropein by detailed analysis of ^1H and ^{13}C NMR spectral data.

INTRODUCTION

Earlier investigations on the iridoid components of *Arbutus unedo* (Ericaceae) led to the isolation of unedoside (**1**) [1, 2], the major iridoid component, and monotropein (**2**) [3]. Careful re-examination of the ethanolic extract of leaves of *Arbutus unedo* collected in winter showed the presence of at least three other iridoids (R_f 0.17, 0.14, 0.10). In this report we describe the structure of the compound with R_f 0.14 which was present in small amounts and named unedide (**3**) by us.

RESULTS AND DISCUSSION

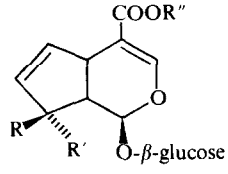
Compound **3** is an amorphous solid with acidic properties analogous to those of **2**, molecular formula $C_{16}H_{24}O_{12}$, $[\alpha]_D$ −83° and it gave a grey-violet reaction with vanillin reagent. The UV absorption (232 nm, log ε = 3.8) was characteristic of a conjugated enol–ether system and the IR spectrum showed weak bands at 1700 (C=O) and 1650 (C=C) cm^{-1}.

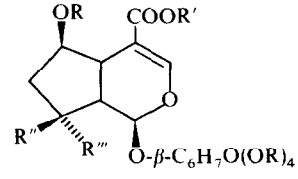
Enzymatic hydrolysis with β-glucosidase followed by isolation of glucose confirmed that **3** was a β-D-glucopyranoside. The ^1H NMR spectrum of **3** also showed the presence of a doublet (δ 4.80, J = 7.5 Hz), characteristic for the anomeric proton of a β-glucopyranose. Furthermore, this spectrum was somewhat similar to that of **2** (see Experimental) except for: (1) the lack of the doublet of doublets at δ 6.21 and 5.68 of the olefinic H-6 and H-7 protons (AB part of an ABX system where X = H-5, absorbing between δ 3.6 and 3.3 as a multiplet masked by glucose signals), (2) the presence at higher field of a pattern of signals easily recognizable as the ABX part of a more complex spin system. The AB part (2H-7), appeared as an eight-line multiplet centred at δ 1.93 and the X part (H-6) as a narrow multiplet (six-line multiplet in 300 Hz expanded scale) centred at δ 4.37 whose multiplicity was clearly due to further coupling with the vicinal H-5.

These assignments were checked by double resonance experiments. The irradiation of the multiplet at δ 4.37 due to the oxymethine H-6 proton (X part) simplified the eight-line multiplet centred at δ 1.93 (AB part) into a double doublet due to a simple AB system with a value of the coupling constant (J_{AB} = 13.3 Hz) typical of geminal protons. During the same irradiation, the broad double doublet centred at δ 2.90 gave rise to a doublet ($J_{5,9}$ = 9.7 Hz) with a residual small splitting caused by allylic coupling with H-3 ($J_{3,5} \simeq$ 1 Hz) and therefore it must be attributed to H-5, which was further coupled with H-9. The resonance of H-5 was clearly seen to be shielded with respect to the corresponding allylic H-5 of **2**.

The possibility of an opposite arrangement (OH-7, 2 H-6) may be easily eliminated if one considers that in such a case the signal multiplicity of the methylene protons would be more complex owing to the further vicinal coupling with the H-5, while the X part would be a simple double of doublets.

The small value of the coupling constant $J_{5,6}$ (3.3 Hz) did not provide useful information on the C-6 stereochemistry owing to the ambiguous data reported





2 R = CH_2OH, R′ = OH, R″ = H
6 R = OH, R′ = CH_2OH, R″ = Me

3 R = R′ = H, R″ = CH_2OH, R‴ = OH
4 R = Ac, R′ = H, R″ = CH_2OAc, R‴ = OH
5 R = H, R′ = Me, R″ = CH_2OH, R‴ = OH
7 R = H, R′ = Me, R″ = OH, R‴ = Me

for proton–proton coupling constants in a saturated five-membered ring [4]: e.g. $J^{cis}_{5,6} = 2.0$ Hz and $J^{trans}_{5,6} = 6.0$ Hz respectively in the C-6 epimeric pair ajugol (αOH-6) [5] and mioporoside (βOH-6) [6]. Moreover, in lamiridoside (βOH-6) $J^{trans}_{5,6} \leq 1$ Hz [7].

The acetylation of **3** under mild conditions afforded the hexaacetate **4** showing in the ^{1}H NMR spectrum the expected deshielding values ($\Delta\delta = 1.05$ and 0.5 respectively) for H-6 and 2H-10. The unchanged value of the H-9 resonance indicated that the OH-8 had not been acetylated as confirmed by the OH absorption in the IR spectrum. The presence of a carboxyl group at C-4 which was responsible for the acidic properties of **3** was proved by the preparation of the methyl ester **5** (COOMe at δ 3.80).

As far as the configuration of **3** is concerned the stereochemistry of the centres C-1, C-6, C-8 and C-9 was established by ^{13}C NMR spectroscopy. The comparison of the ^{13}C NMR spectra of **2** and **3** with that of gardenoside **6** (Table 1) established the identity in **2** and **3** of the stereochemistry of the quaternary C-8 centre on the basis of the C-9 resonance value, which is a sensitive probe to establish the equatorial (α) or axial (β) configuration of the OH-8 [4, 8]. In fact, the C-9 resonance of **3** (44.56 ppm) was in full agreement with that of monotropein (**2**) (44.84 ppm) but, by contrast, was rather different from that (51.38 ppm) of **6** (8-epimonotropein methyl ester).

The shift difference observed for C-4 (γ-effect, ~3 ppm) in C-6 epimers, induced by the axial or equatorial orientation of OH-6 [9], has been utilized as a useful criterion to establish the stereochemistry of the C-6 centre of **3**. In fact, the close coincidence of the C-4 resonance values of shanziside methyl ester **7** [10] as the model compound (110.75 ppm) and of unedide methyl ester **5** (109.79 ppm) clearly indicates a β-configuration for the OH-6 of **3** (a mean value of ~107 ppm was to be expected for the α-epimer).

Indirect chemical support for the β-orientation of the OH-6 was provided, by analogy with the formation of brasoside tetraacetate and asperuloside pentaacetate during the acetylation of 6-α-hydroxy-7-deoxyloganic acid (**8**) [11] and 10-deacetylasperulosidic acid (**9**) [12], respectively, by the non-closure of a lactone ring between OH-6 and COOH-4 during the acetylation of **3**. In fact, inspection of Dreiding models showed that the stereochemical requirements for closure of the lactone ring are identical in **8** and **9**, and in the α-epimer at C-6 of **3**, despite the different degrees of saturation of their cyclopentane rings.

The stereochemistry of the C-1 and C-9 centres of **3** must be identical to that of **2** owing to the close coincidence of both the $J_{1,9}$ coupling constant values and of the ^{1}H and ^{13}C NMR chemical shifts. Thus unedide (**3**) has been assigned the structure and configuration 6,7-dihydro-6β-hydroxymonotropein.

EXPERIMENTAL

CC was on cellulose CF 11 (Whatman) or Si gel 70–230 mesh (Merck). For acidic compounds, Si gel 70–230 mesh (Merck) was treated with dil. HCl, then washed with hot H_2O to eliminate Cl^- ions, dried and activated at 120° for 8 hr. TLC used Si gel SIF_{254} (Erba) and cellulose (Merck) plates. Paper chromatograms (PC) were on Schleicher and Schüll No. 2043 b Mgl paper. Spray reagents: 2 N H_2SO_4, heating at 120° (Si gel plates), vanillin (1 g

Table 1. ^{13}C NMR chemical shifts of compounds **2**–**7***

	3 (D_2O)	**4** ($CDCl_3$)	**5** (D_2O)	**2**† (D_2O)	**6**† (D_2O)	**7**‡ (D_2O)
C-1	95.16 *d*	94.34 *d*	95.14 *d*	95.20 *d*	94.35 *d*	94.82 *d*
C-3	153.86 *d*	154.09 *d*	153.53 *d*	152.44 *d*	151.88 *d*	152.66 *d*
C-4	109.79 *s*	107.41 *s*	109.79 *s*	111.04 *s*	111.13 *s*	110.75 *s*
C-5	40.47 *d*	37.99 *d*	40.46 *d*	37.89 *d*	37.98 *d*	39.85 *d*
C-6	76.32 *d*	78.68 *d*	76.38 *d*	138.01 *d*	134.56 *d*	76.48 *d*
C-7	43.97 *t*	42.49 *t*	43.98 *t*	132.82 *d*	136.04 *d*	48.65 *t*
C-8	81.67 *s*	77.11 *s*	81.65 *s*	85.65 *s*	86.13 *s*	78.91 *s*
C-9	44.56 *d*	44.22 *d*	44.55 *d*	44.84 *d*	51.38 *d*	50.59 *d*
C-10	68.97 *t*	70.69 *t*	69.06 *t*	67.39 *t*	66.11 *t*	24.23 *q*
C-11	171.60 *s*	§	170.03 *s*	171.30 *s*	170.11 *s*	170.41 *s*
			52.66 *q*		52.74 *q*	52.76 *q*
C-1′	99.23	96.87	99.16	99.13	99.23	99.14
C-2′	73.37	71.18	73.37	73.53	73.53	73.52
C-3′	76.32	72.45	76.38	76.47	76.48	76.28
C-4′	70.34	68.26	70.33	70.42	70.49	70.51
C-5′	77.07	72.45	77.07	77.15	77.15	77.16
C-6′	61.45	61.79	61.45	61.54	61.63	61.63

* The spectra were recorded at 20 MHz. Chemical shifts in ppm from TMS (dioxane (67.4 ppm) was used as internal standard).

† In ref. [9] this spectrum was registered in CD_3OD solution.

‡ From ref. [10].

§ This carbon absorbs in the range of C=O acetyls (173.03, 171.96, 171.66, 171.46, 171.06 ppm). Additional signals of Me (21.17, 20.62, 20.33 ppm).

vanillin, 2 ml conc. HCl, 100 ml MeOH) and 3,5-dinitrosalicylic acid (0.5 g 3,5-dinitrosalicylic acid, 4 g NaOH, 100 ml H_2O), heating at 100° (cellulose plates and PC). All evapns of volatile material were performed under red. pres. ^{1}H NMR spectra were recorded at 90 MHz. Chemical shifts are given in δ values and coupling constants in Hz. HDO was used as internal standard at 4.70 ppm for D_2O solns and TMS for $CDCl_3$.

Isolation of iridoid fraction. A reference specimen of *Arbutus unedo* has been deposited at the Botanical Institute Herbarium (University of Rome). Aerial part (7 kg, fr.wt.) of *Arbutus unedo* was harvested in the Botanical Garden of the University of Rome in December 1979 and extracted twice, at room temp., with 90% EtOH (12 l. each) for 2 days. A PC eluted with *n*-BuOH–HOAc–H_2O (63:10:27) and visualized with vanillin showed 5 spots with R_f values: 0.27 (pink, unedoside, **1**), 0.25 (violet, monotropein, **2**), 0.17 (brown, **A**), 0.14 (grey-violet, unedide, **3**), 0.10 (violet, **B**). The ethanolic extracts were concd to an aq. suspension which was washed with petrol, bp 40–70° (3 l.). Decolorizing charcoal (1.2 kg) was added and the suspension stratified on a Gooch funnel (20 cm diam.) containing a layer of Si gel (100 g). Mono- and disaccharides were removed with H_2O (10 l.) and 5% EtOH (7 l.). **1**, **2**, **A** and **B** (fractions Ia 10 g and Ib 40 g) with 15% EtOH (25 l.); **1**, **2**, **3** and **B** (fractions IIa 13 g and IIb 6 g) with 30% EtOH (20 l.); **2** and **B** (fraction III, 34 g) with 50% EtOH (8 l.).

Isolation of unedide (**3**). Fraction IIa (13 g), chromatographed on cellulose (150 g) with *n*-BuOH satd with H_2O, afforded in succession: (a) **1** and **2** (2 g); (b) **2** and **3** (0.4 g); (c) **2**, **3** and **B** (0.2 g); (d) **3** and **B** (0.5 g) and (e) **B** (0.2 g). Fraction (d), rechromatographed on 'acidic' Si gel in CH_2Cl_2–MeOH–H_2O (CMW) (70:30:3), afforded (f) impure **3** (20 mg) and (g) **3** and **B** (200 mg). Fraction (g) rechromatographed on 'acidic' Si gel in CMW (70:30:5) gave pure **3** (85 mg). $[\alpha]_D^{25}$ −83° (MeOH, *c* 4.9); UV λ_{max} nm (log ε): 232 (3.8); IR ν_{max}^{nujol} cm^{-1}: 3300, 1700, 1650. (Found: C, 46.90; H, 5.98. $C_{15}H_{24}O_{12}$ requires: C, 47.06; H, 5.92%). ^{1}H NMR (D_2O): δ 7.54 (1 H, *br. s*, $J_{3,5}$ = 1.0, H-3), 5.63 (1 H, *d*, $J_{1,9}$ = 2.3, H-1), 4.37 (1 H, *m*, H-6), 3.63 (2 H, *s*, 2 H-10), 2.90 (1 H, *br. dd*, $J_{5,6}$ = 3.3, $J_{5,9}$ = 9.7, H-5), 2.66 (1 H, *dd*, $J_{1,9}$ = 2.3, $J_{5,9}$ = 9.7, H-9), 1.93 (2 H, *o*, J_{AB} = 13.3, 2 H-7).

Monotropein (**2**). ^{1}H NMR (these data are from a new 100 MHz spectrum we determined to complete the assignments previously reported [12] (D_2O): δ 7.40 (1 H, *br. s*, $J_{3,5}$ = 1.0, H-3), 6.21 (1 H, *dd*, $J_{5,6}$ = 2.8, $J_{6,7}$ = 5.7, H-6), 5.68 (1 H, *dd*, $J_{5,7}$ = 1.7, $J_{6,7}$ = 5.7, H-7), 5.60 (1 H, *d*, $J_{1,9}$ = 2.0, H-1), 3.63 (2 H, *br. s*, 2 H-10), 3.6–3.3 (1 H, *m*, H-5), 2.66 (1 H, *dd*, $J_{1,9}$ = 2.0, $J_{5,9}$ = 8.0, H-9).

Hexaacetylunedide (**4**). Compound **3** (7 mg) was treated with dry pyridine (0.3 ml) and Ac_2O (0.6 ml) for 2 hr at room temp. MeOH was added, after 15 min the soln was evapd and the residue, chromatographed on 'acidic' Si gel in Et_2O–EtOAc (7:3), gave the hexaacetate **4** (30 mg). ^{1}H NMR ($CDCl_3$): 7.56 (1 H, *s*, H-3), 5.53 (1 H, *d*, $J_{1,9}$ = 2.5, H-1), 5.42 (1 H, *m*, H-6), 4.13 (2 H, *br. s*, 2 H-10), 3.00 (1 H, *br. dd*, $J_{5,9}$ = 9.0, H-5), 2.58 (1 H, *dd*, $J_{1,9}$ = 2.5, $J_{5,9}$ = 9.0, H-9), 1.8–2.1 (2 H-7 hidden by acetyl signals).

Methyl ester (**5**). Compound **3** (85 mg), dissolved in MeOH (5 ml), was methylated with CH_2N_2 in Et_2O at 0° and the soln taken to dryness. The residue, chromatographed on Si gel with $CHCl_3$–MeOH (4:1), afforded pure **5** (30 mg). ^{1}H NMR (D_2O): δ 7.52 (1 H, *br. s*, H-3), 5.66 (1 H, *d*, $J_{1,9}$ = 2.3, H-1), 4,36 (1 H, *m*, H-6), 3.80 (3 H, *s*, COOMe), 3.66 (2 H, *s*, 2 H-10), 2.98 (1 H, *br. d*, $J_{5,9}$ = 9.7, H-5), 2.70 (1 H, *dd*, $J_{1,9}$ = 2.3, $J_{5,9}$ = 9.7, H-9), 1.93 (2 H, *o*, 2 H-7).

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