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A ^{13}C-NMR STUDY OF THE STRUCTURE OF AN ACYL-LINARIN FROM *VALERIANA WALLICHII*

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Key Word Index—*Valeriana wallichii*; Valerianaceae; flavone acyl-glycoside; linarin-*O*-2-methylbutyrate.

Abstract—An acylated acacetin-7-*O*-rutinoside of *Valeriana wallichii* has been shown to be a mixture of its 2‴-*O*- and 3‴-*O*-2-methylbutyryl esters by ^{13}C-NMR, GLC, and MS.

INTRODUCTION

From the rhizomes of a didrovaltratum race of *Valeriana wallachii* DC., Thies isolated a compound to which the structure of linarin-isovalerate was assigned [1]. The isovalerate moiety was indicated tentatively as being esterified with the 3- or 4-hydroxyl group of the L-rhamnose part of linarin (acacetin-7-*O*-β-rutinoside). As part of our program on the structure elucidation and synthesis of naturally occurring acylated phenolic glycosides, we undertook a reinvestigation of the structure of this compound in order to localize the exact position of the acyl residue.

RESULTS AND DISCUSSIONS

The purified glycoside concentrate proved on TLC to be a mixture of two components—(R_f 0.63) and (R_f 0.54) which were named glycosides A and B respectively. Their separation was achieved by preparative TLC on Si gel. The major component—glycoside A—showed the same IR spectrum as the one published by Thies for the compound isolated by him [1]. On deacylation it yielded linarin (R_f 0.36) [2] as the only flavonoid. Transesterification of glycoside A with BF_3–butanol reagent [3] and GLC analysis of the product indicated that the acyl fragment was not isovalerate but the isomeric 2-methylbutyrate. The retention time of the butyl esters of 2-methylbutyric, isovaleric, *n*-valeric acids were 9.3 min, 10 min, and 13.5 min respectively. A comparison of the ^{1}H-NMR spectrum of glycoside A with that of 2-methylbutyric acid in the region upfield of 2.5 ppm showed similarities. The noise decoupled ^{13}C-NMR spectrum of glycoside A, upfield of 39 ppm, showed four signals corresponding to C-3 (26.5 ppm), C-4 (11.4 ppm), and C-5 (16.4 ppm) of the 2-methylbutyrate moiety in addition to the C-6‴ (17.8 ppm) of the rhamnose part. The C-2 of the acyl residue calculated to be at 39.9 ppm [4] was obscured by the solvent (DMSO-d_6: MeOH-d_4) signals. In addition, the signal for the carbonyl carbon was *ca* 2.0 ppm downfield as compared with those of other saturated short chain fatty acid esters. This was indicative of α-branching in the acyl residue.

Permethylation of glycoside A and subsequent MS, along well established lines [5], showed principal fragments at *m/e* 516, 298, 259, 157 and 85. The fragment at *m/e* 259 is the base peak and must be ascribed to di-*O*-methylrhamnose esterified with a saturated C_5-acid. This indicated that the acyl residue was attached to the rhamnose moiety in linarin. The position of the acyl substituent could be determined by a comparison of the ^{13}C-NMR spectra of glycoside A and its desacyl derivative linarin. The assignment of the signals for the sugar carbon atoms were achieved by a consideration of the corresponding spectra of apigenin-7-*O*-β-D-glucoside and hesperetin-7-*O*-β-rutinoside (hesperidin). The signals of the carbon atoms of the aglycone moieties could be easily recognized on the basis of previous work [6, 7]. Analogous with the results obtained on 7-*O*-β-D-glucosyloxycoumarin [8] the glycosidation of the 7-hydroxyl in the above flavonoid glycosides results in a slight upfield shift of the C-7 signal and downfield shift of the C-6 and C-8 signals. The changes are of the order of about 1 ppm only. The only other signal to be affected was that of C-10 which was shifted in each case by *ca* 1.5 to 1.9 ppm. The assignments for the sugar carbon atoms, as indicated in the table, was arrived at by known methods [9, 10]. The presence of two signals at 175.3 ppm and 175.8 ppm assignable to ester carbonyl carbon atoms, and that of 12 signals for sugar carbons in the region 65.0 ppm to 77.0 ppm in the ^{13}C-NMR spectrum of glycoside A indicated that it must be a mixture of two acylated linarins. Comparison of the signals for the glucose carbon atoms in linarin and the glycoside mixture A confirmed that the acyl group was not on the glucose moiety. The signal at 97.7 ppm can be assigned to either C-1″ (glucose) or C-1‴ (rhamnose) in a C-2″-or C-2‴-*O*-acyl-linarin. This is based on the well known fact [11, 12] that acylation of a sugar hydroxyl shifts the signal of the carbon atom bearing the hydroxyl by *ca* +2.0 ppm and those of the two contiguous carbon atoms by *ca* −2.0 ppm. Specific irradiation at the resonance frequency of H-1‴ (rhamnose) at 4.65 ppm did not change the multiplicity of the signal at 97.7 ppm in the ^{13}C-NMR spectrum of glycoside A thus confirming the assignment of this signal to C-1‴. This thus establishes that one site of acylation is C-2‴. That the second component of glycoside A mixture was a 3‴-*O*-acylated linarin was deduced

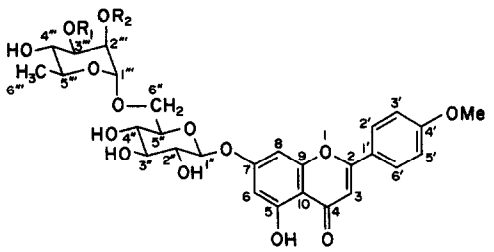
Table 1. ^{13}C-NMR spectra of flavonoid glycosides

Compound	Carbon resonances of sugar carbons (ppm from TMS)											
	$C_{1''}$	$C_{2''}$	$C_{3''}$	$C_{4''}$	$C_{5''}$	$C_{6''}$	$C_{1'''}$	$C_{2'''}$	$C_{3'''}$	$C_{4'''}$	$C_{5'''}$	$C_{6'''}$
glycoside A mixture												
2-*O*-acyl	101.1	74.0	76.6*	69.9	76.0*	66.4	97.8	73.4†	68.4	72.8†	69.0	17.8
3-*O*-acyl	101.1	74.0	76.6*	69.9	76.0*	66.4	100.3	69.2†	72.3	69.5	69.0†	17.8
apigenin-7-*O*-β-D-glucoside	100.2	73.3	77.4	69.8	76.6*	60.9						
hesperetin-7-*O*-β-rutinoside	101.3	73.7	77.0*	70.4	76.4*	66.7	100.3	71.5	71.0	73.0	69.0	17.8
acacetin-7-*O*-β-rutinoside	100.0	73.8	76.5*	69.9	76.0*	66.3	100.4	71.0	70.5	72.3	68.5	17.9

*† Assignments bearing the same superscript in any one spectrum may be reversed.

by the absence of signals at 71.0 ± 0.5 ppm for C-2‴ and C-3‴ and the appearance of the C-4‴ signal at 69.5 ppm. Since acyl migrations [13] between C-3 and C-2 of mannose and glucose derivatives have been observed, the *cis*-relationship of 2-OH and 3-OH in rhamnose as in the case of mannose, should also be favourable to such migrations. Hence it is not possible to decide with certainty whether both components of glycoside A are genuine and/or which of the two isomers is an artefact respectively. On the basis of the above results, glycoside A is a mixture of acacetin-7-*O*-[2‴-*O*- and 3‴-*O*-(2-methyl)-butyryl]-α-L-rhamnopyranosyl-β-D-glucopyranoside. The structure of glycoside B will form the topic of a subsequent publication.

This is the first report of the occurrence in nature of a flavonoid glycoside acylated with 2-methylbutyric acid. Since in most of the acylated flavonoid glycosides isolated till now, the position of the acyl substituent has not been definitively established, the use of ^{13}C-NMR spectroscopy in the future is an elegant method for the determination of such structures [14]. The possibility exists that some of the genuine acyl glycosides found in nature are present as mixtures but could not be identified due to lack of appropriate analytical techniques.



Glycoside A

R_1 = H, R_2 = E†(Me)CHCO

R_2 = H, R_1 = E†(Me)CHCO

Linarin $R_1 = R_2$ = H

* After completion of this manuscript our attention was drawn to a publication by Markham and Ternai [15]. Among others, the ^{13}C-NMR spectrum of hesperidin has been interpreted. The values and assignments are in general agreement with those reported by us in this communication.

EXPERIMENTAL

The ^{13}C-NMR spectra were run on a Bruker WP-60 and Varian XL-100 spectrometers in DMSO-d_6–MeOH-d_4 solution with TMS as internal standard. GLC analyses were performed on a Perkin–Elmer 900 gas chromatograph. SP-1000 (2.5% on Chromosorb GAW-DMCS, 80–100 mesh) was used as the stationary phase in glass columns (3.6 m × 2 mm) with Ar carrier 30 ml/min. Preparative TLC separations of the components of the glycoside concentrate [1] were effected on Si gel plates (Merck 0.25 mm) using EtOAc–MeOH–H_2O (200:33:27). MS of the permethyl ether was carried out on an AEI-MS 30 instrument.

Glycoside A. Mp 140–5° (Lit. [1] 138–40°) $[\alpha]_D^{25} = -91.3°$ (c = 0.6 MeOH) (Lit. [1] $[\alpha]_D^{24} = -80°$ in MeOH) R_f 0.63. ^{1}H-NMR (60 MHz, DMSO-d_6 + CF_3CO_2D) δ = 0.8–2.4 (12H, protons of the fatty acid residue and rhamnose C-C$\underline{H}_3$) 3.3–3.9 (12H, sugar protons and $\underline{CH}_3$O-), 4.65 (*s*, 1H, H-1‴ rhamnose) 4.9–5.2 (broad Wy_2 = 15 Hz, 2H, H81″ glucose and $\underline{CH}$-O-acyl), 6.5 (*d*, J = 3 Hz, 1H, H-6), 6.85 (*d*, J = 3 Hz, 1H, H-8), 6.9 (*s*, 1H, H-3), 7.15 (*d*, J = 9 Hz, 2H, H-3′ and H-5′), 8.1 (*d*, J = 9 Hz, 2H, H-2′ and H-6′).

Transesterification with BF_3–butanol reagent. The glycoside A (5 mg) and the BF_3–butanol (14%) reagent (2 ml) were heated (100°) for 10 min. After cooling, pentane (30 ml) and H_2O (30 ml) were added and vigourously shaken. The organic layer was separated and thoroughly extracted with H_2O (3 × 30 ml) and dried. The pentane soln was evaporated at atmos. pres. and the oily residue was dissolved in CH_2Cl_2 and subjected to GLC. Only the butyl ester of 2-methylbutyric acid was detected, R_t 9.3 min. The R_t for the butyl esters of isovaleric and valeric acids, prepared analogously, were 10.0 min and 13.5 min respectively.

Permethylation. The permethyl ether of glycoside A was prepared with CH_3I and NaH in DMF solution [6].

^{13}C-NMR spectra. Glycoside A (2‴-*O*-acyl- and 3‴-*O*-acyl-linarin) (15.08 MHz). 182.4 (C_4), 175.8 (−CO), 175.3 (−CO), 164.4 (C_7), 163.3 (C_2), 162.8 ($C_{4'}$), 161.5 (C_9), 157.5 (C_5), 128.7 ($C_{2'}$ and $C_{6'}$), 123.1 ($C_{1'}$), 114.9 ($C_{3'}$ and $C_{5'}$), 105.9 (C_{10}), 104.3 (C_3), 100.9 (C_6), 100.3 ($C_{1'''}$), 100.1 ($C_{1''}$), 97.7 ($C_{1'''}$), 95.0 (C_8), 76.6, 76.0, 74.0, 73.4, 72.8, 72.3, 69.9, 69.5, 69.2, 69.0, 68.4, 66.4 (sugar carbons cf Table 1), 55.6 (MeO), 26.5 (C_{3*}), 17.8 ($C_{6'''}$), 16.4 (C_{5*}), 11.4 (C_{4*}). Apigenin-7-*O*-β-D-glucoside (15.08 MHz) 182.3 (C_4), 164.6 (C_7), 163.3 (C_2), 161.9 (C_9), 161.3 ($C_{4'}$), 157.2 (C_5), 128.8 ($C_{2'}$ and $C_{6'}$), 121.1 ($C_{1'}$), 116.3 ($C_{3'}$ and $C_{5'}$), 105.6 (C_{10}), 103.3 (C_3), 100.2 ($C_{1''}$), 99.7 (C_6), 95.1 (C_8), 77.4, 76.6, 73.3, 69.8, 60.9 (sugar carbons cf Table 1). Linarin (acacetin-7-*O*-β-rutinoside) (25.2 MHz) 182.4 (C_4), 164.4 (C_7), 163.4 (C_2), 162.9 ($C_{4'}$), 161.6 (C_9), 157.4 (C_5), 128.8 ($C_{2'}$ and $C_{6'}$), 123.1 ($C_{1'}$), 115.0 ($C_{3'}$ and $C_{5'}$), 105.8 (C_{10}), 104.1 (C_3), 100.8 (C_6), 100.4 ($C_{1''}$), 100.0 ($C_{1'''}$), 95.1 (C_8), 76.5, 75.9, 73.3, 72.3, 71.0, 70.5, 69.9, 68.4, 66.3 (sugar carbons cf Table 1), 55.7 (OMe), 17.8 ($C_{6'''}$). Hesperidin (hesperetin-7-*O*-β-rutinoside)* (15.08 MHz) 197.7

(C_4), 166.0 (C_7), 163.8 (C_5), 163.4 (C_9), 148.6 ($C_{4'}$), 147.0 ($C_{3'}$), 131.7 ($C_{1'}$), 118.6 ($C_{6'}$), 114.5 ($C_{5'}$), 112.3 ($C_{2'}$), 104.1 (C_{10}), 101.3 ($C_{1''}$), 100.3 ($C_{1'''}$), 97.1 (C_6), 96.3 (C_8), 77.0, 76.4, 73.7, 73.0, 71.5, 71.0, 70.4, 69.0, 66.7 (sugar carbons cf Table 1), 56.1 MeO), 18.0 ($C_{6'''}$).

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O''-RHAMNOSOLISOSWERTISIN FROM OATS (*AVENA SATIVA*)

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Key Word Index—*Avena sativa*; Gramineae; oats; *C*-glycosylflavonoid; O''-rhamnosyl-isoswertisin.

Previous studies of oats have shown the presence of avenacin in roots [1], avenacoside in leaves [2] and flavonoids in leaves [3,4]*. A methanolic extract of finely ground primary leaves of Shokan 1 seedlings subjected to Si gel column chromatography has now yielded a pale yellow pigment. *O*-rhamnosyl-*C*-β-D-glucopyranosylgenkwanin or (*O''*-rhamnosylisoswertisin, **1**).

Acidic hydrolysis of **1** gave rhamnose and an aglycone (**2**). The aglycone, **2** was identified as isoswertisin [5,6] from NMR, IR and MS [7] data and confirmed by direct comparison with an authentic sample. The NMR spectrum of the acetate of **1** exhibited two acetyls attached to aromatic ring at δ 2.35 and 2.42, indicating that rhamnose was attached to *C*-glucosyl moiety in **2**. Thus, **1** was identified as *O''*-rhamnosylisoswertisn, although the glycosyl linkage is not yet established.

EXPERIMENTAL

Extraction. Finely ground primary leaves (2.8 kg, fr. wt) of oat cultivar were extracted with 1.5 l. of MeOH. The MeOH soluble fraction was then evaporated to dryness. The resulting solid was successively extracted with 200 ml Et_2O and 200 ml MeOH. To the MeOH soln, 1.6 l. of Et_2O was added to form the Et_2O-insoluble ppt. (2.7 g). The ppt. was then chromatographed over Si gel column (3 × 45 cm) with $CHCl_3$–MeOH–H_2O (7:3:1), **1** separated and the crystallization from MeOH–$CHCl_3$ gave pale yellow powdery crystals (54 mg). Mp 199–204°, $[\alpha]_D^{18}$ − 108 (Py, *c* 0.64), (Found: C, 52.49; H, 5.92. $C_{28}H_{32}O_{14}.2.5\ H_2O$ requires: C, 52.75; H, 5.85%), UV λ_{max}^{EtOH} nm (log ε): 272 (4.18), 340 (4.20); + NaOMe: 260 (4.15), 270 (4.16), 404 (4.41): + NaOAc: no shift; + NaOAc + H_3BO_3: no shift: + $AlCl_3$: 280 (4.17), 306 (4.06), 340 (4.25), 392 (4.15): + $AlCl_3$ + HCl: 278 (4.16), 306 (4.06), 340 (4.25), 392 (4.11); IR ν_{max}^{KBr} cm^{-1}: 3400, 1660, 1600, 1450, 1050; NMR (90 MHz, DMSO-D_6 + D_2O); δ 8.04 (2H, *d*, *J* = 8.5 Hz, C-2', 6'), 6.92 (2H, *d*, *J* = 8.5 Hz, C-3', 5'), 6.82 (1H, *s*, C-6), 6.50 (1H, *s*, C-3), 5.8–3.0 (*m*), 3.92 (3H, *s*, OMe), 0.47 (3H, *d*, *J* = 6.8 Hz, rhamnose MeOH). NMR of the acetate of **1** (Mp 138–143°) (90 MHz, $CHCl_3$): δ 8.00 (2H, *d*, *J* = 9.0 Hz, C-2', 6'), 7.26 (2H, *d*, *J* = 9.0 Hz, C-3', 5'), 6.68 (1H, *s*, C-6), 6.60 (1H. *s*. C-3), 5.5–3.7 (*m*), 4.06 (3H, *s*, OMe), 2.42 (3H, *s*, aromatic acetyl), 2.35 (3H, *s*, aromatic acetyl), 2.1–1.8 (18H, 6 × sugar acetyl), 0.62 (3H, *d*, *J* = 6.8 Hz, rhamnose Me). Acid hydrolysis of **1** gave rhamnose and the aglycone **2**, mp shrinking at 200–230° and melting with decomp. at 290–293° (lit. [5]); MS (80 eV) *m/e* (rel. int.): 326 (11), 311 (10), 297 (100), 179 (30), 121 (9), 118 (5); IR ν_{max}^{KBr} cm^{-1}: 3350, 1650, 1580, 1100–1050; NMR (90 MHz, DMSO-d_6 + hexaduetroacetone): δ 8.03 (2H, *d*, *J* = 8.5 Hz, C-2', 6'), 6.95 (2H, *d*, *J* = 8.5 Hz, C-3', 5'), 6.63 (1H, *s*, C-6), 6.43 (1H, *s*, C-3), 4.87 (1H, *d*, *J* = 10 Hz, C-1''), 3.91 (3H, *s*, OMe), 4.2–3.0 (*m*) was identified as isoswertisin by direct comparison with NMR and IR of authentic sample and mmp (293°).

* The compound described by Weissenboeck as 'apigenin-7-methoxy(?)-8-*C*-β-rhamnosylglucosylglucoside' in *Aventa sativa* L, leaves ('gellhafer') was suggested to be *O''*-rhamnosylisoswertisin i.e. **1** (Prof. J. Chopin, personal communication).

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