Phytochemistry, Vol. 28, No. 1, pp. 247 249, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00
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CHALCONE GLUCOSIDES FROM *BIDENS PILOSA*

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(*Received* 19 *May* 1988)

Key Word Index—*Bidens pilosa*; Asteraceae; chalcones; okanin 4'-diglucoside; okanin 3',4'-diglucoside; okanin 3'-glucoside; okanin 4'-glucoside; okanin 4'-(6''-*O*-acetylglucoside).

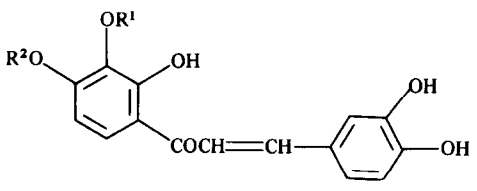
Abstract—Five okanin glucosides and diglucosides have been isolated from the flowers of *Bidens pilosa*. Their structures have been determined by means of UV, FD-MS, ^{1}H and ^{13}C NMR spectroscopy.

INTRODUCTION

In previous papers [1–3] we reported the isolation and structure elucidation of chalcone glucosides from the leaves of *Bidens pilosa* L. (Asteraceae, Heliantheae, Coreopsidinae). Most of them were acylated with *p*-coumaric- and/or acetic acid on the sugar moiety and were relatively non-polar. In the present contribution we are dealing with more polar compounds, okanin mono- and diglucosides, which we have isolated from the flowers of *Bidens pilosa*. Their structures have been determined by spectroscopic methods.

RESULTS AND DISCUSSION

All compounds were okanin glucosides: the ^{1}H NMR spectra exhibited okanin sets of aromatic signals, and acid hydrolysis on TLC yielded glucose. On TLC compound **1** had rather low R_f-values in all three solvent systems, suggesting that it is a diglucoside. The UV spectra after the addition of shift reagents showed that the hydroxyl groups in the 2', 3 and 4 positions were unsubstituted. The ^{1}H NMR and FD mass spectrum ([M + Na]$^+$ at 635) confirmed a diglucoside. The doublet for one anomeric proton appeared at 4.98 ppm, in a range typical of a flavonoid *O*-glucoside [4], whereas the anomeric H-atom of the second glucose moiety gave rise to the doublet at 4.41 ppm. The coupling constants of 7.3 and 7.7 Hz revealed β-configuration in both cases. Moreover, the protons at the 6''-position of one glucose molecule moved downfield to 4.20 and 3.83 ppm, respectively. These data suggest that the two sugar moieties are 1,6-linked, which was confirmed by the ^{13}C NMR spectra. The anomeric carbon of the first glucose in flavonoid-*O*-diglucosides usually resonates between 100 and 102.5 ppm, whereas C-1 of the terminal glucose resonates at *ca* 104 ppm [5]. In the ^{13}C NMR spectrum of **1** the signal for one anomeric carbon appeared at 102.43 ppm and the other at 104.69 ppm. The signal for C-6'' shifted downfield by 7.31 ppm as observed in the literature for β-linked diglucosides [6, 7]. The ^{13}C NMR spectral data for the aglycone were identical with those given for okanin 4'-glucoside [1]. Thus, **1** is okanin 4'-*O*-[β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside]. An okanin 4'-diglucoside was earlier isolated from *Coreopsis petrophiloides* [8], but no attempt was made then to establish the interglycosidic linkage.



1 R¹ = H, R² = gentiobiose
2 R¹ = R² = Glc
3 R¹ = R² = H
4 R¹ = Glc, R² = H
5 R¹ = H, R² = Glc
6 R¹ = H, R² = 6-acetyl Glc

On TLC compound **2** behaved similarly to **1**, suggesting that **2** is a diglucoside, as well. The UV spectra revealed free 2',3 and 4 hydroxyl groups. The ^{1}H NMR spectrum exhibited two glucose molecules which differed only little from each other. The coupling constant of the anomeric proton doublets was consistent with β-D-glucose. The [M + Na]$^+$ peak in the FD-mass spectrum at 635 confirmed an okanin diglucoside.

The position of the sugars could be determined by the ^{1}H NMR spectral data. The δ-values of the anomeric protons at 5.02 and 4.99 ppm suggested that both sugar molecules are linked separately to phenolic hydroxyl groups [9]. On glucosidation of okanin (**3**) at the 4'-OH, the signal for H-5' moves downfield by 0.38 ppm, whereas on glucosidation at 3'-OH the signal for H-6' shifts downfield by 0.25 ppm (see Table 1). In both cases the meta-related coupled protons are hardly affected. In the ^{1}H NMR spectrum for **2** the signals for both A-ring protons appeared at a lower field than the corresponding signals in okanin 3'- and -4'-glucosides, and there is a good agreement between the data measured for **2** and those calculated on the basis of glucosidation shifts (see Table 1). The signals for the sugar protons in the ^{1}H NMR spectrum of **2** could be attributed to the

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Table 1. Effect of glucosidation on the ^{1}H NMR chemical shift values of the A-ring protons in okanin glucosides, ppm

	Okanin	Ok-4'gluc.	Ok-3'gluc.	Ok-3',4' digluc. (calculated)	2
H-5'	6.46	6.84	6.50	6.88	6.93
H-6'	7.53	7.62	7.78	7.87	7.89

respective protons of **4** and **5** (see Table 2). Thus **2** is okanin 3',4'-di-*O*-β-D-glucoside, a new compound.

Okanin 3'-glucoside (**4**) and okanin 4'-glucoside (**5**) could be easily separated by DCCC and were identified with their UV and NMR spectral data [1]. Compound **6** had a faster migration on TLC than **4** and **5**. The ^{1}H NMR data agreed well with those for okanin 4'-glucoside. The signal at 2.08 ppm in the ^{1}H NMR spectrum exhibited an acetyl group attached to the sugar moiety [4]. Compared to okanin 4'-glucoside, the C-6'' protons were markedly deshielded by 0.52 and 0.55 ppm, accompanied by a smaller downfield shift of the H-5'' signal. Thus, the acetyl group must be linked to the C-6'' hydroxyl group [10] and compound **6** is okanin 4'-*O*-β-D-(6''-*O*-acetylglucoside) another new natural compound.

EXPERIMENTAL

Plant material. Flowers of *Bidens pilosa* were collected from plants cultivated in the botanical garden, Universität Marburg in Sept. 1987. Voucher specimen Nr. 72–880 is deposited there.

General procedures. TLC: SiO_2; solvent mixtures: I EtOAc–MeOH–H_2O (100:17:13) II $CHCl_3$–MeOH–H_2O (13:7:4, lower phase), III EtOAc–HCO_2H–H_2O (15:3:4). Prep. HPLC: LiChrosorb^R RP-18 (7 μm) 250–25, 100 bar. NMR: ^{1}H NMR spectra were run at 400 MHz and ^{13}C NMR spectra at 100 MHz with solvent (CD_3OD) as int. standard.

Extraction and isolation. The dried ground flowers (50 g) were successively extracted with petrol and MeOH in a Soxhlet apparatus. The methanolic extract was chromatographed over Sephadex LH-20 with MeOH as eluent. Six fractions were collected. Fraction 3 was further separated in the same system to yield nine fractions. Fraction 6 (second column) was submitted to RLCC in the system EtOAc–*iso*-PrOH–H_2O (5:3:7) in the ascending mode. Final purification by prep. HPLC (MeOH–H_2O–HOAc 49:51:2.5) and over LH-20 (EtOH) furnished 23 mg of **1**. **2** (5 mg) was obtained by HPLC in the system MeOH–HOAc–H_2O (16:24:1). Fraction 4 (first column) was subjected to DCCC ($CHCl_3$–MeOH–H_2O 13:7:4, descending mode, 10 ml-fractions/hr, at fraction 120 change to

Table 2. ^{1}H NMR spectral data of the sugar moieties of okanin glucosides in CD_3OD, δ-values (ppm), *J*(Hz)

	1	2	4	5	6
H-1''	4.98, *d*	5.02, *d*	4.80, *d*	4.98, *d*	4.98, *d*
	$J_{1''2''}=7.3$	$J_{1''2''}=7.7$	$J_{1''2''}=7.7$	$J_{1''2''}=7.5$	$J_{1''2''}=7.5$
H-2''	3.55, *dd*	3.58, *dd*	3.52, *dd*	3.54, *dd*	3.56, *dd*
	$J_{2''3''}=9.2$	$J_{2''3''}=9.3$	$J_{2''3''}=9.3$	$J_{2''3''}=9.3$	$J_{2''3''}=9.3$
H-3''	3.50, *dd*	3.39–3.44	3.41–3.48	3.50, *dd*	3.50, *dd*
	$J_{3''4''}=9.0$	—	—	$J_{3''4''}=8.4$	$J_{3''4''}=8.4$
H-4''	3.40, *dd*	3.39–3.44	3.41–3.48	3.41, *dd*	3.41, *dd*
	$J_{4''5''}=9.4$	—	—	$J_{4''5''}=9.4$	$J_{4''5''}=9.7$
H-5''	3.73, ABX	3.26, ABX	3.28, ABX	3.47, ABX	3.69, ABX
H-6''	4.21, ABX	3.79, ABX	3.78, ABX	3.91, ABX	4.43, ABX
	$J_{6''5''}=-$	$J_{6''5''}=2.1$	$J_{6''5''}=2.4$	$J_{6''5''}=1.9$	$J_{6''5''}=2.2$
	$J_{6''6''}=11.7$	$J_{6''6''}=12.0$	$J_{6''6''}=12.0$	$J_{6''6''}=12.1$	$J_{6''6''}=11.9$
H-6''	3.83, ABX	3.72, ABX	3.73, ABX	3.71, ABX	4.26, ABX
	$J_{6''5''}=6.4$	$J_{6''5''}=5.2$	$J_{6''5''}=4.3$	$J_{6''5''}=5.6$	$J_{6''5''}=6.4$
H-1'''	4.41, *d*	4.99, *d*			
	$J_{1'''2'''}=7.7$	$J_{1'''2'''}=7.7$			
H-2'''	3.25, *dd*	3.54, *dd*			
	$J_{2'''3'''}=8.4$	$J_{2'''3'''}=9.5$			
H-3'''	3.31–3.35	3.49, *dd*			
H-4'''	3.31–3.35	3.39–3.44			
	—	—			
H-5'''	3.22, ABX	3.47, ABX			
H-6'''	3.85, ABX	3.92, ABX			
	$J_{6'''5'''}=2.2$	$J_{6'''5'''}=2.3$			
	$J_{6'''6'''}=11.8$	$J_{6'''6'''}=12.3$			
H-6'''	3.65, ABX	3.72, ABX			
	$J_{6'''5'''}=5.7$	$J_{6'''5'''}=5.2$			

$CHCl_3$–MeOH–*iso*-PrOH–H_2O 26:13:1:8; at fraction 125 change to $CHCl_3$–MeOH–*iso*-PrOH–H_2O 13:6:1:4). Fractions 106–130 (DCCC) were finally purified by HPLC (MeOH–H_2O–HOAc 98:102:5) and LH-20 (*iso*-PrOH) to yield 8 mg of **6**. Fractions 152–171 were chromatographed over LH-20 (*iso*-PrOH) to furnish 51 mg of **4**. Compound **5** (10 mg) was obtained from fractions 216–270 and final purification by HPLC (MeOH–H_2O–HOAc 98:102:5). Okanin (**3**) was obtained after acidic hydrolysis of **4** according to [4].

Okanin 4'-diglucoside (**1**). Mp (uncorr.): 148° R_f I: 0.25; II: 0.0; III: 0.36 UV: λ_{max}(nm): 380, 312sh, 264 (MeOH); 508, 373, 334, 272sh ($AlCl_3$); 422, 351sh, 326sh, 273sh, 244sh, ($AlCl_3$ + HCl); 434, 285, 259sh (NaOAc); 416, 338sh, 290 (NaOAc + H_3BO_3); ^{1}H NMR (aglycone): 7.77 (*d*, 15 Hz, H-β), 7.63 (*d*, 9 Hz, H-6'), 7.57 (*d*, 15 Hz, H-α), 7.22 (*d*, 2 Hz, H-2), 7.12 (*dd*, 2 Hz, 8 Hz, H-6), 6.91 (*d*, 9 Hz, H-5'), 6.81 (*d*, 8 Hz, H-5); ^{13}C NMR: 194.62 (C=O), 153.82 (C-2'), 151.58 (C-4'), 150.49 (C-4), 146.98 (C-3, C-β), 135.70 (C-3'), 128.15 (C-1), 124.12 (C-6), 122.73 (C-6'), 118.14 (C-α), 117.41 (C-1'), 116.64 (C-5), 115.79 (C-2), 108.32 (C-5'), 104.69 (C-1'''), 102.43 (C-1''), 77.99* (C-3''), 77.94* (C-3'''), 77.64* (C-5''), 77.32* (C-5'''), 75.22† (C-2'''), 74.70† (C-2''), 71.64‡ (C-4''), 71.35‡ (C-4'''), 69.75 (C-6''), 62.67 (C-6'''); *,†,‡: interchangeable; FDMS: $[M+Na+H]^+$636 (57.4%), $[M+Na]^+$635 (100%), M^+612 (7.4%).

Okanin 3',4'-diglucoside (**2**). Mp (uncorr.): 150° R_f I: 0.24; II: 0.0; III: 0.34; UV: λ_{max}(nm): 384, 269 (MeOH); 511, 365, 326sh, 306sh ($AlCl_3$); 436, 320, 273sh ($AlCl_3$ + HCl); 477sh, 405, 283 (NaOAc); 417, 328sh, 284 (NaOAc + H_3BO_3); ^{1}H NMR (aglycone): 7.89 (*d*, 9 Hz, H-6'), 7.79 (*d*, 15 Hz, H-β), 7.58 (*d*, 15 Hz, H-α), 7.20 (*d*, 2 Hz, H-2), 7.13 (*dd*, 2 Hz, 8 Hz, H-6), 6.93 (*d*, 9 Hz, H-5'), 6.82 (*d*, 8 Hz, H-5); FDMS: $[M+Na+H]^+$ 636 (36.3%), $[M+Na]^+$ 635 (27.1%).

Okanin 4'-(6''-O-acetyl)glucoside (**6**): mp (uncorr.): 130° R_fI: 0.58; II: 0.27; III: 0.81; UV: λ_{max}(nm): 381, 310sh, 265 (MeOH); 510, 372, 335 ($AlCl_3$); 423, 323sh, 274sh, 245sh ($AlCl_3$ + HCl); 446, 390, 296sh, 262 (NaOAc); 393, 320sh, 274 (NaOAc + H_3BO_3); ^{1}H NMR (aglycone and acetyl): 7.68 (*d*, 15 Hz, H-β), 7.62 (*d*, 9 Hz, H-6'), 7.56 (*d*, 15 Hz, H-α), 7.20 (*d*, 2 Hz, H-2), 7.13 (*dd*, 2 Hz, 8 Hz, H-6), 6.82 (*d*, 8 Hz, H-5), 6.78 (*d*, 9 Hz, H-5'), 2.08 (*s*, MeCO). FDMS: $[M+H]^+$ 493 (2.4%), $[M-(acetylglucosyl)]^+$ 288 (100.0%).

Acknowledgements—We thank Dr Th. Kämpchen and U. Grundey (Institut für Pharmazeutische Chemie der Universität Marburg) for recording the NMR spectra and Dr M. Steinbach (Fachbereich Chemie der Universität Marburg) for running the FD-mass spectra.

REFERENCES

1. Hoffmann, B. and Hölzl, J. (1988) *Planta Med.* 52.
2. Hoffmann, B. and Hölzl, J. (1988) *Planta Med.* (in press).
3. Hoffmann, B. and Hölzl, J. (1988) *Phytochemistry* (in press).
4. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
5. Markham, K. R. and Chari, V. M. (1982) in *The Flavonoids—Advances in Research* (Harborne, J. B. and Mabry, T. J., eds), p. 44. Chapman & Hall, London.
6. Markham, K. R., Ternai, B., Stanley, R., Geiger, H. and Mabry, T. J. (1978) *Tetrahedron* **34**, 1389.
7. Colson, P., Jennings, H. J. and Smith, I. C. P. (1974) *J. Am. Chem. Soc.* **96**, 8081.
8. Crawford, D. J. (1978) *Phytochemistry* **17**, 1680.
9. De Bruyn, A., Anteunis, M. and Verhegge, G. (1975) *Bull. Soc. Chim. Belg.* **84**, 721.
10. Birkofer, L., Kaiser, C., Hillges, B. and Becker, F. (1969) *Liebigs Ann. Chem.* **725**, 196.