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A GERMACRADIENE GLYCOSIDE FROM ROOTS OF *PIMPINELLA SAXIFRAGA*

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Key Word Index—*Pimpinella saxifraga*; Umbelliferae; sesquiterpene; germacradiene; glycoside.

Abstract—A new germacradiene glycoside was found to occur in the roots of *Pimpinella saxifraga*. The structure of its acetylation product was determined by spectroscopic methods, including 2D NMR techniques.

INTRODUCTION

Pimpinella saxifraga L. (Umbelliferae) is a perennial plant widely distributed over Europe and Asia. Roots of this plant, together with those of closely related *P. major* (L.) Hudson, have been used in herbal medicine for their expectorant, broncho-secretolytic and antiphlogistic properties [1]. There are some reports on coumarin and essential oil constituents of the roots of *P. saxifraga* [2]. The present paper describes an acetylation product of naturally occurring compound from this plant material.

RESULTS AND DISCUSSION

Chromatography of the ethanolic root extract of *P. saxifraga* afforded a new sesquiterpene glycoside which was purified after acetylation. The acetyl derivative **1** was obtained as needles of m.p. 178–180°C.

A sharp doublet of the anomeric proton at δ_H 4.53 (J = 8.1 Hz) and remaining signals of the β-glucopyranosyl tetraacetate moiety were readily identifiable in the ^1H and ^{13}C NMR spectra of **1** (Table 1) and its EIMS revealed ion peaks characteristic for the tetraacetyl glucose fragmentation at m/z 331, 169 and 109. Other signals in the spectra fitted well with a germacrane-type sesquiterpene containing two olefinic double bonds at $\Delta^{1(10)}$ and Δ^4, two allylic methyls, two further tertiary methyls, two olefinic methines and two oxygen bearing methines. Moreover, the aglycone contained an acetate group and a tertiary hydroxyl, since acetylation, under usual conditions, gave a pentaacetate (**1**) which still exhibited the hydroxyl band at 3578 cm^{-1} in its IR spectrum. The hydroxyl group together with the two tertiary methyls (singlets at δ_H 1.17 and δ_H 1.32) and a quaternary carbon attached to the oxygen function (δ_C 72.74) constituted a hydroxyisopropyl side chain of the aglycone. This was confirmed by the presence of an ion peak at m/z 59 $[Me_2COH]^+$ and the aglycone fragmentation in the EIMS. As expected, the ESIMS showed an $[M + Na]^+$ peak at m/z 649, consistent with the molecular formula $C_{31}H_{46}O_{13}$.

The entire sequence of protons attached to the sesquiterpene carbon skeleton and protons of glucosyl tetraacetate moiety were established by 2D NMR experiments (^1H–^1H COSY and HMQC). Comparison of the chemical shifts of H-2 (δ_H 5.60) and H-6 (δ_H 4.82) indicated that the acetate was attached to C-2. Thus, the sugar moiety could be assigned to the C-6 position. The proton at C-2 was placed axially on the basis of its interaction with one equatorial ($J_{2,3}$ = 5.5 Hz) and two axial protons ($J_{1,2}$ = 10.4 Hz, $J_{2,3'}$ = 10.3 Hz). The couplings did not allow a clear assignment of the configuration at C-2 as the expected one depends upon the conformation of the sesquiterpene ten-membered ring.

The NMR spectral data indicated that **1** belonged to the class of *trans,trans*-1(10),4-germacradiene-type sesquiterpenes and suggested that conformation of **1** was related to those adopted by ten-

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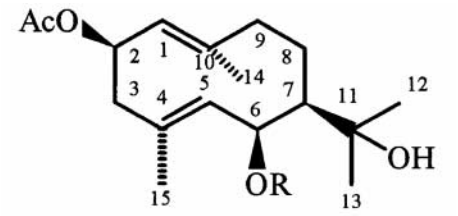

1 R=β-glucopyranosyl tetraacetate

membered rings in tovarol [3], its masked epoxy-equivalent shiromodiol [4] and their numerous esters, which have been reported from various plant sources, mainly from umbelliferous species [5, 6]. The compounds of crossed conformations have their C-14 and C-15 methyls below the ring plane and the side chain placed in the usual β (equatorial) orientation [7]. In particular, the data were similar to those noted for tovarol (1(10),4-germacradiene-6β,8α-diol) [3] and the couplings involving H-5, H-6 and H-7 were identical. Thus, H-5 and H-6 in **1** were assumed to be β and α orientated, respectively, and the distinct diaxial couplings of H-2 required the acetate ester to be in the β (equatorial) position. The observed couplings for H-2 and chemical shifts for H-1 and H-14 were comparable to those of tanacetol B [8], a germacradiene having *trans*-1(10) double bond with α-methyl at C-10 and β-acetoxy group at C-2. These observations support the relative stereochemistry represented by formula **1**.

EXPERIMENTAL

General

Mp: uncorr. CC: Merck silica gel (Art. 7734 and Art. 109385), Sephadex LH 20 (Pharmacia Biotech AB). TLC: Merck silica gel (Art. 5553). ^{1}H NMR: 500.13 MHz. ^{13}C NMR: 125.77 MHz.

Plant material

Roots of *P. saxifraga* were collected in October 1995 from commercially available plants cultivated by KAWON in Gostyń, Poland and a voucher specimen was deposited at the authors' (Z. J. and M. Z.-W.) Department.

Extraction and isolation

Fresh roots (3000 g) were cut into small pieces and exhaustively extracted with EtOH at room temperature. After removal of EtOH, the remaining extract was partitioned between *n*-BuOH and water. Then the *n*-BuOH layer was concentrated to give a residue (100 g) which was passed through a Sephadex LH 20 column. The eluate with water was concentrated and subjected to a silica gel column which was continuously eluted with a mixture of $CHCl_3$–MeOH–H_2O (24:8:1). Combined fractions (98 mg) containing a compound which appeared red on a TLC plate ($CHCl_3$–MeOH, 8:1, R_f 0.56) after spraying with sulphuric acid and changed to blue on heating, were rechromatographed on silica gel using $CHCl_3$–MeOH gradient (up to 20% MeOH) but complete purification could

Table 1. ^{1}H and ^{13}C NMR spectral data of compound **1** ($CDCl_3$, TMS as internal standard, δ values)

C		H		*J* (Hz)
1	128.32	1	5.06 *d*	10.4
2	69.29	2	5.60 *ddd*	10.4, 10.3, 5.5
3	44.78	3	2.51 *m*[a]	
4	130.55	3′	2.19 *dd*	11.4, 10.3
5	133.97	5	5.46 *br d*	7.1
6	81.55	6	4.82 *br dd*	7.1, *ca* 1.0
7	50.06	7	1.17 *m*[b]	
8	25.72	8	2.04 *m*[d]	
9	36.11	8′	1.75 *m*[c]	
10	139.92	9	2.51 *m*[a]	
11	72.74	9′	1.67 *m*	
12	28.24	12	1.17 *s*[b] (4 H)	
13	29.33	13	1.32 *s* (3 H)	
14	22.62	14	1.75 *s*[c] (4 H)	
15	17.24	15	1.53 *s* (3 H)	
Glucose moiety				
1	100.94	1	4.53 *d*	8.1
2	71.52	2	4.96 *dd*	9.5, 8.1
3	73.00	3	5.17 *dd*	9.5, 9.5
4	68.47	4	5.02 *dd*	9.9, 9.5
5	72.01	5	3.64 *m*	
6	62.22	6	4.18 *dd*	12.2, 5.4
		6′	4.08 *br d*	12.2
-O*H*			2.67 *s*	
-CO*Me*	20.51 (2×), 20.71 (2×), 21.24		1.99 *s*, 2.01 *s*, 2.04 *s*[d], 2.07 *s*, 2.09 *s*	
-*CO*Me	169.31, 169.51, 170.20, 170.48, 170.58			

[a,b,c,d]Overlapped signals.

not be achieved. Acetylation (Ac_2O, pyridine) and usual work-up followed by silica gel column chromatography ($CHCl_3$–EtOAc, 2:1) yielded compound **1** (18 mg) which crystallized from MeOH.

Compound **1**

Colourless needles; m.p. 178–180°C; $[\alpha]_D^{20.9}$ −16.8 (MeOH, *c* 1.0); IR υ_{max}^{KBr} cm^{-1}: 3578, 1752, 1734, 1371, 1238, 1080, 1058, 1041, 982, 911; ESIMS (pos. ions) *m/z*: 649 $[M + Na]^+$ (100); EIMS 70 eV (rel. int.) *m/z*: 566 $[M - 60]^+$ (0.3), 331 (21.0), 271 (8.8), 218 [aglycone-60 − 18]$^+$ (8.8), 200 $[218 - 18]^+$ (9.4), 169 (100), 160 $[218 - Me_2CO]^+$ (81.2), 109 (42.3), 59 $[Me_2COH]^+$ (9.4), 43 (42.9); ^1H and ^{13}C NMR spectra: Table 1.

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