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# 2-METHYLBUTAN-1-YL-β-D-GLUCOSIDE, A HEMITERPENE GLUCOSIDE FROM *BYSTROPOGON PLUMOSUS*[1]

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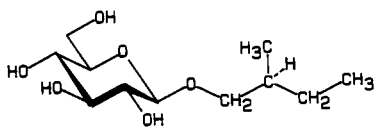
ABSTRACT.—(*S*)-2-Methylbutan-1-yl-β-D-glucopyranoside was isolated in a yield of 0.01% from the leaves of *Bystropogon plumosus*, and its structure was elucidated from its degradation products and $^1$H- and $^{13}$C-nmr spectral data.

*Bystropogon plumosus* (L. fil.) L'Her. (Lamiaceae) is endemic in the Canary Islands and is traditionally used as a remedy against colds (1). Its essential oil, distilled from the leaves, has been shown to consist of ca. 90% C-3 carbonyl compounds of the *p*-methane series of which (+)-pulegone is the main constituent (1). Several plants producing monoterpenes often contain monoterpene glycosides (2,3); however, investigation of the leaves of *B. plumosus* for putative monoterpene glycosides failed, but resulted in the isolation of a glycosidic compound **1** in a yield of 0.01% whose isolation and structure elucidation are presented below.

An aqueous fraction was obtained from the lyophilized leaves after extraction with MeOH, evaporation of the latter, and suspension of the residue in $H_2O$ following mainly the procedure of Mulkens *et al.* (4). The concentrated



**1**

TABLE 1. $^1$H- and $^{13}$C-nmr Spectra of **1** in $CD_3OD/CDCl_3$.

| Position | $^{13}$C nmr | $^1$H nmr |
|---|---|---|
| 1 | 10.91 (q) | 0.90 |
| 2 | 25.98 (t) | A 1.47 $J_{12A}$ = 7.4; $J_{2A2B}$ = ca. 13 |
| | | B 1.15 $J_{12B}$ = 7.4; |
| 3 | 35.00 (d) | 1.69 $J_{35}$ = 6.6; $J_{2A3}$ = 5.2; $J_{2B3}$ = ca. 6 |
| 4 | 75.31 (t) | A 3.78 |
| | | B 3.30 |
| 5 | 16.22 (q) | 0.93 |
| 1′ | 103.21 (d) | 4.27 $J_{1'2'}$ = 7.7 |
| 2′ | 73.74 (d) | 3.28 |
| 3′ | 76.40 (d) | 3.3 ± |
| 4′ | 70.43 (d) | 3.5 |
| 5′ | 75.70 (d) | 3.29 $J_{5'6'A}$ = 3.2 |
| 6′ | 62.03 (t) | A 3.86 $J_{5'6'B}$ = 4.4 |
| | | B 3.78 $J_{6'A6'B}$ = 12 |

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aqueous phase was chromatographed on an $Al_2O_3$ column followed by medium pressure chromatography on RP 8 to

give a chromatographically (tlc) pure substance **1** with $[\alpha]^{20}D$ −22.2°.

Enzymatic and acidic hydrolysis of **1** yielded glucose (tlc) and 2-methylbutan-1-ol (gc). The $^1H$- and $^{13}C$-nmr spectral data of **1** are given in Table 1. The hydroxyl substitution at C-1 and the methyl substitution at C-2 of the butane skeleton are in accordance with the chemical shifts of both carbons and the respective protons attached to C-1 and C-2. The coupling constant, 7.7 Hz, of H-1′ indicates a β configuration of the glycosidic linkage. The complete $^1H$ connectivities of the aglycone were readily elucidated from the cross peaks in the 2D $^1H$ COSY spectrum. Thus, **1** is 2-methylbutan-1-yl-β-D-glucopyranoside. The acetate of 2-methylbutan-1-ol obtained by enzymatic hydrolysis of **1** showed an 8:2 ratio of the *S* to *R* enantiomer using complexation gc (5); thus, at least 80% of **1** possesses the *S* configuration at C-2.

Compound **1** is a genuine constituent because the isolation procedure excludes any potential for artificial formation. A similar compound, 2-methyl-3-buten-2-yl-β-D-glucopyranoside, has recently been isolated in a yield of 0.8% from the dry aerial parts of *Ferula loscosii* Lge. Wk. (Umbelliferae) (6). Compound **1** is part of *O*-β-D-glucopyranosyldihydrozeatin, a metabolite of cytokinin metabolism (7). During preparation of this manuscript, **1** and its 6-*O*-α-arabinofuranosyl derivative were published as glycosidic conjugates of aroma components obtained from apple fruit (8). The aglycone of **1** is a saturated hemiterpene moiety. It may originate biogenetically from isoleucine as in fermentations producing alcoholic beverages (9) or from the DMAPP/IPP pool in plants producing monoterpenes. Its occurrence in a Lamiacean plant that produces monoterpenes may indicate its function as a transport conjugate of a hemiterpene group used for biosynthesis of terpenoids.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The 1D- and 2D-nmr spectra were recorded at ambient temperature on a Bruker AM 300 nmr spectrometer in $CD_3OD$-$CDCl_3$ (1:1) at 300 ($^1H$) and 75.5 ($^{13}C$) MHz; chemical shifts are in ppm relative to TMS. The optical rotation was measured with a Perkin-Elmer 241 polarimeter. (*S*)-(−)-2-Methylbutan-1-ol and racemic 2-methylbutan-1-ol were obtained from Aldrich (Steinheim, FRG).

PLANT MATERIAL.—*B. plumosus* was field grown at the Institute in Münster. A voucher specimen is deposited under MSPB 2.

ISOLATION.—Fresh leaves were pulverized under liquid $N_2$ and subsequently lyophylized. This material (120 g) was extracted with MeOH in a Soxhlet for 6 h, the MeOH extract was taken to dryness, and the residue was suspended in $H_2O$. The filtered aqueous solution was repeatedly extracted with petroleum ether until a light yellow aqueous phase was obtained, which was concentrated and chromatographed on neutral $Al_2O_3$ (Merck Darmstadt 1077; 80 × 3 cm) with 2 liters of $H_2O$. The entire eluate was concentrated under reduced pressure and chromatographed using a medium pressure column (Büchi, Flawil, Switzerland; 26 × 2.6 cm) filled with RP 8 (Lichroprep 40-63 μm, Merck) and 2 liters $H_2O$ (5 ml/min) as the mobile phase followed by a gradient from $H_2O$ to $H_2O$-MeCN (85:15), prepared with communicating vessels with 300 ml $H_2O$ in the first and 300 ml $H_2O$-MeCN (85:15) in the second vessel. Compound **1** eluted between 280 and 330 ml after starting the gradient; these fractions were taken to dryness giving a colorless hygroscopic substance with $[\alpha]^{20}D$ −22.2 ($c$ = 0.135, MeOH).

HYDROLYSIS.—Compound **1** (ca. 1 mg) was dissolved in 1.5 ml McIlvaine buffer pH 5.5, β-glucosidase (ca. 1 mg) (from sweet almonds, Serva, Heidelberg) was added, and the solution was subsequently covered with 2 ml *n*-pentane. The closed vessel was incubated for 14 h at 37°. The aglycone was analyzed by gc from the *n*-pentane phase and the sugar component by tlc from the aqueous phase.

Acidic hydrolysis was performed with ca. 1 mg of **1** in 2 ml 0.1 N HCl covered with 2 ml *n*-pentane at ambient temperature for 24 h. Identification of the products was as above.

ACETYLATION.—(*S*)-(−)-2-Methylbutan-1-ol (2 mg) or (*S*/*R*)-2-methylbutan-1-ol (2 mg) or the product of hydrolysis of **1** after careful removal of *n*-pentane (2 mg) was dissolved in 100 μl pyridine, and 100 μl $Ac_2O$ was added. After 24 h at ambient temperature, 2 ml $H_2O$ was

added and the acetates were extracted into *n*-pentane, dried over anhydrous $Na_2SO_4$, and used for glc.

TLC SYSTEMS.—Compound **1** was chromatographed on Si gel plates with *n*-PrOH–toluene–HOAc–$H_2O$ (25:20:10:10) (4); detection with anisaldehyde/$H_2SO_4$; $R_f$ of **1** 0.43. Carbohydrates on Si gel with MeCN-$CS_2$-$H_2O$-$HCO_2H$ (85:5:10:0.5); developed 3 times; detection with anisaldehyde/$H_2SO_4$; $R_f$ of glucose 0.31, fucose 0.40, xylose 0.55, rhamnose 0.67.

GLC SYSTEMS.—Racemic 2-methylbutan-1-ol: DB-wax capillary column (J&W Scientific, Frankfurt FRG) 30 m × 0.25 mm; 60–70° at 1°/min, then isothermally; He 1.15 ml/min; split injection 1:35; FID; Rt 7.6 min. Separation of (*R/S*)-2-methylbutan-1-ol as the acetates: fused silica capillary column (25 m × 0.25 mm) with OV-101 coated with Ni (II)-bis[3-(heptafluorobutyryl)-(1*R*)-10-methyl-camphorate] 0.1 M (5); 70° isothermally; $N_2$ 1.3 bar.

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LITERATURE CITED

1. A. Nahrstedt, D. Economou, and F.J. Hammerschmidt, *Planta Med.*, **50**, 247 (1985).
2. E. Stahl-Biskup, *Flavour and Fragrance*, **2**, 75 (1987).
3. G. Schulz, "Aetherisches Oel und glykosidisch gebundene flüchtige Pflanzeninhaltsstoffe. Untersuchungen an ausgewählten Arten der Lamiaceae," Ph.D. Thesis, University of Hamburg, Federal Republic of Germany, 1989.
4. A. Mulkens, E. Stephanou, and J. Kapetanidis, *Pharm. Acta Helv.*, **60**, 276 (1985).
5. V. Schurig, A. Ossig, and R. Link, *J. High Res. Chromatogr. Chromatogr. Commun.*, **11**, 89 (1988).
6. M. Pinar and M. Martin-Lomas, *Phytochemistry*, **16**, 281 (1977).
7. R.E. Summons, D.S. Letham, B.I. Gollnow, C.W. Parker, B. Entsch, L.P. Johnson, J.K. McLeod, and B.G. Rolfe, *Phytochemistry*, **18**, 819 (1979).
8. W. Schwab and P. Schreier, *J. Agric. Food Chem.*, **38**, 757 (1990).
9. W.R. Sponholz, in: "Modern Methods of Plant Analysis." Ed. by H.F. Linskens and J.F. Jackson, Springer-Verlag, Berlin, 1988, NS Vol. 6, p. 172.

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