109; UV λ_{max}^{MeOH} nm (log ε): 232 (3.65); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300; ^1H NMR (500 MHz, $CDCl_3$): δ1.00, 1.03, 1.18, 1.21 (3H, each *s*), 1.86 (1H, *d*, *J* = 10 Hz), 3.44 (1H, *d*, *J* = 10 Hz), 3.57 (1H, *dd*, *J* = 12 and 7 Hz), 3.73 (1H, *t*, *J* = 10 Hz), 3.76 (1H, *dd*, *J* = 12 and 3 Hz), 4.61 (1H, *dd*, *J* = 7 and 3 Hz), 5.22 (1H, *br s*), 5.33 (1H, *br s*), 5.78 (1H, *dd*, *J* = 16 and 10 Hz), 6.17 (1H, *d*, *J* = 16 Hz).

Acetylation of sterebin E (**1**). A mixture of sterebin E (**1**) (10 mg), Ac_2O (2 ml) and pyridine (1 ml) was kept at room temp. for 24 hr. The reaction mixture was freed from organic solvents *in vacuo* and purified by silica gel chromatography to yield sterebin E diacetate as amorphous powder (7 mg), EIMS (direct inlet) 70 eV, *m/z*: 422 $[M]^+$; UV λ_{max}^{MeOH} nm (log ε): 235 (3.94); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1725, 1240; ^1H NMR (100 MHz, $CDCl_3$): δ1.01, 1.02, 1.17, 1.24, 1.84, (3H, each *s*), 1.96 (1H, *d*, *J* = 10 Hz), 2.07, 2.18 (3H, each *s*), 3.83 (1H, *dd*, *J* = 8 and 10 Hz), 4.70 (2H, *d*, *J* = 7 Hz), 4.83 (1H, *d*, *J* = 10 Hz), 5.58 (1H, *t*, *J* = 7 Hz), 5.67 (1H, *dd*, *J* = 16 and 10 Hz), 6.20 (1H, *d*, *J* = 16 Hz).

Ozonolysis followed by $NaBH_4$ *reduction of sterebins E* (**1**)*–H* (**4**) *and A* (**5**). Sterebins E (**1**)–H (**4**) or A (**5**) (5 mg each) in MeOH (5 ml) was ozonized at −60° for 10 min and then the reaction mixture was reduced with $NaBH_4$ to give the residue (5 mg each) which was chromatographed over silica gel to yield the tetraol (3 mg each) as colourless powder, $[\alpha]_D$ +4.7° (*c* 0.02, MeOH), ^1H NMR (500 MHz, $CDCl_3$): δ0.86, 1.00, 1.16, 1.33 (3H, each *s*), 3.44 (1H, *d*, *J* = 10 Hz) 3.60 (1H, *t*, *J* = 11 Hz), 3.94 (1H, *dd*, *J* = 11 and 4 Hz), 3.95 (1H, *t*, *J* = 10 Hz).

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DITERPENEBUTENOLIDES IN *SOLIDAGO GIGANTEA*

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Key Word Index—*Solidago gigantea*; Asteraceae; diterpenebutenolides; 6-deoxy-solidagolactone IV-18,19-olide; 2β-*O*-β-D-glucopyranosyl-6-deoxy-solidagolactone IV-18,19-olide.

Abstract—6-Deoxy-solidagolactone IV-18,19-olide and 2β-*O*-βD-glucopyranosyl-6-deoxy-solidagolactone IV-18,19-olide, two new diterpenebutenolides of the *cis*-clerodane type, were isolated from *Solidago gigantea*. Lactones of this type were not detected in *S. virgaurea* and *S. canadensis*.

INTRODUCTION

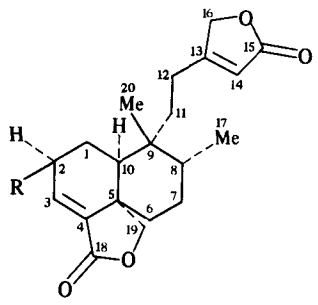
2β-Hydroxy-6-deoxy-solidagolactone IV-18,19-olide (**1**, identical with L in [1]) was reported as a possible artifact of acid hydrolysis of the crude extract of *Solidago gigantea* Ait. [1]. Due to its butenolide ring, **1** is selectively detectable by spraying with Kedde reagent.

As we have shown that the compound is stable to heat and acid treatment, it must be a genuine constituent of *S. gigantea*. To establish if compounds of this type could serve as additional markers for the differentiation of the medicinally used *Solidago* species a TLC comparison of *S. virgaurea* L., *S. canadensis* L. and *S. gigantea* Ait. was performed in addition to a preparative scale investigation of *S. gigantea*.

RESULTS AND DISCUSSION

The crude chloroform–methanol extract of the freeze-dried aerial parts of diploid *S. gigantea* plants, collected at the full flowering stage, was purified and concentrated by column chromatography. In the resulting fractions 12 Kedde-positive spots were detected by TLC, one of them corresponding to **1**. Furthermore, two main components were isolated, and their structures determined by means of EIMS, FABMS, ^1H and ^{13}C NMR spectroscopy as 6-deoxy-solidagolactone IV-18,19-olide (**2**) and 2β-*O*-β-D-glucopyranosyl-6-deoxy-solidagolactone IV-18,19-olide (**3**).

On TLC screening of several different samples of *S. virgaurea* and *S. canadensis* of cytologically defined



	R
1	OH
2	H
3	O-β-gluco-pyranosyl

origins no Kedde-positive compounds could be found, whereas in some samples of *S. gigantea* **1** and **2** were detected. After acid hydrolysis of the methanolic extract the detection of **1** was possible in every sample of *S. gigantea* and gave evidence of the occurrence of **3** in each (di- as well as tetraploid) sample examined. Assuming that the absence of **1** and **2** in many investigated plants is due to the stage of development, it is postulated that **2** may be hydroxylated at C-2 very rapidly and then glucosylated to give **3**.

EXPERIMENTAL

Isolation of **2**. Extraction with $CHCl_3$–MeOH (1:1) of 250 g freeze-dried leaves of *S. gigantea* gave 35 g of extract which was purified by extraction with $CHCl_3$, CC on silica gel 60 (Merck) with various mobile phases [$CHCl_3$–Me_2CO (9:1, 4:1), $CHCl_3$–*n*-hexane (7:3), $CHCl_3$–MeOH–H_2O (80:10:1)] and prep. TLC on silica gel 60 F_{254} (Merck, 2 mm, $CHCl_3$–MeOH–H_2O 80:10:1) to give 13.2 mg colourless needles, mp 218°.

Isolation of **3**. Dry CC of 20 g crude saponin mixture (H.I. = 2500) [1] on silica gel (Woelm TSC, $CHCl_3$–MeOH–H_2O mixtures of increasing polarity) followed by prep. TLC (see above $CHCl_3$–MeOH–H_2O 6:4:1 and BuOH–PrOH–H_2O 3:4:10) gave 2.0 mg amorphous **3**. 1.5 mg were acetylated to **3**-Ac in the usual way [2].

Structure elucidation was performed by EIMS, FABMS, ^1H and ^{13}C NMR spectroscopy, and comparison with available data of similar substances [1, 3, 4].

Compound **2**. EIMS, Finnigan MAT 8200+SS 300, 8 kV, 70 eV, ion source: 150°, sample:250°, *m/z* (rel. int.): 330 $[M]^+$ (96), 300 (10), 219 (59), 189 (24), 163 (16), 161 (20), 159 (18), 133 (28), 117 (30), 111 (42), 105 (40), 98 (58), 79 (48), 55 (70), 41 (100); ^1H NMR (250 MHz, $CDCl_3$, TMS): δ6.80 (*br s*, H-3), 5.9 (*br s*, *J* = *ca* 2 Hz, H-14), 4.90 (*J* = *ca* 2 Hz, H-16), 4.52 (*d*, *J* = 8 Hz, H_a-19), 3.76 (*d*, *J* = 8 Hz H_b-19), 1.00 (*s*, Me-20), 0.87 (*d*, *J* = 6 Hz); ^{13}C NMR (62.9 MHz, $CDCl_3$) C-1–C-20 (ppm): 19.0, 26.3, 135.7, 43.0, 31.0, 27.0, 36.5, 37.9, 42.6, 30.6, 23.4, 169.9, 115.5, 170.1, 73.0, 15.6, 173.6, 76.1, 25.5.

Compound **3**. Acid hydrolysis gave **2** and glucose, which was identified by GC [5]. Spectroscopic measurements were performed with **3**-Ac. FABMS, Varian-MAT 311 A, positive, thioglycerol, *m/z*: 699 $[M-Na]^+$, 807 $[M-Na+thioglycerol]^+$; negative, *m*-NO_2-benzylalcohol *m/z*: 675 $[M-H]^-$, 722 $[M+NO_2]^-$, 829 $[M+m\text{-}NO_2-benzylalcohol]^-$; ^1H NMR (250 MHz, $CDCl_3$, TMS): δ4.50 (*m*, H-2), 6.70 (*d*, *J* = 2 Hz, H-3), 5.88 (*br s*, H-14), 4.74 (*br s*, $H_{a,b}$-16), 4.50 (*d*, *J* = 8 Hz, H_a-19), 3.74 (*d*, *J* = 8 Hz, H_b-19), 1.00 (*s*, Me-20), 0.88 (*d*, *J* = 6 Hz, Me-17); 4.68 (*d*, *J* = 8 Hz, H-1'), 5.00 (*dd*, *J* = 8/8 Hz, H-2'), 5.25 (*dd*, *J* = 8/8 Hz, H-3'), 5.10 (*dd*, *J* = 8/8 Hz, H-4'), 4.24 (*m*, H_2-6'), 2.01, 2.03, 2.05, 2.07 (*s*, MeCO).

Proof of stability of **1**. Wet *S. gigantea* drug was heated to 100° for 4, 12, 20, 27 and 45 hr. Then it was heated with 2M HCl for a further 2 hr. The amount of **1** in the treated sample was the same as that in the 'untreated' drug.

Chromosome numbers. For cytological characterization samples from Lower Austria were used. A list of the exact locations is given in [6, 7]. Voucher specimens were deposited in the herbarium of the Institute of Pharmacognosy, University of Vienna. The chromosome number was determined after colchicination of the root tips and fixation with MeOH–glacial HOAc. Four of 15 samples of *S. gigantea* were tetraploid (2*n* = 36), the others diploid (2*n* = 18). All *S. canadensis* and *S. virgaurea* specimens examined were diploid (2*n* = 18).

Detection of Kedde-positive compounds. 0.1 g powdered drug was refluxed with $CHCl_3$ for 8 hr. The residue was dissolved in 1 ml $CHCl_3$ and 20 and 40 μl, respectively, were used for TLC [silica gel 60 F_{254} (Merck) $CHCl_3$–MeOH–H_2O (80:10:1)]. The substances were detected by spraying with Kedde reagent (0.1 g 3,5-dinitrobenzoic acid in 10 ml MeOH, then 2 M KOH).

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