Flavonolignans from *Avena sativa*

Eva Wenzig,[†] Olaf Kunert,*,[‡] Daneel Ferreira,[§] Martin Schmid,[‡] Wolfgang Schühly,[†] Rudolf Bauer,[†] and Alois Hiermann[†]

Department of Pharmacognosy, Institute of Pharmaceutical Sciences, Karl Franzens University of Graz, Universitätsplatz 4, A-8010 Graz, Austria, Department of Pharmaceutical Chemistry and Pharmaceutical Technology, Institute of Pharmaceutical Sciences, Karl Franzens University of Graz, Universitätsplatz 1, A-8010 Graz, Austria, and Department of Pharmacognosy and National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, Mississippi 38677

Three flavonolignans derived from the flavone tricin were isolated from *Avena sativa* herb. This is the first report of the presence of flavonolignans in *A. sativa*. In the known compounds **1a** and **1b**, a coniferyl alcohol moiety is linked to the flavone by an ether bond; in the new natural product **2**, it is linked by C−C bonds. Structure elucidation of compound **2** was performed by 1D and 2D NMR experiments, and the absolute configuration was determined from circular dichroic data.

Avena sativa L. (Poaceae) is a grain that has been cultivated in the northern hemisphere for thousands of years.[1] Apart from its importance as a nutrient and forage cereal, it is also used as a remedy against several diseases in traditional folk medicine. The aerial parts of *A. sativa* are traditionally used against rheumatism, gout, and liver and skin diseases and because of their suggested diuretic and sedative effects.[2]

Many constituents of this plant are already known. Oat herbs contain saponins,[3] C-glycosylflavones,[4,5] flavonoid-*O*-glycosides,[4] and silicic acid as the main inorganic compound.[2] Flavonolignans have not yet been found in this plant.

As a part of our ongoing phytochemical and pharmacological studies on *A. sativa*, we isolated a new flavonolignan with an unusual skeleton from the dichloromethane extract. Furthermore we obtained the two rare diastereomeric flavonolignans tricin 4′-*O*-(*threo*-β-guaiacylglyceryl) ether and tricin 4′-*O*-(*erythro*-β-guaiacylglyceryl) ether (salcolins A and B, respectively) from oat herbs for the first time. Isolation and structure elucidation of these compounds are described here.

The dichloromethane extract of oat herbs that had been pre-extracted with petroleum ether was subjected to several chromatographic separation steps, which finally led to the isolation of the compounds **1a**, **1b**, and **2**.
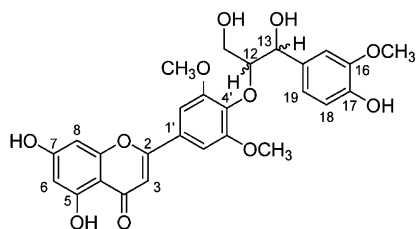
Compound **1a**: *erythro*
Compound **1b**: *threo*

LCESIMS of compounds **1a** and **1b** both yielded the same molecular ion peak at *m/z* 525, [M − H]⁻. The MS^n

fragmentation pattern indicated that the compounds may be derivatives of the flavone tricin (5,7,4′-dihydroxy-3′,5′-dimethoxyflavone). ¹³C NMR shifts of compounds **1a** and **1b** were in accordance with literature values for the two flavonolignans salcolins A and B, two epimers with different configurations at position 13.[6,7] The most significant differences between the ¹H and ¹³C NMR resonances of compounds **1a** and **1b** were observed for the aliphatic part of the guaiacylglyceryl moiety: δ(**1a**) − δ(**1b**) was −0.07 ppm at H-13, +0.12 ppm at H-12, +0.2 and +0.25 ppm at 11-CH₂, and −0.9 ppm at C-12. Such differences are similar to the results of Bouaziz et al.[6] and permitted the identification of compound **1a** as tricin-4′-*O*-(*erythro*-β-guaiacylglyceryl) ether (salcolin B) and compound **1b** as tricin-4′-*O*-(*threo*-β-guajacylglyceryl) ether (salcolin A).

Although compounds **1a** and **1b** showed optical activity, their CD spectra did not exhibit any Cotton effects. Since insufficient sample quantities complicated accurate measurement of their optical rotations, the enantiomeric purity of compounds **1a** and **1b** was examined by CE (capillary electrophoresis) measurements using the method of Schmid et al.[8]

This method has been developed for the chiral separation of compounds containing a vicinal diol functionality using a sodium borate buffer and β-cyclodextrin for chiral complexation. Considering the structures of compounds **1a** and **1b**, this method should be suitable for the separation of their possible enantiomers. When a CE examination was performed using *RR,SS*-hydrobenzoin as a positive control, *RR,SS*-hydrobenzoin was separated as expected, but in the case of **1a** and **1b** no separation occurred. Both compounds appeared as single sharp peaks in the electropherogram, presumably indicating that compounds **1a** and **1b** are enantiomerically pure.

The occurrence of optically active compounds **1a** and **1b** not exhibiting CD Cotton effects is presumably explicable in terms of their conformational mobility. Inspection of the structures indicates that a number of electronic transitions are possible in the observable UV region. The chromophores should also be able to show significant exciton coupling in certain mutual orientations. The overall CD spectrum will depend on the weighted average of the spectra from all the conformers present in solution, which can generate different signs of the rotational strength of a

* Corresponding author. Tel: +43/316/3805367. Fax: +43/316/3809846. E-mail: olaf.kunert@uni-graz.at.
† Department of Pharmacognosy, Karl Franzens University of Graz.
‡ Department of Pharmaceutical Chemistry and Pharmaceutical Technology, Karl Franzens University of Graz.
§ The University of Mississippi.

given transition. Thus, the overlapping spectra may cancel each other, giving no observable CD spectrum.[9]

Compounds **1a** and **1b** have previously been found in three other plants: the first isolation was from *Salsola collina* L. (Chenopodiaceae),[10] and recently the compounds were also isolated from *Hyparrhenia hirta* (L.) Stapf[5] and *Sasa veitchii* (Carr.) Rehder,[6] two members of the Poaceae family. A related compound, tricin 4′-*O*-(β-*p*-hydroxyphenylglyceryl) ether, has been reported as a constituent of *Aegilops ovata* L. (also Poaceae).[11] In these papers only relative configurations were described.
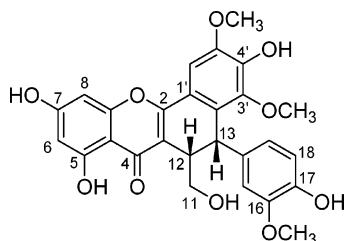
Compound **2**

The molecular formula $C_{27}H_{24}O_{10}$ of compound **2** was deduced from negative mode HRESIMS (m/z [M − H]⁻ = 507.4, calcd [M − H]⁻ = 507.4687). The MS^n fragmentation (ESI negative mode) of the molecular ion led to the loss of CH_3 groups in the first two fragmentation steps {m/z 492 [(M − H) − CH$_3$]⁻ and 477 [(M − H) − 2CH$_3$] as the major ions of MS^2 and MS^3 respectively}. Further fragmentation of m/z 477 yielded two major ions: m/z 461 [(M − H − 2CH$_3$) − 16]⁻ and m/z 446 [(M − H − 2CH$_3$) − OCH$_3$]. Production of m/z 339 in the first fragmentation step may be due to the loss of ring D after a McLafferty rearrangement between the positions 2′, 3′, 13, 14, and 19. The UV/vis spectrum of the compound is typical for a flavonoid derivative. The ^{1}H NMR and HSQC spectra indicated the presence of three *O*-methyl groups, one oxygenated methylene group, two aliphatic methine groups, and six aromatic methine groups.

HMBC was used to obtain the complete assignment of proton and carbon resonances: H-13 showed nine long-range C−H correlations (via 3 bonds with C-11, C-15, C-1′, C-19, C-3′, and C-3 and via 2 bonds with C-12, C-2′, C-14). H-12 showed correlations only with C-2, C-14, C-2′, and C-3. Considering these observations H-12 and H-13 could be differentiated. H-6′ could be assigned because of its HMBC correlations with C-2, C-5′, C-4′, and C-2′. The methoxy groups could be assigned from their NOESY correlations: The protons at δ 3.84 (5′-OCH$_3$) showed NOESY correlation with H-6′ (δ 7.66); the 16-OCH$_3$ protons (δ 3.57) gave a cross signal with H-15 (δ 7.24), and the methoxy protons in position 3′ (δ 3.91) correlated with H-13 (δ 5.70).

The relative configuration of compound **2** was deduced from 1D and 2D NMR data: H-13 (δ 5.70) resonates as a sharp singlet. This indicates that H-12 and H-13 are not diaxially oriented. Due to the overlapping of resonances H-12 and H-11e in pyridine-d_5, a more detailed analysis was not possible using this solvent. However, these resonances were well separated in the proton and HSQC spectra of **2** recorded in acetone-d_6. H-11a (δ 3.24) resonated as a triplet with a large coupling constant (10 Hz). H-12 (δ 3.60) and H-11e (δ 3.79) both resonated as double doublets (J = 10 Hz, 4 Hz), while H-13 appeared as a sharp singlet. From these findings, an axial orientation of H-12 and an equatorial orientation of H-13 was deduced.

The absolute configuration of compound **2** was determined by CD measurement. The negative Cotton effect at

260 nm probably resulted from an overlap of the $\pi \rightarrow \pi^*$ transition of the carbonyl chromophore and the transition of the biphenylmethylidene C-13 chromophore. When applying the aromatic quadrant rule to the biphenylmethylidene chromophore, the recorded negative Cotton effect is reconcilable with *S* absolute configuration at C-13.[12] With this information and the knowledge of relative configuration, the absolute configuration at C-12 could be determined as *S*.

The molecular backbone of this new flavonolignan is rather unusual. Most known flavonolignans contain a *trans* substituted 1,4-dioxane ring which is formed between a flavonoid and a guaiacylglyceryl moiety as in silybin; in salcolins A and B the flavonoid and the guajacylglyceryl moiety are linked by ether bonds. As far as the skeleton of the new compound **2** is concerned, the only related substance described is the flavonolignan neohydnocarpin, which has been isolated from the seeds of the southeast Asian tree *Hydnocarpus wightiana* Blume (Flacourtiaceae).[13,14] Neohydnocarpin comprises a luteolin moiety and a coniferyl alcohol unit, linked by C−C bonds. The authors explained the biogenesis of this structure via a lignan acid derived from conidendrin or substituting cinnamic acid in flavonoid biosynthesis or by a coupling of the flavone 3-position with the β-carbon of a coniferyl alcohol moiety and subsequent modifications.[13,14] Taking this into consideration, compound **2** could probably be composed of a tricin moiety and a coniferyl alcohol moiety. Sharma et al.[13] claimed that the relative configuration at the benzylic positions of neohydnocarpin must be *cis*. We deduced the same relative configuration for compound **2** from 2D NMR data, and we additionally determined the absolute configuration as 12*S*, 13*S* from circular dichroic data.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241 MC polarimeter using a microcuvette (path length 1 dm). CD measurements were carried out on a Jasco J-715 spectropolarimeter using a 0.1 cm path length cell. Spectra were recorded over the wavelength range from 220 to 500 nm using a resolution of 0.2 nm and a scan speed of 50 nm/min at a temperature of 25 °C. Spectra resulted from the averaging of 5 scans. UV/vis spectra were recorded on a Specord 50 photometer (Analytik Jena). NMR spectra were recorded with a Varian Unity Inova (600 MHz) spectrometer using the parameters described by Seebacher et al.[15] Pyridine-d_5 and acetone-d_6 were used as solvents, and TMS was used as an internal standard. LCMS experiments were performed on a Thermo Finnigan Surveyor liquid chromatograph interfaced with a LCQ Deca XP^{PLUS} mass detector using electrospray ionization (ESI). HRESIMS was performed via flow injection analysis on an Agilent 1100 Series HPLC system and an Agilent G1946D 1100 Series single quadrupole mass spectrometer (SL type). Semipreparative HPLC separations were performed with a Waters 616 pump, a Waters 600S controller, and a Waters 996 photodiode array detector. CE experiments were performed on a HP ^{3D}Capillary Electrophoresis System.

Plant Material. Herbs of *A. sativa* were commercial samples purchased from Kottas (Vienna, Austria). The identity of the plant material was verified by microscopy[16] and TLC.[17] A voucher specimen (EW-AS03) is deposited at the herbarium of the Department of Pharmacognosy of the University of Graz.

Extraction and Isolation. Dried, pulverized oat herbs (2000 g) were sequentially extracted in a Soxhlet apparatus with petroleum ether and CH_2Cl_2 to yield 28 g of CH_2Cl_2 extract. Subsequent separation of the crude extract by Sephadex LH 20 (4.8 × 60 cm; mobile phase: EtOAc) and RP-18 column chromatography (2 × 10 cm; gradient: MeOH/H$_2$O, 50/50−100/0) led to subfraction I (MeOH/H$_2$O, 50/50) contain-

Table 1. ^{13}C and ^{1}H NMR Data (solvent: pyridine-d_5) of Compounds **1a**, **1b**, and **2**[18]

	1a		**1b**		**2**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1						
2	164.2		164.2		160.0	
3	105.9	6.93 (1H, s)	106.0	6.94 (1H, s)	112.2	
4	182.7		182.8		181.5	
5	163.6		163.7		163.4	
6	100.3	6.73 (1H, d, 1.6 Hz)	100.4	6.74 (1H, d, 1.6 Hz)	100.2	6.67 (1H, s)
7	166.5		166.7		166.0	
8	95.2	6.81 (1H, d, 1.6 Hz)	95.3	6.82 (1H, d, 1.6 Hz)	95.2	6.83 (1H, s)
9	158.9		158.9		158.2	
10	105.3		105.2		105.4	
11e	61.8	4.59 (1H, dd, 12.4 Hz, 5.1 Hz)	61.9	4.39 (1H, dd, 12.0 Hz, 3.4 Hz)	62.7	4.37 (1H, s)
11a		4.30 (1H, dd, 12.3 Hz, 3.4 Hz)		4.05 (1H, dd, 12.0 Hz, 3.3 Hz)		3.84 (1H, s)
12	88.2	5.03 (1H, q, 5.2 Hz, 3.65 Hz)	89.1	4.91 (1H, q, 5.1 Hz, 3.65 Hz)	42.8	4.38 (1H, s)
13	74.1	5.65 (1H, d, 4.8 Hz)	74.1	5.72 (1H, d, 4.8 Hz)	37.4	5.70 (1H, s)
14	134.5		134.2		136.3	
15	112.0	7.52 (1H, s)	112.1	7.56 (1H, s)	112.9	7.24 (1H, s)
16	148.7		148.6		148.9	
17	147.7		147.9		147.0	
18	116.1	7.24 (1H, d, 8.1 Hz)	116.1	7.25 (1H, d, 8.2 Hz)	116.5	7.01 (1H, d, 7.3 Hz)
19	120.6	7.35 (1H, d, 8.1 Hz)	120.5	7.45 (1H, d, 8.2 Hz)	120.9	6.86 (1H, d, 7.3 Hz)
1′	127.2		127.3		118.5	
2′	105.2	7.29 (2H, s)	105.3	7.30 (1H, s)	129.1	
3′	154.3		154.1		148.0	
4′	140.9		141.8		146.2	
5′	154.3		154.1		149.4	
6′	105.2	7.29 (2H, s)	105.3	7.30 (1H, s)	104.4	7.66 (1H, s)
3′-OCH$_3$	56.6	3.82 (3H, s)	56.6	3.83 (3H, s)	60.5	3.91 (3H, s)
5′-OCH$_3$	56.6	3.82 (3H, s)	56.6	3.83 (3H, s)	56.6	3.84 (3H, s)
16-OCH$_3$	56.0	3.75 (3H, s)	56.0	3.73 (3H, s)	56.0	3.57 (3H, s)

ing compound **2** and subfraction II (MeOH/H$_2$O, 60/40 and 70/30) containing compounds **1a** and **1b**.

Both subfractions were further separated by semipreparative HPLC (column: LiChrosorb RP-18 (7 μm) LiChroCART 250-10 HPLC cartridge, E. Merck, Darmstadt; Cat. Nr. 16817; mobile phase: 20% acetonitrile, 80% H$_2$O:THF 69:5; flow rate: 2.6 mL/min), which yielded compound **2** (t_R = 19.83 min, 1.2 mg), subfraction IIa (t_R = 24.54 min, containing compound **1a**), and subfraction IIb (t_R = 28.54 min, containing compound **1b**). Final purification of subfractions IIa and IIb by Sephadex LH 20 column chromatography (1.8 × 82 cm) led to the isolation of compound **1a** (0.9 mg) and compound **1b** (0.9 mg), respectively.

(1a) Tricin-4′-*O*-(*erythro*-β-guaiacylglyceryl) ether [salcolin B; 5,7-dihydroxy-2-[4-{hydroxy-2-(4-hydroxy-3-methoxyphenyl)-1-(hydroxymethyl)ethoxy}-3,5-dimethoxyphenyl]-4*H*-1-benzopyran-4-one]: yellow, amorphous powder (0.9 mg); UV (MeOH) λ_{max} 271, 285sh, 305sh, 335 nm; $[\alpha]_D^{24}$ +15° (*c* about 0.05, MeOH); ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5), see Table 1; CD $[\theta]_{271,335}$ 0; LCESIMS (positive ion mode) (*m/z*) 527 [M + H]$^+$, 331 [tricin + H]$^+$, 316 [(tricin + H) − CH$_3$]$^+$.

(1b) Tricin-4′-*O*-(*threo*-β-guaiacylglyceryl) ether [salcolin A; 5,7-dihydroxy-2-[4-{2-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-1-(hydroxymethyl)ethoxy}-3,5-dimethoxyphenyl]-4*H*-1-benzopyran-4-one]: yellow amorphous powder (0.9 mg); UV (MeOH) λ_{max} 271, 287sh, 303sh, 333 nm; $[\alpha]_D^{24}$ −10° (*c* about 0.05, MeOH); ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5), see Table 1; CD $[\theta]_{271,333}$ 0; LCESIMS (positive ion mode) (*m/z*) 527 [M + H]$^+$, 331 [tricin + H]$^+$, 316 [(tricin + H) − CH$_3$]$^+$.

(2) (−)-(5*S*,6*S*)-5,6-Dihydro-3,8,10-trihydroxy-5-(4-hydroxy-3-methoxyphenyl)-6-hydroxymethyl-2,4-dimethoxy-7*H*-benzo[*c*]xanthen-7-one: yellow amorphous powder (1.2 mg); UV (MeOH) λ_{max} 271, 365 nm; $[\alpha]_{Hg}^{23}$ −43.5° (*c* 0.1, MeOH); ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5), see Table 1; CD $[\theta]_{261}$ −918, $[\theta]_{298}$ 0, $[\theta]_{322}$ −334, $[\theta]_{394}$ 0; LCESIMS (negative ion mode) (*m/z*) 507 [M − H]$^-$, 492 [(M − H) − CH$_3$]$^-$, 477 [(M − H) − 2CH$_3$]$^-$, 461 [(M − H − 2CH$_3$) − 16]$^-$, 446 [(M − H − 2CH$_3$) − OCH$_3$]$^-$, 339 [(M − H − C$_9$H$_{12}$O$_3$)]$^-$; HRESIMS (negative ion mode) (*m/z*) 507.4 (M − H, calcd for C$_{27}$H$_{23}$O$_{10}$, 507.4687).

CE Examination of Compounds 1a and 1b. Separations were carried out at ambient temperature using a fused silica capillary (58.5 cm, 50 μm i.d., 50 cm effective length (Optronis, Kehl, Germany)). Experiments were performed on an HPCE instrument (Agilent Technologies, Waldbronn, Germany) equipped with a DAD UV detector. Sodium borate and β-cyclodextrin were purchased from Fluka (Buchs, Switzerland). The running electrolyte was a mixture of a 50 mM aqueous sodium borate solution containing 1.8% β-cyclodextrin as a chiral selector and MeOH (85:15). The electrolyte was degassed with He and filtered through a syringe filter (20 μm, Schleicher and Schuell, Dassel, Germany) prior to use. Samples were applied by pressure (20 mbar × 9 s); voltage during measurement was 20 kV.

References and Notes

(1) Hegi, G. In *Illustrierte Flora von Mitteleuropa*; Conert, H. J., Ed.; Parey Buchverlag: Berlin, 1998; Vol. 1, Part 3, p 212.
(2) Schneider, E. *Z. Phytother.* **1985**, *6*, 165−167.
(3) Tschesche, R.; Schmidt, W. *Z. Naturforsch.* **1966**, *21*, 896−897.
(4) Chopin, J.; Dellamonica, G.; Bouillant, M. L.; Besset, A.; Popovici, G.; Weissenböck, G. *Phytochemistry* **1977**, *16*, 2041−2043.
(5) Popovici, G.; Weissenböck, G.; Bouillant, M. L.; Dellamonica, G.; Chopin, J. *Z. Pflanzenphysiol.* **1977**, *85*, 103−115.
(6) Bouaziz, M.; Veitch, N. C.; Grayer, R. J.; Simmonds, M. S. J.; Damak, M. *Phytochemistry* **2002**, *60*, 515−520.
(7) Nakajima, Y.; Yun, Y. S.; Kunugi, A. *Tetrahedron* **2003**, *59*, 8011−8015.
(8) Schmid, M. G.; Wirnsberger, K.; Jira, T.; Bunke, A.; Gübitz, G. *Chirality* **1997**, *9*, 153−156.
(9) Harada, N.; Sato, H.; Nakanishi, K. *J. Chem. Soc., Chem. Commun.* **1970**, 1691−1693.
(10) Syrchina, A. I.; Gorshkov, A. G.; Shcherbakov, V. V.; Zichenko, S. V.; Vereshchagin, A. L.; Zaikov, K. L.; Semenov, K. L. *Khim. Prir. Soed.* **1992**, *2*, 182−186.
(11) Cooper, R.; Gottlieb, H. E.; Lavie, D. *Isr. J. Chem.* **1977**, *16*, 12−15.
(12) DeAngelis, G. G.; Wildman, W. C. *Tetrahedron* **1969**, *25*, 5099−5112.
(13) Sharma, D. K.; Ranganathan, K. R.; Parthasarathy, M. R.; Bhushan, B.; Seshadri, T. R. *Planta Med.* **1979**, *37*, 79−83.
(14) Parthasarathy, M. R.; Ranganathan, K. R.; Sharma, D. K. *Phytochemistry* **1979**, *18*, 506−508.
(15) Seebacher, W.; Simic, N.; Weis, R.; Saf, R.; Kunert, O. *Magn. Reson. Chem.* **2003**, *41*, 636−638.

(16) Hänsel, R.; Keller, K.; Rimpler, H.; Schneider, G. *Hager's Handbuch der Pharmazeutischen Praxis*; Springer-Verlag: Berlin, Heidelberg, 1992; Vol. 4, pp 437−445.

(17) Wagner, H.; Bladt, S. *Plant Drug Analysis*; Springer-Verlag: Berlin, 2001; p 242.

(18) Assignments were made using HSQC and HMBC techniques; the numbers of the positions correlate with the numbers used in Figure 2.

NP049636K