Tyrolobibenzyls – Novel Secondary Metabolites from *Scorzonera humilis*

by **Christian Zidorn**[a]), **Ernst P. Ellmerer-Müller**[b]), and **Hermann Stuppner**[a])*

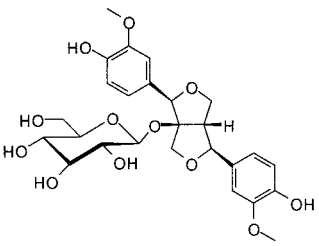
[a]) Institut für Pharmazie, Universität Innsbruck, Innrain 52, A-6020 Innsbruck
[b]) Institut für Organische Chemie, Universität Innsbruck, Innrain 52a, A-6020 Innsbruck

Tyrolobibenzyls A (**2**), B (**3**), and C (**4**) were isolated from *Scorzonera humilis* (Asteraceae) of Tyrolean origin. Compounds **2** and **3** possess a unique phenylethyl-benzofuran backbone and represent a new class of natural compounds. Structural assignments are based on 1D and 2D NMR as well as MS data. Furthermore, peracetylated derivatives of compounds **3** and **4** were synthesized. Pharmacological evaluation of these compounds by the [^{3}H]thymidine assay showed no pronounced cytotoxicity.

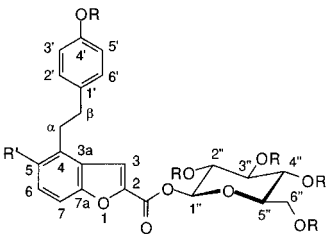
1. Introduction. – *Scorzonera humilis* L. (Asteraceae) is a perennial rosette plant of 10–40 cm height with yellow solitary flowerheads. Its distribution area covers the submediterranean and temperate zone of Europe from Portugal to Russia [1]. In Central Europe, this species is restricted to wet habitats and is considered an endangered plant [2]. In traditional medicine, *S. humilis* has been used as a remedy for wound healing and gastro-intestinal disorders [3]. Knowledge about the secondary metabolite spectrum of the whole genus *Scorzonera* is limited. Prior investigations of *S. hispanica* (black salsify) led to the isolation and identification of 3,4-dimethoxycinnamic acid methyl ester, β-sitosterol, the lignan (1*S*,3a*R*,4*S*,6a*R*)-1,4-bis(4-β-D-glucopyranosyloxy-3,5-dimethoxyphenyl)tetrahydrofuro[3,4-*c*]furan, the bisabolane derivative puliglutone, as well as the sesquiterpene lactone glucosides scorzoneroside, ixerisoside D, and 3-*O*-angeloyl-11β,13-dihydrodesacylcynaropicrin-8-yl β-D-glucoside [4].

2. Results and Discussion. – The MeOH extract (60.0 g) of air-dried subaerial parts (190.8 g) of *S. humilis* was partitioned between H_2O/MeOH and AcOEt, and the AcOEt fraction was subjected to repeated column chromatography (CC) over silica gel and then over *Lobar RP18*, yielding compounds **1** (17.2 mg), **2** (133.8 mg), **3** (158.2 mg), and **4** (93.4 mg). Furthermore, compounds **3** and **4** were acetylated and subsequently purified by means of CC (silica gel) and semi-prep. HPLC to give the pure peracetyl derivatives **3a** and **4a**, respectively.

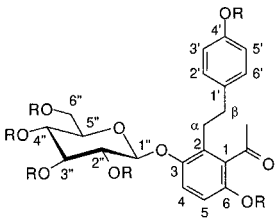
Compound **1** was identified by comparison of its ESI-MS and ^{1}H- and ^{13}C-NMR data with literature values as the lignan pinoresinol-1-yl β-D-glucopyranoside (=(1*S*,3a*R*,4*R*,6a*R*)-tetrahydro-1,4-bis(4-hydroxy-2-methoxyphenyl)-1*H*,3*H*-furo[3,4-*c*]furan-3a-yl β-D-glucopyranoside), formerly isolated from *Stauntonia hexaphylla* DECNE (Lardizabalaceae), *Forsythia suspensa* VAHL. (Oleacae) and *Olea europaea* L. as well as *Olea africana* MILL. (Oleaceae) [5].



1



2 R=R'=H
3 R=H, R'=OH
3a R=R'=AcO



4 R=H
4a R=AcO

The ESI-MS of compound **2** displayed signals at *m/z* 443 ($[M-H]^-$) and 281 ($[M-\text{glucose}-H]^-$), which is consistent with the molecular formula $C_{23}H_{24}O_9$. The ^{1}H-NMR spectrum (*Table 1*) exhibited signals for a β-D-glucose moiety, signals assignable to a *para*-substituted aromatic ring, three vicinal aromatic protons and one isolated methine group (H–C(3)), indicating the presence of a second aromatic ring system, and signals for two methylene groups attributable to an ethanediyl moiety.

These data, together with the ^{13}C-NMR and DEPT spectra (*Table 2*) and the HSQC and HMBC experiments (*Table 3*) established the structure of **2** as 4-[2-(4-hydroxyphenyl)ethyl]benzofuran-2-carboxylic acid (2*S*,3*R*,4*S*,5*S*,6*R*)-tetrahydro-3,4,5-trihydroxy-6-(hydroxymethyl)-2*H*-pyran-2-yl ester (=β-D-glucopyranosyl 4-[2-(4-hydroxyphenyl)ethyl]benzofuran-2-carboxylate). This substance belongs to a new type of natural compounds for which we propose the name tyrolobibenzyls due to the origin of the plant material and one characteristic part of the molecule; thus, **2** is called tyrolobibenzyl A. So far neither natural nor synthesized (phenylethyl)benzofuran-2-carboxylic acid derivatives are known in the literature[1]).

The ^{13}C-NMR and DEPT spectra (*Table 2*) of **2** showed signals of thirteen CH groups, seven quarternary C-atoms, and three CH_2 groups. Two of the aromatic CH signals at δ(C) 130.5 and 116.0 exhibited double intensities and could be assigned to the atoms C(2′), C(6′), and C(5′) of a 4-substituted phenyl ring, respectively. Taking into account the downfield-shifted signal of C(4′) (δ(C) 156.4) and mass spectrometry data, the substituent at C(4′) had to be a OH group.

HSQC and HMBC Experiments (*Table 3*) with **2** established the second aromatic system as a benzofuran-2-carboxylic acid derivative. Accordingly, the HMBC spectrum showed couplings between *H*–C(5) and C(3a), *H*–C(7) and C(3a), *H*–C(6) and C(7a), *H*–C(3) and C(3a), as well as *H*–C(3) and the exocyclic COO group.

The linkages between the 4-hydroxyphenyl ring, the benzofuran, and the sugar moiety of **2** were deduced from the HMBC experiment, too. A cross-peak between the signal of *H*–C(1″) (δ(H) = 5.40) and the signal of the exocyclic COO group (δ(C′) = 168.7) revealed that the anomeric OH group of β-D-glucose was esterified with the 2-carboxy moiety of the benzofuran ring. On the other hand, two- and three-bond couplings between the CH_2 groups of the ethanediyl moiety with C(6′), C(1′), and C(2′), as well as with C(4), C(5), and C(3a) confirmed that the 4-hydroxyphenyl ring was attached at C(4) of the benzofuran ring *via* CH_2CH_2.

The ESI-MS of compound **3** displayed signals at *m/z* 459 ($[M-H]^-$) and 297 ($[M-\text{glucose}-H]^-$), which is consistent with the molecular formula $C_{23}H_{24}O_{10}$. Except for differences due to a missing aromatic CH group at position 5, which in

---

[1]) *Chemical Abstracts Services* online search from April 28, 2000.

Table 1. *^{1}H-NMR Spectra of* **2**, **3**, **3a**, **4**, *and* **4a**[a])[b]). δ in ppm rel. to $SiMe_4$, J in Hz.

	2	**3**	**3a**[c])		**4**	**4a**[d])
H–C(3)	6.04 (*s*)	6.02 (*s*)	5.89 (*s*)	H–C(4)	7.21 (*d*, *J*(4,5) = 9.0)	7.07 (*d*, *J*(4,5) = 8.5)
H–C(5)	6.96 (*dd*, *J*(5,6) = 8.0, *J*(5,7) = 1.0)			H–C(5)	6.73 (*d*, *J*(5,4) = 9.0)	6.99 (*d*, *J*(4,5) = 8.5)
H–C(6)	7.41 (*dd*, *J*(6,5) = 8.0, *J*(6,7) = 8.0)	7.14 (br. *d*, *J* = 9.0)	7.25 (*d*, *J* = 9.0)	MeCO–C(1)	2.33 (*s*)	2.28 (*s*)
H–C(7)	7.18 (*dd*, *J*(7,6) = 8.0, *J*(7,5) = 1.0)	7.10 (br. *d*, *J* = 9.0)	7.21 (*d*, *J* = 9.0)			
2 H–C(α)	3.75 (*m*)	3.58 (*m*)	3.37 (*dt*, *J* = 13.5, 7.0)	2 H–C(α)	2.91 (*m*)	2.70 (*m*[f]))
	3.11 (*m*)	3.33 (*m*)	2.90 (*dt*, *J* = 13.5, 7.0)		2.80 (*m*)	
2 H–C(β)	2.89 (*m*)	2.76 (*m*)	2.90 (*dt*, *J* = 13.5, 7.0)	2 H–C(β)	2.80 (*m*)	2.81 (*m*)
	2.77 (*m*)		2.80 (*dt*, *J* = 13.5, 7.0)		2.80 (*m*)	2.71 (*m*[f]))
H–C(2′)	6.97[e]), 6.98[e])	7.15[e]), 7.14[e])	7.04[e]), 7.03[e])	H–C(2′)	7.06[e]), 7.05[e])	7.18[e]), 7.17[e])
H–C(3′)	6.66[e]), 6.67[e])	6.73[e]), 6.72[e])	6.91[e]), 6.90[e])	H–C(3′)	6.75[e]), 6.74[e])	6.98[e]), 6.97[e])
H–C(5′)	6.66[e]), 6.67[e])	6.73[e]), 6.72[e])	6.91[e]), 6.90[e])	H–C(5′)	6.75[e]), 6.74[e])	6.98[e]), 6.97[e])
H–C(6′)	6.97[e]), 6.98[e])	7.15[e]), 7.14[e])	7.04[e]), 7.03[e])	H–C(6′)	7.06[e]), 7.05[e])	7.18[e]), 7.17[e])
H–C(1″)	5.40 (*d*, ^3J = 8.0)	5.33 (*d*, *J* = 8.0)	5.44 (*d*, *J* = 8.0)	H–C(1″)	4.90 (*d*, *J* = 8.0)	5.12 (*d*, *J* = (8.0)
H–C(2″)	3.59 (*m*[f])	3.62 (*dd*, *J* = 8.0, 8.0)	5.23 (*dd*, *J* = 9.5, 8.0)	H–C(2″)	3.57 (*dd*, *J* = 9.5, 8.0)	5.38 (*dd*, *J* = 9.5, 8.0)
H–C(3″)	3.51 (*dd*, ^3J = 9.0, 9.0)	3.53 (*dd*, *J* = 9.0, 9.0)	5.32 (*dd*, *J* = 9.5, 9.5)	H–C(3″)	3.52 (*m*)	5.31 (*dd*, *J* = 9.5, 9.5)
H–C(4″)	3.44 (*dd*, ^3J = 9.0, 9.0)	3.40 (*m*)	5.16 (*dd*, *J* = 9.5, 9.5)	H–C(4″)	3.46 (*m*)	5.21 (*dd*, *J* = 9.5, 9.5)
H–C(5″)	3.57 (*m*[f])	3.48 (br. *d*, *J* = 9.0)	3.93 (*ddd*, *J* = 9.5, 6.0, 2.0)	H–C(5″)	3.46 (*m*)	3.87 (*ddd*, *J* = 9.5, 5.5, 2.5)
2 H–C(6″)	3.94 (*dd*, ^2J = 12.5, ^3J = 2.5)	3.96 (*dd*, *J* = 12.5, 2.5)	4.30 (*dd*, *J* = 12.5, 6.0)	2 H–C(6″)	3.96 (*dd*, *J* = 12.0, 1.5)	4.29 (*dd*, *J* = 12.5, 5.5)
	3.75 (*dd*, ^2J = 12.5, ^3J = 5.5)	3.78 (*dd*, *J* = 12.5, 5.5)	4.19 (*dd*, *J* = 12.5, 2.0)		3.77 (*dd*, *J* = 12.0, 5.0)	4.18 (*dd*, *J* = 12.5, 2.5)

[a]) All assignments are based on HSQC and HMBC experiments. [b]) Arbitrary numbering (see *Formulae*). *c*) MeCO *s* at 2.28, 2.20, 2.10, 2.08, 2.03, and 2.00. [d]) MeCO *s* at 2.29, 2.26, 2.06 (2×), 2.04, 1.96. [e]) Most intense signals of the *AA′XX′* spin system. [f]) Overlapping signals.

compound **3** is replaced by a quaternary C-atom substituted by an OH group. ^{1}H- (*Table 1*) and ^{13}C-NMR (*Table 2*) data of **3** were nearly identical with those of **2**. Hydroxylation at C(5) was supported by comparison of the ^{13}C-NMR spectrum of **3** with that of **2** (downfield-shifted signal of C(5) ($\Delta\delta = +24.2$ ppm) and upfield-shifted signals for the vicinal C(4) and C(6) ($\Delta\delta = 15.2$ and -12.0 ppm, resp.) in **3**). The assigned structure was confirmed by HSQC and HMBC experiments with **3** (*Table 3*) and the data of peracetylated derivative **3a** (*Tables 1* and *2*). Thus, **3** is 5-hydroxy-4-[2-(4-hydroxyphenyl)ethyl]benzofuran-3-carboxylic acid (2*S*,3*R*,4*S*,5*S*,6*R*)-tetrahydro-3,4,5-trihydroxy-6-(hydroxymethyl)-2*H*-pyran-2-yl ester (= β-D-glucopyranosyl 5-hydroxy-4-[2-(4-hydroxyphenyl)ethyl]benzofuran-2-carboxylate) or tyrolobibenzyl B.

The ESI-MS of compound **4** displayed signals at m/z 433 ($[M-H]^-$) and 271 ($[M-\text{glucose}-H]^-$), which is consistent with the molecular formula $C_{22}H_{26}O_9$. Partial structures could be established by the ^{1}H-NMR spectrum (*Table 1*), which revealed

Table 2. *^{13}C-NMR Spectra of* **2**, **3**, **3a**, **4**, *and* **4a**. Signal assignments by HSQC and HMBC data, δ in ppm rel. to $SiMe_4$.

	2	**3**	**3a**[a])		**4**	**4a**[b])
C(2)	164.8	165.4	162.5	C(1)	131.0	136.4
CH(3)	94.3	94.2	95.1	C(2)	130.0	129.1
C(3a)	114.8	115.4	114.0	C(3)	150.5	152.8
C(4)	142.8	127.6	121.4	CH(4)	119.4	116.1
CH(5) or O–C(5)	129.5	153.7	151.7	CH(5)	114.7	121.5
CH(6)	133.0	121.0	129.5	C(6)	150.4	141.9
CH(7)	116.3	116.2	116.3	Me_3CO	208.4	202.7
C(7a)	156.2	149.5	154.8	*Me*CO	32.1	32.0
COO	168.7	169.2	165.9	$CH_2(\alpha)$	31.0	30.4
$CH_2(\alpha)$	39.2	30.8	27.8	$CH_2(\beta)$	36.6	35.7
$CH_2(\beta)$	38.5	36.7	35.7	C(1′)	134.0	139.3
C(1′)	133.8	135.0	138.8	CH(2′)	129.7	129.7
CH(2′)	130.5	130.4	130.0	CH(3′)	116.0	121.7
CH(3″)	116.0	116.0	121.7	C(4′)	156.4	149.2
C(4′)	156.4	156.2	149.5	CH(5′)	116.0	121.7
CH(5′)	116.0	116.0	121.7	CH(6′)	129.7	129.7
CH(6′)	130.5	130.4	130.0	CH(1″)	103.8	99.2
CH(1″)	101.2	101.1	96.8	CH(2″)	75.3	71.2
CH(2″)	74.4	74.3	72.6	CH(3″)	78.5	73.0
CH(3″)	78.3	78.7	71.2	CH(4″)	71.5	68.4
CH(4″)	70.9	70.9	67.7	CH(5″)	78.2	72.3
CH(5″)	78.8	78.3	72.8	$CH_2(6'')$	62.6	62.0
CH(6″)	62.3	62.3	61.7			

[a]) MeCO: 170.8, 21.2; 171.2, 21.0; 169.8, 20.7; 170.3, 20.7; 170.3, 20.6; 170.3, 20.6. [b]) MeCO: 170.6, 20.8; 170.3, 20.7; 169.9, 21.3; 169.8, 20.7; 169.5, 20.7; 169.3, 21.2.

Table 3. *Important HMBC Cross-Peaks Observed for Compounds* **2**–**4**

2		**4**		**4a**	
Proton	C-Atom	Proton	C-Atom	Proton	C-Atom
H–C(3)	C(2), COO, C(3a)	H–C(4)	C(2), C(6)	H–C(4)	C(2), C(3), C(5), C(6)
H–C(5)	C(3a), C(4), C(7)	H–C(5)	C(1), C(3)	H–C(5)	C(1), C(3), C(4), C(6)
H–C(6)	C(4), C(5), C(7), C(7a)				
H–C(7)	C(3a), C(5), C(7a)	*Me*CO	Me*C*O	*Me*CO	Me*C*O, C(1)
H_a–C(α)	C(3a), C(4), C(5), C(1′)	H_a–C(α)	C(1), C(2), C(3), C(β), C(1′)	H_a–C(α)	C(1), C(2), C(3), C(β), C(1′)
H_b–C(α)	C(3a), C(4), C(5), C(1′)	H_b–C(α)	C(2), C(3), C(β), C(1′)	H_b–C(α)	C(2), C(3), C(β), C(1′)
H_a–C(β)	C(4), C(1′), C(2′)/C(6′)	H_a–C(β)	C(2), C(α), C(1′)	H_a–C(β)	C(2), C(α'), C(1′)
H_b–C(β)	C(4), C(1′), C(2′)/C(6′)	H_b–C(β)	C(2), C(α), C(1′)	H_b–C(β)	C(2), C(α'), C(1′)
H–C(2′)/H–C(6′)	C(β), C(1′), C(2′)/C(6′), C(3′)/C(5′), C(4′)	H–C(2′)/H–C(6′)	C(β), C(2′)/ C(6′), C(4′)	H–C(2′)/ H–C(6′)	C(β), C(2′)/ C(6′), C(4′)
H–C(3′)/H–C(5′)	C(1′), C(2′)/C(4′), C(3′)/C(5′), C(4′)	H–C(3′)/H–C(5′)	C(1′), C(3′)/ C(5′), C(4′)	H–C(3′)/ H–C(5′)	C(1′), C(3′)/ C(5′), C(4′)
H–C(1″)	COO	H–C(1″) C(3)	H–C(1″) C(3)		

signals due to β-D-glucose, a 4-(hydroxyphenyl)ethyl moiety, a tetrasubstituted benzene ring and a downfield-shifted Me group. The ^{13}C-NMR (*Table 2*), HSCQ, and HMBC experiments (*Table 3*) allowed us to establish the structure of **4** as 1-{3-(β-D-glucopyranosyloxy)-6-hydroxy-2-[2-(4-hydroxyphenyl)ethyl]phenyl}ethanone or tyrolobibenzyl C. Compound **4** represents a new natural compound and seems, in spite of the lack of a benzofuran ring system, to be biogenetically closely related to compounds **2** and **3**.

The ^{13}C-NMR and DEPT spectrum (*Table 2*) of **4** showed twenty signals, two of them, at δ 129.7 (C(2′), C(6′)) and 116.0 (C(3′), C(5′)) with double intensity. Twelve of these signals, including the ones with double intensities, were the same as the corresponding signals of **2** and **3** and assignable to the (4-hydroxyphenyl)ethyl and the β-D-glucose moieties. Two of the remaining eight signals were attributable to an ethanone, *i.e.* δ 204.7 (CO) and 32.1 (Me), and the other six signals to the tetrasubstituted benzene ring.

The partial structures established by ^{1}H- and ^{13}C-NMR could be connected by means of the long-range correlations observed in the HMBC spectrum. ^{1}H-NMR Multiplicities and coupling constants ($J(4,5) = J(5,4) =$ 9.0 Hz) of the aromatic H–C(4) and H–C(5) of the acetophenone moiety revealed that these protons had to be located vicinal to each other. The connection of β-D-glucose *via* an ether bond to C(3) of the acetophenone moiety was evidenced by the downfield shift of the anomeric-proton signal (δ 4.90) and HMBC cross-peak with the C(3) signal. Two- and three-bond couplings between the signal of 1 *H*–C(α) of the ethanediyl moiety and the signals of C(1), C(2), and C(3) localized the (4-hydroxyphenyl)ethyl moiety at C(2). The position of the ethanone group at C(1) was established by the HMBC cross-peak between the Me signal at δ 2.33 and that of C(1) at δ 131.0. Finally, three-bond couplings of *H*–C(4) with C(2) and C(6), as well as of *H*–C(5) with C(1) and C(3) revealed that the OH substituent was attached at C(6).

To verify the structure of compound **4**, it was acetylated to **4a**. The molecular mass of **4a** determined by ESI-MS as 686 (*m*/*z* 686) showed the expected increase of 252 mass units in comparison with **4**. Additionally, ^{1}H-NMR (*Table 1*), ^{13}C-NMR (*Table 2*), HSQC, and HMBC (*Table 3*) data were in agreement with the proposed structure **4a**.

In a first attempt to evaluate the new natural products **2**–**4** for a potential pharmacological activity, we tested these substances, as well as the corresponding acetyl derivatives **3a** and **4a** and the known compound **1**, in the (^{3}H)thymidine cytotoxicity assay against leukaemic cell lines GTB and HL60 [6]. None of the tested substances showed any cytotoxic effects in the concentration range between 0.25 and 4.00 μmol/l.

Pharmacological evaluations in other areas, like *e.g.* inflammation and antibiosis, are in progress. Furthermore, we are currently investigating the infrageneric distribution of this unique class of substances within the genus *Scorzonera* to obtain clues about their potential as chemotaxonomic markers.

The authors wish to thank Ms. *M. Stütz* for valuable technical assistance, Mag. pharm. *D. Weigand* for IR measurements, Prof. Dr. *K.-H. Ongania* and Dr. *S. Sturm* for MS measurements, as well as Dr. *R. Hatschenberger* and Prof. Dr. *G. Konwalinka* for cytotoxicity assays.

Experimental Part

General. Column chromatography (CC): *Merck* silica gel *60* (40–63 μm). Semi-prep. HPLC: *Merck* 250 × 10 mm *LiChrospher® RP-18* (10 μm material) column; isocratic MeCN/H_2O 1:1, flow rate 3.5 ml/min; detection at 205 nm; for each run, injection of a 100-μl aliquot of a soln. containing 10 mg/ml of substance. FT-IR: $\tilde{\nu}_{max}^{ZnSe}$ in cm^{-1}; microspectrometry. NMR Spectra: *Varian-Unityplus-500* spectrometer at 500 (^{1}H) and 125 MHz (^{13}C) in CD_3OD (**1**–**4**) or $CDCl_3$ (**3a** and **4a**). MS: ESI, neg. mode; FAB, pos. mode; *m*/*z* (rel. %).

Plant Material. Scorzonera humilis L. was collected in the Leutasch valley near Unterkirchen, Tyrol, Austria, at an altitude of 1020 m. A voucher specimen is deposited in the herbarium of the Institute of Pharmacy.

Extraction and Isolation. Partitioning of the MeOH extract (60.0 g) obtained from freeze-dried subaerial parts (190.8 g) of *S. humilis* between H_2O/MeOH 2:1 and AcOEt yielded 18.4 g from the AcOEt phase.

Subsequent repeated CC (silica gel, gradients of petroleum ether/CH_2Cl_2/Me_2CO, AcOEt/Me_2CO, AcOEt/MeOH, and CH_2Cl_2/MeOH), CC (*Sephadex LH-20*, Me_3OH/AcOEt/H_2O (3:1:1), and consecutive CC (*Lobar RP18*, H_2O/MeCN) yielded pure compounds **1** (17.2 mg), **2** (133.8 mg), **3** (158.2 mg), and **4** (93.4 mg).

Tumor Cell-Growth-Inhibition Assays. [^{3}H]Thymidine incorporation assays were performed as described in [6]. Briefly, 100-µl aliquots of the cell suspensions ($1 \cdot 10^6$ cells/ml) were placed into wells of a microtiter plate and layered with 100 µl of medium containing various concentrations of **1**, **2**, **3**, **3a**, **4**, or **4a** in a concentration range between 0.25 and 4.00 µmol/l. Controls contained 100 µl of pure medium without the test substances. Plates were then incubated for 48 h at 37° under 5% CO_2 atmosphere and high humidity. All cultures were then pulsed for 24 h with 0.5 mCi of [^{3}H]thymidine per well. The samples were collected on glass-filter paper with a multiple automated harvester. The filters were dried at 55° and then transferred to scintillation vials containing 4 ml of scintillation fluid. Radioactivity was measured in a liquid scintillation counter. Three replicate wells were used at each point, and experiments were performed in duplicate on different days ($n = 6$).

Tyrolobibenzyl A (**2**). White amorphous solid. M.p. 119–131° (dec.). FT-IR: 3400 (br.), 2921, 1684, 1606, 1564, 1513, 1460, 1377, 1248, 1172, 1074, 1046, 987. ESI-MS: 443 (4, $[M - H]^-$), 281 (100, $[M - \text{glucose} - H]^-$).

Tyrolobibenzyl B (**3**). White amorphous solid. M.p. 136–146° (dec.). FT-IR: 3400 (br.), 2927, 1670, 1612, 1568, 1514, 1458, 1382, 1249, 1176, 1075, 1029, 991. ESI-MS: 919 (100, $[2M - H]^-$), 459 (12, $[M - H]^-$), 297 (6, $[M - \text{glucose} - H]^-$).

Tyrolobibenzyl C (**4**). White amorphous solid. M.p. 118–122° (dec.). FT-IR: 3400 (br.), 2935, 1678, 1611, 1514, 1485, 1453, 1357, 1258, 1075, 1040, 1019. ESI-MS: 433 (100, $[M - H]^-$), 271 (90, $M - \text{glucose} - H]^-$).

Acetylation of **3** *and* **4**. To **3** (20 mg), aliquots of Ac_2O and pyridine were added. This soln. was mixed with cat. amounts of *N,N*-dimethylpyridin-4-amine and kept at r.t. for 24 h. Subsequently, the mixture was separated by CC (silica gel, gradient $CH_2Cl_2 \rightarrow$ MeOH): 23.0 mg of an acetate mixture. The peracetate **3a** was finally purified by isocratic semi-prep. HPLC (H_2O/MeCN 1:1): 11.2 mg of pure peracetate **3a**.

Likewise **4** was acetylated and the product purified: 38.0 mg of peracetate **4a**.

2,3,4,6-Tetra-O-*acetyl-β*-D-*glucopyranosyl 5-Acetoxy-4-[2-(4-acetoxyphenyl)ethyl]benzofuran-2-carboxylate* (**3a**). Colorless crystals. M.p. 231–245° (dec.). FT-IR: 2924, 2854, 1733, 1644, 1465, 1377, 1368, 1250, 1170, 1148, 1097, 1028, 1013, 970. FAB-MS: 735 ($[M + Na]^+$, 100).

1-{6-Acetoxy-2-[2-(4-acetoxyphenyl)ethyl]-3-(2,3,4,6-tetra-O-*acetyl-β*-D-*glucopyranosyloxy)phenyl}ethanone* (**4a**). Colorless crystals. M.p. 279–287° (dec.). FT-IR: 2949, 2880, 1763, 1749, 1704, 1598, 1508, 1465, 1430, 1372, 1242, 1194, 1068, 1045, 909. ESI-MS: 709 (100, $[M + Na]^+$), 379 (95, $[M - (Ac)_4\text{glucose} + Na]^+$).

REFERENCES

[1] H. Meusel, E. J. Jäger, in 'Vergleichende Chorologie der Zentraleuropäischen Flora', G. Fischer Verlag, Jena, 1992, Vol. 3, pp. 133–134, 188, 308–309, 533.

[2] Ed. H. Niklfeld, in 'Rote Listen gefährdeter Pflanzen Österreichs', 2nd edn., Austria Medien Service, Graz, 1999, p. 106, Ed. E. Jedicke, in 'Die Roten Listen', Ulmer, Stuttgart, 1997.

[3] Eds. R. Asche and E.-D. Schultze, in 'Die Ragginer', Verlag Dr. Friedrich Pfeil, München, 1996, p. 334.

[4] V. V. Tolstikhina, O. V. Bryanskii, A. I. Syrchina, A. A. Semenov, *Khim. Prir. Soedin*. **1988**, *24*, 763; O. V. Bryanskii, V. V. Tolstikhina, A. A. Semenov, *Khim. Prir. Soedin* **1992**, *28*, 591; O. V. Bryanskii, V. V. Tolstikhina, S. V. Zinchenko, A. A. Semenov, *Khim. Pri. Soedin*. **1992**, *28*, 640; C. Zidorn, E. P. Ellmerer-Müller, H. Stuppner, *Pharmazie*, submitted.

[5] T. Nikaido, T. Ohmoto, T. Kinoshita, U. Sankawa, S. Nishibe, S. Hisada, *Chem. Pharm. Bull*. **1981**, *29*, 3586; H. Tsukamoto, S. Hisada, S. Nishibe, *Chem. Pharm. Bull*. **1985**, *33*, 1232; H.-B. Wang, R. Mayer, G. Rücker, M. Neugebauer, *Phytochemistry* **1993**, *34*, 1621.

[6] C. Zidorn, H. Stuppner, M. Tiefenthaler, G. Konwalinka, *J. Nat. Prod.* **1999**, *62*, 984.

Received May 4, 2000