Phytochemistry, Vol. 33, No. 4, pp. 813–816, 1993
Printed in Great Britain.

0031-9422/93 $6.00+0.00

ANTI-LEUKAEMIC COMPOUNDS DERIVED FROM STILBENES IN *PICEA ABIES* BARK

ERKKI MANNILA, ANTTI TALVITIE and ERKKI KOLEHMAINEN

Department of Chemistry, University of Jyväskylä, P.O.B 35, 40351 Jyväskylä, Finland

(*Received in revised form* 14 *December* 1992)

Key Word Index—*Picea abies*; Pinaceae; bark; stilbenes; bibenzyls; hydrogenation; methylation; enzymatic deglycosidation; antileukaemic activity.

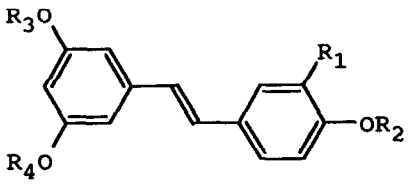
Abstract—Resveratrol was identified and isolated as a minor stilbene derivative from the bark of *Picea abies*. Three derivatives of resveratrol and three additional derivatives of the primary stilbenes from *P. abies* bark were prepared. Improvement over piceatannol in antileukaemic activity was observed in a preliminary test.

INTRODUCTION

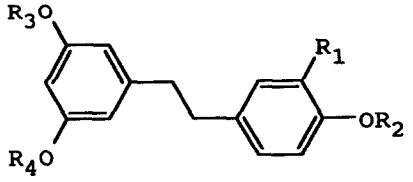
Increasing interest has been paid to primitive medicinal plants to find new substances with potentially useful biological properties. Several stilbene and bibenzyl compounds with such properties have been isolated from, for example, *Combretum caffrum* [1, 2]. In our earlier work we isolated the main stilbene compounds from *Picea abies* bark [3]. Subsequently, we modified the isolated stilbenes hoping to obtain increased biological activity and an indication of the relationship between the structure and activity [4].

RESULTS AND DISCUSSION

Resveratrol, 3,4′,5-trihydroxystilbene (**1**), was isolated as a minor compound. Catalytic hydrogenation of **1** on Pd charcoal catalyst gave 3,4′,5-trihydroxybibenzyl (**2**). Methylation of the hydroxyl groups of **1** with dimethyl



1, 3, 5, 8, 9, 11



2, 4, 6, 7, 10

	R_1	R_2	R_3	R_4	
1	H	H	H	H	**2**
3	H	CH_3	CH_3	CH_3	**4**
5	OH	H	H	H	**6**
-	OH	H	H	Glc	**7**
8	OCH_3	CH_3	CH_3	Glc	-
9	OCH_3	CH_3	CH_3	H	**10**
11	OCH_3	H	H	Glc	

Glc = β-D-Glucopyranosyl

sulphate led to 3,4',5-trimethoxystilbene (**3**), and catalytic hydrogenation of **3** gave 3,4',5-trimethoxybibenzyl (**4**).

Preparing additional stilbene and bibenzyl derivatives from the primary stilbenes from *P. abies* bark by catalytic hydrogenation of the double bond of piceatannol (**5**) was not successful. Therefore, the expected 3,3',4,5'-tetrahydroxybibenzyl (**6**) was produced from 3'-β-D-glucopyranosyloxy-3,4,5'-trihydroxybibenzyl (**7**) via enzymatic hydrolysis of the glycosidic bond. Enzymatic hydrolysis of the glycosidic bond of 3'-β-D-glucopyranosyloxy-3,4,5'-trimethoxystilbene (**8**) gave 3'-hydroxy-3,4,5'-trimethoxystilbene (**9**) also. The corresponding saturated compound, 3'-hydroxy-3,4,5'-trimethoxybibenzyl (**10**), was obtained by catalytic hydrogenation of **9**.

The structural verification of **7** and **8** was carried out in our earlier study [4]. The structures of **1–4**, **6**, **9** and **10** were primarily examined by ^{1}H and ^{13}C NMR spectroscopy (Table 1). Assignment of the ^{13}C NMR signals of **1–4**, **6**, **9** and **10** is based on our earlier work [3, 4]. The second order ^{1}H NMR spectra were resolved using an iterative computation (Table 2) [5]. The ^{1}H NMR para-

Table 1. ^{13}C NMR spectral data of compounds **1–4**, **6**, **9** and **10** (δ values, in acetone-d_6)

C	1	2	3 ($CDCl_3$)	4 ($CDCl_3$)	C	6	9	10
3	159.6	159.3	161.0	160.7	3'	159.6	159.5	159.5
5	159.6	159.3	161.0	160.7	5'	159.6	162.1	162.1
4'	158.2	*	159.4	157.9	3	146.2	150.5	150.6
1	140.9	145.2	139.7	144.3	4	145.6	150.4	149.0
1'	130.1	133.6	130.0	133.9	1'	144.3	140.7	145.5
β	129.1	39.7	128.8	38.5	1	134.9	131.4	135.8
α	126.9	37.5	126.6	36.8	α	38.1	127.5	38.2
2'	128.7	130.1	127.8	129.4	β	39.3	129.5	39.2
6'	128.7	130.1	127.8	129.4	6	120.8	120.8	121.5
3'	116.4	115.9	114.8	113.8	5	116.7	112.8	114.0
5'	116.4	115.9	114.8	113.8	2	116.3	110.5	113.4
2	105.7	107.8	104.8	106.6	6'	108.1	104.1	106.6
6	105.7	107.8	104.8	106.6	2'	108.1	106.8	109.2
4	102.7	101.1	99.7	98.0	4'	101.5	101.5	100.0
Me	—	—	55.4	55.3	Me	—	56.1	56.5
Me	—	—	55.4	55.3	Me	—	56.1	56.4
Me	—	—	55.3	55.3	Me	—	55.4	55.5

* Signal hidden in noise.

Table 2. ^{1}H NMR parameters (δ values in acetone-d_6; J in Hz) for the aromatic and double bond protons of compounds **1–4**, **6**, **9** and **10** calculated with the iterative program MAOCON [5]

	1	2	3 ($CDCl_3$)	4 ($CDCl_3$)		6	9	10
H-2'	6.84	6.74	6.90	6.83	H-2	6.86	7.37	6.80
H-3'	7.42	7.04	7.44	7.10	H-5	6.86	7.22	6.81
H-5'	7.42	7.04	7.44	7.10	H-6	6.69	7.06	6.72
H-6'	6.84	6.74	6.90	6.83	$J_{2,6}$	2.1	2.2	2.0
$J_{2',5'}$	0.1	0.4	0.5	0.0	$J_{2,5}$	0.0	−0.1	0.0
$J_{2',6'}$	2.3	2.5	2.2	2.4	$J_{5,6}$	7.9	8.3	8.1
$J_{3',5'}$	2.5	2.6	2.2	2.4				
$J_{5',6'}$	8.5	8.3	8.3	8.6	H-α	—	7.11	—
$J_{2',3'}$	8.5	8.3	8.3	8.6				
H-α	6.89	—	6.91	—	H-β	—	7.28	—
H-β	7.02	—	7.04	—	$J_{\alpha,\beta}$	—	16.40	—
$J_{\alpha,\beta}$	16.30	—	16.90	—	H-2'	6.36	6.80	6.31
H-2	6.54	6.21	6.65	6.34	H-4'	6.33	6.48	6.23
H-4	6.28	6.19	6.38	6.31	H-6'	6.36	6.79	6.29
H-6	6.54	6.21	6.65	6.34	$J_{2',4'}$	2.1	1.9	2.2
$J_{2,4}$	2.1	2.0	2.0	2.3	$J_{4',6'}$	2.1	1.9	2.2
$J_{4,6}$	2.1	2.0	2.0	2.3	$J_{2',6'}$	2.0	1.6	2.1
$J_{2,6}$	2.1	2.1	2.0	2.2				

meters are in accord with the earlier results [4, 6]. The mass spectra of **1–4**, **6**, **9** and **10** were measured for further characterization of the compounds.

The antileukaemic activity of **1–4**, **6**, **9** and **10** was preliminarily tested on mice leukaemia system L1210 cells using a test substrate concentration of 0.1 mM (Kajander, O., Abcell Oy, Kuopio, Finland, personal communication). Piceatannol (3,3',4,5'-tetrahydroxystilbene) (**5**) has been identified as the antileukaemic principle in the seeds of *Euphorbia lagascae* [7]. The activity of 3,3',4,5'-tetrahydroxybibenzyl (**6**) was found to be higher than, and for 3'-hydroxy-3',4,5'-trimethoxybibenzyl (**10**) equal to that of **5** and isorhapontin (**11**) [8]. The activity of the other compounds **1–4** and **9** was weak. In the case of compounds **6** and **10**, the saturation of the stilbene double bond increased the activity whilst in the case of compounds **2** and **4** it did not.

Tests were made on a 0.1 mM solution of each substrate in H_2O or DMSO. The number of L1210 cells was read after treatment for 48 hr and compared with the control (no addition). The number of cells with resveratrol (**1**) was 71% (s.d.=11%), 2,4',5-trihydroxybibenzyl (**2**) 77% (s.d.=13%), 3,4',5-trimethoxystilbene (**3**) 92% (s.d.=13%), 3,4',5-trimethoxybibenzyl (**4**) 89% (s.d.=11%), 3,3',4,5'-tetrahydroxybibenzyl (**6**) 22% (s.d.=6%), 3'-hydroxy-3,4,5'-trimethoxystilbene (**9**) 72% (s.d.=14%) and 3'-hydroxy-3,4,5'-trimethoxybibenzyl (**10**) 58% (s.d.=8%) with respect to the control.

EXPERIMENTAL

General. EIMS and HRMS at 70 eV. ^{1}H and ^{13}C NMR at 270 and 67.9 MHz, respectively, TMS as int. standard, TLC and flash CC on silica gel. Hydrogenation in EtOH–H_2O (9:1) soln using Pd charcoal catalyst at 25° and 1 atm [9]. Methylation in refluxing dry Me_2CO with Me_2SO_4 in the presence of excess K_2CO_3 [10].

Resveratrol, (E)-3,4',5-*trihydroxystilbene* (**1**). Obtained from the aglycone fr. from *P. abies* bark using flash CC. Elution with CH_2Cl_2–MeOH (9:1) gave **1** between two main aglycones [3], mp 255–260° [11]. In a typical chromatographic run, **1** was obtained in *ca* 20 mg yield from 1 g of oily EtOAc extract of the bark. EIMS *m/z* (rel. int.): 228 ([M]$^+$, 100), 211 (10), 199 (6), 181 (9), 157 (5), 153 (4), 114 (3). ^{1}H NMR (acetone-d_6): δ7.44, 7.43, 7.42, 7.41, 7.40, 7.39, 6.86, 6.85, 6.83, 6.82, 6.81 (A_2B_2, H-2', H-3', H-5', H-6'), 7.05, 6.99, 6.91, 6.85 (AB, α, β), 6.54, 6.53, 6.28, 6.27, 6.26 (A_2X, H-2, H-6, H-4). ^{13}C NMR: Table 1.

3,4',5-*Trihydroxybibenzyl* (**2**). Obtained from **1** (20 mg, 0.09 mmol) by catalytic hydrogenation. Purification by flash CC with $CHCl_3$–EtOH (9:1) elution. Yield 18 mg (89%), mp 153–155° [12]. EIMS *m/z* (rel. int.): 230 ([M]$^+$, 28), 165 (1), 137 (2), 107 (100). ^{1}H NMR (acetone-d_6): δ7.05, 7.04, 7.03, 7.02, 6.75, 6.74, 6.73, 6.72 (A_2B_2, H-2', H-3', H-5', H-6'), 6.22, 6.22, 6.21, 6.19, 6.18, 6.17 (A_2B, H-2, H-6, H-4), 2.79 (CH_2). ^{13}C NMR: Table 1.

(E)-3,4',5-*Trimethoxystilbene* (**3**). Obtained from **1** (25 mg, 0.1 mmol) by methylation. Purification was carried out by flash CC using EtOH elution. Yield 16 mg (59%), crystals, mp 53–54° [12]. EIMS *m/z* (rel. int.): 270 ([M]$^+$, 100), 239 (22), 224 (12), 196 (9), 195 (9), 152 (10). ^{1}H NMR ($CDCl_3$): δ7.45, 7.42, 6.90, 6.89 (A_2B_2, H-2', H-3', H-5', H-6'), 7.07, 7.01, 6.93, 6.87 (AB, α, β), 6.65, 6.54, 6.38, 6.37, 6.36 (A_2X, H-2, H-6, H-4), 3.83 (3 × Me). ^{13}C NMR: Table 1.

3,4',5-*Trimethoxybibenzyl* (**4**). Obtained from **3** (15 mg, 0.06 mmol) by catalytic hydrogenation without chromatographic purification. Yield 14 mg (85%), viscous oil [12]. EIMS *m/z* (rel. int.): 272 ([M]$^+$, 100), 151 (26), 136 (20), 122 (90), 121 (100). ^{1}H NMR ($CDCl_3$): δ7.21, 7.11, 7.10, 7.09, 6.86, 6.85, 6.84, 8.83, 6.82, 6.81 (A_2B_2, H-2', H-3', H-5', H-6'), 6.34, 6.33, 6.32, 6.31, 6.30 (A_2B, H-2, H-6, H-4), 3.79 (Me), 3.76 (2 × Me), 2.84 (CH_2). ^{13}C NMR: Table 1.

Piceatannol, (E)-3,3',4,5'-*tetrahydroxystilbene* (**5**). Isolation and identification described in ref. [3].

3,3',4,5'-*Tetrahydroxybibenzyl* (**6**). Obtained from **7** by enzymatic hydrolysis. Compound **7** (40 mg, 0.1 mmol) was dissolved in Walpole acetate buffer [13] together with β-D-glucuronidase concentrate (Sigma, product no. G 7396). The reaction mixt. was kept at 37° until **7** was no longer observed by TLC (3 days). Purification was performed by flash CC by EtOH elution. Yield 15 mg (65%). HRMS *m/z* 246.0912 requires 246.0892. Viscous oil. EIMS *m/z* (rel. int.): 246 ([M]$^+$, 19), 151 (6), 123 (100), 107 (6). ^{1}H NMR (acetone-d_6): δ6.88, 6.86, 6.85, 6.71, 6.70, 6.68, 6.67 (ABC, H-2, H-5, H-6), 6.37, 6.34, 6.33, 6.32, A_2B, H-2', H-6', H-4'), 3.10, 2.87 (CH_2). ^{13}C NMR: Table 1.

3'-β-D-*Glucopyranosyloxy*-3,4,5'-*trihydroxybibenzyl* (**7**). Prepn and identification described in ref. [4].

(E)-3'-β-D-*Glucopyranosyloxy*-3,4,5'-*trimethoxystilbene* (**8**). Prepn and identification described in ref. [4].

(E)-3'-*Hydroxy*-3,4,5'-*trimethoxystilbene* (**9**). Obtained by enzymatic hydrolysis from **8** (50 mg, 0.1 mmol) as described above. Purification was performed by flash CC with ligroin (40–60°)–EtOH (1:1) elution. Yield 23 mg (80%), needles, mp 124° [14]. EIMS *m/z* (rel. int.): 286 ([M]$^+$, 100), 256 (30), 211 (7), 181 (5), 143 (6). ^{1}H NMR (acetone-d_6): δ7.38, 7.37, 7.25, 7.24, 7.22, 7.21, 7.09, 7.06 (ABC, H-2, H-5, H-6), 7.29, 7.23, 7.18, 7.12 (AB, α, β), 6.81, 6.80, 6.79, 6.50, 6.49, 6.48 (ABX, H-2', H-6', H-4'), 4.03, 4.01, 3.95 (Me). ^{13}C NMR: Table 1.

3'-*Hydroxy*-3,4,5'-*trimethoxybibenzyl* (**10**). Obtained from **9** (20 mg, 0.07 mmol) by catalytic hydrogenation without chromatographic purification. Yield 17 mg (85%). HRMS *m/z* 288.1366 calcd. 288.1362. Light yellow oil. EIMS *m/z* (rel. int.): 288 ([M]$^+$, 22), 258 (6), 151 (100), 121 (36). ^{1}H NMR (acetone-d_6): δ6.83, 6.81, 6.80, 6.74, 6.73, 6.71, 6.70 (ABC, H-2, H-5, H-6), 6.32, 6.31, 6.30, 6.29, 6.25, 6.24, 6.24 (ABC, H-2', H-4', H-6'), 3.76, 3.75, 3.71 (Me), 2.80 (CH_2) [15]. ^{13}C NMR: Table 1.

Antileukaemic activity measurements. Three replicated assays of each substrate and six of the control were done. After 48 hr incubation the number of living cells in a small amount of the dyed sample was counted in a Bürker chamber. Each sample was counted three times and the mean value was calculated. The final average number of living cells is the mean value calculated from the mean values of the three countings of the three replicated assays

and is expressed in percentages from the number in the control. Standard deviations were calculated.

Acknowledgements—We thank Dr O. Kajander for antileukaemic activity measurements and Ms M. Lahtiperä and Mr R. Kauppinen for the mass and NMR spectral measurements. We also thank the Research and Science Foundation of Farmos, the Jenny and Antti Wihuri Foundation and the Finnish Culture Foundation for a grant to E. M.

REFERENCES

1. Pettit, G. R., Singh, S. B., Niven, M. L., Hamel, E. and Schmidt, J. M. (1987) *J. Nat. Prod.* **50**, 119.
2. Pettit, G. R., Singh, S. B. and Schmidt, J. M. (1988) *J. Nat. Prod.* **51**, 517.
3. Talvitie, A., Mannila, E. and Hamunen, A. (1987) *Finn. Chem. Letters* 43.
4. Talvitie, A., Mannila, E. and Kolehmainen, E. (1992) *Liebigs Ann. Chem.* 399.
5. Laatikainen, R. (1977) *J. Magn. Reson.* **27**, 169.
6. Kalabin, G. A., Kushnarev, D. F., Tyukavkina, N. H., Gromova, A. S. and Lutskii, V. J. (1976) *Khim. Prirodn. Soedin.* 3.
7. Ferrigni, N. R., McLaughlin, L. J., Powell, R. G. and Smith Jr, C. R. (1984) *J. Nat. Prod.* **47**, 347.
8. Mannila, E. and Talvitie, A. (1992) *Phytochemistry* **31**, 3288.
9. Augustine, R. L. (1967) *Catalytic Hydrogenation*, p. 57. Marcel Dekker, New York.
10. Fieser, L. F. and Fieser, M. (1976) *Reagents for Organic Synthesis*, p. 295. John Wiley and Sons, New York.
11. Ryu, S. Y., Han, Y. N. and Han, B. H. (1988) *Arch. Pharm. Res.* **11**, 230.
12. Conzalez, H. G., Diaz, J. G., Arancibia, L. and Bermejo, J. (1988) *Planta Med.* **54**, 184.
13. Sober, H. A. (1970) *Handbook of Biochemistry*, p. J-234. The Chemical Rubber Co., Cleveland.
14. Aritomi, M. and Donnelly, D. M. X. (1976) *Phytochemistry* **15**, 2006.
15. Tezuka, Y., Hirano, H., Kikuchi, T. and Xu, G.-J. (1991) *Chem. Pharm. Bull.* **39**, 593.