

Phytochemistry Vol. 49, No. 6, pp. 1777–1778, 1998 © 1998 Elsevier Science Ltd. All rights reserved Printed in Great Britain 0031-9422/98/5 - see front matter

PII: S0031-9422(98)00256-8

(+)-ISOLARISIRESINOL 3a-O-SULPHATE FROM LEAVES OF MYRSINE SEGUINII

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(Received in revised form 2 March 1998)

Key Word Index—Myrsine seguinii; Myrsinaceae; lignan; (+)-isolarisiresinol 3a-O-sulphate.

Abstract—From leaves of *Myrsine seguinii*, (+)-isolarisiresinol $3a-O-\beta$ -D-glucopyranoside and 3a-O-sulphate were isolated. Their structures were elucidated by spectroscopic analyses. © 1998 Elsevier Science Ltd. All rights reserved

In previous papers, we have reported that phytochemical investigation of leaves of *Myrsine seguinii* afforded flavonol glycosides [1] and hydroquinone glycosides [2]. Following further investigation, compounds **1** and **2** were isolated from leaves and their structures were elucidated to be lignan derivatives. Compound **1** was identified as (+)-isolarisiresinol 3a-O- β -D-glucopyranoside by spectroscopic evidence [3].

Compound 2 was isolated as a colourless crystalline compound. The ¹³C NMR spectrum was similar to that of 1 (see Table 1). Although no NMR signals were observed for the sugar unit, compound 2 showed a much more polar nature on TLC (R_f 0.21) than 1 (R_f 0.33). The negative ion FAB mass spectrum indicated that the molecular weight of 2 was 440 which was 80 mass units higher than that calculated for isolarisiresinol (1a). This in increase in mass corresponded with an esterification of one of the hydroxyl groups by -PO(OH)₂ (MW 81) or -SO₂OH (MW 81). This was also indicated by the high polarity of the compound. The acid moiety was presumed to be sulphate, because no couplings $(J_{POC} \text{ and } J_{POCC})$ were observed in the ¹³C NMR spectrum. The HR-FAB-mass spectrum further showed that sulphur was present in compound 2. The site of esterification was determined to be the hydroxyl group at the 3a-position, since the ¹³C NMR chemical shift of C-3a was shifted downfield $(\delta_{\rm C} 67.6)$, when compared with that of compound 1a ($\delta_{\rm C}$ 62.5 [4]). From the sign of the optical ro-

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tation, the absolute structure of the lignan moiety was expected to be (+)-isolarisiresinol (1a) and this was further confirmed by measurement of the CD spectrum [3]. Finally, acid hydrolysis of 2 afforded (+)-isolarisiresinol (1a) and a prepicitate of barium sulphate on addition of barium nitrate (see Experimental). Therefore, the structure of 2 was elucidated to be (+)-isolarisiresinol 3a-O-sulphate.

EXPERIMENTAL

General

Mps.: uncorr.; ¹H NMR and ¹³C NMR: 400 MHz and 100 MHz, respectively; TLC: Merck, precoated (0.25 mm thickness) with CHCl₃–MeOH– H_2O (15:6:1); prep. TLC: Merck, precoated (0.25 mm thickness) with CHCl₃–MeOH (9:1).

Isolation procedure

The plant material, and a part of the extraction and isolation procedures, were described in the preceding paper [1]. Compound 1 was isolated from the 40% MeOH eluate on highly porous synthetic resin (Diaion HP-20) column chromatography and 2 from 20% MeOH by successive separation on silica gel CC, droplet counter-current chromatography and HPLC in yields of 14 mg and 82 mg, respectively.

(+)-Isolarisiresinol 3a-O-β-D-glucopyranoside (1). Colourless needles (MeOH), mp. 135–138°, $[\alpha]_D^{23}$ +45.4° (MeOH, *c* 0.84) [5], other spectroscopic data were essentially the same as those reported [3].



Fable	1.	^{13}C	NMR	data	for	1	and	2
		(CI	$D_3OD, 1$	100 MI	Hz)			

1 33.9 33.6 2 39.6 39.2 2a 65.3 64.9 3 46.0 45.2 3a 69.6 67.6 4 48.0 47.9 5 117.4 117.4 6 145.9 145.8 7 147.2 147.2 8 112.5 112.5 9 129.2 129.3 10 134.5 134.5 1' 138.7 138.5 2' 114.4 114.4 3' 149.0 148.8 4' 145.2 145.2 5' 116.1 116.1 6' 123.2 123.1 1'' 105.2 $2''$ $3''$ 78.2 $4''$ $4''$ 77.9 $6''$ $6''$ 62.8 56.5 56.5 $\cdot 0Me$ 56.6 56.5 56.5		1	2
2 39.6 39.2 2a 65.3 64.9 3 46.0 45.2 3a 69.6 67.6 4 48.0 47.9 5 117.4 117.4 6 145.9 145.8 7 147.2 147.2 8 112.5 112.5 9 129.2 129.3 10 134.5 134.5 1' 138.7 138.5 2' 114.4 114.4 3' 149.0 148.8 4' 145.2 145.2 5' 116.1 116.1 6' 123.2 123.1 1'' 105.2 $2''$ $2''$ 75.2 $3''$ $3''$ 77.9 $6''$ $6''$ 62.8 65.5 $0Me$ 56.6 56.5	1	33.9	33.6
2a 65.3 64.9 3 46.0 45.2 $3a$ 69.6 67.6 4 48.0 47.9 5 117.4 117.4 6 145.9 145.8 7 147.2 147.2 8 112.5 112.5 9 129.2 129.3 10 134.5 134.5 $1'$ 138.7 138.5 $2'$ 114.4 114.4 $3'$ 149.0 148.8 $4'$ 145.2 145.2 $5'$ 116.1 116.1 $6'$ 123.2 123.1 $1''$ 105.2 $2''$ $2''$ 75.2 $3''$ $3''$ 77.9 $6''$ $6''$ 62.8 56.5 $60Me$ 56.6 56.5	2	39.6	39.2
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	2a	65.3	64.9
3a 69.6 67.6 4 48.0 47.9 5 117.4 117.4 6 145.9 145.8 7 147.2 147.2 8 112.5 112.5 9 129.2 129.3 10 134.5 138.7 2' 114.4 114.4 3' 149.0 148.8 4' 145.2 145.2 5' 116.1 116.1 6' 123.2 123.1 1'' 105.2 2'' 2'' 75.2 3'' 6'' 123.2 123.1 1'' 105.2 2'' 2'' 75.2 3'' 3'' 78.2 4'' 4'' 71.7 5'' 6'' 62.8 56.5 .0Me 56.5 56.5	3	46.0	45.2
4 48.0 47.9 5 117.4 117.4 6 145.9 145.8 7 147.2 147.2 8 112.5 112.5 9 129.2 129.3 10 134.5 134.5 1' 138.7 138.5 2' 114.4 114.4 3' 149.0 148.8 4' 145.2 145.2 5' 116.1 116.1 6' 123.2 123.1 1'' 105.2 2'' 2'' 75.2 3'' 5'' 176.1 116.1 6'' 123.2 123.1 1'' 105.2 2'' 2'' 75.2 3'' 6'' 62.8 5'' ·OMe 56.5 56.5 ·OMe 56.6 56.5	3a	69.6	67.6
5 117.4 117.4 6 145.9 145.8 7 147.2 147.2 8 112.5 112.5 9 129.2 129.3 10 134.5 134.5 1' 138.7 138.5 2' 114.4 114.4 3' 149.0 148.8 4' 145.2 145.2 5' 116.1 116.1 6' 123.2 123.1 1'' 105.2 2'' 2'' 75.2 3'' 3'' 78.2 4'' 4'' 71.7 5'' 5'' 77.9 6'' 6'' 62.8 56.5 •OMe 56.5 56.5	4	48.0	47.9
6 145.9 145.8 7 147.2 147.2 8 112.5 112.5 9 129.2 129.3 10 134.5 134.5 1' 138.7 138.5 2' 114.4 114.4 3' 149.0 148.8 4' 145.2 145.2 5' 116.1 116.1 6' 123.2 123.1 1" 105.2 2" 2" 75.2 3" 3" 78.2 4" 4" 71.7 5" 5" 77.9 6" 6" 62.8 56.5 OMe 56.5 56.5 OMe 56.6 56.5	5	117.4	117.4
7 147.2 147.2 8 112.5 112.5 9 129.2 129.3 10 134.5 134.5 1' 138.7 138.5 2' 114.4 114.4 3' 149.0 148.8 4' 145.2 145.2 5' 116.1 116.1 6' 123.2 123.1 1" 105.2 2" 2" 75.2 3" 3" 78.2 4" 4" 71.7 5" 6" 62.8 56.5 \cdot OMe 56.5 56.5 \cdot OMe 56.6 56.5	6	145.9	145.8
8 112.5 112.5 9 129.2 129.3 10 134.5 134.5 1' 138.7 138.5 2' 114.4 114.4 3' 149.0 148.8 4' 145.2 145.2 5' 116.1 116.1 6' 123.2 123.1 1'' 105.2 2'' 2'' 75.2 3'' 3'' 78.2 4'' 4'' 71.7 5'' 6'' 62.8 56.5 •OMe 56.5 56.5 •OMe 56.6 56.5	7	147.2	147.2
9 129.2 129.3 10 134.5 134.5 1' 138.7 138.5 2' 114.4 114.4 3' 149.0 148.8 4' 145.2 145.2 5' 116.1 116.1 6' 123.2 123.1 1'' 105.2 2'' 2'' 75.2 3'' 3'' 78.2 4'' 4'' 71.7 5'' 6'' 62.8 56.5 •OMe 56.5 56.5 •OMe 56.6 56.5	8	112.5	112.5
10 134.5 134.5 1' 138.7 138.5 2' 114.4 114.4 3' 149.0 148.8 4' 145.2 145.2 5' 116.1 116.1 6' 123.2 123.1 1" 105.2 2" 2" 75.2 3" 3" 78.2 4" 4" 71.7 5" 5" 77.9 6" 6" 62.8 56.5 OMe 56.5 56.5 OMe 56.6 56.5	9	129.2	129.3
1' 138.7 138.5 $2'$ 114.4 114.4 $3'$ 149.0 148.8 $4'$ 145.2 145.2 $5'$ 116.1 116.1 $6'$ 123.2 123.1 $1''$ 105.2 2" $2''$ 75.2 3" $3''$ 78.2 4" $4''$ 71.7 5" $5''$ 62.8 56.5 •OMe 56.5 56.5 •OMe 56.6 56.5	10	134.5	134.5
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	1'	138.7	138.5
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	2'	114.4	114.4
4' 145.2 145.2 5' 116.1 116.1 6' 123.2 123.1 1" 105.2 2" 2" 75.2 3" 3" 78.2 4" 4" 71.7 5" 5" 77.9 6" 6" 62.8 56.5 •OMe 56.5 56.5 •OMe 56.6 56.5	3'	149.0	148.8
5' 116.1 116.1 6' 123.2 123.1 1" 105.2 2" 75.2 3" 78.2 4" 71.7 5" 77.9 6" 62.8 -OMe 56.5 56.5 -OMe 56.6 56.5	4'	145.2	145.2
6' 123.2 123.1 1" 105.2 2" 75.2 3" 78.2 4" 71.7 5" 77.9 6" 62.8 •OMe 56.5 56.5 •OMe 56.6 56.5	5'	116.1	116.1
1" 105.2 2" 75.2 3" 78.2 4" 71.7 5" 77.9 6" 62.8 -OMe 56.5 56.5 -OMe 56.6 56.5	6'	123.2	123.1
2" 75.2 3" 78.2 4" 71.7 5" 77.9 6" 62.8 -OMe 56.5 56.5 -OMe 56.6 56.5	1″	105.2	
3" 78.2 4" 71.7 5" 77.9 6" 62.8 -OMe 56.5 56.5 -OMe 56.6 56.5	2″	75.2	
4" 71.7 5" 77.9 6" 62.8 •OMe 56.5 56.5 •OMe 56.6 56.5	3″	78.2	
5" 77.9 6" 62.8 •OMe 56.5 56.5 •OMe 56.6 56.5	4″	71.7	
6" 62.8 -OMe 56.5 56.5 -OMe 56.6 56.5	5″	77.9	
-OMe 56.5 56.5 -OMe 56.6 56.5	6″	62.8	
-OMe 56.6 56.5	-OMe	56.5	56.5
	-OMe	56.6	56.5

(+)-Isolarisiresinol 3a-O-sulphate (2). Colourless crystalline state (MeOH), mp. 185–195° (decomp.), $[\alpha]_{D3}^{23}$ +11.3° (MeOH, *c* 0.53). IR ν_{max}^{KBr} cm⁻¹: 3400, 2900, 1600, 1505, 1450, 1365, 1250, 1200, 1120, 1060, 1020, 945. UV λ_{max}^{MeOH} nm (log ϵ): 209 (4.50), 229sh (4.08), 284 (3.75). ¹H NMR (CD₃OD): δ 1.91, (H, *tt*, *J* = 3 and 10 Hz, H-3), 2.06 (H, *m*. H-2), 2.78–2.90 (2H, *m*, H-1a and 1b), 3.70 (H, *dd*, *J* = 6 and 11 Hz, H-2aa), 3.76 (H, *dd*, *J* = 5 and 11 Hz, H-2ab), 3.80 and 3.83 (each 3H, each *s*, - OMe on C-7 and 3'), 3.88 (H, *dd*, *J* = 3 and 10 Hz, H-3aa), 3.94 (H, *br d*, *J* = 10 Hz, H-4), 4.10 (H, *dd*, *J* = 3 and 10 Hz, H-3ab), 6.20 (H, *br s*, H-5), 6.60 (H, *dd*, *J* = 2 and 8 Hz, H-2'), 6.66 (H, *br s*, H-8), 6.73 (H, *d*, *J* = 8 Hz, H-3'), 6.67 (H, *d*, *J* = 2 Hz, H-6'). ¹³C NMR (CD₃OD): see Table 1; CD

(MeOH, $c 7.22 \times 10^{-5}$ M) $\Delta \epsilon$ (nm); -0.60 (228), +1.98 (239), +2.00 (276), -2.97 (292). HR-FAB-MS (negative centroid) (Xe, matrix: PEG-400) m/z: 439.1049 [M-H]⁻ (C₂₀H₂₃O₉S requires 439.1062, cf. C₂₀H₂₄O₉P requires 439.1158).

Acid hydrolysis of 2. Compound 2 (15 mg) was treated with 2 N HCl (6 ml) at 60° for 48 hr. The reaction mixture was partitioned between Et2O (100 ml) and H₂O (100 ml). The organic layer was concentrated and then the residue was purified by prep. TLC to give (+)-isolarisiresinol (1a, 2.20 mg). CD (MeOH, $c \ 6.11 \times 10^{-5}$ M) $\Delta \epsilon$ (nm): +7.83 (215), +0.86 (226), +4.80 (238), +0.22 (253),+2.62 (275), -3.93 (292); NMR spectral data were essentially the same as those reported for isolarisiresinol [4]. The aq. layer was concentrated to about 10 ml and a soln of $Ba(NO_3)_2$ was added. The precipitate was collected by centrifugation. Since the white precipitate was not soluble on addition of 2 N HCl solution, it was confirmed to be BaSO₄.

Acknowledgements—The authors are grateful for the access to the superconductant NMR instrument in the Research Center for Molecular Medicine of Hiroshima University School of Medicine.

REFERENCES

- Zhong, X.-N., Otsuka, H., Ide, T., Hirata, E., Takushi, A. and Takeda, Y., *Phytochemistry*, 1997, 46, 943.
- Zhong, X.-N., Otsuka, H., Ide, T., Hirata, E., Takushi, A. and Takeda, Y., *Phytochemistry*, 1998, in press.
- Achenbach, H., Löwel, M., Waibel, R., Gupta, M. and Solis, P., *Planta Medica*, 1992, 58, 270.
- Urones, J. G., de Pascal Teresa, J., Sanches Marcos, I. and Diez Martin, D., *Phytochemistry*, 1987, 26, 1540.
- 5. Thieme, H. and Benecke, R., *Die Pharmazie*, 1969, **24**, 567.