Pergamon

0031-9422(94)00556-7

Phytochemistry, Vol. 37, No. 6, pp. 1699–1702, 1994

0031-9422/94 $7.00 + 0.00

4-*O*-DEMETHYLYATEIN FROM THE BRANCH WOOD OF *THUJA OCCIDENTALIS*

SHINGO KAWAI, TAKAO HASEGAWA, MAIKO GOTOH and HIDEO OHASHI

Department of Applied Bioorganic Chemistry, Faculty of Agriculture, Gifu University, Yanagido, Gifu 501-11, Japan

(*Received in revised form* 7 *April* 1994)

Key Word Index—*Thuja occidentalis*; Cupressaceae; lignan; 4-*O*-demethylyatein; 2-(3,5-dimethoxy-4-hydroxybenzyl)-3-(3,4-methylenedioxybenzyl)-butyrolactone.

Abstract—A new lignan, (−)-2-(3,5-dimethoxy-4-hydroxybenzyl)-3-(3,4-methylenedioxybenzyl)-butyrolactone [(−)-3,5-dimethoxy-4-hydroxy-3′,4′-methylenedioxylignanolide], (−)-4-*O*-demethylyatein was isolated from the branch wood of *Thuja occidentalis* and its structure established by spectroscopic analyses. The spectral data of the isolated compound were completely identical with those of the synthesized authentic compound, except for the optical rotation. The proposed significance of this compound on the biosynthetic pathway of *Thuja* lignans is discussed.

INTRODUCTION

There is much interest in the biochemical regulation of the biogenesis of lignin and lignans [1–3]. *Thuja plicata* and *T. standisii* (Cupressaceae) accumulate the lignans, thujaplicatin (**2**) and dihydroxythujaplicatin (**3**), and their methyl ethers (**4** and **5**), which have 3,4-dihydroxy-5-methoxyl- or 3,5-dimethoxy-4-hydroxyphenyl groups [4, 5]. However, these oxidative modifications are not present in gymnosperm lignin. It is of interest to know whether 3,4-dihydroxy-5-methoxyphenyl and 3,5-dimethoxy-4-hydroxyphenyl (syringyl) groups are synthesized before or after dimerization of phenylpropane units.

As preliminary work for a detailed study on the biosynthesis of *Thuja* lignans, we phytochemically examined lignans in the branch wood of *T. occidentalis* L. and found a new lignan, 4-*O*-demethylyatein (**1**) as a major lignan of *T. occidentalis*. It can be considered as a precursor to 4′-demethylpodophyllotoxin [6].

RESULTS AND DISCUSSION

Preliminary examination indicated that a lignan was present as a main phenolic constituent of *T. occidentalis* branch wood. ^{1}H NMR and mass spectra suggested it was a dibenzylbutyrolactone with 3,5-dimethoxy-4-hydroxybenzyl and 3,4-methylenedioxybenzyl groups.

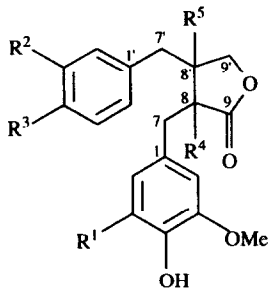
To confirm the detailed structure and optical rotation of this compound, 260 g of *T. occidentalis* branch wood was extracted and fractionated to obtain the phenolic fraction. This fraction was further purified by column chromatography and preparative TLC. Compound **1** (15.3 mg) was isolated and its structure was established as follows.

Compound **1**, $C_{21}H_{22}O_7$, $[\alpha]_D$ −25° (CH_2Cl_2), showed an $[M]^+$ at *m/z* 386.1374 in its high resolution mass spectrum. The IR absorption of 1770 cm^{-1} and the ^{13}C NMR signal at δ178.6 indicated the presence of a carbonyl group, and the appearance of signals at δ2.42–2.65 (2H, *m*, C8, C8′), 3.85 (1H, *dd*, C9′) and 4.14 (1H, *dd*, C9′) in the ^{1}H NMR spectrum verified the presence of a butyrolactone skeleton.

The ^{1}H NMR spectrum of **1** revealed the presence of two equivalent methoxyl groups (δ3.85), one methylenedioxyl group (δ5.93) and five aromatic protons [δ6.35 (2H, *s*), 6.46 (1H, *d*), 6.47 (1H, *d*) and 6.70 (1H, *dd*)]. The fragment ion peaks at *m/z* 167 (86%) and 135 (24%) in the mass spectrum correspond to 3,5-dimethoxy-4-hydroxybenzyl and 3,4-methylenedioxybenzyl cations, respectively (Fig. 1). Furthermore, the appearance of a fragment ion at *m/z* 250 (19%) indicated that the 3,5-dimethoxy-4-hydroxybenzyl group was attached to C-9 and not C-9′ (Fig. 1). Accordingly, the structure was estimated to be (8*R*, 8′*R*)-2-(3,5-dimethoxy-4-hydroxybenzyl)-3-(3,4-methylenedioxybenzyl)butyrolactone (**1**) [(−)-4-*O*-demethylyatein]. ^{13}C NMR spectral data were consistent with the structure of **1**.

For further confirmation of the structure, (±)-4-*O*-demethylyatein (**1**) by the modified method of Brown and Daugan [7]. The spectral data of synthetic **1** were completely identical with those of the isolated **1**, except for the optical rotation.

This is the first report of the isolation of (−)-4-*O*-demethylyatein (**1**) in nature. Tomioka *et al.* reported that the racemic mixture of **1** synthesized by an alternative route, had cytotoxicity against KB cells [8]. Compound **1** was also suggested to account for the biosynthesis of the 4′-demethyl series of *Podophyllum* lignans, e.g. 4′-demethylpodophyllotoxin [9] and it was proved that matairesionl (**6**) was a precursor compound of *Podophyllum* lignans [10]. Recently, it was demonstrated that in *For-*



		R^1	R^2	R^3	R^4	R^5
1	4-*O*-Demethylyatein	OMe	OCH_2O	OCH_2O	H	H
2	Thujaplicatin	OH	OMe	OH	H	H
3	Dihydroxythujaplicatin	OH	OMe	OH	OH	OH
4	Thujaplicatin methyl ether	OMe	OMe	OH	H	H
5	Dihydroxythujaplicatin methyl ether	OMe	OMe	OH	OH	OH
6	Matairesinol	H	OMe	OH	H	H

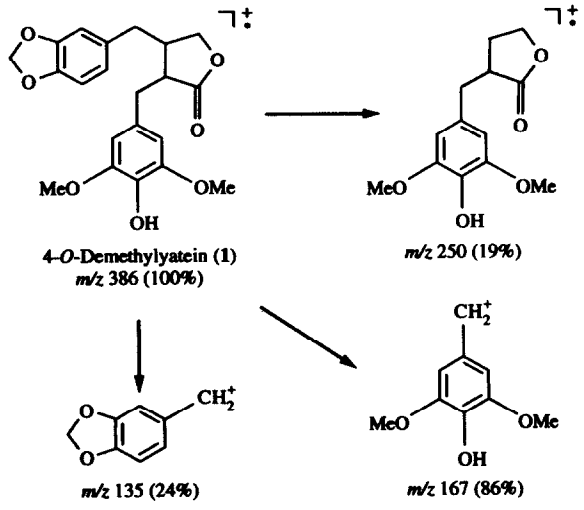


Fig. 1. Fragmentation pattern of 4-*O*-demethylyatein, 1.

sythia spp., (±)-pinoresionl synthesized from two molecules of coniferyl alcohol was converted to (−)-secoisolariciresinol [2, 11], and it was further oxidized to form (−)-matairesinol (**6**) [12, 13]. Therefore, it is likely that in *Thuja* sp. matairesinol (**6**) is first biosynthesized, and hydroxylated [thujaplicatin (**2**)], methylated [thujaplicatin methyl ether (**4**)] and cyclized to form 4-*O*-demethylyatein (**1**) [Fig. 2]. Then, we attempted to detect matairesinol (**6**), thujaplicatin (**2**) and thujaplicatin methyl ether (**4**) in *T. occidentalis* by using synthetic authentic compounds (data not shown), but could not detect them in this tree relative to the amount of **1**.

Kutsuki *et al.* [14] reported that *Thuja O*-methyltransferase could not catalyse the methylation of dihydrothujaplicatin (**3**) to its methyl ether (**5**). They concluded that sinapyl alcohol was first synthesized and then dimerization occurred to form the lignans in *Thuja* species. However, they did not investigate the possibility that thujaplicatin (**2**) was converted into thujaplicatin methyl ether (**3**). To elucidate the order of introduction of the *O*-methyl group we are conducting incorporation experiments using isotopically labelled compounds.

EXPERIMENTAL

General. Analytical and prep. TLC utilized silica gel (Merck Kieselgel 60 F_{254}, solvent system: MeOH–CH_2Cl_2 and EtOAc–*n*-hexane). After development, compounds were located by exposure to UV light (254 nm) and/or spraying with $FeCl_3$ reagent. CC employed Wako gel C-200. IR spectra were recorded with a JASCO A-100 using the film method. UV spectra were measured with a Shimadzu UV-1200. EI-MS was performed on a JEOL JMS DX-303 (direct insertion probe; ionizing voltage, 70 eV). ^1H and ^{13}C NMR spectra were recorded with a JEOL JMM GX-270 using TMS as an int. standard Optical rotation was measured with a Union Automatic Digital Polarimeter.

Extraction and isolation. Thuja occidentalis L. branches grown on the campus of Gifu University were used. The wood meal (260 g) was extracted with hot MeOH for 10 hr. The MeOH solubles were extracted with *n*-hexane, decanted and concd. The extracts were dissolved in EtOAc and partitioned with satd $NaHCO_3$ and 2 N NaOH, successively. The NaOH layer was acidified with 1 N HCl to pH 4 and re-extracted with EtOAc. The combined EtOAc solubles were dried (977 mg), then applied to a silica gel column (2.7 × 18.7 cm) and eluted with a mixt. of MeOH and CH_2Cl_2 (2:98). The desired eluents were collected and further purified by repeated prep. TLC to give **1** (15.3 mg).

(−)-4′-*O*-*Demethylyatein* (**1**). Oils. TLC [solvent; MeOH–CH_2Cl_2 (1:99)]: R_f 0.40; $[\alpha]_D$ −25°; (CH_2Cl_2; *c* 0.32); UV λ_{max}^{EtOH} nm: 284, 236sh, 213; HRMS: found 386.1374, calcd for $C_{21}H_{22}O_7$ 386.1365; EIMS *m/z* (rel.

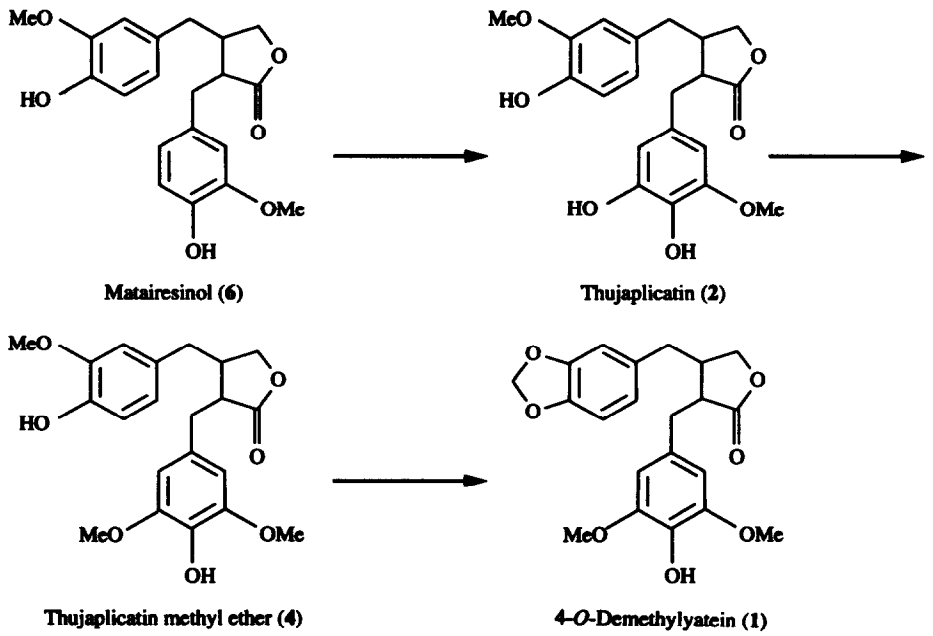


Fig. 2. Possible biosynthetic route to 4-*O*-demethylyatein (1) from matairesinol, 6.

int.): 387 (28), 386 $[M]^+$ (100), 250 (19), 237 (7), 224 (5), 168 (47), 167 $[CH_2C_6H_2$ (OH) $(OMe)_2]^+$ (86), 136 (14), 135 $[CH_2C_6H_2$ $(OCH_2O)]^+$ (24); IR ν_{max} cm^{-1}: 3500 (OH), 2950, 1770 (C=O), 1620, 930; ^1H NMR ($CDCl_3$): δ2.4–2.7 (4H, *m*, C7′-H_2, C8-H, C8′-H), 2.90 (2H, *d*, J = 5.9 Hz, C7-H_2), 3.86 (6H, *s*, C3-OMe, C5-OMe), 3.87 (1H, *dd*, $J_{8',9'}$ = 5.9 Hz, $J_{9',9}$ = 9.3 Hz, C9′-H), 4.15 (1H, *dd*, $J_{8',9'}$ = 6.8 Hz, $J_{9',9'}$ = 9.3 Hz C9′-H), 5.94 (2H, *d*, J = 1.1 Hz, C3′-OCH_2O-C4′), 6.36 (2H, *s*, C2-H, C6-H), 6.46 (1H, *d*, J = 1.5 Hz, C2′-H), 6.47 (1H, *d*, J = 7.0 Hz, C5′-H), 6.70 (1H, *dd*, $J_{2',6'}$ = 1.5 Hz, $J_{5',6'}$ = 7.0 Hz, C6′-H): ^{13}C NMR ($CDCl_3$): δ 35.1 (C7′), 38.4 (C7), 40.9 (C8), 46.6 (C8′), 56.3 (OMe × 2), 71.2 (C9′), 101.0 (OCH_2O), 105.8, 108.2, 108.7, 108.7, 121.5, 128.6, 131.5, 133.5, 146.3, 147.0, 147.8 (aromatic-C), 178.6 (C9).

Chemical syntheses. (±)-4′-*O*-Demethylyatein (1) was synthesized by the modified method of Brown and Daugan [7].

3-(3,4-*Methylenedioxybenzyl*)*butyrolactone.* Synthesized via following 5 steps: (i) piperonal (5.0 g, 33.3 mmol)/diethylsuccinate (1 eq.)/lithium methylate (2 eq.) in refluxing MeOH; (ii) product of (i)/10% Pd-C in MeOH under H_2 at room temp.; (iii) product of (ii) (recrystallized from EtOAc and *n*-hexane)/$CaBH_4$ [prepd from $NaBH_4$ (8 eq.) and $CaCl_2$ (4 eq.)] in absolute THF (freshly distilled from K metal and benzophenone) at room temp.; (iv) product of (iii)/6 N HCl in EtOAc at room temp.; (v) CC [eluent: MeOH–CH_2Cl_2 (1:99)].

4-*Benzyloxy*-3,5-*dimethoxybenzylbromide.* Prepd via following 4 steps: (i) syringaldehyde (1.0 g, 5.5 mmol)/benzyl chloride (1.1 eq.)/K_2CO_3 (1.1 eq.)/KI (0.3 eq.) in DMSO at room temp.; (ii) product of (i)/$NaBH_4$ (1 eq.) in MeOH at 0°, (iii) CC [eluent: EtOAc–*n*-hexane (1:3)]; (iv) product of (iii)/PBr_3 in absolute Et_2O (dried over Na metal) at room temp.

The above lactone (565 mg, 2.57 mmol) and bromide (1.3 eq.) were condensed by the lithium hexamethyldisilazane (LHMD) complex. LHMD was prepd from *n*-BuLi (1.2 eq.) and hexamethyldisilazane (1.5 eq.) in absolute THF at 0°; a THF soln of the lactone, hexamethyl phosphoric triamide (1.5 eq.) and a THF soln of the bromide were added dropwise at −78°, successively. The product was purified by CC [eluent: EtOAc–*n*-hexane (1:4)] (821 mg, 67%). An aliquot of this compound (130 mg, 0.27 mmol) was hydrogenated on 10% Pd-C in MeOH–dioxane (2:1). The product was purified by repeated prep. TLC to give racemic 1 (107 mg, 98%). The spectral data of this were completely identical with the isolated 4-demethylyatein (1) without optical rotation.

Acknowledgements—The authors wish to thank Dr Toshiaki Umezawa, Wood Research Institute, Kyoto University, and Dr Hideharu Ishida, Faculty of Agriculture, Gifu University, for measurements of mass spectra, and IR spectra and optical rotation, respectively. We also thank Dr James L. Davis, Department of General Engineering, University of Wisconsin, for critical reading of this manuscript.

REFERENCES

1. Ayres, D. C. and Loike, J. D. (1990) in *Lignans—Chemical, Biological and Clinical Properties*, pp. 269–302. Cambridge University Press, Cambridge.
2. Davin, L. B., Bedgar, D. L., Katayama, T. and Lewis, N. G. (1992) *Phytochemistry* **31**, 3875.

3. Ozawa, S., Davin, L. B. and Lewis, N. G. (1993) *Phytochemistry* **32**, 643.
4. Maclean, H. and Murakami, K. (1966) *Can. J. Chem.* **44**, 1541.
5. Murakami, K. (1970) *Memoirs of the College of Agriculture, Kyoto Univ.* **96**, 1.
6. Dewick, P. M. (1993) *Nat. Prod. Rep.* **10**, 233.
7. Brown, E. and Daugan, A. (1987) *Heterocycles* **26**, 1169.
8. Tomioka, K., Kawasaki, H. and Koga, K. (1990) *Chem. Pharm. Bull.* **38**, 1899.
9. Kamil, W. M. and Dewick, P. M. (1986) *Phytochemistry* **25**, 2093.
10. Broomhead, A. J., Rahman, M. A., Dewick, P. M., Jackson, D. E. and Lucas, J. A. (1991) *Phytochemistry* **30**, 1489.
11. Katayama, T., Davin, L. B. and Lewis, N. G. (1992) *Phytochemistry* **31**, 3869.
12. Umezawa, T., Davin, L. B. and Lewis, N. G. (1990) *Biochem. Biophys. Res. Commun.* **17**, 1008.
13. Umezawa, T., Davin, L. B. and Lewis, N. G. (1991) *J. Biol. Chem.* **266**, 10 210.
14. Kutsuki, K., Shimada, M. and Higuchi, T. (1981) *Mokuzai Gakkaishi* **27**, 39.