

0031-9422(95)00269-3

FLAVONOIDS AND LIGNANS FROM LEAVES OF CRYPTOMERIA JAPONICA

WEN-CHIUNG SU, JIM-MIN FANG and YU-SHIA CHENG*

Department of Chemistry, National Taiwan University, Taipei, Taiwan 106, Republic of China

(Received in revised form 27 February 1995)

Key Word Index—Cryptomeria japonica; Taxodiaceae; leaves; flavonoids; lignans.

Abstract—Eight flavonoids and 10 lignans were isolated from the leaves of *Cryptomeria japonica*. The new compounds are *cis*-dihydrodehydrodiconiferyl alcohol triacetate and *seco*dihydrodehydrodiconiferyl alcohol tetraacetate. Their structures were determined by chemical and spectral methods.

INTRODUCTION

The Japanese cedar, *Cryptomeria japonica*, is a widely distributed conifer called 'sugi' in Japanese. We recently reported the isolation of sesquiterpenes [1], diterpenes of abietane-, kaurane- and labdane-types [2, 3], and a triterpene, chamaecydin [4], from the ethyl acetate-soluble part of its leaves. We describe herein 18 constituents comprised of flavonoids and lignans, including two novel compounds 16 and 17.

RESULTS AND DISCUSSION

The leaves of C. japonica were extracted with acetone. The ethyl acetate-soluble portion of the extract was chromatographed to give flavonoids and lignans 1-18. The known flavonoids, taxifolin (1) [5], 5-hydroxy-4',7dimethoxyflavone (2) [6], 5-hydroxy-3,4',7-trimethoxyflavone (3) [7], 5-hydroxy-3,3',4',7-tetramethoxyflavone (4) [8], quercetin (5) [9], catechol pentaacetate (6) [10], epicatechol pentaacetate (7) [10] and 4',4"',7,7"-tetramethylamentoflavone (8) [11], the known lignans, matairesinol (9) [12], nortrachelogenin (10) [13], isolariciresinol tetraacetate (11) [14], secoisolariciresinol tetraacetate (12) [14], cedrusinin triacetate (13) [15], dihydrodehydrodiconiferyl alcohol triacetate (14) [16], cedrusin tetraacetate (15) [16] and agatharesinol tetraacetate (18) [17], were identified by comparison of their physical and spectral data (mp, $\lceil \alpha \rceil$, mass, IR, ¹H and ¹³CNMR) with literature data.

Compound 16 $(C_{26}H_{30}O_9)$ was assigned as *cis*-dihydrodehydrodiconiferyl alcohol triacetate because it showed characteristic IR and NMR spectra (Table 1) similar to those of the *trans*-isomer 14. The *cis*-configuration of 16 was established by irradiation of H-7 (at δ 5.82) which caused a 6.5% nOe of H-8 (at δ 3.80). Due to the shielding effects of the adjacent groups, the C-9 protons (at δ 3.82 and 3.92) and the C-9-OAc (at δ 1.81) of 16 appeared at higher fields than those of *trans*-isomer 14, occurring at δ 4.22, 4.38 and 2.05. The CD spectrum of 16 is similar to that of 14, exhibiting a negative Cotton effect at 276.5 nm and a positive Cotton effect at 253 nm. Accordingly, compound 16 has the (75,85)-configuration.

Compound 17 ($C_{28}H_{34}O_{10}$) showed IR absorptions at 1756 and 1730 cm⁻¹ and ¹³C signals at δ 168.8, 168.9, 169.1 and 171.0 attributable to four acetoxy groups. By analysis of the ¹H, ¹³C and H–C correlated spectra, the structure of 17 was determined to be *seco*dihydrodehydrodiconiferyl alcohol tetraacetate. Compound 17 was correlated with 14 by a sequence of hydrogenolysis, giving a tetraol 17a, and acetylation (Scheme 1). This correlation confirms that 17 has the 8S-configuration. Its CD spectrum showed a negative Cotton effect at 286.5 nm and a positive Cotton effect at 264.5 nm.

EXPERIMENTAL

General. Merck silica gel 60F sheets were used for analytical TLC. HPLC was carried out on a Hibar Lichrosorb Si 60 (7 μ m or 10 μ m) column (25 cm × 1 cm).

Plant material. The plant used in this study was introduced from Japan and cultivated in suburban Taipei. A voucher specimen is deposited in our laboratory. Leaves (1.4 kg) of C. japonica D. Don. were exhaustively extracted with Me₂CO. The Me₂CO extract was passed through a pad of charcoal, concd and re-extracted with EtOAc. The EtOAc-sol. portion (45 g) was chromatographed on a silica gel column, eluting with gradients of hexane and EtOAc. Appropriate frs were comb. and purified by HPLC to give 18 (13 mg), 13 (4 mg), 15 (9 mg), 16 (8 mg), 14 (110 mg), 3 (10 mg), 2 (55 mg), 4 (12 mg), 12

^{*}Author to whom correspondence should be addressed.



(17 mg), **17** (20 mg), **6** (13 mg), **7** (15 mg), **11** (15 mg), **8** (14 mg), **9** (15 mg), **10** (12 mg), **5** (10 mg) and **1** (15 mg), in order of increasing polarity, respectively.

 Important of the reasing polarity, respectively.
 20.6, 20

 Cedrusinin triacetae (13).
 13 C NMR (CDCl₃, 75 MHz):

 δ 30.6 (C-8'), 31.6 (C-7'), 50.4 (C-8), 56.0 (OMe), 63.7 (C-9'),
 75 MH

 65.7 (C-9), 87.0 (C-7), 109.5 (C-5'), 109.7 (C-2), 118.0 (C-6),
 63.7 (C-6)

122.9 (C-5), 124.6 (C-6'), 126.0 (C-1'), 129.2 (C-2'), 134.0 (C-3'), 139.5 (C-4), 140.1 (C-1), 151.3 (C-3), 158.0 (C-4'), 20.6, 20.8, 21.0, 168.9, 170.8, 171.1 ($3 \times OAc$).

Cedrusin tetraacetate (15). ¹³C NMR (CDCl₃, 75 MHz): δ 30.3 (C-8'), 31.5 (C-7'), 51.1 (C-8), 56.0 (OMe), 63.7 (C-9'), 65.5 (C-9), 87.9 (C-7), 109.6 (C-2), 117.5 (C-6),

	$\delta_{\rm H}$ (300 MHz)		$\delta_{\rm C}$ (75 MHz)	
	16	17	16	17
1	_		139.5	138.0
2	7.01 (d, J2)	6.60(d, J2)	110.6	113.2
3			151.1	150.6
4			139.3	138.2
5	7.0(d, J8)	6.85 (d, J8)	122.6	122.4
6	6.95 (dd, J2, 8)	6.64(dd, J2, 8)	118.8	121.0
7	5.82(d, J7.5)	2.86 (dd, J7, 11)	86.5	37.9
		2.90 (dd, J7, 11)		
8	3.80 (ddd, J3, 6, 7.5)	3.44 (dddd, J7, 7, 7, 7)	46.0	39.2
9	3.82 (dd, J6, 11.5)	4.16 (dd, J7, 10.5)	63.6	66.4
	3.92 (dd, J3, 11.5)	4.22 (dd, J7, 10.5)		
1′			128.6	139.6
2'	6.66 (<i>d</i> , <i>J</i> 2)	6.54 (d, J2)	117.1	119.2
3′			135.4	134.1
4′		_	146.2	136.5
5′		_	144.2	151.0
6′	6.64 (<i>d</i> , <i>J</i> 2)	6.62(d, J2)	113.0	110.7
7′	2.62(t, J7)	2.59 (t, J7)	32.0	32.3
8′	1.92(tt, J6.5, 7)	1.87 (tt, J6.5, 7)	30.6	30.2
9′	4.08 (t, J 6.5)	4.05 (t, J6.5)	63.7	63.7
OMe	3.80 (s)	3.69 (s)	56.0	55.7
	3.89 (s)	3.77 (s)	56.2	55.9
OAc	1.81 (s)	1.94 (s)	20.5, 168.8	20.5, 168.8
	2.05 (s)	2.05 (s)	20.6, 170.5	20.6, 168.9
	2.28 (s)	2.25 (s)	21.0, 171.1	20.8, 169.1
		2.29 (s)		20.9, 171.0

Table 1. ¹H and ¹³C NMR spectral data of compounds 16 and 17 (CDCl₃ solution, δ , J values in Hz)

Assignments of ¹H and ¹³C resonances confirmed by H-C COSY and HMBC spectra, as well as by NOE experiments.

122.1 (C-6'), 122.4 (C-2'), 122.8 (C-5), 128.0 (C-1'), 133.8 (C-5'), 135.0 (C-3'), 139.5 (C-4), 139.8 (C-1), 149.0 (C-4'), 151.4 (C-3), 20.2, 20.6, 20.8, 20.9, 168.9, 170.6, 170.7, 171.1 (4 × OAc).

Cis-Dihydrodehydrodiconiferyl alcohol triaacetate (16). Gum. $[\alpha]_{D}^{25} - 66^{\circ}$ (CHCl₃; c 0.8). TLC (EtOAc-CH₂Cl₂, 1:9), R_f 0.48. IR ν_{max}^{neat} cm⁻¹: 1757, 1728, 1601, 1494. UV λ_{max}^{MeOH} nm (ε): 281 (5550), 255 (1700), 211 (40250). CD (MeOH): $[\theta]_{318} + 670$, $[\theta]_{276.5} - 2240$, $[\theta]_{253} + 2700$. EIMS (70 eV) m/z (rel. int.) 486 [M]⁺ (4), 426 (5), 384 (25), 369 (5), 265 (6), 165 (10), 43 (100). HRMS for C₂₆H₃₀O₉, requires 486.1890; found [M]⁺ m/z486.1892.

secoDihydrodehydrodiconiferyl alcohol tetraacetate (17). Gum. $[\alpha]_D^{25} - 2.5^{\circ}$ (CHCl₃; c 2.0). TLC (EtOAc-CHCl₃-hexane, 1:1:1) R_f 0.75. IR ν_{max}^{neat} cm⁻¹: 1756, 1730, 1593, 1505. UV λ_{max}^{MeOH} nm (ε): 274 (7700). CD (MeOH): $[\theta]_{264.5} + 670$, $[\theta]_{286.5} - 550$. EIMS (70 eV) m/z (rel. int.) 530 [M]⁺ (12), 488 (75), 446 (25), 428 (60), 386 (42), 189 (65), 137 (90), 43 (100). HRMS for C₂₈H₃₄O₁₀, requires 530.2152; found [M]⁺ m/z 530.2153.

Correlation of 17 with 14. A mixt. of 14 (20 mg) and 10% Pd/C (5 mg) in MeOH (5 ml) was stirred at 20° under an H₂ atmosphere for 16 hr. The mixt. was filtered, the filtrate concd and purified by HPLC and elution with

EtOAc-hexane (4:1) to give secolignan 17a (18 mg). Gum. $[\alpha]_{D}^{25} + 39^{\circ}$ (CHCl₃; c 1.5). TLC (EtOAc-hexane, 4:1) R_f 0.33. IR $v_{\text{max}}^{\text{neat}}$ cm⁻¹: 3375, 1597, 1507, 1487, ¹H NMR (CDCl₃, 300 MHz): δ 1.78 (*tt*, J = 6.5, 7 Hz, H-8'), 2.57 (t, J = 7 Hz, H-7'), 2.85 (dd, J = 7, 11 Hz, H-7), 2.96 (dd, J = 7, 11 Hz, H-7), 3.42 (dddd, J = 7,7,7,7 Hz, H-8), 3.57 (t, J = 6.5 Hz, H-9'), 3.75 (dd, J = 7, 11 Hz, H-9), 3.76 (s, OMe), 3.83 (s, OMe), 3.85 (dd, J = 7, 11 Hz, H-9), 6.51 (d, J = 2 Hz, H-2'), 6.56 (d, J = 2 Hz, H-6'), 6.60 (d, J = 2 Hz, H-2), 6.61 (dd, J = 2, 8 Hz, H-6), 6.73 (d, J = 2, 8 Hz, H-6), 6.J = 8 Hz, H-5). ¹³C NMR (CDCl₃, 75 MHz): δ 31.9 (C-8'), 34.4 (C-7'), 36.6 (C-7), 44.3 (C-8), 55.8 (OMe), 55.9 (OMe), 62.1 (C-9'), 65.2 (C-9), 109.2 (C-6'), 111.7 (C-2), 114.0 (C-2'), 120.7 (C-6), 121.7 (C-5), 127.3 (C-3'), 132.3 (C-1), 133.0 (C-1'), 141.8 (C-4'), 143.6 (C-4), 146.2 (C-3), 146.5 (C-5'). EIMS (70 eV) m/z (rel. int.) 362 [M]⁺ (20), 224 (5), 208 (67), 179 (25), 164 (15), 151 (12), 137 (100). HRMS for $C_{20}H_{26}O_6$ requires 362.1730; found [M]⁺ m/z 362.1731.

Treatment of 17a (18 mg) with Ac_2O (0.5 ml) in pyridine (0.5 ml) for 16 hr gave the corresponding tetraacetate 17 (19 mg) after usual work-up.

Agatharesinol tetraacetate (18). Gum. $[\alpha]_{b}^{55} - 20^{\circ}$ (CHCl₃; c 1.3) [lit. [17] $[\alpha]_{b}^{55} - 19^{\circ}$ (Me₂CO; c 1.0)]. ¹³C NMR (CDCl₃, 75 MHz): δ 50.0 (C-7), 64.0 (C-9), 72.8



Scheme 1.

(C-8), 121.7 (C-3, 5, 3', 5'), 127.3 (C-2', 6'), 128.0 (C-8'), 129.2 (C-2, 6), 131.9 (C-7'), 134.4 (C-1'), 137.0 (C-1), 149.6 (C-4), 150.2 (C-4'), 20.7, 20.7, 21.1, 21.1, 169.3, 169.4, 170.2, 170.6 (4 × OAc).

REFERENCES

- 1. Su, W.-C., Fang, J.-M. and Cheng, Y.-S. (1994) *Phytochemistry* **39**, 603.
- 2. Su, W.-C., Fang, J.-M. and Cheng, Y.-S. (1994) *Phytochemistry* **35**, 1279.
- 3. Su, W.-C., Fang, J.-M. and Cheng, Y.-S. (1994) *Phytochemistry* 37, 1109.
- 4. Su, W.-C., Fang, J.-M. and Cheng, Y.-S. (1993) *Phytochemistry* 34, 779.
- 5. Pew, J. C. (1948) J. Am. Chem. Soc. 70, 3031.
- 6. Chakravarti, D. and Ghosh, R. P. (1944) J. Indian Chem. Soc. 21, 171.
- 7. Guider, J. M., Simpson, T. H. and Thomas, D. B. (1955) J. Chem. Soc. 170.

- Gomm, A. S. and Nierenstein, M. (1931) J. Am. Chem. Soc. 53, 4408.
- Ternai, B. and Markham, K. R. (1976) Tetrahedron 32, 565.
- Hergert, H. L. and Kurth, E. F. (1953) J. Org. Chem. 18, 521.
- 11. Okuyama, T., Ohta, Y. and Shibata, S. (1979) Shoyakugaku Zasshi 33, 185.
- 12. Haworth, R. D. and Richardson, T. (1935) J. Chem. Soc. 633.
- 13. Nishibe, S., Hisada, S. and Inagaki, I. (1971) *Phytochemistry* 10, 2231.
- 14. Fonseca, S. F., Campello, J. P., Barata, L. E. S. and Ruveda, E. A. (1978) *Phytochemistry* 17, 499.
- 15. Agrawal, P. K. and Rastogi, R. P. (1982) *Phytochemistry* 21, 1459.
- Agrawal, P. K., Agrawal, S. K. and Rastogi, R. P. (1980) *Phytochemistry* 19, 1260.
- 17. Enzell, C. R. and Thomas, B. R. (1965) Acta Chem. Scand. 19, 913.