Tetrahedron Letters, Vol.31,No.3,pp393-396, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

WOODFRUTICOSIN, AN INHIBITOR OF DNA TOPOISOMERASE II FROM WOODFORDIA FRUTICOSA KURZ

Shigetoshi Kadota,[a] Yasushi Takamori,[a] Tohru Kikuchi,[a,*] Akiko Motegi,[b] and Hisao Ekimoto[b]

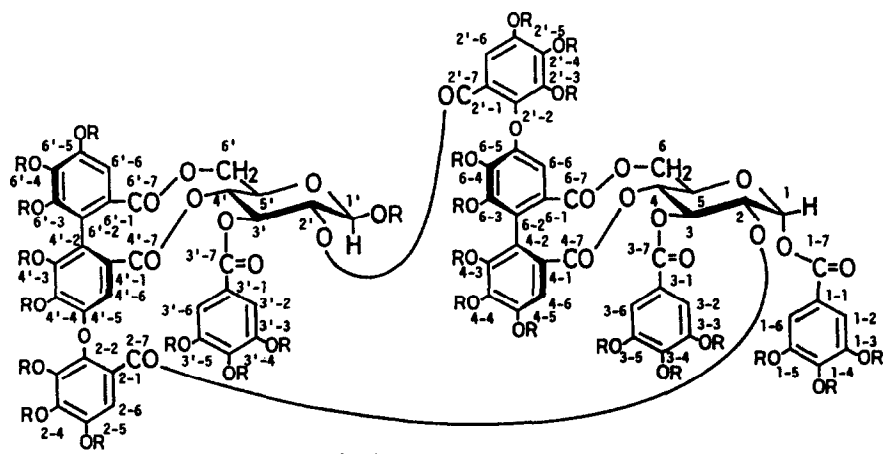
Research Institute for Wakan-Yaku (Oriental Medicines), Toyama Medical and Pharmaceutical University,[a] 2630 Sugitani, Toyama 930-01, Japan and Research Laboratories, Nippon Kayaku Co., Ltd.,[b] 31-12 Shimo 3-chome, Kita-ku, Tokyo 115, Japan

Summary: Woodfruticosin (1), a new dimeric tannin having an inhibitory activity against DNA topoisomerase II, was isolated from the methanol extract of Woodfordia fruticosa Kurz (Lythraceae) and its structure was determined by the use of 2D NMR spectroscopy including HMQC and HMBC techniques.

As a part of our studies on biologically significant substances from medicinal plants in Nepal,[1] we have isolated a new dimeric hydrolysable tannin, named woodfruticosin (1), from the leaves of Woodfordia fruticosa Kurz (Lythraceae)[2] and found that it has an inhibitory activity against DNA topoisomerase II.[3] This paper describes the isolation and structure elucidation of this new tannin.

The methanol extract of dried leaves (590 g) of W. fruticosa, collected in Nepal, was extracted with chloroform and then with ethyl acetate. The insoluble residue was roughly separated by Iatrobeads column chromatography with 25% MeOH/$CHCl_3$, 50% MeOH/$CHCl_3$, and MeOH. The MeOH eluate was further separated by a combination of chromatographies over Diaion HP-20 and Sephadex LH-20 to give woodfruticosin (1)(750 mg).

Woodfruticosin (1), a light brown powder, $C_{75}H_{52}O_{48}$,[4] $[\alpha]_D^{23}$ +114.5° (acetone), showed UV absorptions (MeOH) at 219 and 271 nm (log ε: 5.07 and 4.77) and IR absorptions (KBr)



1: R=H
2: R=CH_3

at 3300 (OH), 1715 (ester carbonyl), and 1610 cm^{-1}(phenyl). The negative ion FAB-mass spectrum of 1 exhibited the $(M-H)^-$ peak at m/z 1719 and the ^1H-(400 MHz) and ^{13}C-NMR (100 MHz) spectra, analyzed with the aid of ^1H-^1H COSY and HMQC,[5] indicated the presence of nine carbonyls, twelve aromatic methines, and two glucose units (see Table I).[6]

Methylation of 1 with dimethyl sulfate and potassium carbonate in dry acetone afforded a hexacosa-O-methylated compound (2), $[\alpha]_D^{23}$ +191.0°(acetone), whose positive ion FAB-mass spectrum exhibited the $(M+H)^+$ peak at m/z 2085. Methanolysis of 2 afforded methyl tri-O-methylgallate, MS m/z: 226 ($C_{11}H_{14}O_5$: Found 226.0829, Calcd 226.0841) and trimethyl (S)-octa-O-methylvaloneate,[7] $[\alpha]_D^{23}$ -15.4° (acetone), MS m/z: 660 ($C_{32}H_{36}O_{15}$: Found 660.2039, Calcd 660.2054), along with glucose and methyl β-glucoside, which were identified by GC comparison with authentic samples after trimethylsilylation. The above result suggested that 1 may be a dimeric tannin having three galloyl and two valoneoyl groups and two D-glucose cores.

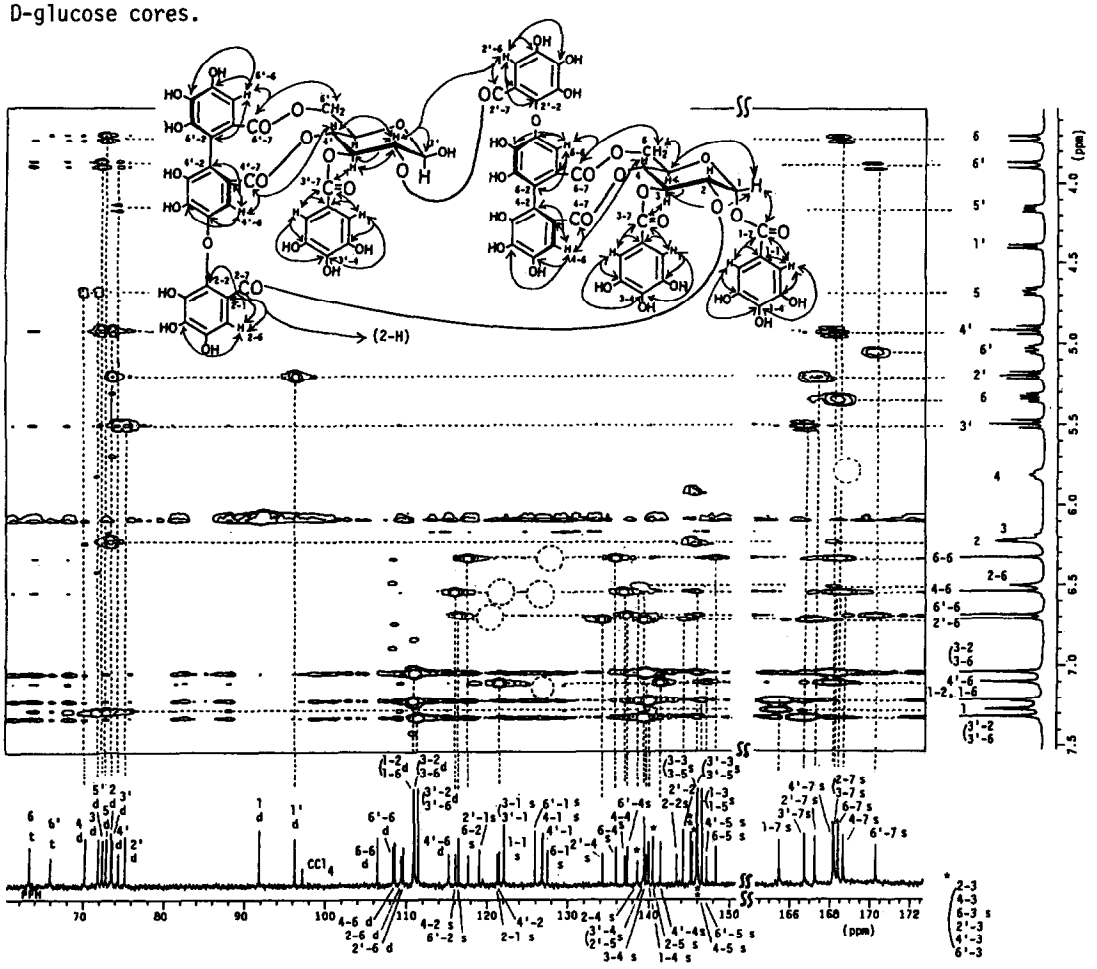


Fig. 1. HMBC Spectrum of Woodfruticosin (1) in Acetone-d_6 (60 mg, 38°C, 64 h run, J_{CH} = 10 Hz)
Dotted circles indicate the expected correlation peaks, which were not observed in this spectrum, but were observed in the spectrum measured with J_{CH} = 4.5 Hz.

Next, we measured the HMBC spectrum[5] to determine the total structure of 1. As shown in Fig. 1, the ^{13}C-signals at δ 165.42 (C-1-7), 166.68 (C-3'-7), and 168.16 (C-3-7)[8] showed long range correlations with the ^{1}H-signals at δ 7.20 (1-2-H and 1-6-H) and 7.27 (1-H), at δ 5.49 (3'-H) and 7.31 (3'-2-H and 3'-6-H), and at δ 6.21 (3-H) and 7.03 (3-2-H and 3-6-H), respectively, indicating the location of the galloyl groups at the C-1, C-3, C-3' positions of glucose cores. On the other hand, the carbonyl ^{13}C-signals at δ 167.20 (C-2'-7), δ 168.35 (C-6-7), and δ 168.61 (C-4-7) were correlated with the ^{1}H-signals at δ 5.19 (2'-H) and 6.71 (2'-6-H), at δ 6.32 (6-6-H), 3.72, and 5.33 (6-H_2), and at δ 5.81 (4-H) and 6.53 (4-6-H), respectively, while the ^{13}C-signals at δ 168.16 (C-2-7),[8] δ 168.11 (C-4'-7), and δ 170.24 (C-6'-7) were correlated with the ^{1}H-signals at δ 6.22 (2-H) and 6.48 (2-6-H), at δ 4.91 (4'-H) and 7.09 (4'-6-H), and at δ 3.88, 5.04 (6'-H_2), and 6.68 (6'-6-H), respectively. These two sets of ^{13}C-signals could be ascribed to the carbonyl carbons of the valoneoyl groups by detailed analysis of the HMBC spectrum, in which long range correlations between the aromatic protons and carbons were observed as indicated by arrows in the formula in Fig. 1. Thus the valoneoyl groups should be linked to glucose cores at the C-4, C-6, and C-2' and at the C-4', C-6', and C-2 positions, respectively, to form a cyclic dimeric tannin (1). The orientation of the valoneoyl groups[9] was deduced by comparing the chemical shift values of the para carbons in the diphenyl moieties: viz., the phenoxy-bearing carbons (C-6-5 and C-4'-5) were expected to resonate at lower field than the hydroxy-bearing carbons (C-4-5 and C-6'-5),[10] respectively (Table I).

The S-configuration of the valoneoyl groups in 1 was evidenced by a positive Cotton effect at 239 nm ($[\theta]_{MeOH}$ $+1.6 \times 10^5$) and a negative one at 261 nm ($[\theta]_{MeOH}$ -5.1×10^4) in the CD spectrum.[11]

These findings led us to conclude that the structure of woodfruticosin should be represented by the formula 1.

An inhibitory effect of woodfruticosin (1) against topoisomerase II[12] is shown in Fig. 2. It is noteworthy that 1 shows dose-dependently a strong inhibitory activity, comparable with those of etoposide and adriamycin which are known as inhibitors of this enzyme.[13] Although several other compounds such as coumermycin A_1 and epipodophylotoxins have been reported as the blocker of topoisomerase II enzyme,[13] our present result provided the first example of a natural tannin having an inhibitory effect against DNA topoisomerase II.

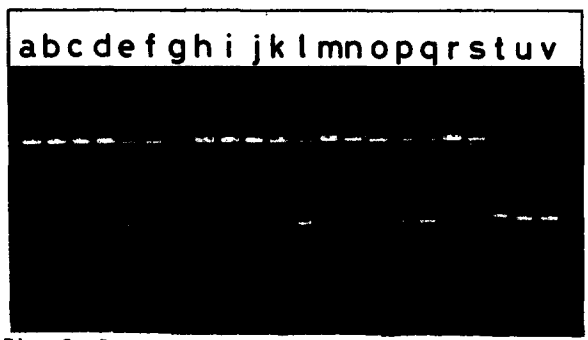


Fig. 2. Inhibition of Kineplast DNA Decatenation Activity of Topoisomerase II by Woodfruticosin (1), Etoposide, and Adriamycin.

lane a-d, control; lane e-g, DNA + topoisomerase II; lane h, DNA + topoisomerase II + 20 μg/ml (1); lane i, DNA + topoisomerase II + 10 μg/ml (1); lane j, DNA + topoisomerase II + 5 μg/ml (1); lane k, DNA + topoisomerase II + 2.5 μg/ml (1); lane l, DNA + topoisomerase II + 1.25 μg/ml (1); lane m, DNA + topoisomerase II + 20 μg/ml etoposide; lane n, DNA + topoisomerase II + 10 μg/ml etoposide; lane o, DNA + topoisomerase II + 5 μg/ml etoposide; lane p, DNA + topoisomerase II + 2.5 μg/ml etoposide; lane q, DNA + topoisomerase II + 1.25 μg/ml etoposide; lane r, DNA + topoisomerase II + 20 μg/ml adriamycin; lane s, DNA + topoisomerase II + 10 μg/ml adriamycin; lane t, DNA + topoisomerase II + 5 μg/ml adriamycin; lane u, DNA + topoisomerase II + 2.5 μg/ml adriamycin; lane v, DNA + topoisomerase II + 1.25 μg/ml adriamycin.

Table I. ^{1}H- and ^{13}C-NMR Data for Woodfruticosin (1) (Acetone-d_6, 38°C)

Position	δ H[a]	δ C	Position	δ H	δ C	Position	δ H	δ C
1	7.27 d (3)	91.81 d	1-1	———	122.01 s			
			1-2	7.20 s	110.83 d			
			1-3	———	146.51 s			
2	6.22 m	73.45 d	1-4	———	139.91 s			
3	6.21 m	71.86 d	1-5	———	146.51 s			
4	5.81 brt (10)	70.18 d	1-6	7.20 s	110.83 d			
			1-7	———	165.42 s			
			2-1	———	121.18 s	2'-1	———	118.93 s
5	4.67 dd (10,7)	72.85 d	2-2	———	143.40 s	2'-2	———	144.14 s
			2-3	———	140.44[b]s	2'-3	———	145.30[b]s
6	3.72 d (13)	63.27 t	2-4	———	138.45 s	2'-4	———	134.23 s
	5.33 dd (13,7)		2-5	———	138.54[b]s	2'-5	———	139.33 s
			2-6	6.48 br s	109.32 d	2'-6	6.71 s	109.58 d
			2-7	———	168.16[c]s	2'-7	———	167.20 s
1'	4.40 d (8)	96.18 d	3-1	———	121.95 s	3'-1	———	121.95 s
			3-2	7.03 s	110.95 d	3'-2	7.31 s	111.43 d
2'	5.19 dd (9.5,8)	75.22 d	3-3	———	145.87 s	3'-3	———	146.01 s
			3-4	———	139.57 s	3'-4	———	139.33 s
3'	5.49 t (9.5)	73.64 d	3-5	———	145.87 s	3'-5	———	146.01 s
			3-6	7.03 s	110.95 d	3'-6	7.31 s	111.43 d
			3-7	———	168.16[c]s	3'-7	———	166.68 s
4'	4.91 t (10)	74.37 d	4-1	———	126.73 s	4'-1	———	126.81 s
			4-2	———	116.02 s	4'-2	———	121.44 s
5'	4.15 dd (10,5.5)	72.38 d	4-3	———	145.08[b]s	4'-3	———	146.01[b]s
			4-4	———	136.98 s	4'-4	———	141.40 s
6'	3.88 d (13)	65.90 t	4-5	———	145.80 s	4'-5	———	147.12 s
	5.04 dd (13,5.5)		4-6	6.53 s	108.53 d	4'-6	7.09 s	115.15 d
			4-7	———	168.61 s	4'-7	———	168.11 s
			6-1	———	127.46 s	6'-1	———	125.85 s
			6-2	———	117.58 s	6'-2	———	116.38 s
			6-3	———	145.11[b]s	6'-3	———	146.07[b]s
			6-4	———	135.87 s	6'-4	———	137.28 s
			6-5	———	148.26 s	6'-5	———	145.96 s
			6-6	6.32 s	106.44 d	6'-6	6.68 s	108.34 d
			6-7	———	168.35 s	6'-7	———	170.24 s

a) Coupling constants (Hz) in parenthesis. b) Assignments may be interchanged. c) The ^{13}C-signals of C-2-7 and C-3-7 in 2 (in acetone-d_6) were observed at δ 167.41 and 167.49, respectively. These signals were correlated with the ^{1}H-signals of 2-H and 2-6-H (δ 5.88 and 6.36) and of 3-H, 3-2-H, and 3-6-H (δ 6.17, 7.34, and 7.34), respectively, in the HMBC spectrum.

References and Notes

1) S. Kadota, N. Lami, Y. Tezuka, and T. Kikuchi, Chem. Pharm. Bull., 36, 834 (1988).
2) His Majesty's Govt. of Nepal, "Medicinal Plants of Nepal," His Majesty's Govt. Press, Kathmandu, 1970, p. 72; S. B. Kalindhar, M. R. Parthasarathy, and P. Sharma, Indian J. Chem., 20B, 720 (1981).
3) G. L. Chen and L. F. Liu, Annu. Rep. Medicinal Chemistry, 21, 257 (1986).
4) Elementary analysis of 1 gave a satisfactory result.
5) A. Bax and M. F. Summers, J. Am. Chem. Soc., 108, 2093 (1986); M. F. Summers, L. G. Marzilli, and A. Bax, ibid., 108, 4285 (1986).
6) The ^{1}H-NMR spectra of 1 obtained in acetone-d_6 (25°C and 38°C) and in methanol-d_4 (25°C) showed that one of the glucose units was in the α-anomeric form and the other in the β-anomeric form.
7) T. Okuda, T. Hatano, K. Yazaki, and N. Ogawa, Chem. Pharm. Bull., 30, 4230 (1982).
8) Since the ^{13}C-signals of C-3-7 and C-2-7 in 1 are overlapped, an alternative structure in which the substituents at C-2 and C-3 are reversed can not be excluded at this stage. However, the structure 1 is supported by the analysis of the HMBC spectrum of 2.
9) T. Hatano, R. Kira, T. Yasuhara, and T. Okuda, Heterocycles, 27, 2081 (1988).
10) E. Pretsch, T. Clerc, J. Seibl, and W. Simon, "Tables of Spectral Data for Structure Determination of Organic Compounds," Springer-Verlag, New York, 1981, p. C120.
11) T. Okuda, T. Yoshida, T. Hatano, T. Koga, N. Toh, and K. Kuriyama, Tetrahedron Lett., 23, 3937 (1982).
12) Assay of the activity against topoisomerase II was done according to Miller's method; see K. G. Miller, L. F. Liu, and P. T. Englund, J. Biol. Chem., 256, 9334 (1981).
13) K. Drlica and R. J. Franco, Biochemistry, 27, 2253 (1988).

(Received in Japan 18 September 1989; accepted 15 November 1989)