Constituents of the Leaves of *Woodfordia fruticosa* KURZ. I. Isolation, Structure, and Proton and Carbon-13 Nuclear Magnetic Resonance Signal Assignments of Woodfruticosin (Woodfordin C), an Inhibitor of Deoxyribonucleic Acid Topoisomerase II[1)]

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Woodfruticosin (woodfordin C), a new cyclic dimeric hydrolyzable tannin having an inhibitory activity toward deoxyribonucleic acid (DNA) topoisomerase II, has been isolated from the leaves of *Woodfordia fruticosa* KURZ (Lythraceae) along with three known flavonol glycosides and three known flavonol glycoside gallates. The structure of woodfruticosin (woodfordin C) was determined by the use of two-dimensional nuclear magnetic resonance (2-D NMR) spectroscopy including heteronuclear multiple quantum coherence (HMQC) and heteronuclear mucltiple bond connectivity (HMBC) techniques. Detailed analyses of the proton and carbon-13 NMR (^{1}H- and ^{13}C-NMR) spectra of six known flavonoids were performed.

Keywords *Woodfordia fruticosa*; woodfruticosin; woodfordin C; tannin; DNA topoisomerase II; Lythraceae; HMQC; HMBC; quercetin 3-*O*-(6″-galloyl)-β-D-glucopyranoside; quercetin 3-*O*-(6″-galloyl)-β-D-galactopyranoside

The leaves of *Woodfordia fruticosa* KURZ (Lythraceae) possess antibiotic properties as well as sedative properties,[2)] and are used as a folk medicine in India and Nepal. In Sri Lanka, the dried flowers of this plant are used for treatment of haemorrhoids, dysentery, and liver diseases.[3)] Chemical investigation of the leaves of this plant has been done by Dan and Dan,[4)] who reported the isolation of lupeol, β-sitosterol, betulin, ursolic acid, betulinic acid, and oleanolic acid.[5)] In the course of our search for biologically significant substances in Ayurvedic traditional medicines, we have found that the methanol and water extracts of the leaves of this plant show an inhibitory effect against deoxyribonucleic acid (DNA) topoisomerase II.[6)] Careful separation of these extracts with monitoring by *in vitro* bioassay[7)] led to the isolation of a new dimeric hydrolyzable tannin designated as woodfruticosin (**9a**), together with gallic acid (**1a**), ellagic acid (**2**), three known flavonol glycosides, and three known flavonol glycoside gallates. Among these, **9a** was found to have a strong inhibitory activity against DNA topoisomerase II. Very recently, Okuda *et al.*[8)] isolated a tannin named woodfordin C from the flowers of *W. fruticosa*, with which woodfruticosin (**9a**) was identified. In this paper, we wish to present full details of the isolation and the structure elucidation of this new tannin (**9a**).

Dried leaves of *W. fruticosa*, collected at Kathmandu (Nepal) in August, 1987, were extracted with hot methanol and then with hot water successively. The water extract was partitioned between butanol and 0.15% aqueous hydrochloric acid and the butanol layer was concentrated to dryness. The residue was separated by centrifugal partition chromatography (CPC)[9)] to give fractions (frs. 1-12, 1-13, 1-J, and 1-K) having an inhibitory activity against DNA topoisomerase II, along with gallic acid (**1a**) and ellagic acid (**2**). These active fractions were combined and further purified by recycling preparative high-performance liquid chromatography (HPLC), giving two compounds, F4 (**6**) and F5 (**7**) (Chart 2).

The major one (F4, **6**), a yellow powder, $[\alpha]_D$ +26.1° (MeOH), showed ultraviolet (UV) absorptions at 257, 268sh, and 360 nm (log ε: 4.16, 4.08, and 4.10, respectively) and infrared (IR) absorptions at 3250 (OH), 1700 (ester carbonyl), 1650 (conjugated ketone), and 1610 cm^{-1} (phenyl). The fast atom bombardment mass spectrum (FAB-MS) and the negative ion FAB-MS exhibited the quasi-molecular ion peak at *m/z* 617 and at *m/z* 615, respectively, corresponding to the molecular formula $C_{28}H_{24}O_{16}$. The proton nuclear magnetic resonance

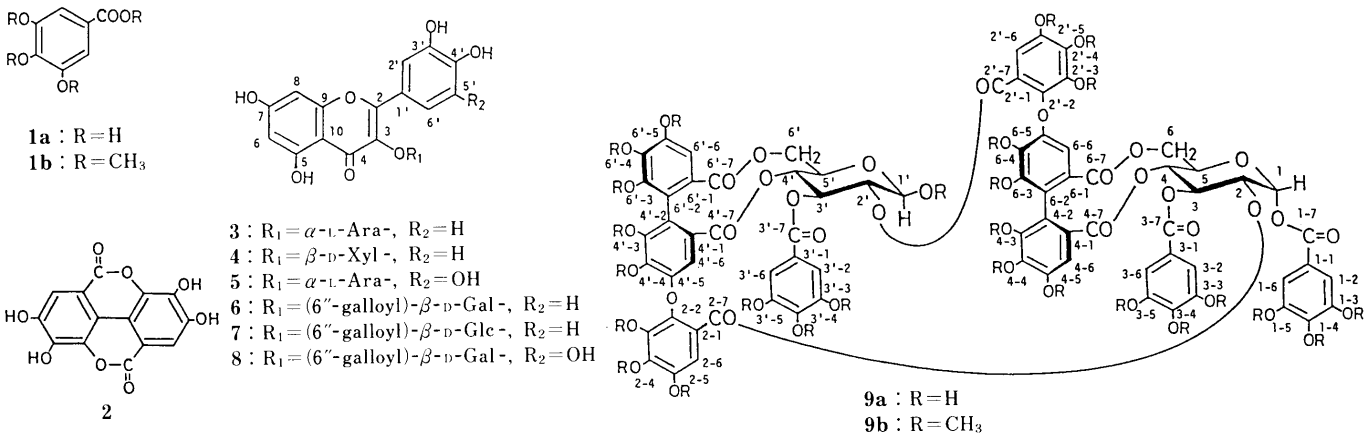

Chart 1

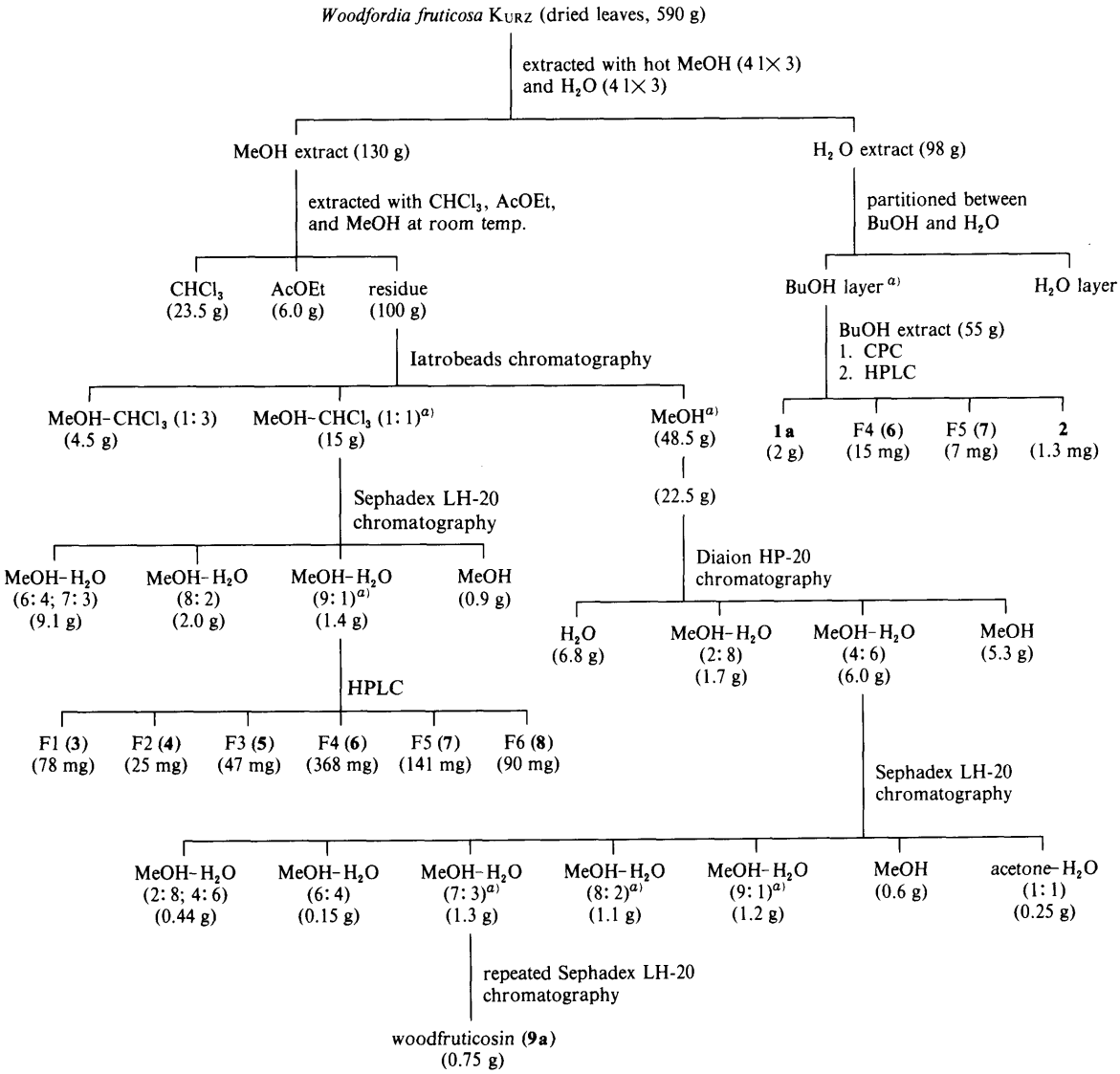

Chart 2. Extraction and Separation of the Constituents of *Woodfordia fruticosa* KURZ

a) These fractions showed a positive inhibitory effect against DNA topoisomerase II.

(^{1}H-NMR) spectrum of compound F4 (**6**), analyzed with the aid of ^{1}H–^{1}H shift correlated spectroscopy (COSY),[10] showed characteristic signals assignable to an anomeric proton at δ_H 5.10 (d, J=8.0 Hz), methylene protons adjacent to an ester grouping at δ_H 4.21 (dd, J=11, 6.0 Hz) and 4.31 (dd, J=11, 7.0 Hz), and four oxygenated methine protons at δ_H 3.59 (dd, J=9.5, 3.5 Hz), 3.79 (t, J=6.5 Hz), 3.84 (dd, J=9.5, 8.0 Hz), and 3.88 (d, J=3.5 Hz), together with aromatic protons at δ_H 6.89 (2H, s) assignable to a galloyl group, suggesting the presence of a 6-*O*-galloylgalactoside residue in F4. Also the ^{1}H-NMR spectrum showed signals due to 1,2,4-trisubstituted benzene protons (δ_H 6.81, 7.55, 7.78) and *meta*-coupled benzene protons (δ_H 6.18 and 6.37) attributable to flavonoid A-ring protons (Table I). The ^{1}H-detected heteronuclear multiple bond connectivity (HMBC) spectrum[11] of F4 (**6**) indicated that the carbon signals at δ_C 136.46 (C-3)[12] and 168.74 (C-7‴) are correlated with the proton signals at δ_H 5.10 (1″-H) and at δ_H 4.21 (6″-H), 4.31 (6″-H), and 6.89 (2‴- and 6‴-H), respectively (Fig. 1 and Table II). Based on the foregoing findings, this compound was determined to be quercetin 3-*O*-(6″-galloyl)-*β*-D-galactopyranoside (**6**),[13] and this was confirmed by comparison of its spectral data with published values.

The minor compound (F5, **7**), a yellow powder, $[\alpha]_D$ −50.6° (MeOH), showed UV and IR spectra similar to those of **6**. The positive and negative ion FAB-MS exhibited the $[M+H]^+$ and $[M-H]^-$ peaks at m/z 617 and at m/z 615, respectively. The ^{1}H- and ^{13}C-NMR spectra, which were analyzed by the use of ^{1}H–^{1}H COSY, ^{1}H-detected heteronuclear multiple quantum coherence (HMQC),[11] and HMBC techniques, confirmed that compound F5 is quercetin 3-*O*-(6″-galloyl)-*β*-D-glucopyranoside (**7**) (Tables I and II).[13]

On the other hand, the methanol extract was extracted with chloroform and then with ethyl acetate. The insoluble residue was roughly separated by Iatrobeads column chromatography and eluted successively with MeOH–$CHCl_3$ (1 : 3 and 1 : 1) and MeOH as shown in Chart 2. The eluate with MeOH–$CHCl_3$ (1 : 1) which has an inhibitory activity against DNA topoisomerase II was further separated by a combination of Sephadex LH-20 column chromatography and recycling preparative HPLC to give compounds F1—F6

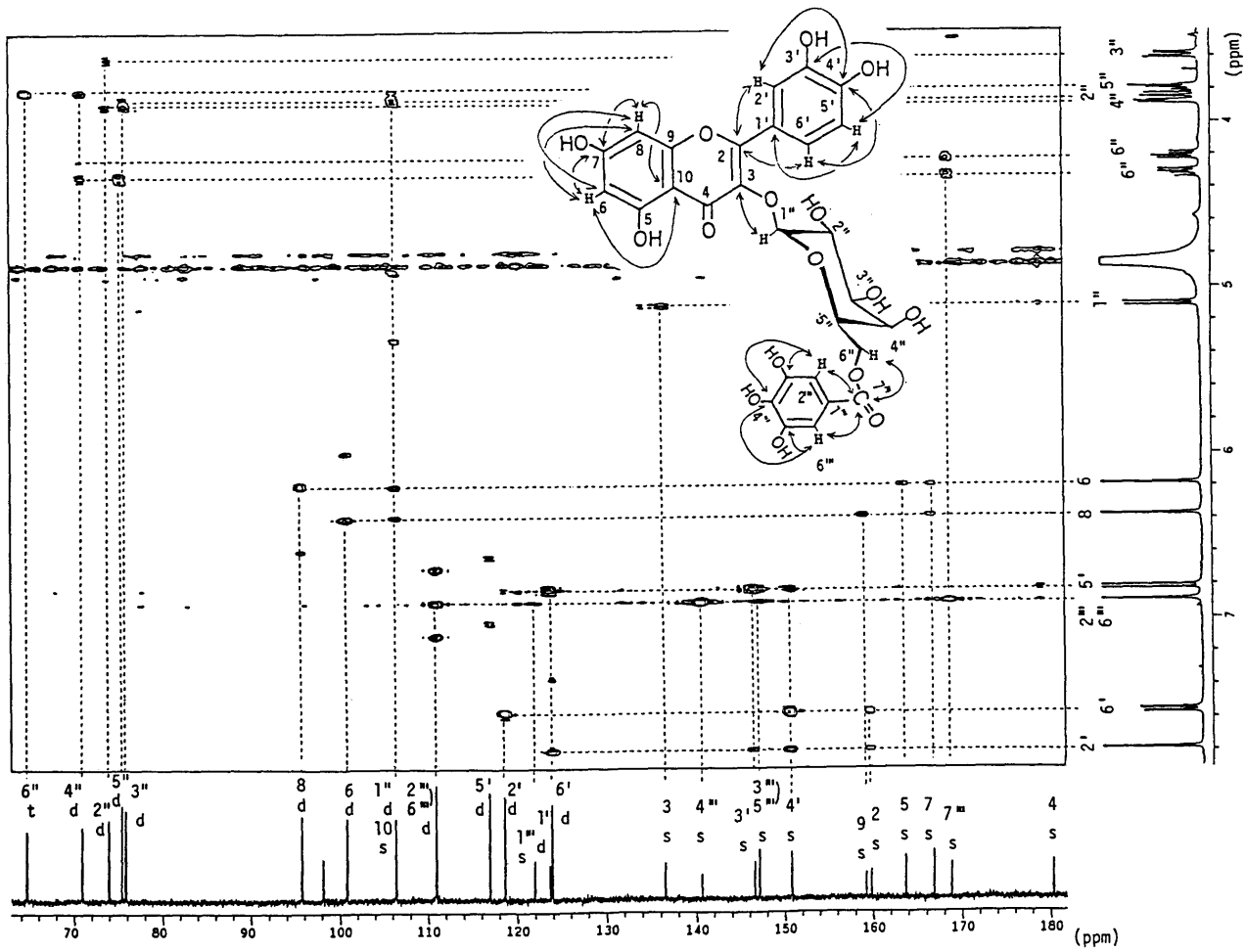

Fig. 1. HMBC Spectrum of Quercetin 3-*O*-(6″-Galloyl)-*β*-D-galactopyranoside (**6**) in Methanol-d_4

TABLE I. ^{1}H-NMR Data for Flavonol Glycosides and Flavonol Glycoside Gallates Obtained from *W. fruticosa*

Position	3[a]	4[a]	5[a]	6[a]	7[b]	8[a]
6	6.19 d (2.0)	6.20 d (2.0)	6.19 d (1.5)	6.18 d (2.0)	6.68 d (2.0)	6.16 d (2.5)
8	6.38 d (2.0)	6.39 d (2.0)	6.37 d (1.5)	6.37 d (2.0)	6.63 d (2.0)	6.35 d (2.5)
2′	7.75 d (2.0)	7.61 d (2.0)	7.32 s	7.78 d (2.0)	8.22 d (2.0)	7.36 s
5′	6.86 d (8.0)	6.86 d (8.5)	—	6.81 d (8.5)	7.32 d (8.5)	—
6′	7.57 dd (8.0, 2.0)	7.59 dd (8.5, 2.0)	7.32 s	7.55 dd (8.5, 2.0)	8.17 dd (8.5, 2.0)	7.36 s
Sugar						
1″	5.15 d (7.0)	5.16 d (7.5)	5.16 d (7.0)	5.10 d (8.0)	6.08 d (7.5)	5.17 d (8.0)
2″	3.90 dd (8.5, 7.0)	3.52 dd (9.0, 7.5)	3.90 dd (8.0, 7.0)	3.84 dd (9.5, 8.0)	4.26 dd (9.0, 7.5)	3.86 dd (9.5, 8.0)
3″	3.64 dd (8.5, 3.0)	3.10 dd (11, 9.0)	3.66 dd (8.0, 2.5)	3.59 dd (9.5, 3.5)	4.32 t (9.0)	3.63 dd (9.5, 3.5)
4″	3.80 t (3.5)	3.78 dd (11, 5.0)	3.84 br s	3.88 d (3.5)	4.23 t (9.0)	3.92 d (3.5)
5″	3.44 dd (13, 3.0)	3.41 d (8.5)	3.48 d (11)	3.79 t (6.5)	4.13 ddd (9.0, 4.0, 2.0)	3.82 t (6.5)
	3.82 dd (13, 4.0)	3.50 dd (8.5, 5.0)	3.86 dd (11, 3.0)	—	—	—
6″	—	—	—	4.21 dd (11, 6.0)	4.87 dd (12, 2.0)	4.26 dd (11, 6.5)
	—	—	—	4.31 dd (11, 7.0)	4.99 dd (12, 4.0)	4.31 dd (11, 6.5)
Galloyl						
2‴,6‴	—	—	—	6.89 s	7.36 s	6.90 s

δ Values in ppm and coupling constants (in parenthesis) in Hz. *a*) In methanol-d_4. *b*) In pyridine-d_5.

(Chart 2). The MeOH eluate was also separated again by chromatography over Diaion HP-20 and the eluate with MeOH–H_2O (4:6) was further separated by repeated chromatography over Sephadex LH-20 to give woodfruticosin (woodfordin C) (**9a**) as shown in Chart 2.

Compound F1 (**3**) was obtained as a yellow powder and showed $[\alpha]_D$ −60.1° (MeOH). It showed UV absorptions at 239 and 314 nm (log *ε*: 4.08 and 4.18, respectively) and IR absorptions at 3400 (OH), 1640 (conjugated ketone), and 1600 cm^{-1} (phenyl). The negative ion FAB-MS exhibited the $[M-H]^-$ peak at *m/z* 433. The ^{1}H-NMR of compound F1 showed signals due to *meta*-coupled benzene protons [δ_H 6.19 (d, J=2.0 Hz) and 6.38 (d, J=2.0 Hz)] and 1,2,4-trisubstituted benzene protons [δ_H 6.86 (d,

Table II. ^{13}C-NMR Data for Flavonol Glycosides and Flavonol Glycoside Gallates Obtained from *W. fruticosa*

Position	3[a,c]	4[a]	5[a]	6[a,c]	7[b,c]	8[a,c]
2	159.42 s	159.68 s	159.38 s	159.76 s	158.03 s	159.42 s
3	136.41 s	136.20 s	136.55 s	136.46 s	135.09 s	136.59 s
4	180.20 s	180.14 s	180.08 s	180.26 s	178.52 s	180.05 s
5	163.78 s	163.81 s	163.70 s	163.63 s	162.50 s	163.54 s
6	100.76 d	100.73 d	100.69 d	100.76 d	99.74 d	100.69 d
7	167.03 s	166.91 s	166.89 s	166.79 s	165.84 s	166.69 s
8	95.57 d	95.55 d	95.49 d	95.64 d	94.53 d	95.55 d
9	159.21 s	159.20 s	159.10 s	159.17 s	157.46 s	159.01 s
10	106.37 s	106.39 s	106.34 s	106.33 s	105.04 s	106.27 s
1′	123.65 s	123.78 s	122.49 s	123.57 s	122.66 s	122.39 s
2′	118.24 d	117.98 d	110.64 d	118.55 d	117.42 d	110.81 d
3′	146.72 s	146.81 s	147.20 s	146.51 s	146.64 s	147.04 s
4′	150.71 s	150.65 s	138.87 s	150.71 s	150.56 s	138.92 s
5′	116.94 d	116.77 d	147.20 s	116.88 d	116.28 d	147.04 s
6′	123.78 d	124.06 d	110.64 d	123.84 d	122.96 d	110.81 d
Sugar						
1″	105.46 d	105.40 d	105.61 d	106.33 d	104.46 d	106.33 d
2″	73.65 d	76.00 d	73.74 d	73.83 d	75.74 d	73.94 d
3″	74.91 d	78.29 d	75.00 d	75.74 d	78.29 d	75.71 d
4″	69.87 d	71.75 d	70.02 d	70.81 d	70.78 d	70.76 d
5″	67.73 t	67.97 t	67.91 t	75.29 d	75.94 d	75.20 d
6″	—	—	—	64.57 t	64.07 t	64.37 t
Galloyl						
1‴	—	—	—	121.87 s	121.02 s	121.81 s
2‴	—	—	—	110.90 d	110.15 d	110.88 d
3‴	—	—	—	147.08 s	147.26 s	147.04 s
4‴	—	—	—	140.54 s	140.63 s	140.54 s
5‴	—	—	—	147.08 s	147.26 s	147.04 s
6‴	—	—	—	110.90 d	110.15 d	110.88 d
7‴	—	—	—	168.74 s	167.04 s	168.71 s

δ Values in ppm. Multiplicities of carbon signals were determined by means of the DEPT method and are indicated as s, d, and t. *a*) In methanol-d_4. *b*) In pyridine-d_5. *c*) HMQC and HMBC spectra were measured.

J=8.0 Hz), 7.57 (dd, J=8.0, 2.0 Hz), and 7.75 (d, J=2.0 Hz)] along with sugar protons (δ_H 3.44, 3.64, 3.80, 3.82, 3.90, 5.15) (Table I). The ^{13}C-NMR of compound F1 showed a carbonyl (δ_C 180.20), five aromatic methine carbons (δ_C 95.57, 100.76, 116.94, 118.24, and 123.78), and five sugar carbons (δ_C 67.73, 69.87, 73.65, 74.91, and 105.46) along with nine quaternary carbons (δ_C 106.37, 123.65, 136.41, 146.72, 150.71, 159.21, 159.42, 163.78, and 167.03) (Table II).

Extensive analysis of the ^{1}H- and ^{13}C-NMR spectra of F1 (**3**) with the aid of ^{1}H–^{1}H COSY, HMQC, and HMBC allowed us to deduce that compound F1 is an α-arabinoside of quercetin. In the HMBC spectrum, the carbon signals due to 3-C (δ_C 136.41) and 5″-C (δ_C 67.73) showed long-range correlation with the signal due to the anomeric proton (δ_H 5.15). Thus, compound F1 was determined to be quercetin 3-*O*-α-L-arabinopyranoside (**3**),[11] and this was confirmed by comparison of its spectral data with the published values.

Similarly, compound F2 (**4**) and compound F3 (**5**) were identified as quercetin 3-*O*-β-D-xylopyranoside (**4**)[14] and myricetin 3-*O*-α-L-arabinopyranoside (**5**),[12] respectively, while compound F6 was identified as myricetin 3-*O*-(6″-galloyl)-β-D-galactopyranoside (**8**).[13] Assignments of ^{1}H- and ^{13}C-NMR signals of these compounds are shown in Tables I and II.

Woodfruticosin (woodfordin C) (**9a**) was obtained as a light brown powder and showed $[\alpha]_D$ +182° (acetone). The molecular formula for **9a** was determined as $C_{75}H_{52}O_{48}$ on the basis of elemental analysis and the negative ion FAB-MS, which exhibited the $[M-H]^-$ peak at m/z 1719. In the UV spectrum it showed absorption bands at 219 and 271 nm (log ε: 5.07 and 4.77, respectively) and in the IR spectrum strong absorptions at 3300 (OH), 1715 (ester carbonyl), and 1610 cm^{-1} (phenyl). The ^{1}H- and ^{13}C-NMR spectra of **9a**, analyzed with the aid of ^{1}H–^{1}H COSY (Fig. 2) and the HMQC technique[11] (Fig. 3), indicated the presence of two glucose units, six isolated aromatic methines, and three pairs of equivalent aromatic methines (Table III). Also, the ^{13}C-NMR spectrum exhibited eight signals due to carbonyl carbons, among which the one at δ 168.16 was shown to correspond to two carbon atoms (C-2-7 and C-3-7).

Methylation of **9a** with dimethyl sulfate and potassium carbonate in dry acetone afforded a hexacosa-*O*-methylated compound (**9b**), light yellow powder, $[\alpha]_D$ +126° (acetone), whose positive ion FAB-MS exhibited the $[M+H]^+$ peak at m/z 2085. Methanolysis of **9b** with sodium methoxide in absolute methanol[15] afforded methyl tri-*O*-methylgallate (**1b**) and trimethyl (*S*)-octa-*O*-methylvaloneate (**10**),[16] $[\alpha]_D$ −15.4° (acetone), along with glucose and methyl β-glucoside, which were identified by gas chromatography (GC) comparison with authentic samples after trimethylsilylation.

The above results suggested that **9a** may by a dimeric tannin having three galloyl and two valoneoyl groups and two D-glucose cores.

Next, we measured the HMBC spectrum in order to determine the total structure of **9a**. As shown in Figs. 4 and 5, the carbonyl carbon signals at δ 165.42 (C-1-7) and 166.68

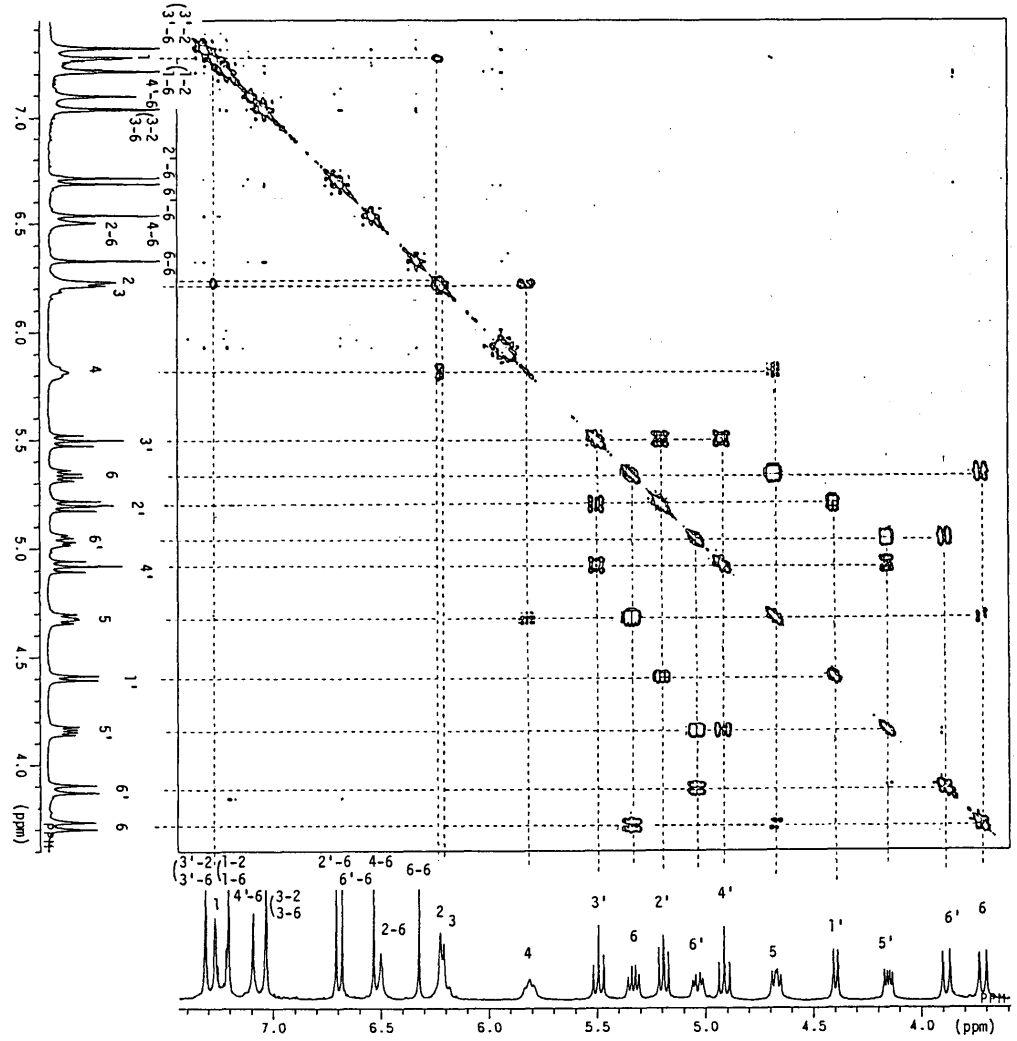

Fig. 2. ^1H–^1H COSY Spectrum of **9a** in Acetone-d_6

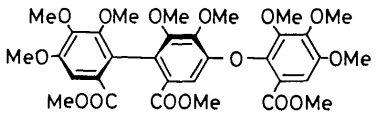

Chart 3

(C-3′-7) showed long-range correlations with the proton signals at δ 7.20 (1-2-H and 1-6-H) and 7.27 (1-H) and at δ 5.49 (3′-H) and 7.31 (3′-2-H and 3′-6-H), respectively, indicating the location of two galloyl groups at the C-1 and C-3′ positions of the glucose cores. Furthermore, another carbon signal at δ 168.16 (C-3-7 or C-2-7) showed long-range correlations with the proton signals at δ 6.21 (3-H) and 7.03 (3-2-H and 3-6-H), suggesting the presence of a galloyl group at the C-3 position. This was verified by detailed analysis of the ^{13}C-NMR spectrum of the *O*-methylated compound **9b**, whose carbonyl carbon signals appeared separately at δ 165.71, 166.56, 166.68, 167.41, 167.49, 167.85, 167.93, 168.63, and 168.72. As seen in Fig. 6, the carbon signals at δ 165.71 (C-1-7), 166.56 (C-3′-7), and 167.49 (C-3-7) showed long-range correlations with the proton signals at δ 7.10 (1-H) and 7.32 (1-2-H and 1-6-H), at δ 5.40 (3′-H) and 7.48 (3′-2-H and 3′-6-H), and at δ 6.17 (3-H) and 7.34 (3-2-H and 3-6-H), respectively.

On the other hand, the carbonyl carbon signals at δ 167.20 (C-2′-7), δ 168.35 (C-6-7), and δ 168.61 (C-4-7) in **9a** were correlated with the proton 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 carbon signals at δ 168.16 (C-2-7),[17] δ 168.11 (C-4′-7), and δ 170.24 (C-6′-7) were correlated with the proton 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 carbon 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. 4. 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 (**9a**).

The orientation of the valoneoyl groups[18] was deduced by comparing the chemical shift values of the *para* carbons in the diphenyl moieties, whose assignments were done by detailed analysis of the HMBC spectrum as shown in Fig. 7. The phenoxy-bearing carbons (C-6-5 and C-4′-5) are expected to resonate at lower field than the hydroxy-bearing carbons (C-4-5 and C-6′-5, respectively).[19]

The absolute configuration of the valoneoyl groups in **9a**

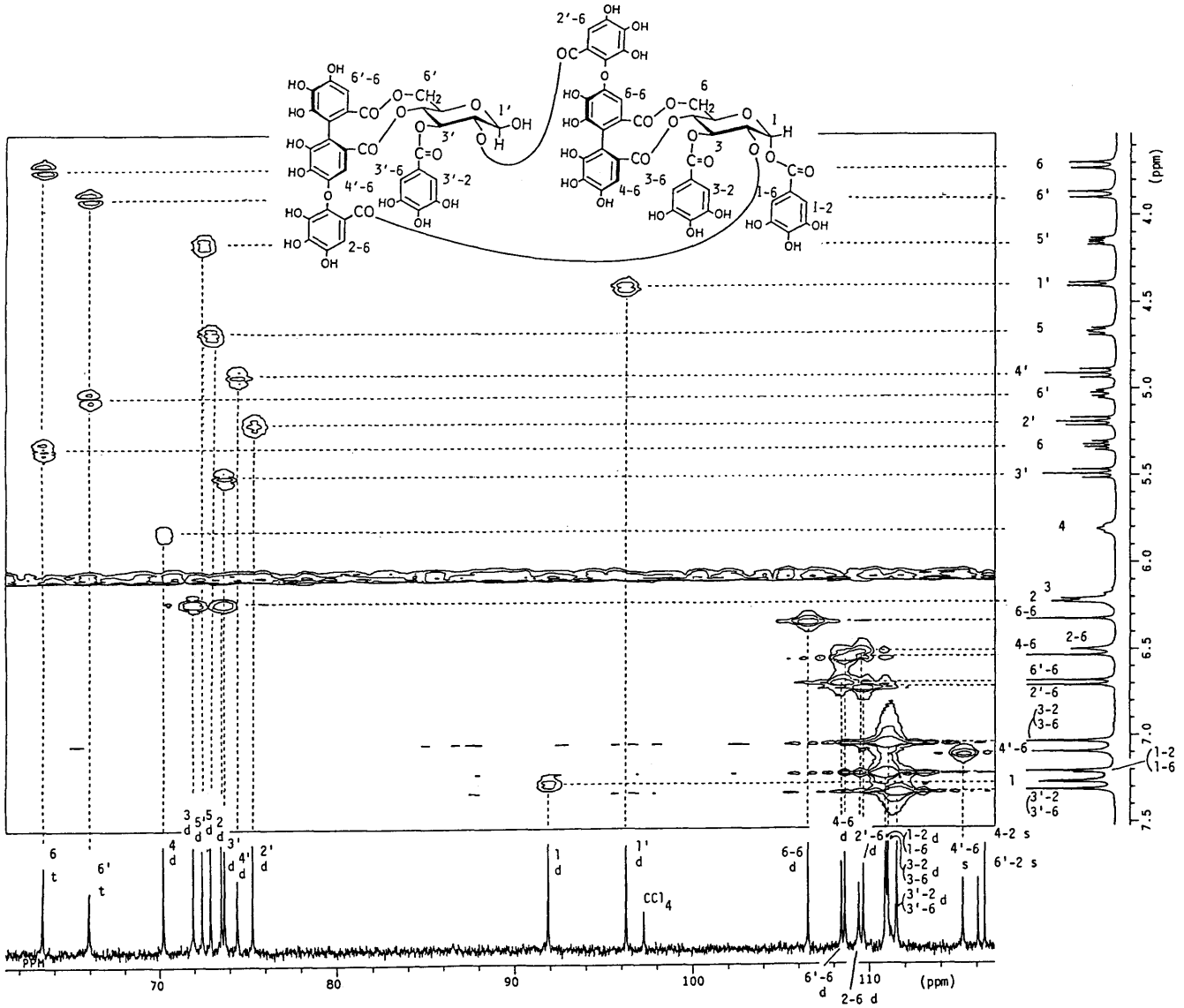

Fig. 3. HMQC Spectrum of **9a** in Acetone-d_6

was determined to be *S* by circular dichroism (CD) measurement,[20] which showed a positive Cotton effect at 239 nm ($[\theta]^{20} + 1.6 \times 10^{16}$, in methanol) and a negative one at 261 nm ($[\theta]^{20} - 5.1 \times 10^{4}$, in methanol).

These findings led us to conclude that the structure of woodfruticosin (woodfordin C) should be represented by the formula **9a**. Our present result is the first example of an exact analysis of ^{1}H- and ^{13}C-NMR signals in dimeric tannins and it will be useful for the structure elucidation of tannins. The inhibitory activity against DNA topoisomerase II of these compounds will be reported elsewhere.

Experimental

Optical rotations were measured on a JASCO DIP-4 automatic polarimeter at 22 °C. Circular dichroism (CD) spectra were recorded on a JASCO J-500C spectropolarimeter in methanol solutions at 20 °C. UV spectra were taken with a Shimadzu 202 UV spectrometer in methanol solutions and IR spectra with a JASCO IRA-2 in KBr discs. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL GX-400 spectrometer in acetone-d_6, in methanol-d_4, or in pyridine-d_5 solutions with tetramethylsilane as an internal standard, and chemical shifts are recorded in δ values. ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, HMQC, and HMBC were obtained with the usual pulse sequence and data processing was performed with the standard JEOL software. MS and high-resolution MS were obtained with a JEOL JMS-D 300 spectrometer (ionization voltage, 70 eV; accelerating voltage, 3 kV) using a direct inlet system and FAB-MS measurements were done with a JEOL SX-102 spectrometer using glycerol or thioglycerol as a matrix. GC analyses were carried out on a Shimadzu gas chromatograph (Model GC-6A) with a flame ionization detector using a 2% OV-17 column (on Gas-chrom Q, 2 m × 3 mm i.d. glass tube) at a column temperature of 180 °C. Nitrogen was used as the carrier gas at a flow rate of 35 ml/min. Column chromatographies were done with Iatrobeads 6RS-8060 (Iatron Laboratories Inc.), Diaion HP-20 (Mitsubishi Chemical Industries Ltd.), or Sephadex LH-20 (Pharmacia Co., Ltd.). Preparative HPLC separations were carried out on a Nippon Bunseki Kogyo liquid chromatograph (LC-908) with JAIGEL GS-310 (column size, 20 × 500 mm; detector setting, UV_{254} nm; solvent, MeOH; flow rate, 6 ml/min) or JAIGEL GS-320 (column size, 20 × 500 mm; detector setting, UV_{254} nm; solvent, CH_3CN : H_2O (45 : 55); flow rate, 5 ml/min). CPC was performed on a centrifugal partition chromatograph model L-90 (Sanki Engineering Ltd.) equipped with twelve column cartridges, and developed with butanol–acetic acid–H_2O (4 : 1 : 5, normal-phase and reversed-phase development) at 1000 rpm; the upper layer was used as a stationary phase for the normal-phase development and was pumped into columns prior to the loading of a sample solution, while the lower layer was used as a stationary phase for the reversed-phase development. Thin layer chromatography (TLC) analyses were done on Merck Kieselgel GF_{254} plates developed with $CHCl_3$–MeOH–H_2O (6 : 4 : 1) and benzene–ethyl formate–formic acid (1 : 5 : 2) and spots were detected by the use of 1%

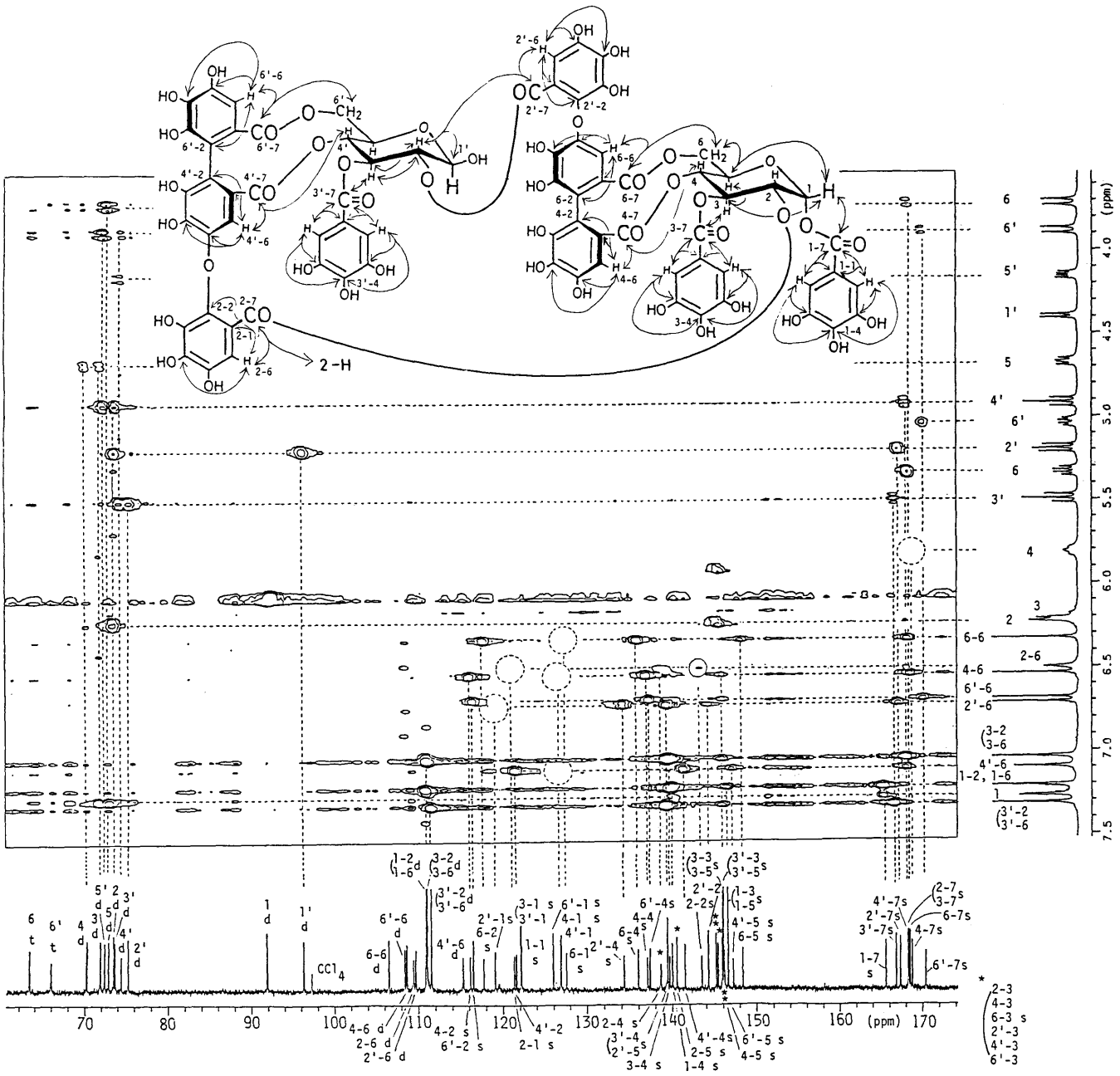

Fig. 4. HMBC Spectrum of **9a** in Acetone-d_6 (60 mg, 38 °C, 64 h Run, $J_{CH}=10$ Hz)

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.

aqueous $FeCl_3$ reagent or UV light. Solutions were concentrated under reduced pressure at a temperature below 40 °C. For drying organic solutions, anhydrous $MgSO_4$ was employed. *In vitro* bioassay of the activity against DNA topoisomerase II was done according to Miller *et al.*[7]

Extraction and Separation of the Constituents of Leaves of *Woodfordia fruticosa* Kurz Dried leaves (590 g) of *W. fruticosa*, collected in Nepal in August, 1987, were pulverized and extracted three times (3 h) with boiling methanol (4 l × 3) and then with boiling water (3 l × 3) to give a methanolic extract (130 g) and a water extract (98 g).

A portion (50 g) of the water extract was dissolved again in water (630 ml), and 5% HCl (20 ml) was added. The acidic aqueous solution was extracted with butanol (1 l × 3) and the combined butanol layers were evaporated to give a residue (28 g). A portion (10 g) of this residue was submitted to CPC to give thirteen normal-phase fractions [fr. 1-1 (0.58 g), fr. 1-2 (1.19 g), fr. 1-3 (0.84 g), fr. 1-4 (4.11 g), fr. 1-5 (1.01 g), fr. 1-6 (0.81 g), fr. 1-7 (0.68 g), fr. 1-8 (0.37 g), fr. 1-9 (0.35 g), fr. 1-10 (0.34 g), fr. 1-11 (0.22 g), fr. 1-12 (0.17 g), and 1-13 (0.05 g)] and eleven reversed-phase fractions [fr. 1-K (0.03 g), fr. 1-J (0.09 g), fr. 1-I (0.15 g), fr. 1-H (0.17 g), fr. 1-G (0.14 g), fr. 1-F (0.11 g), fr. 1-E (0.07 g), fr. 1-D (0.03 g), fr. 1-C (0.04 g), fr. 1-B (0.05 g), and fr. 1-A (0.07 g)]. Among these, fr. 1-11 gave gallic acid (**1a**, 400 mg), mp 238—246 °C, while fr. 1-I gave ellagic acid (**2**, 260 mg), mp >310 °C. On the other hand, fractions 1-12, 1-13, 1-J, and 1-K showed an inhibitory activity against DNA topoisomerase II, and they were combined and again purified by recycling preparative HPLC (column, JAIGEL GS-310F; solvent, MeOH) to give quercetin 3-*O*-(6″-galloyl)-β-D-galactopyranoside (compound F4, **6**) (3 mg) and quercetin 3-*O*-(6″-galloyl)-β-D-glucopyranoside (compound F5, **7**) (1.5 mg).

Quercetin 3-*O*-(6″-Galloyl)-β-D-galactopyranoside (Compound F4, **6**): Light yellow powder, $[\alpha]_D$ +26.1° ($c=0.1$, MeOH). Positive ion FAB-MS: 617 $[M+H]^+$. Negative ion FAB-MS: 615 $[M-H]^-$. UV λ_{max} nm (log ε): 220 (4.57), 260 (4.43), 270 (4.42). IR ν_{max} cm^{-1}: 3250, 1700, 1650, 1610. ^1H- and ^{13}C-NMR: Tables I and II.

Quercetin 3-*O*-(6″-Galloyl)-β-D-glucopyranoside (Compound F5, **7**): Light yellow powder, $[\alpha]_D$ −50.6° ($c=0.3$, MeOH). Positive ion FAB-MS: 617 $[M+H]^+$. Negative ion FAB-MS: 615 $[M-H]^-$. UV λ_{max} nm (log ε): 239 (4.08), 314 (4.18). IR ν_{max} cm^{-1}: 3400, 1700, 1650, 1600. ^1H- and ^{13}C-NMR: Tables I and II.

The methanol extract (130 g) was extracted with chloroform (1 l × 3)

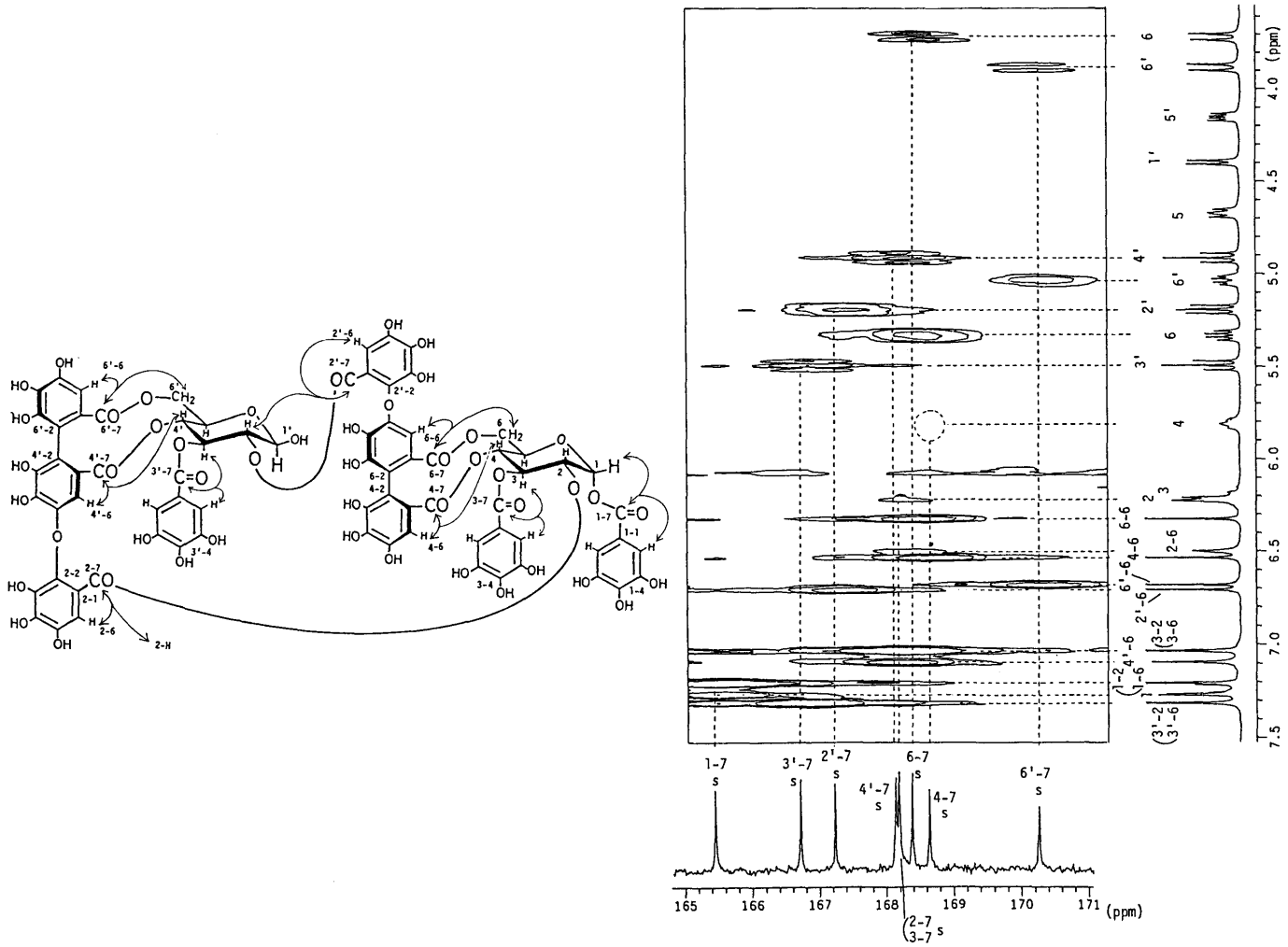

Fig. 5. Enlarged HMBC Spectrum of the Carbonyl Region of **9a** in Acetone-d_6

and then with ethyl acetate (1 l × 3). The insoluble residue (100 g) was dissolved in a minimum amount of MeOH and the insoluble substance (2 g, ellagic acid) was removed by filtration. The MeOH solution was chromatographed on an Iatrobeads (800 g) column to give three fractions: fr. 1 (MeOH–$CHCl_3$ 1 : 3, 2 l) 4.5 g, fr. 2 (MeOH–$CHCl_3$ 1 : 1, 2 l) 15 g, and fr. 3 (MeOH 3 l), 48.5 g. Among these, frs. 2 and 3 had inhibitory activity against DNA topoisomerase II.

Fraction 2 (15 g) was again separated by chromatography over Sephadex LH-20 (350 g) with MeOH–H_2O (6 : 4, 7 : 3, 8 : 2, and 9 : 1; each 1 l) and MeOH (1 l) as the eluents. Each fraction was collected in 10 ml portions and the following fractions were obtained: frs. 1—100 (7.5 g), frs. 101—200 (1.6 g), frs. 201—300 (2.0 g), frs. 301—400 (1.4 g), and frs. 401—500 (0.9 g). Among these, frs. 301—400 were combined and subjected to recycling preparative HPLC with MeOH to give quercetin 3-*O*-α-L-arabinopyranoside (compound F1, **3**) (78 mg), quercetin 3-*O*-β-D-xylopyranoside (compound F2, **4**) (25 mg), myricetin 3-*O*-α-L-arabinopyranoside (compound F3, **5**) (47 mg), quercetin 3-*O*-(6″-galloyl)-β-D-galactopyranoside (compound F4, **6**) (368 mg), quercetin 3-*O*-(6″-galloyl)-β-D-gluopyranoside (compound F5, **7**) (141 mg), and myricetin 3-*O*-(6″-galloyl)-β-D-galactopyranoside (compound F6, **8**) (90 mg).

On the other hand, a portion (22.5 g) of fr. 3 was chromatographed on a Diaion HP-20 (500 ml) column. The column was eluted successively with H_2O (1.5 l), MeOH–H_2O (2 : 8 and 4 : 6, each 1.5 l), and MeOH (1.5 l) and the eluates were separated into four fractions: fr. H-1: H_2O eluate (6.8 g), fr. H-2: MeOH–H_2O (2 : 8) eluate (1.7 g), fr. H-3: MeOH–H_2O (4 : 6) eluate (6.0 g), fr. H-4: MeOH eluate (5.3 g).

Fraction H-3 has an inhibitory activity against DNA topoisomerase II. A portion (5.0 g) of fr. H-3 was again subjected to chromatography over Sephadex LH-20 (300 g) and the eluates were separated into seven fractions: fr. L-1: MeOH–H_2O (2 : 8 and 4 : 6, each 2 l) eluate (0.44 g), fr. L-2: MeOH–H_2O (6 : 4, 3 l) eluate (0.15 g), fr. L-3: MeOH–H_2O (7 : 3, 3 l) eluate (1.30 g), fr. L-4: MeOH–H_2O (8 : 2, 2 l) eluate (1.10 g), fr. L-5: MeOH–H_2O (9 : 1, 2 l) eluate (1.20 g), fr. L-6: MeOH (3 l) eluate (0.60 g), fr. L-7: acetone–H_2O (1 : 1, 5 l) eluate (0.25 g).

Fraction L-3 was further separated by chromatography over Sephadex LH-20 (100 g) with MeOH–H_2O (7 : 3) to give woodfruticosin (woodfordin C) (**9a**) (0.75 g).

Quercetin 3-*O*-α-L-Arabinopyranoside (Compound F1, **3**): Light yellow powder, $[\alpha]_D$ −60.1° (c=0.7, MeOH). Positive ion FAB-MS: 435 $[M+H]^+$. Negative ion FAB-MS: 433 $[M-H]^-$. UV λ_{max} nm (log ε): 257 (4.16), 268sh (4.08), 360 (4.10). IR ν_{max} cm^{-1}: 3400, 1640, 1600. ^1H- and ^{13}C-NMR: Tables I and II.

Quercetin 3-*O*-β-D-Xylopyranoside (Compound F2, **4**): Light yellow powder, $[\alpha]_D$ −38.8° (c=0.1, MeOH). Negative ion FAB-MS: 433 $[M-H]^-$. UV λ_{max} nm (log ε): 240 (4.21), 285 (4.25). IR ν_{max} cm^{-1}: 3300, 1650, 1600. ^1H- and ^{13}C-NMR: Tables I and II.

Myricetin 3-*O*-α-L-Arabinopyranoside (Compound F3, **5**): Light yellow powder, $[\alpha]_D$ −54.2° (c=0.1, MeOH). Positive ion FAB-MS: 451 $[M+H]^+$. Negative ion FAB-MS: 449 $[M-H]^-$. UV λ_{max} nm (log ε): 240 (4.08), 288 (4.19), 297 (4.17). IR ν_{max} cm^{-1}: 3300, 1650, 1600. ^1H- and ^{13}C-NMR: Tables I and II.

Myricetin 3-*O*-(6″-Galloyl)-β-D-galactopyranoside (Compound F6, **8**): Light yellow powder, $[\alpha]_D$ +33.7° (c=0.1, MeOH). Positive ion FAB-MS: 633 $[M+H]^+$. Negative ion FAB-MS: 631 $[M-H]^-$. UV λ_{max} nm (log ε): 240 (4.32), 320 (4.39). IR ν_{max} cm^{-1}: 3300, 1700, 1650, 1600. ^1H- and ^{13}C-NMR: Tables I and II.

Woodfruticosin (Woodfordin C) (**9a**): Light brown powder, $[\alpha]_D$ +182° (c=0.7, acetone). *Anal.* Calcd for $C_{75}H_{52}O_{48}\cdot 6H_2O$: C, 48.22; H, 3.59. Found: C, 48.63; H, 3.43. Negative ion FAB-MS: 1719 $[M-H]^-$. UV λ_{max} nm (log ε): 219 (5.07), 271 (4.77). IR ν_{max} cm^{-1}: 3300, 1715, 1610. CD ($c=5.8\times10^{-5}$, MeOH) [θ] (nm): $+1.6\times10^5$ (239), -5.1×10^4 (261). ^1H- and ^{13}C-NMR: Table III.

Methylation of Woodfruticosin (Woodfordin C) (9a) A mixture of **9a** (10 mg), dimethyl sulfate (90 μl), and K_2CO_3 (100 mg) in dry acetone (2 ml)

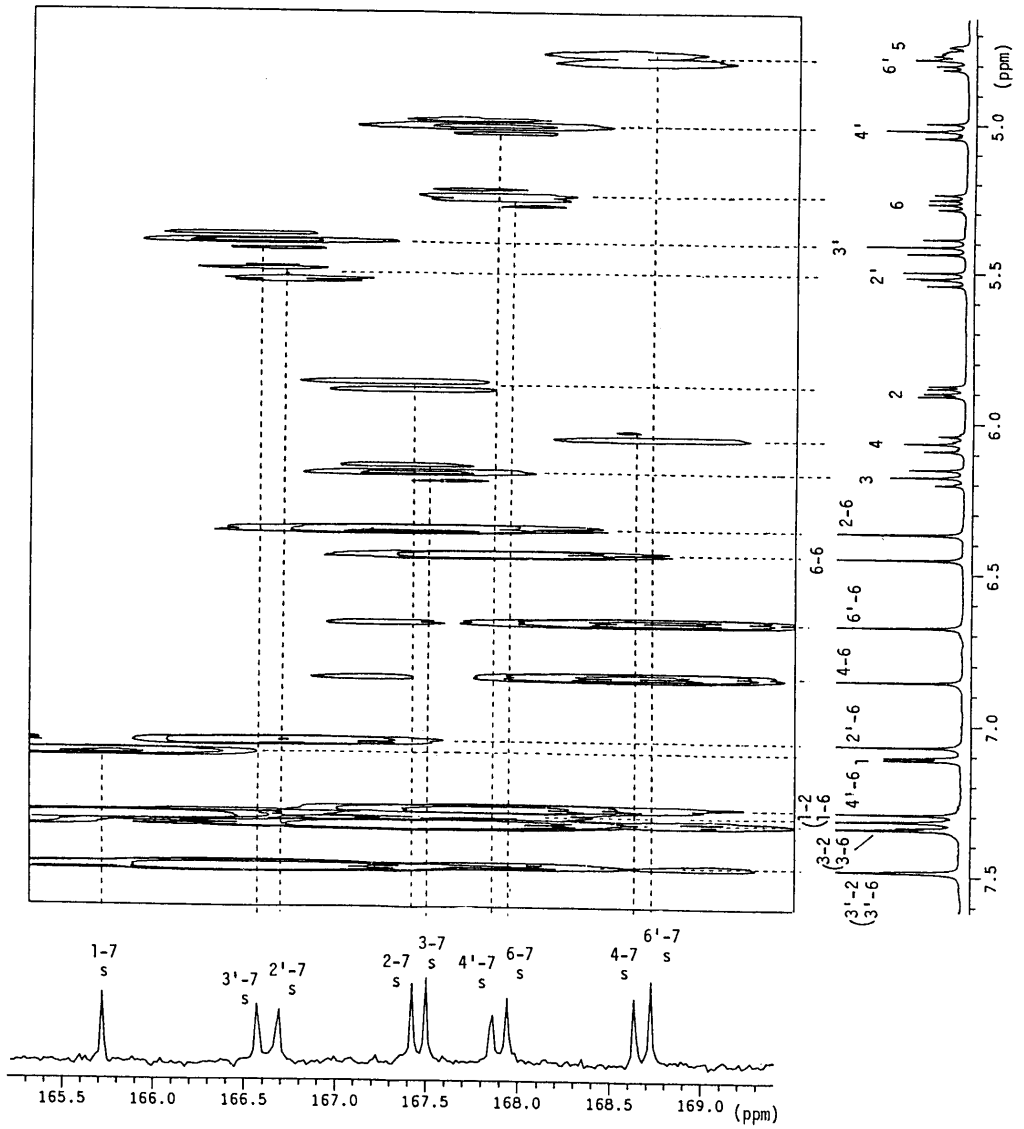

Fig. 6. Enlarged HMBC Spectrum of the Carbonyl Region of the Hexacosa-*O*-methylated Compound (**9b**) in Acetone-d_6 (70 mg, 38 °C, 42 h Run $J_{CH}=4.5$ Hz)

was stirred for 12 h at room temperature, and then refluxed for 3 h. After removal of insoluble material by filtration, the filtrate was concentrated *in vacuo*. The residue was purified by preparative TLC with benzene–acetone (3 : 1) to give the hexacosa-*O*-methylated compound (**9b**) (7 mg), light yellow powder, $[\alpha]_D$ +126° ($c=0.5$, acetone). Positive ion FAB-MS: 2085 $[M+H]^+$. UV λ_{max} nm ($\log\varepsilon$): 255 (5.77), 292sh (5.51). IR ν_{max} cm^{-1} (KBr): 1720, 1580. ^{1}H-NMR (acetone-d_6, 38 °C): δ 3.79 (1H, dd, $J=10$, 2.0 Hz, 6-H), 3.96 (1H, dd, $J=13$, 2.5 Hz, 6′-H), 4.09 (1H, ddd, $J=10$, 5.5, 2.5 Hz, 5′-H), 4.36 (1H, d, $J=8.0$ Hz, 1′-H), 4.75 (1H, ddd, $J=10$, 7.0, 2.0 Hz, 5-H), 4.78 (1H, dd, $J=13$, 5.5 Hz, 6′-H), 5.02 (1H, t, $J=10$ Hz, 4′-H), 5.26 (1H, dd, $J=13$, 7.0 Hz, 6-H), 5.40 (1H, t, $J=10$ Hz, 3′-H), 5.51 (1H, dd, $J=10$, 8.0 Hz, 2′-H), 5.88 (1H, dd, $J=10$, 4.0 Hz, 2-H), 6.05 (1H, t, $J=10$ Hz, 4-H), 6.17 (1H, t, $J=10$ Hz, 3-H), 6.36 (1H, s, 2-6-H), 6.44 (1H, s, 6-6-H), 6.67 (1H, s, 6′-6-H), 6.85 (1H, s, 4-6-H), 7.06 (1H, s, 2′-6-H), 7.10 (1H, d, $J=4.0$ Hz, 1-H), 7.29 (1H, s, 4′-6-H), 7.32 (2H, s, 1-2-H and 1-6-H), 7.34 (2H, s, 3-2-H and 3-6-H), 7.48 (2H, s, 3′-2-H and 3′-6-H). ^{13}C-NMR (acetone-d_6, 38 °C): δ 63.98 (d, C-6), 65.53 (d, C-6′), 70.05 (d, C-4), 71.95 (d, C-5), 72.59 (d, C-3), 72.85 (d, C-5′), 73.79 (d, C-2′), 73.99 (d, C-2), 74.46 (d, C-4′), 74.87 (d, C-3′), 92.12 (d, C-1), 102.99 (d, C-1′), 165.71 (s, C-1-7), 166.56 (s, C-3′-7), 166.68 (s, C-2′-7), 167.41 (s, C-2-7), 167.49 (s, C-3-7), 167.85 (s, C-4′-7), 167.93 (s, C-6-7), 168.63 (s, C-4-7), and 168.72 (s, C-6′-7).

Methanolysis of Hexacosa-*O*-Methylated Compound (9b) A solution of **9b** (7 mg) in absolute methanol (1 ml) and 1% methanolic sodium methoxide (1 ml) was left to stand overnight with stirring at room temperature. Then the reaction mixture was neutralized with acetic acid and concentrated *in vacuo*. The residue was partitioned between chloroform and H_2O. The chloroform layer was concentrated and the residue was purified by preparative TLC with $CHCl_3$, giving two bands. The less polar band afforded methyl tri-*O*-methylgallate (**1b**) (1.5 mg), while the more polar band gave trimethyl (*S*)-octa-*O*-methylvaloneate (**10**) (2.1 mg). The aqueous layer afforded glucose and methyl β-glucoside, which were identified by GC comparison with authentic samples after trimethylsilylation.

Methyl Tri-*O*-methylgallate (**1b**): White powder, MS *m/z*: 226 (M^+), 212, 195, 183, 155. High-resolution MS *m/z*: Found 226.0829, Calcd for $C_{11}H_{14}O_5$ (M^+) 226.0841. ^{1}H-NMR: δ 7.34 (2H, s) and 3.91 (12H, Me × 4).

Trimethyl (*S*)-Octa-*O*-methylvaloneate (**10**): White powder, $[\alpha]_D$ −15.4° ($c=0.3$, acetone). MS *m/z*: 660 (M^+), 308. High-resolution MS *m/z*: Found 660.2039, Calcd for $C_{32}H_{36}O_{15}$ (M^+) 660.2054. UV λ_{max} nm ($\log\varepsilon$): 222 (4.14), 303 (3.85). CD ($c=1.4\times10^{-4}$, MeOH) [θ] nm: -3.9×10^4 (250). ^{1}H-NMR: 6.92 (1H, s), 7.30 (1H, s), 7.35 (1H, s), 3.50—4.07 (33H, Me × 11).

Acknowledgement We are grateful to Prof. T. Okuda and Assoc. Prof. T. Yoshida of Okayama University for valuable discussions. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 63870090) from the Ministry of Education, Science and Culture of Japan. One of the authors (K. N. N.) is grateful to the Japanese Government for a scholarship.

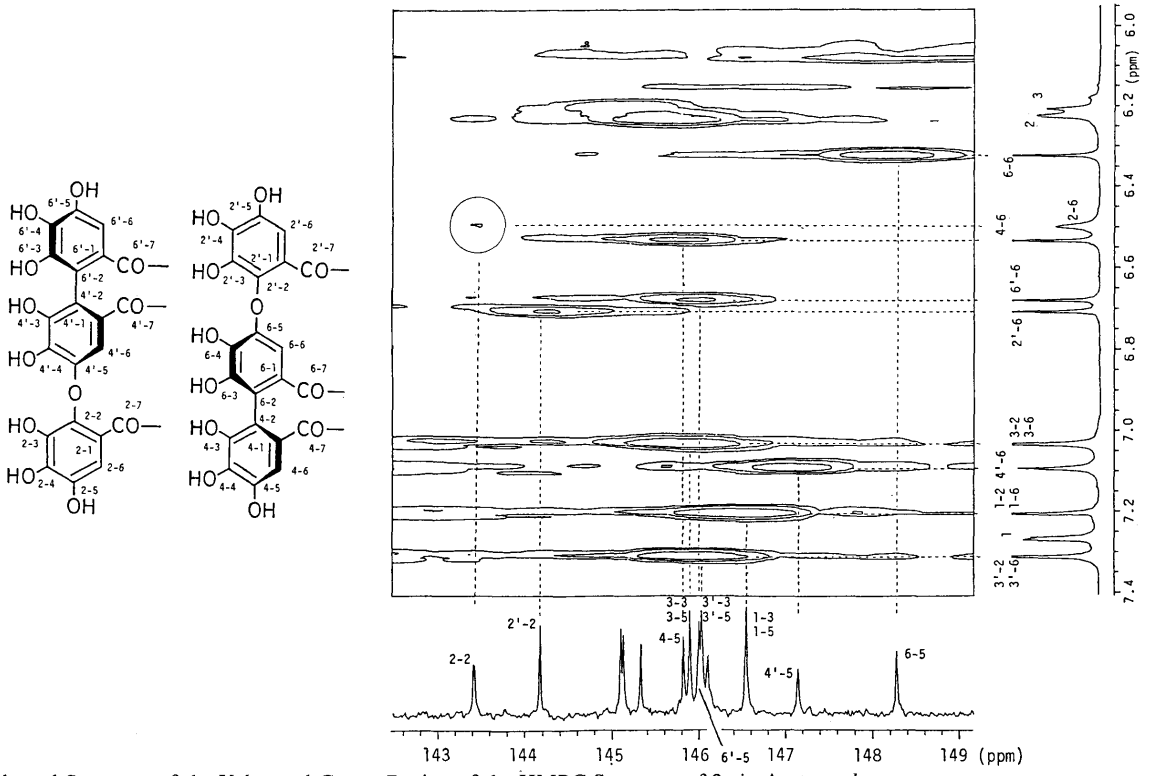

Fig. 7. Enlarged Spectrum of the Valoneoyl Group Region of the HMBC Spectrum of **9a** in Acetone-d_6

TABLE III. ^{1}H- and ^{13}C-NMR Data for Woodfruticosin (Woodfordin C) (**9a**) (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 (3.0)	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			
			1-5	—	146.51 s			
3	6.21 m	71.86 d	1-6	7.20 s	110.83 d			
			1-7	—	165.42 s			
4	5.81 br t (10)	70.18 d	2-1	—	121.18 s	2'-1	—	118.93 s
			2-2	—	143.40 s	2'-2	—	144.14 s
5	4.67 dd (10, 7.0)	72.85 d	2-3	—	140.44[b] s	2'-3	—	145.30[b] s
			2-4	—	138.45 s	2'-4	—	134.23 s
			2-5	—	138.54[b] s	2'-5	—	139.33 s
6	3.72 d (13)	63.27 t	2-6	6.48 br s	109.32 d	2'-6	6.71 s	109.58 d
	5.33 dd (13, 7.0)		2-7	—	168.16[c] s	2'-7	—	167.20 s
			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
1'	4.40 d (8.0)	96.18 d	3-3	—	145.87 s	3'-3	—	146.01 s
			3-4	—	139.57 s	3'-4	—	139.33 s
			3-5	—	145.87 s	3'-5	—	146.01 s
2'	5.19 dd (9.5, 8.0)	75.22 d	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
3'	5.49 t (9.5)	73.64 d	4-1	—	126.73 s	4'-1	—	126.81 s
			4-2	—	116.02 s	4'-2	—	121.44 s
			4-3	—	145.08[b] s	4'-3	—	146.01[b] s
4'	4.91 t (10)	74.37 d	4-4	—	136.98 s	4'-4	—	141.40 s
			4-5	—	145.80 s	4'-5	—	147.12 s
			4-6	6.53 s	108.53 d	4'-6	7.09 s	115.15 d
5'	4.15 dd (10, 5.5)	72.38 d	4-7	—	168.61 s	4'-7	—	168.11 s
			6-1	—	127.46 s	6'-1	—	125.85 s
6'	3.88 d (13)	65.90 t	6-2	—	117.58 s	6'-2	—	116.38 s
	5.04 dd (13, 5.5)		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*) Overlapping of two carbon signals was confirmed by the ^{13}C-NMR measurement without NOE. In the *O*-methylated compound **9b**, the ^{13}C-signals of C-2-7 and C-3-7 (in acetone-d_6) are observed at δ 167.41 and 167.49, respectively, which are 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.

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