[Chem. Pharm. Bull. 36(8)2925—2933(1988)]

^{13}C Nuclear Magnetic Resonance Spectra of Hydrolyzable Tannins. II.[1] Tannins Forming Anomer Mixtures

TSUTOMU HATANO,[a] TAKASHI YOSHIDA,[a] TETSURO SHINGU[b] and TAKUO OKUDA*,[a]

Faculty of Pharmaceutical Sciences, Okayama University,[a] Tsushima, Okayama 700, Japan and Faculty of Pharmaceutical Sciences, Kobe Gakuin University,[b] Ikawadani, Nishi-ku, Kobe 673, Japan

(Received February 6, 1988)

The glucose carbon signals in the ^{13}C nuclear magnetic resonance (NMR) spectra of hydrolyzable tannins forming anomer mixtures, which differ from each other in the locations of acyl groups, were assigned. The utility of these data was demonstrated by application to the structural assignments of recently isolated dimeric hydrolyzable tannins [*e.g.*, camptothin A (**13**) and camptothin B (**14**)].

Keywords——tannin; ^{13}C-NMR; 2D NMR; anomer mixture; ellagitannin; dimeric hydrolyzable tannin; rugosin E; cornusiin A; camptothin A; camptothin B

The use of ^{13}C nuclear magnetic resonance (NMR) spectroscopy is one of the most informative approaches for the structural study of hydrolyzable tannins.[1,2] The determination of the locations of acyl groups on the sugar moieties in the tannin molecules is one of the important applications of this approach. We previously reported full assignments of the glucose carbon signals in the ^{13}C-NMR spectra of hydrolyzable tannins possessing $^4C_1$ glucopyranose cores in which the anomeric centers are acylated.[1] We also investigated the ^{13}C-NMR spectra of tannins existing as anomer mixtures, such as tellimagrandin I (**1**)[3] and pedunculagin (**2**).[3] The diagnostic chemical shifts of anomeric carbons in these tannins and the other tannins forming anomer mixtures allowed ready discrimination of substituents at C-2 of the glucose residues.[1] However, the other sugar carbon resonances in these tannins have not yet been assigned. We now report the full assignments of the sugar carbons of the tannins forming anomer mixtures in which the glucopyranose cores adopt the $^4C_1$ conformation, and application of these NMR data to the characterization of recently isolated dimeric hydrolyzable tannins.

Results and Discussion

Monomeric Tannins Forming Anomer Mixtures

Tannins forming anomer mixtures, especially oligomeric hydrolyzable tannins in which all the anomeric centers are unacylated [*e.g.*, cornusiin A (**3**)[4]], show complicated and less informative proton nuclear magnetic resonance (^1H-NMR) spectra. However, the ^{13}C-NMR spectra are expected to be useful for structure elucidation of such oligomers, since the locations of acyl groups in the dimeric tannins having acylated anomeric centers can be readily determined by comparison of their glucose carbon signals with those of the constituent monomeric tannins.[1] Therefore, we first tried to assign the glucose carbon signals of monomeric tannins forming anomer mixtures, to facilitate the structural study of oligomeric hydrolyzable tannins existing as anomer mixtures.

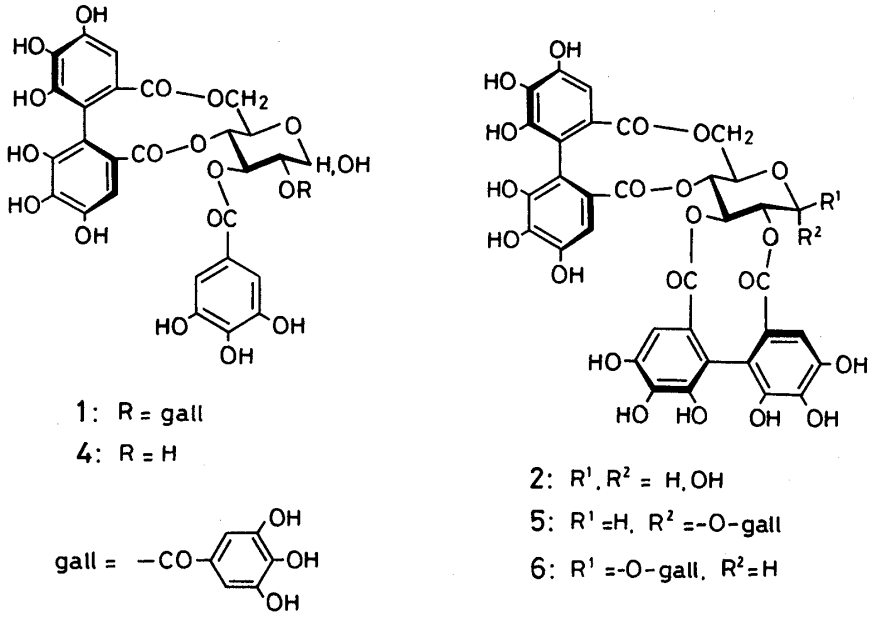
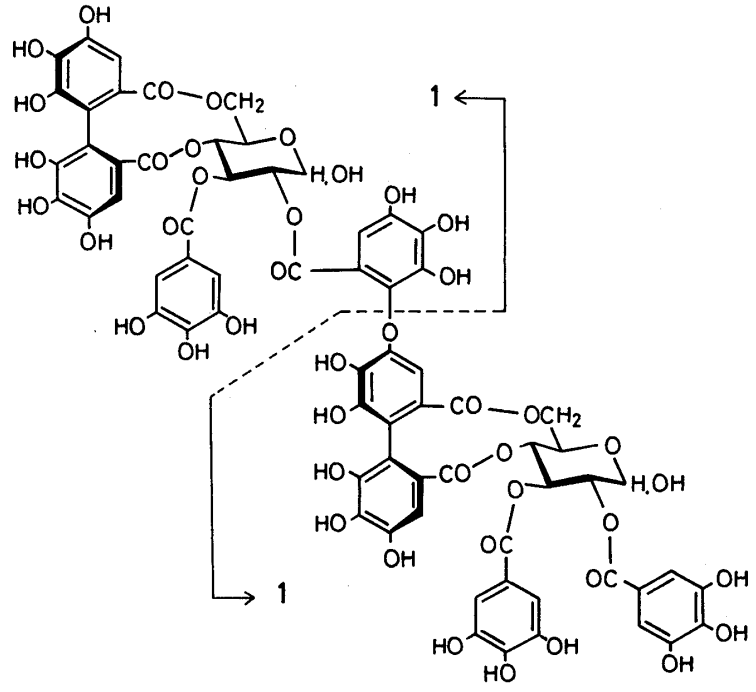


Chart 1



3

Chart 2

In advance of the ^{13}C-NMR measurements, the glucose proton signals of the monomeric tannins, **1**, **2**, and gemin D (**4**),[5] which form anomer mixtures and are frequently found as constituent units of oligomers, were unequivocally assigned based on the ^{1}H–^{1}H correlation spectra, and the results are summarized in Table I.

The proton signals of all of the measured monomeric tannins showed the coupling constants characteristic of $^{4}C_{1}$ glucopyranose. Then the ^{1}H-^{13}C correlation spectra of these tannins were recorded, and the established assignments of the ^{13}C signals of the glucose

Table I. ^{1}H-NMR Spectral Data for Glucose Residue in Monomeric Tannins Forming Anomer Mixtures

	Tellimagrandin I (**1**)[a]	Pedunculagin (**2**)[b]	Gemin D (**4**)[b]
α-Anomer			
H-1	5.57 (d, J=4 Hz)	5.50 (d, J=3.5 Hz)	5.31 (d, J=4 Hz)
H-2	5.11 (dd, J=4, 10 Hz)	5.09 (dd, J=3.5, 10 Hz)	3.86 (dd, J=4, 10 Hz)
H-3	5.88 (t, J=10 Hz)	5.49 (t, J=10 Hz)	5.52 (t, J=10 Hz)
H-4	5.11 (t, J=10 Hz)	5.10 (t, J=10 Hz)	4.96 (t, J=10 Hz)
H-5	4.67 (ddd, J=1.5, 6.5, 10 Hz)	4.63 (ddd, J=1.5, 6.5, 10 Hz)	4.57 (ddd, J=1, 6.5, 10 Hz)
H-6	5.28 (dd, J=6.5, 13 Hz)	5.29 (dd, J=6.5, 13 Hz)	5.25 (dd, J=6.5, 13 Hz)
H-6	3.77 (dd, J=1.5, 13 Hz)	3.80 (dd, J=1.5, 13 Hz)	3.75 (dd, J=1, 13 Hz)
β-Anomer			
H-1	5.13 (d, J=8 Hz)	5.09 (d, J=8 Hz)	4.78 (d, J=7.5 Hz)
H-2	5.24 (dd, J=8, 10 Hz)	4.87 (dd, J=8, 9 Hz)	3.61 (dd, J=7.5, 9 Hz)
H-3	5.61 (t, J=10 Hz)	5.26 (dd, J=9, 10 Hz)	5.35 (dd, J=9, 10 Hz)
H-4	5.11 (t, J=10 Hz)	5.09 (t, J=10 Hz)	4.99 (t, J=10 Hz)
H-5	4.27 (ddd, J=1, 6.5, 10 Hz)	4.24 (ddd, J=1, 6.5, 10 Hz)	4.11 (ddd, J=0.5, 6.5, 10 Hz)
H-6	5.30 (dd, J=6.5, 13 Hz)	5.31 (dd, J=6.5, 13 Hz)	5.27 (dd, J=6.5, 13 Hz)
H-6	3.84 (dd, J=1, 13 Hz)	3.86 (dd, J=1, 13 Hz)	3.81 (dd, J=0.5, 13 Hz)

a) 500 MHz. *b*) 400 MHz.

Table II. ^{13}C-NMR Spectral Data for Glucose Residue in Hydrolyzable Tannins (100 MHz)

	α-Anomer						β-Anomer					
	C-1	C-2	C-3	C-4	C-5	C-6	C-1	C-2	C-3	C-4	C-5	C-6
Tellimagrandin I (**1**)	91.2	72.9	71.1	71.1	67.2	63.5	96.7	74.1	73.5	71.1	72.0	63.5
Pedunculagin (**2**)	91.8	75.6	75.8	69.9	67.4	63.6	95.4	78.3	77.6	69.6	72.5	63.6
Gemin D (**4**)	94.0	72.1	74.0	71.0	67.5	63.8	98.4	74.8	75.9	71.3	72.0	63.8
Potentillin (**5**)[a]	90.7	74.1	76.0	69.1	71.0	63.2						
Casuarictin (**6**)[a]							92.4	76.0	77.3	69.3	73.5	63.1
Penta-*O*-galloyl-β-D-glucose (**10**)[a]							93.4	71.9	73.5	69.5	74.1	62.9
Tellimagrandin II (**11**)[a]							93.8	71.8	73.3	70.8	73.1	63.1
Rugosin E (**12**)												
Glucose core L[b]							93.1	71.6	73.1	70.5	72.8	62.9
Glucose core R[c]	91.1	72.8	71.2	71.0	66.7	63.4	96.5	74.0	73.5	71.0	71.7	63.4

a) Data taken from ref. 1 (50.1 MHz). *b*) Left glucose core in formula **12**. The signals of the glucose core L of both anomers (due to the anomerization of the glucose core R) overlap with each other. *c*) Right glucose core in formula **12**.

moiety are listed in Table II.

Formerly we reported that the C-1, C-3 and C-5 signals of glucose in potentillin (**5**),[6] which possesses an axial acyl group at the anomeric center, are shifted upfield from the signals of casuarictin (**6**),[3] which has an equatorial acyl group at the anomeric center (C-1, −1.7 ppm; C-3, −1.3 ppm; C-5, −2.5 ppm).[1] However, in the tannins forming anomer mixtures, **1**, **2**, and **4**, larger upfield shifts of the signals of C-1, C-3 and C-5 of the α-anomers from the corresponding signals of β-anomers (Table III) are observed. The C-2 signals also show upfield shifts for the α-anomers.

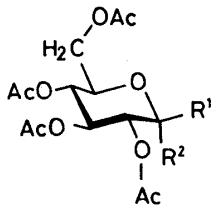
It was reported that the C-1 signal of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucose (**7**) shows a large upfield shift (−3.83 ppm) relative to the signal of the β-anomer of 2,3,4,6-tetra-*O*-acetyl-D-glucose (**8**), and that the C-5 signal of 1,2,3,4,6-penta-*O*-acetyl-α-D-glucose (**9**) shows a significant downfield shift (+2.69 ppm) relative to that of the α-anomer of **8**, upon acylation

TABLE III. Differences (ppm) of the Chemical Shifts of the Glucose Carbons of α-Anomers of Monomeric Tannins Relative to Those of Corresponding Carbons of β-Anomers

	C-1	C-2	C-3	C-4	C-5	C-6
Tellimagrandin I (**1**)	−5.5	−1.2	−2.4	0	−4.8	0
Pedunculagin (**2**)	−3.6	−2.7	−1.8	+0.3	−5.1	0
Gemin D (**4**)	−4.4	−2.7	−1.9	−0.3	−4.5	0

TABLE IV. Effects of Galloylation on the Chemical Shifts of the Glucose Carbons of Monomeric Tannins

	Changes in the chemical shifts (ppm)					
	C-1	C-2	C-3	C-4	C-5	C-6
α-Anomer of **2**→**5**	−1.1	−1.5	+0.2	−0.8	+3.6	−0.4
β-Anomer of **2**→**6**	−3.0	−2.3	−0.3	−0.3	+1.0	−0.5
β-Anomer of **1**→**11**	−2.9	−2.3	−0.2	−0.3	+1.1	−0.4
α-Anomer of **4**→α-anomer of **1**	−2.8	+0.8	−2.9	+0.1	−0.3	−0.3
β-Anomer of **4**→β-anomer of **1**	−1.7	−0.7	−2.4	−0.2	0	−0.3

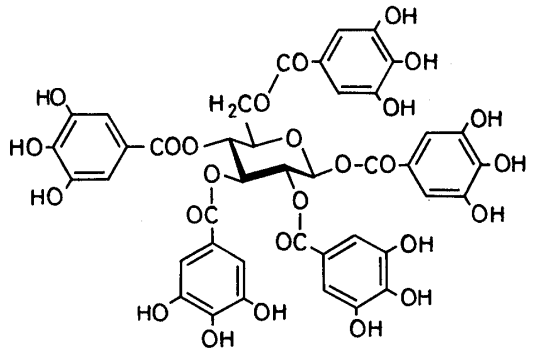


7: R^1 = -OAc, R^2 = H

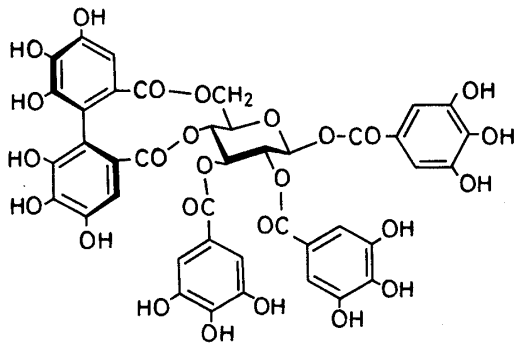
8: R^1, R^2 = H, OH

9: R^1 = H, R^2 = -OAc

Ac = CH_3CO-



10



11

Chart 3

at O-1.[7] Similar effects upon acylation at O-1 were also reported for other acyl groups,[8] and were explained in terms of *exo*-anomeric and anomeric effects.[8] The observed effects upon the galloylation at O-1 of the monomeric hydrolyzable tannins (Table IV) could be interpreted in an analogous way.

The effects of O-2 galloylation can be seen by comparison of the ^{13}C signals between

TABLE V. Sequences of the ^{13}C Signals of the Glucose Residue in Hydrolyzable Tannins Forming Anomer Mixtures

	Sequences (lower field ↔ higher field)
α-Anomer	
1	C-1 ---- C-2 ---- C-3[a] --- C-4[b] --- C-5 ---- C-6
2	C-1 ---- C-3 ---- C-2 ---- C-4 ---- C-5 ---- C-6
4	C-1 ---- C-3 ---- C-2 ---- C-4 ---- C-5 ---- C-6
β-Anomer	
1	C-1 ---- C-2 ---- C-3 ---- C-5 ---- C-4 ---- C-6
2	C-1 ---- C-2 ---- C-3 ---- C-5 ---- C-4 ---- C-6
4	C-1 ---- C-3 ---- C-2 ---- C-5 ---- C-4 ---- C-6

a) δ 71.12. *b*) δ 71.06.

TABLE VI. The Effects of Substitution of a Hexahydroxydiphenoyl (HHDP) Group for Two Galloyl Groups on the Glucose Carbon Signals

	Changes in the chemical shifts (ppm)					
	C-1	C-2	C-3	C-4	C-5	C-6
α-Anomer of **1**→α-anomer of **2**	+0.6	+2.7	+4.7	−1.2	+0.2	+0.1
β-Anomer of **1**→β-anomer of **2**	−1.3	+4.2	+4.1	−1.5	+0.5	+0.1
11→**6**	−1.4	+4.2	+4.0	−1.5	+0.4	0
10→**11**	+0.4	−0.1	−0.2	+1.3	−1.0	+0.2

gemin D (**4**) and tellimagrandin I (**1**) (Table IV). The differences of the chemical shifts of the β-carbon signals (C-1 and C-3) in **1** from the corresponding signals of **4** are almost the same as the galloylation shifts observed for galloylglucoses,[1] although the value of the upfield shift of the C-3 signal of each anomer is large enough to change the sequence of the signals (Table V).

The effects of the substitution of a hexahydroxydiphenoyl (HHDP) group for two galloyl groups at O-2 and O-3 are shown by a comparison of the ^{13}C resonances of pedunculagin (**2**) with those of tellimagrandin I (**1**) (Table VI). The downfield shifts of the C-2 and C-3 signals of both anomers of **2** are significant. The downfield shifts can be explained by the formation of a diphenoyl ester bridge which may result in a constraint of the glucopyranose ring.[9]

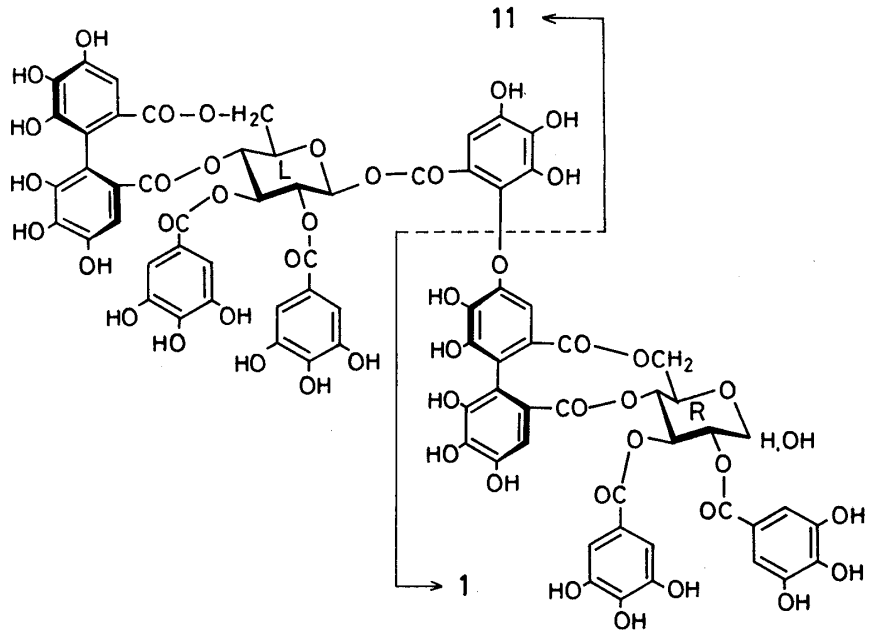
Although conspicuous downfield shifts of the C-3 (+3.7 ppm) and C-5 (+3.1 ppm) signals on substitution of two HHDP groups for four galloyl groups were reported for the ^{13}C signals of casuarictin (**6**) relative to those of penta-*O*-galloyl-β-D-glucose (**10**),[9] our $\{^1H\}$–^{13}C single-frequency selective decoupling experiments on **6** revealed that the reported assignments should be revised.[1] The signals of C-2 and C-3 in **6** exhibit fairly large downfield shifts on the basis of the revised assignments (Table VI), while the effects on the signals of C-4—C-6 were rather small. The difference of these effects may have been induced by the difference of rigidity between the ten-membered ring containing the diphenoyl ester bridge located at C-2 and C-3, and the eleven-membered ring containing the bridge located at C-4 and C-6. In fact, the effects of substitution at C-4 and C-6, as shown by the differences in the chemical shifts of the glucose carbons between tellimagrandin II (**11**)[3,10] possessing an HHDP group at C-4 and C-6, and penta-*O*-galloyl-β-D-glucose (**10**), are small (≤ 1.3 ppm). This is in contrast with the marked effects (up to 4.7 ppm) of the substitution at C-2 and C-3, as indicated by the analogous comparison of **6** and **11** (Table VI).

Dimeric Hydrolyzable Tannins Existing as Anomer Mixtures

Although the ^{13}C-NMR spectra of dimeric hydrolyzable tannins forming anomer

mixtures are complicated, the following examples show that a comparison of the ^{13}C-NMR data with those of monomeric tannins allows assignment of the locations of the acyl groups in the dimeric tannins without degradative experiments.

Rugosin E (**12**),[11] one of the dimeric tannins existing as an anomer mixture, might be biogenetically produced from tellimagrandin I (**1**) and tellimagrandin II (**11**) by C-O oxidative coupling.[1,11] The chemical shifts of the glucose carbons in the ^{13}C-NMR spectrum of **12** show good agreement with those of the merged signals of **1** and **11**, allowing facile assignments of



12

Chart 4

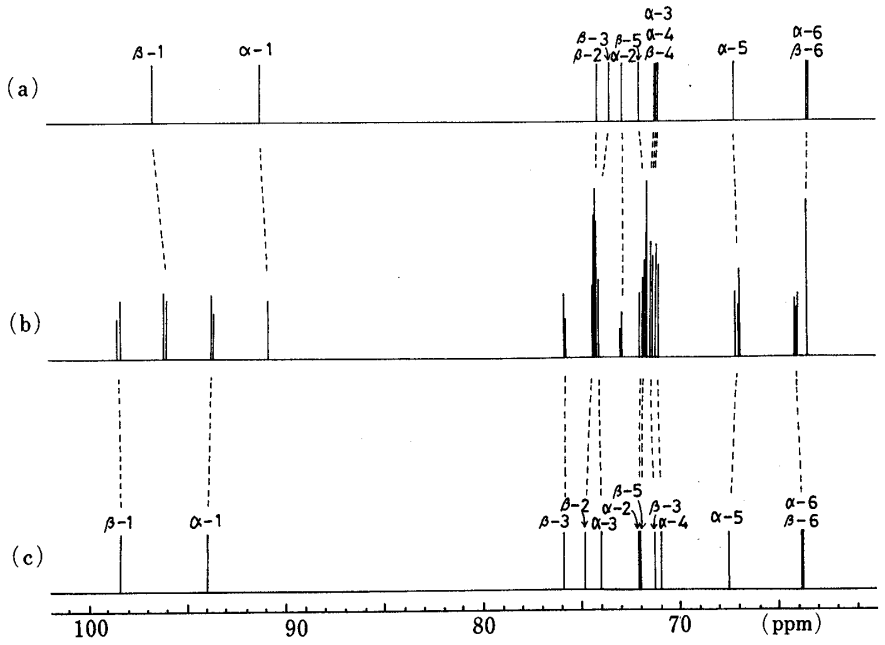
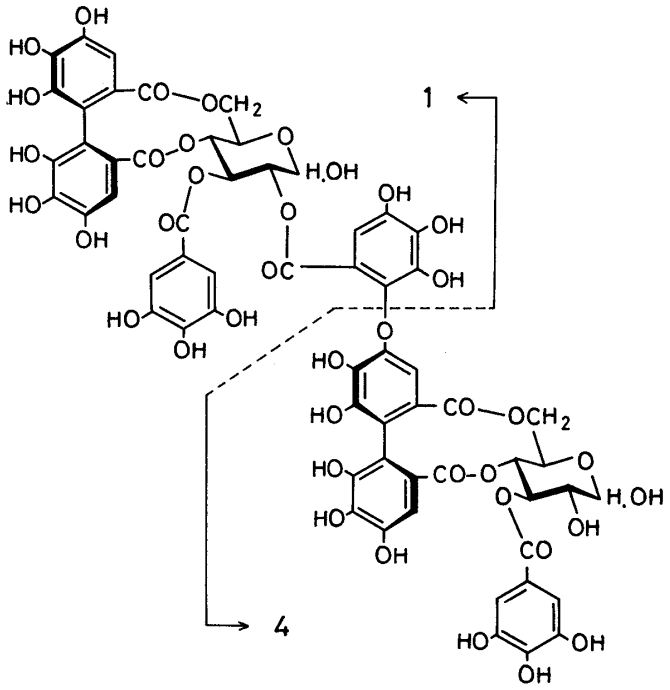


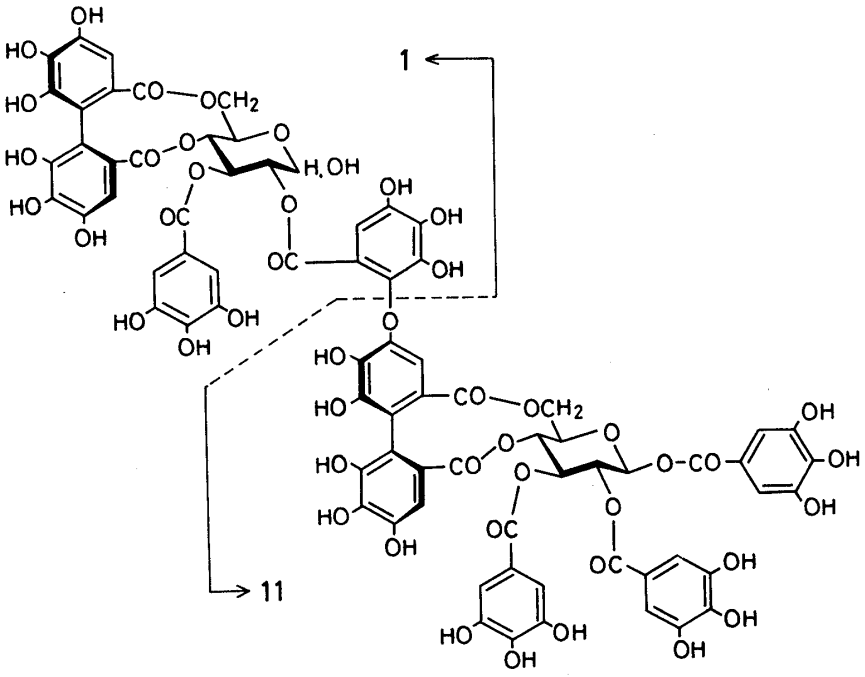
Fig. 1. Stick-Diagrams of the ^{13}C-NMR Spectra for the Glucose Carbons of (a) Tellimagrandin I (**1**), (b) Camptothin A (**13**, 125 MHz) and (c) Gemin D (**4**)

β-1 means C-1 of the glucose core of the β-anomer.



13

Chart 5



14

Chart 6

the glucose carbon resonances of the dimer (Table II). This assignment for rugosin E has been unambiguously confirmed by the ^{1}H–^{13}C correlation spectrum of **12** in combination with the ^{1}H–^{1}H correlation spectrum.

Analogous correspondence of the ^{13}C signals of glucose carbons of a dimeric tannin to

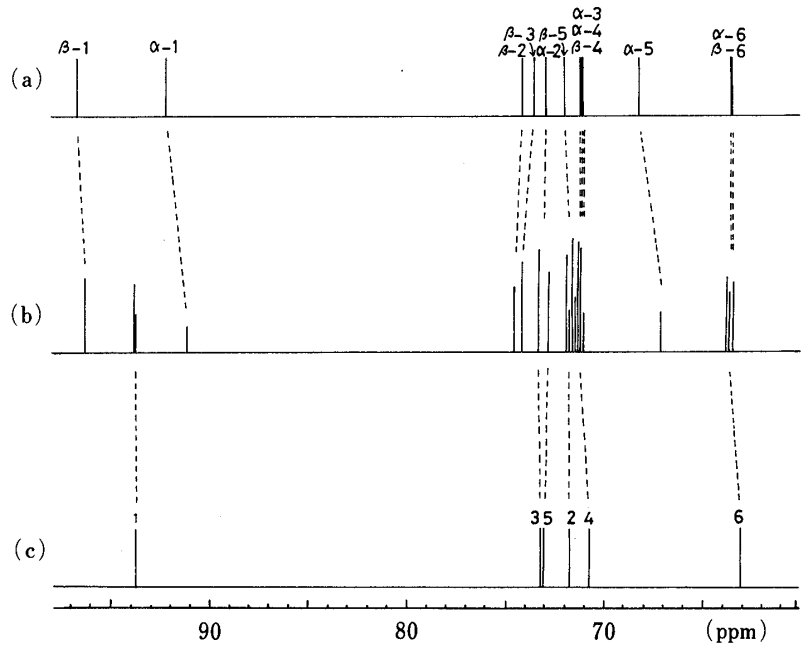


Fig. 2. Stick-Diagrams of the ^{13}C-NMR Spectra for the Glucose Carbons of (a) Tellimagrandin I (**1**), (b) Camptothin B (**14**, 100 MHz) and (c) Tellimagrandin II (**11**)

those of the constituent monomers was observed for cornusiin A (**3**).[4] The ^{13}C signals of glucose carbons of **3**, which could be produced through oxidative coupling of two molecules of tellimagrandin I (**1**) in plant tissues, were found to be almost superimposable upon the glucose carbon signals of **1**.

Then the ^{13}C-NMR data of monomeric tannins were applied to the structural assignments of two new dimeric hydrolyzable tannins forming anomer mixtures, named camptothin A (**13**) and camptothin B (**14**), which were isolated from *Camptotheca acuminata* leaves.[12]

The ^{1}H-NMR and the fast-atom bombardment mass spectra indicate that camptothin A (**13**) possesses two galloyl groups, an HHDP group and a valoneoyl group together with two glucose cores. Although this tannin, existing as four anomeric forms, showed a complicated ^{13}C-NMR spectrum, the chemical shifts of the glucose carbon signals are comparable to those of the combined signals of the constituent anomers, tellimagrandin I (**1**) and gemin D (**4**), as shown in Fig. 1. Therefore the locations of the acyl groups in camptothin A are deduced to be as follows. The two galloyl groups and the galloyl part of the valoneoyl group should be at O-3 of a glucose core and at O-2 and O-3 of the other glucose core, and the HHDP group and the HHDP moiety of the valoneoyl group should be at O-4 and O-6 of the two glucose cores. One of the possible structures, **13**, thus assigned for camptothin A, was consistent with that deduced on the basis of chemical and ^{1}H-NMR spectroscopic data.

The structure of camptothin B (**14**) was also substantiated by analogous correspondence of the glucose carbon signals in **14** to those of the constituent monomeric tannins, tellimagrandin II (**11**) and both anomers of tellimagrandin I (**1**) (Fig. 2).

These applications suggest that the assignment of each signal of the glucose carbons in the ^{13}C-NMR spectra can lead to straightforward assignments of the structures, or selection of possible structures, for some tannins of unknown structures.

Experimental

Materials——All the tannins and derivatives measured in the present study were obtained in the previous

experiments.[3,5,6,10-12]

Instruments——NMR spectra were measured on a Bruker AM-400 spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C), and on a Varian VXR-500 instrument (500 MHz for ^1H and 125 MHz for ^{13}C) in the SC-NMR Laboratory of Okayama University, in acetone-d_6 or in acetone-$d_6 + D_2O$ at ambient temperature. Chemical shifts are given in δ values (ppm) from tetramethylsilane.

References

1) Part I: T. Yoshida, T. Hatano, T. Okuda, M. U. Memon, T. Shingu and K. Inoue, *Chem. Pharm. Bull.*, **32**, 1790 (1984).
2) T. Yoshida and T. Okuda, *Heterocycles*, **14**, 1743 (1980).
3) T. Okuda, T. Yoshida, M. Ashida and K. Yazaki, *J. Chem. Soc., Perkin Trans. 1*, **1983**, 1765.
4) T. Okuda, T. Hatano, N. Ogawa, R. Kira and M. Matsuda, *Chem. Pharm. Bull.*, **32**, 4662 (1984).
5) T. Yoshida, Y. Maruyama, M. U. Memon, T. Shingu and T. Okuda, *Phytochemistry*, **24**, 1041 (1985).
6) T. Okuda, T. Yoshida, M. Kuwahara, M. U. Memon and T. Shingu, *Chem. Pharm. Bull.*, **32**, 2165 (1984).
7) T. Utamura, K. Kuromatsu, K. Suwa, K. Koizumi and T. Shingu, *Chem. Pharm. Bull.*, **34**, 2341 (1986).
8) K. Yoshimoto, Y. Itatani and Y. Tsuda, *Chem. Parm. Bull.*, **28**, 2065 (1980).
9) E. Haslam, *Fortschr. Chem. Org. Naturst.*, **41**, 1 (1982).
10) T. Okuda, T. Hatano and T. Yasui, *Heterocycles*, **16**, 1321 (1981).
11) T. Okuda, T. Hatano and N. Ogawa, *Chem. Pharm. Bull.*, **30**, 4234 (1982).
12) T. Hatano, Y. Ikegami, T. Shingu and T. Okuda, *Chem. Pharm. Bull.*, **36**, 2017 (1988).