OLIGOMERIC HYDROLYZABLE TANNINS — THEIR ^{1}H NMR SPECTRA AND PARTIAL DEGRADATION†

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Abstract — The ^{1}H nmr spectroscopy provides a convenient method of assigning the orientation of the valoneoyl group in the oligomeric hydrolyzable tannins, which is the unit most frequently joining the monomeric constituents in the molecules. The behavior of oligomers upon partial degradation has been found to be dependent on the location of valoneoyl group on the glucose core.

Contents

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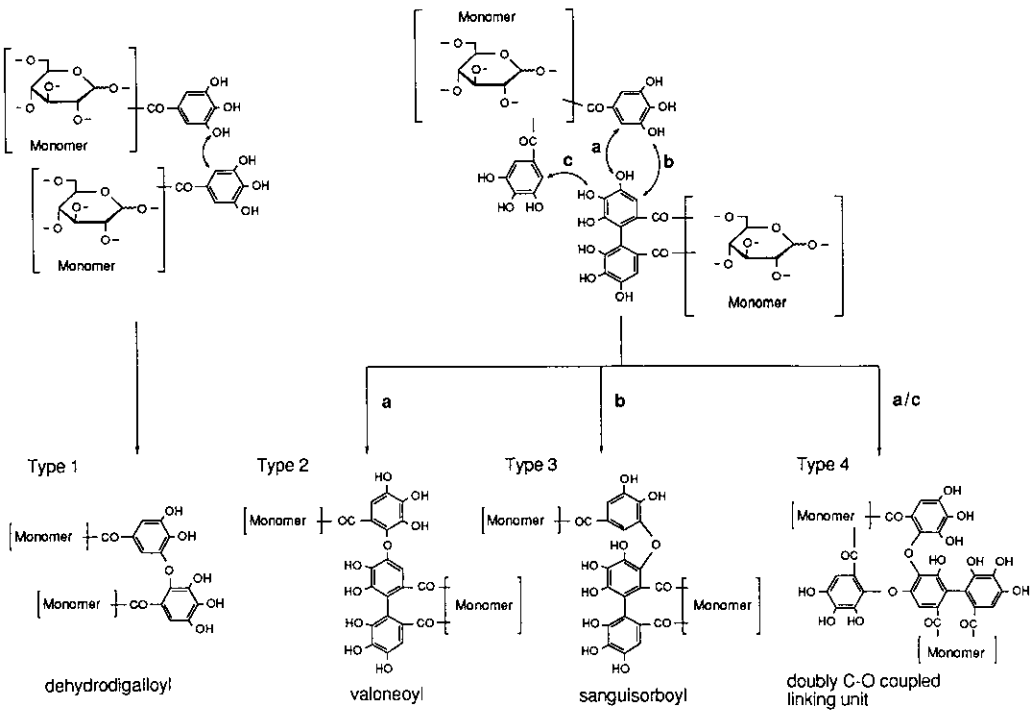
†Dedicated to Professor Masatomo Hamana on the occasion of his 75th birthday.

I. Introduction

About one hundred and twenty oligomeric hydrolyzable tannins of diverse structures, with molecular sizes up to tetramers (molecular weight 1500-3800), have been hitherto isolated from various plants.[1] Their molecules are composed of two or more glucose cores esterified with gallic acid and/or its metabolites such as dehydrodigallic acid, hexahydroxydiphenic acid (bisgallic acid) and valoneic acid (trisgallic acid). These oligomers are biogenetically regarded as the metabolites produced by the intermolecular C-O (or C-C) oxidative coupling among two or more monomeric ellagitannins.[1,2] They are classified into four types of oligomers, according to the structures of the part joining each monomeric constituent, as illustrated in Scheme 1.[1]

About 70% of these oligomers belong to the type 2 oligomers having valoneoyl group as the linking unit, and their distribution in the plant kingdom, and the degree of their condensation (dimer ~ tetramer) are most diverse. This type of oligomers are also of particular interest



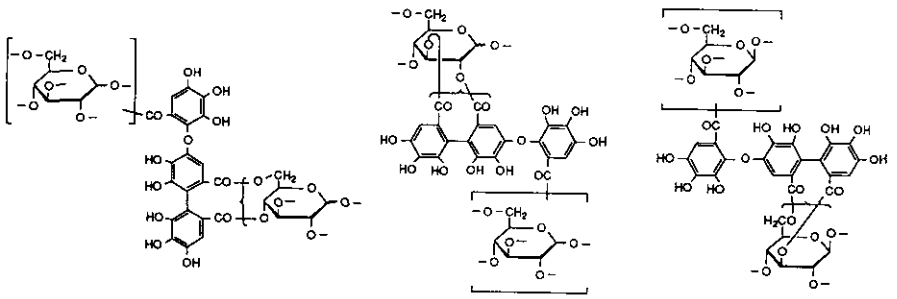
Scheme 1

because of their potent biological activities including host-mediated antitumor activity,[3,4] stimulation of iodination in human polymorphonuclear leukocytes[5] and monocytes,[6] and antiviral activity against human immunodeficiency virus (HIV),[6] which are often specifically exhibited by some oligomers.

We previously developed a convenient method to assign the monomeric constituents and the absolute configuration of chiral hexahydroxydiphenoyl (HHDP) and valoneoyl group in each oligomer molecule, based on the ^{13}C nmr and cd spectral analyses.[7-9] We have recently developed an additional convenient method of determining the orientation (binding mode) of valoneoyl group in a molecule, based on a systematic investigation of the ^{1}H nmr spectra of known oligomers. We have also compiled the behaviors of the oligomers upon their partial hydrolysis, which are different depending on the location of the valoneoyl group on the glucose core, and on pH of the medium, to facilitate the structure determination of newly isolated oligomers of this type.

II. Classification of Oligomeric Hydrolyzable Tannins Having Valoneoyl Group as the Unit Linking Monomeric Constituents

The type 2 oligomeric hydrolyzable tannins are further classified into three subtypes (subtypes 2A, 2B and 2C) according to the location of the HHDP moiety of the valoneoyl group on the glucose core, as shown in Scheme 2.



Subtype-2A Subtype-2B Subtype-2C

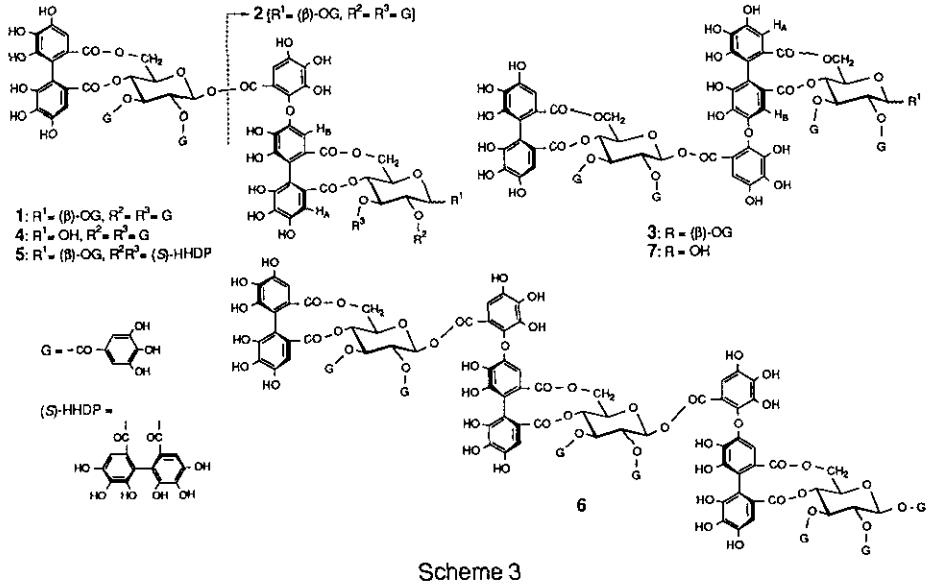
Scheme 2

The glucose core in the oligomers belonging to subtypes 2A and 2B, having the HHDP moiety of the valoneoyl group at O-4/O-6 and O-2/O-3 of the glucose core, respectively, mostly adopts the C1 conformation, while the glucose core of the subtype 2C oligomers, having the HHDP moiety at O-3/O-6, adopts the 1C or skew-boat conformation. The oligomers of the former two subtypes are thus readily discriminated from those of the latter subtype, based on the coupling pattern of the glucose proton signals in the ^{1}H nmr spectrum.

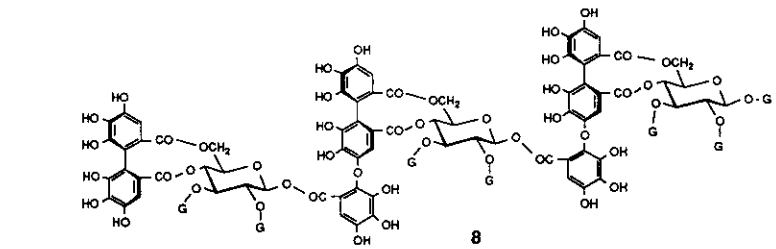
III. ^{1}H Nmr Spectral Features of Each Subtype of Oligomers and Convenient Methods for Assigning Orientation of the Valoneoyl Group

1. Oligomers of Subtype 2A The ellagitannins, having an HHDP (or the HHDP part of a valoneoyl) group bridged on O-4/O-6 of a C1 glucopyranose core, are characterized by a large chemical shift difference ($\Delta\delta$ ca. 1.5 ppm) between the C-6 methylene protons.[10,11] This anomaly can be interpreted in terms of an anisotropic effect of a C-6 ester carbonyl group, which is restrained to be rigidly coplanar with one of the C-6 methylene protons in the eleven-membered diester ring.

The orientation of the valoneoyl group in rugosin D (**1**),[12] a dimer which represents this type of



Scheme 3



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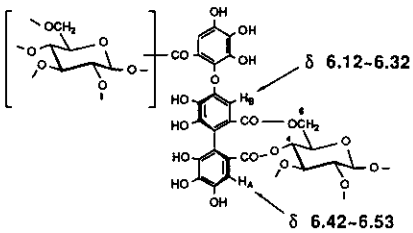
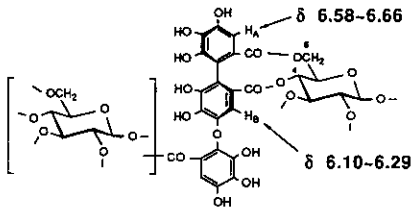
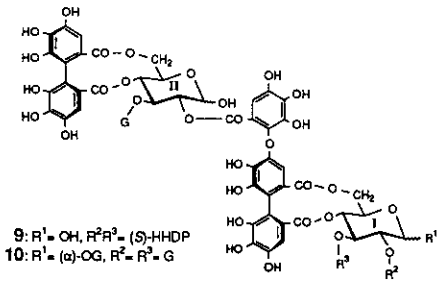


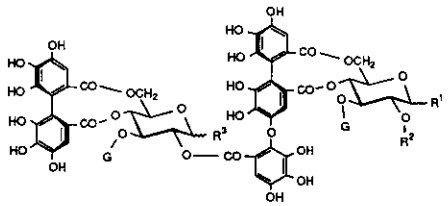
Rugosin-type Oligomers



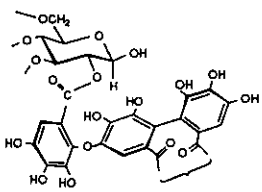
Isorugosin-type Oligomers

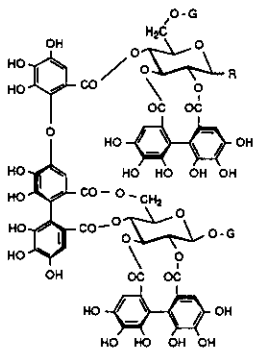


9: R^1 = OH, R^2R^3 = (*S*)-HHDP
10: R^1 = (α)-OG, R^2 = R^3 = G

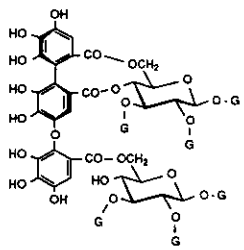


11: R^1 = R^2 = OH, R^2 = G
12: R^1 = OH, R^2 = G, R^3 = (β)-OG
13: R^1 = R^3 = (β)-OG, R^2 = G
14: R^1 = R^2 = OH, R^2 = H
15: R^1 = (β)-OG, R^2 = G, R^3 = OH





16: R = OH
17: R = (β)-OG



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Scheme 4

oligomers, and widely found in a variety of plant species,[1] was determined based on its chemical degradation into rugosin A (**2**), whose structure was unambiguously established with the aid of the ^{1}H-^{13}C long-range COSY.[13,14] The structure of isorugosin D (**3**),[14] which is an isomer of rugosin D (**1**) concerning the orientation of the valoneoyl group, was also confirmed by the ^{1}H-^{13}C long-range COSY. Upon comparison of the valoneoyl proton signals in the ^{1}H nmr spectra between **1** and its isomer (**3**), a significant difference of the chemical shift of the H_A signal (δ6.46 in **1** and δ6.66 in **3**) was observed. The chemical shifts of the valoneoyl H_A signal, similar to those of **1** and **3**, were observed in the spectra of the analogs of rugosin D (**1**) [rugosins E (**4**), F (**5**) and G (**6**), etc. (δ6.42-6.53)][12] and of isorugosin D (**3**) [isorugosins E (**7**)[15] and G (**8**),[16] etc. (δ6.58-6.66)].

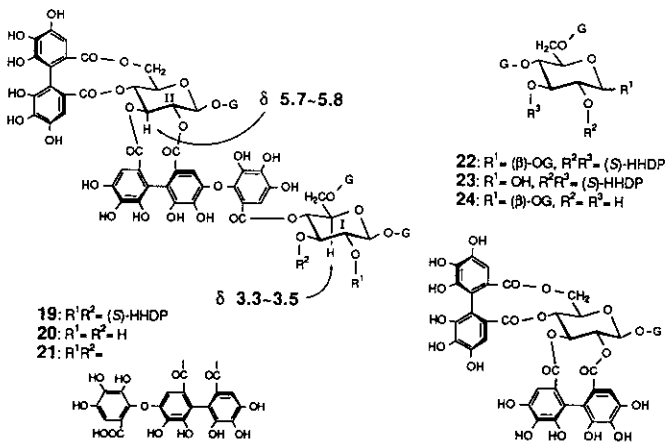
Among the subtype 2A oligomers, more than twenty compounds have the galloyl part of a valoneoyl group at O-2 of a C1 glucopyranose. The characteristic spectral feature of these oligomers is a remarkable upfield shift (Δδ 0.4-1.0 ppm) of an α-oriented H-1 (β-anomer) signal on the glucose-II, relative to that of the analogs possessing a galloyl group at O-2. This upfield shift is ascribable to the anisotropic effect of the valoneoyl group, as illustrated in Scheme 4.[17] The chemical shift of the anomeric proton signal is hence useful for assigning the location of the galloyl part of valoneoyl group.

Camelliin A (**9**)[18] and woodfordin B (**10**)[19] have the rugosin-type valoneoyl group at O-4/O-6 of a glucose core, while the orientation of valoneoyl group in cornusiin A (**11**), C, D (**12**) and E (**13**),[17,20] and camptothins A (**14**) and B (**15**),[17,21] etc., is the same as that of isorugosin D (**3**). The valoneoyl H_A of these oligomers resonates at the region characteristic of the rugosin-type (δ6.42-6.53) and isorugosin-type (δ6.58-6.66) valoneoyl group. Therefore, the orientation of the valoneoyl group at O-4/O-6 of the glucose core is straightforwardly assigned, based on the diagnostic chemical shift of the valoneoyl H_A signal. It is noteworthy that this convenient method is applicable to all of the oligomers of subtype 2A, in which the galloyl part of the valoneoyl group is bound to O-4 or O-6, as well as to O-1 and O-2 of a glucose core, as exemplified by nobotanins A (**16**) and F (**17**),[22] and cornusiin G (**18**).[23]

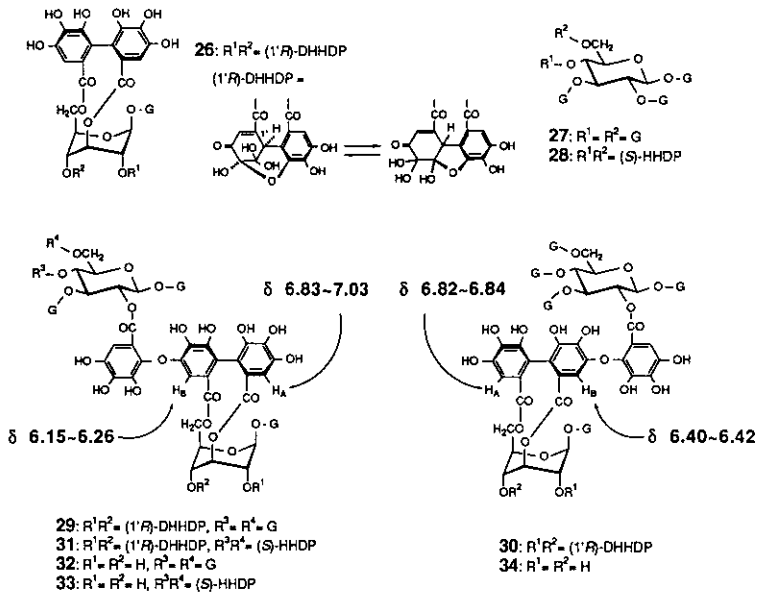
The upfield shift of the H-3 signal (δ ca. 5.5) of glucose-I in isorugosin D (**3**), relative to the

corresponding signal (δ5.82) of rugosin D (**1**), is characteristic of their structural correlation. An analogous shift difference (Δδ ca. 0.3 ppm) is commonly observed between the H-3 signal in the oligomers having the valoneoyl group of isorugosin-type (**7** and **11**, etc.), and that in the oligomers having the rugosin-type valoneoyl group (**4** and **10**, etc.). Therefore, the chemical shift of H-3 of the glucose core having the HHDP part of valoneoyl group at O-4/O-6, gives an additional basis for assigning the orientation of the valoneoyl group in the oligomers of subtype 2A.

2. Oligomers of Subtype 2B Nobotanins B (**19**),[24] G (**20**) and H (**21**),[25] dimers of subtype 2B, have the galloyl part of valoneoyl group bound to O-4 of one of the C1 glucose cores (glucose-I). The orientation of the valoneoyl group at O-2/O-3 of glucose-II in these dimers was unequivocally determined by the ^{1}H-^{13}C long-range COSY spectra.[25] Their isomers differing only in the orientation of the valoneoyl group have not yet been found. This subtype of oligomers has the following ^{1}H nmr spectral features. The H-5 signal of the glucose-I shows a remarkable upfield shift (δ3.3-3.5), from that of related monomers [pterocarinin C (**22**), praecoxin B (**23**) and 1,4,6-tri-O-galloyl-β-D-glucose (**24**), etc.; δ4.0-4.5]. On the other hand, the H-3 signal of the glucose-II shifts lower (δ5.7-5.8) than that of casuarictin (**25**) (δ5.45), another composing monomer. The presence of nobotanin B (or H) moiety, in a trimer (nobotanin J[26]) and a tetramer (nobotanin K[26]), was easily deduced from these signals.



Scheme 5



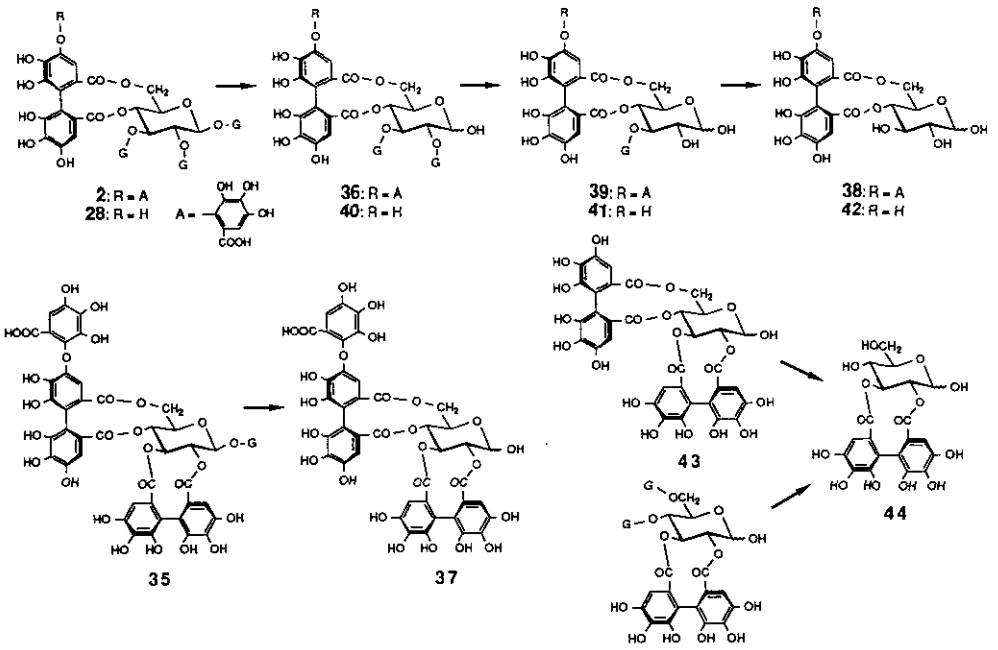
Scheme 6

3. Oligomers of Subtype 2C Dimers of this subtype have been found only in Euphorbiaceous plants.[1] Most of them have geraniin (**26**)[27] as one of the composing monomers, and are biogenetically regarded as the products from intermolecular C-O oxidative coupling, between an (*R*)-HHDP group of geraniin and a galloyl group of the other monomer such as pentagalloylglucose (**27**), tellimagrandin II (**28**), etc. These dimers are thus distinguished from the other types of oligomers, by their nmr signals characteristic of the 1C glucopyranose core, and of dehydrohexahydroxydiphenoyl (DHHDP) group equilibrated between five-membered and six-membered hemiacetal forms.[27] The simpler spectra of the phenazine derivatives, which are obtained by condensation with o-phenylenediamine in a weakly acidic medium, provide clearer structural information.

Euphorbins A (**29**) and B (**30**)[28,29] are isomers concerning the orientation of the valoneoyl group. The H_B signal of the valoneoyl group in the ^1H nmr spectra of euphorbin A-type dimers (**29**, **31**-**33**)[29-31] resonates at a higher field (δ6.15-6.26) than that of the euphorbin B-type dimers (**30** and **34**,[32] δ6.40-6.42). However, the other valoneoyl signals (H_A and H_C) of these dimers are not useful in discriminating them (Scheme 6).

IV. Behaviors of the Type 2 Oligomers upon Hydrolysis

Partial degradation of hydrolyzable tannins is effected generally by the treatment with tannase, hot water or weak acid. Tannase, produced by Aspergillus niger grown in a culture medium containing tannic acid (gallotannin mixture) as a sole organic nutrient,[33] is a hydrolase fundamentally specific to hydrolysis of galloyl esters. Although galloyl ester linkages are generally hydrolyzed with tannase prior to the other ester linkages, the reaction rate is different depending on the locations of the galloyl groups on a glucose core, probably because of the steric requirement. The bridged HHDP and valoneoyl groups are also often hydrolyzed with tannase prepared according to the literature.[33,34] The most susceptible position to tannase is the anomeric position, as have been shown by many examples, including hydrolyses of rugosins A (**2**) and C (**35**), which give rugosin B (**36**) and praecoxin A (**37**).[13,35] Rugosin B (**36**) is further hydrolyzed with tannase to 4,6-valoneoyl-D-glucose (**38**) via coriariin F (**39**).[13] Treatment of tellimagrandin II (**28**) with tannase gives tellimagrandin I (**40**) first, which is further hydrolyzed to gemin D (**41**), and then 4,6-HHDP-D-glucose (**42**).[36] The HHDP ester linkages at

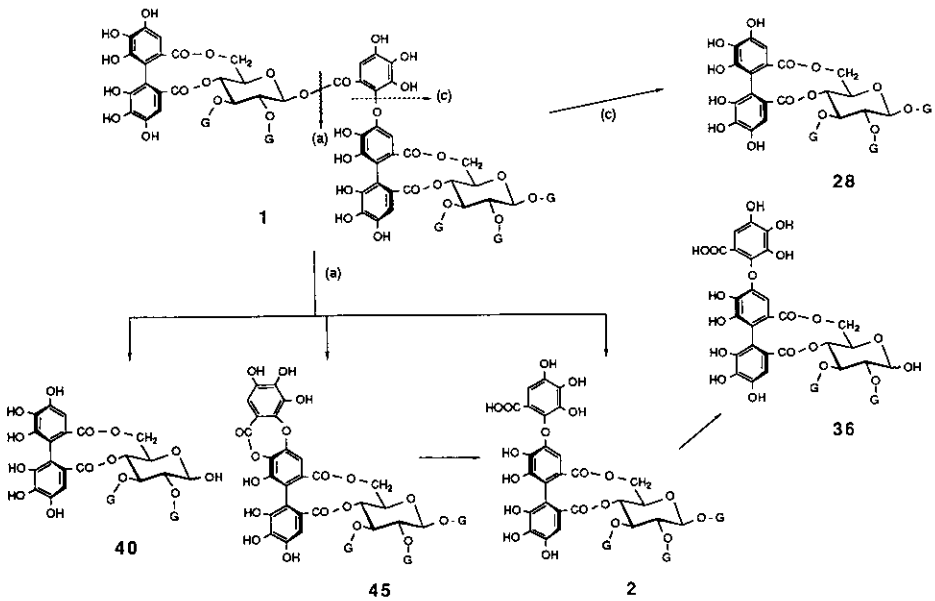


Scheme 7

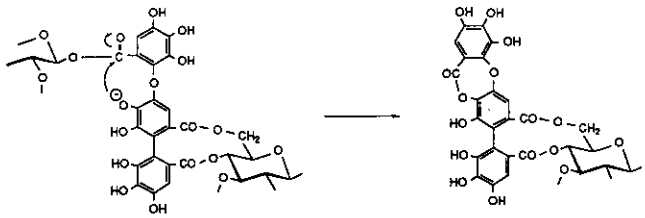
O-4/O-6 are cleaved much faster than those at O-2/O-3, as exemplified by enzymatic hydrolysis of pedunculagin (**43**) giving 2,3-HHDP-D-glucose (**44**).[37] Enzymatic hydrolysis of praecoxin B (**23**) indicated that the hydrolysis of the galloyl groups at O-4 and O-6 occurs prior to that of HHDP group at O-2/O-3.[35] These findings and some other examples show that the order of the trend of these tannins undergoing the enzymatic hydrolysis, induced by the differences of locations of galloyl and HHDP (or valoneoyl) groups on the glucose core, is as follows: O-1 (galloyl) > O-2 (galloyl) > O-3 (galloyl) ≥ O-6, O-4 (galloyl), O-4/O-6 (HHDP) > O-2/O-3 (HHDP).

The partial hydrolytic degradation of the oligomers into monomeric products, in hot aqueous media, is indispensable for the structure determination. The behavior of the oligomers having valoneoyl group as the linking unit, upon the hydrolysis, largely depends on the location of the valoneoyl group on the glucose core, besides the reaction conditions.

At an early stage of the partial hydrolysis in hot water,[38] under monitoring by hplc, rugosin D (**1**) yields tellimagrandin I (**40**) and rugosin A (**2**), and prostratin C (depsidone) (**45**)[39] which is further hydrolyzed into rugosins A (**2**) and B (**36**) upon prolonged reaction (Scheme 8). The



Scheme 8



depsidone

Scheme 9

formation of prostratin C (**45**) is attributable to transesterification in the valoneoyl group, under participation of a neighboring phenolic hydroxyl group (see Scheme 9).

The reaction rate and the products are pH-dependent as shown in Figure 1.

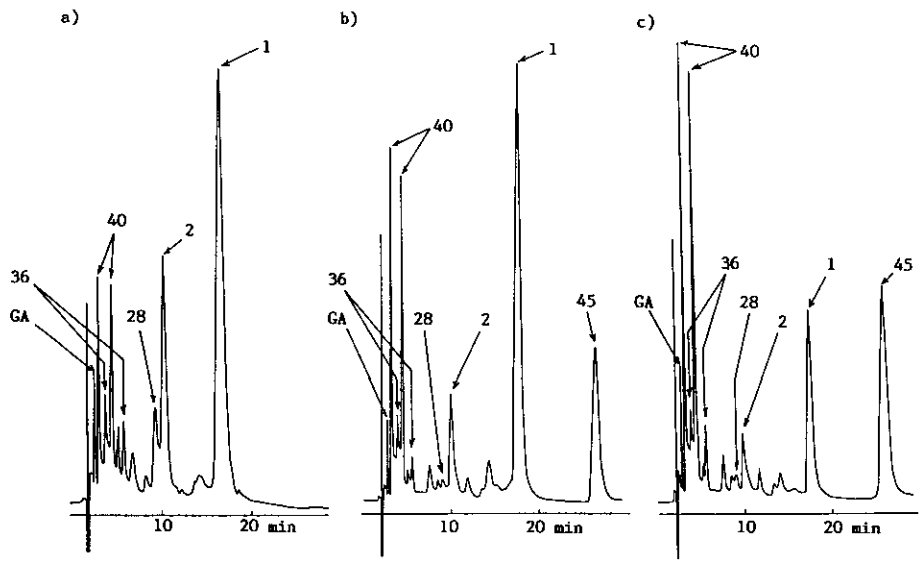


Figure 1. Hplc of the Hydrolyzates of Rugosin D (**1**) at Different pH
a) pH 2.3, 60°C, 48 h. b) pH 5.7, 60°C, 10 min. c) pH 6.5, 60°C, 10 min.
Hplc condition: column, LiChrospher RP-18 (5 μm), 4 x 250 mm; solvent, 0.1M H_3PO_4-0.1M KH_2PO_4-EtOH-EtOAc (17:17:4:2); flow rate, 1.0 ml/min; temperature, 40°C. GA = gallic acid

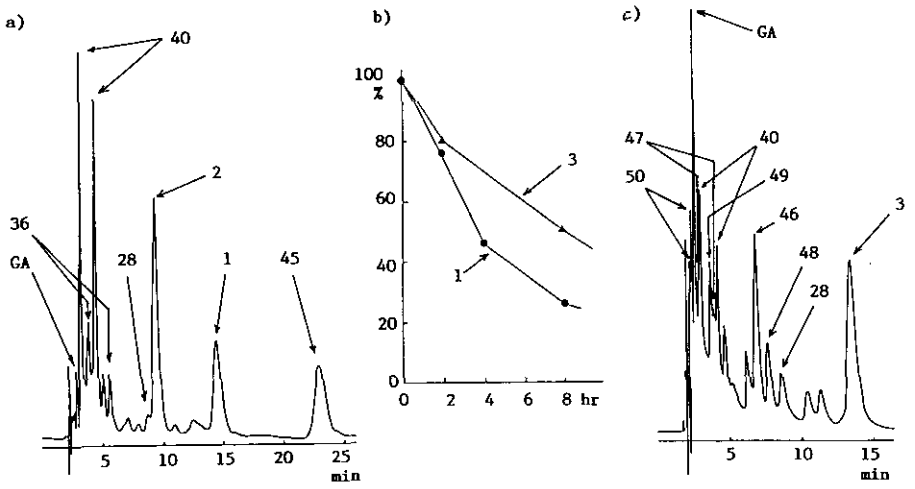
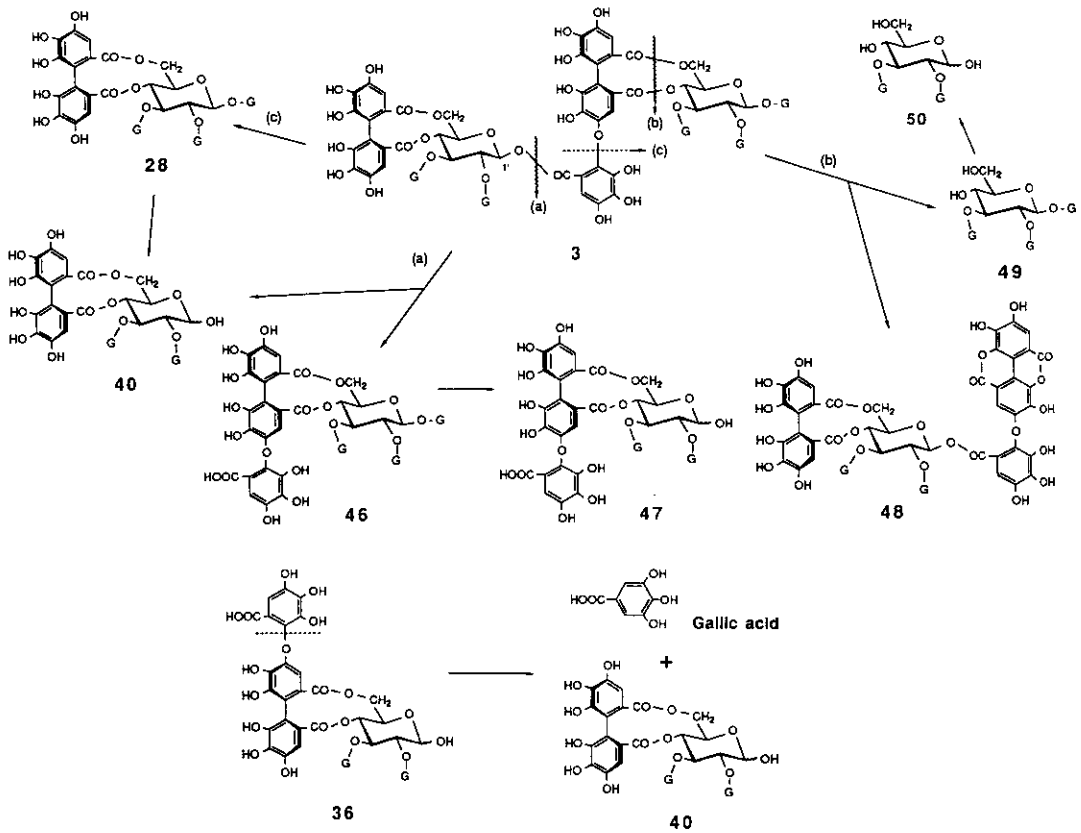


Figure 2. Comparison of the Hydrolysis of Rugosin D (**1**) and Isorugosin D (**3**)

a) Hplc of the hydrolyzates of rugosin D (**1**) (in water, 60°C, 4 h). b) Time course of hydrolysis of rugosin D (**1**) and isorugosin D (**3**) in water (60°C). c) Hplc of the hydrolyzates of isorugosin D (**3**) (in water, 60°C, 8 h). Hplc condition: see, Figure 1. GA = gallic acid

Isorugosin D (**3**) is somewhat stabler ($t_{1/2}$, 8.0 h) than rugosin D (**1**) ($t_{1/2}$, 3.9 h) in water at 60°C (Figure 2), and gives the hydrolyzates different from those of the latter, as shown in Scheme 10. In addition to tellimagrandin I (**40**) and isorugosin A (**46**) [and isorugosin B (**47**)] produced by cleavage at O-1' in a similar way to that of rugosin D (**1**), prostratin A (**48**), 1,2,3-tri-O-galloyl-β-D-glucose (**49**) and 2,3-di-O-galloyl-D-glucose (**50**) are also produced by hydrolysis of the O-4/O-6 ester linkages.

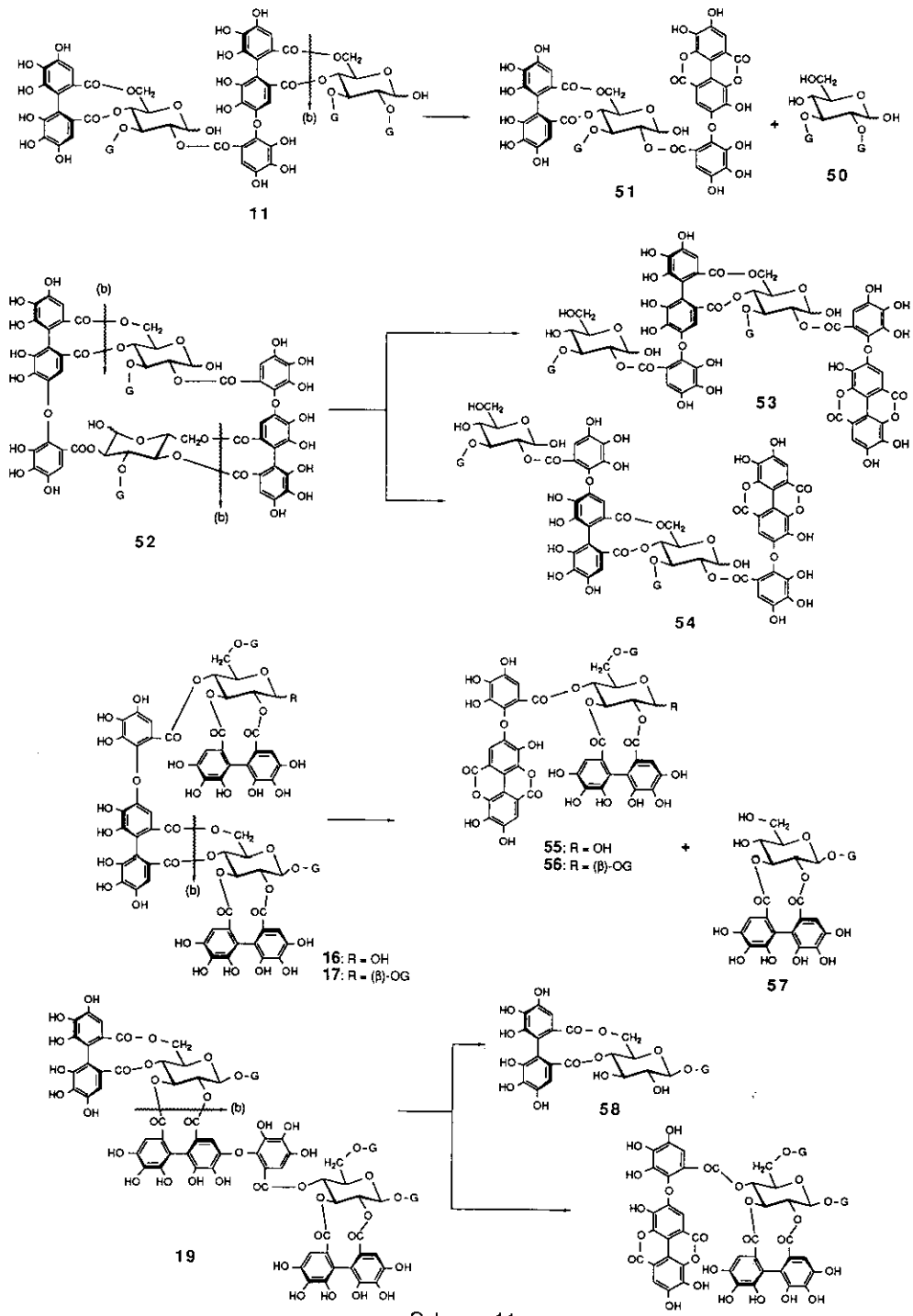
It should be noted that a small amount of tellimagrandin II (**28**), obtained upon these hydrolyses, is regarded as a by-product from cleavage of an ether bond of the valoneoyl group. This kind of the ether-bond cleavage, under similar mild conditions, has been frequently observed upon the partial hydrolysis of oligomers.[28,29,40] A simple monomer, rugosin B (**36**), subjected to this reaction under various conditions, showed that the ether cleavage occurs most easily in weak alkali. Rugosin B (**36**), thus treated with 0.1M KH_2PO_4-Na_2HPO_4 buffer (pH 7.4) at 60°C for 2.5



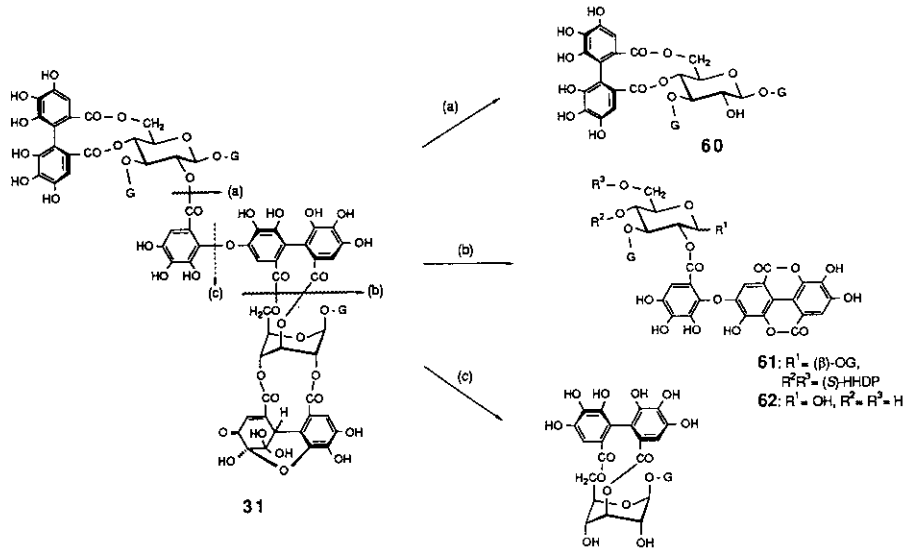
Scheme 10

h, gave the major products identified as tellimagrandin I (**40**) and gallic acid. This reductive cleavage of the ether bond is ascribable to a disproportionation reaction.

The oligomers, in which the galloyl part of the valoneoyl group is bound to O-2 or O-4 of a glucose core, are hydrolyzed predominantly at the HHDP part bound to the other glucose core, to yield a product having a dilactonized valoneoyl group at O-2 or O-4. This is independent of the orientation of the valoneoyl group as found in the following examples. Cornusiin A (**11**) gave, upon hydrolysis in hot water, cornusiin B (**51**) and 2,3-di-O-galloyl-D-glucose (**50**).[17] Oenothein B (**52**),[3,41] an antitumor macrocyclic dimer, which has two valoneoyl groups with mutually reversed orientation, was hydrolyzed in a hot weak acid (0.05M H_2SO_4, 90°C, 13 h) into two dimers (**53** and **54**) possessing a dilactonized valoneoyl group at O-2 of a glucose core.



Scheme 11



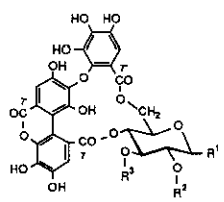
Scheme 12

Similarly, partial hydrolysis of nobotanin A (**16**) [or nobotanin F (**17**)], in hot water (100°C, 15 h), afforded a hydrolyzate (**55**) (or **56**) and isostrictinin (**57**).[22] Nobotanin B (**19**), classified in subtype 2B, also gave **56** and strictinin (**58**), upon analogous hydrolysis (Scheme 11).[24]

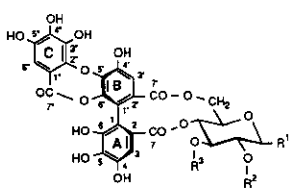
Among the acyl groups in the dimers of subtype 2C, the 2,4-bridged acyl group is usually most unstable and eliminated first under most of hydrolytic conditions. On the other hand, the valoneoyl ester linkages at O-3/O-6 are considerably stable, and their cleavages in hot water are often preceded by that of the ether bond. The phenazine derivatives of euphorbins A (**29**), B (**30**) and F (**31**), thus gave corilagin (**59**) as a major product in boiling water (10 h).[28,29] Hydrolysis of the valoneoyl HHDP part at O-3/O-6 has been found to occur in an acidic medium: Treatment of euphorbin F (**31**) with 0.01% (v/v) sulfuric acid (pH 1.8, 60°C, 3-5 h) gave, along with **59** and **60**, tirucallin A (**61**) and oenothein C (**62**), which have a dilactonized valoneoyl group at O-2 of glucose, in a similar way to the hydrolysis of cornusiin A (**11**) and oenothein B (**52**), etc., described above.

V. Application —Structural Revision of Alnusiin and Bicornin—

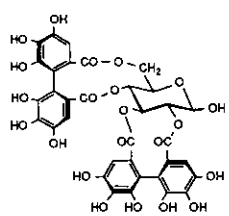
The application of the cleavage of the ether linkage in the valoneoyl group mentioned above, to the other type of acyl group, tergalloyl group, which is an isomer of valoneoyl group, was then attempted. Alnusiin (**63**),[42] with a monolactonized tergalloyl group, was thus treated with 0.1 M KH_2PO_4-Na_2HPO_4 buffer (pH 7.4, 60°C, 20 min) to give gallic acid. Another product from this cleavage was, however, unexpectedly identified as pedunculagin (**43**),[43] which was inconsistent with the proposed structure for alnusiin. The structure of alnusiin (**63**) has thus been reinvestigated and revised to a depsidone structure (**63a**), on the basis of the following evidence: 1) The previous assignments of the ^1H and ^{13}C nmr signals for the monolactonized tergalloyl group were based on the ^1H-^{13}C long-range COSY spectrum of the permethylated derivative of alnusiin, in which the signal at δ163.0 was assigned to C-7'.[42] However, when the signal at δ163.0 is assigned to C-7", the assignments of some ^1H and ^{13}C signals of the tergalloyl group can be corrected to be as follows: δ_H 7.185 (previous assignment: H-3') → H-6"; δ_C 150.1 (C-5') → C-2"; δ_C 145.3 (C-6) → C-6'; δ_C 144.1 (C-2") → C-5'; δ_H 7.18 (H-6") → H-3'; δ_C 167.3 (C-7") → C-7'. The connectivity between the signals at δ_H 7.18 and one of the C-6 methylene protons of the glucose core, through the ester carbonyl carbon at δ167.3 in the ^1H-^{13}C long-range COSY, is consistent with the revised structure **63a**. 2) The rotating-frame



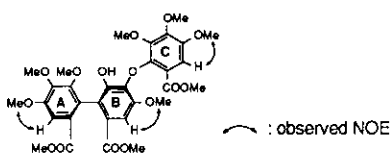
63: R^1 = OH, R^2R^3 = (*S*)-HHDP
65: R^1 = (β)-OG, R^2 = R^3 = G



63a: R^1 = OH, R^2R^3 = (*S*)-HHDP
65a: R^1 = (β)-OG, R^2 = R^3 = G



43



: observed NOE

64

Scheme 13

Overhauser enhancement (ROESY) spectrum of the decamethyl derivative (**64**), which was obtained by methanolysis of trideca-O-methylalnusiin, indicated the presence of methoxyl group at an ortho-position of each aromatic proton.[44] 3) The cd spectrum of alnusiin exhibited a strong positive Cotton effect, [θ] +2.5 x 10^5, at 235 nm, and a negative one, [θ] -5 x 10^4, at 265 nm,[9] which are consistent with the presence of two (S)-biphenyl moieties.

The structure of bicornin, a tannin isolated from Trapa bicornis, which was previously proposed to be (**65**)[42] based on its structural analogy to alnusiin, has also been revised to **65a**.

VI. Concluding Remarks

Since the oligomeric hydrolyzable tannins of the type 2, discussed in this review, are widely distributed in a variety of plant families such as Rosaceae, Cornaceae, Coriariaceae, Betulaceae, Euphorbiaceae, Melastomataceae, Theaceae, Lythraceae, Trapaceae, Onagraceae, Hamamelidaceae, and others,[1] isolation of further new oligomers of this type in the future from various plant species is expectable. The isolation of these compounds is particularly significant when they are found upon the search of biologically active compounds.[45] Combined with the previously reported ^{13}C nmr and cd spectroscopy,[7-9] the application of the ^1H nmr spectra and the partial hydrolytic degradation, described in this review, should facilitate the structure determination of the new oligomers.

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44. The ^{1}H nmr (500 MHz, acetone-d_6) data of **64**: δ 7.33, 7.14, 7.11 (1H each, s, aromatic H), 3.91, 3.87, 3.86, 3.84, 3.82, 3.71, 3.57, 3.55, 3.54, 3.53 (3H each, s, OMe). The methoxyl signals which showed NOE's with the aromatic protons were δ3.91 (7.33), 3.87 (7.11) and 3.71 (7.14).

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