Chemistry of Natural Compounds, Vol. 33, No. 2, 1997

^{1}H AND ^{13}C NMR SPECTROSCOPY IN THE STUDY OF FLAVAN-3-OLS, PROANTHOCYANIDINS, AND THEIR DERIVATIVES
II. PROTON MAGNETIC RESONANCE SPECTROSCOPY OF PROANTHOCYANIDINS

A. D. Vdovin, Z. A. Kuliev, and N. D. Abdullaev

UDC 543.42.25+541.63+541.67

The characteristics of the proton magnetic resonance spectra of proanthocyanidins and their derivatives are considered. An interrelationship is traced between spectral properties and structural, stereochemical, and conformational factors.

We have previously considered the possibility of proton magnetic resonance (PMR) spectroscopy in the investigation of monomeric flavan-3-ols [1]. In the present paper we give an analysis of the PMR spectra of low-molecular-mass proanthocyanidins.

In the study of proanthocyanidin dimers wide use is made of Weinges's nomenclature [2-5]. We present it in Fig. 1, separating the proanthocyanidins into groups on the basis of stereochemical characteristics.

To the first group belong proanthocyanidins in which the "upper" position is occupied by a flavan-3-ol with the 2,3-*trans*-configuration (**1-4**). The second group contains proanthocyanidins in which the "upper" position is occupied by a flavan-3-ol with the 2,3-*cis*-configuration (**5-8**).

CHARACTERISTICS OF THE PMR SPECTRA OF THE PROANTHOCYANIDIN DIMERS OF THE FIRST GROUP AND THEIR DERIVATIVES

At room temperature the dimers of the first and second groups can be distinguished through the SSCCs $J_{2,3}$ of the flavan-3-ols occupying the "upper" positions (Tables 1 and 3). However, in the first group of dimers the interflavan bond may have either the α- or the β-orientation [6-8].

Proanthocyanidins of the first group with the α-orientation of the interflavan bond have long been known. A dimer with the β-orientation of this bond has been synthesized and identified by H. Kolodziej [9]. These isomers can readily be distinguished from one another in spectra obtained at room temperature: with the α-orientation of the interflavan bond, $J_{3,4}$ amounts to 6.7-10.0 Hz [5-7, 10-18, 27], while with the β-orientation it is from 4.2 to 6.2 Hz [8, 12, 17, 19]. According to estimates of the SSCCs [8] in proanthocyanidins with the β-orientation of the interflavan bond at room temperature ring *C* may have a half-boat conformation. This is connected with the fact that the substituent at C-4 strives to assume an equatorial orientation.

In the spectra of peracetates or acetates of methyl ethers obtained with the aim of improving the resolution of the resonance lines at an elevated temperature [12, 19], ring *C* may also assume the relatively low-energy half-boat or C2-pentacoplanar conformation.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 40 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 135-149, March-April, 1997. Original article submitted June 19, 1995.

0009-3130/97/3302-0101$18.00

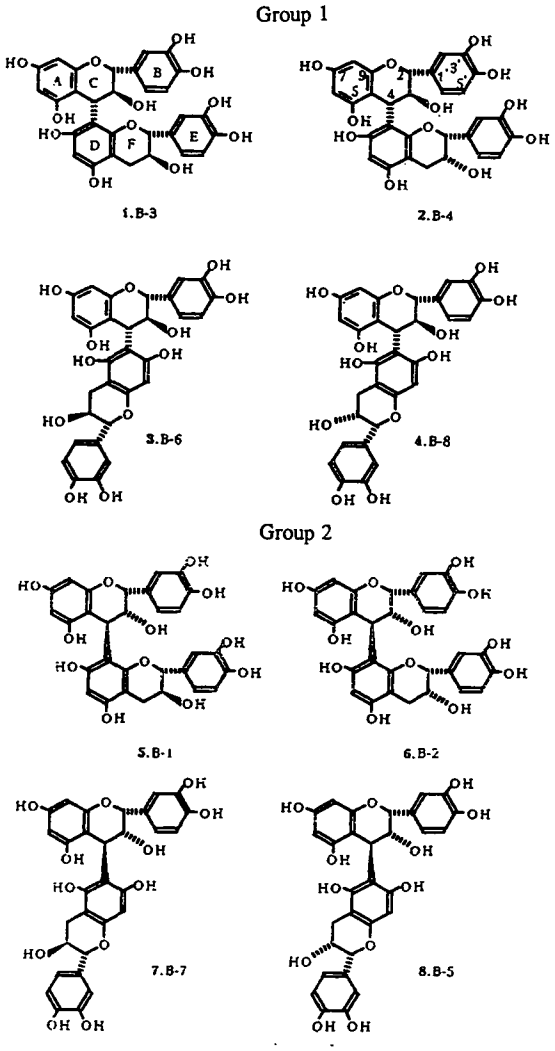


Fig. 1. Structures of proanthocyanidin dimers.

Among synthetic products, dimeric proanthocyanidins are frequently found that include (−)-fisetinidol [12] − the mirror isomer of (+)-fisetinidol. In this case, the considerations relating to the SSCCs of the 4α- and 4β-isomers are reversed. The chemical shifts of the H-2, H-3, and H-4 protons of the "upper" blocks [21-23] are close to the corresponding CSs of their stereochemical analogs, the 4α- [5, 11, 16, 18, 20, 25] and 4β- [8, 18, 25, 26] phloroglucinol derivatives of the flavan-3-ols.

For the stereochemical analogs of proanthocyanidins B-3 and B-6, in which the "upper" position is occupied by fisetinidol, an allyl SSCC of the H-4 axial proton with the H-5 aromatic proton is observed [10-12, 14, 16, 28]. It is known that in butenes the allyl constant is positive for dihedral angles of 90 and 270° and may amount to 1-2 Hz. In this case it is observed in derivatives of aromatic compounds. This phenomenon may arise as a consequence of an overlapping of the H-4 orbitals by the π-electrons of the aromatic system of ring *A*.

W. R. Bergman et al. [29], using the MM2 program, showed that polymeric proanthocyanidins with the 4-6 type of bond have more compact packing than their analogs with 4-8 interflavan bonds. On the basis of bond lengths and valence angles, a system of equations was derived for calculating the values of a vector characterizing the size of the molecule. An important aspect of investigations of the structures of proanthocyanidins is, therefore, the determination of the position of the interflavan bond in the symmetrical aromatic ring *A*: 6 or 8. An interesting method of determining the type of interflavan linkage has been proposed by G. Nonaka et al. [24]. They used the CS of H-2 of the "lower" block with the 2,3-*trans*- configuration. In the case of a 4-6 bond the CS of this proton amounted to 4.58 ppm, while for a 4-8 bond it was 4.91-4.97 ppm.

TABLE 1. CSs (δ, ppm) and SSCCs (J, Hz) of the Protons in the PMR Spectra of the Proanthocyanidins of the First Group and Their Derivatives

H	2	3	4	6	8	3-OX	Solvent	Lit.
*B-3[1,6] (HC)	4.84d $J_{2,3}$=10	6.10 t	βa4.85dd $J_{4,3}$=10.0 $J_{4,5}$=1.5	6.47dd $J_{6,5}$=8.5 $J_{6,8}$=2.5	6.36d $J_{8,6}$=2.5	Ac 1.53s	acetone-d_6 *t* 32°C	16
[2]	4.93d $J_{2,3}$=10	4.97 m	2.7 m	6.46s	–	Gal 7.24s		
B-3 [2] (C2-HB)	4.67d $J_{2,3}$=7	5.92 t J=7	βe4.56d $J_{4,3}$=7	5.73d $J_{6,8}$=2	5.79d $J_{8,6}$=2	Gal 6.75s	DMSO-d_6 *t* 150°C	6
(C2)	4.43d $J_{2,3}$=8	3.58 m	α 2.70dd $J_{4,3}$=5 $J_{4,4}$=16 β 2.36dd $J_{4,3}$=8 $J_{4,4}$=16	5.90s	–			
†'B-6 [1,2] (HB)	4.64 $J_{2,3}$=7.3	4.36	α 4.72 $J_{4,3}$=5.0	6.43 $J_{6,5}$=8.4	6.51 $J_{8,5}$=2.5		–	8
(C2)	4.75 $J_{2,3}$=10.0	–	β4.89 $J_{4,3}$=8.0	–	6.45 $J_{8,5}$<1			
B-6 [4] (HC)	4.78d $J_{2,3}$=10.0	5.68 m	β 4.34d $J_{4,3}$=9.0	6.64	6.64		$CDCl_3$, *t* 30°C	5
	4.86d $J_{2,3}$=9.0	5.06 m	–	–	6.63			
B-6 [5] (C2-HB)	5.27d $J_{2,3}$=8.0	5.49 dd	β 4.85 d $J_{4,3}$=6.25			Ac 1.63	DMSO-d_6 *t* 150°C	12
	5.09d $J_{2,3}$=7.0	5.18 m	2.8 m			1.88		
B-4 (HC)	4.80d $J_{2,3}$=8.0	4.68m	β 4.55d $J_{4,3}$=10.0	5.8–7.4	5.8–7.4		acetone-d_6	7
	5.09s	4.33s	2.7–3.0m	5.8–7.4	–			
Conformer	4.60d $J_{2,3}$=8.0	4.48m	β 4.48m	5.8–7.4	5.8–7.4			
	5.06s	4.19s	–	5.8–7.4	–			
B-4 [5] (HC)	4.73d $J_{2,3}$=10.1	5.89 dd ΣJ=19.5	β 4.86d $J_{4,3}$=9.5	6.03d $J_{6,8}$=2.4	6.15d $J_{8,6}$=2.4		$CDCl_3$, *t* 100°C	14
	5.05br.s $J_{2,3}$<1	5.44 m	2.89 m	6.10 s	–			
B-8 [4] (HC)	4.88d $J_{2,3}$=10.0	5.72 t ΣJ=19.0	β 4.52d $J_{4,3}$=9.0	6.71	6.72		$CDCl_3$, *t* 30°C	5
	5.14s	5.43 m	–	–	6.75			
Rotamer (HC)	4.88d $J_{2,3}$=10.0	5.82 t ΣJ=19.0	β 4.45d $J_{4,3}$=9.0	6.49	6.50			
	5.20s	5.40 m	–	–	6.75			

*H-5 6.68 dd ($J_{5,6}$ = 8.5; $J_{5,4}$ = 1.5; H-2′ 6.73 d ($J_{2',6'}$ = 2.0); H-5′ 6.78 d ($J_{5',6'}$ = 8.2); H-6′ 6.63 dd ($J_{6',5'}$ = 8.2; $J_{6',2'}$ = 2.0).

†H-5 6.78; H-5 6.83.

[1]The marked blocks are fisetinidol. For comments on the long-range SSCCs, $J_{4,5}$, see text. In all the tables the conformations of ring *C* of the corresponding blocks are specified: C3-pentacoplanar – (C3); half-chair – (HC); C2-pentacoplanar – (C2); or half-boat – (HB). (C2-HB) means that, according to SSCC values, ring *C* has a conformation intermediate between half-chair and C2-pentacoplanar.

[2]The hydroxy group at C-3 is galloylated.

[3]The "lower" block is 3β,3′,4α,4′,7-pentahydroxyflavan (leucofisetinidol), and from the SSCC the interflavan bond has the β-orientation.

[4]Peracetate.

[5]"Upper" block is (−)-fisetinidol. Methyl ether acetate.

[6]Methyl ether acetate.

TABLE 2. Difference in the H-2 and H-3 CSs (ppm) of the "Upper" Blocks of the First Group with Different Absolute Configurations

Relative configuration	Absolute configurations in positions			Difference between the H-2 and H-3 CSs
	2	3	4	
4-8-bond with (+)-catechin				
2,3-*trans*-3,4-*trans*-	S	R	R	0.55
	R	S	S	0.14
2,3-*trans*-3,4-*cis*	S	R	S	0.17
	R	S	R	0.61
4-6-bond with (+)-catechin				
2,3-*trans*-3,4-*trans*-	S	R	R	0.17
	R	S	S	0.19
2,3-*trans*-3,4-*cis*-	S	R	S	0.19
	R	S	S	0.16

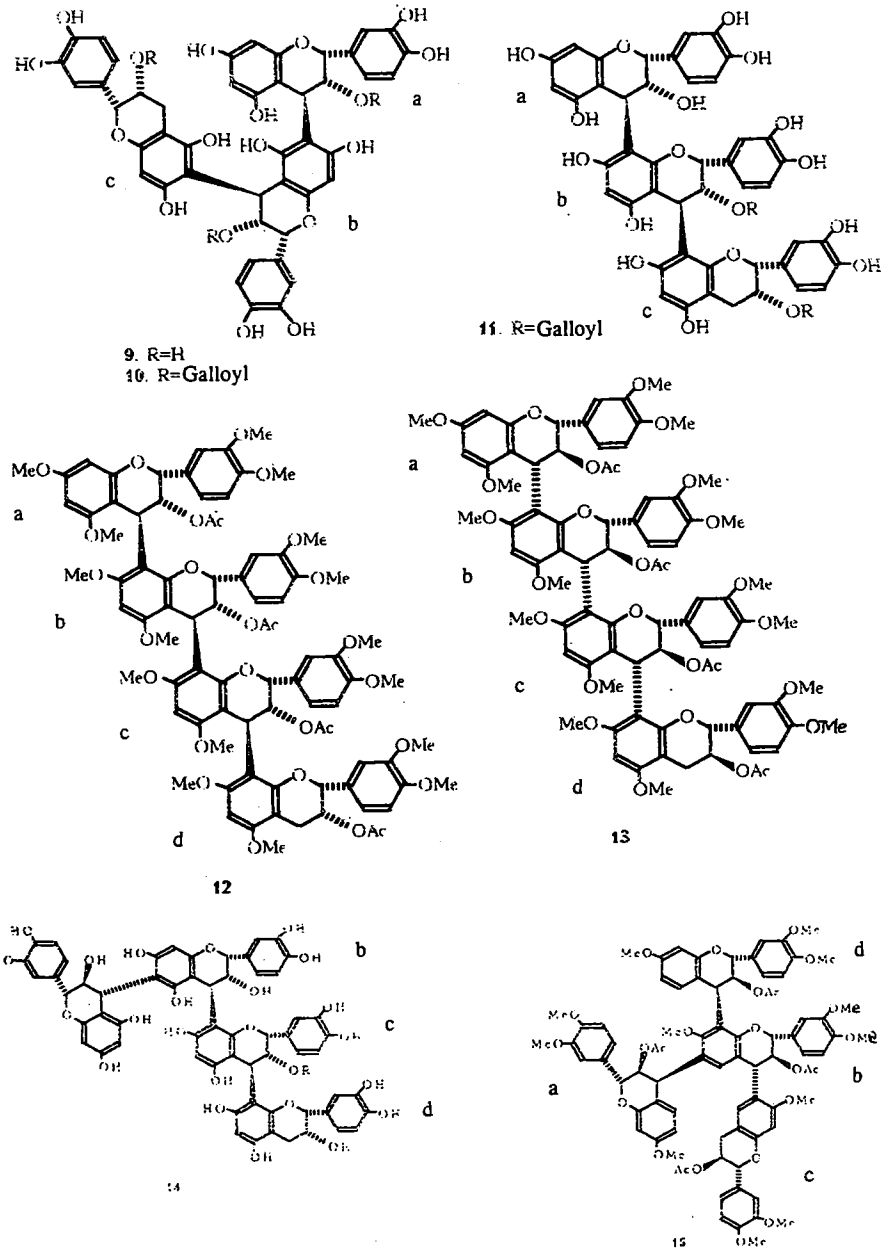
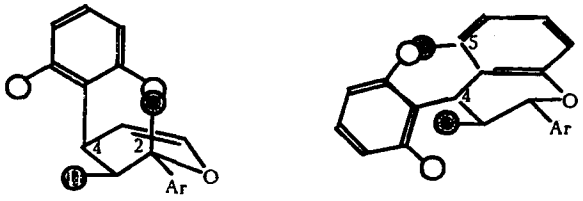
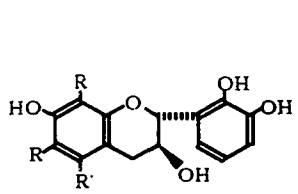


Fig. 2. Structures of compounds (**9-15**).

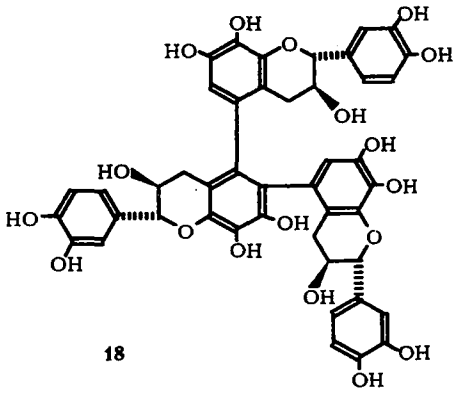


16β 16α

Fig. 3. Factors hindering rotation about the interflavan bond.

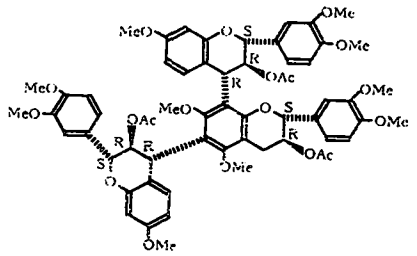


(17). R = 4α-(+)-afzelechin or
4β-(−)-epicatechin
R′ = H or OH

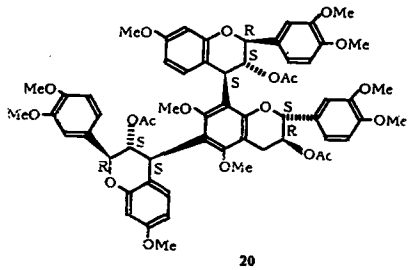


18

Fig. 4. Structures of angular proanthocyanidins.



19



20

Fig. 5. Mirror isomerism in angular proanthocyanidins.

On generalizing a large amount of literature information on the PMR spectroscopy of the dimeric proanthocyanidins [6-8, 30-38], their peracetates [5, 13], and the acetates of their methyl ethers [5, 10-12, 14, 17, 18, 20] obtained in various solvents and in various temperature regimes, we came to the conclusion that the most reliable method of determining the type of interflavan linkage is the use of the chemical shift of the aromatic protons of ring *A* of the "lower" block in the spectra of acetates of methyl ethers of the proanthocyanidins obtained under conditions of rotation about the interflavan bond, i.e., at temperatures of 100-170°C. The signal of the H-6 proton in the 4-8-linked dimers, regardless of their stereochemistry, appears in the 6.06-6.16 ppm region, and the signal of the H-8 protons in the alternative compounds appears in the 6.20-6.38 ppm interval. The ranges of these signals do not overlap. The results from different authors agree well. The rule deduced is valid for all dimeric proanthocyanidins, regardless of their stereochemistries. This method may therefore be considered reliable for the determination of such an important structural feature as the type of interflavan bond of the dimers of the first and second groups.

In PMR spectroscopy a method exists for identifying and determining the ratio of mirror isomers which involves the use of chiral solvents [61]. The method is based on the fact that an optically pure chiral solvent forms a complex predominantly with one of the mirror isomers, inducing in it changes in the CSs of the corresponding protons, while the CSs of the protons of the other mirror isomer remain unchanged. By comparing the integral intensities of the protons of the corresponding chiral centers it is possible to determine the optical purity or the ratio of the mirror isomers of the substance under investigation.

TABLE 3. CSs (δ, ppm) and SSCCs (J, Hz) of the Protons in the PMR Spectra of Proanthocyanidins of the Second Group and Their Derivatives

H	2	3	4	6	8	Gall. 3-OAc	Solvent	Lit.
B-1 (HC)	5.10 br.s	3.96 m	α 4.68 d $J_{4,3}$=2	5.96 d $J_{6,8}$=2	6.03 d $J_{8,6}$=2		acetone-d_6	30
(C2-HB)	4.75 d $J_{2,3}$=8	4.08	α 2.81 dd $J_{4,3}$=6 $J_{4,4}$=16 β 2.56 dd $J_{4,3}$=7 $J_{4,4}$=16	5.96 s	–			
B-1 (HC)	5.46 br.s	5.31 m	α 4.60 d $J_{4,3}$=2	5.80 d $J_{6,8}$=2	5.93 d $J_{8,6}$=2	6.95 s	acetone-d_6	30
(C2-HB)	4.43 d $J_{2,3}$=6	4.04 m	α 2.89 dd $J_{4,3}$=6 $J_{4,4}$=16 β 2.55 dd $J_{4,3}$=8 $J_{4,4}$=16	6.08 s	–			
B-1 [2]	5.12 br.s	3.88 m	α 4.65 m	5.86–5.99 m	5.86–5.99 m		acetone-d_6 + D_2O	36
	4.56 m	4.15 m	β 4.11 d $J_{4,3}$=7	6.07 br.s	–			
B-1 (C2-HC)	5.06 br.s	3.84 m	α 4.56 br.s	5.9 br.s	6.01 d $J_{8,6}$=2		acetone-d_6 + D_2O	36
[3] (C2-HB)	4.98 d $J_{2,3}$=8	4.11 m	α 4.48 dd $J_{4,3}$=4	6.03 s	–			
B-1 [4] (HC-C3)	5.36 s	5.28 t $J_{2,3}$=1.5 $J_{3,4}$=2.0	α 4.58 d $J_{4,3}$=2.0	5.82	5.92	1.74 s	$CDCl_3$, t 100°	5
	4.50 d $J_{2,3}$=8.25	5.12 m	–	6.14	–	1.84 s		
B-1 [5]	5.99 br.s	5.7 br.s	α 4.91 d $J_{4,3}$=1.9	6.43 d	6.57 d	–	$C_6H_5NO_2$, t 30°	15
(C2) Primary rotamer	4.54 d $J_{2,3}$=10	5.44 ddd $J_{2,3}$=10 $J_{3,4\alpha}$=7 $J_{3,4\beta}$=9	α 3.25 dd $J_{4,3}$=7 $J_{4,4}$=18 β2.66 dd $J_{4,3}$=9 $J_{4,4}$=18	6.98 s	–	–		
Secondary rotamer	5.79 br.s	5.58 br.s	α5.36–5.52 m	6.90 d	6.95 d			
	5.05 br.s	5.36–5.52 m	2.66–3.25 m	6.89 s	–			
B-2 (C2)	5.11 br.s	4.30 m	α4.75 br.s	5.97–6.04 m	5.97–6.04 m		acetone-d_6	30
	4.95 br.s	4.00 m	2.76–2.88 m	5.97–6.04 m	–			
B-2 [1] (HC)	5.65 br.s	5.55 m	α 4.79 d $J_{4,3}$=3	5.93 s	5.93 s	6.99 s	acetone-d_6	31
[1]	4.98 br.s	5.55 m	3.0 m	6.13 s	–	7.07 s		
B-2 [5] (HC)	5.60 br.s	5.18 d $J_{3,4}$=1.8	α 4.48 d $J_{4,3}$=1.8	6.00 d $J_{6,8}$=2	6.26 d $J_{8,6}$=2		$CDCl_3$	27
	4.56 d	5.12 m	2.9 m	6.66 s	–			
B-2 [4] (HC)	5.44 s	5.28 m	α 4.61 d $J_{4,3}$=2.2	6.02	6.25		$CDCl_3$, t 100°	5
(HB)	5.03 d $J_{2,3}$=6.5	5.30 m	–	–	6.33			
B-5 [4] (C3-HC)	5.47 br.s $J_{2,3}$=1.5	5.31 t	α 4.59 d $J_{3,4}$=2.2	6.06 d	6.30 d	1.78'	$CDCl_3$, t 100°	5.43
	5.11 d	5.53 m	2.69 m	–	6.38 s	1.91'		
B-5 [3]	5.00 br.s 5.21 br.s	4.0 m 4.0 m	α 4.60 br.s α 4.02 d $J_{4,3}$=2	6.09 s –	6.09 s 6.05 s		acetone-d_6 +D_2O	35
B-5 [1] (C-3)	5.15 br.s	5.44 s	α 4.66 s	6.04 d' J=2	6.16 d' J=2	7.06 s	acetone-d_6	37
[1]	5.15 br.s	5.60 m	4.1 m	–	6.17 s	7.06 s		

TABLE 3 (continued)

H	2	3	4	6	8	Gall. 3-OAc	Solvent	Lit.
D	4.63 s	4.04 m	2.25–2.81 m				acetone-d_6	34
	4.63 s	4.04 m	2.25–2.81 m					
D	4.80 s	5.35 m	2.42–3.02 m			7.00 s	acetone-d_6	34
	4.80 s	5.35 m	2.42–3.02 m			7.00s		

[1]Galloylation.

[2]The "lower" block has the substituent $-\alpha S-CH_2-Ar$ in position 4.

[3]Substituent $-4\beta S-CH_2-Ar$.

[4]Methyl ether acetate.

[5]Peracetate.

D) dimer – (−)-epigallocatechin-2′-2′-(−)-epigallocatechin.

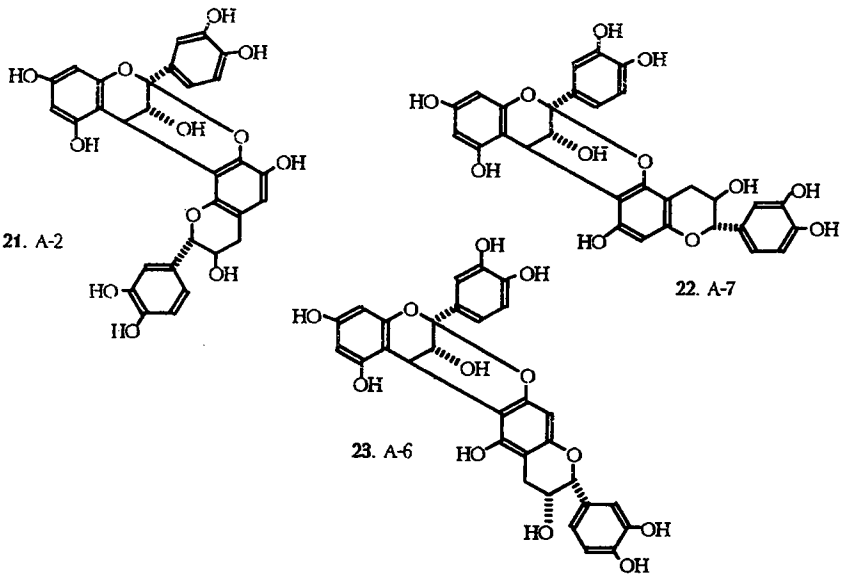


Fig. 6. Structures of proanthocyanidins of group A.

Workers of D. J. Roux's group [12, 39, 40] have proposed an original method of identifying mirror isomers of the proanthocyanidins of the first group that is based on the fact that in acetates of methyl ethers of proanthocyanidins obtained in deuterodimethyl sulfoxide at 150°C characteristic features of the difference in the chemical shifts of the H-2 and H-3 protons in the corresponding pairs of mirror isomers are observed (Table 2). However, the difference in the CSs of H-2 and H-3 that we have calculated from the results of the same authors is valid only for proanthocyanidins with a C4-C8 interflavan bond.

CHARACTERISTICS OF THE PMR SPECTRA OF THE PROANTHOCYANIDINS OF THE SECOND GROUPS

In the proanthocyanidins of the second group, the "upper" position is occupied by a flavan-3-ol with the 2,3-*cis*-configuration. They are readily distinguished from the proanthocyanidins of the first group by the nature of the splitting of the signals of the protons of the "upper" block. In the PMR spectra [7, 30-37, 63], the signals of the protons of ring *C* frequently appear in the form of singlets when ring *C* has the C2-pentacoplanar conformation. Otherwise, the signals of the H-2 and H-3

TABLE 4. CSs (δ, ppm) and SSCCs (J, Hz) of the Protons in the PMR Spectra of Trimers and Tetramers of the Proanthocyanidins and Their Derivatives

H	2	3	4	6	8	Gall. 3-OAc	Solvent	Lit.
9 a (C2)	4.89 br.s	3.96 br.s	α 4.82 br.s	6.00–6.20 m	6.00–6.20 m		acetone-d_6 + D_2O	35
b (C2)	4.89 br.s	3.96 br.s	α 4.46 br.s	–	6.00–6.20 m			
c	4.94 br.s	4.32 m	2.50–3.10 m	–	6.00–6.20 m			
10[2] a (C2)	5.28 s	5.57 s	α 4.70 s	5.80–6.20 m	5.80–6.20 m	6.98[b]	acetone-d_6	35
[2] b (C2)	5.16 br.s	5.57 s	α 4.88 s	–	5.80–6.20 m	7.04[b]		
[2] c (C2)	5.28 s	5.57 s	2.95–3.16 m	–	5.80–6.20 m	7.08[b]		
11 a (C2)	5.68 s	4.06 br.s	α 4.95 br.s	5.95–6.16 m	5.95–6.16 m		acetone-d_6	37
[2] b (C2)	5.28 s	5.65 s	α 4.95 br.s	5.95–6.16 m	–	7.03[b]		
[2] c	5.28 s	5.62 m	2.92–3.12 m	5.95–6.16 m	–	7.10[b]		
12[3] a	5.63 br.s	5.20–5.34 m	α 4.70 s	5.76 d $J_{6,8}$=2	5.96 d $J_{8,6}$=2	1.73[j]	$CDCl_3$, t 100°C	43
b	5.03 br.s	4.92 br.s	α 4.88 s	6.16 s[a]	–	1.69[i]		
(C2) c	5.03 br.s	4.92 s	α 4.88 s	6.09 s[b]	–	1.69[i]		
(C2) d	4.82 d	5.45 d	2.95–3.16 m	6.03 s[b]	–	1.62[i]		
13[3] a (HC)	4.60 d $J_{2,3}$=10.2	5.80 dd ΣJ=19.2	β 4.78 d $J_{4,3}$=9.0	6.08 d $J_{6,8}$=2.5	6.13 d $J_{8,6}$=2.5	1.63[j]	$CDCl_3$, t 100°C	14
b (C2)	4.72 d $J_{2,3}$=10.0	5.74 dd ΣJ=18.9	β 4.83 d $J_{4,3}$=8.5	5.92 s[a]	–	1.73[i]		
c (HC)	4.80 d $J_{2,3}$=10.2	5.56 dd ΣJ=19.0	β 4.98 d $J_{4,3}$=9.0	6.02 s[b]	–	1.80[i]		
d	5.05 br.s $J_{2,3}$<1	5.56 m	2.94 m	6.08 s[b]	–	1.84[i]		
14 a C2-HB	4.62 d $J_{2,3}$=8	4.44 m	β 4.80 d $J_{4,3}$=7	5.90 s	5.90 s		acetone-D_6	36
b(C2)	5.28 br.s	4.08 br.s	α 4.84 s	–	6.12 s			
c(C2)	5.28 br.s	4.14 br.s	α 4.84 s	6.05 s	–			
d	5.07 br.s	4.58 br.s	2.90 m	6.05 s	–			
15[3] a (HB)	5.313 d $J_{2,3}$=4.0	5.429 t $J_{3,2}$=4.0	α 4.420 d $J_{4,3}$=4.0	6.351 dd $J_{6,8}$=8.5 $J_{6,8}$=2.3	6.536 d $J_{8,6}$=2.5	H-5 6.451 d $J_{5,6}$=8.5	D-DMSO t 90°C	47
b (C2)	4.978 d $J_{2,3}$=7.2	5.142 t $J_{3,4}$=9.0	β 4.277 d $J_{4,3}$=9.0	–	–	6.418 br.s		
c (C2)	4.896 d $J_{2,3}$=9.3	5.177 m	α 2.86 dd $J_{4,3}$=5.5 $J_{3,4}$=16 β 2.73 dd $J_{4,3}$=8.0 $J_{4,4}$=16	–	6.406 s	6.638 br.s		
d (HC)	5.000 d $J_{2,3}$=9.8	6.083 t ^3J=10.0	β4.725y d $J_{4,3}$=10.2 $J_{4,3}$=1.0	6.561 dd $J_{6,8}$=8.5 $J_{6,8}$=2.5	6.410 d $J_{8,6}$=2.5	6.729 dd $J_{5,6}$=8.5 $J_{5,4}$=1.5		

[1]The assignment of the signals denoted by the same superscript symbols for one compound may be reversed.

[2]Hydroxy groups at C-3 galloylated.

[3]Methyl ether acetate.

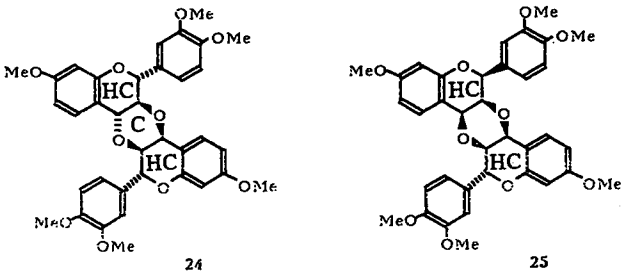


Fig. 7. Structures of proanthocyanidins with bridge interflavan links through oxygen atoms.

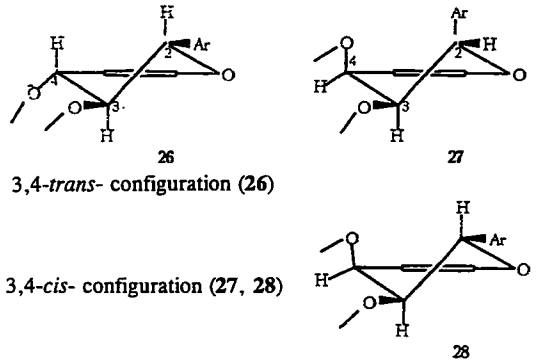


Fig. 8. Conformations of rings *C* in proanthocyanidins with bridge interflavan links.

protons are detected in the form of doublets with SSCCs of about 2 Hz – in this case, ring *C* of the "upper" block is present in the half-chair conformation. The orientation of the interflavan bond is then axial. The CSs of the H-2 protons are approximately 5.1-5.2 ppm, and those of the H-3 proton 3.9-4.3 ppm. The signal of the H-4 proton, which has the α-orientation, appears in the 4.3-4.9 ppm interval. Such scatter is apparently connected with rotational isomerism about the sp^3-sp^2 bonds, as can well be seen for the case of the peracetate of proanthocyanidin B-1 in Table 3.

Practically all proanthocyanidins with the 2,3-*cis*-configuration of the "upper" block have the 3,4-*trans*-configuration. The first natural dimer with the 2,3-*cis*-3,4-*cis*-configuration was identified by L. Y. Foo [41].

The characteristics of the signals of the aromatic protons in the flavan-3-ols occupying the "upper" position are analogous to those that we have given for the proanthocyanidins of the first group. Galloylation [30, 34, 37, 38, 42] induces a paramagnetic shift of more than +1 ppm in the signal of the H-3 proton. The induced CSs (ICSs) of H-2 and H-4 can be either positive or negative. This may be due to the influence of the spatial anisotropy of the ester group.

From the spectra of the acetates of methyl ethers of the proanthocyanidins of the second group, obtained under conditions of rotation about the interflavan bonds (at 100°C) [5, 43], we deduce the following ICSs of the "upper" block (ppm): H-2 +0.3; H-3 +1.3; and H-4 0.1. These values are close to those observed on galloylation. Analysis of the literature on the PMR spectra of the peracetates [5, 13, 15, 27, 44] has shown that the spectral characteristics of the protons of ring *C* are close to those in the methyl ether acetates. That is, the effects of the acylation of a nonaromatic hydroxy group are universal and depend little on the nature of the acid residue.

For the signals of the protons of ring *B*, paramagnetic shifts in the range from 0.2 to 0.5 ppm, as compared with the spectra of the phenols, are observed. This is apparently due to the repulsion of the π-electrons of the aromatic system by the carbonyl groups of the acetic acid residues.

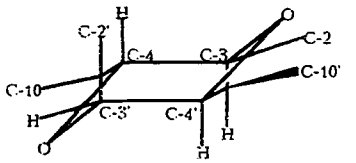
Details of the PMR spectra of proanthocyanidins consisting of two epigallocatechin fragments in which the interflavan linkage is of the C-2′–C-2′ type have been published [34, 45, 46]. The parameters of the spectra of these proanthocyanidins are close to the corresponding magnitudes in the spectra of (–)-epigallocatechin (see Table 3, compound D). However, the signals of the protons of rings *A* and *C* may have a fairly complex nature as a result of superposition. The signal of the unsubstituted H-6′ proton is shifted downfield by ~0.2 ppm in comparison with gallocatechin as a component of proanthocyanidins.

TABLE 5. CSs (δ, ppm) and SSCCs (J, Hz) of the Protons in the PMR Spectra of the Upper Blocks of Proanthocyanidins of Group A[1]

	Block a				Block b				
	H-3	H-4	H-6	H-8	H-2	H-3	H-4	H-6	H-8
A-6[2]	4.14d $J_{3,4}=4$	4.34d $J_{4,3}=4$	6.05[h]	6.12[h]	4.85s	4.20s	2.85m	6.05[h]	–
A-7	4.15d $J_{3,4}=4$	4.30d $J_{4,3}=4$	6.10[i]	6.12[i]	4.87s	4.20br.	2.95m	–	6.10[i]

[1]Solvent – acetone-d_6.

[2]The signals marked with the same superscript letters may be interchanged.



2S

Fig. 9. Conformation of the ring formed by the interflavan bonds in a 3,4-*trans*- isomer.

PMR SPECTRA OF TRIMERS AND TETRAMERS OF PROANTHOCYANIDINS

Trimers and tetramers of proanthocyanidins are compounds transitional between the low-molecular-mass proanthocyanidins and their polymeric analogs. The structures of some oligomers of the proanthocyanidins are given in Fig. 2, and details of the ^{1}H NMR spectra of these compounds in Table 4. The parameters of the PMR spectra of the oligomers are close to those observed in the spectra of the dimers. The spectra of the compounds with the 2,3-*cis*- configuration (**9-12**) can be interpreted relatively easily, since the signals of the protons of ring *C*, H-2, H-3, and H-4, appear either in the form of singlets or with small spin–spin coupling constants. In these cases, the probability of the overlapping of these signals is low. It can be seen from the information on ^{1}H NMR spectroscopy given in Table 4 that proanthocyanidins with the 2,3-*cis*- configuration frequently assume a C2-pentacoplanar conformation in which the interflavan bond has a pseudoequatorial orientation. It can be seen from the SSCCs that this conformation may be retained in methyl ether acetates and at an elevated temperature (see Table 4, compound (**12**)).

For proanthocyanidins including blocks with the 2,3-*trans*-configuration (**13-15**) the PMR spectra are very complex because of the superposition of the signals of the corresponding protons. Therefore, to improve resolution, instruments with a high working frequency are used [35-37, 47]. The spectra of peracetates or of methyl ether acetates are frequently obtained [5, 10-12, 14, 43, 47, 48]. There are reports of PMR spectra of methyl ether acetates obtained at 160-210°C [10-12, 48]. Judging from SSCCs, under these extreme conditions the conformations of the rings *C* of the flavan blocks occupying the "upper" or "lower" positions in a proanthocyanidin may change from half-chair to half-boat (see Table 4, compounds (**12**), (**13**), and (**15**)). In these circumstances the scatter of the CSs of the protons of ring *C* amounts to about 1 ppm. This phenomenon is connected not only with conformational isomerism in ring *C* but also with hindered rotation about the sp^3-sp^2 bonds.

In a study of peracetates of dimers and trimers of proanthocyanidins at room temperature, when the conformation of ring *C* in the "upper" block is retained, differences were observed in the CSs of H-2, H-3, and H-4 [29]. This phenomenon is also due to rotational isomerism about sp^3-sp^2 bonds: C-4–C-6, C-4–C-8, or C-2–C-1′.

For polymeric proanthocyanidins with hindered rotation about the interflavan bonds, one rotamer represents a right-hand, and the other a left-hand, helix [29]. The quantitative ratio of the isomers is about 1:3. The energy of the transition from one conformation to the other amounts to 15-20 kcal/mole [50]. The hindrance to rotation about interflavan bonds is due to the overlapping of the substituents in ring *A* of the "lower" block, which are in the *ortho*-position to the interflavan bond or to the H-2 proton (if the interflavan bond is β-axial) or with the substituent in position 5 (if the interflavan bond is equatorial) [4]. The latter is true both for the α- and the β-orientations of the interflavan bond (Fig. 3).

A. C. Fletcher et al. [49] have used not the integrals but the intensities of the peaks in the ^{1}H NMR spectra to estimate the populations of rotamers.

There are reports of "angular" proanthocyanidins [10, 12, 47, 51, 52] synthesized as the result of a search for new biologically active compounds of this class. Structurally, they may be divided into two groups (Fig. 4). In one group (**17**) the interflavan bond is realized in the usual way (4-6 or 4-8), while in the second (**18**) it is realized between aromatic nuclei. In the first case, therefore, it is possible to observe in the aromatic region of the PMR spectrum two *AB* spin systems, while in the region of nonaromatic protons the picture is analogous to that for the spectra of the linear trimers. In the second case, the signals of the aromatic protons can easily be interpreted, while in the region of the H-2, H-3, and H-4 protons the picture is fairly complex.

Mirror isomerism is also observed among the angular trimers, and (+)- and (−)-fisetinidol blocks are also found [12] (Fig. 5). The method of identifying the enantiomers is analogous to that described for the dimeric proanthocyanidins. Thus, NMR spectroscopy possesses reserves in those regions that have hitherto been considered nontraditional for it.

There are reports in the literature of proanthocyanidins of group A with cyclic (bridge) interflavan links [3, 53-59], these being realized, usually, as three main types (Fig. 6).

The lower block may have the *cis*- or the *trans*-configuration. However, this does not affect the parameters of the upper block, since its conformation is fixed fairly rigidly. The value $J_{3,4} = 4$ Hz for the latter shows that the heterocycle of this block has a conformation close to a half-chair (see Table 5).

Similar structural fragments can form ordinary interflavan bonds of the 4-8 type with (+)-catechin, (−)-epicatchin, and other flavanoids [60], while retaining their own spectral characteristics.

Cyclic interflavan bonds in the proanthocyanidins can also be formed in another way: for example, without the realization of a C−C bond but through oxygen atoms alone (Fig. 7). Here the bridge interflavan link may have either the 3,4-*trans*-3,4-*cis*- or the 3,4-*cis*-3,4-*cis*-configuration (**24**, **25**).

According to PMR spectroscopy [54, 55, 62], rings *C* in these groups of compounds have the half-chair conformation (Fig. 8).

A study of these compounds using Dreiding models shows that even when the conformations of rings *C* of the flavan blocks are stable, the ring formed by the interflavan bonds has the chair conformation in the 3,4-*trans*-isomer (Fig. 9), while in the 3,4-*cis*-analog its conformation is fairly labile.

Summing up the possibilities of proton resonance spectroscopy in the investigation of the structures of flavan-3-ols and proanthocyanidins, it is possible to distinguish the following important points. PMR spectroscopy is capable of giving information on the stereochemistry and the rotational and conformational isomerism of these compounds. For the proanthocyanidins the possibility exists of determining the position of the interflavan bond. For them, an original method of investigating mirror isomers without involving chiral reagents has been found. Perhaps the only defect of PMR spectroscopy is the limited nature of its possibilities in the study of high-molecular-mass proanthocyanidins, which, however, can be overcome by the use of ^{13}C NMR spectroscopy.

REFERENCES

1. A. D. Vdovin, Z. A. Kuliev, and N. D. Abdullaev, Khim. Prir. Soedin., 16 (1997).
2. R. W. Hemingway, L. Y. Foo, and L. J. Porter, J. Chem. Soc., Perkin Trans. I, 1209 (1982).
3. K. Weinges, W. Kaltenhauser, H. Marx, F. Nader, J. Perner, and D. Geiler, Liebigs Ann. Chem., **711**, 184 (1968).
4. A. C. Fletcher, L. J. Porter, and E. Haslam, J. Chem. Soc., Chem. Commun., 627 (1976).
5. H. Kolodziej, Phytochemistry, **25**, 1209 (1986).
6. T. Tanaka, G. Nonaka, and I. Nishioka, Phytochemistry, **22**, 2575 (1983).
7. R. S. Thompson, D. Jacques, E. Haslam, and R. J. N. Tanner, J. Chem. Soc., Perkin Trans. I, 1387 (1972).
8. S. E. Drewes, D. J. Roux, S. H. Egger, and J. Feeney, J. Chem. Soc. (C), 1217 (1967).
9. H. Kolodziej, Phytochemistry, **24**, 2460 (1985).
10. J. J. Bota, P. M. Viviers, D. A. Young, I. C. du Preez, D. Ferreira, D. J. Roux, and W. E. Hull, J. Chem. Soc., Perkin Trans. I, 527 (1982).
11. P. M. Viviers, D. A. Young, J. J. Bota, D. Ferreira, D. J. Roux, and W. E. Hull, J. Chem. Soc., Perkin Trans. I, 535 (1982).
12. P. H. Viviers, H. Kolodziej, D. A. Young, D. Ferreira, and D. J. Roux, J. Chem. Soc., Perkin Trans. I, 2555 (1983).
13. V. K. Sethi, S. C. Taneja, K. L. Dhar, and C. K. Atal, Phytochemistry, **23**, 2402 (1984).

14. K. Von Weinges, K. Göritz, and F. Nader, Liebigs Ann. Chem., **715**, 164 (1968).
15. H. Rauwald, Planta Med., **46**, 110 (1982).
16. K. Weinges, W. Bahr, W. Ebbert, K. Göritz, and H. D. Marx, Fortschr. Chem. Org. Naturst., **27**, 158 (1969).
17. J. C. S. Malan, D. A. Young, A. Steenkamp, and D. Ferreira, J. Chem. Soc., Perkin Trans. I, 2567 (1988).
18. E. Young, E. V. Brandt, D. A. Young, D. Ferriera, and D. J. Roux, J. Chem. Soc., Perkin Trans. I, 1737 (1986).
19. K. Kurasawa, W. D. Ollis, I. O. Sutherland, O. R. Gottlieb, and A. B. de Oliveira, Phytochemistry, **17**, 1389 (1978).
20. A. Pelter, P. I. Amenechi, R. Warren, and S. H. Harper, J. Chem. Soc. (C), 2572 (1969).
21. J. J. Bota, D. Ferreira, and D. J. Roux, J. Chem. Soc., Chem. Commun., 700 (1978).
22. S. E. Drewes, D. J. Roux, H. M. Saayman, H. Eggers, and J. Feeney, J. Chem. Soc. (C), 1302 (1967).
23. A. Delcour, D. Ferreira, and D. J. Roux, J. Chem. Soc., Perkin Trans. I, 1711 (1983).
24. G. Nonaka, F. Hsu, and I. Nishioka, J. Chem. Soc., Chem. Commun., 781 (1981).
25. A. Steenkamp, J. C. S. Malan, and D. Ferreira, J. Chem. Soc., Perkin Trans. I, 2179 (1988).
26. L. Y. Foo and L. J. Porter, J. Chem. Soc., Perkin Trans. I, 1186 (1978).
27. L. Langhammer, H. Rauwald, and G. Schulze, Arch. Pharm., **314**, 424 (1981).
28. Yu. Yu. Samitov, The Stereospecificity of Nuclear Spin−Spin Coupling Constants and Conformational Analysis [in Russian], Izd. Kaz. Un-ta (1990).
29. W. R. Bergman, V. N. Vismanadhan, and W. L. Mattice, J. Chem. Soc., Perkin Trans. II, 45 (1988).
30. G. Nonaka, I. Nishioka, T. Nagasawa, and H. Oura, Chem. Pharm. Bull., **29**, 2862 (1981).
31. G. Nonaka, N. Miwa, and I. Nishioka, Phytochemistry, **21**, 429 (1982).
32. S. Morimoto, G. Nonaka, and I. Nishioka, Chem. Pharm. Bull., **34**, 643 (1986).
33. G. Nonaka, R. Sakai, and I. Nishioka, Phytochemistry, **23**, 1753 (1984).
34. G. Nonaka, O. Kawahara, and I. Nishioka, Chem. Pharm. Bull., **31**, 3906 (1983).
35. F. Hsu, G. Nonaka, and I. Nishioka, Chem. Pharm. Bull., **33**, 3142 (1985).
36. F. Hsu, G. Nonaka, and I. Nishioka, Chem. Pharm. Bull., **33**, 3293 (1985).
37. Y. Kashiwada, G. Nonaka, and I. Nishioka, Chem. Pharm. Bull., **34**, 4083 (1986).
38. G. Nonaka, M. Muta, and I. Nishioka, Phytochemistry, **22**, 237 (1983).
39. A. Delcour, E. J. Serneels, D. Ferreira, and D. J. Roux, J. Chem. Soc., Perkin Trans. I, No. 4, 669 (1985).
40. J. A. Steenkamp, D. Ferreira, and D. J. Roux, Tetrahedron Lett., **26**, 3045 (1985).
41. L. Y. Foo, J. Chem. Soc., Chem. Commun., 236 (1986).
42. G. Nonaka, F. Hashimoto, and I. Nishioka, Chem. Pharm. Bull., **34**, 61 (1986).
43. H. Kolodziej, D. Ferreira, and D. J. Roux, J. Chem. Soc., Perkin Trans. I, 343 (1984).
44. M. Ensemeyer, L. Langhammer, and H. Rauwald, Arch. Pharm., **313**, 61 (1980).
45. H. Kolodziej, J. Chem. Soc., Chem. Commun., 205 (1987).
46. H. Kolodziej, J. Chem. Soc., Perkin Trans. I, 219 (1988).
47. J. A. Steenkamp, D. Ferreira, and D. J. Roux, J. Chem. Soc., Perkin Trans. I, 23 (1983).
48. P. M. Viviers, J. J. Bota, D. Ferreira, D. J. Roux, and H. M. Saayman, J. Chem. Soc., Perkin Trans. I, 17 (1983).
49. A. C. Fletcher, L. J. Porter, E. Haslam, and R. K. Gupta, J. Chem. Soc., Perkin Trans. I, 1628 (1977).
50. E. Haslam, Phytochemistry, **16**, 1625 (1977).
51. D. A. Young, H. Kolodziej, D. Ferriera, and D. J. Roux, J. Chem. Soc., Perkin Trans. I, 2537 (1985).
52. L. Y. Foo and R. W. Hemingway, J. Chem. Soc., Chem. Commun., 85 (1984).
53. D. Jacques, E. Haslam, G. R. Bedford, and D. Greatbanks, J. Chem. Soc., Perkin Trans. I, 2663 (1974).
54. S. Morimoto, G. Nonaka, and I. Nishioka, Chem. Pharm. Bull., **33**, 4338 (1985).
55. G. Nonaka, S. Morimoto, and I. Nishioka, J. Chem. Soc., Perkin Trans. I, 2139 (1983).
56. D. Jacques, E. Haslam, G. F. Bedford, and D. Greatbanks, J. Chem. Soc., Chem. Commun., 518 (1973).
57. S. Morimoto, G. Nonaka, and I. Nishioka, Chem. Pharm. Bull., **36**, 33, (1988).
58. G. Nonaka, S. Morimoto, J. Kinjo, T. Nohara, and I. Nishioka, Chem. Pharm. Bull., **35**, 149 (1987).
59. J. J. Karchesy and R. W. Hemingway, J. Agric. Food Chem., **34**, 966 (1986).
60. H. Hikino, M. Takahashi, and C. Konno, Tetrahedron Lett., **23**, 673 (1982).
61. H. Günther, NMR Spectroscopy: An Introduction, Wiley, New York (1980).
62. D. A. Young, D. Ferreira, and D. J. Roux, J. Chem. Soc., Perkin Trans. I, No. 9, 2031 (1985).