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^{13}C NMR OF FLAVONOLIGNANS FROM *HYDNOCARPUS WIGHTIANA*

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Key Word Index—*Hydnocarpus wightiana*; Flacourtiaceae; flavonolignans; ^{13}C-NMR.

Flavonolignans are a recently discovered group of natural products composed of a flavonoid chromophore combined with a coniferyl alcohol moiety. The earliest member silymarin was reported to possess antihepatotoxic effects, preventing disturbances in liver lipid metabolism induced by phalloidin. In a search for new members of this group, we reported 5 compounds based on a flavone unit rather than a dihydroflavonol unit from *Hydnocarpus wightiana* seed coats [1–4]. So far one flavonolignan silychristin has been analysed by ^{13}C NMR [5]. Data are also available for a xantholignan kielcorin [6].

Table 1 presents the ^{13}C NMR data of compounds **1–6** and their assignments. The assignments have been made in conformity with the published data of flavonoids [7] and peak widths of the signals at half height. In hydnocarpin (**1**) [8], the two units are linked through O atoms to form a dioxane ring. Signal assignment based on a comparison with luteolin and kielcorin for the C_{10} unit, is consistent with structure (**1**). Isohydnocarpin (**2**) (*vide infra*) differs from (**1**) in having a dihydrofuran ring involving the 4′ and 5′ positions instead of a dioxane ring. The signals which are significantly affected by this structural modification are those from C-12, C-13 and C-5′ and to a smaller extent C-4′, the last being *ortho* to the new substituent; the C-12 signal shifts upfield by nearly 24 ppm while those of C-13 and C-5′ shift downfield by nearly 10 and 14 ppm respectively. Methoxyhydnocarpin (**3**) has an additional methoxyl at C-5′ relative to (**1**). This is clearly reflected in the downfield shift of this carbon signal by *ca* 32 ppm and the smaller upfield shift of the *ortho* and *para* carbons viz. C-4′, C-6′ and C-2′. Hydnowightin (**5**) is a further elaboration of (**1**) with an additional C_{10} unit. It was suggested [4] that its formation could be visualized by participation of dehydrodiconiferyl alcohol in place of coniferyl alcohol with luteolin and subsequent ring opening of the dihydrofuran to provide an *o*-hydroxystilbene system. Consistent with this suggestion, the point of attachment of the extra C_{10} unit viz. C-18 gives its signal at δ 132.5, a shift of nearly 17 ppm to low field relative to (**1**). The signals of Me stilbenic carbons at C-12′ and C-13′ are observed at δ 142.6 and 132.4 respectively, the relative deshielding being due to the hydroxymethyl group at C-12′. In the preliminary work dealing with the structure of hydnowightin [4], the 60 MHz PMR spectrum of its Me ether acetate could not be satisfactorily analysed. The spectrum of the parent compound is better resolved in MDSO-d_6 + D_2O at 80 MHz. The flavone A-ring protons H-6 and H-8 and the pyrone ring proton H-3 appeared at δ 6.2, 6.5 and 6.4 respectively, the first two being clear doublets (J = 2.5 Hz) and the last a singlet. The H-5′ also appeared as a doublet at δ 6.3 (J = 9 Hz) ruling out the attachment of the extra C_{10} unit to C-5′ or C-6′.

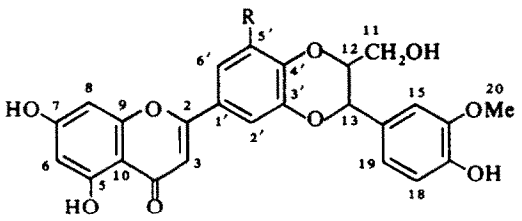
Table 1. Carbon shifts of compounds 1 to 6*

Carbon No.	1	2	3	4	5	6
2	164.2	164.7	164.2	164.5	164.1	152.8
3	103.8	103.8	103.8	104.8	103.9	104.4
4	181.6	181.7	181.7	180.8	180.4	177.7
5	157.3	157.4	157.3	157.3	157.7	153.6
6	98.5	99.0	98.9	97.1	98.8	107.0
7	161.4	161.6	161.4	161.3	161.5	155.7
8	93.9	94.0	94.0	96.8	93.8	104.6
9	162.8	164.1	163.0	161.3	156.9	152.2
10	103.8	103.8	103.8	104.8	103.9	108.3
1′	123.6	123.6	122.3	120.3	127.1	108.9
2′	114.6	114.4	108.1	113.7	112.1	114.8
3′	143.6	145.2	144.3	147.2†	145.3	137.5
4′	147.2	141.6	136.6	148.2	147.3†	150.3
5′	116.7	130.7	149.0	119.2	115.5	105.6
6′	119.3	116.6	104.1	133.9	113.4	140.8
11	59.6	62.9	59.9	69.2	59.8	
12	76.4	52.8	75.7	35.2	76.1	
13	78.1	87.9	78.3	40.9	77.8	
14	127.0	132.0	127.2	132.4	127.1	
15	110.9	110.4	112.0	112.3	113.2	
16	147.7†	147.8†	147.7†	147.9†	147.8†	
17	147.0†	147.7†	147.2†	147.6†	147.2†	
18	115.4	115.7	115.4	115.7	132.5	
19	120.6	119.2	120.6	127.4	119.2	
20	55.8	55.8	56.0 55.8	56.1	55.6	

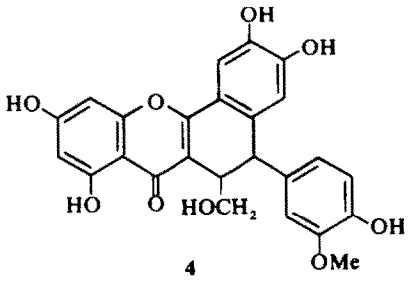
*The δ values are in ppm downfield from TMS.
†Assignments interchangeable.

The remaining aromatic protons appeared as a multiplet around δ 6.8 and could not be analysed. The stilbenic proton gave a singlet at δ 7.7, slightly broadened due to allylic coupling with the methylene protons. The dioxane ring proton on the benzylic carbon C-13 was reminiscent of the corresponding proton of hydnocarpin and its signal was a doublet at δ 5.05 (J = 7.5 Hz) as expected.

Neohydnocarpin (**4**) has a different skeleton from the rest of the compounds studied but yet is composed of the same two units—a luteolin moiety and a coniferyl alcohol moiety, linked by C—C bonds. The structure can be biogenetically visualized as arising from a lignan acid derived from conidendrin or isotaxiresinol taking the place of cinnamic acid in flavonoid biosynthesis or a coupling of the flavone 3-position with the β-carbon of a coniferyl alcohol moiety and subsequent modifications. The lack of oxygenation on C-12 and C-13 is readily substantiated by the upfield shift of C-12 and C-13 to δ 35.2 and 40.9 as compared to δ 76.4 and 78.1 in (**1**). The point of attachment in the flavone B-ring viz. C-6′ is *ca*



1 R = H
3 R = OMe



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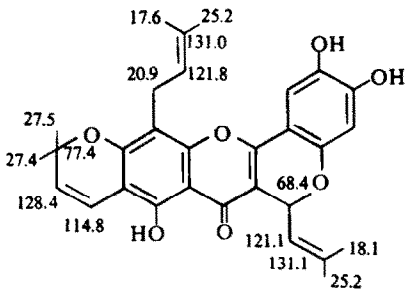

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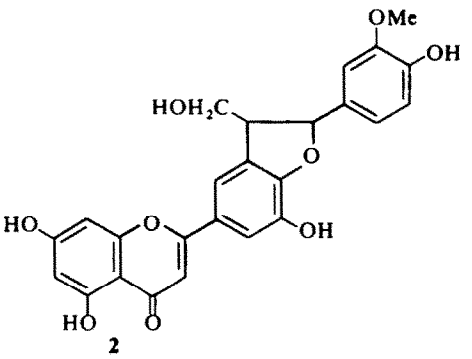

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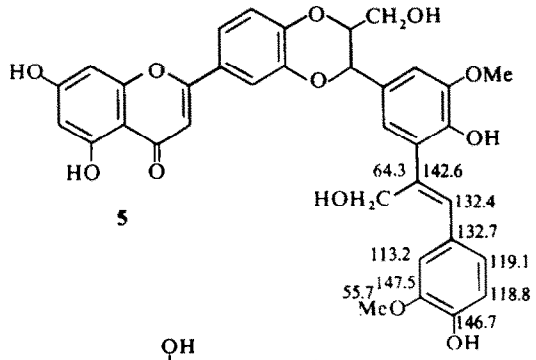

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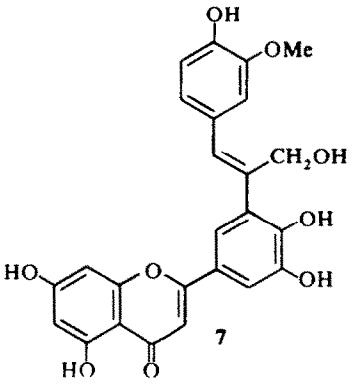

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15 ppm downfield to its chemical shift in (1), a shift comparable to that of C-5′ in going from (1) to (2). The hydroxymethyl carbon is also relatively downfield at δ 69.2, attributable to the proximity to the carbonyl group and would serve to eliminate the alternative formulation with the exchange of the hydroxymethyl and guaiacyl residues. An apparent anomaly seemed to be in the chemical shift of C-3 at δ 104.8. Wenkert *et al.* [9] observed the position of the C-3 signal of flavones to be around δ 120 when the 3-position carried a prenyl group. A similar shift can be expected in the case of neohydnocarpin and its absence in (4) suggests a shielding influence of the extra ring system. As a model compound we studied readily available cycloheterophyllin (6) and its aromatic carbon shifts are also included in Table 1. As can be seen from the data, the C-3 signal is at δ 104.4 which is not very different from that observed in normal flavones.

The structure (2) for isohydnocarpin was based on the alkaline degradation of the Me ether and the PMR data of its acetate and the Me ether acetate [2]. The ^{13}C NMR spectrum of isohydnocarpin contained many additional peaks than those accounted for by structure (2), mentioned in Table 1. As the sample was homogeneous on TLC, the PMR spectrum of the parent compound in DMSO-d_6 was also studied at 80 MHz, which indicated the possible presence of an equilibrium between (2) and its ring opened form (7) having an *o*-hydroxystilbene unit as present in (5). Other examples include oxyresveratrol and chlorophorin. Although doublets attributable to H-6 were not well resolved, H-8 appeared as two separate doublets at δ 6.35 and 6.45 as did H-3 which appeared as two singlets at δ 6.5 and 6.6. The stilbenic proton was a slightly broadened singlet at δ 8.1. The additional signals in the ^{13}C NMR spectrum could not be completely analysed because of a partial overlap but but the signals at δ 146.5 and 132 are close to those observed for the corresponding carbons in (5) and support the proposed equilibrium at least in DMSO solutions.

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FLAVONOIDS OF *ABIES AMABILIS* NEEDLES

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Key Word Index—*Abies amabilis*; Pinaceae; flavonol glycosides; *C*-glycosylflavones; syringetin; laricytrin; dihydroquercetin.

Abstract—Seventeen flavonol glycosides were identified from needles of *Abies amabilis* and these were based on 6 aglycone types: syringetin, isorhamnetin, kaempferol, quercetin, laricytrin and myricetin. Glycosides were 3-*O*-rutinosides, 3-*O*-glucosides, 3-*O*-galactosides or 3-*O*-rhamnosides. Also identified as needle constituents were rhamnosylvitexin and dihydroquercetin.

INTRODUCTION

We have begun a series of investigations to discover patterns of evolution in North America species of *Abies* Mill (true firs). In addition to various anatomical and morphological characters of needles, twigs and cones, we are determining the needle flavonoids of all species for future use as chemotaxonomic characters. This initial study reports the leaf flavonoids of *A. amabilis* (D. Douglas) J. Forbes (Pacific silver fir) which is a common, low-evelation, coniferous species of the Cascade and Coast Mountains of Oregon, Washington and British Columbia.

Although the terpenoids from various tissues of *A. amabilis* have been well studied as a source of chemotaxonomic input [1, 2], the flavonoids of this species (and many other common conifers) have been largely neglected. Mullick [3] has reported cyanidin as a red pigment in the periderm of *A. amabilis*, and Hergert and Goldschmid [4] found the 3′-*O*-glucoside of dihydroquercetin in its wood and bark.

RESULTS AND DISCUSSION

The needles of *A. amabilis* proved to be a rich source of varied flavonoids, although these compounds were present in low concentration relative to angiosperm foliage that we have examined. Three classes of flavonoids are present: flavonol glycosides, *C*-glycosylflavones and a dihydroflavonol (see Table 1).

Table 1. R_f values for flavonoid glycosides of *Abies amabilis*

Compound	R_f (× 100) Solvent 1*	Solvent 2†
Syringetin 3-*O*-rutinoside	47	76
Kaempferol 3-*O*-rutinoside	37	42
Quercetin 3-*O*-rutinoside	39	22
Laricytrin 3-*O*-rutinoside	41	32
Isorhamnetin 3-*O*-rhamnoside	24	56
Isorhamnetin 3-*O*-galactoside	20	45
Isorhamnetin 3-*O*-glucoside	20	45
Kaempferol 3-*O*-rhamnoside	19	33
Kaempferol 3-*O*-galactoside	27	34
Kaempferol 3-*O*-glucoside	27	34
Quercetin 3-*O*-rhamnoside	17	22
Quercetin 3-*O*-galactoside	17	19
Quercetin 3-*O*-glucoside	17	19
Laricytrin 3-*O*-rhamnoside	21	32
Laricytrin 3-*O*-galactoside	21	24
Laricytrin 3-*O*-glucoside	21	24
Myricetin 3-*O*-rhamnoside	17	9
Rhamnosylvitexin	48	28
M‡	36	61
N‡	39	56
Dihydroquercetin	45	61

* Solvent 1 = H_2O–*n*BuOH–Me_2O–HOAc (16:2:2:1) on Polyamide DC 6.6.

† Solvent 2 = $CHCl_3$–*iso*PrOH–butanone–HOAc (10:3:3:4) on Polyamide DC 6.6.

‡ Partially identified as rhamnosyl-*C*-glycosyl derivatives of apigenin.