

STUDIES IN THE THYMELAEACEAE I. NMR SPECTRAL
ASSIGNMENTS OF DAPHNORETIN

GEOFFREY A. CORDELL

*Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy,
University of Illinois at Chicago, Chicago, IL 60612*

ABSTRACT.—The high-field proton and ^{13}C -nmr spectra of daphnoretin (**1**) are assigned unambiguously through polarization transfer and selective decoupling experiments.

Daphnoretin (**1**) is a well-known bis-coumarin derivative found principally in the Thymelaeaceae, but also in the Leguminosae and Rutaceae [Table 1 (1-25)]. The structure was determined by Tschesche *et al.* (10), and confirmed by total synthesis (26). Interest in daphnoretin (**1**) has been rekindled recently because of the *in vivo* antineoplastic activity shown against the Ehrlich ascites carcinoma in mice (21,27), and the ability of **1** to inhibit a number of enzymes involved in DNA synthesis in Ehrlich ascites cells (28). It should be noted that daphnoretin (**1**) is not active in the standard NCI *in vivo* or *in vitro* assays (7, 15, 16, 21).

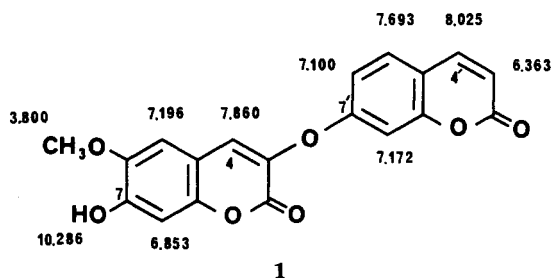


TABLE 1. Occurrence of Daphnoretin in Plants

Plant Source	Family	Reference
<i>Boeninghausenia albiflora</i>	Rutaceae	1,2
<i>Boeninghausenia japonica</i>	Rutaceae	3
<i>Coronilla balansae</i>	Leguminosae	4
<i>Coronilla cretica</i>	Leguminosae	4,5
<i>Coronilla hyrcana</i>	Leguminosae	4,5
<i>Coronilla orientalis</i>	Leguminosae	4,5
<i>Coronilla repanda</i>	Leguminosae	4,5
<i>Coronilla scorpioides</i>	Leguminosae	4-6
<i>Coronilla varia</i>	Leguminosae	4,5,7
<i>Daphne cannabina</i>	Thymelaeaceae	8
<i>Daphne gnidium</i>	Thymelaeaceae	9
<i>Daphne mezereum</i>	Thymelaeaceae	10-13
<i>Daphne tangutica</i>	Thymelaeaceae	13
<i>Daphnopsis racemosa</i>	Thymelaeaceae	10,11
<i>Edgeworthia gardneri</i>	Thymelaeaceae	14,15
<i>Peddiea fischeri</i>	Thymelaeaceae	16
<i>Ruta graveolens</i>	Rutaceae	17
<i>Thymelaea hirsuta</i>	Thymelaeaceae	18,19
<i>Thymelaea tartonraira</i>	Thymelaeaceae	20
<i>Wikstroemia foetida</i> var <i>oahuensis</i>	Thymelaeaceae	21
<i>Wikstroemia indica</i>	Thymelaeaceae	22-24
<i>Wikstroemia viridiflora</i>	Thymelaeaceae	25

Even though **1** has been isolated frequently and sometimes in high-yield, nmr spectral data are not well established. This paper reports the first definitive study of the ^1H -nmr spectrum and a complete and unambiguous assignment of the ^{13}C -nmr spectrum of daphnoretin (**1**).¹

A preliminary study of the ^1H -nmr spectrum of **1** has been published (4,13,25), without discussion or measurement of coupling constants. Our initial work at 60 MHz,² using $\text{DMSO}-d_6$ as the solvent, failed to resolve adequately a three-proton complex in the region δ 7.03-7.20.³ At 360 MHz, each aromatic proton was distinct (Figure 1),⁴ and a number of selective decoupling experiments established the assignments. Thus, irradiation of the doublet at δ 6.363 (H-3') collapsed the doublet at δ 8.025 to a singlet (H-4'), and back irradiation, as well as reducing the multiplicity of H-3', also eliminated a long-range coupling (~ 0.3 Hz) in the proton at δ 7.172, indicating this doublet ($J=2.3$ Hz) to be H-8'. Final confirmation of the assignments on the A unit came through irradiation of the doublet ($J=9.6$ Hz) at δ 7.693, which reduced the doublet of doublets at δ 7.100 to a doublet ($J=2.3$ Hz). Three singlets, at δ 7.860, 7.196 and 6.853, were assigned to H-4, H-5, and H-8, respectively, based on the established data for coumarins (29).

More substantial problems were posed by the ^{13}C -nmr spectrum.⁵ At 90.546

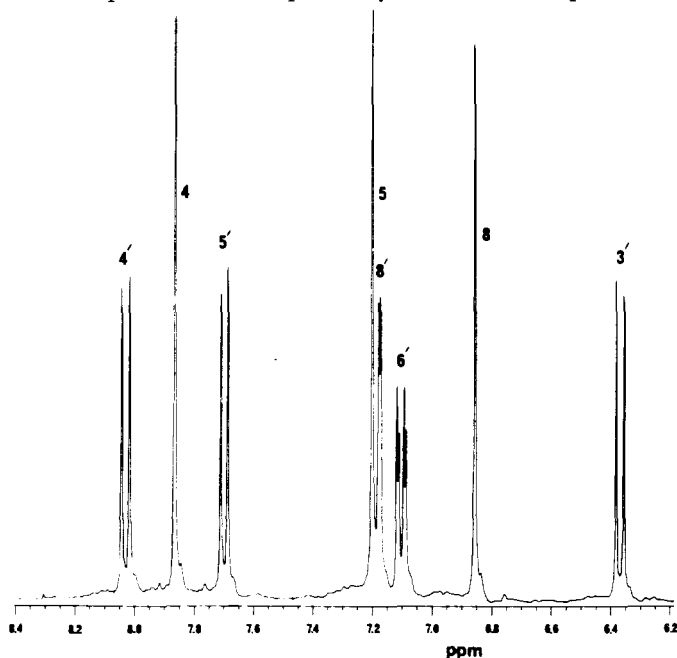


FIGURE 1. 360 MHz ^1H -nmr Spectrum of Daphnoretin (**1**) (aromatic region only).

¹The daphnoretin used in this study was obtained from the stems of *Edgeworthia gardneri* Meisn. (Thymelaeaceae) (15).

²Obtained using a Varian T-60A instrument equipped with a Nicolet TT-7 Fourier Transform attachment.

³The total field dispersion of the H-6, H-8 and H-5' protons at 60 MHz is 5.8 Hz, making unambiguous assignment of H-6 and H-8 impossible.

⁴Obtained using a Nicolet NT 360 spectrometer at the NSF Regional NMR Facility, University of Illinois at Urbana, Urbana-Champaign, IL.

⁵Partial assignments of the ^{13}C -nmr spectrum of **1** have been reported (9) as follows: δ 160.0 (C-2), 159.7 (C-2'), 157.0 (C-7), 155.0 (C-9), 144.1 (C-4'), 130.0 (C-5') and 113.5 (C-3'). The remaining signals were not assigned.

MHz, the 19 carbon atoms were clearly resolved in the proton noise decoupled spectrum, and the INEPT spectrum (30-32) afforded a substantial amount of information concerning the coupling of the individual carbons (Figure 2).⁴

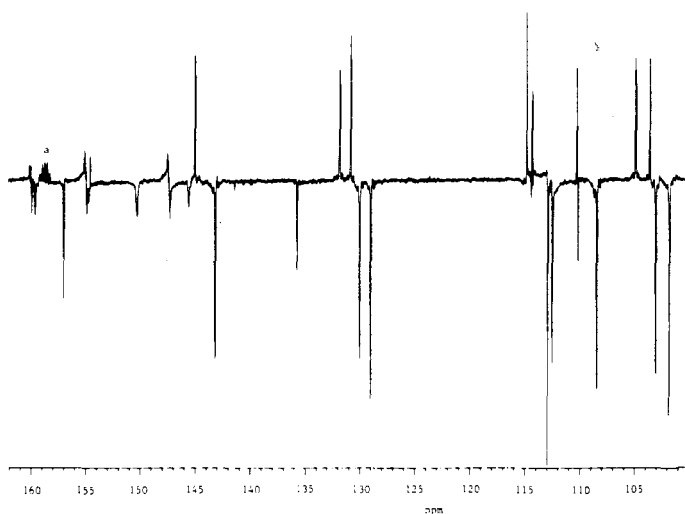


FIGURE 2. INEPT Spectrum of Daphnoretin (**1**) at 90.546 MHz (aromatic region only).

^a“ghost” from DMSO-*d*₆

By comparison with the established data for coumarins (33-35), a number of assignments could be made quite readily. For the purposes of discussion, the carbon atoms of daphnoretin (**1**) may be divided into three groups, (a) those bearing a proton, (b) those attached to oxygen, and (c) those attached only to carbon. Assignments for each of these groups of carbon atoms will be presented in turn.

(a) The INEPT spectrum clearly indicated those carbons bearing a proton (Figure 2) to be at δ 144.11, 130.95, 129.89, 113.81, 113.55, 109.39, 104.03, and 102.84. The signal at δ 113.81 displayed $^1J_{\text{CH}} = 173.9$ Hz permitting unambiguous assignment to C-3' (34), and because δ 104.03, but not δ 102.84, showed a $^3J_{\text{CH}} = 3.9$ Hz, these signals could be assigned to C-8' and C-8, respectively. The most downfield carbon bearing a proton, δ 144.11, was assigned to C-4' (33-35) and was confirmed when heteronuclear decoupling (LPSFORD) at δ 8.05 eliminated the $^1J_{\text{CH}}$ coupling. Irradiation at H-4 (δ 7.86) collapsed the doublet of doublets ($J = 4.9, 165.5$ Hz) at δ 130.95 to a doublet and thus should be C-4, which is further coupled only to H-5. The other signal (δ 129.89) in this region was established to be H-5' also through irradiation at the frequency of the attached proton, δ 7.70.

In the course of irradiating H-8' (δ 7.17), it was apparent that a number of other resonances not associated with H-8' had also been affected, presumably because of the very close chemical shift of H-5 (δ 7.20). However, this did permit the assignment of the resonance at δ 109.39 to C-5, leaving the doublet of doublets at δ 113.55 to be assigned to C-6'.

(b) Eight of the carbon atoms are attached to oxygen, and except for C-4' at δ 144.11, are all of the signals downfield of δ 135 ppm. The most downfield carbons were anticipated to be C-2 and C-2' (33-35), and they were easily distinguished because the signal at δ 160.07 was observed to be a doublet of doublets ($J = 9.9, 4.1$ Hz) and should be C-2', whereas δ 159.75 was a broad doublet ($J = 11.6$ Hz) as anticipated for C-2. Selective decoupling (LPSFORD) experiments irradiating H-4 (δ 7.87) and H-4' (δ 8.05) confirmed these assignments.

Irradiation at δ 7.87 (H-4) removed a number of small coupling constants resulting in some simplification, thus the narrow doublet ($^2J_{\text{CH}}=3.9$ Hz) could be assigned to C-3, and the ddd at δ 147.5 to C-9, tentatively. Since this same signal was also simplified on irradiation of H-5 and H-8, the assignment was confirmed. These irradiations also affected the rather diffuse signal at δ 145.75, which, on the basis of previously published work (34,35) should be C-6, bearing the methoxy group. On the other hand, C-9', being in a 7-substituted coumarin, should resonate at about δ 155 (34,35); indeed, the signal at δ 155.06 was a ddd that simplified on irradiation of either H-4', H-5' or H-8'.

A distinction between C-7' and C-7 was initially made on the basis of their anticipated chemical shift differences (34,35). Confirmation was achieved through irradiation at δ 7.21 (H-5) and δ 6.88 (H-8), which markedly enhanced the resonance at δ 150.44, and at δ 7.71 (H-5'), which simplified the signal at δ 157.07. These signals could therefore be assigned to C-7 and C-7', respectively.

(c) Only two of the carbons in daphnoretin are attached solely to carbon, C-10 and C-10', and may be distinguished on the basis of the complexity of their signals in the coupled spectrum. In this way, the broad signal at δ 114.45 was assigned to C-10' and the broadened doublet ($^3J_{\text{CH}}=4.8$ Hz) at δ 110.25 to C-10.

The observed and calculated values for daphnoretin (**1**) are shown in Table 2, and a number of discrepancies are immediately apparent. Thus, the observed value for C-7 is downfield of the calculated value, and the assignment of C-7 *vs.* C-6 may be questioned. However, it is well established (34,35) that C-7 in a 6,7-dioxygenated coumarin appears in the region 150-152 ppm and C-6 in the region 143-146 ppm.

TABLE 2. Observed and Calculated ^{13}C -Chemical Shifts of Daphnoretin (**1**)

Carbon	Observed Chemical Shift (δ , ppm)	Coupling Constant (J , Hz)	Calculated Chemical Shift (35)
C-2	159.75	11.6	158.5
C-3	135.74	3.9	137.5
C-4	130.95	165.5, 4.9	114.7
C-5	109.39	162.5, 3.8	109.5
C-6	145.75	3.3	145.9
C-7	150.44	6.8, 3.8	144.5
C-8	102.84	162.7	103.0
C-9	147.50	4.6, 5.6, 9.7	145.5
C-10	110.25	4.6	113.7
C-2'	160.07	4.0, 9.9	160.8
C-3'	113.81	173.9	112.7
C-4'	144.11	166.7, 4.5	143.3
C-5'	129.89	165.5, 3.4	128.7
C-6'	113.55	165.5, 4.6	112.1
C-7'	157.07	2.8, 7.8	162.6
C-8'	104.03	165.8, 3.9	100.6
C-9'	155.06	4.2, 5.3, 9.6	155.6
C-10'	114.45	complex	112.3
6'-OCH ₃	56.00	144.4	

Actually, C-4 appears 16 ppm further downfield than predicted, although inspection of the data reveals that this estimated value is based on only one prior observation (35).

A more serious concern involves the relative chemical shifts of C-10 and C-10', but as previously discussed, the coupling observed for these two signals justifies the assignments indicated. Both C-7' and C-8' have also undergone fairly substantial shifts, the

former upfield by 5.6 ppm, and the latter downfield by 3.4 ppm. In these cases, calculated values were obtained through the use of a substituent effect for a methoxy group (35), and this probably is inadequate to account for the interaction between the two nuclei.⁶

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⁶See, for example, the ¹³C-substituent effects of -OCH₃ and OPH for C₆H₆ derivatives (36).