Tetrahedron Letters,Vol.28,No.32,pp 3655-3658,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

USE OF THE SELECTIVE INEPT NMR TECHNIQUE IN THE STRUCTURE ELUCIDATION OF (+)-AFZELECHIN-7-O-β-D-APIOSIDE, A BITTER PRINCIPLE OF POLYPODIUM GLYCYRRHIZA

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Summary: The position of sugar attachment of a novel bitter flavonoid glycoside, (+)-afzelechin-7-O-β-D-apioside, was conveniently established by the application of selective insensitive nuclei enhanced by polarization transfer (INEPT) NMR experiments.

The rhizomes of the fern Polypodium glycyrrhiza D.C. Eaton (Polypodiaceae) exhibit a bittersweet taste[1], and have a history of human consumption both for use as a food and medicinal agent, and during certain religious ceremonies in the Pacific northwest region of North America[2]. From a 1-butanol extract of this plant part a novel bitter flavonoid glycoside, (+)-afzelechin-7-O-β-D-apioside (**1**), was isolated by repeated chromatography over silica gel and Sephadex LH-20[3]. By high-resolution electron-impact mass spectrometry, the elemental formula of **1** was determined as $C_{20}H_{22}O_9$[4]. Assignments of the ^{1}H-NMR and ^{13}C-NMR spectra of **1**[5] were carried out with the use of ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR 2D-NMR studies. In the ^{1}H-NMR spectrum, the doublet of doublets resonating at δ 2.71 and δ 2.42 (1H each) and the doublet at δ 4.60 (J = 7.5 Hz) indicated **1** to be a flavan-3-ol derivative with trans-substitution (2R,3S) of ring C[6]. Furthermore, two 2H doublets centered at δ 7.16 and δ 6.75 (J = 8.5 Hz) were suggestive of 4'-monosubstitution in ring B of **1**. Chemical shifts in the ^{13}C-NMR spectrum supported the assignment of **1** as a flavan-3-ol type of compound[7], and, in addition, provided evidence that the saccharide moiety of the compound was D-apiose[8]. Hydrolysis of **1** afforded (+)-afzelechin and D-apiose, which were identified by direct comparison with authentic samples[9].

Conventional methods to determine the position of attachment of a sugar moiety to a flavonoid aglycone have involved methylation and ^{1}H- or ^{13}C-NMR analysis[10], which are either time-consuming or can lead to ambiguous results. In this investigation, the position of sugar attachment of **1** was determined by a selective INEPT NMR experiments on the intact heteroside. The selective INEPT technique was developed recently[11], and is used to determine connectivity in the molecule by emphasizing three-bond C-H couplings[12]. When 1"-H of **1** was irradiated at δ 5.34 (Δ_1 and Δ_2 for J = 6 Hz), only the C-7 and C-4" carbons were enhanced in the resultant polarization-transfer spectrum (Fig. 1d)[13]. Analogous irradiations of the $4_{equatorial}$- (δ 2.71) and 2β - (δ 4.60) protons of **1** selectively enhanced carbons 5 and 9 (Fig. 1b) and carbons 9, 1', 2', and 6' (Fig. 1c), respectively. Use of this technique therefore demonstrated that the carbon of attachment of D-apiose in the aglycone of **1** was C-7, and also enabled the assignment of all quaternary-carbon chemical shifts in the ^{13}C-NMR spectrum of this compound. The sugar attachment was determined as

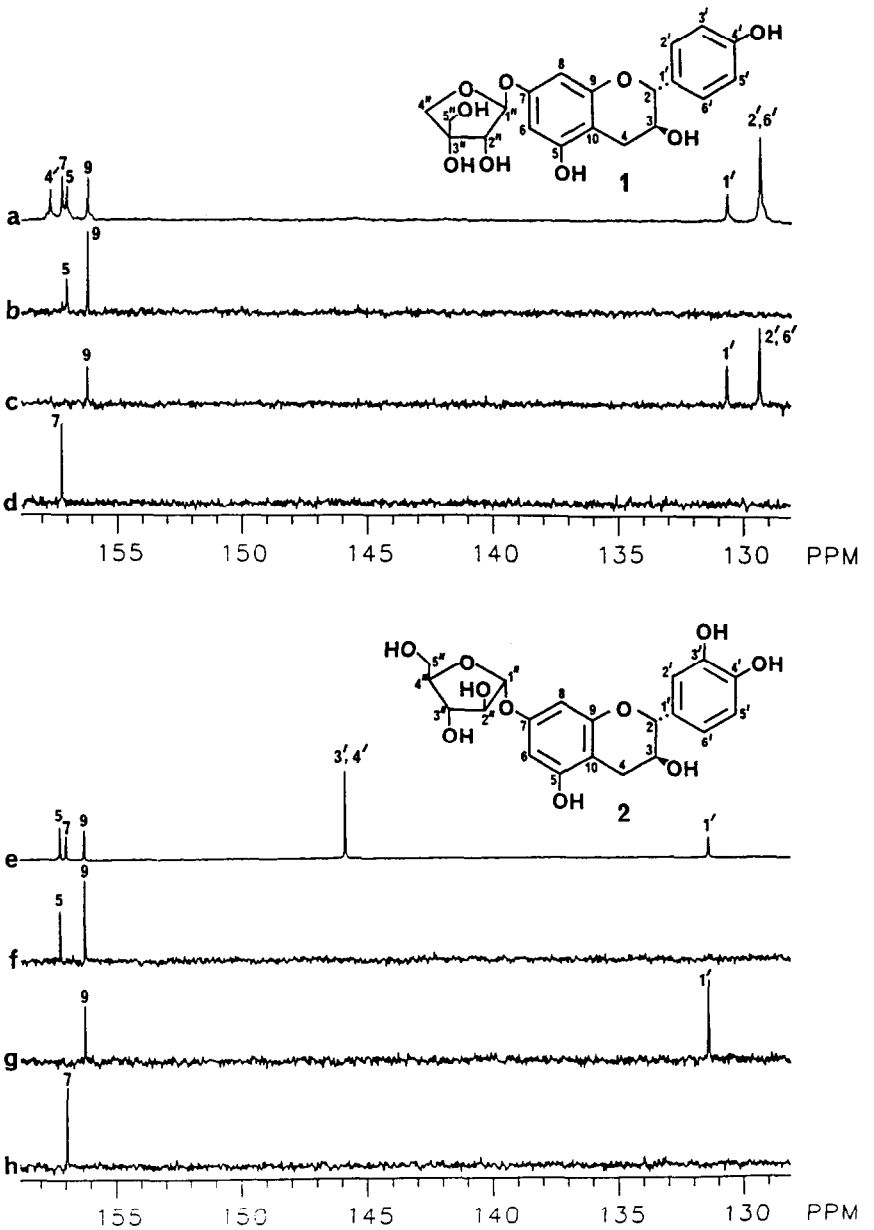

Figure 1. Downfield regions of the ^{13}C-NMR spectra of afzelechin apioside (**1**) (a-d) and polydin (**2**) (e-h). (a,e) Proton-noise decoupled spectra; (b-d; f-h) selective INEPT spectra obtained by irradiation of $^{4}_{eq}$-H, 2-H, and 1"-H, respectively.

β- by the application of Klyne's rule[14]. The structure elucidation of **1** using the selective INEPT technique was completed in only two hours using about 40 mg of material. Compound **1** represents the first isolation of a glycoside of (+)-afzelechin from a natural source.

In order to confirm the methodology proposed herein, we have also investigated a neutral-tasting constituent of P. glycyrrhiza rhizomes using the selective INEPT technique, namely, polydin (**2**), which has the known structure, (+)-catechin-7-O-α-L-rhamnoside[15]. Irradiation of H-1" of **2** at δ 5.27 (Δ_1 and Δ_2, J = 6 Hz) enhanced only the one expected carbon on the aglycone, carbon 7 (Fig. 1h), while similar irradiations of H-4_{eq} (δ 2.66) and H-2β (δ 4.53) again enhanced carbons two or three bonds distant from the sites of irradiation (Fig. 1f and g). The selective INEPT experiment also allowed unambiguous assignments to be made for the first time of quaternary carbons C-5 and C-7 in the ^{13}C-NMR spectra of **2**[16] and its aglycone, (+)-catechin[17]. The present method offers an alternative for the establishment of glycoside sugar attachment to a 2D-COSY NMR procedure, utilizing long-range couplings, that was recently used to confirm the structure of a flavonoid constituent of Gutierrezia grandis[18].

Acknowledgment: This work was supported, in part, by contract N01-DE-02425 with the National Institute of Dental Research, NIH, Bethesda, Maryland. The authors thank the Research Resources Center, University of Illinois at Chicago, for provision of NMR facilities, and Ms. M. Sitt, for typing this manuscript.

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2. Turner, N.C.; Bell, M.A.M. Econ. Bot., **1973**, 27, 257.
3. Compound **1** was obtained as a colorless powder from $CHCl_3$, mp 135-137°; $[\alpha]_D$ -93.3° (c 0.12, MeOH); UV, λ_{max} (MeOH) 274 (log ε 4.06), 224 sh (5.77) nm; IR, ν_{max} (KBr) 3430, 1627, 1618, 1599, 1516, 1145, 1030 cm^{-1}.
4. HR-MS of **1**: Found 406.1270, cald. for $C_{20}H_{22}O_9$, 406.1263. EI-MS (80 eV) 406 (M^+, 2%), 274 (16), 139 (74), 136 (25), 107 (32), 84 (19), 66 (20), 44 (100).
5. ^{1}H-NMR of **1**: (360 MHz, DMSO-d_6) δ 9.59, 9.49 (phenolic OH), 7.16 (2H, d, J = 8.5 Hz, 2'-, 6'-H), 6.75 (2H, d, J = 8.5 Hz, 3'-, 5'-H), 6.10 (1H, d, J = 2.2 Hz, 8-H), 5.91 (1H, d, J = 2.2 Hz, 6-H), 5.34 (1H, d, J = 4 Hz, 1"-H), 4.60 (1H, d, J = 7.5 Hz, 2-H), 4.03 (1H, m, 2"-H), 4.00, 3.69 (2H, AB system, J_{AB} = 9.4 Hz, 4"-H_2), 3.90 (1H, m, 3-H), 3.37 (2H, m, 5"-H_2), 2.71 (1H, dd, J = 16, 5.5 Hz, 4_{eq}-H), 2.42 (1H, dd, J = 16, 8.1 Hz, 4_{ax}-H); ^{13}C-NMR of **1**: (90.8 MHz, DMSO-d_6) δ 156.77 (s, 4'), 156.16 (s, 7), 156.08 (s, 5), 155.15 (s, 9), 129.58 (s, 1'), 128.29 (d, 2',6'), 114.69 (d, 3',5'), 106.78 (d, 1"), 101.68 (s, 10), 95.62 (d, 8), 94.74 (d, 6), 80.88 (d, 2), 78.38 (s, 3"), 75.82 (d, 2"), 73.84 (t, 4"), 65.89 (d, 3), 62.13 (t, 5"), 27.85 (t, 4).
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9. Acid hydrolysis of 1 was carried out with 5% acetic acid at 100 °C for 2.5 hrs, and the products were compared with (+)-afzelechin from Dr. F. Delle Monache, Università Cattolica del Sacro Cuore, Rome, Italy, and with D-apiose generated in situ from frangulin B obtained from Prof. Dr. H. Wagner, University of Munich, Munich, W. Germany.

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16. ^{13}C-NMR of polydin (2): (90.8 MHz, DMSO-d_6) δ 156.07 (s, 5), 155.83 (s, 7), 155.11 (s, 9), 145.71 (s, 3',4'), 130.25 (s, 1'), 118.22 (d, 6'), 115.00 (d, 5'), 114.24 (d, 2'), 106.01 (d, 1''), 101.59 (s, 10), 95.86 (d, 8), 94.76 (d, 6), 84.51 (d, 4''), 81.95 (d, 2''), 80.90 (d, 2), 76.33 (d, 3''), 65.92 (d, 3), 61.03 (t, 5''), 27.47 (t, 4).

17. ^{13}C-NMR of (+)-catechin (commercial sample): (90.8 MHz, DMSO-d_6) δ 156.23 (s, 7), 155.98 (s, 5), 155.15 (s, 9), 144.63 (s, 3',4'), 130.38 (s, 1'), 118.26 (d, 6'), 114.89 (d, 5'), 114.29 (d, 2'), 98.86 (s, 10), 94.91 (d, 8), 93.66 (d, 6), 80.79 (d, 2), 66.13 (d, 3), 27.67 (t, 4). For earlier assignments, see Wenkert, E.; Gottlieb, H.E. Phytochemistry **1977**, 16, 1811, and Refs. 10a and 14.

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(Received in USA 7 May 1987)