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NMR ASSIGNMENT OF BRAZILEIN

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Abstract—The ^1H and ^{13}C NMR signals of brazilein from *Caesalpinia sappan* were assigned by HMBC correlations and the physicochemical data investigated. © 1997 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Sappan Lignum, the heart wood of *Caesalpinia sappan* [1], is used in traditional Chinese medicine as an analgesic and antiinflammatory agent for the treatment of traumatic disease and menstrual disorders.

Brazilin [(6a*S*-*cis*)-7,11b-dihydrobenz[*b*]indeno[1,2-*d*]pyran-3,6a,9,10,(6*H*)-tetrol], the main constituent of Sappan Lignum, has been used as a dye and was found to have a hypoglycemic [2] and antiinflammatory action [3] in experimental animals. Brazilin is oxidized to produce brazilein (6a,7-dihydro-3,6a,10-trihydroxy-benz[*b*]indeno[1,2-*d*]pyran-9(6*H*)-one) by air and light. Micovic and Robinson [4] established the position of the quinonoid group in *O*-trimethyl brazilein and it might plausibly be assumed that brazilein was analogously constituted; however, the full assignment has not yet been directly confirmed. The present work reports on the physicochemical NMR data of brazilein isolated from the methanol extract of the heart wood of *C. sappan*.

RESULTS AND DISCUSSION

Brazilein was isolated as reddish brown crystals (methanol–water). Most of it seemed to be produced during storage and in the process of extraction and isolation. When the extract was placed in air and light, surprisingly large amounts of brazilein were produced (*ca* 8% of the extract after 1 month).

The EI mass spectrum of brazilein showed a $[M]^+$ at *m/z* 284. The UV-visible spectrum showed prominent maximum absorptions at 445 and 556 nm, which were red-shifted by increasing conjugation, compared with brazilin. The optical specific rotation value of brazilein was large, $[\alpha]_D^{20}$ −1012° (DMSO; *c* 0.80) and −700° (MeOH; *c* 0.10).

Table 1. ^1H NMR data of brazilein and brazilin (400 MHz)*

^1H	Brazilein (δ, DMSO-d_6)	Brazilin (δ, acetone-d_6)†
1	7.91, 1H, *d*, *J* = 8.8	7.35, 1H, *d*, *J* = 8.3
2	6.67, 1H, *dd*, *J* = 8.8, 2.3	6.66, 1H, *dd*, *J* = 8.3, 2.4
4	6.47, 1H, *d*, *J* = 2.3	6.46, 1H, *d*, *J* = 2.4
6	4.10, 1H, *d*, *J* = 11.8	3.87, 1H, *d*, *J* = 11.2
	4.56, 1H, *d*, *J* = 11.8	4.10, 1H, *d*, *J* = 11.2
7	2.95, 2H, *s*	2.97, 1H, *d*, *J* = 15.7
		3.18, 1H, *d*, *J* = 15.7
8	6.43, 1H, *s*	6.81, 1H, *s*
11	7.22, 1H, *s*	6.92, 1H, *s*
12	—	4.14, 1H, *s*

*Chemical shifts (δ) expressed relative to TMS.
†Brazilin was assigned by ref. [5].

The ^1H and ^{13}C NMR spectral features of brazilein were similar to those of brazilin, but the data for brazilin is not useful for the signal assignment of brazilein because of the similarity of various olefinic carbons (Tables 1 and 2).

The ^1H NMR spectrum of brazilein exhibited five olefinic methine proton signals and two methylene signals, δ 4.10 (1H) and 4.56 (1H) and δ 2.95 (2H), which were assigned to H-6 and H-7, respectively, with ease. The olefinic proton signal at δ 6.67 was coupled with the signal at δ 7.91 (J = 8.8 Hz) and was *meta*-coupled with one at δ 6.47 (J = 2.3 Hz). Therefore, these three olefinic signals were assigned to H-2, H-1 and H-4, respectively.

H-8 and H-11 were assigned by HMBC correlations as shown in Fig. 1. A carbonyl carbon resonance at δ 79.4 was correlated only to a olefinic proton resonance at δ 7.22 in the HMBC spectrum. Therefore, the proton resonance at δ 7.22 could be ascribed to either H-8 or H-11. However, by detailed observation of

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Table 2. ^{13}C NMR data of brazilein and brazilin (100 MHz)*

^{13}C	Brazilein (δ, DMSO-d_6)	Brazilin (δ, acetone-d_6)†
1	130.6	132.3
1a	110.9	115.8
2	110.9	110.0
3	162.2	155.8
4	102.9	104.3
4a	157.8	157.9
6	73.0	71.0
6a	74.3	78.1
7	39.7	43.1
7a	159.0	131.7
8	117.6	113.0
9	179.4	145.4
10	152.4	145.1
11	104.2	112.6
11a	126.1	137.6
12	151.7	51.3

*Chemical shifts (δ) expressed relative to TMS.
†Brazilin was assigned by ref. [5].

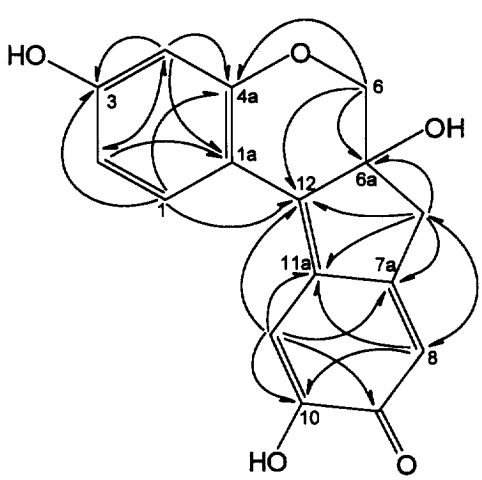

Fig. 1. HMBC correlations of brazilein.

correlations in the HMBC spectrum, the proton resonance was assigned to H-11 because it was long-range-correlated to C-12 at δ 151.65 which was correlated with H-1 at δ 7.91, H-6 at δ 4.10, H-6′ at δ 4.56 and H-7 at δ 2.95. Consequently, the other olefinic proton resonance at δ 6.43 was assigned as H-8, which was correlated with resonances at δ 39.70 (C-7) and δ 152.40 (C-10) in the HMBC spectrum.

The corresponding carbon signals for each proton signal were identified by a ^1H-^{13}C one-bond chemical shift correlation experiment (HMQC). Both of the quaternary carbon resonances at δ 126.1 (C-11a) and at δ 159.0 (C-7a) were correlated with H-11 and H-7, but H-8 was correlated not with the resonance at δ 159.0 but with the resonance at δ 126.1. The long-range C–H coupling was not or just weakly correlated through two bond distances, but strongly correlated through three bonds between proton and carbon in the phenyl ring systems under this experimental condition. The cross-peak between the carbon resonance at δ 126.1 (C-11a) and the proton resonance at δ H-11 was very weak. Finally, it was concluded that the carbon resonance at δ 126.1 could be ascribed to C-11a and the carbon resonance at δ 159.0 to C-7a. From the remaining correlations in the HMBC spectrum, together with the above-mentioned spectral findings, all carbon signals were assigned as shown in Table 2.

EXPERIMENTAL

General. Mp: uncorrs. ^1H (400 MHz) and ^{13}C NMR (100 MHz): Bruker AMX 400. Chromatography: silica gel [Kieselgel 60 (70–230 mesh), Merck], Sephadex LH-20 (Pharmacia) and cellulose (Avicel, Merck).

Extraction and isolation. Dried heartwood (100 g), purchased in Taejon, Korea, was extracted with MeOH (every 3 days at room temp. × 3). The MeOH extract was concd under red. pres. and partitioned between H_2O–MeOH (3:1, 200 ml) and EtOAc (100 ml × 3). The EtOAc extract was concd and applied to a silica gel column eluting with $CHCl_3$–MeOH (15:1 to 5:1), to afford frs containing brazilein (1.7 g) and brazilin (1.5 g), respectively. The brazilein-containing fr. was chromatographed on silica gel eluting with $CHCl_3$–MeOH–H_2O (10:3:1) to afford crude brazilein (730 mg). Crude brazilein (700 mg) was dissolved in hot MeOH (200 ml), concd to *ca* 40 ml, allowed to stand overnight at room temp. and filtered to afford purified brazilein (470 mg). The brazilin-containing fr. was chromatographed on Sephadex LH-20 eluting with MeOH–H_2O (9:1) and subsequently on cellulose eluting with EtOAc. Recrystallized from MeOH–H_2O afforded brazilin (680 mg).

Brazilein. Reddish-brown crystals (MeOH–H_2O), mp 260–265° (dec.). $[\alpha]_D^{20}$ −1012° (DMSO; *c* 0.80); −700.0° (MeOH; *c* 0.10). UV-Vis.: λ_{max}^{DMSO} nm (log ε): 276 (3.82), 445 (4.42), 556 (4.81). EIMS (*m/z*): 284 $[M]^+$. ^1H NMR (400 MHz, DMSO-d_6): Table 1. ^{13}C NMR (100 MHz, DMSO-d_6): Table 2.

Brazilin. Amber-yellow crystals (MeOH–H_2O), mp 145–149°. $[\alpha]_D^{0}$ +80.0° (DMSO; *c* 0.75). UV-Vis.: λ_{max}^{DMSO} nm (log ε): 292 (3.81). EIMS (*m/z*): 286 $[M]^+$. ^1H NMR (400 MHz, acetone-d_6): Table 1. ^{13}C NMR (100 MHz, acetone-d_6): Table 2.

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