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ANTIOXIDANT STUDY AND ASSIGNMENTS OF NMR SPECTRAL DATA FOR 3′,4′,7-TRIHYDROXYFLAVANONE 3′,7-DI-O-β-D-GLUCOPYRANOSIDE (BUTRIN) AND ITS HYDROLYZED PRODUCT

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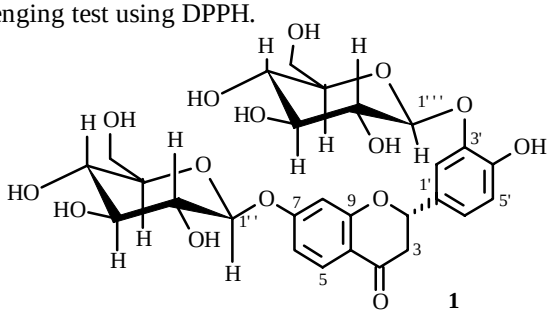
The NMR spectral data including high resolution ^1H, ^{13}C and 2D NMR for butrin, 3′,4′,7-trihydroxyflavanone 3′,7-di-O-β-D-glucopyranoside, isolated from flowers of Butea monosperma, *are reported here for the first time. Butrin was hydrolyzed using b-glucosidase to butin in high yield. They were subjected to free radical scavenging test using 2,2-diphenyl-1-picrylhydrazyl (DPPH) spectrophotometric assay. At a dose of 4×10^{-8} mol of tested compounds, the percentage of reduced DPPH for butin was 14.5% while no reduction was observed for butrin (0%).*

Key words: Butrin, butin, antioxidant, 3′,4′,7-trihydroxyflavanone 3′,7-di-O-β-D-glucopyranoside, *Butea monosperma*, NMR spectra.

Butrin, 3′,4′,7-trihydroxyflavanone 3′,7-di-O-β-D-glucopyranoside (**1**), is a major flavonoid found in *Butea monosperma* [1, 2], *B. frondosa* [3, 4], and *B. superba* [5]. *Butea monosperma* is used in India as a medicinal plant to cure hepatic disorders and viral hepatitis [2]. Isobutrin and butrin (**1**) were reported as the antihepatotoxic components in a butanolic fraction of methanolic extracts of the flower of *B. monosperma* [2]. Despite the early identification of this compound [3], to the best of our knowledge there is no report on its ^{13}C NMR and high resolution 2D NMR in the literature. In this paper we report the detailed NMR assignment of butrin. Butrin is hydrolyzed completely to butin (**2**) and their antioxidant activities were assayed by radical scavenging test using DPPH.



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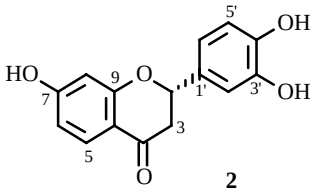

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Butrin was separated as yellow needles from the flowers of *Butea monosperma*. The UV and FAB negative mass spectra were similar to those reported previously [2]. The high resolution ^1H NMR spectrum recorded on a 500 MHz instrument is presented in Table 1. The ^{13}C NMR spectral data are assigned with the help of 2D NMR spectra including HMQC and HMBC spectra. In the ^1H NMR spectrum (in DMSO-d_6) the signals at δ 5.45 (2H, bdd, J = 2.2, 12.5 Hz, H-2, OH-3″ or OH-3‴), 2.67 (1H, dd, J = 2.2, 14.5 H-3β), and 3.2 (m, H-3α) represented the C ring proton signals for a flavanone structure and the signals at δ 7.70 (1H, d, J = 8.6 Hz, H-5), 6.70 (1H, bd, J = 8.6 Hz, H-6), and 6.66 (1H, bs, H-8) are assigned for the A ring. The signals at δ 7.28 (1H, bs, H-2′), 6.83 (1H, d, J = 8.3 Hz, H-5′), and 7.02 (1H, bd, J = 8.3 Hz, H-6′) represent the 3′, 4′ di-substituted B ring. The anomeric protons at 4.97 (1H, d, J = 7.4 Hz) and 4.71 (1H, d, J = 7.1 Hz) represent two β-glycosidic bonds.

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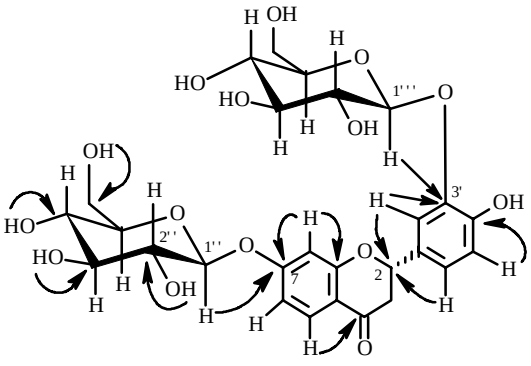
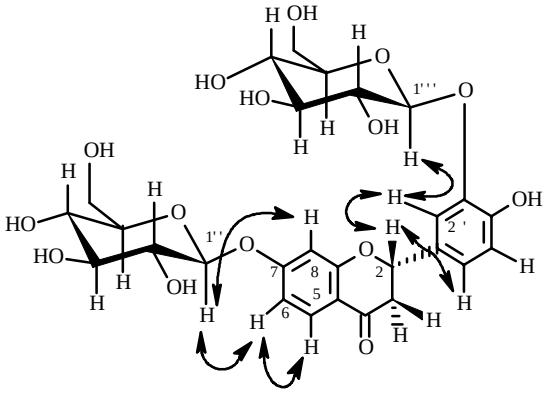
TABLE 1. ^{1}H and ^{13}C NMR Spectral Data for Butrin (δ, ppm, J/Hz)

C atom	H (in DMSO-d_6)	H (in DMSO-d_6+D_2O)	C (in DMSO-d_6)
2	5.45 (bdd, J = 2,2, 12.5)	5.44 (d, J = 2.2, 12.5)	79.2 (CH)
3α	3.2 (m)	3.10-3.45 (m)	42.9 (CH_2)
3β	2.67 (bdd, J = 2.2, 15.5)	2.66 (dd, J = 2.5, 17.5)	
4	-	-	190.4
5	7.70 (d, J = 8.6)	7.69 (d, J = 8.6)	127.9 (CH)
6	6.70 (bd, J = 8.6)	6.70 (dd, J = 2.3, 8.6)	110.9 (CH)
7	-	-	162.8
8	6.66 (bs)	6.62 (d, J = 2.3)	103.5 (CH)
9	-	-	163.5
10	-	-	115.7
1′	-	-	129.7
2′	7.28 (bs)	7.24 (d, J = 1.6)	115.4 (CH)
3′	-	-	145.1
4′	-	-	147.1
5′	6.83 (d, J = 8.3)	6.82 (d, J = 8.2)	115.3 (CH)
6′	7.02 (bd, J = 8.3)	7.00 (dd, J = 1.6, 8.2)	121.7 (CH)
1″	4.97 (d, J = 7.4)	4.94 (d, J = 7.3)	99.7 (CH)
1‴	4.71 (d, J = 7.1)	4.71 (d, J = 7.3)	101.3 (CH)
2″,2‴	3.25 (m)	3.10-3.45 (m)	73.3, 73.1 (CH)
3″,3‴	3.30 (m)	3.10-3.45 (m)	76.4, 76.0 (CH)
4″,4‴	3.30 (m)	3.10-3.45 (m)	77.2, 77.0 (CH)
5″,5‴	3.15 (m)	3.10-3.45 (m)	69.9, 69.4 (CH)
6″,6‴	3.68 (m), 3.43 (m)	3.10-3.45 (m), ca 3.7 (m)	60.7, 60.5 (CH_2)
OH-2″,2‴	5.02 (d, J = 4.4)	-	
OH-3″,3‴	5.35 (bd, J = 4.2), 5.45 (bd)	-	
OH-4″,4‴	5.09 (bd)	-	
OH-6″,6‴	4.54 (t, J = 5.6), 4.59 (t, J = 5.4)	-	
OH-4′	8.78 (bs)	-	



The important HMBC correlations for Butrin.



The important ROESY correlations for Butrin.

Fig. 1. The HMBC and ROESY correlations of Butrin.

The relative position of the sugar parts were deduced according to HMBC and ROESY spectral data (Fig. 1). In the HMBC spectrum the cross peaks between H-1″ and C-7 and H-1‴ and C-3′ suggested the position of the sugar parts at C-7 and C-3′ respectively. On the other hand the ROESY spectrum showed the cross peaks between H-1″ and H-6 and H-8, and H-1‴ and H-2′ which confirmed the position of the glucosidation at C-7 and C-3′ respectively. Table 1 shows the chemical shift values of the ^{13}C NMR spectrum of butrin; the correlation of ^{1}H and ^{13}C NMR signals was achieved by interpretation of its HMQC spectrum.

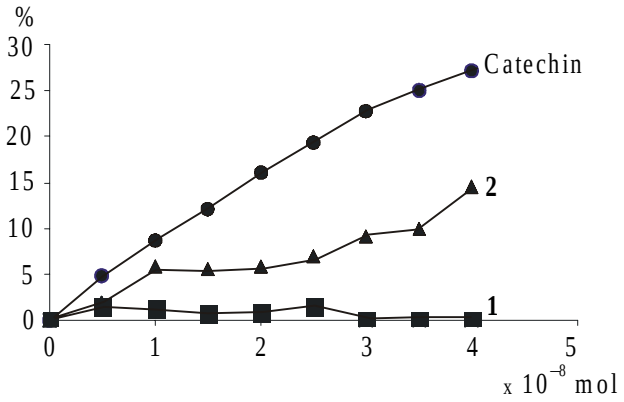


Fig. 2. Scavenging activity of butrin and butin on DPPH radical. The DPPH reduction percentage is proportional to the amount of initial radical scavengers added to the DPPH solution.

The ^{1}H NMR spectrum of **1** was well resolved after addition of a few drops of D_2O to the DMSO-d_6 solution (Table 1). The H-2 signal at δ 5.45 that overlapped with OH-3″ and OH-3‴ was simplified to a double doublet at 5.44 (J = 2.5, 12.5 Hz).

To confirm the stereochemistry at C-2, butrin (**1**) (6 mg) was hydrolyzed with *β*-glycosidase, which gave butin (**2**) and glucose. The ^{1}H NMR spectrum and optical rotation of the aglycone was measured in acetone-d_6 and methanol respectively and compared to those reported in the literature for (–)-butin [6, 7]. The presence of the glucose was confirmed by co-TLC on silica gel using a mobile phase, acetone: 2 mM sodium acetate (17: 3), R_f 0.52 visualized by thymol reagent.

The free radical scavenging activity of compounds **1** and **2** was measured by the method of Blois [8] and compared to the standard compound, (+)-catechin. Figure 2 compares the activity of **1**, **2**, and standard antioxidant, (+)-catechin. At a dose of 4×10^{-8} mol of tested compounds the percentage of reduced DPPH for 2 and (+)-catechin were 14.5 and 27.1% respectively while no reduction was observed for **1** (0%). This indicates that the antioxidant activity of **2** is mostly due to the free 3′ and 4′-hydroxy groups in the C ring, and glucosidation at one of them decreased the activity [9, 10].

EXPERIMENTAL

General Procedures: ^{13}C NMR spectra (broad band and DEPT experiments) were measured on a JEOL, JNM-EX270 instrument at 67.5 MHz. ^{1}H NMR, ^{1}H-^{1}H COSY, ROESY, HMQC, and HMBC experiments were performed on a 500 MHz Bruker AMX500. Sample concentration was 10 mg per 0.6 ml DMSO-d_6 and 2.8 mg per 0.6 ml acetone-d_6 for compounds **1** and **2**, respectively. MS spectra and optical rotations were recorded respectively on a JEOL JMS-SX102A spectrometer and a JASCO DIP-370 digital polarimeter. The absorptions of DPPH solutions were measured at 517 nm on a Spectronic 20D+ UV/VIS spectrophotometer. (+)-Catechin and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained commercially from Extrasynthese and Aldrich chemical companies, respectively.

Isolation of Butrin. *Butea monosperma* (Lam.) Kuntze was collected from Tonk district of Rajasthan-India on March 2001. The plant material was identified by Prof. P. S. Jain at the Department of Botany, University of Rajasthan, Jaipur. A voucher specimen, with herbarium number RUBL 19871, has been deposited in the herbarium of the same university. The air-dried flowers of the plant were extracted with hot methanol. Butrin was separated from the methanolic extract, after concentration followed by recrystallization from methanol, as pale yellow needles, m.p. 190–191°C, $[\alpha]_D^{21}$ –73° (c, 1 in py), lit., $[\alpha]_D^{30}$ –81.7° (c, 1.681 in py) [11].

Hydrolysis of Butrin. Butrin (6 mg) was dissolved in 12 ml distilled water and incubated with about 0.5 mg of *β*-glucosidase from almond (Sigma Co) at 25° for 3 hr. The water layer was concentrated and extracted with *n*-butanol to separate the aglycone, which was identified by ^{1}H NMR and optical rotation as (–)-butin (2.8 mg), $[\alpha]_D^{22}$ –36.1° (c, 1 in MeOH), lit.,$[\alpha]_D^{30}$ –18.7° (c, 0.5 in MeOH) [7].

^{1}H NMR spectral data of butin (270 MHz, in acetone-d_6, δ, ppm, J/Hz): 7.71 (1H, d, J = 8.6, H-5), 7.03 (1H, d, J = 1.3, H-2′), 6.86 (2H, m, H-5′, 6′), 6.56 (1H, dd, J = 2.3, 8.6, H-6), 6.41 (1H, d, J = 2.3, H-8), 5.39 (1H, dd, J = 3.0, 12.5, H-2), 3.00 (1H, dd, J = 12.5, 16.9, H-3α), 2.67 (1H, dd, J = 3.0, 16.9, H-3β).

Determination of the Free Radical Scavenging Activity of 1 and 2 by Spectrophotometric Method. To 5 ml of a 100 mM solution of DPPH in methanol was added 50–400 ml of a solution of (100 mM) compounds **1** and **2** and (+)-catechin. After 30 min shaking of the solutions under dark conditions, the absorptions of the test solutions were measured at 517 nm. Figure 2 shows the results of correlation between the percentage of DPPH reduction after addition of the radical scavenging compounds and the initial amount of the antioxidants ($\times 10^{-8}$ mol). The percentage of the reduced DPPH was calculated by the following equation:

$$\text{Percentage of reduced DPPH} = [(A_0 - A_1)/A_0] \times 100,$$

where A_0 is the absorbance of the control (5 ml DPPH solution + 50–400 ml methanol), and A_1 is the absorbance in the presence of sample.

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