SPECTROSCOPIC DETERMINATION OF A PURE ORGANIC COMPOUND ISOLATED FROM THE STEM BARK OF *Butea monosperma* (LAM.) KUNTZE

Soe Tun Myaing[1], Thein Tun Aung[2], Arnt Win[3]

Abstract

One of Myanmar indigenous medicinal plants, *Butea monosperma* (Lam.) Kuntze, Myanmar name (Pouk) was chosen for chemical analysis. A pure organic compound, pale yellow needle shape compound was isolated from the stem bark of Pouk by applying advanced separation methods such as, Thin-Layer and Column Chromatographic methods. This pure compound was checked by phytochemical test which gave rise to positive for flavonoid test. The yield percent of this pure compound was found to be 0.63 % based upon the ethyl acetate crude extract. The molecular formula of the pure compound was determined as $C_{31}H_{20}O_{10}$ by using some spectroscopic techniques, such as FT-IR, ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), DEPT, HMQC and FAB-mass spectral data respectively. Hydrogen deficiency index of this compound is 22. Finally, the complete structure of pure compound was elucidated by applying DQF-COSY, ^{1}H NMR splitting patterns, coupling constant (J values) and HMBC spectroscopic studies.

Keywords : *Butea monosperma* (Lam.) Kuntze, thin-layer and column chromatographic methods, spectroscopic techniques

Introduction

Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people. They serve as therapeutic agents as well as raw materials for the manufacture of traditional and modern medicine. Substantial amount of foreign exchange can be earned by exporting medicinal plants to other countries. In

[1] Lecturer, Dr.,Department of Chemistry, Magway University
[2] Lecturer, Dr.,Department of Chemistry, Mandalay University of Distance Education
[3] Associate Professor, Dr.,Department of Chemistry, Kyaukse University

this way indigenous medicinal plants play significant role of an economy of a country (Sofowora, 1982).

People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health, but they may not understand the science behind these medicines, but know some medicinal plants are highly effective only when used at therapeutic doses. Traditional use of medicine is recognized as a way to learn about potential future medicines. Researchers have identified number of compounds used in mainstream medicine which were derived from ′ ethnomedical′ plant sources(Elezabeth and Subramanian, 2013).

Butea monosperma (Pouk) is a medium-sized deciduous tree belonging to family Fabaceae . They comprise one of the largest families of flowering plants numbering 630 genera and 18000 species (The Wealth of India, 1988) . This tree grows up to 50 ft high ,with clusters of flowers. *Butea monosperma* is used as tonic, astringent , aphrodisiac and diuretics . All parts of plant have been used as crude drug for the treatment of tumors, piles, skin diseases, wounds and ulcers. The stem bark is used for the treatment of dyspepsia, diarrhoea and dysentery. Stem bark powder is used to apply on injury caused due to axe. Paste of stem bark is applied in case of body swelling (Patil *et al*., 2006). It is also used for the cure of ulcer, sore throat and snake bite (Fageria and Rao,2015**).**

This study aimed to isolate a pure organic compound from stem bark of *Butea monosperma* (Lam.) Kuntze , locally known as Pouk and to elucidate its structure.

Materials and Methods

Commercial grade reagents from; BDH, MERCK, etc, were used in this research work. Analytical and preparative TLC was performed by using precoated silica gel plates (Merck Co Inc., Kiesel gel $60F_{254}$). Silica gel (70 to 230 Mesh ASTM) was used for column chromatography.

The following advanced instruments were used in the characterization of the samples and elucidation of the pure compound.

1. UV-lamp (Lambda-40, Perkin Elmer Co. England)
2. FT IR Spectrophotometer, Shimadzu, Japan
3. ^{1}H NMR Spectrometer, 500 MHz, Japan
4. ^{13}C NMR Spectrometer, 125 MHz, Japan
5. FAB-mass Spectrometer

Sample Collection

The stem barks of Pouk (Figure1) were collected from Khone Su village, Minbu (Sagu) Township, Magway Region. The samples were cut into small pieces and allowed to dry in air. Then the dried pieces were stored in a well-stoppered bottle and used throughout the experiment.

Figure 1. The plant and stem bark of *Butea monosperma* (Lam.) Kuntze

Extraction and Isolation of a Pure Compound

The air dried stem bark sample (1150 g) was percolated with 5 L of 95 % ethanol for two months. The percolated solution was evaporated and then extracted with (350 mL) of ethyl acetate. When ethyl acetate extract was

concentrated, the crude sample (5.87 g) was obtained. This extract (2.94 g) was taken and chromatographed on a silica gel column, eluting with n-hexane and ethyl acetate with various ratios from non-polar to polar to produce (324) fractions. Each and every fractions were checked by TLC. The same R_f value fractions were combined. Thirteen combined fractions were obtained. Then, the combined fraction (X) was found to be main fraction which showed only one spot on TLC and UV active. It was recrystallized by 30 % EtOAc in n-hexane. The yield percent of this pale yellow needle shape pure compound is found to be 0.63 % (18.5 mg) based upon the crude ethyl acetate extract.

The molecular formula and the structure of this isolated compound were assigned by using advanced spectroscopic methods such as FT IR, ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), DEPT, HMQC, DQF-COSY, HMBC and FAB-mass spectral data (Breitmaier, 2002; Crews *et al*., 1998; Hesse *et al*., 1997; Nakanishi, 1962; Silverstein *et al*., 2005).

Results and Discussion

The ethyl acetate crude extract was separated on a silica gel column using gradient elution with n-hexane and ethyl acetate from non-polar to polar to obtain one pure bioactive bi-isoflavonoid compound, 3-(2,4-dihydroxyphenyl)-5-hydroxy-7-((3-(4-hydroxyphenyl)-7-methoxy-4-oxo-4*H*-chromen-5-yl) oxy)-4*H*-chromen-4-one.

Molecular Formula Determination of the Isolated Pure Compound

This isolated compound was obtained as pale yellow needle shape crystal.The FT IR spectrum exhibited absorption bands at 3392.90, 3041.84, 2928.04, 2854.74, 1684.98, 1614.47, 1579.75, 1253.77, 1188.19, 1037.74, 916.22, 835.21 and 779.27 cm^{-1} ascribable to hydroxyl, sp^2 H/C, sp^3 H/C, ether, carbonyl and aromatic ring functional groups respectively (Figure 2). The ^{1}H NMR spectrum (Figure 3 and Table 1) revealed one methoxy singlet at δ_H 3.87 ppm, two same chemical shift of aromatic protons at δ_H 6.82 ppm, another two same chemical shift aromatic protons δ_H 7.37 ppm and two down field chemical shift singlet methine protons δ_H 8.22 ppm and δ_H 8.30 ppm. The DEPT and FT-IR spectral data displayed the presence of one sp^3 methoxy

carbon, thirteen sp^2 methine carbons, fifteen sp^2 quaternary carbons, two carbonyl carbons, OH group and ether group. The molecular formula was determined to be $C_{31}H_{20}O_{10}$ from the observation of ^1H NMR spectrum, ^{13}C NMR (Figure 4) and FAB mass spectrometry (Figure 5).

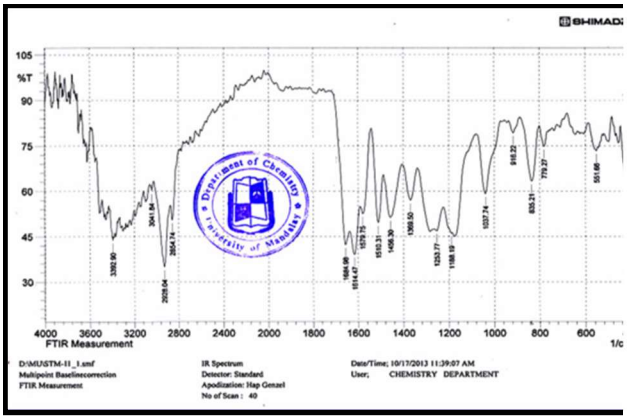


Figure 2. FT IR Spectrum of Pure Compound (KBr)

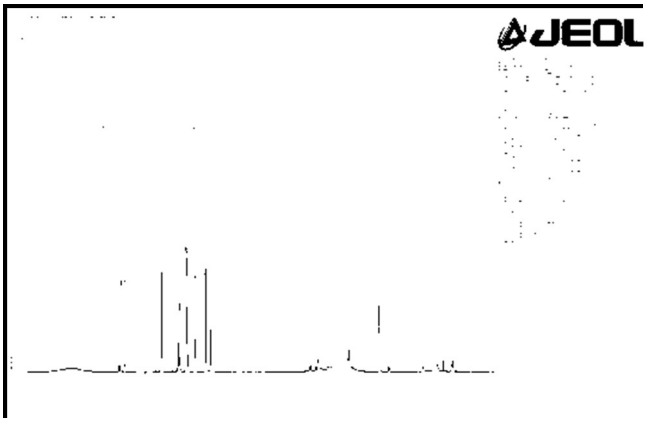


Figure 3. ^1H NMR Spectrum of Pure Compound

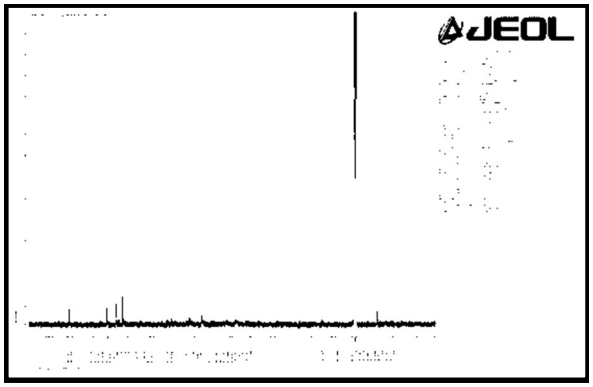


Figure 4. ^{13}C NMR Spectrum of Pure Compound

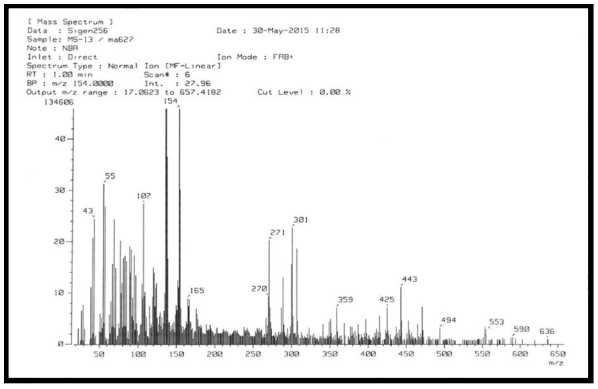


Figure 5. FAB-Mass Spectrum of Pure Compound

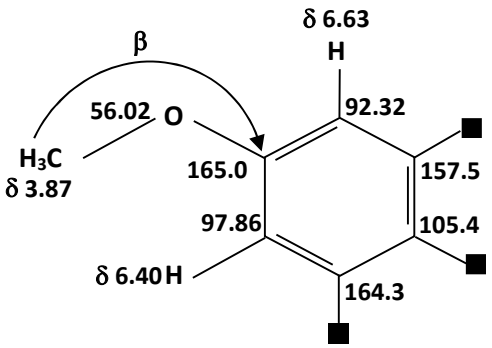
Table 1. ^{1}H NMR Spectral Data of Isolated Pure Compound

No.	Chemical shift (δ/ppm)	No. of protons	Splitting pattern	Coupling constant (J value Hz)	Proton assignment
1.	3.87	3H	s	–	sp^3methoxy proton
2.	6.22	1H	d	2.1	sp^2methine proton
3.	6.28	1H	dd	2.1, 8.2	sp^2methine proton
4.	6.37	1H	d	2.3	sp^2methine proton
5.	6.38	1H	d	2.3	sp^2methine proton
6.	6.40	1H	d	2.2	sp^2methine proton
7.	6.63	1H	d	2.2	sp^2methine proton
8.	6.82	1H	d	8.5	sp^2methine proton
9.	6.82	1H	d	8.5	sp^2methine proton
10.	6.99	1H	d	8.2	sp^2methine proton
11.	7.37	1H	d	8.5	sp^2methine proton
12.	7.37	1H	d	8.5	sp^2methine proton
13.	8.22	1H	s	–	sp^2methine proton
14.	8.30	1H	s	–	sp^2methine proton
Total number of protons					16

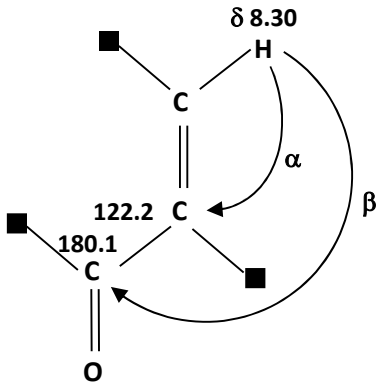
Structure Elucidation of Pure Compound

The structure elucidation of pure compound could be determined by ^{1}H NMR, DQF-COSY, HMQC and HMBC spectral data, respectively.

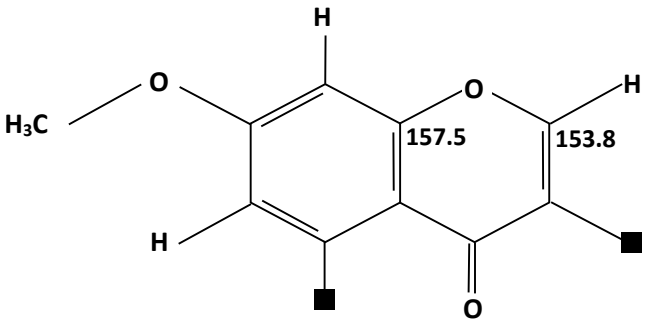
In the structure elucidation, the tri-substituted benzene ring fragments $\underset{\sim}{a}$, $\underset{\sim}{b}$ and $\underset{\sim}{c}$ could be assigned by DQF-COSY, ^{1}H NMR, splitting pattern, coupling constant (J-values), HMQC and HMBC spectra.



a

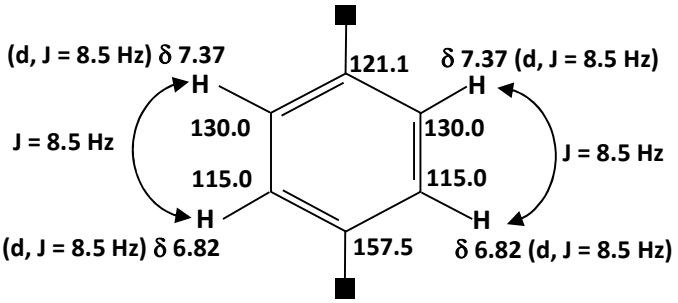


b



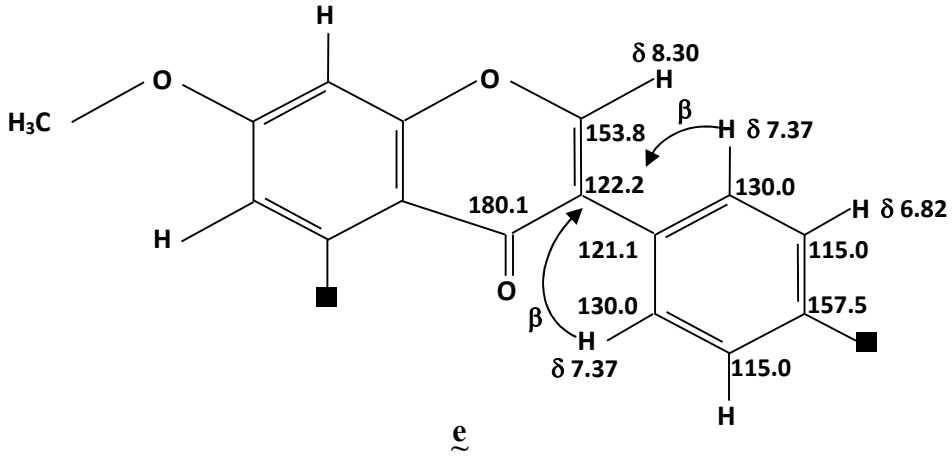
c

Moreover, another symmetrical disubstituted benzene ring fragment d could also be assigned by DQF-COSY, ^{1}H NMR, HMQC and HMBC spectral data.



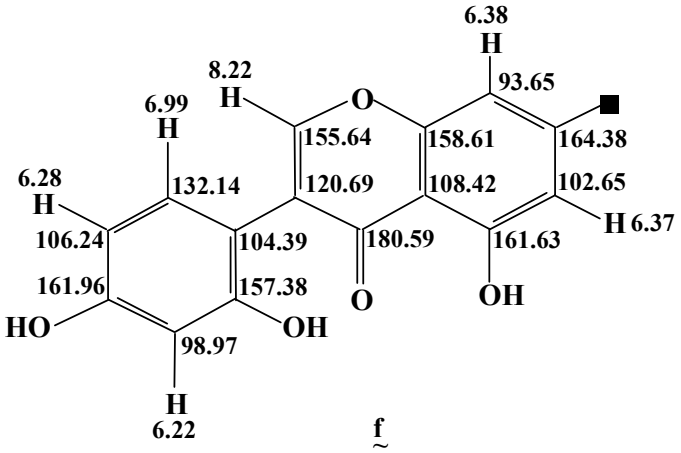
d

Furthermore, the fragments c and d were connected by using HMBC spectrum which produced the extended isoflavonoid partial structure e.



e

In addition, another isoflavonoid skeleton type fragment f with different substituent groups from the former one could be assigned by using spectroscopic data as described below.



f
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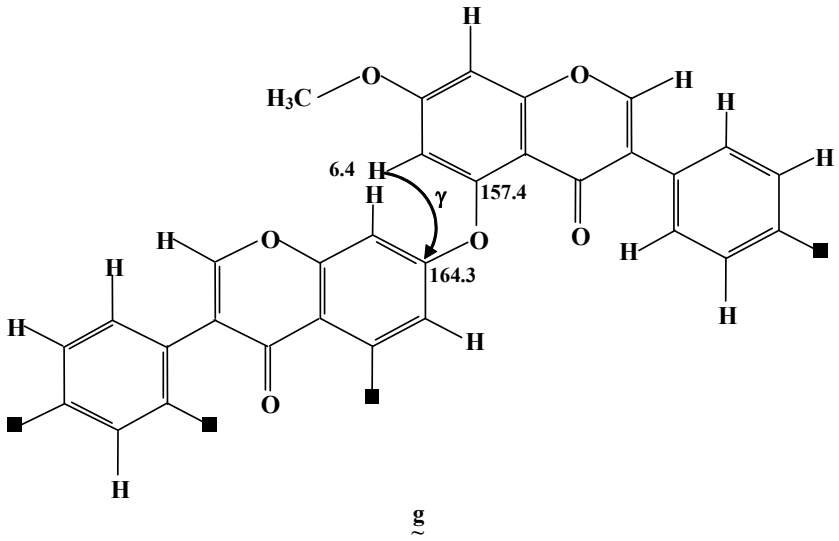
The connection between two different isoflavonoid moiety (e and f) by applying the only HMBC signal leads to the following biflavonoid skeleton type fragment g .



g
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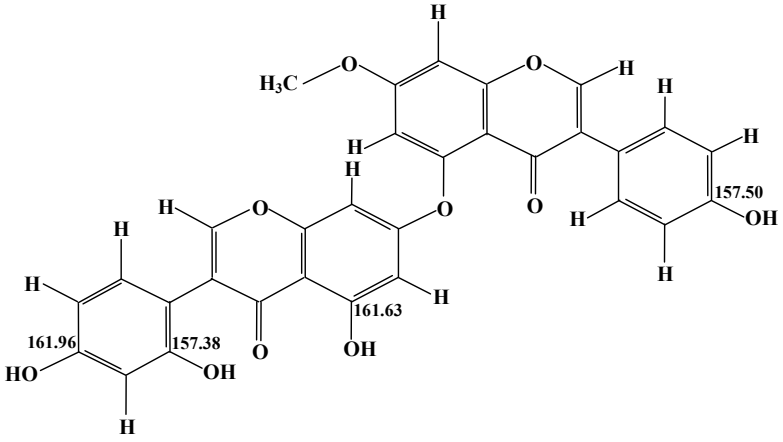
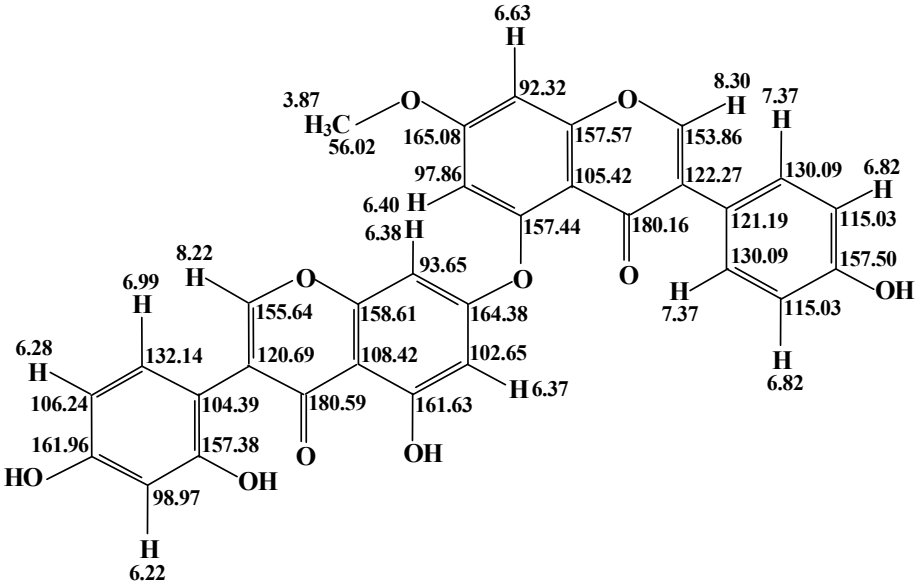
In this stage, the elucidated partial molecular formula is $C_{31}H_{16}O_6$ and remaining molecular formula is ($C_{31}H_{20}O_{10} - C_{31}H_{16}O_6 = H_4O_4$). It must be four hydroxy groups.

Finally, the remaining four hydroxy groups attached to reasonable four downfield chemical shift carbons (161.96 ppm, 157.38 ppm, 161.63 ppm and 157.50 ppm) could be established the complete planar structure of pure compound.



The elucidated structure of pure compound could be confirmed by FAB-mass fragmentation behaviour. The IUPAC name of pure compound is 3-(2,4-dihydroxyphenyl)-5-hydroxy-7-((3-(4-hydroxyphenyl)-7-methoxy-4-oxo-4*H*-chromen-5-yl) oxy)-4*H*-chromen-4-one.



Conclusion

In the present investigation, was described the isolation of pure bioactive bi-isoflavonoid compound which possessed many biological activities, especially anti-inflammatory activity from the stem bark of *Butea monosperma* (Lam.) Kuntze (Pouk). Moreover, the fractionated pure pale yellow needle shape compound could be illustrated by using sophisticated spectroscopic method and confirmed by FAB mass fragmentation behaviour. Further studies are required and are in progress.

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