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ISOLATION OF ATRANORIN, BERGENIN AND GONIOTHALAMIN FROM *HOPEA SANGAL*

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ABSTRACT

A phytochemical investigation was conducted on *Hopea sangal* (Dipterocarpaceae). Three compounds whose names were atranorin, bergenin and goniothalamin were isolated from the ethyl acetate extract of the stem bark of the plant. The structures of these compounds were determined by X-ray and NMR. Studies with the latter involve ^{1}H, ^{13}C and APT spectral data.

Keywords: timber, dipterocarpaceae, *hopea sangal*, atranorin, bergenin, goniothalamin, NMR

INTRODUCTION

Dipterocarpaceae is an important Asiatic tree family containing 13-15 genera and 487-580 species with various species deliminination problems (Ashton *et al.*, 1999). Two morphological views were proposed by Ashton (1980) and Kostermans (1992) on Dipterocarpaceae in Sri Lanka that suggest significant differences in generic, subgenera and species boundaries. Many species are of the large forest emergent, typically reaching heights of 40-70m tall, with the tallest known living specimen over 85m tall (Ridley, 1967). Flowers and fruits of most of the Dipterocarpaceae members are difficult to obtain and a discriminatory tool using vegetative parts such as leaves would help in identification. Furthermore, a chemotaxonomic approach may help in a future revision of the family. The species of this family are of major importance in the timber trade. Their distribution is pantropical, from northern South America to Africa, the Seychelles, India, Indonesia and Malaysia, with the greatest diversity and abundance in western Malaysia. *Hopea* consists of above 100 species (Ridley *et al.*, 1922 and Joshi *et al.*, 2004). It is distributed from mainland South-East Asia toward Peninsular Malaysia, Sumatra, Borneo, the Philippines and New Guinea (Plant Resources of South-East Asia, 1994). *Hopea sangal* is distributed in Peninsular Malaysia, peninsular Thailand and Sumatra. It is the important source of Merawan timber. In this paper, we report the isolation and characterization of atranorin, bergenin and goniothalamin. These compounds have never been reported before from any *Hopea (*Chan K. C., 1969; Geevananda *et al.*, 1980; Tanaka *et al.*, 2000).

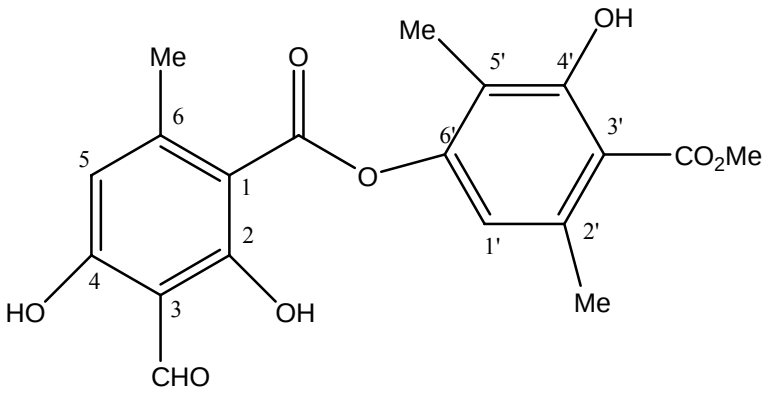

Figure-1.

MATERIALS AND METHODS

TLC and PTLC were performed using pre-coated aluminium and plastic plates with silica gel 60 F_{254}, column chromatography was carried out on silica gel 230-400 mesh, whereas radial chromatography was done using glass plates with Merck's silica gel 1.07749. Spots and bands for compounds on TLC, PTLC and radial chromatography were detected using UV lights. UV spectra were recorded on a UV-1650PC spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on JEOL JNM-ECP 400. Chemical shifts in ppm were referenced to internal acetone-d_6, DMSO-d_6 and $CDCl_3$.

Plant material

The stem bark of *Hopea sangal* was collected from the forest area of the main campus of Universiti Kebangasaan Malaysia. A voucher specimen had been deposited at the Herbarium of Universiti Kebangasaan Malaysia.

Extraction and isolation

The air-dried powder stem bark (250 g) of *Hopea sangal* was extracted (Soxhlet) with ethyl acetate (3X, 16 hours each) and the combined extracts were evaporated to give a brown residue (5g). The extract was subjected to column chromatography on silica gel with hexane containing increasing percentages of EtOAc as eluent and each collected fraction was 20ml. Fractions 3-5 (120mg) were rechromatographed by RC to give atranorin (1) (4.3 mg), R_f 0.52 (hexane-EtOAc 9:1). Fraction 6 and 7 (50mg) were purified by PTLC to give goniothalamin (3) (3.5mg), R_f 0.45 (hexane-EtOAc 7:3).

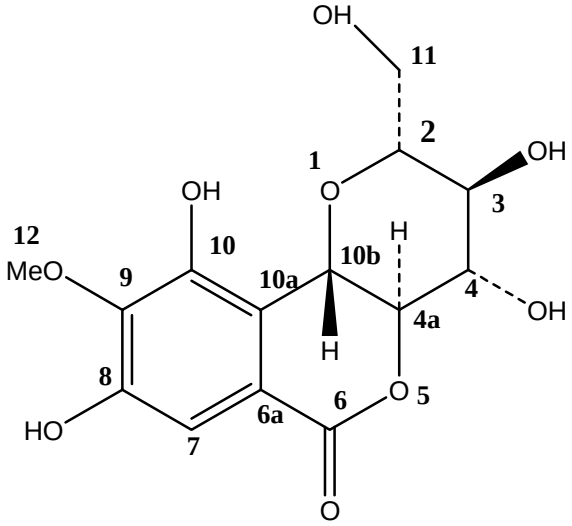

Figure-2.

Fractions 8-11 (120mg) were purified by radial chromatography with chloroform containing increasing percentages of methanol as eluent and each collected fraction was 20ml. Fractions 3-5 contain bergenin (2) (4.2mg), R_f 0.50 ($CHCl_3$-MeOH 8:2). Atranorin, bergenin and goniothalamin were identified by comparison with the previous NMR and mass spectral data (Quilhot *et al.*, 1975; Jahodar *et al.*, 1992 and Yoshida *et al.*, 1982).

Atranorin (1)

Recrystallisation from acetone gives light brown needles (3.5mg), Mp 194 ^oC, UV ($CHCl_3$) λ_{max} nm (logε): 305 (0.29), 265 (1.06). ^1H NMR ($CDCl_3$, 400 MHz): δ 12.57, 12.52, 12.05 (each 1H, Ar-OH), 10.37 (1H, -CHO), 6.53 (1H, Ar-H, H- 6'), 6.40 (1H, Ar-H, H-5), 4.00 (3H, s, $-CO_2Me$), 2.70 (3H, s, Ar-Me, C- 6), 2.55 (3H, s, Ar-Me, C- 5'), 2.10 (3H, s, Ar-Me, C-2'). Apt showed four methyl groups at δ 9.6, 24.3, 25.8 and 52.6 for Me- C2', Me- C5', Me- C6 and $-CO_2Me$. And also showed carbonyl ester at δ 169.3 (C-1) and 169.9 (C-4') and showed eldehyde group at δ 194.1 (C-3).

Bergenin (2)

White needles (4.2mg), mp 235^oC. ^1H NMR (DMSO-d_6, 400 MHz): δ 6.48 (m, arom., H-7), 5.68 (1H, d, H-10b), 4.99 (1H, dd, H-4a), 3.99 (1H, dd, H-4), 3.80 (2H, d, H-11), 3.76 (3H, s, H-12), 3.60 (1H, m, H-2), 3.49 (1H, dd, H-3). ^{13}C NMR (DMSO- d_6, 100 MHz): δ 60.0 (C-12), 61.2 (C-11), 70.8 (C-3), 72.2 (C-10b), 73.8 (C-4), 79.9 (C-4a), 81.8 (C-2), 109.6 (C-7), 116.1 (C-10a), 118.2 (C-6a), 140.7 (C-9), 148.2 (C-10), 151.1 (C-8), 163.5 (C-6).

Goniothalamin (3)

3.5mg. Mp 85^oC, UV (acetone), λ_{max} nm (logε) 259 (0.04), 283 (0.01), 307 (0.17), 326 (0.37). EIMS for $C_{13}H_{12}O_2$ *m/z* (rel. int.): 200 (55 %), 172 (24.8 %), 131 (37 %), 104 (100 %), 91 (33 %), 68 (89 %), 39 (28.5 %). ^1H NMR (acetone- d_6, 400 MHz): δ 2.55-2.68 (m, H-5), 5.11-5.17 (m, H-6), 5.99 (dd, J = 1.21, 1.12 Hz, H-3), 6.4 (dd, J = 6.6, 6.6 Hz, H-7), 6.76 (d, J = 15.8 Hz, H-8), 7.06 (m, 1H, H-4), 7.27-7.52 (m, 5H, arom.). ^{13}C NMR (acetone-d_6, 100 MHz): δ 23.4 (C-5), 78.8 (C-6), 121.8 (C-3), 127.6 (C-7), 127.7 (C-10, C-14), 129.0 (C-12), 129.6 (C-11, C-13), 133.3 (C-8), 137.2 (C-9), 146.5 (C-4) and 164.0 (C-2).

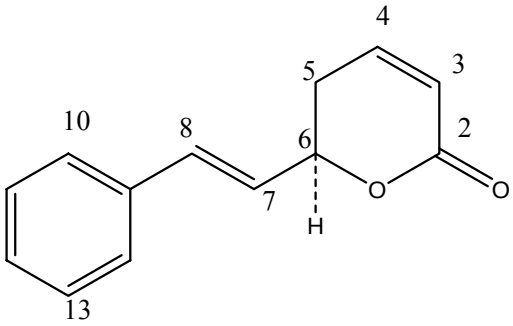

Figure-3.

RESULTS AND DISCUSSIONS

Purification of the extract of the stem bark of *Hopea sangal* afforded compounds namely atranorin (1), bergenin (2) and goniothalamin (3) which were examined.

Compound 1 showed strong absorption in its UV spectrum at 305 (0.29) and 265 (1.06). The ^1H NMR displayed four methyl groups at δ 2.10, 2.55, 2.70 and 4.00. It also showed an aldehydic proton at δ 10.37. ^{13}C NMR spectrum displayed carbonyl ester groups at δ 169.3 and 169.9. It also showed a carbon aldehyde group at δ 194.1. Atranorin is a major component normally found in lichens (Quilhot W. *et al.*, 1975; Culberson C.F. *et al.*, 1977; Faik A. *et al.*, 2008; Lumbsch H. T., 1995). It has never been isolated before from any higher plants. In our work, we are very sure that the compound did not come from lichens living on the bark of *Hopea sangal*. This is because the outer (dry) part of the bark was first removed

by trimming it with a machete. The inner, soft dark-red woody part was chopped into small pieces before letting them dry in the laboratory. The dry powder of this remaining part was extracted and separated to give the atranorin and others.

Compound 2 was isolated as white crystals. The mass spectral data of the compound gave a molecular formula C_{14} $H_{16}O_9$, *m/z* 328 for M^+. The melting point of 235 ^{0}C is consistent with the published melting point of bergenin 238 ^{0}C (Madusolumuo and Okoye, 1995). The ^{1}H spectrum exhibited a signal for one aromatic proton (s, 6.95, 1H) and a signal for methoxy protons (s, 3.7, 3H). ^{13}C NMR spectrum showed a carbonyl group signal at δ 163.5 and a signal for the methoxy at δ 60.0. Bergenin is a common compound isolated from the higher plants but it has never been isolated before from *Hopea sangal* (Aiyar *et al.*, 1964; Ahmad *et al.*, 1972; Izawa *et al.*, 1973; Tomizawa *et al.*, 1976; Bandaranayaka *et al.*, 1977; Yoshida *et al.*, 1982; Mebe and Makuhnga, 1992 and Ito *et al.*, 2003).

Compound 3 m.p. 85^{o}C, molecular formula $C_{13}H_{12}O_2$ by EIMS (M^+, *m/z* 200). Compound 3 showed strong absorptions in its UV spectrum at 259, 283, 307 and 326 nm (log ε) 0.04, 0.01, 0.17 and 0.37. ^{1}H NMR displayed two olefinic protons as doublets at δ 5.99 and 6.4 for H-3 and H-7, which also showed that they are in *a trans* configuration. It also showed aromatic protons at δ 7.72-7.52 (m, 5H). ^{13}C NMR spectrum showed a carbonyl group signal at δ 164.0. Goniothalamin was first isolated from *Goniothalamus* species and found several times from the same genus (Ahmad *et al.*, 1991; Sam *et al.*, 1987; Hasan *et al.*, 1994 and Jewers *et al.*, 1972). And also from other species (Cavalheiro and Yoshida, 2000; Mosaddik *et al.*, 2000).

CONCLUSIONS

The isolation and identification of atranorin (1), bergenin (2) and goniothalamin (3) from the stem bark of *Hopea sangal* was the first ever to be reported from this plant. The work was carried out by means of various physical (solvent extraction, radial chromatography) and spectral techniques.

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