IMMUNOLOGICAL ACTIVITY AND CRYSTALLOGRAPHICAL STUDIES OF THE CARBAZOLE DERIVATIVE FROM ANGOPHORA COSTATA

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ABSTRACT

The present study involved the isolation of natural product that has been identified as carbazole derivative, reported first time from Angophora costata of South Australian origin. Carbazole derivatives have been of special pharmaceutical importance because of their versatile biological aspects including inflammation, antibacterial, antifungal, antiviral, anticancer and protein-kinase inhibition activities. The immunological activity of the carbazole derivative has been performed in the present paper by using T-cell proliferation assay and cytotoxic assay using MTT method and CC1 mouse normal liver cell lines at concentrations 20 and 5.0µg/mL with IC₅₀ value is 1.4 ± 0.14 µg/mL as compared to control.

Keyword: Carbazole derivative, immunology, crystallography

Fig-1: 7-methoxy-1-methyl-9H-carbazol or methyl 8-methyl-9H-carbazol-2-yl
The title compound was previously synthesized by Radhika S. Kusurkar et al., 2003, (7) and it is first report of its isolation from natural source. Carbazole derivative of angophora costata was found to be remarkable inhibiting the proliferation of T-cell with IC50 as 0.7±0.03 µg/mL. Angophora costata showed cell toxicity against CCl mouse normal liver cell line at concentration of 1.0µg/mL as compared to control.

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**INTRODUCTION**

*Angophora costata* (Gaertn.) Britten is a large genus of aromatic trees belonging to the family Myrtaceae. It comprises of over 500 species indigenous to Australia, Tasmania and neighboring islands (8). Various species of angophora is found in South Australia. The genus name Angophora comes from the Greek for 'bearing a goblet', referring to the shape of the fruit. The species epithet costata comes from the Latin for 'ribbed', referring to the ribs on the fruit capsules (1). The genus Angophora is very close to Eucalyptus and can be distinguished easily by its opposite leaves. It is commonly known by a variety of names including smooth-barked apple, rose gum, rusty gum, rose apple or Sydney red gum and is widely distributed along the coast, ranges and tablelands of New South Wales and Queensland through into south and central western Queensland (2). It grows primarily on sandstone soils, usually on headlands, plateaus or other elevated areas. *A. costata* is a plant of medicinally important, wide spreading tree. Usually apparent height found between 15 and 25 m. The trunk is often gnarled and crooked with a pink to pale grey. Sometimes found with rusty-stained bark. Oil glands are not visible. Cream to white colored flowers occurs in clusters at the end of branches, the fruit is a ribbed capsule surrounded by persistent teeth. Flowers in early spring or summer, probably containing tannins and various phenolics substances, while the gum is used in diarrhea (9, 8).

**EXPERIMENTAL SPECIFICATIONS**

**Material**

The red gum (350 gm) was collected from bark *Angophora costata*. The gum was collected by Pederson, S. and identified by Mr. Slee, A.V. voucher specimen has been deposited in the Australian National Herbarium, as catalog number CANB 621590.

**Extraction and Isolation**

The red gum was repeatedly extracted with methanol at room temperature. The concentrated syrupy residue obtain after the removal of solvent from methanolic extract in vacuum, was
partitioned between Hexane, Dichloromethane, Acetone, and Ethanol. After evaporation of the solvent from ethanolic part, the residue was separated by column chromatography on Alumina as stationary phase and using Hexane/Acetone (8:2) as an eluent to obtain fractions SE-1 to SE-8. Fraction SE-5 was loaded on Alumina as a stationary phase and Hexane % Acetone (7:3) as mobile phase to afford reddish needle like crystals of compound SA-2 (15mg) (Fig-1).

Crystallographic study of *Angophora costata*

![Molecular structure of compound SA-2](image)

**Fig-2:** The molecular structure of (I) with displacement ellipsoids drawn at 30% probability level.

![Crystal packing of compound SA-2](image)

**Fig-3:** The crystal packing of compound SA-2. Only hydrogen atoms involved in hydrogen bonding are shown.

**T-cell proliferation assay**

Peripheral blood mononuclear cells (PBMCs) were separated from heparinized venous blood of healthy volunteers reported to be free of medication from 10 days. The blood was mixed with Ficoll-Hypaque as described by Sultana *et al.*, 2010 (10).
Cytotoxicity assay using MTT method and CC1 mouse normal liver cell line
Cytotoxic activity of compounds was evaluated in 96-well flat-bottom microtiter plates by using the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide). The CC-1 cell line (Rat wistar liver cells), were harvested, counted and diluted with MEM. The cells (6 x 10^4 cells/mL) were incubated overnight and the supernatant was carefully removed and compound was added in different concentrations (20, 5.0 and 1.0µg/mL). After 48 hrs, MTT was added and incubated for further 4 hrs. Finally, DMSO was added to each well after proper aspiration of the MTT. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 520 nm; using a micro plate reader (Spectra Max plus 340, Molecular Devices, CA, USA) (11). The cytotoxicity was recorded as concentration causing 50% growth inhibition (IC_{50}). The percent inhibition was calculated by using the following formula:

\[
\% \text{ inhibition} = 100 - \left( \frac{\text{Mean of O.D of test compound}}{\text{Mean of O.D of positive control}} \right) \times 100
\]

Fig-5: Cytotoxicity assay result of *Angophora costata* using MTT method and CC1 mouse normal liver cell line. Control means (without drug).
RESULT AND DISCUSSION

The structure of compound was unambiguously established by single-crystal X-ray diffraction analysis (Fig-1). The structure of compound SA-2 (Fig-1) is almost planner with a dihedral angle of 4.02 Å (11) between the planes of two phenyl rings (C1--C6 and C7--C12) (Fig-1). All the bond angles and lengths were found in normal range and similar to other related structure. In the crystal, the components are linked into a three dimensional framework by intermolecular C3---H3A…N1, C15---H15B…O1 (Fig-3).

General experimental

Single-crystal X-ray diffraction data were collected on a Bruker Smart APEX II, CCD detector diffractometer (3). Data reductions were performed by using SAINT program. The structures were solved by direct methods (4), and refined by full-matrix least squares on F2 by using the SHELXTL-PC package (5). The figures were plotted with the aid of ORTEP program (6).

Crystal data: empirical formula = C14H13NO, Mr = 211.25, orthorhombic, space group P2₁2₁2₁, a = 5.8053 Å (3), b = 9.6217 Å (4), c = 18.9952 Å (8), V = 1061.01Å³ (8), Z = 4, ρcalc = 1.323 mg/m³, F(000) = 448 μ (Mo Kα) = 0.71073 Å, max/min transmission 0.9884 / 0.9682, crystal dimensions 0.39 x 0.18 x 0.14 mm, 2.14° < θ < 25.50°, 6268 reflections were collected, out of which 1,969 reflections were observed (Rint = 0.0209). The R-values were; R1 = 0.0458, W2 = 0.1258 for I > 2σ(I), and R1 = 0.0488, W2 = 0.288 for all data, max/min residual electron density; 0.307 / –0.365e Å⁻³.

T-cell proliferation assay

The suppression of immune system is beneficial in the management of various inflammatory conditions (10). Results showed that Angophora costata at concentration 0.5µg/mL expresses moderate inhibition of proliferation of T-cell however, complete suppression was observed at concentrations 5 and 50µg/mL as compared to control values (Fig-4). No immunomodulatory activity of Angophora costata has been reported till date. The mean IC₅₀ value is (0.7 ± 0.03 µg/mL).

Cytotoxicity assay using MTT method and CC1 mouse normal liver cell line

Natural origin compounds are chemical compounds having toxic effects on plants, animals, bacteria and also on the substructures such as different organs for e.g. liver, kidneys e.t.c (11). The effect of Angophora costata used in this study showed greater cell toxicity at
concentration 1.0µg/mL as compared to control values (Fig-5). It also showed tremendous cytotoxic effect in CC-1 mouse normal liver cell line at concentrations 20 and 5.0µg/mL (Fig-5). The mean IC₅₀ value is 1.4 ± 0.14 µg/mL.

REFERENCES