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ScienceDirect
Chinese Chemical Letters 21 (2010) 593–595
CHINESE CHEMICAL LETTERS
www.elsevier.com/locate/cclet

A novel antimicrobial flavonoidic glycoside from the leaves of *Alstonia macrophylla* Wall ex A. DC (Apocynaceae)

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Received 31 August 2009

Abstract

A new flavonoidic glycoside, tricin-4′-*O*-β-L-arabinoside (**1**) was isolated from the leaves of *Alstonia macrophylla* along with two known flavonoids, vitexin and myricetin-3′-rhamnoside-3-*O*-galactoside. Their structures were established by chemical and spectral evidences. The known compounds were reported for the first time from this plant. Moreover compound **1** was tested for antifungal and antibacterial activities.

Keywords: *Alstonia macrophylla*; Vitexin; Myricetin-3′-rhamnoside-3-*O*-galactoside; Tricin-4′-*O*-β-L-arabinoside; Antibacterial; Antifungal activity

The genus *Alstonia* (Apocynaceae) found in Africa and Asia comprises about 60 species, 6 of which are distributed in India [1]. The phytochemical constituents of the *Alstonia* sp. have been investigated intensively. Until now more than 300 compounds have been reported from this genus. *Alstonia macrophylla* Wall is very promising tall evergreen tree. Ethnobotanical studies indicate that decoction of the leaves and bark is widely used by the tribal of India to treat stomachache [2], skin diseases and urinary infection [3,4]. The bark of the plant also used as febrifuge, tonic, emmenagogue, anti-chloretic, vulnerary [5], anti-dysenteric, anti-periodic [6], vermifuge and anti-diabetic [7]. Oiled and heated leaves of the plant are used to treat sprains, bruises and dislocated joints [8]. Recently antimicrobial [9], antiinflammatory, antioxidant [10], antipyretic [11], CNS [12], antiplasmodial [13] and anticancer [14] activities have been reported. Previous phytochemical studies on the leaves of *A. macrophylla* showed diverse class of compounds such as alkaloids, flavonoids, sterols and terpenoids, justifying their use [15]. Keeping in view the therapeutic utility of *A. macrophylla* it was considered significant to carry out phytochemical research on the leaves of the plant. As part of a continuing effort to discover novel secondary metabolite a new flavonoidic glycoside was isolated together with two known flavonoids, vitexin [16] and myricetin-3′-rhamnoside-3-*O*-galactoside [17]. This paper presents the isolation and structure elucidation of the new compound.

Compound **1** was isolated from mixed ethyl acetate, acetone and methanol extract of leaves by preparative thin layer chromatography (PTLC) crystallized from chloroform–methanol as yellow needles (165 mg), m.p. 310 °C. Elemental analysis agreed to the molecular formula $C_{22}H_{22}O_{11}$. The glycosidic nature of the new natural product was

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1001-8417/$ – see front matter
doi:10.1016/j.cclet.2009.11.022

evidenced by its paper chromatographic behaviour [18], high solubility in water, positive Molish test and by the formation of osazone. It was further supported by the presence of an anomeric proton at δ 5.33 in the ^{1}H NMR spectrum and a broad band in the region 1065 and 1121 cm^{-1} due to *O*-glycosylation in the IR spectrum.

The compound **1** gave positive test with zinc and hydrochloric acid [19] and sodium amalgam followed by acidification [20], indicating its flavone or flavanone nature. A yellow colour with Wilson boric acid reagent [21] and maxima at 240 and 350 nm in the UV spectrum indicated it to be a flavone glycoside. UV spectrum of **1** with diagnostic shift reagents [18,22] confirms the presence of free hydroxyl groups at C-5 and C-7 position. The localization of a free hydroxyl group at C-5 is confirmed by a bathochromic shift of 22 nm in band II with $AlCl_3$. The presence of free hydroxyl group at C-7 is shown by a bathochromic shift of 18 nm in band II. Furthermore the complete acid stability of $AlCl_3$-complex together with negative borate reaction ruled out the presence of *ortho*-dihydroxyl grouping in either A or B ring. No shift with NaOMe showed the absence of free hydroxyl at 4′ position. The IR spectrum displayed strong bands at 3380 (OH), 1670 (C=O) and 1615 cm^{-1} (C=C, aromatic).

Total acid hydrolysis of **1** with 0.2 mol/L HCl gave an aglycone, m.p. 291–93 °C and a sugar. The sugar was identified as L-arabinose by paper chromatography, osazone formation and GLC of its trimethyl silylether derivative. The aglycone showed bathochromic shift of 58 nm in band I without decrease in intensity in the NaOMe, thus showing that sugar is linked to the 4′ position of the aglycone. The aglycone was characterized as 5,7,4′-trihydroxy-3′,5′-dimethoxy flavone (tricin), m.p. 291–93 °C by spectral and chromatographic comparison with authentic sample [23].

Acetylation of **1** with Ac_2O/pyridine afforded a pentaacetate derivative, m.p. 130–132 °C. The ^{1}H NMR spectrum of acetate in $CDCl_3$ showed two phenol acetoxyls at δ 2.45 and δ 2.38, each integrating for three protons and three sugar acetoxyls in the range of δ 2.08–2.19 integrating for nine protons, respectively. A sharp singlet integrated for six protons appeared at δ 3.93 ascribed to two methoxy groups at C-3′ and 5′. The two doublet at δ 6.72 and δ 7.03 (J = 2.0 Hz, each), each with meta coupling (J = 2.0 Hz) are assigned to C-6 and C-8 proton while a C-3 proton resonated as a singlet at δ 6.56. The 2′ and 6′ protons appeared as a singlet at δ 7.05 typical for a symmetrical substituted myricetin type B ring. The sugar protons appeared in the range of δ 4.23–5.45 as multiplet integrating for six protons. The anomeric proton H-1″ of arabinose appeared as a doublet at δ 5.32 (J = 8.0 Hz). The chemical shift confirmed the direct attachment of sugar to the aglycone and diaxial coupling (J = 8.0 Hz) between H-1″ and H-2″ suggested β-configuration of L-arabinose.

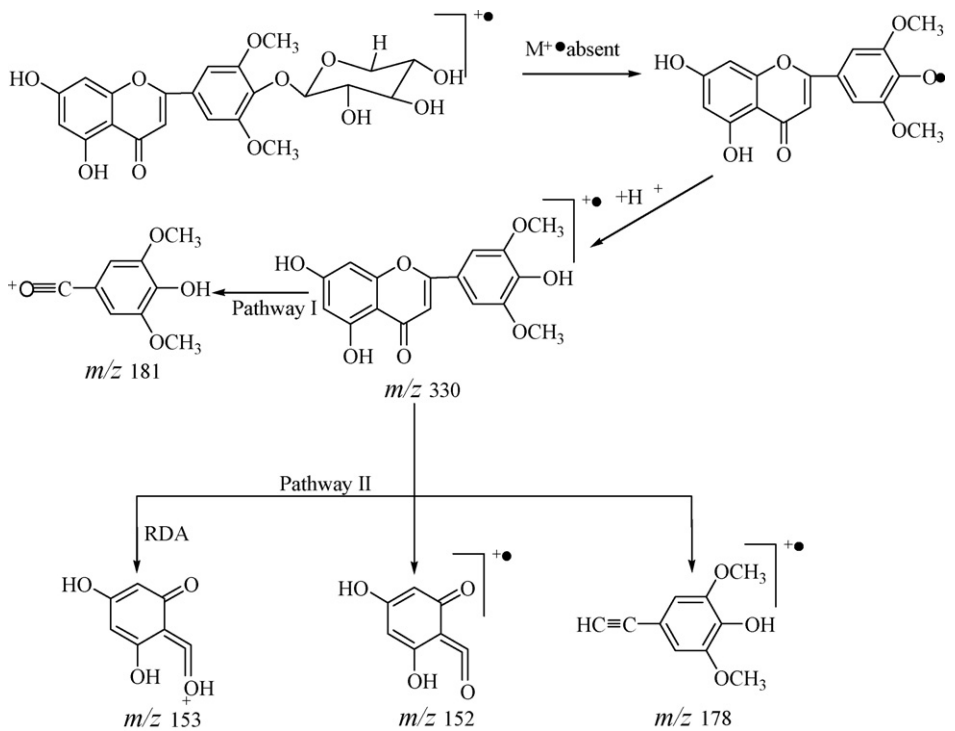

Fig. 1. Mass scheme of compound **1**.

The mass spectrum (Fig. 1) of **1** fully supported the assigned structure of the glycoside as it exhibited 330 $[M\text{-gly}]^{\bullet+}$ as the base peak. This was further supported by the RDA fragmentation [24] respectively. Ring A (*m/z* 153 $[A_1 + H]^+$, 152 $[A_1]^{\bullet+}$) and B (*m/z* 181 $[B_1]^+$, 178 $[B_1]^{\bullet+}$) which are indicative of the presence of two hydroxyl groups in ring A and two methoxyl and one hydroxyl group in ring B of the aglycone. The presence of peaks at *m/z* 259, 199, 169, 157 and 130 in the mass spectrum of acetate, finally established the sugar moiety as pentopyranoside [25].

The position of sugar residue in **1** was confirmed by hydrolysis of methylated glycoside. The methylated aglycone corresponded the molecular formula $C_{19}H_{18}O_7$, showed bathochromic shift of 10 nm in band II with NaOAc, confirming that C-7 hydroxyl which was glycosylated in C-7 had become free. The formation of this partial methyl ether thus proved that the sugar arabinose was linked to C-7 hydroxyl of the aglycone. The methylated sugar was identified as 2,3,4-tri-*O*-methyl-L-arabinose as a colourless liquid $[\alpha]_D$-122 (in water). The quantitative estimation of sugar by Somogyi's copper micro method [26] showed the presence of one mole of arabinose per mole of aglycone. Compound **1** was therefore characterized as tricin-4′-*O*-β-L-arabinoside. It was interesting to note the configuration of arabinose is β which is of rare occurrence.

The compound **1** showed significant antimicrobial activity using agar well diffusion method [27]. The *in vitro* antibacterial activity against *Staphylococcus aureus* (IAO-SA-22), *Escherichia coli* (K-12) and *in vitro* antifungal activity against *Salmonella typhimurium* (MTCC-98), *Candida albicans* (IAO-109) was carried out, with chloramphenicol and nystatin respectively, as positive control. With a concentration of 1 mg/mL, the novel flavonoidic glycoside **1** showed inhibitory activities against growth of *S. aureus*, *E. coli*, *S. typhimurium* and *C. albicans*. The most promising result was observed against *S. typhimurium* with zone of inhibition 20 mm, while *E. coli* ranked next (16 mm) followed by *S. aureus* and *C. albicans* whose zone of inhibition were 13 and 12 mm respectively.

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