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CAFFEINE AND *P*-ANISALDEHYDE FROM THE FRUITS OF *ENTEROLOBIUM SAMAN* PRAIN

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ABSTRACT

Fruits of the plant were air dried for several days and cut into small pieces, which were subjected to oven dried for 24 hours at 45-50°C to effect further chopping. Cold methanol extract of fruits were subjected to acid-base treatment to separate the alkaloids. The treated extract was subjected to column chromatography to isolate the pure compound. The xanthine alkaloid, 1, 3, 7-trimethyl-1*H*-purine-2, 6 (3H, 7H)-dione, caffeine (compound 1) and also *p*-anisaldehyde (compound 2) were isolated from the column fraction of this 'alkaloid containing residue' by elution with ethyl acetate/10% methanol and ethyl acetate/50-100% methanol, respectively. The isolated pure compounds were identified by extensive spectral (^{1}H-NMR, ^{13}C-NMR, DEPT, HMBC, HSQC, Mass) data analysis. Greshoff isolated an alkaloid, pithecolobin from the bark, which is saponin like in its action. Wehmer stated that the seeds also contain pithecolobin. In the Philippines, a decoction of the inner bark (fresh cambium) and the fresh leaves of this tree is used in diarrhea. All these above mentioned study lead us to work on it.

INTRODUCTION: *Enterolobium saman* (Jacq.) Prain (*Leguminosae*, subfamily *Mimosoideae*) is a large tree of 20 to 25 meters high with characteristic umbrella-shapped canopy, native to Latin America where is commonly known as "Rain-tree" and also available in Bangladesh with the bengali name of "Koroi" [1]. The fresh leaves of the plants are used in diarrhea [1].

Previous investigations of *Enterolobium saman* Prain have revealed the presence of a number of secondary metabolites viz., triterpene bisdesmosides enterolosaponins A and B [2], albizzine [3-5]. Fruit sample of *Enterolobium saman* Prain was collected from the University of Dhaka, Dhaka-1000, Bangladesh, in December 2005. The plant was identified by Mr. Monjurul Kader Mia, Principal Scientific Officer, Bangladesh National Herbarium. A voucher specimen for this collection is maintained at the Bangladesh National Herbarium (BNH) under the accession number, DACB- 32081.

MATERIALS AND METHODS: Usually the intact plant/plant part(s) is collected as a whole and sun-dried. In fresh condition, it is then oven-dried at reduced temperature (not more than 50°C) to make suitable for grinding purpose. The coarse powder is then stored in airtight container with marking for identification and kept in cool, dark and dry place for use. Extraction can be done in two ways- Cold Extraction and Hot Extraction. Chromatographic techniques are the most useful in the isolation and purification of compounds from plant extracts. Nuclear magnetic resonance (NMR) spectroscopy is used to determine the structure of the compound.

Extraction of dried and powdered fruits of *E. saman* was done by cold extraction process by using methanol as a solvent [6]. The air dried and pulverized plant material (100 gm) was cold extracted with methanol. Cold methanol extract of fruits were subjected to acid-base treatment to separate the alkaloids. The xanthine alkaloid caffeine [7] (compound **1**) and also *p*-anisaldehyde [8-10] (compound **2**) were isolated from the column fraction of this 'alkaloid containing residue' by elution with ethyl acetate/10% methanol and ethyl acetate/50-100% methanol, respectively. The structures of compounds **1** and **2** were unambiguously elucidated by high-resolution NMR techniques.

^{1}H-NMR spectra were obtained from Bangladesh Council of Scientific and Industrial Research (BCSIR) with Bruker NMR spectrophotometer (400MHz, with TMS as the internal reference). Silica gel (kieselgel G 60, mesh 70-230, particle size 0.043-0.063 mm) was used for column chromatography. PTLC was done on using coated glass plates (kieselgel 60 PF_{254}, Merck).

RESULT AND DISCUSSION: The ^{1}H NMR and ^{13}C NMR spectra of compound **1** have shown four ^{1}H signals (three methyl groups and one methine proton) and eight ^{13}C signals (five ring carbons and three methyl carbons) that assumed to be a substituted xanthine system. The ^{1}H NMR spectrum has shown one singlet integrating for one proton at δ 7.49 typical for an aromatic or olefinic proton.

The three singlets, each integrating for three protons, centered at δ 3.40, δ 3.57 and δ 3.98 could be assigned to three methyl groups each of which is adjacent to an electro negative element as N or O. Again the presence of these three methyl groups was also observed in ^{13}C NMR at δ 27.9, δ 29.8 and δ 33.6 respectively, that tentatively could be assumed the presence of three N-CH_3 groups.

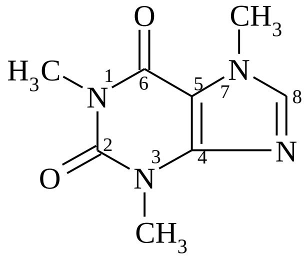
High-resolution ESI-TOFMS of compound **1** showed a $[M+H]^+$ peak at m/z 195, suggesting the molecular formula $C_8H_{10}N_4O_2$ in agreement with the NMR spectra.

These spectral data was fund to be identical to those published for caffeine [6]. On this basis compound **1** was identified as Caffeine (1, 3, 7-trimethyl-1*H*-purine-2, 6 (3*H*, 7*H*)-dione). This is the first report of its occurrence from the *Enterolobium* genus.

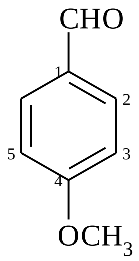
The ^{1}H NMR spectra of compound **2** showed two symmetric pairs of coupled two-protons doublets at δ 7.84 (H-2 and H-6) and at δ 7.0 (H-3 and H-5), assigning to a 1,4-disubstituted benzene ring. The presence of one sharp one-proton singlet at δ 9.88 and another sharp three-proton singlet at δ 3.89 could be attributed to one aldehydic group and one methoxyl group respectively. So it is clear from all of these ^{1}H NMR signals that the aldehydic and methoxyl groups are the substituents at C-1 and C-4, respectively, of the 1, 4-disubstituted benzene ring. On this basis the compound **2** was identified as 4-methoxybenzaldehyde (*p*-anisaldehyde).

Caffeine (1): White crystal; $C_8H_{10}N_4O_2$ (ESI-TOFMS: m/z $[M+H]^+$ 195); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49 (1H, s, H-8), 3.98 (3H, s, 7-CH_3), 3.40 (3H, s, 3-CH_3), 3.57 (3H, s, 1-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.8 (1-CH_3), 155.5 (C-2), 27.9 (3-CH_3), 151.8 (C-4), 107.6 (C-5), 148.8 (C-6), 33.6 (7-CH_3), 141.4 (C-8).

***p*-anisaldehyde (2):** Colorless gum; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.88 (1H, s, 1-CHO), 7.84 (1H, d, J = 9.4 Hz, H-2/H-6), 7.00 (1H, d, J = 9.4 Hz, H-3/H-5), 3.89 (3H, s, 4-OCH_3).



Compound**1**



Compound**2**

CONCLUSION: Compound **1** was previously isolated from *Coffea Arabica, Coffea salvatrix, Coffea eugenioides, Coffea bengalensis, Camellia thea* and *Camellia sinensis* [11]; *Theobroma cacao* and *Camellia assamica* [12]; *Paulinia pachycarpa, Paulinia cupana* and *Paulinia yoco* [13]; *Ilex paraguariensis* [14]; *Mascaro coffea* [15] and *Coffea canephora* [16] whilst the compound **2** has been found from *Decalepis hamiltonii* [17], *Syringa oblate* [18], *Cirsium arvense* [19], *Pimpinella anisum* [20], *Daedalea juniperina* [9], *Citocybe odora* and *Lentinellus cochleatus* [10]. In the present work, compounds **1** & **2** were isolated for the first time from *E. saman* and could be useful as chemotaxonomic markers for the study of genus.

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