

AN ALKYLATED COUMARIN FROM *KAYEA ASSAMICA**

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Key Word Index—*Kayea assamica*; Guttiferae; Calophylloideae; coumarin; assamene.

Abstract—Chemical investigation of *Kayea assamica* afforded one new and two known alkylated coumarin derivatives whose structures were determined through extensive use of NMR spectroscopy and chemical transformations. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

In continuation of our interest on the bioactive principles from the plants of the tropical rain-forest of the south-eastern sub-Himalayan region [1, 2], we undertook the chemical investigation of the plant *Kayea assamica* King & Prains, which is used as a traditional fish poison. The genus *Kayea* belongs to the sub-family Calophylloideae of the family Guttiferae [3, 4]. It consists of three species, namely *K. assamica*, *K. floribunda* and *K. manii*, occurring in India.

K. assamica is a slow-growing tall handsome ever-green tree of 20–25 m height that is found only in the Lakhimpur district of Assam, India [5]. Fruits of *K. assamica* are used in this region by the local people as a fish poison [6] and the aqueous extract of the stem bark is valued for its anti-malarial effects [7]. We have now isolated from this species one new and two known alkylated coumarins from root bark and fruit peel, respectively. The structures of these compounds have been established through extensive use of ¹H and ¹³C NMR (1D and 2D) spectroscopy and chemical transformations.

RESULTS AND DISCUSSION

The concentrated petrol extract of air dried root bark of *K. assamica* afforded, on standing overnight at room temperature a crystalline compound A which was found to be pure by TLC. This compound was

designated by the name assamene, mp 135°, [α]_D²⁵ = (+)38° (c, 0.4, CHCl₃). It was analysed for C₂₂H₂₈O₆ by EI-mass spectrometry (15 eV and 70 eV) giving [M]⁺ at 388, FAB-mass spectrometry (+) giving [M + H]⁺ at 389, DEPT [8] and ¹H-¹³C correlated COSY [9–11]. The UV spectrum of assamene in methanol exhibited absorption maxima at 203, 220, 248, 330 and 372 nm and did not show any shift in the presence of alkali which is very characteristic of 5,7-dihydroxy coumarins [12]. The IR spectrum of assamene showed absorption bands at 3300 cm⁻¹ (–OH), 1695 cm⁻¹ (coumarin carbonyl group), 1590 cm⁻¹ (chelated acyl group) and 1640 cm⁻¹ (carbonyl group) indicative of a coumarin structure. The ferric chloride test with this compound in ethanol showed an olive green colour characteristic of a phenolic hydroxyl group.

The ¹H NMR (400 MHz) spectrum of assamene revealed two three-proton broad singlets at δ 1.77 and 1.70 each assignable to a methyl on a double bond together with three singlets and one broad singlet at δ 5.90, 6.28, 11.92 and 13.71, respectively, each integrating for one proton. The ¹H NMR spectrum also displayed one three-proton doublet with *J* = 6.75 Hz at δ 1.17 and five doublet of doublets at δ 0.99, 1.03, 3.33, 3.41 and 5.10, respectively, the former two signals integrating for three protons and the latter three integrating each for one proton. In addition to these, the ¹H NMR spectrum also revealed six single proton doublet of doublet of quartets at δ 1.45, 1.57, 1.897, 2.01, 3.66 and 4.97. The signals at δ 13.71, 5.90 and 11.92 were found to be D₂O exchangeable and assigned to one hydrogen bonded hydroxyl, and two other hydroxyls, respectively. The proton carbon connectivity was determined by combined use of DEPT and ¹H-¹³C COSY NMR experiments (Fig. 1). The proton-proton coupling network was finally determined by ¹H-¹H COSY recorded by the GRASP [13]

* Part of these results were accepted for presentation in the International Symposium on Natural Products Chemistry, Karachi, Pakistan, 17–22 January 1994 and were presented in the symposium of the First Indian Society of Chemists and Biologists held at the Central Drug Research Institute, Lucknow, India, during 23–24 March 1996.

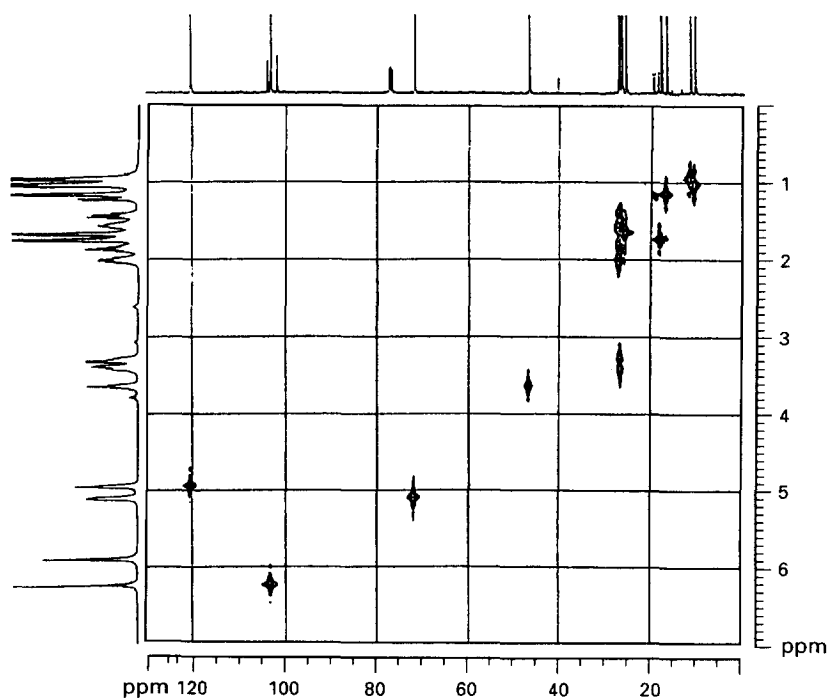


Fig. 1. ^1H - ^{13}C COSY NMR spectra of assamene (1).

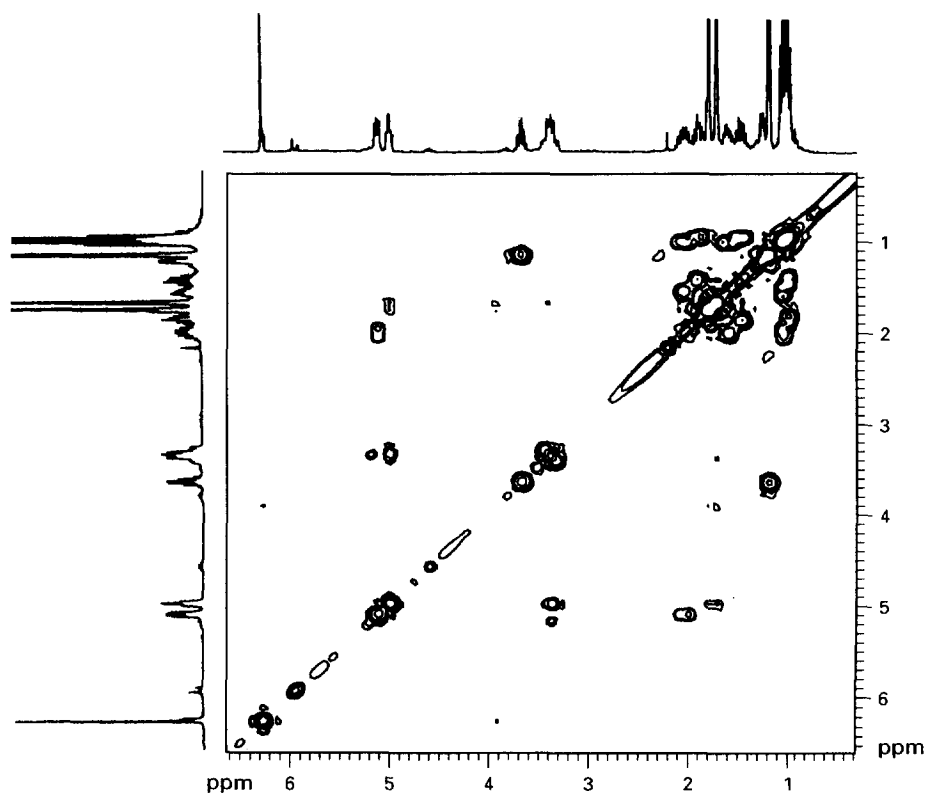


Fig. 2. ^1H - ^1H COSY NMR spectra of assamene (1).

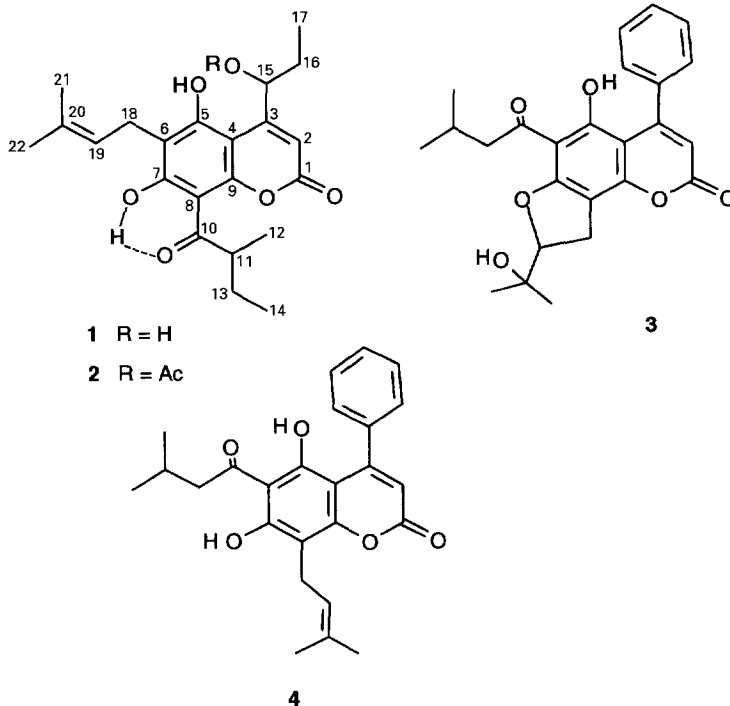
method at 300 MHz, the contour plot of which is shown in Fig. 2. Based on the above evidence the structure of assamene was established as **1**. This structure was further confirmed by the fact that assamene

on acetylation gave an acetate derivative **2** whose spectral data were very similar to an insecticidal coumarin isolated from *Mamea americana* [14].

The examination of the air dried fruit peels of *K.*

assamica afforded two known coumarins **3** [14] and **4** [12]. Compounds **1**, **3** and **4** showed appreciable antifungal properties against pathogenic fungi *Halominthosporium oryzae*, *Phytophthora oryzae*, *Alternaria solani*, *Curvularia eraglostidis* and *Colletotrichum gleosporioides* [15].

Acetylation of Assamene (1). To a soln of 45 mg of assamene (**1**) in 0.5 ml of pyridine was added 1 ml of Ac_2O and the mixt. left overnight. Usual work-up [16] and purification by prep. TLC (EtOAc–petrol, 1:10) gave 40 mg of compound **2** as gum. $[\alpha]_D^{25} = -28^\circ$ (0.8, EtOH); IR $\nu_{\text{max}}^{\text{Nujol}} \text{ cm}^{-1}$: 3 300, 2 980, 1 710, 1 695, 1 590,



EXPERIMENTAL

General. The general experimental procedures were similar to those reported elsewhere [1, 16]. GRASP ^1H – ^1H COSY was recorded with Bruker Avance DPX300 operated at 300 MHz attached with a GRASP II unit and with standard software.

Extraction and isolation of assamene 1. Air dried and powdered bark (200 g) was exhaustively extracted with petrol in a soxhlet extractor for 20 hr. The extract was concd under red. pres. The concd extract was kept overnight at room temp. with a little Et_2O . Yellow crystals of assamene (**1**) (2.1 g) sepd out from the extract that were found to be homogeneous on TLC (EtOAc–petrol, 1:7). Compound **1** was recrystallized from CHCl_3 –EtOAc (3:1) as plates, mp 135° . UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 203, 220, 248, 330, 372; (NaOH): 203, 248, 332, 372; (HCl): 220, 295, 325. IR $\nu_{\text{max}}^{\text{Nujol}} \text{ cm}^{-1}$: 3 300, 2 980, 1 695, 1 640, 1 590, 1 450, 1 400, 1 365, 1 330, 1 270, 1 220, 1 145, 1 130, 1 090, 1 055, 980, 885, 860, 840. EI-MS (15 eV) m/z : 388 $[\text{M}]^+$, 370 $[\text{M} - \text{H}_2\text{O}]^+$, 356, 331, 313, 295, 263 and 257; EI-MS (70 eV) m/z : 388, 370, 355, 341, 331, 313, 295, 285, 275, 257, 245, 229, 217, 205, 190, 176, 165, 147, 128, 105, 91, 77, 69 and 57; positive FAB-MS m/z : 389.2, 331.1, 313.1, 285.1, 231.1, 229.1, 205.1, 165.1, 115.1, 91.1, 69.1 and 41.0 (Found C: 67.99% and H: 7.25%; $\text{C}_{22}\text{H}_{28}\text{O}_6$ requires C: 68.01% and H: 7.27%).

1 450, 1 400. ^1H NMR (400 MHz): δ 0.98 (3H, t , $J = 7$ Hz; H-14), 1.07 (3H, t , $J = 7$ Hz, H-17), 1.24 (3H, d , $J = 7$ Hz; H-12), 1.40 (1H, ddq , $J = 4.7, 13$ Hz, H-13a), 1.60 (1H, ddq , $J = 3, 7, 14$ Hz, H-16a), 1.80 (1H, ddq , $J = 7, 9, 13$ Hz, H-13b), 1.82 (3H, $br s$, H-21), 1.86 (3H, $br s$, H-22), 2.01 (1H, ddq , $J = 7, 8, 14$ Hz, H-16b), 2.19 (3H, s , CH_3COO), 3.34 (1H, dd , $J = 9, 15$ Hz, H-18a), 3.42 (1H, dd , $J = 8, 15$ Hz, H-18b), 3.85 (1H, ddq , $J = 4, 7, 9$ Hz, H-11), 5.20 (1H, dd , $J = 8, 9$ Hz, H-19), 6.28 (1H, s , H-2), 6.51 (1H, dd , $J = 3, 8$ Hz, H-15), 7.27 (1H, $br s$, D_2O exchangeable OH at C-5), and 14.59 (1H, s , D_2O exchangeable OH at C-7). EI-MS m/z : 472 $[\text{M}]^+$, 454, 412, 370, 355, 331, 313, 286, 271, 245, 229, 217, 201, 165, 115, 91, 69, 57. (Found C: 66.93% and H: 6.99%; $\text{C}_{24}\text{H}_{30}\text{O}_7$ requires C: 66.96% and H: 7.02%).

Extraction of fruit peels. Air dried and powdered fruit peels (pericarp) of *K. assamica* (212 g) were extracted with petrol in a soxhlet extractor for 20 hr. The extract was concd and kept at room temp. for 3 days. A crystalline solid sepd out from the concd extract which on recrystallization from petrol–EtOAc (9:1) yielded a pure single compound **3** (27.5 g), mp 105° , whose UV, IR, NMR and MS data are similar to the reported data [14]. (Found C: 73.85% and H: 6.40%; $\text{C}_{25}\text{H}_{26}\text{O}_6$ requires C: 73.87% and H: 6.45%.)

The mother liquor (9.2 g) on gradient chromatography over silica gel with petrol, EtOAc and CHCl_3 gave a yellow crystalline solid of structure **4**

Table 1. NMR data of assamene (1)

C	¹ H NMR (δ ppm 400 MHz)	¹³ C NMR (δ ppm 100 MHz)
1	—	209.987 <i>s</i>
2	6.28 <i>s</i>	103.066 <i>d</i>
3	—	102.034 <i>s</i>
4	—	120.157 <i>s</i>
5	(OH) 11.92 <i>br s</i>	161.490 <i>s</i>
6	—	103.931 <i>s</i>
7	(OH) 13.71 <i>s</i>	160.717 <i>s</i>
8	—	151.289 <i>s</i>
9	—	156.147 <i>s</i>
10	—	167.076 <i>s</i>
11	3.66 <i>ddq</i> (<i>J</i> = 6.53, 6.75, 5.77 Hz)	46.873 <i>d</i>
12	1.17 <i>d</i> (<i>J</i> = 6.75 Hz)	16.727 <i>q</i>
13a	1.45 <i>ddq</i> (<i>J</i> = 6.53, 7.35, 13.56 Hz)	26.533 <i>t</i>
13b	1.897 <i>ddq</i> (<i>J</i> = 5.77, 7.37, 13.43 Hz)	
14	1.03 <i>dd</i> (<i>J</i> = 7.34, 7.35 Hz)	10.580 <i>q</i>
15	5.10 <i>dd</i> (<i>J</i> = 4.8, 9.7 Hz) (OH) 5.90 <i>br</i>	71.858 <i>d</i>
16a	2.01 <i>ddq</i> (<i>J</i> = 7.14, 9.69, 14.40 Hz)	26.745 <i>t</i>
16b	1.57 <i>ddq</i> (<i>J</i> = 4.8, 7.4, 14.4 Hz)	
17	0.99 <i>dd</i> (<i>J</i> = 7.42, 7.33 Hz)	11.655 <i>q</i>
18a	3.33 <i>dd</i> (<i>J</i> = 8.11, 15.3 Hz)	27.246 <i>t</i>
18b	3.41 <i>dd</i> (<i>J</i> = 15.42, 9.01 Hz)	
19	4.97 <i>ddq</i> (<i>J</i> = 1, 8.1, 9.01 Hz)	120.279 <i>d</i>
20	—	133.727 <i>s</i>
21	1.770 <i>br s</i>	25.607 <i>q</i>
22	1.70 <i>s</i>	17.972 <i>q</i>

(110 mg), mp 97° (Bz-EtOAc, 4:1), $[\alpha]_D^{21} = +22^\circ$, whose UV, IR, NMR and MS data are similar to the reported data [12]. (Found C: 71.05% and H: 6.19%; C₂₅H₂₆O₆ requires C: 71.07% and H: 6.20%.)

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