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Long chain 4-hydroxycinnamate esters from *Allamanda neriifolia* Hook

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ABSTRACT

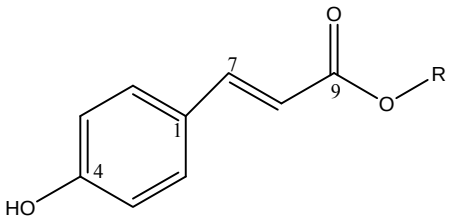
Allamanda neriifolia Hook, locally known as yellow bell is an ornamental plant that grows throughout the country. This study was conducted as part of our research on the chemical constituents of Philippine plants. A mixture of four long chain 4-hydroxycinnamate esters (**1a-1d**) was obtained from the dichloromethane extract of the air-dried flowers of *Allamanda neriifolia* Hook. The structures of **1a-1d** were elucidated by extensive one-dimensional and two-dimensional NMR spectroscopy and mass spectrometry.

Keywords: *Allamanda neriifolia* Hook, Apocynaceae, 4-hydroxycinnamate Esters, Yellow Bell.

1. Introduction

Allamanda neriifolia Hook, locally known as yellow bell, belongs to the family Apocynaceae. It is an ornamental plant that grows abundantly throughout the country. An earlier study reported the isolation of new iridoids, isoallamandicin, allamcin, allamancin, 3-*O*-methyl derivatives of allamcin and allamancin, allamcidin, allamcidin glucoside, 13-*O*-acetylplumieride, plumiepoxide, and plutoplumericin B and the known iridoids, plumericin, isoplumericin, allamandin, allamandicin, deglucosyl-plumieride, 13-*O*-p-coumaroyl plumieride, plumieride, protoplumericin, gardenoside and 10-dehydrogardenoside from the stems and leaves of *Allamanda neriifolia* Hook [1]. Plumericin, isoplumericin, and allamandin were found to be active against KB cell culture [2]. Plumericin and isoplumericin were reported to exhibit algicidal activity [3]. A previous study reported the isolation of a major iridoid, 13-*O*-(β-D-glucopyranosyl-p-coumaroyl) plumieride from the leaves and stems of *Allamanda neriifolia* Hook [4].

We report herein the isolation and structure elucidation of four long chain 4-hydroxycinnamate esters (**1a-1d**) from the dichloromethane extract of the air-dried flowers of *Allamanda neriifolia* Hook. To the best of our knowledge, this is the first report on the isolation of **1a-1d** from *A. neriifolia.*



1a R = -$(CH_2)_8CH{=}CH(CH_2)_{11}CH_3$
1b R = -$(CH_2)_{21}CH_3$
1c R = -$(CH_2)_8CH{=}CH(CH_2)_{13}CH_3$
1d R = -$(CH_2)_{23}CH_3$

2. Materials and Method

2.1 General

The NMR data were recorded in $CDCl_3$ solutions on a Bruker AMX Fourier Transform 400 NMR Spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C with $CDCl_3$ as reference, while two-dimensional NMR experiments were conducted on a Bruker DMX600 spectrometer. High resolution mass spectrum was acquired in methanol using a Bruker Bio Apex IIe 7 T Fourier Transform Ion Cyclotron Resonance mass spectrometer with an Analytica electrospray ionization source. Column chromatography was performed with silica gel 60 (70-230 mesh), while the TLC was performed with plastic backed plates coated with silica gel F254. The plates were visualized by spraying with vanillin-H_2SO_4, followed by warming.

2.2 Plant Material

Flowers of yellow bell were collected from Cavite State University, Indang, Cavite in September, 2005. The flowers were identified as *Allamanda neriifolia* Hook at the Philippine National Museum with a voucher specimen of PNH No. 252635.

2.3 Extraction and Isolation

The air-dried flowers (1.2 kg) of *Allamanda neriifolia* Hook were ground in an osterizer, soaked in CH_2Cl_2 for three days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (125 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The 20% acetone in CH_2Cl_2 fraction from the first column was rechromatographed using 15% EtOAc in petroleum ether to afford sample **1** (**1a**-**1d**, 15 mg] after washing with petroleum ether.

3. Results and Discussions

The dichloromethane extract of the air-dried flowers of *Allamanda neriifolia* Hook afforded 15 mg of a mixture of four long chain 4-hydroxycinnamate esters (**1a**-**1d**) by silica gel chromatography. This mixture was a colorless solid which produced a characteristic blue spot when warmed after spraying with vanillin-sulfuric acid solution. It gave an R_f value of 0.28 in 5% EtOAc in petroleum ether as developing solvent. The structures of **1a**-**1d** were elucidated by extensive 1D and 2D NMR spectroscopy and mass spectrometry as follows.

The ^1H-NMR spectrum of **1a** (Table 1) indicated the presence of aromatic proton doublets resonating at δ 7.43 (J = 8.8 Hz) and 6.83 (J = 8.8 Hz) which integrated for two protons each. The large coupling constant suggested ortho coupling. A broad singlet at δ 5.12 which integrated for one proton suggested an aromatic hydroxyl proton as deduced from its deshielded resonance. Olefinic proton doublets which integrated for one proton each were detected at δ7.62 (J =16.0 Hz) and 6.30 (J = 16.0 Hz). The large coupling constant suggested that these protons were located *trans* to each other. The deshielded nature of these protons revealed that they were conjugated to an aromatic system. Further deshielding was attained through the electron withdrawing effect of the conjugated ester carbonyl. The olefinic proton at δ 7.62 was assigned to H-7 since it was significantly more deshielded than the other olefinic proton at δ 6.30 (H-8). This deshielding of H-7 was due to the ring current of the aromatic π electrons. Thus, **1a** contained a 1,4-disubstituted benzene with a *trans* olefin conjugated to an ester carbonyl attached to C-1 and a hydroxyl attached to C-4. A 4-hydroxycinnamate moiety [5] was deduced from the ^1H NMR spectrum of **1a**.

Table 1: 400 MHz ^1H-NMR and 100 MHz^{13}C-NMR, COSY and HMBC Correlations of 1a in $CDCl_3$.

Position	δ_C	δ_H mult. (J in Hz)[a]	COSY, δ (J Hz)	HMBC correlations, δ
1	127.50	---------	----------	
2	115.83	6.83 (d, J = 8.8)	7.43 (d, J = 8.8)	
3	129.90	7.43 (d, J = 8.8)	6.83 (d, J = 8.8)	
4	157.40	---------	---------	
5	129.90	7.43 (d, J = 8.8)	6.83 (d, J = 8.8)	
6	115.88	6.83 (d, J = 8.8)	7.43 (d, J = 8.8)	
7	144.08	7.62 (d, J = 16.0)	6.30 (d, J = 16.0)	
8	115.95	6.30 (d, J = 16.0)	7.62 (d, J = 16)	
9	167.42	---------	---------	
1'	64.63	4.20 (t, J = 6.8)	1.70	1.70, 1.40
2'	28.77	1.70	1.40, 4.20 (t, J = 6.8)	4.20, 1.40, 1.26
3'	29.33	1.40	1.70, 1.26	1.26, 1.70, 4.20
4'	29.60	1.26	1.40	1.40, 1.70
5'-6'	29.7	1.26		
7'	29.77	1.30	2.01	1.26, 2.01, 5.35
8'	27.22	2.01	5.35 (t, J = 4.4)	1.26, 1.30, 5.35
9'	129.90	5.35 (t, J = 4.4)	2.01	1.26, 1.30, 2.01
10'	129.90	5.35 (t, J = 4.4)	2.01	1.26, 1.30, 2.01
11'	27.22	2.01	5.35 (t, J = 4.4)	1.26, 1.30, 5.35
12'	29.78	1.30	2.01	1.26, 1.30, 5.35
13'-19'	29.7	1.26		
20'	29.66	1.26		1.27, 0.88, 1.26
21'	31.91	1.27	1.26, 0.88 (t, J = 6.4)	0.88, 1.27, 1.26
22'	14.12	0.88 (t, J = 6.4)	1.27	1.26, 1.27

[a] Multiplet unless otherwise indicated

From the shielded region of the ^{1}H-NMR spectrum, long chain fatty alcohols were evident [6]. The characteristic resonances of fatty alcohols are as follows: a methyl triplet at δ0.88 (t, J = 6.4 Hz); methylene protons of long chain fatty alcohols centered at δ1.26; and oxymethylene protons at δ 4.20 (t, *J* = 6.8). The deshielded nature of the oxymethylene protons suggested that these fatty alcohols were esterified to the 4-hydroxycinnamate. There is one remaining resonance in the deshielded region of the spectrum at δ 5.35 which integrated for two protons which was previously unassigned. This chemical shift suggested olefinic protons which are usually found at C_9 and C_{10} in most naturally occurring fatty acids [6]. The presence of allylic methylene protons was indicated by the resonance at δ 2.01 which integrated for about four protons. Thus, the fatty alcohol attached to the 4-hydroxycinnamate is unsaturated.

In the most shielded region of the spectrum, the methyl triplet at δ0.88 integrated for more than five protons. Since this methyl triplet is only found at one end of the fatty alcohol, then it should integrate for only three protons. Thus, sample **1** is actually a mixture of unsaturated and saturated fatty alcohols esterified to the 4-hydroxycinnamate. To verify this assumption and determine the chain lengths of the fatty alcohols, the mass spectrum was obtained. The mass spectrum of sample **1** gave a peak at *m/z* 164, indicating a stable fragment for 4-hydroxycinnamate ($C_9H_7O_3$) and a base peak at *m/z* 147 for the cinnamate ($C_9H_7O_3$ - OH). It also gave molecular ions at *m/z* 470, *m/z* 472, *m/z* 498 and *m/z* 500. Furthermore, the high resolution mass spectrum of sample **1** gave molecular ion peaks at *m/z* 470.3764 corresponding to the molecular formula of $C_{31}H_{50}O_3$ (**1a**) and at *m/z* 472.3916 corresponding to $C_{31}H_{52}O_3$ (**1b**). The removal of 4-hydroxycinnamate ($C_9H_7O_3$) from the molecular ion ($C_{31}H_{50}O_3$) of **1a**, resulted into an unsaturated C_{22} fatty alcohol ($C_{22}H_{43}$) attached to the 4-hydroxycinnamate. Likewise, the removal of 4-hydroxycinnamate ($C_9H_7O_3$) from the molecular ion ($C_{31}H_{52}O_3$) of **1b**, resulted into a saturated C_{22} fatty alcohol ($C_{22}H_{45}$). These data obtained from the mass spectrum supported the structures of **1a** and **1b**. Similarly, the molecular ions at *m/z* 498 and *m/z* 500 detected from the mass spectrum supported the structures of **1c** and **1d** with two methylenes added to **1a** and **1b**, respectively.

The ^{13}C and DEPT NMR spectra of **1a** (Table 1) gave resonances for carbons with the following functionalities: conjugated carbonyl carbon of an ester at δ 167.42; protonated aromatic, olefinic and conjugated olefinic carbons resonating at δ 115.83, 115.95, 129.90, 144.08 and 157.40; non-protonated aromatic carbons at δ 127.50 and 157.40; an oxymethylene carbon at δ 64.63; methylene carbons at δ 22.69, 25.00, 27.22, 28.77, 29.33, 29.53, 29.55, 29.57, 29.60, 29.66, 29.70, 29.78 and 31.91; and a methyl carbon at δ 14.02. It is noted that some of the methylene carbon resonances clustered at about δ 29.7 (29.53, 29.55, 29.57, 29.60, 29.66, 29.70, and 29.78) are overlapping. The relatively shielded nature of the carbonyl carbon of an ester at δ 167.4 confirmed its conjugation to a double bond as suggested in the ^{1}H NMR discussion. The deshielded aromatic carbon at δ 157.4 suggested that it is bonded to the hydroxyl of the cinnamate ester. The almost overlapping methylene carbons centered at δ 29.7 supported the presence of fatty alcohols [6, 7] in **1a** as deduced from the ^{1}H NMR spectrum.

Based on the COSY spectrum (Table 1 and Figure 1), a total of five isolated spin systems were deduced as follows. For the 4-hydroxycinnamate moiety of **1a**, two isolated spin systems were detected. The aromatic protons at δ 7.43 and 6.83; and the olefinic protons at δ 7.62 and 6.30 were respectively coupled to each other. For the fatty alcohol moiety of **1a**, three isolated spin systems were deduced. The oxymethylene protons at δ 4.20 (t, *J* = 6.8) esterified to the 4-hydroxycinnamate were coupled to the methylene protons at δ 1.70, which were in turn coupled to another set of methylene protons at δ 1.40, which were finally coupled to the methylene protons at δ 1.26. The second isolated spin system starts with the methyl triplet at the other end of the fatty alcohol. This methyl triplet at δ 0.88 was coupled to the methylene protons at δ 1.27, which were in turn coupled to another methylene group at δ 1.26. From the ^{1}H NMR spectrum, it was deduced that the fatty alcohol was unsaturated. The olefinic proton at δ 5.35 was coupled to the allylic methylene protons at δ 2.01, which were in turn coupled to the methylene protons at δ 1.30, which were finally coupled to the methylene protons at δ 1.26. Connectivities of these three fragments could not be deduced from the COSY spectrum due to the overlapping methylene protons centered at about δ 1.26.

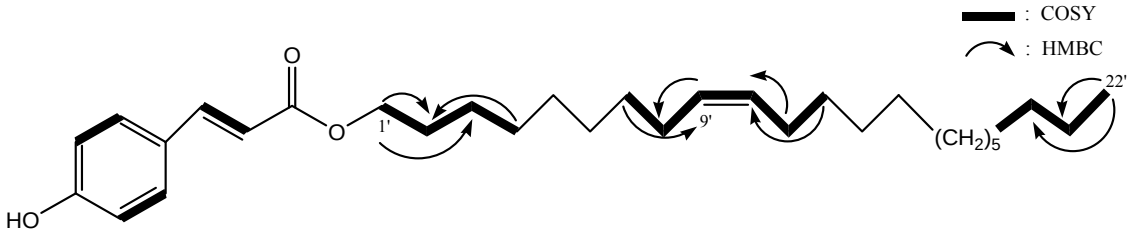


Fig 1: ^{1}H-^{1}H COSY and ^{1}H-^{13}C long-rang correlations of **1a**.

Protons attached to carbons were assigned (Table 1) from HSQC 2D NMR data and the structure of **1a** was supported by analysis of the HMBC 2D NMR data: key HMBC correlations are shown in Figure 1 and Table 1. It is noted that only the HMBC of the shielded region of the spectrum was provided. Thus, only the fatty alcohol moiety of **1a** could be analyzed from the available HMBC spectrum. Long-range correlations were observed between H_2-2′, H_2-3′ and C-1′; H_2-1′, H_2-3′, H_2-4′ and C-2′; H_2-1′, H_2-2′ and C-3′; and H_2-2′, H_2-3′ and C-4. Another set of long-range correlations were detected for the olefinic protons as follows: H_2-7′, H_2-8′ and C-9′; H_2-11′, H_2-12′ and C-10′; H-9′, H-10′ and C-8′; and H-9′, H-10′ and C-11′. At the end of the fatty alcohol chain, H3-22′ was long-range correlated to C-21′ and C-20′. This supported the isolated spin systems of the fatty alcohol portion of **1a** deduced from COSY. However, due to overlapping resonances for both the protons centered at about δ 1.26 and the carbons centered at about δ 29.7, the position of the double bond in the long fatty alcohol chain could not be ascertained based on HMBC. Likewise, the location of the double bond in the long chain fatty alcohol could not be deduced from the fragmentation pattern in the mass spectrum because of its facile migration in the fragments. Thus, the double bond was assigned to C_9 since most naturally occurring fatty acids have double bonds in this position. Literature search revealed that the two saturated long chain fatty alcohols esterified

to 4-hydroxycinnamate (**1b** and **1d**) were previously reported from the leaf fibers of abaca (*Musa textilis*) [8]. Compound **1b** was also isolated from *Psidia punctulata* [9], while **1d** was also reported in another study [10].

4. Conclusion

A mixture of four long chain 4-hydroxycinnamate esters (**1a-1d**) were obtained from the dichloromethane extract of the air-dried flowers of *Allamanda neriifolia* Hook. The structures of **1a-1d** were elucidated by extensive one-dimensional and two-dimensional NMR spectroscopy and mass spectrometry. Compounds **1b** and **1d** were previously reported from the leaf fibers of abaca (*Musa textilis*). To the best of our knowledge, this is the first report on the isolation of **1a-1d** from *Allamanda neriifolia* Hook.

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