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Aromatic Compound Glucopyranoside from New Species *Artocarpus thailandicus*

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Abstract: Problem statement: Many Artocarpus plants belonging to the family Moraceae are found in Thailand and have been used as Thai traditional medicines. These plants are known to produce a variety of phenolic glycoside. *A. thailandicus* is the new species and this prompted us to make further investigations on the occurrence of these types of compounds in the new Artocarpus species. Thus, continuation of our work has resulted in the isolation of two aromatic glycoside compounds from the ethyl acetate extract of the leaves and twigs of *A. thailandicus*. **Approach:** The *A. thailandicus* was selected and percolated with hexane and ethyl acetate. The ethyl acetate extract was purified and elucidated chemical structures. The purified compounds were tested for anti-bacterial activity. **Results:** Two compounds, p-hydroxyphenyl-6-acetoxy-β-D-glucopyranoside (1) and p-hydroxyphenyl-β-D-glucopyranoside (2), were isolated from the leaves and twigs of *A. thailandicus* by means of chromatography. Structural elucidation was achieved by ^{1}H, ^{13}C and 2D-NMR, as well as mass spectral data. **Conclusion:** The results showed that the two aromatic compound glucopyranosides from *A. thailandicus* can be recorded as constituent in the new species.

Key words: *Artocarpus thailandicus*, moraceae, aromatic compound glucopyranoside

INTRODUCTION

The genus Artocarpus (Moraceae) comprises approximately 50 species and is widely distributed in tropical and subtropical regions of Southeast Asia (Nomura *et al*., 1998; Syah *et al*., 2001). Some isolated compounds from genus Artrocarpus have been shown to exhibit interesting biological activities including cytotoxic, anti-inflammatory, antiplatelet, antioxidant, antibacterial and anticomplementary effects (Suhartati *et al*., 2001). *A. thailandicus* is the new species, which was identified by The Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Thailand (BKF no.119030). There are no reports the chemical constituent from *A. thailandicus*.

MATERIALS AND METHODS

General experimental procedure: UV spectra were obtained on a ShimadzuUV-1601 spectrophotometer with EtOH as solvent. Melting point was measured on a BÜchi 322 micro melting point apparatus and has to be uncorrected. IR spectra in KBr disk were recorded on Shimadzu 8900 FTIR spectrophotometer. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were taken on a Bruker DPX spectrometer with CD_3OD solutions. The chemical shifts were recorded in δ values which were referenced to CH_3OH as the internal standard (^{1}H = δ 3.31; ^{13}C = δ 48.99). ^{13}C-^{1}H, COSY, HMBC and 1D spectra were obtained with the usual pulse sequence and the data processing was performed with standard Bruker DPX software. CC was carried out under TLC monitoring using silica gel 60 (Merck 7734, 70-230

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mesh, MERCK) and detection with using UV detector. Mass spectra were recorded on HR-TOF-MS: Micro-mass model VQ-Tof 2 for HRMS and Thermo Finnigan Polaris Q for EIMS.

Plant material: The *A. thailandicus* leaves and twigs part were collected in July, 2010 from Trang province of Thailand and identified by Forest Herbarium. A voucher specimen (BKF no. 119030) has been deposited at the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok, Thailand.

Extraction and isolation: The air-dried powdered leaves and twigs of *A. thailandicus* (1.36 kg) were successively percolated with hexane (4 L×3 days×7 times) and then extracted with ethyl acetate, (4 L×3 days×8 times), at room temperature, respectively and followed by filtration. The filtrates were combined and evaporated to dryness under reduced pressure to afford defatted hexane extract (9.36 g) and ethyl acetate extracts (18.92 g), respectively (Nantapap *et al*., 2010). The ethyl acetate extract was subjected to silica gel chromatography [EtOAc –Hexane, 0:100, 10:90, 50:50, 80:20] to give subfractions B_1, B_2 and B_3. The semi-solids of B_2 were recrystallized with 95% ethanol to give the purified white needle crystals 0.06 g which identified as p-hydroxyphenyl-6-acetoxy-β-D-glucopyranoside or arbutin ester (1). The compound 2 was isolated from F_6 by CC techniques 3 times to afford subfractions C_1-C_5, D_1-D_3 and E_1-E_3, respectively. The solids of subfraction E_3 were crystallized with 95% ethanol to give the purified white needle crystals in 0.03 g and recognized as p-hydroxyphenyl-β-D-glucopyranoside or arbutin.

RESULTS

In the present work, the defatted ethyl acetate extract of the new plant *A. thailandicus* was subjected to phytochemical investigation leading to the isolation of two compounds. Both of them are known natural products showing a *para*-hydroxyphenyl substituent at C-1' and acetoxy unit at C-6 in glucose moiety. However, their accurate structures were intensively established by spectroscopic means and also by comparison with closely related compounds.

DISCUSSION

Compound 1 was obtained as a white needle in ethanol, melting point 205.5-207.9°C. The empirical formula was deduced as $C_{14}H_{18}O_8$ from ion peak at shown an m z^{-1} = 314 in EIMS. The key fragmentation ions in the mass spectrum of compound 1 at m/z 314 [M^+] and 110 (base peak), were useful in obtaining the structure of 1. The ions at mz^{-1} 110 for $C_6H_6O_2^{+\cdot}$, which loss of a stabilized *para*-hydroxyphenol confirmed the structure of phenolic glucopyranoside derivatives. The IR spectrum absorption bands at 3502–3255 (OH), 1735 (C = O of ester), 1600, 1575, 1512, 1450 (C = C of aromatic) and 1280, 1074 (C–O–C linkage) and the UV bands at 286, 223 nm (log ε 3.71 and 4.28, respectively) indicated the π→π* of aromatic and n→π* which belong to carbonyl chromosphore. The ^{1}H and ^{13}C NMR spectral data indicated the presence of one hydroxyphenyl, one acetoxy and β-D-glucopyranosyl group. Furthermore, the HMBC correlation from H-1' (δ4.71) for anomeric proton to C-1 (δ 153.14) gave evidence for the attachment of the p-hydroxyphenyl moiety to C-1 of the central glucose unit which has ^{13}C NMR resemble with previously report (Iossifova *et al*., 1999). The relationship between the dihedral angle and vicinal coupling constant ^{3}J is given theoretically by the Karplus equation: ^{3}Jab = $J^0\cos^2$f-0.28(0°<f<90°) and ^{3}Jab = $J^{180}\cos^2$f-0.28(90°<f<180°). Thus, the relative configuration at H-1' and H-2' could be determined by the $^3J_{1',2'}$, H-C-C-H (7.4 Hz) coupling constant which indicated that the two protons were located opposite side with dihedral angle 180° (Pradupsri *et al*., 2009). Thus, a doublet at δ4.71 ppm (1H, d, J = 7.4 Hz) integrating for a single proton was attributed to H-1' of glucose, indicating a β-linkage (Table 1). Our 2D-NMR spectroscopic investigation strongly supported the p-hydroxyphenyl component at the C-1' position of glucose. The arrangement of this hydroxyphenyl linkage was further confirmed by the HMBC correlation from H-1'-C-1 (δ153.14). On the other hand, the chemical shifts of aromatic carbons were deduced by the DEPT, HMBC and COSY experiments.

The ^{13}C NMR signals (Table 2) at δ120.26 (C-2, 6), 117.29 (C-3,5) and δ153.14 (C-1), 154.89 (C-4) were to be in the right place of methine carbon and quaternary carbon, respectively. Moreover, the aromatic of this compound was found to have a hydroxyl group at C-4 since AA'BB' pattern of H-2, H-6 and H-3, H-5 was observed at δ 6.94 and 6.69, respectively. ($J_{2,3} = J_{3,2} = J_{5,6} = J_{6,5}$ = 6.7 Hz and $J_{2,6} = J_{6,2} = J_{3,5} = J_{5,3}$ = 2.3 Hz). In addition, the position of linkage of the terminal glucopyranoside moiety at 6' of central glucose unit was deduced from the ^{13}C NMR spectrum and HMBC correlation. The relationship from H-6'a, b (δ4.38, 4.25) -C-1'' (δ173.78) gave evidence for the attachment of the acetoxy moiety to C-6'. From the above spectral data, the structure of 1 (Fig. 1) was thus established as p-hydroxyphenyl-6-acetoxy-δ-D-glucopyranoside.

Table 1 ^{1}H NMR spectral data of compound 1 and 2 in CD_3OD (400 MHz)

Position	1 (δ, m, J)		2 (d, m, J)	
p-hydroxyphenyl				
2, 6	6.94	2H, (AA'BB', $J_{2,3}$, $J_{6,5}$ = 6.7, 2.3)	6.97	2H, (AA'BB', $J_{2,3}$, $J_{6,5}$ = 6.7, 2.3)
3, 5	6.69	2H, (AA'BB', $J_{3,2}$, $J_{5,6}$ = 6.7, 2.3)	7.0	2H, (AA'BB', $J_{3,2}$, $J_{5,6}$,= 6.7, 2.3)
Glucose				
1'	4.71	1H, d (7.4)	4.73	1H, d (7.4)
2'	3.41	1H, t-like (7.4)	3.44	1H, t-like (7.4)
3'	3.43	1H, obsc.	3.37	1H, obsc.
4'	4.48	1H, obsc.	3.39	1H, obsc.
5'	3.57	1H, m	3.42	1H, obsc.
6'	4.38	1H, Ha, dd (11.8, 4.3)	3.89	1H, Ha, m
	4.25	1H, Hb, dd (11.8, 6.4)	3.70	1H, Hb, m
Acetoxy 2''	2.05	3H, s		

δ in ppm from TMS [coupling constants (J) in Hz are given in parentheses], obsc. = obscure

Table 2 ^{13}C NMR , ^{1}H – ^{13}C and ^{1}H – ^{1}H correlations spectral data of compound 1 and 2 in CD_3OD (400 and 100 MHz)*

				HMBC		COSY	
C	1 (δ ^{13}C)	2 (δ ^{13}C)	H	1	2	1	2
p-hydroxyphenyl							
1	153.14	152.44	1	-	-	-	-
2	120.29	119.41	2	C-4	C-4	H-3	H-3
3	117.29	116.62	3	C-1	C-1	H-2	H-2
4	154.89	153.81	4	-	-	-	-
5	117.29	116.62	5	C-1	C-1	H-6	H-6
6	120.29	119.41	6	C-4	C-4	H-5	H-5
Glucose							
1'	104.22	103.66	1'	C-1, C-2', C-3'	C-1, C-3', C-5'	H-2'	H-2'
2'	75.36	74.99	2'	C-1'	C-4'	H-1'	H-1', H-3'
3'	78.31	78.05	3'	-	-	-	H-2', H-4'
4'	72.09	71.46	4'	-	C-3', C-5', C-6'	H-5'	H-3', H-6'b
5'	75.70	78.02	5'	C-3', C-4'	-	H-4', H-6'b	-
6'	65.12	62.58	6'a	C-4', C-5', C-6', C-1²	C-4', C-5'	H-6'b	H-6'b
			6'b	C-4', C-5', C-6', C-1²	C-4', C-5'	H-5', H-6¢a	H-6'a
Acetoxy							
1''	173.78		1''	-		-	-
2''	20.84		2''	C-1''		-	-

*: Assignment from DEPT experiments

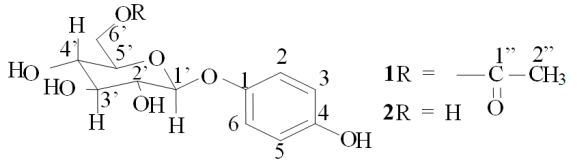

Fig. 1: Structure of 1 and 2

Compound 2 (arbutin) (Yang *et al*., 2010) secluded from the ethyl acetate extract was obtained as white crystals in ethanol (mp 162.3-162.9°C) analyzed for $C_{12}H_{16}O_7$ by means of EIMS measurement on the [M^+] ion (mz^{-1}) 272. The UV spectrum of the compound in methanol showed absorption bands maxima at 286 and 223 nm (log ε3.50 and 4.04) which were characteristic of phenolic part in structure. The mass spectrum of compound 2 was suggested to has the bear a resemblance to structure as in 1, except the position-6 of the acetoxy is different from compound 1. The fragment ion in the mass spectrum at m/z 110 (base peak) belonging to the para-hydroxyphenol ($C_6H_6O_2^{+\cdot}$) was in agreement with those observed in compound 1. On the other hand, the 400 MHz ^{1}H NMR spectrum of 2 (Table 1) demonstrated the presence of four aromatic protons, five methine protons for glucose unit, two alkyl protons for one methylene groups. The five methine protons resonated in the downfield region when comparison with 1 at δ 4.73 (H-1'), 3.44 (H-2'), 3.37 (H-3'), 3.39 (H-4') and 3.42 (H-5'), indicating that they are in the region of the glucose moiety. In addition, the doublet, δ 4.73 H-1' displayed on the glucose region suggesting the presence of anomeric proton coupling

with J = 7.4 Hz, together with two AA'BB' pattern (δ6.97, H-2, 6 and 7.0, H-3, 5) consistent with two ortho related hydrogens in an aromatic of glucopyranoside moiety.

The ^{13}C NMR spectrum (Table 2) of closely resembled that of 1. On the basis of systematic ^{13}C NMR spectroscopic analysis of arbutin systematical structure stated that the resonance for glucoside carbon C-1', C-2', C-3', C-4', C-5' and C-6' were found at δ103.66, 74.99, 78.05, 71.46, 78.02 and 62.58, respectively. Moreover, the anomeric protons at δ4.73 exhibited HMBC correlations to aromatic carbons δ152.44 (C-1) together with the aromatic protons H-2, 6 and H-3, 5 correlated with carbon C-3, 5 and C-2, 6, respectively. Additionally, the HMBC spectrums confirmed the involvement of C-6' as in the glucose ring. This structure was agreement with the related structure, arbutin which was reported by (Pop *et al*., 2009).

Antibacterial activity: Additionally, the isolated compounds were assessed for antibacterial activity against 11 bacteria strains (all obtained from The National Institute of Health, Department of Medical Sciences, Bangkok) by Minimum Inhibitory Concentration (MIC) value. Determination of the MIC was carried out according to the modified resazurin assay described by Sarker *et al*. (2007) with some modifications. The result showed that both two compounds exhibited antibacterial activity with MIC value ranging from 250-500 μg mL^{-1}. Compound 1 was effective against 3 strains of bacteria including *Escherichia coli* (ETEC) DMST 30543, *Salmonella typhi* DMST 22842 and *Proteus mirabilis* DMST 8212. Compound 2 showed antibacterial activity against 5 bacteria strains including *E. coli* (ETEC), *P. mirabilis*, *Enterobacter aerogenes* ATCC 13048, *Ent. cloacae* ATCC 23355 and *Staphylococcus aureus* ATCC 25923.

CONCLUSION

Arbutin is both an ether and a glycoside; a glycosylated hydroquinone extracted from bearberry plant in the genus Arctostaphylos. In adition, arbutin could be inhibited the tyrosinase and thus prevents the formation of melanin. Moreover, arbutin is therefore used as a skin-lightening agent and found in wheat together with also in *Bergenia crassifolia* (Pop *et al*., 2009). These new results stimulated our investigation regarding the composition of glycosidically bound phenolic in the new species *A. thailandicus*. Moreover, the spectroscopic techniques prompted us to gain more information for structural elucidation of these two compounds.

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