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Isolation of Terpenes and Coumarin from the Stem Bark of *Jatropha pandurifolia* (Andr.)

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A total of seven compounds were isolated from the methanolic extract of the stem bark of *Jatropha pandurifolia* Andr. Their structures were identified as 3-O-acetylaleuritolic acid (**1**), jatropholone A (**2**), jatropholone B (**3**), 2α-hydroxyjatropholone (**4**), 2β-ydroxyljatropholone (**5**), scopoletin (**6**) and aleuritolic acid 3-*p*-hydroxycinnamate (**7**) by spectroscopic studies including ^{1}H NMR analyses as well as by comparison with published data. Among them, compound (**7**) was the first occurrence from the genus of the *Jatropha* of *Euphorbiaceae* family and compound (**1**) and (**6**) were isolated for the first time from this plant.

Key Words: *Jatropha pandurifolia*, Euphorbiaceae, 3-O-acetylaleuritolicacid, Jatropholone A, Jatropholone B, 2α-Hydroxy jatropholone, 2β-Hydroxy jatropholone, Scopoletin, Aleuritolic acid 3-*p*-hydroxycinnamate.

INTRODUCTION

The genus *Jatropha* belonging to the family *Euphorbiaceae*, comprises of 200 species, which were distributed mainly in the tropical and subtropical regions of America and Africa. *Jatropha pandurifolia* Andr. (synonyms: *Jatropha integerrima* Jacq.) commonly known as Peregrina or spicy jatropha or fiddlehead jatropha, is a species of flowering plant native to Cuba and Hispaniola[1]. It is a shrub attaining a height of 1-1.5 m, was introduced to the Philippines is now widely spreaded to West Indies especially Cuba[2].

Traditionally this plant is used to treat the conditions like warts, tumors, rheumatism, herpes, purities, toothaches, scabies, eczema and ring worm. The plant also showed significant antioxidant, antimicrobial and cytotoxic activities[3]. Integerrimene, a possible biogenetic precursor of the rhamnofolane diterpenes and a new rhamnofolane endoperoxide 2-epicaniojane were isolated from the roots of *J. integerrima*[4]. Five new compounds and 9 known compounds have been isolated from the roots of *J. integerrima*[5]. Two cyclic heptapeptides named integerrimide A and B were isolated from the latex of *J. integerrima*. These compounds showed antifungal, antimalarial as well as effects on nerve and cancer cells[6]. Present communication reports the isolation of 3-O-acetylaleuritolicacid (**1**), jatropholone A (**2**), jatropholone B (**3**), 2α-hydroxyjatropholone (**4**), 2β-hydroxyjatropholone (**5**), scopoletin (**6**) and aleuritolic acid 3-*p*-hydroxycinnamate (**7**) from the methanolic extract of the stem bark of *J. pandurifolia*.

EXPERIMENTAL

^{1}H NMR spectra were obtained using a Varian Unity 500 spectrometer (500 MHz) in $CDCl_3$. Vacuum liquid chromatography (VLC) and Gel permeation chromatography were conducted on silica gel (Kieselgel 60, mesh 70-230) and sephadex LH-20 respectively. TLC and preparative TLC were carried out using Merck silica gel 60 PF_{254} on glass plates at a thickness of 0.5 mm and spots were visualized under UV light (254 nm and 366 nm) and spraying with 1% vanillin- H_2SO_4 followed by heating at 110° C for 5-10 min.

Plant samples of *J. pandurifolia* were collected from Dhaka in August, 2011. A voucher specimen had been maintained in the herbarium of the Department of Botany, University of Dhaka under the accession number DUH-6297 for future reference.

The stem bark after cutting into small pieces was dried under sun for several days. The plant materials were then oven dried for 24 h at considerably low temperature (40 ºC) for better grinding. The coarse powder was then stored in air tight container and kept in cool, dry and dark place for future use.

Extraction and isolation: About 665 g of powdered stem barks of the plant was soaked in 2 L of methanol for a period of 2 months with occasional shaking and stirring. The whole mixture was then filtered off through a cotton plug followed by Whatman filter paper No. 1 and the filtrate thus obtained was concentrated at 40 ºC with a rotary evaporator. The weight of the crude extract was 24 gm, which was then fractionated with vacuum liquid chromatography (VLC)[7] using *n*-hexane, ethyl acetate and methanol. A total of 24 fractions were eluted

and among them vacuum liquid chromatography fraction 5 (8 % EH, 3.5 g) was further fractionated with gel permeation chromatography[8] using sephadex LH-20 as stationary phase. The column was then eluted with 20 % petroleum ether in chloroform and then with 10 % petroleum ether in chloroform and 100 % chloroform. Finally the column was washed with chloroform and methanol mixtures with increasing polarity. A total of 43 fractions were collected, each 1 mL. Pre-coated thin layer chromatography[9] of column fractions eluted with 20:80 petroleum ether : chloroform (column fractions 2-4) over silica gel using toluene-ethyl acetate (99:1) afforded compound **1** (4 mg). Again, pre-coated thin layer chromatography of column fractions eluted with 20:80 petroleum ether: chloroform (column fractions 14-23) over silica gel using 95 % toluene in 5 % ethyl acetate as the developing solvent gave compound **2** (5 mg) and compound **3** (5 mg) and from column fractions 39-43 eluted with 100 % methanol using pre-coated thin layer chromatography over silica gel using toluene-ethyl acetate (99:5) afforded mixture of two compounds compound **4** (3 mg) and compound **5** (3 mg) and also resulted compound **6** (5 mg) and compound **7** (3.5 mg).

Detection method: 3-O-Acetylaleuritolic acid (**1**): (4 mg) white crystalline form; showed dark quenching spot under UV light (254 nm) as well as it also gave violet colour on the TLC plate after spraying with vanillin-sulfuric acid followed by heating at 110 ºC for 2 min.

^{1}H NMR (500 MHz, $CDCl_3$) δH: 5.52 (1H, dd, *J* = 7.5, 3 Hz, H-15), 4.45 (1H, dd, *J* = 10, 5.2 Hz, H-3), 2.02 (3H, s, OOCMe), 0.95 (3H, s, Me), 0.94 (3H, s, Me), 0.93 (3H, s, Me), 0.91 (3H, s, Me), 0.90 (3H, s, Me), 0.87 (3H, s, Me), 0.83 (3H, s, Me).

Jatropholone A(2): (5 mg) Colourless cubic crystal. It was evident as a black spot under the both 254 nm and 356 nm UV light on TLC and deep blue spot with vanillin-sulfuric acid followed by heating for 1 min. ^{1}H NMR (500 MHz, $CDCl_3$) δH : 3.27 (1H, dd, J = 16.5, 8 Hz, H-1), 2.52 (1H, dd, *J* = 16.5, 4.5 Hz, H-1), 2.71 (1H, m, H-2), 2.71 (1H, m, H-7), 2.62 (1H, m, H-7), 1.82 (1H, m, H-8), 0.90 (1H, dd, *J* = 13, 6 Hz, H-8), 0.94 (1H, ddd, *J* = 12, 8.5, 3.5 Hz, H-9), 1.59 1H, d, *J* = 8 Hz, H-11), 1.29 (3H, d, *J* = 7.5Hz, H-11), 1.29 (3H, d, *J* = 7.5Hz, H-16), 5.20 (1H, bs, H-17), 4.68 (1H, s, H-17), 1.25 (3H, s, H-18), 0.83 (3H, s, H-19), 2.28 (3H, s, H-20).

Jatropholone B (3): (5 mg) Colourless cubic crystal. It gave a black spot under the both 254 nm and 356 nm UV light on TLC and deep blue spot with vanillin-sulfuric acid followed by heating for 1 min. ^{1}H NMR (500 MHz, $CDCl_3$) δH: 3.18 (1H, dd, *J* = 16.5, 8Hz, H-1), 2.52 (1H, dd, *J* = 16.5, 3.5 Hz, H-1), 2.63 (1H, m, H-2), 2.63 (1H, m, H-7), 2.60 (1H, m, H-7), 1.80 (1H, m, H-8), 0.87 (1H, dt, *J* = 13.5, 6.5 Hz, H-8), 0.93 (1H, ddd, *J* = 12.5, 4, 3.5 Hz, H-9), 1.59 (1H, d, *J* = 8.5 Hz, H-11), 1.29 (3H, d, *J* = 7.5 Hz, H-16), 5.23 (1H, bs, H-17), 4.67 (1H, s, H-17), 1.23 (3H, s, H-18), 0.80 (3H, s, H-19), 2.26 (3H, s, H-20).

2α-Hydroxyjatropholone (4): (3 mg) pale yellow oil; showed a black spot under the both 254 nm and 356 nm UV light on TLC and deep blue spot with vanillin-sulfuric acid followed by heating for 1 min. The ^{1}H NMR (500 MHz, $CDCl_3$) δH : 3.10 (1H, d, *J* = 16.3Hz, H-1) 2.99 (1H, d, *J* = 16.3 Hz, H-1), 2.71 (1H, ddd, *J* = 15.0, 6.3, 2.3 Hz H-7) 2.62 (1H, ddd, *J* = 15.0, 7.0,5.0 Hz, H-7), 1.82 (1H, m, H-8), 0.94 (1H, ddd, *J* = 12.5, 8.1, 5.5 Hz, H-9), 1.55 (1H, d, *J* = 8.1 Hz, H-11), 1.40 (3H, s, H-16), 5.17 (1H, s, H-17) and 4.63 (1H,s, Hz H-17), 1.22 (3H, s, H-18), 0.80 (3H, s, H-19), 2.25 (3H, s, H-20).

2β-Hydroxyjatropholone (5): (3 mg) Pale yellow oil; evident as a black spot under the both 254 nm and 356 nm UV light on TLC and deep blue spot with vanillin-sulfuric acid followed by heating for 1 min. The ^{1}H NMR (500 MHz, $CDCl_3$) δH : 3.07 (1H, t, *J* = 17.1 Hz, H-1), 2.61 (1H, obs dd, *J* = 10.4, 4.7 Hz, H-7), 2.62 (1H, ddd, *J* = 15.0, 7, 5 Hz, H-7), 1.81 (1H, ddd, *J* = 10, 7.9, 3.5Hz, H-8), 0.87 (1H, m, H-8), 0.93 (1H, ddd, *J* = 11.1,8.6, 3.9 Hz, H-9), 1.55 (1H, d, *J* = 8.1 Hz, H-11), 1.38 (3H, s, H-16), 5.27 (1H, s, H-17), 4.73 (1H, s, H-17), 1.22 (3H, s, H-18), 0.80 (3H, s, H-19), 2.26 (3H, s, H-20).

Scopoletin (6): (5 mg) white crystal; showed blue fluorescence under UV light (366 nm) on TLC plate and was evident as no colour after spraying with vanillin-sulfuric acid reagent followed by heating at 110 ºC for 2 min. The ^{1}H NMR (500 MHz, $CDCl_3$) δH: 6.26 (1H, d, *J*= 8.8 Hz, H-3), 7.50 (1H, dd, *J* = 8.6, 2.0 Hz, H-4), 6.83 (1H, s, H-5), 3.95 (3H, br. s, OMe-6), 6.09 (1H, br. s, H-7), 6.91 (1H, s, H-8).

Aleuritolic acid 3-*p*-hydroxycinnamate (7): (3.5 mg) white crystal; showed dark quenching spot under UV light (254 nm) as well as it also gave violet colour on the TLC plate after spraying with vanillin-sulfuric acid followed by heating at 110 ºC for 2 min. The ^{1}H NMR (500 MHz, $CDCl_3$) δH: 5.54 (1H, dd, *J* = 7.5, 3 Hz, H-15), 4.60 (1H, dd, *J* = 10, 5.2 Hz, H-3), 2.28 (1H, ddd, *J* = 1.1, 3.2, 13.3 Hz, H-18), 7.43 (1H, J = 8.0 Hz, H-2´), 6.83 (1H, d, *J* = 8 Hz, H-3´), 3.49 (1H, s, H-4´ (phenolic proton), 6.83 (1H, d, *J* = 8 Hz, H-5´), 7.43 (1H, *J* = 8.0 Hz,H-6´), 7.59 (1H, d, *J* = 16.0 Hz, H-7´), 6.29 (1H, d, 16.0 Hz, H-8´), 0.89 (3H, s, Me), 0.92 (3H, s, Me), 0.92 (3H, s, Me), 0.92 (3H, s, Me), 0.94 (3H, s, Me), 0.97 (3H, s, Me), 0.97 (3H, s, Me).

RESULTS AND DISCUSSION

Seven compounds were isolated from the methanol extract of the stem bark of *J. pandurifolia* by repeated chromatographic separation and purification over silica gel. The structure of 1-7 were resolved by ^{1}H NMR and determined to 3-O-acetylaleuritolic acid (**1**), jatropholone A (**2**), jatropholone B (**3**), 2α-hydroxyjatropholone (**4**), 2β-hydroxyjatropholone (**5**), scopoletin (**6**) and aleuritolic acid 3-*p*-hydroxycinnamate (**7**) by comparing the spectral data with those reported for this compounds.

The ^{1}H NMR spectrum of compound **1** showed the presence of seven methyl singlets at δ 0.83, 0.87, 0.90, 0.91, 0.93, 0.94 and 0.95 on an oleanane skeleton *i.e.* the compound must be a pentacyclic triterpenoid. A characteristics double doublet of one olefinic proton at δ 5.52 was assigned to H-15, suggesting an olean-14-ene skeleton. One methine proton exhibited a double doublet at δ 4.45 assigned to H-3. The downfield shift of H-3 suggested that it was esterified and one methyl singlet at δ 2.02 showed that the compound **1** has one acetyl group. The coupling constant of this methine proton indicates that the acetyl functional group must be in axial position. The above spectral features were similar to the ones reported for acetylaleuritolic acid[10,11].

From the ^{1}H NMR spectrum of compound **2** exhibited the presence of an exocyclic methylene group was revealed by methylene proton signals at δ 5.20 and 4.68 (1H each) as well as signal of a methyl group at δ 2.28 indicated a fully substituted aromatic ring with one methyl substituent group. Two mutually coupled methine proton signals at δ 1.59 and δ 0.94 indicating connectivity between a cyclopropane moiety and an aromatic nucleus. A methyl proton singlet at approximately δ 1.29 as well as two sets of AB doublets (benzylic methylene protons) at δ 3.27 and 2.52 as reported for compound **2**. One H each were observed from δ 3.27 (H-1), δ 2.71 (H-2) and 1.29 (H-16) showed the compound was identified as 2β-methylejatropholone or Jatropholone A. ^{1}H NMR spectrum with those existing in the literature indicated that the compound is the jatropholone A[12,13].

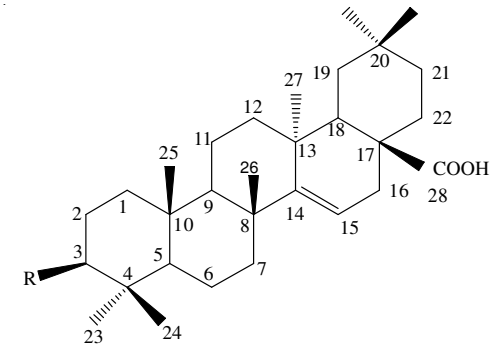
The ^{1}H NMR spectrum of compound **3** displayed resonances similar to those of compound **2**. The difference in the ^{1}H NMR of compound **3** was the stereoisomer at H-2 at δ 2.63 from compound **2**. The identity of compound **3** as jatropholone B was confirmed by comparison of its spectral data with reported values[12,13].

The ^{1}H NMR spectrum of compound **4** showed the presence of an exocyclic methylene group was revealed by methylene proton signals at δ 5.17 and 4.63 (1H each) as well as signal of a methyl group at δ 2.25 (s) indicated a fully substituted aromatic ring with one OH and one methyl substituent group. Two mutually coupled methine proton signals at δ 1.55 and δ 0.94 indicating connectivity between a cyclopropane moiety and an aromatic nucleus as found in jatropholones A and B, also isolated in the present study. The differences were a methyl proton singlet at δ 1.40 instead of a doublet at approximately δ 1.27, as well as two sets of AB doublets (benzylic methylene protons) at δ 3.10 and 2.99 (both with *J* = 16.3 Hz) instead of two sets of doublets at δ 3.25 and 2.50, as reported for jatropholone A and B, indicating the presence of an additional OH group at pentane ring. The one H each were observed from δ 3.10 (H-1) and 1.40 (H-16) showed the compound **4** was identified as 2α-hydroxyjatropholone[14].

The ^{1}H NMR spectrum of compound **5** displayed resonances similar to those of compound **4**. The differences in the proton NMR of compound **5** was the two benzylic methyline protons (H2-1) found as an obscured triplet at δ 3.07 with a coupling constant of 17.1 Hz instead of AB doublets (3.10 and 2.99) as found in compound **4**. Full assignments of ^{1}H NMR are given in below compound **5** was thus 2β- hydroxyjatropholone, the C-2-epimer of 2α-hydroxyjatropholone[14].

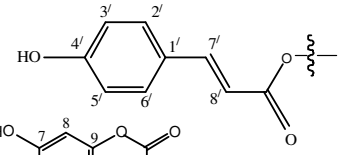
From the ^{1}H NMR spectrum of compound **6**, it was elucidated as scopoletin. The spectrum displayed a clear AB quartet centered at δ 6.26 and 7.50 (1H each), which were typical for H-3 and H-4 of a coumarin nucleus. The relatively short peak at δ 7.50 indicated along range zig zag coupling of H-4 with H-8 appeared at δ 6.91 over five bonds. The spectrum also showed a singlet at δ 6.83 and a broad singlet at δ 6.09, each of one proton intensity. These could be assigned to H-5 and a hydroxyl group proton at C-7. A three proton singlet in the spectrum at δ 3.93 revealed the presence of a methoxyl group at H-6. Comparison of the chemical shifts of the methoxyl and hydroxyl groups allowed to place these substituent's at C-6 and C-7, respectively. On this basis, compound **6** was characterized as 7-hydroxy-6-methoxy coumarin (scopoletin). The identity of compound **6** as scopoletin was confirmed by comparison of its spectral data with reported values[15].

The ^{1}H NMR spectrum of compound **7** showed the presence of seven methyl singlets at δ 0.89, 0.92 (6H, 2CH_3), 0.92, 0.94 and 0.97 (6H, 2CH_3), on an oleanane skeleton *i.e.* the compound must be a pentacyclic triterpenoid. The ^{1}H NMR spectrum displayed a multiplet at δ 5.54, which indicated the presence of an olefinic proton which were assigned at H-15 suggesting an olean-14-ene skeleton. The spectrum also exhibited a double doublet centered at δ 4.60 indicative of H-3 proton of a triterpene nucleus. The downfield shift of H-3 suggested that it was esterified. One methine proton at δ 4.60 assigned to H-3 was esterified that the compound has one moiety on this position. Two doublets centered at δ 6.83 and 7.43 integrating two protons each indicates the presence of disubstituted benzene ring. A broad singlet found at δ 3.49 can be attributed to the presence of hydroxyl proton. Two downfield doublets at δ 7.59 and δ 6.29 revealed the presence of two trans coupled olefinic protons at H-7´ and H-8´ respectively. The coupling constant of this methine proton indicates that the spectral feature are in close agreement to presence of 4-hydroxy cinnamic acid moiety must be in axial position. On this basis, the identity of confirmed of compound **7** as aleuritolic acid 3-*p*-hydroxycinnamate[16].

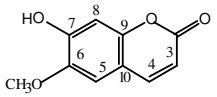


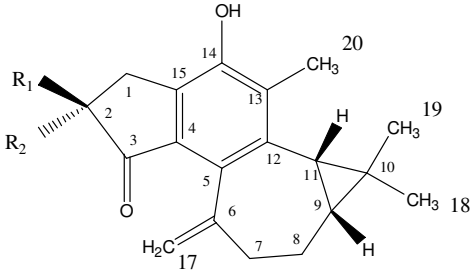
Compound **1**, when R = -OOCH$_3$

Compound **7**, when R =



Compound **6**





Compound **2**, when R_1 = CH_3, R_2 = H
Compound **3**, when R_1 = H, R_2 = CH_3
Compound **4**, when R_1 = CH_3, R_2 = OH
Compound **5**, when R_1 = OH, R_2 = OH

Scheme-I

REFERENCES

1. http://211.114.21.20/tropicalplant/html/print.jsp?rno=479.
2. http://english.scib.cas.cn/plants/201005/t20100521_54403.html.
3. http://www.stuartxchange.org/ShanghaiBeauty.html.
4. S. Sutthivaiyakit, W. Mongkolvisut, P. Ponsitipiboon, S. Prabpai, P. Kongsaeree, S. Ruchirawat and C. Mahidold, *Tetrahedron Lett.*, **44**, 3637 (2003).
5. S. Sutthivaiyakit, W. Mongkolvisut, S. Prabpai and P. Kongsaeree, *J. Nat. Prod.*, **72**, 2024 (2009).
6. W. Mongkolvisut, S. Sutthivaiyakit, H. Leutbecher, S. Mika, I. Klaiber, W. Mollerm, H. Rösner, U. Beifuss and J. Conrad, *J. Nat. Prod.*, **69**, 1435 (2006).
7. J.C. Coll and B.F. Bowden, *J. Nat. Prod.*, **49**, 934 (1986).
8. R.G. Reid and S.D. Sarker, *Nat. Product Isolation*, **4**, 111 (1998).
9. Q.T. Liu and J.L. Kinderlerer, *J. Chromatogr. A*, **855**, 617 (1999).
10. M.O.F. Goulart, A.E.G. Sant'Ana, R.A. de Lima, S.H. Cavalcante, M.G. de Carvalho e Raimundo Braz Filho, *Quimica Nova*, **16**, 95 (1993).
11. R.B. Bates, N.E. Jacobsen, W.N. Setzer and C.C. Stessman, *Magn. Reson. Chem.,* **36**, 539 (1998).
12. K.K. Purushoothaman, S. Chandrasekharan, A.F. Cameron, J.D. Cannolly, C. Labbe, A. Maltz and D.S. Rycroft, *Tetrahedron Lett.*, **11**, 979 (1979).
13. C. Hua, S. Bao, Y. Song and H. Deyu, *Nat. Prod. Res. Dev.*, **19**, 982 (2007).
14. S. Somyote, M. Wantana, P. Samran and K. Palangpon, *J. Nat. Prod.*, **72**, 2024 (2009).
15. T. Begum, M.S. Rahman and M.A. Rashid, *Dhaka Univ. J. Pham. Sci.*, **5**, 21 (2006).
16. J.A. Beutlery, Y. Kashman, M. Tischler, J.H. Cardellina II, G.N. Gray, M.J. Currens, M.E. Wall, M.C. Wani and M.R. Boyd, *J. Nat. Prod.*, **58**, 1039 (1995).