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Flavone glycodsides of *Annona reticulata* (seeds)

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ABSTRACT

3'-O-β-D-glucopyranosyl [2", 3: 7,8] furanoflavone, 3-methoxy-6-O-β-D-glucopyranosyl [2", 3: 7,8] furanoflavone, 3-methoxy- 3', 4'-methylenedioxy-7-O-β-D-glucopyranosylflavone and apigeniin-7-β-glucoronicacid 6"-butyl ester have been isolated first time from the seeds of *Annona reticulata* and identified by spectroscopic data.

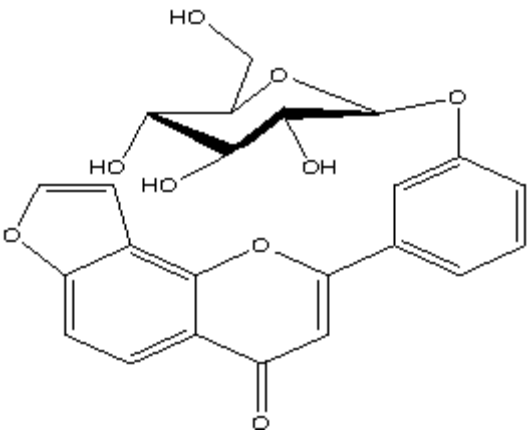
Key words: Flavone glycodsides, *Annona reticulata.*

INTRODUCTION

Annonaceous plants are said to be rich source of bio-active substances[1]. *Annona* is a large genus, with most of its species being confined to tropical climates[2]. About three species are said to be available in India and some of them are known for their medicinal value[3]. *Annona reticulata* or Bullock's heart is commonly known as *Ramphal* and naturalized in Bengal and other parts of India[2]. The unripe fruits is considered to be anthelmintic, the bark of a powerful astringent, the leaves and seeds possess insecticidal properties[3]. The seeds are also highly poisonous[4]. Earlier investigators had reported the isolation of kaurane and kaurene diterpenoids[5-8], isoquinoline[9], benzylisoquinoline[9-10], aporphine[8,10,11] and pyrimidine-β-carboline[11] alkaloids[8,13,14] and N-acyltryptamines. We report herein, the isolation and characterization of flavone glycosides for the first time isolated from this plant source and from family itself.

Isolation and characterization of compounds

The defatted seeds of *Annona reticulata* (5kg) were extracted with MeOH (15 liter, 16 Hours). The methanolic extract was concentrated and then diluted with water to make it aqueosmethanolic extract (7:3). The aqueosmethanolic extract was then partitioned with different solvents in their increasing polarity (Viz: n-hexane, $CHCl_3$, EtOAc and n-buthyl alcohol). The silica gel column chromatography of ethylacetate soluble portion yielded three compounds; Compound I, II and III while butbanol soluble portion yielded only one compound (compound IV) were eluted respectively in $CHCl_3$-MeOH (85:15) early fraction $CHCl_3$-MeO (85:15) later fraction, $CHCl_3$-MeO (80:20) & EtOAc-MeOH (9:1).

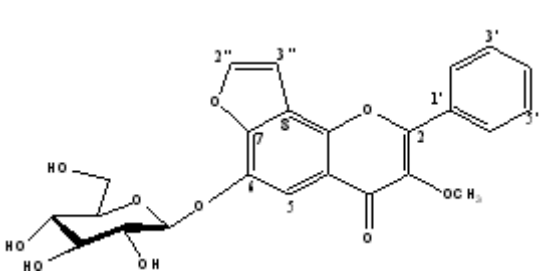


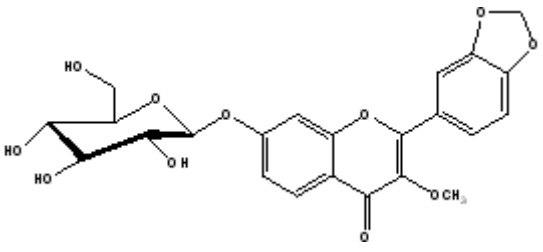
Compound 1

[$C_{23}H_{20}O_9$] Pale yellow needles, m.p. 259-260°C, [α]D-33° (Py), FAB-MS showed (M+H$^+$) ion peak at m/z 441. The positive responds towards Shinoda and Feigal test, absorption band in IR ν_{max} at 3433,1625,1594,1459,1082,785 & 631 cm^{-1} and UV λ_{max} 216,261 and 300 nm collectively suggests the presence of furanoflavone glycoside nucleus in the molecule. ^{1}H & ^{13}CNMR (DMSO-d6) spectrum showed signals at δ 8.25 (1H, d, J=2.1 Hz) δ 7.58 (1H, d, J=1.8H) and δ_c 147.4 & δ_c 107.0 for H-2" & H3" for the presence of furan ring. The signals at δ 7.11 (1H, s) δ_c 104.5 were attributed to H-3 of flavone. ^{1}HNMR shoed two doublets at δ7.99 (1H, J=8.7 Hz) and 7.77 (1H, J=8.7 Hz) for two ortho coupled aromatic hydrogens which may be assigned to the hydrogens at C-5 and C-6 position of the flavone nucleus. The glycosidic nature of the compound was confirmed by signal at δ 5.12 (d, J=6.6 Hz) for anomeric hydrogen and by signals form δ 3.19 to 3.75, altogether six hydrogen atom. The angular position of furan ring at C-7 (oxygenated) and C-8 position was deduced by ^{1}H-^{1}H COSY spectrum which also 1.5 & 7.5Hz. H-4'), 7.52 (1H, t, 7.5 HZ, H=-5) and 7.85 (1H, dd, J=1.5 & 7.5 Hz, H-6'). A brief reflux of compound I in methanolic 2N-HCl yielded the mixture of glycon and aglycone. Glycone part was identified as glucopyranose while aglycon was identified as pongol[16-17] by Co-TLC with authentic samples. The coupling constant of anomeric proton J=6.6 Hz suggested the β-glycosidic nature of glycosidic linkage. ^{13}CNMR and DEPT is also in consistance with structure I which showed altogether 23 carbons of one -CH_2, fourteen -CH-, includeing nine aromatic and five alipathic with eight quarternary carons. In the basis of the above data compound I was characterized as 3'-O-β-D-glucopyranosyl [2", 3": 7,8] furanoflavone. This is the second report of isolations of this compound from nature and first time from *Annona reticulatal* earlier it was isolated from fruint of *Pongamia pinnata*[17-19].

Compound II

[$C_{24}H_{22}O_{10}$] Pale yellow crystal, m.p. 233-234^{0}C, $[\alpha]_D$-32.8^0 FAB-MS [M+1]$^+$ ion peak at m/z 471. The positive responds towards Shinoda test and Feigals test collectively confirmed the flavonide glycoside nucleus in the molecule. The IR υ_{max} 2437 (hydroxyl), 1620 (conjugated carbonyl), 1595, 1480 and 1381 cm^{-1} for (aromatic) functionalities, the UV absorption band at λ_{max} 218, 260 and 306 nm, are indicative of 3, 6, 7- trioxygenated flavonoid molecule. A deep analysis of ^{1}H and ^{13}CNMR revealed the presence of a furan ring fused to ring A, with the characteristic proton doublets at δ 8.24 & 7.52 (1H, *d*, each, J=2.01 Hz) and δ_c 147.4 & 104.8 attributed to H-2" and H-3" of furan ring respectively. Signal appeared for an aromatic proton on ring A [δ7.62 (1H, *s*, H-5)] and unsubstituted ring B [signals at δ 8.14 (2H, *m*, H-2' &-6') and 7.60 (3H, *m*, H-3', -4', -5')]. The signal for one methoxy group at δ 3.85 (3H, *s*) was determined at position 3 with the help of NOE effect between hydrogen of methoxy group and those resonated at δ 8.14 (2H, *m,* for H-2'and H6'). The glycosidic moiety was settled at C-6 position on the basis of absence of any correlation for H-3" in ^{1}H NMR & ^{1}H-^{1}H COSY experiment and an observed NOE between the anomeric proton at δ 5.27 and H-5 δ7.62. ^{13}CNMR and DEPT showed the presence of 24 carbons for: viz, eight aromatic methine carbons six aliphatic oxymethine and oxymethylene carbons of sugar, one carbonyl carbon of flavone and eight aromatic quaternary carbons. The spectral data of aglycon portion agreed well with the reportd data of 6-methoxy karangin[20]. Therefore this compound was identified as 3-methoxy-6-O-β-*D*-glucopyranosyl [2", 3": 7, 8] furanoflavone, named as pongamoside-C[19] earlier reported from *Pongaia pinnata*.



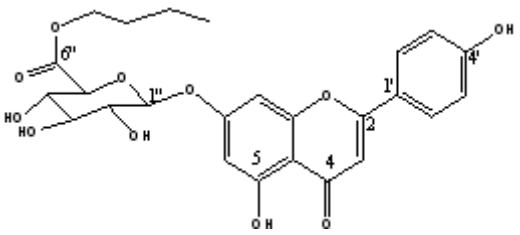


Compound III

[$C_{23}H_{22}O_{11}$] White crystalline solid, m.p. 214-215°C. FAB- mass showed $[M+1]^+$ m/z 475. The IR, UV and colour tests suggests the presence of flavone glycoside skelton of molecule. ^{1}HNMR spectrum showed signals for six aromatic protons at δ7.12 (1H, *dd*, J=9.0 & 2.4 Hz), δ 7.33 (1H, *d*, J=2.4 Hz), δ 7.98 (1H, *d*, J=9.0 Hz), δ 7.11 (1H, *d*, J=8.4 Hz), δ 7.57 (1H, *d*, J=1.5 Hz) and δ 7.65 (1H, *dd*, J=8.4 & 1.5 Hz), one aromatic methoxy group at δ 3.79 (3H, *s*), and for one methylenedioxy group at δ 6.15 (2H, *s*). It accounts nine sites out of ten in a flavone nucleus but spectrum showed several signals in the region from 3.19 to 3.52 for oxymethine and oxymeythylene and a signal at δ 5.06 (H, *d*, J=7.2 Hz) for anomeric proton. The high coupling constant for aromatic hydrogen (J=7.2) suggested that glycosidic linkages is of δ type. The position of glycone molecule in compound was settled on A ring of flavone on the basis of NOE studies. According to this correlation of anomeric proton signal at δ 5.06 with H-6 (δ7.12) and H-8 (7.33) of A ring suggested that glycosidation occured at C-7. The nature of glycon part was settled as glucopyranose by acid hydrolysis followed by comperative Co-TLC with acthentic sample of D-glucose. The methylenedioxy group assumed to be present at C-3' and C-4' position. The only unaccounted position left behind C-3 should posses the methoxy group because of the fact that the nine site of flavone molecule has already accounted for. According to ^{13}CNMR & DEPT experiment the nature of all 23 carbon of compound are as; one methoxy, two methylene, eleven methines (including six aromatic and five aliphatic) nine quaternary carbons. Finally on the basis of above spectral data it was identified as 3-methoxy-3',4'-methylenedioxy-7-O-β-D-glucopyranosylflavone. Earlier it was reported as pongamoside-D[20]. It was further confirmed by comparative study of spectra and CO-TLC with authentic sample.

Compound IV

[$C_{25}H_{26}O_{11}$] White crystals, m.p.220-224°C, FAB–MS spectrum showed molecular ion peak $[M+H]^+$ at m/z 503. IR showed absorption bands υ_{max} at 1746 (ester), 1639 cm^{-1} (enolic) functionalities while the absorption bands at 1605 and 1412 cm^{-1} indicated the presence of aromatic ring. The UV spectrum showed bands at λ_{max} 267 and 371 nm (MeOH). ^{1}H NMR showed signal at δ 0.80 (3H, *t*, J=7.1), 1.23 (2H, *m*), 1.51 (2H, *m*), 4.18-4.00 (2H, *m*) for an ester linked butyl chain. The anomeric proton of glucose appeared at δ 5.29 (*d*, J=6.80 Hz) was evident for its β- configuration. The aromatic proton showed signals at δ 6.92, 7.93 (2H each, *d*, J=8.7Hz) and δ 6.44, 6.83 (1H each, *d*, J=1.9 Hz). The characteristic peak of flavonid H-3 proton appeared at δ 6.85 (1H, *s*). The anomeric and oxymethine proton signal reveals the presence of a sugar moiety and 5,7,4' trisubstituted flavone nucleus. The sugar was first de-esterified with base then hydrolyzed with acid. A comparative Co-TLC of hydrolysed with authentic samples showed glycon as glucoronic acid and aglycon as apigenin. The ester linkage of butyl chain with glucoronic acid (C-6") and anomeric of glucoronic acid with phenolic –OH (C-7) were confirmed by HMQC experiment. The presence of twenty-five carbon was revealed by ^{13}C NMR spectrum while DEPT experiment revealed the presence of one methyl, three methylene, seven aromatic methines, five aliphatic methines and nine quaternary carbons. (The assignment of different carbon signals are shown in Table 1). On the basis of above spectral data and by comparing the data of reported literature[21] it was recognized as apigenin-7-δ-glucoronicacid-6"-butyl ester known as thellunginate earlier reported from *Pimpinella thellungiana*[22] and *Tectona grandis*[23] species. This is the first report of isolation of this compound from *Annona reticulata.*



EXPERIMENTAL

The seeds of *Annona reticulata* were purchased from United Chemicals & Allied work, Civil Row-10, Kolkatta, India. The seeds were milled by conventional method. Defatted seeds of *Annona reticulata* (5 Kg) were extracted with methanol (15L) for 16 hours. The methanolic extract (63.8g) was then fractionated in to four parts according to the

Table 1: ^{1}H and ^{13} CNMR data of compounds I, II, III & IV recorded in $CDCl_3$

Position	δ_H (J in Hz)	δ_c (I)	δ_H (J in Hz)	δ_c(II)	Position	δ_H (J in Hz)	δ_c(III)	δ_H (J in Hz)	δ_c(IV)
2	-,	150.0	-,	153.8	2	-,	155.9	-,	164.6
3	7.11(s),	104.5	-,	141.2	3	-,	139.9	6.85(s)	99.7
4	-,	176.8	-,	173.3	4	-,	172.9	-,	182.3
5	7.99(d, 8.7),	120.9	7.62(s),	103.3	5	7.98(d,9.0),	126.1	-,	161.7
6	7.77(d,8.7),	110.0	-,	140.7	6	7.12(dd,9.0&2.4), 115.3	6.44(d,1.9),		95.0
7	-,	161.6	-,	141.2	7	-,	161.3	-,	162.7
8	-,	118.8	-,	118.8	8	7.33(d,2.4),	99.8	6.83(d,2.0),	95.0
9	-,	157.8	-,	144.5	9	-,	154.2	-,	157.2
10	-,	117.0	-,	119.6	10	-,	118.1	-,	105.7
1'	-,	132.3	-,	130.3	1'	-,	123.9	-,	121.2
2'	7.75(brs),	119.9	8.14(m),	128.0	2'	7.57(d,1.5),	108.4	7.93(d,8.7),	128.9
3'	-,	157.6	7.60(m),	128.6	3'	-,	149.2	6.92(d,8.7),	116.3
4'	7.29(dd,7.5,1.5),	119.9	7.60(m),	130.6	4'	-,	147.5	-,	161.4
5'	7.52(t,7.5),	130.3	7.60(m),	128.6	5'	7.11(d,8.4),	107.9	6.92(d,8.7),	116.3
6'	7.8(d,7.5),	113.6	8.14(m),	128.0	6'	7.65(dd,8.4&1.5),	123.2	7.93(d,8.7),	128.9
1"					1"	5.06(d,7.2),	103.3	5.29(d,6.8),	103.4
2"	8.25(d,2.1),	147.4	8.24(d,2.1),	147.4	2"	3.27-3.52(m),	73.0	4.18-4.00(m),	73.0
3"	7.58(d,1.8),	107.6	7.52(d,2.1),	104.8	3"	3.27-3.52(m),	76.4	4.18-4.00(m),	75.8
1"'	5.12(d,6.6),	100.3	5.27(d,6.9),	100.8	4"	3.27-3.52(m),	69.5	4.18-4.00(m),	71.4
2"'	3.28-3.56(m),	73.2	3.30-3.43(m),	73.2	5"	3.19(m),	77.1	4.18-4.00(m).	75.5
3"'	3.28-3.56(m),	76.4	3.30-3.43(m),	76.4	6"	-,	169.0		
4"'	3.28-3.56(m),	69.7	3.30-3.43(m),	69.3	6"a	3.70(dd,10.2&4.5),	60.5		
5"'	3.19(m),	77.1	3.27(m),	77.2	6"b	3.27-3.52(m),	60.5		
6"'-a	7.75(brd,11.1),	60.7	3.69(dd,11.6,3.6),		1"'	4.18-4.00(m),	64.6		
6"'-b	3.28-3.56(m),	60.7	3.53(dd,11.6,3.6),	60.4	2"'	1.51(m),	30.3		
3-OCH_3			3.85(s),	59.6	3"'		1.23(m),	18.7	
					4"'		0.80(t,7.1),	13.7	
					3-OCH_3	3.79(s),	59.4		
					-OCH_2O-	6.15(s),	101.7		

increasing polarity of solvents. The ethylacetate and butanol soluble fraction was Chromatographed over SiO_2 gel column and eluted with solvents of increasing polarity. The melting poin were measured on a Yazawa hot stage microstageapparatus and are uncorrected. Optical rotations were measured on JASCO DIP-360 Polarimeter (Cell length 5 CM). UV absorption spectra were recorded on JASCO UV/visible spectrophotometer (model no. 7800) while IR on JASCO FT-IR 5300 spectrometer.

Compound I

Pale yellow needles, m.p. 259-260 °C, $[\alpha]_D$ -33° (Py), FAB-MS: $[M+H]^+$ at m/z 441; IR (KBr) υ_{max} 3433,1625,1594,1459,1082,785, & 631 cm^{-1}. UV λ_{max} 216, 261 and 300 nm.

Compound II

Pale yellow crystal, m.p. 233-234 °C, $[\alpha]_D$-32.8° FAB-MS $[M+1]^+$ at m/z 471.IR (KBr) υ_{max} 2437 (hydroxyl), 1620 (conjugated carbonyl), 1595, 1480 and 1381 cm^{-1} (aromatic). UV at λ_{max} 218, 260 and 306 nm.

Compound III

White crystalline solid, m.p. 214-215 °C. FAB- mass showed $[M+1]^+$ m/z 475. IR (KBr) υ_{max} 3426 cm^{-1} (hydroxyl), 1635 cm^{-1} (conjugated carbonyl), 1595, 1448, & 1383 cm^{-1} (aromatic). UV λ_{max} at 211, 241, 306 and 344 nm.

Compound IV

White crystalline solid, m.p. 220-224°C, $[\alpha]^{29}_D$ **(-)** 126.6 °C(c, 0.15 MeOH), FAB-MS: $[M+H]^+$ at m/z 503. IR (KBr) υ_{max} 3447, 1746, 1639, 1605, 1412, 1284, 1121, 1015 and 936 cm^{-1}. UV λ_{max} at 267 and371 nm. For ^1H, ^{13}C NMR data (of compounds I, II, III & IV) see Table.

Table

^1H and ^{13}C NMR data of compounds I, II, III & IV recorded in $CDCl_3$ at 300 MHz and 75 MHz respectively.

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