TWO NEW COMPOUNDS FROM *Phyllanthus niruri*

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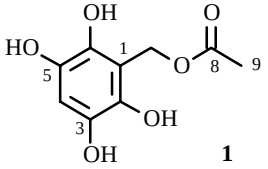
*Three compounds were isolated from Phyllanthus niruri Linn. One effective method, improved dry column liquid chromatography for isolating these compounds, was applied on a silica gel column. It is a combination of conventional dry column chromatographic and flash chromatographic techniques. Structures of those obtained compounds were elucidated by means of spectral techniques including IR, MS, and 1D NMR and 2D NMR. Compound **1** was elucidated as 2,3,5,6-tetrahydroxybenzyl acetate. Compound **2**, 2,4,5-trihydroxy-3-(4,6,7-trihydroxy-3-oxo-1,3-dihydroisobenzofuran-5-yl)-benzoic acid methyl ester, was given the name phyllangin, and compound **3** was named corilagin. Compounds **1** and **2** are new compounds.*

Key words: Dry column chromatography; *Phyllanthus niruri* Linn; 2,3,5,6-tetrahydroxybenzyl acetate; Phyllangin; Corilagin.

Phyllanthus niruri Linn, an annual plant that grows widespread in many countries, has been used as a medicine for treating hepatitis in India for a long time [1]. Many studies on this plant showed that it has many bioactivities [2–12]. The bioactivities of this plant prompted us to continue to investigate its chemical components. We have reported the chemical components of this plant in previous works [13, 14]. In recent work we obtained three compounds, compound **1**, compound **2**, and compound **3**, by applying an improved dry column chromatography technique. Their structures were elucidated by means of spectral techniques including IR, MS, and NMR. Compounds **1** and **2** are new compounds among them. We wish to report here on the isolation and structure determination of these compounds.



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Compound **1**. When sprayed with $FeCl_3$-methanol solution, compound **1** appeared as a blue-black spot in a silica gel TLC plate. This led to the conclusion that this compound consisted of a hydroxylphenyl group. The NMR showed one CH_3 and one CH_2 in the molecule, and the proton of CH_3 does not correlate with the proton of CH_2. The chemical shift of CH_2 (δ 5.12 and δ 67.52) showed that the CH_2 links to one oxygen atom and a phenyl group. A carbon signal at δ 171.76 revealed one C=O in the molecule, and its chemical shift showed that this C=O does not link to the phenyl group. The fact that the four carbon groups contain a phenyl group means the phenyl group is a symmetry structure. Data of the chemical shift of carbon showed four carbons linked to oxygen atoms. Two of them are in the *othro* position of the CH_2O group and showed a chemical shift of 139.95. Another two are in the *meta* position of the CH_2O group and showed a chemical shift of 147.89. We conclude that this phenyl has the structure of the 2,3,5,6-tetrahydroxylphenyl group. The molecular formula $C_9H_{10}O_6$ was established on the basis of the molecular weight obtained from the ESIMS and NMR data as shown in Table 1. HMBC confirmed that the CH_2 group links to the carbon of the phenyl ring directly and consisted of an ester fragment. The proton (δ 1.89) of CH_3 correlating to C (δ 171.76) showed the CH_3 group links to the carboxylic group. Compound **1** was confirmed to have the structure of 2,3,5,6-tetrahydroxyphenyl acetate according to all the data mentioned above. The assignments of NMR signals are shown in Table 1.

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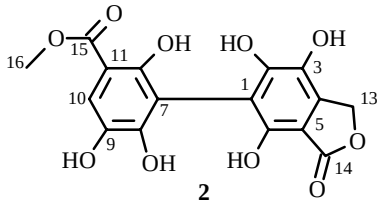
TABLE 1. NMR Data of Compound **1**

Atom (^{13}C)	^{1}H (multiplicity)	^{13}C	HMBC (^{13}C)
9	1.89 (s, 3H)	21.64	8
7	5.12 (s, 2H)	67.52	1, 2 (6), 8
4	6.69 (s, 1H)	108.80	11, 2 (6), 3 (5), 8
1	-	126.56	-
2, 6	-	139.95	-
3, 5	-	147.89	-
8	-	171.76	-

Multiplicity was provided by DEPT (90 and 135) spectra. HMQC provided links of protons and carbons.

Compound **2**. The results of a chemical reaction test revealed that the hydroxylphenyl group is a part of this molecule. On the basis of elemental analysis results and molecular weight, the molecular formula of compound **2** was established as $C_{16}H_{12}O_{10}$. ^{1}H-NMR, dept -NMR, and ^{1}H-^{1}H COSY spectra showed three groups of protons, one CH_3, one CH_2, and one CH, in this compound, and these protons do not correlat with each other. Chemical shifts of carbons and IR spectra showed two carboxylic groups and two phenyl groups in the molecule, and the two carboxylic groups were parts of ester groups. Based on the conclusions mentioned above, three rings, including two phenyl rings, are in this molecule.



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The data of chemical shift also gave the result that CH_3, CH_2, and six aromatic carbons are connected to oxygen atoms. If the two phenyls were linked to each other by an oxygen bridge, the components of the compound would not correspond to the molecular weight. That the two phenyl groups are linked to each other directly by a bond was established on the basis of the molecular formula. If the two phenyl groups were not linked directly by a bond and are linked to each other by an oxygen bridge, the components of the compound would not correspond to the molecular formula. On the basis of molecular weight and chemical shifts of carbons, six hydroxyl groups are connected to these aromatic carbons. HMBC showed a proton signal at δ 3.50 correlated to a carbon signal at δ 166.41 and δ 120.49. This means that the CH_3 consists of a fragment of CO_2CH_3 and this fragment is connected to a phenyl ring (carbon signal of NMR at δ 115.33, 120.49, 144.85, 137.02, 144.15). The HMBC of the proton signal at δ 7.05 also revealed that the carboxylic group (δ 166.41) is connected to a phenyl group (carbon signals at δ 115.33, δ 120.49, δ 144.85 and δ 137.02, δ 144.15). The HMBC of H (δ 7.05) showed strong correlations with carbons (δ 120.49, δ 144.85 and δ 137.02, δ 144.15), and weak correlation with carbon (δ 115.33). These facts led to the conclusion that H (δ 7.05) is at an *ortho*-position of the carboxylic group, and carbon (δ 115.33) is at the *para*-position of the proton (δ 7.05). The other carbons (δ 144.85, 137.02, 144.15) in this phenyl group are connected to hydroxyl groups. Carbon (δ 137.02) is in the *meta*-position of CH_3OCO, and its chemical shift occurs later than the two carbons (δ 144.85, 144.15) in the ortho-position of the CH_3OCO. The HMBC and chemical shift of CH_2) show that it is linked to a carbon signal at δ 125.87 in the phenyl group (carbon signals at δ 113.82, δ 116.27, δ 125.87, δ 137.81, δ 139.18, δ 144.68) and one oxygen atom, and the CH_2 consist, of a fragment CO_2CH_2. The fact that H (δ 5.13, CH_2) does not correlate to carbon δ 116.27 in the HMBC shows that the carbon is in the *para* position of CH_2. According to the molecular formula and chemical shifts of carbons in this phenyl group, a conclusion was made that the three hydroxyl groups are connected to carbons (δ 137.81, 139.18, 144.68) of the phenyl group. Three carbons (δ 113.82, 116.27, 125.87) on this phenyl group are not connected to hydroxyl groups, one carbon (δ 116.27) is connected to another phenyl group, one (δ 125.87) is connected to CH_2, and another one (δ 113.82) is connected to a carboxylic group C=O (δ 170.18). The fact that CO_2CH_2 is connected to the phenyl group led to the conclusion that CO_2CH_2 and the phenyl group form a five-membered lactone ring. The CH_2 is in the *ortho*-position of C=O in the phenyl group. According to the data mentioned above, the structure of this compound is elucidated as 2,4,5-trihydroxy-3-(4,6,7-trihydroxy-3-oxo-1,3-dihydroisobenzofuran-5-yl)-benzoic acid methyl ester (**2**). The assignment of all NMR signals is shown in Table 2.

TABLE 2. NMR Data of Compound **2**

Atom C	^{1}H (multiplicity)	^{13}C	HMBC (^{13}C)
16	3.50 (s, 3H)	51.06	11, 15
13	5.13 (s, 2H)	66.12	4, 14, 5, 3, 2, 6
10	7.05 (s, 1H)	109.13	7, 11, 12, 9, 8, 15
5	-	113.82	-
7	-	115.33	-
1	-	116.27	-
11	-	120.49	-
4	-	125.87	-
9	-	137.02	-
3	-	137.81	-
2	-	139.18	-
8	-	144.15	-
6	-	144.68	-
12	-	144.85	-
15	-	166.41	-
14	-	170.18	-

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Multiplicity was provided by DEPT (90 and 135) spectra. HMQC provided links of protons and carbons.

TABLE 3. Data of NMR of Compound **3**

Atoms C	^{1}H (multiplicity)	^{13}C	HMBC (carbon)
16	3.30 (1H, t, J = 7.41)	52.99	15, 17, 18
14	3.01 (1H, t, J = 9.12)	55.53	15, 13
	3.32 (1H, t, J = 8.23)		
18	2.95 (1H, t, J = 7.26)	59.97	17, 19
15	3.43 (1H, m, J = 7.52)	62.15	16, 17, 14
17	3.67 (1H, t, J = 7.28)	66.72	16, 18, 15, 27
19	5.28 (1H, d, J = 7.23)	85.84	20
11	5.57 (s, H)	98.83	7, 10, 8, 13
2	5.64 (s, H)	100.68	6, 3, 5, 27
22, 26	6.09 (1H)	101.46	21, 24, 23 (25), 20
7	-	107.24	-
6	-	107.73	-
21	-	111.12	-
12	-	115.96	-
1	-	116.00	-
10	-	128.21	-
3	-	128.71	-
24	-	130.92	-
4	-	135.76	-
9	-	135.86	-
8	-	136.14	-
5	-	136.54	-
23, 25	-	136.90	-
20	-	157.21	-
27	-	159.04	-
13	-	160.63	-

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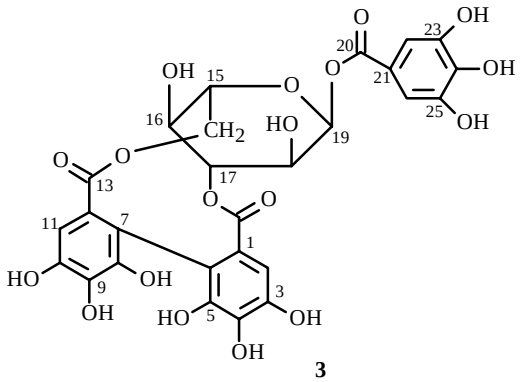
Multiplicity was provided by DEPT (90 and 135) spectra. HMQC provided links of protons and carbons.



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The IR and NMR of compound **3** show that it is a glucoside. Its structure was elucidated on the basis of spectral data. Structural studies show that compound **3** is corilagin [15].

EXPERIMENTAL

General Methods. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) were recorded on a Bruker AM-500 spectrometer. Samples were dissolved in CD_3SOCD_3 (DMSO-d_6) for NMR studies. Chemical shifts are reported in ppm, and coupling constants are in Hz. ESIMS spectrum was recorded using a BRUKER Esquire 3000 *plus* mass spectrometer. IR spectrum was recorded using a Therma Nicolet nexus 470 infrared spectrometer. Melting points were scaled in an X-4 microscopic digit melting point meter. Elementary analysis was performed on a PE2400 CHN elemental analyzer.

Plant Material: The plant material was collected from Longan county, Guangxi province in August 2002 and was identified as *Phllanthus niruri* Linn by Dr Shifeng Ni, and a voucher specimen was kept in the Botany Department of Guangxi University.

Isolation and Purification. The air-dried *P. niruri* (4.5 kg) was extracted with ethanol, with constant mechanical stirring; this procedure was repeated until no further extract bud was obtained .The combined extracts were filtered while warm through glass wool and concentrated. The residue (120 g) was extracted successively with ethyl acetate and butanol. The butanol extract was centrifuged, and then concentrated to dryness to obtain 25 g residue. Chromatography of the 25 g residue was carried out on a silica gel (160–200 mesh) column using a solvent system of ethyl acetate and methanol (v/v) as eluent. Different polarity systems demonstrated after 15 × 500 ml eluent were used. A fraction obtained at a ratio of ethyl acetate to methanol of 90:10 gave 350 mg solid residue. This residue (150 mg) was purified by gel liquid chromatography in a Sephadex LH-20 column by methanol, giving 120 mg discolored residue. After many eluent systems were tested on thin layer silica gel plates, an eluent system with a ratio of ethyl acetate (saturated by water): chloroform: formic acid of 50:10:3 gave an optimal separation effect. Data of R_f of the components gave 0.35, 0.43, and 0.47 respectively in this eluent system. Twenty mg of the discolored residue dissolved in methanol was mixed with 0.5 g of silica gel H and all solvent carefully evaporated. After activation at 110°C for 1 hour, silica gel H was packed in a 2.5 × 20 cm glass column under vacuum and dabbed carefully. The scale of silica gel H in the column was 2.5 × 15 cm. Then the sample mixed with silica gel was put on top of the column under vacuum immediately. An eluent (500 ml) consisting of 50 parts of ethyl acetate (saturated by water), 10 parts of chloroform, and 3 parts of formic acid, as use in the TLC tests, was applied under pressure of 1 atm. A fraction collector was used to collect eluates in 3-ml fractions. The fractions were separated by TLC and then combined. Compound **1** (0.8 mg) was obtained in the 30th to 34th fractions, compound **2** (1.2 mg) in the 61st to 64th fractions, and compound **3** (7.6 mg) in the 101st to 108th fractions. All separation processes were completed in 2 hours. Repetition of this procedure gave enough samples.

Compound 1, brown powder. ESIMS gave a molecular weight of 214. ESIMS gave a molecular weight of 214. H-H COSY showed that there are no protons that correlate with each other. IR (KBr, ν_{max} cm^{-1}): 3295, 3025, 2976, 2885, 1718, 1605, 1510. All NMR data and assignments are shown in Table 1.

Compound 2, brown powder. Colorization by $FeCl_3$–methanol solution on a thin silica gel plate showed a blue black spot. ESIMS showed a molecular weight of 364. Element analysis showed C 52.6%, H 3.3%, and O 44.1%. IR (KBr, ν_{max}, cm^{-1}): 3352, 3055, 2981, 2880, 1725, 1733, 1605, 1513, 1120. Its NMR data are shown in Table 2.

Compound 3, brown powder, m.p. 204–205°C. ESIMS showed M^-633 and M^+ 635 and gave a molecular weight of 634. IR (KBr, ν_{max}, cm^{-1}): 3350, 3150, 2980, 2830, 1720, 1715. NMR data of compound **3** are shown in Table 3.

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