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TRITERPENOID SAPONINS FROM THE LEAVES OF *ILEX KUDINCHA*

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Eight new triterpenoid saponins, kudinosides I-P along with three known saponins were isolated from the leaves of *Ilex kudincha*. The aglycone moiety of kudinosides I-K was determined as a new triterpene (kudinolic acid), a fore-genin for the lactonic triterpene. The structures of all saponins were established on the basis of spectroscopic and chemical evidence. The biogenesis of the triterpene with lactonic moiety was briefly discussed.

Keywords: *Ilex kudincha*; Aquifoliaceae; Triterpenoid saponin; Structural elucidation

INTRODUCTION

We previously reported the isolation and structural elucidation of triterpenoid saponins called kudinosides A-G, a series of kudinlactone saponins, from the leaves of *Ilex kudincha* (Chinese crude drug named "Ku-Ding-Cha", Aquifoliaceae) [1, 2]. In the course of chemical studies on medicinal plants of *Ilex* genus having cardiovascular activities, we have isolated eight new triterpenoid saponins named kudinosides I (**1**) ~ P (**8**), together with three known saponins: latifoloside G (**9**), latifoloside H (**10**) [3] and 3-β-D-glucuronopyranosyl asiatic acid 28-*O*-β-D-glucopyranoside (**11**) [4] from the title plant. Extensive 2D NMR experiments revealed that saponins **1**–**3** have *trans*-bihydroxy groups on the C-19 and C-20 of the

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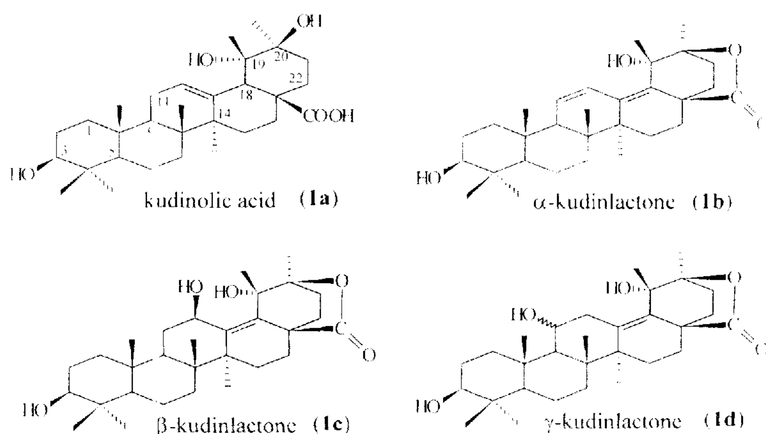

FIGURE 1 The aglycones 1a ~ 1d.

aglycone (**1a**), an intermediate to form the lactonic structure on the E-ring (**1b** ~ **1d**) (Fig. 1). The present paper deals with the isolation and structural elucidation of the new saponins.

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RESULTS AND DISCUSSION

The water soluble fraction of the ethanol extract of *Ilex kudincha* was chromatographed on silica gel columns, followed by repeated RP-8 and MCI columns to yield eleven saponins (**1**–**11**). Some of them are minor saponins. Saponins **1**–**10** have 4 ~ 6 sugar units, but only **11** has two sugar units, which is because **11** has a glucuronic acid unit on the molecule and the glucuronic acid increases its polarity. Saponins **9** and **10** were identified as latifoloside G and latifoloside H, furthermore, saponins **4**, **5**, **8**, **9**, **11** and **6**, 7, **10** were determined as ursolic and oleanolic-type saponins, respectively, by comparison of the NMR data with the reported Ref. [3].

Kudinoside K (**3**) was a colorless powder and showed a [M H] ion peak at *m/z* 1397 in the negative FAB-MS. On acid hydrolysis, **3** afforded glucose, rhamnose and arabinose as the sugar moieties. The ^{1}H NMR spectrum exhibited the presence of seven single methyls at δ: 0.88, 1.08, 1.11, 1.15, 1.30, 1.46, 1.73, a tri-substituted olefinic proton at δ 5.60 in the aglycone moiety, and six anomeric proton signals at δ 4.74 (1H, d, $J=$ 7.0 Hz, H-1-Ara), 5.13 (1H, d, $J=7.6$ Hz, H-1-Glc), 5.28 (1H, d, $J=7.6$ Hz, H-1-Glc′), 6.21 (1H, d, $J=7.9$ Hz, H-1-glc), 6.37 (1H, s, H-1-Rha), and 6.67 (1H, s, H-1-rha′). The configurations of sugar units were

determined by the J_{H1-H2} coupling constant of anomeric protons: β for glucose and α for arabinose and rhamnose. The ^{1}H and ^{13}C NMR spectra revealed that the aglycone of **3** was a derivative of pomolic acid. Comparison of their chemical shifts between the aglycone and pomolic acid indicated that it was the same except for E-ring. Due to the C-20 from δ 42.4 in pomolic acid to δ 76.2 (+33.7) and the other changes of chemical shift of carbons such as C-18 [δ 50.1 (−4.7)], C-21 [δ 33.0 (+6.1)], C-22 [δ 34.3 (−3.0)], and C-30 [δ 23.4 (+6.0)], the C-20 must be an oxygen-bearing quaternary in the aglycone of **3**. These positions were confirmed by HMBC and NOESY experiments. In the HMBC spectrum, the methine proton (δ 3.16, H-18) was correlated with a carboxyl signal (δ 178.0, C-28), an olefinic quaternary carbon signal (δ 138.8, C-13), an aliphatic carbon signal (δ 47.9, C-17) and two oxygen-bearing quaternary carbon signals (δ 74.1, C-19 and δ 76.2, C-20). Furthermore, the methine proton at δ 3.16 the proton at δ 2.02 (H-22) and the proton of methyl at δ 1.46 (H-29) in the NOE spectrum (Fig. 3). These evidences indicated the occurrence of *trans*-bihydroxy groups in the molecule. Hence, the aglycone of **3** was formulated as 3β,19α,20β-trihydroxyurs-12-en-28-oic acid, a new triterpene and named kudinolic acid (**1a**).

The locations of sugar linkages and sequences were deduced from the HMBC correlation. The spectral findings showed correlation between the anomeric proton signal at δ 4.74 (H-1 of Ara) and the carbon signal at δ 88.7 (C-3 of the aglycone), the anomeric proton signal at δ 6.37 (H-1 of Rha) and the carbon signal at δ 74.7 (C-2 of Ara), the anomeric proton signal at δ 5.13 (H-1 of inner Glc) and the carbon signal at δ 82.7 (C-3 of Ara), the anomeric proton signal at δ 5.28 (H-1 of terminal Glc) and the carbon signal at δ 84.5 (C-2 of inner Glc), the anomeric proton signal at δ 6.21 (H-1 of esterified glc) and the carbon signal at δ 178.0 (C-28 of the aglycone), and the anomeric proton signal at δ 6.67 (H-1 of rha) and the carbon signal at δ 76.2 (C-2 of esterified glc). So, the sequence of the C-3 position is S_2, the same sequence as that of kudinoside C [2] (see Fig. 2). From these data, the structure of kudinoside K was determined as 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl (1→3)-[α-L-rhamnopyranosyl(1→2)]-α-L-arabinopyranosyl kudinolic acid 28-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside.

Kudinoside J (**2**) showed a $[M-H]^-$ ion peak at m/z 1251 in negative FAB-MS, consistent with $C_{59}H_{96}O_{28}$. **2** was the same aglycone moiety of **3** by comparing their ^{1}H and ^{13}C NMR spectra. On acid hydrolysis, **2** also gave glucose, rhamnose and arabinose as sugar moieties. The ^{1}H NMR spectrum exhibited five anomeric protons at δ 4.71 (d, $J=6.8$ Hz),

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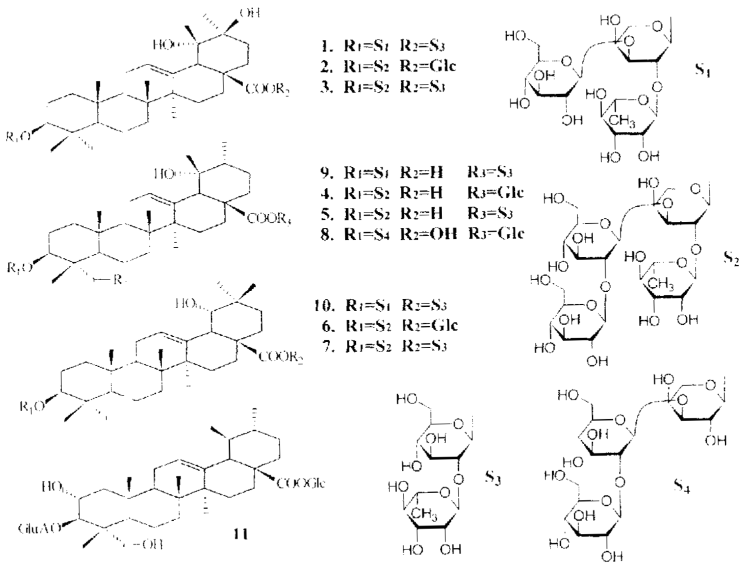

FIGURE 2 The structures of saponins 1 ~ 11.

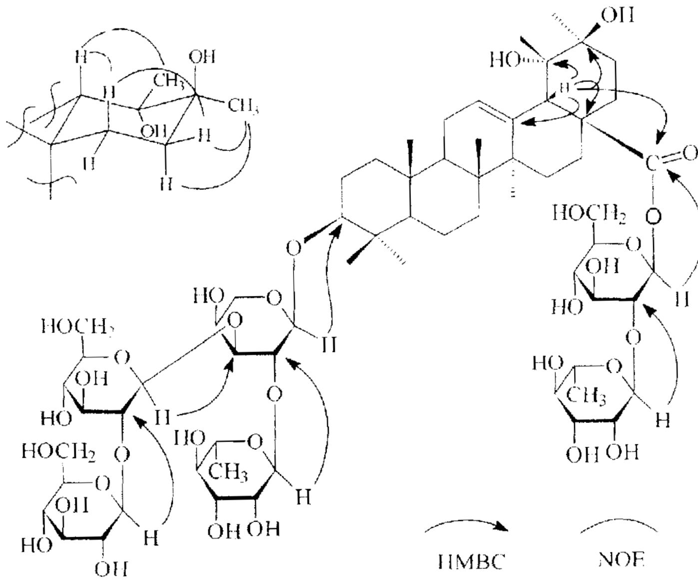

FIGURE 3 The key correlation of kudinoside K in 2D NMR spectra.

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5.14 (d, $J=7.8$ Hz), 5.28 (d, $J=7.8$ Hz), 6.20 (d, $J=8.1$ Hz), 6.35 (brs). The ^{13}C NMR spectrum showed five anomeric carbons (see Tab. I). Sugar moieties showed correlation between C-3 and H-1 of Ara, C-3 of Ara and

TABLE I ^{13}C NMR spectra data for saponins **1** ~ **8** (pyridine-d_5)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	39.3	39.3	39.3	39.4	39.3	39.5	39.5	39.1
2	26.7	26.8	26.9	26.9	26.9	26.9	26.9	26.8
3	88.4	88.5	88.7	88.6	88.8	88.6	88.6	82.5
4	39.7	39.7	39.7	39.8	39.8	39.9	39.8	43.6
5	56.3	56.4	56.5	56.5	56.6	56.5	56.5	48.0
6	18.8	18.8	18.9	18.9	18.9	18.9	19.0	18.5
7	33.8	33.7	33.8	33.7	33.8	33.2	33.1	33.3
8	40.6	40.7	40.7	40.8	40.8	40.5	40.5	39.7
9	48.2	48.2	48.2	48.0	48.0	48.8	48.9	47.8
10	37.2	37.2	37.2	37.2	37.3	37.4	37.4	37.1
11	24.2	24.2	24.2	24.3	24.3	24.6	24.6	24.3
12	128.2	128.3	128.2	128.7	128.7	123.6	123.6	128.7
13	138.8	138.7	138.8	139.5	139.5	144.5	144.6	139.4
14	42.3	42.3	42.3	42.2	42.2	42.6	42.5	42.3
15	29.8	29.8	29.8	29.8	29.9	29.5	29.4	29.4
16	26.5	26.6	26.5	26.4	26.4	28.3	28.3	26.3
17	47.9	47.9	47.9	49.0	49.0	47.0	47.0	48.8
18	50.6	50.3	50.1	54.8	55.0	45.2	45.0	54.6
19	74.2	74.1	74.1	73.0	73.0	81.5	81.6	72.9
20	76.4	76.2	76.2	42.5	42.6	35.9	36.0	42.2
21	32.9	33.0	33.0	26.9	26.9	29.4	29.2	26.2
22	34.3	34.3	34.3	37.3	37.5	33.7	33.8	37.8
23	28.3	28.3	28.4	28.4	28.5	28.4	28.4	64.6
24	17.1	17.2	17.3	16.9	16.9	17.4	17.4	13.6
25	15.9	15.9	15.9	15.9	15.9	16.0	16.0	17.6
26	17.5	17.5	17.5	17.7	17.4	17.7	17.7	17.2
27	24.2	24.4	24.2	24.5	24.6	24.8	24.9	24.7
28	176.7	176.8	178.0	177.3	177.3	177.4	177.5	177.1
29	23.5	23.5	23.6	27.3	27.3	28.9	28.9	27.2
30	23.4	23.4	23.4	17.4	17.4	25.0	25.0	16.8
3-O-Ara								
1	140.7	105.1	105.1	105.2	105.3	105.2	105.2	107.1
2	74.9	74.5	74.7	74.5	74.5	74.6	74.4	71.2
3	82.0	82.5	82.7	82.6	82.8	82.6	82.7	86.0
4	68.1	69.4	69.4	69.4	69.5	69.3	69.1	69.2
5	64.8	65.4	65.6	65.3	65.7	65.5	65.6	67.1
Rha								
1	102.0	101.1	101.1	101.2	101.3	101.2	101.1	
2	72.6	72.3	72.4	72.3	72.4	72.3	72.3	
3	72.4	72.2	72.3	72.2	72.3	72.2	72.3	
4	74.2	73.7	73.9	73.8	73.9	73.6	73.8	
5	70.1	69.8	69.9	69.8	70.0	69.9	70.0	
6	18.6	18.4	18.3	18.3	18.5	18.5	18.6	

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TABLE I (Continued)

	1	2	3	4	5	6	7	8
Glc(inn.)								
1	140.5	103.0	103.1	103.1	103.2	103.1	103.2	104.5
2	75.0	84.4	84.5	84.3	84.2	84.3	84.2	86.0
3	78.6	78.4	78.4	78.4	78.4	78.3	74.3	78.4
4	71.6	71.9	71.1	71.1	71.1	71.0	71.2	71.6
5	78.3	78.8	78.5	78.5	78.6	78.6	78.5	78.6
6	62.7	62.3	62.5	62.4	62.5	62.3	62.4	62.5
Glc(ter.)								
1		106.2	106.3	106.1	106.1	106.1	106.2	106.0
2		76.0	76.2	76.0	76.2	76.1	76.3	76.5
3		78.3	78.3	78.2	78.2	78.2	78.2	77.7
4		70.4	70.7	70.3	70.6	70.3	70.5	70.9
5		78.6	78.8	78.7	78.9	78.5	78.7	77.9
6		62.1	62.1	62.1	62.2	62.2	62.1	62.5
28-O-glc								
1	95.1	96.0	95.1	96.0	95.1	96.0	95.1	95.9
2	76.0	75.1	76.2	75.1	76.2	75.0	76.2	74.2
3	79.8	79.2	78.8	79.2	79.8	79.3	79.7	79.0
4	71.6	71.2	71.7	71.3	71.6	71.1	71.6	71.5
5	78.9	79.0	79.8	78.9	78.7	79.0	78.6	79.2
6	62.4	62.1	62.4	62.2	62.3	62.1	62.2	62.2
Rha								
1	101.5		101.5		101.6		101.6	
2	72.6		72.7		72.7		72.7	
3	72.4		72.6		72.5		72.5	
4	74.0		74.1		74.1		74.0	
5	69.9		69.9		69.9		69.9	
6	18.8		18.8		18.7		18.6	

H-1 of Glc (inn.), C-2 of Glc (inn.) and H-1 of Glc (ter.), C-2 of Ara and H-1 of Rha, C-28 and H-1 of glc in the HMBC spectrum. The sugar moiety (C-3 position) was the same linkages and sequences as that of kudinoside C [2]. The signals of the NMR data δ 6.20 (d, $J = 8.1$ Hz) and δ 96.0 of glucose indicated the esterifing position between the carboxyl (C-28) and the glucose. Based on the evidence, the structure of kudinoside J has been concluded to be 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl(1—3)-[α-L-rhamnopyranosyl(1→2)]-α-L-arabinopyranosyl kudinolic acid 28-*O*-β-D-glucopyranoside.

Kudinoside I (**1**) was obtained as an amorphous powder and exhibited a $[M-H]^-$ ion peak at m/z 1235 in the negative FAB-MS. **1** afforded kudinolic acid, glucose, rhamnose, and arabinose on acid hydrolysis. The ^1H NMR spectrum of **1** showed five anomeric protons at δ 4.85 (1H, d, $J = 6.8$ Hz, H-1-Ara), 5.09 (1H, d, $J = 7.8$ Hz, H-1-Glc), 6.20 (1H, d,

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$J = 8.0$ Hz, H-1-glc), 6.15 (1H, brs, H-1-Rha), 6.65 (1H, brs, H-1-rha). The sugar linkages and sequences were decided by HMBC spectrum. We observed the correlation between the following carbons and protons in the sugar moieties: C-3 and H-1 of Ara, C-2 of Ara and H-1 of Rha, C-3 of Ara and H-1 of Glc, C-28 and H-1 of glc, C-2 of glc and H-1 of rha. These sugar linkages and sequences were the same as those of latifoloside G ~ H [3]. Therefore, the structure of kudinoside I was determined as 3-*O*-α-L-rhamnopyranosyl(1→2)-[β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl kudinolic acid 28-*O*-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranoside.

Kudinoside P (**8**) showed a $[M-H]^-$ ion peak at *m/z* 1105 and was consistent with the molecular formula $C_{53}H_{86}O_{24}$ deduced by the negative FAB-MS. On acid hydrolysis, **8** afforded arabinose and glucose. The ^{1}H and ^{13}C NMR spectra of **8** exhibited a rotundic acid as the aglycone by comparing of the reference NMR data [5]. The ^{1}H NMR spectrum gave four anomeric protons at δ 4.80 (d, $J = 7.4$ Hz), 5.16 (d, $J = 7.8$ Hz), 5.26 (d, $J = 7.6$ Hz) and 6.28 (d, $J = 8.0$ Hz). The four corresponding anomeric carbons were assigned in Table I. On alkaline hydrolysis, **8** afforded glucose, this result implied the glucosyl group should link at the C-28 position. Sugar linkages were observed the long-range correlation between the following carbons and protons in the sugar moieties: C-3 and H-1 of Ara, C-3 of Ara and H-1 of Glc (inn.), C-2 of Glc (inn.) and H-1 of Glc (ter.), C-28 and H-1 of glc. Based on the foregoing evidence, the structure of **8** has been concluded to be 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl (1→3)-α-L-arabinopyranosyl rotundic acid 28-*O*-β-D-glucopyranoside.

Compounds **4** and **6** were the isomers and both assigned the molecular formula $C_{59}H_{96}O_{27}$. The saponins afforded a $[M-H]^-$ ion peak at *m/z* 1235 in the negative FAB-MS. The sugar moieties of the ^{1}H, ^{13}C NMR data of **4**, **6** were the same linkage and sequence as those of **2**. They all gave glucose, rhamnose and arabinose on acid hydrolysis. Compounds **4**, **6** showed a pomolic acid and a siaresinolic acid as the aglycones, respectively, by their NMR spectra. So, the structure of **4** was deduced as 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl(1→3)-[α-L-rhamnopyranosyl (1→2)]-α-L-arabinopyranosyl pomolic acid 28-*O*-β-D-glucopyranoside, named kudinoside L and **6** was 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl(1→3)-[α-L-rhamnopyranosyl (1→2)]-α-L-arabinopyranosyl siaresinolic acid 28-*O*-β-D-glucopyranoside, named kudinoside M.

Compounds **5** and **7** were the isomers, showed a $[M-H]^-$ molecular ion peak at *m/z*: 1381 in the negative FAB-MS and exhibited a pomolic acid and a siaresinolic acid as the aglycones, six anomeric carbon/proton signals at δ 105.3 [4.70 (d, $J = 6.7$ Hz)], 103.2 [5.07 (d, $J = 7.7$ Hz)], 106.1 [5.26

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(d, $J = 7.8$ Hz)], 101.3 [6.28 (brs)], 95.1 [6.14 (d, $J = 8.0$ Hz)] and 101.6 [6.60 (brs)] in the NMR spectra. By comparison of the NMR data of sugar moieties, compounds **5**, **7** had the same sugar and linkages with **3**. Therefore, kudinoside N (**5**) was formulated as 3-*O*-β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl(1→3)-[α-L-rhamnopyranosyl(1→2)]-α-L-arabinopyranosyl pomolic acid 28-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside and kudinoside O(**7**) was done as 3-*O*-β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl(1→3)-[α-L-rhamnopyranosyl(1→2)]-α-L-arabinopyranosyl siaresinolic acid 28-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside.

Thirty triterpenes and saponins isolated from *I. kudincha* [1,2,6], especially saponins **1**, **2** and **3** of having *trans*-bihydroxy groups of their aglycones give an important clue with regard to the biogenesis of forming lactonic structure as the lactonic triterpenes. The lactonic structure should reasonably explain through the key intermediate of kudinolic acid (**1a**). The biogenesis was showed as follows: the C-28 methyl of α-amyrin would be oxidized to form a carboxyl and changed to ursolic acid. The C-19 methine of the ursolic acid would be oxidized to form a hydroxyl on the C-19 position to give the pomolic acid. The C-20 methine of the pomolic acid would be done another hydroxyl to form the kudinolic acid. From the acid, the two positions between the hydroxyl on the C-20 and the carboxyl on the C-28 would be esterified to give a lactone (Fig. 4.) and finally should synthesize a series of kudinlactones (**1b** ~ **1d**).

The geometry of the lactone indicated that the configuration of the hydroxy group on the C-20 of kudinolic acid should be β hydroxyl, which is

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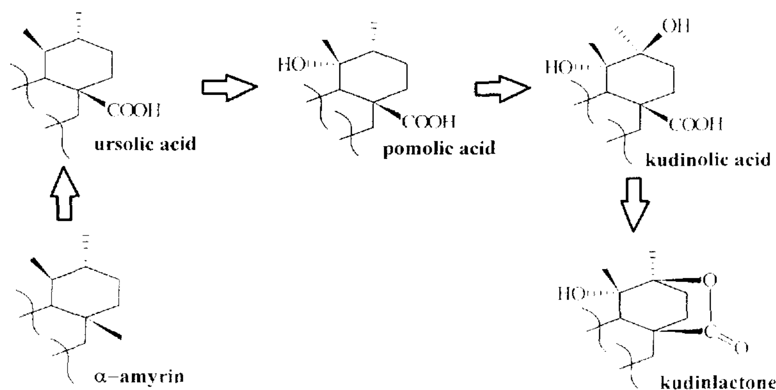

FIGURE 4 Biogenesis of kudinlactone.

the necessary condition of forming the lactone. This assuming result was substantiated by the NOESY experiment of **3**.

EXPERIMENTAL SECTION

General Experimental Procedures

^{1}H and ^{13}C NMR spectra were obtained with Bruker AM-400, DRX-500 spectrometer, FAB-MS were taken on VG Autospec 3000 system spectrometer. Optical rotations were measured on a JASCO-20C digital polarimeter, the IR spectrum was recorded as a KBr pellet on a Perkin-Elmer 1750 FTIR spectrometer, and Gas chromatography (GC) was run on a Hitachi G-3000 gas chromatography. Chromatographic material used RP-8 (40–60 μm, Merck), silica gel (160–200 mesh and 10–40 μm) and MCI-gel CHP20P (75–150 μm, Mitsubishi Chemical Industries, Ltd.). Spot of TLC was detected by spraying with 5% H_2SO_4 followed by heating.

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Extraction and Isolation

Plant Material

Plants were collected in Yinde, Guangdong Province, China in July, 1992 and identified by Prof. Chen. A voucher specimen (No. ICN-34248) is deposited in the Herbarium of the Department of Medicinal Plants, Jinan University, Guangdong, China.

Extraction and Isolation of Saponins

The dry leaves (5 Kg) were extracted (× 3) with 70% EtOH. The extract was chromatographed on silica gel column, eluted with $CHCl_3$–MeOH–H_2O (7:3:0.5) to give five fractions: Fr.I (20 g), Fr.II (68 g), Fr.III (6 g), Fr.IV (2 g), Fr.V (0.5 g), further purified Fr.III, Fr.IV, Fr.V by RP-8 gel (40–60 μm) column chromatography with H_2O containing increasing proportions of MeOH (10% ~ 90%, stepwise elution with 10% increase at each step), then by silica gel (10–40 μm, 7:3:0.5 ~ 65:35:9, $CHCl_3$:MeOH:H_2O) and MCI gel CHP 20P (10% ~ 70% MeOH) columns to yield **1** (60 mg), **2** (50 mg), **3** (210 mg), **4** (40 mg), **5** (35 mg), **6** (60 mg), **7** (51 mg), **8** (46 mg), **9** (74 mg), **10** (43 mg), **11** (30 mg).

Kudinoside K (**3**) Colorless powder, $[\alpha]_D^{27.2}$ −14.8 (MeOH, C 0.047), FAB-MS *m/z*: 1397 $[M-H]^-$, 1235 $[M-H\text{-}162]^-$, 1089 $[M-H\text{-}162-146]^-$,

927 [M–H-146–162 × 2]⁻, $C_{65}H_{106}O_{32}$, ^{1}H NMR δ: 0.88 (3H, s, Me-25), 1.08 (3H, s, Me-24), 1.11 (3H, s, Me-26), 1.15 (3H, s, Me-23), 1.30 (3H, s, Me-30), 1.46 (3H, s, Me-29), 1.73 (3H, s, Me-27), 3.16 (1H, s, H-18), 3.22 (1H, dd, $J = 4.2$, 11.3Hz, H-3), 4.74 (1H, d, $J = 7.0$Hz, H-1-Ara), 5.13 (1H, d, $J = 7.6$ Hz, H-1-Glc), 5.28 (1H, d, $J = 7.6$ Hz, H-1-Glc′), 6.21 (1H, d, $J = 7.9$ Hz, H-1-glc), 6.37 (1H, s, H-1-Rha), 6.67 (1H, s, H-1-rha′).

Kudinoside J (**2**) $[\alpha]_D^{27.0}$ +96.8 (MeOH, C 0.016), $C_{59}H_{96}O_{28}$, FAB-MS *m/z*: 1251 [M–H]⁻, 1089 [M–H-162]⁻, 943 [M–H-162–146]⁻, 927 [M–H-162 × 2]⁻. ^{1}H NMR δ: 0.87 (3H, s, Me-25), 1.15 (3H, s, Me-24), 1.16 (3H, s, Me-26), 1.20 (3H, s, Me-23), 1.29 (3H, s, Me-30), 1.46 (3H, s, Me-29), 1.71 (3H, s, Me-27), 3.16 (1H, s, H-18), 3.23 (1H, dd, $J = 4.1$, 11.2 Hz, H-3), 4.71 (1H, d, $J = 6.8$ Hz, H-1-Ara), 5.14 (1H, d, $J = 7.8$ Hz, H-1-Glc), 5.28 (1H, d, $J = 7.8$ Hz, H-1-Glc′), 6.20 (1H, d, $J = 8.1$ Hz, H-1-glc), 6.35 (1H, s, H-1-rha).

Kudinoside I (**1**) White amorphous powder, $[\alpha]_D^{26.8}$ –5.2 (MeOH, C 0.013), $C_{59}H_{96}O_{27}$, ^{1}H NMR δ: 0.89 (3H, s, Me-25), 1.08 (3H, s, Me-24), 1.13 (3H, s, Me-26), 1.14 (3H, s, Me-23), 1.31 (3H, s, Me-30), 1.46 (3H, s, Me-29), 1.74 (3H, s, Me-27), 3.16 (1H, s, H-18), 3.27 (1H, dd, $J = 4.2$, 11.4 Hz, H-3), 4.85 (1H, d, $J = 6.8$ Hz, H-1-Ara), 5.09 (1H, d, $J = 7.8$ Hz, H-1-Glc), 6.20 (1H, d, $J = 8.0$ Hz, H-1-glc′), 6.15 (1H, s, H-1-Rha), 6.65 (1H, brs, H-1-rha). FAB-MS *m/z*: 1235 [M–H]⁻, 1089 [M–H-162]⁻, 927 [M–H-162–146]⁻, 781 [M–H-162–146 × 2]⁻.

Kudinoside P (**8**) $[\alpha]_D^{27.2}$ –14.3 (MeOH, C 0.015), FAB MS *m/z*: 1105 [M–H]⁻, 943 [M–H-162]⁻, 781 [M–H-2 × 162]⁻, 649 [M–H-2 × 162–132]⁻, 619 [M–H-3 × 162]⁻, 487 [M–H-3 × 162-H-132]⁻. ^{1}H NMR δ: 0.85, 0.86, 1.18, 1.37, 1.64 (s, 5 × CH_3), 1.05 (d, $J = 4.9$ Hz, CH_3), 4.80 (1H, d, $J = 7.4$ Hz, H-1 of Ara), 5.16 (1H, d, $J = 7.8$ Hz, H-1 of Glc), 5.26 (1H, d, $J = 7.4$ Hz, H-1 of Glc′), 6.28 (1H, d, $J = 8.0$ Hz, H-1 of glc).

Kudinoside L (**4**) FAB-MS *m/z*: 1235 [M–H]⁻, 1073 [M–H-162]⁻, 911 [M–H-162 × 2]⁻, 765 [M–H-162 × 2–146]⁻, 749 [M–H-162 × 3]⁻, 603 [M–H-162 × 3–146]⁻, 471 [M–H-162 × 2–146–132]⁻, $C_{59}H_{96}O_{27}$, ^{1}H NMR δ: 0.87 (3H, s), 1.06 (d, $J = 6.5$ Hz, 3H), 1.12, 1.14, 1.17, 1.39, 1.70 (3H × 5, s), 3.24 (1H, dd, $J = 4.3$, 11.5 Hz, H-3), 4.72 (1H, d, $J = 6.7$ Hz, H-1-Ara), 5.15 (1H, d, $J = 7.8$ Hz, H-1-Glc), 5.27 (1H, d, $J = 7.8$ Hz, H-1-Glc′), 6.19 (1H, d, $J = 8.0$ Hz, H-1-glc), 6.32 (1H, brs, H-1-rha).

Kudinoside M (**5**) FAB-MS *m/z*: 1381 [M–H]⁻, 1219 [M–H-162]⁻, 1073 [M–H-162–146]⁻, 911 [M–H-162 × 2–146]⁻, 749 [M–H-146–162 × 3]⁻, 603 [M–H-146 × 2–162 × 3]⁻, $C_{65}H_{106}O_{31}$, ^{1}H NMR δ: 0.88 (3H, s), 1.06 (d, $J = 6.4$ Hz, 3H), 1.12, 1.16, 1.18, 1.40, 1.71 (3H × 5, s), 3.22 (1H, dd, $J = 4.2$, 11.3 Hz, H-3), 4.70 (1H, d, $J = 6.7$ Hz, H-1-ara), 5.07 (1H,

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d, $J = 7.7$ Hz, H-1-Glc), 5.26 (1H, d, $J = 7.8$ Hz, H-1-Glc′), 6.14 (1H, d, $J = 8.0$ Hz, H-1-glc), 6.28 (1H, brs, H-1-Rha), 6.60 (1H, brs, H-1-rha).

Kudinoside N (**6**) FAB-MS *m/z*: 1235 $[M-H]^-$, 1073 $[M-H-162]^-$, 911 $[M-H-162 \times 2]^-$, 765 $[M-H-162 \times 2-146]^-$, 749 $[M-H-162 \times 3]^-$, 603 $[M-H-162 \times 3-146]^-$, 471 $[M-H-162 \times 2-146-132]^-$, $C_{59}H_{96}O_{27}$, ^1H NMR δ: 0.72, 0.72, 1.07, 1.08, 1.12, 1.12, 1.63 (3H × 7, s), 3.23 (1H, dd, $J = 4.3$, 11.4 Hz, H-3), 4.72 (1H, d, $J = 6.7$ Hz, H-1-Ara), 5.15 (1H, d, $J = 7.8$ Hz, H-1-Glc), 5.27 (1H, d, $J = 7.8$ Hz, H-1-Glc′), 6.20 (1H, d, $J = 8.0$ Hz, H-1-glc), 6.32 (1H, brs, H-1-Rha).

Kudinoside O (**7**) FAB-MS *m/z*: 1381 $[M-H]^-$, 1219 $[M-H-162]^-$, 1073 $[M-H-162-146]^-$, 911 $[M-H-162 \times 2-146]^-$, 749 $[M-H-146-162 \times 3]^-$, 603 $[M-H-146 \times 2-162 \times 3]^-$, $C_{65}H_{106}O_{31}$, ^1H NMR δ: 0.70, 0.71, 1.07, 1.08, 1.11, 1.12, 1.64 (3H × 7, s), 3.21 (1H, dd, $J = 4.2$, 11.4 Hz, H-3), 4.71 (1H, d, $J = 6.7$ Hz, H-1-Ara), 5.12 (1H, d, $J = 7.8$ Hz, H-1-Glc), 5.26 (1H, d, $J = 7.8$ Hz, H-1-Glc′), 6.20 (1H, d, $J = 8.0$ Hz, H-1-glc), 6.37 (1H, brs, H-1-Rha), 6.64 (1H, brs, H-1-rha).

Compound **11** FAB MS *m/z*: 825 $[M-H]^-$, 663 $[M-H-162]^-$, 487 $[M-H-162-176]^-$, ^1H NMR δ: 0.70 (3H, S, CH_3), 0.84 (3H, d, $J = 5.3$ Hz, CH_3), 0.90 (3H, S, CH_3), 0.911 (3H, d, $J = 5.5$ Hz, CH_3), 1.06 (3H, S, CH_3), 1.21 (3H, S, CH_3), 5.14 (H-12), 4.27 (1H, d, $J = 7.2$ Hz, H-1 of GluA), 5.16 (1H, d, $J = 7.9$ Hz, H-1 of Glc); ^{13}C NMR δ: 46.8 (C-1), 66.0 (C-2), 84.4 (C-3), 43.6 (C-4), 47.1 (C-5), 17.0 (C-6), 32.2 (C-7), 39.3 (C-8), 45.8 (C-9), 36.8 (C-10), 23.1 (C-11), 125.2 (C-12), 137.8 (C-13), 41.7 (C-14), 27.6 (C-15), 23.7 (C-16), 47.3 (C-17), 52.4 (C-18), 38.3 (C-19), 38.3 (C-20), 30.2 (C-21), 35.8 (C-22), 62.1 (C-23), 13.9 (C-24), 16.8 (C-25), 16.9 (C-26), 20.9 (C-27), 175.0 (C-28), 17.0 (C-29), 23.3 (C-30), C-3-O-gluA δ: 103.2 (C-1), 73.2 (C-2), 73.8 (C-3), 71.9 (C-4), 76.3 (C-5), 173.3 (C-6), C-28-O-glc δ: 94.1 (C-1), 72.3 (C-2), 76.7 (C-3), 69.6 (C-4), 77.5 (C-5), 60.7 (C-6).

Acid hydrolysis The saponin (8 mg) in 1 ml MeOH was refluxed in 10 ml of 4N HCl for 4 hr, and then the mixture was extracted with AcOEt. The aqueous layer was adjusted to pH 6 with $NaHCO_3$. After evaporating to dryness, the sugars were extracted with pyridine from the residue and analyzed by HPTLC (comparing with authentic sugars) on silica gel with $CHCl_3$:MeOH:H_2O:AcOH [7:3:0.5:1]; detection with 4% α-naphthol-EtOH-5% H_2SO_4, in which the presence of arabinose, glucose, rhamnose and glucuronic acid were establish. The pyridine extract was derivatized with thiazolidine as described previously [7]. Monosaccharides were detected by GC and conditions: column, SupelcoSPB-1 0.25 mm × 27 m; column temperature, 230°C; carrier gas, N_2; t_R, L-arabinose (8.3 min), D-arabinose (8.6 min), L-glucose (13.3 min), D-glucose (13.8 min), L-rhamnose

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(9.5 min), D-rhamnose (9.1 min), L-glucuronic acid (10.6 min), and D-glucuronic acid (10.8 min). D-glucose, L-arabinose and L-rhamnose were detected in **1** ~ **10**. D-glucose and D-glucuronic acid were detected in **11**.

Alkaline hydrolysis The saponin (3 mg) was refluxed in 0.5N KOH (2 ml) for 2 hr at 70°C. The mixture was adjusted to pH 6 with 1N HCl and then extracted with AcOEt. The extract was concentrated to dryness and treated to acidic hydrolysis. After neutralizing and evaporating to dryness. The residue was extracted with pyridine and analyzed by HPTLC to detect the sugars.

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