Pergamon

PII: S0031-9422(96)00015-5

Phytochemistry, Vol. 43, No. 2, pp. 443–445, 1996

0031-9422/96 $15.00 + 0.00

TRITERPENOID GLYCOSIDES FROM *ILEX KUDINCHA*

MING-AN OUYANG, HAN-QING WANG,* ZI-LI CHEN† and CHONG-REN YANG‡

Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, Gansu, China; †Department of Chemistry, Jinan University, Guangdong, China; ‡Kunming Institute of Botany, Academia Sinica Kunming, Yunnan, China

(*Received* 3 *November* 1995)

Key Word Index—*Ilex kudincha*; Aquifoliaceae; β-kudinlactone; kudinosides A, B and C; structural elucidation.

Abstract—Three new saponins, named kudinosides A, B and C, were isolated from the leaves of *Ilex kudincha*. On the basis of spectroscopic analysis and chemical transformation their structures were elucidated as 3-*O*-β-D-glucopyranosyl - (1→3) - [α - L - rhamnopyranosyl - (1→2)] - α - L - arabinopyranosyl - β - kudinlactone, 3 - *O* - β - D - glucopyranosyl - (1→2) - β - D - glucopyranosyl - (1→3) - α - L - arabinopyranosyl - β - kudinlactone and 3 - *O* - β - D - glucopyranosyl - (1→2) - β - D - glucopyranosyl - (1→3) - [α - L - rhamnopyranosyl - (1→2)] - α - L-arabinopyranosyl-β-kudinlactone. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Ilex kudincha C. J. Tseng is widely used as a traditional beverage, known as Ku-Ding-Cha, in southern China. It is also used in folk medicine as a diuretic and for the treatment of a sore throat, weight loss and hypertension [1–2]. In a continuation of our chemical investigation on *I. kudincha*, we report here on the isolation and structural elucidation of three new saponins, named kudinosides A (**1**), B (**2**) and C (**3**), respectively.

RESULTS AND DISCUSSION

The butanol extract of the leaves of *I. kudincha* provided three saponins A, B and C (**1**–**3**). Their IR spectra showed ester group absorptions (1730 cm^{-1}) together with strong hydroxyl absorptions (3450–3200 cm^{-1}) and a C=C double bond absorption (1640 cm^{-1}).

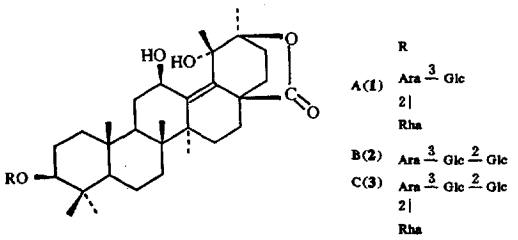
Cellulase treatment of **1**, **2** or **3** gave the same aglycone, designated β-kudinlactone (**4**), $C_{30}H_{46}O_5$, which showed a quasi-molecular ion peak at *m/z* 486 $[M]^+$ in its EI mass spectrum. The ^{13}C NMR (DEPT spectrum) and ^1H NMR data indicated the presence of seven methyls, nine methylenes, four methines and 10 quaternary carbons. When β-kudinlactone was compared with known triterpenes [3–7], it became apparent that it was an ursolic acid derivative. ^1H–^1H COSY and ^1H homodecoupling experiments revealed five isolated spin systems (H-1–2, H-5–7, H-9–11–12, H-15–16 and H-21–22). The structure of **4** was determined by analysis of the NMR data including ^1H–^1H COSY, ^{13}C–^1H COSY and COLOC data. The β-orientation of the C-3 and C-12 hydroxyl groups and the α-orientation of the C-19 hydroxyl group were determined from NOESY experiments. The configuration of the 28,20β-lactone was determined by comparison with the known triterpene compound [7]. Accordingly, β-kudinlactone was represented as 3β,12β,19α-trihydroxyurs-13(18)-en-28,20β-lactone.

In addition, arabinose, glucose and rhamnose were identified by TLC. The ^{13}C NMR spectra indicated the presence of three anomeric carbon signals: δ 105.5, 104.8 and 101.9 in saponin A, δ 107.2, 104.7 and 106.8 in saponin B, and of four anomeric carbon signals: δ 105.1, 103.1, 106.4 and 101.0 in saponin C. The simultaneous presence of a 3-*O*-glycosidic linkage was easily seen by the attendant downfield shift (to about δ 88.7) for C-3.

The anomeric configuration of the sugars were fully defined by the ^1H NMR spectra. Thus, the anomeric proton signals for **1** at δ 4.76 (1H, *d*, *J* = 4.5 Hz), 5.13 (1H, *d*, *J* = 7.9 Hz) and 6.40 (1H, *br s*), for **2** at δ 4.79 (1H, *d*, *J* = 7.5 Hz), 5.17 (1H, *d*, *J* = 7.9 Hz) and 5.25 (1H, *d*, *J* = 8.0 Hz), for **3** at δ 4.76 (1H, *d*, *J* = 7.5 Hz), 5.13 (1H, *d*, *J* = 7.9 Hz), 5.24 (1H, *d*, *J* = 8.0 Hz) and 6.40 (1H, *br s*) led to the assignments of the anomeric configurations of the arabinoside units and the rhamnoside units as α and those of the glucoside units as β. These assignments were supported by the ^{13}C NMR data.

The sites of the sugar linkages (kudinoside C) were established by FAB mass spectrometry, ^{13}C–^1H COSY, TOCSY and NOESY experiments. First, FAB mass spectrometry provided that the terminal sugars were rhamnose and glucose (*m/z* 949 $[M + Li - 146]^+$ and 931 $[M + Li - 162]^+$), and each sugar proton and carbon signals were determined by ^{13}C–^1H COSY and TOCSY. Second, the NOESY experiment on kudinoside

*Author to whom correspondence should be addressed.



C gave four characteristic cross-peaks between the signal assignable to the anomeric proton of Ara (δ 4.76) and the signal assignable to the proton of C-3 (δ 3.30), between the anomeric proton of Rha (δ 6.40) and the anomeric proton of Ara (δ 4.76), between the anomeric proton of Glc (δ 5.13) and the proton H-3 of Ara (δ 4.20), and between the anomeric proton of Glc (δ 5.24) and the anomeric proton of Glc. These assignments were also supported by comparison with the ^{13}C NMR data with that for each monosaccharide [8].

Based on the above evidence, the chemical structure of kudinoside C was elucidated to be 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-β-kudinlactone.

Both kudinoside A and B contained one less sugar moiety than kudinoside C. With the aid of the FAB-mass spectra, we found that the sugar moeity of A contained one less glucose moiety than that of C, and that that of B contained one less rhamnose than that of C. Thus, kudinoside A was identified as 3-*O*-β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-β-kudinlactone, kudinobide B was 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranosyl-β-kudinlactone.

EXPERIMENTAL

Mps; uncorr.; ^1H and ^{13}C NMR: 100 MHz, pyridine-d_5 with TMS as int. standard; CC and chromatography TLC: silica gel, RP-8 and RP-18 using the following solvent systems: (a) $CHCl_3$–MeOH–H_2O (14:6:1), $CHCl_3$–MeOH–H_2O (65:35:9), and MeOH–H_2O (3:2 → 7:3) for saponins; (b) C_6H_6–Me_2CO (5:1) for sapogenin; (c) $CHCl_3$–Me_3OH–H_2O (7:3:1) lower layer (9 ml) + 1 ml HOAc for sugars. Detection: saponins and sapogenin, sprayed with 10% H_2SO_4 followed by heating for 5 min at 105°; for sugar, aniline phthalate reagent.

Plant material. Plants were collected in Yinde, Guangdong Province, China in the summer of 1992 and identified by Prof. Zi-li 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 leaves of *I. kudincha* (5 kg) were pulverized and extracted with EtOH–H_2O (7:3). The extracts were combined and concd *in vacuo* to give a brown residue which was suspended in H_2O and extracted with Et_2O, EtOAc and BuOH, respectively. The *n*-BuOH extract was subjected to TSK gel G3000S CC [eluting with H_2O → MeOH (100% H_2O, 80% H_2O–MeOH, 50% H_2O–MeOH; 30% H_2O–MeOH), silica gel CC [eluting with $CHCl_3$–MeOH-H_2O, 14:6:1 → $CHCl_3$–MeOH–H_2O, 65:35:9], and reversed-phase HPLC [ODS, eluting with MeOH–H_2O, 7:3 → MeOH–H_2O = 14:9, UV: 215 nm, flow rate: 5 ml min^{-1}, injection: 0.4 ml (10 mg ml^{-1})] to afford **1** (80 mg), **2** (70 mg) and **3** (180 mg).

Kudinoside A (**1**). Mp 195–197°, $[\alpha]_D^{20}$ −67.8° (*c* 1.7, pyridine-d_5). *IR* (*KBr*) ν_{max} cm^{-1}: 3450–3200 (OH), 1730 (C=O), 1640 (C=C), 1450, 1380, 1070, 1040. $C_{47}H_{74}O_{18}$; ^1H NMR δ: 0.88 (3H, *s*, CH_3), 0.92 (3H, *s*, CH_3), 0.96 (3H, *s*, CH_3), 1.29 (3H, *s*, CH_3), 1.50 (3H, *s*, CH_3), 1.61 (3H, *s*, CH_3), 1.64 (3H, *s*, CH_3), 1.67 (3H, *d*, *J* = 5.9 Hz, C-5-CH_3 of Rha), 4.76 (1H, *d*, *J* = 4.5 Hz, C-1-H of Ara), 5.13 (1H, *d*, *J* = 7.9 Hz, C-1-H of Glc), 6.40 (1H, *br s*, C-1-H of Rha); ^{13}C NMR: Table 1; FAB-MS *m/z*: 926 [M], 763 $[M-1-162]^-$, 701 $[M-1-H_2O-CO_2-162]^-$, 467 $[M-1-H_2O-162-146-132]^-$.

Kudinoside B (**2**). Mp 218 − 221°, $[\alpha]_D^{20}$ −16.3 (*c* 0.19, pyridine-d_5), $C_{47}H_{74}O_{19}$. IR (KBr) ν_{max} cm^{-1}: 3450–3200 (OH), 1730 (C=O), 1640 (C=C), 1450, 1380, 1070, 1040; FAB-MS *m/z*: 942 $[M]^-$, 779 $[M-1-162]^-$, 717 $[M-1-H_2O-CO_2-162]^-$, 555 $[M-1-H_2O-CO_2-(2\times162)]^-$, 423 $[M-1-H_2O-CO_2-(2\times162)-132]^-$; ^1H NMR δ: 0.85 (3H, *s*, CH_3), 0.89 (3H, *s*, CH_3), 1.14 (3H, *s*, CH_3), 1.23 (3H, *s*, CH_3), 1.50 (3H, *s*, CH_3), 1.60 (3H, *s*, CH_3), 1.64 (3H, *s*, CH_3), 4.79 (1H, *d*, *J* = 7.5 Hz, C-1-H of Ara), 5.17 (1H, *d*, *J* = 7.9 Hz, C-1-H of Glc), 5.25 (1H, *d*, *J* = 8.0 Hz, C-1-H of Glc); ^{13}C NMR: Table 1.

Kudinoside C (**3**). Mp 185–187°, $C_{53}H_{84}O_{23}$, $[\alpha]_D^{20}$ −31.0° (*c* 0.15, pyridine-d_5). IR (KBr) ν_{max} cm^{-1}: 3450–3200 (OH), 1728 (C=O), 1640 (C=C), 1450, 1380, 1070, 1040; FAB-MS *m/z*: 1095 $[M+Li]^+$, 1111 $[M+Na]^+$, 949 $[M+Li-146]^+$, 931 $[M+Li-162]^+$, 607 $[132+146+(2\times162)]^+$; ^1H NMR δ: 0.85 (3H, *s*, CH_3), 0.88 (3H, *s*, CH_3), 1.16 (3H, *s*, CH_3), 1.23 (3H, *s*, CH_3), 1.49 (3H, *s*, CH_3), 1.61 (3H, *s*, CH_3), 1.64 (3H, *s*, CH_3), 4.76 (1H, *d*, *J* = 7.0 Hz, C − 1 − H of Ara), 5.13 (1H, *d*, *J* = 7.9 Hz, C − 1 − H of Glc), 5.24 (1H, *d*, *J* = 8.0 Hz, C − 1 − H of Glc), 6.40 (1H, *br s*, C − 1 − H of Rha); ^{13}C NMR: Table 1.

Acid hydrolysis of kudinoside A, B or C. The sample (kudinoside A, B or C) was dissolved in 7% H_2SO_4 (5 ml) and refluxed on a water bath at 90° for 3 hr. The reaction mixt. was diluted with H_2O and extracted with $CHCl_3$. The aq. layer was neutralized with 1 N NaOH and concd *in vacuo*. The residue was compared with standard sugars on TLC with solvent system (c) and shown to consist of Ara, Glc and Rha in each case.

Enzymatic hydrolysis of kudinoside A, B or C. Kudinoside A (35 mg), B (30 mg) or C (40 mg) was dissolved in EtOH–H_2O (1:9) and 0.01 M Na–Pi

Table 1. ^{13}C NMR chemical data for kudinosides

C	1	2	3	4	Sugar	1	2	3
1	39.2	39.3	39.3	39.2	3-Ara C-1	105.5	107.2	105.1
2	28.5	28.6	28.2	28.3	2	74.4	71.6	74.4
3	88.9	89.5	88.5	78.2	3	82.5	86.0	83.0
4	39.9	39.9	39.6	39.5	4	68.4	69.2	69.4
5	56.4	56.4	56.3	56.1	5	65.0	67.3	65.8
6	18.8	18.7	18.6	18.7	3-Glc C-1	104.8	104.7	103.1
7	35.7	35.6	35.5	35.6	2	75.1	86.2	84.9
8	41.9	42.0	41.7	41.8	3	78.2	78.4	78.4
9	45.1	45.0	44.9	45.0	4	71.5	70.9	70.9
10	37.3	37.3	37.1	37.6	5	78.7	79.3	78.4
11	28.9	28.9	28.9	28.9	6	62.6	62.2	62.5
12	66.5	66.5	66.1	66.2	3-Glc C-1		106.8	106.4
13	146.6	146.7	146.4	146.4	2		76.4	76.2
14	44.3	44.3	43.9	43.9	3		77.7	78.2
15	29.1	28.9	28.9	28.9	4		70.7	70.5
16	26.7	26.9	26.8	26.3	5		77.8	78.9
17	44.6	44.7	44.1	44.1	6		62.2	62.0
18	137.6	137.7	137.8	137.6	3-Rha C-1	101.9		101.0
19	74.3	74.5	74.4	74.4	2	72.4		72.4
20	86.2	86.3	85.7	85.7	3	72.5		72.6
21	28.5	28.6	28.3	28.4	4	74.1		74.0
22	32.8	32.9	32.9	32.9	5	70.3		69.8
23	28.3	28.5	28.2	28.8	6	18.3		18.3
24	17.0	17.1	17.2	16.7				
25	16.7	16.7	16.8	16.5				
26	18.6	18.4	18.2	18.3				
27	23.7	23.6	23.5	23.5				
28	176.1	176.2	175.4	175.4				
29	25.4	25.3	25.2	25.3				
30	19.7	19.8	19.5	19.5				

buffer (pH 4.0) 5 ml each, and incubated with crude cellulase (50 mg, Sigma) for 2 weeks at 37°. After work-up as usual, the crude genin was subjected to CC on silica gel with C_6H_6–Me_2CO (5:1) giving **4** (12 mg), mp 220–222°, $[\alpha]_D^{20}$ +114° (*c* 0.035, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 3300–3200 (OH), 1730 (C=O), 1640 (C=C); EIMS *m/z*: 486 $[M]^+$; ^1H NMR (pyridine-d_5) δ: 0.93 (6H, *br s*, 2 × CH_3), 1.03 (3H, *s*, CH_3), 1.24 (3H, *s*, CH_3), 1.50 (3H, *s*, CH_3), 1.59 (3H, *s*, CH_3), 1.65 (3H, *s*, CH_3), 3.45 (1H, *dd*, $J = 4.4$, 11.2 Hz, H-3), 0.96 (1H, *br s*, H-5), 2.18 (1H, *br d*, H-9), 5.92 (1H, *br s*, H-12); ^{13}C NMR: Table 1.

Acknowledgements—The authors are grateful to the staff of the Laboratory of Kunming Institute of Botany, Academia Sinica, for financial support and their valuable advice. Thanks are also due to Mr Yu-qing Lu and Guan-ping Yu for helpful discussions of the experimental part of the work.

REFERENCES

1. Zhai, P. K. (1985) *Iconographia Cormophytorum Sinicoru*, p. 641. Beijing Science Publishing House, Beijing.
2. Jang Su Medical College (1975) *Chinese Medicine Dictionary*, p. 1288. Shanghai People's Publishing House, Shanghai.
3. Akira, I., Mari, K. and Hiroko, M. (1987) *Chem. Pharm. Bull.* **35**, 841.
4. Munthiro, N., Yuji, M. and Takashi, I. (1989) *Phytochemistry* **28**, 1479.
5. Takashi, K., Kazuko, Y. and Shigenobu, A. (1991) *Tetrahedron* **47**, 7219.
6. Manik, C. D. and Shashi, B. M. (1982) *Phytochemistry* **21**, 2069.
7. Errington, S. G. and Jefferies, P. R. (1988) *Phytochemistry* **27**, 543.
8. Bock, L. (1983) *Adv. Carbohydr. Chem. Biochem.* **41**, 27.