[Chem. Pharm. Bull. 31(11)3865—3871(1983)]

Chemical and Chemotaxonomical Studies on Filices. XLIII.[1] Chemical Studies on the Constituents of *Lindsaea javanensis* BL., *L. japonica* (BAK.) DIELS and *Tapeinidium pinnatum* (CAV.) C. CHR.

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(Received March 2, 1983)

Three new diterpene glycosides (V, VII and IX) were isolated from *Lindsaea javanensis*, together with lindsaea acid, 2β,16α-dihydroxy-*ent*-kaurane 2-*O*-β-D-glucopyranoside and 16α,17,19-trihydroxy-*ent*-kaurane 19-*O*-β-D-glucopyranoside. The structures of the new compounds were elucidated by spectroscopic methods as 16α,19-dihydroxy-*ent*-kaurane 19-*O*-β-D-glucopyranoside, 12β,16α,17,19-tetrahydroxy-*ent*-kaurane 19-*O*-β-D-glucopyranoside and 12β,16α,19-trihydroxy-*ent*-kaurane 19-*O*-β-D-glucopyranoside, respectively. From *L. japonica*, *o*-coumaric acid was isolated, while lindsaea acid and 2,6-dimethoxybenzoquinone were isolated from *Tapeinidium pinnatum*.

Keywords——*Lindsaea javanensis*; *Lindsaea japonica*; *Tapeinidium pinnatum*; Pteridaceae; lindsaea acid; *o*-coumaric acid; 2,6-dimethoxybenzoquinone; *ent*-kaurane-type diterpene glycoside; chemotaxonomy; structural elucidation

As a continuation of our chemical and chemotaxonomical studies of ferns, the constituents of two species of the genus Lindsaea (*Lindsaea javanensis* BL. and *L. japonica* (BAK.) DIELS) and one species of the genus Tapeinidium (*Tapeinidium pinnatum* (CAV.) C. CHR.) were investigated. This paper deals with the structural elucidation of three new compounds.

(1) *Lindsaea javanensis* BL. (Japanese Name: Sankaku-Hongushida)

From this fern, three new glycosides, A (V), B (VII) and C (IX), were isolated along with lindsaea acid (I),[2] *trans*-cinnamic acid, creticoside B[3] (2β,16α-dihydroxy-*ent*-kaurane 2-*O*-β-D-glucopyranoside II) and 16α,17,19-trihydroxy-*ent*-kaurane 19-*O*-β-D-glucopyranoside (III).[4]

Glycoside A (V), $C_{26}H_{44}O_7$, colorless needles, mp 237—238 °C, $[\alpha]_D^{22}$ −64.0° (c=0.35, pyridine), gave D-glucose and 16α,19-dihydroxy-*ent*-kaurane (VI)[5] on acidic hydrolysis. In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of V (in Pyr.-d_5), the signal assignable to C-19 (δ 73.2) was shifted downfield by 9 ppm[6] in comparison with that of VI. The ^{13}C-NMR spectrum of V also showed characteristic signals[7] of the β-D-glucopyranosyl moiety. These data and the large coupling constant (7 Hz) of the anomeric proton signal ($\delta_{\text{Pyr.-}d_5}$ 4.82) in the proton magnetic resonance (^{1}H-NMR) spectrum showed that the D-glucose was linked to the hydroxyl group at C-19 in the β-configuration. Accordingly, glycoside A is 16α,19-dihydroxy-*ent*-kaurane 19-*O*-β-D-glucopyranoside (V).

Glycoside B (VII), $C_{26}H_{44}O_9$ was obtained as colorless needles, mp 257—258 °C, $[\alpha]_D^{26}$

$-46.4°$ ($c=0.3$, pyridine). Enzymatic hydrolysis using β-D-glucosidase (emulsin) afforded D-glucose and an aglycone (VIII), $C_{20}H_{34}O_4$, colorless needles, mp 252—253 °C, $[\alpha]_D^{17}$ $-51.4°$ ($c=0.28$, MeOH). The ^{1}H-NMR spectrum (Pyr.-d_5) and ^{13}C-NMR spectrum (Pyr.-d_5) of VIII are similar to those of 16α,17,19-trihydroxy-*ent*-kaurane (IV, aglycone of III), indicating it to be an *ent*-kaurane type diterpene. The proton signals at δ 3.60 and 3.97 (each 1H, d, $J=11$ Hz) and the carbon signal at δ 64.1 (t) revealed the presence of a hydroxyl group at C-19.[8] Furthermore, the proton signals at δ 4.14 and 4.44 (each 1H, d, $J=11$ Hz) and the carbon signals at δ 68.6 (t) and 81.7 (s) indicated the presence of a 16α,17-glycol system.[9] In the ^{1}H-NMR spectrum, signals due to two tertiary methyl groups at δ 0.99 and 1.19 (each 3H, s), the 13-hydrogen at δ 2.70 (1H, m) and one carbinyl methine at δ 4.28 (1H, m) were observed. These spectral data and the molecular formula ($C_{20}H_{34}O_4$) indicate VIII to be a derivative of 16α,17,19-trihydroxy-*ent*-kaurane bearing an additional secondary hydroxyl group. A comparison of the ^{13}C-NMR spectral data of VIII with those of IV indicated that the signals assignable to C-11, C-12 and C-13 were shifted downfield by 10.7, 43.9 and 7.8 ppm, respectively (Table II), and in the proton nuclear magnetic double resonance experiments on VIII, the multiplet at δ 2.70 (assigned to 13-H) changed into a doublet on irradiation of the signal at δ 4.28, suggesting that the additional secondary hydroxyl group is located at C-12. In the ^{1}H-NMR spectrum of VIII, the signal of the 10-methyl group was not shifted downfield in comparison with that of IV[10] and a one-proton multiplet of $W_{h/2}$ 22 Hz due to 12-H appeared at δ 4.28, indicating that the hydroxyl group at C-12 is oriented in the β-configuration. This conclusion was supported by the fact that the carbon signal due to C-14 of VIII was at almost the same position as that of IV, namely the *gauche* γ-substituent effect was not observed on introduction of the 12-hydroxyl group.[11] Thus, VIII was considered to be 12β,16α,17,19-tetrahydroxy-*ent*-kaurane. A comparison of the ^{13}C-NMR spectrum of VII with that of VIII showed a downfield shift (9.0 ppm) of the carbon signal assignable to C-19. Furthermore, the characteristic signals due to a β-D-glucopyranosyl moiety were observed in the ^{13}C-NMR spectrum of VII. These findings and the large coupling constant (7 Hz) of the anomeric proton signal (δ 4.86) indicated that D-glucopyranose was bound to the hydroxyl group at C-19 in the β-configuration. Therefore, the 12β,16α,17,19-tetrahydroxy-*ent*-kaurane 19-*O*-β-D-glucopyranoside structure was assigned to glycoside B (VII).

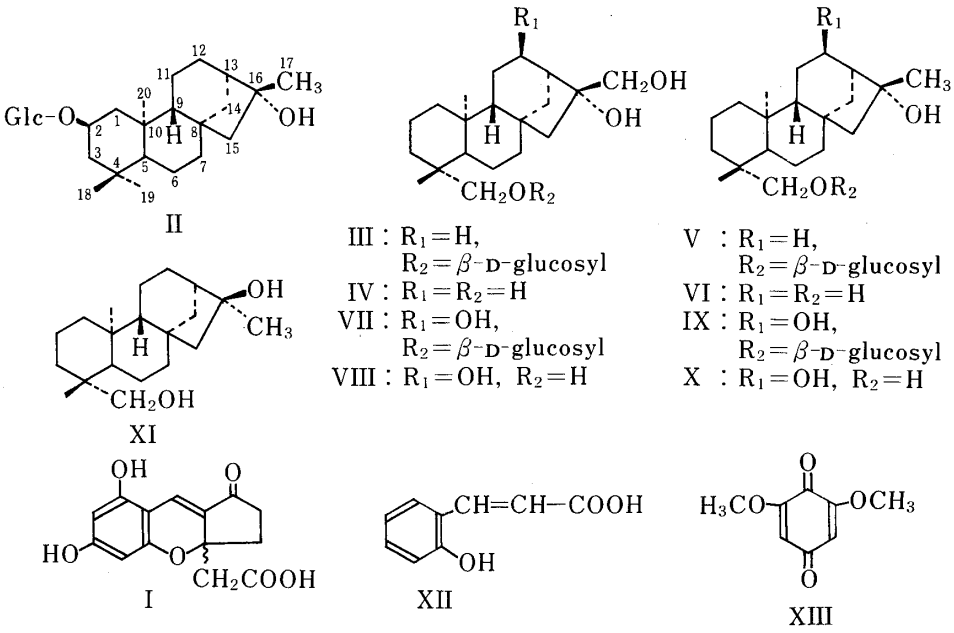

Fig. 1

Table I. ^{1}H-NMR Chemical Shifts in Pyridine-d_5 (ppm)

	IV	VI	VIII	X
4β-CH_3	1.04 (s)	1.04 (s)	0.99 (s)	1.04 (s)
4α-CH_2OH	3.64 (d, J=11 Hz) 4.02 (d, J=11 Hz)	3.57 (d, J=11 Hz) 3.95 (d, J=11 Hz)	3.60 (d, J=11 Hz) 3.97 (d, J=11 Hz)	3.61 (d, J=11 Hz) 3.99 (d, J=11 Hz)
10-CH_3	1.20 (s)	1.16 (s)	1.19 (s)	1.20 (s)
12α-H			4.28 (m, $W_{h/2}$=22 Hz)	4.33 (m, $W_{h/2}$=20 Hz)
13-H			2.70 (m)	2.62 (m)
16β-CH_3		1.53 (s)		2.04 (s)
16β-CH_2OH	4.03 (d, J=10 Hz) 4.17 (d, J=10 Hz)		4.14 (d, J=11 Hz) 4.44 (d, J=11 Hz)	

Table II. ^{13}C-NMR Chemical Shifts in Pyridine-d_5

	II	III	IV	V	VI	VII	VIII	IX	X	XI
C-1	48.0	40.6	40.7	40.6	40.7	40.5	40.7	40.6	40.7	40.8
C-2	72.4	18.7	18.7	18.7	18.7	18.7	18.7	18.6	18.7	18.8
C-3	48.0	36.7	36.2	36.8	36.1	36.6	36.1	36.6	36.2	36.2
C-4	34.6	38.3	39.3	38.4	39.2	38.3	39.3	38.3	39.2	39.3
C-5	56.0	56.9	56.9	57.1	57.0	56.7	56.7	56.7	56.6	57.1
C-6	20.4	21.1	21.1	21.1	21.0	21.0	21.0	21.0	21.0	20.7
C-7	42.2	43.0	43.1	43.0	43.0	42.0	42.0	42.0	42.2	43.0
C-8	45.3	44.8	44.9	45.5	45.4	44.7	44.7	45.0	45.1	44.7
C-9	57.0	57.2	57.3	57.4	57.4	58.2	58.3	58.4	58.5	57.9
C-10	41.0	39.5	39.6	39.5	39.5	39.0	39.1	39.0	39.2	39.6
C-11	18.4	18.7	18.7	18.5	18.6	29.4	29.4	29.0	29.1	18.8
C-12	27.2	26.7	26.7	27.2	27.2	70.5	70.6	71.2	71.2	27.5
C-13	49.3	45.9	46.0	49.3	49.2	53.8	53.8	56.8	56.9	47.7
C-14	38.0	37.5	37.7	37.9	37.9	37.2	37.3	38.0	38.2	38.9
C-15	58.5	53.8	54.0	58.7	58.5	54.0	54.1	59.4	59.6	58.5
C-16	77.7	81.5	81.5	77.9	77.7	81.7	81.7	78.0	78.0	76.3
C-17	25.0	66.3	66.4	25.1	25.0	68.6	68.6	27.4	27.4	33.1
C-18	33.8	28.2	28.1	28.4	28.0	28.3	28.1	28.3	28.1	28.1
C-19	22.4	73.0	64.1	73.2	64.0	73.1	64.1	73.0	64.1	64.1
C-20	18.9	18.4	18.6	18.5	18.4	18.4	18.7	18.7	18.7	18.5
C-1′	102.7	105.3		105.5		105.4		105.4		
C-2′	75.3	75.1		75.3		75.3		75.3		
C-3′	78.3	78.1		78.4		78.4		78.3		
C-4′	71.8	71.7		71.9		71.8		71.7		
C-5′	78.6	78.6		78.8		78.8		78.7		
C-6′	62.9	62.8		62.9		62.9		62.9		

Glycoside C (IX) was obtained as an inseparable mixture of glycosides B (VII) and C (IX), and on acidic hydrolysis, gave only D-glucose as a sugar. After enzymatic hydrolysis with β-D-glucosidase, the aglycone (X), $C_{20}H_{34}O_3$, mp 211—212 °C, $[\alpha]_D^{24}$ −9.5° (c=0.20, MeOH) was obtained in a pure form. The ^{1}H-NMR spectrum (Pyr.-d_5) of X exhibited signals due to three tertiary methyl groups at δ 1.04, 1.20 and 2.04 (each 3H, s), 13-hydrogen at δ 2.62 (1H, m) and the 4α-hydroxymethyl group[8] at δ 3.61 and 3.99 (each 1H, d, J=11 Hz). These spectral data are analogous to those of VI, suggesting X to be an *ent*-kaurane type diterpene. Furthermore, one proton signal at δ 4.33 was ascribed to a carbinyl proton at C-12 on the basis of spin-decoupling experiments. The presence of a hydroxyl group at C-12 was also

supported by the downfield shifts of the ^{13}C-NMR (Pyr.-d_5) signals assignable to C-11, C-12 and C-13 (10.5, 44.0 and 7.7 ppm, respectively) in comparison with those of VI (Table II). In the ^{1}H-NMR spectrum of X, the 10-methyl proton signal remained in the same region as that of VI, and a one-proton multiplet of $W_{h/2}$ 20 Hz due to C-12 was also observed at δ 4.33. In the ^{13}C-NMR spectrum of X, the carbon signal due to C-14 was not shifted upfield in comparison with that of VI.[11] Therefore, the hydroxyl group at C-12 is oriented in the β-configuration, as in the case of VIII. The ^{13}C-NMR signals due to C-16 and C-17 were found at δ 78.0 (s) and 27.4 (q), respectively, indicating the presence of a tertiary hydroxyl group at C-16. The chemical shift values are close to those (δ 77.7 (s), 25.0 (q)) of 16α,19-dihydroxy-*ent*-kaurane (VI), rather than those (δ 76.3 (s), 33.1 (q)) of 16β,19-dihydroxy-*ent*-kaurane (XI),[12] so that the hydroxyl group at C-16 is oriented in the α-configuration. The strong downfield shift of the 16β-methyl signal (δ 2.04) in comparison with that (δ 1.53) of VI is probably due to pyridine-induced shift around the 12β-hydroxyl group. Thus, X was formulated as 12β,16α,19-trihydroxy-*ent*-kaurane. In the ^{13}C-NMR spectrum of IX, the signal assignable to C-19 (δ 73.0) was shifted downfield by 8.9 ppm in comparison with that of X and the characteristic signals due to a β-D-glucopyranosyl moiety were also observed. These observations and the large coupling constant (7 Hz) of the anomeric proton signal (δ 4.81) indicated that the glucopyranosyl moiety had a β-glycosidic linkage with the hydroxyl group at C-19. Consequently, glycoside C was established as 12β,16α,19-trihydroxy-*ent*-kaurane 19-*O*-β-D-glucopyranoside (IX).

As insufficient amounts of materials were available, chemical correlations of VII and IX to known compounds were not possible, but the comparative ^{13}C-NMR data (see Table II) with those of related compounds are sufficient to support the proposed structures.

(2) *L. japonica* (BAK.) DIELS (Japanese Name: Saigoku-Hongushida)

From this fern, *o*-coumaric acid (XII) was isolated.

(3) *Tapeinidium pinnatum* (CAV.) C. CHR. (Japanese Name: Gozadakeshida)

From this fern, 2,6-dimethoxybenzoquinone (XIII)[13] and lindsaea acid (I) were isolated. The former is the first example of a benzoquinone derivative isolated from ferns.

Experimental

The instruments used to obtain physical data, the materials and the experimental conditions were the same as those described in part XXXVII[14] in this series unless otherwise specified.

Isolation Procedure

1) *Lindsaea javanensis* BL.——The air-dried ferns (120 g), collected in August in Yakushima, Kagoshima Prefecture, were extracted 3 times with methanol (each 300 ml) under reflux for 6 h. The combined methanolic extracts (900 ml) were passed over activated charcoal (12 g) in a column of 3 cm diameter and were further eluted with methanol (3 l). The combined solution (3.9 l) was concentrated *in vacuo* to 100 ml and partitioned between $CHCl_3$ (400 ml) and H_2O (300 ml). The lower layer was concentrated *in vacuo* to a syrup (1.2 g), which was subjected to column chromatography on silica gel (35 g). Elution was carried out with $CHCl_3$ (200 ml), 3% MeOH in $CHCl_3$ (200 ml, fr. 1), 10% MeOH in $CHCl_3$ (200 ml, fr. 2) and 15% MeOH in $CHCl_3$ (200 ml, fr. 3) successively. Fraction 1 was subjected to preparative layer chromatography (PLC) (solvent system, $CHCl_3$–MeOH, 15 : 1) to give *trans*-cinnamic acid (30 mg). Fraction 2 was purified by chromatography on silica gel using a mixture of $CHCl_3$ and MeOH (20 : 1) as the eluent to give creticoside B (II, 13 mg) and glycoside A (V, 15 mg). The upper layer was extracted 3 times with ethyl acetate (each 300 ml). The combined ethyl acetate extracts were concentrated *in vacuo* to a syrup (650 mg), which was subjected to droplet counter current chromatography (DCCC, solvent system, $CHCl_3$–MeOH–H_2O, 4 : 4 : 3). The combined fractions 17—33 were chromatographed on silica gel with $CHCl_3$–MeOH (10 : 1), followed by *n*-BuOH–sat. H_2O to afford glycoside B (VII, 20 mg) and glycoside C (IX, 15 mg). The combined fractions 34—43 were dissolved in MeOH, treated with excess ethereal diazomethane for 12 h and then concentrated to dryness. The residue was subjected to PLC (solvent system, $CHCl_3$–ether 3 : 1) to yield di-*O*-methyl-lindsaea acid methyl ester (3 mg). The product from the combined fractions 44—84 was crystallized from a mixture of MeOH and H_2O to give 16α,17,19-trihydroxy-*ent*-kaurane 19-*O*-β-D-glucopyranoside (III, 100 mg).

2) ***L. japonica*** **(BAK.) DIELS**——The air-dried ferns (100 g), collected in August in Yakushima, Kagoshima Prefecture, were extracted 3 times with methanol (each 500 ml) under reflux for 6 h. The combined methanolic extracts (1.5 l) were passed over activated charcoal (10 g) in a column of 3 cm diameter and eluted with methanol (2 l). The combined eluates (3.5 l) were concentrated *in vacuo* to give a residue (300 mg), which was subjected to column chromatography on silica gel (13 g) with $CHCl_3$. The enriched fraction was further chromatographed on silica gel (10 g) with $CHCl_3$–ether (4 : 1) to give *o*-coumaric acid (XI, 20 mg).

3) ***Tapeinidium pinnatum*** **(CAV.) C. CHR.**——The air-dried ferns (300 g), collected in December in Taiwan, were extracted 3 times with methanol (each 1 l) under reflux for 6 h. The combined methanolic extracts were passed over activated charcoal (30 g) in a column of 5 cm diameter and eluted with methanol (5 l). The combined eluates were concentrated to a syrup (14 g), which was partitioned between the upper and lower phases of a mixture of $CHCl_3$ (200 ml), MeOH (200 ml) and H_2O (80 ml). The lower phase was concentrated *in vacuo* to a syrup (1.6 g), which was subjected to column chromatography on silica gel with 10% ether in $CHCl_3$ to afford 2,6-dimethoxybenzoquinone (8 mg). The upper phase was extracted with ethyl acetate (200 ml) and the organic phase was evaporated to a syrup (2.7 g), which was subjected to DCCC to give lindsaea acid (I, 15 mg).

***trans*-Cinnamic Acid**——Colorless needles from a mixture of $CHCl_3$ and *n*-hexane, mp 130—132 °C, UV λ_{max}^{MeOH} nm (log ε): 216 (4.13), 221 (4.07), 272 (4.18), IR ν_{max}^{KBr} cm^{-1}: 2900, 1670, 1625, 1600, 1575, 1495, 1450, 975, 765, 705. ^{1}H-NMR (δ, $CDCl_3$): 6.22 (1H, d, J=16 Hz), 7.05—7.45 (5H, m), 7.62 (1H, d, J=16 Hz). This product was identical with an authentic sample on direct comparison (thin-layer chromatography (TLC), infrared (IR) and mixed fusion).

Creticoside B (II)——Colorless needles from a mixture of MeOH and benzene, mp 269—270 °C (lit.,[3] 258—261 °C), $[\alpha]_D^{22}$ −36.1° (c=0.36, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600, 2930, 2860, 1465, 1390, 1370, 1160, 1075, 1025. MS *m/z*: 450 (M^+-H_2O, 2%), 361 (4%), 306 ($M^+-C_6H_{10}O_5$, 1%), 288 (306−H_2O, 6%), 271 (288−OH, 100%), ^{1}H-NMR (δ, Pyr.-d_5): 0.77 (3H, s), 0.85 (3H, s), 0.94 (3H, s), 1.51 (3H, s), 3.84—4.59 (7H, m), 5.05 (1H, d, J=8 Hz). ^{13}C-NMR (δ, Pyr.-d_5): see Table II. This product was identical with an authentic sample on direct comparison (TLC, IR, ^{1}H-NMR and mixed fusion).

16α,17,19-Trihydroxy-*ent*-kaurane 19-*O*-β-D-Glucopyranoside (III)——Colorless needles from a mixture of MeOH and H_2O, mp 219—220 °C, $[\alpha]_D^{23}$ −61.7° (c=1.66, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3330, 2920, 1450, 1370, 1045, 1020. ^{1}H-NMR (δ, Pyr.-d_5): 1.00 (3H, s), 1.18 (3H, s), 3.52 (1H, d, J=11 Hz), 3.88—4.64 (9H, m), 4.81 (1H, d, J=7 Hz). ^{13}C-NMR (δ, Pyr.-d_5): see Table II.

Enzymatic Hydrolysis of III——A solution of III (80 mg) in acetate buffer (pH=5, 10 ml) was incubated with β-D-glucosidase (emulsin, 100 mg) at 37 °C for 200 h and the hydrolysate was extracted with ethyl acetate. The organic layer was concentrated *in vacuo*. The residue was chromatographed on silica gel with a mixture of $CHCl_3$ and MeOH (30 : 1) to give IV (53 mg).

Aglycone IV——Colorless needles from a mixture of ethyl acetate and *n*-hexane, mp 224—225 °C, $[\alpha]_D^{24}$ −34.2° (c=0.4, ethanol). IR ν_{max}^{KBr} cm^{-1}: 3380, 2920, 1440, 1385, 1370, 1010. MS *m/z*: 322 (M^+, 1%), 304 (M^+-H_2O, 3%), 291 (M^+-CH_2OH, 100%), 273 (291−H_2O, 29%), 123 (68%). Calcd for $C_{20}H_{34}O_3$: 322.2506 (M), Found: 322.2505 (M^+). ^{1}H-NMR (δ, Pyr.-d_5): see Table I. ^{13}C-NMR (δ, Pyr.-d_5): see Table II. This product was assigned as 16α,17,19-trihydroxy-*ent*-kaurane by direct comparison with an authentic sample (TLC, IR, ^{1}H-NMR, ^{13}C-NMR and mixed fusion).

Glycoside A (V)——Colorless needles from a mixture of acetone and *n*-hexane, mp 237—238 °C, $[\alpha]_D^{22}$ −64.0° (c=0.35, pyridine). *Anal.* Calcd for $C_{26}H_{44}O_7$: C, 66.64; H, 9.46. Found: C, 66.48; H, 9.50. IR ν_{max}^{KBr} cm^{-1}: 3600, 2920, 2840, 1440, 1370, 1080, 1030, 870. ^{1}H-NMR (δ, Pyr.-d_5): 1.01 (3H, s), 1.18 (3H, s), 1.53 (3H, s), 3.53 (1H, d, J=10 Hz), 4.22 (d, J=10 Hz), 3.80—4.66 (7H, overlapping with d at δ 4.22), 4.82 (1H, d, J=7 Hz). ^{13}C-NMR (δ, Pyr.-d_5): see Table II.

Acidic Hydrolysis of V——A mixture of V (15 mg) in 50% MeOH (7 ml) containing 5% HCl was heated in a boiling water-bath for 5 h. After cooling, the reaction mixture was poured into water. The whole mixture was extracted with ethyl acetate. The organic phase was washed with water, dried over anhydrous Na_2SO_4 and concentrated to yield VI (8 mg), which was recrystallized from a mixture of $CHCl_3$ and *n*-hexane. The water phase was neutralized with Ag_2CO_3 and filtered. The filtrate was concentrated. The residue was subjected to column chromatography on silica gel with 30% MeOH in $CHCl_3$ to yield D-glucose (3.2 mg), $[\alpha]_D^{23}$ +43.8° (c=0.16, H_2O). Its trimethylsilyl ether gave t_R values of 9′30″ and 14′12″ in GLC (column temp. 180 °C, t_R of D-glucose: 9′30″ and 14′12″).

Aglycone VI——Colorless needles, mp 200—201 °C, $[\alpha]_D^{22}$ −40.5° (c=0.4, ethanol), MS *m/z*: 306 (M^+, 3%), 288 (M^+-H_2O, 30%), 275 (M^+-CH_2OH, 36%), 257 (275−H_2O, 100%). Calcd for $C_{20}H_{34}O_2$: 306.2559 (M), Found: 306.2577 (M^+). IR ν_{max}^{KBr} cm^{-1}: 3600, 2930, 2850, 1485, 1450, 1370, 1125, 1035, 1015, 935. ^{1}H-NMR (δ, Pyr-d_5): see Table I. ^{13}C-NMR (δ, Pyr.-d_5): see Table II. This product was assigned as 16α,19-dihydroxy-*ent*-kaurane by direct comparison with an authentic sample (TLC, IR, ^{1}H-NMR, ^{13}C-NMR and mixed fusion).

Glycoside B (VII)——Colorless needles from MeOH, mp 257—258 °C, $[\alpha]_D^{26}$ −46.4° (c=0.3, pyridine). *Anal.* Calcd for $C_{26}H_{44}O_9$: C, 62.38; H, 8.86. Found: C, 62.24; H, 8.81. IR ν_{max}^{KBr} cm^{-1}: 3330, 2930, 2870, 1450, 1380, 1350, 1170, 1070, 870. ^{1}H-NMR (δ, Pyr.-d_5): 0.96 (3H, s), 1.20 (3H, s), 2.74 (1H, m), 3.54 (1H, d, J=11 Hz), 3.84—4.60 (10H, m), 4.86 (1H, d, J=7 Hz). ^{13}C-NMR (δ, Pyr.-d_5): see Table II.

Enzymatic Hydrolysis of VII——VII (12 mg) was hydrolyzed as described for III to yield VIII (7 mg) from the organic layer. The water layer was concentrated and subjected to PLC (solvent system, $CHCl_3$–MeOH 5 : 2) to give D-glucose.

Aglycone VIII——Colorless needles from a mixture of acetone and *n*-hexane, mp 252—253 °C, $[\alpha]_D^{17}$ −51.4° (c = 0.28, MeOH). *Anal.* Calcd for $C_{20}H_{34}O_4$: C, 70.97; H, 10.12. Found: C, 71.09; H, 10.14. MS *m/z*: 307 ($M^+ - CH_2OH$, 11%), 289 ($M^+ - CH_2OH - H_2O$, 100%), 271 (289 − H_2O, 40%). IR ν_{max}^{KBr} cm^{-1}: 3600, 2940, 1445, 1370, 1350, 1030, 870. ^{1}H-NMR (δ, Pyr.-d_5): see Table I. ^{13}C-NMR (δ, Pyr-d_5): see Table II.

Glycoside C (IX)——Colorless needles contaminated with VII from a mixture of MeOH and benzene, which gave a single spot on TLC (silica gel, $CHCl_3$–MeOH (5 : 2) and *n*-BuOH–sat. H_2O). ^{1}H-NMR (δ, Pyr.-d_5): 0.98 (3H, s), 1.18 (3H, s), 2.02 (3H, s), 3.50 (1H, d, J = 11 Hz), 3.80—4.52 (8H, m), 4.81 (1H, d, J = 7 Hz). ^{13}C-NMR (δ, Pyr.-d_5): see Table II.

Acidic Hydrolysis of IX——IX (3 mg) was hydrolyzed as described for V. D-Glucose was identified by gas-liquid chromatography (GLC).

Enzymatic Hydrolysis of IX——IX (15 mg) was hydrolyzed as described for III and the product (10 mg) was chromatographed on alumina with 3% MeOH in $CHCl_3$ to give X (7 mg) and VIII (2 mg).

Aglycone X——Colorless needles from acetone, mp 211—212 °C, $[\alpha]_D^{24}$ −9.5° (c = 0.20, MeOH). *Anal.* Calcd for $C_{20}H_{34}O_3$: C, 74.49; H, 10.63. Found: C, 74.68; H, 10.60. MS *m/z*: 304 ($M^+ - H_2O$, 15%), 273 (304 − CH_2OH, 38%), 255 (273 − H_2O, 24%), 246 (273 − C_2H_3, 100%). IR ν_{max}^{KBr} cm^{-1}: 3360, 2940, 1480, 1380, 1130, 1060, 930. ^{1}H-NMR (δ, CD_3OD): 0.94 (3H, s), 0.97 (3H, s), 1.46 (3H, s), 3.32 (d, J = 11 Hz, overlapping with C$\underline{H}_3$OH), 3.71 (1H, d, J = 11 Hz), 4.00 (m, overlapping with $CH_3O\underline{H}$). ^{1}H-NMR (δ, Pyr.-d_5): see Table I. ^{13}C-NMR (δ, Pyr.-d_5): see Table II.

Preparation of 16β,19-Dihydroxy-*ent*-kaurane (XI) from IV——HIO_4 Oxidation of IV: A mixture of IV (50 mg), MeOH (7 ml) and HIO_4 (30 mg) was stirred for 1 h at room temperature. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was concentrated to give a residue, which was chromatographed on silica gel with $CHCl_3$–MeOH (70 : 1) to afford 19-hydroxy-*ent*-17-norkauran-16-one (45 mg).[5] Colorless needles from a mixture of benzene and *n*-hexane. mp 155—156 °C. IR ν_{max}^{KBr} cm^{-1}: 3350, 2950, 1740, 1485, 1030, 850. ^{1}H-NMR (δ, Pyr.-d_5): 0.99 (3H, s), 1.20 (3H, s), 3.64 (1H, d, J = 11 Hz), 3.99 (1H, d, J = 11 Hz).

16β,19-Dihydroxy-*ent*-kaurane (XI): 19-Hydroxy-*ent*-17-norkauran-16-one (40 mg), Mg (40 mg) and MeI (0.5 ml) in ether (10 ml) were heated under reflux for 3 h.[15] The reaction mixture was diluted with ethyl acetate, acidified with 25% H_2SO_4 and extracted with ethyl acetate. The organic phase was washed with water, dried over anhydrous Na_2SO_4 and concentrated to give a residue, which was chromatographed on silica gel with $CHCl_3$–MeOH (50 : 1) to afford XI. Colorless needles from MeOH, mp 233—234 °C, $[\alpha]_D^{23}$ −58.4° (c = 0.75, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3300, 2920, 1440, 1370, 1130, 1040. ^{1}H-NMR (δ, Pyr.-d_5): 1.04 (3H, s), 1.21 (3H, s), 1.50 (3H, s), 3.65 (1H, d, J = 11 Hz), 4.03 (1H, d, J = 11 Hz). ^{13}C-NMR (δ, Pyr.-d_5): see Table II.

Di-*O*-methyl Lindsaea Acid Methyl Ester——Colorless needles from ethanol, mp 130—131 °C. MS *m/z*: 318 (M^+), 245, 244, 215. ^{1}H-NMR (δ, $CDCl_3$): 1.70—2.07 (2H, m), 2.46 (1H, d, J = 16 Hz), 2.78 (1H, d, J = 16 Hz), 2.95—3.17 (2H, m), 3.67, 3.82, 3.88 (each 3H, s), 6.24 (1H, s), 6.36 (2H, s). This product was identical with an authentic sample on direct comparison (MS, IR, ^{1}H-NMR and GLC (t_R: 6′25″. column temp., 230 °C, WCOT, SE-30)).

***o*-Coumaric Acid (XII)**——Colorless needles from a mixture MeOH and $CHCl_3$, mp 210—212 °C. UV λ_{max}^{MeOH} nm (log ε): 215 (5.22), 273.5 (5.22), 323.5 (4.94). IR ν_{max}^{KBr} cm^{-1}: 3400, 1660, 1620, 1600, 1460, 1430, 1090, 990, 750. ^{1}H-NMR (δ, DMSO-d_6): 6.50 (1H, d, J = 16 Hz), 6.76 (1H, dd, J = 8, 2 Hz), 6.85 (1H, td, J = 8, 8, 2 Hz), 7.20 (1H, td, J = 8, 8, 2 Hz), 7.54 (1H, dd, J = 8, 2 Hz), 7.81 (1H, d, J = 16 Hz). ^{13}C-NMR (δ, DMSO-d_6): 168.0 (C-1), 118.1 (C-2), 139.5 (C-3), 120.8 (C-4), 156.5 (C-5), 116.0 (C-6), 131.3 (C-7), 119.3 (C-8), 128.5 (C-9). MS *m/z*: 164, 146, 118, 91, 90. Calcd for $C_9H_8O_3$: 164.0472 (M), Found: 164.0471 (M^+). This product was identical with an authentic sample on direct comparison (TLC, IR, ^{1}H-NMR and mixed fusion).

2,6-Dimethoxybenzoquinone (XIII)——Yellow needles from MeOH, mp 221—222 °C. UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 289 (4.20), 379 (2.67). IR ν_{max}^{KBr} cm^{-1}: 3060, 1690, 1640, 1620, 1590, 1320, 1105, 1005, 875. MS *m/z*: 168, 140, 138, 125, 112, 97, 80, 69 (base peak), Calcd for $C_8H_8O_4$: 168.0423 (M), Found: 168.0427 (M^+). ^{1}H-NMR (60 MHz, δ, $CDCl_3$): 3.75 (6H, s), 5.75 (2H, s). ^{13}C-NMR (δ, $CDCl_3$): 186.6, 157.3, 107.4, 56.4. This product was identical with a synthetic sample prepared by warming pyrogallol trimethyl ether with nitric acid (TLC, IR, UV, MS and mixed fusion).[16]

References and Notes

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