Pergamon

PII: S0031-9422(96)00395-0

Phytochemistry, Vol. 43, No. 6, pp. 1319–1323, 1996

0031-9422/96 $15.00 + 0.00

STEROIDAL GLYCOSIDES, INDIOSIDES A–E, FROM *SOLANUM INDICUM**

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((*Received in revised form* 8 *May* 1996))

Key Word Index—*Solanum indicum*; Solanaceae; indiosides; spirostanols; furostanols; (23*S*,25*R*,26*R*)-spirost-5-en-3β,23,26-triol; steroidal saponins; isoanguivine.

Abstract—Together with isoanguivine, protodioscin, solasonine and solamargine, five new steroidal glycosides, named indiosides A–E were obtained from *Solanum indicum* (indiosides A and B from the fruits, indiosides C–E from the roots). Their structures were characterized as: (23*S*,25*R*,26*R*)-spirost-5-en-3β,23,26-triol 3-*O*-{α-L-rhamnopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranoside} (indioside A); (25*R*)-26-*O*-β-D-glucopyranosyl-22α-methoxyfurost-5-en-3β,26-diol 3-*O*-{α-L-rhamnopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranoside} (indioside B); (25*R*)-26-*O*-β-D-glucopyranosyl-furost-5-en-3β,22ξ,26-triol 3-*O*-{α-L-rhamnopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3]-β-D-galactopyranoside} (indioside C); (25*R*)-26-*O*-(β-D-glucopyranosyl)-furost-5-en-3β,22ξ,26-triol 3-*O*-{α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)]-β-D-galactopyranoside} (β-solatrioside) (indioside D); diosgenin 3-*O*-{α-L-rhamnopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-galactopyranoside} (indioside E). Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Plants of the genus *Solanum*, with more than 1700 species, are widespread in the tropical and temperate zones. Some of these plants have long been used as a vegetable and as a medicinal agent. Our systematic studies on the chemical constituents of *Solanum* species from Japan, China, Bangladesh and Brazil have revealed that they contain abundant steroidal saponins. As a part of our programme we have isolated five new steroidal saponins, named indiosides A–E (**1–5**), and four known steroidal glycosides from *Solanum indicum* L. This paper deals with the structure elucidation of the new compounds.

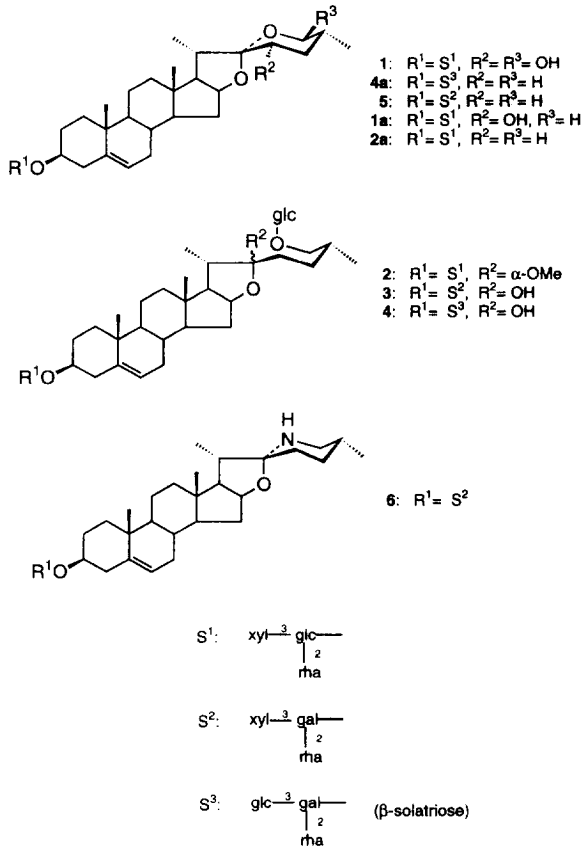
RESULTS AND DISCUSSION

The water soluble parts of the methanolic extract of the fruits and the roots were separated by MCI gel CHP-20P, silica gel, and Chromatorex ODS chromatography to yield **1**, **2** and **7** from fruits and **3–9** from roots.

Compounds **6–9** were known steroidal glycosides, and identified as isoanguivine [1], protodioscin [2, 3], solasonine [4] and solamargine [4], respectively, by spectroscopic data and/or direct TLC comparison with authentic samples.

Indioside A (**1**) showed a pale yellow coloration with Ehrlich reagent and a negative test with the Dragendorff reagent. Its molecular formula was determined as $C_{44}H_{70}O_{18}$ from the FAB-mass spectrum and ^{13}C NMR spectrum. The negative FAB-mass spectrum showed a quasi-molecular ion peak $[M-H]^-$ at *m/z* 885 and a fragment peak [*m/z* M − H − pen]$^-$ at *m/z* 753, and the ^{13}C NMR spectrum displayed 44 carbon signals including those of three anomeric carbons (δ 99.8, 102.3, 105.3), one hemiacetal carbon (δ 96.0, *d*) and one spiroketal carbon (δ 113.4, *s*). The ^1H and ^1H–^1H COSY NMR spectra of **1** exhibited signals due to two tertiary methyl groups (δ 0.98, 6H, *s*), three secondary methyl groups (δ 1.13, 3H, *d*, $J = 5.9$ Hz; 1.24, 3H *d*, $J = 6.6$ Hz and 1.71, 3H, *d*, $J = 5.9$ Hz), three anomeric protons (δ 4.94, 2H, *d*, $J = 7.7$ Hz; 6.27, 1H, *s*), one olefinic proton (δ 5.26, 1H, *brs*) and a hemiacetal methine proton (δ 5.17, 1H, *d*, $J = 7.7$ Hz), indicating **1** to be a steroidal glycoside having three monosaccharides. Acid hydrolysis of **1** afforded D-glucose, L-rhamnose and D-xylose as the sugar components, and inseparable decomposition products arising from the aglycone part. The ^{13}C NMR spectral data of **1** are listed in Table 1. The 27 carbon signals originating from the aglycone part were almost coincident with those of dioscin [2] except for the C-22 to C-26 carbons. The 17 signals due to the sugar residue carbon signals were superimposable on those of the sugar part of anguivine [1]. All signals attributable to C-22 (δ 113.4, *s*), C-23 (δ 67.2, *d*), C-24 (δ 37.4, *t*), C-25 (δ 38.3, *d*) and C-26 (δ 96.0, *d*) were shifted

*Part XXXVI in the series 'Studies on the Solanaceous Plants'. For Part XXXV, see *Phytochemistry* (1996) **43**, 1069.



downfield in comparison with those of dioscin [2]. Since the resonances of C-23 methylene and C-26 oxymethylene carbons were observed at δ 31.8 and 66.8, respectively, in dioscin, the above data indicated that hydroxy groups attached to C-23 and C-26 to be a hemiacetal structure at C-26. $NaBH_4$ reduction of **1** afforded a 23-hydroxydiosgenin glycosine (**1a**), whose structure was determined from its ^1H NMR, ^{13}C NMR and mass spectra. The orientations of the hydroxyls at C-23 and C-26, and the methyl group at C-25 in **1**, were determined to be all equatorial (23*S*,25*R*,26*R*) by the *J* value between the H-26 methine proton (*d*, $J = 9.2$ Hz at δ 5.68) and the H-25 axial proton, and NOE effects between H-25 (δ 1.86) and H-23 (δ 4.87), H-26 (δ 5.68) and H-24 (δ 2.28) in the differential NOE spectra for the peracetate (**1b**) of **1**. Thus, the structure of **1** was formulated as (23*S*,25*R*26*R*)-spirost-5-en-3β,23,26-triol 3-*O*-{α-L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[β-D-xylopyranosyl-(1 $\rightarrow$ 3)]-β-D-glucopyranoside}.

Indioside B (**2**) showed a positive coloration with Ehrlich reagent. The ^{13}C NMR spectrum of **2** showed 51 carbon signals including one methoxy signal and four anomeric carbon signals (δ 99.7, 102.2, 104.7, 105.2), suggesting **2** to be a furostanol tetraglycoside. On enzymatic hydrolysis with β-glucosidase, compound **2** liberated the spirostanol glycoside **2a** and D-glucose. The positive FAB-mass spectrum of **2a** showed a quasi-molecular peak due to $[M + H]^+$ at *m/z* 855. Acid hydrolysis of **2a** afforded diosgenin, D-glucose, L-rhamnose and D-xylose. A comparative study of the ^{13}C NMR data for **2a** with those of dioscin [2] and anguivine [1], showed that the sugar part displayed the same chemical shifts as that of anguivine, and the aglycone part was identical with that of dioscin. Therefore, the structures of **2a** and **2** were determined to be diosgenin 3-*O*-{α-L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[β-D-xylopyranosyl-(1 $\rightarrow$ 3)]-β-D-glucopyranoside} and its 26-*O*-(β-D-glucopyranosyl)-22α-methoxyfurostanol glycoside, respectively.

Indioside C (**3**) showed a positive coloration with Ehrlich reagent, suggesting **3** was also a furostanol glycoside. The negative FAB-mass spectrum of **3** showed a peak due to $[M - H]^-$ at *m/z* 1033, its quasi-molecular ion, and its ^{13}C NMR spectrum showed 50 carbon signals comprising four anomeric carbons (δ 100.4, 102.2, 104.9, 106.8) and a hemiacetal carbon signal (δ 110.6, *d*), indicating **3** to be related to **2**. Acid hydrolysis of **3** gave diosgenin as the aglycone and D-glucose, D-galactose, L-rhamnose and D-xylose as sugar components. A comparative study of the ^{13}C NMR data of **3** with those of protodioscin [2] and isoanguivine [1], revealed that the chemical shifts of the sugar parts were superimposable on those of isoanguivine and the 26-*O*-β-glucopyranosyl moiety of protodioscin, and those due to the aglycone moiety

Table 1. ^{13}C-NMR Data for compounds **1**, **1a**, **2**, **2a**, **3**, **4**, **4a** and **5** in pyridine-d_5

	1	**1a**	**2**	**2a**	**3**	**4**	**4a**	**5**
C-1	37.3	37.4	37.3	37.4	37.5	37.3	37.4	37.4
2	29.9	30.0	29.9	30.0	29.9	29.9	29.9	30.1
3	77.3[a]	77.4[a]	78.3	78.3	78.3[a]	78.4[a]	78.3	78.2
4	40.1	39.8	40.0	39.8	38.7	38.9	38.7	38.7
5	140.6	140.7	140.6	140.7	140.8	140.8	140.8	140.8
6	121.7	121.8	121.7	121.8	121.7	121.7	121.7	121.7
7	32.2	32.3	32.1	32.3	32.3	32.3	32.2	32.1
8	31.4	31.7	31.5	31.7	31.6	31.6	31.6	31.6
9	50.1	50.3	50.1	50.2	50.2	50.9	50.2	50.2
10	37.0	37.2	37.0	37.1	37.1	37.0	37.1	37.1
11	21.0	21.0	20.9	21.1	20.9	21.0	21.0	21.0
12	38.5	38.6	38.5	38.6	40.2	39.8	39.8	39.8
13	40.9	41.1	40.6	40.4	40.9	40.7	40.8	40.4
14	56.6	56.6	56.4	56.6	56.3	56.5	56.6	56.6
15	32.0	32.5	32.1	32.2	32.4	32.4	32.3	32.2
16	81.6	81.3	81.2	81.1	82.2	81.0	81.1	81.6
17	62.5	64.2	64.0	62.9	64.1	63.8	62.9	62.8
18	16.5	16.3	16.1	16.3	16.4	16.4	16.3	16.2
19	19.2	19.3	19.3	19.4	19.3	19.3	19.4	19.3
20	36.1	37.1	40.3	41.9	40.6	40.9	41.9	41.9
21	14.7	16.9	16.2	15.0	16.1	16.4	15.0	15.0
22	113.4	111.8	112.5	109.2	110.6	110.6	109.2	109.2
23	67.3	73.5	30.6	31.8	37.1	37.0	30.1	31.1
24	37.4	35.9	28.0	29.2	28.3	28.3	29.9	29.2
25	38.3	33.6	34.0	30.6	34.3	34.2	30.6	30.5
26	96.0	66.8	75.0	66.8	75.0	74.1	66.8	66.9
27	17.3	19.0	17.2	17.3	17.2	17.4	17.3	17.2
OMe			47.1					
gal C-1					100.4	100.6	100.4	100.4
(or glc C-1)	99.8	99.8	99.7	99.1				
2	77.8[a]	77.9[a]	77.5[a]	77.7[a]	76.4	76.6	76.5	76.4
3	88.6	88.2	88.0	88.1	85.0	84.8	84.8	84.9
4	69.4	69.4	69.3	69.4	70.3	70.8	70.8	70.2
5	78.2	78.4	77.7	77.9	75.2	75.1	74.9	75.0
6	62.2	62.2	62.2	62.3	62.3	62.8	62.5	62.1
rha C-1	102.3	102.4	102.2	102.3	102.2	101.9	102.2	102.2
2	72.3	72.4	72.3	72.4	72.5	72.5	72.5	72.5
3	72.7	72.8	72.6	72.8	72.8	72.8	72.8	72.8
4	73.9	74.1	73.8	74.6	74.1	74.1	74.1	74.6
5	69.5	69.6	69.4	69.6	69.4	69.4	69.4	69.4
6	18.6	18.6	18.6	18.7	18.6	18.5	18.6	18.5
glc C-1						105.7	105.9	
(or xyl C-1)	105.3	105.4	105.2	105.4	106.8			106.7
2	74.5	74.6	74.5	74.0	74.7	74.8	75.0	74.1
3	77.6[a]	77.6[a]	77.2[a]	77.3[a]	77.4	78.2[a]	78.3	77.4
4	70.5	70.6	70.4	70.6	71.0	71.5	71.6	70.9
5	67.1	67.2	67.0	67.2	67.1	78.3[a]	78.5	67.0
6						62.4	62.6	
26-*O*-glc C-1			104.7		104.9	104.8		
2			75.0		75.2	76.6		
3			78.3		78.5[a]	78.5[a]		
4			71.5		71.6	71.6		
5			78.3		78.5[a]	78.7[a]		
6			62.7		62.8	62.2		

[a]Signals may be interchangeable within each column.

were coincident with those of protodioscin. Therefore, the structure of **3** was determined as (25*R*)-26-*O*-(β-D-glucopyranosyl)-furost-5-en-3β,22ξ,26-triol 3-*O*-{α-L-rhamnopyranosyl-(1$\rightarrow$2)-[β-D-xylopyranosyl-(1$\rightarrow$3)]-β-D-galactopyranoside}.

Indioside D (**4**) showed a positive coloration with Ehrlich reagent, suggesting that **4** was a furostanol glycoside. On enzymatic hydrolysis with β-glucosid-

ase, compound **4** yielded the spirostenol glycoside **4a** and D-glucose. The FAB-mass specturm of **4a** showed a peak due to $[M-H]^-$ at *m/z* 885, and its ^{13}C NMR spectrum exhibited 45 carbon signals as listed in Table 1. Its 27 carbon signals due to the aglycone part were the same as those of **2a**, and the 18 carbon signals due to the sugar part showed a good coincidence with those of solasonine (**8**) [4], by comparing the ^{13}C NMR data of **4a** with those of **2a** and **8**. Thus, the structures of **4a** and **4** were determined as diosgenin 3-*O*-β-solatrioside and the corresponding 26-*O*-(β-D-glucopyranosyl)-furost-5-en-22ξ,26-diol glycoside, respectively.

Indioside E (**5**) showed negative coloration with Ehrlich and Dragendorff reagents. The FAB-mass spectrum of **5** showed a peak due to $[M-H]^-$ at *m/z* 853, and the ^{13}C NMR spectrum of **5** showed 44 carbon signals, comprising one spiroketal carbon (δ 109.2, *s*) and three anomeric carbon signals (δ 100.4, 102.2, 106.7), suggesting **5** was a spirostenol trioside. Acid hydrolysis of **5** afforded diosgenin as the aglycone and D-galactose, L-rhamnose and D-xylose as the sugar components. A comparative study of the ^{13}C NMR data of **5** with those of **3** suggested that the 3-*O*-glycosyl sugar part of **5** was identical with that of **3**. Therefore, the structure of **5** was determined as diosgenin 3-*O*-{αL-rhamnopyranosyl-(1$\rightarrow$2) [β-D-xylopranosyl-(1$\rightarrow$3)]-β-D-galactopyranoside}.

EXPERIMENTAL

^1H (400 MHz) and ^{13}C NMR (100 MHz): JEOL GX-400 with TMS as an int. standard; EI- and FAB-MS: JEOL JMS DX-303HF mass spectrometer; $[\alpha]_D$: *l* = 0.5; TLC: precoated silica gel 60 F_{245} (Merck); CC: silica gel (270–400 mesh, Merck) Chromatorex ODS (Fuji Silisia Chemical Ltd) and MCI gel CHP-20P (Mitsubishi Kasei Co.).

Extraction and separation. Fresh fruits of *S. indicum* (3.9 kg) were extracted with MeOH, and the extract (212 g) was shaken with benzene and H_2O. Removal of the solvent from the aqueous phase gave 210 g of a residue, which was subjected to CC on MCI gel CHP-20P eluting sequentially with H_2O, H_2O–MeOH (60:40), H_2O–MeOH (40:60), H_2O–MeOH (20:80), MeOH, NH_4OH–MeOH (2:98) to provide 10 frs (Fr. 1–10). Fr. 5 [10.1 g, H_2O–MeOH (40:60) eluate] was subjected to a combination of various CC on Chromatorex ODS (50–70% aq. MeOH), silica gel ($CHCl_3$–MeOH–H_2O, 9:2:0.1) to give **1** (195 mg), **2** (53 mg) and **7** (185 mg). Dried roots of *S. indicum* (450 g) were extracted with MeOH, and the extract (29 g) was shaken with benzene and H_2O. After removal of the solvent of the aqueous phase, 23 g of residue was obtained. This was subjected to CC on MCI gel CHP-20P eluting with H_2O, H_2O–MeOH (60:40), H_2O–MeOH (40:60), H_2O–MeOH (20:80), MeOH, NH_4OH–MeOH (2.5:97.5) providing 6 frs (Fr. 1–6). Fr. 3 [4.3 g, H_2O–MeOH (40:60) eluate] was subjected to a combination of silica gel ($CHCl_3$–MeOH–H_2O, 8:2.2:0.2–7:3:0.5) and Chromatorex ODS (40–50% aq. MeOH) CC to give **3** (110 mg), **4** (16 mg), **5** (21 mg), **6** (4 mg) and **7** (72 mg). Fr. 5 (537 mg, MeOH eluate) was subjected to silica gel ($CHCl_3$–MeOH–H_2O, 8:2:0.2–7:3:0.5) CC to give **8** (10 mg) and **9** (50 mg).

Indioside A (**1**). Amorphous powder, Ehrlich reagent: pale yellow. $[\alpha]_D^{24}$ −78.9° (*c* = 0.50, pyridine). Negative FAB-MS (*m/z*): 885 $[M-H]^-$. ^1H MNMR (pyridine-d_5) δ: 0.98 (6H, *s*, Me-18, 19), 1.13 (3H, *d*, *J* = 5.9 Hz, Me-27), 1.24 (3H, *d*, *J* = 6.6 Hz, Me-21), 1.71 (3H, *d*, *J* = 5.9 Hz, rha H_3-6), 1.96 (1H, *m*, H-17) 2.00 (1H, *m*, H-25), 2.05 2.13 (each 1H, *m*, H_2-24), 2.70 (2H, *m*, H_2-4), 2.99 (1H, *br t*, *J* = 6.6 Hz, H-20), 3.65 (1H, *br t*, *J* = 10.2 Hz, xyl H-5*a*), 3.81 (1H, *m*, glc H-5), 3.93 (1H, *m*, xyl H-2), 3.96 (1H, *m*, H-23), 3.97 (1H, *m*, H-3), 4.06 (1H, *m*, xyl H-4), 4.12 (1H, *m*, xyl H-3), 4.23 (2H, *m*, xyl H-5*b*, glc H-6*a*), 4.29 (1H, *t*, *J* = 9.3 Hz, rha H-4), 4.42 (1H, *br d*, *J* = 11.0 Hz, glc H-6*b*), 4.52 (1H, *dd*, *J* = 3.0, 9.1 Hz, rha H-3), 4.66 (1H, *br dd*, *J* = 6.9, 15.4 Hz, H-16), 4.85 (1H, *br s*, rha H-2), 4.89 (1H, *m*, rha H-5), 4.94 (2H, *br d*, *J* = 7.7 Hz, glc H-1, xyl H-1), 5.17 (1H, *d*, *J* = 7.7 Hz, H-26), 5.26 (1H, *br s*, H-6), 6.27 (1H, *s*, rha H-1). ^{13}C NMR see Table 1.

$NABH_4$ *reduction of* **1**. $NaBH_4$ (25 mg) was added to a solution of **1** (11 mg) in MeOH (5 ml) at room temp. This mixt. was stirred overnight, then H_2O (5 ml) was added. The mixt. was passed through MCI gel CHP 20P, washed with H_2O and then eluted with MeOH. The MeOH eluate was evapd under red. pres. to give a residue, which was subjected to CC over silica gel ($CHCl_3$–MeOH–H_2O, 8:2:0.2) to afford **1a** (9 mg). Amorphous powder, Ehrlich reagent: pale yellow. $[\alpha]_D^{25}$ −81.9° (*c* = 0.51, pyridine). Negative FAB-MS (*m/z*): 869 $[M-H]^-$. ^1H NMR (pyridine-d_5) δ: 0.94 (3H, *s*, Me-18), 1.05 (3H, *s*, Me-19), 1.29 (3H, *d*, *J* = 7.0 Hz, Me-27), 1.43 (3H, *d*, *J* = 6.6 Hz, Me-21), 1.77 (3H, *d*, *J* = 6.2 Hz, rha H_3-6), 3.69 (1H, *t*, *J* = 10.0 Hz, xyl H-5), 3.85-4.37 (*m*), 4.47 (1H, *br d*, *J* = 11.4, glc H-6), 4.59 (1H, *br d*, *J* = 9.1 Hz, rha H-3), 4.89 (1H, *br s*, rha H-2), 4.96 (1H, *m*, rha H-5), 5.00 (2H, *br d*, *J* = 7.3 Hz, glc H-1 and xyl H-1), 5.31 (1H, *br s*, H-6), 6.34 (1H, *s*, rha H-1). ^{13}C NMR see Table 1.

Acetylation of **1**. A soln of **1** (10 mg) in Ac_2O (1 ml) and pyridine (2 ml) was allowed to stand at room temp. for 15 hr. The reaction mixt. was evapd *in vacuo* and the residue purified by silica gel CC (hexane–Me_2CO, 2:1) to give the decaacetate **1b** (11 mg). ^1H NMR ($CDCl_3$) δ: 0.80 (3H, *s*, Me-18), 0.90 (3H, *d*, *J* = 6.6 Hz, Me-27), 0.96 (3H, *d*, *J* = 7.0 Hz, Me-21), 1.02 (3H, *s*, Me-19), 1.17 (3H, *d*, *J* = 6.2 Hz, rha H_3-6), 1.86 (1H, *m*, H-25), 1.99, 2.01, 2.02, 2.03×2, 2.04, 2.05, 2.06, 2.11, 2.21 (*s*, total 10×Ac), 2.09 (*m*, H-20), 2.28 2.44 (each 1H, *m*, H_2-24), 3.36 (1H, *dd*, *J* = 9, 6, 11.4 Hz, xyl H-5a), 3.59 (2H, *m*, glc H-5, H-3), 3.70 (1H, *br t*, *J* = 8.4 Hz, glc H-2), 3.95 (1H, *t*, *J* = 9.0 Hz, glc H-3), 4.05 (1H, *br d*, *J* = 12.1 Hz, glc H-6a), 4.06 (1H, *dd*, *J* = 5.1, 11.4 Hz, xyl H-5b), 4.20 (1H, *dd*, *J* = 5.1, 12.1 Hz, glc H-6*b*), 4.43 (1H, *m*, rha H-5), 4.46 (1H, *d*, *J* = 7.7 Hz, glc H-1), 4.63 (1H, *d*, *J* = 7.0 Hz,

xyl H-1), 4.76 (2H, *m*, xyl H-2, H-16), 4.87 (3H, *m*, glc H-4, xyl H-4, H-23), 5.08 (1H, *t*, J=10.1 Hz, rha H-4), 5.22 (1H, *t*, J=8.4 Hz, xyl H-3), 5.24 (1H, *dd*, J=3.5, 10.1 Hz, rha H-3), 5.25 (1H, *br s*, rha H-1), 5.34 (1H, *d*, J=3.5 Hz, rha H-2), 5.39 (1H, *br s*, H-6), 5.68 (1H, *d*, J=9.2 Hz, H-26)

Acid hydrolysis of **1**. A soln of **1** (3 mg) in 1N HCl/H_2O–dioxane (0.5 ml) was heated at 80° for 2 hr, then the solvent was removed under N_2. The sugar components were identified by TLC ($CHCl_3$–MeOH–Me_2CO–H_2O, 3:3:3:1); R_f 0.67 (rhamnose), 0.63 (xylose), 0.50 (glucose).

Indioside B (**2**). Amorphous powder, Ehrlich reagent: positive. $[\alpha]_D^{29}$ −55.2° (c=0.50, pyridine). Positive FAB-MS (m/z): 1049 $[M+H]^+$. ^1H NMR (pyridine-d_5) δ: 0.78 (3H, *s*, Me-18), 0.95 (3H, *d*, J=6.6 Hz, Me-27), 1.01 (3H, *s*, Me-19), 1.15 (3H, *d*, J=7.0 Hz, Me-21), 1.70 (3H, *d*, J=5.5 Hz, rha H_3-6), 3.22 (3H, *s*, OMe), 4.79 (1H, *d*, J=7.7 Hz, 26-*O*-glc H-1), 4.91 (2H, *d*, J=8.0 Hz, glc H-1 and xly H-1), 5.29 (1H, *br s*, H-6), 6.24 (1H, *s*, rha H-1). ^{13}C NMR see Table 1.

Enzymatic hydrolysis of **2**. Compound **2** (24 mg) was taken up in H_2O (20 ml), β-glucosidase (20 mg) was added, and then the mixt was incubated at 37°. After 15 hr, the reaction mixt was evapd. The residue was subjected to CC on silica gel to give **2a** (6 mg) and D-glucose. **2a**: amorphous powder. $[\alpha]_D^{28}$ −79.0° (c=0.10, pyridine). Positive FAB-MS m/z 855 $[M+H]^+$. ^1H NMR (pyridine-d_5) δ: 0.71 (3H, *d*, J=5.5 Hz, Me-27), 0.84 (3H, *s*, Me-18), 1.07 (3H, *s*, Me-19), 1.15 (3H, *d*, J=7.0 Hz, Me-21), 1.77 (3H, *d*, J=6.2 Hz, rha H_3-6), 4.89 (1H, *d*, J=7.0 Hz, glc H-1), 4.98 (1H, *br d*, J=4.4 Hz, xly H-1), 5.34 (1H, *br s*, H-6), 6.33 (1H, *s*, rha H-1). ^{13}C NMR see Table 1.

Acid hydrolysis of **2a**. A soln of **2a** (4 mg) in 1N HCl (1 ml) was heated to 80° for 2 hr to furnish diosgenin (benzene–Me_2CO, 2:1; R_f 0.50), glucose, rhamnose and xylose, detected by TLC.

Indioside C (**3**). Amorphous powder, Ehrlich reagent: positive. $[\alpha]_D^{26}$ −37.0° (c=0.29, pyridine). Negative FAB-MS (m/z): 1033 $[M-H]^-$. ^1H NMR (pyridine-d_5) δ: 0.85 (3H, *s*, Me-18), 1.00 (3H, *d*, J=6.6 Hz, Me-27), 1.06 (3H, *s*, Me-19), 1.30 (3H, *d*, J=7.0 Hz, Me-21), 1.70 (3H, *d*, J=6.3 Hz, rha H_3-6), 4.85 (1H, *d*, J=8.0 Hz, glc H-1), 4.87 (1H, *d*, J=8.1 Hz, gal H-1), 5.06 (1H, *d* J=7.3 Hz, xyl H-1), 5.31 (1H, *br s*, H-6), 6.31 (1H, *s*, rha H-1). ^{13}C NMR see Table 1.

Indioside D (**4**). Amorphous powder, Ehrlich reagent: positive. $[\alpha]_D^{26}$ −75.2° (c=0.57, pyridine). Negative FAB-MS (m/z): 1063 $[M-H]^-$. ^1H NMR (pyridine-d_5) δ: 0.82 (3H, *s*, Me-18), 1.01 (3H, *d*, J=7.0 Hz, Me-27), 1.06 (3H, *s*, Me-19), 1.34 (3H, *d*, J=7.0 Hz, Me-21), 1.69 (3H, *d*, J=6.2 Hz, rha H_3-6), 4.65 (1H, *d*, J=7.7 Hz, glc H-1), 4.85 (1H, *d*, J=8.1 Hz, glc H-1), 4.98 (1H, *d*, J=7.7 Hz, gal H-1), 5.31 (1H, *br s*, H-6), 6.30 (1H, *s*, rha H-1). ^{13}C NMR see Table 1.

Enzymatic hydrolysis of **4**. Compound **4** (10 mg) was taken up in 100 mM acetate buffer (pH 5.0, 5 ml), β-glucosidase (5 mg) was added, and then the reaction mixt. was incubated at 37°. After 10 hr, the reaction mixt. was evapd. The residue was chromatographed on silica gel to give **4a** (2 mg) and D-glucose. **4a**: amorphous powder. Positive FAB-MS m/z 885 $[M+H]^+$. ^1H NMR (pyridine-d_5) δ: 0.69 (3H, *br s*, Me-27), 0.83 (3H, *s*, Me-18), 1.06 (3H, *s*, Me-19), 1.15 (3H, *d*, J=6.9 Hz, Me-21), 1.63 (3H, *d*, J=5.9 Hz, rha H_3-6), 5.21 (1H, *br s*, H-6), 6.31 (1H, *s*, rha H-1), gal H-1 and glc H-1 were overlapped DHO signal. ^{13}C NMR see Table 1.

Indioside E (**5**). Amorphous powder. $[\alpha]_D^{26}$ −78.9° (c=0.50, pyridine). Negative FAB-MS (m/z): 853 $[M-H]^-$. ^1H NMR (pyridine-d_5) δ: 0.70 (3H, *d*, J=5.5 Hz, Me-27), 0.83 (3H, *s*, Me-18), 1.07 (3H, *s*, Me-19), 1.15 (3H, *d*, J=7.0 Hz, Me-27), 1.69 (3H, *d*, J=7.2 Hz, rha H_3-6), 5.00 (1H, *d*, J=7.7 Hz, gal H-1) 5.05 (1H, *d*, J=7.3 Hz, xyl H-1), 5.32 (1H, *br s*, H-6), 6.23 (1H, *s*, rha H-1). ^{13}C NMR see Table 1.

Acid hydrolysis of **5**. A soln of **5** (10 mg) in 1N HCl (1 ml) was heated at 80° for 2 hr on a hot bath to furnish diosgenin, galactose, rhamnose and xylose, detected by TLC.

Acknowledgments—We are grateful to Mr K. Takeda and Mr T. Iriguchi of Kumamoto University for NMR and MS measurements. The authors thank Dr S. Kawanobu and K. Fujieda of the Department of Agriculture, Kyushu University, for supplying the seedling of *S. indium*.

REFERENCES

1. Ripperger, H. and Himmelreich, U. (1994) *Phytochemistry* **37**, 1725.
2. Agrawal, P. K., Jain, D. C., Gupta, R. K. and Thakur R. S. (1985) *Phytochemistry* **24**, 2479.
3. Kawasaki, T., Komori, T., Miyahara, K., Nohara, T., Hosokawa, I. and Mihashi, K. (1972) *Chem. Pharm. Bull.* **22**, 2164.
4. Mahato, S. R., Saha, N. P., Ganguly, A. N., Kasai, R. and Tanaka, O. (1980) *Phytochemistry* **19**, 2017.