Phytochemistry, Vol. 27, No. 12, pp. 3919–3924, 1988.
Printed in Great Britain.

0031–9422/88 $3.00+0.00

FOUR PREGNANE GLYCOSIDES, BOUCEROSIDES AI, AII, BI AND BII, FROM *BOUCEROSIA AUCHERIANA**

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(*Received* 7 *April* 1988)

Key Word Index—*Boucerosia aucheriana*; Asclepiadaceae; pregnane glycoside; boucerosides AI, AII, BI, and BII; 2,6-dideoxy sugar.

Abstract—Four new glycosides were isolated from *Boucerosia aucheriana*. The structures of boucerosides AI, AII, BI, and BII were deduced on the basis of chemical and spectral evidence as boucerogenin I 3-*O*-*β*-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranoside, boucerogenin II 3-*O*-*β*-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranoside, boucerogenin I 3-*O*-*β*-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside, and boucerogenin II 3-*O*-*β*-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside, respectively.

INTRODUCTION

In our continuing studies on the plants of the Asclepiadaceae family, a large number of C/D *cis*-pregnane glycosides with 2,6-dideoxy sugars such as cymarose or oleandrose were isolated [1]. Some of the glycosides showed antitumour activities against Ehrlich carcinoma or melanoma B-16 [2–4]. Mitsuhashi and his co-workers [5] isolated two C/D *cis*-pregnanes, dihydroboucerin (**5**) and boucerin (**6**), as their deacyl type aglycones from *Boucerosia aucheriana* which is a plant indigenous to Pakistan and known to have a very bitter taste. However, the glycosides were not isolated. We describe in this paper the isolation and structure elucidation of four new glycosides from this plant.

RESULTS AND DISCUSSION

A crude glycoside mixture, obtained from the chloroform soluble portion of the defatted methanolic extract from the dried aerial parts of *B. aucheriana*, showed positive Keller–Kiliani and Liebermann–Burchard reactions indicating the presence of a steroidal glycoside with 2,6-dideoxy sugar [6]. First, we tried to separate and determine the aglycone in order to obtain information on the ^{1}H and ^{13}C NMR spectra for the whole structure determination of the glycoside. The crude glycoside was hydrolysed with 0.05 N H_2SO_4 in 75% methanol.

The hydrolysate was extracted with chloroform. The organic layer was chromatographed repeatedly over silica gel to give 12-*O*-benzoyl dihydroboucerin (**7**), 12-*O*-benzoyl boucerin (**8**), 12-*O*-benzoyl-20-*O*-acetyl dihydroboucerin (**9**), and 12-*O*-benzoyl-20-*O*-acetyl boucerin (**10**). These four aglycones have a benzoylester moiety on the basis of this UV absorption maxima at 229 nm, IR absorption (1710, 1600 cm^{-1}), and ^{1}H and ^{13}C NMR spectra (Table 1). In addition **9** and **10** have an acetyl ester moiety as shown by the NMR spectra (^{1}H: δ 1.93, ^{13}C: 170.2 and 21.6; ^{1}H: 1.98, ^{13}C: 170.2 and 21.6, respectively). Judging from the molecular formulae of **7** ($C_{28}H_{40}O_5$) and **8** ($C_{28}H_{38}O_5$), and the NMR spectra, the latter had an olefinic bond. A similar relationship was observed between **9** and **10**. This type of difference had been observed between **5** and **6**, namely, 5α-H or Δ^5. Therefore, the olefinic bonds of **8** and **10** are probably located at the same position. An olefinic proton signal at δ 5.44 and carbon signals at δ 140.7 (5-C) and 121.5 (6-C) of **8** supported this assumption. Furthermore, the 19-methyl signal at δ 1.02 in the spectrum of **8** was shifted to up field at δ 0.83 in the spectrum of **7**. Analogous results were observed in the case of **9** and **10**. As shown in Table 1, these four aglycones had four oxygenated carbons which are assignable to positions C-3, C-12, C-14, and C-20 in a C/D *cis*-pregnane system. The ester linkages in these aglycones are located at C-12 on the basis of the chemical shifts of the double doublet signals (J = 12 and 4 Hz, 12-Hα). The signals assignable to the 20-H proton of **7** and **8** were shifted *ca* 1 ppm downfield compared to those of **9** and **10**. Another secondary hydroxyl group was assigned to be in the 3α-position by the signal pattern of the methine proton. The tertiary hydroxyl group was placed at the 14β-position by biogenetic analogy to other asclepiadaceous aglycones. Since the acetate (**11**) of **7** was identical with that of **9**, compounds **9** and **10** must have a 12-*O*-benzoyl and 20-*O*-acetyl functionalities. The stereochemistry at C-20 was deduced to be *R* by Kimura's results [7]. The above arguments lead to the conclusion that the structures of **7**–**10** are 12-*O*-benzoyl dihydroboucerin, 12-*O*-benzoyl boucerin, 12-*O*-benzoyl-20-*O*-acetyl dihydroboucerin, and 12-*O*-benzoyl-20-*O*-acetyl boucerin, respectively.

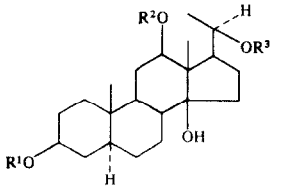
Several chromatographic procedures over silica gel and reversed phase gel gave two fractions. Each fraction

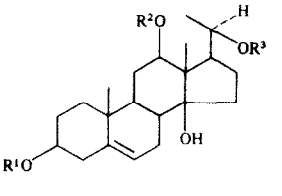
*Part 68 in the series 'Studies on the Constituents of Asclepiadaceae Plants'. For part 67 see Zhang, Z-X., Zhou, J., Hayashi, K. and Kaneko, K. (1988) *Phytochemistry* **27** (in press).

Table 1. ^{13}C NMR chemical shifts of compounds **7**, **8**, **9**, **10**, and **11**

C	7	9	8	10	11
1	37.5	37.4	37.8	37.6	36.8
2	32.2[a]	32.3	32.5[a]	32.4[a]	27.7[a]
3	70.5	70.5	71.2	71.1	73.6
4	39.1	39.1	43.2	43.2	34.2
5	45.1	45.0	140.5	140.7	44.5
6	28.2[b]	28.2[a]	121.8	121.5	28.0
7	29.2[b]	29.2[a]	27.8	27.7	28.9
8	40.4	40.9	36.9	37.6	40.8
9	46.7	46.5	43.9	44.0	46.3
10	36.1	36.1	37.5	37.6	35.9
11	27.0[c]	26.8[b]	26.7[b]	26.7[b]	26.8[a]
12	79.8	79.7	79.3	79.3	79.6
13	53.4	52.5	53.3	52.5	52.5
14	84.9	85.6	85.2	85.3	85.5
15	32.8[a]	32.3	33.4[a]	32.8[a]	32.2
16	26.5[c]	25.9[b]	26.4[b]	25.6[b]	26.0[a]
17	53.1	50.7	53.0	50.6	50.7
18	11.6	10.2	11.5	10.3	10.2
19	12.4	12.2	19.6	19.6	12.0
20	70.8	74.0	70.8	74.0	74.0
21	23.7	19.3	23.7	19.4	19.3
1′	166.7	166.6	166.7	166.7	166.7
2′	131.6	131.7	131.6	131.6	131.8
3′	130.1	130.1	130.0	130.1	130.2
4′	128.9	128.9	128.9	128.9	128.9
5′	133.3	133.4	133.3	133.4	133.5
1″		170.2		170.2	170.3 × 2
2″		21.6		21.6	21.6, 21.3

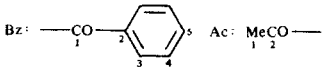
δ values (ppm) from internal TMS in C_5D_5N.
[a–c]Values in each column may be interchangeable.





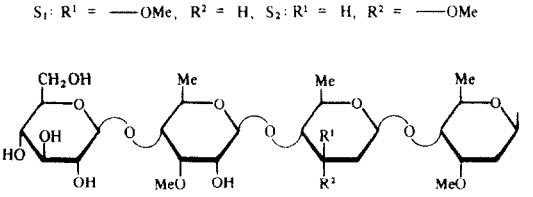
1 R^1 = S_1, R^2 = R^3 = Bz
3 R^1 = S_2, R^2 = R^3 = Bz
5 R^1 = R^2 = R^3 = H
7 R^1 = R^3 = H, R^2 = Bz
9 R^1 = H, R^2 = Bz, R^3 = Ac
11 R^1 = R^3 = Ac, R^2 = Bz
12 R^1 = H, R^2 = R^3 = Bz

3 R^1 = S_1, R^2 = R^3 = Bz
4 R^1 = S_2, R^2 = R^3 = Bz
6 R^1 = R^2 = R^3 = H
8 R^1 = R^3 = H, R^2 = Bz
10 R^1 = H, R^2 = Bz, R^3 = Ac
12 R^1 = R^3 = Ac, R^2 = Bz
13 R^1 = H, R^2 = R^3 = Bz



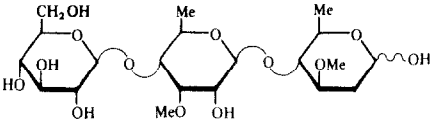
showed one spot on high performance reversed phase TLC (Merck HPTLC RP-18). However, one of the fractions revealed a complex profile on HPLC while the other showed only four peaks which were recovered by preparative HPLC and named boucerosides AI (**1**), AII (**2**), BI (**3**), and BII (**4**) in order of elution.

The abundant glycoside bouceroside AII (**2**) had the molecular formula $C_{62}H_{90}O_{21}$ on the basis of its elemental analysis and FD MS (*m/z*: 1193 $[M+Na]^+$). The peak at *m/z* 1031 [1193 − 162] must arise from the ion of $[M+Na]^+$ through the loss of a terminal hexose (162). The 400 MHz ^1H NMR spectrum of **2** showed methyl peaks of its aglycone moiety at δ 0.78 (H_3-19), 1.14 (H_3-18), and 1.35 (H_3-21) and two esterified methine proton signals at 4.99 (*dd*, *J* = 12, 4 Hz, Hα-12) and 5.25 (*dq*, *J* = 9.2, 6.6 Hz, H-20). The esters were two benzoyls shown the ^1H and ^{13}C NMR spectra and UV absorption maxima (229 nm). The sugar sequence of **1** showed four anomeric proton signals at δ 4.41 (*d*, *J* = 7.3 Hz), 4.46 (*dd*, *J* = 9.6, 2 Hz), 4.78 (*d*, *J* = 8.9 Hz), and 4.85 (*dd*, *J* = 10, 2 Hz) and carbon signals at δ 96.0, 101.8, 101.8, and 106.5. The coupling constants of the anomeric proton signals revealed β-linkage for each sugar unit. From its ^{13}C NMR spectrum, compound **2** contained β-cymaropyranosyl, β-oleandropyanosyl, 6-deoxy-3-*O*-methyl-β-allopyranosyl, and terminal β-glucopyranosyl moieties. Three methoxy and three secondary methyl signals also supported the results mentioned above. Acid hydrolysis of **2** gave an aglycone, boucerogenin II (**13**) and two sugar components which were identified with cymarose (**14**) and neocondurangotriose (**15**). The cymarose from the hydrolysate was determined as the D-series using chiral HPLC column upon comparision with the mobilities of 3,5-dinitrophenyl carbamate of authentic methyl cymarosdides [8]. Since **15** was determined as β-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranose, the sugar sequence of **2** is the same as that of condurangoglycoside. Boucerogenin II (**13**) was determined as 12,20-di-*O*-benzoyl dihydroboucerin from its spectrometric data which were analogous to those of **10** other than for the 20-*O*-acetyl group. The glycosidic linkage was located at the C-3 position from glycosylation shifts at the C-2 (+2.2 ppm), C-3 (−7.2), and C-4 (+4.3) positions. Thus, the structure of **2** was established as boucerogenin II 3-*O*-β-D-glycopyran-



S_1: R^1 = —OMe, R^2 = H, S_2: R^1 = H, R^2 = —OMe

14 cymarose



15 neocondurangotriose

osyl-(1 → 4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranoside.

Bouceroside AI (**1**) had the molecular formula $C_{62}H_{88}O_{21}$ which is two mass units less than that of **2**. From the ^{13}C NMR chemical shifts, the sugar chain of **1** was identified to that of **2**. Acidic hydrolysis of **1** gave an aglycone, boucerogenin I (**12**) which was the Δ^5-derivative of **13**, shown by the presence of an olefinic proton signal at δ5.45 (6H) and carbon signals at 140.8 (5-C) and 121.5 (6-C). The sugar components of the hydrolysate were the same as those obtained from **2**. Therefore, bouceroside AI was deduced to be the Δ^5-derivative of bouceroside AII and is boucerogenin I 3-*O*-β-D-glucopyranosyl -(1→4)-6-deoxy-3-*O*-methyl -β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranoside.

Bouceroside BI (**3**) and bouceroside BII (**4**) had the molecular formulae $C_{62}H_{88}O_{21}$ and $C_{62}H_{90}O_{21}$, respectively. Both the glycosides consisted of two β-cymaropyranosyls, one 6-deoxy-3-*O*-allopyranosyl, and terminal β-glucopyranosyl moieties as shown by their ^{13}C NMR spectra (Table 2). This sequence corresponded to the replacement of the oleandrose found in **1** or **2** with cymarose to give a sugar chain the same as that of dregeoside A_{11}[4]. The anomeric carbon of 6-deoxy-3-*O*-methyl-β-allopyranoside characteristically resonates at δ 104.0 when this sugar is linked to the 4-hydroxyl of D-cymarose, in contrast the resonance at 101.8 when linked to the 4-hydroxyl of D-oleandrose [2–4]. The carbon signals of the aglycone moieties of **3** and **4** agreed with those of **1** and **2**, respectively and consequently, **3** is presumed to be boucerogenin I 3-*O*-β-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside. Bouceroside BII (**4**) is concluded to be boucerogenin II 3-*O*-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

Table 2. ^{13}C NMR chemical shifts of sugar moieties of compounds **1–4**

	1	2		3	4
Cym-1	96.3	96.0	Cym-1	96.3	95.9
2	37.2[a]	37.0[a]		37.0[a]	37.2[a]
3	77.9	77.8		78.0	78.1
4	82.9[b]	83.0[b]		82.8[b]	82.9[b]
5	68.9	68.9		69.1	69.0
6	18.3[c]	18.3[c]		18.2[c]	18.6
3-OMe	58.8	58.8		58.8	58.9
Ole-1	101.7	101.8	Cym-1′	100.3	100.4
2	37.4[a]	37.1[a]		37.2[a]	37.4[a]
3	79.4	79.3		78.1	78.1
4	83.2[b]	83.3[b]		83.3[b]	83.3[b]
5	71.9	71.9		69.1	69.0
6	18.6[c]	18.6[c]		17.9[c]	18.6[c]
3-OMe	57.3	57.3		59.0	59.0
Allo-1	101.8	101.8	Allo-1	104.0	104.0
2	72.6	72.6		72.5	72.5
3	83.3[b]	83.4[b]		83.1[b]	83.0[b]
4	83.5[b]	83.5[b]		83.4[b]	83.4[b]
5	69.5	69.5		71.6	71.6
6	18.9[c]	18,9[c]		18.5[c]	18.6[c]
3-OMe	61.6	61.7		61.7	61.7
Glc-1	106.5	106.5	Glc-1	106.5	106.5
2	75.5.5	75.4		75.5	75.4
3	78.3	78.9		78.7	78.3
4	71.9	71.9		71.9	71.9
5	78.2	78.3		78.2	78.2
6	63.0	63.0		63.0	63.0

δ values (ppm) from internal TMS in C_5D_5N. Cym, β-cymaropyranose. Ole, β-oleandropyranose. Allo, β-6-deoxy-3-*O*-methyl-β-allopyranose.

[a–c] in each column may be interchangeable.

It is of interest that the sugar chains of **1** and **2** are the same as that of the antitumour condurangoglycosides. This suggests the possibility of a structure–activity correlation and bioassays of these glycosides against tumour cells are under investigation.

EXPERIMENTAL

Mps: uncorr. ^1H NMR spectra were run at 100, 270 and 400 MHz with TMS as the int. standard in $CDCl_3$ or pyridine-d_5 solution; ^{13}C NMR spectra were run at 90 MHz spectrometer in pyridine-d_5. UV spectra were obtained in MeOH. CC were carried out on Wakogel C-100, C-200, and C-300 for normal phase, and Fujigel ODs Q-3 for reversed phase. HPLC analyses were performed with JASCO 880-PU machine monitored by 875-UV detector at 254 nm using Senshu Pak ODS-SH 4.6 × 250 mm (solvent: H_2O–MeOH 11:36). TLC was carried out on precoated plate, Kieselgel $60F_{254}$, Merck. Abreviations are used for sugars in this section as follows: cym, cymarose; ole, oleandrose; allo, 6-deoxy-3-*O*-methyl-allose; glu, glucose.

Plant material. Boucerosia aucheriana used in this research was collected and identified by Prof. N. A. Quazilkbash of Peshawar University, Pakistan.

Extraction and isolation. Dried and powdered aerial parts of *B. auchariana* (7 kg) 7 were extracted with MeOH and concd under red. pres. to give an extract (1.02 kg), a part (400 g) of which was re-extracted with $CHCl_3$. The $CHCl_3$ soluble fr. was defatted with hexane to give a crude glycoside mixture (117.5 g) showing positive Keller–Kiliani and Liebermann–Burchard reactions, $CHCl_3$ insoluble fr. (214.2 g), and hexane soluble fr. (54.9 g).

Acid hydrolysis of the glycoside mixture. A soln of the crude glycoside (25.2 g) in 300 ml of MeOH and 0.2 N H_2SO_4 (100 ml) was kept on a water bath at 70° for 60 min, then H_2O (500 ml) was added and the whole was concd to 400 ml *in vacuo*. The soln was warmed at *ca* 60° for a further 60 min. After cooling, the mixture was extracted with $CHCl_3$ (3 × 300 ml). The combined organic layer was washed with satd $NaHCO_3$ soln and satd NaCl soln, and dried over $MgSO_4$. After removal of the solvent, an aglycone mixture was obtained. The water layer of the hydrolysate was neutralized with satd $Ba(OH)_2$. The inorganic ppt. was removed by filtration. The filtrate was evapd to dryness to give a syrup (5.92 g).

The aglycone mixture (10 g) was submitted to CC on silica gel (300 g) with solvents of increasing polarity from 1 to 30% MeOH in $CHCl_3$ to separate fr. 2 (4.11 g: a mixture containing **7** and **8**) and fr. 3 (1.58 g: containing **9** and **10**). Fr. 2 was rechromatographed on 45 g of Wakogel C-200 with AcOEt–hexane (1:4) to give a fr. (525.5 mg) which consisted of mainly **7** and **8**. This mixture was further chromatographed over 23 g of Wakogel C-200 with AcOEt–hexane (2:3) to afford pure **7** (14.3) and **8** (17.0 mg). Fr. 3 was chromatographed on 45 g of Wakogel C-200 with

AcOEt–hexane (21:29) to give fr. **B** (46.8 mg) which gave pure **9** from Me_2CO–hexane and fr. C (490.2 mg) which was rechromatographed over 25 g of Wakogel C-200 with AcOEt–hexane (42:58) to give 36.0 mg of **10**.

12-O-Benzoyl-dihydroboucerin (**7**). Colourless needles (mp 128–132°), $[\alpha]_D$ −40.5° ($CHCl_3$; *c* 0.42). (Found: C, 73.51, H, 8.77. $C_{28}H_{40}O_5$ requires: C, 73.65; H, 8.83%). EIMS *m/z*: 438 $[M-H_2O]^+$, 420 $[M-2H_2O]^+$, 334 [M−122: benzoic acid]$^+$, 316 $[334-H_2O]^+$, 298 $[334-2H_2O]^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1710 (CO–O–). UV λ_{max}^{MeOH} nm (log ε): 229 (3.84). ^1H NMR (270 MHz): δ 0.83 (3H, *s*, H_3-19), 1.23 (3H, *d*, *J* = 6.6 Hz, H_3-21), 1.39 (3H, *s*, H-18), 3.61 (1H, *m*, H_α-3), 3.83 (1H, *dq*, *J* = 9.2, 6.6 Hz, H-20), 4.72 (1H, *dd*, *J* = 12, 4.4 Hz, Hα-12), 7.45 (2H, *t*, *J* = 7 Hz, *m*-H of benzoate), 7.57 (1H, *t*, *J* = 7 Hz, *p*-H), 8.06 (2H, *d*, *J* = 7 Hz, *o*-H). ^{13}C NMR: Table 1.

12-O-*Benzoyl-boucerin* (**8**). Colourless needles (mp 133–137°), $[\alpha]_D$ −61.9° ($CHCl_3$; *c* 0.26). Found: C, 73.88; H, 8.54. $C_{28}H_{38}O_5$ requires: C, 73.98; H, 8.43%). EIMS *m/z*: 454 $[M]^+$, 436 $[M-H_2O]^+$, 418 $[M-2H_2O]^+$ 332 $[M-122]^+$, 314, [332 $-H_2O]^+$ 296 $[341-2H_2O]^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1710. UV λ_{max}^{MeOH} nm (log ε): 229 (4.11). ^1H NMR (270 MHz): ν 1.02 (3H, *s* H_3-19), 1.23 (3H, *d*, *J* = 6.6 Hz, H_3-21), 1.41 (3H, *s*, H_3-18), 3.53 (1H, *m*, Hα-3), 3.83 (1H, *dq*, *J* = 9.2, 6.6 Hz, H-20), 4.78 (1H, *dd*, *J* = 12, 4.4 Hz, Hα-12), 5.44 (1H, *m*, H-6), 7.46 (2H, *t*, *J* = Hz, *m*-H), 7.58 (1H, *t*, *J* = 7 Hz, *p*-H), 8.07 (2H, *t*, *J* = 7 Hz, *o*-H). ^{13}C NMR: Table 1.

12-O-*Benzoyl*-20-O-*acetyl dihydroboucerin* (**9**). Colourless fine needles (mp 120–125°), $[\alpha]_D$ +6.5° ($CHCl_3$; *c* 0.20). (Found: C, 72.24; H, 8.77. $C_{30}H_{42}O_6$ requires: C, 72.26; H, 8.49%). EIMS *m/z*: 438 $[M-AcOH]^+$, 420 $[438-H_2O]^+$, 376 $[M-122]^+$, 358 $[376-H_2O]^+$, 316 $[438-122]^+$, 298 $[316-H_2O]^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1720, 1710, 1250. UV λ_{max}^{MeOH} nm (log ε): 228 (4.11). ^1H NMR (270 MHz): δ 0.82 (3H, *s*, H_3-19), 1.12 (3H, *d*, *J* = 6.6 Hz, H_3-21), 1.15 (3H, *s*, H_3-18), 1.93 (3H, *s*, AcO–), 3.61 (1H, *m* Hα-3), 4.76 (1H, *dd*, *J* = 12, 4.4 Hz, Hα-12), 4.88 (1H, *dq*, *J* = 9.2, 6.6 Hz, H-20), 7.45 (2H, *t*, *J* = Hz, *m*-H), 7.58 (1H, *t*, *J* = 7 Hz, *p*-H), 8.10 (2H, *d*, *J* = 7 Hz, *o*-H). ^{13}C NMR: Table 1.

12-O-*Benzoyl*-20-O-*acetyl boucerin* (**10**). Colourless fine needles (mp 126–130°), $[\alpha]_D$ −7.5° ($CHCl_3$; *c* 0.27). Found: C, 72.67; H, 8.23. $C_{30}H_{40}O_6$ requires: C, 72.55; H, 8.12%). EIMS *m/z*: 496 $[M]^+$, 478 $[M-H_2O]^+$, 460 $[M-2H_2O]^+$, 436 $[M-AcOH]^+$, 418 $[436-H_2O]^+$, 374 $[M-122]^+$, 356 [374 $-H_2O]^+$, 314 $[M-122-60]^+$, 296 $[314-H_2O]^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1730, 1710, 1250. UV λ_{max}^{MeOH} nm (log ε): 228 (4.11). ^1H NMR: δ 1.01 (3H, *s*, H_3-19), 1.14 (3H, *d*, *J* = 6.6 Hz, H_3-21), 1.18 (3H, *s*, H_3-18), 1.98 (3H, *s*, AcO–), 3.53 (1H, *m*, Hα-3–), 4.81 (1H, *dd*, *J* = 12, 4 Hz, Hα-12), 4.92 (1H, *dq*, *J* = 9, 6.6 Hz, H-20), 5.43 (1H, *m*, H-6), 7.46 (2H, *J* = 7 Hz, *m*-H), 7.59 (1H, *t*, *J* = 7 Hz, *p*-H), 8.12 (2H, *d*, *J* = 7 Hz, *o*-H). ^{13}C NMR: Table 1.

Acetylation of **7**. A soln of 34.1 mg of **7** in 1 ml of pyridine and 0.6 ml of Ac_2O was kept overnight at room temp. The mixture was poured into ice-H_2O (60 ml) and extracted with Et_2O (40 ml × 3). The organic layer was washed with 2 N HCl, satd $NaHCO_3$ soln and satd NaCl soln, successively, and dried over Na_2SO_4. After removal of the solvent, the residue was recrystallized from AcOEt–hexane to give 24.4 mg of an acetate (**11**), colourless prisms (mp 179–181°), $[\alpha]_D$ +7.8° ($CHCl_3$; *c* 2.23), (Found: C, 71.13; H, 8.19%. $C_{32}H_{44}O_7$ requires: C, 71.08; H, 8.20%.) IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1730, 1710, 1250. UV λ_{max}^{EtOH} nm (log ε): 228 (4.11). ^1H NMR (270 MHz): δ 0.83 (3H, *s*, H_3-19), 1.13 (3H, *d*, *J* = 6.2 Hz, H_3-21), 1.15 (3H, *s*, H_3-18), 4.69 (1H, *m*, Hα-3), 4.76 (1H, *dd*, *J* = 12, 4 Hz, Hα-12), 4.88 (1H, *dq*, *J* = 9.2, 6.2, H-20), 7.44 (2H, *t*, *J* = 7 Hz, *m*-H), 7.56 (1H, *t*, *J* = 7 Hz, *p*-H), 8.10 (2H, *d*, *J* = 7 Hz, *o*-H). ^{13}C NMR: Table 1.

Acetylation of **9**. To a soln of 10.0 mg of **9** in 0.5 ml of pyridine was added 0.5 ml of Ac_2O and the mixture kept overnight at room temp. After usual work-up, recrystallization from AcOEt–hexane gave 6.5 mg of colourless prisms (mp 179–181°), identified with **11** by mmp (no depression 178–180°).

Isolation of boucerosides AI (**1**), *A II* (**2**), *BI* (**3**), *and BII* (**4**). The crude glycoside mixture (18.3 g) was chromatographed on Wakogel C-100 (300 g) with solvents of increasing polarity from 3 to 100% MeOH–$CHCl_3$ into 7 fractions. Fr. 4 (8.96 g) eluated with 30% MeOH in $CHCl_3$ was rechromatographed on 300 g of Wakogel C-100 column with Me_2CO–AcOEt (1:9) to yield a fraction (2.13 g) which showed one spot with UV absorption on ordinary phase TLC. However, this fr. showed two spots on reversed phase TLC with H_2O–MeOH (1:4). This fr was chromatographed over a reversed phase CC (Fuji gel Q3) eluating with the same solvent system as used for the TLC to obtain substances A (1.14 g) and B (0.48 g). While the more polar substance A showed a complex profile on analytical HPLC, substance B showed four peaks which were separated preparatively using HPLC into each component named boucerosides AI (49.8 mg), AII (107.8 mg), BI (42.3 mg), and BII (29.0 mg) in order of polarity.

Bouceroside AI (**1**). White powder (mp 152–157.5°), $[\alpha]_D$ +9.8° (MeOH; *c* 1.11). (Found: C, 60.65; H, 7.40. $C_{62}H_{88}O_{21}$ $3H_2O$ requires: C, 60.87; H, 7.25%). FDMS *m/z*; 1191 $[M+Na]^+$, 1029 [1191–162: glucosyl group]$^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1705, 1600, UV λ_{max}^{MeOH} nm (log 230 (4.26). ^1H NMR (400 MHz): δ 0.97 (3H, *s*, H_3-19) 1.17 (3H, *s*, H_3-18), 1.23, 1.24, 1.28 (each 3H, *d*, *J* = 6.3, 6.3, 6.0, respectively, 6-Me of sugar moiety), 1.35 (3H, *d*, *J* = 6.6 Hz, H_3-21), 1.98 (3H, *s*, AcO–), 3.38, 3.45, 3.56 (each 3H, *s*, 3-O-Me of sugar moiety), 4.40 (1H, *d*, *J* = 7.7 Hz, glc-1-H), 4.47 (1H, *dd*, *J* = 9, 1.5 Hz, ole-1-H), 4.78 (1H, *d*, *J* = Hz, allo-1-H), 4.85 (1H, *dd*, *J* = 10, 2 Hz, cym-1-H), 4.97 (1H, *dd*, *J* = 12, 4 Hz, Hα-12), 5.29 (1H, *dq*, *J* = 9.2, 6.6 Hz, H-20), 5.44 (1H, *m*, H-6), 7.47, 7.49 (each 2H, *t*, *J* = 7 Hz, *m*-H), 7.56 (2H, *t*, *J* = 7 Hz, *p*-H), 7.87, 8.09 (each 2H, *d*, *J* = 7 Hz, *o*-H). ^{13}C NMR: Tables 2 and 3.

Acid hydrolysis of **1**. To a soln of 33 mg of **1** in 30 ml of MeOH, was added 10 ml of 0.2 N H_2SO_4 and the mixture refluxed on a water bath for 60 min. After the soln was diluted with 30 ml H_2O the MeOH was evapd under red. pres. to leave about 40 ml of soln. The mixture was kept on water bath at 60° for a further 60 min, cooled to room temp, and extracted with Et_2O (3 × 30 ml). The organic layer was washed with satd $NaHCO_3$ soln and satd brine and dried over $MgSO_4$. Removal of the solvent gave 14.9 mg boucerogenin I (**12**); colourless fine needles (mp 93–97°), $[\alpha]_D$ +34.8° (MeOH; *c* 0.46). EIMS *m/z*: 558 $[M]^+$, 540 $[M-H_2O]^+$, 522 $[M-2H_2O]^+$, 314 $[M-122\times2]^+$, 296 [314 $-H_2O]^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1705, 1280. UV λ_{max}^{MeOH} nm (log ε): 227 (4.13). ^1H NMR (100 MHz): δ 0.99 (3H, *s*, H_3-19), 1.14 (3H, *d*, *J* = 6.6 Hz, H_3-21), 1.18 (3H, *s*, $H_3$18), 1.98 (3H, *s*, AcO–), 3.55 (1H, *m*, Hα-3), 4.92 (1H, *dd*, *J* = 12, 4 Hz, Hα-12), 5.28 (1H, *dq*, *J* = 9, 6.6 H-20), 5.45 (1H, *m*, H-6), 7.46 (6H, *m*, *m*-H and *p*-H), 8.12 (4H, *m*, O-H). ^{13}C NMR: Table 3.

The water layer of the hydrolysate was neutralized with satd $Ba(OH)_2$ soln. After the inorganic ppt. was filtered off, the filtrate was concd to dryness to give 19.1 mg of a syrup, which was separated by CC on silica gel with the solvents from 4 to 10% MeOH in $CHCl_3$ to give 2.8 mg of cymarose and 14.7 mg of neocondurangotriose identified by comparisons of TLC with the authentic samples (R_f **14**: 0.27; **15**: 0.04, 4% MeOH in $CHCl_3$). Neocondurangotriose: $[\alpha]_D$ +30.5° (MeOH; *c* 0.30). ^1H NMR (270 MHz, pyridine-d_5): δ 1.54 (3H, *d*, *J* = 6.2 Hz, ole-6-Me), 1.65 (3H, *d*, *J* = 6.2 Hz, allo-6-Me), 3.21 (3H, *s*, ole-3-OMe), 3.44 (1H, *t*, *J* = 9.4 Hz, ole-4-H), 3.74 (1H, *dd*, J = 9.9, 2.9 Hz, allo-4-H), 3.84 (3H, *s*, allo-3-OMe), 4.39 (1H, *dd*, *J* = 12, 6 Hz, glu-6-H), 4.48 (1H, *t*, *J* = 3 Hz, allo-3-H), 4.56 (1H, *dd*, *J* = 12, 2 Hz, glu-6-H), 4.74 (1H, *t*, *J* = 3 Hz, ole-11H*β*), 4.99 (1H, *d*, *J* = 7.7 Hz, glu-1-H), 5.26 (1H, *d*, *J* = 8.1 Hz, allo-1-H).

Table 3. ^{13}C NMR chemical shifts of aglycone moieties of compounds **1–4** and their aglycones, **12** and **13**

C	1	3	13	2	4	12
1	37.6	37.5	37.4	37.3	37.3	37.3
2	30.0(−2.2)	30.0	32.2	30.2	30.3	32.5
3	77.7(+7.2)	77.8	70.5	77.3	77.2	71.1
4	34.8(−4.3)	34.8	39.1	39.3	39.3	43.2
5	44.5	44.5	45.0	139.8	139.8	140.8
6	28.1[a]	28.1[a]	28.1[a]	122.2	121.4	121.5
7	29.1[a]	29.1[a]	29.2[a]	27.7	27.7	27.7
8	40.6	40.5	40.5	37.5	37.5	37.6
9	46.5	46.5	46.6	43.8	43.7	43.8
10	36.0	36.0	36.1	37.6	37.3	37.6
11	26.8[b]	26.9[b]	26.9[b]	26.7[a]	26.6[a]	26.8[a]
12	79.4	79.0	79.0	79.3	79.0	78.5
13	52.9	52.9	52.9	52.7	52.7	52.7
14	85.6	85.6	85.7	85.9	85.9	85.9
15	32.0	32.2	32.2	32.6	31.8	32.7
16	25.5[b]	25.7[b]	25.7[b]	26.2[a]	25.4[a]	25.1[b]
17	50.2	50.3	50.3	50.3	50.2	50.3
18	10.2	10.2	10.3	10.2	10.2	10.3
19	12.0	12.0	12.2	19.7	19.7	19.7
20	74.8	74.8	74.8	74.8	74.8	74.8
21	19.4	19.5	19.7	19.3	19.3	19.7
1′	166.0	166.0	166.1	166.0	166.0	166.1
	166.7	166.7	166.8	166.7	166.6	166.8
2′	131.6	131.6	131.7	131.6	131.6	131.7
	131.7	131.7	131.7	131.7	131.7	131.7
3′	129.9	129.9	129.8	129.9	129.8	129.8
	130.1	130.1	130.1	130.1	130.1	130.1
4′	128.8	128.8	128.8	128.8	128.8	128.8
	129.0	129.0	129.1	129.0	129.0	129.1
5′	133.0	133.3	133.0	133.0	132.9	133.0
	133.4	133.4	133.4	133.4	133.3	134.0

δ values (ppm) from internal TMS in C_5D_5N. [a,b,c] Values in each column may be interchangeable.

Glycosilation shifts are given in parentheses.

Analysis for the chirality of the cymarose. The cymarose (2 mg) obtained from the hydrolysis of **1** above was dissolved in 1 ml of MeOH and 1 ml of 1%H_2SO_4 in MeOH. The mixture was kept for 15 min, then 1 ml of H_2O was added, and neutralized with satd $Ba(OH)_2$ soln and evapd to dryness *in vacuo*. After the residue was dried thoroughly, it was dissolved into 0.5 ml of dry toluene, to which *ca* 3 mg of 3,5-dinitrophenyl isocyanate and 0.05 ml of dry pyridine were added and the mixture kept on a water bath at 70° for 30 min. After evapn of the solvent *in vacuo*, the sample was analysed by a chiral column: SUMIPAX OA-1000, 5 μ, 4.0 mm i.d. × 150 mm, mobile phase, hexane–1,2-dichloroethane–EtOH (30:6:1), flow rate: 1 ml/min, monitored by absorption at 254 nm. R_t: 10 min as Me α-D-cymaropyranoside 4-*O*-3,5-dinitrophenyl carbamate and 14 min as Me β-D-cymaropyranoside 4-*O*-3,5-dinitrophenyl carbamate.

Bouceroside AII (**2**). White powder (mp 153–158.5°), $[\alpha]_D$ +8.6° (MeOH; *c* 0.94), (Found: C, 61.05; H, 7.68. $C_{62}H_{90}O_{21}\cdot 2/5H_2O$ requires: C, 61.22; H, 7.46%. FDMS *m/z*: 1193 $[M+Na]^+$, 1031 [1191 − 162: glucosyl group]$^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1705, 1600, UV λ_{max}^{MeOH} nm (log ε): 230 (4.36). ^1H NMR (400 MHz): δ0.78 (3H, *s*, H_3-19), 1.14 (3H, *s*, H_3-18), 1.23, 1.25, 1.28 (each 3H, *d*, *J* = 6.0, 5.4, 5.4, respectively, 6-Me of sugar moiety), 1.35 (3H, *d*, *J* = 6.6 Hz, H_3-21), 3.38, 3.44, 3.60 (each 3H, *s*, 3-*O*-Me of sugar moiety), 4.41 (1H, *d*, *J* = 7.7 Hz, Glc-1-H), 4.46 (1H, *dd*, *J* = 9, 1.5 Hz, ole-1-H), 4.78 (1H, *d*, *J* = 8.8 Hz, allo-1-H), 4.85 (1H, *dd*, *J* = 10, 2 Hz, cym-1-H), 4.99 (1H, *dd*, *J* = 12, 4 Hz, Hα-12), 5.25 (1H, *dq*, *J* = 9.2, 6.6 Hz, H-20), 7.47, 7.48 (each 2H, *t*, *J* = 7 Hz, *m*-H), 7.63 (2H, *t*, *J* = 7 Hz, *p*-H), 7.84, 7, 8.07 (each 2H, *d*, *J* = 7 Hz, *o*-H). ^{13}C NMR: Tables 2 and 3.

Acid hydrolysis of **2**. Compound **2** (29 mg) was hydrolysed in a similar manner as described in the case of **1** to give boucerogenin II (**13**) as aglycone, and a syrup (14.9 mg) which was separated into 2 mg of **14** and 10.7 mg of **15** by CC in similar manner as in **1** and identified with the authentic samples.

Bouceroside BI (**3**). White powder (mp 161.5–168°), $[\alpha]_D$ +22.4° (MeOH; *c* 0.68). (Found: C, 60.03; H, 7.46%. $C_{62}H_{88}O_{21}\cdot 4H_2O$ requires: C, 59.98; H, 7.15%) FDMS *m/z*: 1191 $[M+Na]^+$, 1029 [1191 − 162: glucosyl group]$^+$, 540 [1191 − sugar chain]$^+$, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1705, 1600. UV λ_{max}^{MeOH} nm (log ε): 230 (4.33). ^1H NMR (400 MHz): δ 0.97 (3H, *s*, H_3-19), 1.17 (3H, *s*, H_3-18), 1.21, 1.24, 1.27 (each 3H, *d*, *J* = 6.2, 6.0, 6.2, respectively, 6-Me of sugar moiety), 1.36 (3H, *d*, *J* = 6.0 Hz, H_3-21), 3.42, 3.44, 3.60 (each 3H, *s*, 3-*O*-Me of sugar moiety), 4.41 (1H, *d*, *J* = 7.4 Hz, Glc-1-H), 4.57 (1H, *d*, *J* = 7.8 Hz, allo-1-H), 4.75 (1H, *dd*, *J* = 9.3, 2, Hz, cym-1-H), 4.85 (1H, *dd*, *J* = 10, 2 Hz, cym-1-H), 4.97 (1H, *dd*, *J* = 12, 4 Hz, Hα-12), 5.29

(1H, *dq*, $J = 9.2$, 6.6 Hz, H-20), 5.43 (1H, *m*, H-6), 7.47, 7.49 (each 2H, *t*, $J = 7$ Hz, *m*-H), 7.64 (2H, *t*, $J = 7$ Hz, *p*-H), 7.86, 8.09 (each 2H, *d*, $J = 7$ Hz, *o*-H). ^{13}C NMR: Tables 2 and 3.

Bouceroside BII (**4**). White powder (mp 157.5–165°), $[\alpha]_D$ +21.0° (*c* 0.57; MeOH). (Found: C, 59.29; H, 7.46. $C_{62}H_{90}O_{21}\cdot 9/2H_2O$ requires: C, 59.46; H, 7.24%). FDMS *m/z*: 1193 $[M+Na]^+$, 1031 [1193 − 162: glucosyl group]$^+$, 949 $[1193 - 122 \times 2]^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1705, 1600. UV λ_{max}^{MeOH} nm (log ε): 230 (4.29). ^{1}H NMR (400 MHz): δ 0.78 (3H, *s*, H_3-19), 1.14 (3H, *s*, H_3-18), 1.22, 1.23, 1.26 (each 3H, *d*, $J = 6.0$. 5.4, respectively, 6-Me of sugar moiety), 1.34 (3H, *d*, $J = 6.6$ Hz, H_3-21), 3.41, 3.43, 3.59 (each 3H, *s*, 3-*O*-Me of sugar moiety), 4.39 (1H, *d*, $J = 7.7$ Hz, glc-1-H), 4.58 (1H, *d*, $J = 7.8$ Hz, allo-1-H), 4.74 (1H, *dd*, $J = 10$, 2 Hz, cym-1-H), 4.85 (1H, *dd*, $J = 10$, 2 Hz, cym-1-H), 4.91 (1H, *dd*, $J = 12$, 4.3 Hz, Hα-12), 5.25 (1H, *dq*, $J = 9.2$, 6.6 Hz, H-20), 7.47, 7.48 (each 2H, *t*, $J = 7$ Hz, *m*-H), 7.64 (2H, *t*, $J = 7$ Hz, *p*-H), 7.86, 7, 8.07 (each 2H, *d*, $J = 7$ Hz, *o*-H). ^{13}C NMR: Tables 2 and 3.

Acknowledgements—We thank Prof. N. A. Quazilbash (Peshawar University, Pakistan) for kind collection and identification of plant material. We are grateful to Prof. emritus H. Mitsuhashi (Hokkaido University) for giving us the opportunity of this investigation. We wish to thank Dr Ikura, Mr Yamada and Miss Misu for NMR measurements, Mr Watanabe and Mrs Fujita for MS measurements, and Mrs Matsumoto and Miss Maeda for elemental analyses.

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