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ATRATOSIDES A, B, C AND D, STEROID GLYCOSIDES FROM THE ROOT OF *CYNANCHUM ATRATUM**

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Key Word Index—*Cynanchum atratum*; Asclepiadaceae; atratosides A–D; atratogenins A–B; cynajapogenin-A; 14,15-secopregnane.

Abstract—The glycoside portions of the Chinese crude 'Pai-Wei', dried root of *Cynanchum atratum* have been investigated. Four new glycosides named atratosides A–D of which the aglycones were novel 14,15-*seco*-pregnanes, were isolated and their structures were determined on the basis of spectroscopic evidence and analyses of their hydrolysates as atratogenin A 3-*O*-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside, atratogenin A 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside, atratogenin B 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside, and cynajapogenin A α-D-oleandropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)β-D-cymaropyranoside.

INTRODUCTION

The Chinese crude drug 'Pai-Wei', dried root of *Cynanchum atratum* Bunge (Asclepiadaceae), has been used as an antifebrile and diuretic in China. On the other hand, 'Pai-Chien', dried root of *C. glaucescens* Hand-Mazz, has been used as an antitussive and expectorant. Since both the plants have close relationship from the taxonomical point of view and similar outward appearance of their roots, some confusion exists in many places in China not only in the therapy but also in the name of the two drugs [1]. Some glycosides with aglycones possessing a novel 13,14;14,15-di*seco*-pregnane-type skeleton have been found in a study of 'Pai-Chien' [2]. In our recent studies on glycosides of 'Pai-Wei' we have isolated glaucoside C and H and cynatratoside A–E [3] and F [4] with similar types of skeleton. In the present paper, we wish to describe the isolation and structural elucidation of four new glycosides, named atratoside A(**1**), B(**2**), C(**3**), and D(**4**), with a novel 14,15-*seco*-pregnane type skeleton as aglycone.

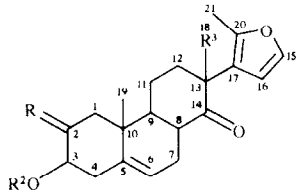
RESULTS AND DISCUSSION

These four glycosides showed positive Liebermann–Burchard and Keller–Kiliani reactions suggesting a Δ^5-steroidal structure and a 2-deoxy sugar residue.

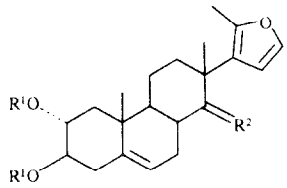
It is convenient to clarify the structures of the aglycones before discussion of the full structures of the glycosides. In order to obtain enough sample for structural elucidation of the new aglycone, hydrolysis of the crude glycoside mixture under acidic conditions and several chromatographic procedures were carried out to afford two new aglycones named atratogenin A(**5**) and atratogenin B(**6**), and cynajapogenin A(**7**) which is an 18-nor-derivative of **5** isolated from *C. japonicum* [5].

Atratogenin A(**5**), corresponded to a molecular formula of $C_{21}H_{28}H_4$ on the basis of its high resolution electron impact mass spectrum (EIMS) with M^+ at *m/z* 344.1979 (calcd: 344.1987). Its IR absorptions at 3500–3400 and 1680 cm^{-1} suggested the presence of hydroxyl and ketone groups which were supported by ^{13}C NMR signals at δ72.4, 76.5, and 212.4 (Table 1). ^1H NMR spectrum of **5** showed three tertiary methyl signals at δ1.15 (C-19 Me), 1.53 (C-18 Me), and 2.17 (C-21 Me) which are in good agreement with those of the glycoside **1**. Other signals are as follows; two carbinyl methine signals at δ3.33 (*ddd*, *J* = 12, 9, 5.5 Hz) and 3.71 (*ddd*, *J* = 12, 9, 4.5 Hz); one olefinic proton at δ5.47 (*t*, *J* = 2 Hz) and two aromatic protons on a furan ring at 6.21 (β-H, *d*, *J* = 2 Hz) and 7.22 (α-H, *d*, *J* = 2 Hz). In the ^{13}C NMR spectrum of **5**, a set of signals at δ147.2 *s* (calcd 148.7), 139.0 *d* (141.0), 123.7 *s* (132.3), and 111.3 *d* (110.1) (Fig. 1) suggested those signals to be ascribed to the carbons of a furan ring with 2,3-disubstituents by the calculations according to Runsink's parameter [6] where the value for the *t*-butyl group was used as the C-13 substituent. The chemical shift of C-17 at δ123.7, which deviated from the calculated value (132.3) seemed to be affected by the C-14 carbonyl group. When the ketone was reduced this signal was shifted to δ128.0. The UV absorption of **5** at 215 nm (ε = 14000) is in good agreement with the presence of the furan ring. Acetylation of **5** provided a diacetate (**8**), whose IR spectrum exhibited no hydroxyl absorption. Treatment of **5** with *p*-ClC_6H_4COCl in pyridine gave **9**, $C_{35}H_{34}O_6Cl_2$. The

*Part 68 in the series 'Studies on the Constitutents of Asclepiadaceae Plants'. For Part 67 see Tsukamoto, S., Hayashi, K., Kaneko, K. and Mitsuhashi, H. (1987) *J. Chem. Soc. Perkin I* (in press).

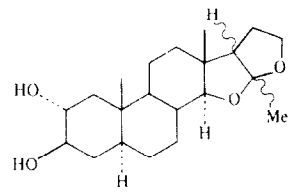


	R^1	R^2	R^3
1	H, HO	S_1	Me
2	H, HO	S_2	Me
3	O=	S_2	Me
4	H, HO	S_3	H
5	H, HO	H	Me
6	O=	H	Me
7	H, HO	H	H
8	H, AcO	AcO	Me
9	H, PCBO	PCBO	Me
16	O=	Ac	Me

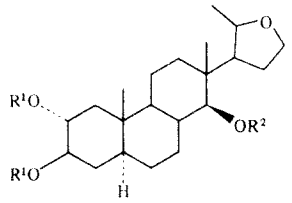


	R^1	R^2
10	H	H, HO
11	H	H, HO

PCB: *p*-Cl-benzoyl



12



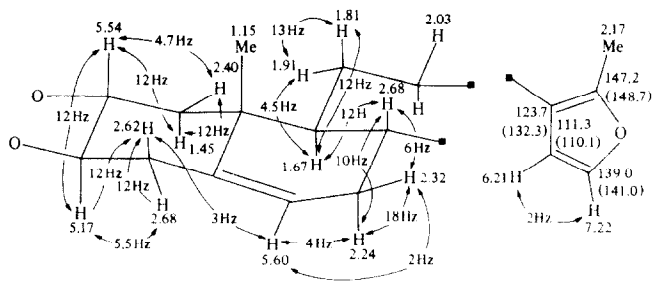
13 $R^1 = R^2 = H$
14 $R^1 = Ac$, $R^2 = H$
15 $R^1 = R^2 = Ac$

proton spin decoupling experiments of **9** were carried out to provide two partial structures (**I** and **II**) shown in Fig. 1.

The reduction of **5** with sodium borohydride in methanol at room temp for 12 hr gave two triols, **10** and **11**. The ^{1}H NMR spectra of **10** and **11** showed an additional carbinyl methine signal at δ3.68 (1H, *d*, $J = 2.5$ Hz) and 3.40 (1H, *d*, $J = 8.8$ Hz), respectively, compared with that of **5**. This fact suggested that this ketone must be located between tertiary and quarternary carbons.

The catalytic hydrogenation of **5** with PtO_2 in AcOH–EtOH (1:1) at room temperature afforded two compounds **12** and **13** which possessed the molecular formulae $C_{21}H_{34}O_4$ (M^+ at *m/z* 350) and $C_{21}H_{36}O_4$(M^+ at *m/z* 352), respectively. Both the compounds showed no carbonyl absorption in their IR spectra. The ^{1}H NMR spectrum of **12** exhibited three tertiary methyl signals at δ0.88, 0.95, and 1.55; five proton signals adjacent to oxygen at δ4.00 (1H, *m*), 3.61 (2H, *m*), 3.40 (1H, *m*) and 3.17 (1H, *d*, $J = 10$ Hz, 8-CH). The FD and EIMS of **13** gave the molecular ion peak at *m/z* 352, indicating fully hydrogenated product of **5**. The presence of spin–spin coupling between protons at δ1.24 (3H, *d*, $J = 7.3$ Hz, 21-Me) and 4.15 (1H, *m*, 20-CH) in the ^{1}H NMR spectrum of **13** were confirmed by decoupling experiments. Acetylation of **13** afforded a diacetate (**14**), $C_{25}H_{40}O_6$, and a triacetate (**15**), $C_{27}H_{42}O_7$.

On the basis of the arguments above, the structure of atratogenin-A(**5**) can be concluded as 15,20-epoxy-2α,3β-dihydroxy-14,15-*seco*-pregna-5,15 (16),17 (20)-trien-14-one. This skeleton might be derived biogenetically from a pregnane type precursor by conversion to the furan derivative via the oxidative cleavage between the C-14–15 bond and dehydrative condensation of the C-15 and C-20 oxo groups. The abnormally low field shift of the C-18 methyl signal at δ1.53 is accounted for by the



I II

Fig. 1.

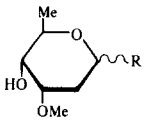
effect of the C-14 carbonyl and the furanyl groups.

Atratogenin-B(**6**) has the molecular formulae $C_{21}H_{26}O_4$ by its high resolution EIMS (M^+ at *m/z* 342.1832; calcd value 342.1822). Acetylation of **6** with acetic anhydride and pyridine provided a monoacetate (**16**), whose IR spectrum exhibited no hydroxyl absorption. The ^1H NMR spectrum of **6** is closely related to **5** except that there is only one carbinyl methine proton signal at δ4.19 (1H, *br dd*, $J = 10.2$, 2.4 Hz 3-CHα) and a set of AB type quartets at 2.69 and 2.25 (each *d*, $J = 13$ Hz, 1-CHα and β), while the triplet signal due to 1-CHα at 1.12 ($J = 12.2$ Hz) in the spectrum of **5** disappeared in the case of **6**. Moreover, by comparing the ^{13}C NMR data with that of **5**, compound **6** contains one more ketone group at the C-2 position istead of a hydroxyl group. Therefore the structure of **6** is deduced to be 15,20-epoxy-3β-hydroxy-14,15-*seco*-pregna-5,15(16), 17(20)-triene-2.14-dione.

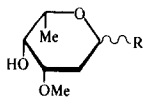
Cynajapogenin-A (**7**) has the molecular formula $C_{20}H_{26}O_4$ from the elemental analysis and high resolution EIMS. The ^1H NMR and ^{13}C NMR spectra of **7** very closely resemble those of **1**. However, these spectra lacked he signals due to the C-18 methyl group. This compound is identified as cynajapogenin A, which was isolated from the aglycone fraction of *Cynanchum japonicum*. [6]

Atratoside-A (**1**) possessed the molecular formula $C_{42}H_{64}O_{13}$ on th basis of its elemental analysis and field desorption mass spectrometry (FDMS). The ^1H NMR spectrum of **1** showed signals due to three tertiary methyls at δ1.13, 1.52 and 2.17, one olefinic at 5.43 (1H, *br s*), and two aromatic protons on a furan ring at 6.21 (β-H in the furan ring, *d*, $J = 2$ Hz) and 7.22 (α-H, *d*, $J = 2$ Hz). Mild acid hydrolysis of **1** afforded an aglycone (**5**), and two sugar components, D-cymarose (**18**) and L-diginose (**19**) by comparison with authentic samples. This is in good accord with the fragmentation pattern : *m/z* 776 $[M]^+$, 632 $[M-144]^+$, 488 $[632-144]^+$, 344 $[488-144]^+$ in its FDMS. Since the glycosidation shifts [7–9] were observed at C-2(−2.4 ppm), C-3(+8.8 ppm) and C-4(−2.8 ppm) in the ^{13}C NMR for the aglycone moiety of **5**, the sugar moiety is linked to the C-3 hydroxyl group of the aglycone (**5**). The ^1H NMR signals due to the anomeric proton at δ4.98 (1H, *br d*, $J = 3.5$ Hz), 4.76 and 4.69 (each 1H, *dd*, $J = 10$, 2 Hz) indicated one α and two β sugar linkages. The terminal sugar is assigned to be β-cymaropyranose and the other two sugars are assigned to be β-cymaropyranose and α-diginopyranose by comparing with ^{13}C NMR chemical shifts of methyl α-(**17a**) and β-cymaropyranosides (**17b**) and methyl α-(**18a**) and β-diginopyranosides (**18b**) [10]. The sequence of these three sugars is assigned by the partially relaxed Fourier transform (PRFT) method [11, 12] as follows. A set of signals with longer spin lattice relaxation time ($T = 0.210$ sec) among signals due to sugar carbons could be assigned to a terminal as β-cymaropyranose followed by α-diginopyranose ($T = 0.185$ sec) and finally the β-cymaropyranose ($T = 0.170$ sec) directly linked to the aglycone. This sugar sequence is the same as that of cynatratoside-F though the aglycone is different [13]. Since the diginose in this drug is in the L-series, as determined by its specific rotation, the structure of **1** was deduced as atratogenin A 3-*O*-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside.

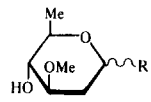
Atratoside-B(**2**), $C_{48}H_{74}O_{18}$, liberated **5**, D-cymarose



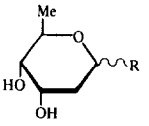
17 R = OH
17a R = α-OMe
17b R = β-OMe



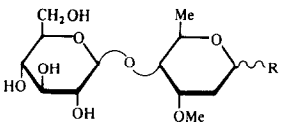
18 R = OH
18a R = α-OMe
18b R = β-OMe



19 R = OH



20 R = OH



21 R = OH

(**17**), L-diginose (**18**), and strophanthobiose (**21**). This glycoside showed four anomeric proton signals at δ4.99 (1H, *br s*), 4.60–4.80 (2H, *m*) and 4.21 (1H, *br d*, $J = 6$ Hz) and four anomeric carbon signals at δ97.4, 99.2, 100.9, and 106.3 in its ^1H NMR and ^{13}C NMR spectra. The other information on the sugar sequence was given by the fragmentation pattern in the FDMS spectrum of **2**: *m/z* 938 $[M]^+$, 664 $[M-\text{glucose}-144]^+$, 520 $[664-144]^+$. Enzymatic hydrolysis of **2** gave **1** as a deglucosyl derivative (**22**) which was identical with an authentic sample in IR, ^1H NMR, ^{13}C NMR, FDMS and $[\alpha]_D$ comparison. Glucose in the hydrolysate was identified by high performance liquid chromatography (HPLC) with an authentic sample. Therefore, the structure of **2** was established as atratogenin-A 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-α-D-diginopyranosyl-(1→4)-β-D-cymaropyranoside.

Atratoside C (**3**) has the formula $C_{48}H_{72}O_{18}$ by FDMS and elemental analysis. ^1H NMR spectrum of **3** showed three tertiary methyl at δ1.11, 1.53, and 2.17, one olefinic proton at 5.68 (H-6) and two furyl proton at 6.21 and 7.27 (each *d*, $J = 2$ Hz). These signals are very closely related to those of atratogenins and suggest that the aglycone of **3** is also the same type as **1** and **2**. The anomeric signals of the sugar portion of **3** at δ4.33 (*d*, $J = 8$ Hz), 4.75 (*dd*, $J = 10$, 2 Hz), 4.85 (*dd*, $J = 10$, 2 Hz), and 4.97 (*br t*, $J = 3$ Hz) resemble those of **2**. The carbon chemical shifts of the sugar moiety are also superimposed to those of **2**. Mild acid hydrolysis of **3** gave **17**, **18**, **21**, and **6**. The presence of **21** suggests terminal D-glucopyranose linked to D-cymaropyranose. By PRFT measurement, the terminal glucopyranose was confirmed in the sugar sequence of **3** which is, therefore, the same as that of **2**. These facts lead to the conclusion that the structure of **3** is atratogenin B 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside.

Atratoside D (**4**) has the formula $C_{40}H_{60}O_{13}$ by FDMS and elemental analysis. The ^1H NMR spectrum of **4** showed three anomeric proton signals at δ4.69 (1H, *dd*, $J = 9$, 2 Hz), 4.89 (1H, *dd*, $J = 9$, 2 Hz), and 5.03 (1H, *br t*, $J = 2$ Hz). From the ^{13}C NMR spectrum, **4** contained **7** as the aglycone moiety, and terminal α-oleandropyranose, β-digitoxopyranose, and β-cymaropyranose. The PRFT measurements of **4** indicated that the terminal sugar was also α-oleandropyranose. The acidic hydrolysis of **4** afforded **7** as an aglycone, and **18**, D-

oleandrose (**19**), and D-digitoxose (**20**) as sugar components. FDMS fragments at *m/z* 748 $[M]^+$, 604 $[M-144]^+$ and 474 $[604-130]^+$, suggested the sugar at the middle position in the sugar chain is digitoxose. Consequently, the structure of **4** was confirmed to be cynajapogenin. An α-D-oleandropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-cymaropyranoside.

EXPERIMENTAL

Mps: uncorr. ^{1}H NMR spectra were run in $CDCl_3$ soln and ^{13}C NMR in C_5D_5N soln with TMS as int. standard. TLC was performed on Merck precoated plates, Kieselgel $60F_{254}$ or RP-$18F_{254}$. CC was carried out on Wakogel C-200 (200 mesh) and reverse phase gel (Fuji, ODS-Q3 column). HPLC for glucose analysis was carried out by a Waters system (A-6000 pump, R-401 RI detecter, Radial PAK NH_2 column).

Plant material. Pai-Wei used in this research was obtained in Kun-Ming market and was identified as the dried root of *Cynanchum atratum* Bunge by Professor Jun Zhou, (President of Kunming Institute of Botany, Kunming, Yunnan, China).

Isolation of **5, 6,** *and* **7.** As reported in the previous papers [3, 4] the crude aglycone fraction (18 g) was subjected to silica gel CC with the solvent of increasing polarity from 3% MeOH–$CHCl_3$ to give four fractions (fr. 1: 7 g, fr. 2: 3 g, fr. 3: 3 g, and fr. 4: 4 g). After separation of glaucogenin A from the fr. 2, the residual mixture was submitted to CC on the reverse phase gel with MeOH–H_2O (7:3) and silica gel with 2% MeOH–$CHCl_3$ and AcOEt–hexane (1:3) to give pure **5** (285 mg) and cynajapogenin A (160 mg). Atratogenin B (**6**; 180 mg) was isolated from fraction 1 (7 g) by silica gel columns with 2% MeOH–$CHCl_3$ and AcOEt–hexane (1:3).

Atratogenin A (**5**). Pale yellow powder, (mp 69–73°), $[\alpha]_D$ −88.2° (MeOH; *c* 1.00). HR EIMS *m/z*: 344.1979 (M^+, calcd for $C_{21}H_{28}O_4$: 344.1987). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550–3400, 1680, 1495, 1430, 1420, 1360, 1340, 1200, UV λ_{max}^{MeOH} nm (ε): 217 (14000). EIMS *m/z*: 344 $[M]^+$, 311, 122 (base peak). ^{1}H NMR: δ1.12 (1H, *t*, *J* = 12.2 Hz, 1-CHα), 1.15 (3H, *s*, 19-Me), 1.53 (3H, *s*, 18-Me), 1.60 (1H, *ddd*, *J* = 12, 12, 5 Hz, 9-CH), 1.82 (2H, *m*, 11-CH_2), 2.03 (2H, *m*, 12-CH_2), 2.17 (3H, *s*, 21-Me), 2.24 and 2.32 (each 1H, *m*, 7-CHα,β), 2.38 (1H, *dd*, *J* = 13, 5 Hz, 1-CHβ), 2.65 (3H, *m*, 4-CH_2 and 8-CH), 3.33 (1H, *ddd*, *J* = 12, 9, 5 Hz, 3-CHα), 3.71 (1H, *ddd*, *J* = 12, 9, 4.5 Hz, 2-CHα), 5.47 (1H, *t*, *J* = 2.5 Hz, 6-CH), 6.21 (1H, *d*, *J* = 2 Hz, 16-CH), 7.22 (1H, *d*, *J* = 2 Hz, 15-CH). ^{13}C NMR: see Table 1.

Acetylation of **5.** Compound **5** (10 mg) was treated with 0.7 ml Ac_2O and 1 ml pyridine at room temp. for 12 hr. Usual work-up gave 7 mg of an acetate (**8**) as colourless fine needles from Me_2CO, (mp 120–122°), $[\alpha]_D$ −50.9° (MeOH; *c* 0.11). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1705, 1690, 1340, 1200, 1060. EIMS *m/z*: 428 $[M]^+$, 368 $[M-AcOH]^+$, 308 $[M-2AcOH]^+$, 43 (base peak). ^{1}H NMR: δ1.18 (3H, *s*, 19-Me), 1.53 (3H, *s*, 18-Me), 2.05 (6H, *s*, 2x−OAc), 2.17 (3H, *s*, 21-Me), 4.77 (1H, *ddd*, *J* = 12, 10, 6 Hz, 3-CHα), 5.16 (1H, *ddd*, *J* = 12, 10, 5 Hz, 2-CHβ), 5.52 (1H, *t*, *J* = 2.5 Hz, 6-CH), 6.22 (1H, *d*, *J* = 2 Hz, 16-CH), 7.22 (1H, *d*, *J* = 2 Hz, 15-CH).

p-*Chlorobenzoylation of* **5.** To a soln of **5** in 2 ml pyridine, *p*-ClC_6H_4COCl (1 ml) was added and the mixture was allowed to stand at room temp. for 12 hr, then to which 50 ml H_2O was added. The resulting mixture was extracted with $CHCl_3$ (30 ml × 5). The organic layer was washed with 2 N HCl (50 ml), satd $NaHCO_3$ soln (50 ml) and satd NaCl soln (50 ml), successively, then dried over Na_2SO_4. After removal of the solvent, the residual mixture was chromatographed over a silica gel column eluating with 10% Me_2CO–hexane to give 25 mg of di-*p*-chlorobenzoate (**9**) as colourless needles from Me_2CO, (mp 79–81°), $[\alpha]_D$ −117.1° ($CHCl_3$; *c* 1.65). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1700, 1580, 1480, 1380, 1320, 1260, 1250, 990, 830. EIMS *m/z*: 620 $[M]^+$, 464 $[M-p\text{-}ClC_6H_4COOH]^+$, 308 $[M-2\times p\text{-}ClC_6H_4COOH]^+$, 139 (base peak, $[p\text{-}ClC_6H_4C{=}O]^+$. ^{1}H NMR: δ1.33 (3H, *s*, 19-Me), 1.45 (1H, *t*, 1-CHα), 1.54 (3H, *s*, 18-Me), 1.67 (1H, *ddd*, *J* = 12, 12, 4.5, 9-CH), 1.81 and 1.91 (each 1H, *m*, 11-CH_2), 2.03 (2H, *m*, 12-CH_2), 2.17 (3H, *s*, 21-Me), 2.24 (1H, *dddd*, *J* = 18, 10, 2, 2 Hz, 7-CHα), 2.32 (1H, *ddd*, *J* = 18, 6, 4 Hz, 7-CHβ), 2.40 (1H, *dd*, *J* = 12, 4.7 Hz, 1-CHβ), 2.62 (1H, *m*, 4-CHβ), 2.68 (2H, *m*, 4-CHα and 8-CH), 5.17 (1H, *ddd*, *J* = 12, 10, 5.5 Hz, 3-CHα), 5.54 (1H, *ddd*, *J* = 12, 10, 4.7 Hz, 2-CHβ), 5.61 (1H, *t*, *J* = 2.5 Hz, 6-CH), 6.21 (1H, *d*, *J* = 2 Hz, 16-CH), 7.22 (1H, *d*, *J* = 2 Hz, 15-CH).

Sodium borohydride reduction of **5.** To a soln of 80 mg of **5** in 10 ml MeOH, 120 mg of $NaBH_4$ was added. The mixture was stirred for 12 hr at room temp. Work-up in the usual manner gave a yellowish powder, which was chromatographed on a silica gel column with 2% MeOH–$CHCl_3$ to afford two triols **10** (10 mg) and **11** (25 mg). Compound **10**, colourless fine plates, (mp 94–96°), $[\alpha]_D$ −46.0° ($CHCl_3$; *c* 0.13). HR EIMS *m/z*: 346.2132 (M^+, calcd for $C_{21}H_{30}O_4$: 346.2144). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 3400, 1440, 1360, 1200, 980, 940, 900, 830. EIMS *m/z*: 346 $[M]^+$, 313, 231 (base peak), 135, 122, 109. ^{1}H NMR: δ1.05 (3H, *s*, 19-Me), 1.13 (1H, *t*, *J* = 12 Hz, 1-CHα), 1.23 (3H, *s*, 18-Me), 2.13 (1H, *d*, *J* = 12, 4.5 Hz, 1-CHβ), 2.39 (1H, *s*, 21-Me), 3.33 (1H, *ddd*, *J* = 12, 9, 5.5 Hz, 3-CHα), 3.68 (1H, *d*, *J* = 2.5 Hz, 14-CHα), 3.70 (1H, *m*, 2-CHβ), 5.46 (1H, *t*, *J* = 2.5 Hz, 6-CH), 6.29 (1H, *d*, *J* = 2 Hz, 16-CH), 7.24 (1H, *d*, *J* = 2 Hz, 15-CH). Compound **11**, colourless fine plates, (mp 109–110°), $[\alpha]_D$ −52.0° (*c* 0.50; $CHCl_3$). HR EIMS *m/z*: 346.2142 (M^+, Calcd for $C_{21}H_{30}O_4$: 346.2144). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 3350, 1440, 1420, 1360, 1200, 1120, 1000, 960. EIMS *m/z*: 346 (M^+), 313, 231 (base peak), 135, 122, 109. ^{1}H NMR: δ1.08 (3H, *s*, 19-Me), 1.31 (3H, *s*, 18-Me), 2.12 (1H, *dd*, *J* = 12, 4.5 Hz, 1-CHβ), 2.40 (3H, *s*, 21-Me), 3.33 (1H, *ddd*, *J* = 12, 9, 5.5 Hz, 3-CHα), 3.40 (1H, *d*, *J* = 8.8 Hz, 14-CHβ), 3.71 (1H, *ddd*, *J* = 12, 9, 4.5 Hz, 2-CHβ-), 5.46 (1H, *br d*, *J* = 5 Hz, 6-CH), 6.31 (1H, *d*, *J* = 2 Hz, 16-CH), 7.22 (1H, *d*, *J* = 2 Hz, 15-CH). ^{13}C NMR: see Table 1.

Hydrogenation of **5.** A mixture of 100 mg of **5** and 100 mg of PtO_2 in 10 ml AcOH–EtOH (1:1) was stirred under a hydrogen atmosphere at room temp. for 3.5 hr. After the uptake of hydrogen stopped, the catalyst was filtered off. The filtrate was concd to dryness, then the residue was separated by silica gel CC with 3% MeOH–$CHCl_3$ and Me_2CO–hexane (1:3) to give **12** (15 mg) and **13** (24 mg). Compound **12**, fine needles, (mp 86–88°), $[\alpha]_D$ −17.7° (*c* 1.40; $CHCl_3$). HR EIMS *m/z*: 350.2449 (M^+, Calcd for $C_{21}H_{34}O_4$: 350.2457). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550–3400, 1420, 1360, 1275, 1190, 1130, 1020, 850. EIMS *m/z*: 350 $[M]^+$, 321, 248, 111 (base peak). ^{1}H NMR: δ0.88 (3H, *s*, 19-Me), 0.95 (3H, *s*, 18-Me), 1.55 (3H, *s*, 21-Me), 3.17 (1H, *d*, *J* = 10.3 Hz, 14-CH), 3.40 (1H, *m*, 3-CHα), 3.61 and 4.00 (2H and 1H, *m*, 15-CH_2 and 2-CHβ). Compound **13**, fine needles, (mp 100–102°) $[\alpha]_D$ −16.8° ($CHCl_3$; *c* 0.50). HR EIMS *m/z*: 352.2613 (M^+, calcd for $C_{21}H_{36}O_4$: 352.2613). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500–3300, 1430, 1360, 1250, 1140, 1040. EIMS *m/z*: 352 (M^+), 280, 266, 84 (base peak). ^{1}H NMR: δ0.83 (3H, *s*, 19-Me), 0.98 (3H, *s*, 18-Me), 1.24 (3H, *s*, 21-Me), 3.05 (1H, *dd*, *J* = 9.3, 3.4 Hz, 14-CHβ), 3.43 (1H, *m*, 3-CHα), 3.59 (1H, *m*, 2-CHβ), 3.75 and 3.93 (each 1H, *m*, 15-CH_2), 4.15 (1H, *m*, 20-CH).

Acetylation of **13.** Compound **13** (15 mg) in 1 ml pyridine was treated with 0.5 ml Ac_2O at room temp. for 6 hr. Usual work-up gave a yellow powder, which was chromatographed on a silica gel column with 2% MeOH–$CHCl_3$ and 10% Me_2CO hexane to give 2 mg of a diacetate (**14**) and 4 mg of a triacetate (**15**). The diacetate (**14**), colourless fine needles, (mp 57–59°), $[\alpha]_D$ −26.0° ($CHCl_3$; *c* 0.30). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1710, 1460–1420, 1360, 1260 1040. FDMS *m/z*: 437 $[M+H]^+$ 436 $[M]^+$. ^{1}H NMR:

δ0.90 (3H, *s*, 18-Me), 0.98 (3H, *s*, 19-Me), 1.26 (2H, *d*, *J* = 7.3 Hz, 21-Me), 2.01 and 2.02 (each 3H, *s*, -OAc), 3.04 (1H, *d*, *J* = 9.8 Hz, 14-CH), 3.75 and 3.93 (2H, *m*, 15-CH_2), 4.12 (1H, *m*, 20-CH), 4.81 (1H, *ddd*, *J* = 12, 9, 6 Hz, 3-CHα), 5.03 (1H, *ddd*, *J* = 12, 9, 5 Hz, 2-CHβ). The triacetate (**15**), colourless fine needles, (mp 70–72°), $[\alpha]_D$ −18.6° ($CHCl_3$; *c* 0.14). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1700, 1400, 1230, 1070, 1020. FDMS *m/z*: 479 $[M+H]^+$ 478 $[M]^+$ ^1H NMR: δ0.90 (3H, *s*, 19-Me), 0.99 (3H, *s*, 18-Me), 1.13 (3H, *d*, *J* = 6.5 Hz), 21-Me), 3.70 and 3.90 (2H, *m*, 15-CH_2), 4.13 (1H, *m*, 20-CH), 4.64 (1H, *d*, *J* = 10.3 Hz, 14-CH), 4.81 (1H, *ddd*, *J* = 12, 9, 6 Hz, 3-CHα), 5.03 (1H, *ddd*, *J* = 12, , 5 Hz, 2-CHβ).

Atratogenin B(**6**). Pale yellow oil, $[\alpha]_D$ −87.4° ($CHCl_3$; *c* 2.10), HR EIMS *m/z*: 342.1832 (M^+, calcd for $C_{21}H_{26}H_4$: 342.1822). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1730, 1480, 1460, 1310, 1260, 1220, 1120, 1110. EIMS *m/z*: 342 (M^+), 135 (base peak), 122. ^1H NMR: δ1.10 (3H, *s*, 19-Me), 1.53 (3H, *s*, 18-Me), 1.70 and 1.85 (2H, *m*, 11-CH_2), 1.79 (1H, *ddd*, *J* = 12, 12, 4 Hz, 9-CH), 2.04 (2H, *m*, 12-CH_2), 2.17 (3H, *s*, 21-Me), 2.25 (1H, *d*, *J* = 13 Hz, 1-CHα), 2.33 and 2.45 (2H, *m*, 7-CH_2), 2.65 (1H, *ddd*, *J* = 12, 10, 6 Hz, 8-CH), 2.69 (1H, *d*, *J* = 13 Hz, 1-CHβ), 2.88 (1H, *dd*, *J* = 12.5, 7.8 Hz, 4-CHβ), 4.19 (1H, *br dd*, *J* = 10.2, 2.4 Hz, 3-CHα), 5.70 (1H, *t*, *J* = 2.5 Hz, 6-CH), 6.21 (1H, *d*, *J* = 2 Hz, 16-CH), 7.22 (1H, *d*, *J* = 2Hz, 15-CH). ^{13}C NMR: see Table 1.

Acetylation of **6**. atratogenin B(**6**) (10 mg) in 1 ml pyridine was treated with 0.5 ml Ac_2O at room temp. for 12 hr. The mixture was poured into 50 ml H_2O, extracted with 3 × 20 ml Et_2O, washed with 2 N hydrochloric acid, satd $NaHCO_3$ soln and brine, and dried over $MgSO_4$. Evapn of the solvent gave 8 mg of an acetate (**16**), pale yellow noncrystallized material, $[\alpha]_D$ −47.8° (MeOH; *c* 0.08). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710, 1680, 1400, 1340, 1260, 1180, 1060. EIMS *m/z*: 384 $[M]^+$, 324 $[M-AcOH]^+$ 171, 43. ^1H NMR: δ1.15 (3H, *s*, 19-Me), 1.53 (3H, *s*, 18-Me), 2.17 (3H, *s*, 21-Me), 2.18 (3H, *s*, -OAc), 2.28 (1H, *d*, *J* = 12.5 Hz, 1-CHα), 2.63 (1H, *d*, *J* = 12.5 Hz, 1-CHβ), 5.19 (1H, *br t*, *J* = 10 Hz, 3-CH), 5.71 (1H, *br s*, 6-CH), 6.21 (1H, *d*, *J* = 2 Hz, 16-CH), 7.23 (1H, *d*, *J* = 2 Hz, 15-CH).

Cynajapogenin A (**7**). An amorphous powder, (mp 71–74°), $[\alpha]_D$ −45.3° (MeOH; *c* 0.8), HR EIMS *m/z*: 330.1817 (M^+, calcd for $C_{20}H_{26}O_4$: 330.1824). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600-300, 1710, 1500, 1480, 1360, 1200. UV λ_{max}^{MeOH} nm (ε): 217.5 (5500). EIMS m/z: 330 (base peak), 297, 186, 108. ^1H NMR: δ1.15 (3H, *s*, 19-Me), 1.78 (2H, *m*, 11-CH_2), 2.04 (2H, *m*, 12-CH_2), 2.17 (3H, *s*, 21-Me), 2.22 and 2.32 (each 1H, *m*, 7-$CH_{\alpha,\beta}$), 2.38 (1H, *dd*, *J* = 12.5, 5 Hz, 1-CH_β), 3.35 (1H, *ddd*, *J* = 12, 9, 5 Hz, 3-CH_α), 3.41 (1H, *dd*, *J* = 12, 5 Hz, 13-CH_β), 3.70 (1H, *ddd*, *J* = 12, 9, 4.5 Hz, 2-CH_β), 5.46 (1H, *br d*, *J* = 4.5 Hz, 6-CH), 6.20 (1H, *d*, *J* = 2 Hz, 16-CH), 7.27 (1H, *d*, *J* = 2 Hz, 15-CH). ^{13}C NMR: see Table 1.

Isolation of **1**, **2**, **3** *and* **4**. The benzene soluble portion of the crude glycoside reported previously [4], was submitted to CC on silica gel and eluted with 2% MeOH–$CHCl_3$ to give five fractions. From fr. 3 (20 g), cynatratoside B, C, F and glaucoside C were separated by CC [4]. The residual mixture of fr. 3 was further separated on reverse phase and a silica gel column with MeOH–H_2O (7:3) and 1% MeOH–$CHCl_3$, respectively, to give pure **1** (150 mg) and **4** (120 mg). The benzene-insoluble portion of the crude glycosides was applied to a silica gel column and eluted with solvent of increasing polarity from $CHCl_3$ to $CHCl_3$–MeOH (3:2) to afford six fractions. After isolation of cynatratoside D, E [3] and glaucoside H [4] from fraction 5 (20 g), the residue was continually separated on a reverse phase column (RP-18) with 20% H_2O–MeOH to give pure **2** (200 mg) and **3** (140 mg).

Atratoside-A (**1**). An amorphous powder, (mp 105–110°), $[\alpha]_D$ −65.9° (MeOH; *c* 1.60). Found: C, 63.05; H, 8.30. $C_{42}H_{64}O_{13}\cdot 3/2H_2O$ requires: C, 62.76; H, 8.34. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1700, 1450, 1370, 1200, 1160, 1140, 1090, 1060, 1020, 1000, 980, 970, 940, 910, 860. FDMS *m/z*: 776 $[M]^+$, 632 $[M-144]^+$ 488 $[632-144]^+$, 344 $[488-144]^+$. ^1H NMR: δ1.13 (3H, *s*, 19-Me), 1.23–1.30 (9H, *m*, 6′-, 6″- and 6‴-Me), 1.52 (3H, *s*, 18-Me), 2.17 (3H, *s*, 21-Me), 3.41, 3.43, 3.44 (each 3H, *s*, 3′-, 3″- and 3‴-OMe), 4.69 and 4.76 (each 1H, *dd*, *J* = 10, 2 Hz, 1′- and 1‴-CH), 4.98 (1H, *d*, *J* = 3.5 Hz, 1″-CH), 5.43 (1H, *br s*, 6-CH), 6.21 (1H, *d*, *J* = 2 Hz, 16-CH), 7.22 (1H, *d*, *J* = 2 Hz, 15-CH). ^{13}C NMR: see Tables 1 and 2.

Table 1. ^{13}C NMR chemical shifts of the aglycone moieties

C	1	2	3	4	5	6	7
1	45.3	45.2	52.0	45.4	46.0	51.2	46.2
2	70.0 (−2.4)	70.2 (−2.2)	205.6 (−4.6)	70.1 (−2.4)	72.4	210.2	72.5
3	85.3 (+8.8)	85.2 (+8.7)	78.3 (+3.1)	85.4 (+8.9)	76.5	75.2	76.5
4	37.6 (−2.8)	37.5 (−2.9)	39.4 (−3.3)	37.7 (−2.9)	40.4	42.7	40.6
5	139.2	139.0	136.8	138.9	140.1	137.3	140.1
6	121.6	121.6	123.8	121.5	120.9	123.2	120.8
7	27.0	26.9	27.0	25.9	26.9	27.0	25.8
8	50.7	50.6	50.1	52.2	50.7	50.1	52.2
9	41.6	41.5	41.7	45.6	41.6	41.7	45.6
10	39.0	38.9	43.4	39.8	39.5	43.7	39.4
11	20.4	20.4	19.7	25.5	20.4	20.4	25.5
12	38.3	38.3	38.2	33.8	38.3	38.7	34.0
13	47.7	47.7	47.7	47.9	47.6	47.7	47.9
14	212.2	212.2	211.8	209.3	214.4	211.8	209.3
15	139.3	139.3	139.4	140.1	139.3	139.4	139.9
16	111.4	111.3	111.4	112.0	111.3	111.4	112.0
17	123.7	123.3	123.8	117.9	123.7	123.7	117.9
18	23.9	23.9	23.9	—	23.9	23.9	—
19	19.8	19.8	19.7	20.2	20.0	19.9	20.2
20	148.1	148.0	148.1	148.3	148.1	148.1	148.0
21	14.6	14.6	14.7	11.9	14.6	14.7	11.9

δvalues (ppm) from internal TMS in C_5D_5N. Glycosylation shifts are given in parentheses.

Table 2. ^{13}C NMR chemical shifts of the sugar moieties

	1	2	3	4
	β-D-Cym	β-D-Cym	β-D-Cym	β-D-Cym
C-1	97.5	97.4	95.6	97.9
C-2	35.4	35.5	34.6	35.8
3	77.5	77.4	77.1	77.9
4	82.1	81.9	81.8	82.3
5	69.6	69.5	69.6	69.4
6	18.8	18.4	18.6	18.6
3-OMe	57.4	57.3	57.2	57.1
	β L-Dgn	α-L-Dgn	α-L-Dgn	β-D-Dgt
C-1	101.1	100.9	100.8	100.5
2	32.4	32.3	32.4	38.8
3	73.8	73.5	73.6	68.7
4	74.6	74.5	74.6	82.2
5	67.7	67.5	67.5	67.6
6	17.9	17.8	17.9	18.1
3-OMe	55.3	55.3	55.4	
	β-D-Cym	β-D-Cym	β-D-Cym	α-D-Ole
C-1	99.5	99.2	99.3	100.3
2	35.3	36.1	36.1	35.8
3	78.9	78.1	78.1	78.8
4	74.2	82.9	82.9	76.9
5	71.1	69.5	69.6	69.4
6	18.4	18.3	18.5	18.4
3-OMe	58.0	58.5	58.5	57.1
		β-D-Glc	β-D-Glc	
C-1		106.3	106.5	
2		75.2	75.4	
3		78.1	78.3	
4		71.7	71.8	
5		78.1	78.3	
6		62.9	62.9	

See legand to Table 1.

Cym: cymaropyranosyl, Dgn: diginopyranosyl, Dgt: digitoxopyranosyl, Glc: glucopyranosyl.

Acidic hydrolysis of **1**. To a soln of 70 mg of **1** in 10 ml MeOH was added 10 ml of 0.1 N H_2SO_4 and the mixture was kept at 50° for 1 hr. Then the soln was diluted with H_2O (10 ml) and concd to 20 ml, it was kept at 70° for 30 min again, neutralized with aq satd $Ba(OH)_2$ soln, and the ppts were filtered off. The filtrate was concd to dryness and chromatographed on a column of silica gel (16 g of Wakogel C-200) with 1% MeOH–$CHCl_3$ to give 4.2 mg of D-cymarose: $[\alpha]_D$ +51.6° (H_2O; *c* 0.40), 2.7 mg of L-diginose: $[\alpha]_D$ −58.8° (H_2O; *c* 0.4) and 17.3 mg of atratogenin A (**5**): (mp 68–72°), $[\alpha]_D$ −75° (MeOH; *c* 0.66) identified by TLC with the authentic sample. The R_f values of **5** and diginose are 0.49 and 0.40 with solvent $CHCl_3$–MeOH (9:1); 0.22 and 0.11 with solvent AcOEt–hexane (3:1); 0.61 and 0.33 with solvent Me_2CO petrol (2:3), respectively.

Atratoside-B (**2**). An amorphous powder, (mp 153–158°), $[\alpha]_D$ −48.3° (MeOH; *c* 2.10). Found: C, 58.52; H, 7.67. $C_{48}H_{74}O_{18}\cdot 5/2H_2O$ requires: C, 58.60; H, 8.03. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1700, 1520, 1470, 1420, 1360, 1200, 1080, 1050, 1020, 920. FDMS *m/z*: 938 $[M]^+$, 664 $[M^+ -$ strophanthobiose$]^+$ 500 $[644-144]^+$. ^{1}H NMR: δ1.13 (3H, *s*, 19-Me), 1.23–1.30 (9H, *m*, 6′-, 6″- and 6‴-Me), 1.52 (3H, *s*, 18-Me), 2.17 (3H, *s*, 21-Me), 3.40, 3.43 (6H, and 3H, *s*, 3′-, 3″- and 3‴-OMe), 4.21 (1H, *br d*, *J* = 6 Hz, 1⁗-CH), 4.60–4.80 (2H, *m*, 1′-and 1‴-CH), 4.99 (1H, *br s*, 1″-CH), 5.45 (1H, *br s*, 6-CH), 6.22 (1H, *d*, *J* = 2 Hz, 16-CH), 7.22 (1H, *d*, *J* = 2 Hz, 15-CH). ^{13}C NMR: see Tables 1 and 2.

Acidic hydrolysis of **2**. To a soln of 90 mg of **2** in 10 ml of MeOH was added 10 ml of 0.1 N H_2SO_4 and the mixture kept at 50° for 1 hr. After work-up atratogenin A (**5**), **17**, **18** and **21** were identified by TLC with the authentic samples. The cymarose was separated over silica gel CC to give 2.2 mg of D-cymarose, $[\alpha]_D$ +57.6° (*c* 0.20; H_2O).

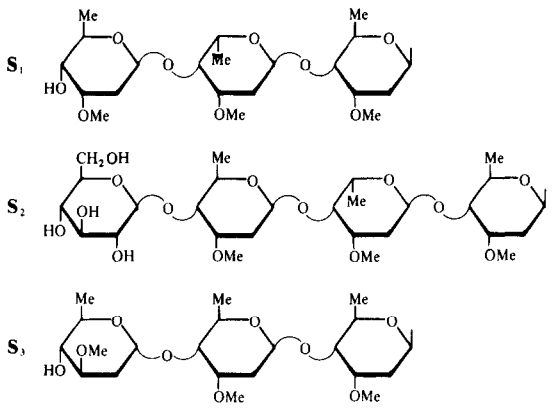
Enzymatic hydrolysis of **2** *with snail enzyme (β-glucosidase)*. A suspension of **2** (80 mg) and powdered snail digestive juice (80 mg) in 10 ml of 0.3 M NaOAc buffer adjusted to pH 5.5 was allowed to stand at 37° for 120 hr. TLC analysis with MeOH–$CHCl_3$ (1:9) revealed the formation of **1**. The soln was concd and the residue was subjected to silica gel CC with 2% MeOH–$CHCl_3$ to give compound **22** (25 mg). Further elution with MeOH–$CHCl_3$ (1:1) gave a fraction in which glucose was detected by HPLC (column, radial PAK NH_2 with 10% H_2O–MeCN).

Compound **22**, an amorphous powder, (mp 108–113°), $[\alpha]_D$ −62.0° (MeOH; *c* 0.50). (Found: C, 63.03; H, 8.21. $C_{42}H_{64}O_{13}\cdot 3/2H_2O$ requires: 62.76; H, 8.34). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1700, 1450, 1370, 1200, 1160, 1140, 1090, 1060, 1020, 1000, 980, 970, 940, 910, 860. FDMS *m/z*: 776 $[M]^+$, 632 $[M-144]^+$, 488 $[632-144]^+$ 344 $[488-144]^+$. ^{1}H NMR: δ1.14 (3H, *s*, 19-Me), 1.23–1.32 (9H, *m*, 6′-, 6″-and 6‴-Me), 1.52 (3H, *s*, 18-Me), 2.17 (3H, *s*, 21-Me), 3.42 and 3.43 (3H and 6H, *s*, 3′-, 3″- and 3‴-Me), 4.69, 4.76 (each 1H, *d*, *J* = 10, 2 Hz, 1′-and1‴-CH), 4.99 (1H, *d*, *J* = 3 Hz, 1″-CH), 5.44 (1H, *br s*, 6-CH), 6.21 (1H, *d*, *J* = 2 Hz, 16-CH), 7.22 (1H, *d*, *J* = 2 Hz, 15-CH). ^{13}C NMR: see tables 1 and 2. These data were identical with those of atratoside A (**1**).

Atratoside-C (**3**). An amorphous powder, (mp 148–153°), $[\alpha]_D$ −58.8° (*c* 2.2; MeOH). Found: C, 58.45; H, 7.84. $C_{48}H_{72}O_{18}\cdot 3H_2O$ requires: C, 58.18; H, 8.08. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1735, 1705, 1450, 1360, 1200, 1050. FDMS *m/z*: 936 $[M]^+$. ^{1}H NMR: δ1.10 (3H, *s*, 19-Me), 1.22–1.30 (9H, *m*, 1′, 2″, and 3‴-Me), 1.53 (3H, *s*, 18-Me), 2.17 (3H, *s*, 21-Me), 3.39 (6H, *s*, -OMe ×2), 3.43 (3H, *s*, -OMe), 4.33 (1H, *d*, *J* = 8 Hz, 1⁗-CH), 4.75 (1H, *dd*, *J* = 10, 2 Hz, 1‴ or 1′-CH), 4.85 (1H, *dd*, *J* = 10, 2 Hz, 1′ or 1‴-CH), 4.97 (1H, *br t*, *J* = 3 Hz, 1″-C), 5.43 (1H, *br s*, 6-CH), 6.21 (1H, *d*, *J* = 2 Hz, 16-CH), 7.23 (1H, *d*, *J* = 2 Hz, 15-CH). ^{13}C NMR: see Tables 1 and 2.

Acidic hydrolysis of **3**. To soln of **3** (90 mg) in MeOH (15 ml) was added 5 ml of 2 N H_2SO_4 at 50° on a water bath and the mixture was warmed for 1 hr, then 15 ml H_2O was added to the mixture and MeOH removed under red. pres. The mixture was warmed again at 50° for 30 min, neutralized with aq. satd $Ba(OH)_2$ and the ppt. formed was filtered off. The filtrate was concd to dryness. The residual syrup was chromatographed over silica gel (18 g) with the solvent of 2% MeOH–$CHCl_3$ to obtain atratogenin B (15 mg), $[\alpha]_D$ −85 ($CHCl_3$; *c* 1.1), EIMS *m/z*: 342 $[M]^+$, 135 (base peak), 122, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1730, 1480, 1460, 1310, 1260, 1220, 1120. cymarose (**17**, 3 mg), $[\alpha]_D$ +51.5° (*c* 0.3; H_2O), diginose (**18**, 2.3 mg); $[\alpha]_D$ −58.8° (*c* 0.4, H_2O), and strophanthobiose (**21**, 2.2 mg), a syrup, $[\alpha]_D$ +30.8° (*c* 0.5; H_2O). They were identified with authentic samples by TLC R_f values. Compound **18** R_f 0.41 with $CHCl_3$–MeOH 9:1, and R_f 0.11 (AcOEt–hexane, 3:1), and 0.33 (petrol–Me_2CO, 3:2). Compound **21**; R_f 0.25 ($CHCl_3$–MeOH–H_2O, 7:3:0.5), R_f 0.30 (benzene Me_2CO–MeOH, 2:2:1).

Atratoside-D (**4**). An amorphous powder, (mp 92–94°), $[\alpha]_D$ −52.3° (*c* 0.8; MeOH). Found: C, 62.50; H, 8.21. $C_{40}H_{60}O_{13}\cdot H_2O$ requires: C, 62.66; H, 8.09. FDMS *m/z*: 748 $[M]^+$, 604 $[M-144]^+$ 474 $[604-130]^+$ IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1710, 1520, 1470, 1420, 1380, 1220, 1050, 930. UV λ_{max}^{MeOH} nm (ε): 217 nm (5000), ^{1}H NMR: δ1.13 (3H, *s*, 19-Me), 1.23–1.30 (9H, *m*,



6′-, 6″, 6‴-Me), 2.17 (3H, *s*, 21-Me), 3.41 and 3.46 (each 3H, *s*, -OMe), 4.69 and 4.89 (each 1H, *dd*, $J = 10$, 2 Hz, 1′-and or 1″-CH), 5.03 (1H, *br d*, $J = 2$ Hz, 1‴-CH), 5.46 (1H, *br d*, $J = 4$ Hz, 6-CH), 6.20 (1H, *d*, $J = 2$ Hz, 16-CH), 7.30 (1H, *d*, $J = 2$ Hz, 15-CH). ^{13}C NMR: see Table 1.

Acidic hydrolysis of **4**. To a soln of **4** (90 mg) in 15 ml MeOH was added 30 ml of 0.1 N H_2SO_4. The mixture was kept at 50° for 50 min, then diluted with H_2O (30 ml) and MeOH removed under red. pres. to about the volume of 60 ml. The soln was kept 60° for further 30 min, then neutralized with aq. satd $Ba(OH)_2$ and the ppt. filtered off. The filtrate was concd to dryness and chromatographed on a column of silica gel with the solvent 1% MeOH–$CHCl_3$ to give 15.3 mg of cynajapogenin A (**7**), (mp 67–73°), $[\alpha]_D$ −42.5 ($CHCl_3$; *c* 0.5), 5.2 mg of **17**, $[\alpha]_D$ +50.8° (H_2O; *c* 0.5), 2.1 mg of **19**, $[\alpha]_D$ −8.8° (MeOH; *c* 0.2), and 2.2 mg of **20**, $[\alpha]_D$ +45.5° (H_2O; *c* 0.2).

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