New Acetylcholinesterase-Inhibitory Pregnane Glycosides of *Cynanchum atratum* Roots

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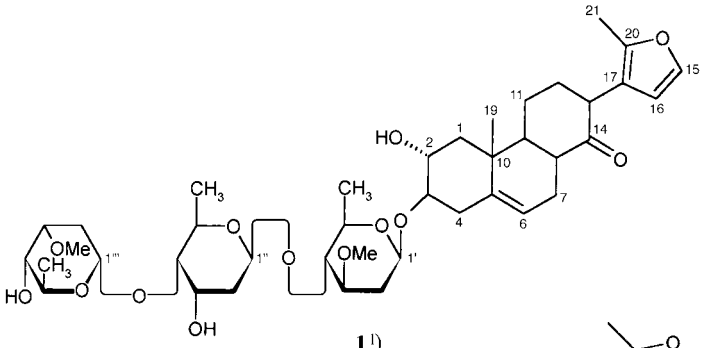
Three new pregnane glycosides, cynatroside A (**1**), cynatroside B (**2**), and cynatroside C (**3**), isolated from the roots of *Cynanchum atratum* (Asclepiadaceae), were characterized as 7*β*-{[*O*-*α*-L-cymaropyranosyl-(1 → 4)-*O*-*β*-D-digitoxopyranosyl-(1 → 4)-*β*-D-oleandropyranosyl]oxy}-3,4,4a,4b,5,6,7,8,10,10a-decahydro-6*α*-hydroxy-4b-methyl-2-(2-methyl-3-furyl)phenanthren-1(2*H*)-one (**1**), 7*β*-{[*O*-*β*-D-cymaropyranosyl-(1 → 4)-*O*-*α*-L-diginopyranosyl-(1 → 4)-*β*-D-cymaropyranosyl]oxy}-3,4,4a,4b,5,6,7,8,10,10a-decahydro-2,6*α*-dihydroxy-4b-methyl-2-(2-methyl-3-furyl)phenanthren-1(2*H*)-one (**2**), and 7*β*-{[*O*-*α*-L-cymaropyranosyl-(1 → 4)-*O*-*β*-D-digitoxopyranosyl-(1 → 4)-*β*-L-cymaropyranosyl]oxy}-3,4,4a,4b,5,6,7,8,10,10a-decahydro-2,6*α*-dihydroxy-4b-methyl-2-(2-methyl-3-furyl)phenanthren-1(2*H*)-one (**3**), respectively. In addition, ten known constituents were identified, *i.e.*, cynascyroside D (**4**), glaucoside C (**5**), glaucoside D (**6**), atratoside A (**7**), 2,4-dihydroxyacetophenone (**8**), 4-hydroxyacetophenone (**9**), syringic acid (**10**), azelaic acid (**11**), suberic acid (**12**), and succinic acid (**13**). Among these compounds, **1**–**4** significantly inhibit acetylcholinesterase activity.

Introduction. – *Alzheimer*'s disease (AD) is a neurodegenerative process associated with the deposition of amyloid plaques and fibrillary tangles as well as neurotransmitter alterations in the brain [1]. One of the main functional deficits in AD involves cholinergic neurons, which provide the rationale for a potential therapeutic approach to the treatment of AD [2]. Several therapeutic strategies have been explored to enhance cholinergic neurotransmission to alleviate some of the symptoms of AD. These include the use of acetylcholinesterase (AChE) inhibitors, the administration of acetylcholine precursors, acetylcholine releasers, and direct agonists for both muscarinic and/or nicotinic receptors [3]. Among these strategies, inhibition of AChE has proven to be the most successful means of balancing the cholinergic deficit and stabilizing the symptoms [4].

As part of our continuing research for AChE-inhibitory compounds from natural resources [5–7], we recently found that 80%-MeOH extract of the roots of *Cynanchum atratum* BUNGE (Asclepiadaceae) significantly inhibited AChE activity. This plant has been used as an antifebrile and diuretic in Korea and China [8]. Previous chemical studies of *C. atratum* revealed the presence of C_{21}-substituted pregnane glycosides such as glaucoside C and H, cynatratosids A–F, atratosides A–C, cynajapogenin A, atratoside D, and acetophenones such as acetovanillone, 3,4-dihydroxyacetophenone, and 4-hydroxyacetophenone [8–11]. However, to date, few reports on the biological activity of this plant are available. In the present study, we have attempted to isolate AChE-inhibitory components of *C. atratum* by subsequent bioactivity-guided fractionations. As a result, we have isolated the three new pregnane glycosides **1**–**3**, along with the ten known compounds **4**–**13**, from the 80%-MeOH extract of the roots of *C. atratum* (see *Figs. 1* and *2*).



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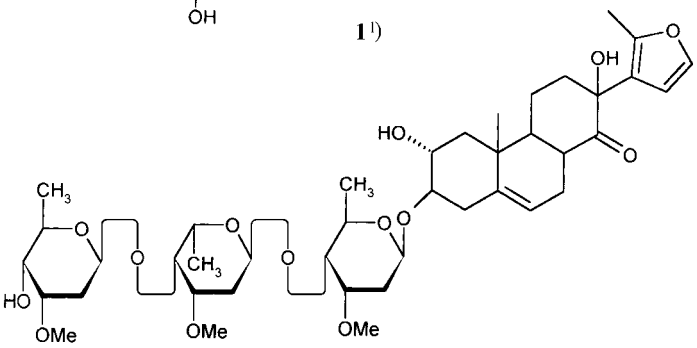

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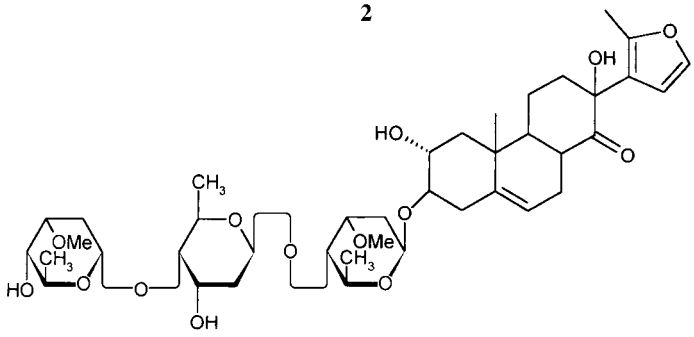

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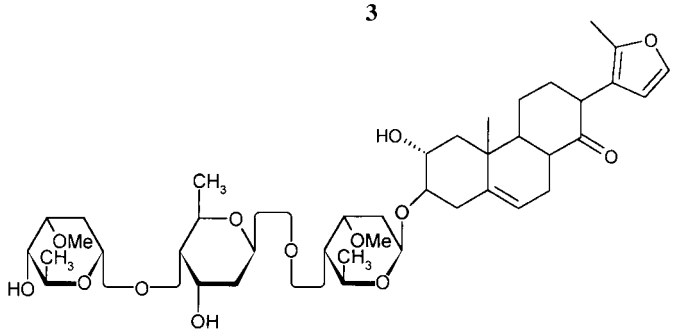

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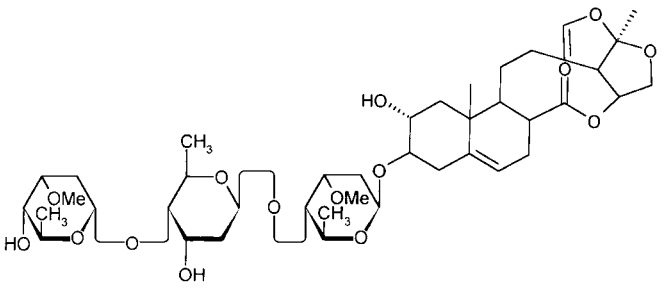
Fig. 1. *Structures of compounds* **1**–**4**



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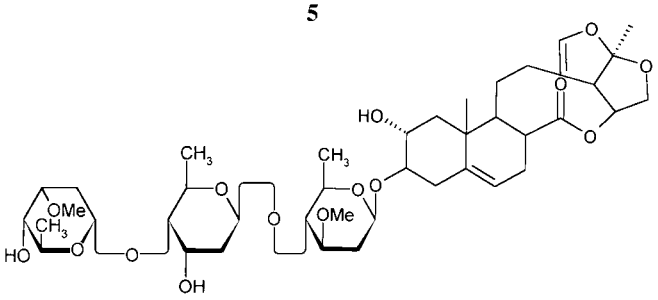

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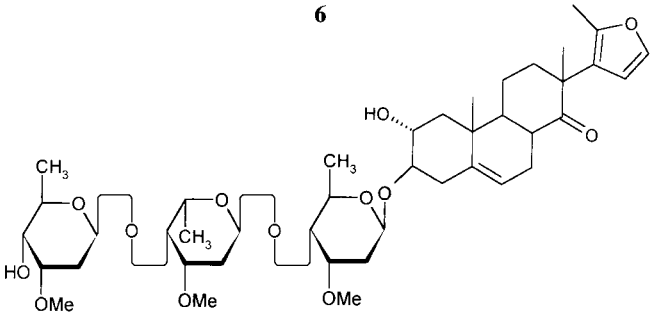

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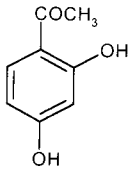

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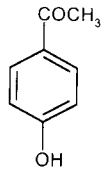

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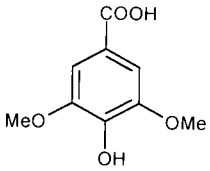

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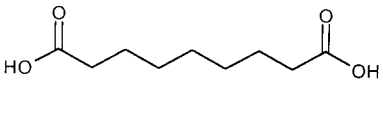

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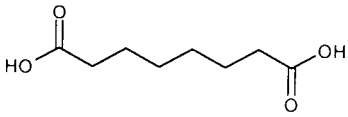

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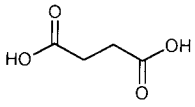

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Fig. 2. *Structures of compounds* **5**–**13**

Results and Discussion. – The 80%-MeOH extract of the roots of *C. atratum* was found to exhibit a significant inhibitory activity against AChE. To isolate AChE-inhibitory constituents of *C. atratum*, the 80%-MeOH extract was suspended in H_2O and then subsequently partitioned with hexane, AcOEt, and BuOH. The AChE-inhibitory activities of these fractions were evaluated; the most prominent activity was found in the AcOEt fraction. By means of several chromatographic techniques, three new pregnane glycosides **1**–**3**, which we call cynatrosides A–C, and ten known compounds **4**–**13** were isolated from the AcOEt fraction. Compounds **1**–**7** were positive in *Liebermann-Burchard* and *Keller-Kiliani* reactions, suggesting the presence of a pregn-5-ene moiety and 2-deoxy sugar residues in their structure.

Cynatroside A (**1**) was obtained as a white powder. The positive-ion HR-FAB-MS showed the $[M+H]^+$ at *m/z* 749.4104 ($C_{40}H_{61}O_{13}^+$; calc. 749.4112). The fragment ions at *m/z* 749 ($[M+H]^+$), 605 ($[M+H-144]^+$), 475 ($[M+H-144-130]^+$), and 331 ($[M+H-144-130-144]^+$) indicated the loss of three 2-deoxy sugars. The IR absorptions at 3444 and 1716 cm^{-1} suggested the presence of OH and keto groups in **1**. The ^{1}H- and ^{13}C-NMR (cf. *Tables 1* and *2*), COSY, and HMBC data of cynatroside A established the structure 7β-{[*O*-α-L-cymaropyranosyl-(1 → 4)-*O*-β-D-digitoxopyranosyl-(1 → 4)-β-D-oleandropyranosyl]oxy}-3,4,4a,4b,5,6,7,8,10,10a-decahydro-6α-hydroxy-4b-methyl-2-(2-methyl-3-furyl)phenanthren-1(2*H*)-one (**1**).

Table 1. *^{13}C-NMR Chemical Shifts of the Aglycone Moieties of Compounds* **1**–**3** (100 MHz)[1])

	1 ($CDCl_3$)	**2** (C_5D_5N)	**3** ($CDCl_3$)
C(1)	44.3	45.1	44.3
C(2)	69.7	69.3	69.6
C(3)	86.5	84.8	86.3
C(4)	37.4	37.2	37.4
C(5)	137.9	138.6	138.3
C(6)	121.5	121.2	120.6
C(7)	25.3	25.5	25.0
C(8)	51.8	52.1	52.4
C(9)	45.4	42.7	42.0
C(10)	38.5	39.5	39.3
C(11)	25.2	22.1	21.8
C(12)	33.6	38.5	38.5
C(13)	47.9	76.0	75.3
C(14)	209.3	212.8	213.5
C(15)	139.9	140.6	140.5
C(16)	110.7	110.2	109.4
C(17)	116.4	120.9	119.4
C(18)	–	–	–
C(19)	19.8	19.5	19.8
C(20)	147.9	Overlap	149.3
C(21)	11.8	12.5	12.6

The ^{1}H-NMR spectrum of **1** showed a characteristic cynajapogenin A type pattern due to two tertiary Me signals at δ 1.14 (*s*, Me(19)) and 2.19 (*s*, Me(21)) and two aromatic protons of a furan moiety at δ 6.22 and 7.28 (each *d*, $J = 2$ Hz, H–C(16) and H–C(15))[1]. In addition, three secondary Me-groups at δ 1.26–1.35, two MeO groups at δ 3.43, and three anomeric protons at δ 4.53 (br. *d*, $J = 8.5$ Hz, H–C(1″)), 4.93 (*d*, $J = 3.7$ Hz,

[1]) Steroid numbering.

Table 2. *^{13}C-NMR Chemical Shifts of the Sugar Moieties of Compounds* **1**–**3** (100 MHz)[a])

	1 ($CDCl_3$)	**2** (C_5D_5N)	**3** ($CDCl_3$)
	D-Ole	D-Cym	L-Cym
C(1′)	98.5	97.9	97.4
C(2′)	36.4	34.3	35.5
C(3′)	78.9	77.0	76.8
C(4′)	82.1	81.7	82.1
C(5′)	71.4	69.3	69.0
C(6′)	18.1	18.1	17.8
MeO	56.8	57.1	58.2
	D-Dig	L-Dgn	D-Dig
C(1″)	99.8	101.0	99.5
C(2″)	36.9	31.6	36.7
C(3″)	68.7	72.2	68.2
C(4″)	79.3	74.2	79.2
C(5″)	67.5	67.0	67.4
C(6″)	18.0	17.5	17.8
MeO	–	55.7	–
	L-Cym	D-Cym	L-Cym
C(1‴)	97.4	99.1	98.2
C(2‴)	30.9	33.0	30.9
C(3‴)	75.0	77.6	75.0
C(4‴)	71.9	73.9	71.9
C(5‴)	65.8	71.1	65.9
C(6‴)	17.8	18.0	18.1
MeO	56.3	57.2	56.3

[a]) D-Ole = D-oleandropyranosyl, D- and L-Cym = D- and L-cymaropyranosyl, D-Dig = D-digitoxopyranosyl, D-Dgn = D-diginopyranosyl.

H–C(1‴), and 5.02 (br. *d*, *J* = 8.5 Hz, H–C(1′)) were observed for its sugar moiety. The splitting patterns of the anomeric-proton signals indicated that **1** had three sugar units with one α-linkage and two β-linkages. Upon acidic hydrolysis, this glycoside liberated L-cymarose (=2,6-dideoxy-3-*O*-methyl-L-*ribo*-hexose), D-digitoxose (=2,6-dideoxy-D-*ribo*-hexose), and D-oleandrose (=2,6-dideoxy-3-*O*-methyl-D-*arabino*-hexose) as sugar components, which were identified by comparison with authentic samples.

The ^{13}C-NMR spectrum of **1** showed a total of 40 ^{13}C-resonances, of which 20 C-atoms belong to the aglycone and 20 C-atoms to the oligosaccharide moiety. The 20 ^{13}C-resonances were identified as arising from the aglycone cynajapogenin A, a 14,15-seco-18-norpregnane isolated from *C. atratum*, by comparison of its physical and spectroscopic data [10][12–13]. Moreover, glycosidation shifts [14] of δ(C) were observed for C(2) (−2.8 ppm), C(3) (+10.0 ppm), and C(4) (−3.2 ppm) of the aglycone moiety of **1**, suggesting that the sugar moiety was linked at C(3) of the aglycone. The ^{1}H- and ^{13}C-NMR spectra of **1** were compatible with the three sugar components α-L-cymaropyranose, β-D-digitoxopyranose, and β-D-oleandropyranose, which were also supported by the ^{1}H,^{13}C COSY data. The sugar sequence was the same as that of glaucoside D (**6**), but the aglycone was different [15].

Cynatroside B (**2**) was obtained as white powder. The positive-ion HR-FAB-MS showed the $[M+H]^+$ at *m*/*z* 779.4181 ($C_{14}H_{63}O_{14}^+$; calc. 779.4218). The fragmentation pattern at *m*/*z* 780 ($[M+H]^+$), 762 ($[M+H-H_2O]^+$), 618 ($[M+H-H_2O-144]^+$), 474 ($[M+H-H_2O-2(144)]^+$), and 330 ($[M+H-H_2O-3(144)]^+$) indicated the loss of three 2-deoxy sugars. The IR absorptions at 3445 and 1715 cm^{-1} suggested the presence of OH and keto groups in **2**. The ^{1}H- and ^{13}C-NMR (see *Tables 1* and *2*), COSY, and HMBC data of cynatroside B established the structure 7β-{[*O*-β-D-cymaropyranosyl-(1 → 4)-*O*-α-L-diginopyranosyl-(1 → 4)-β-D-cymaropyranosyl]-

oxy}-3,4,4a,4b,5,6,7,8,10,10a-decahydro-2,6α-dihydroxy-4b-methyl-2-(2-methyl-3-furyl)-phenanthren-1(2*H*)-one (**2**).

The ^{1}H-NMR spectrum of **2** showed the characteristic 14,15-seco-18-norpregnane-type pattern due to two tertiary Me at δ 0.88 (*s*, Me(19)) and 2.06 (*s*, Me(21)) and two aromatic protons of a furan moiety at δ 6.36 and 7.25 (each *d*, $J = 2.0$ Hz, H–C(16) and H–C(15))[1]. In addition, three secondary Me groups at δ 1.20–1.28 and three MeO groups at δ 3.38, 3.39, and 3.40 (each *s*, MeO–C(3′), MeO–C(3″), MeO–C(3‴)) were observed for its sugar moiety. The ^{1}H-NMR signals, due to the anomeric protons at δ 4.67 (br. *d*, $J = 8.2$ Hz, H–C(1‴)), 4.74 (br. *d*, $J = 8.0$ Hz, H–C(1′)), and 4.95 (*d*, $J = 3.2$ Hz, H–C(1″)), indicated that one α and two β sugar linkages were present. Also, acidic hydrolysis of **2** afforded an aglycone **2a** and the two sugar components D-cymarose and L-diginose (=2,6-dideoxy-3-*O*-methyl-L-*lyxo*-hexose), as established by comparison with authentic samples.

The ^{13}C-NMR spectrum of **2** showed a total of 41 ^{13}C-resonances, of which 20 C-atoms belong to the aglycone **2a** and 21 C-atoms to the oligosaccharide moiety. The ^{13}C-NMR signals of isolated **2a** at δ 72.1, 76.0, and 76.2 (see *Table 3*) suggested the presence of one more OH group than in the aglycone cynajapogenin A. The position of this additional OH group was established by the HMBC spectrum where H_α–C(12) and H_β–C(12) showed long-range heteronuclear interactions with C(13) and C(17) (*Fig. 3*). Therefore, the structure of **2a** was deduced to be 15,20-epoxy-2α,3β,13β-trihydroxy-14,15-seco-18-norpregna-5,15(16),17(20)-trien-14-one (=13-hydroxycynajapogenin A). Since glycosidation shifts were observed for δ(C) of C(2) (−2.8 ppm), C(3) (+8.6 ppm), and C(4) (−3.0 ppm) of the aglycone moiety of **2**, the sugar moiety was most likely linked to C(3) of the aglycone. The ^{1}H- and ^{13}C-NMR spectra of **2** suggested that the three sugar components were β-D-cymaropyranose, α-L-diginopyranose, and β-D-cymaropyranose, as also supported by the ^{1}H,^{13}C COSY data. The sugar sequence was the same as that of atratoside A (**7**), but the aglycone (see **2a**) was different [10].

The ^{1}H- and ^{13}C-NMR spectra (see *Tables 1* and *2*) of cynatorside C (**3**) closely resembled that of cynatroside A (**2**). The difference between **3** and **2** concerned only the sugar moiety. Thus, the structure of **3** could be assigned as 7 β-{[*O*-α-L-

Table 3. *^{13}C-NMR* (125 MHz) *and ^{1}H-NMR* (500 MHz) *Data of 13-Hydroxycynajapogenin A* (**2a**) *in (D_5) Pyridine from 1D- and 2D-NMR Experiments.* δ in ppm, J in Hz

	δ (C)	δ (H)
CH_2(1)	45.9	2.37 (*dd*, $J = 12.7$, 4.4, H_β), 1.43 (*t*, $J = 12.2$, H_α)
CH(2)	72.1	4.12 (*ddd*, $J = 13.0$, 8.8, 4.4)
CH(3)	76.2	3.81 (*dd*, $J = 17.0$, 8.5)
CH_2(4)	40.2	2.63 (*d*, $J = 7.4$)
C(5)	139.6	
CH(6)	120.2	5.41 (*d*, $J = 4.5$)
CH_2(7)	25.5	2.68 (*m*), 2.21 (*m*)
CH(8)	52.3	1.62 (*td*, $J = 11.6$, 4.8)
CH(9)	42.8	2.73 (*td*, $J = 11.1$, 4.8)
C(10)	39.2	
CH_2(11)	22.2	1.82 (*m*)
CH_2(12)	39.6	2.89 (*dt*, $J = 13.4$, 3.1, H_β), 1.99 (*td*, $J = 13.4$, 4.1, H_α)
C(13)	76.0	
C(14)	212.9	
CH(15)	140.6	7.51 (*d*, $J = 1.9$)
CH(16)	110.2	6.67 (*d*, $J = 1.9$)
C(17)	121.2	
C(18)	–	
Me(19)	19.8	0.91 (*s*)
C(20)	149.2	
Me(21)	12.5	2.16 (*s*)

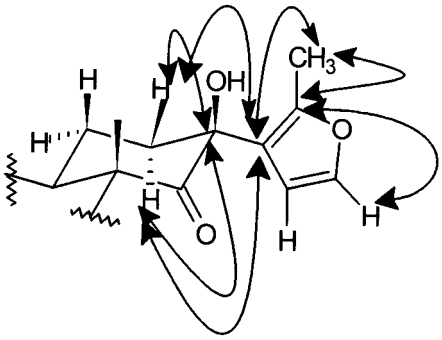

Fig. 3. *Selected HMBC interactions in compound* **2a**

cymaropyranosyl-(1 → 4)-*O*-*β*-D-digitoxopyranosyl-(1 → 4)-*β*-L-cymaropyranosyl]oxy}-3,4,4a,4b,5,6,7,8,10,10a-decahydro-2,6*α*-dihydroxy-4b-methyl-2-(2-methyl-3-furyl)phenanthren-1(2*H*)-one.

Cynatroside C (**3**) showed three anomeric-proton signals at δ 4.71 (br. d, $J = 8.0$ Hz, H–C(1′)), 4.82 (br. d, $J = 8.0$ Hz, H–C(1″)), and 4.88 (d, $J = 4.0$ Hz, H–C(1‴)) and three anomeric C-atom signals at δ 97.4, 98.2, and 99.5 in its ^{1}H- and ^{13}C-NMR spectra, respectively. Further information about the sugar sequence was given by the fragmentation pattern in the FAB-MS of **3** with peaks at *m*/*z* 765 ($[M+H]^+$), 747 ($[M+H-H_2O]^+$), 603 ($[M+H-H_2O-144]^+$), 473 ($[M+H-H_2O-144-130]^+$), and 329 ($[M+H-H_2O-144-130-144]^+$). The sugar sequence was also the same as that of cynascyroside D (**4**), but the aglycone was different [13].

The MS, IR, and ^{1}H- and ^{13}C-NMR spectra of **4**–**13** were identical to those reported previously for cynascyroside D (**4**) [13], glaucoside C (**5**) [8][15], glaucoside D (**6**) [15], atratoside A (**7**) [10], 2,4-dihydroxyacetophenone (**8**) [16], 4-hydroxyacetophenone (**9**) [16], syringic acid (**10**) [17], azelaic acid (**11**) [18], suberic acid (**12**) [19], and succinic acid (**13**) [20], respectively. Compounds **4**, **6**, **8**, and **10**–**13** were reported in the present study for the first time from *C. atratum*.

The isolated thirteen compounds were tested for inhibitory activity against AChE (*Table 4*). The inhibition degree of pregnane glycosides against AChE differed

Table 4. IC_{50} *Value of AChE-Inhibitory Pregnane Glycosides Isolated from* C. atratum

Compound	IC_{50} [μM]
1	6.4
2	3.6
3	52.3
4	152.9
5	> 300
6	246.8
7	> 300
8	> 300
9	> 300
10	> 300
11	> 300
12	> 300
13	> 300
velnacrine	0.4

depending on the aglycone type of the pregnane glycosides and the sequences of carbohydrate moieties. Among the thirteen compounds, **1** and **2** showed the most-potent inhibitory activity against AChE. Compounds **3** and **4** were also found to be active in the inhibition of AChE. However, compounds **5** and **6** with a 13,14:14,15-disecopregnane-type aglycone showed no inhibitory activity against AChE. The mechanism of compound **2** responsible for the inhibitory activity against AChE *in vitro*, and whether the compound has an anti-amnestic activity *in vivo*, is now being studied in our laboratory.

Experimental Part

General. Column chromatography (CC): silica gel *60* (*Merck*, 0.040–0.063 mm). High-pressure liquid chromatography (HPLC): *Hitachi L-6200* equipped with a UV/VIS detector; *YMC*-pack *C8* column. TLC: precoated silica gel *60* F_{254} (*Merck*); detection at 254 and 356 nm and by spraying with anisaldehyde/sulfuric acid reagent. UV Spectra: *Shimadzu-UV-1601PC* spectrophotometer. IR Spectra: *Perkin-Elmer-1710* spectrophotometer; in cm^{-1}. ^1H- and ^{13}C-NMR, COSY, and HMBC Spectra: *Jeol-GSX-400* spectrometers; chemical shift δ in ppm rel. to $SiMe_4$ as internal standard and coupling constants *J* in Hz. EI-MS: *VG-Trio-II* spectrometer. FAB-MS: glycerol matrix; *Jeol-JMS-AX-505-WA* spectrometer.

Enzyme Assay. AChE Activity was determined by the modified method of *Ellman* and co-workers [5–7][21]. AChE was diluted in 0.1M phosphate buffer (pH 8.0) at 4.3 units/ml. The color reagent DTNB (=5,5′-dithiobis[2-nitrobenzoic acid]), sample, and authentic AChE were added to a spectrophotometric cuvette and preincubated for 5 min at 25°. Acetylthiocholine iodide was added as a substrate and the incubation continued for 3 min. AChE activity was terminated by the addition of 100 μM neostigmine. Finally, the absorbance at 412 nm was measured.

Plant Material. The roots of *C. atratum* were purchased from Kyungdong Oriental Herbal Market, Seoul, Korea, and identified by Dr. Dae S. Han, an emeritus professor of the College of Pharmacy, Seoul National University. A voucher specimen (SNUPH-00929) was deposited in the Herbarium of the Medicinal Plant Garden, College of Pharmacy, Seoul National University.

Extraction and Isolation. The dried roots of *C. atratum* (7.5 kg) were cut into pieces and extracted with 80% MeOH (3×) in an ultrasonic apparatus. Upon evaporation, the MeOH extract yielded 1.5 kg. This extract was then suspended in dist. H_2O and subsequently partitioned with hexane and AcOEt. The AcOEt fraction (290 g) was fractionated by extensive CC (silica gel, hexane/AcOEt mixture of increasing polarity) to yield fifteen major fractions (*Fr. 1–15*). *Fr. 11* was resubmitted to CC (silica gel, AcOEt/hexane): twelve subfractions (*Fr. 11-1* to *11-12*). Among these subfractions, *Fr. 11-6* was submitted to CC (silica gel, hexane/AcOEt/MeOH 50:50:3): sixteen subfractions (*Fr. 11-6-1* to *11-6-16*). *Fr. 11-6-6* was submitted to CC *RP-18*, H_2O/MeOH: fourteen subfractions (*Fr. 11-6-6-1* to *11-6-6-14*). Compounds **2** and **7** were isolated from *Fr. 11-6-6-10* by semiprep. HPLC (RP_8 AcCN/MeOH/H_2O 50:5:45). Compound **5** was isolated from *Fr. 11-6-6-13* by semiprep. HPLC (RP_8, AcCN/MeOH/H_2O 49:5:46). *Fr. 11-7* was submitted to CC (silica gel, hexane/AcOEt/MeOH 50:50:3): twelve subfractions (*Fr. 11-7-1* to *11-7-12*). *Fr. 11-7-2* was submitted to CC (silica gel, hexane/AcOEt/MeOH 50:50:3): 18 fractions (*Fr. 11-7-2-1* to *11-7-2-18*). Compounds **1** and **4** were isolated from *Fr. 11-7-2-13* by Demiprep. HPLC (RP_8, AcCN/MeOH/H_2O 44:4:52). Compounds **3** and **6** were isolated from *Fr. 11-7-2-18* by semiprep. HPLC (RP_8, AcCN/MeOH/H_2O 45:5:50). Compounds **8**, **9**, and **11** were isolated from *Fr. 6*, *Fr. 7*, and *Fr. 8*, resp., by crystallization from hexane/AcOEt 1:1. CC (silica gel, gradient of MeOH in $CHCl_3$) of *Fr. 9* yielded fifteen subfractions (*Fr. 9-1* to *9-15*). Compounds **10** and **12** were isolated from *Fr. 9-7* and *Fr. 9-12*, resp., by crystallization from hexane/AcOEt 5:1. *Fr. 10* was submitted to CC (silica gel, hexane/AcOEt/MeOH 50:50:3): sixteen fractions (*Fr. 10-1* to *10-16*). Compound **13** was isolated from *Fr. 10-8* by crystallization from hexane/AcOEt 2:1.

Cynatroside A (=*(6*R,7R*)-7-{[*O*-*2,6-Dideoxy-3*-O-*methyl*-α-L-ribo-*hexopyranosyl*-*(1*→*4)*-O-*2,6-dideoxy*-β-D-ribo-*hexopyranosyl*-*(1*→*4)*-*2,6-dideoxy-3*-O-*methyl*-β-D-arabino-*hexopyranosyl]oxy}-3,4,4a,4b,5,6,7,8,10,10a-decahydro-6-hydroxy-4b-methyl-2-(2-methyl-3-furyl)phenanthren-1(2*H*)-one*; **1**): $[\alpha]_D^{20} = -9.1$ ($c = 0.26$, $CHCl_3$). IR (KBr): 3444, 2932, 1716, 1653, 1059, 750. ^1H-NMR (400 MHz, $CDCl_3$)[1]): 1.14 (*s*, Me(19)); 1.26–1.35 (*m*, Me(6′), Me(6″), Me(6‴)); 2.19 (*s*, Me(21)); 3.43 (*s*, 2 MeO); 4.53, 5.02 (2 br. *d*, *J* = 8.5, H–C(1′), H–C(1″)); 4.93 (*d*, *J* = 3.7, H–C(1‴)); 6.22 (*d*, *J* = 1.5, H–C(16)); 7.28 (*d*, *J* = 1.5, H–C(15)). ^{13}C-NMR

(100 MHz, $CDCl_3$): *Tables 1* and *2*. FAB-MS (pos.): 771 ($[M+Na]^+$), 749 ($[M+H]^+$), 605 ($[M+H-144]^+$), 475 ($[M+H-144-130]^+$), 331 ($[M+H-144-130-144]^+$). HR-FAB-MS (pos.): 749.4104 ($[M+H]^+$, $C_{40}H_{61}O_{13}^+$; calc. 749.4112).

Cynatroside B (=*(6*R,*7*R*)-7-{[*O*-*2,6-Dideoxy-3*-O-*methyl-β*-D-ribo-*hexopyranosyl-(1 → 4)*-O-*2,6-dideoxy-3*-O-*methyl-α*-L-lyxo-*hexopyranosyl-(1 → 4)-2,6-dideoxy-3*-O-*methyl-β*-D-ribo-*hexopyranosyl]oxy}-3,4,4a,4b,5,6,7,8,10,10a-decahydro-2,6-dihydroxy-4b-methyl-2-(2-methyl-3-furyl)-phenanthren-1(2*H*)-one*; **2**): $[\alpha]_D^{20} = -47.7$ ($c = 1.00$, $CHCl_3$). IR (KBr): 3445, 2933, 1715, 1653, 1058, 752. ^{1}H-NMR (300 MHz, $CDCl_3$)[1]): 0.88 (*s*, Me(19)); 1.20–1.28 (*m*, Me(6′), Me(6″), Me(6‴)); 2.06 (*s*, Me(21)); 3.38, 3.39, 3.40 (3*s*, MeO–C(3′), MeO–C(3″), MeO–C(3‴)); 4.67 (br. *d*, *J* = 8.2, H–C(1‴)); 4.74 (br. *d*, *J* = 8.0, H–C(1′)); 4.95 (*d*, *J* = 3.2, H–C(1″)); 5.39 (br. *d*, H–C(6)); 6.36 (*d*, *J* = 2.0, H–C(16)); 7.25 (*d*, *J* = 2.0, H–C(15)). ^{13}C-NMR (75 MHz, $CDCl_3$): *Tables 1* and *2*. FAB-MS (pos.): 802 ($[M+H+Na]^+$), 779 ($[M+H]^+$), 761 ($[M+H-H_2O]^+$), 617 ($[M+H-H_2O-144]^+$), 473 ($[M+H-H_2O-2(144)]^+$), 329 ($[M+H-H_2O-3(144)]^+$). HR-FAB-MS (pos.): 779.4181 ($[M+H]^+$, $C_{41}H_{63}O_{14}^+$; calc. 779.4218).

13-Hydroxycynajapogenin A (=*(6*R,*7*R*)-3,4,4a,4b,5,6,7,8,10,10a-Decahydro-2,6-7-trihydroxy-4b-methyl-2-(2-methyl-3-furyl)-phenanthren-1(2*H*)-one*; **2a**): ^{1}H-NMR (300 MHz, (D_5)pyridine): *Table 3*. ^{13}C-NMR (125 MHz, (D_5)pyridine): *Table 3*. FAB-MS (pos.): 369 ($[M+Na]^+$), 347 ($[M+H]^+$), 329 ($[M+H-H_2O]^+$).

Cynatroside C (=*6*R,*7*R*)-7-{[*O*-*2,6-Dideoxy-3*-O-*methyl-α*-L-ribo-*hexopyranosyl-(1 → 4)*-O-*2,6-dideoxy-β*-D-ribo-*hexafuranosyl-(1 → 4)-2,6-dideoxy-3*-O-*methyl-β*-L-ribo-*hexopyranosyl]oxy}-3,4,4a,4b,5,6,7,8,10,10a-decahydro-2,6-dihydroxy-4b-methyl-2-(2-methyl-furan-3-yl)-phenanthren-1(2*H*)-one*; **3**): $[\alpha]_D^{20} = -40.2$ ($c = 0.60$, $CHCl_3$). IR (KBr): 3444, 2932, 1715, 1653, 1056, 754. ^{1}H-NMR (300 MHz, $CDCl_3$)[1]): 0.88 (*s*, Me(19)); 1.18–1.24 (*m*, Me(6′), Me(6″), Me(6‴)); 2.06 (*s*, Me(21)); 3.38, 3.43 (*s*, 2 MeO), 4.71 (br. *d*, *J* = 8.0, H–C(1‴)); 4.82 (br. *d*, *J* = 8.0, H–C(1′)); 4.88 (*d*, *J* = 4.0, H–C(″)); 5.39 (br. *d*, H–C(6)); 6.36 (*d*, *J* = 1.7, H–C(16)); 7.25 (*d*, *J* = 1.7, H–C(15)). ^{13}C-NMR (75 MHz, $CDCl_3$): *Tables 1* and *2*. FAB-MS (pos.): 765 ($[M+H]^+$), 747 ($[M+H-H_2O]^+$), 603 ($[M+H-H_2O-144]^+$), 473 ($[M+H-H_2O-144-130]^+$), 329 ($[M+H-H_2O-144-130-144]^+$). HR-FAB-MS (pos.): 765.4048 ($[M+H]^+$, $C_{40}H_{61}O_{14}^+$; calc. 765.4061).

Acid Hydrolysis of Cynatroside A (**1**), *B* (**2**), *and C* (**3**). To a soln. of each compound (10 mg) in MeOH (5 ml) was added 0.1N H_2SO_4 (10 ml). Each mixture was kept at 50° for 50 min and then diluted with H_2O (10 ml). After concentration to 20 ml, the mixture was kept for 60° for further 30 min and then neutralized with aq. sat. $Ba(OH)_2$ soln. The precipitate was filtered off. The filtrate was evaporated and the residue submitted to CC (silica gel, hexane/AcOEt/MeOH 50 : 50 : 3). Thus, cynajapogenin A [15] was obtained from **1**, and **2a** from **2** and **3**. The sugar components from each hydrolysate were identified by TLC comparison with authentic samples.

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