New Secondary Metabolites from *Allium victorialis*

by **Sadia Khan**[a]), **Itrat Fatima**[b]), **Mehdi Hussain Kazmi**[a]), and **Abdul Malik***[b])

[a]) Department of Applied Chemistry, University of Karachi, Karachi-75270, Pakistan
[b]) International Centre for Chemical and Biological Sciences, HEJ Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan (phone: +92-21-4824926; e-mail: abdul.malik@iccs.edu)

Allumines A and B (**1** and **2**, resp.), two new steroidal alkaloids, and a new cyclopentene derivative, **3**, were isolated from the $CHCl_3$-soluble fraction of the whole plant of *Allium victorialis*. Their structures were elucidated by spectroscopic techniques, including 1D- and 2D-NMR spectroscopy.

Introduction. – The plant family Alliaceae comprises of 21 genera. The largest genus of this family is *Allium* with *ca.* 600 species distributed in Asia, Europe, and North West America. In Pakistan, 41 species of this genus have so far been discovered [1]. Various *Allium* species are used for the treatment of a variety of ailments [2–5]. One of the species is *Allium victorialis*, a shrub occurring in Europe, temperate Asia to Japan, and North West America. It grows in northern mountainous regions of Pakistan [1]. It is used by local population as antithrombotic [6] and antiscorbutic agent [1], and to treat profuse menstruation and cold. Previously, a cyclopentane derivative has been reported by us from this plant [7]. The chemotaxonomic and ethnopharmacological importance of the genus *Allium* prompted us to carry out further phytochemical studies on *A. victorialis*. As a result, we herein report two new steroidal alkaloids, named allumines A and B (**1** and **2**, resp.), along with a new cyclopent-1-enecarboxylate **3**.

Results and Discussion. – The EtOH extract of the whole plant was partitioned into fractions soluble in hexane, $CHCl_3$, AcOEt, BuOH, and H_2O. Column chromatography of the $CHCl_3$-soluble fraction provided compounds **1**–**3** as described in the *Exper. Part* (*Fig. 1*).

Allumine A (**1**) was obtained as a colorless gummy solid. The molecular formula $C_{36}H_{53}NO_3$ was deduced from HR-EI-MS with M^+ peak at m/z 547.4025. The IR spectrum exhibited the absorption bands of OH/NH (3450–3330 cm^{-1}) and ester C=O groups (1700 cm^{-1}), C=C bond (1660 cm^{-1}), and aromatic moiety (1602, 1540, and 1500 cm^{-1}). In the EI-MS, the fragment-ion peak at m/z 398 resulted from the loss of a hydroxy-dimethyl-benzoyl moiety. The base peak at m/z 98 originated from a bond cleavage between C(20) and C(22) of 20,26-epiminocholestane [8], and a further intense peak at m/z 126 was due to bond cleavage between C(17) and C(20). These data indicated that the compound **1** was a dihydroverazine-type alkaloid [9]. The ^{13}C-NMR and DEPT spectra (*Table 1*) showed 36 signals for six Me, eleven CH_2, and eleven CH groups, and eight quaternary C-atoms. The ester C=O resonated at δ(C) 170.0, while

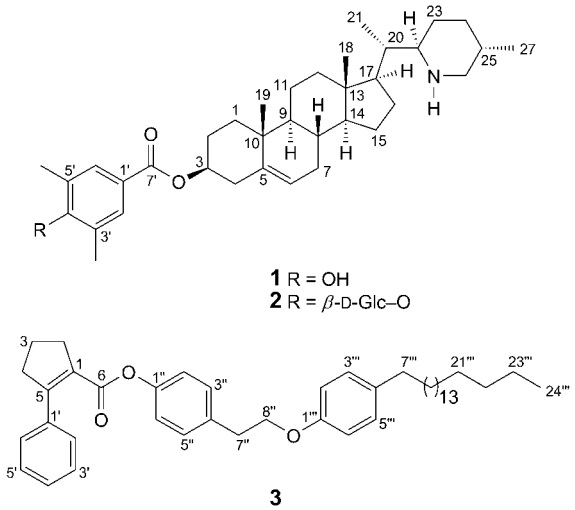

Fig. 1. *Structures of allumines A and B* (**1** and **2**, resp.)*, and compound* **3**

the olefinic C-atom signal appeared at δ(C) 137.9 and 122.1. The CH–O C-atom resonated at δ(C) 79.0, and the signals of four Me groups of the steroidal nucleus were detected at δ(C) 11.9, 13.6, 19.3, and 19.6, respectively. Two aromatic Me signals appeared together as a *singlet* at δ(C) 16.9. In the ^{1}H-NMR spectrum, signals for Me(18) and Me(19) groups of a 'normal' steroid ring system with a C=C bond were observed as *singlet*s at δ(H) 0.71 and 1.02, and a vinyl H-atom at C(6) resonated at δ(H) 5.24. In addition, two Me signals appeared as *doublet*s at δ(H) 0.90 ($J = 6.5$) and 0.81 ($J = 6.5$), which were attributed to Me(21) and Me(27), respectively. The CH–O H-atom resonated as a *multiplet* at δ(H) 3.18–3.20, and the $w_{1/2}$ value of 23 Hz confirmed its axial orientation. The relative configuration of the Me-substituted piperidine moiety was determined as (22*R*,25*S*) by NOEs (*Fig. 2*).

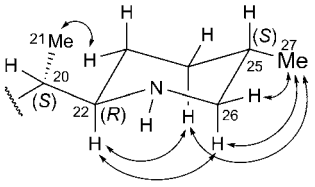

Fig. 2. *NOE Correlations* (H ↔ H) *of piperdine moiety of* **1**

Basic hydrolysis of **1** provided 4-hydroxy-3,5-dimethylbenzoic acid and a base which gave a positive digitonine test, characteristic of a genuine steroid with a 3β-OH group [10]. Its physical and NMR spectral data showed complete agreement with those of oblonginine [11] which is a 22-epimer of veramiline [10]. Thus, compound **1** is a 3-*O*-benzoyl derivative of oblonginine. The presence of 4-hydroxy-3,5-dimethylbenzoyloxy group at C(3) was concluded by the downfield shift of C(3) in ^{13}C-NMR spectrum compared to that of oblonginine, and also by HMBC experiment showing ^3J correlation

Table 1. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; $CDCl_3$) *of Compounds* **1** *and* **2**. Atom numbering as indicated in *Fig. 1*; δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	1.48–1.50 (*m*)	38.5	1.48–1.49 (*m*)	38.2
2	1.51–1.52 (*m*)	29.7	1.50–1.52 (*m*)	29.2
3	3.18–3.20 (*m*)	79.0	3.17–3.18 (*m*)	79.2
4	1.58–1.61 (*m*)	39.0	1.57–1.58 (*m*)	39.3
5	–	137.9	–	138.1
6	5.24 (br. *s*)	122.1	5.24 (br. *s*)	122.3
7	1.61–1.62 (*m*)	31.9	1.61–1.63 (*m*)	32.0
8	1.88–1.89 (*m*)	32.9	1.87–1.88 (*m*)	33.1
9	1.06–1.08 (*m*)	52.7	1.07–1.08 (*m*)	52.5
10	–	35.0	–	35.2
11	0.80–0.81 (*m*)	22.6	0.79–0.81 (*m*)	22.6
12	0.98–0.99 (*m*)	39.6	0.98–0.99 (*m*)	40.0
13	–	42.0	–	42.6
14	1.98–2.00 (*m*)	56.6	1.96–1.97 (*m*)	56.9
15	0.92–0.93 (*m*)	24.1	0.91–0.93 (*m*)	24.3
16	1.87–1.88 (*m*)	28.0	1.86–1.87 (*m*)	28.4
17	1.15–1.17 (*m*)	54.1	1.15–1.17 (*m*)	54.4
18	0.71 (*s*)	11.9	0.73 (*s*)	11.9
19	1.02 (*s*)	19.3	1.03 (*s*)	19.5
20	0.96–0.98 (*m*)	39.5	0.96–0.97 (*m*)	39.8
21	0.90 (*d*, J = 6.5)	13.6	0.90 (*d*, J = 6.5)	13.8
22	2.50 (*dt*, J = 10.8, 2.0)	59.8	2.50 (*dt*, J = 11.0, 2.2)	60.0
23	1.44–1.45 (*m*)	24.6	1.46–1.47 (*m*)	24.6
24	1.86–1.88 (*m*)	33.2	1.88–1.89 (*m*)	33.8
25	1.41–1.43 (*m*)	30.9	1.41–1.42 (*m*)	31.2
26	2.24–2.26 (*m*)	54.2	2.25–2.26 (*m*)	54.4
27	0.81 (*d*, J = 6.5)	19.6	0.83 (*d*, J = 6.5)	19.7
1′	–	124.4	–	12.4
2′,6′	8.08 (*s*)	129.6	8.08 (*s*)	129.7
3′,5′	–	130.0	–	130.0
4′	–	160.7	–	162.1
7′	–	170.0	–	170.1
Me–C(3′,5′)	2.01 (*s*)	16.9	2.02 (*s*)	16.9
1″			5.85 (*d*, J = 7.5)	102.3
2″			4.35–4.36 (*m*)	75.4
3″			4.21–4.23 (*m*)	79.1
4″			4.36–4.38 (*m*)	71.2
5″			4.38–4.39 (*m*)	78.5
6″			4.51 (*dd*, J = 2.0, 11.0), 4.60 (*dd*, J = 6.1, 11.0)	61.8

of H–C(3) with the C=O C-atom of the ester resonating at δ(C) 170.0. The structure of allumine **1** was, therefore, deduced as 3β-*O*-(4-hydroxy-3,5-dimethylbenzoyl)oblonginine (= (3β,17β)-17-{(1*S*)-1-[(2*R*,5*S*)-5-methylpiperidin-2-yl]ethyl}androst-5-en-3-yl 4-hydroxy-3,5-dimethylbenzoate).

Allumine B (**2**) was obtained as a colorless gummy solid. Its molecular formula was deduced as $C_{42}H_{63}NO_8$ by a quasimolecular-ion peak at m/z 708.4475 in negative-ion

mode HR-FAB-MS. The IR spectrum was similar to that of **1**. The ^{13}C-NMR and DEPT spectra showed 42 signals for six Me, twelve CH_2, and 16 CH groups, and eight quaternary C-atoms. They showed close similarities to those of **1** except additional peaks due to a hexose moiety (anomeric C-atom at δ(C) 102.3, and further signals of CH–O and CH_2O C-atoms in the range of 79.1 – 61.8). The ^{1}H-NMR spectrum was also similar to that of compound **1** with an additional signal of an anomeric H-atom of the hexose moiety as a *doublet* at δ(H) 5.85 ($J = 7.5$), and further signals of CH–O and CH_2O H-atoms in the range of 4.60 – 4.21. The larger value of coupling constant of the anomeric H-atom allowed us to assign β-configuration to the hexose moiety. The EI-MS showed the base peak at *m*/*z* 98, and intense peaks at *m*/*z* 398 and 126 were common to compound **1**, revealing the presence of hexose moiety in the aromatic ester. Basic hydrolysis and extraction with $CHCl_3$ furnished oblonginine and glycoside of an aromatic compound, which, on subsequent acid hydrolysis, provided 4-hydroxy-3,5-dimethylbenzoic acid, and the glycone which could be identified as D-glucose by sign of its optical rotation and co-TLC with an authentic sample. The anomeric H-atom resonating at δ(H) 5.85 showed ^3J correlation with C(4′) in HMBC spectrum of **2**, confirming the presence of the glucose moiety at C(4′). Allumine B (**2**) is therefore, 3β-*O*-[4-(β-D-glucopyranosyloxy)-3,5-dimethylbenzoyl]oblonginine (=(3β,17β)-17-{(1*S*)-1-[(2*R*,5*S*)-5-methylpiperidin-2-yl]ethyl}androst-5-en-3-yl 4-(β-D-glucopyranosyloxy)-3,5-dimethylbenzoate).

Compound **3** was obtained as a white crystalline solid. Its HR-EI-MS showed M^+ peak at *m*/*z* 636.4542 consistent with the molecular formula $C_{44}H_{60}O_3$. The IR spectrum evidenced the presence of a conjugated C=O group (1665 cm^{-1}), conjugated olefinic C=C bond (1625 cm^{-1}), and aromatic moieties (1540, 1500 cm^{-1}). The ^{13}C-NMR and DEPT spectra showed 44 signals for one Me, 22 CH_2, and 13 CH groups, and eight quaternary C-atoms (*Table 2*). The most downfield signal at δ(C) 164.0 was assigned to the ester C=O group, while the signals of a disubstituted cyclopentene moiety were observed at δ(C) 37.6, 32.0, and 23.2. The signals of olefinic C-atom appeared at δ(C) 155.6 and 123.4. The signals between δ(C) 155.4 and 115.1 were due to aromatic C-

Table 2. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; $CDCl_3$) *of Compound* **3**. Atom numbering as indicated in *Fig. 1*; δ in ppm, *J* in Hz.

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
1	–	123.4	4″	–	137.2
2	–	155.6	7″	1.27 – 1.28 (*m*)	37.3
3	1.27 – 1.28 (*m*)	37.6	8″	3.63 (*t*, *J* = 6.0)	70.5
4	1.54 – 1.56 (*m*)	23.2	1‴	–	155.4
5	1.22 – 1.23 (*m*)	32.0	2‴,6‴	6.74 (*d*, *J* = 8.5)	115.1
6	–	164.0	3‴,5‴	7.15 (*d*, *J* = 8.5)	128.7
1′	–	125.6	4‴	–	133.2
2′,6′	7.64 – 7.69 (*m*)	134.1	7‴	1.24 (br. *s*)	35.0
3′,5′	7.91 – 7.95(*m*)	130.1	8‴	1.24 (br. *s*)	31.8
4′	7.89 – 7.90 (*m*)	125.9	9‴ – 21‴	1.24 (br. *s*)	29.6
1″	–	150.0	22‴	1.24 (br. *s*)	31.9
2″,6″	7.07 (*d*, *J* = 9.0)	119.5	23‴	1.24 (br. *s*)	22.6
3″,5″	7.52 (*d*, *J* = 9.0)	129.2	24‴	0.85 (*t*, *J* = 7.0)	14.1

atoms, while CH_2–O C-atom signal was observed at δ(C) 70.5. An octadecyl moiety was evident by the terminal Me resonance at δ(C) 14.1, and signals of 17 CH_2 groups in the range of 35.0–22.6. In the ^{1}H-NMR spectrum, The H-atoms of two *para*-substituted phenyl rings formed two *AA′BB′* systems resonating at δ(H) 7.07 (*d*, $J = 9.0$, 2 H), 7.52 (*d*, $J = 9.0$, 2 H), 6.74 (*d*, $J = 8.5$, 2 H), and 7.15 (*d*, $J = 8.5$, 2 H), respectively. The signals of octadecyl moiety were observed at δ(H) 1.24 (br. *s*, 17 CH_2), and a *triplet* of terminal Me group at δ(H) 0.85 ($J = 7.0$). The CH_2–O H-atoms resonated at δ(H) 3.63 (*t*, $J = 6.0$). The assignments of various signals were accomplished with the help of ^{1}H,^{1}H-COSY and HMQC experiments, and supported by HMBC features, in which H–C(8″) exhibited ^2J correlation with C(7″) (δ(C) 37.3), as well as ^3J correlations with C(4″) (δ(C) 137.2) and C(1‴) (155.4) (*Fig. 3*). The H–C(5) ((δ(H) 1.22) showed ^2J correlation with C(1) (δ(C) 123.4) and C(4) (23.2), as well as ^3J correlations with C(2) (δ(C) 155.6) and C(3) (37.6). Further HMBC features were in complete agreement with the assigned structure of compound **3** as 4-{[2-(4-octadecylphenyl)oxy)]ethyl}-phenyl 2-phenylcyclopent-1-ene-1-carboxylate (*Fig. 3*).

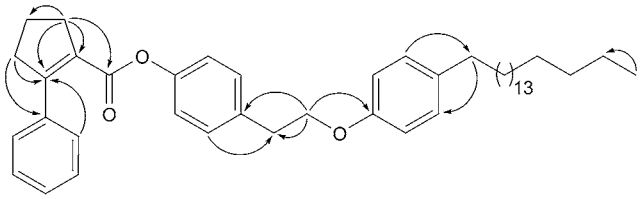

Fig. 3. *Key HMBCs* (H → C) *of compound* **3**

Experimental Part

General. Arachidonic acid and sodium citrate were purchased from the *Sigma Chemical Co.* (St. Louis, Mo. USA). All other chemicals used were of the highest purity grade available. TLC: Silica-gel *60* F_{254} plates (SiO_2; *E. Merck*, D-Darmstadt). Column chromatography (CC): SiO_2 (250–400 mesh; *E. Merck*, D-Darmstadt). Optical rotations: *Jasco DIP-360* digital polarimeter. IR Spectra: *Jasco 302-A* spectrophotometer; in KBr; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker 500* MHz instrument; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. EI- and HR-FAB-MS: *Jeol JMS-HX-110* and *JMS-DA-500* mass spectrometers with glycerol as matrix; in *m*/*z* (rel. %).

Plant Material. The whole plant material of *Allium victorialis* L. was collected from northern areas of Pakistan in 2004 and identified by Dr. *Surraiya Khatoon*, Plant Taxonomist, Department of Botany, University of Karachi, Karachi, Pakistan, where a voucher specimen has been deposited with the herbarium (voucher specimen No. 202/KUH).

Extraction and Isolation. The freshly collected whole plants of *A. victorialis* (20 kg) were shade-dried, ground, and extracted with EtOH (3 × 40 l, 10 d each) at r.t. The combined EtOH extract was evaporated under reduced pressure to yield a residue (800 g) which was suspended in H_2O (1.0 l), and successively extracted with hexane (80 g), $CHCl_3$ (170 g), AcOEt (220 g), and BuOH (150 g). The $CHCl_3$-soluble fraction (80 g) was subjected to CC (SiO_2; hexane, hexane/$CHCl_3$, $CHCl_3$, and $CHCl_3$/MeOH in increasing order of polarity) to obtain 20 subfractions. The subfraction which eluted with hexane/$CHCl_3$ 3.0 : 7.0 gave one major spot on TLC. It was purified by prep. TLC (hexane/$CHCl_3$ 3.5 : 6.5) to furnish compound **3** (16 mg). The subfraction which eluted with $CHCl_3$/MeOH 9.8 : 0.2 was rechromatographed and eluted with same solvent system to obtain *allumine A* (**1**; 18 mg). The subfraction which eluted with $CHCl_3$/MeOH 9.5 : 0.5 was rechromatographed and eluted with $CHCl_3$/MeOH 9.0 : 1.0 to provide *allumine B* (**2**; 15 mg).

Allumine A (=*(3β,17β)-17-{(1*S*)-1-[(2*R,5S*)-5-Methylpiperidin-2-yl]ethyl}androst-5-en-3-yl 4-Hydroxy-3,5-dimethylbenzoate* = *3β*-O-*(4-Hydroxy-3,5-dimethylbenzoyl)oblonginine*; **1**). Colorless gummy solid. $[\alpha]_D^{20} = -53$ ($c = 0.02$, $CHCl_3$). IR (KBr): 3450–3330, 1700, 1660, 1602, 1540, 1500. ^{13}C- and ^{1}H-NMR: see *Table 1*. EI-MS: 547 (9, M^+), 398 (20), 126 (28), 98 (100). HR-EI-MS: 547.4025 (M^+, $C_{36}H_{53}NO_3^+$; calc. 547.4029).

Allumine B (=*(3β,17β)-17-{(1*S*)-1-[(2*R,5S*)-5-Methylpiperidin-2-yl]ethyl}androst-5-en-3-yl 4-(β-*D*-Glucopyranosyloxy)-3,5-dimethylbenzoate* = *3β*-O-*[4-(β-*D*-Glucopyranosyloxy)-3,5-dimethylbenzoyl]-oblonginine*; **2**). Colorless gummy solid. $[\alpha]_D^{20} = -60$ ($c = 0.03$, $CHCl_3$). IR (KBr): 3450–3330, 1700, 1660, 1602, 1540, 1500. ^{13}C- and ^{1}H-NMR: see *Table 1*. EI-MS: 546 (7, $[M-162-H]^+$), 398 (18), 126 (30), 98 (100). HR-FAB-MS (neg.): 708.4475 ($[M-H]^-$, $C_{42}H_{62}NO_8^-$; calc. 708.4479).

4-{[2-(4-Octadecylphenyl)oxy)]ethyl}phenyl 2-Phenylcyclopent-1-ene-1-carboxylate (**3**). White crystalline solid. M.p. 82–83°. IR (KBr): 1665, 1625, 1540, 1500. ^{13}C- and ^{1}H-NMR: see *Table 2*. HR-EI-MS: 636.4542 (M^+, $C_{44}H_{60}O_3^+$; calc. 636.4545).

Alkaline Hydrolysis of **1**. The compound **1** (8 mg) was added to a soln. of 4% NaOH (2 ml), MeOH (6 ml), and H_2O (1.5 ml). The suspension was warmed gently on a steam-bath, until a vigorous exothermic reaction started, and in 10 min the entire solid had dissolved. The soln. was then heated under reflux for further 5 min. H_2O (3 ml) was then added, the MeOH was removed *in vacuo* and repeatedly extracted with $CHCl_3$. The residue from the org. phase crystallized from acetone/hexane, m.p. 220°, $[\alpha]_D^{23} = -40.3$ ($c = 0.14$; $CHCl_3$). Its physical and spectral data were in complete agreement with those reported in literature for oblonginine [11].

The alkaline soln. and H_2O washings were combined and acidified to pH 2 with 0.1N HCl, and the resulting precipitate was filtered and crystallized from benzene, m.p. 226°. Its physical and spectral data corresponded to those reported in literature for 4-hydroxy-3,5-dimethylbenzoic acid [12].

Hydrolysis of Compound **2**. Alkaline hydrolysis of compound **2** was carried out as described for compound **1** to obtain oblonginine. The basic soln. was acidified with 0.1N HCl to pH 2 and freeze-dried. The residue was subjected to acid hydrolysis by refluxing with 10% aq. HCl for 3 h at 100°. On cooling, the aq. fraction was extracted with $CHCl_3$. The $CHCl_3$ fraction was repeatedly washed with H_2O, dried (Na_2SO_4), and freed of solvent. The residue crystallized from benzene to furnish 4-hydroxy-3,5-dimethylbenzoic acid. The aq. phase was neutralized with Ag_2CO_3 and concentrated. The sugar was identified as D-glucose through co-TLC with an authentic sample and sign of its optical rotation ($[\alpha]_D^{23} = +51$).

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