Three New Cyclolanostane Triterpenoids from the Ethanol Extract of the Stems of *Kadsura heteroclita*

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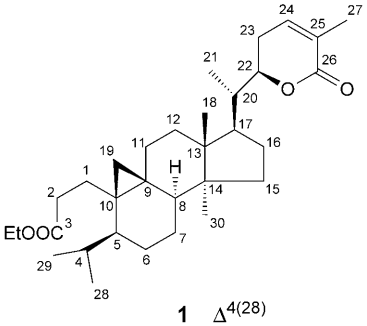
Three new cyclolanostane triterpenoids, **1**–**3**, were isolated from the EtOH extract of the stems of *Kadsura heteroclita*. Their structures and configurations were determined by extensive 1D- and 2D-NMR spectroscopy, high-resolution mass spectrometry (HR-MS), and circular dichroism (CD) spectroscopy. The three new compounds are likely to be artificial products formed during the extraction process, and might be derived from schisanlactone E (**4**) and two related double-bond isomers, respectively.

1. Introduction. – *Kadsura heteroclita* (ROXB.) CRAIB. (Schisandraceae) is a well-known traditional Chinese medicine (TCM) used for long time especially in the folk medicine of Southern China. The stem of *Kadsura heteroclita* is said to promote vital energy and blood circulation, to expel wind-evil and to remove wetness-evil (in terms of TCM), and had been used for the treatment of gastric and duodenal ulcers, acute and chronic gastroenteritis, dysmenorrhea, postpartum abdominal pain, and trauma [1]. It is also the main component of famous TCM preparations such as '*jixueteng gao*' [2] and '*zhonghua dieda wan*' [3].

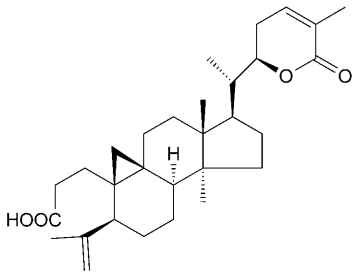
Some triterpenoids and lignans have been hitherto isolated from *K. heteroclita*, with biological activities such as cholesterol-biosynthesis inhibition and anti-lipid peroxidation [4–10]. In one of our previous phytochemical investigations, 13 triterpenoids and ten lignans were isolated from the stems of *K. heteroclita* [11–13].

In continuation of our work on the constituents of the title plant, we herein describe the isolation and structure elucidation of three new cyclolanostane triterpenoid esters, **1**–**3**, from the EtOH extract of the stems of *K. heteroclita*. Their structures were elucidated based on extensive spectroscopic (CD, ^{1}H- and ^{13}C-NMR, DEPT, ^{1}H,^{1}H-COSY, HSQC, HMBC) and mass-spectrometric (MS) analyses.

2. Results and Discussion. – The petroleum-ether-soluble part of the EtOH extract of powdered stems of *K. heteroclita* was purified by repeated chromatography on silica gel, followed by preparative reverse-phase HPLC to afford compounds **1**–**3**.



1 $\Delta^{4(28)}$
2 $\Delta^{4(5)}$
3 $\Delta^{4(28),6}$



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Compound **1** was obtained as a colorless, optically active powder (m.p. 71–72°; $[\alpha]_D^{20} = +95.8$ ($c = 0.41$, MeOH)). HR-EI-MS indicated the molecular formula $C_{32}H_{48}O_4$ (*m/z* 496.3560 (M^+, calc. 496.3553). A 9,19-cyclo-3,4-secolanostane skeleton was deduced from the ^{1}H-NMR data (*Table*), with typical signals for the geminal cyclopropyl H-atoms [δ(H) 0.41 (*d*, $J = 4.4$ Hz); 0.72 (*d*, $J = 4.4$ Hz)], signals for four angular Me groups [δ(H) 1.91, 1.68, 0.99, 0.92], as well as an olefinic, terminal CH_2 moiety [δ(H) 4.73 and 4.80 (2*s*)] [14] . A six-membered α,β-unsaturated lactone ring was assigned to the peripheral side chain, based on the presence of an MS fragment ion at *m/z* 111.0432 (*Fig. 1*) in combination with an HMBC experiment.

In the HMBC spectrum of **1** (*Fig. 2*), the Me(27) H-atoms were correlated with C(24), C(25), and C(26); and Me(21) was correlated with C(17), C(20), and C(22). Compared with the known schisanlactone E [15], the main differences in the ^{1}H- and ^{13}C-NMR spectra (*Table*) were the appearance of an EtO group [δ(H) 4.06, 1.22; δ(C) 60.24, 14.21) in **1**, and the fact that the C(3)=O resonance of **1** was shifted upfield to δ(C) 173.93. Further, the EtO group at δ(H) 4.06 showed HMBC cross-peaks with the C=O and Me C-atoms at δ(C) 173.93 and 14.21, respectively. All these data suggested that **1** was the ethyl ester of schisanlactone E (**4**), as supported by the MS fragment at *m/z* 451.3197 (*Fig. 1*).

The absolute configuration at C(22) of the lactone moiety was (*R*), based on a positive *Cotton* effect at 258 nm in the CD spectrum of **1** [16][17]. From the above data, the structure of **1** was determined as (8*R*,9*S*,22*R*)-3-ethoxy-3-oxo-9,19-cyclo-3,4-secolanosta-4(28),24-dien-26-oic acid 22,26-lactone.

Compound **2** was isolated as a colorless, optically active powder (m.p. 73–74°; $[\alpha]_D^{20} = +160°$ ($c = 0.10$, MeOH)). It had the same molecular formula ($C_{32}H_{48}O_4$) as compound **1**, as revealed by HR-EI-MS (*m/z* 496.3565 (M^+; calc. 496.3553)).

The main differences in the ^{1}H- and ^{13}C- NMR spectroscopic data of **2** (*Table*) was the absence of the terminal, olefinic CH_2 resonances of **1** (δ(H) 4.73, 4.80; δ(C) 111.59, 149.33), as well as the appearance of two quaternary olefinic C-atoms (δ(C) 129.11, 130.94) and an angular Me group (δ(H) 1.56). These changes suggested a C=C bond between C(4) and C(5) in **2**, as supported by the HMBC correlations of C(5) with Me(28), Me(29), H–C(1), and CH_2(19).

Table. *^{1}H- and ^{13}C-NMR Spectroscopic Data of* **1**–**3**. At 400 and 100 MHz, resp., in $CDCl_3$; δ in ppm, J in Hz.

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	1.38–1.41 (*m*)	28.92	1.37–1.40 (*m*)	29.16	1.49–1.51 (*m*)	28.18
	2.42–2.44 (*m*)		2.20–2.23 (*m*)		2.14–2.17 (*m*)	
2	2.22–2.24 (*m*)	31.64	2.20–2.22 (*m*)	33.18	2.31–2.33 (*m*)	30.44
	2.50–2.53 (*m*)		2.21–2.24 (*m*)		2.50–2.52 (*m*)	
3		173.93		173.97		173.40
4		149.33		129.11		147.94
5	2.43–2.46 (*m*)	45.77		130.94	2.99 (*s*)	45.66
6	1.11–1.15 (*m*)	25.00	1.01–1.04 (*m*)	25.40	5.36 (*s*)	127.50
	1.30–1.33 (*m*)		2.11–2.14 (*m*)			
7	1.08–1.12 (*m*)	27.65	2.04–2.07 (*m*)	28.23	5.36 (*s*)	129.85
	1.52–1.54 (*m*)		2.25–2.27 (*m*)			
8	1.57–1.60 (*m*)	47.66	0.94–0.97 (*m*)	48.49	2.63 (*s*)	43.71
9		21.15		25.15		20.55
10		27.04		26.75		28.72
11	1.29–1.31 (*m*)	26.76	1.41–1.43 (*m*)	27.29	1.46–1.49 (*m*)	25.73
	2.11–2.14 (*m*)		1.76–1.79 (*m*)		2.02–2.05 (*m*)	
12	1.66–1.69 (*m*)	32.84	1.68–1.71 (*m*)	33.38	1.62–1.64 (*m*)	32.95
	1.68–1.72 (*m*)		1.70–1.73 (*m*)		1.67–1.71 (*m*)	
13		45.58		45.58		45.45
14		48.55		48.64		49.46
15	1.32–1.35 (*m*)	35.60	1.33–1.36 (*m*)	36.65	1.20–1.22 (*m*)	32.72
	1.34–1.36 (*m*)		1.40–1.42 (*m*)		1.46–1.49 (*m*)	
16	1.39–1.42 (*m*)	26.94	1.37–1.40 (*m*)	25.52	1.44–1.47 (*m*)	26.44
	1.74–1.76 (*m*)		1.42–1.44 (*m*)		1.76–1.78 (*m*)	
17	1.60–1.63 (*m*)	48.06	1.58–1.61 (*m*)	44.97	1.57–1.60 (*m*)	46.50
18	0.99 (*s*)	17.82	1.03 (*s*)	18.88	0.96 (*s*)	13.91
19	0.41 (*d*, J=4.4)	29.90	0.29 (*d*, J=2.8)	34.44	0.92 (*d*, J=4.3)	19.59
	0.72 (*d*, J=4.4)		0.82 (*d*, J=3.2)		0.92 (*d*, J=4.3)	
20	2.20–2.22 (*m*)	39.09	2.04–2.07 (*m*)	38.98	2.07–2.09 (*m*)	38.85
21	0.96 (*d*, J=6.8)	13.04	0.97 (*d*, J=6.4)	13.10	0.99 (*d*, J=6.5)	13.18
22	4.44 (*dt*, J=13.2, 3.6)	80.57	4.45 (*dt*, J=12.0, 3.6)	80.59	4.46 (*dt*, J=13.2, 3.6)	80.24
23	2.12–2.14 (*m*)	23.43	2.08–2.10 (*m*)	23.55	2.05–2.08 (*m*)	23.12
	2.38–2.40 (*m*)		2.40–2.42 (*m*)		2.38–2.41 (*m*)	
24	6.60 (*d*, J=6.4)	139.46	6.60 (*d*, J=6.0)	139.51	6.61 (*d*, J=6.3)	139.13
25		128.23		128.23		127.94
26		166.62		166.68		166.30
27	1.91 (*s*)	17.00	1.92 (*s*)	17.02	1.92 (*s*)	16.69
28	4.73 (*s*)	111.59	1.56 (*s*)	21.00	4.78 (*s*)	112.36
	4.80 (*s*)				4.82 (*s*)	
29	1.68 (*s*)	19.41	1.77 (*s*)	20.16	1.67 (*s*)	19.38
30	0.92 (*s*)	19.73	0.91 (*s*)	19.72	0.78 (*s*)	16.80
EtO	4.06 (*q*, J=7.2)	60.24	4.06 (*q*, J=7.2)	60.21	4.09 (*q*, J=7.3)	60.05
	1.22 (*t*, J=6.8, 7.2)	14.21	1.22 (*t*, J=6.4, 7.2)	14.22	1.20 (*t*, J=6.3, 7.2)	13.91

The CD spectrum of compound **2** showed a positive *Cotton* effect at 253 nm, similar to that of compound **1**. Therefore, compound **2** was assigned the (22*R*)-configuration.

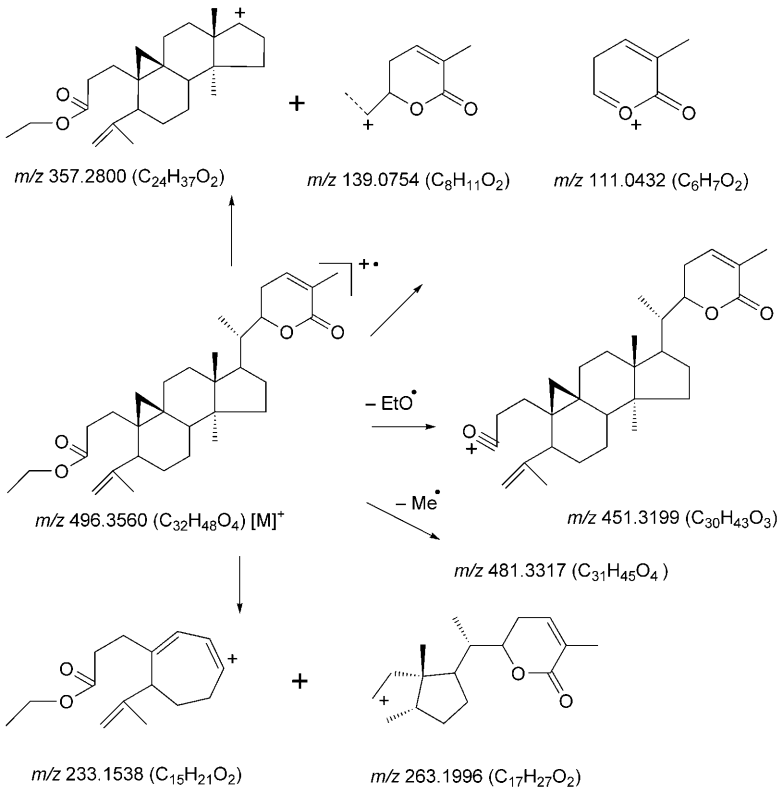

Fig. 1. *HR-EI-MS Fragmentation of* **1**

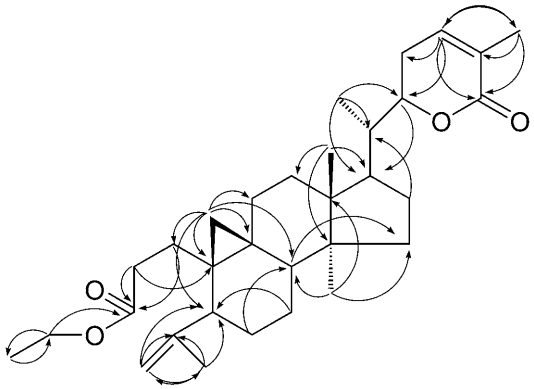

Fig. 2. *Key HMBC correlations for* **1**

From the above data, the structure of **2** was deduced as (8*R*,9*S*,22*R*)-3-ethoxy-3-oxo-9,19-cyclo-3,4-secolanosta-4,24-dien-26-oic acid 22,26-lactone.

Compound **3** was obtained as a colorless, optically active powder from MeOH (m.p. 84–85°; $[\alpha]_D^{20} = -14$ ($c = 0.12$, MeOH)). Its molecular formula was determined as $C_{32}H_{46}O_4$ by HR-EI-MS (*m/z* 494.3396 (M^+; calc. 494.3396)). Compared with **1**, the main differences in ^{1}H- and ^{13}C-NMR spectroscopic data (*Table*) was the appearance of two olefinic resonances at δ(H) 5.36 (δ(C) 127.50, 129.85), indicating that **3** had one more C=C bond than **1**, consistent with the molecular formula of **1** compared to **3**. In the HMBC spectrum, both olefinic H-atoms showed correlations with H–C(4), H–C(5), H–C(8), H–C(9), and H–C(10). Hence, the additional C=C bond was placed between C(6) and C(7). Again, we observed a positive *Cotton* effect at 254 nm in the CD spectrum of **3**, indicating (*R*)-configuration at C(22). Thus, from the above data, compound **3** was identified as (8*R*,9*S*,22*R*)-3-ethoxy-3-oxo-9,19-cyclo-3,4-secolanosta-4(28),6,24-trien-26-oic acid 22,26-lactone.

To our knowledge, no genuine *ethyl* ester has ever been isolated from a natural source. Therefore, compound **1** might be an artifact of schisanlactone E (**4**), a compound we reported earlier [11]; similarly, compounds **2** and **3** are likely to be derived from the corresponding carboxylic acids during the extraction procedure in refluxing EtOH.

The authors thank Mr. *Guangzheng Wang* for collecting the plants. Prof. *Nairong Zhang* (Hunan Institute for Control of Pharmaceutical Products) and Prof. *Hubiao Chen* (School of Pharmaceutical Sciences, Peking University) are kindly acknowledged for the identification of the plant material. We also thank the Department of Analytical Chemistry, Shanghai Institute of Materia Medica, for recording MS, NMR, IR, UV and CD spectra.

Experimental Part

General. Column chromatography (CC): silica gel *H* (200–300 mesh, *Qingdao Marine Chemical Group, Co.*). Thin-layer chromatography (TLC): silica gel GF_{254} plates; visualization under UV light and by spraying with vanillin/H_2SO_4, followed by heating. HPLC Separations were performed on an *Agilent* system equipped with a diode-array detector, using an *Alltima C18 ODS* column (5 μm, 250 × 10.0 mm) and a mobile phase consisting of MeOH/H_2O. Melting points (m.p.): *Büchi B-540* apparatus; uncorrected. UV Spectra: *Varian CARY-300-Bio* UV/VIS spectrophotometer; in MeOH soln.; λ_{max} (log ε) in nm. Optical rotations: *Perkin-Elmer 341-MC* polarimeter. CD Spectra: *JASCO J-810* spectropolarimeter; $\Delta\varepsilon$ in mdeg (λ in nm). IR Spectra: *Nicolet Magna-750* IR spectrometer; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Varian Mercury-Plus-400* spectrometer; at 400 (^{1}H) and 100 MHz (^{13}C), resp.; δ in ppm rel. to Me_4Si (= 0 ppm). EI- and HR-EI-MS (70 eV): *Varian MAT-711* mass spectrometer; in *m/z*.

Plant Material. The stems of *Kadsura heteroclita* (ROXB.) CRAIB. were collected from Shimen County, Hunan Province, P. R. China, and identified by Prof. *Nairong Zhang* (Hunan Institute for Control of Pharmaceutical Products) and Prof. *Hubiao Chen* (School of Pharmaceutical Sciences, Peking University). A voucher specimen (KH-0403) was deposited at the Department of Pharmacognosy, School of Pharmaceutical Sciences, Peking University , Beijing, P. R. China.

Extraction and Isolation. The powdered stems of *K. heteroclita* (3 kg) were extracted with 95% EtOH (15 l) for 2 h at reflux (3×). The pooled EtOH solns. were concentrated *in vacuo* to give a residue (237 g), which was extracted with petroleum ether (PE) and then AcOEt. The PE-soluble fraction afforded, upon evaporation, a waxy residue (70 g), which was further separated by CC (1.5 kg SiO_2; PE/AcOEt 97 : 3, 95 : 5, 93 : 7, 92 : 8, 91 : 9, 89 : 11, 86 : 14, 84 : 16, 70 : 30, 60 : 40, 50 : 50, 40 : 60, 30 : 70): fractions *Fr. 1–Fr. 13*. *Fr. 5* (2 g) was subjected to RP-HPLC (*Alltima C18*; MeOH/H_2O 95 : 5, 2.0 ml/min) to afford **3** (2.4 mg), **1** (30.1 mg), and **2** (2.6 mg) at t_R 11.3, 12.3, and 13.6 min, resp.

*(8*R*,9S,22*R*)-3-Ethoxy-3-oxo-9,19-cyclo-3,4-secolanosta-4(28),24-dien-26-oic Acid 22,26-Lactone* (**1**). Colorless powder (from MeOH). M.p. 71–72° (MeOH). UV (MeOH): 201 (4.05). $[\alpha]_D^{20} = +95.8$ (c = 0.41,

MeOH). CD (MeOH): +3.33 (238), +6.09 (258). IR (KBr): 2939, 1716, 1637, 1450, 1377, 1356, 1238, 1155, 1121, 1030, 895, 860. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 496 (100, M^+), 481, 451, 397, 357, 341, 263, 233, 215, 147, 139, 121, 111, 107, 95, 81, 69. HR-EI-MS: 496.3560 (M^+, $C_{32}H_{48}O_4^+$; calc. 496.3553).

*(8*R*,9*S*,22*R*)-3-Ethoxy-3-oxo-9,19-cyclo-3,4-secolanosta-4,24-dien-26-oic Acid 22,26-Lactone* (**2**). Colorless powder (from MeOH). M.p. 73–74°. UV (MeOH): 205 (3.84). $[\alpha]_D^{20} = +160$ ($c = 0.10$, MeOH). CD (MeOH): +1.59 (239), +2.82 (253). IR (KBr): 2928, 1724, 1637, 1450, 1375, 1232, 1157, 1121, 1032, 953, 849. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 496 (100, M^+), 481, 453, 451, 395, 357, 263, 235, 233, 222, 199, 121, 111, 107, 95, 81, 69. HR-EI-MS: 496.3565 (M^+, $C_{32}H_{48}O_4^+$; calc. 496.3553).

*(8*R*,9*S*,22*R*)-3-Ethoxy-3-oxo-9,19-cyclo-3,4-secolanosta-4(28),6,24-trien-26-oic Acid 22,26-Lactone* (**3**). Colorless powder (MeOH). M.p. 84–85° (MeOH). UV (MeOH): 201 (4.15). $[\alpha]_D^{20} = -14$ ($c = 0.12$, MeOH). CD (MeOH): +6.05 (221), +4.63 (254). IR (KBr): 2947, 1727, 1630, 1452, 1377, 1242, 1119, 1036, 897. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 494 (M^+), 451, 393 (100), 375, 339, 261, 231, 211, 185, 173, 159, 145, 111, 105, 95, 81, 69. HR-EI-MS: 494.3396 (M^+, $C_{32}H_{46}O_4^+$; calc. 494.3396).

REFERENCES

[1] New Medical College of Jiangsu, 'Great Dictionary of Chinese Materia Medica', Shanghai Science & Technology Press, Shanghai, 1977, p. 814.
[2] J.-S. Yang, *Chin. Pharm. J.* **1963**, *9*(3), 1271.
[3] National Commission of Chinese Pharmacopoeia, 'Pharmacopoeia of the People's Republic of China', Chemical Industry Press, Beijing, 2005, Vol. 1, p. 376.
[4] K. Kunio, M. Toshio, K. Akira, I. Akiko, M. Taku, O. Sudafumi, L.-N. Li, H. Xue, *Planta Med*. **1989**, *55*, 297.
[5] L.-N. Li, H. Xue, D.-L. Ge, K. Kunio, M. Toshio, O. Sadafumi, *Planta Med.* **1989**, *55*, 300.
[6] L.-N. Li, H. Xue, K. Kunio, K. Akira, O. Sadafumi, *Planta Med*. **1989**, *55*, 548.
[7] X.-W. Yang, H. Miyashiro, M. Hattori, T. Namba, Y. Tezuka, T. Kikuchi, D.-F. Chen, G.-J. Xu, T. Hori, M. Extine, H. Mizuno, *Chem. Pharm. Bull.* **1992**, *40*, 1510
[8] D.-F. Chen, G.-J. Xu, X.-W. Yang, M. Hattori, Y. Tezuka, T. Kikuchi, T. Namba, *Phytochemistry* **1992**, *31*, 629.
[9] Y.-P. Chen, Z.-W. Lin, H.-J. Zhang, H.-D. Sun, *Phytochemistry* **1990**, *29*, 3358.
[10] X.-W. Yang, M. Hattori, T. Namba, D.-F. Chen, G.-J. Xu, *Chem. Pharm. Bull.* **1992**, *40*, 406.
[11] W. Wang, J.-Z. Liu, J. Han, Z.-R. Xu, R.-X. Liu, P. Liu, X.-C. Ma, S.-H. Guan, D.-A. Guo, *Planta Med*. **2006**, *72*, 450.
[12] W. Wang, J.-Z. Liu, R.-X. Liu, Z.-R. Xu, M. Yang, W.-X. Wang, P. Liu, G. Sabia, X.-M. Wang, D.-A. Guo, *Planta Med.* **2006**, *72*, 284.
[13] W. Wang, X.-C. Ma, P. Liu, R.-X. Liu, S.-H. Guan, D.-A. Guo, *Nat. Prod. Commun.* **2006**, *1*(2), 109.
[14] R. Tan, H. Xue, L.-N. Li, *Planta Med.* **1991**, *57*, 87.
[15] J.-S. Liu, M.-F. Huang, *Acta Chim. Sin.* **1991**, *49*, 502.
[16] J.-S. Liu, M.-F. Huang, G. F. Arnold, E. Arnold, J. Clardy, W. A. Ayer, *Tetrahedron Lett.* **1983**, *24*, 2351.
[17] J.-S. Liu, M.-F. Huang, *Acta Chim. Sin.* **1984**, *42*, 464.

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