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SECOABIETANE DITERPENES FROM *COLEUS BARBATUS*

A. KELECOM*, T. C. DOS SANTOS* and W. L. B. MEDEIROS†

*Laboratory of Marine Natural Products, Department of General Biology, Universidade Federal Fluminense, C.P. 183, 24.000 Niterói, RJ, Brazil; †Núcleo de Pesquisas de Produtos Naturais, CCS, bloco H, Universidade Federal do Rio de Janeiro, Ilha do Fundão, 21.941 Rio de Janeiro, RJ, Brazil

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Key Word Index—*Coleus barbatus*; Labiatae; diterpene; secoabietane; *abeo*-abietane.

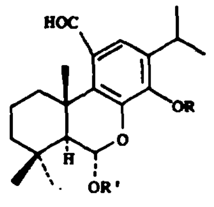
Abstract—Two new 6,7-secoabietane diterpenes have been isolated from *Coleus barbatus*. Their structures were deduced from chemical and spectral data. One of these compounds exhibits an unprecedent double rearrangement of the abietane skeleton.

INTRODUCTION

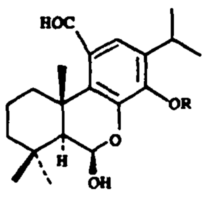
During our continuing investigations [1–6] on the stem constituents of the Labiate, *Coleus barbatus* Bentham, we isolated several new abietane-derived diterpenes which are all characterized by the presence of a free or substituted catechol moiety in the C-ring, and by a functionalized C-20 methyl group. In this paper, we wish to report on the isolation and structure determination of two minor constituents, seco-diterpenes **1** and **2**. Both compounds are related to cariocal (**3**) [1], but maintain the C-20 methyl group unsubstituted.

RESULTS AND DISCUSSION

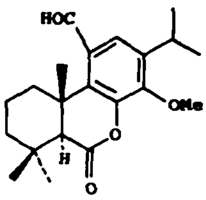
Diterpenes **1** and **2** were each found in minute amounts, the former in the mother liquors of crystallization of (−)-20-deoxocarnosol [2, 5], and the latter as a contaminant of a barbatusol-related compound [3]. Usual chromato-



1 R = R' = H
4 R = R' = Ac
7 R = Me ; R' = H
11 R = Me ; R' = Ac



6 R = H
8 R = Me



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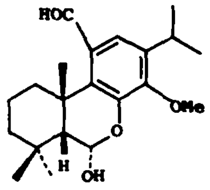

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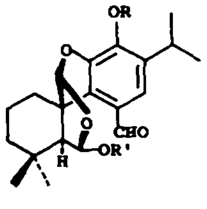
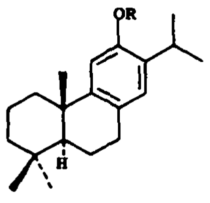
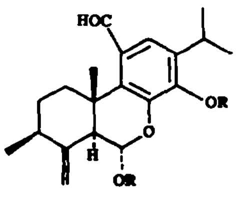


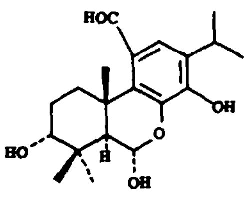
3 R = R' = H
12 R = R' = Ac
13 R = Me ; R' = H



10a R = Me
10b R = Ac



2 R = H
5 R = Ac



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graphic processes were inefficient, in our hands, to isolate the trace compounds, **1** and **2**. These could only be purified after acetylation and conventional silica gel column chromatography. This afforded pure **4** (mp 124–125°: $[\alpha]_D + 64.6°$ and **5** (mp 250–252°; $[\alpha]_D + 155.3°$), each of them devoid of a hydroxy band in the IR spectrum.

High resolution mass spectrometry of **4** established the empirical formula $C_{24}H_{32}O_6$ (416.2200, required 416.2199). General spectral data indicated the presence of an isopropyl group (6H, *d*, $J = 7$ Hz, at δ 1.20; 1H *hept* $J = 7$ Hz at δ 3.02) on a pentasubstituted aromatic ring (1H *s* at δ 7.27), of an α,β-unsaturated aldehyde moiety (UV: 258 and 309 nm; IR: 2725 and 1675 cm^{-1}; ^{1}H NMR: 1H *s* at δ 10.59), of a phenol acetate IR: 1754 cm^{-1}; ^{1}H NMR: 3H *s* at δ 2.30), of a secondary acetate (3H *s* at δ 2.08) and of three tertiary methyl groups (Table 1). These preliminary data rationalized seven of the nine unsaturations of **4**, and five of the six oxygen atoms. The sixth one is believed to participate in an (acetylated) hemiacetal function. This would explain not only the low field position of the carbinol acetate hydrogen (at δ 6.42), but also the sensitivity of **4** to alkaline medium. Indeed, base hydrolysis of **4** afforded an inseparable mixture of **1** and **6** (Table 1). Similarly, attempts to isolate **1**, from the crude extract, as its methyl ether derivative (CH_3I, K_2CO_3, acetone) yielded a 2:3 mixture of **7** and **8** (ν_{OH} 3425 cm^{-1}). The ^{1}H NMR spectrum of the latter mixture (Table 1) suggested that these compounds are epimers at the hemiacetal carbon. This was clearly demonstrated by PCC oxidation of mixture **7** + **8** to a single compound, ester **9** (–COOR at δ 166.68). The ^{13}C NMR spectrum of **9** contained signals for 5 =C, 1 =CH, 2C, 2CH, $3CH_2$, $5CH_3$ together with one ester, one methoxy and one conjugated aldehyde carbon, and defined the tricyclic nature of **9**. The IR ($\nu_{C=O}$ 1770 cm^{-1}) and ^{13}C NMR (no $C_{sp^3}(O)$) spectra supported a phenyl ester function present as a δ-lactone, as deduced from what follows. The doublet nature of the hemiacetal hydrogen of **4** suggested that there is only one methine vicinal to it. The hydrogen of this methine was observed as a *d* at δ 1.75 in **4** (confirmed by double irradiation experiments) and as a *s* at 2.31 in **9**. This showed that the methine was not vicinal to methylene groups and must be substituted by the two quaternary carbons of **9**. Since no benzylic hydrogen is observed in the ^{1}H NMR spectra of **4** or **9** (Table 1), one of the quaternary carbons must be attached to the aromatic nucleus, necessarily *ortho* to the phenyl ester position, defining a δ-lactone moiety. On the other hand, the aromatic substitution pattern was established in the following way. First, the upfield position of the aromatic carbon substituted by the methoxy group (at δ 148.98 in **9**, and at 154.79 in **10a**[7]) suggested the *ortho* relationship between the methoxy and the phenyl ester groups. Second, the absence of deshielding effects on the aromatic hydrogen during acetylation (**1** → **4**) or methylation (**1** → **7** + **8**) proved that this hydrogen was *meta* to the phenolic hydroxyl of **1** (Table 1). Third, the UV spectra of **1** + **6**, **4** and **11** (the only pure product obtained by acetylation of **7** + **8**) were practically identical to those of **3**, **12** and **13**, respectively [1], supporting a *para*-hydroxybenzaldehyde moiety in **1**. All these considerations left only one position for the isopropyl group, and determined completely the aromatic substitution pattern, 15 of the 21 carbon atoms and two of the three rings of **9**. The third ring could *a priori* be either a 4- or a 6-membered one, *i.e.* including the three remaining CH_2 groups and one or both quaternary carbon atoms. The fairly good agreement between the ^{13}C NMR data of **9** and **10b**[7] supported a 6-membered **A**-ring. The **A**/**B** *trans*-fusion resulted from the 1,3-diaxial interactions, observed in the ^{1}H NMR spectra of **6** and **8**, between Me-19, Me-20 and the 6β-OH group, and was confirmed by epimerization at C-5 (alkaline treatment) of **7** + **8** to yield pure **14** in which the **A**/**B** rings are now *cis*-fused (Table 1). Hence, **9** was derived from a 6,7-secoabietane skeleton, and the relative structure of the parent diterpene is correctly depicted by **1**, where the stereochemistry at C-6 was deduced from the coupling constant $J_{5\alpha/6\beta} = 8$ Hz.

High resolution mass spectrometry of **5** established the empirical formula $C_{24}H_{30}O_6$ (414.2027, required 414.2042). The mass spectral fragmentation pattern of **5**, which was almost identical to that of **4** with the ions shifted downwards by two mass units, strongly suggested the close structural relationship of **4** and **5**. Indeed, UV, IR and ^{1}H NMR data indicated the identity of the **B** and **C**

Table 1. ^{1}H NMR (100 MHz, $CDCl_3$)

H	mult.	**1**	+	**6**	**4**	**7**
H-5	*d*†	1.72		n.o.	1.75	n.o.
H-6	*d*†	5.66		5.91 *d*§	6.42	5.66
H-7	*s*		10.46		10.59	10.53
H-14	*s*	7.34		7.45	7.27	7.19
H-15	*h*‖		3.25		3.02	
Me-16	*d*‖		1.24		1.20	
Me-17	*d*‖		1.24		1.20	
Me-18	*s*	1.17		1.09	1.17	1.14
Me-19	*s*	1.10		1.29	0.85	1.06
Me-20	*s*	1.52		1.91	1.48	1.51
OR(6)	*s*	n.o.		n.o.	2.08	n.o.
OR(12)	*s*	n.o.		n.o.	2.30	3.90

*Signals may be reversed in any vertical column;
n.o.: not observed.
†$J = 8$ Hz; ‡$J = 9.5$ Hz; §$J = 1.5$ Hz; ¶finer on D_2O addition; ‖$J = 7$ Hz.

rings in **4** and **5**, and the substitution of the *gem* dimethyl group of **4** by one secondary methyl group (3H, *d*, *J* = 7 Hz at δ 1.09) and one exomethylene moiety (IR: 896 cm^{-1}; ^{1}H NMR: two 1H, broad *s* at δ 4.58 and 4.95). The deshielded position of H-5 (at δ 2.45 in **5** and at δ 1.75 in **4**, Table 1) fixed the exomethylene at C-4. This showed that **5** was a rearranged 6,7-secoabietane diterpene in which one of the C-4 methyl groups has migrated to the C-3 position. Both 19 (4β → 3β)-*abeo* and 18(4α → 3α)-*abeo*-abietanes have already been described [8–10]. The stereochemistry at C-3 of **5** was determined by comparison with literature data. In 3α-Me structures, the equatorial 3β-H is observed in the ^{1}H NMR spectrum as a *ddq* (*J* = 7, 15 and 7 Hz) at δ 2.66 [8], whereas in 3β-Me structures, the axial 3α-H appears as a multiplet at about 2.05 [9, 10]. The absence of a proton signal between 2.5 and 3.0 as well as the 4H integration under the secondary acetate signal (δ 2.12) indicated the axial orientation of H-3 and established the structure of **5**, and hence of **2**.

Diterpenes **1** and **2** are remarkable structures: as far as we know, **1** is the second 6,7-secoabietane with a free C-20 methyl group, and **2** is the first 6,7-secoabietane also rearranged in the A-ring. It has been proposed [9] that the rearrangement of Me-19 results from migration of a 3α-OH group onto the C-4 position and subsequent dehydration. Hence, one may postulate that a derivative such as **15** is a highly probable intermediate in the transformation of **1** into **2**. We are now actively looking for the presence of such a missing link in the extracts of *Coleus barbatus*.

EXPERIMENTAL

All the equipment used for this work has been previously described [3]. In the text, the sentence 'the reaction medium was treated in the usual way' means: diluted with water, extracted exhaustively with $CHCl_3$ or CH_2Cl_2, the organic layer washed with water, then dried over dry $MgSO_4$, filtered off and evaporated to dryness under reduced pressure.

Preparation of derivatives **4** *and* **5**. The extraction of air-dried stems of *Coleus barbatus* and partial purification work of the hexane crude extract have been described previously [2]. At that time, we claimed that "silica gel column chromatography (eluted with a gradient of EtOAc from 10 to 25% in hexane) afforded (−)-20-deoxocarnosol still contaminated with another diterpene" Crystallization afforded pure 20-deoxocarnosol and mother liquors (**A**) containing the former compound and **1** in relative proportions 3:1 (by ^{1}H NMR). Fractions eluted from the same column with 25–35% EtOAc in hexane (**B**) yielded a reddish gum, homogenous on TLC, composed of a diterpene related to barbatusol [3] contaminated by **2** (by ^{1}H NMR). Acetylation in the normal conditions (Ac_2O–pyridine 1:1, 3–4 hr, at room temp.) of **A** and **B** furnished 75 mg of crude acetylated **A** (AcA) and 350 mg of crude acetylated **B** (AcB). Silica gel column chromatography of AcA (eluent: hexane–EtOAc from 100:0 to 75:25) afforded pure **4** (18 mg, yield: 0.0006% from dry plant material) and the acetylated derivative of 20-deoxocarnosol (53 mg) identified by spectral comparison with an authentic sample [2].

Compound **4**. mp 124–125° (semicrystalline); $[\alpha]^{25}$: +64.6° (589), +67.4° (578), +76.6° (546) and +122.6° (436 nm), *c* = 0.73 in $CHCl_3$; UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm (ε): 224 (8000), 258 (7350) and 309 (2500) shifted to 224 (8485), 258 (7400) and 350 (2380) in alkaline MeOH; IR $\nu_{\max}^{\mathrm{film}}$ cm^{-1}: 2915, 2725, 1754, 1675, 1597, 1449, 1355, 1286, 1274, 1212, 1185, 1147, 1119, 1083, 1067, 1010, 958, 935 and 900; MS *m/z* (rel. int): 416.2200 $[M]^+$ (7, $C_{24}H_{32}O_6$ requires 416.2199), 374.2103 (13, $C_{22}H_{30}O_5$ requires 374.2093, loss of CH_2CO, metastable ion at *m/z* 336.24), 357 $[M^+ - AcO]^+$ (6), 341 $[M^+ - 75]$ (7), metastable ion at *m/z* 279.52), 330 (3), 314.1874 ($C_{20}H_{26}O_3$ requires 314.1882, 374 − AcOH, metastable ion at *m/z* 263.63) (100), 299.1642 ($C_{19}H_{23}O_3$ requires 299.1647, 314-Me) (20), 286 (314 − 28, metastable ion at *m/z* 260.50) (7), 285 (314 − 29, metastable ion at *m/z* 258.68) (5), 271.1335 $C_{17}H_{19}O_3$ requires 271.1334, 314 − *i*Pr, metastable ion at 233.89) (14), 244 (13), 231 (21), 218 (27), 206 (7), 205 (6), 203 (8) and 43 (51); ^{1}H NMR: Table 1.

Silica gel CC of Ac-**B** (eluent:hexane–EtOAc from 95:5 to 70:30) afforded pure **5** (29 mg, yield: 0.001% from dry plant material) and the acetylated new *abeo*-abietane derivative the structure of which is still under study.

Compound **5**. mp 250–252°; $[\alpha]^{25}$: +155.3° (589), +162.2° (578), +185.3 (546) and +333.1 (436 nm), ($CHCl_3$, *c* 0.36); UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm (ε): 222 (6460), 262 (2440) and 309 (988); IR $\nu_{\max}^{\mathrm{film}}$ cm^{-1}: 2995, 2775, 1770, 1705, 1655, 1610, 1570, 1460, 1428, 1360, 1285, 1220, 1180, 1140, 1111, 1085, 1050, 1020, 965, 896, and 853; MS *m/z* (rel. int.): M^+ 414.2027 ($C_{24}H_{30}O_6$ requires

data of compounds **1**, **4**–**9**, **11** and **14**.

+	**8**	**9**	**11**	**14**	**5**
	n.o.	2.31 *s*	1.64	n.o.	2.45 *d*‡
	5.97 *br s*¶	—	6.55	5.20 *br s*	6.56 *d*‡
	10.57	10.38	10.51	9.89	10.40
	7.35	7.35	7.24	6.80	7.29
3.28		3.33	3.26	3.21	3.04
1.23		1.22*	1.21	1.23*	1.19*
1.23		1.25*	1.21	1.24*	1.20*
	1.09	1.37	1.18	1.31	4.58 *br s* 4.95 *br s*
	1.29	1.19	0.87	1.07	1.09 *d*‖
	1.94	1.54	1.54	1.78	1.38
	n.o.	—	2.07	5.70 *br s*	2.12
	3.83	3.97	3.87	3.75	2.28

414.2042) (3), 372 ($[M-CH_2CO]^+$ metastable ion at *m/z* 334.26) (29), 355 $[M-AcO]^+$ (14), 339.1191 ($C_{20}H_{19}O_5$ requires 339.1232, metastable ion at *m/z* 277.58 (22), 328 (12), 312 (372 − AcOH, metastable ion at *m/z* 261.67) (100), 297 (312 − Me, metastable ion at *m/z* 282.72 (40), 284 (312 − 28, metastable ion at *m/z* 258.51) (2), 283 (312 − 29, metastable ion at *m/z* 256.70) (18), 279 (339 − AcOH) (1), 269 (312 − *i*Pr, metastable ion at *m/z* 231.93) (1), 255 (1), 243 (10), 241 (9), 218 (22), 206 (20), 205 (18), 176 (6), 109 (18), 108 (21) and 43 (60); ^{1}H NMR: Table 1.

Preparation of mixture **7** + **8**. The above-cited fraction A (235 mg), in dry Me_2CO (5 ml), was treated with MeI (1 ml) and K_2CO_3 at room temp. for 5.5 hr. The solids were then filtered off, washed with Me_2CO and the filtrate evaporated to dryness. The residue was purified by silica gel CC (eluent: hexane–EtOAc from 95:5 to 80:20). This afforded the dimethyl ether derivative of 20-deoxocarnosol (147 mg) identified by spectral comparison with an authentic sample [5], and the mixture of **7** + **8** (55 mg) analysed as such.

Mixture **7** + **8**: yellowish gum; UV $\lambda_{\max}^{\text{MeOH}}$ nm (ε): 228 (11970), 276 (7480) and 310 (sh); IR $\gamma_{\max}^{\text{film}}$ cm^{-1}: 3425, 2941, 2725, 1680, 1592, 1550, 1460, 1412, 1387, 1360, 1330, 1287, 1220, 1167, 1145, 1105, 1089, 1036, 1026, 997, 963, 930 and 889; MS *m/z* (rel. int.): 346 $[M]^+$ ($C_{21}H_{30}O_4$) (45), 331 (41), 317 (8), 313 (63), 285 (13), 261 (20), 247 (22), 245 (16), 233 (61), 219 (20) and 43 (100); ^{1}H NMR: Table 1.

Hydrolysis of **4**. Compound **4** (15 mg) was treated at room temp. with a saturated methanolic solution of K_2CO_3 (5 ml). The reaction was monitored by TLC. After complete consumption of starting material (± 2.5 hr), the reaction medium was neutralized with 1N HCl and treated in the usual way. This afforded a yellowish gum homogeneous on TLC of identical R_f as **1** (*i.e.* as fraction A) in various solvent systems, shown by ^{1}H NMR to be a 2:3 mixture of **1** and **6**. Mixture **1** + **6**: UV $\lambda_{\max}^{\text{MeOH}}$ nm (ε): 220 (7300), 234 (7320) and 287 (4415) shifted to 220 (8450), 256 (5640) and 362 (5350) in alkaline MeOH; IR $\nu_{\max}^{\text{film}}$ cm^{-1}: 3508, 2985, 1685, 1618, 1585, 1475, 1445, 1370, 1350, 1298, 1250, 1180, 1005, 972, 905 and 855; MS *m/z* (rel. int.): 332 $[M]^+$ ($C_{20}H_{28}O_4$) (33), 317 (37), 299 (100), 296 (5), 285 (13), 271 (20), 247 (25), 231 (24), 219 (68) and 43 (98); ^{1}H NMR: Table 1.

PCC oxidation of mixture **7** + **8**. Mixture **7** + **8** (35 mg), in dry CH_2Cl_2 (3 ml) was stirred vigourously at room temp. with PCC (1.8 eq). After 3 hr, the reaction medium was filtered over a small silica gel column eluted with pure CH_2Cl_2. Evaporation of the solvent under red. pres. afforded a yellow material that was purified by prep. TLC (eluent: hexane–EtOAc 85:15, two elutions). This afforded pure **9** (28 mg).

Compound **9**. gum; $[\alpha]_D^{25}$: −28° ($CHCl_3$; *c* 1.00); UV $\lambda_{\max}^{\text{MeOH}}$ nm (ε): 232 (9160) and 272 (7820); IR $\nu_{\max}^{\text{film}}$ cm^{-1}: 2924, 2747, 1770, 1685, 1593, 1545, 1450, 1395, 1375, 1333, 1283, 1212, 1165, 1145, 1115, 1095, 1030, 1015, 1000, 975, 895, 845 and 820; MS *m/z* (rel. int.): 344 $[M]^+$ $C_{21}H_{28}O_4$ (56), 330 (10), 317 (43), 301 (100), 298 (9), 283 (11), 273 (15), 261 (18), 256 (6), 248 (21), 244 (7), 234 (23), 153 (42) and 114 (21); ^{1}H NMR: Table 1; ^{13}C NMR ($CDCl_3$, 25.2 MHz): 39.32 (*t*, C-1), 18.65 (*t*, C-2), 41.53 (*t*, C-3), 32.80 (*s*, C-4), 54.72 (*d*, C-5), 166.68 (*s*, C-6), 191.01 (*d*, C-7), 130.64 (*s*, C-8), 39.92 (*s*, C-10), 141.04 and 143.75 (each *s*, C-9 and C-11), 148.98 (*s*, C-12), 136.20 (*s*, C-13), 124.13 (*d*, C-14), 26.92 (*d*, C-15), 21.09 (2 *q*, C-16 and C-17), 32.99 (*q*, C-18), 22.86 and 23.04 (each *q*, C-19 and C-20) and 61.66 (*q*, OMe).

Acetylation of the mixture **7** + **8**. Mixture **7** + **8** (25 mg) in pyridine (1 ml) was treated with freshly distilled Ac_2O (1 ml) at room temp. for 3.5 hr. The reaction medium was then treated in the usual way. This furnished a complex mixture from the which acetate **11** (11 mg) was obtained pure by prep. silica gel TLC (eluent: hexane–EtOAc 92:8, three elutions).

Compound **11**. mp. 96–99°; $[\alpha]_D^{25}$: +99° ($CHCl_3$; *c* 1.00); UV $\lambda_{\max}^{\text{MeOH}}$ nm (ε): 224 (14300) and 270 (6800); IR $\nu_{\max}^{\text{film}}$ cm^{-1}: 2924, 2710, 1745, 1675, 1587, 1543, 1460, 1404, 1381, 1364, 1351, 1326, 1285, 1264, 1233, 1210, 1163, 1119, 1099, 1082, 1066, 1017, 1003, 980, 944, 930, 917, 887, 850 and 832; MS (20 eV) *m/z* (rel. int.): 388 $[M]^+$ $C_{23}H_{32}O_5$ (9), 346 (2), 331 (3), 313 (100), 295 (7), 285 (6), 271 (6), 260 (4), 258 (3), 245 (12), 234 (5) and 153 (4); ^{1}H NMR: Table 1.

Alkaline treatment of mixture **7** + **8**. Mixture **7** + **8** (14 mg) was treated with a saturated methanolic solution of K_2CO_3 (5 ml), at room temp. for 1 day. The reaction medium was then acidified with 1N aq. HCl and treated in the usual way. Pure **14** (11 mg) was obtained.

Compound **14**. mp 199–200°; $[\alpha]_D^{25}$: +4°, ($CHCl_3$; *c* 0.50); UV $\lambda_{\max}^{\text{MeOH}}$ nm (ε): 218 (9390) and 274 (1670); IR $\nu_{\max}^{\text{film}}$ cm^{-1}: 3509, 2967, 2890, 2770, 1715, 1630, 1595, 1451, 1420, 1389, 1300, 1221, 1166, 1149, 1103, 1087, 1074, 1005 and 877; MS *m/z* (rel. int.): 346 $[M]^+$ $C_{21}H_{30}O_4$ (36), 331 (34), 313 (45), 285 (14), 261 (18), 247 (23), 233 (50), 219 (18), 194 (19) and 43 (100); ^{1}H NMR: Table 1.

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