Biosynthetic Origin of the Carbon Skeleton and Oxygen Atoms of the Avermectins

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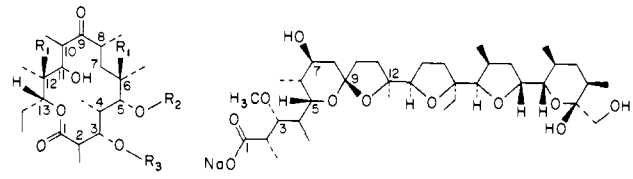
Within the last 5 years, investigations carried out in several laboratories have begun to unravel the complexities of the fascinating sequence of reactions by which branched-chain, polyoxygenated fatty acid metabolities such as macrolide[2] and polyether[3] antibiotics are biosynthesized. For example, incorporation of [1-$^{18}O_2$,1-^{13}C]propionate and analysis by high-field ^{13}C NMR has established the origin of the oxygen atoms of erythromycin A (**1a**, Chart I) and supported the conclusion that the oxygenation level eventually observed at each site in the parent aglycone, 6-deoxyerythronolide B (**1b**), is established during the process of carbon-chain elongation.[4] Specifically, the four secondary hydroxyl or ether functions of the macrolide, independent of their individual D (C-13) or L (C-3, -5, and -11) configuration, each bear excess oxygen isotope derived from the carboxyl oxygens of the propionate precursor. Similar studies have also been reported for the polyether antibiotics monensin A (**2**)[5] and lasalocid A (**3**).[6] The fact that the structural and stereochemical features of the vast majority of macrolides can be fitted to a single configurational model[7,8] has emphasized the generality of the biosynthetic results obtained to date. An analogous set of stereochemical prototypes has recently been described for polyether antibiotics.[8,9] We have now examined the biosynthesis of the avermectins (**4**), a group of macrolide metabolites with potent antiparasitic activity[10] whose

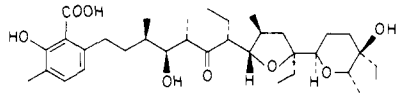
Chart I



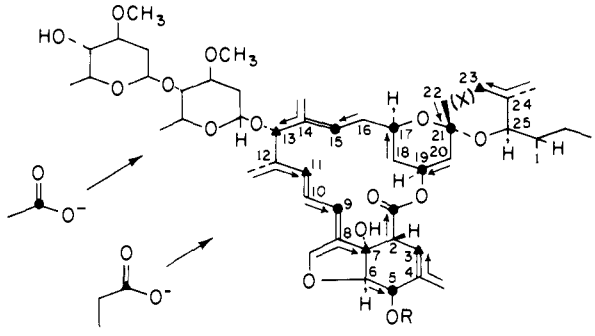
1a R_1 = OH, R_2 = desosaminyl, R_3 = cladinosyl

1b $R_1 = R_2 = R_3 = H$

2



Scheme I

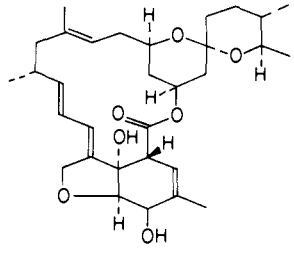


4a (A_{1a}) R = CH_3, X = CH=CH

4b (A_{2a}) R = CH_3, X = CH_2-C(OH)

4c (B_{1a}) R = H, X = CH=CH

4d (B_{2a}) R = H, X = CH_2-C(OH)



5 (a_1)

structures are not simply reconciled with any of the above stereochemical models. Our results are reported below.

Avermectins, and the closely related milbemycin antibiotics (**5**),[11] display a novel combination of several structural features. In addition to a 16-membered lactone, there is a characteristic spiroketal (C-17–25), a feature common to many polyethers, while the cyclohexene ring corresponding to C-2–7 is a typical example of a nonaromatic, alicyclic polyketide. In order to determine the origin of the avermectin carbon skeleton, we have carried out incorporations of [1-^{13}C]acetate and [1-^{13}C]propionate using growing cultures of *Streptomyces avermitilis* 5192 and have analyzed the isolated and purified avermectins A_{1a} (**4a**), A_{2a} (**4b**), B_{1a} (**4c**), and B_{2a} (**4d**) by 62.9-MHz ^{13}C NMR. The observed distribution of label in each metabolite was in accord with the expected derivation of **4** from seven acetates and five propionates, as illustrated in Scheme I. The *sec*-butyl substituent in **4** was

(1) Fellow of the Alfred P. Sloan Foundation, 1978–1982; National Institutes of Health Research Career Development Award, 1978–1983.

(2) For recent reviews of macrolide biosynthesis see: Corcoran, J. W. In "Antibiotics IV. Biosynthesis"; Corcoran, J. W., Ed.; Springer-Verlag: New York, 1981; pp 132–174. Omura, S.; Nakagawa, A. *Ibid.* pp 175–192.

(3) For a recent review of polyether biosynthesis see: Westley, J. W. In ref 2, pp 41–73.

(4) Cane, D. E.; Hasler, H.; Liang, T.-C. *J. Am. Chem. Soc.* **1981**, *103*, 5960. Cane, D. E.; Hasler, H.; Taylor, P. B.; Liang, T.-C *Tetrahedron*, in press.

(5) Cane, D. E.; Liang, T.-C.; Hasler, H. *J. Am. Chem. Soc.* **1981**, *103*, 5962. Cane, D. E.; Liang, T.-C; Hasler, H. *Ibid.* **1982**, *104*, 7274.

(6) Hutchinson, C. R.; Sherman, M. M.; Vederas, J. C.; Naskashima, T. T. *J. Am. Chem. Soc.* **1981**, *103*, 5953. See also: Hutchinson, C. R.; Sherman M. M.; McInnes, A. G.; Walter, J. A.; Vederas, J. C. *Ibid.* **1981**, *103*, 5956.

(7) (a) Celmer, W. D. *J. Am. Chem. Soc.* **1965**, *87*, 1801. (b) Celmer, W. D. *Pure Appl. Chem.* **1971**, *28*, 413.

(8) Cane, D. E.; Celmer, W. D.; Westley, J. W. *J. Am. Chem. Soc.* **1983**, *105*, 3594.

(9) See also: Hutchinson, C. R. *Acc. Chem. Res.* **1983**, *16*, 7.

(10) Albers-Schonberg, G.; Arison, B. H.; Chabala, J. C.; Douglas, A. W.; Eskola, P.; Fisher, M. H.; Lusi, A.; Mrozik, H.; Smith, J. L.; Tolman, R. L. *J. Am. Chem. Soc.* **1981**, *103*, 4216. Springer, J. L.; Arison, B. H.; Hirshfield, J. M.; Hoogsteen, K. *Ibid.* **1981**, *103*, 4221. Burg, R. W.; Miller, B. M.; Baker, E. .; Birnbaum, J.; Currier, S. A.; Hartman, R.; Kong, Y.-L.; Monaghan, R. L.; Olson, G.; Putter, I.; Tunac, J. B.; Wallick, H.; Stapley, E. O.; Oiwa, R.; Omura, S. *Antimicrob. Agents Chemother.* **1979**, *15*, 361. Miller, T. W.; Chaiet, L.; Cole, D. J.; Cole, L. J.; Flor, J. E.; Goegelman, R. T.; Gullo, V. P.; Joshua, H.; Kempf, A. J.; Krellwitz, W. R.; Monaghan, R. L.; Ormond, R. E.; Wilson, K. E.; Albers-Schonberg, G.; Putter, I. *Ibid.* **1979**, *15*, 638. Kass, I. S.; Wang, C. C.; Walrond, J. P.; Stretton, A. O. W. *Proc. Natl. Acad. Sci.* U. S. A. **1980**, *77*, 6211.

(11) Mishima, H.; Kurabayashi, M.; Tamura, C.; Sato, S.; Kuwano, H.; Saito, A. *Tetrahedron Lett.* **1975**, 711. Takiguchi, Y.; Mishima, H.; Okuda, M.; Terao, M.; Aoki, A.; Fukuda, R. *J. Antibiot.* **1980**, *33*, 1120.

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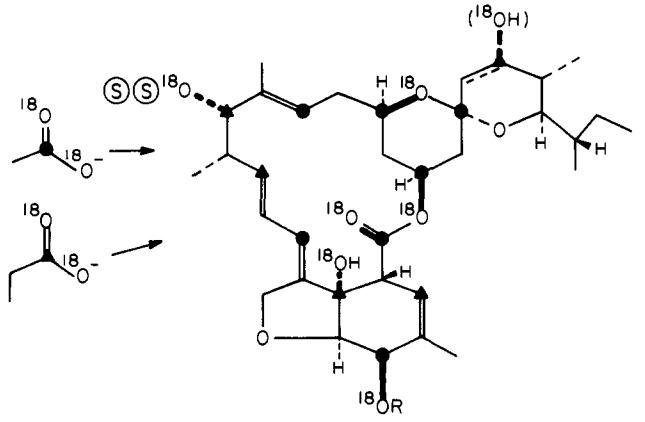
Scheme II



4 a, b, c, d

not labeled by either precursor, consistent with the previously established role of L-isoleucine as the precursor of the starter unit of this series of avermectins.[12]

With the basic building blocks of the avermectin aglycone firmly established, we turned our attention to the origin of the oxygen atoms of these metabolites. To this end, sodium [1-$^{18}O_2$,1-^{13}C]propionate[13] (125 mg), diluted with an equal quantity of unlabeled sodium propionate, was adminstered in lots of 25.0 and 6.25 mg at 48 and 72 h, respectively, to each of eight flasks containing 20 mL of *S. avermitilis* 5192 growing in a complex medium. After an additional 96 h at 28 °C (220 rpm), the contents of the flasks were pooled and centrifuged, and the avermectins were extracted from the cells and purified by established procedures.[10,14] ^{13}C NMR analysis of the isotopically shifted resonances[4-6,15] revealed the presence of excess oxygen-18 at C-7 and C-13 in all four avermectins examined, while avermectins A_{2a} and B_{2a} each bore oxygen isotope at C-23 as well. (Scheme II, Table I) Analogous incorporation of sodium [1-$^{18}O_2$,1-^{13}C]-acetate[16] resulted in ^{18}O labeling at C-1, -5, -17, and -19, on the basis of the observation of characteristic ^{18}O–^{13}C isotope shifts in the spectra of each of the four separately analyzed avermectins. By contrast, only a single unshifted peak corresponding to the spiroketal carbon, C-21, was observed in each sample.

The pattern of oxygen-18 labeling in the avermectins is thus fully consistent with the results of earlier studies of macrolide and polyether antibiotic biosynthesis.[4-6] Moreover, several noteworthy conclusions emerge from the present results: (1) The methyl and ether substituents at C-12 and -13 of **4** are present in an erythro relationship, in contrast to the prevalent threo pattern characteristic of methyl/hydroxyl substituents attached to adjacent biogenetic units in erythromycin (C-2,3; -4,5; -10,11; and -12,13) and monensin (C-2,3; -4,5; and -6,7). The fact that C-13 of the avermectins is nonetheless derived from the propionate precursor indicates that the oxygen labeling results are independent of not only the absolute (D or L) but also the relative (erythro or threo) configuration at any given site.[17] (2) The absence of oxygen-18 label at C-21 suggests that the spiroketal has been generated by

Table I. Incorporation of [1-$^{18}O_2$,1-^{13}C]Acetate and [1-$^{18}O_2$,1-^{13}C]Propionate into Avermectins

C	4	^{13}C shift, ppm[a]	Δδ, ppm[b] [1-$^{18}O_2$, ^{13}C]Ac[c]	Δδ, ppm[b] [1-$^{18}O_2$, ^{13}C]Pr[d]	^{16}O:^{18}O[e]
1	A_1	173.96	0.035		50:40:10[f]
	A_2	173.76	0.035		60:35:5[f]
	B_1	173.69	0.035		65:35
	B_2	173.54	0.035		70:30
5	A_1	77.03	0.023		60:40
	A_2	77.03	0.017		60:40
	B_1	67.76	0.023		70:30
	B_2	76.72[g]	0.023		70:30
7	A_1	80.64		0.023	60:40
	A_2	80.66		0.023	60:40
	B_1	80.44		0.023	60:40
	B_2	80.52		0.023	70:30
13	A_1	82.06		0.023	55:45
	A_2	81.82		0.023	60:40
	B_1	81.99		0.023	60:40
	B_2	81.77		0.023	60:40
17	A_1	68.49	*h*		*h*
	A_2	68.42	0.029		60:40
	B_1	68.42	0.029		60:40
	B_2	68.37	0.029		60:40
19	A_1	68.44	0.023		60:40
	A_2	67.72	0.035		60:40
	B_1	68.32	*h*		*h*
	B_2	67.61[g]	*h*		*h*
21	A_1	95.84	0.0		100:0
	A_2	99.73	0.0		100:0
	B_1	95.83	0.0		100:0
	B_2	99.70	0.0		100:0
23	A_2	69.93		0.017	60:40
	B_2	69.93		0.023	60:40

[a] Bruker WM 250, 62.9 MHz; spectral width 12 000 Hz; 64 K points; quadrature detection; 55° pulse; repetition rate 2.7 s; resolution enhancement by Lorentz–Gauss multiplication,[4,5] −1.5-Hz line broadening, 0.4 Gaussian multiplier; 0.006 ppm/data point. [b] $^{13}C^{18}O$ isotope shift, ±0.006 ppm. [c] Average ^{13}C enrichment 2%. [d] Average ^{13}C enrichment 3%. [e] Uncorrected for contribution of natural abundance ^{13}C to $^{13}C^{16}O$ peak; ±5. [f] $^{13}C^{18}O_2$ peak due to excess intramolecular multiple labeling by 90% acetate precursor. [g] These assignments may be reversed. [h] Signal obscured by overlap with other peaks.

an unexceptional ketalization of a C-21 carbonyl with secondary hydroxyl groups at C-17 and C-25. Earlier studies of monensin biosynthesis,[5] on the other hand, have indicated that the C-9 spiroketal of the polyether is formed by intramolecular alkylation of a hemiketal initially generated by attack of the C-5 hydroxyl at the C-9 carbonyl carbon. (3) The fact that the C-7 tertiary hydroxyl group in **4** retains the carbonyl oxygen of the corresponding propionate precursor strongly implies that the C-2,7 bond of the cyclohexene ring has been formed by a simple aldol condensation, thereby ruling out alternative mechanisms involving electrophilic cyclization of polyolefinic intermediates. It should be noted that little is known at present about the mechanisms by which cyclic saturated, as distinguished from aromatic, polyketides are generated. In the present case, it is not yet possible to specify whether cyclohexene ring formation precedes or follows lactonization. (4) The presence of priopionate-derived oxygen at C-13 of the avermectins rules out the possibility that these metabolites are biosynthesized by late-stage oxidation of a milbemycin or any analogously reduced precursor. Instead the observed labeling at C-13 implies that the biosyntheses of **4** and **5** diverge during the stage of carbon-chain elongation, with milbemycin biosynthesis requiring an additional two steps for dehydration and reduction prior to condensation with the malonate unit corresponding to C-9 and -10. Interestingly, the configuration of the C-12 methyl in both the avermectins and in milbemycins is D, in spite of the different mechanisms by which these centers are apparently introduced. A similar phenomenon is frequently observed among the polyether antibiotics.[8] In the latter cases, however, the cor-

(12) The isopropyl side chain which characterizes the b components of **4**[10] was similarly shown to be derived from L-valine. Albers-Schonberg, G.; Douglas, A. W.; Goegelman, R. T.; Kaplan, L.; Kempf, A.; Tunac, J. B., unpublished results.

(13) 54.9% $^{18}O_2{}^{13}C$, 32.2% $^{18}O^{13}C$, 3.6% $^{16}O^{13}C$. Prepared as previously described.[4,5]

(14) No attempt was made to separate the minor b components, which were present in sufficiently low concentrations so as not to interfere with the ^{13}C NMR analysis.

(15) Vederas, J. C. *J. Am. Chem. Soc.* **1980**, *102*, 374. Risley, J. M.; Van Etten, R. L. *Ibid.* **1980**, *101*, 252, 4609, 6699.

(16) 73.4% $^{18}O_2{}^{13}C$, 14.6% $^{18}O^{13}C$, 0.8% $^{16}O^{13}C$. Prepared as previously described.[4,5]

(17) The methyl/ether substituents at C-24,25 of 4 also have an erythro relationship. The origin of the C-25 oxygen atom is under investigation.

responding methyl groups most often have the L configuration.

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Registry No. **4a**, 65195-51-9; **4b**, 65195-53-1; **4c**, 65195-55-3; **4d**, 65195-57-5; acetic acid, 64-19-7; propionic acid, 79-09-4; carbon, 7440-44-0; oxygen, 7782-44-7.

Flexible New Synthetic Route to Daunomycinone, Adriamycinone, and Their 6-Deoxy Analogues[1]

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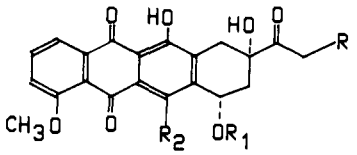
The total synthesis of naturally occurring anthracyclines and related analogue structures has been the subject of intense study since 1970, especially efforts directed toward preparation of the aglycones daunomycinone (**1**) and adriamycinone (**2**), which are

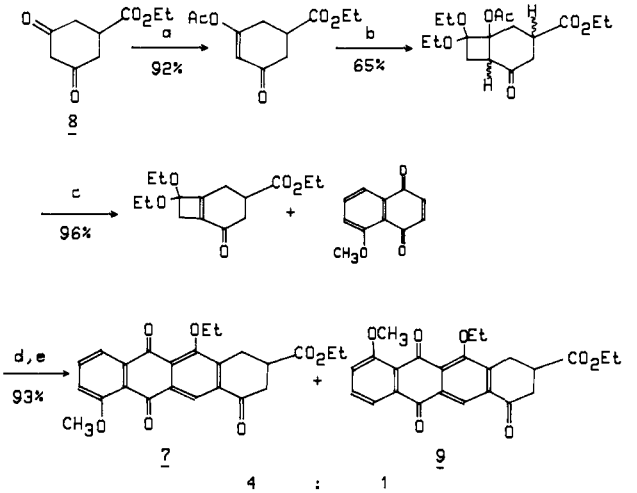


1, R = R_1 = H; R_2 = OH
2, R = OH; R_1 = H; R_2 = OH
3, R = H; R_1 = daunosamine; R_2 = OH
4, R = OH; R_1 = daunosamine; R_2 = OH
5, R = R_1 = R_2 = H
6, R = OH; R_1 = R_2 = H

components of the clinically useful antitumor agents daunorubicin (**3**) and adriamycin (**4**).[2,3] There remains, however, the need for synthetic routes that incorporate the oxygen function at C-7 at an early stage, since the existing methodology for this functionalization is inadequate especially with regard to scaleup to preparative levels.[3,4] It is, furthermore, desirable that these routes be inherently flexible permitting preparation of analogue structures differing in the substitution pattern in the anthraquinone nucleus.[4]

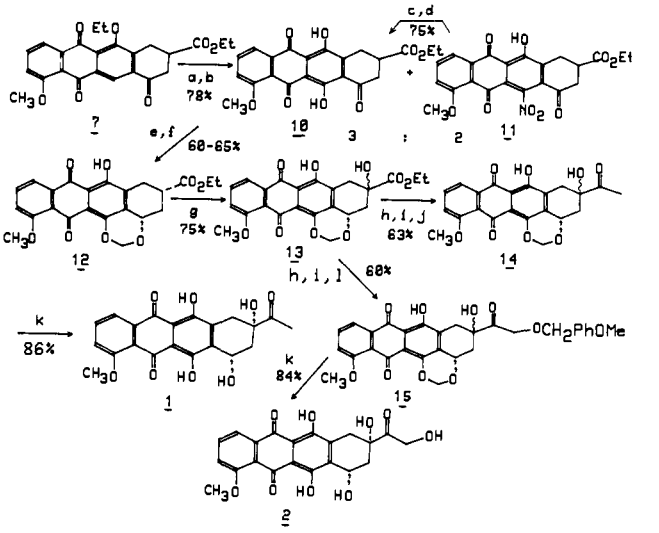
We have previously reported preliminary studies that defined the elements of a solution that meets the aforementioned criteria.[5] In the present paper, we describe the completion of our studies

Scheme I[a]



[a] Reagents: (a) Ac_2O, 70-80 °C, 16 h; (b) $CH_2=C(OEt)_2$ (4 equiv), Et_2O, *hν* (450-W Hanovia medium-pressure Hg lamp), room temperature, 40-48 h; (c) LiOEt (1 equiv), THF, room temperature, 1 h; (d) Δ (185-190 °C), mesitylene, 1.5 h; (e) NaOEt (catalyst), O_2, EtOH, room temperature, 10 min.

Scheme II[a]



[a] Reagents: (a) SbF_5 (10 equiv)-HF (100 equiv), CH_2Cl_2, -78 °C, 10 min; (b) anhydrous NH_4NO_3 (5 equiv), $(CF_3CO)_2O$ (20 equiv), CH_2Cl_2, room temperature, 1 h; (c) H_2, Pd-C, EtOAc; (d) $NaNO_2$, H_2SO_4, H_2O then Et_2NOH; (e) BH_3-THF (8 equiv), THF, 0 °C, 0.2 h; (f) $AgOSO_2CF_3$ (3 equiv), $ClCH_2OCH_3$ (3 equiv), CH_2Cl_2, room temperature, 0.5 h; (g) LDA (10 equiv), O_2, THF, -78 °C then $(EtO)_3P$ (2 equiv), -78 °C, 2 h; (h) 0.1% KOH, EtOH-H_2O (2:1), reflux, 0.5 h; (i) EtSH (3 equiv), 1,1'-carbonyldiimidazole (1.5 equiv), $Mg(OEt)_2$ (catalyst), DMF, room temperature, 16 h; (j) $LiCu(CH_3)_2$ (25 equiv), Et_2O-THF, 0 °C, 3 h; (k) CF_3COOH, room temperature, 4 h; then CH_3OH, reflux, 1 h; (l) $LiCu(CH_2OCH_2PhOCH_3)_2$ (25 equiv), THF, 0 °C, 3 h.

in this area which have culminated in the development of a highly flexible route to **1**, **2**, and related structures such as the potentially significant 6-deoxy series of aglycones **5** and **6**. The derived glycosides of **5** and **6**, along with their 11-deoxy counterparts, may possess reduced dose-dependent cardiotoxicity, which is a major problem associated with the clinical application of **3** and **4**.[6,7]

[†] Fellow of the A. P. Sloan Foundation 1976-1980; recipient of a Career Development Award (CA-00702) from the National Cancer Institute of the National Institutes of Health.

(1) Preliminary stages of this investigation were conducted in the Department of Chemistry, Wayne State University, Detroit, MI 48202.

(2) For a comprehensive referencing in this area, see: Kelly, T. R.; Vaya, J.; Anathasubramanian, L. *J. Am. Chem. Soc.* **1980**, *102*, 5983.

(3) (a) Dolson, M. G.; Chenard, B. L.; Swenton, J. S. *J. Am. Chem. Soc.* **1981**, *103*, 5263. (b) Broadhurst, M. J.; Hassall, C. H.; Thomas, G. J. *J. Chem. Soc., Chem. Commun.* **1982**, 158.

(4) Several such routes now exist; cf. ref 2 and 3 and papers cited therein. None permit access to 6-deoxy systems and other analogue series from common intermediates, and direct introduction of the C-7 and C-9 oxygen functions generally remains a problem. The recent work of Johnson et al. also utilizes a C-9 ketone as a means to introduce the C-9 oxygen at the stage of the tetracyclic system; however, only in the 7,11-deoxy series: Kimball, S. D.; Walt, D. R.; Johnson, F. *J. Am. Chem. Soc.* **1981**, 103, 1561.

(5) (a) Boeckman, R. K., Jr.; Delton, M. H.; Nagasaka, T.; Watanabe, T. *J. Org. Chem.* **1977**, *42*, 2946. (b) Boeckman, R. K., Jr.; Delton, M. H.; Dolak, T. R.; Watanabe, T.; Glick, M. D. *Ibid.* **1979**, *44*, 4781.

(6) Chlewbowski, R. T. *West. J. Med* **1979**, *131*, 364 and references therein.

(7) For a related application of this strategy to the preparation of (±)-alkavinone see: Boeckman, R. K., Jr.; Sum, F.-W *J. Am. Chem. Soc.* **1982**, *104*, 4604.

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