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PREPARATION AND CHARACTERIZATION OF NEW C-11 OXYGENATED ARTEMISININ DERIVATIVES

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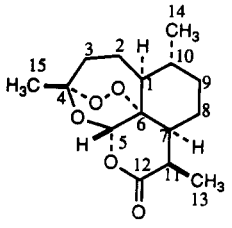
ABSTRACT.—Anhydrodihydroartemisinin [**2**], a potent antimalarial sesquiterpene endoperoxide, was converted to 11-hydroxyartemisinin [**4**], 11-hydroxy-11-*epi*-artemisinin [**5**], a ketone derivative **7** of artemisinin, and an enol ether epoxide **8**. Compounds **4**, **5**, **7**, and **8** were characterized completely by spectral methods including ^{1}H-nmr and ^{13}C-nmr spectroscopy. The stereochemistry of the epoxide **8** was established unequivocally by X-ray crystallographic analysis.

It has been recently suggested (1,2) that the introduction of an additional oxygen substituent at the polar edge of the molecule of artemisinin [**1**] would enhance its antimalarial activity. Recent publications (2,3) in this area have prompted us to report on our own findings. While the published work has focussed on epoxidizing anhydrodihydroartemisinin [**2**] as a means of reaching this goal, the present work has been extended to include also the hydroxylation of the same compound.

RESULTS AND DISCUSSION

Hydroxylation of anhydrodihydroartemisinin [**2**] by treatment with OsO_4 proceeded smoothly and quantitatively to produce the diol mixture **3**. That this mixture consisted, apparently, of the two C-11 hydroxy epimers in nearly 1:1 ratio was shown by tlc on Si gle G plates where it exhibited two pink spots, R_f 0.33 and 0.35, when sprayed with anisaldehyde spray reagent (4). Attempts to separate the mixture by flash



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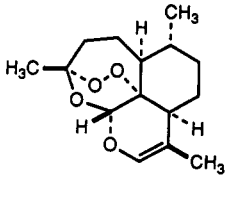

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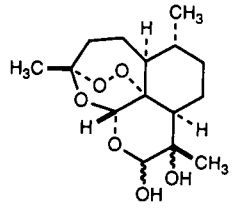

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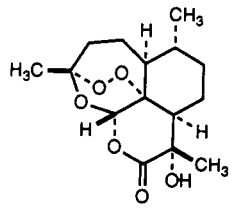

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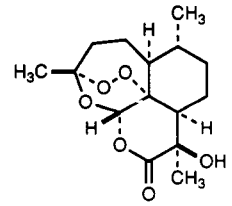

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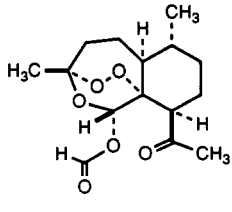

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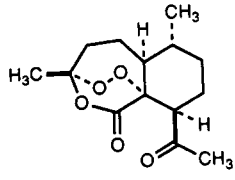

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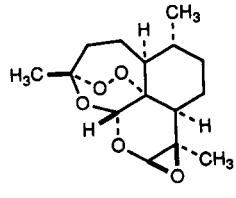

8

chromatography were unsuccessful; therefore, it was subjected to oxidation without further purification. The use of Jones reagent (5) at room temperature yielded primarily **4** in 51% yield, without any of the other epimer **5**, but several minor spots were observed including those of the unreacted starting material. When the oxidation was run at 0° to −5°, instead of at room temperature, the yield of **4** was unchanged, but **5** was obtained in about 7% yield and its production was not reproducible. It should be noted that oxidation of the diol mixture **3** using Jones reagent in Et_2O instead of Me_2CO produced the major product **7**, together with a trace of the previously reported ketone **6** (6). This suggested that the diol **3** had undergone a periodate-type cleavage under these conditions to produce **6** as an intermediate. The latter is then oxidatively converted to **7**. This hypothesis was confirmed by subjecting **6** to oxidation by Jones reagent to give **7** in 64% yield. Oxidation with Moffat reagent (7), on the other hand, yielded **4** and **5** in nearly equal yields (35%).

The other C-11 oxygenated artemisinin derivative obtained was the epoxide **8**, which was generated by treating the enol ether **2** with *m*-chloroperbenzoic acid in an $NaHCO_3$-buffered heterogeneous medium. The product was identical to a compound previously obtained by a different procedure (2). However, an attempt to confirm the stereochemistry of the epoxide group by a 2D nOe experiment was inconclusive. Furthermore, the C-13 methyl protons resonated at δ 1.31, a decidedly shielded position relative to that associated with the corresponding moiety in related compounds (2). It thus became imperative to confirm the stereochemical assignments at positions 11 and 12 by single-crystal X-ray analysis. The crystal structure was solved by direct methods (Multan11/82). Full matrix least-squares refinement of atomic positional and thermal parameters converged (max shift, ESD = 0.04) at $R = \Sigma||Fo|-|F_c||/\Sigma|F_o| = 0.040$, $R_w = [\Sigma w(|F_o|-|F_c|)^2/\Sigma w|F_o|^2]^{1/2} = 0.057$, $GOF = [\Sigma w(|F_o|-|F_c|)^2/(N_{observations}-N_{parameters})]1/2 = 1.67$. A view of the solid-state conformation is provided in Figure 1. Bond lengths are in accord with expectations (8).[1]

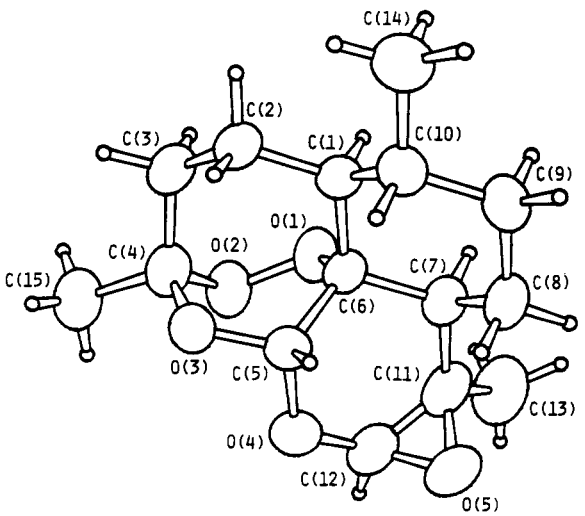

FIGURE 1. Atom numbering scheme and solid-state conformation of epoxide [**8**]; small circles represent hydrogen atoms.

[1]Atomic coordinates for epoxide **8** have been deposited at the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

The present report constitutes the first on the formation of the two epimeric 11-hydroxy derivatives of artemisinin [**1**]. Hydroxylation of **2**, unlike epoxidation, produces more of the isomer which retains stereochemistry at C-13. This could be necessary for maintaining a high level of antimalarial activity, since it is known that 11-*epi*-artemisinin is much less active than artemisinin itself (9).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Ir spectra were obtained on Perkin-Elmer 580 IR, and specific rotations were obtained on 241 MC or Jasco digital polarimeter model DIP-370 instruments. Nmr spectra were determined on a Varian XL 200 spectrometer or a Varian VXR-300 spectrometer, and chemical shift values are given in δ (ppm) with TMS as internal standard. Standard Varian pulse sequences were used for DEPT, APT, and HETCOR spectra, which aided nmr assignments. Low-resolution eims (70 eV) was obtained using an E.I. Finnigan model 4600 quadrupole system, while cims was obtained using CH_4 as ionizing gas.

Hrfabms was carried out at the University of Kansas. X-ray crystallographic measurements were made on an Enraf-Nonius CAD-4 diffractometer (Cu-Kα radiation, λ = 1.5418 Å; incident-beam graphite monochromator). Tlc was performed on Si gel G plates using Et_2O–*n*-hexane (4:1), unless otherwise specified, and visualized by spraying with anisaldehyde reagent (4). *Artemisia annua* L. (Asteraceae) was grown in Riyadh, Saudi Arabia at the medicinal plant garden. Greenhouse-grown plants were planted in early November 1987. Leaves were picked at the pre-flowering stage in early April 1988. A voucher specimen is preserved at the herbarium of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Artemisinin [**1**] used in preparing **2** was isolated from the plant material using a literature procedure (10).

OsO_4 HYDROXYLATION OF **2** TO THE DIOL MIXTURE **3**.—Anhydrodihydroartemisinin [**2**] (1.0 g) (6,11) was dissolved in pyridine (5 ml), OsO_4 (1.5 g) was added, and the mixture was stirred for 2 h. A 10% solution of sodium metabisulfite was added (100 ml), and the mixture was stirred for 35 min. The mixture was extracted with CH_2Cl_2 (500 ml × 3) and washed with 10% HCl (200 ml × 2), $NaHCO_3$ (200 ml × 2), and finally H_2O (400 ml × 3). Drying over anhydrous Na_2SO_4 and evaporation yielded 1.1 g of a crystalline mixture **3** of the diols which showed two spots of almost equal intensity when analyzed on Si gel G tlc plates, R_f 0.33 and 0.35.

OXIDATION OF THE DIOL MIXTURE **3** TO 11-HYDROXYARTEMISININ [**4**].—The diol mixture **3** (270 mg) was dissolved in Me_2CO (10 ml), and Jones reagent (5) (1.5 ml) was added. The reaction mixture was stirred at room temperature for 1.2 h, and MeOH (8 ml) was added to quench excess reagent. After additional stirring for 10 min, H_2O (70 ml) was added and the mixture was extracted with $CHCl_3$ (300 ml × 3). The $CHCl_3$ phase was washed with 1% $NaHCO_3$ (100 ml × 2) followed by H_2O, dried over anhydrous Na_2SO_4, and evaporated to leave 234 mg of an oily residue. Tlc analysis revealed the presence of several minor spots and a major spot, R_f 0.55, corresponding to **4**. Flash chromatography on Si gel using *n*-hexane–Et_2O (7:3) as a solvent provided **4** (140 mg) as colorless needles from Et_2O–*n*-hexane, mp 137–138°; $[\alpha]_D$ +81° (*c* = 0.1, in MeOH); ir (KBr) ν max (cm^{-1}) 3565 (free OH), 3480 (bonded OH), 1740 (lactone CO); ^1H nmr ($CDCl_3$) δ 5.92 (1H, s, H-5), 4.24 (1H, s, exchangeable, OH), 2.41 (1H, ddd, *J* = 4.0, 13.0, 15.0 Hz, H-3α), 2.08 (1H, m, H-3β), 1.92–2.02 (3H, m, H-2α, H-7, H-8α), 1.78 (1H, dddd, *J* = 3.0, 3.0, 13.0, 13.0 Hz, H-9β), 1.52 (1H, m, H-1), 1.49 (1H, m, H-2β), 1.47 (3H, s, Me-15), 1.41 (3H, s, Me-13), 1.39 (1H, m, H-10), 1.21 (1H, m, H-8β), 1.08 (1H, m, H-9α), 1.00 (3H, d, *J* = 6.0 Hz, Me-14); ^{13}C nmr see Table 1; cims (CH_4) *m/z* $[MH]^+$ 299 (10%) calcd C 60.39, H 5.07; found C 60.41, H 5.05.

Repeating the same reaction at −5 to 0° gave 137 mg of **4** and 7 mg of **5**. However, the production of **5** was not reproducible.

OXIDATION OF THE DIOL MIXTURE **3** TO **6** AND **7**.—Repeating the reaction (50 mg of **3**) with Jones Reagent using Et_2O as a solvent, instead of Me_2CO, and with stirring at room temperature for 6 h, gave upon workup as before, 33 mg of a mixture of products that was separated by flash chromatography on Si gel, using Et_2O–*n*-hexane (1:9) as a solvent to give 2 mg of **6** R_f 0.72, [physical and spectral data identical to those reported (6)] and 17 mg of **7**: R_f 0.61; mp 128–129°, $[\alpha]_D$ 83° (*c* = 0.109, $CHCl_3$); ir ν max (KBr) (cm^{-1}) no OH bands, 1710 (Me-CO), 1745 (lactone CO); cims (NH_3) (% rel. int.) *m/z* $[M + NH_3]^+$ 286 (92) with the base peak at *m/z* $[M + NH_3\text{-}16]^+$ 270; ^1H nmr ($CDCl_3$) δ 2.47 (3H, s, Me-13), 2.44 (1H, m, H-7), 2.36 (1H, m, H-3α), 2.27 (1H, m, H-8α), 2.22 (1H, m, H-3β), 2.20 (1H, m, H-8β), 2.01 (1H, m, H-2α), 1.77 (1H, dddd, *J* = 13.0, 13.0, 3.0, 3.0 Hz, H-9β), 1.62 (3H, s, Me-

TABLE 1. ^{13}C-nmr Chemical Shift Assignments.[a]

Carbon	Compound				
	2	**4**	**5**	**7**	**8**
C-1	51.5(1)	49.9(1)	49.5(1)	49.9(1)	51.7(1)
C-2	24.4(2)	24.7(2)	24.7(2)	24.4(2)	24.3(2)
C-3	36.2(2)	35.7(2)	35.8(2)	34.8(2)	36.2(2)
C-4	104.5(0)	105.5(0)	105.4(0)	108.8(0)	104.5(0)
C-5	89.7(1)	94.0(1)	94.8(1)	165.5(0)	90.5(1)
C-6	79.0(0)	82.4(0)	80.9(0)	85.7(0)	78.2(0)
C-7	44.5(1)	50.2(1)	50.7(1)	58.1(1)	45.1(1)
C-8	30.0(2)	25.2(2)	24.4(2)	26.6(2)	24.2(2)
C-9	34.1(2)	33.6(2)	33.4(2)	33.6(2)	33.4(2)
C-10	37.5(1)	37.6(1)	37.7(1)	34.1(1)	37.0(1)
C-11	108.1(0)	71.8(0)	71.4(0)	204.6(0)	56.2(0)
C-12	135.0(1)	172.2(0)	175.7(0)	—	82.6(1)
C-13	16.2(3)	24.3(3)	31.5(3)	28.0(3)	22.1(3)
C-14	20.3(3)	19.7(3)	19.8(3)	19.2(3)	20.1(3)
C-15	25.9(3)	25.2(3)	24.9(3)	24.3(3)	26.0(3)

[a]The number in parentheses indicates the number of hydrogens attached to the corresponding carbon and was determined by DEPT GL experiments. Assignments are based on 1D and 2D nmr experiments and literature comparison with published artemisinin assignments (13,14).

15), 1.39 (1H, m, H-1), 1.37 (1H, m, H-10), 1.24 (1H, m, H-2β), 1.12 (1H, m, H-9α), 0.93 (3H, d, J = 6.0 Hz, Me-14); ^{13}C nmr see Table 1.

MOFFAT OXIDATION OF THE DIOL MIXTURE **3** TO 11-HYDROXYARTEMISININ [**4**] AND 11-HYDROXY-11-*epi*-ARTEMISININ [**5**].—The diol mixture **3** (0.60 g) was dissolved in C_6H_6 (4 ml) and added to a stirring solution of dicyclohexylcarbodiimide (2.5 g) in C_6H_6 (4 ml) containing anhydrous phosphoric acid (225 mg) and DMSO (8 ml). After 24 h, the reaction mixture was worked up by adding 10% MeOH oxalic acid solution (10 ml) and stirred with H_2O (100 ml) for 90 min. The mixture was extracted with $CHCl_3$ (500 ml × 3), washed with H_2O (200 ml × 2), dried over anhydrous Na_2SO_4, and evaporated. The residue was dissolved in CH_2Cl_2 (100 ml) and filtered to remove the urea. The residue left after evaporating the CH_2Cl_2 was found to show two main spots upon tlc as before, R_f 0.55 and 0.65, due to **4** and **5**, respectively, together with some unreacted diol. Flash chromatography yielded 210 mg of **5** and 207 mg of **4** in addition to 42 mg of unreacted diol mixture. Compound **4** was identified by comparison with the product of Jones oxidation obtained above (same mp and mmp and indistinguishable spectra). Compound **5** was obtained as colorless needles: mp 128.5–129°; $[\alpha]_D$ +62° (c = 0.1, MeOH); ir (KBr) ν max (cm^{-1}) 3570 (free OH); 3250 and 3460 (two bands for bonded OH), 1741 (lactone CO); ^{1}H nmr ($CDCl_3$) δ 5.90 (1H, s, H-5), 2.96 (1H, br s, exchangeable, OH), 2.43 (1H, m, H-3α), 2.31 (1H, m, H-8α), 2.08 (1H, m, H-3β), 2.02 (1H, m, H-2α), 1.77 (1H, m, H-9β), 1.75 (3H, s, Me-13), 1.48 (1H, m, H-2β), 1.46 (3H, s, Me-15), 1.36 (2H, m, H-1, H-10), 1.03–1.10 (2H, m, H-8β, H-9α), 0.99 (3H, d, J = 6.0 Hz, Me-14); ^{13}C nmr see Table 1; cims (CH_4) m/z $[M+1]^+$ 299 (9%). Calculated for $C_{15}H_{22}O_6$, C 60.39, H 5.07; found C 60.28, H 5.11.

OXIDATIVE CONVERSION OF **6** TO **7**.—Compound **6** (50 mg) (6) was oxidized with Jones reagent as in the preceding expeirment to give 32 mg of **7** (indistinguishable physical and spectral data).

EPOXIDATION OF **2** TO ENOL ETHER EPOXIDE **8**.—Anhydrodihydroartemisinin [**2**] (200 mg) was dissolved in CH_2Cl_2 (15 ml), and *m*-chloroperbenzoic acid (190 mg in 0.5 N $NaHCO_3$) was added. The reaction mixture was vigorously stirred at room temperature for 75 min, after which the reaction was complete as shown by tlc. The reaction mixture was diluted to 300 ml with CH_2Cl_2 and washed with 5% Na_2SO_3 (100 ml × 3), 5% $NaHCO_3$ (100 ml × 3), and finally with H_2O (100 ml × 3). After drying over anhydrous Na_2SO_4 and evaporation, the tlc analysis using *n*-hexane–Et_2O (3:2) revealed the presence of a major spot, R_f 0.70, corresponding to **8**. Flash chromatography on Si gel using *n*-hexane–Et_2O (8:2) as a solvent provided **8** (141 mg) as colorless crystals from Et_2O/*n*-hexane: mp 118°; $[\alpha]_D$ +1.625° (c = 0.032, MeOH), hrfabms $[M-H]^+$ 283.1561 (consistent with the formula $C_{15}H_{22}O_5 + H^+$, calcd 283.1546); ^{1}H nmr ($CDCl_3$) δ 5.27 (1H, s, H-5), 4.94 (1H, s, H-12), 2.31 (1H, ddd, J = 4.0, 13.0, 14.5 Hz, H-

3α), 2.02 (1H, ddd, 5.0, 3.0, 14.5 Hz, H-3β), 1.74–1.94 (3H, m, H-8α, H-7, H-2α), 1.40–1.59 (2H, m, H-8β, H-2β), 1.69 (1H, m, H-9β), 1.42 (3H, s, Me-15), 1.32 (1H, m, H-1), 1.31 (3H, s, Me-13), 1.29 (1H, m, H-10), 1.08 (1H, m, H-9α), 0.95 (3H, d, J = 6.0 Hz, Me-14); ^{13}C nmr see Table 1.

X-RAY CRYSTAL STRUCTURE ANALYSIS OF COMPOUND **8**.—Crystal data: $C_{15}H_{22}O_5$, MW = 282.34, orthorhombic, space group $P2_12_12_1(D_2^4)$, No. 19, a = 9.092 (1), b = 25.250 (2), c = 6.273 (1) Å (from 25 orientation reflections, 36°<θ<40°), V = 1440.1 (5) Å^3, Z = 4, D_c = 1.302 g·cm^{-3}, μ (CuKα radiation, λ = 1.5418 Å) = 7.6 cm^{-1}. Crystal dimensions 0.36 × 0.40 × 0.44 mm. One octant of intensity data was recorded on an Enraf-Nonius CAD-4 diffractometer [CuKα radiation, ω–2θ scans, θ_{max} = 75°, scanwidth (0.80 + 0.14 tan θ)°]. The intensities of four reference reflections, remeasured every 2 h during data collection to monitor crystal stability, indicated that significant deterioration occurred (overall intensity loss = 7%). From a total of 1732 measurements, those 1436 reflections with $I>3.0\sigma(I)$ were retained for the analysis. In addition to the usual Lorentz and polarization corrections, a linear decay correction was also applied.

The crystal structure was solved by direct methods (MULTAN11/82). Approximate coordinates for all carbon and oxygen atoms were derived from an E map. Hydrogen atoms were all located in a difference Fourier synthesis evaluated following several rounds of full-matrix least-squares refinement of non-hydrogen atom positional and thermal parameters (at first isotropic, then anisotropic). In the subsequent least-squares iterations, hydrogen atom positional and isotropic thermal parameters, and latterly also an extinction correction (g), were included as variables. The parameter refinement converged at R = 0.40 [R_w = 0.057, GOF = 1.67, g = 5.9(3) × 10^{-6}]. A final difference Fourier synthesis contained no unusual features (max. $\Delta\rho$ = 0.21e/Å^3).

Crystallographic calculations were performed on PDP11/44 and Micro-VAX computers by use of the Enraf-Nonius Structure Determination Package (SDP). For all structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from literature (12). In the least-squares iterations, $\Sigma w\Delta^2[w = 1/\sigma^2(|F_o|), \Delta = (|F_o| - |F_o|)]$ was minimized.

LITERATURE CITED

1. D.S. Ellis, Z.L. Li, H.M. Gu, W. Peters, B.L. Robinson, G. Tovey, and D.C. Warhurst, *Ann. Trop. Med. Parasitol.*, **79**, 1367 (1985).
2. O. Petrov and I. Ognyanov, *Collect. Czech. Chem. Commun.*, **56**, 1037 (1991).
3. A.J. Lin, L. Li, W.K. Milhous, and D.L. Klayman, *Med. Chem. Res.*, **1**, 20 (1991).
4. F.S. El-Feraly and C.D,. Hufford, *J. Org. Chem.*, **47**, 1527 (1982).
5. C. Djerassi, R.R. Engle, and A. Bowers, *J. Org. Chem.*, **32**, 1249 (1989).
6. F.S. El-Feraly, A. Ayalp, M.A. Al-Yahya, D.R. McPhail, and A.T. McPhail, *J. Nat. Prod.*, **53**, 66 (1990).
7. K.E. Pfitzner and J.G. Moffat, *J. Am. Chem. Soc.*, **85**, 3027 (1963).
8. F.H. Allen, O. Kennard, D.G. Watson, L. Brammer, A.G. Orpen, and R. Taylor, *J. Chem. Soc., Perkin Trans. 2*, S1 (1987).
9. N. Acton and Daniel L. Klayman, *Planta Med.*, **53**, 266 (1987).
10. H.N. ElSohly, E.M. Croom, F.S. El-Feraly, and M.M. El-Sherei, *J. Nat. Prod.*, **53**, 1560 (1990).
11. A.J. Li, M. Lee, and D.L. Klayman, *J. Med. Chem.*, **32**, 1249 (1989).
12. J.A. Ibers and W.C. Hamilton, Eds., "International Tables for X-Ray Crystallography," The Kynoch Press, Birmingham, U.K., 1974, Vol. IV.
13. G. Blasko, G.A. Cordell, and D.C. Lankin, *J. Nat. Prod.*, **51**, 1273 (1988).
14. W. Zhongshan, T.T. Nakashima, K.R. Kopecky, and J. Molina, *Can. J. Chem.*, **63**, 3070 (1985).

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