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SYNTHESIS AND *IN VITRO* ANTI-HUMAN IMMUNODEFICIENCY VIRUS ACTIVITY OF ARTEMISININ (QINGHAOSU)-RELATED TRIOXANES

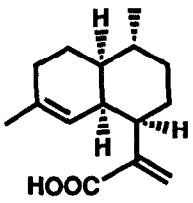
Mankil Jung*[a] and Raymond F. Schinazi[b]

[a]Research Institute of Pharmaceutical Sciences & Department of Medicinal Chemistry, School of Pharmacy, University of Mississippi, University, MS 38677
[b]Veterans Affairs Medical Center & Department of Pediatrics, Emory University School of Medicine, Decatur, GA 30033

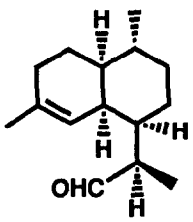
Abstract: A series of artemisinin (qinghaosu)-related trioxanes has been prepared and assayed *in vitro* for anti-HIV activity. One of these compounds, 12-n-butyldeoxoartemisinin shows a good antiviral activity against HIV-1.

Artemisinin (Qinghaosu), **5**, isolated from *Artemisia annua*, L., is a sesquiterpene lactone bearing an unusual cyclic peroxide function[1]. This natural product is of special interest because of its outstanding anti-malarial activity[2a], *in vitro* activity against *Pneumocystis carinii*[2b] and novel structure[2a, c-d]. Unique endoperoxide within the molecule has prompted us to prepare artemisinin-related trioxanes and evaluate their *in vitro* anti-HIV activity. In this communication, we would like to report, for the first time, the structure-activity relationship of artemisinin derivatives.

A series of artemisinin-related compounds has been prepared from artemisinic acid, **1**[3-8]. Thus, reduction of artemisinic acid, **1** with sodium borohydride ($NiCl_2$, CH_3OH)[9] to dihydroartemisinic acid, followed by photooxygenative cyclization and acidic treatment of photo adducts afforded artemisinin, **5** in 21 % yield. Direct reduction of artemisinin, **5** with $NaBH_4$ in the presence of $BF_3.Et_2O$ in THF afforded deoxoartemisinin, **6** in 75 % yield[4]. Compound **6** was also synthesized in 35 % yield *via* chiral photooxygenative cyclization as a penultimate step from dihydroartemisinyl alcohol **3a**, prepared from artemisinic acid, **1**[5]. Treatment of artemisinin, **5** with $NaBH_4$ in methanol (0°C, 1h., 89 % yield) to dihydroartemisinin **7** and subsequent etherification in ethanol under acidic catalysis ($BF_3.Et_2O$) in anhydrous benzene (reflux, 1h.) afforded ß-arteether **8** according to the literature[6]. Reaction of dihydroartemisinyl aldehyde **2**, prepared from **1** by a literature procedure[7], with n-butylmagnesium chloride gave alcohol **3b**. Aldehyde **2** was also used to prepare **4**[7]. Photooxygenative cyclization, as previously mentioned, of **3b** and **4** provided 12-n-butyldeoxoartemisinin **9**[8] and homodeoxoartemisinin **10**[7] in 12 % and 21 % yields, respectively. Compound **13** was prepared in 86 % yield from (R)-(+)-3-methylcyclohexanone with (methoxymethyl)triphenylphosphonium chloride in the presence of phenyllithium in anhydrous ether



1



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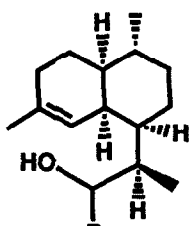
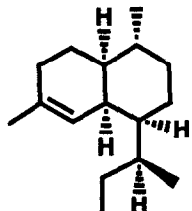


3a: R = H
b: R = n-butyl



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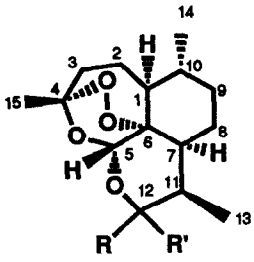
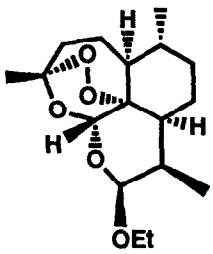


5: R = R' = O
6: R = R' = H
7: R = H, R' = OH



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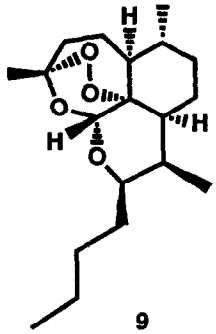

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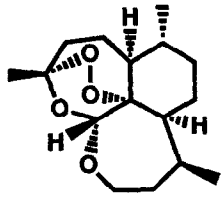

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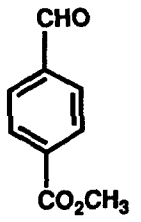
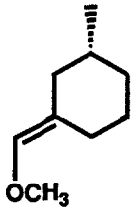
Scheme 1

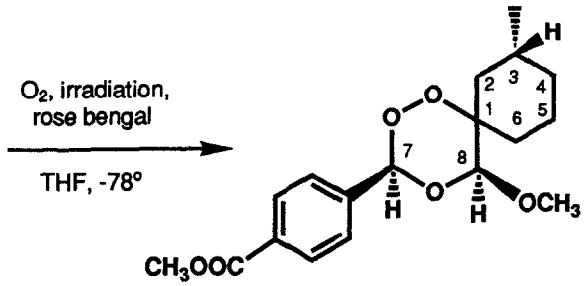


12

+



13



11

(r.t., 15 h.). Chiral photooxygenative cyclization of **13** with **12** in the presence of oxygen (irradiation with 450 watts high pressure mercury arc lamp, 3 h., rose bengal as a sensitizer, -78 °C in THF) afforded trioxane **11**[10,11] in 51 % yield (Scheme 1). The assignment of ^{1}H NMR and ^{13}C NMR signals were made on the basis of 2D-COSY and HETCOR spectra of **11**. The relative configuration at the new chiral centers (C-7 and C-8) was unambiguously determined as depicted in **11** by utilization of two dimensional NOE (NOSEY) techniques.

The anti-HIV activity of artemisinin and its related trioxanes was determined in human peripheral blood mononuclear (PBM) cells[12] acutely infected with HIV-1 LAI and presented in Table 1. Compounds **5**, **6**, and **11** do not exhibit any significant anti-HIV activity (EC_{50}>100 μM) while **8** and **10** show a moderate anti-HIV activity. 12-n-Butyldeoxoartemisinin **9** shows a modest *in vitro* antiviral activity against HIV-1 LAI (EC_{50} = 4.7 μM) comparable to that of 2',3'-dideoxyinosine (EC_{50} = 5.5 μM), but significantly less than 3'-azido-3'-deoxythymidine (AZT). However, this compound was toxic to human PBM cells with an IC_{50} value of 1.3 μM, suggesting that the antiviral effect could be secondary to the toxic effect. The mechanism of activity of artemisinin-related trioxanes against HIV is unknown. However, it is known that artemisinin and its derivatives act as free-radical generators[13]. We propose activated oxygen may mediate the anti-HIV activity of the trioxanes.

Table 1

Median Effective (EC_{50}) and Inhibitory (IC_{50}) Concentrations of Various Trioxanes in acutely HIV-1 infected PBM cells

Compound	EC_{50} (μM)	IC_{50} (μM)[a]
5	>100	>100
6	>100	>100
8	41.2	30.9
9	4.7	1.3
10	50.2	>100
11	>100	>100
AZT	0.004	>100

[a]Cytotoxicity was measured using ^{3}H-thymidine uptake in human PBM cells.

In conclusion, we found 12-n-butyldeoxoartemisinin possesses a modest anti-HIV activity. This is the first report on anti-HIV activity of artemisinin derivatives, although the compound is not selective against this virus. Artemisinin is virtually non-toxic (LD_{50} = 4228 mg/Kg orally administered to mice) and without carcinogenicity[2a] suggesting that this class of compounds, and in particular 12-n-

butyldeoxoartemisinin deserves further evaluation as a potential antiviral agent for treatment of HIV infections, especially if non-toxic congeners can be synthesized.

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11. For **11**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.05 and 7.62 (m, 4H, aromatic), 6.24 (s, 1H, 7-H), 4.65 (s, 1H, 8-H), 3.92 (s, 3H, CO_2CH_3), 3.56 (s, 3H, OCH_3), 2.53-2.35 (m, 1H, 2-Hα), 2.05-1.85 (m, 1H, 3-H), 1.71-1.61 (m, 7H), 0.98 (d, J=9 Hz, 3H, CH_3 at C3). IR ($CHCl_3$): max 3005, 2960, 2850, 1720, 1620, 1440, 1280, 1120, 1070, 1050, 1020, 970, 910. MS (70 eV): m/e 336 (M^+).

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