Antimalarial Activity of 1,4-Epidioxy-bisabola-2,12-diene Derivatives[1] ☆

Gerhard Rücker[a]*, Eberhard Breitmaier[b], Detlef Manns[a], Walter Maier[c], Anne Marek[a], Berta Heinzmann[a], Klemens Heiden[a], and Stephan Seggewies[a]

[a] Institut für Pharmazeutische Chemie der Universität Bonn, Kreuzbergweg 26, D-53115 Bonn

[b] Institut für Organische Chemie und Biochemie der Universität Bonn, Gerhard-Domagk-Str. 1, D-53121 Bonn

[c] Institut für Medizinische Parasitologie der Universität Bonn, Sigmund-Freud-Str. 25, D-73127 Bonn

Key Words: *1,4-Epidioxy-bisabola-2,12-diene derivatives; aromatic hydroperoxides; in vitro antimalarial activity; photoxidation of γ-curcumene*

Summary

1,4-Epidioxy-bisabola-2,12-diene (**3**) and aromatic hydroperoxides (**4**, **5**) were prepared by photoxidation of γ-curcumene (**1**). Reduction and esterification of **6** and **7** afforded compounds **9** to **10**. All compounds were tested *in vitro* for antimalarial activity. The activity could not be increased significantly, compared with **3**. The most active compounds, **3** and **9**, did not show *in vivo* antimalarial activity in mice.

Introduction

There has been a rapid increase in the resistance of malaria parasites, in particular of *Plasmodium falciparum*, to currently used drugs[2]. In previous studies the ascaridol homologue 1,4-epidioxy-bisabola-2,12-diene (**3**), isolated from *Heterothalamus psiadioides, Heterothalamus alienus*[3], *Kaunia rufescens* (syn. *Eupatorium rufescens* Lund ex de Candolle)[15] and *Senecio selloi*[8] plants from Brazil, showed an effective antimalarial activity *in vitro*. This prompted us to derivatize the molecule in order to increase the activity[3,5].

Results

γ-Curcumene (**1**), isolated from the essential oil of *Helichrysum italicum*, was used as starting material for the preparation of ascaridol analogue peroxides. According to the reaction constants determined by Monroe[6] for terpinene (100×10^6 L/(Ms)) and isopren (3.7×10^4 L/(Ms)), the cyclohexadiene structure reacts more readily than the olefinic structure in the side chain. Photooxidation of **1** with daylight, air, and bengalrose affords 1,4-epidioxy-bisabola-2,12-diene (**3**) only, while the use of an UV-radiating lamp and oxygen results in **3** as well as the products **4**–**7**. The diene **1** is sensitive to aromatization, giving α-curcumene (**2**) during the reaction, which on photooxidation affords the hydroperoxides **4** and **5**, due to ene reactions[7]. The aromatic hydroperoxides **4** and **5** were identified by TLC also as constituents of the Brazilian plant *Senecio selloi* Spreng. by photooxidation of **2** isolated from the plant[8].

Chromatographic separation of **6** and **7** with silver nitrate impregnated silica gel affords another side product, the ketone **8**. Reduction of **6** and **7** with triphenylphosphine (PPh_3)[9] leads to the alcohols **9** and **10a**. If the reduction of **6** and **7** is carried out in the mixture, and **9** and **10a** are separated by CC on silica gel, no decomposition to **8** occurred and the yield is increased. Alcohol **10a** was transformed into the esters **10b–10d**[5].

The structures of the compounds were characterized by MS, 1D and 2D ^{1}H NMR- and ^{13}C NMR spectroscopy. Due to the chiral atoms C-1, C-4, and C-8, two diastereomeric pairs of enantiomers are built, which on hydroperoxidation at the carbon atoms C-12 and C-13 can form several isomers (see Tables 2 and 3). No stereochemistry studies were carried out so far.

Table 1. EC_{50}-values determined for compounds **3–11d**, chloroquinediphosphate, and artemisinin against *Plasmodium falciparum*, strain NF-54.

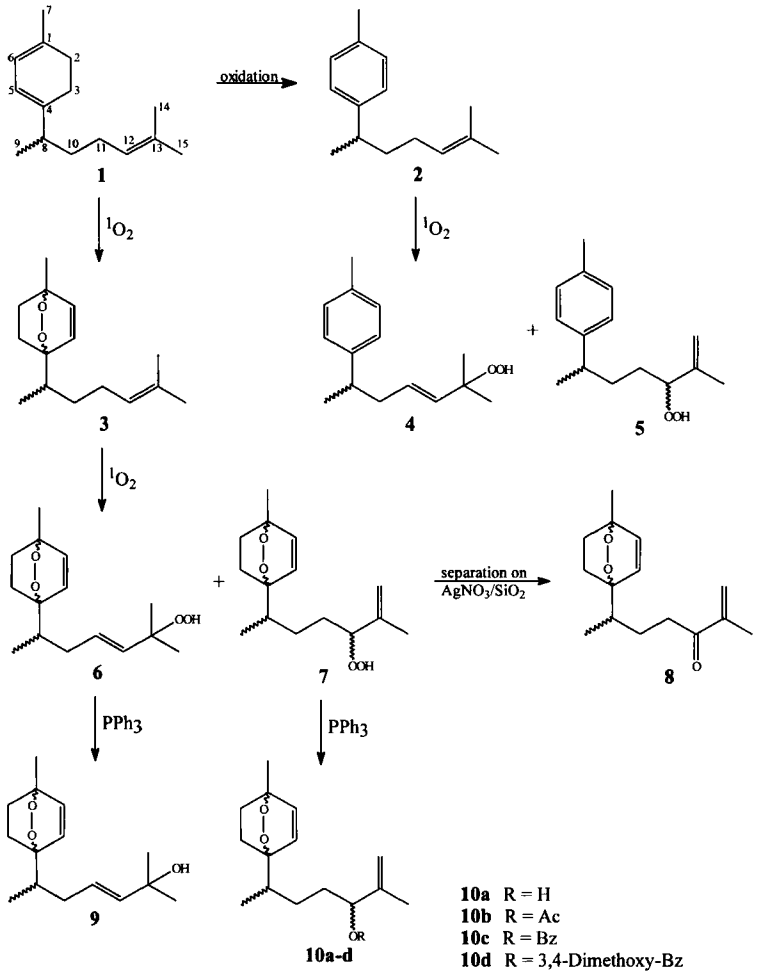
Compound	EC_{50} [μg/ml]	EC_{50} [nmol/ml]	95 % C.L.	Std. dev.
3	0.05	0.21	8.86×10^{-3}	5.57×10^{-3}
4	>1	>4.3	–	–
5	0.10	0.43	1.08×10^{-2}	3.38×10^{-3}
6	0.07	0.30	1.08×10^{-2}	6.81×10^{-3}
7	0.10	0.37	1.39×10^{-2}	8.75×10^{-3}
8	0.25	1.0	1.75×10^{-2}	1.10×10^{-2}
9	0.04	0.15	2.00×10^{-3}	1.25×10^{-3}
10a	0.11	0.43	1.74×10^{-2}	1.09×10^{-2}
10b	0.06	0.20	0.017	1.06×10^{-2}
10c	0.08	0.29	–	6.32×10^{-4}
10d	0.09	0.31	–	1.26×10^{-2}
chloroquine × 2 PO_4	0.01	0.02	3.61×10^{-3}	2.27×10^{-3}
artemisinin	0.005	0.017	1.80×10^{-4}	1.13×10^{-4}

Compounds **3–10d** were tested *in vitro* against *Plasmodium falciparum* and their EC_{50} values were determined (Table 1). The best results, compared to artemisinin[2] (EC_{50} < 0.005 μg/ml) were obtained for compounds **3** and **9** (EC_{50} = 0.05 and 0.04 μg/ml respectively). The activity could not be increased significantly by derivatization of **3**. **3** and **9** were tested on mice, but were found to be inactive.

Acknowledgments

Financial support given by the *Fonds der Chemischen Industrie*, Frankfurt is gratefully appreciated.

  0365-6233/97/0102-0012 $17.50 +.50/0



Experimental Section

General

CC was executed with silica gel (0.063–0.200 mm, Merck, Darmstadt) or silica gel impregnated with 20% of $AgNO_3$[10]. Analytical TLC: precoated glass plates (60 F_{254}, layer thickness 0.25 mm, Merck, Darmstadt); detection UV – 254 nm, R-1 = anisaldehyde/H_2SO_4[11], R-2 = peroxide reagent[12]. FAB-MS: 70eV; rel. intensities in %; if not indicated otherwise; ^{1}H NMR were measured at 300 MHz and ^{13}C NMR at 75 MHz in $CDCl_3$.

γ-Curcumene (**1**)

The separation of 3 g of the essential oil of *Helichrysum italicum* (Melchers, Bremen) by means of CC on silica gel (light petroleum) yielded 350 mg of a mixture, which was separated with $AgNO_3$ impregnated silica gel (light petroleum:diethyl ether:toluene (98:2:1)), affording 220 mg of **1**: colourless oil; R_f 0.6–0.7 (light petroleum). Spectroscopic data are identical with ref.[13]. $[\alpha]_D^{22} = -76.5°$ ($c = 0.996$ in $CHCl_3$); literature values of optical rotation of **1** are reported different[13].

Photoxidation

To 500 mg of **1** in 250 ml $CHCl_3$, 50 mg of bengal rose, dissolved in 10 ml of methanol, were added. The solution was exposed to an UV-radiating lamp (Hg-Dampf-Hochdruckstrahler TQ 150 (Hanau) or HPK 125 (Philips)) for 15 min at –10 °C with oxygen bubbling vividly through it. This mixture was evaporated *in vacuo* at room temp. and separated by CC on silica gel (light petroleum:acetone (88:12)), giving, after evaporation *in vacuo* at room temp., the fractions I–V.

1,1-Dimethyl-5-(4-methyl-2,3-dioxabicyclo[2.2.2]oct-5-en-1-yl)-1-hexene (1,4-Epidioxy-bisabola-2,12-diene) (**3**)

Fraction II (R_f 0.56; light petroleum:ethyl acetate (90:10)) gave 10 mg of colourless oil. Spectroscopic data identical to ref. [3,14].

(E)-1-Hydroperoxy-1,1-dimethyl-5-(4-methylphenyl)-2-hexene (**4**)

Fraction III of the separation of the photoxidation mixture contained 60 mg of a mixture of **4** and **5**, which were separated by CC on silica gel impregnated with 10% of $AgNO_3$ (light petroleum:acetone (95:5)), giving 24 mg of **4**, slightly impure because of fast decomposition. Yellow oil.– TLC: R_f 0.15 (light petroleum:acetone (95:5)), R-1: violet, R-2: immediately blue.– FAB-MS (70 eV, m-NBA + NaOAc); *m/z* (%) = 257 (3, $M+Na^+$). 217 (2), 201 (11, M^+–OOH), 176 (12), 119 (100. $C_9H_{11}^+$). ^{1}H NMR and ^{13}C NMR data Table 2 and 3.

2-Hydroperoxy-1-(1-methylethenyl)-4-(4-methylphenyl)-pentane (**5**)

Separation of Fraction III (see **4**) gave 30 mg of **5**. Colourless oil.– TLC: R_f 0.18 (*n*-hexane:diethyl ether (90:10)), R-1: red-orange, R-2: immediately blue.– $[\alpha]_D^{20}$: –17.57° ($c = 0.64$, $CHCl_3$).– FAB-MS (70 eV, m-NBA) = 257 (27, $M+Na^+$), 217 (4), 201 (17, M^+–OOH), 176 (31), 119 (100, $C_9H_{11}^+$). ^{1}H NMR and ^{13}C NMR data Table 2 and 3.

(E)-1-Hydroperoxy-1,1-dimethyl-5-(4-methyl-2,3-dioxabicyclo[2.2.2]oct-5-en-1-yl)-2-hexene (**6**)

Fraction IV of the separation of the photoxidation mixture contained 300 mg of a mixture of **6** and **7**, which were separated by CC on silica gel, impregnated with $AgNO_3$ (light petroleum:ethyl acetate (82:18)). 168 mg of **6** were obtained. Colourless oil.– TLC: R_f 0.22 (light petroleum:acetone (90:10)), R-1: brown-yellow, R-2: immediately blue.– $[\alpha]_D^{20}$: –6.08° ($c = 1.3$,

Table 2. ^{1}H NMR ($CDCl_3$) spectroscopic data of compounds **4–10d** (δ [ppm]).

H	**4**	**5**	**6**	**7**	**8**	**9**	**10a**	**10b**	**10c**	**10d**
			isomer a (isomer b)	isomer a (isomer b)	isomer a (isomer b)	isomer a (isomer b)	isomer a (isomer b)	isomer a (isomer b)	isomer a (isomer b)	isomer a (isomer b)
2	7.08	7.09	6.39 (6.4)	6.4 (6.42)	6.41 (6.415)	6.39 (6.4)	6.40 (6.41)	6.41 (6.42)	6.37 (6.39)	6.35 (6.36)
3	7.08	7.09	6.46 (6.49)	6.45 (6.48)	6.54 (6.51)	6.47 (6.49)	6.47 (6.50)	6.45 (6.47)	6.43 (6.45)	6.41 (6.43)
5(′)	7.08	7.09	1.98–2.08	1.96–2.8	1.5–1.56	1.98–2.08	1.98–2.07	1.97–2.07 (1.99)	1.98	1.97 (1.95–2.04)
5″			1.47–1.56	1.48–1.56	2.0–2.07	1.49–1.56	1.50–1.56	1.48–1.54	1.47–1.52 (1.47–1.52)	1.48–1.50 (1.45–1.52)
6(′)	7.08	7.09	1.98–2.08	1.96–2.8	1.5–1.56	1.98–2.08	1.98–2.07	1.97–2.07	2.03	2.01
6″			1.47–1.56	1.48–1.56	2.0–2.07	1.49–1.56	1.50–1.56	1.48–1.54	1.47–1.52	1.48–1.52
7	2.32	2.33	1.36	1.39	1.37	1.37	1.37	1.37	1.35	1.33
8	2.79	2.65	1.78–1.87	1.64–1.78	1.7–1.8	1.74–1.86	1.62–1.78	1.48–1.85	1.77–1.80	1.75–1.80
9	1.27	1.22 (1.23)	0.97	0.99	1.00 (1.00)	0.98	0.99 (1.00)	0.99 (1.00)	1.02 (1.01)	1.00 (0.99)
10(′)	2.33	1.3–1.5*	1.91 (1.94)	1.16–1.32	1.4–1.5	1.66 (1.74)	1.05–1.34	1.05–1.34	1.17–1.23	1.68–1.74
10″			2.41 (2.415)	1.38–1.48	1.89–2.0	2.39	1.50–1.70	1.48–1.85	1.81–1.91 (1.71–1.91)	1.88
11(′)	5.6	1.5–1.66*	5.65	1.66–1.7	2.74	5.56	1.45–1.7	1.48–1.85	1.19–1.23	168–1.74
					(2.77)				(1.19–1.25)	
11″									1.65–1.77	1.12–1.22
12	5.37	4.27 (4.28)	5.53 (5.56)	4.29		5.64 (5.65)	4.05	5.13	5.39	5.34
14(′)	1.26	4.99	1.31	5.02	5.75 (5.77)	1.31	4.83 (4.84)	4.87 (4.90)	4.91 (4.93)	4.90
14″		5.01			5.95 (5.96)		4.94	4.93 (4.94)	5.02 (5.03)	5.00
15	1.24	1.65 (1.69)	1.31	1.73	1.85 (1.86)	1.31	1.72 (1.71)	1.71 (1.72)	1.79 (1.78)	1.76
OOH	6.83	7.8	7.5 (7.55)	7.8 (7.82)						
OH						1.46	1.57			
–Ac								2.06		
(2′)									8.05	7.53
(3′)									7.43	
(4′)									7.55	
(5′)									7.43	7.66
(6′)									8.05	6.86
(8′)										3.9
(9′)										3.9

* assignment interchangeable

Table 3. ^{13}C NMR ($CDCl_3$) spectroscopic data of compounds **4–10d** (δ [ppm]).

C	4	5	6	7	8	9	10a	10c	10d
			isomer a (isomer b)	isomer a (isomer b)	isomer a (isomer b)	isomer a (isomer b)	isomer a (isomer b)	isomer a (isomer b)	isomer a (isomer b)
1	135.5	135.3	74.5	74.5	74.5	74.5	74.5	74.4	74.3
2	128.9	128.9	136.5 (136.2)	136.5 (136.3)	136.4 (136.2)	136.4 (136.2)	136.4 (136.3)	136.2 (136.4)	136.3 (136.4)
3	126.8	126.7	132.5 (133.4)	133.4 (132.8)	133.0 (133.5)	132.8 (133.4)	133.1 (133.5)	133.3 (132.8)	133.4 (132.9)
4	143.4	143.8	79.8	80.0	80.2	79.7	80.0 (79.9)	79.7 (79.8)	79.7 (79.8)
5	126.8	126.7	26.7 (25.3)	26.3*	25.5* (25.9*)	26.3 (26.5)	26.1 (25.5)	25.3 (26.1)	25.2 (25.9)
6	128.9	128.9	29.4 (29.5)	29.3# (29.4#)	29.4 (29.6)	29.4 (29.6)	29.5 (29.6)	29.5 (29.4)	29.4 (29.3)
7	21.0	21.0	21.43	21.5	21.5	21.5	21.5	21.4	21.3
8	39.6	39.6 (39.3)	37.5 (37.4)	37.6 (37.5)	37.1	37.6 (37.5)	37.5 (37.4)	37.4 (37.3)	37.3 (37.2)
9	21.7	22.7 (22.4)	14.3 (14.27)	14.3 (14.2)	14.5 (14.4)	14.0 (13,9)	14.3 (14.2)	14.2	14.0 (14.1)
10	1.5	34.2 (34.1)	34.6 (34.3)	27.1# (27.6#)	26.0* (26.7*)	34.0 (33.8)	33.3 (33.1)	31.0 (30.7)	30.9 (30.7)
11	130.6	28.8 (28.9)	130.0 (129.8)	25.3*	36.0 (35.7)	125.10 (125.0)	27.5 (27.0)	26.6 (27.0)	26.6 (26.9)
12	134.6	89.8 (89.6)	134.9 (135.2)	89.6 (89.5)	201.7 (201.6)	139.9 (139.8)	75.8	78.0 (77.8)	77.5 (77.8)
13	81.9	143.5 (143.4)	81.97	143.4	144.2 (144.3)	70.64	147.4	142.4 (142.6)	142.7 (142.8)
14	24.5*	114.4* (114.1*)	24.5 (24.4)	114.9 (114.3)	124.5	29.9 (29.8)	110.9 (111.0)	113.4 (112.9)	113.2 (112.7)
15	23.8*	17.2* (17.0*)	24.32 (24.36)	17.4	17.7	29.9 (29.8)	17.8	18.0 (18.3)	17.9 (18.2)
1′								130.3	148.5
2′								129.4	111.9
3′								128.2	152.8
4′								132.7	165.5
5′								128.2	123.4
6′								129.4	110.2
7′								165.5	>200
8′									55.9
9′									55.9

*,# assignment interchangeable

$CHCl_3$).– FAB-MS (70 eV, m-NBA + NaOAc); *m/z* (%) = 291 (88, $M+Na^+$), 259 (20), 235 (100, M^+–OOH). ^1H NMR and ^{13}C NMR data see Tables 2 and 3.

2-Hydroperoxy-1-(1-methylethenyl)-4-(4-methyl-2,3-dioxabicyclo[2.2.2]-oct-5-en-1-yl)-pentane (**7**)

Separation of fraction IV (see **6**) gave 36 mg of **7**. Colourless oil.– TLC: R_f 0.22 (light petroleum:acetone (90:10)), R-1: brown-red, R-2: immediately blue.– $[\alpha]_D^{20}$: +12.25° (*c* = 1.02, $CHCl_3$).– FAB-MS (70 eV, m-NBA + NaOAc); *m/z* (%) = 291 (100, $M+Na^+$), 273 (34, 291–H_2O^+), 217 (18, M^+–OOH-H_2O).– ^1H NMR and ^{13}C NMR data Tables 2 and 3.

1-(1-Methylethenyl)-4-(4-methyl-2,3-dioxabicyclo [2.2.2]oct-5-en-1-yl)pentan-2-one (**8**)

This substance was obtained as a side product during the separation of the mixture of **6** and **7**. Colourless oil.– TLC: R_f 0.16 (toluene:acetone (98:2)),

R-1: grey-brown, R-2: blue after 5–8 min.– $[\alpha]_D^{20}$: –4.27° (c = 0.8, $CHCl_3$).– FAB-MS (70 eV, m-NBA + NaOAc); *m/z* (%) = 273 (55, $M+Na^+$), 251 (M^+–H), 217 (80, 251–$H_2O_2^+$), 132 (100, $C_{10}H_{12}^{+\bullet}$). ^{1}H NMR and ^{13}C NMR data Tables 2 and 3.

(E)-1-Hydroxy-1,1-dimethyl-5-(4-methyl-2,3-dioxabicyclo[2.2.2]oct-5-en-1-yl)-2-hexene (**9**)

A solution of 35 mg of **6** in diethyl ether was added dropwise to an ice-cooled solution of 38 mg of triphenylphosphine in 10 ml of diethyl ether. After stirring for 15 min at 0° C, the temperature was slowly raised to room temp. and stirred for another hour. The reaction mixture was evaporated *in vacuo* at room temp. and purified by CC on silica gel (light petroleum:ethyl acetate (80:20)), giving 20 mg of a colourless oil.– TLC: R_f 0.2 (light petroleum:ethyl acetate (80:20)) R-1: violet, R-2: blue after 5–8 min.– $[\alpha]_D^{20}$: +11.5° (c = 0.27, $CHCl_3$).– ^{1}H NMR and ^{13}C NMR data Tables 2 and 3.

2-Hydroxy-1-(1-methylethenyl)-4-(4-methyl-2,3-dioxabicyclo[2.2.2]oct-5-en-1-yl)-pentane (**10a**)

30 mg of **7** were submitted to the same procedure described above for the reduction of **6**. After stirring at room temp. for 1 h, the reaction mixture was evaporated *in vacuo* and purified by CC on silica gel (light petroleum: ethyl acetate (80:20)), affording 17 mg colourless oil. – TLC: R_f 0.28 (light petroleum:ethyl acetate (80:20)), R-1: brown-violet, R-2: blue after 5–8 min.– $[\alpha]_D^{20}$: +15.9° (c = 0.33, $CHCl_3$). ^{1}H NMR and ^{13}C NMR data Tables 2 and 3.

[1-(1-Methylethenyl)-4-(4-methyl-2,3-dioxabicyclo[2.2.2]oct-5-en-1-yl)-pentyl] acetate (**10b**)

To 10 mg of **10a** in 4 ml pyridine 2 ml of acetic anhydride were added and the reaction mixture stirred over night at room temp., excluding humidity. The solution was evaporated *in vacuo* at 40° C until dryness and purified by CC on silica gel (light petroleum:ethyl acetate (93:7)). 5 mg of a slightly yellow oil were obtained.– TLC: R_f 0.43 (light petroleum:ethyl acetate (93:7)), R-1: brown-violet, R-2: blue after 5–8 min.– $[\alpha]_D^{20}$: +9,3° (c = 0.86, $CHCl_3$). ^{1}H NMR data Table 2.

[1-(1-Methylethenyl)-4-(4-methyl-2,3-dioxabicyclo[2.2.2]oct-5-en-1-yl)-pentyl]-benzoate (**10c**)

318 mg **10a** were dissolved in 5 ml diethyl ether and 331 mg triphenylphosphine, dissolved in 5 ml diethyl ether, were added. The mixture was stirred and a solution of 154 mg benzoic acid and 221 mg diethylazodicarboxylate in diethyl ether was added dropwise. It was stirred for several hours; then it was allowed to stand for 6 days at room temp., excluding humidity. The solution was evaporated *in vacuo* at room temp. and purified two times by CC on silica gel (light petroleum:diethyl ether (85:15)). 154 mg of an oily product were obtained. – TLC: R_f 0.17 (light petroleum:diethyl ether (85:15)), R-1: violet, R-2: blue reaction after 2 min. ^{1}H and ^{13}C NMR data Table 2 and 3.

[1-(1-Methylethenyl)-4-(4-methyl-2,3-dioxabicyclo[2.2.2]oct-5-en-1-yl)-pentyl]-3,4-dimethoxybenzoate (**10d**)

311 mg **10a**, 252 mg 3,4-dimethoxybenzoic acid, and 33 mg 4-(*N,N*-dimethylamino)pyridine were dissolved in 22 ml dichloromethane. It was cooled in an ice bath, stirred and 300 mg dicyclohexylcarbodiimide were added. After 2 h, the ice was removed and the mixture was allowed to stand for 15 days at room temp., excluding humidity. The solution was evaporated *in vacuo* at room temp. and purified two times by CC on silica gel (light petroleum:ethyl acetate (75:25)). 392 mg of highly viscous, slightly yellow oil were obtained.– TLC: R_f 0.23 (light petroleum:ethyl acetate (75:25)), R-1: intensive violet, R-2: blue after 4–5 min. ^{1}H and ^{13}C NMR data Table 2 and 3.

In Vitro Antimalarial Activity

To determine the antimalarial activity of compounds **3–10d** *in vitro*, tests against *Plasmodium falciparum*, strain NF-54, were carried out (Table 1). 1% ethanolic solutions of the compounds were diluted serially with medium and prepared in 24 well-plates. The growth rate was followed up during a 96 h period in RPMI 1640 medium, supplemented with 10% human plasma and 0.2% $NaHCO_3$ at 5% hematocrit level without antibiotics. Parasitaemia was counted from Giemsa-stained smears. EC_{50} values see Table 1. Results are means of two independent experiments done in duplicate, each compared with a positive and a negative (chloroquinediphosphate) control, as well as with experiments undertaken to determine the EC_{50} of artemisinin and chloroquinediphosphate. Calculations are carried out by Probit analysis. The compounds **4–10d** turned out to be active, but the activity could not be increased significantly, compared with **3**.

Blood Schizontocidal Test Protocol

Female, random-bred NMRI albino mice, weighting 25–32 g, were inoculated peritoneally with 10^6 parasitized blood cells of *Plasmodium yoellii nigeriensis*. Animals were then dosed intraperitoneally once daily for three consecutive days, beginning on the day of infection (day 0). Compounds **3** and **9** were dissolved in ethanol 96% and diluted with sterile 0.9% NaCl solution to give concentrations of 40, 80, 160, 320, and 640 mg/kg mice. The parasitaemia was determined on days 2, 4, 6, 8, and 10 by Giemsa-stained blood smears and compared with untreated controls. A compound was supposed to have an activity, if lifetime of the treated mice exceeded the lifetime of the untreated mice at least twice. Both compounds were inactive.

References

☆ Dedicated to Prof. Dr. Hans Achenbach, Erlangen, on the occasion of his 65th birthday.

[1] Peroxides as Constituents of Plants – Part 22; for Part 21 see *Planta Med.* **1996**, *62*, 565–566.

[2] G. Rücker, *Pharmazie in unserer Zeit* **1995**, *24*, 189–195.

[3] A. Marek, *Ph. D. Thesis*, Bonn, **1994**

[4] G. Rücker, E. Schenkel, D. Manns, M. Falkenberg, A. Marek, *Phytochemistry* **1996**, *41(1)*, 297–300.

[5] S. Seggewies, *Diploma Thesis*, Bonn, **1996**

[6] B. M. Monroe, *J. Am. Chem. Soc.* **1981**, *103*, 7253–7256.

[7] A. Frimer, *Chem. Rev.* **1979**, 359–387.

[8] B. Heinzmann, *Ph. D. Thesis*, Bonn, **1996**.

[9] L. Horner, W. Jurgeleit, *Liebigs Ann. Chem.* **1955**, *591*, 138–152.

[10] B. de Vries, *Chem. Ind.* **1962**, 1049.

[11] E. Merck, *Anfärbereagenzien für Dünnschicht- und Papier-Chromatographie*, Darmstadt, **1970** (reagent No. 21).

[12] W. Huber, E. Fröhlke, *Chromatographia* **1972**, *5*, 256.

[13] N. H. Andersen, D. D. Syrdal, *Phytochemistry* **1970**, *9*, 1325–1340.

[14] F. Bohlmann, J. Jakupovic, C. Zdero, *Phytochemistry* **1978**, *17*. 2034–2036.

[15] K. Heiden, *Ph. D. Thesis*, Bonn 1996.

Received: November 21, 1996 [FP159]