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Synthesis and trichomonacidal activity of perketals and hydroperoxides

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Abstract

Some perketals were synthesized by the Dussault procedure using simple bromides and 2-methoxyprop-2-yl hydroperoxide. Treatment with acetic acid gave the corresponding hydroperoxides. Both perketals and hydroperoxides were tested in vitro as trichomonacidal agents. Most of them exhibited very good activities. The most powerful compound was 2-methoxyprop-2-yl hexadec-1-yl peroxide which exhibited an IC_{50} value of 0.51 μM being 10 times more effective than the reference compound Metronidazole. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Perketals; Peroxides; Hydroperoxides; *Trichomonas vaginalis*

1. Introduction

Trichomonas vaginalis is a pathogenic protozoa responsible for vaginitis and acute inflammatory disease of the genital mucosa [1]. This human parasitic disease affects at least 170 million individuals globally. It may also increase the risk of transmission of HIV and predispose pregnant women to premature rupture of membranes and early labour [2]. Current therapy uses 5-nitro-imidazoles like Metronidazole (Flagyl®) but is somewhat limited by the resistance of certain strains [3]. Thus the search of alternative therapies to circumvent the appearance of totally resistant strains appeared to us of great interest.

T. vaginalis is microaerophilic and does not have any efficient system to resist against oxidative stress (peroxidase, catalase), therefore it shows complete incapacity to get rid of the so-formed peroxides without subsequent generation of radicals, and does not have any radical scavenger source neither [4]. As a consequence, *T. vaginalis* is inhibited by a too high O_2 concentration [5]. Lipidic hydroperoxides formed during oxidative stress of the parasite are lethal [6]. We thought that exogenous peroxides could also be lethal for *T. vaginalis*. We recently reported that synthetic *t*-butyl alkyl peroxide and perketals are effectively trichomonacidal in vitro [7]. Some of the tested products were more active than the reference molecule Metronidazole, for example 2-methyl-prop-2-yl-2-methoxy-eth-1-yl-peroxide was about six times more potent. In connection with this precedent work, we reported herein the synthesis and in vitro trichomonacidal activity of other perketals and their corresponding hydroperoxides. We aimed to prepare long alkyl chain hydroperoxides that could show structural similitude with lethal endogenous lipid hydroperoxide.

2. Results and discussion

2.1. Chemistry

Perketals **4a**–**k** (Table 1) and hydroperoxides **5a**–**k** (Table 2) were prepared according to the Dussault procedure (Scheme 1) [8]. Ozonolysis of dimethyl but-2-ene (**1**) in methanol gave 2-methoxyprop-2-yl hydroperoxide (**2**). Further reaction of **2** with alkylbromides **3a**–**k**, in the presence of cesium hydroxide afforded

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Table 1
Physicochemical data of perketals **4**

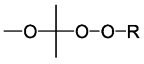
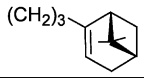
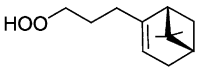
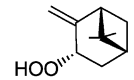
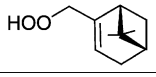
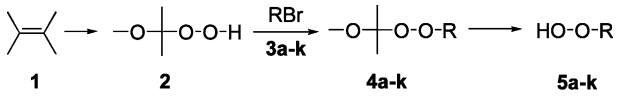
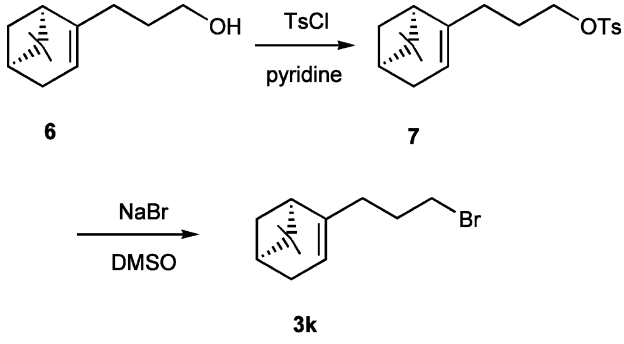
Comp. **4**  R:		Yield (%)	Formula	Analysis calculated / found C %	H %
4a	$(CH_2)_5CH_3$	44	$C_{10}H_{22}O_3$	63.12 / 63.49	11.65 / 11.92
4b	$(CH_2)_6CH_3$	77	$C_{11}H_{24}O_3$	64.67 / 64.50	11.84 / 11.71
4c	$(CH_2)_7CH_3$	86	$C_{12}H_{26}O_3$	63.81 / 63.49	12.00 / 12.26
4d	$(CH_2)_9CH_3$	62	$C_{14}H_{30}O_3$	68.25 / 68.59	12.27 / 12.45
4e	$(CH_2)_{11}CH_3$	54	$C_{16}H_{34}O_3$	70.02 / 69.78	12.49 / 12.22
4f	$(CH_2)_{15}CH_3$	40	$C_{20}H_{42}O_3$	72.67 / 73.01	12.81 / 12.98
4g	$(CH_2)_{17}CH_3$	38	$C_{22}H_{46}O_3$	73.69 / 73.38	12.93 / 12.75
4h	$(CH_2)_3OPh$	80	$C_{13}H_{20}O_4$	64.98 / 64.81	8.39 / 8.28
4i	$(CH_2)_2Ph$	36	$C_{12}H_{18}O_3$	68.54 / 68.20	8.63 / 8.48
4j	$(CH_2)_3CN$	70	$C_8H_{15}NO_3$	55.47 / 55.21	8.73 / 8.50
4k		69	$C_{16}H_{28}O_3$	71.60 / 71.92	10.51 / 10.78

Table 2
Physicochemical data of hydroperoxides **5**

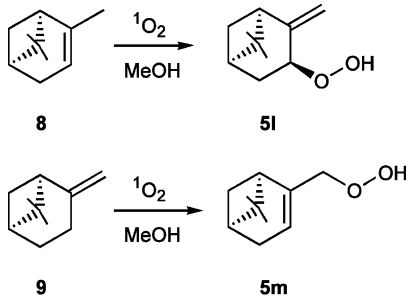
Comp. **5**	HO–O–R	Yield (%)	Formula	Analysis calc. / found C %	H %
5b	$HOO(CH_2)_6CH_3$	82	$C_7H_{16}O_2$	63.60 / 63.27	12.20 / 11.86
5c	$HOO(CH_2)_7CH_3$	87	$C_8H_{18}O_2$	65.71 / 65.98	12.41 / 12.67
5d	$HOO(CH_2)_9CH_3$	91	$C_{10}H_{22}O_2$	68.92 / 68.63	10.27 / 9.95
5e	$HOO(CH_2)_{11}CH_3$	85	$C_{12}H_{26}O_2$	71.23 / 71.61	12.95 / 13.09
5f	$HOO(CH_2)_{15}CH_3$	83	$C_{16}H_{34}O_2$	74.36 / 74.69	13.26 / 13.52
5g	$HOO(CH_2)_{17}CH_3$	79	$C_{18}H_{38}O_2$	75.46 / 75.14	13.37 / 13.22
5h	$HOO(CH_2)_3OPh$	77	$C_9H_{12}O_3$	64.27 / 63.95	7.19 / 6.98
5i	$HOO(CH_2)_2Ph$	86	$C_8H_{10}O_2$	69.55 / 69.17	7.29 / 6.94
5j	$HOO(CH_2)_3CN$	92	$C_4H_7NO_2$	47.52 / 47.15	6.98 / 6.73
5k		97	$C_{12}H_{20}O_2$	73.43 / 73.05	10.27 / 9.88
5l		65	$C_{10}H_{16}O_2$	71.39 / 71.03	9.59 / 9.33
5m		50	$C_{10}H_{16}O_2$	71.39 / 71.13	9.59 / 9.41



Scheme 1.



Scheme 2.



Scheme 3.

perketals **4a**–**k** in moderate to good yields (Table 1). Alkyl bromides **3a**–**j** used for this reaction sequence were all commercially available. The perketal **4k** was synthesized in order to explore more constrained and lipophilic peroxides. Compound **4k** was obtained starting from non-commercial bromide **3k**. Thus, homonopylbromide **3k** was obtained from known homonopol **6** [9] (Scheme 2). Subsequent tosylation of alcohol **6** in pyridine gave the tosylate **7** which was substituted with NaBr in DMSO to give homonopylbromide (**3k**). Deprotection of perketals **4b**–**k** performed with aqueous acetic acid gave the corresponding hydroperoxides **5b**–**k** (Table 2).

For completion of the pinene series, known hydroperoxides **5l** and **5m** were prepared by photooxygenation of, respectively α-pinene **8** and β-pinene **9** according to the Brill procedure [10] (Scheme 3).

All perketals **4a**–**k** or hydroperoxides **5b**–**m** had sufficient stability required for biological tests. However, *n*-hexyl hydroperoxide (**5a**) was too unstable for significant use.

2.2. Trichomonacidal activity

The most active compound of the present work was compound **4f**, a perketal with an IC_{50} of 0.51 μM (Table 3). This compound was about 11 times more active than metronidazole ($IC_{50} = 5.8$ μM) and about two times more active than the best trichomonacidal peroxide previously reported [7]. Three other perketals exhibited IC_{50} less than 2 μM (compounds **4c**, **4d** and **4h**) and six others had IC_{50} values less than 100 μM. There is no clear-cut correlation between biological activity and the length of the alkyl chain since compound **4f** with $R = (CH_2)_{15}CH_3$ was the most active and compound **4e** with $R = (CH_2)_{11}CH_3$ was inactive; compound **4c** with $R = (CH_2)_7CH_3$ was very active ($IC_{50} = 0.82$ μM) and compound **4b** with $R = (CH_2)_6CH_3$ was slightly active ($IC_{50} = 73.5$ μM).

Concerning the hydroperoxide series, the two best compounds were **5c** and **5d** exhibiting an IC_{50} of about 2.5 μM. All other compounds had an IC_{50} superior to 50 μM. For this series, the optimal length of the alkyl chain was $R = (CH_2)_7CH_3$ and $(CH_2)_9CH_3$.

Perketals were more active than their corresponding hydroperoxides except for the pair **4e** and **5e**. In our previous work, we reported that perketals were less active than their corresponding *t*-butyl alkyl peroxides [7]. We can here complete our study, by comparaison of perketals **4b**,**h**,**i**,**j** and their corresponding hydroperoxides **5b**,**h**,**i**,**j** that have also been evaluated as *t*-butyl alkyl peroxide [7]. It clearly appeared that *t*-butyl alkyl peroxides are more active than their perketals analogues, themselves being more potent than their hydroperoxide counterparts, except for compound **4h** which was more active than its *t*-butyl peroxide analogue.

As expected, long chain alkyl hydroperoxides had good trichomonacidal activity presumably due to structural similitude with lipidic hydroperoxides formed during lethal oxidative stress [6]. Some perketals exhibited more potency, one of them being 11 times more effective than the reference compound Metronidazole.

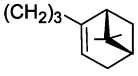
3. Experimental

3.1. Chemistry

The compounds were all identified by usual physical methods, i.e. ^1H, ^{13}C NMR and elemental analysis.

^1H and ^{13}C NMR spectra were measured with a Bruker ARX 400 (400 and 100.6 MHz, for ^1H and ^{13}C, respectively). ^1H chemical shifts were reported in ppm from an internal standard TMS or of residual $CHCl_3$ (7.27 ppm). The following abbreviations were used: m (multiplet), s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quadruplet) and qt (quintuplet). ^{13}C chemical shifts were reported in ppm from the central peak of deuteriochloroform (77.14). Optical rotations were measured at 20 °C on a Perkin–Elmer 241 MC polarimeter in a 1 dm cell. Elemental analyses were performed with a Perkin–Elmer 240 analyser. Analytical

Table 3
In vitro trichomonacidal activities of perketals **4** and hydroperoxides **5** on *T. vaginalis* CMP strain

Perketals:			Hydroperoxides :	
—O—(—)—O—O—R	Trichomonacidal activities on *T. vaginalis* IC_{50} (μM) ± SD [a]	R	HO—O—R	Trichomonacidal activities on *T. vaginalis* IC_{50} (μM) ± SD [a]
4a	52.6 ± 6.8	$(CH_2)_5CH_3$		
4b	73.5 ± 8.8 [b]	$(CH_2)_6CH_3$	**5b**	60.6 ± 7.0
4c	**0.82 ± 0.11**	$(CH_2)_7CH_3$	**5c**	**2.4 ± 0.3**
4d	**1.5 ± 0.2**	$(CH_2)_9CH_3$	**5d**	**2.6 ± 0.3**
4e	> 365	$(CH_2)_{11}CH_3$	**5e**	59.4 ± 7.0
4f	**0.51 ± 0.07**	$(CH_2)_{15}CH_3$	**5f**	> 387
4g	39.1 ± 5.0	$(CH_2)_{17}CH_3$	**5g**	349
4h	**1.4 ± 0.2** [b]	$(CH_2)_3OPh$	**5h**	> 595
4i	57.1 ± 6.9 [b]	$(CH_2)_2Ph$	**5i**	> 725
4j	57.8 ± 6.9 [b]	$(CH_2)_3CN$	**5j**	98.9 ± 11.9
4k	33.5 ± 4.4	$(CH_2)_3$ 	**5k**	306 ± 43
			**5l**	59.4 ± 7.0
			**5m**	297 ± 41
Metronidazole	5.8 ± 0.6	/		

[a]Values inferior to Metronidazole reference (IC_{50} = 5.8 μM) are in bold.
[b]Previous work [7].

TLC was performed on Merck precoated silica gel 60F plates. Merck silica gel 60 (230–400 mesh) was used for column chromatography.

3.1.1. Preparation of homonopylbromide **3k**

*3.1.1.1. (1S)-4-(6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-yl)-but-1-yl-tosylate (**7**).* Tosylchloride (16.21 g, 85.1 mmol, 1.2 equiv.) was slowly added to a solution of (1*S*)-homonopol **6** [10] (13.78 g, 70.91 mmol, 1 equiv.) in pyridine (17.2 ml, 213 mmol, 3 equiv.) at 0 °C. After stirring for 4 h at room temperature, water (2 ml) was added to destroy the excess of tosylchloride. Then, the reaction mixture was poured into an aq. solution of 6 N HCl (35 ml) at 0 °C and extracted with Et_2O (5 × 60 ml). The organic layers were washed with aq. solution of 1 N HCl (2 × 30 ml), saturated $NaHCO_3$ (2 × 20 ml), dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (Et_2O/C_6H_{14}: 75/25) to give 19.03 g of the tosylate **7**.

Yield: 77%. *Anal.* $C_{20}H_{28}O_3S$: Found (Calc.): C, 68.52 (68.93); H, 7.91% (8.10). ^1H NMR ($CDCl_3$): δ 7.78 (d, 2H, *J* = 8.2 Hz), 7.33 (d, 2H, *J* = 8.2 Hz), 5.15–5.06 (m, 1H), 4.02 (t, 2H, *J* = 6.4 Hz), 2.44 (s, 3H), 2.32 (dt, 1H, *J* = 8.4, 5.6 Hz), 2.24–2.13 (m, 2H), 2.12–1.98 (m, 1H), 1.98–1.79 (m, 3H), 1.63 (qt, 2H, *J* = 7.0 Hz), 1.44–1.28 (m, 2H, *J* = 7.1, 5.25 Hz), 1.25 (s, 3H), 1.08 (d, 1H, *J* = 8.4 Hz), 0.78 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 147.5, 144.6, 133.3, 129.8 (2C), 127.8 (2C), 116.3, 70.6, 45.6, 40.8, 37.8, 36.0, 31.6, 31.2, 28.5, 26.3, 22.9, 21.6, 21.1. $[\alpha]_D = -9.6$ (*c* 2.08, CH_2Cl_2).

*3.1.1.2. (1S)-4-(6,6-dimethyl-bicyclo[3.1.1]hept-2-ene-2-yl)-but-1-yl-bromide (**3k**).* Tosylate **7** (1.04 g, 2.98 mmol, 1 equiv.) was added to a dry suspension of NaBr (915 mg, 8.94 mmol, 3 equiv.) in DMSO. The reaction was stirred for 20 min at r.t. and then 2 h at 70 °C. Brine (15 ml) and water (15 ml) were added and the mixture was extracted with petroleum ether/Et_2O 1:1 (6 × 25 ml). The organic layers were washed with brine (2 × 10 ml), dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography on silica gel (petroleum ether) to give 510 mg of bromide **3k** as an oil.

Yield: 66%. *Anal.* $C_{13}H_{21}Br$: Found (Calc.): C, 60.50 (60.70); H, 8.15% (8.23). ^1H NMR ($CDCl_3$): δ 5.23–5.13 (m, 1H), 3.40 (t, 3H, *J* = 6.8 Hz), 2.35 (dt, 1H, *J* = 8.4 5.6 Hz), 2.27–2.15 (m, 2H), 2.15–1.94 (m, 4H),

1.85 (qt, 2H, $J = 7.3$ Hz), 1.58–1.39 (m, 2H), 1.27 (s, 3H), 1.13 (d, 1H, $J = 8.4$ Hz), 0.82 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 147.7, 116.3, 45.8, 40.9, 38.0, 35.9, 33.8, 32.6, 31.7, 31.3, 26.4, 25.7, 21.2. $[\alpha]_D = -22.1$ (c 1.49, CH_2Cl_2).

*3.1.2. Preparation of perketals **4a–k***

Perketals **4a–k** were synthesized following the Dussault procedure [8] from corresponding bromides **3a–k**.

*3.1.2.1. 2-Methoxyprop-2-yl-hex-1-yl-peroxide (**4a**).* ^{1}H NMR ($CDCl_3$): δ 3.94 (t, 2H, $J = 6.7$ Hz), 3.24 (s, 3H), 1.65–1.4 (m, 2H), 1.32 (s, 6H), 1.3–1.1 (m, 6H), 0.82 (t, 3H, $J = 6.3$ Hz). ^{13}C NMR ($CDCl_3$): δ 104.4, 74.9, 48.9, 31.6, 27.7, 25.7, 22.6, 13.8.

*3.1.2.2. 2-Methoxyprop-2-yl-hept-1-yl-peroxide (**4b**).* Spectral data were identical to those previously reported [7].

*3.1.2.3. 2-Methoxyprop-2-yl-oct-1-yl-peroxide (**4c**).* ^{1}H NMR ($CDCl_3$): δ 3.93 (t, 2H, $J = 6.6$ Hz), 3.29 (s, 3H), 1.7–1.4 (m, 2H), 1.31 (s, 6H), 1.25–1.1 (m, 10H), 0.81 (t, 3H, $J = 6.7$ Hz). ^{13}C NMR ($CDCl_3$): δ 104.1, 74.7, 48.6, 31.6, 29.2, 29.0, 27.7, 25.9, 22.4, 13.7.

*3.1.2.4. 2-Methoxyprop-2-yl-dec-1-yl-peroxide (**4d**), 2-methoxyprop-2-yl-dodec-1-yl-peroxide (**4e**), 2-methoxyprop-2-yl-hexadec-1-yl-peroxide (**4f**).* Spectral data were identical to those previously reported [8].

*3.1.2.5. 2-Methoxyprop-2-yl octadec-1-yl-peroxide (**4g**).* ^{1}H NMR ($CDCl_3$): δ 3.95 (t, 2H, $J = 6.4$ Hz), 3.30 (s, 3H), 1.75–1.5 (m, 2H), 1.38 (s, 6H), 1.3–1.2 (m, 30H), 0.87 (t, 3H, $J = 6.1$ Hz). ^{13}C NMR ($CDCl_3$): δ 94.1, 75.2, 49.1, 31.9, 29.4, 27.8, 26.1, 22.7, 14.1.

*3.1.2.6. 2-Methoxyprop-2-yl-3-phenoxyprop-1-yl-peroxide (**4h**).* Spectral data were identical to those previously reported [7].

*3.1.2.7. 2-Methoxyprop-2-yl-2-phenyleth-1-yl-peroxide (**4i**).* Spectral data were identical to those previously reported [7].

*3.1.2.8. 2-Methoxyprop-2-yl-3-cyano-prop-1-yl-peroxide (**4j**).* Spectral data were identical to those previously reported [7].

*3.1.2.9. 2-Methoxyprop-2-yl-3-[(1S)-6,6-dimethyl-bicyclo[3.1.1]hept-2-en-2-yl]-prop-1-yl-peroxide (**4k**).* ^{1}H NMR ($CDCl_3$): δ 5.27–5.10 (m, 1H), 3.99 (t, 2H, $J = 6.6$ Hz), 3.30 (s, 3H), 2.33 (dt, 1H, $J = 8.5$, 5.7 Hz), 2.26–2.14 (m, 2H), 2.14–1.88 (m, 4H), 1.82–1.51 (m, 2H), 1.38 (s, 6H), 1.28 (s, 3H), 1.12 (d, 1H, $J = 8.5$ Hz), 0.81 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 147.3, 116.1, 104.2, 74.5, 48.8, 45.6, 40.7, 37.8, 33.1, 31.5, 31.1, 26.2, 25.4, 22.6, 21.0. $[\alpha]_D = -1.9$ (c 1.53, $CHCl_3$).

*3.1.3. Hydroperoxides **5b–k***

Hydroperoxides **5b–k** were synthesized following the Dussault method [8] from, respectively perketals **4b–k**.

*3.1.3.1. Heptyl hydroperoxide (**5b**).* ^{1}H NMR (C_6D_6): δ 8.10 (bs, 1H), 3.95 (t, 2H, $J = 6.2$ Hz), 1.75–1.05 (m, 10H), 0.96 (t, 3H, $J = 7.7$ Hz). ^{13}C NMR (C_6D_6): δ 76.8, 32.0, 29.4, 27.9, 26.2, 22.9, 14.2.

*3.1.3.2. Octyl hydroperoxide (**5c**).* Spectral data were identical to those previously reported [11].

*3.1.3.3. Decyl hydroperoxide (**5d**), dodecyl hydroperoxide (**5e**), hexadecyl hydroperoxide (**5f**).* Spectral data were identical to those previously reported [8].

*3.1.3.4. Octadecyl hydroperoxide (**5g**).* ^{1}H NMR ($CDCl_3$): δ 7.08 (bs, 1H), 3.95 (t, 2H, $J = 6.6$ Hz), 1.70–1.05 (m, 32H), 0.97 (t, 3H, $J = 5.5$ Hz). ^{13}C NMR ($CDCl_3$): δ 76.7, 32.3, 30.1, 29.8, 28.0, 26.3, 23.0, 14.3.

*3.1.3.5. 3-Phenoxypropyl hydroperoxide (**5h**).* ^{1}H NMR (C_6D_6): δ 9.01–8.08 (bs, 1H), 7.10 (t, 2H, $J = 8.0$ Hz), 6.81 (dt, 3H, $J = 8.1$, 1.2 Hz), 3.94 (dt, 2H, $J = 6.2$, 1.75 Hz), 3.68 (t, 2H, $J = 6.2$ Hz), 1.83 (qt, 2H, $J = 6.2$ Hz). ^{13}C NMR (C_6D_6): δ 159.2, 129.7, 121.1, 114.9, 73.6, 64.7, 28.0.

*3.1.3.6. 2-Phenylethyl hydroperoxide (**5i**).* ^{1}H NMR (C_6D_6): δ 9.45 (bs, 1H), 7.42–7.07 (m, 5H), 4.20 (t, 2H, $J = 6.9$ Hz), 2.96 (t, 2H, $J = 6.9$ Hz). ^{13}C NMR (C_6D_6): δ 138.6, 129.2, 128.6, 126.5, 77.4, 34.4.

*3.1.3.7. 3-Cyanopropyl hydroperoxide (**5j**).* ^{1}H NMR ($CDCl_3$): δ 9.2 (bs, 1H), 3.66 (t, 2H, $J = 5.7$ Hz), 1.76 (t, 2H, $J = 7.3$ Hz), 1.41 (qt, 2H, $J = 5.7$ Hz). ^{13}C NMR ($CDCl_3$): δ 119.6, 74.0, 23.8, 13.5.

*3.1.3.8. 3-[(1S)-6,6-dimethyl-bicyclo[3.1.1]hept-2-en-2-yl]-propyl-hydroperoxide (**5k**).* ^{1}H NMR (C_6D_6): δ 7.87 (bs, 1H), 5.23–5.11 (m, 1H), 3.83 (t, 2H, $J = 6.4$ Hz), 2.29 (dt, 1H, $J = 8.4$, 5.7 Hz), 2.23–2.12 (m, 2H), 2.09–1.84 (m, 4H), 1.71–1.45 (m, 2H), 1.23 (s, 3H), 1.18 (d, 1H, $J = 8.4$ Hz), 0.86 (s, 3H). ^{13}C NMR (C_6D_6): δ 148.0, 116.7, 76.8, 46.1, 41.3, 38.1, 33.4, 32.0, 31.6, 26.5, 25.7, 21.5.

Hydroperoxides **5l–m** were obtained following literature [8].

*3.1.3.9. (1S,3S)-6,6-dimethyl-2-methylene-bicyclo-[3.1.1]heptan-3-yl hydroperoxide (**5l**) and [(1S)-6,6-dimethyl-bicyclo[3.1.1]hept-2-en-2-yl]-methyl hydroperoxide (**5m**).* Spectral data were identical to those previously reported [11].

3.2. Antiparasitic activity

T. vaginalis strain CMP (Châtenay-Malabry, parasitologie) was isolated in year 1987 from a woman suffering from a STD and stored as stabilate in liquid nitrogen with 6% DMSO as cryoprotectant. It was Metronidazole-sensitive; 15 mg of tested compounds were dissolved in 500 μl of pure DMSO; 5 μl added in a 1.5 ml volume of culture medium provided a 100 mg l^{-1} final concentration.

Culture tubes with fresh sterile TYM enriched with filtered horse serum 10% alone or with (in triplicate) 1, 10, 100 mg l^{-1} of the tested compound, were inoculated with 10^4 Trichomonas. The tubes were incubated for 48 h at 35 °C and the number of parasites ml^{-1} in each tube determinated with a haemocytometer (Kova slide 10, Boeringer). The results were estimated as the percentage of growth inhibition compared to untreated controls and plotted as probit value as a function of drug concentration ($n = 9$). The IC_{50} and 95% confidence limits were interpolated from the corresponding dose–response curve. Metronidazole (No. 8823 R.P.) was used as reference compound.

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