51. Synthesis, Structure, and Antimalarial Activity of Some Enantiomerically Pure, *cis*-Fused Cyclopenteno-1,2,4-trioxanes

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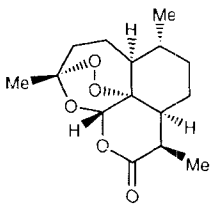
Two pairs of enantiomerically pure *cis*-fused cyclopenteno-1,2,4-trioxanes (**7**, *ent*-**7** and **8**, *ent*-**8**) are prepared (*Schemes 1–3*). Their identities are established by dye-sensitized photo-oxygenation of *ent*-**7** and **8** to the allylic hydroperoxides, reduction to the corresponding alcohols, and conversion to the (1*S*)-camphanoates (*Scheme 4*), the structures of which are determined by X-ray analysis. The dynamic properties of *ent*-**7** are investigated by NMR spectroscopy and PM3 calculations. Evidence for an easily accessible twist-boat conformation is obtained. The *in vitro* and *in vivo* antimalarial activities of **7**, *ent*-**7**, **8**, and *ent*-**8** as well as those of the racemic mixtures are evaluated against *Plasmodium falciparum*, *P. berghei*, and *P. yoelii*. No correlation is observed between configuration and activity. Racemates and pure enantiomers have commensurate activities. The mode of action on the intraerythrocytic parasite is rationalized in terms of close docking by the twist-boat conformer of the trioxane on the surface of a molecule of heme, single-electron transfer to the O–O σ^* orbital, and scission to the acetal radical which then irreversibly isomerizes to a C-centered radical, the ultimate lethal agent (*Scheme 5*).

Introduction. – Artemisinin (**1**) is a naturally occurring tetracyclic 1,2,4-trioxane which has provided a valuable lead for a new class of peroxidic antimalarial agents [1]. Much effort has been expended in effecting minor changes to **1** which, unsurprisingly, has led to derivatives endowed with similar efficacy as the parent molecule [2]. Typical examples are artemether (**2**), arteether (**3**), and the sodium salts of artesunic (**4**) and artelinic acids (**5**) [3]. We were of the opinion that a far simpler, synthetically accessible trioxane is required which would be as efficacious as chloroquine used to be before resistance developed and as nontoxic as certain artemisinin derivatives. In quest of this goal, we undertook a study of the synthesis and structure-activity relations of several tricyclic 1,2,4-trioxanes which mimic parts of the artemisinin skeleton [4]. We found that certain rings in **1** were redundant and concluded that high artemisinin-like activity might be conferred by a *cis*-fused bicyclic 1,2,4-trioxane entity such as **6** and reinforced by an aryl substituent attached to the heterocycle.



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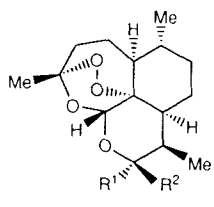
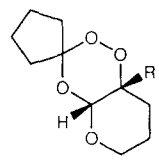


2 R^1 = H, R^2 = MeO
3 R^1 = H, R^2 = EtO
4 R^1 = $NaOOC(CH_2)_2COO$, R^2 = H
5 R^1 = H, R^2 = 4-$NaOOC-C_6H_4CH_2O$



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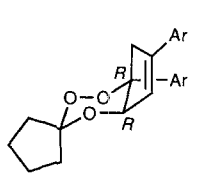
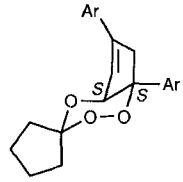
We now describe the synthesis, structure analysis, and antimalarial activity of the pure enantiomers of the diphenyl-substituted *cis*-fused cyclopenteno-1,2,4-trioxanes **7** and *ent*-**7** and their *p*-fluoro analogues **8** and *ent*-**8**. Both molecules apparently fulfil most of the structural criteria embodied in **6**. However, it remains to be seen which absolute configuration, *e.g.* **7** or *ent*-**7**, will correlate best in terms of antiparasitic activity with the natural configuration of the trioxane ring in artemisinin (**1**).



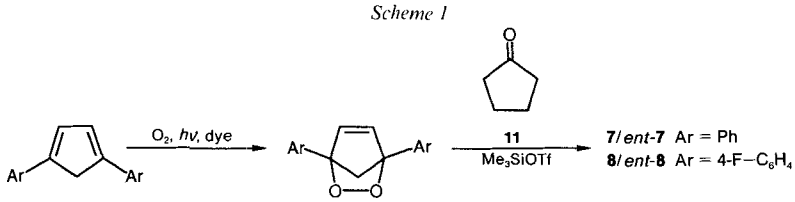
7 (+) Ar = Ph
8 (+) Ar = 4-F–C_6H_4



ent-**7** (–) Ar = Ph
ent-**8** (–) Ar = 4-F–C_6H_4

Chemistry. – The racemic trioxane **7**/*ent*-**7** was prepared from 1,4-diphenylcyclopenta-1,3-diene (**9**) in two steps. The dye-sensitized photo-oxygenation of **9** gave the endoperoxide **10** which by condensation *in situ* with cyclopentanone (**11**) in the presence of a catalytic amount of trimethylsilyl trifluoromethanesulfonate (Me_3SiOTf) afforded **7**/*ent*-**7** in 89% overall yield (*Scheme 1*).

Scheme 1



9 Ar = Ph
12 Ar = 4-F–C_6H_4

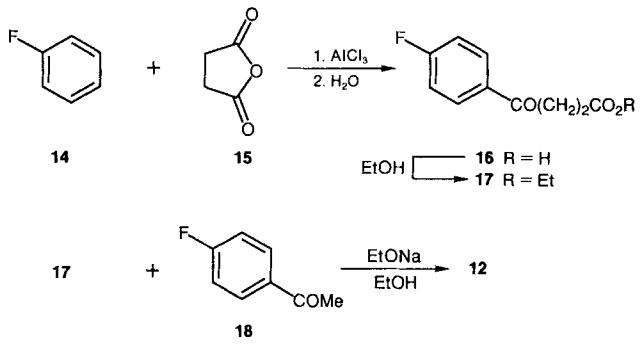
10 Ar = Ph
13 Ar = 4-F–C_6H_4

7/*ent*-**7** Ar = Ph
8/*ent*-**8** Ar = 4-F–C_6H_4

Racemic **8**/*ent*-**8** was similarly obtained from 1,4-bis(4-fluorophenyl)cyclopenta-1,3-diene (**12**) in 66% yield *via* the analogous non-isolated intermediate peroxide **13** (*Scheme 1*). However, three extra steps were required for preparing **12**. *Friedel-Crafts* acylation of

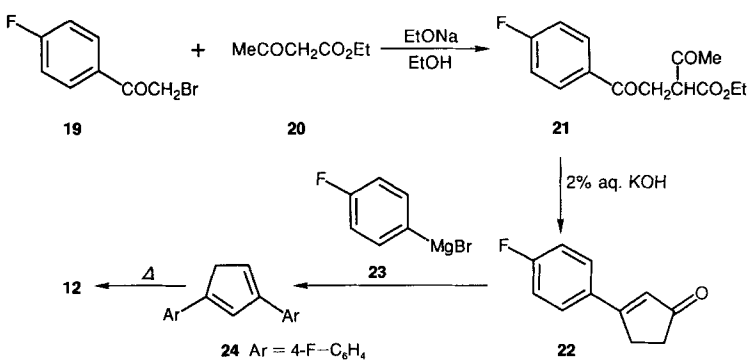
fluorobenzene (**14**) with succinic anhydride (**15**) gave 3-(4-fluorobenzoyl)propionic acid (**16**) and thence **17** by esterification (*Scheme 2*). Next, the condensation of **17** and 4-fluoroacetophenone (**18**) was effected with NaOEt in EtOH. Different sequences of addition were tried, but did little to improve yields of **12** which varied widely from 31–70%.

Scheme 2



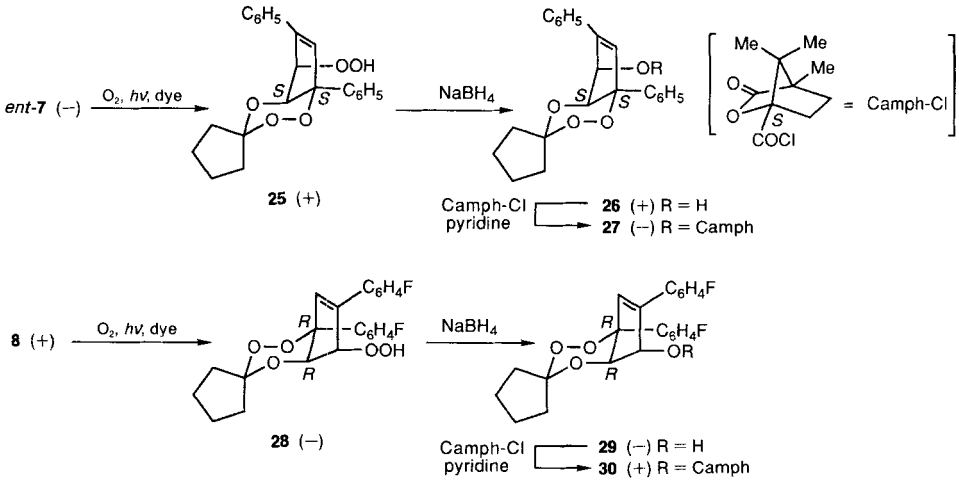
In view of the unreliable aldol coupling of **17** and **18**, the cyclopentadiene **12** was prepared by adapting an earlier procedure [5] (*Scheme 3*). The 4-fluorophenacyl bromide (**19**) was condensed with ethyl acetoacetate (**20**). The resulting ethyl 2-acetyl-3-(4-fluorobenzoyl)propionate (**21**), on treatment with base, cyclized to the cyclopentenone **22**. The addition of 4-fluorophenylmagnesium bromide (**23**) to **22** furnished, on workup, 1,3-bis(4-fluorophenyl)cyclopenta-1,3-diene (**24**) which, by heating in boiling EtOH, readily isomerized to **12**. The overall yield of **12** from **19** was 25%.

Scheme 3



After trying many different chiral columns, resolution of the racemic mixtures **7**/*ent*-**7** as well as **8**/*ent*-**8** was successfully achieved by chromatography over a *Chiracel OG* column. The (+)-enantiomers were assigned the absolute configurations represented by **7** and **8**. Proof was secured by the functionalization of the optically pure olefins and characterization of diastereoisomeric derivatives (*Scheme 4*). The dye-sensitized photo-

Scheme 4



oxygenation of *ent*-**7** and **8** occurred exclusively on the least hindered '*exo*'-face of the double bond to give the '*exo*'-hydroperoxides **25** and **28**, respectively. Reduction to the alcohols **26** and **29** and treatment with (–)-camphanoyl chloride in the presence of base gave the crystalline camphanoates **27** and **30**, respectively, the configurations of which were determined by X-ray analysis (see below).

The aforementioned racemic cyclopentenes were also resolved kinetically by osmium-catalyzed asymmetric dihydroxylation [6].

Structure Analysis. – The absolute configurations of the (–)-enantiomer *ent*-**7** and the (+)-enantiomer **8**, namely (*S*,*S*) and (*R*,*R*), respectively, were unambiguously established from the crystal structures of the camphanoate derivatives **27** and **30** (*Fig. 1*). By

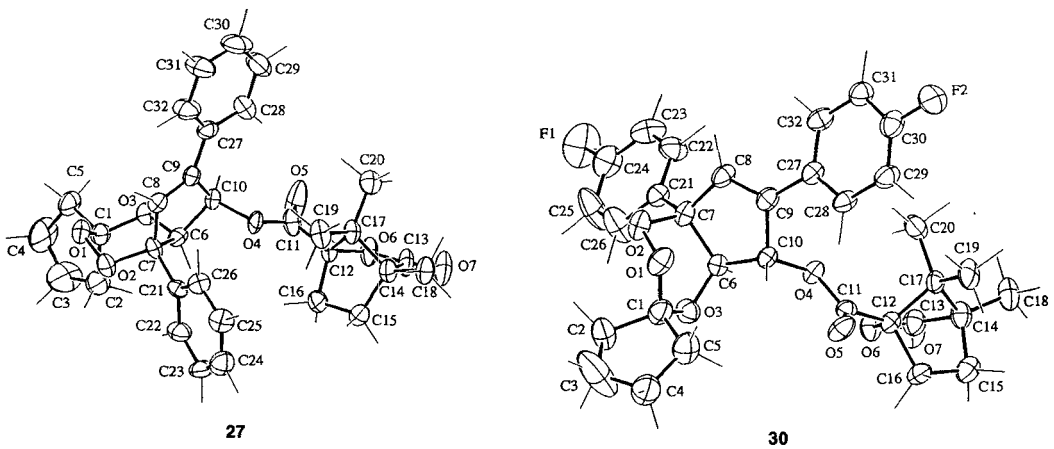


Fig. 1. *Perspective views of the crystal structures of* **27** *and* **30** *with arbitrary atomic numbering.* Ellipsoids are represented with 40% probability.

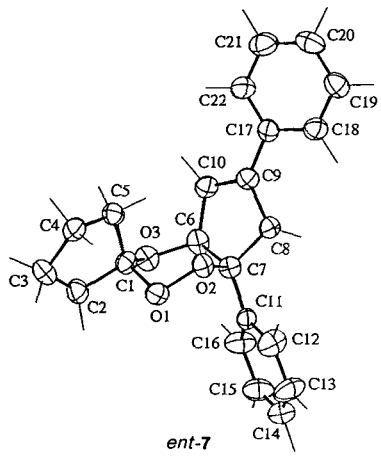


Fig. 2. *Perspective view of the crystal structure of* ent-**7** *with arbitrary atomic numbering.* Ellipsoids are represented with 40% probability.

way of comparison, the structure of the racemic olefin, depicted as *ent*-**7** for reasons of conformity, was also determined by X-ray analysis (*Fig. 2*). In all three molecules, the cyclopenteno-1,2,4-trioxane moiety displays significant conformational differences. The 1,2,4-trioxane rings of *ent*-**7** and **27** adopt a slightly flattened chair conformation defined by the asymmetry parameters [7] which have the following minimum values, $\Delta C_2(O(1)-O(2)) = 0.019(2)$ (*ent*-**7**) and $\Delta C_s(O(1)) = 0.016(6)$ (**27**) (*Fig. 3*). In contrast,

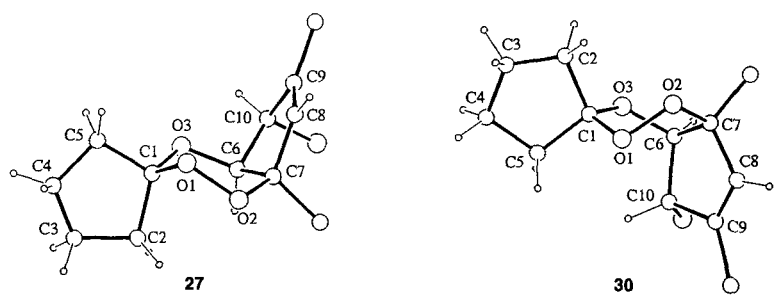


Fig. 3. *Perspective views of partial structures of* **27** *and* **30** *showing the ring fusions and 1,2,4-trioxane ring conformations*

the trioxane ring in **30** takes up a twist-boat conformation in which the O(1)–O(2) bond is bisected by a pseudo-C_2 axis ($\Delta C_2(O(1)-O(2)) = 0.052(3)$) (*Fig. 3*). It is also worth noting that the angular aryl substituent, regardless of the ring conformation, occupies equatorial-like positions in the camphanoates **27** and **30** (*Fig. 3*). However, the parent olefin *ent*-**7** prefers, at least in the solid state, the chair conformation where the angular Ph substituent is axially disposed (*Fig. 2*). Furthermore, the endocyclic torsion angles about the O(1)–O(2) bond of all three trioxanes are quite large and range from 69 to 82.4° (see *Table 5* in the *Exper. Part*).

The aforementioned divergences are not exceptional and can be ascribed to an inherent property of the *cis*-fused bicyclic entity, namely its conformational mobility[1]). This conclusion was confirmed by a dynamic NMR spectroscopic study of *ent*-**7**. Its general constitution was entirely concordant with the ^{1}H- and ^{13}C-NMR spectra obtained at room temperature in $CDCl_3$ solution. A NOESY spectrum [9], recorded at room temperature, indicated that the bridgehead *H*–C(4a) and the Ph group at C(7a) are contiguous, in keeping with the *cis*-fusion of the trioxane and cyclopentene rings (*Fig. 4*). More importantly, the NOESY spectrum also revealed strong dipolar coupling between the spirocyclopentane protons and *H*–C(4a) and *H*–C(5). Clearly, these NOESY cross-peaks are explicable in terms of fast conformational inversion of the trioxane ring. Indeed, low-temperature ^{1}H-NMR spectroscopy of *ent*-**7** in $CDCl_3$ in the range 223 to 273 K showed rapid equilibration, apparently between the chair-like conformers (**C** and **C′**) bearing axial and equatorial angular Ph groups, respectively (*Fig. 4*). On lowering the

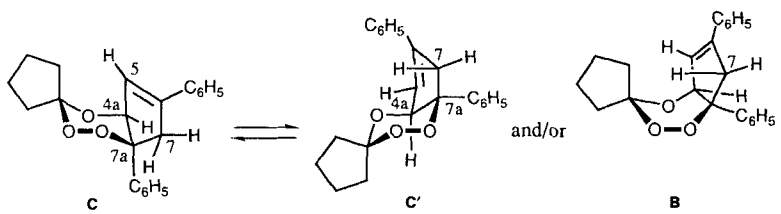


Fig. 4. *Inversion of chair* (**C**) *and boat-like* (**B**) *conformers of* ent-**7**

temperature to 223 K, the equilibrium became frozen on the NMR time scale to favor either **C** or **C′** in a ratio of 5.4:1. Thus the difference in free energy between the ground states of the conformers (ΔG_0(223)) is only 0.75 kcal/mol. Despite this slight difference, it is important to identify which of the two is the more stable. The small scalar coupling between *H*–C(4a) and *H*–C(5) ($^3J_{major} < 1.5$ Hz) and the similarity of the chemical shifts of the geminal protons at C(7) ($\Delta\delta_{major} = 0.13$ ppm) observed for the major conformer suggest that it is **C**, similar to the X-ray structure of *ent*-**7**.

Although the foregoing assignment is tentative, it is nonetheless corroborated by the marked difference in chemical shift between the geminal protons at C(7) which is displayed by the minor conformer ($\Delta\delta_{minor} = 0.75$ ppm). This difference can be attributed to the strong anisotropic field exerted by the O–O bond which in **C′** selectively affects the '*endo*'-disposed proton at C(7) (*Fig. 4*). The minor conformer cannot be represented by **C**, since neither of the methylene protons lies above the O–O bond. However, a boat-like conformer **B** is also possible, since it too would exhibit the same differential shielding on the C(7) protons.

The dynamic equilibrium was further monitored by a complete line-shape analysis [10]. The CH_2(7) resonances were examined in the temperature range 223–273 K by making use of the DNMR5 program [11] and afforded the relevant activation parameters: $\Delta H^{\neq}_{223} = 15.2$ kcal/mol, $\Delta S^{\neq} = 6.6$ cal/mol K, and $\Delta G^{\neq}_{273} = 13.4$ kcal/mol[2]).

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[1]) The present results show that, contrary to a previous suggestion [8], the *cis*-fused cyclopentene ring does not automatically force the trioxane ring into a twist-boat conformation.

[2]) These values are remarkably similar to those determined for *cis*-decalin: $\Delta H^{\neq} = 13.6 \pm 0.7$ kcal/mol, $\Delta S^{\neq} = 3.5$ cal/mol K, and $\Delta G^{\neq} = 13.0$ kcal/mol [12].

The preceding values are consonant with either the proposed chair-to-chair or chair-to-boat inversions. It can be supposed that during pseudorotation, the trioxane ring becomes flattened in the transition state, and that its evolution would not only regenerate either of the two chair conformers but flexible twist-boat conformations as well. Support for this supposition was obtained by exploring the potential-energy surface of *ent*-**7**. Full geometry optimization was performed with the CHEM-X [13] and MOPAC programs by using the semi-empirical PM3 method [14]. Several minima were located. The global minimum was computed to have a heat of formation of 0.9 kcal/mol and a geometry which can be described as a slightly flattened and twisted chair (**TC**) reminiscent of the X-ray structure (*Table 1*). Close by, the twist-boat conformer (**TB**) is berthed at a local minimum 1.1 kcal/mol higher in energy. Calculation also showed that passage between **TC** and **TB** is obstructed by a barrier of 9.8 kcal/mol, somewhat lower than that obtained experimentally. The transition-state structure, t.s.-1, was fully characterized by vibrational-frequency analysis as a half-chair conformer in which five atoms of the trioxane ring are almost coplanar (*Fig. 5*).

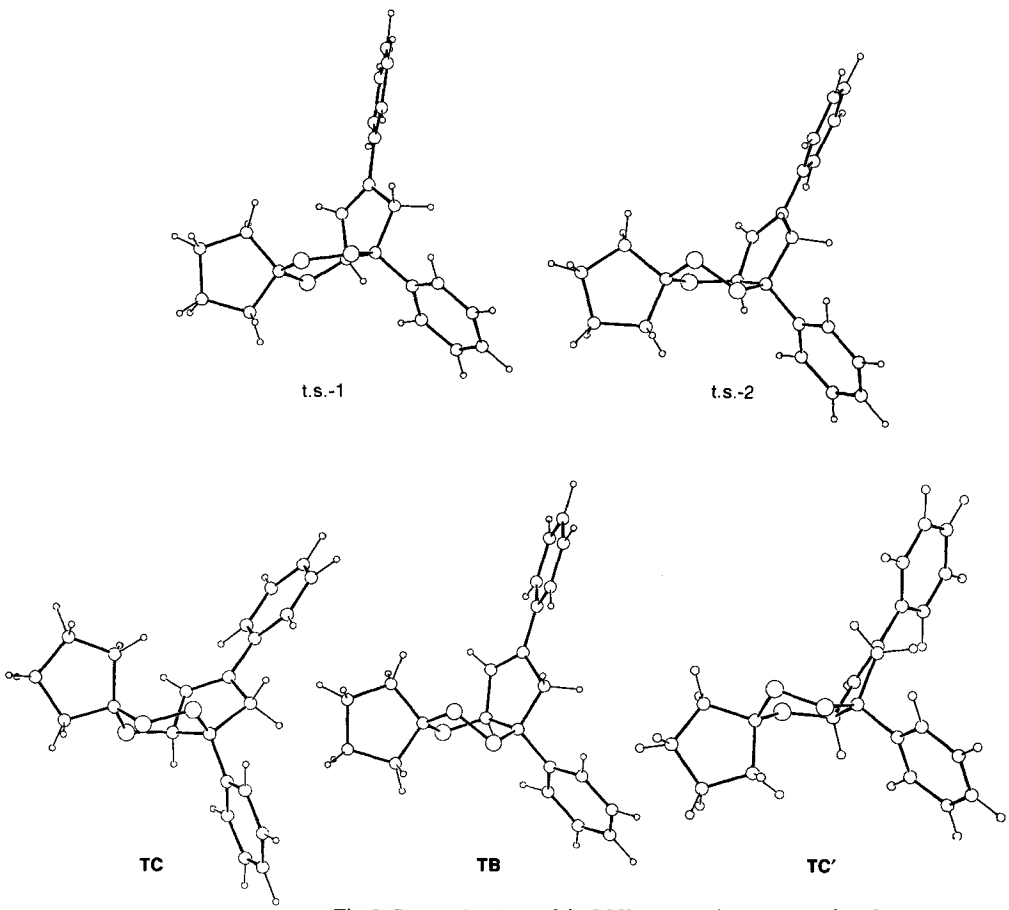


Fig. 5. *Perspective views of the PM3-optimized geometries of conformers* **TC**, **TB**, *and* **TC'** *and of the transition-state structures t.s.-1 and t.s.-2 of* ent-**7**

Table 1. *Total and Relative Heats of Formation of Conformers and Transition States of* ent-**7** *as Computed at the PM3 Level*

	TC	t.s.-1	**TB**	t.s.-2	**TC′**
Heat of formation [kcal/mol]	0.9	10.7	2.0	4.3	3.9
Relative heat of formation [kcal/mol]	0.0	9.8	1.1	3.4	3.0

Other local minima were discovered, the energetically lowest of which is the twist-chair conformer **TC′** (*Fig. 5*) having a heat of formation of 3.9 kcal/mol. The conformers **TC** and **TC′** are distinguished one from the other by the pseudoaxial and pseudoequatorial orientations of the angular Ph substituent. The potential-energy surface connecting **TB** to **TC′** was also examined and showed that a low barrier, amounting to only 2.3 kcal/mol, lies between them (*Table 1*). The transition-state structure t.s.-2 was found to be a half-chair with a heat of formation of 4.3 kcal/mol. It is, therefore, possible that the minor conformer of *ent*-**7** observed in the dynamic NMR experiment may well be a time-averaged mix of **TC′** and **TB**.

The assignment of trioxane-ring conformations for all minima (*Fig. 5*) was based on the analysis of displacement asymmetry parameters [7]. Finally, the geometries of the trioxane ring for the different minima of *ent*-**7** were systematically compared with the X-ray structure of artemisinin (**1**) [15] by using the MODEL program [16]. The best fit was obtained for **TC′**, as evidenced by the average deviation of atoms between the two trioxane rings which amounted to 0.213 Å; the match for **TB** was only marginally worse. These findings indicate that **TB** and **TC′** are easily accessible from the global minimum. It is, therefore, reasonable to expect that the (*S*,*S*)-enantiomers *ent*-**7** and *ent*-**8** would be the most biologically active. However, it will be seen shortly that this is not the case.

Antimalarial Activity. – Samples of **8** and *ent*-**8**, both racemic and enantiomerically pure, together with appropriate reference compounds, were tested *in vitro* against *Plasmodium falciparum* clones by using the method developed by *Desjardins* and coworkers [17]. The effectiveness of the sample in inhibiting the growth of DNA in the Indochina W2 and Sierra Leone D6 clones was determined. Inhibitory concentrations (*IC*) are expressed in ng/ml. The W2 clone is resistant to chloroquine (CLQ), pyrimethamine, and sulfadoxine but susceptible to mefloquine, whereas the D6 clone is susceptible to chloroquine, pyrimethamine, and sulfadoxine but resistant to mefloquine. It is immediately seen that no significant difference exists between **8**, *ent*-**8**, and the racemic mixture

Table 2. In vitro *Antimalarial Activity*[a]) *of Some Cyclopenteno-1,2,4-trioxanes against* P. falciparum *Clones*

	8/*ent*-**8** (racemic)	**8**	*ent*-**8**	Artesunate	CLQ
W2 Clone	3.57	2.01	3.93	0.57	54.55
D6 Clone	1.85	2.07	3.95	0.97	3.30

[a]) All values are IC_{50} and expressed in ng/ml.

(*Table 2*). The (+)-enantiomer **8** is slightly more active, but no more so than the racemate. No discrimination is seen between the sensitive (D6) and resistant (W2) clones.

Samples of **7**, *ent*-**7**, **8**, *ent*-**8**, and the usual reference compounds were tested *in vivo* against *P. berghei* N and *P. yoelii* NS. The method used was the four-day test [18]

where the effective dose (*ED*) is expressed in mg/kg. The dose was administered daily to infected mice for four days, and parasitemia was read on the fifth day. The NS strain is chloroquine-resistant, whereas the N strain is chloroquine-sensitive. Two administration routes were used, subcutaneous (sc) and oral (*p.o.*). In the latter route, samples were taken up in dimethyl sulfoxide (DMSO) and diluted serially with *Tween 80* in H_2O. The *in vivo* results (*Table 3*) reinforce those obtained *in vitro*. This time, the (+)-enantiomers **7**

Table 3. In vivo *Antimalarial Activity*[a]) *of Some Cyclopenteno-1,2,4-trioxanes against* P. berghei *N and* P. yoelii *ssp. NS*

		P. berghei N		*P. yoelii* NS	
		ED_{50}	ED_{90}	ED_{50}	ED_{90}
7/*ent*-**7** (racemic)	sc	4.0	8.0	5.8	13.0
	p.o.	25.0	75.0	10.0	28.0
ent-**7**	sc	4.2	8.0	6.0	11.0
	p.o.	40.0	400.0	23.0	68.0
7	sc	2.3	5.5	5.0	9.0
	p.o.	20.0	25.0	6.0	19.0
8/*ent*-**8** (racemic)	sc	2.5	6.0	4.5	7.6
	p.o.	2.5	6.0	5.6	10.0
8	sc	2.1	3.6	1.8	3.4
	p.o.	2.6	4.8	1.4	5.0
ent-**8**	sc	1.8	3.2	1.5	2.8
	p.o.	2.1	3.6	1.1	3.1
Chloroquine	sc	1.8	3.1	2.4	56.0
Quinine	sc	65.0	170.0	128.0	290.0
Artemisinin	sc	0.9	2.3	5.8	10.0

[a]) Values are expressed as *ED* in mg/kg/day × 4.

and **8** are slightly less active than the (−)-enantiomers *ent*-**7** and *ent*-**8** but insignificantly so. The non-fluorinated trioxanes **7** and *ent*-**7**, enantiomerically pure and racemic, display almost identically high activities sc against the sensitive line *P. berghei* and manifest the same trend with slightly less activity sc against the resistant line *P. yoelii*. The *p.o.* values are typically bigger and show greater variations, but again these are not a function of chirality. On passing to the fluoro derivatives **8** and *ent*-**8**, a net improvement in activity is evident. The values of the racemic and individual enantiomers are remarkably similar for both the sc and *p.o.* administration routes against *P. berghei*. The same indifference to chirality is demonstrated in the chloroquine-resistant line *P. yoelii*. It is significant in all cases that the ED_{50} and ED_{90} values are not only commensurate with each other but also strikingly similar for both administration routes as well as being just about the same for all chiral forms.

Discussions. – The *in vitro* and *in vivo* results confirm that the synthetic trioxanes are endowed with powerful antimalarial activity, particularly against the chloroquine-resistant parasites. Of note is the superior efficacy of the fluorinated derivatives **8** and *ent*-**8** when administered by the oral route, which even surpasses that of the reference compound, artemisinin[3]). Notwithstanding these remarkable activities, the second most im-

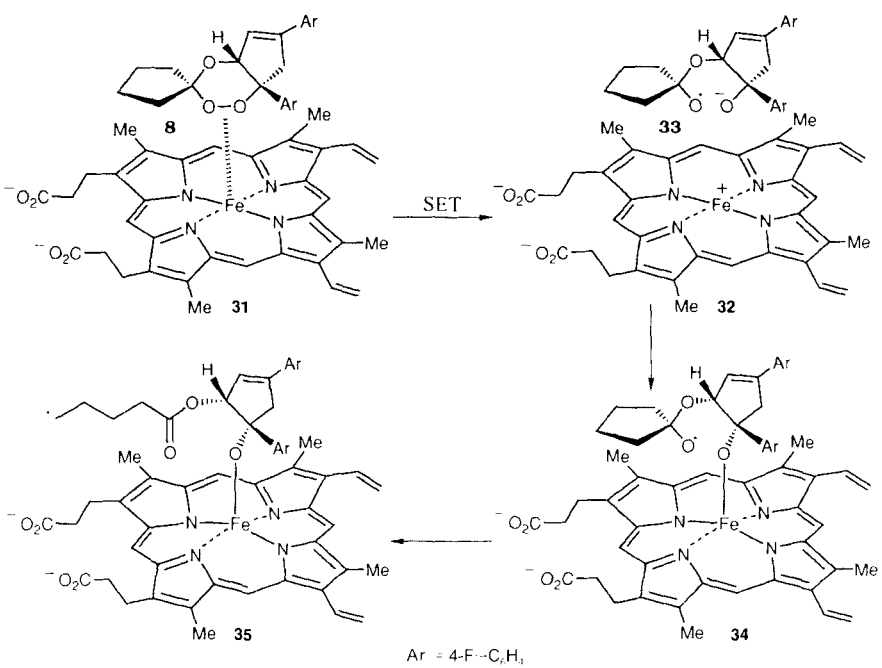
[3]) Observations on racemic, related trioxanes, including racemic **8**/*ent*-**8**, have been reported [19].

portant finding to emerge is the total irrelevance of the configuration of the *cis*-fused bicyclic entity to its mode of action.

It can be assumed that the present trioxanes and artemisinin kill the malarial parasite by the same mechanism, but differently from that of chloroquine [20]. *Plasmodium* in the intraerythrocytic stage, in order to thrive, digests hemoglobin. The prosthetic group, heme, remaining after proteolysis, being toxic to the parasite, normally is removed by enzyme-catalyzed oxidative polymerization to hemozoin which deposits as an insoluble pigment [21]. Evidence has accumulated to suggest that chloroquine [22] and artemisinin [23] in their separate ways interrupt this detoxification process either by inhibiting the polymerase supposedly responsible or by potentiating heme. Artemisinin is reported to form an adduct with hemin [23] or heme [24] after generating an unidentified oxyl radical [25]. Experiments with artemisinin-like tricyclic trioxanes have shown that the Fe^{2+} ion or heme cleaves the peroxide link to give an initial oxyl radical which by [1,5]-H rearrangement affords a C-centered radical, the entity responsible for antimalarial activity [26]. It has also been found that the decomposition of racemic mixtures **7**/*ent*-**7** and **8**/*ent*-**8** by $FeCl_2 \cdot 4\ H_2O$ entails unraveling of the trioxane and spirocyclopentane rings through oxyl and C-centered radicals [27]. The Fe^{2+} ion operates as an electron shuttle and isomerizes the trioxane ring directly to an ester derivative obviating the need for H-atom abstraction. Further, the earlier speculation [20] that 1,2,4-trioxanes would extrude an O-atom to create a ferryl species from a ferrous salt was not vindicated.

In the light of these considerations, it is safe to assume that free heme confined within the parasite does not behave like cytochrome P450 [28]. It does not become activated as

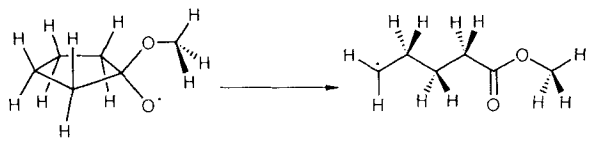
Scheme 5



oxo-heme, but deploys instead its electronic redox properties to convert the trioxane ring into the *ad hoc* parasiticidal agent. It can now be postulated that the Fe^{2+} ion in heme targets the peroxide bond of **8** or *ent*-**8** for example. It is not surprising that configuration is not an issue since the receptor, the heme molecule, is itself achiral. Nevertheless, conformation may be crucial. Fortunately, **8** with little expenditure of energy can flip into the twist-boat conformer and nestle on the surface of heme (**31**) so that the O–O bond lies atop the Fe^{2+} center (*Scheme 5*). Single-electron transfer (SET) to the O–O σ^* orbital then sunders the trioxane ring to form the radical anion **33**. In reality, the process is probably one of coordination in which the newly created hemin cation **32** binds directly to the cyclopentenyl-oxide ion **33**. This latter sequence is akin to the *Haber-Weiss* reaction [29], a relevant illustration being the reactivation of native ferrous lipoxygenase to the catalytic ferric form by priming with substrate hydroperoxide [30]. The attached spirocyclic acetal radical **34** spontaneously rearranges to the ester **35**, placing the radical on the end of the side chain. Thereafter, the δ-radical of the pentanoyl substituent can disable the parasite by deadly alkylation. If **35** fails to find a parasite as a victim, it presumably reacts with itself possibly on a vinyl substituent to yield a more stable radical which eventually evolves to an adduct.

Credence for ester formation as the driving force for antimalarial action was provided by a consideration of the model structures, the 1-methoxycyclopentyl-1-oxyl radical **36** and the δ-radical **37** of methyl pentanoate (*Scheme 6*). The geometries of **36** and **37** were optimized by semi-empirical unrestricted *Hartree-Fock* calculations according to the PM3 method [14]. The heats of formation, −59.5 and −76.6 kcal/mol, so obtained confirm that the conversion of **36** to **37** is a strongly exothermic process.

Scheme 6



36 **37**

Heats of formation: −59.49 kcal/mol −76.57 kcal/mol

Conclusion. – The present findings provide a coherent rationale for the mode of action of the *cis*-fused cyclopenteno-1,2,4-trioxanes **7**, *ent*-**7**, **8**, and *ent*-**8** and indicate that a C-centered radical, rather than an oxidant species, is the lethal antiparasitic agent. The sequence of close docking on heme, rupture of the peroxide bond by electron transfer, and thermodynamically driven rearrangement to an active radical, is undoubtedly manifested by other structurally related peroxidic antimalarials such as artemisinin, yingzhaosu [31], and certain 3,3,6,6-tetrasubstituted 1,2,4,5-tetroxanes [32]. Finally, it should be possible to apply these mechanistic principles and design new bicyclic peroxides of even greater potency and synthetic availability.

Experimental Part

General. See [4]. 1D- and 2D-NMR Spectra: in $CDCl_3$; *Bruker-AMX-400* spectrometer operating at 9.4 Tesla and equipped with a probe-head for variable-temp. experiments.

1. *3-(4-Fluorobenzoyl)propanoic Acid* (**16**). To a stirred soln. of fluorobenzene (**14**; 160 g, 1.66 mol) and succinic anhydride (**15**; 46.8 g, 0.47 mol) under N_2 was added $AlCl_3$ (167 g, 1.25 mol) in portions over 1 h. After stirring for 4 more h, the resulting mixture was poured onto crushed ice (500 g). Next, conc. aq. HCl soln. (65 ml) was added with stirring over 30 min. The resulting precipitate was filtered, washed with hexane (3 × 100 ml), and dried for 12 h in a desiccator: **16** (92 g, 99%). Colorless solid, m.p. 108–110°, which was directly esterified.

2. *Ethyl 3-(4-Fluorobenzoyl)propanoate* (**17**). A mixture of EtOH (130 ml), CH_2Cl_2 (360 ml), conc. H_2SO_4 soln. (3.5 ml), and **16** (90 g) under N_2 was heated under reflux for 8 h. After cooling to 22°, H_2O (250 ml) was added. The org. phase was separated, washed with aq. $NaHCO_3$ soln., dried (Na_2SO_4), and evaporated: Washing (hexane) gave **17** (94 g, 91%). Colorless solid. M.p. 41–43°.

3. *1,4-Bis(4-fluorophenyl)cyclopenta-1,3-diene* (**12**). Na (5 g, 0.22 mol) was dissolved piecemeal in dry EtOH (100 ml) with vigorous stirring under N_2. After complete evaporation under reduced pressure and cooling, dry toluene (100 ml) was added with stirring. Next, **17** (23 g) and then 4-fluoroacetophenone (**18**; 140 ml) were quickly added. The resulting mixture was stirred at 40° for 16 h. On cooling to 22°, H_2O (400 ml) was added. Separation of the aq. layer and heating at 80° resulted in the precipitation of **12** which by successive rinsing with H_2O (40 ml), EtOH (20 ml), and hexane (10 ml) gave **12** (8 g, 31%) as pale yellow crystals (m.p. 156–158°) in sufficient purity for photo-oxygenation. The addition of **18** prior to **17** did not noticeably improve the yield of **12** which varied from 31–70% regardless of the order of addition. ^{1}H-NMR: 3.70 (*t*, *J* = 0.8, 2 H); 6.83 (*t*, *J* = 0.8, 2 H); 7.05 (*m*, 4 H); 7.50 (*m*, 4 H).

4. *3-(4-Fluorophenyl)cyclopent-2-enone* (**22**) [5]. Ethyl acetoacetate (**20**; 13 g, 0.1 mol) was added to a stirred soln. of EtONa (7.5 g, 0.11 mol) in EtOH (80 ml). After heating the resulting soln. for 15 min, 4-fluorophenacyl bromide (**19**; 21.7 g, 0.1 mol) was added and heating continued for 3 more h. The mixture was cooled, poured into ice-H_2O (250 ml) and extracted with Et_2O (2 × 150 ml). After drying ($MgSO_4$), Et_2O was removed giving *ethyl 2-acetyl-3-(4-fluorobenzoyl)propanoate* (**21**; 23.2 g, 87%) as colorless crystals, m.p. 33–34°. Next, **21** (8.0 g, 0.03 mol) was added to 2% aq. KOH soln. (180 ml) at 50° with stirring for 10 min. The clear soln. was heated under reflux for 30 min and then cooled to 0° in an ice bath. On standing for 30 min, a precipitate formed, which on filtration, washing with H_2O, drying, and recrystallization from EtOH/Et_2O gave **22** (2.55 g, 42%). Pale yellow crystals. M.p. 87–89°. ^{1}H-NMR: 2.60 (*t*, *J* = 5.1, 2 H); 3.04 (*td*, *J* = 5.1, 1.5, 2 H); 6.32 (*t*, *J* = 1.5, 1 H); 7.15 (*m*, 2 H); 7.65 (*m*, 2 H). Anal. calc. for $C_{11}H_9FO$: C 75.00, H 5.11; found: C 74.83, H 5.05.

5. *1,3-Bis(4-fluorophenyl)cyclopenta-1,3-diene* (**24**) [5]. A soln. of (4-fluorophenyl)magnesium bromide (**23**; prepared from Mg (267 mg, 0.011 mol) and 4-fluorophenyl bromide (1.93 g, 0.011 mol)) in Et_2O (8 ml) was added dropwise to a stirred soln. of **22** (1.76 g, 0.01 mol) in Et_2O (40 ml). The mixture was heated under reflux for 1 h with stirring, then cooled to 20°, and poured into ice-cold 10% aq. H_2SO_4 soln. (10 ml). After stirring for 30 min, the Et_2O layer was separated, washed with aq. $NaHCO_3$ soln. and H_2O, and evaporated: **24** (1.73 g, 68%). Colorless crystals. M.p. 156–157°. ^{1}H-NMR: 3.56 (*s*, 2 H); 6.56 (*s*, 1 H); 7.05 (*m*, 4 H); 7.13 (*s*, 1 H); 7.50 (*m*, 4 H). Anal. calc. for $C_{17}H_{12}F_2$: C 80.41, H 4.72; found: C 80.04, H 4.64.

Isomerization of **24**. A suspension of **24** in EtOH on heating under reflux for 1 h underwent complete conversion to **12**, identical to that prepared in *Exper. 3*.

6. *(4'aRS,7'aRS)-4'a,7'a-Dihydro-6',7'a-diphenylspiro[cyclopentane-1,3'-7'H-cyclopenta[1,2-e][1,2,4]trioxine]* (**7**/*ent*-**7**). A soln. of 1,4-diphenylcyclopenta-1,3-diene (**9**; 3.0 g, 13.4 mmol) [33] in CH_2Cl_2 (60 ml) and CCl_4 (15 ml) containing tetraphenylporphyrin (TPP; 20 mg) as sensitizer was photo-oxygenated at 5–10° for 30 min according to a previously described procedure [4]. The yield of endoperoxide **10** was quantitative. The solvent was evaporated, the residue dissolved in CH_2Cl_2 (120 ml), and the soln. cooled to −78°. Next, cyclopentanone (**11**; 5 ml) and Me_3SiOTf (0.3 ml) were added with stirring which was continued for 4 h. Finally, Et_3N (0.5 ml) was added and the soln. allowed to warm to 22°. After addition of H_2O (30 ml), the org. phase was separated, dried (Na_2SO_4), and evaporated and the brownish oil purified by CC (SiO_2, hexane/CH_2Cl_2 2:1): **7**/*ent*-**7** (4.0 g, 89%). Pale yellow crystals. M.p. 85–86°. ^{1}H-NMR: 1.6–2.0 (*m*, 7 H); 2.40 (*m*, 1 H); 3.04 (br. *d*, *J* = 16, 1 H); 3.29 (br. *d*, *J* = 16, 1 H); 5.23 (br. *s*, 1 H); 6.35 (br. *ddd*, *J* = 2, 2, 2, 1 H); 7.30–7.7 (*m*, 10 H). ^{13}C-NMR: 23.0, 24.3, 35.9, 36.2, 44.3, 80.1, 87.3, 113.2, 124.7, 125.9, 126.0, 127.6, 128.3, 128.4, 128.5, 134.9, 141.9, 143.1. Anal. calc. for $C_{22}H_{22}O_3$: C 79.02, H 6.63; found: C 78.80, H 6.71.

7. *(4'aRS,7'aRS)-6',7'a-Bis(4-fluorophenyl)-4'a,7'a-dihydrospiro[cyclopentane-1,3'-7'H-cyclopenta[1,2-e][1,2,4]trioxine]* (**8**/*ent*-**8**). A soln. of **12** (3.5 g, 13.8 mmol) was subjected to the procedure described in *Exper. 6*: **8**/*ent*-**8** (3.4 g, 66%). Colorless crystals. M.p. 104–105°. ^{1}H-NMR: 1.6–1.95 (*m*, 7 H); 2.30 (*m*, 1 H); 2.95 (*dt*, *J* = 16.0, 2.0, 1 H); 3.2 (br. *d*, *J* = 16.0, 1 H); 5.1 (br. *s*, 1 H); 6.2 (br. *s*, 1 H); 7.1–7.6 (*m*, 8 H). ^{13}C-NMR: 22.9, 24.3, 35.9, 36.1, 44.2, 80.0, 86.9, 113.3, 115.2 (*J* = 21), 115.5 (*J* = 21), 124.3, 127.6 (*J* = 8), 127.9 (*J* = 8), 130.9 (*J* = 3), 137.5 (*J* = 3), 141.9, 162.3 (*J* = 245), 162.8 (*J* = 245). Anal. calc. for $C_{22}H_{20}O_3F_2$: C 71.34, H 5.45; found: C 71.27, H 5.48.

8. *Resolution of Racemic Trioxanes* **7**/ent-**7** *and* **8**/ent-**8**. The action of *N*-methylmorpholine *N*-oxide in the presence of catalytic amounts of potassium osmate and 1,4-bis(dihydroquinidine)phthalazine in aq. acetone at 25° brought about selective dihydroxylation of **7** and **8** so that at 60% conversion, the remaining *ent*-**7** and *ent*-**8** were essentially optically pure (98% enantiomeric excess) [6]. By effecting asymmetric dihydroxylation with the reagent containing the ligand of opposite chirality, namely 1,4-bis(dihydroquinine)phthalazine, **7** and **8** were obtained with high enantiomeric purity (96% ee).

Submission of racemic **7**/*ent*-**7** or **8**/*ent*-**8** (maximum loading of 19 mg) to HPLC (*Chiracel OG* column, 25 × 2 (*Daicel Industries Ltd.*, Tokyo-100, Japan), 2% i-PrOH in hexane) at 25° resulted in clean separation of both enantiomers.

Optical rotations $[\alpha]_D^{22}$ ($CHCl_3$, *c* = 1.1): +128 (**7**), −129 (*ent*-**7**), +115 (**8**), −133.3 (*ent*-**8**).

9. *(+)-(4'aS,5'R,7'S)-4'a,7'a-Dihydro-6',7'a-diphenylspiro[cyclopentane-1,3'-5'H-cyclopenta[1,2-e][1,2,4]trioxin]-5'-ol* (**26**) *and (−)-(4'aR,5'S,7'aR)-6',7'a-Bis(4-fluorophenyl)-4'a,7'a-dihydrospiro[cyclopentane-1,3'-5'H-cyclopenta[1,2-e][1,2,4]trioxin]-5'-ol* (**29**). Solns. of *ent*-**7** (0.97, 2.9 mmol) and **8** (1.08, 2.91 mmol) in CCl_4 (80 ml) and CH_2Cl_2 (20 ml) containing TPP (10 mg) were individually photo-oxygenated at 8° for 4 h according to the previous procedure [4] [34]. The hydroperoxides **25** and **28**, respectively, were obtained, but not isolated, by evaporation of solvent *in vacuo* without heating. In each case, dry THF (20 ml) was added to the hydroperoxide, followed by $NaBH_4$ (133 mg, 3.52 mmol) in portions over 20 min. After 4 h (reaction complete), H_2O (20 ml) was added dropwise, then 5M aq. HCl (3 ml). The org. phase was separated, the aq. phase extracted with CH_2Cl_2 (2 × 10 ml), the combined org. phase dried ($MgSO_4$) and evaporated, and the crude oil purified by CC (SiO_2, CH_2Cl_2).

Recrystallization (hexane/Et_2O 1:1) gave **26** (0.76 g, 75%). Pale yellow crystals. M.p. 111–112°. $[\alpha]_D^{20}$ = +50.7 (*c* = 1.0, $CHCl_3$). ^{1}H-NMR: 1.59–2.21 (*m*, 9 H); 4.40 (*d*, *J* = 1.5, 1 H); 5.25 (*dd*, *J* = 6.8, 1.5, 1 H); 6.36 (*s*, 1 H); 7.24–7.66 (*m*, 10 H). ^{13}C-NMR: 23.8, 23.9, 34.8, 37.2, 79.8, 81.8, 91.3, 113.5, 126.7, 126.8, 127.8, 128.5, 128.6, 128.7, 128.9, 133.1, 140.5, 147.1. Anal. calc. for $C_{22}H_{22}O_4$: C 75.41, H 6.33; found: C 74.31, H 6.41.

Similarly, **29** (0.84 g, 74%) was obtained as pale yellow crystals. M.p. 102–104°. $[\alpha]_D^{20}$ = −42.6 (*c* = 1.1, $CHCl_3$). ^{1}H-NMR: 1.76–2.16 (*m*, 9 H); 4.34 (*d*, *J* = 1.5, 1 H); 5.24 (br. *d*, *J* = 4.3, 1 H); 6.25 (*s*, 1 H); 7.10–7.66 (*m*, 8 H). ^{13}C-NMR: 23.7, 24.0, 34.8, 37.2, 79.9, 82.1, 91.0, 113.7, 115.5 (*J* = 21), 115.7 (*J* = 21), 127.3, 128.5 (*J* = 8), 128.7 (*J* = 8), 129.3, 136.3, 146.2, 162.7 (*J* = 246), 163.1 (*J* = 246). Anal. calc. for $C_{22}H_{20}F_2O_4$: C 68.39, H 5.22; found: C 68.56, H 5.45.

10. *(1S)-Camphanoates* **27** *and* **30**. Solns. of **26** (0.37 g, 1.05 mmol) and **29** (0.4 g, 1.05 mmol) in pyridine (10 ml) were individually treated with portions of (1*S*)-camphanoyl chloride (0.25 g, 1.2 mmol) under Ar at 0°. Thereafter, the temp. was allowed to rise to 22°. After stirring for 3 days, the mixture was poured into H_2O (10 ml) to which CH_2Cl_2 (10 ml) was added. The org. phase was decanted, the aq. phase extracted with CH_2Cl_2 (2 × 5 ml), the combined org. phase washed with aq. $NaHCO_3$ soln. (5 ml), dried ($MgSO_4$), and evaporated, and the oil purified by CC (SiO_2, CH_2Cl_2/AcOEt 19:1).

Recrystallization (hexane/CH_2Cl_2 3:1) gave **27** as colorless crystals (0.39 g, 70%). M.p. 169–170°. $[\alpha]_D^{20}$ = −29.2 (*c* = 1, $CHCl_3$). ^{1}H-NMR: 0.65 (*s*, 3 H); 0.67 (*s*, 3 H); 1.00 (3 H); 1.58–2.03 (*m*, 12 H); 4.39 (*dd*, *J* = 2, 0.6, 1 H); 6.58 (*d*, *J* = 2.7, 1 H); 7.26–7.58 (10 H).

Similarly, **30** was obtained as colorless crystals (0.45 g, 75%). M.p. 154–160°. $[\alpha]_D^{20}$ = +21.0 (*c* = 1, $CHCl_3$). ^{1}H-NMR: 0.62 (*s*, 3 H); 0.82 (*s*, 3 H); 1.03 (*s*, 3 H); 1.55–2.06 (*m*, 12 H); 4.39 (br. *s*, 1 H); 6.36 (*s*, 1 H); 7.05–7.58 (8 H).

Crystallographic Data of ent-**7**, **27**, *and* **30**. Cell parameters and diffracted intensities were measured at r.t. on a *Nonius CAD4* (**27** and **30**) and *Philips PW1100* (*ent*-**7**) diffractometer with graphite-monochromated MoK_α (λ = 0.71069 Å; **27** and *ent*-**7**) and CuK_α (λ = 1.5418 Å; **30**) radiations. Data were corrected for *Lorentz* and polarization effects. Anal. absorption corrections [35] were applied for **30**. Two reference reflections (100 refl. measured in each case) showed variations of less than 3.5 σ (*I*) and a decrease of *ca.* 4.5% for *ent*-**7**; all intensities were corrected for this drift. The structures were solved by direct methods using MULTAN 87 [36]; all other

Table 4. *Crystal Data, Intensity Measurement, and Structure Refinement for* ent-**7**, **27**, *and* **30**

	ent-**7**	**27**	**30**
Formula	$C_{22}H_{22}O_3$	$C_{32}H_{34}O_7$	$C_{32}H_{32}O_7F_2$
Mol. wt.	334.4	530.6	566.6
Crystal system	monoclinic	monoclinic	monoclinic
Space group	$P2_1/c$	$P2_1$	$P2_1$
a [Å]	6.445(1)	9.474(3)	7.822(1)
b [Å]	21.942(7)	8.377(1)	8.2526(6)
c [Å]	12.343(1)	17.864(5)	22.250(2)
β [°]	93.705(7)	91.53(1)	91.799(5)
V [Å^3]	1741.9(6)	1417.3(6)	1435.6(3)
Z	4	2	2
$F(000)$	712	564	596
D_c [g cm^{-3}]	1.28	1.24	1.31
$\mu(MoK_\alpha)$ [mm^{-1}]	0.078	0.081	0.839
A^* min., max.	–	–	1.039, 1.150
$(\sin\theta/\lambda)_{max}$ [Å^{-1}]	0.53	0.55	0.56
No. measured reflns.	2233	2197	2323
No. observed reflns.	1276	1643	2272
Criterion for observed	$\lvert F_o\rvert > 4\sigma(F_o)$	$\lvert F_o\rvert > 4\sigma(F_o)$	$\lvert F_o\rvert > 4\sigma(F_o)$
Refinement (on F)	full-matrix	full-matrix	full-matrix
No. parameters	226	352	369
Weighting scheme	$\omega = 1/\sigma^2(F_o)$	$\omega = 1$	$\omega = 1/\sigma^2(F_o)$
Max. and average Δ/σ	0.0016, 0.0003	0.041, 0.006	0.0001, 0.00003
Max. and min. $\Delta\rho$ [e Å^{-3}]	0.43, −0.64	0.39, −0.38	0.30, −0.33
S	2.00	1.15	3.17
R, ωR	0.058, 0.043	0.062, 0.062	0.062, 0.041

Table 5. *Selected Bond Lengths* [Å], *Bond Angles* [°], *and Torsional Angles* [°] *for* ent-**7**, **27**, *and* **30**

	ent-**7**	**27**	**30**
O(1)–O(2)	1.477(5)	1.473(8)	1.472(6)
O(1)–C(1)	1.439(7)	1.43(2)	1.387(2)
O(2)–C(7)	1.447(7)	1.44(1)	1.453(7)
O(3)–C(1)	1.413(8)	1.42(2)	1.429(9)
O(3)–C(6)	1.446(7)	1.435(9)	1.416(7)
O(4)–C(10)	–	1.47(1)	1.479(6)
C(6)–C(7)	1.548(8)	1.55(2)	1.545(8)
C(6)–C(10)	1.502(9)	1.52(1)	1.55(1)
C(7)–C(8)	1.532(8)	1.50(2)	1.50(1)
C(8)–C(9)	1.493(8)	1.33(2)	1.330(9)
C(9)–C(10)	1.345(9)	1.51(2)	1.489(9)
C(1)–O(1)–O(2)	105.6(4)	107(1)	106.8(4)
O(1)–O(2)–C(7)	107.0(4)	108.4(7)	105.4(4)
C(1)–O(3)–C(6)	114.7(4)	114.1(8)	115.4(6)
O(1)–C(1)–O(3)	108.8(5)	107.4(9)	109.2(5)
O(3)–C(6)–C(7)	113.7(5)	112(1)	112.4(4)
C(6)–C(7)–O(2)	106.9(4)	111.1(8)	108.2(4)
C(6)–C(7)–C(8)	102.7(5)	102.1(9)	103.3(5)
C(7)–C(8)–C(9)	105.0(4)	112(1)	112.1(6)
C(8)–C(9)–C(10)	110.1(5)	109.8(9)	112.5(6)
C(9)–C(10)–C(6)	111.3(5)	104(1)	103.5(5)

Table 5 (cont.)

	ent-7	27	30
C(1)–O(1)–O(2)–C(7)	–74.9(5)	69(1)	–82.4(6)
O(1)–O(2)–C(7)–C(6)	61.6(5)	–53(1)	37.9(7)
O(2)–C(7)–C(6)–O(3)	–46.5(6)	42(1)	23.1(9)
C(7)–C(6)–O(3)–C(1)	42.9(6)	–45(1)	–53.1(8)
C(6)–O(3)–C(1)–O(1)	–53.6(6)	60(1)	12.8(7)
O(3)–C(1)–O(1)–O(2)	68.0(5)	–70.6(8)	53.1(6)
C(10)–C(6)–C(7)–C(8)	–26.9(5)	29(1)	15.3(6)
C(6)–C(7)–C(8)–C(9)	25.4(6)	–21(1)	–8.4(7)
C(7)–C(8)–C(9)–C(10)	–14.5(7)	4(1)	–2.5(8)
C(8)–C(9)–C(10)–C(6)	–3.6(7)	15.8(9)	12.2(7)
C(9)–C(10)–C(6)–C(7)	19.8(6)	–27.6(9)	–16.6.(6)

calculations used the XTAL [37] and ORTEP [38] programs. All coordinates of the H-atoms were calculated. A summary of crystal data, intensity measurement, and structure refinement is given in *Table 4*, and selected geometrical parameters are reported in *Table 5*. Crystallographic data have been deposited with the *Cambridge Crystallographic Data Center*, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, England.

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