

Crystal Structure of Crispolide Diacetate,† a 4,14-Cyclogermacranolide with a Secondary Peroxy Ester Moiety‡

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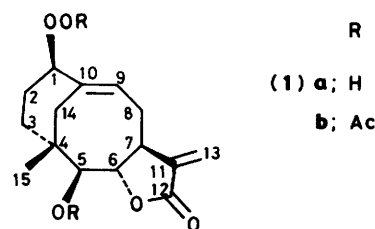
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The crystal structure of crispolide diacetate (**1b**), a 4,14-cyclogermacranolide bearing a secondary peroxy ester group, was determined by direct methods from 1612 reflections, and refined to $R = 0.041$. The crystals are orthorhombic, space group $P2_12_12_1$. The methyl group at C(4) and the hydroxy group at C(5) are *cis*-oriented on the β -face of the eight-membered ring, in accordance with the proposed biogenetic derivation for the unique terpenoid skeleton. The secondary peroxy ester group adopts a conformation different from that found in solution and in the solid state for tertiary peroxy esters.

Crispolide (**1a**) is a sesquiterpene lactone with a unique§ 4,14-cyclogermacran skeleton, isolated from *Tanacetum vulgare* L. v. *crispum* Fiori and from two chemotypes of *T. vulgare* L.¹ Crispolide bears a secondary hydroperoxyl group, and is one of the very few naturally occurring hydroperoxides not formally derivable from the photo-oxygenative modification of a known olefinic precursor.

For the biogenesis of the carbon skeleton of (**1a**), Scheme 1, involving acid-catalysed transannular opening of an oxirane ring by an exocyclic methylene group in a 4 α ,5 β -epoxygermacr-10(14)-ene precursor, has been proposed.¹ Whereas acid-catalysed transannular cyclizations involving an exocyclic double bond as nucleophilic species are known reactions in the nine-membered-ring caryophyllene derivatives (*cf.* the formation of clovene and caryolanol from caryophyllene² and of clovane-2,9-diol from caryophyllene oxide³), reactions of this type have never been reported for the homologous germacran derivatives. A biogenesis of this type for crispolide requires configurational inversion at C(4) relative to the epoxyolefin precursor, with a *cis*- β -orientation of the C(4) methyl and the C(5) hydroxy groups with respect to the eight-membered ring.¶ The stereochemical relationship between these two groups was not however firmly derivable on the basis of spectral data alone, owing to ambiguities in the assessment of the stereochemistry at C(4).¹



To establish the configuration at this centre and to elucidate the conformation of the bicyclic carbon skeleton of crispolide, an *X*-ray diffraction study on the diacetate (**1b**) was undertaken. In spite of the presence of the secondary peroxy ester group, (**1b**) was stable under the conditions of *X*-ray analysis, and appears to be the first compound bearing this moiety to be studied by diffraction techniques.

Results and Discussion

The results of the *X*-ray analysis establish that crispolide diacetate (**1b**) has the constitution and relative stereochemistry shown.

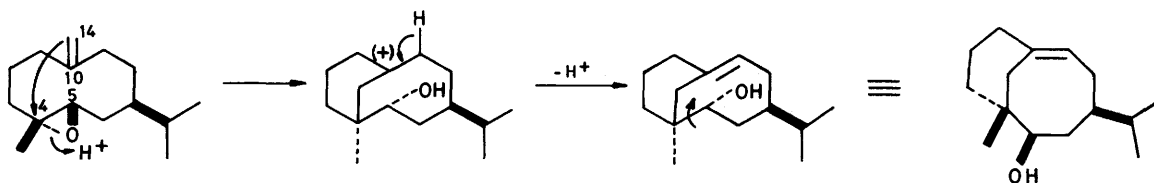
The main skeletal feature of crispolide is the fusion of the methylene- γ -lactone ring to a bicyclo[5.3.1]undecene. The lactone ring is *trans*-closed at C(6), the peroxy ester group is β -axial, and the configuration at C(4) is that expected from the biogenetic hypothesis depicted in Scheme 1 [methyl at C(4) and hydroxy group at C(5) on the β -face of the eight-membered ring]. The stereochemistry at the endocyclic double bond must necessarily be *cis* in order to allow the bridged fusion of the six- and eight-membered rings. The resulting system is large enough to accommodate a bridgeheaded double bond (*S* number⁴ 9), and most bond distances and angles over the individual rings are in fact within normal ranges. The endocyclic torsion angle round the olefinic double bond [C(14)-C(10)=C(9)-C(8)] is close to the unstrained value of 0°; however, the corresponding exocyclic torsion angle [C(1)-C(10)=C(9)-C(8)] deviates significantly from planarity (163.0°). Besides torsional strain associated with the out-of-plane bending of the allylic carbon C(1), the molecule of crispolide diacetate also presents strain due to non-bonded interactions between *pro-R*-H(14) (the one pointing towards the lactone ring) and the quasiaxial protons H(6) and *pro-R*-(β)H(8) [distance H(14) \cdots H(6) = 2.134 Å; distance H(14) \cdots H(8) = 2.301 Å]. In particular, the transannular separation H(14) \cdots H(6) is distinctly shorter

† Crispolide is (1*R*, 4*R*, 5*S*, 6*S*, 7*S*) (*E*)-1-hydroperoxy-5-hydroxy-4,14-cyclogermacra-9,11(13)-dien-12,6-olactone.

‡ Supplementary data available (SUP 56402, 3 pp.): H-co-ordinates, temperature factors. For details of Supplementary Publications see Instructions for Authors, *J. Chem. Soc., Perkin Trans. 2*, Issue no. 1, 1986. Structure factor tables are available on request from the editorial office.

§ The germacran skeleton shows a C_2 axis through C(2) and C(7); this makes numbering ambiguous unless the absolute configuration is known. From the constitutional point of view, the carbon skeleton of crispolide is shared by disyhamifolide. On biogenetic grounds the latter was, however, formulated as a 10,15-cyclogermacranolide (F. Bohlmann, A. K. Dhar, J. Jakupovic, R. M. King, and H. Robinson, *Phytochemistry*, 1981, **20**, 1077). According to c.d. studies crispolide is to be considered a 4,14-cyclogermacranolide (see later).

¶ The bidimensional representation of crispolide does not follow the conventional representation of germacranolides, which has a re-entrant angle at C(5). In (**1a**) this carbon atom is in fact tetrahedral, and not trigonal or part of a *trans*-epoxide ring, (for a more detailed discussion of this problem, see D. Rogers, G. P. Moss, and S. Neidle, *J. Chem. Soc., Chem. Commun.*, 1972, 142; N. H. Fischer, E. J. Olivier, and H. D. Fischer, *Prog. Chem. Org. Nat. Prod.*, 1979, **38**, 59; P. J. De Clercq, *Tetrahedron*, 1981, **37**, 4277).



Scheme 1. Possible biogenetic route to 4,14-cyclogermacrane derivatives

Table 1. Atom co-ordinates ($\times 10$) and temperature factors ($\text{\AA}^2 \times 10$) (e.s.d.s in parentheses)

Atom	x	y	z	U
O(1)	8 957(2)	10 132(1)	318(1)	42(1)*
O(2)	8 157(2)	10 759(2)	-1 002(1)	62(1)*
O(3)	13 142(2)	10 694(2)	4 095(2)	59(1)*
O(4)	14 472(2)	11 025(2)	3 778(1)	57(1)*
O(5)	14 309(3)	12 251(2)	4 856(2)	75(1)*
O(6)	8 456(2)	9 553(1)	1 964(1)	38(1)*
O(7)	7 365(2)	11 071(2)	1 882(2)	64(1)*
C(1)	12 139(3)	11 193(2)	3 535(2)	48(1)*
C(2)	10 781(3)	10 723(3)	3 861(2)	53(1)*
C(3)	10 528(3)	9 619(2)	3 524(2)	45(1)*
C(4)	10 785(3)	9 460(2)	2 483(2)	36(1)*
C(5)	9 743(2)	10 093(2)	1 927(2)	33(1)*
C(6)	10 128(3)	10 280(2)	910(2)	37(1)*
C(7)	10 632(3)	11 386(2)	687(2)	40(1)*
C(8)	12 133(3)	11 599(3)	896(2)	50(1)*
C(9)	12 326(3)	11 745(2)	1 909(2)	48(1)*
C(10)	12 323(3)	10 990(2)	2 523(2)	40(1)*
C(11)	10 147(3)	11 509(2)	-281(2)	46(1)*
C(12)	8 998(3)	10 792(2)	-402(2)	44(1)*
C(13)	10 578(4)	12 113(3)	-941(2)	72(1)*
C(14)	12 234(3)	9 866(2)	2 284(2)	39(1)*
C(15)	10 713(3)	8 314(2)	2 238(2)	47(1)*
C(16)	14 954(3)	11 835(2)	4 279(2)	51(1)*
C(17)	16 348(4)	12 074(4)	3 976(3)	73(1)*
C(18)	7 334(3)	10 147(2)	1 888(2)	44(1)*
C(19)	6 091(4)	9 515(4)	1 800(3)	67(1)*

* Equivalent isotropic U defined as one third of the trace of the orthogonalised U tensor.

Table 2. Bond lengths (\AA) (e.s.d.s in parentheses)

O(1)-C(6)	1.456(3)	O(1)-C(12)	1.357(3)
O(2)-C(12)	1.206(4)	O(3)-O(4)	1.456(3)
O(3)-C(1)	1.437(4)	O(4)-C(16)	1.366(4)
O(5)-C(16)	1.185(4)	O(6)-C(5)	1.451(3)
O(6)-C(18)	1.353(3)	O(7)-C(18)	1.200(4)
C(1)-C(2)	1.547(4)	C(1)-C(10)	1.510(4)
C(2)-C(3)	1.535(5)	C(3)-C(4)	1.555(4)
C(4)-C(5)	1.546(4)	C(4)-C(14)	1.551(4)
C(4)-C(15)	1.530(4)	C(5)-C(6)	1.550(4)
C(6)-C(7)	1.554(4)	C(7)-C(8)	1.538(4)
C(7)-C(11)	1.500(4)	C(8)-C(9)	1.501(5)
C(9)-C(10)	1.329(4)	C(10)-C(14)	1.502(4)
C(11)-C(12)	1.477(4)	C(11)-C(13)	1.313(5)
C(16)-C(17)	1.477(6)	C(18)-C(19)	1.481(5)

Table 3. Bond angles ($^\circ$) (e.s.d.s in parentheses)

C(6)-O(1)-C(12)	110.6(2)	O(4)-O(3)-C(1)	107.9(2)
O(3)-O(4)-C(16)	111.9(2)	C(5)-O(6)-C(18)	116.0(2)
O(3)-C(1)-C(2)	104.1(2)	O(3)-C(1)-C(10)	113.2(2)
C(2)-C(1)-C(10)	109.6(2)	C(1)-C(2)-C(3)	114.2(3)
C(2)-C(3)-C(4)	114.3(2)	C(3)-C(4)-C(5)	109.4(2)
C(3)-C(4)-C(14)	106.7(2)	C(5)-C(4)-C(14)	109.6(2)
C(3)-C(4)-C(15)	110.5(2)	C(5)-C(4)-C(15)	111.3(2)
C(14)-C(4)-C(15)	109.3(2)	O(6)-C(5)-C(4)	107.9(2)
O(6)-C(5)-C(6)	109.0(2)	C(4)-C(5)-C(6)	114.9(2)
O(1)-C(6)-C(5)	110.6(2)	O(1)-C(6)-C(7)	104.6(2)
C(5)-C(6)-C(7)	115.1(2)	C(6)-C(7)-C(8)	115.6(2)
C(6)-C(7)-C(11)	101.1(2)	C(8)-C(7)-C(11)	118.4(2)
C(7)-C(8)-C(9)	109.9(3)	C(8)-C(9)-C(10)	124.7(3)
C(1)-C(10)-C(9)	122.1(3)	C(1)-C(10)-C(14)	112.9(2)
C(9)-C(10)-C(14)	124.1(3)	C(7)-C(11)-C(12)	106.9(2)
C(7)-C(11)-C(13)	130.6(3)	C(12)-C(11)-C(13)	122.5(3)
O(1)-C(12)-O(2)	121.2(3)	O(1)-C(12)-C(11)	109.1(2)
O(2)-C(12)-C(11)	129.6(3)	C(4)-C(14)-C(10)	109.9(2)
O(4)-C(16)-O(5)	122.9(3)	O(4)-C(16)-C(17)	109.1(3)
O(5)-C(16)-C(17)	128.0(3)	O(6)-C(18)-O(7)	123.3(3)
O(6)-C(18)-C(19)	111.7(3)	O(7)-C(18)-C(19)	124.9(3)

than the sum of the normal van der Waals radii, and is probably responsible for the fact that the $C(sp^3)-C(sp^3)$ bonds associated with C(6) and C(14) are longer than usual (Table 3). The 1,3-diaxial contact between H(6) and H(8) is within the range (2.629 \AA) corresponding to a normal van der Waals separation.

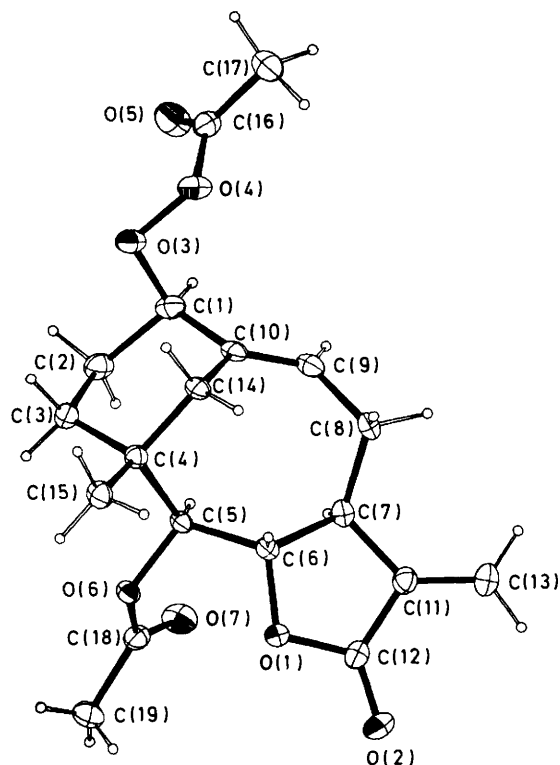
The torsion angle sequence around the eight-membered ring (Table 4) shows that this ring adopts the 'chair-boat' conformation.⁵ According to force-field calculations, this should be the most stable conformation of *cis*-cyclo-octene.⁵ The modules of the endocyclic torsion angles, however, differ from those calculated for the parent hydrocarbon,⁵ the major differences being, as expected, at the carbon atoms involved in fusion with the γ -lactone and cyclohexane rings. The latter adopts a chair conformation, with C(1) above and C(4) below the mean plane through the other four atoms. The bridged fusion of the six- and eight-membered rings therefore takes place without altering the most stable conformation of the individual rings. The γ -lactone ring is distinctly non-planar, and the sum of the five endocyclic torsion angles is 90.4° . The puckering parameters⁶ ($Q_2 = +0.2701$; $\varphi_2 = -98.69^\circ$) show that the lactone ring adopts a conformation intermediate between a $C(7)\alpha$ envelope and a $C(7)\alpha, C(11)\beta$ half-chair. Overall the molecule of crispolide is roughly L-shaped, with the mean plane through the γ -lactone

and the eight-membered ring approximately perpendicular to the plane of the six-membered ring.

The c.d. spectra of crispolide derivatives display a negative Cotton effect for the $n-\pi^*$ transition of the conjugated lactone chromophore around 250 nm, showing that in solution the methylene- γ -lactone group has left-handed chirality [negative ω_2 , torsion angle $C(13)=C(11)-C(12)=O(2)$].⁷ The proton n.m.r. parameters for the γ -lactone ring are in good accord with the geometry found in the solid state, showing that the conformations in solution and in the solid state are similar: both $J_{7,13a/b} (> 3 \text{ Hz}^1)$ and the torsion angle $H(7)-C(7)-C(11)=C(13)$ ($+93.2^\circ$) show that the γ -lactone ring belongs to the pseudorotational *S*-type.⁸ The structure depicted in the Figure, displaying a negative value for ω_2 (-14.4°), should thus also represent the absolute configuration of the molecule. The sign of ω_2 is paired with that of ω_3 [torsion angle $O(1)-C(6)-$

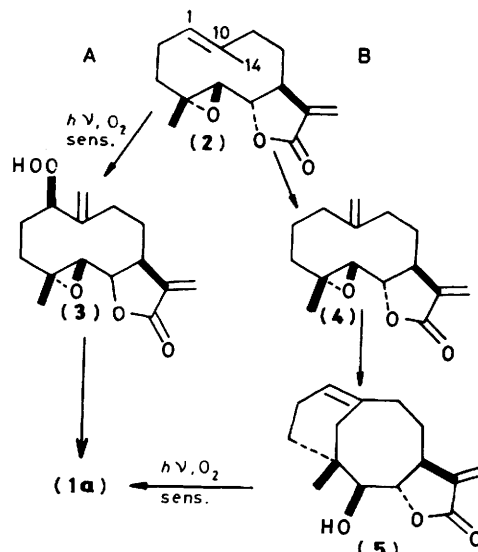
Table 4. Relevant torsion angles ($^{\circ}$) (e.s.d.s 0.2–0.3 $^{\circ}$)

C(2)–C(1)–C(10)–C(14)	–55.4
C(10)–C(1)–C(2)–C(3)	46.3
C(1)–C(2)–C(3)–C(4)	–47.9
C(2)–C(3)–C(4)–C(5)	–65.5
C(2)–C(3)–C(4)–C(14)	53.0
C(14)–C(4)–C(5)–C(6)	45.5
C(3)–C(4)–C(14)–C(10)	–60.4
C(5)–C(4)–C(14)–C(10)	58.0
C(4)–C(5)–C(6)–C(7)	–104.8
C(5)–C(6)–C(7)–C(8)	82.4
C(6)–C(7)–C(11)–C(12)	23.9
C(8)–C(7)–C(11)–C(13)	–29.1
C(7)–C(8)–C(9)–C(10)	75.4
C(8)–C(9)–C(10)–C(1)	–163.0
C(8)–C(9)–C(10)–C(14)	5.0
C(1)–C(10)–C(14)–C(4)	65.0
C(9)–C(10)–C(14)–C(4)	–104.0
C(7)–C(11)–C(12)–O(1)	–12.5
C(13)–C(11)–C(12)–O(2)	–14.4
C(12)–O(1)–C(6)–C(7)	21.2
C(6)–O(1)–C(12)–C(11)	–5.9
C(1)–O(3)–O(4)–C(16)	95.3
O(3)–O(4)–C(16)–O(5)	–4.0
O(3)–O(4)–C(16)–C(17)	175.2
C(5)–O(6)–C(18)–O(7)	–7.2

**Figure.** ORTEP II drawing of the molecule of crispolide diacetate (**1b**) with the thermal ellipsoids at the 20% probability level

C(7)–C(11)], the modulus of which is larger, as frequently encountered in methylene- γ -lactones.⁹

Considerable attention has been given recently to the conformational aspects of peroxy compounds.¹⁰ Peroxy esters are, however, the least investigated compounds of this group, on account of their instability. Dipole moment measurements have

**Scheme 2.** Possible biogenetic routes to crispolide (**1a**)

shown that these compounds adopt in solution a conformation entirely different from that of the parent peroxy acids, with values of the peroxide torsion angle (τ) ranging from 110 to 180 $^{\circ}$.¹¹ In crispolide diacetate τ is 95.3 $^{\circ}$, a value very similar to that found in the solid state for hydrogen peroxide by neutron diffraction (90.2 $^{\circ}$),¹² but different from the values reported for tertiary peroxy esters.¹³ These differences might be due to a flattening of the potential energy curve around this bond.¹¹ The dramatic increase in steric demands in passing from secondary to tertiary substrates should, however, be taken into account: in crispolide diacetate the torsion angle O–O–C=O is close to 0 $^{\circ}$ (Table 4), whereas *t*-butyl peroxy esters, probably for steric reasons,¹⁴ have values for this angle ranging from 30 to 45 $^{\circ}$.¹⁴ In spite of the coplanarity of the carbonyl oxygen and the peroxide group, which should favour conjugation, the bond length between the peroxide and the carbonyl group is normal [1.456(3) Å], and not considerably shorter as in *t*-butyl peroxy esters.¹³

The carbonyl group of the acetate at C(5) is *syn*-oriented with respect to H(5) [C(5)–O(6)–C(18)–O(7) – 7.2 $^{\circ}$]. This represents the most favoured geometry of secondary esters.¹⁵ As for the peroxy ester group, H(1) also is *syn*-oriented with respect to the carbonyl; their separation distance, however, is much longer; and acylation shifts, supposedly due to magnetic anisotropy of the carbonyl group,¹⁶ were not observed for H(1) in the ¹H n.m.r. spectrum of (**1b**).¹

In the crystals of crispolide diacetate the packing of the molecules is controlled mainly by van der Waals forces; no other intermolecular contacts are present.

The outcome of the present crystallographic study is in accordance with the hypothesis that the bridged carbon skeleton of crispolide results from the transannular cyclization of a 4 α ,5 β -epoxygermacr-10(14)-ene derivative. The precise nature of the ultimate precursor, however, is not known. Parthenolide (**2**) is the main terpenic constituent of both *T. vulgare* L. v. *crispum* Fiori and the two chemotypes of *T. vulgare* L. from which crispolide was isolated.¹ Two routes are conceivable from parthenolide (**2**) to crispolide (**1a**) (Scheme 2); route A involves photo-oxygenation to peroxy parthenolide (**3**), and then cyclization to crispolide; route B requires isomerization of parthenolide (**2**) to its $\Delta^{10(14)}$ -analogue (**4**), cyclization to compound (**5**) and then photo-oxygenation of (**5**).

Whereas peroxy parthenolide is a known natural com-

pound,¹⁷ compounds (4) and (5) have never been reported. Furthermore, *in vivo* isomerization of the endocyclic 1(10)-double bond to its exocyclic form [$\Delta^{10(14)}$] appears to involve functionalization of one of the carbons allylic to the exocyclic methylene as well, since, to the best of our knowledge, all but two the naturally occurring $\Delta^{10(14)}$ -germacrenolides also bear an allylic oxygenated function.* Route A appears therefore biogenetically more plausible than route B, even though it implies survival of the labile hydroperoxy group during a further biogenetic step.

Experimental

Crystal Data.— $C_{19}H_{24}O_7$, M 364, orthorhombic, $a = 9.869(3)$, $b = 12.979(5)$, $c = 14.587(5)$ Å, $U = 1868$ Å³ (by least-squares refinement on diffractometer angles for 21 automatically centred reflections, $\lambda = 0.71069$ Å), space group $P2_12_12_1$, $Z = 4$, $D_c = 1.76$ g cm⁻³, colourless, air-sensitive crystals of ellipsoidal form (dimensions $0.3 \times 0.4 \times 0.7$ mm), $\mu(\text{Mo-K}\alpha) 0.9$ cm⁻¹.

Data Collection and Processing.¹⁸—Nicolet R3 diffractometer, ω mode with ω scan width 1° , ω scan speed varying from 1.5 for the weakest to 15° min⁻¹ for the strongest reflections, graphite-monochromated Mo-K α radiation; 1864 reflections measured ($1.5 \leq \theta \leq 25^\circ$), 1847 unique [merging $R 0.044$ after empirical absorption correction (max., min. transmission factors 1.14, 1.00)], giving 1612 with $I \geq 2\sigma(I)$. No evidence of crystal decay.

Structure Analysis and Refinement.—Direct methods (RANTAN; $E > 1.65$) gave all but three non-hydrogen atoms, which were located in the next difference Fourier map. Cascade block diagonal least-squares refinement (103 parameters per block) was performed with all non-hydrogen atoms anisotropic

* The two exceptions are artevasin (R. O. Asplund, M. McKnee, and P. Balasubramanian, *Phytochemistry*, 1972, 11, 3542) and $4\alpha,5\beta$ -epoxy-1-deoxochrysanolide (F. Bohlmann, A. Adler, J. Jakupovic, R. M. King, and H. Robinson, *Phytochemistry*, 1982, 21, 1349).

and hydrogen atoms located in the ΔF map and refined under the constraint C—H = 0.98 ± 0.02 Å, with three overall u_{iso} values for tertiary and secondary H and methyl groups [0.035(3), 0.054(3), and 0.091(4) Å², respectively]. The weighting scheme $w = 1/(\sigma^2|F_o| + 0.001|F_o|^2)$, with $\sigma|F_o|$ from counting statistics¹⁸ gave satisfactory agreement analyses. Final R and R_w values are 0.041 and 0.044, goodness of fit 1.34, maximum residual peak in final ΔF 0.16 e Å⁻³. Programs, computer used, and sources of scattering factor data are given in ref. 18.

References

- G. Appendino, P. Gariboldi, and G. M. Nano, *Phytochemistry*, 1982, 21, 1099.
- D. H. R. Barton and P. de Mayo, *Quart. Rev.*, 1957, 11, 189.
- A. Aebi, D. H. R. Barton, and A. S. Lindsey, *J. Chem. Soc.*, 1953, 3124.
- F. S. Fawcett, *Chem. Rev.*, 1950, 47, 219.
- O. Ermer and S. Lifson, *J. Am. Chem. Soc.*, 1973, 95, 4121.
- M. Nardelli, *Acta Crystallogr., Sect. C*, 1983, 39, 1141.
- A. F. Beecham, *Tetrahedron*, 1972, 28, 5543.
- Z. Samek, *Collect. Czech. Chem. Commun.*, 1978, 43, 3210.
- A. T. McPhail and G. A. Sim, *Tetrahedron*, 1973, 29, 1751.
- O. Exner in 'The Chemistry of Functional Groups: Peroxides,' ed. S. Patai, Wiley, New York, 1983, pp. 85—96.
- B. Plesnicar and O. Exner, *Collect. Czech. Chem. Commun.*, 1981, 46, 490.
- W. R. Busing and H. A. Levy, *J. Chem. Phys.*, 1965, 42, 3045.
- (a) L. S. Silbert, L. P. Witnauer, D. Swern, and C. Ricciuti, *J. Am. Chem. Soc.*, 1959, 81, 3244; (b) quoted by J. Z. Gougoutas in 'The Chemistry of Functional Groups: Peroxides,' ed. S. Patai, Wiley, New York, 1983, p. 405.
- F. D. Verderame and J. C. Miller, *J. Phys. Chem.*, 1962, 66, 2185.
- A. McL. Mathieson, *Tetrahedron Lett.*, 1965, 4137.
- Z. Samek and M. Buděšínský, *Collect. Czech. Chem. Commun.*, 1979, 44, 558.
- F. S. El-Ferally, Y. M. Chan, E. Fairchild, and R. W. Doskotch, *Tetrahedron Lett.*, 1977, 1973.
- G. Appendino, M. Calleri, G. Chiari, and D. Viterbo, *J. Chem. Soc., Perkin Trans. 2*, 1984, 903.

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