# Crystal and Molecular Structures and Absolute Configuration of Triptolide and Tripdiolide, Two Anti-leukaemic Diterpenoid Triepoxides

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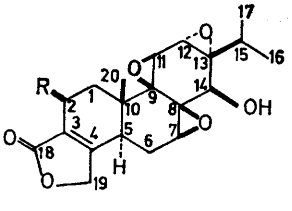
The crystal and molecular structures and absolute configuration of the title compounds, have been determined by three-dimensional, single-crystal, $X$-ray analysis. Crystals of triptolide (Ia) are monoclinic, space group $P2_1$, with $a$ = 13·420(1), $b$ = 6·256(1), $c$ = 11·593(1) Å, $\beta$ = 118·09(1)°, and $Z$ = 2. Crystals of tripdiolide (Ib) have the same space group, $a$ = 13·680(2), $b$ = 6·253(1), $c$ = 11·684(1) Å, $\beta$ = 119·05(1)°, and $Z$ = 2. The structure of (Ia) was determined by direct methods and refined to $R$ 0·086, by least-squares methods, for 1044 independent reflections measured by counter diffractometry. The structure of (Ib) was found by direct application of the phase angles for (Ia), and refined by least-squares to $R$ 0·082 for 1399 independent reflections.

Each molecule has an 18(4 ⟶ 3)*abeo*-abietane skeleton, and tripdiolide is 2-hydroxytriptolide.

TRIPTOLIDE, $C_{20}H_{24}O_6$ (Ia), and tripdiolide, $C_{20}H_{24}O_7$ (Ib), are two new diterpenoid epoxides whose isolation from extracts of *Tripterygium wilfordii*, and structural characterization by $X$-ray diffraction methods, have been reported briefly.[1] Both compounds show significant antileukaemic activity against the L-1210 and P-388 leukaemias in the mouse, *in vivo*, and against cell cultures from human carcinoma of the nasopharynx (KB).[1]

We give here the results of the $X$-ray analyses which led to the characterization of the two molecular structures. The compounds are the first recognised diterpenoid triepoxides, and the first reported natural products containing the 18(4 → 3)*abeo*-abietane skeleton.



(I) a; R = H
b; R = OH

EXPERIMENTAL

Crystals of both compounds are transparent colourless needles, elongated along $b$.

*Crystal Data*.—(i) *Triptolide* (Ia). $C_{20}H_{24}O_6$, $M$ = 360·4. Monoclinic, $a$ = 13·420(1), $b$ = 6·256(1), $c$ = 11·593(1) Å, $\beta$ = 118·09(1)°, $U$ = 858·7 Å$^3$, $D_m$ = 1·40(1) (flotation), $Z$ = 2, $D_c$ = 1·39, $F(000)$ = 384. Space group $P2_1$ ($C_2^2$, No. 4). Cu-$K_\alpha$-radiation, $\lambda$ = 1·5418 Å, $\mu$(Cu-$K_\alpha$) = 8·6 cm$^{-1}$. Single-crystal diffractometry.

(ii) *Tripdiolide* (Ib). $C_{20}H_{24}O_7$, $M$ = 376·4. Monoclinic. $a$ = 13·680(2), $b$ = 6·253(1), $c$ = 11·684(1) Å, $\beta$ = 119·05(1)°, $U$ = 873·7 Å$^3$, $Z$ = 2 (isostructural with triptolide), $F(000)$ = 400. Space group $P2_1$ ($C_2^2$, No. 4). Mo-$K_\alpha$-radiation, $\lambda$ = 0·71069 Å, $\mu$(Mo-$K_\alpha$) = 0·71 cm$^{-1}$. Single-crystal diffractometry.

*Intensity Measurements*.—These were made by single-crystal diffractometry, by use of a Picker four-circle instrument controlled by an XDS Sigma 2 computer. The experimental techniques used have been described in detail.[2]

For triptolide, a single quadrant of reciprocal space was examined out to sin $\theta/\lambda$ 0·52 and yielded 1071 independent reflections significantly above background. Copper $K_\alpha$-radiation, made monochromatic by Bragg reflection from the (002) planes of a highly oriented graphite crystal, was used with pulse-height analysis and scintillation counting.

Tripdiolide, examined in the same way to sin $\theta/\lambda$ 0·59 with Mo-$K_\alpha$-radiation, yielded 1399 significant reflections. Both crystals were stable to irradiation and no significant changes were noted in the intensities of reference reflections. Both $(0kl)$ and $(0k\bar{l})$ reflections were measured for each compound to check the accuracy of the crystal alignment. For each compound the average agreement between the structure amplitudes of symmetry-related reflections was to within 1%. No absorption corrections were applied.

*Structure Determination and Refinement*.—(i) *Triptolide* (Ia). A starting set of phase angles was obtained by use of the reflections 7,0,4, 6,1,5, and 1,0,5 to define the origin ($\phi$ = 0). Symbols were assigned to the reflections 1,4,0, 1,3,$\bar{6}$, 5,2,4, 4,0,$\bar{4}$, and 5,0,0. Symbolic addition[3] among the 60 largest $E$ values suggested $\phi = \pi/2, \pi/2, \pi, \pi$, and 0, respectively, for these reflections, and this starting set was expanded and refined by use of the weighted tangent formula procedure of Woolfson *et al.*[4] to give phases for 351 $E$ values $>$1·0. The resulting $E$ map had 26 peaks, 25 of which corresponded to physically acceptable atomic sites. The remaining atom was found from a three-dimensional electron-density synthesis calculated with phases obtained by structure-factor calculation ($R$ 0·35). With all atoms treated as carbon, and with individual isotropic thermal parameters assigned, least-squares refinement by the block-diagonal approximation reduced $R$ to 0·22. Six of the atoms had thermal parameters clearly lower than the remaining 20, and could be assigned as oxygen in a chemically reasonable way. Continued least-squares refinement gave $R$ 0·11 at convergence.

The positions of 14 of the 24 hydrogen atoms were established from a three-dimensional difference electron-density synthesis. Of the remaining hydrogen atoms, those on the methyl groups were not resolved (an observation in keeping with n.m.r. measurements[1]) and that on the C(14) hydroxy-group could not be found. The hydrogen atoms were included in fixed positions and with $B$ held at 2·0 Å$^2$ in the final least-squares cycles. At convergence, $R$ was 0·086 and $R'$ 0·112 for all 1044 reflections. The

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[1] S. M. Kupchan, W. A. Court, R. G. Dailey, jun., C. J. Gilmore, and R. F. Bryan, *J. Amer. Chem. Soc.*, 1972, **94**, 7194.

[2] R. F. Bryan, P. T. Greene, P. F. Stokely, and E. W. Wilson, jun., *Inorg. Chem.*, 1971, **10**, 1468.

[3] I. L. Karle and J. Karle, *Acta Cryst.*, 1963, **16**, 969.

[4] G. Germain, P. Main, and M. M. Woolfson, *Acta Cryst.*, 1971, *A*, **27**, 368.

standard deviation of an observation of unit weight was 0·96. The exclusion of 27 reflections with $(|F_o| - |F_c|) > 3{\cdot}0\sigma(|F_o|)$, which were given zero weight in the final refinement, gave $R$ 0·078.*

A list of observed and calculated structure amplitudes is given as Supplementary Publication No. SUP 20651 (4 pp., 1 microfiche).†

Scattering factors used were for neutral atoms.[5] The weighting scheme used was based on counting statistics, with some allowance being made for errors of a non-statistical kind in the stronger low-order reflections.[6] The tangent formula refinement program used was MULTAN;[4] all other programs used were written in this laboratory for the Sigma 2 computer.

(ii) *Tripdiolide* (Ib). Because of the obvious similarity in their cell parameters and diffraction patterns, (Ia) and (Ib) were assumed to be isostructural. Direct application of the phase angles for (Ia) to the corresponding structure amplitudes for (Ib) by structure-factor calculation gave $R$ 0·26. The extra oxygen atom in (Ib) was then located from a three-dimensional difference electron-density synthesis.

Block-diagonal least-squares refinement of the 27 sets of atomic parameters as before gave $R$ 0·11. A second difference electron-density map showed the locations of 15 of the hydrogen atoms. The remaining 9 methyl hydrogen atoms were again unresolved. Inclusion of these additional 15 atoms with fixed parameters in the least-squares calculations gave $R$ 0·078 and $R'$ 0·108 at convergence. $\sigma$ For an observation of unit weight was 1·1.

*Determination of Absolute Configuration*.—The absolute configuration of triptolide was established in two ways by taking into account the anomalous scattering of the oxygen atoms.[7] In separate structure-factor calculations for the set of 1017 reflections $R$ values of 0·0785 and 0·0783 were obtained for the two possible enantiomeric structures. By Hamilton's $R$ ratio test[8] this difference indicates a distinction between the two enantiomers at the 90% confidence level. This choice of absolute configuration was confirmed by measurement of the intensities of Friedel pairs of reflections for which the calculated difference in structure amplitude was >0·15.[9] The signs of the observed differences in the structure amplitudes are the same as those calculated in each case but the actual numerical agreement is indifferent. The results of the comparison are given in the Supplementary Publication.

We did not try to determine the absolute configuration of tripdiolide. It is reasonable to assume that it is the same as that of triptolide.

## RESULTS AND DISCUSSION

The $X$-ray analyses have established the molecular structure and absolute configuration of triptolide (Ia), and have confirmed that tripdiolide (Ib) is the 2-hydroxy-derivative of triptolide.[1]

Parameters defining the crystal structures are given, together with their standard deviations,[10] in Table 1. A view of the molecular structures as found in the crystalline state is shown in Figure 1. Bond lengths, bond angles, and torsion angles are shown in Figures 2 and 3.

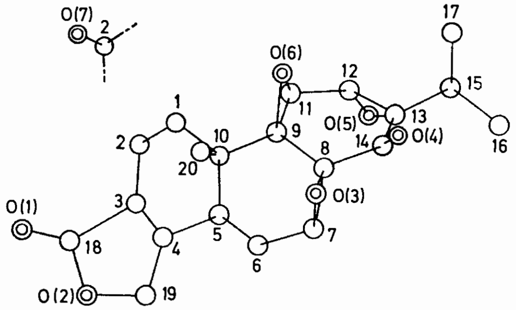

FIGURE 1 View of the molecular structure of triptolide (Ia) as found in the crystal. Atoms are carbon unless otherwise indicated. The inset shows the orientation of the hydroxy-group in the isostructural tripdiolide (Ib)

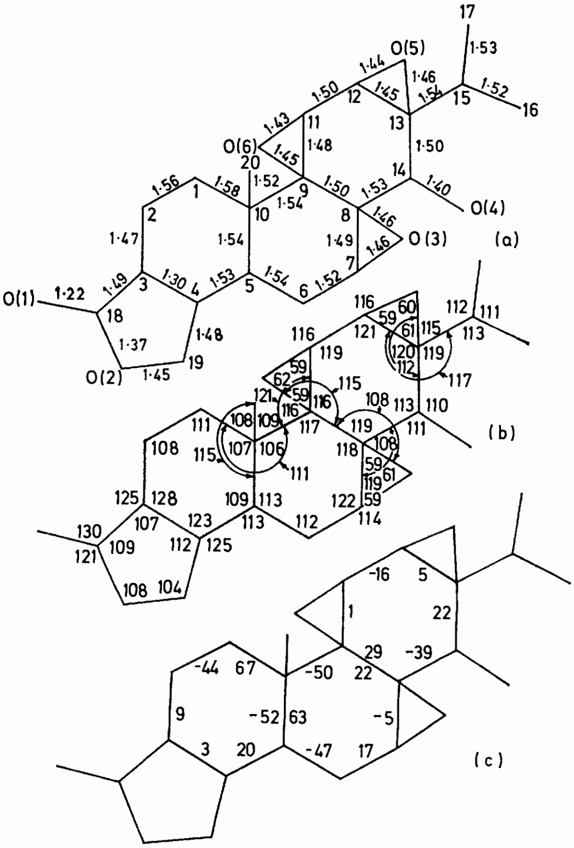

FIGURE 2 (a) Bond lengths, σ(C–C) 0·015, σ(C–O) 0·012 Å; (b) bond angles, σ(C–C–C) 1°; and (c) torsion angles, σ *ca*. 2°, for triptolide (Ia)

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* Details of the refinement procedures are given in ref. 2. The use of anisotropic thermal parameters did not significantly reduce $R$.

† See note about Supplementary Publications in Notice to Authors, No. 7, in *J.C.S. Dalton*, 1972, index issue.

[5] H. P. Hanson, F. Herman, J. D. Lea, and S. Skillman, *Acta Cryst.*, 1964, **17**, 1040.

[6] W. R. Busing and H. A. Levy, *J. Chem. Phys.*, 1957, **26**, 563; D. F. Grant, R. C. G. Killean, and J. L. Lawrence, *Acta Cryst.*, 1964, **17**, 781.

[7] D. T. Cromer and D. Liberman, *J. Chem. Phys.*, 1970, **53**, 1891.

[8] J. A. Ibers and W. C. Hamilton, *Acta Cryst.*, 1964, **17**, 781; W. C. Hamilton, *ibid.*, 1965, **18**, 502.

[9] J. M. Bijvoet, A. F. Peerdeman, and A. J. van Bommel, *Nature*, 1951, **169**, 271.

[10] L. I. Hodgson and J. S. Rollett, *Acta Cryst.*, 1963, **16**, 329.

Table 1

Atomic co-ordinates ($\times 10^4$) and isotropic thermal parameters, with standard deviations, in parentheses

| | Triptolide (Ia) | | | | Tripdiolide (Ib) | | | |
|---|---|---|---|---|---|---|---|---|
| Atom | $x/a$ | $y/b$ | $z/c$ | $B/Å^2$ | $x/a$ | $y/b$ | $z/c$ | $B/Å^2$ |
| C(1) | 1922(4) | 3647(10) | 6880(5) | 2·49(9) | 1841(4) | 3649(10) | 6886(5) | 2·27(8) |
| C(2) | 3217(5) | 3344(11) | 7420(5) | 2·67(10) | 3101(4) | 3636(10) | 7405(5) | 2·41(9) |
| C(3) | 3754(4) | 3368(10) | 8857(5) | 2·56(10) | 3694(4) | 3547(10) | 8873(5) | 2·33(8) |
| C(4) | 3270(4) | 3335(10) | 9601(5) | 2·39(9) | 3201(4) | 3396(9) | 9611(5) | 2·24(8) |
| C(5) | 1995(4) | 3142(10) | 9069(5) | 2·20(9) | 1954(4) | 3159(9) | 9064(4) | 1·85(8) |
| C(6) | 1664(4) | 1882(11) | 9983(5) | 2·55(10) | 1658(4) | 1911(11) | 9984(5) | 2·68(9) |
| C(7) | 405(5) | 1432(12) | 9351(12) | 2·70(10) | 422(5) | 1404(10) | 9363(5) | 2·55(9) |
| C(8) | −331(4) | 1624(11) | 7907(5) | 2·23(9) | −313(4) | 1509(9) | 7938(5) | 1·93(8) |
| C(9) | 186(4) | 2439(9) | 7093(5) | 1·92(9) | 159(4) | 2363(9) | 7091(4) | 1·84(7) |
| C(10) | 1478(4) | 2222(9) | 7671(4) | 1·78(9) | 1438(4) | 2237(9) | 7666(5) | 1·88(7) |
| C(11) | −434(4) | 4187(11) | 6169(5) | 2·33(9) | −489(4) | 4079(9) | 6158(5) | 2·35(9) |
| C(12) | −1519(4) | 4989(11) | 6087(5) | 2·46(10) | −1568(4) | 4819(10) | 6069(5) | 2·24(8) |
| C(13) | −2120(4) | 3761(10) | 6622(4) | 2·12(9) | −2133(4) | 3600(10) | 6632(5) | 2·28(8) |
| C(14) | −1613(4) | 1740(11) | 7361(5) | 2·06(9) | −1581(4) | 1600(10) | 7409(5) | 2·32(8) |
| C(15) | −3410(4) | 4029(11) | 5962(5) | 2·63(10) | −3391(5) | 3865(10) | 6000(5) | 2·65(9) |
| C(16) | −3923(5) | 3209(13) | 6798(6) | 3·60(11) | −3857(6) | 3059(14) | 6902(7) | 4·08(13) |
| C(17) | −3982(5) | 3028(15) | 4598(6) | 3·96(13) | −3995(6) | 2736(13) | 4662(6) | 3·72(12) |
| C(18) | 4992(4) | 3467(11) | 9740(5) | 2·90(11) | 4904(5) | 3606(10) | 9725(5) | 2·54(9) |
| C(19) | 4108(10) | 3413(10) | 11008(5) | 2·80(10) | 4058(5) | 3443(11) | 11007(5) | 2·80(9) |
| C(20) | 1762(4) | −103(11) | 7574(6) | 2·59(10) | 1743(4) | −140(10) | 7609(5) | 2·42(9) |
| O(1) | 5772(3) | 3460(8) | 9483(4) | 3·28(7) | 5670(4) | 3657(9) | 9467(4) | 3·76(8) |
| O(2) | 5184(3) | 3488(8) | 11008(4) | 3·14(7) | 5117(3) | 3585(9) | 10996(4) | 3·18(7) |
| O(3) | 51(3) | −470(8) | 8519(3) | 2·96(7) | 93(3) | −511(8) | 8566(4) | 2·86(7) |
| O(4) | −2142(3) | −38(8) | 6571(3) | 3·49(8) | −2111(4) | −172(8) | 6588(4) | 3·48(8) |
| O(5) | −1484(3) | 5605(7) | 7316(3) | 2·57(7) | −1488(3) | 5486(7) | 7304(3) | 2·44(6) |
| O(6) | −466(3) | 2047(7) | 5705(3) | 2·66(7) | −498(3) | 1903(7) | 5712(3) | 2·56(6) |
| O(7) | | | | | 3452(4) | 1911(9) | 6860(4) | 3·55(8) |
| H(11) | 1499 | 3221 | 5858 | 2·00 | 1429 | 3073 | 5888 | 2·00 |
| H(12) | 1764 | 5299 | 6976 | † | 1564 | 5255 | 6890 | |
| H(21) | 3497 | 4601 | 7033 | | 3342 | 5099 | 7103 | |
| H(22) | 3351 | 1833 | 7067 | | | | | |
| H(51) | 1670 | 4739 | 8969 | | 1595 | 4762 | 8932 | |
| H(61) | 2072 | 359 | 10198 | | 2133 | 436 | 10251 | |
| H(62) | 1929 | 2745 | 10882 | | 1917 | 2822 | 10251 | |
| H(71) | −167 | 1750 | 9875 | | 42 | 1667 | 10042 | |
| H(111) | 84 | 5417 | 5875 | | −125 | 5625 | 6000 | |
| H(121) | −1916 | 6459 | 5417 | | −2083 | 6000 | 5437 | |
| H(141) | −1742 | 1689 | 8234 | | −1750 | 1667 | 8208 | |
| H(151) | −3621 | 5717 | 5889 | | −3583 | 5542 | 5917 | |
| H(191) | 4075 | 2011 | 11575 | | 4004 | 2073 | 11553 | |
| H(192) | 4022 | 4803 | 11546 | | 3965 | 4867 | 11504 | |
| H(04) | | | | | −1917 | −1625 | 7104 | |
| H(07) | | | | | 3042 | 708 | 7041 | |

† All hydrogen atoms included in fixed positions and with $B$ 2·0 Å$^2$.

The bond distances in each molecule are as expected, except that the C–C bonds involved in the epoxide rings are shorter than the usual C–C single-bond distance. Strain in the ring systems is shown by the closure of bond angles in the planar five-membered ring, and by the increase in bond angles over the regular tetrahedral value at the epoxide ring junctions with the six-membered rings.

The equations of least-squares mean planes through the rings and the deviations of individual atoms from these planes are given in Table 2. From Table 2 and the torsion angles (Figure 2) it is seen that the conformation of the cyclohexene ring A is an envelope, of ring B a half-chair, and of ring C an unusual 1,3-diplanar conformation.[11] This last is normally only encountered in cyclohexenes, and is presumably due to the constraints imposed on ring C by the epoxide rings. The nature of the A/B and B/C ring junctions is shown in Figure 4.

Table 2

(*a*) Equations, in the form $aX + bY + cZ = d$, for the least-squares mean planes through the rings *

| Ring | $a$ | $b$ | $c$ | $d$ |
|---|---|---|---|---|
| A | 0·1665 | −0·9859 | 0·0145 | −2·0042 |
| | 0·2205 | −0·9753 | 0·0111 | −2·1673 |
| B | −0·2770 | 0·9590 | 0·0606 | 2·7543 |
| | −0·2995 | 0·9532 | 0·0416 | 2·6961 |
| C | 0·0232 | 0·6393 | 0·7686 | 6·5349 |
| | 0·0199 | 0·6552 | 0·7552 | 6·4317 |
| D | 0·0256 | −0·9993 | 0·0257 | −1·8619 |
| | 0·0466 | −0·9989 | −0·0016 | −2·2067 |

(*b*) Deviations (Å) from the planes

A: C(1) 0·34, 0·28; C(2) −0·09, −0·04; C(3) −0·09, −0·11; C(4) 0·05, 0·03; C(5) 0·18, 0·20; C(10) −0·38, −0·36

B: C(5) −0·32, −0·31; C(6) 0·11, 0·12; C(7) 0·05, 0·04; C(8) −0·03, −0·02; C(9) −0·15, −0·15; C(10) 0·33, 0·33

C: C(8) −0·22, −0·21; C(14) 0·20, 0·19; C(13) −0·03, −0·02; C(12) −0·12, −0·11; C(11) 0·10, 0·09; C(9) 0·07, 0·07

D: C(3) 0·01, 0·02; C(18) 0·01, 0·01; O(2) 0·01, 0·02; C(19) 0·00, −0·01; C(4) −0·01, −0·02, O(1) 0·01, −0·02

* $X$, $Y$, and $Z$ in Å refer to an orthogonal axial system with $X = x + z \cos \beta$, $Y = y$, $Z = z \sin \beta$. Values for triptolide precede those for tripdiolide.

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[11] R. Bucourt and D. Hainaut, *Bull. Soc. chim. France*, 1965, 1366.

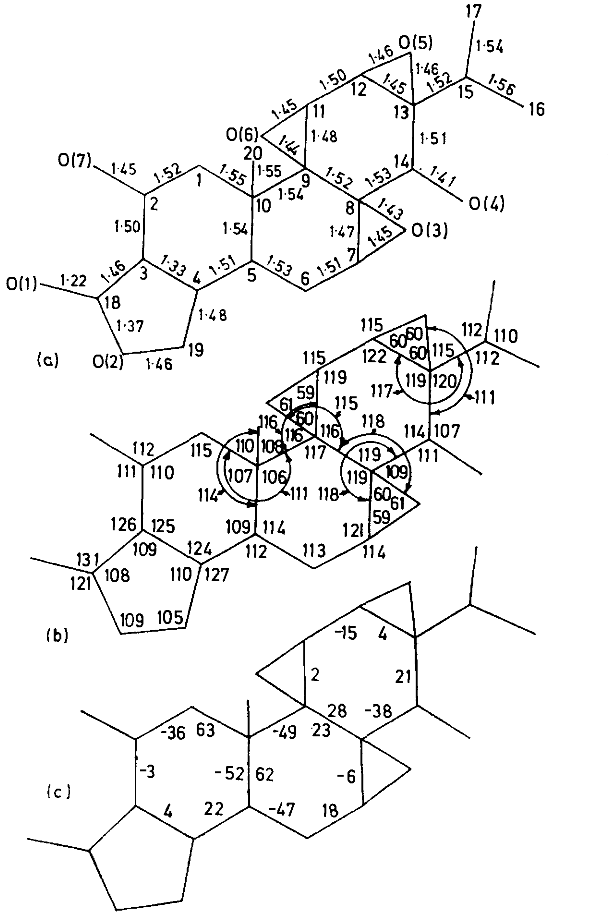

FIGURE 3 (a) Bond lengths (b) bond angles, and (c) torsion angles for tripdiolide (Ib); σ values as for Figure 2

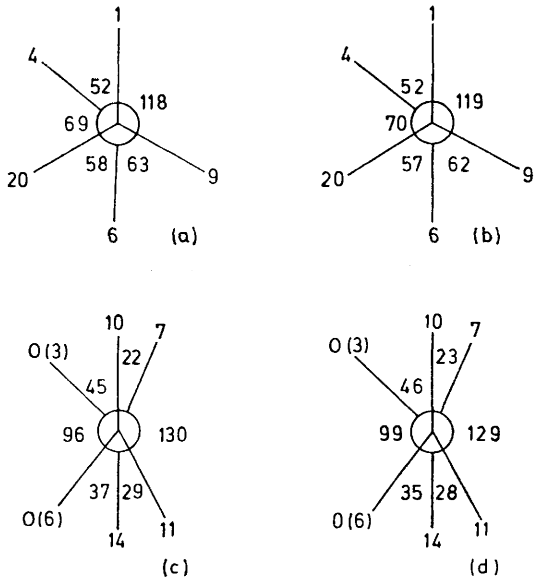

FIGURE 4 Newman projections showing the stereochemistry of the ring junctions: (a) and (b) C(10)–C(5); (c) and (d) C(9)–C(8); (a) and (c) triptolide, (b) and (d) tripdiolide

These two compounds, together with triptonide,[1] are apparently the first recognised diterpenoid triepoxides, and the first natural products found to contain the 18 (4 → 3)*abeo*-abietane skeleton.

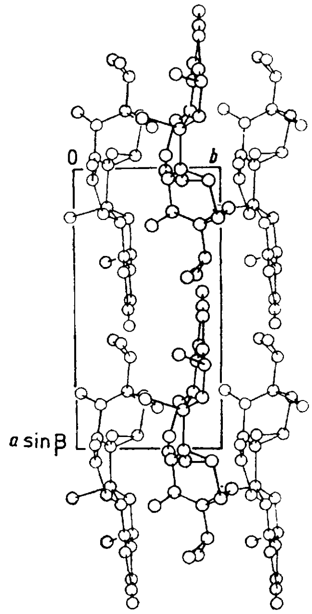

FIGURE 5 Molecular packing in the crystal structure of tripdiolide

The molecular packing in the crystal structure of triptiolide is shown in Figure 5, and the shorter intermolecular contacts are listed in Table 3. There is a hydrogen bond linking the hydroxy-group O(4) to the epoxide oxygen O(5) of a neighbouring molecule giving a helical arrangement of molecules along an axis parallel to *b*.

TABLE 3

Selected shorter intermolecular contacts (Å)

| Contact | (Ia) | (Ib) |
|---|---|---|
| $O(1)\cdots C(16^{I})$ | 3·33 | 3·38 |
| $C(1)\cdots O(6^{II})$ | 3·30 | 3·36 |
| $O(7)\cdots C(12^{III})$ | * | 3·39 |
| $O(7)\cdots C(17^{III})$ | * | 3·44 |
| $O(3)\cdots O(5^{IV})$ | 3·08 | 3·16 |
| $O(5)\cdots H(04^{V})$ | * | 1·88 |
| $O(4)\cdots O(5^{VI})$ | 2·87 | 2·85 |

* Contact not present or not measured.

Roman numerals as superscripts refer to the following equivalent positions relative to the reference molecule at *x*, *y*, *z*:

| | | | |
|---|---|---|---|
| I | $1 + x, y, z$ | IV | $x, y - 1, z$ |
| II | $-x, 0·5 + y, 1 - z$ | V | $x, 1 + y, z$ |
| III | $-x, y - 0·5, 1 - z$ | VI | $x, y - 0·5, z$ |

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