

X-Ray Analysis of Pristimerol Bis-*p*-bromobenzoate, a Derivative of the Triterpene Quinone Methide Pristimerin

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Chemical studies on the structure of pristimerin, an unusual triterpenoid quinone methide, have been confirmed by an X-ray study of pristimerol bis-*p*-bromobenzoate, which crystallises in space group $P2_1$, with $Z = 2$ in a unit cell of dimensions $a = 20.43 \pm 0.02$, $b = 6.35 \pm 0.01$, $c = 17.76 \pm 0.02$ Å, $\beta = 118.5 \pm 0.2^\circ$. The structure was solved by the heavy-atom method and refined to R 0.14, for 1686 observed reflections. The data suggest the absolute configuration to be that expected on biogenetic grounds.

PRISTIMERIN, an orange crystalline pigment with both antibiotic and antitumour activity, has been isolated from *Pristimera indica*¹ (Willd.) and other members of the Celastraceae.² Although diagnosed as an unusual quinone methide its structure remained problematic until its recognition³ as a triterpene, when extensive

¹ S. S. Bhatnagar and P. V. Divekar, *J. Sci. Ind. Res., India*, 1951, **10**, B, 56.

² P. K. Grant and A. W. Johnson, *J. Chem. Soc.*, 1957, 4079.

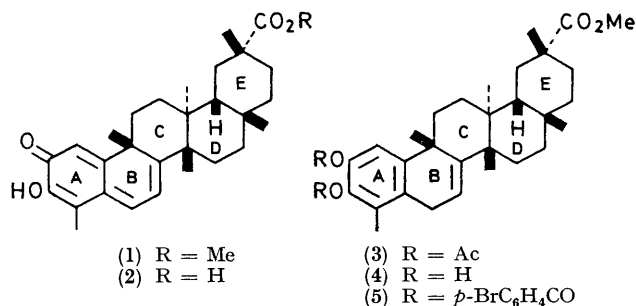
³ R. G. Cooke and R. H. Thomson, *Rev. Pure Appl. Chem.*, 1958, **8**, 85.

chemical and spectroscopic studies^{4,5} culminated in the suggestion of structure (1) for pristimerin. The

⁴ (a) K. Nakanishi, Symposium on Phytochemistry, ed. H. R. Arthur, Oxford, Hong Kong, 1961, p. 97; (b) R. Harada, H. Kakisawa, S. Kobayashi, M. Musya, K. Nakanishi, and T. Takahashi, *Tetrahedron Letters*, 1962, 603; (c) K. Nakanishi, H. Kakisawa, and Y. Hirata, *J. Amer. Chem. Soc.*, 1955, **77**, 3169, 6729; *Bull. Chem. Soc. Japan*, 1956, **29**, 7.

⁵ (a) A. W. Johnson, P. F. Juby, T. J. King, and S. W. Tam, *J. Chem. Soc.*, 1963, 2884; (b) P. K. Grant, A. W. Johnson, P. F. Juby, and T. J. King, *J. Chem. Soc.*, 1960, 549; (c) P. K. Grant and A. W. Johnson, *J. Chem. Soc.*, 1957, 4669.

related pigment celastrol^{4,6} was shown to be the corresponding acid (2). Pristimerin has been assumed to be derived from a β -amyryn-like precursor, and on this biogenetic analogy rests the absolute and relative



stereochemistry shown in (1), although models indicate possible unfavourable interactions [C(27)-CO₂Me] with the *cis*-DE ring fusion. Some points in the chemistry of pristimerin are not yet clear. Thus, the product of reductive acetylation^{2,4c} differs from the acetate⁷ (3) of reduced pristimerin [pristimerol (4)]. The former remains unformulated, as do various manganese dioxide oxidation products⁸ of pristimerin. Further, isopristimerin-II, a product of acid-catalysed rearrangement of pristimerin has been formulated in two ways.^{5a,9} It was therefore considered desirable to establish the structure of pristimerin by X-ray crystallography, and secure the foundation for any further chemical study.

A suitable heavy-atom containing derivative of pristimerin was then sought, and found, after many trials, in the bis-*p*-bromobenzoate (5) of pristimerol (4), which is unambiguously related to pristimerin. Low availability of pristimerin restricted the quantity of derivative (5), and few crystals of sufficient size could be obtained. After collection of diffractometer data, the structure was solved *via* location of the bromine positions from a three-dimensional Patterson synthesis. The non-hydrogen light atoms appeared on subsequent Fourier summations. Refinement of the provisional structure finally reduced *R* to 0.14, with isotropic temperature factors for carbon and oxygen atoms, anisotropic parameters for the two bromines, and exclusion of hydrogen atoms. The structure (5) was thus demonstrated for pristimerol bis-*p*-bromobenzoate, and the complete structure (1) confirmed for pristimerin. Derivative (5) has five six-membered carbocyclic rings in a triterpenoid skeleton of the cerin type, with ring fusions CD and DE *trans* and *cis* respectively. Ring A is planar, while rings C, D, and E adopt classical chair conformations. Ring E is slightly flattened to enable the carboxymethyl group to avoid unusually close contacts with C(27). A general view of the molecule is shown in Figure 1, and the arrangement in the cell in Figure 2. Bond lengths and angles, which are all

chemically reasonable within the given errors, are presented in Figures 3 and 4, and the final atomic

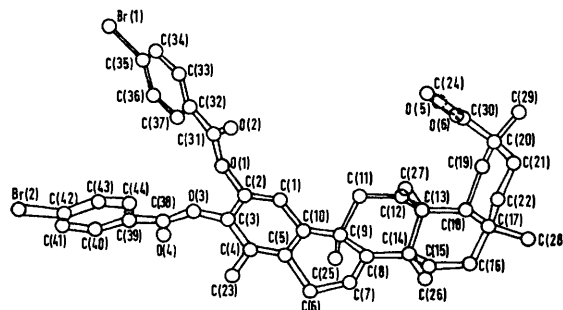


FIGURE 1 Pristimerol bis-*p*-bromobenzoate. A general view of the molecule along *b*, showing the numbering system used

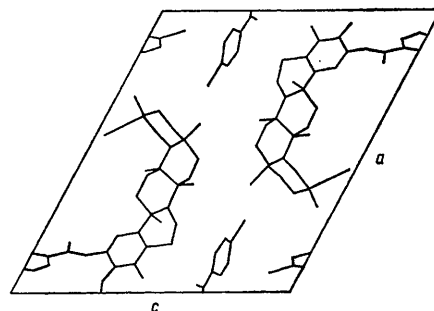


FIGURE 2 A projection along *b*, showing the arrangement of the molecules in the cell

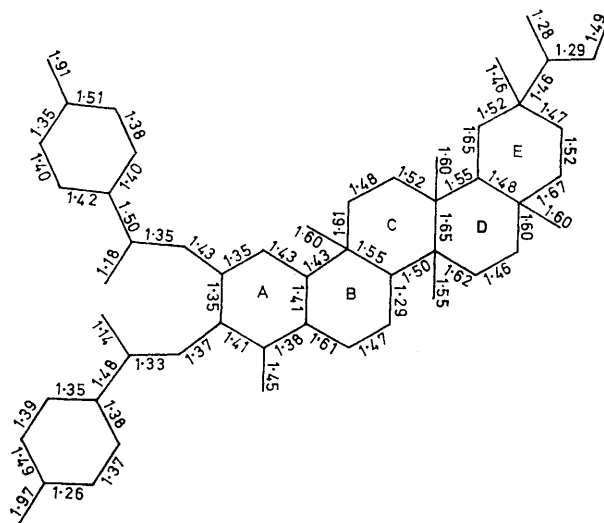


FIGURE 3 Bond lengths (Å); mean σ 0.05 Å

parameters listed in the Table. The absolute configuration of the bis-*p*-bromobenzoate (5) was investigated¹⁰ at the state of refinement with *R* 15.47%. Structure factors were computed by use of atomic co-ordinates for the enantiomer shown in (5), and for its mirror

⁷ S. Seshadri, V. V. Mhaskar, A. B. Kulkarni, and R. C. Shah, *J. Sci. Ind. Res., India*, 1958, **17**, B, 111.

⁸ J. L. Taylor, Ph.D. Thesis, University of London, 1956.

⁹ K. Nakanishi, Y. Takahashi, and H. Budzikiewicz, *J. Org. Chem.*, 1965, **30**, 1729.

¹⁰ J. Ibers and W. C. Hamilton, *Acta Cryst.*, 1964, **17**, 781.

⁶ O. Givold, *J. Amer. Pharm. Assoc.*, 1939, **28**, 440; 1940, **29**, 12; 1942, **31**, 529; M. S. Schechter and H. L. Haller, *J. Amer. Chem. Soc.*, 1942, **64**, 182; P. N. Bhargava, *Proc. Indian Acad. Sci.*, 1946, **24**, 506.

image (y co-ordinates reversed), by use of anomalous scattering factors for bromine. R values of 15.5 and 15.6 were obtained (the overall increase in R was occasioned by the change in scale on introduction of new

Final atomic co-ordinates, with estimated standard deviations, and isotropic temperature factors

| Atom | x/a | y/b | z/c | $B/\text{\AA}^2$ |
|-------|------------|-------------|-----------|------------------|
| Br(1) | 0.0674(4) | -0.0292(14) | 1.1181(3) | * |
| Br(2) | -0.2591(3) | 1.0722(11) | 0.6964(3) | * |
| C(1) | 0.205(2) | 0.333(6) | 0.729(2) | 3.86 |
| C(2) | 0.149(2) | 0.418(6) | 0.739(2) | 3.83 |
| C(3) | 0.098(2) | 0.554(6) | 0.683(2) | 3.45 |
| C(4) | 0.108(2) | 0.630(6) | 0.615(2) | 3.90 |
| C(5) | 0.165(1) | 0.540(5) | 0.604(2) | 2.93 |
| C(6) | 0.174(2) | 0.603(6) | 0.522(2) | 3.31 |
| C(7) | 0.253(1) | 0.575(5) | 0.544(2) | 1.93 |
| C(8) | 0.305(1) | 0.463(5) | 0.602(2) | 2.42 |
| C(9) | 0.279(2) | 0.311(5) | 0.651(2) | 3.11 |
| C(10) | 0.219(2) | 0.398(5) | 0.660(2) | 2.91 |
| C(11) | 0.347(2) | 0.233(6) | 0.740(2) | 4.75 |
| C(12) | 0.420(2) | 0.221(6) | 0.741(2) | 4.49 |
| C(13) | 0.445(1) | 0.416(5) | 0.711(2) | 1.86 |
| C(14) | 0.382(2) | 0.457(5) | 0.611(2) | 3.11 |
| C(15) | 0.405(2) | 0.675(5) | 0.584(2) | 3.71 |
| C(16) | 0.478(2) | 0.653(8) | 0.589(2) | 6.10 |
| C(17) | 0.546(1) | 0.572(6) | 0.677(2) | 3.31 |
| C(18) | 0.521(2) | 0.399(6) | 0.712(2) | 4.19 |
| C(19) | 0.587(2) | 0.349(7) | 0.811(2) | 4.65 |
| C(20) | 0.632(2) | 0.531(7) | 0.869(2) | 4.90 |
| C(21) | 0.648(2) | 0.696(7) | 0.821(3) | 5.70 |
| C(22) | 0.586(3) | 0.782(9) | 0.738(3) | 8.68 |
| C(23) | 0.053(2) | 0.775(6) | 0.555(2) | 4.55 |
| C(24) | 0.561(2) | 0.934(9) | 0.976(3) | 7.77 |
| C(25) | 0.239(2) | 0.096(7) | 0.599(2) | 5.18 |
| C(26) | 0.387(2) | 0.279(6) | 0.554(2) | 4.44 |
| C(27) | 0.436(1) | 0.617(5) | 0.760(2) | 2.48 |
| C(28) | 0.603(2) | 0.470(8) | 0.649(3) | 5.75 |
| C(29) | 0.701(2) | 0.450(8) | 0.937(3) | 6.88 |
| C(30) | 0.600(2) | 0.613(8) | 0.921(3) | 6.69 |
| C(31) | 0.151(2) | 0.435(7) | 0.876(2) | 4.64 |
| C(32) | 0.132(1) | 0.314(5) | 0.936(1) | 2.19 |
| C(33) | 0.103(2) | 0.110(6) | 0.914(2) | 4.77 |
| C(34) | 0.081(2) | -0.010(7) | 0.963(2) | 5.20 |
| C(35) | 0.095(2) | 0.111(6) | 1.042(2) | 4.35 |
| C(36) | 0.125(2) | 0.305(7) | 1.064(2) | 5.75 |
| C(37) | 0.146(2) | 0.423(6) | 1.012(2) | 4.20 |
| C(38) | -0.018(1) | 0.535(5) | 0.677(1) | 2.28 |
| C(39) | -0.075(2) | 0.674(5) | 0.679(1) | 3.57 |
| C(40) | -0.060(1) | 0.867(5) | 0.714(1) | 2.57 |
| C(41) | -0.112(2) | 1.004(6) | 0.718(2) | 4.50 |
| C(42) | -0.184(2) | 0.892(6) | 0.689(2) | 4.56 |
| C(43) | -0.196(2) | 0.705(7) | 0.662(2) | 5.90 |
| C(44) | -0.144(2) | 0.584(6) | 0.654(1) | 4.01 |
| O(1) | 0.136(1) | 0.329(4) | 0.804(1) | 5.21 |
| O(2) | 0.187(2) | 0.591(6) | 0.891(1) | 7.94 |
| O(3) | 0.045(1) | 0.640(3) | 0.700(1) | 3.59 |
| O(4) | -0.030(1) | 0.373(5) | 0.645(1) | 5.77 |
| O(5) | 0.572(2) | 0.501(6) | 0.958(1) | 7.42 |
| O(6) | 0.590(2) | 0.811(5) | 0.928(1) | 8.00 |

* Final anisotropic temperature factors ($\times 10^4$) for the bromine atoms, in the form $\exp [-(B_{11}h^2 + B_{22}k^2 + B_{33}l^2 + B_{23}hl + B_{13}hl + B_{12}hk)]$.

| | B_{11} | B_{22} | B_{33} | B_{23} | B_{13} | B_{12} |
|-------|----------|----------|----------|----------|----------|----------|
| Br(1) | 93 | 990 | 47 | 92 | 79 | -151 |
| Br(2) | 47 | 682 | 75 | 61 | 59 | 242 |

scattering factors). The significance of this difference can be judged by Hamilton's test.¹¹ With two models differing in only one parameter, and 1467 degrees of freedom [*i.e.* 1606(measurements) - 219(atomic parameters)], an R ratio of 1.006 suggests that the probability that the enantiomer giving the higher R index is the

correct one is not significant (significance level ≈ 0.01). In view of the small R difference, and the dependence of the significance test on the absence of systematic errors in the data, it is not possible to be quite certain of this conclusion. However, although doubt must remain, it seems probable that pristimerin has the

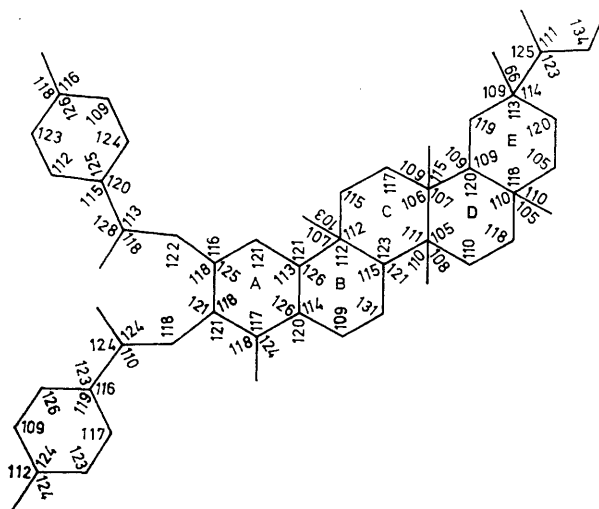


FIGURE 4 Bond angles (deg.); mean σ 3°

Some addition angles:

| | | | |
|-------------------|-----|-------------------|-----|
| C(8)-C(9)-C(25) | 110 | C(13)-C(14)-C(26) | 109 |
| C(10)-C(9)-C(11) | 113 | C(16)-C(17)-C(22) | 108 |
| C(12)-C(13)-C(18) | 116 | C(18)-C(17)-C(28) | 106 |
| C(14)-C(13)-C(27) | 103 | C(19)-C(20)-C(30) | 113 |
| C(8)-C(14)-C(15) | 113 | C(21)-C(20)-C(29) | 109 |

absolute stereochemistry (1) expected from its biogenetic connections.

EXPERIMENTAL

Pristimerol (Dihydropristimerin).²—Sodium borohydride was added to a stirred solution of pristimerin (100 mg) in ethanol (4 ml) until the colour of the solution was discharged. Excess of borohydride was destroyed with acetic acid, and hot water was added to the boiling ethanolic solution until a slight turbidity resulted. On cooling, pristimerol crystallised as needles (89 mg) from ethanol, m.p. 238 °C (lit.,² m.p. 241 °C).

Pristimerol Bis-p-bromobenzoate.—Pristimerol (89 mg) was dried overnight at 120° *in vacuo* and mixed with *p*-bromobenzoyl chloride (89 mg). The mixture was dissolved in dry pyridine (1 ml) and heated under reflux for 1 h. The cooled product was pipetted with stirring into dilute sulphuric acid (5%; 20 ml). The precipitate was collected by centrifugation and washed successively with water, aqueous 2% sodium hydroxide, and water. In each case the solid was removed by centrifugation. Finally, crystallisation from methanol afforded *pristimerol bis-p-bromobenzoate* (5), m.p. 213° (60 mg) [Found: M (mass spectrum), 830.183. $C_{44}H_{48}Br_2O_6$ requires M , 830.182]; ν_{\max} (mull) 1735 and 1094 cm^{-1} .

Crystallographic Experimental.—Pristimerol bis-*p*-di-bromobenzoate crystallised in a feather-like habit from most common organic solvents. Very slow cooling (vacuum flask method) of solutions in benzene, chloroform, carbon

¹¹ W. C. Hamilton, *Acta Cryst.*, 1965, **18**, 502.

tetrachloride, ethyl acetate, methanol, or various mixtures, failed to provide suitable crystals. After many attempts, fast cooling of a hot concentrated benzene-ethanol solution, gave, in the microcrystalline mass, a few crystals sufficiently large for crystallographic work. Most of these were twinned, but one single crystal was finally discovered, *ca.* $0.1 \times 0.2 \times 0.5$ mm, which was used in all subsequent work. Oscillation and Weissenberg photographs were taken about the *b* (needle) axis, and for intensity measurements the crystal was transferred to a Stoe two-circle paper-tape controlled diffractometer. By use of Cu- K_{α} radiation, intensity data were collected on the layers $h0-5l$, by the moving-crystal moving-counter method. The crystal rotated through 2.5° at $1^{\circ} \text{ min}^{-1}$, while the counter scanned 5° at $2^{\circ} \text{ min}^{-1}$. Background was measured for 75 s on each side of each reflection. 2615 independent reflections were measured, once only. No absorption corrections were made. Paper tape for diffractometer control was generated on a Stantec Zebra computer, using a program written by Dr. J. P. G. Richards (University of Wales). Diffractometer data on tape were transferred to cards using a program by Dr. T. J. King (Nottingham). Data reduction and all subsequent crystallographic calculations were performed using the N.R.C. (Ottawa) programs of F. R. Ahmed, S. R. Hall, M. E. Pippy, and C. P. Saunders.

Crystal Data: $\text{C}_{44}\text{H}_{48}\text{Br}_2\text{O}_6$, $M = 830.182$, $a = 20.43 \pm 0.02$, $b = 6.35 \pm 0.01$, $c = 17.76 \pm 0.02$ Å, $\beta = 118.5 \pm 0.2^{\circ}$, $U = 2024$ Å³, $D_m = 1.40$, $Z = 2$, $D_c = 1.36$, $F(000) = 860$. Space group $P2_1$ from systematic absences: $0k0$ when $k = 2n + 1$ (detected on the diffractometer). Cu- K_{α} radiation, $\lambda = 1.542$ Å; $\mu(\text{Cu-}K_{\alpha}) = 32 \text{ cm}^{-1}$.

Structure Determination.—1790 Reflections with net count >25 were used in structure determination, except in final refinement when 104 weak reflections of doubtful validity (uneven background) were excluded. A three-dimensional Patterson summation allowed the x and z co-ordinates, and the difference in y co-ordinates, to be found for the two bromine atoms, one of which was set at $y = 0$. These co-ordinates were refined once by least-squares (block-diagonal), with isotropic temperature factors. A structure-factor calculation with the bromines only led

to R 42%. A Fourier map using F_0 and the phases calculated from the bromine atoms enabled 34 non-hydrogen atom positions to be found. The new parameters were refined once. With phases calculated from the improved model, a second Fourier map was then computed, from which provisional positions of all the remaining non-hydrogen atoms were measured. R had then been reduced to 26% with these atomic co-ordinates after one round of least-squares refinement. The temperature factors of two atoms (of the carbomethoxy-group) shifted to a high value; refinement was thus continued (3 cycles) without these atoms. A difference Fourier then gave new positions for the two atoms, and two more rounds of refinements, with all non-hydrogen atoms and with anisotropic temperature factors for the bromines (only), reduced R to 19%. However, the temperature factors of the atoms of the carbomethoxy-group still diverged rapidly in refinement. Structure factors were therefore calculated, omitting the carbomethoxy atoms, and a second difference Fourier summed. Two new oxygen positions were found, and included in three cycles of refinement; a final difference Fourier permitted location of the remaining two carbon atoms. It was now clear that C(24) (methyl of CO_2Me) was bonded to O(6), and not O(5) as indicated in the earlier Fourier maps. Refinement was now resumed, and a total of six cycles performed with all atoms except hydrogen, and anisotropic temperature factors for bromine atoms only. During this refinement 104 of the weakest reflections, of uncertain status (*v.s.*) were excluded, and inter-layer rescaling carried out. A weighting scheme ($\sqrt{w} = 1$, if $|F_0| \leq 40$; $\sqrt{w} = 40/|F_0|$ if $|F_0| > 40$) was also introduced. R converged to 14.0%. Further improvement required introduction of anisotropic temperature factors for carbon and oxygen atoms. However it was considered that insufficient observed reflections were available (1686 measurements, 469 parameters for anisotropic vibration). A list of observed and calculated structure factors (R 0.14, not using anomalous scattering) is listed in Supplementary Publication No. 20260 (19 pp., 1 microfiche).*

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* For details of Supplementary Publications see Notice to Authors No. 7 in *J. Chem. Soc. (A)*, 1970, Issue No. 20.