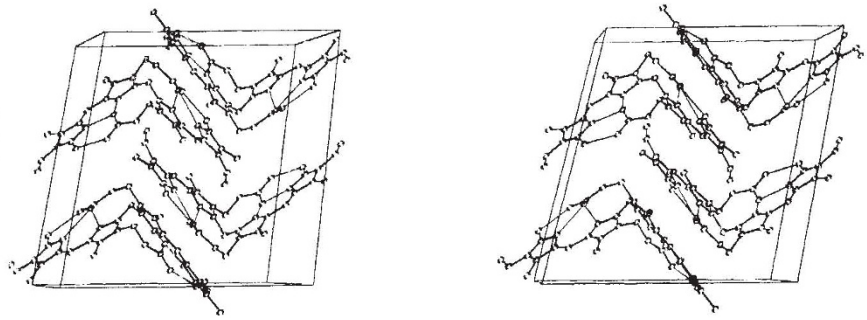

Fig. 6 Packing of bilirubin molecules in the unit cell. The *b* axis is approximately perpendicular to the page, and the *c* axis is horizontal.

determined one: estimated standard deviations of individual bond lengths were generally in the range 0.02–0.05 Å.

The two independent molecules of bilirubin in the asymmetric unit of the structure both have the conformation shown in Fig. 3 and represented in a simplified way in Fig. 4. The molecule takes the form of a ridge tile, the ridge being along the line $C8^1$–$C10$–$C12^1$. Thus rings A and B lie in one plane, and rings C and D in another, the interplanar angle being about 98°. Six intramolecular hydrogen bonds (two sets, each involving a carboxylic acid group, a pyrrole-imino hydrogen, and a terminal lactam system) stabilise this conformation. The average N···O and O···O distances in these hydrogen bonds are 2.70 and 2.53 Å, respectively. There is no evidence for intermolecular hydrogen bonds: the low solubility of bilirubin in water, which has important biological consequences, can readily be understood in terms of this pattern of hydrogen bonding. Although the hydrogen bonding, and therefore the conformation, may well be altered in very polar solvents (such as dimethylformamide and methanolic ammonia), it seems likely that in less polar solvents (such as chloroform) the conformation with six hydrogen bonds (Fig. 3) will be retained. Unfortunately, the X-ray data are neither sufficiently numerous nor sufficiently accurate to allow determination of the position of the hydrogen atoms in these hydrogen bonds and we are not able to make a clear cut assignment of either the lactam or the lactim formulations.

The X-ray result provides independent proof of the correctness of the gross chemical structure assigned to bilirubin by Fischer *et al.*[4] The stereochemistry about the double bonds at the *meso*-bridges (C5 and C15) is frequently, even in modern work[18,19], represented as *E*. The *Z* configuration (Fig. 4) is now shown to be correct. This type of structure seems to have been first portrayed by Blauer and King[14]; the first discussion in detail was by Kuenzle *et al.*[15].

The observed bond lengths suggest that delocalisation over an individual conjugated system of two pyrrole rings (that is, A+B or C+D) is rather limited since C4–C5 and C15–C16 seem to be essentially double bonds (average 1.30 Å) whereas C5–C6 and C14–C15 resemble single bonds (average 1.48 Å). This supports the view that bilirubin can be regarded as a 2,2′-dipyrrolylmethane (that is, rings B + C) with conjugating substituents at the α positions (compare with the model compound shown in Fig. 5)[18].

The packing of the four bilirubin molecules in the unit cell is shown in Fig. 6. Taking the original analogy further, we now have a stack of ridge tiles interleaved with a similar but inverted stack. Figure 6 suggests an explanation for the diffuse *h*-odd reflections observed in the diffraction patterns of most of our crystals: enantiomeric conformations of bilirubin (related by inversion centres) have very similar ridge tile shapes and the orientation of any ridge tile is virtually unaltered by a rotation of 180° about a direction approximately parallel to the a^* axis (vertical in Fig. 6). On the basis of Fig. 6, it is not difficult to imagine disordered structures which would give an apparent halving of the *a* axis. We have, indeed, collected a third set of data from a disordered crystal, and successfully refined it to $R = 19.7\%$ by assuming that either enantiomer may occupy any molecular site, the packing of the ridge tiles remaining constant.

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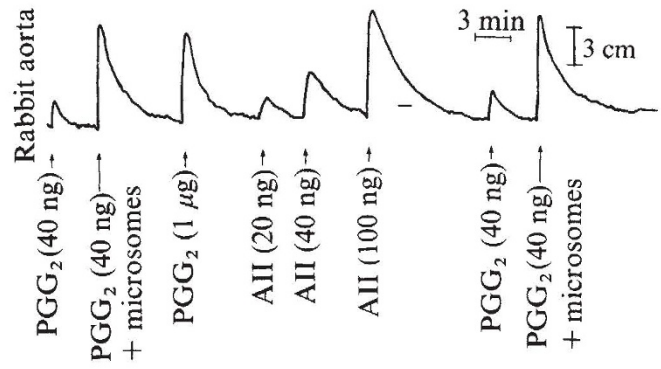
1 Lathe, G. H., *Essays in Biochemistry*, **8**, 107–148 (CRC, Cleveland, Ohio, 1972).
2 Schmid, R., and McDonagh, A. F., *Ann. N.Y. Acad. Sci.*, **244**, 533–552 (1975).
3 Fischer, H., and Orth, H., *Die Chemie des Pyrrols*, **2.1**, 626 (Akademische. Verlagsgesellschaft, Leipzig, 1937).
4 Fischer, H., Plieninger, H., and Weissbarth, O., *Hoppe-Seyler's Z. Physiol. Chem.*, **268**, 231–260 (1941).
5 Falk, H., Grubmayr, K., Herzig, U., and Hofer, O., *Tetrahedron Lett.*, 559–562 (1975).
6 Gray, C. H., Kulczycka, A., and Nicholson, D. C., *J. chem. Soc.*, 2276–2285 (1961).
7 Kuenzle, C. C., *Biochem. J.*, **119**, 395–409 (1970).
8 Hutchinson, D. W., Johnson, B., and Knell, A. J., *Biochem. J.*, **123**, 483–484 (1971).
9 Manitto, P., Ricca, G. S., and Monti, D., *Gazzetta Chim. Ital.*, **104**, 633–637 (1974).
10 Nichol, A. W., and Morell, D. B., *Biochim. biophys. Acta*, **177**, 599–609 (1969).
11 Fog, J., and Jellum, E., *Nature*, **198**, 88–89 (1963).
12 Fog, J., and Bugge-Asperheim, B., *Nature*, **203**, 756–757 (1964).
13 Brodersen, R., Flodgaard, H., and Hansen, J. K., *Acta scand. Chim.*, **21**, 2284–2285 (1967).
14 Blauer, G., and King, T. E., *J. biol. Chem.*, **245**, 372–381 (1970).
15 Kuenzle, C. C., Weibel, M. H., Pelloni, R. R., and Hemmerich, P., *Biochem. J.*, **133**, 364–368 (1973).
16 Knell, A. J., Hancock, F., and Hutchinson, D. W., in *Metabolism and Chemistry of Bilirubin and Related Tetrapyrrols* (edit. by Bakkan, A. F. and Fog, J.), 234–239 (Pediatric Research Institute, Oslo, 1975).
17 Manitto, P., and Monti, D., *J. chem. Soc. Chem. Comm.*, 112–123 (1976).
18 Bonnett, R., Hursthouse, M. B., and Neidle, S., *J.C.S. Perkin Trans.*, **II**, 902–906, 1335–1340 (1972).
19 Rüdiger, W., *Fortschr. Chem. Organ. Natur.*, **29**, 60–139 (1971).



Erratum

In the article "Identification of an enzyme in platelet microsomes which generates thromboxane A_2 from prostaglandin endoperoxides" by P. Needleman, S. Moncada, S. Bunting, J. R. Vane, M. Hamberg and B. Samuelsson (*Nature*, **261**, 558; 1976) microsomes were added to the wrong dose in Fig. 5 and the vertical scale (3 cm) was too large. Both are correct in the version reprinted below.