appears to pass through or near the purines, while pyrimidines at the other end of the pairs stack O2-on-ring as with Y-R steps. R-Y steps tend to stack ring-on-ring, with little contribution from exocyclic atoms.

El Hassan & Calladine (1997) have recently examined roll, slide and twist behaviour at 400 different steps observed in crystal structures of 24 A- and 36 B-DNA oligomers. The author has carried out a similar analysis of 1137 steps from 86 sequence-specific protein–DNA complexes (Dickerson, 1998*a*,*c*; Dickerson & Chiu, 1997). A striking feature is that trends in local parameters are just the same in DNA crystals and in protein–DNA complexes. The frequently invoked nightmare of 'crystal packing deformations' appears to be of only minor significance. In both studies (El Hassan & Calladine, 1997; Dickerson, 1998*b*), roll *versus* slide, slide *versus* twist and twist *versus* roll plots are presented for all ten possible base-pair steps. Fig. 23.3.4.9 illustrates roll *versus* slide plots for two Y-R, two R-R and two R-Y steps.

Table 23.3.4.2 summarizes observations from these roll/slide/twist plots. These are labelled the 'Minor Canon' since they are recent, approximate and not well understood. However, they provide goals for future investigations of helix behaviour.

23.3.4.2. *A-tract bending*

It has long been known that introduction of short A-tracts into general-sequence B-DNA in phase with the natural 10–10.5 base-pair repeat produced overall curvature that could be detected *via* eletrophoretic gel retardation, ring-cyclization kinetics and other physical measurements in solution (Marini *et al.*, 1982; Wu & Crothers, 1984; Koo *et al.*, 1986; Crothers & Drak, 1992). However, the microscopic source of the observed macroscopic curvature remained unclear. Solution measurements alone cannot discriminate between three alternative curvature models: (1) local bending within the A-tracts themselves; (2) bending at junctions between A-tract B-DNA and general-sequence B-DNA; or (3) inherently straight and unbent A-tracts, with curvature resulting from removal of the normal writhe expected in general-sequence B-DNA (Koo *et al.*, 1990; Crothers *et al.*, 1990). The three curvature models are compared schematically in Fig. 10 of reference B77.

X-ray crystallographic results for DNA oligomers come down unequivocally in favour of model (3) above. Short A-tracts of four to six base pairs are straight and unbent in C-G-C-G-A-A-T-T-C-G-C-G (B1–B6), C-G-C-A-A-A-A-A-A-G-C-G (B20), C-G-C-A-A-A-A-A-T-G-C-G (B31), C-G-C-A-A-A-T-T-T-G-C-G (B17, B52), C-G-C-G-A-A-A-A-A-A-G-C (B64) and C-A-A-A-G-A-A-A-A-G (B105) (A-tracts are double-underlined). It has been claimed (Sprous *et al.*, 1995) and disputed (Dickerson *et al.*, 1994, 1996) that the observed straightness of crystalline A-tracts was only an artifact of crystal packing, or of the high levels of methyl-2,4-pentanediol (MPD) used in the crystallization. This concern now is put to rest by the observation that B-DNA packed against a protein molecule in its biological working environment behaves exactly the same as B-DNA packed against other DNA molecules in the crystal, as borne out by the roll/slide/twist studies of El Hassan & Calladine (1997) for DNA and of Dickerson (1998*a*,*b*,*c*) and Dickerson & Chiu (1997) for protein–DNA complexes. Added support has come from recent molecular-dynamics simulations by Beveridge and co-workers (Sprous *et al.*, 1999), who have demonstrated that the duplex of sequence GGGGGGAA-AATTTTCGAAAATTTTCCCCCC is severely curved because of a roll kink at the double-underlined central CG step, whereas the duplex GGGGGGTTT-

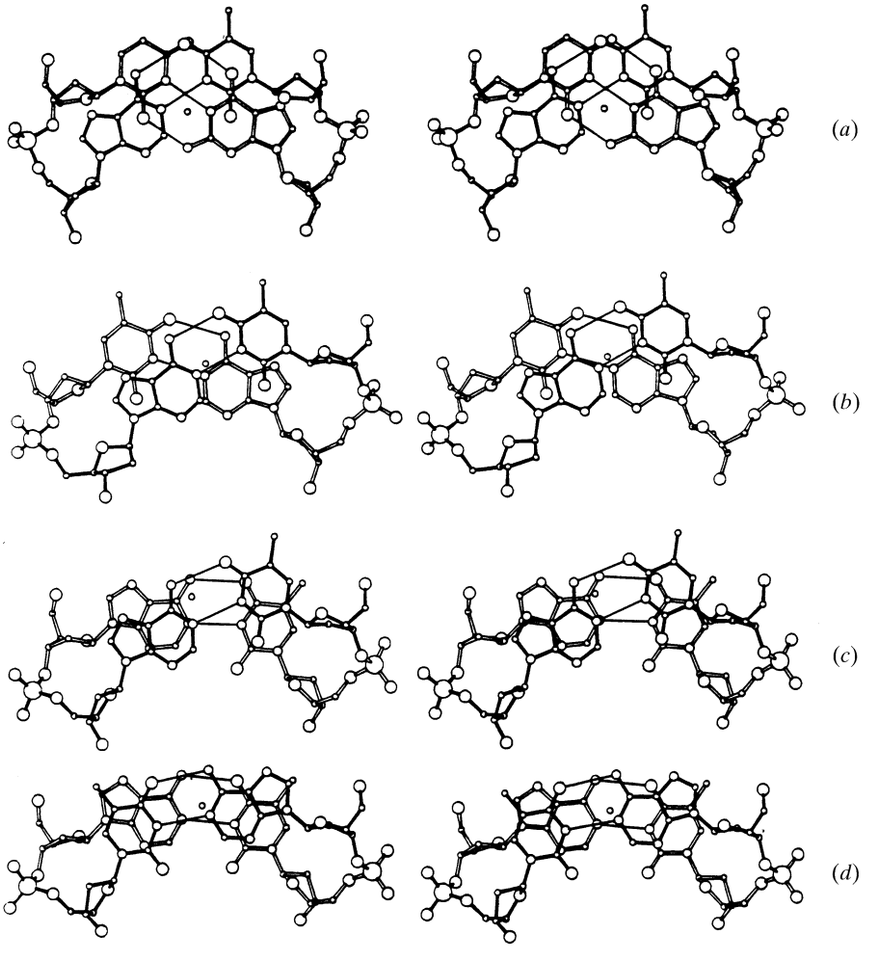

Fig. 23.3.4.8. Representative base-pair steps from B-DNA single-crystal X-ray analyses. (*a*) Pyrimidine-purine C-A step from C-C-A-A-G-A-T-T-G-G (B22, B46) (roll/slide/twist = $-7.4°/2.6$ Å$/49.9°$). Note the lack of ring-on-ring stacking, replaced by the stacking of pyrimidine O2 and purine N6 or O6, on aromatic rings of the adjacent base pair. This stacking opens up the twist angle to an unusual 50°. Note also the large +2.6 Å slide, which positions pyrimidine O2 over the six-membered rings of the neighbouring purines. (*b*) Pyrimidine-purine T-A step from C-G-A-T-A-T-A-T-C-G (B62) (roll/side/twist = $3.8°/-0.2$ Å$/39.5°$). The stacking is similar to C-A, except that a near-zero slide positions pyrimidine O2 over the five-membered rings of purines. (*c*) Purine-purine A-A step from C-C-A-A-C-G-T-T-G-G (B46, B50) (roll/slide/twist = $8.8°/0.5$ Å$/28.7°$). Ring-on-ring overlap now predominates, with consequently lowered twist angle and essentially zero slide. Note that purines are more extensively stacked than pyrimidines, which appear to be approaching the O2-on-ring stacking of Y-R steps. (*d*) Purine-pyrimidine A-T step from C-G-A-T-A-T-A-T-C-G (B62) (roll/slide/twist = $5.2°/0.0$ Å$/25.2°$). Ring-on-ring stacking again lowers the twist angle and keeps slide around zero. Now there is no stacking of exocyclic N or O on neighbouring rings.

references