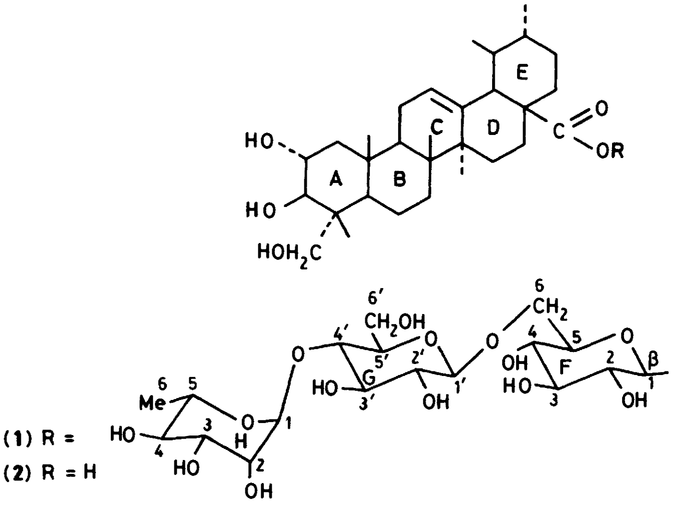
Stereochemistry of a Triterpenoid Trisaccharide from *Centella asiatica*. *X*-Ray Determination of the Structure of Asiaticoside

Shashi B. Mahato* and Niranjan P. Sahu
Indian Institute of Chemical Biology, Jadavpur, Calcutta 700032, India
Peter Luger and Eveline Müller
Freie Universität Berlin, Fachbereich Chemie, Institut für Kristallographie, 1000 Berlin 33, West Germany

The molecular geometry of asiaticoside (**1**), a trisaccharide triterpene isolated from *Centella asiatica*, has been determined by a single-crystal *X*-ray analysis. The glycoside, $C_{48}H_{78}O_{19}$, is prismatic with space group monoclinic, *C*2 and lattice constants $a = 41.46$ (1), $b = 7.563$ (4), $c = 17.988$ (5) Å, $\beta = 99.66(5)°$, cell volume $V = 5\,602.3$ Å^3, and $Z = 4$. Diffractometer intensity measurements were made with Cu-K_α radiation and least-squares adjustment of the atomic parameters converged to a final *R* value of 0.073. ^{13}C N.m.r. and f.a.b.-m.s. are satisfactorily interpreted within terms of the structure.

Centella asiatica Linn. (syn. *Hydrocotyl asiatica* Linn.) (Umbelliferae) is a prostrate, perennial, faintly aromatic herb found wild throughout India. The plant enjoys a considerable reputation in Indian medicine as a diuretic and tonic. An infusion of the plant is used in India and Madagascar in the treatment of leprosy.[1] The major glycoside, asiaticoside, is known to have antileprotic activity.[2–4] Phytochemical studies[5–8] and elucidation of the planar structure of asiaticoside have been reported.[9,10] However, the complete stereochemistry remains to be established. In view of the potential of this glycoside in the treatment of leprosy we now report the single-crystal *X*-ray determination of the complete structure and stereochemistry of asiaticoside together with ^{13}C n.m.r. and fast-atom-bombardment mass spectra (f.a.b.-m.s.).[11–13]



Experimental

M.p.s are uncorrected. ^1H N.m.r. spectra were recorded on a JEOL FX100 (99.6 MHz) instrument for $CDCl_3$ or C_5D_5N solutions. ^{13}C N.m.r. spectra were recorded on a JEOL FX100 Fourier transform spectrometer operating at 25.5 MHz, for $CDCl_3$ or C_5D_5N solutions with tetramethylsilane as internal standard. Electron impact mass spectra (e.i.-m.s.) were recorded on a Hitachi Model RMU-6L mass spectrometer. Optical rotations were measured on a Perkin-Elmer automatic polarimeter. Fast-atom-bombardment mass spectra (f.a.b.-m.s.) were obtained on a ZAB–SE mass spectrometer operating with a potential of 5—8 kV applied to the xenon gun with 1-thioglycerol (for negative-ion f.a.b.) or diethanolamine (DEA) (for positive-ion f.a.b.) as matrices.

Isolation of Asiaticoside.—The air-dried powdered leaves of *C. asiatica* (1 kg) were successively extracted with light petroleum (60—80 °C), chloroform, and 90% ethanol at ambient temperature. Evaporation of the solvent under reduced pressure gave a viscous dark mass which was extracted with butanol, and washed (water) to remove inorganic impurities. The butanol extract on removal of the solvent under reduced pressure gave a viscous dark brown mass (65 g). A portion of this extract (50 g) was chromatographed on silica gel (900 g). Graded elution was effected with light petroleum (60—80 °C), chloroform, and

Table 1. ^{13}C N.m.r. chemical shifts δ_C (±0.1) of asiatic acid (**2**) and asiaticoside (**1**) (C_5D_5N)

Carbon	(2)	(1)	Carbon	(2)	(1)
1	47.9	48.0	27	24.2	23.7
2	69.1	68.8	28	179.9	176.2
3	78.9	78.6	29	24.2	23.7
4	42.9	42.5	30	21.6	21.2
5	48.3	48.0	G-1		95.4
6	18.9	18.1	G-2		73.5
7	33.5	33.0	G-3		78.3
8	40.4	40.1	G-4		70.1
9	48.5	48.0	G-5		76.6[b]
10	38.6	38.2	G-6		70.8
11	24.0	24.6[a]	G-1′		104.4
12	125.8	126.0	G-2′		74.9
13	139.5	138.3	G-3′		77.5
14	43.7	43.4	G-4′		78.4
15	29.0	28.4	G-5′		76.3[b]
16	25.2	24.8[a]	G-6′		61.4
17	48.3	48.4	R-1		102.3
18	53.8	53.1	R-2		72.4[c]
19	39.7	39.0	R-3		72.1[c]
20	39.7	40.1	R-4		73.5
21	31.4	30.8	R-5		69.4
22	37.6	36.7	R-6		18.1
23	67.5	66.0			
24	14.2	14.0			
25	17.7	17.7[b]			
26	17.7	17.4[b]			

G = Glucose, R = rhamnose. [a,b,c] May be interchanged in each vertical column.

Table 2. Final atomic parameters and standard deviations (in parentheses) for non-hydrogen atoms in asiaticoside

Atom	*x*	*y*	*z*
C(1)	0.168 8(1)	0.397 0(7)	0.342 5(3)
C(2)	0.141 4(1)	0.392 4(6)	0.392 7(3)
O(2)	0.114 78(8)	0.501 3(5)	0.362 7(2)
C(3)	0.130 4(1)	0.203 0(7)	0.402 5(3)
O(3)	0.103 82(7)	0.199 4(5)	0.447 9(2)
C(4)	0.157 9(1)	0.078 7(7)	0.434 8(3)
C(5)	0.187 0(1)	0.096 7(6)	0.387 7(2)
C(6)	0.216 1(1)	−0.021 0(7)	0.413 6(3)
C(7)	0.238 8(1)	−0.033 6(8)	0.351 9(3)
C(8)	0.251 4(1)	0.143 8(7)	0.327 0(3)
C(9)	0.222 6(1)	0.276 9(7)	0.312 2(3)
C(10)	0.198 7(1)	0.288 9(6)	0.374 5(3)
C(11)	0.234 6(1)	0.456 4(9)	0.289 4(4)
C(12)	0.261 3(2)	0.444(1)	0.239 2(5)
C(13)	0.276 6(1)	0.301 2(8)	0.222 7(3)
C(14)	0.267 4(1)	0.119 5(7)	0.250 9(3)
C(15)	0.297 7(1)	−0.001 4(8)	0.265 7(3)
C(16)	0.321 3(1)	0.007 3(9)	0.206 0(3)
C(17)	0.332 6(1)	0.199 2(9)	0.197 0(3)
C(18)	0.303 1(1)	0.316(1)	0.169 7(3)
C(19)	0.290 9(1)	0.282(2)	0.085 2(4)
C(20)	0.318 6(2)	0.303(2)	0.036 9(4)
C(30)	0.306 8(2)	0.262(4)	−0.046 4(5)
C(21)	0.346 0(2)	0.180(2)	0.062 3(4)
C(22)	0.359 6(1)	0.208(1)	0.143 2(3)
C(23)	0.144 4(1)	−0.109 9(7)	0.427 6(3)
O(23)	0.136 0(1)	−0.161 0(6)	0.351 7(2)
C(24)	0.166 6(1)	0.110 4(8)	0.519 7(3)
C(25)	0.214 0(1)	0.383 0(8)	0.446 2(3)
C(26)	0.277 4(1)	0.215 2(8)	0.388 7(3)
C(27)	0.243 6(1)	0.030(1)	0.189 7(3)
C(28)	0.348 7(1)	0.269 7(8)	0.271 0(3)
O(28)	0.342 3(1)	0.408 1(7)	0.300 3(3)
C(29)	0.263 0(2)	0.413(2)	0.059 4(9)
O(100)	0.373 30(8)	0.164 6(5)	0.301 2(2)
C(101)	0.389 7(1)	0.220 9(7)	0.371 0(2)
C(102)	0.407 2(1)	0.062 9(8)	0.406 9(3)
O(102)	0.384 4(1)	−0.061 3(7)	0.426 2(3)
C(103)	0.430 0(1)	0.118 8(8)	0.476 3(3)
O(103)	0.451 2(1)	−0.022 9(7)	0.500 1(3)
C(104)	0.451 0(1)	0.273 7(8)	0.457 7(3)
O(104)	0.471 05(8)	0.330 0(6)	0.523 0(2)
C(105)	0.429 2(1)	0.421 8(7)	0.423 6(3)
O(105)	0.412 01(7)	0.354 8(5)	0.354 8(2)
C(106)	0.445 8(1)	0.594 4(7)	0.408 3(3)
O(200)	0.473 39(8)	0.563 8(6)	0.367 9(2)
C(201)	0.488 7(1)	0.722 1(8)	0.354 7(3)
C(202)	0.521 6(1)	0.682 6(9)	0.329 4(3)
O(202)	0.542 50(9)	0.607 2(8)	0.389 8(3)
C(203)	0.537 0(1)	0.853 0(8)	0.304 5(3)
O(203)	0.567 93(8)	0.815 5(6)	0.279 6(2)
C(204)	0.514 1(1)	0.947(1)	0.245 4(3)
C(205)	0.482 0(2)	0.979(1)	0.278 1(5)
C(206)	0.457 1(3)	1.069(3)	0.223(1)
O(206)	0.452 2(2)	0.994(3)	0.156 5(5)
O(205)	0.469 02(9)	0.811 7(9)	0.296 7(3)
O(300)	0.527 2(1)	1.115 3(7)	0.228 9(3)
C(301)	0.531 7(2)	1.140(1)	0.152 3(5)
C(302)	0.530 6(4)	1.337(2)	0.138 0(7)
C(303)	0.560 1(8)	1.431(1)	0.177 7(6)
O(302)	0.529 0(2)	1.365(1)	0.058 0(4)
O(303)	0.555 7(5)	1.613(1)	0.157 7(5)
C(304)	0.589 6(4)	1.350(2)	0.158 9(5)
O(304)	0.616 2(4)	1.430(2)	0.198 3(6)
C(305)	0.588 9(2)	1.152(1)	0.176 3(4)
C(306)	0.617 9(3)	1.058(3)	0.153 8(8)
O(305)	0.561 0(1)	1.072 0(7)	0.135 8(2)
O(1W)	0.375 1(3)	0.727(2)	0.299 4(7)
O(2W)	0.533 2(5)	1.799(4)	0.031(1)
O(3W)	0.427 5(5)	1.135(4)	0.027(1)
O(4W)	0.437 6(8)	1.281(6)	0.051(2)

chloroform–methanol (90:10, 85:15, 82:18, 80:20). A total of 108 fractions of 250 ml each were collected and fractions giving similar spots on t.l.c. were combined. Fractions 58—70 eluted with $CHCl_3$–MeOH (82:18) furnished crude asiaticoside which on further purification by rechromatography on silica gel and crystallisation from dioxane gave pure asiaticoside (1.5 g), m.p. 230—232 °C (decomp.); $[\alpha]_D$ −16° (*c* 0.52 in MeOH); [lit.,[9] m.p. 230—233 °C (decomp.); $[\alpha]_D$ −14° (EtOH)]; f.a.b.-m.s. (negative ion) (1-thioglycerol) 993 $[M - H + 2H_2O]^-$, 957 $[M - H]^-$, 487 $[M - H -$ sugar entity$]^-$, 471 $[M -$ genin$]^-$, 453, and 409; f.a.b.-m.s. (positive ion) (DEA) 1 064 $[M + DEAH]^+$, 651, 594 $[M +$ DEAH − sugar entity$]^+$, 570, 550, 526, 509, 465, 443, and 421. ^{13}C N.m.r. assignments of asiaticoside and its genin, asiatic acid, are shown in Table 1. Assignments of the signals of the genin were made by comparison with those of triterpenes with similar skeletons.[14—16] ^{13}C N.m.r. signals of (1) were assigned by comparison with those of asiatic acid and sugar entities using glycosylation shifts.[17,18]

Crystal Data.—$C_{48}H_{78}O_{19} + 2H_2O + C_4H_4O_2$, lattice constants (Å, degrees) $a = 41.46(1)$, $b = 7.563(4)$, $c = 17.988(5)$, $\beta = 96.66(5)$, cell volume (Å^3) $V = 5\,602.3$, formula units/cell $Z = 4$, *X*-ray density (g cm^{-3}) $\rho_x = 1.279$, space group *C*2, total number of reflections 5 019, unobserved ($I < 2\sigma$) 379, linear absorption coefficient $\mu(\text{Cu-}K_\alpha) = 8.46$ cm^{-1}, no absorption correction, *R* value = 0.073. $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w\text{-}F_o^{\,2}]^{\frac{1}{2}} = 0.103$.

Crystallographic Measurements.—Suitable single crystals of asiaticoside were obtained from solution in dioxane. A prismatic specimen with dimensions 0.1 × 0.2 × 0.45 mm was selected for the *X*-ray measurements. From preliminary rotation and Weissenberg photographs the space group was determined to be monoclinic, *C*2. Because of the chirality of the molecule, *Cm* and *C*2/*m* could definitely be excluded.

Precise lattice constants and the intensity data of a quadrant ($-h$, k, l and $-h$, k, $-l$) were measured on a Stoe four-circle diffractometer with Ni-filtered Cu-K_α radiation. Orientation matrix and lattice constants were refined from 50 high-order reflections. The reflection intensities were recorded by the θ–2θ scan technique with variable scan range and variable scan speed. Two standard reflections which were measured every 90 min showed no significant variations during the whole data collection.

Structure Determination and Refinement.—Phase determination was carried out successfully with direct methods (MITHRIL).[19] From the phase set with the best combined figure of merit 53 of the 67 non-hydrogen atoms of the asiaticoside molecule could be identified, the missing atoms were located in subsequent difference syntheses. However, the least-squares refinements (with the corresponding programs of the *X*-Ray 76[20] system) did not proceed in a straightforward manner.

First, remarkable influence of secondary extinction was discovered so that an isotropic extinction coefficient was

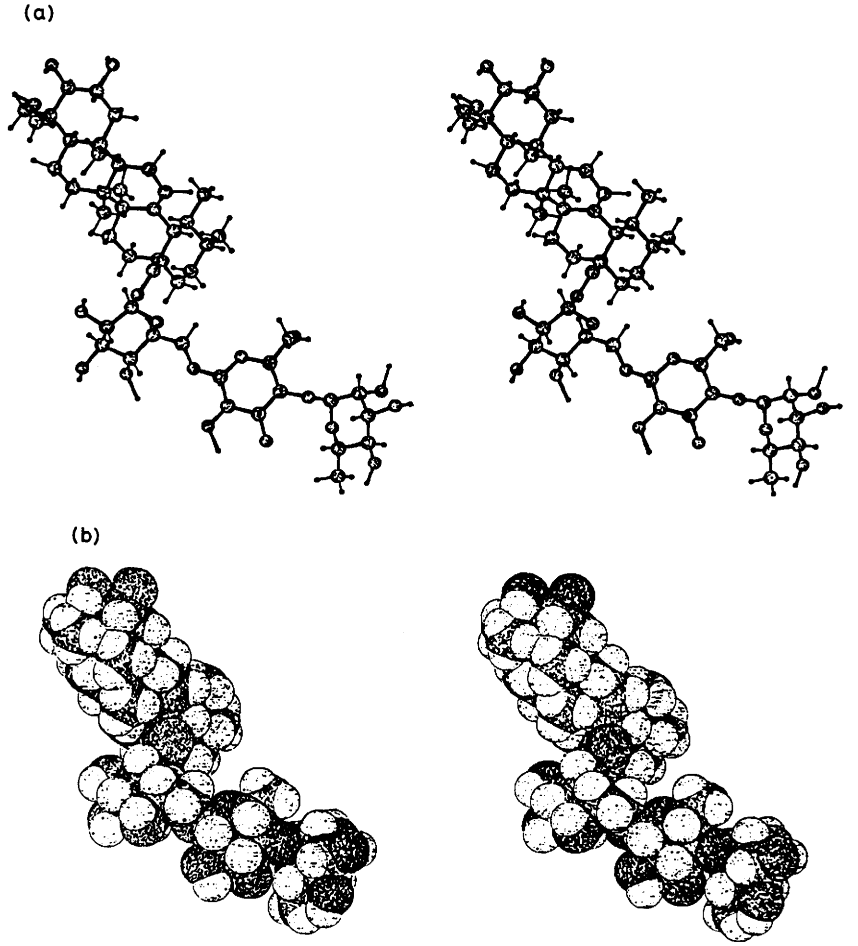


Figure 1. (a) Stereoscopic pair of drawings of the molecular structure of asiaticoside[4]; (b) stereo pair of drawings of the van der Waals surface of asiaticoside

introduced in the further refinement. Second, during the anisotropic refinement of the C and O atoms the thermal parameters of a number of atoms, especially at the terminal rhamnose monosaccharide unit, tended to be unusually large. Attempts to redetermine the atoms in question failed to improve results. Thus partial disorder must be assumed for these atom groups, although no multiple positions could be resolved from a difference synthesis. Third, after completion of the molecular structure (without H) and even after its anisotropic refinement a total of 16 peaks were found in a difference synthesis with peak heights more or less in the range of a half carbon or oxygen atom. Two groups of six peaks each having approximately the geometry of a dioxane ring (the solvent used for crystallization) were present. It was thus assumed that one dioxane molecule (disordered on two positions) and two water molecules (each also disordered on two positions) exist in the crystal structure of asiaticoside. These solvent molecules were added to the further refinement. The dioxane fragments were fitted by a proper dioxane molecule using the co-ordinates of our previous *X*-ray structure determination of dioxane itself.[21] The fitted geometry was then treated as a rigid group of which only orientation and translation was refined.

All hydrogens of the asiaticoside molecule [except for those at O(104) and O(302)] were included in the final refinement cycles. About 50% could be located from difference syntheses, all further hydrogens were calculated from chemical considerations. Some of the hydrogens were selected as to fit with intermolecular hydrogen bonds. Since this selection was ambiguous the positions of some hydroxy hydrogens may be in question. Isotropic thermal parameters obtained from their parent heavy atoms (plus 20%) were assigned to the hydrogens. All their positional and thermal parameters were kept unrefined. No hydrogens were determined nor calculated for the solvent molecules.

Using the atomic parameters as described above the final refinements were calculated with a matrix subdivision into four blocks, one for the triterpene entity, two for the glucose residues, and one for the rhamnose residue and the solvent parameters. A weighting scheme was used that made $w\,\Delta F$ almost independent from F_0 and sinθ. Unobserved reflections were included in the refinement only if $|F_c| > |F_0|$. After convergence of all parameters a final *R* value of 7.3% was obtained. The maximum and average shift/error ratios at the end of the refinement were 0.53 and 0.25. A final electron density map showed all residual density below 0.5 e Å^{-3}. The final atomic parameters are given in Table 2. A complete atomic parameters list and the anisotropic thermal parameters are deposited at the Cambridge Crystallographic Data Centre.*

Results and Discussion

The molecular structure of asiaticoside is shown in the stereoscopic drawing[22] in Figure 1. The atomic numbering

---

* For details see Instruction for Authors in *J. Chem Soc., Perkin Trans. 2*, 1987, Issue 1, section 5.6.3.

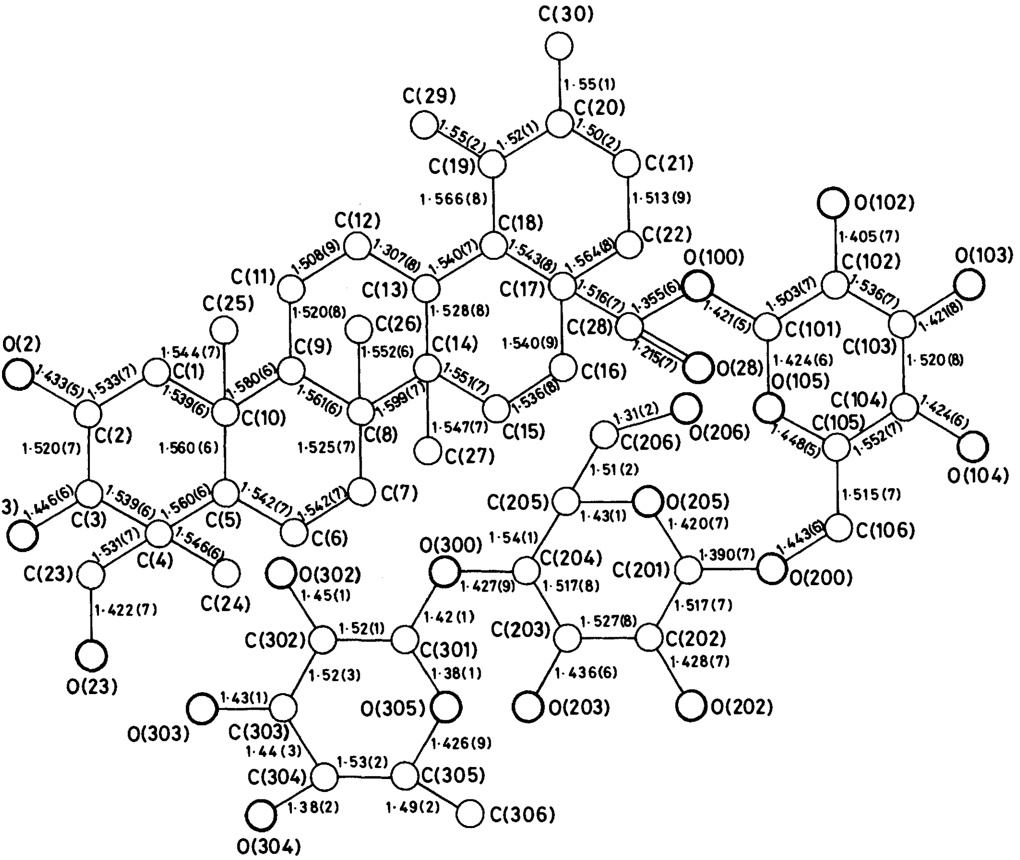


Figure 2. Bond lengths (Å) (e.s.d.s in parentheses) and atom-numbering scheme for the asiaticoside molecule

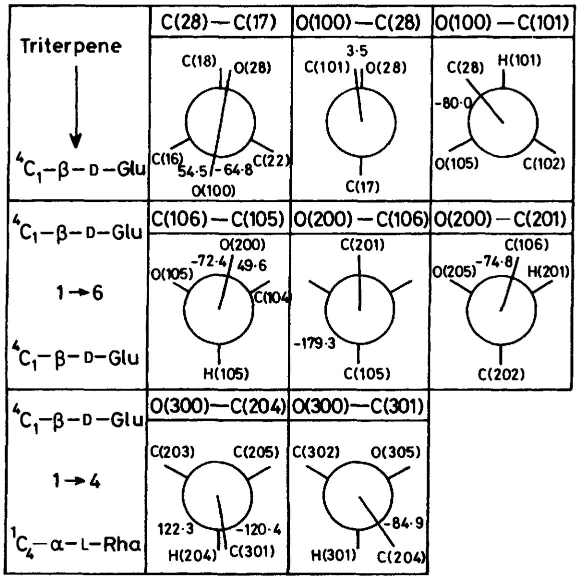


Figure 3. A number of Newman projections for illustration of the glycosidic linkages

scheme and bond lengths are given in Figure 2. Bond angles and selected torsion angles are listed in Tables 3 and 4.

The triterpene entity consists of five six-membered rings **A**—**E**, with a *trans*-arrangement for the rings **A**, **B**, **C**, and **D** and a *cis*-fusion between **D** and **E**. It follows from the Cremer–Pople puckering parameters[23,24] (see Table 5) that **A**, **B**, **D**, and **E** have chair conformations (**B** and **E** are slightly distorted, **D** is remarkably distorted towards a half-chair form) whereas **C** adopts an envelope conformation [due to the double bond C(12)–C(13)]. It is interesting to note that in 4-methoxyursan-3-yl benzoate,[25] having a comparable triterpene ring system, the distribution of six-membered-ring conformations is almost the same.

The triterpene substituents OH and CH_2OH of ring **A** are in equatorial positions, and the methyl groups on rings **A**—**D** occupy axial positions. The two methyl groups on ring **E** are in equatorial positions and the carboxyl group at C(17), which bears the sugar chain, is axial. The axial methyl groups and the *cis*-fusion between rings **D** and **E** causes a number of 1,3-diaxial contacts which may contribute to some strain in this ring system and which may be responsible for the unusually large C–C bond lengths C(9)–C(10) and C(8)–C(14).

The triterpene skeleton and the trisaccharide sugar chain are connected *via* the carboxyl group at C(17) to the asiaticoside molecule. The first two monosaccharide components are β-D-glucose residues (rings **F** and **G**) and the third is α-L-rhamnose. The sugar rings **F** and **G** are in a $^4C_1$ chair conformation, whereas ring **H** of the L-rhamnose residue adopts a $^1C_4$ conformation (see Table 5). In both glucopyranosyl residues the CH_2OH group is in a gauche-gauche position.

Of special interest is the linkage of the sugar chain to the triterpene entity of asiaticoside, and the glycosidic linkages between the sugar residues. Newman projections for all such bonds are given in Figure 3.

The linkage between the sugar chain and the triterpene

Table 3. Valence angles (°) (e.s.d.s in parentheses)

C(2)–C(1)–C(10)	112.8(4)
C(1)–C(2)–O(2)	110.9(4)
C(1)–C(2)–C(3)	110.1(4)
O(2)–C(2)–C(3)	110.8(4)
C(2)–C(3)–O(3)	110.1(4)
C(2)–C(3)–C(4)	113.5(4)
O(3)–C(3)–C(4)	110.7(4)
C(3)–C(4)–C(5)	109.1(4)
C(3)–C(4)–C(23)	106.9(4)
C(3)–C(4)–C(24)	110.9(4)
C(5)–C(4)–C(23)	109.4(4)
C(5)–C(4)–C(24)	114.5(4)
C(23)–C(4)–C(24)	105.6(4)
C(4)–C(5)–C(6)	114.6(4)
C(4)–C(5)–C(10)	116.0(4)
C(6)–C(5)–C(10)	109.9(4)
C(5)–C(6)–C(7)	110.1(4)
C(6)–C(7)–C(8)	114.6(5)
C(7)–C(8)–C(9)	109.6(4)
C(7)–C(8)–C(14)	109.9(4)
C(7)–C(8)–C(26)	109.1(4)
C(9)–C(8)–C(14)	108.3(4)
C(9)–C(8)–C(26)	110.6(4)
C(14)–C(8)–C(26)	109.2(4)
C(8)–C(9)–C(10)	116.5(4)
C(8)–C(9)–C(11)	110.6(4)
C(10)–C(9)–C(11)	113.1(4)
C(1)–C(10)–C(5)	107.5(4)
C(1)–C(10)–C(9)	108.0(4)
C(1)–C(10)–C(25)	107.6(4)
C(5)–C(10)–C(9)	106.6(4)
C(5)–C(10)–C(25)	114.0(4)
C(9)–C(10)–C(25)	112.9(4)
C(9)–C(11)–C(12)	113.2(6)
C(11)–C(12)–C(13)	126.5(8)
C(12)–C(13)–C(14)	121.5(6)
C(12)–C(13)–C(18)	118.7(6)
C(14)–C(13)–C(18)	119.6(5)
C(8)–C(14)–C(13)	109.0(4)
C(8)–C(14)–C(15)	109.3(4)
C(8)–C(14)–C(27)	111.5(4)
C(13)–C(14)–C(15)	111.0(4)
C(13)–C(14)–C(27)	108.7(4)
C(15)–C(14)–C(27)	107.4(4)
C(14)–C(15)–C(16)	115.1(4)
C(15)–C(16)–C(17)	109.8(5)
C(16)–C(17)–C(18)	109.5(4)
C(16)–C(17)–C(22)	110.8(6)
C(16)–C(17)–C(28)	110.4(5)
C(18)–C(17)–C(22)	112.0(5)
C(18)–C(17)–C(28)	109.4(5)
C(22)–C(17)–C(28)	104.7(4)
C(13)–C(18)–C(17)	110.9(5)
C(13)–C(18)–C(19)	114.7(4)
C(17)–C(18)–C(19)	111.7(6)
C(18)–C(19)–C(20)	110.9(5)
C(18)–C(19)–C(29)	109.3(8)
C(20)–C(19)–C(29)	110.4(9)
C(19)–C(20)–C(30)	110.8(7)
C(19)–C(20)–C(21)	110.9(9)
C(30)–C(20)–C(21)	108.0(1)
C(20)–C(21)–C(22)	113.1(8)
C(17)–C(22)–C(21)	112.2(5)
C(4)–C(23)–O(23)	112.4(4)
C(17)–C(28)–O(28)	125.9(5)
C(17)–C(28)–O(100)	112.4(5)
O(28)–C(28)–O(100)	121.7(5)
C(28)–O(100)–C(101)	116.0(4)
O(100)–C(101)–C(102)	107.4(4)
O(100)–C(101)–O(105)	106.7(4)
C(102)–C(101)–O(105)	111.2(4)
C(101)–C(102)–O(102)	109.5(4)
C(101)–C(102)–C(103)	110.6(5)
O(102)–C(102)–C(103)	110.6(4)
C(102)–C(103)–O(103)	109.8(5)
C(102)–C(103)–C(104)	110.3(4)
O(103)–C(103)–C(104)	107.3(5)
C(103)–C(104)–O(104)	110.0(4)
C(103)–C(104)–C(105)	109.2(4)
O(104)–C(104)–C(105)	112.0(4)
C(104)–C(105)–O(105)	107.3(4)
C(104)–C(105)–C(106)	116.6(4)
O(105)–C(105)–C(106)	109.6(4)
C(101)–O(105)–C(105)	110.0(3)
C(105)–C(106)–O(200)	110.9(4)
C(106)–O(200)–C(201)	110.7(4)
O(200)–C(201)–C(202)	109.1(5)
O(200)–C(201)–O(205)	107.5(4)
C(202)–C(201)–O(205)	109.5(5)
C(201)–C(202)–O(202)	109.7(5)
C(201)–C(202)–C(203)	110.0(5)
O(202)–C(202)–C(203)	108.9(4)
C(202)–C(203)–O(203)	110.1(5)
C(202)–C(203)–C(204)	110.7(5)
O(203)–C(203)–C(204)	112.5(5)
C(203)–C(204)–C(205)	107.6(5)
C(203)–C(204)–O(300)	110.0(5)
C(205)–C(204)–O(300)	108.0(6)
C(204)–C(205)–C(206)	111.9(9)
C(204)–C(205)–O(205)	108.5(7)
C(206)–C(205)–O(205)	107.8(9)
C(205)–C(206)–O(206)	115.0(2)
C(201)–O(205)–C(205)	113.2(5)
C(204)–O(300)–C(301)	114.4(6)
O(300)–C(301)–C(302)	106.7(8)
O(300)–C(301)–O(305)	112.1(6)
C(302)–C(301)–O(305)	109.8(9)
C(301)–C(302)–C(303)	112.0(1)
C(301)–C(302)–O(302)	108.1(9)
C(303)–C(302)–O(302)	110.0(1)
C(302)–C(303)–O(303)	105.0(2)
C(302)–C(303)–C(304)	111.0(1)
O(303)–C(303)–C(304)	116.0(2)
C(303)–C(304)–O(304)	110.0(1)
C(303)–C(304)–C(305)	109.0(1)
O(304)–C(304)–C(305)	111.0(1)
C(304)–C(305)–C(306)	112.0(1)
C(304)–C(305)–O(305)	110.2(8)
C(306)–C(305)–O(305)	106.8(9)
C(301)–O(305)–C(305)	114.3(6)

skeleton is *via* the axial carboxyl group at C(17). The torsion angles along C(28)–C(17) and O(100)–C(28) are close to zero, *i.e.* O(28) is eclipsed to C(18) and C(101). From this it follows that the six atoms C(18), C(17), C(28), O(28), O(100), and C(101) are almost in a common plane. For the torsion angle at O(100)–C(101), C(28) and O(105) are at an angle of −80.0° which is close to the −60° value of the *exo*-anomeric effect.

The two glucopyranosyl residues are connected by a β-1-6 linkage, which, as far as we know, has not been investigated by *X*-ray analysis. Thus the present data are the first results for a linkage of this type. As already mentioned, O(200) is in gauche-gauche position with respect to ring **F**. The torsion angle along O(200)–C(106) is about 180° so that C(201) and C(105) are in a pure *trans*-position. Comparable data for a β-1-6 linkage

Table 4. Dihedral angles (°) (e.s.d.s in parentheses)

C(1)–C(2)–C(3)–C(4)	57.1(5)
C(2)–C(3)–C(4)–C(5)	-51.6(5)
C(3)–C(4)–C(5)–C(25)	80.9(4)
C(3)–C(4)–C(5)–C(6)	-179.7(4)
C(3)–C(4)–C(5)–C(10)	50.5(5)
C(4)–C(5)–C(6)–C(1)	-137.4(8)
C(4)–C(5)–C(6)–C(7)	164.0(4)
C(5)–C(6)–C(1)–C(2)	43.1(6)
C(5)–C(6)–C(7)–C(8)	57.3(5)
C(6)–C(1)–C(2)–C(3)	-39.0(4)
C(6)–C(1)–C(2)–O(2)	-162.0(3)
C(6)–C(1)–C(23)–O(23)	-99.4(3)
C(5)–C(6)–C(1)–C(23)	9.8(6)
C(5)–C(6)–C(1)–C(24)	58.4(6)
O(2)–C(2)–C(3)–C(4)	-179.8(4)
O(2)–C(2)–C(3)–O(3)	-55.1(5)
O(3)–C(3)–C(4)–C(5)	-175.9(4)
C(2)–C(1)–C(6)–C(7)	168.9(4)
C(1)–C(6)–C(7)–C(8)	39.0(4)
C(5)–C(6)–C(7)–C(8)	57.3(5)
C(6)–C(7)–C(8)–C(9)	-47.5(5)
C(7)–C(8)–C(9)–C(10)	47.3(5)
C(8)–C(9)–C(10)–C(5)	-53.7(5)
C(8)–C(9)–C(10)–C(11)	129.9(6)
C(7)–C(8)–C(9)–C(26)	120.4(5)
C(7)–C(8)–C(9)–C(14)	-119.9(5)
C(8)–C(9)–C(14)–C(27)	121.8(5)
C(8)–C(9)–C(14)–C(15)	-6.8(6)
C(9)–C(10)–C(11)–C(12)	-15.2(8)
C(9)–C(10)–C(5)–C(6)	60.0(4)
C(9)–C(14)–C(13)–C(12)	1.5(6)
C(9)–C(14)–C(13)–C(18)	-174.8(4)
C(9)–C(14)–C(15)–C(16)	-158.3(5)
C(10)–C(5)–C(6)–C(7)	-63.3(5)
C(10)–C(11)–C(12)–C(13)	1.0(1)
C(12)–C(13)–C(14)–C(9)	1.5(6)
C(13)–C(14)–C(9)–C(10)	-153.8(6)
C(14)–C(9)–C(10)–C(11)	145.6(8)
C(13)–C(14)–C(15)–C(16)	-42.2(6)
C(11)–C(12)–C(13)–C(14)	3.0(1)
C(11)–C(12)–C(13)–C(18)	179.6(6)
C(26)–C(9)–C(14)–C(27)	156.4(4)
C(26)–C(9)–C(14)–C(13)	-95.0(3)
C(14)–C(15)–C(16)–C(17)	56.7(6)
C(15)–C(16)–C(17)–C(18)	-62.3(5)
C(15)–C(16)–C(17)–C(22)	173.6(4)
C(16)–C(17)–C(18)–C(19)	-73.8(7)
C(16)–C(17)–C(18)–C(13)	55.4(6)
C(17)–C(18)–C(19)–C(20)	-54.0(1)
C(17)–C(18)–C(19)–C(29)	-175.8(7)
C(17)–C(18)–C(13)–C(14)	-44.6(6)
C(18)–C(13)–C(14)–C(15)	36.8(6)
C(18)–C(19)–C(20)–C(21)	57.0(1)
C(18)–C(19)–C(20)–C(30)	178.0(1)
C(19)–C(20)–C(21)–C(22)	-58.0(1)
C(30)–C(20)–C(21)–C(22)	-180.0(9)
C(20)–C(21)–C(22)–C(17)	54.0(1)
C(21)–C(22)–C(17)–C(18)	-49.0(1)
C(21)–C(22)–C(17)–C(28)	-167.7(8)
C(22)–C(17)–C(28)–O(28)	111.8(7)
C(22)–C(17)–C(18)–C(19)	49.5(8)
C(22)–C(17)–C(28)–O(100)	-64.8(6)
C(17)–C(28)–O(100)–C(101)	-179.8(4)
C(16)–C(17)–C(28)–O(28)	-129.0(6)
C(16)–C(17)–C(28)–O(100)	54.5(6)
O(28)–C(28)–O(100)–C(101)	3.5(7)
C(28)–O(100)–C(101)–C(102)	160.7(4)
C(28)–O(100)–C(101)–O(105)	-80.1(5)
O(100)–C(101)–C(102)–C(103)	170.7(4)
O(100)–C(101)–C(102)–O(102)	-67.2(5)
O(100)–C(101)–O(105)–C(105)	178.7(4)
C(101)–C(102)–C(103)–C(104)	-49.5(6)
C(101)–C(102)–C(103)–O(103)	-167.6(5)
O(102)–C(102)–C(103)–O(103)	70.9(6)
C(102)–C(103)–C(104)–C(105)	54.1(6)
C(102)–C(103)–C(104)–O(104)	177.4(4)
O(103)–C(103)–C(104)–O(104)	-63.0(6)
C(103)–C(104)–C(105)–O(105)	-62.5(5)
C(103)–C(104)–C(105)–C(106)	174.3(4)
O(104)–C(104)–C(105)–C(106)	52.1(6)
C(104)–C(105)–O(105)–C(101)	67.8(4)
C(104)–C(105)–C(106)–O(200)	49.6(6)
O(105)–C(105)–C(106)–O(200)	-72.5(5)
C(105)–O(105)–C(101)–C(102)	-64.5(5)
C(105)–C(106)–O(200)–C(201)	-179.3(4)
O(105)–C(101)–C(102)–O(102)	176.4(4)
O(105)–C(101)–C(102)–C(103)	54.3(5)
C(106)–O(200)–C(201)–C(202)	166.6(4)
C(106)–O(200)–C(201)–O(205)	-74.7(5)
O(200)–C(201)–C(202)–C(203)	172.5(4)
O(200)–C(201)–C(202)–O(202)	-67.9(6)
O(200)–C(201)–O(205)–C(205)	179.3(5)
C(201)–C(202)–C(203)–C(204)	-54.3(6)
O(202)–C(202)–C(203)–O(203)	60.5(6)
C(202)–C(203)–C(204)–C(205)	56.1(7)
C(202)–C(203)–C(204)–O(300)	173.5(5)
O(203)–C(203)–C(204)–C(205)	179.7(5)
O(203)–C(203)–C(204)–O(300)	-62.9(7)
C(203)–C(204)–C(205)–C(205)	-179.0(1)
C(203)–C(204)–C(205)–O(205)	-59.8(7)
C(203)–C(204)–O(300)–C(301)	122.3(6)
C(205)–C(204)–O(300)–C(301)	-120.5(7)
C(204)–C(205)–C(206)–O(206)	51.0(2)
C(204)–C(205)–O(205)–C(201)	65.0(7)
C(205)–O(205)–C(201)–C(202)	-62.3(7)
C(205)–O(205)–C(201)–O(200)	179.3(5)
O(205)–C(201)–C(202)–O(202)	174.7(5)
O(205)–C(205)–C(206)–O(206)	-68.0(2)
O(205)–C(201)–C(202)–C(203)	55.0(6)
C(206)–C(205)–C(204)–O(300)	63.0(1)
C(204)–O(300)–C(301)–C(302)	154.9(8)
C(204)–O(300)–C(301)–O(305)	-84.9(8)
O(300)–C(301)–C(302)–C(303)	69.0(1)
O(300)–C(301)–C(302)–O(302)	-169.5(9)
C(301)–C(302)–C(303)–C(304)	53.0(1)
C(301)–C(302)–C(303)–O(303)	179.0(1)
O(302)–C(302)–C(303)–O(303)	59.0(2)
C(302)–C(303)–C(304)–C(305)	-55.0(1)
C(302)–C(303)–C(304)–O(304)	-177.0(1)
O(303)–C(303)–C(304)–O(304)	64.0(1)
C(303)–C(304)–C(305)–C(306)	177.0(1)
O(303)–C(303)–C(304)–C(305)	-174.0(1)
C(303)–C(304)–C(305)–O(305)	58.0(1)
O(304)–C(304)–C(305)–O(305)	179.3(9)
O(304)–C(304)–C(305)–C(306)	-62.0(1)
C(304)–C(305)–O(305)–C(301)	-60.4(9)
C(306)–C(305)–O(305)–C(301)	178.0(1)
C(305)–O(305)–C(301)–C(302)	57.1(9)
C(305)–O(305)–C(301)–O(300)	-61.3(9)
O(305)–C(301)–C(302)–O(302)	69.0(1)
O(305)–C(301)–C(302)–C(303)	-52.0(1)

are not available; however, some examples for an α-1-6 linkage exist where values around 180° (antiperiplanar arrangement) also dominate for this torsion angle. Numerical values are −173.9° for α-malibiose,[26] 172.5° for planteose,[27] and −169.5° for raffinose.[28] For the bond O(200)–C(201) the torsion angle C(106)–O(200)–C(201)–O(205) = −74.8° is again close to the −60° value of the *exo*-anomeric effect.

The glycosidic linkage between the final two monosaccharide

Table 5. Cremer–Pople puckering parameters of the eight six-membered rings (e.s.d.s in parentheses)

Ring	Q[Å]	θ[°]	Φ[°]
A	0.563(5)	5.2(5)	263(5)
B	0.579(5)	11.2(5)	4(3)
C	0.525(6)	48.8(6)	50.2(8)
D	0.534(6)	18.6(6)	152(2)
E	0.550(9)	174.8(9)	76(10)
F	0.599(5)	8.9(5)	318(3)
G	0.593(7)	4.0(7)	298(9)
H	0.55(1)	177(1)	93(24)

Table 6. Hydrogen bonding distances (Å)

Sequence	O ··· O (Å)	Symmetry operations for second O
O(3)–H(3O) ··· O(102)	2.896(6)	$\frac{1}{2} - x, \frac{1}{2} + y, 1 - z$
O(2)–H(2O) ··· O(203)	2.703(5)	$-\frac{1}{2} + x, -\frac{1}{2} + y, z$
O(23)–H(23O) ··· O(2)	2.716(6)	$x, -1 + y, z$
O(102)–H(102O) ··· O(1W)	2.78(1)	$x, -1 + y, z$
O(104) ··· O(104)	2.629(5)	$1 - x, y, 1 - z$
O(104) ··· O(202)	2.716(7)	$1 - x, y, 1 - z$
O(202)–H(202O) ··· O(3)	2.725(5)	$\frac{1}{2} + x, \frac{1}{2} + y, z$
O(203)–H(203O) ··· O(303)	2.675(9)	$x, -1 + y, z$
O(302) ··· O(302)	2.994(9)	$1 - x, y, -z$
O(302)–H(302O) ··· O(2W)	2.75(2)	x, y, z
O(304)–H(304O) ··· O(23)	2.87(1)	$\frac{1}{2} + x, \frac{3}{2} + y, z$
O(1W) ··· O(28)	2.77(1)	x, y, z
O(3W) ··· O(206)	2.66(2)	x, y, z

components is an α-1-4 linkage between a $^4C_1$ and a $^1C_4$ chair conformation. The C(301) atom is in an anticlinal conformation relative to C(203) and C(205), *i.e.* it is eclipsed by H(204). Along the bond O(300)–C(301) the torsion angle between C(204) and O(305) (−84.9°) is remarkably larger than expected for an ideal gauche orientation. However, it has been shown[29] that this torsion angle is close to 60° according to the *exo*-anomeric effect only in methyl pyranosides. In oligosaccharides it is always found to be $>60°$ and even reaches 122° in β-maltose.[30]

A number of intermolecular contacts exist in the crystal lattice due to the large number of solvent molecules. Because of the disorder of these molecules and some positional uncertainties of the hydrogens the hydrogen bonding scheme could not undergo complete determination. However, the hydrogen bonds given in Table 6 surely exist. Hydroxy groups of the asiaticoside molecule as possible donors contribute to intermolecular hydrogen bonds, with the exception of O(103). Further contacts, especially with the solvent molecules, may exist, but were not considered.

Acknowledgements

We thank Dr. B. N. Pramanik, Schering Plough Pharmaceutical Research Division, Bloomfield, New Jersey, U.S.A. for f.a.b.-m.s.

References

1 B. N. Sastri (ed.) 'The Wealth of India, Raw Materials,' vol. II C.S.I.R., New Delhi, 1950, p. 116.
2 P. Baiteau, A. Buzas, E. Lederer, and J. Polonosky, *Nature*, 1949, **163**, 258.
3 *Anon.*, *Nature*, 1945, **155**, 601.
4 J. Polonsky, *Cienc. Cult.* (*Maracaibo*), 1968, **20**, 19.
5 S. C. Bhattacharya, *J. Indian Chem. Soc.*, 1956, **33**, 893.
6 R. P. Rastogi, B. Sarkar, and M. L. Dhar, *J. Sci. Ind. Res.*, 1960, **19B**, 2527.
7 T. Dutta and U. P. Basu, *J. Sci. Ind. Res.*, 1962, **21B**, 239.
8 R. P. Rastogi and M. L. Dhar, *Indian J. Chem.*, 1963, **1**, 267.
9 J. Polonosky, E. Sach, and E. Lederer, *Bull. Soc. Chim. Fr.*, 1959, 880.
10 J. Polonosky and M. Jean Zylber, *Bull. Soc. Chim. Fr.*, 1961, 1586.
11 D. H. Williams, C. Bradley, G. Bojesen, S. Santikarn, and L. C. E. Taylor, *J. Am. Chem. Soc.*, 1981, **103**, 5700.
12 M. Barber, R. S. Bordoli, R. D. Sedgwick, and A. N. Tyler, *J. Chem. Soc., Chem. Commun.*, 1981, 325.
13 C. Fenselau, *J. Nat. Prod.*, 1984, **47**, 215.
14 S. Seo, Y. Tomita, and K. Tori, *Tetrahedron Lett.*, 1975, 7.
15 T. Tsuyuki, Y. Hamada, T. Honda, T. Takahashi, and K. Matsushita, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 3127.
16 S. Shigenaga, I. Kouno, and N. Kawano, *Phytochemistry*, 1985, **24**, 115.
17 S. B. Mahato, N. P. Sahu, A. N. Ganguly, R. Kasai, and O. Tanaka, *Phytochemistry*, 1980, **19**, 2017.
18 S. Seo, Y. Tomita, K. Tori, and Y. Yashimura, *J. Am. Chem. Soc.*, 1978, **100**, 333.
19 C. J. Gilmore, MITHRIL, 'A Computer Program for the Automatic Solution of Crystal Structures from *X*-Ray Data,' University of Glasgow, Scotland, 1983.
20 J. M. Stewart (ed.) 'The *X*-Ray Program System,' Version 1976, University of Maryland, 1976.
21 J. Buschmann, E. Müller, and P. Luger, *Acta Crystallogr., Sect. C*, 1986, **42**, 873.
22 E. Keller, *Chem. Unserer Zeit.*, 1980, **14**, 56.
23 D. Cremer and J. A. Pople, *J. Am. Chem. Soc.*, 1975, **97**, 1354.
24 P. Luger and R. Bülow, *J. Appl. Crystallogr.*, 1983, **16**, 431.
25 M. Laing, M. E. Burke-Laing, R. Bartok, and R. C. M. Weeks, *Tetrahedron Lett.*, 1977, 3839.
26 J. A. Kanters, G. Roelofsen, H. M. Doesburg, and T. Koops, *Acta Crystallogr.. Sect. B*, 1976, **32**, 2830.
27 D. C. Rohrer, *Acta Crystallogr., Sect. B*, 1972, **28**, 425.
28 H. M. Berman, *Acta Crystallogr.. Sect. B*, 1970, **26**, 290.
29 G. A. Jeffrey, J. A. Pople, J. S. Binkley, and S. Vishweshwara, *J. Am. Chem. Soc.*, 1978, **100**, 373.
30 M. E. Gress and G. A. Jeffrey, *Acta Crystallogr.. Sect. B*, 1977, **33**, 2490.

Received 18*th November* 1986; *Paper* 6/2221